Supplementary Information

Automated Modular Synthesis of Aptamer-Drug Conjugates for Targeted Drug Delivery

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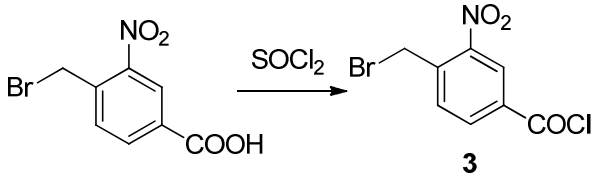
Synthesis protocols:

Unless otherwise noted below, all commercially available reagents and solvents were purchased from Aldrich and used without further purification. ^{1}H NMR (TMS as the internal standard) and ^{19}F NMR spectra ($CFCl_3$ as the external standard and low field designated positive) were recorded on a Bruker AM300 or Bruker AM400 spectrometer. ^{13}C NMR spectra were recorded on a Bruker AM400 spectrometer. Chemical shifts (δ) are reported in ppm, and coupling constants (J) are in Hertz (Hz).

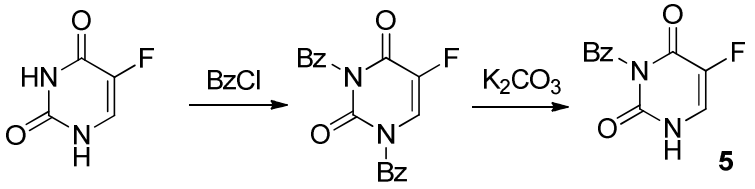
Synthesis of compound **3**



3

4-Bromomethyl-3-nitrobenzoic acid (3.30g, 12.69 mmol) was dissolved in $SOCl_2$ (50 mL), and the solution was heated under reflux for 3 hours. Removal of extra $SOCl_2$ gave yellow solid **3**, which was used in the next step without further purification.

Synthesis of compound **5**

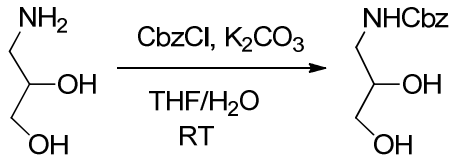


5

To a solution of 5-FU (1.30g, 10.0 mmol) in pyridine/acetonitrile (10 mL/4mL) at 0 ^{o}C was added benzoyl chloride dropwise, and the reaction was stirred at room temperature overnight. The reaction solution was poured into a mixture of CH_2Cl_2 and H_2O (1:1, 120 mL), and the organic extract was collected. The organic solution was concentrated *in vacuo*, and the residue was dissolved in a mixture of dioxane (20 mL) and aqueous potassium carbonate (0.5 mM, 10mL). The mixture was stirred at room temperature for two hours, and acetic acid was added to adjust the pH to 5. The mixture was concentrated *in vacuo*, followed by addition of saturated aqueous sodium bicarbonate. The white solid which precipitated from the solution after 1 hour of stirring was collected and washed with cold water 3

times giving compound **5** as a white solid (1.68g, 70% yield in two steps). m.p.:140 ^{o}C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 9.40 (br, 1 H), 7.95 (d, *J* = 7.8 Hz, 2 H), 7.72 (t, *J* = 8.1 Hz, 1 H), 7.55 (t, *J* = 7.8 Hz, 2 H), 7.28 (d, *J* = 6.1 Hz, 1 H); ^{19}F NMR (282 MHz, $CDCl_3$) δ -171.81 (d, *J* = 8.2 Hz, 1 F); IR (thin film) ν_{max} 3099, 1961, 1771, 1671, 1433, 1253 cm^{-1}.

