# Ring-Opening Polymerization Reaction of Polyhedral Oligomeric Silsesquioxanes (POSSs) for Preparation of Well-controlled 3D Skeletal Hybrid Monoliths

**Supporting Information**

**Hui Lin[a,b], Junjie Ou[a,*], Zhenbin Zhang[a,b], Jing Dong[a], Hanfa Zou[a,*]**

[a] CAS Key Laboratory of Separation Science for Analytical Chemistry, National Chromatographic R & A Center, Dalian Institute of Chemical Physics, Chinese Academy of Sciences (CAS), Dalian 116023, China

[b] Graduate School of Chinese Academy of Sciences, Beijing 100049, China

[*] To whom correspondence should be addressed:

Prof. Hanfa Zou

Tel: +86-411-84379610

Fax: +86-411-84379620

E-mail: hanfazou@dicp.ac.cn

Dr. Junjie Ou

Tel: +86-411-84379576

Fax: +86-411-84379620

E-mail: junjieou@dicp.ac.cn

# Experimental section

## Materials

Octaglycidyldimethylsilyl POSS (PSS-octa[(3-glycidyloxypropyl)dimethylsiloxy] substituted) (POSS-epoxy), hexamethylene diamine (HDA), 1,8-diaminooctane (DAO), 1,10-diaminodecane (DAD), 1,12-diaminododecane (DADD), poly(ethylene glycol) (PEG, Mn=10,000) and (3-aminopropyl)triethoxysilane (APTES) were purchased from Aldrich (Milwaukee, WI, USA). The fused-silica capillaries with dimension of 50 and 75 μm i.d. and 365 μm o.d. were obtained from the Refine Chromatography Ltd. (Yongnian, Hebei, China). Trypsin was purchased from Promega (Madison, WI, USA). Bovine serum albumin (BSA), thiourea, benzene, toluene, phenol and other standard compounds were all obtained from Sigma (St Louis, MO, USA). Dithiothreitol (DTT) and iodoacetamide (IAA) were purchased from Sino-American Biotechnology Corporation (Beijing, China). HPLC-grade acetonitrile (ACN) was used for the preparation of mobile phases. The water used in all experiments was doubly distilled and purified by a Milli-Q system (Millipore Inc., Milford, MA, USA). C18-particles (5 μm, 120 Å pore) were purchased from DAISO (Osaka, Japan). Other chemical reagents were all of analytical grade.

## Preparation and modification of the POSS-based hybrid monoliths

Before the preparation of the monolithic columns, the fused-silica capillary was pretreated and rinsed by 1.0 M NaOH for 4 h, water for 30 min, 1.0 M HCl for 14 h, and water for another 30 min, successively, and then dried by a nitrogen stream at room temperature. The mixture of (3-aminopropyl)triethoxysilane (APTES)/methanol (50%, v/v) was used to introduce the $-NH_2$ group onto the inner surface of capillary for anchoring monolith matrices to the capillary inner wall, as described in the previous report,[1] and then the prepolymerization mixture with the feed recipes listed in Table 1 was injected into the modified capillaries with a syringe. The fulfilled capillaries were then sealed with rubber stoppers and immersed in a water bath at 50 $^{o}$C for 24 h. After that, the capillaries were flush with methanol. For bulk hybrid monoliths, the

prepolymerization mixture was placed in a centrifuge tube and reacted under the same conditions. After polymerization, the bulk hybrid monoliths were cut into smaller pieces, extracted with ethanol overnight in a Soxhlet apparatus and dried in a vacuum. For the modification of the resulting hybrid capillary monolithic column, a $H_2O$/ACN (50%, v/v) mixture contain 2.5% (v/v) ammonia was pumped through the column and reacted at 50 $^oC$ overnight, then the columns were washed with methanol.

**Instrumentation**

The chromatographic evaluation of hybrid monolithic columns was performed on an LC system equipped with an Agilent 1100 (Hewlett-Packard) micropump and a UV detector (K-2501, Knauer, Germany). Data was collected at 214 nm, and processed by a chromatography workstation (Beijing Cailu Scientific Instrument Ltd., Beijing, China). A 7725i injector with a 20 μL sample loop was used. A T-union connector served as a splitter with one end connected to the capillary monolithic column and the other end to a blank capillary (95-cm long, 50 μm i.d. and 365 μm o.d.). The split ratio was controlled at about 1/240. The outlet of the hybrid monolithic column was connected with a Teflon tube to a empty fused-silica capillary (75 μm i.d. and 365 μm o.d.), where a detection window was made by removing a 2 mm length of the polyimide coating in a position of 5.5 cm from the separation monolithic column outlet.

SEM images were obtained by using a JEOL JSM-5600 scanning electron microscope (JEOL, Tokyo, Japan). FT-IR spectra were measured with a Bruker Tensor 27 FT-IR spectrometer (Bruker Daltonics, Ettlingen, Germany). Pore size measurement was performed on an Autopore IV 9500 (Micromeritics, Norcross, USA). Thermal gravimetric analysis was carried out on a Setsys 16/18 (Setaram, Caluire, France). Nitrogen adsorption/desorption measurements of dry bulk monoliths were performed on a Quadrasorb SI surface area analyzer and pore size analyzer (Quantachrome, Boynton Beach, USA).

