Supporting Information for:

Tracking nucleic acid nanocapsule assembly, cellular uptake and disassembly using a novel fluorescently labeled surfactant

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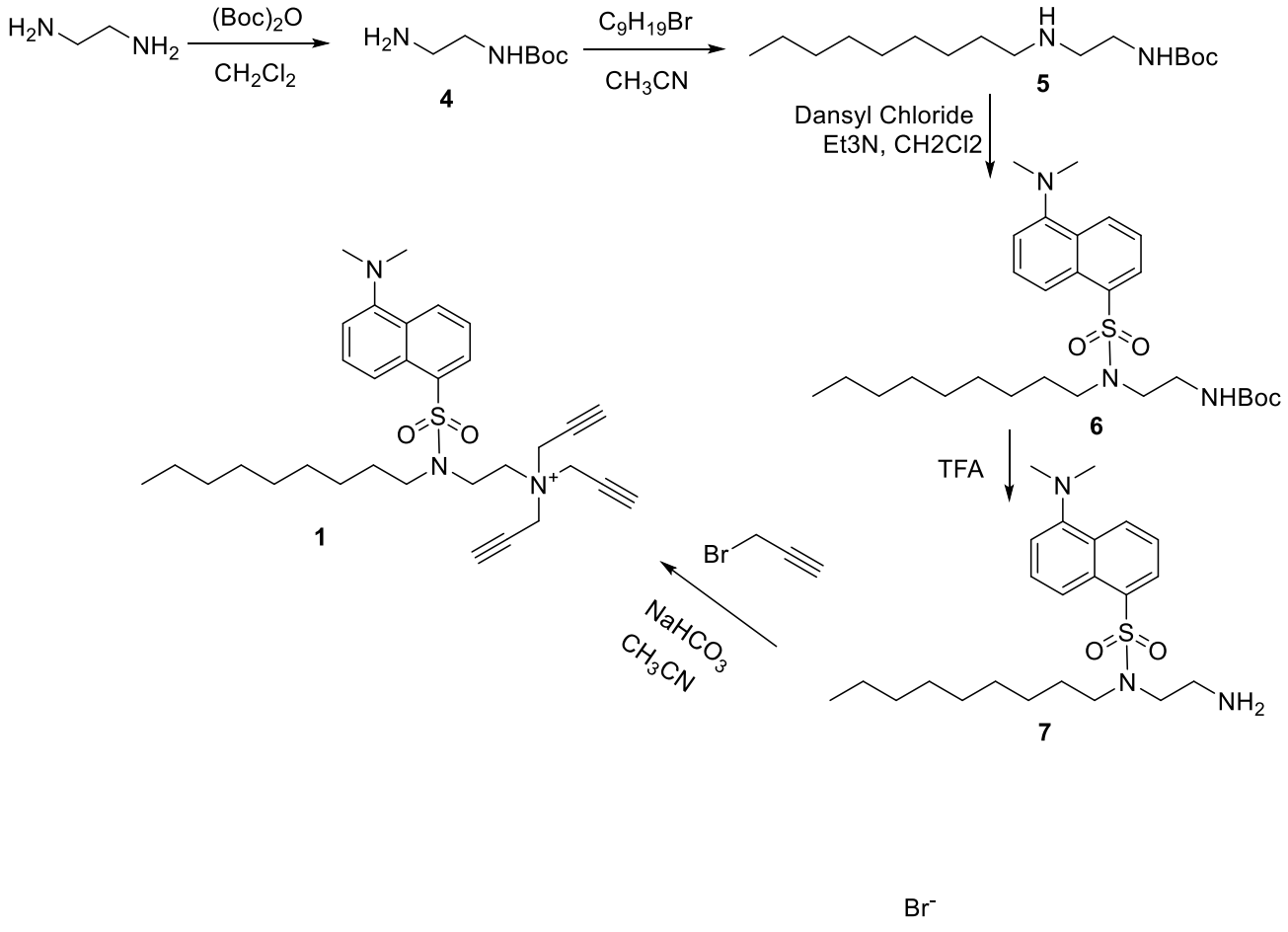
General Method

For the purposes of synthesis and spectroscopic analyses, methylene chloride, methanol, hexanes, tetrahydrofuran, dimethylformamide, acetonitrile, and ethyl acetate were of HPLC grade. All other reagents and solvents were of ACS-certified grade or higher, and were used as received from commercial suppliers. ^{1}H and ^{13}C NMR spectra were recorded on a Bruker DRX-300 spectrometer. Mass spectrometry analysis was recorded on a Sciex QSTAR Elite mass spectrometer.

Representative Schemes



Scheme S1. Synthesis of surfactant **1.**

Syntheses

Syntheses of compounds **2**[1] is previously reported.

Compound **4.**[2] To a stirred and cooled (0°C) solution of ethylenediamine (4.45 mL, 67 mmol) in CH_2Cl_2 (60 mL) was added Di-tert-butyl dicarbonate (1.45 g, 6.7 mmol) in CH_2Cl_2 (20 mL) dropwise for 1h. After stirring at room temperature for 24 h, the solvent was removed under reduced pressure. The crude product was dissolved in sat. $NaHCO_3$ (50 mL) and was extracted with CH_2Cl_2 (3×30 mL). The organic phase was dried, filtered and concentrated under reduced pressure to give a colorless oil as a pure product (0.66 g, 62 %).^{1}H NMR (400 MHz, $CDCl_3$, δ): 5.10 (br, 1H), 3.15 (t, *J* = 6.0 Hz, 2H), 2.77 (d, *J* = 6.0 Hz, 2H), 1.51 (br, 2H), 1.42 (s, 9H).

