Electronic Supplementary Material (ESI) for ChemComm.

Supporting Information

Controlled Drug Release to Cancer Cells from Modular One-Photon Visible Light-Responsive Micellar System

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Contents

General Methods

All chemicals were purchased from Sigma-Aldrich (St. Louis, MO) unless otherwise specified. Nile Red was purchased from Acros Organics. Analytical thin-layer chromatography (TLC) was carried out on Merck silica gel 60 F_{254} glass plates and flash column chromatography was performed on Merck silica gel 60 (70 – 230 mesh). Calcium and magnesium free, Dulbecco's Phosphate Buffered Saline solution (DPBS) and 10× PBS were purchased from Thermo Fisher Scientific (Waltham, MA). Dulbecco's Modified Eagle's Medium (DMEM) was purchased from American Type Culture Collection (ATCC, Manassas, VA), and Fetal bovine serum (FBS) and penicillin/streptomycin were from Life Technologies (Carlsbad, CA). Corning Costar TC-treated, cell culture plates (24 well, clear flat bottom) and black microplates (96 well, clear flat bottom) were purchased from Fischer Scientific (Waltham, MA). For cell dissociation, TrypLE express reagent was used, and for cell viability assay, LIVE/DEAD Viability/Cytotoxicity Kit for mammalian cells from was used as received from Life Technologies (Carlsbad, CA). ^{1}H and ^{13}C solution-state NMR were recorded on a Varian VNMRS 600 (600 MHz for ^{1}H and 150 MHz for ^{13}C) spectrometer or Varian Inova-500 (500 MHz for ^{1}H, and 125 MHz for ^{13}C). Chemical shifts are reported relative to residual solvent peaks (δ 7.26 for $CDCl_3$ in ^{1}H NMR and δ 77.2 for $CDCl_3$ in ^{13}C NMR). IR spectra were recorded on a Perkin Elmer Spectrum 2 FT/IR or a Perkin Elmer Spectrum 100 employing a Universal ATR Sampling Accessory and are reported in terms of frequency of absorption (cm^{-1}). Mass spectral data were collected on a Micromass QTOF2 Quadrupole/Time-of Flight Tandem mass spectrometer (ESI-MS). Irradiations were performed with a Dolan-Jenner Fiber-Lite Model 190, using an EKZ halogen bulb (10.8V, 30 Watt, 3100K) via a Dolan-Jenner BGT1826 fiber optic gooseneck, on high output setting. UV-vis spectral data were collected on a Shimadzu UV-3600 UV-Vis-NIR Spectrophotometer with a quartz spectrasil UV-vis cuvette (1 x 1 x 4 cm) using direct detection at a slit width of 2 nm. Fluorescence measurements were obtained using a Cary Eclipse spectrophotometer (Varian, Inc.) with a quartz fluorescence cuvette (1 x 1 x 0.2 cm) with the following settings: excitation wavelength of 550 nm, excitation slit width = 5 nm, emission slit width = 10 nm, range = 560 nm to 700 nm and scan speed = 30 nm/min. Transmission electron microscopy (TEM) was performed on a FEI Tecnai G2 Sphera operating at 200kV. The sample was prepared at 250 μM and deposited onto a carbon-coated 300 mesh TEM grid for 30 seconds before being wicked away with filter paper.

Synthetic Methods

1,3-didodecylpyrimidine-2,4,6(1H,3H,5H)-trione (2) – To a solution of n-dedecyl amine (2.00 mL, 8.70 mmol) in 36 mL dichloromethane was added a solution of n-dodecyl isocyanate (2.10 mL, 8.70 mmol) in 36 mL dichloromethane under argon. The mixture was heated to reflux for 1 h. After malonyl chloride (0.85 mL, 8.70 mmol) was added, the mixture was heated at reflux for an additional hour. The mixture was allowed to cool to room temperature and was quenched with 1 N HCl (50 mL). The crude product was extracted with dichloromethane (3 x 50 mL) and the combined organic phases were washed with H_2O (1 x 30 mL), dried over $MgSO_4$ and filtered. Solvent was removed under vacuo. Purification by flash column chromatography (hexane:EtOAc = 9:1 to 4:1) gave 4.01 g (99% yield) of the product as yellow solid. Characterization of this compound matched that of the previously reported compound.[1]

1,3-didodecyl-5-(furan-2-ylmethylene)pyrimidine-2,4,6(1H,3H,5H)-trione – 1,3-Didodecylpyrimidine-2,4,6(1H,3H,5H)-trione (2.00 g, 4.3 mmol) in H_2O (8.6 mL) was treated with 2-furaldehyde (0.36 mL, 4.3 mmol). The mixture was stirred at room temperature for 21 h. The yellow waxy solid was extracted by dichloromethane (3 x 30 mL). Combined organic phases was dried over $MgSO_4$, filtered and the solvent was removed in vacuo to give 2.17 g of pale yellow solid in 93 % yield; ^{1}H NMR (500 MHz, Chloroform-*d*) δ 8.62 (d, *J* = 3.8 Hz, 1H), 8.42 (s, 1H), 7.83 (d, *J* = 1.5 Hz, 1H), 6.74 – 6.70 (m, 1H), 3.95 (ddd, *J* = 10.1, 5.8, 2.8 Hz, 4H), 1.67 – 1.58 (m, 4H), 1.36 – 1.23 (m, 36H), 0.87 (t, *J* = 6.9 Hz, 6H). ^{13}C NMR (125 MHz, $CDCl_3$) δ 162.4, 160.8, 151.4, 151.1, 150.3, 141.0, 127.9, 115.2, 112.1, 42.7, 41.9, 32.1, 29.9, 29.8, 29.8,

