Rigid Cyanine Dye Nucleic Acid Labels

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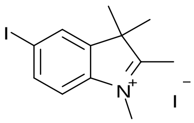
SUPPORTING INFORMATION for Chemical Communications

1.0 General Experimental

All solvents and reagents, except where mentioned, were purchased from Aldrich and used without further purification. Copper(I) iodide was purified as described by Dieter *et al.*[1] Anhydrous Et_3N was obtained by distillation over KOH. HPLC purification of small molecules was carried out on a Varian Prostar using a Varian Pursuit C18 column using 0.1% TFA:H_2O (solvent A) and 0.1% TFA:MeCN (solvent B) with the gradients as described for each compound. Purification of the oligonucleotides in was carried out on a HP 1100 Series with a Hichrom HIRPB-100A column (10 cm × 4.6 mm) with the solvent system aq. 50 mM TEAA (solvent C, Glen Research) and 20% H_2O:MeCN (solvent D) with the gradients as described. Mass spectra were recorded on Micromass Q-Tof (ES) spectrometer**.** Samples of oligonucleotides for mass spectrometry were desalted using MicroSpin G-25 columns (GE Healthcare) and dissolved in 50:50 isopropanol:H_2O with 1% triethylamine at approximately 50 μM. Spectra were recorded on a 4700 Proteomics Analyzer (Applied Biosystems). (E)-2-(2-(4-Methoxyphenylamino)vinyl)-1,3,3-trimethyl-3*H*-indolium iodide[2] and 1,3,3-trimethyl-2-((1E,3E)-4-(N-phenylacetamido)buta-1,3-dienyl)-3*H*-indolium[3] iodide were synthesised as described in the literature.

2.0 Synthesis

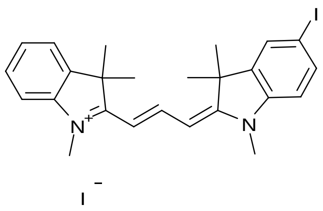
5-Iodo-1,2,3,3-tetramethyl-3*H*-indolium iodide



Adopting the procedure of Zimmermann and Hennig,[4] 4-iodophenylhydrazine (1.90 g, 8.1 mmol) was added to a mixture of 3-methylbutan-2-one (871 μL, 8.1 mmol), perchloric acid (1.90 mL, 32.4 mmol) and ethanol (20 mL) and was refluxed overnight. The reaction was concentrated to an oily residue, dissolved in DCM and washed with aq. $NaHCO_3$. After drying over $MgSO_4$ the solvent was evaporated, the residue dissolved in iodomethane (5 mL) and the resulting mixture was heated to reflux overnight. The reaction was cooled, filtered and washed with ether to give a brown solid (1.71 g, 5.7 mmol, 70% yield).

^{1}H-NMR (400 MHz, DMSO-d$_6$) δ: 8.25 (*d*, *J* = 1.5 Hz, 1H, Ar*H*), 7.97 (*dd*, *J* = 1.5 Hz and 8.5 Hz, 1H, Ar*H*), 7.69 (*d*, *J* = 8.5 Hz, 1H, Ar*H*), 3.91 (*s*, 3H, N^+CH_3), 2.71 (*s*, 3H, C-CH_3), 1.49 (s, 6H, $C(CH_3)_2$). Agrees with the literature.[4]

5-Iodo-1,3,3-trimethyl-2-((1E,3E)-3-(1,3,3-trimethylindolin-2-ylidene)prop-1-enyl)-3*H*-indolium iodide (1)



5-Iodo-1,2,3,3-tetramethyl-3*H*-indolium iodide (600 mg, 1.4 mmol) and (E)-2-(2-(4-methoxyphenylamino)vinyl)-1,3,3-trimethyl-3*H*-indolium iodide (610 mg, 1.4 mmol) were heated at 50 °C in a mixture of acetic anhydride (260 μL, 2.8 mmol) and pyridine (6 mL) for 5 h. The reaction mixture was cooled to room temperature and the solid, obtained by addition of ether, was filtered and purified by RP-HPLC (A:B 50:50 to 0:100 over 22 min. R_t = 17.1 min) to give 676 mg of a dark red solid (1.1 mmol, 79% yield).

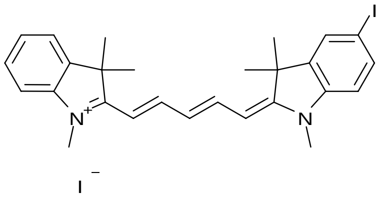
^{1}H-NMR (400 MHz, CDCl$_3$) δ: 8.36 (*t*, *J* = 13.5 Hz, 1H, polyene CH=C*H*-CH), 7.70 (*dd*, *J* = 1.5 Hz and 8.5 Hz, 1H, Ar*H*), 7.63 (*d*, *J* = 1.5 Hz, 1H, Ar*H*), 7.44-7.18 (*m*, 4H, Ar*H*), 6.90 (*d*, *J* = 8.5 Hz, 1H, Ar*H*), 6.54 (*d*, *J* = 13.5 Hz, 1H, polyene C*H*=CH-CH), 6.44 (*d*, *J* = 13.5 Hz, 1H, polyene CH=CH-C*H*), 3.70 (*s*, 3H, N$^+$C*H*$_3$), 3.62 (*s*, 3H, NC*H*$_3$), 1.72 (*s*, 6H, C(C*H*$_3$)$_2$), 1.70 (*s*, 6H, C(C*H*$_3$)$_2$).