**Preparation of BSA Tryptic Digest and cHPLC-MS Analysis.**

The 2 mg BSA was dissolved in 1 mL of denaturing buffer containing 8 M urea and 100 mM

ammonium bicarbonate. After the addition of 20 μL of DTT (20 mM) solution, the mixture was incubated at 60 °C for 1 h to reduce the disulfide bonds of the protein. Subsequently, 7.48 mg IAA was added, and then the mixture was incubated at room temperature in the dark for 40 min. After that, the mixture was diluted 8-fold with 100 mM ammonium bicarbonate buffer (pH 8.2) and digested at 37 °C for 16 h with trypsin at enzyme-to-substrate ratio of 1:25 (w/w). After digesting, the pH value of the tryptic digestion solution was adjusted to 2-3 by 10% trifluoroacetic acid aqueous solution. Solid-phase extraction (SPE) was performed with a homemade C18 cartridge. The collected peptides were dried under vacuum and dissolved in a 0.1% formic acid aqueous solution (2 mL), and then stored in a -20 °C freezer before cLC-MS/MS analysis.

The cLC-MS/MS analysis was carried out on a Finnigan LTQ ion trap mass spectrometer (Finnigan, San Jose, USA) which interfaced with a surveyor MS pump. Buffer A was water (containing 0.1% formic acid), and buffer B was 100% ACN (containing 0.1% formic acid). Tryptic digests were automatically injected onto the column with buffer A for 3 min at the flow rate of 5 μL/min, the trapped peptides were then separated at a flow rate of ca. 200 nL/min on the POSS monolith (40 cm in length × 75 μm i.d.) or the C18-particles packed column (12 cm in length × 75 μm i.d.) with an integrated emitter, which was prepared by directly tapering the tip from the outlet of the capillary. The separation was performed with gradient elution from 5 to 80% ACN (containing 0.1% formic acid) within 65 min. The LTQ linear ion trap mass spectrometer was equipped with a nanospray ion source, and the temperature of the ion transfer capillary was set at 200 °C. The spray voltage was set at 1.8 kV, and the normalized collision energy was set at 35.0%. One microscan was set for each MS and MS/MS scan. All MS and MS/MS spectra were acquired in the data dependent mode. The mass spectrometer was set such that one full MS scan was followed by six MS/MS scans on the six most intense ions. The dynamic exclusion function was set as follows: repeat count 2, repeat duration 30 s, and exclusion duration 90 s. System control and data collection were done by Xcalibur software version 1.4 (Thermo, USA). The scan range was set from m/z 400 to m/z 1600.

The acquired MS/MS spectra were searched on a database using the SEQUEST (version 0.28) against an IPI_bovine_BOVIN_3.32 (32946 sequences; 16109453 residues). Cysteine residues were searched as fixed modification of 57.0215 Da, and methionine residues as variable modification of 15.9949 Da. Peptides were searched using fully tryptic cleavage constraints and

up to two internal cleavage sites were allowed for tryptic digestion. The mass tolerances were 2 Da for parent mass and 1 Da for fragment masses.

**References**

**1.** L. Ren, Z. Liu, Y. Liu, P. Dou and H.-Y. Chen, *Angew. Chem. Int. Ed.* 2009, **48**, 6704-6707.

**2.** J. Choi, J. Harcup, A. F. Yee, Q. Zhu and R. M. Laine, *J. Am. Chem. Soc.* 2001, **123**, 11420-11430.

## Supplementary Figures

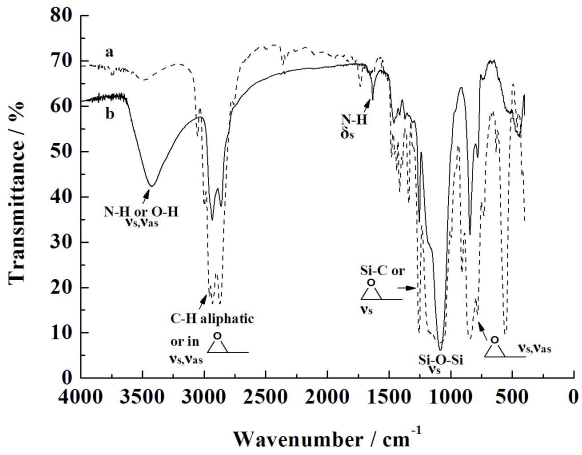


**Fig. S1** FT-IR spectra of a) octaglycidyldimethylsilyl POSS (POSS-epoxy), b) POSS-HDA monolith. $\nu_a$: symmetric vibration, $\nu_{as}$: asymmetric vibration, $\delta_s$: in-plane bending (scissoring).[2]

As shown in Fig. S1, a strong band at about 1085 $cm^{-1}$ is almost unchanged before and after reaction, whilst the peak at 1255 $cm^{-1}$ and the peaks range from 725 $cm^{-1}$ to 910 $cm^{-1}$ (assigned to the epoxy groups) are decreased. The broad peak at 3428 $cm^{-1}$ (assigned to the N-H and O-H groups) is increased remarkably increased, which was the product were production of the ring-opening reaction. In addition, a N-H peak at 1630 $cm^{-1}$ is observed only on the spectra of the hybrid materials. All these results demonstrated the occurrence of the ring-opening reaction forcefully.

