Electronic Supporting Information

New peptide-silica bio-inspired stationary phase with an improved approach for hydrophilic interaction liquid chromatography

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Experimental

Reagents and materials

L-Tyrosine and L-Alanine were purchased from Wako (Osaka, Japan), DCC was purchased from Wako chemicals. HOBt was purchased from Dojindo chemicals, Japan. YMC silica (SIL-120-S5) having diameter 5µm, pore size 12 nm and surface area 300m^2 g^{-1} (YMC-gel, Kyoto, Japan) was used for modification. HPLC-grade methanol, acetonitrile was obtained from Wako (Osaka, Japan) and Nacalei Inc. Co. (Tokyo, Japan) respectively. Ammonium acetate was purchased from Wako Chemicals, Japan. All nucleosides and nucleobases were commercially available and used without further purification. Sulfamethoxazole and Sulfamethoxypyridazine were obtained from TCI (Tokyo, Japan). Sulfadiazine was obtained from Sigma (St Louis, MO, USA). Sulfamonomethoxine and Sulfaquinoxaline were obtained from Wako (Osaka, Japan).

Immobilization of APS on silica surface

3-aminopropyltrimethoxysilane (APS) grafted silica was prepared by refluxing porous silica gel (3.0 g) and (1.5 ml) APS in toluene for 24 hr. After successive washing with toluene, ethanol and diethyl ether the particles were dried in vacuum. The dried particles were characterized by elemental analysis.

Synthesis of Tripeptide Boc-YAY-OH

The tripeptide molecule was newly designed and synthesized by solution phase methodology (Scheme S1) for grafting with silica.

1 Boc-Tyr(1)-OH (1)
A solution of tyrosine 9.05 g (50 mmol) in a mixture of dioxan (100 mL), water (50 mL) and 1M NaOH (50 mL) was stirred and cooled in an ice-water bath. Di-*tert*-butylpyrocarbonate 10.4 g (52 mmol) was added and stirring was continued at room temperature for 6 hrs. Then the solution was concentrated *in vacuo* to about 15 to 20 mL, cooled in an ice-water bath, covered with a layer of ethyl-acetate (about 100 mL) and acidified with a dilute solution of $KHSO_4$ to pH 2-3 (Congo red). The aqueous phase was extracted with ethyl acetate and this operation was done repeatedly. The ethyl acetate extracts were pooled, washed with water and dried over anhydrous Na_2SO_4 and evaporated *in vacuo*. The pure material was obtained.

Yield = 11.2 g (40 mmol, 80%). (Found: C, 59.47; H, 6.52; N, 4.7%. $C_{14}H_{19}NO_5$ (281) requires: C, 59.78; H, 6.76; N, 4.98%).

2 Boc-Tyr(1)-Ala(2)-OMe (2)
9.85 g (35 mmol) of Boc-Tyr(1)-OH was dissolved in a mixture of 20 mL dichloromethane (DCM) in an ice-water bath. H-Ala-OMe was isolated from 7.21 g (70 mmol) of the corresponding methyl ester hydrochloride by neutralization, subsequent extraction with ethyl acetate and concentration to 10 mL and this was added to the reaction mixture, followed immediately by 7.21 g (35 mmol) of di-cyclohexaylcarbodiimide (DCC). The reaction mixture was allowed to come to room temperature and stirred for 24 hrs. DCM was evaporated, residue

was taken in ethyl acetate (60 mL), and dicyclohexylurea (DCU) was filtered off. The organic layer was washed with 2M HCl (3 × 50 mL), brine (2 × 50 mL), 1M sodium carbonate (3 × 50 mL) and brine (2 × 50 mL) respectively. Then dried over anhydrous sodium sulfate, and evaporated *in vacuo* to yield **2** as a solid sample.
Yield = 8.9 g (24.5 mmol, 70%). (Found: C, 58.98; H, 7.2; N, 7.49%. $C_{18}H_{26}N_2O_6$ (366) requires: C, 59.01 H, 7.10; N, 7.65%). ^{1}H NMR (400 MH$_Z$, $CDCl_3$) δ 6.98-6.96 (ring Hs of Tyr, 2H, d, J = 8Hz); 6.72-6.68 (ring Hs of Tyr, 2H, m); 6.39-6.37 (Ala NH, 1H, d, J = 8Hz); 4.98-4.96 (Tyr NH, 1H, d, J = 8Hz); 4.36-4.32 (C^{α}H of Tyr, 1H, m); 4.25 (C^{α}H of Ala, 1H, m); 3.73 (-OCH_3, 3H, s); 2.95-2.93 (C^{β}Hs of Tyr, 2H, m); 2.90-2.86(C^{β}H of Ala, 1H, m); 1.42 (Boc-CH_3s, 9H, s).

3 Boc-Tyr(1)-Ala(2)-OH (3)

To 8.05 g (22 mmol) of Boc-Tyr(1)-Ala(2)-OMe, 30 mL MeOH and 20 mL of 2M NaOH were added. The reaction mixture was stirred and the progress of saponification was monitored by thin layer chromatography (TLC). After 10 h methanol was removed under *vacuo*, the residue was taken in 50 mL of water, washed with diethyl ether (2 × 50 mL). Then the pH of the aqueous layer was adjusted to 2 using 1M HCl and it was extracted with ethyl acetate (3 × 50 mL). The extracts were pooled, dried over anhydrous sodium sulfate, and evaporated *in vacuo* to yield **3** as a solid compound.

Yield = 4.6 g (13 mmol, 60%). Found: C, 57.72; H, 6.61; N, 7.63%. $C_{17}H_{24}N_2O_6$ (352) requires C, 57.95, H, 6.81, N, 7.95%. ^{1}H NMR (400 MH$_Z$, DMSO-d_6) δ 7.93-7.91 (Phenolic OH of Tyr(1), 1H, d, J = 8Hz); 6.84-6.82 (Tyr(2) NH, 1H, d, J = 8Hz); 6.24-6.22 (Ala NH, 1H, d,); 4.14-4.12 (Tyr(1) NH, 1H, d, J = 8Hz); 4.05-3.99 (C^{α}H of Tyr(1) and C^{α}H of Ala, 2H, m); 2.85-2.80 (C^{β}Hs of Tyr(1), 2H and C^{β}H of Ala, 2H, m); 1.32 (Boc-CH_3s, 9H, s).

4 Boc-Tyr(1)-Ala(2)-Tyr(3)-OMe (4)
4.2 g (12 mmol) of Boc-Tyr(1)-Ala(2)-OH in 10 mL of DMF was cooled in an ice-water bath and H-Tyr-OMe was isolated from 4.68 g (24 mmol) of the corresponding methyl ester hydrochloride by neutralization, subsequent extraction with ethyl acetate and concentration to 10 mL and it was added to the reaction mixture, followed immediately by 2.47 g (12 mmol) DCC and 1.62 g (12 mmol) of HOBt. The reaction mixture was stirred for three days. The residue was taken in ethyl acetate (60 mL) and the DCU was filtered off .The organic layer was washed with 2M HCl (3 × 50 mL), brine (2 × 50 mL), 1M sodium carbonate (3 × 50 mL) and brine (2 × 50 mL) respectively. Then dried over anhydrous sodium sulfate and evaporated *in vacuo* to yield peptide **4** as yellowish-white solid.

Yield = 5.0 g (9.5 mmol, 80%). (Found: C, 61.2; H, 6.58; N, 7.83%. $C_{27}H_{35}N_3O_8$ (529) requires C, 61.24; H, 6.61; N, 7.93%.). ^{1}H NMR (400 MH$_Z$, $CDCl_3$) δ 6.99-6.97 (ring Hs of Tyr(3), 2H, d, J = 8Hz); 6.92-6.92 (ring Hs of Tyr(1), 2H, d, J = 8Hz); 6.72-6.68 (ring Hs of Tyr(1) and Tyr(3), 4H, m); 6.46-6.43 (Tyr(3) NH, 1H, d, J = 12Hz); 6.39-6.37 (Ala NH, 1H, d, J = 8Hz); 4.98-4.96 (Tyr(1) NH, 1H, d, J = 8Hz); 4.80-4.75 (C^{α}H of Tyr(3), 1H, m); 4.36-4.32 (C^{α}H of Tyr(1), 1H, m); 4.25 (C^{α}H of Ala, 1H, m); 3.73 (-OCH_3, 3H, s); 3.14-3.09 (C^{β}Hs of Tyr(3), 2H, m); 2.95-2.93 (C^{β}Hs of Tyr(1), 2H, m); 2.90-2.86(C^{β}H of Ala, 1H, m); 1.42 (Boc-CH_3s, 9H, s). ESI-HR-Mass $[M+Na]^+$ = 552.60, $[M+K]^+$ = 567.75, $M_{[Calcd]}$ = 529. FT-IR (KBr) 3332, 2986, 2938, 1760, 1683, 1656, 1518, 1448 cm^{-1}.

