Non-fouling NTA-PEG-based TEM Grid Coatings for Selective Capture of Histidine-tagged Protein Targets from Cell Lysates

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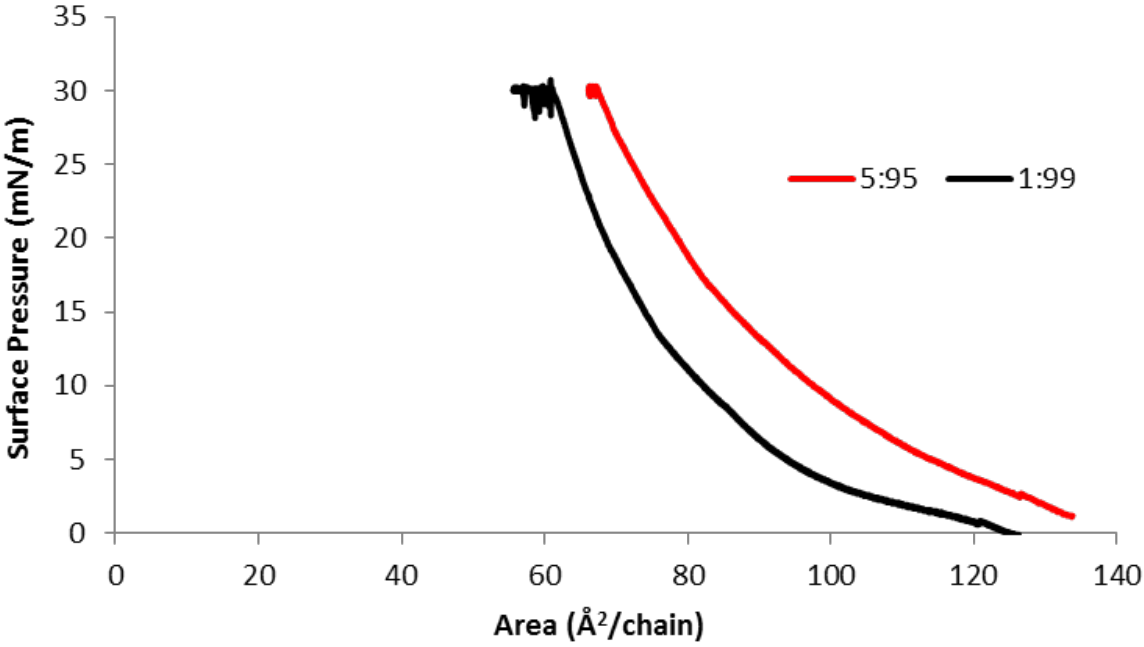


Figure S1. Pressure-area isotherm for 1:99 (black) and 5:95 (red) NTA-PEG2000-DSPE:mPEG350-DTPE monolayer films at 20 °C. The mushroom to brush regime is estimated to be approximately 75 $Å^2$/molecule for mPEG350-DTPE-rich monolayers.

TEM Grid Carbon Coating Procedure: A Formvar solution was prepared by dissolving 100 mg Formvar powder in 50 mL DCM. Copper grids (400 mesh) were purchased from Ted Pella and sonicated in acetone for 30 min before drying overnight in a dust free environment. Glass microscope slides were freshly cleaned with detergent, rinsed exhaustively with distilled water, dried overnight in a vacuum oven, and stored in a dust free environment until coated with Formvar solution for 30 sec by partial immersion before drying for 5 min. The edges of the glass slides then were scraped with a razor before floating the Formvar film on water by submersion of the glass slide into a darkly tinted Pyrex glass dish. The pre-cleaned copper grids were placed atop the floating Formvar film (15 - 20 grids, shiny side down) and picked up with a pre-cleaned glass slide before transfer to a Petri dish to dry overnight. Then, a carbon film was evaporated onto the glass slide with the TEM grids facing up before removal of the Formvar film by placement of the grids on top of filter paper soaked with $CHCl_3$ in a glass Petri dish for 30 min. Finally, the filter paper was removed and the grids dried overnight in a dust free environment before transfer to a standard TEM grid storage box.

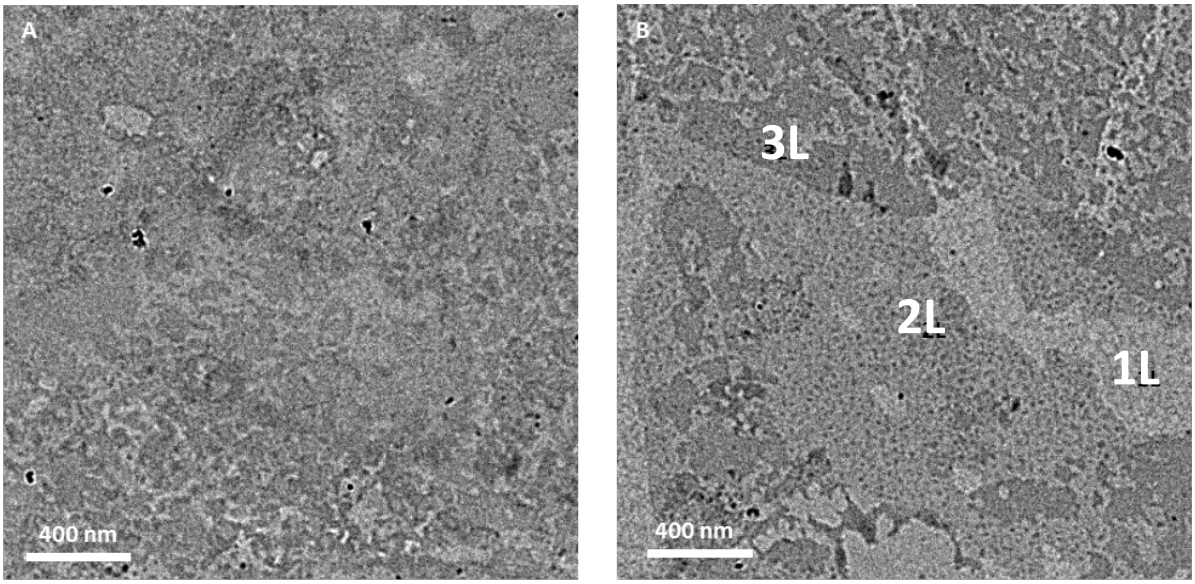


Figure S2. (A) **Negative Stain TEM Images of Grids Coated with 1:99 NTA-PEG2000-DSPE:mPEG350-DTPE Monolayers.** (B) Some sections of the monolayer-coated grid display areas with stepwise contrast that is suggestive of monolayer (1L), bilayer (2L) and trilayer (3L) lipid films. Scale bar: 400 nm.

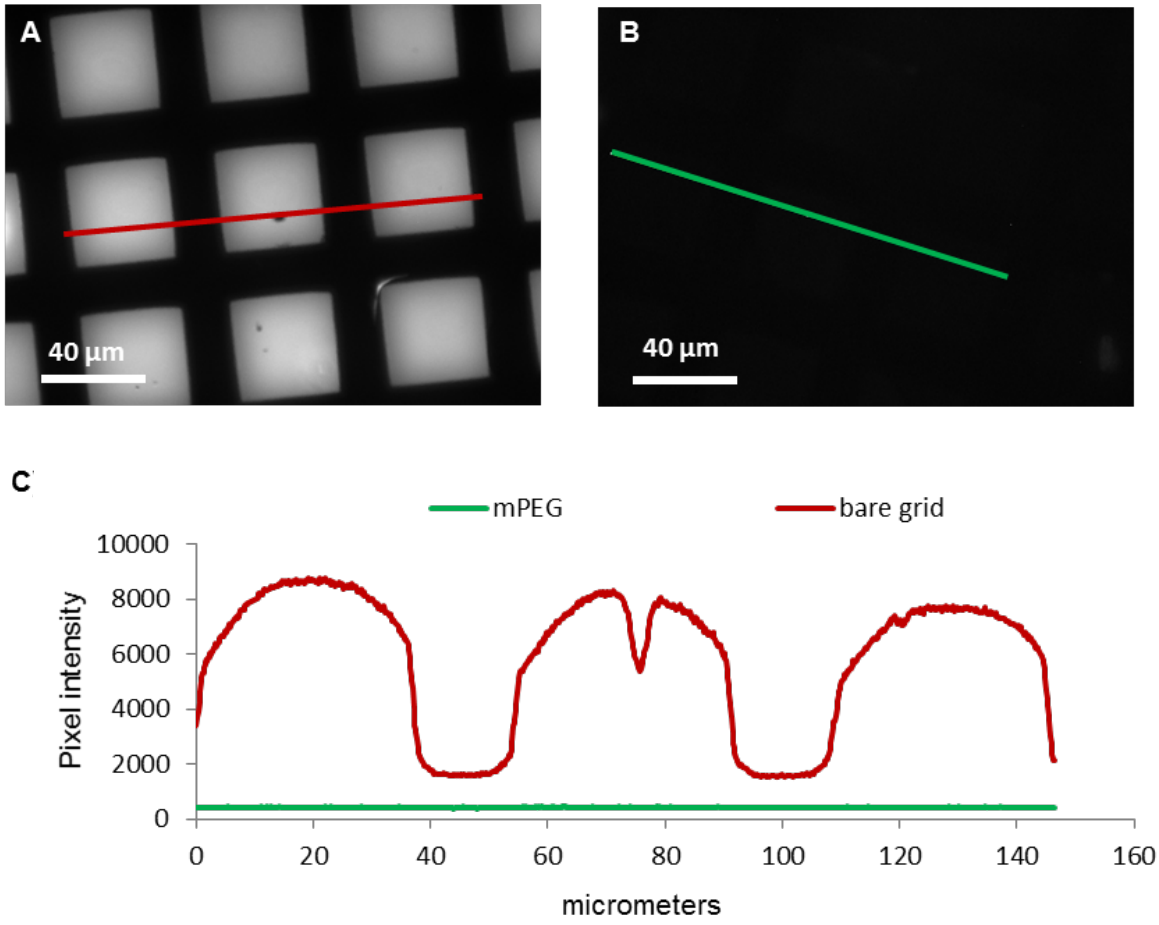


Figure S3. Fluorescence Microscopy of Uncoated and Stabilized Monolayer-coated TEM Grids. Fluorescence microscopy analysis of EM grids exposed to 2.0 mg/mL His_6-GFPuv for 2 min, followed by three MilliQ water rinses to remove unbound protein. (A) Bare carbon coated grids (glow discharged) and (B) 100% mPEG350-DTPE coated grids prepared by compression to the brush regime of mPEG350, LS transfer onto carbon coated grids, and photopolymerization as described in the Experimental Section. (C) Line scans of the regions highlighted in (A) and (B), showing CCD pixel intensity as a function of position along the grid using the same protein source, protein deposition time, rinsing conditions, and fluorescence imaging settings. Substantially lower His_6-GFPuv fluorescence was found on the mPEG350-DTPE coated grids than in the bare carbon coated grids. The abrupt reduction in pixel intensity centered at 76 µm in the bare carbon surface line scan is attributed to an absence of carbon coating on this section of the TEM grid.

