

Supporting Information

Syntheses and Evaluation of Photoresponsive Quencher for Fluorescent Hybridization Probes

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1. Absorption of dU^{DAB} in different solvents.

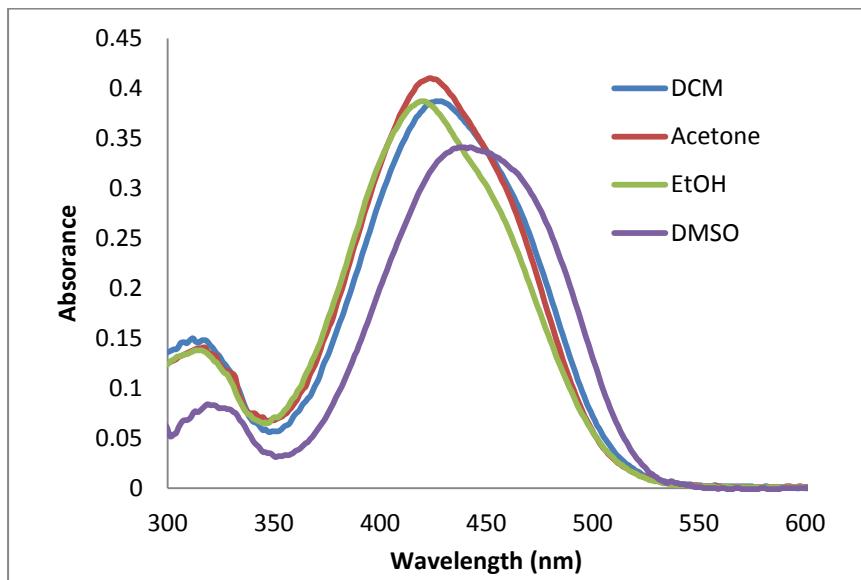


Figure S1. Absorption spectra of dU^{DAB}, **1**, in different solvents at 10 μM concentration.

2. AT-IR of dU^{DAB} nucleoside.

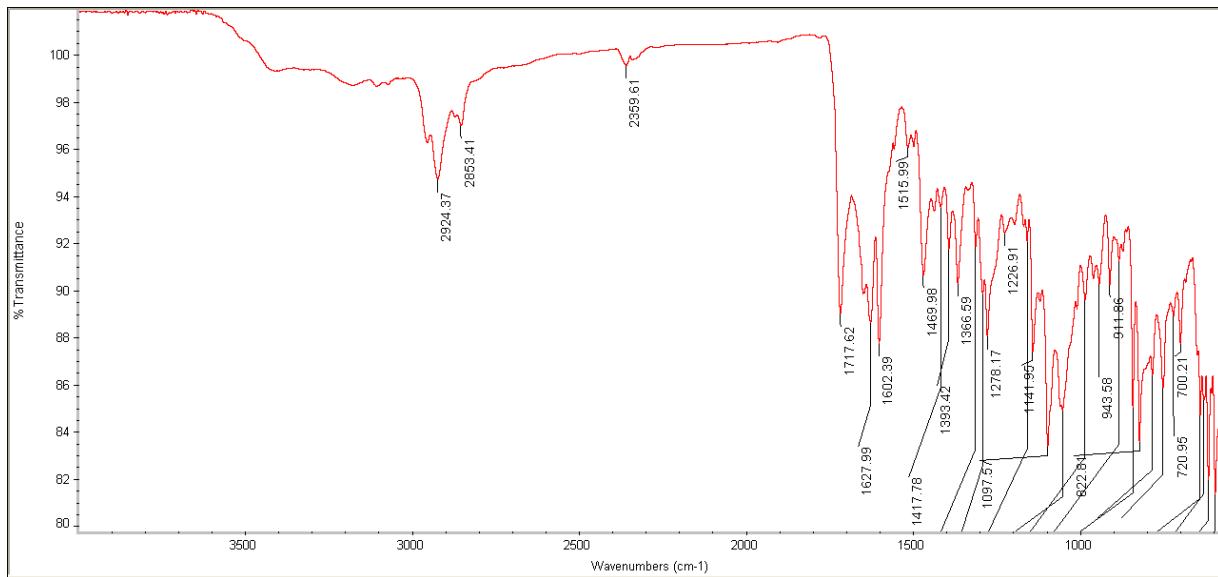


Figure S2. IR spectrum of dU^{DAB}, **1**.

**3. Crystal data, data collection and structure refinement details for 1
(dU^{DAB}).**

Table S1. Crystal data and structure refinement for 1 (dU^{DAB})

Chemical formula	C ₂₃ H ₂₅ N ₅ O ₅
M _r	451.48
Crystal system, space group	Orthorhombic, P2 ₁ 2 ₁ 2 ₁
Temperature (K)	100
a, b, c (Å)	5.5457 (3), 8.9659 (3), 42.4254 (13)
V (Å ³)	2109.48 (15)
Z	4
Radiation type	Mo Kα
μ (mm ⁻¹)	0.10
Crystal size (mm)	0.30 × 0.20 × 0.02
Diffractometer	Bruker KappaApexII CCD diffractometer
Absorption correction	Multi-scan Bruker SADABS
T _{min} , T _{max}	0.970, 0.998
No. of measured, independent and observed [I > 2σ(I)] reflections	9463, 4002, 3551
R _{int}	0.022
(sin θ/λ) _{max} (Å ⁻¹)	0.624
R[F ² > 2σ(F ²)], wR(F ²), S	0.039, 0.103, 1.05
No. of reflections	4002
No. of parameters	302
No. of restraints	0
H-atom treatment	H atoms treated by a mixture of independent and constrained refinement
Δρ _{max} , Δρ _{min} (e Å ⁻³)	0.22, -0.19
Absolute structure	Flack H D (1983), Acta Cryst. A39, 876-881
Absolute structure parameter	-1.1 (11)

Table S2. Fractional atomic coordinates and isotropic or equivalent isotropic displacement parameters (\AA^2)

	<i>x</i>	<i>y</i>	<i>z</i>	$U_{\text{iso}}^*/U_{\text{eq}}$
C1'	-0.5983 (4)	0.4023 (2)	0.95682 (4)	0.0161 (4)
H1	-0.7364	0.3783	0.9711	0.019*
C2'	-0.4684 (4)	0.5412 (2)	0.96883 (4)	0.0178 (4)
H2A	-0.4599	0.5429	0.9921	0.021*
H2B	-0.3036	0.5492	0.9600	0.021*
C3'	-0.6316 (4)	0.6629 (2)	0.95634 (4)	0.0180 (4)
H3A	-0.5431	0.7594	0.9540	0.022*
C4'	-0.7117 (4)	0.6017 (2)	0.92443 (4)	0.0185 (4)
H4	-0.8850	0.6276	0.9209	0.022*
C5'	-0.5631 (5)	0.6565 (2)	0.89663 (5)	0.0267 (5)
H5A	-0.6075	0.5981	0.8777	0.032*
H5B	-0.6040	0.7622	0.8925	0.032*
O1	-0.6872 (3)	0.44114 (14)	0.92653 (3)	0.0199 (3)
O2	-0.8295 (3)	0.67637 (15)	0.97801 (3)	0.0214 (3)
H2	-0.9313	0.7367	0.9708	0.032*
O3	-0.3081 (3)	0.64452 (18)	0.90158 (4)	0.0322 (4)
H3B	-0.2632	0.7107	0.9143	0.048*
O4	-0.6189 (3)	0.15793 (14)	0.99587 (3)	0.0212 (3)
O5	-0.0723 (3)	-0.10843 (15)	0.94045 (3)	0.0240 (4)
N1	-0.4468 (3)	0.27064 (16)	0.95300 (3)	0.0156 (4)
N3	-0.3358 (3)	0.03225 (17)	0.96774 (3)	0.0161 (4)
H3N	-0.3521	-0.0438	0.9807	0.019*
N4	0.5242 (4)	0.1440 (2)	0.82006 (4)	0.0273 (4)
N5	0.4820 (4)	0.2367 (2)	0.79835 (4)	0.0275 (4)
N6	1.0478 (5)	0.2062 (2)	0.68934 (4)	0.0418 (6)
C2	-0.4780 (4)	0.1545 (2)	0.97393 (4)	0.0171 (4)

C4	-0.1701 (4)	0.0145 (2)	0.94359 (4)	0.0168 (4)
C5	-0.1338 (4)	0.1459 (2)	0.92385 (4)	0.0171 (4)
C6	-0.2728 (4)	0.2659 (2)	0.93019 (4)	0.0161 (4)
H6	-0.2479	0.3533	0.9179	0.019*
C7	0.0374 (4)	0.1459 (2)	0.89724 (4)	0.0176 (4)
C8	-0.0126 (4)	0.2337 (2)	0.87044 (4)	0.0224 (5)
H8	-0.1568	0.2908	0.8697	0.027*
C9	0.1449 (4)	0.2382 (2)	0.84515 (5)	0.0252 (5)
H9	0.1088	0.2987	0.8274	0.030*
C10	0.3563 (4)	0.1541 (2)	0.84566 (5)	0.0222 (5)
C11	0.4071 (4)	0.0673 (2)	0.87190 (5)	0.0224 (5)
H11	0.5509	0.0098	0.8725	0.027*
C12	0.2497 (4)	0.0638 (2)	0.89732 (4)	0.0199 (5)
H12	0.2880	0.0041	0.9151	0.024*
C13	0.6381 (5)	0.2259 (3)	0.77213 (5)	0.0271 (5)
C14	0.8309 (5)	0.1291 (3)	0.76968 (5)	0.0323 (6)
H14	0.8695	0.0659	0.7869	0.039*
C15	0.9683 (5)	0.1227 (3)	0.74264 (5)	0.0368 (6)
H15	1.0995	0.0548	0.7414	0.044*
C16	0.9154 (5)	0.2164 (3)	0.71677 (5)	0.0297 (6)
C17	0.7228 (5)	0.3165 (3)	0.71977 (5)	0.0328 (6)
H17	0.6854	0.3823	0.7029	0.039*
C18	0.5868 (5)	0.3200 (3)	0.74706 (5)	0.0329 (6)
H18	0.4561	0.3881	0.7487	0.039*
C19	1.0060 (6)	0.3082 (3)	0.66372 (5)	0.0405 (7)
H19A	1.0505	0.4094	0.6703	0.061*
H19B	0.8350	0.3061	0.6579	0.061*
H19C	1.1040	0.2787	0.6455	0.061*
C20	1.2310 (6)	0.0917 (3)	0.68554 (6)	0.0423 (7)

