Electronic Supporting Information

Remarkable enhancement of selectivity towards ultraversatile analytes by a strategically integrated H-bonding site containing phase†

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Materials and methods: L-Glutamic acid, stearylamine, diethylphosphorocyanidate (DPEC, peptide synthesis reagent), triethylamine (TEA), and β-alanine were purchased from Wako (Japan) and used without further purification. Standard reference material SRM 869b, Column Selectivity Test Mixture for Liquid Chromatography was obtained from the Standard Reference Materials Program (NIST, Gaithersburg, MD, USA). Polycyclic aromatic hydrocarbons (PAHs) were purchased from TCI, Japan. β-Carotene was purchased from Sigma, USA. Nonpolar, polar, and basic compounds set (uracil, propranolol, butyl paraben, dipropyl phthalate, naphthalene, amitriptylene, and acenaphthene) was obtained from Wako and TCI, Japan. Bases and nucleosides (thymine, uracil, 4,6-diaminopyrimidine, uridine, adenosine, cytosine, and cytidine) were purchased either from Sigma-Aldrich, USA or from TCI, Japan. Sulfa drugs (sulfanilamide, sulfapyridine, sulfadimethoxine, sulfamethazine, sulfamethoxypyridazine, sulfamerazine, sulfisoxazole, sulfadoxine, sulfadiazine, sulfamethoxazole, sulfathiazole, sulfamonomethoxine, sulfectamide, sulfaquinoxaline, sulfamethizole) were obtained from TCI, Japan or Wako, Japan. Pentapeptides, namely [D-Ala^2]-Leucin enkephalinamide, [D-Ala^2]-Leucine enkephalin, [Met^5]-Enkephalin acetate salt hydrate, Leucine enkephalin acetate were purchased from Sigma–Aldrich (St. Louis, USA). Salicylamides and its acids, namely salicylamide, salicylic acid, acetylsalicylic acid (aspirin), 4-aminosalicylic acid were purchased from TCI, Japan. 3-Aminopropylsilane (APS) was obtained from TCI, Japan. Other nucleosides including 5-methyluridine, sodium *p*-toluenesulfonate, theobromine, theophylline, 2'-deoxyuridine, *N,N,N*-trimethylphenylammonium chloride, and vidarabine were obtained from TCI, Japan. 2'-Deoxyguanosine, 3'-deoxyguanosine, 4-nitrophenyl α-D-glucopyranoside, and 4-nitrophenyl β-D-glucopyranoside were purchased from Sigma (Sigma, St. Louis, MO, USA). Porous silica particles (YMC-GEL) was purchased from YMC (Kyoto, Japan); their average diameter, pore size, and surface area were 5 mm, 12 nm, and 330 m^2g^{-1}, respectively. HPLC-grade methanol was obtained from Nacalai Tesque (Japan). IR measurements were conducted on a JASCO (Japan) FTIR-4 100 Plus instrument in KBr. For DRIFT measurements, the accessory DR PRO410-M (JASCO, Japan) was used. TGA was performed on a Seiko EXSTAR 6000 TG/DTA 6300 thermobalance in N_2 flow (200 $mLmin^{-1}$) from 30 to 900 °C at a heating rate of 10 °C min^{-1}. For characterization of synthesis, ^{1}H NMR spectra were recorded on a JEOL JNM-LA400 (Japan) instrument.

Solid-state ^{13}C CP/MAS NMR and suspended-state ^{1}H NMR measurements: NMR spectra were measured by a Varian UnityInova AS400 instrument at a static magnetic field of 9.4 T by using a GHX nanoprobe for suspended-state NMR and a solid probe for CP/MAS NMR spectroscopy at a spin rate of 2000–3500 Hz for suspended-state NMR and 4000–4500 Hz for solid-state NMR measurements, respectively. Solid-state ^{13}C CP/MAS, the NMR measuring parameters were: spectral width 50000 Hz, proton pulse width = 11.6 ms, contact time for cross polarization 5 ms, and delay before acquisition 2 s. High-power proton decoupling of 63 dB with fine attenuation of dipole r = 2500 was used only during detection periods. In suspended-state ^{1}H NMR spectra were recorded at room temperature using a GHX Varian AS400 nanoprobe. The parameters used for measurement were delay time 1.5 s, pulse width 2.2 ms, transient number 32, and spectral width 6000 Hz. For assigning peaks, after determination of a pulse width of 90 °, simple RELAY COSY (correlation spectroscopy test) was carried out and the chemical shifts of the terminal methyl and methylene proton of the alkyl chain were determined.

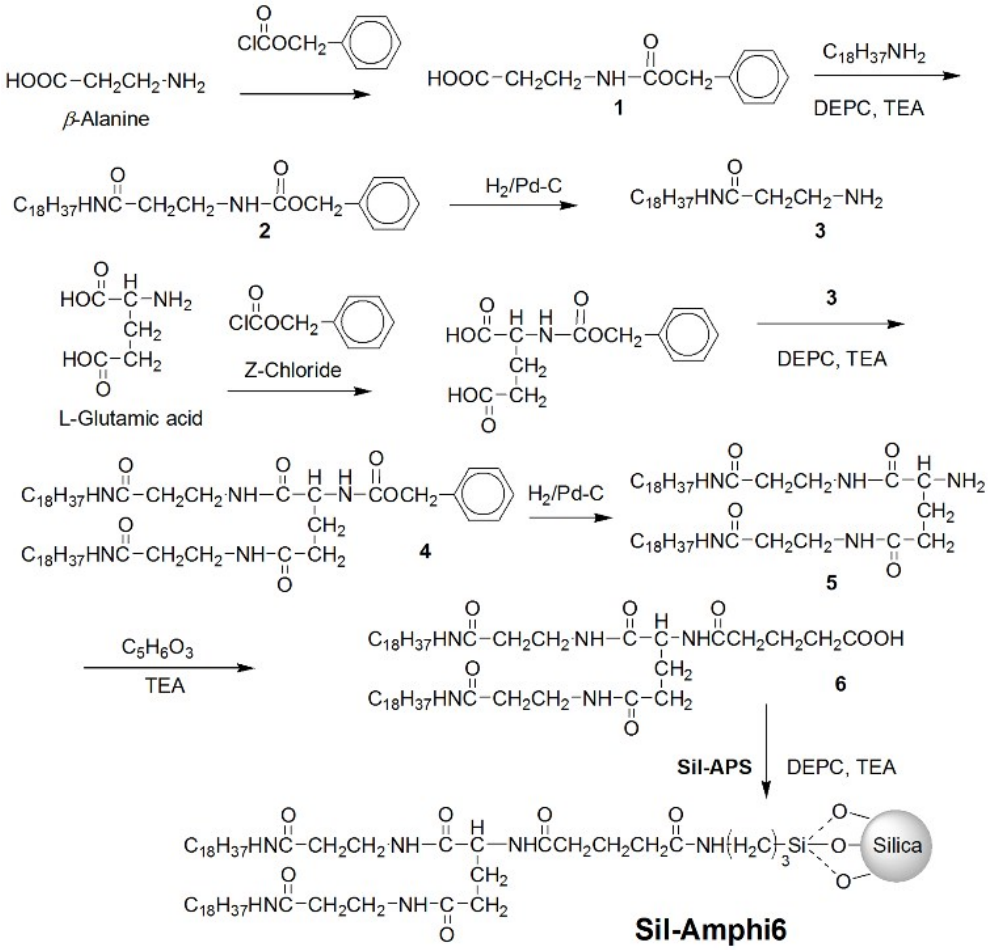
Liquid chromatographic measurements: After the complete characterization, Sil-Amphi**6** and Sil-APS was packed into a stainless-steel column (150 × 4.6 mm i.d.) using methanol/water (80:20, v/v) as the propulsive solvent (slurry packing method). In RPLC, a commercial ODS column (Inertsil ODS-3, 150 × 4.6 mm i.d.) used as the reference was obtained from GL Science (Tokyo, Japan). HPLC-grade methanol and acetonitrile were used as components of the mobile phase. Millipore water was used during the experiments. The chromatographic system consisted of a Gulliver PU-1580 intelligent HPLC pump, a Rheodyne sample injector with a 20 mL loop, and a JASCO multiwavelength UV detector MD 2010 plus. The column temperature was maintained by using a column jacket that had a circulator with a heating and a cooling system. A personal computer connected to the detector with JASCO ChromNAV (Ver 1.17 or later) software was used for system control and data analysis. As the sensitivity of the UV detector was high, 5 μL of sample solution was used for each injection. To avoid overloading effects, special attention was given to the selection of optimum experimental conditions. All most all separations were performed at a flow rate of 1.00 mL min^{-1}. Measurement of the retention factor (k) was carried out under isocratic elution conditions. The separation factor (α) is the ratio of the retention factor of two solutes that are being analyzed. The retention time of D_2O was used as the void volume (t_0) marker (the absorption of D_2O was measured at 400 nm). All data points were derived from at least triplicate measurements. The other chromatographic conditions for the Tanaka RPLC characterization of the phases were as reported previously.[1] In this protocol, six variables reflecting different chromatographic properties such as retention factor for pentylbenzene, $k_{PB;}$ hydrophobic selectivity, $\alpha(CH_2)$; retention factor ratio between pentylbenzene and butylbenzene; shape selectivity, $\alpha_{T/O;}$ retention factor ratio between triphenylene and *o*-terphenyl; hydrogen bonding capacity, $\alpha_{C/P;}$ retention factor ratio between caffeine and phenol; total ion-exchange capacity at pH 7.6, $\alpha_{A/P;}$ retention factor ratio between benzylamine and phenol; acidic ion-exchange capacity at pH 2.7, $\alpha_{A/P;}$ and retention factor ratio between benzylamine and phenol were used for the characterization. Water/1-octanol partition coefficient (P) was measured by the retention studies with ODS (monomeric; Inertsil ODS, 250 × 4.6 mm i.d., GL Science, Tokyo, Japan): $\log P = 3.579 + 4.207 \log k$ ($r = 0.999997$).[2] For the separation of polar and basic analytes in the HILIC mode, an ammonium acetate (NH_4Ac) buffer and acetonitrile (ACN) mixture were used. In HILIC, most of the experiments were carried out at room temperature. The other chromatographic conditions for the HILIC characterization of the phase (and variables reflecting different chromatographic properties) were used as described by Y. Kawachi et al.[3]

