SUPPORTING INFORMATION

Monitoring Eukaryotic and Bacterial UDG Repair Activity with DNA-Multifluorophore Sensors

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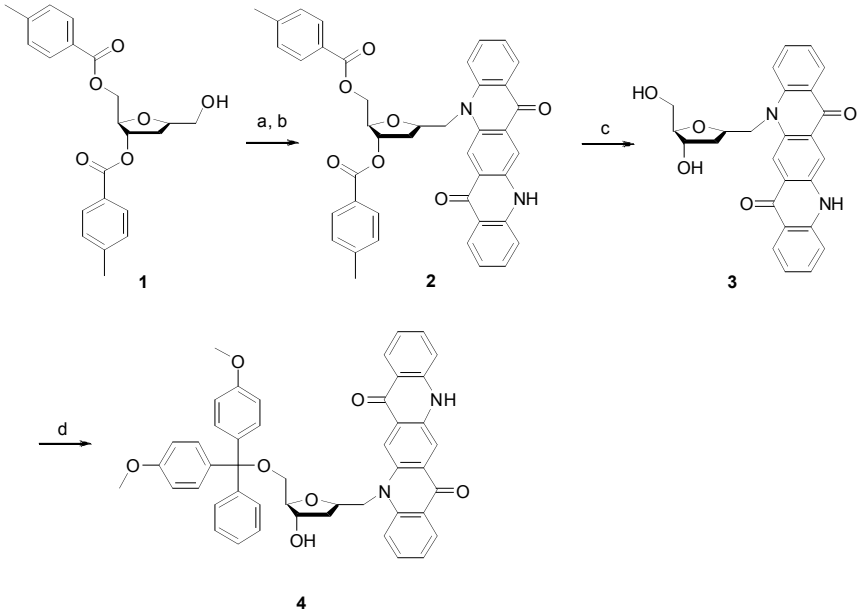


Reagents and conditions: (a) Tf_2O, poly(4-vinylpyridine), CH_2Cl_2, 0°C; (b) Quinacridone, NaH, DMA, 0°C; (c) CH_3ONa/CH_3OH, CH_2Cl_2, r.t.; (d) DMT-Cl, DIPEA, CH_2Cl_2, r.t.

3', 5'-Di-(p-toluoyl)-1'-β-Quinacridonylmethyl-2'-deoxyriboside (2)

3', 5'-Di-(p-toluoyl)-1'-β-hydroxymethyl-2'-deoxyriboside (**1**) was prepared by following the reported procedure[1]. ^{1}H-NMR ($CDCl_3$, 400 MHz) δ = 7.95-7.91 (m, 4H), 7.25-7.21 (m, 4H), 5.47 (d, 1H, *J* = 6.0 Hz), 4.60 (q, 1H, *J* = 5.6 Hz), 4.46-4.38 (m, 3H), 3.90 (dd, 1H, *J* = 2.8, 12 Hz), 3.57 (dd, 1H, *J* = 3.2, 12 Hz), 2.41 (s, 3H), 2.39 (s, 3H), 2.38-2.33 (m, 1H), 2.15-2.09 (dd, 1H, *J* = 5.6, 13.6 Hz). ^{13}C-NMR ($CDCl_3$, 100 MHz) δ = 166.7, 166.1, 144.1, 144.0, 129.7, 129.6, 129.2, 126.9, 126.8, 83.1, 80.0, 64.7, 63.0, 33.2, 21.7, 21.6.

Poly(4-vinylpyridine) (0.76 g, 7.18 mmol) was suspended in anhydrous CH_2Cl_2 (30 mL) and trifluoromethanesulfonic anhydride (0.62 mL, 3.64 mmol) was added via syringe at 0 °C. The reaction mixture was stirred at 0 °C for 30 min, followed by addition of compound **1** (1.4 g, 3.64 mmol) in CH_2Cl_2 (5 mL) dropwise. The reaction was further stirred at 0 °C for 2 h. After filtering off the polymer, the product in DCM was concentrated and subjected to a short flash column chromatography (ethyl acetate/hexanes 1:3). The solvent was evaporated *in vacuo* and the product was used directly for the next step without further characterization.

Quinacridone (726 mg, 2.32 mmol) was suspended in anhydrous dimethylacetamide (20 mL) and NaH (94 mg, 2.32 mmol, 60% dispersion in mineral oil) was added in one portion under an atmosphere of argon at 0 °C. Then reaction was stirred at r.t. for 30 min. The triflate intermediate (1.2 g, 2.32 mmol) obtained from the previous step was dissolved in 2 mL anhydrous CH_2Cl_2 and added to the reaction dropwise via syringe. The reaction was stirred overnight under an atmosphere of argon at r.t. and the solvent was evaporated *in vacuo*. The crude product was purified by flash column chromatography (ethyl acetate/hexanes 1:1) to give compound **2** as a red solid (220 mg, 14%). ^{1}H-NMR (CDCl3, 500 MHz) δ = 11.79 (s, 1H), 8.85 (s, 1H), 8.42 (s, 1H) 8.32 (d, 1H, *J* = 10 Hz), 8.15 (d, 1H, *J* = 10 Hz),7.91-7.87 (m, 3H), 7.73-7.69 (m, 2H), 7.47-7.43 (m, 3H), 7.32 (d, 1H, *J* = 10 Hz), 7.26 (t, 1H, *J* = 9.5 Hz), 7.18 (t, 1H, *J* = 9.5 Hz), 7.02 (d, 1H, *J* = 10 Hz) 5.46 (d, 1H, *J* = 7.5 Hz); 4.97-4.84 (m, 3H), 4.42-4.28 (m, 4H), 2.36 (s, 3H), 2.29-2.26 (m, 5H); ^{13}C-NMR (DMSO-d_6, 125 MHz) δ = 176.9, 176.7, 165.4, 165.2, 143.9, 143.3, 141.3, 136.0, 134.5, 134.2, 133.7, 129.4, 129.3, 128.9, 128.8, 126.7, 126.3,

126.2, 125.5, 124.3, 120.6, 120.3, 120.0, 119.0, 117.0, 116.5, 114.0, 113.9, 82.0, 77.9, 76.8, 64.6, 48.9, 35.6, 21.2. HRMS (ESI, m/z): $[M+H]^+$ calculated for $C_{42}H_{35}N_2O_7$, 679.2444, found 679.2430.

1'-β- Quinacridonylmethyl-2'-deoxyriboside (3)

To **2** (204 mg, 0.3 mmol) dissolved in CH_2Cl_2 (15 mL) was added $NaOCH_3$ (0.5 M in MeOH, 2 mL) dropwise at r.t.. The reaction mixture was stirred at r.t. for 1 h and the completion was monitored by TLC. The solvent was evaporated under vacuum and the crude product was purified by flash column chromatography ($MeOH/CH_2Cl_2$ 1: 10) to give compound 3 as a red solid (126 mg, 95%). ^{1}H-NMR ($CDCl_3$, 500 MHz) δ = 11.93 (s, 1H), 8.78 (s, 1H), 8.56 (s, 1H), 8.35 (dd, 1H, *J* = 2, 8 Hz), 8.25 (dd, 1H, *J* = 1.5, 8.5 Hz), 7.93-7.91 (m, 1H), 7.81 (1H, td, *J* = 1.5, 8.0 Hz), 7.75 (1H, td, *J* = 1.5, 8.5 Hz), 7.52 (d, 1H, *J* = 8.5 Hz), 7.30 (t, 1H, *J* = 8.0 Hz), 7.24 (td, 1H, *J* = 1.5, 8.0 Hz), 5.02 (d, 1H, *J* = 4.0 Hz), 4.81 (t, 1H, *J* = 5.5 Hz), 4.75 (d, 2H, *J* = 5.0 Hz), 4.68-4.63 (m, 1H), 4.24-4.22 (m, 1H), 3.68-3.66 (m, 1H), 3.48-3.36 (m, 2H), 2.10-1.97 (m, 2H). ^{13}C-NMR (DMSO-d_6, 125 MHz) δ = 177.1, 176.6, 142.5, 141.4, 135.8, 134.4, 134.3, 133.9, 126.6, 126.2, 125.5, 124.1, 120.6, 120.5, 119.9, 118.9, 117.2, 116.5, 114.2, 88.1, 76.5, 72.3, 62.4, 50.2, 38.3. HRMS (ESI, m/z): $[M+H]^+$ calculated for $C_{26}H_{23}N_2O_5$, 443.1607, found 443.1603.

