Supporting Information

New L-lysine derived high molecular-shape selective organic phase with ordered functional groups for reversed-phase liquid chromatography

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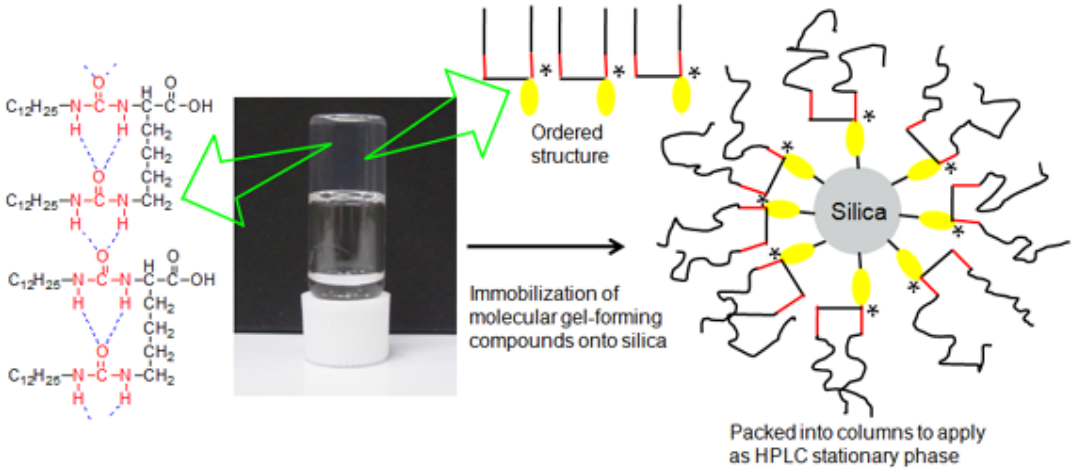
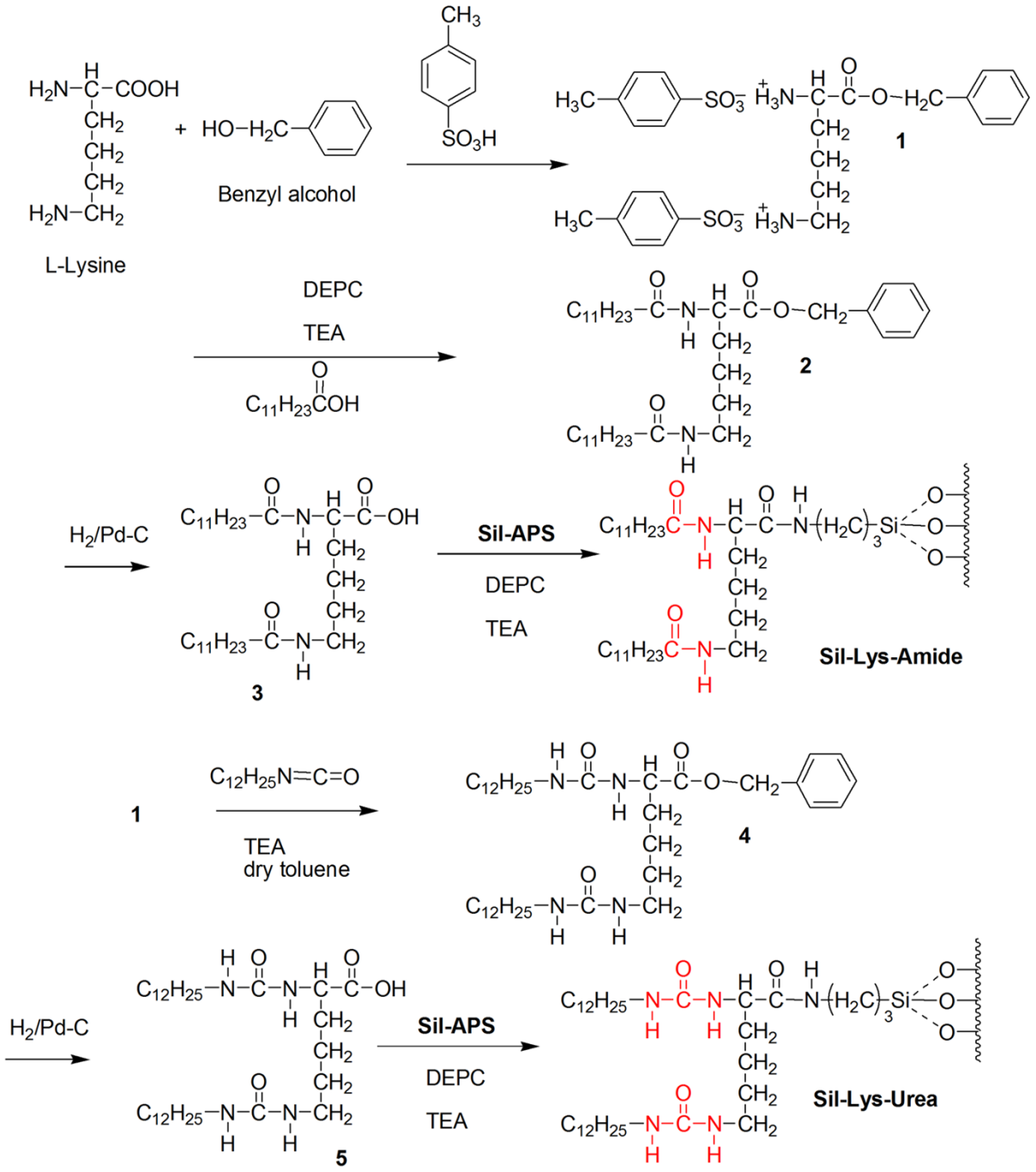


Scheme S1. Schematic illustration of the urea containing organic phase.