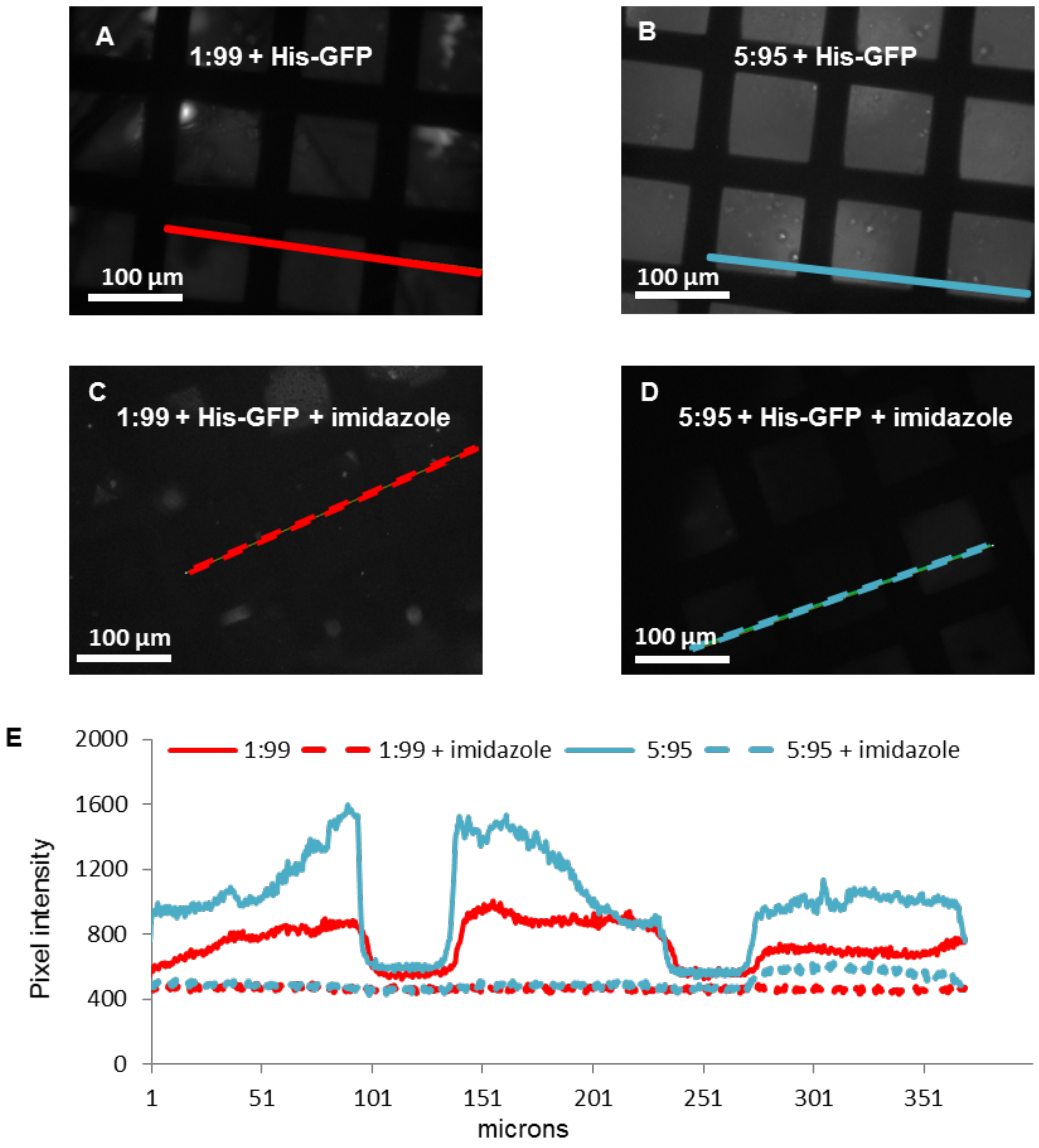


Figure S4. Fluorescence Microscopy of TEM Grids Coated with Stabilized NTA-PEG2000-DSPE:mPEG350-DTPE Monolayers. Fluorescence microscopy analysis of EM grids coated with 1:99 or 5:95 NTA-PEG2000-DSPE:mPEG350-DTPE after compression to the brush regime of mPEG350, LS transfer onto carbon coated grids, photopolymerization, and Ni^{2+} activation as described in the Experimental Section. The grids were then exposed to 2.0 mg/mL His_6-GFPuv for 2 min, followed by three MilliQ water rinses to remove unbound protein. (A) Appearance of 1:99 coating before and (C) after 500 mM imidazole, pH 7.2 treatment. (B) Appearance of 5:95 coating before and (D) after 500 mM imidazole, pH 7.2 treatment. (E) Line scans of the regions highlighted in (A) - (D), showing CCD pixel intensity as a function of position along the grid using the same protein source, protein deposition time, rinsing conditions, and fluorescence imaging settings.

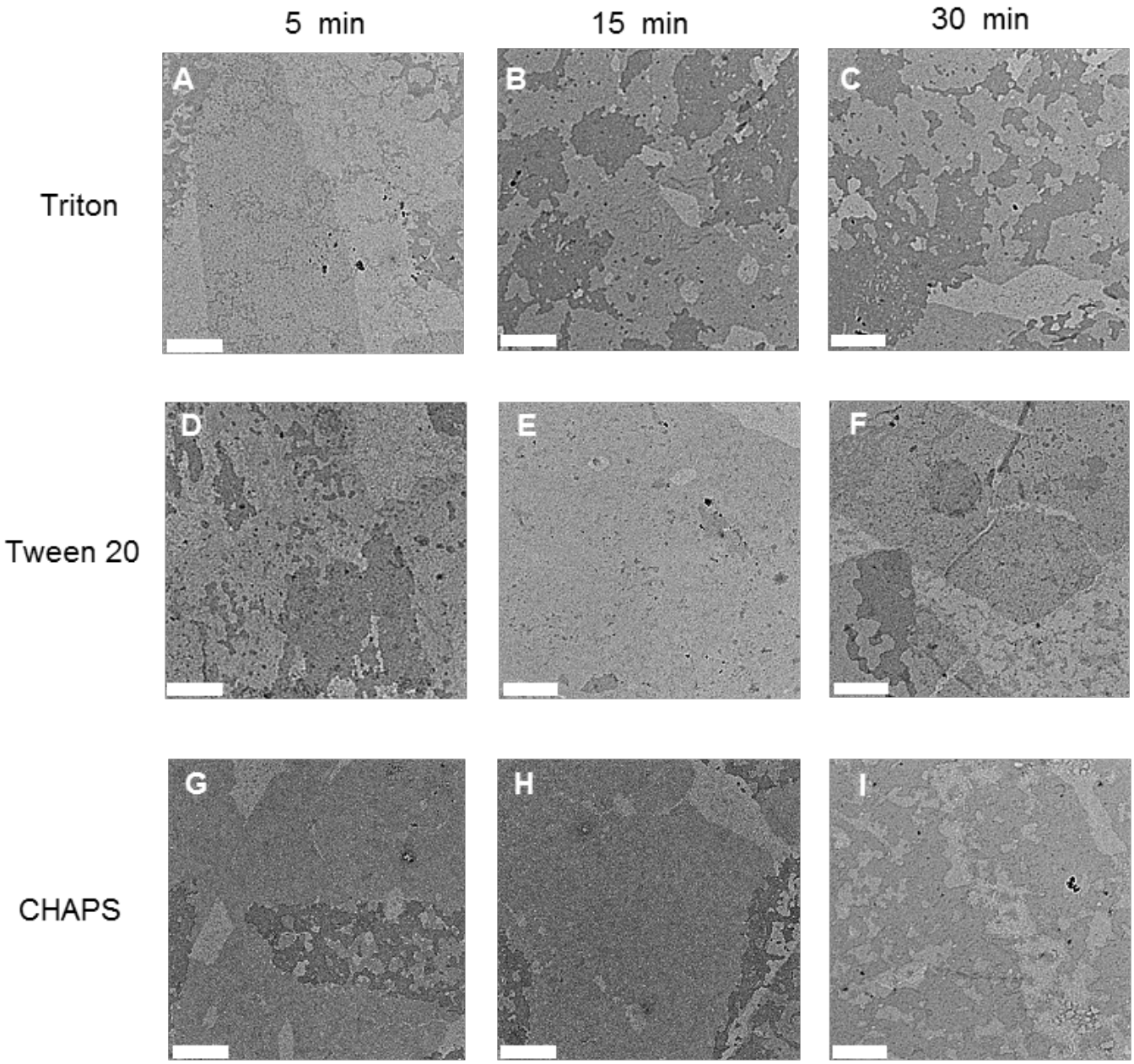


Figure S5. Negative Stain TEM Images of TEM Grids Coated with 1:99 NTA-PEG2000-DSPE:mPEG350-DTPE After Exposure to Different Detergents. Negative Stain TEM analysis of EM grids coated with 1:99 NTA-PEG2000-DSPE:mPEG350-DTPE after compression to the brush regime of mPEG350, LS transfer onto carbon coated grids, photopolymerization, and exposure to different detergent solutions for varying periods of time prior to TEM analysis. (A – C): A 0.03% Triton X-100 solution (10 μL) was placed in contact with the grids for (A) 5, (B) 15, or (C) 30 min. (D – F): A 0.014% Tween 20 solution (10 μL) was placed in contact with the grids for (D) 5, (E) 15, or (F) 30 min. (G – I): A 0.5% CHAPS solution (10 μL) was placed in contact with the grids for (G) 5, (H) 15, or (I) 30 min. The scale bars in all images are 400 nm. A defocus of > 10 μm was used to improve visualization of the monolayer film coatings.

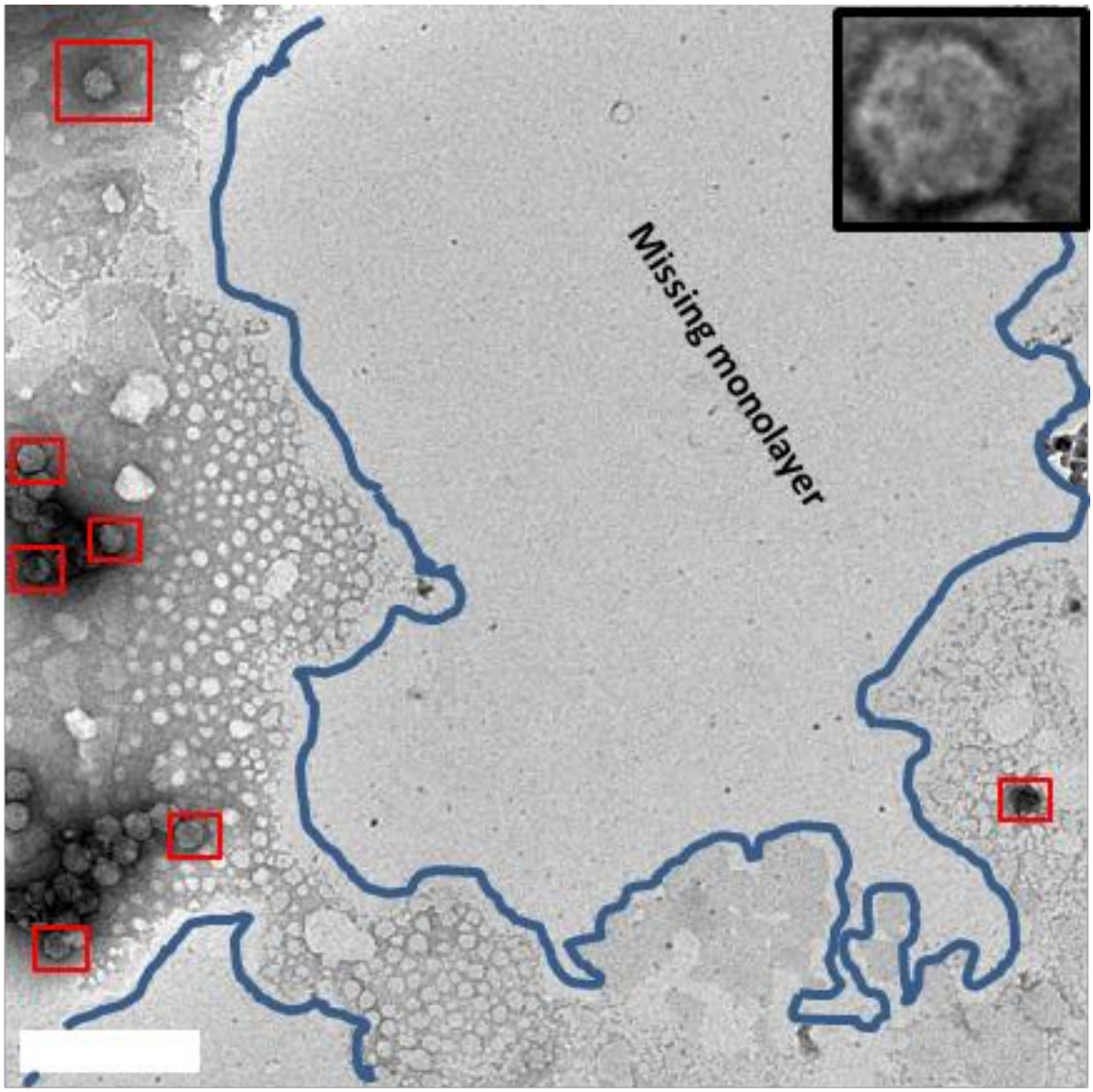


Figure S6. Negative Stain TEM Images of TEM Grids Coated with 1:99 NTA-PEG2000-DSPE:mPEG350-DTPE After Exposure to Tween 20. Negative Stain TEM analysis of EM grids coated with 1:99 NTA-PEG2000-DSPE:mPEG350-DTPE after compression to the brush regime of mPEG350, LS transfer onto carbon coated grids, photopolymerization, and Ni^{2+} activation, prior to treatment with 0.014% Tween 20 solution for 20 min. The grids were then treated with purified His_6-T7 bacteriophage before negative staining and TEM analysis. The area in the red box has been enlarged in the inset shown in the top right. Although large areas of the surface have been stripped free of lipid monolayer, the material remaining still shows the ability to capture His_6-T7 bacteriophage. Scale bar = 400 nm.