H20A	1.1627	-0.0057	0.6910	0.063*
H20B	1.3675	0.1134	0.6995	0.063*
H20C	1.2863	0.0903	0.6636	0.063*

Table S3 Atomic displacement parameters (\AA^2)

	U^{11}	U^{22}	U^{33}	U^{12}	U^{13}	U^{23}
C1'	0.0185 (11)	0.0147 (9)	0.0152 (8)	0.0028 (9)	0.0018 (9)	-0.0001 (7)
C2'	0.0169 (11)	0.0151 (9)	0.0215 (9)	0.0025 (9)	-0.0016 (9)	-0.0018 (7)
C3'	0.0202 (12)	0.0139 (9)	0.0199 (9)	-0.0008 (9)	0.0020 (9)	0.0020 (8)
C4'	0.0212 (12)	0.0142 (9)	0.0200 (9)	0.0030 (9)	-0.0005 (9)	0.0023 (7)
C5'	0.0304 (14)	0.0280 (11)	0.0217 (10)	0.0057 (11)	0.0016 (10)	0.0044 (9)
O1	0.0248 (9)	0.0145 (6)	0.0204 (6)	0.0032 (7)	-0.0056 (7)	-0.0005 (5)
O2	0.0258 (9)	0.0174 (7)	0.0210 (6)	0.0095 (7)	0.0046 (7)	0.0030 (5)
O3	0.0285 (10)	0.0368 (9)	0.0313 (8)	0.0008 (8)	0.0077 (8)	0.0011 (7)
O4	0.0231 (9)	0.0189 (7)	0.0214 (7)	0.0022 (7)	0.0072 (7)	0.0036 (5)
O5	0.0315 (10)	0.0165 (7)	0.0241 (7)	0.0082 (7)	0.0050 (7)	0.0029 (5)
N1	0.0186 (9)	0.0124 (7)	0.0159 (7)	0.0003 (8)	0.0007 (7)	0.0011 (6)
N3	0.0205 (10)	0.0117 (7)	0.0162 (7)	0.0002 (8)	0.0012 (7)	0.0031 (6)
N4	0.0265 (12)	0.0339 (10)	0.0216 (8)	-0.0010 (10)	0.0059 (9)	-0.0018 (8)
N5	0.0283 (12)	0.0312 (10)	0.0230 (8)	-0.0049 (10)	0.0046 (8)	-0.0011 (8)
N6	0.0500 (16)	0.0463 (12)	0.0291 (10)	0.0101 (13)	0.0154 (11)	0.0089 (9)
C2	0.0171 (11)	0.0155 (9)	0.0187 (9)	-0.0014 (9)	-0.0009 (9)	-0.0014 (7)
C4	0.0203 (11)	0.0178 (9)	0.0125 (8)	0.0007 (10)	-0.0026 (8)	-0.0019 (7)
C5	0.0172 (11)	0.0158 (9)	0.0184 (9)	0.0001 (9)	-0.0007 (9)	0.0002 (7)
C6	0.0187 (11)	0.0148 (9)	0.0147 (8)	-0.0007 (9)	0.0003 (9)	0.0010 (7)
C7	0.0191 (12)	0.0162 (9)	0.0176 (9)	-0.0008 (9)	0.0003 (9)	-0.0021 (7)
C8	0.0216 (12)	0.0235 (10)	0.0220 (9)	0.0023 (10)	0.0024 (10)	0.0024 (8)
C9	0.0294 (13)	0.0263 (10)	0.0198 (9)	0.0005 (11)	0.0017 (10)	0.0033 (8)
C10	0.0191 (12)	0.0258 (10)	0.0216 (9)	-0.0036 (10)	0.0041 (9)	-0.0025 (8)
C11	0.0162 (12)	0.0251 (11)	0.0259 (10)	-0.0005 (10)	0.0018 (10)	-0.0037 (8)
C12	0.0216 (12)	0.0192 (9)	0.0189 (9)	-0.0005 (9)	0.0003 (9)	-0.0006 (8)
C13	0.0290 (14)	0.0294 (11)	0.0230 (10)	-0.0080 (11)	0.0059 (10)	-0.0031 (8)
C14	0.0305 (14)	0.0434 (13)	0.0231 (10)	0.0008 (13)	0.0053 (11)	0.0050 (9)
C15	0.0321 (15)	0.0459 (14)	0.0325 (12)	0.0066 (13)	0.0091 (12)	0.0053 (10)
C16	0.0355 (15)	0.0314 (11)	0.0223 (10)	-0.0051 (12)	0.0072 (11)	-0.0002 (9)
C17	0.0417 (16)	0.0328 (12)	0.0240 (10)	-0.0010 (12)	0.0047 (11)	0.0049 (9)
C18	0.0362 (15)	0.0310 (12)	0.0314 (11)	0.0018 (12)	0.0076 (12)	0.0029 (9)
C19	0.0556 (19)	0.0427 (13)	0.0232 (10)	0.0015 (15)	0.0103 (12)	0.0002 (10)
C20	0.0465 (19)	0.0488 (15)	0.0315 (12)	0.0012 (15)	0.0132 (13)	0.0003 (11)

Table S4 Geometric parameters (Å, °)

C1'—O1	1.420 (2)	C4—C5	1.459 (3)
C1'—N1	1.458 (3)	C5—C6	1.351 (3)
C1'—C2'	1.527 (3)	C5—C7	1.476 (3)
C1'—H1	1.0000	C6—H6	0.9500
C2'—C3'	1.513 (3)	C7—C12	1.388 (3)
C2'—H2A	0.9900	C7—C8	1.411 (3)
C2'—H2B	0.9900	C8—C9	1.384 (3)
C3'—O2	1.437 (3)	C8—H8	0.9500
C3'—C4'	1.526 (3)	C9—C10	1.394 (3)
C3'—H3A	1.0000	C9—H9	0.9500
C4'—O1	1.449 (2)	C10—C11	1.387 (3)
C4'—C5'	1.520 (3)	C11—C12	1.388 (3)
C4'—H4	1.0000	C11—H11	0.9500
C5'—O3	1.434 (3)	C12—H12	0.9500
C5'—H5A	0.9900	C13—C14	1.381 (3)
C5'—H5B	0.9900	C13—C18	1.387 (3)
O2—H2	0.8400	C14—C15	1.379 (3)
O3—H3B	0.8400	C14—H14	0.9500
O4—C2	1.216 (2)	C15—C16	1.413 (3)
O5—C4	1.236 (2)	C15—H15	0.9500
N1—C6	1.367 (3)	C16—C17	1.400 (4)
N1—C2	1.380 (2)	C17—C18	1.382 (3)
N3—C2	1.376 (3)	C17—H17	0.9500
N3—C4	1.385 (3)	C18—H18	0.9500
N3—H3N	0.8800	C19—H19A	0.9800
N4—N5	1.263 (2)	C19—H19B	0.9800
N4—C10	1.433 (3)	C19—H19C	0.9800
N5—C13	1.413 (3)	C20—H20A	0.9800

N6—C16	1.379 (3)	C20—H20B	0.9800
N6—C19	1.439 (3)	C20—H20C	0.9800
N6—C20	1.453 (4)		
O1—C1'—N1	107.35 (14)	C4—C5—C7	121.85 (17)
O1—C1'—C2'	105.41 (15)	C5—C6—N1	124.61 (17)
N1—C1'—C2'	115.21 (18)	C5—C6—H6	117.7
O1—C1'—H1	109.6	N1—C6—H6	117.7
N1—C1'—H1	109.6	C12—C7—C8	117.69 (19)
C2'—C1'—H1	109.6	C12—C7—C5	122.96 (17)
C3'—C2'—C1'	100.91 (16)	C8—C7—C5	119.34 (19)
C3'—C2'—H2A	111.6	C9—C8—C7	121.1 (2)
C1'—C2'—H2A	111.6	C9—C8—H8	119.4
C3'—C2'—H2B	111.6	C7—C8—H8	119.4
C1'—C2'—H2B	111.6	C8—C9—C10	120.20 (18)
H2A—C2'—H2B	109.4	C8—C9—H9	119.9
O2—C3'—C2'	107.06 (15)	C10—C9—H9	119.9
O2—C3'—C4'	112.06 (18)	C11—C10—C9	119.12 (19)
C2'—C3'—C4'	103.05 (15)	C11—C10—N4	116.1 (2)
O2—C3'—H3A	111.4	C9—C10—N4	124.68 (19)
C2'—C3'—H3A	111.4	C10—C11—C12	120.6 (2)
C4'—C3'—H3A	111.4	C10—C11—H11	119.7
O1—C4'—C5'	108.55 (17)	C12—C11—H11	119.7
O1—C4'—C3'	105.97 (15)	C11—C12—C7	121.28 (19)
C5'—C4'—C3'	114.47 (18)	C11—C12—H12	119.4
O1—C4'—H4	109.2	C7—C12—H12	119.4
C5'—C4'—H4	109.2	C14—C13—C18	118.9 (2)
C3'—C4'—H4	109.2	C14—C13—N5	125.23 (19)
O3—C5'—C4'	113.37 (18)	C18—C13—N5	115.9 (2)
O3—C5'—H5A	108.9	C15—C14—C13	121.1 (2)