Materials and Methods

The tocopherol isomers were obtained from CalBiochem, USA. β-Carotene was purchased from Sigma, USA. L-lysine, lauric acid, dodecyl isocyanate, diethylphosphorocyanidate (DPEC, peptide synthesis reagent), triethylamine (TEA), and β-alanine were purchased from Wako (Japan) and used without further purification. Polycyclic aromatic hydrocarbons (PAHs) were purchased from TCI (Japan). L-lysine-based organic phases on silica (Sil-Lys-Urea and Sil-Lys-Amide) stationary phases were synthesized, characterised, and packed into a stainless steel column (150 mm × 4.6 mm i.d.). YMC silica (YMC SIL-120-S5 having a 5 μm diameter, and a 12 nm pore size) was used. In contrast, we used commercial C_{18} (Inertsil, ODS 3, column size 150 mm × 4.6 mm i.d. with a 5.5 μm particle size, and a 10 nm pore size from GL Science), and C_{30} (column size 150 mm × 4.6 mm i.d., a 5 μm particle size, and a 10 nm pore size from Nomura Chemical Co., Ltd.) columns for the comparison of chromatographic results. The conformational structure and mobility of the alkyl chain of the stationary phase was determined by using solid-state ^{13}C CP/MAS NMR spectra. Surface bonding chemistry of silica particles was studied by using solid-state ^{29}Si CP/MAS NMR spectra. NMR spectra data were obtained by a Varian Unity^{Inova} AS400 at a static magnetic field of 9.4 T using a solid probe for CP/MAS NMR at a spin rate of 4000-4500 Hz for solid-state NMR. The chromatographic system included a Gulliver PU-1580 intelligent HPLC pump with a Rheodyne sample injector. A JASCO multiwavelength UV detector MD 1510 plus was also used. The chromatography was performed under isocratic elution conditions. The retention factor (k) was determined by using the formula $(t_e - t_o)/t_o$, where t_e and t_o were the retention time of the samples and of methanol, respectively. The separation factor (α) was given by the ratio of retention factors.

Synthesis of amide and urea type low-molecular-weight organogelators (compound 3 & 5)



Scheme S2. Synthesis of $N^{\alpha},N^{\varepsilon}$-Bis(lauroyl)-L-lysine (**3**) and $N^{\alpha},N^{\varepsilon}$-Bis(dodecylaminocarbonyl)-L-lysine (**5**) .

*L-Lysine benzyl ester bis(toluene-p-sulfonate) (**1**):* **1** was prepared according to the literature, [1] white powder, yield 2.0 g (13%); mp 148–151 °C; IR (KBr): ν = 3436, 3034, 1752, 1526, 1218, 1176 cm^{-1}.

*$N^{\alpha},N^{\varepsilon}$-Bis(lauroyl)-L-lysine benzyl ester (**2**)*: **1** (5.00 g, 8.61 mmol) and lauric acid (3.45 g, 17.20 mmol) were dissolved in dry chloroform (600 ml) by stirring. Triethylamine (TEA) (7.17 g, 21.5 mmol) was added to the mixture followed by diethylphosphorocyanidate (DEPC) (3.50 g, 21.5 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 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 pressure, recrystallized from methanol, and dried *in vacuo* to give a white powder (6.10 g, yield: 70.84%). mp: 80–82 ˚C; ^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.35 (m, 5H; C_6H_5), 6.19 (s, 1H; $N^{\alpha}H$), 5.61 (s, 1H; $N^{\varepsilon}H$), 5.18 (m, 2H; $C_6H_5CH_2$) 4.61 (s, 1H; *C*H*), 3.18 (m, 2H; $N^{\varepsilon}HCH_2$), 2.22 (m, 2H; $N^{\alpha}HCOCH_2$) 2.14 (m, 2H; $N^{\varepsilon}HCOCH_2$) 1.25-1.62 (m, 42H; $CH_3(CH_2)_9 \times 2$, *$CH(CH_2)_3$), 0.86-0.89 ppm (t, 6H; $CH_3 \times 2$); IR (KBr): ν = 3322, 2921, 2850, 1721, 1642, 1542, 1469 cm^{-1}. Elemental analysis (Anal. Found: H, 10.59; C, 72.94; N, 4.67 Cal. For $C_{37}H_{64}N_2O_4$: H, 10.73; C, 73.95; N, 4.66%).