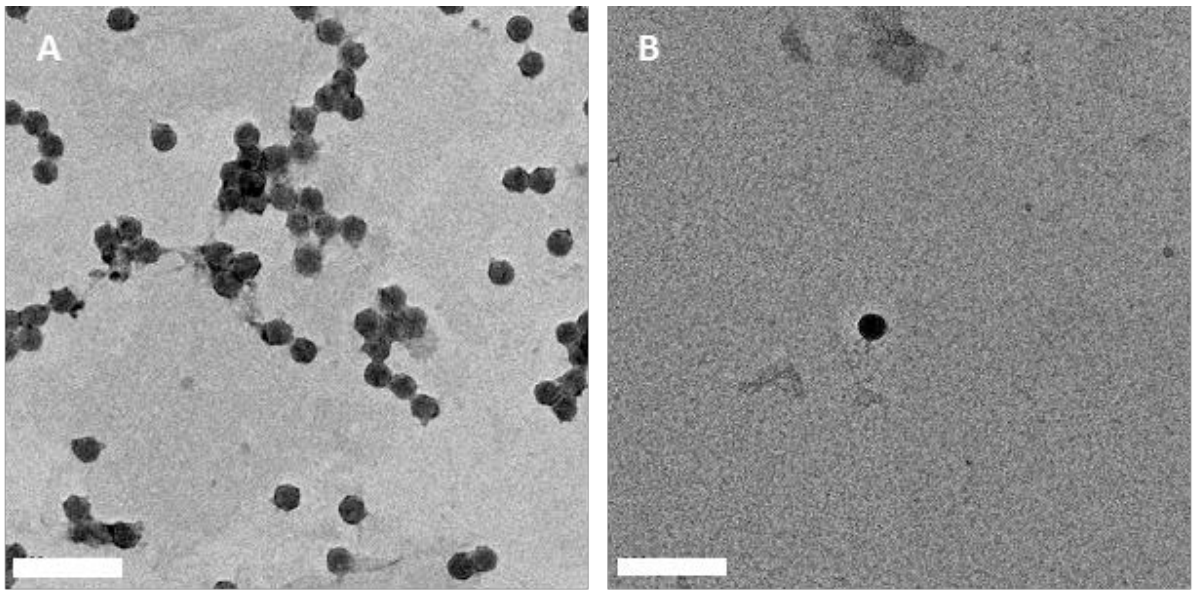


Figure S7. Negative Stain TEM Images of Purified T7 Bacteriophage on TEM Grids Coated with (A) 20:80 NTA-PEG2000-DSPE:mPEG350-DTPE and (B) 100% mPEG-DTPE. Scale bar = 200 nm. Comparison of (A) with Figure 4B in the main text reveals a significantly greater surface density of T7 phage particles captured on the grid possessing an increased NTA-PEG2000-DSPE concentration.

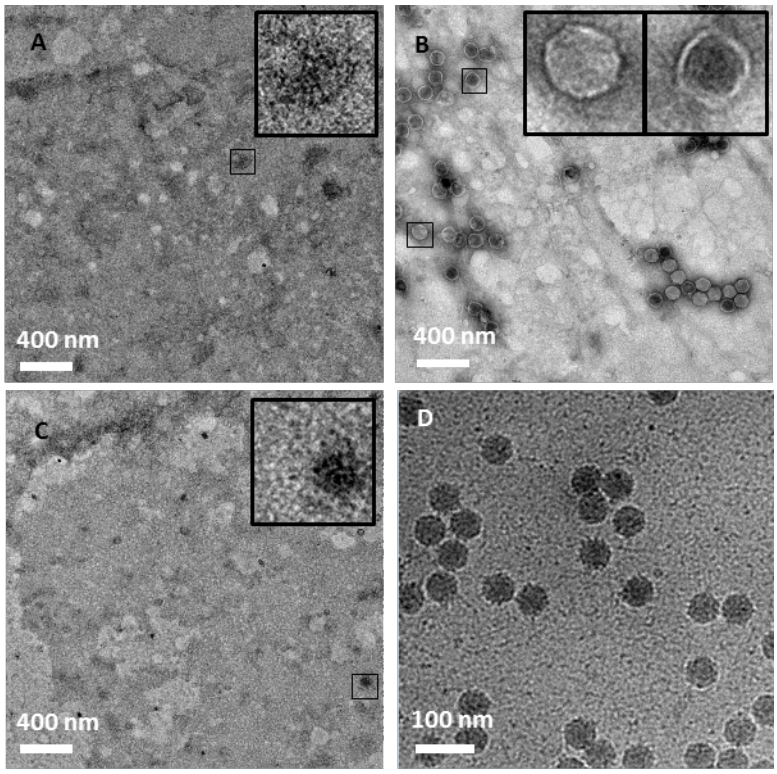
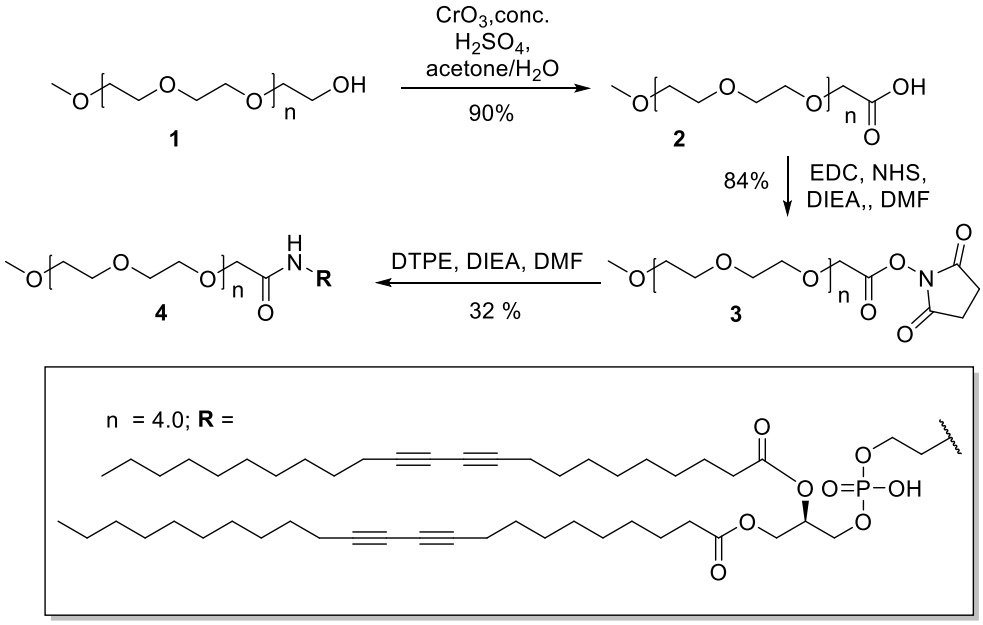


Figure S8. Effect of NTA Surface Density on His-T7 Bacteriophage Captured from Cell Lysates Using Negative Stain and cryoEM Analysis. (*A*) Negative stain TEM appearance of grid coated with stabilized 100% mPEG350-DTPE monolayer after 2 min exposure to cell lysate containing His-T7 bacteriophage. The particle in this panel is not phage; **(*B*)** same as in (***A***), except that the grid was coated with stabilized 1:99 Ni^{2+}:NTA-PEG2000-DSPE:mPEG350-DTPE monolayer with mature and immature T7 phage highlighted in the left and right insets respectively; **(*C*)** same as in (***B***), except that the grid was rinsed with 500 mM imidazole, pH = 7.4 after the 2 min lysate exposure step. The particle in this inset is not phage, and **(*D*)** cryoEM appearance of grid coated with stabilized 5:95 Ni^{2+}:NTA-PEG2000-DSPE:mPEG-350-DTPE monolayer after 2 min exposure to cell lysate containing His-T7 bacteriophage.

Materials. Solvents were purchased from Mallinckrodt/Baker and used without further purification unless noted. Toluene was purchased from Fisher. N-(3-dimethylaminopropyl)-N'-ethylcarbodiimide hydrochloride (EDC) and N^{ε}-((benzyloxy)carbonyl)-l-lysine (H-Lys(Z)-OH) were purchased from Advanced Chemtech. Heterobifunctional PEG derivatives were purchased from JenKem technology USA. 1,2-distearoyl-*sn*-glycero-3-phosphoethanolamine (DSPE) and 1,2-(tricosa-10',12'-diynoyl)-*sn*-glycero-3-phosphoethanolamine (DTPE) were purchased from Avanti Polar Lipids. All other chemicals were purchased from Sigma Aldrich and used without further purification. Dichloromethane (DCM), and toluene were distilled from CaH_2. Triethylamine (TEA) was distilled from CaH_2 and stored over BaO. Tetrahydrofuran (THF) was distilled from sodium-benzophenone ketyl. α-methoxy-polyethylene glycol (mPEG350) was purchased from Sigma Aldrich and purified by azeotropic drying with toluene. Jones' reagent (1.25 M in CrO3) was prepared by dissolving 17.5 g CrO_3 in 125 mL water plus 16 mL conc. H_2SO_4.

Experimental Methods. Nuclear magnetic resonance spectroscopy (NMR) was performed on a Bruker Avance ARX-400 NMR spectrometer using deuterated chloroform ($CDCl_3$) as NMR solvent and internal standard unless otherwise noted.



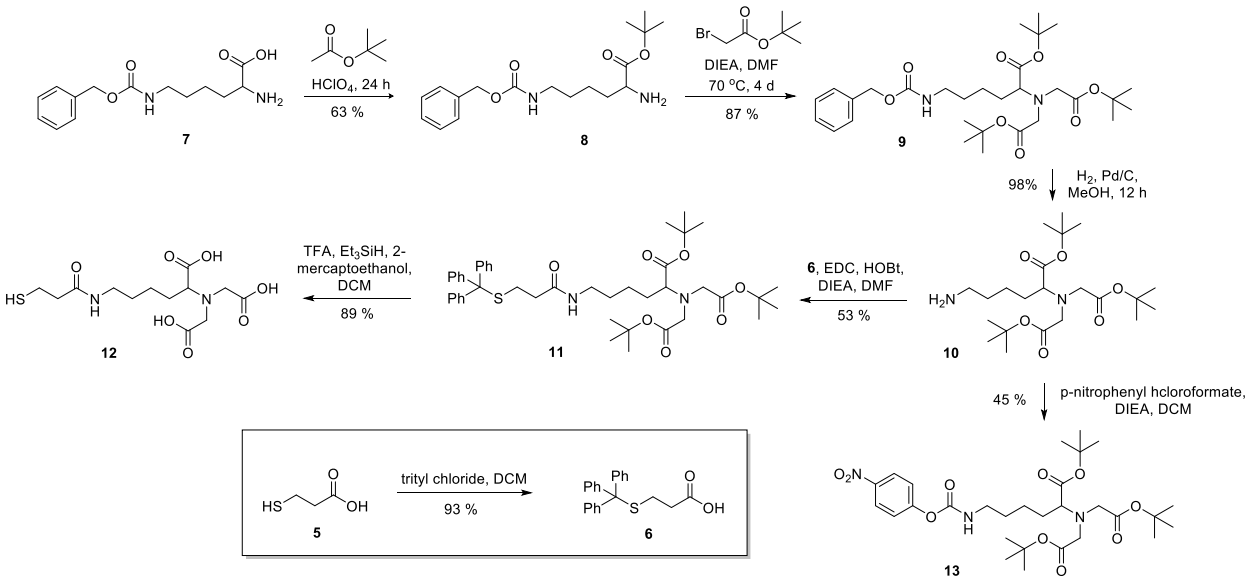
Scheme S1. Synthesis of $mPEG_{350}$-DTPE (**4**).

mPEG350-CO_2H (2). mPEG350 (**1**, 5.00 g, 14.3 mmol) was dissolved in 280 mL acetone. Jones' Reagent (15 mL, 1.25 M, 18.75 mmol) was added to a 500 mL round bottom flask and the mPEG solution was added to this flask dropwise over 1 h via addition funnel. The resulting solution was stirred at 20 °C for 1 h before quenching the excess Jones reagent with 10 mL iPrOH. The resulting green precipitate was removed by decantation of the liquid solution. The volatiles were removed under reduced pressure and the residue dissolved in 100 mL H_2O. The aqueous phase was extracted with DCM (3 x 120 mL). The organic layers were combined and dried over anhydrous Na_2SO_4, filtered, and evaporated under reduced pressure. The clear oily residue was used without further purification. Yield: 4.52 g (90 %); TLC: R_f= 0.26 (3:17 MeOH:DCM); ^{1}H NMR (400 MHz, $CDCl_3$): δ 3.35 (s 3H), 3.4-3.8 (m 30H), 4.01 (s 2H), 10.1-12.2 (br 1H).

mPEG350-NHS (3). Compound **2** (274 mg, **0**.753 mmol), and NHS (217 mg, 1.885 mmol) were dissolved in 15 mL DCM and the resulting solution was cooled in an ice bath. EDC (173 mg,

0.902 mmol) was then added followed by DIEA (393 µL, 2.259 mmol), and the solution was stirred while slowly warming from 4 to 20 °C over 18 h. Volatiles were removed under reduced pressure and the residue was dissolved in 50 mL DCM. The organic filtrate was washed with 50 mL H_2O twice before combining and extracting the aqueous phase with DCM (2 x20 mL). The combined organic phases were dried over anhydrous Na_2SO_4 before removing the solvent under reduced pressure and purifying the residue by flash chromatography on silica (3:17 MeOH:DCM). Yield: 230 mg (84 %); TLC: R_f= 0.66 (3:17 MeOH:DCM); ^{1}H NMR (400 MHz, $CDCl_3$): δ 2.65 (s 4H), 3.35 (s 3H), 3.4-3.8 (m 30H), 4.01 (s 2H).

mPEG350-DTPE (4). Compound **3** (100 mg, 0.210 mmol) and DTPE (183 mg, 0.210 mmol) were dissolved in DMF (5 mL) in a 100 mL round bottom flask covered in aluminum foil. DIEA (37 µL) was added and the solution was stirred at 20 °C for 36 h under N_2. The volatiles were removed under reduced pressure and the residue was purified by chromatography on silica (3:17 MeOH:DCM). Yield: 82 mg (32%); TLC: R_f= 0.29 (3:17 MeOH:DCM). ^{1}H NMR (400 MHz,



Scheme 2. Synthesis of NTA derivatives.

