Supporting Information

[12]aneN₃-based Gemini-type Amphiphiles with Two-photon Absorption Properties for Enhanced Non-viral Gene Delivery and Bioimaging

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1. Synthesis and Measurements

1.1. Synthesis of Compounds 1-11

Scheme 1 Schematic route for DEDPP-4, DEDPP-8 and DEDPP-12: (i) NaN₃, DMF, 65 °C, 8 h; (ii) KCO₃, Acetonitrile, Reflux, 24 h; (iii) NaOH (6 M), Reflux, 24 h; (iv) HCl (2 M); (v) N-methylmorpholine, EDCI, HOBT, r.t., 8 h; (vi) Propargyl-[12]aneN₃, CuBr, CHCl₃, r.t., 30 min; (vii) HCl-EtOAc, DCM, r.t., 2h.

**Synthesis of 1.** Under argon atmosphere, to the dry DMF (30 mL) solutions of 8-Bromo-1-octanol (10 mmol) were slowly added sodium azide (1.30 g, 20 mmol, 2.0 equiv.) at 0 °C. The mixture was slowly warmed up to 65 °C and stirred for overnight. After cooling to room temperature and filtering, saturated brine solution (50 mL) added to the system, and then the mixture solution was extracted with ethyl acetate (20 mL ×3). The collected organic layers were dried over anhydrous Na₂SO₄. After filtration and vacuum distillation, the crude product was purified by a silica gel column chromatography with PE/EtOAc (v/v = 15:1) as eluent to obtained compounds 1(1.54
g, 9 mmol) as colorless oil. Yield: 65%. $^1$H NMR (400 MHz, CDCl$_3$) δ 3.55 (t, $J = 6.6$ Hz, 2H), 3.21 (t, $J = 6.9$ Hz, 2H), 2.36 (s, 1H), 1.62 – 1.46 (m, 4H), 1.33 (dt, $J = 7.0$, 3.7 Hz, 4H). $^{13}$C NMR (101 MHz, CDCl$_3$): δ 62.57, 51.42, 32.54, 28.84, 26.57, 25.38.

**Synthesis of 3-5.**

Compound 2 (1.00 g, 3.18 mmol) were prepared according to literature methods and dissolved in 30 ml of anhydrous acetonitrile, and then 1-bromo-hydrocarbon (3.0 equiv.), K$_2$CO$_3$ (1.32 g, 9.55 mmol) and catalytic amount KI were added into the solution, under stirring at refluxing temperature for 48 h. After cooling to room temperature and filtering, the solvent was removed under reduced pressure, then the crude product was purified by column chromatography on silica gel with PE/EtOAc (v/v =10:1 ~ 20:1) as eluent to obtained corresponding compounds 3, 4, 5 as white solid in 60 ~ 75% yields.

**Compound 3:** 1.02 g, 2.31 mmol; Yield: 75%. $^1$H NMR (600 MHz, CDCl$_3$) δ 7.51 (d, $J = 8.5$ Hz, 4H), 6.83 (d, $J = 8.5$ Hz, 4H), 3.98 (t, $J = 6.6$ Hz, 4H), 1.77 (t, $J = 7.3$ Hz, 4H), 1.49 (q, $J = 7.5$ Hz, 4H), 0.98 (t, $J = 7.4$ Hz, 6H). $^{13}$C NMR (151 MHz, CDCl$_3$) δ 161.80, 154.29, 131.52, 128.60, 127.52, 114.87, 114.85, 113.60, 68.08, 31.23, 19.28, 13.92. HRMS-ESI (m/z): calcd. [M+H]$^+$ for [C$_{26}$H$_{27}$N$_4$O$_2$]$^+$, 427.2129; found 427.2131.

**Compound 4:** 1.16 g, 2.19 mmol; Yield: 68%. $^1$H NMR (600 MHz, CDCl$_3$) δ 7.52 (d, $J = 8.5$ Hz, 4H), 6.84 (d, $J = 8.3$ Hz, 4H), 3.97 (t, $J = 6.7$ Hz, 4H), 1.79 (t, $J = 7.4$ Hz, 4H), 1.45 (t, $J = 7.8$ Hz, 4H), 1.49 – 0.95 (m, 16H), 0.88 (t, $J = 6.8$ Hz, 6H). $^{13}$C NMR (151 MHz, CDCl$_3$) δ 161.79, 154.34, 131.52, 128.65, 127.56, 114.86, 113.58, 68.39, 31.89, 29.41, 29.30, 29.20, 26.08, 22.74, 14.19. HRMS-ESI (m/z): calcd. [M+H]$^+$ for [C$_{34}$H$_{43}$Na$_2$O$_2$]$^+$, 539.3381; found 539.3378.
**Compound 5**: 1.25 g, 1.92 mmol; Yield: 60%. $^1$H NMR (600 MHz, CDCl$_3$) $\delta$ 7.53 (d, $J = 8.5$ Hz, 4H), 6.84 (d, $J = 8.5$ Hz, 4H), 3.98 (t, $J = 6.6$ Hz, 4H), 1.79 (p, $J = 6.9$ Hz, 4H), 1.45 (p, $J = 7.4$ Hz, 4H), 1.40 – 1.16 (m, 36H), 0.88 (t, $J = 7.0$ Hz, 4H). $^{13}$C NMR (151 MHz, CDCl$_3$) $\delta$ 161.81, 154.37, 131.53, 128.69, 127.59, 114.88, 113.58, 68.40, 32.01, 29.80, 29.75, 29.73, 29.69, 29.66, 29.47, 29.44, 29.21, 26.09, 22.78, 14.20. HRMS-ESI (m/z): calcd. [M+Na]$^+$ for [C$_{42}$H$_{59}$N$_4$NaO$_2$]$^+$, 673.4452; found 673.4449.