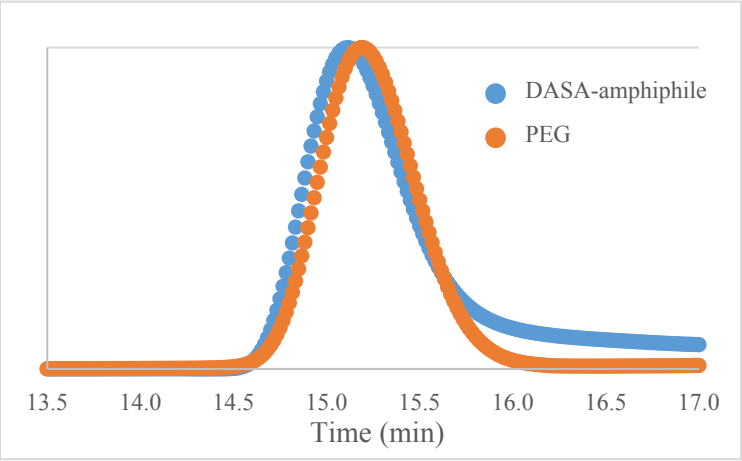
29.8, 29.8, 29.8, 29.6, 29.5, 29.5, 29.5, 28.3, 28.2, 27.1, 27.1, 22.9, 14.3. IR (ATR) 3145, 2917, 2850, 1723, 1655, 1575, 1466, 1448, 1407, 1385, 1358, 1299, 1255, 1199, 1164, 1128, 1095, 1029, 883, 792, 755, 711 cm^{-1}. HRMS (ESI+) *m/z* 565.3976 +Na (565.3981 calc'd for $C_{33}H_{54}N_2O_4Na^+$ [M + Na]$^+$))

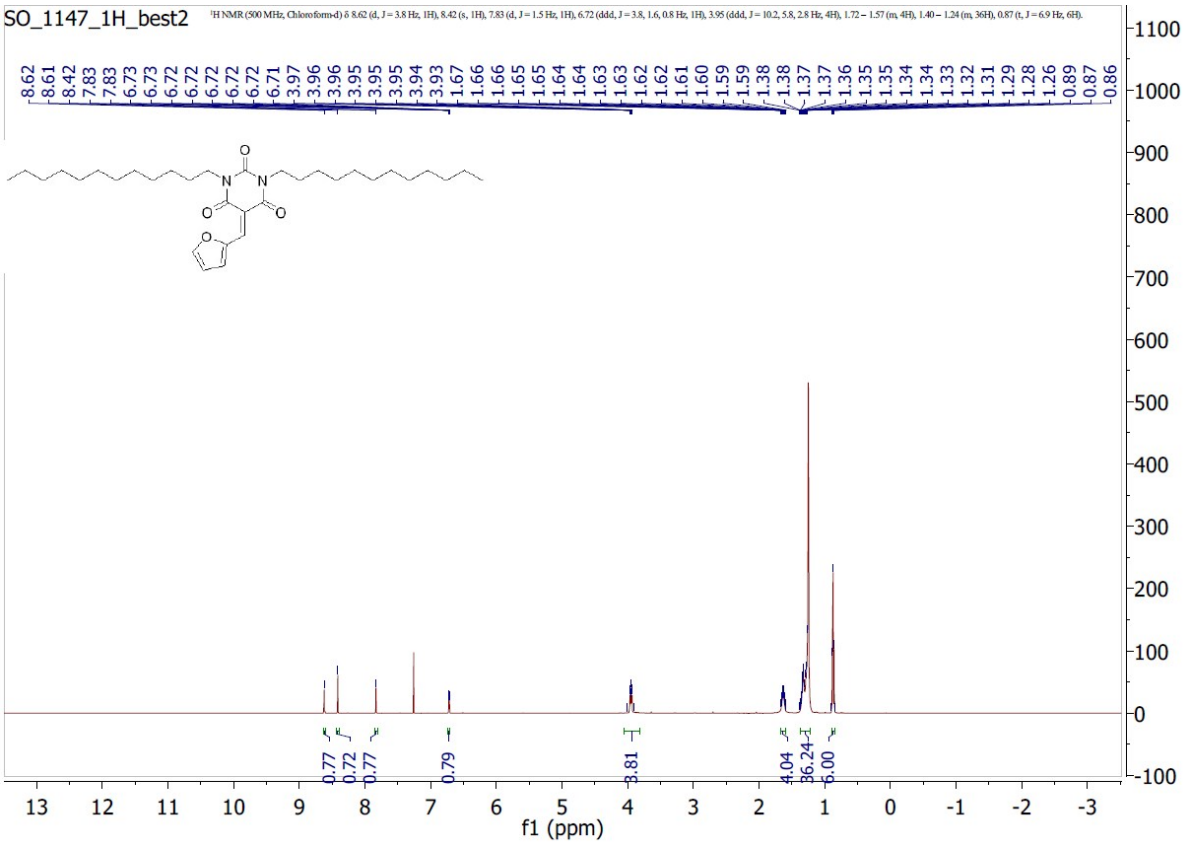
5-((2Z,4E)-5-((6-azidohexyl)(ethyl)amino)-2-hydroxypenta-2,4-dien-1-ylidene)-1,3-didodecylpyrimidine-2,4,6(1H,3H,5H)-trione (3) – To a stirring solution of 1,3-didodecyl-5-(furan-2-ylmethylene)pyrimidine-2,4,6(1H,3H,5H)-trione (500 mg, 0.921 mmol) in THF (1 mL) was added a solution of 6-azido-N-ethylhexan-1-amine (173 mg, 1.013 mmol) in THF (1mL) and the mixture was stirred at rt for 20 minutes. Solvent was removed in vacuo and the product was purified by column chromatography using DCM:MeOH (100:0 to 99:1) system. Dark purple solid (397 mg) was obtained in 61 % yield; ^{1}H NMR (400 MHz, Methylene Chloride-d_2) δ 12.55 (s, 1H), 7.26 (dd, J = 22.1, 12.3 Hz, 1H), 7.08 (s, 1H), 6.78 (d, J = 12.4 Hz, 1H), 6.05 (td, J = 12.3, 2.9 Hz, 1H), 3.86 (td, J = 9.5, 6.5 Hz, 4H), 3.54 – 3.35 (m, 4H), 3.28 (q, J = 6.8 Hz, 2H), 1.69 (q, J = 8.0 Hz, 2H), 1.64 – 1.55 (m, 6H), 1.45 – 1.37 (m, 4H), 1.36 – 1.20 (m, 40H), 0.88 (t, J = 6.6 Hz, 6H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 165.2, 163.2, 155.5, 151.5, 149.5, 146.9, 141.0, 105.2, 51.4, 42.0, 41.8, 32.1, 29.8, 29.8, 29.8, 29.8, 29.7, 29.7, 29.6, 29.5, 29.4, 28.9, 28.4, 27.2, 27.1, 26.5, 22.8, 14.2. IR (ATR) 2922, 2853, 2094, 1691, 1620, 1597, 1555, 1492, 1456, 1428, 1403, 1365, 1338, 1265, 1199, 1138, 1087, 968, 909, 838, 780, 759, 728, 687 cm^{-1}.

