Electronic Supplementary Information (ESI)

Structural Diversity Induced by Pyrene Intercalators in Homogeneous Oligodeoxyguanylates

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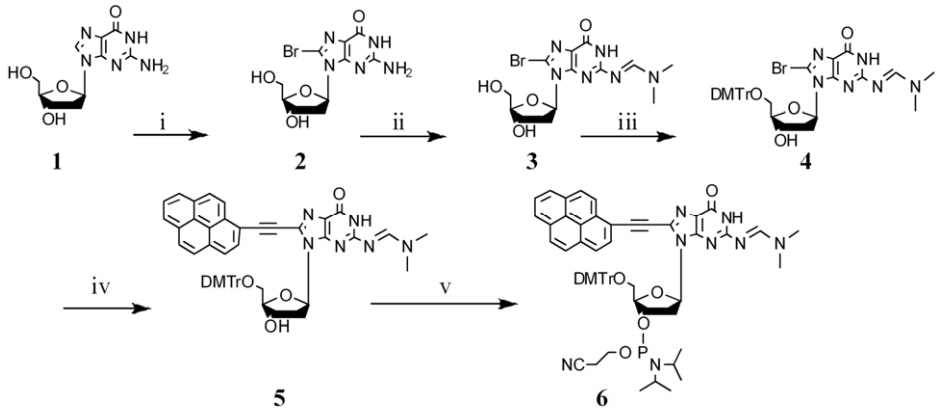

Scheme S1. Synthesis of pyrene-labeled deoxyguanosine derivatives. i) *N*-Bromosuccinimide, H_2O, r.t., 3 h, 93%; ii) *N*,*N*-dimethylformamide dimethylacetal, DMF, r.t., 3 h, in situ reaction; iii) DMTrCl, pyridine, r.t., overnight, 52% (2 steps); iv) 1-ethynylpyrene, $Pd(PPh_3)_4$, CuI, DMF, 50 °C, 3 h, 94%; v) 2-cyanoethyldiisopropyl-chlorophosphoramidite, 4-methylmorpholine, CH_2Cl_2, r.t., 1 h, 85%

Table S1. MALDI-TOF mass spectral data ($[M^+]$) for the ODNs

Sequences	Calculated m/z	Found m/z
G1	7616.1	7621.6
G2	7842.3	7848.5
G3	8068.6	8064.7
G4	8294.9	8297.3
G5	8521.1	8515.6
G6	7389.8	7379.4
G7	6709.3	6709.9
G8	8294.9	8295.1
G9	8219.8	8217.2
G10	7427.3	7426.5

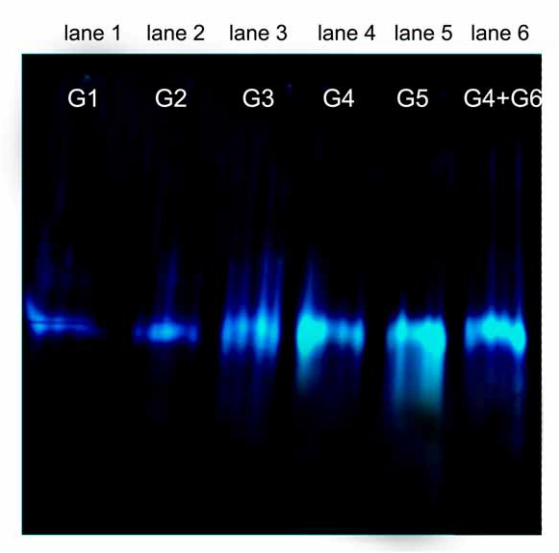

Fig. S1 Gel electrophoresis of ODNs on a denaturing polyacrylamide gel. The image was obtained using a UV analyzer. See the Experimental Section for details of the sample preparation procedure.

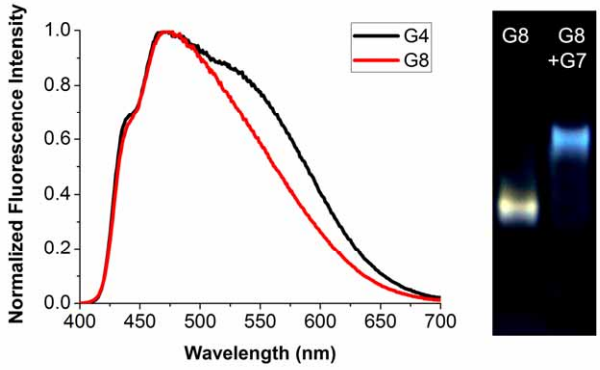

Fig. S2 Properties of ODN G8, which features pyrene intercalator units in a 1,4 relationship. (Left) Normalized fluorescent spectra of G4 and G8, recorded at 20 °C in a buffer of 100 mM Tris-HCl, 10 mM $MgCl_2$, and 100 mM NaCl (pH 7.2). Each ODN concentration was 1.0 μM. (Right) Gel electrophoresis of ODNs on a non-denaturing polyacrylamide gel. The image was obtained using a UV analyzer. See the Experimental Section for details of the sample preparation procedure.

