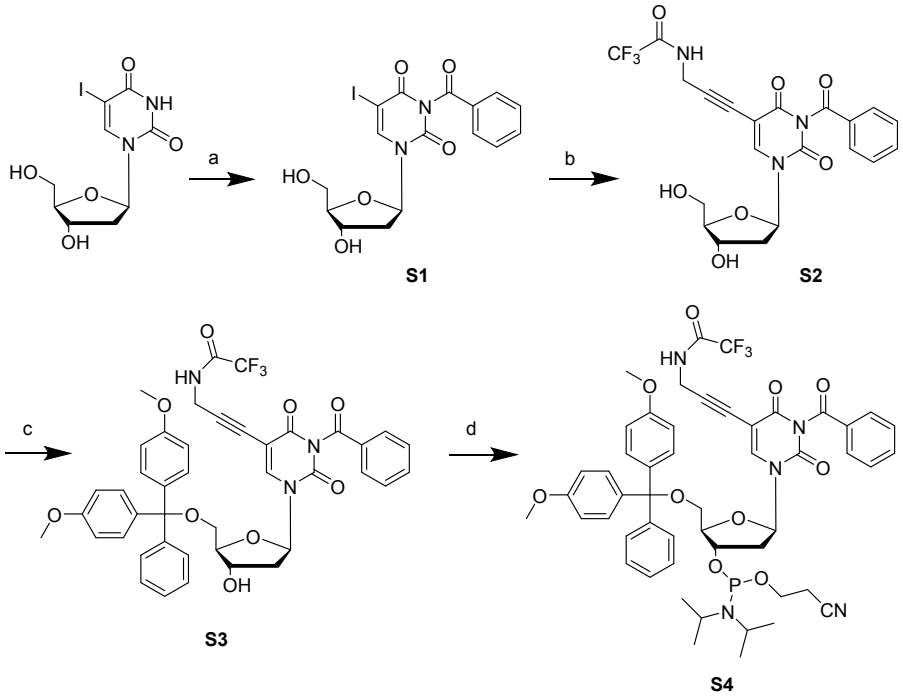
Supplementary Information

Propargyl amine-selective dual fluorescence turn-on method for post-synthetic labeling of DNA

Synthesis of 5´-protected propargylamino-deoxyuridine phosphoramidite



Scheme S1. Synthesis of 5´-protected propargylamino-deoxyuridine phosphoramidite. (a) benzoyl chloride, TEA, ACN, room temperature, overnight. (b) 2,2,2-trifluoro-N-(prop-2-yn-1-yl)acetamide, $(PPh_3)_4Pd$, CuI, TEA, DMF, 45–50 °C, 5 h. (c) DMT-Cl, pyridine, room temperature, 3h. (d) 2-Cyanoethyl N,N-diisopropylchlorophosphoramidite, DIPEA, CH_2Cl_2, 0°C, 1h.

*Compound **S1***

Triethylamine (5 mL, 35.3 mmol) was added to a solution of 5-iodo-2´-deoxyuridine (2.50 g, 7.06 mmol) in MeCN (50 mL). The mixture was degassed with N_2 and then benzoyl chloride (0.860 mL, 7.41 mmol) was slowly added dropwise. After stirring overnight at room temperature, the solvent was evaporated under reduced pressure and the crude residue purified through column chromatography (SiO_2; CH_2Cl_2/MeOH, 10:1) using a Combiflash machine to afford **S1** (2.05 g, 4.38 mmol, 62%) as a white foam.**^{1}H NMR** (400 MHz, DMSO-d_6): δ 8.67 (s, 1H), 8.05 (d, J = 7.6 Hz, 2H), 7.80 (t, J = 7.2, 6.4 Hz, 1H), 7.61 (t, J = 7.6, 8.0 Hz, 2H), 6.07 (t, J = 6.4 Hz, 1H), 5.33–5.28 (m, 2H), 4.31–4.27 (m, 1H), 3.86–3.83 (m, 1H), 3.71–3.58 (m, 2H), 2.31–2,26 (m, 1H), 2.21–2.15 (m, 1H). **^{13}C NMR** (100 MHz, DMSO-d_6): δ 8.92, 45.76, 60.95, 68.64, 70.03, 85.99, 88.25, 129.97, 131.09, 131.16, 146.35,

149.20, 159.74, 169.34. **MS (Low resolution FAB+)**: *m*/*z* (%) calcd $[M + Na]^+$ 480.98, found 481.29 (100.00)

*Compound **S2***

CuI (160 mg, 0.840 mmol) and $Pd(PPh_3)_4$ (970 mg, 0.840 mmol) were added to a solution of **S1** (2.00 g, 4.36 mmol) in DMF (20 mL), and then the mixture was degassed. Triethylamine (1.20 mL, 8.46 mmol) and *N*-trifluoroacetylpropynylamide (676 mg, 4.80 mmol) were added and then the solution was stirred for 4 h at 55 °C under N_2. Upon completion of the reaction, the mixture was concentrated and the residue purified through column chromatography (SiO_2; CH_2Cl_2/MeOH, 10:1) using a Combi-flash machine to obtain **S3** (1.65 g, 3.42 mmol, 78.5%) as a light-yellow solid. **^{1}H NMR** (400 MHz, DMSO-d_6): δ 8.47 (s, 1H), 8.07 (d, *J* = 7.6 Hz, 2H), 7.81 (t, *J* = 7.2, 7.6 Hz, 1H), 7.61 (t, *J* = 7.6, 8.4 Hz, 2H), 6.09 (t, *J* = 6, 6.4 Hz, 1H), 5.35 (br s, 1H), 5.26 (br s, 1H), 4.29–4.24 (m, 3H), 3.87–3.84 (m, 1H), 3.69–3.59 (m, 2H), 2.32–2.56 (m, 1H), 2.22–2.16 (m, 1H). **^{13}C NMR** (100 MHz, DMSO-d_6): δ 8.98, 29.84, 61.16, 70.26, 74.80, 86.25, 88.43, 88.86, 97.96, 114.80, 117.66, 129.96, 131.10, 131.21, 136.21, 145.39, 148.52, 161.02, 169.23. **MS (High resolution FAB+)**: *m*/*z* (%) calcd 482.1175, found 482.1178 (23.14).

*Compound **S3***

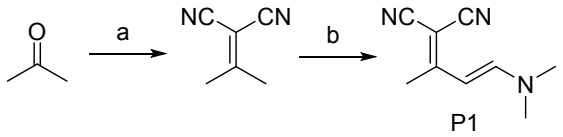
S2 (500 mg, 1.04 mmol) was co-evaporated with pyridine (2 × 5 mL) and suspended in pyridine (5.0 mL). A solution of DMT-Cl (458 mg, 1.35 mmol) in pyridine (5 mL) was added to the stirring solution. The clear light-yellow mixture was stirred at room temperature. Upon completion of the reaction, saturated aqueous $NaHCO_3$ (20 mL) was added. The aqueous phase was extracted with CH_2Cl_2 and the combined organic phases were dried (Na_2SO_4) and concentrated under reduced pressure. The residue was purified through column chromatography (SiO_2; hexane/EtOAc, 1:3) using a Combi-flash machine to obtain **S3** (692 mg, 0.88 mmol, 85 %) as a light-yellow foam. **^{1}H NMR** (400 MHz, DMSO-d_6): 8.35 (s, 1H), 7.98 (s, 1H), 7.90 (d, *J* = 7.6 Hz, 2H), 7.64 (t, *J* = 7.6, 7.2 Hz, 1H), 7.50–7.43 (m, 4H), 7.36–7.22 (m, 8H), 6.87 (d, *J* = 8.8 Hz, 4H), 6.26 (t, *J* = 6.8, 6.4 Hz, 1H), 4.59 (t, *J* = 2.8 Hz, 1H), 4.09 (d, *J* = 2.8 Hz, 1H), 3.97–3.82 (m, 2H), 3.79 (s, 6H) 3.34 (d, *J* = 2.8 Hz, 2H), 2.93 (s, 3H), 2.86 (s, 3H), 2.52–2.46 (m, 1H), 2.35–2.28 (m, 1H). **^{13}C NMR** (100 MHz, DMSO-d_6): 30.24, 31.51, 36.59, 41.80, 55.29, 63.39, 71.95, 74.78, 86.33, 86.88, 87.09, 87.81, 98.77, 113.36, 127.03, 127.85, 128.09, 129.28, 129.95, 130.52, 130.91, 135.40, 135.49, 143.47, 144.48, 147.96, 158.62, 158.64, 161.24, 162.75, 167.74. **MS (High resolution FAB+)**: *m*/*z* (%) calcd 783.2404, found 783.2410 (100.0).

*Compound **S4***

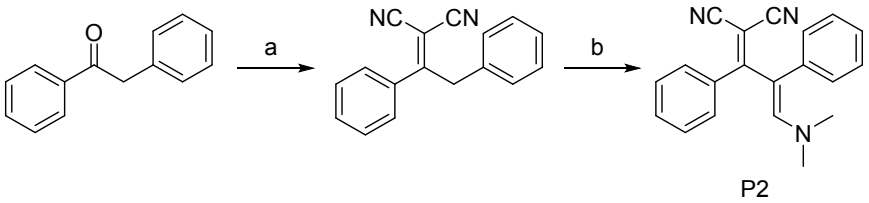
N-Methylmorpholine (140 µL, 1.28 mmol) was added to a stirred solution of **S3** (500 mg, 0.64 mmol) in anhydrous CH_2Cl_2 (5 mL) at 0 °C and then the mixture was stirred 15 min at 0 °C. 2-Cyanoethyl *N*,*N*-diisopropylchlorophosphoramidite (186 µL, 0.832 mmol) was added, and then the solution was stirred at room temperature for 1 h. The mixture was purified through column chromatography (SiO_2;

hexane/EtOAc) to give the **S4** (478.6 mg, 0.49 mmol, 76%, diastereoisomeric mixture) as a light-yellow foam. **^{31}P NMR** (160 MHz, D_2O) δ 156.73; 152.77. **MS (High resolution FAB+)**: *m*/*z* (%) calcd 984.3560, found 984.3567 (29.12)

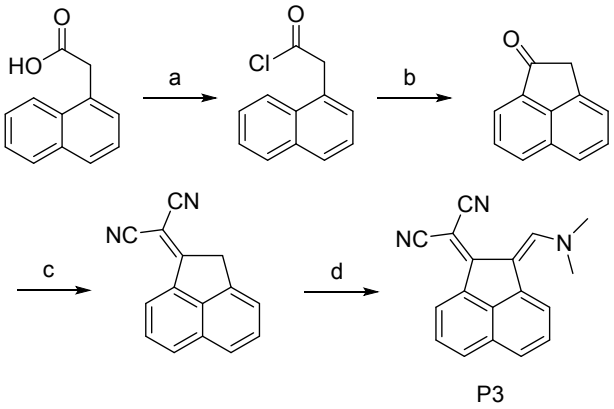
Synthesis of ylidenemalononitrile enamines



Scheme S2. Synthesis of **Probe P1**. (a) malononitrile, Al_2O_3 (activated basic), $CHCl_3$, 1h; (b) Dimethylformamid-dimethylacetal, Ac_2O, toluene, 45°C, 1h.



Scheme S3. Synthesis of **Probe P2**. (a) malononitrile, AcOH, NH_4OAc, toluene, reflux, 2h; (b) Dimethylformamid-dimethylacetal, Ac_2O, toluene, room temperature, overnight.