$CDCl_3$): δ 0.88 (t 6H), 1.25-1.35 (m 44H), 1.49 (m 8H), 2.22 (m 8H), 2.53 (br 2H), 2.61 (br 2H), 3.36 (s 3H), 3.54-4.32 (m, 40H), 512 (br 1H), 7.64 (br 1H).

***S*-Trityl-3-mercaptopropionic acid (6).** 3-Mercaptopropionic acid (**5**, 6.00 g, 56.5 mmol) was dissolved in DCM (50 mL) in a 250 mL round bottom flask. Trityl chloride (17.34 g, 62.2 mmol) in DCM (30 mL) was added dropwise to this solution over 1 h before stirring for an additional 12 h. The white precipitate was filtered and washed with diethyl ether (2 x 50 mL) and dried under a 50 μm vacuum to give a fine white powder. Yield: 18.25 g (93%); ^{1}H NMR (400 MHz, $CDCl_3$): δ 2.25 (t, 2H, J = 8 Hz), 2.47 (t, 2H, J = 8 Hz), 7.2-7.3 (m, 9H), 7.43 (d, 6H).

tert-Butyl N^6-((benzyloxy)carbonyl)-l-lysinate (8). N^{ε}-((benzyloxy)carbonyl)-l-lysine (12.03 g, 42.92 mmol) was mixed with t-butyl acetate (120 mL) in a 250 mL round bottom flask and concentrated $HClO_4$ (3.90 mL) added to this mixture, producing a clear solution. This solution was stirred for 12 h before extracting with 200 mL H_2O, 200 mL 5% HCl, then 200 mL H_2O. The aqueous extracts were combined and extracted with diethyl ether (3 x 200 mL) after addition of 30% NaOH solution until the aqueous layer was pH 11. The ether extracts were combined and dried over anhydrous $MgSO_4$. The ether was then filtered and concentrated under reduced pressure and dried under a 50 μm vacuum overnight giving a colorless oil. Yield: 9.25 g (63 %). ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.30 (s 9H), 1.23-1.50 (m 8H), 2.99 (t 2H), 3.11 (t (1H), 4.91 (s 2H), 5.61 (br 1H), 7.14-7.16 (m 5H). ^{13}C NMR (101 MHz, $CDCl_3$): δ 175.18, 156.34, 142.38, 136.60, 128.31, 127.88, 108.60, 80.74, 77.46, 77.14, 76.82, 66.29, 54.66, 40.66, 34.36, 31.08, 29.53, 27.90, 22.64.

Di-t-butyl 2,2'-((6-(((benzyloxy)carbonyl)amino)-1-(t-butoxy)-1-oxohexan-2-yl)azanediyl)diacetate (9). N^{ε}-Benzyloxycarbonyl-L-lysine-t-butyl ester (**8**, 9.25, 27.5 mmol) was dissolved in DMF (70 mL) prior to the addition of t-butyl bromoacetate (12.2 mL, 16.10 g, 82.6 mmol) and DIEA (16.8 mL, 11.9 g, 92.1 mmol) by syringe. The solution was stirred under N_2 at 70 °C for 72 h. The solvent was evaporated under reduced pressure and the residue was extracted with 200 mL of ethyl acetate and filtered. The ethyl acetate extract was purified by flash chromatography on silica (4:1 hexane:EtOAc) to give **9** as a slightly yellow oil. Yield: 12.56 g (87%); TLC: R_f = 0.48 (4:1 hexane:EtOAc); ^{1}H NMR ($CDCl_3$): δ 1.25-1.50 (m 6H), 1.30 (s 18H), 1.32 (s 9H), 3.04 (m 2H), 3.16 (t 1H), 3.33 (q 4H), 4.93 (s 2H), 5.39 (br 1H), 7.15-7.19 (m 5H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 172.07, 170.41, 156.32, 136.69, 128.15, 127.79, 127.64, 80.70, 80.33, 77.54, 77.22, 76.90, 66.03, 64.91, 60.04, 53.62, 40.56, 29.93, 29.02, 27.97, 27.86, 27.73, 22.80, 20.71, 13.97.

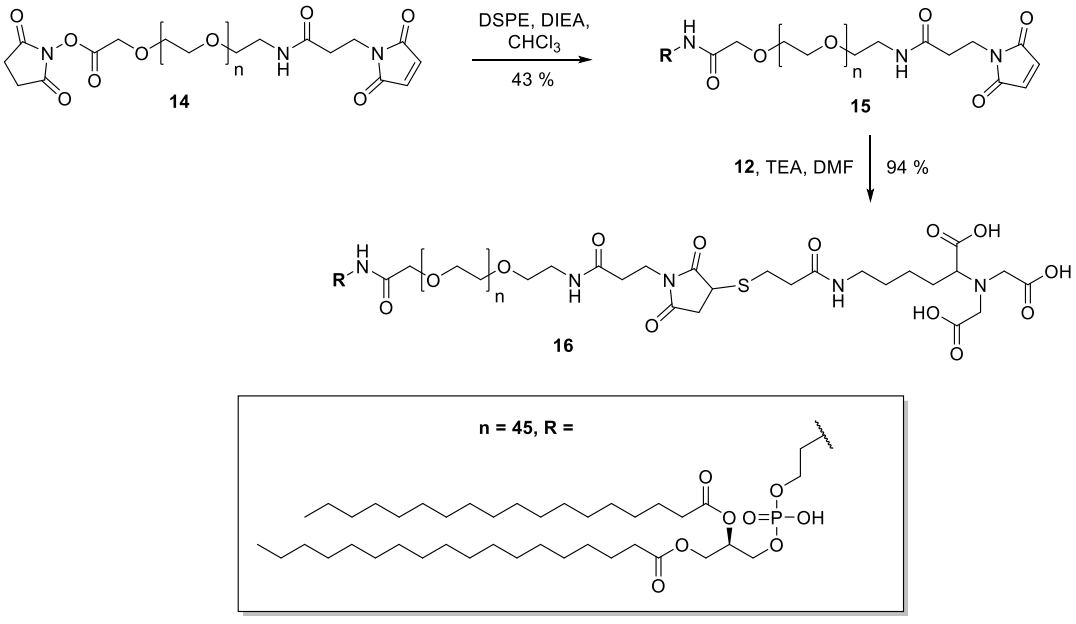
Di-t-butyl 2,2'-((6-amino-1-(tert-butoxy)-1-oxohexan-2-yl)azanediyl)diacetate (10). Compound **9 (**9.84 g, 17.4 mmol) was dissolved in MeOH (90 mL) in a 500 mL round bottom flask. To this solution was added 40 mg of 10% Pd/C. The flask was evacuated and purged with H_2 three times and then stirred for 12 h under 1 atm H_2. The heterogeneous solution was then filtered through a pad of Celite, with further washing of the Celite cake with 50 mL MeOH. The filtrate was concentrated under reduced pressure to give **10** as a clear oil. Yield: 7.35 g (98%); TLC: R_f = 0 (4:1 hexane:EtOAc); ^{1}H NMR ($CDCl_3$): δ 1.29 (s 18H), 1.30 (s 9H) 1.1-1.5 (m 6H), 2.63 (t 2H), 3.16 (t 1H), 3.30 (q 4H), 3.96 (br 3H)^{13}C NMR (101 MHz, $CDCl_3$) δ 172.05, 170.46, 80.78, 80.42, 77.41, 77.09, 76.77, 64.94, 53.52, 49.54, 40.90, 31.09, 30.09, 27.95, 27.87, 22.91.

Di-t-butyl 2,2'-((1-(t-butoxy)-1-oxo-6-(3-(tritylthio)propanamido)hexan-2-yl)azanediyl)diacetate (11). Compound **10** (1.46 g, 4.19 mmol) and Compound **6** (1.80 g, 4.19 mmol) were dissolved in DMF (100 mL) in a 250 mL round bottom flask. This solution was cooled on an ice bath before addition of EDC (0.962 g, 5.02 mmol), HOBt (0.678 g, 5.02 mmol), and 1.86 mL of DIEA (1.35 g, 10.4 mmol). The solution was stirred under Ar for 48 h while allowing the mixture to gradually warm from 4 → 20 °C. The DMF was evaporated under reduced pressure and the residue was dissolved in EtOAc (80 mL). This solution was washed with H_2O (2 x 100 mL) before combining the aqueous phases and back extracting with EtOAc (100 mL). The EtOAc layers were combined and dried over anhydrous Na_2SO_4. The solvent was evaporated and the residue purified by flash chromatography on silica using 1:1 hexane:EtOAc as eluent yielding **11** as a colorless oil. Yield: 1.70 g (53%).; TLC: R_f= 0.54 (1:1 hexane:EtOAc); ^{1}H NMR ($CDCl_3$): δ 1.22-1.61 (m 6H), 1.39 (s 18H), 1.43 (s 9H), 2.09 (t 2H), 2.46 (t 2H), 3.11 (m 2H), 3.25 (t 1H), 3.43 (m 4H), 5.98 (br 1H), 7.14-7.4 (m 15H); MS (ESI+). Expected: 762.02 [M+H]; Found 762.71.

2,2'-((1-Carboxy-5-(3-mercaptopropanamido)pentyl)azanediyl)diacetic acid (12). Compound **11** (0.800 g, 1.05 mmol) was dissolved in DCM (10 mL), followed by addition of Et_3SiH (0.367 g, 3.16 mmol) and 2-mercaptoethanol (.246 g, 3.15 mmol). This solution was cooled on an ice bath before addition of TFA (15 mL) dropwise over 10 min. The solution was stirred for 1 h at 4 °C before removal of the volatiles under reduced pressure. Diethyl ether (20 mL) and 4 drops of concentrated HCl were added to the residue before decanting the organic phase and repeating the process two more times. Toluene (30 mL) was then added to the residue and evaporated under reduced pressure three times to give compound **12** as a white

powder. Yield: 0.327 g (89%); ^{1}H NMR (D_2O): δ 1.41 (m 2H), 1.49 (m 2H), 1.80-1.90 (m 2H), 2.43 (t 2H), 2.67 (t 2H), 2.73 (m 1H), 3.13 (m 2H), 3.93 (s 4H).

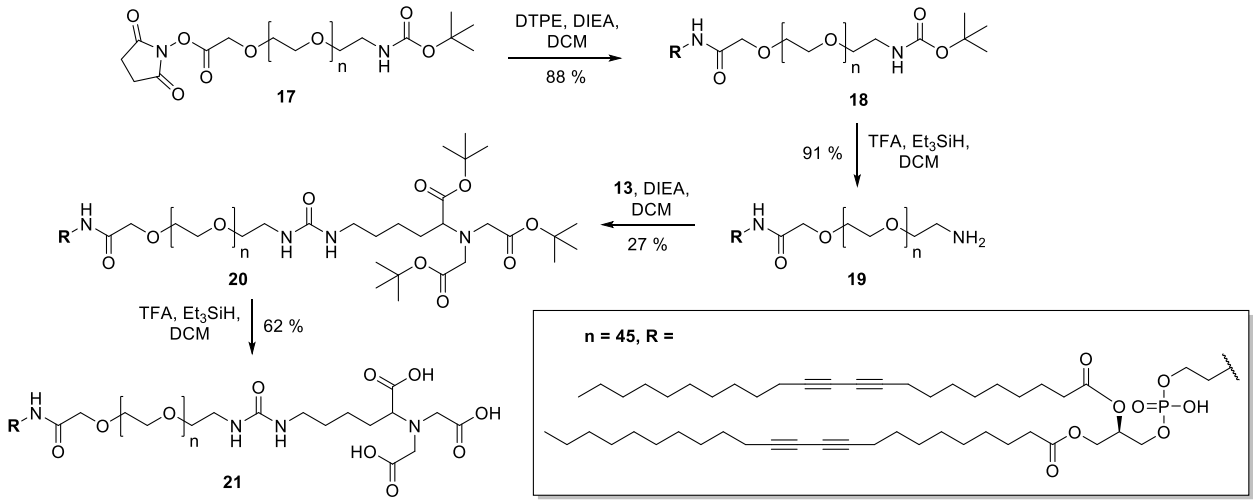
Di-tert-butyl-2,2'-((1-(tert-butoxy)-6-(((4-nitrophenoxy)carbonyl)amino)-1-oxohexan-2-yl)azanediyl)diacetate (13). p-nitrophenyl chloroformate (PNP-Cl, 0.552 g, 2.74 mmol) was dissolved into 20 mL DCM in a 100 mL round bottom flask equipped with a stir bar and addition funnel and was cooled to 4˚C in an ice bath. Compound **12** (0.983 g, 2.28 mmol) in 20 mL DCM was added to the addition funnel. The system was evacuated and flashed with nitrogen gas. The solution of **12** was added over a one hour period at 4˚C and stirred for an additional 12 hours warming to room temperature. The solution was concentrated in vacuo and purified by flash chromatography using 4:1 hexanes: EtOAc as eluent. Yield: 0.609 g (45%).; TLC: R_f= 0.24 (4:1 hexanes: EtOAc); ^{1}H NMR ($CDCl_3$): δ 1.38 (s 18H), 1.40 (s 9H), 1.18-1.59 (m 6H), 3.18-3.29 (m 3H), 3.40 (q 4H), 6.09 (br 1H), 7.24 (d 2H, J = 9 Hz), 8.14 (d 2H, J = 9 Hz); MS (ESI+). Expected: 596.68 [M+H]; Found 596.59 ([M+H], 618.54 [M+Na].



