

Supporting information

Strategic achievement for the baseline separation of tocopherol isomers by integration of weak interaction sites on alternating copolymer

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Experimental details

Materials

Alternating copolymer, poly(octadecyl acrylate-*alt*-N-octadecyl- β -alanyl-maleimide)-grafted silica (Sil-*alt*-P) stationary phase was synthesized, characterized, and packed into stainless steel column (150 × 4.5 mm i.d.). A YMC silica (YMC SIL-120-S5 having diameter 5 μm , pore size 12 nm, and surface area 300 $\text{m}^2 \text{ g}^{-1}$) was used. For comparing chromatographic results, we have used two commercial monomeric and polymeric C₁₈ columns and C₃₀ column. The monomeric C₁₈ column (Inertsil, ODS 3, column size 250 × 4.6 mm i.d. with particle size 5.5 μm , pore size 10 nm, and surface area of silica particles 450 $\text{m}^2 \text{ g}^{-1}$) was purchased from G. L. Sciences (Tokyo, Japan). This contains 13.8% C in the bonded octadecyl phase. The polymeric C₁₈ column (250 × 4.6 mm i.d., Shodex, C18 P, particle size 5 μm , pore size 10 nm, and surface area 300 $\text{m}^2 \text{ g}^{-1}$ with end cap of the unreacted silanol group) containing 17.5% C was obtained from Shodex (Tokyo, Japan). The C₃₀ column (150 × 4.6 mm i.d., Develosil, particle size 5 μm , pore size 13 nm, and surface area 297 $\text{m}^2 \text{ g}^{-1}$) containing 17.8% C was obtained from Nomura Chemical Co. Ltd. (Japan).

The tocopherol isomers were obtained from CalBiochem, USA. N-Octadecyl amine was purchased from Sigma, USA. Octadecyl acrylate (ODA) was obtained from TCI, Japan and used after removing polymerization inhibitor. Maleic anhydride was obtained from TCI, Japan. The new monomer N-octadecyl- β -alanylmaleimide (OAMI) was synthesized with different steps and the compounds synthesized in every step were characterized by melting point, FT-IR, and ¹H-NMR measurements.

Synthesis of N-octadecyl- β -alanylmaleimide (OAMI) monomer

N-Carbobenzoyl- β -alanine (**I**): 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; ^1H NMR (400 MHz, CDCl_3): δ = 7.34 (m, 5H; C_6H_5), 5.29 (s, 1H; $\text{CH}_2\text{NHC(O)}$), 5.15 (s, 2H; $\text{C(O)CH}_2\text{C}_6\text{H}_5$), 3.47 (t, 2H; CH_2CH_2), 2.60 ppm (t, 2H; CH_2CH_2); IR (KBr): ν = 3336, 3150, 3036, 1686, 1536, 1496, 1454 cm^{-1} .

*N'-Octadecyl-N $^\alpha$ -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 diethylphosphorocyanide (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

