Supporting Information

Fluorescent water-soluble perylenediimide-cored cationic dendrimers: synthesis, optical properties, and cell uptake

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Materials

2-Hydroxyethylmetharylate (97%), acryloyl chloride (98%), triethylamine (Et_3N, 99.5%), 2,6-diisopropylaniline (90+%), N-methyl-2-pyrrolidone (NMP, 99+%), cysteamine (CA, 98%), anhydrous dimethyl sulfoxide (DMSO, 99.9%) were purchased from Alfa Aesar and used without further purification. 1, 6, 7, 12-Tetrachloroperylene-3, 4, 9, 10-tetracar-boxylic acid dianhydride was obtained from Beijing Wenhaiyang Perylene Chemistry (Beijing, China). All other solvents and reagents were purchased from commercial suppliers and were used as received. S2 cells were propagated in Schneider Drosophila Medium supplemented with 10% FBS, 100 U/mL penicillin and 100 μg/mL streptomycin at 25 ℃ without CO_2.

Instruments

Nuclear magnetic resonance (NMR) spectra were recorded on Bruker 400 (400 MHz ^{1}H; 100 MHz ^{13}C) or Bruker 600 (600 MHz ^{1}H; 150 MHz ^{13}C) spectrometer using $CDCl_3$ and CF_3COOD as solvent at room temperature. Chemical shifts were reported downfield from 0.00 ppm using TMS as internal reference. Matrix-assisted laser-desorption ionization time-of-flight mass spectrometry (MALDI-TOF MS) were determined on Bruker Daltonics Inc. BIFLEX III MALDI-TOF (MW<3000) and AXIMA-CFR plus MALDI-TOF (MW>3000) mass spectrometer. The molecular weights (Mn) and molecular weight distributions (Mw/Mn) of dendrimers were determined by gel permeation chromatography (GPC), GPC measurements were performed on a Waters GPC system equipped with Waters Styragel columns, a Waters-2487 dual wavelength (λ) UV detector, and a Waters-2414 refractive index detector. Monodispersed dextran standards were used to obtain a calibration curve. The UV-Vis absorption spectra were recorded on a spectrophotometer (Cintra 20, GBC, and Australia). The corrected Fluorescence spectroscopic studies were performed on a fluorescence spectrophotometer (Horiba Jobin Yvon FluoroMax-4 NIR, NJ, USA) at room temperature (25 ℃). Fluorescence quantum yields (FQYs) were measured at room temperature by using cresyl violet in methanol as reference ($Ø_f$ =0.54).[1] Dynamic light scattering (DLS) was performed with an argon ion laser (Stabilite 2060-04, λ 637.2 nm, Spectra-Physics), a SP-125 goniometer, and an ALV-5000 multiple-tau digital correlator. The temperature was kept constant at 293 K for light scattering

measurements. Excited state life-time measurement data were collected using a Horiba Jobin Yvon Fluromax-4 spectroflurometer at 560 nm excitation for **G1**, **G2**, and **G3**.

Cytotoxicity Assay

Cell viability was monitored using Tali^{TM} viability kit-Dead Cell Green (Invitrogen, Catalog A10787) that was a green-fluorescent nuclear and chromosome stain. It does not penetrate intact membranes, but easily penetrate compromised membranes characteristic of dead cells. The measurement was performed at 48 h post-incubation of dendrimer or dendrimer/DNA complexes. Replace the fresh cell medium after 48 h of incubation and then add 1 μL Dead Cell Green into 100 μL cell medium for 0.5 h incubation.

Cellular Uptake

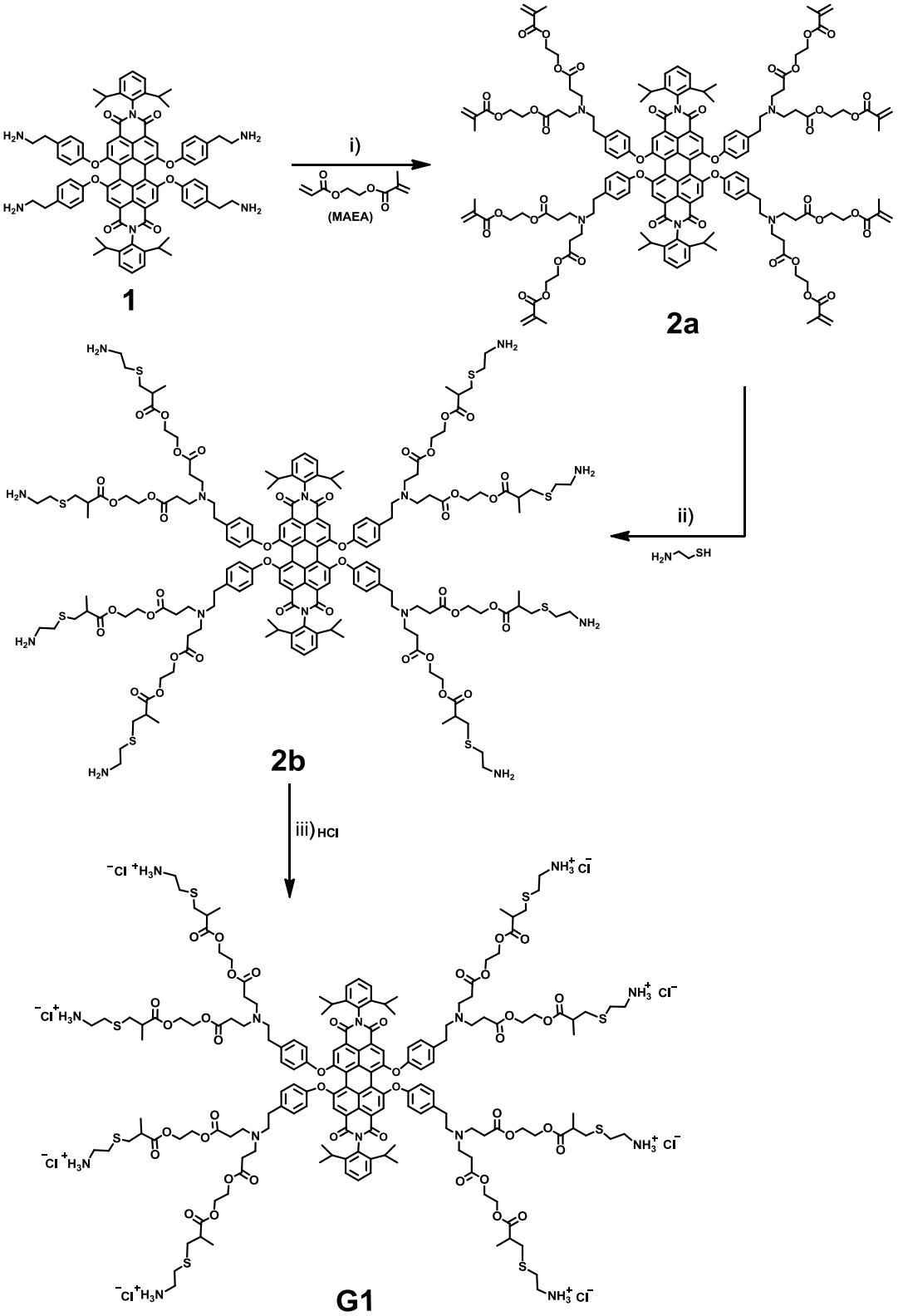
Cellular uptake experiment was performed in 35 mm × 35 mm cell culture dish, 2.5×10^5 cells per well. After 6 h of cell seeding, making the cells adhere to the bottom of the dish, add the dendrimer to the cell culture dish. Cellular uptake was imaged by fluorescent microscope. The fluorescence intensity of uptaken dendrimers was calculated by Image-J Program.

Transfection Efficiency

Single-strand DNA (20 bp, 100 μM) was labled with 30 μM CXR Reference Dye (Promega, Catalog C5411) that could bind with DNA at a final concentration of 0.3-0.5 μM. DNA and dendrimer at different N/P ratios of 1:1, 2:1, 4:1 and 8:1 were pre-incubated in culture medium at room temperature and then treated with cells. After 24 h, 36 h and 48 h, the fluorescence images of dendrimers binding to DNA were obtained by fluorescent microscopy.

