Supplementary information (ESI) for *Chemical Communications*

Fluorometric sensing of the salt-induced B-Z DNA transition by combination of two pyrene-labeled nucleobases

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Experimental

General. ^{1}H NMR spectra were measured with Varian Mercury 400 (400 MHz) spectrometer. ^{13}C NMR spectra were measured with JEOL JMN α-500 (500 MHz) spectrometer. Coupling constant (J value) are reported in hertz. The chemical shifts are expressed in ppm downfield from tetramethylsilane, using residual chloroform (δ= 7.24 in ^{1}H NMR) and dimethylsulfoxide (δ= 2.48 in ^{1}H NMR, δ= 39.5 in ^{13}C NMR) as an internal standard. FAB Mass spectra were recorded on JEOL JMS DX-300 spectrometer or JEOL JMS SX-102A spectrometer. Wakogel C-200 was used for silica gel column chromatography. Pre-coated TLC plates Merck silica gel 60 F_{254} was used for monitoring reactions. TLC spots were visualized with UV light or anisaldehyde (a solution of 9.0 mL *p*-anisaldehyde, 3.5 mL acetic acid and 10 mL sulfuric acid in 330 mL ethanol). The reagents for the DNA synthesizer such as A, G, C, T-β-cyanoethyl phosphoramidite, and CPG support were purchased from GLEN Research.

8-Bromo-2′-deoxyguanosine. A mixture of 2′-deoxyguanosine (3.0 g, 11.2 mmol) and *N*-bromosuccinimide (2.2 g, 12.4 mmol) in water (200 mL) was stirred for 5 min at room temperature. The precipitated solid was collected by filtration and washed with acetone and dried to give 8-bromo-2′-deoxyguanosine (2.7 g, 69%). ^{1}H NMR (DMSO-d_6) δ= 10.78 (s, 1H), 6.47 (s, 2H), 6.16 (t, 1H, J = 7.30 Hz), 4.40 (ddd, 1H, J = 3.0, 3.1, 6.0 Hz), 3.80 (ddd, 1H, 3.1, 5.4, 5.5 Hz), 3.63 (dd, 1H, J = 5.3, 11.6 Hz), 3.50 (dd, 1H, J = 5.8, 11.6 Hz), 3.16 (ddd, 1H, J = 6.6, 7.6, 13.7 Hz), 2.10 (ddd, 1H, J = 3.0, 6.5, 13.3 Hz).

3′,5′-*O*,*O*′-bis(*tert*-Butyldimethylsilyl)-8-bromo-2′-deoxyguanosine (1). To a solution of 8-bromo-2′-deoxyguanosine (1.0 g, 2.9 mmol) in DMF (10 mL) was added imidazole (1.2 g, 17 mmol) and *tert*-butyldimethylsilyl chloride (1.2 g, 8.62 mmol) at room temperature, and the reaction mixture was stirred at room temperature for 12 h. The solvent was evaporated *in vacuo*. The oil was added to a saturated aqueous $NaHCO_3$. The precipitated white solid was collected by filtration, and washed with water and ethanol. The white solid was dried to give **1** (1.56 g, 94%). ^{1}H NMR (DMSO-d_6) δ= 10.78 (s, 1H), 6.38 (s, 2H), 6.15 (dd, 1H, J = 7.0, 7.1 Hz), 4.59 (ddd, 1H, J = 3.2, 3.3, 6.2 Hz), 3.76 (m, 2H), 3.65 (m, 1H), 3.38 (ddd, 1H, J = 6.5, 6.6, 13.4 Hz), 2.15 (ddd, 1H, J = 3.6, 7.0, 13.2 Hz), 0.89 (s, 9H), 0.83 (s, 9H), 0.11 (s, 6H), –0.01 (s, 3H), –0.02 (s, 3H)

8-(1-Pyrenyl)ethynyl-3′,5′-*O*,*O*′-bis(*tert*-Butyldimethylsilyl)-2′-deoxyguanosine (2). To a solution of **1** (250 mg, 0.44 mmol), 1-ethynylpyrene (100 mg, 0.53 mmol), and triethylamine (0.25 mL, 1.78 mmol) in

