Supplementary Materials: Cationic Nanoparticles Assembled from Natural-Based Steroid Lipid for Improved Intracellular Transport of siRNA and pDNA

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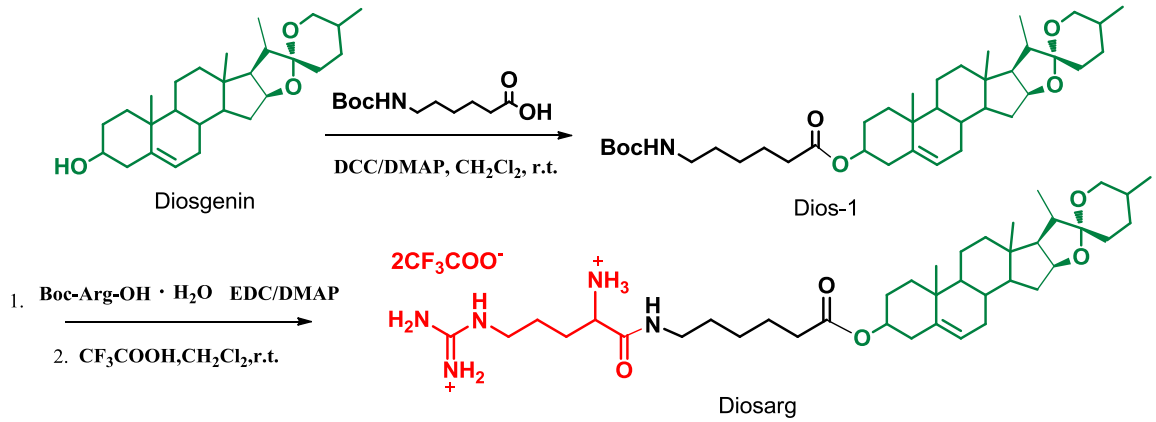
S1. Synthesis and Characterization of the Cationic Lipid Diosarg



Scheme S1. The synthesis routes of the cationic lipid Diosarg. (DCC: N,N'-dicyclohexylcarbodiimide, EDC: 1-Ethyl-3-(3-dimethylaminopropyl)carbodiimide, DMAP: 4-Dimethylaminopyridine)

The synthesis procedures of the cationic lipid Diosarg:

Step 1: In a 100-mL flask were added diosgenin (414 mg, 1.0 mmol), Boc-N-caproic acid (231 mg, 1.5 mmol) and N,N'-dicyclohexylcarbodiimide (DCC)/4-Dimethylaminopyridine (DMAP) (300 mg/100 mg) dissolved in 50 mL of dichloromethane, and the mixture was stirred at ambient temperature for 24 h. Then, the dicyclohexylurea (DCU) solids were removed by filtration; the residual solution was further concentrated and then purified by flash column chromatography (EtOAC/hexane = 1/3 *v*/*v*) to achieve Dios-1 as a white solid (isolated yield: 66.1%).

Step 2: In a 50-mL flask, the Dios-1 (320 mg, 0.5 mmol) was dissolved in the mixture solution of CH_2Cl_2/CF_3COOH (20 mL/5 mL) and stirred at ambient temperature for 2 h. Then, the solvent was removed under reduced pressure, and then Boc-protected arginine Boc-Arg·HCl·H_2O (250 mg, 0.75 mol), 1-Ethyl-3-(3-dimethylaminopropyl)carbodiimide (EDC)/DMAP (300 mg/100 mg), which was dissolved in the mixture solution of CH_2Cl_2/triethylamine (50 mL/5 mL), were added; then, the mixture was stirred at ambient temperature for 24 h and then washed with distilled water (3 × 100 mL), then concentrated under reduced pressure and purified by flash column chromatography (eluent: EtOAC/MeOH = 10/1 *v*/*v*) to get the Boc-protected Diosarg lipid precursor as a yellowish solid. Finally, the Boc groups were removed in CH_2Cl_2/CF_3COOH (5 mL/5 mL) after a 3-h reaction at room temperature, then the solvent was removed and precipitated with diethyl ether. The final product, cationic lipid Diosarg, was obtained as a white solid (isolated yield: 59.6%).