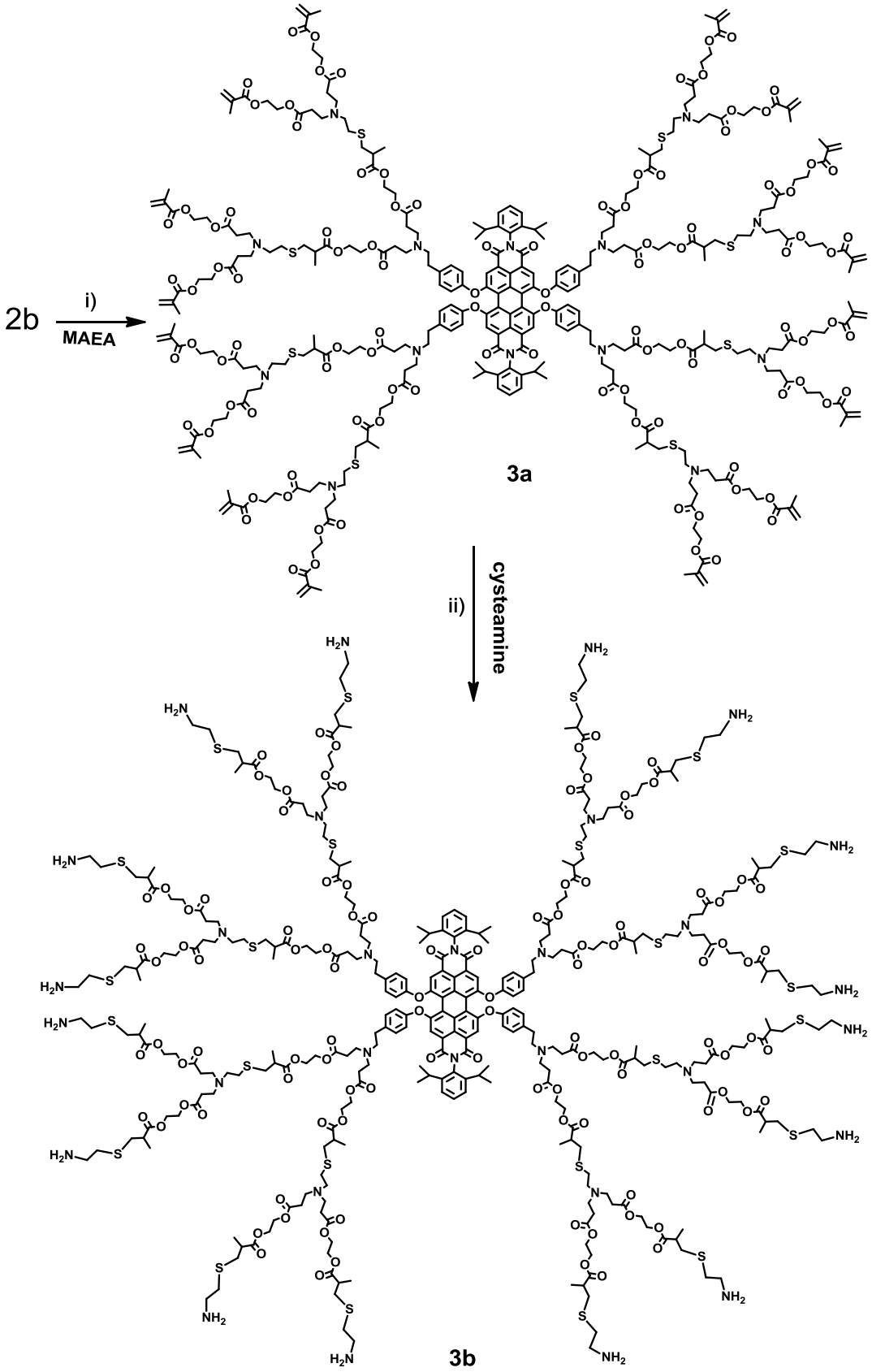
Synthesis Schemes

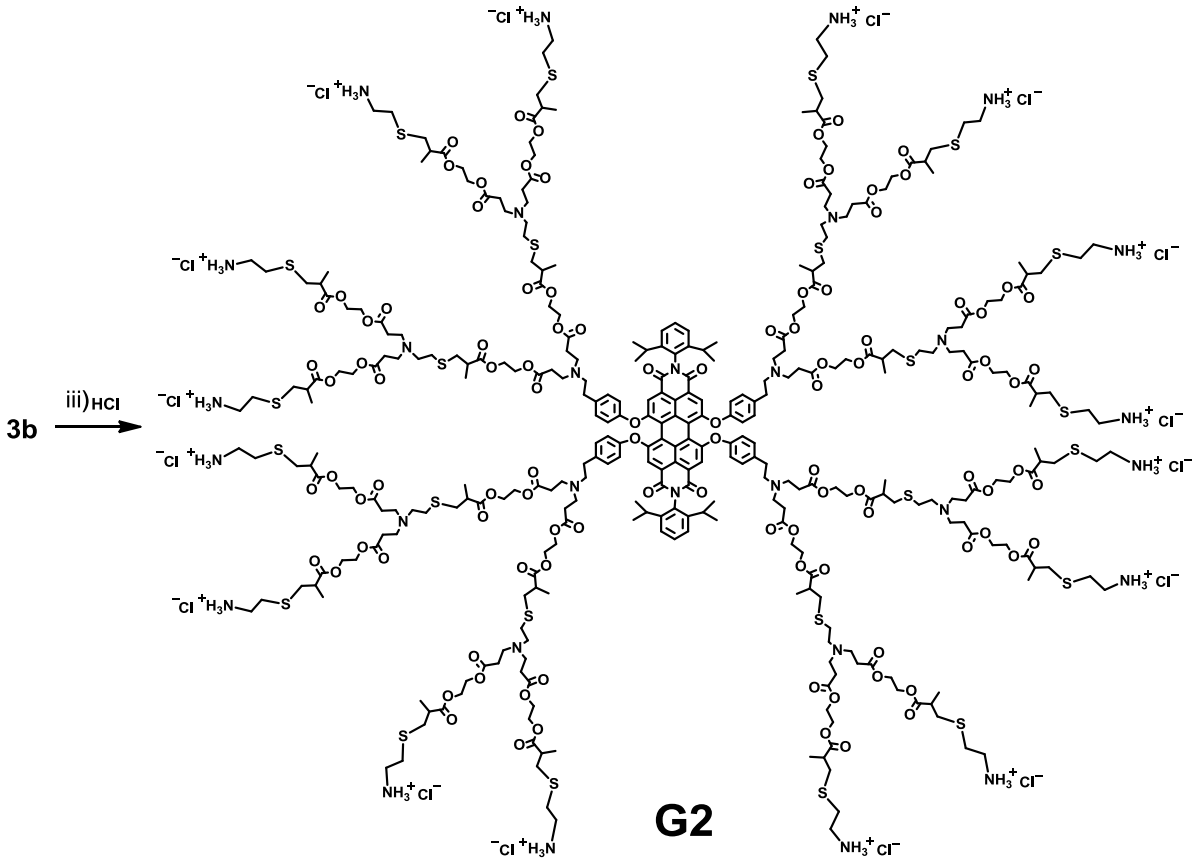
1. Synthesis of G1



Scheme S1 Synthesis of **G1**: i) MAEA, first at room temperature for 24 h and then at 50℃ for another 50 h; ii) Cysteamine, DMSO, room temperature (r. t.), 45 min; iii) 2M HCl, CH_2Cl_2, r. t., 5 min.

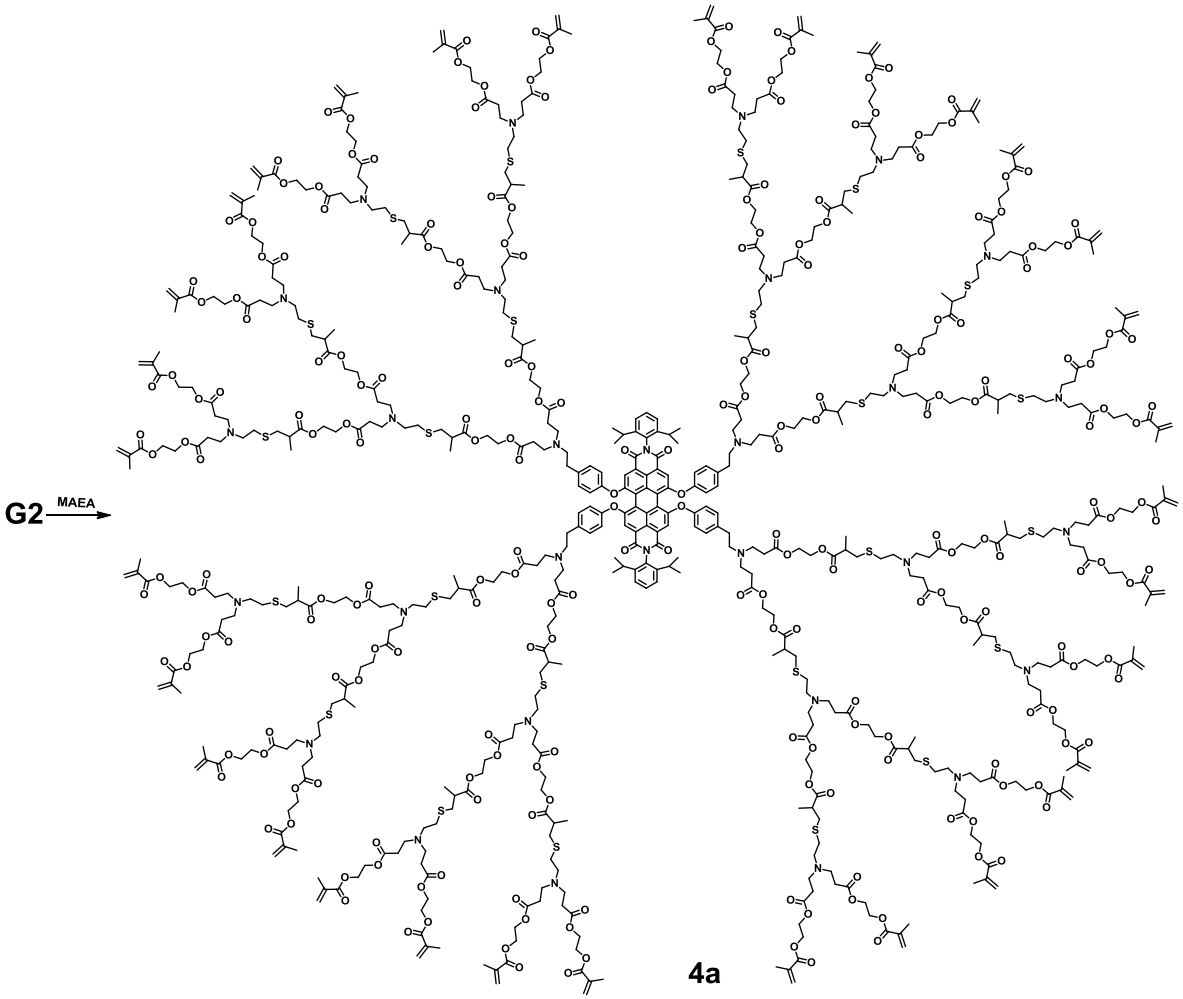
2. Synthesis of G2



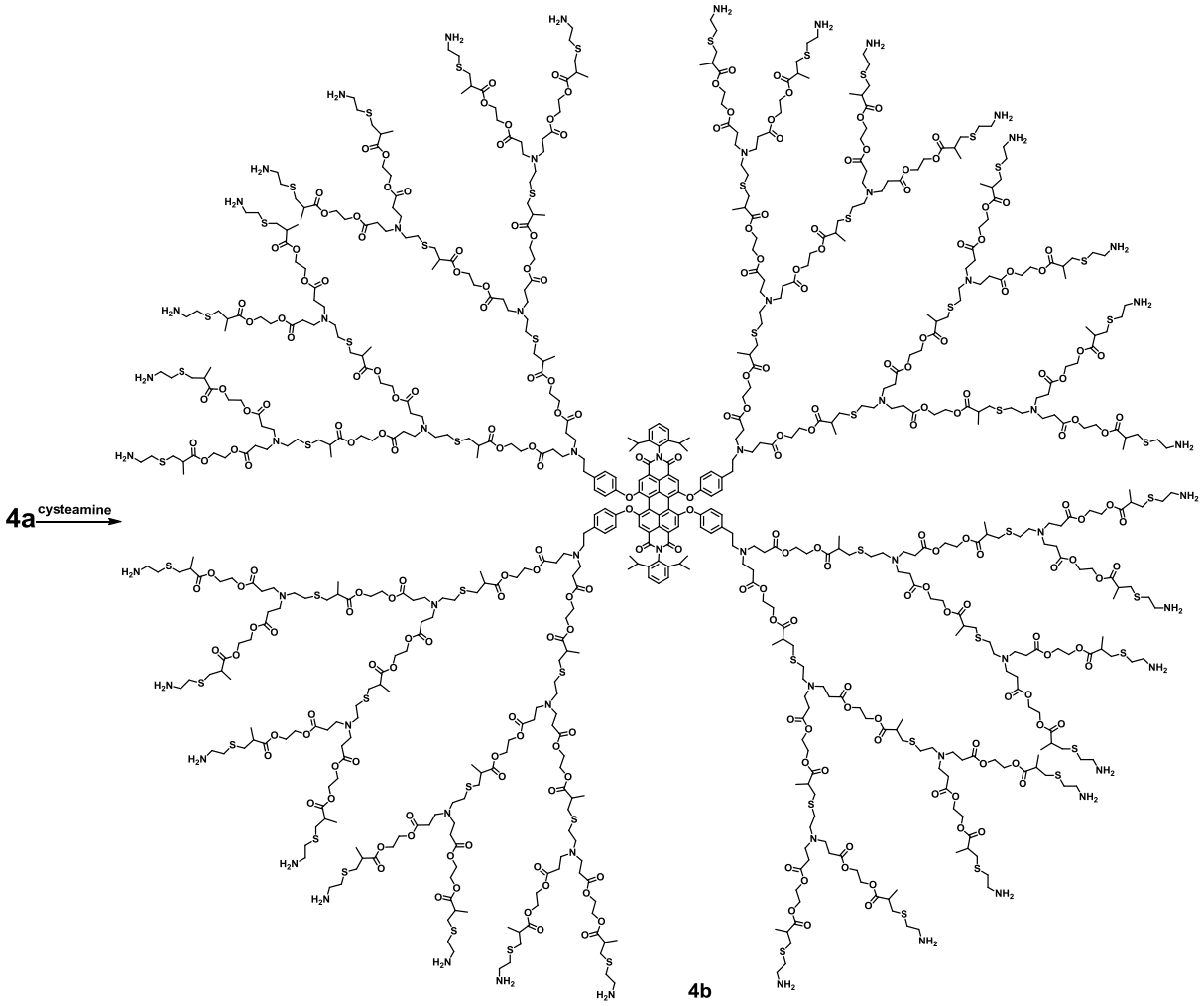


Scheme S2 Synthesis of **G2**: i) MAEA, first at room temperature for 24 h and then at 50℃ for another 72 h; ii) Cysteamine, DMSO, r. t., 45 min; iii) 2M HCl, CH_2Cl_2, r. t., 5 min.