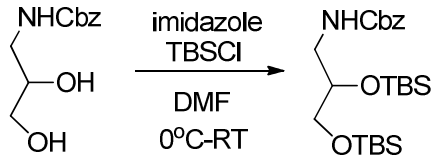
Synthesis of Compound **10**:



10

To a solution of 3-amino-1,2-propandiol (3.74 g, 41 mmol) in THF (140 mL) were added water (70 mL) and potassium carbonate (17.00 g). The solution was cooled to 0 ^{o}C, and benzyl chloroformate (7.00 g, 41.0 mmol) was added dropwise. The reaction was then warmed to room temperature and stirred for three hours. The reaction mixture was then extracted with ethyl acetate (3 x 50 mL), and the combined organic phase was washed with saturated saline and dried over anhydrous sodium sulfate. The organic solution was concentrated *in vacuo*, and the residue was purified by recrystallization giving compound **10** as a white solid (8.34 g, 90% yield). m.p.: 69 ^{o}C;^{1}H NMR (300 MHz; $CDCl_3$)δ 7.36 (m, 5 H), 5.2 (bs,1 H), 5.12 (s, 2 H), 3.78 (m, 1 H), 3.62 (m, 2 H), 3.35 (m, 2 H), 2.27 (br, 2 H); IR (thin film) ν_{max} = 3328, 2940, 1686, 1557, 1495, 1270, 1152 cm^{-1}; HRMS (ESI-) calculated for $C_{11}H_{15}NO_4Na$: 248.0899; found: 248.0899.

Synthesis of compound **1**:

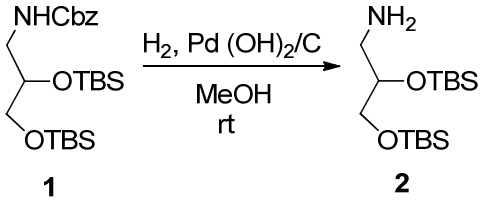


10 **1**

To a solution of compound **10**(5.3 g, 23.6 mmol) and imidazole (14.2 g, 94 mmol) in DMF (35 mL) at 0^{o}C was added the solution of TBSCl (1.808 g, 12 mmol) in DMF (80 mL). The reaction was warmed to room temperature and stirred overnight. Water (100 mL) was added to the reaction, and the mixture was extracted with CH_2Cl_2 (3 x 50 mL). The combined organic phase was washed

with saturated ammonium chloride solution (3 x 60 mL) and dried over anhydrous sodium sulfate. The solution was concentrated *in vacuo*, and the residue was purified by flash column to give compound **1** (8.9 g, 84% yield) as a colorless oil; R_f = 0.8 (ethyl acetate/petroleum ether = 1/3); ^{1}H NMR (300 MHz; $CDCl_3$) δ7.40-7.28 (m, 5 H), 5.10 (s, 3 H), 3.79 (m, 1 H), 3.59-3.47 (m, 2 H), 3.36-3.24 (m, 2 H), 0.88 (s, 18 H), 0.05 (s, 12 H); ^{13}C NMR (100 MHz; $CDCl_3$) δ156.4, 136.7, 128.4, 128.0, 127.9, 71.34, 66.54, 65.56, 44.49, 25.87, 25.76, 18.24, 18.02, -3.60, -4.62, -4.94; IR (thin film) ν_{max} 2954, 2857, 1729, 1510, 1472, 1255, 1104, 837 cm^{-1}; HRMS (ESI+) calculated for $C_{11}H_{15}NO_4Na$: 476.2628; found: 476.2614.

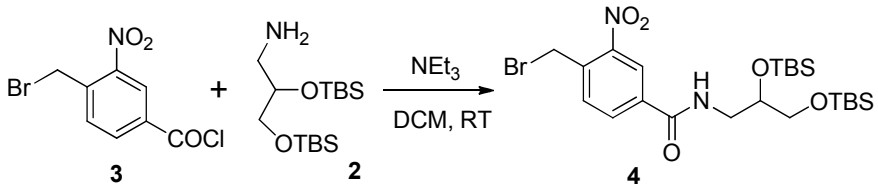
Synthesis of compound **2**



1 **2**

To a solution of compound **1**(5 g, 11 mmol) in methanol (180 mL) was added palladium hydroxide on carbon, (20wt.%, 1.0 g), and the mixture was stirred under 1 atm H_2 at room temperature for two hours. Filtration of the solid catalyst followed by concentration gave compound **2** (3.5 g, 99% yield) as a colorless oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ 6.19 (s, 2 H), 3.90-3.87 (m, 1 H), 3.62 (m, 1 H), 3.55-3.51 (m, 1 H), 3.06-3.03 (m, 1 H), 2.92-2.90 (m, 1 H), 0.88 (s, 18 H), 0.22-0.01 (s, 12 H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 70.44, 65.20, 43.41, 25.85, 25.83, 18.22, 17.95, 4.47, -4.60, -5.47; IR (thin film) ν_{max} 2955, 2858, 1472, 1361, 1255, 1111, 837 cm^{-1}; HRMS (ESI+) calculated for $C_{15}H_{38}NO_2Si_2$: 320.2441; found: 320.2434.

