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Supporting Information

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Nucleic acid with guanidinium modification exhibits efficient cellular uptake**

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Experimental details

Materials. Mass spectral analysis for nucleoside analogues was done on an *ABI* MDS-Sciex API-100 spectrometer under atmospheric pressure ionization conditions. UV analyses were performed on a *Shimadzu* UV-1200 spectrometer. ^{1}H- and ^{31}P-NMR spectra were recorded on a *JEOL* JNM-AL300 or a JNM-LA500 FT-NMR spectrometer. Asymmetric polymerase chain reactions for preparation of modified DNAs were performed on a *Techne* TC-312 gene amplifier. *KOD Dash* DNA polymerase was from *Toyobo*. The primer oligonucleotide (P#1: 5'-FAM-labeled-TGT GAA GTG TCC CAG CCT GT-3') and template oligonucleotides (T#A3: 5'-AAA ACA GGC TGG GAC ACT TCA CA-3', T#A5: 5'-AAA AAA CAG GCT GGG ACA CTT CAC A-3', T#A10: 5'-AAA AAA AAA AAC AGG CTG GGA CAC TTC ACA-3') were purchased from *Hokkaido System Science Co., Ltd.*.

5-[(6-Guanidino-hexylcarbamoyl)-methyl]-2'-deoxyuridine-5'-triphosphate (TgTP). The triethylammonium salt of 5-[(6-amino-hexylcarbamoyl)-methyl]-2'-deoxyuridine-5'-triphosphate (**TaTP**; 100 OD_{260nm}, 11 μmol) was dissolved with 1.0 M *S*-ethylthiourea hydrobromide in DMF (2.4 mL). To the above solution, triethylamine (660 μL, 4.7 mmol) was added and stirred at room temperature for 8 h. After evaporation, the residue was purified by reversed-phase HPLC (C18, 20 × 250 mm) with the gradient 3.5-14 % acetonitrile in 50 mM triethyl ammonium acetate buffer (pH 7.2) over 45 min at a flow rate of 8.0 mL/min to give 5-[(6-guanidino-hexylcarbamoyl)-methyl]-2'-deoxyuridine-5'-triphosphate in 70 % yield: ^{1}H NMR (300 MHz, D_2O)= δ 7.82 (s, 1H), 6.23 (t, 1H), 4.59 (m, 1H), 4.18-3.99 (m, 3H), 3.26 (s, 2H), 3.08 (m, 4H), 2.30 (m, 2H), 1.43 (m, 4H), 1.22 (m, 4H); ^{31}P NMR (500 MHz, D_2O)= δ -5.6 (d), -10.6 (d), -21.4 (t): Negative ion ES-MS *m/z* [assignment] 665.1 [$(M-H)^-$].

Preparation of modified DNAs by one-primer PCR. PCR experiments were performed using a 100 μL reaction containing 7.5-10 μM the template (T#A3, T#A5 or T#A10), 5.0 μM the primer P#1,

5.0 U *KOD Dash* DNA polymerase, a reaction buffer supplied with enzyme (at 1 × concentration) and 200 μM a modified dUTP (TgTP). For all preparations, a hot start (1 min at 94°C) was used, followed by one-primer PCR and a final incubation (5 min at 74°C). One-primer PCR conditions (denaturing, annealing, extension) were set as follows: condition 1 (10 cycles, 0.5 min at 94°C, 0.5 min at 52°C, 5 min at 74°C); condition 2 (20 cycles, 0.5 min at 94°C, 0.5 min at 52°C, 5 min at 74°C). Under condition 1, reactions using templates T#A3 and T#A5, with Tg, gave core-$(Tg)_3$ and core-$(Tg)_4$ as a major product, respectively (Table S1). Reaction using T#A10 with Tg gave a mixture of core-$(Tg)_5$ and core-$(Tg)_6$ in the ratios of 3:4 under condition 1 and 5:11 under condition 2, while core-$(Ta)_4$ was obtained with T#A5 with TaTP under condition 2. The reaction mixture was desalted with a Sep-Pak cartridge column (*Waters*; C18), then purified by denaturing PAGE and eluted from the gel into TBE buffer (45 mM tris-borate, 1 mM EDTA). The eluted sample was passed through a Sep-Pak cartridge column again and lyophilized to yield the sequence-modified oligonucleotide with uridine at the 3'-end.