C4'—C5'—H5A	108.9	C15—C14—H14	119.4
O3—C5'—H5B	108.9	C13—C14—H14	119.4
C4'—C5'—H5B	108.9	C14—C15—C16	120.5 (2)
H5A—C5'—H5B	107.7	C14—C15—H15	119.8
C1'—O1—C4'	109.38 (13)	C16—C15—H15	119.8
C3'—O2—H2	109.5	N6—C16—C17	121.7 (2)
C5'—O3—H3B	109.5	N6—C16—C15	120.4 (2)
C6—N1—C2	121.41 (17)	C17—C16—C15	117.9 (2)
C6—N1—C1'	120.69 (15)	C18—C17—C16	120.5 (2)
C2—N1—C1'	117.84 (16)	C18—C17—H17	119.8
C2—N3—C4	127.80 (16)	C16—C17—H17	119.8
C2—N3—H3N	116.1	C17—C18—C13	121.1 (2)
C4—N3—H3N	116.1	C17—C18—H18	119.5
N5—N4—C10	112.99 (19)	C13—C18—H18	119.5
N4—N5—C13	114.56 (19)	N6—C19—H19A	109.5
C16—N6—C19	120.6 (2)	N6—C19—H19B	109.5
C16—N6—C20	120.85 (19)	H19A—C19—H19B	109.5
C19—N6—C20	118.5 (2)	N6—C19—H19C	109.5
O4—C2—N3	122.33 (17)	H19A—C19—H19C	109.5
O4—C2—N1	123.68 (18)	H19B—C19—H19C	109.5
N3—C2—N1	113.98 (17)	N6—C20—H20A	109.5
O5—C4—N3	118.25 (17)	N6—C20—H20B	109.5
O5—C4—C5	126.71 (18)	H20A—C20—H20B	109.5
N3—C4—C5	115.03 (16)	N6—C20—H20C	109.5
C6—C5—C4	116.77 (17)	H20A—C20—H20C	109.5
C6—C5—C7	121.31 (17)	H20B—C20—H20C	109.5

4. Comparison of the X-Ray structure of dU^{DAB} with Thymidine.

Table S5: Selected bond distances (Å) and angles (°) for 1

Bond lengths [Å]			Angles [°]	
	1	Thymidine	1	Thymidine
N4-N5	1.263		N5-N4-C10	112.9
N4-C10	1.433		N4-C10-C11	116.1
N5-C13	1.413		N4-N5-C13	114.6
C1'-O1	1.420	1.434	O1-C1'-N1	107.6
C1'-N1	1.458	1.480	C1'-N1-C6	120.7
C1'-C2'	1.527	1.515	O1-C1'-C2'	105.4
C2'-C3'	1.513	1.523	C1'-C2'-C3'	100.9
C3'-O2	1.437	1.428	O2-C3'-C2'	107.1
C3'-C4'	1.526	1.529	O2-C3'-C4'	112.1
C4'-O1	1.434	1.425	C4'-O1-C1'	109.4
O4-C2	1.216	1.206	O4-C2-N3	122.3
O5-C4	1.236	1.230	O5-C4-C5	126.7
N1-C6	1.367	1.374	C2-N1-C6	121.4
N1-C2	1.380	1.385	N3-C2-N1	113.9
N3-C2	1.376	1.381	C2-N3-C4	127.8
N3-C4	1.385	1.378	N3-C4-C5	115.0
C4-C5	1.459	1.453	C4-C5-C6	116.8
C5-C6	1.351	1.343	C5-C6-N1	124.6
C5-C7	1.476	1.504	C6-C5-C7	121.9
				123.5

5. Analysis of thermal melting curves of double-stranded probes.

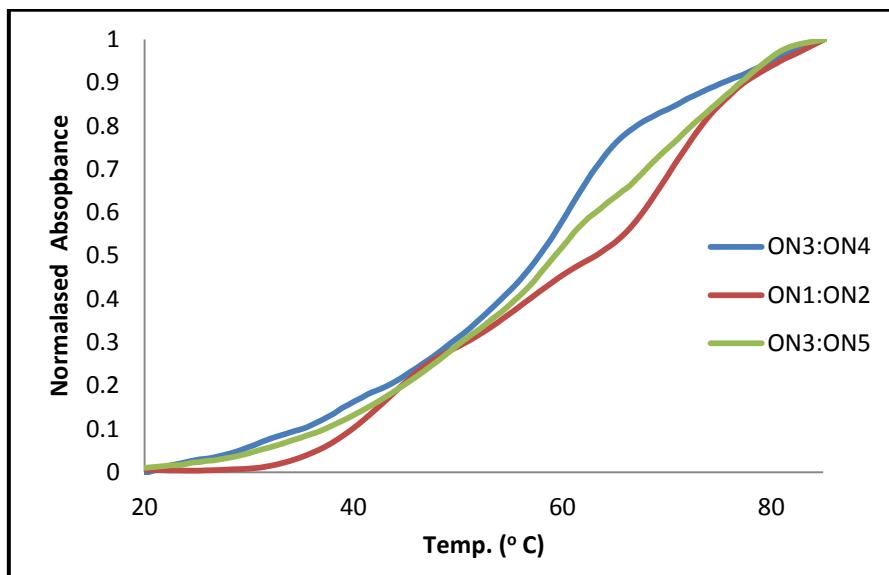
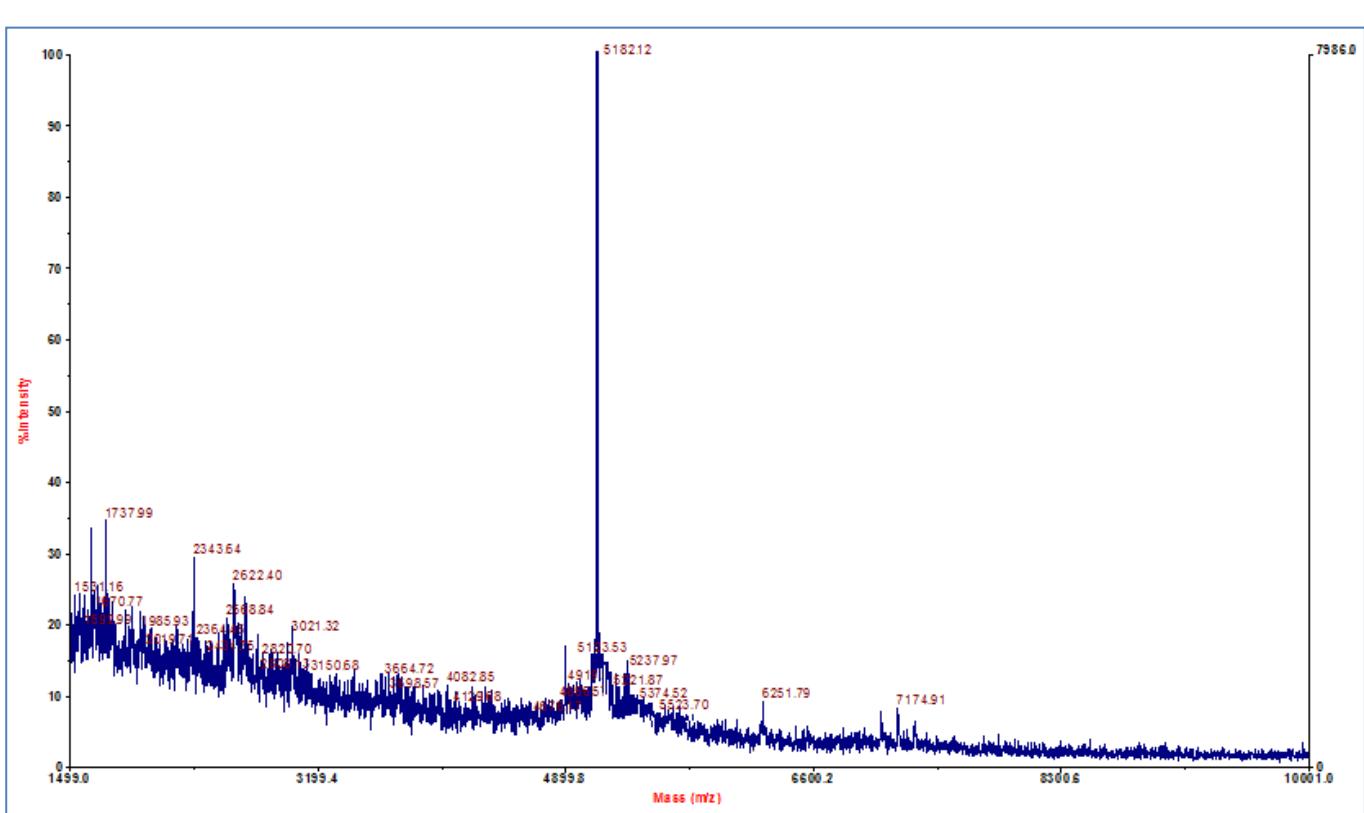
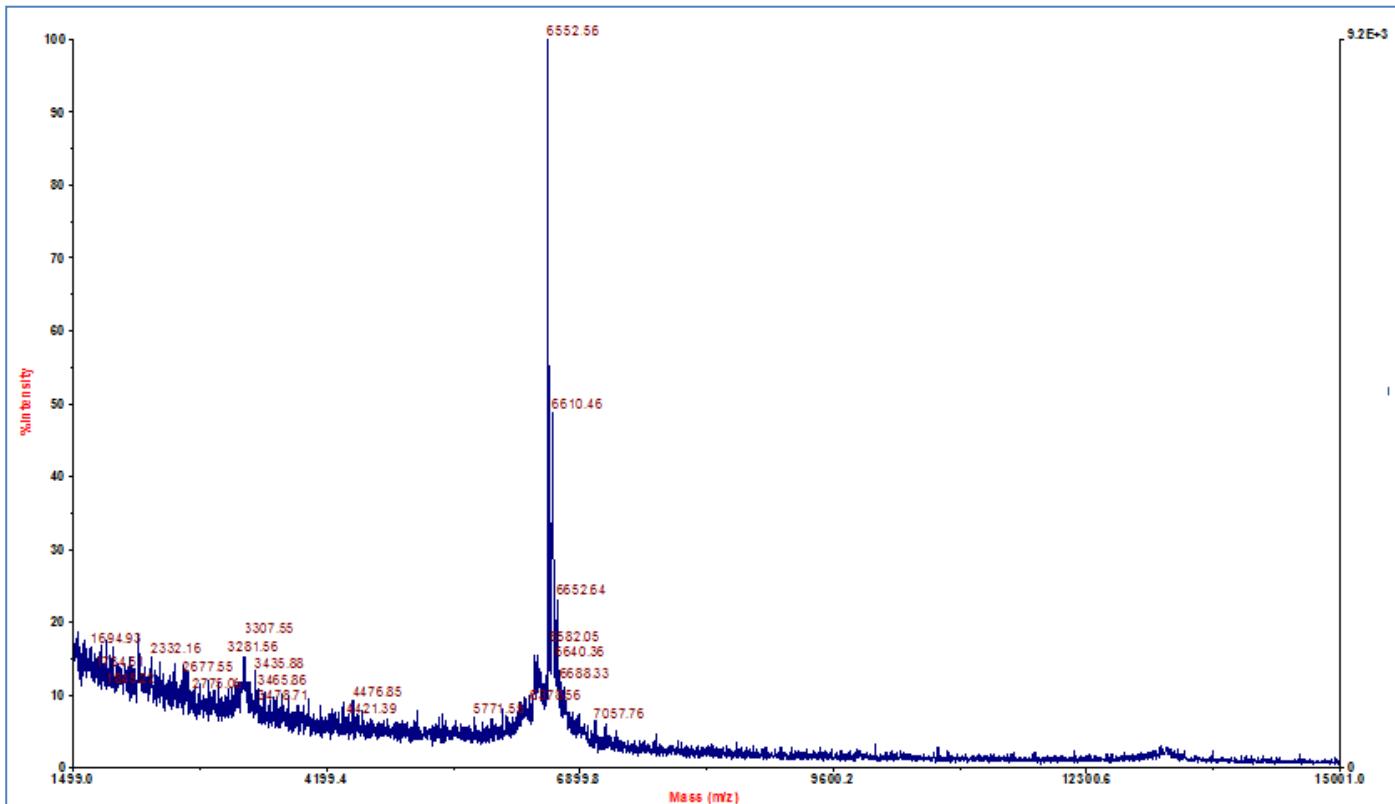
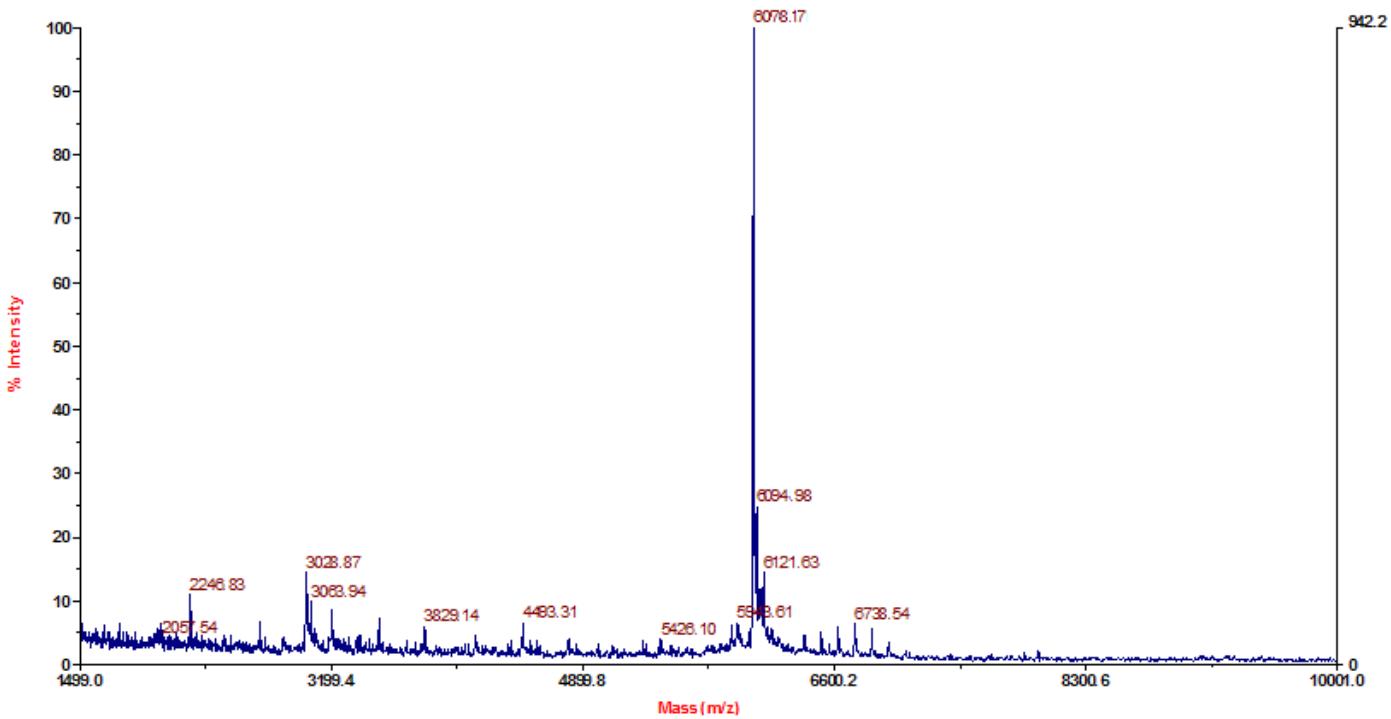


