

Thiol-yne 'Click' Chemistry as a Route to Functional Lipid Mimetics

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Supplemental Information

Materials. Dimethylformamide (DMF, Alfa Aesar) and tetrahydrofuran (THF, Fisher) were purified over molecular sieves and stored under nitrogen till use. H-Glu(oBzl)-OH and H-Lys(Z)-OH were purchased from EMD Novabiochem and their N-carboxyanhydrides (NCA) were synthesized by method described below. Triphosgene (Acros), dodecylthiol (Sigma Aldrich) Propargyl amine (Acros), trifluoroacetic acid (Acros) and HBr in acetic acid (30 wt%, Acros) were used without purification. 1-[4-(2-Hydroxyethoxy)-phenyl]-2-hydroxy-2-methyl-1-propane-1-one (Irgacure 2959) were purchased from Ciba Specialty Chemicals. All other solvents were obtained from Fisher or Aldrich and used without further purification.

Synthesis.

Synthesis of NCA. The synthesis of Glu(oBzl)-NCA and Lys(Z)-NCA was performed using a method described previously.¹⁻³ Briefly, H-Glu(oBzl)-OH (10 g, 42.2 mmol) was introduced into a flame dried flask and dissolved into THF to a final concentration ca. 0.1 g/ml. The slurry was stirred at 55-60 °C for 20 minutes under nitrogen. Triphosgene (5.12 gm, 17.25 mmol), dissolved in 10 ml of THF was introduced into the H-Glu(oBzl)-OH slurry under a steady flow of nitrogen and a drying tube was attached to the reactor. After about one hour, the reaction became clear. The reaction mixture was concentrated and precipitated into hexanes overnight. The precipitate was then redissolved in THF, and 1 g of decolorizing charcoal was added and stirred overnight to eliminate residual hydrochloric acid. The reaction mixture was

then passed through a celite column to remove charcoal, concentrated, and reprecipitated into hexanes overnight at low temperature. The white powder formed is then dried under vacuum overnight to obtain Glu(oBzl)-NCA. Typical yields were between 85 – 92%. Synthesis of (Lys(Z)-NCA) was performed using the same procedure.

Synthesis of acetylene terminated PBLG. Propargylamine (5.3 μ l, 0.082 mmol) was added to a suspension of Glu(oBzl)-NCA (5gm, 16.34 mmol) in DMF at 25 $^{\circ}$ C. The reaction was stirred under argon for 5 days. The reaction mixture was then precipitated into ether and dried under vacuum at room temperature for 24 hours.

Synthesis of 2,3-bis-dodecylsulfanyl-propylamine via thiol-alkyne click reaction. The thiol-yne click reaction between propargylamine (1 eq.) and dodecanethiol (2 eq.) was carried out in presence of photo initiator, 1-[4-(2-Hydroxyethoxy)-phenyl]-2-hydroxy-2-methyl-1-propane-1-one (Irgacure 2959, 1 wt% of combined amine and thiol) under UV light (365 nm) for 30 minutes at atmospheric conditions.

Synthesis of DDT₂-PBLG and DDT₂-P(Z-Lys) using the divergent approach. Divergent synthesis was carried out by the ring opening polymerization of Glu(oBzl)-NCA using 2,3-bis-dodecylsulfanyl-propylamine as a macroinitiator in DMF for 5 days at room temperature. The polymer was precipitated in ether, redissolved in chloroform and reprecipitated in cold ether to remove any unreacted dodecanethiol and photo initiator from the click reaction. DDT₂-P(Lys(Z))-NCA was synthesized similarly, using Lys(Z) NCAs.

Synthesis of DDT₂-PBLG using the convergent approach. A solution of PBLG (1 eq. in THF, 8 wt %) was mixed with dodecane thiol (4 eq.) The thiol-yne click reaction was carried out in the presence of Irgacure 2959, 2 wt% of combined amount of PBLG and thiol) under UV light (365 nm) for 2 hrs at atmospheric conditions. The reaction mixture was then concentrated,

resuspended in chloroform and precipitated in cold ether to remove unreacted thiol and photoinitiator.

Deprotection chemistry. The benzyl glutamate and Z-lysine side chains were deprotected by stirring in TFA or 30 wt% HBr in glacial acetic acid respectively for two hours at room temperature. The resulting slurry was then diluted with deionized water and neutralized with 6 M NaOH and dialyzed against water using 3500 MW cut-off membrane for 24 hours. In case of lysine, the mixture was further acidified by adding a small amount of 6 M HCl and dialyzing against water for a day to replace bromide with chloride as the counter ion. Aqueous solutions were finally adjusted to pH = 6 using HCl or NaOH.