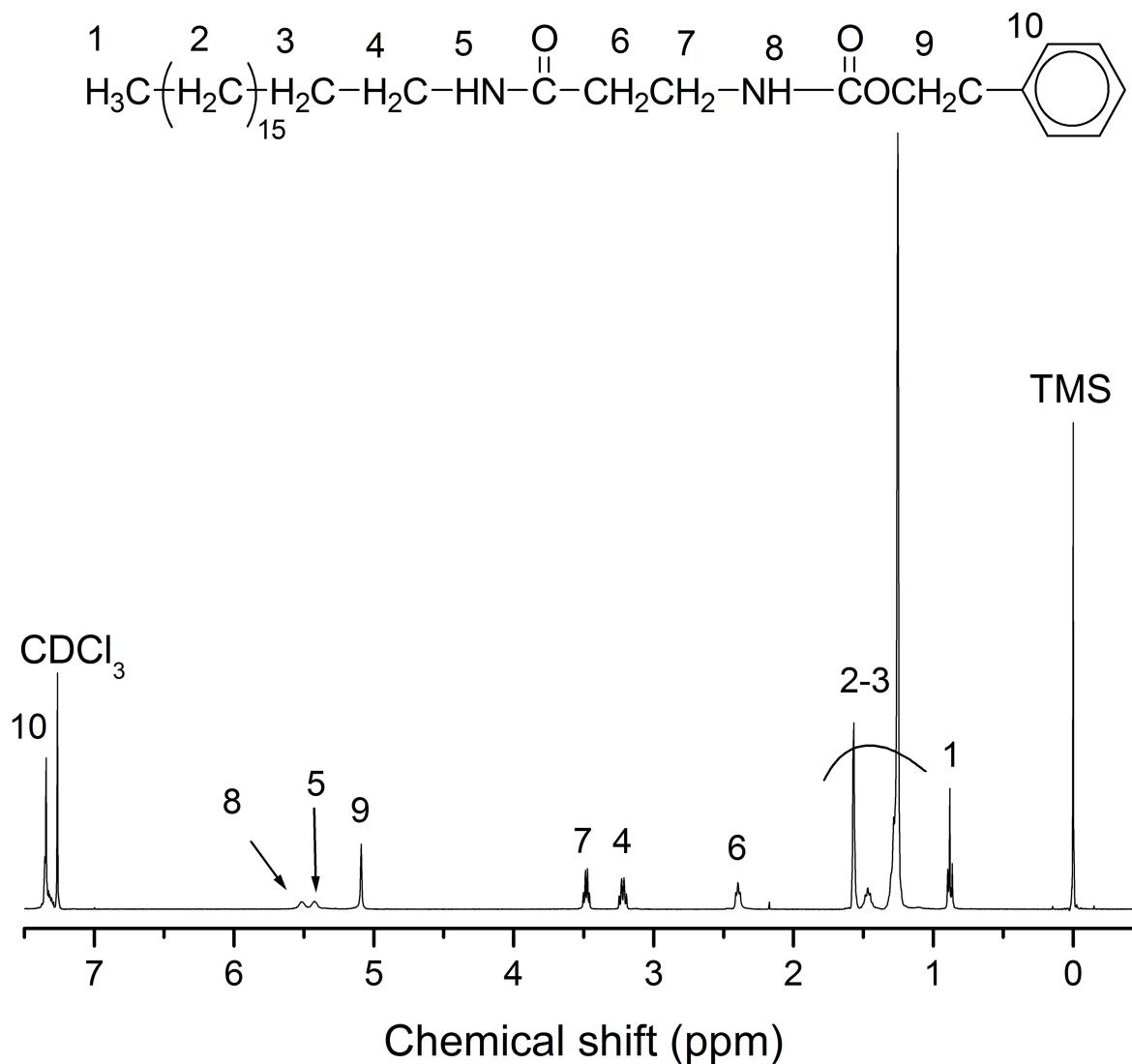


Fig. S1 ^1H NMR spectrum of compound **2**

pressure, recrystallized from methanol, and dried in vacuo to give a white powder (14.1 g, yield: 63.63%). mp: 124–126 °C; ^1H NMR (400 MHz, CDCl_3): δ = 7.34 (m, 5H; C_6H_5), 5.52 (s, 1H; NHC(O)CH_2), 5.41 (s, 1H; $\text{CH}_2\text{NHC(O)}$), 5.09 (s, 2H; $\text{C(O)CH}_2\text{C}_6\text{H}_5$)), 3.48 (q, 2H; $\text{CH}_2\text{CH}_2\text{NHC(O)}$), 3.22 (q, 2H; $\text{CH}_2\text{CH}_2\text{NHC(O)}$), 2.39 (t, 2H; $\text{C(O)CH}_2\text{CH}_2\text{NH}$), 1.46 (m, 2H; $\text{CH}_2\text{CH}_2\text{CH}_2\text{NHC(O)CH}_2$), 1.25 (m, 30H; $\text{CH}_3\text{CH}_2 \times 15$), 0.86 ppm (t, 3H; CH_3); IR (KBr): ν = 3333, 3295, 2918, 2849, 1683, 1635, 1539, 1468, 1442 cm^{-1} .

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: 65.13%). mp: 101–102 °C; ^1H NMR (400 MHz, CDCl_3): δ =