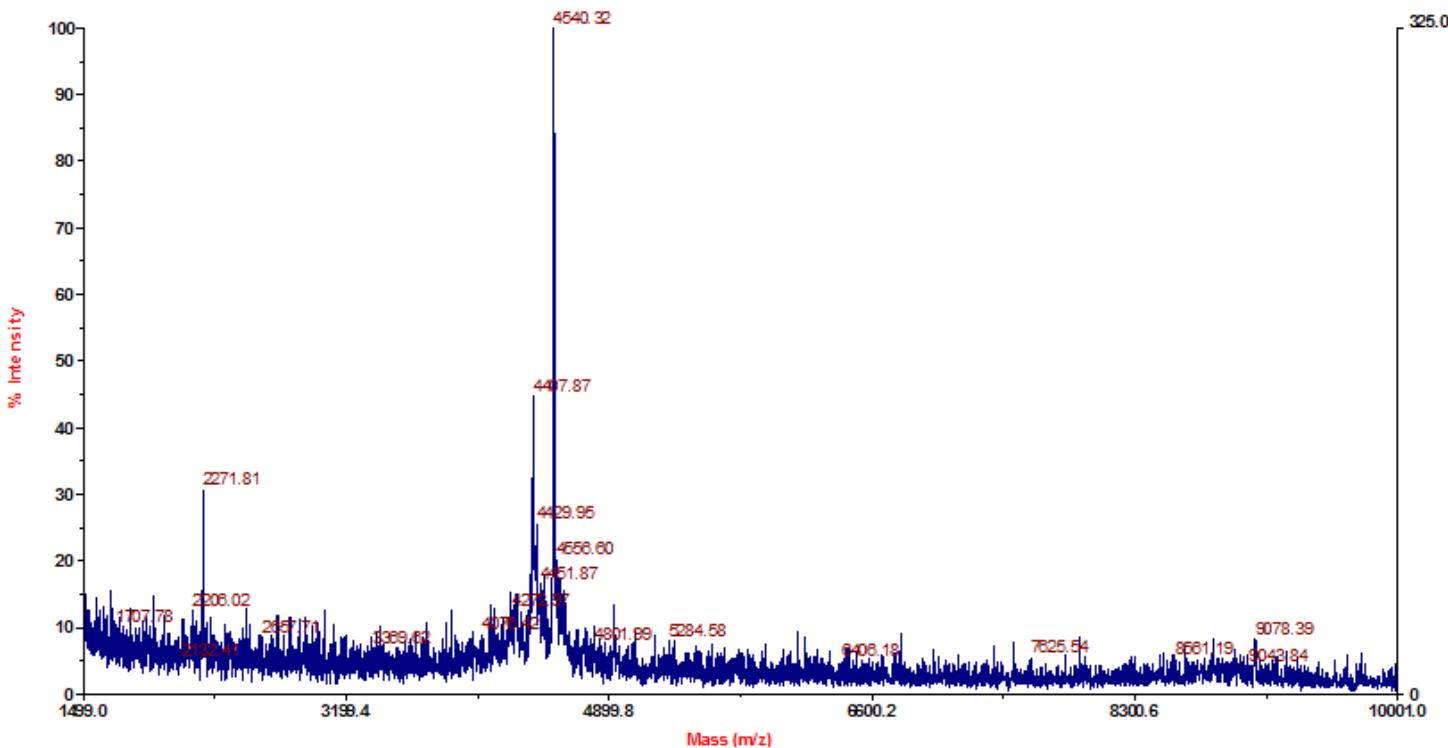
Figure S3. Thermal melting curves of double stranded probes (1 μ M, in PBS buffer)