5'-(4, 4'-Dimethoxytrityl)-1'-β-quinacridonylmethyl-2'-deoxyriboside (4)

To **3** (120 mg, 0.27 mmol) dissolved in anhydrous pyridine (10 mL) was added DIPEA (0.28 mL, 1.6 mmol), followed by 4, 4'-dimethoxytrityl chloride (390 mg, 1.1 mmol) under an atmosphere of argon. The reaction was stirred at r.t. for 2 h and the completion was monitored by TLC. Then methanol (10 mL) was added to quench the excess 4,4'-dimethoxytrityl chloride for 20 minutes. After evaporation of the solvents, the product was purified by flash column chromatography (ethyl acetate/hexanes 3:1 to pure ethyl acetate) to give the product as a red solid (168 mg, 85%). ^{1}H-NMR ($CDCl_3$, 500 MHz) δ = 11.7 (s, 1H), 8.55 (s, 1H), 8.38 (s, 1H), 8.26 (dd, 1H, *J* = 1.5, 7.5 Hz), 8.15 (d, 1H, *J* = 7.5 Hz), 7.76 (d, 1H, *J* = 9.0 Hz), 7.61-7.55 (m, 2H), 7.38 (d, 1H, *J* = 8.5 Hz), 7.16-7.06 (m, 7H), 7.03-7.00 (m, 4H), 6.70-6.67 (m, 4H), 5.10 (s, 1H), 4.78-4.75 (m, 1H), 4.67-4.64 (m, 2H), 4.02 (s, 1H), 3.75-3.73 (m, 1H), 3.64 (s, 3H), 3.63 (s, 3H), 2.89 (s, 1H), 2.02-2.00 (m, 1H), 1.79-1.76 (m, 1H). ^{13}C-NMR (DMSO-d_6, 125 MHz) δ = 176.8, 176.7, 157.8, 144.7, 142.8, 141.4, 135.8, 135.5, 135.4, 134.5, 134.0, 133.7, 129.6, 129.5, 127.6, 127.5, 126.6, 126.4, 126.1, 125.4, 124.2, 120.5, 120.4, 119.9, 119.0, 117.1, 116.6, 114.2, 113.6, 112.9, 85.7, 85.2, 76.7, 72.2, 63.6, 54.8, 49.0, 38.3, 33.3. HRMS (ESI, m/z): $[M+Na]^+$ calculated for $C_{47}H_{40}N_2O_7Na$, 767.2733, found 767.2715. The product was further characterized by 2D ROESY experiment as the β-anomer.

Phosphoramidite derivative of 4. Prior to the synthesis of ODF, the prepared Q-DMT were transformed to 3'-O-phosphoramidite derivatives by a standard method. Briefly, Q-DMT compound (1 eq.) was dissolved in anhydrous DCM under an atmosphere of argon, to which DIPEA (6 eq.) and 2-cyanoethyl N,N-diisopropylchlorophosphoramidite (2 eq.) were added sequentially. The reaction mixture was stirred at room temperature for 2 h and then concentrated *in vacuo*. The residue was purified by flash column chromatography (ethyl acetate : hexanes = 3:1 with 5% triethylamine). ^{31}P NMR (100 MHz, $CDCl_3$) δ = 149.14, 149.05.

Table S1. Mass spectrometry data for quenched probes and controls.

Compound	Probe sequence	Calc'd Mass	Found
Probe **1**	5'- A A U Y U A A	2231.5	2231.6
Probe **2**	5'- A A U Y Y U A A	2611.8	2611.3
Probe **3**	5'- A A U Y Y U A Q	2803.1	2802.4
Probe **4**	5'- A_o A U Y Y U A_o A[a]	2671.9	2670.1
Probe **5**	5'- A A T Y T A A	2259.6	2258.9
Probe **6**	5'- A A T Y Y T A A	2639.9	2639.4
Probe **7**	5'- A_o A T Y Y T A_o A	2618.7	2618.5
Reference **8**	5'- Y S[b]	578.4	578.7
Reference **9**	5'- Y Y S	958.8	959.8

[a] Monomer "A_o" is 2'-O-methyladenosine, incorporated to increase nuclease resistance.
[b] Monomer "S" is a tetrahydrofuran (abasic) spacer, added to enhance solubility.

