Supporting Information

Synthesis and Application of ^{19}F-labeled Fluorescent Nucleoside as a Dual-mode Probe for i-Motif DNAs

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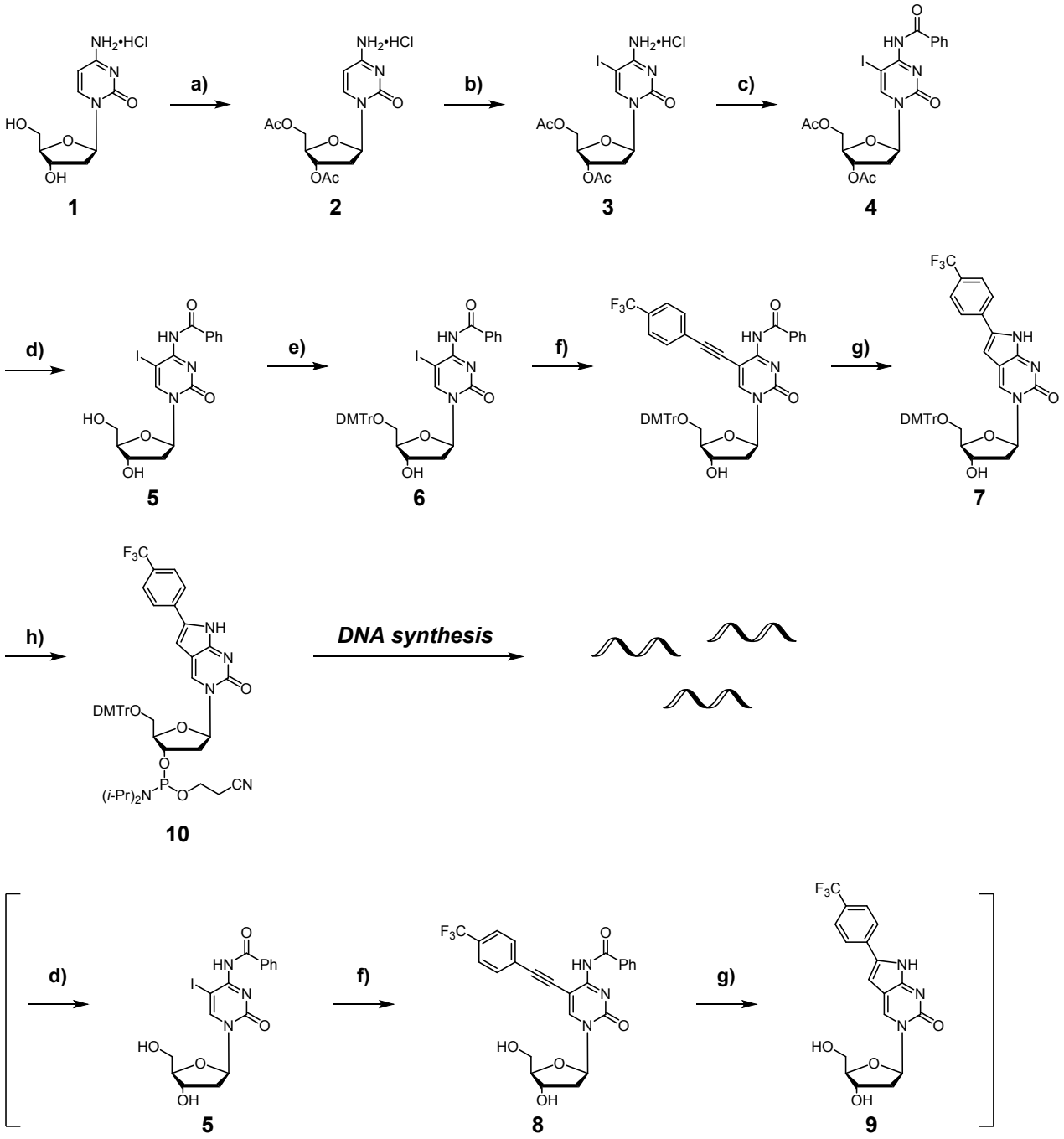
Materials

Acetyl chloride was received from Kishida Chemical Co. Ltd. Iodine, potassium carbonate, phosphoramidochloridous acid, 4-(dimethylamino)pyridine were received from Wako Chemicals and used without further purification. Iodic acid, 4-ethynyl trifluorotoluene, bis(triphenylphosphine)palladium(II) dichloride, copper(I) iodide, 2-cyanoethyl *N,N*-diisopropylchloro phosphoramidite were purchased from Sigma-Aldrich Chemicals Co. and used as received. Acetic acid, pyridine, trimethylamine, *N,N*-diisopropylethylamine, sodium cacodylate trihydrate were purchased from Nacalai and used as received. Benzoic anhydride, 4,4'-dimethoxytrityl chloride were obtained from TCI. Di-tert-butyl dicarbonate was obtained from Peptide Institute Inc. and used as received. Glen-Pak™ DNA and RNA cartridges columns were purchased at Glen Research. All other chemicals and solvents were purchased from Sigma-Aldrich Chemicals Co., Wako Pure Chemical Ind. Ltd., TCI, or Kanto Chemical Co. Inc. and used without further purification. Synthetic oligonucleotides not containing ^{FP}dC were obtained from Sigma Genosys. Water was deionized (specific resistance of $\geq$ 18.0 MW cm at 25^{o}C) by a Milli-Q system (Millipore Corp.). All reactions were carried out under an argon atmosphere unless otherwise stated.

Methods and Equipment

NMR spectra were obtained on a JEOL JNM ECA-600 spectrometer operating at 600 MHz for ^{1}H NMR and 150 MHz for ^{13}C NMR in $CDCl_3$ unless otherwise noted. Flash column chromatography was performed employing Silica Gel 60 (70–230 mesh, Merck Chemicals). Silica-gel preparative thin-layer chromatography (PTLC) was performed using plates from Silica gel 70 PF_{254} (Wako Pure Chemical Ind. Ltd.). DNA concentrations were measured by NanoDrop ND-1000 spectrophotometer. Stock 100 mM Na cacodylate buffers were prepared by dissolving sodium cacodylate trihydrate (1.07 g, 5.0 mmol) in milliQ water (25 mL) before adjusting to the desired pH with hydrochloric acid and minimal sodium hydroxide, then topping up to 50 mL with milliQ water. Measurement of pH was conducted with a LAQUA F-72 pH/ion meter (HORIBA Ltd., Kyoto, Japan).

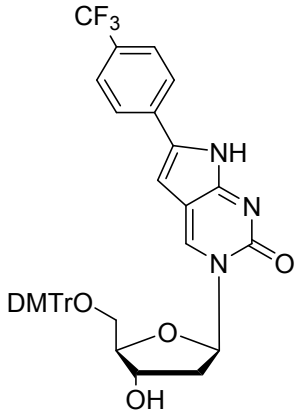
Scheme S1. Synthetic route of ^{FP}dC products



Reagents and conditions: (a) CH_3COCl, AcOH, $CHCl_3$, 50-60 °C, 1-3 days; (b) I_2, HIO_3, AcOH, 40 °C, 24 h; (c) Bz_2O, pyridine, 85 °C, 3 h; (d) K_2CO_3, MeOH, r.t., 1 h; (e) DMTrCl, pyridine, r.t., 4 h; (f) 4-Ethynyl trifluorotoluene, $Pd(PPh_3)Cl_2$, CuI, Et_3N, THF, 50-55 °C, 18 h; (g) Et_3N, EtOH, 55°C 18 h; (h)) $(iPr_2N)_2PO(CH_2)_2CN$, DIPEA, CH_3CN, 0°C to r.t, 1.5 h

Synthesis and characterization of ^{FP}dC products

Figure S1. Compound **7**



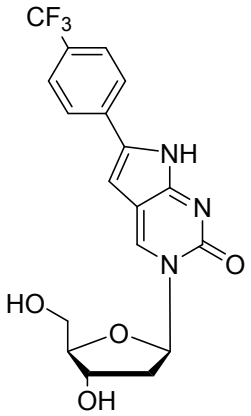
3-((2R,4S,5R)-5-((bis(4-methoxyphenyl)(phenyl)methoxy)methyl)-
4-hydroxytetrahydrofuran-2-yl)-6-(4-(trifluoromethyl)phenyl)-
3,7-dihydro-2H-pyrrolo[2,3-d]pyrimidin-2-one

The detailed synthetic route and characterization data for Compounds **2**-**6** were reported by Cho, S. J. and co-workers.[S1] Compound **6** was evaporated thrice with CH_2Cl_2 before being dissolved in THF. CuI (0.3 equiv.) followed by Et_3N (10 equiv.) were added to the solution before introduction of 4-ethynyl trifluorotoluene (2.3 equiv.) then $Pd(PPh_3)Cl_2$ (0.1 equiv.). The resultant reaction mixture was stirred in the dark at 50-55 °C for 18 h and the solvent was removed. After filtering the mixture through Celite and washing with ethyl acetate, the filtrate was collected and the solvent removed. The crude compound was dissolved in EtOH and Et_3N (10 equiv.) was added to the solution. The resultant reaction mixture was stirred at 55 °C for 18 h and the solvent was removed. Purification of the mixture by silica gel column chromatography (DCM/MeOH=14/1)

afforded compound **7** (72 %).

^{1}H NMR ($CDCl_3$): δ 8.97 (s, 1H), 7.72 (d, J_{HH} = 8.2 Hz, 2H), 7.45 (q, J_{HH} = 7.9 Hz, 4H), 7.35 (d, J_{HH} = 8.6 Hz, 5H), 7.29 (t, J_{HH} = 7.5 Hz, 2H), 7.22 (t, J_{HH} = 7.1 Hz, 1H), 6.82 (dd, J_{HH} = 8.5 Hz, 5.8 Hz, 4H), 6.65 (s, 1H), 5.66 (s, 1H), 4.72 (q, J_{HH} = 5.2 Hz, 1H), 4.24 (s, 1H), 3.71 (d, J_{HH} = 12.2 Hz, 6H), 3.55 (d, J_{HH} = 8.8 Hz, 1H), 3.50 (d, J_{HH} = 8.9 Hz, 1H), 3.11 (t, J_{HH} = 6.5 Hz, 1H), 2.43 (t, J_{HH} = 6.8 Hz, 1H). ^{13}C NMR ($CDCl_3$): δ 159.7, 158.6, 155.1, 144.3, 138.8, 137.3, 135.8, 135.4, 133.5, 130.2, 130.0, 129.5 (q, J_{CF} = 33.0 Hz), 128.2, 128.1, 127.9, 125.5, 125.3, 123.9 (q, J_{CF} = 272 Hz), 113.3, 110.3, 98.6, 88.1, 87.0, 86.4, 70.5, 62.6, 55.1, 42.5. ^{19}F NMR ($CDCl_3$): δ -62.4880. HRMS (ESI-TOF) calculated for $C_{39}H_{33}F_3N_3O_6$ $[M-H]^-$ 696.2327, found 696.2322.

Figure S2. Compound **9**



3-((2R,4S,5R)-4-hydroxy-5-(hydroxymethyl)tetrahydrofuran-2-yl)-
6-(4-(trifluoromethyl)phenyl)-3,7-dihydro-2H-pyrrolo[2,3-d]pyrimidin-2-one

Compound **5** was evaporated thrice with CH_2Cl_2 before being dissolved in THF. CuI (0.3 equiv.) followed by Et_3N (10 equiv.) were added to the solution before introduction of 4-ethynyl trifluorotoluene (2.3 equiv.) then $Pd(PPh_3)Cl_2$ (0.1 equiv.). The resultant reaction mixture was stirred in the dark at 50-55 °C for 18 h and the solvent was removed. After filtering the mixture through Celite and washing with ethyl acetate, the filtrate was collected and the solvent removed. The crude state compound **6** was dissolved in EtOH and Et_3N (10 equiv.) was added to the solution. The resultant reaction mixture was stirred at 55 °C for 18 h and the solvent was removed. Purification of the mixture by preparatory thin layer chromatography (DCM/MeOH=7/1) afforded compound **9** (30 %).

^{1}H NMR (MeOD): δ 8.98 (s, 1H), 7.94 (d, J_{HH} = 8.1 Hz, 2H), 7.75 (d, J_{HH} = 8.5 Hz, 2H), 6.88 (s, 1H), 6.38 (t, J_{HH} = 6.1 Hz, 1H), 4.42 (dd, J_{HH} = 10.5 Hz, 4.4 Hz, 1H), 4.07 (q, J_{HH} = 3.7 Hz, 1H),

3.92 (dd, J_{HH} = 12.1 Hz, 3.3 Hz, 1H), 3.82 (dd, J_{HH} = 12.1 Hz, 3.9 Hz, 1H), 2.62 (ddd, J_{HH} = 13.8 Hz, 6.3 Hz, 4.6 Hz, 1H), 2.23 (qunit, J_{HH} = 6.5 Hz, 1H). ^{13}C NMR ($(CD_3)_2CO$): δ 165.1, 154.9, 146.7, 132.5, 130.0 (q, J_{CF} = 32.2 Hz), 127.9, 126.0, 125.0 (q, J_{CF} = 273 Hz), 93.6, 90.2, 88.8, 87.6, 84.7, 71.2, 62.1, 42.2. ^{19}F NMR (MeOD): δ -64.0909. HRMS (ESI-TOF) calculated for $C_{18}H_{16}F_3N_3NaO_4$ $[M+Na]^+$ 418.0991, found 418.0990.