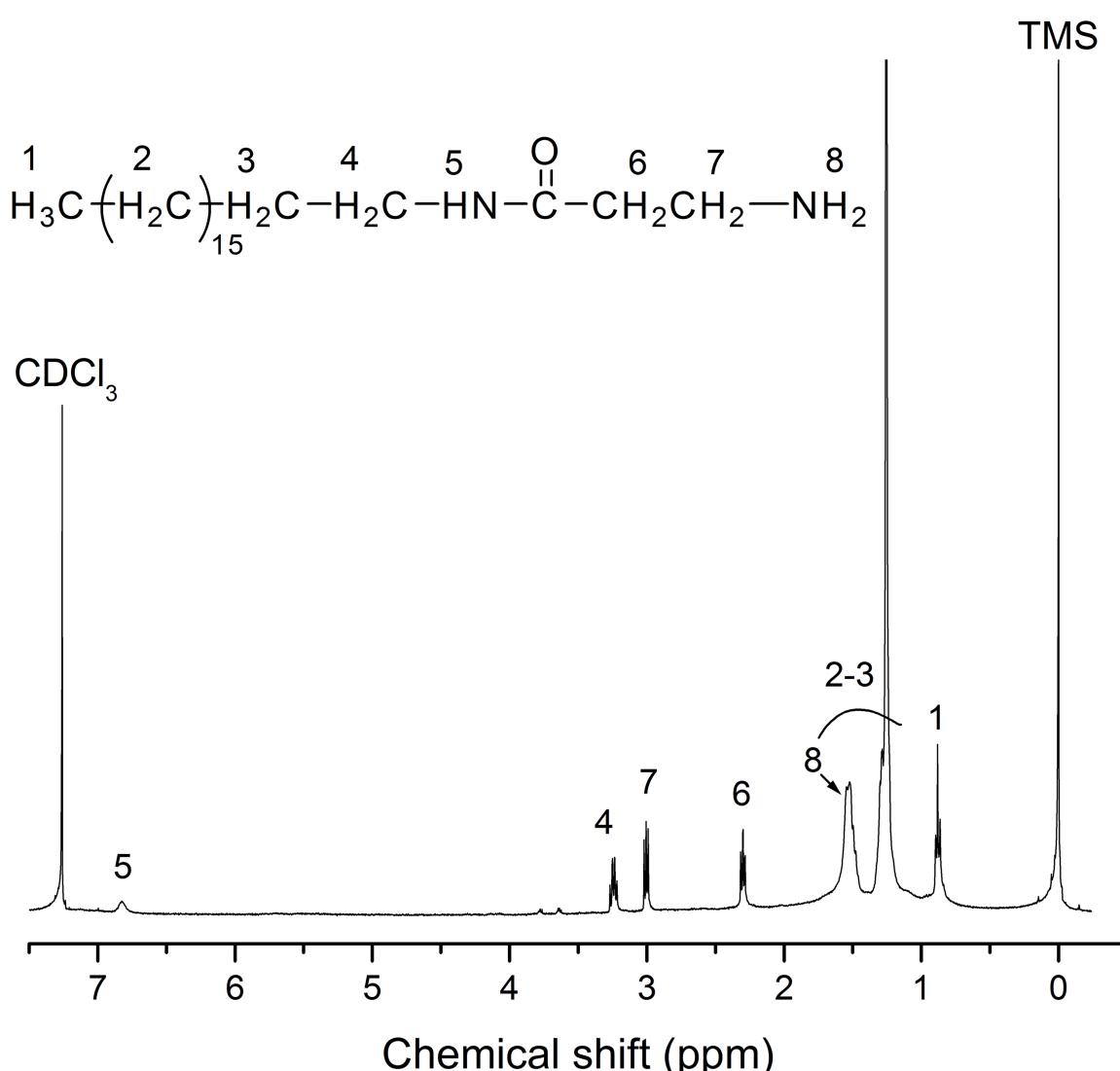


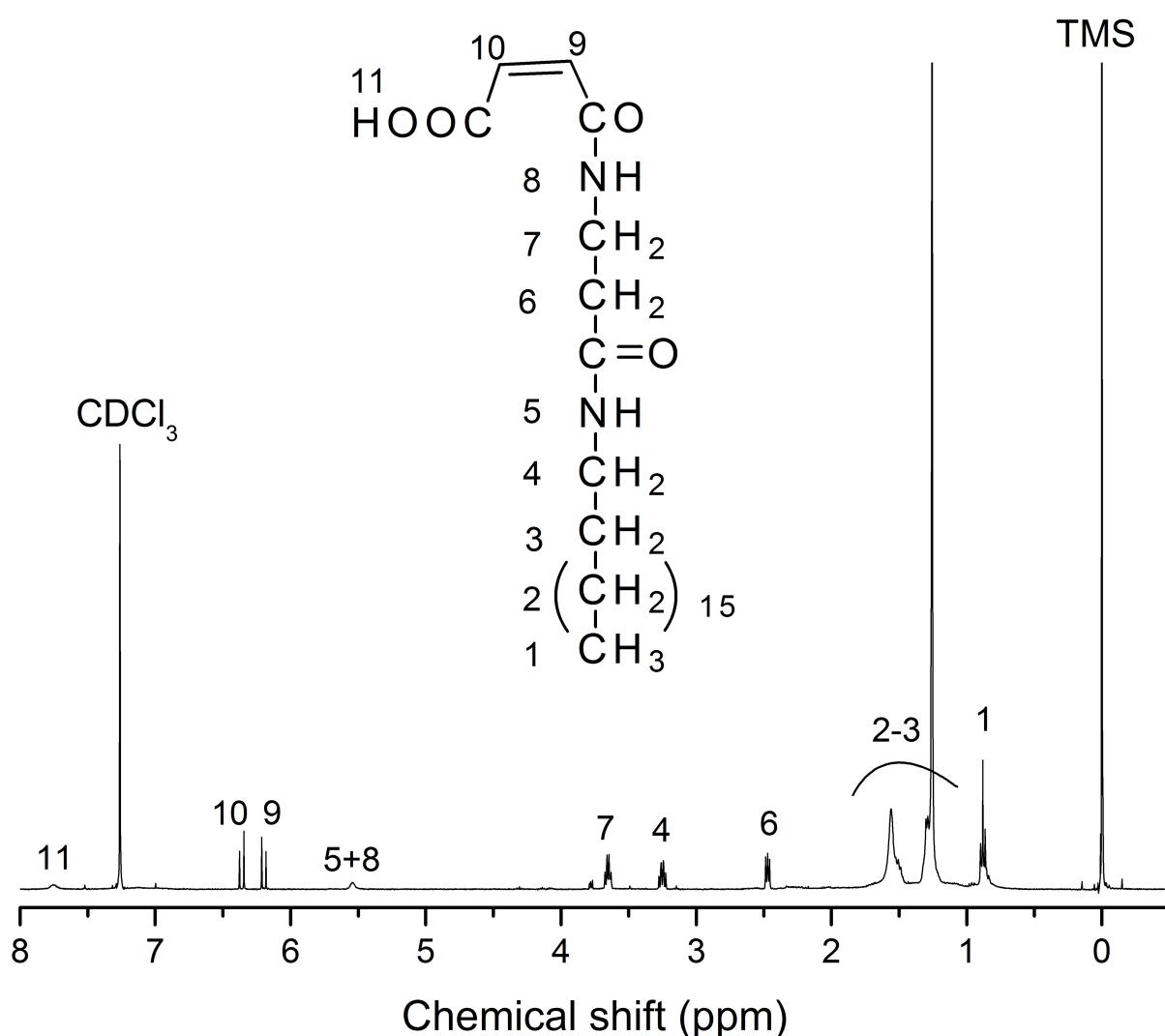
Fig. S2 ¹H NMR spectrum of compound 3

6.84 (s, 1H; CH₂NHC(O)), 3.23 (q, 2H; CH₂CH₂NHC(O)) 2.98 (t, 2H; CH₂CH₂NH₂), 2.28 (t, 2H; C(O)CH₂CH₂NH₂), 1.47 (m, 2H; CH₂CH₂CH₂NHC(O)CH₂, CH₂NH₂), 1.13 (m, 30H; CH₃CH₂ × 15), 0.86 ppm (t, 3H; CH₃); IR (KBr): ν = 3302, 2918, 2849, 1683, 1635, 1541, 1471 cm⁻¹.

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N-Octadecyl- β -alanymaleimic acid (**4**): A solution of *N*'-Octadecyl- β -alanineamide (**3**) (12.51 g, 36.74 mmol) in chloroform (100 ml) was added dropwise to a solution of maleic anhydride (6.49 g, 66.14 mmol) in chloroform (100 ml), and the mixture was stirred for 30 min. The mixture was vacuum filtered, and the solid was washed with CHCl₃ (100 ml) and hexane (100 ml) to yield **4** as a white solid (12.09 g, 75%). mp 136–138 °C; ¹H NMR (400 MHz, CDCl₃): δ = 10.50 (s, 1H; CHCOOH), 6.34 (d, 1H; CHCHCOOH), 6.18 (d, 1H; CHCHC(O)), 5.53 (s, 1H; C(O)NHCH₂, CH₂NHC(O)), 3.63 (q, 2H; CH₂CH₂NHC(O)), 3.22

