Electronic Supplementary Information

Linear Dendronized Polyols as a Multifunctional Platform for a Versatile and Efficient Fluorophore Design

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I. General Methods

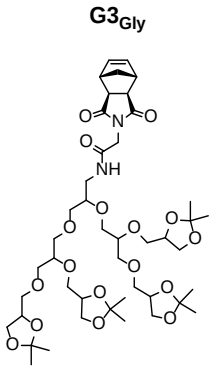
Materials. All reagents were purchased from Acros Organics, Fisher Scientific, AK Scientific, TCI America, or Sigma-Aldrich and used without further purification unless noted otherwise. Dichloromethane (DCM), pyridine, tetrahydrofuran (THF), toluene, dimethyl sulfoxide (DMSO), and *N*,*N*-dimethylformamide (DMF) were stored over activated 4 Å molecular sieves. All other solvents, such as ethanol (EtOH), methanol (MeOH), ethyl acetate (EtOAc), and hexanes (Hx) were reagent grade and used without further purification. The photophysical characterization and photostability study were carried out in aqueous 0.1 M phosphate buffer (PB, 4.11 g/L Na_2HPO_4, 1.60 g/L NaH_2PO_4) at pH 7.4. 3rd-generation Grubbs catalyst (pyridine-modified 2nd-generation Grubbs catalyst) for ROMP polymerization[1] and *N*-glycine *cis*-5-norbornene-*exo*-2,3-dicarboximide[2] were synthesized according to reported procedures. Linear dendronized polyols (LDPs) denoted LDPs-$\mathbf{Gx_LY}$ were synthesized with monomers $\mathbf{Gx_L}$ (x = 2 or 3; L = Gly or Cys) and **Y** with **Y** = **C**, **F**, **B**, **PGx** (x = 2, 3, or 4), **P**, and **R** with a feed ratio of 20 : 2, which gave a molecular weight of 12 kDa for 2nd-generation and 20 kDa for 3rd-generation acetal-protected LDPs. Previously reported crosslinked dendronized polyols (CDPs)[3] denoted CDPs-$\mathbf{G3_{Gly}Y}$ were synthesized with monomers $\mathbf{G3_{Gly}}$, $\mathbf{tris_{crossl.}}$, and **Y** with **Y** = **C**, **F**, **B**, **P**, and **R** with a feed ratio of 50 : 25 : 2, which gave a molecular weight of 79 kDa for acetal-protected CDPs. Their linear precursors pCDPs-$\mathbf{G3_{Gly}Y\text{-}Z}$ were synthesized with monomers $\mathbf{G3_{Gly}}$, **Z** = **NHS** or **tris** (for later crosslinking), and **Y** with a feed ratio of 50 : 25 : 2. For the FRET study, LDPs, pCDPs, and CDPs were synthesized with two different kinds of fluorophore monomers $\mathbf{Y_1}$ and $\mathbf{Y_2}$ with a feed ratio of 2 : 2 while maintaining the feed ratios of the other monomers mentioned above (see Scheme S8 and Figure S6). For the determination of the polymers' molar concentrations the above mentioned molecular weights of LDPs and CDPs determined by GPC were utilized.

All reactions were carried out under anhydrous conditions under an N_2 atmosphere. The progress of reactions was monitored by thin layer chromatography (TLC) using plastic sheets with a 0.2 mm thick layer of silica 60 coated with indicator F_{254}. Flash and gravity chromatography were performed using 230 - 400 mesh (40 - 63 µm) silica gel. Ratios of solvents for NMR and flash chromatography are reported as volume ratios. For optical measurements, quartz glass cuvettes with a path length of 1 cm for absorption and 0.4 cm for emission measurements were utilized.

Instrumentation. NMR spectra were recorded using a Varian U400, UI400, U500, VXR500, or UI500NB spectrometer in the NMR Laboratory, School of Chemical Sciences, University of Illinois. The data were processed in MestReNova 10.0.2. NMR data are reported in the following order: chemical shift, multiplicity (s = singlet, d = doublet, t = triplet, q = quartet, p = quintet, m = multiplet), and integration. Chemical shifts (δ) are reported in parts per million (ppm) and coupling constants (*J*) in hertz (Hz). ^{1}H NMR chemical shifts were referenced to the residual solvent peak of chloroform-*d* ($CDCl_3$) at 7.26 ppm. ^{13}C NMR chemical shifts were referenced to the solvent peak of $CDCl_3$ at 77.0 ppm. Mass spectral analyses were provided by the Mass Spectrometry Laboratory, School of Chemical Sciences, University of Illinois, using ESI 3 on a Waters Micromass Q-TOF spectrometer, or MALDI-TOF on an Applied Biosystems Voyager-DE STR spectrometer. Analytical gel permeation chromatography (GPC) experiments were performed on a hybrid system equipped with a Waters 1515 isocratic pump, Waters 2414 refractive index detector, Waters 2998 photodiode array detector, and miniDAWN TREOS 3-angle laser light scattering detector (MALLS, Wyatt Technology, CA). The MALLS detector collects wavelengths of light in the range of 500 - 800 nm. The laser wavelength of the TREOS instrument was set to 658 nm. The MALLS detector was used for the determination of absolute molecular weights M_n and calibrated with toluene. Measurements were performed with a polystyrene standard at 50 °C using DMF containing 0.1 M LiBr as mobile phase (flow rate = 1.0 mL/min). The absolute molecular weights M_n of LDPs were given as number-average molar weights and determined based on the *dn/dc* value of each sample. In the ratio, *dn* is the slope of the polymer solution's refractive index which is plotted against the polymer concentration *dc*. The *dn/dc* value of the polymer was calculated using ASTRA software (version 6.1, Wyatt Technology CA) assuming 100% mass recovery. DLS analysis was performed on a Brookhaven ZetaPALS instrument. The wavelength of the DLS laser was set to 633 nm (red laser). UV/Vis absorption spectra were recorded on a Shimadzu UV-2501PC spectrophotometer. Fluorescence spectra were recorded on a Horiba Jobin Yvon Fluoromax-3 spectrophotometer. Relative florescence quantum yields (FQY) were determined using fluorescein (Φ_r = 0.95 in 0.1 M aqueous NaOH solution) as reference according to a previously reported method (see Equation S2).[4-6]

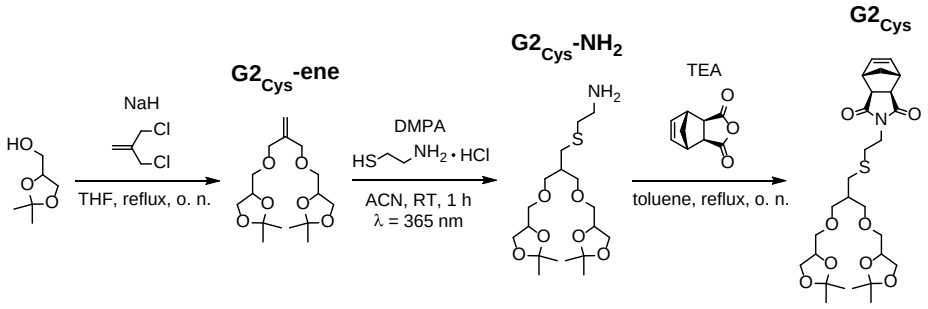
II. Monomer Synthesis

Dendron Monomers



Scheme S1. Chemical structure for monomer **G3$_{Gly}$** with an unsymmetrical 3rd-generation polyglycerol (PG) dendron connected to norbornene via a *N*-glycine linker.

G3$_{Gly}$. Dendron monomer **G3$_{Gly}$** was synthesized according to a previously reported literature procedure.[3]

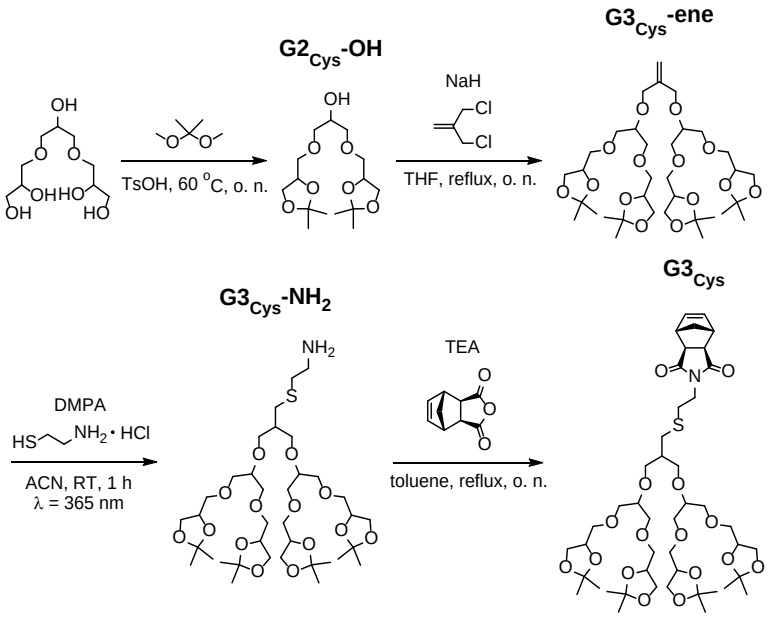


Scheme S2. Synthetic strategy for monomer **G2$_{Cys}$** with a 2nd-generation PG dendron connected to norbornene via a cysteamine linker.

G2$_{Cys}$-ene. Compound **G2$_{Cys}$-ene** was prepared according to a previously reported literature procedure.[7]

G2$_{Cys}$-NH$_2$. G2$_{Cys}$-ene (860 mg, 2.72 mmol, 1 equiv.), DMPA (34.8 mg, 0.14 mmol, 0.05 equiv.), and cysteamine hydrochloride (310 mg, 2.73 mmol, 1 equiv.) were added into a 20 mL vial and dissolved in acetonitrile (ACN, 10 mL). The suspension was stirred under irradiation with 365 nm light at room temperature for 1 h. The mixture became homogeneous and was concentrated in vacuo. The remaining residue was dissolved in DCM, washed with saturated NaCl solution, and dried over Na_2SO_4. After evaporation of the solvent in vacuo, crude product **G2$_{Cys}$-NH$_2$** was used without further purification.

$G2_{Cys}$. $G2_{Cys}$-NH_2 (1.0 g, 2.54 mmol, 1 equiv.) and *cis*-5-norbornene-*exo*-2,3-dicarboxylic anhydride (890 mg, 5.42 mmol, 2.13 equiv.) were added into a 20 mL vial, dissolved in toluene (13.5 mL), and treated with TEA (1.5 mL, 10.77 mmol, 4.24 equiv.). The mixture was refluxed with stirring for 12 h and concentrated in vacuo. The remaining residue was dissolved in DCM, washed with saturated NaCl solution, and dried over Na_2SO_4. After evaporation of the solvent in vacuo, the crude product was purified by gradient column chromatography (DCM / MeOH 19:1 to 5:1) to give product **$G2_{Cys}$** (560 mg, 1.04 mmol, 39% over two steps) as a light-yellow oil. ^{1}H NMR (500 MHz, $CDCl_3$) δ = 6.27 (s, 2 H, C<u>H</u>=C<u>H</u>), 4.23 (m, 2 H, H_{PG}, H_{linker}), 4.03 (m, 2 H, H_{PG}, H_{linker}), 3.85 (m, 2 H, H_{PG}), 3.73-3.40 (m, 11 H, H_{PG}, H_{linker}), 3.26 (m, 2 H, -C<u>H</u>-C<u>H</u>-$_{imide}$), 2.71-2.54 (m, 6 H, H_{PG}, H_{linker}), 1.51-1.49 (m, 1 H, C<u>H_2</u>$_{bridge}$), 1.40 (m, 7 H, C<u>H_2</u>$_{bridge}$, H_{acetal}), 1.34 ppm (m, 6 H, H_{acetal}). ^{13}C NMR (126 MHz, $CDCl_3$) δ = 178.17, 137.95, 109.53, 74.72, 72.76, 72.26, 72.20, 71.16, 71.09, 70.69, 70.67, 66.76, 64.03, 63.98, 53.56, 51.93, 50.87, 47.98, 47.37, 47.22, 45.86, 45.59, 45.49, 45.28, 43.05, 39.58, 37.45, 31.05, 30.54, 29.69, 26.86, 25.51 ppm. ESI-HRMS (m/z): calcd for $[M+Na]^+$ 562.2445; found, 562.2484.