1 K. Kimata, K. Iwaguchi, S. Onishi, K. Jinno, R. Eksteen, K. Hosoya, M. Arki, N. Tanaka, *J. Chromatogr. Sci.* 1989, **27**, 721; Euerby, P. Petersson, *J. Chromatogr. A* 2003, **994**, 13.

2 H. Ihara, T. Sagawa, Y. Goto, S. Nagaoka, Polymer 1999, 40, 2555

3 Y. Kawachi, T. Ikegami, H. Takubo, Y. Ikegami, M. Miyamoto, N. Tanaka, *J. Chromatogr. A* 2011, **1218**, 5903.

Synthesis of *N′,N″*-Bis(octadecylaminocarbonylethyl)-N^{α}-[(4-carboxy)butanoyl]-L-glutamide (6) and its immobilization onto silica

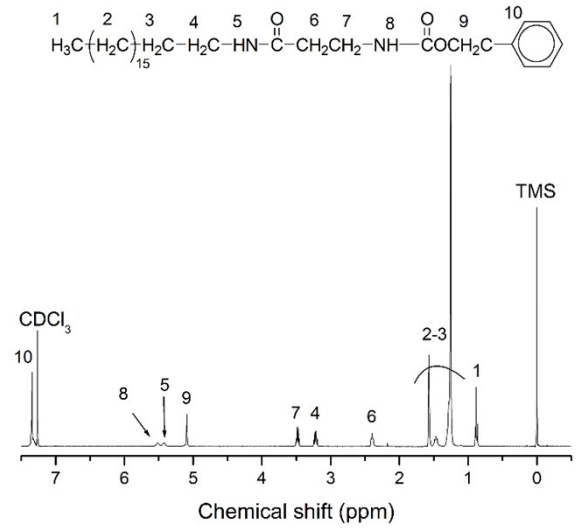


Scheme S1. Synthesis of L-glutamic acid derivative-grafted silica stationary phase.

*N-Carbobenzoyl-β-alanine (**1**)*: *β*-Alanine (18 g, 205 mmol) was dissolved in NaOH solution (2 M, 250 ml) and stirred in an ice bath at 0 ˚C and pH around 12. Carbenzoxy chloride (Z-Cl) (32.5 ml, 205 mmol) was added dropwise and keep pH 12 followed by the addition of NaOH (2 M). After completion of Z-Cl addition the mixture was stirred for 1 h at 0 ˚C and 12 h at room temperature. The reaction mixture was extracted with diethyl ether (3 × 100 ml) to remove unreacted Z-Cl and the aqueous layer was separated. HCl (2 M) was added to the aqueous layer until the pH reached 2. The white solid obtained was isolated by filtration, washed with HCl and dried *in vacuo* to give **1** (42.2 g, yield: 83.56%). mp: 104–106 ˚C; ^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.34 (m, 5H; C_6H_5), 5.29 (s, 1H; $CH_2NHC(=O)$), 5.15 (s, 2H; $C(=O)CH_2C_6H_5$), 3.47 (t, 2H; $NHCH_2CH_2$), 2.60 ppm (t, 2H; $C(=O)OCH_2CH_2$); IR (KBr): ν = 3336, 3150, 3036, 1686, 1536, 1496, 1454 cm^{-1}. Elemental analysis (Anal. Found: H, 5.85; C, 59.11; N, 6.25 Cal. For $C_{11}H_{13}NO_4$: H, 5.87; C, 59.19; N, 6.27%).

*N′-Octadecyl-N^{α}-carbobenzoyl-β-alanineamide (**2**)*: *N*-Carbobenzoyl-*β*-alanine (**1**) (10.0 g, 44.48 mmol) and stearylamine (13.29 g, 49.32 mmol) were dissolved in dry THF (300 ml) by stirring. Triethylamine (TEA) (11.30 g, 112.10 mmol) was added to the mixture followed by diethylphosphorocyanidate (DEPC) (8.04 g, 49.32 mmol) and stirring was continued for 1 h at 0 ˚C. The ice bath was removed and the mixture was stirred overnight at room temperature. The mixture was concentrated under reduced pressure and the residue was dissolved in $CHCl_3$ (300 ml). The chloroform solution was washed with 10%

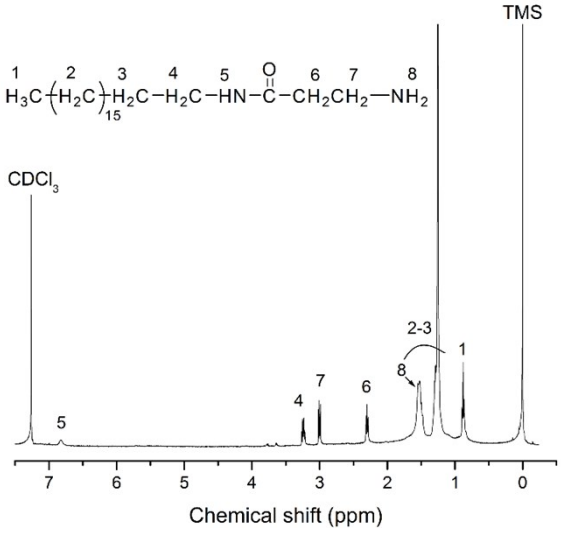
$NaHCO_3$ solution, HCl (0.2 M), and distilled water. The solution was dried over Na_2SO_4, concentrated under reduced



^{1}H NMR spectrum of compound **2**

pressure, recrystallized from methanol, and dried in vacuo to give a white powder (14.1 g, yield: 63.63%). mp: 124–126 ˚C; ^{1}H NMR (400 MHz, $CDCl_3$): δ =7.34 (m, 5H; C_6H_5), 5.52 (s, 1H; N*H*C(O)CH_2), 5.41 (s, 1H; CH_2N*H*C(=O)), 5.09 (s, 2H; C(=O)C$H_2C_6H_5$)), 3.48 (q, 2H; CH_2CH_2NHC(=O)), 3.22 (q, 2H; CH_2CH_2NHC(=O)), 2.39 (t, 2H; C(=O)CH_2CH_2NH), 1.46 (m, 2H; $CH_2CH_2CH_2$NHC(O)CH_2), 1.25 (m, 30H; CH_3CH_2 × 15), 0.86 ppm (t, 3H; CH_3); IR (KBr): ν = 3333, 3295, 2918, 2849, 1683, 1635, 1539, 1468, 1442 cm^{-1}. Elemental analysis (Anal. Found: H, 10.59; C, 73.30; N, 5.89 Cal. For $C_{29}H_{50}N_2O_3$: H, 10.62; C, 73.37; N, 5.90%).