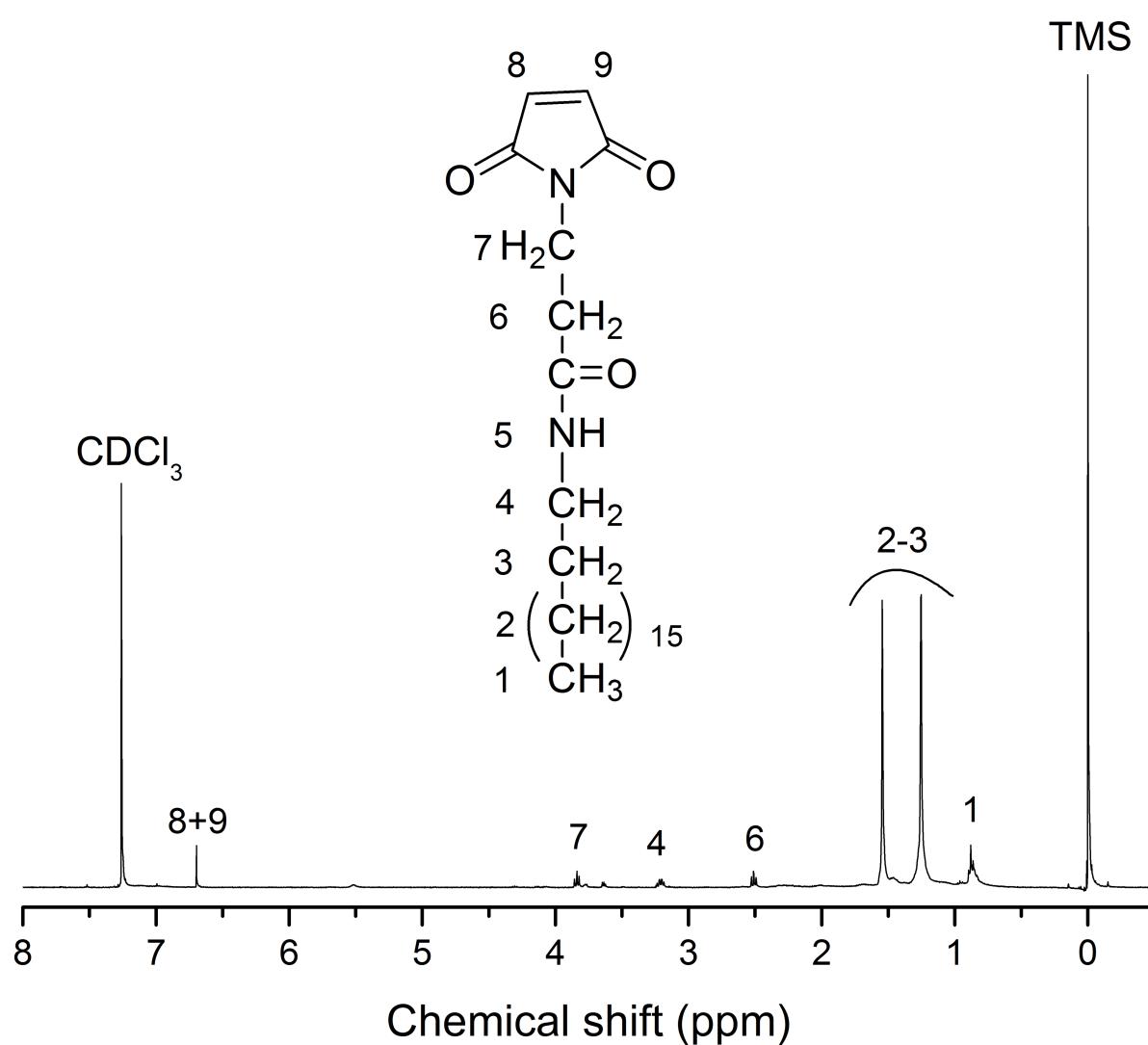
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4 **Fig. S3** ¹H NMR spectrum of compound 4

6 (q, 2H; CH₂CH₂NHC(O)), 2.47 (t, 2H; C(O)CH₂CH₂NH), 1.55 (m, 2H;
8 CH₂CH₂CH₂NHC(O)CH₂), 1.25 (m, 30H; CH₃CH₂ × 15), 0.86 ppm (t, 3H; CH₃); IR
(KBr): ν = 3331, 3328, 3074, 2919, 2849, 1711, 1644, 1535, 1467 cm⁻¹.

