Supporting Information

A highly fluorescent DNA toolkit; Synthesis and properties of oligonucleotides containing new Cy3, Cy5 and Cy3B monomers

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ESI.1 Supplementary figures and tables

Table ESI.1. T_m data from UV melting for Cy3dT and Cy5dT duplexes [a]

Duplex	Oligo 1	Oligo 2	T_m/ °C	Δ T_m/ °C
Unmodified control	ODN-14	ODN-15	54.8	---
Cy3dT control	ODN-12	ODN-15	52.4	-2.4
Cy5dT control	ODN-14	ODN-13	51.7	-3.1
Cy3dT+Cy5dT in opposite-strands	ODN-12	ODN-13	48.9	-5.9
Cy3dT+Cy5dT in same-strand	ODN-16	ODN-17	48.6	-6.2
Unmodified control	ODN-18	ODN-19	57.2	---
Cy3BdT control (2 additions)	ODN-7	ODN-19	50.4	-6.8

[a]Average T_m value was calculated from three successive melting curves. Standard deviation for all T_m data is ± 0.1 ºC.

Table ESI.2. Oligonucleotide sequences used in UV melting analysis. **b** = Cy3BdT; **3** = Cy3dT; **5** = Cy5dT; **p** = propanol;

ODN	Sequence
ODN-12	CGTATATTC**3**TTATTTTTAAAAGCC
ODN-13	GGCTTT**5**AAAAATAAAGAATATACG
ODN-14	CGTATATTCTTTATTTTTAAAAGCC
ODN-15	GGCTTTTAAAAATAAAGAATATACG
ODN-16	GGA**5**TTTCG**3**TTTTATAATTGCC
ODN-17	GGCAATTATAAAAACGAAAATCC
ODN-7	CGCTTC**b**GTATC**b**ATATTCATC**p**
ODN-18	CGCTTCTGTATCTATATTCATC
ODN-19	CTATGATGAATATAGATACAGAAGCGTCAT

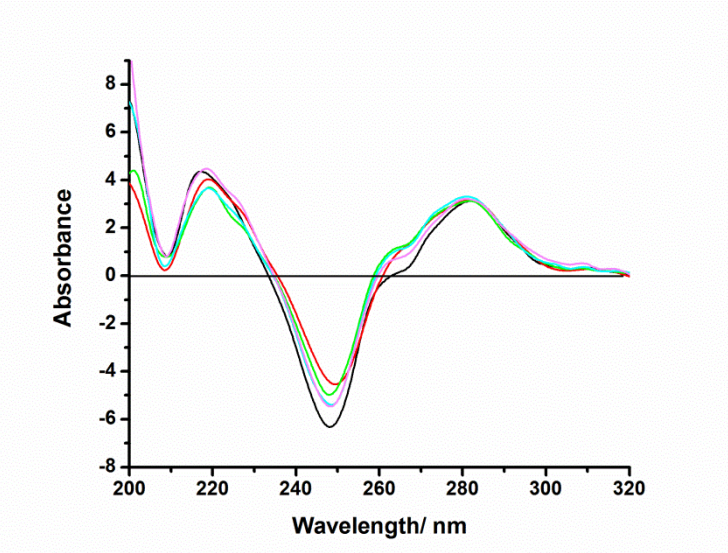


Figure ESI.1. Circular dichroism spectra of Cy3dT and Cy5dT oligonucleotides (sequences as in Table ESI.1 and ESI.2) shows that the duplexes containing the CydT monomers still adopt the B-DNA conformation. Ten successive spectra were recorded and averaged. Unmodified control (black), Cy3dT and Cy5 dT in the same strand (red), Cy3dT and Cy5dT in opposite strands (green), Cy5dT control (blue) and Cy3dT control (pink).

Table ESI.3. Photophysical properties; UV-Vis absorbance maxima (A_{max}), fluorescence emission maxima (Em_{max}), extinction coefficient (ε) and quantum yield (Φ). (recorded in MeOH).

Compound	A_{max} (nm)	Em_{max} (nm)	ε ($M^{-1}cm^{-1}$)	Φ
Cy3-NHS ester*	550	570	150,000	>0.15
Cy5-NHS ester*	649	670	250,000	>0.28
Cy3B-NHS ester*	559	570	130,000	>0.70
ICy3	551	567	114,000	0.04
Cy3dT	565	591	112,000	0.05
Cy5dT	660	685	208,000	0.06
ICy3B	562	575	141,000	0.96
5-ethynyl-Cy3B	566	579	118,000	0.88
Cy3BdT	576	596	122,000	0.50
β-Cy3BdR	571	585	130,000	0.80

*Data from GE Healthcare.

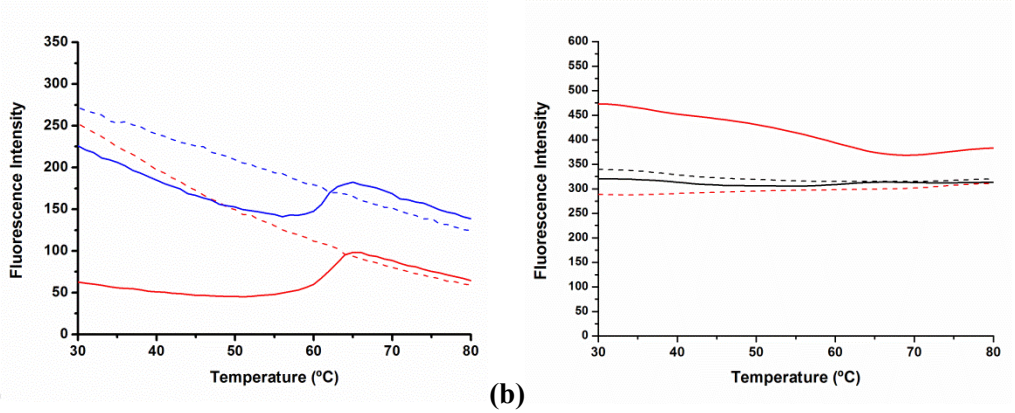


Figure ESI.2. Fluorescence melting of (a) Cy3dT oligonucleotides (ODN-20 ss (red, dashed), ODN-20_ODN-22 ds (red, solid line)) and Cy5dT oligonucleotides (ODN-21 ss (blue, dashed), ODN-21_ODN-22 ds (blue, solid line)) compared to that of (b) 5´Cy3B oligonucleotides (ODN-11 ss (black, dashed), ODN-11_ODN-25 ds (black, solid line)) and Cy3BdT oligonucleotides (ODN-23 ss (red, dashed), ODN-23_ODN-24 ds (red, solid line)) oligonucleotides (sequences in Table ESI.4). The oligonucleotides containing Cy3dT (ODN-20) and Cy5dT (ODN-21) show significant temperature dependence; in the single-strand the fluorescence decreases dramatically as the temperature increases. For Cy3dT and Cy5dT the shape of the duplex melting curves for these examples is unusual. The examples containing 5´-Cy3B have virtually no temperature dependence in either the single-strand or duplex form. The example containing Cy3BdT shows no temperature dependence in the single-strand form and in the duplex form exhibits the melting profile of a HyBeacon probe.

Table ESI.4. Oligonucleotide sequences used in fluorescence melting study. **b** = Cy3BdT; **3** = Cy3dT; **5** = Cy5dT; **h** = 5-(hexyn-1-ol)-6-Cy3B phosphoramidite; **p** = propanol.

ODN	Sequence
ODN-20	CACCAAAGATGATATTT**3**CTTTAATGG**p**
ODN-21	CACCAAAGATGATATTT**5**CTTTAATGG**p**
ODN-22	CCATTAAAGAAAATATCATCTTTGGTG
ODN-23	TTGCGTACA**b**TCTCCGTTTTTAATAGCCAT
ODN-24	ATGGCTATTAAAAACGGAGAATGTACGCAA
ODN-11	**h**TCAGTTTTCCTGGATTATGC
ODN-25	GCATAATCCAGGAAAACTGA

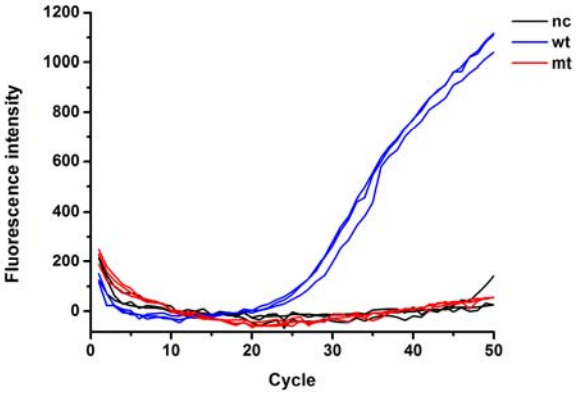


Figure ESI.3. Fluorescence accumulation of RT-PCR using a Molecular Beacon probe containing Cy3B and DABCYL (ODN-8). The sample containing wild-type template has a C_T value of 20 whereas the samples containing the mutant-type template show no real-time fluorescence accumulation. This is because at the monitoring temperature (58 °C), the mutant template:probe duplexes are very unstable.

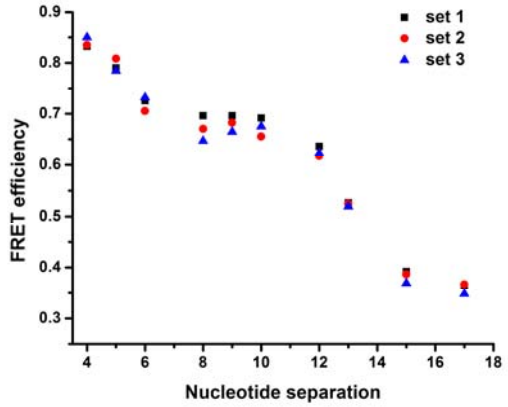


Figure ESI.4. FRET efficiency *vs*. number of nucleotides separating dyes (not including CydT nucleobases). Sequences as in Table ESI.5. 3 experimental sets. FRET efficiency calculated according to the equation:

$$\text{FRET efficiency} = (I_{FRET} \times Abs_{615})/(I_{direx} \times Abs_{530})$$

Where I_{FRET} is the emission intensity of the Cy5 peak when excited *via* FRET (ex 530 nm), I_{direx} is the emission intensity of the Cy5 peak when directly excited (ex 615 nm), Abs_{615} is the absorbance of the sample at 615 nm and Abs_{530} is the absorbance of the sample at 530 nm.

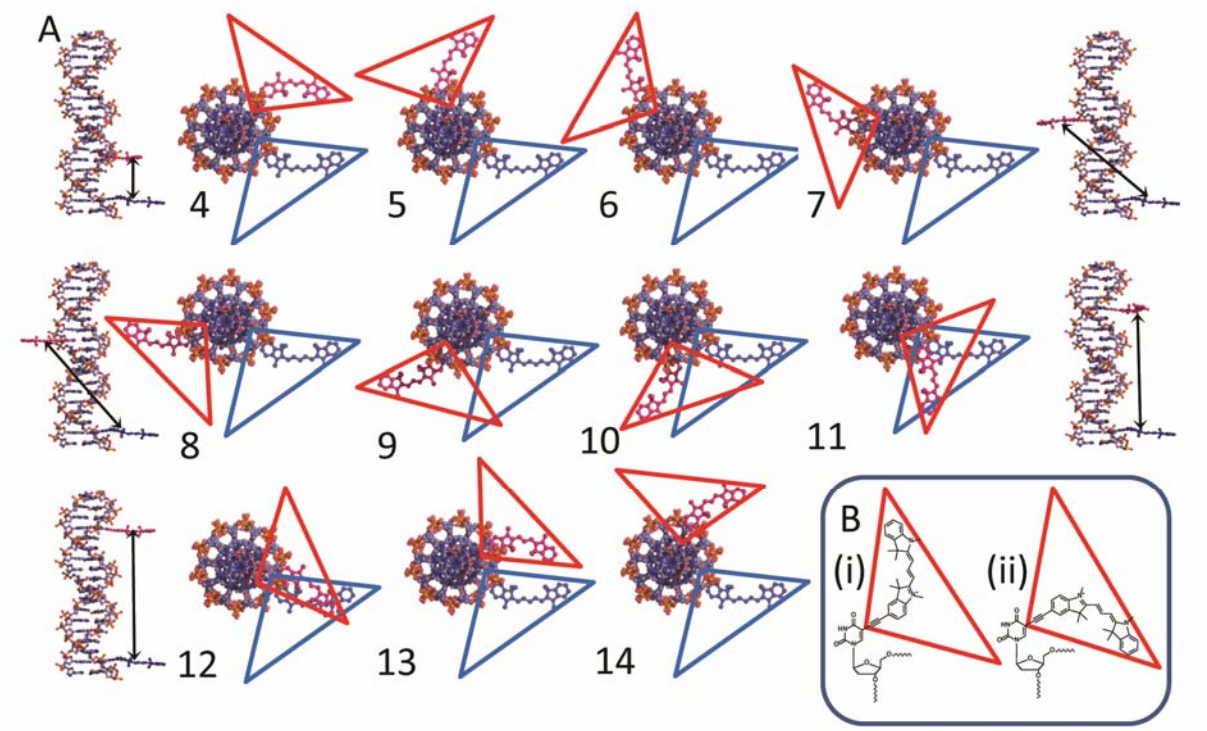


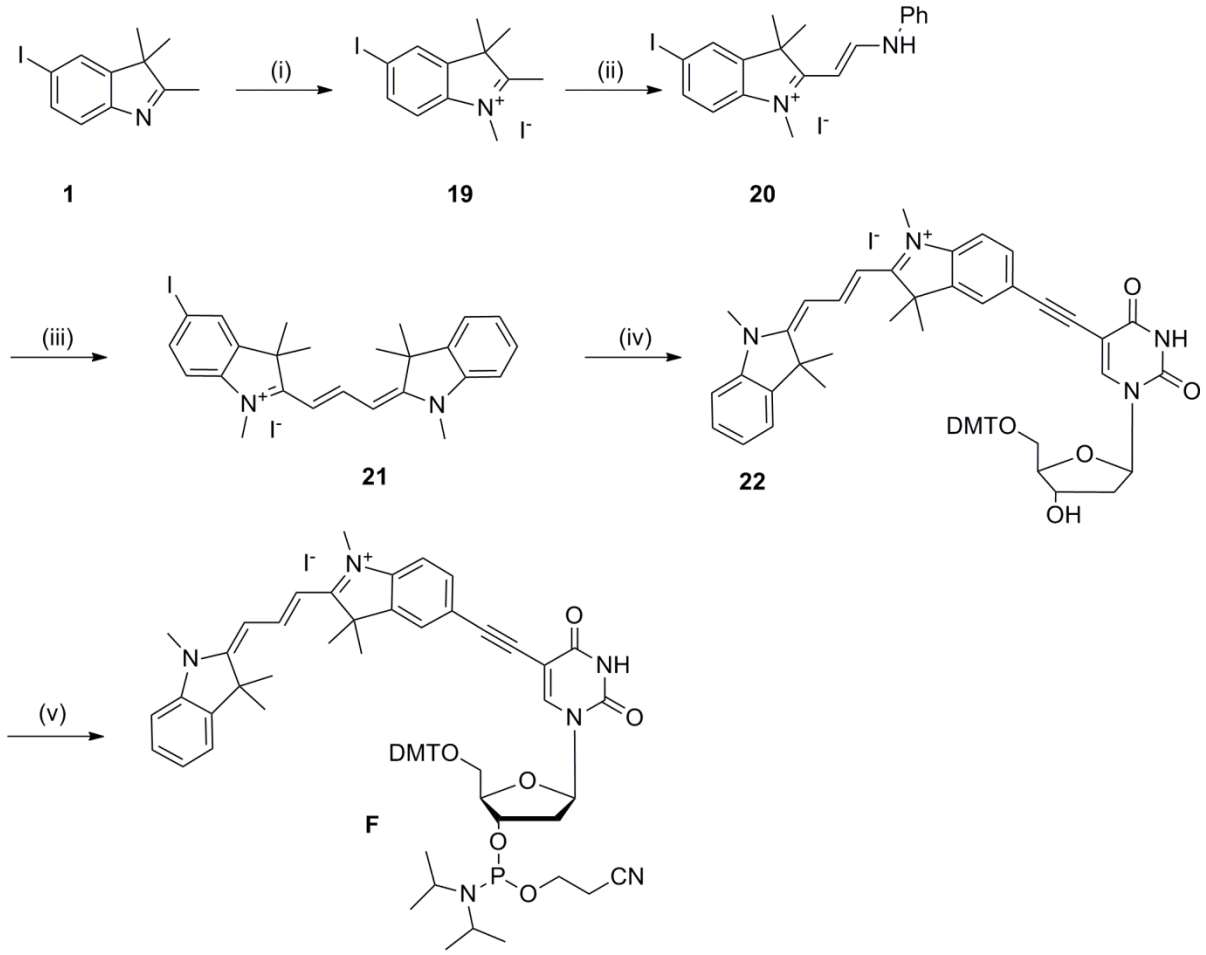
Figure ESI.5. (A) Separation between Cy3dT (pink structure in red triangle) and Cy5dT (blue structure in blue triangle) in the DNA helix. Change in helical turn is shown for separations of 4 to 14 nucleotides (viewed down the helical axis) and change in helical length is shown for separations of 4, 8 and 12 nucleotides (side-on view). An increase in dye separation from 4 to 7 nucleotides and also from 12 to 14 takes the dyes further from one another both along and around the helix axis. In contrast, an increase in dye separation from 8 to 11 nucleotides results in an increase in distance along the helical axis which is counteracted by a decrease in distance around the helical axis. These schematic representations qualitatively fit the data plotted in Figure ESI.4. (B) The crescent-shaped dye component of the Cy-dT monomers is expected to rotate about the single bonds linking it to the thymine base and occupy a volume in space described by a cone. B(i) shows one extreme position of the CyDye (in this case Cy3) and B(ii) shows the other extreme. In the models in ESI.5A the dyes are shown in position B(ii).

Table ESI.5. Oligonucleotide sequences used in FRET experiment. **3**= Cy3dT; **5**= Cy5dT.