*N^{α},N^{ε}-Bis(lauroyl)-L-lysine (**3**)*: N^{α},N^{ε}-Bis(lauryl)-L-lysine benzyl ester (**2**) (5.00 g, 8.32 mmol) was dissolved in 600 ml of ethanol with heating and Pd carbon black (1.5 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 (4.10 g, yield: 82.01%). mp: 111–112 ˚C; ^{1}H NMR (400 MHz, $CDCl_3$): δ = δ = 5.72 (s, 1H; $N^{\alpha}H$), 4.74 (s, 1H; $N^{\varepsilon}H$), 4.44 (s, 1H; *C*H*), 3.19 (m, 2H; $N^{\varepsilon}HCH_2$), 2.27 (m, 2H; $N^{\alpha}HCOCH_2$) 2.17 (m, 2H; $N^{\varepsilon}HCOCH_2$) 1.25-1.62 (m, 42H; $CH_3(CH_2)_9 \times 2$, *$CH(CH_2)_3$), 0.86-0.89 ppm (t, 6H; $CH_3 \times 2$); IR (KBr): ν = 3305, 2917, 2848, 1717, 1641, 1556 cm^{-1}. Elemental analysis (Anal. Found: H, 11.04; C, 69.56; N, 5.32 Cal. For $C_{30}H_{58}N_2O_4$: H, 11.45; C, 70.54; N, 5.48%).

*N^{α},N^{ε}-Bis(dodecylaminocarbonyl)-L-lysine benzyl ester (**4**)*: To a dry toluene solution of **1** (5.00 g, 8.85 mmol), dodecylisocyanate (3.64 g, 17.20 mmol), and triethylamine (3.60 g, 26.00) were added with stirring. After heating the solution at 100 °C for 10 min, the excess toluene was evaporated off. The residue was recrystallized from methanol and dried *in vacuo*, which gives a white solid powder (4.50 g, yield: 90%).
mp: 111–112 ˚C; ^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.31 (m, 5H; C_6H_5), 5.32 (s, 1H; $N^{\alpha}H$), 5.18 (m, 2H; $C_6H_5CH_2$) 4.78 (s, 1H; $N^{\varepsilon}H$), 4.64 (s, 1H; $N^{\alpha}HCONH$) 4.47 (m, 1H; $N^{\varepsilon}HCONH$) 4.46 (s, 1H; *C*H*), 3.13-3.14 (m, 6H; $N^{\varepsilon}HCONHCH_2$, $N^{\varepsilon}HCH_2$, $N^{\alpha}HCONHCH_2$), 1.26-1.78 (m, 46H; $CH_3(CH_2)_{10} \times 2$, *$CH(CH_2)_3$), 0.86-0.95 ppm (t, 6H; $CH_3 \times 2$); IR (KBr): ν = 3340, 2922, 2850, 1733, 1628, 1577, 1467 cm^{-1}. Elemental analysis (Anal. Found: H, 10.68; C, 70.26; N, 8.78 Cal. For $C_{39}H_{70}N_4O_4$: H, 10.71; C, 71.08; N, 8.50%).

*N^{α},N^{ε}-Bis(dodecylaminocarbonyl)-L-lysine (**5**)*: *N^{α},N^{ε}-Bis(dodecylaminocarbonyl)-L-lysine benzyl ester (**4**)*(4.00 g, 6.07 mmol) was dissolved in 500 ml of ethanol with heating and Pd carbon black (1.5 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 (3.40 g, yield: 85.01%). mp: 141–142 ˚C; ^{1}H NMR (400 MHz, $CDCl_3$): δ = 6.17 (s, 1H; $N^{\alpha}H$), 5.54 (s, 1H; $N^{\varepsilon}H$), 4.95 (s, 1H; $N^{\alpha}HCONH$) 4.18 (s, 2H; $N^{\varepsilon}HCONH$, *C*H*) 3.10-3.25 (m, 6H; $N^{\varepsilon}HCONHCH_2$, $N^{\varepsilon}HCH_2$, $N^{\alpha}HCONHCH_2$), 1.26-1.80 (m, 46H; $CH_3(CH_2)_{10} \times 2$, *$CH(CH_2)_3$), 0.86-0.89 ppm (t, 6H; $CH_3 \times 2$); IR (KBr): ν = 3332, 2922, 2851, 1719, 1627, 1577, 1467 cm^{-1}. Elemental analysis (Anal. Found: H, 11.09; C, 67.62; N, 9.72 Cal. For $C_{32}H_{64}N_4O_4$: H, 11.34; C, 67.56; N, 9.85%).

Immobilization of LMWG onto Silica

(3-Aminopropyl)trimethoxysilane (APS) grafted silica (Sil-APS) was prepared by refluxing porous silica gel (3.00 g) and 1.5 ml of 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 L-lysine derivatives **3** and **5**, respectively. Sil-APS (3.0 g) and **3** and **5** (3.00 g, 5.72 mmol and 3.25 g, 5.72 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 lipid molecule and dried in vacuo to get Sil-Lys-Amide and Sil-Lys-Urea stationary phases (Scheme S1).