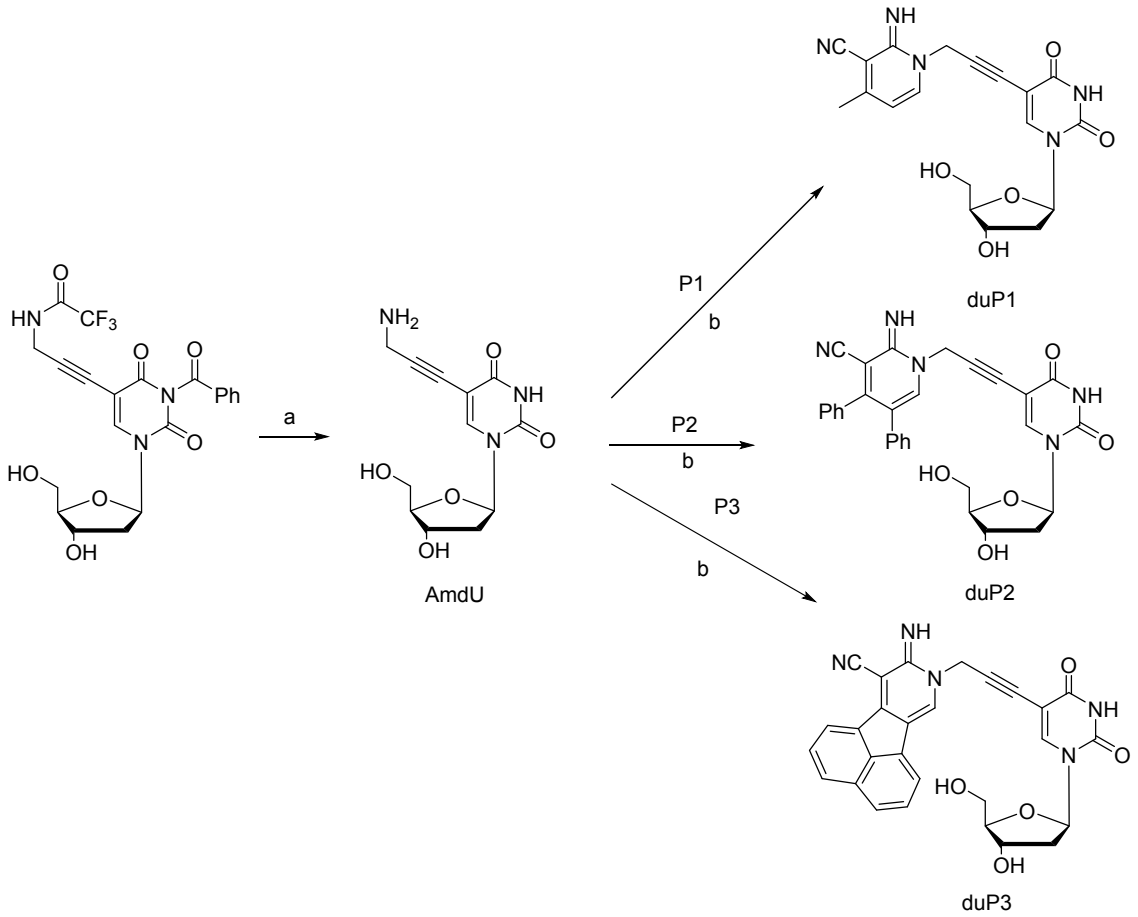
Scheme S4. Synthesis of **Probe P3**. (a) oxalyl chloride, DMF, DCM, 1h; (b) $AlCl_3$, DCM, 3h; (c) malononitrile, AcOH, NH_4OAc, toluene, reflux, 2h; (d) Dimethylformamid-dimethylacetal, Ac_2O, toluene, room temperature, overnight.

P1, P2, P3 were prepared according to the previously reported procedure[1].

P1: **^{1}H NMR**: (400 MHz, $CDCl_3$) δ 7.21 (d, *J* = 12.4 Hz, 1H), 5.67 (d, *J* = 12.8 Hz, 1H), 3.23 (s, 3H), 3.00 (s, 3H), 2.25 (s, 3H). **^{13}C NMR**: (100 MHz, $CDCl_3$) δ 17.24, 37.53, 45.87, 45.51, 97.09, 116.10, 116.85, 152.40, 168.22.

P2: **^{1}H NMR**: (400 MHz, $CDCl_3$) δ 7.44–7.18 (m, 10H), 6.85 (br s, 1H), 2.76 (br s, 6H). **^{13}C NMR**: (100 MHz, $CDCl_3$) δ 44.12, 65.33, 112.36, 116.10, 117.98, 128.12, 128.36, 128.47, 130.16, 132.48, 135.92, 137.34, 154.66, 172.82.

P3: **^{1}H NMR**: (400 MHz, $CDCl_3$) δ 8.71 (d, *J* = 7.6 Hz, 1H), 8.63 (s, 1H), 8.00 (d, *J* = 8 Hz, 1H), 7.70–7.63 (m, 2H), 7.53 (t, *J* = 7.6 Hz, 1H), 7.30 (d, *J* = 6.8 Hz, 1H), 3.49 (s, 6H). **^{13}C NMR**: (100 MHz, $CDCl_3$) δ 45.47, 106.71, 118.05, 118.28, 119.47, 122.71, 125.35, 127.80, 128.12, 129.91, 130.99, 132.57, 135.20, 135.62, 153.03, 162.16.



Scheme S5. Synthesis of **fluorescent nucleosides**. (a) aq NH_3, room temperature, overnight; (b) TEA, DMF, room temperature, overnight.

Synthesis of fluorescent nucleosides

AmdU

The protected base **S2** (1.50 g, 3.12 mmol) was dissolved in ammonia solution (20 mL) and stirred for 12 h. Evaporation of the solvent provided the deprotected base **S17** as a yellowish oil. This product was used without any further purification.

duP1

In a dry, N_2-flushed flask, **S17** (200 mg, 0.710 mmol) was dissolved in anhydrous DMF (5 mL). **P1** (637 mg, 2.13 mmol) and triethylamine (0.49 mL, 3.55 mmol) were added. After the mixture had been stirred at room temperature overnight, the solvent and triethylamine were evaporated *in vacuo*, and the residue was purified through column chromatography (SiO_2; CH_2Cl_2/MeOH, 8:1). The product was obtained in 27% yield (75.5 mg, 0.19 mmol). **^{1}H NMR**: (400 MHz, DMSO-d_6) δ 11.41 (s, 1H), 8.65 (d, J = 6.8 Hz, 1H), 7.84 (s, 1H), 7.74 (s, 1H), 6.94 (d, J = 6.8 Hz, 1H), 6.17 (t, J = 7.2, 6.8 Hz, 1H), 5.31 (br, 1H), 4.97 (br, 1H), 4.21 (s, 1H), 3.76 (q, J = 2.8, 4.0 Hz, 1H), 3.67 (s, 2H), 3.48 (br, 2H), 2.54 (s, 3H), 2.10–2.06 (m, 2H). **^{13}C NMR**: (100 MHz, DMSO-d_6) δ 20.39 26.48 61.81 70.93 84.589 87.89 98.75 111.66 112.04 116.00 118.97 130.06 138.24 158.63 158.94 158.25 163.58. **MS (ESI+)**: *m*/*z* (%) calcd 398.14, found 398.20 (100.0).

duP2

In a dry, N_2-flushed flask, **S17** (200 mg, 0.71 mmol) was dissolved in anhydrous DMF (5 mL). **P2** (637 mg, 2.13 mmol) and triethylamine (mL, mmol, equiv) were added. After the mixture had been stirred at room temperature overnight, the solvent and triethylamine were evaporated *in vacuo*, and the residue purified through column chromatography (SiO_2; CH_2Cl_2/MeOH, 8:1). The product was obtained in 35% yield (133.8 mg, 0.25 mmol). **^{1}H NMR**: (400 MHz, DMSO-d_6) δ 11.44 (s, 1H), 8.85 (s, 1H), 7.87 (d, J = 4.4 Hz, 2H), 7.36–7.33 (m, 3H), 7.25–7.24 (m, 5H), 7.12–7.09 (m, 2H), 6.19 (t, J = 7.2, 6.4 Hz, 1H), 5.25 (d, J = 4.0 Hz, 1H), 4.95 (t, J = 5.2 Hz, 1H), 4,23 (quint, J = 3.2, 4.0, 4.4, 4.0 Hz, 1H), 3.79–3.76 (m, 3H), 3.56–3.46 (m, 2H), 2.14–2.09 (m, 2H). **^{13}C NMR**: (100 MHz, DMSO-d_6) δ 26.72, 61.89 70.96 84.61 87.89 99.70 111.80 112.48 115.75 126.16 127.93 128.56 129.08, 130.19, 130.31, 130.40, 136.11, 136 29, 138.24, 142.05, 145.06, 147.51, 150.82, 163.47. **MS (High resolution FAB+)**: *m*/*z* (%) calcd 536.1934, found 536.1936 (100.0)

duP3

In a dry, N_2-flushed flask, **S17** (200 mg, 0.71 mmol) was dissolved in anhydrous DMF (5 mL). **P3** (637 mg, 2.13 mmol) and triethylamine (mL, mmol, equiv) were added. After the mixture had been stirred at room temperature overnight, the solvent and triethylamine were evaporated *in vacuo*, and the residue purified through column chromatography (SiO_2; CH_2Cl_2/MeOH, 8:1). The product was obtained in 63% yield (223.3 mg, 0.44 mmol). **^{1}H NMR**: (400 MHz, DMSO-d_6) δ 11.46 (s, 1H), 9.45 (s, 1H), 8.39 (d, J = 7.2 Hz, 1H), 8.17 (d, J = 8.4 Hz, 1H), 8.12 (d, J = 7.2 Hz, 1H), 8.04 (d, J = 8.4 Hz, 1H), 7.91–7.88

(m, 3H), 7.80 (t, *J* = 7.2, 8.0 Hz, 1H). 6.20 (t, *J* = 7.2, 6.4 Hz, 1H), 5.24 (d, *J* = 4.4 Hz, 1H), 4.88 (t, *J* = 5.2, 5.6, 1H), 4.21 (d, *J* = 3.2, 4.4 Hz), 3.77–3.75 (m, 3H), 3.51–3.47 (m, 2H), 2.12–2.09 (m, 2H). **^{13}C NMR**: (100 MHz, DMSO-d_6) δ 26.64, 61.87, 70.97, 84.61, 87.97, 91.75, 111.74, 113.87, 115.43, 120.67 122.87, 124.73, 124.76, 127.02, 129.37, 129.80, 130.50, 132.44, 134.87, 138.26, 141.29, 142.03, 146.96, 150.83, 163.66. **MS (High resolution FAB+)**: *m*/*z* (%) calcd 508.1621, found 536.1624 (100.0).

Solid-Phase Oligonucleotide Synthesis

The phosphoramidites were introduced to produce **ODN1** on a controlled-pore glass (CPG) solid support (1-*O*-dimethoxytritylpropyldisulfide, 1´-succinyl-lcaa-CPG), using a standard phosphoramidite approach and an automated DNA synthesizer (MERMADE DNA-Synthesizer). The synthesized oligonucleotide was cleaved from the solid support under the influence of 30% aqueous NH_4OH (1.0 mL) for 10 h at 60 °C. The crude product obtained from the automated ODN synthesis was lyophilized and diluted with distilled water (1 mL). The ODN was purified through high-performance liquid chromatography (HPLC; Merck LichoCART C18 column; 10 × 250 mm; 10 μm; pore size: 100 Å). The HPLC mobile phase was held isocratic for 10 min [5% MeCN/0.1 M triethylammonium acetate (TEAA); pH 7.0] at 2.5 mL/min. The gradient was increased linearly over 10 min from 5 to 50% MeCN/0.1 M TEAA at the same flow rate. The fractions containing the purified ODN were cooled and lyophilized. Subsequently, 80% aqueous AcOH was added to the ODN. After 1 h at ambient temperature, the AcOH was lyophilized under reduced pressure. The residue was diluted with water (1 mL); this solution was purified through HPLC using the conditions described above.

