Electronic Supplementary Material (ESI) for Chemical Communications

Environmentally Sensitive Fluorescent Purine Nucleoside That Changes Emission Wavelength upon Hybridization

Yoshio Saito,*[a] Azusa Suzuki[a] Yuji Okada[a] Yuki Yamasaka[a] Nobukatsu Nemoto[a] Isao Saito,*[b]

[a]Department of Chemical Biology and Applied Chemistry, School of Engineering, Nihon University, Koriyama, 963-8642, Japan, [b]NEWCAT Institute, School of Engineering, Nihon University, Koriyama, 963-8642, Japan

Email Address: saitoy@chem.ce.nihon-u.ac.jp

List of Contents

Experimental

General: ^{1}H-NMR spectra (400 MHz) and ^{13}C-NMR spectra (100 MHz) were measured with Bruker Avance 400F spectrometer. Coupling constant (*J* value) are reported in hertz. The chemical shifts are shown in ppm downfield from tetramethylsilane, using residual dimethyl sulfoxaide (δ 2.50 in ^{1}H-NMR, δ 39.5 in ^{13}C-NMR) and chloroform (δ 7.26 in ^{1}H-NMR, δ 77.0 in ^{13}C-NMR) as an internal standard. ESI-TOF masses were recorded on a JMS-T100LC "AccuTOF", Applied DATUM Solution Business Operations.

Oligonucleotides synthesis and characterization: The reagents for DNA synthesis were purchased from Glen Research. Mass spectra of oligodeoxynucleotides were determined with a MALDI-TOF MS (Shimadzu AXIMA-LNR, positive mode) with 2',3',4'-trihydroxyacetophenone as a matrix. Calf intestinal alkaline phosphatase (Promega), Crotalus adamanteus venom phosphodiesterase I (USB), and *Penicillium citrinum nuclease* P1 (Roche) were used for the enzymatic digestion of ODNs. All aqueous solutions utilized purified water (Millipore, Milli-Q sp UF). Reversed-phase HPLC was performed on CHEMCOBOND 5-ODS-H columms (10 × 150 mm, 4.6 × 150 mm) with a JASCO Chromatograph, Model PU-2080, using a UV detector, Model UV-2075 plus at 260 nm.

Procedure for the synthesis of modified nucleosides

6-Amino-1-(2-deoxy-*β*-D-erythro-pentofuranosyl)-1,5-dihydro-3-(2-naphthylethynyl)-4*H*-pyrazolo[3,4-*d*]pyrimidin-4-one (1) A mixture of 7-iodo-8-aza-7-deaza-2'-deoxyguanosine **2** (120 mg, 0.31 mmol), $Pd(PPh_3)_4$ (18.0 mg, 0.02 mmol), CuI (6.0 mg, 0.03 mmol) and 2-ethynylnaphthalene (60.0 mg, 0.39 mmol) in DMF (5 ml) and Et_3N (0.1 ml) was stirred at 50 ^{o}C at under argon atmosphere for 30 min. After allowing to cool to room temperature, The reaction solution was concentrated in *vacuo*. The residue was purified by silica gel column chromatography with chloroform-methanol mixture (12 : 1 to 8 : 1) to give **1** as a colorless solid (87.2 mg, 68 %); ^{1}H-NMR (DMSO-d_6, 400 MHz) δ 2.20 (ddd, *J* = 4.1, 6.6, 13.2 Hz, 1H), 2.72 (m, 1H), 3.40 (m, 1H), 3.51 (m, 1H), 3.79 (m, 1H), 4.40 (m, 1H), 4.77 (m, 1H), 5.27 (d, *J* = 4.3 Hz, 1H), 6.34 (m, 1H), 6.80 (br, 2H), 7.59-7.63 (complex, 3H), 7.97-8.01 (complex, 3H), 8.23 (m, 1H), 10.84 (s, 1H); ^{13}C-NMR (DMSO-d_6, 100 MHz) δ 37.7, 62.4, 70.9, 82.2, 83.3, 87.6, 91.9, 100.4, 118.9, 127.0, 127.4, 127.8, 127.8, 127.9, 128.5, 129.7, 131.6, 132.5, 132.7, 155.4, 155.7, 157.1; HRMS (ESI) *m/z* 440.1335 calcd for $C_{22}H_{19}N_5O_4Na$ $[M + Na]^+$, found 440.1362.

