Supporting Information

One-pot Modular Assembly Strategy for Triple-play Enhanced Cytosolic siRNA Delivery

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1. Synthesis of amino acid based cationic lipid ($LG2C_{14}$)

1.1 Synthesis of ditetradecyl L-glutamate ($G2C_{14}$)

$G2C_{14}$ was synthesized as described in our previous study.[1] L-Glutamic acid (11.8 g, 80.2 mmol) and *p*-toluene sulfonic acid (TsOH) (18.3 g, 96.2 mmol) were dissolved in 350 mL of methylbenzene and stirred for 1 h at 110 °C. Tetradecylalcohol (47.8 g, 176.7 mmol) was added, and 12 h later, the reaction mixture was evaporated to remove methylbenzene and then dissolved in dichloromethane (DCM). The solution was washed by 5% (w:v) sodium carbonate solution (100 mL × 2) and distilled water (100 mL × 1). Evaporated organic layer obtained the oil, recrystallized the oil by 100 mL methanol to get white powder, yield 55%.

$G2C_{14}$: ^{1}H-NMR (300 MHz, $CDCl_3$): δ 4.11 (t, J = 6.8 Hz, 2H, $COOCH_2$), 4.07 (t, J = 6.8 Hz, 1H, $COOCH_2$), 3.50 (dd, J = 5.4, 8.0 Hz, 1H, NH_2CH), 2.46 (t, J = 7.5 Hz, 2H, CH_2CO),

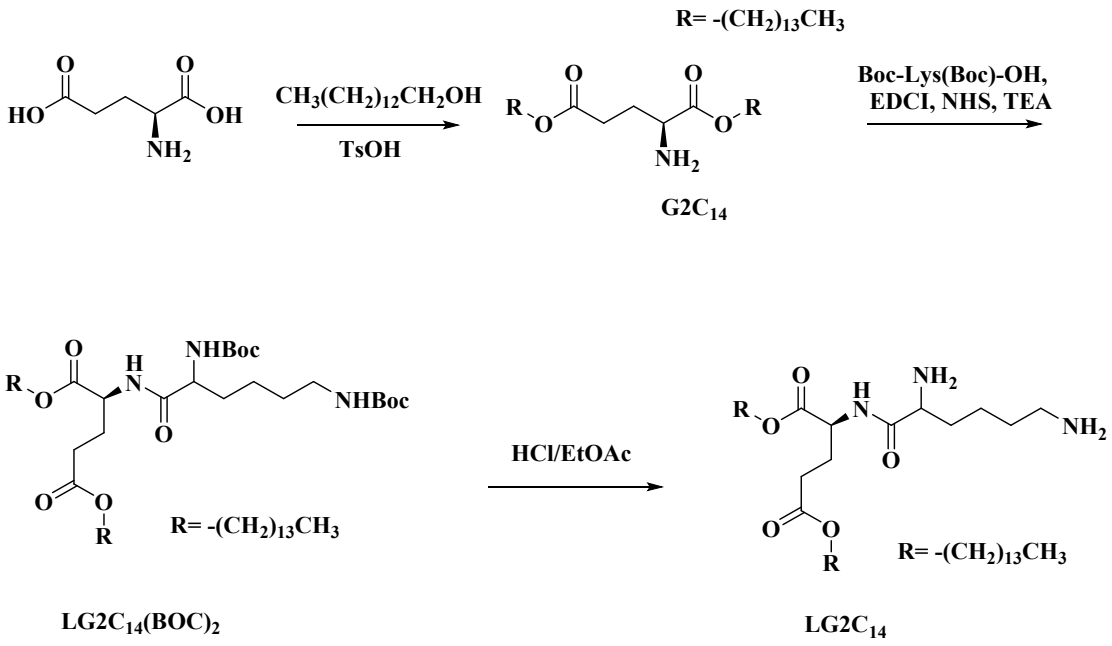
2.12-2.05 (m, 1H, NH_2CHCH_2), 1.90-1.79 (m, 2H, NH_2CHCH_2), 1.65-1.54 (m, 4H, $COOCH_2CH_2$), 1.31-1.26 (m, 44 H, $CH_{2\ (myristoyl)}$), 0.88 (t, J = 7.0 Hz, 6H, CH_2CH_3).

1.2 Synthesis of $LG2C_{14}$

Boc-Lys(Boc)-OH (3 g, 8.7 mmol), (3-dimethylaminopropyl) ethyl-carbodiimid monohydrochloride 3-propanediamine (EDC) (3.3 g, 17.3 mmol) and *N*-hydroxysuccinimide (NHS) (2.0 g, 17.3 mmol) were dissolved in 60 mL chloroform ($CHCl_3$) and stirred for 3 h at room temperature. $G2C_{14}$ (4.7 g, 8.7 mmol) and triethylamine (TEA) (0.88 g, 8.7 mmol) were dissolved in 40 mL $CHCl_3$ and stirred for 1 h at room temperature. Subsequently, two solutions above were mixed and stirred for 12 h at room temperature. The solution was washed with brine and dried over anhydrous Na_2SO_4, filtered and concentrated. The residue was purified by column chromatography with dichloromethane / methanol (150/1, v/v) to give compound $LG2C_{14}(BOC)_2$, yield 55%.

