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Supplementary Information

Detection of a single DNA base-pair mismatch using an anthracene-tagged fluorescent probe

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1. Monomer synthesis

Anthracen-9-yloxy acetic acid ethyl ester (**1**) and anthracen-9-yloxy acetic acid (**2**) were prepared as described by Molard *et al.* in *J. Org. Chem.*, 2006, **71**, in press (Web release date: 30/9/2006).

2-(Anthracen-9-yloxy)-N-(1-hydroxymethyl-2-hydroxy-ethyl)-acetamide (**3**)

Anthracen-9-yloxy acetic acid (**2**) (1.52 g, 6 mmol) was dissolved in anhydrous DMF (30 ml). HOBt (0.88 g, 6.5 mmol) was added to the soln. followed by DIPC (0.86 ml, 5.5 mmol) and the soln. stirred under N₂ at r.t. in the absence of light for 15 mins. Serinol (0.5 g, 5.5 mmol) and DIEA (0.95 ml, 5.5 mmol) were added and the reaction left to stir at 40 °C for 40 hours. The soln. was diluted in MeOH/DCM (1:2) and washed with H₂O (3 x 50 ml) and dried over MgSO₄. Purification by silica column chromatography (DCM with 5 % MeOH) gave the desired product as a pale yellow solid (1.21 g, 67 %). (*R*_f 0.17 in DCM with 5 % MeOH); M.p. 149 °C (found: C, 70.3; H, 5.9; N, 4.2 %. C₁₉H₁₉O₄N requires C, 70.1; H, 5.9; N, 4.3 %); ¹H (400 MHz, CDCl₃/CD₃CN, 1:1) 8.11 (1H, s, *H*₁₀), 8.01 (2H, d, *J* = 7.9, *H*₁), 7.84 (2H, d, *J* = 7.3, *H*₄), 7.53 (1H, s, *NH*), 7.31 (4H, m, *H*₂ and *H*₃), 4.45 (2H, s, OCH₂CO), 3.95 (1H, m, CHNH), 3.64 (4H, m, CHCH₂OH), 3.20 (2H, s, 2 x OH); ¹³C (100 MHz, CDCl₃/CD₃CN, 1:1) 169.0 (*C* of C₁₄H₉), 149.2 (*C* of C₁₄H₉), 132.5 (*C* of C₁₄H₉), 128.8 (CH of C₁₄H₉), 126.2 (CH of C₁₄H₉), 126.0 (CH of C₁₄H₉), 124.4 (CO), 123.5 (CH of C₁₄H₉), 121.8 (CH of C₁₄H₉), 73.8 (OCH₂CO), 62.1 (2 x CH₂OH), 52.9 (NHCH); *m/z* (EI) calcd for C₁₉H₁₉O₄N (*M*⁺) 325.1314, found 325.1308.

2-(Anthracen-9-yloxy)-N-(1-methoxy-dimethoxytrityl-2-hydroxy-ethyl)-acetamide (4)

2-(Anthracen-9-yloxy)-N-(1-hydroxymethyl-2-hydroxy-ethyl)-acetamide (**3**) (1 g, 3 mmol) was dissolved in anhydrous pyridine (20 ml). Dimethoxytritylchloride (1.04 g, 3 mmol) was added to the soln. followed by DMAP (0.038 g, 0.3 mmol) and the reaction left to stir under N₂ at r.t. in the absence of light for 24 hours. The reaction mixture was poured onto H₂O (50 ml), extracted with DCM (2 x 50 ml) and dried over MgSO₄. Column chromatography on silica (Hexane/EtOAc/TEA, 40:59:1) afforded the desired compound as a pale yellow crystalline solid (0.81 g, 42 %). (R_f 0.38 in DCM with 5 % MeOH); M.p. 89 °C (found: C, 75.9; H, 6.1; N, 2.1 %. C₄₀H₃₇O₆N.0.5H₂O requires C, 75.5; H, 6.0; N, 2.2 %); ¹H (400 MHz, CD₃CN) 8.32 (1H, s, H₁₀), 8.22 (2H, d, *J* = 7.6, H₁), 8.03 (2H, d, *J* = 7.3, H₄), 7.66 (1H, s, NH), 7.48 (4H, m, H₂ and H₃), 7.40 (9H, m, trityl), 6.79 (4H, m, trityl), 4.63 (2H, s, OCH₂CO), 4.32 (1H, m, CHNH), 3.81 (2H, m, CHCH₂OH), 3.67 (6H, s, 2 x OCH₃), 3.39 (1H, s, OH), 3.31 (2H, m, CH₂ODMTr); ¹³C (100 MHz, CD₃CN) 168.2 (C of C₁₄H₉), 158.6 (C of trityl), 149.1 (C of C₁₄H₉), 145.2 (C of trityl), 136.1 (C of trityl), 136.0 (C of trityl), 132.3 (C of C₁₄H₉), 130.0 (CH of trityl), 129.9 (CH of trityl), 128.5 (CH of C₁₄H₉), 128.0 (C of trityl), 127.9 (C of trityl), 126.8 (CH of C₁₄H₉), 126.0 (CH of C₁₄H₉), 125.8 (C of trityl), 124.1 (CO), 123.2 (CH of C₁₄H₉), 121.6 (CH of C₁₄H₉), 113.1 (NHCH), 73.6 (OCH₂CO), 62.3 (CH₂OH), 61.5 (CH₂ODMTr), 54.8 (OCH₃), 51.4 (OCH₃); *m/z* (EI) calcd for C₄₀H₃₇O₆N (M⁺) 627.2621, found 627.2614.