6-[[(Dimethylamino)methylidene]amino]-1-(2-deoxy-*β*-D-erythro-pentofuranosyl)-1,5-dihydro-3-(2-naphthylethynyl)-4*H*-pyrazolo[3,4-*d*]pyrimidin-4-one (3) A solution of **1** (75.0 mg, 0.18 mmol) in DMF (5 ml) was stirred with *N*,*N*-dimethylformamide diethyl acetal (45 μl, 0.27 mmol) at 50 ^{o}C for 30 min. After completion of the reaction, the solvent was evaporated, and the residue was purified by silica gel column chromatography with chloroform-methanol mixture (9 : 1) to give compound **3** as a colorless solid (43.4 mg, 51 %); ^{1}H-NMR (DMSO-d_6, 400 MHz) δ 2.20 (ddd, *J* = 4.0, 6.7, 13.2 Hz, 1H), 2.75 (m, 1H), 3.08 (s, 3H), 3.21 (s, 3H), 3.42 (m, 1H), 3.53 (m, 1H), 3.82 (m, 1H), 4.44 (m, 1H), 4.78 (m, 1H), 5.30 (d, *J* = 4.2 Hz, 1H), 6.51 (m, 1H), 7.58-7.64 (complex, 3H), 7.97-8.01 (complex, 3H), 8.24 (m, 1H), 8.75 (s, 1H), 11.45 (s, 1H); ^{13}C-NMR (DMSO-d_6, 100

MHz) δ 34.9, 37.9, 40.8, 62.4, 71.0, 82.2, 83.4, 87.7, 92.0, 102.8, 118.9, 127.0, 127.4, 127.8, 127.8, 127.9, 128.5, 129.6, 131.6, 132.5, 132.7, 154.8, 157.9, 158.7, 159.6; HRMS (ESI) *m/z* 495.1757 calcd for $C_{25}H_{24}N_6O_4Na$ [M + Na]$^+$, found 495.1786.

6-[[(Dimethylamino)methylidene]amino]-1-[2-deoxy-5-*O*-(4,4'-dimethoxytriphenylmethyl)-*β*-D-erythro-pentofuranosyl]-1,5-dihydro-3-(2-naphthylethynyl)-4*H*-pyrazolo[3,4-*d*]pyrimidin-4-one (4) To a solution of **3** (36.0 mg, 0.08 mmol) in anhydrous pyridine (3 ml) was added 4,4'-dimethoxytrityl chloride (39.0 mg, 0.11 mmol), and the stirring continued at room temperature for 1 h. After completion of the reaction, the solvent was evaporated, and the residue was diluted with ethyl acetate, washed with saturated $NaHCO_3$ and brine. The combined organic layer was dried with anhydrous Na_2SO_4, filtered and concentrated. The purification by silica gel column chromatography with $CHCl_3$-MeOH-Et_3N mixture (30 : 1 : 0.5) yields compound **4** as a colorless solid (42.8 mg, 72 %); ^{1}H-NMR ($CDCl_3$, 400 MHz) δ 2.37 (m, 1H), 3.02-3.09 (complex, 4H), 3.16 (s, 3H), 3.31 (m, 1H), 3.38 (m, 1H), 3.70 (s, 6H), 4.05 (m, 1H), 4.83 (m, 1H), 6.63 (m, 1H), 6.78 (m, 4H), 7.16-7.33 (complex, 7H), 7.42 (m, 2H), 7.47-7.52 (complex, 2H), 7.67 (m, 1H), 7.79-7.81 (complex, 3H), 8.17 (m, 1H), 8.70 (s, 1H), 8.72 (s, 1H); ^{13}C-NMR ($CDCl_3$, 100 MHz) δ 35.2, 38.0, 41.5, 55.1 (×2), 64.5, 73.4, 81.5, 83.5, 85.6, 86.4, 93.3, 103.7, 113.1 (×4), 119.9, 126.5, 126.8, 126.8, 127.8, 127.8 (×2), 127.9, 128.0, 128.2 (×2), 128.6, 129.1, 130.0 (×2), 130.1 (×2), 132.4, 132.9, 133.1, 136.0, 136.0, 144.8, 155.2, 158.0, 158.4, 158.4, 158.6, 158.7; HRMS (ESI) *m/z* 797.3064 calcd for $C_{46}H_{42}N_6O_6Na$ [M + Na]$^+$, found 797.3043.