6. MALDI-TOF mass spectra of labeled oligonucleotides

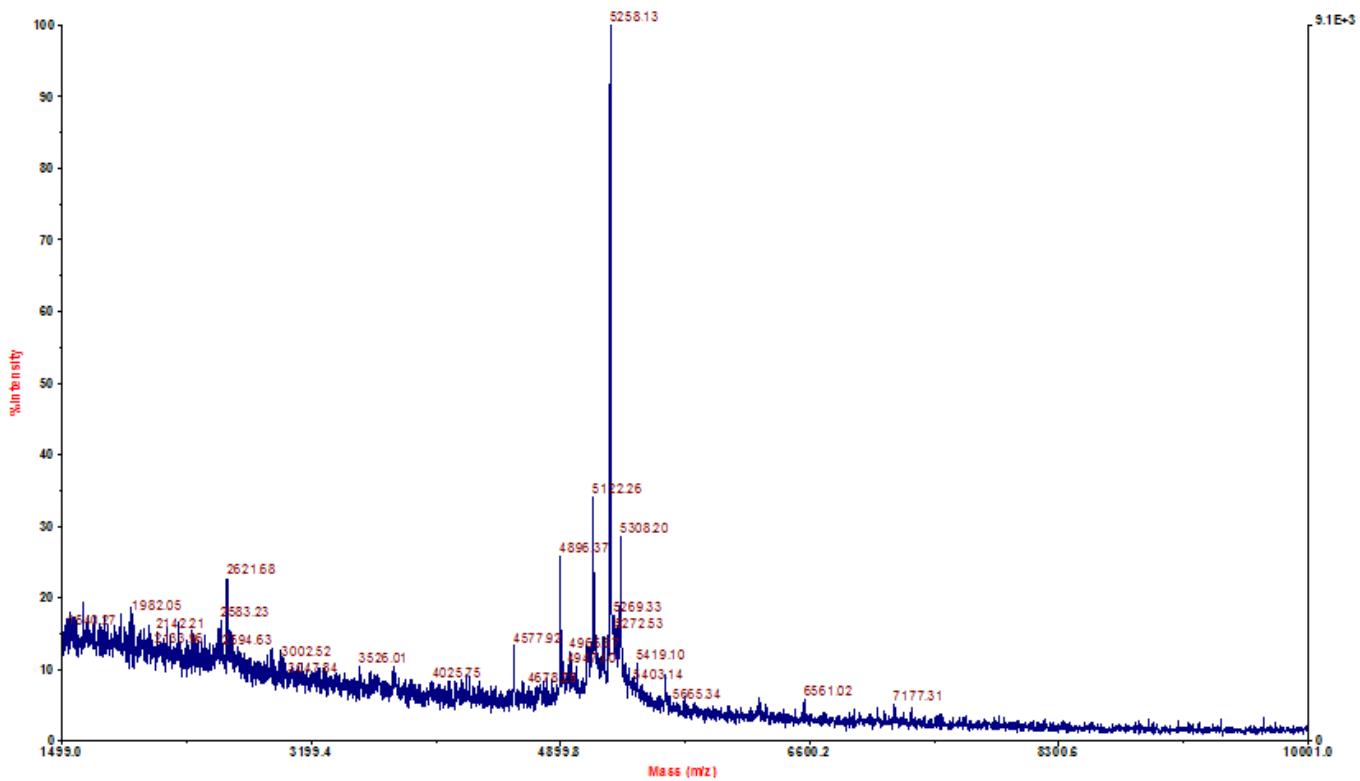




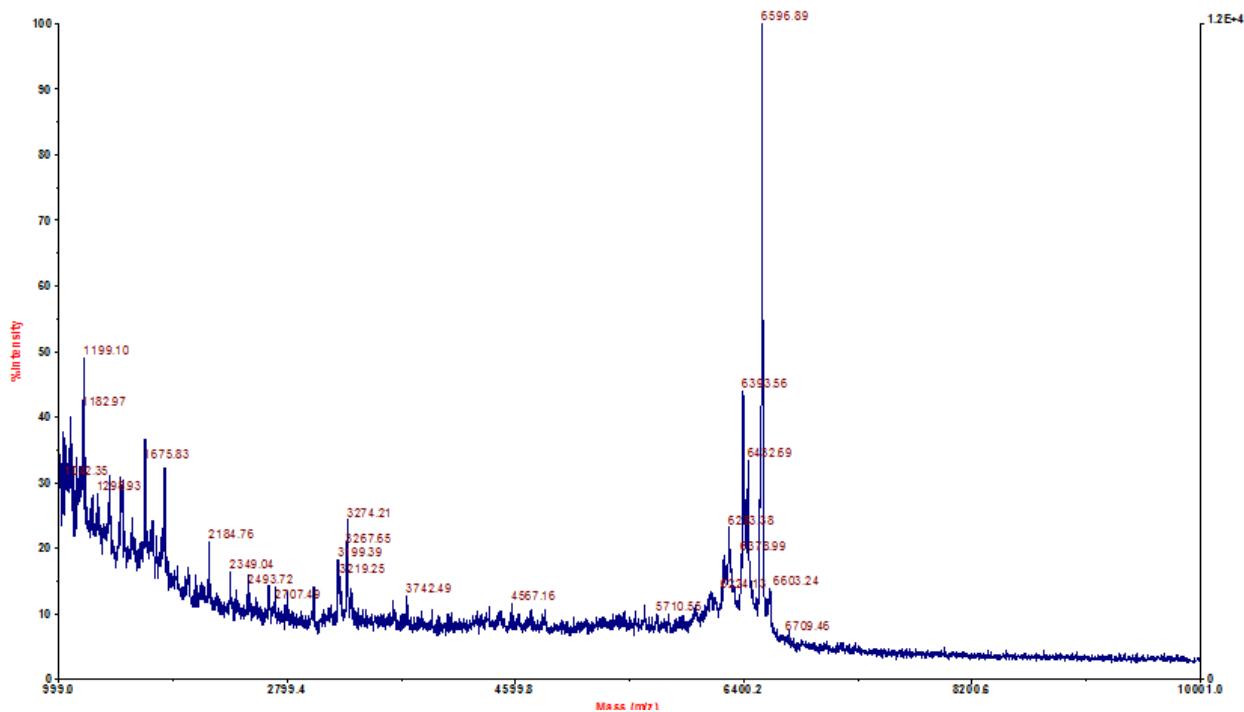
ON3 calcd *m/z* for C₂₀₀H₂₂₃N₆₈O₁₁₈P₁₉Na 6075.84, found 6078.17



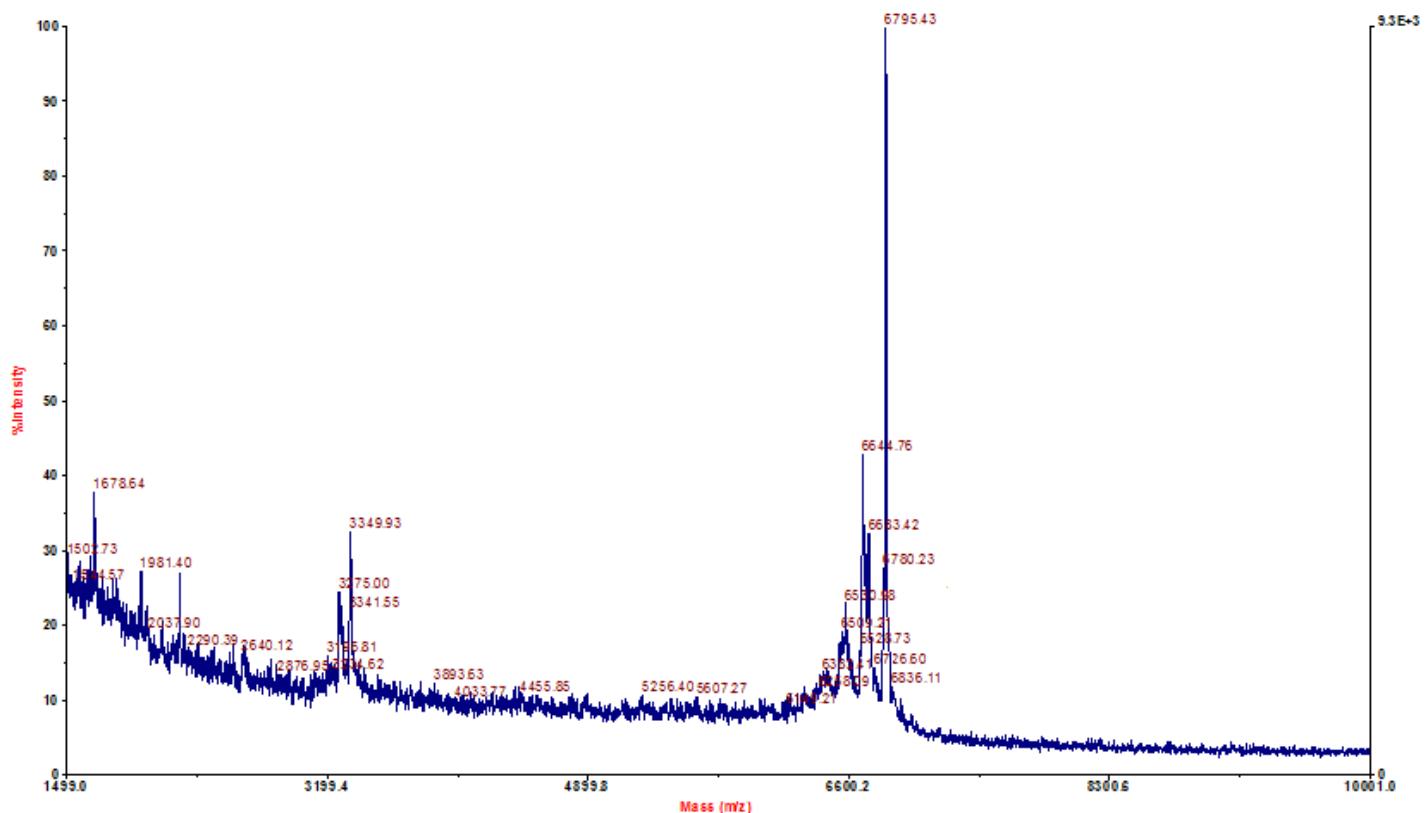
ON4 calcd *m/z* for C₁₄₈H₁₇₃N₅₄O₈₆P₁₄Na 4538.70, found 4540.32