*N′-Octadecyl-β-alanineamide (**3**)*: *N′*-Octadecyl-N^{α}-carbobenzoyl-*β*-alanineamide (**2**) (11.50 g, 23.66 mmol) was dissolved in ethanol (600 mL) with heating and Pd carbon black (1 g) was added to the solution. H_2 gas was bubbled slowly into the solution for 6 h at 60 ˚C. The Pd carbon black was removed by filtration, then the solution was concentrated under reduced pressure, recrystallized from methanol, and dried in vacuo to give a white powder (7.49 g, yield: 80.13%). mp: 101–102 ˚C; ^{1}H NMR (400 MHz, $CDCl_3$): δ = 6.84 (s, 1H; CH_2N*H*C(=O)), 3.23 (q, 2H; CH_2CH_2NHC(=O)) 2.98 (t, 2H; $CH_2CH_2NH_2$), 2.28 (t, 2H; C(=O)C$H_2CH_2NH_2$), 1.47 (m, 2H; $CH_2CH_2CH_2$NHC(O)CH_2, CH_2NH_2), 1.13 (m, 30H; CH_3CH_2 × 15), 0.86 ppm (t, 3H; CH_3); IR (KBr): ν = 3302, 2918, 2849, 1683, 1635, 1541, 1471 cm^{-1}. Elemental analysis (Anal. Found: H, 12.98; C, 73.99; N, 8.15 Cal. For $C_{21}H_{44}N_2O$: H, 13.02; C, 74.06; N, 8.23%).



^{1}H NMR spectrum of compound **3**

*N',N''-bis(octadecylaminocarbonylethyl)-N$^{\alpha}$-benzyloxycarbonyl-L-glutamide (**4**)*: *N*-benzyloxycarbonyl-L-glutamic acid (L-Glu-Z) (1.04 g, 3.55 mmol), *N'*-Octadecyl-*β*-alanineamide (**3**) (2.54 g, 14.91 mmol), and triethylamine (1.10 g, 10.8 mmol) were dissolved in 500 mL of THF and stirred at room temperature. DEPC (1.45 g, 8.87 mmol) was added to the mixture and stirred for 1 day at room temperature, the solution was concentrated *in vacuo* and the residue was recrystallized from ethanol to give white solid *N',N''*-bis(octadecylaminocarbonylethyl)-*N*$^{\alpha}$-benzyloxycarbonyl-L-glutamide (**4**): yield 2.95 g (84.29 %); mp: 225-227 °C; ^{1}H-NMR (400 MHz, $CDCl_3$): δ = 7.30-7.44 (m, 5H, C_6H_5), 6.96-7.10 (m, 1H, N*H*), 6.70-6.90 (m, 1H, N*H*), 6.50-6.70 (m, 1H, N*H*), 5.87-6.10 (m, 2H, N*H* × 2), 5.02-5.14 (s, 2H, $CH_2C_6H_5$), 4.07-4.27 (m, 1H, *C*H*), 3.13-3.28 (m, 4H, C(=O)CH_2CH_2NH × 2), 2.98-3.13 (q, 4H, $CH_3(CH_2)_{16}CH_2$ × 2), 2.15-2.47 (m, 6H, C(=O)CH_2 × 3), 1.41-1.57 (m, 6H, $CH_3(CH_2)_9CH_2$ × 2, *CHCH_2), 1.00-1.41 (m, 60H, $CH_3(CH_2)_{15}$ × 2), 0.79-0.97 (m, 6H, CH_3 × 2); IR (KBr): ν = 3298, 2919, 2850, 1686, 1655, 1632, 1541, 1470 cm^{-1}; Elemental analysis (Anal. Found: C, 70.66; H, 10.79; N, 7.43. Calc. for $C_{55}H_{99}N_5O_6$: C, 71.31; H, 10.77; N, 7.56 %).

*N',N''-bis(octadecylaminocarbonylethyl)-L-glutamide (**5**)*: **4** (2.91 g, 3.18 mmol) was dissolved in 500 mL of ethanol and THF mixture (1:1) with heating and Pd black (1 g) was added to the solution. H_2 gas was bubbled slowly into the solution for 8 hours. After confirming the removal of Z-group by FTIR measurement, Pd black was removed by filtration. The filtrate was concentrated and dried *in vacuo* to give white solid *N',N''*-bis(octadecylaminocarbonylethyl)-L-glutamide (**5**): yield 2.29 g (95 %); mp: 183-185 °C; ^{1}HNMR (400 MHz, $CDCl_3$): δ = 7.70-7.83 (m, 1H, N*H*), 6.09-6.33 (m, 1H, N*H*), 5.87-6.10 (m, 2H, N*H* × 2), 3.16-3.29 (m, 4H, $CH_3(CH_2)_{16}CH_2$ × 2), 2.37-2.46 (m, 4H, C(=O)CH_2CH_2NH × 2), 2.17-2.28 (m, 2H, *CHCH_2CH_2), 1.87-2.01 (m, 2H, *CHCH_2), 1.57-1.80 (m, 2H, NH_2), 1.43-1.57 (m, 4H, $CH_3(CH_2)_{15}CH_2$ × 2), 1.13-1.37 (m, 60H, $CH_3(CH_2)_{15}$ × 2), 0.83-0.93 (t, 6H, CH_3 × 2); IR (KBr): ν = 3308, 2919, 2849, 1635, 1540, 1469 cm^{-1}; Elemental analsis (Anal. Found: C, 71.13; H, 11.83; N, 8.70. Calc. For $C_{47}H_{93}N_5O_4$: C, 71.25; H, 11.83; N, 8.84 %).

*N',N''-Bis(octadecylaminocarbonylethyl)-N$^{\alpha}$-[(4-carboxy)butanoyl]-L-glutamide (**6**)*: **5** (2.24 g, 2.83 mmol) was dissolved in 300 mL of chloroform with heating. Glutaric anhydride (0.54 g, 4.50 mmol) and triethylamine (0.77 g, 7.70 mmol) were added to the solution and stirred with heating. After being stirred for 1 day at room temperature, the solution was concentrated *in vacuo*. The residue was recrystallized from ethanol and dried *in vacuo* to give a white solid (**6**): yield 2.50 g (90 %); mp: 191-194 °C; ^{1}H-NMR (400 MHz, $CDCl_3$): δ = 6.96-7.10 (m, 1H, N*H*), 6.70-6.90 (m, 1H, N*H*), 6.50-6.70 (m, 1H, N*H*), 5.87-6.10 (m, 2H, N*H* × 2), 4.07-4.27 (m, 1H, *C*H*), 3.38-3.63 (m, 4H, (C=O)CH_2CH_2NH × 2), 3.08-3.28 (m, 4H, $CH_3(CH_2)_{16}CH_2$ × 2), 2.37-2.62 (m, 10H, (C=O)CH_2 × 5), 1.59-2.11 (m, 4H, *CHCH_2, CH_2CH_2(C=O)O), 1.42-1.53 (m, 4H, $CH_3(CH_2)_{15}CH_2$ × 2), 1.03-1.38 (m, 60H, $CH_3(CH_2)_{15}$ × 2), 0.79-0.95 (t, 6H, CH_3 × 2); IR (KBr): ν = 3299, 3082, 2918, 2850, 1713, 1637, 1549, 1469 cm^{-1}; Elemental analsis (Anal. Found: C, 69.01; H, 11.20; N, 7.85 Calc. For $C_{52}H_{99}N_5O_7$: C, 68.91; H, 11.01; N, 7.73 %).

Immobilization of 6 onto Silica

(3-Aminopropyl)trimethoxysilane (APS) grafted silica (Sil-APS) was prepared by refluxing porous silica gel (3.00 g) and 1.5 mLof APS in toluene for 24 h. After successive washing with toluene, ethanol, and diethyl ether, the particles were dried *in vacuo*. The dried particles were characterized by elemental analysis and TGA. Sil-APS was then coupled with amphiphile **6**. Sil-APS (3.0 g) and **6** (2.4 g, 2.64 mmol) were taken in 300 mL of dry THF and stirred. DEPC (1.5 g, 9.60 mmol) and TEA (1.1 g, 10.60 mmol) were added to the solution and stirred at 60 °C. After being stirred for 1 day, the grafted particles were centrifuged with hot THF and hot chloroform, methanol, and diethylether several times to remove the unreacted amphiphile molecule and dried *in vacuo* to get Sil-Amphi**6** stationary phases (Scheme S1).

