Electronic Supplementary Material (ESI) for Chemical Communications

Supplementary Information

Monitoring i-motif transitions through the exciplex emission of a fluorescent probe incorporating two ^{Py}A units

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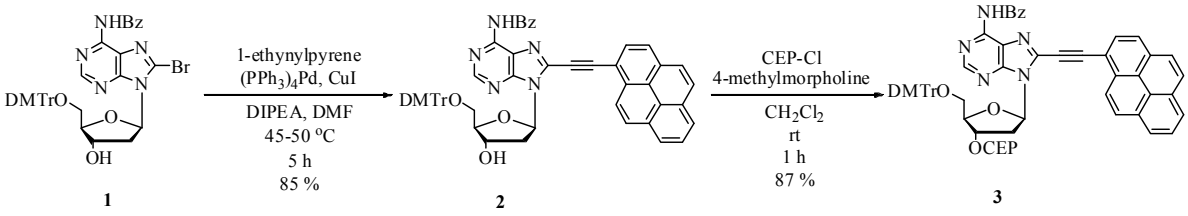
Synthesis of ^{Py}A



Scheme 1 Synthesis of pyrene-labeled deoxyadenosine derivatives.

***N*$_6$-Benzoyl-5′-*O*-[bis(4-methoxyphenyl)phenylmethyl]-8-(1-ethynylpyrenyl)-2′-deoxyadenosine (2).** $(PPh_3)_4Pd$ (18 mg, 0.026 mmol) and CuI (10 mg, 0.053 mmol) were added to a solution of **1** (190 mg, 0.26 mmol) in DMF (3 mL) under Ar and the mixture was stirred for 5 min at room temperature. 1-Ethynylpyrene (60 mg, 0.26 mmol) and *N*,*N*-diisopropylethylamine (68 µL, 0.39 mmol) were added to this solution. After degassing, the reaction mixture was stirred at 45–50 °C for 5 h and monitored by TLC. Water was added to the solution and the product was extracted with excess EtOAc. The organic phase was washed twice with water. After evaporation of solvent in vacuo, the residue was subjected to chromatography (SiO_2; hexane/EtOAc, 1:1) to yield **2** (188 mg, 85 %). M.p. 140–141 °C. $[\alpha]^{23}_D$ = +33° (*c* = 0.525, CH_2Cl_2). IR (film): ν 3366, 2923, 2203, 1684, 1582, 1508, 1464, 1339, 1174, 846, 702 cm^{-1}. ^{1}H NMR (300 MHz, $CDCl_3$): δ 9.15 (s, 1H; NH), 8.64 (s, 1H; H-2), 8.25–7.15 (m, 23H; ArH), 6.89 (t, *J* = 6.9 Hz, 1H; H-1'), 6.68–6.73 (m, 4H; ArH), 5.10 (m, 1H; H-3'), 4.22 (dd, *J* = 10.1, 6.0 Hz, 1H; H-4'), 3.70 (s, 6H; OCH_3), 3.71–3.65 (m, 1H; H-2'), 3.47–3.44 (m, 2H; H-5'), 2.45–2.34 (m, 1H; H-2'), 2.34 (br s, 1H; OH). ^{13}C NMR (75 MHz, $CDCl_3$): δ 163.9, 158.0, 157.9, 152.5, 148.7, 144.2, 137.4, 135.5, 135.3, 133.3, 132.5, 132.3, 130.5, 130.3, 129.6, 129.5, 129.4, 129.0, 128.4, 127.7, 127.4, 127.3, 127.1, 126.7, 126.6, 126.3, 126.2, 125.9, 124.4, 124.0, 123.7, 123.4, 113.5, 112.6, 96.8, 85.9, 85.0, 82.5, 72.7, 63.6, 54.7, 36.9. HRMS–FAB (*m/z*): $[M + H]^+$ calcd for $C_{56}H_{44}N_5O_6$, 882.3294; found, 882.3288.

