Supporting Information

Cellular Uptake Evaluation of Amphiphilic Polymer Assemblies: Importance of Interplay between Pharmacological and Genetic Approaches

Ziwen Jiang,[†] *Huan He*,[†] *Hongxu Liu*,[†] *S. Thayumanavan*[*,†,‡,§]

[†]Department of Chemistry, [‡]Molecular and Cellular Biology Program, [§]Center for Bioactive Delivery at the Institute for Applied Life Sciences, University of Massachusetts Amherst, Massachusetts 01003, United States

[*]Email: thai@chem.umass.edu (S.T.)

Table of Contents

1. Supplementary Figures and Table

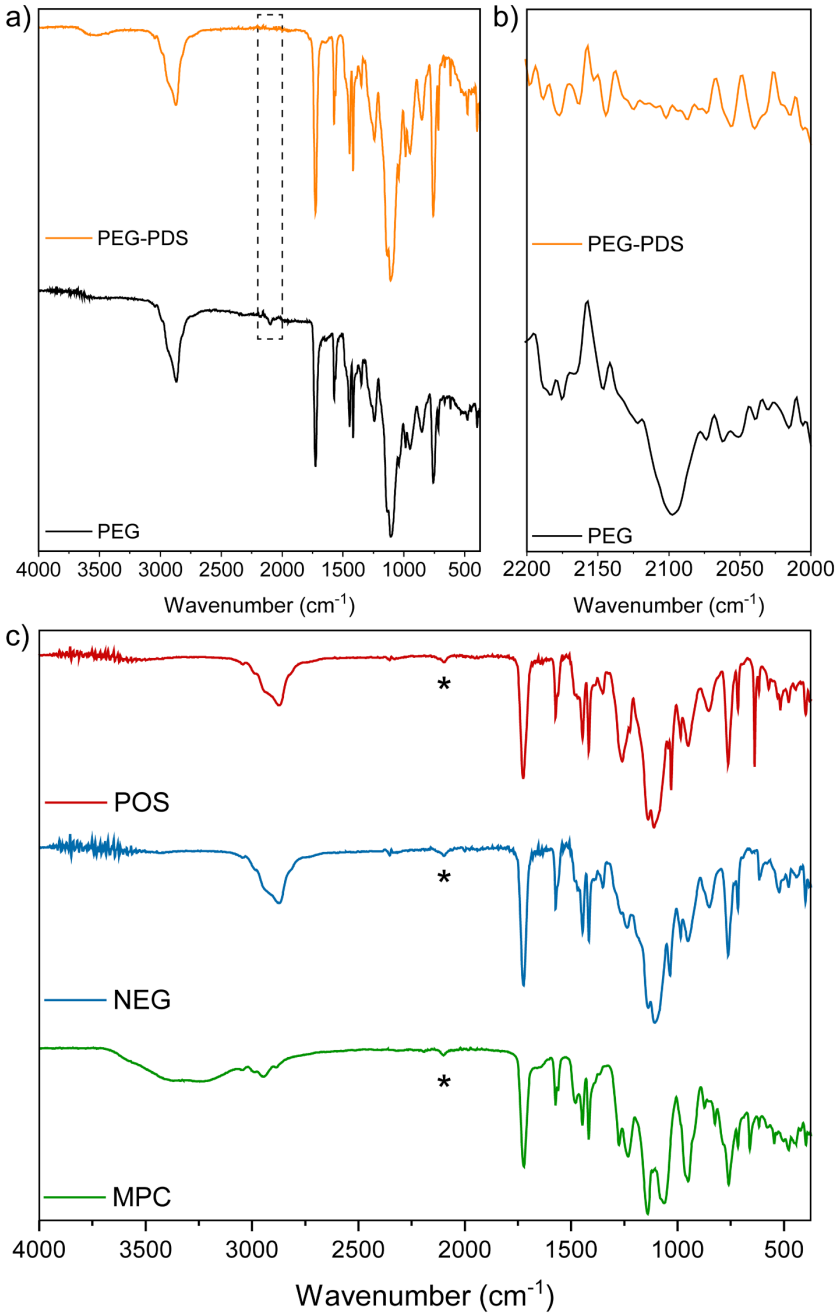


Figure S1. FTIR spectra of (a,b) **PEG-PDS** and **PEG**, (c) **POS**, **NEG**, and **MPC**. The comparison between **PEG-PDS** and **PEG** demonstrated the appearance of azido group (2100 cm^{-1}) in the polymer. **PEG-PDS** was synthesized without azido-modifications on the chain transfer agent and radical initiator. The strategy ensures the appearance of azido group on amphiphilic polymers with different surface charge.

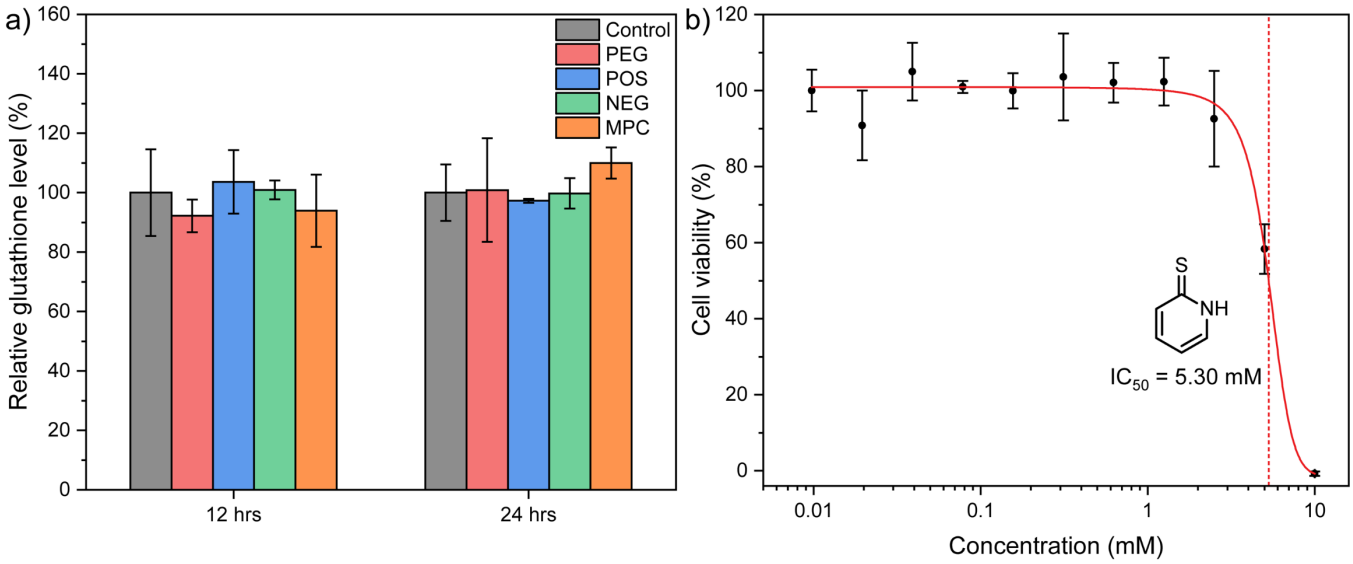


Figure S2. (a) Relative level of intracellular glutathione (GSH) in HeLa cells after incubating with each amphiphilic polymer (0.2 mg·mL^{-1}) at different time points. No significant change in GSH level was observed after incubating amphiphilic polymers with cells for 24 hours. The GSH level in HeLa cells that treated in complete growth medium without polymer added was normalized as 100%. *N* = 3. (b) Half maximal inhibitory concentration (IC_{50}) of 2-mercaptopyridine in HeLa cells at 24-hour time point. From alamarBlue assay, 2-mercaptopyridine has an IC_{50} value at (5.30 ± 0.22) mM, which is equal to degrading PDS homopolymers at a concentration of 1.35 mg·mL^{-1}. *N* = 4. In each figure, error bars represent the standard deviation of replicates.

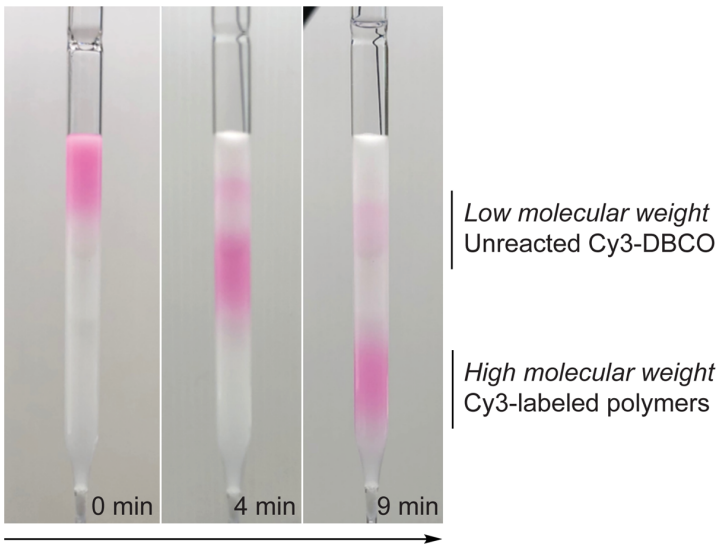


Figure S3. A representative purification process for Cy3-labeled amphiphilic polymers by size-exclusion chromatography over SorbaDex 20-LH matrix.

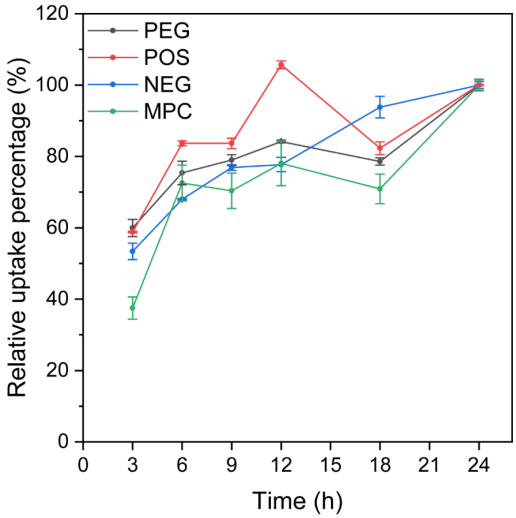


Figure S4. Cellular uptake of amphiphilic polymers at different time point. The median fluorescence Cy3-labeled polymer intensity of the HeLa cells is used as the reference and normalized as 100%. The decrease in cellular uptake efficiency from 12-hour to 18-hour is possibly related to cell proliferation. For the experiment, a total number of 30 k HeLa cells were seeded 24 hours before the experiment. Next, cells were incubated with polymer-containing (0.05 mg • mL^{-1} for **PEG**, **POS**, and **MPC**, 0.01 mg·mL^{-1} for **NEG**) complete DMEM growth medium for different time length and measured with flow cytometry. *N* = 3. Error bars represent the standard deviation of replicates.

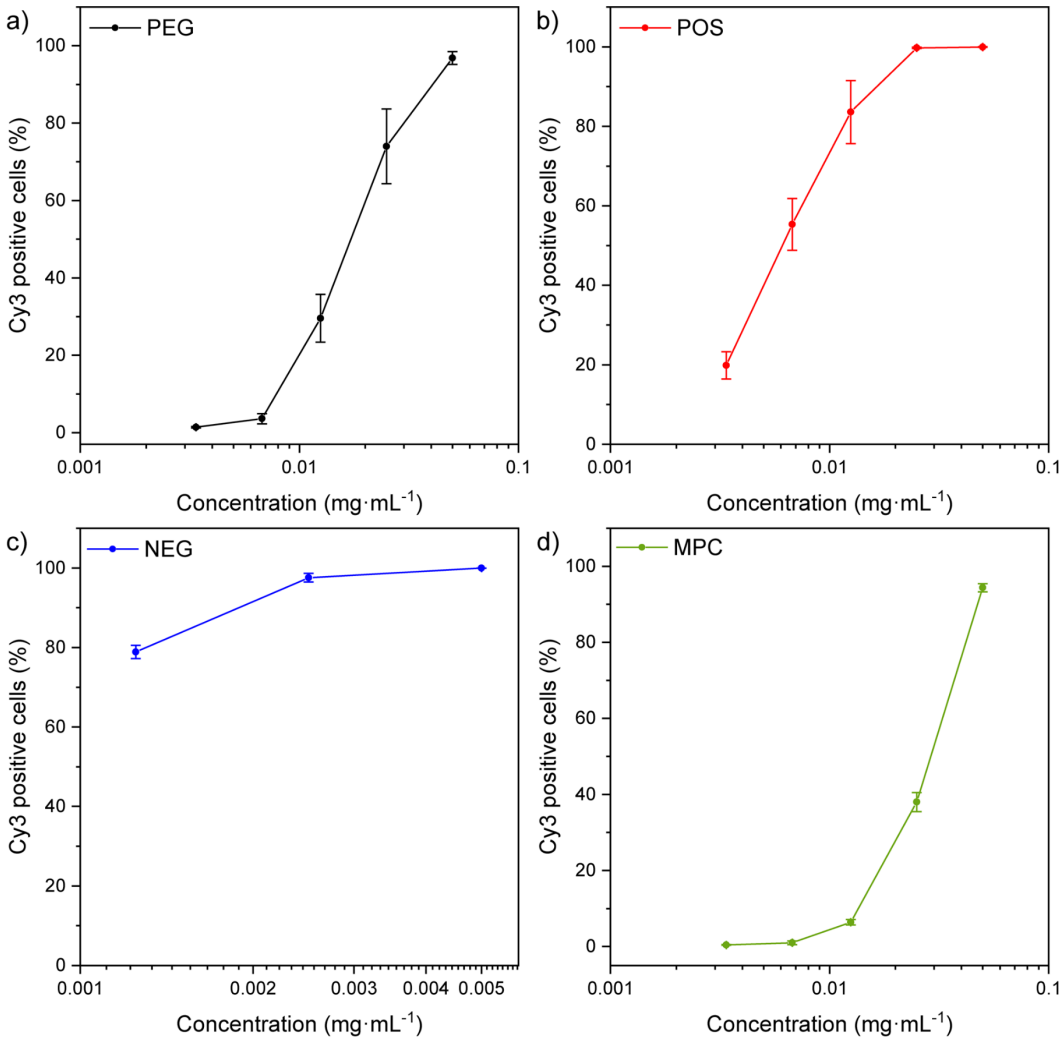


Figure S5. Cellular uptake evaluation of Cy3-labeled polymers at different concentrations in HeLa cells. For the experiment, a total number of 10 k HeLa cells were seeded 24 hours before the experiment. Next, cells were incubated with polymer-containing DMEM at different concentrations for 3 hours. The percentage of Cy3 positive cells was measured with flow cytometry. The highly efficient cellular uptake of **NEG** is currently under investigation. *N* = 4. Error bars represent the standard deviation of replicates.

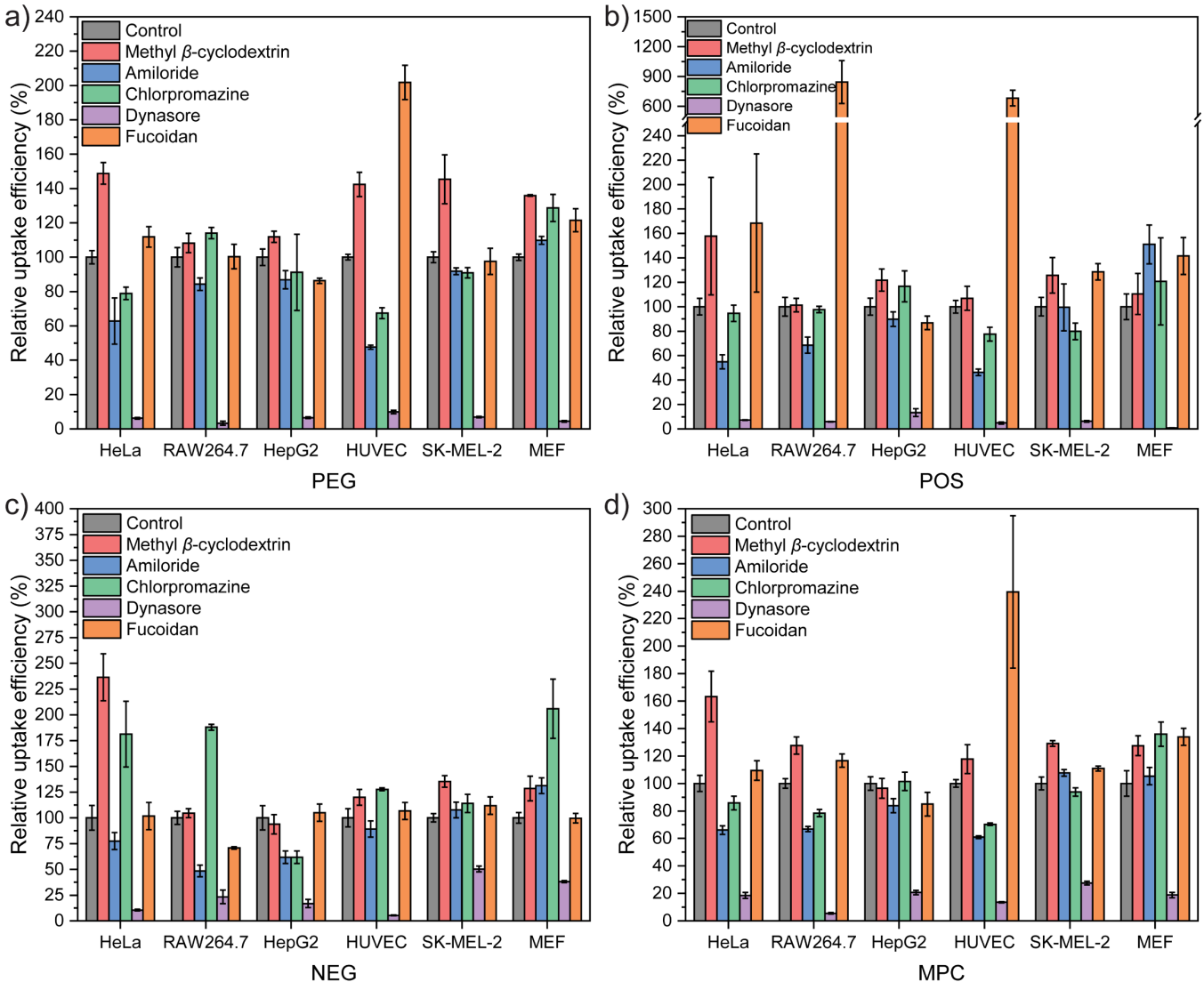


Figure S6. Cellular uptake efficiency of amphiphilic polymers in the presence of pharmacological inhibitors. The evaluation was performed in six different types of cells: HeLa, RAW264.7, HepG2, HUVEC, SK-MEL-2, and MEF. *N* = 4. Error bars represent the standard deviation of replicates. AMI, amiloride. MβCD, methyl-β-cyclodextrin. CPZ, chlorpromazine. DYN, dynasore. FCD, fucoidan.

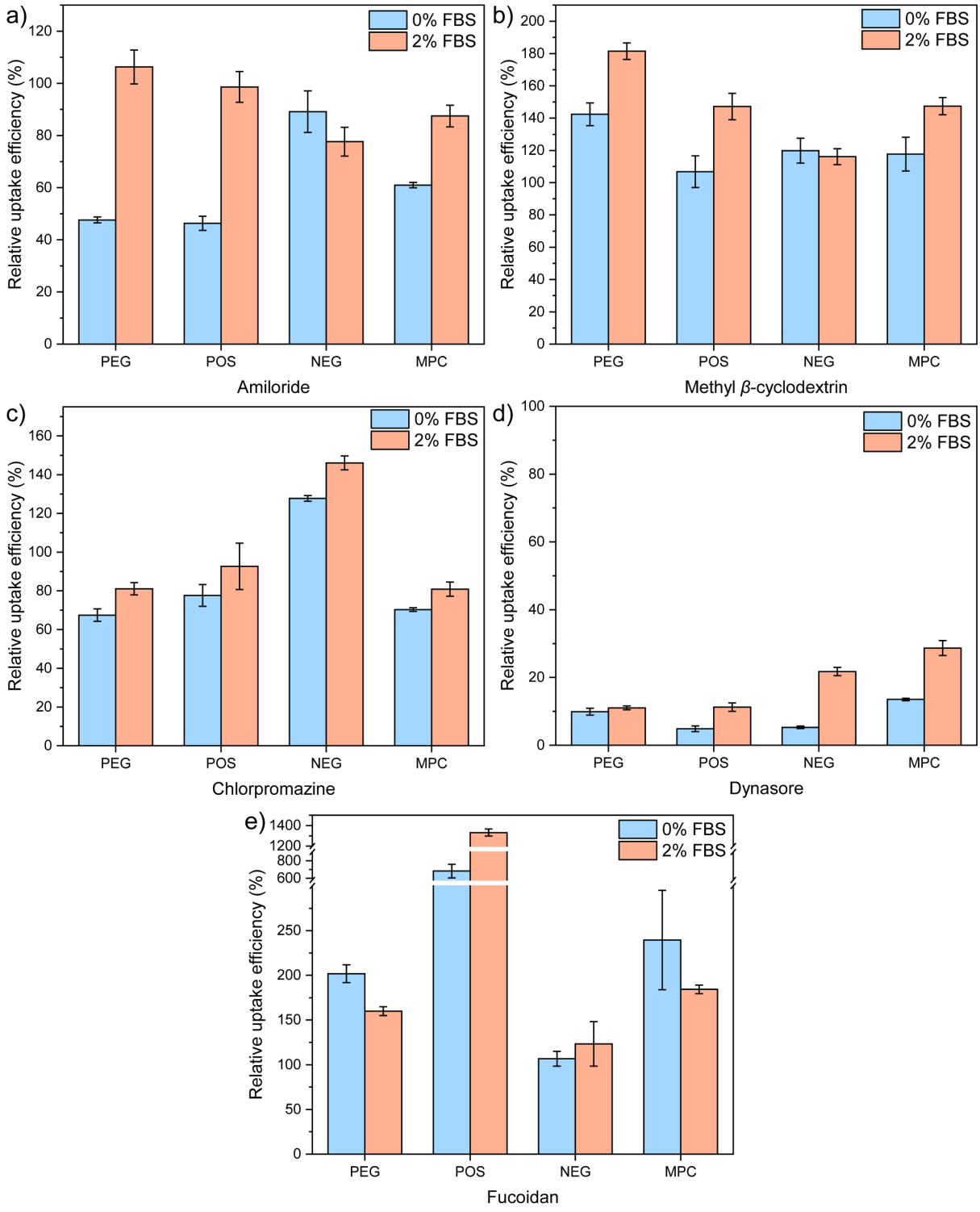


Figure S7. Effect of fetal bovine serum on the cellular uptake efficiency of amphiphilic polymers in the presence of pharmacological inhibitors. The evaluation was conducted in HUVEC. *N* = 4. Error bars represent the standard deviation of replicates.

