Supplementary Material (ESI) for Chemical Communications

Supporting Information

"Clickable", Polymerized Liposomes as a Versatile and Robust Platform for Rapid Optimization of Their Peripheral Compositions

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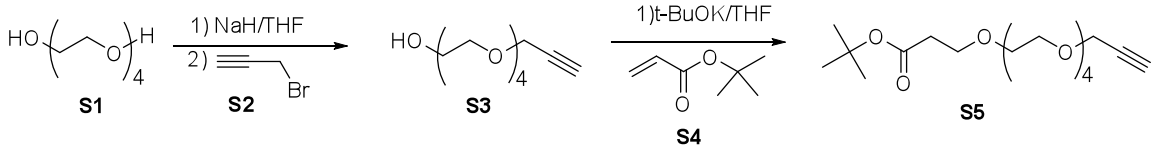
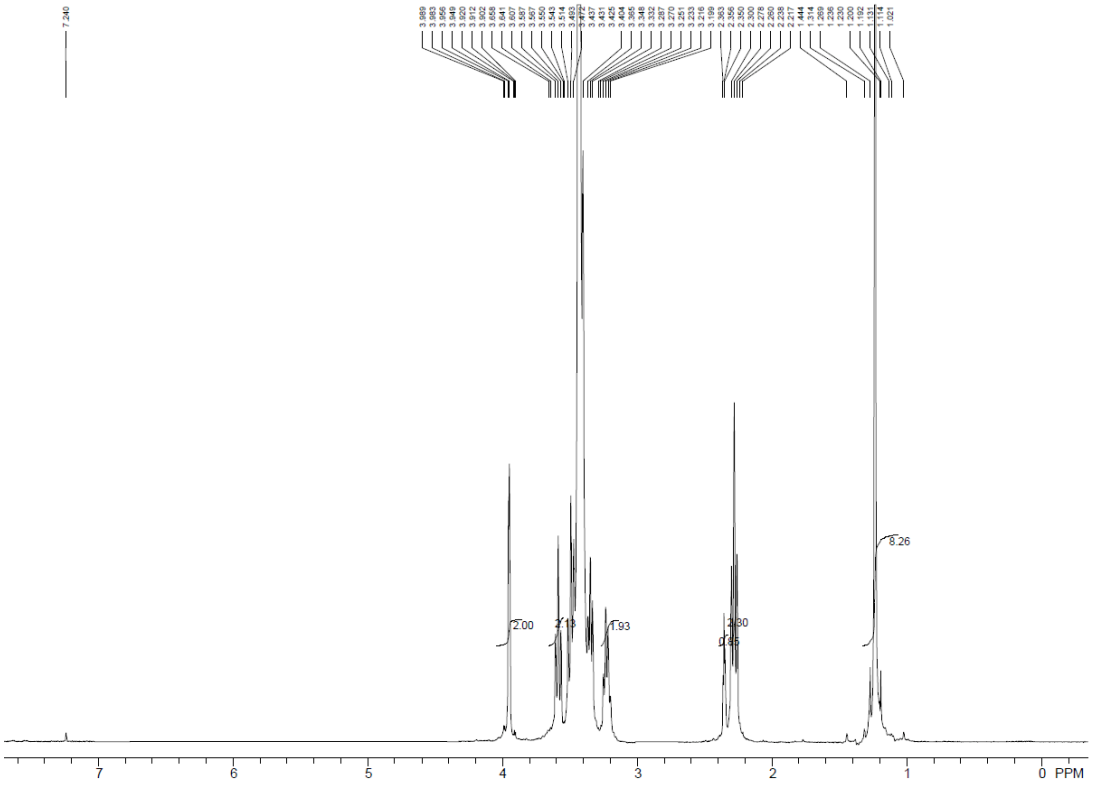
A. Synthesis

General: Air sensitive reactions were performed under a nitrogen atmosphere using Schlenk technique. **S4** and **S7** were purchased from Sigma-Aldrich (St. Louis, MO), **S16** from Thermo-Scientific (Pittsburgh, PA), **S14** from Quanta BioDesign Ltd. (Powell, OH), **S9, 2** from Avanti Polar Lipids Inc. (Alabaster, Al) and **S1** from Alfa Aesar (Ward Hill, MA), and used without further purification. Flash chromatography was carried out on silica gel (60Å, Sorbent Technologies). All ^{1}H and ^{13}C-NMR spectra were recorded in GE QE-300 in $CDCl_3$ (Cambridge Isotope Laboratories Inc.) using residual protons in the solvent as an internal standard. Mass spectroscopy (MS) measurements were carried out using electrospray ionization (ESI) technique on Deca XP Plus from Thermo Finnigan.



To a solution of the alkyne **S3**[1] (500 mg, 2.15 mmol) and ^{t}BuOK (12 mg, 0.11 mmol) under nitrogen in dry THF (1.0 mL) was drop-wise added tert-butyl acrylate (**S4**, 358 mg, 2.79 mmol). The mixture was stirred overnight at room temperature. The solution was neutralized with 1 N HCl, mixed with saturated brine solution, and extracted three times with CH_2Cl_2 (25 mL). The combined organic layers were dried over $MgSO_4$, filtered, and the solvent was removed under vacuum affording a crude product, which was purified by flash chromatography (ethyl acetate/methanol 9:1) to give the alkyne **S5** (442 mg, 1.22 mmol, 57%) as a yellow oil. ^{1}H NMR (300 MHz, $CDCl_3$): δ 3.95 (t, J = 2.2 Hz, 2H), 3.58 (t, J = 6 Hz, 2H), 3.52-3.29 (m, 14H), 3.23 (t, J = 5.4 Hz, 2H), 2.35 (t, J = 2.2 Hz, 2H), 2.27 (t, J = 5.4 Hz, 2H). 1.23 (s, 9 H). ^{13}C NMR (75 MHz, $CDCl_3$): δ 28.5, 36.7, 58.6, 66.5, 67.2, 70.2, 70.6, 70.7, 70.9, 75.3, 79.9, 80.7, 171.2. MS (ESI) m/z calcd for $C_{18}H_{32}O_7$: 360.2; found: 383.4 ($[M + Na]^+$).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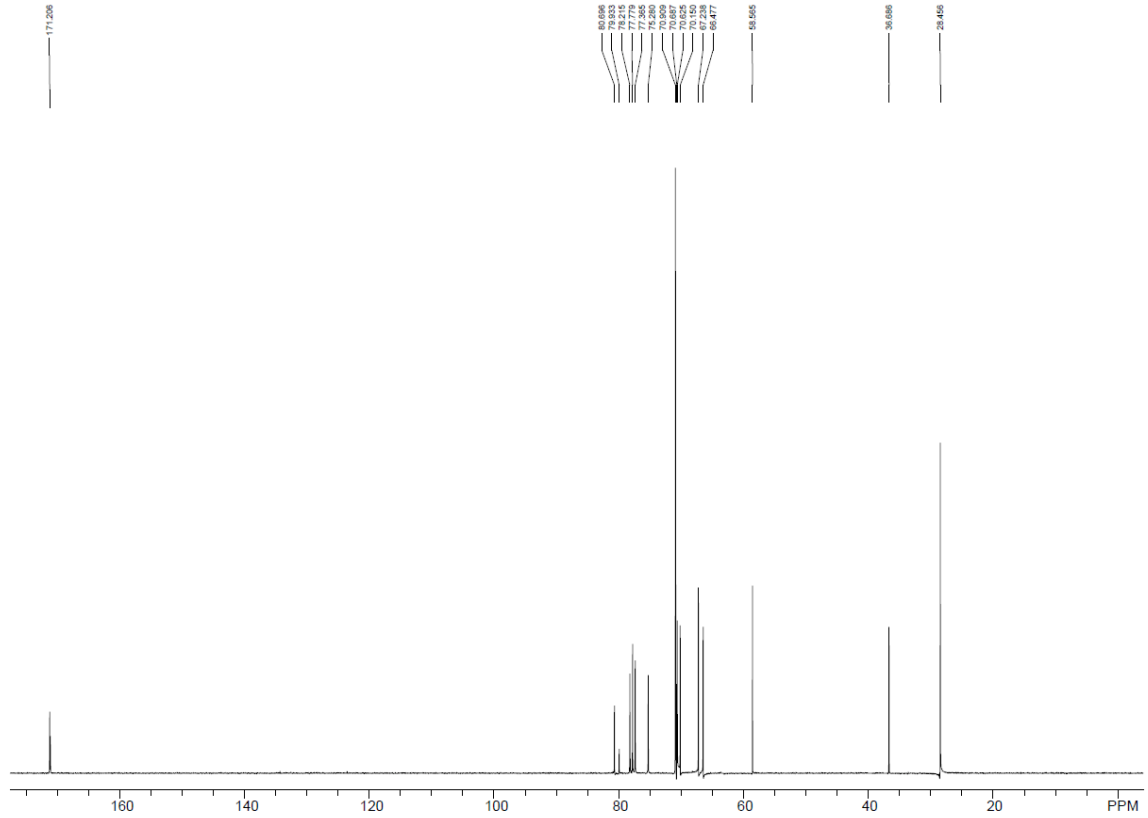
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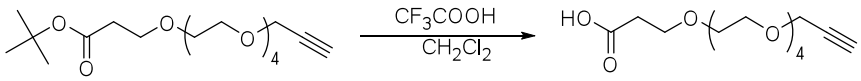
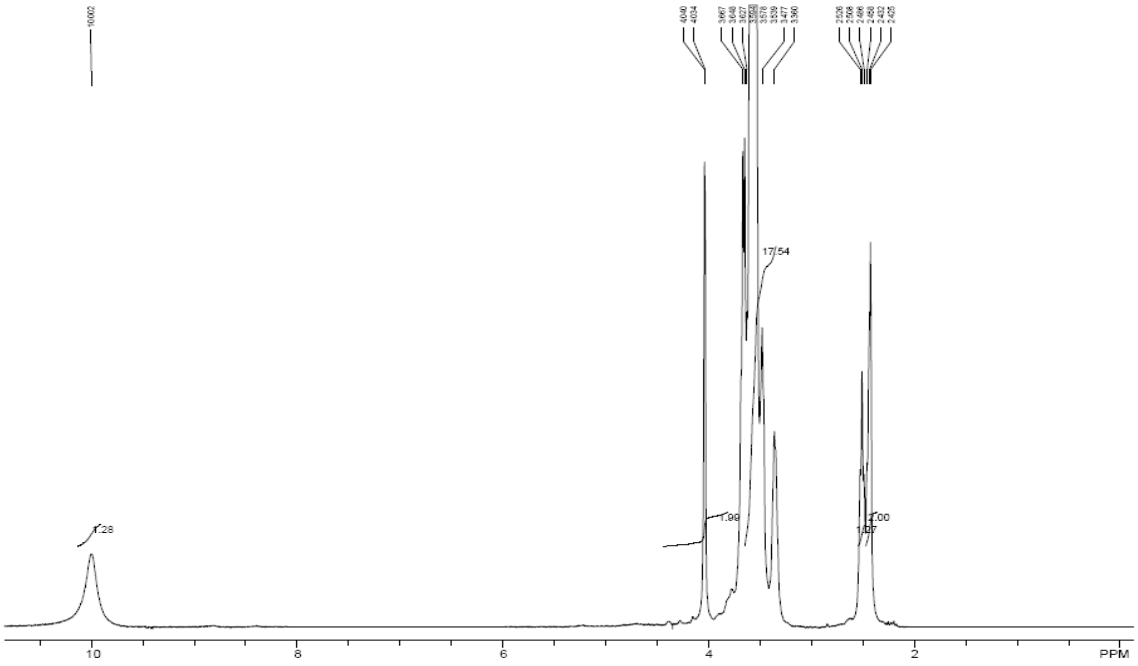
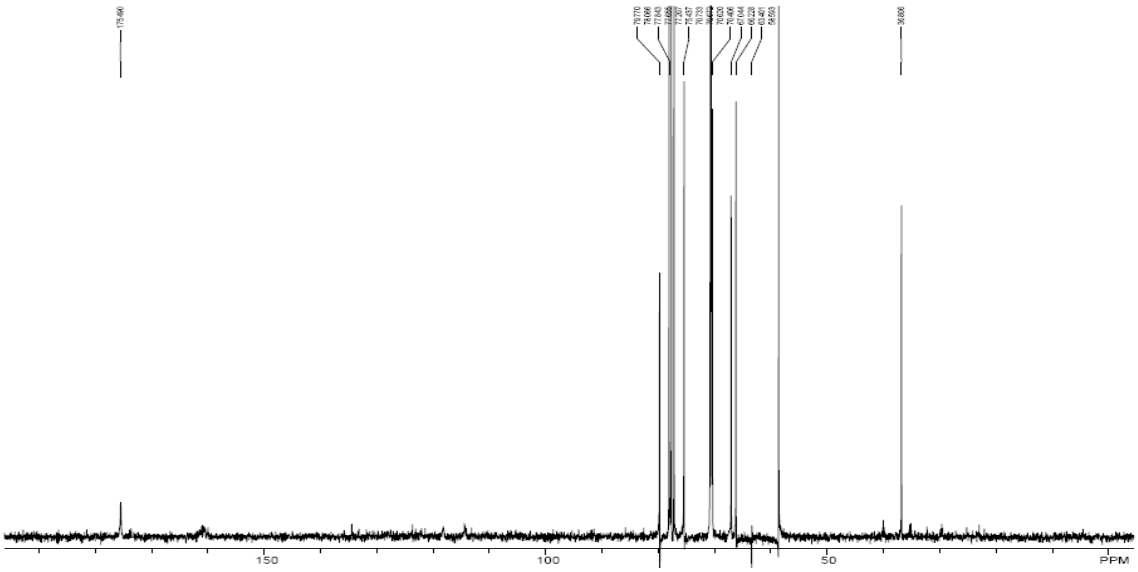
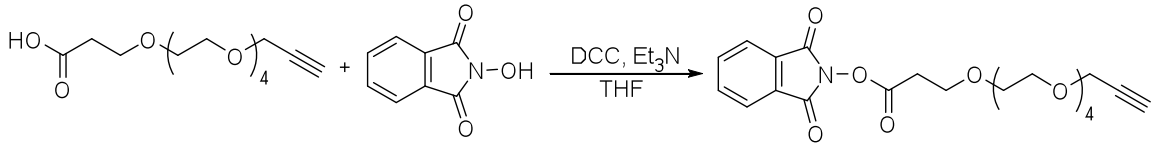