Characterization.

^1H and ^{13}C NMR spectra were recorded on Varian Mercury 300 (300 MHz) spectrometer using $\text{d}_6\text{-DMSO}$ as a solvent. Variable-angle DLS and SLS measurements were made using incident light at 633 nm from a Spectra Physics Model 127 HeNe laser operating at 40 mW. The angular dependence of the autocorrelation functions was measured using a Brookhaven Instruments BI-200SM goniometer with an avalanche photodiode detector and TurboCorr correlator. Correlation functions were analyzed according to the method of cumulants using the companion software. Real time FTIR was recorded on Bruker 88 spectrometer. Typical sample thickness was around 200 microns and the light intensity of the high pressure mercury lamp delivered to the sample via light pipe was $\sim 20 \text{ mW/cm}^2$. Molecular weight distributions of the polymers synthesized were determined using size exclusion chromatography (SEC) with a Viscotek TDA 302, fitted with two low molecular weight columns connected in series. TEM images were taken on a JEOL JEM-2100 transmission electron microscope. Detailed protocols and results from these techniques are given below.

NMR. ¹H NMR spectra were recorded on Varian Mercury 300 (300 MHz) spectrometer using d₆-DMSO as a solvent. The NMR and peak assignments for the divergent synthesis of DDT₂-PBLG is given in Figure S1 below.