Table S1. The yields (%) of modified DNA

Condition	1	2	3	4	5	6	7
core-(**Tg**)$_3$	89 (67)	80	NA	NA	NA	NA	NA
core-(**Tg**)$_4$	52 (30)	62	80	60	82	92	90
core-(**Tg**)$_5$	29 (11)	20	NA	NA	NA	NA	NA
core-(**Tg**)$_6$	39 (14)	44 (39)	51	8	61	61	64
core-(**Ta**)$_4$	64	84 (41)	NA	NA	NA	NA	NA

The products were resolved by denaturing PAGE, and the gel images were recorded with Molecular Imager® FX. The yields were calculated by measuring the intensity of each band corresponding to the product. The number in parentheses indicates the isolated yield. One-primer PCRs were performed using 5.0 μM the primer, 7.5-15 μM the template, 0.05 U/μL DNA polymerase. Primer extension reactions were performed using 0.4 μM the primer, 1.2 μM the template, 0.05 U/μL DNA polymerase.

Condition

1: *KOD Dash*, One-primer PCR, 10 cycles, denaturing 0.5 min at 94 °C, annealing 0.5 min at 52 °C, extension 5 min at 74 °C

2: *KOD Dash*, One-primer PCR, 20 cycles, denaturing 0.5 min at 94 °C, annealing 0.5 min at 52 °C,

extension 5 min at 74 °C
3: *KOD Dash*, Primer extension reaction, denaturing 1.5 min at 94 °C, annealing 0.5 min at 52 °C, extension 30 min at 74 °C
4: *Vent(exo-)*, One-primer PCR, 10 cycles, denaturing 0.5 min at 94 °C, annealing 0.5 min at 52 °C, extension 5 min at 74 °C
5: *Vent(exo-)*, Primer extension reaction, denaturing 1.5 min at 94 °C, annealing 0.5 min at 52 °C, extension 30 min at 74 °C
6: *Vent(exo-)*, Primer extension reaction, denaturing 1.5 min at 94 °C, annealing 0.5 min at 52 °C, extension 60 min at 74 °C
7: *Vent(exo-)*, Primer extension reaction, denaturing 1.5 min at 94 °C, annealing 0.5 min at 52 °C, extension 120 min at 74 °C

UV Melting measurements. Absorbance measurements in the UV region were made on Shimadzu UV–1700 spectrophotometers. Melting curves (absorbance *vs.* temperature curves) were measured at 260 nm with these spectrophotometers connected to a *Shimadzu* TMSPC–8 thermoprogrammer. A heating rate of 0.5˚C/min was used for all measurements. Water condensation on the cuvette exterior in the low temperature range was avoided by flushing with a constant stream of dry N_2 gas. Prior to the experiment, the buffer was degassed with an ultrasonic wave for 5 min.

Cell culture. Cell cultivation was carried out at 37˚C in a humidified atmosphere of 5 % carbon dioxide and 95 % air. Cell counting was conducted with a Coulter counter (Model Z-1, Beckman Coulter, Inc., Fullerton, CA, U.S.A.).

HeLa cells were cultivated in 10-cm plastic plates, each of which contained 8 mL of 10 % fetal bovine serum (FBS)-supplemented Eagle's minimum essential medium (MEM). Subcultivation was performed at a 10:1 splitting ratio with 0.25 % trypsin in calcium- and magnesium-free phosphate buffered saline at pH 7.4 (PBS). The medium was changed every 2 days after inoculation.