**Synthesis of 6-8**

Compounds 3-5 (3 mmol) were dissolved in 30 ml of propanol, and then 60 ml of 6M KOH solution was added. The mixture was refluxed during 48 h and then cooled to room temperature. The pH was adjusted to 6–7 with 2 M HCl, a yellow color precipitate of colloidal appearance being obtained. The solid was filtered off, washed with ethanol and dried under high vacuum to give corresponding compounds 6, 7, 8 as pale yellow solid in 50 ~ 73%.

**Compound 6**: 1.70 g, 3.66 mmol; Yield: 73%. $^1$H NMR (600 MHz, CDCl$_3$) $\delta$ 7.49 (d, $J = 8.8$ Hz, 4H), 6.82 (d, $J = 8.8$ Hz, 4H), 1.48 (q, $J = 7.5$ Hz, 4H), 1.76 (dq, $J = 8.6$, 6.6 Hz, 4H), 0.97 (t, $J = 7.4$ Hz, 6H). $^{13}$C NMR (151 MHz, CDCl$_3$) $\delta$ 167.01, 160.64, 152.63, 139.89, 131.42, 128.59, 114.47, 67.88, 31.29, 19.29, 13.94. HRMS-ESI (m/z): calcd. [M-H]$^-$ for [C$_{26}$H$_{27}$N$_2$O$_6$]$^-$, 463.1875; found 463.1872.

**Compound 7**: 2.03 g, 3.52 mmol; Yield: 70%. $^1$H NMR (600 MHz, CDCl$_3$) $\delta$ 7.52 (d, $J = 8.2$ Hz, 4H), 6.84 (d, $J = 8.2$ Hz, 4H), 3.96 (t, $J = 6.5$ Hz, 4H), 1.77 (q, $J = 7.6$ Hz, 4H), 1.44 (q, $J = 7.4$ Hz, 4H), 1.38 – 1.21 (m, 16H), 0.88 (t, $J = 6.9$ Hz, 6H). $^{13}$C NMR (151 MHz, CDCl$_3$) $\delta$ 167.03, 160.75, 152.61, 139.55, 131.44, 128.49, 114.53, 68.29,
Compounds 6-8, 2.5 mmol; Yield: 50%. \[^1\text{H}\text{ NMR (600 MHz, CDCl}_3\text{)}\] δ 7.53 (d, \(J = 6.5\text{ Hz, 4H}\)), 6.84 (d, \(J = 7.5\text{ Hz, 4H}\)), 3.97 (t, \(J = 6.1\text{ Hz, 4H}\)), 1.78 (p, \(J = 6.6\text{ Hz, 4H}\)), 1.44 (p, \(J = 6.8\text{ Hz, 4H}\)), 1.38 – 1.03 (m, 36H), 0.87 (t, \(J = 7.0\text{ Hz, 4H}\)). \[^{13}\text{C NMR (151 MHz, CDCl}_3\text{)}\] δ 166.22, 159.99, 151.86, 138.64, 130.63, 127.67, 113.74, 67.47, 31.24, 28.98, 28.93, 28.90, 28.74, 28.65, 28.51, 25.35, 22.00, 13.39. HRMS-ESI (m/z): calcd. [M-H] \(^-\) for \([\text{C}_{34}\text{H}_{43}\text{N}_2\text{O}_6]^-\), 575.3127; found 575.3130.

**Synthesis of 9-11**

Under argon atmosphere, compounds 6-8 (2.5 mmol), HOBt (0.85 g, 6.3 mmol), N-methylmorpholine (1.01 g, 10.0 mmol), and EDCI (1.44 g, 7.5 mmol) were dissolved in anhydrous DMF (10 mL). The solution was kept stirring for 30 minutes at 0 °C, then compound 1 (1.28 g, 7.5 mmol) in 5 mL anhydrous DMF was added dropwise, the mixture allowed to warm up to room temperature and stirred for overnight. 40 mL water added slowly to quench the reaction and a yellow solid precipitated, after filtration and vacuum distillation, the crude product was purified by column chromatography on silica gel with PE/EtOAc (v/v = 10:1 ~ 20:1) as eluent to give corresponding compounds 9, 10, 11 as yellow solid in 52 ~ 62% yields.