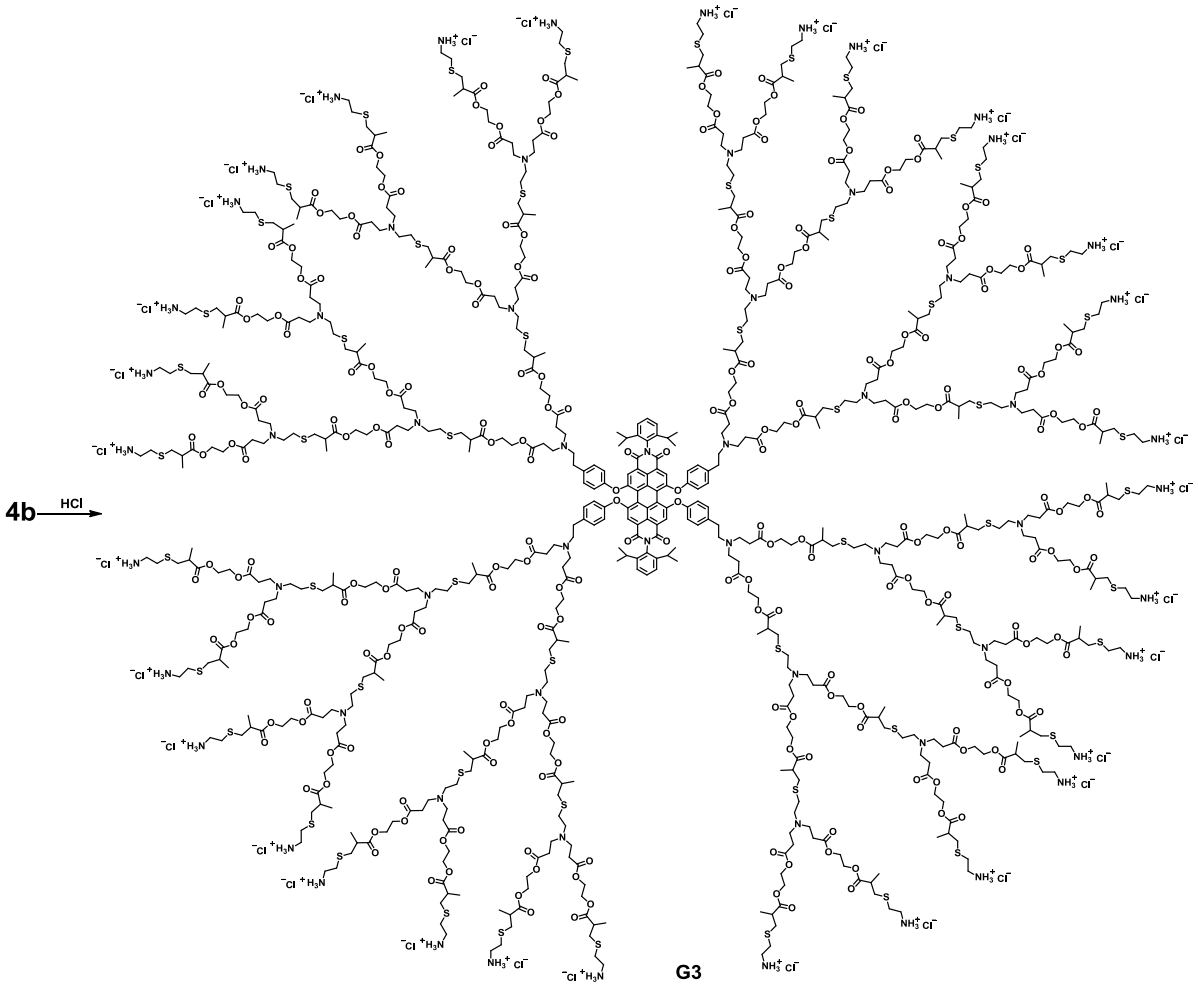
3. Synthesis of G3



Scheme S3 Synthesis of **4a**.



Scheme S4 Synthesis of **4b**.



Scheme S5 Synthesis of **G3.**

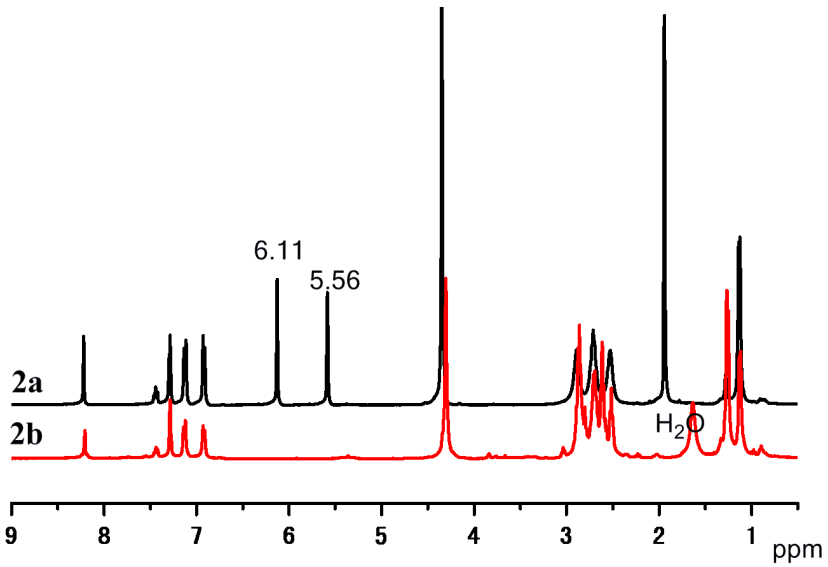


Fig. S1 ^{1}H NMR spectra of **2a** and **2b** in $CDCl_3$ (Note: the characteristic peaks of methylene group (CH_2=) at 5.56 and 6.11 ppm disappeared completely, indicating the completion of the click reaction of **2a**).

UV-Vis Absorption and Fluorescence Emission Properties

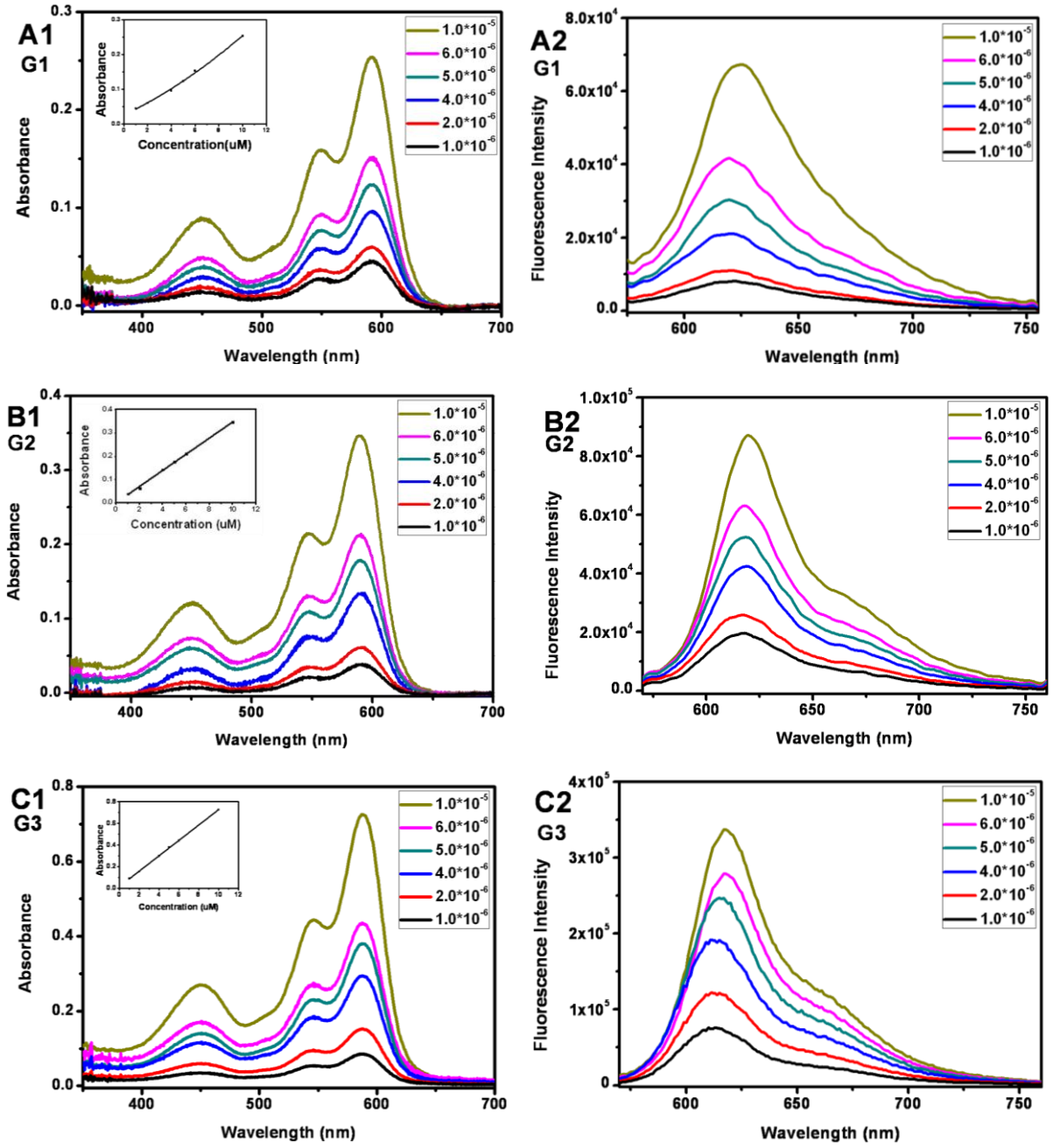


Fig. S2 Concentration-dependent absorbance (A1, B1, and C1) and fluorescence (A2, B2, and C2) spectra in aqueous solutions of **G1-G3** (concentration: 1.0×10^{-6} M to 1.0×10^{-5} M, λ_{ex} = 545 nm).

From the inset of B1 and C1 (Fig. S2), a linear dose-dependent absorbance was observed over the whole concentration range of **G2** and **G3**. But in case of **G1**, when the concentration was higher than 5 μM, a nonlinear curve was observed (Fig. S2A1). These indicate that the aggregation of central PDI in aqueous solution was inhibited by the increase of dendron generations. Therefore, all the dendrimers exist in a non-aggregated state in aqueous solution at concentrations below 5 μM.

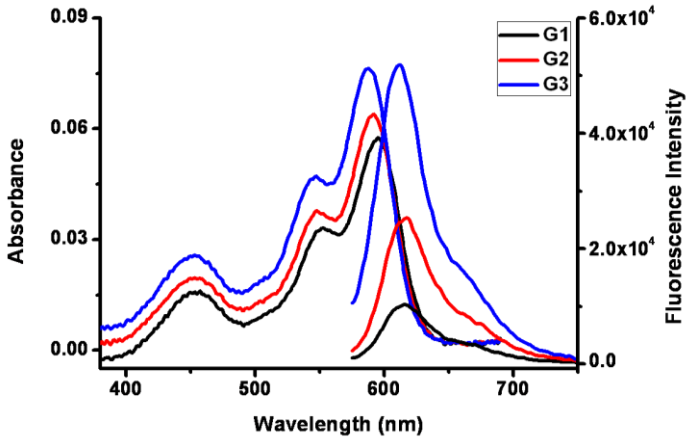


Fig. S3 Absorption and emission spectra of **G1**, **G2**, and **G3** in water (concentration: 2×10^{-6} M, λ_{ex} = 545 nm).

Table S1. Excited state life-time date of **G1**, **G2**, and **G3** in water.

molecule no.	λ_{abs} /nm	λ_{em} /nm	τ /ns
G1	560	615	0.03
G2	560	615	0.22
G3	560	615	1.37

Table S2. Dynamic light scattering (DLS) data of **G1**, **G2**, and **G3** in water.

molecule no.	number of $NH_3^+Cl^-$ groups	radius (nm) in water
G1	8	1.1±0.5
G2	16	1.8±0.3
G3	32	3.2±0.2

Table S3. The complex sizes of dendrimer and DNA (Dendriplex) at N/P=8:1 in water.

Dendriplex no.	Average size (nm)
G1/DNA	129.9±1.1
G2/DNA	136.5±3.4
G3/DNA	136.6±2.1

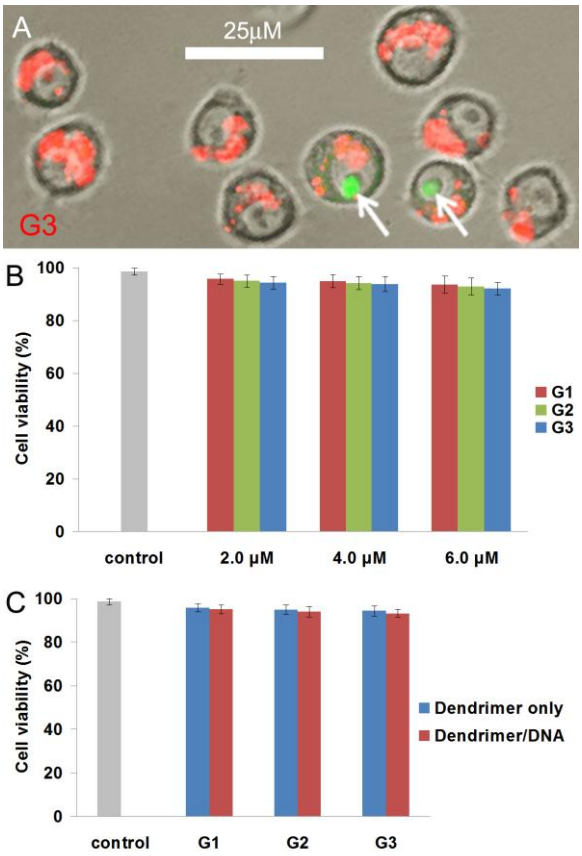
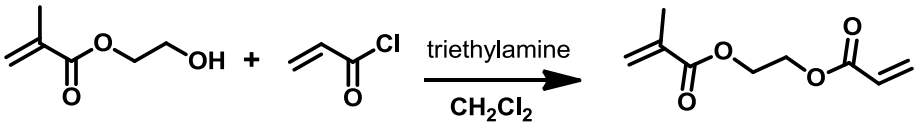


Fig. S4 Cell viability assays. (A) Fluorescence image of cells incubated with **G3** and then subjected to the staining of Dead Cell Green. Arrows indicate dead cells. (B) Cell viability with 48h incubation of 2 μM **G1**, **G2**, and **G3**. (C) Cell viability with 48h incubation of dendrimers and dendrimer/DNA complexes (2 μM). The data were mean±SEM.