10 *N-Octadecyl-β-alanyl-maleimide (OAMI)*: A mixture of **4** (12.00 g, 27.34 mmol), sodium
12 acetate (3.81 g, 46.46 mmol), and acetic anhydride (200 ml) was heated to 100°C for 1 h.
14 Then the mixture was cooled to room temperature and poured into an ice/water slurry. The obtained solid was vacuum filtered and purified by column chromatography (CHCl₃, silica gel) to get *N*-Octadecyl-β-alanyl-maleimide (**OAMI**) as a white solid (10.09 g, 84%). mp 140–142 °C; ¹H NMR (400 MHz, CDCl₃): δ = 6.69 (s, 1H; C(O)CHCH, CHCHC(O)), 5.51



2 **Fig. S4** ¹H NMR spectrum of **OAMI**

4 (s, 1H; C(O)NHCH₂), 3.82 (t, 2H; CH₂CH₂N), 3.20 (q, 2H; CH₂CH₂NHC(O)), 2.47 (t, 2H;
C(O)CH₂CH₂N), 1.55 (m, 2H; CH₂CH₂CH₂NHC(O)CH₂), 1.25 (m, 30H; CH₃CH₂ × 15),
6 0.86 ppm (t, 3H; CH₃); IR (KBr): ν = 3309, 3108, 2920, 2850, 1767, 1703, 1636, 1546, 1468,
1450, 1416 cm⁻¹.

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Polymerization

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Immobilization of 3-mercaptopropyltrimethoxysilane (MPS) onto Silica

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Dried silica gel (4.00 g) was placed to a dried flask and dispersed in dry toluene (30 ml).
14 Then MPS (1.2 g, 6.11 mmol) was added and the reaction mixture was refluxed for 72 h. The suspension was filtered and the solid was washed with toluene, methanol, water, methanol

and diethyl ether successively and collected by centrifugation to obtain the MPS-modified
2 silica (Sil-MPS). After drying the particles were characterized by elemental analysis (%H
1.20, %C 4.69, %N 0).

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Copolymerization by Surface-Initiated Chain Transfer Reaction

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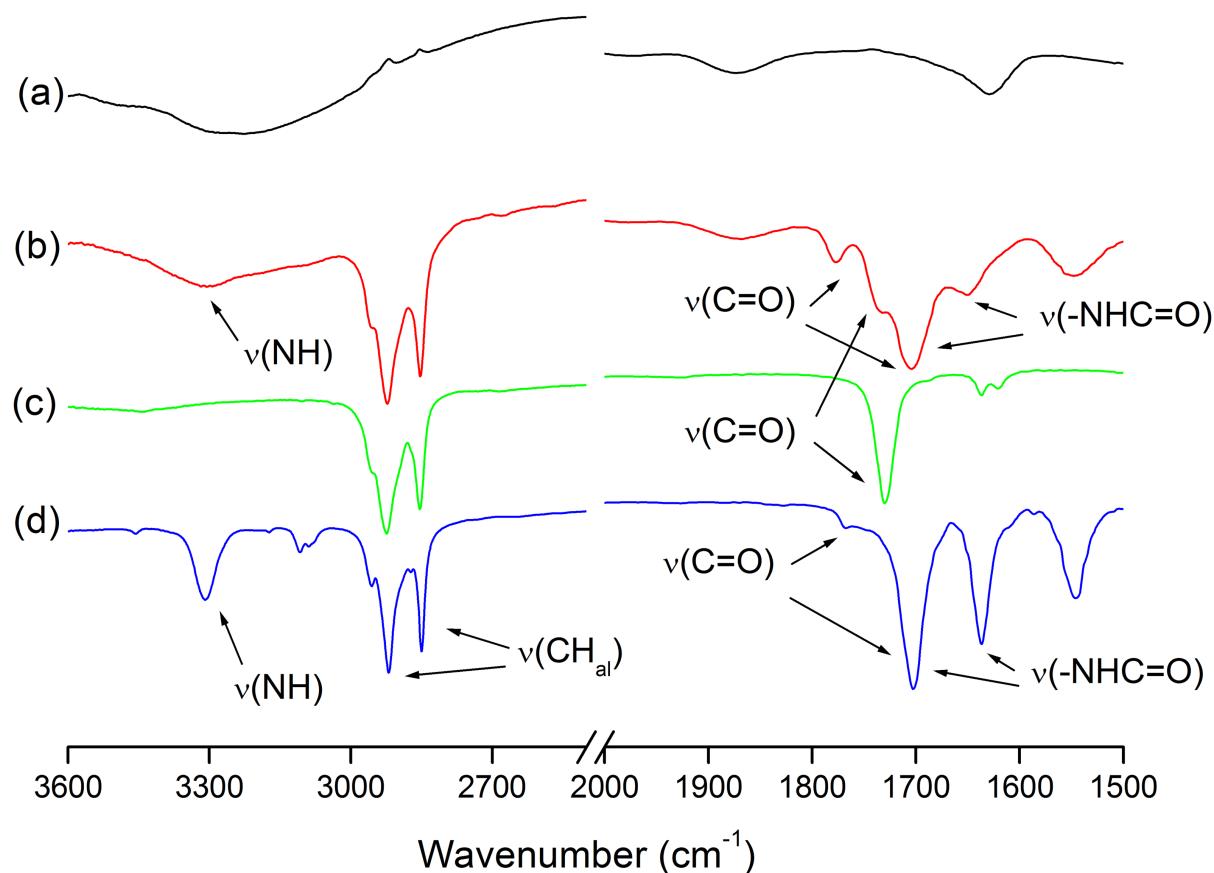
To a reaction flask containing MPS-modified silica (4.00 g) a mixture of ODA (3.50 g,
8 12.33 mmol), OAMI (4.53 g, 12.33 mmol), 100 mg of radical initiator
10 2,2'-azobisisobutyronitrile (AIBN) and dry toluene (10 ml) was added. The polymerization
mixture was gently stirred for 24 h at 60 °C. The white reaction mixture was filtered and
12 washed successively with toluene, chloroform, and methanol and collected by centrifugation
characterized by elemental analysis (%H 3.81, %C 22.68, %N 0.91) and DRIFT
14 spectroscopy.