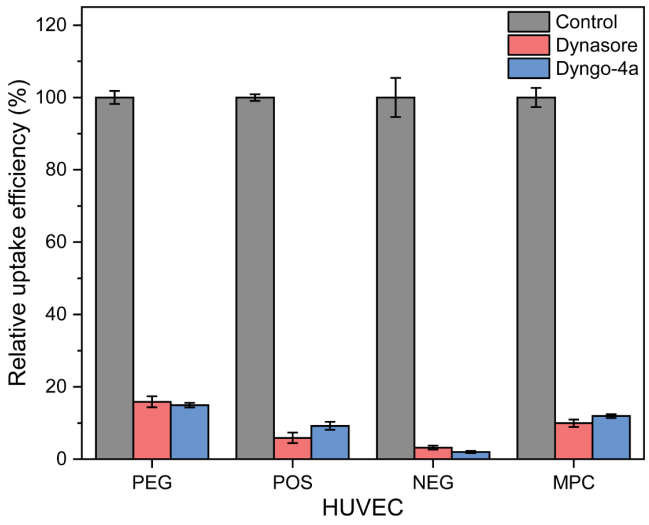


Figure S8. Cellular uptake efficiency of amphiphilic polymers in HUVEC in the presence of Dyngo-4a. *N* = 4. Error bars represent the standard deviation of replicates.

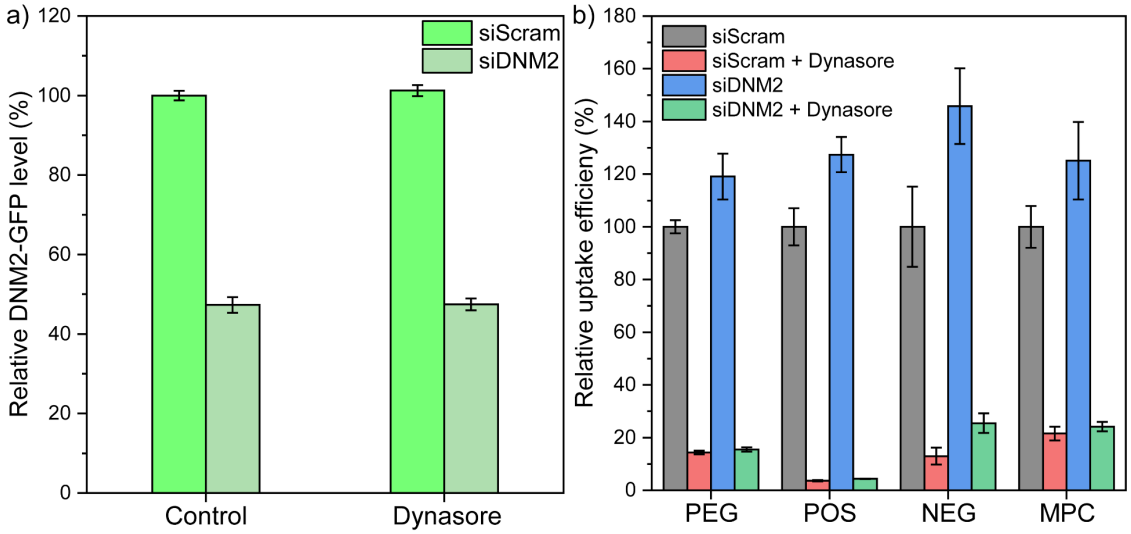


Figure S9. (a) Relative level of DNM2-GFP expression in SK-MEL-2 cells. Cells were treated with either scrambled siRNA or siRNA of dynamin-2 for 48 hours, with or without the subsequent treatment using dynasore. The GFP intensity of scrambled siRNA-treated cells without subsequent dynasore treatment was normalized as 100%. *N* = 16. (b) Cellular uptake efficiency of amphiphilic polymers in SK-MEL-2 cells. The cellular uptake intensity of polymers (Cy3 intensity) in scrambled siRNA-treated cells without subsequent dynasore treatment was normalized as 100%. *N* = 4. In each figure, error bars represent the standard deviation of replicates.

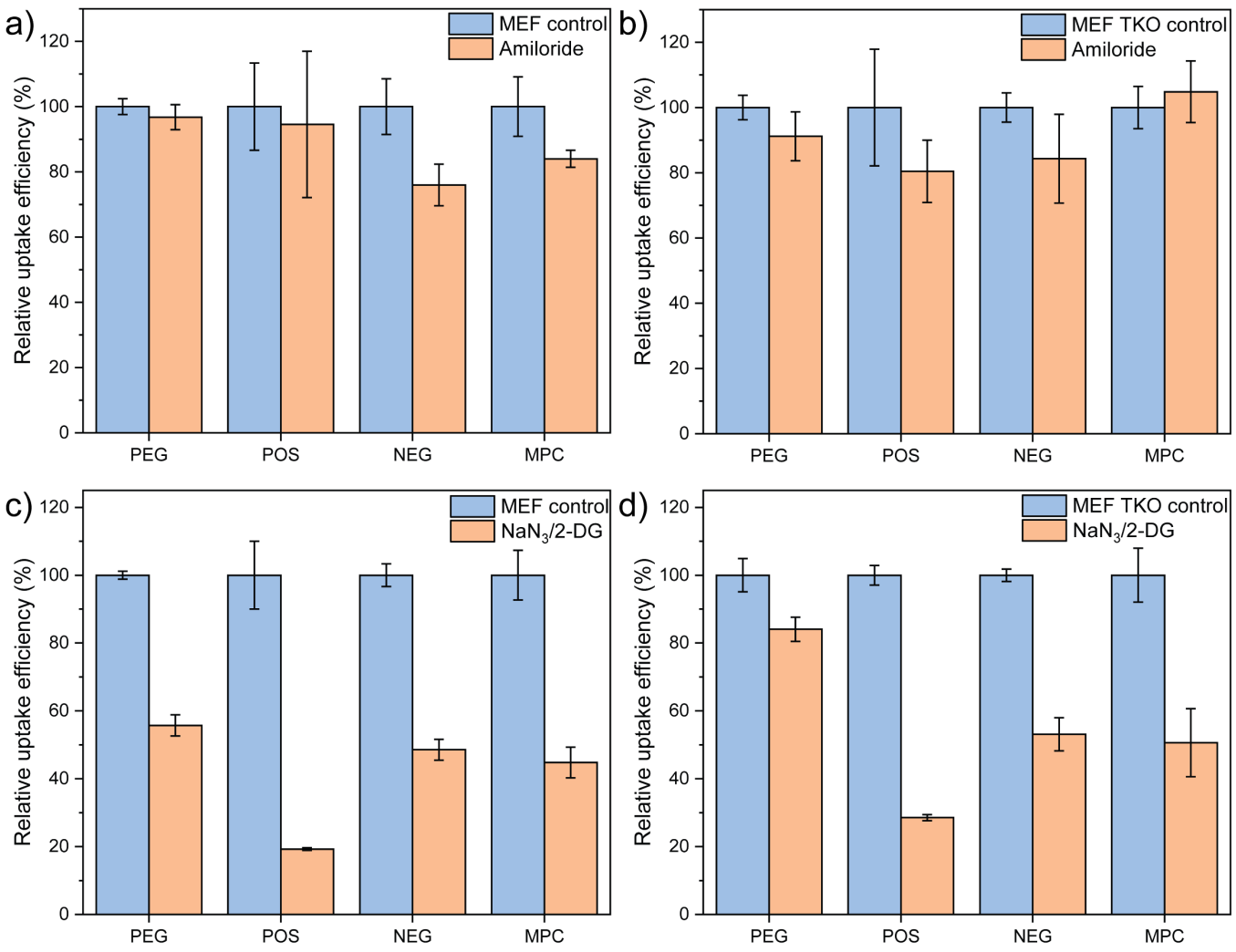


Figure S10. (a~b) Cellular uptake efficiency of amphiphilic polymers in (a) dynamin-containing mouse embryo fibroblasts (MEF), (b) dynamin triple knockout mouse embryo fibroblasts (MEF TKO) in the presence of amiloride. *N* = 4. (c~d) Cellular uptake efficiency of amphiphilic polymers in (c) MEF, (d) MEF TKO in the presence of NaN_3/2-DG, an ATP-depleted condition. Previous reports have shown that the intracellular ATP can be depleted by treating cells with NaN_3[1] or 2-deoxy-D-glucose (2-DG).[2] *N* = 4. In each figure, error bars represent the standard deviation of replicates.

Table S1. Resource and tolerated dose of pharmacological inhibitors used in the study.

Inhibitor	Source	Identifier	Tolerated dose*
Amiloride	Sigma-Aldrich	Cat# A7410	1 mM[†,3]
Bafilomycin A1	Santa Cruz Biotechnology	Cat# sc-201550	500 nM[‡]
Chlorpromazine	TCI America	Cat# C2481	10 μM[‡]
Cytochalasin B	Abcam	Cat# ab143482	1 μM[§]
Cytochalasin D	Sigma-Aldrich	Cat# C8273	1 μM[§]
2-Deoxy-D-glucose	Santa Cruz Biotechnology	Cat# sc-202010	50 mM[†,4]
Dynasore	Santa Cruz Biotechnology	Cat# sc-202592	80 μM[†,5]
Dyngo 4a	Abcam	Cat# ab120689	30 μM[†,6]
Dynole 34-2	Santa Cruz Biotechnology	Cat# sc-362731	6 μM[‡]
Fucoidan	Carbosynth	Cat# YF01606	0.1 mg·mL^{-1}[†,7]
Genistein	Santa Cruz Biotechnology	Cat# sc-3515	200 μM[‡]
Latrunculin A	Abcam	Cat# ab144290	1 μM[§]
16-epi-Latrunculin B	Abcam	Cat# ab144292	1 μM[§]
Mdivi-1	Sigma-Aldrich	Cat# M0199	120 μM[‡]
Methyl-*β*-cyclodextrin	TCI America	Cat# M1356	3.5 mg·mL^{-1}[†,8]
Nocodazole	Santa Cruz Biotechnology	Cat# sc-3518	20 μM[†,9]
Nystatin	Sigma-Aldrich	Cat# N4014	27 μM[†,10]
OcTMAB	Sigma-Aldrich	Cat# 359246	6 μM[‡]
Sodium azide	Sigma-Aldrich	Cat# 71289	10 mM[†,4]
Pitstop 2	Sigma-Aldrich	Cat# SML1169	6 μM[‡]

***** Tolerated dose represents the concentration of the inhibitor that was used to treat HeLa cells. It was chosen either based on literature report or integrating the result from microscopic visualization of cell morphology and alamarBlue assay.

[†] The concentration was chosen based on previous reports.

[‡] The concentration was determined by applying the inhibitor to cells for 4 hours and subsequently checking the cell viability.

[§] The concentration was determined by applying the inhibitor to cells for 1 hour and subsequently checking the cell viability.

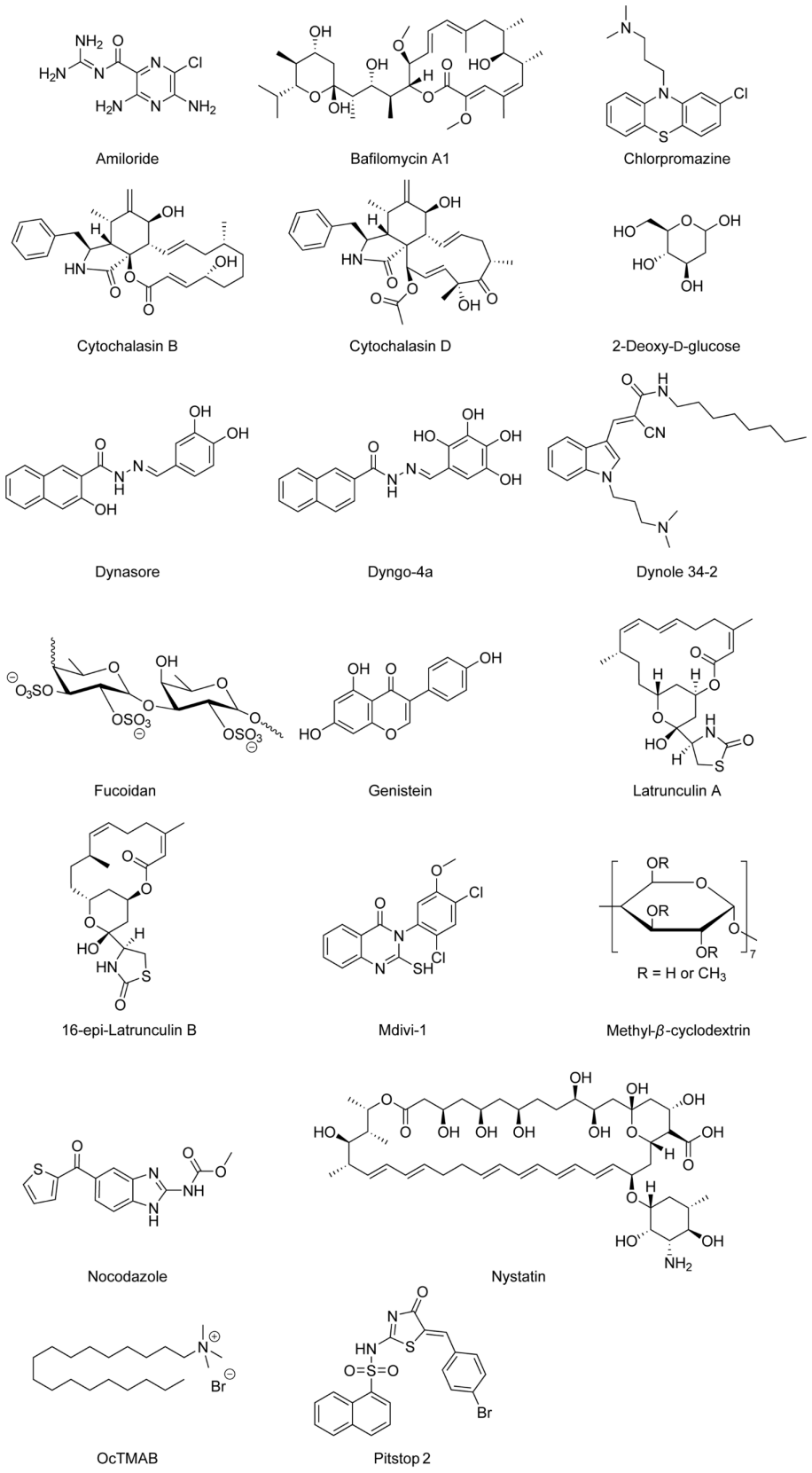


Figure S11. Structure of the pharmacological inhibitors used in the study.

Table S2. Summary on endocytic pathways of materials with different surface charge.*,†

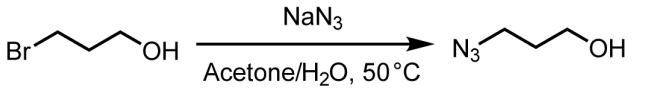
Material	Cationic	Anionic	Neutral
PEG-PLA[11]	CME, CaME, MP	MP	N/A
PEG-PLA[12]	CME, MP	CME, MP	N/A
PEG-oligo(cholic acid)[13]	CME, CaME, MP	CME, CaME, MP	CME, CaME, MP
PAMAM dendrimer[14]	Non-CME, Non-CaME	CaME	Non-CME, Non-CaME
Poly(styrene)[15]	CME, MP	CaME, MP	N/A
Poly(styrene)	CME[16]	CME, MP[17]	N/A
Poly(styrene)[18]	CaME, MP	CaME, MP	N/A
Polymer-DNA complex[19]	CME	CME	N/A
Chitosan[20]	CME, CaME	CME, CaME	N/A
Chitosan[21]	CME, MP	CME, MP	N/A
Liposome[22]	CME, CaME, MP	CME, CaME, MP	CME, CaME, MP
Graphene[23]	CME, MP	CME, MP	N/A
Quantum Dot[24]	N/A	CaME	N/A
Quantum Dot[25]	N/A	N/A	CME, MP
Quantum Dot[20, 26]	CME	CME	N/A
Gold nanoparticle[4]	CME, CaME	CaME	CaME
Gold nanoparticle[27]	N/A	N/A	MP
Upconversion NP[21, 28]	CME, CaME	CME, CaME	N/A
Upconversion NP[29]	CME	N/A	N/A
Silica nanoparticle[18]	CaME	CME, MP	N/A
Titanium dioxide NP[30]	CME, MP	CaME, MP	N/A

* MP, macropinocytosis. CME, clathrin-mediated endocytosis. CaME, lipid raft/caveolae-mediated endocytosis. N/A, not available.

† The summary is concluded based on either redirecting the conclusion from the reference or estimating from the results of the corresponding reference.

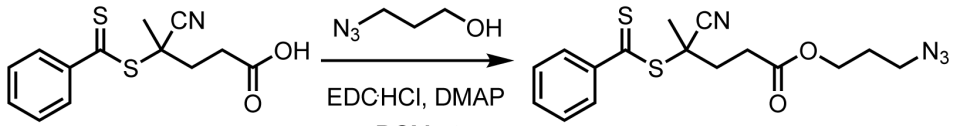
2. Experimental Section

2.1. Synthesis of chain transfer agent, radical initiator, and monomer(s)



3-Azido-1-propanol

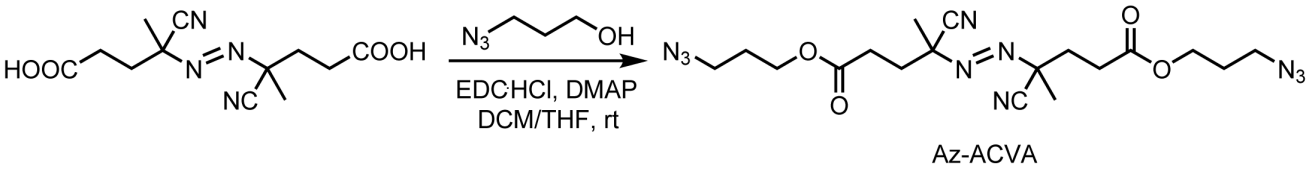
Synthesis of 3-azido-1-propanol. The compound was synthesized based on a previous report.[31] 3-Bromo-1-propanol (10 g, 71.9 mmol) and NaN_3 (10.6 g, 163 mmol) were dissolved in a mixture of H_2O/acetone (*v*:*v* = 3:10, 200 mL in total) and stirred at 50 °C overnight. The mixture was filtered and concentrated *in vacuo*. Dichloromethane (DCM, 200 mL) was added into the concentrated mixture and washed with deionized H_2O for three times. The organic layer was dried over $MgSO_4$ and concentrated *in vacuo*. The product was obtained as pale-yellow oil (6.5 g, 64.2 mmol, 89%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 3.81 – 3.71 (m, 2H), 3.46 (t, *J* = 6.6 Hz, 2H), 1.97 (s, 1H), 1.84 (p, *J* = 6.3 Hz, 2H) (Figure SP1). ^{13}C NMR (100 MHz, $CDCl_3$) δ 59.98, 48.61, 31.57 (Figure SP2).



Az-CTAP

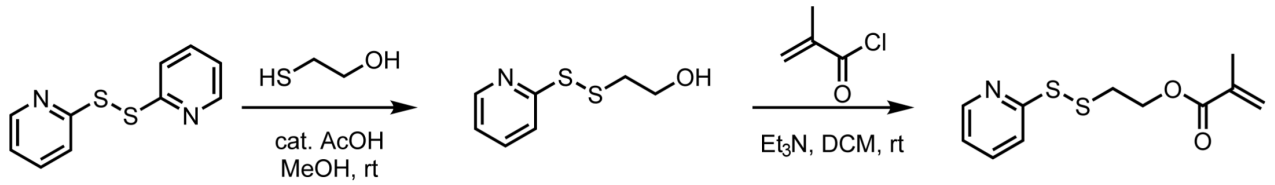
Synthesis of chain transfer agent (Az-CTAP). The compound was synthesized according to a previous report.[32] 4-Cyano-4-(phenylcarbonothioylthio)pentanoic acid (0.96 g, 3.42 mmol), 3-azido-1-propanol (1.73 g, 17 mmol), and 4-dimethylaminopyridine (DMAP, 20 mg, 0.17 mmol) were dissolved in 60 mL DCM. While the solution was cooling in an ice bath, *N*-(3-dimethylaminopropyl)-*N*′-ethylcarbodiimide hydrochloride (EDC·HCl, 1.96 g, 10 mmol) was added. Then the reaction mixture was stirred in an ice bath for 2 hours and warmed up to room temperature to continuously stir overnight. Subsequently, the reaction mixture was washed with saturated NaCl solution three times, dried with anhydrous Na_2SO_4 and

concentrated *in vacuo*. Finally, the concentrated mixture was purified by column chromatography over silica gel with *n*-hexanes–EtOAc (*v*:*v* = 5:1). The product was obtained as dark-red oil (0.87 g, 70%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.93 – 7.88 (m, 2H), 7.60 – 7.54 (m, 1H), 7.43 – 7.37 (m, 2H), 4.21 (t, *J* = 6.2 Hz, 2H), 3.41 (t, *J* = 6.6 Hz, 2H), 2.74 – 2.58 (m, 3H), 2.49 – 2.38 (m, 1H), 1.97 – 1.88 (m, 5H) (Figure SP3).



Az-ACVA

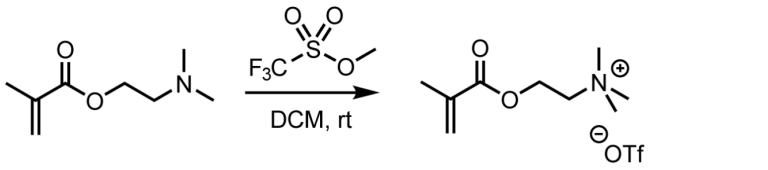
Synthesis of radical initiator (Az-ACVA). 4,4'-Azobis(4-cyanovaleric acid) (2 g, 7.1 mmol), 3-azido-1-propanol (2.16 g, 21.4 mmol), and DMAP (695 mg, 5.7 mmol) were dissolved in a mixture of DCM/THF (*v*:*v* = 1:1, 100 mL in total) and cooled in an ice bath. Subsequently, EDC·HCl (3.29 g, 17.1 mmol) was suspended in 10 mL DCM and added into the reaction mixture dropwise. The reaction mixture was stirred in the ice bath for another 3 hours and warmed up to room temperature to stir for 1 hour. Next, the reaction was quenched by washing with saturated NaCl solution for three times. The organic layer was dried over $MgSO_4$ and concentrated *in vacuo*. The mixture was then purified by column chromatography over silica gel with $CHCl_3$–EtOAc (*v*:*v* = 94:6). The product was obtained as transparent oil (2.27 g, 5.1 mmol, 72%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 4.21 (tdd, *J* = 6.1, 3.8, 1.8 Hz, 4H), 3.40 (td, *J* = 6.6, 2.9 Hz, 4H), 2.60 – 2.30 (m, 8H), 1.93 (pd, *J* = 6.4, 2.7 Hz, 4H), 1.73 (s, 3H), 1.68 (s, 3H) (Figure SP4). ^{13}C NMR (100 MHz, $CDCl_3$) δ 171.35, 117.62, 72.07, 62.26, 48.34, 33.30, 29.18, 28.20, 24.13 (Figure SP5). ESI-MS *m/z* calculated for $C_{18}H_{26}N_{10}NaO_4$ $[M + Na]^+$ 469.20, found 469.20.