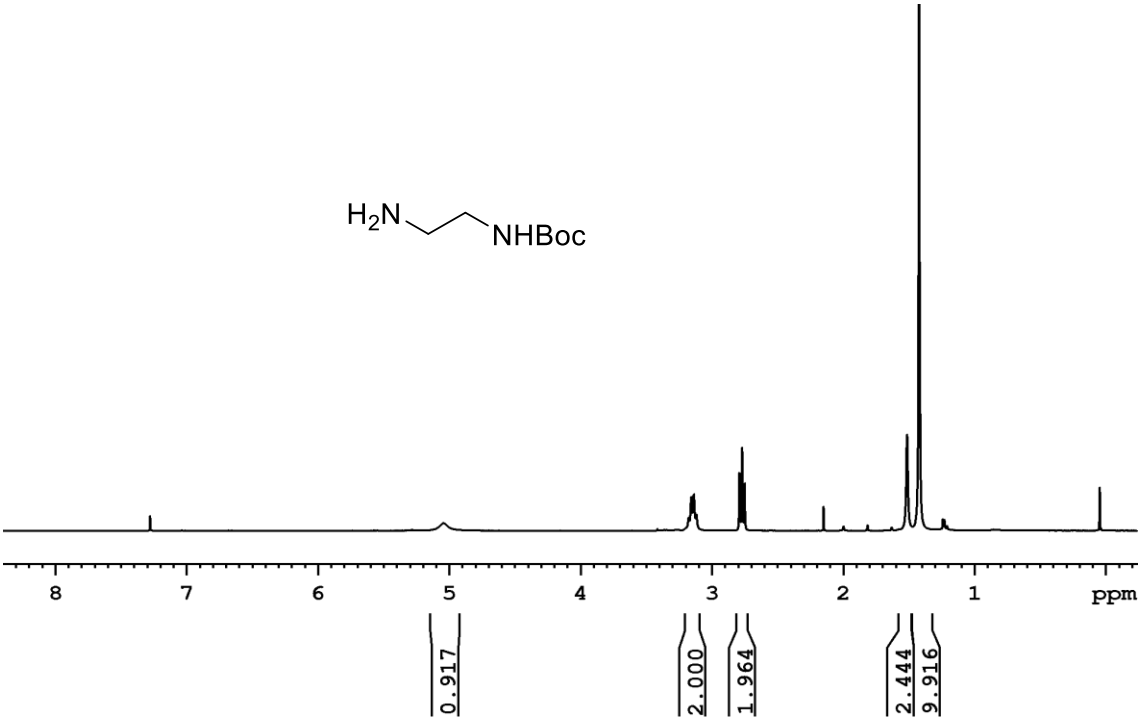


Figure S1: ^{1}H NMR for compound **4** in $CDCl_3$

Compound **5**.[3] 1-bromononane (0.43 g, 2.1 mmol) and tert-butyl (2-aminoethyl)carbamate (0.66 g, 4.12 mmol) were placed in a reaction flask. Anhydrous acetonitrile (30 mL) was added via syringe. The reaction mixture was stirred under reflux for 8 h. Purification step was done using recsytallization from acetonitrile, which furnished 0.5 g (83%) of the title compound 5 as a white powder. ^{1}H NMR (300 MHz, $CDCl_3$, δ): 5.59 (br, 1H), 3.59 (t, *J* = 8.0 Hz, 2H), 3.18 (t, *J* = 8.0 Hz, 2H), 3.00 (t, *J* = 8.0 Hz, 2H), 1.86 (m, 2H), 1.43 (s, 9H), 1.31 (m, 11H), 0.87 (t, *J* = 8.0 Hz, 3H).

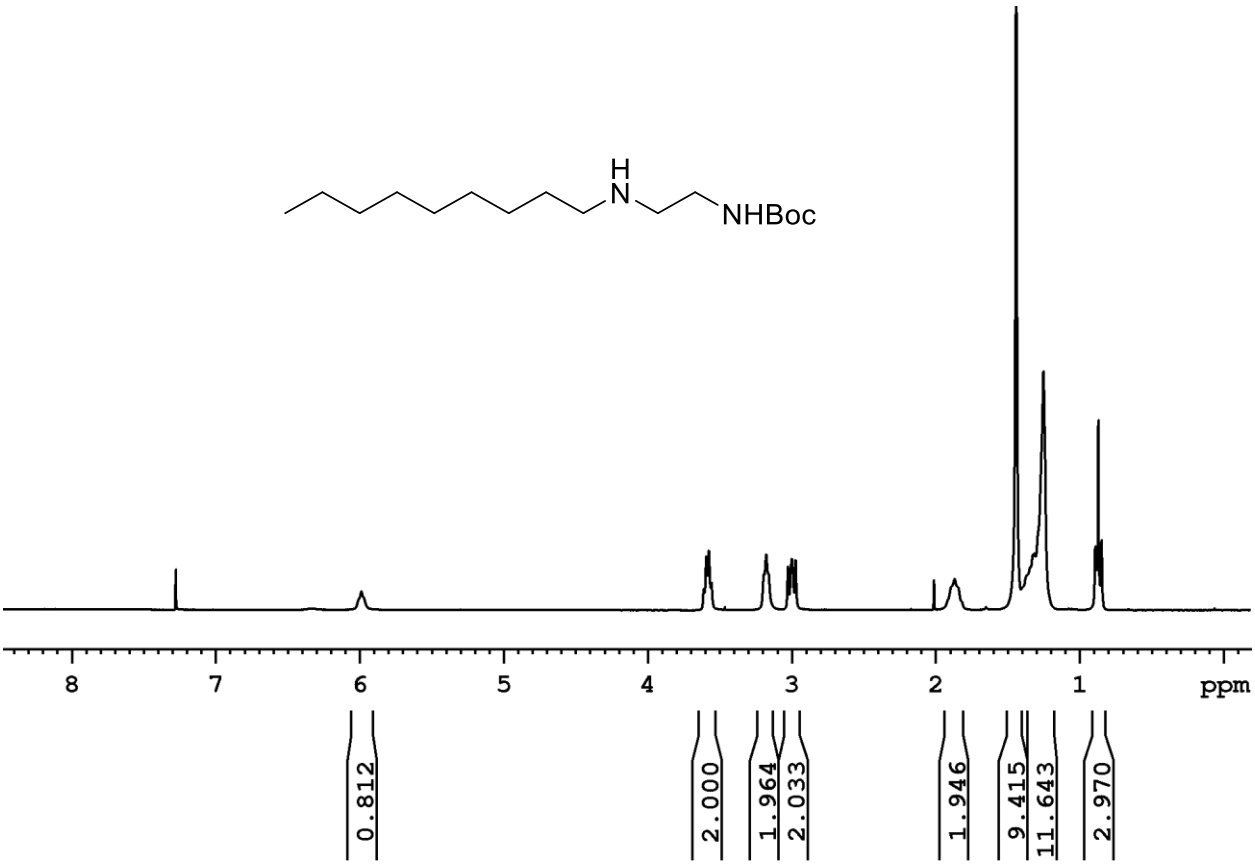


Figure S2: ^{1}H NMR for compound **5** in $CDCl_3$

Compound **6.**[4] To a solution of dansyl chloride (0.06 g, 0.22 mmol) in dry dichloromethane (5.0 mL) was added a solution of compound **5** (0.065 g, 0.22 mmol) and triethylamine (0.05 g, 0.44 mmol) in 1.0 mL of dry dichloromethane. The mixture was stirred at room temperature for 6 h under N_2. After the solvent was removed by rotary evaporation, the residue was purified by column chromatography over silica gel using 2:1 ethyl acetate/hexane as the eluent to afford a yellow oily product **6** (0.09 g,

77%). ^{1}H NMR (400 MHz, $CDCl_3$, δ): 8.56 (d, *J* = 8.5 Hz, 1H), 8.30 (d, *J* = 8.8 Hz, 1H), 8.18 (d, *J* = 7.3 Hz, 1H), 7.55 (dd, *J* = 8.5 and 7.3 Hz, 2H), 7.20 (d, *J* = 7.5 Hz, 1H), 4.84 (br, 1H), 3.39 (t, *J* = 5.7 Hz, 2H), 3.30 (t, *J* = 7.6 Hz, 4H), 2.90 (s, 6H), 1.44 (s, 9H), 1.28 (m, 2H), 1.20 (m, 4H), 1.13 (m, 4H), 0.89 (t, *J* = 7.1 Hz, 3H). ^{13}CNMR (400 MHz, CDCl3, δ): 156, 152, 135, 131, 130, 129, 128, 123, 119, 118, 115, 79.4, 48.1, 46.7, 45.4, 39.2, 31.8, 29.4, 29.1, 29.0, 28.4, 28.3, 26.6, 22.6, 14.1.