Scheme S3. Synthesis of NTA-PEG2000-DSPE (**16**).

Maleimide-PEG2000-DSPE (15). DSPE (0.072 g, 0.096 mmol) and NHS-PEG2000-maleimide (0.200 g, 0.095 mmol) were dissolved in $CHCl_3$ (15 mL) in a 50 mL round bottom flask with stir bar and DIEA (0.062g, 0.480) mmol was added via syringe. The flask was evacuated and flushed with nitrogen and stirred for 72 hours at ambient temperature. The volatiles were evaporated under reduced pressure and the residue purified by flash chromatography on silica using a gradient starting with 85:15 DCM:MeOH and increasing in polarity to 80:20 DCM:MeOH. Yield: 0.118 g (43%).; TLC: R_f= 0.48 (4:1 DCM:MeOH); ^{1}H NMR ($CDCl_3$): δ 0.84 (t 6H), 1.15-1.40 (m 64H), 2.24 (m 4H), 2.48 (t 2H) 2.99 (m 2H), 3.37-4.10 (m 180 H), 3.80-3.96 (m 4H), 4.12 (m 2H), 4.34 (d 2H), 5.17 (m 1H), 6.27 (br 1H), 6.67 (s 1H), 7.38 (br 1H).

NTA-PEG2000-DSPE (16). Compounds **15** (20.0 mg, 0.007 mmol) and **12** (18.0 mg, 0.051 mmol) were dissolved in DMF (4 mL) in a 25 mL round bottom flask with stir bar. TEA (15.0 µL, 0.119 mmol) was added and the flask was evacuated and flushed with nitrogen. The solution was stirred at 40˚C for 24 hours monitoring the consumption of starting material by TLC. Volatiles were removed *in vacuo* at 45˚C. the residue was dissolved in 6 mL PBS buffer (pH = 7.2) plus 4 mL MeOH. This solution was extracted with $CHCl_3$ (3x15 mL). The organic extracts were combined and dried over anhydrous Na_2SO_4, filtered and concentrated *in vacuo* to give **16**. Yield: 21.2 mg (94 %).; TLC: R_f= 0.0 (4:1 DCM:MeOH); ^{1}H NMR ($CDCl_3$): δ 0.84 (t 6H), 1.15-1.40 (m 64H), 2.25 (s 4H), 2.28-2.32 (m 2H), 2.48-2.53 (m 2H), 2.75-2.78 (m 4H), 2.48-3.7 (m 180H), 3.70 (s 4H), 3.88 (br 2H), 4.00 (br 2H), 4.16 (br 2H), 4.37-4.40 (br 1H), 5.30 (s 1H), 6.96 (s 1H).



Scheme S4. Synthesis of NTA-PEG2000-DTPE (**21**).

NHBoc-PEG2000-DTPE (18). NHS-PEG2K-NHBoc (**17**, 190 mg, 0.095 mmol) and DTPE (82.8 mg, 0.095 mmol) were dissolved in DCM (10 mL) in a 25 mL round bottom flask with stir bar. DIEA (83 µL, 0.474 mmol) was added and the flask was evacuated, flushed with nitrogen and covered with aluminum foil. The solution was stirred at ambient temperature for 48 hours in the dark. Evapoarated volatiles and purified by flash chromatography on silica using an eluting system of DCM and MeOH starting with 95:5 then 90:10 then 85:15. Fractions containing product were combined and dried in vacuo to give **18**. Yield: 0.209 g (88 %).; TLC: R_f= 0.72 (80:20 DCM:MeOH); ^{1}H NMR ($CDCl_3$): δ 0.88 (m 6H), 1.23-1.49 (m 50H), 1.42 (s 9H), 2.21 (m 8H), 2.66 (t 4H), 3.26-3.96 (m 180 H), 4.11-4.12 (m 2H), 4.33-4.36 (m 2H), 5.17 (br 1H).

NH_2-PEG2000-DTPE (19). Compound **18** (209 mg, 0.076 mmol) and triethylsilane (200 µL, 1.25 mmol) was dissolved in 30 % TFA in DCM solution (20 mL) and stirred for 1.5 hours under ambient temperature and atmosphere. Volatiles were removed *in vacuo* and the residue

was evaporated with 15 mL DCM twice more. The product was dried *in vacuo* and used without further purification. Yield: 0.191 g (91 %).; TLC: R_f= 0.56 (80:20 DCM:MeOH).

NTA-(OtBu)3-PEG2000-DTPE (20). Compound **19** (95.5 mg, 0.034 mmol) and compound **13** (205 mg, 0.344 mmol) were dissolved in DCM (5 mL) in a 25 mL round bottom flask with stir bar. The flask was evacuated and flushed with nitrogen. DIEA (60 µL, 0.348 mmol) was added and the solution stirred for 48 hours at ambient temperature under a nitrogen atmosphere. Volatiles were evaporated under reduced pressure and the product was purified by flash chromatography on silica using a gradient of DCM:MeOH as eluent starting with 90:10 moving to 85:15 then finally 80:20. Fractions containing product were pooled, concentrated and dried *in vacuo* to give compound **20**. Yield: 29.0 mg (27 %).; TLC: R_f= 0.65 (80:20 DCM:MeOH).

NTA-PEG2000-DTPE (21). Compound **20** (29 mg, 0.009 mmol) and triethylsilane (100 µL, 0.625 mmol) were dissolved in 30% TFA in DCM solution (10 mL) in a 25 mL round bottom flask with stir bar. The solution was stirred under ambient temperature and atmosphere for 1.5 hours. Volatiles were evaporated and the residue was dissolved in 5mL PBS buffer (pH = 7.2) plus 5 mL MeOH. The solution was extracted with $CHCl_3$ (3x8 mL). The organic extracts were dried over anhydrous Na_2SO_4, filtered, concentrated and dried *in vacuo* to give compound **21**. Yield: 18.0 mg (62 %).; TLC: R_f= 0.0 (80:20 DCM:MeOH).

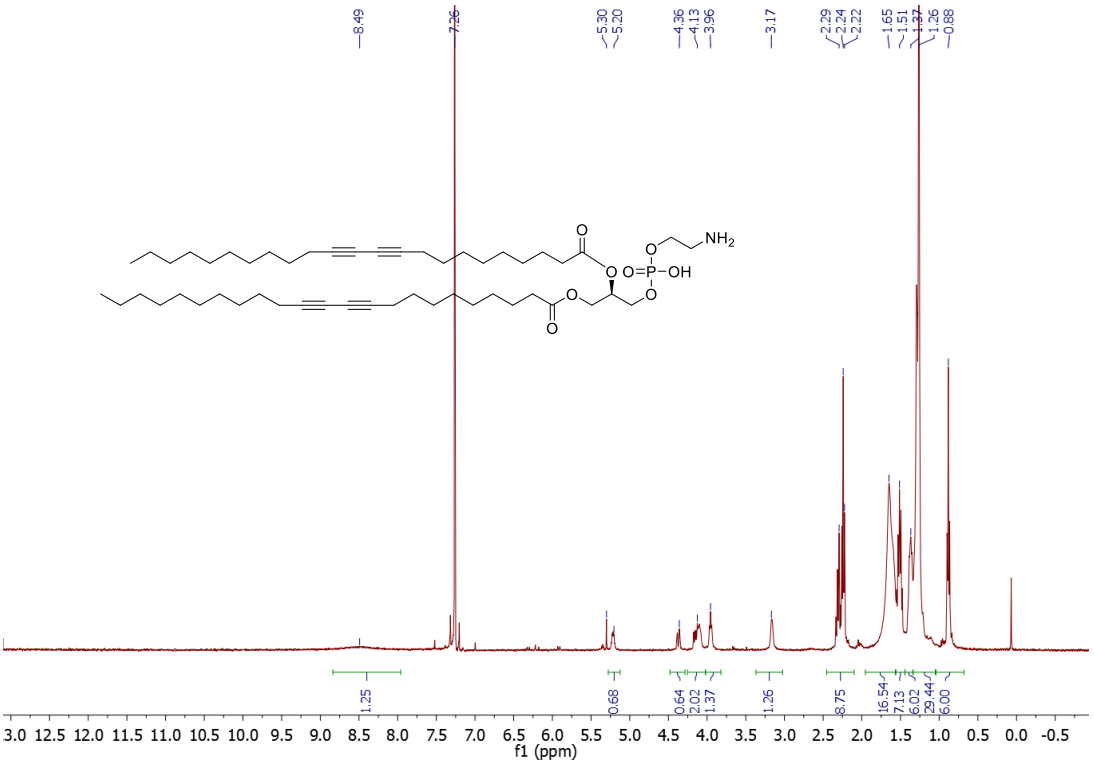


Figure S9. ^{1}H-NMR spectrum (400 MHz) of 1,2-(tricosa-10',12'-diynoyl)-*sn*-glycero-3-phosphoethanolamine (DTPE).

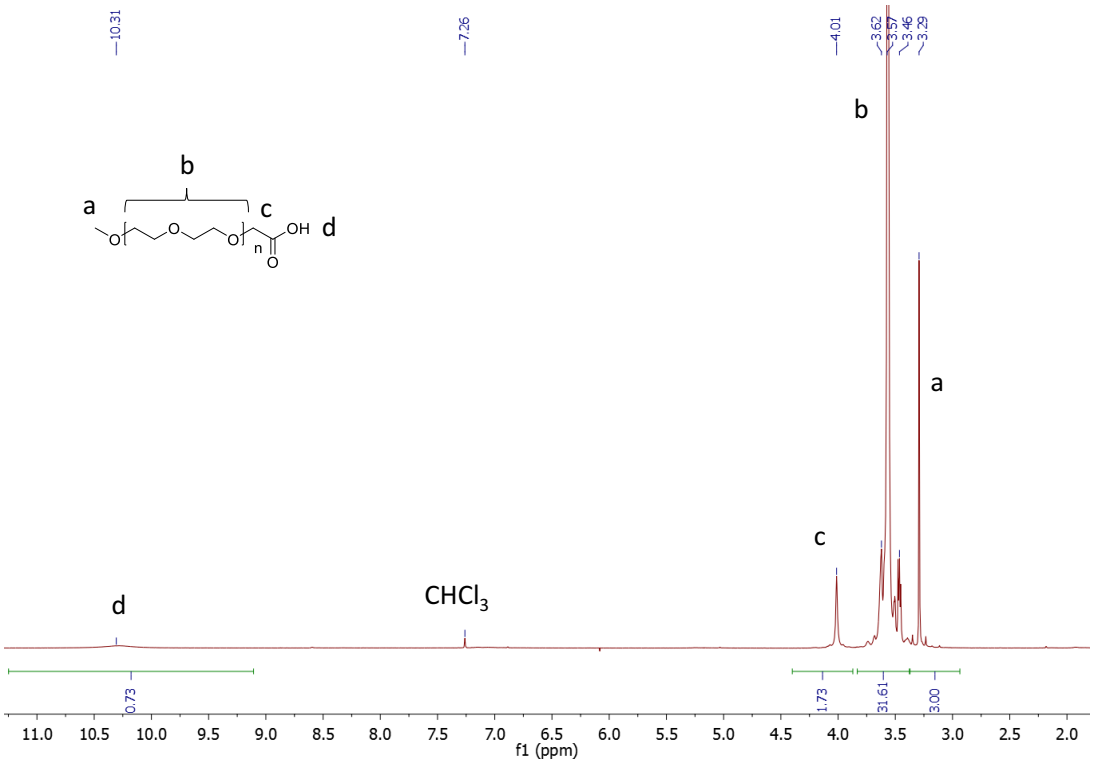


Figure S10. ^{1}H-NMR spectrum (400 MHz) of mPEG350-CO_2H (**2**).