Oligonucleotide (ODN) Synthesis

ODNs were synthesized on solid supports using ^{FP}dC phosphoramidite (compound **10**) and commercially available *O*$^{5'}$-dimethoxytrityl -2'-deoxyribonucleoside *O*$^{3'}$-phosphoramidites. Compound **10** was prepared by dropwise addition of DIPEA at 0 °C to a solution of compound **7** in CH_2Cl_2 followed by stirring at 0 °C for 10 min. Phosphoramidochloridous acid was subsequently added and the mixture was stirred for a further 10 min at 0 °C before warming to room temperature and stirring for another 1 h. After removal of the solvent, the residue was dissolved in MeCN and used immediately without purification for solid-phase DNA synthesis. Solid-phase oligonucleotide synthesis was performed on an ABI DNA synthesizer (Applied Biosystem, Foster City, CA) or M-2-MX DNA/RNA synthesizer (Nihon Techno Service Co., Ltd., Tsukuba, Japan). The modified phosphoramidite was chemically synthesized as described above and without purification incorporated into oligonucleotide through coupling reaction for 10 minutes. The coupling yields of ^{FP}dC phosphoramidite were around 10 % less than the ones obtained with standard phosphoramidite building blocks. Cleavage from the solid support and deprotection were accomplished with 50:50 of $MeNH_2$ in 40 wt. % in water and NH_3 in 28 wt. % in water at RT for 15 min and then at 65 °C for 15 min. The synthesized oligonucleotides were eluted from Glen-Pak™ DNA purification cartridges with purification steps are performed as per

procedure. The final elution was subjected to normal-phase HPLC purification (2 % to 70 % ACN in 50 mM TEAA (pH 7.0) buffer, flow rate of 3.0 mL/min). After purification by HPLC, the products were confirmed by MALDI-TOF MS using a Bruker microflex-KSII (Bruker Corporation, Billerica, MA) (Table S2). DNA concentrations were determined by using NanoDrop ND-1000 (NanoDrop Technologies, Wilmington, DE).

For HPLC analysis, COSMOSIL 5C18 AR-II (Nacalai Tesque, Inc., Kyoto, 150 × 10 mm id), a linear gradient of 2 % to 30 % acetonitrile (in 50 mM TEAA (pH 7.0) buffer) over 30 min at a flow rate of 3.0 mL/min and detection at 254 nm were used.

Table S1. Analytical HPLC profile of synthesized ODNs

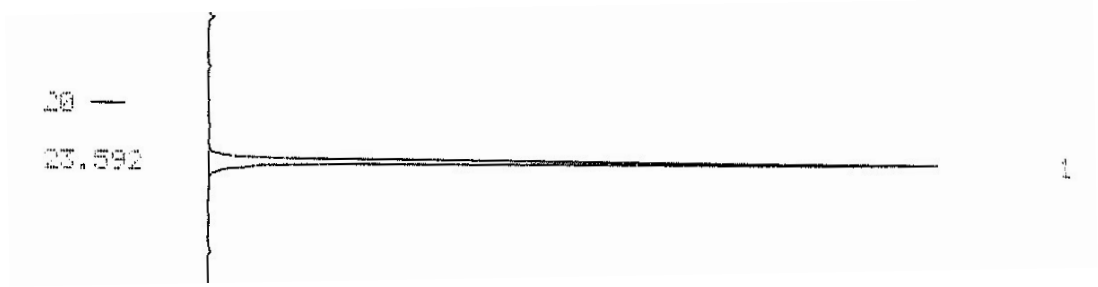
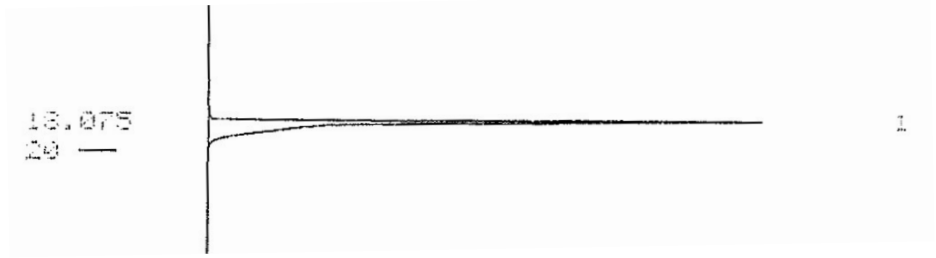
ODN	HPLC Profile
1C	
IM-CT	
2IM-CT	

Table S2. MALDI-TOF-Mass data of ODNs

Name	DNA oligomers	Calcd.	Found
1C	5'-^{FP}**dC**CC CGC CCC GCC CCG CCC CA-3'	6032.02	6032.75
IM-CT	5'-^{FP}**dC**CC TTT CCC TTT CCC TTT CCC-3'	6312.03	6312.51
2IM-CT	5'-C^{FP}**dC**C TTT CCC TTT CCC TTT CCC-3'	6312.03	6312.72

Native DNA oligomers (1C native and IM-CT native) were purchased from Sigma Genosys.

Table S3. Sequences of native ODNs

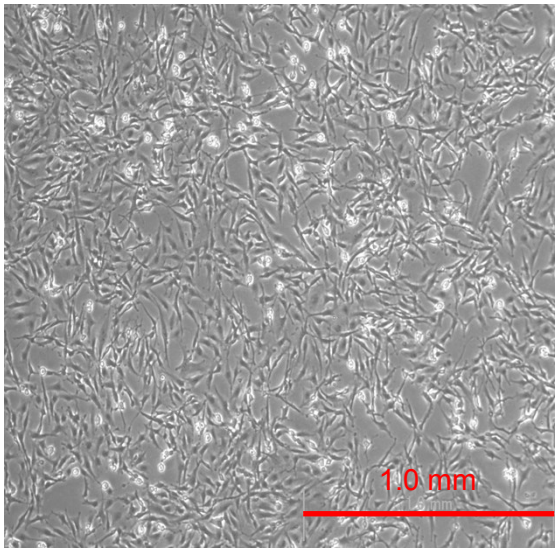
Name	Sequence
1C native	5'-**C**CC CGC CCC GCC CCG CCC CA-3'
IM-CT native	5'-**C**CC TTT CCC TTT CCC TTT CCC-3'

Preparation of Cell Lysate

Cultured HeLa cell was purchased from ATCC and maintained in DMEM (Thermo Fisher Scientific) supplemented with 10 % (v/v) fetal bovine serum (FBS, Sigma Aldrich) at 37 °C with 5% CO_2. For compound treatment, the cells were seeded on 9 cm plates (ca. 2×10^7 cells). After three days, the cells (90 % confluence) were prepared for nucleic extraction (**Figure S3**). 270 μL of 37% formaldehyde and 500 μL of 2.5 M glycine were applied to DMEM medium, and the cells were incubated at room temperature for 5 minutes. After removing the medium, the cells were washed twice with 10 mL of the cold PBS (-) (purchased from Nacalai Tesque). The cells were subsequently collected with a cell scraper and transferred to sterilized 15 mL tubes. Following centrifugation at 1500 rpm for 5 minutes at 4 °C centrifuge, the cell pellet containing nuclei, and supernatant containing cytoplasm, membrane and mitochondria were obtained. The cell pellet was collected and vigorously resuspended with 300 μL of nuclear lysis buffer (10 mM Tris-HCl pH 7.5, 10 mM EDTA, 200 mM NaCl, and 1% SDS) on ice. The resultant solution was moved to

sterilized 1.5 mL protein low-binding tubes for ultra-sonication on ice. Finally, the solution was centrifuged at 16000*g for 10 minutes and the supernatant containing the nucleic components was collected. For acidification of the nuclear lysate (pH 7.8 to pH 5.5), 10 µL of 6 M HCl was added to 300 µL of nuclei lysate buffer based on calculation.

Figure S3. Hela cell image after 3 days culture (4× magnification)

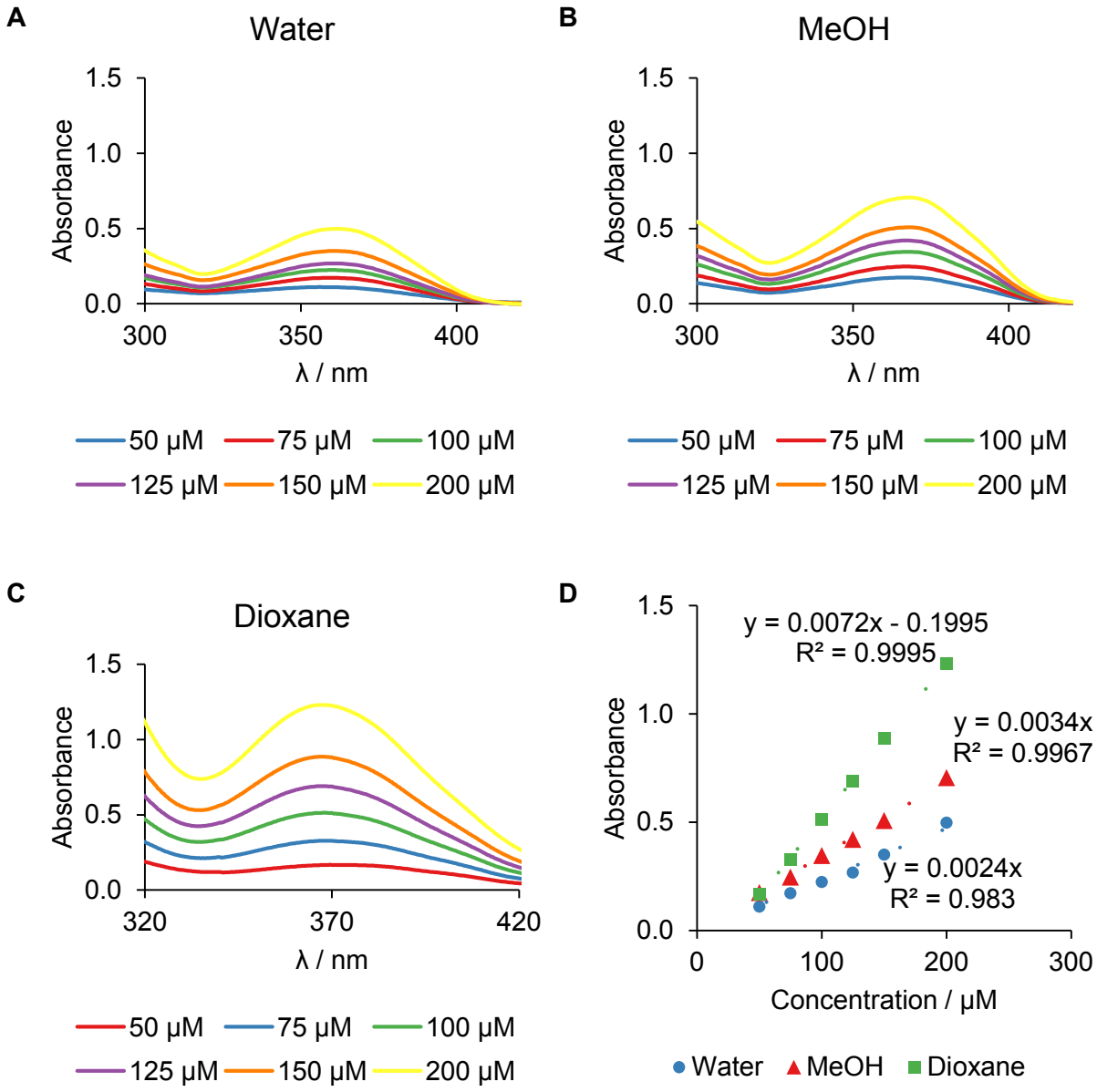


UV-Visible Absorbance

UV-vis absorbance spectra were measured from 220 nm to 500 nm at 20 °C on a JASCO V-750 spectrophotometer equipped with a JASCO PAC-743R thermocontrolled cell changer and a JASCO CTU-100 thermocirculator. All samples were prepared in a total volume of 110 µL containing various concentrations of ^{FP}dC in milliQ water, methanol or dioxane.

Figure S4. Absorbance curves of ^{FP}dC in various solvents [a]

[a] Solution conditions: Various concentrations of ^{FP}dC in water, MeOH or dioxane.