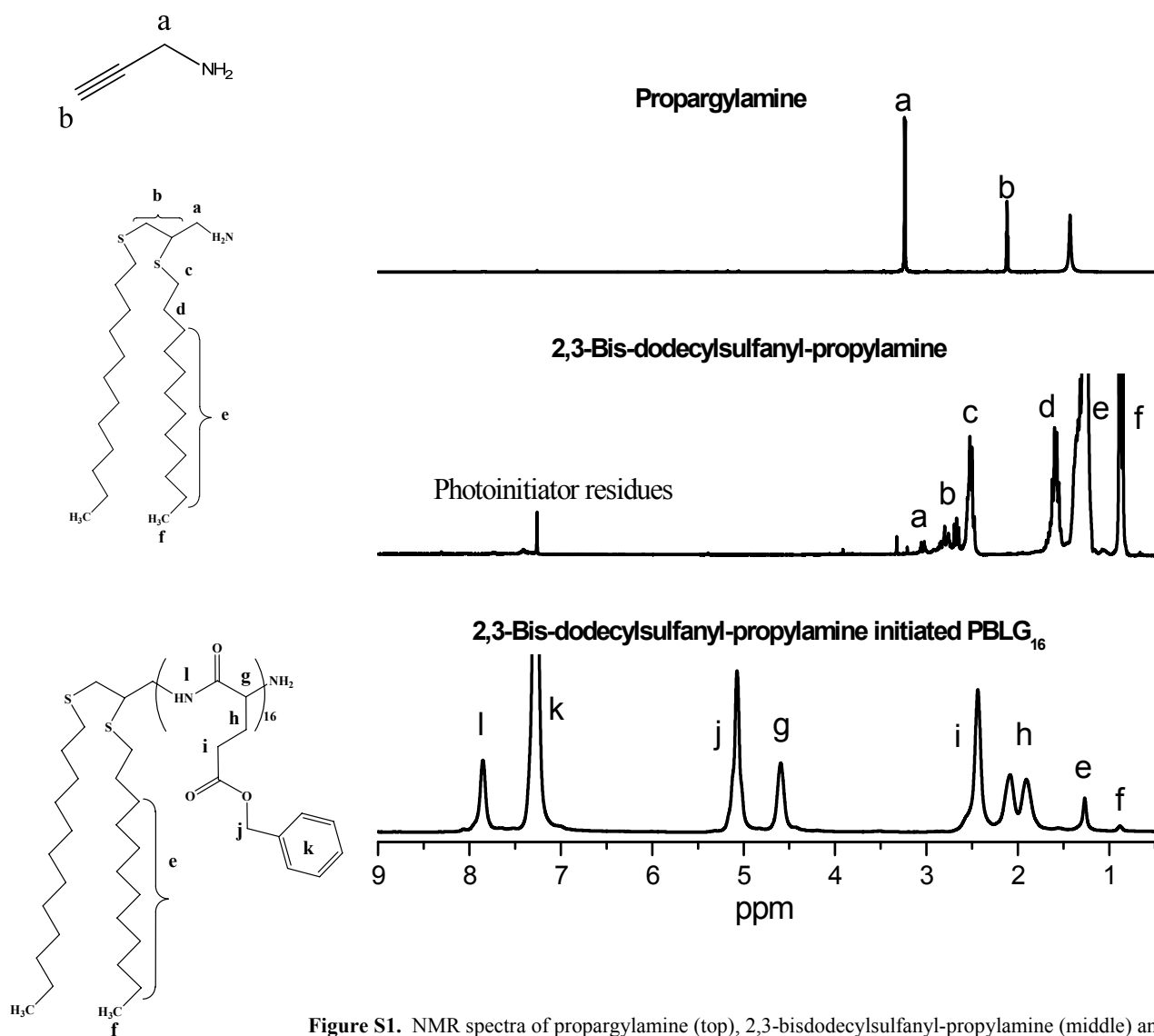


Figure S1. NMR spectra of propargylamine (top), 2,3-bisdodecylsulfanyl-propylamine (middle) and DDT₂-PBLG product (bottom.)

The first step in the divergent synthesis is the thiol-yne reaction with propargylamine. From the NMR spectra, it is clear that the addition was successful due to the presence of aliphatic chains in the 1-2 ppm region. In the second step of the scheme, the PBLG chain is grown from the DDT₂-NH₂ macroinitiator. The NMR confirms the presence of the phenyl protecting groups in the region around 7-7.5 ppm, and a suppression of the aliphatic chains in intensity relative to the PBLG peaks. The degree of polymerization for PBLG was calculated using end group analysis by comparing the integration of d,e and f with k.

The NMR and peak assignments for the convergent synthesis of DDT₂-PBLG is given in Figure S2 below.