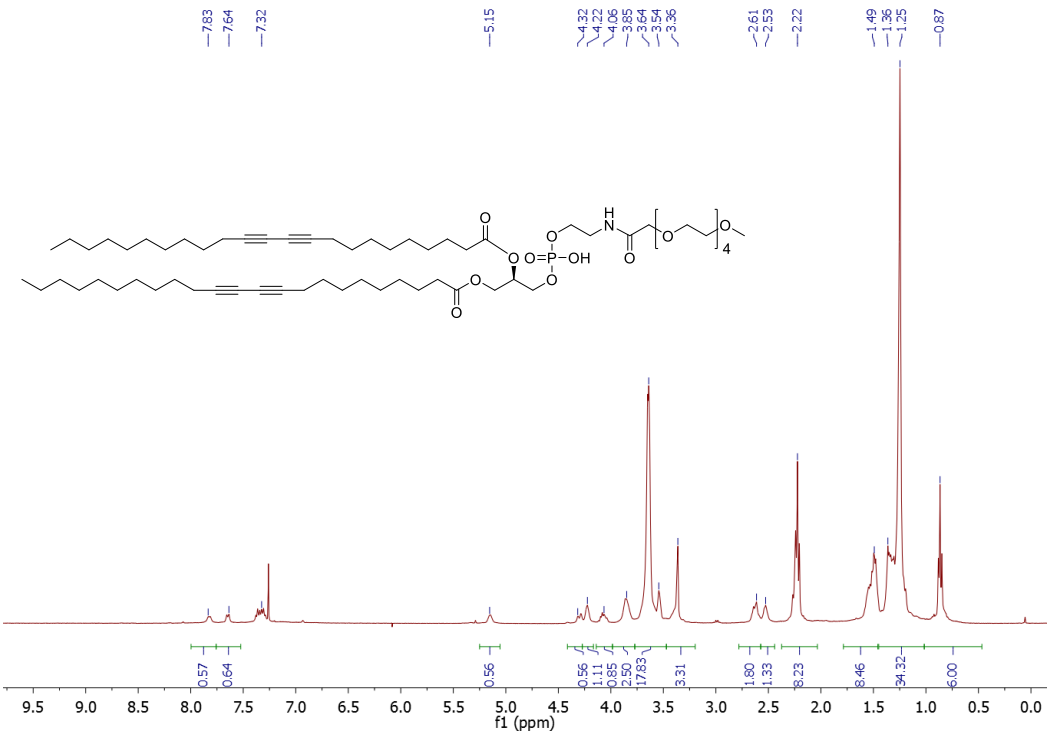


Figure S11. ^{1}H-NMR spectrum (400 MHz) of mPEG350-DTPE (**4**).

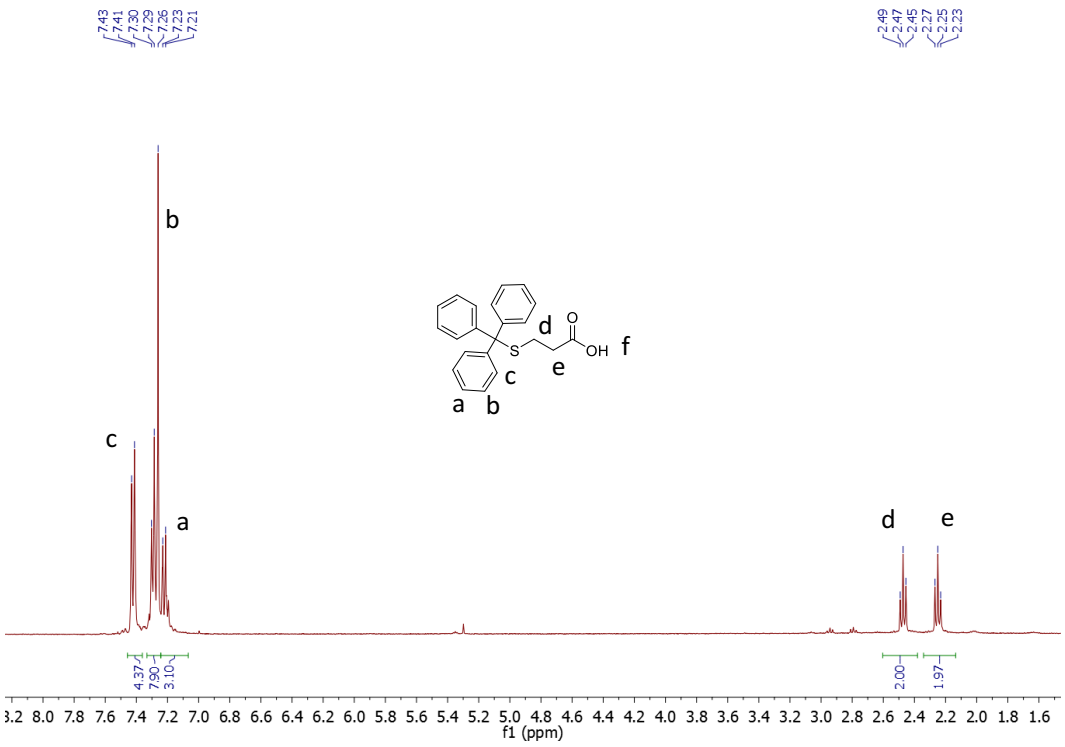


Figure S12. ^{1}H-NMR spectrum (400 MHz) of **6.**

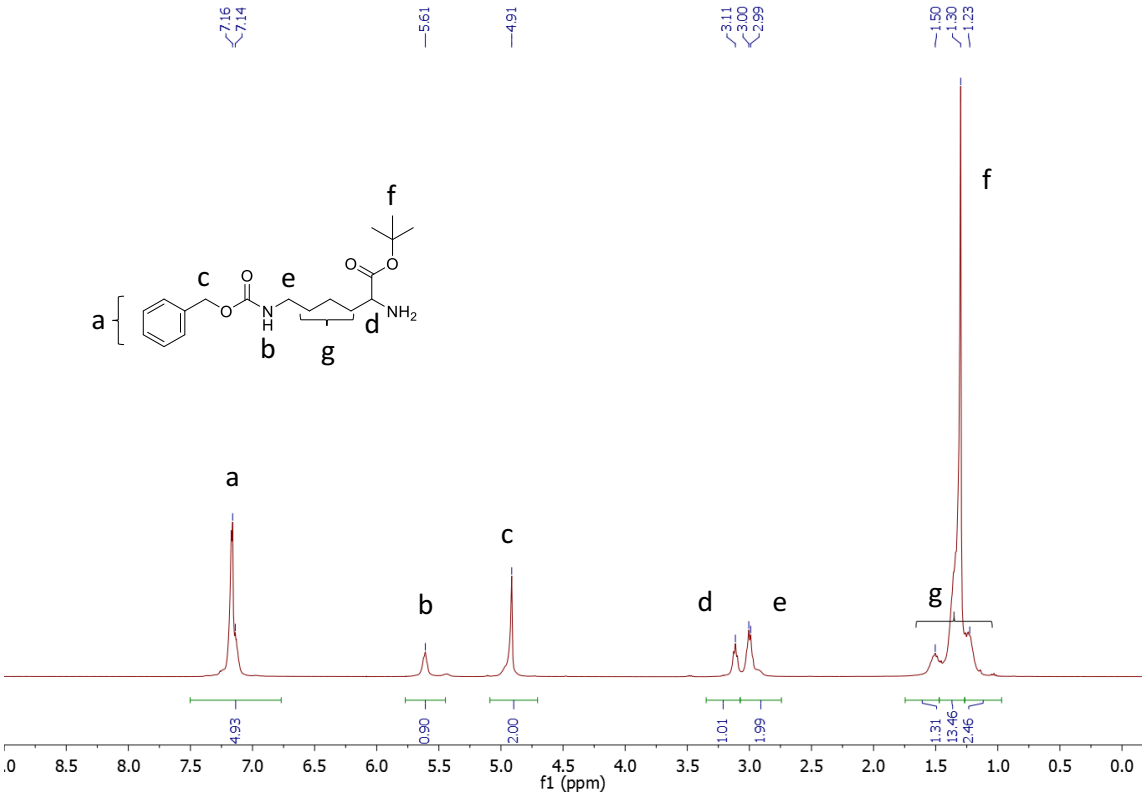


Figure S13. ^{1}H-NMR spectrum (400 MHz) of **8.**

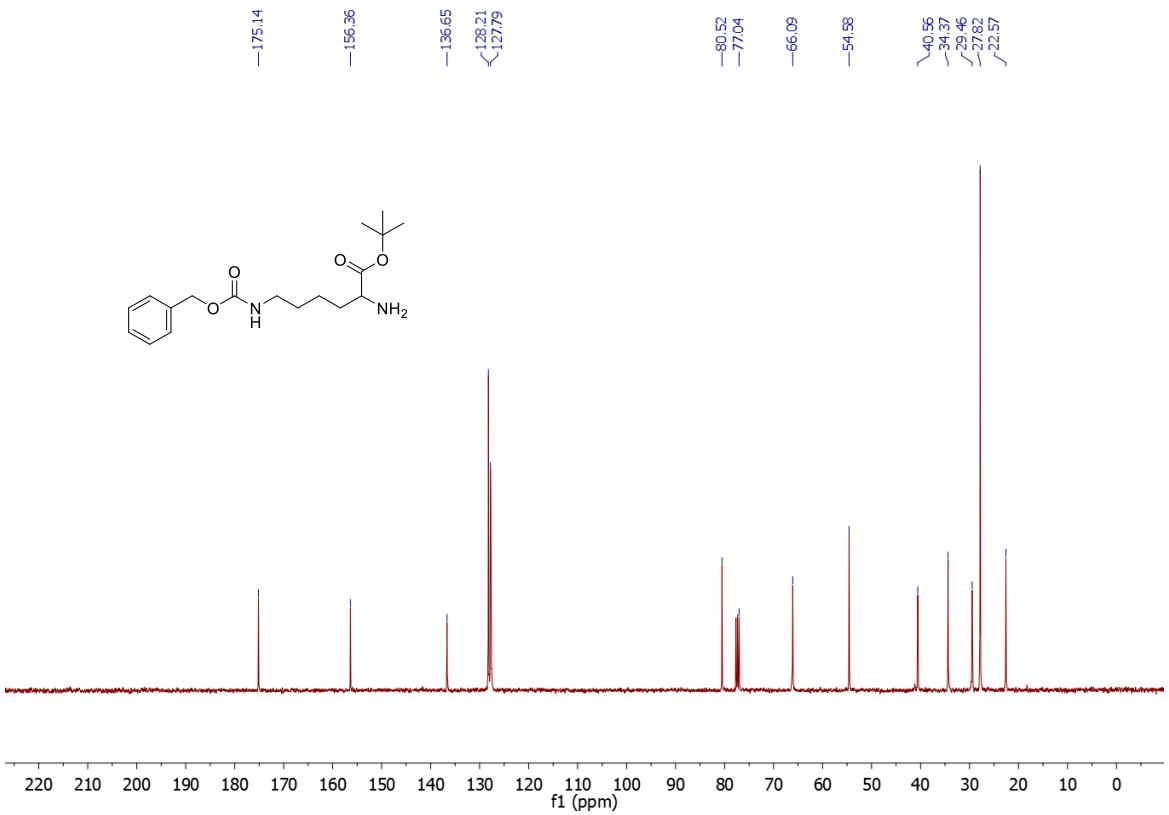


Figure S14. ^{13}C-NMR spectrum (101 MHz) of **8.**

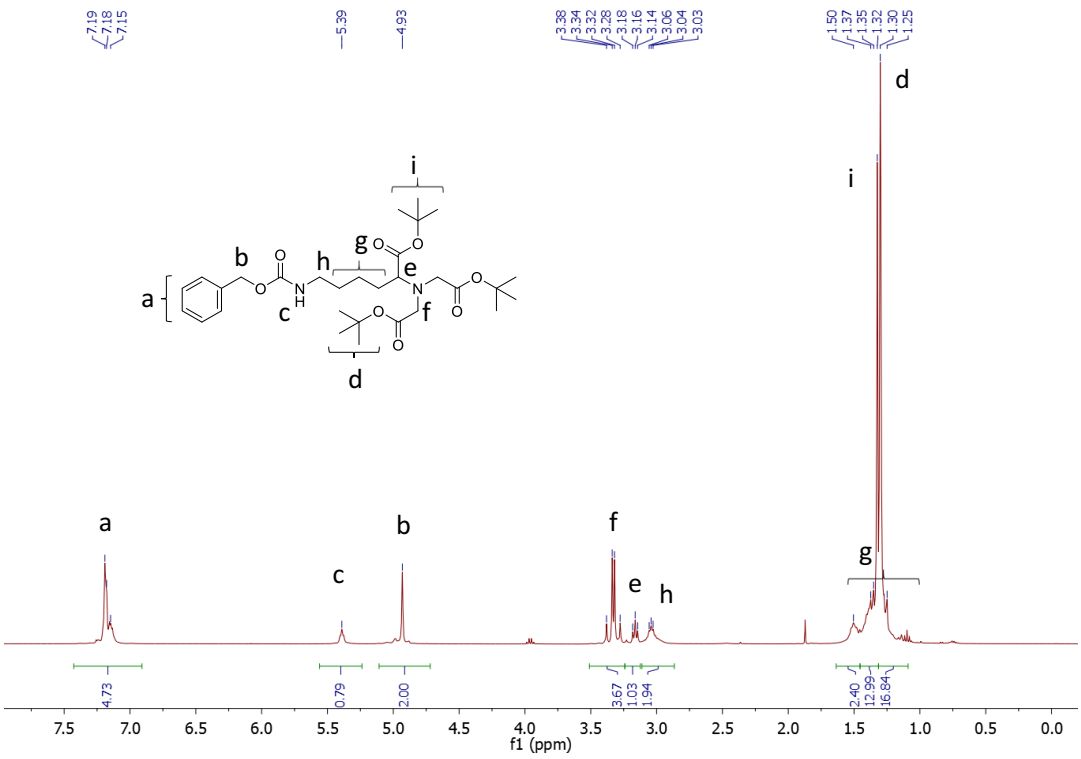


Figure S15. ^{1}H-NMR Spectrum (400 MHz) of **9**.