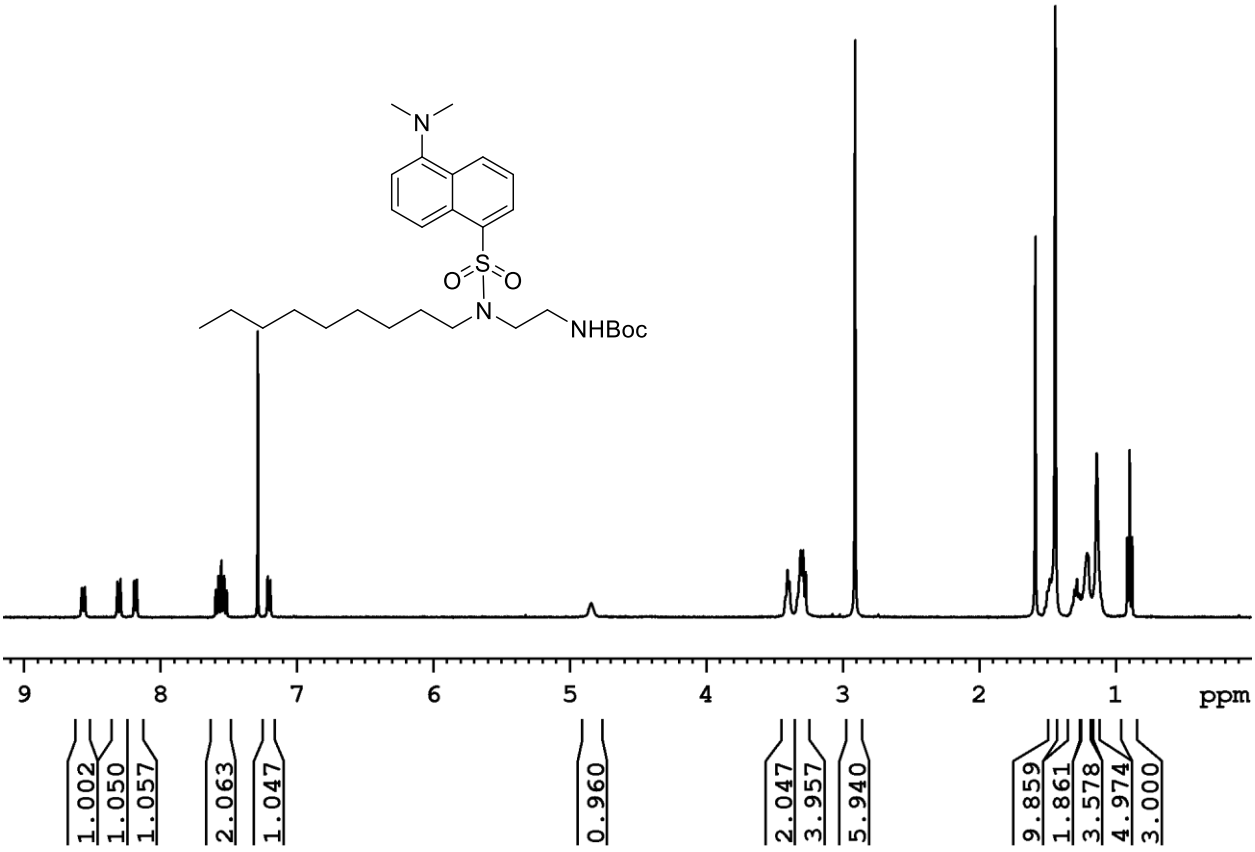


Figure S3: ^{1}H NMR for compound **6** in $CDCl_3$

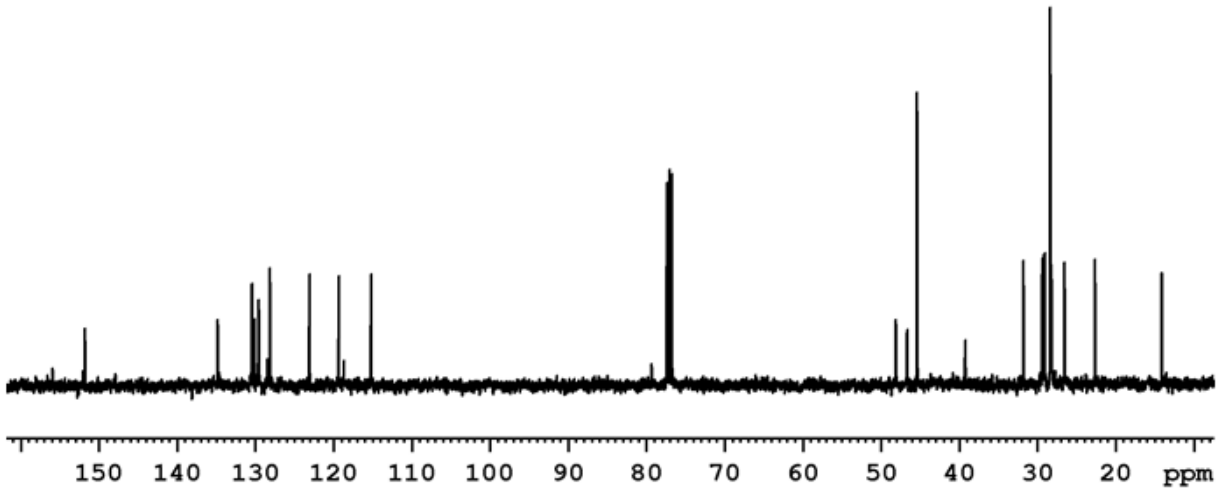


Figure S4: ^{13}C NMR for compound **6** in $CDCl_3$

Compound **7.** Compound **6** was treated with **25%** TFA/DCM at room temperature for 2h, after which time the volatiles were removed **in vacuo** to provide the product **7** as pure product (0.09 g, 99%).^{1}H NMR (400 MHz, $CDCl_3$, δ): 8.60 (dd, *J* = 8.6 & 7.2 Hz, 2H), 8.25 (d, *J* = 7.3 Hz, 1H), 7.74 (dd, *J* = 6.9 and 7.2 Hz, 2H), 7.65 (d, *J* = 7.2 Hz, 1H), 3.62 (t, *J* = 6.8 Hz, 2H), 3.33 (m, 4H), 3.15 (s, 6H), 1.44 (m, 2H), 1.29 (m, 8H), 1.13 (m, 6H), 0.89 (t, *J* = 7.1 Hz, 3H). ^{13}CNMR (400 MHz, CDCl3, δ): 130, 131, 129.5, 129, 128, 127.9, 127.8, 125, 122,116, 45.2, 44.4, 38.1, 31.6, 29.1, 28.9, 28.8, 27.9, 26.1 22.3, 13.0.

