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Supporting Information

Dendritic Polyglycerolsulfate-SS-Poly(ester amide) Micelles for the Systemic Delivery of Docetaxel: Pushing the Limits of Stability through the Insertion of π-π Interactions

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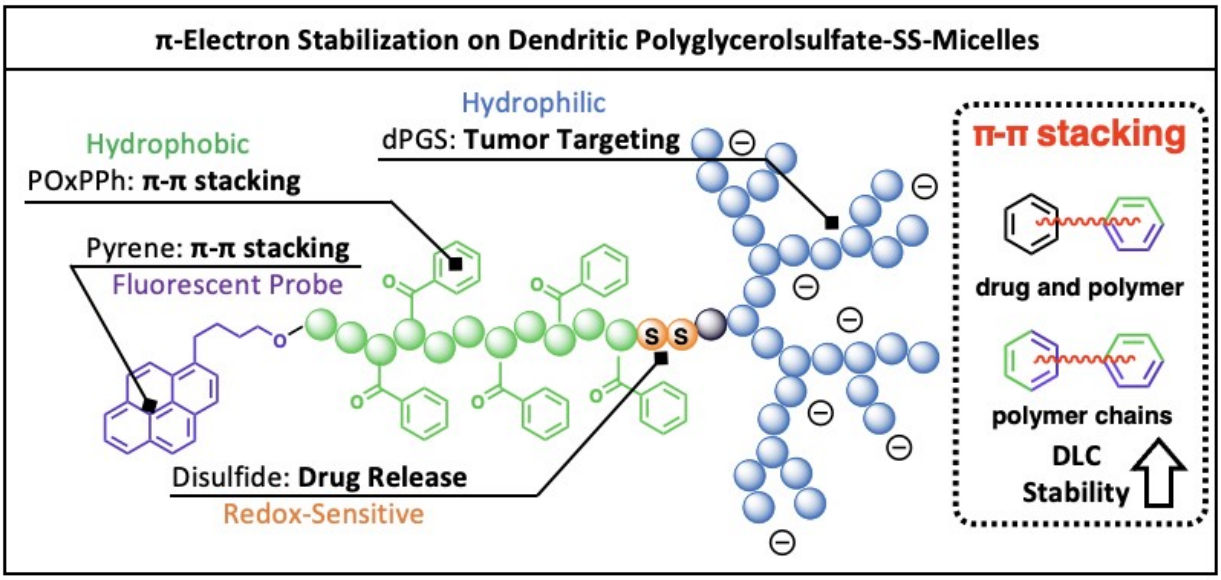
Graphical Abstract:



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Materials and Methods

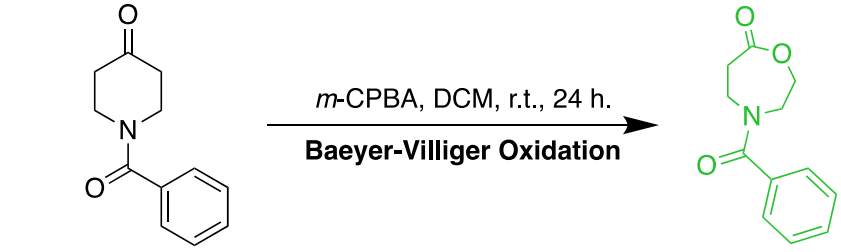
The monomers caprolactone and glycidol were purchased from Acros Organics, purified by distillation, and stored over molecular sieves. 1-Benzoyl-4-piperidone and *m*-CPBA were bought from Sigma-Aldrich. Docetaxel was ordered from MedChemExpress and stored in a freezer. Dialysis membranes were commercially available at Sigma-Aldrich. Deuterated solvents were obtained from Deutero. All the other chemicals were purchased from Sigma-Aldrich, TCI, or Acros Organics and used without purification. ^{1}H NMR spectra and ^{1}H-DOSY NMR spectra were recorded on a Bruker ECX 500 spectrometer operating at 500 MHz using $CDCl_3$, DMF-d_7, CD_3OD-d_4, or D_2O as a solvent. For ^{13}C NMR spectra, the measurements were performed on a Bruker AVANCE700 operating at 700 MHz using $CDCl_3$, DMF-d_7, CD_3OD-d_4, or D_2O as a solvent. The chemical shifts were calibrated against the residual solvent signal.

The molecular weight and polydispersity of the polymers were determined by a Waters 1515 gel permeation chromatography (GPC) instrument equipped with two linear PLgel columns (Mixed-C) following a guard column and a differential refractive index detector. The measurements were performed using tetrahydrofuran (THF; hydrophobic segments), water (hydrophilic components), or dimethylformamide (DMF; amphiphilic segments) as the eluent at a flow rate of 1.0 mL/min at 30 °C and a series of narrow polystyrene standards (THF), Pullulan (water), and poly(methyl methacrylate) (PMMA; DMF) for the calibration of the columns.

Elemental analysis was performed with a VARIO EL III (Elementar). IR spectra were recorded with a Nicolet AVATAR 320 FT-IR 5 SXC (Thermo Fisher Scientific, Waltham, MA) with a deuterated triglycine sulfate (DTGS) detector from 4000 to 650 $cm^{-1.}$ Sample measurements were performed by dropping a solution of the compound and letting the solvent evaporate for a few seconds.

All cell experiments were conducted according to German genetic engineering laws and German biosafety guidelines in the laboratory (safety level 1). According to the manufacturer's instructions, cell viability was determined using a Cell Counting Kit (Hycultec, HY-K0301). A549, HeLa, and McF7 cells were obtained from Leibniz-Institut DSMZ - Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH and cultured in DMEM supplemented with 10% (v/v) FBS, 100 U/mL penicillin and 100 µg/mL streptomycin. A549, HeLa, and McF7 cells were seeded in a 96-well plate at a density of 5 x 104 cells/mL in 90µl DMEM Medium per well overnight at 37°C and 5% CO2. 10 µl of the sample (solved in deionized water) were added in serial dilutions including positive (1% SDS) and negative controls (Medium, H_2O) and incubated for another 48 h at 37°C and 5% CO2.For background subtraction, also wells containing no cells but only sample were used. After 48h incubation, the CCK8 solution was added (10µl/well) and absorbance (450nm/650nm) was measured after approximately 3h incubation of the dye using a Tecan plate reader (Infinite pro200, TECAN-reader Tecan Group Ltd.) Measurements were performed in triplicates and repeated three times. The cell viability was calculated by setting the non-treated control to 100% and the non-cell control to 0% after subtracting the background signal using the Excel software.

Synthesis of 4-benzoyl-1,4-oxazepan-7-one (OxPPh)



1-Benzoyl-4-piperidone

4-benzoyl-1,4-oxazepan-7-one (OxPPh)

The monomer was synthesized following a slightly modified protocol.[1] Briefly, In a 250 mL round bottom flask equipped with a stir bar and a septum, 1-benzoyl-4-piperidone (4.23 g, 20.8 mmol, 1 eq.) was dissolved in 50 mL DCM. Then, *m*-CPBA (6.18 g, 30.9 mmol, 1.5 eq.) was dissolved in 70 mL of DCM and dropwise added to the stirred solution of 1- benzoyl-4-piperidone under ice-cooling. The ice bath was removed, and the mixture was stirred at room temperature for 24 hours. The solution was extracted with sodium thiosulfate, sodium bicarbonate, and sodium chloride saturated solutions (each 100 mL, 3 times). Afterward, the organic layer was collected and dried using magnesium sulfate. The solution was filtered, and the solvent was removed. Next, the received compound was purified by column chromatography on silica (pentane: ethyl acetate, 4:1). The obtained white product (1.42 g, 6.48 mmol, 31.2%) was dried under vacuum and characterized using ^{1}H NMR and mass spectrometry.

^{1}H NMR (500 MHz, CD_3OD, δ, ppm) = 7.60-7.40 (m, 5H), 4.57-4.34 (m, 2H), 4.18-3.89 (m, 2H), 3.86-3.62 (m, 2H), 3.09-2.86 (m, 2H).

MS (EI) m/z: $[M]^+$ Calculated for $C_{12}H_{13}NO_3$ 219.090; Found 219.087.

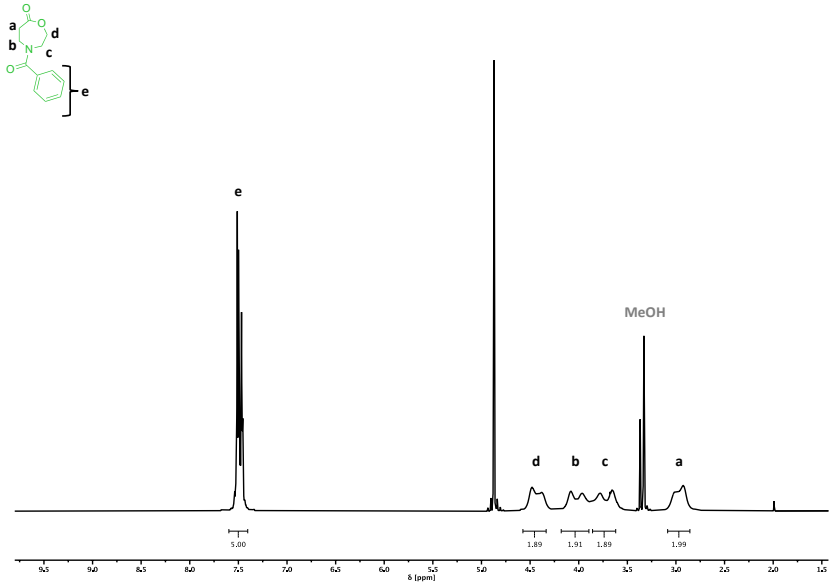
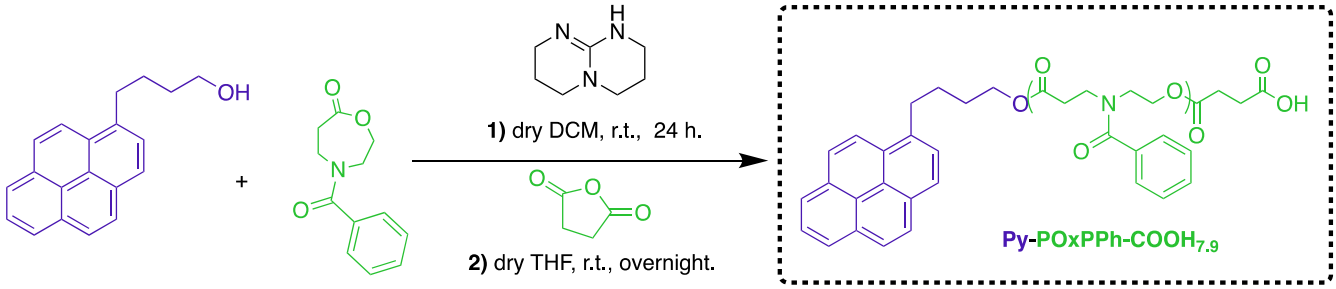


Figure S 1. *^{1}H NMR spectrum (500 MHz) of OxPPh in CD_3OD.*

Synthesis of Py-POxPPh-COOH



To synthesize Py-POxPPh-COOH, in a flame-dried Schlenk flask, pyrenebutanol (38 mg, 0.14 mmol) and TBD (23 mg, 0.17 mmol) were placed, and under stirring, a 1M solution of OxPPh (3.2 mL, 3.2 mmol) in dry DCM was added (1M). The reaction solution was stirred for 24 hours at room temperature. The reaction was quenched with a 0.5M solution of succinic anhydride (0.68 mL, 0.34 mmol) in dry THF. After 24 hours at room temperature, the solution was concentrated, and the product was purified by precipitation into cold methanol three times. The product was obtained as a white crystalline substance (711.6 mg). For characterization, ^{1}H and ^{13}C NMR, and GPC were performed.

^{1}H NMR (500 MHz, $CDCl_3$, δ, ppm) = 8.23-7.84 (m, 9H), 7.60-7.08 (m, 182H), 4.44-3.94 (m, 73H), 3.84-3.42 (m, 145H), 2.85-2.34 (m, 4H, 74H).

Table S 1. *Organocatalytic Ring-Opening Polymerization of OxPPh mediated by TBD.*

Monomer (M)	Catalyst (C)	$[M]_0/[Pyrenebutanol]_0/[C]_0$	Time [h]	$conv.^{NMR}$ (%)	$M_n^{theo.}$ (kDa)	M_n^{NMR} (kDa)	$M_n^{GPC,\ THF}$ (kDa)	$Đ$
OxPPh	TBD	36/1/1.2	24	99.4	8	7.9	3.9	1.3