Incorporation of oligonucleotides. HeLa cells were inoculated at a density of 4.0×10^4 cells$\cdot$cm^{-2} in 96-well plastic plates, each well of which contained 0.1 mL FBS supplemented MEM, and cultivated overnight. After removal of the medium, the cells were washed with 0.1 mL PBS, and incubated at 37˚C for 48 h in 0.1 mL FBS supplemented MEM containing 6-FAM-labeled oligonucleotides. After removal of medium with oligonucleotides, the cells were washed twice with 0.1 mL PBS. For flow cytometer analysis, the cells were incubated with 0.25 % trypsin for 3 min at 37˚C and suspended in

0.5 mL PBS and transferred into the 5-mL polystyrene round-bottom tube. Flow cytometric analysis was performed using a BD LSR Flow cytometer equipped with a 488-nm argon laser (*Becton Dickinson Biosciences Co*., San Jose, CA, U.S.A.). The cells were analyzed at an excitation wavelength of 488 nm and an emission wavelength of 530 nm (FL-1 channel).

Microscopic studies. HeLa cells were cultured on a small piece of cover slip in 96-well plastic plates. Culture conditions were same as above. For fixation, cells were fixed with 0.1 mL of 4 % formaldehyde in PBS at room temperature and then washed three times with 0.1 mL PBS.

For the fluorescence microscopy, both living cell and fixed specimens were observed under the *OLYMPUS* IX-71 equipped with epifluorescence optics (*Olympus Optical Co., Ltd.*, Tokyo). The excitation light was attenuated with a neutral density filter and reflected onto the cells with a dichroic mirror (488 nm) during the emission of fluorescence (> 500 nm).

The images of confocal microscope were collected with a Bio-Rad Radiance 2100 laser scanning confocal system (*Bio-Rad Laboratories*, Hercules, CA, U.S.A.) and an Olympus UPlanApo 40x objective (0.85 NA). FITC were excited at 488 nm with an argon laser, and the emission light was collected using an LP 500 filter. The iris was adjusted to 5.6 mm. The images were collected in 1 μm steps. Data depth for the images was 8 bit.

Hoechst 33342 nuclear staining. HeLa cells were inoculated at a density of 4.0×10^4 cells$\cdot$cm^{-2} in 96-well plastic plates, each well of which contained 0.1 mL FBS supplemented MEM, and cultivated overnight. After removal of the medium, the cells were washed with 0.1 mL PBS, and incubated at 37˚C 48 h in 0.1 mL FBS supplemented MEM containing oligonucleotides with 6-FAM. After removal of the medium and washing of the cells twice with 0.1 mL PBS, the cells were incubated at 37˚C for 15 min in 0.1 mL FBS supplemented MEM containing 0.1 μg / mL Hoechst 33342.

