Supporting Information

Creation of Single Molecular Conjugate of Metal-organic Cage and DNA

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General methods

All chemicals and solvents used in the syntheses were of reagent grade and were used without further purification. Cp*CpZrCl$_2$ was prepared by following a synthesis procedure described in the previous reports (Cp is the cyclopentadienyl ligand and Cp* is the pentamethylcyclopentadienyl ligand).[1] Azide-terephthalic acid was prepared by following a synthesis procedure described in the previous reports.[2] The ^{1}H NMR spectra were recorded with a BRUKER Av300 (300 MHz) NMR spectrometer. Single crystal X-ray diffraction data were collected on a RIGAKU XtaLab P200 CCD system with VariMax Mo Optic with MoKα radiation (λ = 0.71073 Å) and a confocal monochromator, with the constant temperature of 293 K by flowing nitrogen gas. The structure was solved by direct methods and refined by full-matrix least-squares cycles using SHELX.[3] All non-hydrogen atoms were refined with anisotropic thermal parameters. Hydrogen atoms attached to carbon atoms were located at geometrically calculated positions and refined with isotropic thermal parameters. TGA was performed using a Rigaku TP-EVO2 SL DyTG/DTA TypeJ under nitrogen flow scanning from 40 °C to 500 °C with a temperature ramp of 5 °C /min. All IR measurements were performed with a FT/IR 4200 spectrometer (JASCO) with neat samples. The adsorption isotherms of N_2 at 77 K were obtained on a BELSORP-Max volumetric-adsorption instrument from microtrac-BEL, Inc. The measurement temperature was controlled by cryostat. ESI-MS data were recorded by compact QTOF (Bruker Daltonics) in a positive mode. MALDI-TOF-MS data were obtained using a microflex MALDI-TOF mass spectrometer (Bruker Daltonics) by positive mode using a mixture of 3-hydroxypicolinic acid (HPA) and ammonium citrate as a matrix. LC-MS analysis was performed by Agilent 1260 Infinity II LC/MSD with column of ACQUITY UPLC BEH C18, 130 Å, 1.7 μm, 2.1 mm × 50 mm (Waters). Analysis conditions is as follow: column temp, 60 °C; Solvent A, 8.6 mM TEA / 100 mM HFIP; Solvent B, MeOH; Solvent gradient, 0 to 60% B over

30 min; Flow rate, 0.3 mL/min. Temperature-dependent UV absorbance was recorded by V-650 spectrophotometer (JASCO) with a temperature gradient of 0.5 °C min^{-1}, and melting temperature (*T*m) was calculated by the equipped analysis program.

High-speed atomic force microscopy (HS-AFM)

High-speed AFM imaging was performed in tapping mode using a laboratory-built instrument. A miniaturized microcantilever (Olympus: BL-AC7; 6–7-μm long, 2-μm wide, and 90-nm thick) with a spring constant of about 0.1 N/m, a Q value of about 1, and a resonant frequency of about 800 kHz in a solution was used. The free oscillation amplitude of the cantilever was roughly 2 nm, and the feedback was set at the oscillation amplitude to 1.5 nm. Mica coated with spermine was used as the solid substrate. First, 0.1 mg/ml of spermine diluted with milli-Q water was dropped onto the mica immediately after cleavage. After 3-min incubation, the substrate was washed well with milli-Q water. Then, a sample solution of 2 ml was dropped onto that substrate and incubated for 5 minutes, after which it was washed with observation buffer (10 mM Tris, pH 7.5, 75 mM NaCl). The sample was then immersed in the observation buffer for AFM imaging.

Supplementary movie legends

Supplementary Movie S1: HS-AFM movie of **MOC-DNA** captured at a scanning speed of 0.1 s/frame. Scan area: 130 nm × 90 nm. Pixel size: 60 × 40 pixels.

Cell culture

HeLa cells (RIKEN Cell Bank, Japan) were cultured in high glucose Dulbecco's Modified Eagle's Medium (DMEM) (Fujifilm Wako Chem., USA) supplemented with inactivated 10% fetal bovine serum (FBS) (GE Healthcare, USA). Cells were cultured at 37 ºC under humidified atmosphere

of 5% CO_2 and 90% humidity.

Flow cytometry

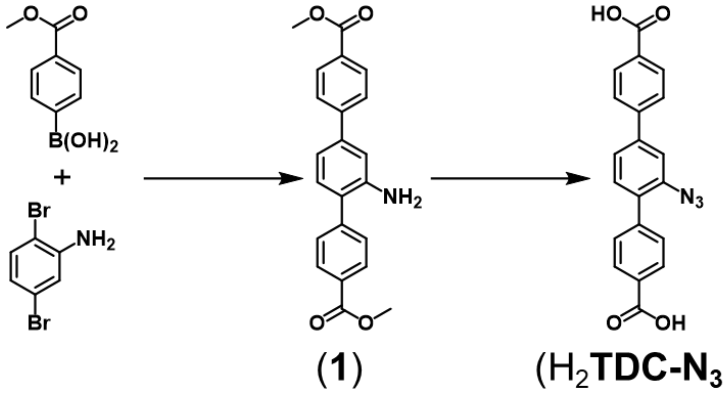
HeLa cells were seeded at 2×10^4 cells/well on 96-well culture plate and cultured for 24 h. After cell condition became stable, cells were washed with PBS twice and incubated for 3 h at 37 ºC with FBS-free DMEM containing each oligonucleotide. For time-dependent cellular uptake estimation, cells were soaked in FBS-free DMEM containing **MOC-DNA** and then they were incubated at 37℃ for 0, 20, 40, 60, 120 and 180 min. Cells incubated for 180 min at 4 ºC were also prepared to estimate the uptake mechanism. In all cases, the concentrations of oligonucleotides were 1 μM. After incubation for each duration, D-MEM containing oligonucleotide was removed and cells were washed with PBS for one time. Then, cells were treated with 0.05% trypsin-EDTA solution (nacalai tesque, INC., Japan) at 37℃ for 2-3 mins and suspended in colorless D-MEM supplemented with 10% FBS and 20 mM HEPES-KOH to stop reaction. Filtrated cell suspension was analyzed by SA3800 flow cytometer (Sony, Japan) to measure the FAM fluorescence intensity which is detected by laser excitation at 488 nm.

Confocal laser scanning microscopy

HeLa cells were seeded at 2×10^4 cells/well on 10φ 35 mm glass-bottomed dishes (Mat Tek Corp., USA) and cultured for 24 h. After cell condition became stable, cells were washed with PBS twice and incubated for 3 h at 37 ºC with FBS-free DMEM containing each oligonucleotide. Concentrations of oligonucleotides were 1 μM. For nucleus staining, 200 μg/mL Hoechst 33342 (Dojindo Mol. Tech. Inc., USA) was added to cell cultures and incubated for 30 min at 37 ºC. After incubation, cells were washed with PBS for one time and filled with colorless DMEM supplemented with 10% FBS and 20 mM HEPES-KOH for observation.

Images were acquired with C2-Si (Nikon) using 60× oil immersion objective lens (PlanApoVC 60×/1.40 NA, Nikon). HeNe laser was used as a source for providing 440-nm wavelength lights. Diode-pumped laser was used for providing 488 nm wavelength lights. Used filters are 447/60-nm bandpass filter, 482-nm long-path dichroic filter and 525/50-nm bandpass filter. Acquired images were analyzed by Fiji software (ver. 2.0.0-rc-69/1.52p, https://fiji.sc[4]).

Sample preparation



Scheme S1. Synthetic procedure for 2'-azido-[1,1':4',1''-terphenyl]-4,4''-dicarboxylic acid (H_2**TDC-N$_3$**).

Synthesis of dimethyl 2'-amino-[1,1':4',1''-terphenyl]-4,4''-dicarboxylate (1)

A mixture of (4-(methoxycarbonyl)phenyl)boronic acid (2.25 g, 12.50 mmol), 2,5-dibromoaniline (1.25 g, 5.00 mmol), tetrakis(triphenylphosphine)palladium (0) (0.13 g, 0.18 mmol) and an aqueous solution of Na_2CO_3 (1 M, 2.25 ml), toluene/EtOH = 4:1 (15 ml) was sonicated, then heated in the microwave (150 ºC, 15 min). After cooling to room temperature, the reaction mixture was added ethyl acetate and washed with brine 3 times, dried over Na_2SO_4, and filtrated. Dimethyl 2'-amino-[1,1':4',1''-terphenyl]-4,4''-dicarboxylate (**1**) appeared as crystalline solids (0.37 g, 20% yield). ^{1}H NMR (DMSO-d_6): δ 8.06-8.03 (dd, 4H), 7.79-7.76 (d, 2H), 7.66-7.63 (d, 2H), 7.18-7.16 (m, 2H), 7.04-7.00 (dd, 1H), 5.13 (s, 2H), 3.88 (s, 6H).

Synthesis and Characterization of 2'-azido-[1,1':4',1''-terphenyl]-4,4''-dicarboxylic acid (H_2TDC-N$_3$)

1 (1.20 g, 3.33 mmol) was dissolved in THF (50 ml) and the mixture was cooled to 0 ºC. To the mixture, ^{t}Bu-nitrite (1.2 ml, 10 mmol) was added, and the mixture was stirred for 15 min. Then

trimethylsilyl azide (0.86 ml, 6.6 mmol) was slowly dropped into a solution, and the mixture was stirred overnight. A solution of LiOH (spatulafull) in THF/EtOH/H2O = 3/2/1 (150 ml) was added to the mixture. After stirring for 6 h, the mixture was acidified with aq. HCl. The resulting solid was collected by filtration and washed with H_2O and acetone. 2'-azido-[1,1':4',1"-terphenyl]-4,4"-dicarboxylic acid (H_2**TDC-N$_3$**) was obtained as a pale-yellow powder (1.05 g, 87% yield). ^{1}H NMR (DMSO-d_6): δ 13.07 (s, 2H), 8.08-8.05 (d, 2H), 8.04-8.01 (d, 2H), 7.94-7.92 (d, 2H), 7.70-7.65 (m, 4H), 7.57-7.54 (d, 2H), IR 2110 cm^{-1}(s,$\nu_{C\equiv N3}$).