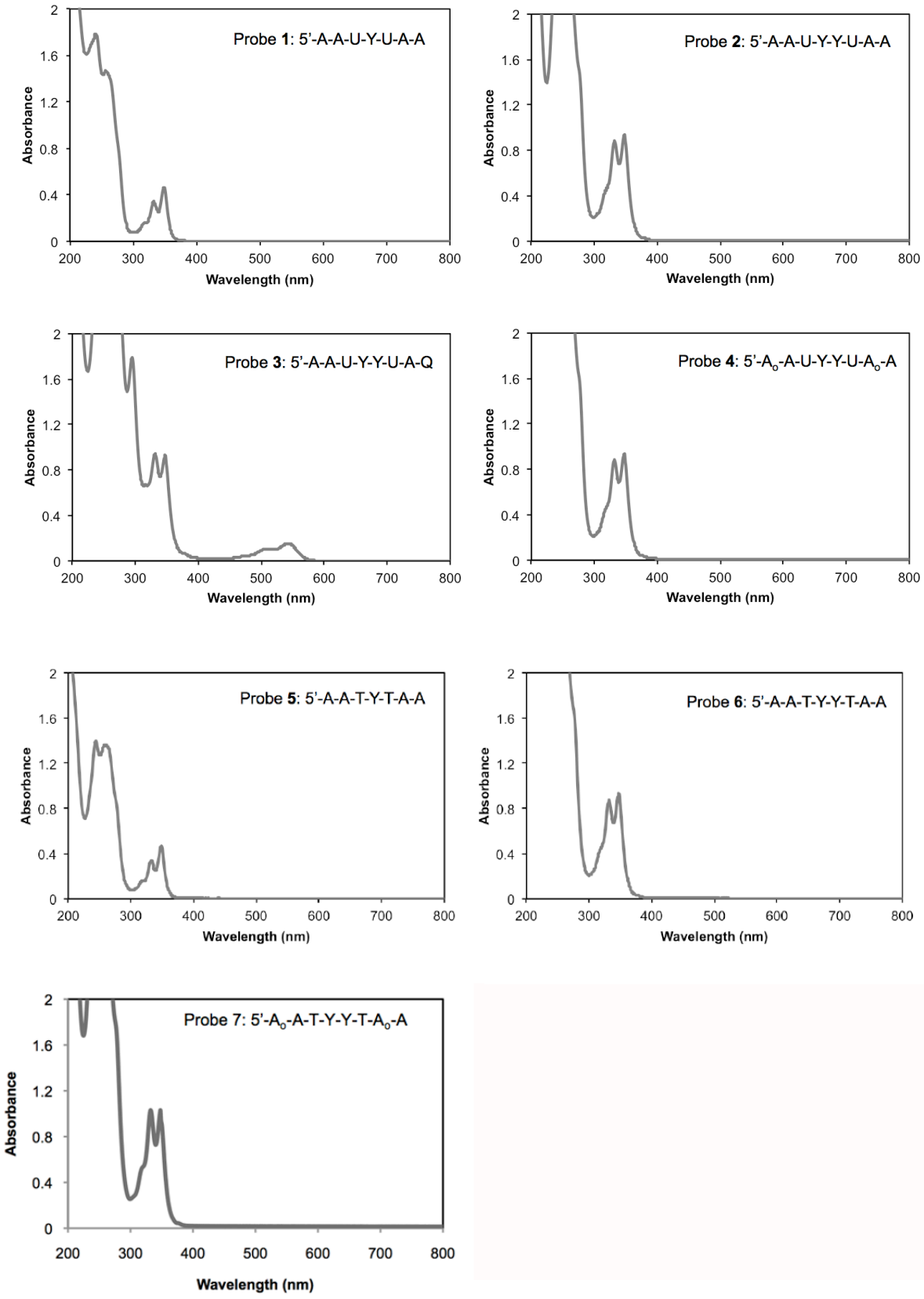


Figure S1. Absorption spectra of probes. [probe] = 10 μM, in water

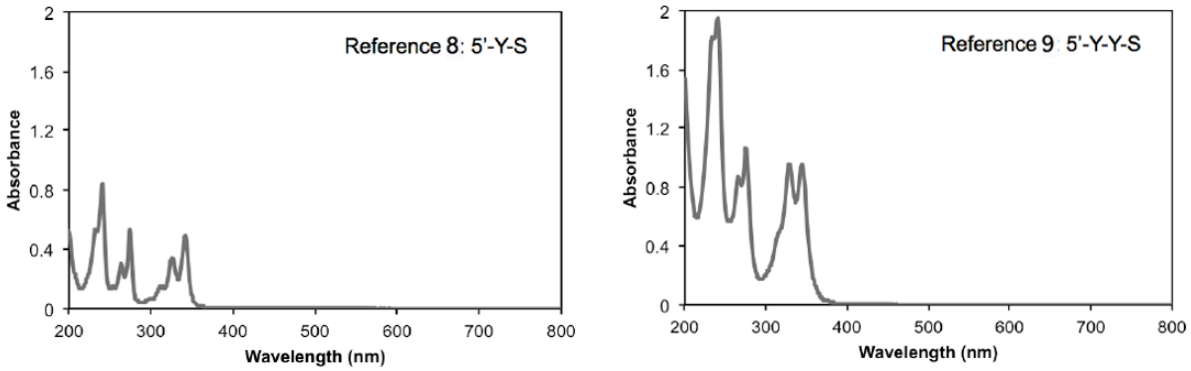


Figure S1 (contd). Absorption spectra of probes. [probe] = 10 μM, in water

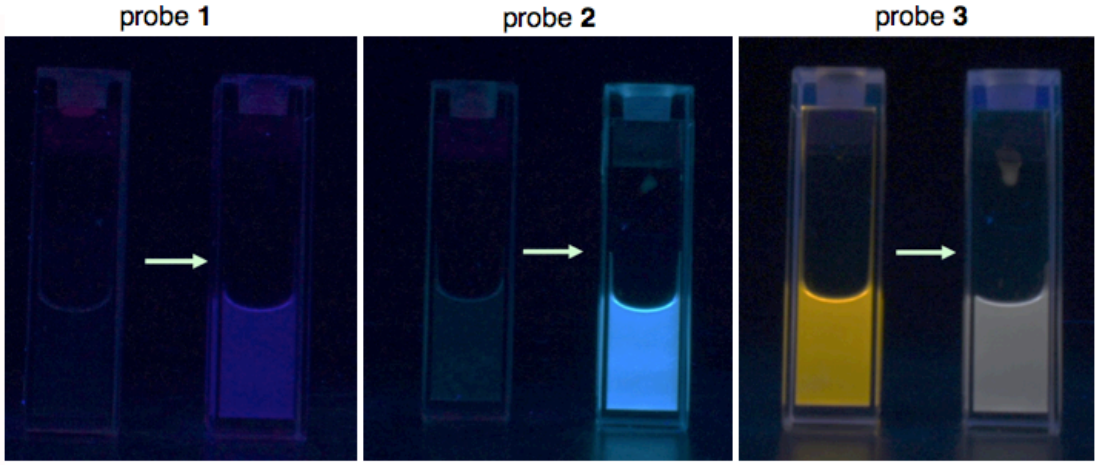


Figure S2: Visible changes in probe fluorescence, by comparison of probe solutions before (left) and after (right) incubation with bacterial uracil deoxyglycosylase. Probe **1** yields a signal in the UV and barely detectable in the visible spectrum; probe **2** yields a cyan signal (480 nm); and probe **3** yields a ratiometric color change from 540 nm to 480+540 nm. *E. coli* UDG 50U/mL (4 nM), probe 4 μM in 1x UDG buffer, 25 °C, 1 h. Photos taken with UV lamp excitation (365 nm).

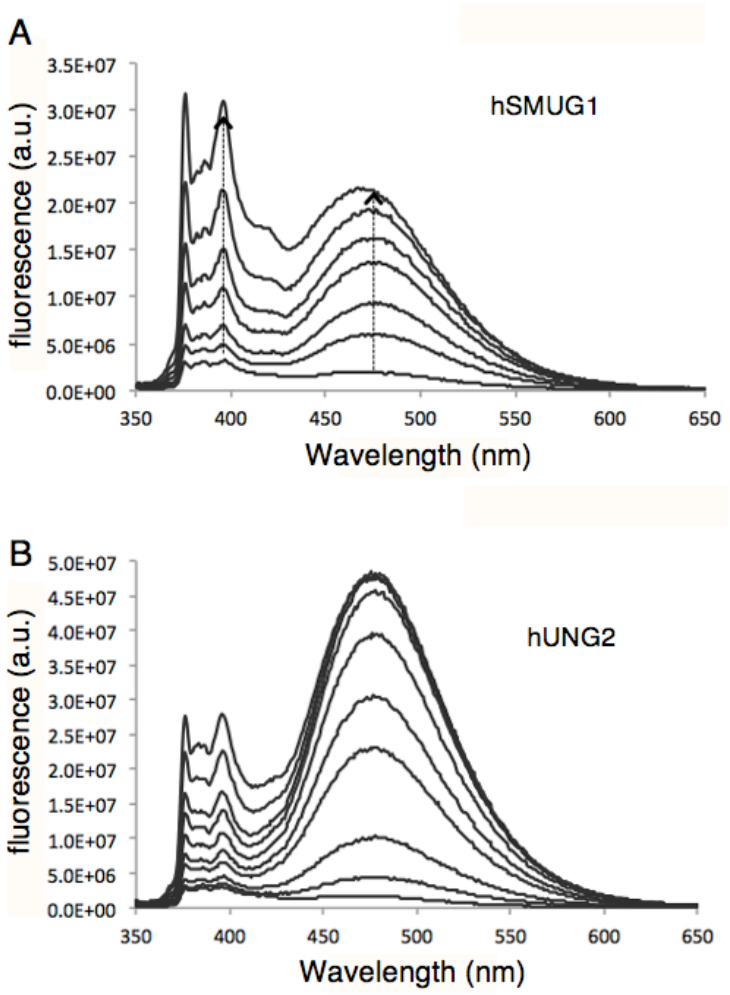


Figure S3: Spectral responses of probe **4** ($A_oAUYYUA_oA$), containing 2'-O-methyl groups for nuclease resistance, to human enzymes SMUG1 and UNG2. See main text, Figures 3,4 for responses without 2'-O-methyl groups. Excitation 340 nm. Conditions: [hSMUG1] = 500 U/mL (400 nM), [UNG2] = 9.7 μg/mL (90 nM) in 1x NEBuffer 1, 37 °C.

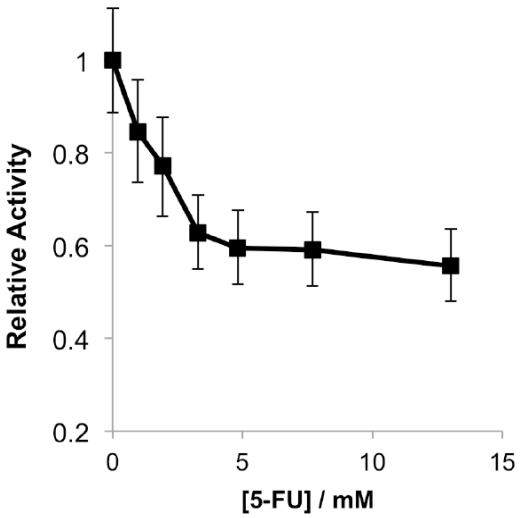


Figure S4. Inhibition of UNG2 by 5-fluorouracil, as monitored by probe **2**. Shown is a plot of relative initial velocities of probe **2** signal with various 5-FU concentrations. Conditions: [probe] = 400 nM, 37 °C, excitation 340 nm, emission 480 nm. Error bars reflect ±15% uncertainty of line slope determinations at each concentration. Initial rates were measured by linear line fit over first 10-30% of reaction. Reactions at each concentration point were carried out as described in Materials and Methods, with the addition of 5FU at the concentrations shown.

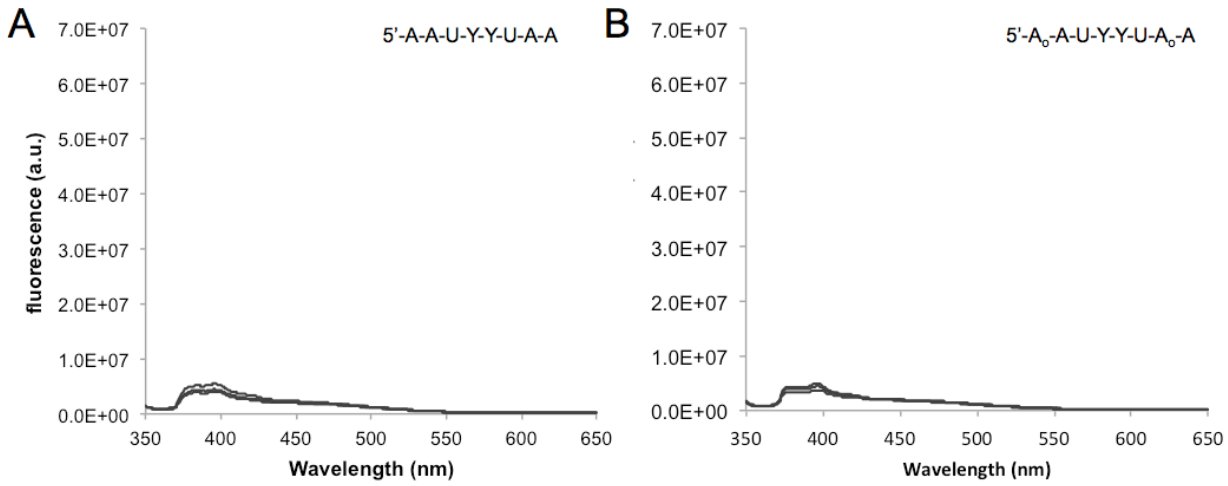


Figure S5. Testing stability of probes **2** and **4** against 10 % human serum. (A) Spectral changes in response of probe **2** with various incubation time (0, 120 and 360 min). (B) Spectral changes in response of probe **4** with various incubation time (0, 120 and 360 min). Conditions: [probe] = 400 nM, in 10 % human serum, 37 °C, excitation 340 nm.