Table S1: Designed sequence

Sequence	5´–3´
ODN1	TTT TTT T AmdU T TTT TTT

Photophysical Properties of Functional Nucleotides

CALCULATED QUANTUM YIELD OF FLUOROPHORE COMPOUNDS in water:

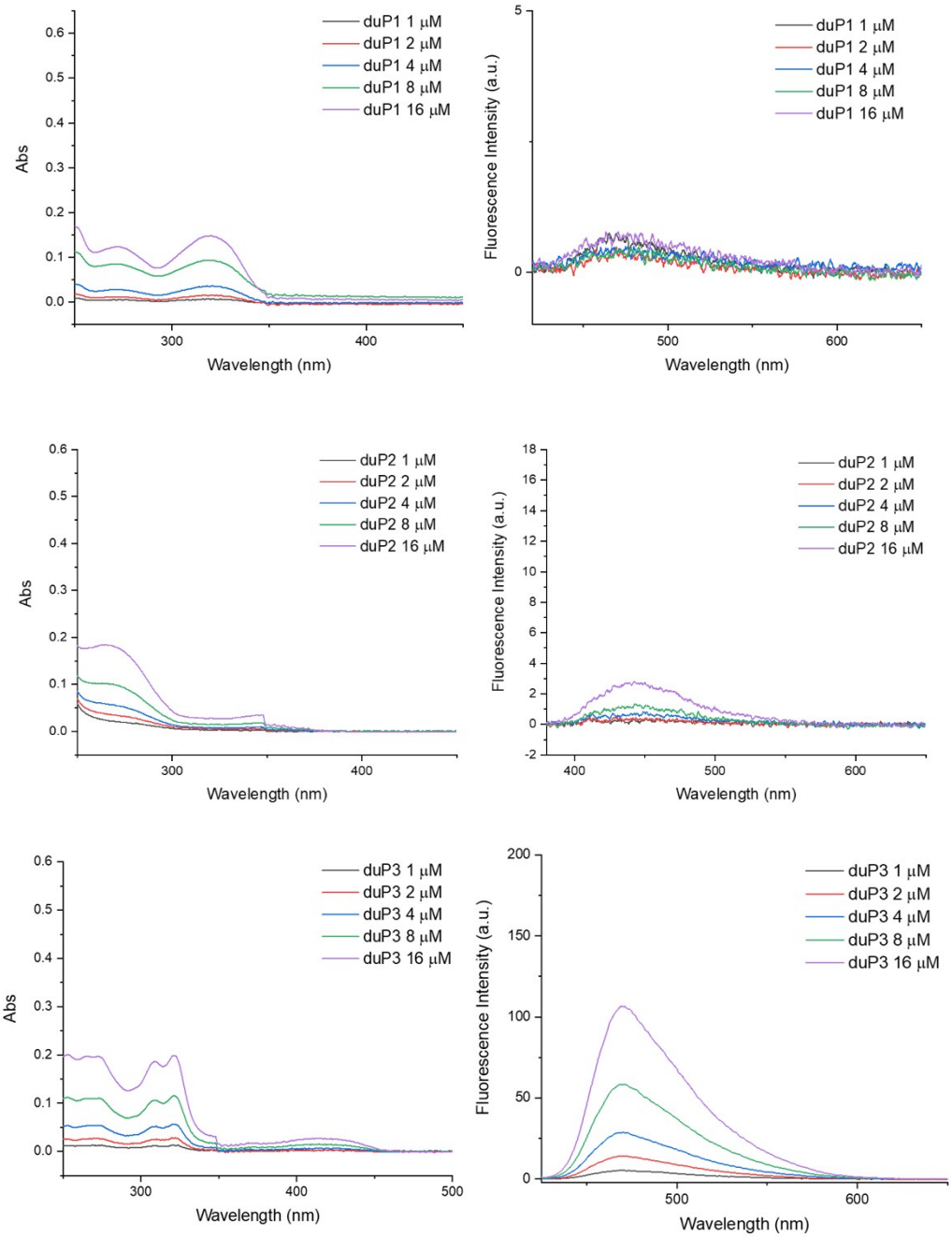
$$Q = Q_R \left(\frac{Grad}{Grad_R}\right)\left(\frac{n^2}{n_R^2}\right)$$

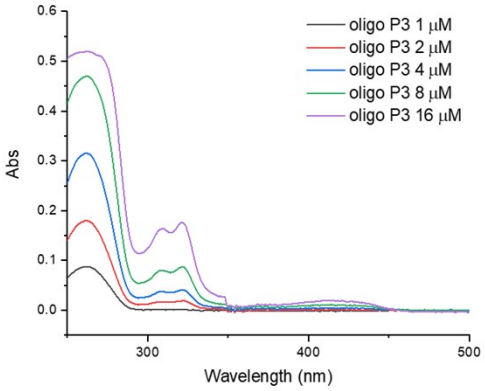
- Q is the fluorescence quantum yield of the compound.
- Q_R is the fluorescence quantum yield of the reference compound (9,10-diphenylanthracence)
- *Grad* is the gradient obtained from the plot of the integrated fluorescence intensity and absorption.

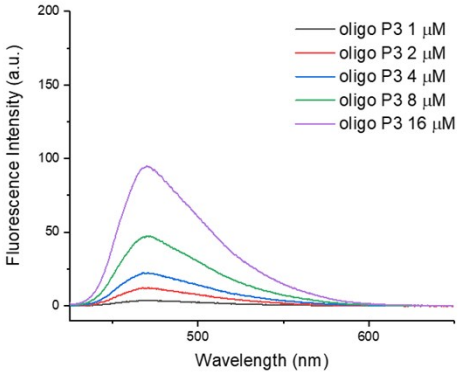
- n the refractive index of the solvent (H_2O).

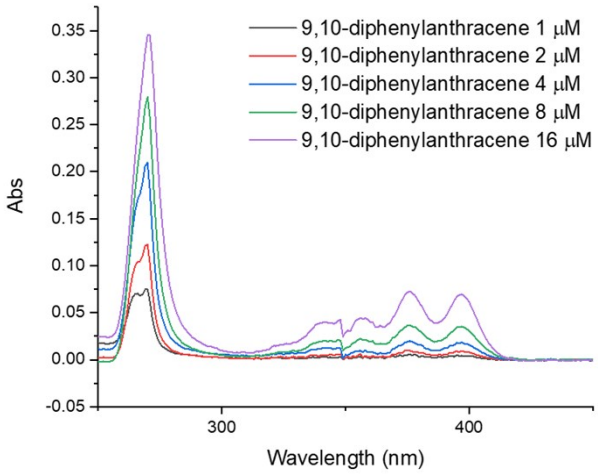
- The subscript “R” denotes the reference compound.

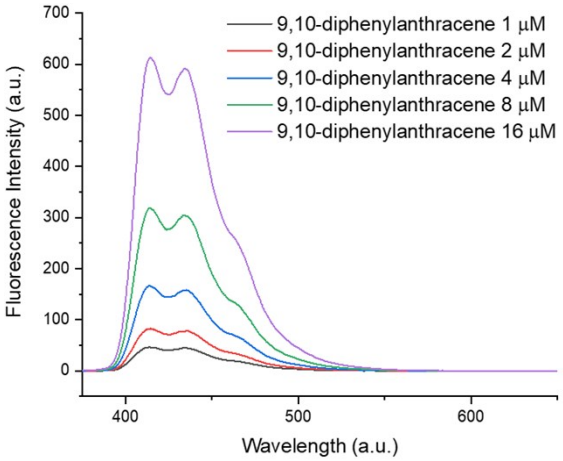
Using the literature values: Q_R (ethanol) = 0.95; n (water) = 1.33; n (ethanol) = 1.36



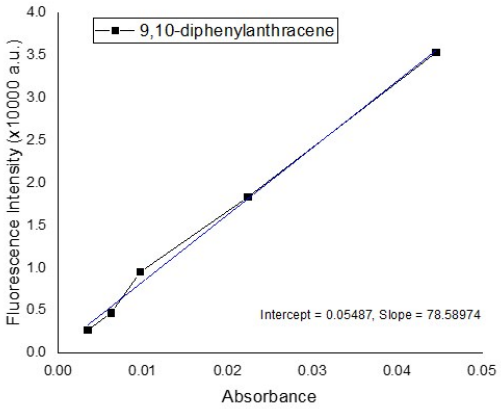


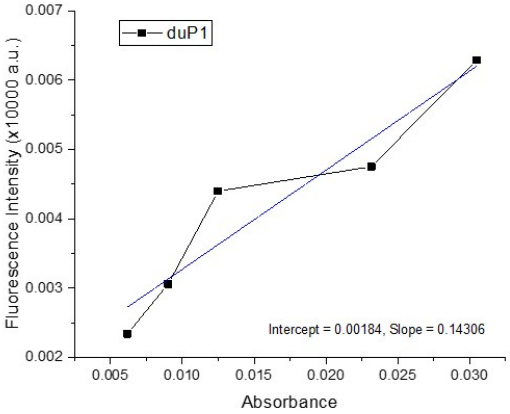


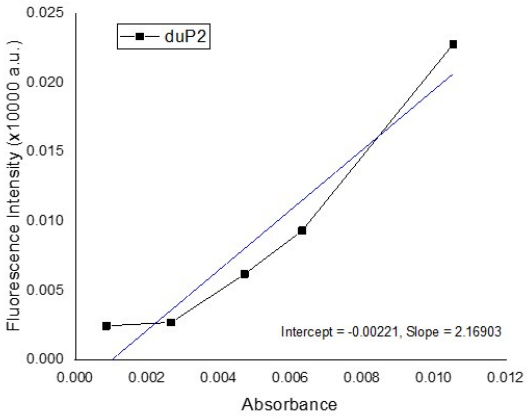


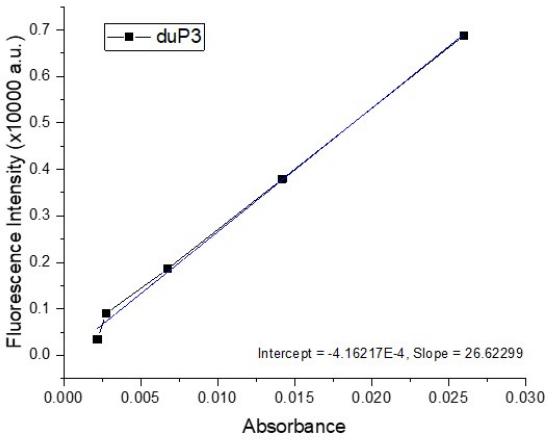


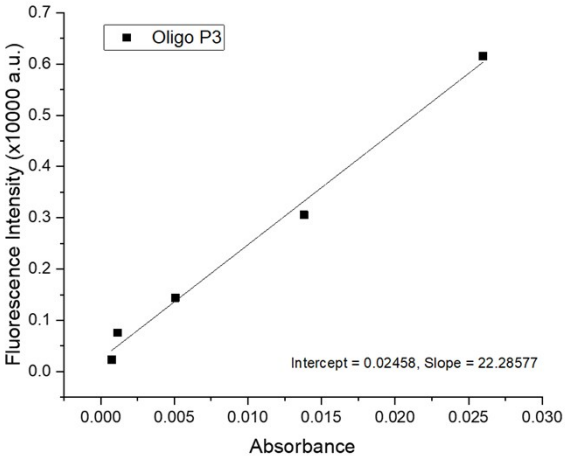
Conc (μM)	9,10-Diphenylanthracence		duP1		duP2		duP3		Oligo P3	
	Absorbance	Area of Fluorescence	Absorbance	Area of Fluorescence	Absorbance	Area of Fluorescence	Absorbance	Area of Fluorescence	Absorbance	Area of Fluorescence
1	0.0035	2707.78689	-0.006180	23.39129	-0.000858	24.46966	0.00276	344.00755	0.000746	233.58443
2	0.00628	4756.85971	-0.003340	30.58532	0.000957	26.93063	0.00219	904.48655	0.001140	757.49709
4	0.00971	9549.38959	0.000130	43.98119	0.003000	61.83974	0.00674	1857.92727	0.005070	1439.5342
8	0.02238	18334.71715	0.010800	47.52716	0.004610	93.23984	0.01419	3794.69322	0.013810	3058.03803
16	0.04461	35359.08279	0.018140	62.92623	0.008820	227.88246	0.02598	6884.75847	0.025380	6158.07985











$$duP1 = 0.95\left(\frac{0.14306}{78.58974}\right)\left(\frac{1.33^2}{1.36^2}\right) = 0.0017$$

$$dUP2 = 0.95\left(\frac{2.16903}{78.58974}\right)\left(\frac{1.33^2}{1.36^2}\right) = 0.025$$

$$duP3 = 0.95\left(\frac{26.62299}{78.58974}\right)\left(\frac{1.33^2}{1.36^2}\right) = 0.308$$

$$Oligo\ P3 = 0.95\left(\frac{22.28577}{78.58974}\right)\left(\frac{1.33^2}{1.36^2}\right) = 0.258$$