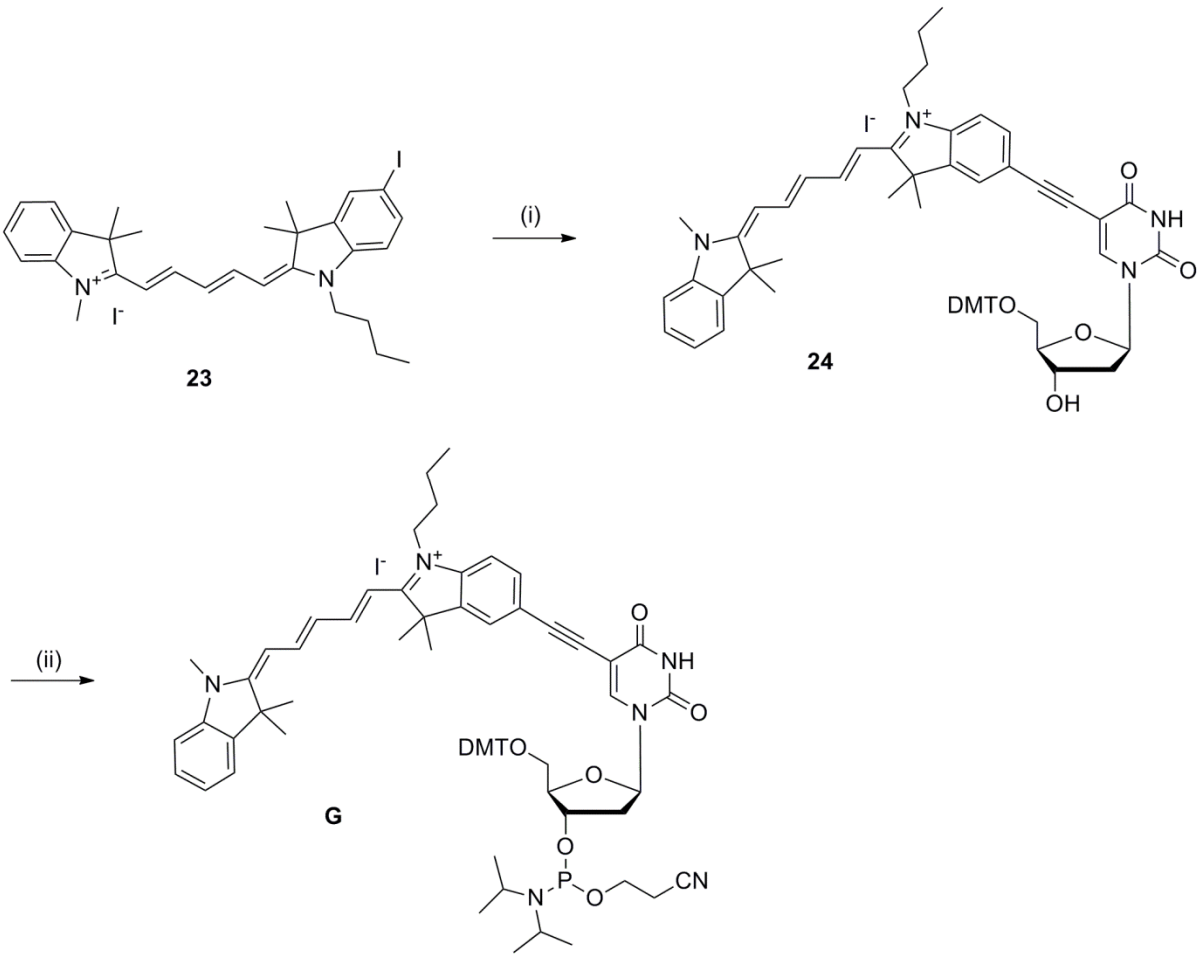
ODN	**Sequence**	**Nucleotide separation between dyes**
ODN-27	CGTATATTCTTTATT**3**TTAAAAGCC	4
ODN-28	CGTATATTCTTTAT**3**TTTAAAAGCC	5
ODN-29	CGTATATTCTTTA**3**TTTTAAAAGCC	6
ODN-30	CGTATATTCTT**3**ATTTTTAAAAGCC	8
ODN-31	CGTATATTCT**3**TATTTTTAAAAGCC	9
ODN-12	CGTATATTC**3**TTATTTTTAAAAGCC	10
ODN-33	CGTATAT**3**CTTTATTTTTAAAAGCC	12
ODN-34	CGTATA**3**TCTTTATTTTTAAAAGCC	13
ODN-35	CGTA**3**ATTCTTTATTTTTAAAAGCC	15
ODN-36	CG**3**ATATTCTTTATTTTTAAAAGCC	17
ODN-37	GGCT**5**TTAAAAATAAAGAATATACG	---
ODN-14	CGTATATTCTTTATTTTTAAAAGCC	---
ODN-15	GGCTTTTAAAAATAAAGAATATACG	---

ESI.2 Materials and methods

ESI.2.1 Supplementary schemes



Scheme ESI.1. Synthesis of Cy3dT-phosphoramidite. Reagents and conditions: (i) MeI, MeCN, reflux, 16 h, 78 %; (ii) *N,N´*-diphenylformamidine, triethylorthoformate, ethanol, reflux, 2.5 h, 95 %; (iii) 1,2,3,3-tetramethyl-3*H*-indolium iodide, Ac_2O, pyridine, 50 °C, 48 h, 61 %; (iv) 1-[2´-(deoxy)-5´-*O*-(4,4´-dimethoxytrityl)-*β*-D-erythro-pentafuranosyl]-5-(eth-1-ynyl)uridine, CuI, $Pd(PPh_3)_4$, Et_3N, DMF, rt, 24 h, 42 %; (v) 2-cyanoethyl-*N,N*-diisopropylchloro-phosphoramidite, DIPEA, DCM, rt, 2 h 45 min, 96 %.



Scheme ESI.2. Synthesis of Cy5dT-phosphoramidite. Reagents and conditions: (i) 1-[2′-(deoxy)-5′-*O*-(4,4′-dimethoxytrityl)-*β*-D-erythro-pentafuranosyl]-5-(eth-1-ynyl)uridine, CuI, $Pd(PPh_3)_4$, Et_3N, DMF, rt, 26 h, 91 %; (ii) 2-cyanoethyl-*N,N*-diisopropylchlorophosphoramidite, DIPEA, DCM, rt, 1 h 45 min, 84 %.

ESI.2.2 Dye Synthesis

ESI.2.2.1 General procedures

Chemicals were purchased from Sigma-Aldrich, Fisher Scientific, Alfa Aesar, Apollo Scientific and Link Technologies. The following solvents were purified by distillation (over calcium hydride) before use: pyridine, Et_3N, DCM, MeCN and MeOH. THF was purified by distillation over sodium and benzophenone. Anhydrous DMF was purchased from Sigma-Aldrich. When mentioned, deoxygenated solutions/solvents were prepared by bubbling argon through the solution for 15 min. Reactions were carried out using air-sensitive techniques, under a degassed atmosphere of argon in cleaned, dry glassware.

Reactions were monitored by thin layer chromatography, which was carried out on Merck Kieselgel 60 F_{254} plates (0.22 mm thickness, aluminium backed). They were visualised by UV irradiation at 254/365 nm. Column chromatography was carried out under air-pressure using Fisher Scientific DAVISIL 60 Å (35-70 micron) silica gel. Silica was pre-equilibrated with Et_3N or pyridine before purification of acid-sensitive compounds.

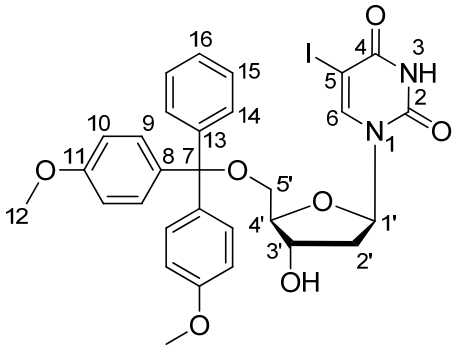
^{1}H NMR spectra were measured on a Bruker AC300 spectrometer at 300 MHz or Bruker DPX400 spectrometer at 400 MHz. ^{13}C spectra were measured at 75 or 100 MHz respectively. ^{31}P spectra were measured at 121 MHz. All shifts were referenced to the residual solvent peak and are given in ppm (1). *J* coupling values are correct to 0.5 Hz. Assignment was aided by DEPT-135, ^{1}H-^{1}H COSY, HMQC and HMBC experiments. Experiments were carried out using $CDCl_3$, d_6-DMSO, CD_3OD or CD_3CN. Low-resolution mass spectra were measured on a Waters ZMD quadrupole mass spectrometer for +/- electrospray ionisation (ESI). These were carried out in HPLC grade MeOH or MeCN.

High-resolution mass spectra were recorded in MeOH or MeCN on a Bruker APEX III FT-ICR mass spectrometer (ESI) or a VG Analytical 70-250-SE mass spectrometer (EI).
Melting points were determined on a Gallenkamp Electrothermal melting point apparatus.

Common procedures including tritylation (2), palladium cross-coupling(3,4), trimethylsilyl deprotection (5,6) and phosphitylation (7) were based on literature procedures. I-Cy5 was synthesised according to the literature (8). 1-[2′-(deoxy)-5′-*O*-(4,4′-dimethoxytrityl)-*β*-D-erythro-pentafuranosyl]-5-(eth-1-ynyl)uridine was prepared according to the published procedures (9,10). Quantities of reagents are quoted to either 3 significant figures or 2 decimal places as appropriate.

ESI.2.2.2. Synthetic procedures

Synthesis of 2′-Deoxy-5′-*O*-(4,4′-dimethoxytrityl)-5-iodouridine (11)



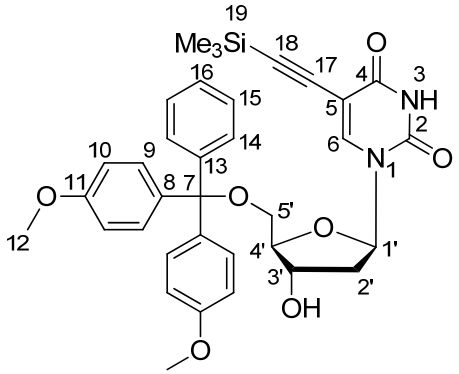
5-Iodo-2′-deoxyuridine (10.2 g, 28.9 mmol) was co-evaporated with distilled pyridine (3 x 10 mL) and dissolved in distilled pyridine (80 mL). To this was added drop-wise a solution of DMTCl (9.79 g, 28.9 mmol) in distilled pyridine (50 mL) over a period of 20 min and the reaction was stirred at rt for 3 h. An additional 0.2 eq DMTCl was added and the reaction stirred for a further 2.5 h. The reaction was quenched by the addition of MeOH (80 mL) and was stirred for 20 min. The reaction volume was reduced by half *in vacuo*, diluted with DCM (250 mL) and washed with H_2O (250 mL) and sat. aq. $NaHCO_3$ (2 x 200 mL) then the solvent was removed *in vacuo*. Following purification by column chromatography (DCM with 0.5 % pyridine → MeOH/DCM, 4:96 with 0.5 % pyridine) the product (16.6 g, 25.3 mmol, 88 %) was afforded as a beige foam.

R_f : 0.44 (MeOH/DCM, 1:9)
LRMS: [ESI+, MeOH] m/z (%): 679 ([M+Na]$^{+}$, 100).
^{1}H (300 MHz, d_6-DMSO): δ 11.84 (s, 1H, NH), 8.13 (s, 1H, H^{6}), 7.53-7.33 (m, 9H, H^{Ar}), 7.02 (d, *J*=12.0, 4H, H^{10}), 6.23 (t, *J* = 6.7 Hz, 1H, $H^{1'}$), 5.42 (d, *J* = 4.3 Hz, 1H, $OH^{3'}$), 4.35

(m, 1H, $H^{3'}$), 4.03 (m, 1H, $H^{4'}$), 3.86 (s, 6H, H^{12}), 3.30-3.27 (m, 2H, $H^{5'}$), 2.37-2.30 (m, 2H, $H^{2'}$) ppm.
^{13}C (75 MHz, d_6-DMSO): δ 160.2 (C^{2}), 157.8 (C^{4}), 149.8 (CH^{6}), 149.3 (C^{Ar}), 144.5 (CH^{Ar}), 144.0 (C^{Ar}), 135.8 (CH^{Ar}), 135.2 (C^{Ar}), 135.1 (C^{Ar}), 129.4 (CH^{Ar}), 127.7 (CH^{Ar}), 127.4 (CH^{Ar}), 126.4 (CH^{Ar}), 123.6 (CH^{Ar}), 113.0 (CH^{10}), 85.6 ($CH^{4'}$), 84.62 ($CH^{1',7}$), 70.2 ($CH^{3'}$), 69.5 (CH^{5}), 63.4 ($CH_2^{5'}$), 54.8 (CH_3^{12}), 39.9 ($CH_2^{2'}$) ppm.

Characterisation data recorded matches previously reported literature values (11).

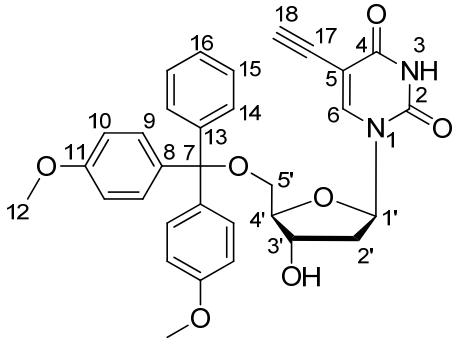
Synthesis of 2′-Deoxy-5′-*O*-(4,4′-dimethoxytrityl)-5-(trimethylsilyl eth-1-ynyl)uridine



To a mixture of 2′-deoxy-5′-*O*-(4,4′-dimethoxytrityl)-5-iodouridine (1.19 g, 1.81 mmol) and CuI (0.07 g, 0.38 mmol) in anhydrous DMF (4.5 mL) under an argon atmosphere, was added trimethylsilylacetylene (0.77 mL, 5.42 mmol) and Et_3N (0.75 mL, 5.42 mmol) and the reaction was stirred for 10 min at rt. $Pd(PPh_3)_4$ (0.21 g, 0.18 mmol) was added and the reaction stirred at rt for 16 h. The solvent was removed *in vacuo* and purification by column chromatography (MeOH/DCM, 4:96 with 1 % pyridine) afforded the product (1.06 g, 1.69 mmol, 94 %) as a light brown foam.

R_f : 0.46 (EtOAc/MeOH/aq. NH_3, 5:1:1)
LRMS [ESI+, MeOH] m/z (%): 649 ($[M+Na]^+$, 78).
HRMS [ESI+, MeOH] for $C_{35}H_{38}N_2O_7SiNa$ $[M+Na]^+$: calcd 649.2340, found 649.2343.
^{1}H (300 MHz, $CDCl_3$): δ 7.99 (s, 1H, H^{6}), 4.45-7.18 (m, 9H, H^{Ar}), 6.83 (d, J = 8.8 Hz, 4H, H^{Ar}), 6.26 (dd, J = 7.7, 5.7 Hz, 1H, $H^{1'}$), 4.45 (dt, J = 6.0, 2.0 Hz, 1H, $H^{3'}$), 4.08 (dt, J = 3.0, 2.0 Hz, 1H, $H^{4'}$), 3.77 (s, 6H, H^{12}), 3.40 (dd, J = 11.0, 4.0 Hz, 1H, $H^{5'}$), 3.30 (dd, J = 11.0, 4.0 Hz, 1H, $H^{5''}$), 2.48 (ddd, J = 13.6, 5.6, 2.4 Hz, 1H, $H^{2'}$), 2.20 (ddd, J = 13.7, 7.6, 6.1 Hz, 1H, $H^{2''}$), 0.00 (s, 9H, H^{19}) ppm.
^{13}C (75 MHz, $CDCl_3$): δ 160.8 (C^{2}), 158.2 (C^{Ar}), 148.8 (C^{4}), 144.1 (C^{Ar}), 142.3 (CH^{6}), 135.2 (C^{Ar}), 129.6 (CH^{Ar}), 127.7 (CH^{Ar}), 127.6 (CH^{Ar}), 126.6 (CH^{Ar}), 113.0 (CH^{Ar}), 100.2 (C^{17}), 99.4 (C^{18}), 94.4 (C^{5}), 86.6 (C^{7}), 86.1 ($CH^{4'}$), 85.4 ($CH^{1'}$), 72.0 ($CH^{3'}$), 63.1 ($CH_2^{5'}$), 54.9 (CH_3^{12}), 45.9 ($CH_2^{2'}$), 0.7 (CH_3^{19}) ppm.
Mp: 86-88 °C (decomposes)
IR ν_{Max}/cm^{-1}: 3360 (br, O-H alcohol), 3050 (w, C-H aromatic), 2953, 2835 (m, C-H alkyl), 2161 (m, C≡C alkyne), 1682 (s, C=O carbonyl).

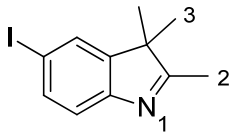
Synthesis of 2′-Deoxy-5′-*O*-(4,4′-dimethoxytrityl)-5-(eth-1-ynyl)uridine



To a solution of 2′-deoxy-5′-*O*-(4,4′-dimethoxytrityl)-5-(trimethylsilyl eth-1-ynyl)uridine (0.11 g, 0.17 mmol) in distilled THF (2 mL) under an argon atmosphere was added 1 M TBAF in THF (0.26 mL, 0.25 mmol) and the reaction was stirred at rt for 20 min. The reaction was partitioned between EtOAc (100 mL) and brine (100 mL). The organic phase was washed with brine and dried (Na_2SO_4). The solvent was removed *in vacuo* and the product (0.09 g, 0.17 mmol, 99 %) was afforded as a light yellow/brown foam.

R_f : 0.38 (MeOH/DCM, 7.5:92.5)
LRMS [ESI+, MeOH] m/z (%): 577 ($[M+Na]^+$, 100).
HRMS [ESI+, MeOH] for $C_{32}H_{30}N_2O_7Na$ $[M+Na]^+$: calcd 577.1945, found 577.1951.
^{1}H (300 MHz, $CDCl_3$): δ 8.00 (s, 1H, H^6), 7.36-7.12 (m, 9H, H^{Ar}), 6.77 (d, *J* = 8.3 Hz, 4H, H^{Ar}), 6.20 (t, *J* = 6.6, 6.6 Hz, 1H, $H^{1'}$), 4.47 (m, 1H, $H^{3'}$), 4.05 (m, 1H, $H^{4'}$), 3.71 (s, 6H, H^{12}), 3.35-3.27 (m, 2H, $H^{5'}$), 2.83 (s, 1H, H^{18}), 2.45 (ddd, *J* = 13.6, 5.6, 2.8 Hz, 1H, $H^{2'}$), 2.20 (ddd, *J* = 13.6, 6.7, 6.7 Hz, 1H, $H^{2'}$) ppm.
^{13}C (75 MHz, $CDCl_3$): δ 161.4 (C^2), 158.6 (C^{Ar}), 149.1 (C^4), 144.4 (C^{Ar}), 143.6 (CH^6), 135.5 (C^{Ar}), 135.3 (C^{Ar}), 130.0 (CH^{Ar}), 128.0 (CH^{Ar}), 127.9 (CH^{Ar}), 127.0 (CH^{Ar}), 113.4 (CH^{Ar}), 99.2 (C^{17}), 87.1 (C^5), 86.5 ($CH^{4'}$), 85.8 ($CH^{1'}$), 82.0 (CH^{18}), 74.1 (C^7), 72.2 ($CH^{3'}$), 63.4 ($CH_2{}^{5'}$), 55.2 ($CH_3{}^{12}$), 41.5 ($CH_2{}^{2'}$) ppm.
Mp: 95-96 °C (decomposes)
IR ν_{Max}/cm^{-1}: 3425 (br,w, O-H alcohol), 3272 (m, H-C alkyne), 2930, 2836 (m, C-H alkyl), 2162 (w, C≡C alkyne), 1682 (s, C=O carbonyl).

Synthesis of 5-iodo-2,3,3-trimethyl-3*H*-indole



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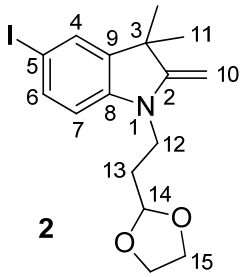
To a solution of 4-iodophenylhydrazine (1.51 g, 6.46 mmol) in AcOH (120 mL) was added 3-methyl-2-butanone (0.75 mL, 7.05 mmol) and the reaction was stirred at reflux for 2.5 h. The reaction mixture was allowed to cool to rt, diluted with H_2O (300 mL) and washed with DCM (2 x 200 mL). The organic phase was collected and concentrated *in vacuo*. The oily residue was diluted with DCM (16 mL). The product was extracted by addition of hexane (300 mL) which was decanted from the resultant purple oily precipitate. The yellow hexane layer was dried *in vacuo* to afford the product **1** (1.48 g, 5.19 mmol, 81 %) as an orange oil.

R_f : 0.41 (DCM/Et_3N, 99:1).
LRMS [ESI+, MeOH] m/z (%): 286 ($[M + H]^+$, 100).
^{1}H (300 MHz, $CDCl_3$) δ 7.63-7.59 (m, 2H, H^{Ar}), 7.31 (t, *J* = 8.0 Hz, 1H, H^{Ar}), 2.26 (s, 3H, H^2), 1.29 (s, 6H, H^3) ppm.

Characterisation data recorded matches previously reported literature values (12).

Synthesis of 5-iodo-1-ethyldioxolane-3,3-dimethyl-2-methyleneindoline

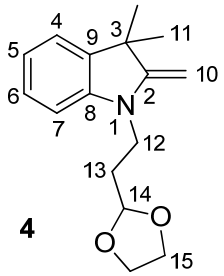


2

To a suspension of KI (15.4 g, 92.7 mmol) in distilled MeCN (60 mL) under an argon atmosphere was added bromoethyldioxolane (7.25 mL, 61.7 mmol) and the yellow suspension was stirred at 50 °C for 1 h. 5-Iodo-2,3,3-trimethylindolenine **1** (8.33 g, 29.2 mol) was added in distilled MeCN (20 mL) and the reaction was stirred at reflux for 45 h. The reaction was allowed to cool to rt and the precipitate was removed by filtration (KBr). The solvent was removed *in vacuo* and following purification by column chromatography (DCM with 0.5 % Et_3N), the product was afforded as a brown oil (3.28 g, 8.52 mmol, 29 %).