6-[[(Dimethylamino)methylidene]amino]-1-[2-deoxy-5-*O*-(4,4'-dimethoxytriphenylmethyl)-*β*-D-erythro-pentofuranosyl]-1,5-dihydro-3-(2-naphthylethynyl)-4*H*-pyrazolo[3,4-*d*]pyrimidin-4-one-3'-[(2-cyanoethyl)-*N*,*N*-(diisopropyl)]phosphoramidite (5) To a solution of **4** (60.0 mg, 0.08 mmol) in anhydrous acetonitrile (1 ml) was added 2-cyanoethyldiisopropyl- chlorophosphoramidite (70 μl) in the presence of Et_3N (1 ml), and stirred at room temperature under an argon atmosphere for 1 h. After completion of the reaction, the solution was diluted with ethyl acetate, washed with saturated $NaHCO_3$ and brine. The combined organic layer was dried with anhydrous Na_2SO_4, filtered and concentrated. The residue was incorporated into oligonucleotides without further purification.

Fluorescence spectra: The fluorescence spectra ware obtained with a Shimadzu RF-5300PC spectrofluorophotometer at 25 ^{o}C using a cell with a 1 cm path length. The excitation and emission bandwidths were 1.5 nm. The fluorescence quantum yields (Φ_{fl}) were determined using 9,10-diphenylanthracene as a reference with the known Φ_{fl} (0.95) in ethanol.[1]

UV absorption measurements: Absorption spectra were obtained using a Shimadzu UV-2550 spectrophotometer at room temperature using 1 cm length cell.

Melting temperature (T_m) measurements: All T_ms of the ODNs (2.5 μM, final concentration) were measured in 50 mM sodium phosphate buffers (pH 7.0) containing 100 mM sodium chloride. Absorbance *vs* temperature profiles ware measured at 260 nm using a Shimadzu UV-2550 spectrophotometer equipped with a Peltier temperature controller using 1 cm path length cell. The absorbance of the samples was monitored at 260 nm from 4 to 90 ^{o}C with a heating rate of 1 ^{o}C/min. From these profiles, first derivatives were calculated to determine T_m values.

Circular dichroism (CD) measurements: CD spectra were recorded with a JASCO J-805 CD spectrophotometer. CD spectra of oligonucleotides solutions (2.5 μM ODNs in 50 mM sodium phosphate buffers (pH 7.0) containing 100 mM sodium chloride at 20 ^{o}C) were measured using 2 mm path length cell.

Reference

1. J. V. Morris, M. A. Mahaney, J. R. Huber, J. Phys. Chem., 1976, 80, 969-974.

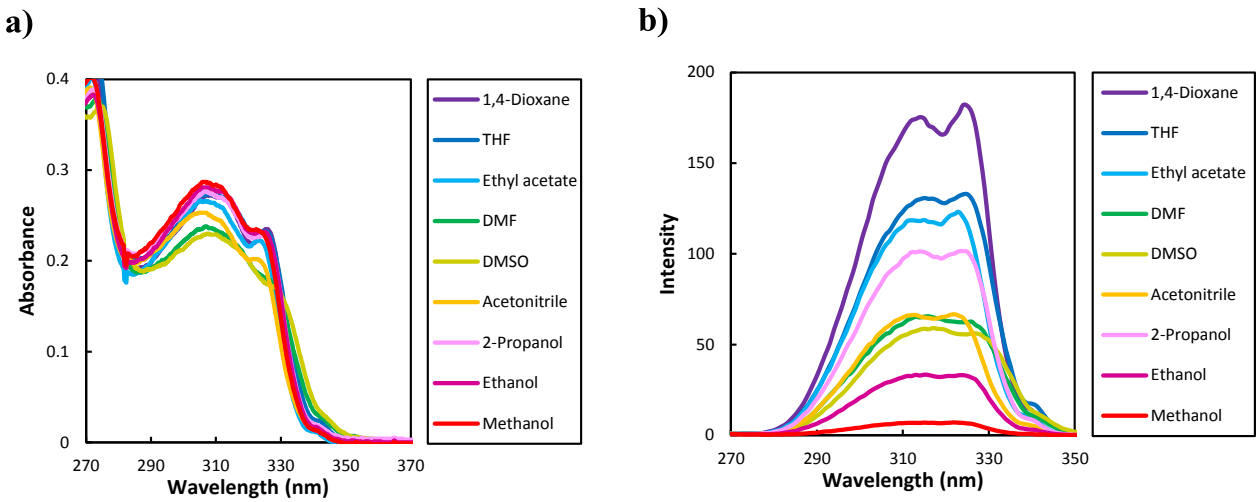


Fig. S1 UV absorption (a) and excitation (b) spectra of ^{na}**G** (**1**) (10 μM) in various solvents.[a]

[a] Solvent is used in the presence of 1 % v/v of DMF because of its low solubility.

