Supporting Information

Development of a Visible Nano-thermometer with a Highly Emissive 2'-O-Methylated Guanosine Analogue

Seigi Yamamoto,[1] Soyoung Park,[1] and Hiroshi Sugiyama*[,1,2]*

[†]Department of Chemistry, Graduate School of Science, Kyoto University, Kitashirakawa-oiwakecho, Sakyo-ku, Kyoto 606-8502, Japan

[‡]Institute for Integrated Cell-Material Sciences (iCeMS), Kyoto University, Yoshida-ushinomiyacho, Sakyo-ku, Kyoto 606-8501, Japan

**Corresponding author:* Dr. Soyoung Park, Prof. Dr. Hiroshi Sugiyama

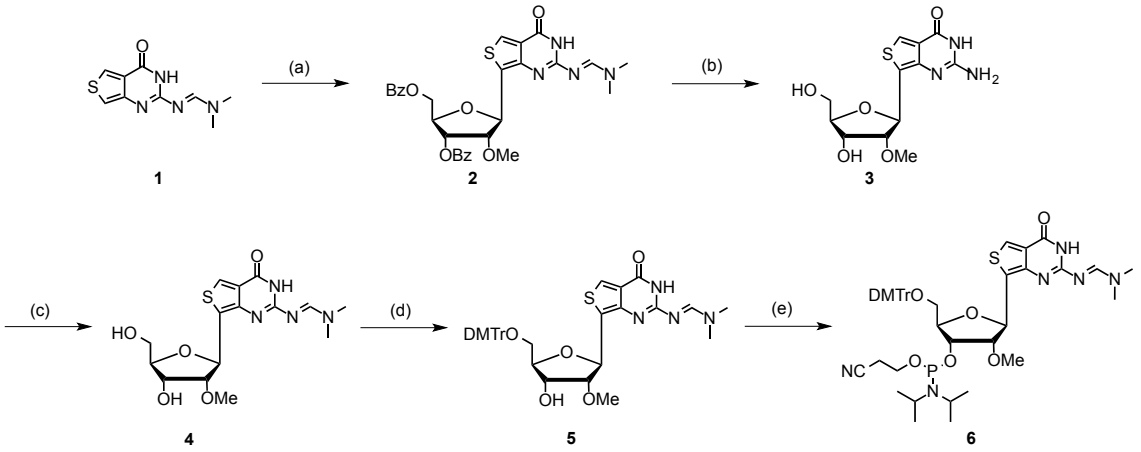
Tel.: (+)81-75-753-4002; Fax: (+)81-75-753-3670

E-mail: oleesy@kuchem.kyoto-u.ac.jp (S. P.), hs@kuchem.kyoto-u.ac.jp (H. S.)

Received Date: (to be automatically inserted after your manuscript is accepted if required according to the journal that you are submitting your paper to)

Materials used for synthesis of 2'-OMe-thG

$DMSO_2$, $MeNO_2$, *N,N*-dimethylformamide dimethyl acetal, 2-cyanoethyl *N,N*-diisopropylchloro phosphoramidite and dimethoxytrityl chloride were received from Wako Chemicals and used without further purification. $SnCl_4$ was purchased from Sigma-Aldrich Chemicals Co. (Milwaukee, WI). 2.0 M ammonia in methanol was received from TCI. Methyl 4-aminothiophene-3-carboxylate hydrochloride was purchased from Apollo Scientific Ltd. All other chemicals and solvents were purchased from Sigma-Aldrich Chemicals Co., Wako Pure Chemical Ind. Ltd., TCI, or Kanto Chemical Co. Inc. 1-acethyl-3,5-di-*O*-benzoyl-2-*O*-methyl-ß-D-ribofuranose and Chloroformamidine hydrochloride were prepared by following the literature procedures [1, 2]. Water was deionized (specific resistance of ≥18.0 MΩ cm at 25°C) by a Milli-Q system (Millipore Corp.).



Methods used for synthesis of 2'-OMe-thG

NMR spectra were obtained on a JEOL JNM ECA-600 spectrometer operating at 600 MHz for ^{1}H NMR and 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.).

Synthesis of 2'-OMe-thG and its phosphoramidite

Reagents and conditions: (a) β-D-deoxyribofuranose 1-acetate 4,5-dibenzoate, $SnCl_4$, $MeNO_2$, 0 ºC to RT,; (b) NH_3/MeOH, 65 ºC,; (c) dimethylformamide dimethyl acetal, DMF/MeOH,; (d) DMTrCl, Py,; (e) 2-cyanoethyl *N,N*-diisopropylchloro phosphoramidite, *i*Pr_2NEt, DCM/MeCN, 0 ºC to RT

***N2*-DMF 2-aminothieno[3,4-*d*]pyrimidine G mimic 2'-O-methyl-3',5'-di-*O*-benzoyl deoxyribonucleoside (2)**

N2-DMF 2-aminothieno[3,4-*d*]pyrimidin-4(3*H*)-one (**1**) was prepared from methyl 4-aminothiophene-3-

carboxylate hydrochloride as reported previously.[1] To a suspension of N_2-DMF 2-aminothieno[3,4-*d*]pyrimidin-4(3*H*)-one (500 mg, 2.25 mmol) and 1-acethyl-3,5-di-*O*-benzoyl-2-*O*-methyl-ß-D-ribofuranose [2] (1.06 mg, 2.56 mmol) in dry $MeNO_2$ (50 mL) was dropwisely added $SnCl_4$ (640 µL, 5.5 mmol) over 1 hour at 0 ºC and stirred for 30 min at the same temperature. Warm up to room temperature gradually and stirred for 3 hour, 1-acethyl-3,5-di-*O*-benzoyl-2-*O*-methyl-ß-D-ribofuranose (532.6 mg, 1.29 mmol) was added to the reaction mixture and then was stirred 24 hour. Reaction was quenched with 25 mL of sat. aq.$NaHCO_3$ The resulting mixture was vigorously stirred for 1.5 h and the preticipate was filtered over a Celite cake. The separated aqueous layer was extracted with CH_2Cl_2. The organic layer were dried over $MgSO_4$ and evaporated. The residue was purified by column chromatography with CH_2Cl_2:MeOH = 99:1 to 90:10 to afford a brown product (1.07 g, 83%,). ^{1}H NMR (600 MHz, $CDCl_3$)
8.78 (s, 1H), 8.12-7.99 (m, 6H), 7.61-7.37 (m, 7H), 5.73 (t, J=6.12, 1H), 5.56 (d, J=4.75, 1H), 4.70-4.59 (m, 4H), 3.41 (s 3H), 3.14 (s, 3H), 3.05 (s, 3H)
^{13}C NMR (150 MHz, CDCl3) 166.41, 166.08, 159.41, 158.10, 154.19, 147.23, 133.49, 133.18, 129.94, 129.79, 129.65, 129.56, 128.58, 128.43, 126.65, 126.01, 125.73, 83.79, 78.81, 78.58, 73.32, 63.88, 58.96, 41.21, 35.15