S5 **S6**

To a solution of the alkyne **S5** (150 mg, 0.42 mmol) under nitrogen in dry CH_2Cl_2 (1 mL) was added drop-wise trifluoroacetic acid (1 mL). The mixture was stirred for 4 hours at room temperature. The solvent was removed under vacuum affording the acid **S6** in quantitative yields. ^{1}H NMR (300 MHz, $CDCl_3$): δ 9.99 (s, 1H), 4.03 (t, J = 2.2 Hz, 2H), 3.82-3.26 (m, 18H), 2.49 (t, J = 2.2 Hz, 2H), 2.41(t, J = 5.4 Hz, 2H). ^{13}C NMR (75 MHz, $CDCl_3$): δ175.5, 79.8, 75.4, 70.7, 70.6, 70.4, 67.0, 66.2, 63.4, 58.6, 36.8. MS (ESI) m/z calcd for $C_{14}H_{24}O_7$: 304.1; found: 327.4 ($[M + Na]^+$).

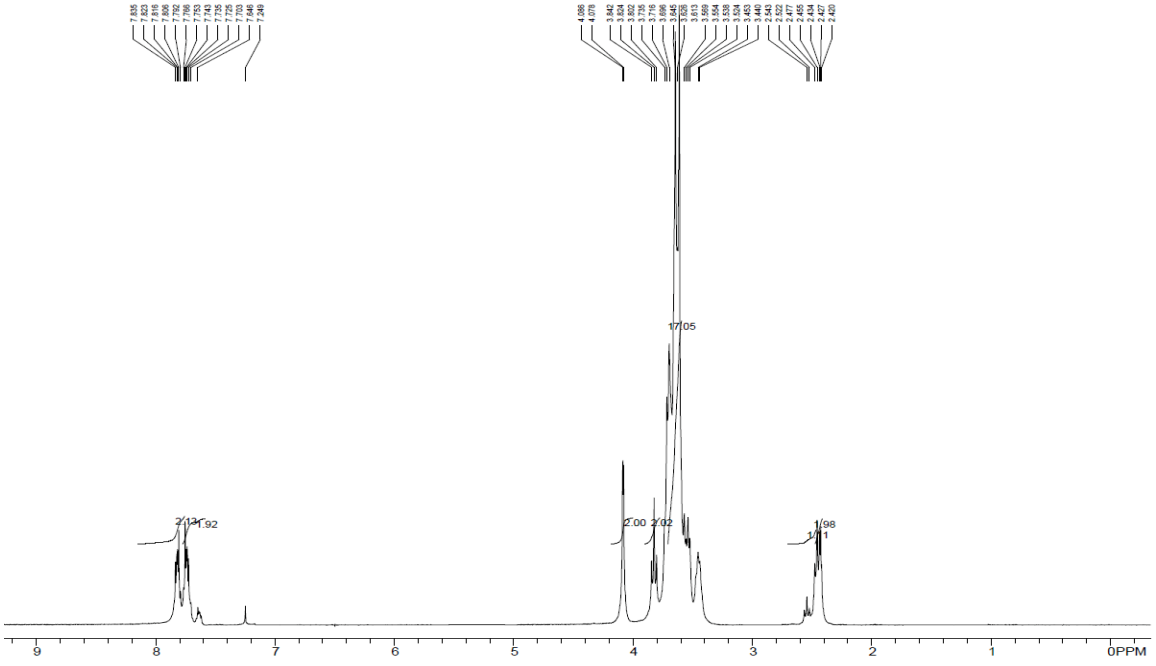


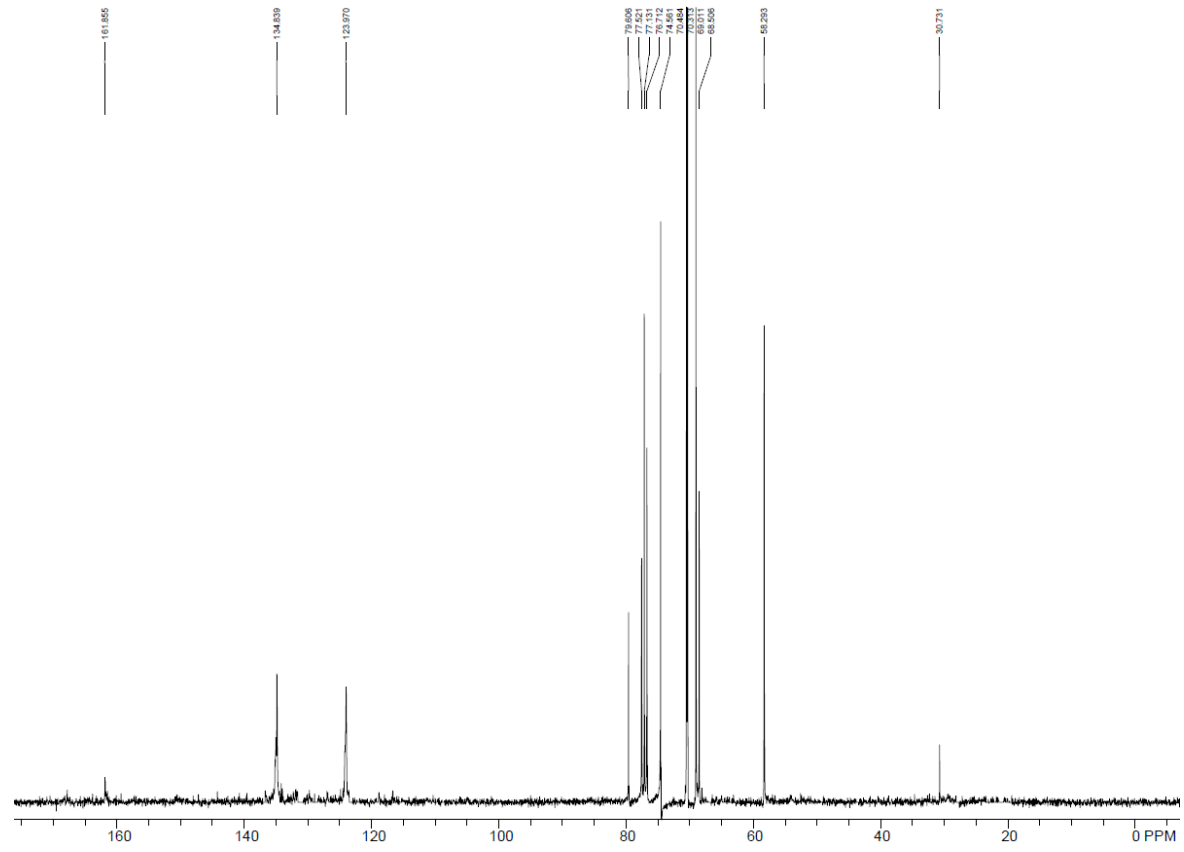


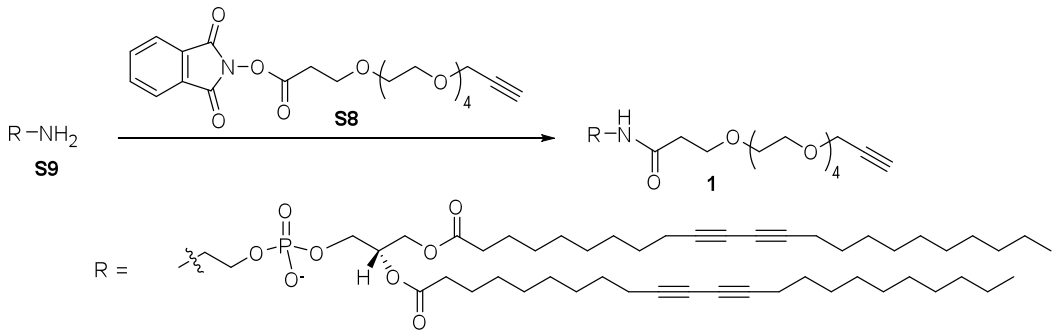


S6 **S7** **S8**

Et_3N (59 µL, 0.43 mmol), and DCC (89 mg, 0.43 mmol) were added to a solution of the acid **S6** (100 mg, 0.33 mmol) in THF (1 mL). A solution of **S7** (70 mg, 0.43 mmol) in THF (1 mL) was added, and the reaction mixture was stirred at room temperature for 12 h. The solution was filtered and the solvent was removed in vacuo, the residue was purified by flash chromatography (ethyl acetate/methanol 9:1) to give **S8** (104 mg, 0.23 mmol, 70%) as a colorless semisolid. ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.90-7.75 (m, 2 H), 7.77 -7.70 (m, 2 H), 4.08 (t, J = 2.2 Hz, 2H), 3.82 (t, 2 H, J = 5.4 Hz) 3.76-3.37 (m, 16 H), 3.57-3.27 (m, 2 H), 2.42-2.48 (m, 3H). ^{13}C NMR (75 MHz, $CDCl_3$): δ161.9, 134.8, 123.9, 79.6, 74.6, 70.7, 70.6, 70.4, 69.0, 68.5, 58.2, 30.7. MS (ESI) m/z calcd for $C_{22}H_{27}NO_9$: 449.2; found: 472.3 ([M + Na]$^+$).

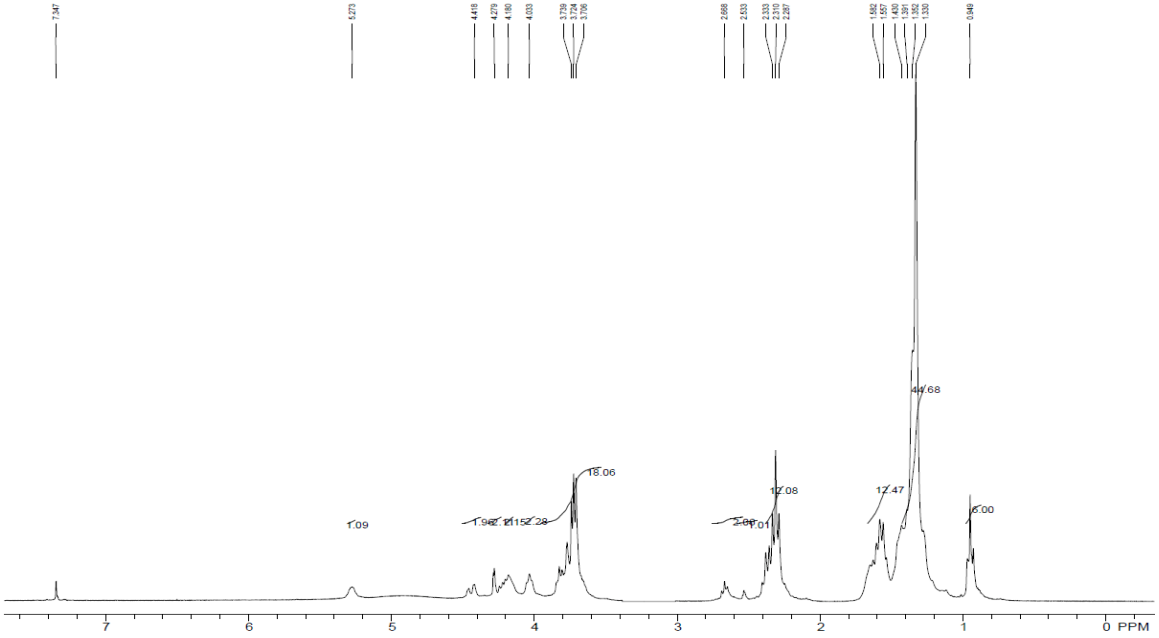


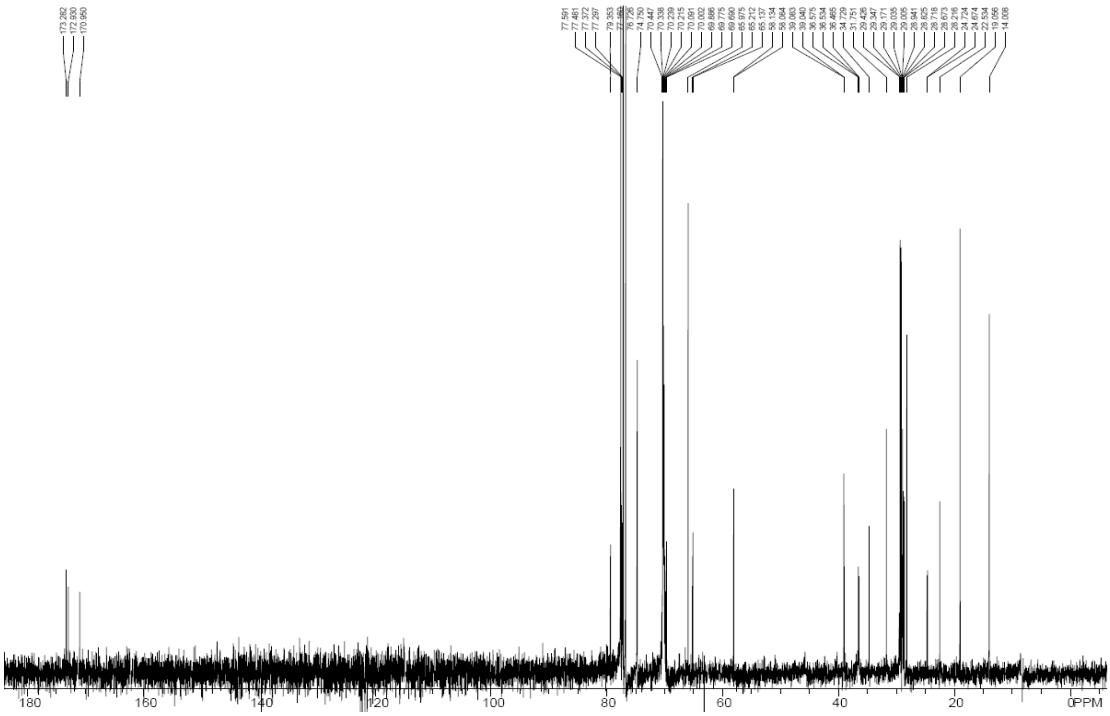


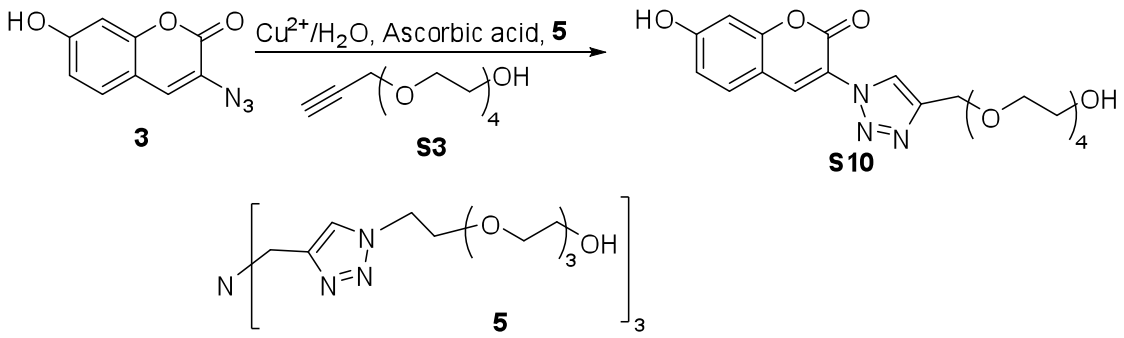


To a solution of the amine **S9** (50 mg, 0.06 mmol) and triethylamine (17 µL, 0.12 mmol) in dry $CHCl_3$ (1 mL) at 0 ˚C under nitrogen was drop-wise added **S8** (35 mg, 0.09 mmol) dissolved in dry $CHCl_3$ (1 mL). After being stirred for 1.5 h at 0 ˚C, the solution was allowed to warm up and stirred overnight at room temperature. Saturated aqueous NH_4Cl (3 mL) was added, and the mixture was extracted three times with $CHCl_3$. The combined organic layers were washed with water, brine, and dried over Na_2SO_4. The solvent was removed under vacuum affording a crude product, which was purified by flash chromatography (chloroform) to give the alkyne **1** (53 mg, 0.05 mmol, 77%) as a yellow oil. ^{1}H NMR (300 MHz, $CDCl_3$): δ 5.27 (m, 1H), 4.50-4.35 (m, 2H), 4.34-4.25 (m, 2H), 4.25-4.10 (m, 2 H), 4.03 (t, 2 H, J = 5.4 Hz), 3.90-3.40 (m, 18H), 2.67 (t, 2 H, J = 5.4