**Compound 9:** 0.81 g, 1.05 mmol; Yield: 52%. \[^1\text{H}\text{ NMR (600 MHz, CDCl}_3\text{)}\] δ 7.48 (d, \(J = 8.8\text{ Hz, 4H}\)), 6.79 (d, \(J = 8.8\text{ Hz, 4H}\)), 4.38 (t, \(J = 6.8\text{ Hz, 4H}\)), 3.93 (t, \(J = 6.5\text{ Hz, 4H}\)), 3.20 (t, \(J = 6.9\text{ Hz, 4H}\)), 1.81 – 1.67 (m, 8H), 1.55 (p, \(J = 6.9\text{ Hz, 4H}\)), 1.44 (tt, \(J = 14.8, 6.6\text{ Hz, 8H}\)), 0.93 (t, \(J = 7.4\text{ Hz, 6H}\)). \[^{13}\text{C NMR (151 MHz, CDCl}_3\text{)}\] δ 165.10, 165.10, 165.10, 165.10, 165.10, 165.10, 165.10, 165.10, etc.
160.49, 152.24, 141.09, 131.37, 129.49, 114.50, 67.80, 66.41, 51.46, 31.29, 29.14, 29.09, 28.87, 28.51, 26.68, 25.85, 19.28, 13.90. HRMS-ESI (m/z): calcd. [M+H]^+ for [C_{42}H_{59}N_{8}O_{6}]^+, 771.4552; found 771.4556.

**Compound 10:** 1.10 g, 1.25 mmol; Yield: 62%. $^1$H NMR (600 MHz, CDCl$_3$) $\delta$ 7.49 (d, $J = 8.7$ Hz, 4H), 6.80 (d, $J = 8.7$ Hz, 4H), 4.38 (t, $J = 6.9$ Hz, 4H), 3.93 (t, $J = 6.6$ Hz, 4H), 3.21 (t, $J = 7.0$ Hz, 4H), 1.76 (dt, $J = 14.4$, 7.1 Hz, 8H), 1.55 (q, $J = 7.4$ Hz, 4H), 1.42 (q, $J = 7.3$ Hz, 8H), 1.38 – 1.24 (m, 28H), 0.87 (t, $J = 7.4$ Hz, 6H). $^{13}$C NMR (151 MHz, CDCl$_3$) $\delta$ 165.10, 160.49, 152.25, 141.09, 131.38, 129.50, 114.50, 68.14, 66.41, 51.47, 31.88, 29.42, 29.30, 29.26, 29.15, 29.10, 28.89, 28.52, 26.69, 26.10, 25.86, 22.72, 14.16. HRMS-ESI (m/z): calcd. [M+Na]^+ for [C_{50}H_{74}N_{8}NaO_{6}]^+, 905.5624; found 905.5620.

**Compound 11:** 1.16 g, 1.17 mmol; Yield: 58%. $^1$H NMR (600 MHz, CDCl$_3$) $\delta$ 7.49 (d, $J = 8.7$ Hz, 4H), 6.80 (d, $J = 8.7$ Hz, 4H), 4.39 (t, $J = 6.8$ Hz, 4H), 3.93 (t, $J = 6.6$ Hz, 4H), 3.22 (t, $J = 7.0$ Hz, 4H), 1.76 (tt, $J = 14.2$, 6.8 Hz, 8H), 1.57 (p, $J = 6.9$ Hz, 4H), 1.43 (dt, $J = 14.6$, 7.0 Hz, 8H), 1.39 – 1.19 (m, 44H), 0.86 (t, $J = 7.0$ Hz, 6H). $^{13}$C NMR (151 MHz, CDCl$_3$) $\delta$ 165.11, 160.49, 152.25, 141.09, 131.38, 129.50, 114.50, 68.14, 66.41, 51.48, 32.00, 29.74, 29.72, 29.68, 29.65, 29.47, 29.43, 29.27, 29.16, 29.10, 28.89, 28.52, 26.70, 26.11, 25.86, 22.77, 14.19. HRMS-ESI (m/z): calcd. [M+H]^+ for [C_{58}H_{91}N_{8}O_{6}]^+, 995.7056; found 995.7060.
1.2. Photo-physical Characterization and Assembly of DEDPP Derivatives

1.2.1. Fluorescence Measurements.

DEDPP-4/8/12 were dissolved in DMSO to obtain the stock solutions (1 mM). The stock solutions were diluting to certain volumes using deionized Milli-Q water and organic solvent to get different concentrations. Trace-volume (100 or 50 μL) quartz cuvettes were used to perform DNA fluorescence titrations. All of the samples were vortex mixed and allowed to stand for 6 - 10 min before testing.