Synthesis of compound **4**

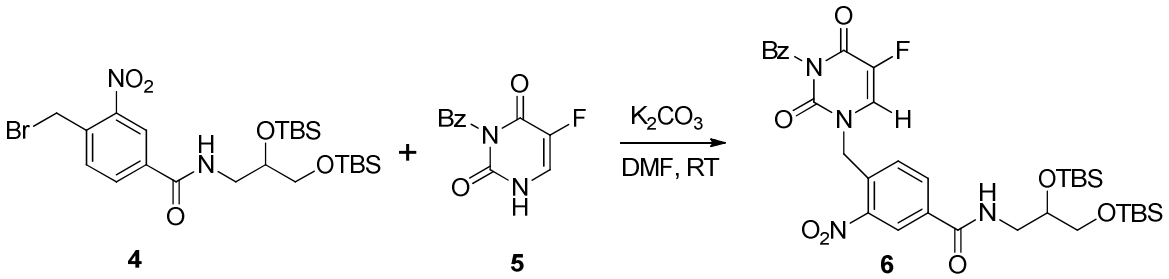


3 **2** **4**

To the solution of compound **2** (3.6 g，11.61 mmol) in CH_2Cl_2 (100 mL) at 0^{o}C were added NEt_3 (2.5 mL, 16.7 mmol) and freshly prepared compound **3** in CH_2Cl_2 (30 mL). The reaction was

warmed to room temperature and stirred for 3 hours. Forty mL of water was added to terminate the reaction, and the resulting mixture was extracted with CH_2Cl_2 (2 x 50 mL). The organic phase was washed with saturated saline and dried over anhydrous sodium sulfate. The residue of concentrated solution was purified by flash column to give compound **4**(3.4 g, 52 % yield) as a yellow oil; R_f = 0.6 (ethyl acetate/petroleum ether=1/10); ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.41 (s, 1 H), 8.05 (d, *J* = 6.0 Hz, 1 H), 7.73 (d, *J* = 6.0 Hz, 1 H), 5.00 (s, 2 H), 3.98-3.93 (m, 1 H), 3.72-3.59 (m, 4 H), 0.93 (s, 18 H), 0.13 (s, 12 H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 164.24, 148.02, 136.32, 135.04, 131.90, 131.85, 123.48, 70.81, 66.43, 44.03, 42.16, 25.78, 18.27, 17.97, -4.58, -4.85, -5.50; IR (thin film) ν_{max} 3334, 2954, 2857, 1642, 1534, 1348, 1258, 1105, 836 cm^{-1}; HRMS (ESI+) calculated for $C_{23}H_{41}N2O_5Si_2BrNa$: 583.1635; found: 583.1634.