The $LG2C_{14}(BOC)_2$ (4.1 g, 4.7 mmol) was dissolved in 45 mL of ethyl acetate solution saturated with HCl for 12 h at 0 ^oC and the $LG2C_{14}$ was obtained with a yield of 75%.

$LG2C_{14}$: ^{1}H-NMR (300 MHz, $CDCl_3$): δ 4.15-4.09 (m, 4H, $COOCH_2CH_2$), 4.02-3.97 (m, 1H, CONHCHCOO), 3.11-2.97 (m, 2H, $NH_2CH_2CH_2$), 2.52-2.5 (m, 2H, CH_2CO), 2.47-2.22 (m, 1H, $CONHCHCH_2$), 2.10-2.08 (m, 1H, $CONHCHCH_2$), 2.05-1.88 (m, 2H, $OCONHCHCH_2$), 1.84-1.72 (m, 2H, NH2CHCH2), 1.65-1.54 (m, 6H, $OCONHCHCH_2$+$COOCH_2CH_2$), 1.31-1.26 (m, 46 H, $NH_2CH_2CH_2CH_2$+$CH_{2\ (myristoyl)}$), 0.88 (t, J = 7.0 Hz, 6H, CH_2CH_3).



Scheme S1. Synthesis of $LG2C_{14}$

2. Synthesis of cyclooctyne-distearoyl phosphatidylethanolamine (ADIBO-DSPE)

2.1 Synthesis of ADIBO-COOH

Aza-dibenzocyclooctyne (220 mg, 0.80 mmol) was dissolved in 15 mL of chloroform, and triethylamine (0.2 mL, 1.43 mmol) was added dropwise and the mixture was stirred at room temperature for 1 h. Succinic anhydride (105 mg, 1.05 mmol) was added to the system and reacted for 12 h at room temperature. After the reaction was completed, the solution was washed tby 1 M aqueous HCl solution (20 mL × 2), and brine and dried over anhydrous Na_2SO_4, filtered and concentrated. The residue was purified by column chromatography with dichloromethane / methanol (25/1, v/v) to give compound ADIBO-COOH. yield 75%.

ADIBO-COOH: ^{1}H NMR (300 MHz, $CDCl_3$) : δ 7.57 (1H, d, J=7.6), 7.33-7.18 (7H, m), 6.56 (1H, m), 5.05 (1H, d, J=13.9 Hz), 3.63 (1H, d, J=13.9 Hz), 3.30 (1H, m), 3.11 (1H, m), 2.52 (1H, m), 2.42 (2H, m), 2.28 (2H, m), 2.01 (1H, m). ESI-HRMS calcd for $C_{22}H_{20}N_2O_4$ $[M+H]^+$ 377.1423, found 377.1498.

2.2 Synthesis of ADIBO-NHS

ADIBO-COOH (124 mg, 0.33 mmol) and N-hydroxysuccinimide (37.9 mg, 0.33 mmol) were dissolved in 8 mL of chloroform. After stirring at room temperature for 5 min, dicyclohexylcarbodiimide (68.1 mg, 0.33 mmol) was added and stirred at room temperature for 12 h. After the reaction was completed, the solution was filteredto removed insoluble 1,3-dicyclohexylurea (DCU) and the solution was concentrated. The residue was purified by column chromatography with dichloromethane / methanol (200/1, v/v) to give compound ADIBO-NHS as yellow oil, yield 65%.