1.2.2. Gel Retardation Assay

The samples were prepared by adding condensing agents with different concentrations to pUC18 DNA (9 μg/mL) in Tris-HCl buffer (50 mM, pH 7.4) with a total volume of 20 μL at room temperature. After interaction with negatively supercoiled pUC18 DNA at 37° C for 45 min, 2 μL of 6× loading buffer were added to the above solution. The samples were electrophoresed at 120 V on a 0.7% agarose gel (stained with 2 μL of 5000× Goldview II) in 1× TAE running buffer for 40 min. The result was visualized under on a UVP EC3 visible imaging system.

1.2.3. Assembly of DEDPP Derivatives

The morphologies of micelles and complexes were investigated by scanning electronic microscopy (SEM), transmission electron microscopy (TEM) and dynamic light scattering (DLS) techniques. The DLS sample were prepared similar to gel retardation assay, the solution was diluted to 200 μL after interaction with negatively supercoiled pUC18 DNA at 37°C for 45 min. The diluted solution 5 μL dropped on the surface of wafer and ultrathin carbon film, then completely evaporated to obtain the SEM and TEM samples.

1.3. In Vitro and in Vivo Experiments
1.3.1. Cell Toxicity

The cytotoxicity of lipoplexes measured by MTT assay in four different cell lines (HeLa, A549, HepG2 and HEK293T). All cells were cultured in complete medium in a humid atmosphere containing 5% CO₂ at 37 ºC. After 48 h of incubation, the cells were seeded in 96-well plate at 5000 cells and 100 μL medium per well and cultured for another 24 h. Different concentrations of lipoplexes in 100 μL DMEM were added into six parallel wells. After 4 h cultured, 10 μL DMEM and 10% FBS were added to each well and cultured for 20 h. The medium was replaced with 20 μL of MTT (5 mg/mL) and incubated for another 4 h. Finally, MTT was replaced with 200 μL of DMSO, and the plates were oscillated for 10 min to fully dissolve the formazan crystals formed by living cells in the wells. The optical density (OD) at 490 nm was recorded at 490 nm using a Thermo Scientific Multiskan GO. The viability of the cells was calculated as cell viability by using the following formula:

\[
\text{Cell viability} = \frac{OD_{490}(\text{Sample}) - OD_{490}(\text{Blank})}{OD_{490}(\text{Control}) - OD_{490}(\text{Blank})} \times 100\%
\]

1.3.2. Endocytosis Inhibition Studies.

Three endocytosis inhibitors (50 μM CPZ, 2.5 mM MβCD or 75 μM AM) preincubated with HeLa cells for 1h, then the complexes (1 mL) in DMEM was added to each well. After 2 h incubation at 37 ºC, Cy5 positive cells were compared, determining which inhibitor played a critical role in decreasing the cellular uptake. In addition, to study whether the cellular uptake of DEDPP-8/DOPE is energy-dependent, the polyplex was added to the cells, and cultured at 4 ºC for 2h.

1.3.3. RFP Transfections in Vivo.
Zebrafish embryos were provided by the China Zebrafish Resource Center. The **DEDPP-8/DOPE-DNA** complexes formed by 10 µg/mL pDsRed-DNA and 20 µM of **DEDPP-8/DOPE** in water for 30 min. Zebrafish embryos were incubated with 1 mL solution containing the complexes for 5 h, then 1 mL fresh water was added and further incubated for 24 h. The fluorescence intensity of RFP was measured by confocal imaging, and Lipo2000 was used as a control. The relevant ethical protocols used for the *in vivo* study for zebrafish embryos were followed by the relevant laws.
2. Characterizations of DEDPP Derivatives

2.1. Uv-Visible Spectra of the Compounds in Different Solvents

Figure S1 UV-vis spectra of DEDPP-4 (A), DEDPP-8 (B) and DEDPP-12 (C) in different solutions. Concentration: 10 μM.
**Table S1** Summarized photophysical data of DEDPP-4, DEDPP-8 and DEDPP-12.