2-Aminothieno[3,4-*d*]pyrimidine G mimic 2'-O-methyl deoxyribonucleoside (3)

A solution of **3** (1.07 g, 1.86 mmol) in 2.0 M ammonia (60 mL) in methanol was heated at 65 ºC overnight. In brown reaction mixture, white solid appeared and then dissolved. The mixture was evaporated and purified by column chromatography with CH_2Cl_2:MeOH = 19:1 to 4:1 to afford a light brown foam. (299.4 mg, 51%)1H NMR (600 MHz, DMSO-d6) 10.58 (brs, 1H), 8.17 (s, 1H), 6.15 (s, 2H), 5.29 (d, J=6.77, 1H), 4.12 (d, J=4.75, 1H), 4.09 (d, J=4.75, 1H), 3.77 (q, J=4.46, 8.53, 1H), 3.71 (dd, J=4.81, 6.80, 1H), 3.52 (dd, J=4.04, 11.56, 1H), 3.47 (dd, J=4.75, 11.53, 1H), 3.29 (s, 3H), ^{13}C NMR (150 MHz, DMSO-d6), 126.46, 87.10, 85.95, 75.50, 70.94, 63.08, 58.42

***N2*-DMF-2-aminothieno[3,4-*d*]pyrimidine G mimic 2'-O-methyl deoxyribonucleoside (4)**

A solution of **1**(131.5 mg, 0.42 mmol) and *N,N*-dimethylformamide dimethyl acetal (0.09 mL) in DMF/MeOH (1/1,6 mL) was stirred overnight at RT. All volatiles were evaporated. ^{1}H NMR (600 MHz, DMSO-d6) 10.98 (brs, 1H), 8.55 (s, 1H), 8.20 (s, 1H), 5.39 (d, J=6.12, 1H) , 4.99 (brs, 1H), 4.09 (t, J=4.75, 1H), 3.80 (t, J=5.47, 2H). 3.56 (dd, J=4.10, 11.59, 1H), 3.49 (dd, J=4.78, 11.57, 1H), 3.37 (s, 3H), 3.28 (s, 1H), 3.14 (s, 3H), 3.03 (s, 3H) 13C NMR (150 MHz, DMSO-d6) 222.33, 192.40, 160.22, 158.34, 155.97, 147.38, 129.62, 126.32, 125.91, 87.29, 86.71, 86.27, 85.76, 75.91, 71.16, 70.98, 63.30, 63.06, 58.40, 58.35, 41.55, 41.00, 35.55

***O5'*-Dimethoxytrityl-*N2*-DMF-2-aminothieno[3,4-*d*]pyrimidine G mimic 2'-O-methyl deoxyribonucleoside (5)**

5 (50 mg, 0.14mmol) was coevaporated with dry pyridine. DMTrCl (57 mg, 0.17 mmol) in dry pyridine (0.3 mL) was added and the solution was stirred at RT for 3 hours. The reaction mixture was directly loaded on silica pad for column chromatography with CH_2Cl_2:MeOH = 1:0to 9:1 containing 0.5 % triethylamine to afford an off-white solid (77.3mg, 75%). ^{1}H NMR (600 MHz, $CDCl_3$) 8.68 (s, 1H), 8.40 (s, 1H), 8.13 (s, 1H), 7.52-6.80, (m, 13H), 5.71 (d, J=6.12, 1H) , 4.29 (d, J=4.75, 1H), 4.11 (q, J=4.43, 7.81, 1H), 4.06 (t, J=5.44, 1H), 3.79 (s, 3H), 3.78 (s, 3H), 3.52 (s, 3H), 3.45-3.42 (m, 1H), 3.26 (dd, J=4.07,10.19, 1H) , 3.14 (s, 3H), 3.07 (s, 3H), 2.73 (d, J=4.75, 1H), 1.69 (brs, 2H) ^{13}C NMR (150 MHz, $CDCl_3$) 159.44, 158.52, 157.87, 153.92, 146.95, 145.03, 136.23, 136.15, 130.29, 130.26, 129.09, 128.39, 127.87, 126.79, 125.62, 113.18, 86.40, 86.25, 83.39, 75.93, 71.42, 64.12, 58.57, 55.30, 41.35, 35.10, 0.077