5 Boc-Tyr(1)-Ala(2)-Tyr(3)-OH (5)

To 4.76 g (9 mmol) of Boc-Tyr(1)-Ala(2)-Tyr(3)-OMe, 30 mL MeOH and 20 mL of 2M NaOH were added. The reaction mixture was stirred and the progress of saponification was monitored by thin layer chromatography (TLC). After 10 h methanol was removed under *vacuo,* the residue was taken in 50 mL of water, washed with diethyl ether (2 × 50 mL). Then the pH of the aqueous layer was adjusted to 2 using 1M HCl and it was extracted with ethyl acetate (3 × 50 mL). The extracts were pooled, dried over anhydrous sodium sulfate, and evaporated *in vacuo* to yield **5** as a solid compound.

Yield = 3.01 g (5.9 mmol, 65%). Found: C, 60.36; H, 6.21; N, 8.03%. $C_{26}H_{33}N_3O_8$ (515) requires C, 60.58, H, 6.40, N, 8.15%. ^{1}H NMR (400 MH_Z, DMSO-d_6) δ 9.27 (-COOH, 1H, b), 8.01 (Phenolic OH of Tyr(3), 1H, d), 7.94-7.92 (Phenolic OH of Tyr(1), 1H, d, J = 8Hz); 6.99-6.89 (ring Hs of Tyr(3) and Tyr (1), 4H, m); 6.85-6.83 (Tyr(3) NH, 1H, d, J = 8Hz); 6.68-6.62 (ring Hs of Tyr(3) and Tyr(1), 4H, m); 6.24-6.22 (Ala NH, 1H, d, J = 8Hz); 4.29 (C^{α}H of Tyr(3), 1H, m); 4.13 (Tyr(1) NH, 1H, d); 4.05-3.99 (C^{α}H of Tyr(1) and C^{α}H of Ala, 2H, m); 2.94-2.91(C^{β}Hs of Tyr(3), 2H, m); 2.85-2.80 (C^{β}Hs of Tyr(1), 2H, m and C^{β}H of Ala, 1H, m); 1.30 (Boc-CH_3s, 9H, s). C^{13} NMR (100 MH_Z, DMSO-d_6) δ 173.07, 172.15, 171.49, 155.89, 155.02, 114.94, 114.87, 114.77, 77.99, 40.01, 39.9-39.08, 38.86, 35.91, 31.3, 28.13. ESI-HR-Mass $[M+Na]^+$ = 538.41, $[M+K]^+$ = 553.38, $M_{[Calcd]}$ = 515. FT-IR (KBr) 3318, 2979, 2935, 1655, 1520, 1449 cm^{-1}.

Immobilization of Boc-YAY-OH on Silica-APS

Boc-YAY-OH was immobilized onto APS grafted silica (Sil-APS) by covalent linkages using DEPC as coupling reagent. APS-modified silica (3.0 g) was added to a 100 ml three-neck round-bottomed container. Boc-YAY-OH (2.5 g) was dissolved in 30 ml of THF and added into the container. Then DEPC (1.5g, 9.6 mmol) and TEA (1.1 g, 10.6 mmol) were added to the solution and stirred at 60 ºC. After being stirred for 1 day the grafted particles were centrifuged and washed with THF, methanol and diethyl ether several times to remove the unreacted lipid molecule and dried in vacuum. The obtained Sil-YAY was then packed into a stainless steel column. The Sil-YAY phase was characterized by elemental analysis, thermogravimetric analysis (TGA), DRIFT-mode FT-IR spectroscopy and Solid State C^{13} NMR spectroscopy.

Instruments and Chromatographic condition

NMR experiments: All NMR studies of the lipid molecules in CDCl3 and DMSO-D6 at 25 ºC were carried out with JEOL JNM-LA 400 (Japan) spectrometers at 400MHz. Chemical shifts (δ) of 1H expressed in parts per million (ppm) with use of the internal standards Me_4Si (δ = 0.00 ppm).

Solid state ^{13}C NMR experiment

Adamantine was used for adjusting the magic angle before experiment. NMR frequency referencing was performed by adjusting carbon peak of adamantine to 38.5 ppm. Representative samples of 200-250 mg were spun at 4000-4500 Hz using 7 mm double bearing ZrO_2 rotors.

Other important parameters were relaxation delay of 4.0 s, pulse of 85.5 degrees, acquisition time of 0.05 s, and spectral width of 40241.4 Hz. High power proton decoupling of 63db with fine attenuation of dipole r = 2500 was used only during detection period.

DRIFT mode Fourier-transform Infrared spectroscopy and Elemental Analysis

FT-IR measurements were conducted with JASCO FT/IR-4100 (Japan). For DRIFT measurement accessory DR PRO410-M (JASCO, Japan) was used. Samples were prepared by mixing the corresponding dried samples with KBr in a 1:100 (wt/wt) ratio. Elemental analyses were carried out on a Yanaco CHN Corder MT-6 Apparatus (Japan).

Scanning Electron Microscopic Data

Very small amount of dried Sil-YAY particles were placed on glass coverslip and allowed to vacuum dry. It was then coated with osmium by using Filgen OPC6ON. Then the micrographs were observed under HITACHI scanning electron microscope.

Chromatographic conditions

Sil-YAY material was packed into stainless-steel columns (150 × 4.6 mm i.d.) by the company, Masis, Inc. (Aomori, Japan). HPLC-grade methanol, acetonitrile and Milipore water were used as components of the mobile phase for HILIC. The chromatographic system consisted of a JASCO Gulliver PU-980 intelligent HPLC pump with a Rheodyne sample injector with a 20 µL sample loop and a JASCO multi wavelength UV detector MD 2010 plus was used. All chromatographic data were obtained by using a JASCO ChromNAV Chromatography Data System. The column temperature was controlled by using a column jacket with a circulator with a heating and cooling system. The chromatography was done under isocratic elution conditions. The flow rate was 1.0 mL min^{-1}, the detection wavelength was UV 254 nm, and the injection volume was 5 µL. The retention factor (k) was determined by $(t_e - t_0)/t_0$ where t_e and t_0 were the retention time of the samples and acetonitrile, respectively. The separation factor (α) was given by the ratio of retention factors for the two solutes being analyzed: $\alpha = k_2/k_1$.

Characterization of short peptide based stationary phase

The elemental analysis results for Sil-APS and Sil-YAY are shown in Table S1. The result of the elemental analysis clearly showed that nitrogen content has a significant increase. The C/N value of Sil-APS is 2.95, which indicates that almost all of the methoxy groups of APS were consumed for silanation to silica for cross linking, or both. The surface concentration of bonded APS was calculated from the nitrogen percentage (N%) of Sil-APS to be 6.34 µmol m^{-2}. Surface concentration of Sil-YAY stationary phases was calculated by using the equation given below by Unger et al using the nitrogen percentage.

$$\text{Amount of bonded phase } (\mu\text{mol/m}^2) = \frac{\%\ X \times 10^6}{(AM)n100(1 - \%\ X(MW)/(AM)n100)S} \quad \ldots.(1)$$

Where % X is the percent carbon or nitrogen increase in the bonded support as determined by elemental analysis, AM is the atomic mass of carbon or nitrogen, MW is the molecular weight of the species bonded to the silica surface, n is the number of carbon or nitrogen atoms present in

the bonded species, and *S* is the specific surface area of the silica support in meters squared per gram.

FT-IR measurements

Infrared spectrometry is one of the useful tools to identify the chemical modifications of compounds. From Fig. S1, it was observed that for Sil-Aps, Si-O-Si bands appeared at 1300-1000 cm^{-1} (broad). The band at 1564 cm^{-1} was attributed to the N-H bending vibrations of Sil-Aps. After grafting with small peptides two new bands amide I and amide II clearly observed. For Sil-YAY amide I band appears at 1654 cm^{-1} and amide II band appears at 1517 cm^{-1}. These results confirmed that tri-peptide Boc-Y-A-Y-OH was grafted on silica surface successfully.