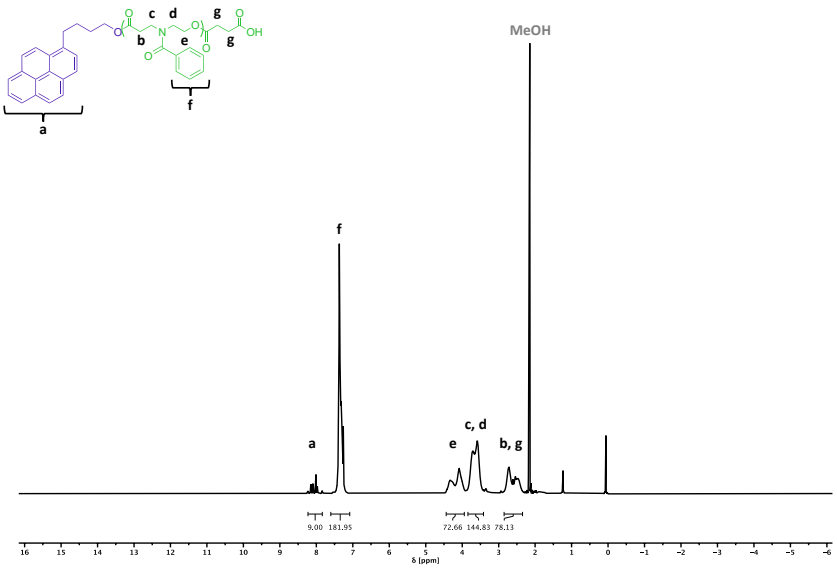


Figure S 2. *^{1}H NMR spectrum (500 MHz) of Py-POxPPh-COOH in $CDCl_3$.*

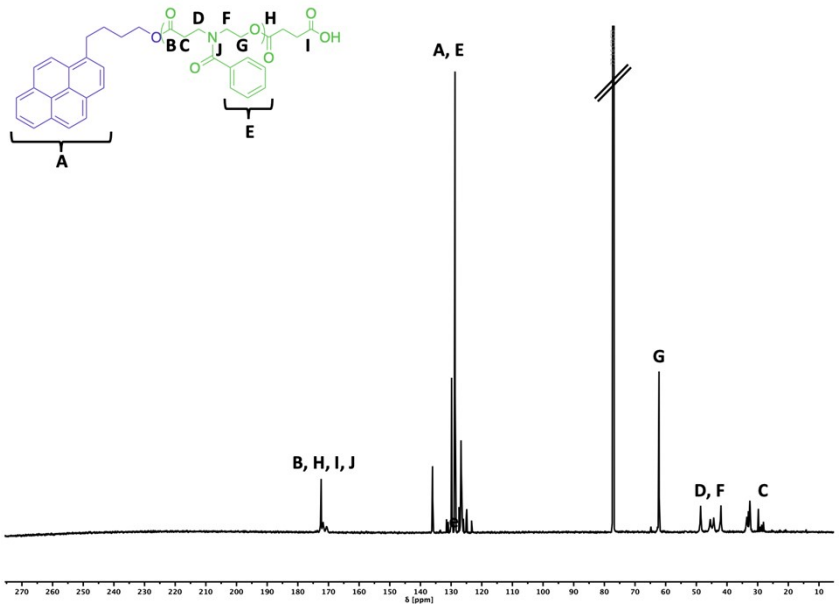
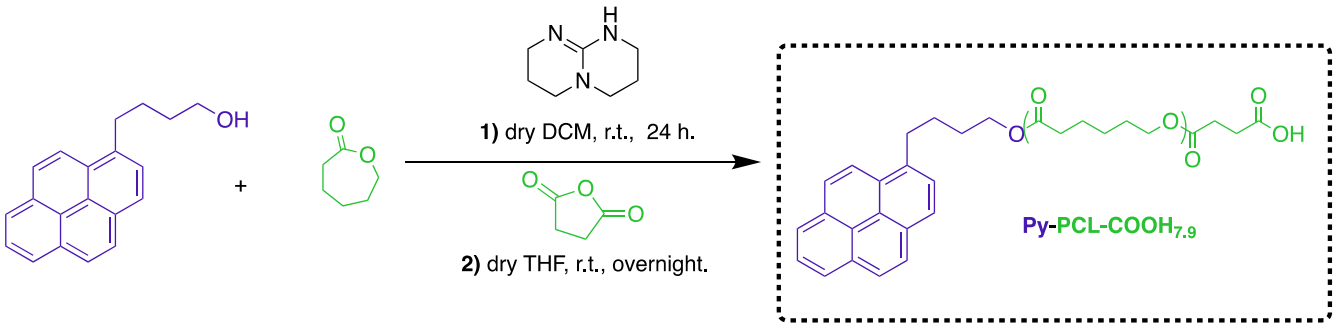


***Figure S 3**. ^{13}C NMR spectrum (700 MHz) of Py-POxPPh-COOH in $CDCl_3$.*

Synthesis of Py-PCL-COOH



To synthesize Py-PCL-COOH, in a flame-dried Schlenk flask, pyrenebutanol (38 mg, 0.14 mmol) and TBD (23 mg, 0.17 mmol) were placed, and under stirring, CL (8.8 mL, 8.8 mmol) was added (plus dry DCM 8.8 mL). The reaction solution was stirred for 24 hours at room temperature. The reaction was quenched with a 0.5M solution of succinic anhydride (0.68 mL, 0.34 mmol) in dry THF. After 24 hours at room temperature, the solution was concentrated, and the product was purified by precipitation into cold methanol three times. The product was obtained as a white crystalline substance (711.6 mg). For characterization, ^{1}H and ^{13}C NMR, and GPC were performed.

^{1}H NMR (500 MHz, $CDCl_3$, δ, ppm) = 8.21-7.82 (m, 9H), 4.22-3.87 (m, 138H), 2.55-2.47 (m, 4H), 2.39-2.18 (m, 138H), 1.78-1.47 (m, 280H), 1.45-1.25 (m, 137H).

***Table S 2.** Organocatalytic Ring-Opening Polymerization of Caprolactone mediated by TBD.*

Monomer (M)	Catalyst (C)	$[M]_0/[Pyrenebutanol]_0/[C]_0$	Time [h]	$conv.^{NMR}$ (%)	$M_n^{theo.}$ (kDa)	M_n^{NMR} (kDa)	$M_n^{GPC,\ THF}$ (kDa)	$Đ$
Caprolactone	TBD	70/1/1.2	24	99.3	8	7.9	6.9	1.1

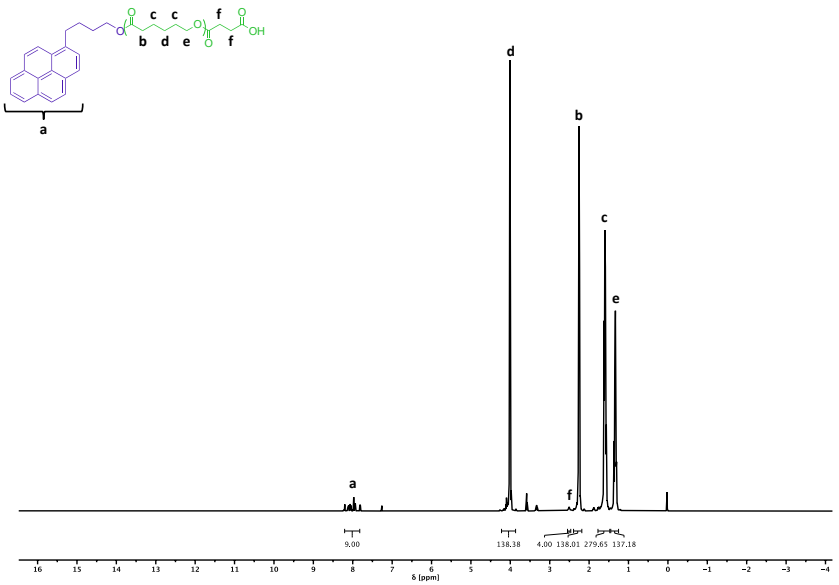


Figure S 4. *^{1}H NMR spectrum (500 MHz) of Py-PCL-COOH in $CDCl_3$.*

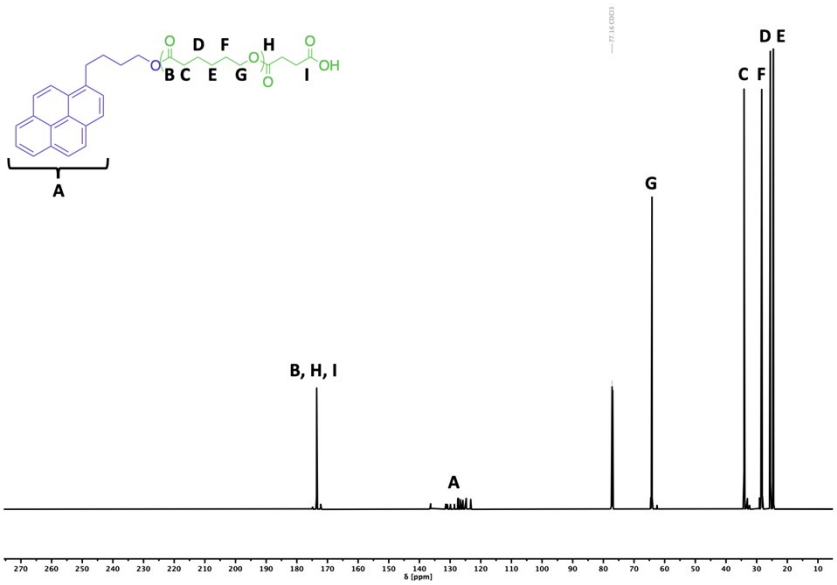
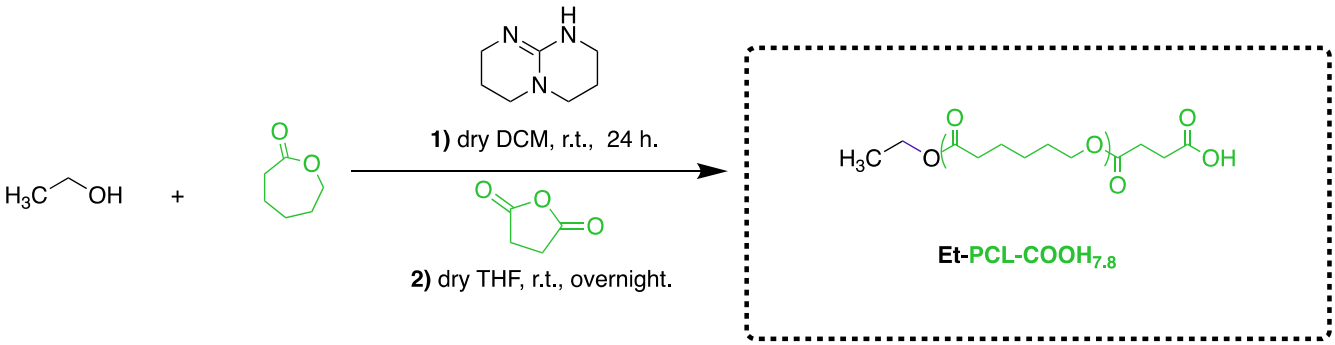


Figure S 5*. ^{13}C NMR spectrum (500 MHz) of Py-PCL-COOH in $CDCl_3$.*

Synthesis of Et-PCL-COOH



The synthesis, ^{1}H NMR, and GPC characterization of Et-PCL-COOH are described in more detail elsewhere.[2] In brief, in a flame-dried Schlenk flask, freshly distilled caprolactone (15 g) was placed. To the flask, dry DCM was added to obtain a 1M solution. Then, the initiator dry ethanol and the catalyst

TBD (1.2 eq.) were added. The reaction was stirred at room temperature for 24 hours. Then, succinic anhydride (4 eq., dry THF, 0.5M) was added, and the reaction was further stirred for 24 hours at room temperature. Then, the solvent was removed under vacuum. The polymer was purified by precipitation from acetone in cold methanol three times. The obtained white polymers were dried under vacuum and further characterized by ^{1}H and ^{13}C NMR, and GPC.

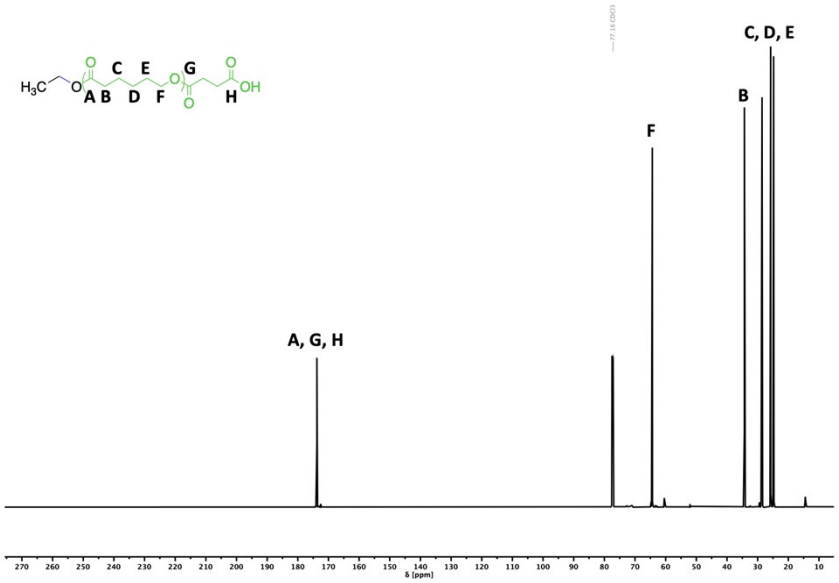
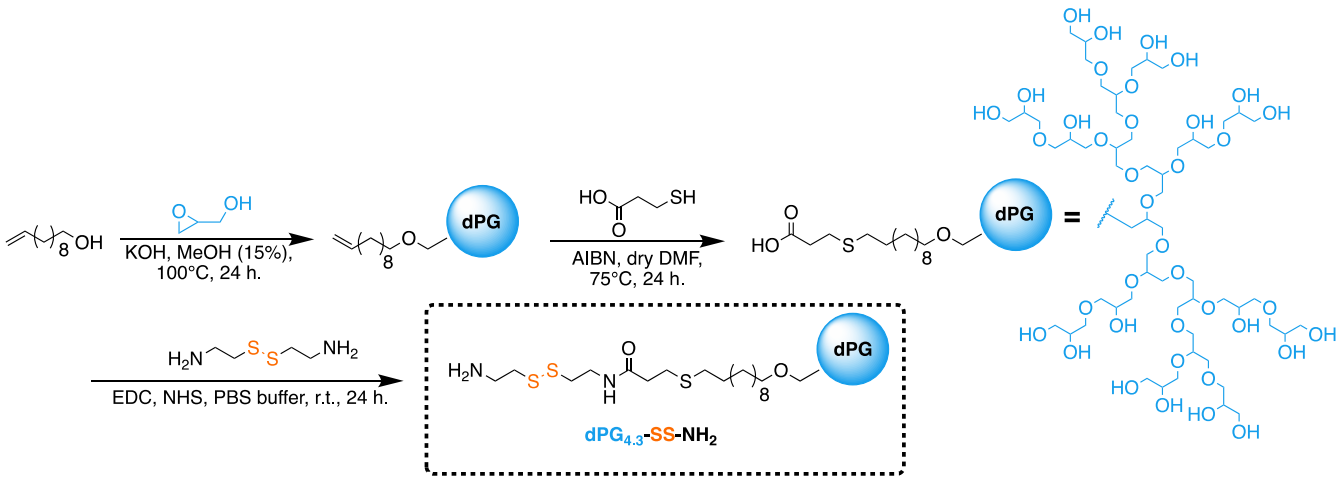


***Figure S 6**. ^{13}C NMR spectrum (700 MHz) of Et-PCL-COOH in $CDCl_3$.*

Synthesis of dPG-SS-NH_2



The synthesis of dPG-SS-NH_2 was performed according to an already published protocol.[2] In short, the reaction was performed in a synthesis reactor (HiTEC). To synthesize dPG-SS-NH_2, 10-undecenol (20.66 g, 0.12 mmol) was loaded into the reactor flask. *In situ* potassium methoxide (KOH 0.31 g, 5 mL MeOH, MeOK, 15% deprotonation) was formed and dried at 60 °C under vacuum. The reactor was filled with an argon atmosphere, heated to 100 °C, and freshly distilled glycidol (200 g, 2.7 mmol) was added over a period of 24 h. After 26 h, the reaction temperature was reduced to 75 °C, and subsequently dry DMF (600 mL), mercaptopropionic acid (29.50 g, 278.0 mmol), and azobisisobutyronitrile (AIBN; 4.56 g, 27.8 mmol) were added to the reaction. The reaction was stirred for 4 h at a constant temperature of 75 °C. The crude product was purified by precipitation in acetone with subsequent tangential flow filtration (TFF) dialysis (Sartocon Slice 200 Stainless Steel Holder) in water/ethanol 10:1 (MWCO: 1 kDa) for 3 days. The solvent was removed under reduced pressure, and after lyophilization (Alpha 3-4 LSC basic), a yellow viscous polymer was obtained. In a Flask, cystamine (7.41 g, 32.9 mmol) was placed, and phosphate-

buffered saline (PBS) buffer (1 L, pH 7.4, 100 mM) was added until the cystamine was dissolved. At the same time, dPG-COOH (25 g, 6.6 mmol) was dissolved in 2-(N-morpholino) ethanesulfonic acid (MES)-buffer (500 mL, pH 5.0, 50 mM). Next, EDC*HCL (6.31 g, 32.9 mmol) and NHS (3.8 g, 32.9 mmol) were added. At room temperature, the reaction solution was stirred for 30 min, and then the cystamine solution was slowly added. The reaction was stirred over 16 h, and the product was purified by precipitation into cold acetone. Then the product was further purified by TFF dialysis in water for 3 days (MWCO: 1 kDa) and then lyophilized. A yellow, oily polymer (16.8 g) was obtained. The obtained product was characterized by ^{1}H and ^{13}C NMR, IR, and GPC.