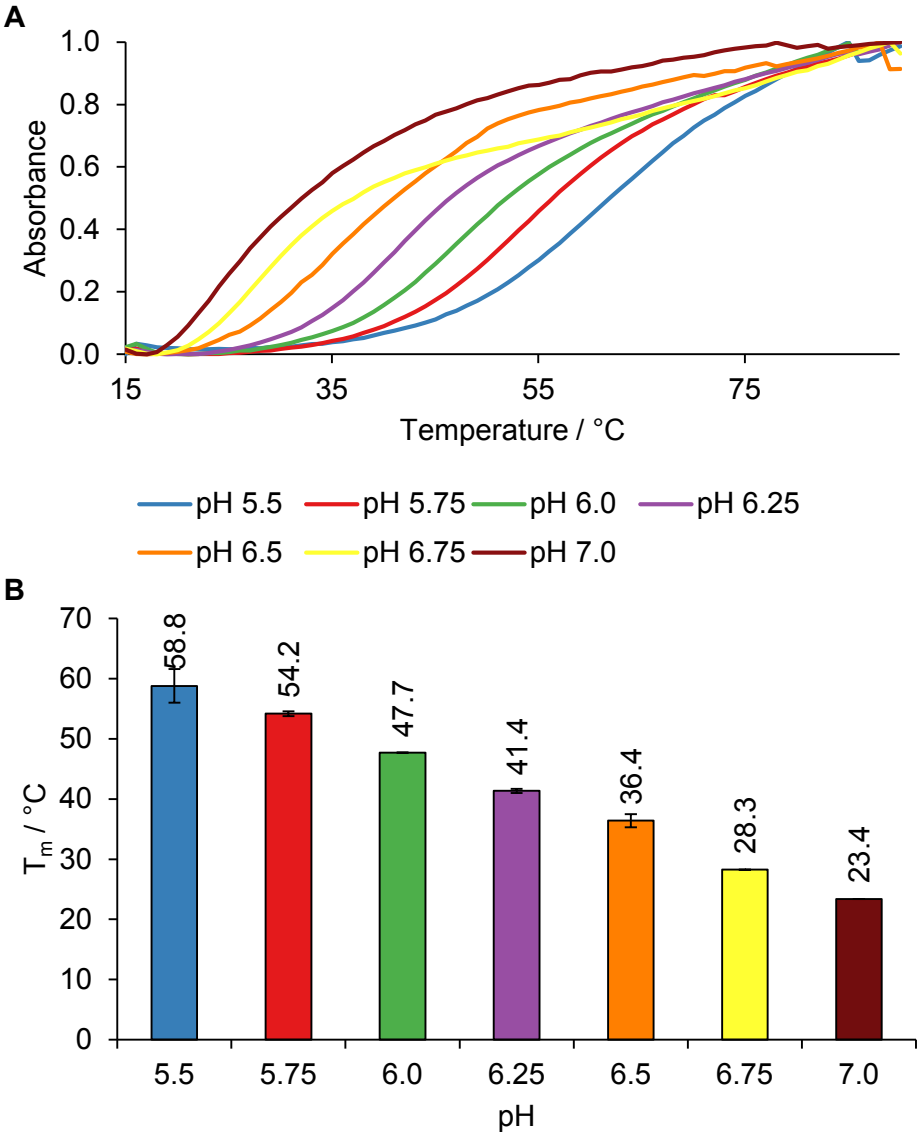


UV-Melting

Melting temperatures were determined by measuring changes in absorbance at 260 nm as a function of temperature on a JASCO V-750 spectrophotometer equipped with a JASCO PAC-743R thermocontrolled cell changer and a JASCO CTU-100 thermocirculator. Absorbance was recorded from 15 to 90 °C at a rate of 1 °C/min. The melting samples were denatured at 95 °C for 5 min and annealed slowly to room temperature then stored at 4 °C until experiments were initiated. All melting samples were prepared in a total volume of 120 μL containing 2.5 μM oligonucleotide and 10 mM Na cacodylate buffer (various pH) for i-motif-forming sequences. All the experiments were duplicated with means and standard errors as indicated.

Figure S5. Melting curves and T_m values of 1C at various pH [a]

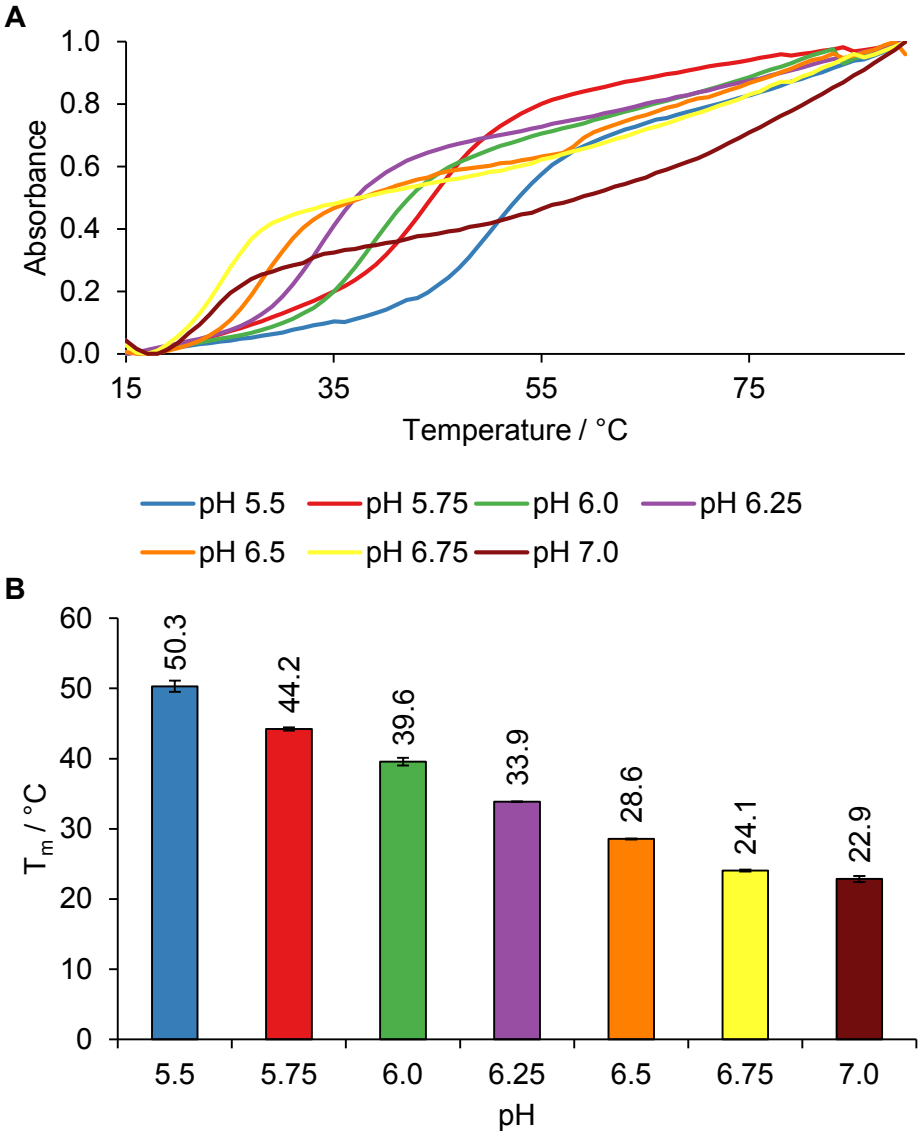
[a] Solution conditions: 2.5 µM DNA in 10 mM Na cacodylate buffer (pH 5.5 – pH 7.0).



(A) Representative UV-melting curve of 1C; (B) T_m values of 1C (n = 2, error bars represent standard error).

Figure S6. Melting curves and T_m values of 1C native at various pH [a]

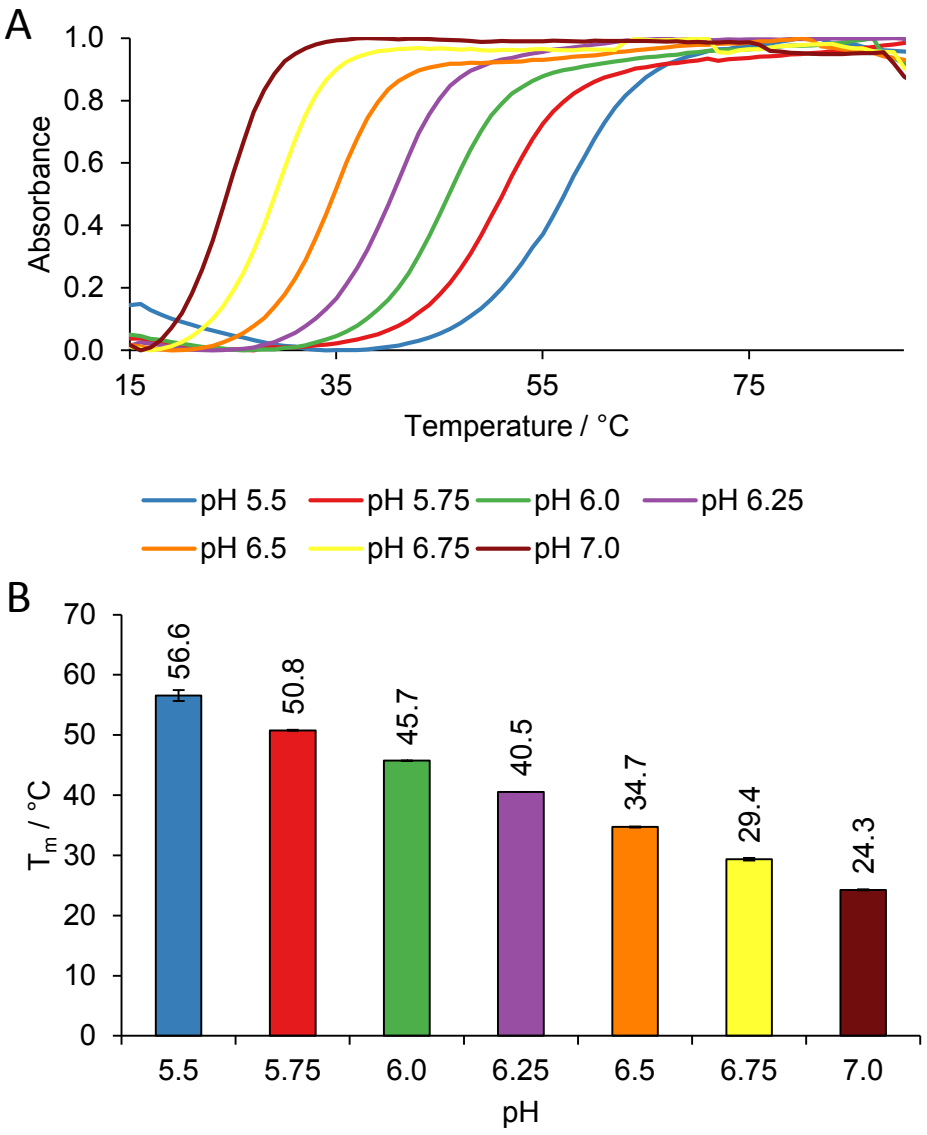
[a] Solution conditions: 2.5 µM DNA in 10 mM Na cacodylate buffer (pH 5.5 – pH 7.0).



(A) Representative UV-melting curve of 1C native; (B) T_m values of 1C native (n = 2, error bars represent standard error).

Figure S7. Melting curves and T_m values of IM-CT at various pH [a]

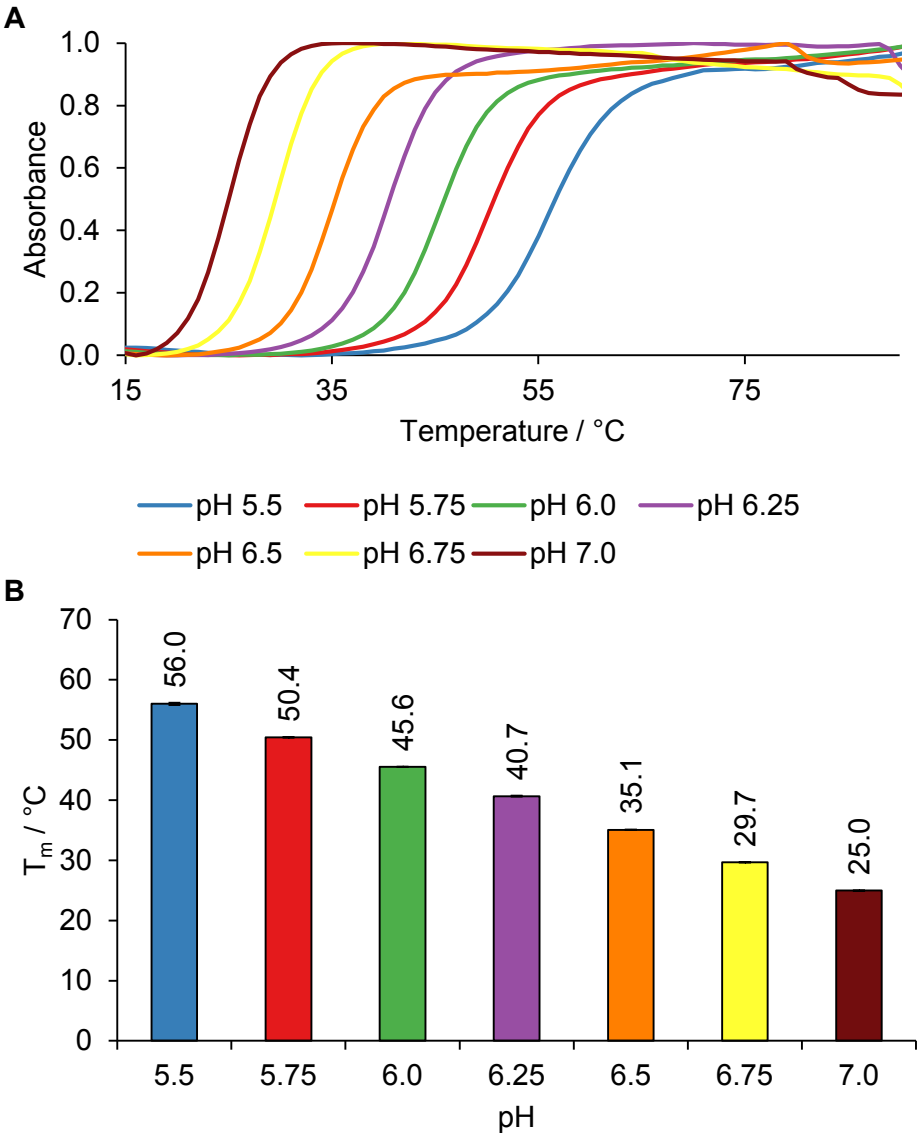
[a] Solution conditions: 2.5 µM DNA in 10 mM Na cacodylate buffer (pH 5.5 – pH 7.0).



(A) Representative UV-melting curve of IM-CT; (B) T_m values of IM-CT (n = 2, error bars represent standard error).

Figure S8. Melting curves and T_m values of IM-CT native at various pH [a]

[a] Solution conditions: 2.5 µM DNA in 10 mM Na cacodylate buffer (pH 5.5 – pH 7.0).



(A) Representative UV-melting curve of IM-CT; (B) T_m values of IM-CT (n = 2, error bars represent standard error).