Thermogravimetric analysis

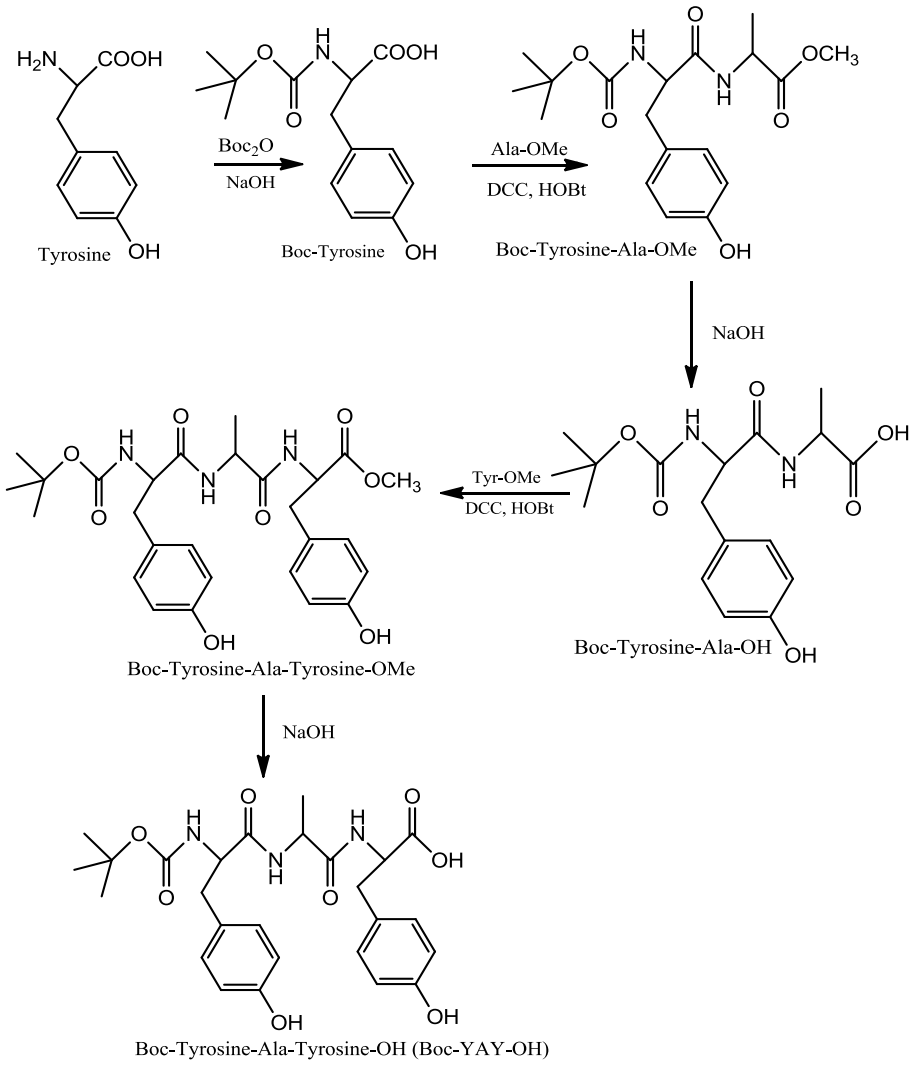
Thermogravimetric curves are usually used to determine thermal stability and to confirm the amount of immobilized compounds. The weight loss observed between 40-800 ºC can be associated with loss of the organic groups attached to the silica surface. From Fig. S2, it has been observed that, Aps modified silica shows amass loss of about 7.99% from 200 to 800 ºC. After bonded with tripeptide Boc-YAY-OH, Sil-YAY shows a mass loss of about 14.04 %, that indicates the increased attachment of organic content in the grafted phase. These data are consistent with the immobilized amounts estimated by the elemental analyses.

Solid state ^{13}C NMR experiment

Solid-state ^{13}C NMR gives useful information about the chemical composition of the peptide grafted silica. In addition, it gives us evidence of successful graftation of peptide molecule over silica. From Fig. S12 it has been observed that the successful graftation of peptide Boc-YAY-OH has occurred.

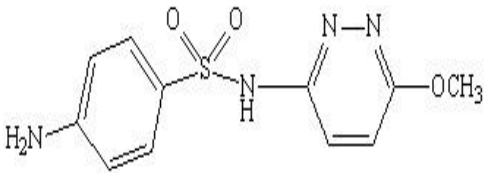
Scanning Electron Microscopic Study

From this study we observe the uniform-ness of the synthesized peptide grafted silica particle. It has been observed from Fig. S13, that the synthesized Sil-YAY particles are very uniform in size.

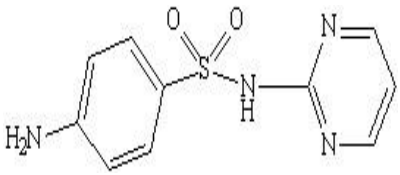


Scheme S1 Synthetic route for preparation of Boc-YAY-OH

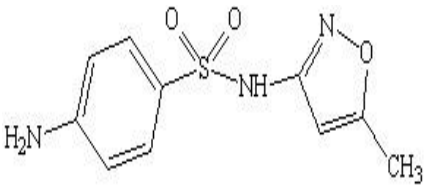
Structures of Sulfur based drug compound studied



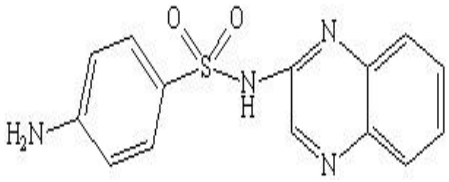
Sulfamethoxypyridazine (SMP)



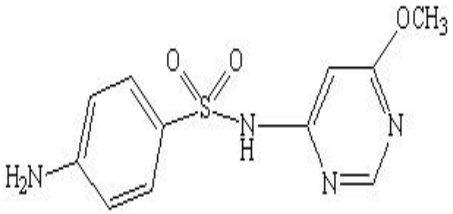
Sulfadiazine (SD)



Sulfamethoxazole (SM)



Sulfaquinoxaline (SQ)



Sulfamonomethoxine (SMM)

Table S1. Elemental analysis data of Sil-APS and Sil-YAY.

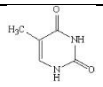
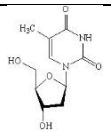
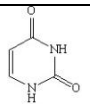
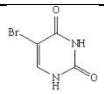
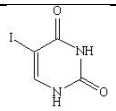
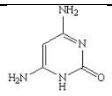
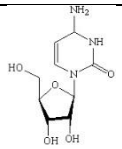
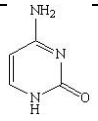
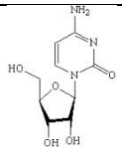
Sample	% C	% H	% N	C/N	Amount of bonded phase (μmol m^{-2})
Sil-APS	7.1	2.0	2.4	2.95	6.34
Sil-YAY	13.37	2.75	2.80	4.77	2.20

Table S2. Comparative Chromatographic data for Hypersil APS2[a], diol-modified silica[a], Sugar – silica-1[a], Sugar-silica-2[a], Sugar-sil-3[a] and Sil-YAY columns.

Sample	Hypersil APS2		Diol-modified silica		Sugar silica-1		Sugar silica-2		Sugar silica-3		Sil-YAY	
	k	α	*k*	α	*k*	α	*k*	α	*k*	α	*k*	α
Napthalene	0.00		0.00		0.00		0.00		0.00		0.00	
Uracil	0.47	1.47	0.23	2.43	0.24	3.62	0.76	2.64	0.79	2.87	0.49	2.98
Adenosine	0.69	1.27	0.56	1.68	0.87	1.36	2.01	1.27	2.27	1.29	1.46	1.69
Cytosine	0.88		0.94		1.18		2.55		2.93		2.48	

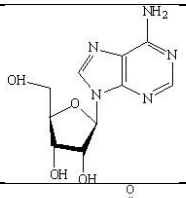
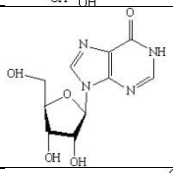
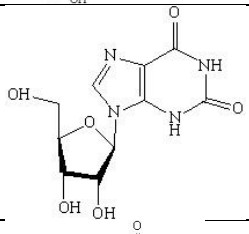
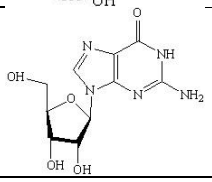
[a] Data taken from ref. *Chem. Eur. J.* 2010, **16**, 5721-5722, Eluent: acetonitrile/water = 80:20 (v/v); flow rate: 1.00 mL min^{-1}; T = 25 ºC; UV detection at λ = 254 nm. It has been observed Sil-YAY shows comparable selectivity as diol-modified silica.

Table S3. The retention factors of Pyrimidine bases with Sil-YAY column.