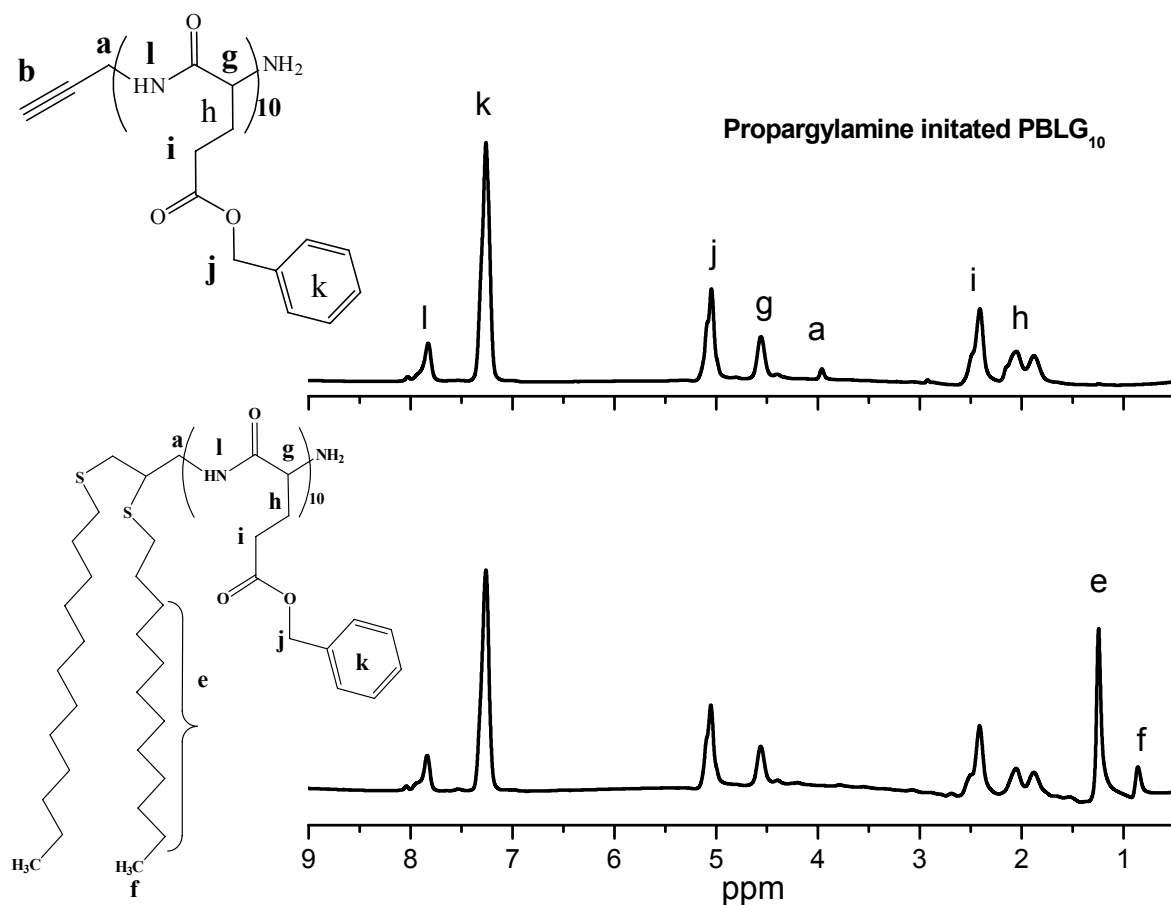


Figure S2. NMR spectra of propargylamine-initiated PBLG (top) and DDT₂-PBLG product (bottom.)

The first step in the convergent synthesis is the polymerization of PBLG from propargylamine. From the NMR spectra, it is clear that we are forming the polymer due to the presence of benzyl peaks in the region 7-7.5 ppm. At this point, the degree of polymerization is calculated using end group analysis by comparing the integrations a and j.

The ¹³C NMR for the divergent and convergent syntheses are shown in Figure S3 below. For the divergent synthesis (left), the disappearance of PA shifts and the presence of shifts at 39.5, 43.9 and 49.3 ppm indicates formation of bis-addition product. The absence of shifts at 128.6, 116.8, 109.6 and 151.6 indicates there is no formation of mono-addition adduct. For the convergent synthesis (right) the peaks due to terminal acetylene group are small compared to

main chain carbons of PBLG. The disappearance of peak at 70.3 ppm and appearance of peak at 44.7 and 41 indicates formation of bis-addition product from thiol-yne reaction. It is difficult to directly probe if mono-addition product is formed because shifts from aromatic group and TFA are centered around 116 and 128 ppm, but there are no peaks at 151 ppm where the mono-addition product would be expected.

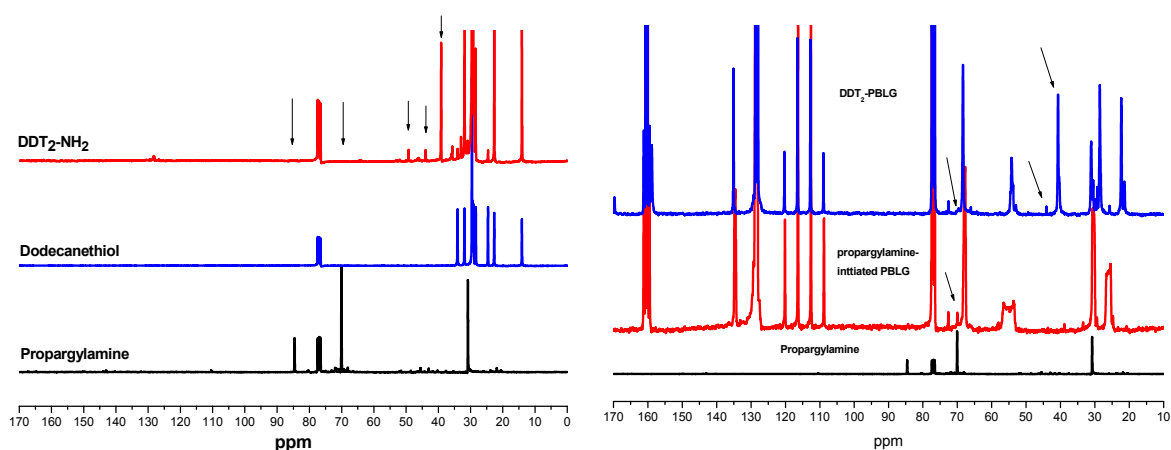


Figure S3. ¹³C NMR spectra for divergent (left) and convergent (right) syntheses. For the convergent synthesis, the resonances in the region 170-90 ppm correspond to the PBLG chain. The relevant resonances for comparison between species are indicated with arrows.