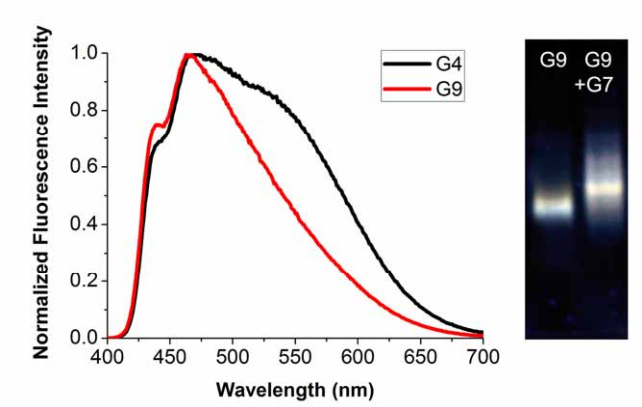

Figure S3. Properties of ODN G9, which features a heterogeneous sequence. (left) Normalized fluorescence spectra of G4 and G9, recorded at 20 °C in a buffer of 100 mM Tris-HCl, 10 mM $MgCl_2$, and 100 mM NaCl (pH 7.2). Each ODN concentration was 1.0 μM. (Right) Gel electrophoresis of ODNs on a non-denaturing polyacrylamide gel. The image was obtained using a UV analyzer. See the Experimental Section for details of the sample preparation procedure.

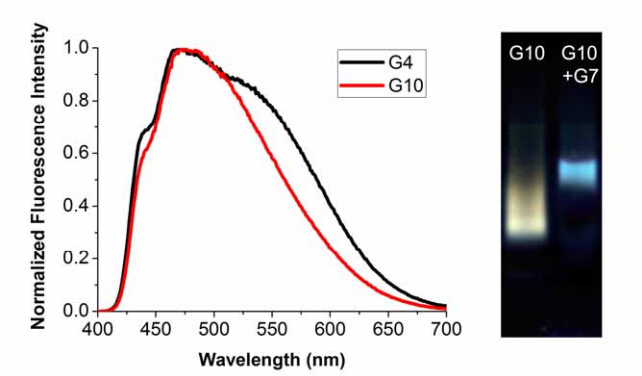

Figure S4. Properties of ODN G10, which lacks the terminal cytosine sequence. (Left) Normalized fluorescence spectra of G4 and G10, recorded at 20 °C in a buffer of 100 mM Tris-HCl, 10 mM $MgCl_2$, and 100 mM NaCl (pH 7.2). Each ODN concentration was 1.0 μM. (Right) Gel electrophoresis of ODNs on a non-denaturing polyacrylamide gel. The image was obtained using a UV analyzer. See the Experimental Section for details of the sample preparation procedure.

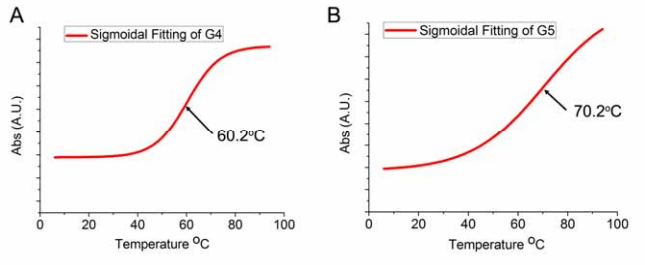

Figure S5. Melting temperature spectra of ODNs G4 and G5. We obtained melting temperature spectra of only G4 and G5 because other ODNs did not provide exact melting temperature curved. The spectra were recorded at 20 °C in a buffer of 20mM Tris-HCl, 100mM NaCl and 20mM $MgCl_2$ (pH 7.2). Each ODN concentration was 1μM and the recorded absorption wavelength was 260nm.

Time resolved spectroscopy in up-conversion mode

A home-made cavity-dumped Kerr lens mode-locked Ti:Sapphire laser was used as a light source. The center wavelength was 780 nm, the repetition rate was 380 kHz, the energy per pulse was 55 nJ, and the second harmonic pulse (390 nm) was generated by using a 100 μm LBO crystal. The time-resolved profilse were obtained by using a fluorescence upconversion that was previously described. To compensate the group velocity dispersion (GVD) and minimize the group velocity mismatch (GVM) effect, we used fused silica prism pairs and non-collinear phase-matching type upconversion setup. Then, its time-resolution was below ~100 fs by using a 500 μm BBO crystal.