Table S2: Photophysical properties of fluorescent nucleosides in PBS buffer

Compound	λ_{ab} (nm)	λ_{em} (nm)	Φ
duP1	350	475	0.0017
duP2	360	515	0.025
duP3	420	475	0.308
Oligo P3	420	475	0.258

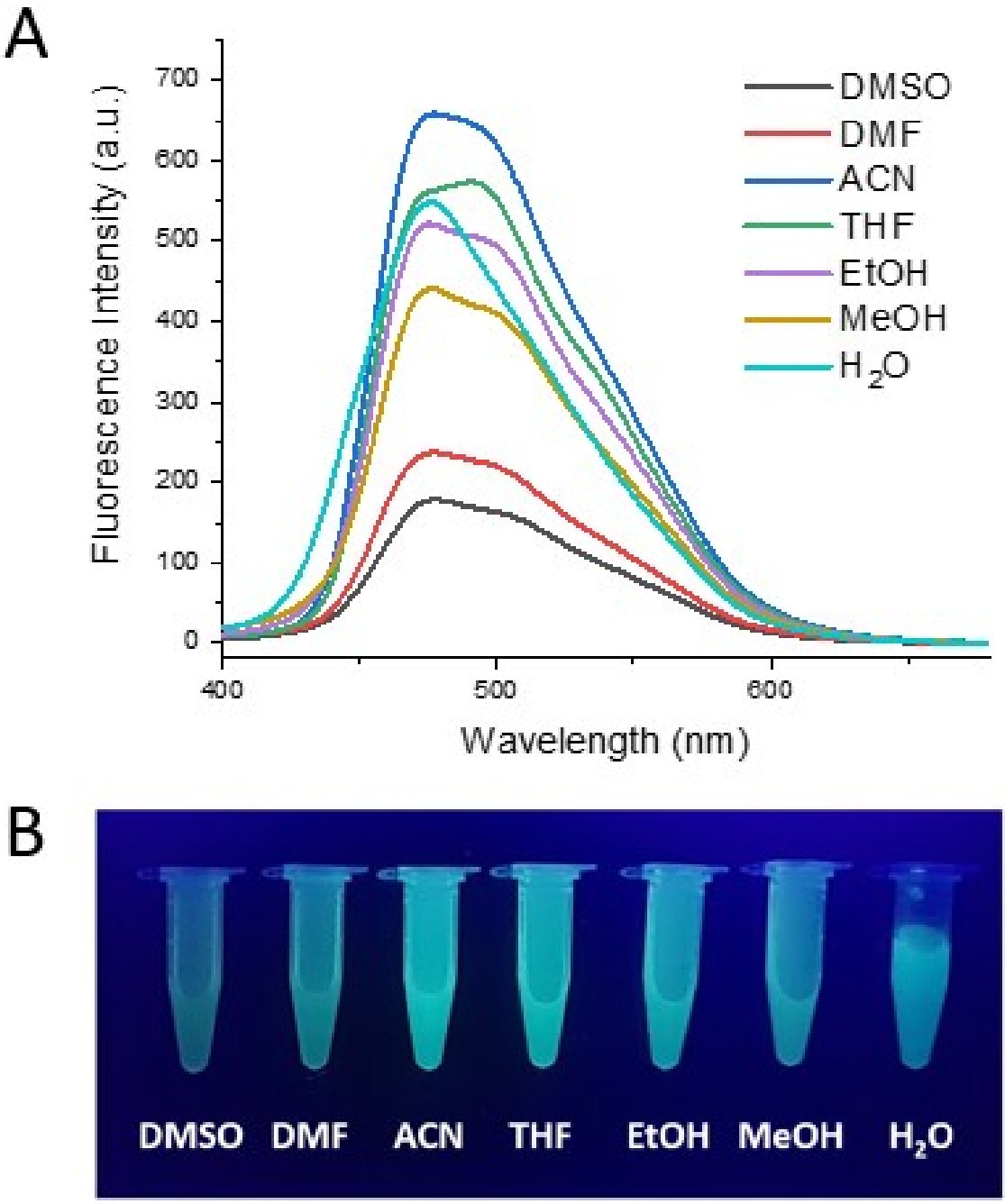


Figure S1. Solvent-dependent fluorescence property of **duP1**. (A) Emission spectra in different solvents. Excitation wavelength is 350 nm. (B) Photographs shown are of 1 mM solutions of **duP1** in different solvents under 365 nm irradiation.

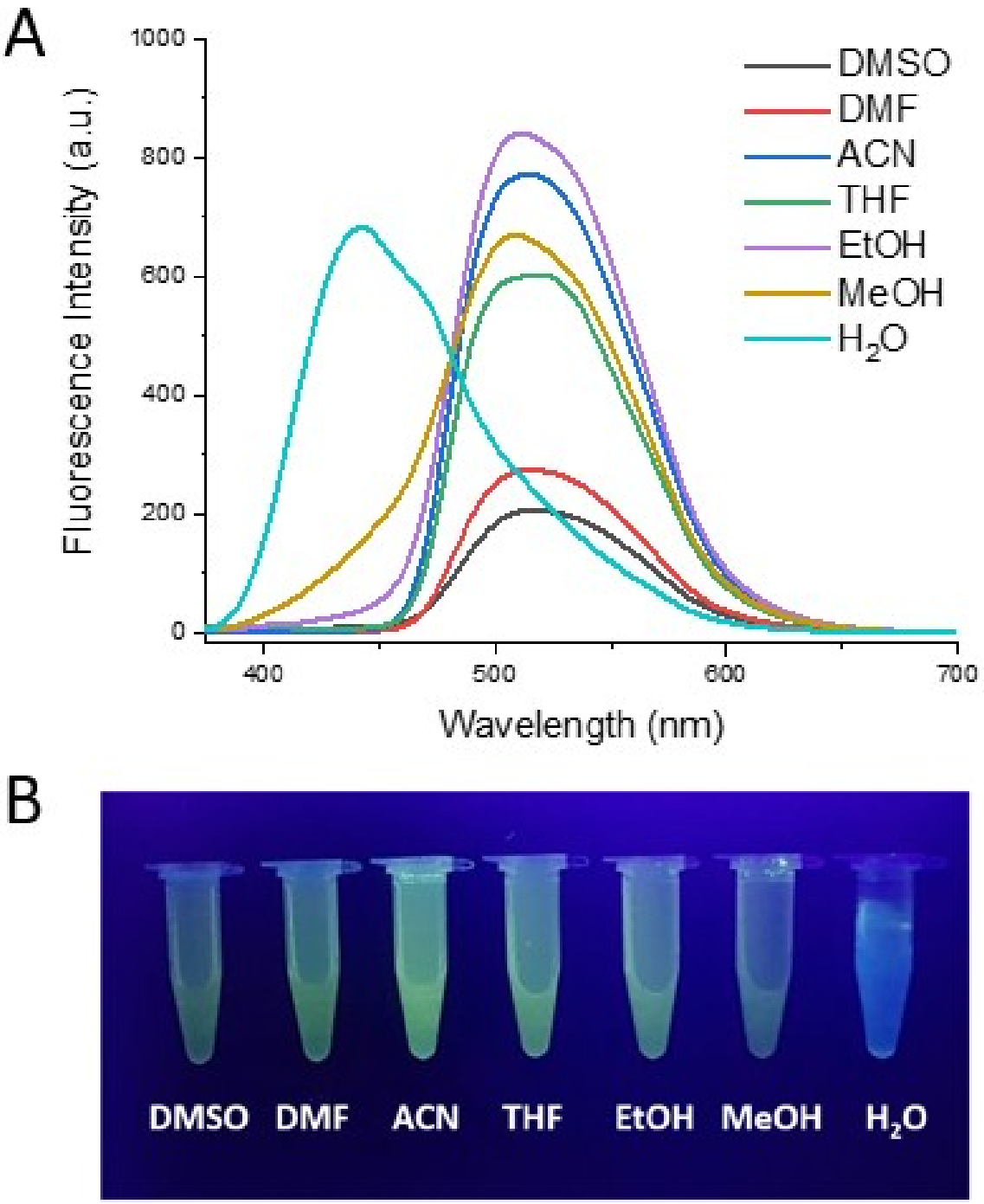


Figure S2. Solvent-dependent fluorescence property of **duP2**. (A) Emission spectra in different solvents. Excitation wavelength is 360 nm. (B) Photographs shown are of 1 mM solutions of **duP2** in different solvents under 365 nm irradiation.

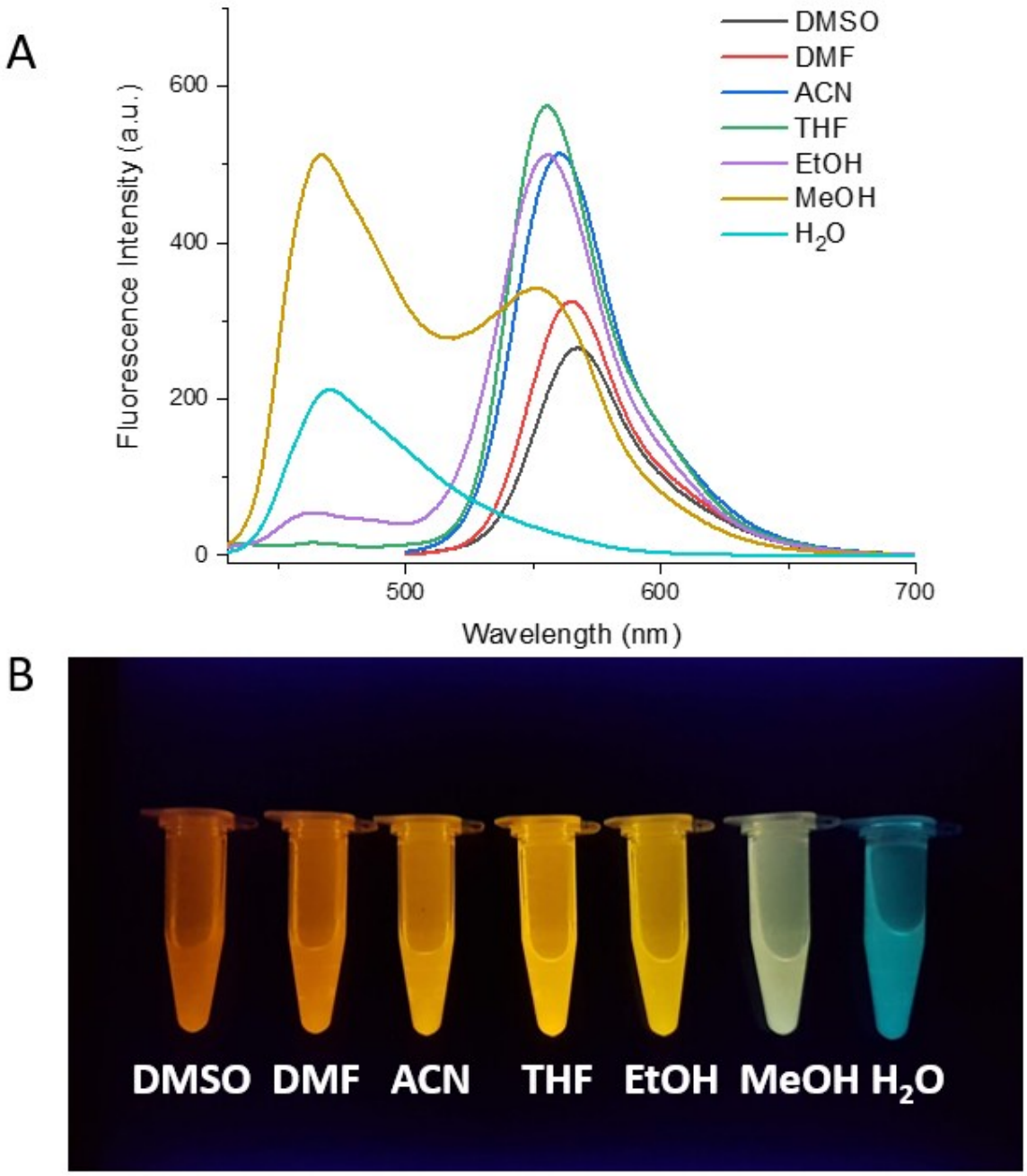


Figure S3. Solvent-dependent fluorescence properties of **duP3**. (A) Emission spectra recorded in various solvents. Excitation wavelength: 420 nm. (B) Photographs of 1 mM solutions of **duP3** in various solvents, under irradiation at 365 nm.