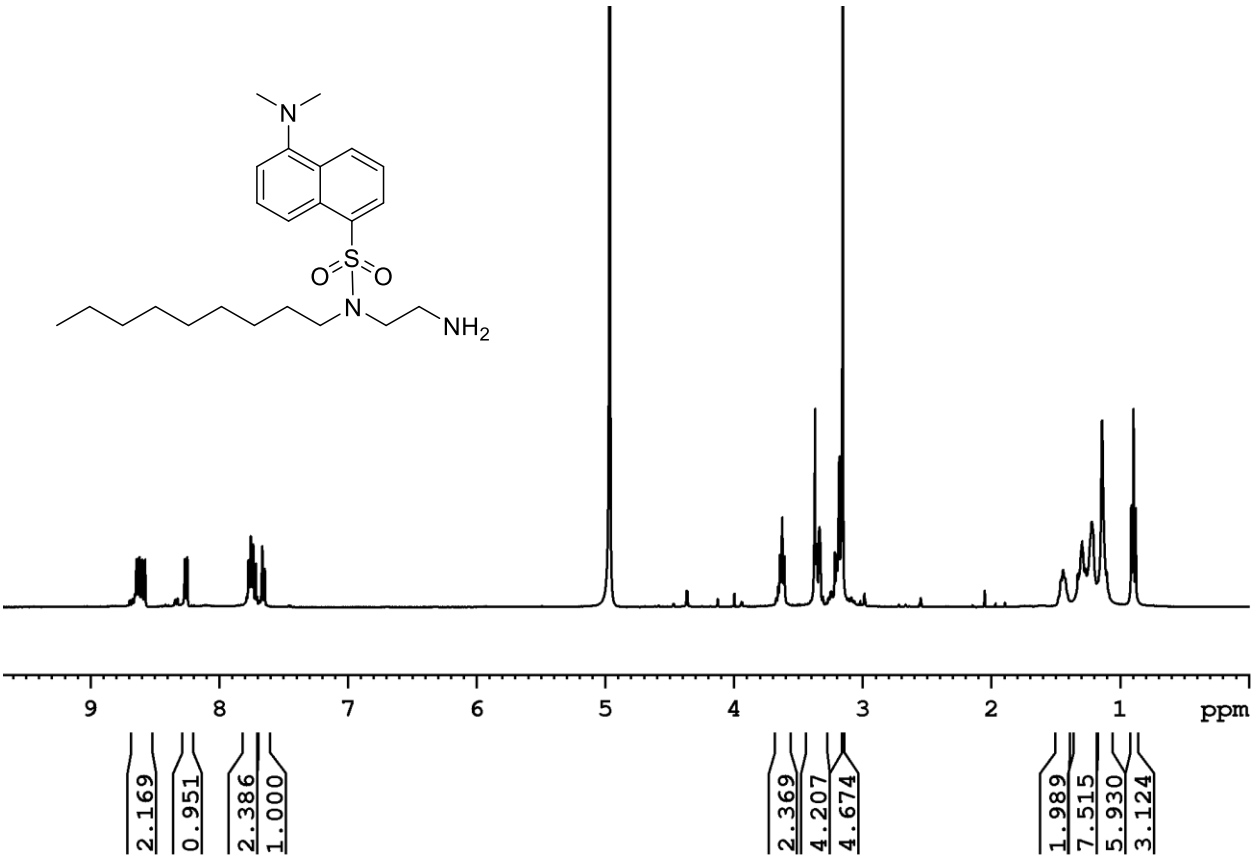


Figure S5: ^{1}H NMR for compound **7** in MeOD

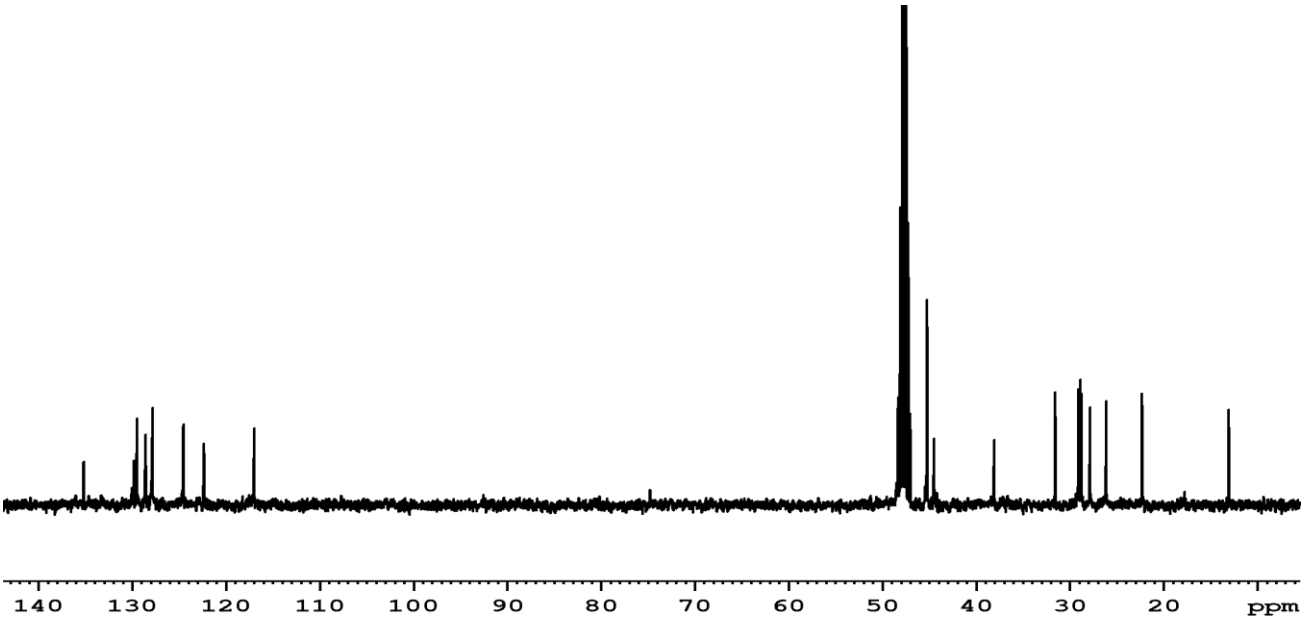


Figure S6: ^{13}C NMR for compound **7** in MeOD

Compound **1**.[4] A mixture of **7** (0.09 g, 0.21 mmol) and $NaHCO_3$ (0.03 g, 0.32 mmol) in CH_3CN (5.0 mL) was stirred at 60 °C, while a solution of propargyl bromide (0.10 g, 0.85 mmol) in dry CH_3CN (1.0 mL) was added dropwise over 10 min. The mixture was stirred for 16 h at 60 °C before another batch of propargyl bromide (0.05 g) and $NaHCO_3$ (0.01 g) were added. The mixture was stirred for another 6 h. The inorganic salts were removed by filtration. After the solvent was removed by rotary evaporation, the residue was purified by column chromatography over silica gel using 20:1 dichloromethane/methanol as the eluent to afford a yellowish oil (0.50 g, 40%). ^{1}H NMR (400 MHz, CD_3OD, δ): 8.65 (d, *J* = 8.5 Hz, 1H), 8.28 (dd, *J* = 7.4, 8.5 Hz, 2H), 7.65 (dd, *J* = 7.5 and 8.6 Hz, 2H), 7.230 (d, *J* = 7.5 Hz, 1H), 4.60 (d, *J* = 2.5 Hz, 6H), 3.90 (s, 4H), 3.71 (t, *J* = 2.4 Hz, 3H), 3.36 (t, *J* = 2.4 Hz, 2H), 2.91 (s, 6H), 1.44 (m, 2H), 1.29 (m, 2H), 1.20 (m, 2H), 1.10 (m, 6H), 0.90 (t, *J* = 7.1 Hz, 3H). ^{13}CNMR (400 MHz, CD_3OD, δ): 152, 134, 131, 130, 129, 128, 123, 119, 115, 83.2, 69.6, 56.3, 49.9, 44.1, 39.9, 31.6, 29.0, 28.6, 27.5, 25.9, 22.3, 13.0. ESI-HRMS (m/z): [M]+ calcd for C32H44N3O2S , 534.3154; found 534.3159.