ON5 calcd m/z for $C_{184}H_{203}N_{62}O_{86}P_{15}Na$ 5255.90, found 5258.13



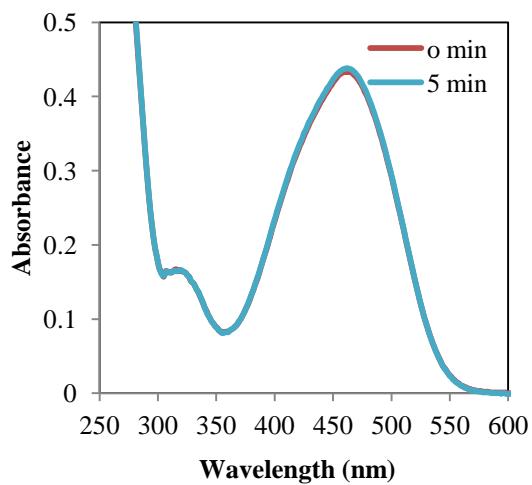
ON6 calcd m/z for $C_{223}H_{255}N_{73}O_{125}P_{20}Na$ 6597.05, found 6596.89



ON7 calcd m/z for $C_{236}H_{258}N_{76}O_{125}P_{20}Na$ 6798.08, found 6795.43.

7. Absorption and Photoisomerisation of ON5

A



B

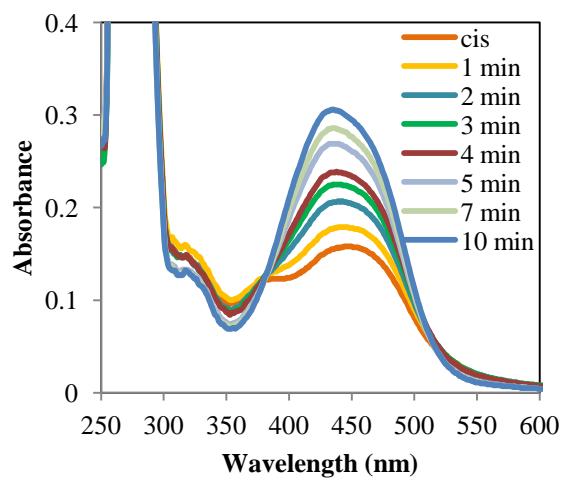


Figure S4 (A) Absorption spectra of 10 μM **ON5** in water, before irradiation (blue line), after irradiation (red line). (B) Absorption spectra of 10 μM **ON5** in DMSO, undergoing thermal relaxation as a function of time (before irradiation - blue line, after irradiation - orange line).

8. Fluorescence emission spectra of FAM labeled ONs and the corresponding double stranded probes

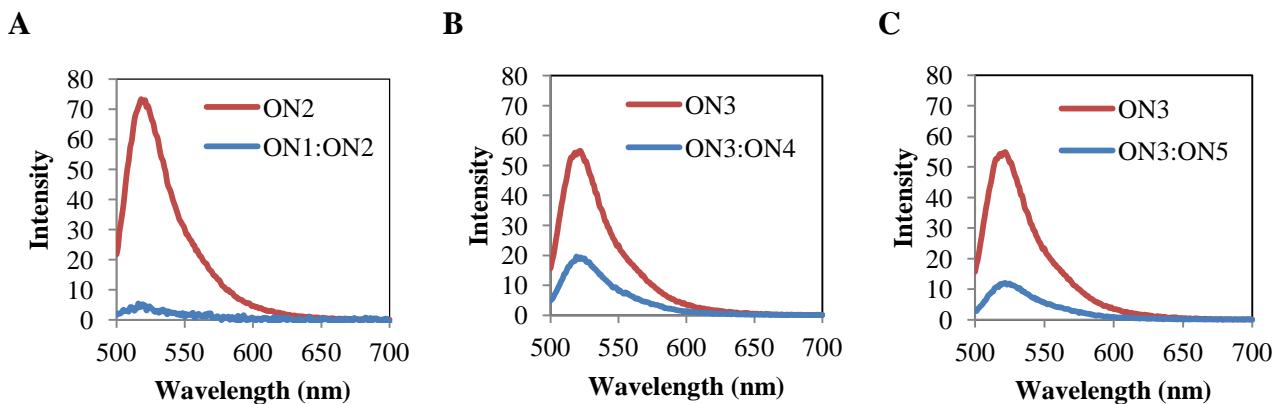


Figure S5. Fluorescence emission of FAM labeled ONs and the corresponding double stranded probes (1 μ M in PBS buffer at room temperature, λ_{ex} 495 nm). (A) **ON1:ON2** probe (**ON1-2'-OMe** labeled with dU^{DAB} at the 5'-end, and **ON2** labeled with FAM at the 3'-end). (B) **ON3:ON4** probe (**ON3** labeled with FAM at 5'-end and **ON4** labeled with dU^{DAB} at the 3'-end). (C) **ON3:ON5** probe (**ON5** labeled with three dU^{DAB}, the short strand).

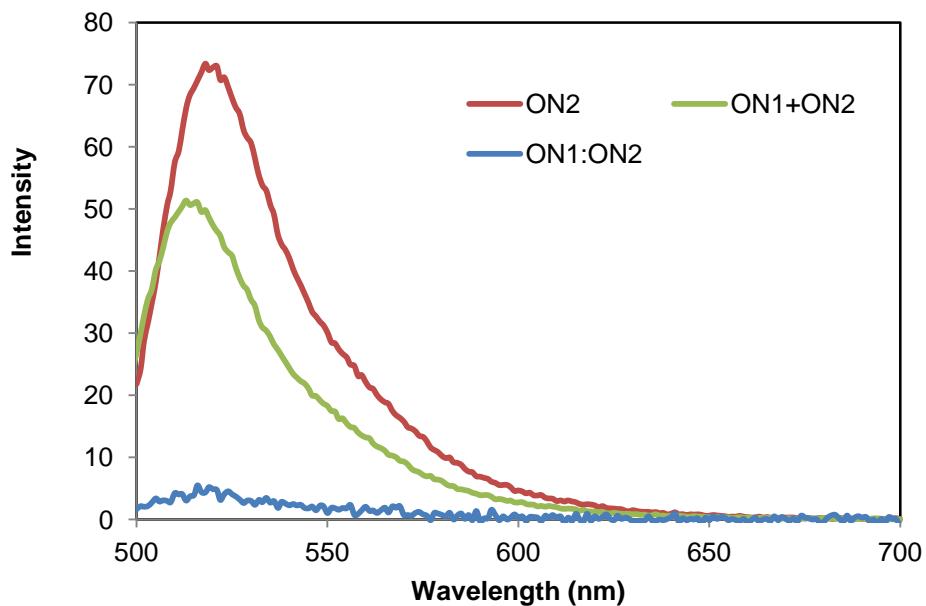


Figure S6. Fluorescence emission of FAM labeled **ON1:ON2** probe and the corresponding double stranded probes (1 μ M in PBS buffer at room temperature, λ_{ex} 495 nm). (**ON1-2'-OMe** labeled with dU^{DAB} at the 5'-end, and **ON2** labeled with FAM at the 3'-end). Red – **ON2** alone, green - **ON2** before the formation of the duplex (**ON1+ON2**), blue –duplex (**ON1:ON2**).

9. Emission spectra of modified TaqMan probes ON6, ON7

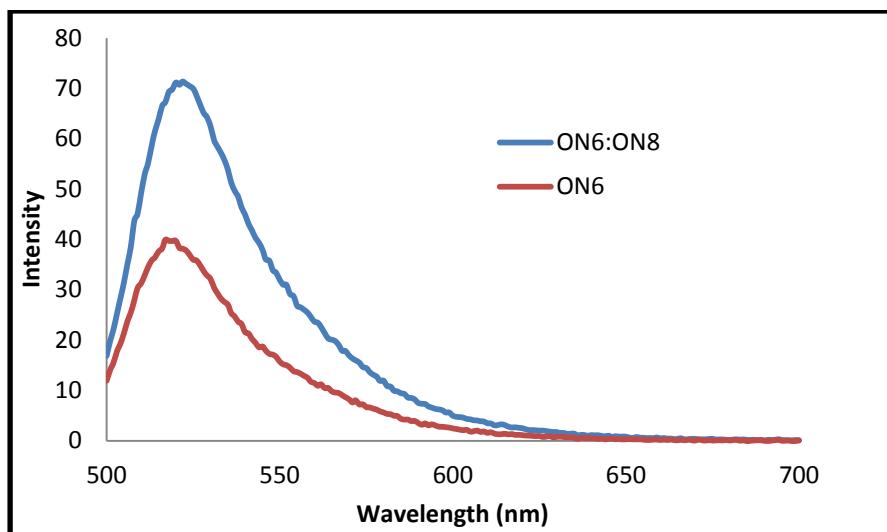


Figure S7. Emission spectra of modified TaqMan probe **ON6** alone and after hybridization with the target DNA **ON8** (1 μ M) measured in PBS buffer (pH 7.4, 25 °C), $\lambda_{\text{ex}} = 495$ nm.