Synthesis and characterizations

1. Synthesis of 2-methacryloyloxyethyl acrylate (MAEA)



Scheme S6 Synthesis of MAEA.

2-Hydroxyethyl methacrylate (39 g, 0.30 mol) and triethylamine (50 mL, 0.36 mol) were dissolved in 200 mL CH_2Cl_2. Acryloyl chloride (26.2 mL, 0.33 mol) was added dropwise into the solution at 0 °C under nitrogen. The solution was then stirred at room temperature overnight. The precipitate was filtered and the filtrate was washed with water (100 mL×3). The organic phase was evaporated under reduced pressure. The further purification was done by column chromatography on silica gel (EtOAc/pentane=3/8 v/v) to afford the product as a colorless oil in a yield of 90.8% (50.1g).^{1}H-NMR (400 Hz, $CDCl_3$): δppm: 6.42 (m, 1H), 6.27-6.06 (m, 2H), 5.85 (m, 1H), 5.58 (m, 1H), 4.40 (m, 4H), 1.94 (s, 3H).

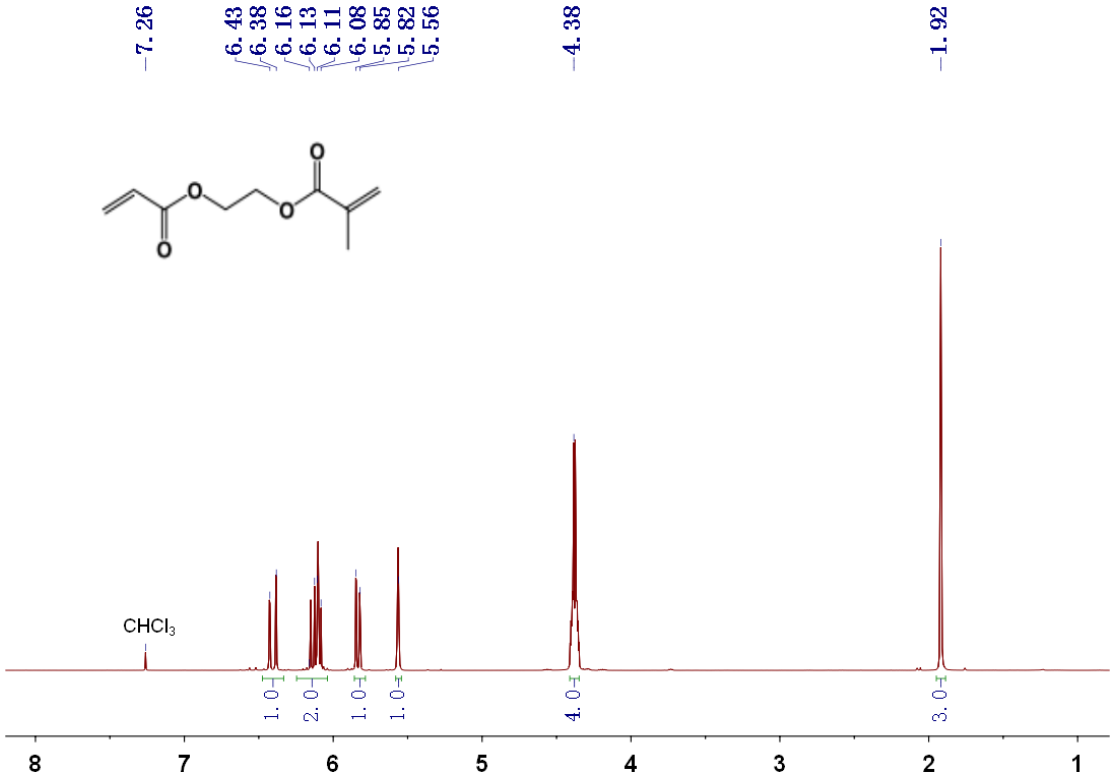


Fig. S5 ^{1}H NMR spectrum of MAEA in $CDCl_3$.

2. Synthesis of G1

Synthesis of compound 1

Compound **1** was synthesized according to the literature.[2] ^{1}H-NMR (400 MHz, CF_3COOD): δppm: 8.50 (s, 4H), 7.60 (d, *J* = 7.8 Hz, 2H), 7.47 (d, *J* = 7.9 Hz, 4H), 7.33 (d, *J* = 8.2 Hz, 8H), 7.20 (d, *J* = 8.3 Hz, 8H), 3.60 (s, 8H), 3.19 (s, 8H), 2.79-2.71 (m, 4H), 1.22 (d, *J* = 6.4 Hz, 24H). ^{13}C-NMR (151 MHz, CF_3COOD), δppm: 166.20, 156.76, 154.78, 145.54, 132.63, 131.82, 130.92, 130.29, 128.47, 124.88, 122.16, 121.43, 121.09, 120.77, 120.14, 42.02, 31.96, 29.09, 22.41.MS (MALDI-TOF, m/z) Calc. for $C_{80}H_{78}N_6O_8$: 1251.51, found: 1250.6.

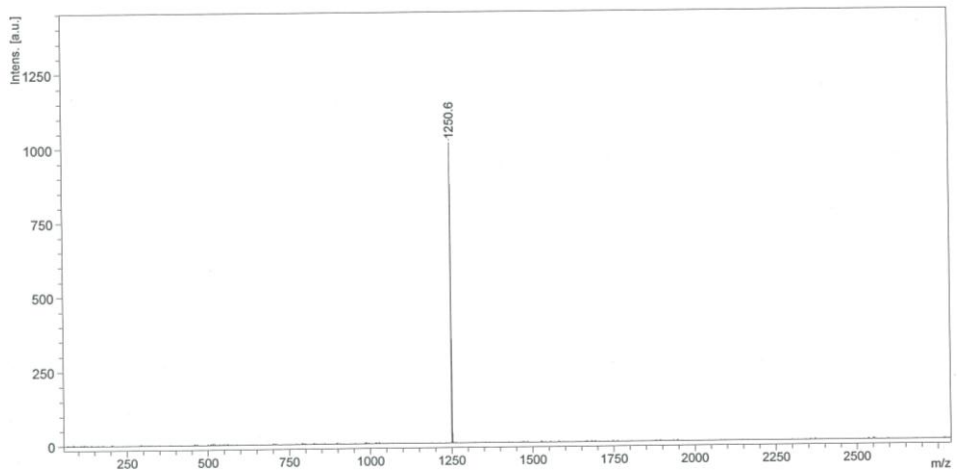


Fig. S6 MALDI-TOF MS spectrum of compound **1**.

Synthesis of compound 2a

Compound **1** (0.2 g, 0.16 mmol) and MAEA (0.58 g, 3.15 mmol) were added in a 10-mL, two-necked reaction tube at room temperature under an N_2 atmosphere. The solution was first stirred overnight and then stirred at 50 °C for 48 h. After cooling down to room temperature, the solution was washed with hexane (30 mL × 3). The residue was dried under vacuum to give **2a** as a red oil in a yield of 98%. ^{1}H-NMR (400 Hz, $CDCl_3$): δppm: 8.20 (s, 4H, *perylene*), 7.40 (t, 2H, *Ph-H*), 7.27 (d, 4H, *Ph-H*), 7.09 (d, 8H, *Ph-H*), 6.91 (d, 8H, *Ph-H*), 6.11 (s, 8H, *HCH=C(CH$_3$)CO*), 5.56 (d, 8H, *HCH=C(CH$_3$)CO*), 4.32 (br, 32H, *COOCH$_2$CH$_2$OCO*), 2.87 (t, 16H, *CH$_2$CH$_2$CO*), 2.69 (m, 20H, *CH$_2$* & *CH isopropyl*), 2.51 (t, 16H, *CH$_2$CO*), 1.9 (s, 24H, *CH2=C(CH$_3$)CO*), 1.12 (d, 24H, *CH$_3$ isopropyl*). ^{13}C-NMR ($CDCl_3$, 400 Hz), δppm: 172.57, 167.26, 163.34, 156.48, 153.58, 145.80, 136.18, 133.31, 130.42, 126.26, 124.46, 122.94, 120.72, 120.22, 62.46, 56.05, 49.63, 32.90, 29.14, 24.18, 18.28. MS (MALDI-TOF, m/z) Calc. for $C_{152}H_{174}N_6O_{40}$, 2725.02; found: 2724.8.

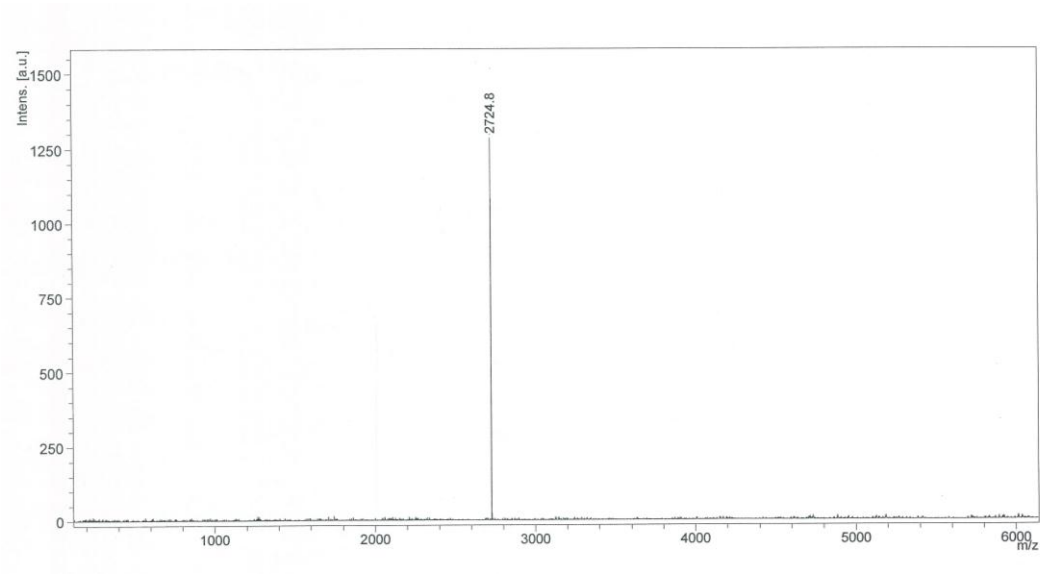


Fig. S7 MALDI-TOF MS spectrum of compound **2a**.

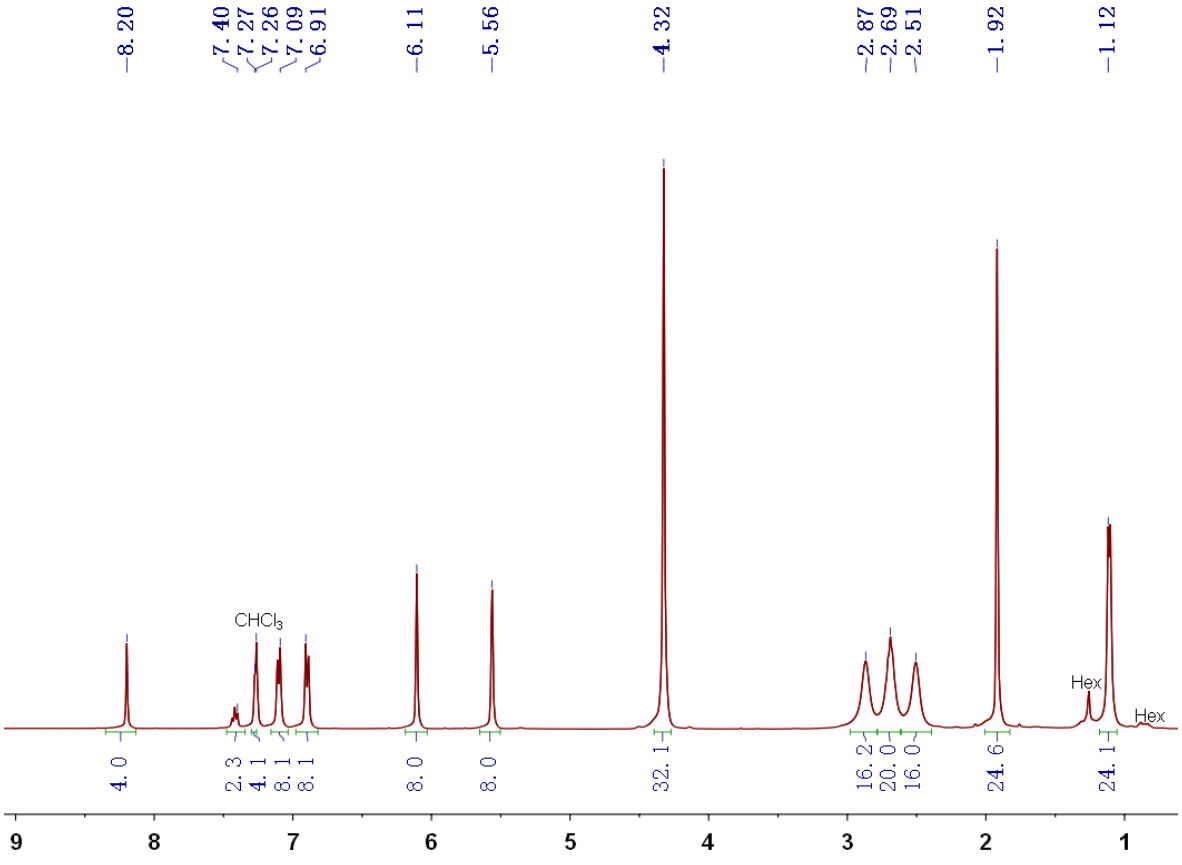


Fig. S8 ^{1}H NMR spectrum of compound **2a** in $CDCl_3$.