R_f : 0.62 (MeOH/DCM, 5:95)
LRMS [ESI+, MeCN] m/z (%): 386 ($[M+H]^+$, 100).
HRMS [ESI+, MeCN] for $C_{16}H_{21}I_1N_1O_2$ $[M+H]^+$: calcd 386.0611, found 386.0607.
^{1}H (400 MHz, CD_3CN): δ 7.40 (m, 2H, H^{Ar}), 6.46 (d, *J* = 9.0 Hz, 1H, H^{Ar}), 4.85 (t, *J* = 4.0 Hz, 1H, H^{14}), 3.96-3.77 (m, 6H, $H^{10,15}$), 3.60 (t, *J* = 7.3 Hz, 2H, H^{12}), 1.89 (dt, *J* = 7.2, 4.8 Hz, 2H, H^{13}), 1.27 (s, 6H, H^{11}) ppm.
^{13}C (100 MHz, CD_3CN): δ 161.6 (C^2), 146.9 (C^{Ar}), 141.9 (C^{Ar}), 137.5 (CH^{Ar}), 131.8 (CH^{Ar}), 109.0 (CH^{Ar}), 103.5 (CH^{14}), 79.9 (C^5), 75.5 ($CH_2{}^{10}$), 65.9 ($CH_2{}^{15}$), 45.2 (C^3), 38.3 ($CH_2{}^{12}$), 31.1 ($CH_2{}^{13}$), 30.3 ($CH_3{}^{11}$) ppm.
IR ν_{Max}/cm^{-1}: 2960 (m, C-H aromatic), 2928 (m, =C-H), 2871 (m, -C-H).

Synthesis of 1-ethyldioxolane-2,3,3-trimethylindolenine

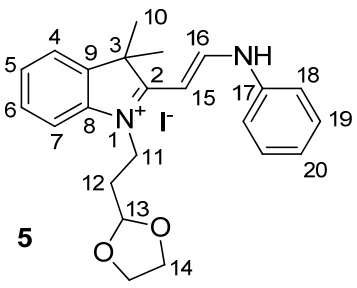


4

To a suspension of KI (3.98 g, 0.02 mol) in distilled MeCN (20 mL), under an argon atmosphere, was added bromoethyldioxolane (1.55 mL, 13.2 mmol) and the yellow suspension was stirred at 50 °C for 1 h. 2,3,3-Trimethylindolenine (2 mL, 12.0 mmol) was added and the reaction stirred at reflux for 45 h. The reaction was allowed to cool to rt and filtered to remove KBr salt. The solvent was removed *in vacuo* and purified by column chromatography (twice in 100 % DCM with 0.5 % Et_3N) to afford the product **4** (1.73 g, 6.68 mmol, 56 %) as an orange oil.

R_f : 0.42 (MeOH/DCM, 5:95)
LRMS [ESI+, MeCN] m/z (%): 260 ($[M+H]^+$, 100).
HRMS [ESI+, MeCN] for $C_{16}H_{22}N_1O_2$ $[M+H]^+$: calcd 260.1645, found 260.1645.
^{1}H (400 MHz, CD_3CN): δ 7.11 (m, 2H, H^{Ar}), 6.74 (t, *J* = 8.0 Hz, 1H, H^{Ar}), 6.63 (d, *J* = 12.0 Hz, 1H, H^{Ar}), 4.88 (t, *J* = 8.0 Hz, 1H, H^{14}), 3.96-3.78 (m, 6H, $H^{10,15}$), 3.64 (t, *J* = 12.0 Hz, 2H, H^{12}), 1.90 (m, 2H, H^{13}), 1.30 (s, 6H, H^{11}) ppm.
^{13}C (100 MHz, CD_3CN): δ 162.2 (C^2), 146.7 (C^{Ar}), 138.6 (C^{Ar}), 128.6 (CH^{Ar}), 122.9 (CH^{Ar}), 119.5 (CH^{Ar}), 106.4 (CH^{Ar}), 103.4 (CH^{14}), 74.2 (CH_2^{10}), 65.7 (CH_2^{15}), 45.0 (C^3), 38.1 (CH_2^{12}), 31.0 (CH_2^{13}), 30.3 (CH_3^{11}) ppm.
IR ν_{Max}/cm^{-1}: 2960 (m, C-H aromatic), 2924 (m, =C-H), 2881 (m, -C-H).

Synthesis of 2-[(E)-2-(phenylamino)vinyl]-1-ethyldioxolane-,3,3-dimethyl-3*H*-indolium iodide



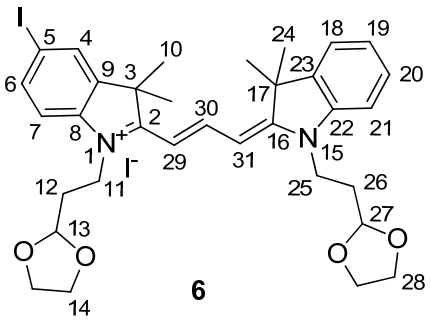
5

To a solution of 1-ethyldioxolane-3,3-dimethyl-2-methyleneindoline **4** (0.21 g, 0.82 mmol) in EtOH (2 mL), under an argon atmosphere, was added *N,N′*-diphenylformamidine (0.16 g, 0.82 mmol) and triethylorthoformate (0.14 mL, 0.82 mmol) and the reaction refluxed at high temperature (97 °C) in the dark for 16 h. The reaction solvent was removed *in vacuo* and following purification by column chromatography (DCM with 0.5 % Et_3N) the product **5** (0.26 g, 0.53 mmol, 65 %) was isolated as an orange foam.

R_f : 0.51 (MeOH/DCM, 1:9)

LRMS [ESI+, MeCN] m/z (%): 363 ([M]$^+$, 100).
HRMS [ESI+, MeCN] for $C_{23}H_{27}N_2O_2$ [M]$^+$: calcd 363.2067, found 363.2068.
^{1}H (400 MHz, CD_3CN): δ 8.60 (d, *J* = 12.0 Hz, 1H, H^{16}), 7.51-7.26 (m, 9H, H^{Ar}), 6.59 (d, *J* = 12.0 Hz, 1H, H^{15}), 5.02 (t, *J* = 4.0 Hz, 1H, H^{13}), 4.17 (t, *J* = 8.0 Hz, 2H, H^{11}), 3.92-3.78 (m, 4H, H^{14}), 2.19 (dt, *J* = 8.0, 4.0 Hz, 2H, H^{12}), 1.68 (s, 6H, H^{10}) ppm.
^{13}C (100 MHz, CD_3CN): δ 177.9 (C^2), 153.3 (CH^{16}), 143.1 (C^{Ar}), 142.0 (C^{Ar}), 141.5 (C^{Ar}), 130.8 (CHAr), 129.6 (CHAr), 127.1 (CHAr), 126.4 (CHAr), 123.3 (CHAr), 119.4 (CH^{Ar}), 112.3 (CH^{Ar}), 102.7 (CH^{13}), 92.8 (CH^{15}), 65.8 (CH_2^{14}), 50.5 (C^3), 40.7 (CH_2^{11}), 31.3 (CH_2^{12}), 28.6 (CH_3^{10}) ppm.
IR ν_{Max}/cm^{-1}: 3419 (w, br, N-H), 2965 (m, =C-H), 2885 (m, -C-H), 2358 (w, =N$^+$-).

Synthesis of 5-iodo-1-ethyldioxolane-3,3-dimethyl-2-((1E,3E)-3-(1-ethyldioxolane-3,3-dimethylindolin-2-ylidene)prop-1-enyl)-3*H*-indolium iodide

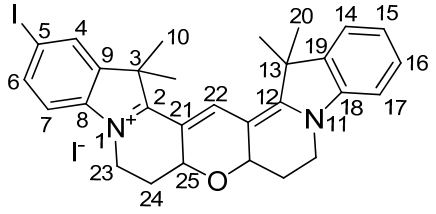


6

To a solution of 2-[(E)-2-(phenylamino)vinyl]-1-ethyldioxolane-3,3-dimethyl-3*H*-indolium iodide **5** (0.35 g, 0.72 mmol) in distilled pyridine (8 mL) was added 5-iodo-1-ethynyldioxolane-3,3-dimethyl-2-methyleneindoline **2** (0.55 g, 1.44 mmol) and Ac_2O (0.68 mL, 7.18 mmol) and the reaction was stirred under an argon atmosphere in the dark at 50 °C for 24 h. The reaction solvent was removed *in vacuo* and the crude material was purified by column chromatography (MeOH/EtOAc, 5:95with 0.1 % pyridine → DCM with 0.1 % pyridine) to afford the product **6** (0.45 g, 0.58 mmol, 79 %) as a dark red solid.

R_f : 0.44 (MeOH/DCM, 1:9)
LRMS [ESI+, MeCN] m/z (%): 655 ([M]$^+$, 100).
HRMS [ESI+, MeCN] for $C_{33}H_{40}I_1N_2O_4$ [M]$^+$: calcd 655.2027, found 655.2027.
^{1}H (400 MHz, CD_3CN): δ 8.44 (t, *J* = 12.0 Hz, 1H, H^{30}), 7.86 (d, *J* = 4.0 Hz, 1H, H^{Ar}), 7.76 (dd, *J* = 8.0, 4.0 Hz, 1H, H^{Ar}), 7.54-7.31 (m, 4H, H^{Ar}), 7.07 (d, *J* = 8.0 Hz, 1H, H^{Ar}), 6.45 (d, *J* = 16.0 Hz, 1H, $H^{29/31}$), 6.36 (d, *J* = 12.0 Hz, 1H, $H^{29/31}$), 4.98 (m, 2H, $H^{13,27}$), 4.21 (t, *J* = 8.0 Hz, 2H, $H^{11/25}$), 4.13 (t, *J* = 8.0 Hz, 2H, $H^{11/25}$), 3.91-3.78 (m, 8H, $H^{14,28}$), 2.18-2.11 (m, 4H, $H^{12/26}$), 1.69 (s, 12H, $H^{10,24}$) ppm.
^{13}C (100 MHz, CD_3CN): δ 176.8 ($C^{2/16}$), 174.8 ($C^{2/16}$), 152.0 (CH^{30}), 144.6 (C^{Ar}), 143.7 (C^{Ar}), 143.4 (C^{Ar}), 142.6 (C^{Ar}), 138.9 (CH^{Ar}), 132.8 (CH^{Ar}), 130.1 (CH^{Ar}), 127.2 (CH^{Ar}), 123.8 (CH^{Ar}), 114.6 (CH^{Ar}), 113.1 (CH^{Ar}), 104.9 ($CH^{29/31}$), 103.8 ($CH^{29/31}$), 103.0 ($CH^{13,27}$), 89.3 (C^5), 66.2 ($CH_2^{14,28}$), 51.0 ($C^{3/17}$), 50.5 ($C^{3/17}$), 41.0 ($CH_2^{11/25}$), 40.7 ($CH_2^{11/25}$), 31.9 ($CH_2^{12/26}$), 31.7 ($CH_2^{12/26}$), 28.4 ($CH_3^{10,24}$) ppm.
Mp: 80 °C (decomposes)
IR ν_{Max}/cm^{-1}: 2968 (m, =C-H), 2885 (m, -C-H), 2359 (m, =N$^+$-).

Synthesis of 5-iodo-Cy3B 7 (13)

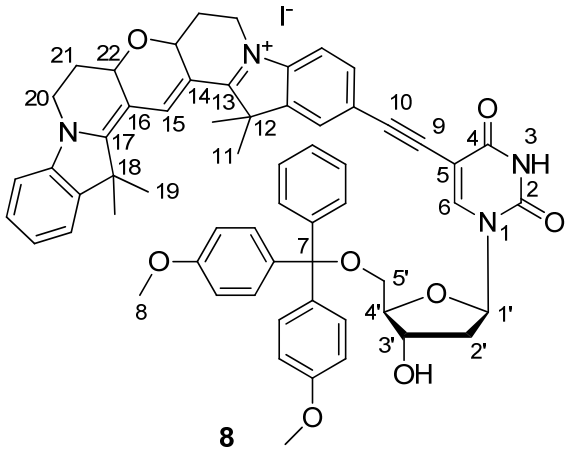


7

To a solution of 5-iodo-1-ethyldioxolane-3,3-trimethyl-2-((1E,3E)-3-(1-ethyldioxolane-3,3-trimethylindolin-2-ylidene)prop-1-enyl)-3*H*-indolium iodide **6** (0.58 g, 0.74 mmol) in $CHCl_3$ (58 mL) was added 50 % aq. H_2SO_4 (12 mL) and the mixture was stirred vigorously for 20 min. The reaction mixture was diluted with $CHCl_3$ (60 mL) and washed with H_2O (3 x 60 mL). The aqueous phases were combined, washed with $CHCl_3$ (2 x 60 mL) and the combined organic phase was dried (Na_2SO_4) then the solvent was removed *in vacuo* to afford the product **7** (0.48 g, 0.71 mmol, 96 %) as a dark pink solid.

R_f : 0.30 (MeOH/DCM, 1:9)
LRMS [ESI+, MeCN] m/z (%): 549 ($[M]^+$, 100).
HRMS [ESI+, MeCN] for $C_{29}H_{30}I_1N_2O_1$ $[M]^+$: calcd 549.1397, found 549.1402.
^{1}H (400 MHz, $CDCl_3$): δ 8.06 (s, 1H, H^{22}), 7.71 (dd, *J* = 8.3, 1.5 Hz, 1H, H^{Ar}), 7.64 (d, *J* = 1.5 Hz, 1H, H^{Ar}), 7.45-7.40 (m, 2H, H^{Ar}), 7.35-7.22 (m, 2H, H^{Ar}), 7.00 (d, *J* = 8.3 Hz, 1H, H^{Ar}), 4.99 (dt, *J* = 11.6, 5.7 Hz, 2H, H^{25}), 4.30-4.19 (m, 4H, H^{23}), 2.72-2.68 (m, 2H, H^{24}), 2.06-1.93 (m, 2H, H^{24}), 1.79 (s, 3H, $H^{10/20}$), 1.77 (s, 3H, $H^{10/20}$), 1.74 (s, 3H, $H^{10/20}$), 1.72 (s, 3H, $H^{10/20}$) ppm.
^{13}C (100 MHz, $CDCl_3$): δ 168.5 ($C^{2/12}$), 166.3 ($C^{2/12}$), 142.3 (C^{Ar}), 141.4 (C^{Ar}), 141.3 (C^{Ar}), 140.4 (C^{Ar}), 138.3 (CH^{22}), 137.8 (CH^{Ar}), 131.1 (CH^{Ar}), 129.1 (CH^{Ar}), 126.1 (CH^{Ar}), 122.2 (CH^{Ar}), 112.6 (CH^{Ar}), 111.3 (C^{21}), 111.1 (CH^{Ar}), 110.3 (CH^{Ar}), 88.9 (C^5), 69.5 (CH^{25}), 48.9 ($C^{3/13}$), 48.4 ($C^{3/13}$), 42.1 (CH_2^{23}), 42.0 (CH_2^{23}), 28.4 ($CH_3^{10/20}$), 27.8 ($CH_3^{10/20}$), 26.5 (CH_2^{24}), 26.4 (CH_2^{24}) ppm.
Mp: 110 °C (decomposes).
IR ν_{Max}/cm^{-1}: 2960 (m, =C-H), 2923 (m, -C-H), 2359 (m, $=N^+$-).
UV/Vis (MeOH): A_{max}= 565 nm, ε_{max}= 141,000 $M^{-1}cm^{-1}$, Em_{max}= 575 nm.

Synthesis of Cy3B-dT

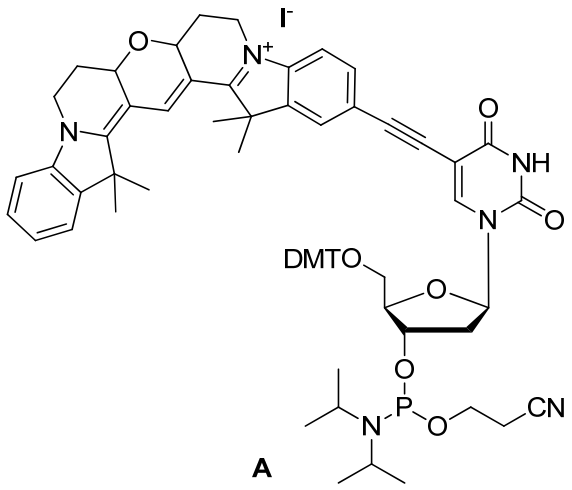


8

To a mixture of 5-iodo-Cy3B **7** (0.48 g, 0.71 mmol), 2′-deoxy-5′-*O*-(4,4′-dimethoxytrityl)-5-(eth-1-ynyl)uridine (0.59 g, 1.06 mmol) and CuI (0.03 g, 0.14 mmol) in anhydrous DMF (4 mL), under an argon atmosphere, was added distilled Et_3N (2.5 mL) and the reaction was stirred for 10 min. $Pd(PPh_3)_4$ (0.08 g, 0.07 mmol) was added and the solution was stirred in the dark for 1.5 h. The reaction mixture was diluted with DCM (100 mL) and washed with 5 % aq. disodium EDTA (100 mL). The organic phase was evaporated to dryness *in vacuo* and purified by column chromatography (DCM to MeOH/DCM, 1:9 with 0.1 % pyridine) affording the product **8** (0.33 g, 0.30 mmol, 42 %) as a dark pink solid.

R_f : 0.24 (MeOH/DCM, 1:9)
LRMS [ESI+, MeCN] m/z (%): 975 ($[M]^+$, 100).
HRMS [ESI+, MeCN] for $C_{61}H_{59}N_4O_8$ $[M]^+$: calcd 975.4327, found 975.4325.
^{1}H (400 MHz, d_6-DMSO): δ 11.78 (s, 1H, NH), 8.13 (s, 1H, H^6), 7.98 (s, 1H, H^{15}), 7.45-7.13 (m, 16H, H^{Ar}), 6.86 (dd, 4H, J = 8.0, 4.0 Hz, H^{Ar}), 6.16 (t, J = 4.0 Hz, 1H, $H^{1'}$), 5.35 (d, J = 4.0 Hz, 1H, $OH^{3'}$), 4.64 (m, 2H, H^{22}), 4.35-4.29 (m, 3H, $H^{3',20}$), 3.97-3.84 (m, 3H, $H^{4',20}$), 3.68 (s, 6H, H^8), 3.22-3.19 (m, 2H, $H^{5'}$), 2.51-2.49 (m, 2H, H^{21}), 2.32-2.27 (m, 2H, $H^{2'}$), 1.99-1.90 (m, 2H, H^{21}), 1.71 (s, 6H, $H^{11/19}$), 1.64 (s, 6H, $H^{11/19}$) ppm.
^{13}C (100 MHz, d_6-DMSO): δ 174.0 (C^{Ar}), 168.3 ($C^{17/13}$), 166.5 ($C^{17/13}$), 161.4 ($C^{2/4}$), 158.0 (C^{Ar}), 149.3 ($C^{2/4}$), 144.7 (C^{Ar}), 142.8 (CH^6), 141.7 (C^{Ar}), 140.7 (C^{Ar}), 140.6 (C^{Ar}), 137.1 (CH^{15}), 135.5 (C^{Ar}), 135.3 (C^{Ar}), 131.9 (CH^{Ar}), 129.7 (CH^{Ar}), 128.7 (CH^{Ar}), 127.9 (CH^{Ar}), 127.57 (CH^{Ar}), 126.7 (CH^{Ar}), 125.6 (CH^{Ar}), 124.8 (CH^{Ar}), 122.4 (CH^{Ar}), 118.4 (C^{Ar}), 113.2 (CH^{Ar}), 111.3 ($C^{14/16}$), 110.8 ($C^{14/16}$), 110.4 (CH^{Ar}), 109.3 (CH^{Ar}), 98.5 (C^5), 91.9 (C^{10}), 86.1 (C^9), 85.9 ($CH^{4'}$), 85.3 ($CH^{1'}$), 82.3 (C^7), 70.5 ($CH^{3'}$), 69.3 (CH^{22}), 63.5 ($CH_2{}^{5'}$), 55.0 ($CH_3{}^8$), 48.5 ($C^{12/18}$), 47.9 ($C^{12/18}$), 41.3 ($CH_2{}^{20}$), 41.0 ($CH_2{}^{2'}$), 27.5 ($CH_3{}^{11/19}$), 26.8 ($CH_3{}^{11/19}$), 26.1 ($CH_2{}^{21}$) ppm.
Mp: >200 °C (decomposes).
IR ν_{Max}/cm^{-1}: 3307 (w, br, N-H and –OH), 2960 (m, =C-H), 2929 (m, -C-H), 2359 (m, $=N^+$-), 1689 (s, C=O).
UV/Vis (MeOH): A_{max}= 581 nm, ε_{max} = 122,000 $M^{-1}cm^{-1}$, Em_{max}= 596 nm.