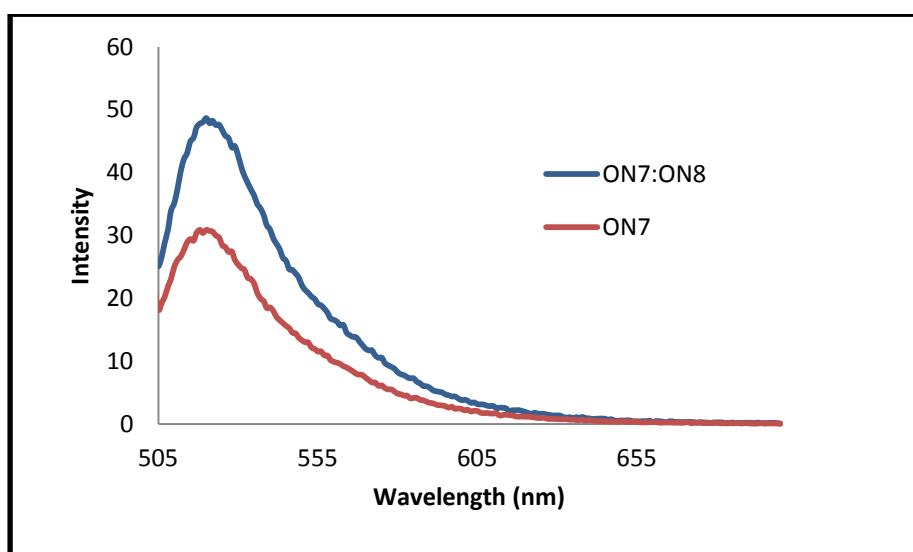


Figure S8. Emission spectra of modified TaqMan probe **ON7** alone and after hybridization with the target DNA **ON8** (1 μ M) measured in PBS buffer (pH 7.4, 25 °C), $\lambda_{\text{ex}} = 495$ nm.

10. Amplification curves of double stranded probes

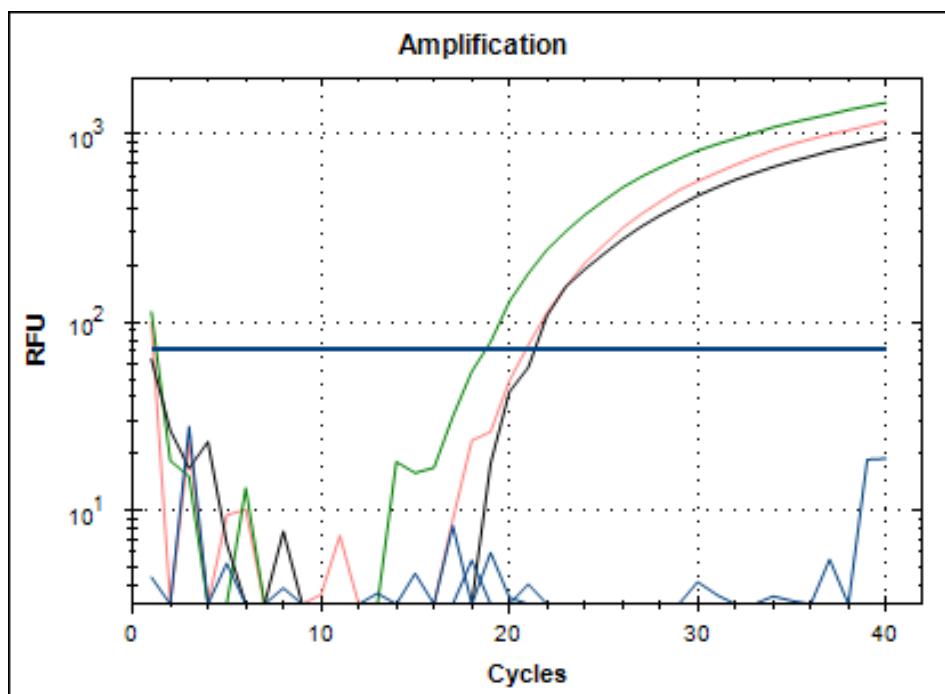


Figure S9. Comparison of ON3:ON4 (black line) and ON3:ON5 (pink line) double stranded probe labeled with dU^{DAB} quenchers in PCR assay to that of the standard TaqMan probe labeled with DABCYL,ON11 (green line).

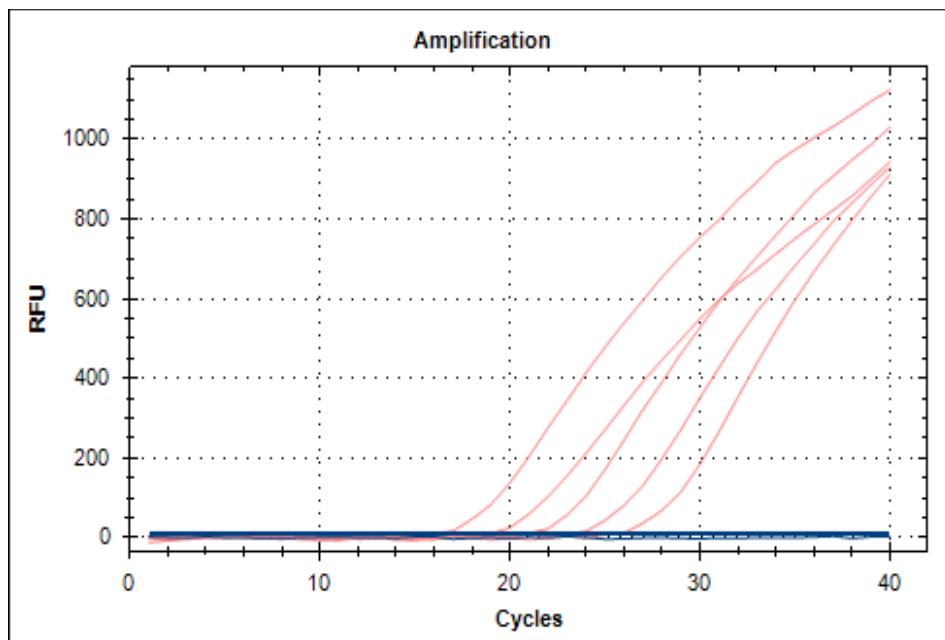


Figure S10. ON3:ON5 double stranded probe in qPCR analysis of cDNA (20 ng, 4 ng, 0.8 ng, 0.16 ng and 0.032 ng, pink lines) obtained from human Hep G2 liver cells; no template control (blue line).

11. Amplification curves of TaqMan probes

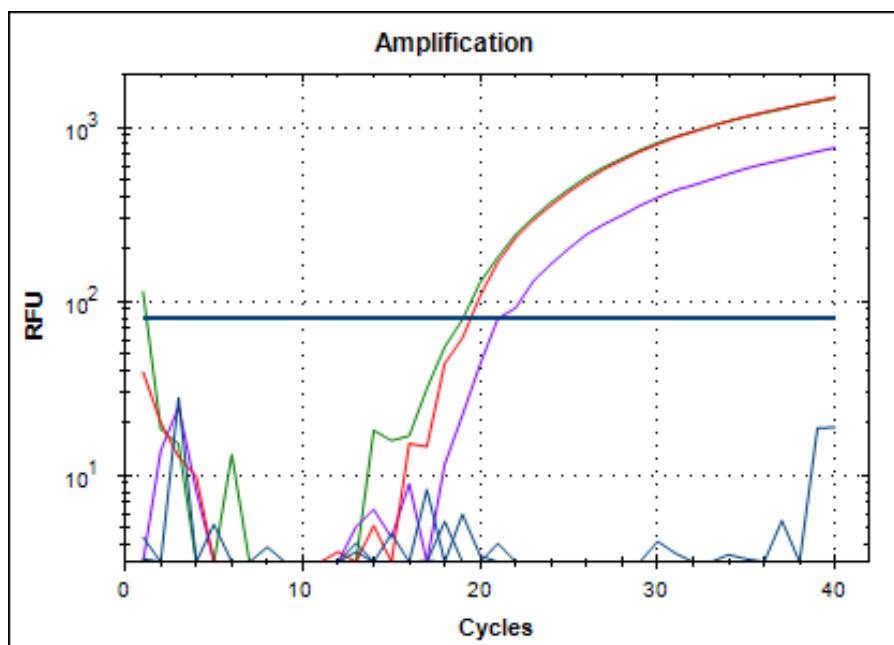


Figure S11. Comparison of **ON6** (red line) and **ON7** (purple line) TaqMan probes labeled with dU^{DAB} quenchers in PCR assay to that of the standard TaqMan probe labeled with DABCYL, **ON11** (green line).

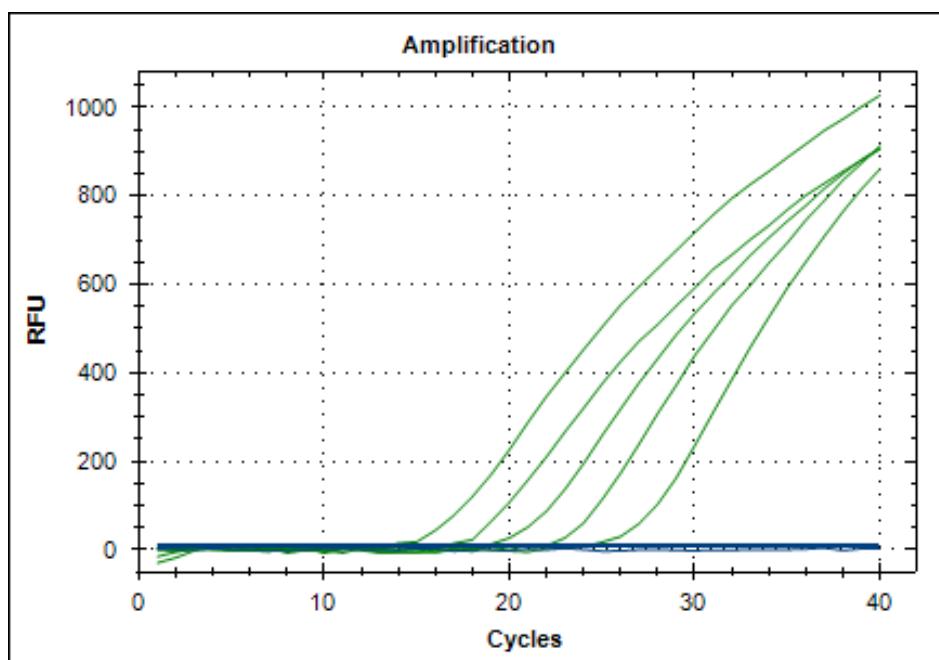


Figure S12. C. **ON11** in qPCR analysis of cDNA (20 ng, 4 ng, 0.8 ng, 0.16 ng and 0.032 ng, pink lines) obtained from human Hep G2 liver cells; no template control (blue line).