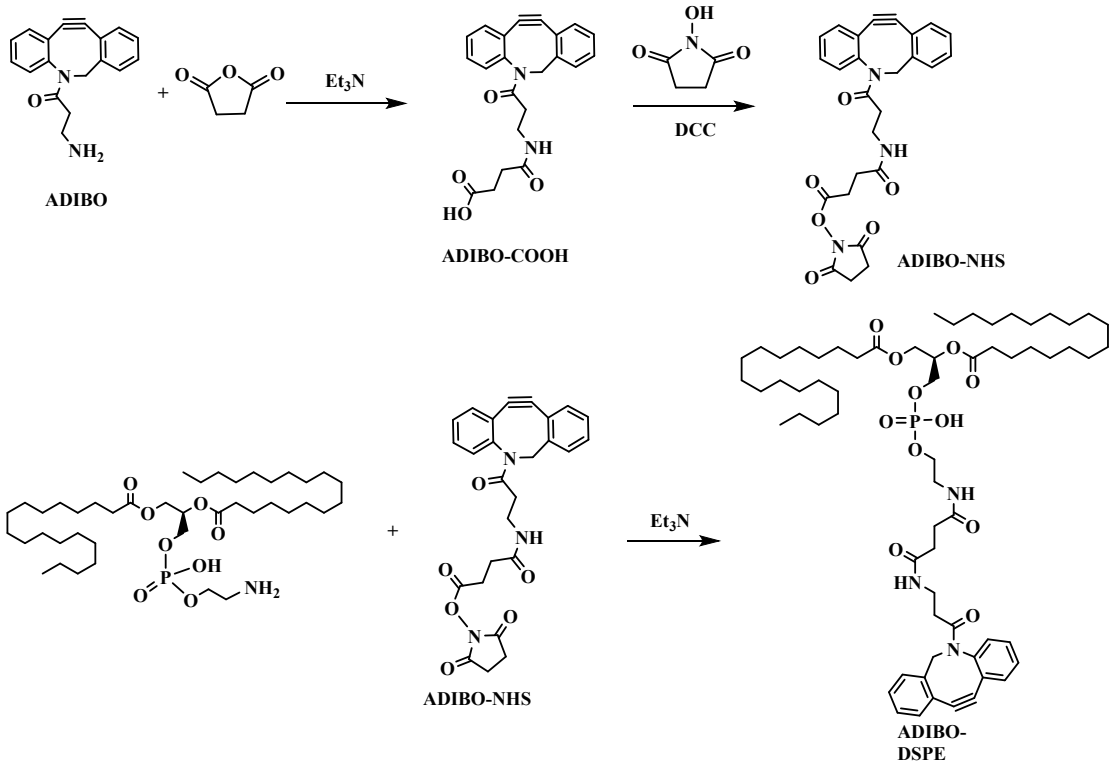
ADIBO-NHS: ^{1}H NMR (300 MHz, $CDCl_3$) : δ 7.66 (1H, d, J=7.7), 7.45-7.23 (7H, m), 5.14 (1H, d, J=13.9 Hz), 3.69 (1H, d, J=13.9 Hz), 2.89-2.78 (2H, m), 2.73-2.66 (3H, m), 2.43 (2H, m). ESI-HRMS calcd for $C_{26}H_{23}N_3O_6$ $[M+H]^+$ 474.1587, found 474.1729.

Synthesis of ADIBO-DSPE

2.3 Synthesis of ADIBO-DSPE

Distearoyl phosphatidylethanolamine (74.8 mg, 0.10 mmol) and triethylamine (41.8 μL, 0.30 mmol) were dissolved in 8 mL of chloroform. After stirring at room temperature for 5 min, ADIBO-NHS (47.34 mg, 0.10 mmol) was added into the above solution at room temperature for 12 h. ADIBO-DSPE was purified by column chromatography with dichloromethane / methanol (20/1, v/v), yield 71%.

ADIBO-DSPE: ^{1}H NMR (300 MHz, $CDCl_3$) : δ 7.69 (1H, m), 7.54-6.97 (7H, m), 5.16 (1H, d, J=13.9 Hz), 4.32 (2H, m), 3.94 (4H, m), 3.66 (1H, m), 3.61 (1H, d, J=13.9 Hz), 3.43 (2H, m), 2.49 (2H, m), 2.25 (4H, m), 1.55 (4H, m), 1.25 (54H, m), 0.88 (6H, m).



Scheme S2. Synthesis of ADIBO-DSPE

3. Synthesis of functional modules

3.1 Synthesis of azide-modified octreotide (N_3-Oct)

3.1.1 Synthesis of 3-azidopropan-1-ol

3-Chloro-1-propanol (3.2 g, 33.8 mmol) and sodium azide (6.5 g, 100 mmol) were dissolved in 100 mL of water, stirred at 90 °C for 20 h, then extracted with ether (100 mL×5). And dried over anhydrous Na_2SO_4, filtered and concentrated to obtain 3-azido-1-propanol, yield 72%.

3-azidopropan-1-ol: ^{1}H-NMR (300 MHz, $CDCl_3$): δ 3.76 (t, J = 6.0 Hz, 2H, CH_2OH), 3.46 (t, J = 6.0 Hz, 2H, CH_2N_3), 1.85 (m, 2H, CH_2).