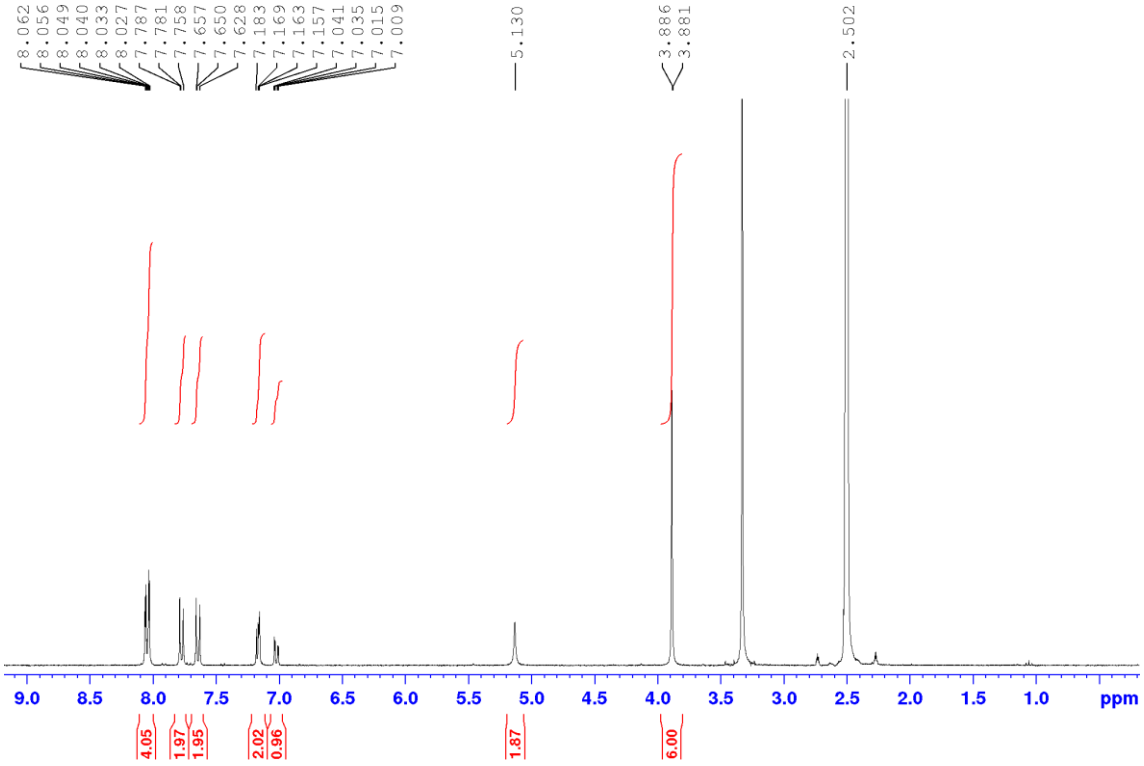


Figure S1. ^{1}H NMR spectrum of **1**.

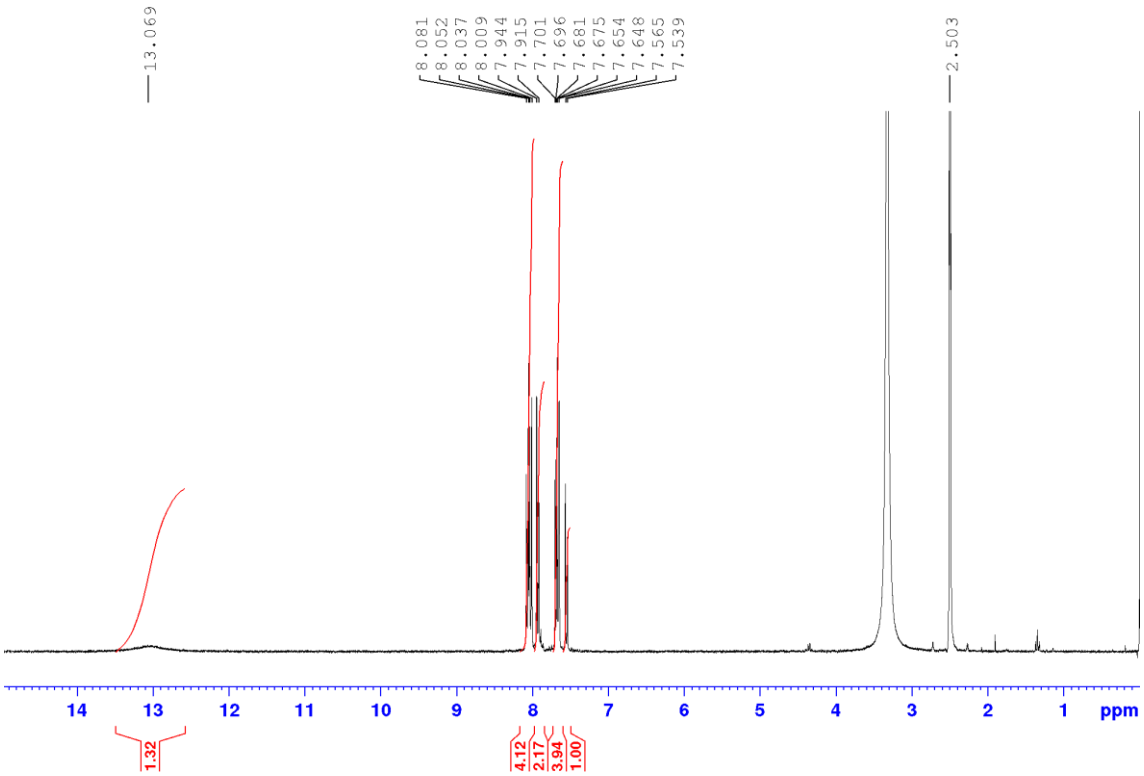


Figure S2. ^{1}H NMR spectrum of H_2**TDC-N$_3$**.

Synthesis of Cp-terephthalic-N_3-MOC (MOC-1)

Zirconocene dichloride (350.0 mg, 1.20 mmol) and azide terephthalic acid (124.0 mg, 0.60 mmol) were dissolved in diethylformamide (DEF, 20 mL). The mixture was transferred to a vial and to the solution 3.0 mL of H_2O and 0.1 mL of 1M HNO_3 were added. The vial was heated in an oven at 60 ºC for 12 h. After cooling to room temperature, the cubic crystals were corrected by filtration and washed with DEF and CH_2Cl_2. The powder was soaked in fresh CH_2Cl_2 to remove residual DEF. The CH_2Cl_2 was replaced twice every 24 h. Then the powder was filtrated and dried in a vacuum at 40 ºC overnight. **MOC-1** was obtained as yellow powder. (169.5 mg, 48% yield).

Synthesis of Cp*-terephthalic-N_3-MOC (MOC-2)

Cp*CpZrCl_2 (110.0 mg, 0.30 mmol) and azide terephthalic acid (31.0 mg, 0.15 mmol) were dissolved in DEF (5 ml) and 1.75 ml of H_2O in a glass vial, which was heated in an oven at 80 ºC for 12 h. After cooling to room temperature, the precipitate was corrected by filtration and washed with DEF. Then the residue was dissolved in CH_2Cl_2 and the solution was filtrated. After evaporation of the solvent, **MOC-2** was obtained as yellow powder. (17.3 mg, 17% yield).

Synthesis of Cp*-terphenyl-N_3-MOC (MOC-3)

Cp*CpZrCl_2 (440.0 mg, 1.20 mmol) and H_2**TDC-N_3** (214 mg, 0.60 mmol) were dissolved in DEF (20 ml) and 7 ml of H_2O in a glass vial, which was heated in an oven at 80 ℃ for 12 h. After cooling to room temperature, the precipitate was corrected by filtration and washed with DEF. Then the residue was dissolved in CH_2Cl_2 and the solution was filtrated. After evaporation of the solvent, **MOC-3** was obtained as yellow powder. (250 mg, 49% yield).

Synthesis of oligonucleotides

Oligo DNA was synthesized at 0.2 μmol or 1.0 μmol scale by DNA/RNA synthesizer MR-2A 7 MX (Nihon Techno Service Co., Japan). 5-(Benzylthio)-1H-tetrazole was used as an activator reagent for coupling. Oxidizing 80 solution is 0.01 mol/L iodine in 64% acetonitrile (ACN) / 30% water / 6% pyridine (Honeywell, BR667AA-4). To introduce the fluorescein unit, 6-FAM lcaa CPG (DMT) 500A (ChemGenes, N-9986-05) was used for 3′ terminus. For the deprotection and cleavage of ONs, controlled pore glass was extruded into a 1.5 mL screw cap (Sarstedt, Germany) and was treated with the 28% ammonium hydroxide for 12 h at room temperature.

After the deprotection, the crude sample of oligonucleotide was concentrated by a centrifugal evaporator (Centrifugal Evaporator CVE-3100, EYELA Co. Japan), then analyzed and purified by a reversed phase HPLC. The mobile phase system is (A) 50 mM triethylamine acetate solution (pH 7.0) containing 5% ACN and (B) ACN in all experiments. The analysis and purifications of oligonucleotides were performed on YMC Hydrosphere C-18 (250 x 4.6 mm, particle size: S-5 μm) column and YMC Hydrosphere 95 C-18 (250 x 10.0 mm, particle size: S-5 μm) under the flow rate of 1 mL/min and 3 mL/min respectively at room temperature. The gradient program is as follows: 0% - 60% B (0-20 min), 95% B (20.1-25 min) and 0% B (25.1-30 min). After the purification, the existence of the target oligonucleotides was confirmed by MALDI-TOF mass or LC-MS.

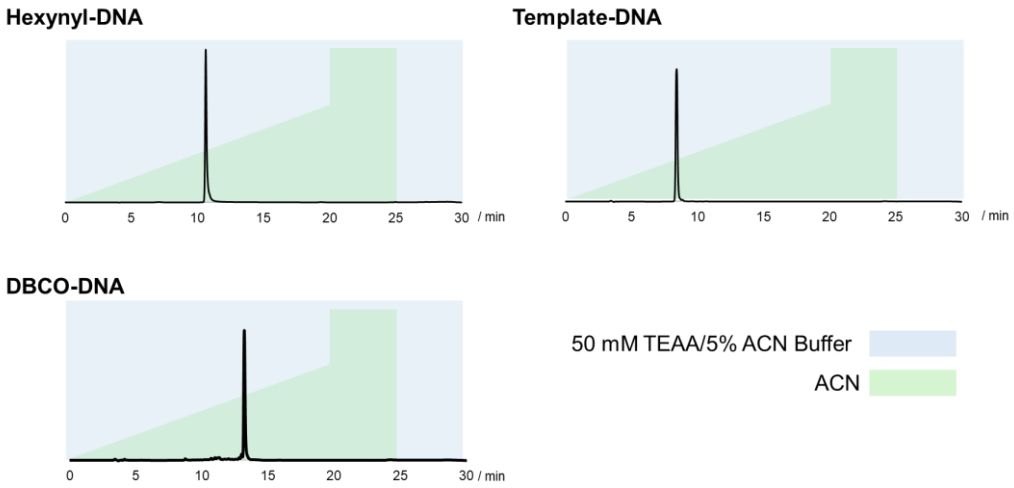
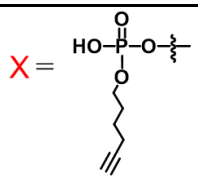
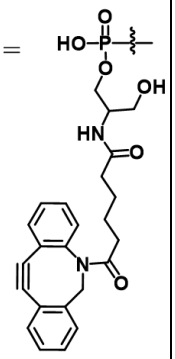


Figure S3. HPLC chart of purified oligonucleotides. The mobile phase system is (A) 50 mM triethylamine acetate (TEAA) solution (pH 7.0) containing 5% ACN and (B) ACN in all experiments. The gradient program is as follows: 0% - 60% B (0-20 min), 95% B (20.1-25 min) and 0% B (25.1-30 min).