Donor-Acceptor Stenhouse Adduct - Poly(ethylene glycol) Conjugate (DASA-amphiphile) (4) – To a stirring mixture of alkyne-terminated poly(ethylene glycol) (150 mg, 0.050 mmol), $CuSO_4$-$5H_2O$ (1.25 mg, 0.005 mmol) and sodium ascorbate (2.0 mg, 0.010 mmol) in 2.0 mL water was added 5-((2Z,4E)-5-((6-azidohexyl)(ethyl)amino)-2-hydroxypenta-2,4-dien-1-ylidene)-1,3-didodecylpyrimidine-2,4,6(1H,3H,5H)-trione (107 mg, 0.150 mmol) dissolved in minimum amount of acetone. The mixture was heated to 50 ºC in a water bath for 16 h. After the mixture was cooled to room temperature, the product was extracted with dichloromethane (4 x 20 mL). The crude product was precipitated twice into diethyl ether, yielding 123 mg (66 % yield) of DASA-amphiphile **4** as a purple solid.

Overlaid GPC Traces of DASA-Amphiphile and PEG

As seen in the below overlaid spectra of GPC, the molecular weight change from approximately 3000 Da (PEG, orange trace) to approximately 3700 Da (DASA-amphiphile, blue trace) has resulted in the peak shift to an earlier elution time.