Dynamic and static light scattering:

Variable-angle DLS and SLS measurements were made using incident light at 633 nm from a Spectra Physics Model 127 HeNe laser operating at 40 mW. The angular dependence of the autocorrelation functions was measured using a Brookhaven Instruments BI-200SM goniometer with an avalanche photodiode detector and TurboCorr correlator. Correlation functions were analyzed according to the method of cumulants using the companion software. All data reported correspond to the average decay rate obtained from the second cumulant fit. Apparent diffusion coefficients (D_{app}) were obtained from the slope of the relaxation frequency (Γ) versus q^2 where

$$q = \frac{4\pi n}{\lambda} \sin\left(\frac{\theta}{2}\right), \quad (1)$$

λ is the wavelength of the incident laser (633 nm), θ is the scattering angle, and n is the refractive index of the media (Figure X in manuscript). The hydrodynamic radius (R_h) was then calculated from the Stokes-Einstein equation (Eq. 2)

$$R_h = \frac{k_B T}{6\pi\eta D_{app}} \quad (2)$$

where k_B is the Boltzmann constant, T is the temperature, and η is the viscosity of the medium.

Angular-dependent static light scattering (SLS) experiments were performed on aqueous polymer solutions with the same instrument as described above. The radius of gyration (R_g) of the assemblies was determined from the angular dependence of the scattering intensity. A Zimm plot of the inverse scattering intensity (I_{ex}) versus the square of the scattering vector (q^2) was used to determine the radius of gyration (Figure S4).

Solutions were prepared by dissolving deprotected polymers into deionized water (0.01 wt. % solution), adjusting the pH to 6 and sonicating for about an hour. Samples were agitated to ensure complete dissolution and then filtered through a 0.45 μ m PVDF syringe-driven filter (Millipore) directly into the scattering cell. Samples were allowed to reach thermal equilibrium prior to measurements.

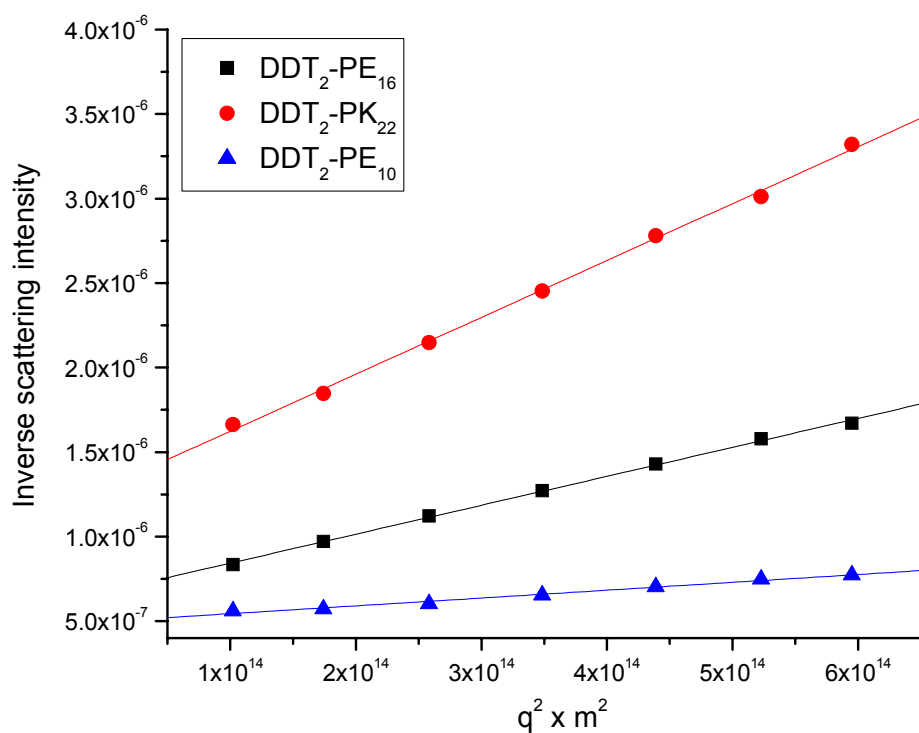


Figure S4. Static light scattering data for A_2B vesicles. From the angular dependence of the scattering, and using Zimm analysis, we obtain R_g .

Kinetics of the thiol addition to propargylamine in the divergent synthesis of DDT₂-PBLG.