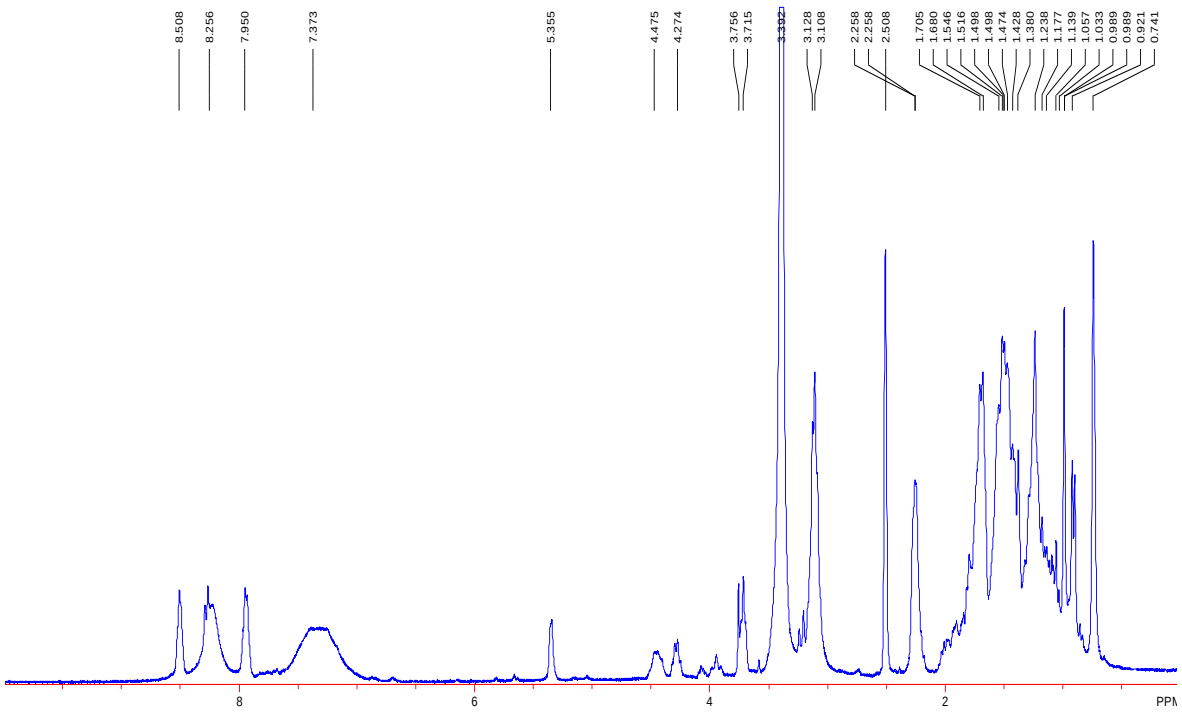


Figure S1. ^{1}H NMR spectra of the cationic lipid Diosarg.

Characterization data of the cationic lipid Diosarg:

^{1}H NMR (d_4-MeOD, 300 Hz): δ 7.70–8.50 (guanidyl-H, arginine), 7.0–7.60 (H, NH_3^+), 5.40 (1H, –CH=C, diosgenin), 4.51 (1H, COO–CH–), 4.18 (2H, OCO–CH_2, diosgenin), 3.11 (2H, Boc-NH–CH_2), 2.30 (2H, –CH_2COO–), 1.42 (9H, Boc), 2.23–0.61 (45H, diosgenin).

^{13}C NMR (d_4-MeOD, 75 Hz): δ 172.7, 168.4, 159.2, 157.3, 139.7, 119.1, 116.0, 108.7, 80.5, 73.9, 66.4, 65.8, 56.2, 52.2, 49.5, 41.5, 40.5, 40.1, 39.8, 39.2, 38.4, 36.8, 36.6, 34.8, 34.1, 32.0, 31.3, 31.1, 28.9, 28.7, 25.9, 25.0, 24.2, 19.6, 18.7, 17.5, 15.1.