***N*$_6$-Benzoyl-5′-*O*-[bis(4-methoxyphenyl)phenylmethyl]-8-(1-ethynylpyrenyl)-3′-{bis[1-(methylethyl)phosphoramidyl]cyanoethyl}-2′-deoxyadenosine (3).** 4-Methylmorpholine (55 µL, 0.501 mmol) was added to a solution of **2** (150 mg, 0.16 mmol) in CH_2Cl_2 (2 mL) under nitrogen and the mixture was stirred at room temperature for 5 min. 2-Cyanoethyldiisopropylaminochlorophosphoramidite (48 µL, 0.217 mmol) was added and then the mixture was stirred for 1 h and monitored by TLC. The solvent was evaporated in vacuo and the residue was purified by chromatography on a short column (SiO_2; hexane/EtOAc/Et_3N, 97:97:6) to yield **3** (160 mg, 87%). M.p. 110–112 °C. $[\alpha]^{23}_D$ = +81° (*c* = 1.06, $CHCl_3$). IR (film): ν 3447, 2968, 2932, 1702, 1608, 1580, 1508, 1463, 1250, 1178, 1117, 1087, 979, 849, 829, 706 cm^{-1}. ^{1}H NMR (300 MHz, $CDCl_3$): δ 9.10 (s, 1H; NH), 8.65–8.05 (m, 9H; $Ar_{py}H$), 7.56–7.14 (m, 14H; ArH), 6.68 (dd, *J* = 11.9, 6.3 Hz, 1H; H-1'), 6.71–6.65 (m, 4H; ArH), 5.27–4.98 (m, 1H; H-3'), 4.43–4.40 (m, 1H; H-4'), 3.74–3.41 (m, 13H; OCH_3, H-2', NCH, OCH_2, H-5'), 2.67–2.54 (m, 1H; H-2'), 2.52–2.36 (m, 2H; CH_2CN), 1.15–1.06 (m, 12H; NCHCH_3). ^{13}C NMR (75 MHz, $CDCl_3$): δ 163.9, 157.9,

152.5, 150.4, 148.7, 144.3, 137.7, 135.5, 135.4, 133.4, 132.6, 132.4, 132.3, 130.7, 130.4, 129.9, 129.6, 129.5, 129.0, 128.4, 127.7, 127.4, 127.2, 126.7, 126.2, 125.9, 124.5, 124.1, 123.9, 123.5, 122.8, 117.0, 113.6, 112.5, 96.8, 85.7, 85.4, 85.3, 82.7, 73.5, 63.4, 63.2, 59.9, 58.2, 57.9, 54.7, 42.9, 24.0, 19.7. ^{31}P NMR (121 MHz, $CDCl_3$): δ 151.6, 151.3. MS (FAB) *m/z*: 1082.3 $[M + H]^+$.

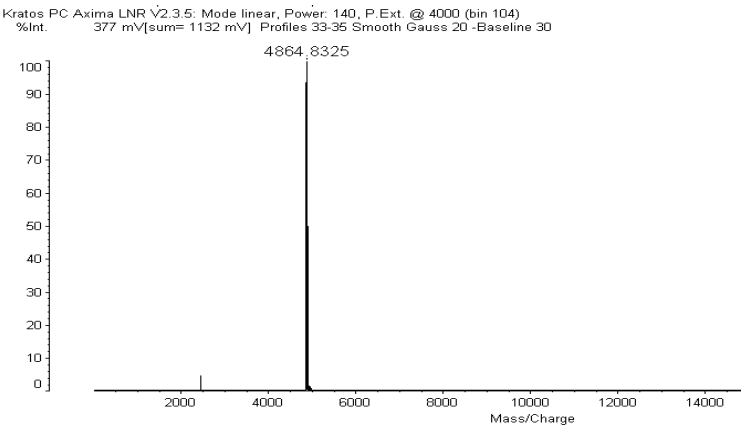
Solid-phase oligonucleotide synthesis

The phosphoramidite ^{Py}**A** was introduced as building block to produce fluorescent oligodeoxynucleotides (ODNs) on a controlled-pore glass (CPG) solid support by using the phosphoramidite approach and an automated DNA synthesizer (POLYGEN DNA-Synthesizer). For comparison, the unmodified ODNs were also prepared. The synthesized oligonucleotides were cleaved from the solid support upon treatment with 30% aqueous NH_4OH (1.0 mL) for 10 h at 55 °C. The crude products from the automated ODN synthesis were lyophilized and diluted with distilled water (1 mL). The ODNs were purified by HPLC (Merck LichoCART C18 column; 10 X 250 mm; 10 μm; pore size: 100 Å). The HPLC mobile phase was held isocratically for 10 min with 5% acetonitrile/0.1 M triethylammonium acetate (TEAA) (pH 7.0) at a flow rate of 2.5 mL/min. The gradient was then increased linearly over 10 min from 5% to 50% acetonitrile/0.1 M TEAA at the same flow rate. The fractions containing the purified ODN were cooled and lyophilized. 80% aqueous AcOH was added to the ODN. After 30 min at ambient temperature, the AcOH was evaporated under reduced pressure. The residue was diluted with water (1 mL), and this solution was then purified by HPLC using the same conditions as those described above. The ODNs were analyzed by HPLC (Hewlett-Packard, ODS Hypersil; 4.6 X 200 mm; 5 m; 79916OD-574) using almost the same eluent system.