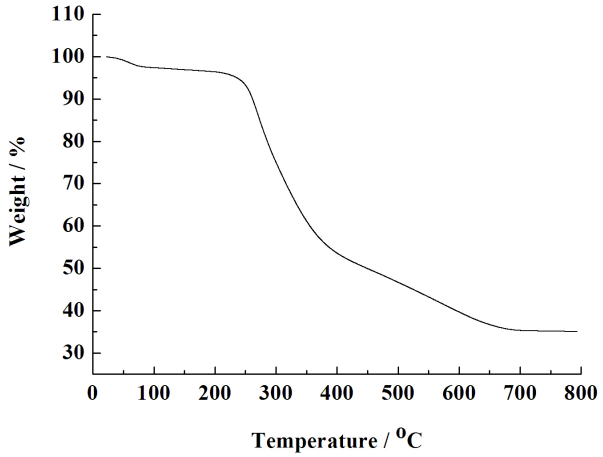


**Fig. S2** Thermal gravimetric analysis of the monolith POSS-HDA at a heating rate of 10 °C/min in air. The significant weight lost indicates the occurrence of the ring-opening polymerization.

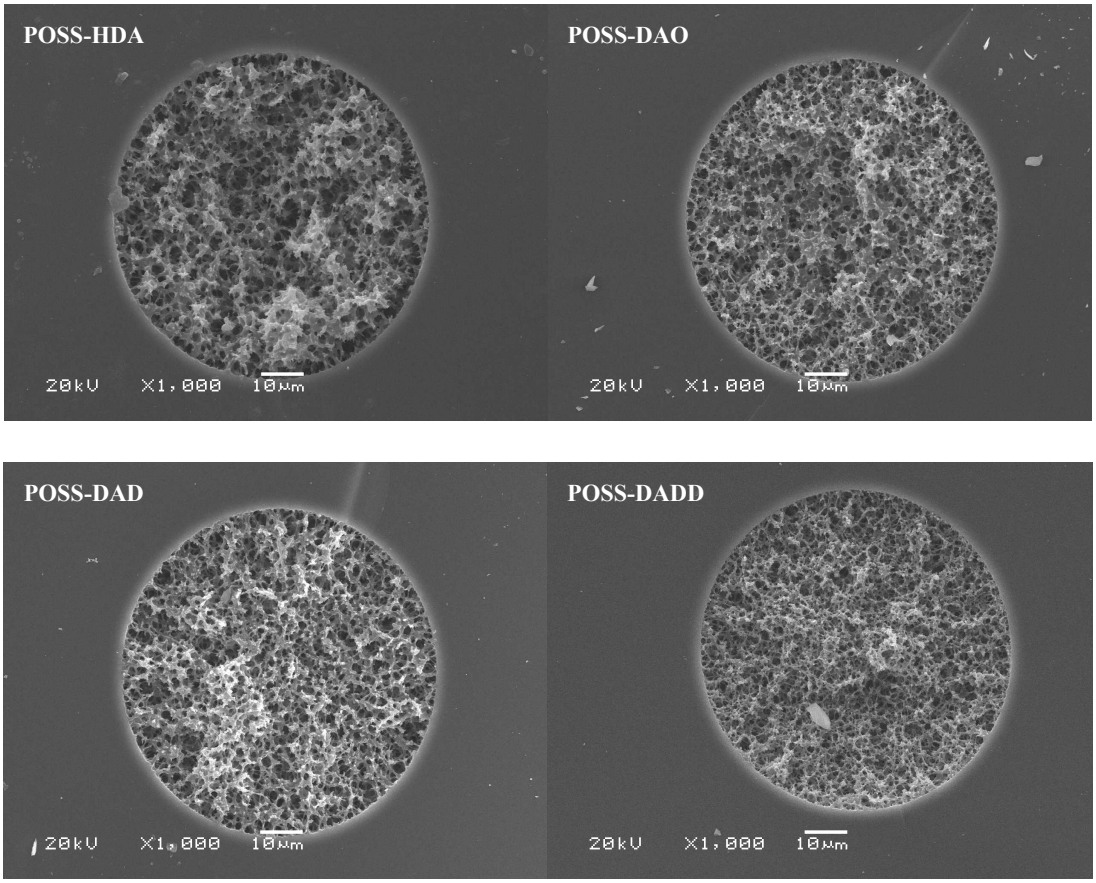


**Fig. S3** The cross-section of the monolithic columns prepared with different diamines. Magnification: 1000×.

The monolithic matrices anchored to the inner wall of the capillaries tightly, and well-controlled 3D skeleton was all achieved on these four monolithic columns.

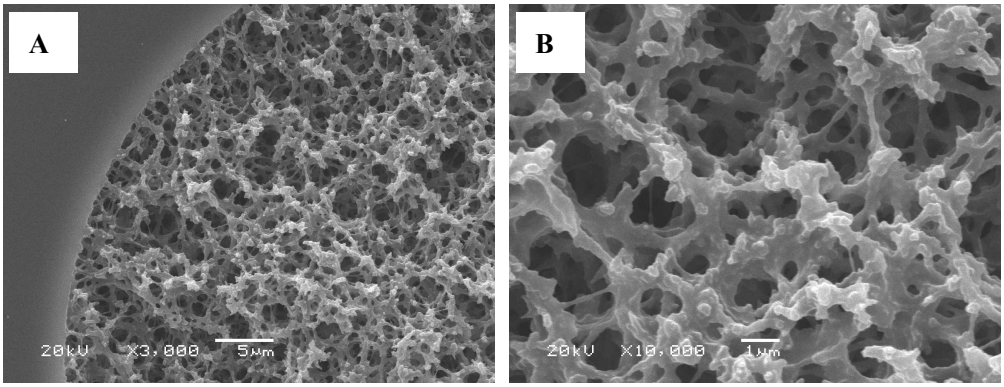


**Fig. S4** SEM images of the cross-section of the hybrid POSS-DADD monolithic column. Magnification: (A) 3000× and (B) 10000×.