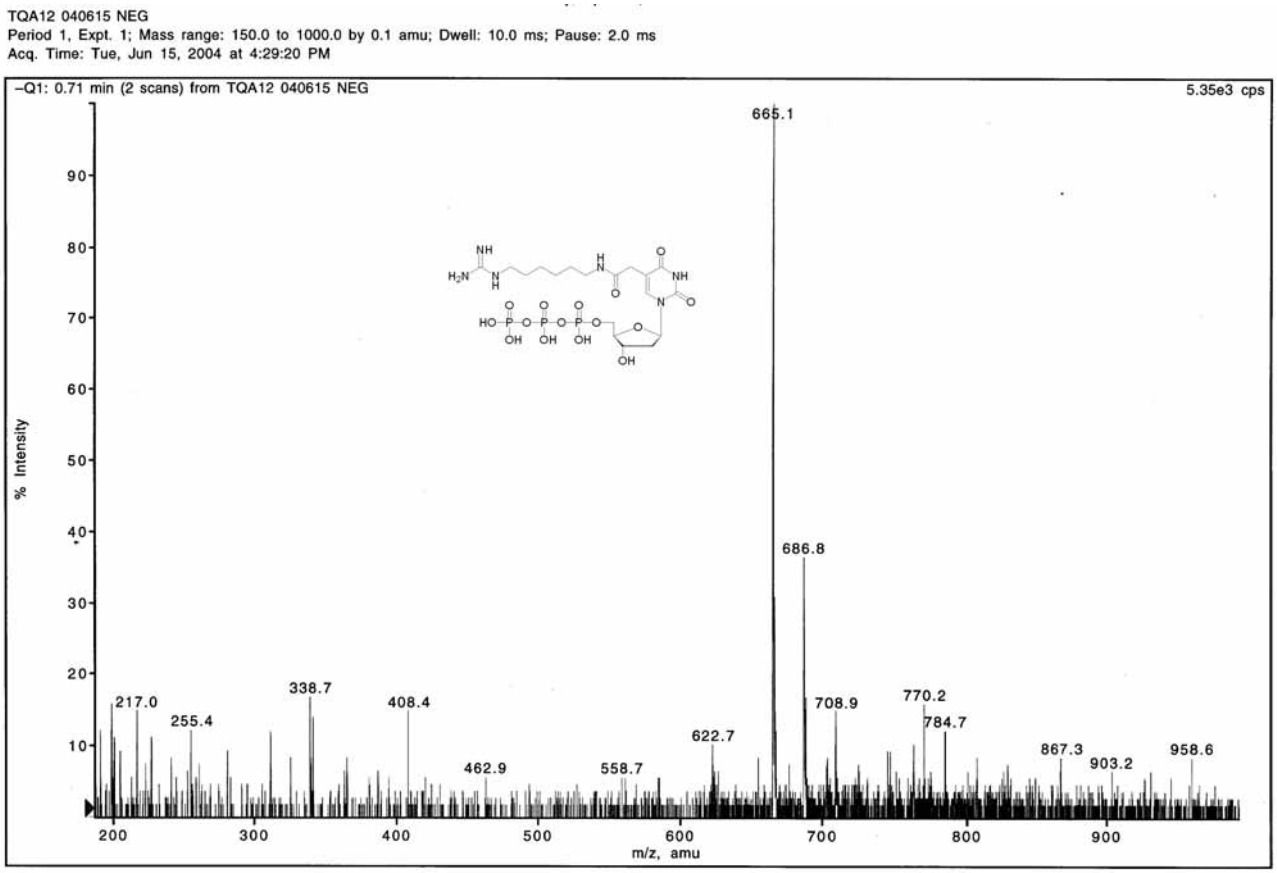

Figure S1. Mass spectrum of 5-[(6-Guanidino-hexylcarbamoyl)-methyl] -2'-deoxyuridine-5'-triphosphate (TgTP).