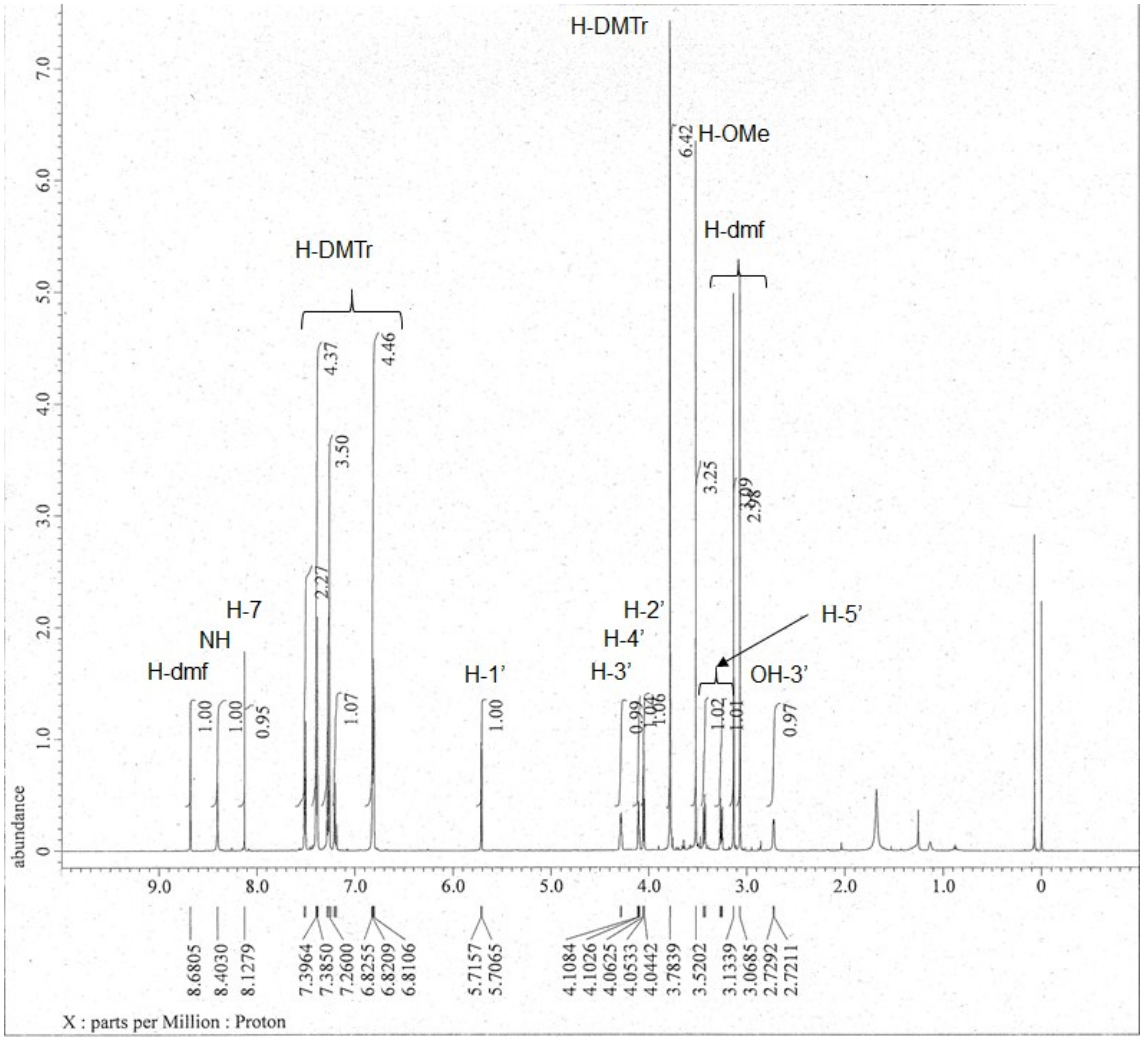
(3'-(2-Cyanoethyldiisopropylphosphoramidite)-*O5'*-dimethoxytrityl-*N2*-DMF-2-aminothieno [3,4-*d*]pyrimidine G mimic 2'-O-methyl deoxyribonucleoside (6)

6 (50 mg, 0.012 mmol) was coevaporated with dry pyridine, dried under vacuum for 3 hours, and dissolved in 1 mL of dry CH_2Cl_2. *N,N*-diisopropylethylamine (63 μL, 0.36 mmol) and 2-cyanoethyl *N,N*-diisopropylchlorophosphoramidite (54 μL, 0.24 mmol) were successively added to the solution at 0 ºC, and the reaction mixture was allowed to warm up and stirred 3 hours at RT. All volatiles were then evaporated and without further purification the residue dissolved in CH_2Cl_2/MeCN (1/4, 0.5 mL) for DNA solid synthesis.

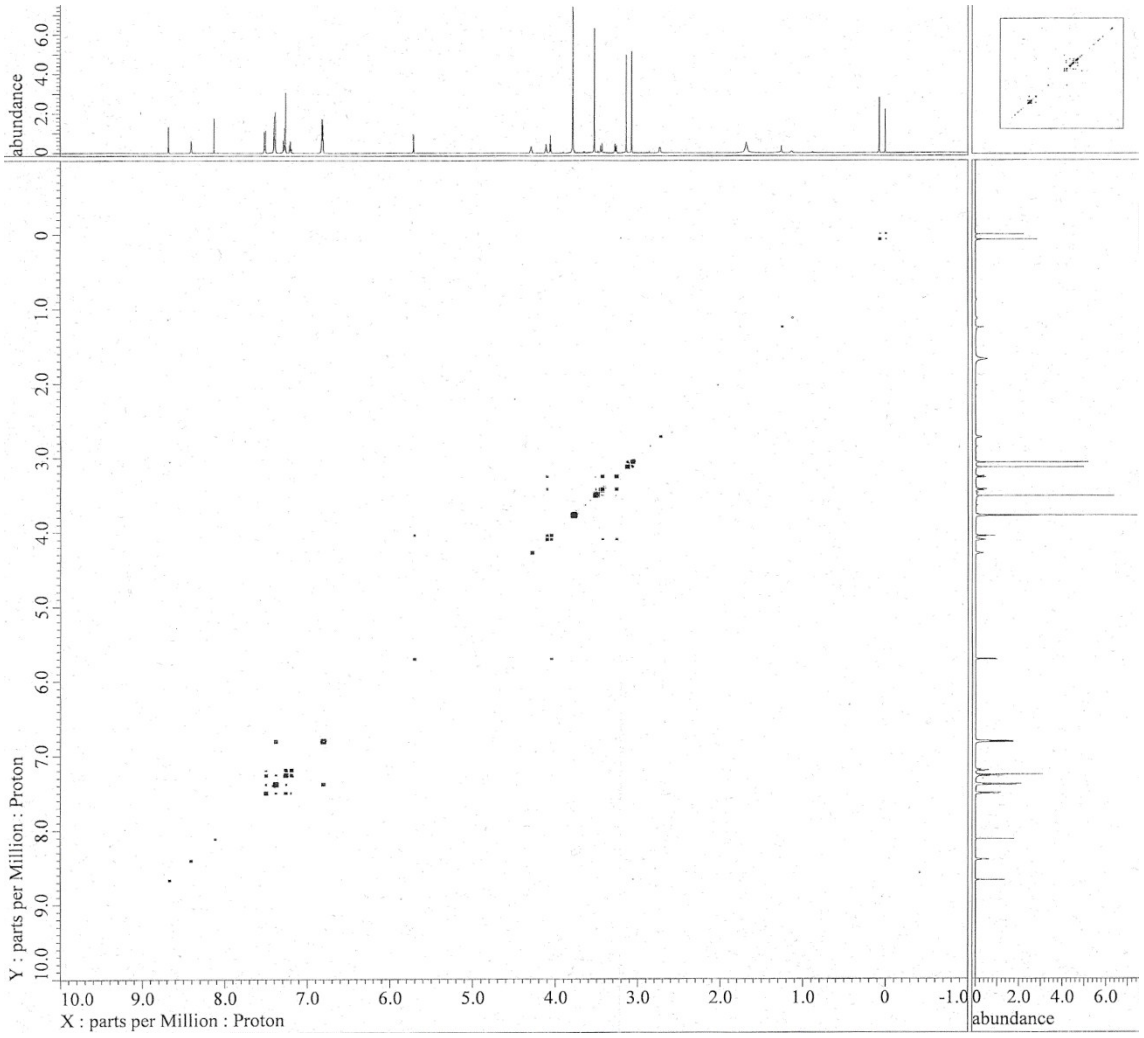
Figure S1. NMR spectra of **6**(β-anomer). (a) 1H NMR spectrum of **6**(β-anomer) (b) COSY spectrum of