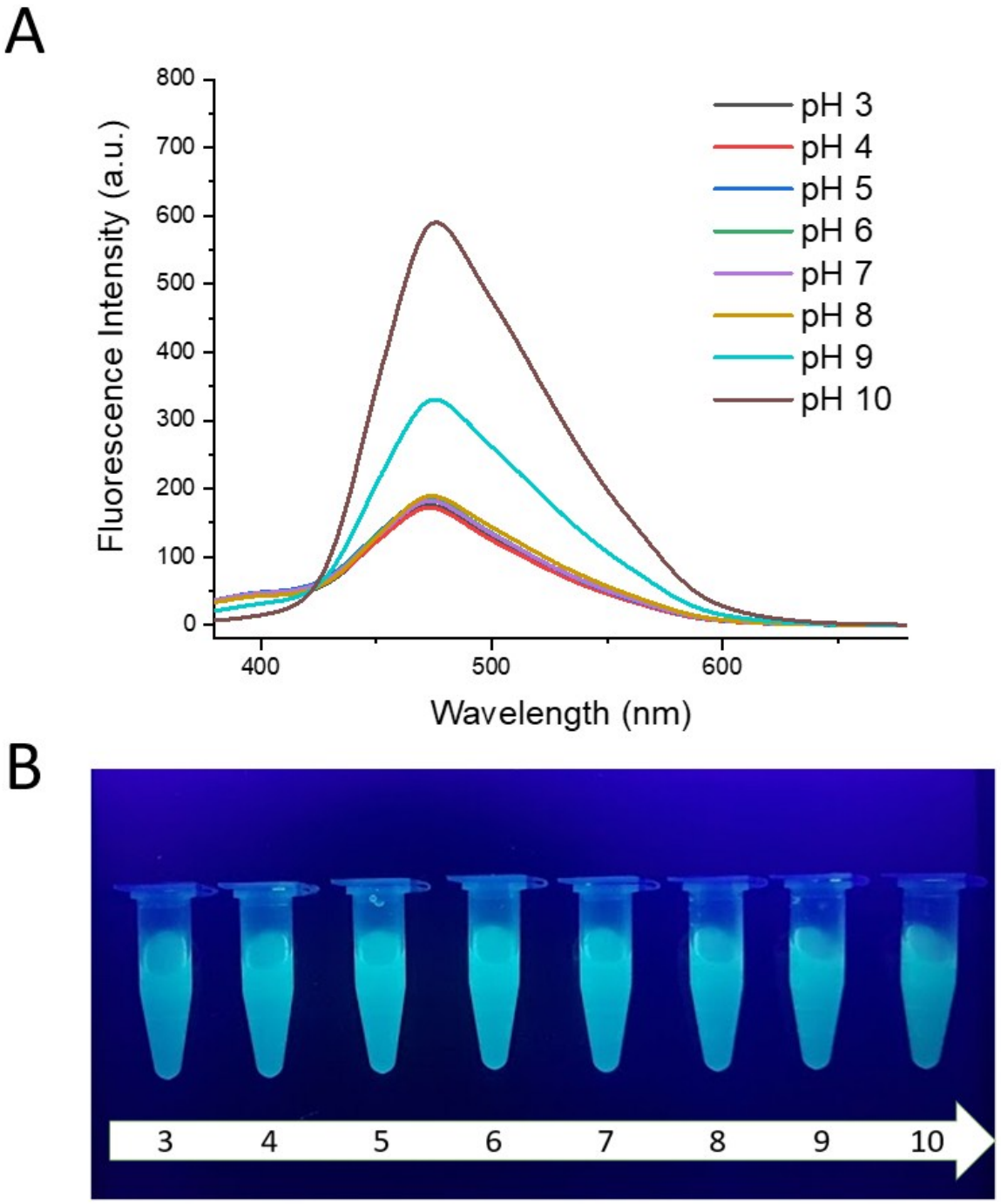


Figure S4. pH-dependent fluorescence property of **duP1**. (A) Emission spectra in different pH. Excitation wavelength is 420 nm. (B) Photographs shown are of 1 mM solutions of **duP1** in different pH under 365 nm irradiation.

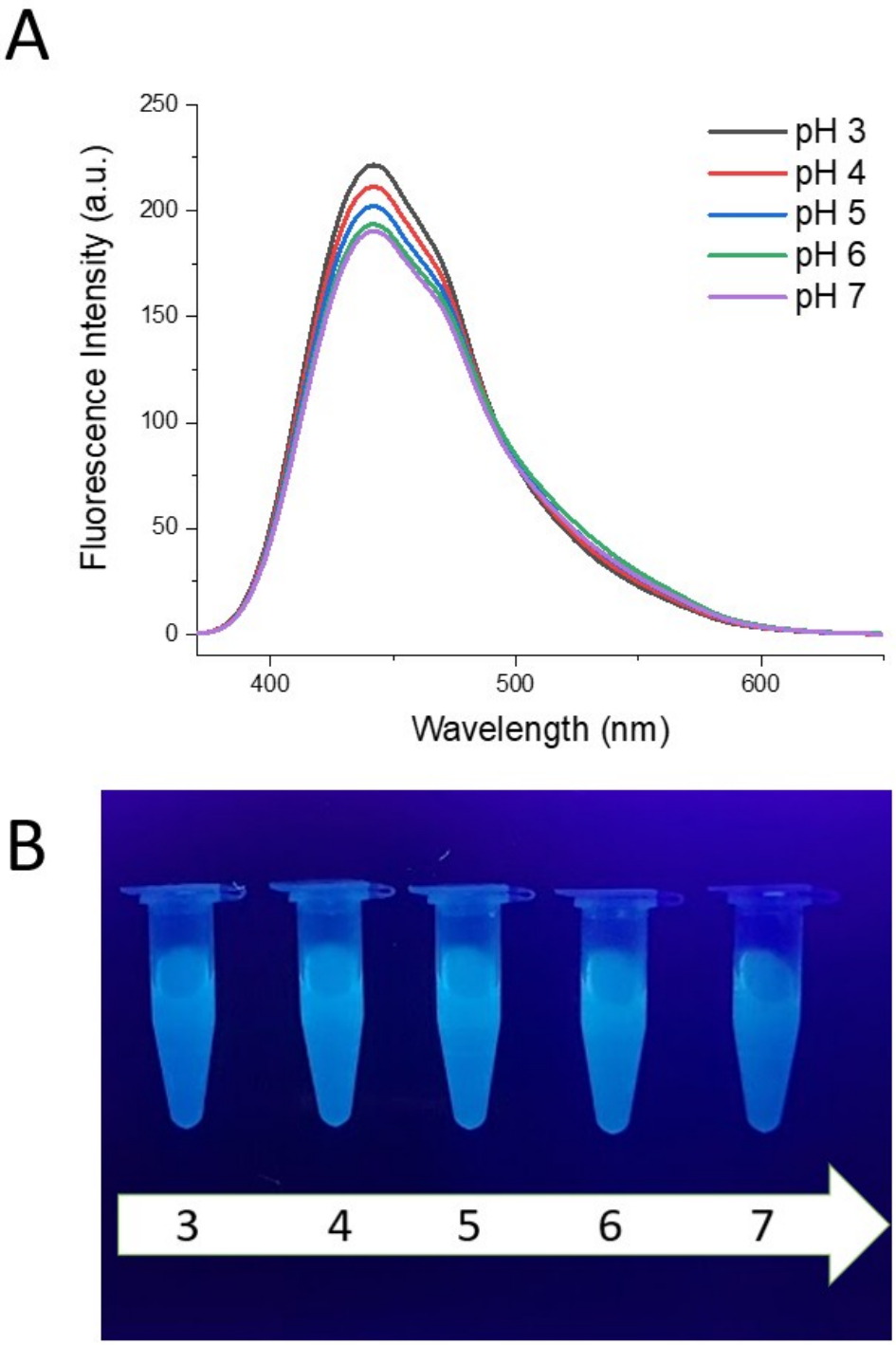


Figure S5. pH-dependent fluorescence property of **duP2**. (A) Emission spectra in different pH. Excitation wavelength is 420 nm. (B) Photographs shown are of 1 mM solutions of **duP2** in different pH under 365 nm irradiation.