Synthesis of Cy3B-dT-phosphoramidite

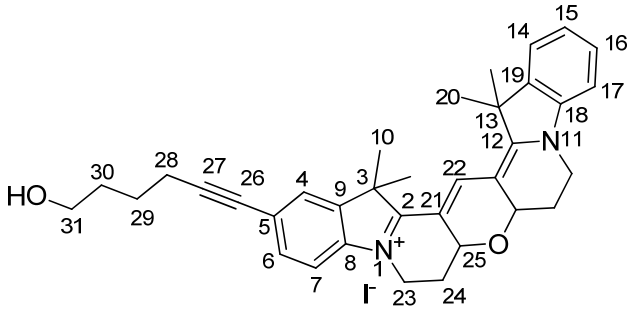


A

Cy3B-dT **8** (0.67 g, 0.61 mmol) was co-evaporated with distilled pyridine (3 x 5 mL) and distilled DCM (3 x 5 mL) then dissolved in distilled DCM (6 mL), with activated molecular sieves (3 Å), under an argon atmosphere. Distilled DIPEA (0.29 mL, 1.53 mmol) was added followed by 2-cyanoethyl *N,N*-diisopropyl-chlorophosphoramidite (0.15 mL, 0.67 mmol) drop-wise and the reaction stirred at rt for 45 min.
The reaction was diluted with distilled DCM (10 mL) and washed with deoxygenated sat. aq. KCl (20 mL). The organic phase was dried (Na_2SO_4) and the solvent was removed *in vacuo*. The crude product was precipitated 3 times consecutively in deoxygenated hexane (3 x 100 mL) from a minimum of distilled DCM (2 mL). The product was co-evaporated with distilled DCM (5 mL) and trace solvents removed *in vacuo* to afford the product **A** (0.50 g, 0.38 mmol, 63 %) as a dark pink solid.

R_f : 0.44 (MeOH/DCM, 1:9 with 1 % pyridine)
LRMS [ESI+, MeCN] m/z (%): 1176 ($[M]^+$, 100).
^{31}P (121 MHz, CD_3CN): δ 149.3 (s, P^{III}), 149.1 (s, P^{III}) ppm.

Synthesis of 5-(hexyn-1-ol)-6-Cy3B



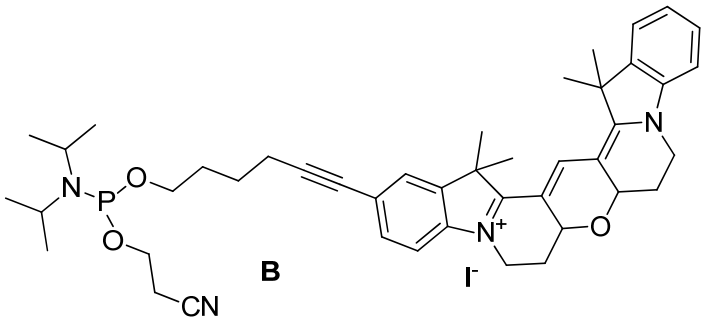
9

To a solution of 5-iodo-Cy3B **7** (0.50 g, 0.70 mmol) and CuI (0.03 g, 0.14 mmol) in anhydrous DMF (3.5 mL), under an argon atmosphere, was added distilled Et_3N (2.5 mL) and 5-hexyn-1-ol (0.08 mL, 0.70 mmol) and the reaction was stirred in the dark at rt for 10 min.

$Pd(PPh_3)_4$ (0.08 g, 0.07 mmol) was added and the solution stirred for 3.5 h. An additional portion of 5-hexyn-1-ol (0.07 mL) was added and the reaction stirred for a further 1.5 h. The reaction was diluted with DCM (40 mL) and washed with 5 % aq. disodium EDTA (3 x 50 mL) and 5 % aq. KI solution (2 x 50 mL). The organic phase was dried (Na_2SO_4) and the solvent was removed *in vacuo*. The crude product was dissolved in DCM (2 mL), filtered through celite and precipitated in hexane (100 mL). The material was filtered through alumina and trace solvents were removed *in vacuo* to afford the product **9** (0.37 g, 0.57 mmol, 82 %) as a deep pink solid.

R_f : 0.25 (MeOH/DCM, 1:9)
LRMS [ESI+, MeCN] m/z (%): 519 ($[M]^+$, 100).
HRMS [ESI+, MeCN] for $C_{35}H_{39}N_2O_2$ $[M]^+$: calcd 519.3006, found 519.3000.
^{1}H (400 MHz, $CDCl_3$): δ 8.00 (s, 1H, H^{22}), 7.62-7.02 (m, 7H, H^{Ar}), 5.00 (m, 2H, H^{25}), 4.20-4.13 (m, 4H, H^{23}), 3.65 (t, *J* = 6.0 Hz, 2H, H^{31}), 2.66-2.62 (m, 2H, H^{24}), 2.41 (t, *J* = 7.0 Hz, 2H, H^{28}), 1.97-1.92 (m, 2H, H^{24}), 1.72-1.65 (m, 16H, $H^{10,20,29,30}$) ppm.
^{13}C (100 MHz, $CDCl_3$): δ 168.5 ($C^{2/12}$), 167.5 ($C^{2/12}$), 141.9 (C^{Ar}), 141.2 (C^{Ar}), 140.8 (C^{Ar}), 140.7 (C^{Ar}), 138.6 (CH^6), 132.4 (CH^{Ar}), 128.8 (CH^{Ar}), 126.4 (CH^{Ar}), 125.7 (CH^{Ar}), 122.6 (CH^{Ar}), 121.7 (C^{Ar}), 111.6 (C^{21}), 111.4 (CH^{Ar}), 111.0 (C^{21}), 110.9 (CH^{Ar}), 91.9 (C^{27}), 80.8 (C^{26}), 70.0 (CH^{25}), 62.7 ($CH_2{}^{31}$), 49.2 ($C^{3/13}$), 48.8 ($C^{3/13}$), 42.3 ($CH_2{}^{23}$), 32.3 ($CH_2{}^{29/30}$), 28.8 ($CH_3{}^{10/20}$), 28.3 ($CH_3{}^{10/20}$), 27.0 ($CH_2{}^{29/30}$), 25.4 ($CH_2{}^{24}$), 19.7 ($CH_2{}^{28}$) ppm.
Mp: >130 °C (decomposes).
IR ν_{Max}/cm^{-1}: 3363 (br, OH), 2927 (m, =C-H), 2860 (m, -C-H), 2350 (m, C≡C).
UV/Vis (MeOH): A_{max}= 573 nm, Em_{max}= 585 nm

Synthesis of 5-(hexyn-1-ol)-6-Cy3B phosphoramidite

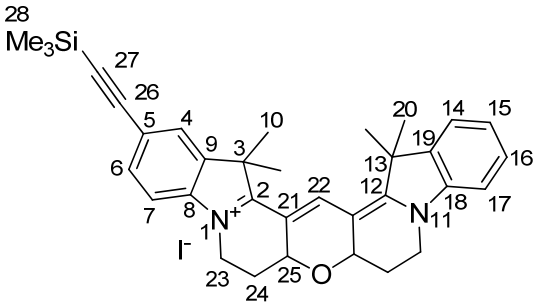


5-(Hexyn-1-ol)-6-Cy3B **9** (0.35 g, 0.54 mmol) was co-evaporated with distilled pyridine (5 mL) and distilled DCM (3 x 5mL) then dissolved in distilled DCM (5 mL), with activated molecular sieves (3 Å), under an argon atmosphere. To this was added distilled DIPEA (0.24 mL, 1.35 mmol) followed by 2-cyanoethyl *N,N*-diisopropyl-chlorophosphoramidite (0.13 mL, 0.60 mmol) and the reaction was stirred at rt for 45 min. The reaction was diluted with distilled DCM (10 mL) and washed with deoxygenated sat. aq. KCl (10 mL) then dried (Na_2SO_4). The solvent was removed *in vacuo* then the crude material was purified by precipitation in deoxygenated hexane (150 mL) from a minimum of distilled DCM (2 mL). The precipitation was repeated a further two times. The precipitate was co-evaporated with distilled DCM (3 mL) and trace solvents were removed under high vacuum to afford the product **B** (0.42 g, 0.50 mmol, 93 %) as a purple foam.

R_f : 0.46 (MeOH/DCM, 1:9 with 1 % pyridine)

LRMS [ESI+, MeCN] m/z (%): 720 ($[M]^+$, 100).
HRMS [ESI+, MeCN] for $C_{44}H_{56}N_4O_3P_1$ $[M]^+$: calcd 719.4085, found 719.4076.
^{31}P (121 MHz, CD_3CN): δ 148.2 (s, P^{III}) ppm.

Synthesis of 5-trimethylsilylethynyl-Cy3B

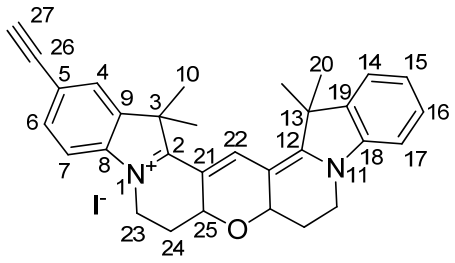


10

To a solution of 5-iodo-Cy3B **7** (0.29 g, 0.43 mmol) and CuI (0.02 g, 0.09 mmol) in anhydrous DMF (2.5 mL), under an argon atmosphere was added distilled Et_3N (1.5 mL) and trimethylsilylacetylene (0.09 mL, 0.64 mmol) and the reaction was stirred at rt for 10 min. $Pd(PPh_3)_4$ (0.05 mg, 0.04 mmol) was added and the reaction was stirred for 16 h.
The reaction was diluted with DCM (100 mL) and washed with 5 % aq. disodium EDTA solution (100 mL) and 5 % aq. sat. KI solution (100 mL). The organic phase was dried (Na_2SO_4) and the solvent was removed *in vacuo*. Following purification by column chromatography (7 M NH_3:MeOH/DCM, 1:99 up to 1:9), the product **10** (0.20 g, 0.31 mmol, 71 %) was afforded as a purple iridescent solid.

R_f : 0.35 (MeOH/DCM, 1:9)
LRMS [ESI+, MeCN] m/z (%): 519 ($[M]^+$, 100).
HRMS [ESI+, MeCN] for $C_{34}H_{39}N_2O_1Si_1$ $[M]^+$: calcd 519.2826, found 519.2822.
^{1}H (400 MHz, $CDCl_3$): δ 7.92 (s, 1H, H^{22}), 7.36-6.95 (m, 7H, H^{Ar}), 4.87 (dd, 1H, *J* = 11.0, 5.0 Hz, H^{25}), 4.82 (dd, *J* = 11.0, 5.0 Hz, 1H, H^{25}), 4.15-4.01 (m, 4H, H^{23}), 2.56-2.53 (m, 2H, H^{24}), 1.89-1.83 (m, 2H, H^{24}), 1.64 (s, 3H, $H^{10/20}$), 1.61 (s, 3H, $H^{10/20}$), 1.59 (s, 3H, $H^{10/20}$), 1.56 (s, 3H, $H^{10/20}$), 0.11 (s, 9H, H^{28}) ppm.
^{13}C (100 MHz, $CDCl_3$): δ 168.6 (C^2), 166.7 (C^{12}), 141.5 (C^{Ar}), 141.4 (C^{Ar}), 140.5 (C^{Ar}), 140.1 (C^{Ar}), 138.2 (CH^{22}), 133.2 (CH^{Ar}), 129.1 (CH^{Ar}), 126.2 (CH^{Ar}), 125.5 (CH^{Ar}), 122.2 (CH^{Ar}), 120.1 (C^{Ar}), 111.6 (C^{21}), 111.2 (CH^{Ar}), 110.5 (C^{21}), 110.4 (CH^{Ar}), 104.3 (C^{26}), 95.6 (C^{27}), 69.5 (CH^{25}), 48.9 ($C^{3/13}$), 48.2 ($C^{3/13}$), 42.2 ($CH_2{}^{23}$), 41.9 ($CH_2{}^{23}$), 28.4 ($CH_3{}^{10}$), 28.3 ($CH_3{}^{10}$), 27.8 ($CH_3{}^{20}$), 27.8 ($CH_3{}^{20}$), 26.5 ($CH_2{}^{24}$), 26.5 ($CH_2{}^{24}$), 0.1 ($CH_3{}^{28}$) ppm.
Mp: 210-211 °C.
IR ν_{Max}/cm^{-1}: 2963 (m, =C-H), 2930 (m, -C-H), 2149 (m, C≡C).

Synthesis of 5-ethynyl-Cy3B

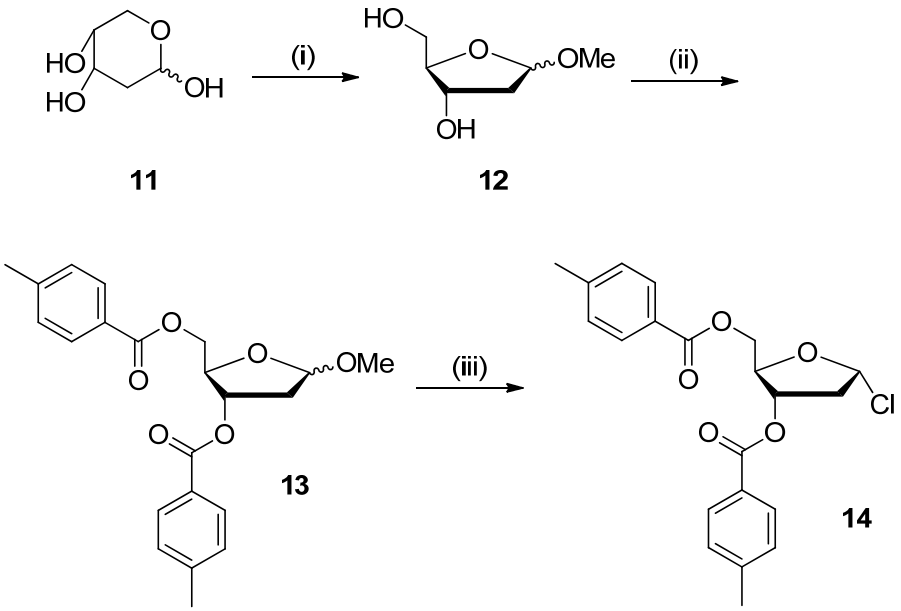


E

To a solution of 5-trimethylsilylethynyl-Cy3B **10** (0.13 g, 0.20 mmol) in distilled THF (3 mL), under an argon atmosphere, was added 1 M TBAF in THF (0.31 mL, 0.31 mmol) and the reaction was stirred for 5 min at rt. The reaction was diluted with DCM (100 mL) and washed with H_2O (2 x 500 mL) and 5 % aq. sat. KI solution (250 mL). The organic phase was dried (Na_2SO_4), the solvent removed *in vacuo* and the crude material purified by column chromatography (7M NH_3:MeOH/DCM, 1:99) to afford the product **E** (0.09 g, 0.16 mmol, 75 %) as a purple iridescent solid.

R_f : 0.32 (MeOH/DCM, 1:9)
LRMS [ESI+, MeCN] m/z (%): 447 ($[M]^+$, 100).
HRMS [ESI+, MeCN] for $C_{31}H_{31}N_2O_1$ $[M]^+$: calcd 447.2431, found 447.2422.
^{1}H (400 MHz, $CDCl_3$): δ 8.08 (s, 1H, H^{22}), 7.53-7.13 (m, 7H, H^{Ar}), 5.01 (dd, *J* = 11.0, 5.0 Hz, 1H, H^{25}), 4.96 (dd, *J* = 11.0, 5.0 Hz, 1H, H^{25}), 4.29-4.17 (m, 4H, H^{23}), 3.16 (s, 1H, H^{27}), 2.73-2.68 (m, 2H, H^{24}), 2.03-1.95 (m, 2H, H^{24}), 1.79 (s, 3H, $H^{10/20}$), 1.77 (s, 3H, $H^{10/20}$), 1.74 (s, 3H, $H^{10/20}$), 1.72 (s, 3H, $H^{10/20}$) ppm.
^{13}C (100 MHz, $CDCl_3$): δ 168.7 ($C^{2/12}$), 166.7 ($C^{2/12}$), 141.8 (C^{Ar}), 141.4 (C^{Ar}), 140.5 (C^{Ar}), 140.2 (C^{Ar}), 138.4 (CH^{22}), 133.3 (CH^{Ar}), 129.1 (CH^{Ar}), 126.2 (CH^{Ar}), 125.7 (CH^{Ar}), 122.2 (CH^{Ar}), 119.0 (C^5), 111.7 (C^{21}), 111.2 (CH^{Ar}), 110.5 (CH^{Ar}), 110.5 (C^{21}), 83.0 (C^{26}), 78.3 (CH^{27}), 69.6 (CH^{25}), 49.0 ($C^{3/13}$), 48.2 ($C^{3/13}$), 42.2 (CH_2^{23}), 41.9 (CH_2^{23}), 28.4 (CH_3^{10}), 28.3 (CH_3^{10}), 27.8 (CH_3^{20}), 27.7 (CH_3^{20}), 26.5 (CH_2^{24}), 26.5 (CH_2^{24}) ppm.
Mp: >230 °C.
IR ν_{Max}/cm^{-1}: 3167 (w, C≡C-H), 2969 (m, =C-H), 2928 (m, -C-H).
UV/Vis (MeOH): A_{max}= 567 nm, ε_{max}= 118,000 $M^{-1}cm^{-1}$, Em_{max}= 584 nm.

Synthesis of 1´-α-chloro-2´-deoxy-3,5-di-*O*-*p*-toluoyl-D-ribose (14)



To a solution of 2´-deoxy-D-ribose (10.0 g, 74.5 mmol) in MeOH (120 mL) was added 1 % methanolic HCl (20 mL: made by adding 1.7 mL acetyl chloride to 100 mL MeOH). The resulting yellow solution was stirred for 30 min. The reaction was neutralised by the addition of solid sodium bicarbonate (4.0 g). The solid was removed by filtration, washed with MeOH and the filtrate was evaporated. Residual MeOH was removed by co-evaporation with distilled pyridine (1 x 50 mL, 2 x 25 mL) and the golden coloured syrup was dried under high vacuum for 2.5 h.

To the residue, dissolved in distilled pyridine (60 mL), cooled to 0 °C, was added *p*-toluoylchloride (22.0 mL, 160 mmol) drop-wise. The cloudy solution was stirred at rt for 16 h. The reaction was diluted with cold water (150 mL) and extracted with DCM (1 x 150 mL, 2 x 100 mL).The organic phase was washed with $NaHCO_3$ (2 x 150 mL), 2 M HCl (150 mL) and H_2O (200 mL), then dried (Na_2SO_4) and the solvent was removed *in vacuo*.

To the oil (30.8 g) dissolved in AcOH (40 mL) at 0 °C, was slowly added sat. HCl in AcOH (63 mL; prepared by adding 16.3 mL acetyl chloride to a mixture of cold AcOH, 81 mL, and water, 4 mL). An additional amount of acetyl chloride (7 mL) was added and the flask was swirled for 10 min resulting in the formation of a thick crystalline precipitate. The precipitate was rapidly filtered and washed thoroughly with cold anhydrous Et_2O (prepared over molecular sieves, 3 Å). Following drying *in vacuo* the product **14** (18.7 g, 48.1 mmol, 64 %) was afforded as a white solid, which was stored under argon.