CD Spectroscopy

CD spectra of oligonucleotide solutions collected in 1 nm steps from 360 nm or 320 nm to 220 nm were measured using JASCO J-805LST Spectrometer in a 1 cm quartz cuvette. Each spectrum shown is the average of two individual scans. The samples were denatured at 95 °C for 5 min and annealed slowly to RT then stored at 4 °C until experiments were initiated. All samples were prepared in a total volume of 110 μL containing 2.5 μM oligonucleotide and 10 mM Na cacodylate buffer (various pH) for i-motif-forming sequences.

Figure S9. CD spectra of 1C and IM-CT at various pH [a]

[a] Solution conditions: 2.5 µM DNA in 10 mM Na cacodylate (various pH).

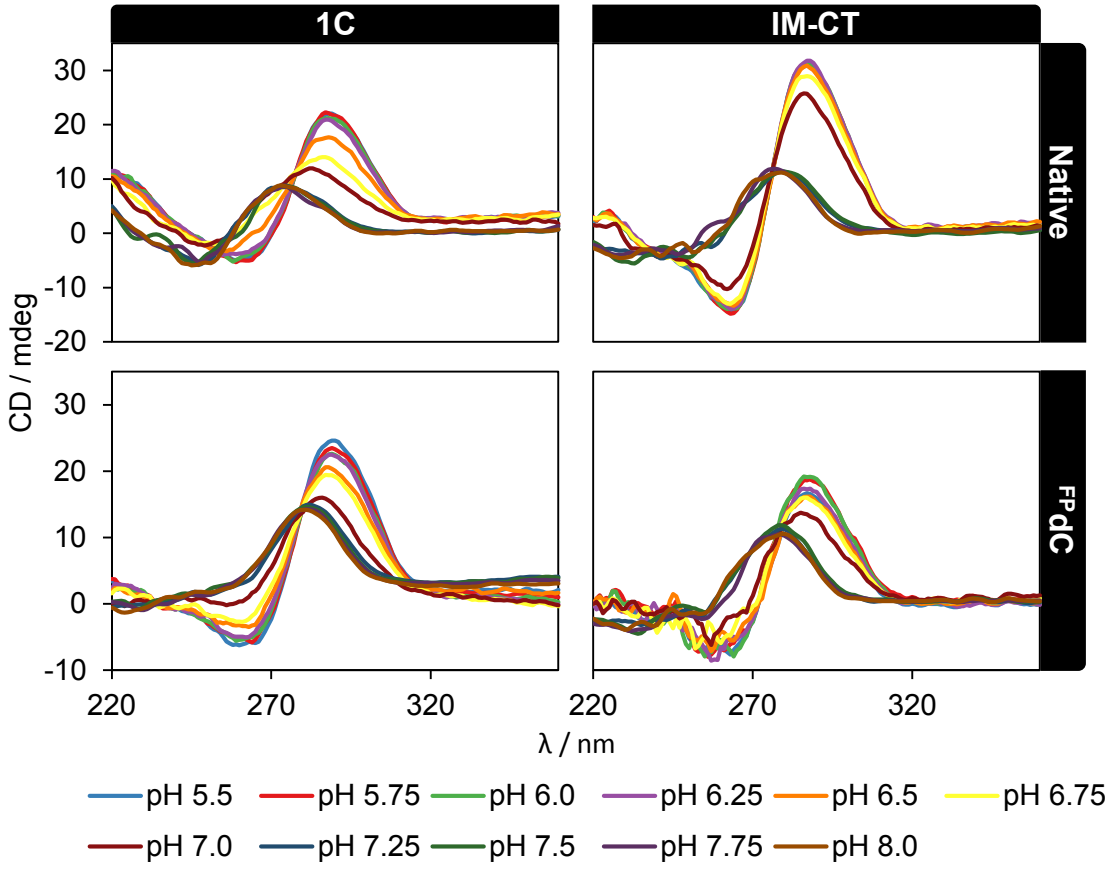


Figure S10. pK_a of 1C and IM-CT [a]

[a] pK_a was calculated based on the CD spectra of 1C and IM-CT at 290 nm with the equation shown below as described by Wadkins *et al.* using the ellipticity value at 290 nm.[S2]

$$signal_{total} = \frac{signal_{folded} - signal_{unfolded}}{1 + 10^{cooperativity \times (pH - pK_a)}} + signal_{unfolded}$$

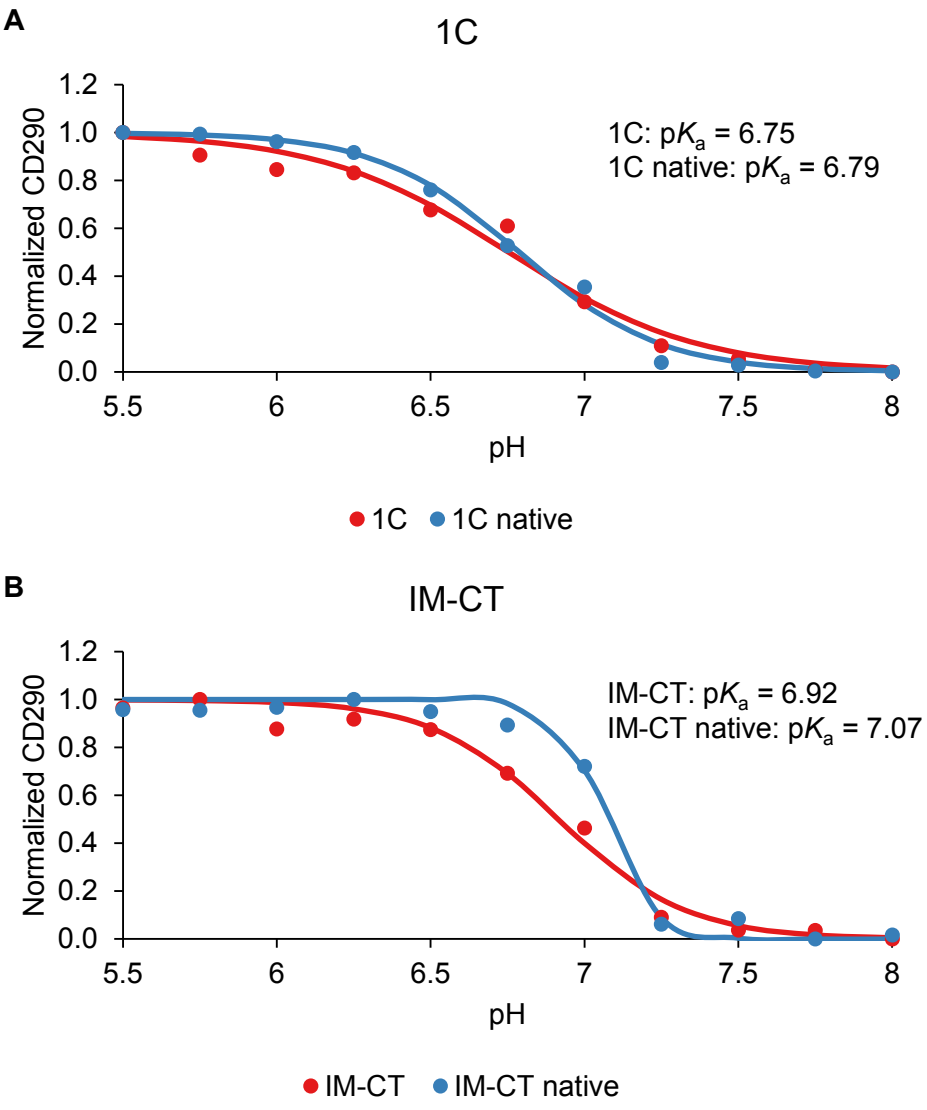


Figure S11. Ag^+ and cysteine switching of IM-CT at pH 6.5 [a]

[a] Solution conditions: 2.5 μM DNA in 10 mM Na cacodylate (pH 6.5). 25 μM $AgNO_3$ was added at the first cycle and excess cysteine or $AgNO_3$ were added for subsequent cycles.

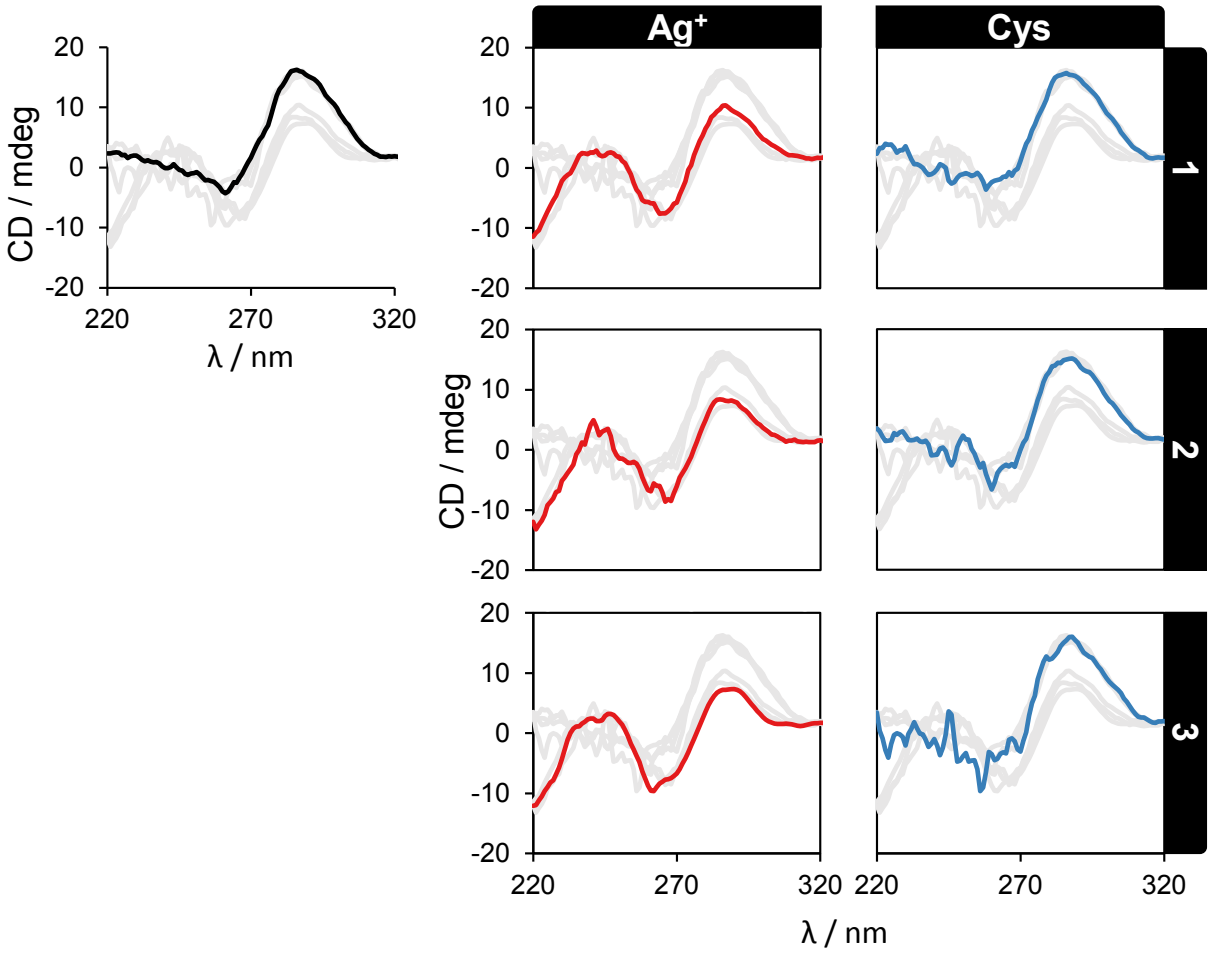
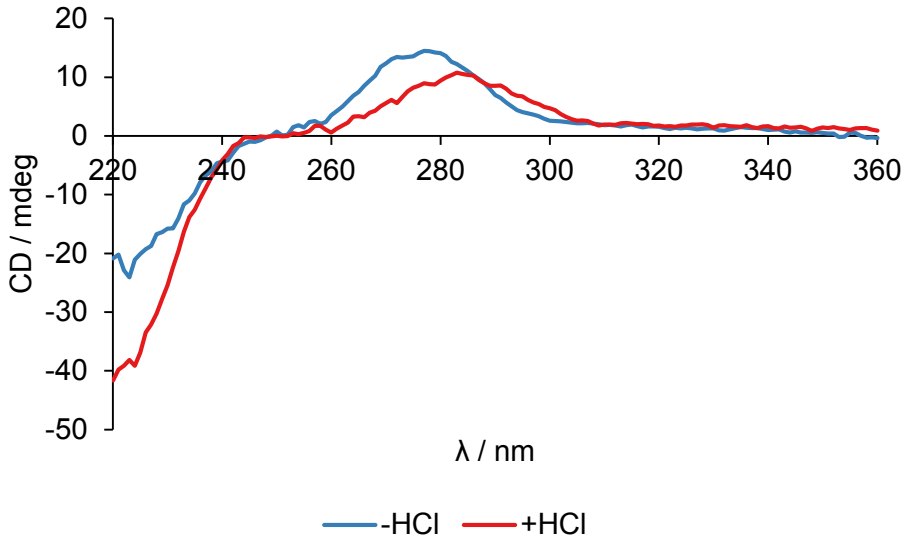


Figure S12. CD spectra of IM-CT in cell lysate [a]

[a] Solution conditions: 20× dilution of cell lysate NMR samples in milliQ water



Fluorescence Measurements

Fluorescence measurements were obtained using 3 mm path length JASCO FMM-100 quartz microcells on a JASCO FP-6300 Spectrofluorometer equipped with a JASCO EHC-573 temperature controller. The emission spectra were recorded from 220 nm to 600 nm at a scan rate of 100 nm/min with an excitation wavelength of 374 nm for ^{FP}dC and 1C, and 365 nm for IM-CT.