Characterizations

TGA

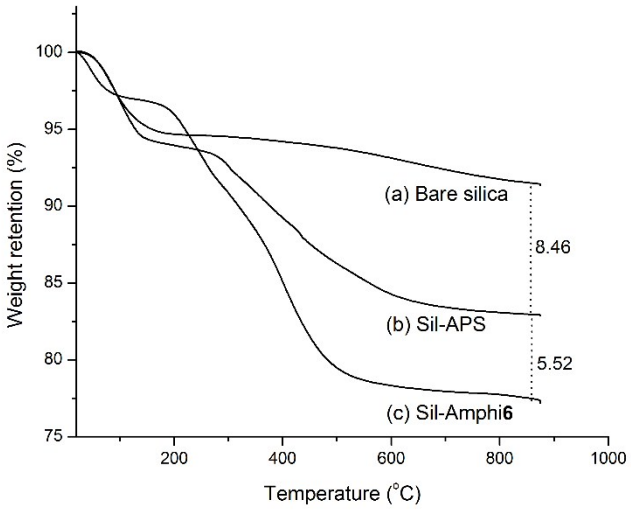


Figure S1. TGA curves of (a) bare silica, (b) Sil-APS, and (c) Sil-Amphi**6**.

To assess the organic contents of Sil-APS and Sil-Amphi**6**, thermogravimetric analysis (TGA) were carried out in addition to elemental analyses. TGA measurements were run at a constant heating rate of 10 °C min^{-1} in 200 mL min^{-1} N_2 by using an empty crucible as a reference. The heating process was carried out up to 900 °C, which has been demonstrated to be sufficiently high to degrade all surface-bonded organosilanes.[1] Typical TGA curves for the bare silica, Sil-APS, and Sil-Amphi**6** are depicted in Figure 1. The weight retention profile of Sil-Amphi**6** reached a plateau at 110 °C (drying period), indicating the removal of surface water. After the thermal degradation of the APS the weight of the sample was constant from 650 to 900 °C. A plateau in the weight retention curve of Sil-Amphi**6** was also observed as the temperature reached 600 °C, confirming that there is no amphiphilic material remaining on the silica at 900 °C. As shown in Figure S1, Sil-APS presented a weight loss of about 8.46% from 200 to 900 °C. After the immobilization compound **6** (Amphi**6**) with silica, it (Sil-Amphi**6**) showed a weight loss of about 5.52 % over the same temperature range, which indicates that the organic content increased slightly. These weight losses are consistent with the immobilized amounts estimated by elemental analyses.

1 J. A. A. Sales, F. P. Faria, A. G. S. Prado, C. Airoldi, Polyhedron 2004, 23, 719; S. Radi, A. Attayibat, A. Ramdani, M. Bacquet, J. Appl. Polym. Sci. 2010, 117, 3345; M. Chamberg, R. Parnas, Y. Cohen, J. Appl. Polym. Sci. 1989, 37, 2921-2931.

DRIFT

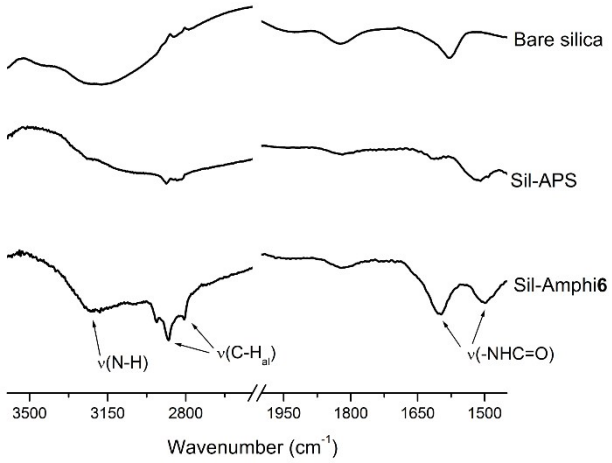


Figure S2. DRIFT spectra of (a) bare silica, (b) Sil-APS, and (c) Sil-FIP; (d) FT-IR spectrum of **1**.

To identify the chemical modifications of compounds, infrared spectroscopy is another useful tool that can be used. Therefore, grafting of organic molecules on a silica surface was also confirmed by diffuse reflectance infrared Fourier transform (DRIFT) measurement as shown in Figure 2. A group of peaks at 2926 and 2857 cm^{-1}, respectively, were attributed to the C−H bond stretching of the long alkyl chain for Sil-Amphi**6**. The spectrum of Sil-Amphi**6** showed intense bands at 1648 and 1549 cm^{-1}, indicating the presence of grafted amide bonded amhiphiles on the silica surface. Equally important is the appearance of N−H stretching (3235 cm^{-1}) in the spectrum for Sil-Amphi**6**, providing further evidence that Amphi**6** was successfully grafted onto the silica surface.[1]

1 A. K. Mallik, H. Qiu, T. Sawada, M. Takafuji, H. Ihara, Anal. Chem. 2012, 84, 6577.

Solid-state ^{13}C CP/MAS NMR and suspended-state ^{1}H NMR

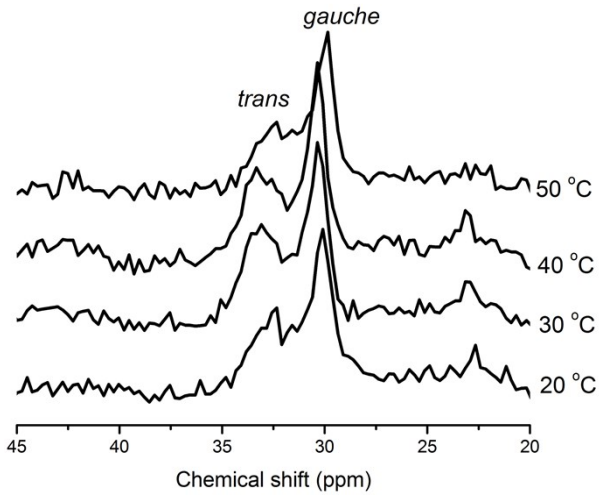


Figure S3. Partial solid-state ^{13}C CP/MAS NMR spectra of Sil-Amiphi**6** at variable temperature.

NMR experiments can be designed to probe conformational structure and dynamic aspects as well as bonding chemistry of immobilized alkyl ligands through observation of ^{1}H, ^{13}C, and ^{29}Si nuclei present in the interphases.[1] The combination of CP[1] with MAS allows acquisition of high-resolution NMR spectra of low-abundance heteronuclei (e.g., ^{13}C and ^{29}Si) in reasonable measuring times. The solid-state ^{13}C

CP/MAS NMR[1] and suspended-state ^{1}H NMR[a] measurements were carried out at different temperatures from 20 ˚C to 50 ˚C to investigate the conformations and mobility of the long alkyl chains, respectively of the grafted organic phase. Under the condition of magic angle spinning and dipolar coupling of protons, the chemical shift of methylene groups in ^{13}C CP/MAS NMR spectroscopy depends largely on the conformation of alkyl chains $-(CH_2)_n-$. It is reported that the ^{13}C signals for alkyl chains is observed at two resonances, one is at 32.6 ppm attributed to *trans* conformation, indicating crystalline and rigid state, and the other at 30.0 ppm corresponding to *gauche* conformation, indicating disordered and mobile state.[1]

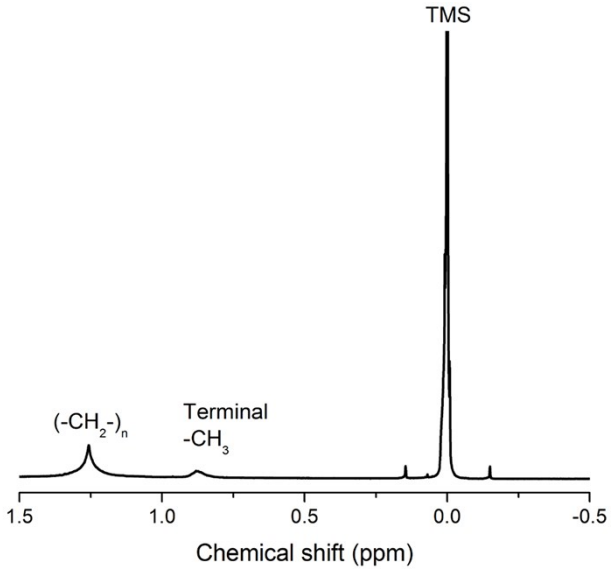


Figure S4. Partial suspended-state ^{1}H NMR spectrum of Sil-Amphi**6** in methanol.