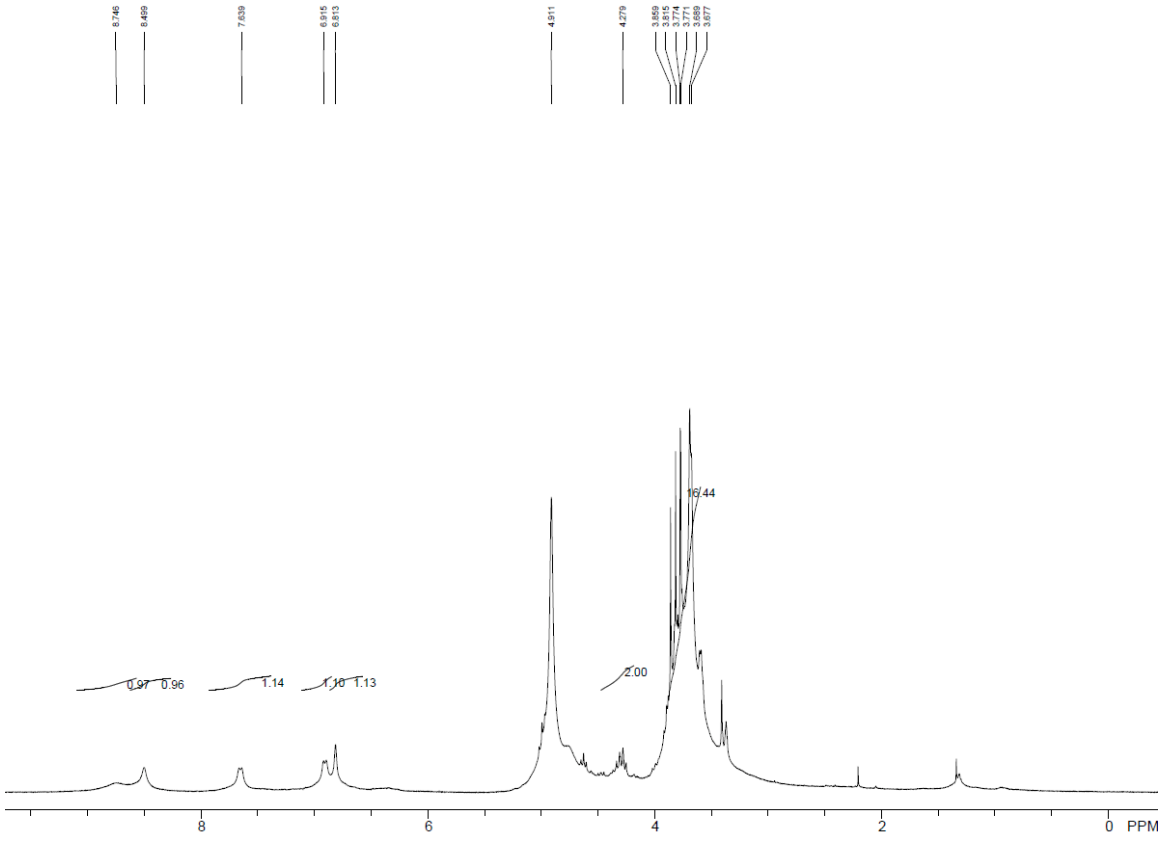
Hz), 2.53 (t, 1 H, J = 2.2 Hz), 2.46-2.05 (m, 12H), 1.75-1.50 (m, 12H), 1.50-1.20 (m, 44H), 0.95 (t, 6 H, J = 5.4 Hz) ^{13}C NMR (75 MHz, $CDCl_3$): δ 173.3, 172.9, 170.9, 79.3, 74.8, 70.4, 70.3, 70.2, 70.1, 70.0, 69.9, 69.8, 69.7, 65.9, 65.2, 56.1, 39.0, 36.5, 34.7, 31.7, 29.4, 29.3, 29.2, 29.1, 29.0, 28.9, 28.8, 28.7, 28.6, 28.5, 28.4, 28.3, 28.2, 24.7, 22.5, 19.1, 14.0. MS (ESI) m/z calcd for $(C_{65}H_{107}NO_{14}P)^-$: 1156.7; found: 1180.9 ($[M + Na + H]^+$).