Table S1. Name, sequences and MS values of oligonucleotides.

Name	Sequences	MS (M+H$^+$)	
		Calc.	Found
Hexynyl-DNA	5'-X-d*ATT TCG GCT CTT CCT*-6FAM-3' X = 	5215.56	5215.13[1)]
DBCO-DNA	5'-Y-d*ATT TCG GCT CTT CCT*-6FAM-3' Y = 	5523.89	5524.21[1)]
Templated-DNA	5'-d*TAA AGC CGA GAA GGA TAA AGC CGA GAA GGA TAA AGC CGA GAA GGA TAA AGC CGA GAA GGA TAA AGC CGA GAA GGA TAA AGC CGA GAA GGA-3'*	28265.4	28253.4[2)]

1) Data was corrected by MALDI-TOF-MS, 2) Data was corrected by LC-MS.

Synthesis of MOC-DNA

The mixture of **DBCO-DNA** (in H_2O 100 μM, 100 μL) and a solution of **MOC-3** in DEF (500 μM, 100 μL) were put into a 1.5 mL screw cap tube and was heated in an aluminum bath at 40 ℃ overnight. Then, the reaction mixture was added 1.0 mL of H_2O and centrifugated. The supernatant was purified to obtain **MOC-DNA** by a reversed phase HPLC (Column: YMC Triart Bio C-4 (250 x 4.6 mm, particle size: S-5 μm), flow rate: 0.75 mL/min, temperature: 50 ºC. The mobile phase system is (A) 50 mM sodium acetate solution (pH 7.0), (B) ACN and (C) water. The gradient program is as follows: 0% - 60% B, 100% - 40% A (0-25min), 95% B, 5% A (25.1-30 min), 100% C (30.1-40 min), 50% B and C (40.1-55 min), 100% B (55.1-65 min), 100% C (65.1-70 min) and 100% A (70.1-80 min).)

Quantification method of the concentration of DBCO-DNA and MOC-DNA

1 μL of **DBCO-DNA** or **MOC-DNA** solution was diluted with 99 μL of 1 M aq. NaOH. Then the fluorescence intensity at λ= 535 nm of the solution was measured by TrisStar 5 Research Plus (BERTHOLD, Germany) with excitation at λ= 495 nm. The concentration of the solution was calculated from the fluorescence intensity and a calibration curve was prepared using **Hexynyl-DNA**.

Structure of MOC-1

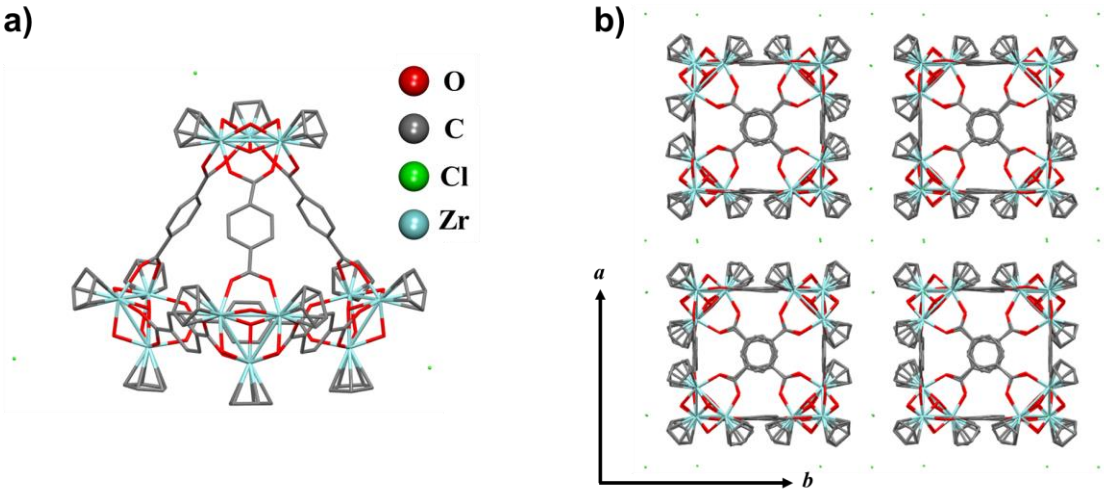


Figure S4. Crystal structure of **MOC-1**. a) The tetrahedral cage structure of **MOC-1**. b) The packing of the structure of **MOC-1** viewed along the c axis. Hydrogen atoms are omitted for clarity. Azide groups were not assigned due to severe disorder.

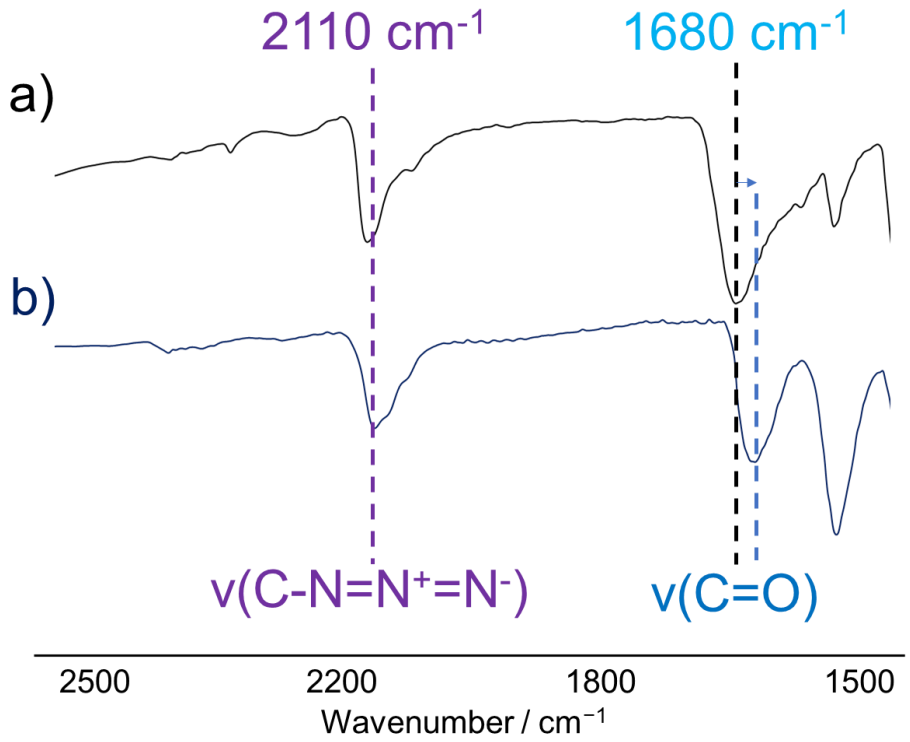


Figure S5. IR spectra of (a) Azide-terephthalic acid and (b) **MOC-1**. The presence of IR peak at 2110 cm^{-1} indicates the existence of N_3 group on the tetrahedral cage.

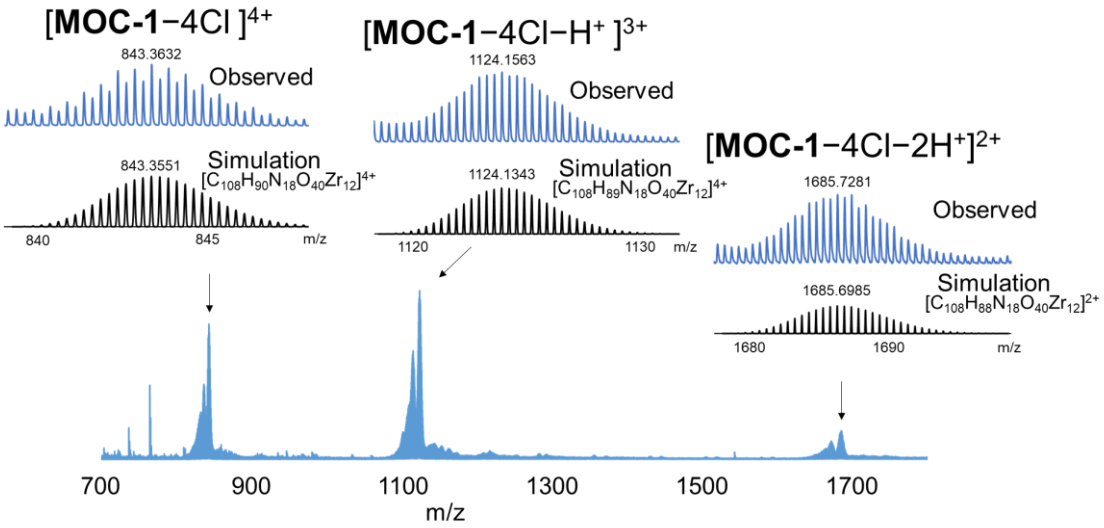


Figure S6. ESI-MS spectra of **MOC-1** dissolved in acetonitrile (blue) and the isotope simulation patterns of [**MOC-1**−4Cl]$^{n+}$ ([$C_{108}O_{40}N_{18}H_{90}Zr_{12}$−(4−n)$H^+$]$^{n+}$) (black).

Stability test of MOC-1 against DNA

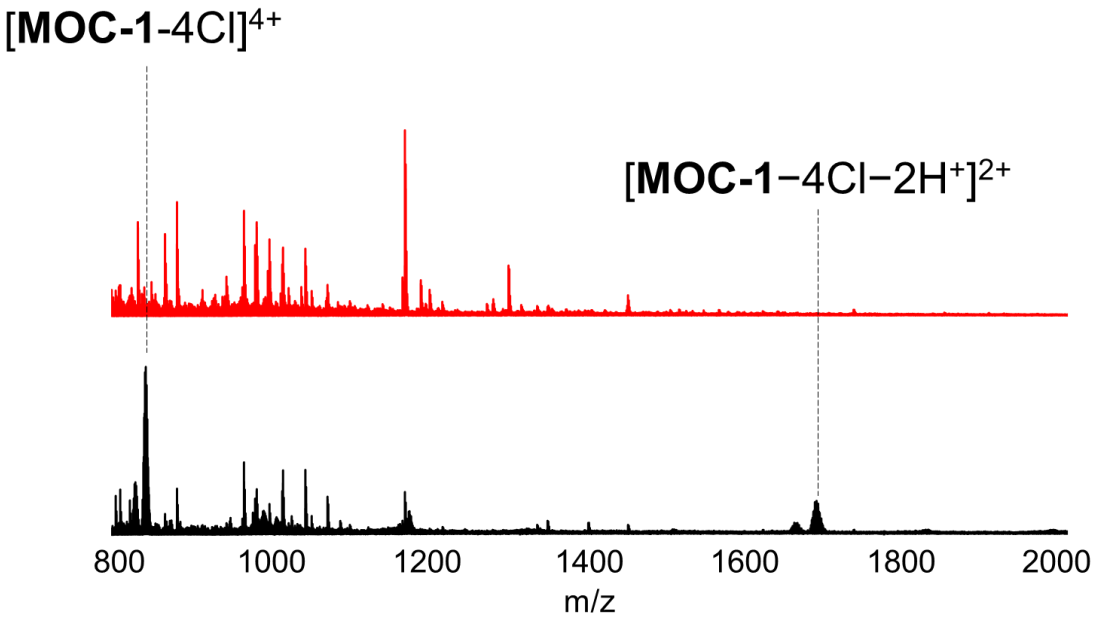


Figure S7. ESI-MS spectra of **MOC-1**. Black: Control. Red: **MOC-1** treated with **Hexynyl-DNA**.