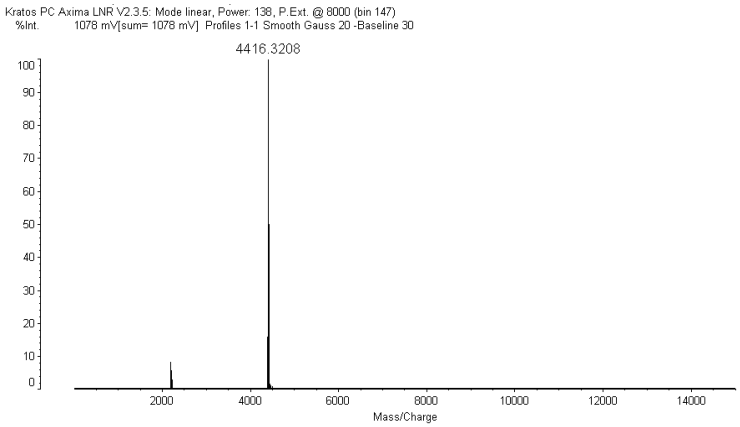
Table S1 MALDI-TOF mass spectral data.

Sequence	MALDI-TOF signal [M^+]	
	Calc. *m*/*z*	**Found *m*/*z***
ODN I	4864.79	4864.8325
ODN N	4416.97	4416.3208
ODN G	4728.15	4728.9314

(a)



(b)



(c)

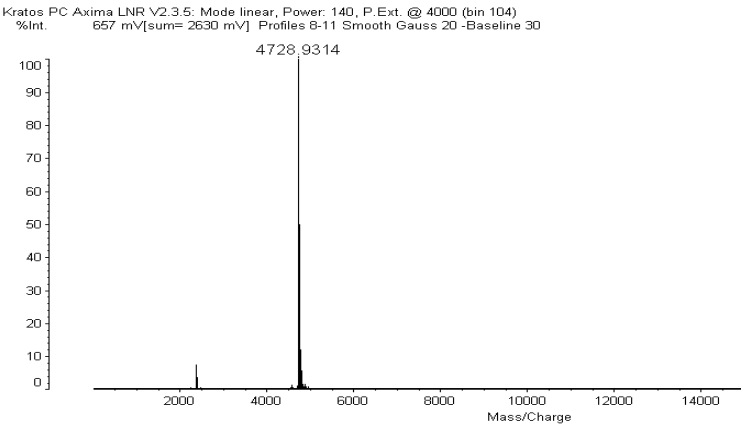


Figure S1 MALDI-TOF mass spectra of (a) **ODN I**, (b) **ODN N**, (c) **ODN G**.

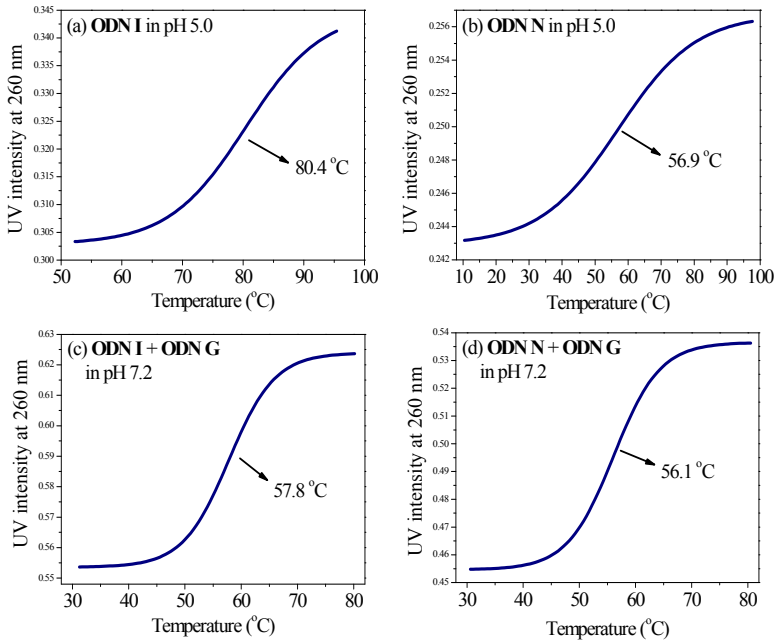


Figure S2 UV melting curves of (a) **ODN I** at pH 5.0 (i-motif structure), (b) **ODN N** at pH 5.0 (i-motif structure), (c) **ODN I** + **ODN G** at pH 7.2 (duplex), and (d) **ODN N** + **ODN G** at pH 7.2 (duplex). All DNA samples were prepared at a concentration of 1.5 μM in 100 mM Tris-HCl buffer and irradiated at 260 nm.