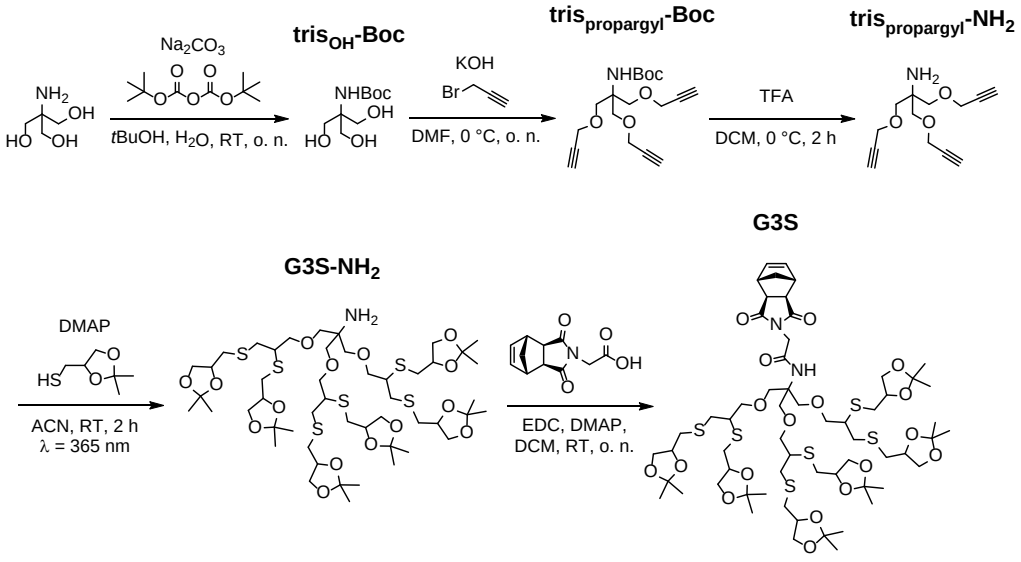
Scheme S3. Synthetic strategy for monomer **$G3_{Cys}$** with a 3rd-generation PG dendron connected to norbornene via a cysteamine linker.

$G2_{Cys}$-OH and **$G3_{Cys}$-ene.** Compounds **$G2_{Cys}$-OH** and **$G3_{Cys}$-ene** were prepared according to a previously reported literature procedure.[7]

$G3_{Cys}$-NH_2. $G3_{Cys}$-ene (1 g, 1.44 mmol, 1 equiv.), 2,2-dimethoxy-2-phenylacetophenone (DMPA, 74 mg, 0.29 mmol, 0.2 equiv.), and cysteamine hydrochloride (329 mg, 2.9 mmol, 2.01 equiv.) were placed into a 20 mL vial and dissolved in ACN (5 mL). The suspension was stirred under irradiation with 365 nm light at room temperature for 1 h. The mixture became homogeneous and was concentrated in vacuo.

The remaining residue was dissolved in DCM, washed with saturated NaCl solution, and dried over Na_2SO_4. After evaporation of the solvent in vacuo, crude product **$G3_{Cys}$-NH_2** was used without further purification.

$G3_{Cys}$. $G3_{Cys}$-NH_2 (1.2 g, 1.56 mmol, 1 equiv.) and *cis*-5-norbornene-*exo*-2,3-dicarboxylic anhydride (460 mg, 2.8 mmol, 1.8 equiv.) were placed into a 20 mL vial, dissolved in toluene (9 mL), and treated with TEA (1 mL). The mixture was refluxed with stirring for 12 h and concentrated in vacuo. The remaining residue was dissolved in DCM, washed with saturated NaCl solution, and dried over Na_2SO_4. After evaporation of the solvent in vacuo, the crude product was purified by gradient column chromatography (DCM / MeOH 19:1 to 5:1) to give product **$G3_{Cys}$** (520 mg, 1.56 mmol, 41% over two steps) as a light-yellow oil. ^{1}H NMR (500 MHz, $CDCl_3$) δ = 6.28 (s, 2 H, C<u>H</u>=C<u>H</u>), 4.23 (m, 4 H, H_{PG}, H_{linker}), 4.04 (m, 4 H, H_{PG}, H_{linker}), 3.72 (m, 4 H, H_{PG}), 3.67-3.44 (m, 25 H, H_{PG}, H_{linker}), 3.26 (s, 2 H, -C<u>H</u>-C<u>H</u>-$_{imide}$), 2.71-2.61 (m, 6 H, H_{PG}, H_{linker}), 1.51-1.49 (m, 1 H, C<u>H</u>$_2$ $_{bridge}$), 1.42-1.40 (m, 13 H, C<u>H</u>$_2$ $_{bridge}$, H_{acetal}), 1.34 ppm (m, 12 H, H_{acetal}). ^{13}C NMR (126 MHz, $CDCl_3$) δ = 177.90, 137.98, 109.47, 78.77, 78.35, 78.29, 74.92, 74.77, 72.68, 72.63, 71.77, 71.58, 71.38, 70.94, 69.77, 67.09, 66.98, 66.95, 62.78, 47.98, 45.32, 43.09, 40.37, 40.07, 37.64, 30.51, 29.76, 26.94, 25.58, 25.54 ppm. ESI-HRMS (m/z): calcd for $[M+H]^+$ 916.4723; found, 916.4698.



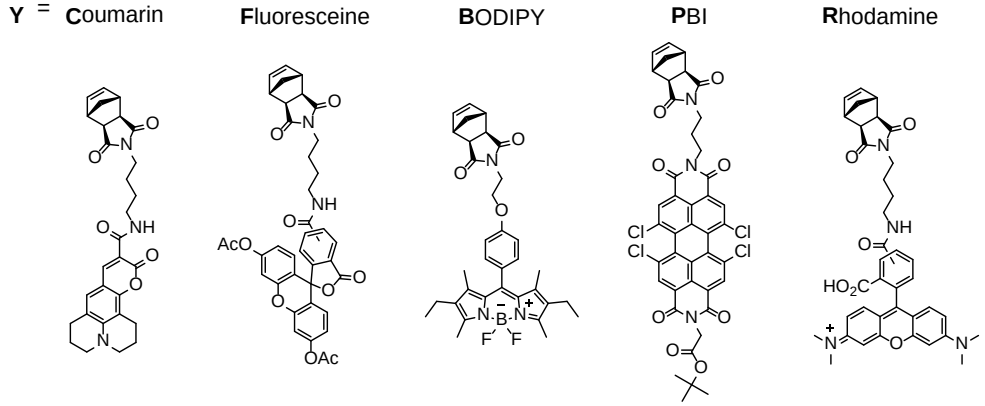
Scheme S4. Synthetic strategy for monomer **G3S** with a tris-cored 3rd-generation sulfide-containing dendron connected to norbornene via a *N*-glycine linker.

$Tris_{propargyl}$-NH_2. Compound **$tris_{propargyl}$-NH_2** was prepared according to a previously reported literature procedure via intermediate products **$tris_{OH}$-Boc** and **$tris_{propargyl}$-Boc**.[8-9]

G3S-NH_2. $Tris_{propargyl}$-NH_2 (235 mg, 1 mmol, 1 equiv.), DMPA (50 mg, 0.2 mmol, 0.2 equiv.), and (2,2-dimethyl-1,3-dioxolan-4-yl)methanethiol (2.96 g, 20 mmol, 20 equiv.) were added into a 20 mL vial and dissolved in ACN (5 mL). The suspension was stirred under irradiation with 365 nm light at room temperature for 2 h and concentrated in vacuo. The remaining residue was dissolved in DCM, washed with saturated NaCl solution, and dried over Na_2SO_4. After evaporation of the solvent in vacuo, crude product **G3S-NH_2** was used without further purification.

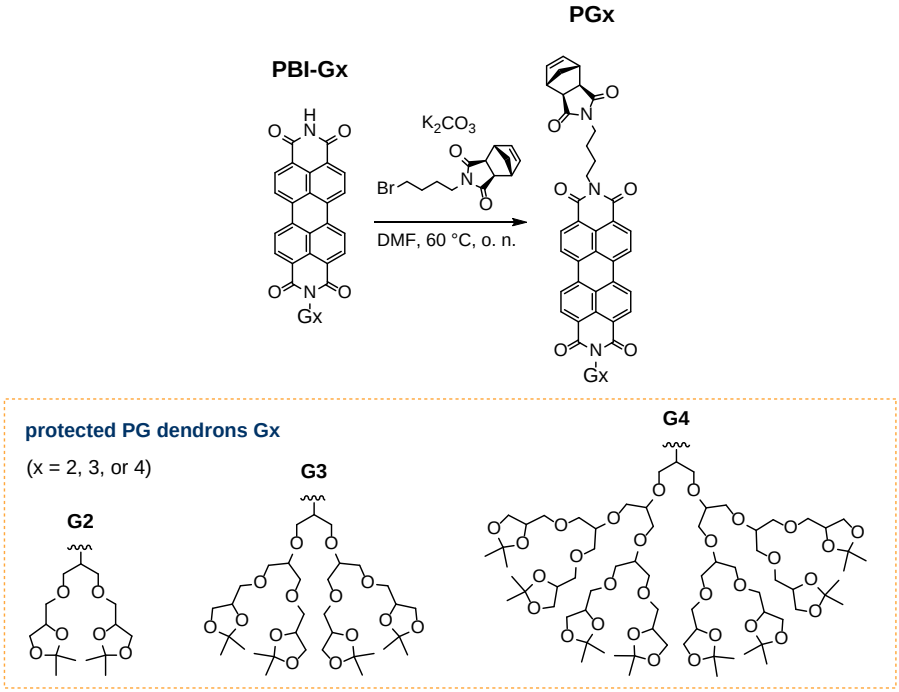
G3S 13. To a solution of **G3S-NH_2** (1.1 g, 0.98 mmol, 1 equiv.) and *N*-glycine *cis*-5-norbornene- *exo*-2,3-dicarboximide (242 mg, 1.09 mmol, 1.12 equiv.) in DCM (10 mL) coupling reagents DMAP (12 mg, 0.1 mmol, 0.1 equiv.) and EDC (400 mg, 2.58 mmol, 2.63 equiv.) were added. The solution was stirred at room temperature for 12 h, during which the same quantity of EDC (400 mg, 2.58 mmol, 2.63 equiv.) was added once again. Subsequently, the mixture was washed with saturated NaCl solution, the organic layer dried over Na_2SO_4, and the solvent removed in vacuo. The crude product was purified by gradient column chromatography (DCM / MeOH 19:1 to 5:1) to give product **G3S** (390 mg, 0.29 mmol, 29% over two steps) as a light-yellow oil. ^{1}H NMR (500 MHz, $CDCl_3$) δ = 6.30-6.29 (m, 2 H, C<u>H</u>=C<u>H</u>), 4.25 (m, 6 H, $H_{dendron}$, H_{linker}), 4.10 (m, 6 H, $H_{dendron}$, H_{linker}), 3.71-3.61 (m, 18 H, $H_{dendron}$), 3.31-2.65 (m, 28 H, $H_{dendron}$, H_{linker}), 1.57 (m, 1 H, C<u>H</u>$_{2\ bridge}$), 1.42 (m, 19 H, C<u>H</u>$_{2\ bridge}$, H_{acetal}), 1.35 ppm (m, 18 H, H_{acetal}). ^{13}C NMR (126 MHz, $CDCl_3$) δ = 177.40, 138.10, 109.75, 77.41, 77.16, 76.91, 75.94, 75.86, 75.77, 73.06, 69.98, 69.55, 68.99, 60.54, 48.17, 46.44, 45.58, 45.49, 43.20, 43.05, 41.37, 36.47, 36.20, 35.73, 35.52, 34.85, 28.61, 27.15, 25.74 ppm. ESI-HRMS (m/z): calcd for $[M+H]^+$ 1327.5212; found, 1327.5151.

Fluorophore Monomers



Scheme S5. Chemical structures of fluorophore monomers **Y** with **Y** = **C**oumarin, **F**luorescein, **B**ODIPY, **P**BI, and **R**hodamine, which are hereinafter abbreviated by the first letter of their name.

Fluorophore Monomers Y with Y = C, **F**, **B**, **P**, and **R.** Fluorophore monomers **Y** = **C**oumarin, **F**luorescein, **B**ODIPY, **P**erylene bisimide (**P**BI), and **R**hodamine were synthesized according to previously reported literature procedures.[2-3]



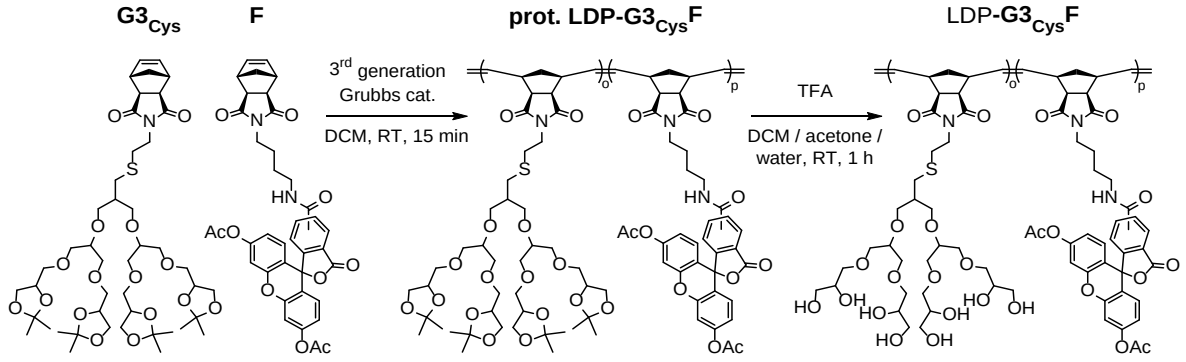
Scheme S6. Synthetic procedure for PG-dendronized PBI monomers **PGx** in three different dendron generations Gx (x = 2, 3, or 4). The chemical structures of acetal-protected PG dendrons are shown for G2 - G4.