Figure S13. Fluorescence spectra of IM-CT and IM-CT native with 10 equivalents of $AgNO_3$ [a]

[a] Solution conditions: 2.5 μM DNA and 25 μM $AgNO_3$ in 10 mM Na cacodylate (pH 6.5).

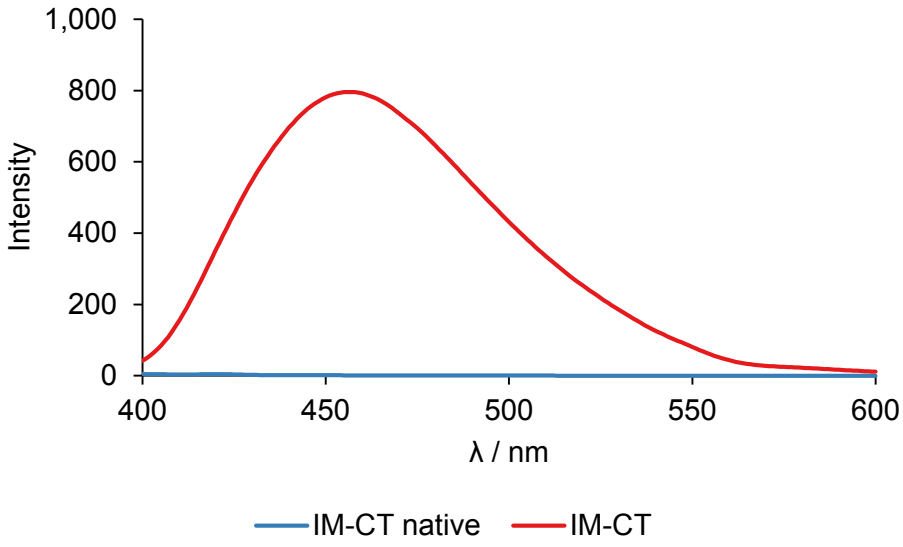


Figure S14. Fluorescence intensity of IM-CT with increasing addition of $AgNO_3$ [a]

[a] Solution conditions: 2.5 μM DNA and $AgNO_3$ in 10 mM Na cacodylate (pH 6.5).

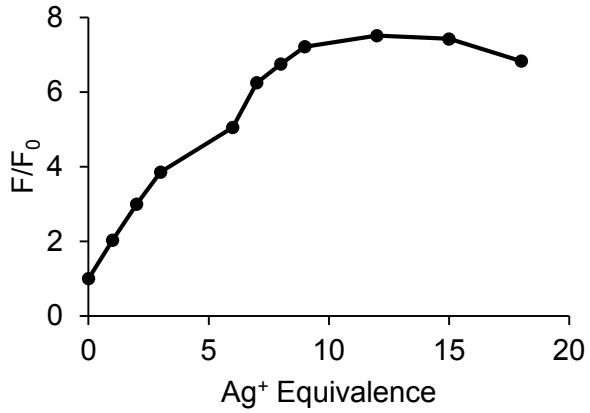


Figure S15. Fluorescence intensity of ^{FP}dC at various pH [a]

[a] Solution conditions: 2.5 μM ^{FP}dC in 10 mM Na cacodylate (various pH).

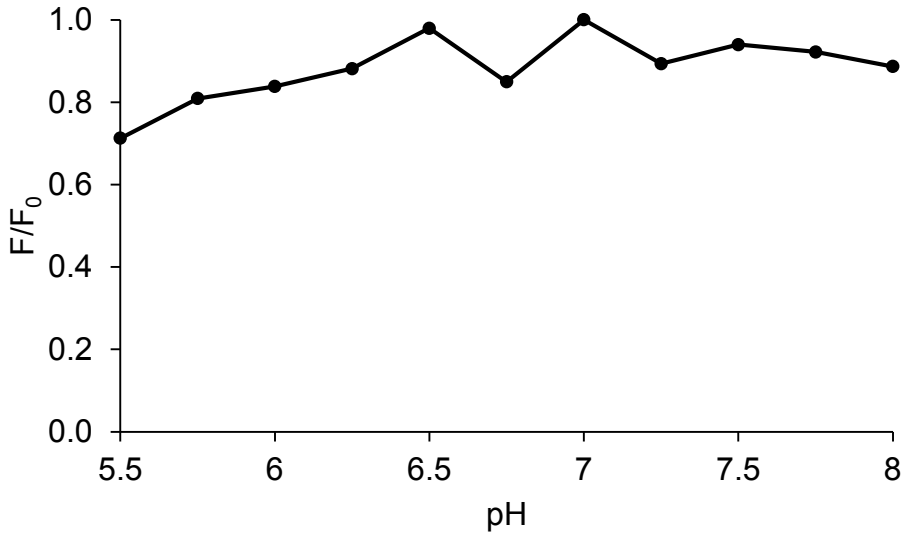
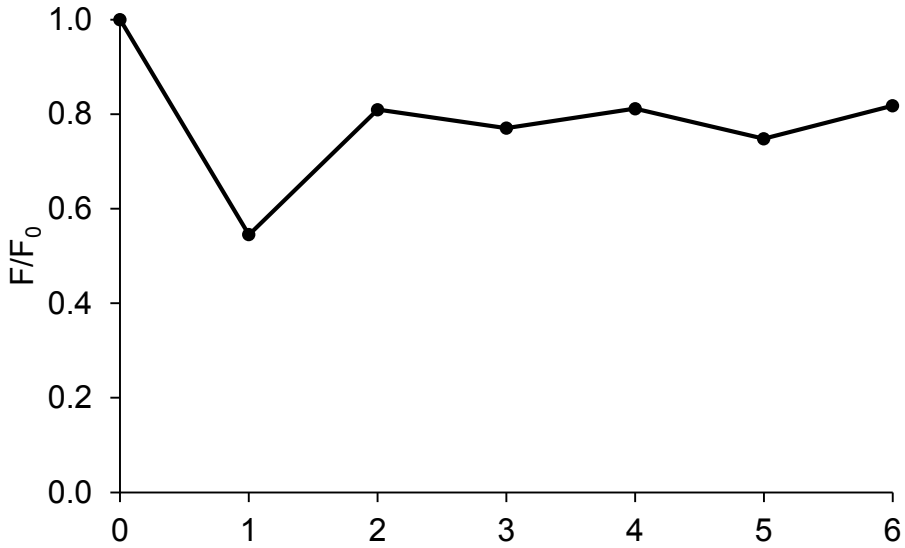


Figure S16. Ag^+ and cysteine switching of ^{FP}dC at pH 6.5 [a]

[a] Solution conditions: 2.5 μM ^{FP}dC in 10 mM Na cacodylate (pH 6.5). 25 μM $AgNO_3$ was added at the first cycle and excess cysteine or $AgNO_3$ were added for subsequent cycles.

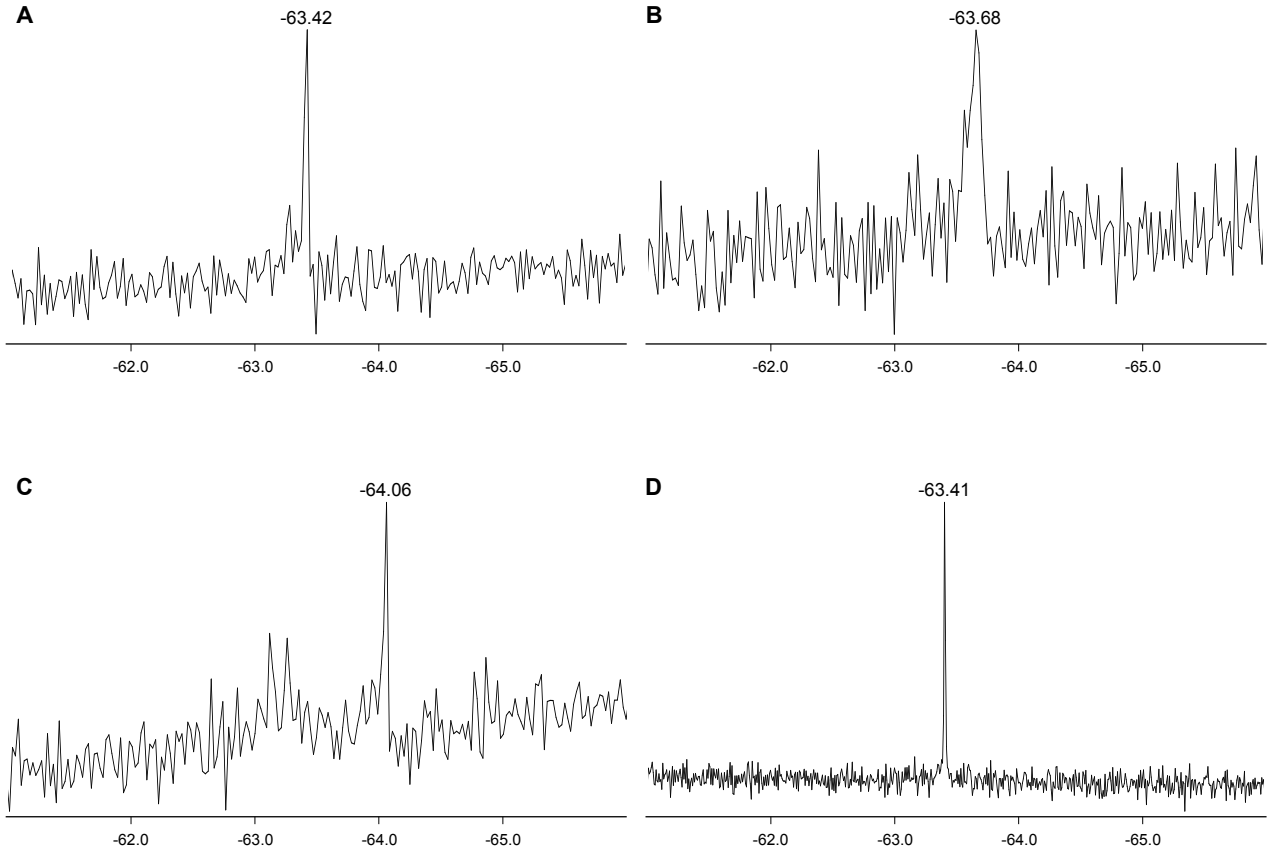


^{19}F NMR of ODNs

NMR spectra were obtained on a JEOL JNM ECA-600 spectrometer operating at 565 MHz for ^{19}F NMR. Samples were prepared with 50 μM oligonucleotide and 20 % D_2O in 10 mM Na cacodylate buffer (pH as indicated) doped with trifluoroacetic acid as an internal standard for i-motif-forming sequences. Spectra were obtained over 512 scans (approximately 45 min) unless otherwise indicated. For cell lysate samples, samples were prepared with 50 μM IM-CT and 20 % D_2O in cell lysate doped with trifluoroacetic acid as an internal standard. Spectra were obtained over 8192 scans for the non-acidified and acidified cell lysate samples.

Figure S17. ^{19}F NMR of 1C and IM-CT [a]

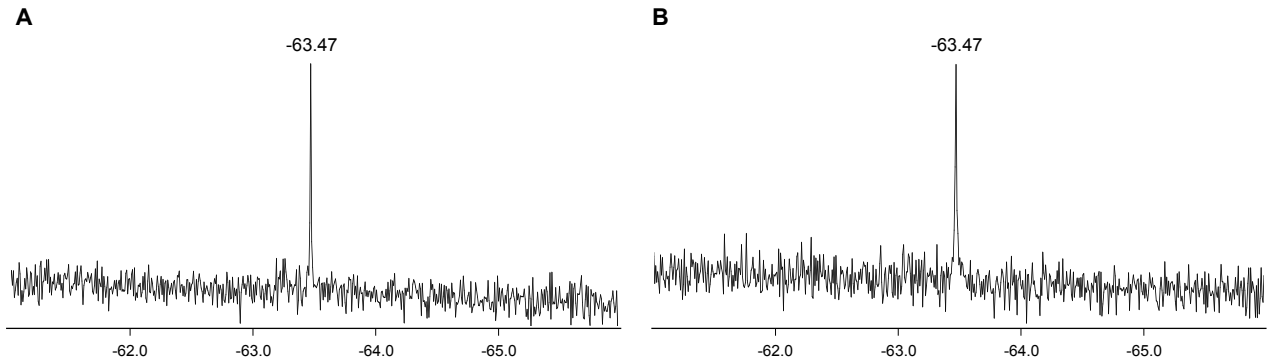
[a] Solution conditions: 50.0 μM DNA and 20 % D_2O in 10 mM Na cacodylate (pH 5.5/pH 7.5) with TFA as an internal standard.



(A) ^{19}F NMR spectrum of 1C at pH 5.5; (B) ^{19}F NMR spectrum of 1C at pH 7.5; (C) ^{19}F NMR spectrum of IM-CT at pH 5.5; (D) ^{19}F NMR spectrum of IM-CT at pH 7.5.