3.1.2 Synthesis of 3-azidopropanal

3-azido-1-propanol (0.5 g, 5.0 mmol) and Dess-Martin periodinane (3.15 g, 7.5 mmol) were dissolved in 50 mL dichloromethane, stirred at room temperature for 1 h. After filtration to

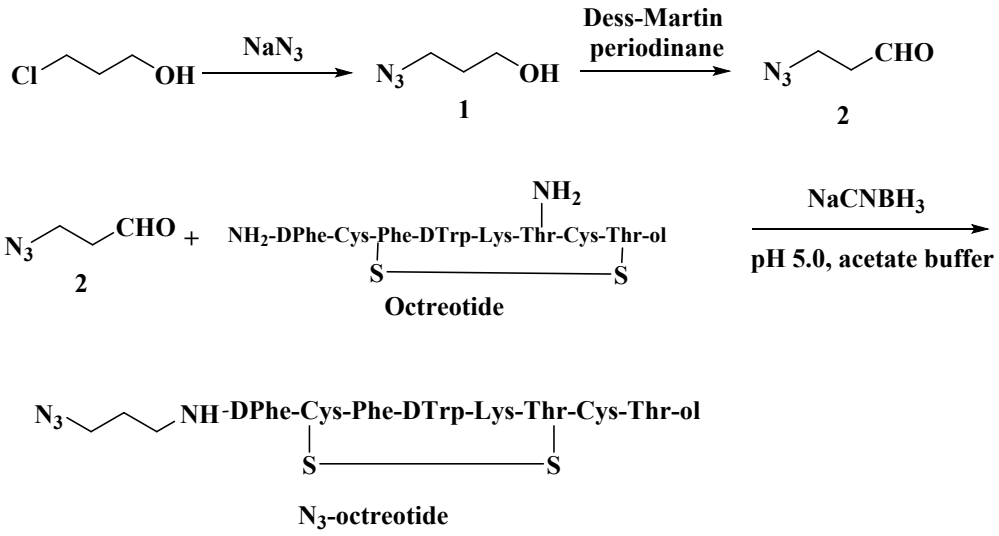
remove the insoluble substance and then evaporated under vacuum, 3-azidopropyne is obtained with yield of 34% after purification by column chromatography.

3-azidopropanal: ^{1}H-NMR (300 MHz, $CDCl_3$): δ3.59 (t, J = 6.0 Hz, 2H, CH_2N_3), 2.76 (m, 2H, CH_2).

3.1.3 Synthesis of N_3-Oct

Octreotide acetate (20 mg, 20 nmol) and sodium cyanoborohydride (25 mg, 4 mmol) were dissolved in 20 mL of pH 5.0 acetate-sodium acetate buffer (0.1 mM), stirred at 4 °C until dissolved completely. 3-azidopropyne (5 mg, 50 nmol) was added, and maintained the reaction at 4 °C for 10 h. The solution was lyophilized to obtain a white powder.

N_3-Oct: ESI-HRMS calcd for $C_{52}H_{70}N_{13}O_{10}S_2$ $[M+H]^+$ 1102.4304, found 1102.4907.

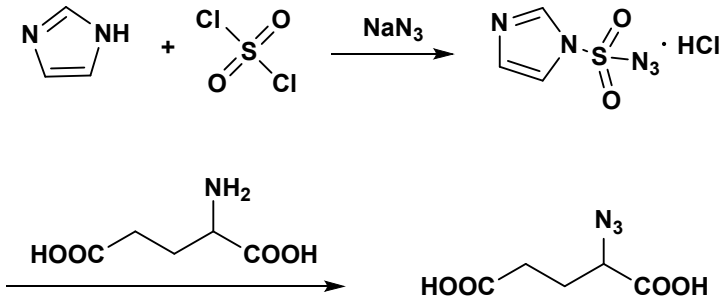


Scheme S3. Synthesis of N_3-Oct

3.2 Synthesis of azide-modified octreotide (N_3-Glu)

As shown in Scheme S4, N_3-Glu was synthesized according to the previous reported synthesis.[2]

N_3-Glu: ESI-HRMS calcd for $C_5H_7N_3O_4Na$ $[M+Na]^+$, 196.0334, found 196.0333.



Scheme S4. Synthesis of N_3-Glu

Reference

[1] Q. Sun, Z. S. Kang, L. J. Xue, Y. K. Shang, Z. G. Su, H. B. Sun, Q. N. Ping, R. Mo, C. Zhang, *J Am Chem Soc*. **2015**, *137*, 6000.

[2] J. Zhang, W. Jin, X. Wang, J. Wang, X. Zhang, Q. Zhang, *Mol. Pharm.* **2010**, *7*, 1159.