PBI-Gx (x = 2, 3, or 4). PG-dendronized PBI fluorophores **PBI-Gx** (x = 2, 3, or 4) were prepared according to a previously reported literature procedure.[10]

General procedure for PGx (x = 2, 3, or 4). To a solution of **PBI-Gx** (15 µmol, 1 equiv.) in DMF (0.5 mL), *N*-(4-bromo-butyl) *cis*-5-norbornene-*exo*-2,3-dicarboximide (4.92 mg, 16.5 µmol, 1.1 equiv.) and anhydrous K_2CO_3 (6.91 mg, 50 µmol, 3.3 equiv.) were added. The mixture was stirred at 60 °C overnight. The suspension was filtered and the solvent removed in vacuo. The crude products were resuspended in DCM and purified through a silica plug (EtOAc / MeOH 19:1 to 9:1). **PG2**. **PBI-G2** (10.39 mg) yielded product **PG2** (8.9 mg, 9.78 µmol, 65%). ^{1}H NMR (500 MHz, $CDCl_3$) δ = 8.51-8.30 (m, 8 H, H_{PBI}), 6.28 (s, 2 H, CH=CH), 5.68 (m, 1 H, $H_{α\text{-}PG}$), 4.43-4.17 (m, 2 H, H_{PG}, H_{linker}), 4.05-3.98 (m, 2 H, H_{PG}, H_{linker}), 3.73-3.47 (m, 14 H, H_{PG}, H_{linker}), 3.27 (s, 2 H, -C<u>H</u>-C<u>H</u>-$_{imide}$), 2.69-2.67 (m, 2 H, C<u>H</u>-$CH_{2\ bridge}$-C<u>H</u>), 1.81-1.50 (m, 5 H, $C\underline{H}_{2\ bridge}$, H_{linker}), 1.79-1.17 ppm (m, 13 H, $C\underline{H}_{2\ bridge}$, H_{acetal}). ESI-HRMS (m/z): calcd for $[M+H]^+$ 910.3546; found, 910.3578. **PG3**. **PBI-G3** (16 mg), yielded product **PG3** (11 mg, 8.55 µmol, 57%). ^{1}H NMR (500 MHz, $CDCl_3$) δ = 8.58-8.48 (m, 8 H, H_{PBI}), 6.26 (s, 2 H, CH=CH), 5.62 (m, 1 H, $H_{α\text{-}PG}$), 4.22 (m, 4 H, H_{PG}, H_{linker}), 4.02-3.86 (m, 4 H, H_{PG}, H_{linker}), 3.71-3.27 (m, 30 H, H_{PG}, H_{linker}), 3.25 (s, 2 H, -C<u>H</u>-C<u>H</u>-$_{imide}$), 2.67-2.65 (m, 2 H, C<u>H</u>-$CH_{2\ bridge}$-C<u>H</u>), 1.80-1.48 (m, 5 H, $C\underline{H}_{2\ bridge}$, H_{linker}), 1.39-1.19 ppm (m, 25 H, $C\underline{H}_{2\ bridge}$, H_{acetal}). ESI-HRMS (m/z): calcd for $[M+H]^+$ 1286.5643; found, 1286.5619. **PG4**. **PBI-G4** (27.3 mg) yielded product **PG4** (18 mg, 8.83 µmol, 59%). ^{1}H NMR δ = 8.67-8.62 (m, 8 H, H_{PBI}), 6.22 (m, 2 H, C<u>H</u>=C<u>H</u>), 5.60 (p, *J* = 7.3, 6.7 Hz, 1 H, $H_{α\text{-}PG}$), 4.47 (m, 2 H, H_{PG}, H_{linker}), 4.21-4.14 (m, 8 H, H_{PG}, H_{linker}), 4.02-3.97 (m, 8 H, H_{PG}, H_{linker}), 3.71-3.33 (m, 60 H, H_{PG}, H_{linker}), 3.17 (s, 2 H,

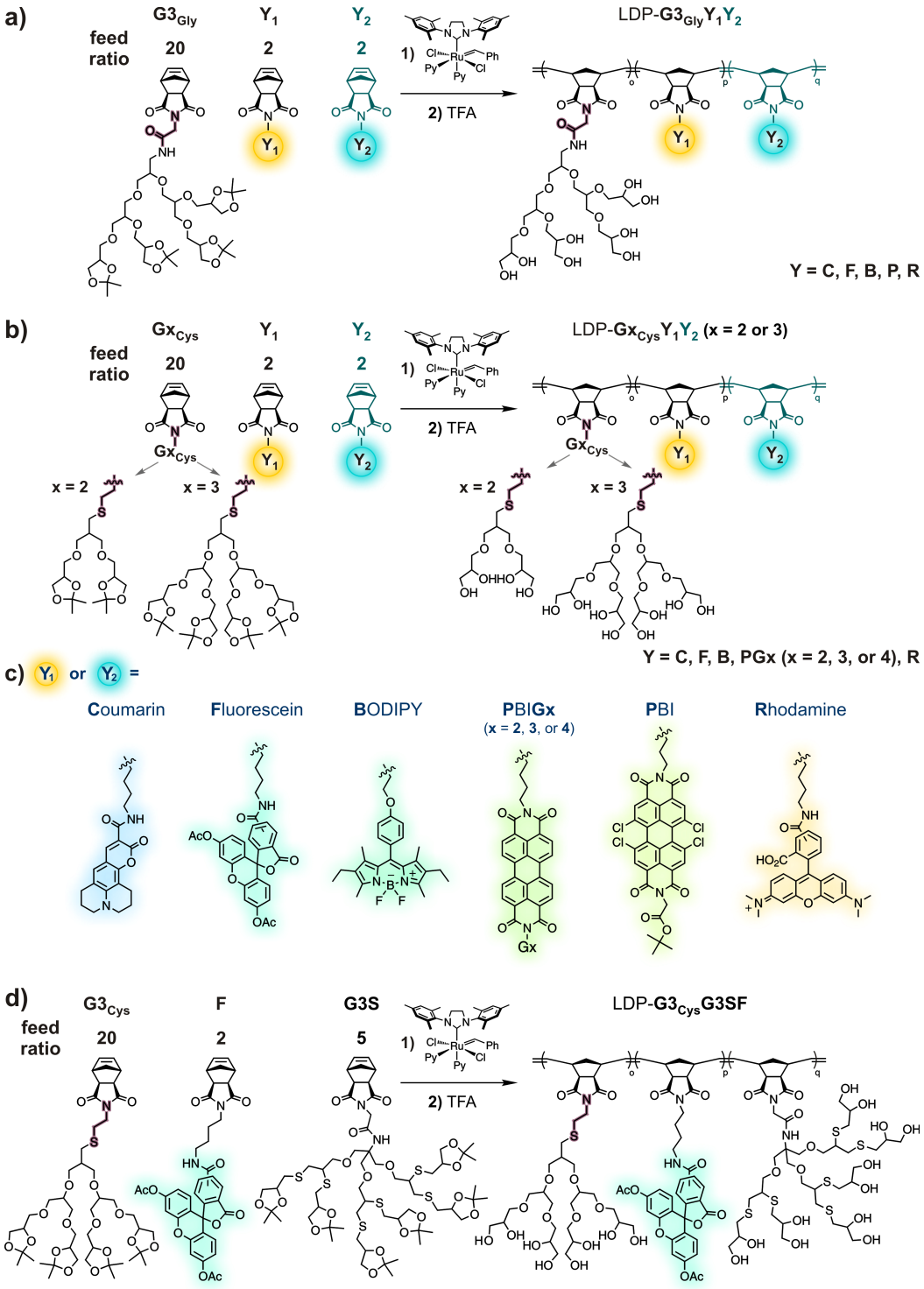
-C<u>H</u>-C<u>H</u>-$_{imide}$), 2.63 (m, 2 H, C<u>H</u>-CH$_{2\ bridge}$-C<u>H</u>), 1.77-1.47 (m, 5 H, C<u>H</u>$_{2\ bridge}$, H$_{linker}$), 1.39-1.21 ppm (m, 49 H, C<u>H</u>$_{2\ bridge}$, H$_{acetal}$). MALDI (m/z): calcd for $[M+K]^+$ 2077.9429; found, 2077.8159.

III. Polymer Synthesis



Scheme S7. Synthesis of representative LDP-**G3$_{Cys}$F** with dendron monomer **G3$_{Cys}$** and fluorophore monomer **F**luorescein with a feed ratio of 20 : 2 via acetal-protected LDP (see Scheme S8 for the entire LDP series).

General procedure for LDPs-Gx$_L$Y. Dendron monomer **Gx$_L$** (240 nmol, 20 equiv.) and fluorophore monomer **Y$_1$** (24 nmol, 2 equiv.), plus optional fluorophore monomer **Y$_2$** (24 nmol, 2 equiv.) or multi-thioether monomer **G3S** (60 nmol, 5 equiv.), respectively, were dissolved in anhydrous DCM (5 mL). 3rd-generation Grubbs catalyst (0.03 M in DCM, 0.04 mL, 12 nmol, 1 equiv.) was then added. The solution was stirred at room temperature for 15 min before butyl vinyl ether (1 mL) was added to quench the catalyst. The solvent was then removed in vacuo. The remaining solid residue was dissolved in DCM, precipitated in 14 mL of hexane, and centrifuged for 10 min at 5000 rpm. This process was repeated two times. The precipitate was dried to give a brown transparent film. The resulting polymer (23 mg) was dissolved in a mixture of DCM (2 mL), acetone (2 mL), water (1 mL) and treated with TFA (0.2 mL). The solution was stirred at room temperature for 1 h and concentrated under reduced pressure. The viscous residue was dissolved in water (1 ml) and dialyzed against aqueous 0.1 M $NaHCO_3$ solution (1 L) for 6 h and water (1 L) for another 6 h. The resulting aqueous polymer solution was lyophilized to give the final product LDP-**Gx$_L$Y**. **LDP-G3$_{Cys}$F.** LDP-**G3$_{Cys}$F** (representative) 15 mg (65%) obtained as white foam (Scheme S7). ^{1}H NMR (500 MHz, $CDCl_3$) δ = 8.42-8.09 (m, 2 H, H$_{fluorescein}$), 7.47-7.36 (m, 2 H, H$_{fluorescein}$), 7.13-6.81 (m, 5 H, H$_{fluorescein}$), 5.75-5.45 (m, 20 H, -C<u>H</u>=C<u>H</u>-$_{backbone}$), 4.24 (m, 40 H, H$_{PG}$), 4.11-4.04 (m, 57 H, H$_{PG}$, H$_{linker}$), 3.71-3.49 (m, 263 H, H$_{PG}$, H$_{linker}$), 3.29-2.95 (m, 20 H, H$_{PG}$, H$_{linker}$, -C<u>H</u>-C<u>H</u>-$_{imide}$), 2.79 (m, 9 H, H$_{linker}$), 2.47-2.15 (m, 22 H, -C<u>H</u>-CH$_{2bridge}$-C<u>H</u>-), 1.65 (m, 65 H, H$_{acetyl,}$ C<u>H</u>$_{2\ bridge}$, H$_{linker}$), 1.56 (m, 18 H, C<u>H</u>$_{2\ bridge}$, H$_{linker}$), 1.40 (m, 120 H, H$_{acetal}$), 1.35 ppm (m, 120 H, H$_{acetal}$). ^{1}H NMR spectrum of LDP-**G3$_{Cys}$F** shows 1 : 10 ratio of **F**luorescein and dendron **G3$_{Cys}$**, which corresponds to feed ratio of mono dye-conjugated LDPs-**Gx$_L$Y** (x = 2 or 3; L = Gly or Cys) in Scheme S8.



Scheme S8. General synthesis and feed ratios for different types of LDPs-$\mathbf{Gx_LY_1Y_2}$ consisting of dendron monomer $\mathbf{Gx_L}$ (x = 2 or 3; L = Gly or Cys) and one or two fluorophore monomers **Y**. The second fluorophore $\mathbf{Y_2}$ shown in dark green is optional and was exclusively introduced for FRET study. The Gly- and Cys-linkers are shaded in purple. 1) 3rd-generation Grubbs catalyst, DCM, RT, 15 min and 2) TFA, DCM / acetone / water, RT, 1 h. a) LDPs-$\mathbf{G3_{Gly}Y}$ with previously reported 3rd-generation dendron monomer[3] $\mathbf{G3_{Gly}}$ and **Y** with a feed ratio of 20 : 2. b) LDPs-$\mathbf{Gx_{Cys}Y}$ with new 2nd- or 3rd-generation dendron monomer $\mathbf{Gx_{Cys}}$ (x = 2 or 3) and **Y** with a feed ratio of 20 : 2. c) Chemical structures of fluorophore monomers[2-3, 10] **Y** with **Y** = **C**oumarin, **F**luorescein, **B**ODIPY, dendronized **PBIGx**, **P**BI, and **R**hodamine. d) LDP-$\mathbf{G3_{Cys}G3SF}$ with new 3rd-generation dendron monomer $\mathbf{G3_{Cys}}$, fluorescein **F**, and multi-thioether-containing monomer **G3S** with a feed ratio of 20 : 2 : 5.