Synthesis of pyridyl disulfide ethyl methacrylate. The compound was synthesized based on a previous report with modifications.[33] 2,2'-Dipyridyl disulfide (15 g, 68 mmol), 2-mercaptoethanol (4.4 g, 56.7 mmol), and acetic acid (1 mL) were dissolved in 100 mL MeOH and stirred at room temperature for 3 hours. The solvent was evaporated *in vacuo* and the yellow oil mixture was dissolved in 50 mL ethyl acetate. Next, *n*-hexanes was added into the ethyl acetate solution until small amount of yellow solid recrystallized from the solution. The mixture was placed in -20 °C overnight until the majority of 2-pyridinthione byproduct recrystallized. The yellow crystal was filtered and the filtrate was collected. After evaporating the solvent of the filtrate, the mixture was purified by flash chromatography over silica gel with *n*-hexanes–EtOAc (4:1 to 3:2). The product, 2-(pyridin-2-yldisulfanyl)ethanol was obtained as pale-yellow oil (7.7 g, 41 mmol, 72%). ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.51 (ddd, *J* = 5.0, 1.8, 0.9 Hz, 1H), 7.61 – 7.54 (m, 1H), 7.40 (dt, *J* = 8.1, 1.0 Hz, 1H), 7.15 (ddd, *J* = 7.4, 5.0, 1.1 Hz, 1H), 5.80 – 5.65 (s, 1H), 3.80 (t, *J* = 5.0 Hz, 2H), 2.98 – 2.92 (m, 2H) (Figure SP6).

In the second step, 2-(pyridin-2-yldisulfanyl)ethanol (7.54 g, 40.3 mmol) and triethylamine (4.9 g, 6.7 mL, 48.3 mmol) were dissolved in 30 mL anhydrous DCM and stirred in an ice bath. The mixture was purged with argon for 5 mins. Methacryloyl chloride (5.5 g, 5.1 mL, 52.3 mmol) was dissolved in 5 mL DCM and added into the reaction mixture dropwise. The mixture was then stirred at 4 °C for 2 hours under an argon atmosphere and then warmed up to room temperature to stir for 1 hour. Next, the mixture was washed with 10% NaCl solution for five times. The organic layer was dried over $MgSO_4$ and concentrated *in vacuo*. The concentrated mixture was purified by column chromatography over silica gel with *n*-hexanes–EtOAc (9:1 to 7:3). The product, pyridyl disulfide ethyl methacrylate was obtained as pale-yellow oil (6.95 g, 27.2 mmol, 67%). ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.47 (ddd, *J* = 4.8, 1.8, 0.9 Hz, 1H), 7.69 (dt, *J* = 8.3,

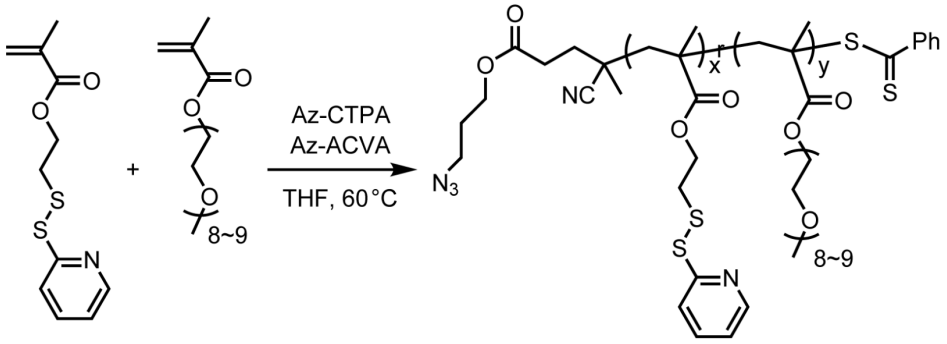
1.1 Hz, 1H), 7.62 (ddd, *J* = 8.1, 7.3, 1.8 Hz, 1H), 7.09 (ddd, *J* = 7.4, 4.8, 1.1 Hz, 1H), 6.12 (dt, *J* = 1.8, 1.0 Hz, 1H), 5.58 (p, *J* = 1.6 Hz, 1H), 4.40 (t, *J* = 6.4 Hz, 2H), 3.09 (t, *J* = 6.4 Hz, 2H), 1.94 (s, 3H) (Figure SP7). ^{13}C NMR (100 MHz, $CDCl_3$) δ 167.23, 159.94, 149.92, 137.20, 136.14, 126.20, 121.01, 119.94, 62.58, 37.62, 18.45 (Figure SP8).



2-(Methacryloyloxy)ethyltrimethylammonium triflate

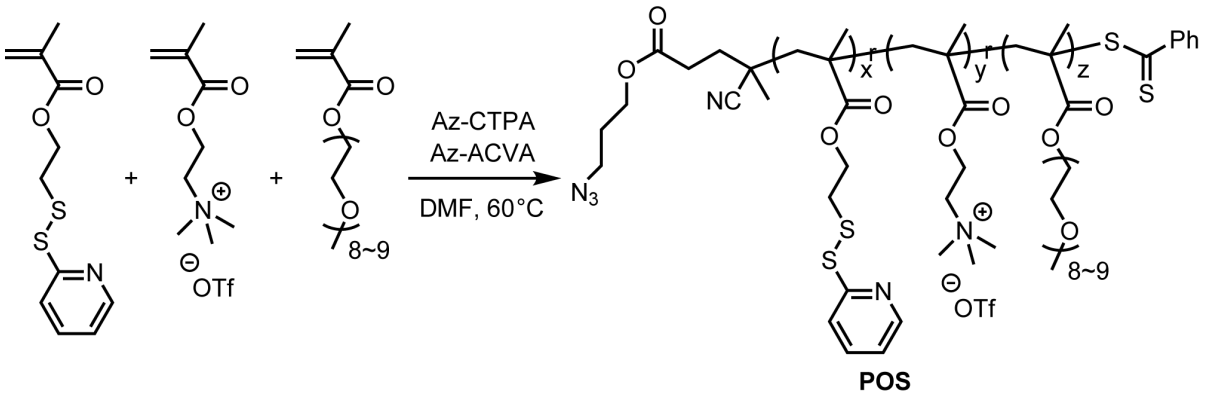
Synthesis of 2-(methacryloyloxy)ethyltrimethylammonium triflate. 2-(Dimethylamino)ethyl methacrylate (4.35 g, 4.66 mL, 27.7 mmol) was dissolved in 12 mL DCM and purged with argon for 5 mins. The solution was cooled in an ice bath. Methyl trifluoromethanesulfonate (5 g, 3.45 mL, 30.5 mmol) was added into the reaction mixture dropwise. The reaction mixture was warmed up to room temperature and stirred for 3 hours under an argon atmosphere. Next, the reaction mixture was added into cold diethyl ether dropwise. The white precipitate was collected and dried under vacuum. The product was obtained as white powder (8.66 g, 27 mmol, 97%). ^{1}H NMR (400 MHz, D_2O) δ 6.19 (d, *J* = 1.4 Hz, 1H), 5.80 (p, *J* = 1.6 Hz, 1H), 4.69 – 4.63 (m, 2H), 3.84 – 3.79 (m, 2H), 3.26 (s, 9H), 1.96 (s, 3H) (Figure SP9). ^{13}C NMR (125 MHz, D_2O) δ 168.42, 135.15, 127.63, 119.62 (q, *J* = 317.1 Hz), 64.61, 58.56, 53.73, 17.20 (Figure SP10). ^{19}F NMR (376 MHz, D_2O) δ -78.82 (Figure SP11).

2.2. Reversible addition–fragmentation chain transfer (RAFT) polymerization

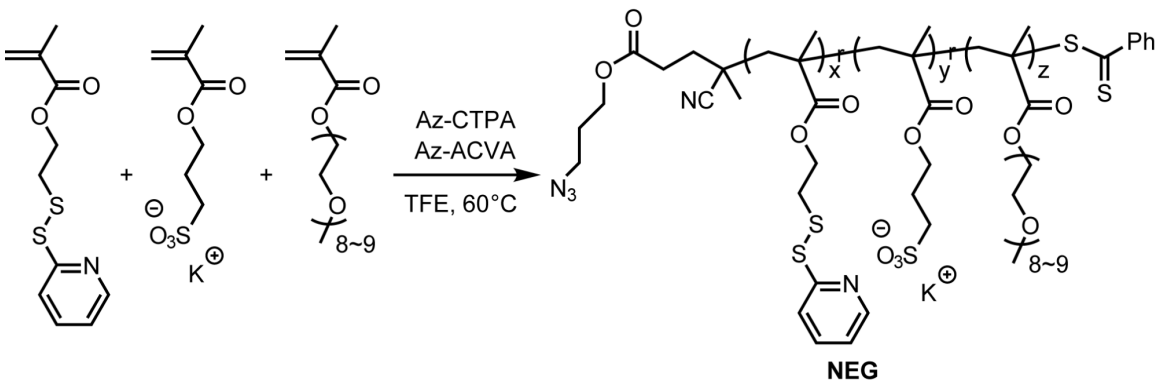


PEG

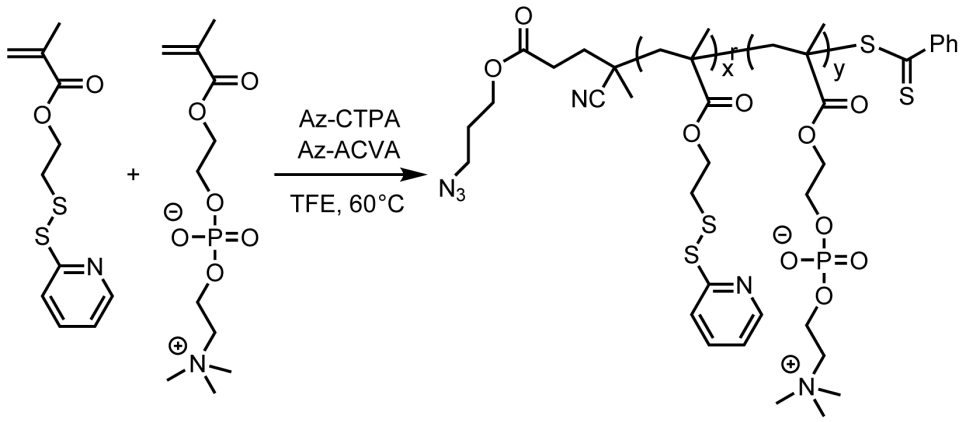
Synthesis of PEG, charge-neutral amphiphilic random copolymer. Pyridyl disulfide ethyl methacrylate (822 mg, 3.22 mmol, 31.9 equiv.), poly(ethylene glycol) methyl ether methacrylate (690 mg, 1.38 mmol, 13.7 equiv., M_n 500), Az-CTAP (36.6 mg, 0.1 mmol, 1.0 equiv.), and Az-ACVA (9 mg, 0.02 mmol, 0.2 equiv.) were dissolved in 3 mL anhydrous tetrahydrofuran (THF) and transferred to a 10-mL Schlenk flask. The flask was sealed and the reaction mixture was degassed with four freeze-pump-thaw cycles. After refilling the flask with an argon atmosphere, the reaction was dipped into a prewarmed oil bath at 60 °C and stirred for 20 hours. Next, the polymerization was quenched by freezing the mixture with liquid nitrogen and exposing to air. The polymer was purified by dissolving in $CHCl_3$ and subsequently precipitating in cold diethyl ether for three times. The product was dried under vacuum and obtained as viscous pink oil (1.45 g). GPC (THF), M_n: 10.3 K, $Đ$: 1.10. ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.45 (s), 7.66 (s), 7.09 (s), 4.21 (s), 4.06 (s), 3.71 – 3.56 (m), 3.54 (dd), 3.36 (s), 3.02 (s), 1.91 (s), 1.83 (s), 1.04 (s), 0.89 (s) (Figure SP12). The molar ratio between three repeating units was determined by integrating the aromatic proton in the pyridine and the methoxy proton (δ 3.3) in the polyethylene glycol side chain (x:y = 0.63:0.37). ^{13}C NMR (100 MHz, $CDCl_3$) (Figure SP13).



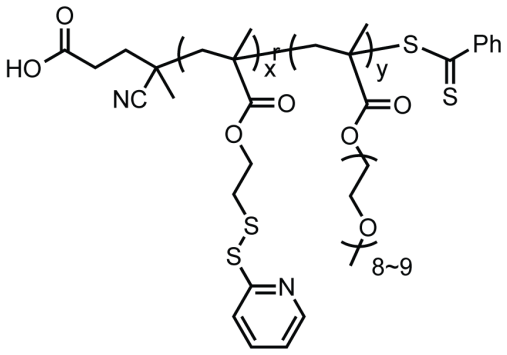
Synthesis of POS, cationic amphiphilic random copolymer. Pyridyl disulfide ethyl methacrylate (500 mg, 1.96 mmol, 33.8 equiv.), 2-(methacryloyloxy)ethyltrimethylammonium triflate (90 mg, 0.28 mmol, 4.8 equiv.), poly(ethylene glycol) methyl ether methacrylate (280 mg, 0.56 mmol, 9.7 equiv., M_n 500), Az-CTAP (21.0 mg, 0.058 mmol, 1.0 equiv.), and Az-ACVA (5.2 mg, 0.012 mmol, 0.2 equiv.) were dissolved in 1.8 mL anhydrous dimethylformamide (DMF) and transferred to a 10-mL Schlenk flask. The flask was sealed and the reaction mixture was degassed with four freeze-pump-thaw cycles. After refilling the flask with an argon atmosphere, the reaction was dipped into a prewarmed oil bath at 60 °C and stirred for 20 hours. Next, the polymerization was quenched by freezing the mixture with liquid nitrogen and exposing to air. The polymer was purified by dialyzing against acetone using 1 kD MWCO dialysis tubing for 24 hours. The product was dried under vacuum and obtained as viscous pink oil (731 mg). GPC (TFE), M_n: 28.6 k, $Đ$: 1.12. ^{1}H NMR (400 MHz, $(CD_3)_2CO$) δ 8.49 (s), 7.81 (s), 7.23 (s), 4.59 (s), 4.29 (s), 4.11 (s), 4.00 (s), 3.72 (d), 3.65 – 3.53 (m), 3.47 (t), 3.29 (s), 3.17 (s), 2.78 (d), 2.08 (s), 1.98 – 1.83 (m), 1.16 (d), 0.97 (s) (Figure SP14). The molar ratio of three repeating units was determined by integrating the aromatic proton in the pyridine, the methylene proton (δ 4.0) next to the quaternary amine, and the methoxy proton (δ 3.3) in the polyethylene glycol side chain (x:y:z = 0.65:0.11:0.24). ^{13}C NMR (100 MHz, $(CD_3)_2CO$) (Figure SP15).



Synthesis of NEG, anionic amphiphilic random copolymer. Pyridyl disulfide ethyl methacrylate (531 mg, 1.96 mmol, 34.6 equiv.), 3-sulfopropyl methacrylate potassium salt (73 mg, 0.30 mmol, 4.9 equiv.), poly(ethylene glycol) methyl ether methacrylate (297 mg, 0.59 mmol, 9.9 equiv., M_n 500), Az-CTAP (21.8 mg, 0.060 mmol, 1.0 equiv.), and Az-ACVA (5.4 mg, 0.012 mmol, 0.2 equiv.) were dissolved in 1.8 mL trifluoroethanol (TFE) and transferred to a 10-mL Schlenk flask. The flask was sealed and the reaction mixture was degassed with four freeze-pump-thaw cycles. After refilling the flask with an argon atmosphere, the reaction was dipped into a prewarmed oil bath at 60 °C and stirred for 20 hours. Next, the polymerization was quenched by freezing the mixture with liquid nitrogen and exposing to air. The polymer was purified by dialyzing against a mixture of acetone/MeOH (*v*:*v* = 1:1) using 1 kD MWCO dialysis tubing for 24 hours. The product was dried under vacuum and obtained as viscous pink oil (485 mg). GPC (TFE), M_n: 27.6 k, $Đ$: 1.18. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.45 (s), 7.67 (s), 7.10 (s), 4.22 (s), 4.08 (s), 3.95 (q), 3.74 – 3.57 (m), 3.57 – 3.51 (m), 3.38 (s), 3.02 (s), 1.92 (s), 1.83 (s), 1.05 (s), 0.89 (s) (Figure SP16). The molar ratio of three repeating units was determined by integrating the aromatic proton in the pyridine, the methylene proton (δ 2.9) next to the sulfonate group, and the methoxy proton (δ 3.3) in the polyethylene glycol side chain (x:y:z = 0.59:0.16:0.25). ^{13}C NMR (125 MHz, $CDCl_3$) (Figure SP17).



Synthesis of MPC, zwitterionic amphiphilic random copolymer. Pyridyl disulfide ethyl methacrylate (514 mg, 2.01 mmol, 39.3 equiv.), 2-methacryloyloxyethyl phosphorylcholine (255 mg, 0.86 mmol, 16.8 equiv.), Az-CTAP (18.6 mg, 0.051 mmol, 1.0 equiv.), and Az-ACVA (4.5 mg, 0.010 mmol, 0.2 equiv.) were dissolved in 1.6 mL trifluoroethanol (TFE) and transferred to a 10-mL Schlenk flask. The flask was sealed and the reaction mixture was degassed with four freeze-pump-thaw cycles. After refilling the flask with an argon atmosphere, the reaction was dipped into a prewarmed oil bath at 60 °C and stirred for 20 hours. Next, the polymerization was quenched by freezing the mixture with liquid nitrogen and exposing to air. The polymer was purified by dialyzing against a mixture of acetone/MeOH/H_2O (*v*:*v*:*v* = 1:1:1) using 1 kD MWCO dialysis tubing for 24 hours. The product was dried under vacuum and obtained as pale-pink powder (746 mg). GPC (TFE), M_n: 25.0 k, $Đ$: 1.30. ^{1}H NMR (400 MHz, CF_3CD_2OD) δ 8.11 (s), 7.80 – 7.49 (m), 7.03 (s), 4.25 – 3.84 (m), 3.44 (s), 3.02 (s), 2.94 – 2.78 (m), 1.82 (br), 0.99 (s), 0.84 (s) (Figure SP18). The molar ratio of two repeating units was determined by integrating the aromatic proton in the pyridine and the methylene proton (δ 2.9) next to the quaternary amine (x:y:z = 0.65:0.35). ^{13}C NMR (100 MHz, CF_3CD_2OD) (Figure SP19). ^{31}P NMR (162 MHz, CF_3CD_2OD) (Figure SP20).



PEG-PDS

Synthesis of PEG-PDS, charge-neutral amphiphilic random copolymer without azido-tag. The random copolymer was synthesized following a previous report,[34] using pyridyl disulfide ethyl methacrylate and poly(ethylene glycol) methyl ether methacrylate (M_n 500) as co-monomers. 4-Cyano-4-(phenylcarbonothioylthio)pentanoic acid was used as the chain transfer agent. 2,2′-Azobis(2-methylpropionitrile) was used as the radical initiator. The polymer was utilized to compare with the FTIR spectrum of the azido-tagged polymer (**PEG**) (Figure S1).

2.3. General procedure for end-group labeling *via* copper-free click chemistry

Amphiphilic polymers and DBCO-Cy3 (Lumiprobe, Cat# E10F0, 1.05 equiv. vs. the azido-end-group on the polymer) were dissolved in 2 mL trifluoroethanol and stirred at room temperature for 24 hours (Figure S3). The solvent was evaporated and the mixture was purified by gel permeation chromatography over SorbaDex 20-LH gel filtration matrix (Cat# 801009). The SorbaDex 20-LH matrix is compatible with organic eluent and was dispersed in the eluent for at least 1 hour before packing the column. A mixture of $CHCl_3$/MeOH (*v*:*v* = 3:1) was used as the eluent for **PEG**, **POS**, and **NEG**. A mixture of $CHCl_3$/TFE (*v*:*v* = 3:1) was used as the eluent for **MPC**. The fraction that runs faster represents the molecules with higher molecular weight (Figure S4). The high-molecular-weight fraction was respectively collected and dried under vacuum. The yield of the dye-conjugation is quantitative.

2.4. Preparation of the amphiphilic polymer stock solution

Amphiphilic polymer (1 mg) was first dissolved in 40 μL organic solvent (acetone for **PEG** and **POS**; trifluoroethanol for **NEG** and **MPC**). Deionized water (1 mL, pre-filtered with 0.22 μm syringe filter unit) was added dropwise into the organic solution of amphiphilic polymers while stirring. The mixture was continuously stirred at room temperature for 2 hours. Subsequently, a calculated amount of dithiothreitol (0.1 equiv. vs. the pyridine disulfide unit in polymer) stock solution was added into the mixture to crosslink ~20% pyridine disulfide unit. The mixture was then stirred overnight. Next, the mixture was purified and concentrated with deionized water using Amicon centrifugal filters with 3 k MWCO. The volume of the concentrated polymer solution was adjusted to afford the polymer stock solution at a concentration of 10 $mg \cdot mL^{-1}$. The stock solution would be directly spiked into cell culture medium for future studies.

2.5. General procedure for cell culture and viability assays

Cell culture. All cells used in the study were cultured in a humidified atmosphere (5% CO_2) at 37 °C. HeLa (Cat# CCL-2) and SK-MEL-2 (Cat# HTB-68) cell lines were purchased from ATCC. Human umbilical vein endothelial cell (HUVEC) was purchased from Lonza (Cat# C2519A). RAW264.7 and HepG2 cell lines were a gift from Dr. Vincent M. Rotello (University of Massachusetts Amherst). Primary mouse embryo fibroblasts (MEF) were a gift from Dr. Jesse Mager (University of Massachusetts Amherst). The DNM2-GFP SK-MEL-2 cell line was provided by Dr. David G. Drubin (University of California, Berkeley). Conditional dynamin triple knockout mouse embryo fibroblasts were a gift from Dr. Pietro De Camilli (Yale University). Except HUVEC, all cells were grown and passaged in Dulbecco's modified eagle medium: Nutrient mixture F-12 + 1× GlutaMAX (DMEM, ThermoFisher, Cat# 10565018) supplemented with 10% fetal bovine serum (FBS) and 1% antibiotics (100 $U \cdot mL^{-1}$ penicillin-streptomycin). HUVEC was grown and passaged in EGM-2 endothelial cell growth medium-2 BulletKit (Lonza, Cat# CC-3162). Cell viability assays were conducted with unlabeled amphiphilic polymers. Cellular uptake evaluation was performed with amphiphilic polymers respectively labeled with Cy3.

AlamarBlue assay to determine the biocompatible range of polymers. A total of 10k HeLa cells were cultured in a 96-well plate for 24 h prior to the experiment. To evaluate the cytotoxicity of amphiphilic polymers, a stock solution of the unlabeled amphiphilic polymers was made before the experiment. The stock solution was serially 2-fold diluted into full DMEM growth medium. Next, the complete growth medium for HeLa cells was replaced by the polymer-containing medium and incubated for 24 hours. After washing with phosphate buffer saline (PBS), the cells were incubated with 220 μL full DMEM/F12 growth medium containing 10% alamarBlue reagent for 60 min. Cell viability was calculated by measuring the fluorescence intensity of alamarBlue at 590 nm, with an excitation wavelength at 535 nm.

Glutathione fluorometric assay. The intracellular glutathione level of HeLa cells was evaluated using glutathione fluorometric assay kit (BioVision, Cat# K264). A total of 100k HeLa cells were cultured in a 6-well plate for 72 h prior to the experiment. The stock solution of unlabeled amphiphilic polymers was added into fresh complete growth medium to result in a concentration of 0.2 $mg \cdot mL^{-1}$. The cells were respectively incubated for 12 hours and 24 hours. After washing with phosphate buffer saline, the cells were dissociated from the plate and processed according to the manual of the kit. The fluorescence readout from each group was normalized based on the value of the control group in which no polymer was presented (Figure S2a).

