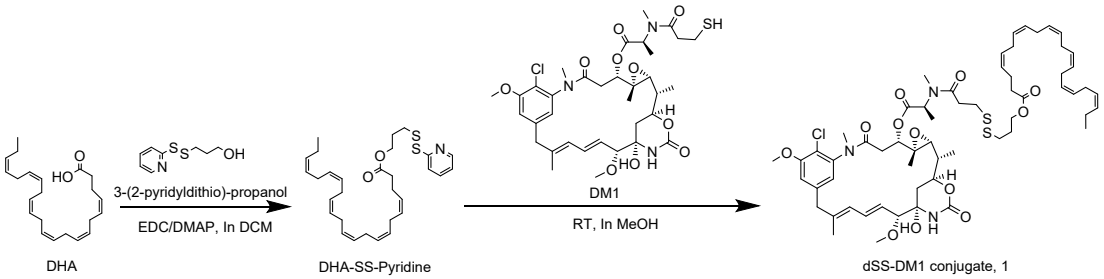
Supporting Information for **Transforming a Highly Toxic Agent into Injectable Safe Nanomedicines *via* Prodrug Self-Assembly for the Treatment of Taxane-Resistant Cancer**

Synthesis of dSS-DM1 Conjugate



Supplementary Scheme 1. Synthetic scheme of conjugate 1

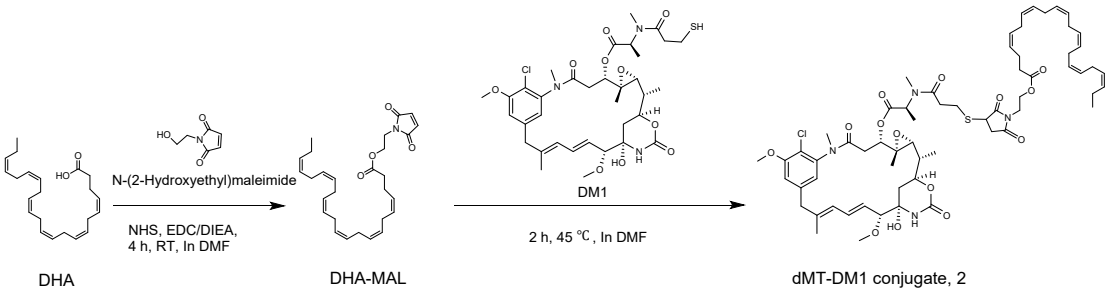
Docosahexaenoic acid (DHA, 50.0 mg, 0.159 mmol) and 3-(2-pyridyldithio)-propanol (96.0 mg, 0.477 mmol) were dissolved in 2 mL of anhydrous dichloromethane (DCM). Next, 1-ethyl-3-(3-dimethylaminopropyl)-carbodiimide (EDC, 36.6 mg, 0.191 mmol) and 4-dimethylamino pyridine (DMAP, 23.3 mg, 0.191 mmol) was added to the solution. The reaction mixture was stirred at 45 °C for 4 h and then washed with 5% citric acid, saturated $NaHCO_3$ and brine. The organic layer was dried over anhydrous Na_2SO_4, filtered, and evaporated under vacuum. The crude residue was purified by flash column chromatography on silica gel (DCM:MeOH = 100:1) to obtain the DHA-SS-Pyridine (48 mg, yield: 60.65%) and further characterized by ^{1}H NMR spectroscopy.

^{1}H NMR (400 MHz, $CDCl_3$) δ 8.47 (d, J = 4.6 Hz, 1H), 7.82 – 7.59 (m, 2H), 7.16 – 7.00 (m, 1H), 5.37 (dt, J = 11.4, 5.9 Hz, 10H), 4.17 (t, J = 6.2 Hz, 3H), 2.91 – 2.69 (m, 10H), 2.48 – 2.27 (m, 5H), 2.13 – 1.80 (m, 6H), 1.26 (s, 3H), 0.97 (t, J = 7.5 Hz, 3H).