^{1}H NMR (500 MHz, CD_3OD, δ, ppm) = 4.02-3.36 (m, 288H), 3.03-2.32 (m, 12H), 1.65-1.50 (m, 4H), 1.46-1.25 (m, 14H).
FTIR (*v*, cm^{-1}) = 1647 (C=O, sec. amide)

Table S 3. *Anionic Polymerization of Glycidol initiated by 10-undecenol.*

Monomer (M)	Catalyst (C)	$[M]_0/[10\text{-undecenol}]_0/[C]_0$	Time [h]	$M_n^{theo.}$ (kDa)	M_n^{NMR} (kDa)	$M_n^{GPC,\ water}$ (kDa)	Đ
Glycidol	MeOK	22.5/1/0.15	24	4	4.3	4.4	1.5

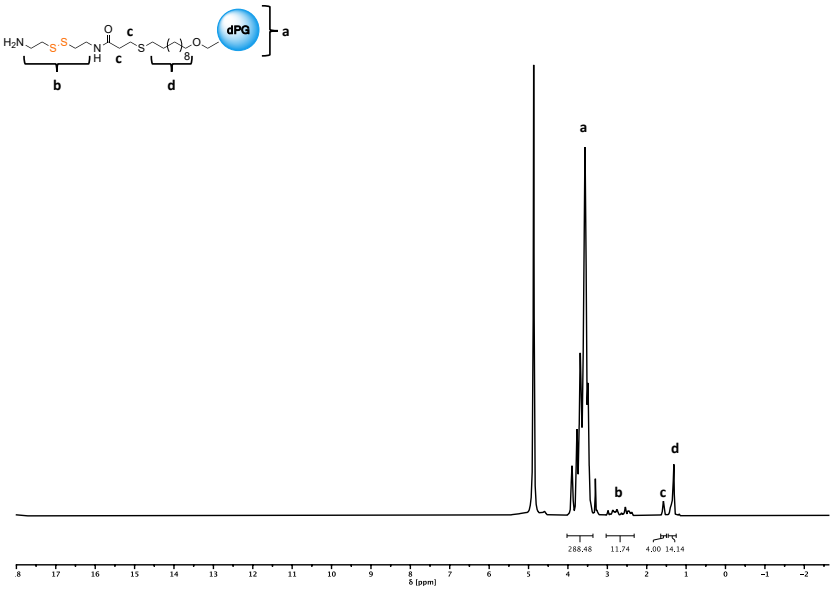


Figure S 7. *^{1}H NMR spectrum (500 MHz) of dPG-SS-NH_2 in CD_3OD.*

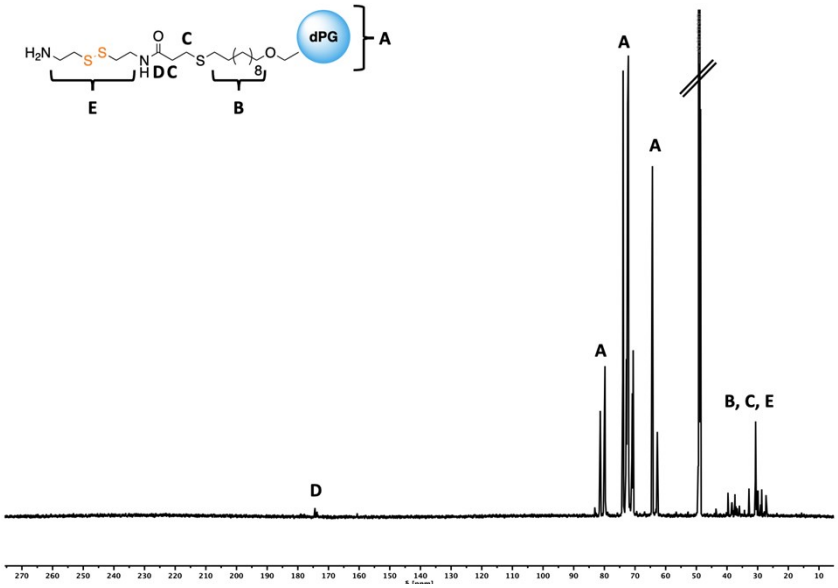


Figure S 8. *^{13}C NMR (700 MHz) of dPG-SS-NH_2 in CD_3OD.*

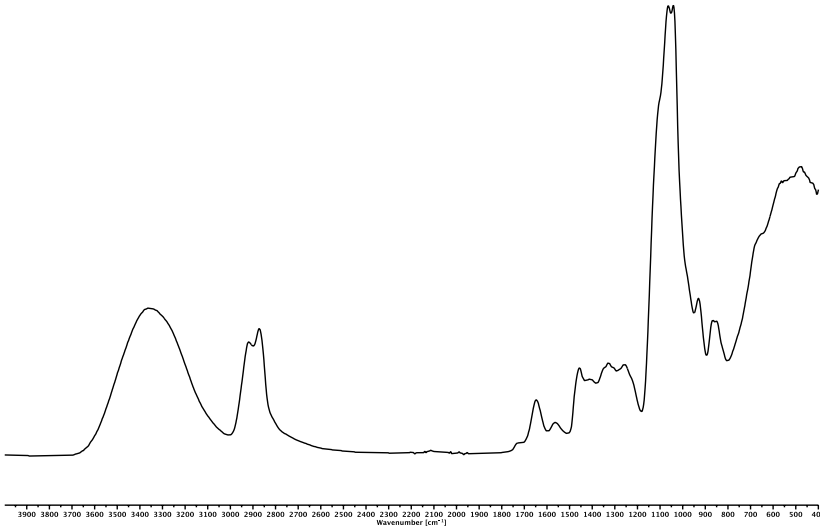
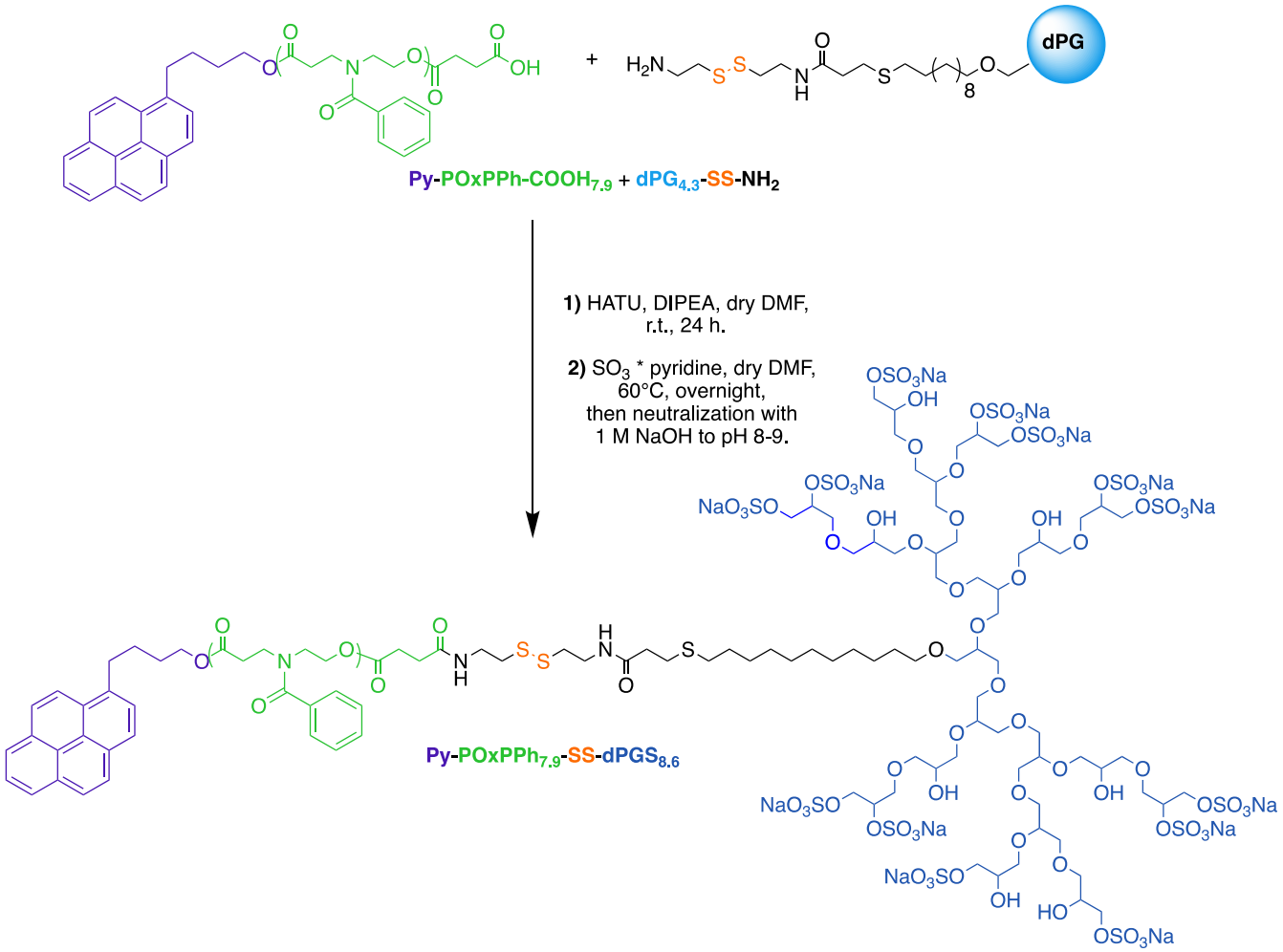


Figure S 9. *FTIR spectrum of dPG-SS-NH_2.*

Synthesis of dPGS-SS-POxPPh-Py



First, the hydrophobic block, Py-POxPPh-COOH, and the hydrophilic block, dPG-SS-NH_2, were coupled using an amide coupling protocol. In a flame-dried Schlenk-flask Py-POxPPh-COOH (0.50 g, 0.065 mmol), HATU (0.06 g, 0.15 mmol), and DIPEA (0.02 g, 0.03 mL, 0.17 mmol) were added. The reactants were dissolved in 5 mL of dry DMF. The acid was then activated under stirring at room temperature for 2 hours. Then, dPG-SS-NH_2 (0.30 g, 0.075 mmol) was dissolved in 5 mL of dry DMF and was slowly added to the solution. The reaction was stirred at room temperature overnight. For purification and separation of uncoupled polymer chains, the crude product was dialyzed against methanol for 3 days (MWCO 3.5 kDa). Then, the methanol was changed to water. The product was obtained after lyophilization (453 mg). For characterization, ^{1}H NMR and DOSY NMR were performed.

Next, the dPG-SS-POxPPh-Py (400 mg) was dissolved in 5 mL of dry DMF and heated to 60°C. SO3*pyridine (2.15 g, 13.48 mmol) dissolved in 10 mL was added over 5 hours to this solution. The solution was further stirred overnight at 60°C. Then, the solution was quenched by adding 1M NaOH until a pH of 8 was reached. The amphiphilic copolymer was dialyzed against brine with an ever-decreasing salt content over a period of 5 days. After lyophilization, a yellow, crystalline polymer was obtained (650 mg). The product was characterized by ^{1}H and ^{13}C NMR, GPC, elemental analysis, and FTIR. Degree of Sulfation (EA): 87%.

^{1}H NMR$_{\text{before sulfation}}$ (500 MHz, DMF-d_7, δ, ppm) = 8.49-8.12 (m, 9H), 7.62-7.26 (m, 177H), 4.98-4.51 (m, 76H), 4.42-4.04 (m, 70H), 3.82-3.45 (m, 582H), 2.89-2.58 (m, 75H+DMF), 1.60-1.53 (m, 5H), 1.41-1.28 (m, 19H).

GPC$_{\text{after sulfation}}$ (H_2O) = M_w: 1.33 kg/mol; *Đ: 2.00*
FTIR (ν, cm^{-1}) = 1732 (C=O, ester); 1631 (C=O, tert. amide)