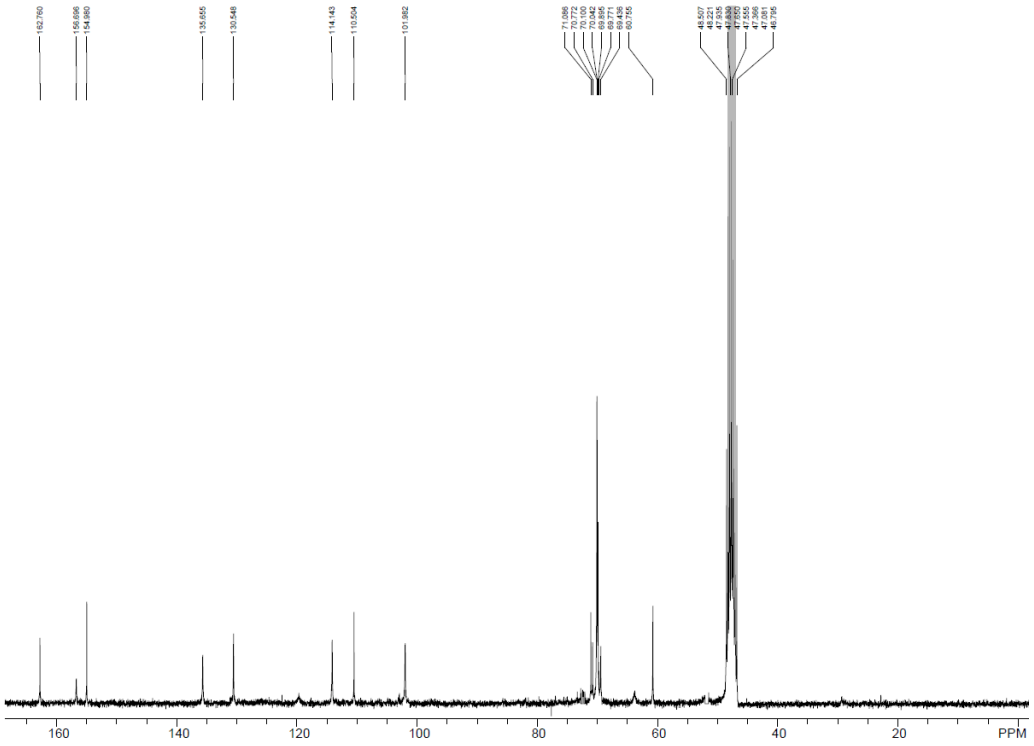


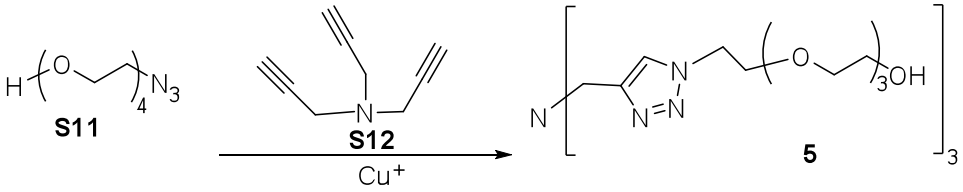




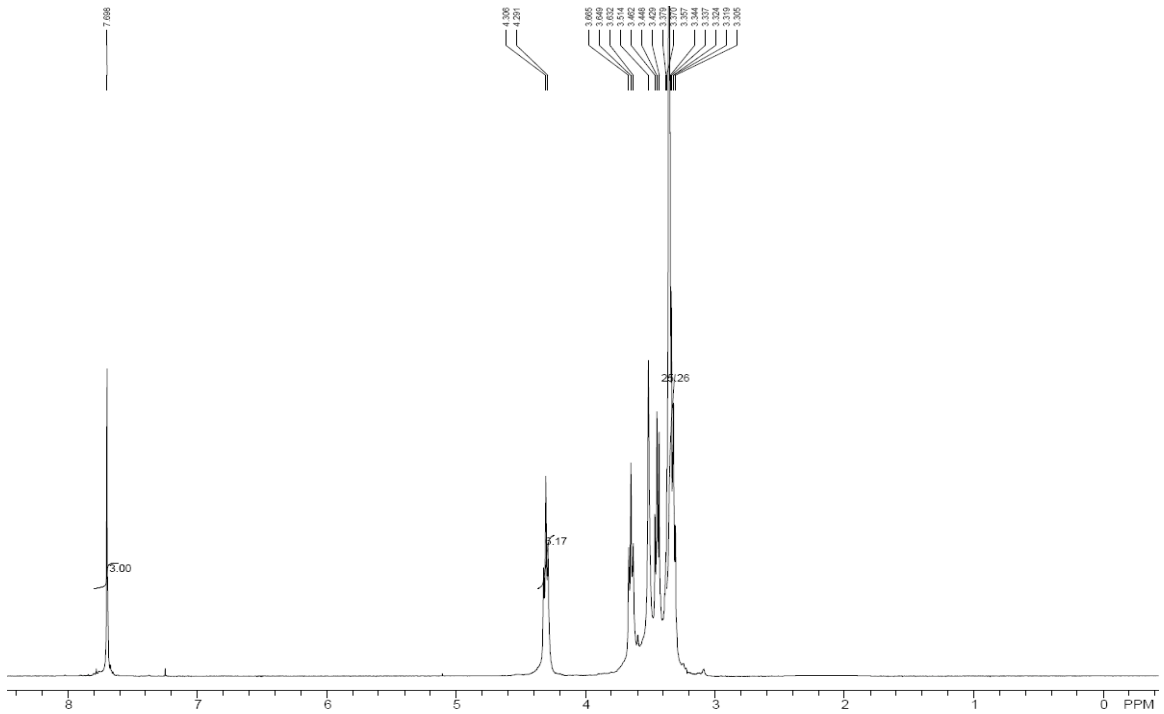
To a stirred solution of **3**[2] (500 mg, 2.46 mmol) in H_2O (2.0 mL) was treated sequentially with the alkyne **S3** (600 mg, 2.58 mmol), ascorbic acid (114 mg, 650 μmol), $CuSO_4$ (20.8 mg, 130 μmol) and ligand **5** (309 mg, 260 μmol). After the mixture was stirred at room temperature for 6 hours, the solvent was evaporated, and the residue was purified by flash chromatography (ethyl acetate/methanol 9:1) to give **S10** (0.86 g, 1.96 mmol, 80%) as a light brown viscous liquid. ^{1}H NMR (300 MHz, CD_3COCD_3): δ 8.75 (s, 1 H), 8.42 (s, 1 H), 7.60 (d, 1 H, J = 7.1 Hz), 6.89 (d, 1 H, J = 7.1 Hz), 6.79 (s, 1 H), 4.27 (m, 2 H), 4.06-3.42 (m, 16 H); ^{13}C NMR (75 MHz, CD_3OD): δ 162.8, 156.7, 154.9, 135.7, 130.5, 114.1, 110.5, 101.9, 71.1, 70.8, 70.1, 70.0, 69.9, 69.8, 69.4, 60.6; MS (ESI) *m*/*z* calcd for $C_{12}H_{24}N_4O_4$: 435.1; found: 458.3 ($[M + Na]^+$).