16 FT-IR and DRIFT spectroscopy

18 Presence of grafted copolymer on silica particles can also be confirmed by diffuse
reflectance infrared Fourier transform (DRIFT) spectroscopy. Figure S5 shows the DRIFT
20 spectra, for (a) bare silica and (b) Sil-*alt*-P. DRIFT spectrum of alternating copolymer-grafted
silica (Sil-*alt*-P) demonstrated characteristics signals at 3304, 2923, 2853, 1777, 1733, 1704,
22 and 1651. The appearance of N–H stretching (3305 cm^{-1}) in spectrum (b) derived from
poly(OAMI), providing the evidence that monomer OAMI successfully polymerized from the
24 silica surface. The signals at 2923 and 2853 cm^{-1} arise from C–H stretches of long alkyl
chains from both of the monomers. The peaks near 1777 and 1704 cm^{-1} attribute to
26 symmetrical C=O stretch and asymmetrical C=O stretch of the imide linkage of the polymer
respectively. The band at 1733 (partially overlapped) arises from ester carbonyl stretching of
28 the monomer ODA confirms both of the monomer on the copolymer-grafted silica. The FT-IR
spectra of the monomers are fully compatible with the copolymer-grafted silica. These results
30 indicate the copolymer grafted onto silica, which are in good agreement with elemental
analyses data.

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2 **Fig. S5** DRIFT spectra of (a) bare silica and (b) Sil-*alt*-P and FT-IR spectra of (c) monomer
4 ODA and (d) monomer OAMI.

6 **Proton NMR**

8 ¹H NMR were recorded with a JEOL JNM-LA400 (Japan) instrument at 400 MHz in CDCl₃
10 solutions at 25 °C. Chemical shifts (δ) of 1H, expressed in parts per million (ppm) with use
12 of the internal standards Me₄Si (δ = 0.00 ppm).

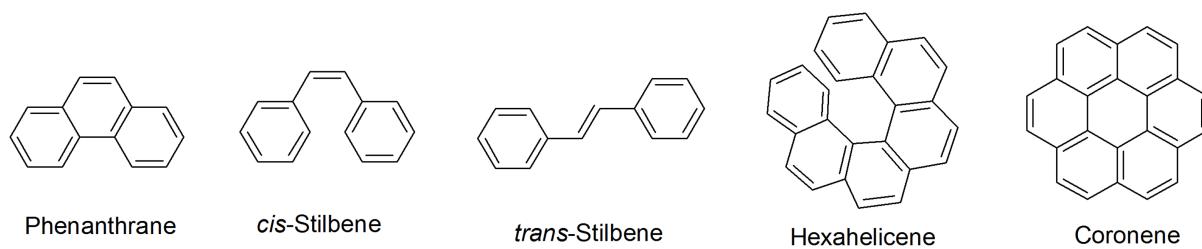
14 **FT-IR, DRIFT and Elemental Analysis.**

16 FT-IR measurements were conducted with JASCO FT/IR-4100 (Japan). For DRIFT
18 measurement accessory DR PRO410-M (JASCO, Japan) was used. Elemental analyses were
20 carried out on a Yanaco CHN Corder MT-6 Apparatus (Japan).