No	Compound	Structure	pk_a	Retention Factor (k)
1	Thymine		pk_a=9.9	0.375
2	Thymidine		pk_{a1}=9.8, pk_{a2}=12.85	0.58
3	Uracil		pk_a=9.5	0.490
4	5-fluorouracil		pk_a=8.0	0.618
5	5-bromo uracil		pk_a=7.93	0.672
6	5-iodouracil		pk_a=8.12	0.527
7	4,6 diamino pyrimidine		pk_{a1}=*, pk_{a2}=*	0.745
8	uridine		pk_{a1}=9.30, pk_{a2}=12.5	1.13
9	Cytosine		pk_{a1}=4.68, pk_{a2}=12.16	2.49
10	Cytidine		Pka_1=4.15, Pka_2=12.5	3.91

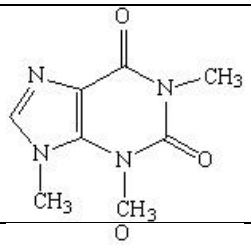
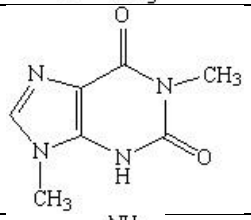
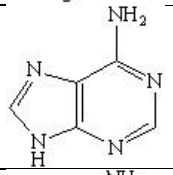
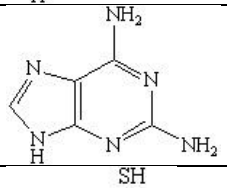
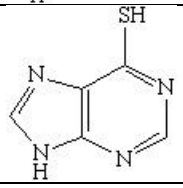
Eluent: acetonitrile/water = 90:10 (v/v) with 10 mM NH_4Ac, flow rate: 1.00 mL min^{-1}; T = 25 ºC; UV detection at λ = 254 nm. It has been observed that structures and pk_a values of analytes are directly related with their retention factor in Sil-YAY column. * Without available data.

Table S4. The retention factors of nucleosides with Sil-YAY column.

No	Compound name	Structure	pK_a	Retention Factor (k)
1	Adenosine		pk_{a1}=3.15, pk_{a2}=12.5	1.47
2	Inosine		pk_{a1}=8.8, pk_{a2}=12.5	4.02
3	Xanthosine		pk_{a1}=2.5, pk_{a2}=12.85	4.61
4	Guanosine		pk_{a1}=1.5, pk_{a2}=9.2	5.99

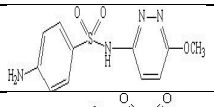
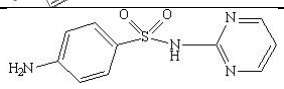
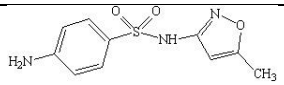
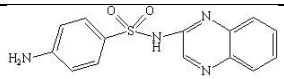
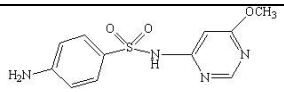
Eluent: acetonitrile/water = 90:10 (v/v) with 10 mM NH_4Ac, flow rate: 1.00 mL min^{-1}; T = 25 ºC; UV detection at λ = 254 nm.

Table S5. The retention factors of Purine bases with Sil-YAY column.

No	Compound name	Structure	pKa	Retention Factor (k)
1	Caffeine		pk_{a1}=*, pk_{a2}=>8.8	-0.13
2	Theophylline		pk_{a1}=<1, pk_{a2}=8.8	0.95
3	Adenine		pk_{a1}=4.15, pk_{a2}=9.8	1.7
4	Guanine		pk_{a1}=3.2, pk_{a2}=9.6	2.6
5	6-mercapto Purine		pk_{a1}=*, pk_{a2}=*	2.75

Eluent: acetonitrile/water = 90:10 (v/v) with 10 mM NH_4Ac, flow rate: 1.00 mL min^{-1}; T = 25 ºC; UV detection at λ = 254 nm. * Without available data.

Table S6. The retention factors of sulfur-based drug molecules with Sil-YAY column.

No	Compound name	Structure	pk_a	Retention Factor (k)
1	Sulfamethoxypyridazine (SMP)		pk_a=7.19	0.677
2	Sulfadiazine (SD)		pk_{a1}=4.5 pk_{a2}=6.1	1.96
3	Sulfamethoxazole (SM)		pk_{a1}=1.7 pk_{a2}=5.61	2.33
4	Sulfaquinoxaline (SQ)		pk_{a1}=6.4 pk_{a2}=5.5	4.29
5	Sulfamonomethoxine (SMM)		pk_{a1}=6.3 pk_{a2}=*	4.9

Eluent: acetonitrile/water = 90:10 (v/v) with 10 mM NH_4Ac, flow rate: 1.00 mL min^{-1}; T = 25 ºC; UV detection at λ = 254 nm. * Without available data.

Table S7. The chromatographic parameters collected at the separation of sulphur based molecules and nucleoanalytes.

Analytes	*k*	As	N/m
SMP	0.677	1.25	31 400
SM	1.96	1.08	30 200
SD	2.33	1.15	33 200
SMM	4.29	1.05	38 000
SQ	4.9	1.11	33 700
Thymine	0.375	0.93	32 500
Uracil	0.49	1.2	36 200
Thymidine	0.58	1.21	31 400
Uridine	1.13	1.08	30 400
Adenosine	1.45	1.26	33 200
Cytidine	3.91	1.05	38 200
Inosine	4.02	1.11	31 200
Guanosine	5.99	1.13	46 800

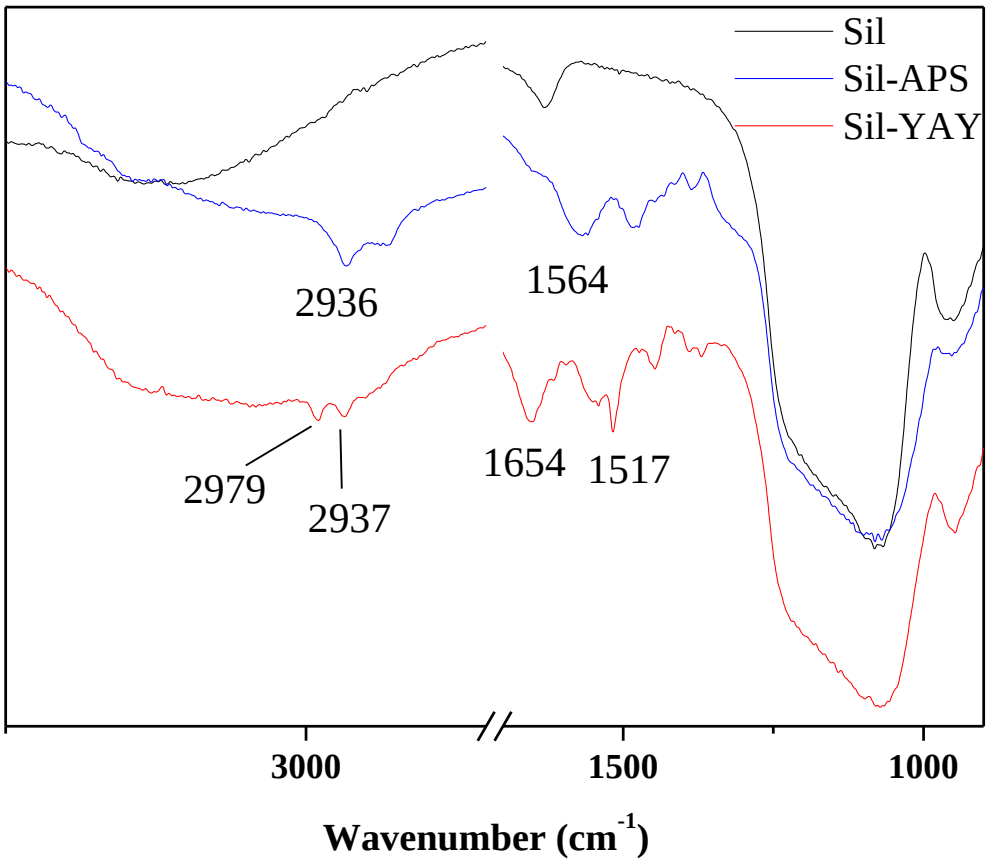


Fig. S1 DRIFT-mode FT-IR spectra of bare silica, silica-APS and silica-YAY.

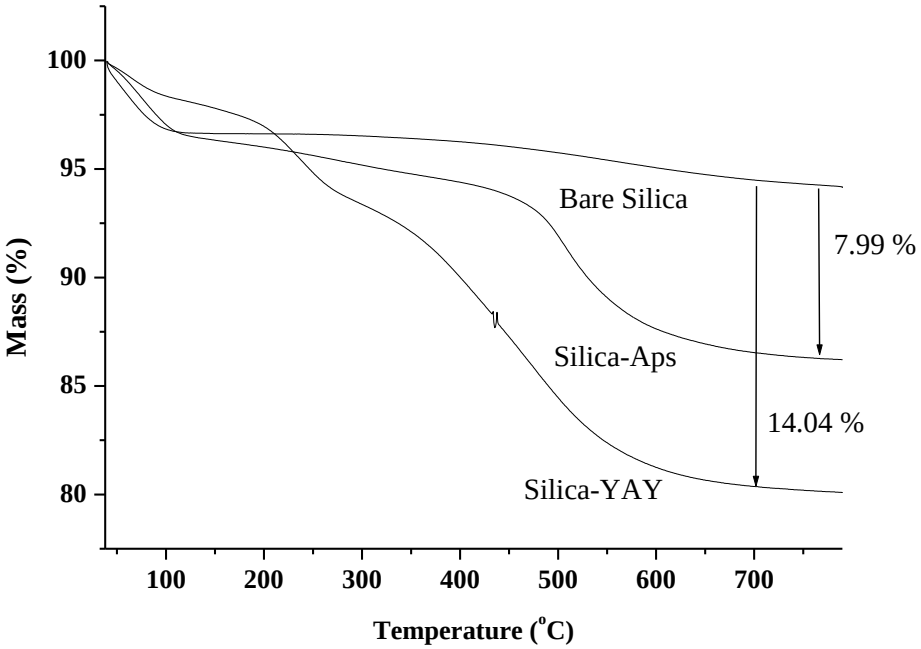


Fig. S2 Thermogravimetric curves of bare silica, silica-Aps and silica-YAY.