6(β-anomer) (c)NOESY spectrum of **6**(β-anomer)

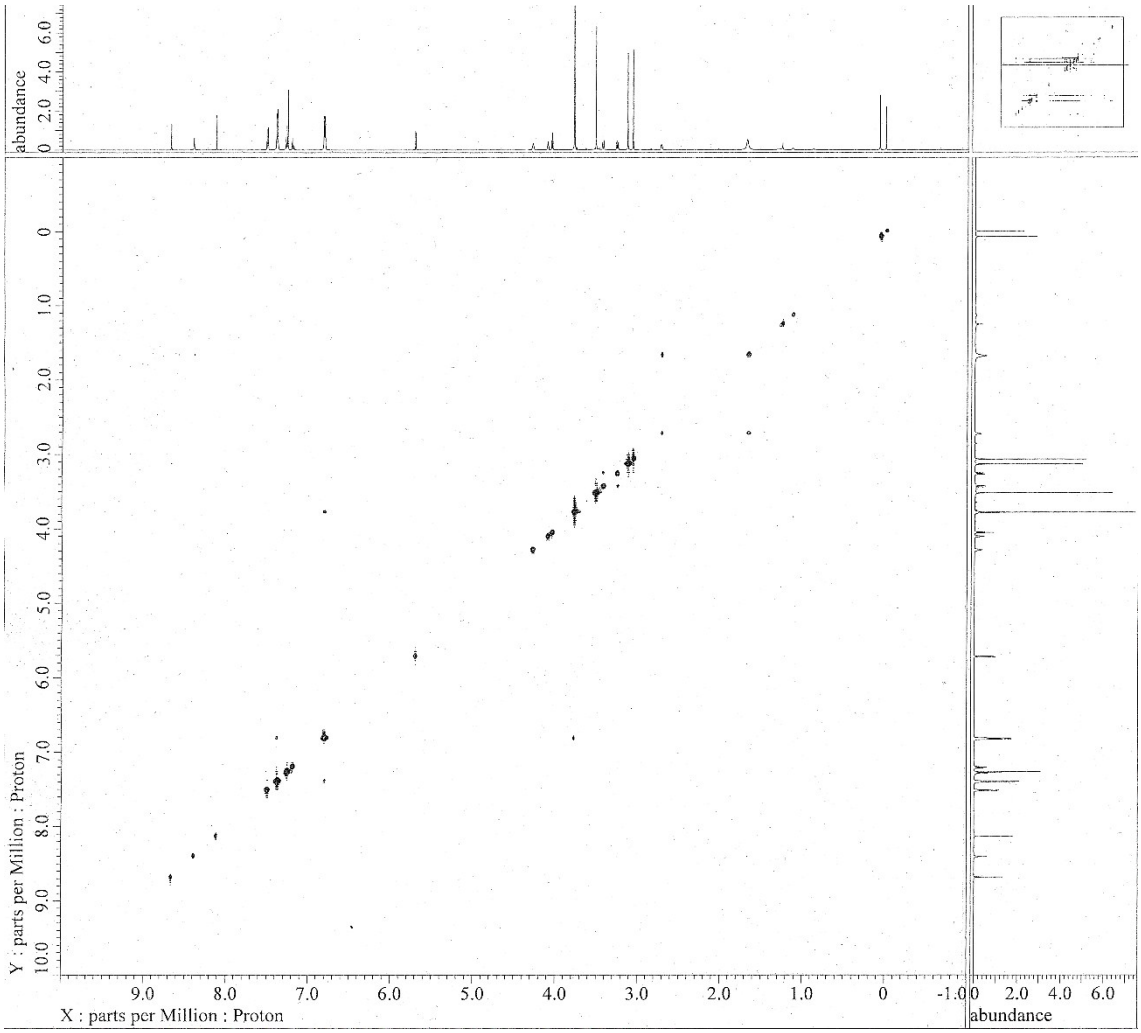
a) 1H NMR spectrum of **6**(β-anomer)



(b) COSY spectrum of **6**(β-anomer)



(c)NOESY spectrum of **6**(β-anomer)


abundance
0
2.0
4.0
6.0
Y : parts per Million : Proton
10.0
9.0
8.0
7.0
6.0
5.0
4.0
3.0
2.0
1.0
0
X : parts per Million : Proton
-1.0
abundance

Photophysical data for 2'-OMe-thG monomer

Samples were measured in water, dioxane, and MeOH at 20 °C. All samples were prepared from a DMSO stock solution. Measurement was conducted with10 μM 2'-OMe-thG monomer in the each solvent containing trace DMSO. All experiments were performed in duplicate with negligible differences; hence only one series is shown.