Sample preparation for gel electrophoresis

Gel electrophoresis of samples (6 μL) of the same concentration in the loading buffer was performed under non-denaturing or denaturing conditions using a 15% polyacrylamide gel [non-denaturing conditions: 30% acrylamide (4 mL), 5XTBE buffer (2 mL), $(NH_4)_2S_2O_8$ (0.1 g), *N*,*N*,*N*′,*N*′-tetramethylethylenediamine (10 μL), and distilled water (2.4 mL); denaturing conditions: 30% acrylamide (4 mL), 5XTBE buffer (2 mL), $(NH_4)_2S_2O_8$ (0.1 g), urea (4.2 g), *N*,*N*,*N*′,*N*′-tetramethylethylenediamine (10 μL)]. Separation conditions: 200 V; 20 mA; 200 W; room temperature. To obtain the images displayed in the Figures, the gel was displayed on a DNA image visualizer without staining. The photographic images were taken using a camera and the images were analyzed using Photoshop CS2 software.

Experimental procedures for the synthesis of pyrene-labeled deoxyguanosine derivatives

8-Bromo-2'-deoxyguanosine (2)

N-**B**romosuccinimide (4.9 g, 28 mmol, 1.5 eq) was added to a solution of 2´-deoxyguanosine (**1**, 5.00 g, 18.7 mmol) in water (200 mL). After stirring for 2 h at room temperature, the solution was filtered through suction filtration and the filter cake washed with cold water and EtOAc. The filter cake was precipitated with excess acetone and then filtered to obtain a yellow solid (6.02 g, 93%). TLC: R_f 0.32 (MeOH/$CHCl_3$, 1:4). ^{1}H NMR (300 MHz, DMSO-d_6): δ 10.78 (s, 1H; N_1-H), 6.48 (s, 2H, C_2-NH_2), 6.16 (dd, J=6.9, 7.5, 1H, C_1'-H) 4.38-4.36 (ddd, J=3.0, 3.0, 6.0, 1H, C_3'-H), 3.80-3.78 (ddd, J=3.1, 5.5, 5.5, 1H, C_4'-H), 3.50 (dd, J=5.8, 11.6, 1H, C_5'-H), 3.16-3.14 (d, J=6.9, 1H, C_2'-H), 2.11-2.08 (ddd, J=3.6, 6.5, 13.3, 1H, C_3'-H). ^{13}C NMR (75 MHz, $CDCl_3$): δ 156.3, 154.2, 152.9, 121.4, 118.4, 112.7, 88.8, 85.9, 71.9, 62.9, 37.3, 31.6). FAB MS (m/z): [M + Na]+ calcd for 368.00; found, 368.00.

5'-Dimethoxytrityl-N-dimethylformamidine-8-Bromo-2'-deoxyguanosine (4)

N,*N*-Dimethylformamide dimethylacetal (0.580 µL, 4.34 mmol) was added to a solution of **2** (1.00 g, 2.89 mmol) in DMF under Ar and then the mixture was stirred for 2 h at room temperature, until TLC revealed the presence of the nonpolar product. The DMF was evaporated under high vacuum. The residue was dissolved in pyridine (15 mL) and dimethyltritylchloride (1.08 g, 3.18 mmol) was added; the mixture was stirred for 6 h at room temperature. 5% $NaHCO_3$ was added to the solution and the product was extracted with excess EtOAc. The organic phase was washed twice with water. After evaporation of the solvent under reduced pressure, the residue was subjected to column chromatography (SiO2; only ethyl acetate) to yield product 2 (1.38g, 69%). ^{1}H NMR (300 MHz, $CHCl_3$-d_6): δ 8.33 (s, 1H; N_1-H, formamidine), 7.41-7.15 (m, 9H, DMT), 6.84-6.73 (m, 4H: DMT), 6.37 (d, J=7.3, 1H, C_1'-H) 4.12-4.10 (m, 1H, C_3'-H), 3.78 (ddd, J-3.1, 5.5, 5.5, 1H, C_4'-H), 3.75 (s, 6H, OCH_3), 3.45 (dd, J=5.8, 11.6, 1H, C_5'-H), 3.22 (m, 1H, C_2'-H), 3.07 (s, 6H, N_2-H), 2.03 (ddd, J=3.6, 6.5, 13.3, 1H, C_3'-H). ^{13}C NMR (75 MHz, $CDCl_3$): δ 158.9, 137.90, 130.4, 128.9, 128.5, 113.5, 72.8, 55.6, 41.80, 31.35, 20.19). FAB MS (m/z): [M + Na]+ calcd for 725.17; found, 725.17.