IV. NMR Spectra

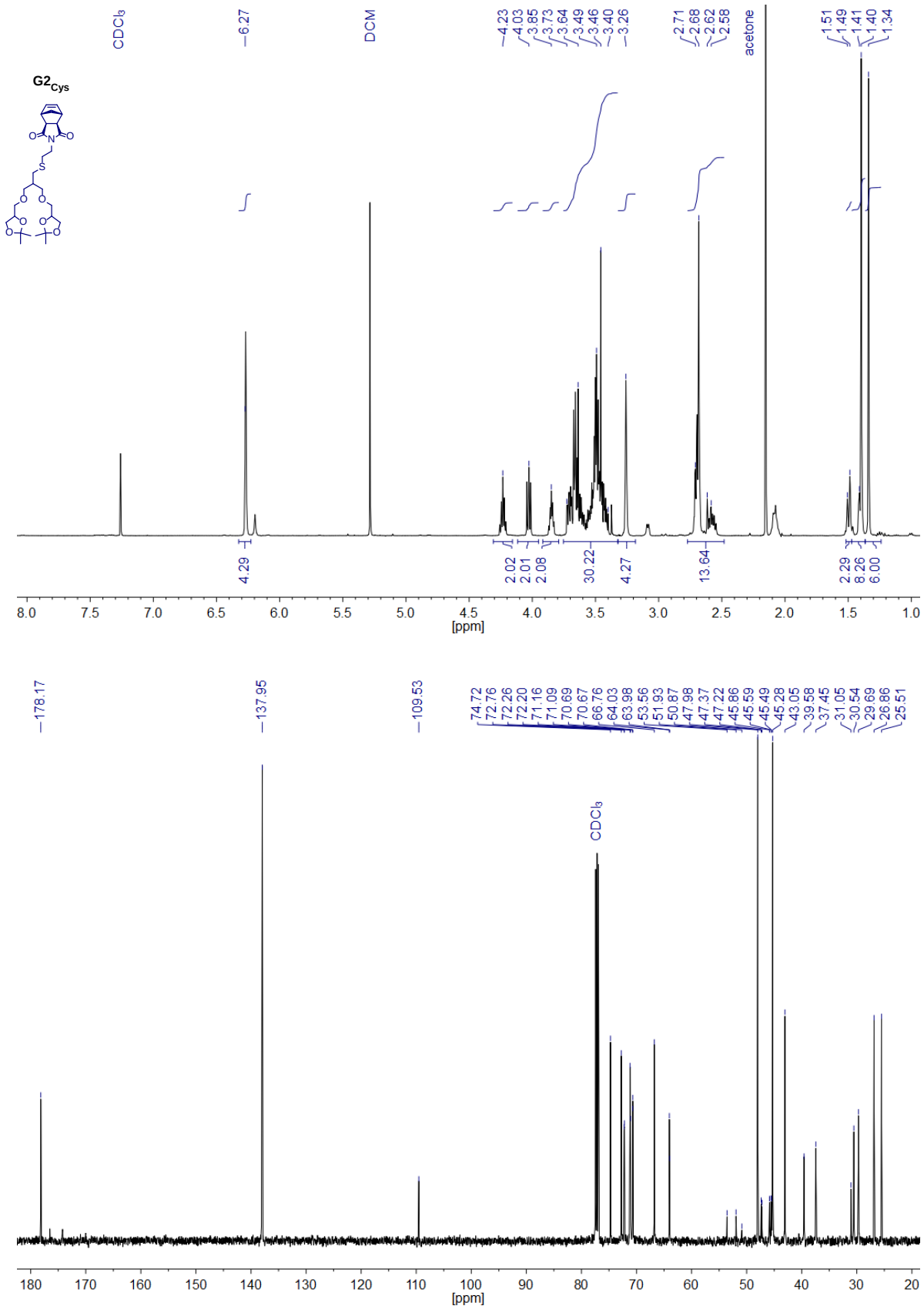


Figure S1. ^{1}H NMR (500 MHz, $CDCl_3$, top) and ^{13}C NMR spectra (126 MHz, $CDCl_3$, bottom) of dendron monomer **G2$_{Cys}$**.

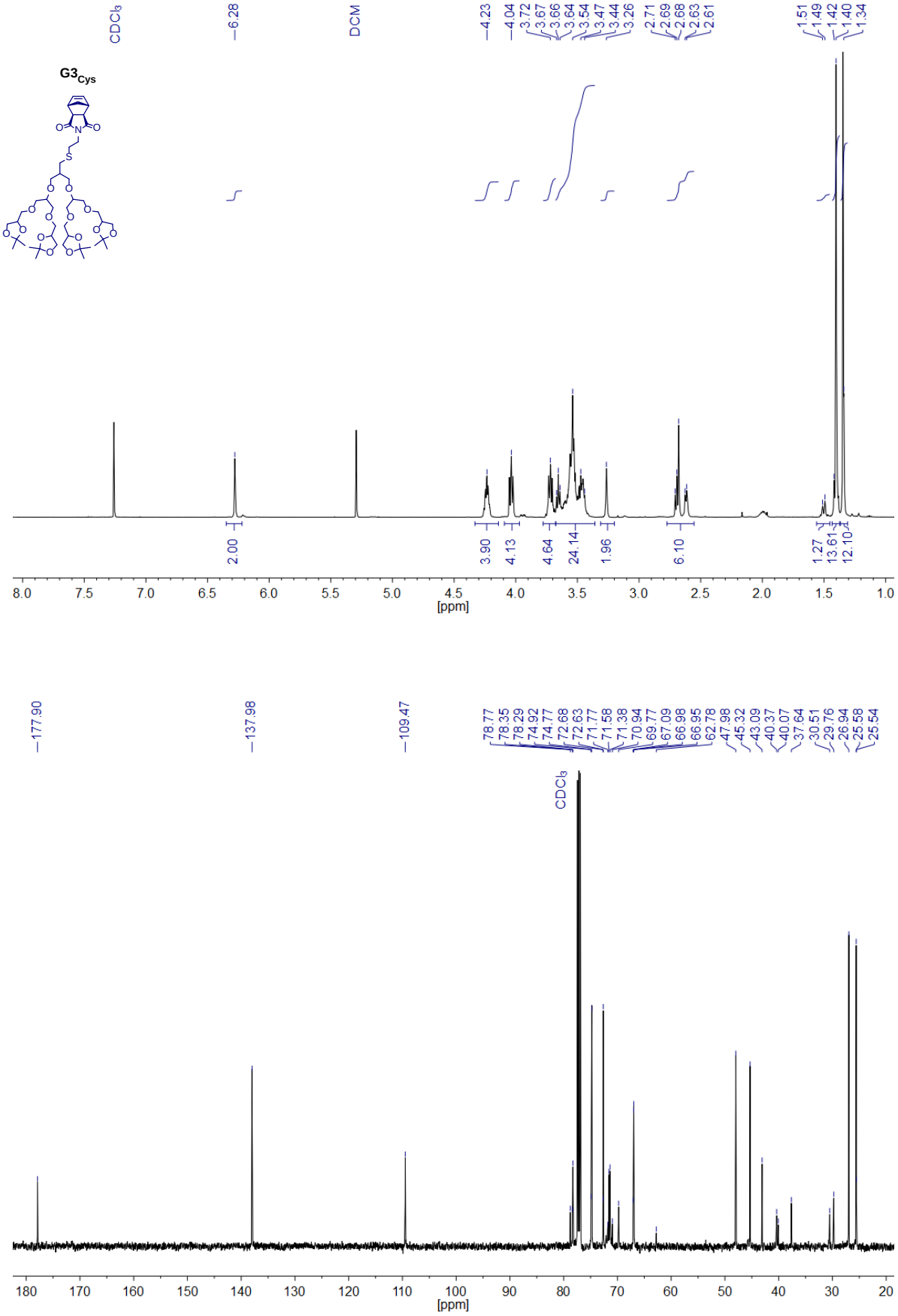


Figure S2. ^{1}H NMR (500 MHz, $CDCl_3$, top) and ^{13}C NMR spectra (126 MHz, $CDCl_3$, bottom) of dendron monomer **G3$_{Cys}$**.

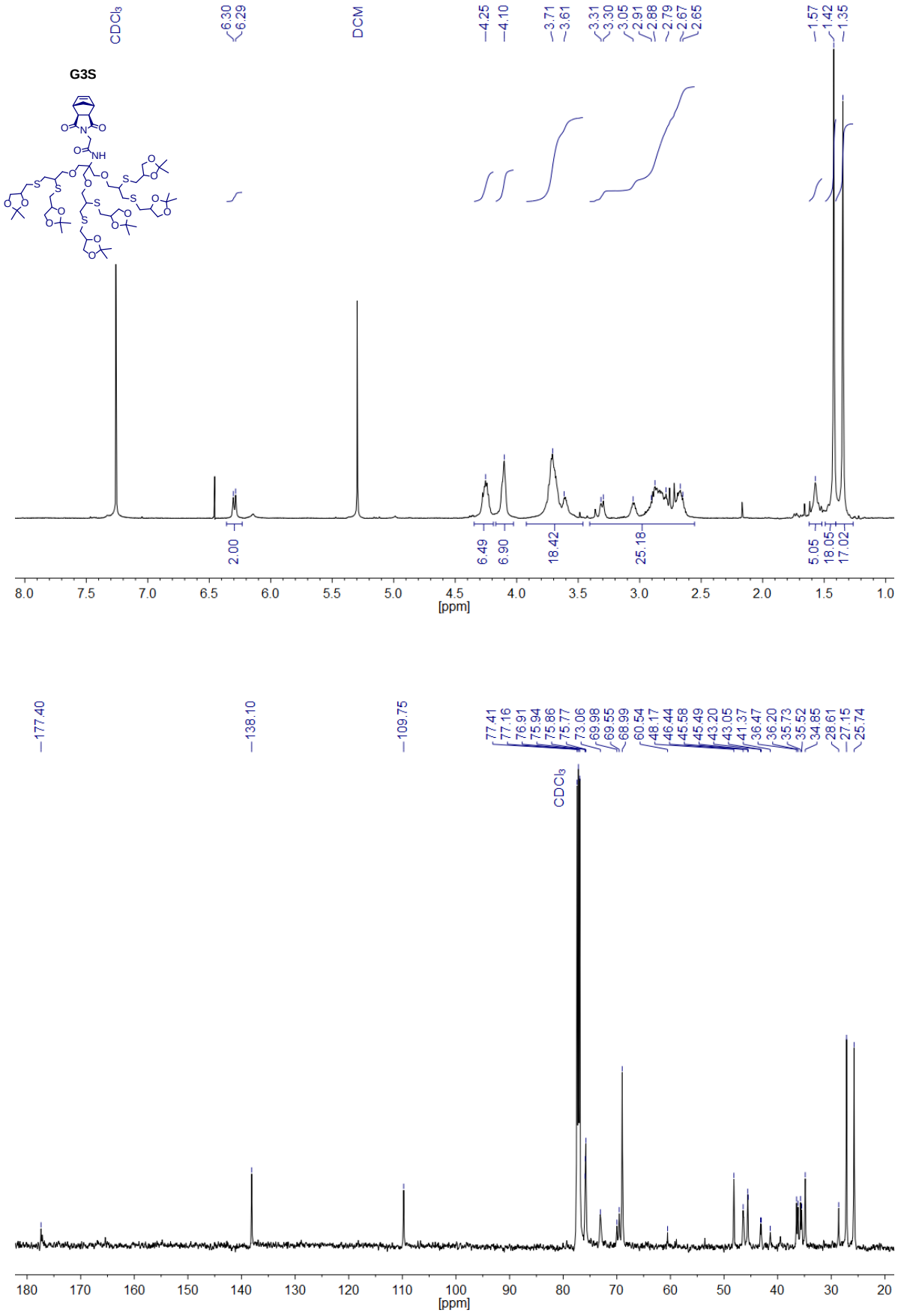


Figure S3. ^{1}H NMR (500 MHz, $CDCl_3$, top) and ^{13}C NMR spectra (126 MHz, $CDCl_3$, bottom) of dendron monomer **G3S**.