1 H. R. Ansarian, M. Derakhsan, M. M. Rahman, T. Sakurai, M. Takafuji, I. Taniguchi, H. Ihara, *Anal. Chim. Acta* **2005**, *547*, 179-187; S. R. Hartmann, E. L. Hahn, *Phys. Rev.* **1962**, *128*, 2042-2053; A. Pines, M. G. Gibby, J. S. J. Waugh, *Chem. Phys.* **1972**, *56*, 1776-1777; E. R. Andrew, A. Bradbury, R. G. Eades, *Nature* **1959**, *183*, 1802-1803; J. Schaefer, E. O. Steijskal, *J. Am. Chem. Soc.* **1976**, *98*, 1031-1032; M. Raitza, J. Wegmann, S. Bachmann, K. Albert, *Angew. Chem. Int. Ed.* **2000**, 39, 3486-3489; A. E. Tonelli, F. C. Schiling, F. A. Bovey, *J. Am. Chem. Soc.* **1984**, *106*, 1157-1158.

Chromatographic evaluation

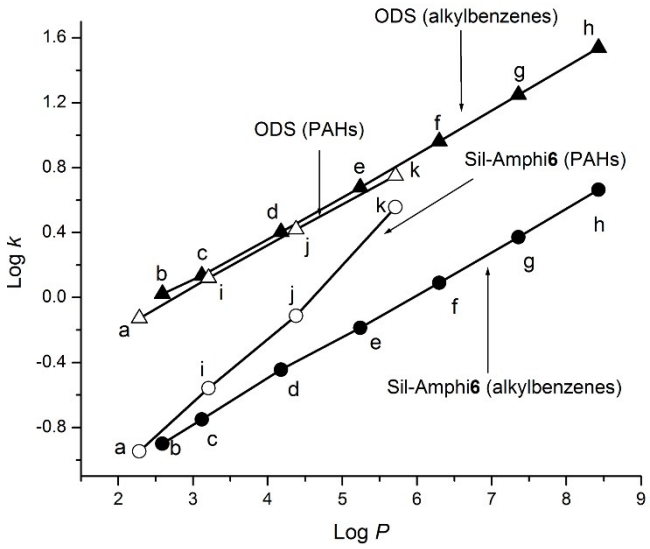


Figure S5. Log *k* versus log *P* plots for ODS and Sil-Amphi**6** stationary phases. Mobile phase: methanol-water (90:10, v/v). Column temperature: 30 °C. Key: a: benzene, b: toluene, c: ethylbenzene, d: butylbenzene, e: hexylbenzene, f: octylbenzene, g: decylbenzene, h: dodecylbenzene, i: naphthalene, j: anthracene, k: naphthacene.

In RPLC, conventional octadecylsilylated silica (ODS) or alkyl phases can recognize the hydrophobicity of analytes, and this hydrophobicity is usually measured by the methylene activity of the stationary phases. It reflects the possibility of the phase being able to separate two molecules (e. g., amylbenzene and butylbenzene) that differ only in methylene groups. Alkylbenzenes can be used as test analytes to understand the retention mode, as well as the extent of hydrophobic interaction among the analytes and the stationary phase in RPLC.[1] The correlation between log *k* (capacity factor) and log *P* (water/1-octanol partition coefficient) for Sil-Amphi**6** and ODS phase showed the retention mode of Sil-Amphi**6**, a RP mode to that of ODS phase (Figure S5). The relationship between log *k* and log *P* is used to determine the hydrophobicity recognition ability or retention mode of a stationary phase. It was observed that log k and log *P* plots of alkylbenzenes and polycyclic aromatic hydrocarbons (PAHs) in ODS were parallel and almost coincided with each other, providing evidence that ODS phase can recognize only the hydrophobicity of analytes. On the other hand, it has been found that Sil-Amphi**6** showed higher retention for PAHs compared to its values for alkylbenzenes (Figure S5).

1 M. M. Rahman, M. Czaun, M. Takafuji, H. Ihara, Chem. Eur. J. 2008, 14, 1312-1321; A. Shundo, T. Sakurai, M. Takafuji, S. Nagaoka, H. Ihara, J. Chromatogr. A 2005, 1073, 169-174; H. A. Claessens, M. A. Van Straten, C. A. Cramers, M. Jezierska, B. Buszewski, J. Chromatogr. A 1998, 826, 135-156.

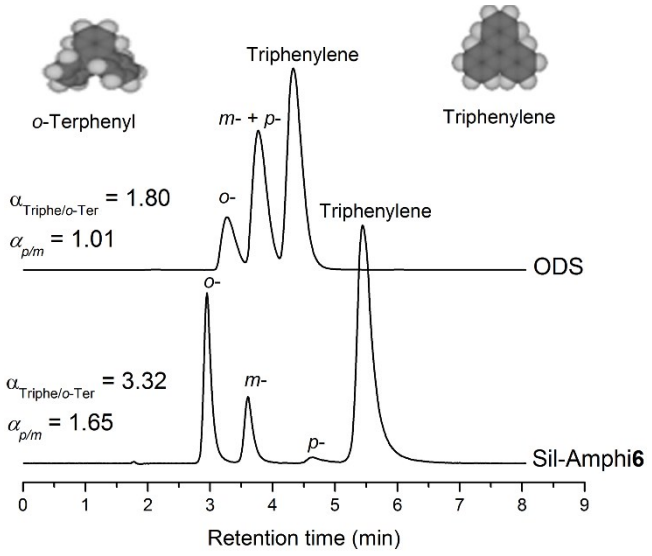


Figure S6. Separation of *o*-, *m*-, *p*-terphenyl and triphenylene on ODS and Sil-Amphi**6** columns. Mobile phase: methanol (100%) and methanol-water (80:20, v/v), respectively; column temperature = 15 °C and 20 °C, respectively; flow rate = 1 mL min^{-1}; detection: λ = 254 nm.

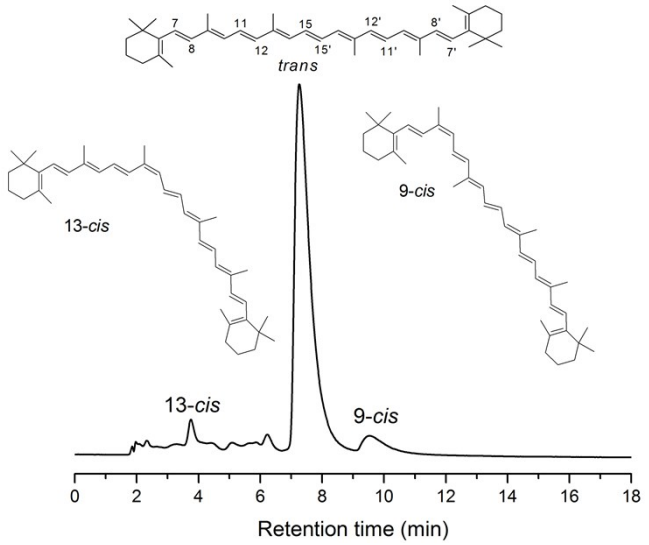


Figure S7. Separation of major β-carotene isomers on Sil-Amphi**6**. Mobile phase: methanol (100%); column temperature = 10 °C; flow rate = 1 mL min^{-1}; detection: λ = 450 nm.

Table S1. Chromatographic Characterization (Tanaka Column Characterization Protocol) of the Newly Synthesized and Commercial Reference Column in This Study (in RPLC)

	k_{PB}	$\alpha(CH_2)$	$\alpha_{T/O}$	$\alpha_{C/P}$	$\alpha_{A/P}$ at pH 7.5	$\alpha_{A/P}$ at pH 2.7
ODS	6.85	1.46	1.30	0.47	0.12	0.47
Sil-Amphi**6**	0.43	1.17	2.68	0.21	0.18	0.99