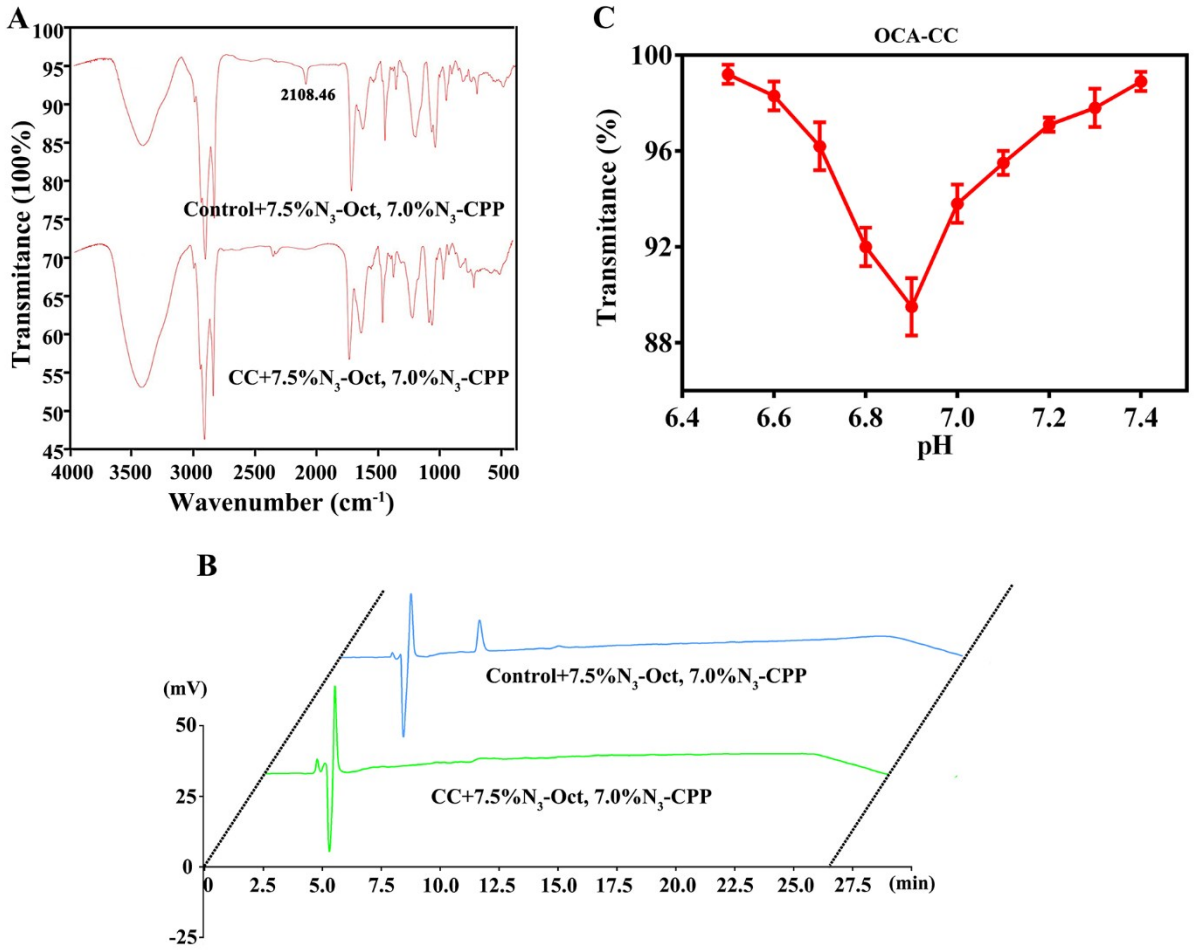


Figure S1. (A) FT-IR spectra of OC-CC. The disappearance of azide peak at 2108 cm^{-1} indicates the complete reaction of N_3-Oct and N_3-CPP with ADIBO. (B) HPLC image of OC-CC. The disappearance of ultraviolet absorption peak of free N_3-Oct at about 6 min further confirms the complete reaction of N_3-Oct with ADIBO. (C) The transmittance of OCA-CC during the pH variation from pH6.5 to pH7.4.

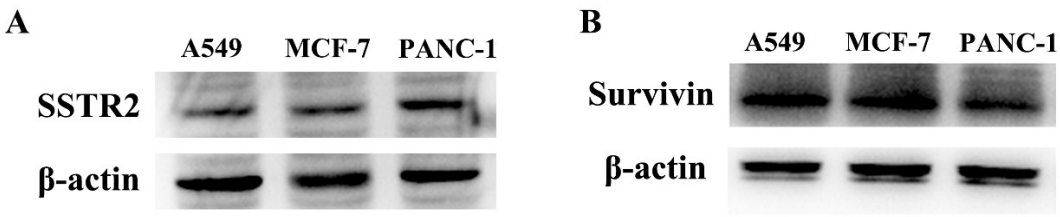


Figure S2. Expression of SSTR2 protein (A) and survivin protein (B) in A549, MCF-7 and PANC-1 cells. Both of SSTR2 and survivin proteins are high expressed in A549, MCF-7 and PANC-1 cells.