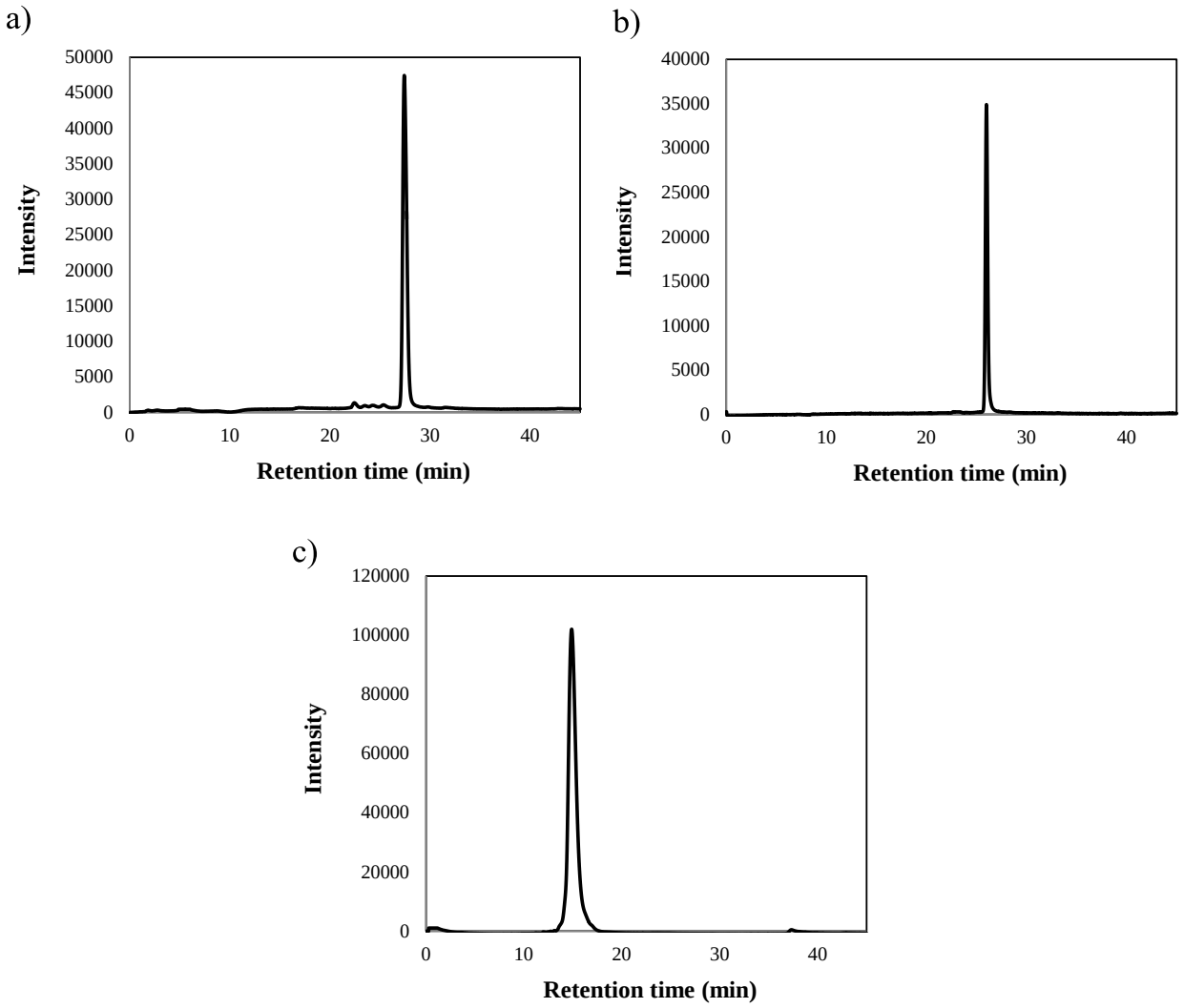
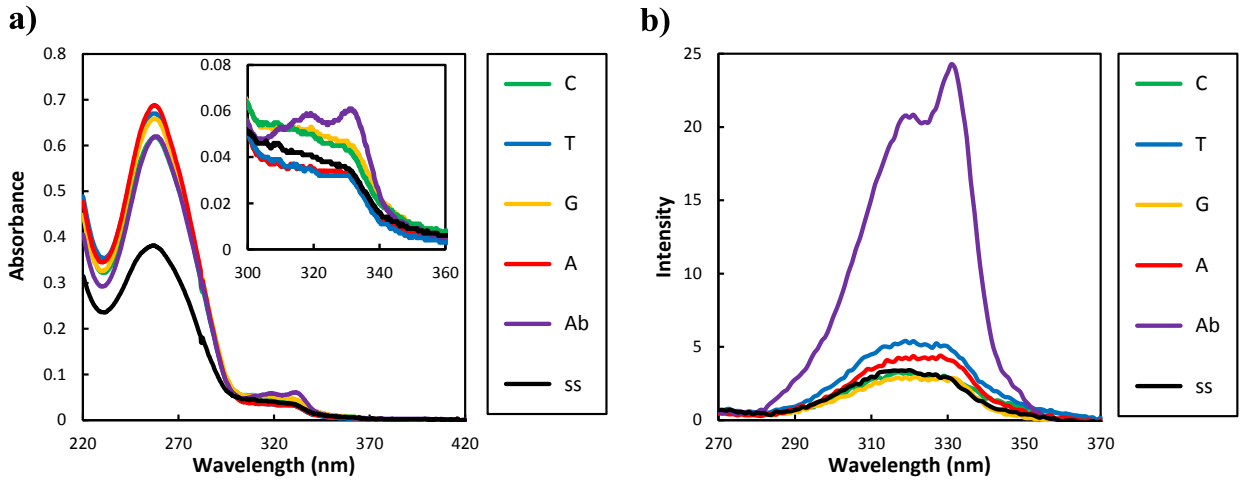