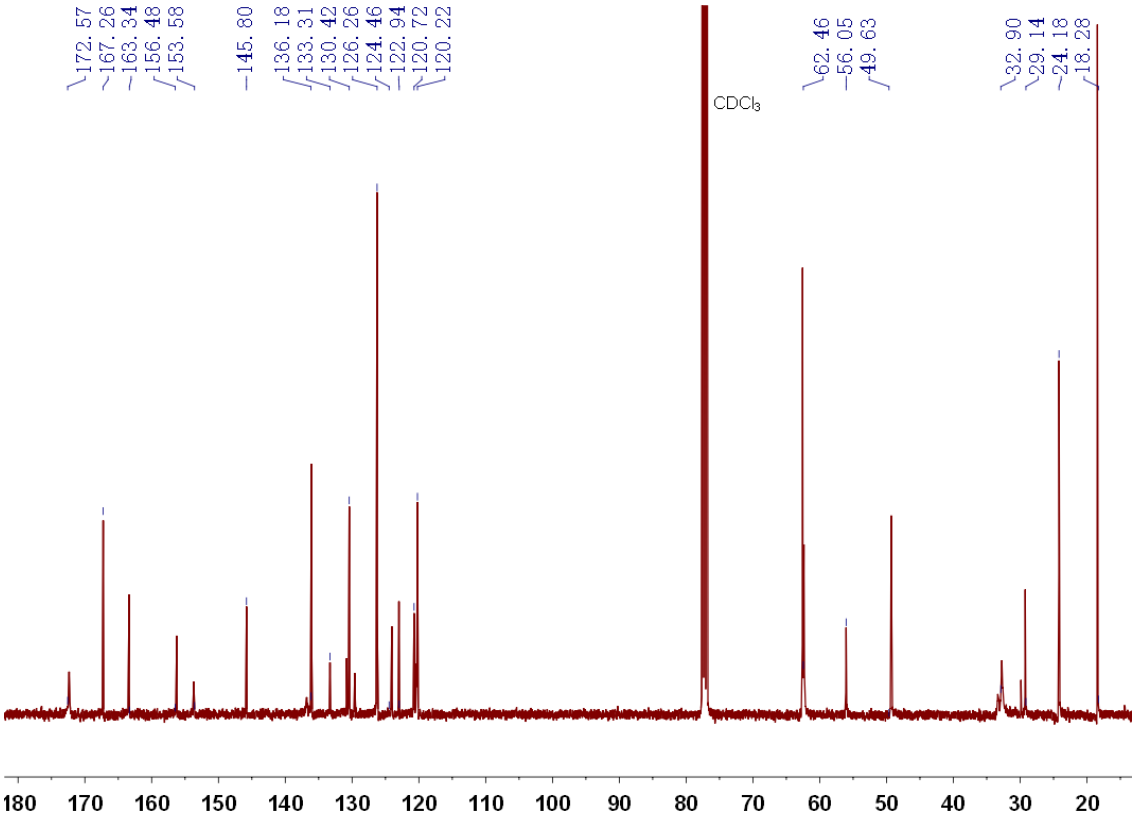


Fig. S9 ^{13}C NMR spectrum of compound **2a** in $CDCl_3$.

Synthesis of compound 2b

Compound **2a** (0.218 g, 0.08 mmol) and cysteamine (0.062 g, 0.81 mmol) were dissolved in DMSO (1 mL). The solution was stirred at room temperature under N_2 atmosphere for 45 min. Dichloromethane (20 mL) was added to dilute the solution followed by washing with cold brine (30 mL×4). Evaporation of the solvent under reduced pressure afforded dendrimer **2b** as a red oil in 98.0% yield. ^{1}H-NMR ($CDCl_3$, 400 Hz): δ ppm: 8.12 (s, 4H, *perylene*), 7.35 (t, 2H, *Ph-H*), 7.20 (d, 4H, *Ph-H*), 7.03 (d, 8H, *Ph-H*), 6.84 (d, 8H, *Ph-H*), 4.22 (m, 32H, *COOCH$_2$CH$_2$OCO*), 2.81 (t, 16H, *SCH$_2$CH$_2$NH$_2$*), 2.77 (m, 32H, *CH$_2$SCH$_2$*), 2.61 (m, 24H, *CH$_2$CH$_2$CO* & *COCH(CH$_3$)CH$_2$*), 2.53 (m, 20H, *CH$_2$* & *CH isopropyl*), 2.43 (t, 16H, *CH$_2$CO*), 1.18 (s, 24H, *CH$_2$=C(CH$_3$)CO*), 1.03 (d, 24H, *CH$_3$ isopropyl*). ^{13}C-NMR ($CDCl_3$, 400 Hz), δppm: 174.60, 172.26, 163.07, 155.96, 153.36, 145.56, 136.84, 133.04, 130.45, 124.26, 122.66, 120.73, 120.10, 62.36, 60.85, 55.97, 49.15, 41.53, 40.11, 36.86, 35.12, 32.56, 29.74, 28.80, 24.07, 16.75. MS (MALDI-TOF, m/z) Calc. for $C_{168}H_{230}N_{14}O_{40}S_8$: 3342.21, found: 3343.57 ($M+H^+$), 3364.75 ($M+Na^+$), 3381.66 ($M+K^+$).

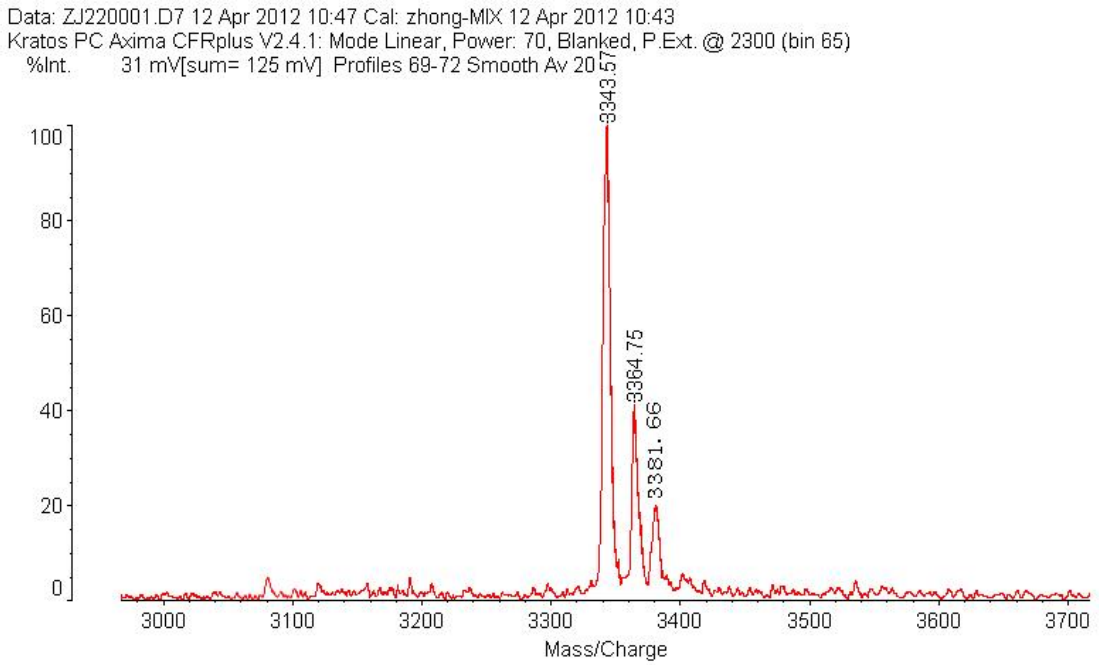


Fig. S10 MALDI-TOF MS spectrum of **2b**.

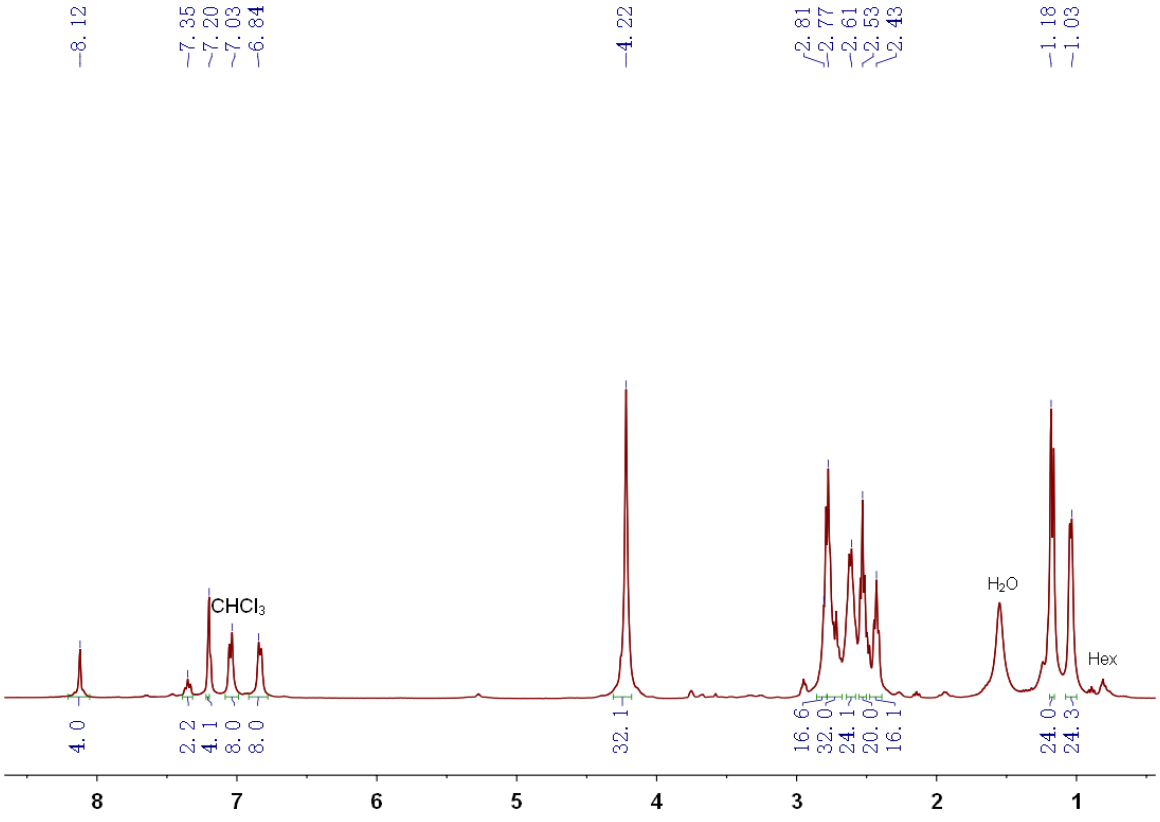


Fig. S11 ^{1}H NMR spectrum of **2b** in $CDCl_3$.

