Supplementary Material (ESI) for Chemical Communications

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 N_2 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 H_2O (3 x 50 ml) and dried over $MgSO_4$. 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 %. $C_{19}H_{19}O_4N$ requires C, 70.1; H, 5.9; N, 4.3 %); ^{1}H (400 MHz, $CDCl_3/CD_3CN$, 1:1) 8.11 (1H, s, *H10*), 8.01 (2H, d, *J* = 7.9, *H1*), 7.84 (2H, d, *J* = 7.3, *H4*), 7.53 (1H, s, N*H*), 7.31 (4H, m, *H2* and *H3*), 4.45 (2H, s, OCH_2CO), 3.95 (1H, m, C*H*NH), 3.64 (4H, m, CHCH_2OH), 3.20 (2H, s, 2 x O*H*); ^{13}C (100 MHz, $CDCl_3/CD_3CN$, 1:1) 169.0 (*C* of $C_{14}H_9$), 149.2 (*C* of $C_{14}H_9$), 132.5 (*C* of $C_{14}H_9$), 128.8 (*C*H of $C_{14}H_9$), 126.2 (*C*H of $C_{14}H_9$), 126.0 (*C*H of $C_{14}H_9$), 124.4 (*C*O), 123.5 (*C*H of $C_{14}H_9$), 121.8 (*C*H of $C_{14}H_9$), 73.8 (OCH_2CO), 62.1 (2 x *C*H_2OH), 52.9 (NH*C*H); *m/z* (EI) calcd for $C_{19}H_{19}O_4N$ (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 N_2 at r.t. in the absence of light for 24 hours. The reaction mixture was poured onto H_2O (50 ml), extracted with DCM (2 x 50 ml) and dried over $MgSO_4$. 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 %. $C_{40}H_{37}O_6N.0.5H_2O$ requires C, 75.5; H, 6.0; N, 2.2 %); ^{1}H (400 MHz, CD_3CN) 8.32 (1H, s, H_{10}), 8.22 (2H, d, J = 7.6, H_1), 8.03 (2H, d, J = 7.3, H_4), 7.66 (1H, s, NH), 7.48 (4H, m, H_2 and H_3), 7.40 (9H, m, *trityl*), 6.79 (4H, m, *trityl*), 4.63 (2H, s, OCH_2CO), 4.32 (1H, m, CHNH), 3.81 (2H, m, CHCH_2OH), 3.67 (6H, s, 2 x OCH_3), 3.39 (1H, s, OH), 3.31 (2H, m, CH_2ODMTr); ^{13}C (100 MHz, CD_3CN) 168.2 (*C* of $C_{14}H_9$), 158.6 (*C* of trityl), 149.1 (*C* of $C_{14}H_9$), 145.2 (*C* of trityl), 136.1 (*C* of trityl), 136.0 (*C* of trityl), 132.3 (*C* of $C_{14}H_9$), 130.0 (*C*H of trityl), 129.9 (*C*H of trityl), 128.5 (*C*H of $C_{14}H_9$), 128.0 (*C* of trityl), 127.9 (*C* of trityl), 126.8 (*C*H of $C_{14}H_9$), 126.0 (*C*H of $C_{14}H_9$), 125.8 (*C* of trityl), 124.1 (*C*O), 123.2 (*C*H of $C_{14}H_9$), 121.6 (*C*H of $C_{14}H_9$), 113.1 (NH*C*H), 73.6 (O*C*H_2CO), 62.3 (*C*H_2OH), 61.5 (*C*H_2ODMTr), 54.8 (O*C*H_3), 51.4 (O*C*H_3); *m/z* (EI) calcd for $C_{40}H_{37}O_6N$ (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 N_2 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 Na_2CO_3 (a.q.) soln. (2 x 50 ml), H_2O (1 x 50 ml) and Brine (1 x 50 ml) and dried over Na_2SO_4. 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 %). ^{31}P (100 MHz, CD_3CN) 148.7, 148.3; ^{1}H (400 MHz, CD_3CN) 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, OCH_2CO), 4.37 (1H, m, C*H*NH), 3.87 (2H, m, CH_2OP), 3.62 (6H, s, 2 x OCH_3), 3.45 (2H, m, CH_2CH$_2$CN), 3.25 (2H, m, CH_2ODMTr), 2.59 (2H, m, C*H*(CH$_3$)$_2$), 2.48 (2H, m, CH$_2$CH_2CN), 1.20 (12H, m, CH(CH_3)$_2$); ^{13}C (100 MHz, CD_3CN) 167.7 (*C* of $C_{14}H_9$), 158.7 (*C* of trityl), 158.6 (*C* of $C_{14}H_9$), 145.2 (*C* of trityl), 136.1 (*C* of trityl), 136.0 (*C* of trityl), 132.3 (*C* of $C_{14}H_9$), 130.1 (*C*H of trityl), 130.0 (*C*H of trityl), 128.0 (*C*H of $C_{14}H_9$), 127.9 (*C* of trityl), 126.8 (*C* of trityl), 126.1 (*C*H of $C_{14}H_9$), 126.0 (*C*H of $C_{14}H_9$), 125.8 (*C* of trityl), 124.1 (*C*O), 123.2 (*C*H of $C_{14}H_9$), 121.6 (*C*H of $C_{14}H_9$), 113.1 (*C*N), 113.0 (NH*C*H), 85.9 (O*C*H$_2$CO), 73.6 (*C*H$_2$CH$_2$CN), 62.1 (CH$_2$*C*H$_2$CN), 62.0 (*C*H$_2$OP), 61.5 (*C*H$_2$ODMTr), 58.3 (O*C*H$_3$), 54.9 (O*C*H$_3$), 46.8 (CH*C*H$_3$), 42.9 (CH*C*H$_3$), 42.8 (CH*C*H$_3$), 42.8 (CH*C*H$_3$), 18.6 (*C*HCH$_3$), 17.4 (*C*HCH$_3$).