Half maximal inhibitory concentration (IC_{50}) of 2-mercaptopyridine. The reaction byproduct between glutathione and the pyridine disulfide units in polymers is 2-mercaptopyridine. To evaluate the IC_{50} of 2-mercaptopyridine, a total of 10k HeLa cells were cultured in a 96-well plate for 24 hours prior to the experiment. The yellow solid (Sigma-Aldrich, Cat# M5852) was dissolved in complete DMEM/F12 growth medium and incubated with cells for 24 hours. After washing with phosphate buffer saline, the cells were incubated with 220 μL full DMEM growth medium containing 10% alamarBlue reagent for 60 min. Cell viability was calculated by measuring the fluorescence intensity of alamarBlue at 590 nm, with an

excitation wavelength at 535 nm. The IC_{50} value was obtained after fitting the dose-dependent cell viability data with DoseResp function in OriginPro 2018.

2.6. General procedure for the cellular uptake evaluation of amphiphilic polymers

Tolerated dose of pharmacological inhibitors. The tolerated dose for each inhibitor was chosen based on integrating the result from microscopic visualization of cell morphology and alamarBlue assay. A total of 10k HeLa cells were cultured in a 96-well plate for 24 hours prior to the experiment. To evaluate the cytotoxicity of pharmacological inhibitors, a stock solution of the inhibitor in DMSO was made before the experiment. Note that the water-soluble compounds (MβCD and fucoidan) were directly dissolved in DMEM. The stock solution was diluted into DMEM medium by 1,000 times, resulting in 0.1% DMSO residue in the medium. Next, the complete growth medium for HeLa cells was replaced by the inhibitor-containing DMEM and incubated for the time of interest (Table S1). After washing with phosphate buffer saline, the cells were incubated with 220 μL full DMEM growth medium containing 10% alamarBlue reagent for 60 min. Cell viability was calculated by measuring the fluorescence intensity of alamarBlue at 590 nm, with an excitation wavelength at 535 nm. The tolerated dose for each inhibitor was chosen based on integrating the result from alamarBlue assay and microscopic visualization of cell morphology.

Cellular uptake of amphiphilic polymers in ATP-depleted condition. A total of 20k HeLa cells were cultured in a 48-well plate for 24 hours prior to the experiment. For ATP depletion, cells were cultured for 1 hour in DMEM containing 10 mM NaN_3 and 50 mM 2-deoxy-D-glucose (2-DG). For temperature control, the 37 °C condition was maintained in the incubator and the 4 °C condition was maintained in the fridge. Subsequently, in the presence of inhibitors, the cells were incubated with Cy3-labelled amphiphilic polymers that spiked into the medium for additional 3 hours. The final concentration of each amphiphilic polymer was maintained at 10 $\mu g \cdot mL^{-1}$. After washing the cells with cold PBS, the fluorescence intensity of Cy3 within the cells was measured using flow cytometry with an excitation wavelength of 561 nm. For the positive control, HeLa cells were cultured in DMEM for 1 hour and incubated with Cy3-labelled

polymers for another 3 hours. For the blank group, HeLa cells were cultured in DMEM for 4 hours. After subtracting the fluorescence signal from the blank group, Cy3 fluorescence of the positive control group was normalized as 100%.

Cellular uptake of amphiphilic polymers in the presence of pharmacological inhibitors. *HeLa cells*. A total of 10k HeLa cells were cultured in a 96-well plate for 24 hours prior to the experiment. For inhibiting different endocytic pathways, cells were cultured for 1 hour in DMEM containing amiloride (1 mM), methyl-*β*-cyclodextrin (3.4 mg·mL^{-1}), chlorpromazine (10 μM), dynasore (80 μM), and fucoidan (0.1 mg·mL^{-1}), respectively. Subsequently, in the presence of inhibitors, the cells were incubated with Cy3-labelled amphiphilic polymers that spiked into the medium for additional 3 hours. The final concentration of amphiphilic polymers was maintained at 20 μg·mL^{-1} for **PEG**, 10 μg·mL^{-1} for **POS**, 2 μg·mL^{-1} for **NEG**, and 50 μg·mL^{-1} for **MPC**. After washing the cells with cold PBS, the fluorescence intensity of Cy3 within the cells was measured using flow cytometry with an excitation wavelength of 561 nm. For the positive control, HeLa cells were cultured in DMEM for 1 hour and incubated with Cy3-labelled polymers for another 3 hours. For the blank group, HeLa cells were cultured in DMEM for 4 hours. After subtracting the fluorescence signal from the blank group, Cy3 fluorescence of the positive control group was normalized as 100%.

RAW264.7 cells. A total of 20k RAW264.7 cells (less than 6 passages) were cultured in a 96-well plate for 24 hours prior to the experiment. For inhibiting different endocytic pathways, cells were cultured for 1 hour in DMEM containing amiloride (1 mM), methyl-*β*-cyclodextrin (1 mg·mL^{-1}), chlorpromazine (10 μM), dynasore (80 μM), and fucoidan (0.1 mg·mL^{-1}), respectively. Subsequently, in the presence of inhibitors, the cells were incubated with Cy3-labelled amphiphilic polymers that spiked into the medium for additional 3 hours. The final concentration of amphiphilic polymers was maintained at 20 μg·mL^{-1} for **PEG**, 10 μg·mL^{-1} for **POS**, 2 μg·mL^{-1} for **NEG**, and 50 μg·mL^{-1} for **MPC**. After washing the cells with cold PBS, the fluorescence intensity of Cy3 within the cells was measured using flow cytometry with an excitation

wavelength of 561 nm. For the positive control, RAW264.7 cells were cultured in DMEM for 1 hour and incubated with Cy3-labelled polymers for another 3 hours. For the blank group, RAW264.7 cells were cultured in DMEM for 4 hours. After subtracting the fluorescence signal from the blank group, Cy3 fluorescence of the positive control group was normalized as 100%.

HepG2 cells. A total of 20k HepG2 cells were cultured in a 96-well plate for 24 hours prior to the experiment. For inhibiting different endocytic pathways, cells were cultured for 1 hour in DMEM containing amiloride (1 mM), methyl-*β*-cyclodextrin (2 $mg \cdot mL^{-1}$), chlorpromazine (10 μM), dynasore (80 μM), and fucoidan (0.1 $mg \cdot mL^{-1}$), respectively. Subsequently, in the presence of inhibitors, the cells were incubated with Cy3-labelled amphiphilic polymers that spiked into the medium for additional 3 hours. The final concentration of amphiphilic polymers was maintained at 20 $\mu g \cdot mL^{-1}$ for **PEG**, 10 $\mu g \cdot mL^{-1}$ for **POS**, 2 $\mu g \cdot mL^{-1}$ for **NEG**, and 50 $\mu g \cdot mL^{-1}$ for **MPC**. After washing the cells with cold PBS, the fluorescence intensity of Cy3 within the cells was measured using flow cytometry with an excitation wavelength of 561 nm. For the positive control, HepG2 cells were cultured in DMEM for 1 hour and incubated with Cy3-labelled polymers for another 3 hours. For the blank group, HepG2 cells were cultured in DMEM for 4 hours. After subtracting the fluorescence signal from the blank group, Cy3 fluorescence of the positive control group was normalized as 100%.

HUVEC cells. A total of 25k HUVEC (less than 3 passages) were cultured in a 96-well plate for 24 hours prior to the experiment. For inhibiting different endocytic pathways, cells were cultured for 1 hour in FBS-free EGM-2 medium containing amiloride (1 mM), methyl-*β*-cyclodextrin (1 $mg \cdot mL^{-1}$), chlorpromazine (10 μM), dynasore (80 μM), and fucoidan (0.1 $mg \cdot mL^{-1}$), respectively. Subsequently, in the presence of inhibitors, the cells were incubated with Cy3-labelled amphiphilic polymers that spiked into the medium for additional 3 hours. The final concentration of amphiphilic polymers was maintained at 20 $\mu g \cdot mL^{-1}$ for **PEG**, 10 $\mu g \cdot mL^{-1}$ for **POS**, 2 $\mu g \cdot mL^{-1}$ for **NEG**, and 50 $\mu g \cdot mL^{-1}$ for **MPC**. After washing the cells with cold PBS, the fluorescence intensity of Cy3 within the cells was measured using flow cytometry with an

excitation wavelength of 561 nm. For the positive control, HUVEC cells were cultured in FBS-free EGM-2 medium for 1 hour and incubated with Cy3-labelled polymers for another 3 hours. For the blank group, HUVEC cells were cultured in FBS-free EGM-2 medium for 4 hours. After subtracting the fluorescence signal from the blank group, Cy3 fluorescence of the positive control group was normalized as 100%. To evaluate the effect of serum on the cellular uptake of polymers in HUVEC cells, the FBS-free EGM-2 medium was replaced with full EGM-2 growth medium in the above procedures.

SK-MEL-2 cells. A total of 20k SK-MEL-2 cells were cultured in a 96-well plate for 24 hours prior to the experiment. For inhibiting different endocytic pathways, cells were cultured for 1 hour in DMEM containing amiloride (1 mM), methyl-*β*-cyclodextrin (1 $mg \cdot mL^{-1}$), chlorpromazine (10 μM), dynasore (80 μM), and fucoidan (0.1 $mg \cdot mL^{-1}$), respectively. Subsequently, in the presence of inhibitors, the cells were incubated with Cy3-labelled amphiphilic polymers that spiked into the medium for additional 3 hours. The final concentration of amphiphilic polymers was maintained at 20 $\mu g \cdot mL^{-1}$ for **PEG**, 10 $\mu g \cdot mL^{-1}$ for **POS**, 2 $\mu g \cdot mL^{-1}$ for **NEG**, and 50 $\mu g \cdot mL^{-1}$ for **MPC**. After washing the cells with cold PBS, the fluorescence intensity of Cy3 within the cells was measured using flow cytometry with an excitation wavelength of 561 nm. For the positive control, SK-MEL-2 cells were cultured in DMEM for 1 hour and incubated with Cy3-labelled polymers for another 3 hours. For the blank group, SK-MEL-2 cells were cultured in DMEM for 4 hours. After subtracting the fluorescence signal from the blank group, Cy3 fluorescence of the positive control group was normalized as 100%.

Primary mouse embryo fibroblasts. A total of 10k MEF cells (less than 3 passages) were cultured in a 96-well plate for 24 hours prior to the experiment. Note that the cells were collected from mouse embryos by Dr. Jesse Mager (UMass Amherst). For inhibiting different endocytic pathways, cells were cultured for 1 hour in DMEM containing amiloride (1 mM), methyl-*β*-cyclodextrin (1 $mg \cdot mL^{-1}$), chlorpromazine (10 μM), dynasore (80 μM), and fucoidan (0.1 $mg \cdot mL^{-1}$), respectively. Subsequently, in the presence of inhibitors, the cells were incubated with Cy3-labelled amphiphilic polymers that spiked into the medium for additional

3 hours. The final concentration of amphiphilic polymers was maintained at 20 μg·mL^{-1} for **PEG**, 10 μg·mL^{-1} for **POS**, 2 μg·mL^{-1} for **NEG**, and 50 μg·mL^{-1} for **MPC**. After washing the cells with cold PBS, the fluorescence intensity of Cy3 within the cells was measured using flow cytometry with an excitation wavelength of 561 nm. For the positive control, MEF cells were cultured in DMEM for 1 hour and incubated with Cy3-labelled polymers for another 3 hours. For the blank group, MEF cells were cultured in DMEM for 4 hours. After subtracting the fluorescence signal from the blank group, Cy3 fluorescence of the positive control group was normalized as 100%.

2.7. Cellular uptake evaluation of amphiphilic polymers in the presence of dynasore analog

Flow cytometry. *HeLa cells*. A total of 10k HeLa cells were cultured in a 96-well plate for 24 hours prior to the experiment. For inhibiting different endocytic pathways, cells were cultured for 1 hour in DMEM containing dynasore (80 μM) and Dyngo-4a (30 μM), respectively. Subsequently, in the presence of inhibitors, the cells were incubated with Cy3-labelled amphiphilic polymers that spiked into the medium for additional 3 hours. The final concentration of amphiphilic polymers was maintained at 20 μg·mL^{-1} for **PEG**, 10 μg·mL^{-1} for **POS**, 2 μg·mL^{-1} for **NEG**, and 50 μg·mL^{-1} for **MPC**. After washing the cells with cold PBS, the fluorescence intensity of Cy3 within the cells was measured using flow cytometry with an excitation wavelength of 561 nm. For the positive control, HeLa cells were cultured in DMEM for 1 hour and incubated with Cy3-labelled polymers for another 3 hours. For the blank group, HeLa cells were cultured in DMEM for 4 hours. After subtracting the fluorescence signal from the blank group, Cy3 fluorescence of the positive control group was normalized as 100%.

HUVEC cells. A total of 25k HUVEC (less than 3 passages) were cultured in a 96-well plate for 24 hours prior to the experiment. For inhibiting different endocytic pathways, cells were cultured for 1 hour in FBS-free EGM-2 medium containing DMEM containing dynasore (80 μM) and Dyngo-4a (30 μM), respectively. Subsequently, in the presence of inhibitors, the cells were incubated with Cy3-labelled amphiphilic polymers that spiked into the medium for additional 3 hours. The final concentration of amphiphilic

polymers was maintained at 20 μg·mL^{-1} for **PEG**, 10 μg·mL^{-1} for **POS**, 2 μg·mL^{-1} for **NEG**, and 50 μg·mL^{-1} for **MPC**. After washing the cells with cold PBS, the fluorescence intensity of Cy3 within the cells was measured using flow cytometry with an excitation wavelength of 561 nm. For the positive control, HUVEC cells were cultured in FBS-free EGM-2 medium for 1 hour and incubated with Cy3-labelled polymers for another 3 hours. For the blank group, HUVEC cells were cultured in FBS-free EGM-2 medium for 4 hours. After subtracting the fluorescence signal from the blank group, Cy3 fluorescence of the positive control group was normalized as 100%.

Confocal microscopy. A total of 80k RAW264.7 cells (less than 6 passages) were seeded into a glass bottom dish (Cellvis, #D35C4-20-0-N) for 24 hours prior to the experiment. To evaluate the effect of dynasore, cells were cultured for 1 hour in DMEM containing dynasore (80 μM). Subsequently, in the presence of inhibitors, the cells were incubated with Cy3-labelled amphiphilic polymers that spiked into the medium for additional 3 hours. The final concentration of amphiphilic polymers was maintained at 100 μg·mL^{-1} for **PEG**, 80 μg·mL^{-1} for **POS**, 25 μg·mL^{-1} for **NEG**, and 100 μg·mL^{-1} for **MPC**. After washing with phosphate buffer saline, the cells were incubated with NucBlue (Hoechst 33342, 1 drop in 500 μL medium) for 5 min in FluoroBrite DMEM at 37 °C to stain the nucleus. The intracellular distribution was measured by confocal microscopy with excitation wavelengths of 405 nm (Hoechst 33342) and 561 nm (Cy3-labeled polymers).

2.8. Cellular uptake evaluation of amphiphilic polymers in DNM2-GFP SK-MEL-2 cells

Dynamin-2 knockdown in DNM2-GFP SK-MEL-2 cells. A total of 10k DNM2-GFP SK-MEL-2 cells were cultured in a 96-well plate in complete DMEM growth medium for 24 hours prior to the experiment. Note that these SK-MEL-2 cells with GFP tagged on one endogenous DNM2 allele[35] were provided by Dr. David G. Drubin (University of California, Berkeley). Lipofectamine RNAiMAX (ThermoFisher, Cat# 13778030) was used to transfect either scrambled siRNA (siScram, Sigma-Aldrich, Cat #SIC001) or

siRNA of dynamin-2 (siDNM2, ThermoFisher, Cat# S4212) into SK-MEL-2 cells. The sequence of siDNM2 is 5′-ACAUCAACACGAACCAUGA-3′. Lipofectamine RNAiMAX and siDNM2 (or siScram) were complexed for transfection based on the ThermoFisher manual. Briefly, each siRNA was diluted with nuclease-free water to afford 10 μM stock solution. The stock solution of siRNA (2 μL) was added into 100 μL Opti-MEM reduced serum medium (Opti-MEM, ThermoFisher, Cat# 51985034). Meanwhile, 6 μL of Lipofectamine was separately added into another 100 μL fresh Opti-MEM. Afterwards, 25 μL of lipid and siRNA was separately taken out from their Opti-MEM solution and mixed for 5 minutes at room temperature. DMEM was removed from cells and 100 μL fresh Opti-MEM was added to each well. The lipid-RNA complex containing Opti-MEM (10 μL) was added into each well and incubated for 48 hours or 72 hours. After washing with phosphate buffer saline, the siScram- or siDNM2-treated SK-MEL-2 cells are ready to be further evaluated.

Cellular uptake evaluation of amphiphilic polymers. The medium for siScram- or siDNM2-treated (48-hour or 72-hour) cells was replaced with DMEM. Cy3-labelled amphiphilic polymers were spiked into the medium to incubate for 3 hours. In parallel, to evaluate the effect of dynasore, the siScram- or siDNM2-treated cells were cultured for 1 hour in DMEM containing dynasore (80 μM). Subsequently, in the presence of dynasore, the cells were incubated with Cy3-labelled amphiphilic polymers that spiked into the medium for additional 3 hours. The final concentration of amphiphilic polymers was maintained at 20 $\mu g \cdot mL^{-1}$ for **PEG**, 10 $\mu g \cdot mL^{-1}$ for **POS**, 2 $\mu g \cdot mL^{-1}$ for **NEG**, and 50 $\mu g \cdot mL^{-1}$ for **MPC**. After washing the cells with cold PBS, the fluorescence intensity of GFP and Cy3 within the cells was measured using flow cytometry with excitation wavelengths of 488 nm and 561 nm. For the positive control, the fluorescence signal in siScram-treated SK-MEL-2 cells without dynasore treatment was normalized as 100%. For the blank group, SK-MEL-2 cells were cultured in DMEM for 3 hours. After subtracting the fluorescence signal from the blank group, GFP or Cy3 fluorescence of the positive control group was normalized as 100%.

2.9. Cellular uptake evaluation of amphiphilic polymers in dynamin triple knockout mouse embryo fibroblasts

Generation of dynamin triple knockout fibroblasts. The conditional dynamin triple-knockout mouse embryo fibroblasts (MEF TKO) were provided by Dr. Pietro De Camilli (Yale University). The deletion of dynamin in these cells is mediated by a tamoxifen-inducible knockout strategy. These cells express a Cre-estrogen receptor mutant knock-in transgene from the ROSA26 locus. Only in response to tamoxifen exposure, Cre is shuttled into the nucleus. The conditional MEF TKO cells were seeded in T75 cell culture flask and cultured in full DMEM growth medium containing 3 μM 4-hydroxytamoxifen (4-OHT, Sigma-Aldrich, Cat# H6278) for 2 days. Next, these cells were split into a 48-well plate. A total of 5k cells were seeded into each well and cultured in full DMEM growth medium containing 300 nM 4-OHT. The medium was replaced every two days with fresh full DMEM growth medium containing 300 nM 4-OHT. The TKO cells used for the experiment were between 6-8 days since the initial 3 μM 4-OHT treatment. The simultaneous loss of dynamin 1,2,3 was confirmed by Western blot.[36] These dynamin triple knockout cells were denoted as MEF TKO cells.

Cellular uptake evaluation of amphiphilic polymers. A total of 10k conditional MEF TKO without 4-OHT treatment were seeded in a 48-well plate and cultured in complete DMEM growth medium for 48 hours prior to the experiment. These dynamin-containing cells were denoted as MEF. Note that these MEF cells were immortalized by the 3T3 method of serial passaging, different from the primary MEF cells provided by Dr. Jesse Mager. The medium for MEF and MEF TKO cells was replaced with DMEM. Cy3-labelled amphiphilic polymers were spiked into the medium to incubate for additional 3 hours. The final concentration of amphiphilic polymers was maintained at 20 $\mu g \cdot mL^{-1}$ for **PEG**, 10 $\mu g \cdot mL^{-1}$ for **POS**, 2 $\mu g \cdot mL^{-1}$ for **NEG**, and 50 $\mu g \cdot mL^{-1}$ for **MPC**. In parallel, to evaluate the effect of pharmacological inhibitors, the MEF or MEF TKO cells were cultured for 1 hour in DMEM containing dynasore (80 μM), amiloride (1 mM), and NaN_3/2-DG (10 mM / 50 mM), respectively. Subsequently, in the presence of inhibitors, the cells were incubated with Cy3-labelled amphiphilic polymers that spiked into the medium for additional 3

hours. After washing the cells with cold PBS, the fluorescence intensity of Cy3 within the cells was measured using flow cytometry with an excitation wavelength of 561 nm. For the comparison between MEF and MEF TKO cells, the fluorescence signal of Cy3-labelled polymers in MEF cells were normalized as 100%. In the experiments with inhibitors, For the positive control, the fluorescence signal in cells without dynasore treatment was normalized as 100%. For the blank group, MEF or MEF TKO cells were cultured in DMEM for 3 hours. After subtracting the fluorescence signal from the blank group, Cy3 fluorescence of the positive control group was normalized as 100%.

2.10. Evaluation of dynasore off-target effects

Flow cytometry. A total of 10k HeLa cells were cultured in a 96-well plate for 24 hours prior to the experiment. For inhibiting different endocytic pathways, cells were cultured for 1 hour in DMEM containing OctTAB (6 μM), Dynole 34-2 (6 μM), Mdivi-1 (30 μM), bafilomycin A1 (400 nM, 200 nM, or 100 nM), nystatin (27 μM), nocodazole (20 μM), genistein (100 μM or 50 μM), and Pitstop 2 (6 μM), respectively. Subsequently, in the presence of inhibitors, the cells were incubated with Cy3-labelled amphiphilic polymers that spiked into the medium for additional 3 hours. Note that the actin inhibitors were conducted with a different time scale. For actin inhibitors, cells were cultured for 30 minutes in DMEM containing cytochalasin B (20 μM, 2 μM, or 0.2 μM), cytochalasin D (20 μM, 2 μM, or 0.2 μM), latrunculin A (1 μM, 0.5 μM, or 0.1 μM), and 16-epi-latrunculin B (1 μM, 0.5 μM, or 0.1 μM), respectively. Subsequently, in the presence of inhibitors, the cells were incubated with Cy3-labelled amphiphilic polymers that spiked into the medium for additional 30 minutes. The final concentration of amphiphilic polymers was maintained at 20 $\mu g \cdot mL^{-1}$ for **PEG**, 10 $\mu g \cdot mL^{-1}$ for **POS**, 2 $\mu g \cdot mL^{-1}$ for **NEG**, and 50 $\mu g \cdot mL^{-1}$ for **MPC**. After washing the cells with cold PBS, the fluorescence intensity of Cy3 within the cells was measured using flow cytometry with an excitation wavelength of 561 nm. For the positive control, HeLa cells were cultured in DMEM for 1 hour and incubated with Cy3-labelled polymers for another 3 hours. For the blank group, HeLa cells were cultured in DMEM for 4 hours. The time scale for control groups of actin inhibitors

were correspondingly adjusted. After subtracting the fluorescence signal from the blank group, Cy3 fluorescence of the positive control group was normalized as 100%.