The stability test was done as follows; A mixture of **MOC-1** solution (5 nmol in 50 μL DMSO), **Hexynyl-DNA** (5 nmol in 37 μL H_2O) and 50 μL of ACN was put into a 1.5 mL screw cap tube and was heated in an aluminum bath at 40 ℃ overnight. To estimate the stability, the control sample was also prepared by the same way except using 37 μL H_2O instead of **Hexynyl-DNA** solution. Then both solutions of control and **MOC-1** with **Hexynyl-DNA** were diluted with 500 μL of ACN, filtrated, and measured by ESI-MS.

Synthesis and the structures of MOC-2 and MOC-3

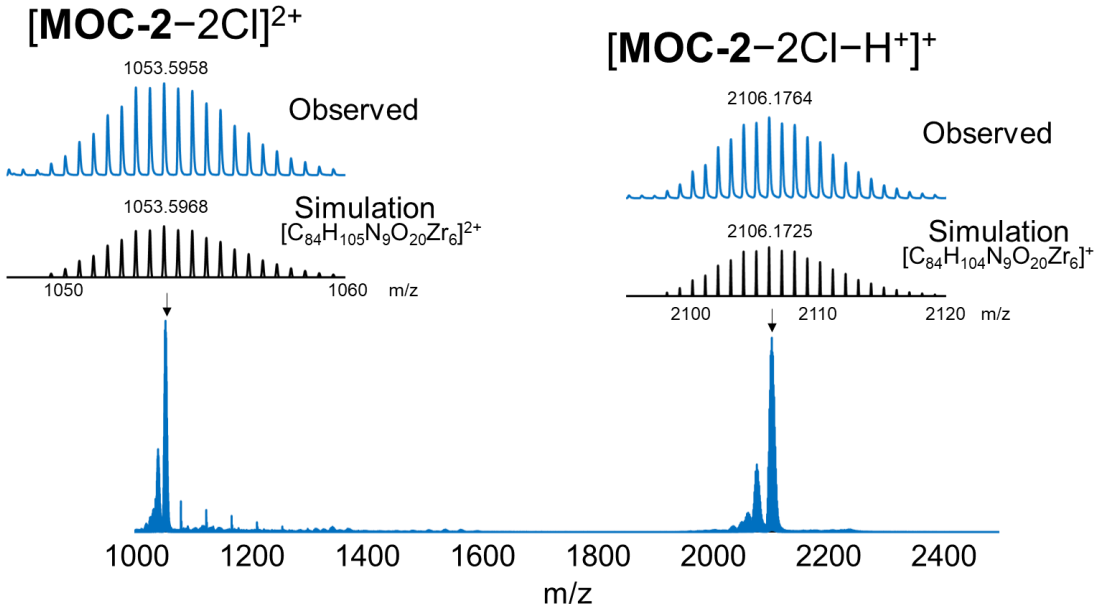


Figure S8. ESI-MS spectra of as-synthesized **MOC-2** in CH_2Cl_2.

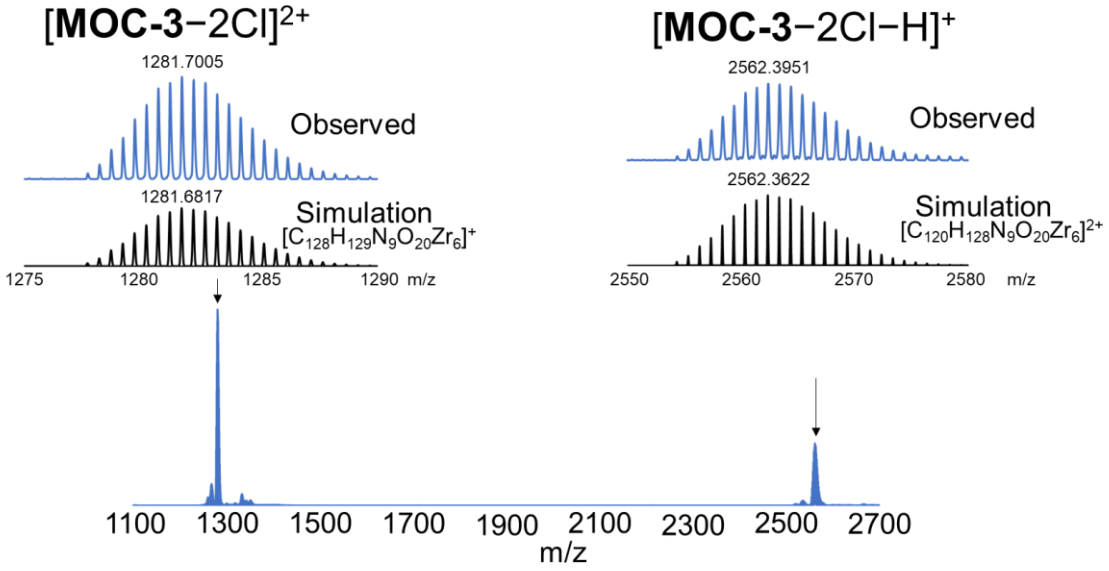


Figure S9. ESI-MS spectra of as-synthesized **MOC-3** in CH_2Cl_2.

The reason why **MOC-2** and **MOC-3** have different structures from **MOC-1** is probably due to the steric hindrance of methyl groups of Cp*, suggested by the larger dihedral angle between a Zr_3 plane and a carboxylate group in **MOC-2** and **MOC-3** than in **MOC-1** (Fig. S10).

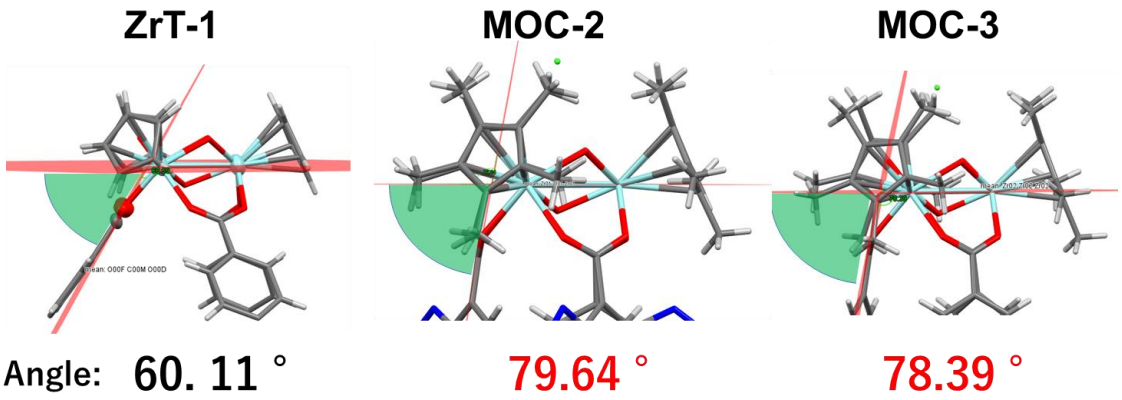


Figure S10. Comparison of dihedral angles between the plane consisting of the carbon and oxygen atoms of the carboxylic acid and the plane consisting of the three zirconium atoms.

*__ZrT-1__ is a reported tetrahedral Zr-MOC which is consisted of Cp and terephthalic acid. The dihedral angle of **ZrT-1** was calculated using cif-file deposited in CCDC as number 950330[5].

Porosity of MOC-3

The porosity of **MOC-3** was evaluated by N_2 adsorption isotherm at 77 K. Approximately 20 mL (STP)·g^{-1} of N_2 was adsorbed at the low-pressure region (P/P_0 = ~10^{-3}) representing the micropore (Fig. S11a), which corresponds to two molecules/cage and is consistent with the presence of two internal voids in **MOC-3** (Fig. S11b). In addition, the pore size estimated by the non-linear density functional theory method shows a sharp distribution at ~3.46 Å (Fig. S11c), which is comparable to the expected pore size of **MOC-3** (Fig. S11d). Moreover, the previously reported isoreticular MOCs had internal pores.[6] These results prove that **MOC-3** has internal nanopores that can trap small molecules.

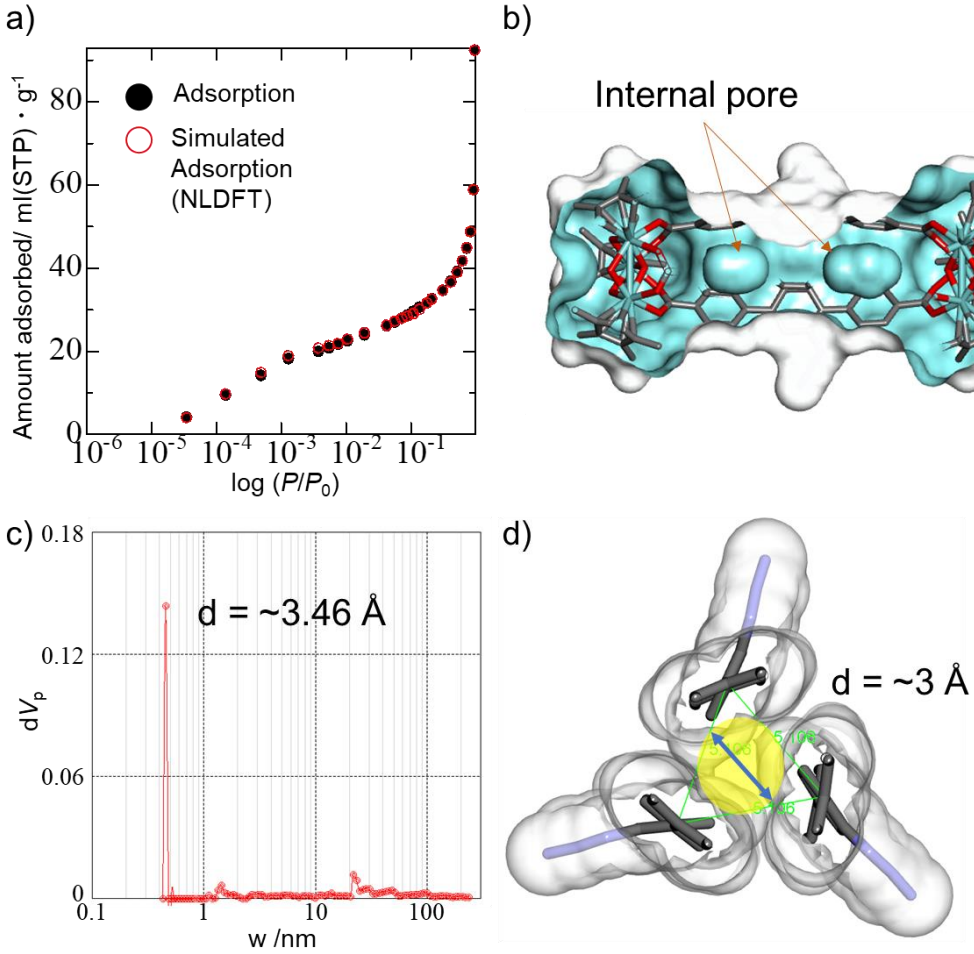


Figure S11. (a) N_2 adsorption isotherm of **MOC-3** measured at 77 K. Black circle: experiment. Red circle: simulated isotherm by NLDFT method. (b) Solvent accessible surface of **MOC-3** calculated with the probe radius of 1.20 Å. (c) Pore size distribution. (d) The cross-section image of the crystal structure of **MOC-3** using CPK model. The yellow circle represents the inscribed circle of the van der Waals surface of three benzene rings.