Fig. S2 HPLC profiles of single-stranded (a) ODN 1: 5'-CGCAAT^{na}**G**TAACGC-3', (b) ODN 3: 5'-CGCAAT^{na}**G**AAACGC-3', and (c) MB 1: 5'-CCACATGTGAAGGGCTTTT^{na}**G**AACTCTGCATGTGG-3'. HPLC analysis was carried out on a CHEMCOBOND 5-ODS-H columm (10 × 150 mm) eluted with 0.05 M ammonium formate buffer containing acetonitrlie. Gradient: from 3 to 20 % acetonitrile for (a) and (b), from 3 to 40 % acetonitrile for (c) at a flow rate 2.0 ml/min over 45 min.

Table S1 MALDI-TOF mass spectral data for the ODNs

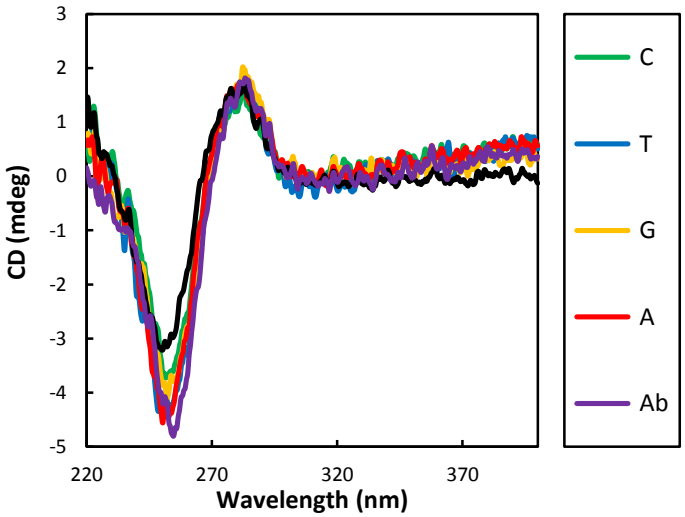
ODNs	MALDI-TOF mass	
	calcd. $[M + H]^+$	found $[M + H]^+$
ODN 1: 5'-CGCAAT^{na}**G**TAACGC-3'	4093.83	4094.09
ODN 3: 5'-CGCAAT^{na}**G**AAACGC-3'	4102.84	4102.60
MB 1: 5'-CCACATGTGAAGGGCTTTT^{na}**G**AACTCTGCATGTGG-3'	10639.08	10640.58