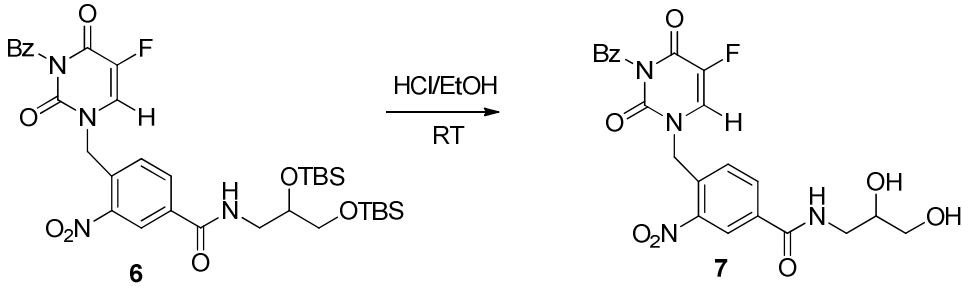
Synthesis of compound **6**



A mixture of compound **5** (351 mg, 1.5 mmol), potassium carbonate (415 mg, 3 mmol) and DMF (15 mL) was stirred for 2 hours at room temperature. The mixture was cooled to 0 ^{o}C, and a solution of compound **4** (421 mg, 0.75mmol) in DMF (5 mL) was added. The reaction was stirred at room temperature for 24 hours. Forty mL of water was then added to the reaction, and the mixture was extracted with ethyl acetate (3 x 50 mL). The combined organic phase was washed with saturated saline and dried over anhydrous sodium sulfate. Solvents were removed, and the residue was purified by flash column to give compound **6** (279 mg, 52% yield) as a pale yellow power; R_f = 0.3 (ethyl acetate/petroleum ether=1/3); m.p.: 74~75 ^{o}C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.44 (s, 1 H), 7.99 (d, *J* = 7.6 Hz, 1 H), 7.89 (d, *J* = 7.5 Hz, 2 H), 7.69-7.60 (m, 2 H), 7.53-7.48(m, 3 H), 6.91 (s, 1 H), 5.30 (s, 2 H), 3.93 (m, 1 H), 3.70-3.51 (m, 4 H), 0.89 (s, 18 H), 0.22-0.01 (m, 12 H); ^{19}F NMR (282 MHz, $CDCl_3$) δ -171.81 (d, *J* = 5.4 Hz, 1 F); ^{13}C NMR (100 MHz, $CDCl_3$) δ 166.93, 164.11, 156.27(d, *J* = 270 Hz), 148.60, 147.86, 141.33, 138.93, 136.39, 135.67, 132.65, 132.13, 130.62, 130.51, 129.35, 129.03, 128.70, 124.09 , 70.67, 66.15, 49.51, 43.89, 29.65, 25.86,

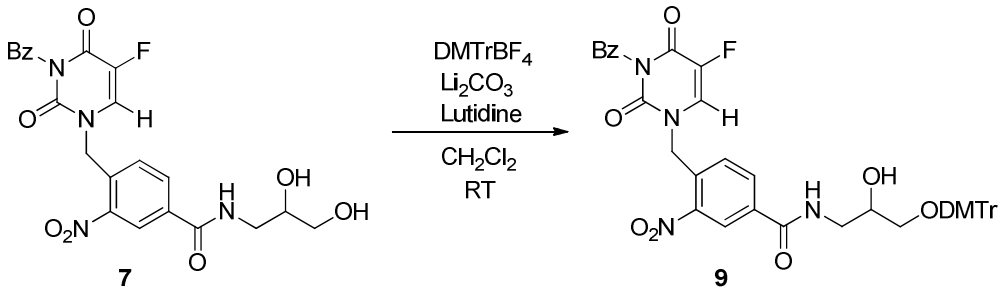
25.75, 18.30, 18.00, -4.56, -4.82, -5.45; IR (thin film) ν_{max} 3375, 2955, 2857, 1757, 1718, 1671, 1533, 1353, 1251, 1102, 836 cm^{-1}; HRMS (ESI-) calculated for $C_{34}H_{47}NO_8Si_2Na$: 737.2814; found: 737.2801.