ESI-MS: [M^+] = 684.5 (*m*/*z*), calculated: 684.5

S2. Hydrodynamic Average Particle Sizes of the Dios-DOPE (2:1, 1:1, 1:2) NPs in Distilled Water at Room Temperature Measured by the Dynamic Light Scattering Instrument

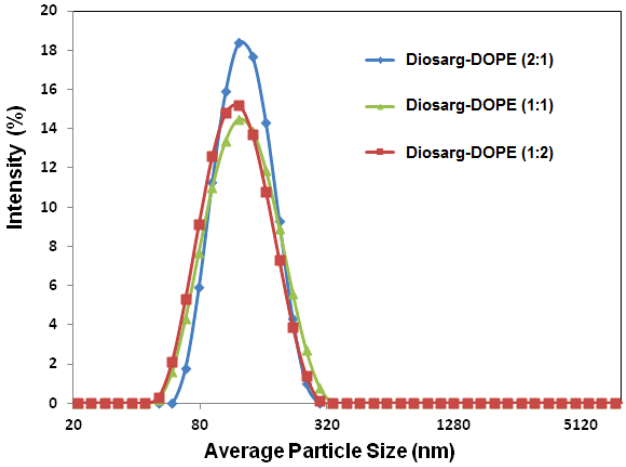


Figure S2. Hydrodynamic average particle sizes of the Diosarg-1,2-dioleoyl-sn-glycero-3-phosphor-ethanolamine (DOPE) (2:1, 1:1, 1:2) NPs in distilled water at room temperature measured by the dynamic light scattering instrument.

S3. Agarose-Gel pDNA Retardation Assay of the pDNA Binding Affinity for the Diosarg Lipid and Diosarg-DOPE NPs

Experimental procedures of the agarose-gel pDNA retardation assay:

Before the assay, the cationic lipid samples (Diosarg and Diosarg-DOPE NPs) were preliminarily dissolved in pure water to prepare the solution with the total Diosarg concentration of 1.7×10^{-3} M (same Diosarg concentration for all of the Diosarg and Diosarg-DOPE NPs); the Diosarg/pDNA and Diosarg-DOPE NPs/pDNA complexes were prepared by mixing pDNA (1 μg) with a predetermined amount of the cationic lipid sample stock solutions under a pre-set ± charge ratio in 50 μL PBS (0.01 M, pH = 7.4) buffer solution. After incubation at 37 °C for 20 min, the Diosarg/pDNA and Diosarg-DOPE NPs/pDNA complex solution were loaded onto a 1% agarose gel containing 0.5 μg/mL ethidium bromide dye (EB). Then, the gel electrophoresis was conducted in 1 × TAE running buffer under 100 mV for 30 minutes, and the pDNA migration bands were thus observed and recorded on a UVP benchtop 2UV transilluminator system.