18 **Chromatography**

The chromatographic system consists of a JASCO X-LC 3185 PU intelligent HPLC pump a 2 Rheodyne sample injector having 20 μ l loop. The chromatograph included a JASCO X-LC 3110 MD UV-vis photodiode array detector. As the sensitivity of UV detector is high, 5 μ l 4 sample was injected. The column temperature was maintained by using a column jacket with a circulator having heating and cooling system. A personal computer connected to the 6 detector with JASCO X-LC software was used for system control and data analysis. The retention factor (k) measurement was done under isocratic elution conditions. The separation 8 factor (α) is the ratio of the retention factor of two solutes that are being analyzed. The chromatography was done under isocratic elution conditions. The retention time of D₂O was 10 used as the void volume (t_0) marker (The absorption for D₂O was measured at 400 nm, which actually considered as injection shock). All data points were derived from at least 12 triplicate measurements; with retention time (t_R) value varying $\pm 1\%$.

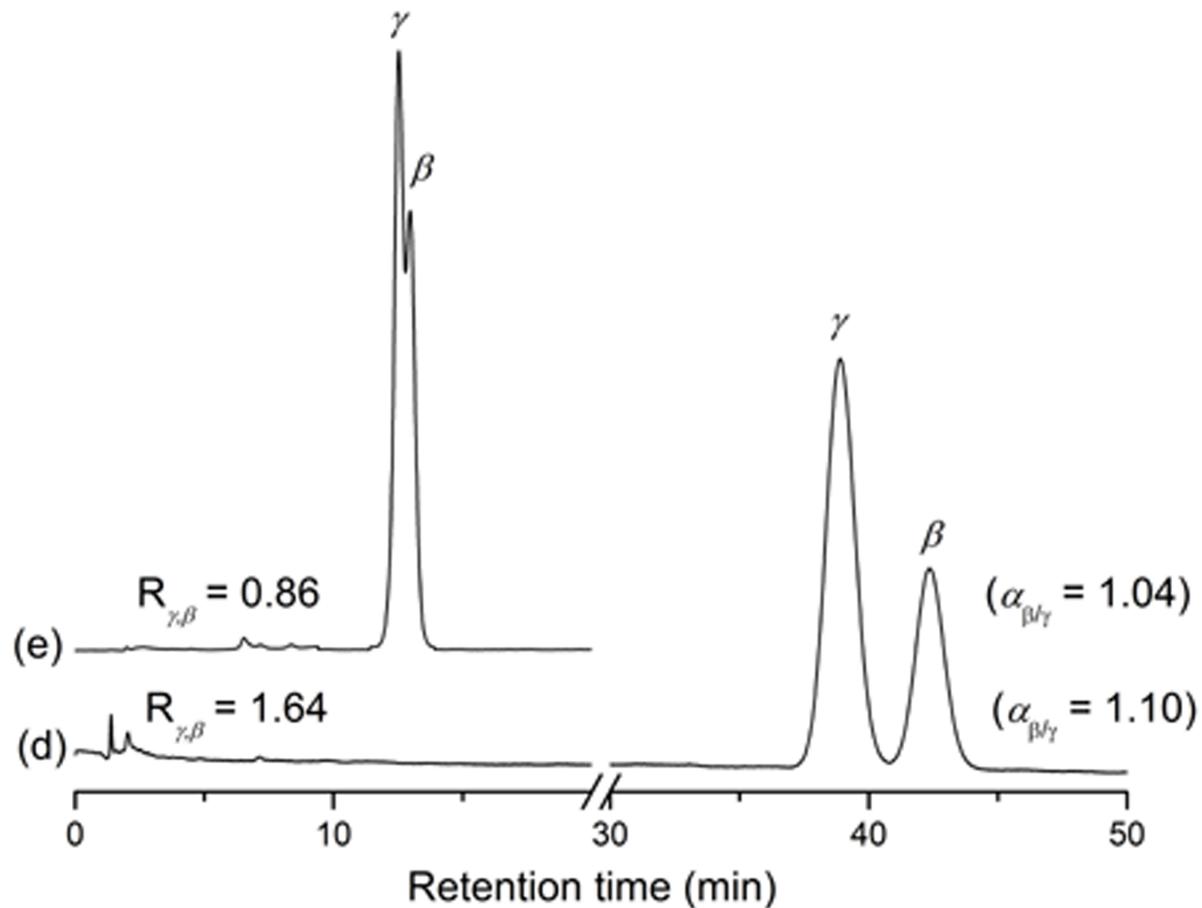
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Fig. S6 Chemical structures of the polycyclic aromatic hydrocarbons (PAHs).

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2 **Fig. S7** Separation of β - and γ -tocopherol isomers with (d) Sil-alt-P and (e)
4 Sil-poly(ODA-alt-OMI). Mobile phase: methanol:water (9 : 1), flow rate: 1 ml/min, detection:
UV at 285 nm, temperature: 40 °C.