Confocal microscopy. A total of 80k RAW264.7 cells (less than 6 passages) were seeded into a glass bottom dish (Cellvis, #D35C4-20-0-N) for 24 hours prior to the experiment. (a) To evaluate the effect of latrunculin A, cells were cultured for 30 minutes in DMEM containing latrunculin A (1 μM). Subsequently, in the presence of inhibitors, the cells were incubated with Cy3-labelled amphiphilic polymers that spiked into the medium for additional 30 minutes. (b) To evaluate the effect of genistein, cells were cultured for 1 hour in DMEM containing genistein (100 μM). Subsequently, in the presence of inhibitors, the cells were incubated with Cy3-labelled amphiphilic polymers that spiked into the medium for additional 3 hours. The final concentration of amphiphilic polymers was maintained at 100 $\mu g \cdot mL^{-1}$ for **PEG**, 80 $\mu g \cdot mL^{-1}$ for **POS**, 25 $\mu g \cdot mL^{-1}$ for **NEG**, and 100 $\mu g \cdot mL^{-1}$ for **MPC**. After washing with phosphate buffer saline, the cells were incubated with NucBlue (Hoechst 33342, 1 drop in 500 μL medium) for 5 min in FluoroBrite DMEM at 37 °C to stain the nucleus. The intracellular distribution measured by confocal microscopy with excitation wavelengths of 405 nm (Hoechst 33342) and 561 nm (Cy3-labelled polymers).

Actin filament staining. A total of 80k RAW264.7 cells (less than 6 passages) were seeded into a glass bottom dish (Cellvis, #D35C4-20-0-N) for 24 hours prior to the experiment. To evaluate the effect of latrunculin A, cells were cultured for 60 minutes in DMEM containing latrunculin A (1 μM). After washing with PBS, the cells were fixed with fixation/permeabilization solution (BD Biosciences, Cat# 554714) at room temperature for 15 minutes. The cells were washed with PBS and stained with phalloidin-iFluor 488 working solution (diluted from 1000× into 1× with PBS that contains 1% FBS, Abcam, Cat# ab176753) at room temperature for 90 minutes. The cells were washed with PBS and incubated with NucBlue (Hoechst 33342, 1 drop in 500 μL medium) for 5 min in FluoroBrite DMEM at 37 °C to stain the nucleus. The

intracellular distribution of nucleus and actin filaments was measured by confocal microscopy with excitation wavelengths of 405 nm (Hoechst 33342) and 488 nm (phalloidin-iFluor 488).

3. Spectral data

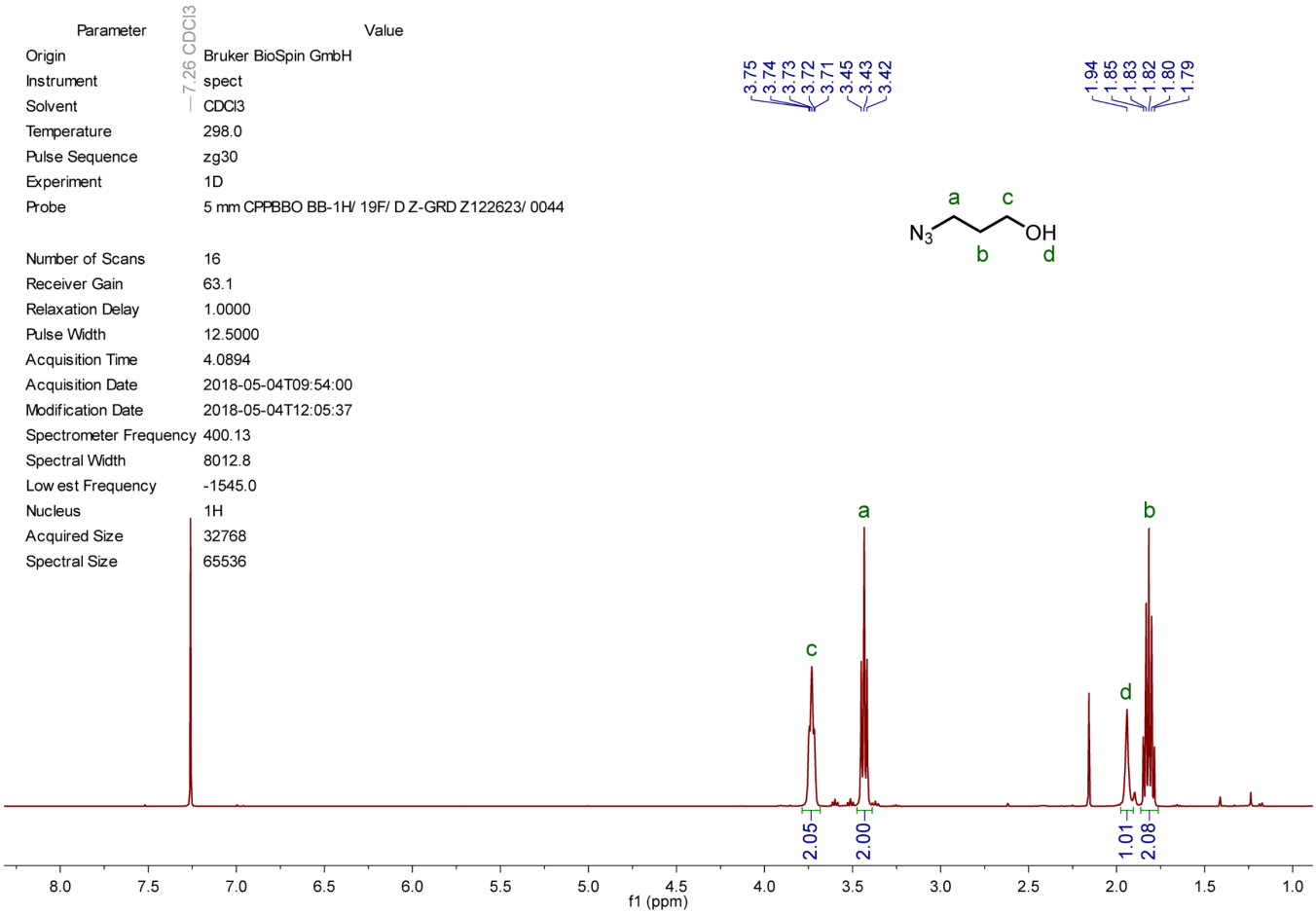


Figure SP1. ^{1}H NMR spectrum (400 MHz) of 3-azido-1-propanol in $CDCl_3$.

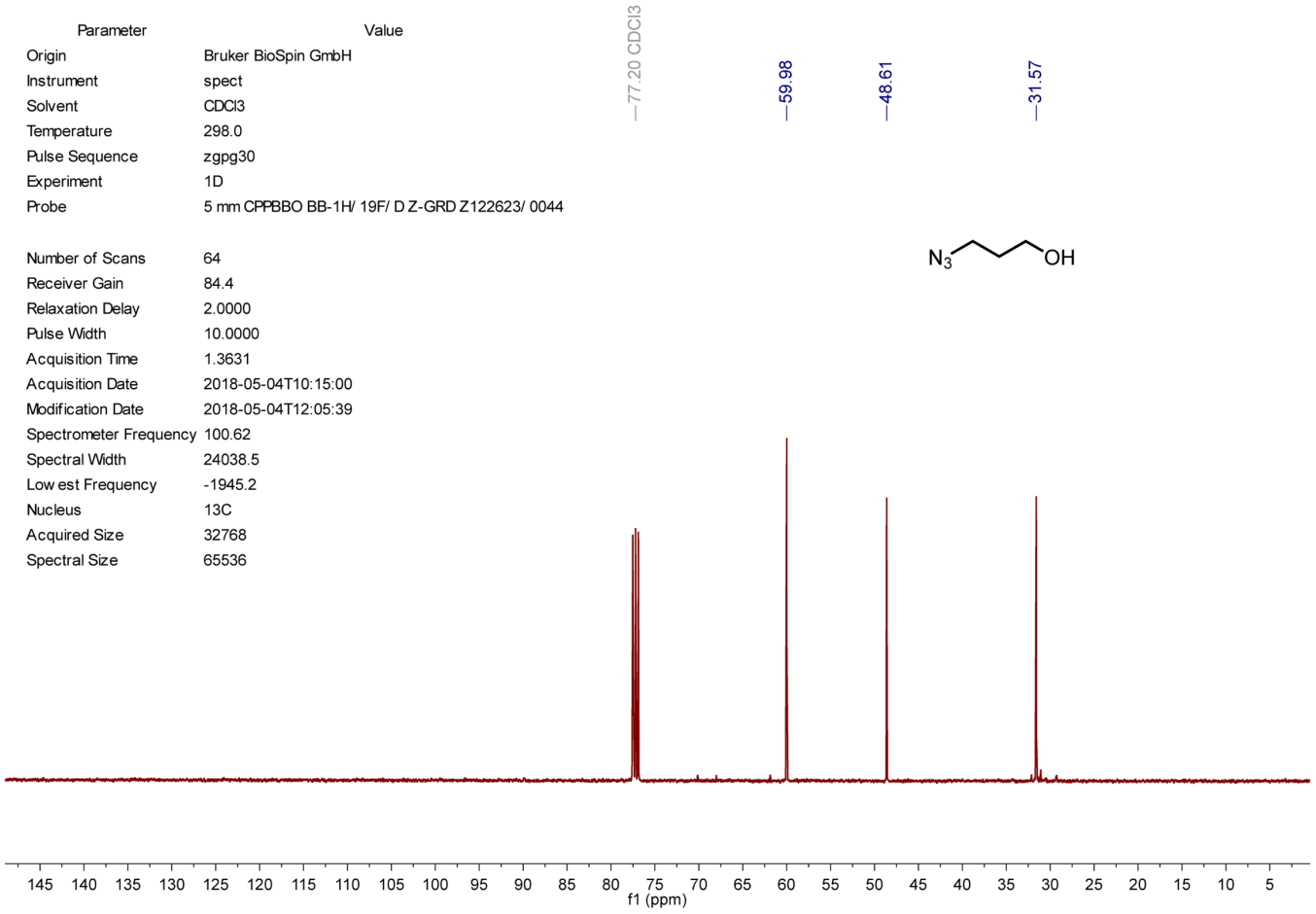


Figure SP2. ^{13}C NMR spectrum (100 MHz) of 3-azido-1-propanol in $CDCl_3$.

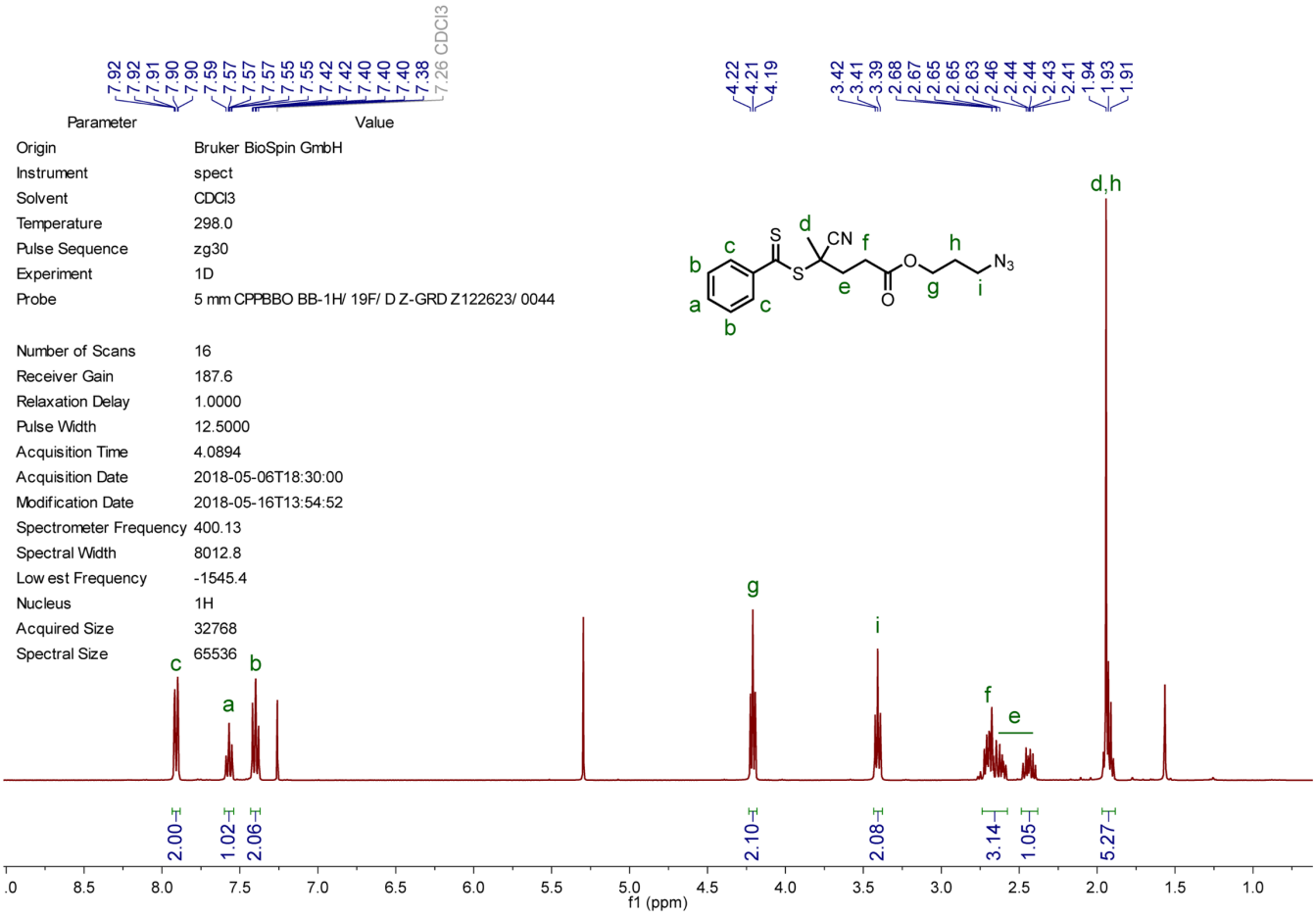


Figure SP3. ^{1}H NMR spectrum (400 MHz) of **Az-CTAP** in $CDCl_3$.

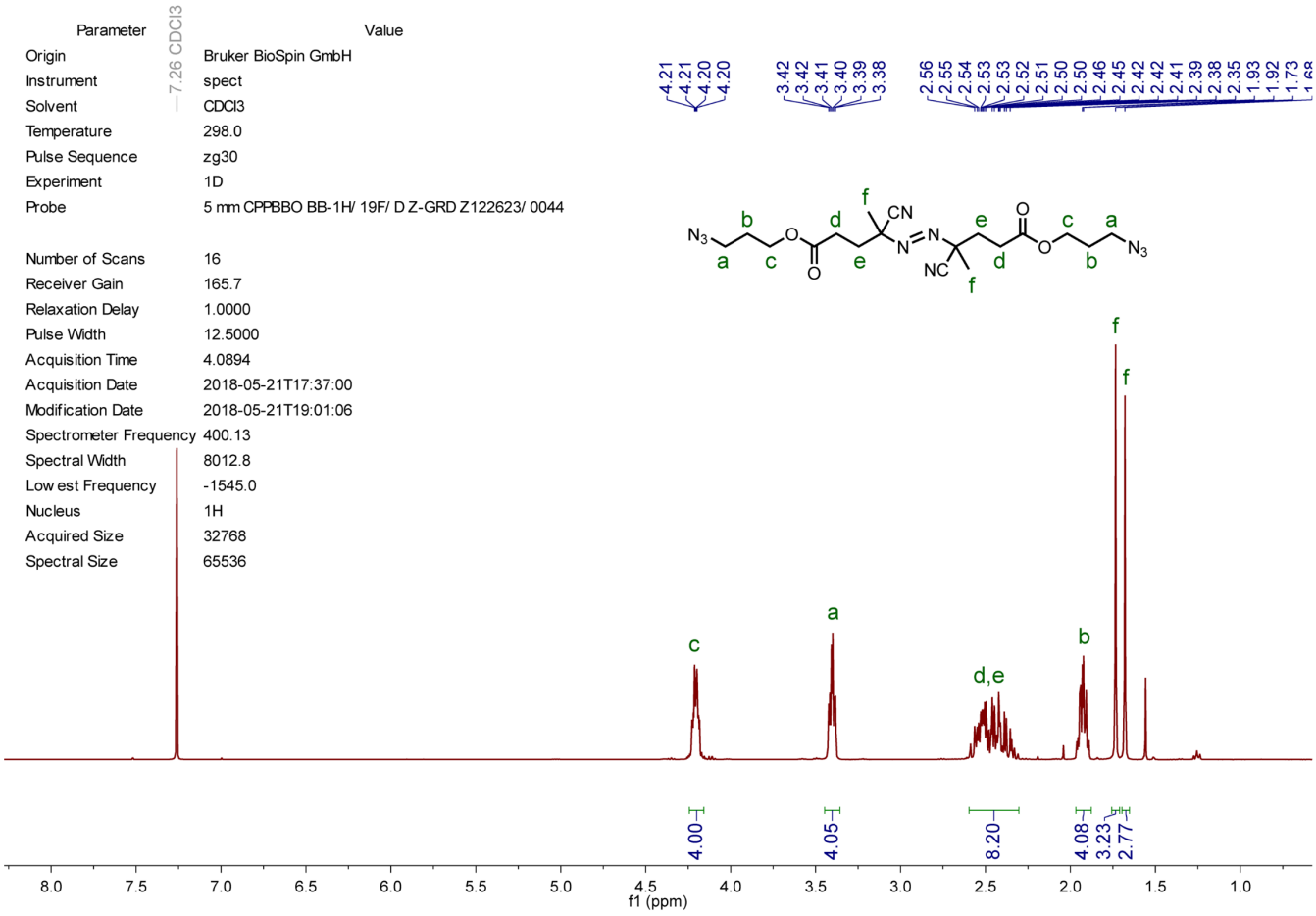


Figure SP4. ^{1}H NMR spectrum (400 MHz) of **Az-ACVA** in $CDCl_3$.

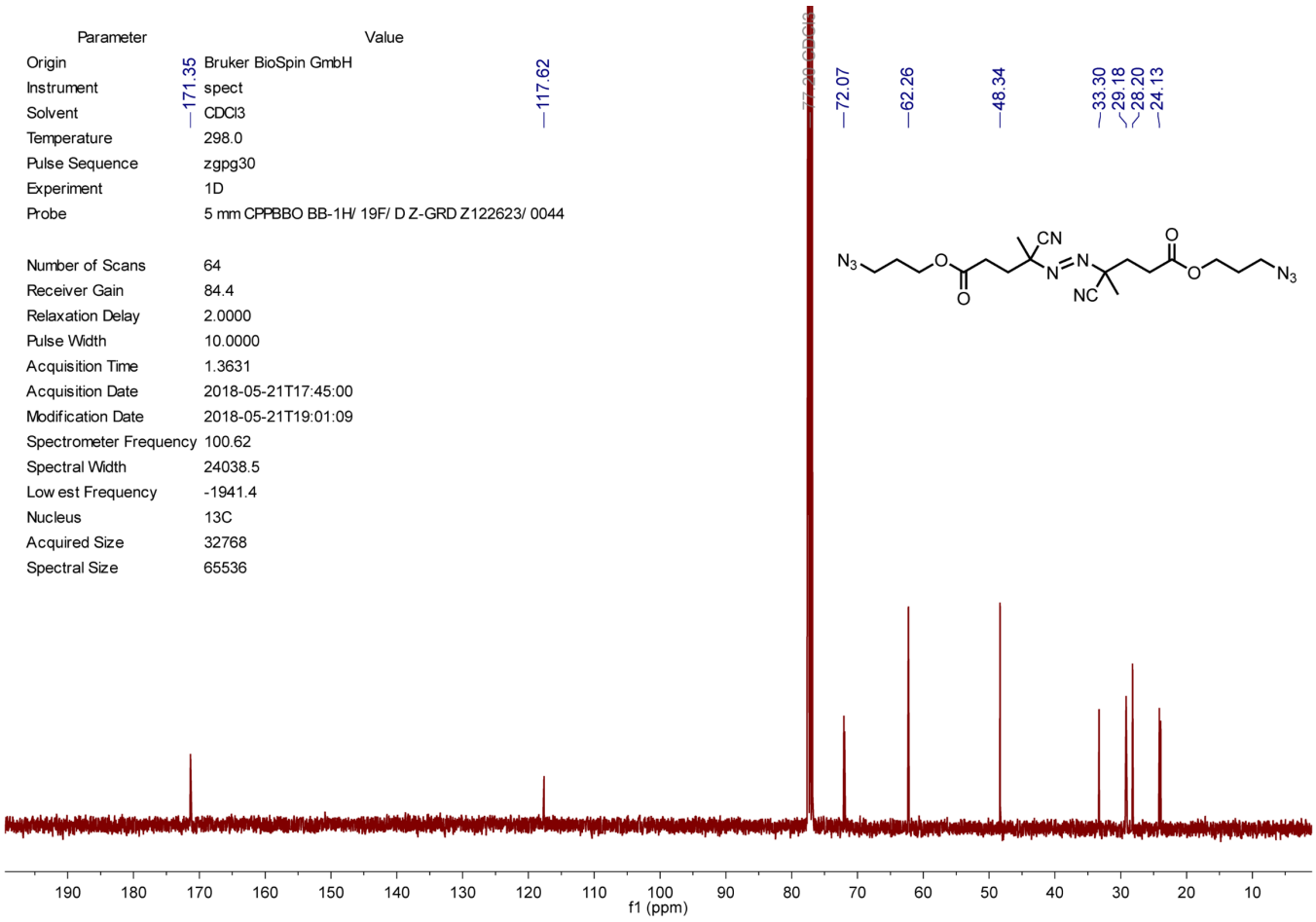


Figure SP5. ^{13}C NMR spectrum (100 MHz) of **Az-ACVA** in $CDCl_3$.

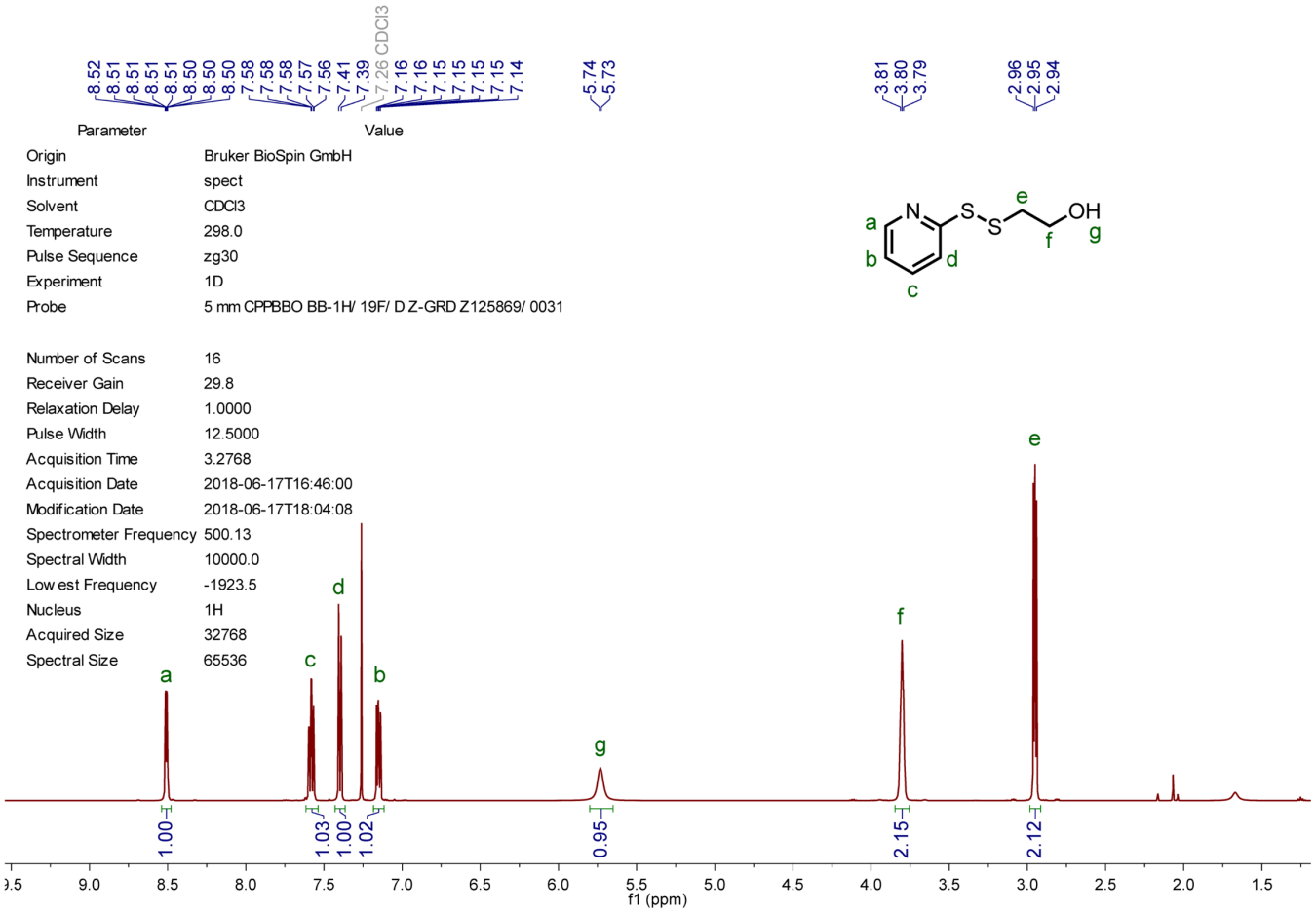


Figure SP6. ^{1}H NMR spectrum (400 MHz) of 2-(pyridin-2-yldisulfanyl)ethanol in $CDCl_3$.