Figure S18. ^{19}F NMR of ^{FP}dC [a]

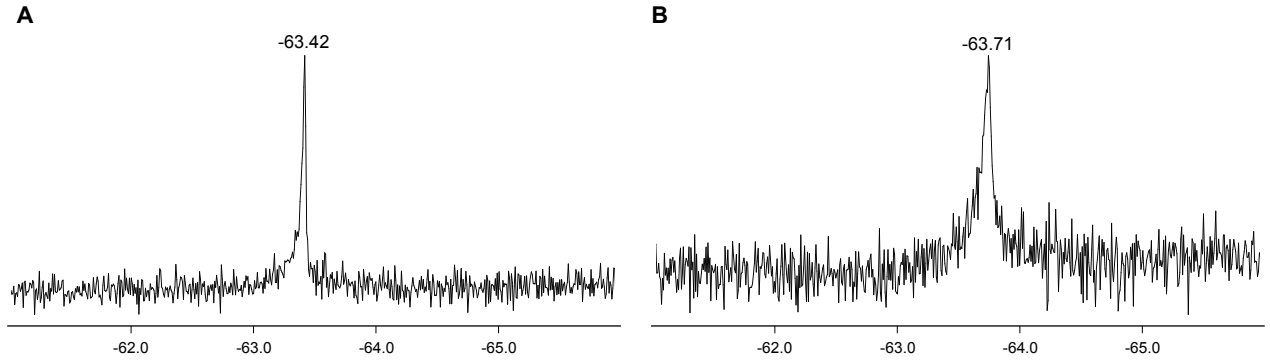
[a] Solution conditions: 200 μM ^{FP}dC and 20 % D_2O in 10 mM Na cacodylate (pH 5.5/pH 7.5) with TFA as an internal standard.



(A) ^{19}F spectrum of ^{FP}dC at pH 5.5; (B) ^{19}F spectrum of ^{FP}dC at pH 7.5.

Figure S19. ^{19}F NMR of IM-CT in different pH of Hela cell nuclei lysate [a]

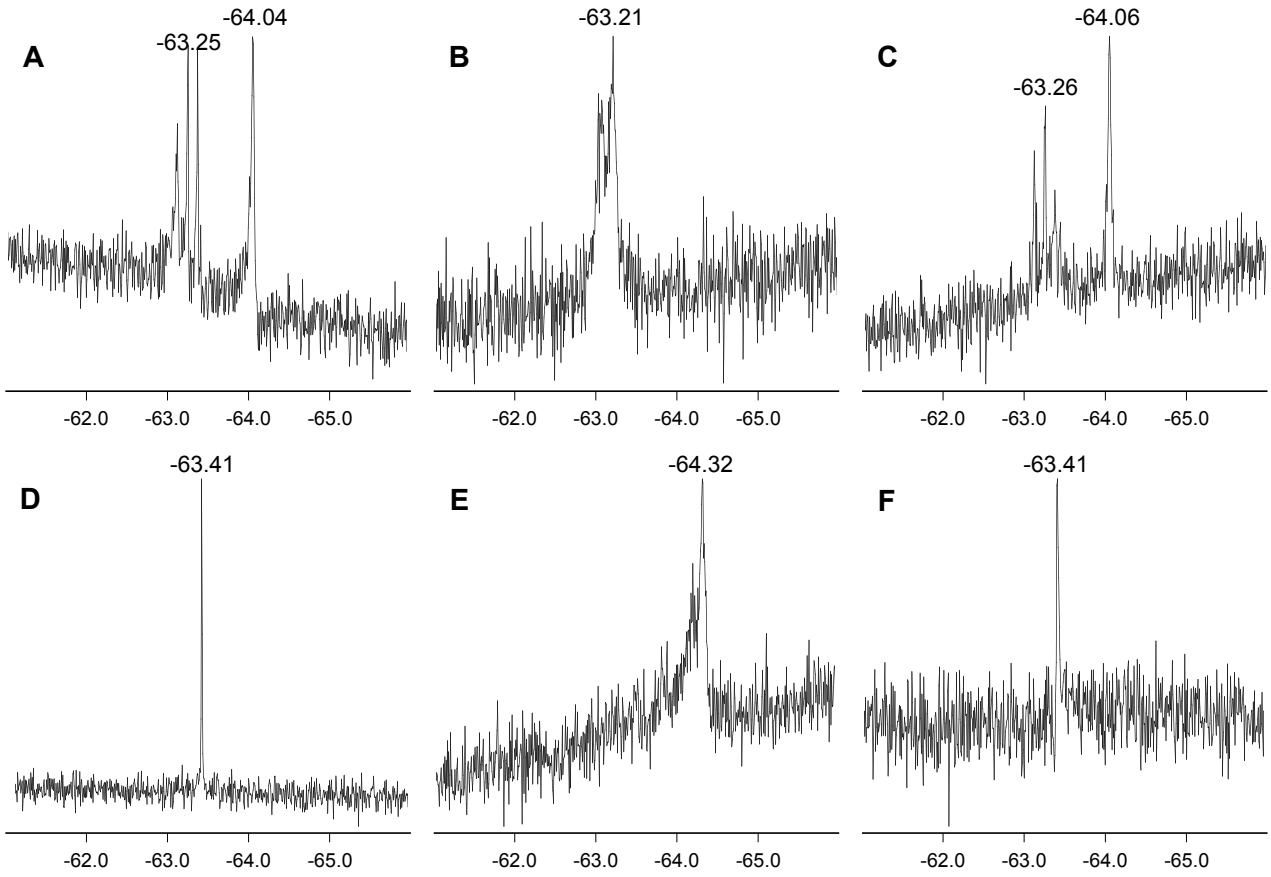
[a] Solution conditions: 50.0 μM DNA and 20 % D_2O in Hela cell nuclei lysate with TFA as an internal standard. Same sample was used for the acidification.



(A) ^{19}F spectrum of IM-CT in cellular conditions ca. pH 7.8; (B) ^{19}F spectrum of IM-CT at acidified nuclear extracted lysate ca. pH 5.5.

Figure S20. ^{19}F NMR of IM-CT with Ag^+ and cysteine switching [a]

[a] Solution conditions: 50.0 μM DNA and 20 % D_2O in 10 mM Na cacodylate (pH 6.5/pH 7.5) with TFA as an internal standard. Same sample was used for the switching.



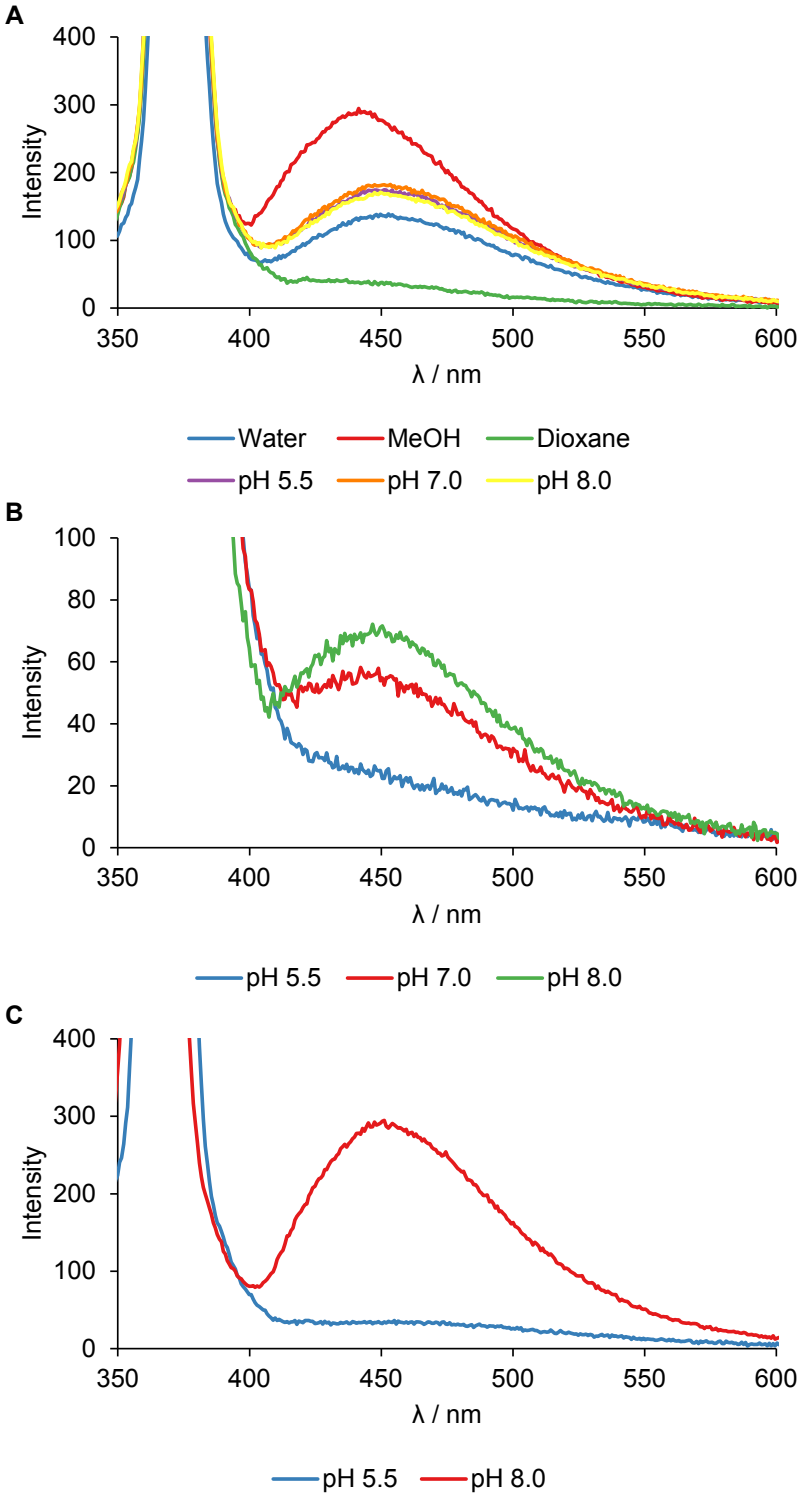
(A) ^{19}F spectrum of IM-CT at pH 6.5 (7521 scans); (B) ^{19}F spectrum of IM-CT at pH 6.5 with addition of Ag^+ (6638 scans); (C) ^{19}F spectrum of IM-CT at pH 6.5 with addition of Ag^+ followed by cysteine (7506 scans); (D) ^{19}F spectrum of IM-CT at pH 7.5; (E) ^{19}F spectrum of IM-CT at pH 7.5 with addition of Ag^+ (8192 scans); (F) ^{19}F spectrum of IM-CT at pH 7.5 with addition of Ag^+ followed by cysteine.

Quantum Yield Measurements

Quantum yields were measured with a Hamamatsu Photonics Quantaurus-QY C11347-11 Absolute PL Quantum Yield Spectrometer and calculated with the integrated measurement software.

Figure S21. Quantum yields of ^{FP}dC, 1C and IM-CT [a]

[a] Solution conditions: 10 µM ^{FP}dC or 2.5 µM DNA in water, MeOH, dioxane or 10 mM Na cacodylate (pH 5.5, pH 7.5 or pH 8.0).



Quantum yield spectra of (A) ^{FP}dC, (B) 1C and (C) IM-CT.

Figure S22. Quantum yield of ^{FP}dC in various solvents [a]

[a] Solution conditions: 2.5 μM ^{FP}dC in various solvents.

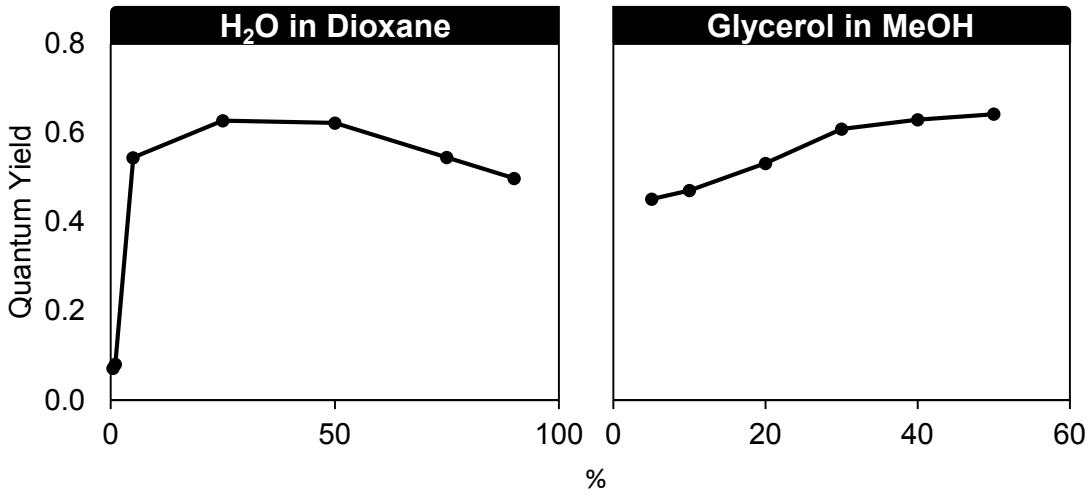


Table S4. Photophysical properties of ^{FP}dC at various pH [a]

[a] Solution conditions: 2.5 μM ^{FP}dC in 10 mM Na cacodylate (pH 5.5, pH 7.0 or pH 8.0).