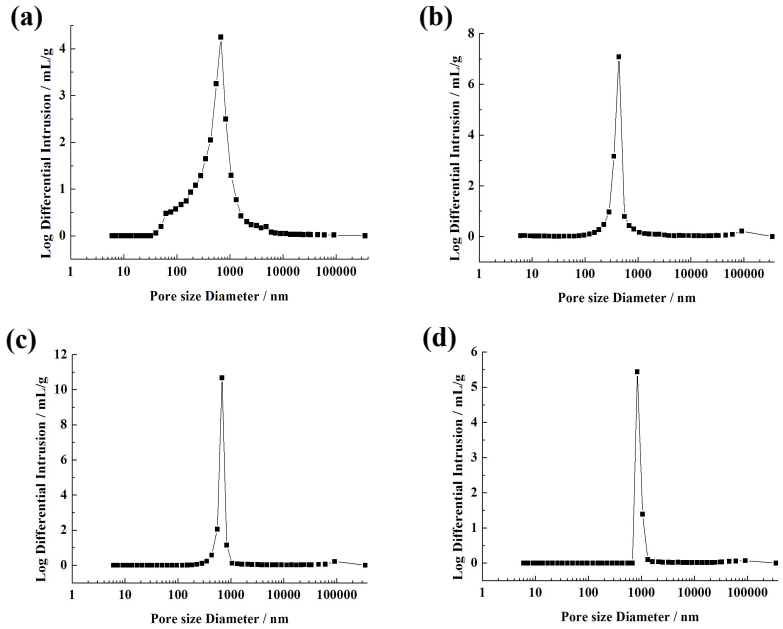


**Fig. S5** Pore size distribution of the monolith POSS-DADD (a) and other monoliths 1 (b), 4 (c) and 5 (d) in Table S2 in the Supporting Information by the mercury intrusion method.

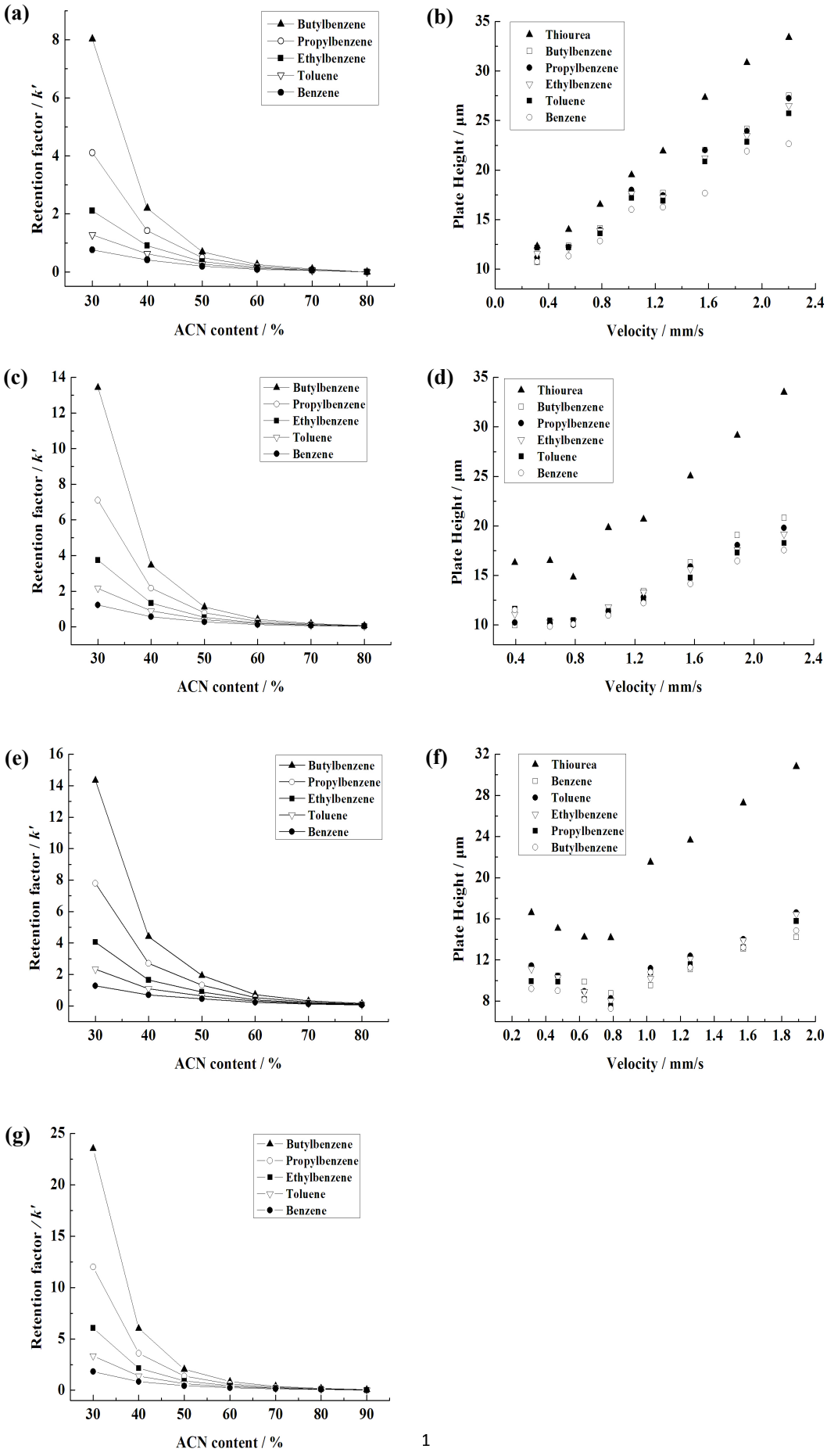
(a)
Retention factor / k'
ACN content / %
Butylbenzene
Propylbenzene
Ethylbenzene
Toluene
Benzene
(b)
Plate Height / μm
Velocity / mm/s
Thiourea
Butylbenzene
Propylbenzene
Ethylbenzene
Toluene
Benzene
(c)
Retention factor / k'
ACN content / %
Butylbenzene
Propylbenzene
Ethylbenzene
Toluene
Benzene
(d)
Plate Height / μm
Velocity / mm/s
Thiourea
Butylbenzene
Propylbenzene
Ethylbenzene
Toluene
Benzene
(e)
Retention factor / k'
ACN content / %
Butylbenzene
Propylbenzene
Ethylbenzene
Toluene
Benzene
(f)
Plate Height / μm
Velocity / mm/s
Thiourea
Benzene
Toluene
Ethylbenzene
Propylbenzene
Butylbenzene
(g)
Retention factor / k'
ACN content / %
Butylbenzene
Propylbenzene
Ethylbenzene
Toluene
Benzene