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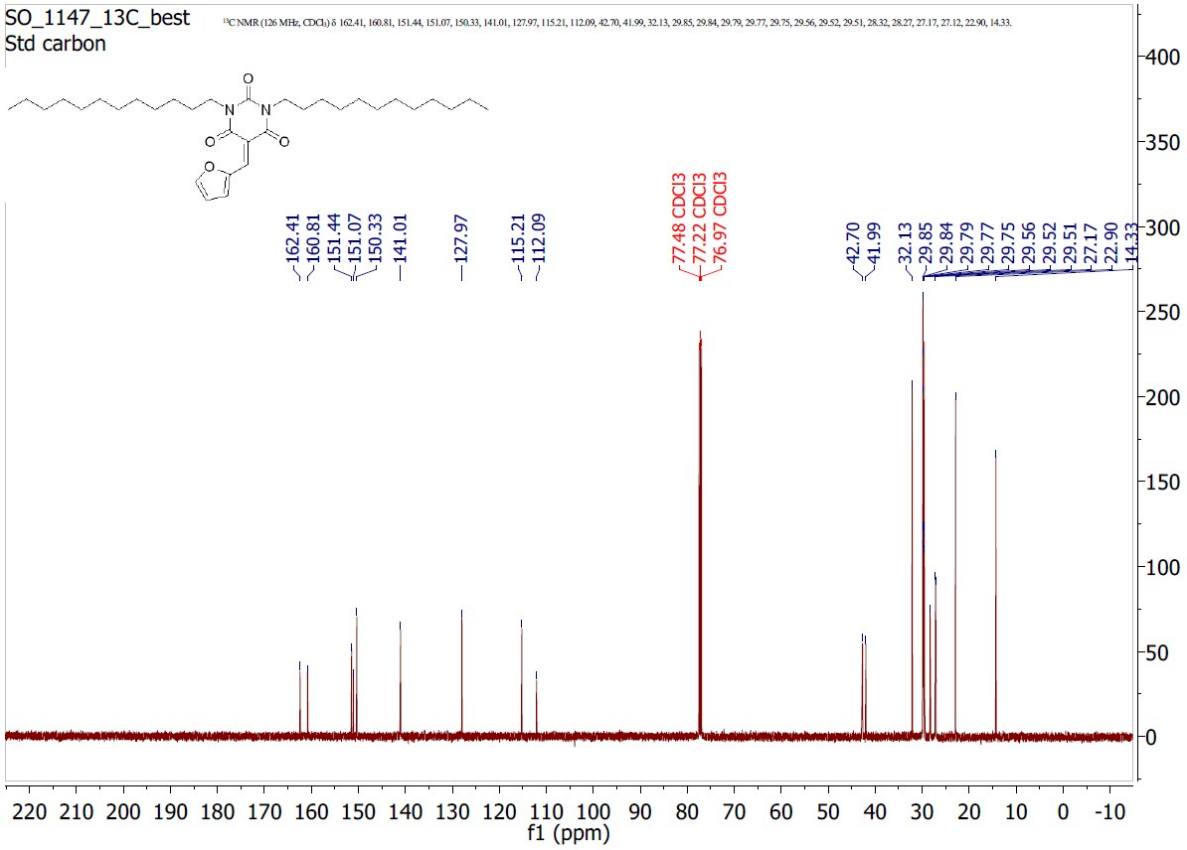
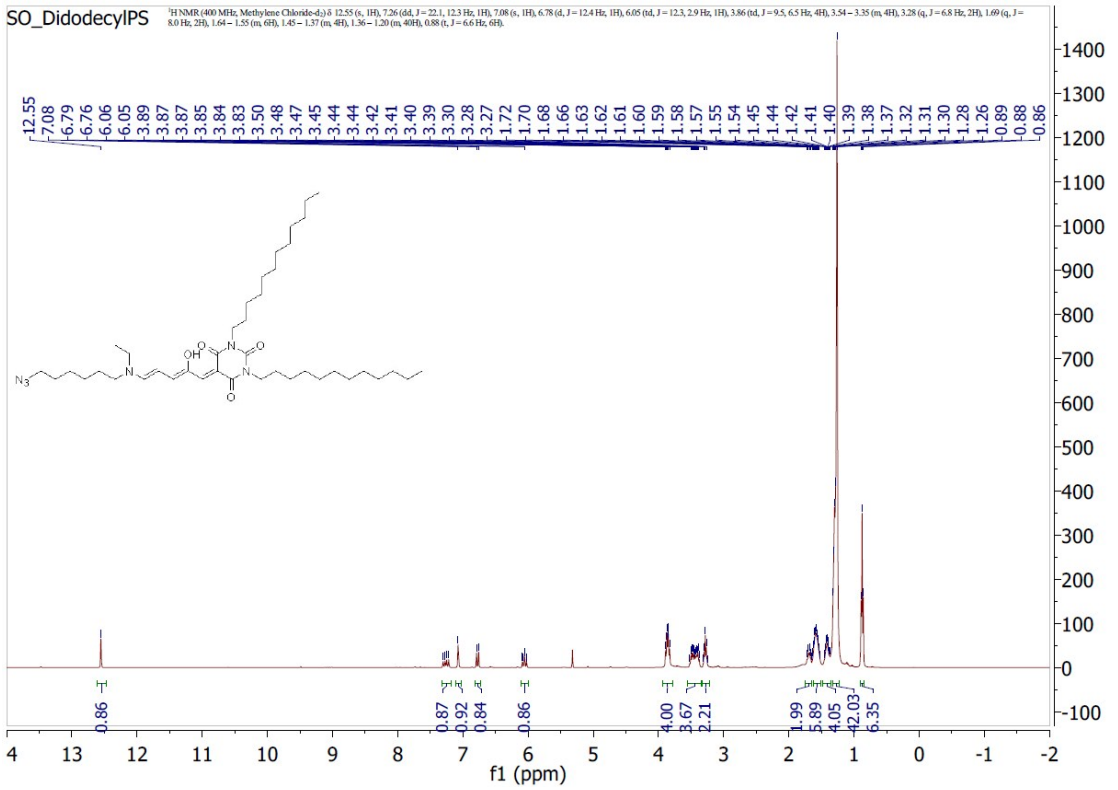
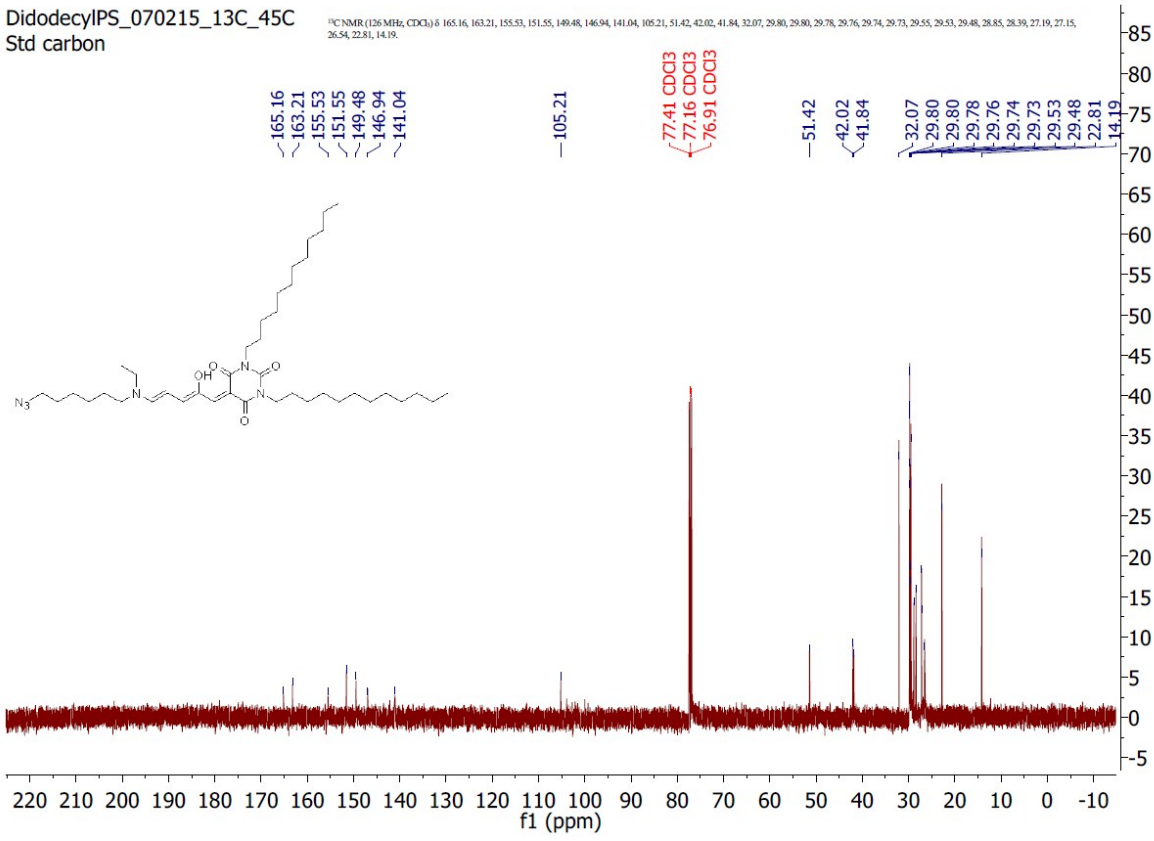
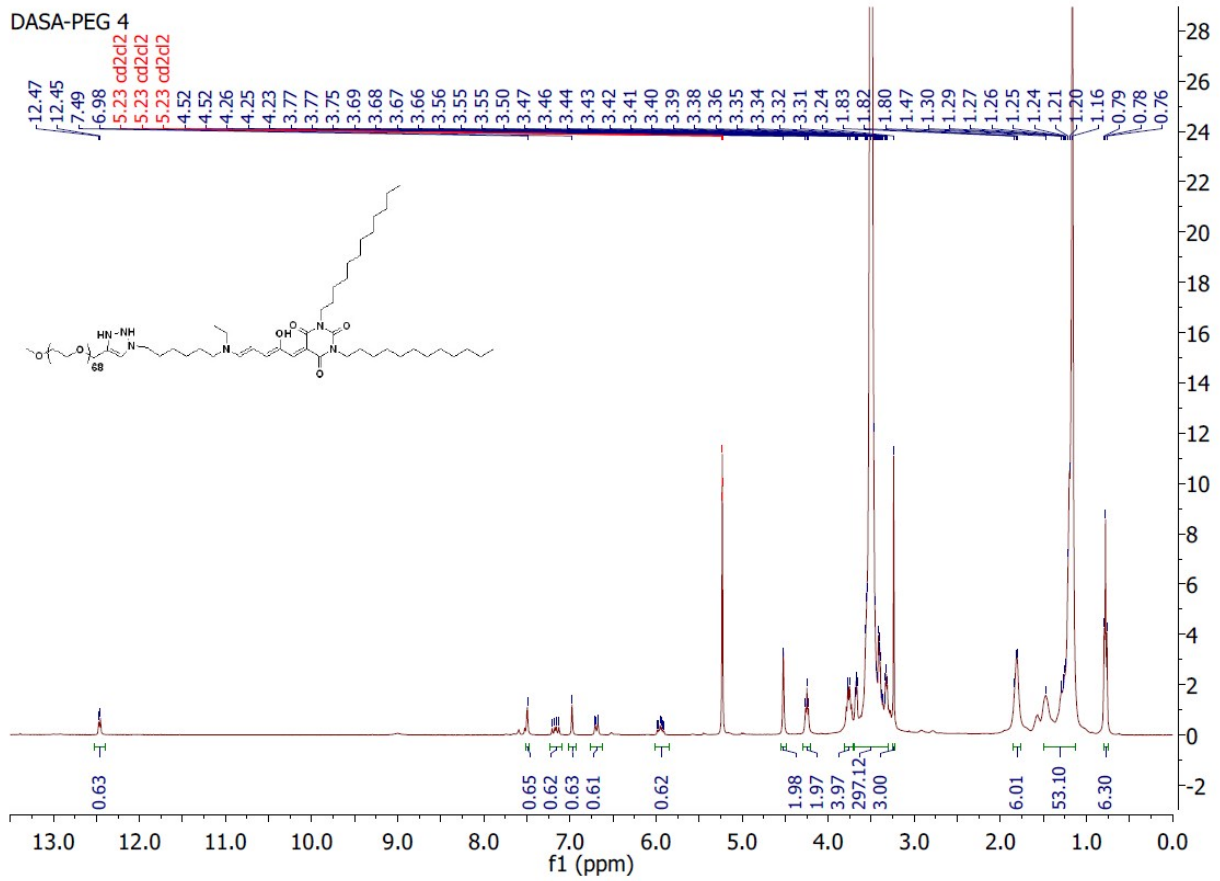
f1 (ppm)