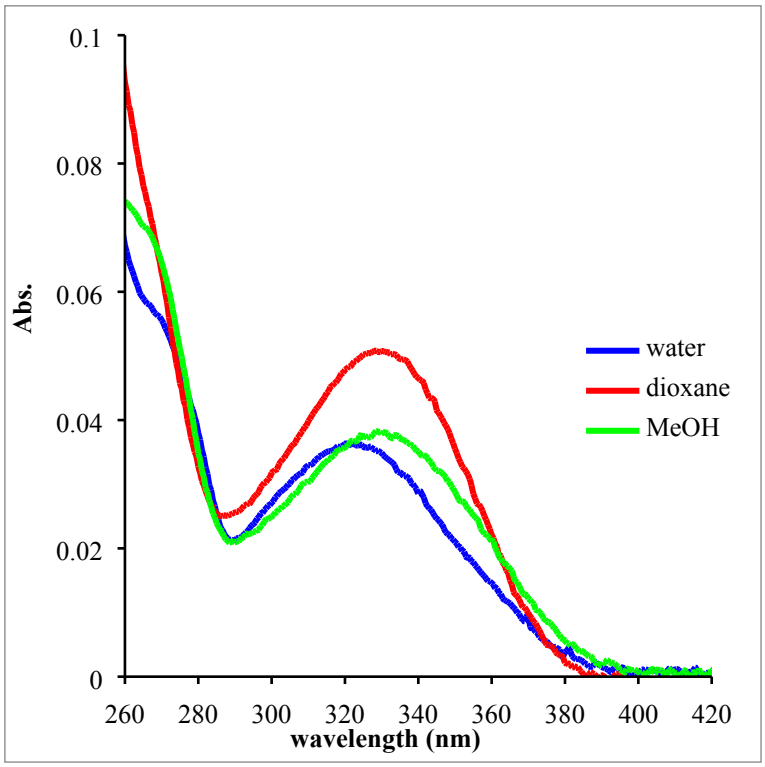


Figure S2. JASCO V-650 UV/VIS spectrophotometer was used to record absorption spectra with a 0.5 nm resolution. The cuvette temperature was kept at 25°C by JASCO PAC-743R. Samples were prepared with 10 μM in H2O, dioxane, or MeOH (blue, red, or green line).

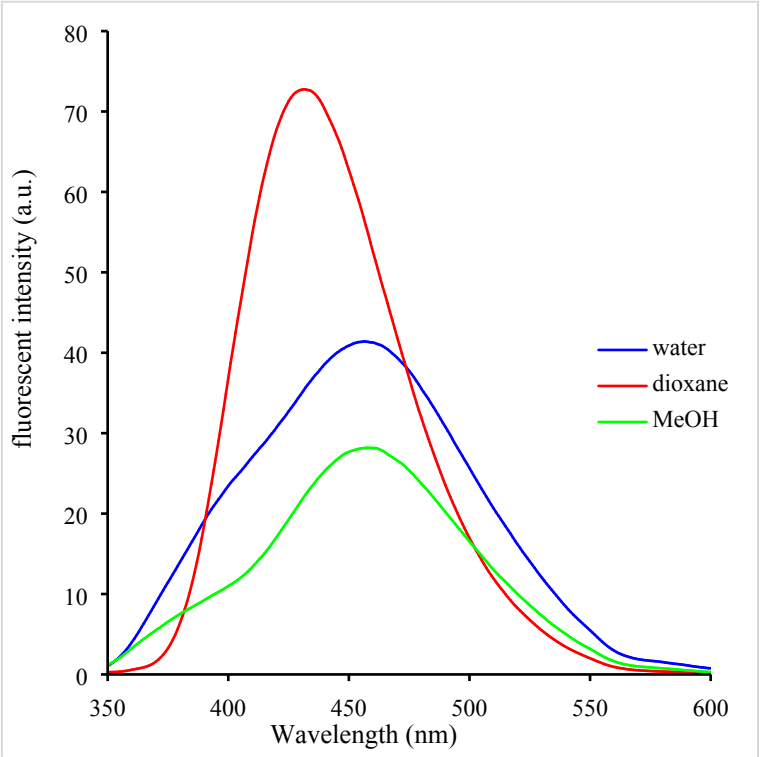
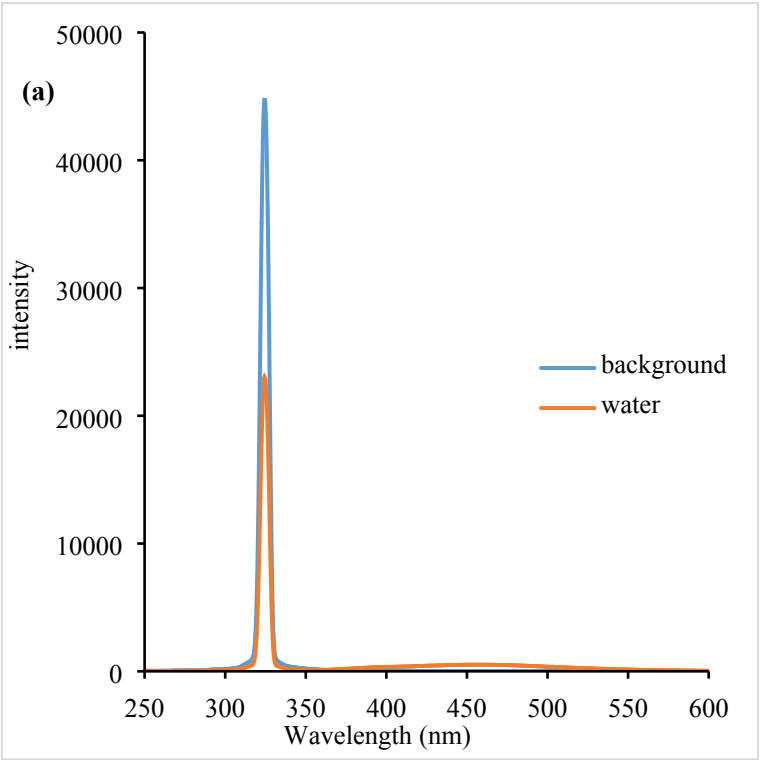


Figure S3. Fluorescence measurements were conducted using a JASCOFP-6300 spectrofluorometer. The sample temperature was controlled with a JASCO EHC-573 at 20 °C. Measurements were performed using fluorescence cells with a 0.5-cm path length. The result of the sample dissolved in H2O is shown as a blue line, dioxane is red line, and MeOH is green line.