Stability test of MOC-3 against DNA

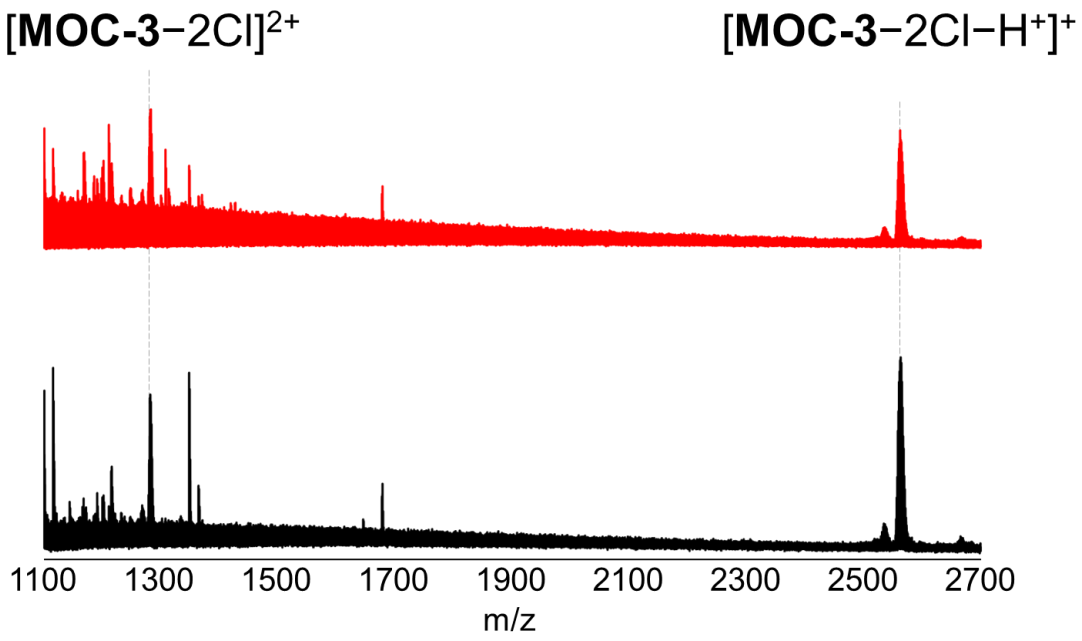


Figure S12. ESI-MS spectra of **MOC-3**. Black: Control sample. Red: **MOC-3** treated with **Hexynyl-DNA**.

The stability test was done as follows; A mixture of **MOC-3** solution (5 nmol in 50 μL DEF), **Hexynyl-DNA** (5 nmol in 37 μL H_2O) and 50 μL of ACN was put into a 1.5 mL screw cap tube and was heated in an aluminum bath at 40 ℃ overnight. To estimate the stability, the control sample was also prepared by the same way except using 37 μL H_2O instead of **Hexynyl-DNA** solution. Then both solutions of control and **MOC-3** with **Hexynyl-DNA** were diluted with 500 μL of ACN, filtrated, and measured by ESI-MS.

Copper-catalyzed click reaction

We tried to conjugate **Hexynyl-DNA** and **MOC-3** via a copper-catalyzed click reaction. After mixing **Hexynyl-DNA**, **MOC-3**, $CuSO_4$, Tris(3-hydroxypropyltriazolylmethyl)amine, and sodium ascorbate in a DEF/H_2O = 1:1 solution, the mixture was incubated for 24 h, at room temperature. After that, we analyzed the reaction mixture by LC-MS. As a result, the peak derived from the adduct of H_2**TDC-N$_3$** and DNA was observed (Fig. S13). Furthermore, the stability of **MOC-3** against Cu^{2+} was investigated. **MOC-3** in DEF solution (500 μM, 19.25 μl) was mixed with aqueous $CuSO_4$ solution (500 mM, 0.76 μl). After incubating for 24 h, at room temperature, the mixture was washed with water and CH_2Cl_2. The organic layer was separated, concentrated, and analyzed by ESI-MS. No mass peak of **MOC-3** was observed (Fig. S14). These results suggest that **MOC-3** was decomposed or became unstable against DNA by Cu^{2+} ion.

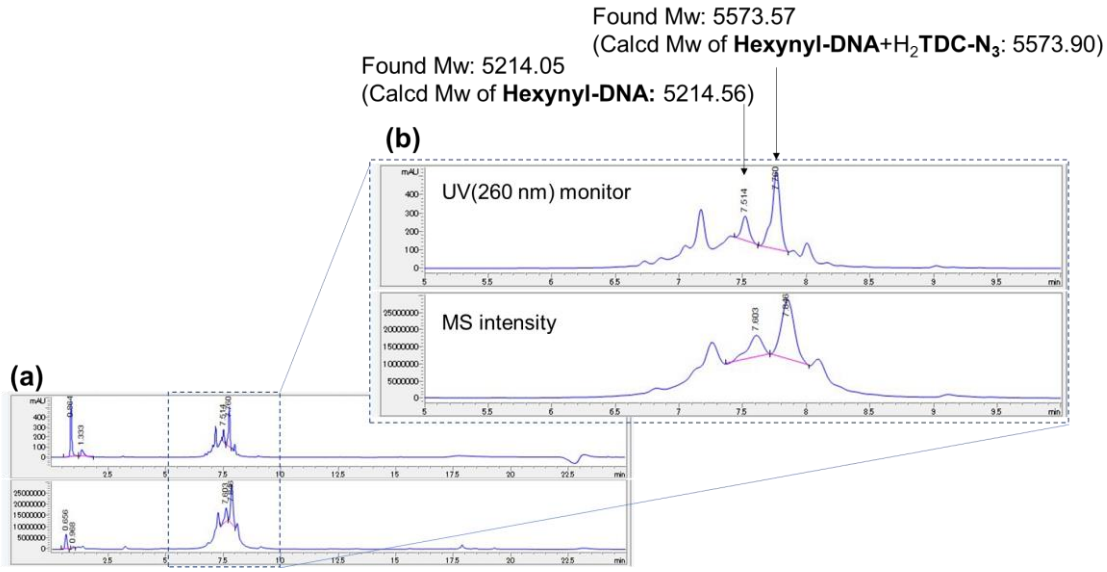


Figure S13. LC-MS charts of the reaction mixture of Cu-catalyzed click reaction of **Hexynyl-DNA** and **MOC-3**. (a) Whole range and (b) Enlarged view.

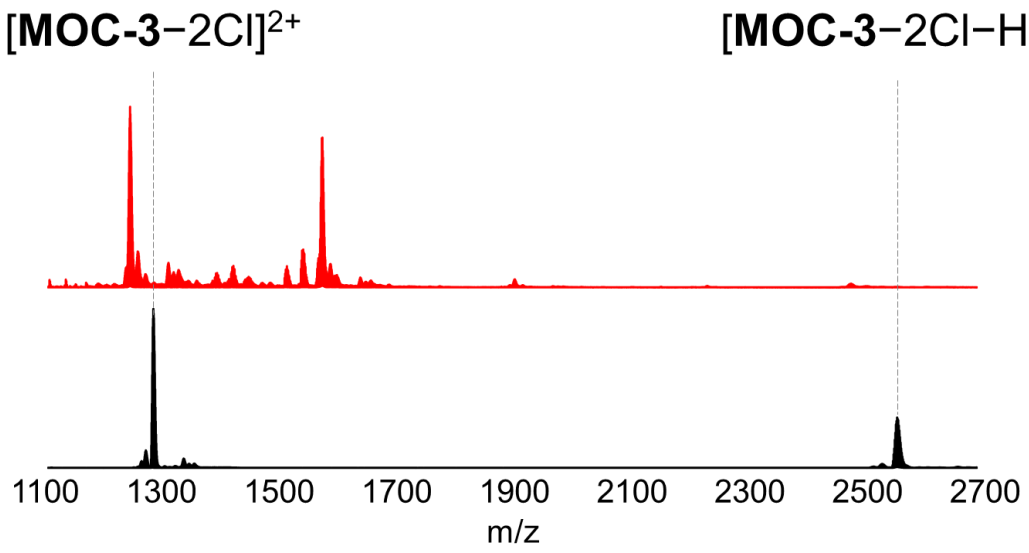


Figure S14. ESI-MS spectra of **MOC-3**. Black: As-synthesized **MOC-3**. Red: **MOC-3** treated with Cu^{2+} ion.

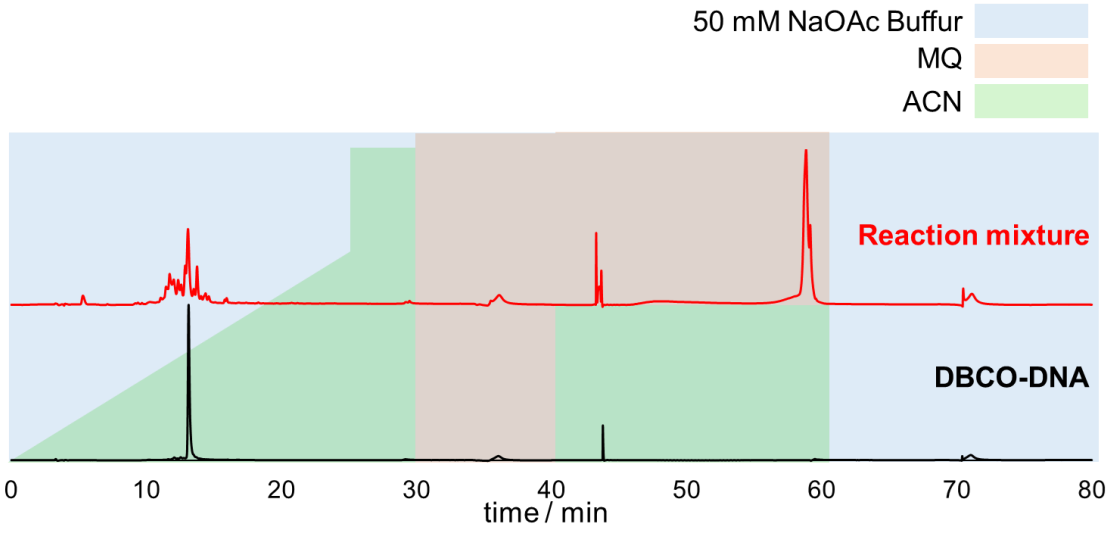


Figure S15. Illustration of the gradient conditions used for **MOC-DNA** purification. The mobile phase system is (A) 50 mM sodium acetate solution (pH 7.0), (B) ACN and (C) water.The gradient program is as follows: 0% - 60% B, 100% - 40% A (0-25min), 95% B, 5% A (25.1-30 min), 100% C (30.1-40 min), 50% B and C (40.1-55 min), 100% B (55.1-65 min), 100% C (65.1-70 min) and 100% A (70.1-80 min).