**Fig. S6** The influence of ACN concentration on the retention factors of alkylbenzenes (a, c, e, g) and the dependence of their plate heights on the linear velocity of the mobile phase (b, d, f) by the monoliths POSS-HDA (a, b), POSS-DAO (c, d), POSS-DAD (e, f) and POSS-DADD (g) in cLC. Experimental conditions: column dimension for (a, b), 41 cm × 75 μm i.d., for (c, d), 49 cm × 75 μm i.d., for (e, f), 49 cm × 75 μm i.d., for (g), 48 cm × 75 μm i.d.; flow rate for (a, e), 50 μL/min (before split), for (c), 80 μL/min (before split), for (g), 100 μL/min (before split); mobile phase for (b, f), ACN/water (50/50, v/v), for (d), ACN/water (45/55, v/v); injection volume, 2.5 μL in split mode; detection wavelength, 214 nm.

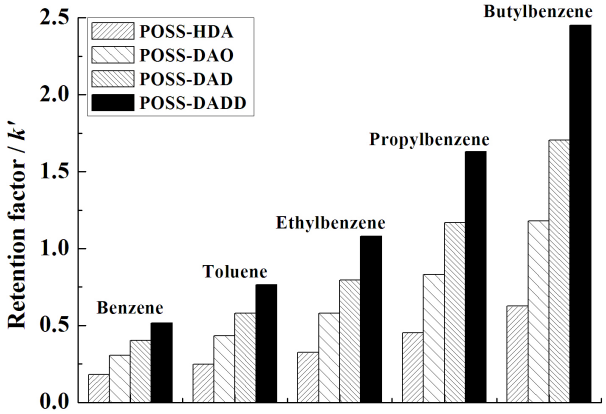


**Fig. S7** Comparison of the hydrophobicity of the hybrid monolithic columns prepared with different diamines. Experimental conditions: flow rate, 50 μL/min (before split); mobile phase, ACN/water (50/50, v/v); injection volume, 2.5 μL in split mode; detection wavelength, 214 nm.

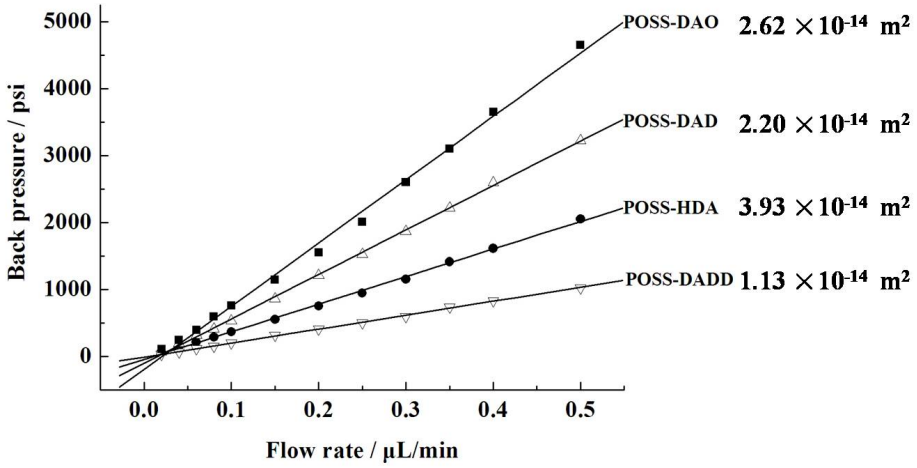


**Fig. S8** The relationships between flow rate and the back pressure drop of the monolithic columns. Experimental condition: mobile phase, ACN/water (40/60, v/v).