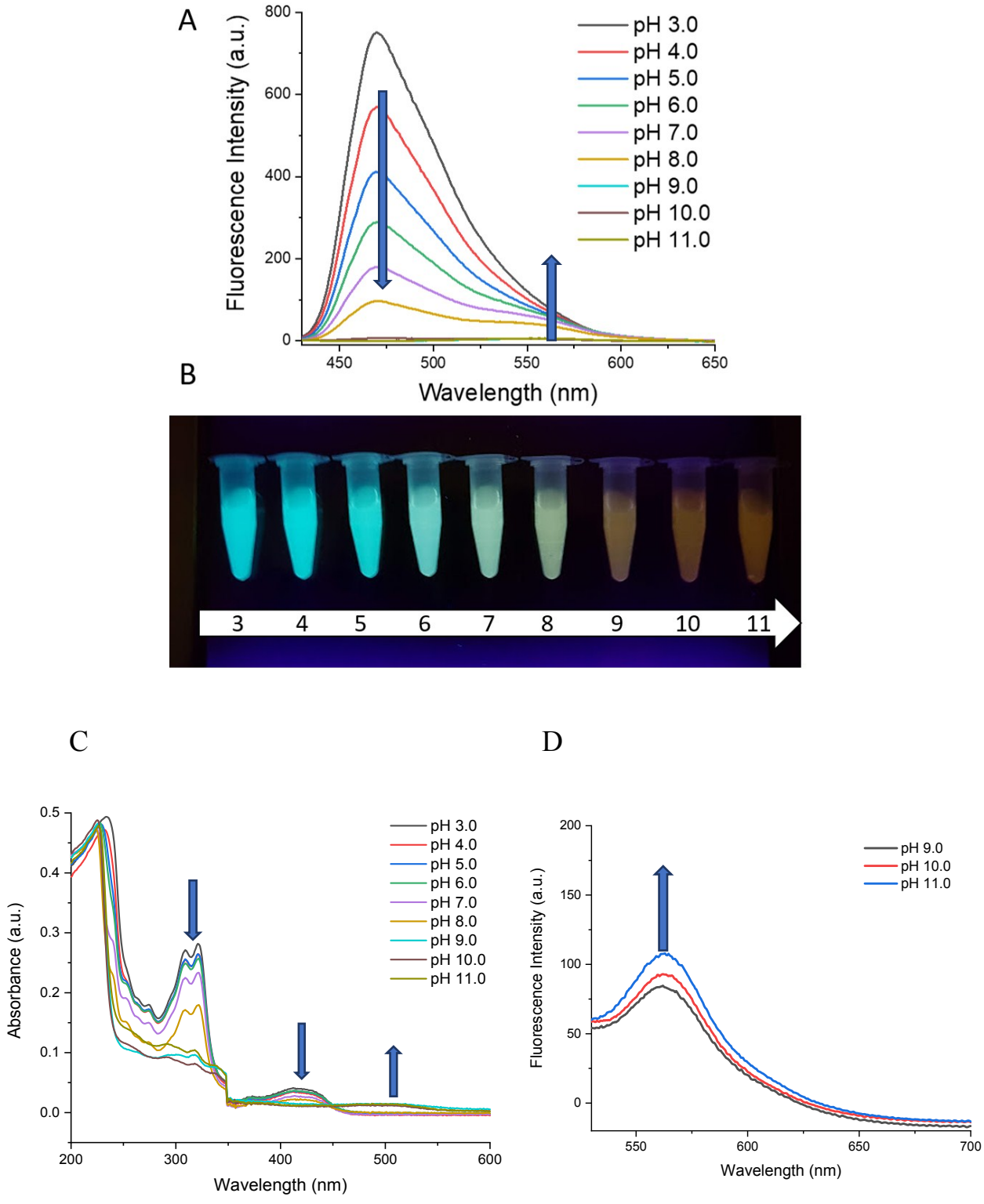


Figure S6. pH-Dependent fluorescence properties of **duP3**. (A) Emission spectra (excitation wavelength: 420 nm) and (B) photographs of 1 mM solutions of **duP3** irradiated at 365 nm. (C) Absorption spectra depends on the pH change. (D) Emission spectra (excitation wavelength:510nm) depends on the pH change.

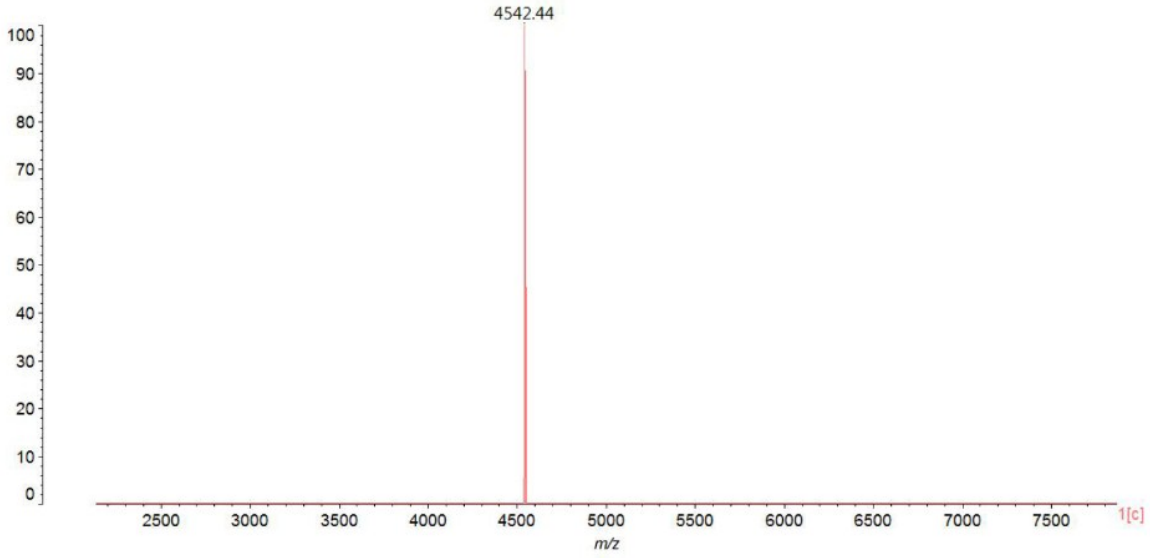


Figure S7. MALDI-TOF mass spectrum of **ODN1**. Calculated MW of **ODN1**: 4540.01; found: 4542.44.

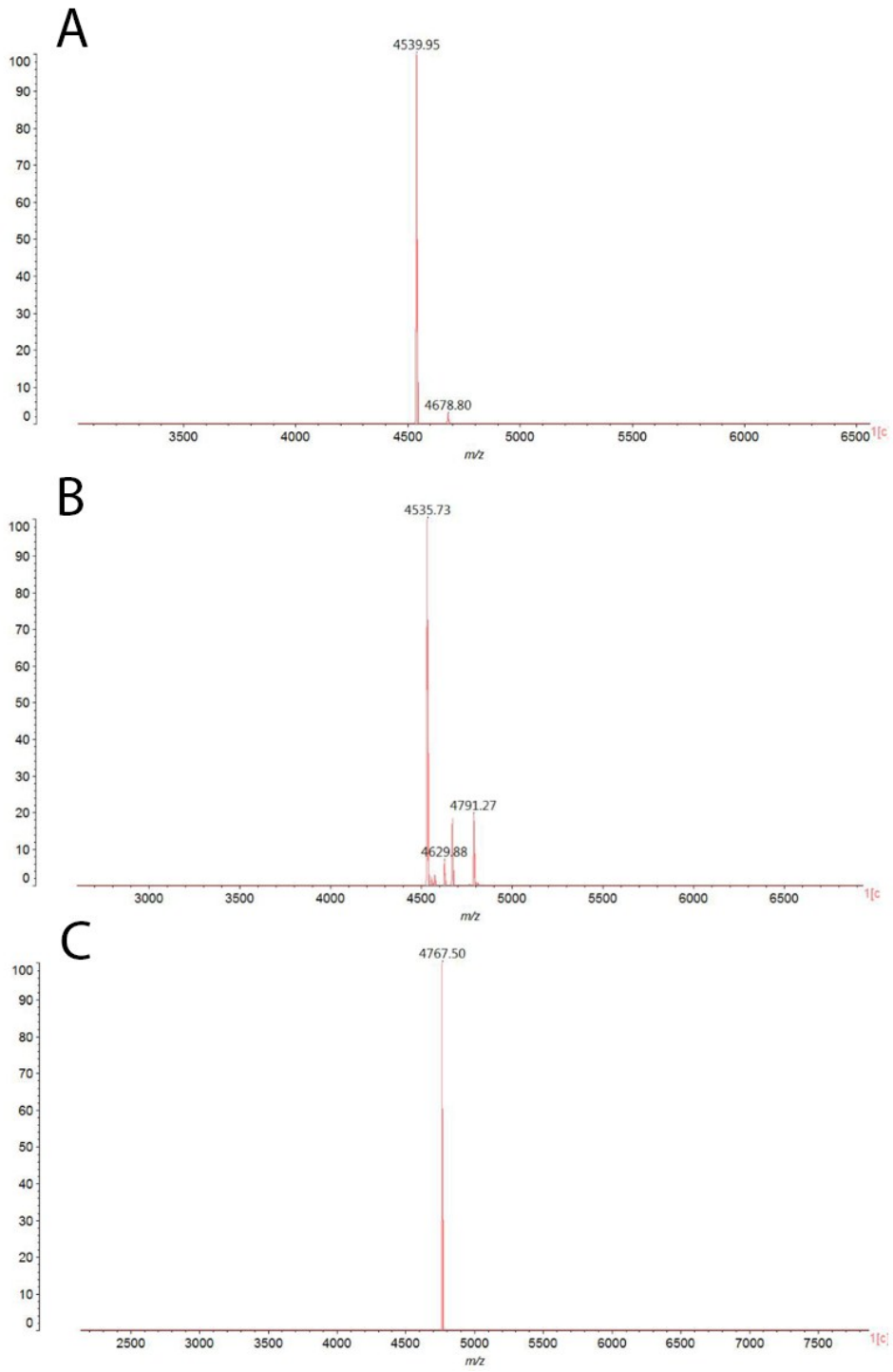


Figure S8. MALDI-TOF mass spectrum to confirm reaction between ODN1 with ylidenemalononitrile enamines compounds. (A) **ODN1** reacted with **Probe P1**: Calculated 4656.16 found 4678.80; (B) **ODN1** reacted with **Probe P2**: Calculated 4794.33 found 4791.27; (C) **ODN1** reacted with **Probe P3**: Calculated 4766.28 found 4767.50.

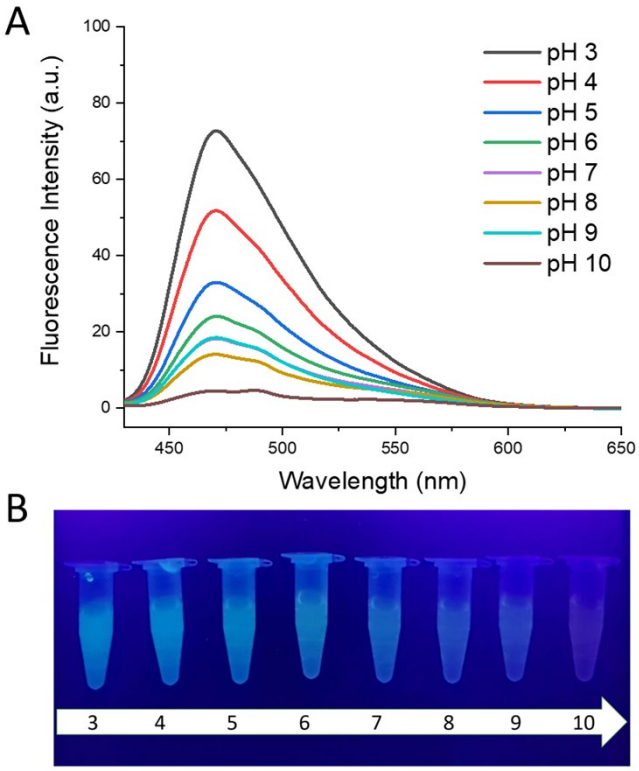


Figure S9. pH-dependent fluorescence properties of **Oligo P3**. (A) Emission spectra recorded at various values of pH (excitation wavelength: 420 nm). (B) Photographs of 0.1 mM solutions of **Oligo P3** at various values of pH.

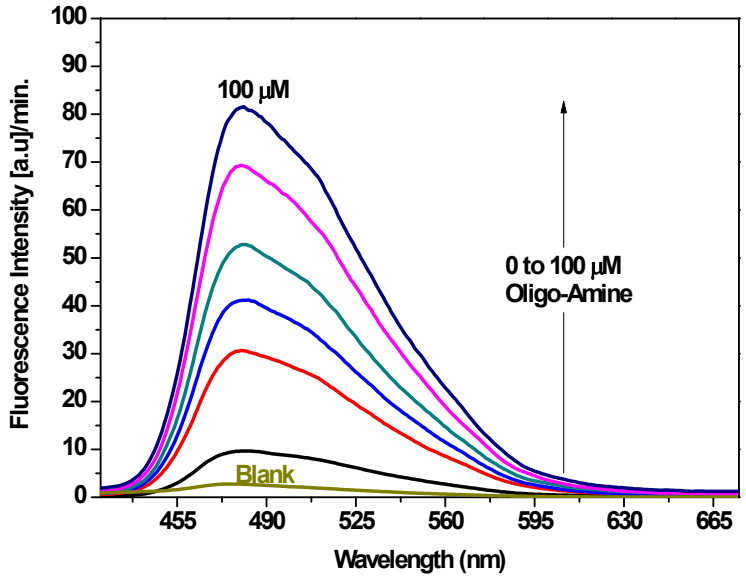


Figure. S10A. Fluorescence spectra of probe **3** (1 mM) with various concentration of amine modified oligonucleotides (0 – 100 µM) in PBS buffer (137 mM NaCl, 2.7 mM KCl, 10 mM Na_2HPO_4, 1.8 mM KH_2PO_4, pH 7.4).

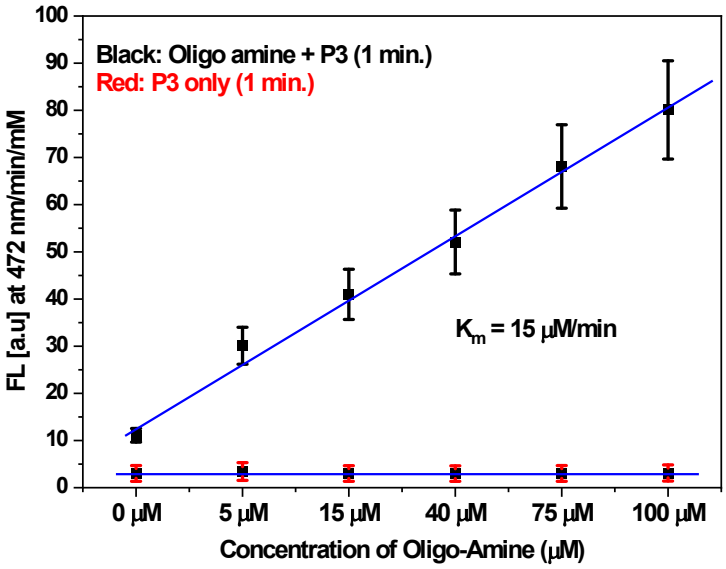


Figure. S10B. Fluorescence intensity plot of probe **3** (1mM) with various concentration of amine modified oligonucleotides (0 – 100 µM) in PBS buffer (137 mM NaCl, 2.7 mM KCl, 10 mM Na_2HPO_4, 1.8 mM KH_2PO_4, pH 7.4) upon excitation at λ_{ex} 420 and emission was recorded at λ_{em} 472 nm.

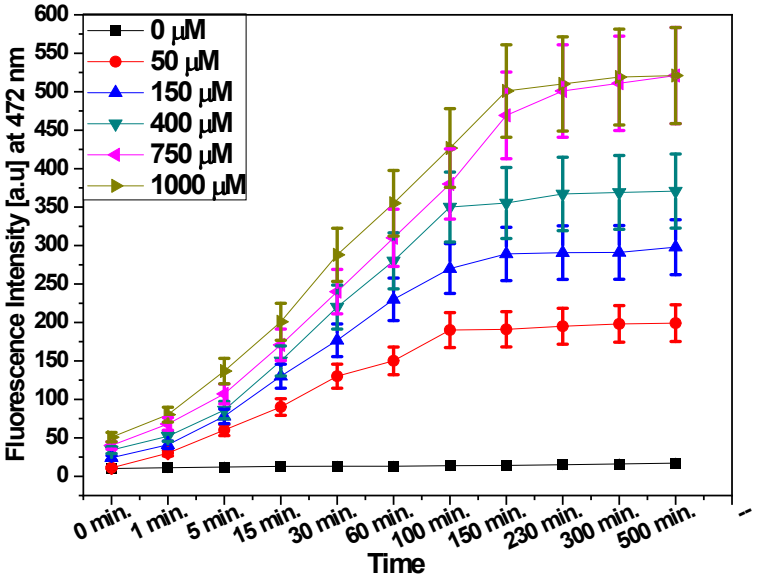


Figure S10C. Fluorescence intensity diagram of various concentration of probe **3** (0 – 1000 μM) with specified concentration of amine modified oligonucleotides (100 μM) in PBS buffer (137 mM NaCl, 2.7 mM KCl, 10 mM Na_2HPO_4, 1.8 mM KH_2PO_4, pH 7.4) upon excitation at λ_{ex} 420 and emission was recorded at λ_{em} 472 nm.

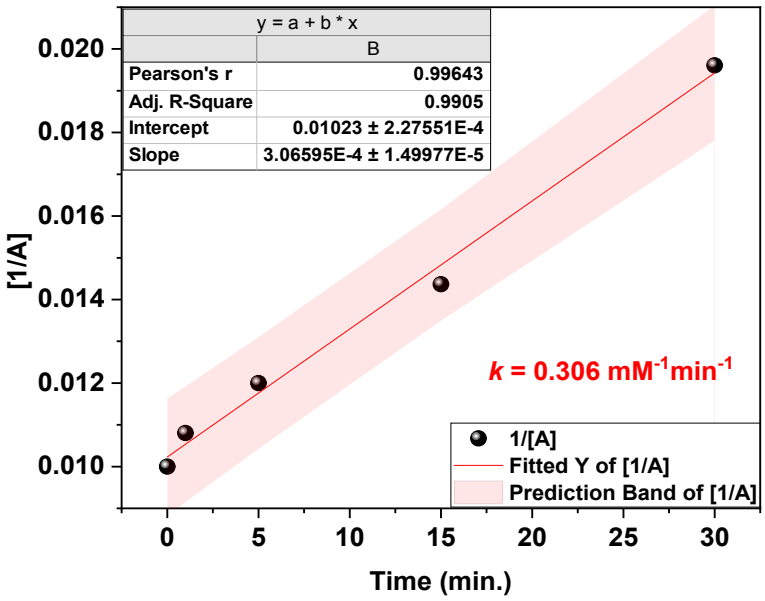


Figure S10D. Reaction Kinetics of probe **P3** (1 mM) and Amine modified Oligonucleotides (100 μM) in H_2O at pH 7.2 (PBS buffer). A is concentration of probe **P3** which is reacted with fixed concentration of Amine modified oligonucleotide upon prolonged time calculated based on emission intensity collected at 472 nm upon excitation at 420 nm.

Note: Recognition is based on fluorescence intensity so, Probe **P3** considered as conversion factor to identify the product/reactant consumption. Accordingly, amount of reactant was calculated and plotted versus time scale (min.) followed by linear data fitting furnished excellent Pearson's coefficient >0.99 with high precision. We followed below references for this assays.

References

1) Pollard TD, De La Cruz EM. Take advantage of time in your experiments: a guide to simple, informative kinetics assays. *Mol Biol Cell*. 2013;24(8):1103–1110. doi:10.1091/mbc.E13-01-0030

2) https://www.sciencedirect.com/topics/neuroscience/chemical-kinetics.

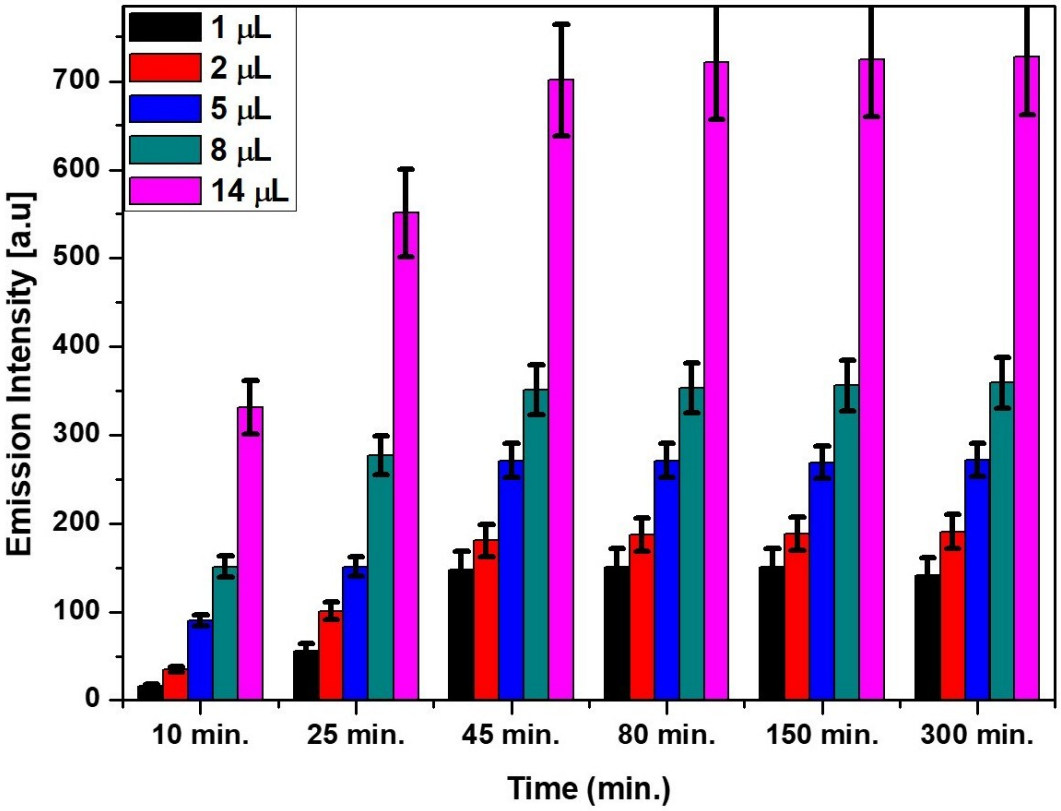


Figure S11. Concentration (according to volume/μL per plate of cells) and time dependent relative emission intensity histogram of **P3** (3 μM) in DMEM medium. Note: Each fluorescence confocal images were processed and calibrated with negative controls and emission intensities were collected from images using *Image-J* software to identify the amount of diffused transfected probe **3** in cytoplasm. Accordingly, least amount of **P3** were utilized to notify the background significant *turn-on* signal in cells. Note: Average intensities were notified from triplicates in green channel. Error range under 15.15±6.5% at green channels. Approximately 2000 cells per plate.

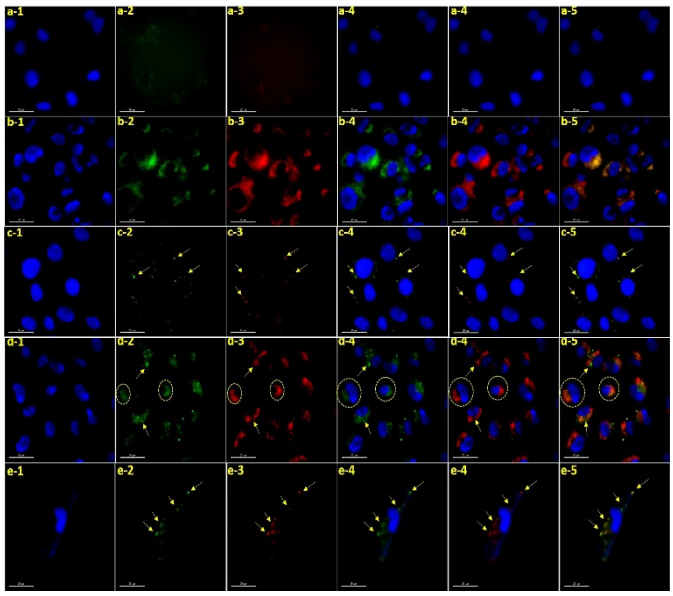


Figure S12. Fluorescence confocal laser live cell imaging studies of **P3** (5 μM) in fibroblast sarcoma-HT1080 cancer cell lines; a) Cells incubated only with **P3**, b) cells incubated with Propargyl amine (10 eq.), 60 min. followed by sequential addition of **P3** (5 μM) 60 min., c) Cells were incubated with propargylamino-presenting oligonucleotide with aid of Lipofectin (TM) (1:5 ratio), about 14 h followed by sequential addition of **P3** (3 μM) d) Cells were incubated with propargylamino-presenting oligonucleotide with aid of Lipofectin(TM) (1:5 ratio), about 14 h followed by sequential addition of increased amount of **P3** (15 μM). (validations of the background and diffused signals from the **P3** in cell cytosol); e) Cells were incubated with propargylamino-presenting oligonucleotide with aid of Lipofectin (TM) (1:5 ratio), about 14 h followed by sequential addition of **P3** (3 μM) in low cell density cell culture plate (cell counts approximately 800 to 1200 per plates). Note: Images were collected at 20 μm scale, and incubation temperature was 37^{o} C. Each image was notified with minimum 6 consecutive readings in various locations of each plate with ~2000 cells. Images numbered from 1-6 represented blue, green, red, blue-green (merge 50:50%), blue-red (merge 50:50%) and blue-green-red channels (merge 33:33:33%) respectively.