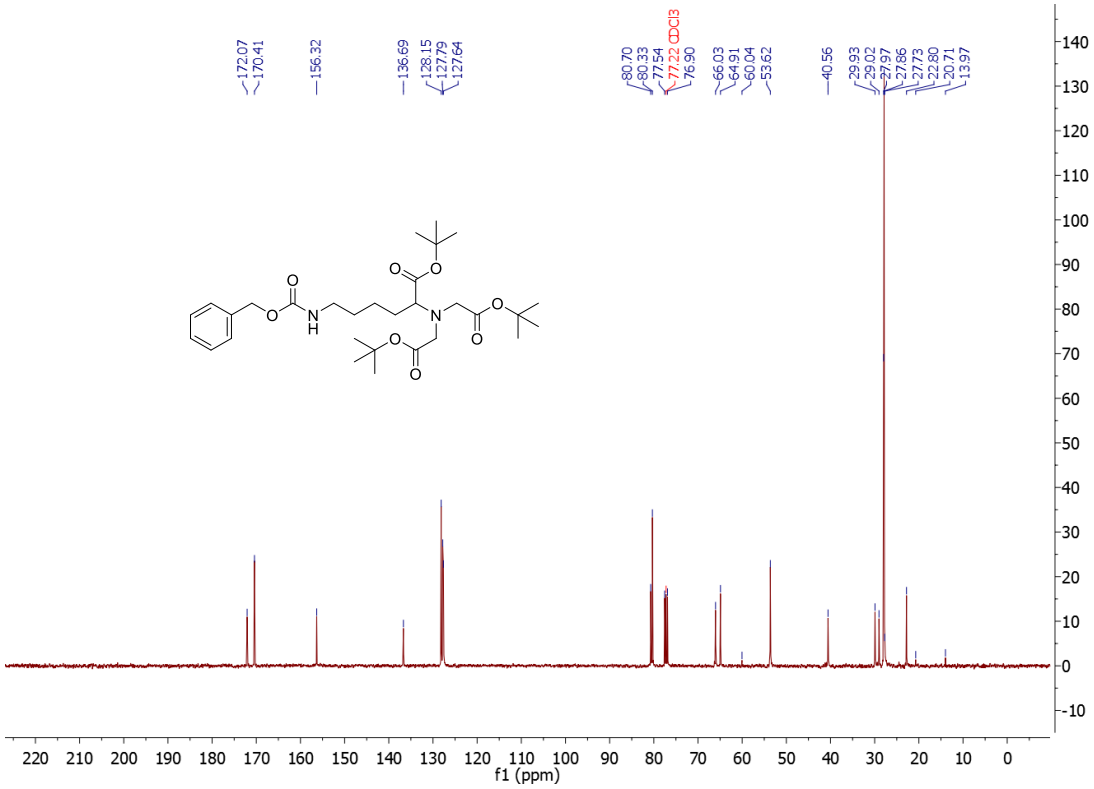


Figure S16. ^{13}C-NMR spectrum (101 MHz) of **9.**

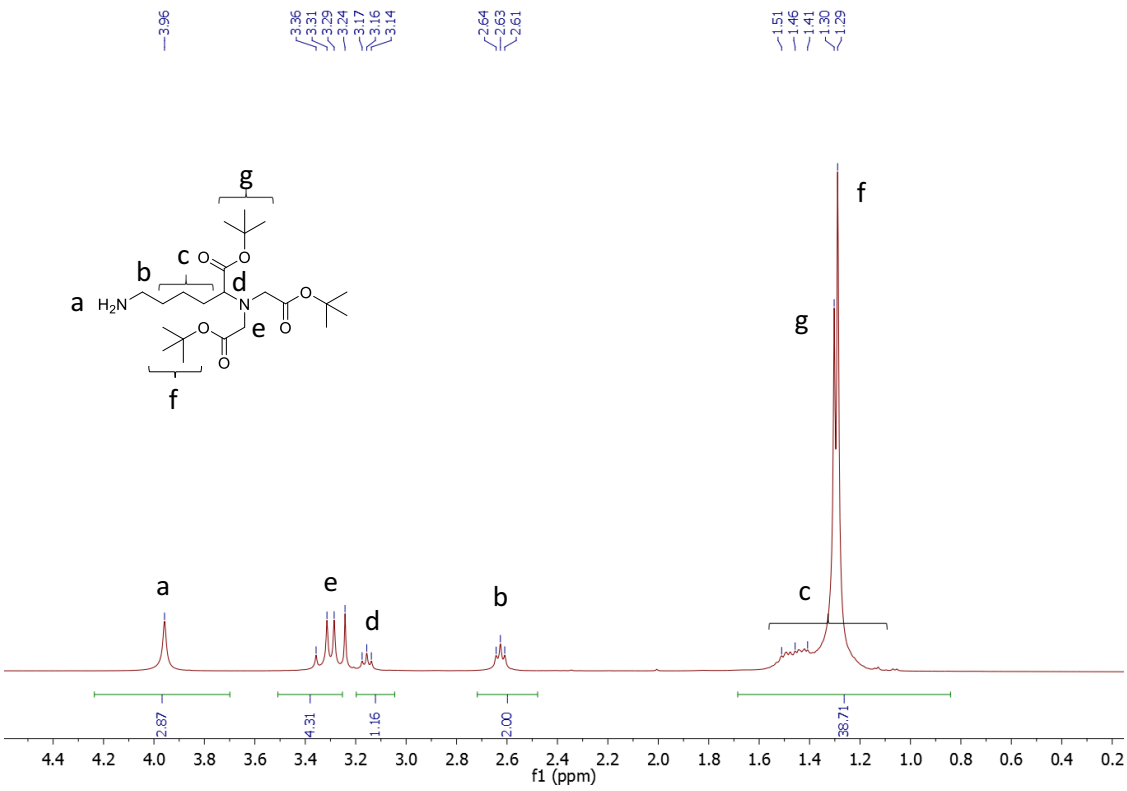


Figure S17. ^{1}H-NMR Spectrum (400 MHz) of **10**.

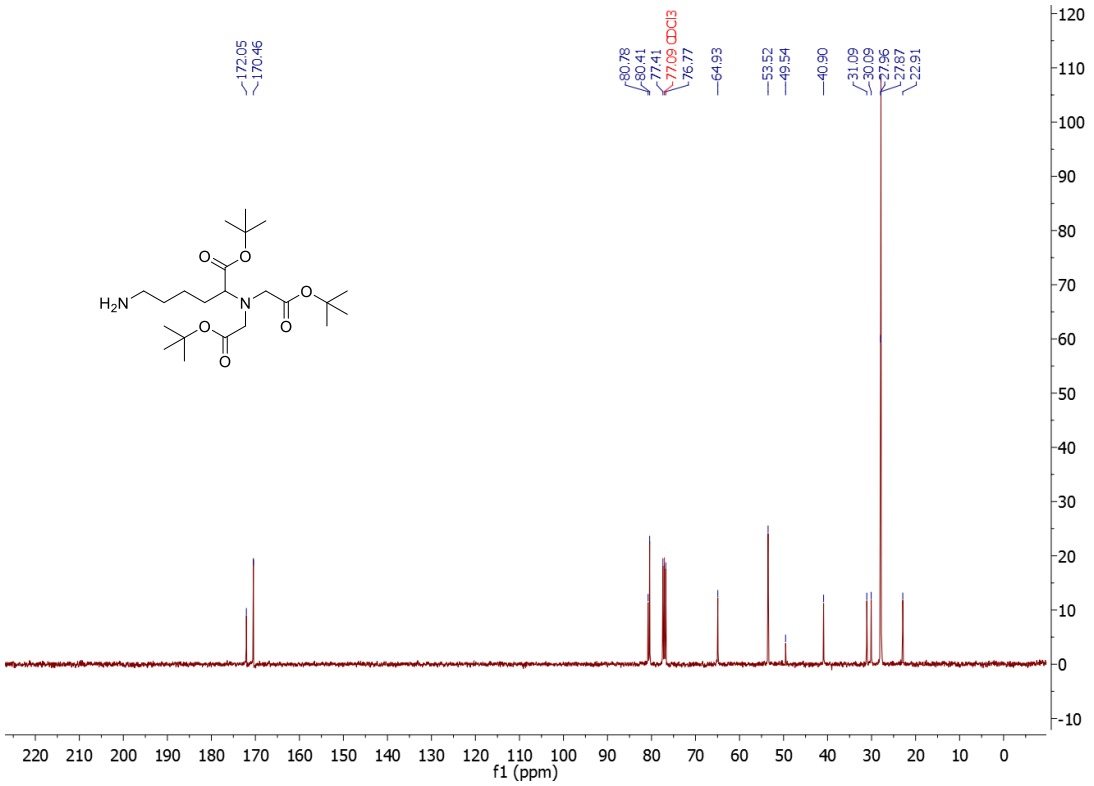


Figure S18. ^{13}C-NMR spectrum (101 MHz) of **10.**

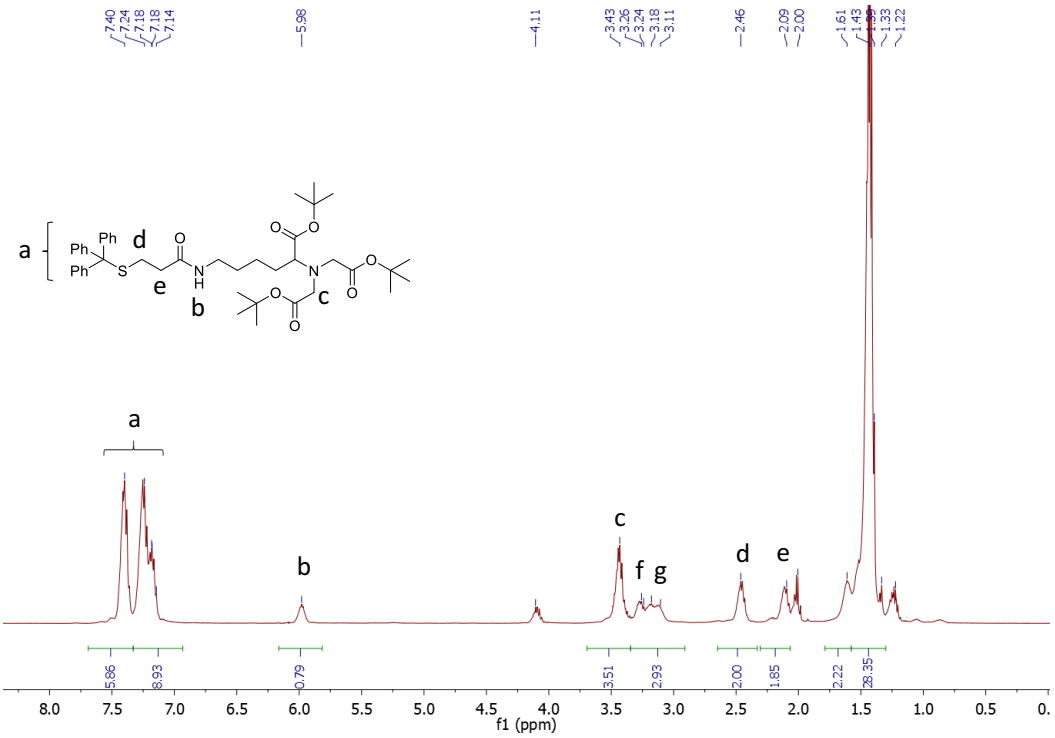


Figure S19. ^{1}H-NMR Spectrum (400 MHz) of **11**.