^{13}C-NMR (100 MHz, CDCl$_3$) δ: 175.2, 173.0, 150.3, 142.4, 142.2, 140.4, 137.6, 130.9, 128.8, 125.8, 121.9, 112.2, 110.9, 104.4, 103.3, 88.3, 49.2, 48.6, 31.4, 31.1, 27.9, 27.7.

HRMS (ES): Calc. for $(C_{25}H_{28}N_2I)^+$ 483.1297. Found 483.1315.

UV/Vis (MeOH): λ_{max} = 550 nm, ε = 78700 M^{-1}cm^{-1}, λ_{em} = 568 nm.

5-Iodo-1,3,3-trimethyl-2-((1E,3E,5E)-5-(1,3,3-trimethylindolin-2-ylidene)-penta-1,3-dienyl)-3*H*-indolium iodide



5-Iodo-1,2,3,3-tetramethyl-3*H*-indolium iodide (400 mg, 0.94 mmol) and 1,3,3-trimethyl-2-((1E,3E)-4-(N-phenylacetamido)buta-1,3-dienyl)-3*H*-indolium (442 mg, 0.94 mmol) were heated at 80 °C in pyridine (4 mL) for 2 h. The product was precipitated with ether, filtered and purified by RP-HPLC (A:B 50:50 to 0:100 over 23 min. R$_t$ = 21.5 min) to give a blue solid (450 mg, 0.71 mmol, 75% yield).

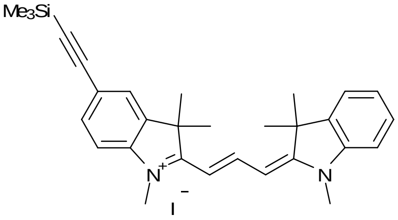
^{1}H-NMR (400 MHz, CDCl$_3$) δ: 7.98-7.85 (*m*, 2H, polyene CH-C*H*=CH-C*H*=CH), 7.66 (*dd*, *J* = 1.5 Hz and 8.0 Hz, 1H, Ar*H*), 7.61 (*d*, *J* = 1.5 Hz, 1H, Ar*H*), 7.43-7.38 (m, 2H, Ar*H*), 7.30-7.26 (*m*, 1H, Ar*H*), 7.17 (*d*, *J* = 8.0 Hz, 1H, Ar*H*), 6.83 (*d*, *J* = 8.5 Hz, 1H, Ar*H*), 6.62 (*t*, *J* = 13.0 Hz, 1H, polyene CH-CH=C*H*-CH=CH), 6.28 (*d*, *J* = 14.0 Hz, 1H, polyene C*H*-CH=CH-CH=CH), 6.10 (*d*, *J* = 13.0 Hz, 1H, polyene CH-CH=CH-CH=C*H*), 3.66 (*s*, 3H, N^{+}C*H*$_3$), 3.54 (*s*, 3H, NC*H*$_3$), 1.70 (s, 6H, (C*H*$_3$)$_2$), 1.67 (s, 6H, (C*H*$_3$)$_2$).

^{13}C-NMR (100 MHz, CDCl$_3$) δ: 174.7, 171.4, 154.4, 152.9, 142.8, 142.7, 142.5, 141.1, 137.4, 131.2, 128.8, 126.4, 125.8, 122.2, 111.8, 110.9, 104.8, 103.1, 87.8, 49.6, 48.8, 31.6, 31.1, 27.9, 27.7.

HRMS (ES) Calc. for $(C_{27}H_{30}N_2I)^+$ 509.1454. Found 509.1452.

UV/Vis (MeOH): λ_{max} = 644 nm, ε = 136300 M^{-1}cm^{-1}, λ_{em} = 665 nm.

1,3,3-Trimethyl-2-((1E,3E)-3-(1,3,3-trimethylindolin-2-ylidene)prop-1-enyl)-5-((trimethylsilyl)ethynyl)-3*H*-indolium iodide



A mixture of anhydrous DMF (6 mL) and anhydrous triethylamine (365 µL, 2.6 mmol) was degassed by freezing and thawing under vacuum before flushing with argon (three times). Added successively to this mixture was **1** (400 mg, 0.66 mmol), dichlorobis(triphenylphosphine)palladium(II) (46 mg, 0.07 mmol), copper(I) iodide (24 mg, 0.14 mmol) and trimethylsilylacetylene (280 µL, 1.98 mmol ol). The mixture was then heated at 45 °C for 1 h, the solvent was removed and the residue dissolved in DCM. This mixture was washed with sat. aq. disodium EDTA and brine, evaporated and Purification by FC (DCM to 10% MeOH/DCM) gave the product as a red solid (257 mg, 0.44 mmol, 67% yield).