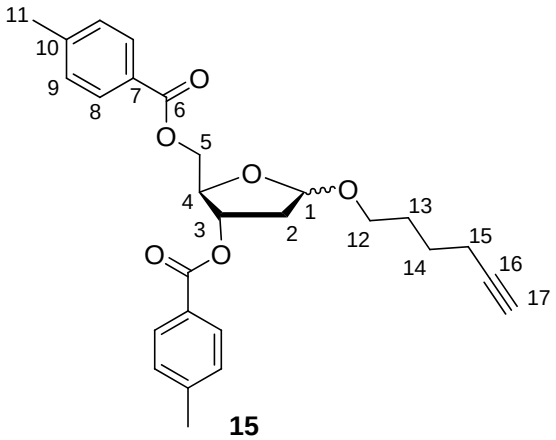
R_f : 0.48 (EtOAc/ Pet ether, 1:1)

LRMS [ESI+, MeCN direct probe application] m/z (%): 452 ([M(^{35}Cl)+Na+CH_3CN]$^+$, 100), 454 ([M(^{37}Cl)+Na+CH_3CN]$^+$, 35).

^{1}H (300 MHz, CD_3CN): δ 8.02 (d, *J* = 12.0 Hz, 2H, H^{Ar}), 7.92 (d, *J* = 8.0 Hz, 2H, H^{Ar}), 7.30-7.24 (m, 4H, H^{Ar}), 6.50 (d, *J* = 4.0 Hz, 1H, $H^{1´}$), 5.59 (dd, *J* = 8.0, 4.0 Hz, 1H, $H^{3´}$), 4.88 (dt, *J* = 8.0, 4.0 Hz, 1H, $H^{4´}$), 4.66 (2xdd, *J* = 16.0, 4.0 Hz, 2H, $H^{5´}$), 2.94-2.74 (m, 2H, $H^{2´}$), 2.44 (2xs, 6H, H^6) ppm.

Characterisation data recorded matches previously reported literature values (14).

Synthesis of 1-α/β-*O*-hexyne-2´-deoxy-3,5-di-*O*-*p*-toluoyl-D-ribose

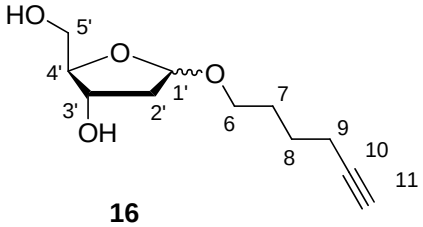


15

To a solution of 1´-α-chloro-2´-deoxy-3,5-di-*O*-*p*-toluoyl-D-ribose **14** (9.28 g, 23.9 mmol) in distilled THF (50 mL), with molecular sieves (3 Å), under an argon atmosphere, was added DMAP (1.12 g, 9.2 mmol) and distilled DCM (10 mL). Meanwhile, 5-hexyn-1-ol (2.03 mL, 18.4 mmol) was stirred in distilled THF (5 mL) over molecular sieves (3 Å). Both solutions were stirred for 30 min before the 5-hexyn-1-ol solution was added to the sugar mixture and the reaction was stirred for 3.5 h. The reaction solvent was removed *in vacuo* then the crude material was purified by column chromatography (100 % DCM) to afford the product **15** (4.43 g, 9.84 mmol, 53 %) as a colourless oil.

R_f : 0.39, 0.47 (DCM)
LRMS [ESI+, MeCN] m/z (%): 473 ($[M+Na]^+$, 100).
HRMS [ESI+, MeCN] for $C_{27}H_{30}Na_1O_6$ $[M+Na]^+$: calcd 473.1935, found 473.1935.
^{1}H (400 MHz, $CDCl_3$): δ (both α and β isomers) 8.02-7.95 (m, 8H, $H^{8\alpha,\beta}$), 7.32-7.25 (m, 8H, $H^{9\alpha,\beta}$), 5.63 (ddd, J = 7.1, 4.6, 2.5 Hz, 1H, $H^{3\alpha}$), 5.48 (ddd, J = 8.2, 2.5, 2.5 Hz, 1H, $H^{3\beta}$), 5.38 (dd, J = 5.6, 2.5 Hz, 1H, $H^{1\alpha}$), 5.33 (m, 1H, $H^{1\beta}$), 4.70-4.45 (m, 6H, $H^{4\alpha,4\beta,5\alpha,5\beta}$), 3.81 (m, 2H, $H^{12\alpha}$), 3.49 (m, 2H, $H^{12\beta}$), 2.64-2.17 (m, 20H, $H^{11\alpha,11\beta,2\alpha,2\beta,15\alpha,15\beta}$), 1.98 (t, J = 2.5 Hz, 2H, $H^{17\alpha,17\beta}$), 1.79-1.56 (m, 8H, $H^{13\alpha,13\beta,14\alpha,14\beta}$) ppm.
^{13}C (100 MHz, $CDCl_3$): δ (both α and β isomers) 166.1 (C^{Ar}), 165.9 (C^{Ar}), 165.8 (C^{Ar}), 143.7 (C^{Ar}), 143.6 (C^{Ar}), 143.5 (C^{Ar}), 143.4 (C^{Ar}), 129.4, (CH^{Ar}), 128.8 (CH^{Ar}), 126.8 (C^{Ar}), 104.3 ($CH^{1\alpha}$), 103.5 ($CH^{1\beta}$), 84.0 ($C^{16\alpha,\beta}$), 81.5 ($CH^{4\alpha}$), 80.8 ($CH^{4\beta}$), 75.3 ($CH^{3\alpha}$), 74.4 ($CH^{3\beta}$), 68.0 ($CH^{17\alpha,\beta}$), 67.2 ($CH_2^{12\alpha}$), 66.7 ($CH_2^{12\beta}$), 64.9 ($CH_2^{5\alpha}$), 64.0 ($CH_2^{5\beta}$), 39.0 ($CH_2^{2\alpha,2\beta}$), 28.4 ($CH_2^{13\alpha}$), 28.3 ($CH_2^{13\beta}$), 25.0 ($CH_2^{14\alpha}$), 24.8 ($CH_2^{14\beta}$), 21.3 ($CH_3^{11\alpha,\beta}$), 17.8 ($CH_2^{15\alpha,15\beta}$) ppm.
IR ν_{Max}/cm^{-1}: 3296 (alkynyl ≡C-H, m), 2946, 2920, 2869 (alkyl C-H, m), 2116 (alkynyl C≡C, w), 1714 (ester C=O, s).

Synthesis of 1-α/β-*O*-hexyne-2′-deoxy-D-ribose

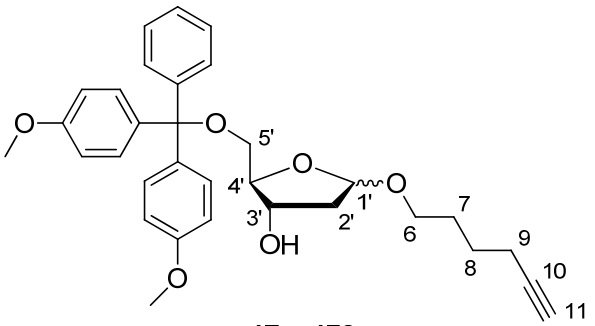


16

To a solution of 1-α/β-*O*-hexyne-2′-deoxy-3,5-di-*O*-*p*-toluoyl-D-ribose **15** (4.23 g, 9.39 mmol) in 7 M NH_3:MeOH (47 mL) was added conc. aq. NH_3 (5 mL) and the reaction was stirred at 45 °C for 16 h. The reaction solvent was removed *in vacuo* and the crude material was purified by column chromatography (EtOAc/DCM, 1:1) to afford the product **16** (1.65 g, 7.71 mmol, 82 %) as a colourless oil.

R_f : 0.34, 0.40 (MeOH/DCM, 1:9)
LRMS [ESI+, MeCN] m/z (%): 237 ($[M+Na]^+$, 100).
HRMS [ESI+, MeCN] for $C_{11}H_{18}Na_1O_4$ $[M+Na]^+$: calcd 237.1097, found 237.1098.
^{1}H (400 MHz, d_6-DMSO): δ (both α and β isomers) 5.09 (dd, J = 5.6, 2.5 Hz, 1H, $H^{1'\beta}$), 5.01 (dd, J = 5.6, 2.5 Hz, 1H, $H^{1'\alpha}$), 4.96 (d, J = 4.5 Hz, 1H, $OH^{3'\beta}$), 4.82 (d, J = 5.1 Hz, 1H, $OH^{3'\alpha}$), 4.60 (2t, J = 5.6 Hz, 2H, $OH^{5'\beta}$, $OH^{5'\alpha}$), 4.11 (m, 1H, $H^{3'\beta}$), 3.91 (dddd, J = 8.3, 5.3x3 Hz, 1H, $H^{3'\alpha}$), 3.72-3.66 (m, 2H, $H^{4'\beta,4'\alpha}$), 3.63-3.56 (m, 2H, $H^{6\alpha}$), 3.48-3.29 (m, 6H, $H^{5'\beta,5'\alpha,6\beta}$), 2.75 (m, 2H, $H^{11\alpha,\beta}$), 2.28 (ddd, J = 13.6, 8.1, 5.6 Hz, 1H, $H^{2'\alpha}$), 2.16 (dddd, J = 6.8x3, 2.8 Hz, 4H, $H^{9\alpha,\beta}$) 2.01-1.86 (m, 2H, $H^{2'\beta}$), 1.63-1.44 (m, 9H, $H^{2'\alpha,7\alpha,7\beta,8\alpha,8\beta}$) ppm.
^{13}C (100 MHz, d_6-DMSO): δ (both α and β isomers) 103.4 ($CH^{1'\beta}$), 102.8 ($CH^{1'\alpha}$), 86.9 ($CH^{4'\alpha}$), 84.7 ($CH^{4'\beta}$), 84.4 ($C^{10\alpha,\beta}$), 71.2 ($CH^{11\alpha,\beta}$), 70.9 ($CH^{3'\alpha}$), 70.0 ($CH^{3'\beta}$), 66.2 ($CH_2{}^{6\alpha,\beta}$), 63.2 ($CH_2{}^{5'\alpha}$), 61.3 ($CH_2{}^{5'\beta}$), 41.2 ($CH_2{}^{2'\beta}$), 41.1 ($CH_2{}^{2'\alpha}$), 28.3 ($CH_2{}^{7\alpha,\beta}$), 24.8 ($CH_2{}^{8\alpha,\beta}$), 17.4 ($CH_2{}^{9\alpha,\beta}$) ppm.
IR ν_{Max}/cm^{-1}: 3377 (alcohol O-H, br, s), 3291 (alkynyl ≡C-H, s), 2936, 2870 (alkyl C-H, m), 2114 (alkynyl C≡C, w).

Synthesis of 1′-α/β-*O*-hexyne-5′-*O*-(4,4′-dimethoxytrityl)-2′-deoxy-D-ribose



17α, 17β

1-α/β-*O*-Hexyne-2′-deoxy-D-ribose **16** (1.51 g, 7.06 mmol) was co-evaporated with distilled pyridine (3 x 10 mL) and dissolved in distilled pyridine (20 mL) under an argon atmosphere.

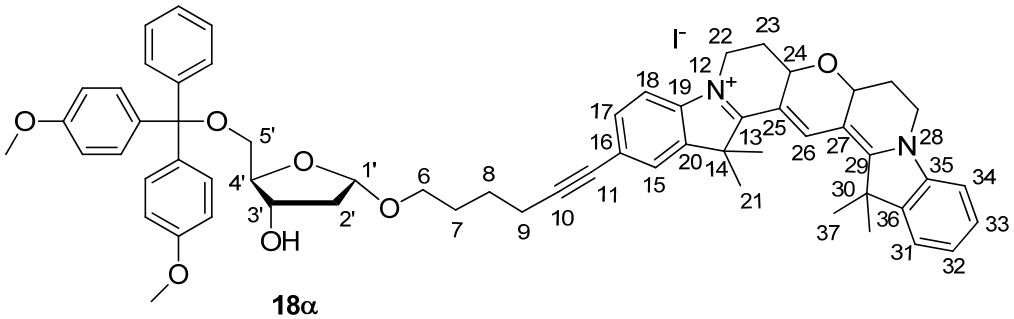
To the solution was added DMAP (0.17 g, 1.40 mmol). A solution of DMTCl (3.59 g, 10.6 mmol, in 15 mL distilled pyridine) was added drop-wise over 20 min and the reaction was stirred at rt for 1 h. MeOH (20 mL) was added to quench the reaction and the solution was stirred for 30 min. The reaction volume was reduced by half *in vacuo*, diluted with DCM (100 mL) and washed with H_2O (100 mL) and $NaHCO_3$ (2 x 100 mL). The organic phase was dried (Na_2SO_4) and the solvent removed *in vacuo*. Following purification by column chromatography (EtOAc/ Pet ether, 1:4 with 0.1 % pyridine) the separated anomeric products, **17α** (1.68 g, 3.25 mmol, 47 %) and **17β** (1.51 g, 2.92 mmol, 41 %), were afforded as colourless oils in a total of 88 % yield.

α-anomer:
R_f : 0.18 (EtOAc/ Pet ether, 1:3)
LRMS [ESI+, MeOH] m/z (%): 1056 ($[2M+Na]^+$, 25), 539 ($[M+Na]^+$, 100).
HRMS [ESI+, MeCN] for $C_{32}H_{36}Na_1O_6$ $[M+Na]^+$: calcd 539.2404, found 539.2412.
^{1}H (400 MHz, d_6-DMSO): δ 7.42-6.88 (m, 13H, H^{Ar}), 5.09 (dd, *J* = 5.6, 2.5 Hz, 1H, $H^{1'}$), 4.92 (d, *J* = 5.1 Hz, 1H, $OH^{3'}$), 3.90-3.88 (m, 2H, $H^{3',4'}$), 3.75 (s, 6H, OCH_3), 3.68 (m, 1H, H^6), 3.44 (m, 1H, H^6), 3.10 (dd, *J* = 9.9, 2.3 Hz, 1H, $H^{5'}$), 2.98 (dd, *J* = 9.6, 5.6 Hz, 1H, $H^{5'}$), 2.76 (m, 1H, H^{11}), 2.34 (m, 1H, $H^{2'}$), 2.22 (dt, *J* =7.0, 2.8 Hz, 2H, H^9), 1.68-1.62 (m, 3H, $H^{2',7}$), 1.57-1.53 (m, 2H, H^8) ppm.
^{13}C (100 MHz, d_6-DMSO): δ 158.0 (C^{Ar}), 145.0 (C^{Ar}), 135.7 (C^{Ar}), 129.7 (CH^{Ar}), 127.8 (CH^{Ar}), 127.7 (CH^{Ar}), 126.6 (CH^{Ar}), 113.1 (CH^{Ar}), 103.0 ($CH^{1'}$), 85.2 (C^{Ar3}), 84.4 (C^{10}), 82.6 ($CH^{4'}$), 71.2 (CH^{11}), 70.6 ($CH^{3'}$), 66.2 ($CH_2{}^6$), 63.9 ($CH_2{}^{5'}$), 55.0 (OCH_3), 41.1 ($CH_2{}^{2'}$), 28.3 ($CH_2{}^7$), 24.9 ($CH_2{}^8$), 17.4 ($CH_2{}^9$) ppm.
IR ν_{Max}/cm^{-1}: 3525 (alcohol O-H, br m), 3290 (alkynyl ≡C-H, m), 2932, 2868, 2836 (alkyl C-H, m), 2114 (alkynyl C≡C, w).

β-anomer:
R_f : 0.1 (EtOAc/Pet ether, 1:3)
LRMS [ESI+, MeOH] m/z (%): 1056 ($[2M+Na]^+$, 7), 539 ($[M+Na]^+$, 100).
HRMS [ESI+, MeCN] for $C_{32}H_{36}Na_1O_6$ $[M+Na]^+$: calcd 539.2404, found 539.2410.
^{1}H (400 MHz, d_6-DMSO): δ 7.44-7.20 (m, 9H, H^{Ar}), 6.89 (d, *J* = 9.1 Hz, 4H, H^{Ar}), 5.09 (m, 1H, $H^{1'}$), 5.04 (d, *J* = 5.1 Hz, 1H, $OH^{3'}$), 4.10 (dd, *J* = 5.1, 5.1 Hz, 1H, $H^{3'}$), 3.87 (ddd, *J* = 4.7x3 Hz, 1H, $H^{4'}$), 3.74 (s, 6H, OCH_3), 3.54 (m, 1H, H^6), 3.29 (m, 1H, H^6), 3.04 (dd, *J* = 12.0, 4.0 Hz, 1H, $H^{5'}$), 2.96 (dd, *J* = 12.0, 4.0 Hz, 1H, $H^{5'}$), 2.71 (t, *J* = 2.3 Hz, 1H, H^{11}), 2.09-2.04 (m, 2H, H^9), 1.90 (ddd, *J* = 12.0, 8.0, 4.0 Hz, 1H, $H^{2'}$), 1.88 (m, 1H, $H^{2'}$), 1.48-1.43 (m, 2H, H^7), 1.36-1.31 (m, 2H, H^8) ppm.
^{13}C (100 MHz, d_6-DMSO): δ 158.0 (C^{Ar}), 149.6 (CH^{Ar}), 145.0 (C^{Ar}), 135.8 (C^{Ar}), 129.7 (CH^{Ar}), 127.7 (CH^{Ar}), 126.6 (CH^{Ar}), 113.1 (CH^{Ar}), 103.3 ($CH^{1'}$), 85.2 (C^{10}), 84.6 ($CH^{4'}$), 84.3 (C^{Ar3}), 71.1 (CH^{11}), 70.8 ($CH^{3'}$), 66.2 ($CH_2{}^6$), 65.1 ($CH_2{}^{5'}$), 55.0 (OCH_3), 41.1 ($CH_2{}^{2'}$), 28.2 ($CH_2{}^7$), 24.8 ($CH_2{}^8$), 17.4 ($CH_2{}^9$) ppm.
IR ν_{Max}/cm^{-1}: 3426 (alcohol O-H, br m), 3291 (alkynyl ≡C-H, m), 2934, 2868, 2836 (alkyl C-H, m), 2114 (alkynyl C≡C, w).

The assignment of the anomers was accomplished by NMR experiments (including NOE) and by agreement with literature data of α-nucleosides (15) and with above reported β-nucleoside NMR data.

Synthesis of 2′-deoxyribose-1′-α-hexynyl-Cy3B (α-Cy3BdR)

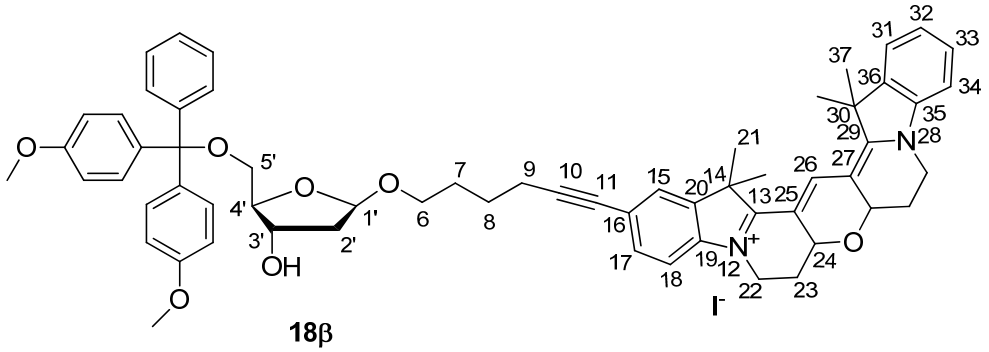


18α

To 1′-α-*O*-hexyne-5′-*O*-(4,4′-dimethoxytrityl)-2′-deoxy-D-ribose **17α** (1g, 1.93 mmol) was added 5-iodo-Cy3B **7** (1.3 g, 1.93 mmol) and CuI (74 mg, 0.386 mmol) in anhydrous DMF (10 mL) under an argon atmosphere. To this, distilled Et_3N (7 mL) was added and the reaction was stirred for 10 min in the dark at rt. $Pd(PPh_3)_4$ (0.22 g, 0.19 mmol) was added and the reaction was stirred for 52 h. The reaction was diluted with DCM (200 mL) and washed with 5 % aq. disodium EDTA (2 x 200mL) and 5 % aq. sat. KI solution (200 mL). The organic phase was dried (Na_2SO_4) and the solvent removed under high vacuum. Following purification by column chromatography (twice in 7 M NH_3:MeOH/DCM, 1:9) the product, **18α** (1.37 g, 1.29 mmol, 66 %) was afforded as a purple foam.