To a stirred solution of tripropargylamine (**S12**, 250 mg; 1.91 mmol) in CH_3CN (2.5 mL) was treated sequentially with the azide **S11**[3] (1.50 g, 6.87 mmol), 2,6-lutidine (735 mg, 6.87 mmol), and $Cu(MeCN)_4PF_6$ (27.7 mg, 0.07 mmol). After the mixture was stirred at room temperature for 24 hours, the solvent was evaporated, and the residue was purified by flash chromatography (ethyl acetate/methanol 9:1) to give **5** (0.93 g, 1.18 mmol, 62%) as a light brown viscous liquid. ^{1}H NMR (300 MHz, $CDCl_3$): 7.69 (s, 3 H), 4.30 (t, 6 H, J = 5.0 Hz), 3.64 (t, 6 H, J = 5.0 Hz), 3.46 (s, 6 H), 3.44 (t, 6 H, J = 5.0 Hz), 3.37-3.30 (m, 30 H); ^{13}C NMR (75 MHz, $CDCl_3$): δ 143.6, 124.4, 72.3, 70.2, 70.0, 69.2, 61.1, 49.6, 47.2; MS (ESI) m/z calcd for $C_{33}H_{60}N_{10}O_{12}$: 788.4; found: 811.4 ($[M + Na]^+$).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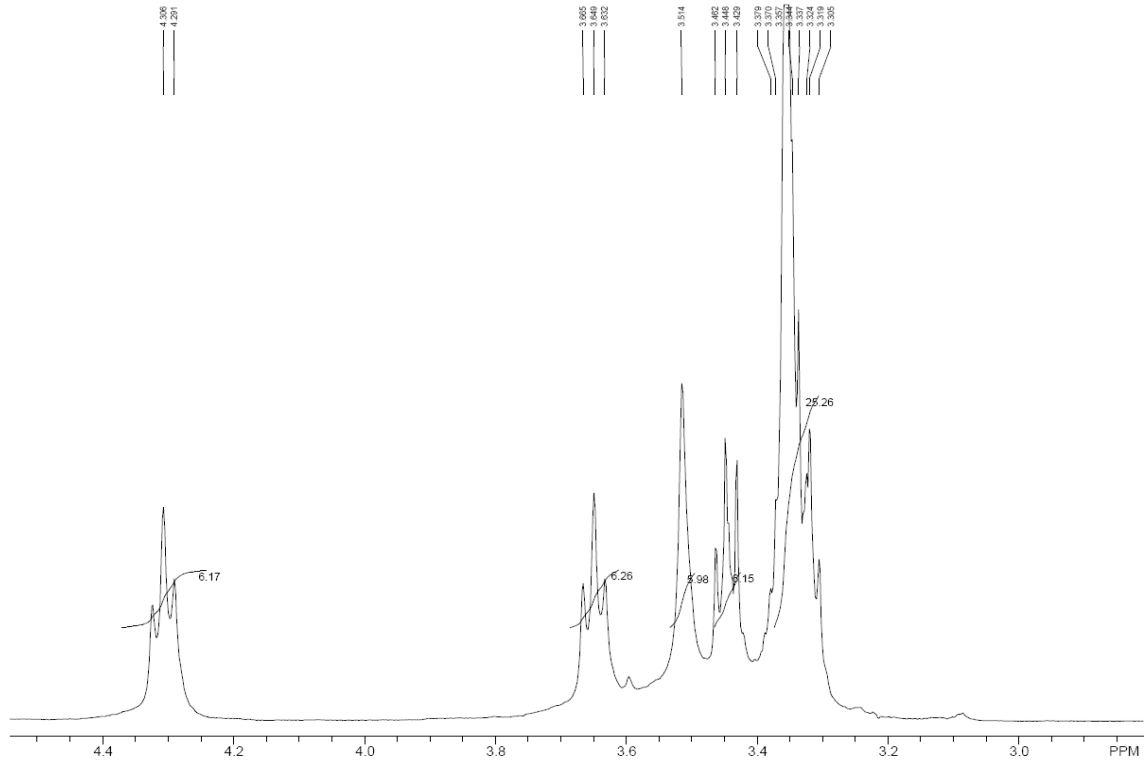
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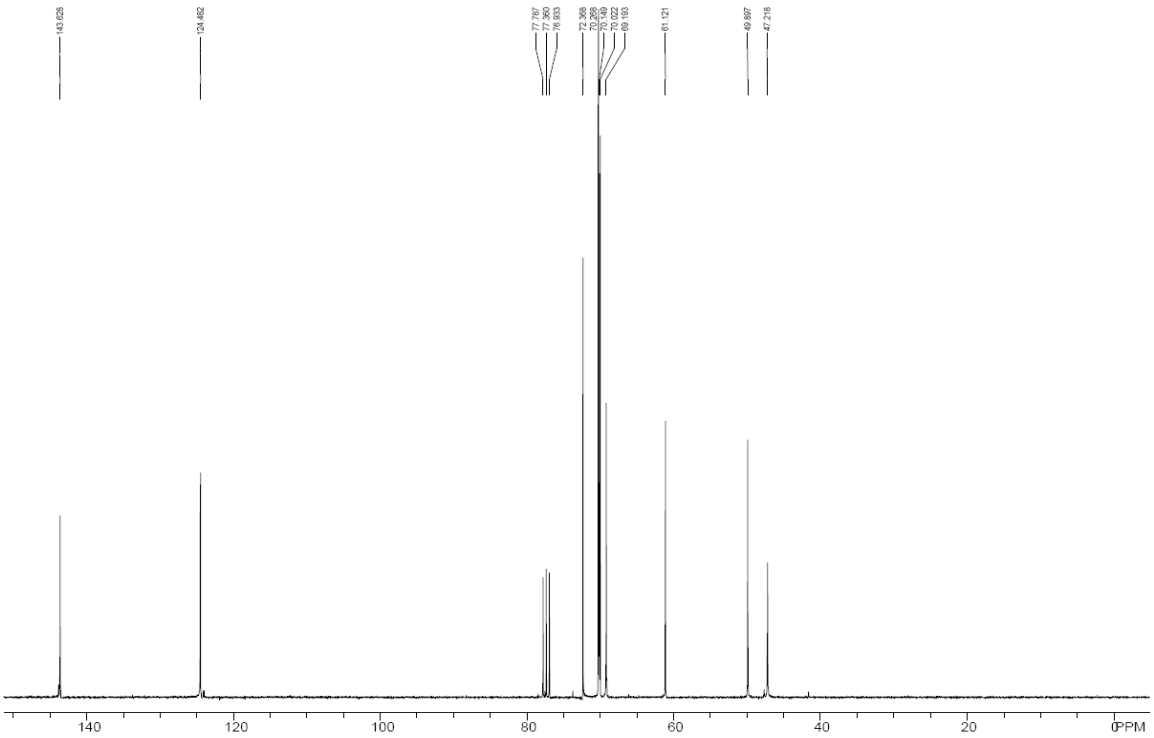
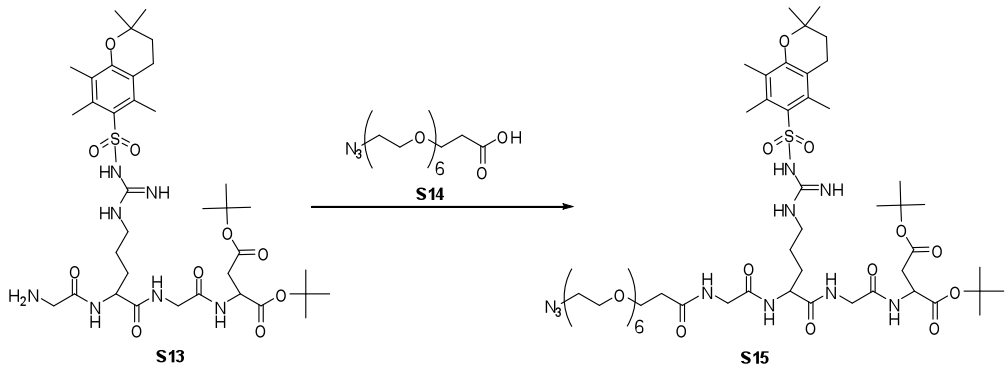
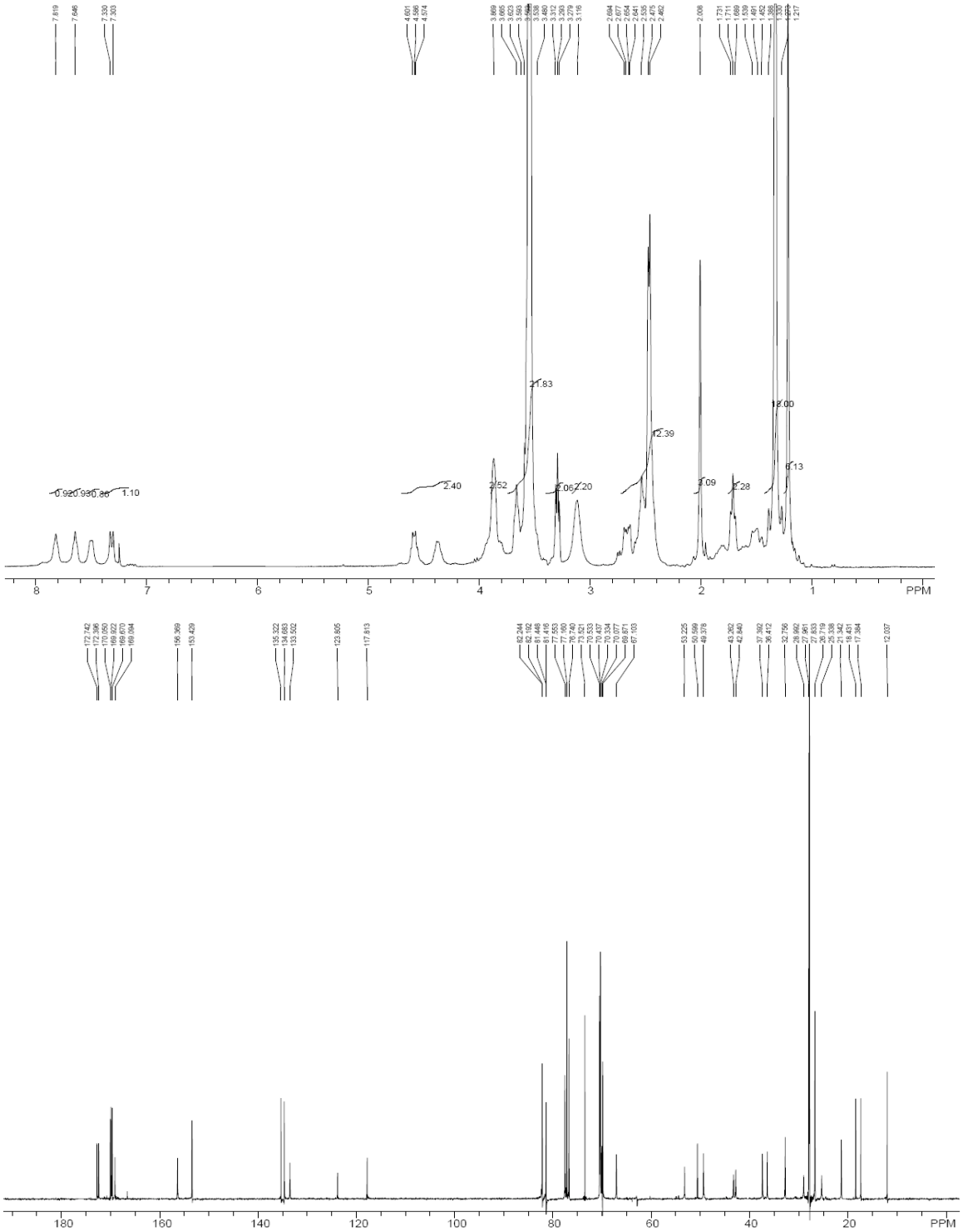
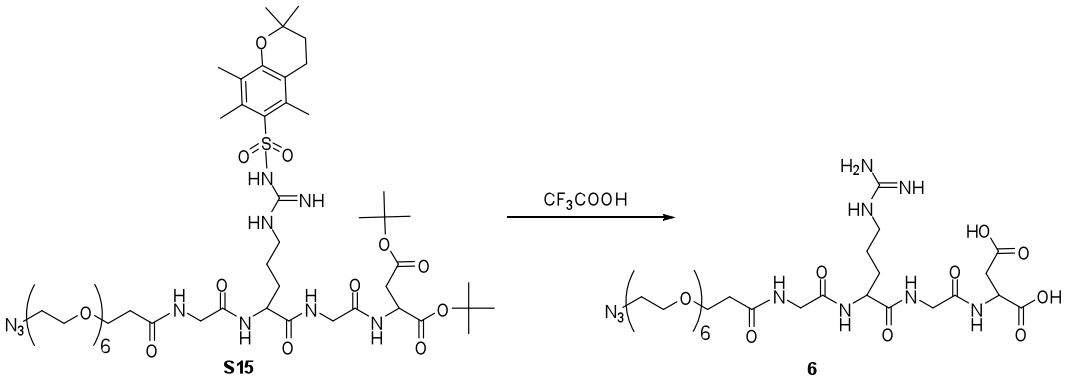