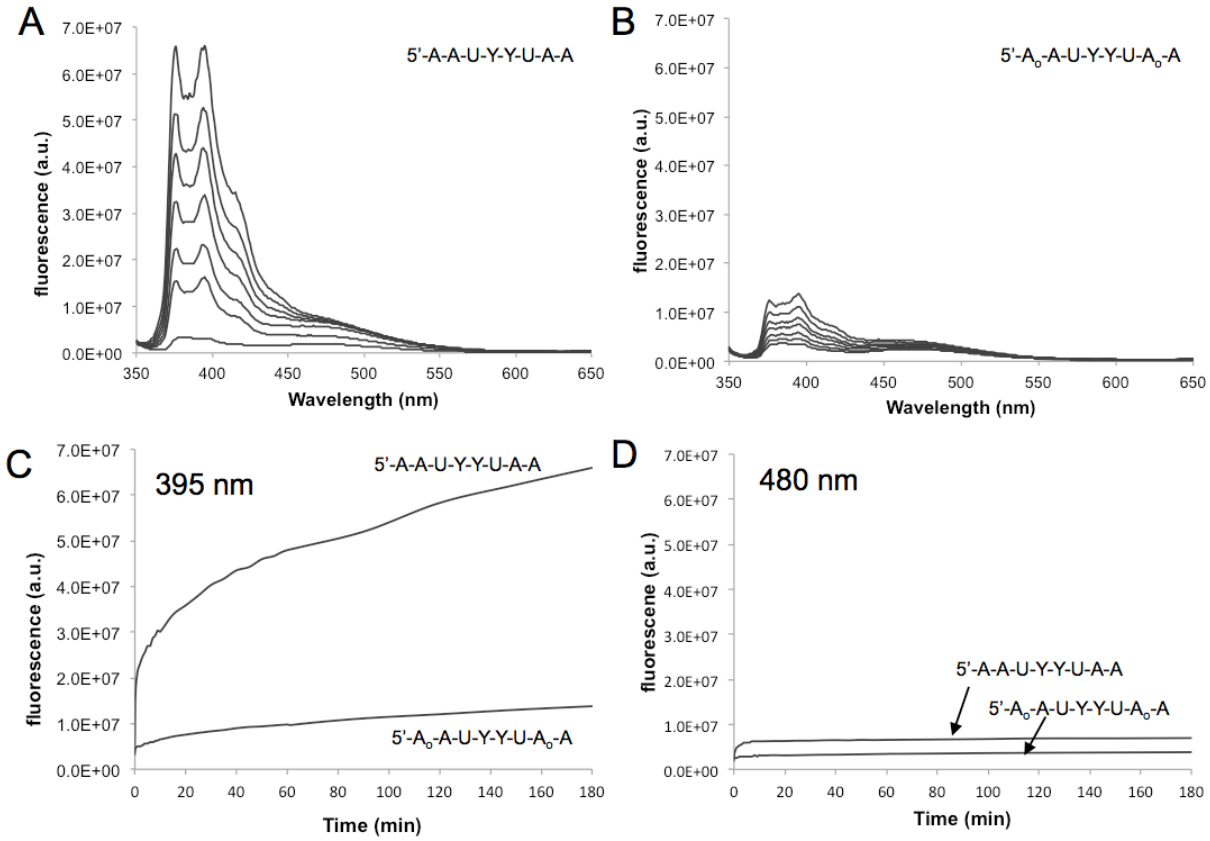


Figure S6. Testing stability of probes **2** and **4** against degradation by T4 DNA polymerase. (A) Spectral changes in response of probe **2**. (B) Spectral changes in response of probe **4**. (C) and (D) Time courses of fluorescence response are shown at the indicated wavelength. Conditions: [probe] = 400 nM, [T4 DNA polymerase] = 120 U/mL, 37^{o}C, excitation 340 nm.

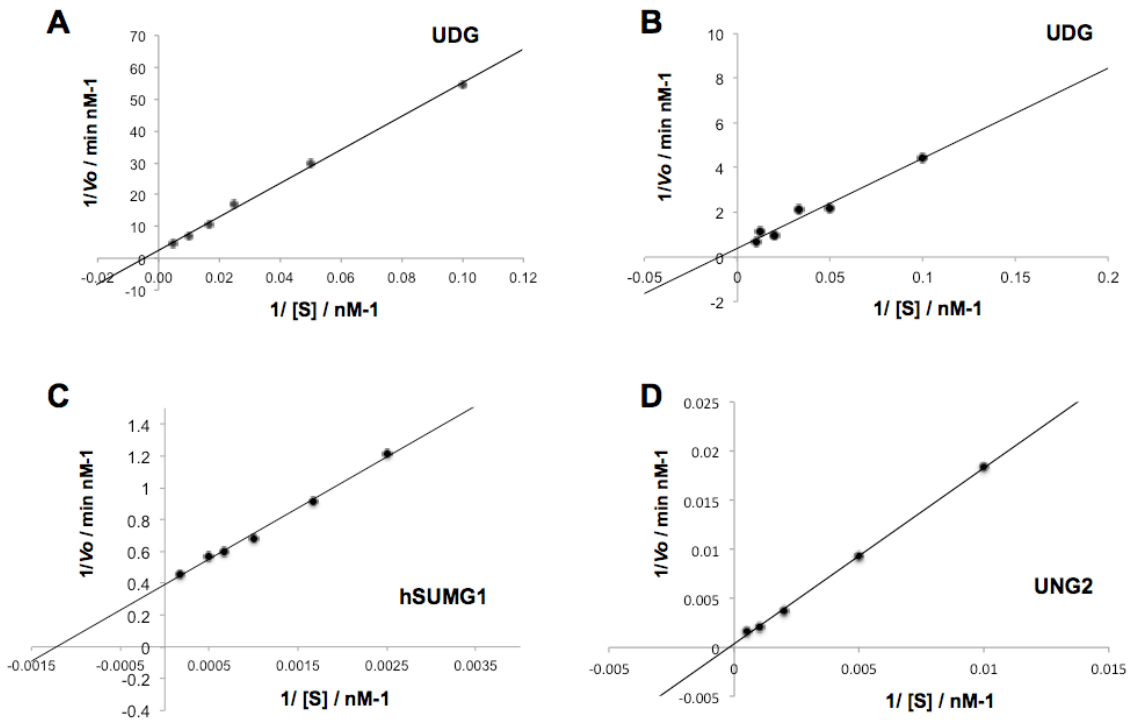
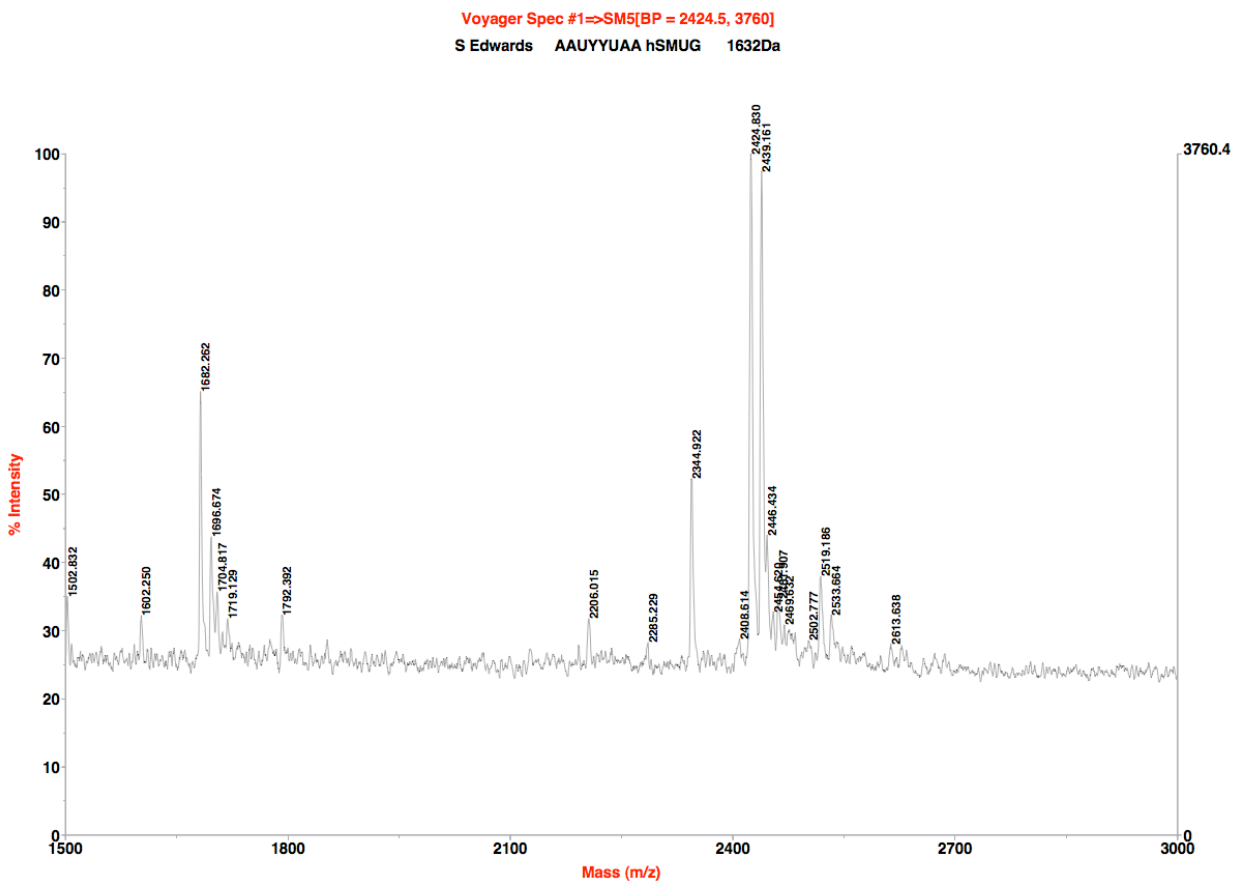