SO_1147_13C_best
Std carbon
f1 (ppm)


SO_DidodecylPS
f1 (ppm)


DidodecylPS_070215_13C_45C
Std carbon
f1 (ppm)


DASA-PEG 4
12.47
12.45
7.49
6.98
5.23 cd2d2
5.23 cd2d2
5.23 cd2d2
4.52
4.52
4.26
4.25
4.23
3.77
3.77
3.75
3.69
3.68
3.67
3.66
3.56
3.55
3.55
3.50
3.47
3.46
3.44
3.43
3.42
3.41
3.40
3.39
3.38
3.36
3.35
3.34
3.32
3.31
3.24
1.83
1.82
1.80
1.47
1.30
1.29
1.27
1.26
1.25
1.24
1.21
1.20
1.16
0.79
0.78
0.76
0.63
0.65
0.62
0.63
0.61
0.62
1.98
1.97
3.97
297.12
3.00
6.01
53.10
6.30
f1 (ppm)

Preparation of Micelle Solutions with Nile Red

The stock solutions were prepared according to the procedure used by Fréchet and coworkers.[2] Briefly, 12.5 µL of Nile Red stock solution in dichloromethane (0.05 mg/mL) was added to a 4 mL vial. This vial was placed under vacuum until the solvent was completely removed. An aqueous DASA-PEG amphiphile stock solution at concentration of 2 mg/mL was prepared and added to this vial. Deionized water was added to dilute the solution to 2 mL with desired concentration. This solution was stirred vigorously for 10 minutes and then allowed to equilibrate at room temperature for 1 hour.

CMC Determination

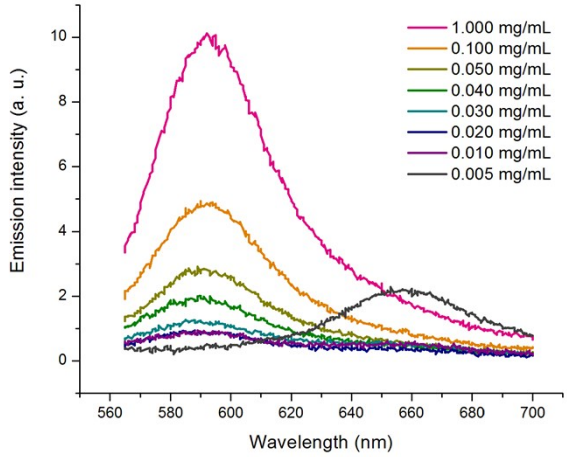


Figure S1. Fluorescence emission spectrum of Nile red with varying concentrations of DASA-amphiphile **4** in water. At low concentrations, maximum emission occurs around 660 nm. As concentrations increase, maximum emission blue shifts indicating encapsulation of Nile red within the core of micelles.

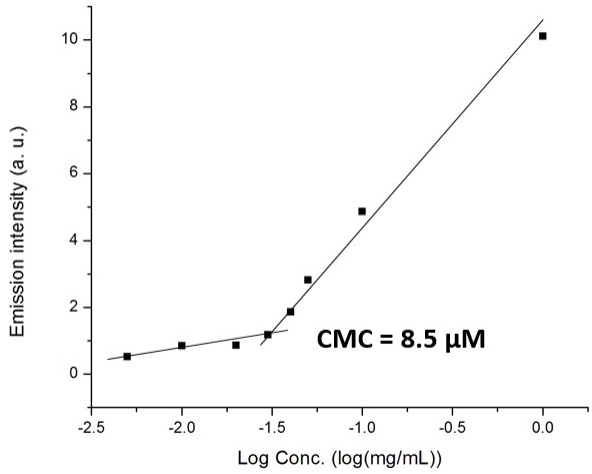


Figure S2. A plot of relative intensity as a function of log of concentration of DASA-amphiphile **4**. Non-linear relationship suggests that the inflection point (8.5 µM) is the critical micelle concentration (CMC).