Synthesis of compound **7**



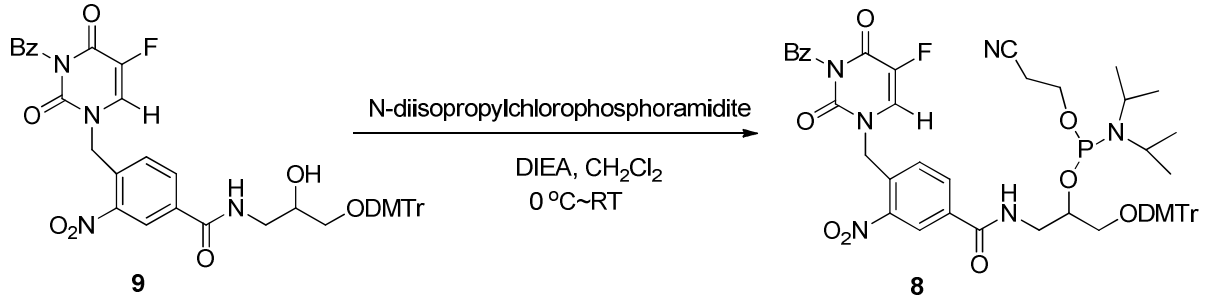
To a solution of compound **6** (1.564 g, 1.44 mmol) in ethanol (150 mL) was added hydrochloric acid (10 wt %, 15 mL), and the reaction was stirred at room temperature for 2 hours. Water (200 mL) was added to the reaction, and the mixture was extracted with ethyl acetate (5 x 150mL). The combined organic phase was washed with saturated saline solution and dried over anhydrous sodium sulfate. Concentration and purification of the residue by flash column gave compound **7** (0.9 g, 90% yield) as a white power; R_f = 0.3 (CH_3OH/CH_2Cl_2 = 1/10); m.p.: 47 ^oC; ^1H NMR (400 MHz, MeOD) δ 7.31 (d, *J* = 1.5 Hz, 1 H), 6.88 (dd, *J* = 8.1, 1.5 Hz, 1 H), 6.81 (d, *J* = 6.2 Hz, 1 H), 6.73 (d, *J* = 7.4 Hz, 2 H), 6.45 (t, *J* = 7.4 Hz, 1 H), 6.29 (m, 3 H), 4.10 (s, 2 H), 2.58-2.53 (m, 1 H), 2.31-2.26 (m, 3 H), 2.16-2.11 (m, 1 H); ^{19}F NMR (282 MHz, MeOD) δ -168.02 (d, *J* = 6.2 Hz, 1 F); ^{13}C NMR (100 MHz, MeOD) δ 167.30, 166.15, 156.99(d, *J* = 270 Hz), 148.86, 147.85, 141.46, 139.11, 135.31, 133.94, 132.16, 131.06, 130.27, 130.08, 129.11, 128.76, 123.98, 70.48, 63.80, 49.66, 42.88; IR (thin film) ν_{max} 3380, 2900, 1752, 1717, 1560, 1352, 1247 cm^{-1}; HRMS (ESI+) calculated for $C_{22}H_{19}N_4O_8FNa$: 509.1085; found: 509.1079.

Synthesis of compound **9**



To a suspension of compound **7** (240 mg, 0.49 mmol) in anhydrous CH_2Cl_2 (40 mL) were added Li_2CO_3 (400 mg) and Lutidine (630 mg). $DMTBF_4$ (~230 mg, 0.56 mmol) was added in portions. CH_2Cl_2 (50 mL) was added to the reaction, and the diluted solution was washed with saturated saline solution and dried over anhydrous sodium sulfate. After removal of solvent, the residue was purified by flash column giving compound **9** (208 mg, 68% yield) as a white powder; R_f = 0.6 (CH_3OH/CH_2Cl_2 = 1/20); m.p.: 99~100 ^oC; ^1H NMR (300 MHz, $CDCl_3$) δ 8.44 (s, 1 H), 8.07-7.90 (m, 3 H), 7.70-7.68 (m, 1 H), 7.58-7.50 (m, 3 H), 7.42-7.38 (m, 3 H), 7.31-7.26 (m, 8 H), 6.82-6.79 (s, 4 H), 5.30 (s, 2 H), 4.00 (m, 1 H), 3.77 (s, 6 H), 3.35 (m, 4 H); ^{19}F NMR (282 MHz, CD_2Cl_2) δ -166.72 (d, J = 18.8 Hz，1 F); ^{13}C NMR(100 MHz, CD_2Cl_2) δ 167.16, 164.81, 158.66, 156.27 (d, J = 270 Hz), 148.60, 147.94, 144.80, 141.50, 139.11, 136.14, 135.73, 132.59, 132.24, 130.76, 130.41, 129.98, 129.42, 128.97, 128.64, 127.92, 126.84, 124.47, 113.12, 86.30, 69.71, 65.13, 59.19, 55.20, 49.60, 46.03, 43.70, 10.63; IR (thin film) ν_{max} 3338, 3066, 2932, 1755, 1717, 1670, 1532, 1248, 1155 cm^{-1}; HRMS (ESI-) calculated for $C_{43}H_{36}FN_4O_{10}$: 788.2494; found: 788.2421.