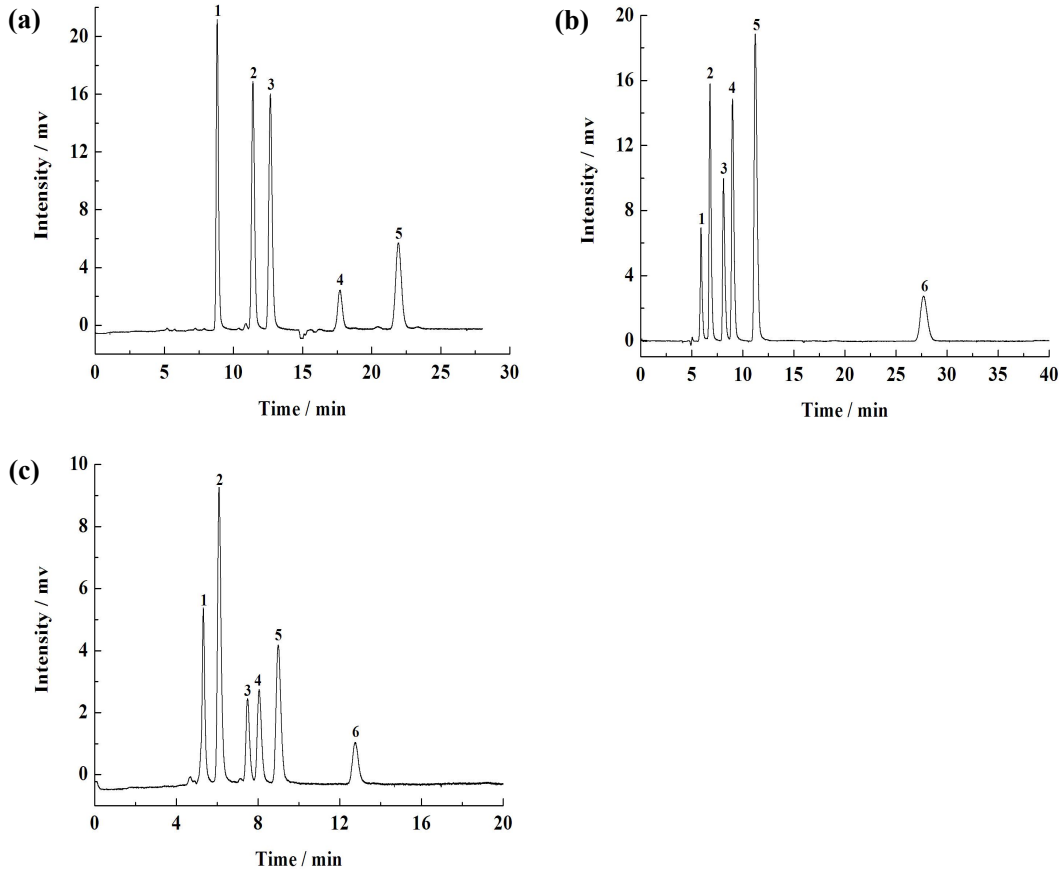


**Fig. S9** Separations of polycyclic aromatic hydrocarbons (PAHs) (a), phenols (b) and anilines (c) on the POSS-DADD monolithic column in cLC. Analytes: (a), (1) naphthalene, (2) acenaphthene, (3) 4,4'-dimethylbiphenyl, (4) p-terphenyl, (5) pyrene; (b), (1) hydroquinone, (2) resorcinol, (3) pyrocatechol, (4) phenol, (5) 4-cresol, (6) 4-tert-butylphenol; (c), (1) 2,4-diaminotoluene, (2) benzidine, (3) 2-nitroaniline, (4) 2,4-dinitroaniline, (5) 4-aminobiphenyl, (6) 2,6-dichloro-4-nitroaniline; Experimental conditions: column dimensions, 47 cm × 75 μm i.d.; mobile phase for (a), ACN/water (60/40, v/v), for (b), ACN/water (35/65, v/v), for (c), ACN/water (50/50, v/v); flow rate, 60 μL/min (before split); injection volume, 2.5 μL in split mode; detection wavelength, 214 nm.