Real time FTIR was recorded on Bruker 88 spectrometer. Typical sample thickness was around 200 microns and the light intensity of the high pressure mercury lamp delivered to the sample via light pipe was $\sim 20 \text{ mW/cm}^2$. Kinetics of the addition of DDT to propargylamine were measured using FTIR by tracking the disappearance of peaks related to thiol (2570 cm^{-1}) and alkyne (2120 cm^{-1} & 3300 cm^{-1}) moieties. Figure S5(A) shows the kinetics of PA with neat thiol. Figure S5(B) shows a 2:1 thiol to alkyne with a small amount of solvent and Figure 5(C) compares the 2:1 thiol to alkyne neat reaction with a 4:1 thiol to alkyne reaction containing a small amount of solvent.

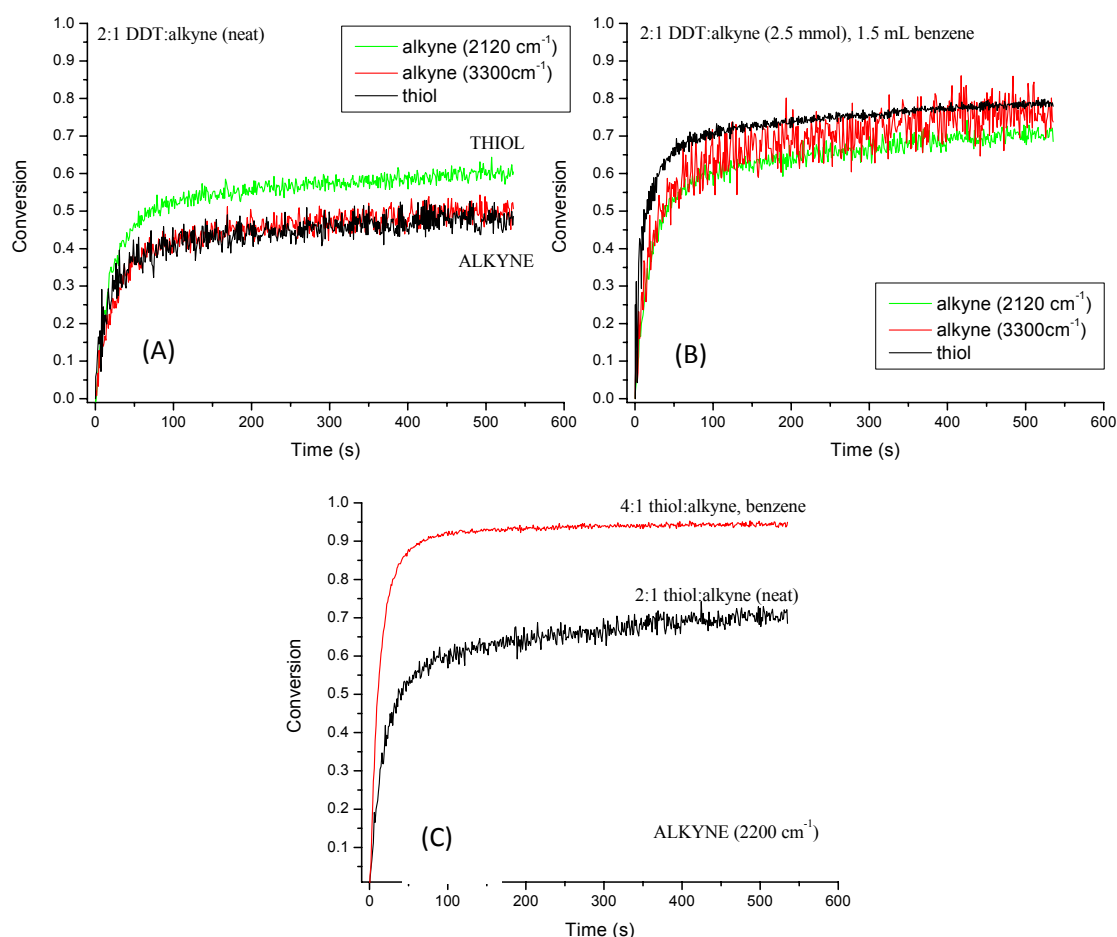


Figure S5. Real time FTIR kinetic data for the addition of DDT to propargylamine using click chemistry. (A) 2:1 thiol:alkyne (neat), (B) 2:1 thiol:alkyne with benzene and (C) comparison of 2:1 neat reaction with 4:1 thiol:alkyne in solvent. The 4:1 reaction reaches $\sim 95\%$ conversion in about 3 minutes.

GPC. Molecular weight distributions of the polymers synthesized were determined using size exclusion chromatography (SEC) with a Viscotek TDA 302, fitted with two low molecular weight columns connected in series. DMF with 0.02 M LiBr was used as eluent at a flow rate of 1 ml/min, operating at 35 °C. DMF GPC traces are shown in Figure S3 for DDT₂-P(Z-Lys)₂₂ and DDT₂-PBLG₁₆ synthesized from the divergent method.

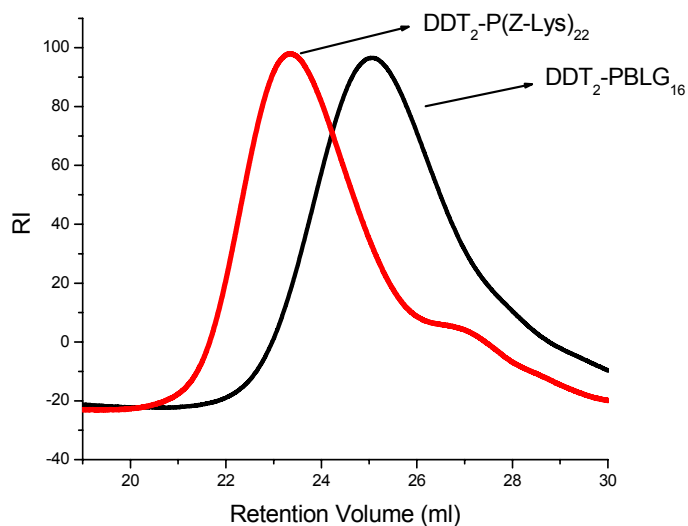


Figure S6. DMF GPC traces of two materials synthesized using the divergent approach. Polydispersities were on the order of 1.3-1.5, typical of NCA ring-opening polymerizations.

TEM. TEM images were taken on a JEOL JEM-2100 transmission electron microscope. The samples were spotted on SiO coated 300 mesh copper grids for 20 minutes followed by staining with 1 wt% aq. solution of phosphotungstic acid.

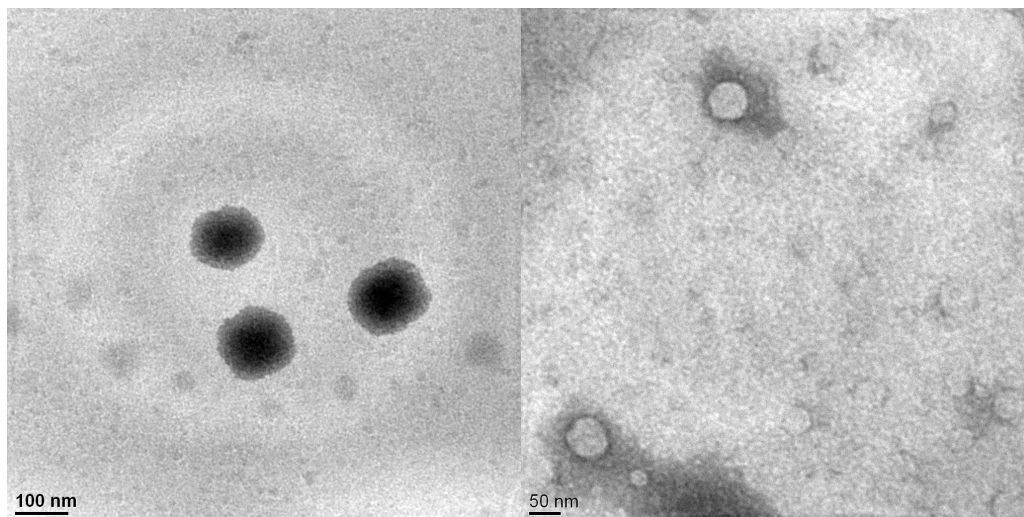


Figure S7. TEM images of DDT₂-PE₁₆ (left) and DDT₂-PK₂₂ (right). Samples were stained with phosphotungstic acid.

References:

- (1) Gebhardt, K. E.; Ahn, S.; Venkatachalam, G.; Savin, D. A. *Langmuir* **2007**, *23*, 2851-2856.
- (2) Gebhardt, K. E.; Ahn, S.; Venkatachalam, G.; Savin, D. A. *J. Col. Int. Sci.* **2008**, *317*, 70-76.
- (3) Naik, S. S.; Savin, D. A. *Macromolecules* **2009**, *42*, 7114-7121.