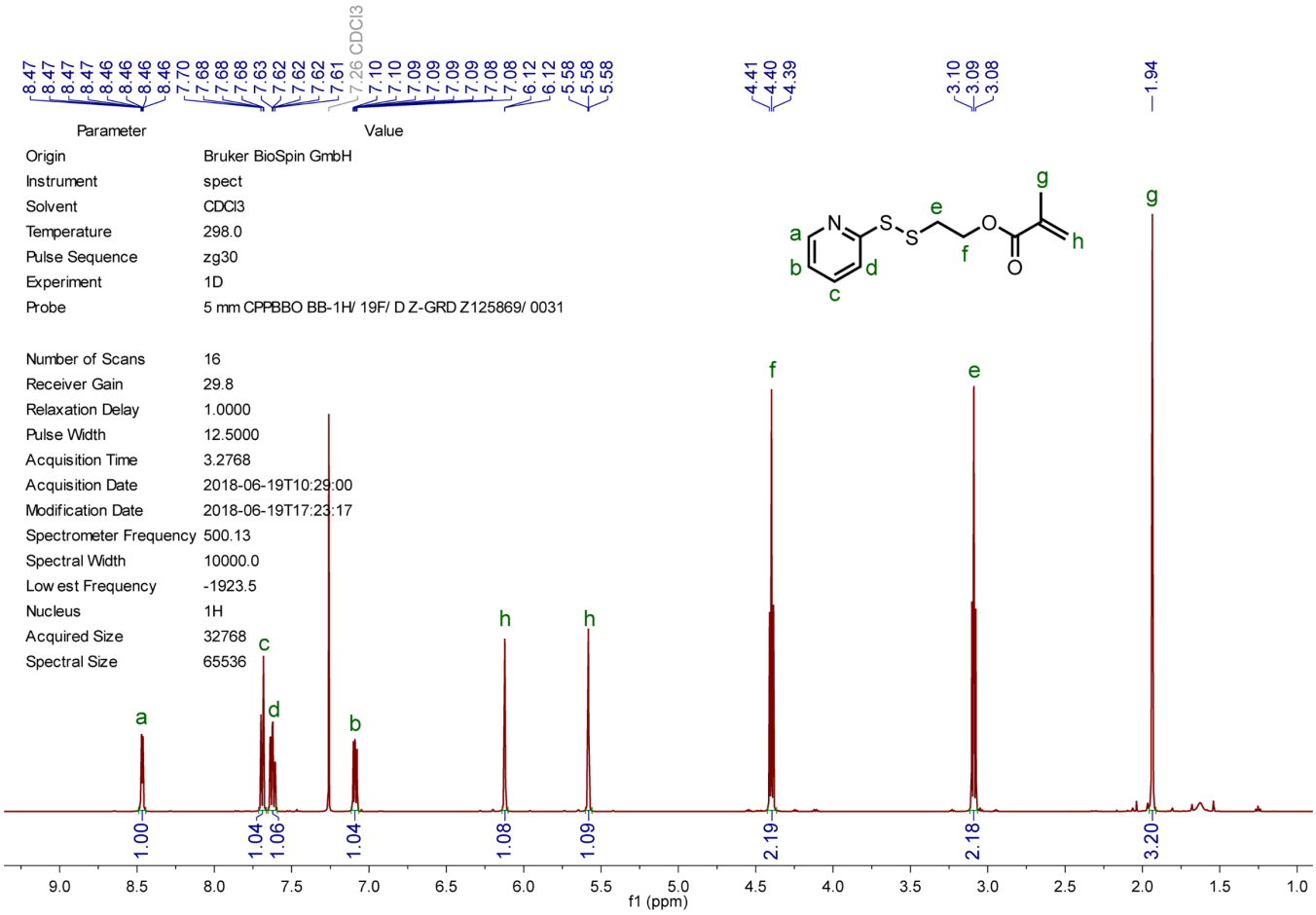


Figure SP7. ^{1}H NMR spectrum (500 MHz) of pyridyl disulfide ethyl methacrylate in $CDCl_3$.

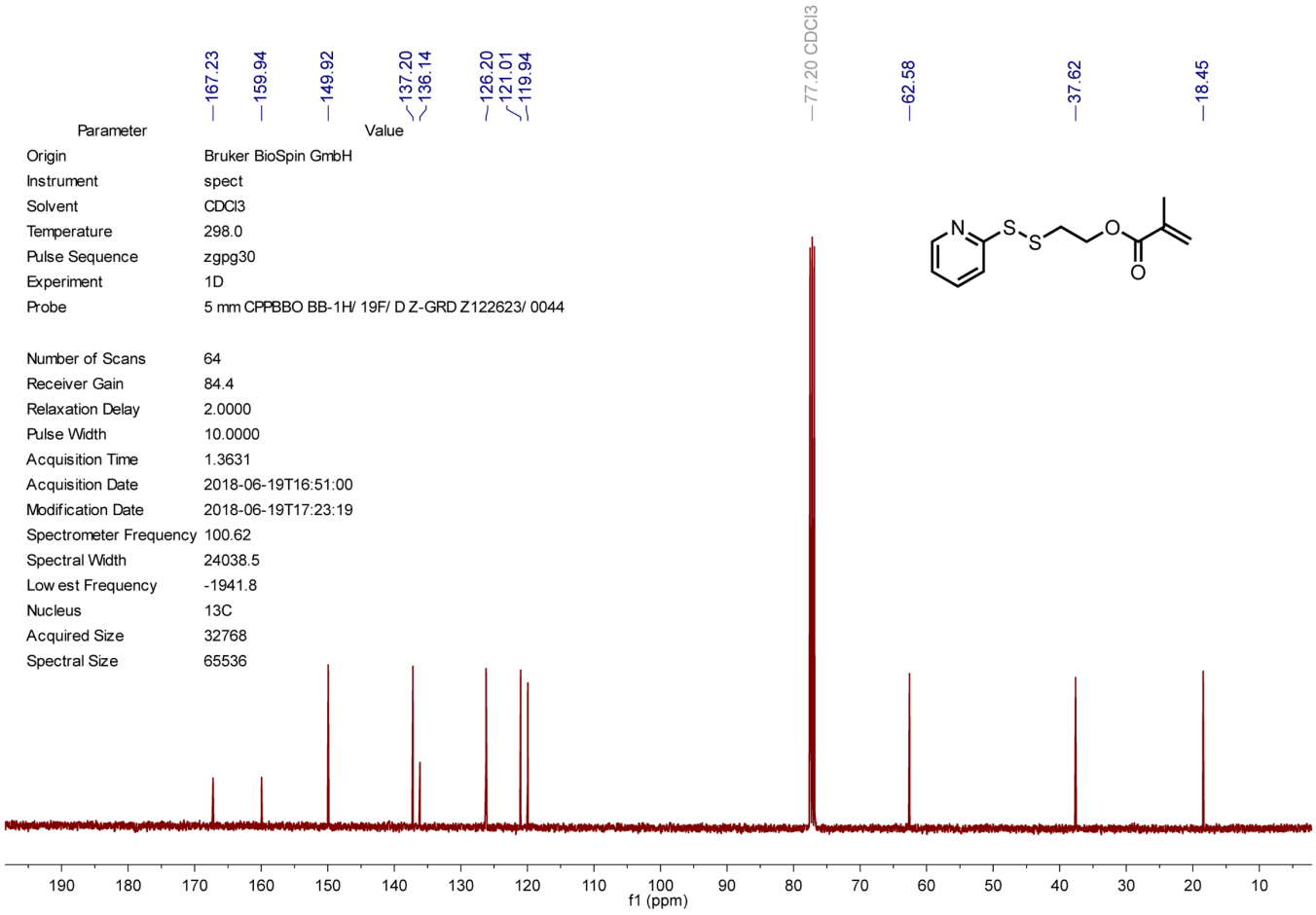


Figure SP8. ^{13}C NMR spectrum (100 MHz) of pyridyl disulfide ethyl methacrylate in $CDCl_3$.

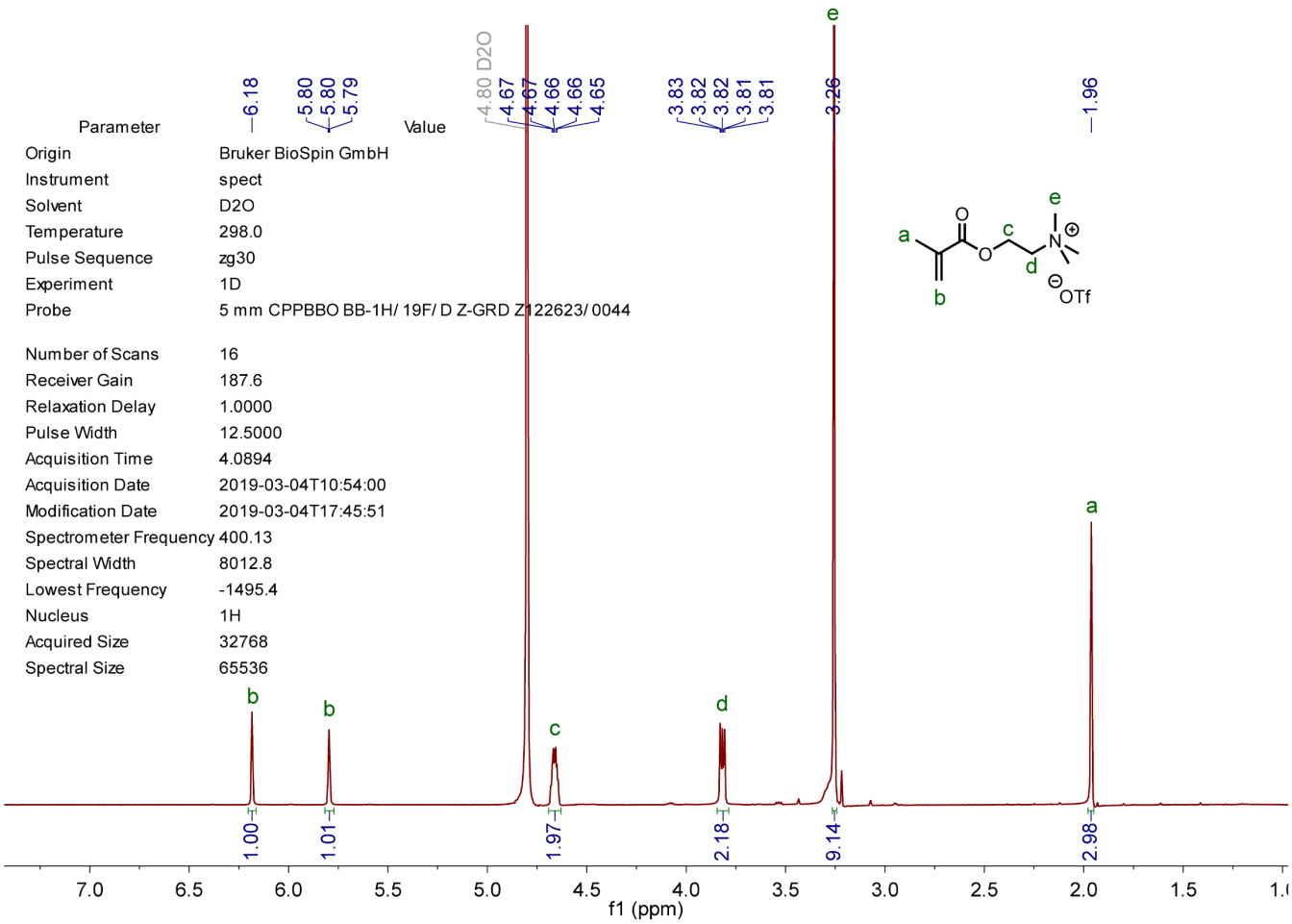


Figure SP9. ^{1}H NMR spectrum (400 MHz) of 2-(methacryloyloxy)ethyltrimethylammonium triflate in D_2O.

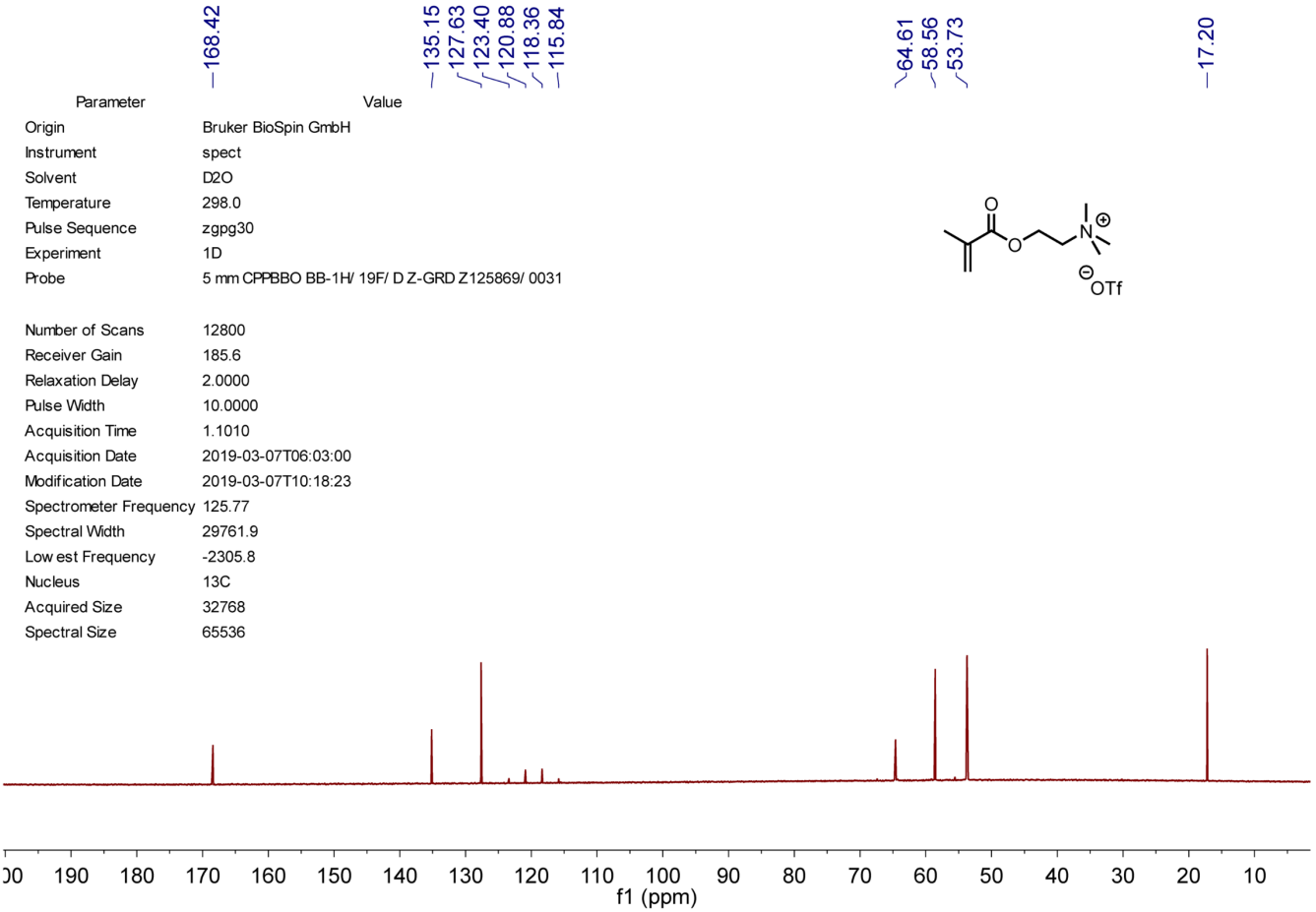


Figure SP10. ^{13}C NMR spectrum (125 MHz) of 2-(methacryloyloxy)ethyltrimethylammonium triflate in D_2O.

Parameter	Value
Origin	Bruker BioSpin GmbH
Instrument	spect
Solvent	D2O
Temperature	298.0
Pulse Sequence	zgflqn
Experiment	1D
Probe	5 mm CPPBBO BB-1H/ 19F/ D Z-GRD Z122623/ 0044
Number of Scans	32
Receiver Gain	5.1
Relaxation Delay	2.0000
Pulse Width	15.0000
Acquisition Time	1.1010
Acquisition Date	2019-03-04T11:18:00
Modification Date	2019-03-04T17:45:56
Spectrometer Frequency	376.47
Spectral Width	59523.8
Low est Frequency	-53869.9
Nucleus	19F
Acquired Size	65536
Spectral Size	131072

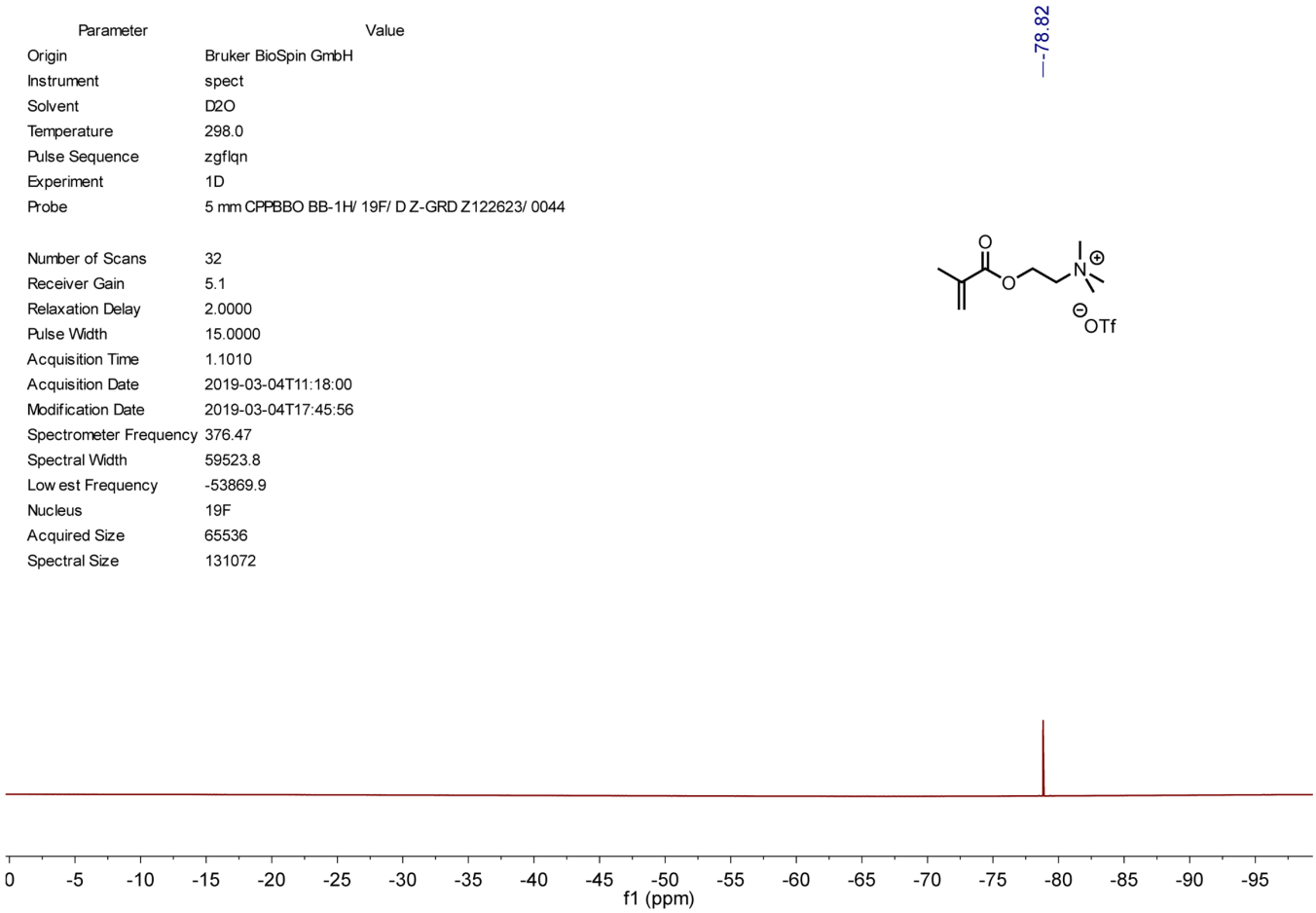


Figure SP11. ^{19}F NMR spectrum (376 MHz) of 2-(methacryloyloxy)ethyltrimethylammonium triflate in D_2O.