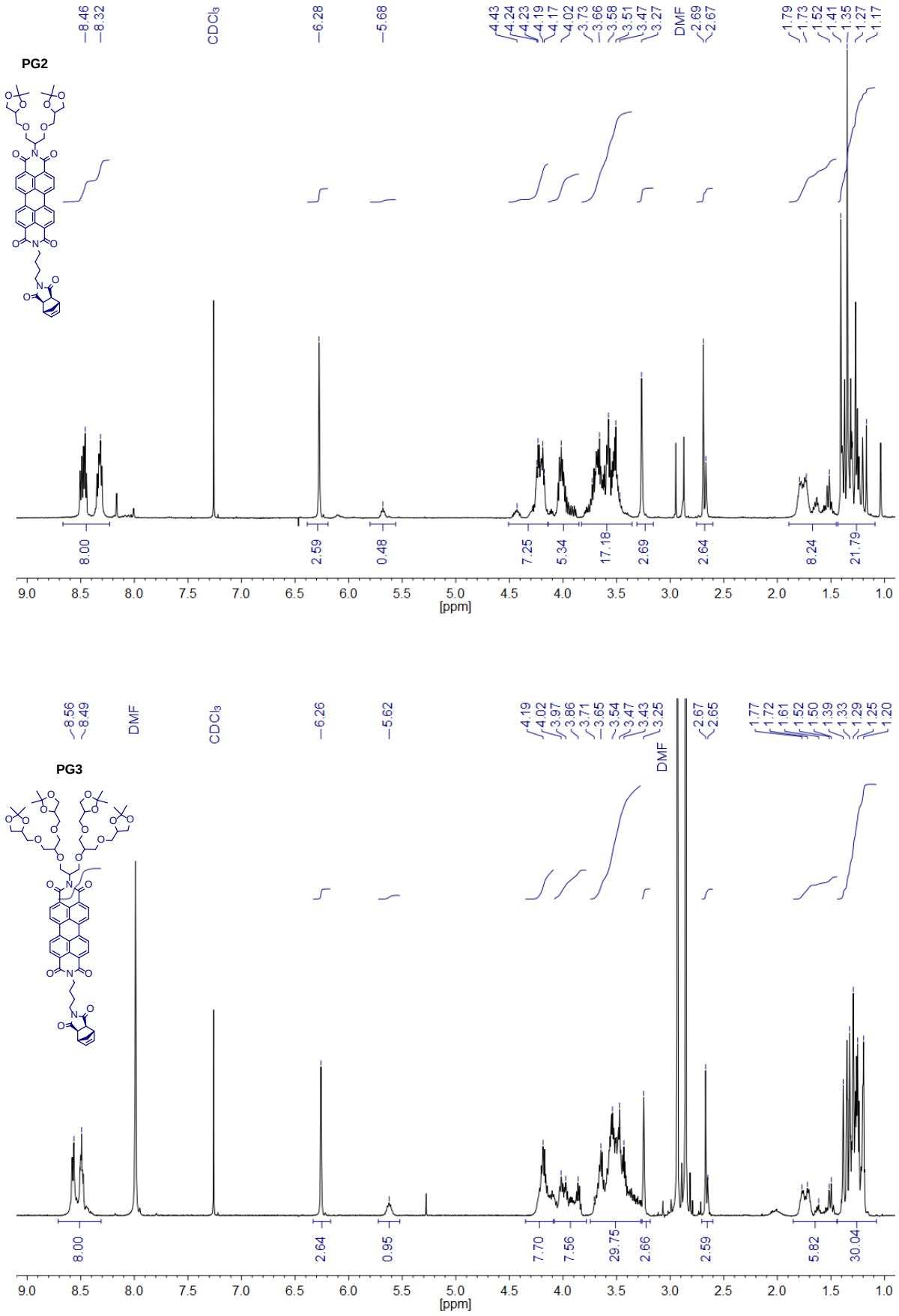


Figure S4. ^{1}H NMR (500 MHz, $CDCl_3$) spectra of dendronized fluorophore monomers **PG2** (top) and **PG3** (bottom).

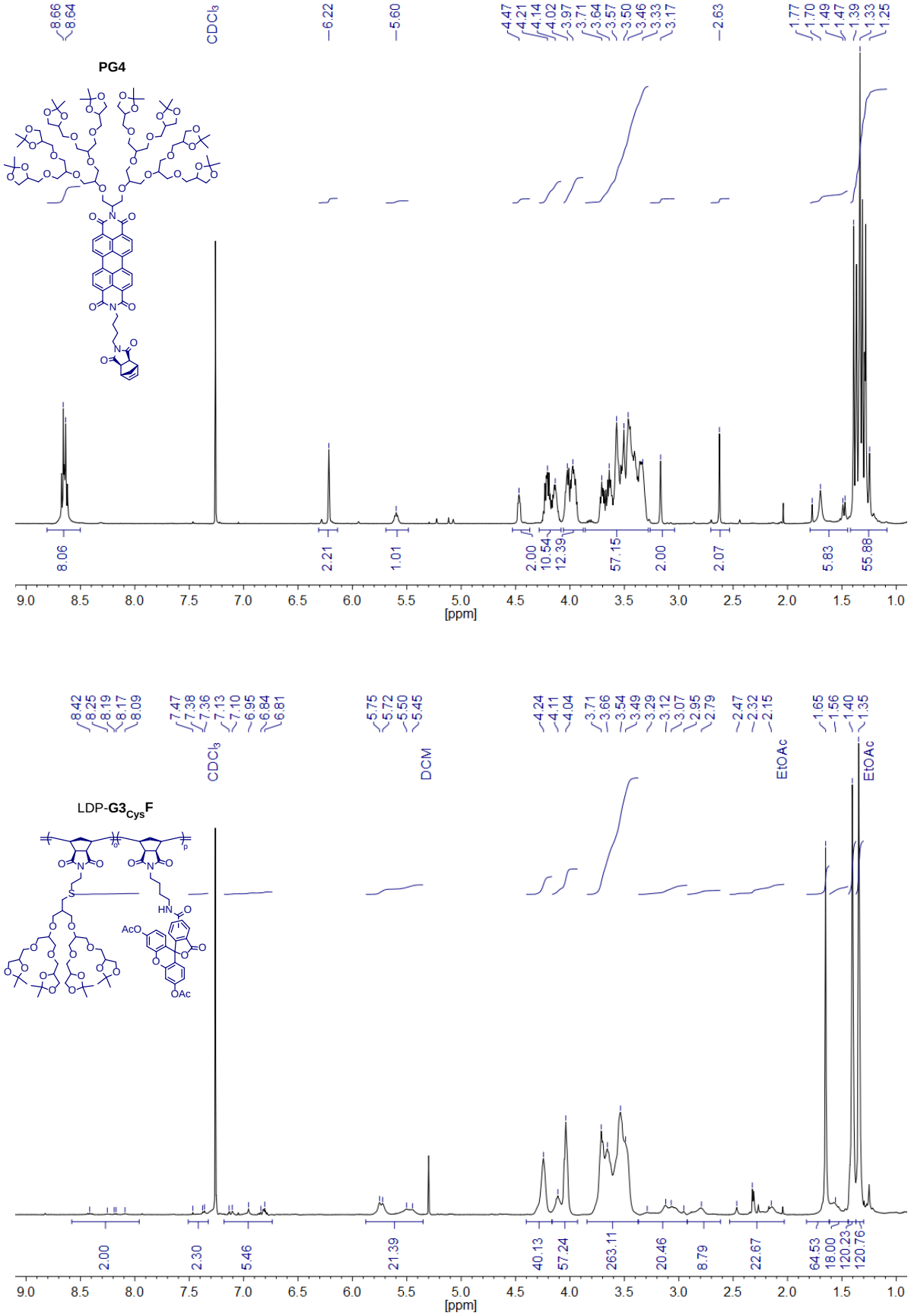


Figure S5. ^{1}H NMR (500 MHz, $CDCl_3$) spectra of dendronized fluorophore monomer **PG4** (top) and representative LDP-**G3$_{Cys}$F**.

V. Structures of pCDPs and CDPs

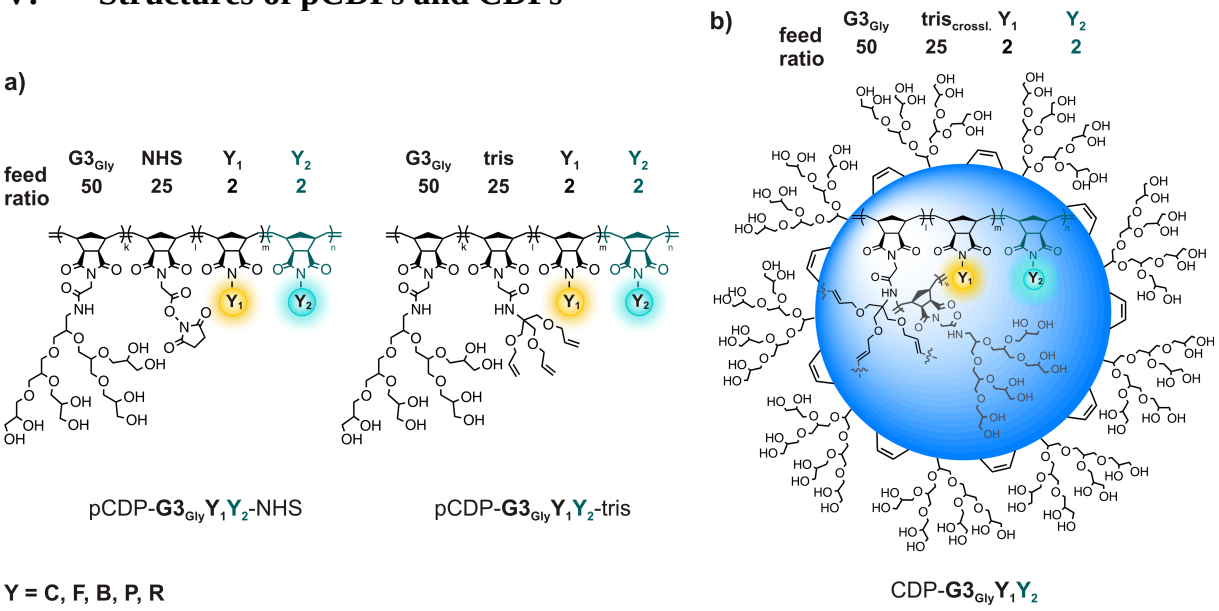


Figure S6. Chemical structures of previously reported crosslinked dendronized polyols[3] (CDPs). a) linear CDP precursors pCDPs-**G3$_{Gly}$Y-Z** with Z = **NHS** (left) or **tris** (right) function and b) globular CDP-**G3$_{Gly}$Y** equipped with unsymmetrical dendron monomer **G3$_{Gly}$** and one or two fluorophore monomers **Y**. The second fluorophore **Y$_2$** shown in dark green is optional and was exclusively introduced for FRET study. For structures of fluorophore monomers **Y** with **Y** = **C**oumarin, **F**luorescein, **B**ODIPY, **P**BI, or **R**hodamine see Scheme S8.

VI. Polymer Characterization

Dynamic Light Scattering (DLS)

Figure S7. DLS analysis of LDP-**G3$_{Gly}$F** measured with a DLS laser of λ_{DLS} = 633 nm in 0.1 M PB (pH 7.4) revealed a hydrodynamic molecular diameter of 5.53 nm. In case of the LDPs of BODIPY, PBI, and rhodamine the wavelength of the DLS laser interfered with the fluorescence of the conjugated fluorophore. Therefore, representative LDP-**G3$_{Gly}$F** serves as an example for the entire LDP series.

Gel Permeation Chromatography (GPC)

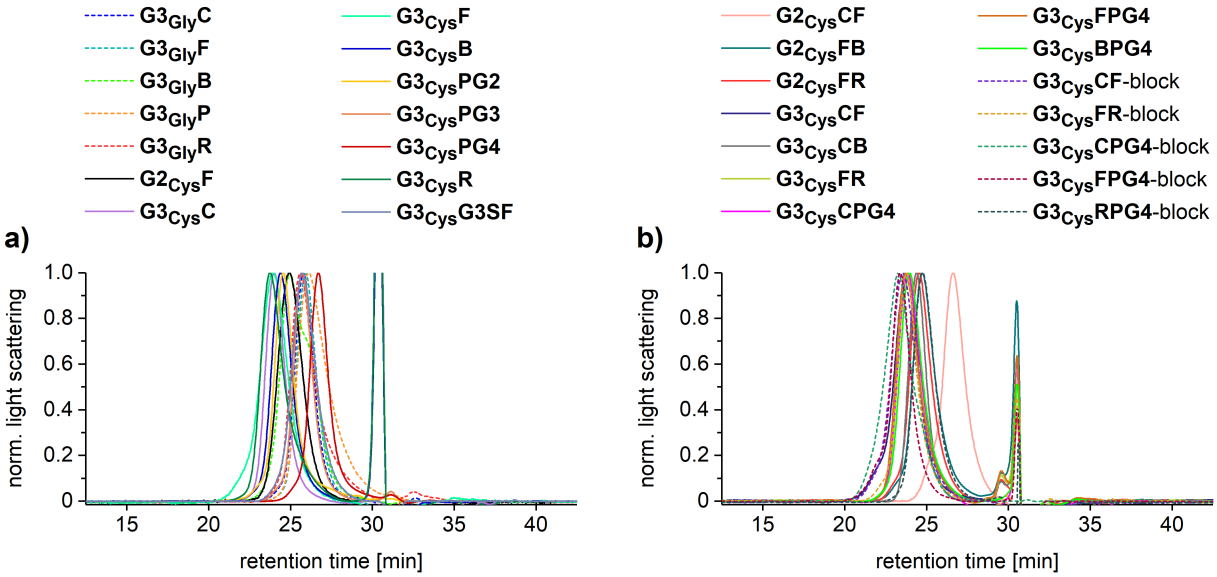


Figure S8. Normalized GPC traces of LDPs a) **Gx$_L$Y** and b) **Gx$_{Cys}$Y$_1$Y$_2$** (x = 2 or 3; L = Gly or Cys) and block copolymers **G3$_{Cys}$Y$_1$Y$_2$**-block measured at a concentration of 50 µM in DMF at 50 °C. The polymers are equipped with an acetal-protected G2 or G3 PG dendron and one or two fluorophores **Y** with **Y** = **C**oumarin, **F**luorescein, **B**ODIPY, **P**BI**Gx**, **P**BI, or **R**hodamine. The signal at a retention time of > 30 min belongs to the solvent peak used as reference.