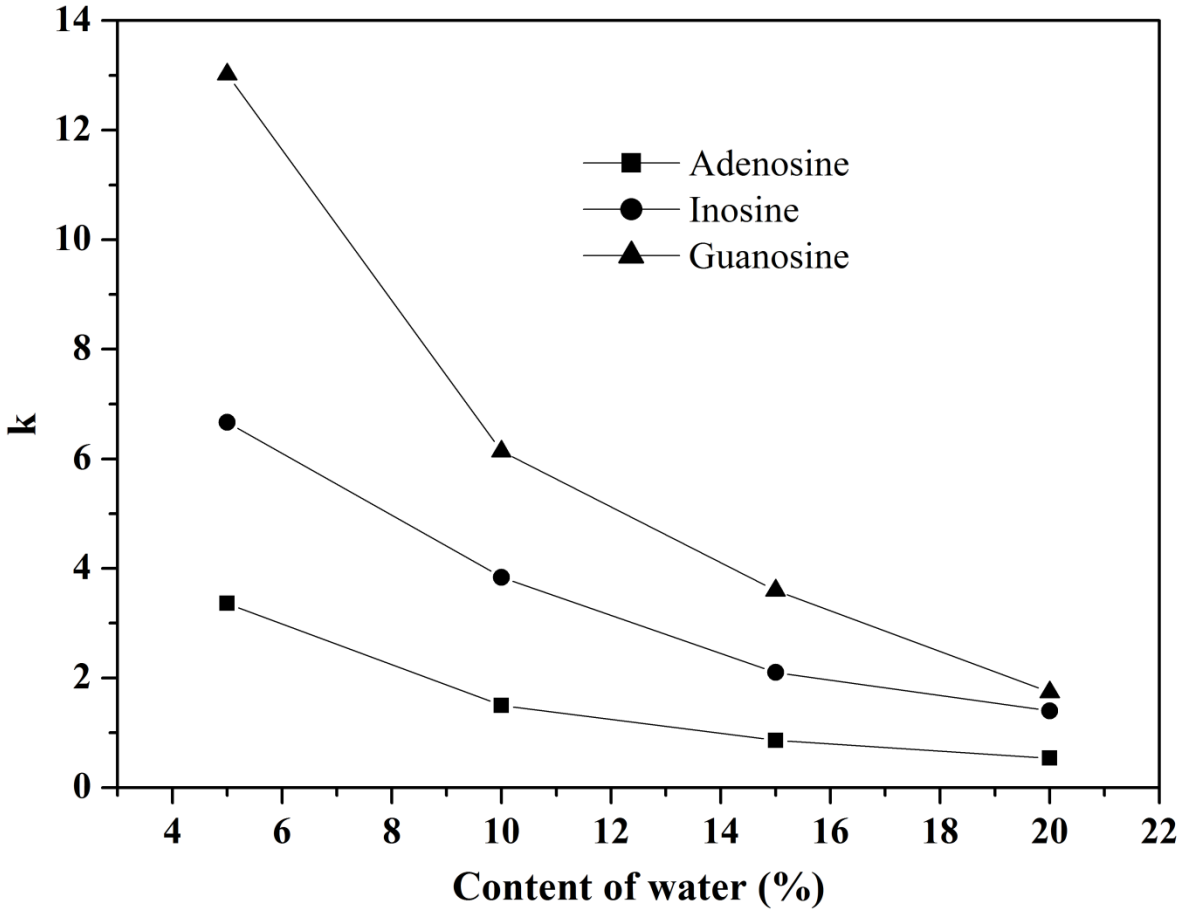
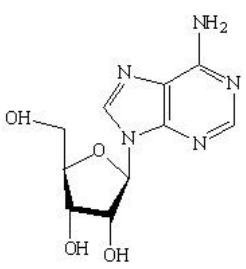
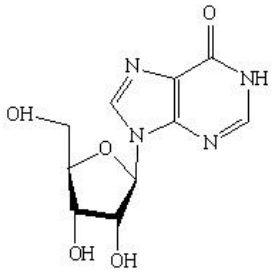


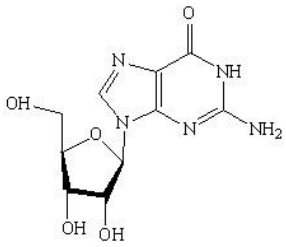
Fig. S3. Effect of the content of water on the retention factors of nucleosides.



Adenosine



Inosine



Guanosine

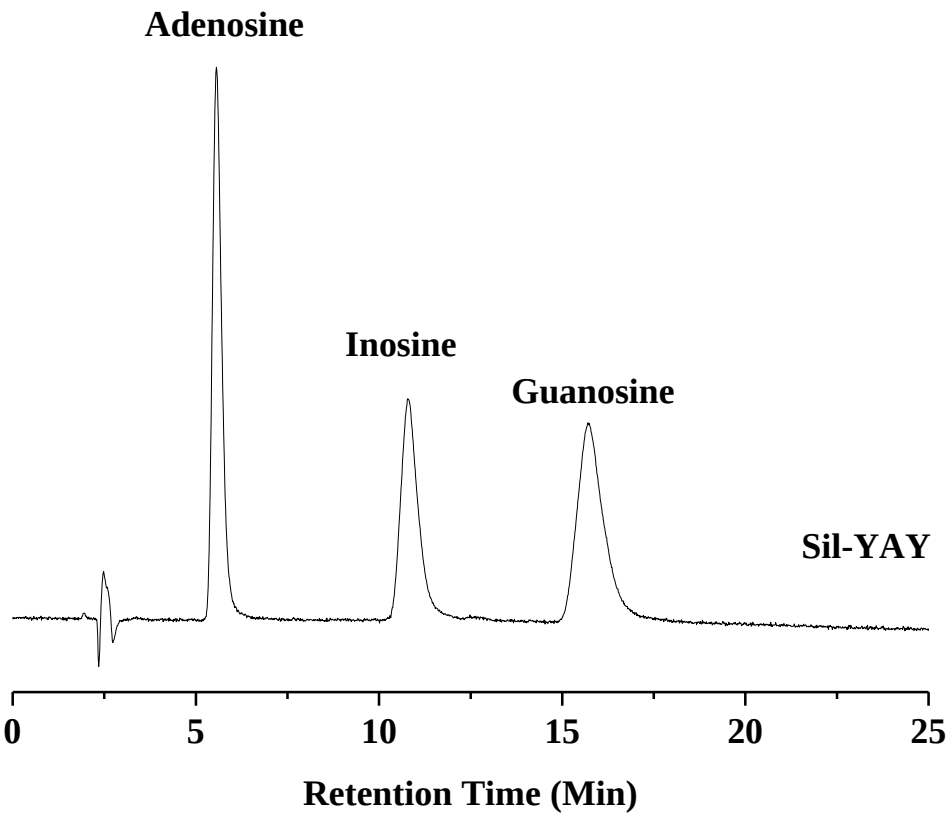


Fig. S4. Separation of nucleosides with 20 mM ammonium acetate: acetonitrile (10:90, v/v) as eluent. Other chromatographic conditions are the same as in Fig. 2.