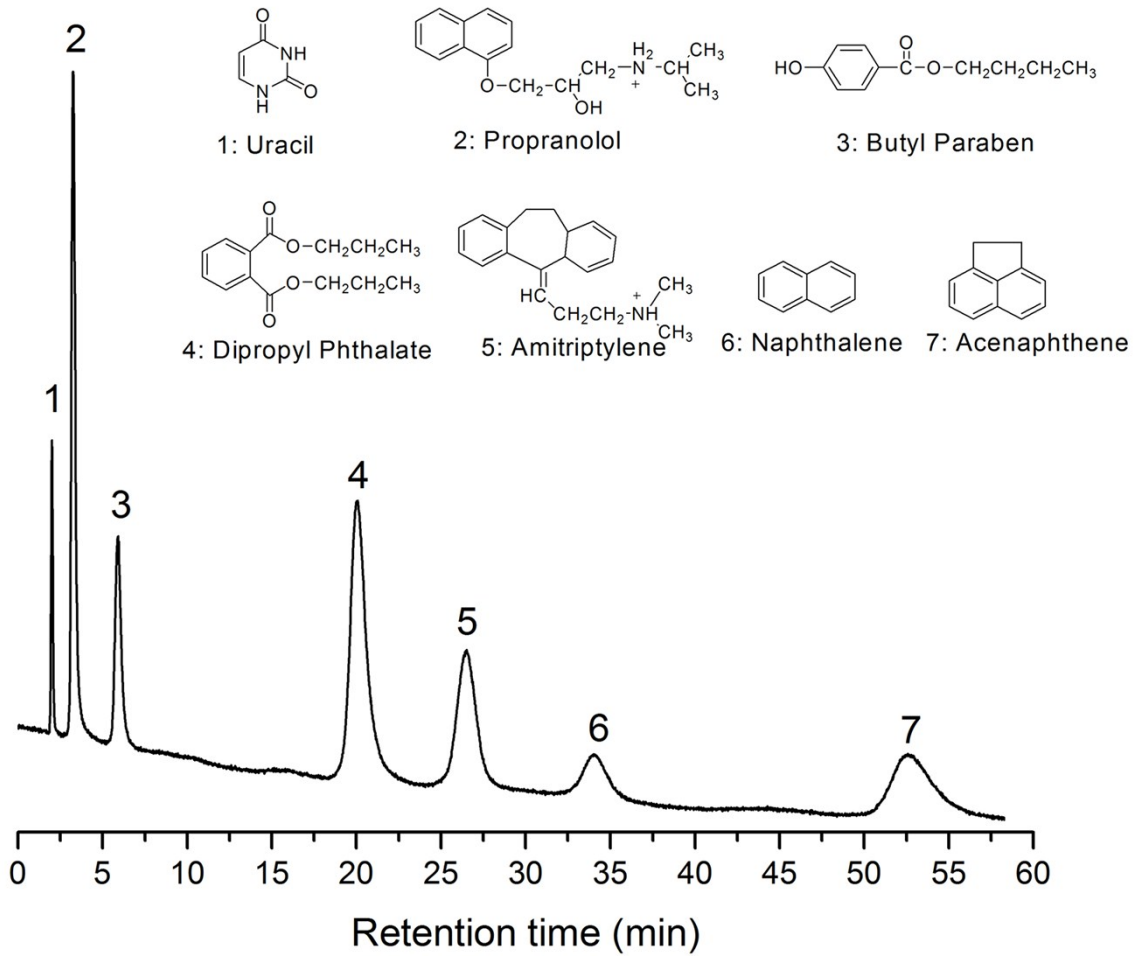


Figure S8. Separation of nonpolar, polar, and basic compounds on Sil-Amphi**6**. Mobile phase = 30:70 (v/v) methanol/20 mM KH_2PO_4/K_2HPO_4 at pH 7; column temperature = 25 °C; flow rate = 1 mL min^{-1}; detection: λ = 254 nm.

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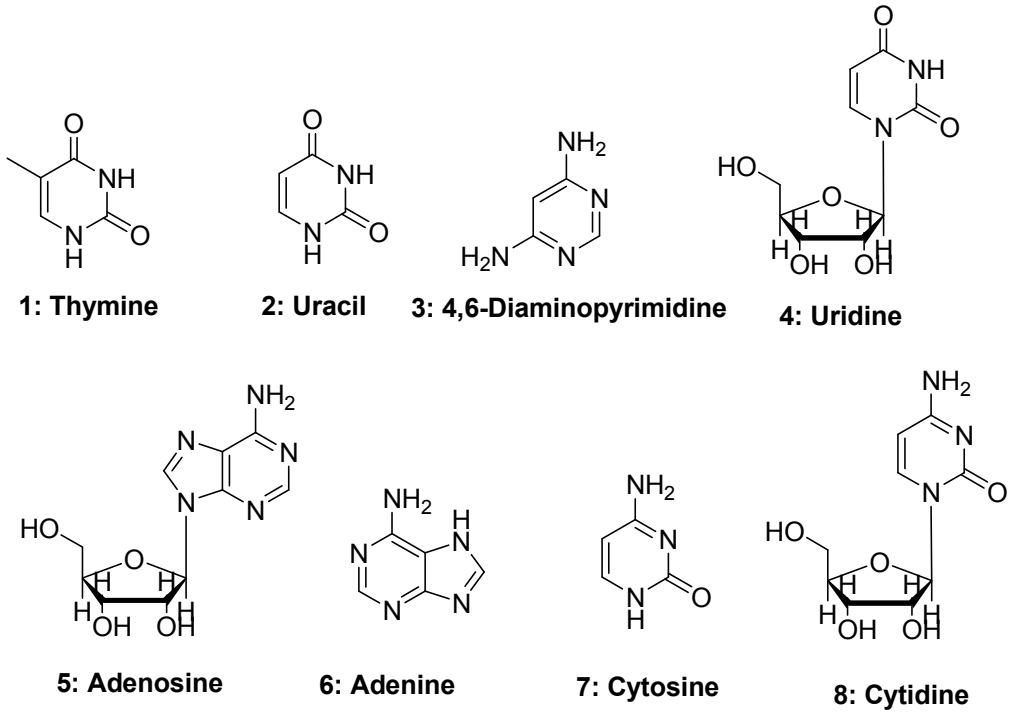


Figure S9. Chemical structures of the nucleobases and nucleosides.

Table S2. Retention factors, selectivity, efficiency, resolution, and asymmetry of sulfa drugs for Sil-Amphi6 stationary phase

Solutes	k	α	N/m	R_s	A_s
Thymine	0.99		29 686		0.90
		1.28		2.26	
Uracil	1.27		37 500		0.83
		1.24		2.33	
4,6-Diaminopyridine	1.57		42 620		0.94
		2.25		10.7	
Uridine	3.54		43 393		0.91
		1.16		2.34	
Adenosine	4.09		45 186		0.94
		1.27		3.77	
Adenine	5.19		42 133		1.23
		1.45		6.75	
Cytosine	7.53		48 046		0.96
		2.03		13.3	
Cytidine	15.31		57 080		1.18

Mobile phase: Acetonitrile: 10 mM NH_4Ac (95:5, v/v). Column temperature: 25 °C. Flow rate: 1.00 mL/min.

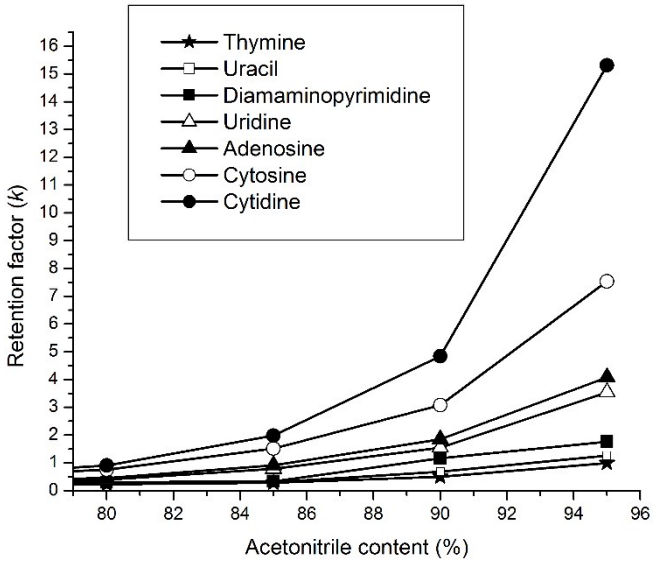


Figure S10. Effect of acetonitrile content on the retention factors of nucleosides and nucleobases. Mobile phase of acetonitrile and 10 mM ammonium acetate with acetonitrile content as indicated. Column temperature = 25 °C; flow rate = 1 mL min^{-1}; detection: λ = 254 nm..