(a) (b)

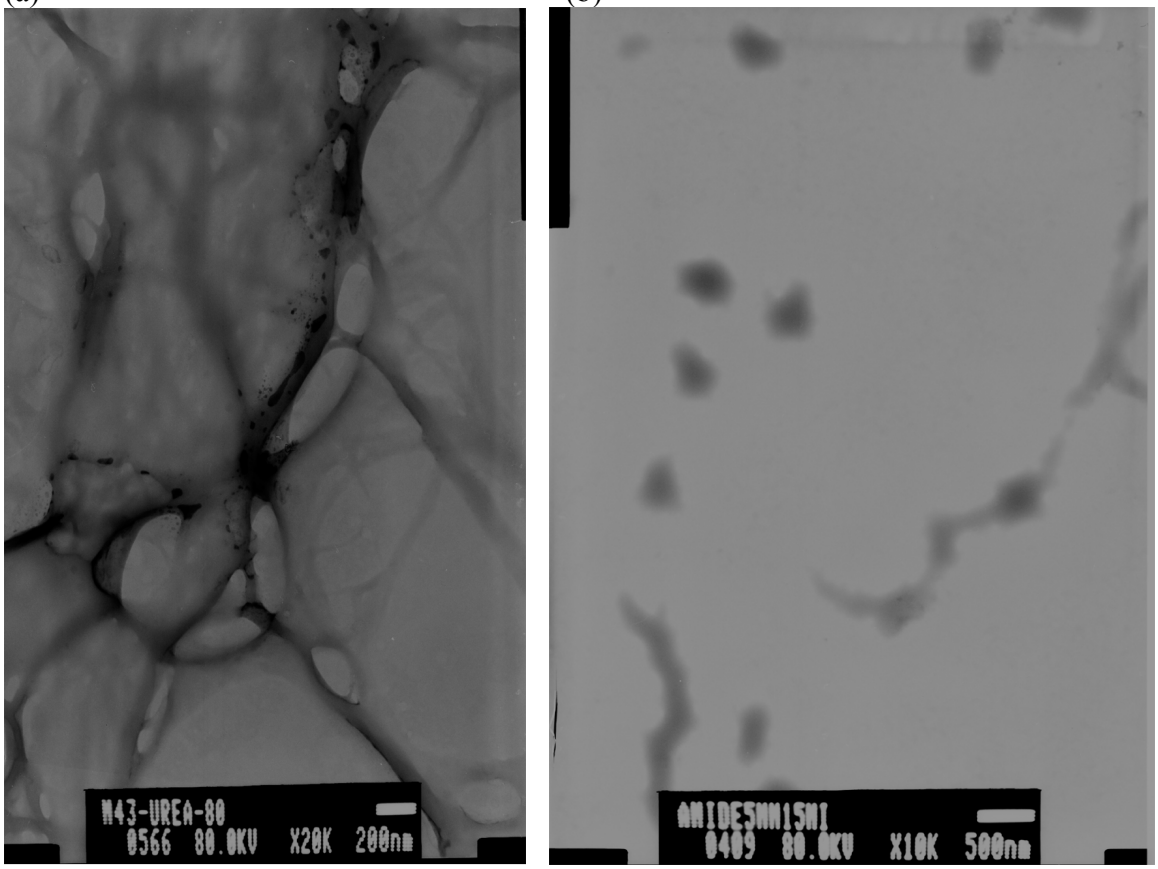


Figure S1. The TEM images of (a) compound **5** and (b) compound **3** in chloroform at a concentration of 1mmol.

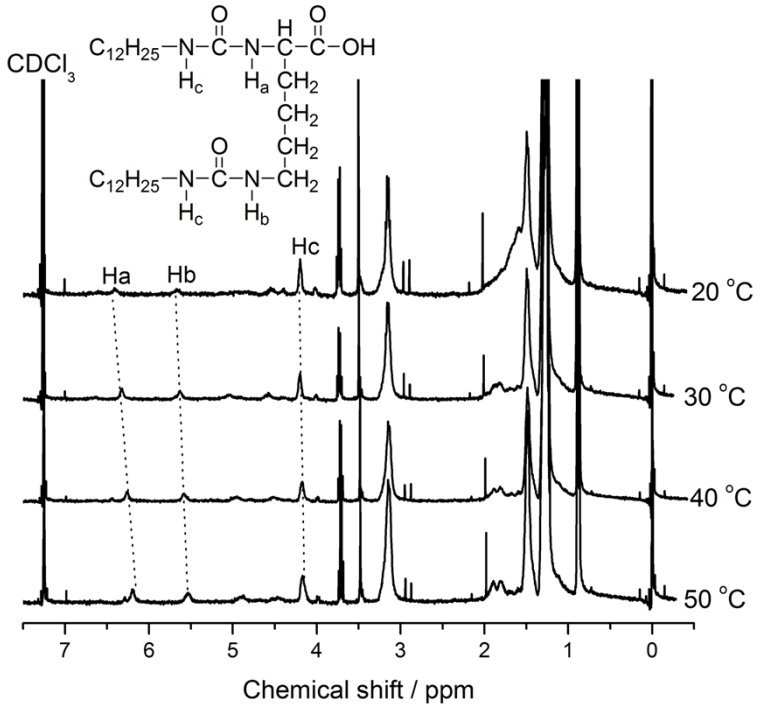


Figure S2. The ^{1}H NMR spectra of the gel of **5** at different temperatures.