Visible Light Induced Micellar Disruption

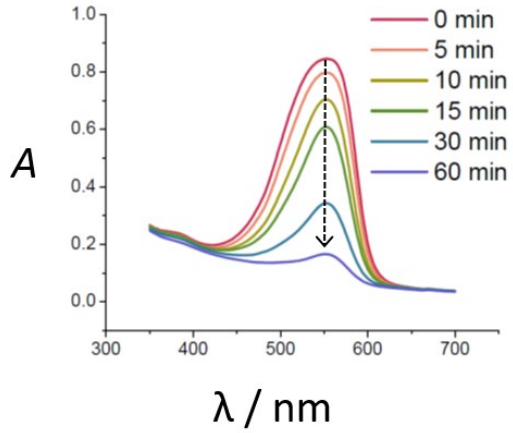


Figure S3. UV-Vis spectra of DASA-amphiphile **4** (0.5 mg/mL) in water after varying times of visible light irradiation

To verify that the disruption of micelle is being induced by the photoisomerization of the DASA segments, UV-Vis spectroscopy was employed (Figure S3). A micellar solution was prepared and the change in absorbance of the DASA moiety λ_{max} = 553 nm) was monitored at different time intervals of visible light irradiation. After 1 hour of irradiation, the DASA absorbance decreased by ~80%. This observation confirms that the primary reason for micelle disruption is indeed the visible light-triggered photoisomerization of the DASA segments from **4** to **5**. As the triene form of the DASA (the hydrophobic core of the micelles) becomes isomerized to a hydrophilic zwitterion by visible light, the amphiphilic character of the DASA-amphiphile **4** is lost, and thus the micellar structure is disintegrated. Of note, no special precautions are required to handle this material because ambient light does not trigger micelle disassembly without extended exposure (> 3 hours).

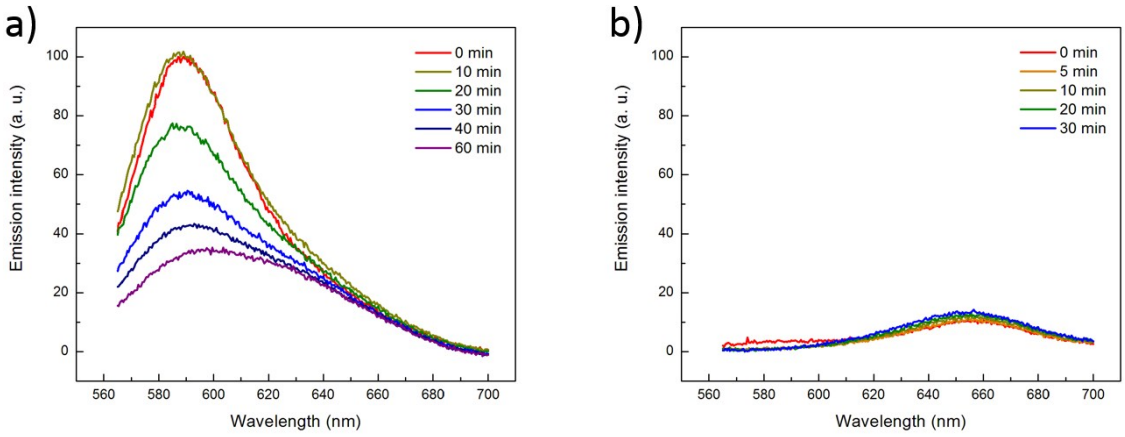


Figure S4. Fluorescence emission spectra of Nile Red in a) 0.50 mg/mL and b) 0.005 mg/mL of DASA-amphiphile **4** in water at various times of visible light irradiation. A noticeable red shift and decrease in emission intensity were observed as light irradiation continued (left), whereas minimum change occurred at the concentration below CMC (right).

In order to demonstrate the disassembly of the micelles with visible light, fluorescence spectroscopy was used to monitor the release of cargo with respect to irradiation time. Nile Red, a solvatochromic and hydrophobic dye that fluoresces at 660 nm with low emission intensity in water and at < 600 nm with enhanced emission intensity in hydrophobic environments, was chosen as the cargo. Upon irradiation, the intensity of fluorescence steadily decreased with a concurrent red-shift of the emission λ_{max}. This confirms that Nile Red is slowly released into the aqueous solution from the micelle core as the DASA segments of DASA-amphiphile **4** isomerize to their hydrophilic form of DASA-nonamphiphile **5** in response to visible light.

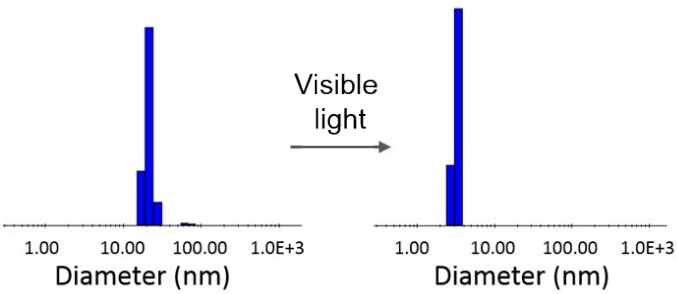


Figure S5. Dynamic light scattering (DLS) measurements of aqueous solution of DASA-amphiphile **4** (0.5 mg/mL) before (left) and after (right) irradiation.

We used dynamic light scattering (DLS) to verify that the micelles are disrupted when we shine light on them. Prior to light irradiation, solutions of DASA-amphiphile **4** above the critical micelle concentration (CMC) (0.5 mg/mL, see Supporting Information for determination of CMC) successfully formed micelles, whose average size was ~22 nm in diameter (Figure S5, left). Upon irradiation with visible light, the micelles rapidly disassembled because DASA moieties isomerize into hydrophilic zwitterions. After irradiation no particle greater than 3 nm could be detected by DLS (Figure S5, right).