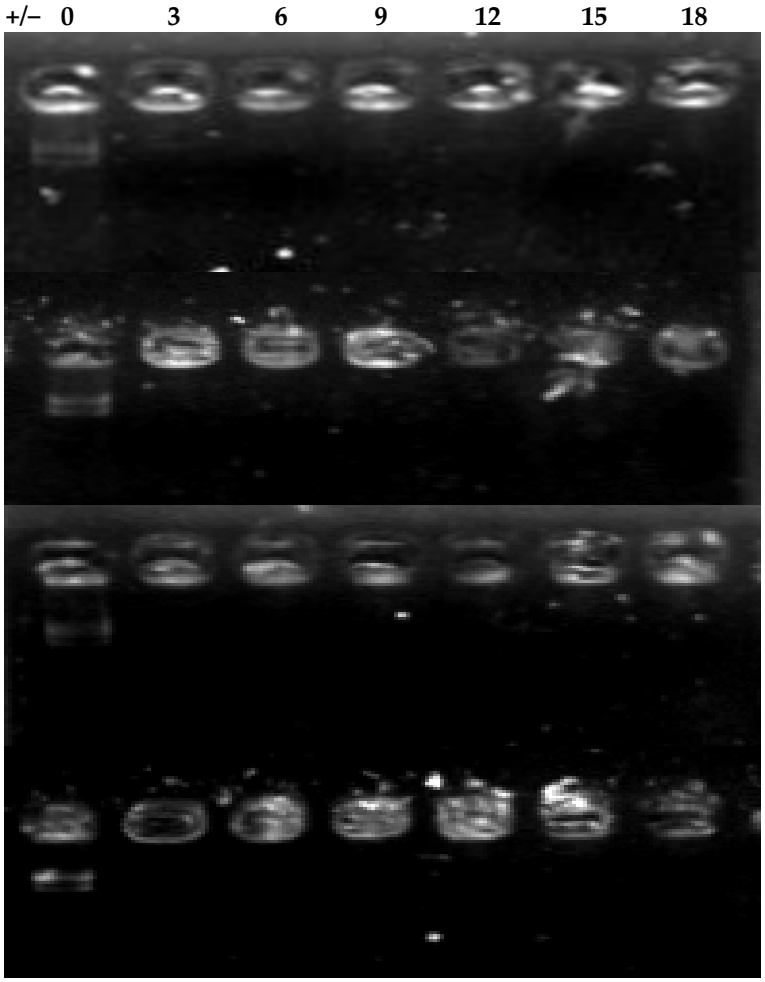


Figure S3. Agarose-gel pDNA retardation assay of the pDNA binding affinity for the Diosarg lipid and Diosarg-DOPE NPs; in each well, pDNA (1 μg) mixed with the Diosarg lipids and Diosarg-DOPE (2:1, 1:1, 1:2) NPs under various ± charge ratios (0–12), respectively.

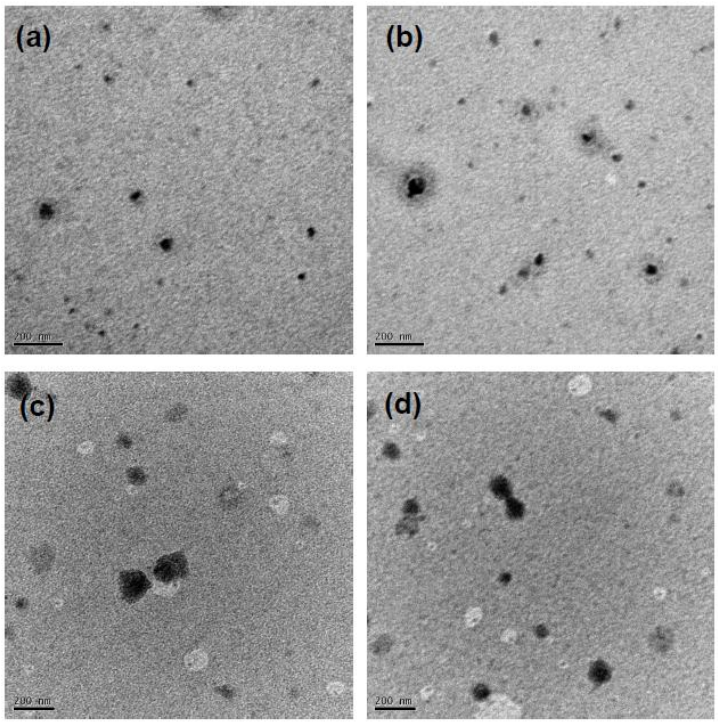
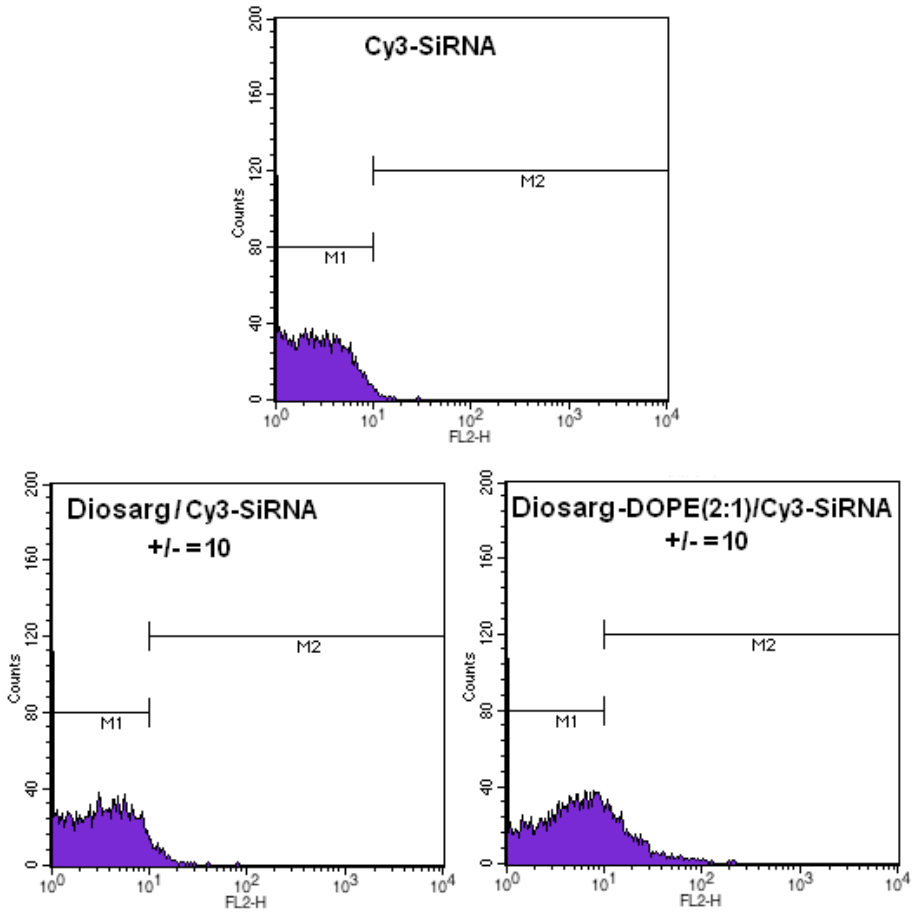