Proof of the bonding style of MOC-DNA

To check if MOC can bind to DNA via noncovalent interactions, we mixed **Hexynyl-DNA**, which cannot undergo click reaction without a catalyst, and **MOC-3**, as the same conditions used to synthesize **MOC-DNA**. After heating, the reaction mixture was diluted with water, and unreacted **MOC-3** was removed by filtration. Then, MALDI-TOF-MS measurements of the filtrate were performed. As a result, only the peak at *m*/*z* = 5213.9753 corresponding to unreacted **Hexynyl-DNA** was observed (Figure S16). The result strongly indicates that click reaction, neither ionic interaction nor coordination bond between phosphate and Zr ion, is the key to the formation of **MOC-DNA**.

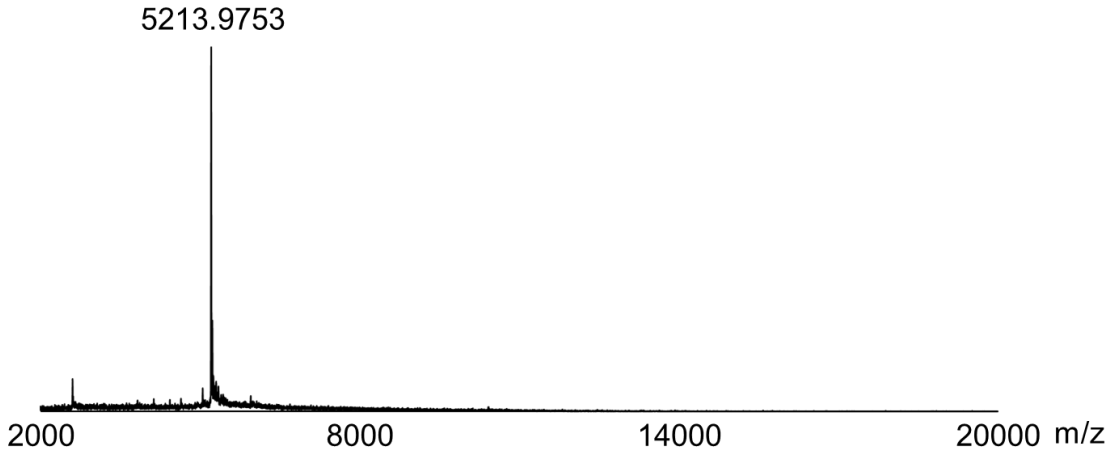


Figure S16. MALDI-TOF-MS spectrum of the mixture of **Hexynyl-DNA** and **MOC-3**.

Evaluation of the temperature-dependent binding ability of DNAs

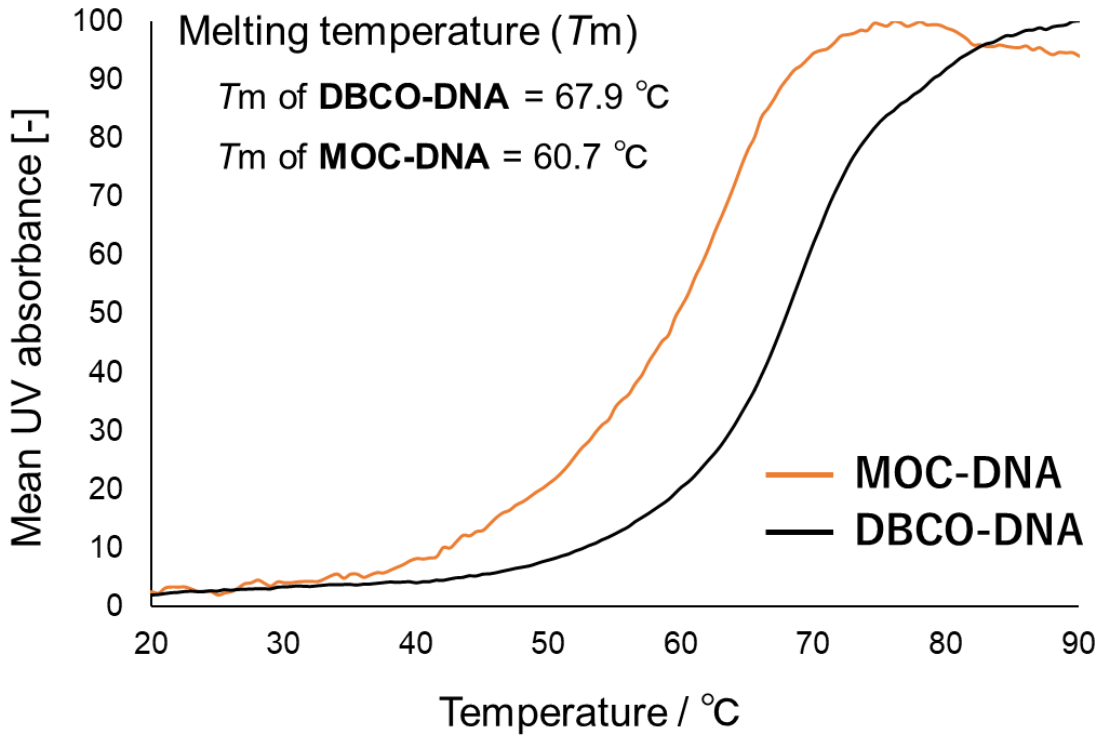


Figure S17. Temperature-dependent UV absorbance (λ=260 nm) changes of the solution containing **Template-DNA** (0.3 μM), **MOC-DNA** or **DBCO-DNA** (1.8 μM), NaCl (75 mM) and Tris-HCl (10 mM). All solutions were heated at 90 ˚C for 3 min and then gradually cooled to room temperature. After that, the UV absorbance of the solution was recorded upon increasing the temperature (0.5 °C min^{-1}).

The melting temperature (*T*m) of **MOC-DNA** shifted to lower than **DBCO-DNA**, probably due to the modification of the 5' end with **MOC-3**, but still clearly higher than room temperature.

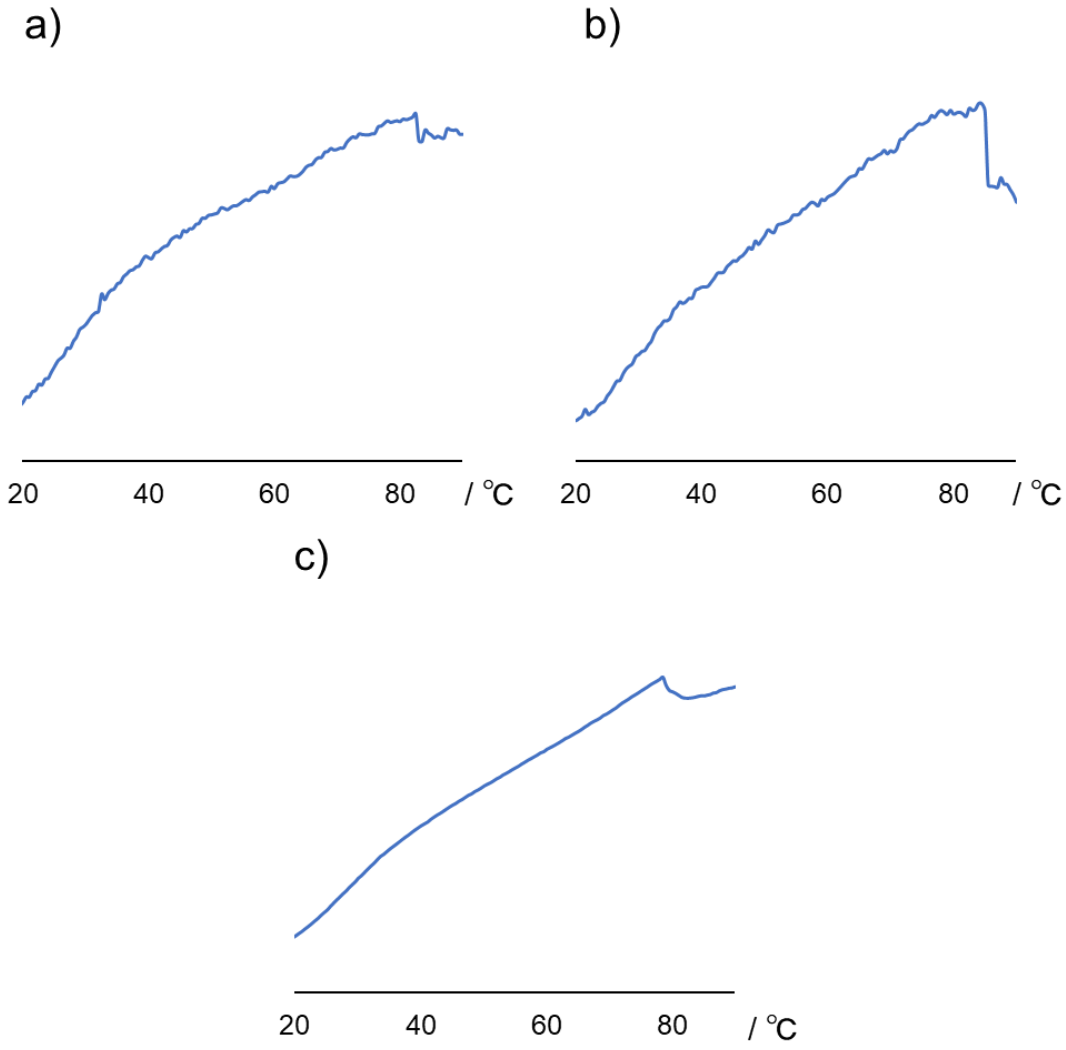


Figure S18. Temperature-dependent UV absorbance (λ=260 nm) changes of the solution containing NaCl (75 mM) and Tris-HCl (10 mM), and (a) **DBCO-DNA** (0.3 μM), (b) **MOC-DNA** (0.3 μM), (c) **Template-DNA** (0.3 μM). All solutions were heated at 90 ˚C for 3 min and then gradually cooled to room temperature. After that, the UV absorbance of the solution was recorded upon increasing the temperature (0.5 °C min^{-1}).