DHA-SS-Pyridine (45.0 mg, 0.0879 mmol) and Maytansinoid (DM1, 39.3 mg, 0.0532 mmol) were dissolved in 2 mL of anhydrous DCM. After stirred at room temperature over 2 h, the reaction mixture was evaporated under vacuum. The residue was purified by flash column chromatography on silica gel (DCM:MeOH = 100:1) to produce the desired conjugate **1** (25.7 mg, yield: 42.4%). The conjugate **1** was characterized by ^{1}H NMR spectroscopy.

^{1}H NMR (400 MHz, C_2D_6SO) δ 6.86 – 6.77 (m, 1H), 6.66 (d, J = 15.7 Hz, 2H), 6.43 (dd, J = 15.3, 11.2 Hz, 1H), 6.26 (s, 1H), 6.02 – 5.88 (m, 1H), 5.64 (dd, J =15.2, 9.0 Hz, 1H), 5.45 – 5.30 (m, 13H), 4.78 (dd, J = 11.9, 2.2 Hz, 1H), 4.31 –4.25 (m, 1H), 3.99 (s, 3H), 3.75 – 3.59 (m, 3H), 3.54 (ddd, J = 15.1, 6.9, 4.0 Hz,3H), 3.35 (s, 3H), 3.20 (s, 3H), 3.14 – 3.05 (m, 2H), 3.01 (dt, J = 10.3, 7.7 Hz, 2H),2.91 – 2.73 (m, 14H), 2.70 – 2.55 (m, 2H), 2.40 – 2.28 (m, 3H), 2.17 (ddd, J =14.6, 9.4, 5.1 Hz, 3H), 2.07 (dd, J = 14.6, 7.4 Hz, 2H), 1.82 – 1.68 (m, 1H), 1.64 (s, 3H), 1.34 – 1.29 (m, 6H), 0.97 (t, J = 7.5 Hz, 3H), 0.80 (s, 3H).

Synthesis of dMT-DM1 Conjugate



Supplementary Scheme 2. Synthetic scheme of conjugate 2

DHA (50.0 mg, 0.159 mmol) and N-(2-Hydroxygethyl) maleimide (MAL, 48.3 mg, 0.191 mmol) were dissolved 1 mL of anhydrous N, N-Dimethylformamide (DMF). Next, N-Hydroxy succinimide (NHS, 22.0 mg, 0.191 mmol), EDC (36.6 mg, 0.191 mmol) and N, N-Diisopropylethylamine (DIEA, 24.6 mg, 0.191mmol) were added to the solution. The reaction mixture was stirred at 45 °C for 4 h and then washed with 5% citric acid, saturated $NaHCO_3$ and brine. The organic layer was dried over anhydrous $NaSO_4$, filtered, and evaporated under vacuum. The residue was purified by flash column chromatography on silica gel (DCM:MeOH = 100:1) to obtain the DHA-MAL (57 mg, yield: 84.5%) and further characterized by ^{1}H NMR spectroscopy.

^{1}H NMR (400 MHz, $CDCl_3$) δ 6.72 (s, 2H), 5.45 – 5.26 (m, 12H), 3.69 (dd, J = 6.4, 4.4 Hz, 2H), 3.46 (dd, J = 11.0, 5.6 Hz, 2H), 2.82 (dd, J = 12.7, 6.1 Hz, 10H), 2.37 (dd, J = 14.3, 7.4 Hz, 2H), 2.21 – 2.17 (m, 2H), 2.08 (p, J = 7.3 Hz, 2H), 0.97 (t, J = 7.5 Hz, 3H).

DHA-MAL (54.0 mg, 0.120 mmol) and DM1 (87 mg, 0.118 mmol) were dissolved in 2 mL of anhydrous DMF. After stirred at 45 °C for 2 h, the reaction mixture was evaporated under vacuum. The residue was purified by flash column chromatography on silica gel (DCM:MeOH = 20:1) to produce the desired conjugate **2** (95.1 mg, yield: 67.4 %). The conjugate **2** was characterized by ^{1}H NMR spectroscopy.