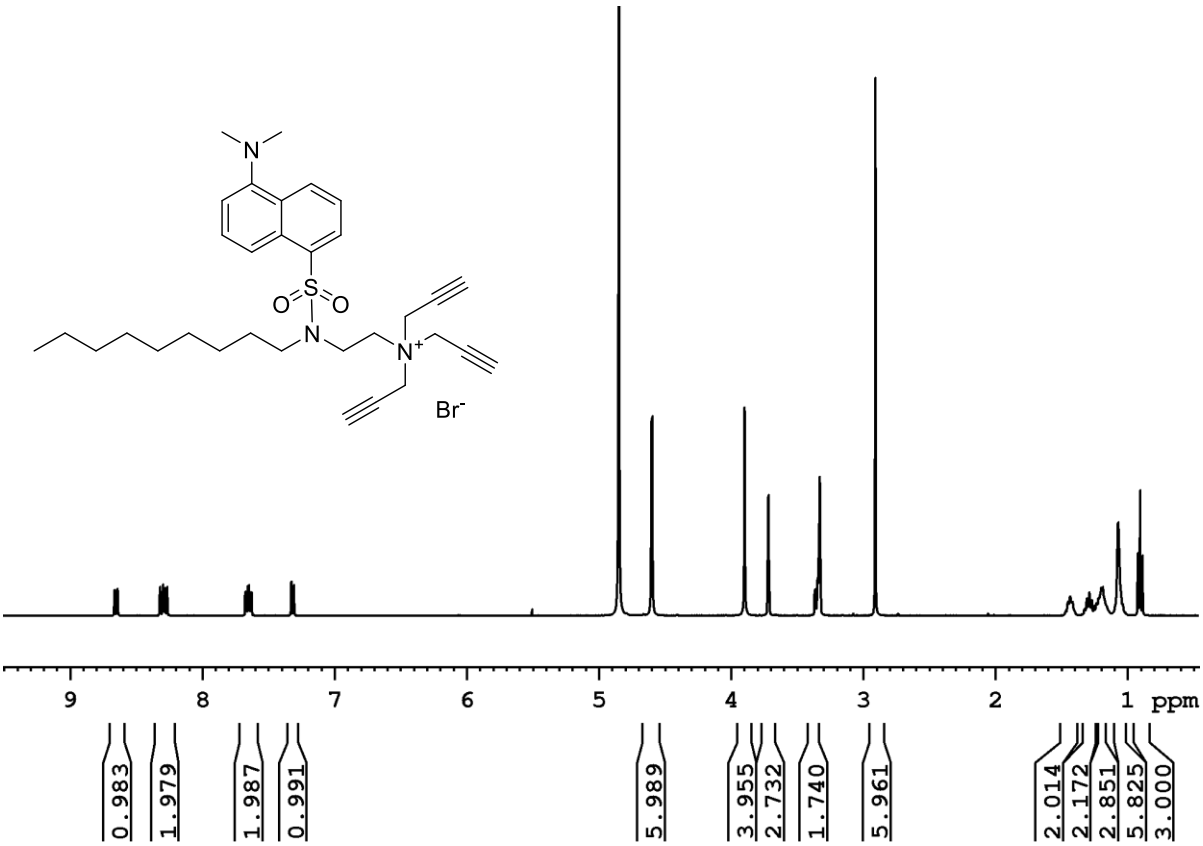


Figure S7: ^{1}H NMR for compound **1** in MeOD

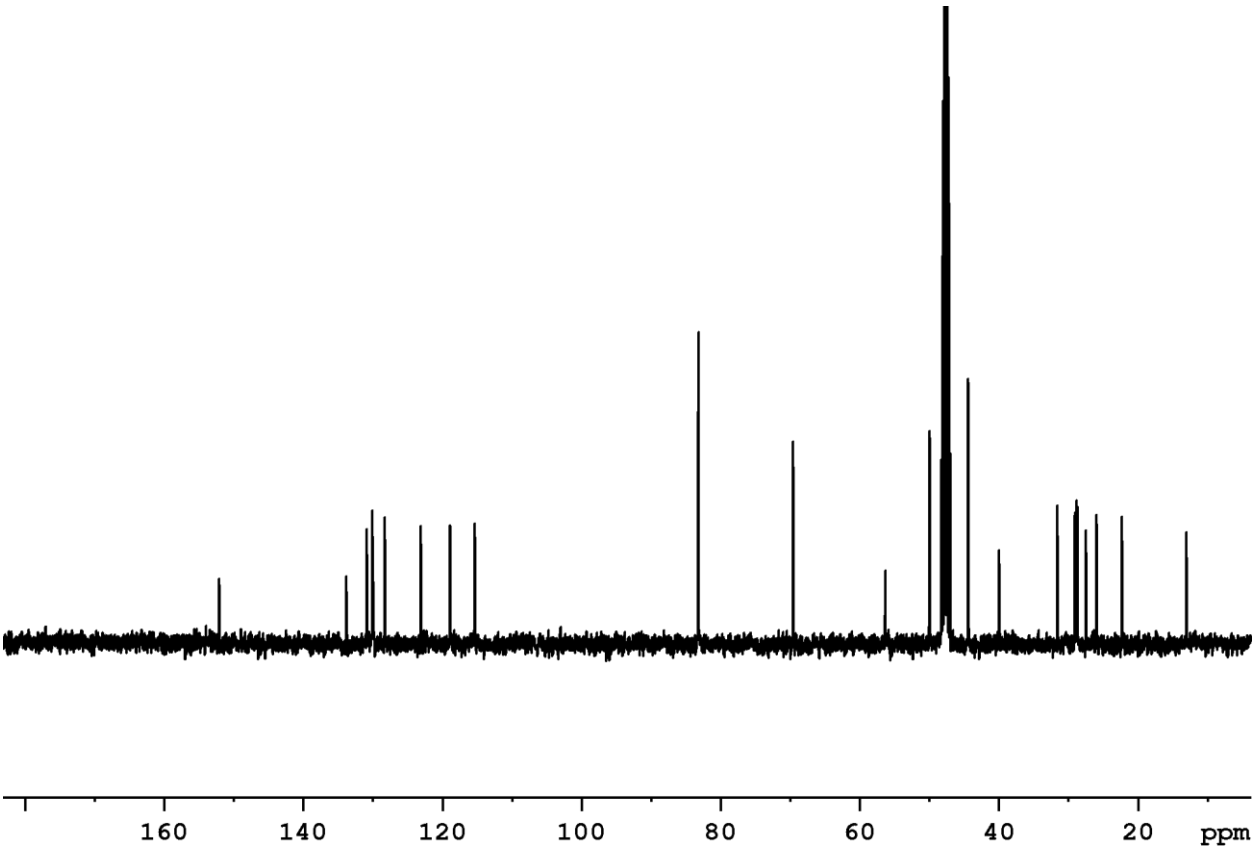


Figure S8: ^{13}C NMR for compound **1** in MeOD

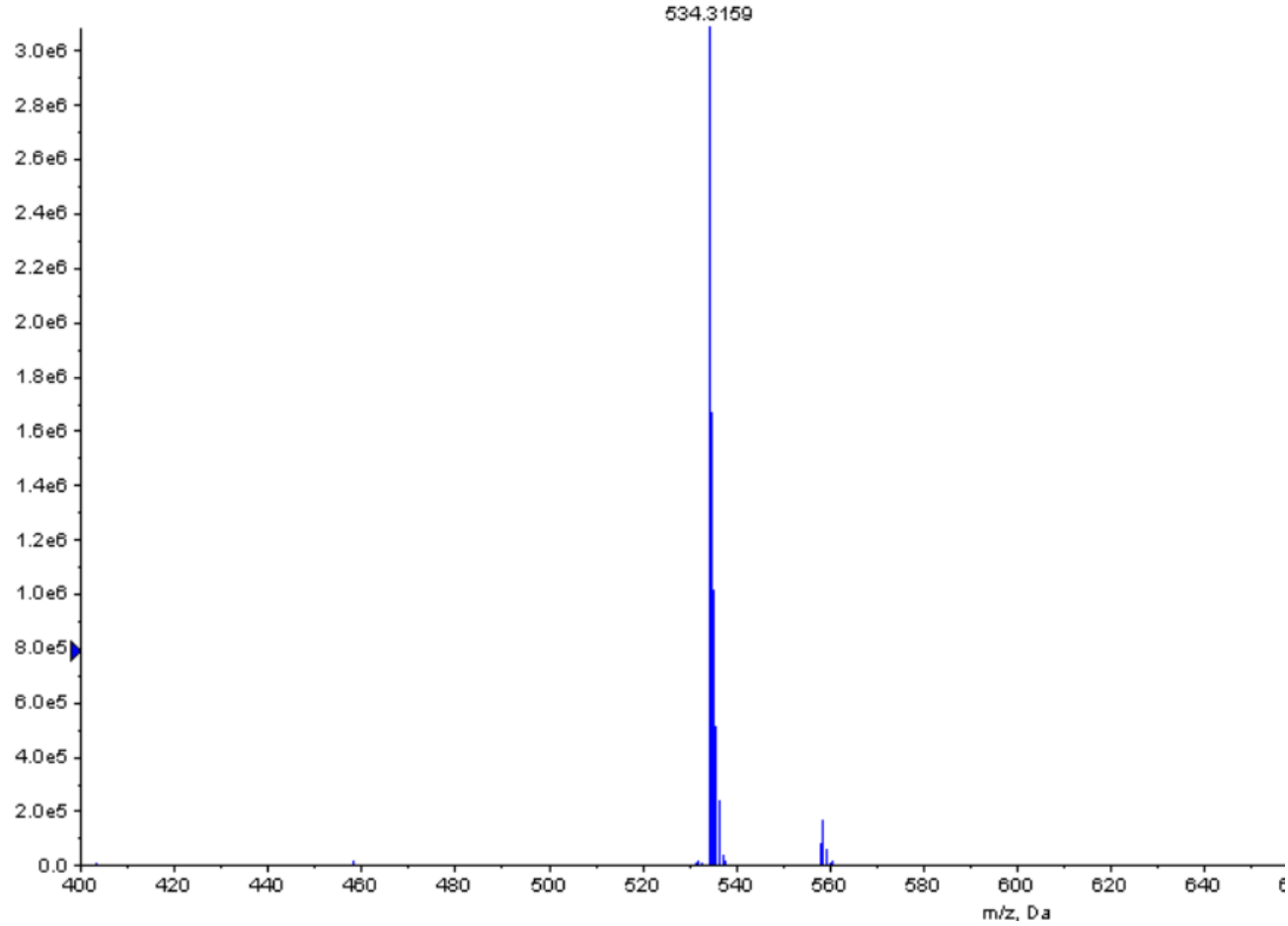


Figure S9: HRMS for compound **1**

Preparation of NANs. In the typical procedure, micelle solution of surfactant **1** (3.0 mg, 0.005 mmol) was prepared in Millipore water (1 mL). To the solution, diazide crosslinker (1.32 mg, 0.006 mmol) dissolve in 5 μL of DMSO, THPTA-Cu complex (0.00012 mmol), and sodium ascorbate (2.5 μL of a 99 mg/mL solution in water, 0.0012 mmol) were added. The reaction mixture was stirred slowly at room temperature. After 6-8 hours, the sample was purified by Sephadex G-25 Nap-5 column and the fractions containing crosslinked micelles (CM) were analyzed by UV-Vis, dynamic light scattering (DLS, and zeta potential. In a disposable cuvette, a solution containing the crosslinked micelles (100 μM) was prepared. To this solution, DNA anchor (30 μM) and 2-hydroxy-4-(2-hydroxyethoxy)-2-methylpropiophenone (2.5 μM) were added for a total volume of 500 μL in Millipore water. The mixture was placed in a Rhyonet reactor for 30 minutes. The thiol-yne photocrosslinking was monitored by dynamic light scattering (DLS) and agarose gel electrophoresis.