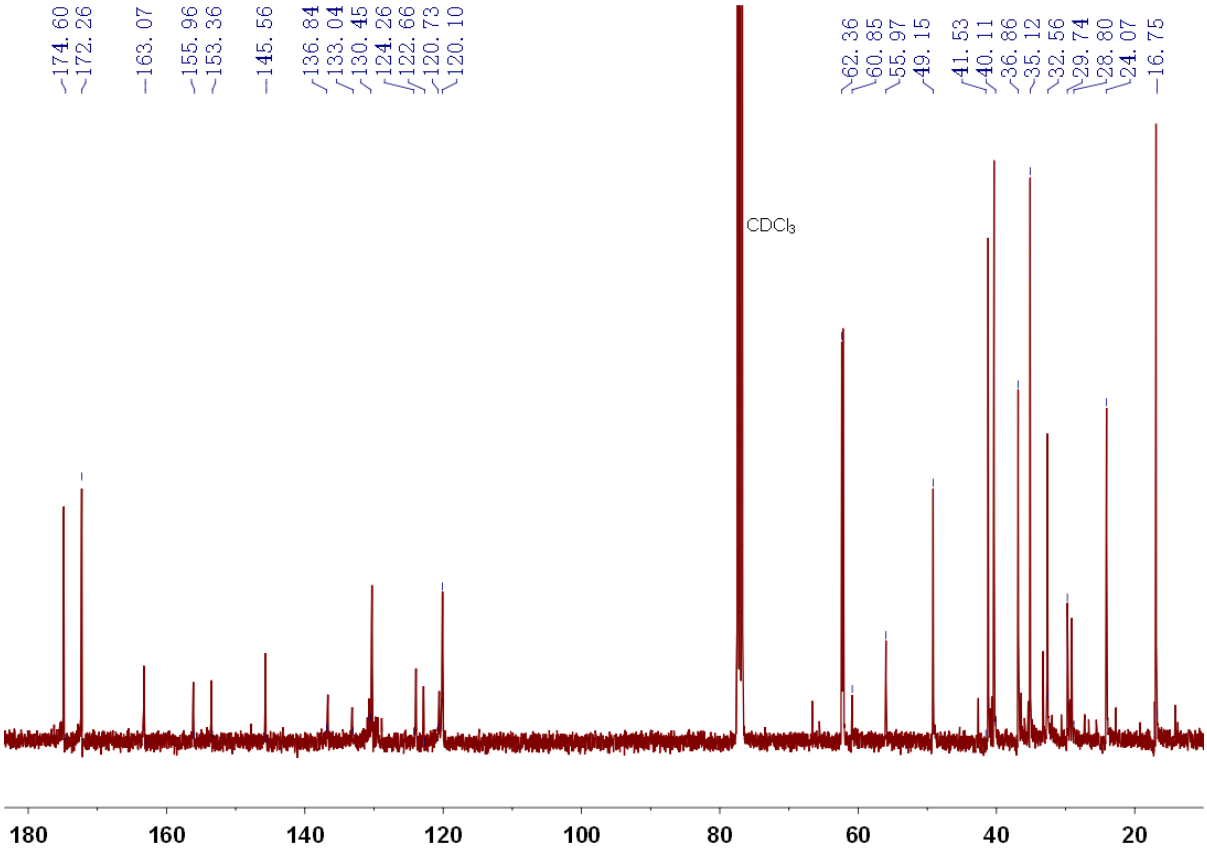


Fig. S12 ^{13}C NMR spectrum of **2b** in $CDCl_3$.

Synthesis of the first-generation dendrimer G1

Dendrimer **2b** was dissolved in CH_2Cl_2 (2 mL), and then 2M HCl (2 mL) was added under stirring. After 5 min of stirring, the solvent was evaporated under reduced pressure. The obtained residue was precipitated by pouring into diethyl ether and re-dissolved in water (2 mL). The further dialysis and lyophilization afforded red solid **G1**.

3. Synthesis of G2

Synthesis of compound 3a

MAEA (0.5 g, 2.71 mmol) was added dropwise to **2b** (0.243 g, 0.072 mmol) in a 10-mL, two-necked reaction tube at room temperature under N_2 atmosphere. After stirring for 24 h, the solution was warmed gradually to 50 °C and stirred for another 72 h. The reaction mixture was washed with hexane (30 mL × 4). The crude product was re-dissolved in dichloromethane (20 mL), and then evaporation of solvent under reduced pressure resulted in the product **3a** as a red oil in 90% yield. ^{1}H-NMR (400 Hz, $CDCl_3$): δppm: 8.18 (s, 4H, *perylene*), 7.41 (t, 2H, *Ph-H*), 7.25 (d, 4H, *Ph-H*), 7.10 (d, 8H, *Ph-H*), 6.92 (d, 8H, *Ph-H*), 6.12 (s, 16H, *HCH=C(CH$_3$)CO*), 5.59 (d, 16H, *HCH=C(CH$_3$)CO*), 4.33-4.27 (br, 96H, *COOCH$_2$CH$_2$OCO*),

2.90-2.42 (m, 172H, *$CH_2NCH_2CH_2CO$* & *$COCH(CH_3)CH_2$* & *SCH_2CH_2N* & *$CH(CH_3)CH_2S$* & *CH_2CH_2CO* & *CH_2* & *CH isopropyl*), 1.94 (s, 48H, *$CH_2=C(CH_3)CO$*), 1.25 (d, 24H, *$COCH(CH_3)CH_2$*), 1.11 (d, 24H, *CH_3 isopropyl*). ^{13}C-NMR ($CDCl_3$, 400 Hz), δppm: 175.30, 173.03, 167.29, 163.65, 156.76, 154.02, 146.06, 136.36, 133.63, 130.86, 126.50, 124.20, 123.08, 120.98, 120.54, 62.84, 56.40, 54.31, 49.32, 40.57, 36.05, 33.08, 30.98, 30.30, 29.59, 24.38, 18.35, 17.25. MS (MALDI-TOF, m/z) Calc. for $C_{312}H_{422}N_{14}O_{104}S_8$: 6289.24, found: 6290.33 ($M+H^+$), 6314.61 ($M+Na^+$), 6328.42 ($M+K^+$).

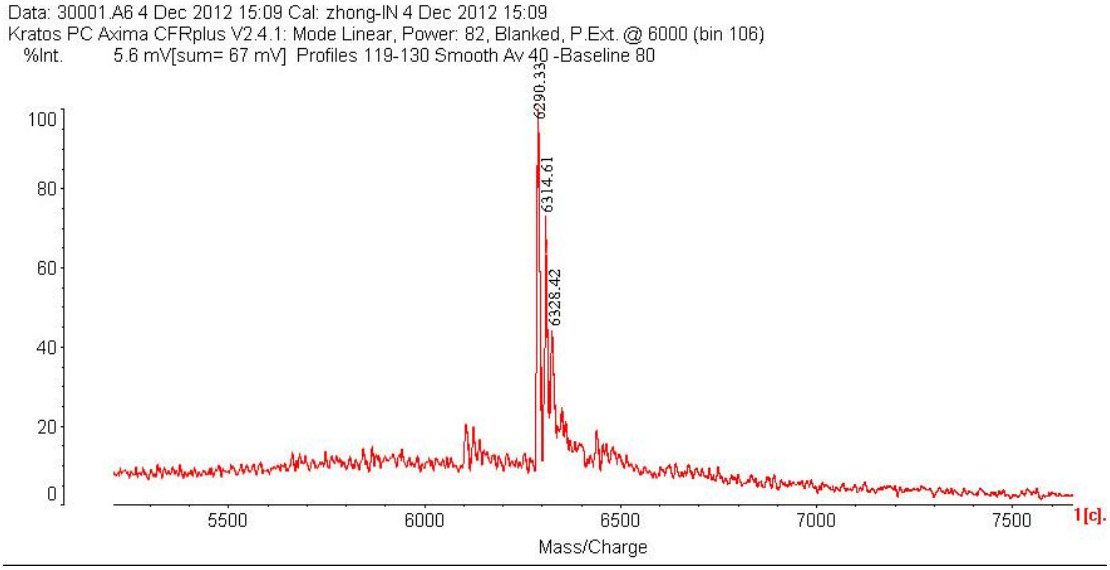


Fig. S13 MALDI-TOF MS spectrum of compound **3a**.

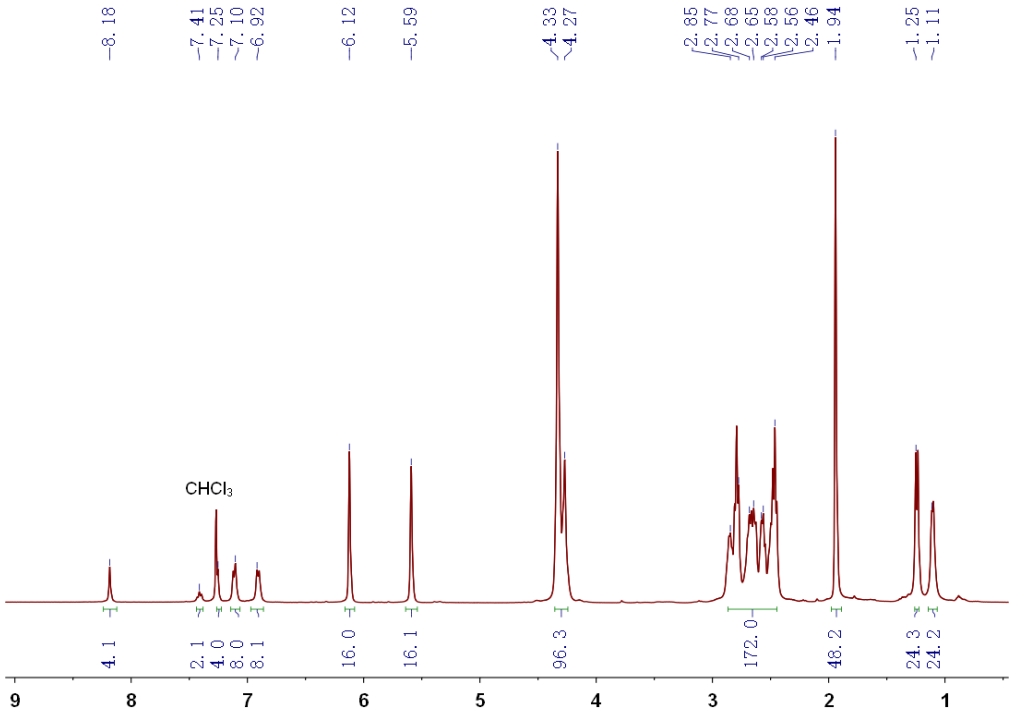


Fig. S14 ^{1}H NMR spectrum of compound **3a** in $CDCl_3$.

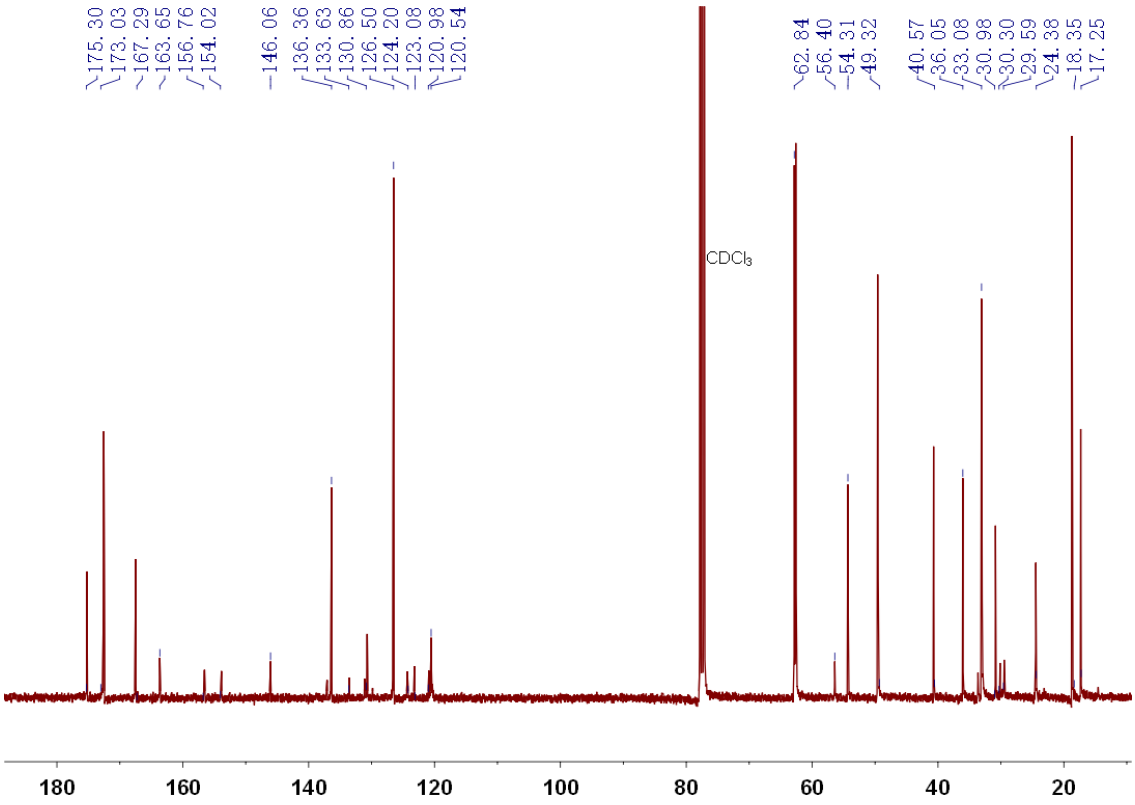


Fig. S15 ^{13}C NMR spectrum of compound **3a** in $CDCl_3$.