<table>
<thead>
<tr>
<th></th>
<th>DEDPP-4</th>
<th></th>
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<th></th>
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<tr>
<td></td>
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<td>$\lambda_{em}$ (nm)</td>
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<td>$\Phi_F$</td>
<td>$\lambda_{ab}$ (nm)</td>
<td>$\lambda_{em}$ (nm)</td>
<td>Stoke’s shift (10$^3$ cm$^{-1}$)</td>
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Abbreviation: $\lambda_{ab}$ = absorption maximum, $\lambda_{em}$ = emission maximum, $\phi_F$ = fluorescence quantum yield, concentration: 10 $\mu$M.
Figure S2 Plot of the emission maximum of DEDPP-4(A), DEDPP-8(B), DEDPP-12(C) in different solvents versus $E_T(30)$, $E_T(30)$ was the empirical parameter for solvent polarity. Concentrations: 10 $\mu$M, $\lambda_{ex} = 340$ nm.
2.2. Two-photon Effect Measurement

Figure S3 TPEF spectra of DEDPP-4 (A), DEDPP-8 (B) and DEDPP-12 (C) at different excitation wavelengths of 700-680 nm. Concentration: 10 μM.
Figure S4 TPEF spectra of DEDPP-4 (A), DEDPP-8 (B) and DEDPP-12 (C) at different excitation energies at 640 nm. Concentration: 10 μM.
2.3. Spectroscopic characterizations and Size distributions of micelles

Figure S5 Fluorescence spectra of DEDPP-4 (A), DEDPP-8 (B), and DEDPP-12 (C) in water at varying concentrations. $\lambda_{ex} = 340$ nm.
Figure S6 TEM images of DEDPP-4 (A), DEDPP-8 (B), DEDPP-12 (C), DEDPP-4/DOPE (D), DEDPP-8/DOPE (E), and DEDPP-12/DOPE (F) in water. Scale bars: 1.00 μm.
Figure S7 Size distributions of micelles determined by DLS, DEDPP-4 (A), DEDPP-8 (B), and DEDPP-12 (C). Concentrations = 5 µM.
Figure S8 SEM images of DEDPP-4/DOPE (A), DEDPP-8/DOPE (B), and DEDPP-12/DOPE (C) in water. Scale bars: 5.00 μm.
Figure S9 Size distributions of micelles determined by DLS, DEDPP-4/DOPE (A), DEDPP-8/DOPE (B), and DEDPP-12/DOPE (C). Concentrations = 5 µM.
Figure S10 Fluorescence intensities of DEDPP-4 (A), DEDPP-8 (B) and DEDPP-12 (C) upon UV 365 nm bleaching for different time. Concentrations = 10 µM, λ<sub>ex</sub> = 340 nm. Data represent mean ± SD (n = 3).
2.4. N/P ratio, TEM, DLS and Zeta Potentials for DEDPP derivatives with pDNA

Table S2 N/P ratio for DEDPP-4/DOPE, DEDPP-8/DOPE, and DEDPP-12/DOPE with pDNA. DEDPP-4/DOPE, 14 μM; (B) DEDPP-8/DOPE, 12 μM; (C) DEDPP-12/DOPE, 10 μM. [DNA] = 9 μg/mL.

<table>
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<tr>
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<th>DEDPP-4/DOPE-DNA</th>
<th>DEDPP-8/DOPE-DNA</th>
<th>DEDPP-12/DOPE-DNA</th>
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<tr>
<td>N/P</td>
<td>4.04</td>
<td>3.46</td>
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Figure S11 SEM images of condensed pUC18 DNA by **DEDPP-4** (14 µM) (G), **DEDPP-8** (12 µM) (H), **DEDPP-12** (10 µM) (I), scale bars: 1.00 µm.
Figure S12 TEM images of condensed pUC18 DNA by DEDPP-4 (14 μM) (A), DEDPP-8 (12 μM) (B), DEDPP-12 (10 μM) (C), DEDPP-4/DOPE (12 μM) (D), DEDPP-8/DOPE (12 μM) (E), DEDPP-12/DOPE (10 μM) (F), scale bars: 200 nm.
Figure S13 Particle size distributions of condensed pUC18 DNA (9 μg/mL) in Tris-HCl buffer (5 mM, pH 7.4): (A) DEDPP-4, 14 μM; (B) DEDPP-8, 12 μM; (C) DEDPP-12/DOPE, 10 μM. [DNA] = 9 μg/mL, 37 °C.
Figure S14 Zeta potentials of pUC18 DNA complexes in 50 mM Trips-HCl (pH 7.4): (A) **DEDPP-4**, 14 μM; (B) **DEDPP-8**, 12 μM; (C) **DEDPP-12**, 10 μM. (D) Zeta potential histogram of pUC18 DNA complex. [DNA] = 9 μg/mL, 37 °C. Data represent mean ± SD (n = 3).
Figure S15 Particle size distributions of condensed pUC18 DNA (9 μg/mL) in Tris-HCl buffer (5 mM, pH 7.4): (A) DEDPP-4/DOPE, 12 μM; (B) DEDPP-8/DOPE, 12 μM; (C) DEDPP-12/DOPE, 10 μM. The molar ratio of DEDPP derivatives/DOPE was 1: 2, [DNA] = 9 μg/mL, 37 °C.
Figure S16 Zeta potentials of pUC18 DNA complexes in 50 mM Trips-HCl (pH 7.4):

