Electronic Supplementary Material (ESI) for RSC Advances.

Electronic Supplementary Information

Liposomes formed from photo-cleavable phospholipids: In-situ formation and photo-induced enhancement in permeability

Dawei Zhang[a,b], Zhenzhen Liu[a], Danielle Konetski[a], Chen Wang[a], Brady. T. Worrell[a], Christopher N. Bowman[*a]

[a]Department of Chemical and Biological Engineering, University of Colorado, UCB 596, Boulder, Colorado 80309, United States
[b] Materials Science and Engineering Program

*E-mail: christopher.bowman@colorado.edu

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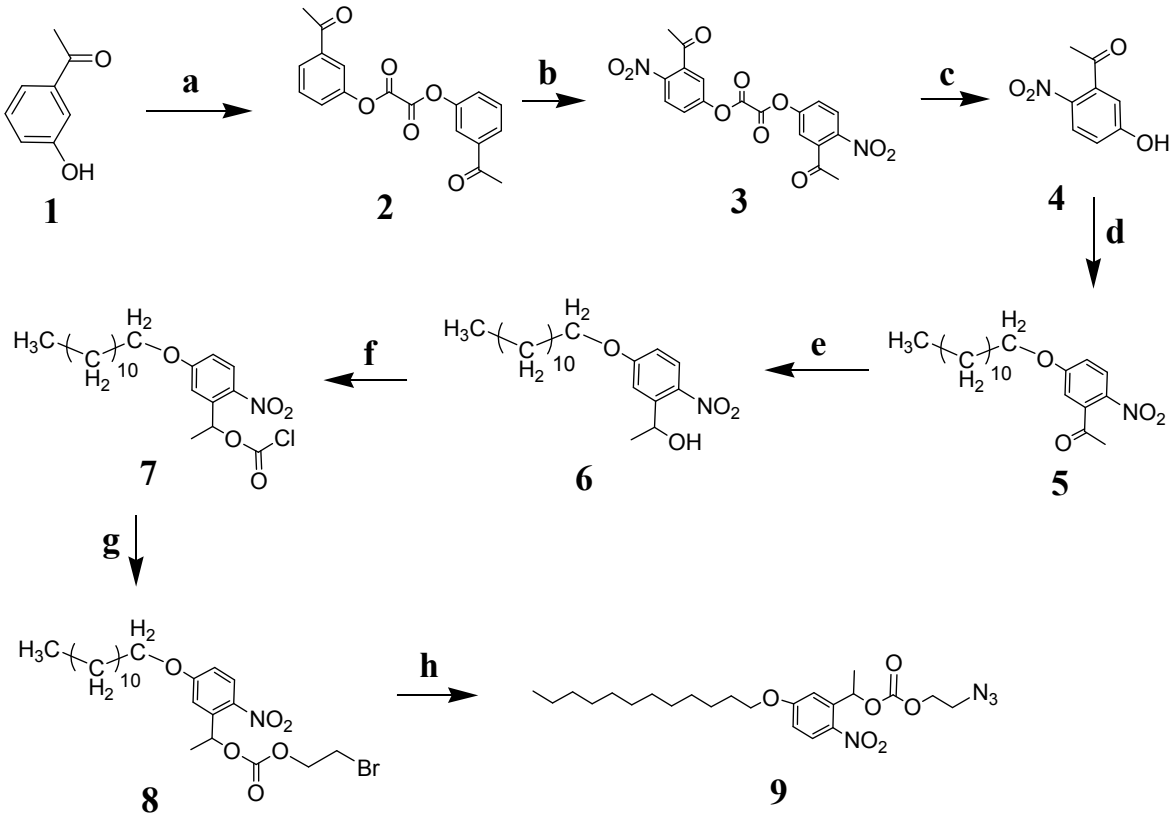
1.General Information

Unless otherwise noted, all reactions were run under air. All chemical reagents were obtained commercially without further purification. ^{1}H and ^{13}C NMR spectra were recorded on BrukerAvance-III 400 spectrometers. Fourier Transform Infrared (FTIR) spectra were recorded on a NIicolet 670 FT-IR spectrometer. Ultraviolet-Visible spectra were recorded on a UV-Vis spectrophotometer (Thermo-Fischer Scientific). Fluorescence excitation ratios were recorded on a QM-6 steady-state spectrofluorimeter (Photon Technology International). Fluorescence images were recorded on a Zeiss Axiovert 200m Widefield microscope equipped with a EMCCD Camera.

2.Experimental

2.1 Synthesis of 1-(5-dodecyloxy-2-nitrophenyl)ethyl 2-azidoethyl carbonate（NBN_3）



Stepa-c: From compound 1 to compound 4, synthesis followed literature[1].