Crosslinker	SCM		DNA-NAN	
	Size (nm)	Zeta potential (mV)	Size (nm)	Zeta potential (mV)
2	24.5 ± 2.71	(+) 40.7 ± 1.48	41.8 ± 2.69	(-) 40.7 ± 1.48
3	19.6 ± 1.15	(+) 37.4 ± 1.28	39.8 ± 2.68	(-) 33.0 ± 1.68

Table S1: DLS (Size and Zeta Potential) data for each step of CM and NAN assembly.

By Azide click reaction	
Size (nm)	Zeta potential (mV)
41.8 ± 2.83	(-) 21.7 ± 2.69

Table S2: Size and Zeta Potential data for azide DNA attachment to a dansyl CM.

Esterase cleavage assay. A solution of NANs (3 μM) was prepared in Tris HCl buffer in a total volume of 400 μl. The solution was placed into an external Peltier unit and allowed to come to 37 ºC. Esterase (Porcine liver esterase, Sigma Aldrich, 5 units) was added 1 hr prior to dilution and agarose gel analysis.

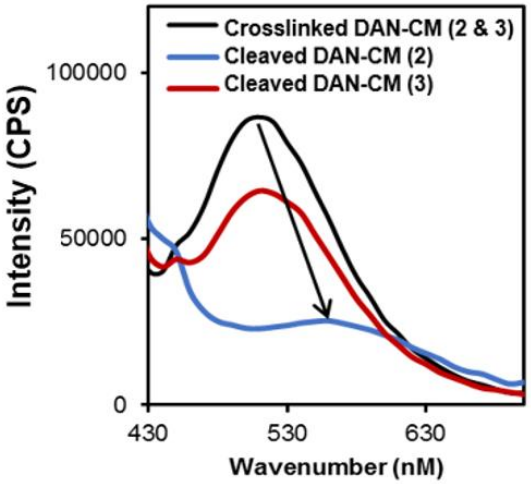


Figure S10. Cleavage study comparison between ester (2) and PEG (3) crosslinked CM (100 μM) using NaOH within 10 min. Decrease in fluorescence in red line (PEG CMs) attributed to dilution by NaOH. No shift in emission noted for red line indicating PEG crosslinked CMs are still intact after NaOH treatment. This is in contrast to the blue line which indicates that the micelles have degraded as indicated by the red shift in emission (indicated by the black arrow). (B) using porcine liver esterase within 1 hr (10 mM Tris-HCl buffer at pH 7 and 37 °C).

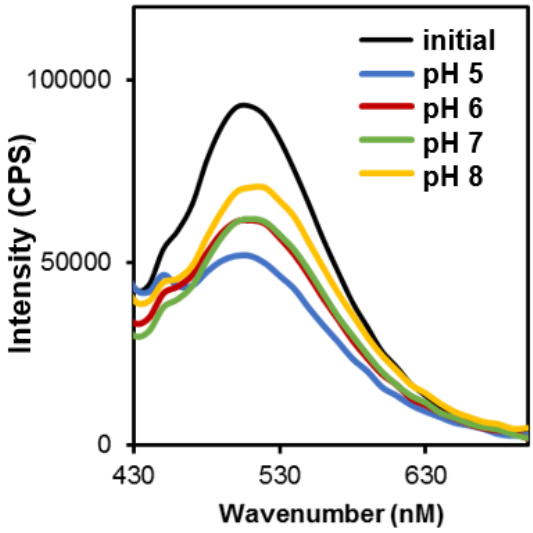


Figure S11. pH study of ester crosslinked CM (100 μM) using esterase for 1 hr at 37 °C.

Cellular uptake and confocal microscopy. HeLa cells were grown in DMEM supplemented with 10% FBS and 1% Penicillin/Streptomycin. Confluent cells were placed in an 8-well confocal chamber at 85,000 cells/mL and incubated overnight. Cells were then treated with 1 μM sample (DAN-surfactant, ester crosslinked NAN, or PEG crosslinked NAN) for 1 hr, washed with 1x PBS, and imaged at various time points using a Leica SP8 confocal microscope. Calculation of R values for images was done using Image J software colocalization analysis tools. The corrected total cell fluorescence (CTCF) was determined using the following equation:

CTCF = (integrated density) - (area of selected cell * mean fluorescence of background)

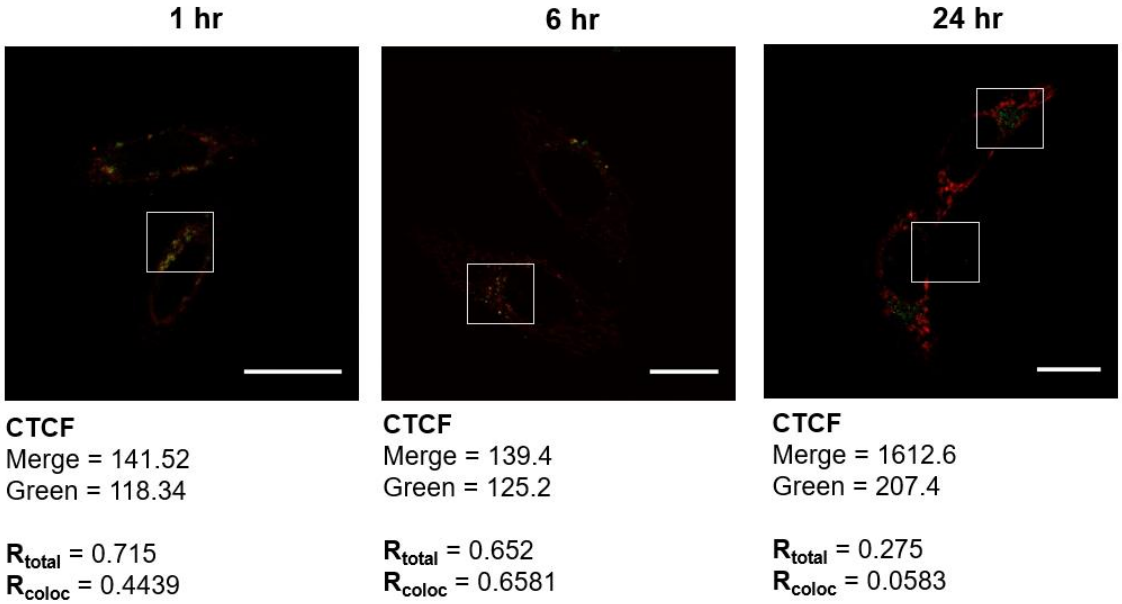


Figure S12. ImageJ analysis of representative confocal images of ester crosslinked NANs post endocytosis into HeLa cells.