(A) DEDPP-4/DOPE, 14 μM; (B) DEDPP-8/DOPE, 12 μM; (C) DEDPP-12/DOPE, 10 μM. (D) Zeta potential histogram of pUC18 DNA complex. The molar ratio of DEDPP derivatives/DOPE was 1: 2, [DNA] = 9 μg/mL, 37 °C. Data represent mean ± SD (n = 3).
Figure S17 Average particle size of pUC18 DNA complexes in medium (with 20% FBS) for different time. (A) DEDPP-4/DOPE, 14 μM; (B) DEDPP-8/DOPE, 12 μM; (C) DEDPP-12/DOPE, 10 μM. The molar ratio of DEDPP derivatives/DOPE was 1: 2. [DNA] = 9 μg/mL, 37 °C. Data represent mean ± SD (n = 3).
2.5. Fluorescence titrations with ctDNA

Figure S18 Linearly fitting functions deduced from the plots of emission intensity changes at 466 nm: (A) DEDPP-4/DOPE, (B) DEDPP-8/DOPE, (C) DEDPP-12/DOPE. Concentrations = 10 \( \mu \text{M} \), \( \lambda_{ex} = 340 \text{ nm} \). The molar ratio of DEDPP derivatives/DOPE was 1:2.
Table S3. Emission enhancement calculated from the fluorescent titration of ctDNA.

<table>
<thead>
<tr>
<th>ctDNA (μg/mL)</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>7</th>
</tr>
</thead>
<tbody>
<tr>
<td>DEDPP-4/DOPE</td>
<td>11.2</td>
<td>20.5</td>
<td>28.8</td>
<td>38.6</td>
<td>49.0</td>
<td>56.0</td>
<td>64.7</td>
</tr>
<tr>
<td>DEDPP-8/DOPE</td>
<td>1.20</td>
<td>1.40</td>
<td>1.66</td>
<td>1.92</td>
<td>2.26</td>
<td>2.57</td>
<td>2.93</td>
</tr>
<tr>
<td>DEDPP-12/DOPE</td>
<td>1.14</td>
<td>1.26</td>
<td>1.42</td>
<td>1.54</td>
<td>1.69</td>
<td>1.96</td>
<td>2.21</td>
</tr>
</tbody>
</table>
2.6. Size distributions of DNA complexes

**Figure S19** Particle size distributions of DEDPP derivatives addition of 7 μg ctDNA at a concentration of 10 μM in Tris-HCl buffer (5 mM, pH = 7.4); (A) DEDPP-4/DOPE, (B) DEDPP-8/DOPE, (C) DEDPP-12/DOPE. Concentrations = 10 μM; The molar ratio of DEDPP derivatives/DOPE was 1: 2.
2.7. EB competition experiment

Figure S20 Fluorescence quenching curves of EB-bounded ctDNA by DEDPP-4/DOPE (A), DEDPP-8/DOPE (B), and DEDPP-12/DOPE (C). The molar ratio of DEDPP derivatives/DOPE was 1:2. $\lambda_{ex} = 537$ nm, [EB] = 20 $\mu$M, [DNA] = 100 $\mu$M, 25 $^\circ$C, Tris-HCl = 5 mM, NaCl = 50 mM, pH = 7.4.
Figure S21 Linearly fitting functions deduced from the plots of emission intensity changes at 610 nm by quenching curves of EB-bounded ctDNA: DEDPP-4/DOPE (A), DEDPP-8/DOPE (B), DEDPP-12/DOPE (C). The molar ratio of DEDPP derivatives/DOPE was 1: 2.
2.8. IC₅₀ of the DEDPP derivatives

Table S4 Potency of DEDPP-4/8/12-DOPE for the tested cell lines.

<table>
<thead>
<tr>
<th>Anti-proliferative activities (IC₅₀ µM) Æ</th>
<th>HeLa</th>
<th>A549</th>
<th>HepG2</th>
<th>HCK293T</th>
</tr>
</thead>
<tbody>
<tr>
<td>DEDPP-4/DOPE</td>
<td>74.3</td>
<td>171.2</td>
<td>164.6</td>
<td>322.5</td>
</tr>
<tr>
<td>DEDPP-8/DOPE</td>
<td>767.4</td>
<td>&gt;1000</td>
<td>97.9</td>
<td>&gt;1000</td>
</tr>
<tr>
<td>DEDPP-12/DOPE</td>
<td>510.3</td>
<td>&gt;1000</td>
<td>243.8</td>
<td>&gt;1000</td>
</tr>
</tbody>
</table>

Æ IC₅₀ values are reported as means of at least three independent experiments, and SD values are lower than 10%. Over 100 expressed as inactive.
2.9. Cellular transfection assays