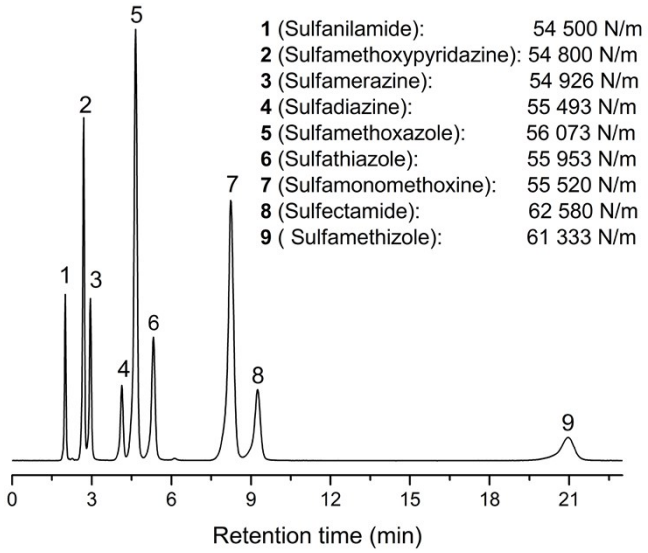
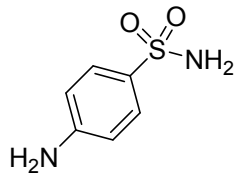
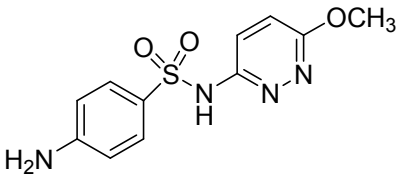


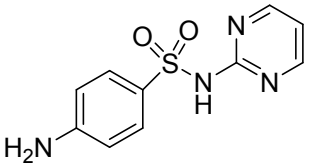
Figure S11. Separation of nine sulfa drugs on Sil-Amphi**6**. Mobile phases: 10 mmol L^{-1} ammonium acetate/acetonitrile (10:90, v/v); column temperature = 25 °C; flow rate = 1 mL min^{-1}; detection: λ = 227 nm.



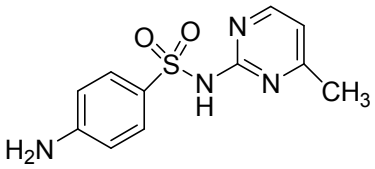
1: Sulfanilamide



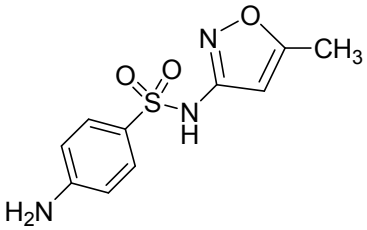
2: Sulfamethoxypyridazine



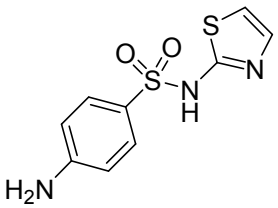
3: Sulfadiazine



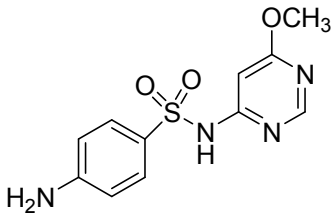
4: Sulfamerazine



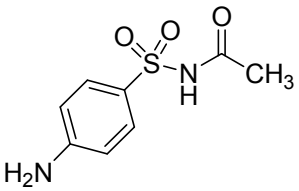
5: Sulfamethoxazole



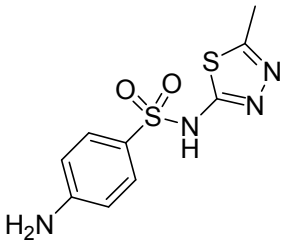
6: Sulfathiazole



7: Sulfamonomethoxine



8: Sulfectamide



9: Sulfamethizole

Chemical structures of the sulfa drugs.

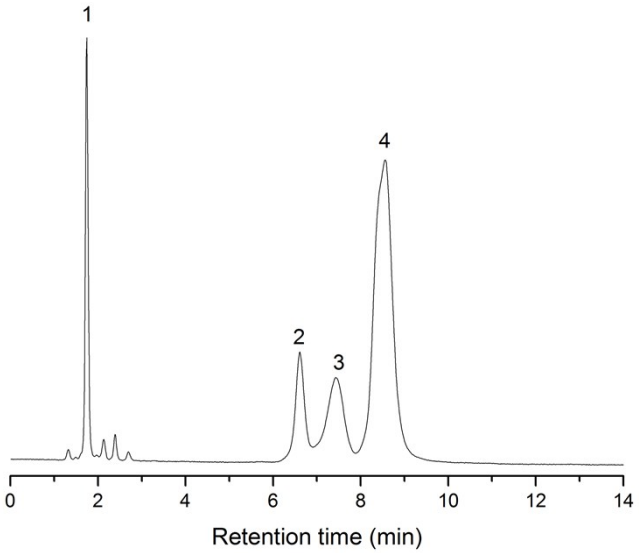
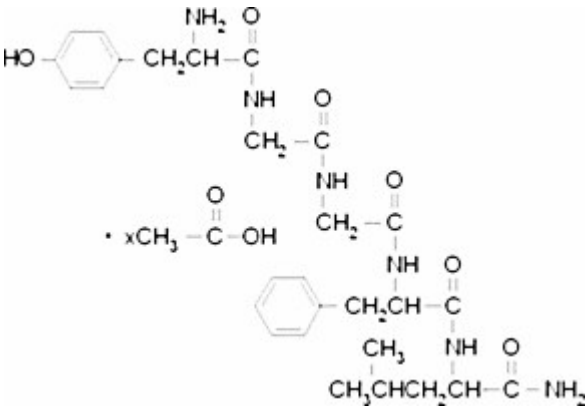
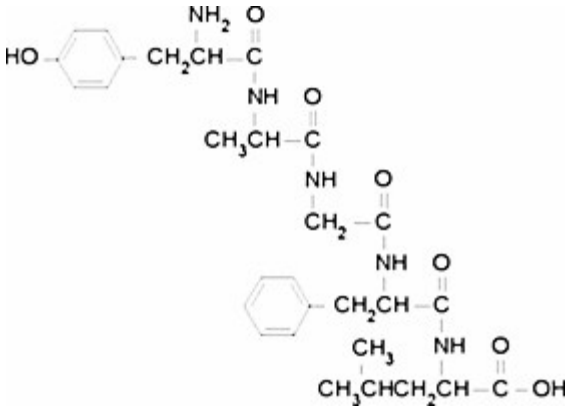


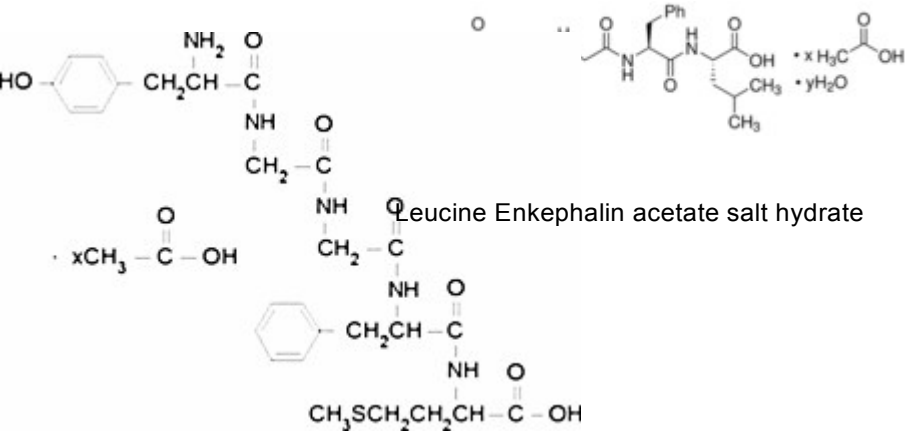
Figure S12. Separation of four pentapeptides (1: [D-Ala^2]-Leucin enkephalinamide, 2: [D-Ala^2]-Leucine enkephalin, 3: [Met^5]-Enkephalin acetate salt hydrate, 4: Leucine enkephalin acetate) on Sil-Amphi**6**. Mobile phases: 10 mmolL^{-1} ammonium acetate/acetonitrile (30:70, v/v); column temperature = 25 °C; flow rate = 1 mL min^{-1}; detection: λ = 200 nm.