Size comparison

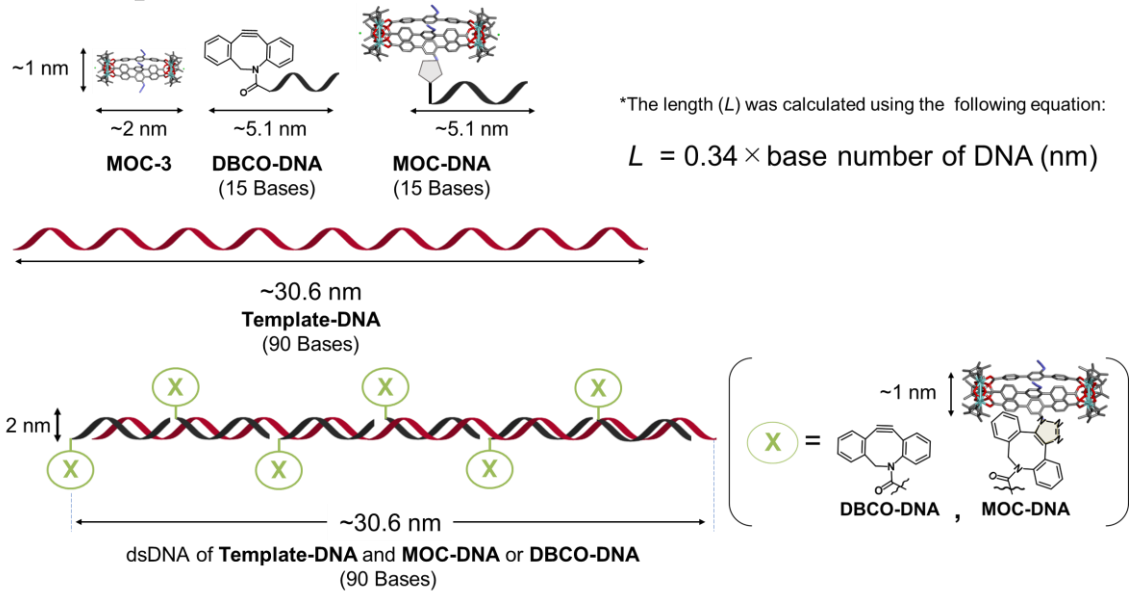


Figure S19. Approximate size of **MOC-3** and DNAs; the size of **MOC-3** was estimated from the crystal structure.

MOC-DNA aggregation observed in HS-AFM

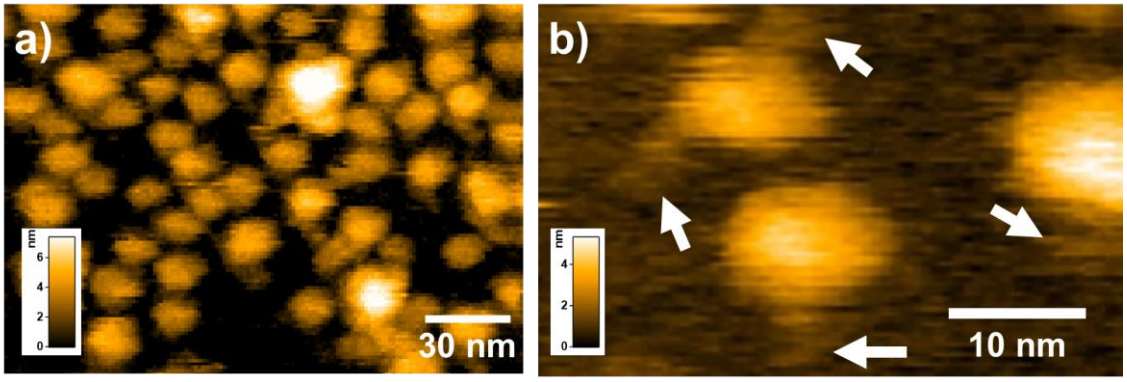


Figure S20. (a), (b) the view of bare **MOC-DNA** (30 μM) in H_2O. String structures are highlighted by arrows. The substrate is spermine/mica.

Fluorescence spectra of DNAs

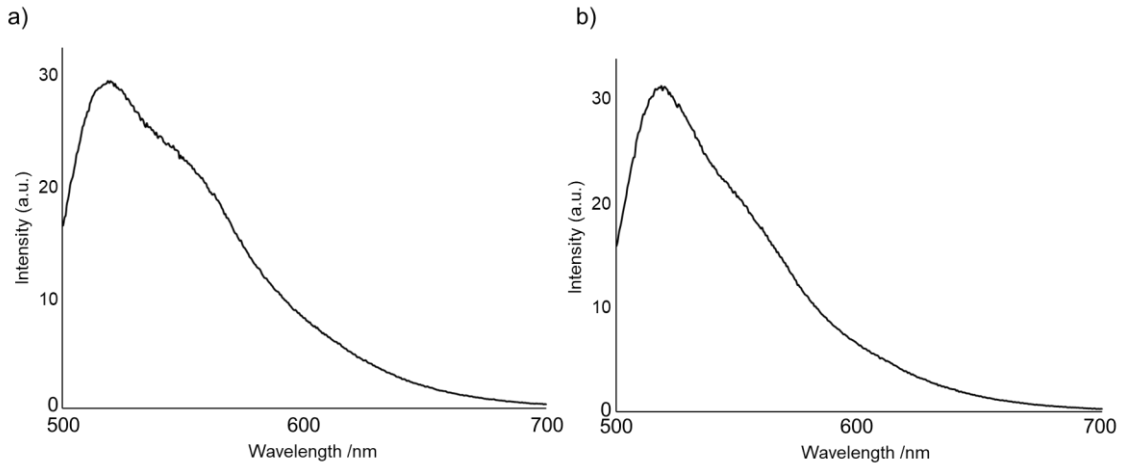


Figure S21. Fluorescence spectra of (a) **DBCO-DNA** and (b) **MOC-DNA**. The concentration of each aqueous solution was 3 μM. Excitation wavelength was 455 nm. The observed spectra were in good agreement with that of 6-FAM[7], showing that the fluorescence originates from the FAM moiety bound to DNAs.

Time-dependent uptake of MOC DNA into HeLa cells

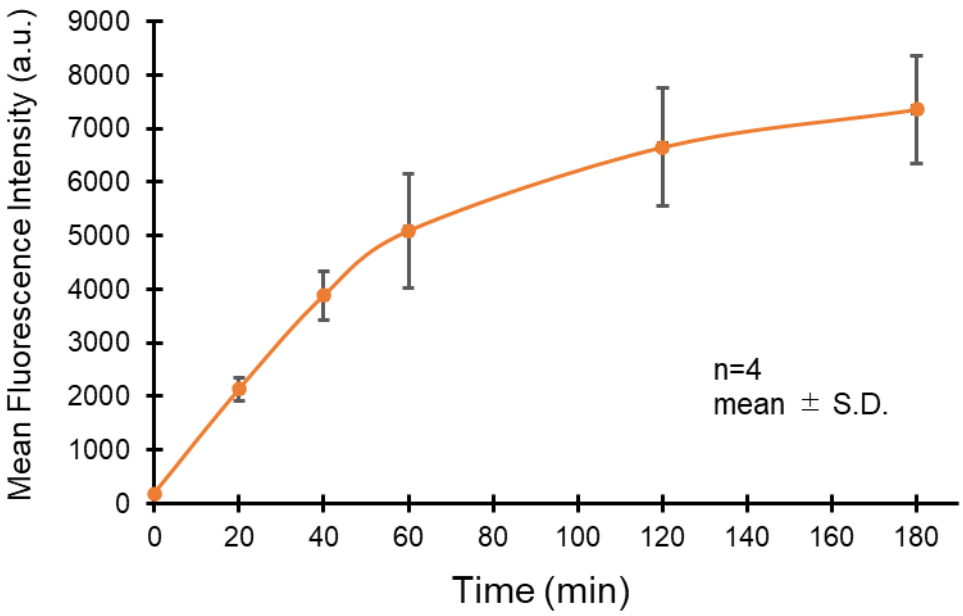


Figure S22. Time-dependent cellular uptake of **MOC-DNA** measured by flow cytometry. HeLa cells were treated with a 1 μM solution of **MOC-DNA**. The experiment was performed as four independent experiments. Error bar represents standard deviation.

Evaluation of cell permeability of each DNA

The cell permeability of each DNA was compared as follows; First, as the background, five cell-free regions (10 μm × 10 μm) were extracted in the brightfield images and their fluorescence intensities were averaged. Next, regions with a cell cross-section length of 12 μm were selected in the brightfield image. At that location, fluorescence intensity was measured in an area 24 μm in length (i.e., a region extending 6 μm outward from both cell membranes) and 5 μm in width (see the yellow bar in Figure S23a). The fluorescence intensity derived from the area was calculated by subtracting the background from the raw fluorescence intensity. Finally, the fluorescence intensity was plotted at the corresponding point (Figure S23b). Statistical processing of the results from different locations is shown in Figure S24. The fluorescence intensity of the cytoplasm was obviously higher than the untreated and **DBCO-DNA-**treated cells (Figure S24d).

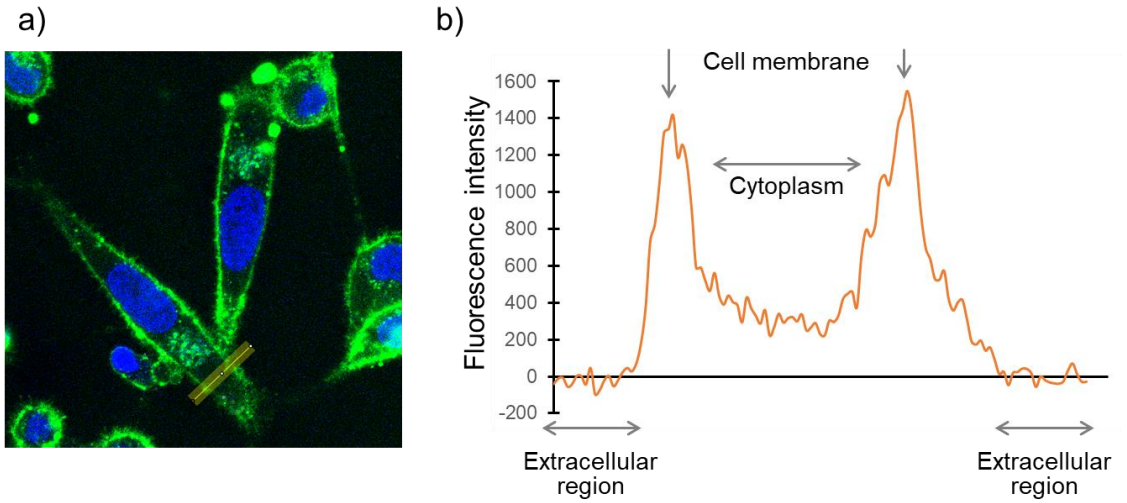


Figure S23. The figure represents how to measure and plot the fluorescence intensity.