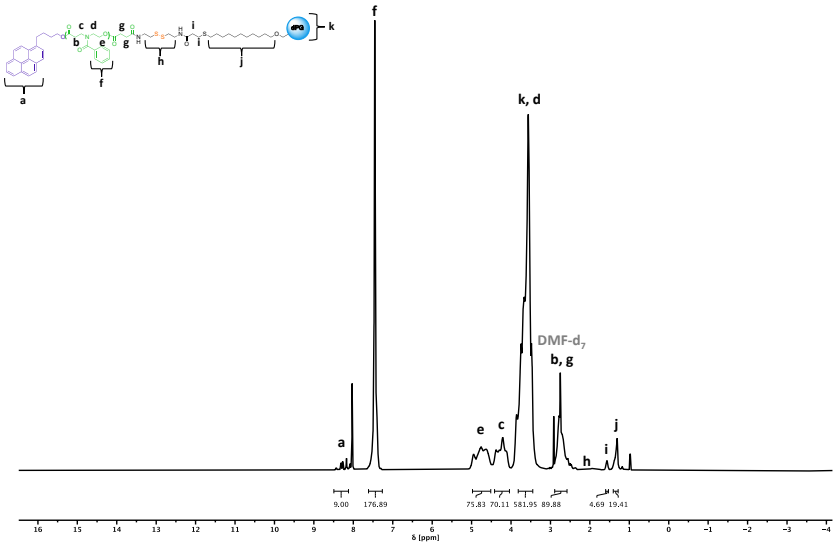


Figure S 10. *^{1}H NMR spectrum (500 MHz) of dPG-SS-POxPPh-Py in DMF-d_7.*

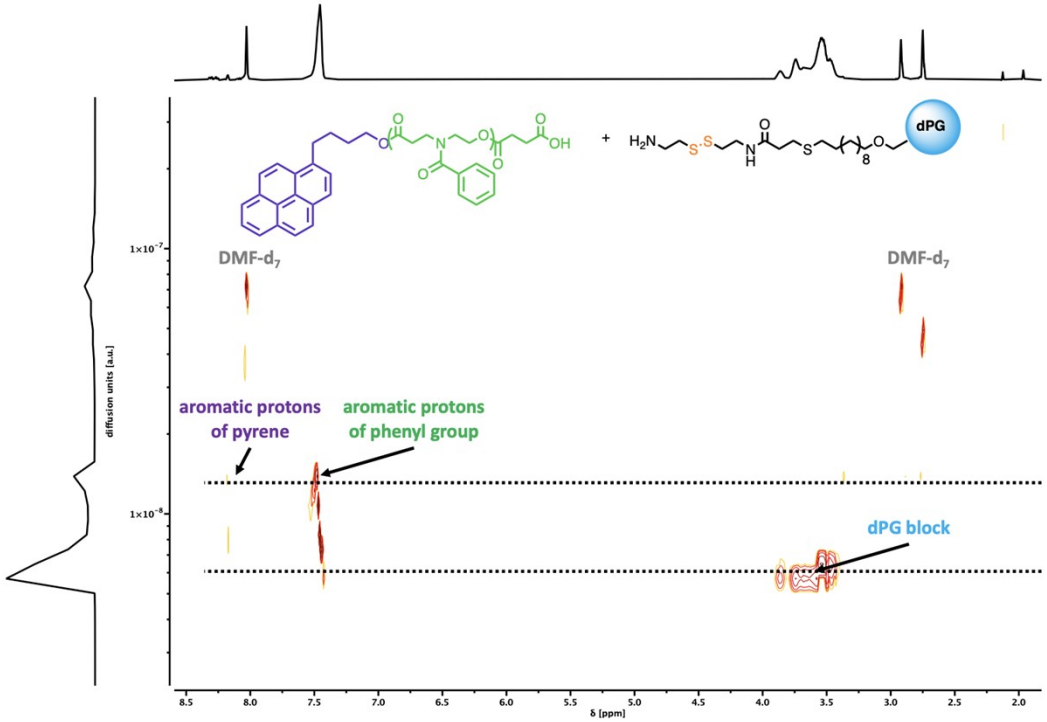


Figure S 11. *^{1}H-derived DOSY NMR spectrum (500 MHz) of non-coupled Py-POxPPh-COOH and dPG-SS-NH_2 in DMF-d_7 showing two distinguished diffusion species.*

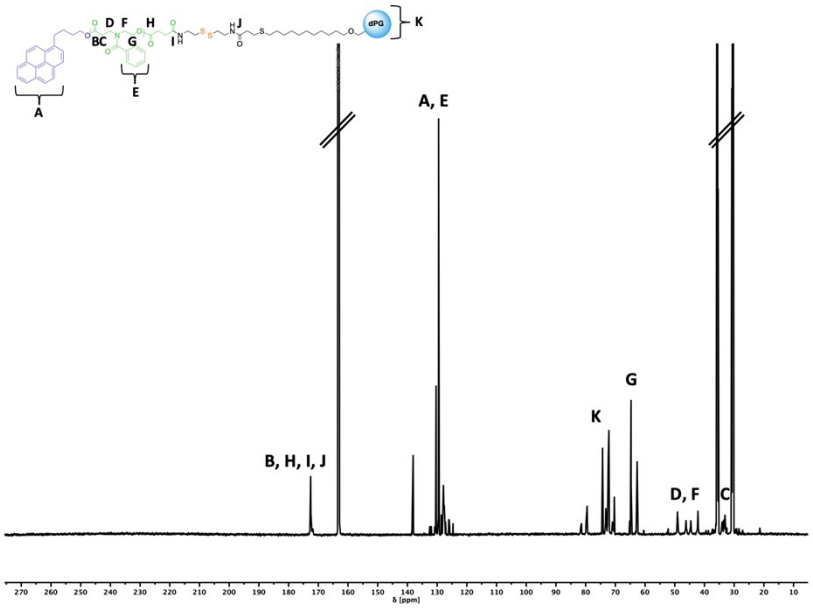


Figure S 12. *^{13}C NMR (700 MHz) of dPG-SS-POxPPh-Py in DMF-d_7.*

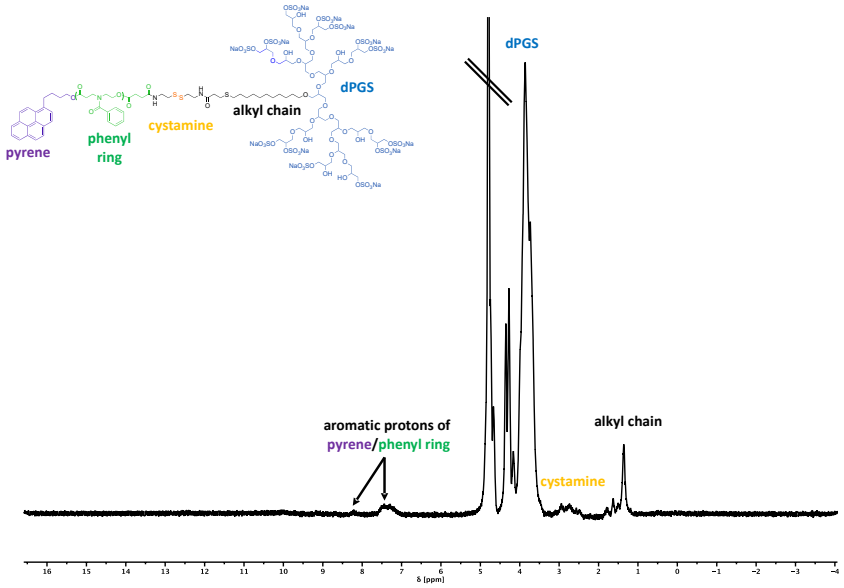


Figure S 13. *^{1}H NMR spectrum (500 MHz) of dPGS-SS-POxPPh-Py in D_2O.*

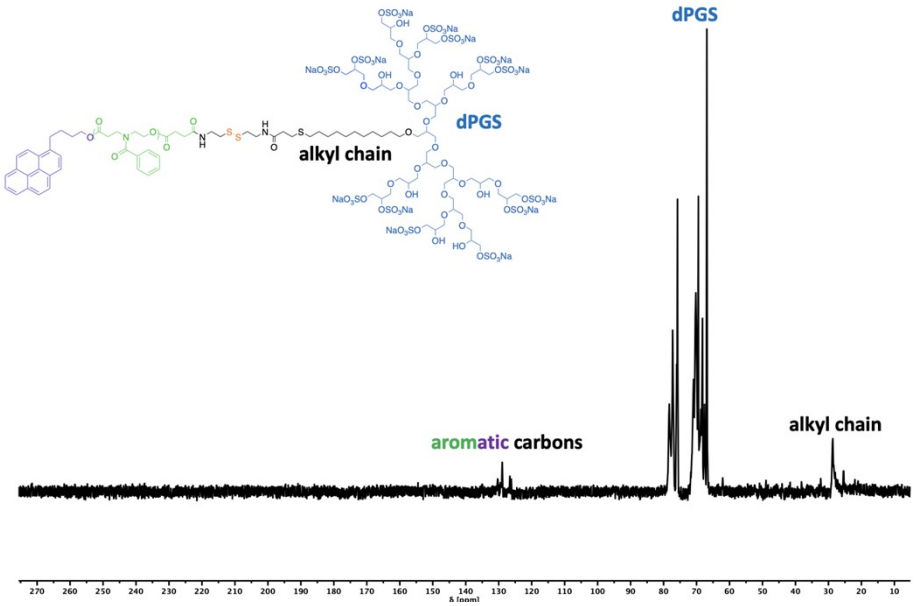


***Figure S 14**. ^{13}C NMR spectrum (700 MHz) of dPGS-SS-POxPPh-Py in D_2O.*

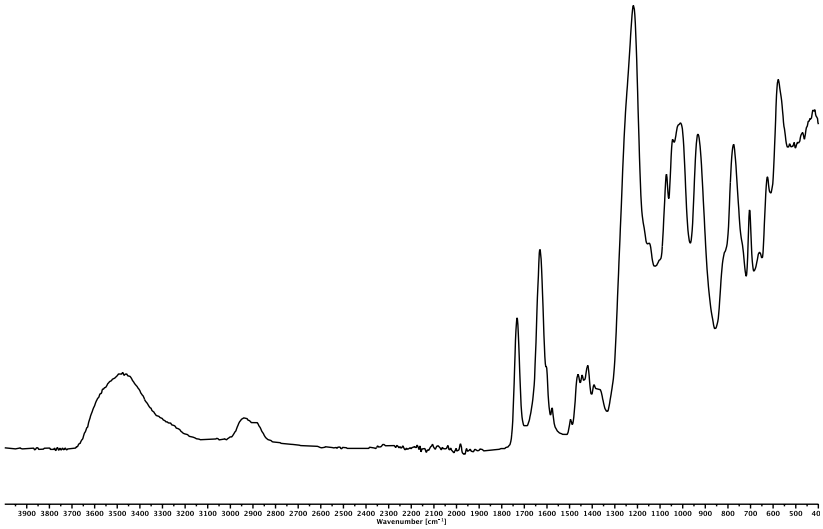
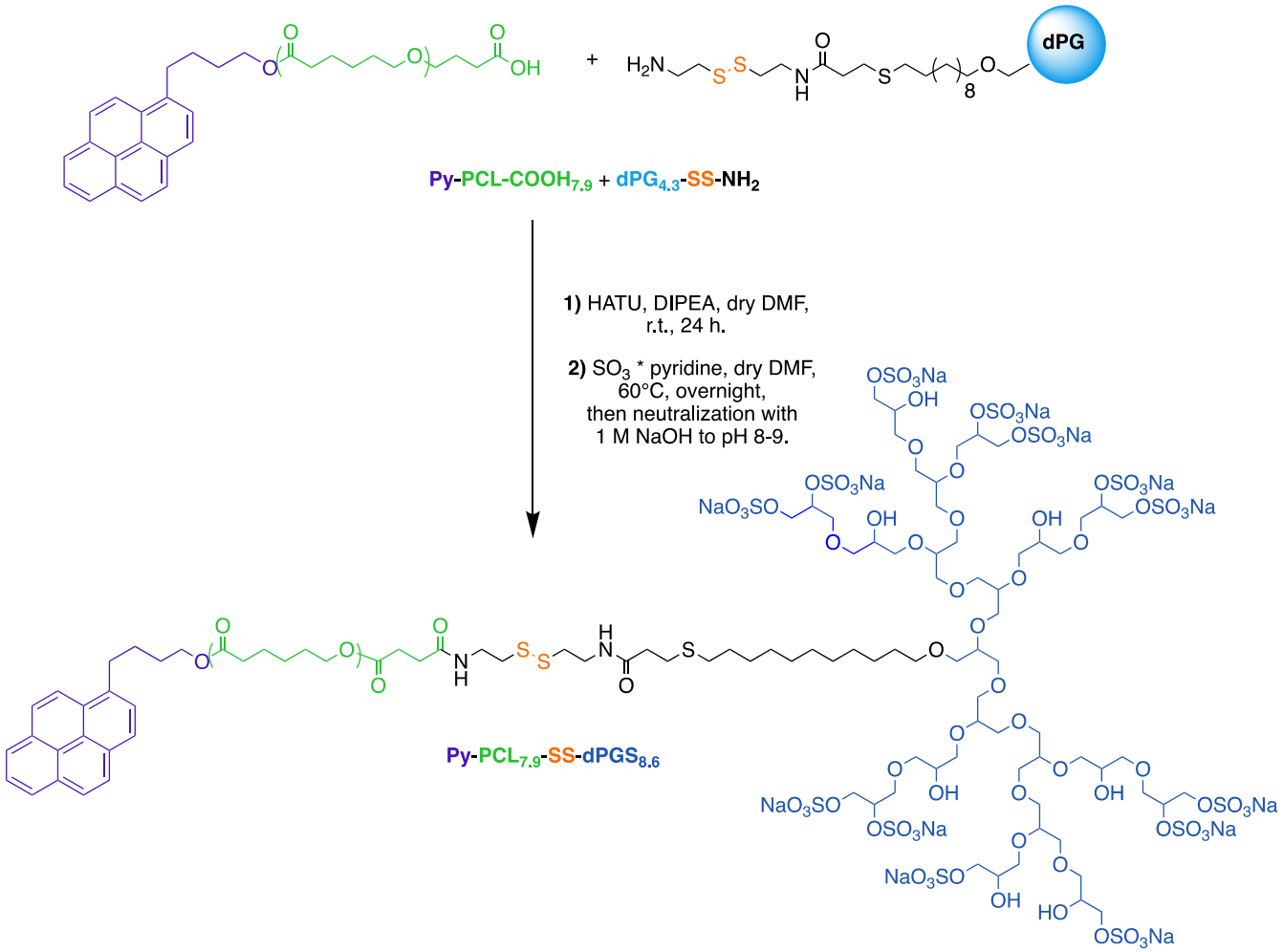


***Figure S 15.** FTIR spectrum of dPGS-SS-POxPPh-Py.*

Synthesis of dPGS-SS-PCL-Py



First, the hydrophobic block, Py-PCL-COOH, and the hydrophilic block, dPG-SS-NH_2, were coupling using an amide coupling protocol. In a flame-dried Schlenk-flask Py-PCL-COOH (0.32 g, 0.040 mmol), HATU (0.04 g, 0.09 mmol), and DIPEA (0.01 g, 0.02 mL, 0.10 mmol) were added. The reactants were dissolved in 5 mL of dry DMF. The acid was then activated under stirring at room temperature for 2 hours. Then, dPG-SS-NH_2 (0.19 g, 0.047 mmol) was dissolved in 5 mL of dry DMF and was slowly added to the solution. The reaction was stirred at room temperature overnight. For purification and separation of uncoupled polymer chains, the crude product was dialyzed against methanol for 3 days (MWCO 3.5 kDa). Then, the methanol was changed to water. The product was obtained after lyophilization (426 mg). For characterization, ^{1}H NMR was performed.

Next, the dPG-SS-PCL-Py (400 mg) was dissolved in 5 mL of dry DMF and heated to 60°C. SO3*pyridine (2.15 g, 13.48 mmol) dissolved in 10 mL was added over 5 hours to this solution. The solution was further stirred overnight at 60°C. Then, the solution was quenched by adding 1M NaOH until a pH of 8 was reached. The amphiphilic copolymer was dialyzed against brine with an ever-decreasing salt content over a period of 5 days. After lyophilization, a yellow, crystalline polymer was obtained (480 mg). The product was characterized by ^{1}H and ^{13}C NMR, GPC, elemental analysis, and FTIR. Degree of Sulfation (EA): 84%.