Step d:5-dodecyloxy-2-nitroacetophenone (5)
Compound **4**(2g, 11mmol) was dissolved in DMF(20mL), and potassium carbonate (5g, 36mmol) was added. Bromododecane (8.5g, 28.7mmol) was added dropwise to the DMF solution. The temperature was increased to 90℃ with oil-bath. The mixture was stirred for 24 hours before evaporation of DMF. The residual solid was dissolved in ethyl acetate, and washed with HCl

(1mol/L), $NaHCO_3$ (1mol/L), water and brine. Organic phase was dried with anhydrous Na_2SO_4. After evaporation of ethyl acetate and purification *via* column chromatography using hexane:ethyl acetate (10:1), pure product is viscous yellow oil (3.5g, 10mmol, 92%).^{1}H NMR (400MHz $CDCl_3$): δ 8.13-8.11 (d, 1H), 6.97-6.94 (m, 1H), 6.76-6.74 (d, 1H), 4.05-4.02 (t, 2H), 2.51 (s, 3H), 1.83-1.76 (m, 2H), 1.46-1.41 (m, 2H), 1.35-1.23 (m, 16H), 0.88-0.85 (t, 3H).

Step e:α-methyl-2-nitro-5-(dodecyloxy)-benzenemethanol (6)

Compound **5**(3.5g, 10mmol) was dissolved in methanol (100mL) at 0℃ with an ice-bath. Sodium borohydride (3.8g, 100mmol) was added in batches. After temperature increased to room-temperature, the reaction mixture was stirred for 24 hours. Methanol was evaporated and the residual was dissolved in dichloromethane, then washed with HCl (1mol/L), $NaHCO_3$ (1mol/L), water and brine. Organic phase was dried with anhydrous Na_2SO_4. After evaporation of solvent, crude product was purified by column chromatography using hexane: ethyl acetate (10:1), obtaining transparent viscous oil (3.4g, 9.7mmol, 97%).^{1}H NMR (400MHz $CDCl_3$): δ 8.04-8.02 (d, 1H), 7.31-7.3 (d, 1H), 6.85-6.82 (m, 1H), 5.58-5.52 (m, 1H), 4.07-4.03 (t, 2H), 2.41-2.4 (d, OH), 1.84-1.77 (m, 2H), 1.56-1.54 (d, 3H), 1.48-1.42 (m, 2H), 1.27-1.26, (m, 16H), 0.89-0.86 (t, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 163.64, 144.74, 140.23, 127.63, 113.38, 112.48, 68.8, 65.97, 31.92, 29.66, 29.64, 29.59, 29.55, 29.36, 29.33, 29.01, 25.93, 24.1, 22.7, 14.14.

Step f, g, h:1-(5-dodecyloxy-2-nitrophenyl)ethyl 2-azidoethyl carbonate (9)

Compound **6** (1g, 2.85mmol) and triphosgene (1.7g, 5.72mmol) were dissolved in dichloromethane. Triethylamine (0.606g, 6mmol) was slowly added into the mixture at 0℃ with an ice-bath. After stirring for 12 hours, the reaction solution was dried using N2-stream, exhaust was absorbed by NaOH solution (5mmol/L), then the residual was further dried in vacuum at room temperature to get crude compound 1-(5-dodecyloxy-2-nitrophenyl)ethylchloroformate (**7**). To a dichloromethane solution of 2-bromoethanol (0.4g, 3.2mmol) and diisopropylethylamine (0.45g, 3.48mmol) was added dropwise dichloromethane solution of crude compound **7** at 0℃. The mixture was stirred for 24 hours after recovering to room temperature. Reaction solution was washed with $NaHCO_3$ (1mol/L), HCl (1mol/L), water and brine. Organic phase was dried with anhydrous Na_2SO_4. After evaporation of dichloromethane, column chromatography using hexane: ethyl acetate (10:1) gave the pure compound as a light yellow viscous oil (1.1g, 2.2mmol, 77%).Compound **8** (0.3g, 0.6mmol) and Sodium azide (0.78g, 12mmol) were dissolved in DMF (5mL), and stirred for 24 hours. After evaporation of DMF, the residual was dissolved in dichloromethane (40mL), then washed with water (20mL×3). Organic phase was dried with anhydrous Na_2SO_4. Compound **9** was obtained after filtering and evaporation of the solvent (0.273g, 0.587mmol, 98%). ^{1}H NMR (400MHz $CDCl_3$): δ 8.09-8.07 (d, 1H), 7.12-7.11 (d, 1H), 6.88-6.85 (m, 1H), 6.44-6.39 (q, 1H), 4.26-4.24 (m, 2H), 4.05-4.01 (t, 2H), 3.53-3.49 (m, 2H), 1.84-1.77 (m, 2H), 1.68-1.66 (d, 3H), 1.47-1.42 (m, 2H), 1.34-1.26, (m, 16H), 0.89-0.86 (t, 3H). ^{13}C NMR (100MHz CDCl3): δ163.69, 153.78, 140.77, 139.93, 127.66, 113.67, 112.08, 72.62, 68.92, 66.23, 49.61, 31.92, 29.66, 29.63, 29.58, 29.54, 29.35, 29.34, 28.98, 25.91, 22.7, 22.11, 14.13. [M] calculated: 464.2; found: 487.7[M+Na^+].

2.3 Synthesis of alkyne-lysolipid

1-(3-Dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride(EDAC.HCl, 310mg, 1.616mmol) was dissolved in dichloromethane, and triethylamine(163mg, 1.616mmol) was added. 4-pentynoic acid(158mg, 1.616mmol) and 4-dimethylaminopyridine(5mg, 0.04mmol) were added with stirring. The mixture was stirred for 20 minutes, and hydroxy-lysolipid(200mg, 0.404mmol) was added. After stirring for 12 hours, dichloromethane was completely removed under low pressure, and remaining slurry compound was dissolved into water and transferred into dialysis bag(cutting off MW=2000).