Synthesis of compound 3b

3b was synthesized by using compound **3a** (0.075 g, 0.012 mmol) and cysteamine (0.0195 g, 0.25 mmol) according to the same procedure as for the synthesis of **2b. 3b** was received as a red oil in 92% yield. ^{1}H-NMR (400 MHz, $CDCl_3$): δppm: 8.18 (s, 4H), 7.42 (t, *J* = 7.5 Hz, 2H), 7.28 (s, 4H), 7.12 (d, *J* = 7.5 Hz, 8H), 6.91 (d, *J* = 7.1 Hz, 8H), 4.30 (s, 96H), 2.88-2.47 (m, 284H), 1.26 (d, *J* = 5.1 Hz, 72H), 1.11 (d, *J* = 4.9 Hz, 24H). ^{13}C-NMR ($CDCl_3$, 400 Hz), δppm: 174.21, 171.61, 162.39, 155.41, 152.82, 145.32, 135.82, 130.31, 129.45, 123.67, 122.21, 119.87, 119.37, 66.15, 61.59, 55.79, 52.77, 52.02, 51.68, 48.50, 40.63, 39.40, 34.38, 31.86, 29.08, 23.31, 21.06, 16.16.

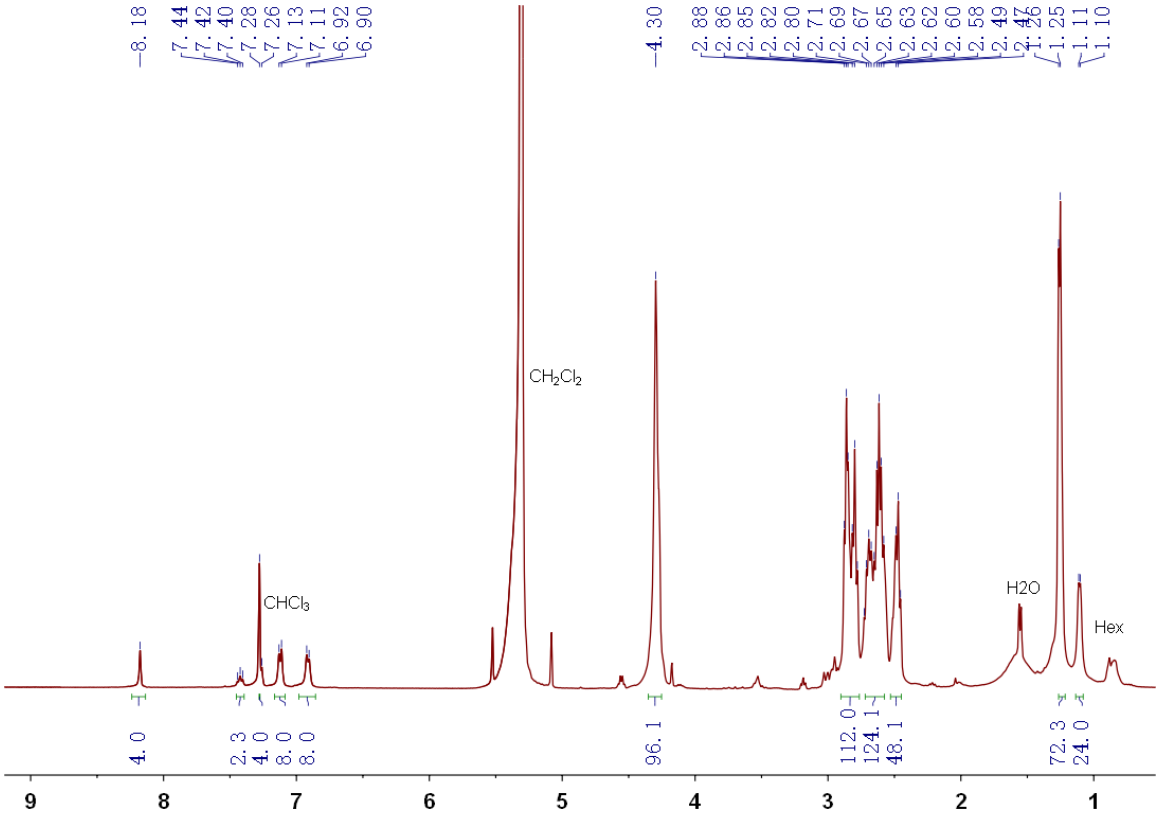


Fig. S16 ^{1}H NMR spectrum of **3b** in $CDCl_3$.

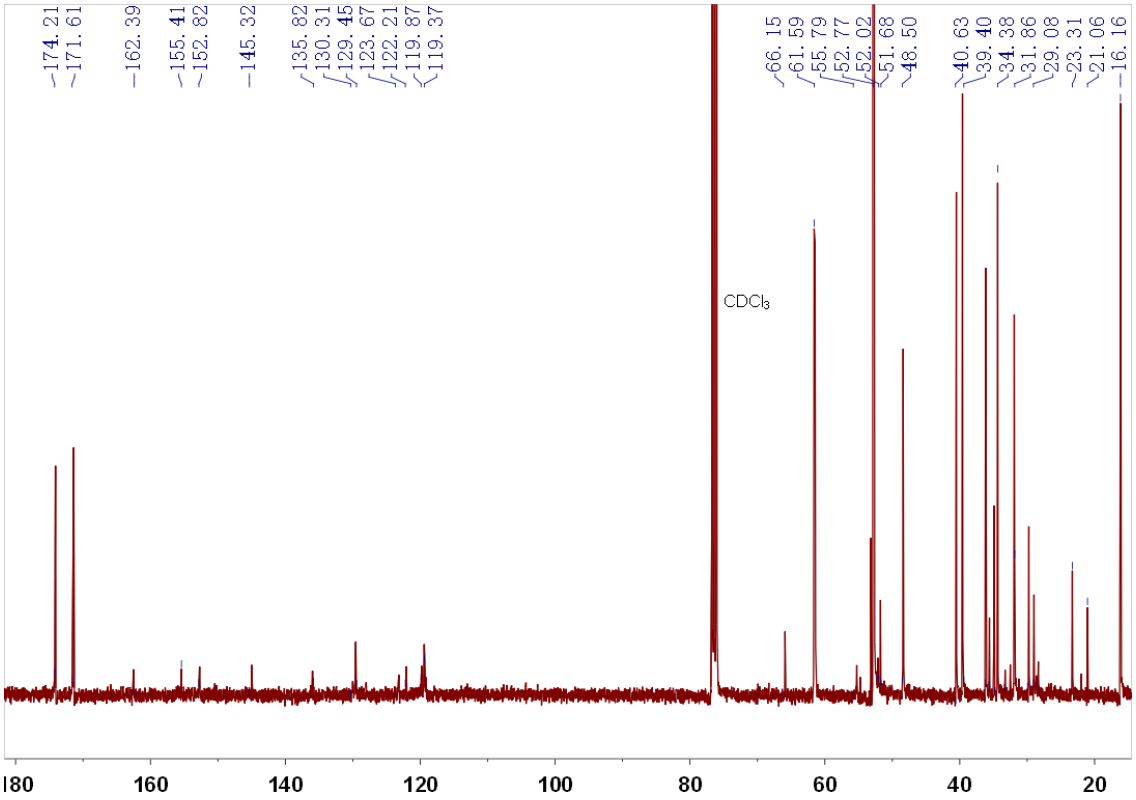


Fig. S17 ^{13}C NMR spectrum of **3b** in $CDCl_3$.

Synthesis of the second generation G2

G2 was synthesized from dendrimer **3b** according to the same procedure as for the synthesis of **G1.**

4. Synthesis of G3

Synthesis of compound 4a

Compound **4a** was synthesized by using **3b** (0.09 g, 0.012 mmol) and MAEA (1.3 g, 7.06 mmol) according to the same procedure as for the synthesis of **2a.** Compound **4a** was received as a red oil in 95% yield. ^{1}H-NMR (400 MHz, $CDCl_3$): δppm: 8.18 (s, 4H), 7.42 (s, 2H), 7.27-7.24 (m, 4H), 7.11 (d, *J* = 7.1 Hz, 8H), 6.92 (s, 8H), 6.13 (s, 32H), 5.60 (s, 32H), 4.31 (d, *J* = 22.1 Hz, 224H), 2.88-2.44 (m, 412H), 1.95 (s, 96H), 1.25 (d, *J* = 6.5 Hz, 72H), 1.10 (s, 24H). ^{13}C-NMR ($CDCl_3$, 400 Hz), δppm: 174.55, 172.49, 167.30, 163.26, 156.05, 153.17, 145.65, 135.84, 130.31, 125.73, 123.41, 123.08, 120.21, 119.95, 62.32, 62.18, 62.07, 53.79, 49.06, 48.96, 40.17, 35.53, 32.56, 32.47, 31.49, 30.34, 29.60, 28.98, 23.94, 22.56, 18.20, 16.80.

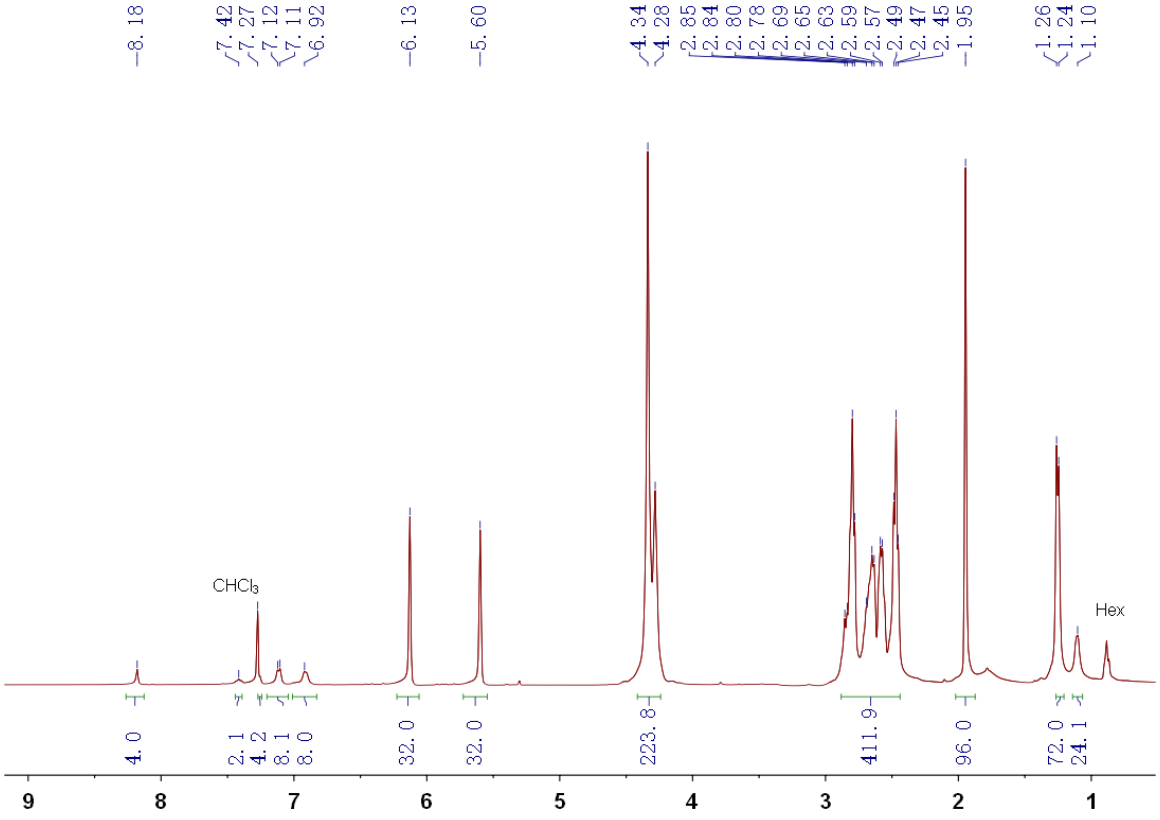


Fig. S18 ^{1}H NMR spectrum of compound **4a** in $CDCl_3$.