ODN 1: 5'-CGCAAT ^{na}**G** TAACGC-3'

ODN 2: 3'-GCGTTA **N** ATTGCG-5' (**N** = C, T, G, A, Abasic site)

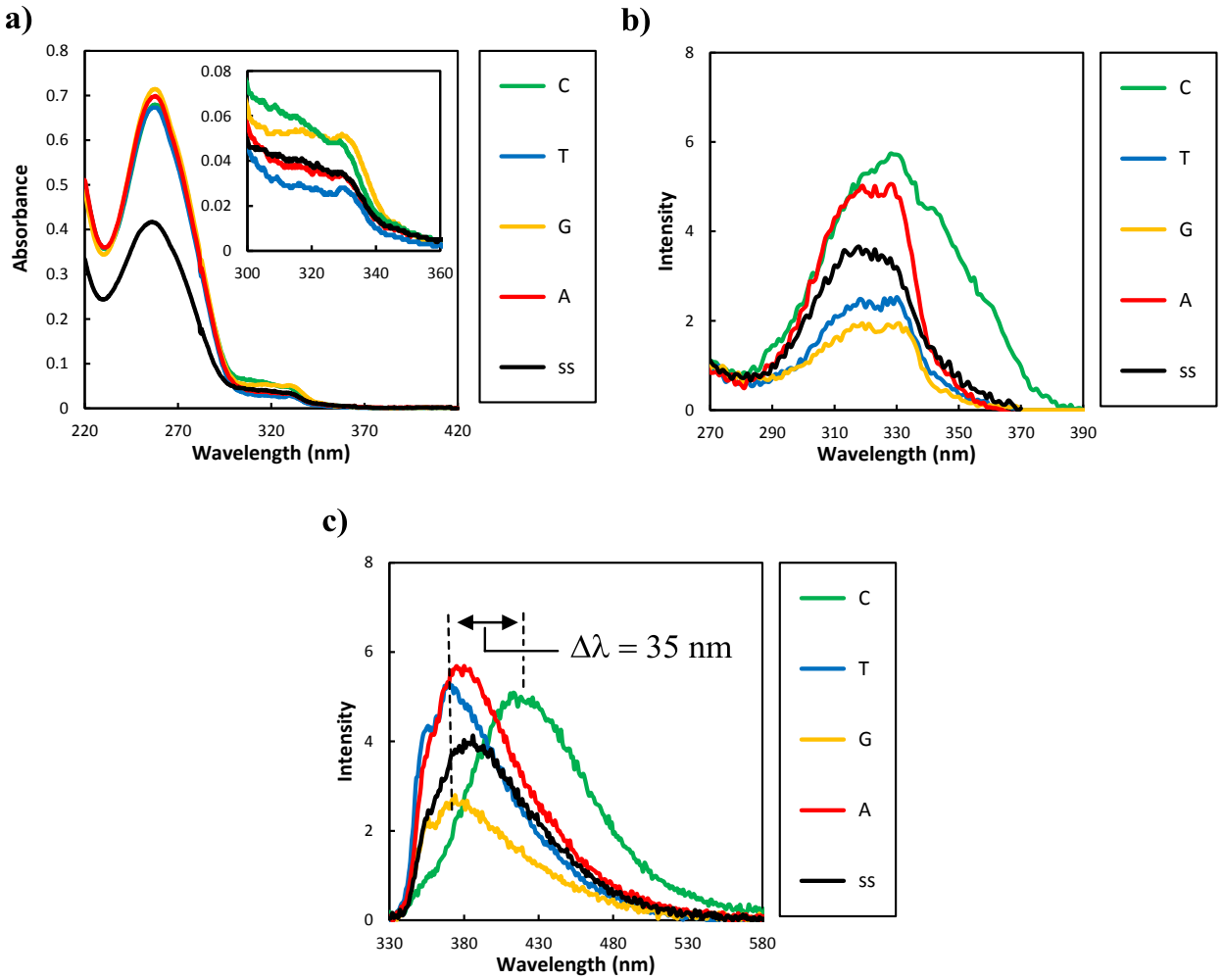
Fig. S3 UV absorption (a) and excitation (b) spectra of ODN 1 hybridized with ODN 2 (**N** = C, T, G, A, Ab). "Ab" denotes abasic site and "ss" denotes a single-strand ODN 1 (2.5 μM ODNs, 0.1 M sodium chloride, 50 mM sodium phosphate buffer, pH 7.0, rt).