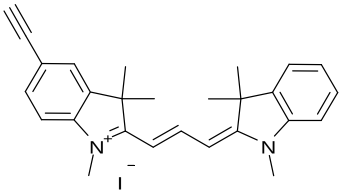
^{1}H-NMR (400 MHz, DMSO-d$_6$) δ: 8.30 (*t*, *J* = 13.5 Hz, 1H, polyene CH=C*H*-CH), 7.73 (*d*, *J* = 1.5 Hz, 1H, Ar*H*), 7.62 (*d*, *J* = 7.0 Hz, 1H, Ar*H*), 7.52-7.42 (*m*, 3H, Ar*H*), 7.38 (*d*, *J* = 8.0 Hz, 1H, Ar*H*), 7.30 (*t*, *J* = 7.0 Hz, 1H, Ar*H*), 6.49 (*d*, *J* = 13.5 Hz, 1H, polyene C*H*=CH-CH), 6.39 (*d*, *J* = 13.5 Hz, 1H, polyene CH=CH-C*H*), 3.66 (*s*, 3H, N^+CH_3), 3.58 (*s*, 3H, NCH_3), 1.66 (*s*, 6H, $C(CH_3)_2$), 1.65 (*s*, 6H, $C(CH_3)_2$) 0.22 (*s*, 9H, $Si(CH_3)_3$).

^{13}C-NMR (100 MHz, DMSO-d$_6$) δ: 175.2, 173.8, 149.7, 143.2, 142.6, 140.8, 132.5, 128.7, 125.7, 122.5, 118.2, 114.9, 111.9, 111.4, 105.3, 103.9, 102.7, 94.4, 55.0, 49.2, 48.6, 31.7, 31.4, 27.4, 27.2, 0.0.

HRMS (ES): Calc. for $(C_{30}H_{37}N_2Si)^+$ 453.2726. Found 453.2718

UV/Vis(MeOH): λ_{max} = 557 nm, ε = 15340 $M^{-1}cm^{-1}$, λ_{em} = 574 nm

5-Ethynyl-1,3,3-trimethyl-2-((1E,3E)-3-(1,3,3-trimethylindolin-2-ylidene)prop-1-enyl)-3*H*-indolium iodide (2)



1,3,3-Trimethyl-2-((1E,3E)-3-(1,3,3-trimethylindolin-2-ylidene)prop-1-enyl)-5-((trimethylsilyl)ethynyl)-3*H*-indolium iodide (1.0 g, 1.7 mmol) and K_2CO_3 (2.3 mg, 17 mmol) were dissolved in mixture of MeOH (10 mL) and DCM (10 mL) and stirred for 2 h at room temperature. The mixture was partitioned between water and DCM and the DCM evaporated to give the product as a dark red solid (596 mg, 1.2 mmol, 69% yield).

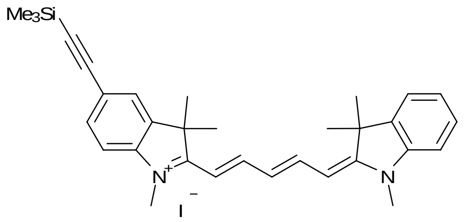
^{1}H-NMR (400 MHz, $CDCl_3$) δ: 8.34 (*t*, *J* = 13.5 Hz, 1H, polyene CH=C*H*-CH), 7.46-7.29 (*m*, 5H, Ar*H* and 2 polyene CH=CH-C*H*), 7.23-7.19 (*m*, 2H, Ar*H*), 7.12 (*d*, *J* = 8.0 Hz, 1H, Ar*H*), 7.01 (*d*, *J* = 8.0 Hz, 1H, Ar*H*), 3.79 (*s*, 3H, N^+CH_3), 3.71 (*s*, 3H, NCH_3), 3.08 (*s*, 1H, ethynyl *H*), 1.65 (*s*, 6H, $C(CH_3)_2$), 1.63 (*s*, 6H, $C(CH_3)_2$).

^{13}C-NMR (100 MHz, $CDCl_3$) δ: 173.4, 171.7, 149.3, 141.5, 140.9, 139.0, 138.8, 131.6, 127.3, 124.1, 123.9, 120.4, 116.8, 109.5, 108.8, 104.8, 103.8, 81.4, 76.4, 47.4, 46.7, 31.0, 30.7, 26.5, 26.4.

HRMS (ES): Calc. for $(C_{27}H_{29}N_2)^+$ 381.2331. Found 381.2327

UV/Vis (MeOH): λ_{max} = 552 nm, ε = 57760 $M^{-1}cm^{-1}$, λ_{em} = 571 nm.

1,3,3-Trimethyl-2-((1E,3E,5E)-5-(1,3,3-trimethylindolin-2-ylidene)penta-1,3-dienyl)-5-((trimethylsilyl)ethynyl)-3*H*-indolium iodide



A mixture of anhydrous DMF (6 mL) and anhydrous triethylamine (350 µL, 2.5 mmol) was degassed by freezing and thawing under vacuum before flushing with argon (three times). Added successively to this mixture was 5-iodo-1,3,3-trimethyl-2-((1E,3E,5E)-5-(1,3,3-trimethylindolin-2-ylidene)-penta-1,3-dienyl)-3*H*-indolium iodide (400 mg, 0.63 mmol), dichlorobis(triphenylphosphine)palladium(II) (44 mg, 0.06 mmol), copper(I) iodide (23 mg, 0.12 mmol) and trimethylsilylacetylene (267 µL, 1.89 mmol). The mixture was then heated at 45 °C for 1 h, the solvent was removed and the residue dissolved in DCM. This mixture was washed with sat. aq. disodium EDTA and brine, evaporated and the product purified by FC (DCM to 10% MeOH/DCM) to give a blue solid (293 mg, 0.48 mmol, 76% yield).