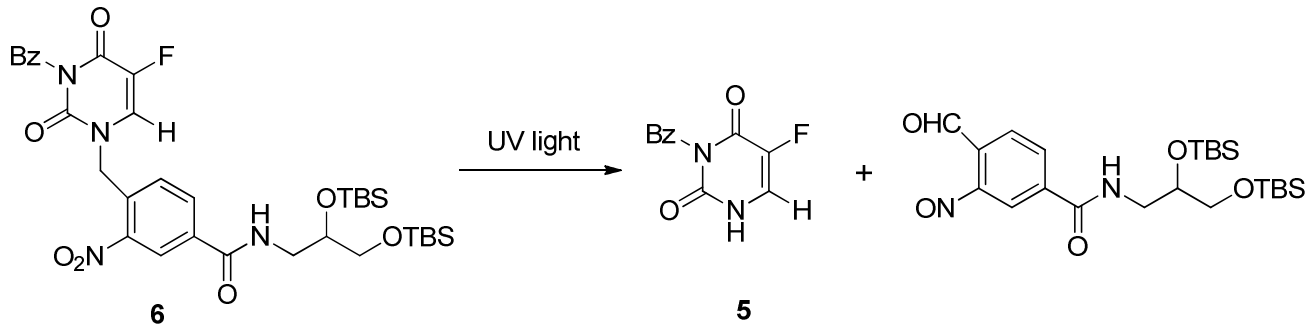
Synthesis of phosphoramidite **8**



To a solution of compound **9** (155 mg，0.2 mmol) and DIEA (289 mg, 2.23 mmol) in CH_2Cl_2 (18 mL) at 0 ^oC was added *N*-diisopropylchlorophosphoramidite (288 mg, 1.22 mmol), and the reaction was monitored by TLC. When the starting material was consumed, as shown by TLC, the reaction was diluted with CH_2Cl_2 (40 mL) and washed with saturated sodium bicarbonate and saturated saline. After drying over anhydrous sodium sulfate, the solution was concentrated, and the residue was purified by flash column giving phosphoramidite **8** (150 mg, 82% yield) as a white powder; R_f = 0.5 (ethyl acetate/petroleum ether = 1/1); ^1H NMR (400 MHz, CD_2Cl_2) δ 8.37 (s, 1 H), 7.92-7.78 (m, 2 H), 7.63 (s, 1 H), 7.46 (s, 3 H), 7.36 (s, 2H), 7.24-7.07 (m, 6 H), 7.01 (s, 1 H), 6.72-6.64 (m, 4 H), 5.23 (s, 2 H), 4.06-3.98 (m, 2 H), 3.78-3.45 (m, 10 H), 3.23-3.06 (m, 2 H), 3.46-3.41 (m, 2 H),

1.91 (s, 2 H), 1.13-1.06 (m, 12 H); ^{19}F NMR (282 MHz, MeOD) δ -164.53 (d, J = 6.2 Hz) 164.65(d, J = 6.2 Hz); ^{31}P NMR (162 MHz, CD_2Cl_2) δ 150.03 (s), 148.26 (s).

Photo-controllable release of drug moiety from compound 6:



The solution of compound **6** (30μM) in MeCN/H_2O (4/1) was subjected to the irradiation of UV light (λ=365 nm, 40W) for 1 hour. Both TLC (thin layer chromatography) and LC/MS results confirmed the efficient release (more than 50%) of benzoyl 5FU (**5**).

ApDCs synthesis and purification

Table S1.Detailed sequence data for **MT-I**, **MT-II**, **sgc8-5FU** and **LIB**.