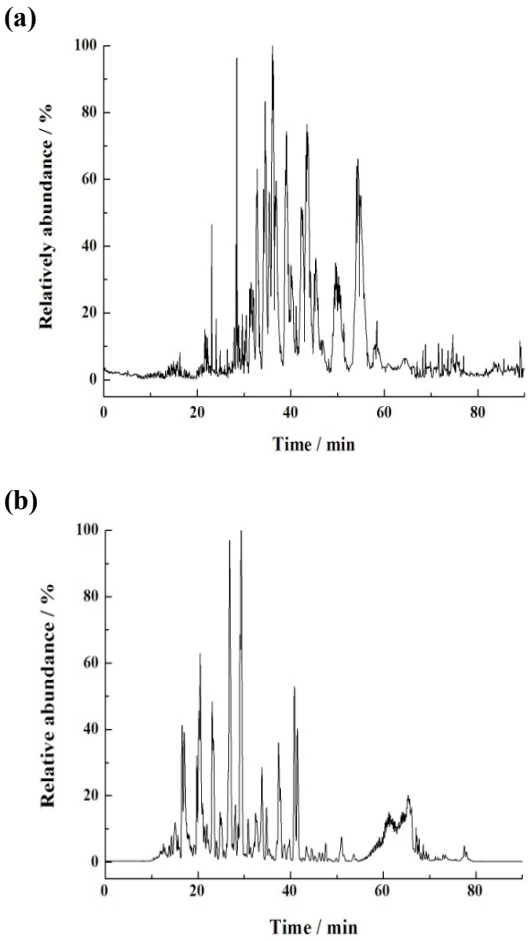


**Fig. S10** Base-peak chromatograms of cLC-MS/MS analysis of a BSA tryptic digest on monolith POSS-DADD (a) and C18-particles packed column (b). Experimental conditions: column dimensions for (a), 40 cm × 75 μm i.d., for (b), 12 cm × 75 μm i.d.; mobile phase: buffer A, 100% water (containing 0.1% formic acid), buffer B, 100% ACN (containing 0.1% formic acid); separation gradient, buffer B from 5% to 80% in 65 min, flow rate, 200 nL/min (after split).

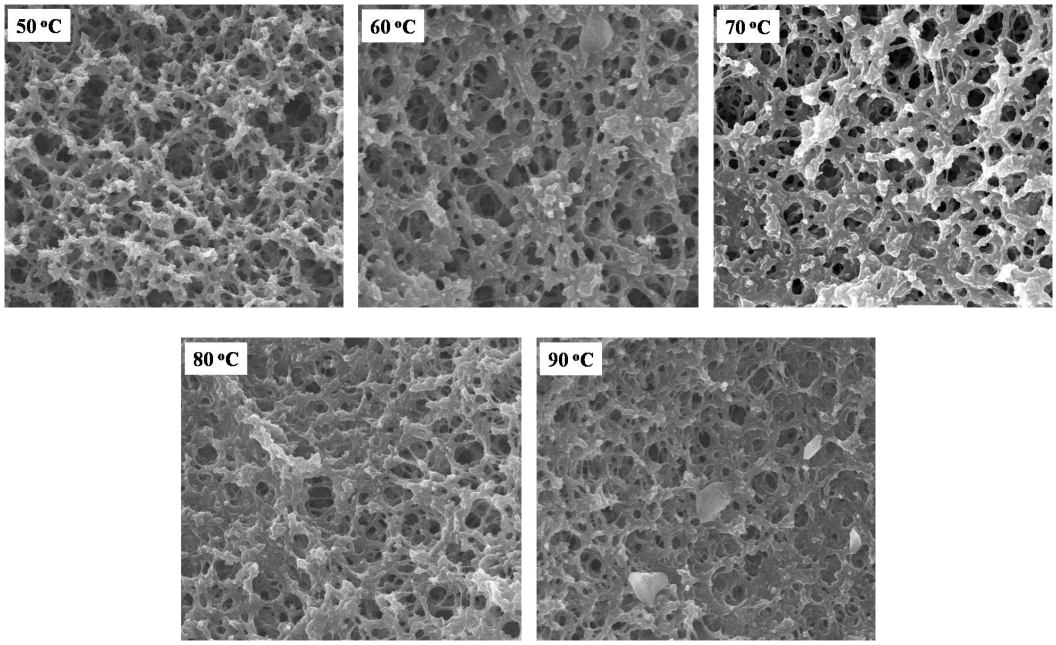


**Fig. S11** The SEM images of the monolith POSS-DADD prepared at different temperatures. Magnification: 3000×.

By comparing the monoliths prepared at different temperatures, it could be found that the pore size decreased as the polymerization temperatures increased.