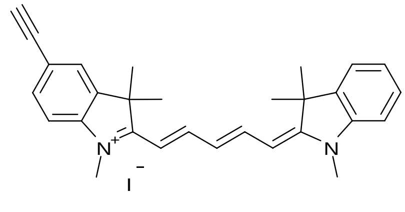
^{1}H-NMR (400 MHz, $CDCl_3$) δ: 8.10-7.97 (*m*, 2H, polyene CH-C*H*=CH-C*H*=CH), 7.45 (*dd*, *J* = 1.0 Hz and 8.0 Hz, 1H, Ar*H*), 7.40-7.36 (*m*, 3H, Ar*H*), 7.25 (*t*, *J* = 6.0 Hz, 1H, Ar*H*), 7.15 (*d*, *J* = 8.0 Hz, 1H, Ar*H*), 6.96 (*d*, *J* = 8.5 Hz, 1H, Ar*H*), 6.91 (*t*, *J* = 12.5 Hz, 1H, polyene CH-CH=C*H*-CH=CH), 6.46 (*d*, *J* = 13.0 Hz, 1H, polyene C*H*-CH=CH-CH=CH), 6.27 (*d*, *J* = 13.0 Hz, 1H, polyene CH-CH=CH-CH=C*H*), 3.74 (*s*, 3H, N^+CH_3), 3.62 (*s*, 3H, NCH_3), 1.73 (*s*, 6H, $(CH_3)_2$), 1.69 (*s*, 6H, $(CH_3)_2$), 0.25 (*s*, 9H, $Si(CH_3)_3$).

^{13}C-NMR (125 MHz, $CDCl_3$) δ: 174.5, 171.9, 154.4, 152.9, 142.9, 142.5, 141.2, 140.7, 132.8, 128.7, 127.1, 125.7, 122.2, 119.1, 110.8, 109.7, 105.3, 104.5, 103.9, 95.1, 53.4, 49.6, 48.7, 32.8, 28.1, 27.9, -0.1.

HRMS (ES) Calc. for $(C_{32}H_{39}N_2Si)^+$ 479.2883. Found 479.2885.

UV/Vis (MeOH/DCM): λ_{max} = 658 nm, ε = 29500 $M^{-1}cm^{-1}$, λ_{em} = 671 nm.

5-Ethynyl-1,3,3-trimethyl-2-((1E,3E,5E)-5-(1,3,3-trimethylindolin-2-ylidene)-penta-1,3-dienyl)-3*H*-indolium iodide (3)



1,3,3-Trimethyl-2-((1E,3E,5E)-5-(1,3,3-trimethylindolin-2-ylidene)penta-1,3-dienyl)-5-((trimethylsilyl)ethynyl)-3*H*-indolium iodide (1.0 g, 1.65 mmol) and K_2CO_3 (2.28 g, 16.5 mmol) were dissolved in mixture of MeOH (10 mL) and DCM (10 mL) and stirred for 2 h at room temperature. The mixture was partitioned between water and DCM and the DCM evaporated to give the product as a dark blue solid (642 mg, 1.2 mmol, 73% yield).

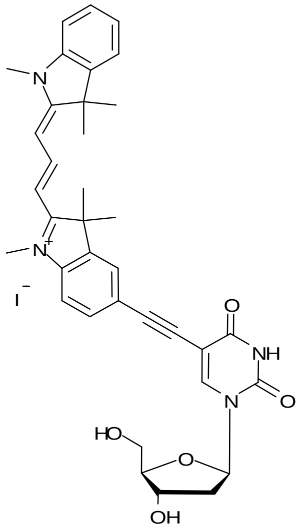
^{1}H-NMR (500 MHz, CD_3OD) δ: 8.21 (*t*, *J* = 13.0 Hz, 1H, polyene CH-C*H*=CH-CH=CH), 8.14 (*t*, *J* = 13.0 Hz, 1H, polyene CH-CH=CH-C*H*=CH), 7.57-7.47 (*m*, 3H, Ar*H*), 7.42 (*t*, *J* = 8.0 Hz, 1H, Ar*H*), 7.33-7.28 (m, 2H, Ar*H*), 7.15 (*d*, *J* = 8.0 Hz, 1H, Ar*H*), 6.61 (*t*, *J* = 13.0 Hz, 1H, polyene CH-CH=C*H*-CH=CH), 6.32 (*d*, *J* = 13.5 Hz, 1H, polyene C*H*-CH=CH-CH=CH), 6.15 (*d*, *J* = 13.5 Hz, 1H, polyene CH-CH=CH-CH=C*H*), 3.66 (*s*, 3H, N^+CH_3), 3.55 (*s*, 3H, NCH_3), 3.43 (*s*, 1H, ethynyl *H*), 1.72 (*s*, 6H, $(CH_3)_2$), 1.72 (*s*, 6H, $(CH_3)_2$).