Figure S7. Lineweaver–Burk plots of reciprocals of initial rates (*Vo*) versus probe concentration for the determination of kinetic parameters $K_{M(app)}$ and V_{max} with UNG enzymes**.** From the linear fit of the data, we determined: (A) $K_{M(app)}$ = 0.21 μM , $k_{cat(app)}$ = 2040 min^{-1}, k_{cat}/K_M = 9710 min^{-1} μM^{-1}. Reaction conditions: [probe **1**] = 10, 20, 40, 60, 100, 200 nM, [UDG] = 1 unit/mL (0.4 nM) at 37 ºC. (B) $K_{M(app)}$ = 0.30 μM , $k_{cat(app)}$ = 882 min^{-1}, k_{cat}/K_M = 2940 min^{-1} μM^{-1}. Reaction conditions: [probe **2**] = 10, 20, 30, 50, 80, 100 nM, [UDG] = 1 unit/mL ([UDG] = 0.4 nM) at 37 ºC. (C) $K_{M(app)}$ = 0.82μM , $k_{cat(app)}$ = 35 min^{-1}, k_{cat}/K_M = 43 min^{-1} μM^{-1}. Reaction conditions: [probe **2**] = 0.4, 0.6, 1.0, 1.5, 2.0, 6.0 μM, [hSUMG1] = 500 unit/mL ([hSMUG1] = 400 nM)) at 37 ºC. (D) $K_{M(app)}$ = 4.67 μM , $k_{cat(app)}$ = 4.6 min^{-1}, k_{cat}/K_M = 0.99 min^{-1} μM^{-1}. Reaction conditions: [probe **2**] = 0.1, 0.2, 0.5, 1.0, 2.0 μM, [UNG2] = 500 unit/mL ([UNG2] = 90 nM) at 37 ºC.

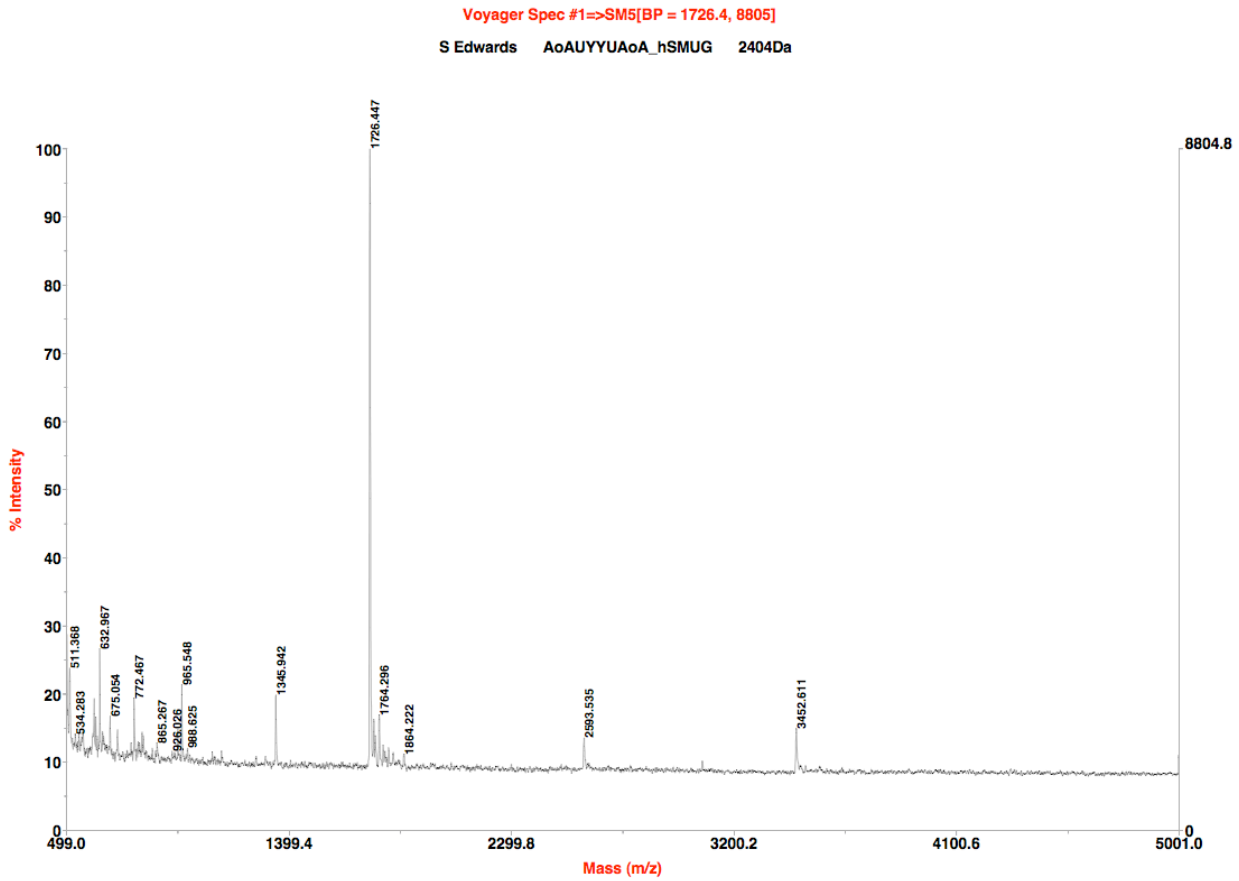
(A) probe 2



Expected/observed products:

- excise 5' uracil or 3’ uracil:
calculated: 2438; found: 2439
- excise 5' uracil and 3’ uracil:
calculated: 2344; found: 2344
- excise 5' uracil, cleave backbone on the 3' side of the abasic site:
calculated: 1696; found: 1696
- excise 3' uracil, cleave backbone on the 3' side of the abasic site:
calculated: 1794; found: 1792
- excise 3' uracil, cleave backbone on the 5' side of the abasic site:
calculated: 1695; found: 1696
- excise 5’ and 3' uracil, cleave backbone on the 5' side of the 5’ abasic site:
calculated: 1716; found: 1719
- excise 5’ and 3' uracil, cleave backbone on the 3' side of the 5’ abasic site:
calculated: 1602; found: 1602
- no cleavage or excision:
calculated: 2533; found: 2533

(B) probe 4



- excise 5' uracil, cleave backbone on the 3' side of the abasic site:
calculated: 1726, 772; found: 1726, 772
- excise 3' uracil, cleave backbone on the 5' side of the abasic site:
calculated: 1726, 772; found: 1726, 772
- excise 5' uracil, cleave backbone on the 5' and 3' sides of the abasic site:
calculated: 1726, 674; found: 1726, 675
- excise 3' uracil, cleave backbone on the 5' and 3' sides of the abasic site:
calculated: 1726, 674; found: 1726, 675
- no cleavage or excision:
calculated: 2593; found: 2594

Figure S8. MALDI-MS data for products of reactions of SMUG1 with probes **2** (A) and **4** (B). 4 uM probe was reacted with 500 units/mL hSMUG1 in 1x NEB reaction buffer 1 (10 mM Bis Tris Propane–HCl, 1 mM $MgCl_2$, 1 mM dithiothreitol) for 24 hours at 37 °C. The enzyme was heat inactivated by heating at 65°C for 20 minutes. The sample was concentrated 3-fold by speed vac, and was characterized by MALDI-TOF following a ziptip clean-up.