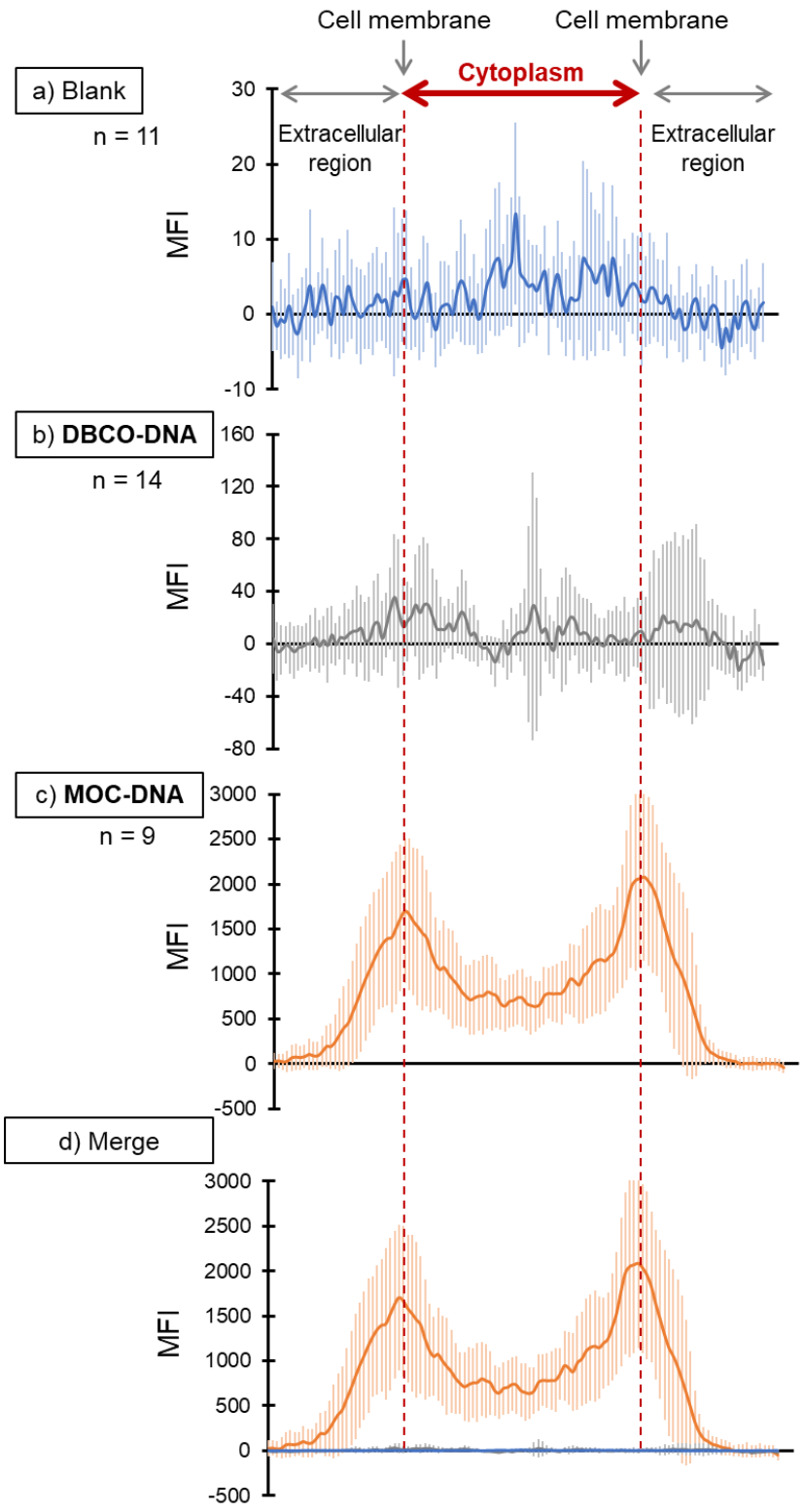


Figure S24. The comparison of the mean fluorescence intensity of cells treated with (a) only buffer (blank), (b) **DBCO-DNA**, (c) **MOC-DNA**. (d) Merged image of the above three figures. Each "n" represents the number of samples used.

Colocalization analysis of MOC-DNA and cell nuclei

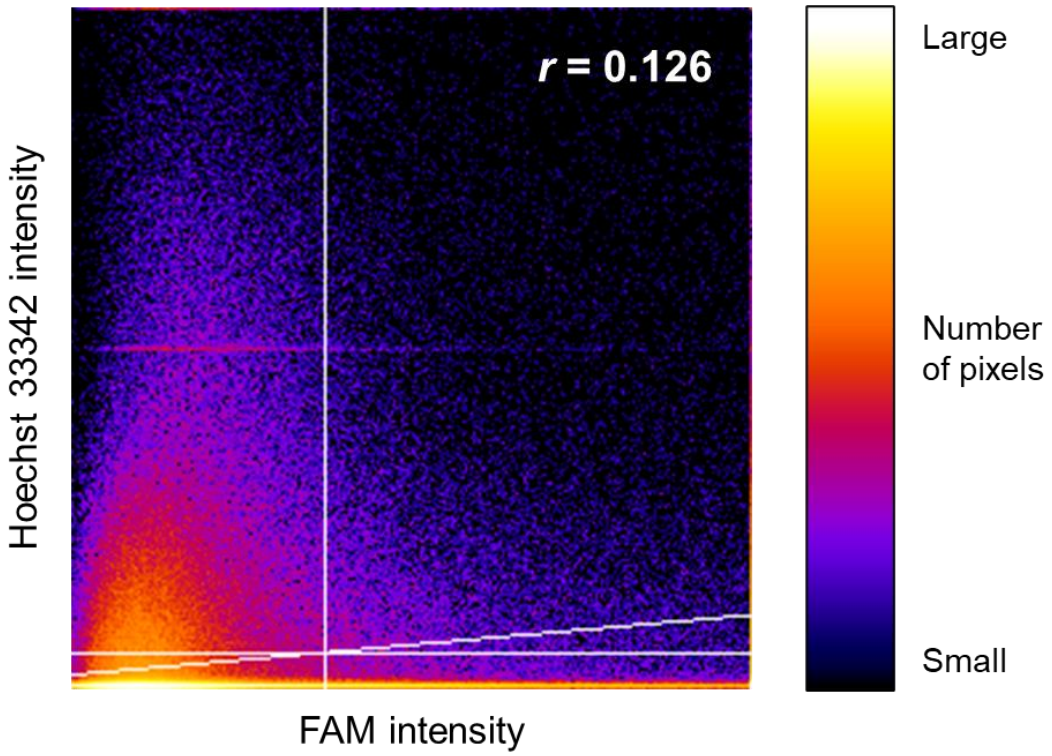


Figure S25. Scatterplot of blue (derived from Hoechst 33342, representing the location of cell nuclei) and green (derived from FAM, representing the location of **MOC-DNA**) intensities in each pixel of the right image shown in Fig.5d. Color represents the number of pixels with corresponding fluorescence intensities of Hoechst 33342 and FAM. From the image, the Pearson's correlation coefficient (r) was estimated as 0.126 by "Correlation" function of Fiji software, showing that there was almost no correlation between the location of **MOC-DNA** and cell nuclei.

Temperature-dependent uptake of MOC DNA in HeLa cells

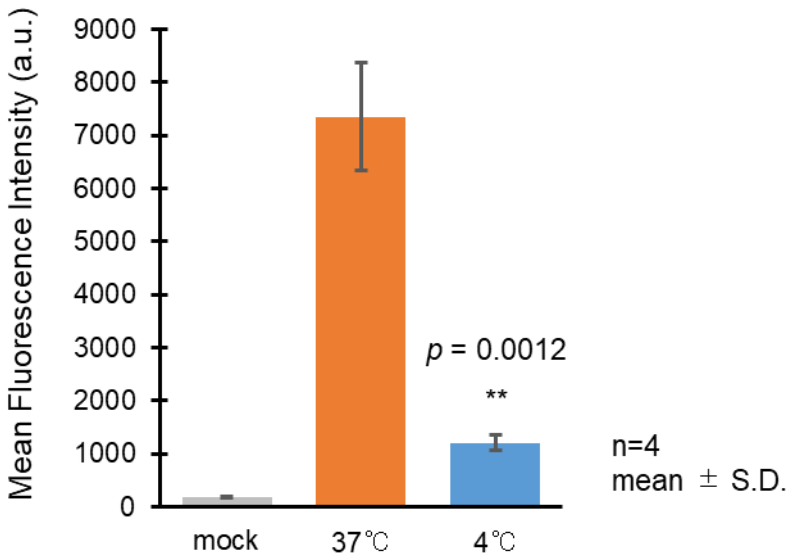


Figure S26. Temperature-dependent cellular uptake of **MOC-DNA** measured by flow cytometry. HeLa cells were incubated with a 1 μM solution of **MOC-DNA** for 3 h at 37 or 4 ℃. The experiment was performed as four independent experiments. Error bar represents standard deviation.

Crystallographic data

Table S2. Crystallographic data and structural summary for **MOC-2**

	MOC-2
Formula	$C_{84}H_{96}N_9O_{20}Zr_6Cl_2$
Crystal system	Trigonal
Space group	*R*−*3*
a (Å)	18.9840(10)
b (Å)	18.9840(10)
c (Å)	51.415(2)
α (°)	90
β (°)	90
γ (°)	120
V (Å^3)	16047.1
Z	6
Temperature (K)	297
R_1	0.0772
wR_2	0.2389
GOF on F^2	0.928

Table S3. Crystallographic data and structural summary for **MOC-3**

	MOC-3
Formula	$C_{120}H_{120}N_9O_{20}Zr_6Cl_2$
Crystal system	Trigonal
Space group	*R*−*3*
a (Å)	17.7435(7)
b (Å)	17.7435(7)
c (Å)	71.981(2)
α (°)	90
β (°)	90
γ (°)	120
V (Å^3)	19625.8
Z	6
Temperature (K)	93
R_1	0.0758
wR_2	0.2533
GOF on F^2	1.045

Author Contributions

R. Matsuda and H. Abe conceived and supervised the project; T. Nakajo mainly conducted experiments and wrote the original manuscript. S. Kusaka and H. Iguchi reviewed and edited the manuscript. K. Nakamoto and M. Honma designed DNAs. H. Hiraoka carried out cell experiments and analysis. K. Nomura, N. Matsubara, R. Baba and Y. Yoshida helped in the synthesis, purification and characterization of DNAs. T. Uchihashi conducted HS-AFM measurement and analysis. All authors have given approval for the final version of the manuscript.

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