^{13}C-NMR (125 MHz, CD_3OD) δ: 175.2, 172.4, 154.9, 153.3, 143.2, 142.5, 141.3, 140.9, 132.6, 128.5, 125.8, 125.6, 125.4, 122.0, 118.3, 110.9, 109.9, 104.3, 102.8, 102.6, 82.8, 78.0, 48.4, 30.7, 30.2, 26.8, 26.6.

HRMS (ES): Calc. for $(C_{29}H_{31}N_2)^+$ 407.2487. Found 407.2503.

UV/Vis (MeOH): λ_{max} = 647 nm, ε = 35000 $M^{-1}cm^{-1}$, λ_{em} = 669 nm.

5-((2′-Deoxyuridine)ethynyl)-1,3,3-trimethyl-2-((1E,3E)-3-(1,3,3-trimethylindolin-2-ylidene)prop-1-enyl)-3*H*-indolium iodide



A mixture of anhydrous DMF (1 mL) and anhydrous triethylamine (20 μL, 0.14 mmol) was degassed by freezing and thawing under vacuum before flushing with argon (three times). Added successively to this mixture was 5-iodo-2′-deoxyuridine (13 mg, 0.04 mmol), dichlorobis(triphenylphosphine)palladium(II) (2.5 mg, 3.6×10^{-3} mmol), copper(I) iodide (1.3 mg, 7.1×10^{-3} mmol) and **2** (20 mg, 0.04 mmol). The mixture was then heated at 45 °C 1 h, the solvent was removed and the residue dissolved in DCM. This mixture was washed with sat. aq. disodium EDTA and brine, evaporated and the product purified by RP-HPLC (A:B 20:80 to 0:100 over 18 min. R_t = 16.1 min) to give a red solid (18 mg, 0.025 mmol, 64% yield).

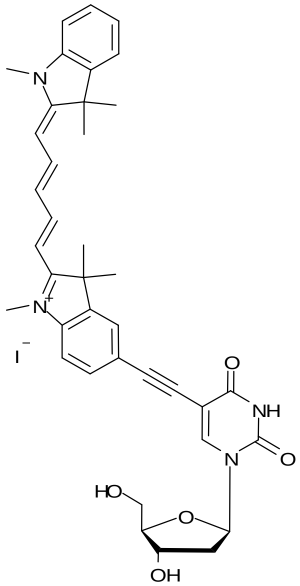
^{1}H-NMR (500MHz, CD_3OD) δ: 8.52 (*t*, *J* = 13.5 Hz, 1H, polyene CH=C*H*-CH), 8.45 (*s*, 1H, *H*-6), 7.64 (*d*, *J* = 1.5 Hz, 1H, Ar*H*), 7.56-7.53 (*m*, 2H, Ar*H*), 7.47-7.44 (*m*, 1H, Ar*H*), 7.38 (*d*, *J* = 8.0 Hz, 1H, Ar*H*), 7.33 (*t*, *J* = 7.5 Hz, 1H, Ar*H*), 7.28 (*d*, *J* = 8.0 Hz, 1H, Ar*H*), 6.44 (*d*, *J* = 13.5 Hz, 1H, polyene CH=CH-C*H*), 6.36 (*d*, *J* = 13.5 Hz, 1H, polyene C*H*=CH-CH), 6.27 (*t*, *J* = 6.5 Hz, 1H, *H*-1′), 4.44-4.42 (*m*, 1H, *H*-3′), 3.97-3.95 (*m*, 1H, *H*-4′), 3.86 (*dd*, *J* = 3.0 and 12.0 Hz, 1H, *H*-5′), 3.72 (*dd*, *J* = 3.0 and 12.0 Hz, 1H, *H*-5″), 3.70 (*s*, 3H, N^+CH_3), 3.64 (*s*, 3H, NCH_3), 2.37-2.23 (*m*, 2H, *H*-2′), 1.76 (2×s, 12H, $(CH_3)_2$).

^{13}C-NMR (125 MHz, MeOD) δ: 177.3, 175.9, 164.3, 152.0, 151.1, 145.1, 144.1, 144.0, 142.3, 142.2, 133.4, 130.0, 127.1, 126.3, 123.4, 121.1, 112.6, 112.1, 104.6, 103.8, 100.4, 93.7, 89.2, 87.1, 82.7, 72.0, 62.6, 50.9, 50.2, 41.8, 31.9, 30.9, 28.2, 28.1, 28.0.

HRMS (ES) Calc. for $(C_{36}H_{39}N_4O_5)^+$ 607.2920. Found 607.2935.

UV/Vis (MeOH): λ_{max} = 563 nm, ε = 124000 $M^{-1}cm^{-1}$, λ_{em} = 588 nm.