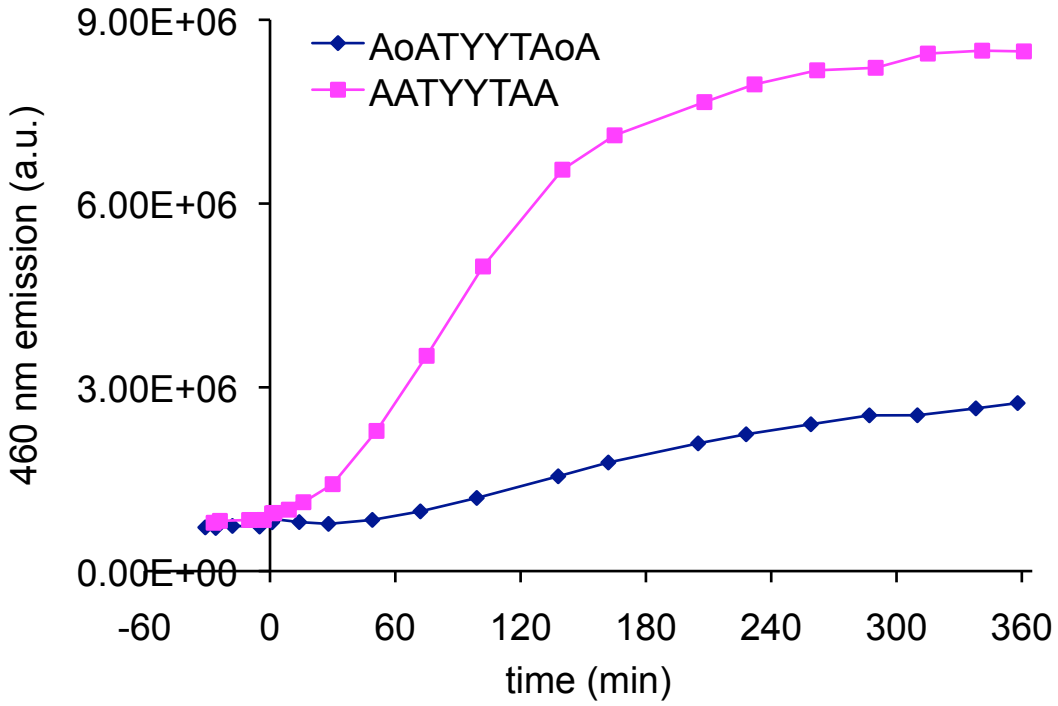
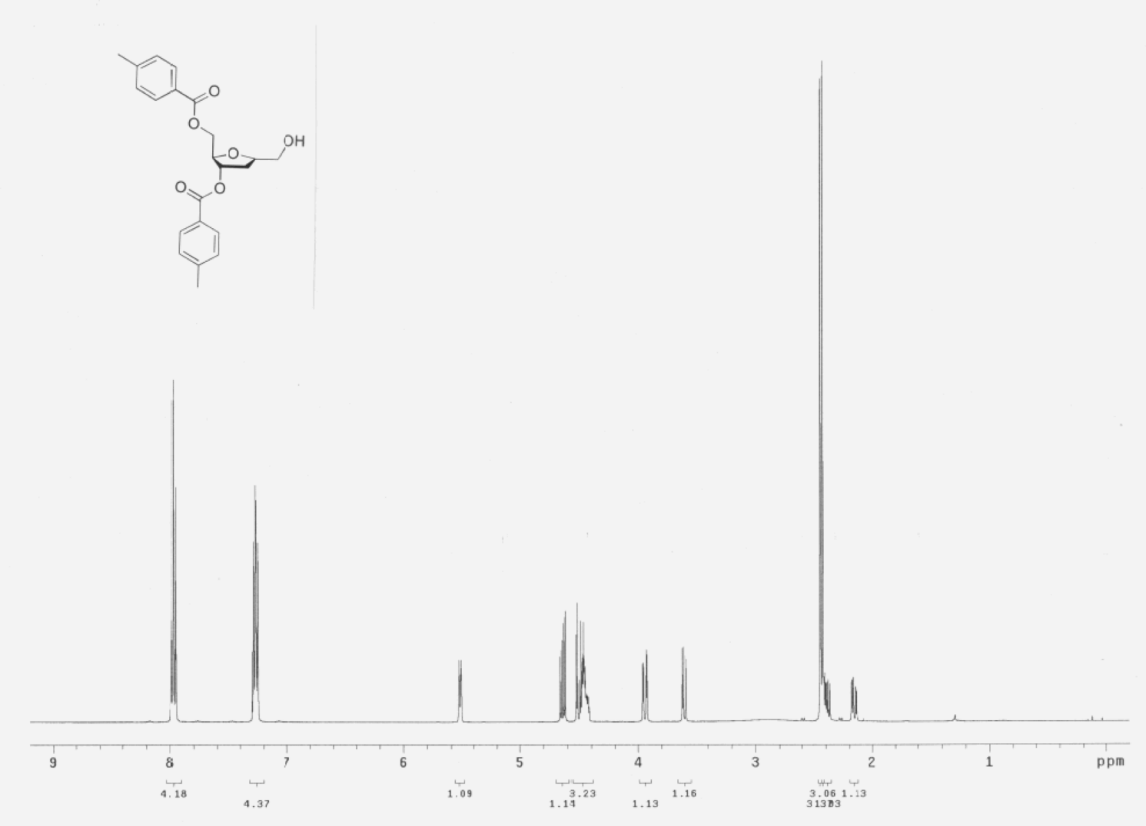