Figure S4. The morphology of the Diosarg-DOPE (2:1)/siRNA (**a**), Diosarg-DOPE (1:1)/siRNA (**b**), Diosarg-DOPE (2:1)/pDNA (**c**) and Diosarg-DOPE (1:1)/pDNA (**d**) complexes under a ± charge ratio of 15 was observed by transmission electron microscopy (TEM).



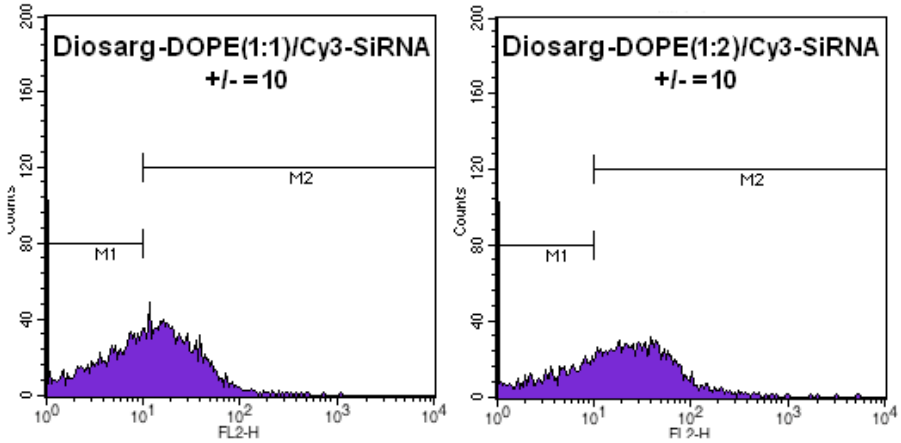


Figure S5. Intracellular uptake of the Diosarg lipid and Diosarg-DOPE (2:1, 1:1, 1:2) NPs/Cy3-siRNA complexes (± = 10) in H1299 cells measured by flow cytometry (10,000 cells for each sample).