**Figure S22** Luciferase gene expressions transfected by **DEDPP-4/8/12** in four cell lines with Lipo2000 as control. HeLa cell lines (A), A549 cell lines (B), HepG2 cell lines (C), HEK293T cell lines (D); [pGL-3] = 10 μg/mL. Data represent mean ± SD (n = 3).
Figure S23 Luciferase expressions transfected by liposomes DEDPP-4/DOPE (A), DEDPP-8/DOPE(B), DEDPP-12/DOPE(C), with molar ratios of 1:1:1:3 at varying concentrations in HeLa cell lines. [pGL-3] = 10 μg/mL. Data represent mean ± SD (n = 3).
Figure S24 Luciferase gene expressions transfected by DEDPP-4/8/12 in the present of DOPE (molar ratio 1:2) in HeLa cell lines (A), A549 cell lines (B), HepG2 cell lines (C), HEK293T cell lines (D). Data represent mean ± SD (n = 3).
Table S5 Optimal luciferase expressions of **DEDPP-4/8/12** in the absent and present of DOPE in different cell lines (% of lipo2000). The molar ratio of **DEDPP** erivatives and DOPE was 1:2.

<table>
<thead>
<tr>
<th></th>
<th>DEDPP-4</th>
<th>DEDPP-8</th>
<th>DEDPP-12</th>
<th>DEDPP-4/DOPE</th>
<th>DEDPP-8/DOPE</th>
<th>DEDPP-12/DOPE</th>
</tr>
</thead>
<tbody>
<tr>
<td>HeLa</td>
<td>40</td>
<td>190</td>
<td>90</td>
<td>720</td>
<td>2350</td>
<td>1250</td>
</tr>
<tr>
<td>A549</td>
<td>45</td>
<td>60</td>
<td>50</td>
<td>310</td>
<td>650</td>
<td>240</td>
</tr>
<tr>
<td>HepG2</td>
<td>15</td>
<td>130</td>
<td>12</td>
<td>220</td>
<td>810</td>
<td>170</td>
</tr>
<tr>
<td>HEK293T</td>
<td>85</td>
<td>120</td>
<td>60</td>
<td>860</td>
<td>1760</td>
<td>950</td>
</tr>
</tbody>
</table>
Figure S25 Luciferase gene expressions transfected by DEDPP-8 (A) and DEDPP-8/DOPE (B) in the media containing 20% serum in four cell lines; [pGL-3] = 10 μg/mL. Data represent mean ± SD (n = 3).
Table S6 Optimal luciferase expressions of **DEDPP-8** and **DEDPP-8/DOPE** in the media containing 20% serum in four cell lines (% of lipo2000). The molar ratio of **DEDPP-8** and DOPE was 1:2,

<table>
<thead>
<tr>
<th></th>
<th>HeLa</th>
<th>A549</th>
<th>HepG2</th>
<th>HEK293T</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>DEDPP-8</strong></td>
<td>40</td>
<td>20</td>
<td>30</td>
<td>50</td>
</tr>
<tr>
<td><strong>DEDPP-8/DOPE</strong></td>
<td>550</td>
<td>120</td>
<td>110</td>
<td>420</td>
</tr>
</tbody>
</table>
2.10. Cellular uptake studies

Figure S26 Cellular uptake of DEDPP-8/DOPE-DNA complexes incubated at 37 °C (A) and 4 °C (B) in HeLa cell lines. [DEDPP-8/DOPE] = 25 μM, [pDsRed-N1] = 10 μg/mL.
Figure S27. Cellular uptake of DEDPP-8/DOPE-DNA complexes in the presence of methyl-β-cyclodextrin (A), amiloride hydrochloride (B) and chlorpromazine (C) in HeLa cell lines. \([\text{DEDPP-8/DOPE}] = 25 \, \mu\text{M}, \, [\text{pDsRed-N1}] = 10 \, \mu\text{g/mL}.\)
2.11. Confocal microscopy images for the delivery process

Figure S28 CLSM images of transport process into HeLa cells after treated with DEDPP-8/DOPE (1:2) and Cy5-labeled DNA for 0.5, 1, 3, and 6 h. The nuclei were stained with KFS147. (a) The nucleus (green channel). (b) **DEDPP-8/DOPE** (blue channel). (c) Cy5-labeled DNA (red channel). (d) Bright field. (d) Merge of a, b, c, d.

[**DEDPP-8**] = 20 μM, [Cy5-DNA] = 10 μg/mL, scale bar: 20 μm.
Figure S29 CLSM images of DEDPP-8/DOPE incubated with HeLa cells for 10 min, 0.5 h, 1 h, 3 h, and 6 h. The nuclei were stained with KFS147. (a) DEDPP-8/DOPE (blue channel). (b) The nucleus (green channel). (c) Bright field. (d) Merge of a, b, c. [DEDPP-8/DOPE] = 20 μM, scale bar: 20 μm.
Figure S30 Two-photon CLSM images of DEDPP-8/DOPE incubated with HeLa cells for 10 min, 0.5 h, 1 h, 3 h, and 6 h. The nuclei were stained with KFS147. (a) DEDPP-8/DOPE (blue channel). (b) The nucleus (green channel). (c) Merge of a, b. [DEDPP-8/DOPE] = 20 μM, λ_ex = 800 nm, scale bar: 20 μm.
2.12. Elemental analysis of DEDPP-4, DEDPP-8 and DEDPP-12

Table S7 Elemental analysis of the obtained DEDPP-4, DEDPP-8 and DEDPP-12 for their hydrochloride salts.