Table S1 Gelation properties of compound **3** (5 mmol) and compound **5** (5 mmol) in organic solvents at room temperature.

	Compound **3**	Compound **5**
Benzene	PG	G
THF	S	PG
Chloroform	S	G
Cyclohexane	S	PS
Methanol	S	PG
Toluene	PG	G
Acetonitrile	I	PG
n-Hexane	VS	PS
Diethyl ether	I	I
Ethyl acetate	S	PG

G, gel; S, solution; PS, partially soluble; VS, viscous solution; PG, partial gel; I, insoluble.

Characterization of Sil-Lys-Urea and Sil-Lys-Amide

Table S2 Elemental analysis and TGA data of Sil-APS, Sil-Lys-Urea, and Sil-Lys-Amide.

	C (%)	H (%)	N (%)	C/N	Surface coverage (μmol/m^2)	Grafting (%) TGA
Sil-APS	8.33	2.23	2.81	2.96	4.86	8.49
Sil-Lys-Urea	15.77	3.30	3.14	5.02	0.72	8.21
Sil-Lys-Amide	14.99	3.05	3.01	4.98	0.68	8.21
[a]C_{30}	17.80	-	-	-	1.66	-
[b]C_{18}	13.80	-	-	-	1.72	-

[a] and [b] indicates carbon content and surface coverage of two commercial columns.

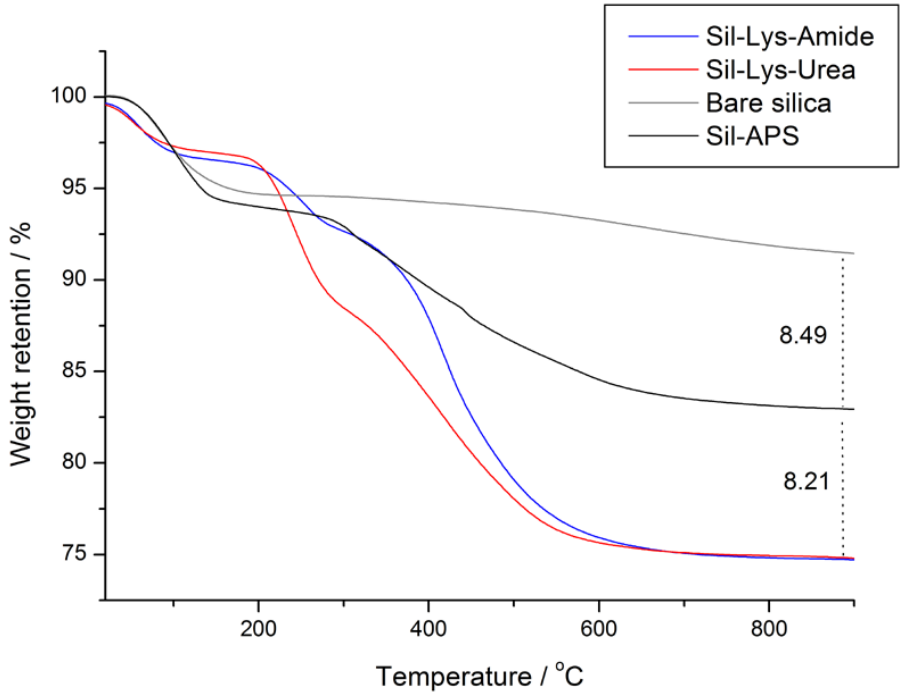


Figure S3. TGA curves of bare silica, Sil-APS, Sil-Lys-Amide, and Sil-Lys-Urea.