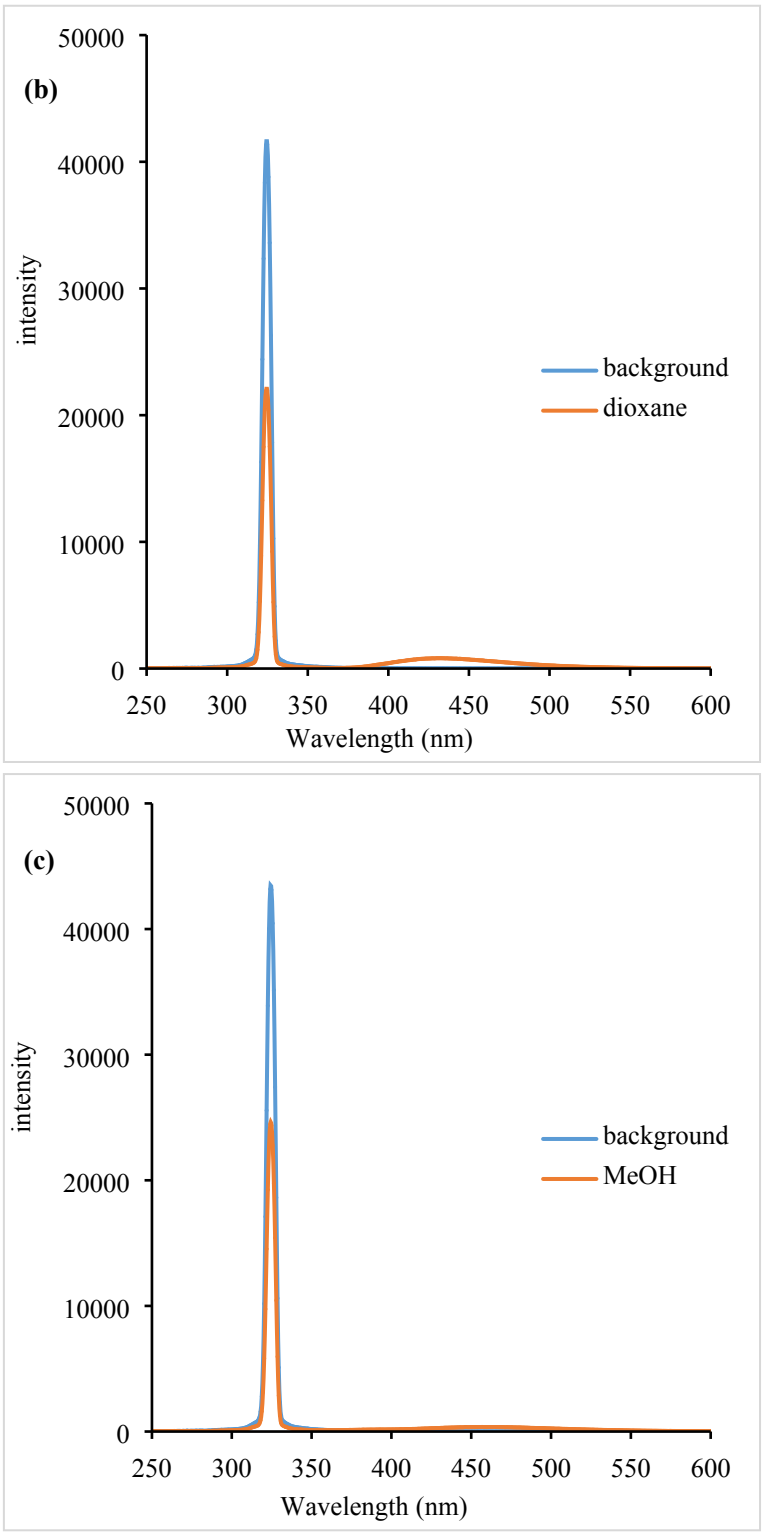
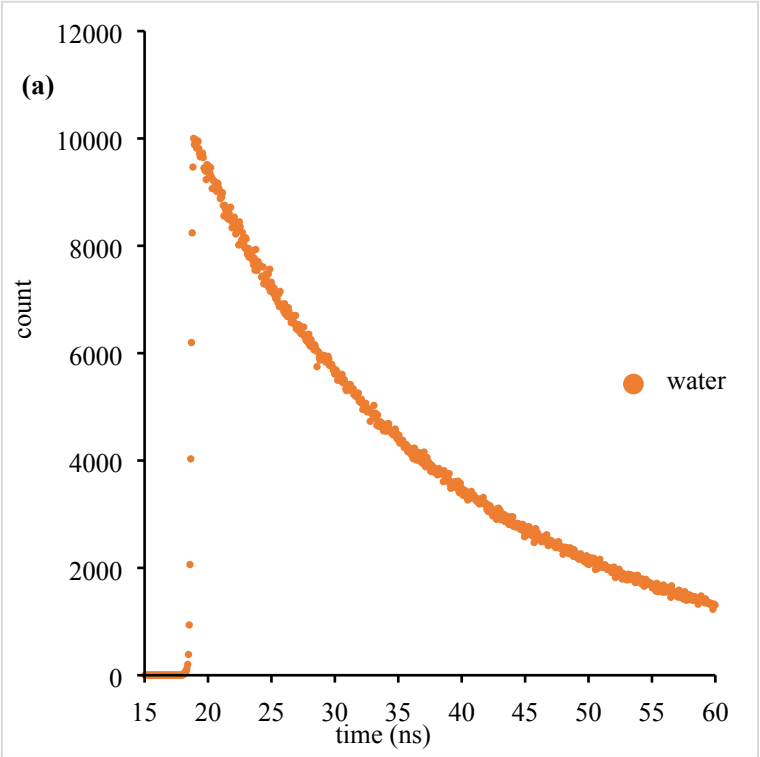
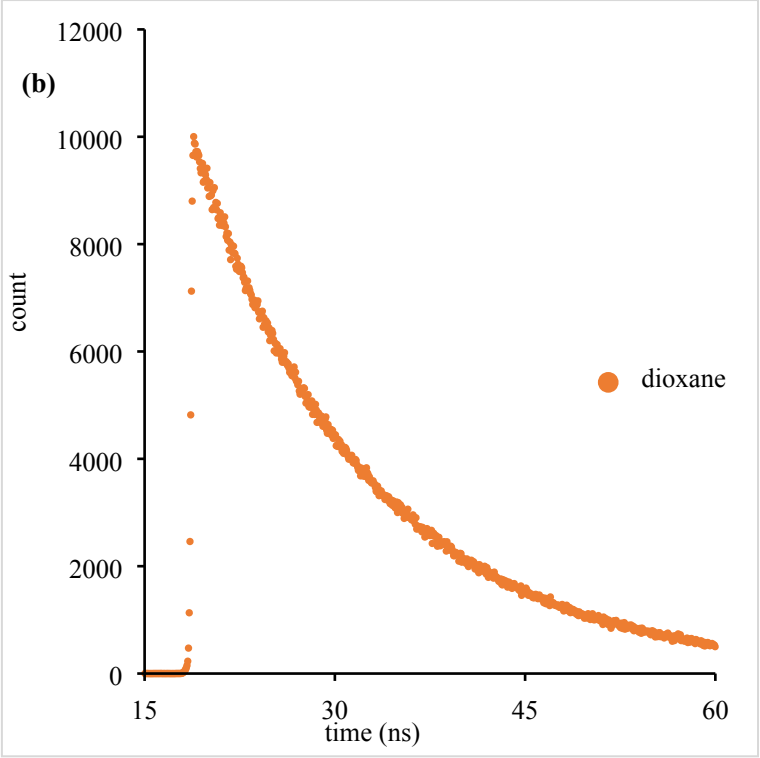