After dialysis against water for 24 hours(outside water was changed every 6 hours), the inside aqueous solution was frozen and lyophilized to remove water. The product was a white solid. ^{1}H NMR (400MHz CDCl3/MeOD=4:1): δ 5.26 (m, 1H), 4.43-4.31 (m, 3H), 4.18-4.13 (q, 1H), 3.99-3.96 (t, 2H), 3.82-3.80 (t, 2H), 3.36 (s, 9H), 2.61-2.47 (m, 4H), 2.32-2.28 (t, 2H), 2.14 (s, 1H), 1.61-1.57 (t, 2H), 1.27 (s, 26H), 0.91-0.88 (t, 3H). ^{13}C NMR (100MHz CDCl3): δ 173.60, 171.31, 82.45, 69.56, 62.71, 54.49, 34.10, 33.31, 31.94, 29.73, 29.71, 29.68, 29.55, 29.39, 29.34, 29.18, 24.88, 22.71, 14.31, 14.16. [M] calculated: 575.7; found: 575.6[M].

2.3Synthesis of TNBPC

Alkyne lysolipid and compound **9** (**NBN_3**) were dissolved in DMF/H_2O (1:1 v/v). $CuSO_4$ and sodium ascorbate were added. The reaction vial was sealed and stirred under N_2-stream overnight. DMF was evaporated and H_2O was lyophilized, and residual was purified using silica column chromatography (chloroform: methanol: H_2O = 5:1:0.1).^{1}H NMR (400MHz CDCl3): δ 8.05-8.02 (m, 1H), 7.63 (s, 1H), 7.06-7.05 (d, 1H), 6.89-6.86 (m, 1H), 6.33-6.28 (q, 1H), 5.23 (m, 1H), 4.65-4.46 (m, 4H), 4.33-4.26 (m, 3H), 4.15-4.02 (m, 4H), 3.76-3.72 (m, 2H), 3.31 (s, 9H), 3.03-3 (m, 2H), 2.73-2.71 (m, 2H), 2.29-2.25 (t, 2H), 1.83-1.79 (m, 2H), 1.65-1.54 (d, 3H), 1.58-1.54 (m, 2H), 1.48-1.44 (m, 2H), 1.26-1.22 (m, 38H), 0.89-0.85 (t, 6H). ^{13}C NMR (100MHz CDCl3): δ172.78, 163.79, 153.65, 140.19, 127.26, 113.8, 112.65, 72.23, 68.86, 66.03, 62.67, 54.83, 48.63, 34.02, 31.8, 29.55, 28.97, 26.18, 25.03, 22.82, 21.67, 21.13, 14.36. [M] calculated: 1039.6; found: 1040.5[$M+H^+$].

2.4 Synthesis of 1-(5-dodecyloxyphenyl)ethyl 2-azidoethyl carbonate (BN_3)—control group

Synthesis of **BN_3** began from step d, using compound **1** as starting material. The operations and treatment were similar with synthesis of **NBN_3**.

2.5 Infrared spectrum

Samples were dissolved in chloroform and a droplet was placed on the surface of a potassium bromide plate. After drying by N_2-stream, samples were tested.

2.6 UV-vis spectrum

Samples were dissolved in chloroform and placed into a 10mm×10mm quartz-cuvette. Spectra were recorded in the range of 200-600nm in absorbance mode with a bandwidth of 2nm, 240nm/min scanning speed.

2.7 In-situ preparation of liposomes

1-(5-dodecyloxy-2-nitrophenyl)ethyl 2-azidoethyl carbonate (NBN_3) (1.16mg, 2.5umol) and alkyne lysolipid (1.44mg, 2.5umol) were completely dispersed in water (500uL) by ultrasound and stirring. $CuSO_4$ (0.12mg, 0.75umol) and sodium ascorbate (0.5mg, 2.5umol) were added into the dispersion, and vortexed for 30 seconds, then the reaction vial was placed statically for 24 hours.

2.8 LC-MS monitoring of CuAAC coupling

CuAAC coupling was performed as described above. A 10uL sample was taken from the reaction system at different time points. After dilution with 50uL methanol, samples were analyzed by LC-MS coupled with an evaporative light scattering detector (ELSD). For all LC-MS runs, the flow-rate was 1mL/min and elution-phase consisted of HPLC-grade water with 0.1% formic acid and HPLC-grade methanol with 0.1% formic acid.

2.9 NMR monitoring of triazole-phospholipid formation and self-assembly of liposomes

CuAAC coupling and simultaneous assembly of liposomes were performed in deuterium oxide, increasing the concentration of the two precursors to 20mmol/L, and NMR-spectra were recorded before adding $CuSO_4$ and sodium ascorbate. After addition of catalysts, NMR-spectra were recorded at various time points. The NMR-scanning mode was water-suppression.