^{1}H NMR$_{\text{before sulfation}}$ (500 MHz, DMF-d_7, δ, ppm) = 8.43-8.11 (m, 9H), 4.27-3.97 (m, 117H), 3.90-3.42 (m, 196H), 2.64-2.53 (m, 4H), 2.49-2.22 (m, 227H), 1.78-1.52 (m, 229H), 1.48-1.28 (m, 123H).
GPC$_{\text{after sulfation}}$ (H_2O) = M_w: 1.24 kg/mol; *Đ: 1.93*
FTIR (*ν*, cm^{-1}) = 1723 (C=O, ester); 1643 (C=O, sec. amide)

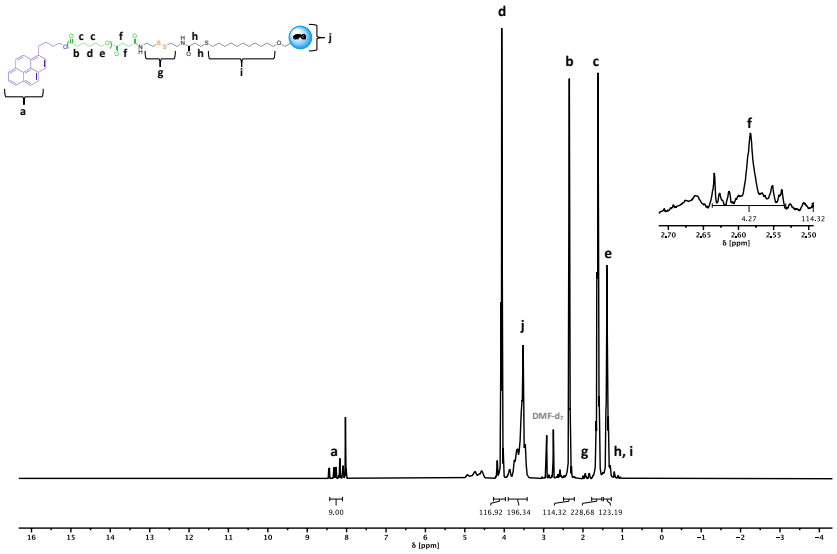


***Figure S 16**. [1]H NMR spectrum (500 MHz) of dPG-SS-PCL-Py in DMF-d_7.*

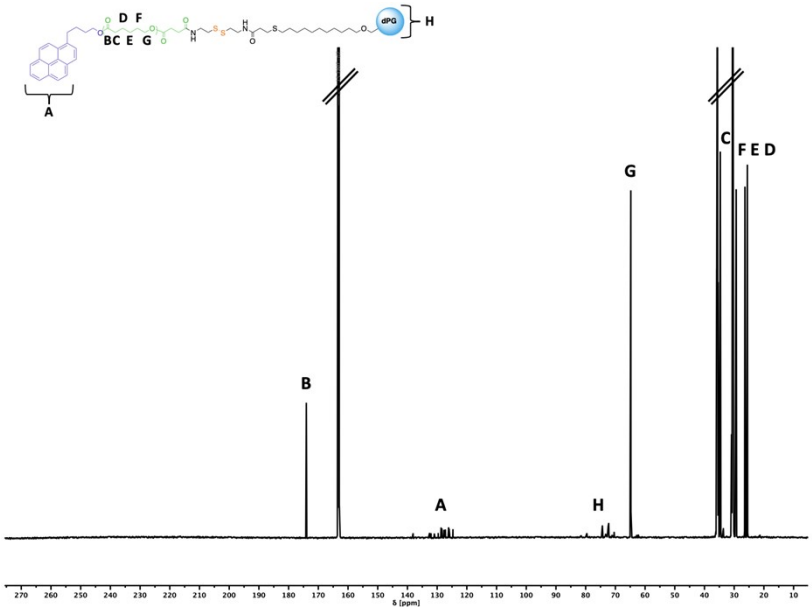


***Figure S 17**. [13]C NMR (700 MHz) of dPG-SS-PCL-Py in DMF-d_7.*

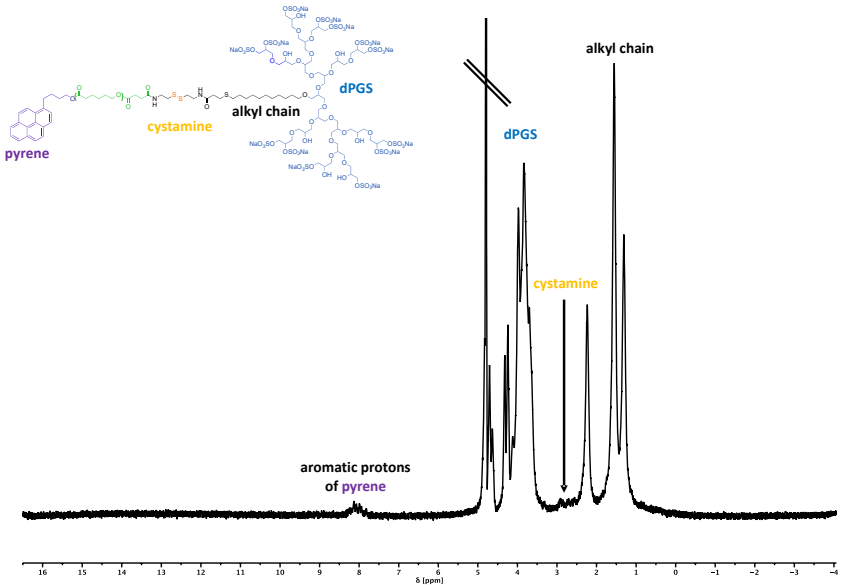


Figure S 18. *^{1}H NMR spectrum (500 MHz) of dPGS-SS-PCL-Py in D_2O.*

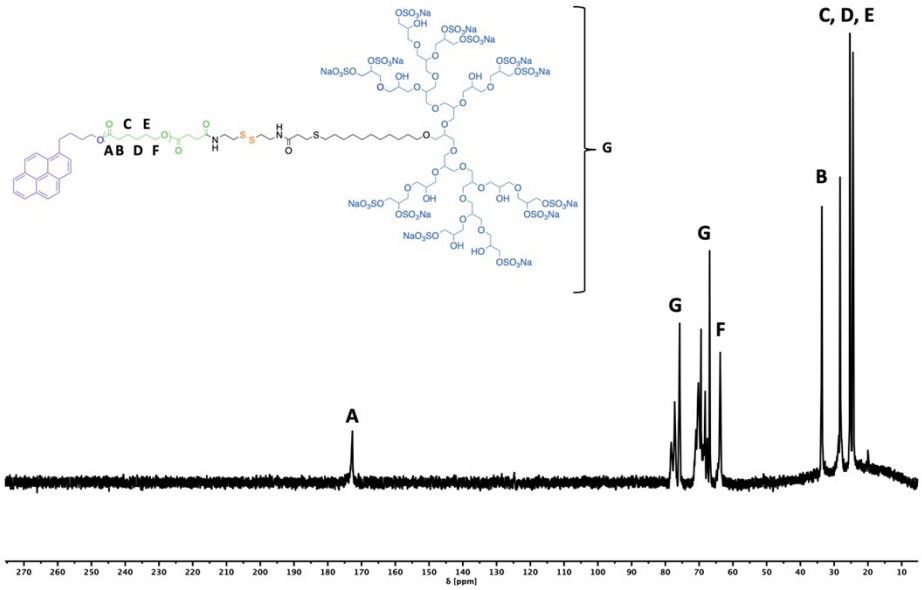


Figure S 19. *^{13}C NMR spectrum (700 MHz) of dPGS-SS-PCL-Py in D_2O.*

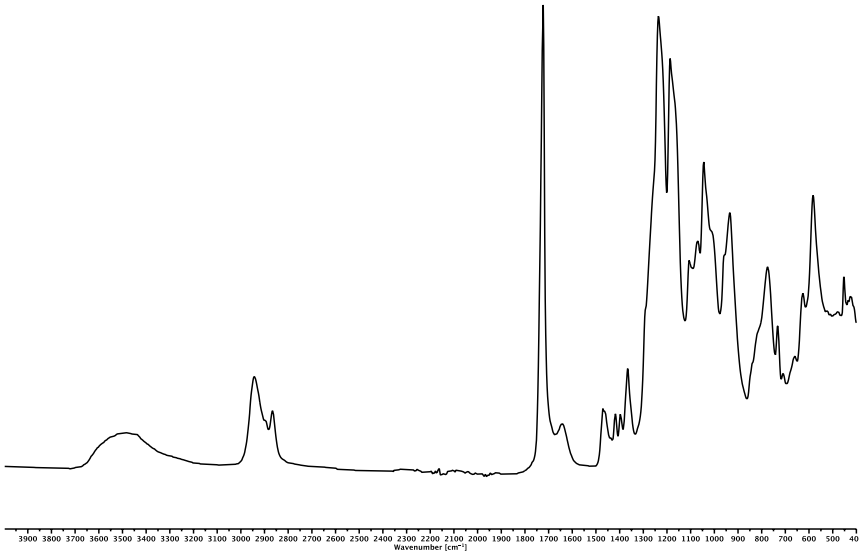
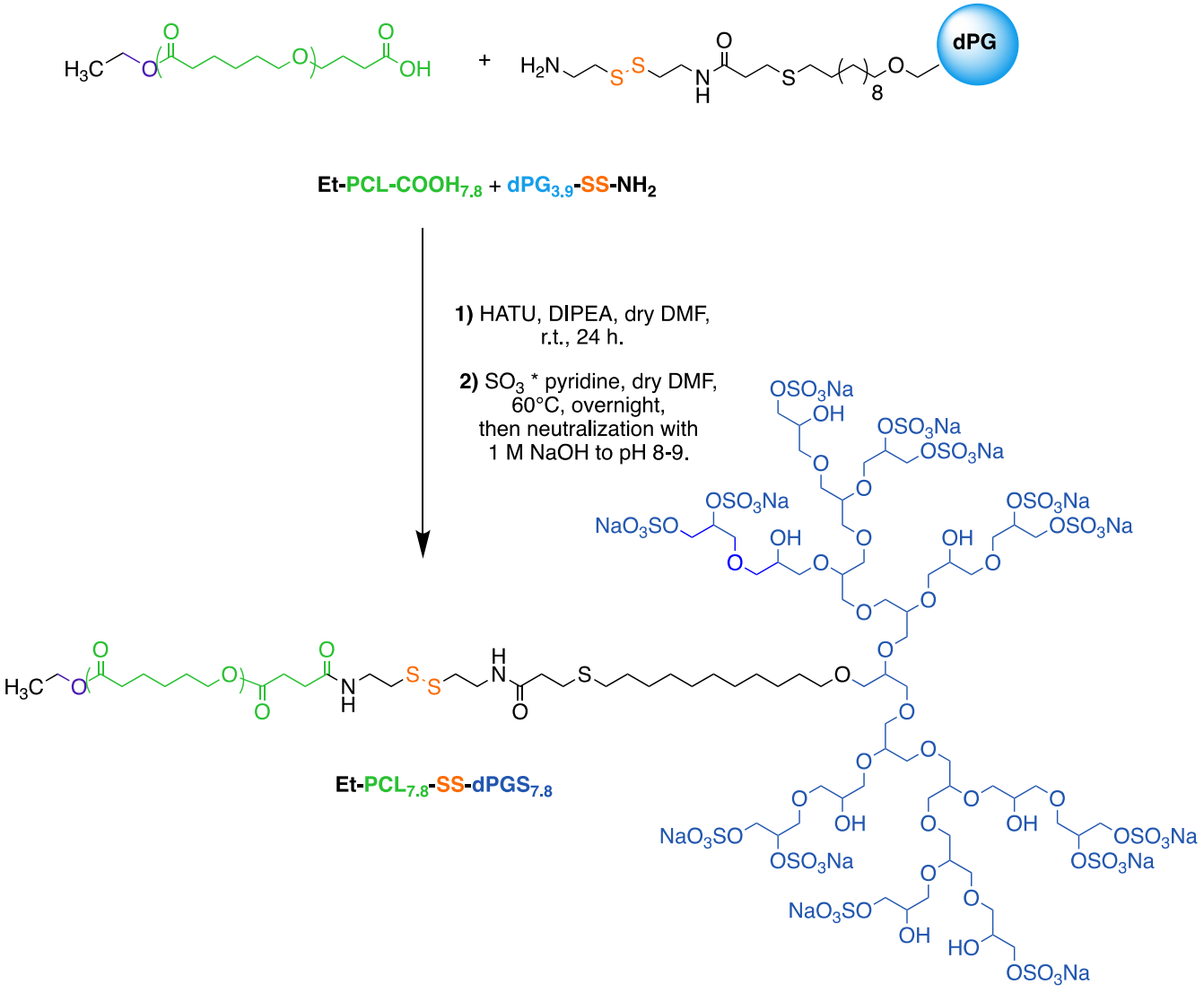


Figure S 20. *FTIR spectrum of dPGS-SS-PCL-Py.*

Synthesis of dPGS-SS-PCL-Et



The synthesis, ^{1}H NMR, GPC, and EA analysis are described elsewhere in more detail.[2]

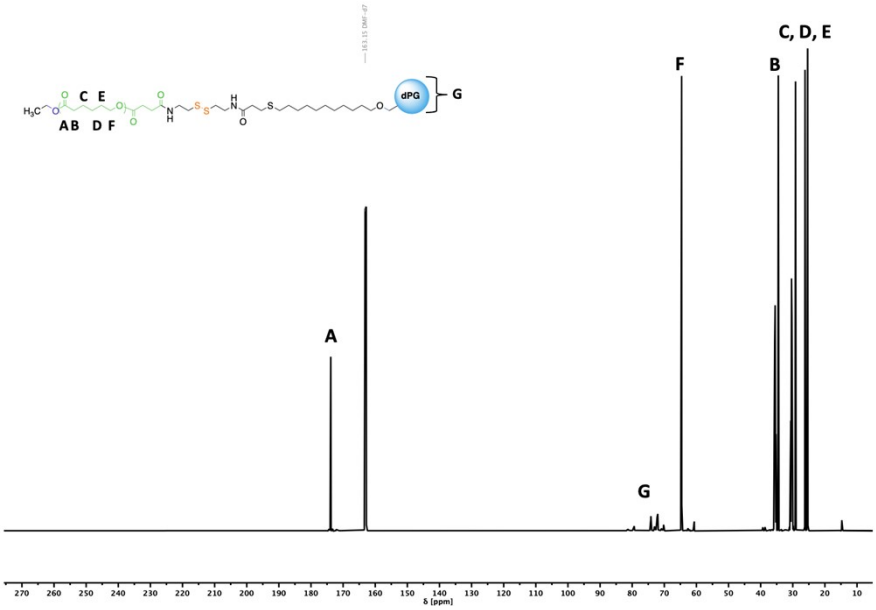


***Figure S 21**. ^{13}C NMR (700 MHz) of dPG-SS-PCL-Et in DMF-d_7.*

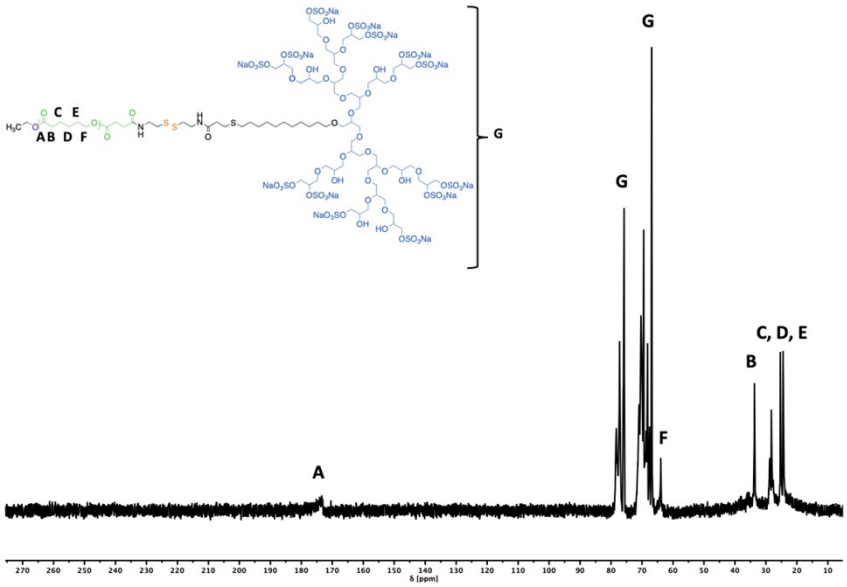


***Figure S 22**. ^{13}C NMR (700 MHz) of dPGS-SS-PCL-Et in D_2O.*

FTIR (*v*, cm^{-1}) = 1725 (C=O, ester); 1643 (C=O, sec. amide)