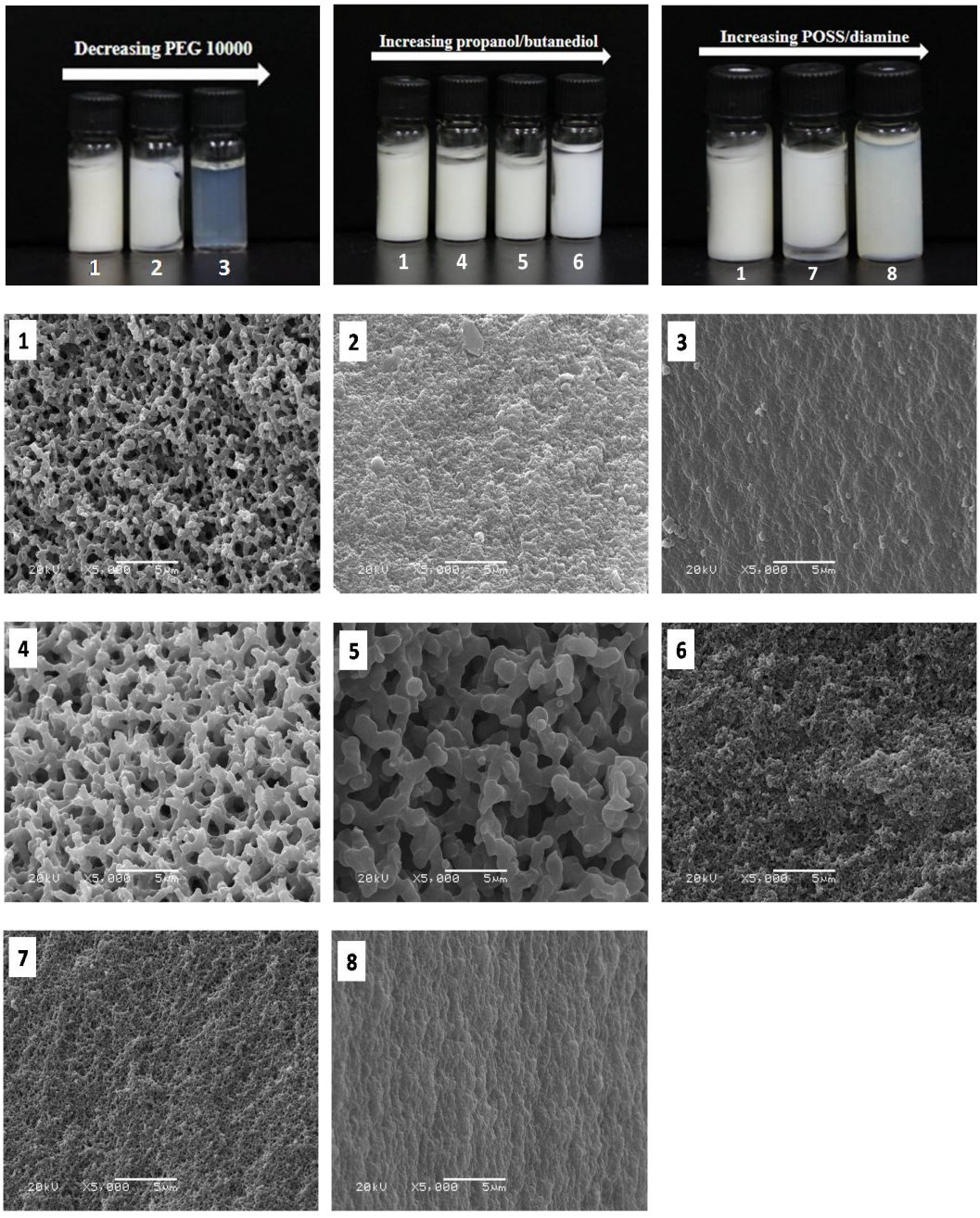


**Fig. S12** The bulk hybrid materials prepared with different feed recipes and their SEM images. Magnification: 5000×.

As shown above, the pore size of the hybrid monoliths decreased with either a decrease of the fraction of PEG 10,000 or an increase of the fraction of propanol. It is promising that hybrid materials with different high surface areas and high ordered 3D skeleton could be achieved conveniently by slightly adjusting the component of the porogenic solvents (see Table S2 for the detail preparation information).

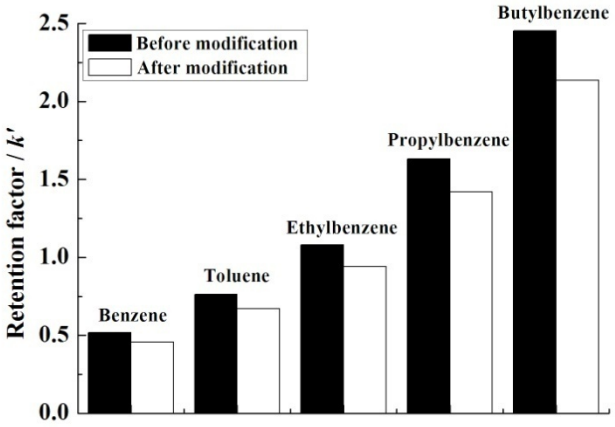


**Fig. S13** The retention factors of alkylbenzenes on the monolith POSS-DADD before and after $NH_3 \cdot H_2O$ modification. Experimental conditions: column dimension, 38 cm × 75 μm i.d.; flow rate, 50 μL/min (before split); mobile phase, ACN/water (50/50, v/v); injection volume, 2.5 μL in split mode; detection wavelength, 214 nm.

The decrease in retention indicated the existence of the residual epoxy groups, and demonstrated the good tailorability of the prepared hybrid materials.

## Supplementary Tables

**Table S1.** Comparison of the performance of the monolithic column and C18-particles packed column in cLC-MS/MS.

| Column | Unique peptides | Sequence coverage / % |
|---|---|---|
| **POSS-DADD monolith** | **51** | **57.0** |
| **C18-particles packed column** | **44** | **59.3** |

503

504 **Table S2.** Components of the prepolymerization solution of the bulk hybrid materials.

| Monolith | POSS (mg) | HDA (mg) | Propanol (µL) | 1,4-Butanediol (µL) | PEG10,000 (mg) | Temp ($^{o}$C) | BET Surface area ($m^2$/g) | Total pore volume (mL/g) | Average pore size (nm) |
|---|---|---|---|---|---|---|---|---|---|
| **1** | **50** | **15** | **280** | **40** | **30** | **70** | **11.46** | **1.63** | **336** |
| **2** | **50** | **15** | **280** | **40** | **15** | **70** | **0.57** | **/** | **/** |
| **3** | **50** | **15** | **280** | **40** | **0** | **70** | **0.47** | **/** | **/** |
| **4** | **50** | **15** | **280** | **80** | **30** | **70** | **6.78** | **1.57** | **802** |
| **5** | **50** | **15** | **280** | **0** | **30** | **70** | **3.75** | **0.79** | **1109** |
| **6** | **50** | **15** | **560** | **0** | **30** | **70** | **46.53** | **0.64** | **64** |
| **7** | **50** | **5** | **280** | **40** | **30** | **70** | **79.09** | **0.25** | **47** |
| **8** | **100** | **5** | **280** | **40** | **30** | **70** | **43.82** | **0.07** | **94** |

505

506

507

508

509

510

511

512

513

514

515

516

517

518

519

520

521

522