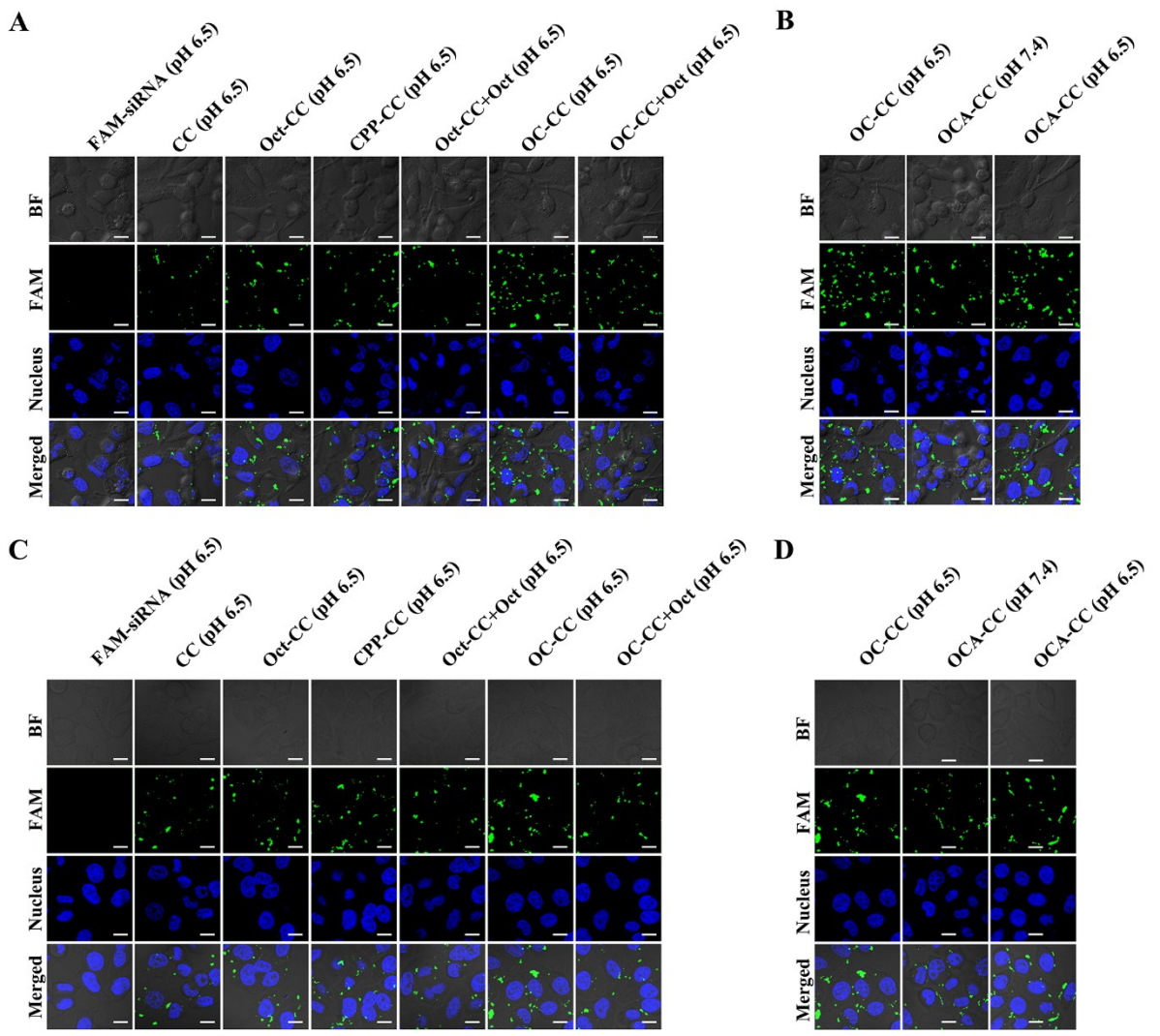


Figure S3. (A) Cellular uptake of FAM-siRNA (green) within A549 cells treated with different formulations at pH6.5 for 6 h observed using LSCM. The nuclei were stained with Hoechst 33342 (blue). Scale bar: 20 μm. (B) Cellular uptake of FAM-siRNA (green) within A549 cells treated with OC-CC and OCA-CC at pH7.4 and 6.5 for 6 h observed using LSCM. The nuclei were stained with Hoechst 33342 (blue). Scale bar: 20 μm. (C) Cellular uptake of FAM-siRNA (green) within PANC-1 cells treated with different formulations at pH6.5 for 6 h observed using LSCM. The nuclei were stained with Hoechst 33342 (blue). Scale bar: 20 μm. (D) Cellular uptake of FAM-siRNA (green) within PANC-1 cells treated with OC-CC and OCA-CC at pH7.4 and 6.5 for 6 h observed using LSCM. The nuclei were stained with Hoechst 33342 (blue). Scale bar: 20 μm.