Figure S4. Quantum yields were measured with HAMAMATSU Absolute PL Quantum Yield spectrometer C11347.Samples were dissolved in (a) H2O (b) dioxane (c) MeOH. All samples were excited at 325nm.


(a)
12000
10000
8000
6000
4000
2000
0
count
water
15
20
25
30
35
40
45
50
55
60
time (ns)


(b)
12000
10000
8000
6000
4000
2000
0
count
dioxane
15
30
45
60
time (ns)

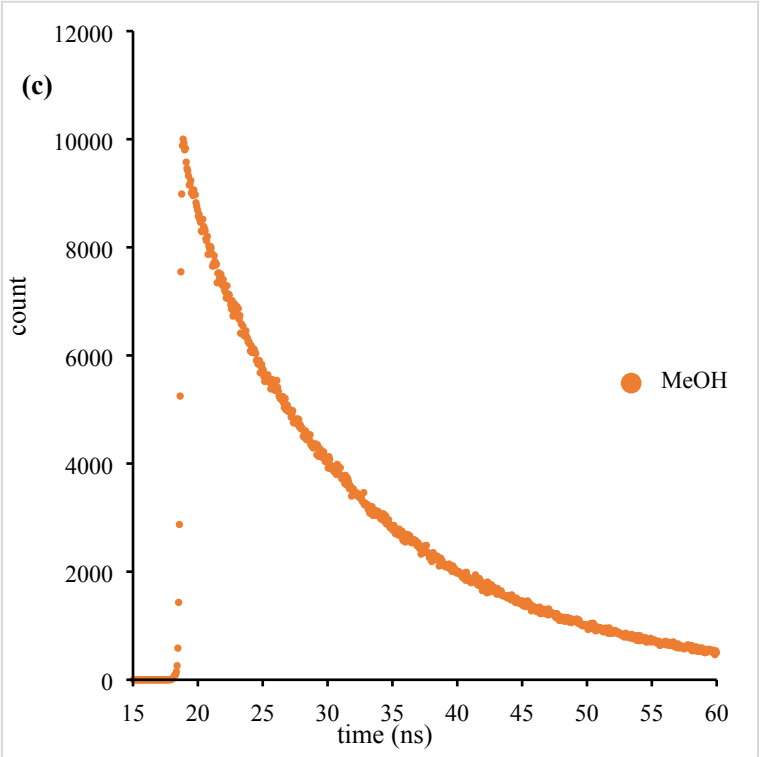


Figure S5. Fluorescence decay curves were collected on a HORIBA Fluorocube 3000U -SHK using an LED laser source using an LED for excitation. All samples were excited at 325 nm and the fluorescence decay was observed at 455 nm. Samples were dissolved dissolved in (a) H_2O (b) dioxane (c) MeOH.

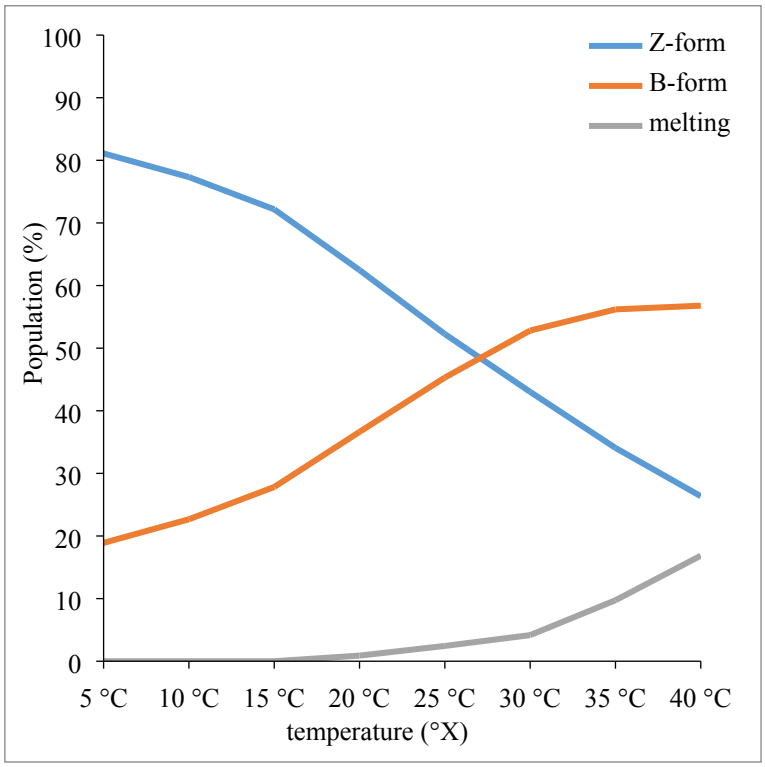


Figure S6. The proportion of Z-DNA, B-DNA and single-strand DNA. Population was calculated from the results of Tm measurement and fluorescent measurement of ODN9.