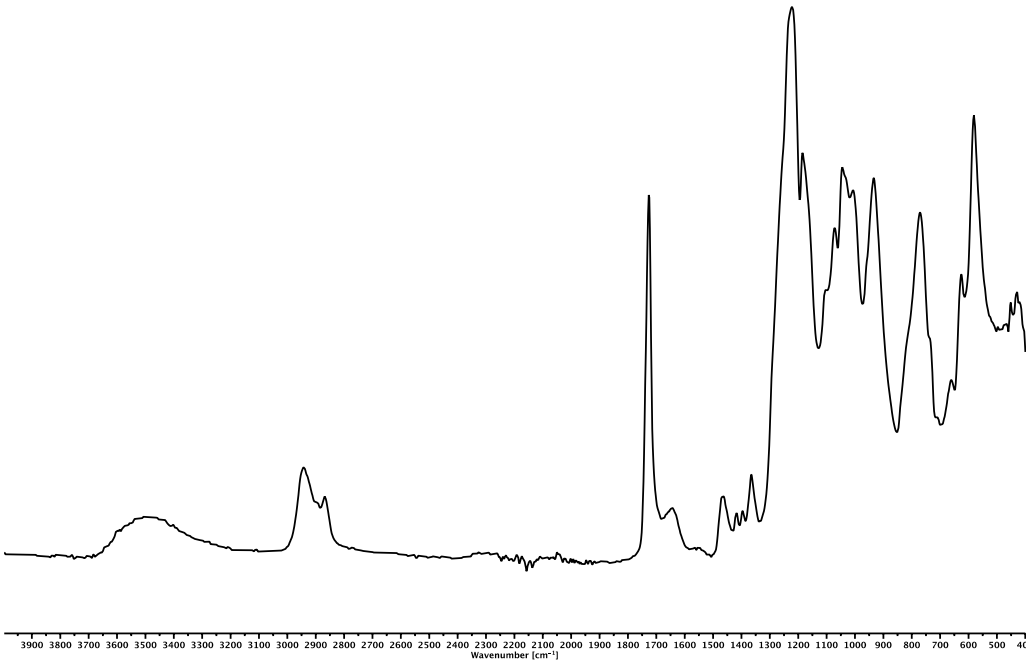
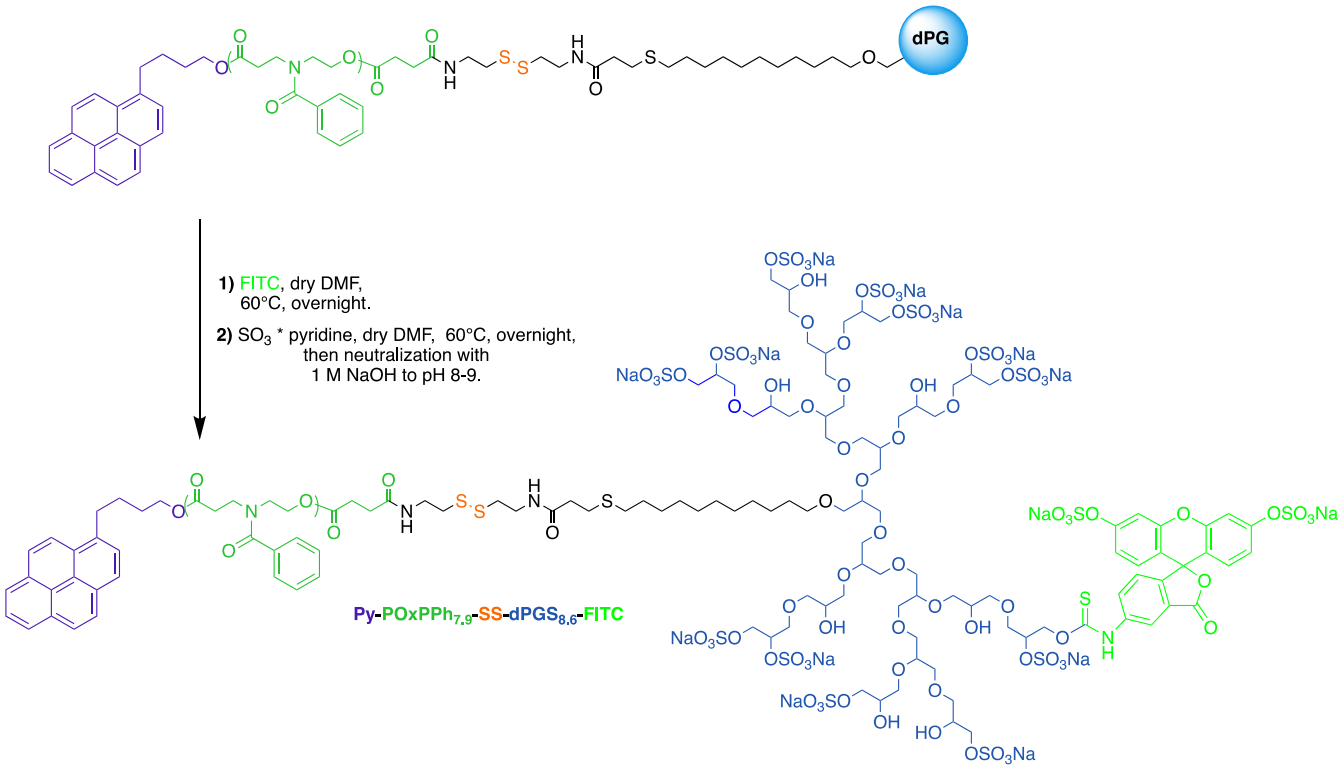


Figure S 23. *FTIR spectrum of dPGS-SS-PCL-Et.*

Synthesis of FITC-dPGS-SS-POxPPh-Py



FITC-labeling of the copolymer, FITC (0.018 g, 0.045 mmol), and dPG-SS-POxPPh-Py (150 mg) were dissolved in 5 mL of dry DMF. The solution was heated to 60°C and stirred overnight in the dark. Next, slowly added SO_3*pyridine (0.35 g, 2.22 mmol) dissolved in 10 mL of dry DMF. After further stirring at 60°C overnight, the solution was quenched by adding 1M NaOH until a pH of 8 was reached. The labeled polymer was dialyzed against brine with an ever-decreasing salt content over a period of 5 days. The solution was concentrated, and free dye was removed with an SEC Sephadex G-25 column, where the

yellow band of the labeled polymer was collected. After lyophilization, the polymer obtained was a yellow powder (252 mg). ^{1}H NMR and UV spectroscopy characterized the product. Degree of Sulfation (EA): 84%.

According to the calibration curve, the coupling efficiency of the FITC-labeling was 30% (6.72 µg/mL (17 µM) of FITC in 1 mg/ml of copolymer solutions).

FTIR (*v*, cm^{-1}) = 1734 (C=O, ester); 1633 (C=O, tert. amide)

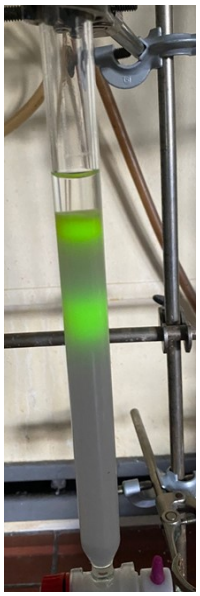


Figure S 24*. Picture of the Sephadex G-25 column of FITC-dPGS-SS-POxPPh-Py showing free dye on top of the column; the polymer ran as a sharp band and was collected. The sample was irradiated by UV light at 366 nm).*

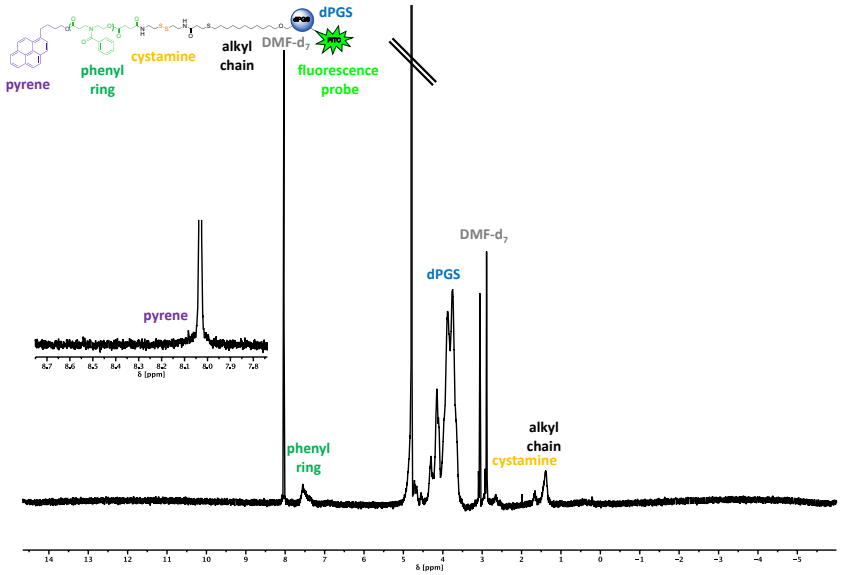


Figure S 25. *^{1}H NMR spectrum (500 MHz) of FITC-dPGS-SS-POxPPh-Py in DMF-d_7:D_2O 1:1.*

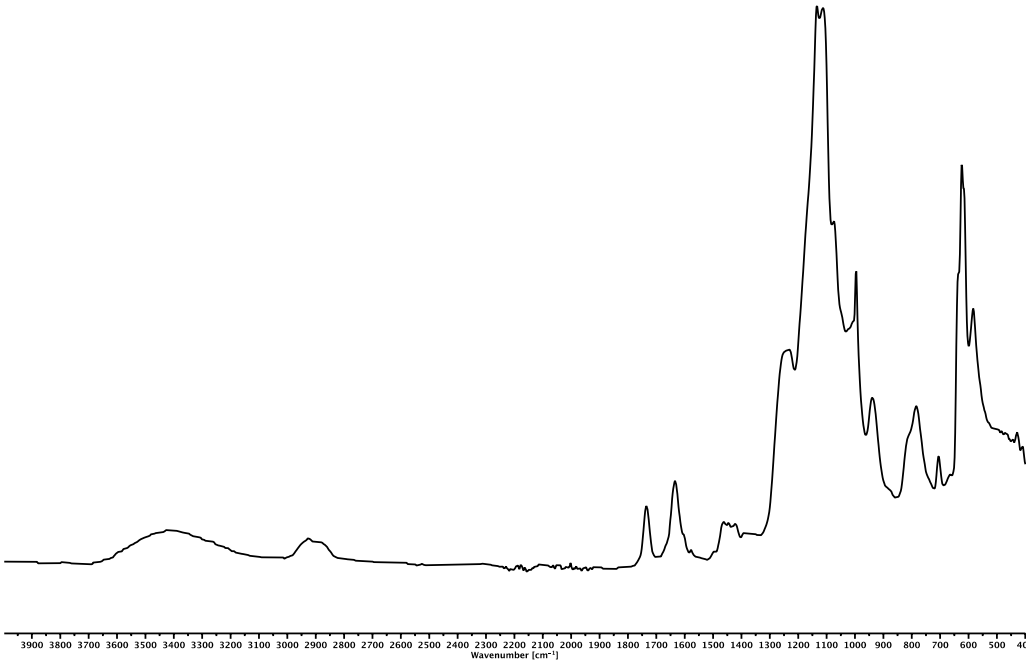


Figure S 26. *FTIR spectrum of FITC-dPGS-SS-POxPPh-Py.*

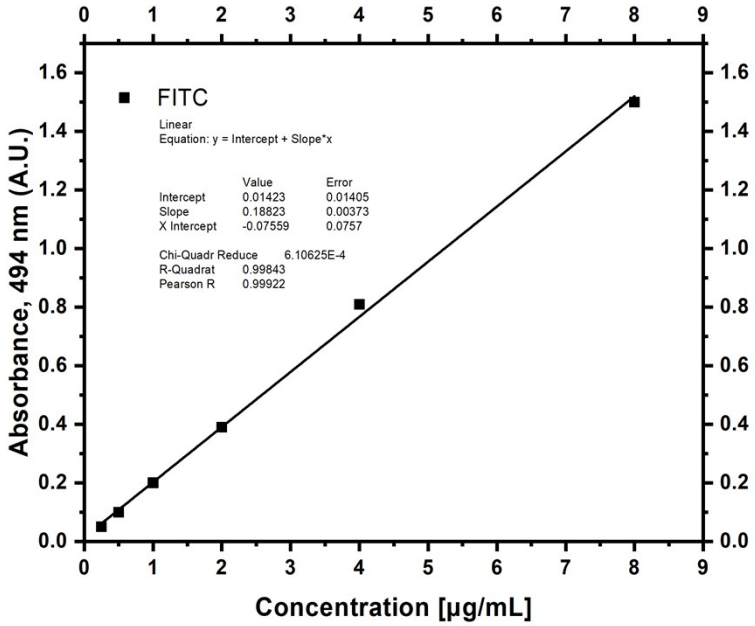


Figure S 27. *UV-VIS Calibration curve of FITC in PBS using different concentrations of the dye.*