2.10 Microscopy-observation of liposome's in-situ formation

200uL precursor water-dispersion (AL&NBN_3 or AL&BN_3) with rhodamine DHPE (5umol/L) as the fluorescent dye were injected into a chamber on a glass slide and placed under fluorescence microscopy. Certain amounts of $CuSO_4$and sodium ascorbate aqueous solutions were injected into the chamber in sequence. Fluorescence images were recorded every 30s at various points in the scope.

2.11 Microscopy-observation of liposome's change under UV-irradiation

10uL of liposome dispersion (TBNPC-or TBPC-liposomes) were dropped onto a slide and covered by glass-slip, half of which was totally shielded by foil-paper. On the microscope, two points were selected separately in the covered and uncovered areas. After turning on 365nm-light, fluorescence images were recorded in sequence every 1 minute at the selected points.

2.12Ratiometric fluorescence assay[2, 3]

Preparation of liposomes loading HPTS Precursors were dispersed in HEPES buffer (10mM, pH=7.04) containing 1mM HPTS as well as $CuSO_4$ and sodium ascorbate to trigger CuAAC coupling and liposome assembly. After 20 hours, extraliposomal components were removed by size exclusive chromatography (Sepharose 4B, Sigma-Aldrich) with 10mM HEPES, pH=7.04. The eluted fraction containing HPTS-loaded liposomes was collected and used within 48 hours.

Measurement of membrane permeability with HPTS assay 50uL of liposomes loaded with HPTSwas added to 1900uL HEPES buffer in a fluorescence cuvette (10mm×10mm)with gentle vibration. After measurements of excitation-ratio R (λ_{em}=510nm, λ_{ex}=410nm, λ_{ex}=453nm, R = $(I_{410ex}/I_{453ex})_{510em}$) were collected 5 times over 8 minutes, 10uL NaOH (0.5M) was added into the cuvette with gentle vibration. Cuvettes were irradiated with 320-390nm UV-light and measured at fixed time points. After 80-minutes of consistent measurements, samples were sonicated for 20 minutes, then the final excitation-ratio was taken. The permeability increase at each time point was calculated using the equation:

$$PERMEABILITY\ INCREASE = \frac{R_t - R_0}{R_\infty - R_0} \times 100\%$$

where R_0 = average R before NaOH addition, R_∞ = R after 20-minutes of sonication, R_t = R at certain time point.