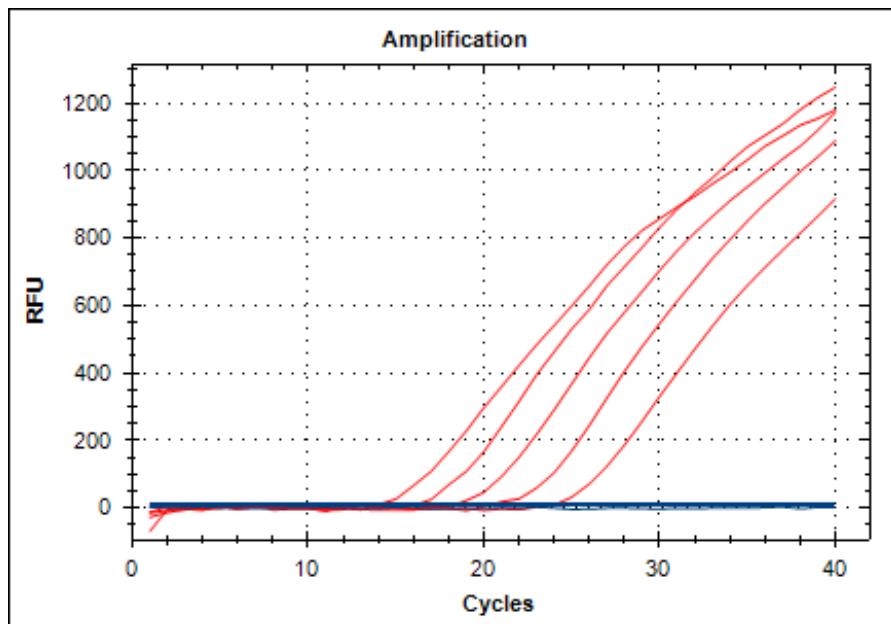


Figure S13. ON6 probe in qPCR analysis of cDNA (20 ng, 4 ng, 0.8 ng, 0.16 ng and 0.032 ng, pink lines) obtained from human Hep G2 liver cells; no template control (blue line).

12. ^1H -NMR spectra of *trans* and *cys* isomers during photoimerisation of dU^{DAB} by irradiation in Acetone- d_6 and DMSO- d_6 (600 MHz).

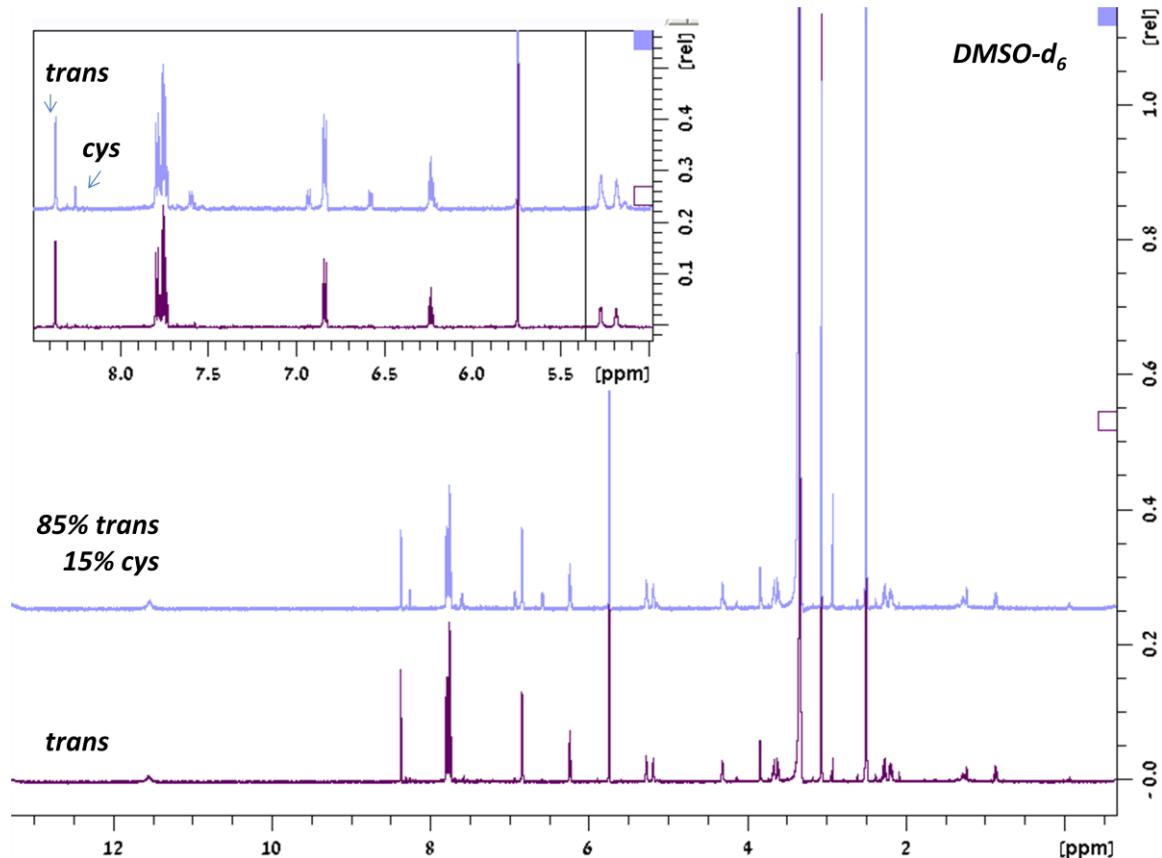


Figure S14. ^1H -NMR spectra 85% *trans* and 15% *cys* during photoimerisation of dU^{DAB} by irradiation in 365 nm in $\text{DMSO}-d_6$ (600 MHz).

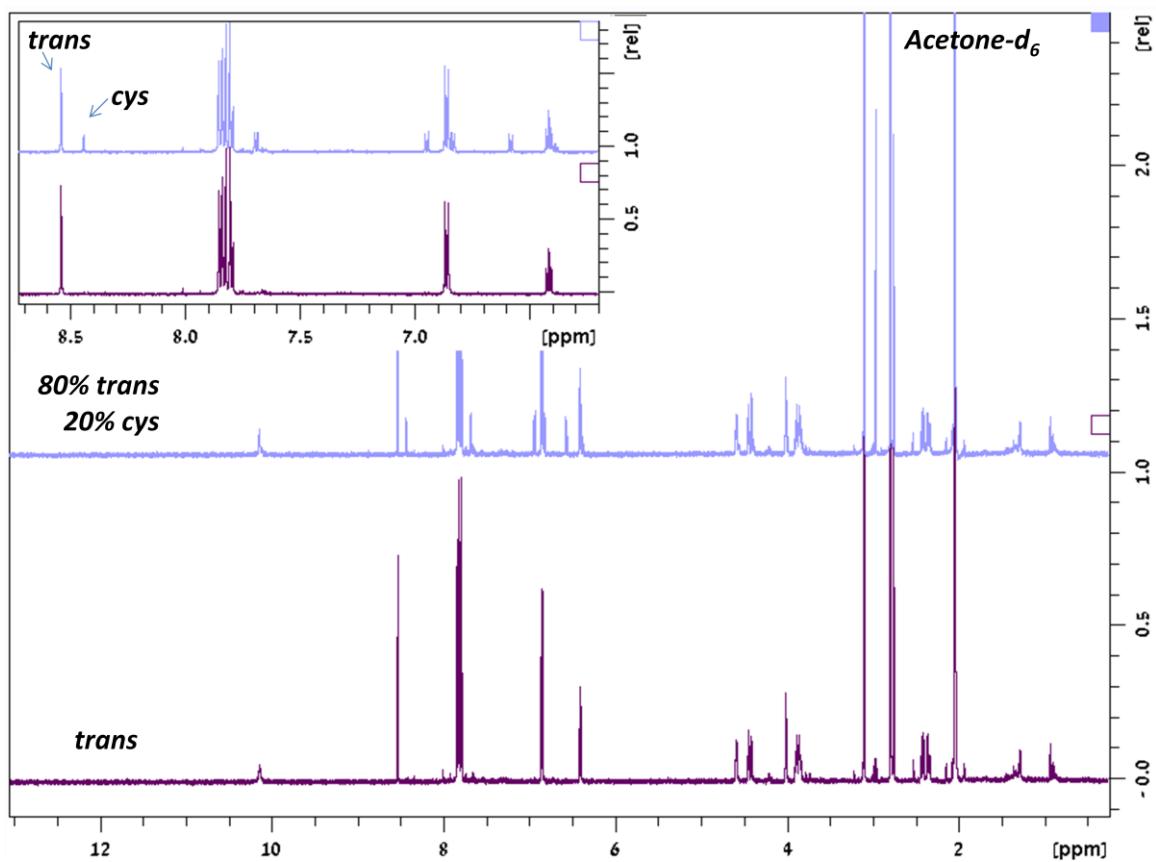


Figure S15. ¹H-NMR spectra 80% *trans* and 20% *cys* during photoimerisation of dU^{DAB} by irradiation in 365 nm in Acetone-*d*₆ (600 MHz).

13. NMR spectra of compound 1, 5, 7 and 8