<table>
<thead>
<tr>
<th>Name</th>
<th>Nitrogen [%]</th>
<th>Carbon [%]</th>
<th>Hydrogen [%]</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>calcd.</td>
<td>found</td>
<td>calcd.</td>
</tr>
<tr>
<td>DEDPP-4</td>
<td>13.24</td>
<td>13.11</td>
<td>53.52</td>
</tr>
<tr>
<td>DEDPP-8</td>
<td>12.31</td>
<td>11.98</td>
<td>55.78</td>
</tr>
<tr>
<td>DEDPP-12</td>
<td>11.50</td>
<td>11.36</td>
<td>57.74</td>
</tr>
</tbody>
</table>
3. $^1$H, $^{13}$C-NMR spectra and HR-MS of synthesized compounds

**Figure S31** $^1$H NMR spectrum of 1 (400 MHz, CDCl$_3$).

**Figure S32** $^{13}$C NMR spectrum of 1 in CDCl$_3$ (101 MHz, CDCl$_3$).
Figure S33 $^1$H NMR spectrum of 3 (600 MHz, CDCl$_3$).

Figure S34 $^{13}$C NMR spectrum of 3 in CDCl$_3$ (151 MHz, CDCl$_3$).
Figure S35 HR-MS spectrum of 3.

Figure S36 $^1$H NMR spectrum of 4 (600 MHz, CDCl$_3$).
Figure S37 $^{13}$C NMR spectrum of 4 in CDCl$_3$ (151 MHz, CDCl$_3$).

Figure S38 HR-MS spectrum of 4.
Figure S39 $^1$H NMR spectrum of 5 (600 MHz, CDCl$_3$).

Figure S40 $^{13}$C NMR spectrum of 5 in CDCl$_3$ (151 MHz, CDCl$_3$).
Figure S41 HR-MS spectrum of 5.

Figure S42 $^1$H NMR spectrum of 6 (600 MHz, CDCl$_3$).
Figure S43 $^{13}$C NMR spectrum of 6 in CDCl$_3$ (151 MHz, CDCl$_3$).

Figure S44 HR-MS spectrum of 6.
Figure S45 $^1$H NMR spectrum of 7 (600 MHz, CDCl$_3$).

Figure S46 $^{13}$C NMR spectrum of 7 in CDCl$_3$ (151 MHz, CDCl$_3$).
Figure S47 HR-MS spectrum of 7.

Figure S48 $^1$H NMR spectrum of 8 (600 MHz, CDCl$_3$).
Figure S49 $^{13}$C NMR spectrum of 8 in CDCl$_3$ (151 MHz, CDCl$_3$).

Figure S50 HR-MS spectrum of 8.
Figure S51 $^1$H NMR spectrum of 9 (600 MHz, CDCl$_3$).

Figure S52 $^{13}$C NMR spectrum of 9 in CDCl$_3$ (151 MHz, CDCl$_3$).
Figure S53 HR-MS spectrum of 9.

Figure S54 $^1$H NMR spectrum of 10 (600 MHz, CDCl$_3$).
Figure S55 $^{13}$C NMR spectrum of 10 in CDCl$_3$ (151 MHz, CDCl$_3$).

Figure S56 HR-MS spectrum of 10.
**Figure S57** $^1$H NMR spectrum of 11 (600 MHz, CDCl$_3$).

**Figure S58** $^{13}$C NMR spectrum of 11 in CDCl$_3$ (151 MHz, CDCl$_3$).
Figure S59 HR-MS spectrum of 11.

Figure S60 $^1$H NMR spectrum of DEDPP-4 (600 MHz, DMSO).
Figure S61 $^{13}$C NMR spectrum of DEDPP-4 in DMSO (151 MHz, DMSO).

Figure S62 HR-MS spectrum of DEDPP-4.
Figure S63 $^1$H NMR spectrum of DEDPP-8 (600 MHz, DMSO).

Figure S64 $^{13}$C NMR spectrum of DEDPP-8 in DMSO (151 MHz, DMSO).
Figure S65 HR-MS spectrum of DEDPP-8.

Figure S66 $^1$H NMR spectrum of DEDPP-12 (600 MHz, DMSO).
Figure S67 $^{13}$C NMR spectrum of DEDPP-12 in DMSO (151 MHz, DMSO).

Figure S68 HR-MS spectrum of DEDPP-12.

References: