Supporting Information

Abasic site-binding ligands conjugated with cyanine dyes for "off-on" fluorescence sensing of orphan nucleobases in DNA duplexes and DNA-RNA hybrids

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Experimental

General Information

All of the DNA and RNA samples were custom synthesized and HPLC purified (>97 %) by Nihon Gene Research Laboratories Inc. (Sendai, Japan) and Sigma-Genosys (Cambridge, UK), respectively. The other reagents were commercially available analytical grade and were used without further purification. The concentration of DNA and RNA were determined from the molar extinction coefficient at 260 nm (ε_{260}) according to the previous report.[1]

Water was deionized (≥18.0 MΩ cm specific resistance) by an Elix 5 UV Water Purification System and a Milli-Q Synthesis A10 system (Millipore Corp., Bedford, MA). The other reagents were purchased from standard suppliers and used without further purification. ^{1}H NMR spectra were measured with a JEOL ECA-600 spectrometer at 500 MHz. High-resolution ESI-MS spectra were measured with a Bruker APEX III mass spectrometer.

Unless otherwise mentioned, all measurements were performed in 10 mM sodium cacodylate buffer solutions (pH 7.0) containing 100 mM NaCl, 1.0 mM EDTA, and ethanol (< 1.0%). Before measurements, DNA-containing samples were annealed as follows: heated at 75°C for 10 min, and gradually cooled to 5°C (3°C /min), after which the solution temperature was raised again to 20°C. The conjugate was then added to the annealed DNA-containing samples.

Fluorescence measurements Fluorescence spectra were measured with a JASCO model FP-6500 spectrofluorophotometer equipped with a thermoelectrically temperature-controlled cell holder (Japan Spectroscopic Co. Ltd., Tokyo, Japan) using a 3 × 3 mm quartz cell.

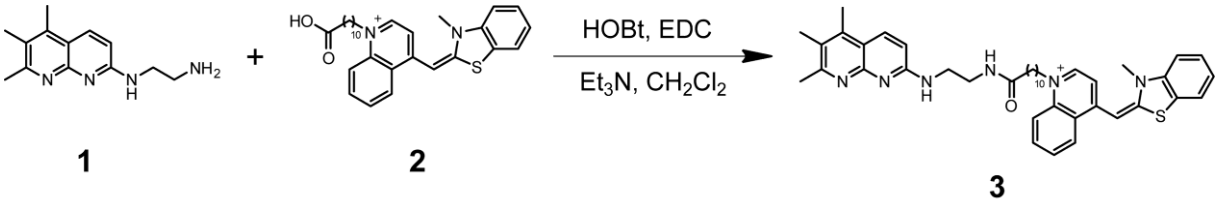
Fluorescence quantum yield (ϕ_{fl}) of TO moiety in **ATMND-TO** (100 nM) in the absence and presence of DNA duplexes (100 nM) was determined using Quantaurus-QY C11347-01 (Hamamatsu Photonics Co., Japan).

The binding affinity with the dissociation constant (K_d) of **ATMND-TO** was determined at 20°C by fluorescent titration experiments, in which the probe concentration was fixed at 100 nM, and the concentration of DNA duplex ranged from 0 to 500 nM. The changes in fluorescence intensity at 530 nm was analyzed by

nonlinear least-squares regression based on a 1:1 binding isotherm.[2]

CD measurements: CD spectra were measured with a JASCO model J-800 spectropolarimeter equipped with a thermoelectrically temperature-controlled cell holder (Japan Spectroscopic Co. Ltd., Tokyo, Japan) using a 2 × 10 mm quartz cell (optical path length: 10 mm).

Synthesis of ATMND-TO (3)



1 2 3

To a solution of aminoethyl group-modified ATMND (**1**; 160 mg, 0.69 mmol, ref. 13 in the main text) and carboxylate-terminated C10 spacer-having TO derivative (**2**; 310 mg, 0.65 mmol, ref. 14 in the main text) in 15 mL CH_2Cl_2 with 0.3 mL triethylamine at 0°C, 1-hydroxybenzotriazole (HOBt; 110 mg, 0.81 mmol) and N-ethyl-N'-(3-dimethylaminopropyl)-carbodiimide (EDC; 100 mg, 0.71 mmol) were added. After stirring for 1h at 0°C, the solution was stirred at room temperature for 24h. The solvents were evaporated and the residue was dissolved in CH_3Cl. The organic phase was washed successively with brine and water, then dried over Na_2SO_4. The crude product was purified by MPLC (CH_3Cl/MeOH), leading to **ATMND-TO** as a red powder (**3**; 53 mg, 0.077 mmol, 8.1%).

^{1}H NMR ($CDCl_3$) : δ (ppm) = 9.17 (d, 1H, *J* = 7.5 Hz), 8.46 (d, 1H, *J* = 8.5 Hz), 8.33 (m, 1H), 7.85 (t, 1H, *J* = 8.5 Hz), 7.79 (t, 2H, *J* = 7.8 Hz), 7.73 (d, 1H, *J* = 9.0 Hz), 7.67 (t, 1H, *J* = 7.0 Hz), 7.49 (d, 2H, *J* = 7.5 Hz), 7.34-7.26 (m, 2H), 7.01 (d, 1H, *J* = 9.0 Hz), 6.64 (s, 1H), 4.71 (t, 2H, *J* = 7.3 Hz), 3.21 (t, 2H), 2.63 (s, 3H), 2.37 (s, 3H), 2.32 (t, 2H), 2.24 (s, 3H), 1.94 (m, 2H), 1.65 (m, 2H), 1.38 (m, 2H), 1.20 (m, 14H)

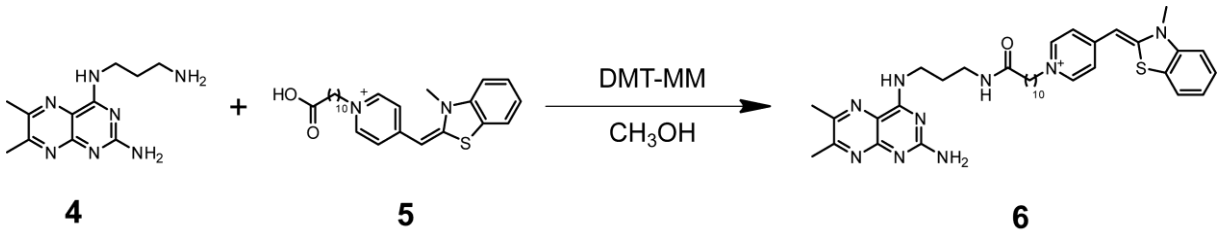
HRMS (ESI) calcd for $C_{42}H_{51}N_6OS^+$ 687.3840, found 687.3840.

Synthesis of ATMND-TO analogues having different spacer lengths

These conjugates were prepared according to the same scheme as synthesized for **ATMND-TO**, in which carboxylate-terminated C7, C8, C9 or C11 spacer-having TO derivatives were utilized instead of C10 spacer-having derivative.

HRMS (ESI) for Cn spacer-containing analogue: n = 7, calcd for $C_{41}H_{49}N_6OS^+$ 645.3370, found 645.3370; n = 8, calcd for $C_{40}H_{47}N_6OS^+$ 659.3527, found.659.3528; n = 9, calcd for $C_{41}H_{49}N_6OS^+$ 673.3683, found.673.3684; n = 11, calcd for $C_{43}H_{53}N_6OS^+$ 701.3996, found. 701.3998.

Synthesis of DMP-BO (6)



4 **5** **6**

To a solution of 2-amino-4-(3-aminopropyl)amino-6,7-dimethylpteridine[3] (**4**; 108 mg, 0.44 mmol) and carboxylate-terminated C10 spacer-having BO derivative (**2**; 224 mg, 0.44 mmol, ref. 14 in the main text) in 15 mL methanol with 4-(4,6-dimethoxy-1,3,5-triazin-2yl)-4-methylmorpholinium chloride (DMT-MM; 137 mg, 0.49 mmol)4 were added. After stirring at room temperature for 24h, the solvents were evaporated and the obtained residues were purified by MPLC (CH_3Cl/MeOH), leading to **DMP-BO** as a yellow powder (**6**; 33 mg, 0.044 mmol, 10%).

HRMS (ESI) calcd for $C_{36}H_{48}N_9OS^+$ 654.3697, found 654.3698.

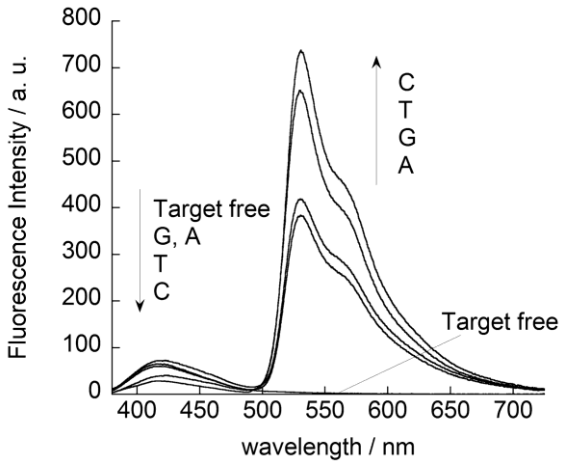


Figure S1. Fluorescence spectra of **ATMND-TO** (100 nM) in the absence (Target free) and presence of 21-mer AP site-containing DNA duplexes (500 nM; 5'-ATT TGG GTG A**X**A TTG CTC ACA-3'/3'-TAA ACC CAC T**N**T AAC GAG TGT-5', **X** = AP site (dSpacer) , **N** = G, C, A, or (T). Solution conditions were the same as those shown in Figure 2A in the main text. Excitation: 370 nm (the maximum absorption wavelength of the ATMND moiety). Temperature, 20°C.

Fluorescence quenching of the ATMND moiety (380-480 nm) is more significant for target C than that for other target nucleobases, which indicates the binding selectivity of ATMND-TO for the target C. On the other hand, fluorescence emissive response of the TO moiety is also observed upon excitation of the ATMND moiety, which is probably due to Förster resonance energy transfer (FRET) from the ATMND moiety to the TO moiety of ATMND-TO that binds to the AP site-containing DNA duplexes. However, this simultaneous excitation results in the decreased selectivity of TO fluorescence for the target C over other target nucleobases, as is seen from the comparison between Figure S1 and Figure 2A in the main text. This reduced selectivity results from large emission from the TO moiety for target G and A, because the ATMND moiety shows large emission forG and A than C and T in the FRET system.

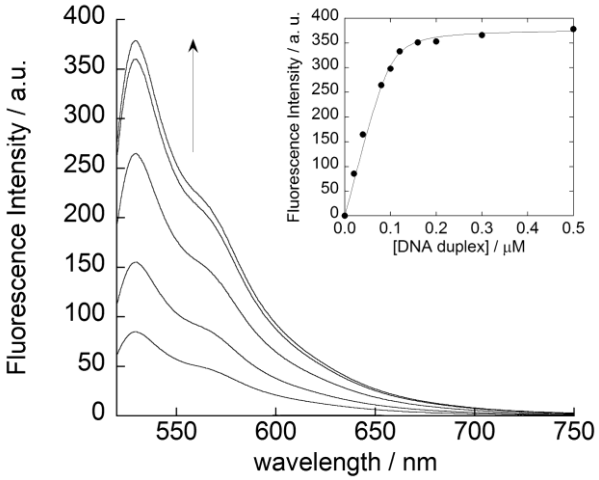


Figure S2. Fluorescence response of **ATMND-TO** (100 nM) to the target C in 21-mer AP site-containing DNA duplexes (5'-ATT TGG GTG A**X**A TTG CTC ACA-3'/3'-TAA ACC CAC T**C**T AAC GAG TGT-5', **X** = AP site (dSpacer)). Inset: Nonlinear regression analysis of the changes in the fluorescence intensity at 530 nm based on a 1:1 binding isotherm model. Other solution conditions were the same as those given in Figure 2A in the main text. Excitation: 506 nm. Temperature, 20°C.

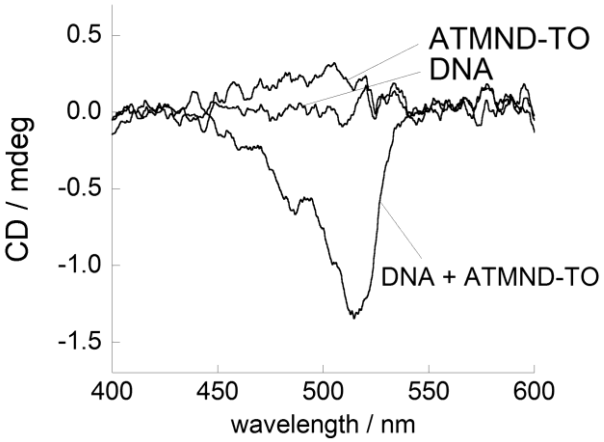


Figure S3. Changes in CD spectra of **ATMND-TO** (5.0 μM) upon binding to the target C in the 21-mer DNA duplex (5.0 μM; 5'-ATT TGG GTG A**X**A TTG CTC ACA-3'/3'-TAA ACC CAC T**C**T AAC GAG TGT-5'). For the comparison, CD spectra of **ATMND-TO** and DNA duplex were also shown. Other solution conditions were the same as those in Figure 2A in the main text. Temperature: 20°C.

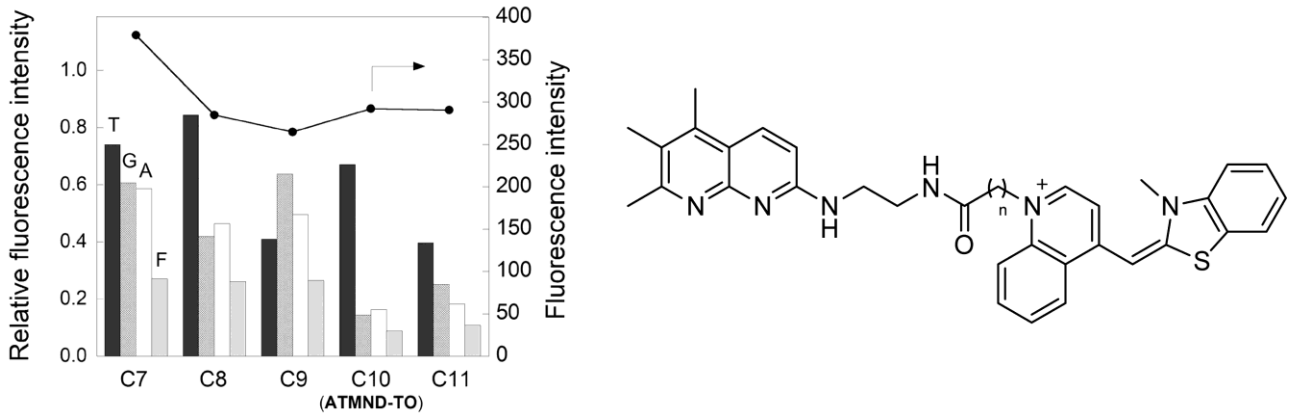


Figure S4. Effect of the spacer length in **ATMND-TO** conjugates on the fluorescence response of the conjugate for the target nucleobases in the AP site-containing DNA duplexes and the fully-matched DNA duplex. Conjugate analogue containing different spacers (n = 7, 8, 9, and 11) corresponds to C7, C8, C9 and C11, respectively. Right ordinate: Fluorescence intensity of the conjugates in the presence of the DNA duplex containing target C. Left ordinate: Relative fluorescence response to DNA duplexes containing target G, A, T and the fully-matched DNA duplex (F). Measurement conditions are the same as given those in Fig. 2A in the main text.

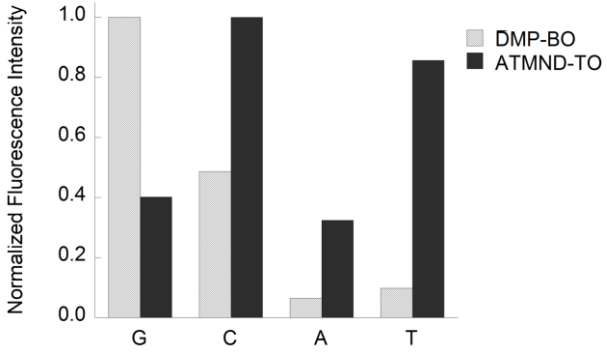


Figure S5. Multiplex analysis of the target nucleobases in the 21-mer AP site-containing DNA duplexes (100 nM; 5'-ATT TGG GTG A**X**A TTG CTC ACA-3'/3'-TAA ACC CAC T**N**T AAC GAG TGT-5', **X** = AP site (dSpacer) , **N** = target nucleobase) by the simultaneous use of **DMP-BO** (100 nM) and **ATMND-TO** (100 nM) in a single solutions. Other solution conditions are the same as those given in Figure 2A in the main text. **DMP-BO**: Excitation, 448.5 nm; Analysis, 478 nm. **ATMND-TO**: Excitation, 506 nm; Analysis, 530 nm. Temperature: 20°C.

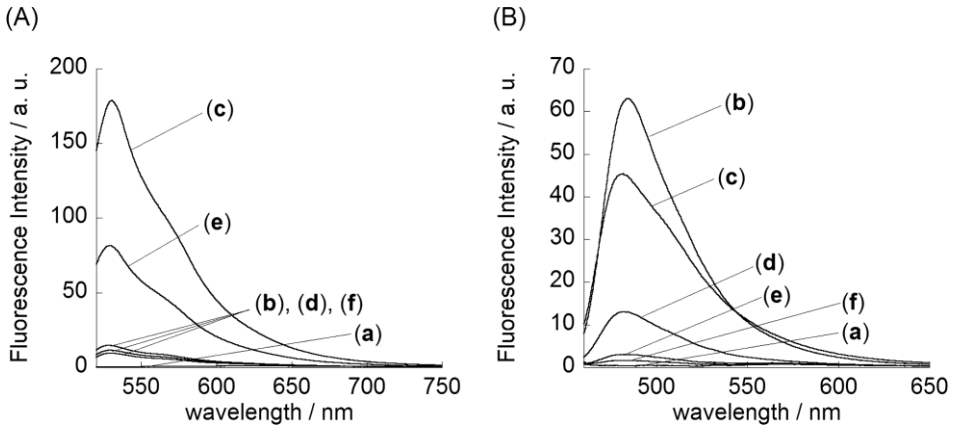


Figure S6. Fluorescence response of (A) **ATMND-TO** and (B) **DMP-BO** to the target nucleobase in the AP site-containing DNA/RNA hybrids (5'-d(ATT TGG GTG A**X**A TTG CTC ACA)-3'/3'-r(UAA ACC CAC U**N**U AAC GAG UGU)-5', **X** = AP site (dSpacer) , **N** = target nucleobase). (a) hybrid free, N = (b) G, (c) C, (d) A, or (e) U, (f) the fully-matched hybrid. Measurement conditions are the same as those given in (A) Figure 2A and (B) Figure 3, respectively.

References

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Complete reference 14 in the main text

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