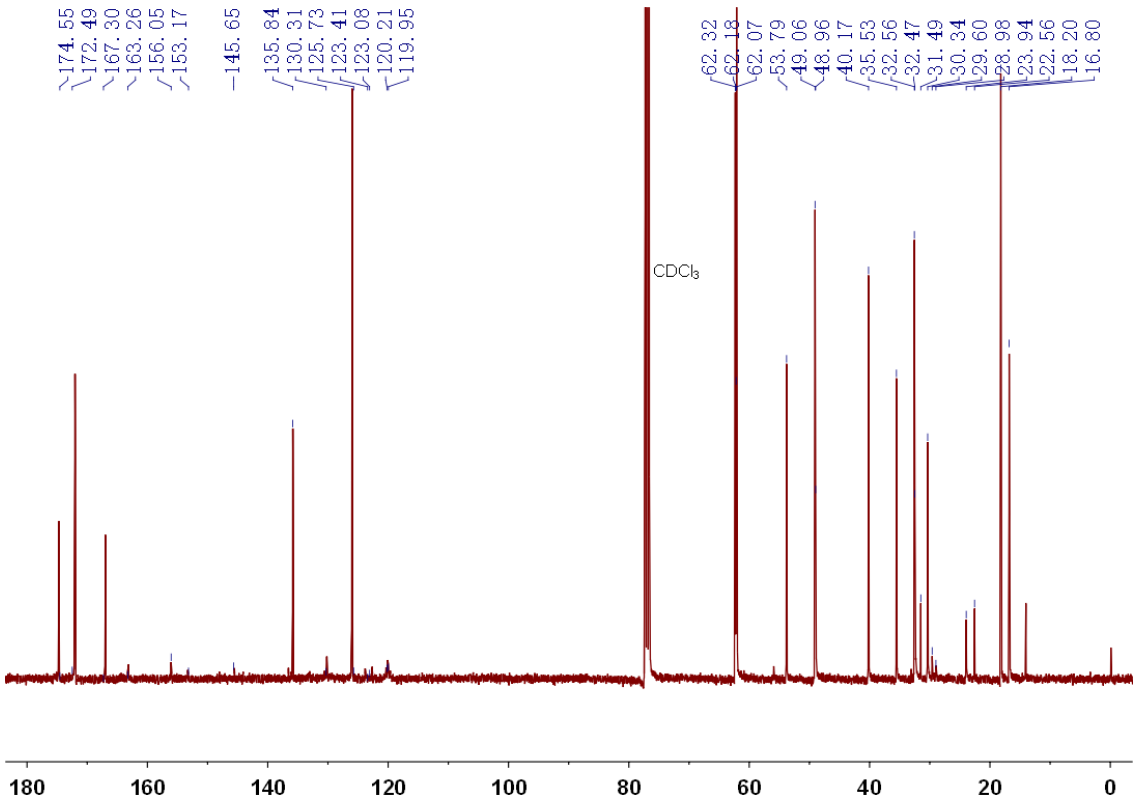


Fig. S19 ^{13}C NMR spectrum of compound **4a** in $CDCl_3$.

Synthesis of compound 4b

4b was synthesized by using compound **4a** and cysteamine according to the same procedure as for the synthesis of **2b. 4b** was received as a red oil in 90% yield. ^{1}H-NMR (400 MHz, $CDCl_3$): δppm: 8.18 (s, 4H), 7.41 (d, *J* = 7.3 Hz, 2H), 7.26 (s, 4H), 7.11 (d, *J* = 7.2 Hz, 8H), 6.92 (s, 8H), 4.30 (s, 224H), 2.89-2.48 (m, 636H), 1.43 (s, 96H), 1.27 (s, 72H), 1.11 (s, 24H). ^{13}C-NMR ($CDCl_3$, 400 Hz), δppm: 173.81, 170.80, 161.88, 154.85, 152.08, 144.33, 134.49, 128.81, 126.96, 123.98, 122.86, 121.44, 118.52, 65.52, 61.18, 54.55, 52.59, 52.16, 51.15, 47.75, 39.90, 39.02, 35.55,34.34, 33.79, 32.93, 31.33, 29.03, 25.32, 22.74, 20.49, 19.89.

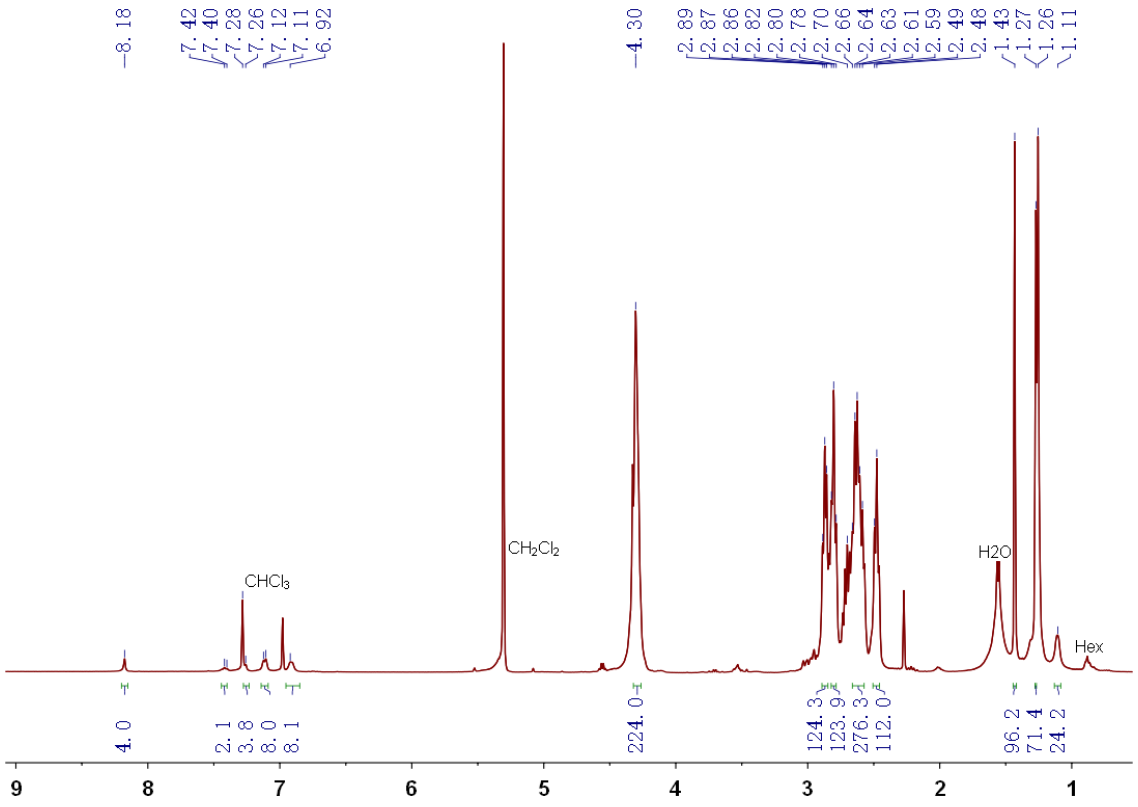


Fig. S20 ^{1}H NMR spectrum of **4b** in $CDCl_3$.

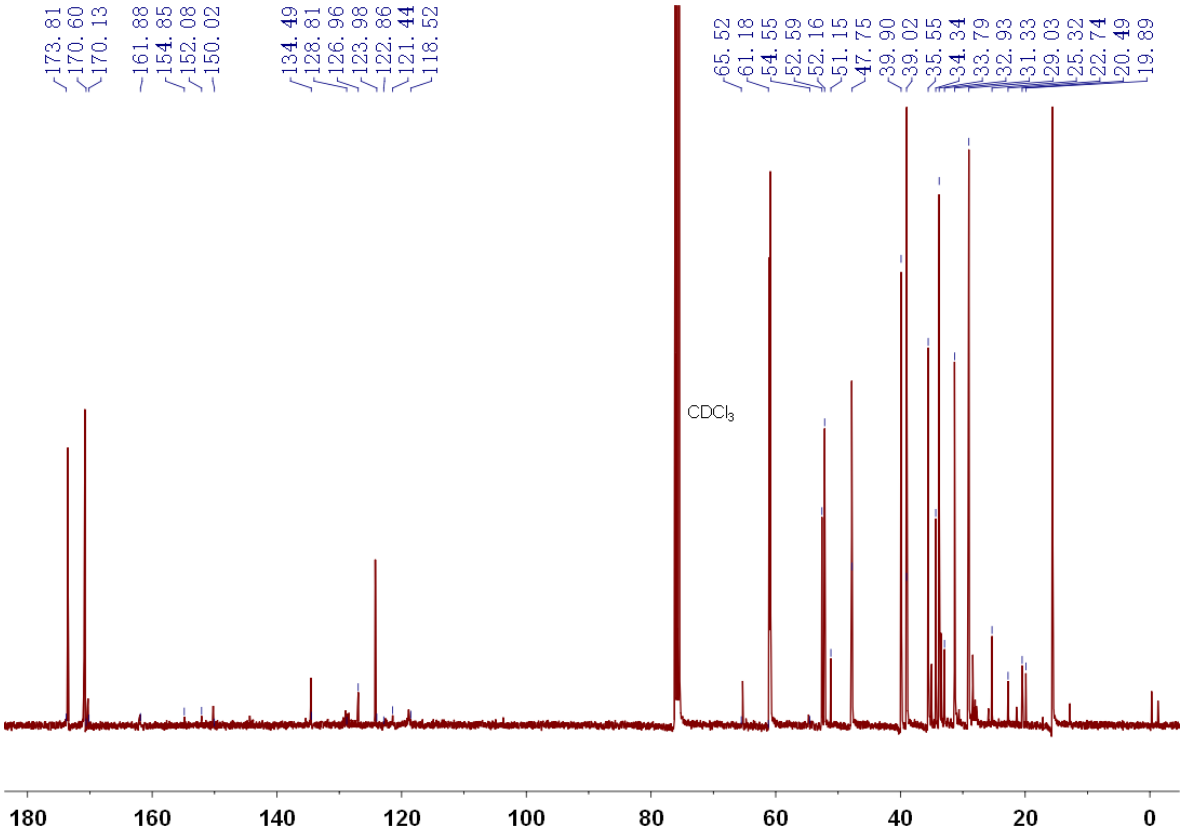


Fig. S21 ^{13}C NMR spectrum of **4b** in $CDCl_3$.

Synthesis of the third generation (G3)

G3 was synthesized from dendrimer **4b** according to the same procedure as for the synthesis of **G1.**

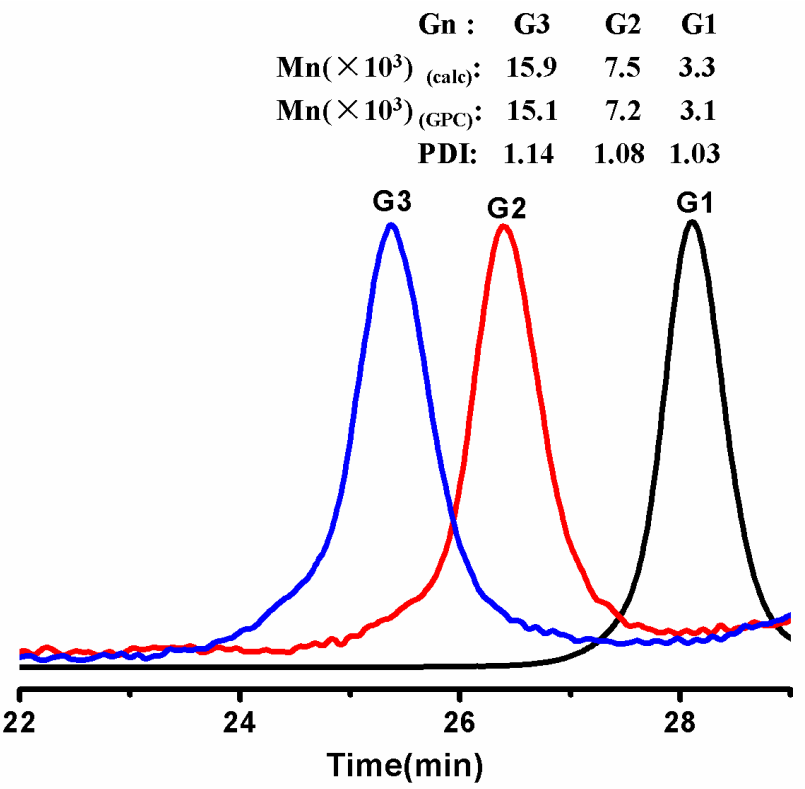


Fig. S22 Molecular weight progress of **G1-G3** measured by GPC.

G1-G3 molecular weights were characterized by gel permeation chromatography (GPC) in aqueous solution (Fig. S22). The molecular weights increased with increasing generations and their distributions were very narrow (all the polydispersity index (M_w/M_n) were less than 1.15).

References

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2. H. A. Klok, J. R. Hernánedz, S. Becker and K. Müllen, *Journal of Polymer Science: Part A: Polymer Chemistry,* 2001, **39**, 1572.