R_f : 0.45 (MeOH/DCM, 1:9)
LRMS [ESI+, MeCN] m/z (%): 938 ($[M]^+$, 100).
HRMS [ESI+, MeCN] for $C_{61}H_{65}N_2O_7$ $[M]^+$: calcd 937.4786, found 937.4781.
^{1}H (400 MHz, d_6-DMSO): δ 7.98 (s, 1H, H^{26}), 7.66-7.19 (m, 16H, H^{Ar}), 6.88 (d, J = 12.0 Hz, 4H, H^{Ar}), 5.11 (dd, J = 5.8, 2.5 Hz, 1H, $H^{1'}$), 4.95 (d, J = 4.9 Hz, 1H, $OH^{3'}$), 4.67-4.62 (m, 2H, H^{24}), 4.37-4.28 (m, 2H, H^{22}), 3.93-3.85 (m, 4H, $H^{3',4',22}$), 3.73-3.70 (m, 7H, OCH_3,H^6), 3.48 (m, 1H, H^6), 3.10 (m, 1H, $H^{5'}$), 2.98 (dd, J = 9.8, 5.5 Hz, 1H, $H^{5'}$). 2.53-2.50 (m, 4H, $H^{23,9}$), 2.35 (m, 1H, $H^{2'}$), 1.96 (dt, J = 11.9, 4.6 Hz, 2H, H^{23}), 1.70-1.67 (m, 17H, $H^{21,37,2',7,8}$) ppm.
^{13}C (100 MHz, d_6-DMSO): δ 168.0 ($C^{13/29}$), 166.8 ($C^{13/29}$), 158.0 (C^{Ar}), 145.0 (C^{Ar}), 141.6 (C^{Ar}), 141.2 (C^{Ar}), 140.8 (C^{Ar}), 140.7 (C^{Ar}), 137.0 (C^{Ar}), 135.7 (CH^{26}), 131.9 (CH^{Ar}), 129.7 (CH^{Ar}), 128.6 (CH^{Ar}), 127.8 (CH^{Ar}), 127.7 (CH^{Ar}), 126.6 (CH^{Ar}), 125.5 (CH^{Ar}), 125.3 (CH^{Ar}), 122.4 (CH^{Ar}), 119.7 (C^{16}), 113.1 (CH^{Ar}), 111.2 (CH^{Ar}), 110.9 (CH^{Ar}), 110.1 ($C^{25/27}$), 109.3 ($C^{25/27}$), 103.0 ($CH^{1'}$), 91.1 ($C^{10/11}$), 85.2 (C^{Ar_3}), 82.6 ($CH^{4'}$), 80.6 ($C^{10/11}$), 70.6 ($CH^{3'}$), 69.3 (CH^{24}), 66.3 (CH_2^6), 64.0 ($CH_2^{5'}$), 55.0 (OCH_3), 48.5 ($C^{14/30}$), 48.0 ($C^{14/30}$), 41.2 ($CH_2^{2'}$), 41.1 (CH_2^{22}), 41.0 (CH_2^{22}), 28.6 (CH_2^7), 27.5 (CH_3^{21}), 26.9 (CH_3^{37}), 26.1 (CH_2^8), 25.1 (CH_2^{23}), 18.5 (CH_2^9) ppm.
Mp: >150 °C (decomposes)
IR ν_{Max}/cm^{-1}: 3336 (alcohol O-H, br m), 2929, 2866, 2835 (alkyl C-H, m).

Synthesis of 2´-deoxyribose-1´-β-hexynyl-Cy3B (β-Cy3BdR)

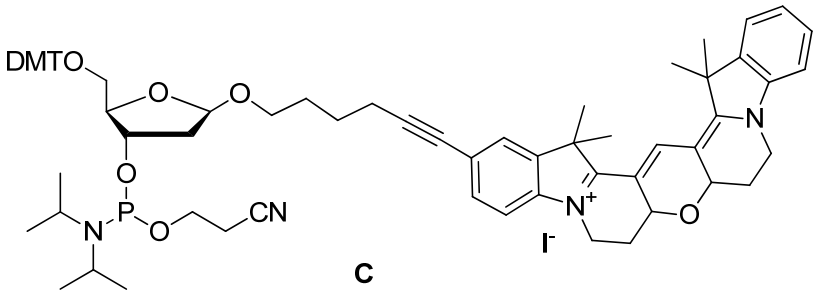


18β

This was prepared using the procedure as detailed above for **18α** from 1´-β-*O*-hexyne-5´-*O*-(4,4´-dimethoxytrityl)-2´-deoxy-D-ribose **17β** (1g, 1.93 mmol) to give the product **18β** (1.51 g, 1.42 mmol, 73 %) as a purple foam.

R_f : 0.34 (MeOH/DCM, 1:9)
LRMS [ESI+, MeCN] m/z (%): 938 ($[M]^+$, 100).
HRMS [ESI+, MeCN] for $C_{61}H_{65}N_2O_7$ $[M]^+$: calcd 937.4786, found 937.4788
^{1}H (400 MHz, d_6-DMSO): δ 7.99 (s, 1H, H^{26}), 7.65-7.18 (m, 16H, H^{Ar}), 6.88 (d, *J* = 8.1 Hz, 4H, H^{Ar}), 5.12 (d, *J* = 3.7 Hz, 1H, $H^{1´}$), 5.04 (d, *J* = 5.1 Hz, 1H, $OH^{3´}$), 4.65 (2xt, *J* = 5.7 Hz, 2H, H^{24}), 4.36-4.28 (m, 2H, H^{22}), 4.10 (m, 1H, $H^{3´}$), 3.97-3.85 (m, 3H, $H^{4´,22}$), 3.73 (2xs, 6H, OCH_3), 3.61 (m, 1H, H^6), 3.36 (m, 1H, H^6), 3.06 (dd, *J* = 8.0, 4.0 Hz, 1H, $H^{5´}$), 2.97 (dd, *J* = 12.0, 8.0 Hz, 1H, $H^{5´}$), 2.50-2.49 (m, 2H, H^{23}), 2.36 (t, *J* = 6.6 Hz, 2H, H^9), 1.97-1.88 (m, 4H, $H^{2´,23}$), 1.70 (s, 6H, H^{21}), 1.68 (s, 6H, H^{37}), 1.55-1.45 (m, 4H, $H^{7,8}$) ppm.
^{13}C (100 MHz, d_6-DMSO): δ 168.0 ($C^{13/29}$), 166.8 ($C^{13/29}$), 158.0 (C^{Ar}), 145.0 (C^{Ar}), 141.6 (C^{Ar}), 141.2 (C^{Ar}), 140.7 (C^{Ar}), 140.7 (C^{Ar}), 137.0 (CH^{26}), 135.7 (C^{Ar}), 131.8 (CH^{Ar}), 129.7 (CH^{Ar}), 128.6 (CH^{Ar}), 127.7 (CH^{Ar}), 126.6 (CH^{Ar}), 125.5 (CH^{Ar}), 125.3 (CH^{Ar}), 122.4 (CH^{Ar}), 119.7 (C^{16}), 113.1 (CH^{Ar}), 111.3 (CH^{Ar}), 110.9 (CH^{Ar}), 110.1 ($C^{25/27}$), 109.3 ($C^{25/27}$), 103.4 ($CH^{1´}$), 91.0 ($C^{10/11}$), 85.2 (C^{Ar_3}), 84.6 ($CH^{4´}$), 80.5 ($C^{10/11}$), 70.8 ($CH^{3´}$), 69.3 (CH^{24}), 66.3 ($CH_2{}^6$), 65.1 ($CH_2{}^{5´}$), 55.0 (OCH_3), 48.5 ($C^{14/30}$), 48.0 ($C^{14/30}$), 41.2 ($CH_2{}^{2´}$), 41.1 ($CH_2{}^{22}$), 41.1 ($CH_2{}^{22}$), 28.5 ($CH_2{}^7$), 27.5 ($CH_3{}^{21}$), 26.9 ($CH_3{}^{37}$), 26.1 ($CH_2{}^8$), 25.1 ($CH_2{}^{23}$), 18.5 ($CH_2{}^9$) ppm.
Mp: >150 °C (decomposes)
IR ν_{Max}/cm^{-1}: 3336 (alcohol O-H, br m), 2929, 2865, 2835 (alkyl C-H, m).
UV/Vis (MeOH): A_{max}= 571 nm, ε= 130,000 $M^{-1}cm^{-1}$, Em_{max}= 585 nm.

Synthesis of β-Cy3BdR phosphoramidite

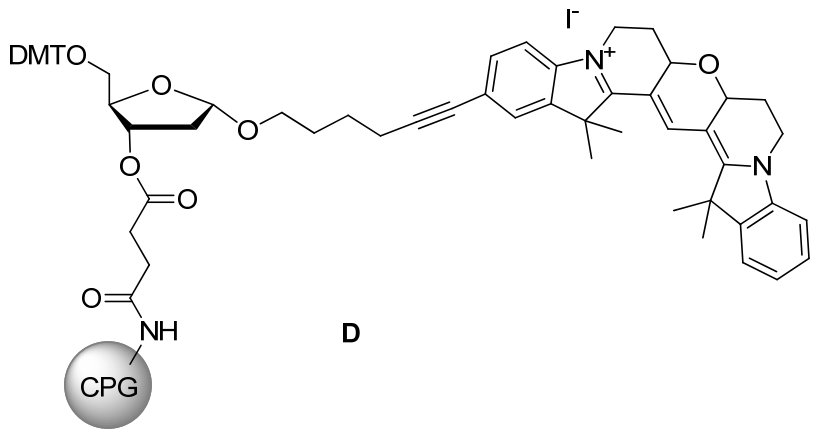


C

2′-Deoxyribose-1′-β-hexynyl-Cy3B (β-Cy3BdR) **18β** (0.50 g, 0.47 mmol) was co-evaporated with distilled pyridine (3 x 5 mL) and distilled DCM (3 x 5 mL) then dissolved in distilled DCM (2 mL), with activated molecular sieves (3 Å), under an argon atmosphere. To this was added distilled DIPEA (0.20 mL, 1.18 mmol) and the reaction was stirred for 10 min. 2-Cyanoethyl *N,N*-diisopropyl-chlorophosphoramidite (0.13 mL, 0.56 mmol) was added drop-wise and the reaction was stirred for 45 min. The reaction mixture was diluted with distilled DCM (10 mL) and washed with deoxygenated sat. KCl (10 mL). The organic phase was dried (Na_2SO_4) and the solvent was removed *in vacuo*. The crude material was precipitated in deoxygenated hexane (100 mL) from a minimum of distilled DCM (1 mL). The precipitation procedure was repeated twice and the final precipitate was co-evaporated with distilled DCM (2 mL) and trace solvents were removed *in vacuo* affording the product **C** (0.56 g, 0.44 mmol, 94 %) as a dark pink iridescent solid.

R_f : 0.51 (MeOH/DCM, 1:9 with 1 % pyridine)
LRMS [ESI+, MeCN] m/z (%): 1138 ($[M]^+$, 100).
^{31}P (300 MHz, CD_3CN): δ 148.8 (s, P^{III}) ppm.

Synthesis of α-Cy3BdR resin (experimental procedure based on literature (16,17))



D

The resin used for coupling was AminoSynBase 1000 Å (loading 59 μmol/g) purchased from Link Technologies. All steps were carried out under argon using dry solvents.

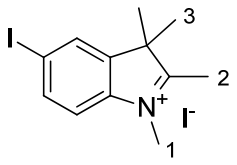
Resin (250 mg, 0.015 mmol) was activated by washing with 3 % TCA in DCM (3 mL) and suspending in 3 % TCA in DCM (3 mL) for 4 h at rt. The TCA was removed by filtration and the resin was washed with Et_3N:DIPEA (9:1) (3 x 3 mL), DCM (3 x 3 mL) and Et_2O (3 x 3 mL). The resin was dried under high vacuum for 1 h then soaked in pyridine (2 mL) for

10 min. To the suspension were added succinic anhydride (112 mg, 1.10 mmol) and 4-DMAP (23.0 mg, 0.189 mmol) in pyridine (2 mL) under an argon atmosphere and the vessel was rotated at rt for 20 h. The reaction solvent was removed and the resin was washed with pyridine (3 x 3 mL), DCM (3 x 3 mL) and Et_2O (3 x 3 mL). The resin was dried under high vacuum for 1 h before soaking in pyridine (3 mL) for 10 min. This was removed and a solution of EDC (46.0 mg, 0.30 mmol), 4-DMAP (2.0 mg, 0.015 mmol), Et_3N (0.01 mL) and 2′-deoxyribose-1′-α-hexynyl-Cy3B (α-Cy3BdR) **39α** (60.0 mg, 0.056 mmol) in pyridine (3 mL) was added. The vessel was rotated under argon for 20 h at rt.

Pentachlorophenol (20.0 mg, 0.07 mmol) was added to the existing mixture which was rotated for a further 3 h. The solvent was removed and the resin was washed with pyridine (3 x 3 mL), DCM (3 x 3 mL) and Et_2O (3 x 3 mL). To the resin was added piperidine (10 % in DMF, 3 mL) and the vessel was rotated for 1.5 min. The solvent was rapidly removed and the resin washed with DCM (3 x 3 mL), Et_2O (3 x 3 mL) and THF (3 x 3 mL). Capping reagent (Ac_2O/pyridine/THF:*N*-methylimidazole 1:1, 2 mL) was added and the vessel was rotated for 1 h. The solution was removed and the resin was washed with THF (3 x 3 mL), pyridine (3 x 3mL), DCM (3 x 3 mL) and Et_2O (3 x 3 mL) before drying *in vacuo* to afford the resin **D** with a loading of 13 μmol/g.

Loading was calculated by the trityl yield method (16).

Synthesis of 5-iodo-1,2,3,3-tetramethyl-3*H*-indolium iodide (18,19)



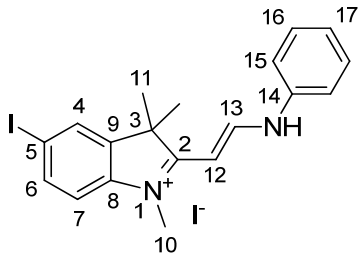
19

To a solution of 5-iodo-2,3,3-trimethyl-3*H*-indole **1** (1.42 g, 4.99 mmol) in MeCN (10 mL) was added MeI (0.34 mL, 5.44 mmol) and the reaction was stirred under reflux for 16 h. The reaction mixture was allowed to cool to rt and the orange precipitate was collected by filtration, washed with Et_2O and the solvent removed *in vacuo* to afford the product **19** (1.67 g, 3.91 mmol, 78 %) as a light orange powder.

R_f : 0.74 (MeOH/DCM, 1:9)
LRMS [ESI+, MeOH] m/z (%): 300 ($[M]^+$, 100).
^{1}H (300 MHz, d_6-DMSO): δ 8.28 (s, 1H, H^{Ar}), 8.00 (d, J = 8.4 Hz, 1H, H^{Ar}), 7.72 (d, J = 8.4 Hz, 1H, H^{Ar}), 3.93 (s, 3H, H^1), 2.73 (s, 3H, H^2), 1.52 (s, 6H, H^3) ppm.

Characterisation data recorded matches previously reported literature values (18,19).

Synthesis of 2-[(E)-2-(phenylamino)vinyl]-5-iodo-1,3,3-trimethyl-3*H*-indolium iodide (experimental method based on literature (20))

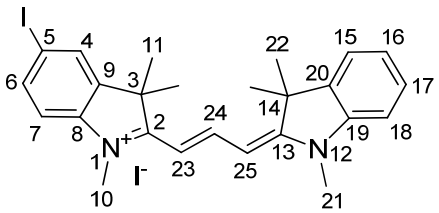


20

To a mixture of 5-iodo-1,2,3,3-tetramethyl-3*H*-indolium iodide **19** (1.10g, 2.58 mmol) and *N,N′*-diphenylformamidine (0.51g, 2.58 mmol) in ethanol (10 mL) was added triethylorthoformate (0.43 mL, 2.58 mmol) and the reaction was heated under reflux for 2.5 h.The mixture was allowed to cool to rt, the solvent removed *in vacuo* and the residue resuspended in ice-cold $CHCl_3$. The resulting orange precipitate was filtered and washed with ice-cold $CHCl_3$. A further 2 isolations of precipitate were made from the filtrate by repeating the procedure, affording the product **20** (1.30 g, 2.45 mmol, 95 %) as an orange powder.

R_f : 0.31 (MeOH/DCM, 5:95)
LRMS [ESI+, MeOH] m/z (%): 403 ($[M]^+$, 100).
HRMS: [ESI+, MeCN] for $C_{19}H_{20}IN_2$ $[M]^+$: calcd 403.0666, found 403.0659.
^{1}H (400 MHz, d_6-DMSO): δ 11.99 (s, 1H, NH), 8.67 (d, *J* = 12.0 Hz, 1H, H^{13}), 8.08 (d, *J* = 1.0 Hz, 1H, H^4), 7.80 (dd, *J* = 8.0, 1.0 Hz, 1H, H^6), 7.54-7.48 (m, 4H, $H^{15/16/17}$), 7.36 (d, *J* = 8.0 Hz, 1H, H^7), 7.30 (t, *J* = 7.0 Hz, 1H, $H^{15/16/17}$), 6.09 (d, *J* = 12.0 Hz, 1H, H^{12}), 3.63 (s, 3H, H^{10}), 1.68 (s, 6H, H^{11}) ppm.
^{13}C (100 MHz, d_6-DMSO): δ 177.5 (C^2), 151.9 (CH^{13}), 143.2 (C^9), 142.2 (C^8), 138.2 (C^{14}), 136.9 (CH^6), 131.1 (CH^4), 129.8 ($CH^{15/16/17}$), 126.2 ($CH^{15/16/17}$), 118.3 ($CH^{15/16/17}$), 113.9 (CH^7), 91.1 (CH^{12}), 90.1 (C^5), 49.5 (C^3), 32.3 (CH^{10}), 27.2 (CH^{11}) ppm.
Mp: 190 °C (decomposes).
IR ν_{Max}/cm^{-1}: 3129 (w-m) (N-H), 2944, 2875 (m) (C-H, C-H_3), 1628 (s) (C=N).

Synthesis of 5-iodo-1,3,3-trimethyl-2-((1E,3E)-3-(1,3,3-trimethylindolin-2-ylidene)prop-1-enyl)-3*H*-indolium iodide (5-iodo-Cy3) (18)



21

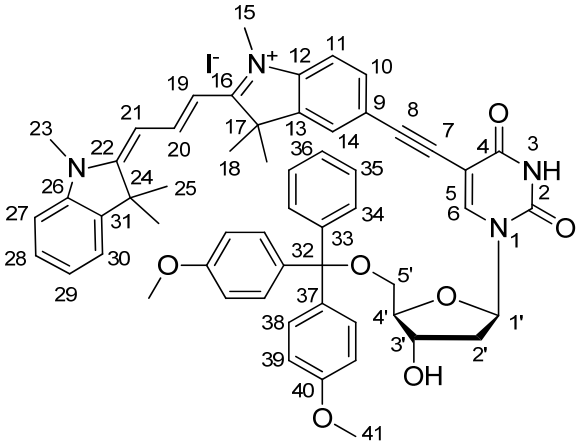
To a mixture of 2-[(E)-2-(phenylamino)vinyl]-5-iodo-1,3,3-trimethyl-3*H*-indolium iodide **19** (1.88g, 3.54 mmol) and 1,2,3,3-tetramethyl-3*H*-indolium iodide (1.07 g, 3.54 mmol) in distilled pyridine (20 mL) was added Ac_2O (0.67 mL, 7.09 mmol) and the reaction was stirred at 50 °C for 24 h. Additional portions of 1,2,3,3-tetramethyl-3*H*-indolium iodide

(0.43g, 1.42 mmol) and Ac_2O (1.11 mL, 11.9 mmol) were added to the reaction and it was stirred at 50 °C for a further 24 h. The mixture was allowed to cool to rt and the addition of Et_2O (20 mL) afforded a brown precipitate. The precipitate was isolated by filtration and following purification by column chromatography (DCM to MeOH/DCM, 5:95) the product **21** (1.31 g, 2.15 mmol, 61 %) was afforded as a dark purple solid.