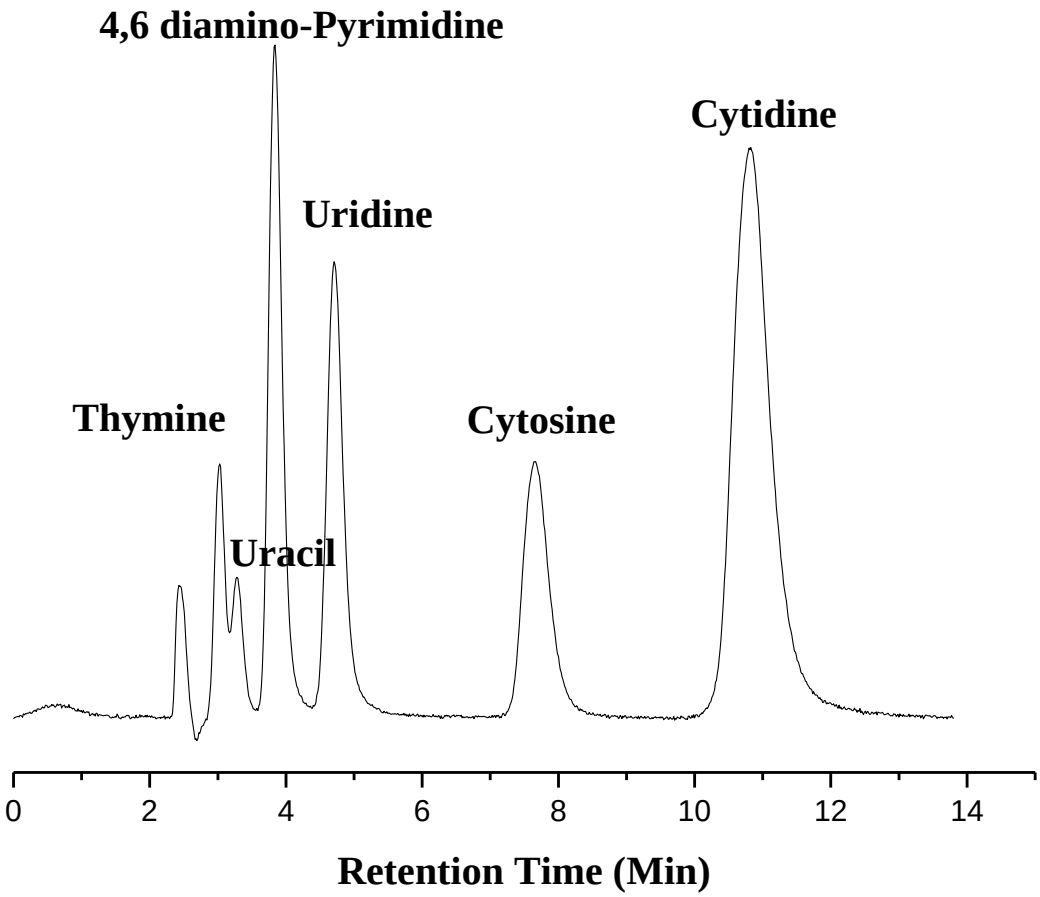


Fig. S5. Separation of pyrimidine bases with 20 mM ammonium acetate: acetonitrile (10:90, v/v) as eluent. Other chromatographic conditions are the same as in Fig. 2.

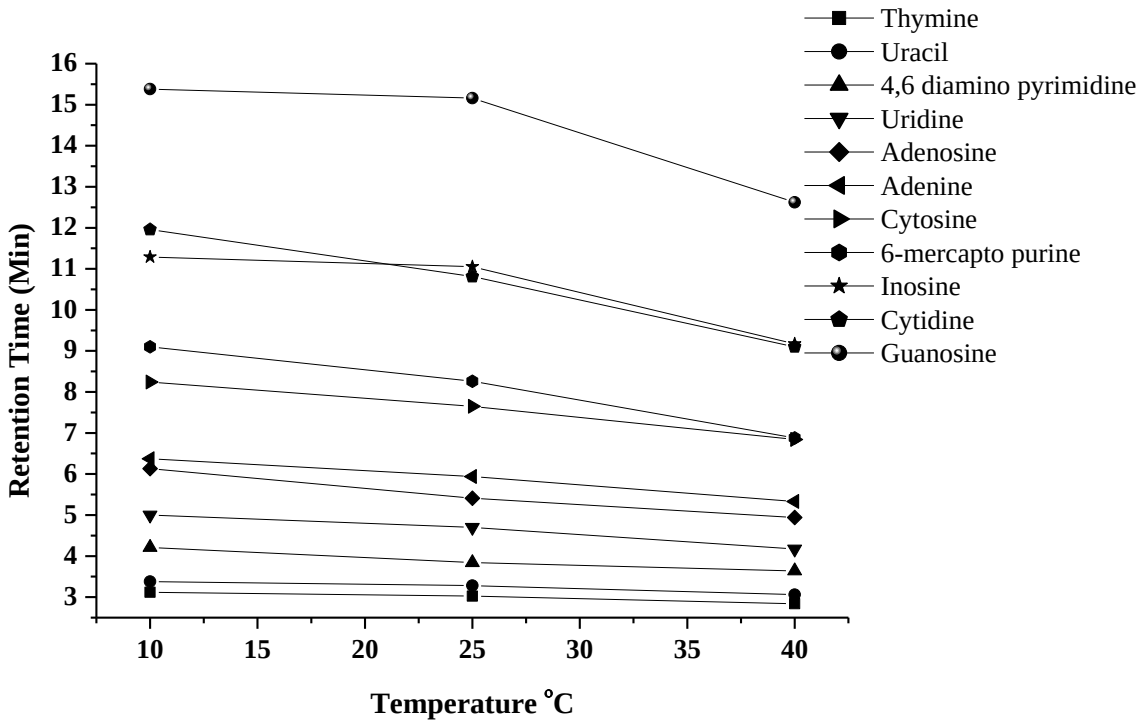


Fig. S6. Effect of the temperature on the retention factors of nucleo-analytes.

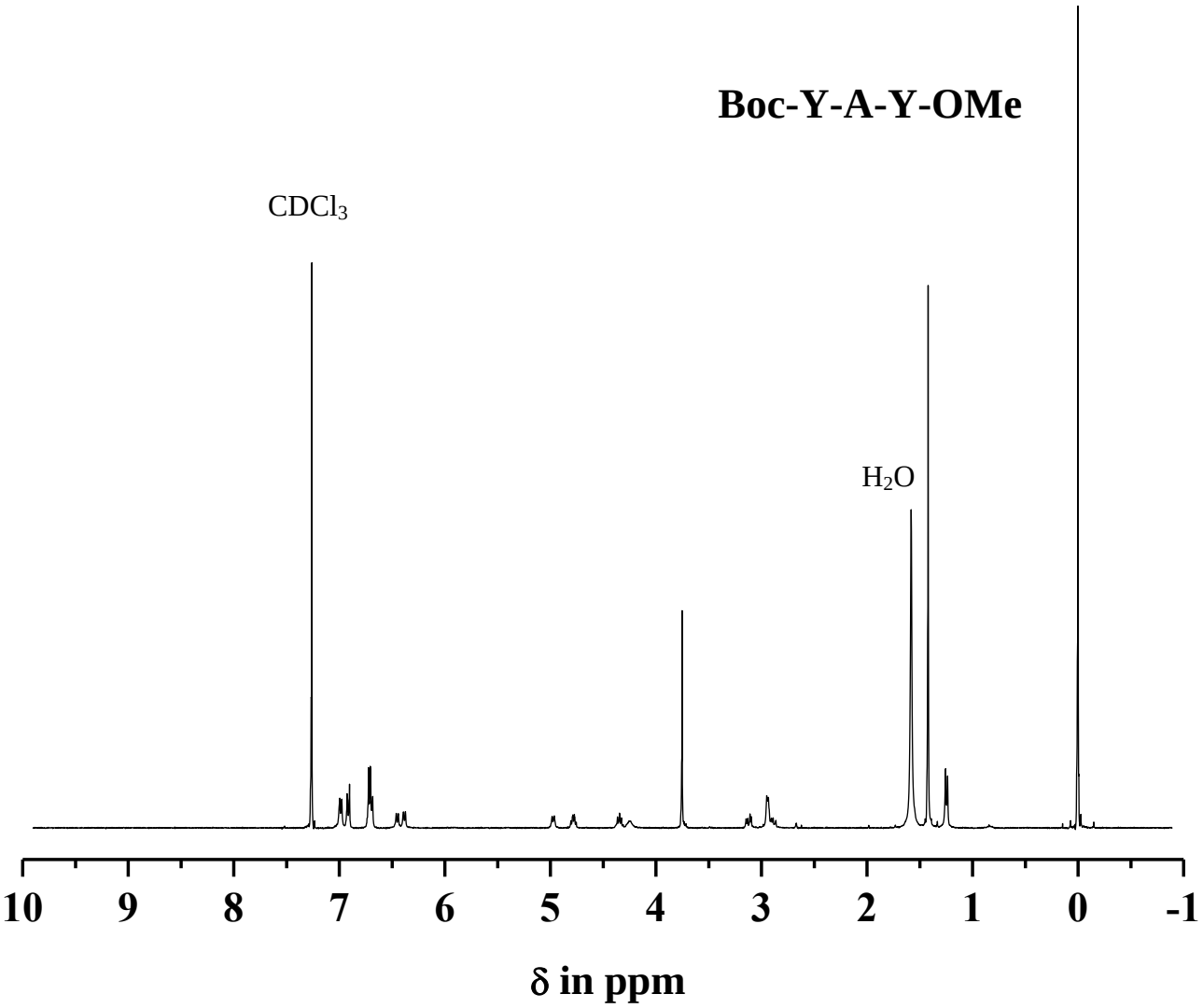


Fig. S7. 400 MHz H^1 NMR spectra of Boc-YAY-OMe in $CDCl_3$.