2-(Anthracen-9-yloxy)-N-(1-methoxy-dimethoxytrityl-2-cyanoethyl(diisopropylamino) -ethyl)-acetamide (5)

All glassware dried in an oven prior to use. Basic alumina activated in furnace at 250 °C and allowed to cool in a vacuum dessicator.

2-(Anthracen-9-yloxy)-*N*-(1-methoxy-dimethoxytrityl-2-hydroxy-ethyl)-acetamide (**4**) (0.69 g, 1.1 mmol) placed in a 25 ml round-bottomed flask with a stirrer bar and a septum. The flask was evacuated and filled with N₂ three times and the solid dissolved in anhydrous DCM (15 ml). DIEA (0.96 ml, 5.5 mmol) was added and the solution stirred at r.t. in the absence of light. 2-Cyanoethyl-diisopropylchlorophosphoramidite (0.3 ml, 1.3 mmol) was added *via* a disposable syringe dropwise and the reaction stirred for 15 hr. The soln. was then transferred *via* a schlenk needle to a 25 ml round-bottomed flask containing a stirrer bar and solid-supported BnOH (0.044 g, 0.11 mmol) and left to stir for 1 hr in the absence of light. The soln. was diluted with EtOAc (10 ml), filtered and then washed with 2 M Na₂CO₃ (a.q.) soln. (2 x 50 ml), H₂O (1 x 50 ml) and Brine (1 x 50 ml) and dried over Na₂SO₄. The soln. was then filtered through activated basic alumina with MeOH and the filtrate evaporated. Co-evaporation with acetonitrile three times removed final traces of DIEA to give the phosphoramidite as a yellow solid (0.53 g, 76 %). ³¹P (100 MHz, CD₃CN) 148.7, 148.3; ¹H (400 MHz, CD₃CN) 8.29 (1H, s, *H*₁₀), 8.14 (2H, m, *H*₁), 7.98 (2H, d, *J* = 8.3, *H*₄), 7.43 (4H, m, *H*₂ and *H*₃), 7.29 (9H, m, *trityl*), 6.74 (4H, m, *trityl*), 4.56 (2H, s, OCH₂CO), 4.37 (1H, m, CHNH), 3.87 (2H, m, CH₂OP), 3.62 (6H, s, 2 x OCH₃), 3.45 (2H, m, CH₂CH₂CN), 3.25 (2H, m, CH₂ODMTr), 2.59 (2H, m, CH(CH₃)₂), 2.48 (2H, m, CH₂CH₂CN), 1.20 (12H, m, CH(CH₃)₂); ¹³C (100 MHz, CD₃CN) 167.7 (*C* of C₁₄H₉), 158.7 (*C* of *trityl*), 158.6 (*C* of C₁₄H₉), 145.2 (*C* of *trityl*), 136.1 (*C* of *trityl*), 136.0 (*C* of *trityl*), 132.3 (*C* of C₁₄H₉), 130.1 (CH of *trityl*), 130.0 (CH of *trityl*), 128.0 (CH of C₁₄H₉), 127.9 (*C* of *trityl*), 126.8 (*C* of *trityl*), 126.1 (CH of C₁₄H₉), 126.0 (CH of C₁₄H₉), 125.8 (*C* of *trityl*), 124.1 (CO), 123.2 (CH of C₁₄H₉), 121.6 (CH of C₁₄H₉), 113.1 (CN), 113.0 (NHCH), 85.9 (OCH₂CO), 73.6 (CH₂CH₂CN), 62.1 (CH₂CH₂CN), 62.0 (CH₂OP), 61.5 (CH₂ODMTr), 58.3 (OCH₃), 54.9 (OCH₃), 46.8 (CHCH₃), 42.9 (CHCH₃), 42.8 (CHCH₃), 42.8 (CHCH₃), 18.6 (CHCH₃), 17.4 (CHCH₃).

2. DNA synthesis and purification

Oligonucleotides **Probe 1a** and **Probe 1b** were synthesized on a Beckmann 1000M DNA synthesizer at Queen's University, Belfast, using 0.2 μM CPG columns, on a 1.0 μM scale synthesis. A high purity setting was used with extra coupling time for amidite **5** with the final DMTr group "OFF". The oligonucleotides were purified *via* reverse-phase HPLC using a linear gradient of Buffer A (0.1 M TEA acetate pH 6.5) and Buffer B (0.1 M TEA acetate in 65 % MeCN pH 6.5).

Probe 1a 5'-TGGACTCXCTCAATG-3'

m/z (MALDI-Tof) calcd for $\text{C}_{155}\text{H}_{192}\text{N}_{51}\text{O}_{89}\text{P}_{14}^+$ ($[\text{M} + 15\text{H}]^+$) 4624.8, found single broad cluster centred at 4630 m/z , retention time 19.49 mins, 0.23 mg, 5.0×10^{-8} mol, 12.4 % yield.

Probe 1b 5'-TGGACTCXCTCAATG-3'

m/z (MALDI-Tof) calcd for $\text{C}_{155}\text{H}_{192}\text{N}_{51}\text{O}_{89}\text{P}_{14}^+$ ($[\text{M} + 15\text{H}]^+$) 4624.8, found single broad cluster centred at 4629 m/z , retention time 18.32 mins, 0.27 mg, 6.0×10^{-8} mol, 14.9 % yield.

Data from HPLC analysis:

Figure 1S. C18 RP-HPLC chromatogram of crude mixture from synthesis.

Figure 2S. UV profiles of (a) Probe 1a and (b) Probe 1b, showing anthracene incorporation (1L_a band).

3. DNA Melting Studies

Each duplex was made up at $1\mu\text{M}$ in aqueous phosphate buffer (0.01 M phosphate, 0.2 M NaCl, pH 7.0).

Fig. 3S. Normalized thermal melt curves of duplexes studied (Temperature/ $^{\circ}\text{C}$)