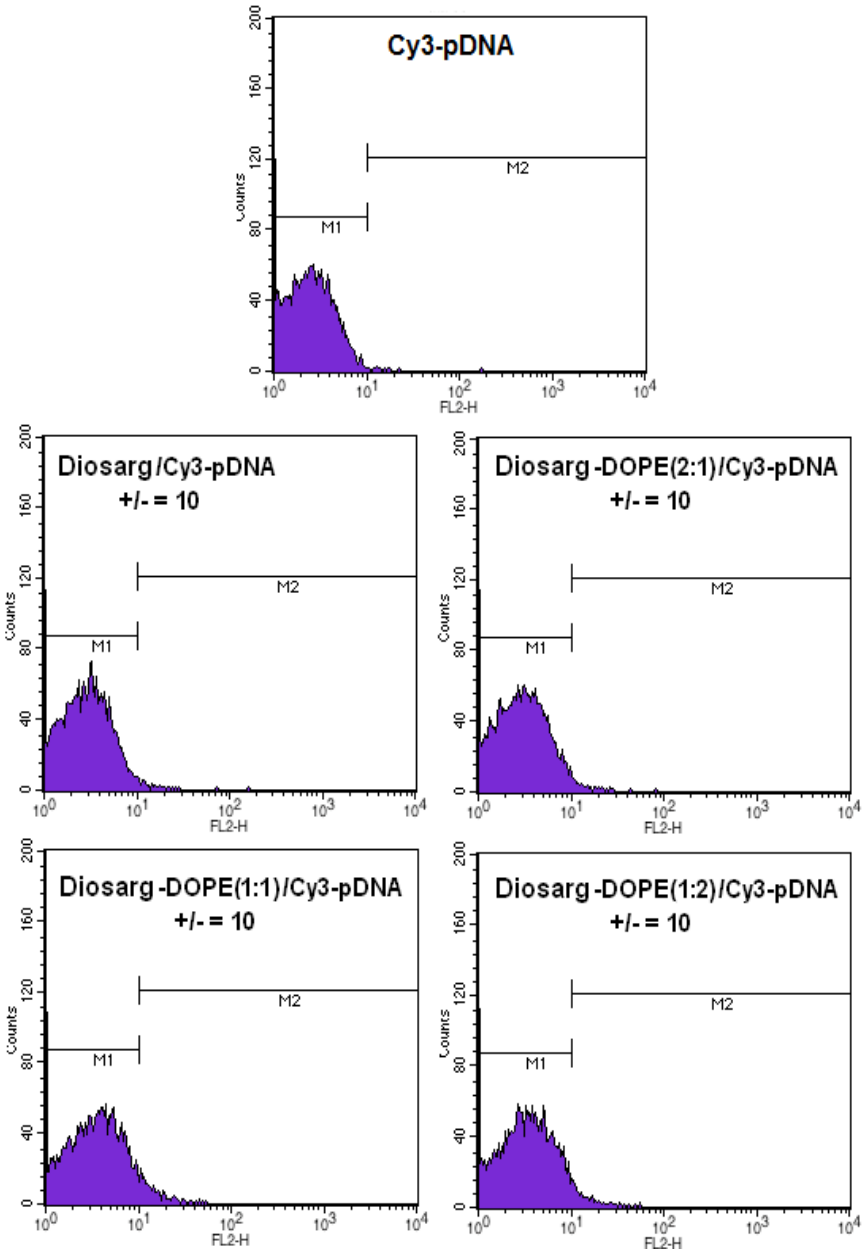


Figure S6. Intracellular uptake of the Diosarg lipid and Diosarg-DOPE (2:1, 1:1, 1:2) NPs/Cy3-pDNA complexes (± = 10) in H1299 cells measured by flow cytometry (10,000 cells for each sample).