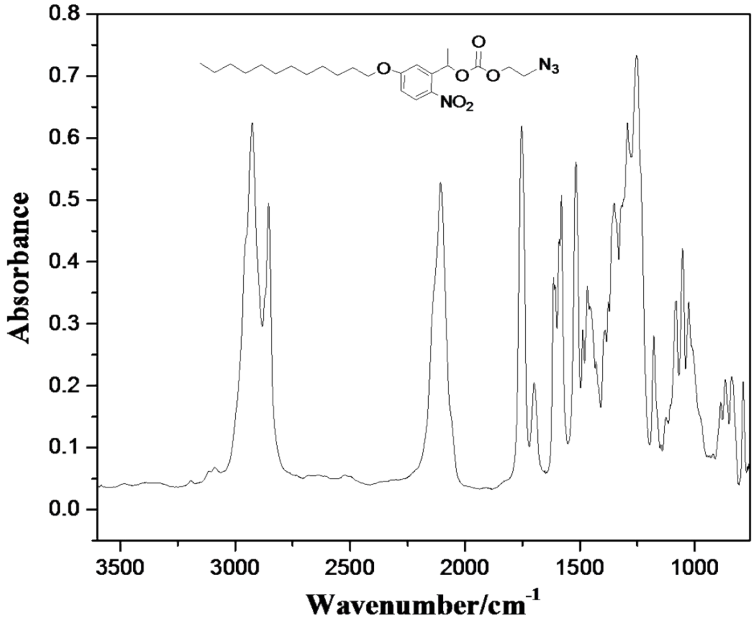


Fig. S1 Infrared spectrum of NBN_3

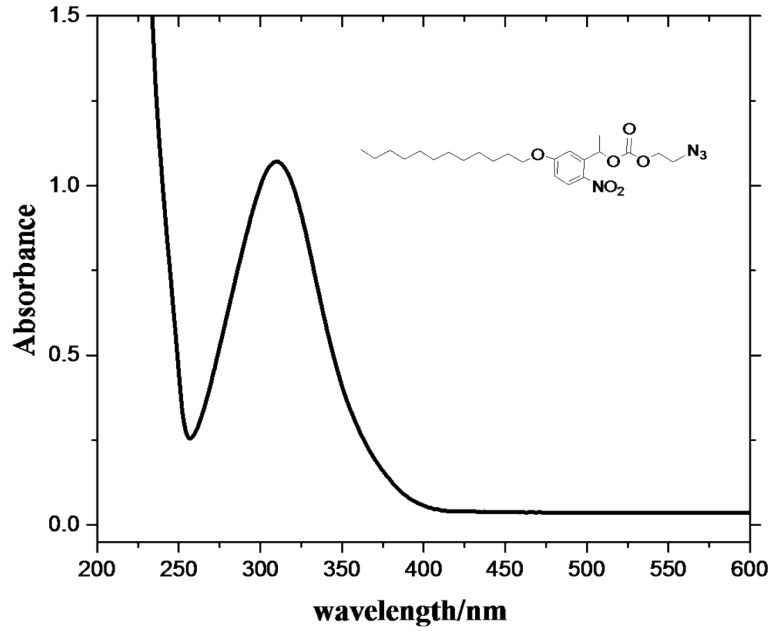


Fig. S2 UV-vis spectrum of NBN_3

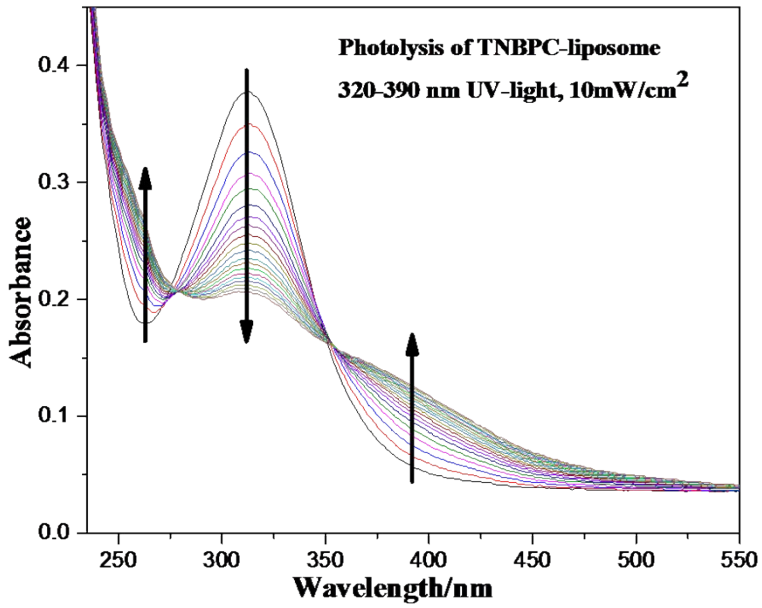


Fig. S3 TNBPC liposome UV-vis spectrumchange under irradiation

Ratiometric fluorescence assay data:

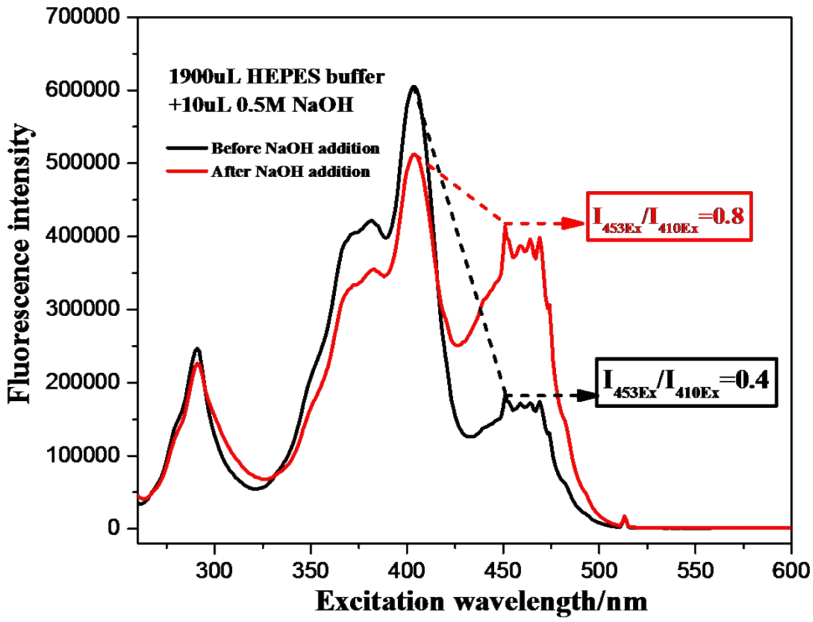


Fig. S4 Spectral change of HPTS in HEPES buffer; before and after NaOH addition

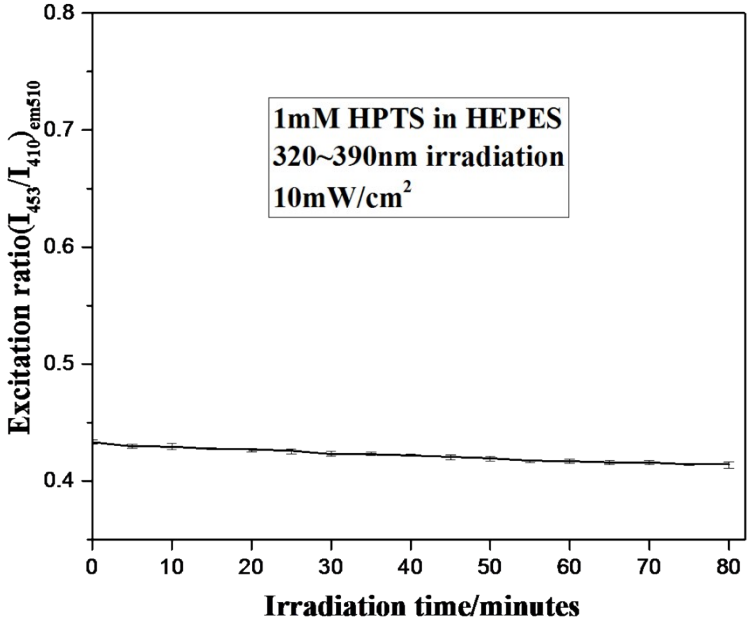


Fig. S5 Excitationratio change of HPTS and HEPES solution (1mM) during irradiation

Movie S1. Time lapse fluorescence microscopy of TNBPC liposome formation, using membrane staining dye (Rh-DHPE, 5μM).