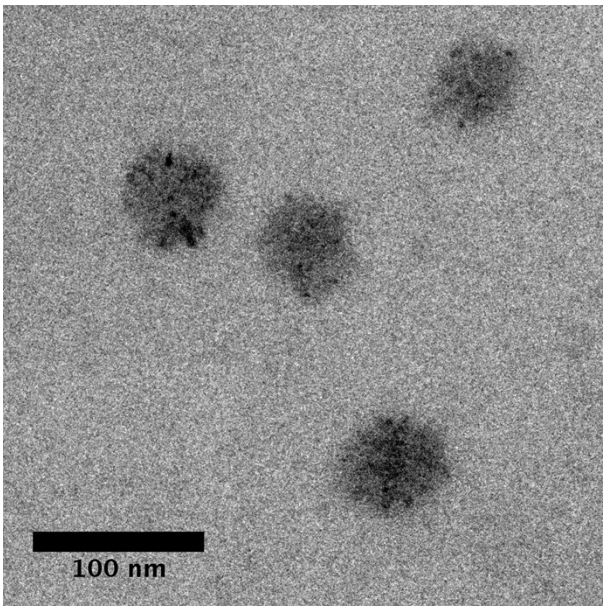


Figure S6. TEM image of micelles formed by DASA-amphiphile **4** in dH_2O

Preparation of a micelle solution with 1 μM of paclitaxel

A stock solution of paclitaxel in dichloromethane was prepared at 0.9 mg/mL concentration. 20 μL of this stock solution was added to a 1-dram vial equipped with a magnetic stir bar and the solvent was removed in vacuo. To this vial was added 1.0 mg of DASA-amphiphile **4** and 2.0 mL of deionized water. The mixture was stirred vigorously for 10 minutes and was allowed to equilibrate at room temperature for 1 hour.

Paclitaxel Content and Loading Efficiency

In this experiment, 0.135 mM (0.5 mg/mL) of DASA-amphiphile **4** and 1 μM (0.85 μg/mL) of paclitaxel was used, which is equivalent to 0.17 wt. % drug loading. After preparing the micelle solution loaded with paclitaxel, the solution was filtered through a molecular cut-off filter (EMD Millipore Amicon Ultra-4 Centrifugal Filter Units; Ultra-4; w/Ultracel-3 membrane; 3000 NMWL; Dia. x L: 17.2 x 73.4mm). The filtered solution was then analyzed to determine the efficiency of paclitaxel loading by reverse-phase HPLC on a preparative C_{18} column (300 Å, 5 μm, 4.6 mm i.d. x 250 mm) with isocratic elution of water-acetonitrile (55:45 v/v) with a flow rate of 1.0 mL/min at 25 °C. First, a calibration curve was established using samples with known concentrations of paclitaxel ranging from 0 to 0.6 μM as shown below. It was found that the loading efficiency was greater than 99% (Integration was 264 for the sample and elution time was 8.8 min). It is known that with low drug content (below 30 wt.%) the drug loading efficiency is generally high (>90%).[3]

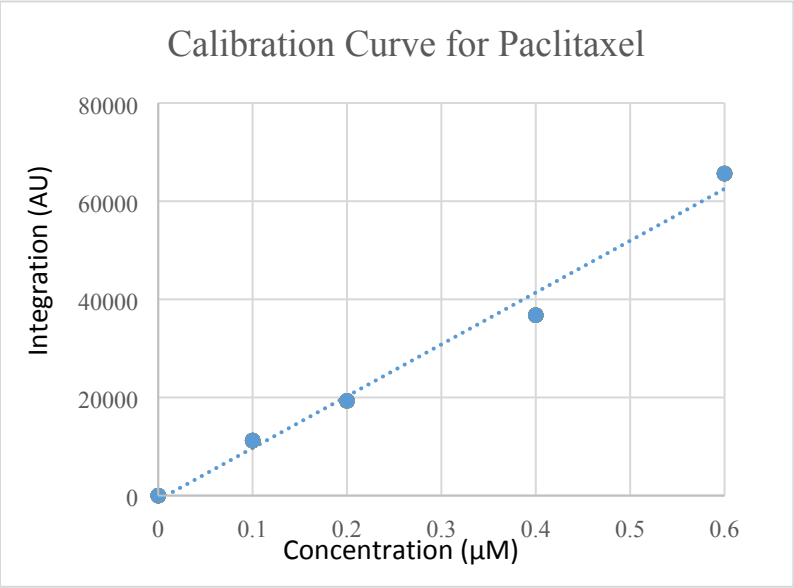


Figure S7. HPLC calibration curve for paclitaxel.

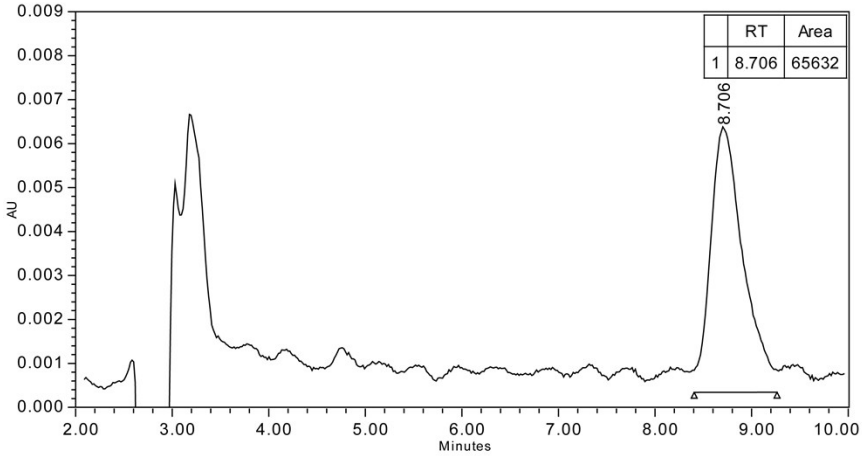


Figure S8. HPLC trace of paclitaxel at 0.6 μM.

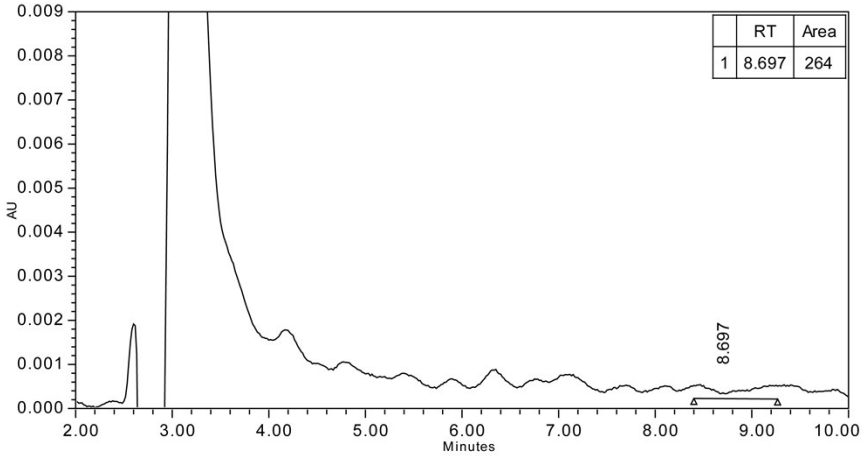


Figure S9. HPLC trace of paclitaxel from micelle loading sample.