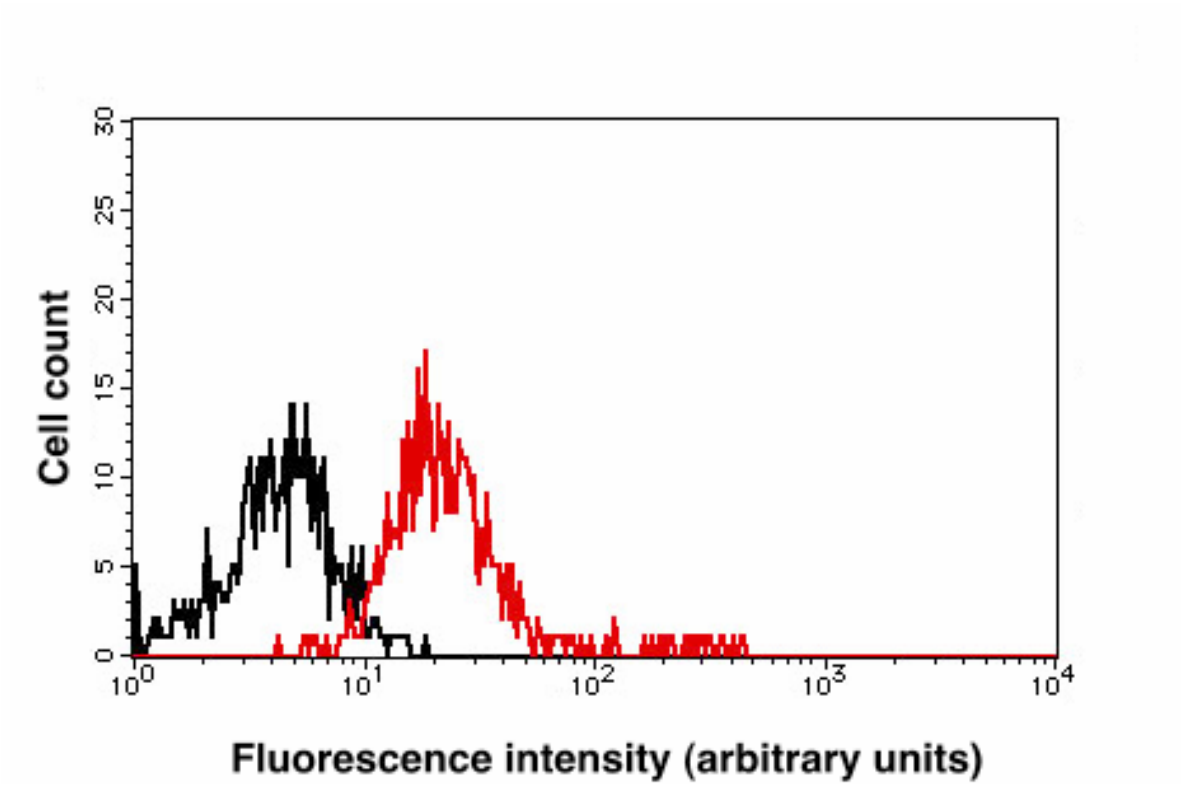

Figure S2. Typical histograms of increase in 6-FAM intensity in HeLa cells induced by induction of modified DNA. After 48 h incubation, the cells were collected and analyzed using flow cytometry. The red line indicates the population of cells incubated in the presence of DNA oligomer with $(\mathbf{Tg})_4$. The black line indicates the population of cells in the absence of DNA oligomer with $(\mathbf{Tg})_4$.

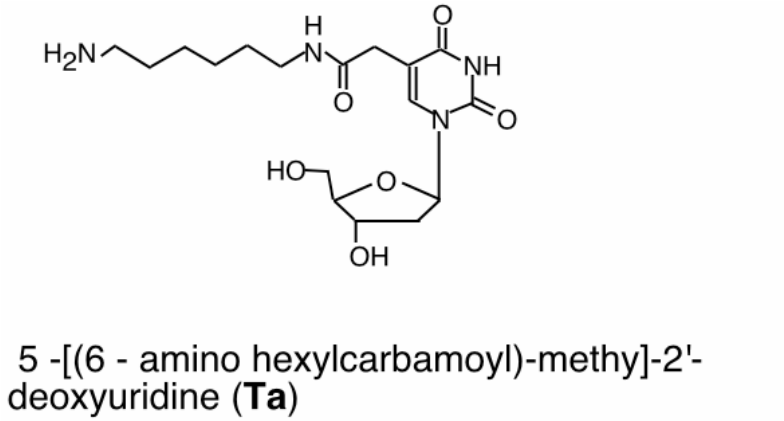

Figure S3. Structure of 5 –[(6 –amino-hexylcarbamoyl)-methy]-2’-deoxyuridine (**Ta**).