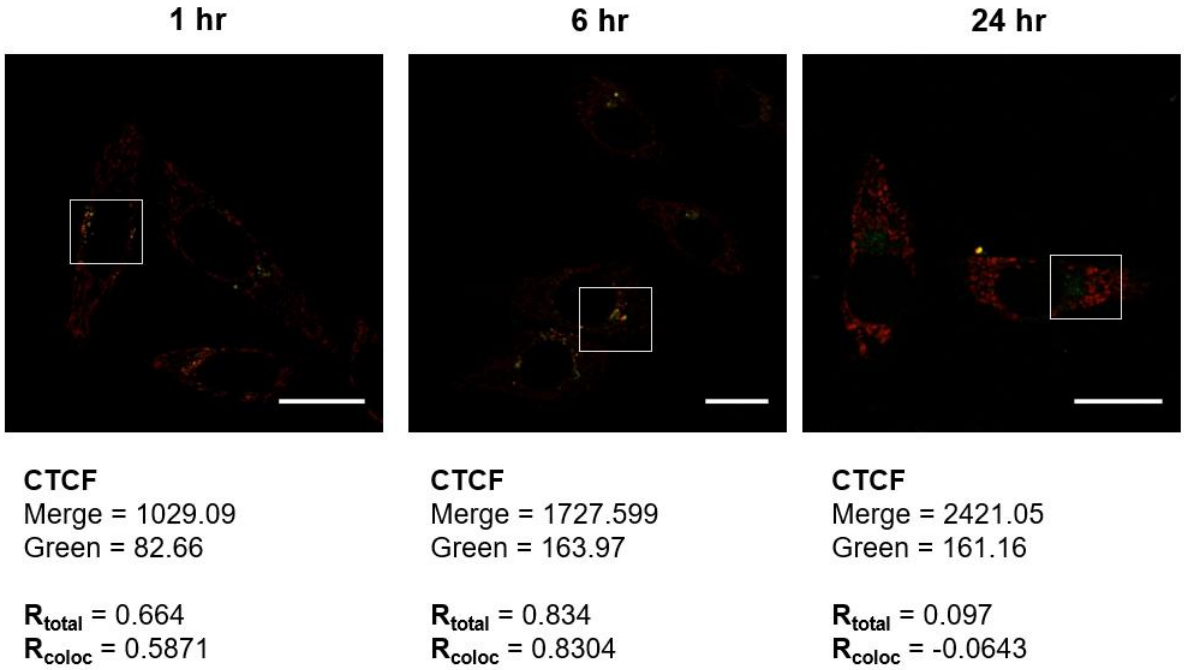


Figure S13. ImageJ analysis of representative confocal images of PEG crosslinked NANs post endocytosis into HeLa cells.

Ligation of TYE665-labeled DNA. 10 μM TYE665-labeled DNA and 20 μM bridge (see **Table S3** for sequences) were added to 5 μM NANs. Water was added to total volume of 250 μL. The solution was heated at 70 °C for 10 minutes and cooled to room temperature. In a separate tube, a solution of 5 mM ATP, 10 μL of 1 U/μL T4 DNA Ligase (Invitrogen), and 1x ligase buffer were added to water in a total volume of 250 μL. The two solutions were combined and the resulting mixture was placed on a 25 °C heat block for 2 hours. Ligase was heat inactivated at 70 °C for 10 minutes, and the reaction was purified using a Sephadex G-25 NAP-10 column. Fractions were analyzed via agarose gel electrophoresis.

Ligation of Bcl-2 DNAzyme. 20 μM Bcl-2 DNAzyme and 40 μM DNAzyme bridge (see **Table S3** for sequences) were added to 10 μM NANs in water in a total volume of 250 μL. The resulting solution was heated at 70 °C for 10 minutes and cooled to room temperature. In a separate tube, a solution of 5 mM ATP, 15 μL of 1 U/μL T4 DNA Ligase (Invitrogen), and 1x ligase buffer were added to water in a total volume of 250 μL. The two solutions were combined and the resulting mixture was placed on a 25 °C heat block for 2 hours. Ligase was heat inactivated at 70 °C for 10 minutes, and the reaction was purified using a Sephadex G-25 NAP-10 column. Fractions were analyzed via dynamic light scattering measurements.

Bcl2 mRNA knockdown. HeLa cells were grown in DMEM supplemented with 10% FBS and 1% Penicillin/Streptomycin. Confluent cells were placed in a 6-well plate at 100,000 cells/mL and incubated overnight. Cells were then washed with 1x PBS and treated with 250 nM Bcl-2 DNAzyme-NANs (PEG or ester) in OptiMEM for 4 hours. Total cellular RNA was isolated using a Qiagen RNeasy Kit following protocol provided by manufacturer. Reverse transcription was carried out using Bio-Rad iScript Supermix. qPCR was performed using iTaq Universal SYBR Green Supermix (Bio-Rad) in the presence of appropriate primers (**Table S3**) on a BioRad CFX Connect Real-Time PCR Detection System. GAPDH mRNA expression was used to normalize data.

DNA sequences	
AH Anchor	5’-SH- TTT TTT TTT TCA CGT CCA GCA G-3’
TYE665 DNA	5’- GTG GAT GGA GGC TAG CTA CAA CGA GTC TTG GAG-TYE665-3’
TYE665 DNA bridge	5’-GCC TCC ATC CAC CTG CTG GAC GTG-3’
Bcl-2 DNAzyme	5’- CAC AGC CAA GGC TAG CTA CAA CGA GTG CCA TGT-3’
Bcl-2 DNAzyme Bridge	5’- GCC TTG GCT GTG CTG CTG GAC GTG-3’
Bcl-2 qPCR Forward Primer	5'- CTG GTG GAC AAC ATC GCC CT-3’
Bcl-2 qPCR Reverse Primer	5'-TCT TCA GAG ACA GCC AGG AGA AAT-3’
GAPDH qPCR Forward Primer	5'-TGC ACC ACC AAC TGC TTA G-3'
GAPDH qPCR Reverse Primer	5'-GAT GCA GGG ATG ATG TTC-3'

Table S3: DNA sequences. Blue represents monophosphorylation for ligation.

References

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