Cell Culture and Sample Preparation

As recommended by the ATCC, MCF-7 human breast cancer cells were cultured in DMEM supplemented with 10% FBS and 1% penicillin/streptomycin, and these cells were grown in a humidified incubator maintained at 37 °C and 5% CO_2. Before live-cell imaging, cancer cells were harvested with TrypLE express reagent (Life Technologies) following the manufacturer's protocol. The harvested cells were then seeded on a 24-well culture plate or a 96-well black microplate. After 24-hour incubation, the medium was aspirated and discarded, and the cells were washed with pre-warmed DPBS twice. Immediately, the washed cells were incubated with each sample either in presence or absence of visible light for an hour. The treated cells were twice washed with pre-warmed DPBS gently and added by a phenol red-free, fresh medium before following experiments.

Quantification of Nile Red Delivery

To quantify the amount of Nile Red delivered in cancer cells, the MCF-7 adherent cells transferred in a black 96-well microplate were treated with Nile Red-loaded DASA-amphiphile **4** samples in presence or absence of visible light for an hour and subsequently washed with DPBS to remove undelivered cargos. After addition of fresh DPBS, the fluorescence of Nile red delivered in each cell sample was measured

with a microplate reader (Infinite M1000, Tecan, San Jose, CA). For calibration, the cancer cells were incubated with free Nile Red with the concentration ranging from 1 to 10 μm at the same time, and the fluorescence of free Nile Red absorbed in each cell sample was detected as described above. Based on the relationship between the Nile Red concentration and the fluorescent signal, the quantity of Nile Red delivered to the cells were calculated, and all measurements were performed in triplicate.

Cell Viability Measurement

The viabilities of treated MCF-7 cancer cells were measured at each time point via LIVE/DEAD Viability/Cytotoxicity Kit for mammalian cells (Life Technologies) according to the manufacturer's protocol. At each time point, cell samples in a black 96-well microplate were stained by calcein acetoxymethyl ester specific for viable cells and ethidium momodimer-1 specific for nonviable cells. The cell viabilities were subsequently analyzed with a microplate reader (Infinite M1000, Tecan, San Jose, CA), and all measurements were performed in triplicate.

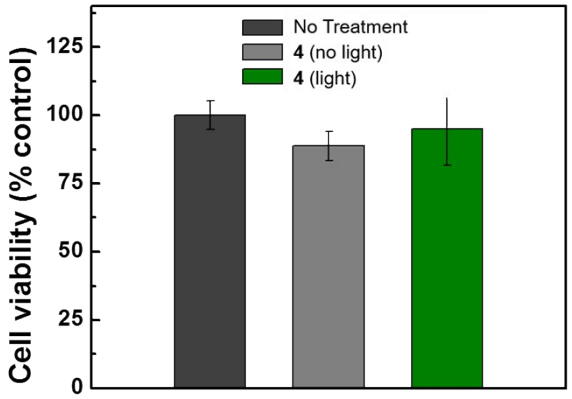


Figure S10. Effect of DASA-amphiphile **4** on cell viability. Relative to non-treated control cells (Black), the DASA-amphiphile **4** treated MCF-7 cells both in the absence and presence of visible light (grey and green, respectively) exhibited minimal decrease of cell viability after 24 hours, confirming the biocompatibility of DASA-amphiphile **4**.

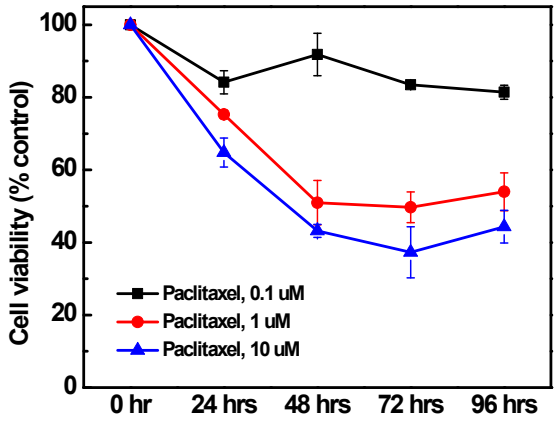


Figure S11. Effect of paclitaxel concentration ranging from 0.1 to 10 μM on cell viability over 4 days. When 0.1 μM of paclitaxel was used, cell viability remained at 80 %. In contrast, 1 μM of paclitaxel led to about 50 % of cell viability with 10 μM of paclitaxel leading to the similar result. Thus, 1 μM of paclitaxel was chosen for the following drug unloading experiments.

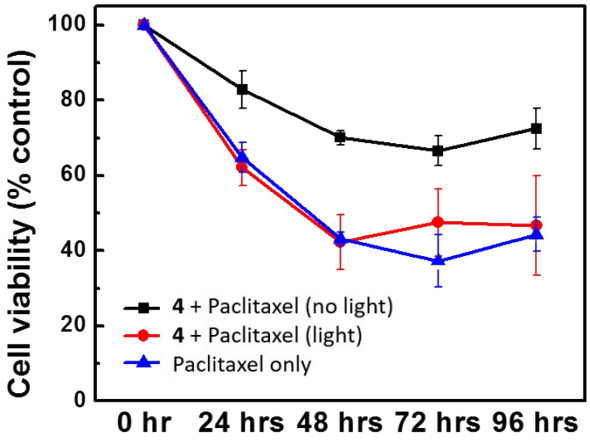


Figure S12. Visible light-mediated cytotoxicity of cancer cells by DASA-amphiphile **4** micelles at a paclitaxel concentration of 10 μM. Compared to the cells incubated with only paclitaxel (blue), the cells incubated with paclitaxel-loaded DASA-amphiphile **4** on exposure to visible light (red) showed comparable death of cells while the cells without visible light irradiation (black) displayed higher cell viabilities over 4 days.

References

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3) K. M. Huh, S. C. Lee, Y. W. Cho, J. Lee, J. H. Jeong, K. Park Hydrotopic polymer micelle system for delivery of paclitaxel. *J. Control. Release, **2005**, 101*, 59-68.