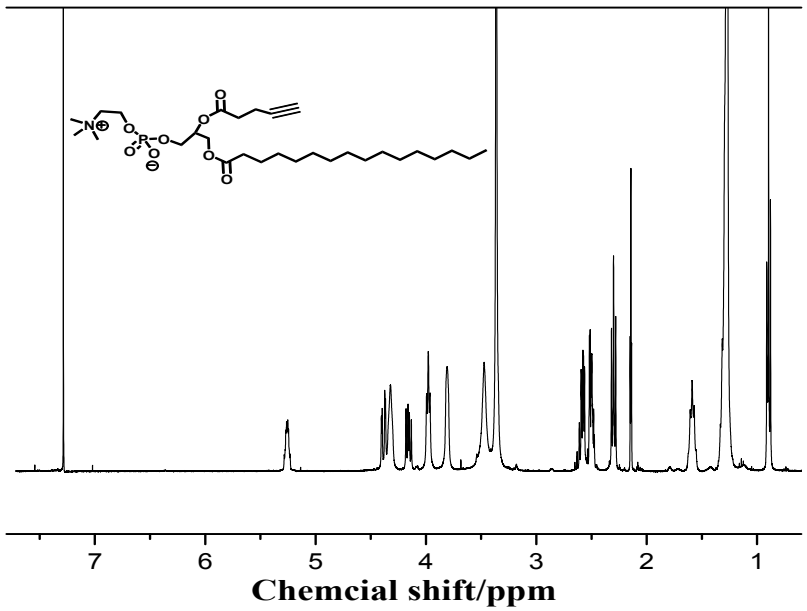
Movie S2. Time lapse fluorescence microscopy of TBPC liposome formation, using membrane staining dye (Rh-DHPE, 5μM).

Movie S3. Time lapse fluorescence microscopy of TNBPC liposome disruption induced by 365nm UV-light (10mW/cm^2), using membrane staining dye (Rh-DHPE, 5μM).

Movie S4. Time lapse fluorescence microscopy of TNBPC liposomes where no UV-light was applied, using membrane staining dye (Rh-DHPE, 5μM).

Movie S5. Time lapse fluorescence microscopy depicting fusion of a TBPC liposome cluster, induced by 365nm UV-light (10mW/cm^2), using membrane staining dye (Rh-DHPE, 5μM).

