7-Deaza-7-[3-(1-pyrenecarboxamido)propynyl]-2'-deoxyadenosine (2)

To a solution of 7-iodo-deazaadenosine 1 (60.0 mg, 0.160 mmol) and pyrene derivative (90.6 mg, 0.320 mmol) in dry DMF (4.0 ml) was added copper(I) iodide (3.0 mg, 0.016 mmol), tetrakis(triphenylphosphine)palladium(0) (18.5 mg, 0.016 mmol) and triethylamine (32.3 mg, 0.320 mmol). The reaction mixture was stirred at room temperature for 6 h. After concentration of the mixture to dryness, the residue was purified by silica gel column chromatography (CHCl₃/MeOH 8/1) to afford 2 (88 %, 74.4 mg, 0.140 mmol) as a pale yellow solid. ¹H NMR (CDCl₃, 400MHz) δ 2.34 (ddd, 1H, J = 2.8, 6.0, 13.6 Hz), 2.64 (ddd, 1H, J = 6.0, 8.0, 13.6 Hz), 3.73 (dd, 1H, J = 3.6, 12.0 Hz), 3.81 (dd, 1H, J = 3.2, 12.0 Hz), 4.01 (m, 1H), 4.52 (m, 1H), 4.55 (s, 2H), 6.49 (dd, 1H, J = 6.0, 8.0 Hz), 7.65 (s, 1H), 8.04-8.28 (complex, 10H), 8.52 (d, 1H, J = 9.2Hz); ¹³C NMR (pyridine-d₅, 100 MHz) δ 30.7, 41.1, 62.5, 71.5, 76.2, 79.2, 84.7, 88.6, 89.1, 95.5, 103.8, 124.1, 124.4, 124.9, 125.3, 125.4, 125.6, 126.0, 126.2, 127.0, 128.2, 128.3, 128.7, 130.5, 130.9, 131.2, 132.2, 149.7, 153.1, 158.5, 170.1; UV λ_max (MeOH) 266 nm (ε = 23100), 275 nm (ε = 35700), 326 nm (ε = 15700), 340 nm (ε = 22000); FABMS (NBA/CH₃OH), m/z 532 ([M+H]^+), HRMS calcd. for C₃₁H₂₆N₅O₄ ([M+H]^+) 532.1985, found 532.1981.

7-Deaza-7-(1-pyrenecarboxamido)propyl-2'-deoxyadenosine (3)

A mixture of 2 (71.1 mg, 0.134 mmol) and 10% Pd/C (14.2 mg) in MeOH (4.0 ml) was stirred under hydrogen atmosphere for 2 h. The reaction mixture was filtered through a celite pad and washed with MeOH (10 mLx2), the filtrate and the washings were combined. After evaporation of the solvent, the residue was purified by silica gel column chromatography (CHCl₃/MeOH 8/1) to afford 3 (86 %, 61.8 mg, 0.115 mmol) as a colorless waxy solid. ¹H NMR (CD₃OD, 400MHz) δ 2.13 (tt, 2H, J = 7.2, 7.2 Hz), 2.25 (ddd, 1H, J = 2.8, 6.0, 13.6 Hz), 2.26 (ddd, 1H, J = 5.6, 6.0, 13.6 Hz), 3.01 (t, 2H, J = 7.2 Hz), 3.67 (t, 2H, J = 7.2 Hz), 3.70 (dd, 1H J = 3.6, 12.0 Hz), 3.78 (dd, 1H, J = 3.2 12.0 Hz), 3.98 (ddd, 1H, J = 3, 12.8 Hz), 4.50 (m, 1H), 6.49 (dd, 1H, J = 6.0, 8.4 Hz), 7.27 (s, 1H), 8.06-8.30 (complex, 9H), 8.46 (d, 1H, J = 9.2 Hz); ¹³C NMR (pyridine-d₅, 100 MHz) δ 18.5, 38.7, 41.0, 49.0, 56.7, 62.7, 71.6, 85.0, 88.6, 102.9, 111.1, 124.1, 124.5, 125.1, 125.2, 125.3, 125.5,
4-N-(N,N-Dimethylaminomethylidienyl)-7-deaza-7-(1-pyrenecarboxamido)propyl-2'-deoxyadenosine (4)