R_f : 0.29 (MeOH/DCM, 5:95)
LRMS [ESI+, MeOH] m/z (%): 483 ($[M]^+$, 100).
^{1}H (400 MHz, CD_3CN): δ 8.43 (t, *J* = 13.6 Hz, 1H, H^{24}), 7.85 (d, *J* = 1.9 Hz, 1H, H^{Ar}), 7.77 (dd, *J* = 8.3, 1.5 Hz, 1H, H^{Ar}), 7.53 (d, *J* = 7.5 Hz, 1H, H^{Ar}), 7.46 (m, 1H, H^{Ar}), 7.33 (t, *J* = 7.6 Hz, 2H, H^{Ar}), 7.05 (d, *J* = 8.5 Hz, 1H, H^{Ar}), 6.39 (d, *J* = 13.6 Hz, 1H, H^{23}), 6.29 (d, *J* = 13.1 Hz, 1H, H^{25}), 3.61 (s, 3H, H^{10}), 3.52 (s, 3H, H^{21}), 1.69 (s, 6H, H^{11}), 1.67 (s, 6H, H^{22}) ppm.
UV/Vis (MeOH): A_{max}= 551 nm, ε_{max}= 114,000 $M^{-1}cm^{-1}$, Em_{max}= 568 nm.

Characterisation data recorded matches previously reported literature values (18).

Synthesis of Cy3dT



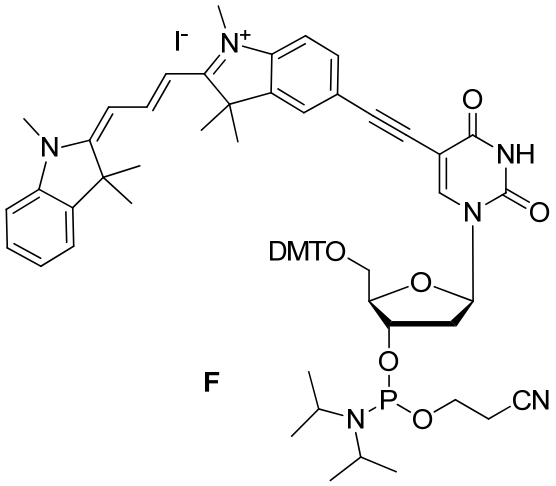
22

To a mixture of 5-iodo-Cy3 **21** (1.24 g, 2.03 mmol), 2′-deoxy-5′-*O*-(4,4′-dimethoxytrityl)-5-(eth-1-ynyl)uridine (1.24 g, 2.23 mmol) and CuI (0.08 g, 0.41 mmol) in anhydrous DMF (10 mL) under an argon atmosphere was added distilled Et_3N (7 mL) and the mixture stirred for 10 min at rt. $Pd(PPh_3)_4$ (0.24 g, 0.20 mmol) was added and the reaction stirred at rt in the dark for 24 h. The reaction solvent was removed *in vacuo*. Following purification by column chromatography ([1] MeOH/DCM, 5:95 with 1 % pyridine. [2] EtOH/EtOAc, 1:9 to 2:8 with 1 % pyridine. [3] MeOH/DCM, 1:9 with 1 % pyridine) the product **22** (0.88 g, 0.85 mmol, 42 %) was afforded as a purple/gold iridescent solid.

R_f : 0.55 (MeOH/DCM, 1:9)
LRMS [ESI+, MeCN] m/z (%): 910 ($[M]^+$, 100).
HRMS [ESI+, MeCN] for $C_{57}H_{57}N_4O_7$ $[M]^+$: calcd 909.4222, found 909.4237.
^{1}H (300 MHz, CD_3CN): δ 8.41 (t, *J* = 13.6 Hz, 1H, H^{20}), 8.20 (s, 1H, H^6), 7.55-7.09 (m, 16H, H^{Ar}), 6.82 (t, *J* = 8.5 Hz, 4H, H^{Ar}), 6.43 (d, *J* = 13.6 Hz, 1H, H^{19}), 6.34 (d, *J* = 13.6 Hz, 1H, H^{21}), 6.19 (t, *J* = 6.5 Hz, 1H, $H^{1'}$), 4.54 (m, 1H, $H^{3'}$), 4.03 (d, *J* = 2.9 Hz, 1H, $H^{4'}$), 3.69 (s, 3H, H^{41}), 3.68 (s, 3H, H^{41}),3.62 (s, 3H, H^{15}), 3.53 (s, 3H, H^{23}), 3.31 (d, *J* = 3.0 Hz, 2H, $H^{5'}$), 2.39-2.34 (m, 2H, $H^{2'}$), 1.71 (s, 6H, $H^{18/25}$), 1.61 (s, 3H, $H^{18/25}$), 1.60 (s, 3H, $H^{18/25}$) ppm.

^{13}C (75 MHz, CD_3CN): δ 177.3 ($C^{16/22}$), 175.8 ($C^{16/22}$), 162.8 (C^{2}), 160.1 (C^{40}), 151.8 (CH^{20}), 151.1 (C^{4}), 146.3 (CH^{6}), 144.3 (C^{Ar}), 142.5 (C^{Ar}), 142.2 (C^{Ar}), 137.4 (C^{Ar}), 137.2(C^{Ar}), 133.6 (CH^{Ar}), 131.5 (CH^{38}), 131.4 (CH^{38}), 130.2 (CH^{Ar}), 129.4 (CH^{Ar}), 127.2 (CH^{Ar}), 126.3 (CH^{Ar}), 125.2 (CH^{Ar}), 123.7 (CH^{Ar}), 120.4 (C^{Ar}), 114.7 (CH^{39}), 112.9 (C^{Ar}), 112.2 (C^{Ar}), 104.9 (CH^{19}), 103.9 (CH^{21}), 100.4 (C^{5}), 93.4 ($C^{8,32}$), 88.1 ($CH^{4'}$), 87.3 ($CH^{1'}$), 83.3 (C^{7}), 72.4 ($CH^{3'}$), 64.7 ($CH_2^{5'}$), 56.4 (CH_3^{41}), 50.9 ($C^{17,24}$), 50.2 ($C^{17,24}$), 42.5 ($CH_2^{2'}$), 33.0 ($CH_3^{15,23}$), 28.5 ($CH_3^{18,25}$), 28.4 ($CH_3^{18,25}$) ppm.
Mp: >130 °C (decomposes).
IR ν_{Max}/cm^{-1}: 3306 (br. w. O-H), 3040 (w. N-H), 2930, 2872 (w. C-H, C-H_3), 1704 (s. C=O).
UV/Vis (MeOH): A_{max}= 565 nm, ε_{max}= 112,000 $M^{-1}cm^{-1}$, Em_{max}= 592 nm.

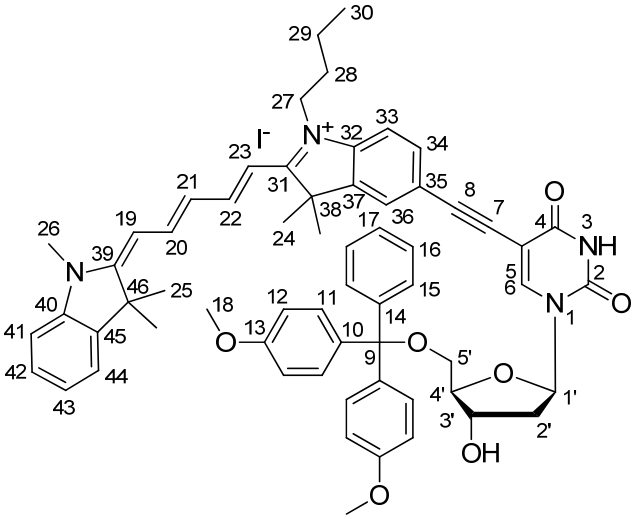
Synthesis of Cy3dT-phosphoramidite



Cy3dT **22** (0.63 g, 0.61 mmol) was co-evaporated with distilled pyridine (3 x 5 mL) and distilled DCM (5 x 5 mL) before suspending in distilled DCM (10 mL), with activated molecular sieves (3 Å), under an argon atmosphere. To this was added distilled DIPEA (0.27 mL, 1.53 mmol) followed by the drop-wise addition of 2-cyanoethyl *N,N*-diisopropyl-chlorophosphoramidite (0.14 mL, 0.61 mmol) and the reaction was stirred at rt for 45 min. An additional portion of 2-cyanoethyl *N,N*-diisopropyl-chlorophosphoramidite (0.1 eq) was added and the reaction stirred for a further 2 h. The solution was diluted with distilled DCM (10 mL) and washed with deoxygenated aq. sat. KCl (20 mL). The organic phase was dried (Na_2SO_4) and the solvent removed *in vacuo* before purification by precipitation in deoxygenated hexane (250 mL) from a minimum of DCM (2 mL). The precipitation process was repeated twice. The precipitate was dried *in vacuo* to afford the product **F** (0.73 g, 0.59 mmol, 96 %) as a dark pink solid.

R_f : 0.39 (MeOH/DCM, 1:9 with 1 % pyridine)
LRMS [ESI+, MeCN] m/z (%): 1110 ($[M]^+$, 100).
^{31}P (121 MHz, CD_3CN): δ 149.3 (s, P^{III}), 149.2 (s, P^{III}) ppm.

Synthesis of Cy5dT

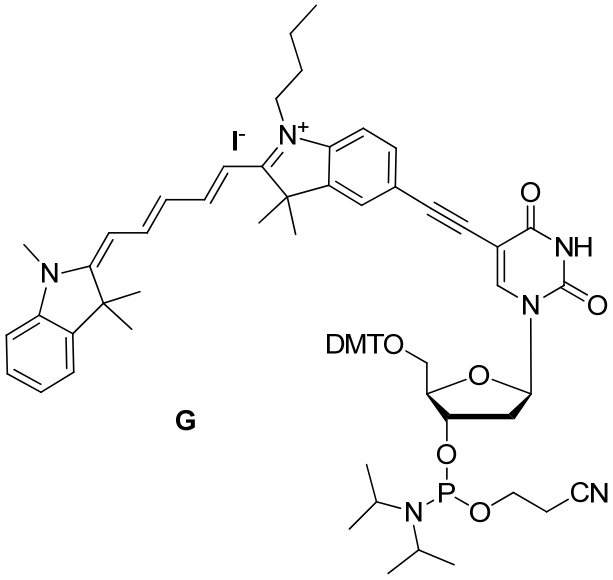


23

To a mixture of iodo-Cy5 (1.00 g, 1.47 mmol), 2′-deoxy-5′-*O*-(4,4′-dimethoxytrityl)-5-(eth-1-ynyl)uridine (1.50 g, 2.70 mmol) and CuI (0.06 g, 0.29 mmol) in anhydrous DMF (7.5 mL) under an argon atmosphere was added distilled Et_3N (5.2 mL) and the mixture stirred at rt for 10 min. $Pd(PPh_3)_4$ (0.17 g, 0.15 mmol) was added and the reaction stirred at rt in the dark for 24 h. Additional CuI (0.2 eq) and $Pd(PPh_3)_4$ (0.1 eq) were added and the reaction stirred at rt for a further 2 h. The reaction solvent was removed *in vacuo* and the product purified by column chromatography ([1] MeOH/DCM, 2:8 with 1 % pyridine. [2] EtOH/EtOAc, 3:7 with 1 % pyridine) to afford the product **23** (1.49 g, 1.35 mmol, 91 %) as a blue/red iridescent solid.

R_f : 0.39 (MeOH/DCM, 1:9)
LRMS [ESI+, MeCN] m/z (%): 978 ($[M]^+$, 100).
HRMS [ESI+, MeCN] for $C_{62}H_{65}N_4O_7$ $[M]^+$: calcd 977.4848, found 977.4859.
^{1}H (400 MHz, CD_3CN): δ 8.19 (s, 1H, H^6), 8.13 (t, *J* = 13.8 Hz, 1H, $H^{20/22}$), 8.05 (t, *J* = 13.1 Hz, 1H, $H^{20/22}$), 7.52-7.00 (m, 16H, H^{Ar}), 6.81 (dd, *J* = 7.04, 7.04 Hz, 4H, H^{12}), 6.57 (t, *J* = 12.5 Hz, 1H, H^{21}), 6.27 (d, *J* = 14.0 Hz, 1H, H^{23}), 6.20-6.14 (m, 2H, $H^{1',19}$), 4.53 (ddd, *J* = 5.5, 3.2, 3.2 Hz, 1H, $H^{3'}$), 4.03 (m, 1H, $H^{4'}$), 3.93 (t, *J* = 7.5 Hz, 2H, H^{27}), 3.67 (s, 3H, H^{18}), 3.66 (s, 3H, H^{18}), 3.59 (s, 3H, H^{26}), 3.30 (d, *J* = 3.0 Hz, 2H, $H^{5'}$), 2.38-2.31 (m, 2H, $H^{2'}$), 1.74-1.61 (m, 8H, $H^{25,28}$), 1.58 (s, 3H, H^{24}), 1.57 (s, 3H, H^{24}), 1.45-1.40 (m, 2H, H^{29}), 0.99-0.94 (m, 3H, H^{30}) ppm.
^{13}C (100 MHz, CD_3CN): δ 176.2 (C^{39}), 172.7 (C^{31}), 162.4 ($C^{2/4}$), 159.6 (C^{13}), 155.4 ($CH^{20/22}$), 154.0 ($CH^{20/22}$), 150.3 ($C^{2/4}$), 145.9 (CH^6), 143.9 (C^{Ar}), 143.8 (C^{Ar}), 143.5 (C^{Ar}), 142.6 (C^{Ar}), 142.3 (C^{Ar}), 137.0 (C^{10}), 136.8 (C^{10}), 133.0 (CH^{Ar}), 131.1 (CH^{Ar}), 129.6 (CH^{Ar}), 129.1 (CH^{Ar}), 129.0 (CH^{Ar}), 127.9 (CH^{Ar}), 126.6 (CH^{Ar}), 125.9 (CH^{21}), 123.3 (CH^{Ar}), 114.3 (C^{12}), 112.4 (C^{Ar}), 111.4 (C^{Ar}), 105.4 (CH^{23}), 103.7 (CH^{19}), 100.1 (C^5), 93.3 ($C^{8,9}$), 87.7 ($CH^{4'}$), 86.9 ($CH^{1'}$), 82.6 (C^7), 72.0 ($CH^{3'}$), 64.3 ($CH_2^{5'}$), 55.9 (CH_3^{18}), 50.6 (C^{46}), 49.6 (C^{38}), 44.7 (CH_2^{27}), 42.1 ($CH_2^{2'}$), 32.5 (CH_3^{26}), 30.0 (CH_2^{28}), 27.9 (CH_3^{25}), 27.5 (CH_3^{24}), 20.8 (CH_2^{29}), 14.2 (CH_3^{30}) ppm.
Mp: >130 °C (decomposes).
IR ν_{Max}/cm^{-1}: 3325 (br. w. O-H), 2959 (w. N-H), 2929, 2871 (w. C-H, C-H_3), 1704 (s. C=O).
UV/Vis (MeOH): A_{max}= 660 nm, ε_{max}= 208,000 $M^{-1}cm^{-1}$,Em_{max}= 690 nm.

Synthesis of Cy5dT-phosphoramidite



G

Cy5dT **23** (0.24 g, 0.22 mmol) was co-evaporated with distilled pyridine (3 x 5 mL) followed by distilled DCM (5 x 5 mL) before suspending in distilled DCM (5 mL), with activated molecular sieves (3 Å), under an argon atmosphere. To the solution was added distilled DIPEA (0.10 mL, 0.55 mmol). 2-Cyanoethyl *N,N*-diisopropyl-chlorophosphoramidite (0.05 mL, 0.22 mmol) was added drop-wise and the reaction was stirred for 45 min. Additional 2-cyanoethyl *N,N*-diisopropyl-chlorophosphoramidite (0.1 eq) was added before stirring for a further 1 hour. The mixture was diluted with distilled DCM (10 mL) and washed with deoxygenated aq. sat. KCl (10 mL). The organic phase was dried (Na_2SO_4) and the solvent removed *in vacuo*. The crude product was purified by precipitation in deoxygenated hexane (50 mL) from a minimum of distilled DCM (2 mL). The precipitation procedure was repeated twice more. The precipitate was dried *in vacuo* to afford the product **G** (0.24 g, 0.18 mmol, 84 %) as a dark blue solid.

R_f : 0.17 (MeOH/DCM, 0.5:9.5with 1% pyridine)
LRMS [ESI+, MeCN] m/z (%): 1178 ($[M]^+$, 100).
^{31}P (121 MHz, CD_3CN): δ 149.34 (s, P^{III}), 149.22 (s, P^{III}) ppm.

ESI.3. Oligonucleotide synthesis and purification

DNA reagents including standard DNA phosphoramidites and solid supports were purchased from Link Technologies Ltd. Oligonucleotides were synthesised on an Applied Biosystems 394 automated DNA/RNA synthesiser. Phosphoramidite cycles, including acid-catalysed detritylation, coupling, capping and iodine oxidation steps, were undertaken in 0.2 or 1.0 µmole scale. Standard DNA phoshoramidites were used for the majority of oligonucleotide sequences, however fast-deprotecting monomers, dmf-dG and Ac-dC, were used for those sequences containing Cy5dT. Coupling efficiencies and overall oligonucleotide yields were determined by the automated trityl cation conductivity monitoring facility of the synthesiser and were ≥98.0 % for all cases. Phosphoramidite monomers were dissolved in anhydrous acetonitrile to a concentration of 0.1 M immediately prior to use. The coupling time for A, G, C and T monomers was set to 25 s, however, the coupling time for the modified monomers was extended to 360 s. The cleavage of oligonucleotides from the solid support and subsequent deprotection was undertaken by suspending the resin in concentrated aqueous ammonia for 60 min at rt followed by heating in a sealed tube for 5 h at 55 °C. For oligonucleotides containing Cy5dT the deprotection time was reduced to 1 h at 55 °C.

A Gilson HPLC system with ABI Aquapore C8 column (8 mm x 250 mm, pore size 300 Å) was used to purify the oligonucleotides by reversed-phase. The following protocol was used for the majority of oligonucleotides: run time 20 min, flow rate 4 mL per min, gradient: time in min (% buffer B);0 (0); 3 (0); 3.5 (25); 15 (70); 16 (100); 17 (100); 17.5 (0); 20 (0). For the hydrophobic Cy3B oligonucleotides a modified protocol was used: run time 25 min, flow rate 4 mL per min, gradient: time in min (% buffer B); 0 (0); 3 (0); 3.5 (15); 15 (60); 16 (100); 22 (100); 22.5 (0); 25 (0). Elution buffer A: 0.1 M ammonium acetate, pH 7.0, buffer B: 0.1 M ammonium acetate with 50 % acetonitrile pH 7.0. Elution of the oligonucleotides was monitored by ultraviolet absorption at 295 nm and the main peak was collected then desalted using NAP-10 gel filtration columns (GE Healthcare), aliquoted into eppendorf tubes and stored at –20 ºC. All oligonucleotides were characterised either by MALDI-TOF mass spectrometry or MicrOTOF electrospray mass spectrometry and capillary gel electrophoresis.

ESI.4. Biophysical studies

ESI.4.1 UV analysis

ESI.4.1.1 Extinction coefficient calculation

Measurements were made on a Cary 4000 UV-Vis spectrometer with Cary temperature controller using Cary Win UV Scan software. Extinction coefficients of compounds (in MeOH or EtOH) were calculated from four readings of UV absorbance at the UV absorbance maxima using the Beer-Lambert law, $A=\varepsilon cl$ (where A is absorbance at UV A_{max}, c is concentration in M, l is pathlength 1 cm and ε is extinction coefficient $M^{-1}cm^{-1}$). Samples were analysed in a 1 mL cuvette (Hellma synthetic quartz 'precision cell QG'; 1 mL volume, 10 mm pathlength).
In the case of the CyDyes, samples were dissolved in EtOH and passed through Dowex-1-chloride (Dowex 1x2-200 ion exchange resin) to convert all counter ions to Cl^-. Samples were then dried in a heating pistol over P_2O_5 at 50 ºC for 48 h before a stock solution was

prepared of each (1 mg / 10 mL MeOH). Compounds were diluted from the stock solution to give four readings below an absorbance value of 1. Absorbance values were plotted against concentration and a straight line was drawn through the points intercepting the Y-axis at 0. The extinction coefficient was taken as the gradient of the line.