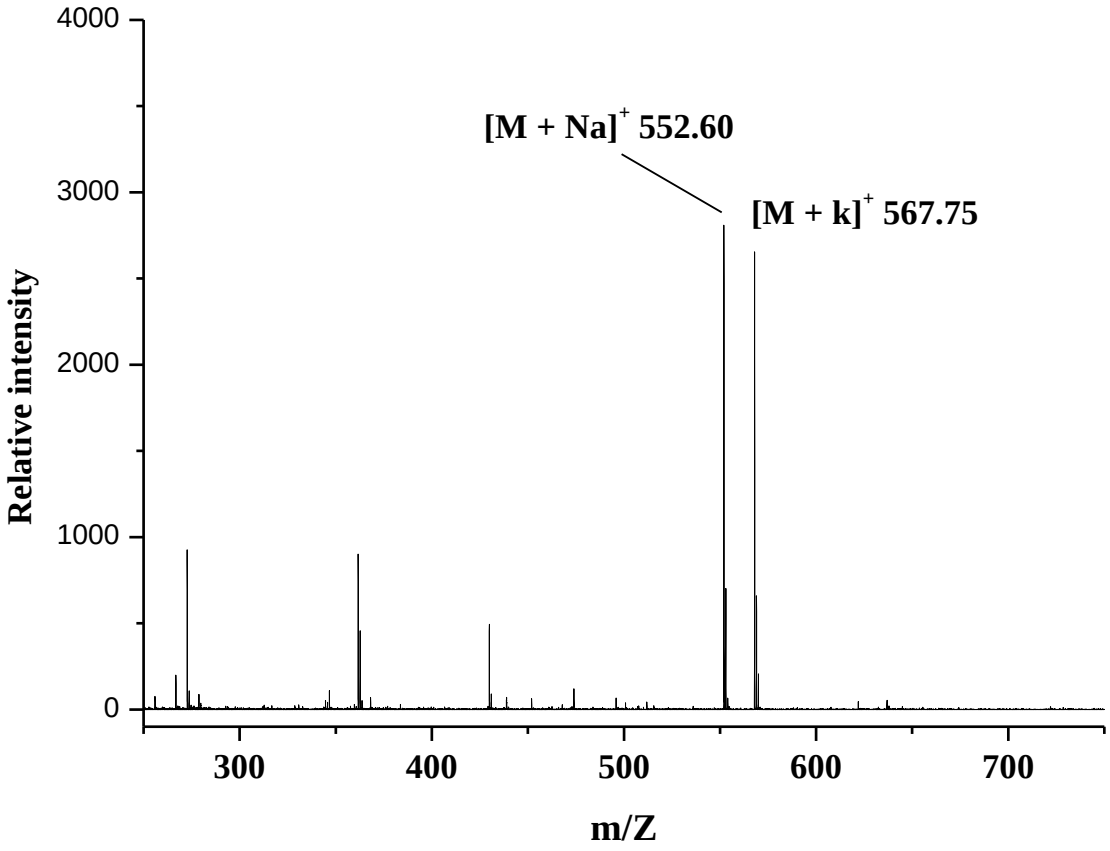


Fig. S8. HR-ESI-Mass spectra of Boc-YAY-OMe.

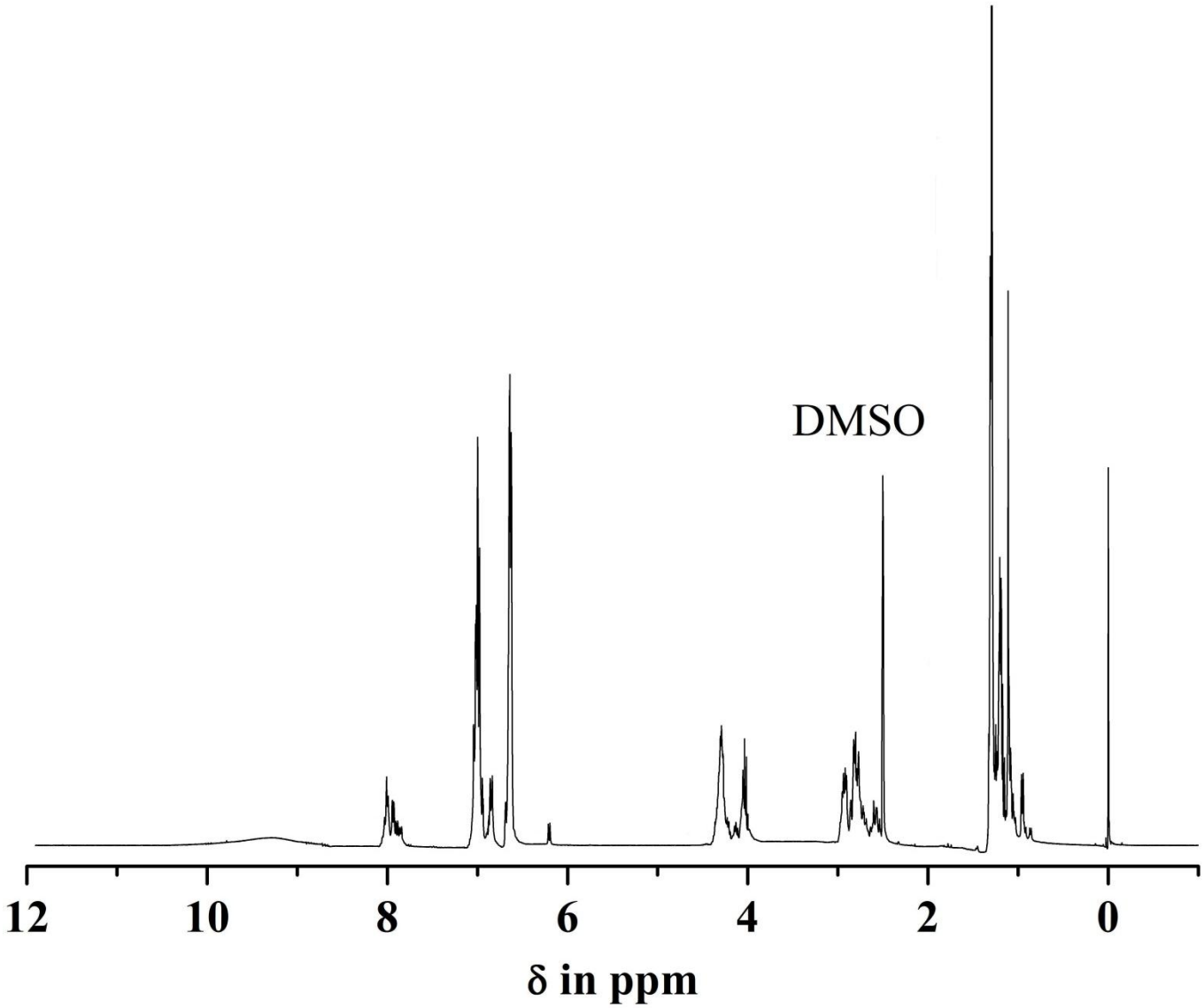


Fig. S9. 400 MHz H^1 NMR spectra of Boc-YAY-OH in DMSO-D_6.