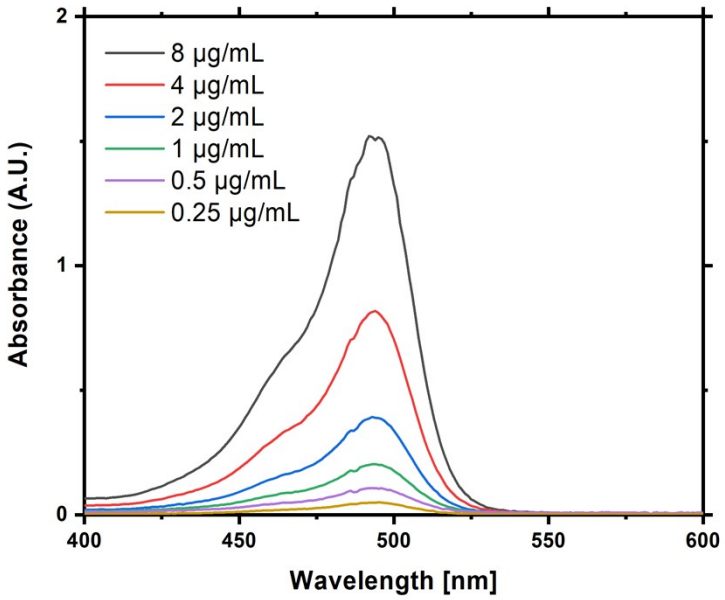


***Figure S 28**. UV-VIS calibration curve of FITC in PBS using different concentrations of the dye.*

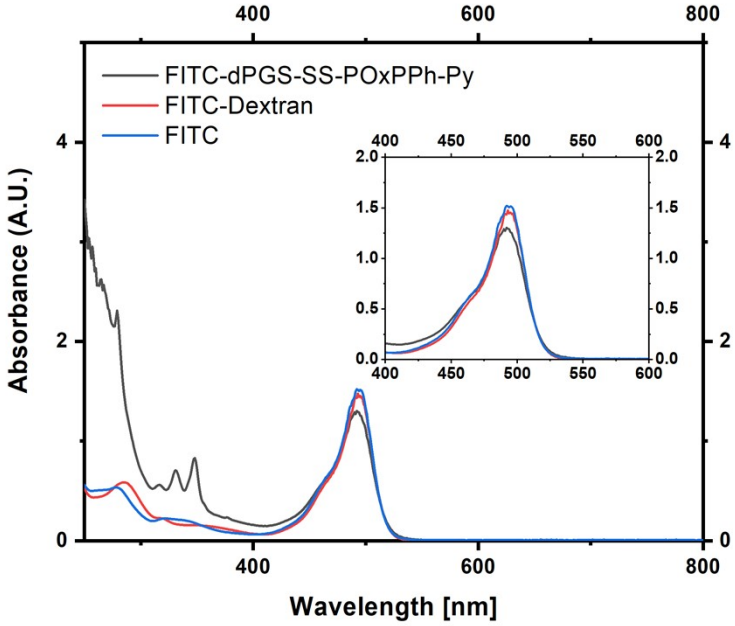


***Figure S 29.** UV-VIS spectra of FITC-labeled dPGS-SS-POxPPh-Py, FITC-labeled Dextran as control, and free FITC in PBS at 37 °C.*

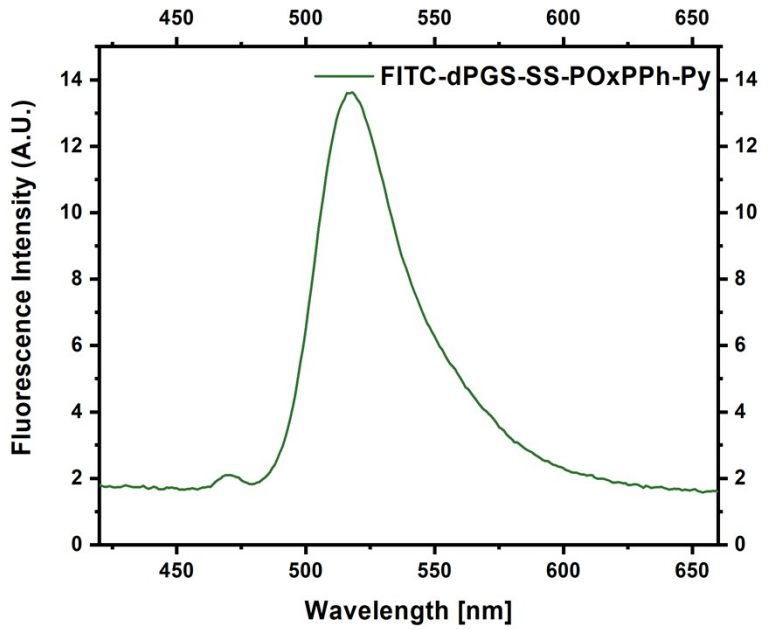


***Figure S 30**. Fluorescence spectrum of FITC-dPGS-SS-POxPPh-Py (λ_{ex} 405 nm) in PBS at 37 °C at 65 µg/mL.*

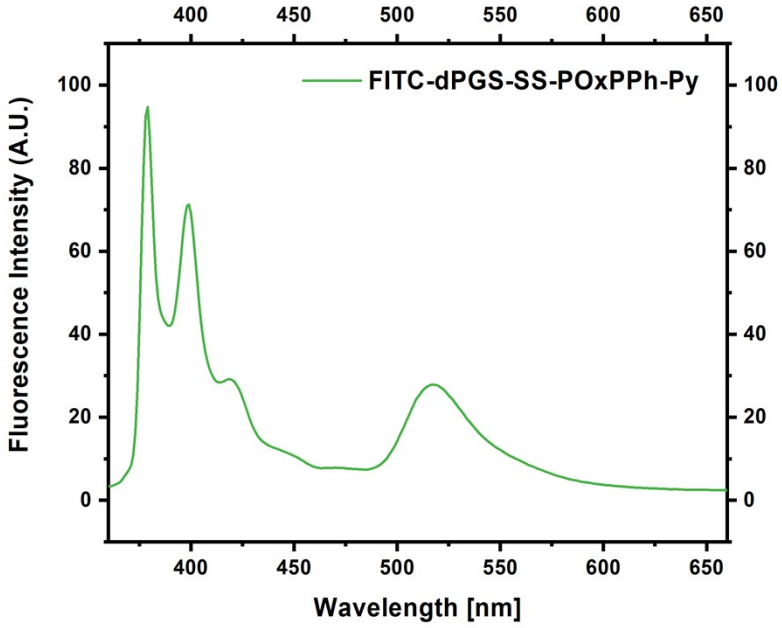


***Figure S 31**. Fluorescence spectrum of FITC-dPGS-SS-POxPPh-Py (λ_{ex} 350 nm) in PBS at 37 °C at 65 µg/mL.*

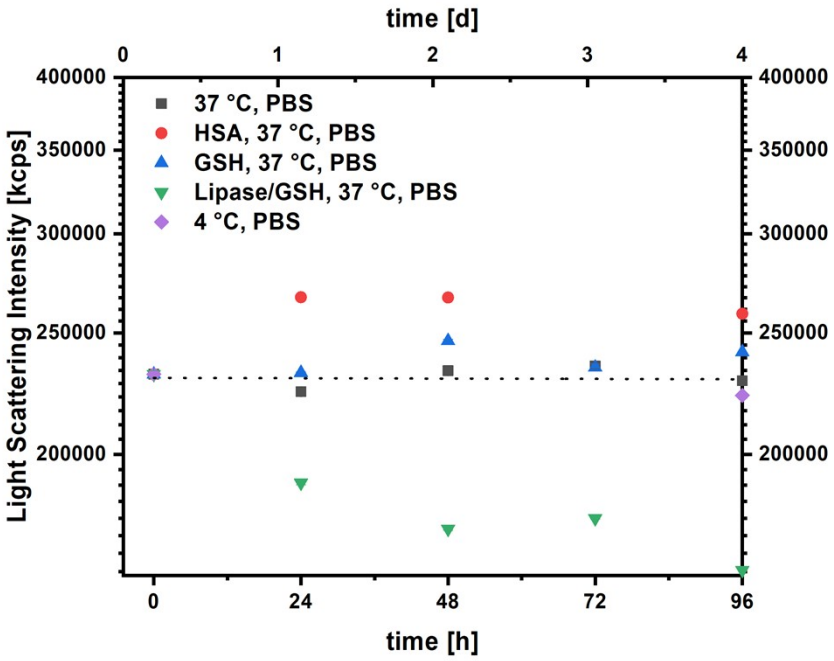


Figure S 32. *Time-dependent DLS measurements on the evolution of the light scattering intensity of DTX@dPGS-SS-POxPPh-Py in different conditions: in PBS at 37 °C, pH 7.4 (black), and 4 °C, pH 7.4 (purple) as control; in the presence of HSA (red); GSH, pH 5.0 (blue); Lipase/GSH (green); according to* $I_{SLS} \sim c_{polymer}$ *the light scattering intensity allows the determination of the concentration of scattering species in solution; as for the controls, constant intensities were detected, the particle´s concentration was stable over 4 days; also for HSA, the intensity kept constant (notably, the initial increase is due to the addition of light scattering protein); GSH addition does not influence the concentration with no breakdown of the aggregates, however, by enzymatic degradation (lipase), the light scattering intensity drops constantly displaying the decrease of scattering species (disruption of micelles).*

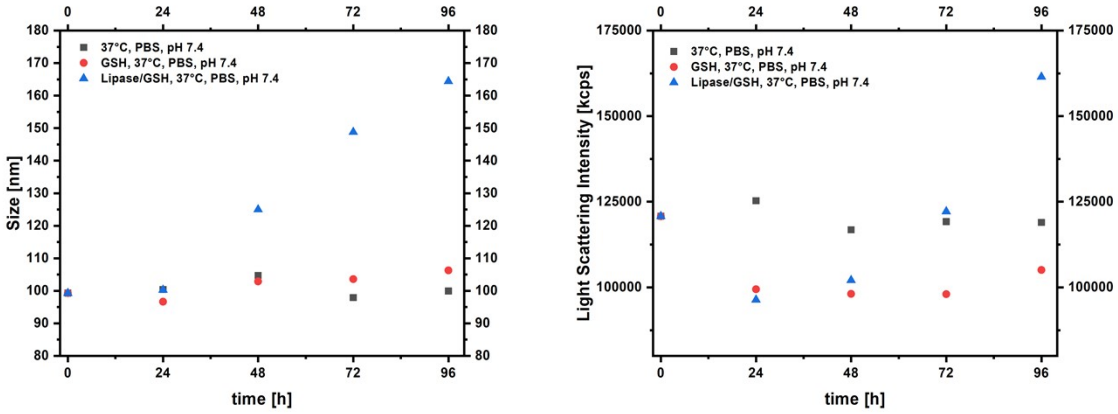


Figure S 33*. Time-dependent DLS measurements on the evolution of the size (left) and the light scattering intensity (right) of DTX@dPGS-SS-POxPPh-Py in different conditions: in PBS at 37 °C, pH 7.4 (black, as control); in the presence of GSH, pH 7.4 (red); Lipase/GSH, pH 7.4 (blue). For the control sample, the size and light scattering intensity remained constant over 4 days; for GSH, pH 7.4, an initial decrease of the light scattering intensity was detected; however, after one day, it also remained constant, the size remained constant over 4 days; For Lipase/GSH, after an initial light scattering intensity decrease, the*

size and light scattering intensity have shown to increase, indicating swelling of the micelles upon enzymatic/reductive degradation.

Dynamic Light Scattering Experiments (DLS) Measurements – Size and Size Distribution

The size and size distribution of the micelles was investigated by DLS measurements as described above. In short, the micelles were measured at a fixed concentration of 1 mg/mL at 37 °C. A series of three measurements were performed to check the reproducibility of the experiments.

ζ-Potential Measurements

The ζ-potential of the samples was measured using a Malvern Zetasizer Ultra (Malvern Instruments Limited, U.K.) in folded capillary cells (Malvern Analytics) at a constant temperature of 25 °C in 10 mM PB buffer solution at a fixed concentration of 1 mg/mL. The samples were calibrated for 2 min before the measurement was performed.

Dynamic Light Scattering (DLS) Measurements - Human Serum Albumin (HSA) Interaction with Micellar Surface

First, the micelle was formed in PBS using the method described above. Next, the micellar solution got incubated with HSA with a fixed protein concentration of 10 mg/mL. The samples were incubated at 37 °C for various periods, and then, their size and distribution were measured as described above.

Dynamic Light Scattering (DLS) Measurements - Enzymatic Hydrolysis with Novozyme 435

The micelles were incubated with 200 wt % Novozyme 435 concerning the polymer amount. For example, 1 mL of 1 mg/mL micelles solution in PBS was set with 2 mg of Novozyme and shaken at 37 °C for a predetermined time interval. The micelle integrity was investigated by DLS as described above.

Dynamic Light Scattering (DLS) Measurements - GSH- Triggered Shedding of Micelles

In the case of GSH samples, the micelle was first formed using the described method above and then incubated in a solution containing 10 mM GSH with the same buffer. The samples were incubated at 37 °C for various periods, and then, their size and distribution were measured as described above.

Dynamic Light Scattering Experiments (DLS) – Critical Micelle Concentration (CMC)

The CMC was investigated following an established protocol.[2] The critical micelle concentration (CMC) was determined by measuring the light scattering intensity using a Malvern Zetasizer Ultra (Malvern Instruments Limited, U.K.) equipped with a 10 mW He−Ne laser operating at a wavelength of 632.8 nm. The scattered light was detected using the backscattering setting at an angle of 173° (NIBS, noninvasive backscatter). The measurements were carried out in 12 mm square glass cuvettes (Hellma Analytics) at a constant temperature of 37 °C, respectively. In the case of sizes at 37°C, the samples were incubated for at least 2 h at 37 °C. All samples were calibrated for 2 min before the experiment was performed. Briefly, the light scattering intensity was measured at various concentrations ranging from 0.015 µg/mL up to 1000 µg/mL starting from a 1 mg/mL stock solution by serial dilution with the respective buffer

solution at 25 °C. A series of three measurements were performed to check the reproducibility of the experiments.

Determination of the CMC

The CMC was determined following an established protocol.[2] The CMC was determined by the use of the DLS technique, and aqueous polymer solutions were prepared in respective buffer solutions. The concentration was not higher than 20-folds of the CMC to hinder any interaggregates of the micelles. The light scattering intensity (in kcps) was plotted against the concentration (in mg/L). A linear trend could be observed as the intensity of scattered light is proportional to the number of scattering particles. The linear behavior was no longer detected when the concentration was too high, indicating interaggregation occurred, and these concentrations were neglected. Subsequently, the light scattering intensity (in kcps) was plotted against the concentration (in mg/L) to determine the CMC where both axes were scaled logarithmically. Again, as the intensity of scattered light is proportional to scattering particles, a sharp increase in scattering intensity can be understood that amphiphilic unimers start forming aggregates. This point can be considered as CMC. Also, the derived count rate was approximated as a horizontal line when under-reaching the CMC since scattering is predominantly from the buffer system and can be correlated to be constant.