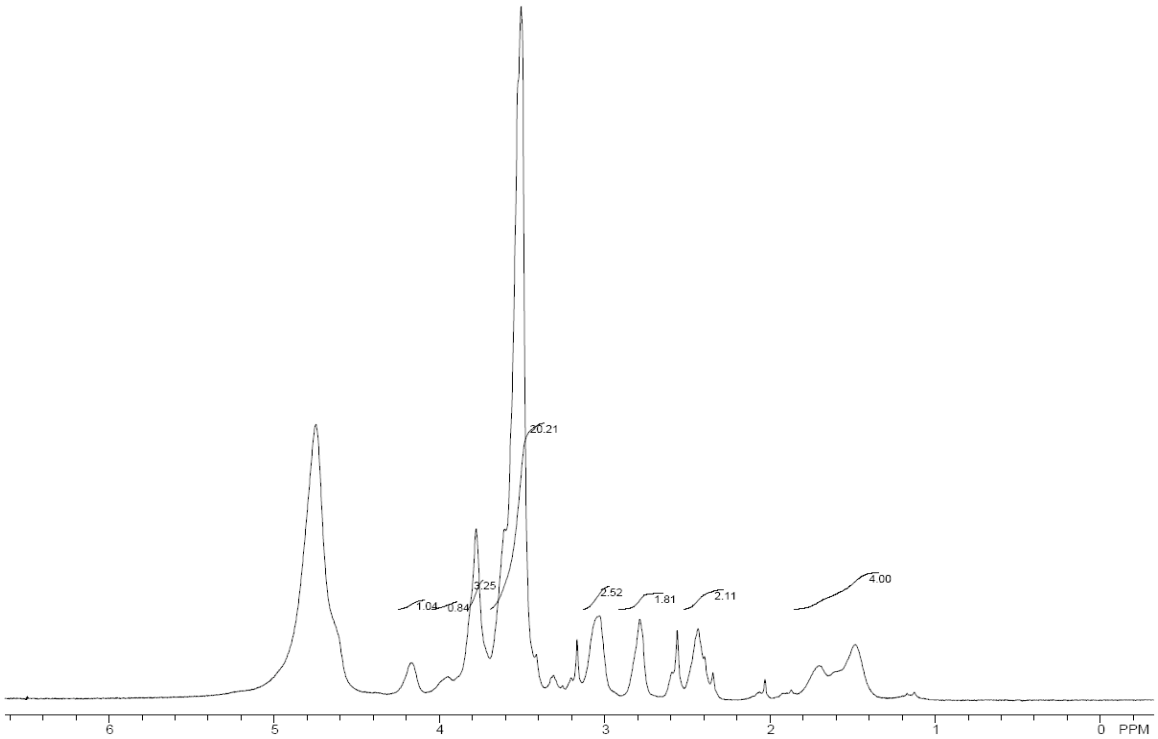
A solution of azide **S14** (39.8 mg, 0.100 mmol) and protected GRGD **S13**[4] (100 mg, 0.080 mmol) in dry DMF (1 mL) was purged with N_2. The solution was stirred and cooled to 0 °C. Sodium 1,2-dihexadecanoyl-*sn*-glycero-3-phosphate (DPPA, 33 μL, 0.100 mmol) was added, followed by a solution of *N,N*-diisoproylethylamine (DIPEA, 16 μL, 0.10 mmol) in DMF (1 mL), and the stirring was continued at 0 °C for 10 h. The mixture was diluted with EtOAc (5 mL) and washed successively with H_2O, 5% aqueous $NaHCO_3$, and brine. The organic phase was dried (Na_2SO_4), and the solvent was removed in vacuo to give crude product which was purified by flash chromatography to give the tetrapeptide derivative **S15** (1.53 g, 4.33 mmol, 90%) as a colorless liquid. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.82 (s, 1 H), 7.65 (s, 1 H),7.33 (s, 1 H), 7.18 (s, 1 H), 7.16 (s, 1 H), 4.67-4.57 (m, 1 H), 4.49-4.28 (m, 1 H), 3.67 (m, 2 H), 3.62 (m, 2 H), 3.59-3.48 (m, 22 H), 3.29 (t, 2 H, J = 5.4 Hz), 3.11 (m, 2 H), 2.80-2.30 (m, 12 H), 2.00 (s, 3 H), 1.71 (t, 2 H, J = 7.0 Hz), 1.33 (s, 18 H), 1.21 (s, 6 H); ^{13}C NMR (75 MHz, CDCl3) δ 172.7, 172.4, 170.1, 169.9, 169.7, 169.1, 156.4, 153.4, 135.3, 134.7, 133.5, 123.8, 82.2, 81.4, 73.5, 70.5, 70.4, 70.3, 70.1, 69.9, 67.1, 53.2, 50.6, 49.4, 37.4, 36.4, 32.8, 28.9, 27.9, 26.7, 25.3,