DNA	Sequence [a]
MT-I	5’ DTTTT TTT**ATCTAACTGCTGCGCCGCCGCCGGGAAAATACTGTACGGTTAGA-**Biotin 3’
MT-II	5’ DTDTDTDTDT TTTTTT**ATCTAACTGCTGCGCCGCCGCCGGGAAAATACTGTACGGTTAGA-**Biotin 3’
sgc8-5FU	5’ (5FU) (5FU) (5FU) (5FU) (5FU) TTTTTTT**ATCTAACTGCTGCGCCGCCGCCGGGAAAATACTGTACGGTTAGA-**Biotin 3’
LIB	5’ DTTTT TTTNN NNNNN NNNNN NNNNN NNNNN NNNNN NNNNN NNNNN NN-Biotin 3’

[a] Bold letters indicate the sgc8 aptamer sequence. **D** represents the unit synthesized from phosphoramidite **8**; 5FU represents the unit synthesized from 5FU phosphoramidite.

DNA preparation.

All DNA probes were synthesized on an ABI3400 DNA/RNA synthesizer (Applied Biosystems, Foster City, CA, USA), and synthesis reagents were purchased from Glen Research. The completed sequences were then deprotected in AMA (ammonium hydroxide/40% aqueous methylamine 1:1) at 65°C for 30 min and further purified by reversed-phase HPLC (ProStar, Varian, Walnut Creek, CA, USA) on a C-18 column using 0.1 M triethylamine acetate (TEAA Glen Research Corp.) and acetonitrile (Sigma Aldrich, St. Louis, MO) as the eluent. The collected DNA products were dried and detritylated by dissolving and incubating DNA products in 200 μL 80% acetic acid for 20 minutes. The detritylated DNA product was precipitated with NaCl (3 M, 25 μL) and ethanol (600 μL). UV-Vis measurements were performed with a Cary Bio-100 UV/Vis spectrometer (Varian) for probe quantification.

Cell culture.

HCT116 cells were obtained from the American Type Culture Collection (Manassas, VA). Cells were cultured in McCoy's 5A medium supplemented with 10% fetal bovine serum (FBS) (heat-inactivated, GIBCO) and 100 IU/mL penicillin-streptomycin (Cellgro) at 37 °C in a humid atmosphere with 5% CO_2. The cell density was determined prior to each experiment using a hemocytometer.

Study of specific binding ability

The binding abilities of aptamers or MTs (final DNA concentrations: 200 nM) were determined using flow cytometry. Cells (2×10^5) were incubated in binding buffer (200 μL, 4.5 g/L glucose, 5 mM $MgCl_2$, 0.1 mg/mL yeast tRNA (Sigma Aldrich) and 1 mg/mL BSA (Fisher Scientific) in Dulbecco's PBS (Sigma)) on ice for 30 min, followed by washing twice with washing buffer (1 mL, 4.5 g/L glucose and 5 mM $MgCl_2$ in Dulbecco's PBS (Sigma)). Precipitated cells were suspended in binding buffer (200

μL) prior to flow cytometric analysis on a FACS can cytometer (BD Immunocytometry Systems). Data were analyzed using FlowJo software. Random DNA sequences (lib) were used as negative controls.

Confocal microscopy

All cellular fluorescenc images were collected on a Leica TCS SP5 confocal microscope (Leica Microsystems Inc., Exton, PA) with a 63x oil immersion objective and Leica Confocal Software. HCT116 cells were observed in DIC mode. Cells were treated with LIB or MTs, respectively, in serum-free cell culture medium, incubated in a cell culture incubator for 2.5 h, followed by staining with Hoechst 33342 (10 μg/mL. Molecular Probes, Inc., Eugene, OR) 0.5 h before washing with Dulbecco's PBS. The resultant cells were then observed by confocal microscopy.

***In vitro* cytotoxicity.**

Cytotoxicity was evaluated using CellTiter 96 cell proliferation assay (Promega, Madison, WI, USA). Cells (5×10^4 /well) were treated in serum-free medium. After incubation for 1 h (37°C, 5% CO_2), medium was removed, and fresh medium (10% FBS, 100 IU/mL penicillin-streptomycin, 200 μL) was added, followed by UV irradiation for 1 h. Then medium was replaced with cell culture medium and incubated for further cell growth (48 h). Medium was removed again, and CellTiter reagent (20 μL) diluted in fresh medium (100 μL) was added to each well and incubated for 1-2 h. The absorbance (490 nm) was recorded using a microplate reader (Tecan Safire microplate reader, AG, Switzerland). Cell viability was determined according to the manufacturer's description.