[D-Ala^2]-Leucine enkephalin



[D-Ala^2]-Leucine enkephalinamide acetate salt



Leucine Enkephalin acetate salt hydrate

[Met^5]Enkephalin acetate salt hydrate

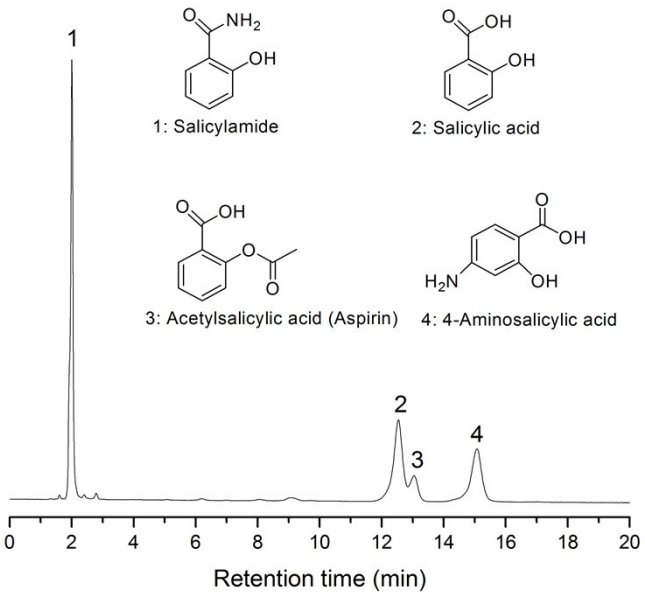


Figure S13. Separation of salicylamides and its acids on Sil-Amphi**6**. Mobile phases: 10 mmolL^{-1} ammonium acetate/acetonitrile (30:70, v/v); column temperature = 25 °C; flow rate = 1 mLmin^{-1}; detection: λ = 200 nm.

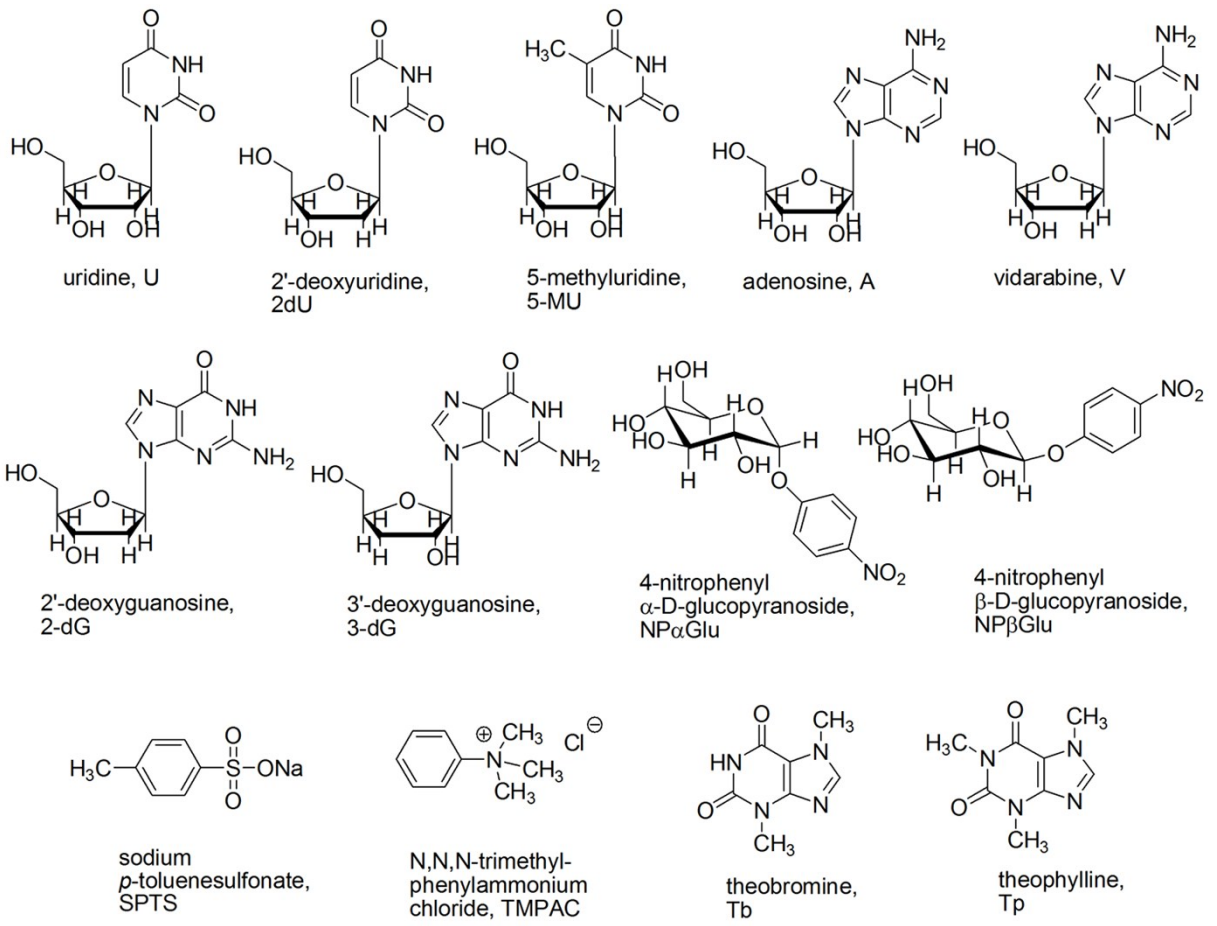


Figure S14. Chemical structure of test samples.

Table S3. Selectivity for methylene groups α(CH_2) and hydroxyl groups α(OH)

Column	U			2dU			α(U/2dU)	5MU			α(U/5U)
	k	*H* (μm)	As	*k*	*H*(μm)	As		*k*	*H* (μm)	As	
Sil-APS	8.09	119	1.34	7.83	120	1.37	1.03	7.53	123	0.85	1.07
Sil-Amphi**6**	1.22	28	0.95	0.68	33	0.91	1.79	1.02	28	0.85	1.20

Mobile phase: ACN-ammonium acetate buffer (20 mM in the aqueous portion, pH 4.7) (90:10, v/v). Column temperature: 30 °C. Flow rate: 0.5 mL/min.

Table S4. Selectivity for configurational isomers

Column	A			V			α(V/A)
	k	*H* (μm)	Asym	*k*	*H* (μm)	Asym	
Sil-APS	2.51	22	0.99	3.70	26	0.89	1.48
Sil-Amphi**6**	1.44	31	0.86	2.01	23	0.86	1.39

Mobile phase: ACN-ammonium acetate buffer (20 mM in the aqueous portion, pH 4.7) (90:10, v/v). Column temperature: 30 °C. Flow rate: 0.5 mL/min.

Table S5. Selectivity for region isomers

Column	3dG			2dG			α(2dG/3dG)
	k	*H* (μm)	Asym	*k*	*H* (μm)	Asym	
Sil-APS	16.7	22	0.86	17.8	30	1.76	1.05
Sil-Amphi**6**	4.04	15	0.86	4.31	14	1.12	1.07

Mobile phase: ACN-ammonium acetate buffer (20 mM in the aqueous portion, pH 4.7) (90:10, v/v). Column temperature: 30 °C. Flow rate: 0.5 mL/min.

Table S6. Selectivity for molecular shapes

Column	NPαGlu			NPβGlu			α(α/β)
	k	*H* (μm)	Asym	*k*	*H* (μm)	Asym	
Sil-APS	1.96	22	1.01	1.65	23	1.01	1.18
Sil-Amphi**6**	1.35	25	0.92	1.13	25	0.81	1.19

Mobile phase: ACN-ammonium acetate buffer (20 mM in the aqueous portion, pH 4.7) (90:10, v/v). Column temperature: 30 °C. Flow rate: 0.5 mL/min.

Table S7. Selectivity for ionic compounds

Column	SPTS		TMPAC	
	k	α(SPTS/U)	*k*	α(TMPAC/U)
Sil-APS	14.1	1.74	0.02	0.002
Sil-Amphi**6**	−[a](0.07)	−[a](0.06)	13.9	11.5

Mobile phase: ACN-ammonium acetate buffer (20 mM in the aqueous portion, pH 4.7) (90:10, v/v). Column temperature: 30 °C. Flow rate: 0.5 mL/min.

[a] SPTS eluted faster than the t_0 marker, toluene.

Table S8. Test for pH on the surface of stationary phases

Column	Theophylline			Theobromine			α(Tb/Tp)
	k	*H* (μm)	Asym	*k*	*H* (μm)	Asym	
Sil-APS	7.18	132	1.47	0.70	42	1.19	0.10
Sil-Amphi**6**	0.40	42	1.06	0.25	57	0.95	0.62

Mobile phase: ACN-ammonium acetate buffer (20 mM in the aqueous portion, pH 4.7) (90:10, v/v). Column temperature: 30 °C. Flow rate: 0.5 mL/min.