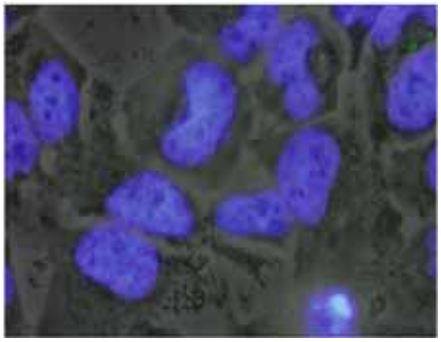

Figure S4. Microscopy images of living HeLa cells cultured in the presence of 4 μM FAM-labeled 20 mer DNA oligomer without $(\mathbf{Tg})_4$ at the 3'-end: overlay images of DNA oligomer, nucleus and phase contrast.

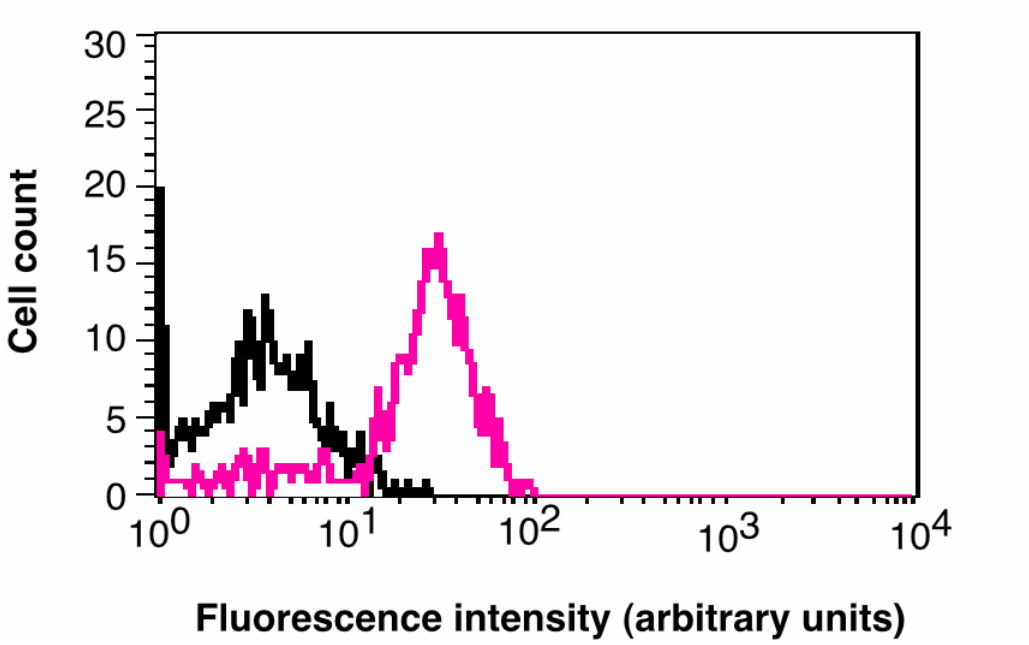

Figure S5. Typical histograms of increase in 6-FAM intensity in RAW 264.7 cells induced by induction of modified DNA. After 48 h incubation, the cells were collected and analyzed using flow cytometry. The pink line indicates the population of cells incubated in the presence of DNA oligomer with $(\mathbf{Tg})_4$. The black line indicates the population of cells in the absence of DNA oligomer with $(\mathbf{Tg})_4$.

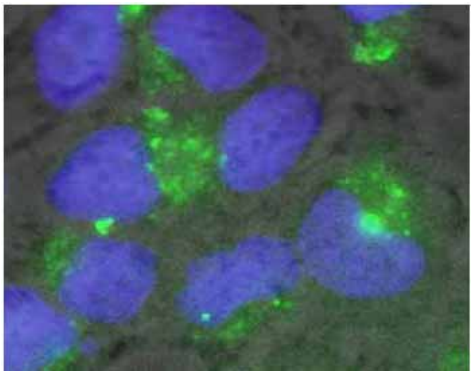

Figure S6. Microscopy images of fixed HeLa cells cultured in the presence of 4μM core-Tg_4: the fluorescence overlay images of DNA oligomer, nuclei, and phase contrast.