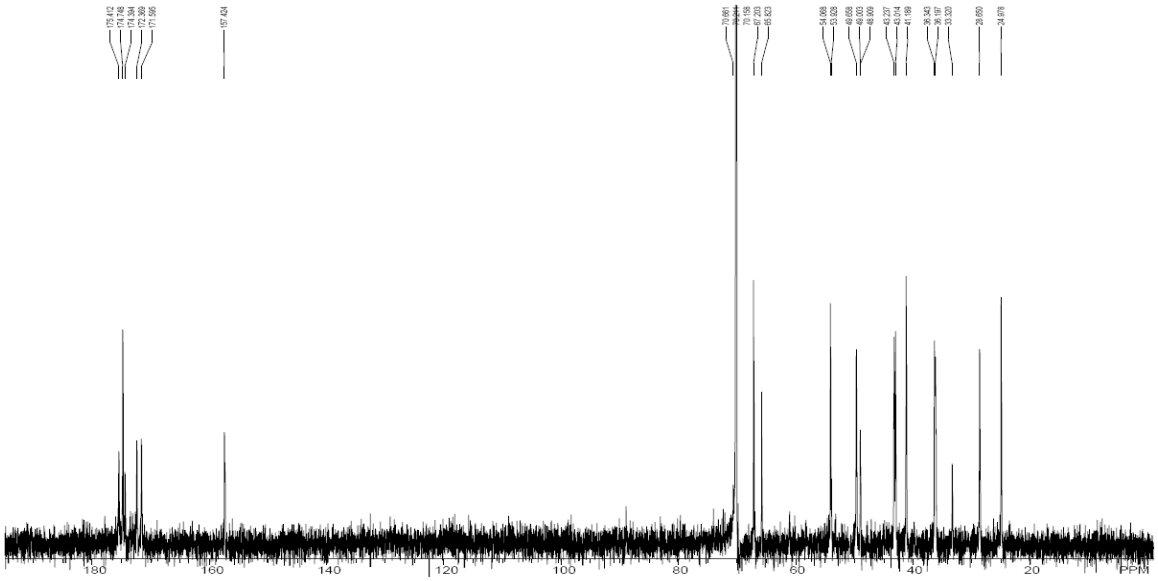
21.3, 18.4, 17.4, 12.0; MS (ESI) *m/z* calcd for $C_{51}H_{86}N_{10}O_{17}S$: 1142.5; found: 1165.7 ([M + Na]$^+$).





A stirred solution of GRGD-EG_6 **S15** (60 mg, 0.05 mmol) in CH_2Cl_2 (0.5 mL) was cooled to 0 °C, and TFA (0.5 mL) was added to it. The obtained solution was stirred at 0 °C for 1 h, allowed to warm to room temperature, and stirred for 2 h at room temperature. The mixture was concentrated in vacuo to give de-protected GRGD derivative **6** (38.2 mg, 100%). ^{1}H NMR (300 MHz, D_2O) δ 4.21 (s, 1 H), 3.80 (s, 5 H), 3.73-3.40 (m, 22 H), 3.08 (s, 2 H), 2.83 (s, 2 H), 2.60 (s, 1 H), 2.47 (s, 2 H), 1.73 (s, 2 H), 1.52 (s, 2 H); ^{13}C NMR (75 MHz, D_2O) δ 175.4, 174.7, 174.4, 172.3, 171.6, 157.4, 157.3, 70.2, 67.2, 65.8, 54.1, 49.6, 49.5, 49.0, 43.2, 43.0, 41.2, 36.3, 36.2, 28.6, 24.9; MS (ESI) *m*/*z* calcd for $C_{29}H_{52}N_{10}O_{14}$: 764.3; found: 787.3 ($[M + Na]^+$).





B. Synthesis of clickable polymerized liposomes (CPL)

A mixture of 5 mg of the alkynyl-terminated lipid **1** and 25 mg of 23:2 Diyne PC (**2**) (Scheme 2) were dissolved in $CHCl_3$ (10 ml) in a 25 ml conical flask. The solvent was evaporated with a rotary evaporator and dried under vacuum pump for 10 min. The transparent lipid film on the wall of the flask was dispersed in 10 ml of milli-Q water at room temperature. The resultant aqueous suspension was sonicated for 1 h at 60 ˚C. The sample was extruded 20 times at 60 ˚C using an Avanti mini extruder with a 100 nm Whatman Nuclepore Track-Etch Membrane filter. The vesicles were polymerized using UV lamp at 254 nm for 1 hour at 0 ˚C in a petri dish yielding a faint brick red solution. The size of the particles in Millipore water was measured by dynamic light scattering (Brookhaven 200 SM goniometer and correlator). The liposomes prepared from various batches were homogeneous in size and exhibited an average diameter of about 110 nm (Fig. S1).

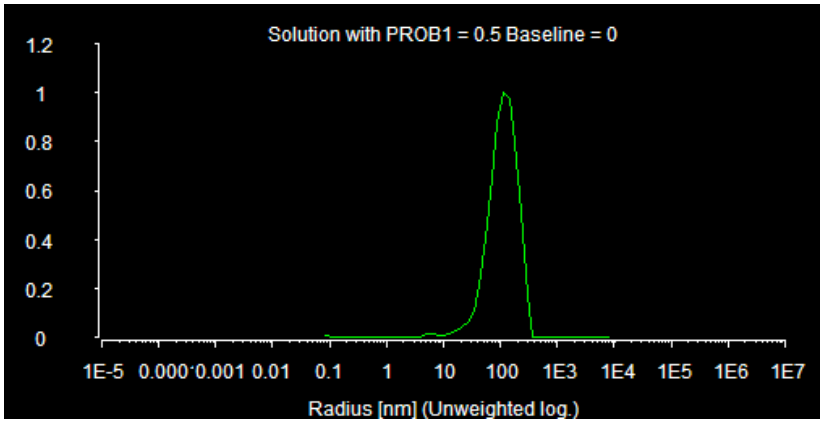


Figure S1. A dynamic light scattering diagram of **CPL** in Millipore water.

C. Stability of the terminal alkyne under UV irradiation

Five milligrams of the alkyne **S6** was dissolved in 10 ml of Millipore water and exposed to UV lamp at 254 nm for 1 hour at 0 ˚C in a petri dish. The solution was subsequently evaporated and the resulting compound checked by TLC and ^{1}H-NMR for any degradation. The results showed that terminal alkyne **S6** was intact under the UV irradiation conditions.

D. Optimization of click reaction conditions on CPL using the coumarin azide 3 (Scheme 3)

Reactants were separately and freshly prepared in 50 mM HEPES buffer (Invitrogen). They were added to and mixed with a pipette in a 96 well plate at a fixed concentration of the alkynyl lipids on the **CPL** and coumarin azide **3**, whereas the concentrations of $CuSO_4$, ligand, and ascorbic acid were varied (see below). The ratio of ligand/copper was always fixed at 2:1. While in the 96 well plate each column was fed with different copper concentration, every row was fed with different ascorbic acid concentration. The overall volume in each well of the plate was 250 μL containing a solution of the coumarin derivative **3**[2] (5.0 mM), **CPL** (0.5 mM derived from the precursor lipid **1**), $CuSO_4$ (2.5 – 0.039 mM), ligand **5** (5.0 – 0.078 mM, $CuSO_4$/**5** 1:2), and ascorbic acid (50.0 – 3.125 mM). The reactions were allowed to stand under ambient conditions (without stirring) to obtain the highly fluorescent product **4** (Scheme 3) and the reactions were monitored by measuring the fluorescence intensity (excitation, 365 nm; emission, 460 nm) over time using a Fusion plate reader (Perkin-Elmer/Packard, Wellesley, MA).

E. Estimation of the yields of CuAAC reactions on CPL

The amount of coumarine-triazole moieties formed after the CuAAC reaction was obtained by measurement of fluorescence intensity and correlation with the calibration curve (Figure S2). This calibration curve was obtained using the analogous coumarine-triazole **S10** with an absorption maximum at λ_{ex} = 360 nm and emission maximum at λ_{em} = 465 nm). Note that the self-quenching of adjacent fluorophores in the liposome **4** was ignored. To estimate the yield of the reaction, the total amount of ethynyl groups on the CPL that is available for the CuAAC reaction with the coumarine azide **3** was measured by the maximum fluorescence intensity achieved during the reaction under optimal reaction conditions. The optimized conditions for click reaction on **CPL** (0.5 mM of alkynyl-terminated lipid (assuming the same as the precursor lipid **1**) are the following: 50 μl each of 312.5 μM $CuSO_4$, 6.25 mM ascorbic acid, 625 μM the ligand, and the azide **3** (5 mM) mixed and allowed to stand under ambient conditions (without stirring) for 1 hour followed by purification via size exclusion column (PD SpinTrap™ G-25 from GE Healthcare).