Fabrication of Empty Micelles

5 mg of the respective polymer were suspended in 500 µL of acetone (HPLC grade). Next, 100 µL of the separate buffer, e.g., PBS or PB buffer, was added, and the sample was placed in an ultrasonic bath until a turbid solution without precipitation was obtained. This solution was slowly added to a stirred solution of the same buffer (4.90 mL) as used in the step before. Finally, the acetone was removed under vacuum. MilliQ again adjusted the volume to a final volume of 5 mL ($c_{polymer}$ = 1 mg/mL). In all cases, clear solutions without precipitation were obtained. As described above, the formed micelles were analyzed in terms of their size, stability, size distribution, and ζ-Potential. The prepared micelles were stored at 4°C in the fridge.

Fabrication of Docetaxel-loaded Micelles

1 mg of Docetaxel and 5 mg of the respective polymer were suspended in 500 µL of acetone (HPLC grade). Next, 100 µL of the buffer, e.g., PBS or PB buffer, was added, and the sample was placed in an ultrasonic bath until a turbid solution without precipitation was obtained. This solution was slowly added to a stirred solution of the same buffer (4.90 mL) as used in the step before. Finally, the acetone was removed under vacuum. MilliQ again adjusted the volume to a final volume of 5 mL ($c_{polymer}$ = 1 mg/mL). To remove the non-encapsulated drug, the solution was passed through a 0.2 µm RC syringe filter. In all cases, clear solutions without precipitation were obtained. As described above, the formed micelles were analyzed in terms of their size, stability, size distribution, and ζ-Potential. The prepared micelles were stored at 4°C in the fridge. For the detection of the drug-loading content of Docetaxel, the micelle formulations were freeze-dried, and the dry powder was resuspended in 70:30 Acetonitrile/water. The DTX concentration was then detected by HPLC analysis (83 bar, UV detection: 227 nm). The standard curve was prepared by serial dilution of a 1 mg/mL stock solution of DTX ranging from 250 µg/mL to 16 µg/mL Acetonitrile/water 70:30.. The Drug Loading Efficiency (DLE%) and Drug Loading Capacity (DLC in wt%) were calculated accordingly:

$$DLC\% = \frac{Weight\ of\ DTX\ in\ Nanoformulation}{Weight\ of\ Sample} \times 100\%$$

$$DLE\% = \frac{Weight\ of\ DTX\ in\ Nanoformulation}{Weight\ of\ total\ DTX\ added}$$

Cell Viability Tests (CCK-8 Assay, Dulbecco's Modified Eagle's Medium (DMEM), 48 h).

Cells were seeded in a transparent 96-well plate with a density of 10 000 cells per well and cultured for 24 h. The medium (DMEM) was removed and replaced with a medium containing micelle (empty or loaded), followed by 48 h of incubation. Subsequently, 10 µL of premixed Cell Counting Kit-8 (CCK-8) solution (Dojindo Molecular Technologies, Inc., Rockville), containing the proprietary WST-8 tetrazolium salt, was added to each well. Viable cells reduce this salt to a formazan dye whose absorbance can be measured in the medium. The absorbance was measured at 450 nm using a Tecan Infinite 200 Pro microplate reader after 2 h. Three independent experimental runs with triplicates were performed (n = 3).

Cryo-TEM measurements

Perforated carbon film-covered microscopical 200 mesh grids (R1/4 batch of Quantifoil, MicroTools GmbH, Jena, Germany) were cleaned with chloroform and hydrophilized by 60 s glow discharging at 10 mA in a Safematic CCU-010 device (safematic GmbH, Zizers, Switzerland). Subsequently, 4 µl aliquots of the sample solution were applied to the grids. The samples were vitrified by automatic blotting and plunged to freezing with an FEI Vitrobot Mark IV (Thermo Fisher Scientific Inc., Waltham, Massachusetts, USA) using liquid ethane as a cryogen. The vitrified specimens were transferred to the autoloader of an FEI TALOS ARCTICA electron microscope (Thermo Fisher Scientific Inc., Waltham, Massachusetts, USA). This microscope is equipped with a high-brightness field-emission gun (XFEG) operated at an acceleration voltage of 200 kV. Micrographs were acquired on an FEI Falcon 3 direct electron detector (Thermo Fisher Scientific Inc., Waltham, Massachusetts, USA) using a 100 µm objective aperture.

Spectral studies: UV-VIS Measurements

For UV-VIS measurements, the samples were prepared as described above. The samples were measured using an Agilent Cary 8454 in 12 mm square glass cuvettes (Hellma Analytics) at a constant temperature of 37 °C. Before every measurement, a background measurement was performed. All samples were calibrated for 2 minutes at 37 °C.

Spectral studies: Fluorescence Measurements

For fluorescence measurements, the samples were prepared as described above. The samples were measured using a JASCO FP-6500 spectrometer in 12 mm square glass cuvettes (Hellma Analytics) at a constant temperature of 37 °C. Before every measurement, a background measurement was performed. All samples were calibrated for 2 minutes at 37 °C. For pyrene detection, the samples were excited at a wavelength of λ_{ex} = 350 nm. For FITC detection, the samples were excited at a wavelength of λ_{ex} = 480 nm.

CMC determinations of empty/DTX-loaded dPGS-SS-PCL-Et, dPGS-SS-PCL-Py, and dPGS-SS-POxPPh-Py

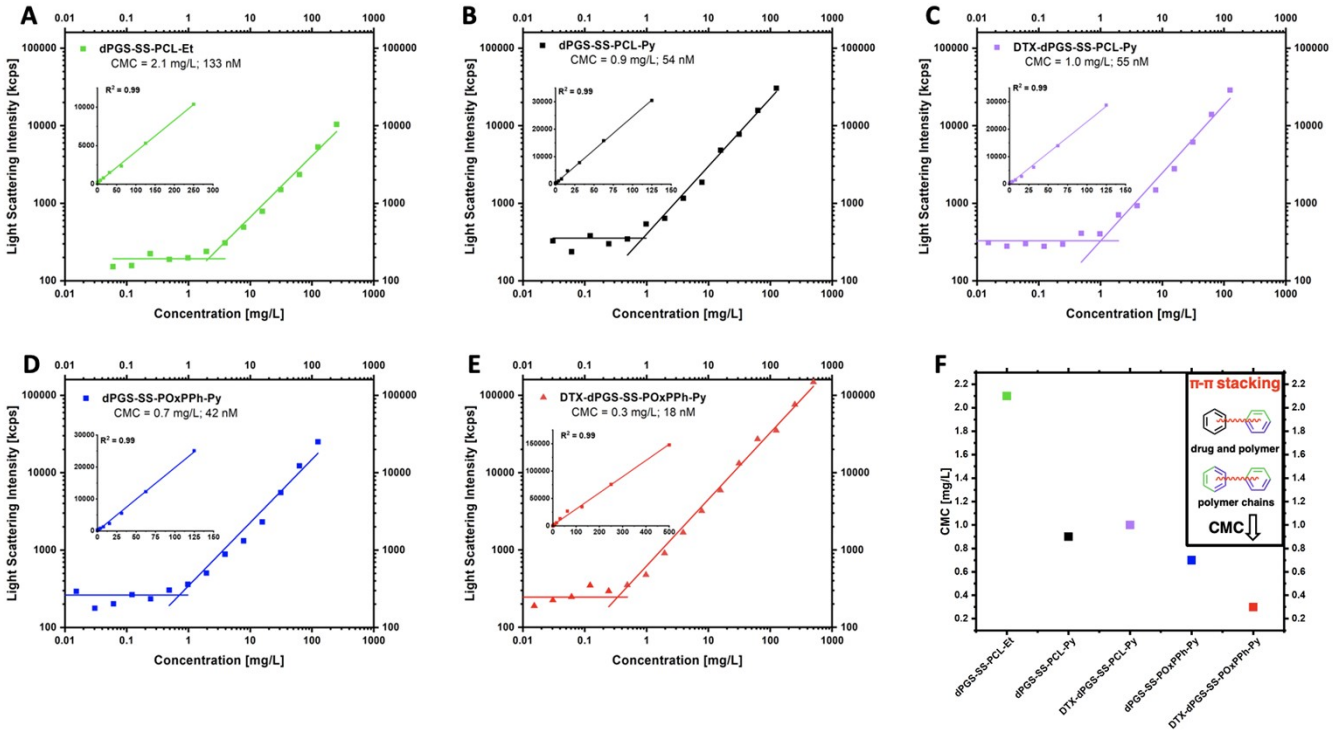


***Figure S 34**. CMC determinations of **(A)** dPGS-SS-PCL-Et **(B)** dPGS-SS-PCL-Py **(C)** DTX-dPGS-SS-PCL-Py **(D)** dPGS-SS-POxPPh-Py **(E)** DTX-dPGS-SS-POxPPh-Py; in PBS at 37 °C by a light scattering experiment with different concentrations (inset: linear dependencies of the count rate and concentration; R^2 = 0.99) **(F)** influence of the π-electron density on the CMC; π-π interactions significantly decrease the CMC.*

Cross-Polarization Solid-State ^{13}C NMR of free DTX, Empty dPGS-SS-POxPPh-Py, and dPGS-SS-POxPPh-Py@DTX

The ^{13}C CP solid-state measurements were conducted at 8.8 T using a JEOL ECZ600 spectrometer operating at 600 MHz proton resonance. The samples were freshly prepared in aqueous solutions as described above, followed by lyophilization to obtain dry powders. The powders were mortared, and 30 mg of the respective sample was loaded onto the MAS rotors (diameter: 3.2 mm). All chemical shifts are reported relative to Adamantane (^{13}C) as an external reference.

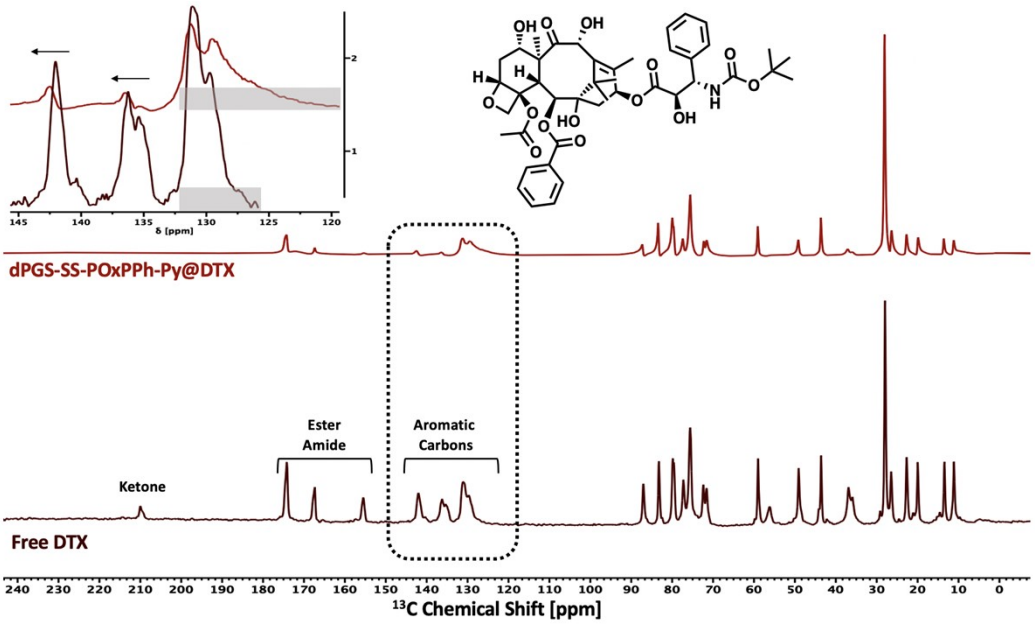


Figure S 35. *Solid-State ^{13}C CP NMR spectra of free DTX (below, dark red), and dPGS-SS-POxPPh-Py@DTX formulation (above, light red). An enlarged section from the stacked spectra shows the aromatic carbons of DTX as they appear mainly to be affected by the interaction with the polymer. A general trend of broader (grey areas) and slightly shifted signals (indicated by arrows to the left) in the formulation compared to the free drug can be found.*

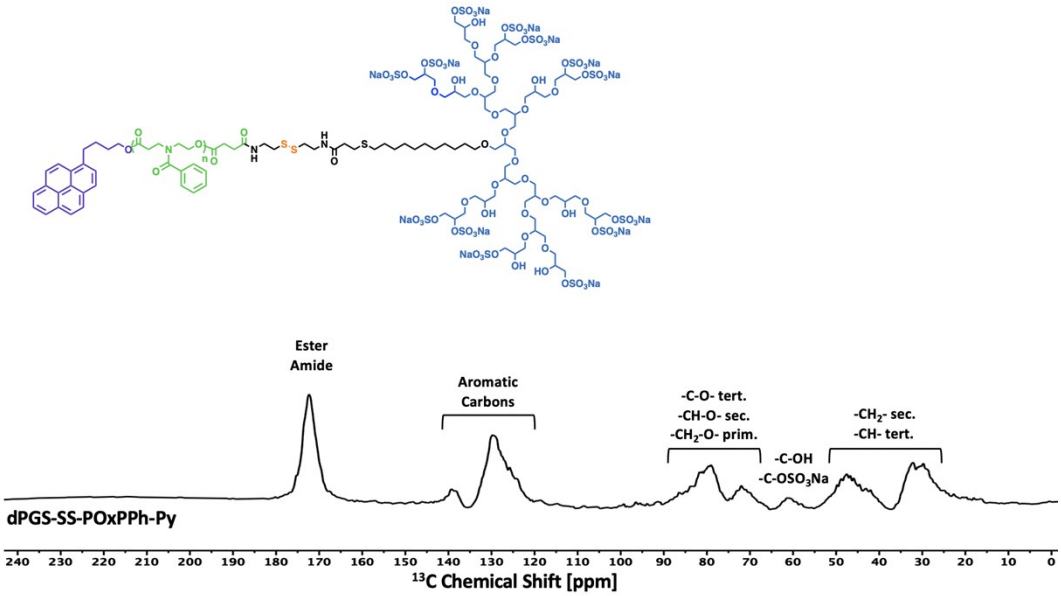


Figure S 36. *Solid-State ^{13}C CP spectrum of empty dPGS-SS-POxPPh-Py.*

References

[1] X. Wang, N. Hadjichristidis, *ACS Macro Letters* **2020**, *9*, 464-470.

[2] D. Braatz, M. Dimde, G. Ma, Y. Zhong, M. Tully, C. Grötzinger, Y. Zhang, A. Mavroskoufis, M. Schirner, Z. Zhong, M. Ballauff, R. Haag, *Biomacromolecules* **2021**, *22*, 2625-2640.