5-((2′-Deoxyuridine)ethynyl)-1,3,3-trimethyl-2-((1E,3E,5E)-5-(1,3,3-trimethyl-indolin-2-ylidene)penta-1,3-dienyl)-3*H*-indolium iodide



Anhydrous DMF (1 mL) and anhydrous triethylamine (33 µL, 0.24 mmol) was degassed and added successively to this mixture was 5-iodo-2′-deoxyuridine (28 mg, 0.05 mmol), dichlorobis(triphenylphosphine) palladium(II) (3.5 mg, 5.0×10^{-3} mmol), copper(I) iodide (1.9 mg, 0.01 mmol) and **3** (29 mg, 0.06 mmol). The mixture was then heated at 45 °C for 1 h, the solvent was removed and the residue dissolved in DCM. This mixture was washed with sat. aq. disodium EDTA and brine, evaporated and the product purified by RP-HPLC (A:B 5:95 to 0:100 over 20 min. R_t = 12.4 min) to give a blue solid (27 mg, 0.036 mmol, 72% yield).

^{1}H-NMR (400MHz, CD_3OD) δ: 8.42 (*s*, 1H, *H*-6), 8.29-8.16 (*m*, 2H, polyene CH-CH=CH-C*H*=CH), 7.58 (*d*, *J* = 1.5 Hz, 1H, Ar*H*), 7.54-7.29 (*m*, 5H, Ar*H*), 7.20 (*d*, *J* = 8.5 Hz, 1H, Ar*H*), 6.61 (*t*, *J* = 12.5 Hz, 1H, polyene CH-CH=C*H*-CH=CH), 6.33 (*d*, *J* = 13.5 Hz, 1H, polyene C*H*-CH=CH-CH=CH), 6.26 (*t*, *J* = 6.5 Hz, 1H, *H*-1′), 6.19 (*d*, *J* = 13.5 Hz, 1H, polyene CH-CH=CH-CH=C*H*), 4.43-4.41 (*m*, 1H, *H*-3′), 3.97-3.94 (*m*, 1H, *H*-4′), 3.85 (*dd*, 1H, *J* = 3.0 and 12.0 Hz, *H*-5′), 3.76 (*dd*, 1H, *J* = 3.0 and 12.0 Hz, *H*-5″), 3.67 (*s*, 3H, N^+CH_3), 3.56 (*s*, 3H, NCH_3), 2.37-2.22 (*m*, 2H, *H*-2′), 1.72 (*s*, 6H, $(CH_3)_2$), 1.71 (*s*, 6H, $(CH_3)_2$).

^{13}C-NMR (125 MHz, MeOD) δ: 176.1, 174.1, 164.4, 163.1, 151.1, 144.9, 144.6, 144.1, 142.8, 141.9, 133.2, 129.8, 127.4, 127.1, 126.8, 126.3, 123.3, 120.2, 112.4, 111.4, 105.6, 104.0, 100.5, 93.9, 89.2, 87.1, 82.4, 71.9, 62.5, 55.8, 50.9, 41.8, 31.8, 31.4, 27.9, 27.8, 27.6.

HRMS (ES) Calc. for $(C_{38}H_{41}N_4O_5)^+$ 633.3077. Found 633.3102.

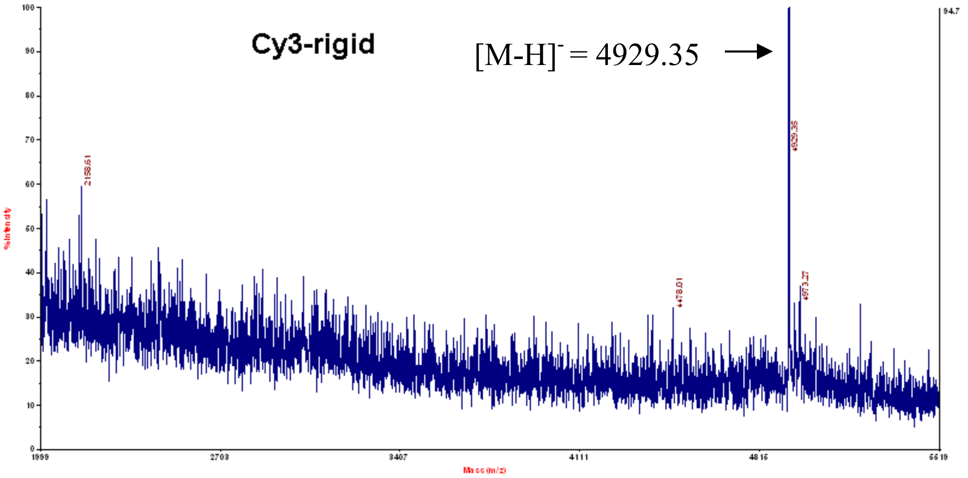
UV/Vis (MeOH/DCM): λ_{max} = 656 nm, ε = 139000 $M^{-1}cm^{-1}$,λ_{em} = 681 nm.