DMF (20 mL) were added tetrakis(triphenylphosphine)palladium(0) (91 mg, 0.08 mmol) and copper(I) iodide (15 mg, 0.07 mmol) under argon. The mixture was stirred at room temperature for 12 h. The reaction mixture was concentrated *in vacuo*, and diluted with ethyl acetate. The solution was washed with 5% EDTA solution and 5% sodium bisulfite solution, dried over Na_2SO_4, filtered and evaporated. The crude product was purified by silica gel column chromatography (chloroform : methanol = 40 : 1) to yield **2** (221 mg, 70%) as a brown solid. ^{1}H NMR (DMSO-d_6) δ= 10.16 (br, 1H), 8.54–8.16 (m, 9H), 6.51 (t, 1H, J = 6.8 Hz), 4.60–4.54 (m, 1H), 3.90–3.80(m, 1H), 3.49–3.40 (m, 1H), 3.18–3.14 (m, 1H), 2.31–2.27 (m, 1H), 0.83 (s, 9H), 0.67 (s, 9H), 0.06 (s, 3H), 0.04 (s, 3H), –0.19 (s, 3H), –0.22 (s, 3H); HRMS (FAB) calcd for $C_{40}H_{49}N_5O_4Si_2$ [M^+] 719.3323, found 719.3335.

8-(1-Pyrenyl)ethynyl-2′-deoxyguanosine (3). To a solution of **2** (100 mg, 0.14 mmol) and 1 M tetrabutylammonium fluoride in THF (0.18 ml 0.18 mmol) in 10 ml of THF was stirred for 12 h at room temperature. The precipitated orange solid was collected by filtration, and washed with THF. The orange solid was dried to give **3** (61 mg, 90%). ^{1}H NMR (DMSO-d_6) δ= 8.55–8.12 (m, 9H), 6.60 (dd, 1H, J = 6.2, 9.0 Hz), 4.46 (d, 1H, J = 5.1 Hz), 3.98 (br, 1H), 3.60–3.58 (m, 2H), 2.22–2.17 (m, 1H), 1.75–1.70 (m, 1H), 2.31–2.27 (m, 1H); HRMS (FAB) calcd for $C_{28}H_{21}N_5O_4$ [M^+] 491.1954, found 491.1594.

2-*N*-(*N*,*N*-Dimethylaminomethylidenyl)-8-(1-pyrenyl)ethynyl-2′-deoxy-5′-*O*-(4,4′-dimethoxytrityl)guanosine (5). To a solution of **3** (1.09 g, 2.22 mmol) in DMF (5 mL) was added *N*,*N*-dimethylformamide diethylacetal (0.43 mL, 2.66 mmol), and the mixture was stirred for 5 h at room temperature. The mixture was evaporated *in vacuo*. The crude product **4** was used for the next reaction without further purification.

To a solution of **4** in pyridine (40 mL) was added 4,4′-dimethoxytrityl chloride (0.90 g, 2.66 mmol), and the mixture was stirred for 6 h at room temperature. The reaction mixture was evaporated *in vacuo*. The crude product was purified by silica gel column chromatography (chloroform : methanol = 50 : 1) to yield **5** (1.12 g, 60%). ^{1}H NMR ($CDCl_3$) δ= 9.29 (br, 1H), 8.49–7.89 (m, 9H), 8.13 (s, 1H), 7.28–7.01 (m, 9H), 6.67 (t, 1H, J = 6.8 Hz), 4.92–4.86 (m, 1H), 4.23–4.16 (m, 1H), 3.76–3.71 (m, 1H), 3.54 (s, 3H), 3.55 (s, 3H), 3.52–3.48 (m, 1H), 3.30–3.28 (m, 1H), 2.80 (s, 3H), 2.77 (s, 3H), 2.76–2.73 (m, 1H); ^{13}C NMR (DMSO-d_6) δ = 158.0, 157.7, 157.7, 157.6, 157.1, 156.8, 149.5, 144.8, 135.6, 135.4, 131.5, 131.5, 130.6, 130.5, 130.3, 129.5, 129.4, 129.2, 128.8, 128.8, 127.6, 127.4, 127.4, 127.3, 127.1, 126.8, 126.3, 126.2, 126.2, 124.8, 124.3, 123.5, 123.1, 120.5, 114.6,113.1, 112.7, 112.7, 92.3, 85.8, 85.5, 85.0, 83.6, 79.1, 71.0, 70.7, 64.2, 54.7, 40.8, 37.9, 34.6; HRMS (FAB) calcd for $C_{52}H_{44}N_6O_6$ [M^+] 848.3332, found 848.3318.