^{1}H NMR (400 MHz, C_2D_6SO) δ 6.86 – 6.77 (m, 1H), 6.66 (d, J = 15.7 Hz, 2H), 6.43 (dd, J = 15.3, 11.2 Hz, 1H), 6.26 (s, 1H), 6.02 – 5.88 (m, 1H), 5.64 (dd, J =15.2, 9.0 Hz, 1H), 5.45 – 5.30 (m, 13H), 4.78 (dd, J = 11.9, 2.2 Hz, 1H), 4.31 –4.25 (m, 1H), 3.99 (s, 3H), 3.75 – 3.59 (m, 3H), 3.54 (ddd, J = 15.1, 6.9, 4.0 Hz,3H), 3.35 (s, 3H), 3.20 (s, 3H), 3.14 – 3.05 (m, 2H), 3.01 (dt, J = 10.3, 7.7 Hz, 2H),2.91 – 2.73 (m, 14H), 2.70 – 2.55 (m, 2H), 2.40 – 2.28 (m, 3H), 2.17 (ddd, J =14.6, 9.4, 5.1 Hz, 3H), 2.07 (dd, J = 14.6, 7.4 Hz, 2H), 1.82 – 1.68 (m, 1H), 1.64 (s, 3H), 1.34 – 1.29 (m, 6H), 0.97 (t, J = 7.5 Hz, 3H), 0.80 (s, 3H).