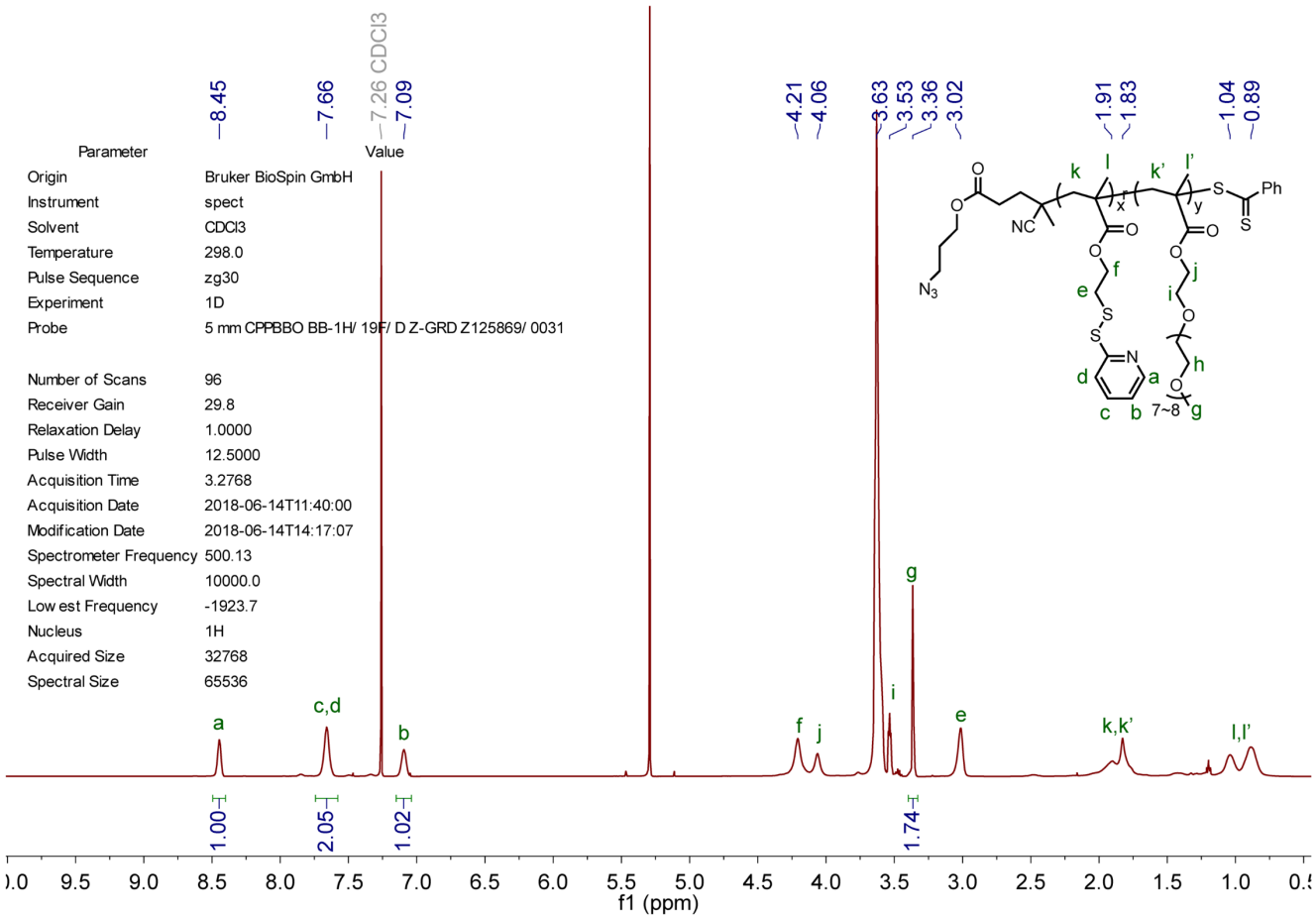


Figure SP12. ^{1}H NMR spectrum (500 MHz) of **PEG** in $CDCl_3$.

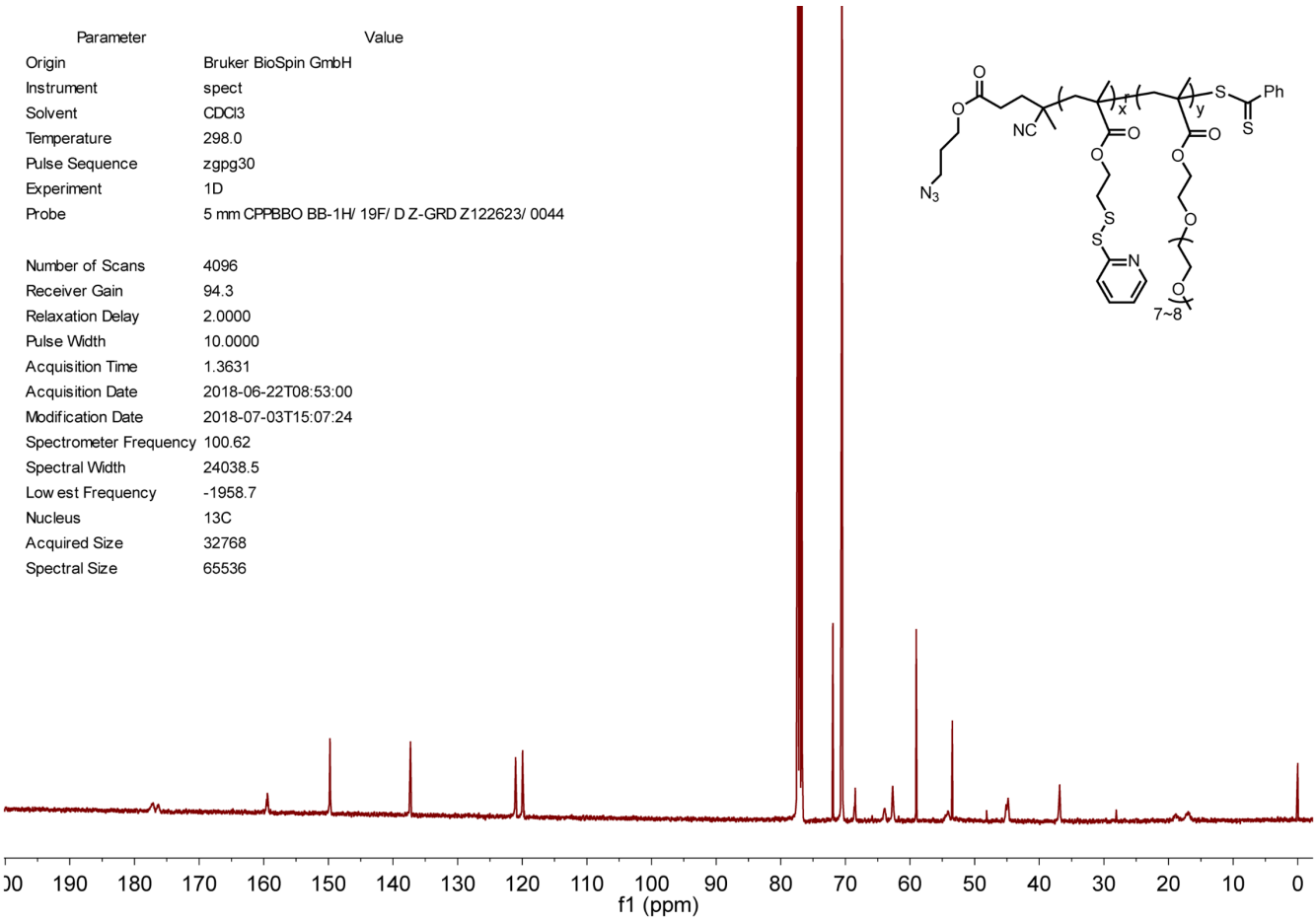


Figure SP13. ^{13}C NMR spectrum (100 MHz) of **PEG** in $CDCl_3$.

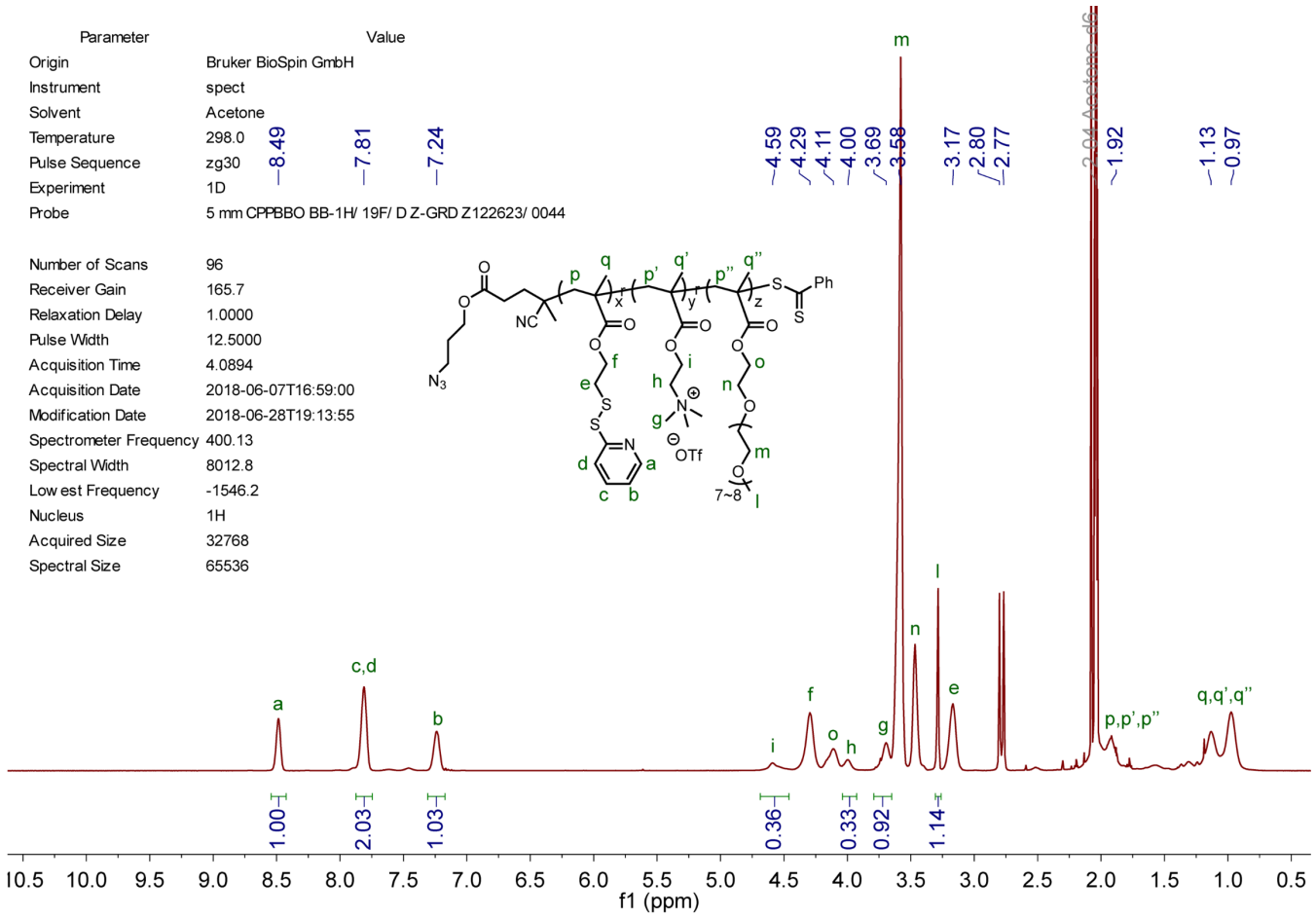


Figure SP14. ^{1}H NMR spectrum (400 MHz) of **POS** in acetone-d_6.

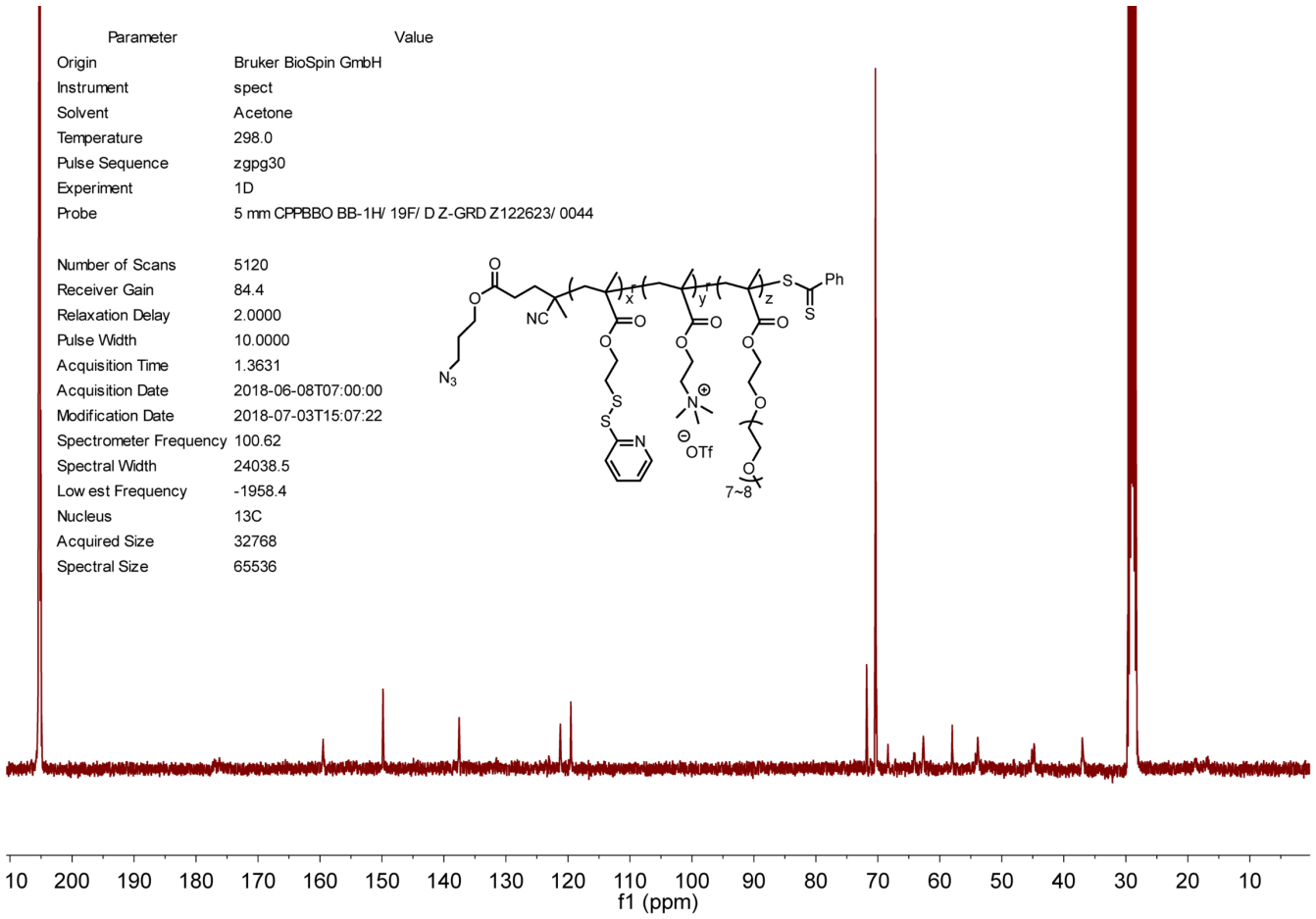


Figure SP15. ^{13}C NMR spectrum (100 MHz) of **POS** in acetone-d_6.

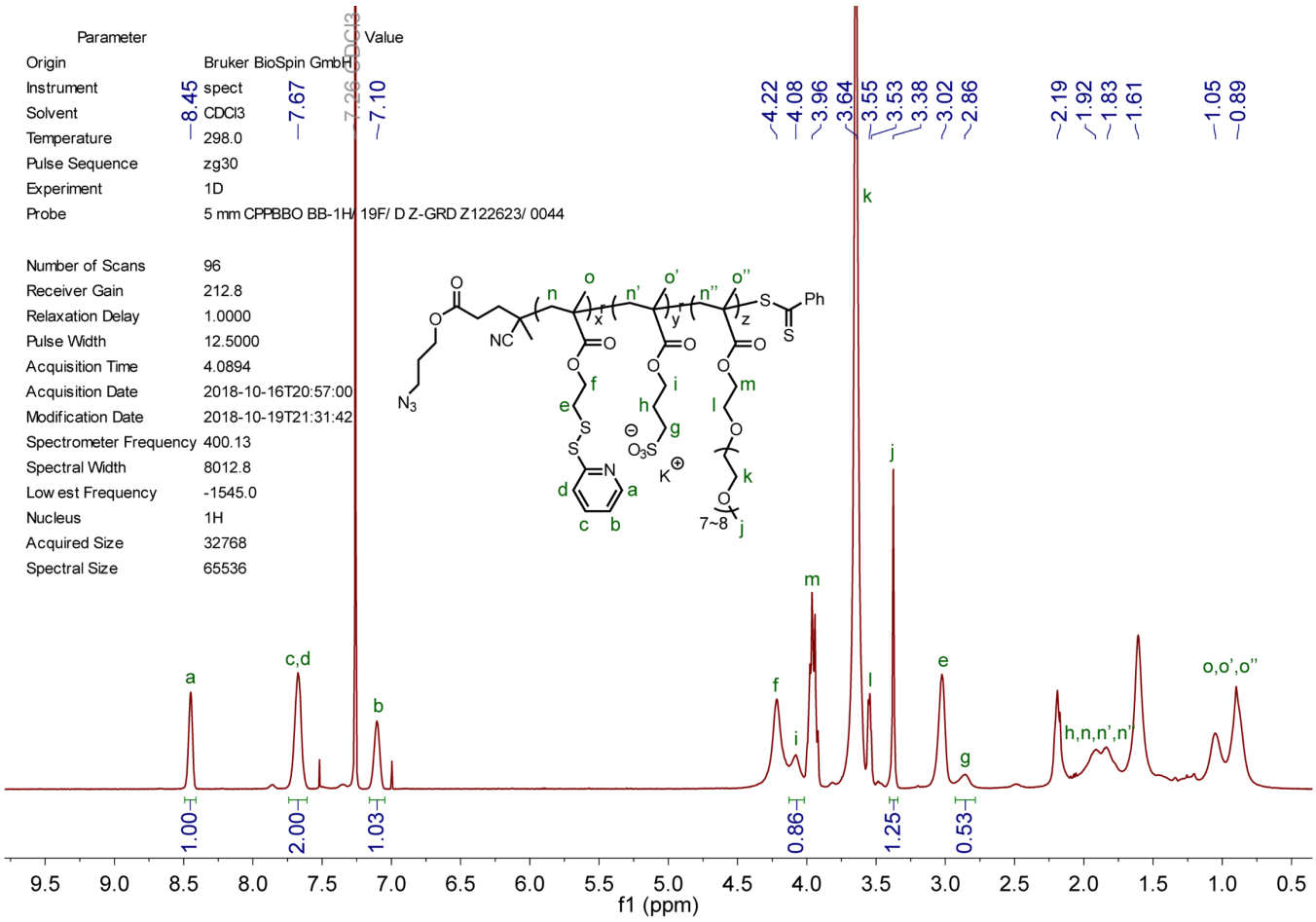


Figure SP16. ^{1}H NMR spectrum (400 MHz) of **NEG** in $CDCl_3$.

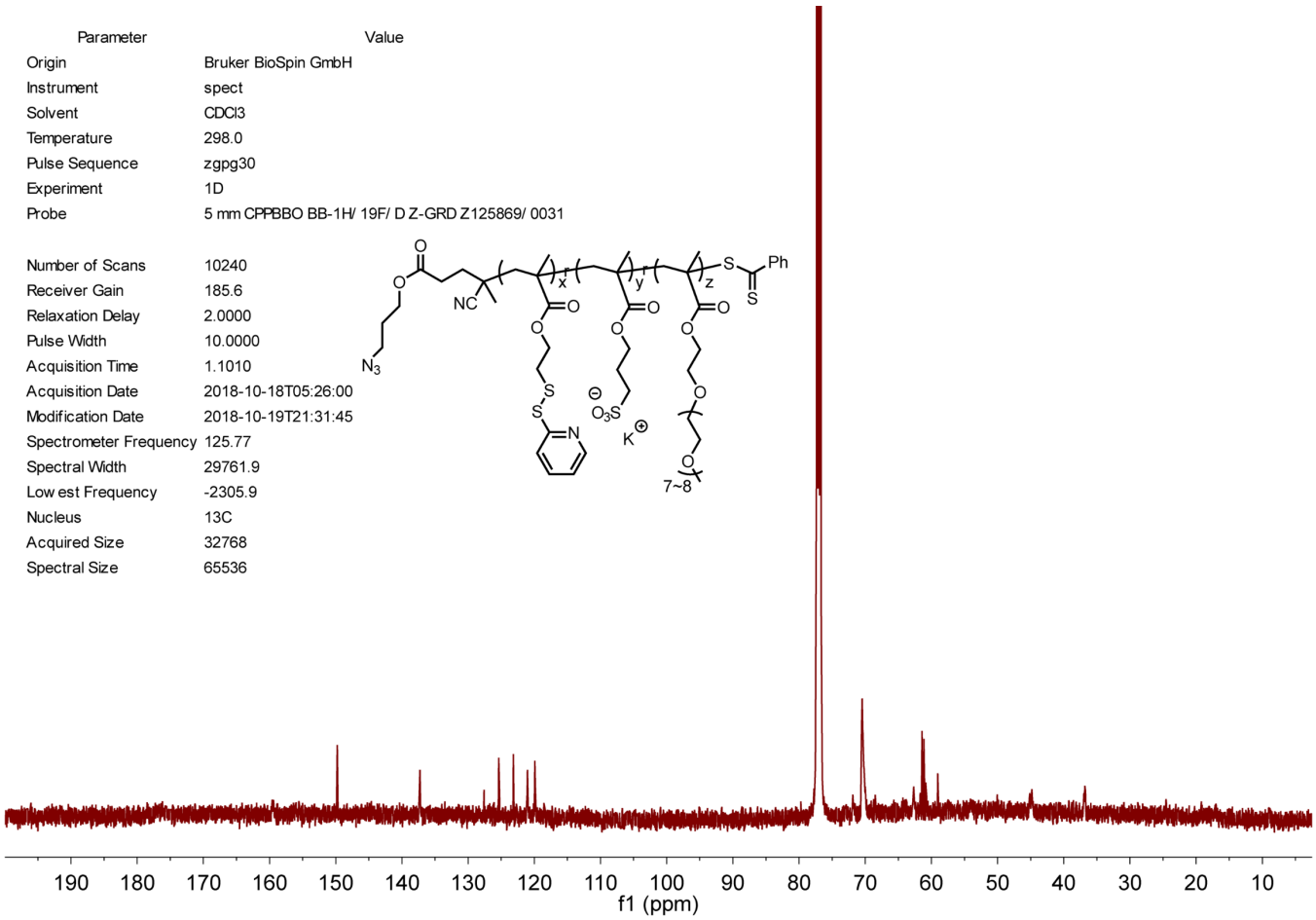


Figure SP17. ^{13}C NMR spectrum (125 MHz) of **NEG** in $CDCl_3$.