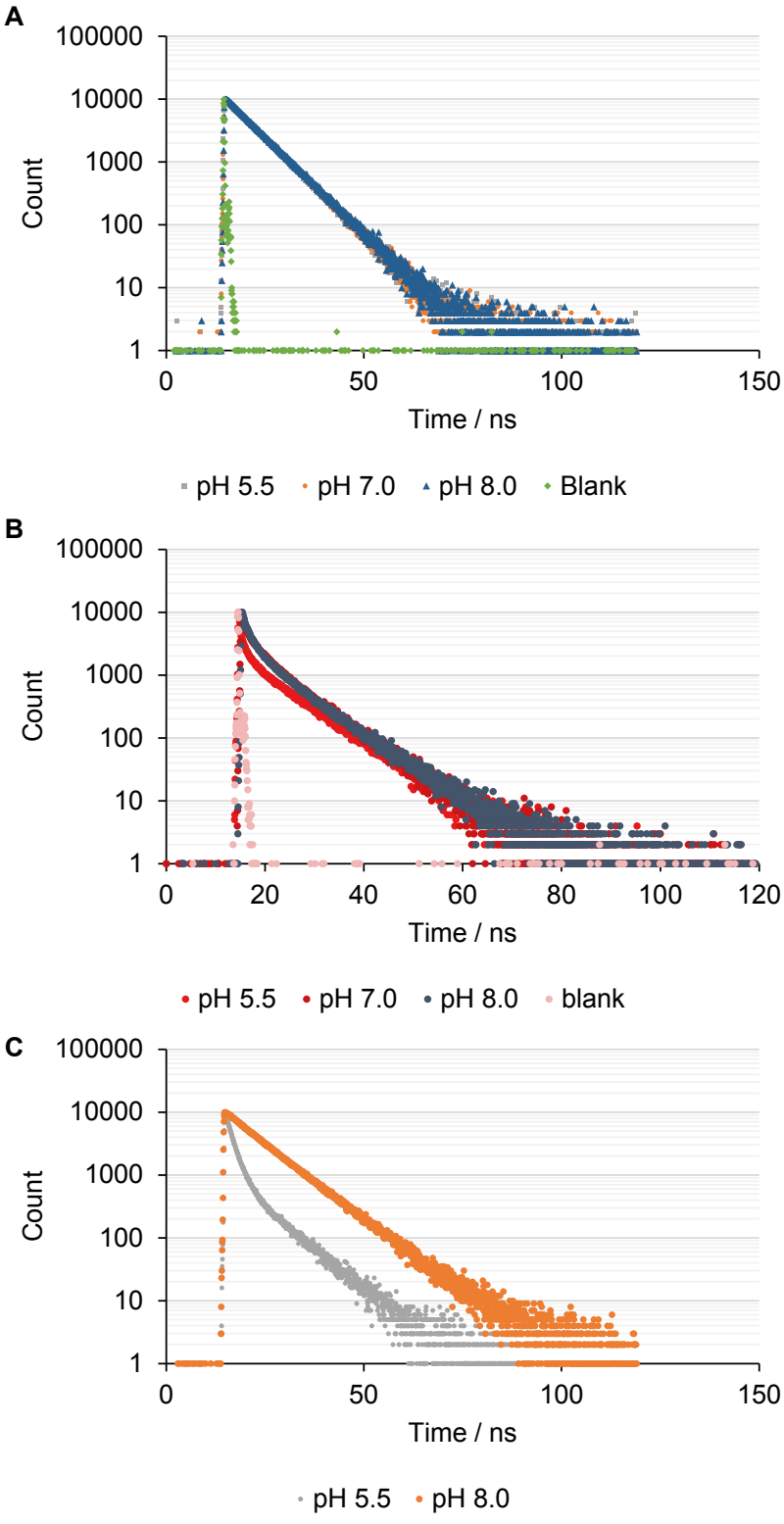
	τ_1 / ns	α_1	τ_{avg} / ns	χ^2	Φ	ε / mol^{-1} dm^3 cm^{-1}	εΦ
pH 5.5	6.91	1.000	6.91	1.14	0.49	24 000	11 712
pH 7.0	6.95	1.000	6.95	1.23	0.49	24 000	117 48
pH 8.0	7.05	1.000	7.05	1.14	0.50	n.d.	n.d.

Time-Resolved Fluorescence Lifetime Measurements

Fluorescence lifetime measurements were obtained using TCSPC (time-correlated single photon counting) on a Horiba Scientific Fluorohub equipped with a Horiba Scientific FluoroCube 3000U-SHK and a 375 nm Horiba Scientific NanoLED pulsed diode light source. Emission wavelength was set at the emission maximums found through fluorescence spectroscopy of each ODN. TAC range was 200 ns and Repetition rate was 1 MHz. The data was fitted to exponential functions using DAS6 (HORIBA).

Figure S23. Lifetimes of ^{FP}dC, 1C and IM-CT [a]

[a] Solution conditions: 10 μM ^{FP}dC or 2.5 μM DNA in 10 mM Na cacodylate (pH 5.5, pH 7.5 or pH 8.0).

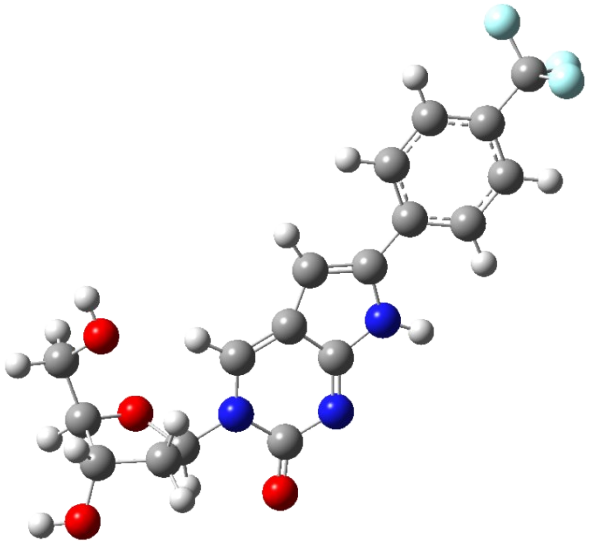


Lifetime plots of (A) ^{FP}dC, (B) 1C and (C) IM-CT.

DFT Calculations

The geometry of modified nucleobases/nucleoside are optimized using DFT, and their optical spectra are calculated using DFT methods as implemented in the Gaussian 16W program package. All the calculations are done using B3LYP (Becke, three-parameter, Lee–Yang–Parr) hybrid exchange and correlation energy functional, default spin, with the 6-311G+(d,2p) basis set for all atoms. The DFT calculations were performed in the gas phase. After geometry optimization, frequency calculations were done to remove any vibrational unstable modes.

Figure S24. Optimized formation of ^{FP}**dC** [a]



[a] Obtained energy of ^{FP}**dC** is -916491.86 kcal/mol.

Figure S25. HOMO-LUMO level and electron distributions of ^{P}**C**

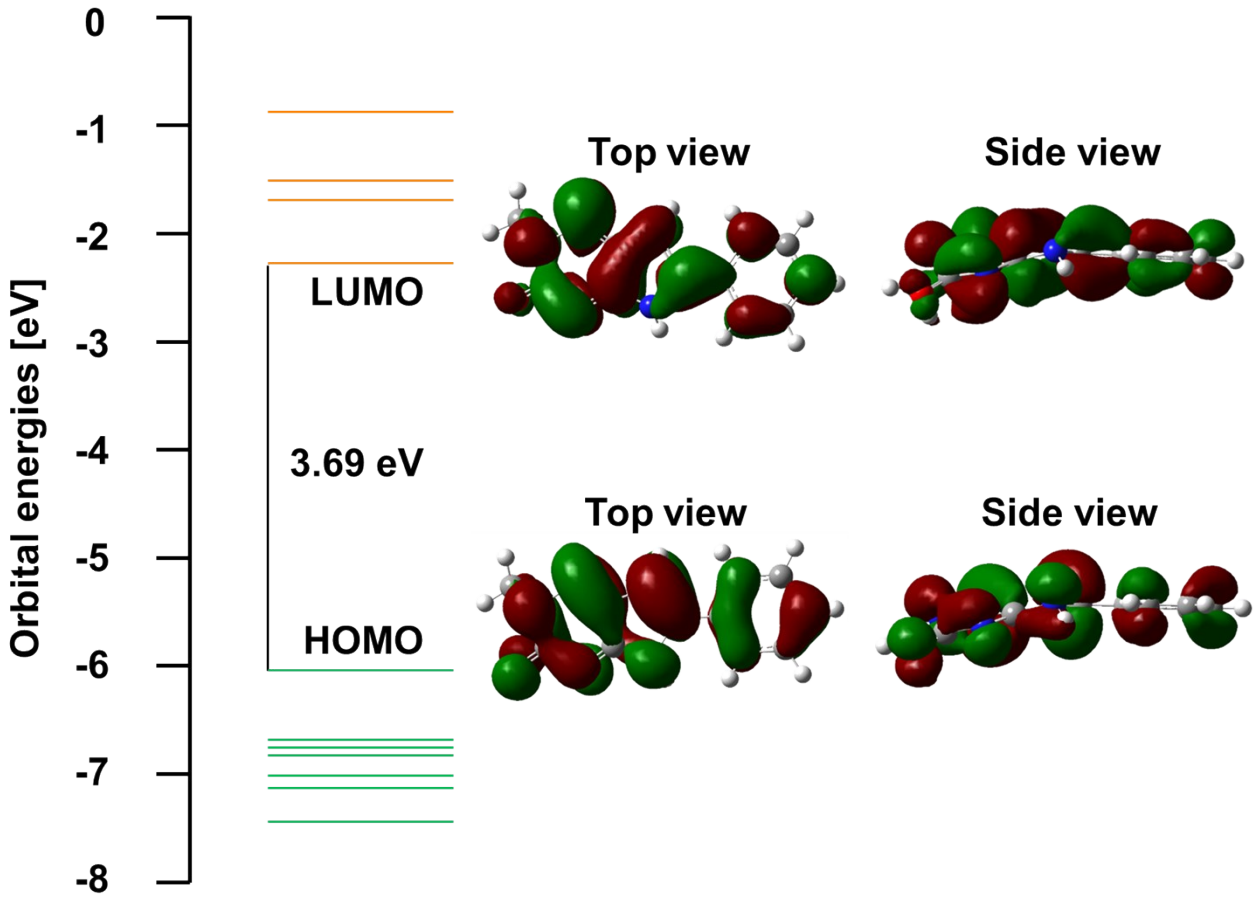
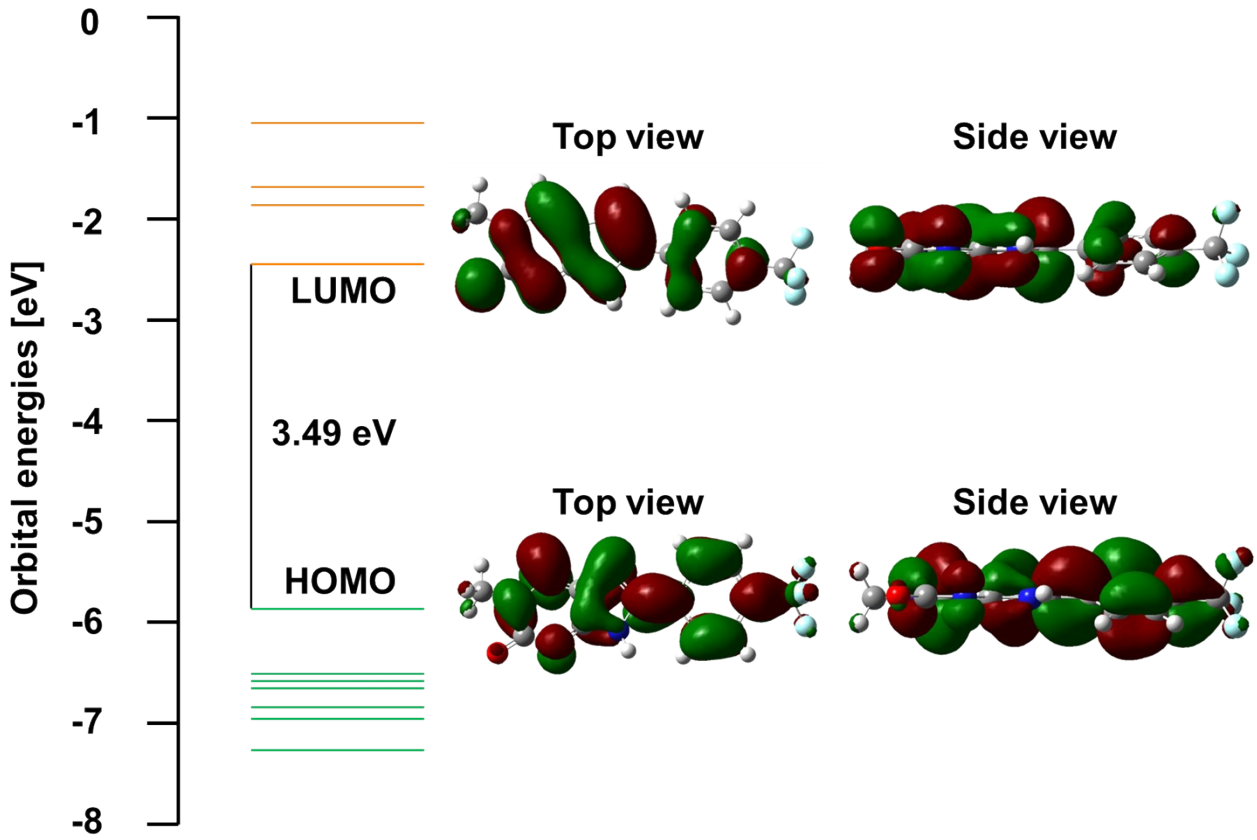