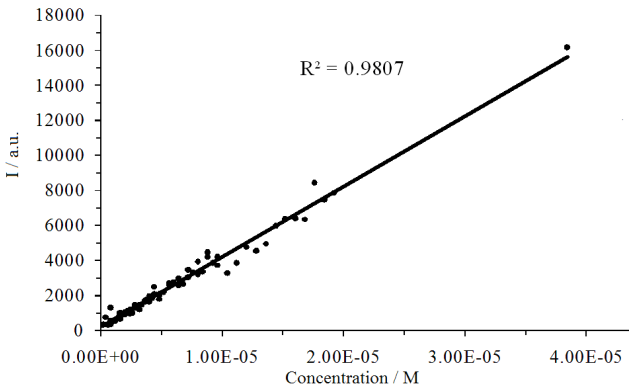


Figure S2. Fluorescence (λ_{ex} = 360 nm, λ_{em} = 465 nm) intensity (I) vs concentration of the coumarine-triazole **S15** mixed with 312.5 μM $CuSO_4$, 6.25 mM ascorbic acid in HEPES (pH = 7.2), served as a calibration curve for quantification of the coumarine-triazole moieties formed on the liposomes during or after the CuAAC reaction.

F. Liposome stability during CuAAC reaction conditions

To test the effect of experimental conditions employed for the CuAAC reaction on the stability of liposomes, we prepared alkynyl-terminated, polymerized and unpolymerized liposomes that encapsulated 5,6-carboxyfluorescein at a self-quenching concentration.[5] Since carboxyfluorescein self-quenches at this concentration, the dye encapsulated inside the liposome displays a low fluorescence. As the liposome becomes leaky, the dye is released from the liposome and gets diluted, leading to an intense fluorescent signal. The amount of change in fluorescence signal of the liposome solution over time gives a measure of the stability of the liposome.

Dye-encapsulated **CPL** was prepared using the same procedure as for **CPL** except the aqueous suspension of **1** and **2** also contained 40 mM of 5,6-carboxyfluorescein (Sigma Aldrich). Non-encapsulated dye was eliminated by gel filtration (PD-10 Desalting column). Half of the resulting solution was then subjected to UV polymerization. The polymerized and the non-polymerized, dye-loaded liposomal suspensions were subjected to the same CuAAC reaction with the non-fluorescent OH-OEG_4-azide (**S11**) under the optimized CuAAC conditions (mentioned in D). Increase in fluorescence intensity during the reaction is attributed to the release (dequenching) of the encapsulated dye. The fluorescence intensity at λ_{ex} 490 nm and λ_{em} 520 nm over time was measured using a Fusion plate reader (Perkin-Elmer/Packard, Wellesley, MA). To measure the total fluorescence intensity corresponding to 100% dye release, Triton X-100 (0.1% w/v final) was added to both solutions of lipsomes after 12 hours. The percentage of dye release caused by the peroxy radical produced from reduction of Cu(II) to Cu(I) by ascorbic acid during click reaction was calculated using the equation $(F - F_0) \times 100/(F_t - F_0)$, where F is the fluorescence intensity measured after exposing the liposomes to the CuAAC reaction conditions and F_0 and F_t are the intensities obtained before the reaction and after Triton X-100 treatment,[5] respectively. Since polymerized and unpolymerized liposomes contain the same amount of dye and the polymerized vesicles cannot be fully ruptured using Triton X-100, hence the F_t values for unpolymerized liposomes were used for calculation. Values were corrected for dilutions caused by the Triton X-100 addition.

Figure S3A shows that unpolymerized liposomes readily decomposed; 70% dequenching occurred within 12 hours even under the optimized reaction conditions. In contrast, **CPL** was quite stable; only 30% dequenching was measured in 12 hours under the same conditions. The increased stability of **CPL** towards peroxy radical generated from the reduction of Cu(II) to Cu(I) by ascorbic acid arises from the extensive cross linking of internal alkynes present in lipid constituting the liposome when subjected to UV irradiation. Unpolymerized liposome lacks of the robust cross-linking and hence succumbs to the peroxy radicals. **CPL** without copper and ascorbic acid is highly stable at ambient conditions and show minimal decomposition in comparison to unpolymerized liposome which is susceptible to air oxidation and fusion with other liposomes.

When the carboxyfluorescein encapsulated polymerized liposomes underwent click reaction at a fixed ratio of copper to ligand varying the concentrations of ascorbic acid it was observed that an increase in the amount of ascorbic acid lead to an increase of decomposition of liposome. The decomposition was attributed to the peroxy radicals formed by the reduction of Cu^{2+} to Cu^{+} via ascorbic acid. These radicals reacted with the unsaturated liposome surface thereby destroying it. Increase in amount of ascorbic acid lead to increase in peroxy radicals thereby accelerating the decomposition of liposomes.

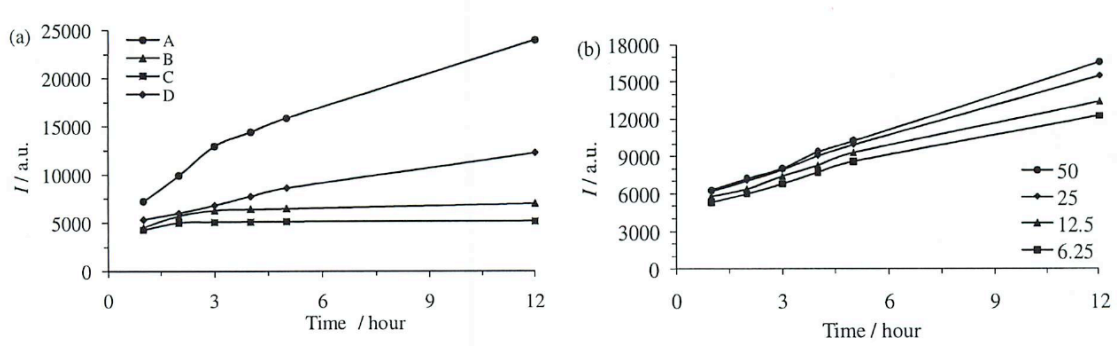


Figure S3. (a) Time dependent fluorescence (λ_{ex} 490 nm; λ_{em} 520 nm) intensities (*I*) as a measure of the stability of (A, C) polymerized and (B, D) unpolymerized liposome solutions prepared from the diacetylene lipid mixture of **1** and **2** (5.0 mg of **1** and 25 mg of **2** in 10 ml water). The solutions A and B also contained 312.5 μM $CuSO_4$, 6.25 mM ascorbic acid, and 625 μM ligand **5**, whereas the solutions C and D served as controls without copper and ascorbic acid. The maximum fluorescence intensity of 34852 units signifying full decomposition, achieved in presence of Triton X-100 (0.1% w/v final). **(b)** Fluorescence (λ_{ex} 490 nm; λ_{em} 520 nm) intensities (*I*) of polymerized liposome CPL solutions prepared from the diacetylene lipid mixture of **1** and **2** (5.0 mg of **1** and 25 mg of **2** in 10 ml water) solutions over time subjected to CuAAC reaction conditions with 312.5 μM $CuSO_4$, 625 μM ligand **5**, and 6.25 (not shown in figure), 12.5, 25, and 50 mM of ascorbic acid.

G. Verifying the ratio of GRGD and FITC on the CPL (Scheme 4)

Reactants were separately and freshly prepared in 50 mM HEPES buffer (pH 7.2). They were added and mixed in a 96 well plate at a fixed concentration of **CPL** (0.5 mM of

alkynyl-terminated lipid) and FITC-EG_6N_3 (**7**) (0.5 mM). The concentration of ligand (650 μM)/$CuSO_4$ (325 μM) was fixed at 2:1. The concentrations of GRGD-EG_6-N_3 (**6**) were varied throughout the row of the 96 well plate. The overall volume in each well of the plate was 250 μL containing a solution of FITC-EG_6N_3 (**7**) (0.5 mM), GRGD-EG_6-N_3 (**6**) (0.0125-1 mM), alkyne terminated polymerized liposome **CPL** (1.0 mM), $CuSO_4$ (325 uM), ligand **5** (650 μM), and ascorbic acid (6.25 mM) (Scheme 4). The reactions were allowed to stand under ambient conditions (without stirring) for 1 hour, and monitored by measuring the fluorescence intensity (excitation, 495 nm; emission, 520 nm) over time using a Fusion plate reader. The reaction mixture was passed through a size exclusion column (PD SpinTrap™ G-25 from GE Healthcare) to obtain the FITC modified liposome.

H. Anti-adhesion assay

Cells were grown to a subconfluent state and harvested using 0.025% trypsin-EDTA mixture (VWR). The harvested cells (2 x 10^5 cells/100 μL/well in a 96-well plate) were then incubated with various concentrations of GRGD-liposome **8** (in serum-free DMEM, VWR) for 15 min in an ice bath and then the solutions were transferred into another 96-well plate, which was coated with vitronectin (96-well Millicoat™ Human Vitronectin Coated Strips). The cells were incubated on the vitronectin-laden plates for 1 hr. at 37 °C with 5% CO_2. After the incubation, the unattached cells were gently removed by rinsing the wells 3 times with HBSS (VWR). One hundred microliters of 0.2% crystal violet in 10% ethanol were added to each well and the cells were incubated for 5 minutes at room temperature. The strips were gently washed for 5 times with PBS (300μL/well) to remove the excess stain. 100 μL of solubilization buffer (50/50 mixture of 0.1M NaH_2PO_4 pH 4.5, and 50% ethanol) was added to each well. Strips were incubated and gently shaken at room temperature until the cell-bound stain was completely solubilized. The absorbance of the resulting wash was determined by a microplate reader (GeneTAC UC 4 Array Scanner with an excitation wavelength at 518 nm and emission wavelength at 532 nm).

References

1. K. Fujiwara, Y. Murata, T. S. M. Wan, K. komatsu, *Tetrahedron,* 1998, **54**, 2049-2058.
2. K. Sivakumar, F. Xie, B. M. Cash, S. Long, H. N. Barnhill and Q. Wang, *Org. Lett.*, 2004, **6**, 4603-4606.
3. Y. Gong, Y. M. Luo and D. Bong, *J. Am. Chem. Soc.*, 2006, **128**, 14430-14431.
4. C. Roberts, C. S. Chen, M. Mrksich, V. Martichonok, D. E. Ingber and G. M. Whitesides, *J. Am. Chem. Soc.*, 1998, **120**, 6548-6555.
5. J. Barbet, P. Machy, A. Truneh and L. D. Leserman, *Biochim. Biophys. Acta*, 1984, **772**, 347-356.