Table S1. Molecular weights M_n and polydispersity indices PDIs of acetal-protected LDPs-**Gx$_L$Y** (x = 2 or 3; L = Gly or Cys) at a concentration of 50 µM in DMF at 50 °C.

LDP-**Gx$_L$Y**[a]	M_n (kDa)[b]	PDI[c]	LDP-**Gx$_L$Y$_1$Y$_2$**[d]	M_n (kDa)[b]	PDI[c]
G3$_{Gly}$C	30.0	1.07	**G2$_{Cys}$CF**	24.8	1.05
G3$_{Gly}$F	22.2	1.03	**G2$_{Cys}$FB**	n. d.	1.54
G3$_{Gly}$B	n. d.	1.15	**G2$_{Cys}$FR**	n. d.	1.08
G3$_{Gly}$P	n. d.	1.10	**G3$_{Cys}$CF**	34.6	1.03
G3$_{Gly}$R	n. d.	1.00	**G3$_{Cys}$CB**	n. d.	1.04
G2$_{Cys}$F	11.6	1.00	**G3$_{Cys}$FR**	n. d.	1.09
G3$_{Cys}$C	23.7	1.02	**G3$_{Cys}$CPG4**	n. d.	1.07
G3$_{Cys}$F	18.9	1.11	**G3$_{Cys}$FPG4**	n. d.	1.01
G3$_{Cys}$B	n. d.	1.08	**G3$_{Cys}$BPG4**	n. d.	1.03
G3$_{Cys}$PG2	n. d.	1.03	**G3$_{Cys}$CF**-block[e]	41.8	1.03
G3$_{Cys}$PG3	n. d.	1.01	**G3$_{Cys}$FR**-block[e]	n. d.	1.08
G3$_{Cys}$PG4	n. d.	1.01	**G3$_{Cys}$CPG4**-block[e]	n. d.	2.08
G3$_{Cys}$R	n. d.	1.09	**G3$_{Cys}$FPG4**-block[e]	n. d.	1.01
G3$_{Cys}$G3SF	33.5	1.03	**G3$_{Cys}$ RPG4**-block[e]	n. d.	1.09

[a] Feed ratio of LDPs-**Gx$_L$Y**: 20 **Gx$_L$**, 2 **Y** and **G3$_{Cys}$G3SF**: 20 **Gx$_{Cys}$**, 5 **G3S**, 2 **F**. [b] Molecular weight M_n of LDPs determined by GPC. [c] Polydispersity index PDI determined by GPC. [d] Feed ratio of LDPs-**Gx$_{Cys}$Y$_1$Y$_2$**: 20 **Gx$_{Cys}$**, 2 **Y$_1$**, 2 **Y$_2$**. [e] Monomers and feed ratios of block copolymers LDPs-**G3$_{Cys}$Y$_1$Y$_2$**-block are the same as the ones of LDPs-**G3$_{Cys}$Y$_1$Y$_2$** with a different monomer feed order: i) 5 **G3$_{Cys}$**, ii) 10 **G3$_{Cys}$**, 2 **Y$_1$**, 2 **Y$_2$**, iii) 5 **G3$_{Cys}$**. n. d. = not determinable, due to interference of the fluorophores' emission with the MALLS detector.

The entire series of acetal-protected LDPs reveals similar molecular weights M_n in the range of 11.6 to 34.6 kDa with narrow polydispersity indices (PDI) between 1.00 to 1.11 (with the exception of LDP-**G2$_{Cys}$FB** and **G3$_{Cys}$CPG4**-block) determined by GPC. Due to the interference of the emission of fluorophores **B**, **P**, and **R** with the MALLS detector,[3] the molecular weights of their corresponding LDPs could not be accurately determined. However, the similarity in retention time and distribution of all measured LDPs suggest similar molecular weights and, thus, comparable polymeric structures.

VII. Equations

Molar Absorption Coefficients $\boldsymbol{\varepsilon}$ were calculated employing the Lambert-Beer law.

$$\varepsilon = \frac{A}{c\,l} \tag{S1}$$

Variable A is the absorption of the sample, c the molar concentration in solution, and l the optical path length of the cuvette.

Relative Fluorescence Quantum Yields $\boldsymbol{\phi_f}$ (FQYs) were calculated using the equation:

$$\phi_f = \phi_r \frac{I}{I_r} \frac{A_r}{A} \frac{\eta^2}{\eta_r^2} \qquad \text{with} \quad \eta = \eta_r \tag{S2}$$

Variable η is the refractive index of the solvent, A the absorbance at the selected excitation wavelength λ_{ex}, I the integrated intensity under the emission curve with an area of ± 11 nm around the maximum for **C**, **F**, **B**, **R** and an area of - 4 nm / + 18 nm around the maximum for **PGx** and **P** due to the small stokes shift of PBIs (the reference was integrated accordingly), and ϕ_r the FQY of the reference, footnote r indicates reference. The reference fluorescein was measured at a concentration of 0.1 µM in 0.1 M aqueous NaOH solution (ϕ_r = 0.95) at an excitation wavelength of 492 nm.[4-6]

Brightness $\boldsymbol{B}$ was calculated as one-thousandth of the product of the molar absorption coefficient and FQY with the following formula:

$$B = \frac{\varepsilon\,\phi_f}{1000} \tag{S3}$$

The variable ε is the molar absorption coefficient and ϕ_f the fluorescence quantum yield.

Cell Viability was calculated using the following formula:

$$cell\ viability = \frac{A_{sample} - A_{blank}}{A_{control} - A_{blank}} \tag{S4}$$

Variable A is the absorption of the compound. Footnote *sample* represents the cells treated with polymer, *control* the cells incubated with cell medium, and *blank* the pure cell medium without cells.

VIII. Photophysical Characterization

Absorption and Emission Properties

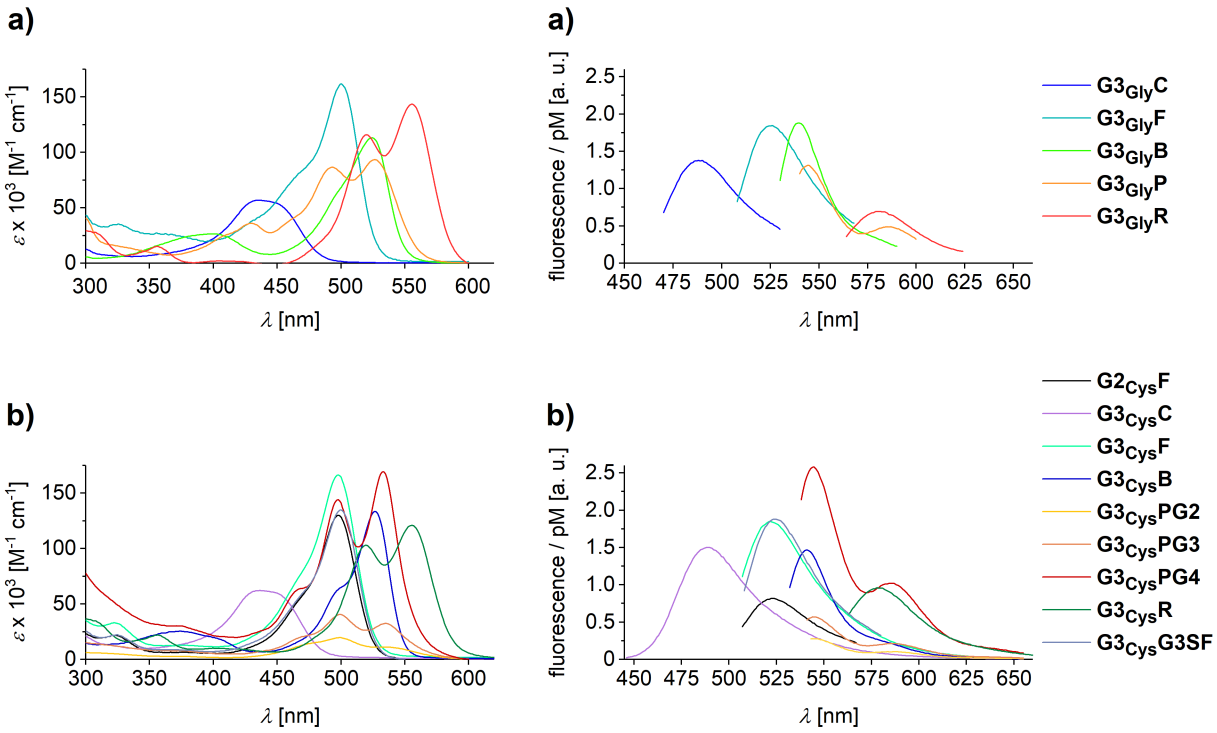


Figure S9. Absorption (left) and emission (right) spectra for LDPs a) **G3$_{Gly}$Y** and b) **Gx$_{Cys}$Y** (x = 2 or 3) measured at a concentration of c_{abs} = 1.5 µM and 10-fold dilution c_{flu} = 0.15 µM in 0.1 M PB (pH 7.4) at 20 °C. The polymer backbone is equipped with a 3rd-generation dendron and a fluorophore **Y** with **Y** = **C**oumarin, **F**luorescein**,** **B**ODIPY, **P**BI**Gx** (x = 2, 3, or 4), **P**BI, or **R**hodamine. Excitation wavelengths λ_{ex} (**Y**) = 444 nm (**C**), 498 nm (**F**), 525 nm (**B**), 527 nm (**P**), 535 nm (**PGx**), 552 nm (**R**).

The absorption spectra of LDPs exhibit an absorption band ranging from 400 to 600 nm. Particularly interesting are the absorption spectra of the **P**BI**Gx** species, LDPs-**G3$_{Cys}$PGx**. In the case of **G3$_{Cys}$PG4**, the absorption maximum is located at the long wavelength peak indicating that the molecule exists in a monomeric state. In the case of **G3$_{Cys}$PG2** and **G3$_{Cys}$PG3**, the maximum undergoes a pronounced blue-shift due to the formation of aggregates. The aggregation tendency is also reflected by decreased molar absorption coefficients and FQYs for LDPs with lower dendronized **P**BI**Gx** monomers, Gx ≤ 3 (see Table 2 and Figure S10). As shown in Figure S9, the emission of the fluorophore-conjugated LDPs covers a spectral range from 450 nm to 650 nm.

Table S2. Photophysical properties of LDPs-**Gx$_L$Y** (x = 2 or 3; L = Gly or Cys) at a concentration of 1.5 µM for absorption and 0.15 µM for emission measurements in 0.1 M PB (pH 7.4) at 20 °C.

LDP-**Gx$_L$Y**[a]	λ_{abs} (nm)	λ_{em} (nm)	ε[b] (M^{-1} cm^{-1})	Φ_f[c] (%)	B[d] (M^{-1} cm^{-1})	Stokes shift (nm)
G3$_{Gly}$C	435	489	55 800	40	22	54
G3$_{Gly}$F	500	526	159 000	51	81	26
G3$_{Gly}$B	524	539	113 000	53	60	15
G3$_{Gly}$P	527	544	93 400	4	3.7	17
G3$_{Gly}$R	555	579	140 000	9	13	24
G2$_{Cys}$F	498	524	130 000	61	79	26
G3$_{Cys}$C	436	489	61 200	40	25	53
G3$_{Cys}$F	497	522	166 000	46	74	25
G3$_{Cys}$B	527	541	132 000	40	53	14
G3$_{Cys}$PG2	498	544	11 100	7.8	0.86	46
G3$_{Cys}$PG3	499	544	32 600	14	4.5	45
G3$_{Cys}$PG4	533	544	167 000	23	38	11
G3$_{Cys}$R	555	578	119 000	8	9.7	23
G3$_{Cys}$G3SF	499	524	131 000	67	88	25

[a] Feed ratio of LDP-**Gx$_L$Y**: 20 **Gx$_L$**, 2 **Y** and **G3$_{Cys}$G3SF**: 20 **Gx$_{Cys}$**, 5 **G3S**, 2 **F**. [b] Molar absorption coefficient ε, Equation S1. [c] Fluorescence quantum yield Φ_f, Equation S2. [d] Brightness B, Equation S3.