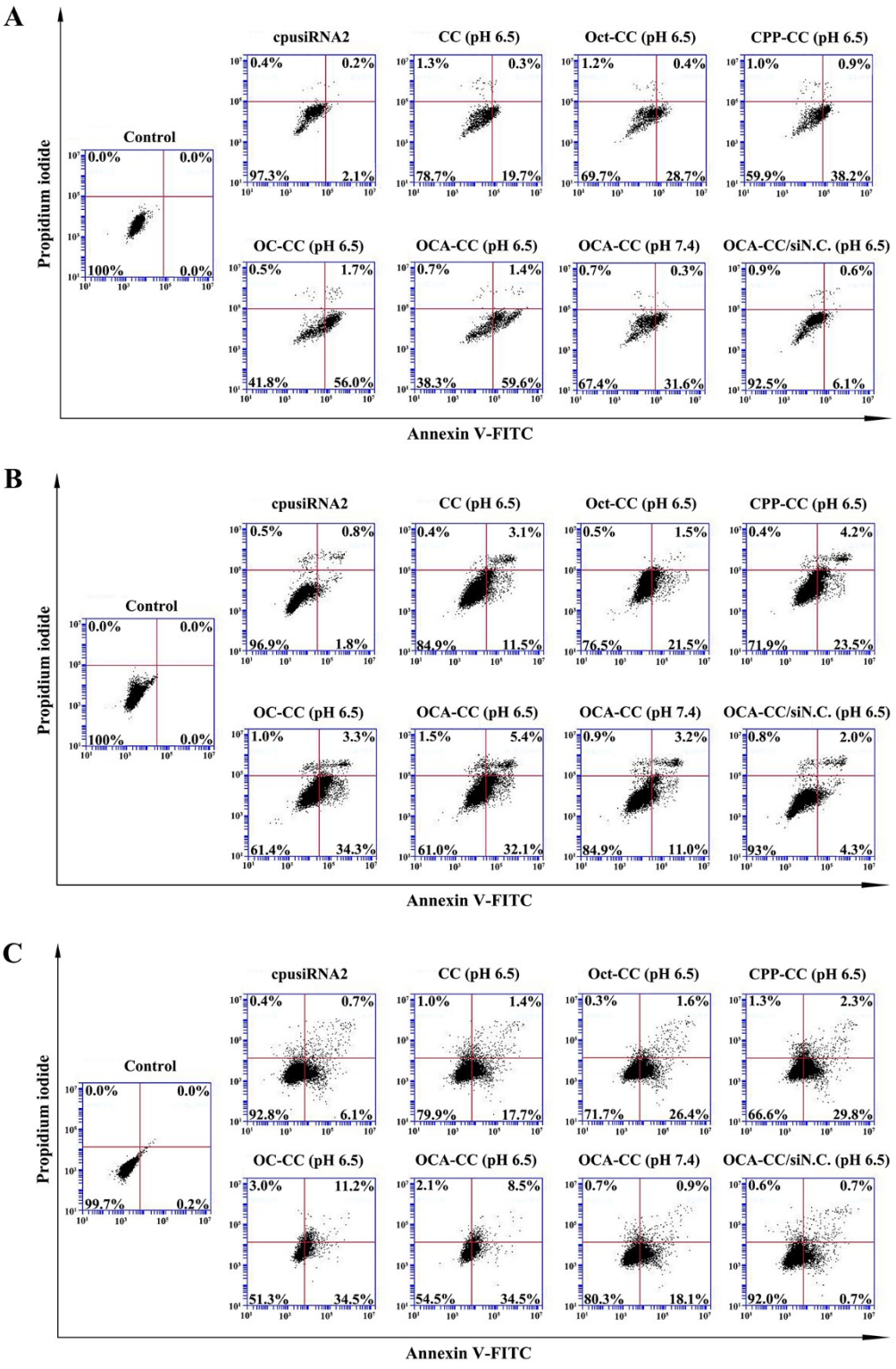


Figure S4. Cell apoptosis of MCF-7 (A), A549 (B) and PANC-1 (C) cells after transfection with different formulations determined by the Annexin V-FITC/PI assay. The viable, early apoptotic, and late apoptotic cell populations (%) are shown in the lower left, lower right and upper right quadrants, respectively.