NMR data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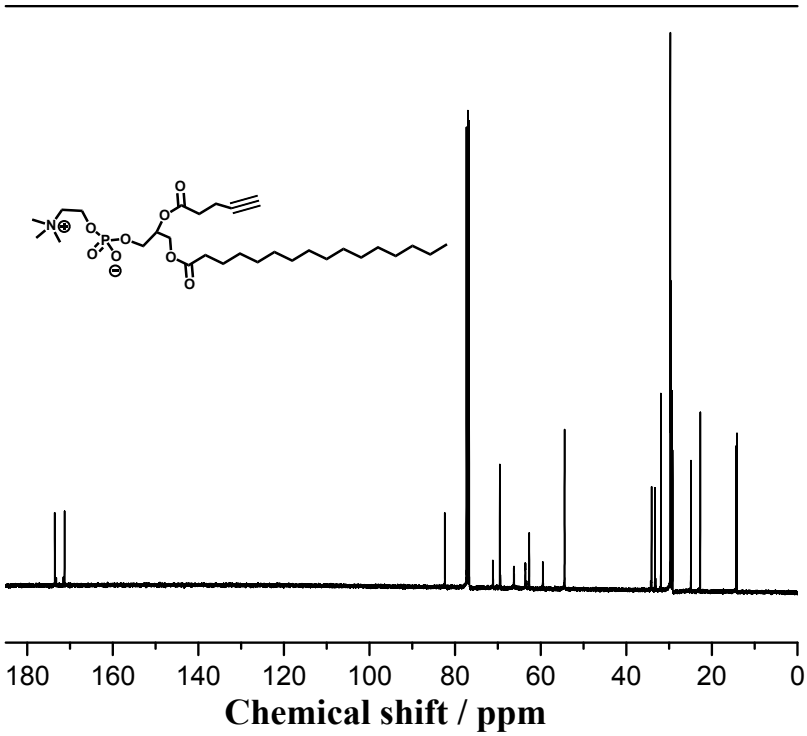
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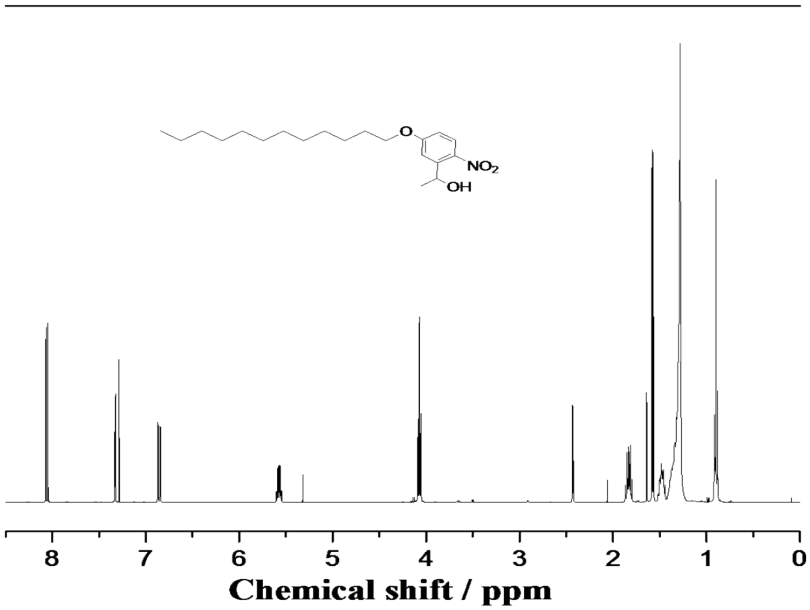
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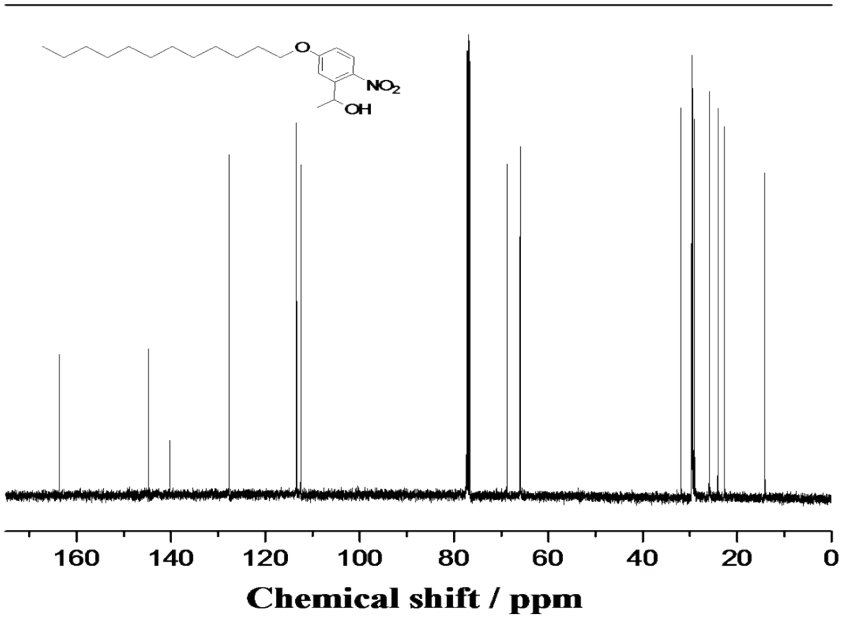
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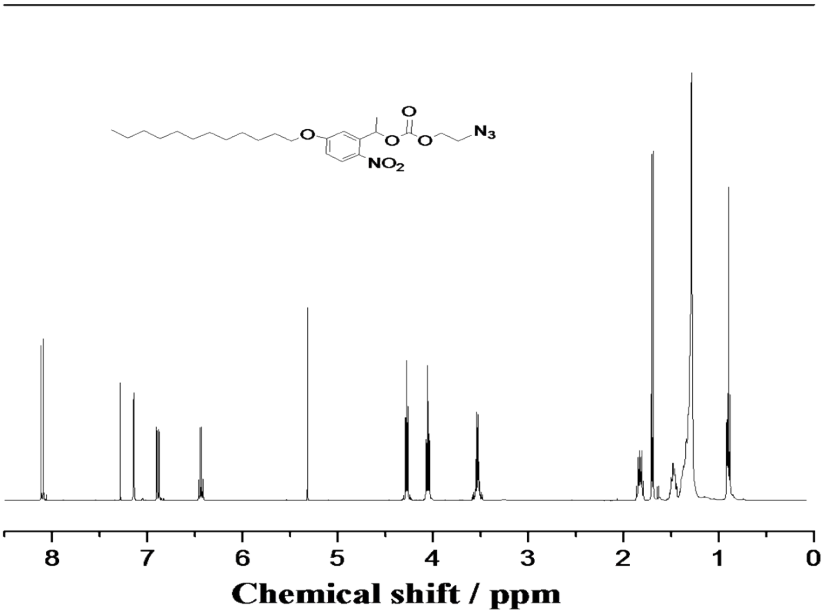
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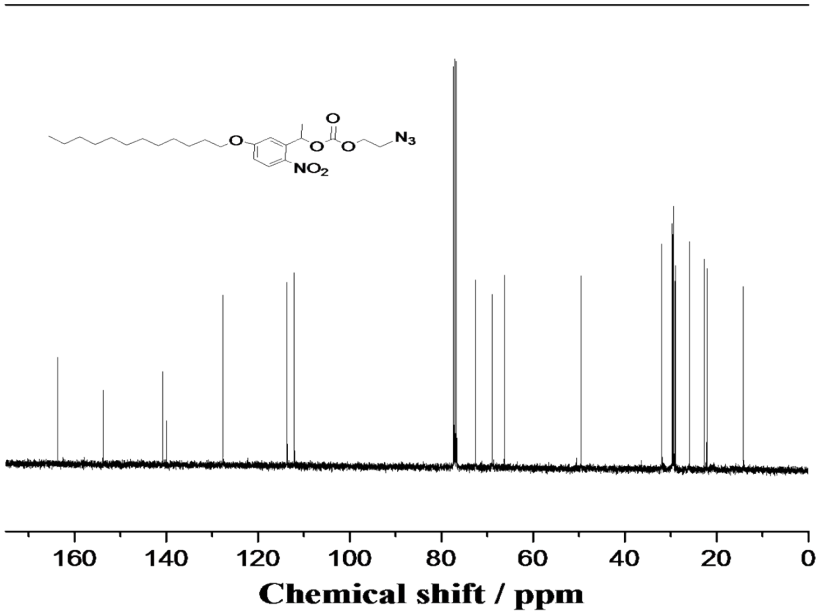
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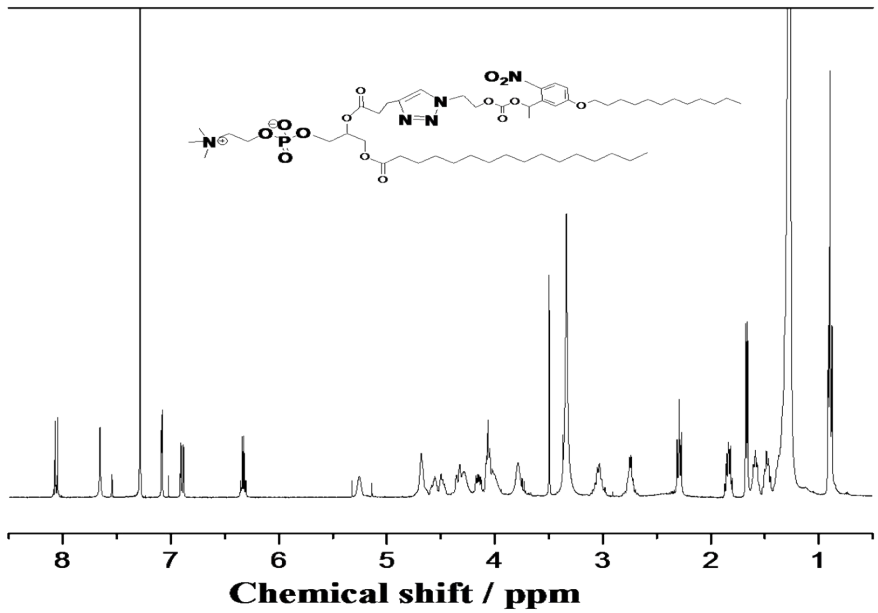
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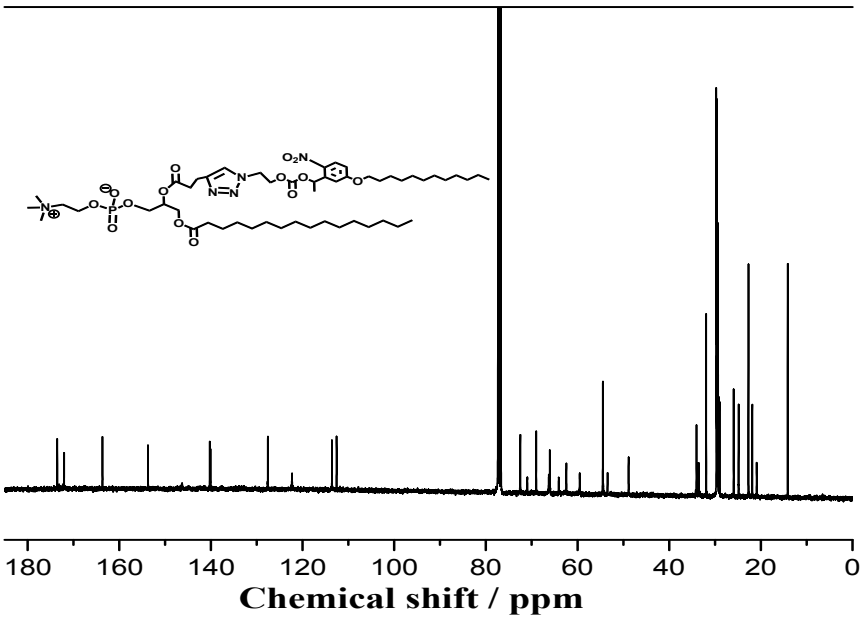
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Reference:

1. D. R. Griffin and A. M. Kasko, *J. Am. Chem. Soc*, 2012, **134**, 13103-13107.
2. A. Hennig, L. Fischer, G. Guichard and S. Matile, *J. Am. Chem. Soc*, 2009, **131**, 16889-16895.
3. T. Liu, C. Bao, H. Wang, L. Fei, R. Yang, Y. Long and L. Zhu, *New J. Chem.*, 2014, **38**, 3507-3513.