FQY and Brightness

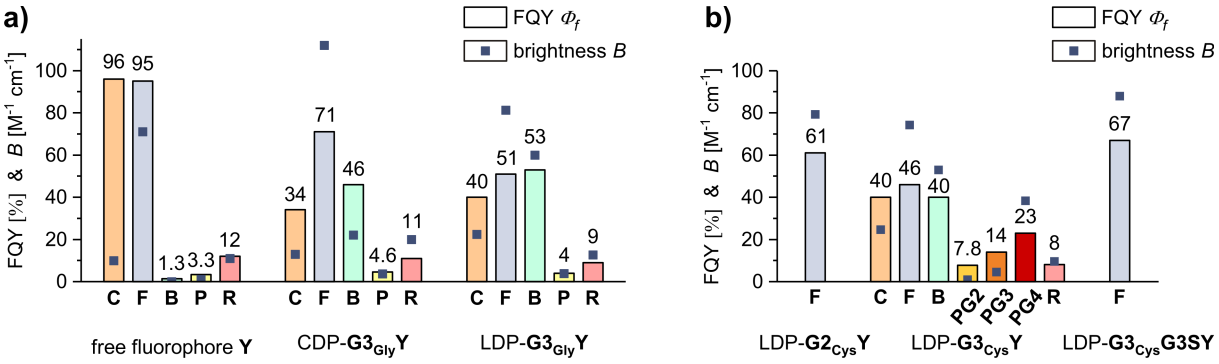


Figure S10. FQYs Φ_f (columns) and brightness B (symbols) of a) free fluorophores, CDPs, and LDPs (see Table 1), and b) LDPs (see Table S2) measured at a concentration of 0.15 µM in 0.1 M PB (pH 7.4) at 20 °C. Fluorophores **Y** = **C**oumarin, **F**luorescein**, B**ODIPY, **P**BI**Gx** (x = 2, 3, or 4), **P**BI, or and **R**hodamine.

As shown in Figure S10, the FQYs and brightness of LDPs with dendron monomer **G3$_{Gly}$** behave similarly to LDPs with dendron monomer **Gx$_{Cys}$** (x = 2 or 3). In case of coumarin, the molar absorption coefficients ε, FQYs Φ_f, and brightness B of LDP-**G3$_{Gly}$C** and LDP-**G3$_{Cys}$C** are higher than those of CDP-**G3$_{Gly}$C**. In addition to the possibility that the short-chain LDPs are sufficient to solubilize and

stabilize coumarin, we hypothesize that the elimination of 1st-generation Grubbs catalyst used in the CDP synthesis helps to reduce catalyst-induced interference with the fluorophore. In case of PBI, two different kinds of PBI monomers were utilized in the polyol synthesis. While the FQYs of bay-substituted **P**BI remain consistently lower than 5%, the FQY significantly improved when imide-substituted dendronized **P**BI**Gx** was incorporated into the LDP platform. Although the FQY of LDP-**G3$_{Cys}$PG4** is still nowhere close to 1, it is about five times higher than the one of previously reported CDP-**G3$_{Gly}$P**.[3] As predicted earlier, chemical modifications on PBI help improving the dyes' FQY. Considering that the new LDPs could be prepared more efficiently than CDPs, we anticipate LDPs could be more widely used in the future than CDPs.

Photostability Study

Protocol for Photostability Study. Free fluorophores and fluorescent nanoparticles were dissolved in 0.1 M PB (pH = 7.4) to give 0.15 μM solutions in 1 mL cuvettes. The cuvettes were placed in a black box equipped with a power-adjustable LED with 470 nm. The distance between the vials and LED was set at 20 cm. After the LED was turned on (t = 0), the fluorescence intensity of the solution was examined every 30 min by a fluorescence spectrometer for 4 h.

Table S3. Absolute fluorescence intensities / pM (a. u.) during the photostability study of the free fluorophores, CDPs, and LDPs at a concentration of 0.15 μM in 0.1 M PB (pH 7.4), irradiated at λ = 470 nm at 20 °C.

	Compound	0 h	1 h	2 h	3 h	4 h
a)	**C**oumarin	0.29	0.099	0.044	0.025	0.016
	CDP-**G3$_{Gly}$C**	0.37	0.33	0.27	0.24	0.21
	LDP-**G3$_{Gly}$C**	0.63	0.57	0.49	0.43	0.37
b)	**F**luorescein	1.37	0.61	0.28	0.12	0.05
	CDP-**G3$_{Gly}$F**	2.74	1.95	1.40	1.04	0.77
	LDP-**G3$_{Gly}$F**	1.90	1.30	0.96	0.74	0.59
c)	**B**ODIPY	0.001	0.0009	0.0006	0.0008	0.0004
	CDP-**G3$_{Gly}$B**	0.55	0.52	0.47	0.43	0.39
	LDP-**G3$_{Gly}$B**	1.73	1.31	1.09	0.93	0.81
d)	**P**BI	0.017	0.0009	0.0006	0.0006	0.0006
	CDP-**G3$_{Gly}$P**	0.072	0.048	0.040	0.039	0.036
	LDP-**G3$_{Gly}$P**	0.075	0.063	0.064	0.063	0.063
e)	**R**hodamine	0.15	0.13	0.15	0.13	0.13
	CDP-**G3$_{Gly}$R**	0.46	0.47	0.46	0.46	0.46
	LDP-**G3$_{Gly}$R**	0.28	0.27	0.26	0.27	0.26
f)	LDP-**G3$_{Gly}$F**	1.90	1.30	0.96	0.74	0.59
	LDP-**G3$_{Cys}$G3SF**	2.07	1.76	1.56	1.42	1.26

The normalized fluorescence intensities of the tested compounds are shown in Figure 1a-e and Figure 2b. The absolute fluorescence intensities are listed in Table S3 and shown in Figure S11. The improvement of the photostability mentioned in the manuscript was calculated by the ratio of the absolute fluorescent intensities of the respective compounds after the final time of 4 h of irradiation.

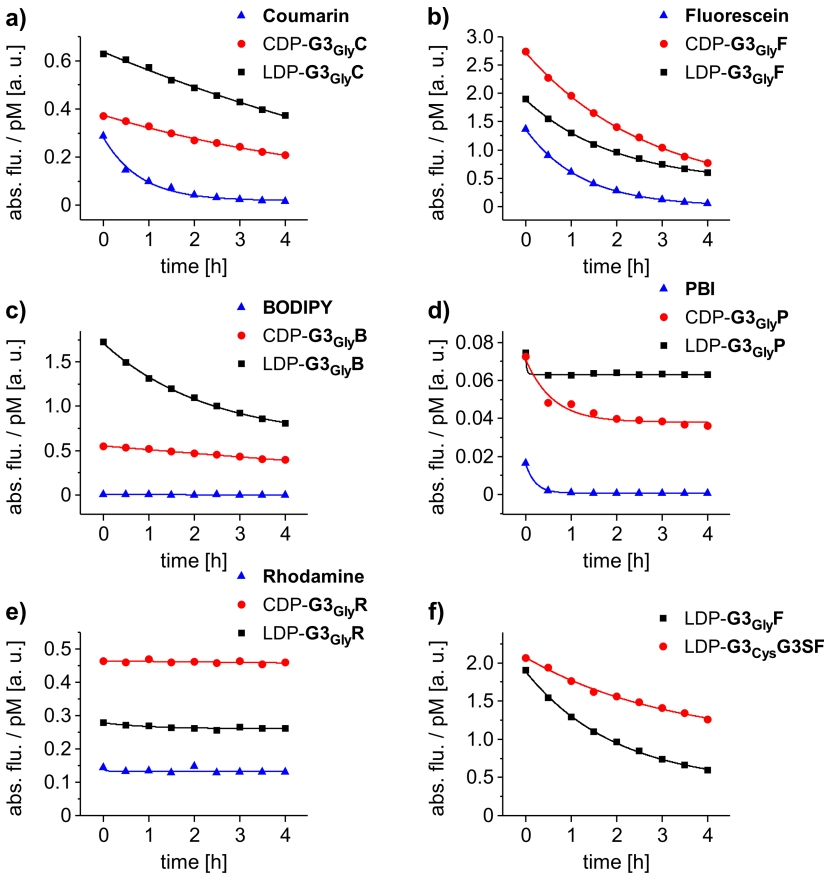


Figure S11. a) - e) Absolute fluorescence intensities over time during the photostability study with free fluorophores, CDPs, and LDPs. PB solutions (0.1 M, pH 7.4) of the corresponding compounds (0.15 µM) were irradiated with blue light at 470 nm over 4 h while the fluorescence intensities were measured. f) Absolute fluorescence intensities of fluorescein-conjugated LDP-**G3$_{Cys}$F** and multi-thioether-containing LDP-**G3$_{Cys}$G3SF** were measured at the same conditions.

FRET Study

Different combinations of two fluorophores (**CF**, **CB**, **FB**, **FPG4**, etc.) were incorporated into **Gx$_{Cys}$** (x = 2 or 3) equipped LDPs. We hypothesized that the excitation of the donor fluorophore can lead to an excitation of the acceptor fluorophore when the two fluorophores incorporated feature strong dipole-dipole interactions. The FRET efficiency can be estimated by the ratio between the maximum emission intensity of the acceptor and donor fluorophore ($I_{acceptor}$ / I_{donor}) with a higher value indicating a higher FRET efficiency. As shown in Figure S12 and listed in Table S4, FRET was observed for different combinations of two fluorophores. Several of them e.g. LDP-**G2$_{Cys}$CF**, LDP-**G2$_{Cys}$FB**, LDP-**G3$_{Cys}$CB**, and LDP-**G3$_{Cys}$CF**-block gave $I_{acceptor}$ / I_{donor} ratios ≥ 1. The corresponding $I_{acceptor}$ / I_{donor} ratios of the entire FRET-polymer series are depicted in Figure S13. When looking at the ratios of the FRET pair of **C**oumarin and **F**luorescein (depicted in Figure 3, S12, and S13), globular CDP afforded more efficient FRET than its linear precursors pCDPs, presumably because of its compact structure caused by cross-linking (for polymer structures see Figure S6). To further improve the FRET efficiency in LDPs, we tried polymerization with a lower 2nd generation dendron monomer and block copolymerization with a

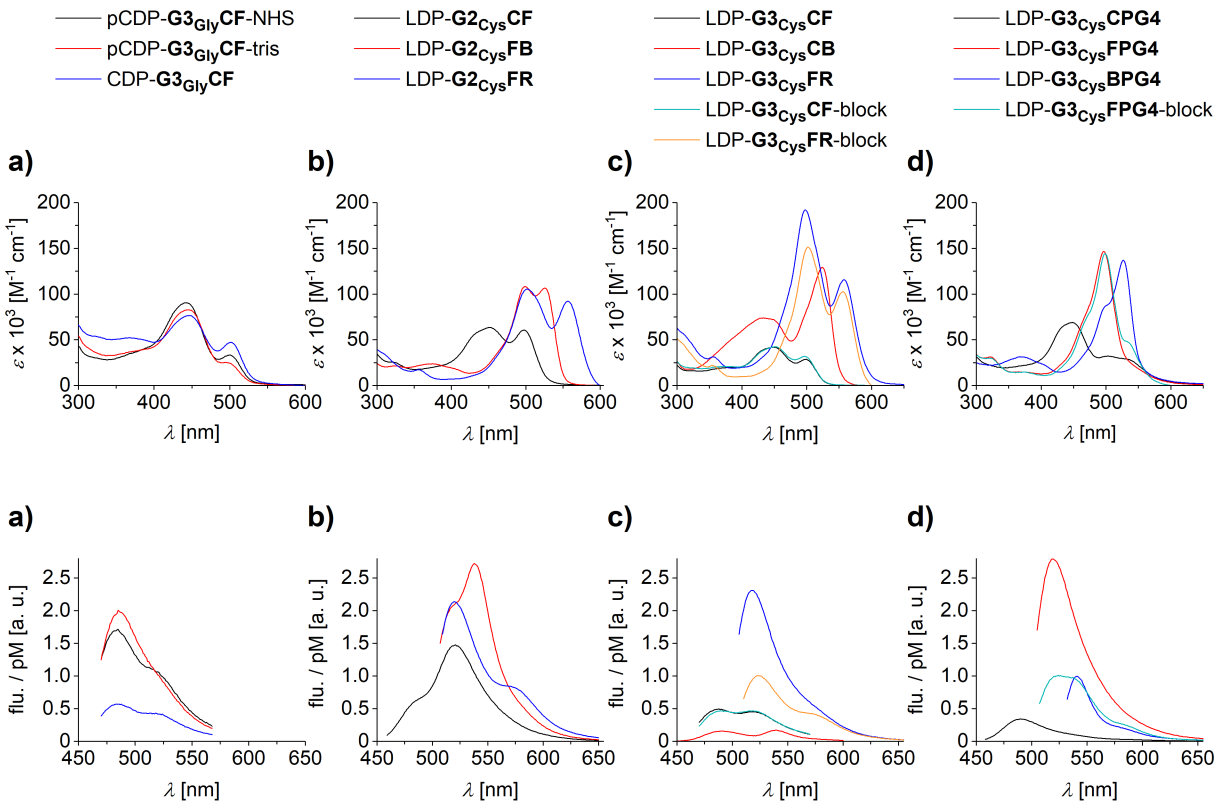


Figure S12. Absorption (top) and emission (bottom) spectra of different FRET polymers measured at a concentration of c_{abs} = 5 µM and 10-fold dilution c_{flu} = 0.5 µM in 0.1 M PB (pH 7.4) at 20 °C. a) linear CDP-precursors pCDP-**G3$_{Gly}$Y$_1$Y$_2$-Z** with **Z** = **NHS** or **tris** function and globular CDP-**G3$_{Gly}$CF**. b) - d) LDPs-**Gx$_{Cys}$Y$_1$Y$_2$** and block copolymers LDPs-**Gx$_{Cys}$Y$_1$Y$_2$**-block. Excitation wavelengths λ_{ex} (**Y$_1$Y$_2$**) = 444 nm (**CY**), 492 nm (**FY**), 525 nm (**BY**), 535 nm (**PY**), and 552 nm (**RY**).