ODN 1: 5'-CGCAAT **X** TAACGC-3' (**X** = ^{na}**G** or G)

ODN 2: 3'-GCGTTA **N** ATTGCG-5' (**N** = C, T, G, A, Abasic site)

Fig. S4 CD spectra of ODN 1 (**X** = G) hybridized with ODN 2 (**N** = C) (black line), and of ODN 1 (**X** = ^{na}**G**) hybridized with ODN 2 (**N** = C, T, G, A, Ab). "Ab" denotes abasic site (2.5 uM ODNs, 50 mM sodium phoshate, 0.1 M sodium chloride, pH 7.0, rt).



ODN 3: 5'-CGCAAT ^{na}**G** AAACGC-3'

ODN 4: 3'-GCGTTA **N** TTTGCG-5' (**N** = C, T, G, A)

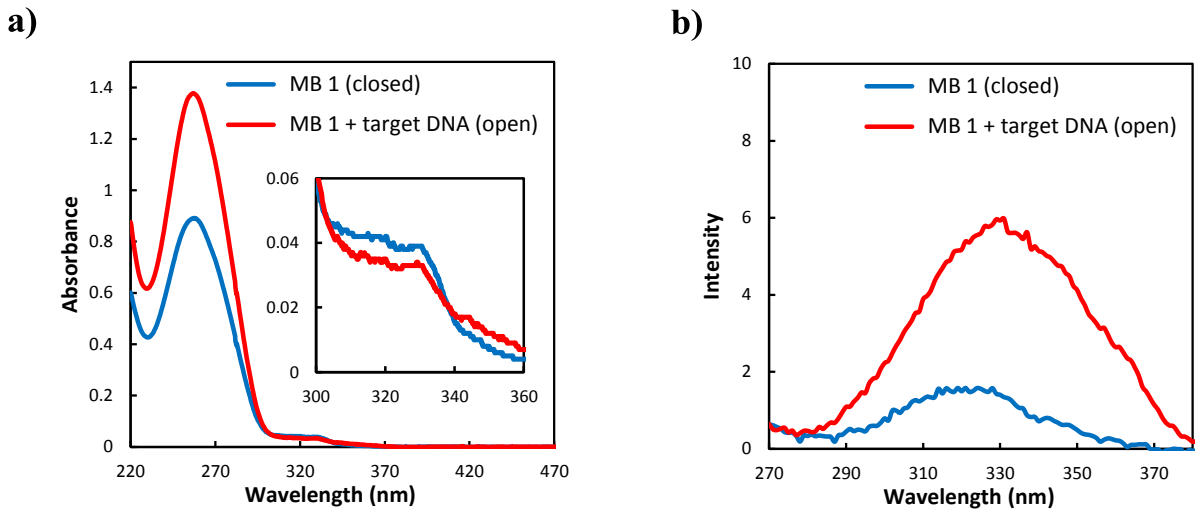
Fig. S5 UV absorption (a), excitation (b) and fluorescence (c) spectra of ODN 3 hybridized with ODN 4 (**N** = C, T, G, A). "ss" denotes a single-strand ODN 3 (2.5 μM ODNs, 0.1 M sodium chloride, 50 mM sodium phosphate buffer, pH 7.0, rt).

Table S2 Thermal melting temperatures (T_m) and fluorescence maximum of duplexes ODN3/ODN4.

ODN 3 / ODN 4(**N**)	T_m (°C)	λ_{max}^{fl} (nm)
ODN 3 / ODN 4 (C)	55.3	410
ODN 3 / ODN 4 (T)	42.4	378
ODN 3 / ODN 4 (G)	43.7	375
ODN 3 / ODN 4 (A)	41.3	379
ODN 3	-	386

ODN 3: 5'-CGCAAT ^{na}**G** AAACGC-3'

ODN 4: 3'-GCGTTA **N** TTTGCG-5' (**N** = C, T, G, A)



MB 1: 5'-CCACATGTGAAGGGCTTTT ^{na}**G** AACTCTGCATGTGG-3'

target DNA($ODN_{bcr/abl}$): 3'-ACTTCCCGAAAACTTGAGAC-5'

Fig. S6 UV absorption (a) and excitation (b) spectra of hairpin MB 1 and the duplex formed by hybridization with target DNA (2.5 μM ODNs, 0.1 M sodium chloride, 50 mM sodium phosphate buffer, pH 7.0, rt).