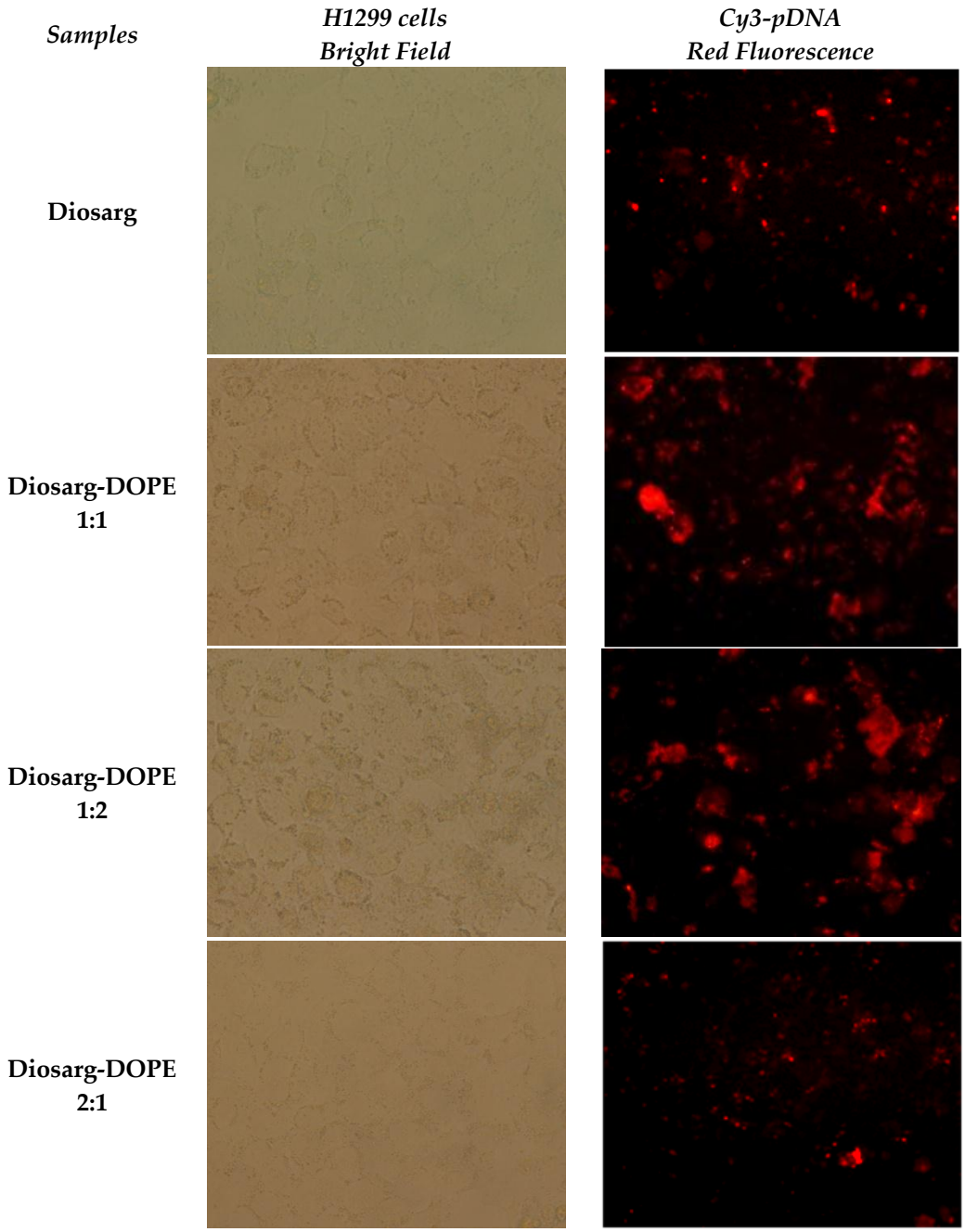


Figure S7. Fluorescence imaging of H1299 cells after six hours of incubation of the Diosarg/Cy3-pDNA and Diosarg-DOPE (2:1, 1:1, 1:2) NPs/Cy3-pDNA under a ± ratio of 15 indicated that the co-assembly of DOPE with the cationic Diosarg lipid could enhance the uptake of Cy3-pDNA.