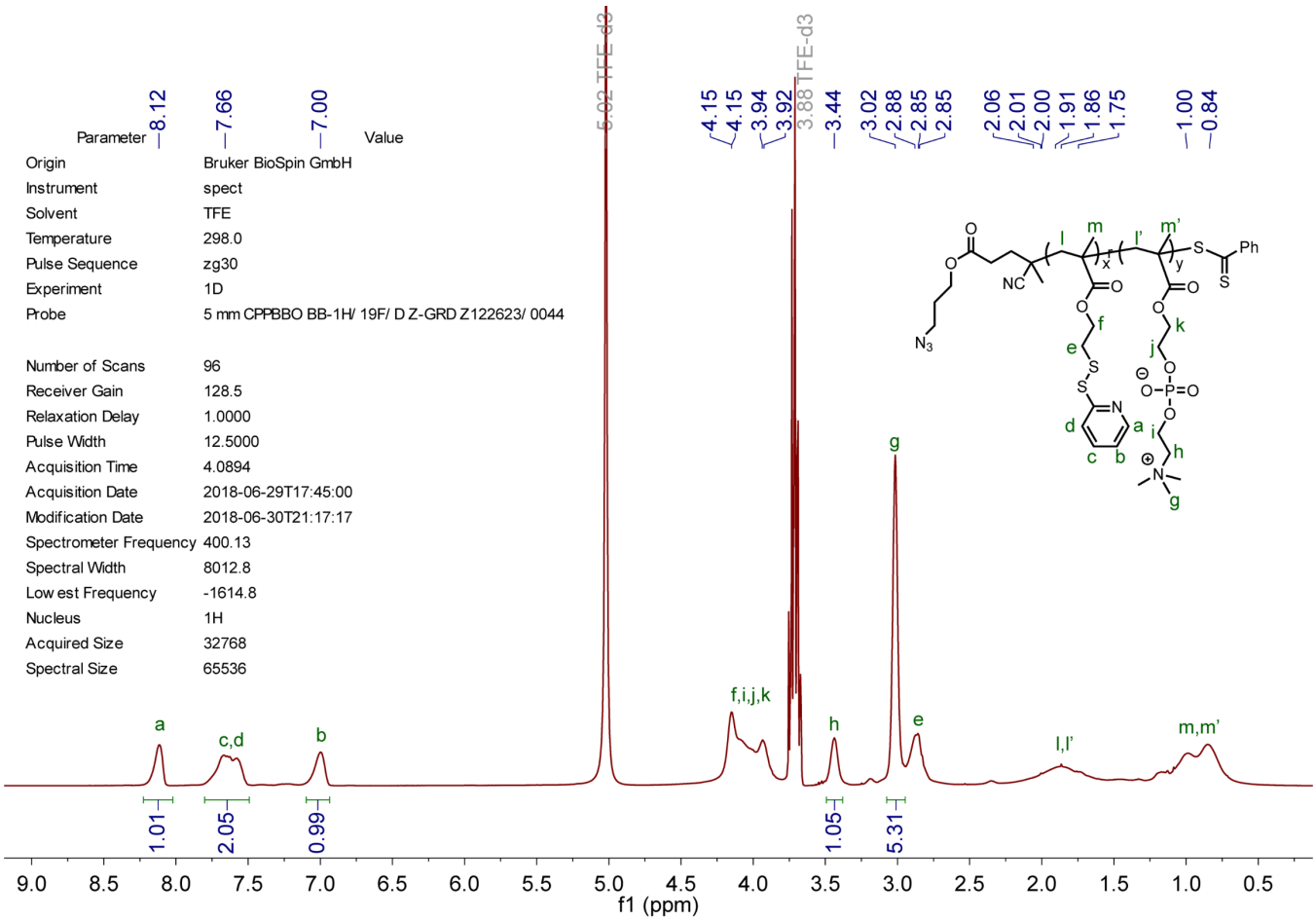


Figure SP18. ^{1}H NMR spectrum (400 MHz) of **MPC** in trifluoroethanol-d_3.

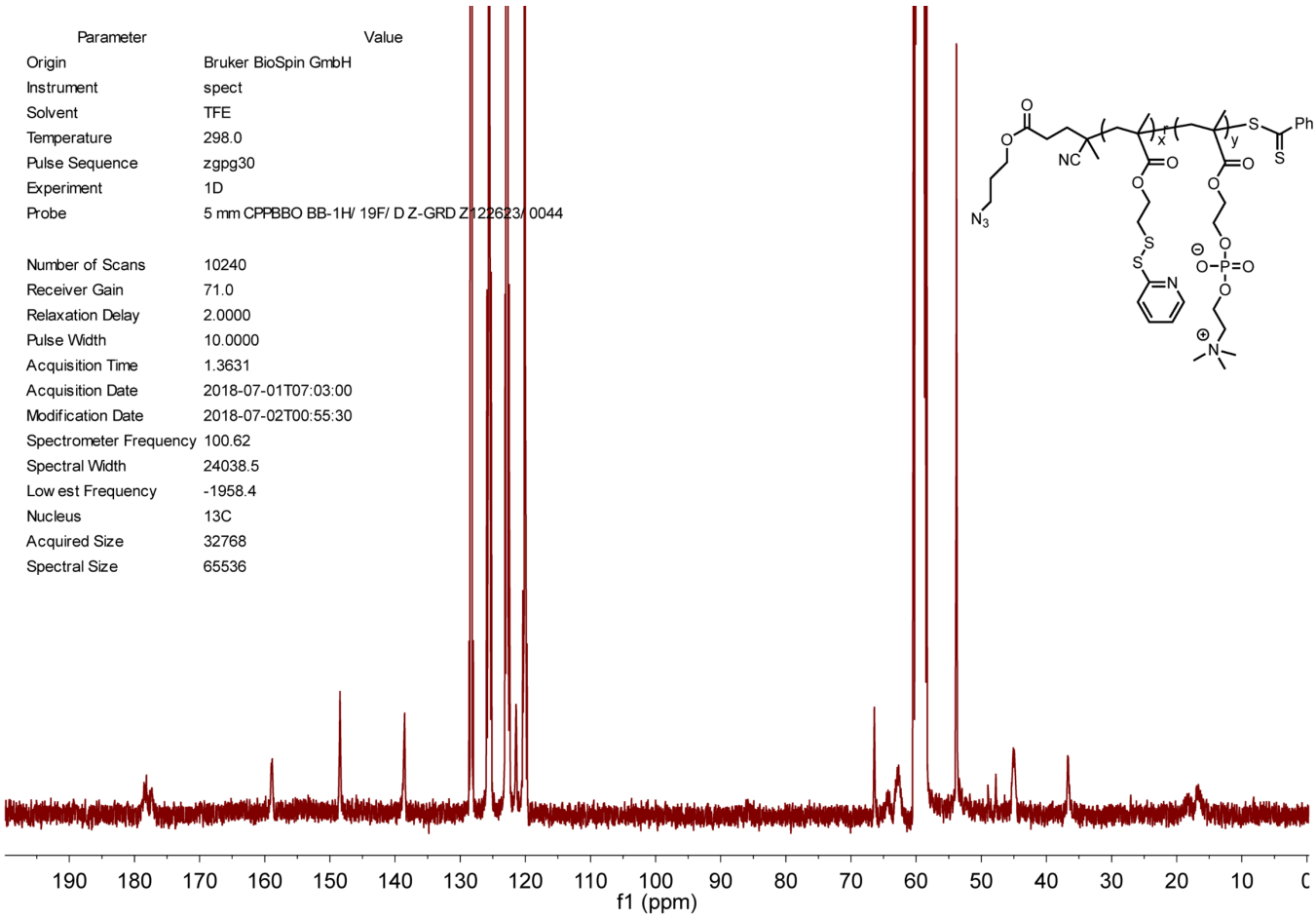
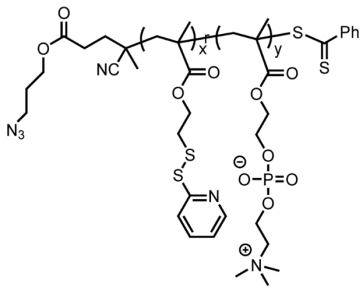


Figure SP19. ^{13}C NMR spectrum (100 MHz) of **MPC** in trifluoroethanol-d_3.

Parameter	Value
Origin	Bruker BioSpin GmbH
Instrument	spect
Solvent	TFE
Temperature	298.0
Pulse Sequence	zgpg30
Experiment	1D
Probe	5 mm CPPBBO BB-1H/ 19F/ D Z-GRD Z122623/ 0044
Number of Scans	5120
Receiver Gain	103.2
Relaxation Delay	2.0000
Pulse Width	12.0000
Acquisition Time	0.5112
Acquisition Date	2018-06-30T17:16:00
Modification Date	2018-06-30T21:17:23
Spectrometer Frequency	161.97
Spectral Width	64102.6
Low est Frequency	-40150.1
Nucleus	31P
Acquired Size	32768
Spectral Size	65536



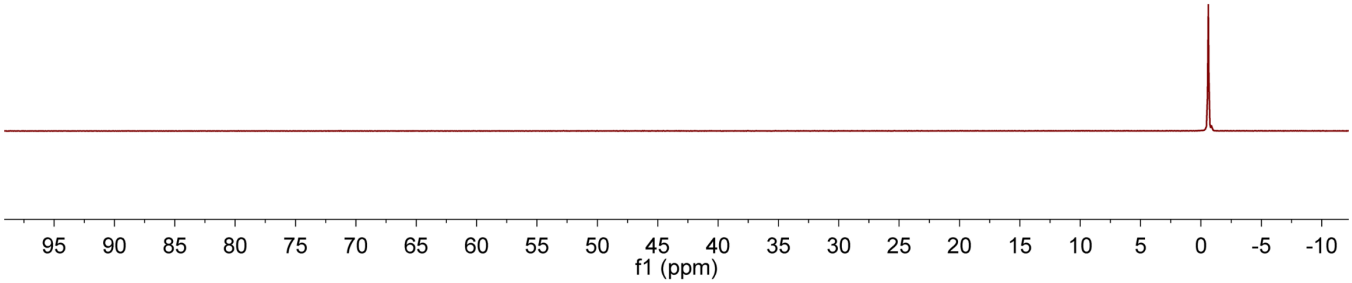


Figure SP20. ^{31}P NMR spectrum (161 MHz) of **MPC** in trifluoroethanol-d_3.

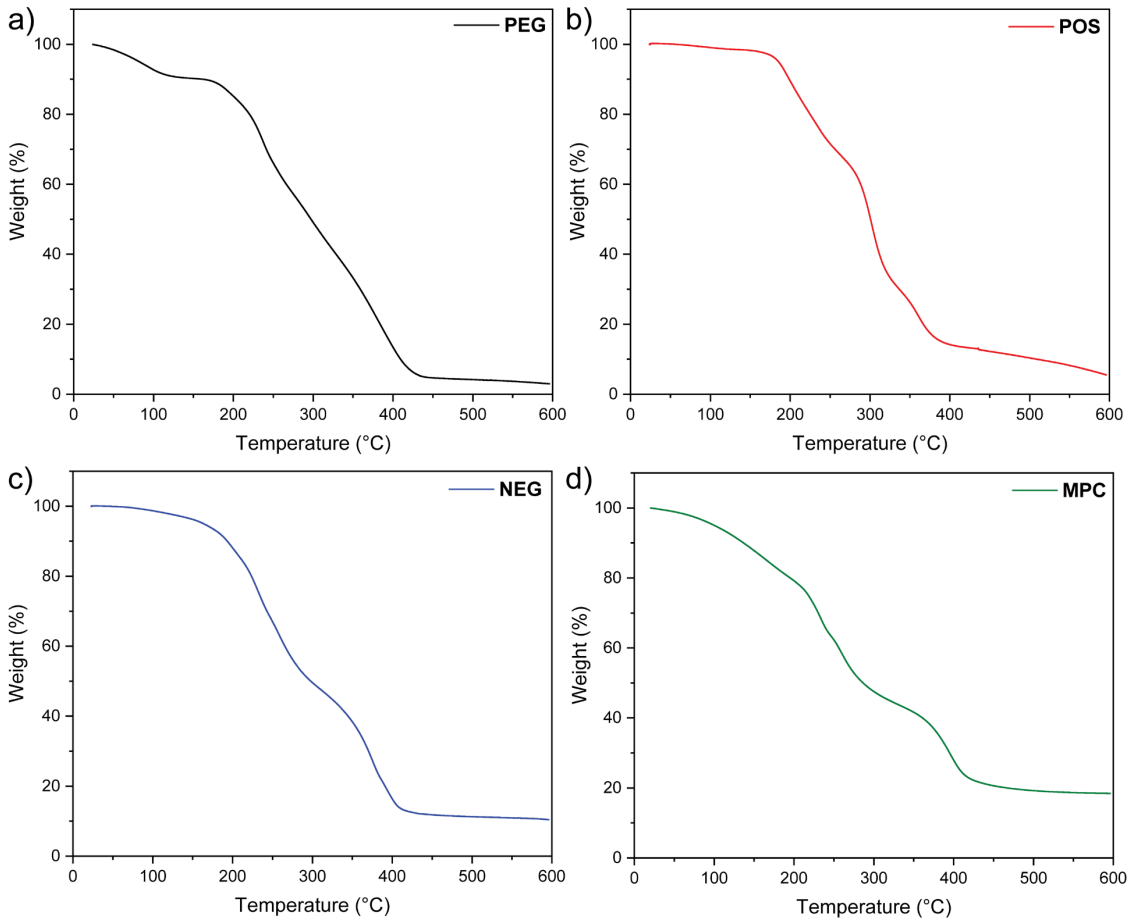


Figure SP21. TGA curves of (a) **PEG**, (b) **POS**, (c) **NEG**, and (d) **MPC**.

4. References

1. Harvey, J.; Hardy, S. C.; Ashford, M. L. J., Dual actions of the metabolic inhibitor, sodium azide on KATP channel currents in the rat CRI-G1 insulinoma cell line. *Br. J. Pharmacol.* **1999,** 126 (1), 51-60.
2. McComb, R. B.; Yushok, W. D., Metabolism of Ascites Tumor Cells. *IV. Enzymatic Reactions Involved in Adenosinetriphosphate Degradation Induced by 2-Deoxyglucose* **1964,** 24 (2 Part 1), 198-205.
3. Koivusalo, M.; Welch, C.; Hayashi, H.; Scott, C. C.; Kim, M.; Alexander, T.; Touret, N.; Hahn, K. M.; Grinstein, S., Amiloride inhibits macropinocytosis by lowering submembranous pH and preventing Rac1 and Cdc42 signaling. *J. Cell Biol.* **2010,** 188 (4), 547.
4. Jiang, Y.; Huo, S.; Mizuhara, T.; Das, R.; Lee, Y.-W.; Hou, S.; Moyano, D. F.; Duncan, B.; Liang, X.-J.; Rotello, V. M., The Interplay of Size and Surface Functionality on the Cellular Uptake of Sub-10 nm Gold Nanoparticles. *ACS Nano* **2015,** 9 (10), 9986-9993.
5. Kirchhausen, T.; Macia, E.; Pelish, H. E., Use of Dynasore, the Small Molecule Inhibitor of Dynamin, in the Regulation of Endocytosis. In *Methods Enzymol.*, Academic Press: 2008; Vol. 438, pp 77-93.
6. McCluskey, A.; Daniel, J. A.; Hadzic, G.; Chau, N.; Clayton, E. L.; Mariana, A.; Whiting, A.; Gorgani, N. N.; Lloyd, J.; Quan, A.; Moshkanbaryans, L.; Krishnan, S.; Perera, S.; Chircop, M.; von Kleist, L.; McGeachie, A. B.; Howes, M. T.; Parton, R. G.; Campbell, M.; Sakoff, J. A.; Wang, X.; Sun, J.-Y.; Robertson, M. J.; Deane, F. M.; Nguyen, T. H.; Meunier, F. A.; Cousin, M. A.; Robinson, P. J., Building a Better Dynasore: The Dyngo Compounds Potently Inhibit Dynamin and Endocytosis. *Traffic* **2013,** 14 (12), 1272-1289.
7. Patel, P. C.; Giljohann, D. A.; Daniel, W. L.; Zheng, D.; Prigodich, A. E.; Mirkin, C. A., Scavenger Receptors Mediate Cellular Uptake of Polyvalent Oligonucleotide-Functionalized Gold Nanoparticles. *Bioconjugate Chem.* **2010,** 21 (12), 2250-2256.

8. Saha, K.; Kim, S. T.; Yan, B.; Miranda, O. R.; Alfonso, F. S.; Shlosman, D.; Rotello, V. M., Surface Functionality of Nanoparticles Determines Cellular Uptake Mechanisms in Mammalian Cells. *Small* **2013,** 9 (2), 300-305.
9. Brito, D. A.; Rieder, C. L., The ability to survive mitosis in the presence of microtubule poisons differs significantly between human nontransformed (RPE-1) and cancer (U2OS, HeLa) cells. *Cell Motil. Cytoskeleton* **2009,** 66 (8), 437-447.
10. Rehman, Z. u.; Hoekstra, D.; Zuhorn, I. S., Protein kinase A inhibition modulates the intracellular routing of gene delivery vehicles in HeLa cells, leading to productive transfection. *J. Controlled Release* **2011,** 156 (1), 76-84.
11. Harush-Frenkel, O.; Debotton, N.; Benita, S.; Altschuler, Y., Targeting of nanoparticles to the clathrin-mediated endocytic pathway. *Biochem. Biophys. Res. Commun.* **2007,** 353 (1), 26-32.
12. Harush-Frenkel, O.; Rozentur, E.; Benita, S.; Altschuler, Y., Surface Charge of Nanoparticles Determines Their Endocytic and Transcytotic Pathway in Polarized MDCK Cells. *Biomacromolecules* **2008,** 9 (2), 435-443.
13. Xiao, K.; Li, Y.; Luo, J.; Lee, J. S.; Xiao, W.; Gonik, A. M.; Agarwal, R. G.; Lam, K. S., The effect of surface charge on in vivo biodistribution of PEG-oligocholic acid based micellar nanoparticles. *Biomaterials* **2011,** 32 (13), 3435-3446.
14. Perumal, O. P.; Inapagolla, R.; Kannan, S.; Kannan, R. M., The effect of surface functionality on cellular trafficking of dendrimers. *Biomaterials* **2008,** 29 (24), 3469-3476.
15. Dausend, J.; Musyanovych, A.; Dass, M.; Walther, P.; Schrezenmeier, H.; Landfester, K.; Mailänder, V., Uptake Mechanism of Oppositely Charged Fluorescent Nanoparticles in HeLa Cells. *Macromol. Biosci.* **2008,** 8 (12), 1135-1143.
16. Jiang, X.; Dausend, J.; Hafner, M.; Musyanovych, A.; Röcker, C.; Landfester, K.; Mailänder, V.; Nienhaus, G. U., Specific Effects of Surface Amines on Polystyrene Nanoparticles in their Interactions with Mesenchymal Stem Cells. *Biomacromolecules* **2010,** 11 (3), 748-753.

17. Jiang, X.; Musyanovych, A.; Röcker, C.; Landfester, K.; Mailänder, V.; Nienhaus, G. U., Specific effects of surface carboxyl groups on anionic polystyrene particles in their interactions with mesenchymal stem cells. *Nanoscale* **2011,** 3 (5), 2028-2035.
18. Schrade, A.; Mailänder, V.; Ritz, S.; Landfester, K.; Ziener, U., Surface Roughness and Charge Influence the Uptake of Nanoparticles: Fluorescently Labeled Pickering-Type Versus Surfactant-Stabilized Nanoparticles. *Macromol. Biosci.* **2012,** 12 (11), 1459-1471.
19. Tian, H.; Guo, Z.; Lin, L.; Jiao, Z.; Chen, J.; Gao, S.; Zhu, X.; Chen, X., pH-responsive zwitterionic copolypeptides as charge conversional shielding system for gene carriers. *J. Controlled Release* **2014,** 174, 117-125.
20. He, C.; Hu, Y.; Yin, L.; Tang, C.; Yin, C., Effects of particle size and surface charge on cellular uptake and biodistribution of polymeric nanoparticles. *Biomaterials* **2010,** 31 (13), 3657-3666.
21. Zaki, N. M.; Nasti, A.; Tirelli, N., Nanocarriers for Cytoplasmic Delivery: Cellular Uptake and Intracellular Fate of Chitosan and Hyaluronic Acid-Coated Chitosan Nanoparticles in a Phagocytic Cell Model. *Macromol. Biosci.* **2011,** 11 (12), 1747-1760.
22. Kang, J. H.; Jang, W. Y.; Ko, Y. T., The Effect of Surface Charges on the Cellular Uptake of Liposomes Investigated by Live Cell Imaging. *Pharm. Res.* **2017,** 34 (4), 704-717.
23. Tu, Z.; Achazi, K.; Schulz, A.; Mülhaupt, R.; Thierbach, S.; Rühl, E.; Adeli, M.; Haag, R., Combination of Surface Charge and Size Controls the Cellular Uptake of Functionalized Graphene Sheets. *Adv. Funct. Mater.* **2017,** 27 (33), 1701837.
24. Zhang, L. W.; Monteiro-Riviere, N. A., Mechanisms of Quantum Dot Nanoparticle Cellular Uptake. *Toxicol. Sci.* **2009,** 110 (1), 138-155.
25. Jiang, X.; Röcker, C.; Hafner, M.; Brandholt, S.; Dörlich, R. M.; Nienhaus, G. U., Endo- and Exocytosis of Zwitterionic Quantum Dot Nanoparticles by Live HeLa Cells. *ACS Nano* **2010,** 4 (11), 6787-6797.

26. Tan, S. J.; Jana, N. R.; Gao, S.; Patra, P. K.; Ying, J. Y., Surface-Ligand-Dependent Cellular Interaction, Subcellular Localization, and Cytotoxicity of Polymer-Coated Quantum Dots. *Chem. Mater.* **2010,** 22 (7), 2239-2247.
27. Ho, L. W. C.; Yung, W.-Y.; Sy, K. H. S.; Li, H. Y.; Choi, C. K. K.; Leung, K. C.-F.; Lee, T. W. Y.; Choi, C. H. J., Effect of Alkylation on the Cellular Uptake of Polyethylene Glycol-Coated Gold Nanoparticles. *ACS Nano* **2017,** 11 (6), 6085-6101.
28. Zhang, D.; Wei, L.; Zhong, M.; Xiao, L.; Li, H.-W.; Wang, J., The morphology and surface charge-dependent cellular uptake efficiency of upconversion nanostructures revealed by single-particle optical microscopy. *Chem. Sci.* **2018,** 9 (23), 5260-5269.
29. Jin, J.; Gu, Y.-J.; Man, C. W.-Y.; Cheng, J.; Xu, Z.; Zhang, Y.; Wang, H.; Lee, V. H.-Y.; Cheng, S. H.; Wong, W.-T., Polymer-Coated NaYF4:Yb3+, Er3+ Upconversion Nanoparticles for Charge-Dependent Cellular Imaging. *ACS Nano* **2011,** 5 (10), 7838-7847.
30. Xie, J.; Pan, X.; Wang, M.; Ma, J.; Fei, Y.; Wang, P.-N.; Mi, L., The role of surface modification for TiO2 nanoparticles in cancer cells. *Colloids Surf. B. Biointerfaces* **2016,** 143, 148-155.
31. Li, Z.; Qu, L.; Zhu, W.; Liu, J. a.; Chen, J.-Q.; Sun, P.; Wu, Y.; Liu, Z.; Zhang, K., Self-accelerating click reaction for preparing cyclic polymers from unconjugated vinyl monomers. *Polymer* **2018,** 137, 54-62.
32. Quémener, D.; Davis, T. P.; Barner-Kowollik, C.; Stenzel, M. H., RAFT and click chemistry: A versatile approach to well-defined block copolymers. *Chem. Commun.* **2006,** 48, 5051-5053.
33. Ghosh, S.; Basu, S.; Thayumanavan, S., Simultaneous and Reversible Functionalization of Copolymers for Biological Applications. *Macromolecules* **2006,** 39 (17), 5595-5597.
34. Ryu, J.-H.; Chacko, R. T.; Jiwpanich, S.; Bickerton, S.; Babu, R. P.; Thayumanavan, S., Self-Cross-Linked Polymer Nanogels: A Versatile Nanoscopic Drug Delivery Platform. *J. Am. Chem. Soc.* **2010,** 132 (48), 17227-17235.

35. Grassart, A.; Cheng, A. T.; Hong, S. H.; Zhang, F.; Zenzer, N.; Feng, Y.; Briner, D. M.; Davis, G. D.; Malkov, D.; Drubin, D. G., Actin and dynamin2 dynamics and interplay during clathrin-mediated endocytosis. *J. Cell Biol.* **2014,** 205 (5), 721-735.

36. Park, R. J.; Shen, H.; Liu, L.; Liu, X.; Ferguson, S. M.; De Camilli, P., Dynamin triple knockout cells reveal off target effects of commonly used dynamin inhibitors. *J. Cell Sci.* **2013,** 126 (22), 5305-5312.