2-*N*-(*N*,*N*-Dimethylaminomethylidenyl)-8-(1-pyrenyl)ethynyl-2′-deoxy-5′-*O*-(4,4′-dimethoxytrityl)guanosine 3′-*O*-(2-cyanoethyl)-*N*,*N*-diisopropylphosphoramidite (6). Compound **5** (62 mg, 0.12 mmol) and tetrazole (14.4 mg, 0.36 mmol) was dissolved in acetonitrile and dichloromethane, and coevaporated three times *in vacuo*. After substituted with nitrogen, 2-cyanoethyl *N*,*N*,*N*′,*N*′-tetraisopropylphosphorodiamidite (40.4 μL, 0.14 mmol) in acetonitrile (0.5 mL) was added, and the reaction mixture was stirred at room temperature for 1.5 h. After the completion of the reaction, the

crude product **6** was used for automated DNA synthesizer without further purification.

Oligodeoxynucleotide synthesis and characterization. Oligodeoxynucleotide (ODN) sequences were synthesis by conventional phosphoramidite method by using an Applied Biosystems 392 DNA/RNA synthesizer. After a deprotection in 28% ammonia at 55 °C for 8 h, ODNs were purified by reverse phase HPLC on a 5-ODS-H column (10 × 150 mm, elution with a solvent mixture of 0.1 M triethylamine acetate (TEAA), pH 7.0, linear gradient over 30 min from 5 % to 20 % acetonitrile at a flow rate 3.0 mL/min). ODNs containing $d^{Pet}G$ and $d^{Py}C$ were fully digested with calf intestine alkaline phosphatase (50 U/mL), snake venom phosphodiesterase (0.15 U/mL) and P1 nuclease (50 U/mL) at 37 °C for 3 h. Digested solution were analyzed by HPLC on a Cosmosil 5C-18AR or CHEMCOBOND 5-ODS-H column (4.6 × 150 mm), elution with a solvent mixture of 0.1 M triethylamine acetate (TEAA), pH 7.0, linear gradient over 20 min from 0 % to 10 % acetonitrile at a flow rate 1.0 mL/min). Concentration of ODNs was determined by comparing peak areas with standard solution containing dA, dC, dG, and dT at a concentration of 0.1 mM. **ODN(^{Pet}G^{Py}C)** 5′-d(CGCGCGCGC^{Pet}G^{Py}CGCG)-3′ (MALDI-TOF $[M - H]^-$ calcd 4771.31, found 4770.92).

UV measurement. UV spectra of DNA (5 μM) were taken in 50 mM sodium phosphate buffer (pH 7.0) and 0.1 or 4.5 M sodium chloride using a JASCO V-550 UV/VIS spectrometer.

Fluorescence measurement. All fluorescence spectra of DNA (5 μM) were taken in 50 mM sodium phosphate buffer (pH 7.0) and 0.1 or 4.5 M sodium chloride at room temperature. Fluorescence spectra were obtained using a SHIMADZU RF-5300PC spectrofluorophotometer.

Circular dichroism (CD) spectra measurement. All CD spectra of DNA (5 μM) were taken in 50 mM sodium phosphate buffer (pH 7.0) and 0.1 or 4.5 M sodium chloride at room temperature. CD spectra were record on a JASCO J-805 instrument.