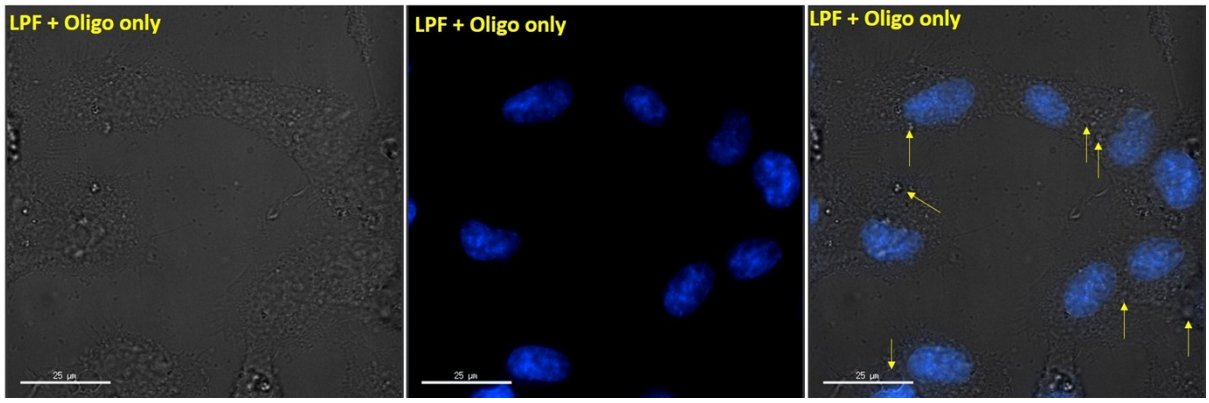


Figure. S13. Fluorescence confocal laser live cell imaging studies in fibroblast sarcoma-HT1080 cancer cell lines with various additives such as **P3** (15 μM) followed by sequential addition of propargyl amine (10.0 eq.), **P3**●Propargyl amine complex (**3** μM) followed by sequential addition of Lipofectin(TM). Transfection of propargyl amine presenting oligo (100 nM/μL) in the presence of Lipofectin(TM). First two images were merged images of blue-green, red-blue and blue-green-red channels. Third image was bright field and blue and merged images of blue-bright field images respectively to show the location of transfected propargylamine presenting oligonucleotide with lipofectamine.

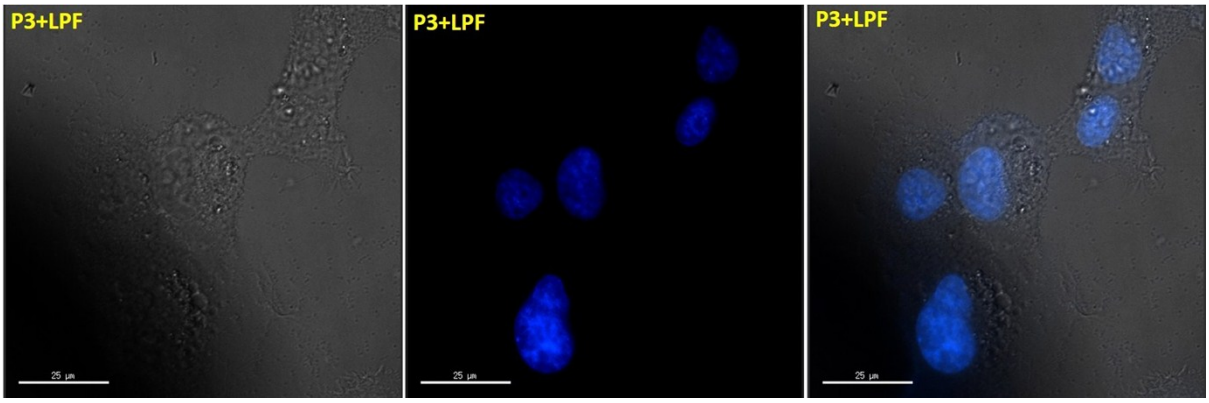


Figure. S14. Fluorescence confocal laser live cell imaging studies in fibroblast sarcoma-HT1080 cancer cell lines with Lipofectin(TM) followed by sequential addition of probe **P3** (3 μM). All other conditions are same. Image scale- 20 μm; Note: Based on time dependent studies, residual amines and Lipofectin(TM), were identified after 14 h which were subtracted in green and red channels.

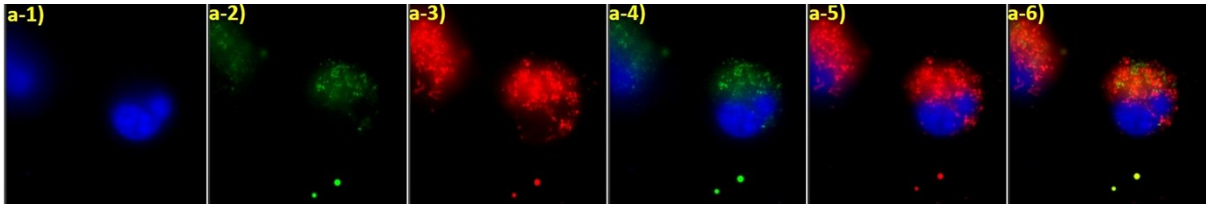


Figure. S15. Fluorescence confocal laser live cell imaging studies in fibroblast sarcoma-HT1080 cancer cell lines, **P3** with high concentration of propargylamine presenting oligonucleotide in the presence of Lipofectin(TM) (1:3 ratio) added in two batches. Images a-(1-6) signifies blue, green, red, merged image from, blue-green (50:50%), blue-red (50:50%) and blue-green-red (33:33:33%) channels respectively. Images were collected from 20 μm size file.

Experimental Section (live Cell Imaging)

Human fibrosarcoma HT1080 cells were obtained from the American Type Culture Collection (ATCC, Manassas, VA, USA). The HT1080 cells were cultured in Dulbecco's modified Eagle's medium (DMEM) and the HSG cells were cultured in Roswell Park Memorial Institute (RPMI 1640) medium containing 10% (v/v) fetal bovine serum (FBS) supplemented with 100 U/mL penicillin with 100 μg/mL and streptomycin followed by incubation at 37 °C in a humidified atmosphere containing 5% CO_2. Cells were seeded in 96-well plate and grew overnight followed by live cell imaging were performed with Hoechst 33342 as nuclear staining dye. Digital fluorescence images were collected at blue, green and red channels with appropriate excitation filters which were analyzed with confocal microscope Olympus software system. Based on calculations appropriate amount of probe **3 (P3)** solution were made in DMSO which were diluted in DMEM medium. Thus, obtained solutions were utilized for the bioimaging. High content imaging platform were used for concentration dependent 3 μM of (1, 2, 5, 8 and 14 μL/Per plate) and quantification emission intensity in cells. Thus, obtained images were processed for further image based statistical analysis.

All oligo transfections were performed with Lipofectin(TM) (Invitrogen USA), for over 14 h before staining with probe **3** with appropriate experimental conditions [1]. Transfection agent Lipofectin(TM):oligo ratios (1:1, 1:2 and 1:5) were performed accordingly 1:5 ratios were standardized for further experiments. In order to avoid the endogenous extracellular DNase activity sterile EDTA

(10 μM, 20 μL) was twice (each 7 h), $ZnSO_4$ (1 mM, 20 μL/plate) and methylchloroethylamine hydrochloride (1 mg/10 mL, 2 μL/plate) during transfection process [2]. Standard protocol was performed for cellular transfection of amine modified oligonucleotides (100 ng/μL). The data acquired from approximately 1500 cells per plate (Beckman Cell counter). Cells treated with only probe **3 (P3)** in various concentrations in specified time after transfection of only Lipofectin(TM) collected images were normalized, which were further used for quantification of intensity based on oligo concentrations.

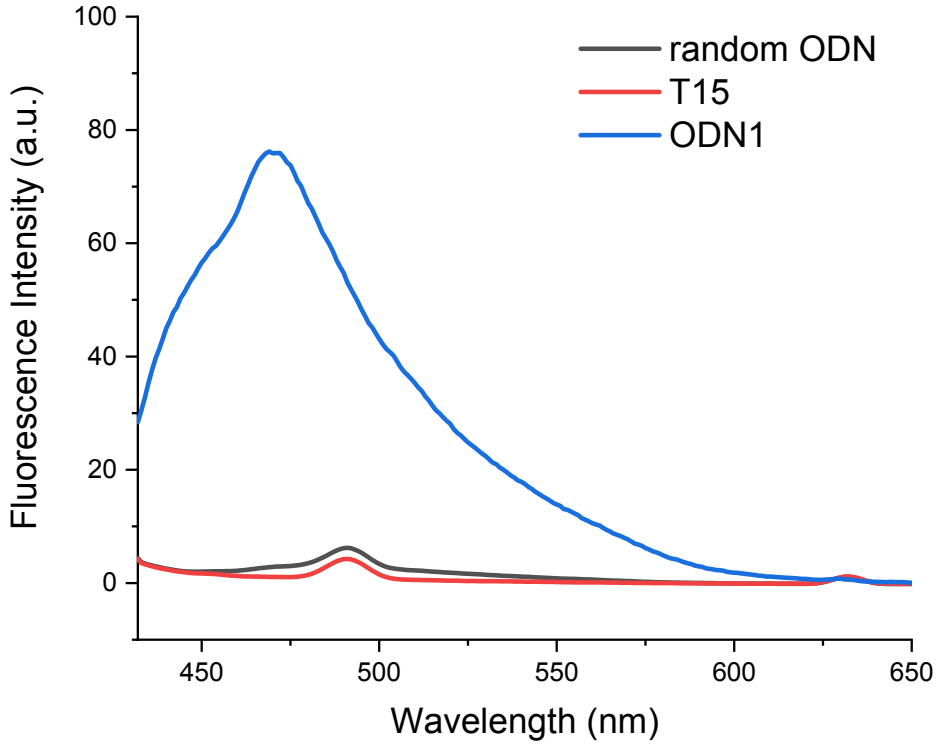


Figure S16 . Emission spectra of the products of the reactions of **ODN 1, T15** (5'-TTT TTT TTT TTT TTT-3') and **random ODN** (5'-GCT CCC TAT AGT GAG-3') separately (0.1 mM) in PBS buffer (137 mM NaCl, 2.7 mM KCl, 10 mM Na_2HPO_4, 1.8 mM KH_2PO_4, pH 7.4) with Probe **P3** (1 mM in MeCN). These reactions were stirred overnight. Excitation wavelength 420nm.

References

[1] Q. Zhai, C. Gao, J. Ding, Y. Zhang, B. Islam, W. Lan, H. Hou, H. Deng, J. Li, Z. Hu, H. I. Mohamed, S. Xu, C. Cao, S. M. Haider, D. Wei, Selective recognition of c-MYC Pu22 G-quadruplex by a fluorescent probe, Nucleic Acids Research, 2019, 47, 2190–2204. doi: 10.1093/nar/gkz059.

[2] G. B. Barra, T. H. S. Rita, J. de A. Vasques, C. F. Chianca, L. F. A. Nery, S. S. S. Costa, EDTA-mediated inhibition of DNases protects circulating cell-free DNA from ex vivo degradation in blood samples, Clinical Biochemistry 48 (2015) 976-981. http://dx.doi.org/10.1016/j.clinbiochem.2015.02.014; A. Kolarevic, D. Yancheva, G. Kocic, A. Smelcerovic, Deoxyribonuclease inhibitors, European Journal of Medicinal Chemistry 88 (2014) 101-111.