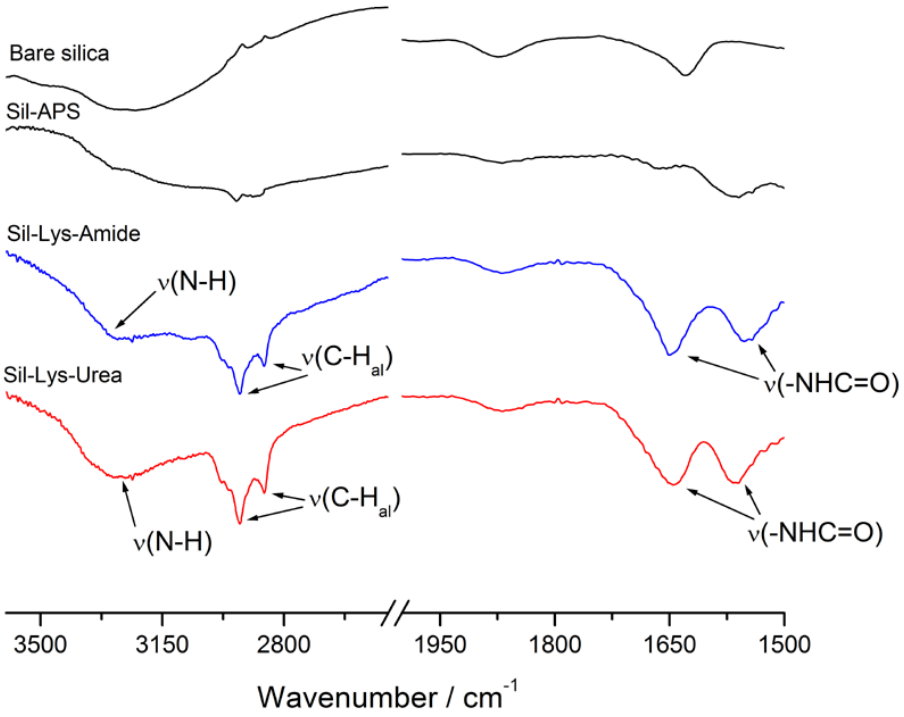


Figure S4. DRIFT spectra of bare silica, Sil-APS, Sil-Lys-Amide, and Sil-Lys-Urea.

A group of peaks at 2927 and 2856 cm^{-1}, respectively, were attributed to the C–H bond stretching of the long alkyl chains for both Sil-Lys-Urea and Sil-Lys-Amide. The spectrum of Sil-Lys-Urea showed intense bands at 1645 and 1560 cm^{-1}, indicating the presence of grafted urea bonded organic phase onto silica surface. Similarly, the spectrum of Sil-Lys-Amide showed intense bands at 1651 and 1552 cm^{-1}, indicating the presence of grafted amide bonded organic phase onto silica surface.

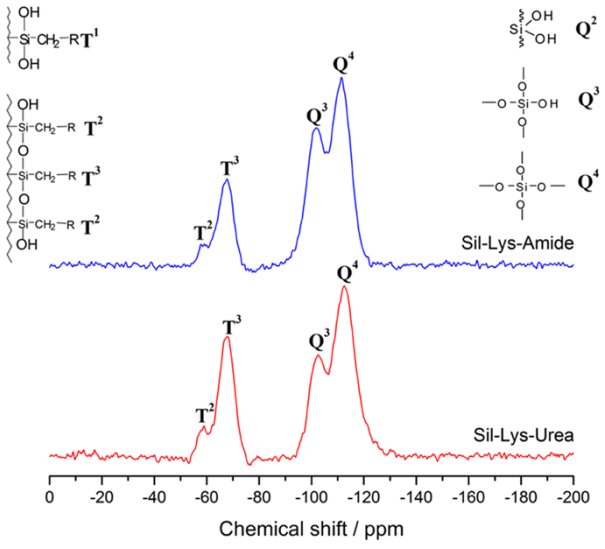


Figure S5. ^{29}Si CP/MAS NMR specta of Sil-Lys-Amide and Sil-Lys-Urea at room temperature.

It is well known that the signals of trifunctional species (T^n) appear in the range of −49 to −66 ppm and signals from the native silica (Q^n) from −91 to −110 ppm.[2a] Obviously Sil-Lys-Urea and

Sil-Lys-Amide phases possess high cross-linking according to the signals at −56 ppm (T^2) and at −65 ppm (T^3), while there is no signal for T^1 species visible in the spectrum (Figure S5). The intensity of signal at −56 ppm for T^2 is very low compared to T^3, which reveals that these stationary phase contain trifunctional species with an extremely high degree of cross-linking.[2b] The absence of T^1 groups on the grafted material proves the successful grafting and furthermore implies the high stability of the phase. In ^{29}Si spectrum of native bare silica, the Q^4 (tetrasiloxane), Q^3 (hydroxysiloxane), and Q^2 (dihydroxysiloxane) were detected from intense signals at −110, −101 and −92 ppm, respectively.[2b] However, in the spectra of Sil-Lys-Urea and Sil-Lys-Amide dihydroxysiloxane (Q^2) or geminal silanol groups are almost undetectable indicating low degree of silanol activity for the grafted materials. The reduced signal for Q^3 species compared to native silica gave an insight about the lower amount of free OH-groups on the surface, which lead to less silanophilic interactions in high-performance liquid chromatography (HPLC).[2a-c]

Chromatographic evaluation

Table S3 Separation factors (α) of poly cyclic aromatic hydrocarbons (PAHs) and their isomers for various phases.

Organic Phases	Phenanthrene/*cis*-Stilbene	*trans*-/*cis*-Stilbene	Coronene/Hexahelicene
Sil-Lys-Urea	**2.06**	**1.66**	**27.3**
Sil-Lys-Amide	1.65	1.58	3.31
C_{30}	1.43	1.37	7.66
C_{18}	1.10	1.10	0.93