ESI.4.1.2 UV Melting Analysis

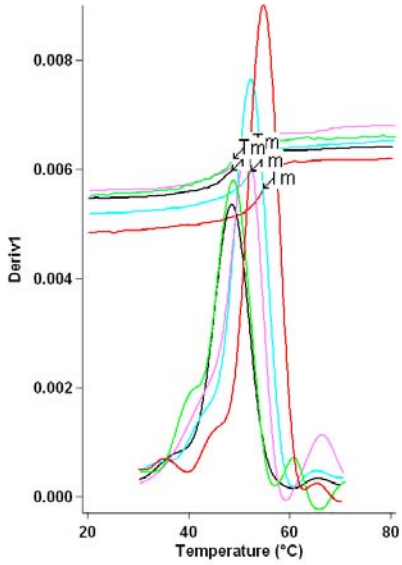
Measurements were made on a Cary 4000 UV-Vis spectrometer with Cary temperature controller. Cary Win UV Thermal software was used with an absorption wavelength of 260 nm. Samples were analysed in 1 mL cuvettes (Hellma synthetic quartz 'precision cell QG'; 1 mL volume, 10 mm pathlength) and were made to 1 μM oligonucleotide concentration in phosphate buffer (10 mM) with NaCl (100 mM) at pH 7. Three successive melting curves were measured, and average T_m values were calculated with Cary Win UV Thermal application software.
The thermal protocol used was as follows:
Start T 20 °C, end T 20 °C.

Thermal protocol for UV melting experiment

Stage	Data interval (°C)	Rate (°C/min)	End Temp (°C)	Hold time (min)
1	1.00	10.00	84.00	2.00
2	0.10	1.00	20.00	2.00
3	0.10	1.00	84.00	2.00
4	0.10	1.00	20.00	2.00
5	0.10	1.00	84.00	2.00
6	0.10	1.00	20.00	2.00
7	0.10	1.00	84.00	2.00
8	0.10	10.00	20.00	0.00

Representative oligonucleotides were chosen to test stability by UV melting and circular dichroism.

Duplex name	Oligo 1	Oligo 2	Line colour
Unmodified control	ODN-14	ODN-15	red
Cy3dT/Cy5dT in the same strand	ODN-16	ODN-17	black
Cy3dT/Cy5dT in opposite strands	ODN-12	ODN-13	green
Cy5dT control	ODN-14	ODN-13	pink
Cy3dT control	ODN-12	ODN-15	blue



Example UV melting data.

ESI.4.1.3 Circular Dichroism Analysis

Circular dichroism spectra were recorded on a Jasco J-720 spectropolarimeter. Samples were made to 1 μM oligonucleotide concentration in phosphate buffer (10 mM) with NaCl (100 mM) at pH 7. Spectra were recorded between 200-320 nm at a rate of 100 nm/min, with step resolution of 0.2 nm, bandwidth of 1.0 nm and sensitivity of 50 mdeg. Ten successive spectra were recorded and an average was taken. A blank (buffer) baseline was subtracted from each spectrum.

ESI.4.2 Fluorescence analysis

ESI.4.2.1 Fluorescence melting analysis

For single-wavelength analysis, measurements were made on a Perkin Elmer LS50B fluorimeter equipped with a Perkin Elmer PTP-1 Peltier system. FLWinlab TempScan software was used with optimum excitation/emission slit widths, excitation wavelength and emission wavelength settings for each individual sample. In cases where multiple samples were compared, the slit width and excitation wavelength settings were kept constant. Where multiple dyes were compared, excitation wavelength was taken as the absorption maxima or ¾ absorbance maxima as specified in experimental discussion.

Samples were analysed in a 200 μL cuvette (Hellma quartz 'SUPRASIL QS'; 200 μL volume, 10 mm pathlength) with a collection angle of 90°. A total sample volume of 200 μL was used, with labelled oligonucleotide concentration of 0.15 μM (0.5 μM for target strand, or 0.15 μM in cases where target strand was also dye-containing). Samples were prepared in GoTaq colourless PCR buffer (Promega) with a total of 3 mM $MgCl_2$ at pH 8.5. The oligonucleotide samples were heated to 70 °C to denature for 3 min before being allowed to slowly cool to rt to anneal.

Samples were heated from 30 °C to 80 °C with a step size of 1 °C and 30 s equilibration.The emission intensity was recorded at each step.

For fixed-wavelength analysis, fluorescence melting was carried out using a RotorGene-3000 with settings of excitation 530 nm and detection 585 nm with a gain of 5. Samples were analysed in RotorGene thin-walled PCR tubes (0.1 mL. Qiagen). A total sample volume of 20 μL was used, with labelled oligonucleotide concentration of 0.15 μM (0.5 μM for target strand). Samples were prepared in GoTaq colourless PCR buffer with a total of 3 mM $MgCl_2$ at pH 8.5.

Samples were heated to 95 °C for 2 min then cooled to 30 °C. After holding at 30 °C for 1 min the samples were heated from 30 °C - 95 °C with a 0.5 °C step (15 sec for first step, 5 sec per step thereafter). Fluorescence emission intensity was recorded at each step.

ESI.4.2.2 Quantum yield calculation

Quantum yields were measured in a 1 mL cuvette (Hellma quartz 'SUPRASIL QS'; 1 mL volume, 10 mm pathlength) and were measured in reference to Rh101 (QY = 1.0 at 25 °C in EtOH in range λ_{ex} 450-565 nm) (21). Samples were initially scanned for OD at the single wavelength of 545 nm using a Cary 50 Bio UV-Vis spectrophotometer. All samples were prepared by dissolving the fluorescent compound in MeOH (analytical grade) and then sequentially diluting with MeOH until a concentration of 0.01 ± 0.008 OD (545 nm) was reached and recorded.

Fluorescence emission measurements were made on a Perkin Elmer LS50B fluorimeter equipped with a Perkin Elmer PTP-1 Peltier system. FL Winlab Scan software was used with an emission scan range of 545-800 nm and excitation of 545 nm. Slit widths of excitation slit 3.5 nm and emission slit 4 nm were used together with a scan speed of 300 nm/min. Emission readings were taken at 25 °C and the area of each sample was calculated relative to its own baseline. Sample and reference measurements were repeated three times, with an average taken for each compound. The following equation was used to calculate the final quantum yield values. (QY = quantum yield, I = fluorescence emission area, η = refractive index of solvent, R = reference and S = sample).

$$QY = QY_R \times (I_S/I_R) \times (OD_R/OD_S) \times (\eta_S^2/\eta_R^2)$$

ESI.4.2.3 FRET experiment

Duplexes between Cy5dT oligonucleotide (ODN-37) and Cy3dT oligonucleotides (ODN-27, ODN-28, ODN-29, ODN-30, ODN-31, ODN-12, ODN-33, ODN-34, ODN-35, ODN-36) were prepared in a 1:1 ratio at 0.2 μM concentration of each oligonucleotide. Control samples were also prepared to show the oligonucleotides against an unmodified complement (ODN-14 with ODN-37, ODN-15 with ODN-26-ODN-36).
A total sample volume of 200 μL was used with sample buffer of 25 mM sodium phosphate with 100 mM NaCl at pH 7.5.

Samples were scanned for UV absorbance from 200-800 nm (a background of buffer only was subtracted automatically from each spectrum). FRET samples were scanned for fluorescence emission from 535-800 nm (excitation 530 nm and 615 nm) with scan speed of

300 nm/min, slit widths of 15 nm excitation slit and 20 nm emission slit. Control samples were scanned with the same conditions.
FRET was calculated by the equation:

$$\text{FRET efficiency} = (I_{FRET} \times Abs_{615})/(I_{direx} \times Abs_{530})$$

I_{FRET} is the emission intensity of the Cy5 peak when excited *via* FRET (ex 530 nm), I_{direx} is the emission intensity of Cy5 peak when directly excited (ex 615 nm), Abs_{615} is the absorbance of the sample at 615 nm and Abs_{530} is the absorbance of the sample at 530 nm.

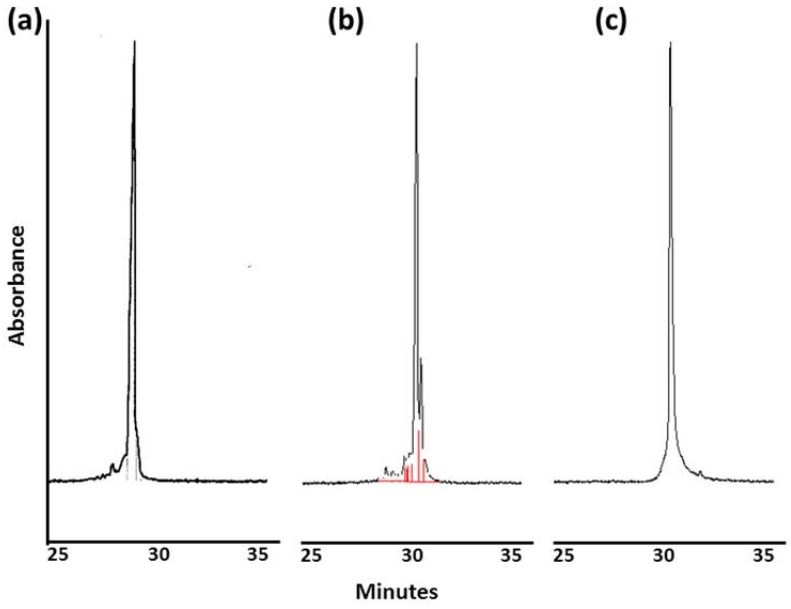
ESI.5. Oligonucleotide data

Cy3dT and Cy5dT Oligonucleotide data

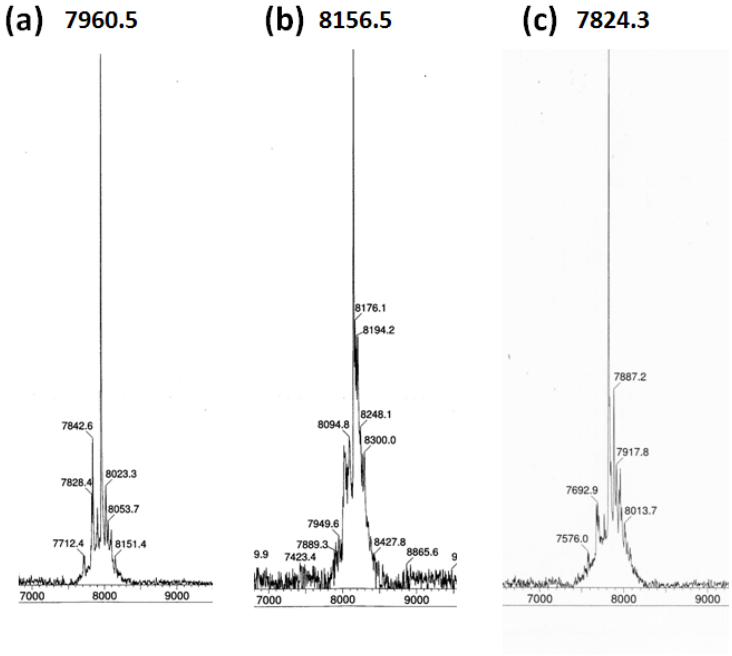
Data (for modified oligonucleotides) and sequences. **3** = Cy3dT phosphoramidite, **5** = Cy5dT phosphoramidite, **p** = propanol. MALDI-TOF Mass spectra$^{(M)}$ of oligonucleotides were recorded on a ThermoBio-Analysis Dynamo MALDI-TOF mass spectrometer in positive ion mode using oligonucleotide (oligo-dT) standards (22) or on a Bruker micrOTOF™ II focus ESI-TOF MS instrument in ES− mode$^{(E)}$ as specified.

Oligonucleotide code	**Sequence (5´ to 3´)**	**Calculated mass (Da)**	**Found mass (Da)**
ODN-16	GGA **5**TT TCG **3**TT TTA TAA TTG CC	7823	7824.3^{M}
ODN-20	CAC CAA AGA TGA TAT TT**3** CTT TAA TGG **p**	8775	8780.0^{E}
ODN-21	CAC CAA AGA TGA TATTT**5** CTT TAA TGG **p**	8845	8849.0^{E}
ODN-27	CGT ATA TTC TTT ATT **3**TT AAA AGC C	7960	7962.1^{M}
ODN-28	CGT ATA TTC TTT AT**3** TTT AAA AGC C	7960	7960.3^{M}
ODN-29	CGT ATA TTC TTT A**3**T TTT AAA AGC C	7960	7962.4^{M}
ODN-30	CGT ATA TTC TT**3** ATT TTT AAA AGC C	7960	7961.4^{M}
ODN-31	CGT ATA TTC T**3**T ATT TTT AAA AGC C	7960	7960.2^{M}
ODN-12	CGT ATA TTC **3**TT ATT TTT AAA AGC C	7960	7960.5^{M}
ODN-33	CGT ATA T**3**C TTT ATT TTT AAA AGC C	7960	7960.9^{M}
ODN-34	CGT ATA **3**TC TTT ATT TTT AAA AGC C	7960	7961.4^{M}
ODN-35	CGT A**3**A TTC TTT ATT TTT AAA AGC C	7960	7961.1^{M}
ODN-36	CG**3** ATA TTC TTT ATT TTT AAA AGC C	7960	7960.5^{M}
ODN-13	GGC TTT **5**AA AAA TAA AGA ATA TAC G	8153	8156.5^{M}
ODN-37	GGC T**5**T TAA AAA TAA AGA ATA TAC G	8153	8153.3^{E}
ODN-38	TTG CGT ACA T**3**A **3**A**3** A**3**A **3**AT AAT AGC CAT	10997	10999.3^{E}

Sample oligonucleotide analyses



Capillary electrophoresis analyses of; (a) ODN-12 (containing one Cy3dT monomer); (b) ODN-13 (containing one Cy5dT monomer); (c) ODN-16 (containing one Cy3dT monomer and one Cy5dT monomer).



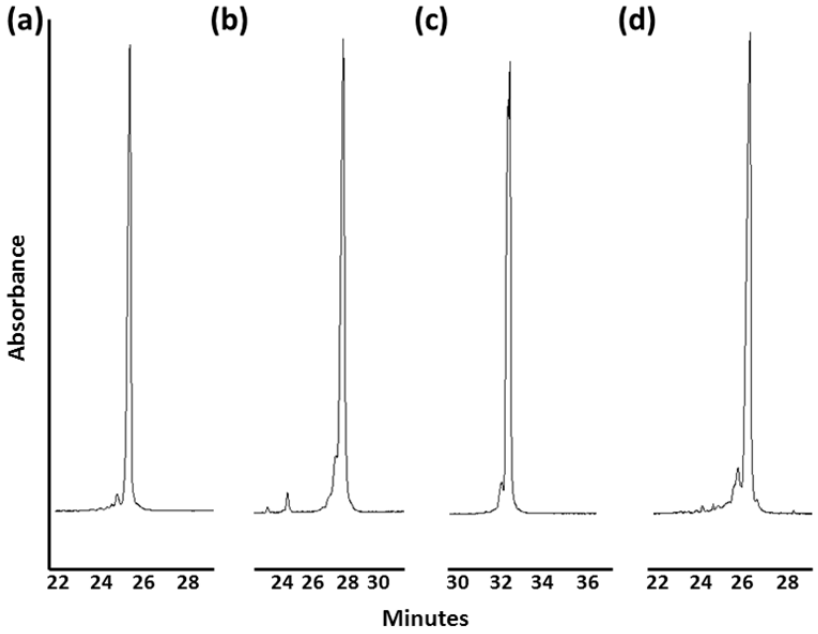
MALDI mass analysis of; (a) ODN-12 (containing one Cy3dT monomer); (b) ODN-13 (containing one Cy5dT monomer); (c) ODN-16 (containing one Cy3dT monomer and one Cy5dT monomer).

Cy3B oligonucleotide data

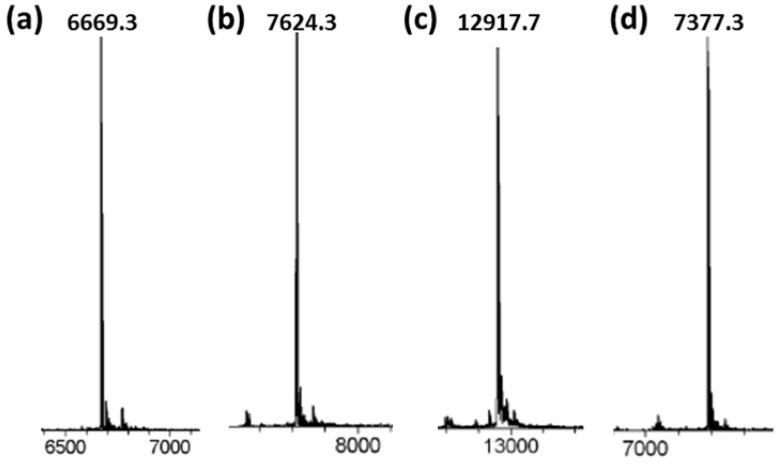
Data (for modified oligonucleotides) and sequences. **b** = Cy3BdT phosphoramidite; **h** = 5-(hexyn-1-ol)-6-Cy3B phosphoramidite; **s** = β-Cy3BdR phosphoramidite; **r** = α-Cy3BdR resin; **q** = BHQ2-dT phosphoramidite; **Q** = Dabcyl-dT phosphoramidite; **d** = Dabcyl resin; **p** = propanol; **H** = hexaethylene glycol. MALDI-TOF Mass spectra[(M)] of oligonucleotides were recorded on a ThermoBio-Analysis Dynamo MALDI-TOF mass spectrometer in positive ion mode using oligonucleotide (oligo-dT) standards (22) or on a Bruker micrOTOF™ II focus ESI-TOF MS instrument in ES− mode[(E)] as specified.

Oligonucleotide code	**Sequence (5′ to 3′)**	**Calculated mass (Da)**	**Found mass (Da)**
ODN-6	**h**TT CCT ATG A**q**G AAT ATA GAT ACA GAA GCG **p**	10326	10325.8[E]
ODN-7	CGC TTC **b**GT ATC **b**AT ATT CAT C**p**	7625	7624.3[E]
ODN-8	**s**CC TAG CAT GAT GAA TAT AGA TAC AGA AGC GTC GCT AGG **d**	12918	12917.7[E]
ODN-9	**s**CC TAG CAT GAT GAA TAT AGA TAC AGA AGC GTC GCT AGG **r**	13153	13151.7[E]
ODN-10	**s**CC GCG GGA TGA ATA TAG ATA CAG AAG CGC CGC GG**Q H**TC TTC TAG TTG GCA TGC T	17835	17834.4[E]
ODN-11	**h**TC AGT TTT CCT GGA TTA TGC	6671	6669.3[E]
ODN-23	TTG CGT ACA **b**TC TCC GTT TTT AAT AGC CAT	9544	9545.4[M]
ODN-39	AAT ATC ATC TTT GGT GTT TCC T**r**	7378	7377.3[E]

Sample oligonucleotide analyses



Capillary electrophoresis analyses of; (a) ODN-11 (containing one 5-(hexyn-1-ol)-6-Cy3B monomer); (b) ODN-7 (containing two CyBdT monomers); (c) ODN-8 (containing one β-Cy3BdR monomer with dabcyl resin); (d) ODN-39 (with α-Cy3BdR resin).



Electrospray mass analysis of; (a) ODN-11 (containing one 5-(hexyn-1-ol)-6-Cy3B monomer); (b) ODN-7 (containing two CyBdT monomers); (c) ODN-8 (containing one β-Cy3BdR monomer with dabcyl resin); (d) ODN-39 (with α-Cy3BdR resin).

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