To a solution of 3 (104.7 mg, 0.195 mmol) in anhydrous DMF (3.0 ml) was added N,N-dimethylformamide diethyl acetal (0.4 ml). The reaction mixture was stirred at 50 °C for 3h. After concentration of the solution to dryness, the residue was purified by silica gel column chromatography (CHCl₃/MeOH 8/1) to afford 4 (84 %, 96.8 mg, 0.163 mmol) as a pale yellow waxy solid. ¹H NMR (CD₃OD, 400MHz) δ 2.18 (tt, 2H, J = 6.8, 7.2 Hz), 2.27 (ddd, 1H, J = 2.8, 6.0, 13.2 Hz), 2.69 (ddd, 1H, J = 2.8, 8.0, 13.2 Hz), 3.06 (s, 3H), 3.08 (s, 3H), 3.12 (t, 2H, J = 7.2 Hz), 3.63 (t, 2H, J = 6.8 Hz), 3.72 (dd, 1H J = 4.0, 12.0 Hz), 3.79 (dd, 1H, J = 3.6 12.0 Hz), 3.99 (m, 1H), 4.51 (m, 1H), 6.54 (dd, 1H, J = 6.0, 8.0 Hz), 7.31 (s, 1H), 7.98-8.30 (complex, 9H), 8.39 (d, 1H, J = 9.2 Hz), 8.55 (s, 1H). ¹³C NMR (pyridine-d₅, 100 MHz) δ 24.3, 31.5, 33.9, 39.6, 39.6, 40.6, 62.9, 71.8, 84.6, 88.4, 111.3, 116.5, 121.1, 124.1, 124.2, 124.4, 125.0, 125.1, 125.3, 125.4, 126.1, 127.0, 127.9, 128.0, 128.4, 130.5, 131.0, 131.8, 132.8, 151.1, 152.3, 156.3, 161.2, 169.5; FABMS (DTT-TG/CH₃OH), m/z 591 ([M+H]+), HRMS calcd. for C₃₄H₃₅N₆O₄ ([M+H]+) 591.2720, found 591.2725.

4-N-(N,N-Dimethylaminomethylidienyl)-5'-O-(4,4'-dimethoxytrityl)-7-deaza-7-(1-pyrenecarboxamido)propyl-2'-deoxyadenosine (5)

To a solution of 4 (60.8 mg, 0.103 mmol) in pyridine (3.0 ml) was added 4,4'-dimethoxytrityl chloride (38.3 mg, 0.113 mmol) and dimethylaminopyridine (2.5 mg, 0.020 mmol). The reaction mixture was stirred at room temperature for 8 h. After concentration of the solution to dryness, the residue was purified by silica gel column chromatography (CHCl₃/MeOH/Et₃N 100:10:1) to afford 5 (75 %, 69.0 mg, 0.077 mmol) as a pale yellow foam. ¹H NMR (CD₃OD, 400MHz) δ 1.86 (tt, J = 6.8, 6.8 Hz), 2.40 (ddd, 1H, J = 3.6, 6.4, 13.2 Hz), 2.60 (s, 3Hx2), 2.92-3.07 (complex, 3H), 3.36 (dd, 1H, J = 3.2, 10.0 Hz) 3.41 (dd, 1H, J = 4.0, 10.0 Hz), 3.69 (s, 3Hx2), 4.07
Oligonucleotide (ODN) synthesis and characterization.

ODNs were synthesized by a conventional phosphoramidite method by using Applied Biosystems 392 DNA/RNA synthesizer. ODNs were purified by reverse phase HPLC on a 5-ODS-H column (10 X 150 mm, elution with 50 mM ammoniumformate buffer, linear gradient over 45 min from 3 % to 20 % acetonitrile at a flow rate 2.0 ml/min). ODNs containing modified nucleotides 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 3h. Digested solutions were analyzed by HPLC on a CHEMCOBOND 5-ODS-H column (4.6x150 mm, elution with a solvent mixture of 50 mM ammoniumformate buffer, linear gradient over 60 min from 3 % to 50 % acetonitrile at a flow rate of 1.0 ml/min). Concentration of each ODN was determined by comparing peak areas with standard solution containing dA, dC, dG, and dT at a concentration of 0.1 mM. **ODN(PyA)** 5’-d(CGCAATPyATAACGC)-3’: MALDI-TOF [(M-H)−] calcd. 4211.00, found 4211.66. **ODNBRCA1(PyA)** 5’-d(GGTACCAPyATGAAAA)-3’: MALDI-TOF [(M-H)−] calcd. 4892.46, found 4893.27.
Fig. 1(sup.) UV and excitation spectra of ODN(PyA). a) UV spectra of ODN(PyA) hybridized with 2.5 µM ODN (A), ODN (C), ODN (G), or ODN (T) (50 mM sodium phosphate, 0.1 M sodium chloride, pH 7.0, room temperature). Excitation was at 350 nm. b) Excitation spectra of ODN(PyA) hybridized with 2.5 µM ODN (A), ODN (C), ODN (G), or ODN (T).
Fig. 2(sup.) UV and excitation spectra of ODN\textsubscript{BRCA1}(PyA). a) UV spectra of ODN\textsubscript{BRCA1}(PyA) hybridized with 2.5 µM ODN\textsubscript{BRCA1} (T) or ODN\textsubscript{BRCA1} (C) (50 mM sodium phosphate, 0.1 M sodium chloride, pH 7.0, room temperature). Excitation was at 350 nm. b) Excitation spectra of ODN\textsubscript{BRCA1}(PyA) hybridized with 2.5 µM ODN\textsubscript{BRCA1} (T) or ODN\textsubscript{BRCA1} (C).
Fig. 3 (sup.) UV-vis spectra of 2.5 µM ODN(PyA) hybridized with 2.5 µM ODN(T) at different temperatures (20, 30, 40, 50, 60, 70, 80 °C). UV-vis spectra were recorded under the same conditions described in Figure 1.