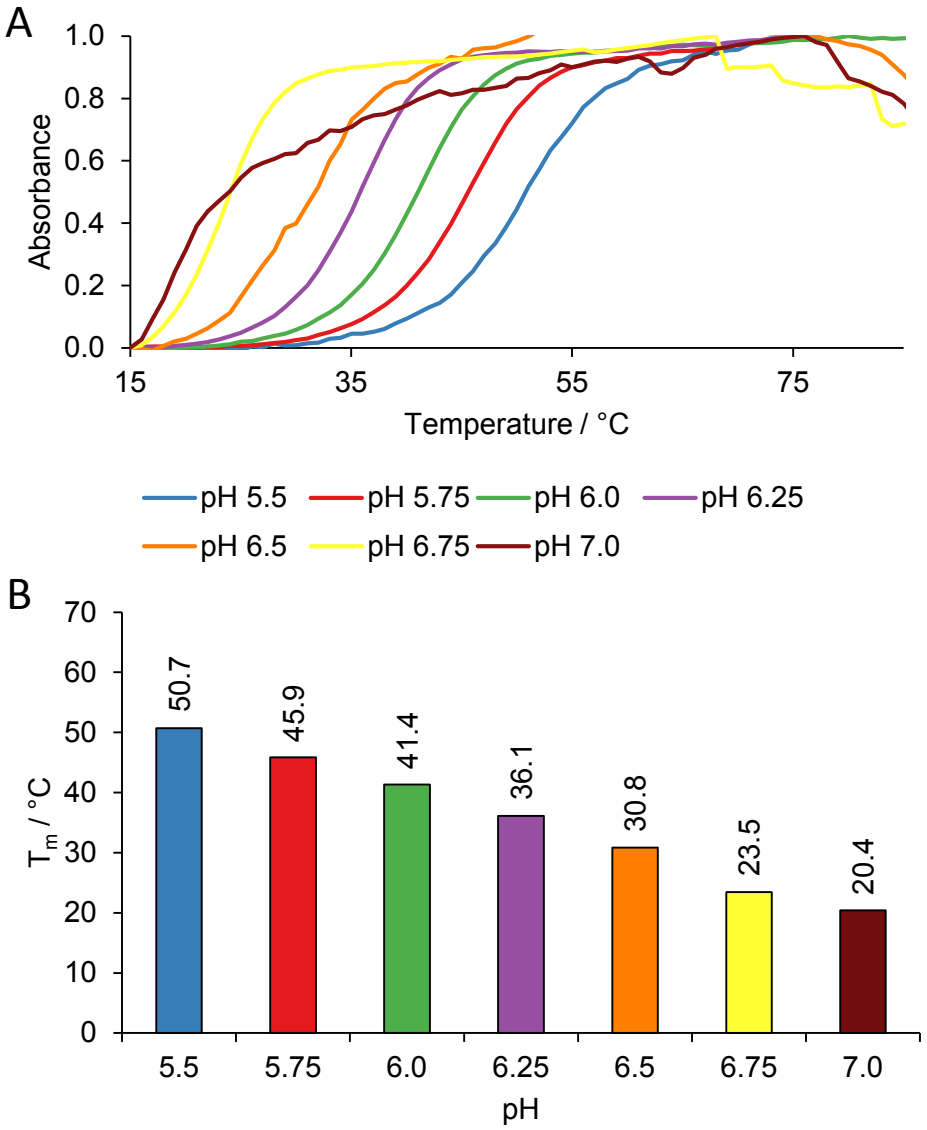
Figure S26. HOMO-LUMO level and electron distributions of ^{FP}**C**



Miscellaneous Data

Figure S27. Melting curves and T_m values of 2IM-CT at various pH [a]

[a] Solution conditions: 2.5 μM DNA in 10 mM Na cacodylate buffer (pH 5.5 – pH 7.0).



(A) Representative UV-melting curve of 2IM-CT; (B) T_m values of 2IM-CT.

Figure S28. CD spectra of 2IM-CT at various pH [a]

[a] Solution conditions: 2.5 μM DNA in 10 mM Na cacodylate (pH 5.5 – pH 8.0).

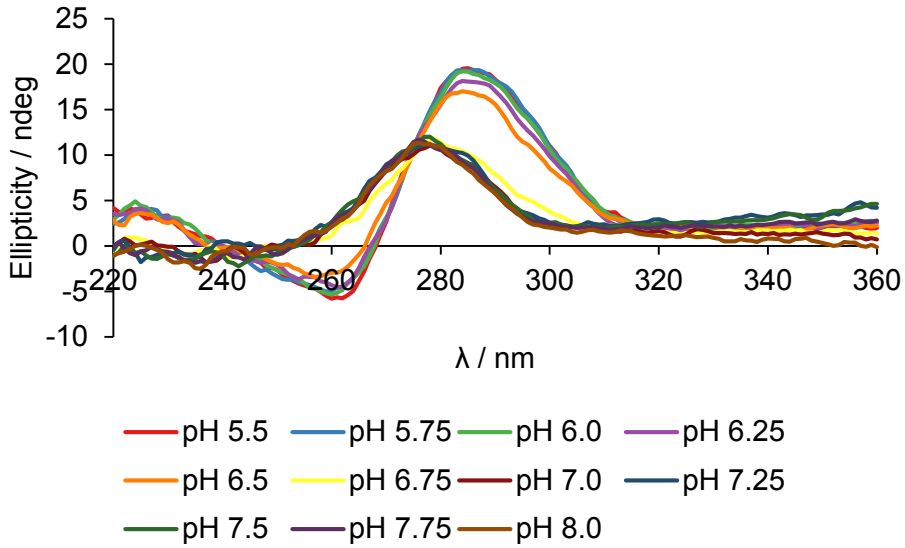


Figure S29. Fluorescence spectra of 2IM-CT at various pH [a]

[a] Solution conditions: 2.5 μM DNA in 10 mM Na cacodylate (pH 5.5 – pH 8.0).

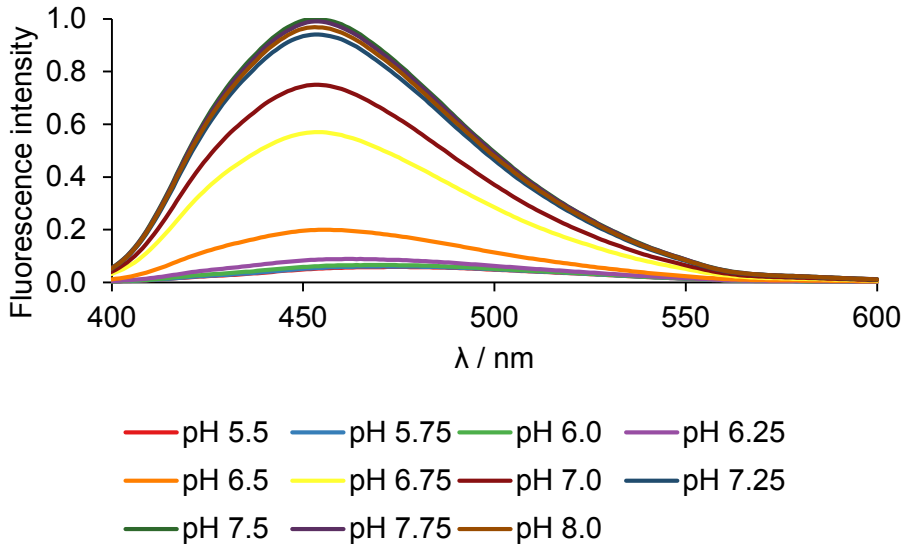
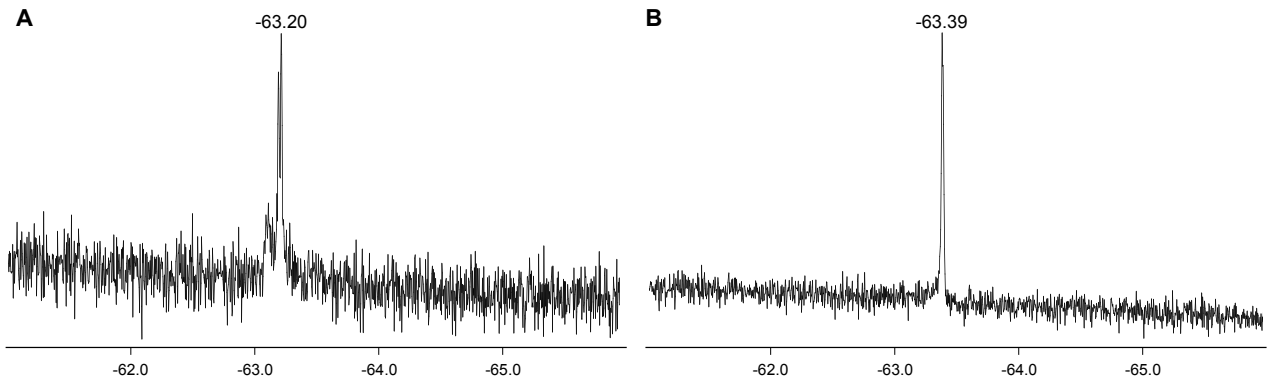


Figure S30. ^{19}F NMR of 2IM-CT [a]

[a] Solution conditions: 50.0 μM DNA and 20 % D_2O in 10 mM Na cacodylate (pH 5.5/pH 8.0) with TFA as an internal standard.



(A) ^{19}F NMR spectrum of 2IM-CT at pH 5.5; (B) ^{19}F NMR spectrum of 2IM-CT at pH 8.0.

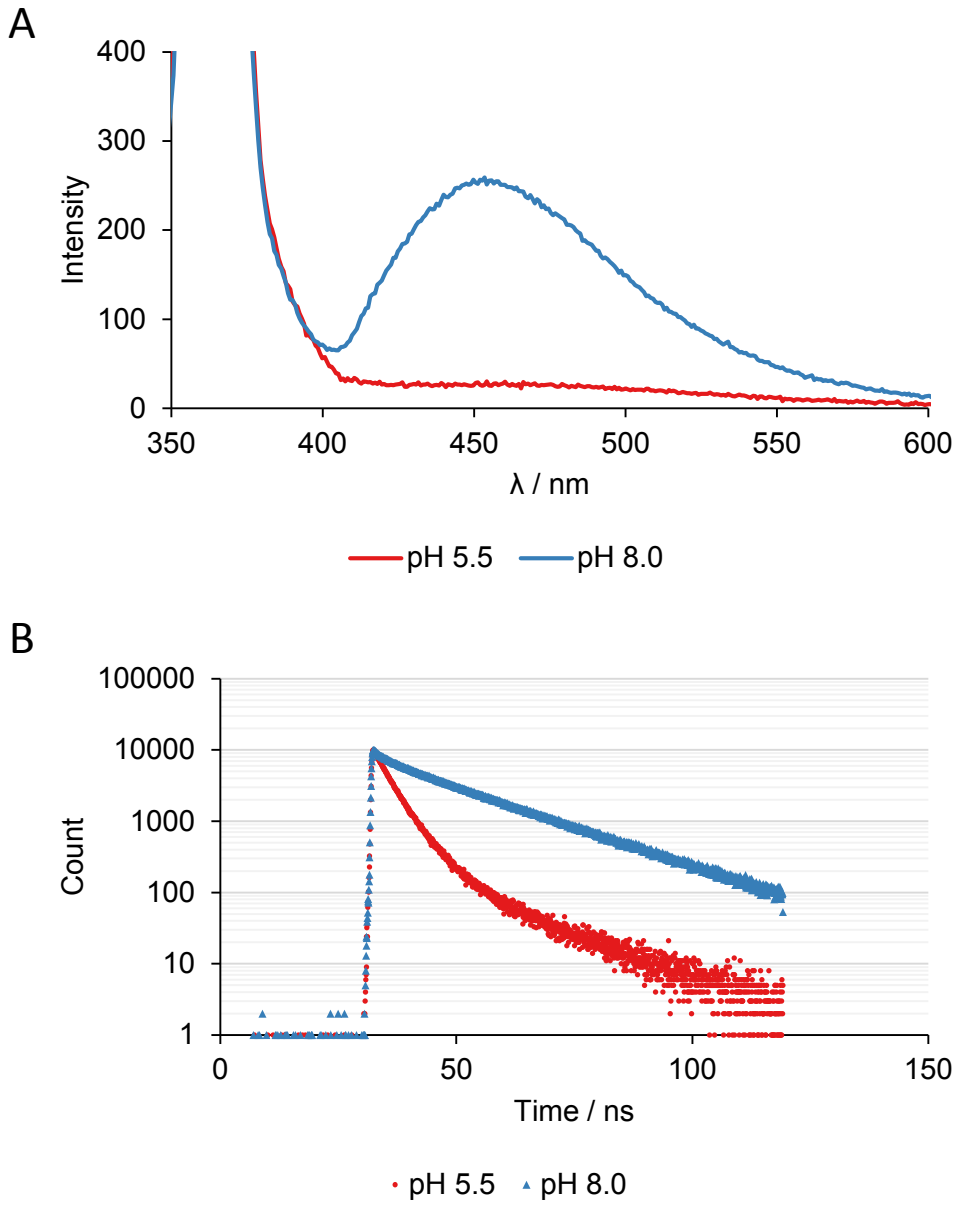
Table S5. Photophysical properties of 2IM-CT at various pH [a]

[a] Solution conditions: 2.5 μM 2IM-CT in 10 mM Na cacodylate (pH 5.5 or pH 8.0).

	τ_1 / ns	α_1	τ_2 / ns	α_2	τ_3 / ns	α_3	τ_{avg} / ns	χ^2	Φ
pH 5.5	0.79	0.365	2.07	0.609	8.25	0.026	1.76	1.23	0.07
pH 8.0	2.53	0.138	9.78	0.585	0.47	0.278	6.20	1.27	0.57

Figure S31. Quantum yield and lifetime of 2IM-CT [a]

[a] Solution conditions: 2.5 μM DNA in 10 mM Na cacodylate (pH 5.5 or pH 8.0).



References

S1) Cho, S. J.; Ghorbani-Choghamarani, A.; Saito, Y.; Hudson, R. H. E. *Curr. Protoc. Nucleic Acid Chem.* **2019**, e75.

S2) Reilly, S. M.; Morgan, R. K.; Brooks, T. A. Wadkins, R. M. *Biochemistry* **2015**, *54*, 1364-1370.