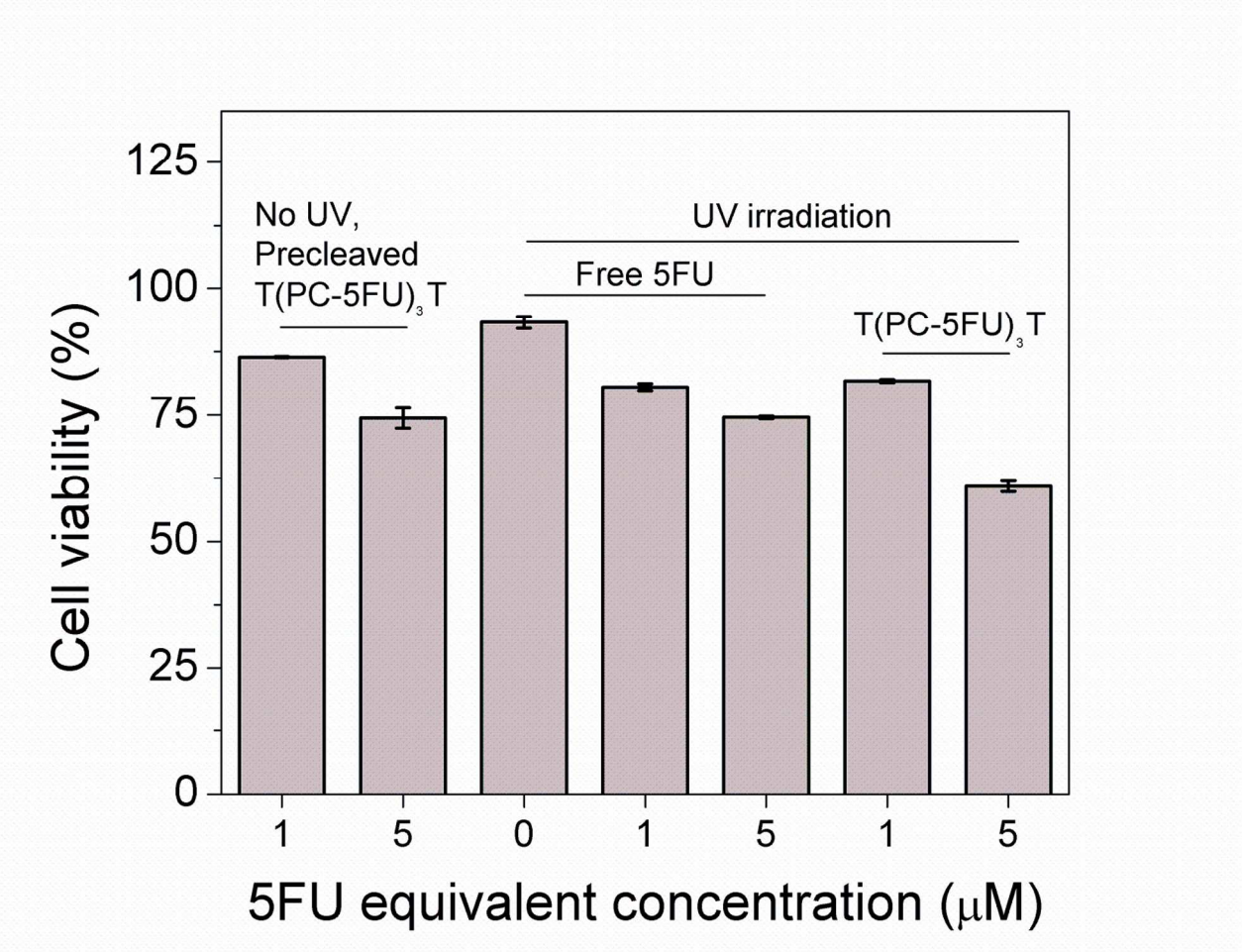


Figure S1. Cytotoxicity of 5FU released from an oligonucleotide ($T(PC\text{-}5FU)_3T$) incorporated into phosphoramidite **8**. 5FU was released from $T(PC\text{-}5FU)_3T$ by either prior UV irradiation (precleaved), or by UV irradiation after incubation with cells.