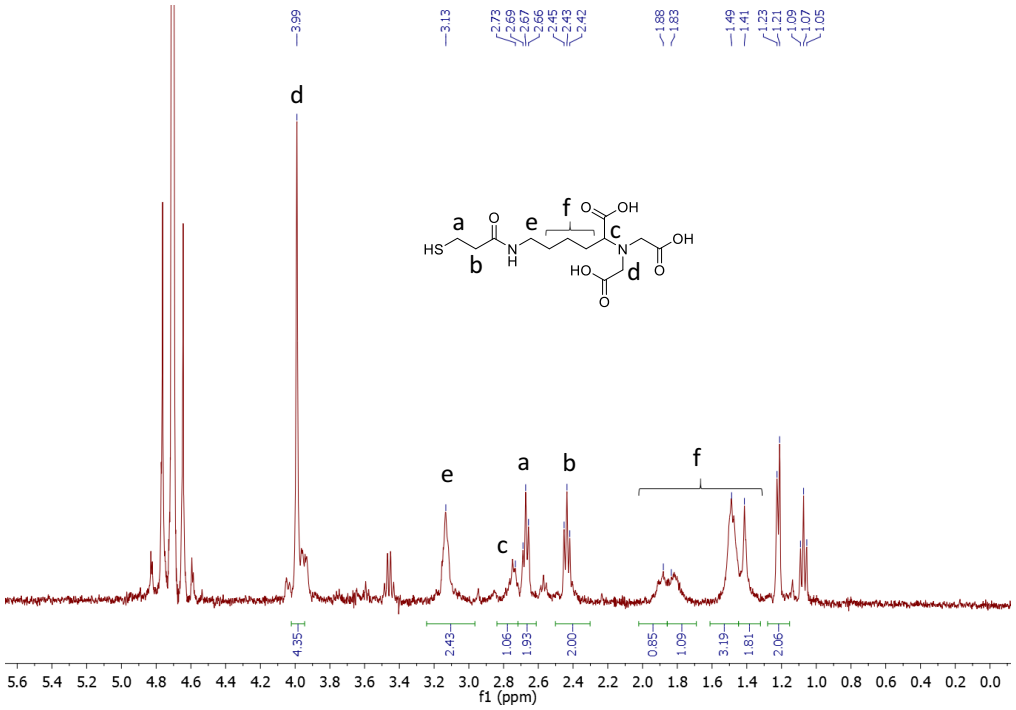


Figure S20. ^{1}H-NMR Spectrum (400 MHz) of **12**.

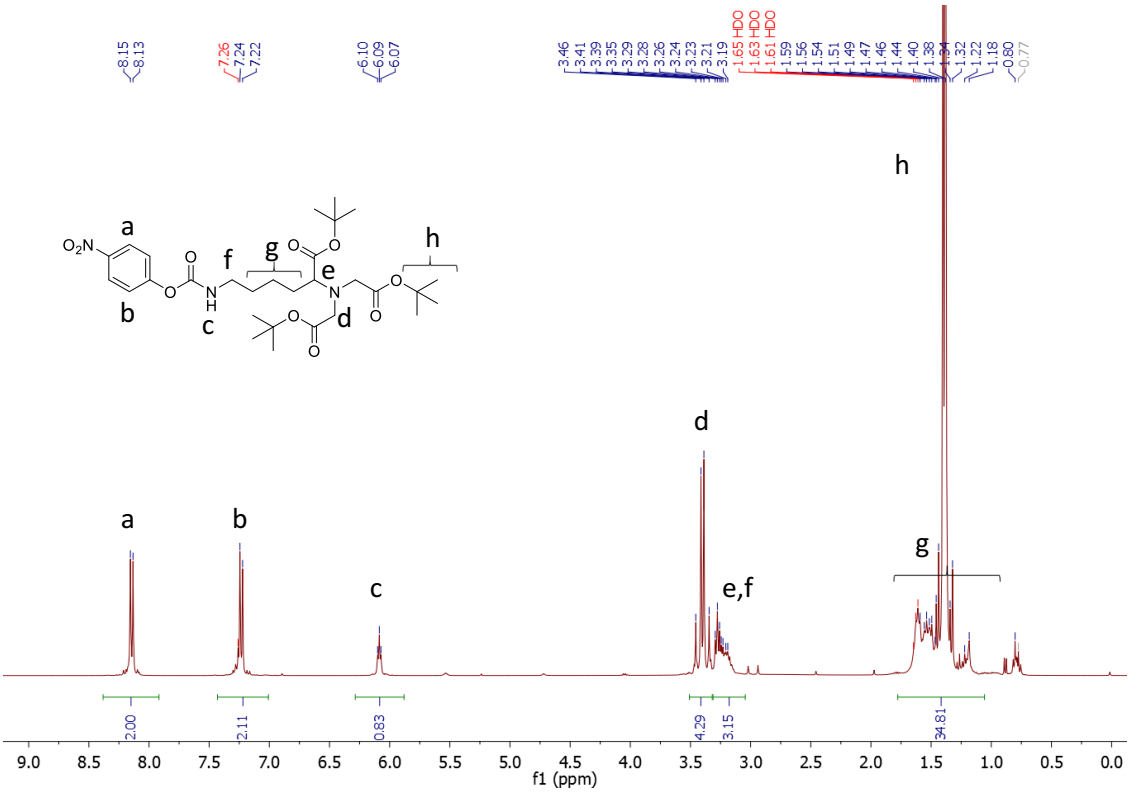


Figure S21. ^{1}H-NMR Spectrum (400 MHz) of **13**.

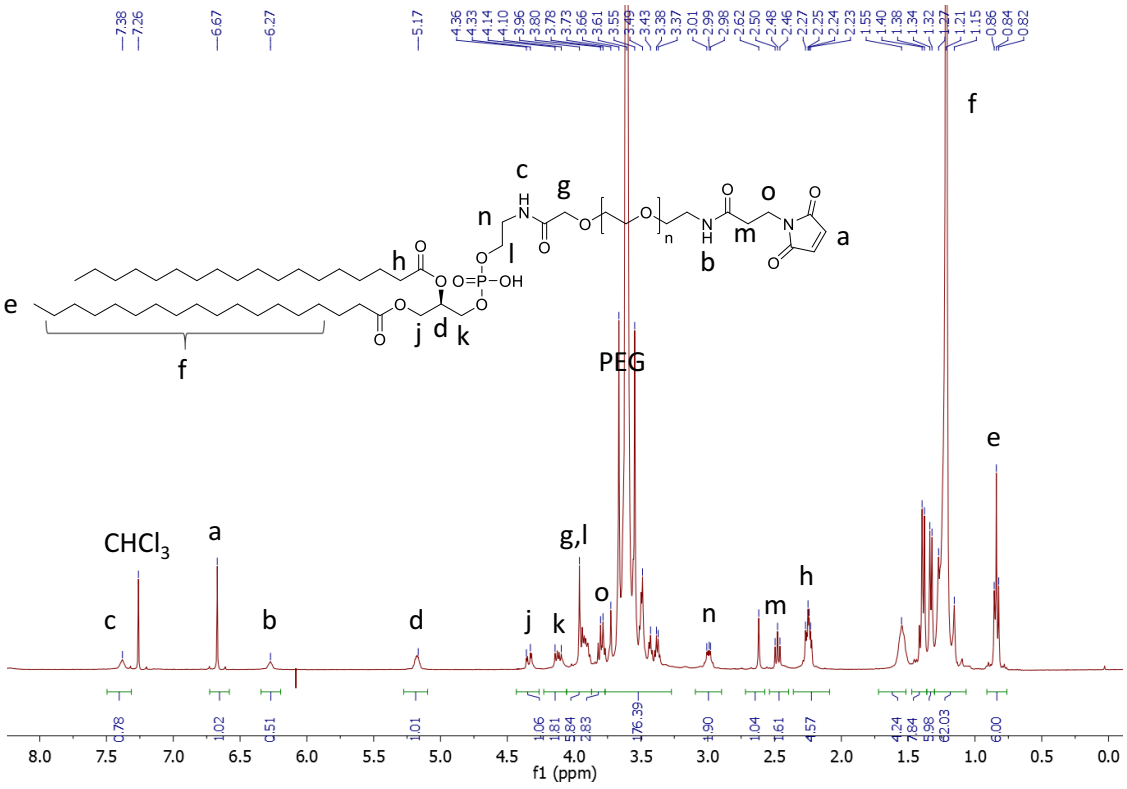


Figure S22. ^{1}H-NMR Spectrum (400 MHz) of **15.**

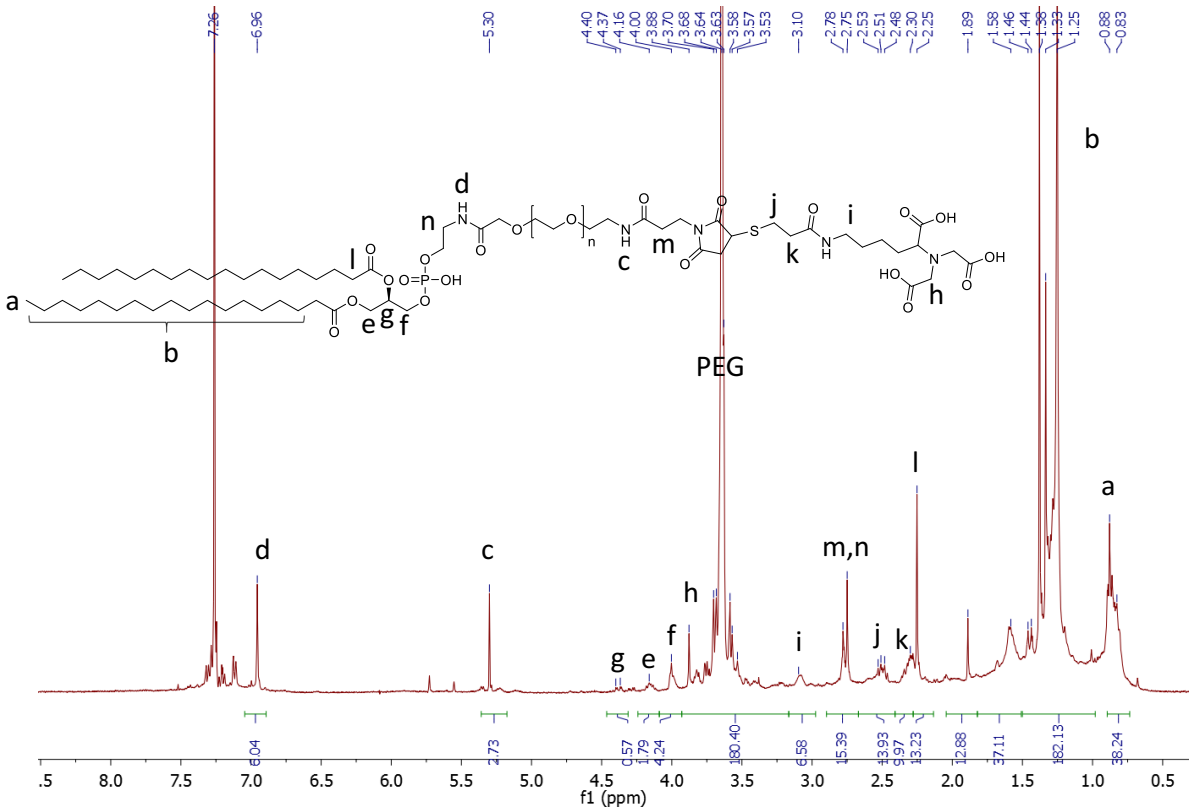


Figure S23. ^{1}H-NMR Spectrum (400 MHz) of **16.**

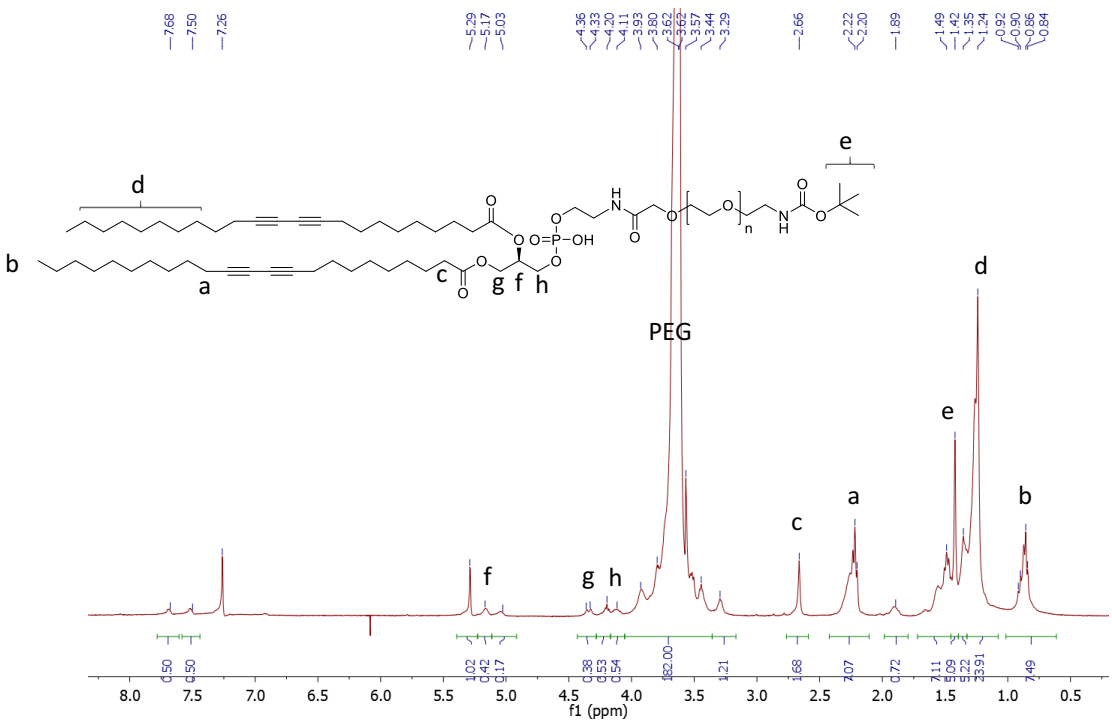


Figure S24. ^{1}H-NMR Spectrum (400 MHz) of **18.**