3rd generation dendron monomer. The block copolymerization was performed with the same feed ratio of monomers (20 **G3$_{Cys}$**, 2 **C**, and 2 **F**) but different feed order (see Table S4) so that **C** and **F** were supposed to be confined in a more compact polymer environment. The $I_{acceptor}$ / I_{donor} ratios show that the most efficient FRET was observed for 2nd-generation dendronized LDPs-**G2$_{cys}$**. Among the different G2-LDPs, LDP-**G2$_{Cys}$CF** afforded the largest stokes shift.

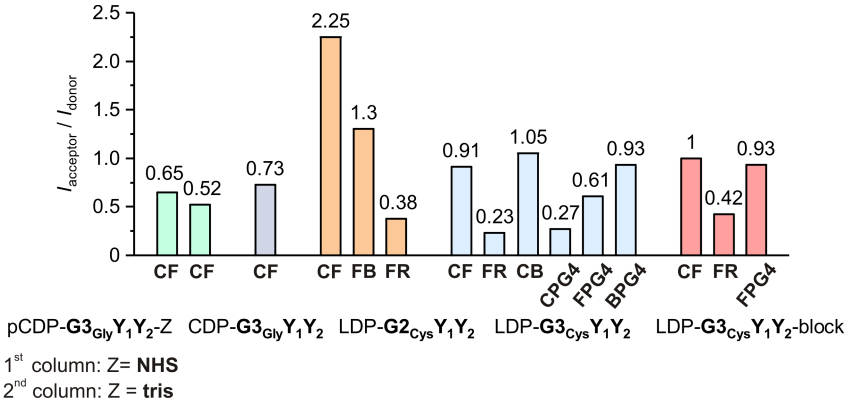


Figure S13. FRET efficiency of linear CDP precursors pCDPs, globular CDPs, and short-chain LDPs measured at 0.5 µM in 0.1 M PB (pH 7.4) at 20 °C. The FRET efficiency was determined by the ratio of the maximum emission intensity ($I_{acceptor}$ / I_{donor}) of the acceptor and donor fluorophores incorporated into different FRET polymers.

Table S4. Photophysical properties of CDPs-**G3$_{Gly}$Y$_1$Y$_2$** and LDPs-**Gx$_{Cys}$Y$_1$Y$_2$** (x = 2 or 3) at a concentration of 5 µM for absorption and 0.5 µM for emission measurements in 0.1 M PB (pH 7.4) at 20 °C.

Gx$_L$Y$_1$Y$_2$	λ_{abs} (nm)	λ_{em} (nm)	ε^d (M^{-1} cm^{-1})	Stokes shift (nm)	$I_{acceptor}$ / I_{donor} [e]
pCDP-**G3$_{Gly}$CF**-NHS[a]	443	485	90 500	42	0.65
pCDP-**G3$_{Gly}$CF**-tris[a]	445	485	82 800	40	0.52
CDP-**G3$_{Gly}$CF**[a]	446	483	76 400	37	0.73
LDP-**G2$_{Cys}$CF**[b]	451	521	63 400	70	2.25
LDP-**G2$_{Cys}$FB**[b]	499	538	104 500	39	1.30
LDP-**G2$_{Cys}$FR**[b]	502	520	105 500	18	0.38
LDP-**G3$_{Cys}$CF**[b]	449	488	41 800	39	0.91
LDP-**G3$_{Cys}$CB**[b]	524	539	129 100	15	1.05
LDP-**G3$_{Cys}$FR**[b]	498	518	192 000	20	0.23
LDP-**G3$_{Cys}$CPG4**[b]	448	490	69 000	42	0.27
LDP-**G3$_{Cys}$FPG4**[b]	496	519	146 400	23	0.61
LDP-**G3$_{Cys}$BPG4**[b]	523	541	136 800	18	0.93
LDP-**G3$_{Cys}$CF**-block[c]	451	518	42 400	67	1.00
LDP-**G3$_{Cys}$FR**-block[c]	502	524	151 400	22	0.42
LDP-**G3$_{Cys}$FPG4**-block[c]	499	524	144 300	25	0.93

[a] Feed ratio of linear pCDPs-**G3$_{Gly}$Y$_1$Y$_2$-Z** with **Z** = **NHS** or **tris** function and globular CDP-**G3$_{Gly}$CF**: 50 **G3$_{Gly}$**, 25 **NHS** or **tris**, 2 **C**, 2 **F**. [b] Feed ratios of LDPs-**Gx$_{Cys}$Y$_1$Y$_2$**: 20 **Gx$_{Cys}$**, 2 **Y$_1$**, 2 **Y$_2$**. [c] Monomers and feed ratios of block copolymers LDPs-**G3$_{Cys}$Y$_1$Y$_2$**-block are the same as the ones of LDPs-**G3$_{Cys}$Y$_1$Y$_2$** with a different monomer feed order: i) 5 **G3$_{Cys}$**, ii) 10 **G3$_{Cys}$**, 2 **Y$_1$**, 2 **Y$_2$**, iii) 5 **G3$_{Cys}$**. [d] Molar absorption coefficient ε, Equation S1. [e] Ratio between the maximum emission intensity I of the acceptor and donor fluorophore incorporated into the polymer.

IX. *In vitro* Cell Studies

Cytotoxicity

Protocol for Sulforhodamine B (SRB) Assay. HeLa cells were seeded on 96-well plates at 1×10^4 cells/well and cultured in serum-containing media for 24 h. The medium was replaced with fresh DMEM containing serum (100 µL/well), into which the polymers were added at the final concentrations of 10, 20, 50, and 100 µg/mL, respectively. After incubation at 37 °C for 4 h, the medium was changed to fresh serum-containing DMEM and the cells were further cultured for 24 h before a viability assessment by SRB assay. Results are represented as a percentage viability of control cells that did not receive polymer treatment according to Equation S4.

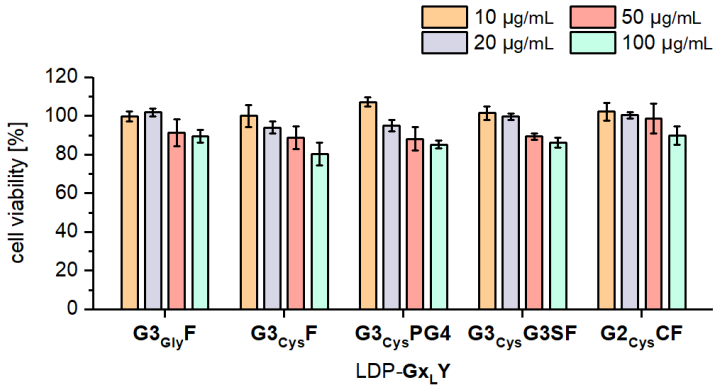


Figure S14. SRB cytotoxicity study of LDP at indicated concentrations on HeLa cells after 24 h of incubation. The bars represent the cell viability (%) of three independent measurements ± standard deviation. LDPs-**Gx$_L$Y** (x = 2 or 3; L = Gly or Cys) with fluorophores **Y** = **C**oumarin, **F**luorescein, and **P**BI**Gx**.

The cytotoxicity study via sulforhodamine B (SRB) assay showed good cell viability at the tested concentration range between 10 - 100 µg/mL after 4 h of incubation. At low concentrations ≤ 20 µg/mL, the cells even proceeded in cell division indicating a good biocompatibility.

Cellular Uptake

Protocol for live-cell imaging. HeLa cells cultured on coverslips in a 6-well plate were incubated with polymers in DMEM (2 mL) at a concentration of 7.5 µg/mL per well. After an incubation for 4 h, cells were washed three times with PBS, and stained with Hoechst 33258 (2 µg/mL) before an observation by confocal laser scanning microscopy (CLSM, LSM700, Zeiss, Germany).

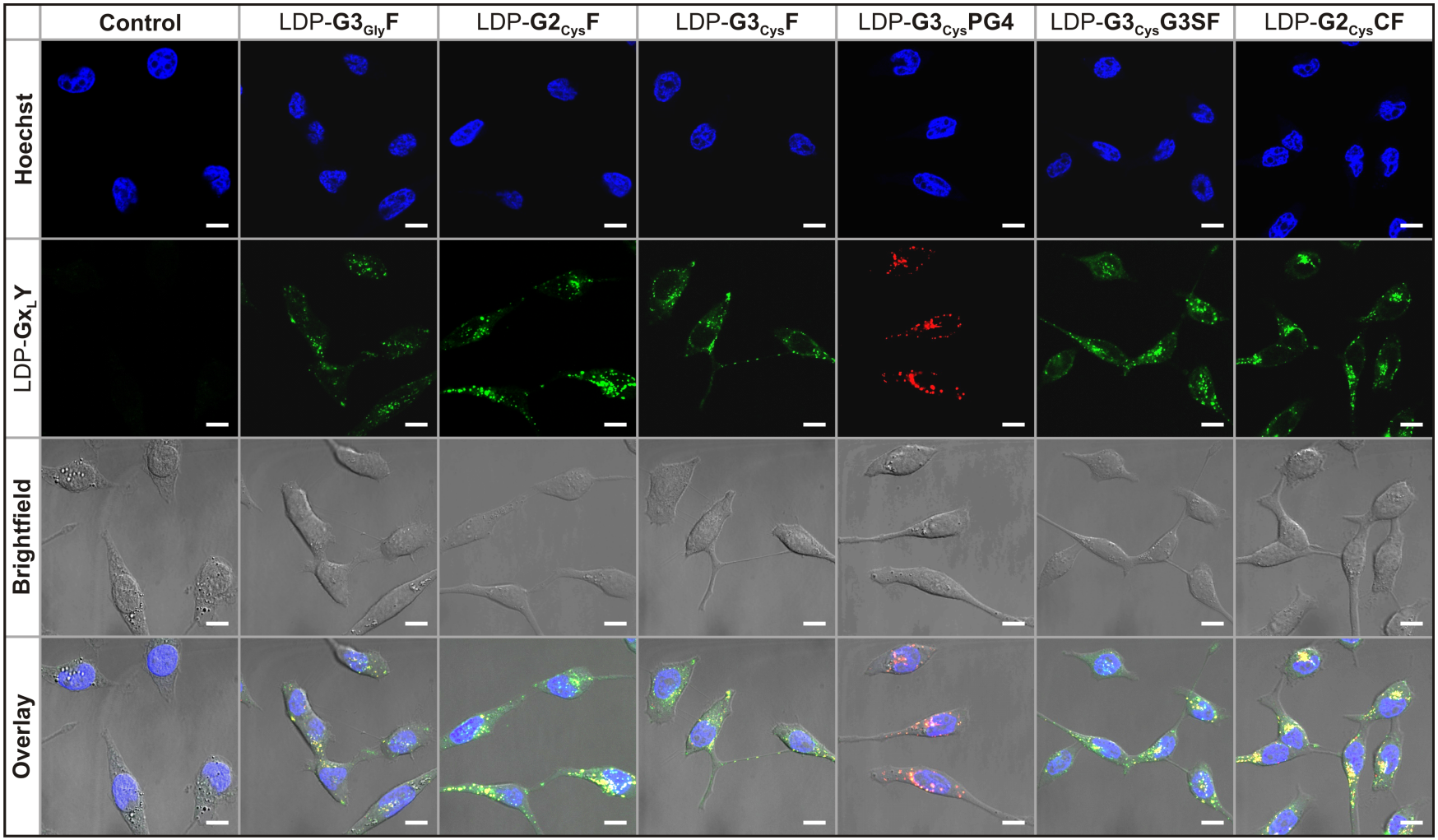


Figure S15. *In vitro* cellular uptake studies via live-cell microscopy of LDPs at a concentration of 7.5 µg/mL into HeLa cells after 4 h of incubation. First row: cell nuclei stained with Hoechst 33342 (blue). Second row: fluorescence from LDPs-**Gx$_L$Y** (x = 2 or 3; L = Gly or Cys) with **Y** = **C**oumarin (green), **F**luorescein (green), and **P**B**I**-**Gx** (red). Third row: brightfield. Fourth row: overlay of cell nuclei, fluorescence from LDPs, and brightfield. Scale bar: 10 µm. The control represents the untreated cell population.

X. References

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