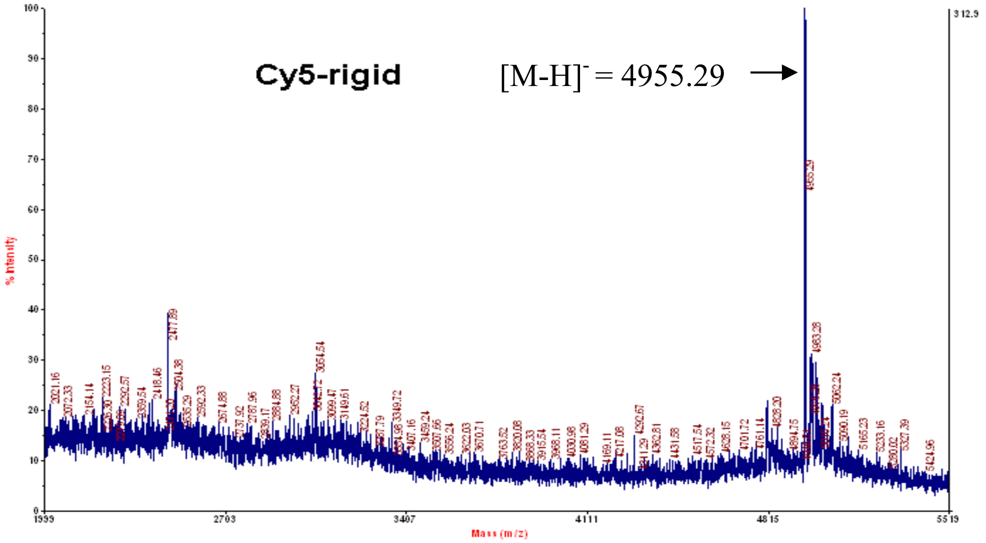
3.0 General procedure for Sonogashira Cross Coupling on Resin

Resin with oligonucleotide containing a 5-iodo-2′-deoxyuracil (supplied by ATDBio Ltd.) was placed in a small eppendorf with **2** or **3** (60 eq with respect to loading of resin), copper(I) iodide (30 eq) and tetrakis (triphenylphosphine)palladium(0) (30 eq). The eppendorf was flushed with argon while a mixture of anhydrous DMF and anhydrous Et_3N (3.5/1.5) was degassed by freezing and thawing under vacuum. The mixture was added to give a solution of 60 mM dye and the reaction was gently agitated under Ar for 3 hours. The reaction mixture was pippetted off and the resin washed with the DMF/Et_3N mixture and then MeCN until colourless. The oligonucleotide was cleaved from the resin with concentrated $NH_{3(aq)}$ at 60 °C for 30 min. The ammonia was removed and the resin washed with water and 50:50 water/MeCN and the solution evaporated. The residue was dissolved in 50 mM TEAA (Glen Research) and initially purified using Poly-Pak cartridges (Glen Research), the DMTr was also removed from the 5′-OH while the oligonucleotide was on the resin by washing with 2% TFA/water. The oligonucleotide was eluted using 50:50 MeCN/water and evaporated to dryness. The oligonucleotide was then purified by RP-HPLC (E:F 95:5 to 20:80 over 40 min with **Cy3-rigid-end** $R_t = 11.3$ min and **Cy5-rigid-end** $R_t = 13.0$ min, E:F 95:5 to 60:30 over 40 min with **Cy3-rigid B** $R_t = 34.1$). Samples were quantitated by UV absorption at 260 nm. The extinction coefficients of the strands were calculated by determining the extinction coefficient for the strands without the modified base, using the nearest neighbour calculation, and then adding the extinction coefficient measured for the free dye base conjugates: ε_{260} for Cy3-nucleobase conjugate 8500 M^{-1} cm^{-1}; ε_{260} for the Cy5-nucleobase conjugate 13500 M^{-1} cm^{-1}.

Table S1 Extinction coefficients, calculated masses and observed masses for the oligonucleotides synthesised.

Construct	ε_{260} (M^{-1} cm^{-1})	Calculated Mass $[M-H]^-$	Mass Observed $[M-H]^-$
Cy3-rigid	140200	4929.0	4929.35
Cy5-rigid	145200	4955.0	4955.29





4.0 Oligonucleotide Sequences

Table S2 Sequences of the oligonucleotides used.

Name	Sequence (5′ to 3′)
Comp	CGC TTC AGG AGC TAA
Unmodified	TTA GCT CCT GAA GCG
Cy3-flexible	T(**Cy3**)TA GCT CCT GAA GCG
Cy5-flexible	T(**Cy5**)TA GCT CCT GAA GCG
Template	CGC TTC AGG AGC TAA TTC GGA GCT AA
Cy3-rigid B	T(**Cy3**)TA GCT CCG AA
Cy3-flexible B	T(**Cy3**)TA GCT CCG AA

5.0 UV Melting

UV data was recorded on a Cary UV-Visible 100Bio spectrophotometer (Varian, UK). Absorption data was recorded at 295 nm, for quadruplex melting, and at 260 nm for duplex melting. Samples were prepared with final concentrations of 2.5 μM DNA, 10 mM sodium phosphate, pH 7.0, 100 mM NaCl and 1 mM disodium EDTA. The samples were annealed by heating at 95 °C for 5 min then slowly cooled to 5 °C at 0.2 °C min^{-1}. Samples were covered with mineral oil to reduce evaporation and dry nitrogen was passed through the sample chamber to prevent condensation at low temperatures. T_m values were determined by the first derivative method, 50% folded and/or Van’t Hoff analysis.