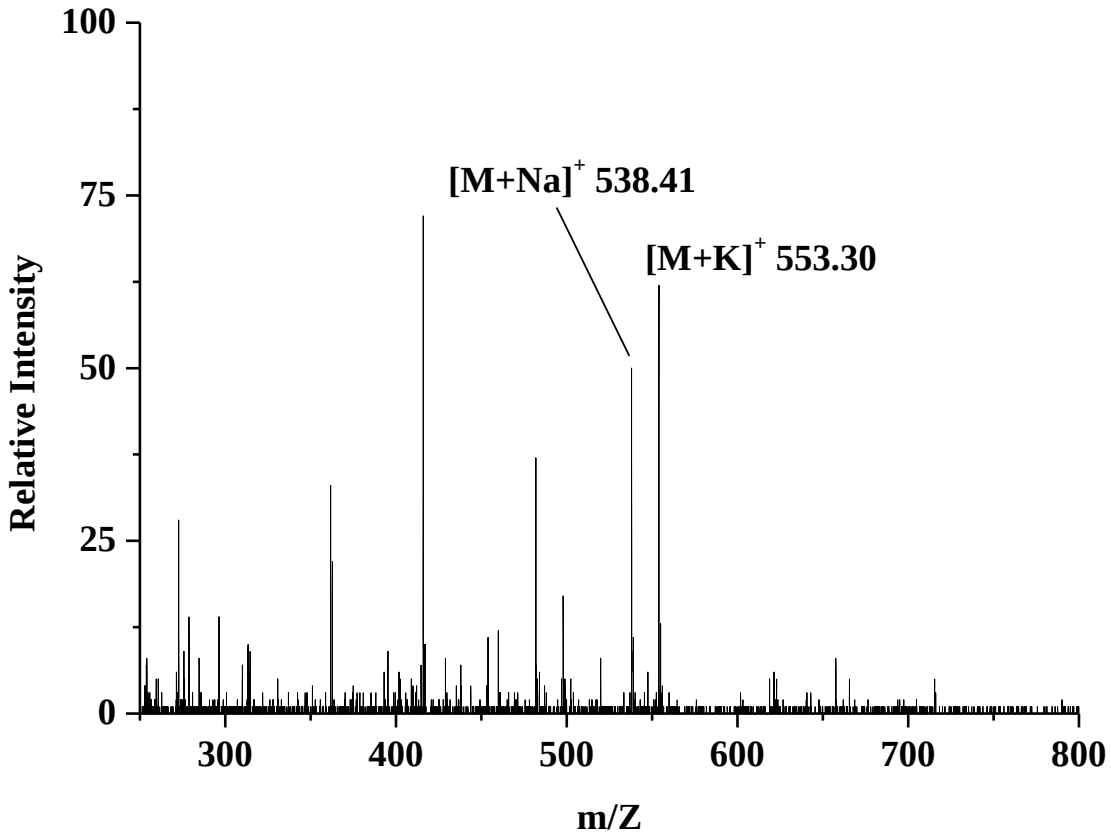


Fig. S10. HR-ESI Mass spectra of Boc-YAY-OH.

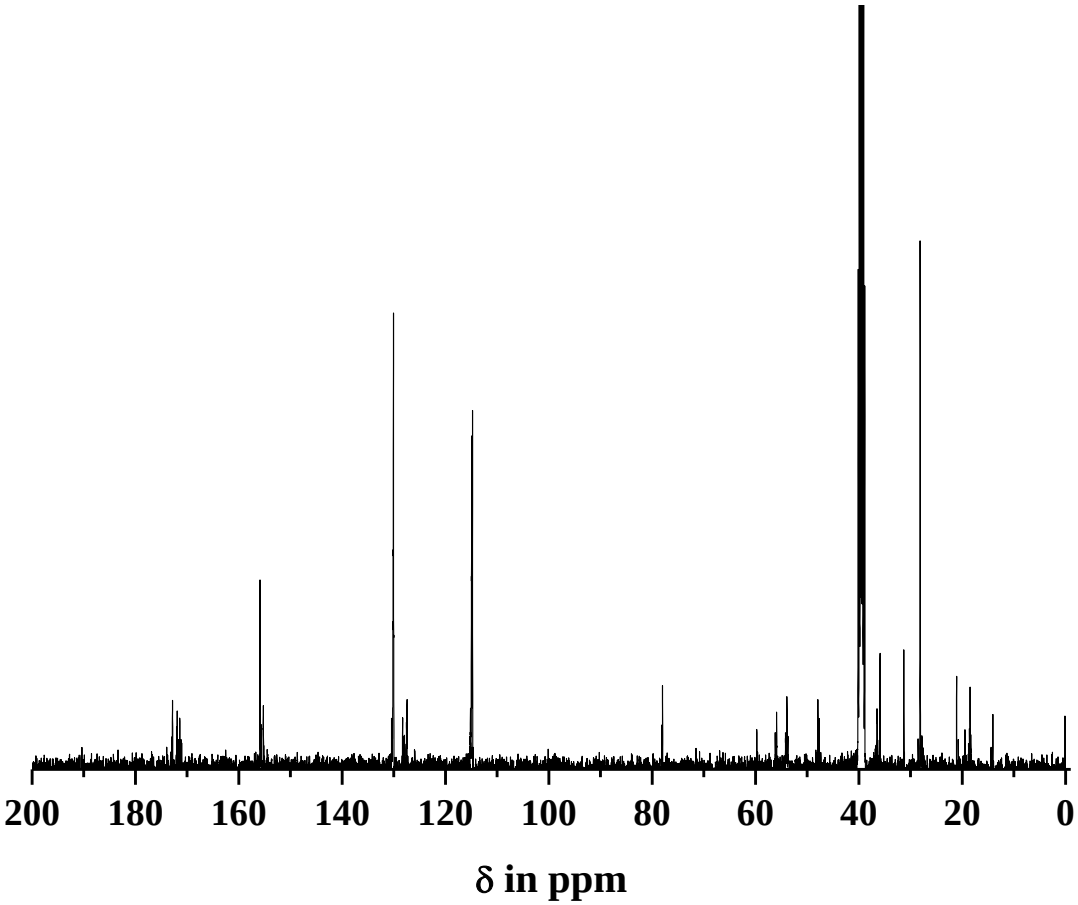


Fig. S11. C^{13} NMR spectra of Boc-YAY-OH in DMSO-D_6.

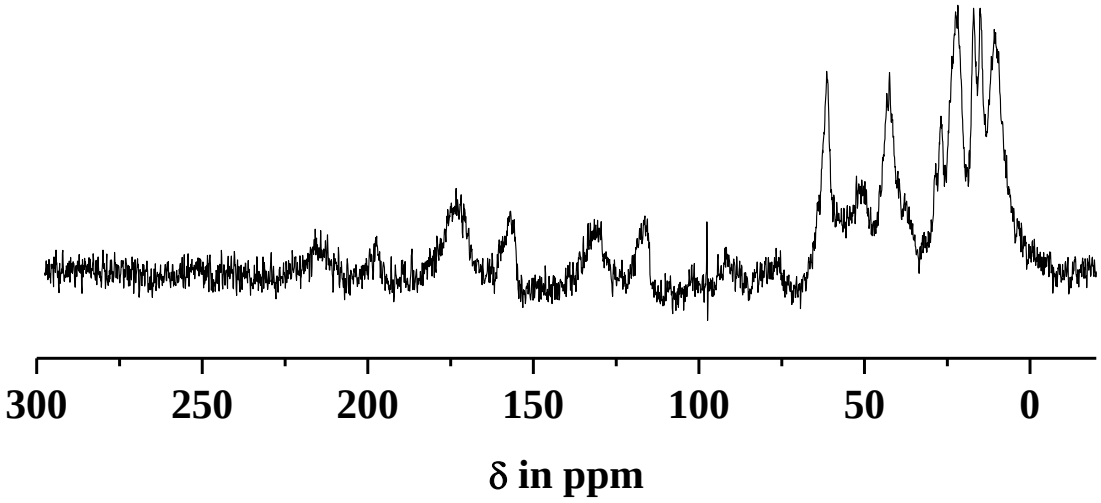


Fig. S12. Solid state C^{13} NMR spectra of Silica-YAY.

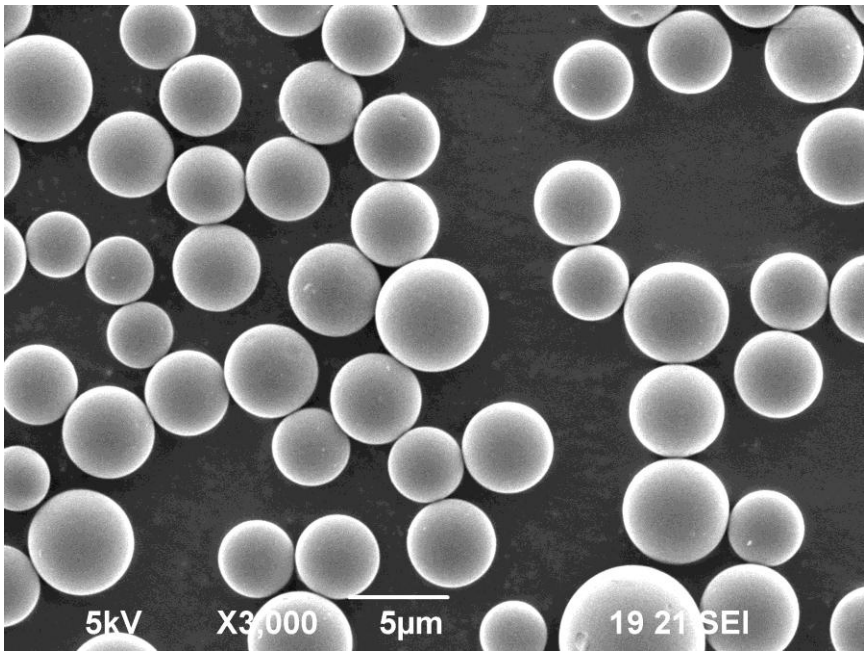


Fig. S13. SEM picture of Silica-YAY particle.