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′*-TGGACTC**X**CTCAATG-*3′*

m/z (MALDI-Tof) calcd for $C_{155}H_{192}N_{51}O_{89}P_{14}^{+}$ ($[M + 15H]^{+}$) 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′*-TGGACTC**X**CTCAATG-*3′*

m/z (MALDI-Tof) calcd for $C_{155}H_{192}N_{51}O_{89}P_{14}^{+}$ ($[M + 15H]^{+}$) 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:

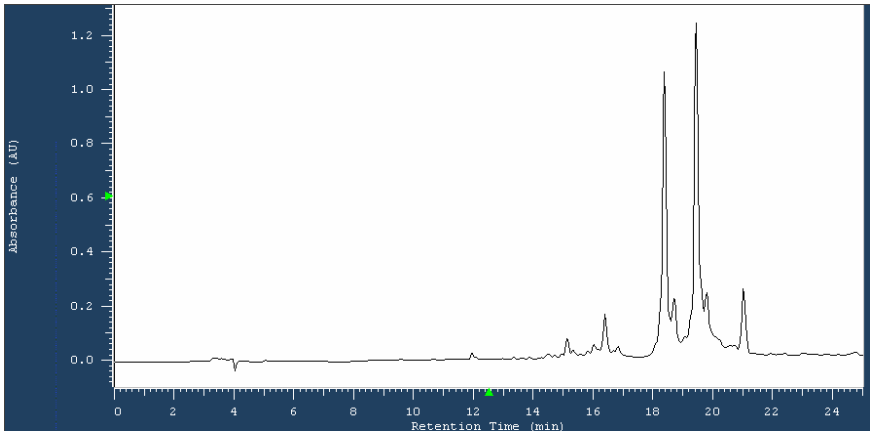
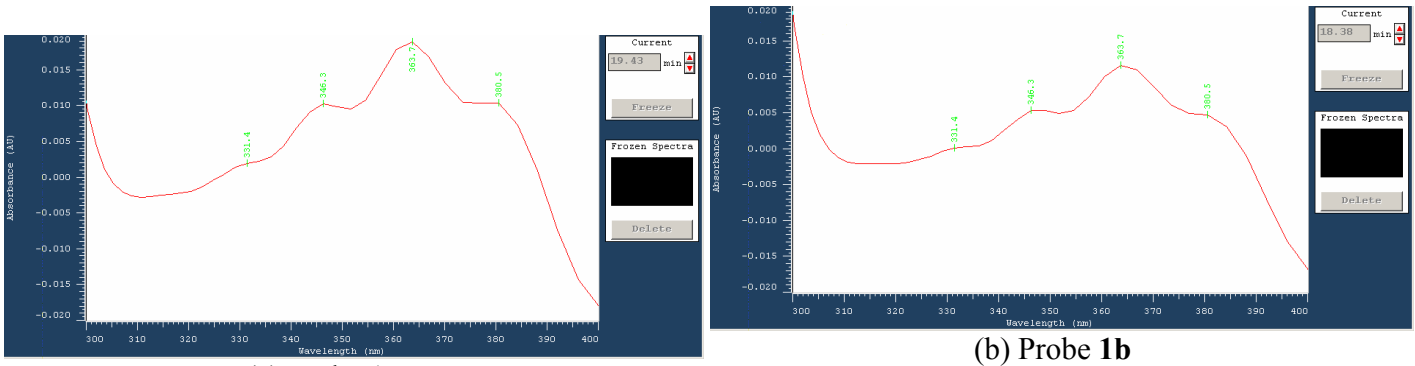


Figure 1S. C18 RP-HPLC chromatogram of crude mixture from synthesis.



(a) Probe **1a** (b) Probe **1b**

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 1μM in aqueous phosphate buffer (0.01 M phosphate, 0.2 M NaCl, pH 7.0).

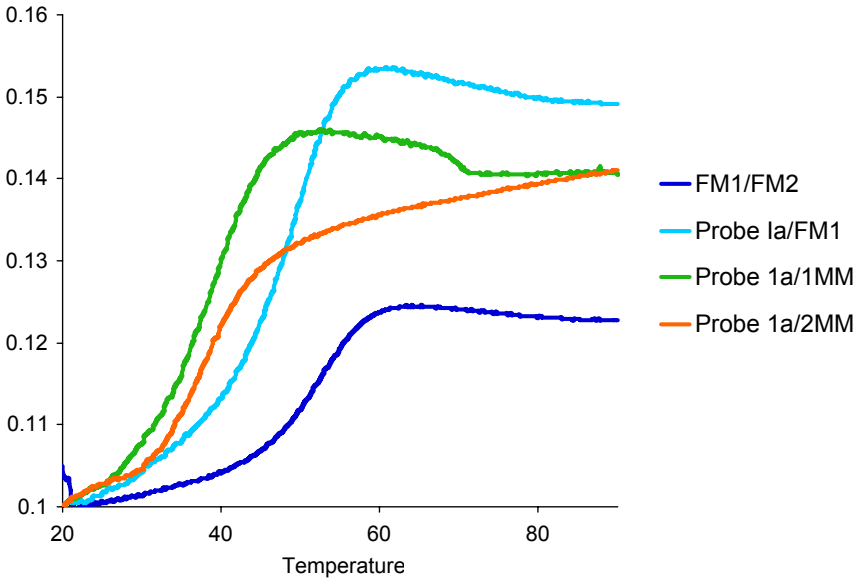


Fig. 3S. Normalized thermal melt curves of duplexes studied (Temperature/˚C)