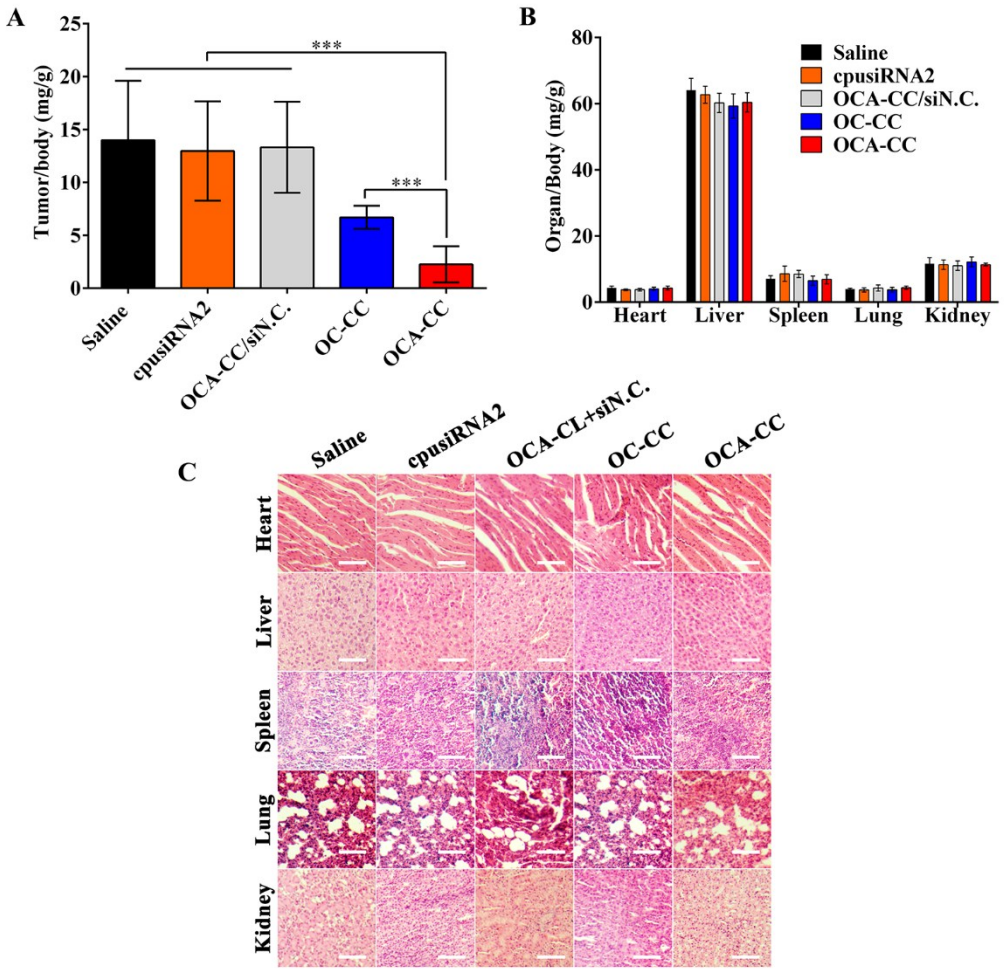


Figure S5. Mass ratio of tumors (A) and major organs (B) to body weight of orthotopic luci-MCF-7 tumor-bearing mice treated with different formulations at a dose of 1.2 mg/kg via tail vein injection. *** P <0.001. (C) Histological images of the HE-stained heart, liver, spleen, lung, kidney and tumor harvested from orthotopic luci-MCF-7 tumor-bearing mice after treatment. Bar: 100 μm.

Table S1. Sample nomenclature for OCA-CC and control formulations prepared with different clickable modules.

Formulations	Oct (mol%)	CPP (mol%)	Glu (mol%)
Oct-CC	7.5	0	0
CPP-CC	0	7.0	0
Glu-CC	0	0	50
OC-CC	7.5	7.0	0
OCA-CC	7.5	7.5	50

Table S2. Particle sizes and zeta potentials of all formulations at pH7.4 and pH6.5.

Formulations	pH7.4			pH6.5		
	Size (nm)	PDI	Zeta potential (mV)	Size (nm)	PDI	Zeta potential (mV)
CC	302.01±8.13	0.206±0.010	18.49±4.48	116.31±1.05	0.151±0.009	19.78±4.22
Oct-CC	147.95±1.58	0.147±0.008	19.45±0.88	132.46±0.91	0.132±0.010	20.90±2.93
CPP-CC	186.63±1.89	0.164±0.015	24.46±4.92	132.06±0.00	0.132±0.013	23.07±2.63
Glu-CC	179.75±2.68	0.166±0.017	-22.80±4.81	178.51±2.63	0.153±0.012	-17.80±2.44
OC-CC	320.68±6.83	0.182±0.032	24.78±2.84	125.06±2.55	0.169±0.010	26.27±3.11
OCA-CC	288.20±9.50	0.196±0.034	-12.00±1.10	149.50±2.40	0.135±0.018	10.80±3.20