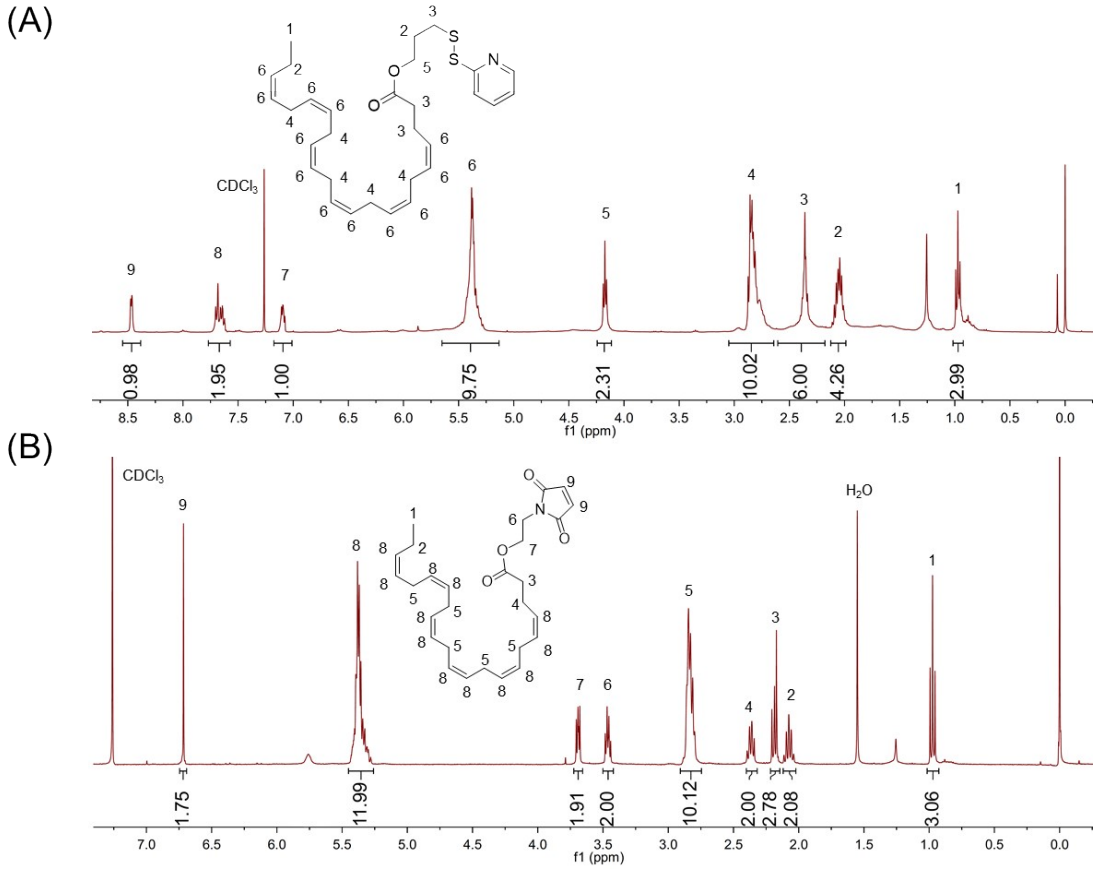


Figure S1. ^{1}H NMR spectra and peak assignment of (A) DHA-SS-Pyridine and (B) DHA-MAL in $CDCl_3$, respectively.

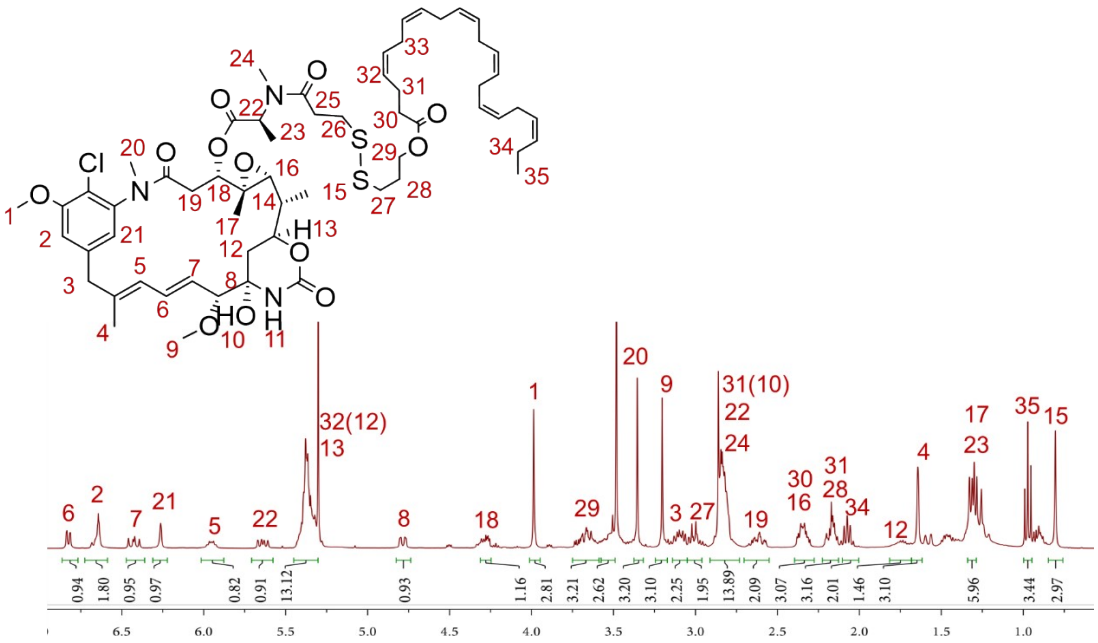


Figure S2. Synthetic spectrum and ^{1}H NMR of **dSS-DM1 in C_2D_6SO**.

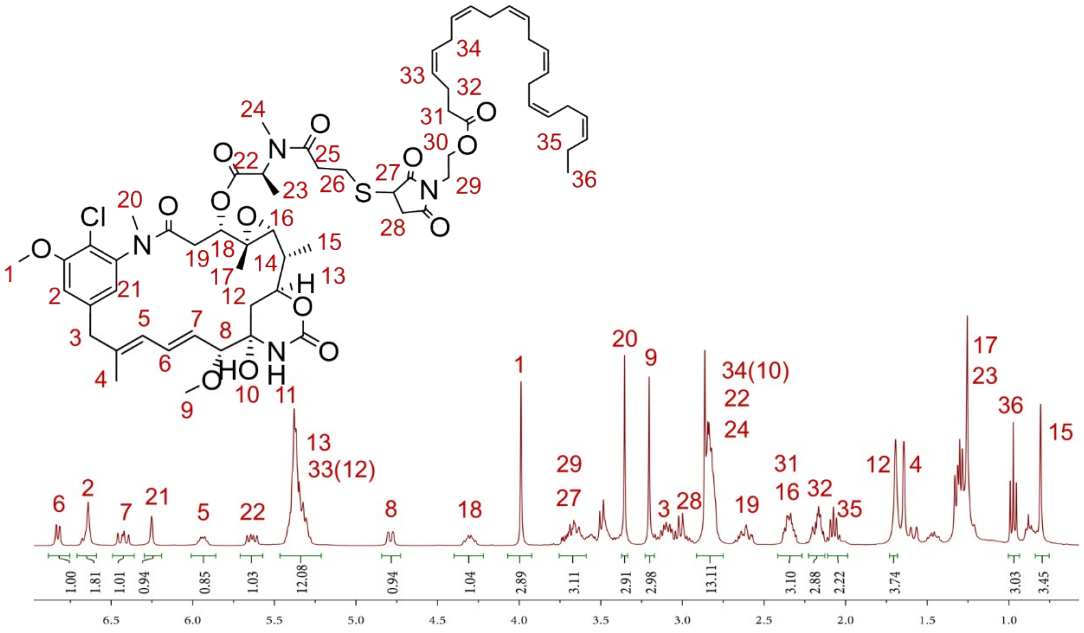


Figure S3. Synthetic spectrum and ^{1}H NMR of **dMT-DM1 in C_2D_6SO**.

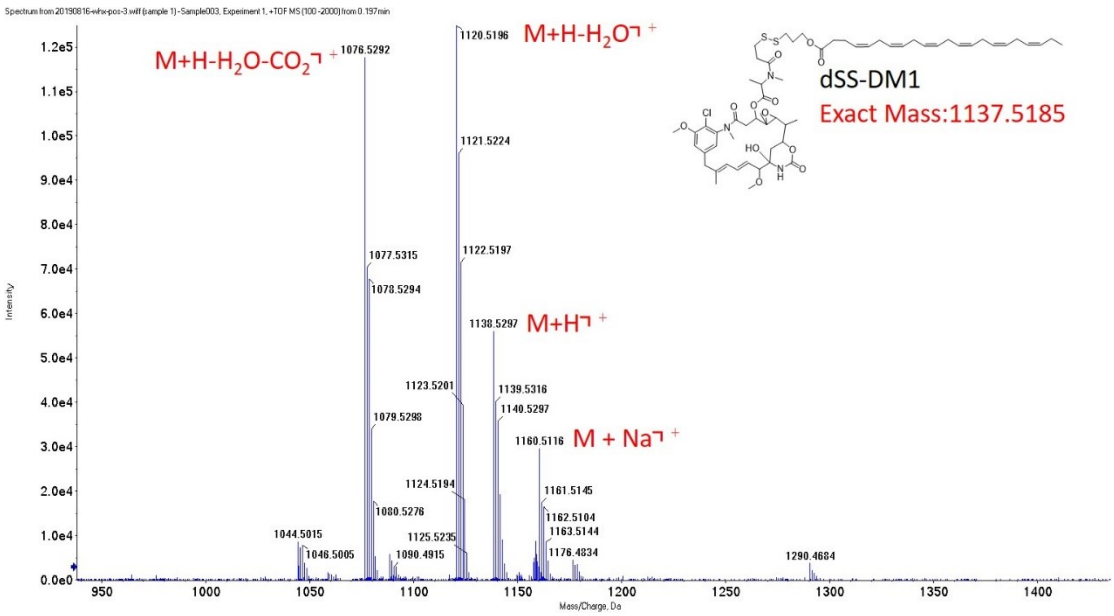


Figure S4. MALDI-TOF-MS of **dSS-DM1**.

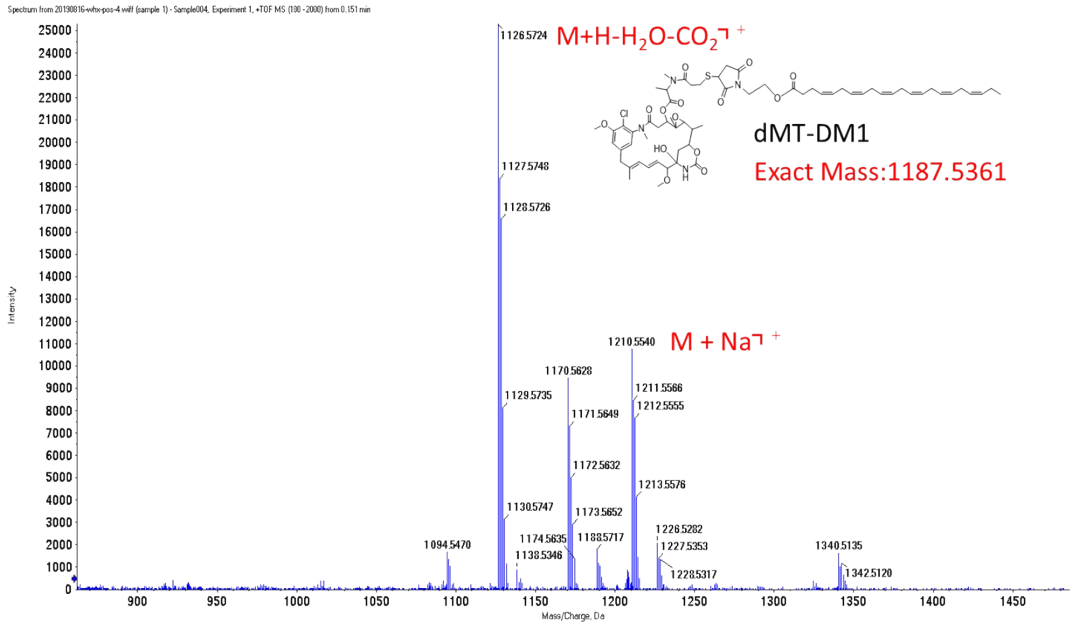


Figure S5. MALDI-TOF-MS of **dMT-DM1**.

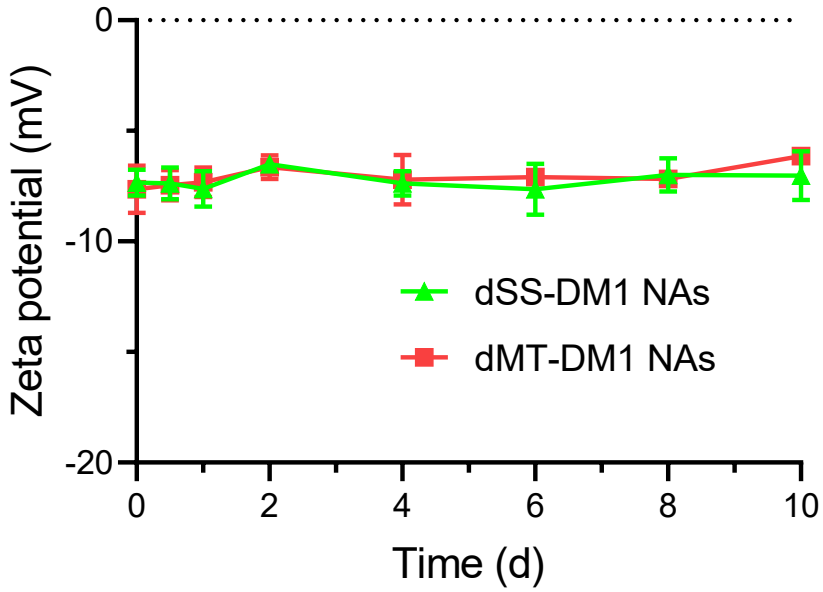


Figure S6. Time-dependent studies on the stability of the nanoparticles in phosphate-buffered saline (PBS, pH 7.4) containing 10% fetal bovine serum (FBS) at 4°C.

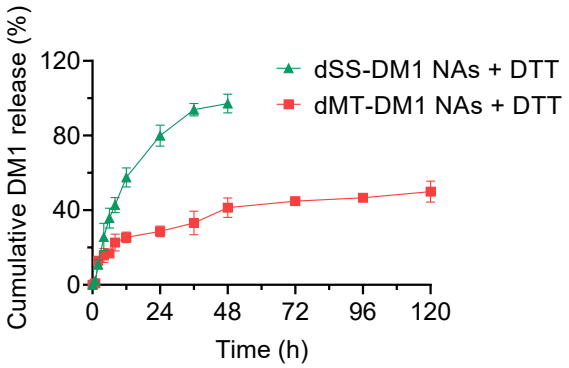


Figure S7. Accumulative drug release profiles from **dSS-DM1 NAs** and **dMT-DM1 NAs** incubated with or without 10 mM DTT at 37 °C.

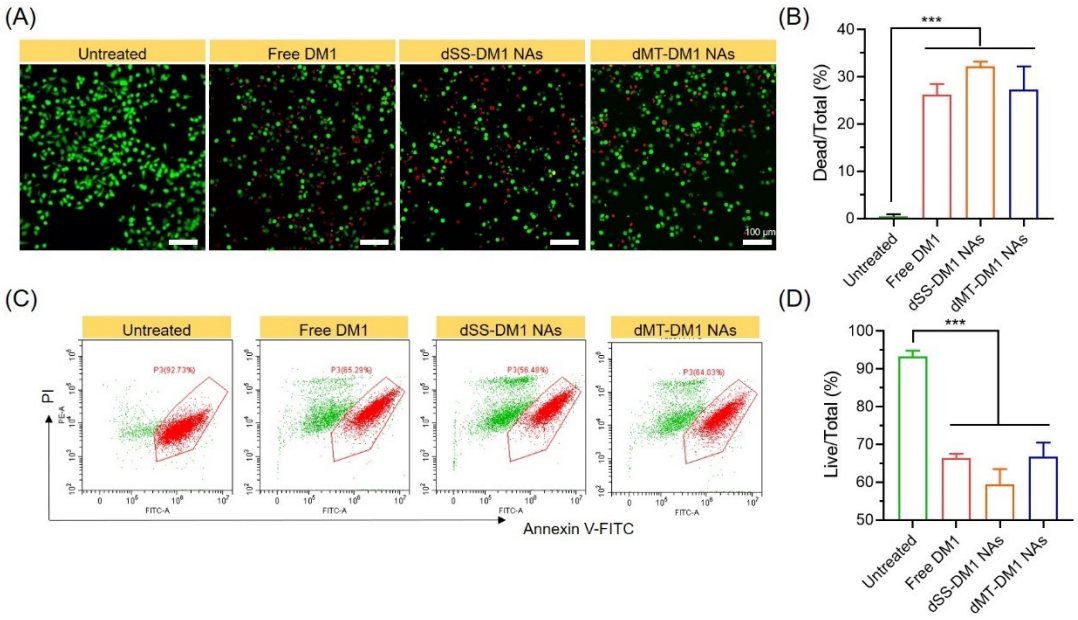


Figure S8. (A, B) Fluorescence microscopy images of HeLa/PTX cells costained with calcein-AM (green, live cells) and propidium iodide (PI, red, dead cells). (C, D) Flow cytometry analysis of HeLa/PTX cell mortality rate induced by different formulations at 30 nM DM1-equivalent concentrations. The data are presented as the mean ± SD (n = 3). * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, # $p > 0.05$.

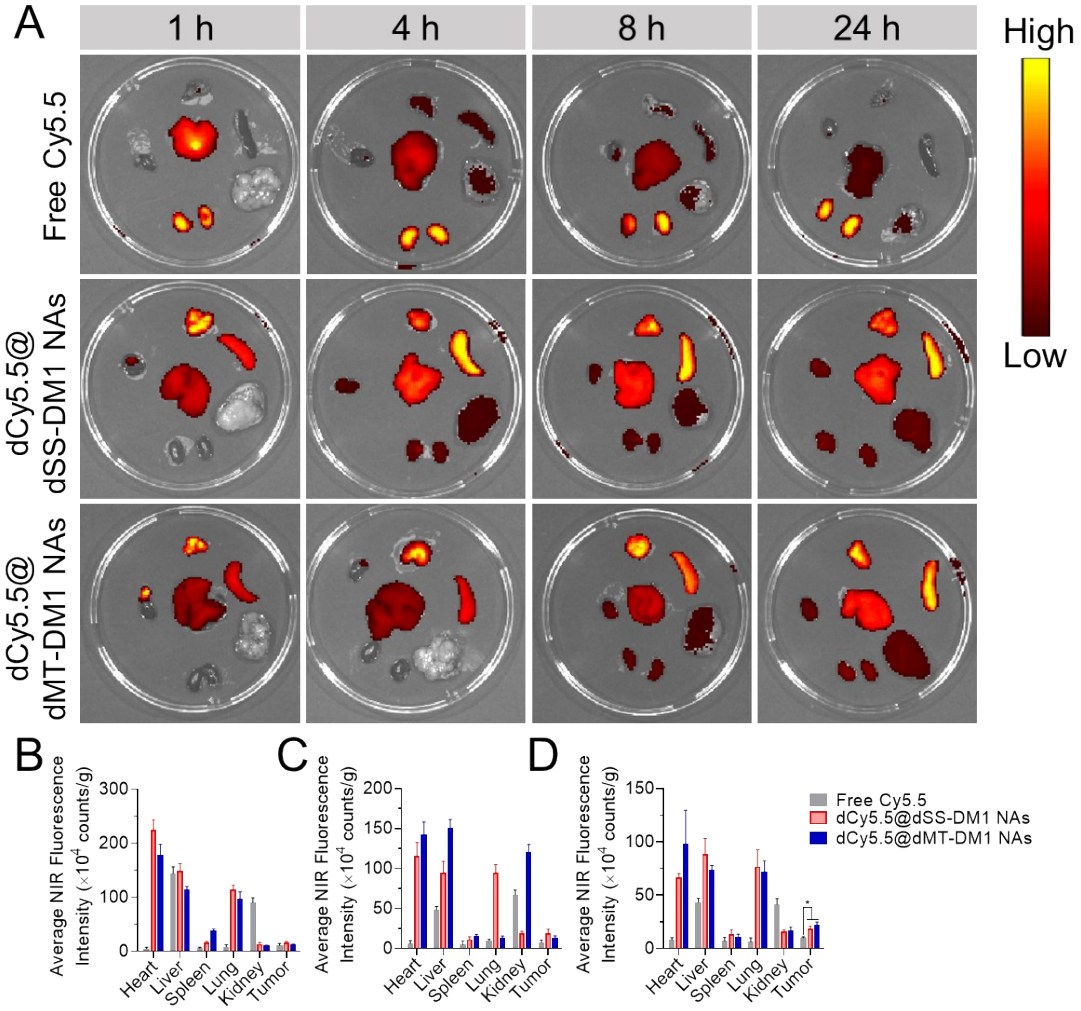


Figure S9. Biodistribution of dCy5.5@NAs in a HeLa/PTX xenograft-bearing mouse model (n = 3). (A) *Ex vivo* fluorescence images of tumors and major organs. Free Cy5.5, **dCy5.5@dSS-DM1 NAs** and **dCy5.5@dMT-DM1 NAs** (20 μg of Cy5.5 per mouse) were intravenously injected into bearing nude mice. Quantitative analysis of average NIR fluorescence intensity at 1 h (B), at 4 h (C) and at 8 h (D) after post-injection. The data are presented as mean ± SD (n = 3). #$p > 0.05$, *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$.

Table S1. Drug loading (DL) of **dSS-DM1 NAs** and **dMT-DM1 NAs (*n* = 3)**

Drug formulation	**dSS-DM1 NAs**	**dMT-DM1 NAs**
DM1	64.1% ± 0.3%	62.5% ± 2.0%