Figure S9. Stabilization of probes by 2'-O-methyl groups in HeLa cell lysate. 2 μM probe **6** (AATYYTAA) or 2 μM probe **7** (AoATYYTAoA) were incubated with complete cell lysate at a concentration of 1.2×10^6 lysed cells/mL at 37 °C. Emission at 460 nm over time is shown.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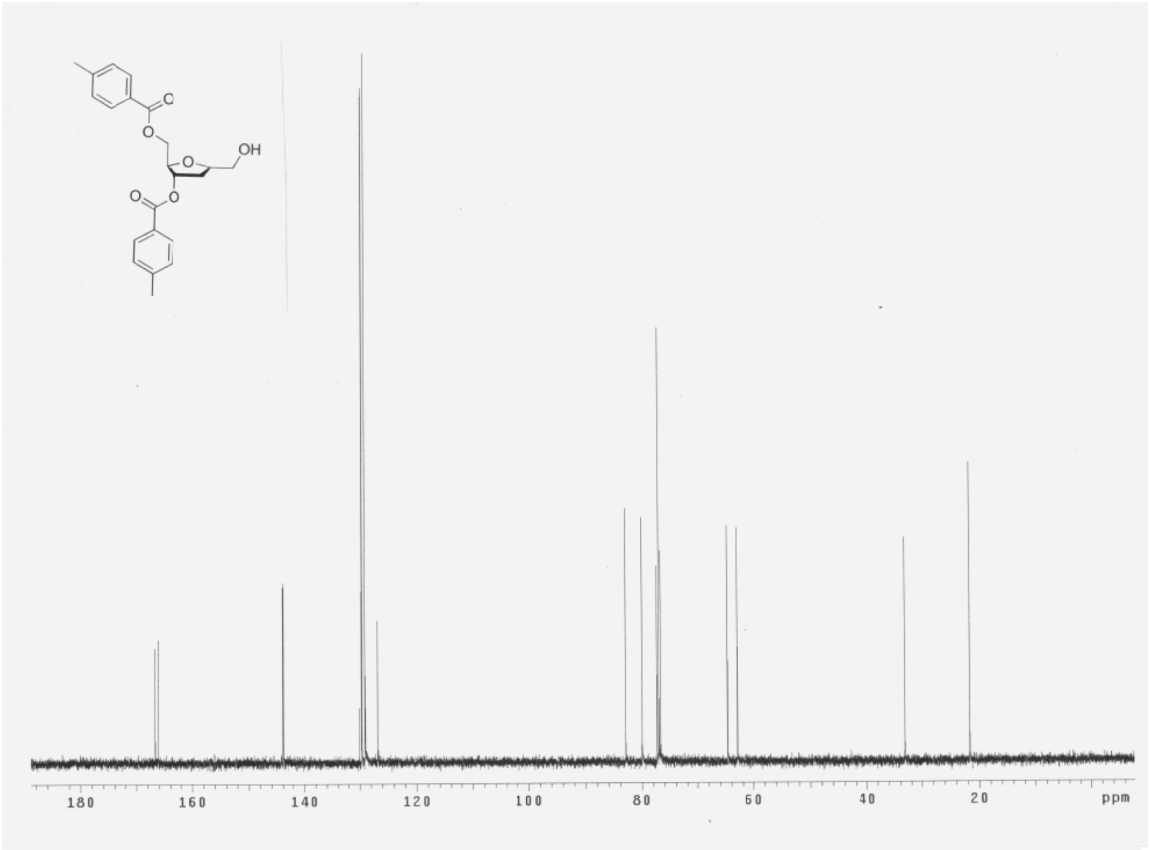
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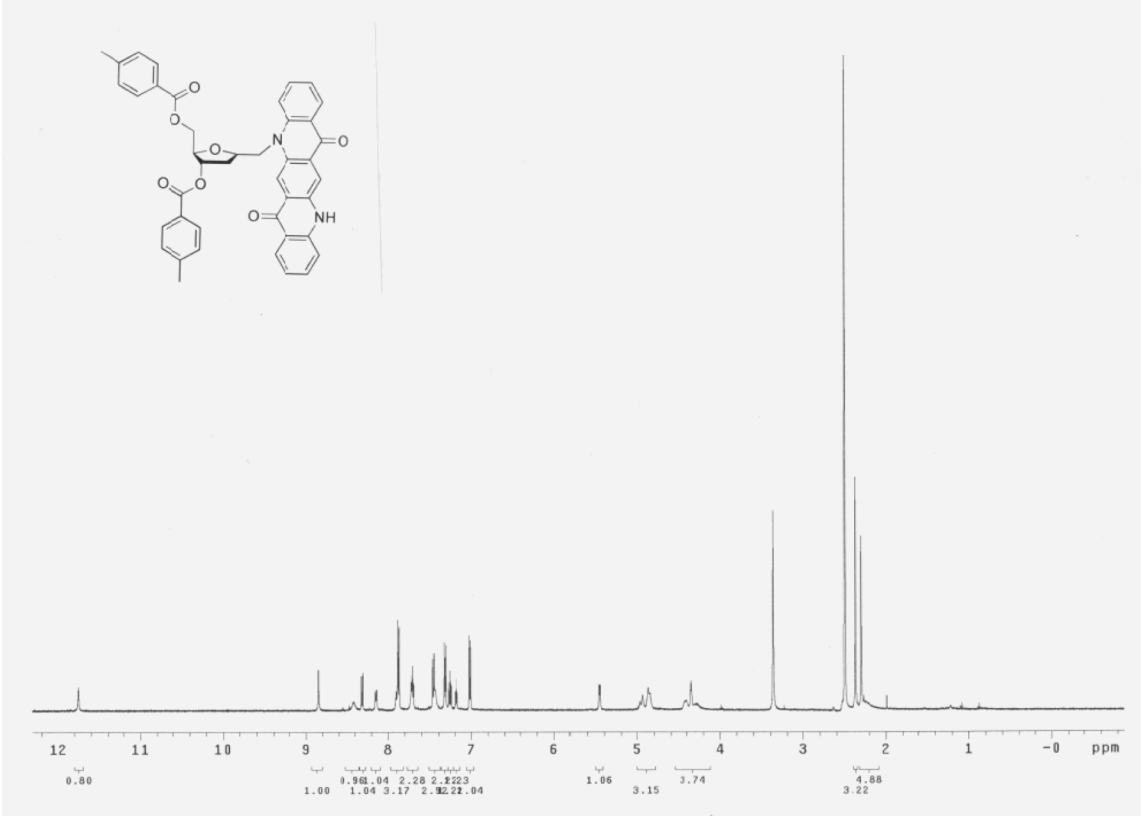
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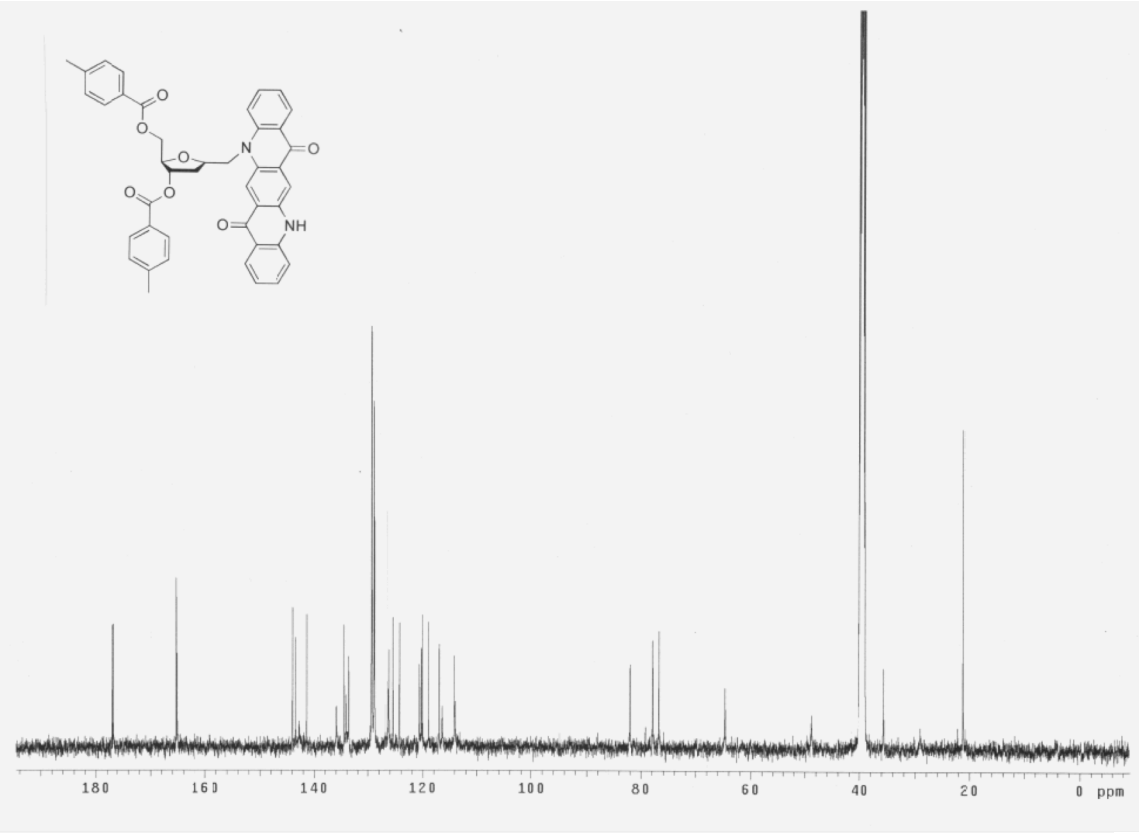
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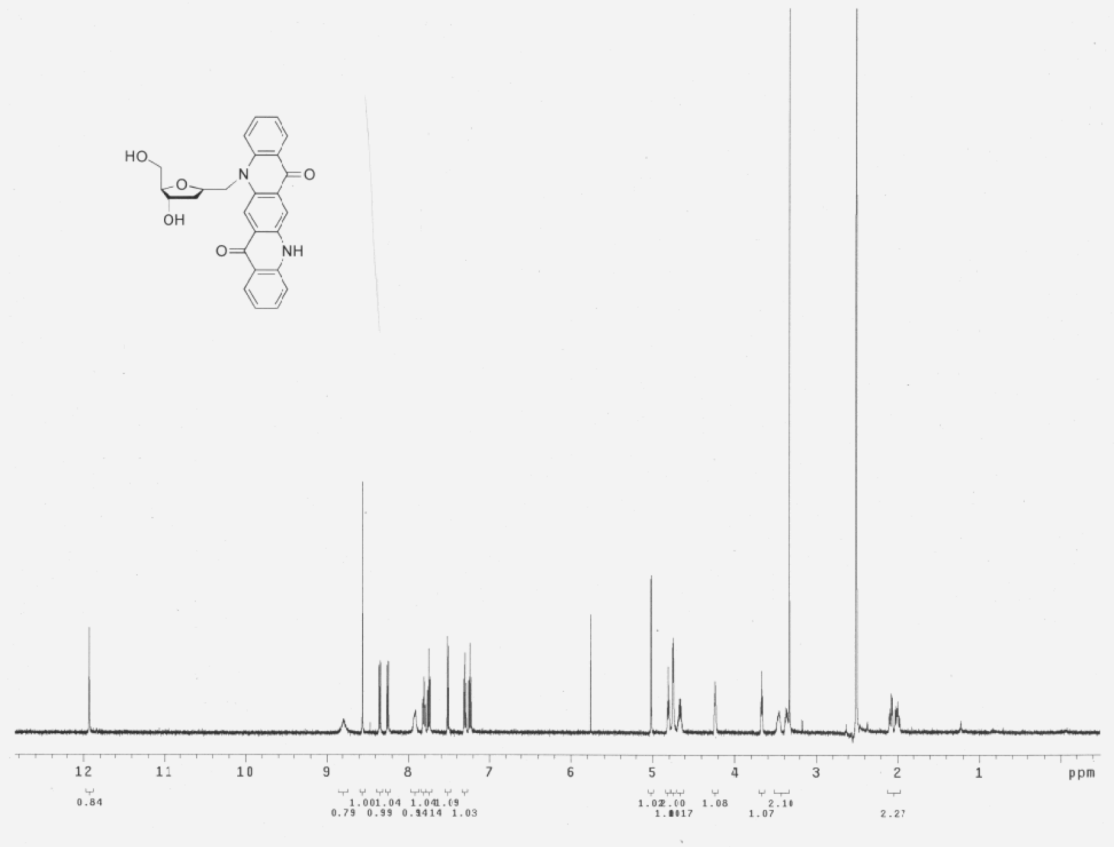