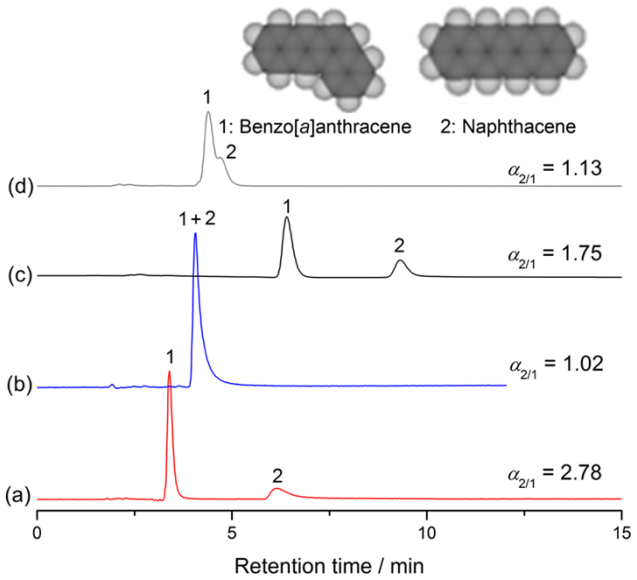


Figure S6. Separation of benzo[*a*]anthracene and naphthacene on (a) Sil-Lys-Urea, (b) Sil-Lys-Amide, (c) C_{30}, and (d) C_{18} phases. Mobile phase: methanol-water (100:0 for (a), (c), and (d) and 55:45 for (b)), column temperature 15 ˚C, flow rate: 1.0 mL / min. UV detection: 265 nm.

Shape selectivity is defined by a column's ability to resolve solutes on the basis of molecular shape, and this property can be evaluated in terms of a selectivity factor for compounds with dissimilar, constrained molecular shapes. Several tests for this purpose have been proposed based on the retention of planar and nonplanar solutes. SRM 869a column selectivity test mixture for liquid chromatography utilizes three PAHs (phenanthro[3,4-*c*]phenanthrene, PhPh; tetrabenzonapthalene, TBN; and benzo[a]pyrene, BaP), two of which are nonplanar (PhPh and TBN). The use of SRM 869a (SRM 869b) provides a way to objectively assess shape selectivity in terms of a numerical descriptor ($\alpha_{TBN/BaP}$). The selectivity factor $\alpha_{TBN/BaP}$ is indicative of how well a particular sorbent will separate isomer mixtures and related shape-constrained solutes. Values of $\alpha_{TBN/BaP} < 1$ are typical of polymeric C_{18} stationary phases and other shape-selective sorbents, whereas values of $\alpha_{TBN/BaP} \geq 1.7$ are more typical of monomeric C_{18} columns, which exhibit reduced shape recognition.

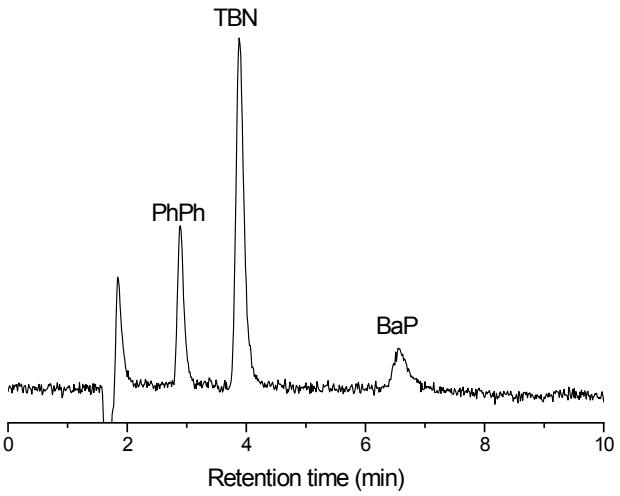


Figure S7. Separation of SRM 869b on a Sil-Lys-Urea phase. Mobile phase: methanol-water (100:0), column temperature 25 ˚C, flow rate: 1.0 mL / min. UV detection: 254 nm.