6.0 CD Spectroscopy

CD spectra were recorded on a Chirascan (Applied Photophysics). Scans were recorded at 20 °C with the wavelength varied from 220 to 320 nm at a rate of 50 nm min^{-1}. The spectra are an average of three scans with a background scan of the buffer subtracted. The spectra were corrected to 0 at 320 nm and are presented as molar ellipticity [θ] (deg dm^2

dmol^{-1}). Molar ellipticity was calculated using *Equation E1* where θ is the corrected ellipticity (mdeg), *c* is the concentration (M) and *l* is the pathlength (cm).

$$[\theta] = \frac{\theta}{10.c.l} \qquad \textbf{E1}$$

Samples were prepared with a final concentration of 4 μM DNA, 10 mM sodium phosphate, pH 7.0, 100 mM NaCl and 1 mM disodium EDTA and were annealed by heating at 95 °C for 5 min and slowly cooled to room temperature overnight before being stored at 4 °C.

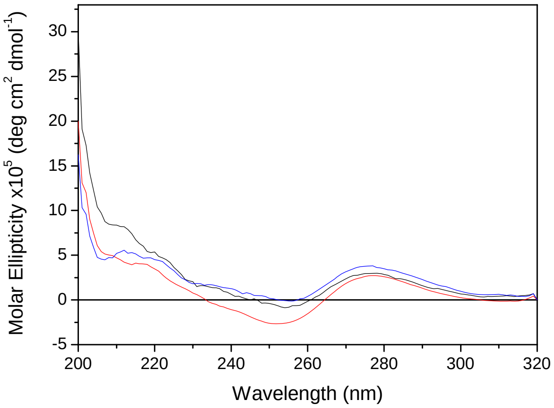


Figure S1 CD spectra recorded for the **Unmodified:Comp** (red line), **Cy3-rigid:Comp** (black line), **Cy5-rigid:Comp** (blue line) in 100 mM Na$^+$, 10 mM sodium phosphate pH 7.0 and 1 mM disodium EDTA.

7.0 Fluorescence Spectroscopy

Fluorescence data was recorded on a Cary Eclipse Fluorescence Spectrometer fitted with a Peltier temperature controller (Varian, UK). Stock solutions of 500 nM oligonucleotide strand concentration were prepared with 10 mM sodium phosphate, pH 7.0, 100 mM

NaCl and 1 mM disodium EDTA. Samples were heated at 95 °C for 5 min and allowed to cool to room temperature and then stored at 4 °C. A sample of the stock solution was diluted to 100 nM with 10 mM sodium phosphate, pH 7.0, 100 mM NaCl and 1 mM disodium EDTA. Emission spectra were recorded between 540 and 750 nm with excitation at 530 nm for Cy3 samples containing Cy3 fluorophores; while samples containing Cy5 fluorophores were excited at 630 nm and emission recorded from 640 nm to 800 nm. Excitation spectra were recorded between 540 and 590 nm with emission at 600 nm for Cy3 samples containing Cy3 fluorophores; while samples containing Cy5 fluorophores were scanned between 540 and 690 nm with the emission at 700 nm. Background fluorescence was removed from samples by scanning the buffer and subtracting from the fluorescence spectra.

Table S4 The excitation and emission wavelengths as recorded for the Cy3 series of molecules and oligonucleotides.

Construct	λ_{ex} (nm)	λ_{em}(nm)
Cy3 iodinated dye	551	566
Cy3-nucleobase conjugate	565	592
Cy3-rigid	567	585
Cy3-rigid:Comp	568	582
Cy3-flexible	553	565
Cy3-flexible:Comp	556	567

Table S5 The excitation and emission wavelengths as recorded for the Cy5 series of molecules and oligonucleotides.

Construct	λ_{ex}(nm)	λ_{em}(nm)
Cy5 iodinated dye	648	664
Cy5-nucleobase conjugate	662	682
Cy5-rigid	664	681
Cy5-rigid:Comp	665	679
Cy5-flexible	652	665
Cy5-flexible:Comp	653	666

Anisotropy measurements were performed on a sample of the stock solution, diluted to 100 nM with 10 mM sodium phosphate, pH 7.0, 100 mM NaCl and 1 mM disodium EDTA using automated polarisers and the Eclipse Advanced Reads Application (ADL Shell). The Cy3 fluorophore was excited at 560 nm with emission recorded at 580 nm. The Cy5 fluorophore was excited at 660 nm and emission recorded at 680 nm.

Table S6 Anisotropy as measured for each of the dyes, dye base conjugates and oligonucleotides.

	r		r
Cy3 iodinated dye	0.16 ± 0.02	**Cy5 iodinated dye**	0.09 ± 0.02
Cy3-nucleobase conjugate	0.16 ± 0.01	**Cy5-nucleobase conjugate**	0.15 ± 0.01
Cy3-rigid	0.29 ± 0.01	**Cy5-rigid**	0.28 ± 0.02
Cy3-rigid:Comp	0.29 ± 0.01	**Cy5-rigid:Comp**	0.29 ± 0.01
Cy3-flexible	0.22 ± 0.01	**Cy5-flexible**	0.20 ± 0.01
Cy3-flexible:Comp	0.25 ± 0.01	**Cy5-flexible:Comp**	0.23 ± 0.01

FRET scans were recorded by exciting at 530 nm and recording the emission between 540 and 800 nm. Fluorescence resulting from the direct excitation of Cy5 was subtracted by scanning a system containing only the relevant Cy5 dye. Scans were corrected for background.

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