5'-Dimethoxytrityl-N-dimethylformamidine-8-(1-pyrenylethynyl)-2'-deoxyguanosine (5)

$(PPh_3)_4Pd$ (72 mg, 0.062 mmol) and CuI (12 mg, 0.062 mmol) were added to a solution of **4** (877 mg, 1.25 mmol) in DMF under Ar and then the mixture was stirred for 5 min at room temperature. 1-Ethynylpyrene (310 mg, 1.37 mmol) was added to this solution. After degassing, the reaction mixture was stirred at 50 °C for 5 h and monitored by TLC. 5% $NaHCO_3$ was added to the solution and the product was extracted with excess EtOAc. The organic phase was washed twice with water. After evaporation of the solvent under reduced pressure, the residue was subjected to column chromatography (SiO_2; EtOAc) to yield the title compound (963 mg, 91%). ^{1}H NMR (300 MHz, DMSO-d_6): δ 8.40-7.87 (m, 9H; Ar_{PY}-H), 7.41-7.15 (m, 9H, DMT), 6.84.-6.73 (m, 4H: DMT), 6.47 (d, J=7.3, 1H, C_1'-H) 4.15-4.12 (m, 1H, C_3'-H), 3.70 (m, 1H, C_4'-H), 3.60 (s, 6H, OCH_3), 3.50 (dd, J=5.8, 11.6, 1H, C_5'-H), 3.30 (m, 1H, C_2'-H), 2.98 (s, 6H, N_2-H), 2.03 (m, 1H, C_3'-H). ^{13}C NMR (75 MHz, $CDCl_3$): δ 158.7,158.3, 145.2, 136.3, 131.7, 128.9, 126.9, 113.2, 96.3, 86.3, 84.5, 60.8, 55.7, 41.9, 30.9, 14.6). FAB MS (m/z): [M + Na]+ calcd for 873.34; found, 873.34.

5'-Dimethoxytrityl-N-dimethylformamidine-8-(1-pyrenylethynyl)-2'-deoxyguanosine 3'-[(2-cyanoethyl)-(N,N-diisopropyl)]-phosphoramidite (6)

4-Methylmorpholine (197 □L, 1.763 mmol) was added to a solution of **5** (250 mg, 0.294 mmol) in CH_2Cl_2 (10 mL) under N_2 and then the mixture was stirred at room temperature for 30 min. 2-Cyanoethyldiisopropyl-aminochlorophosphoramidite (194 □L, 0.881 mmol) was added and the mixture was stirred for 1 h and monitored by TLC. The solution was washed with 5% $NaHCO_3$ and the residue from the organic phase was purified chromatographically through a short column (SiO_2; EtOAc/pyridine, 99:1) to yield a yellow powder (290 mg, 85%). ^{1}H NMR (300 MHz, DMSO-d_6): δ 9.31 (s, 1H; N_1-H), 8.58-8.57 (t, *J* = 6.8 Hz, 2H; $Ar_{PY}H$), 8.40 (s, *J* = 9.2 Hz, 1H; $Ar_{PY}H$), 8.09-7.98 (m, 4H; $Ar_{PY}H$) 7.26–7.06 (m, 9H; DMT), 6.60–6.53 (m,4H; DMT), 5.85 (s, 1H, C_1'-H) 4.16-4.09 (m, 3H, C_1'-H, C_4'-H, C_3'-H), 3.73-3.60(NCH,OCH2, C_5'-H), 3,53-3.42 (s, 3H,OCH_3 + s, 3H,OCH_3), 3.40 (d, 2H, *J* = 9.2 Hz, H5). 2.71 (m, 2H, CH2CN), 1.24–1.15 (2d, 12H, *J* = 6.8 Hz; NCHCH3), 0.93 (s, 9H, t-Bu). ^{13}C NMR (75 MHz, $CDCl_3$): δ 157.8, 157.4 156.3, 149.3, 144.3, 135.3, 130.6, 129.5, 127.7, 127.2, 126.7, 126.0, 124.0, 116.0, 57.7, 54.6, 44.8, 42.9, 40.7, 34.7, 24.0, 22.5,19.9. ^{31}P NMR (121 MHz, $CDCl_3$), δ 150.2, 150.0, FAB MS (m/z): [M + Na]+ calcd for 1073.45; found, 1073.45.