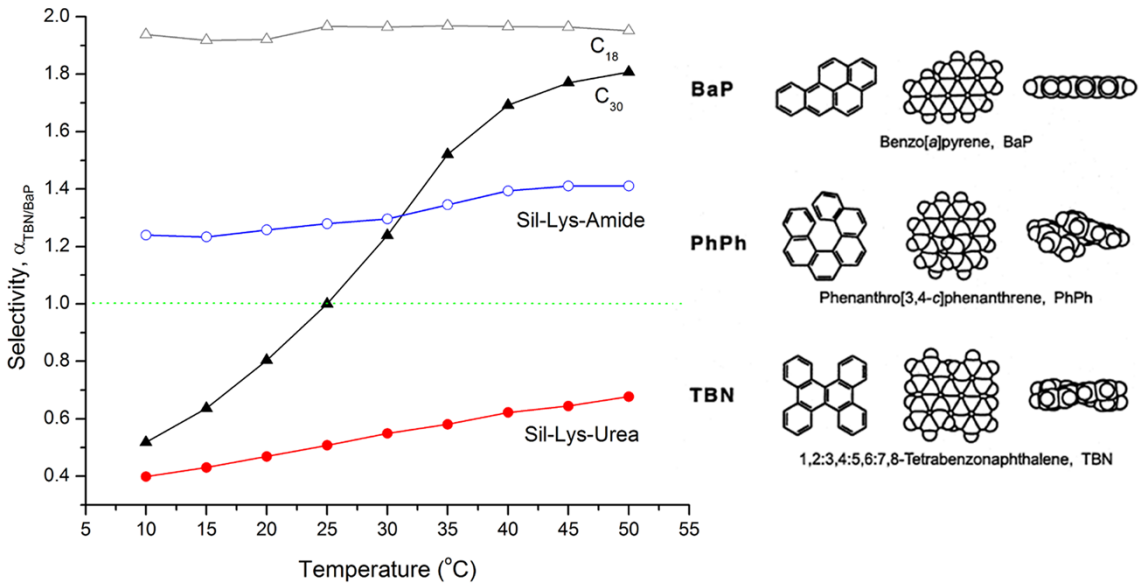


Figure S8. Phase selectivity ($\alpha_{TBN/BaP} = k_{TBN}/k_{BaP}$) plotted as a function of temperature. Columns, Sil-Lys-Urea, Sil-Lys-Amide, C_{30}, and C_{18}.

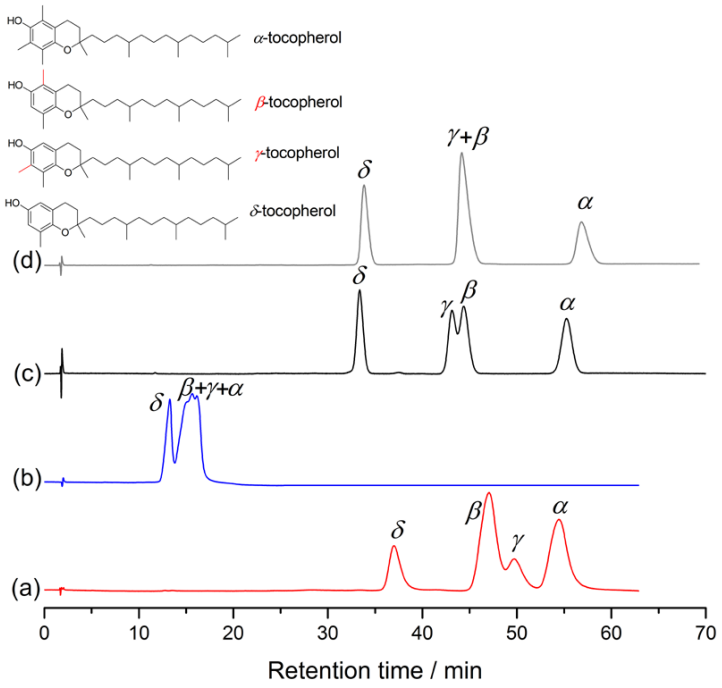


Figure S9. Separation of tocopherol isomers on (a) Sil-Lys-Urea, (b) Sil-Lys-Amide, (c) C_{30}, and (d) C_{18} phases. Mobile phase: methanol-water (75:25) for (a), (55:45) for (b), and (90:10) for (c) and (d); flow rate: 1 ml/min; column temperature: 40 ˚C; UV detection: 285 nm.

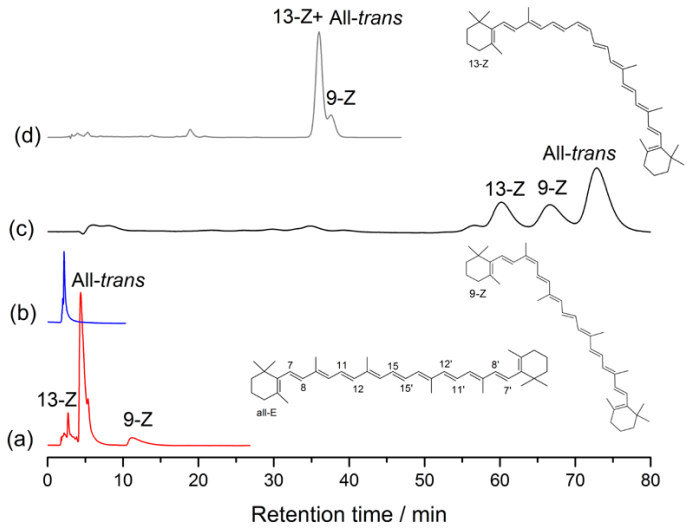


Figure S10. Separation of β-carotene isomers on (a) Sil-Lys-Urea, (b) Sil-Lys-Amide, (c) C_{30}, and (d) C_{18}. Mobile phase: methanol (100%) for (a), (c), and (d), and methanol-water (75:25) for (b); flow rate: 1 ml/min, column temperature: 40 ˚C, UV detection: 450 nm.

Reference

[1] H. Hachisako, Y. Murata, H. Ihara, J. Chem. Soc., Perkin Trans. 2, 1999, 2569–2577.

[2]a) D. W. Sindorf, G. E. Maciel, J. Am. Chem. Soc. 1983, 105, 1487-1493; b) M. Pursh, L. C. Sander, H. -J. Egelhaaf, M. Raitza, S. A. Wise, D. Oelkrug, K. Albert, J. Am. Chem. Soc. 1999, 121, 3201-3213; c) K. Albert, E. Bayer, J. Chromatogr. 1991, 544, 345-370.