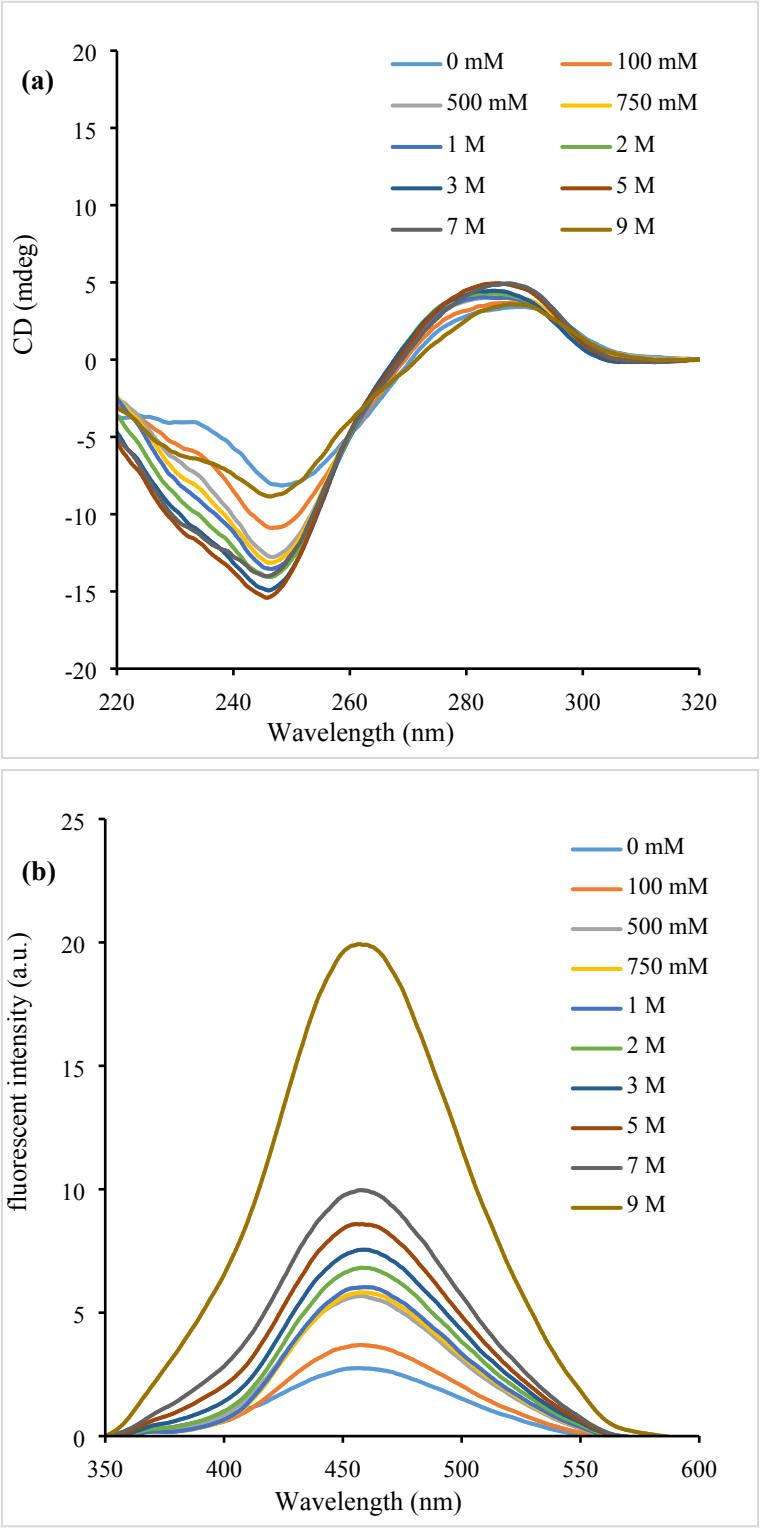


Figure S7. Conformational changes of ODN10 from B-DNA to Z-DNA and fluorescence intensity at various $NaClO_4$ concentrations. (a) Observation of the B–Z transition by CD spectroscopy. (b) Change in fluorescence intensity. All samples contained 5 μM of ODN10 in 20 mM sodium cacodylate buffer (pH 7.0) at 5 °C.

Solid-Phase Synthesis

ODNs having 2'-OMe-thG (ODN1, 9, 10, 12, 17, 18) were synthesized on solid supports using (3'-(2-Cyanoethyldiisopropylphosphoramidite)-*O5'*-dimethoxytrityl-*N2*-DMF-2-aminothieno [3,4-*d*]pyrimidine G mimic 2'-O-methyl deoxyribonucleoside (7)and commercially available *O*5'-dimethoxytrityl-2'-deoxyribonucleoside *O*3'-phosphoramidites. Solid-phase oligonucleotide synthesis was performed on an ABI DNA synthesizer (Applied Biosystem, Foster City, CA). The modified phosphoramidite was chemically synthesized as described above and without purification incorporated into oligonucleotide through coupling reaction for 10 minutes. 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. After purification by HPLC, products were confirmed by ESI-TOFMS (Table S1). DNA concentrations were determined by using the Nano drop ND-1000 (Nano-drop Technologies, Wilmington, DE).

Table S1. ESI-TOF-Mass data of ODNs.

	Calcd.	found
ODN1[M-3H]	1368.98	1367.73
ODN8[M-3H]	1023.84	1024.23
ODN9[M-3H]	1435.90	1435.06
ODN10[M-3H]	1039.16	1037.85

Other ODNs are received from SIGMA-Genosys or JBios.

UV-melting

Melting temperatures were determined by measuring changes in absorbance at 260 nm as a function of temperature using a JASCO V-650 UV/VIS spectrophotometer. JASCO PAC-743R equipped with a high performance temperature controller and micro auto eight-cell holder. Absorbance was recorded in the forward and reverse direction at temperatures from 5 to 95 °C at a rate of 0.5 °C/min. The melting samples were denatured at 95 °C for 5 min and annealed slowly to RT then stored at 5 °C until experiments were initiated. All melting samples were prepared in a total volume of 100 μL containing 5 μM of each strand oligonucleotide, 20 mM Na cacodylate (pH 7.0) and 100 mM NaCl. Synthetic oligonucleotides were obtained from Sigma-Aldrich Chemicals Co.

Fluorescence Measurement

Fluorescence measurements of 2'-OMe-**thG** -containing DNA were conducted using a JASCO FP-6300 spectrofluorometer. The sample temperature was controlled with a JASCO EHC-573. Measurements were

performed using fluorescence cells with a 0.5-cm path length. All samples are containing 5 μM of each strand oligonucleotide in 20 mM sodium cacodylate buffer (pH 7.0) and various concentrations of $NaClO_4$ at 5 °C.

CD Spectroscopy

CD spectra of oligonucleotide solutions collected in0.5-nm steps from 320 to 220 nm were measured using JASCO J-805LST Spectrometer in a 1-cm quartz cuvette. The buffer and concentrations of $NaClO_4$ were the same as for fluorescence measurement. Each spectrum shown is the average of two individual scans.