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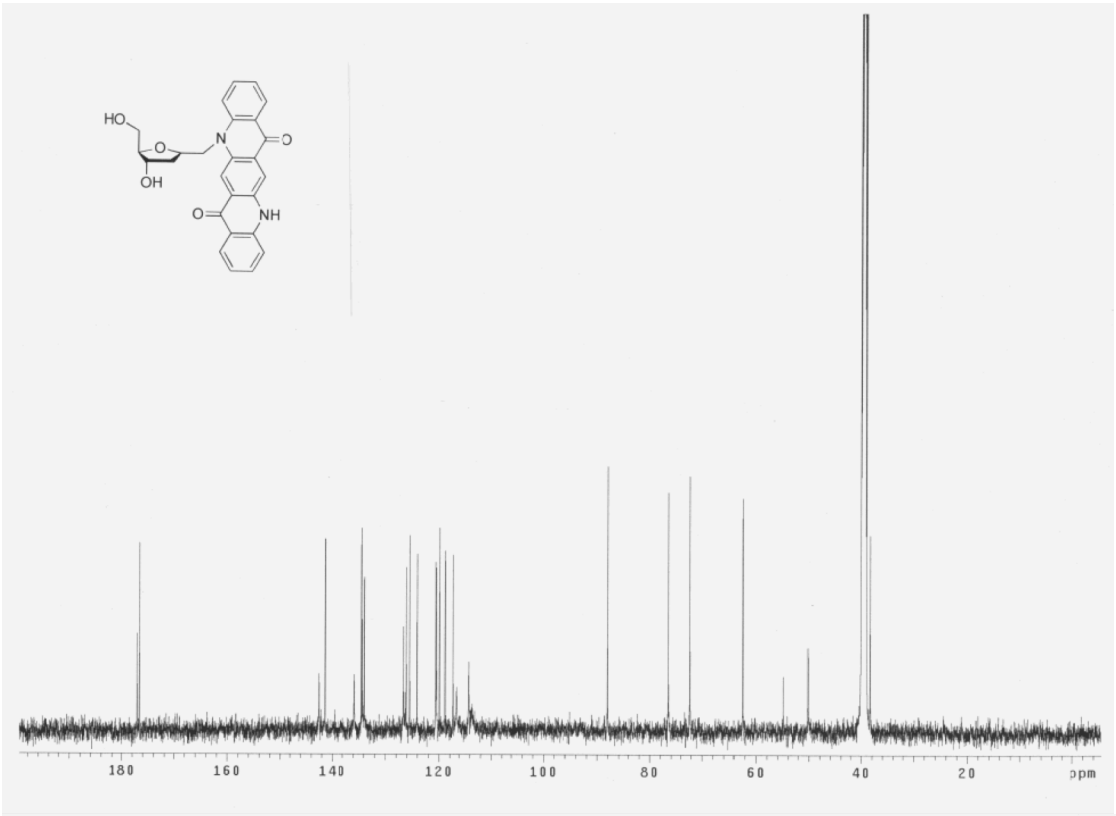
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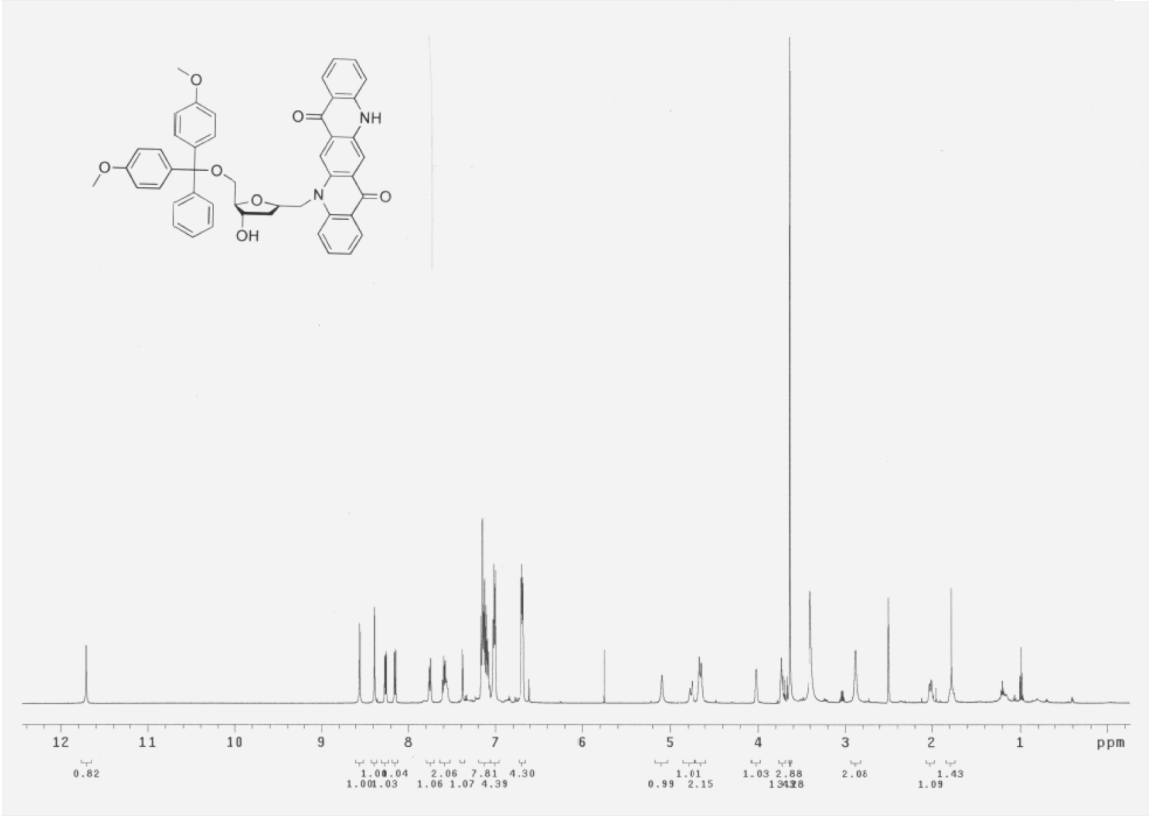
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1.06 1.07
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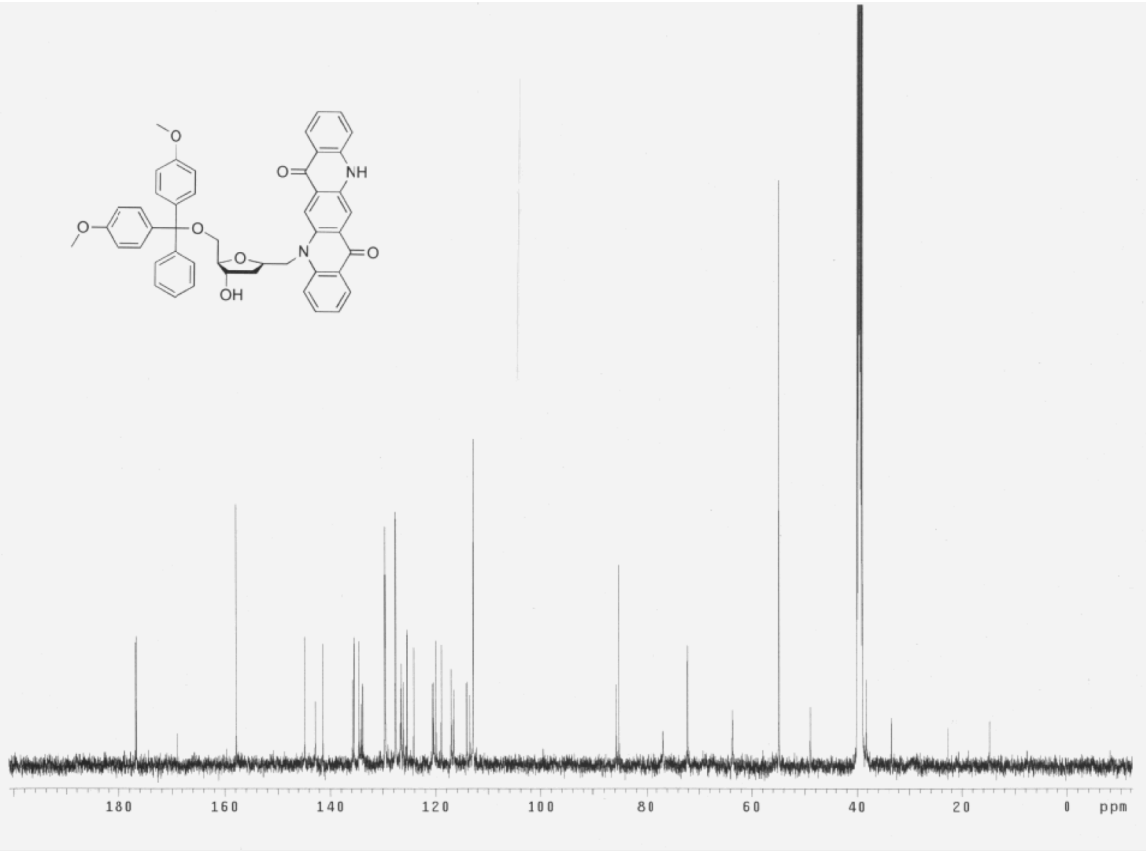
4.39
4.30
0.99
1.01
2.15
1.03
2.88
2.08
1.09
1.43



References

[1] Shigdel, U. K. and He, C. (2008) A new 1'-methylenedisulfide deoxyribose that forms an efficient cross-link to DNA cytosine-5 methyltransferase (DNMT). *J. Am. Chem. Soc.*, **130**, 17634-17635.