Supporting Information

Contents:

General methods.

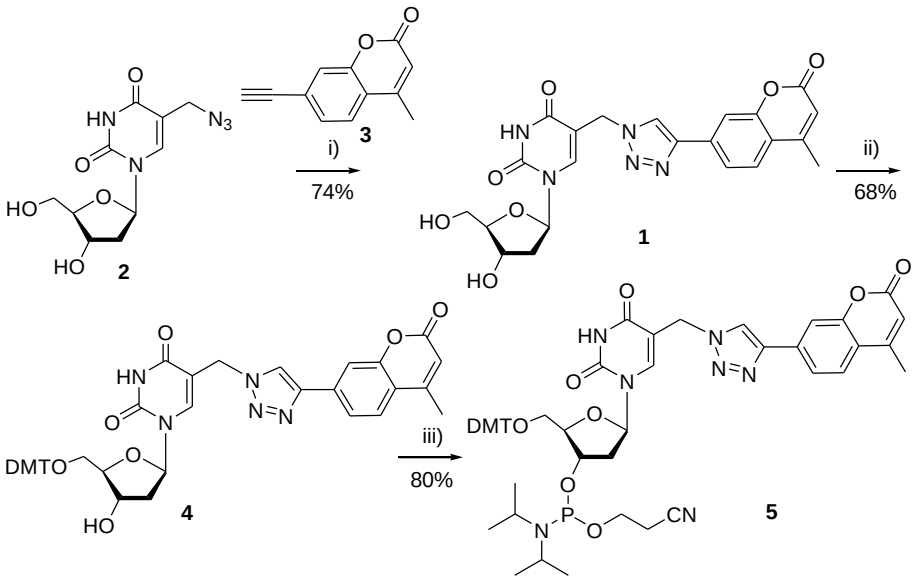
Unless otherwise specified, all chemicals were purchased from Sigma-Aldrich or Fisher Scientific. Water was purified with a Milli-Q purification system. Oligodeoxynucleotides (ODNs) were synthesized via standard automated DNA synthesis techniques using an Applied Biosystems model 394 instrument. The Pac-dA and ^{i}Pr-Pac-dG phosphoramidites were employed for the synthesis of Coumarin-dT containing ODNs. 28% aq. NH_3 was used for the deprotection of the nucleobases and phosphate moieties at room temperature for 30 min. ODNs were subjected to 20% denaturing PAGE (polyacrylamide gel electrophoresis) analysis for purification. The ^{32}P-labelled ODN (1.0 μM) was annealed with 1.5 equiv. of the complementary strand by heating to 65 °C for 3 min in a buffer containing 10 mM potassium phosphate, pH 7.0, and 100 mM NaCl, followed by slow-cooling to room temperature overnight. Radiolabeling was carried out according to the standard protocols. γ-^{32}P-ATP and α-^{32}P-ATP was purchased from Perkin-Elmer Life Sciences. Quantification of radiolabeled ODNs was carried out using a Molecular Dynamics Phosphorimager equipped with ImageQuant Version 5.2 software. DNA duplexes were irradiated with 350 nm UV light; Cross-linked DNA was isolated and analyzed by enzymatic digestion reaction.

^{1}H-NMR and ^{13}C-NMR spectra were recorded on Bruker DRX 300 spectrometer operating at room temperature. Chemical shifts are reported in parts per million (ppm) relative to the residual solvent peak. Multiplicities are reported as singlet (s), doublet (d), doublet of doublets (dd), triplet (t) or multiplet (m). High resolution mass spectrometry was performed at the Department of Chemistry, University of California-Riverside.

References

1. Hong, I. S.; Ding, H.; Greenberg, M. M. *J. Am. Chem. Soc.* **2006,** *128,* 485-491.

Procedure of Monomer Synthesis:



Scheme 1. Synthesis of compound **1** and its phosphoramidite building block **5**. Reagents: i) Cu_2SO_4 and sodium ascorbate; ii) Dimethoxytrityl chloride (DMTCl), pyridine; iii) 2-cyanoethyl-*N*,*N*-diisopropylchlorophosphoramidite, *N*,*N*-diisopropylethylamine and CH_2Cl_2.

5-(4-methylchromen-2-one-1,2,3-triazol-1-yl)methyl-2'-deoxyuridine(1). To a solution of 7-ethynyl-4-methyl-chromen-2-one (**3**, 200 mg, 1.08 mmol) and 5-azido-methyl-2'-deoxyuridine (**2**)[1] (270 mg, 0.954 mmol) in MeOH (8 mL), a 0.296 M aqueous solution of Na-ascorbate (6 mL) was added followed by a 0.368 M aqueous solution of Cu_2SO_4 (3.6 mL). After one hour, TLC confirmed completion of the reaction. The reaction mixture was evaporated to dryness and subjected to flash chromatography (DCM/MeOH, 20:1-7:1) yielding compound **1** (329 mg, 74%) as a white foam. ^{1}H NMR (DMSO-*d*6, 300 MHz): δ 11.60 (s, NH), 8.69 (s, 1H), 8.23 (s, 1H), 7.91-7.90 (d, J = 3 Hz, 1 H), 7.88-7.87 (d, J = 3 Hz, 1H), 7.82 (s, 1H), 6.39 (s, 1H), 6.19-6.15 (t, J = 12 Hz, 1H), 5.29-5.27 (m, 1H), 5.26 (s, 2H), 5.06-5.03 (t, J = 9 Hz, 1H), 4.28-4.25 (m, 1H), 3.81-3.80 (d, J = 3Hz, 1H), 3.62-3.56 (d, J =18, 2H), 3.33 (s, 3H), 2.19-2.15 (t, J = 12Hz, 2H): ^{13}C NMR (DMSO-*d*6, 300 MHz): δ 163.05, 160.23, 153.98, 153.50, 150.74, 145.07, 141.89, 134.77, 126.59, 123.17, 121.43, 119.45, 114.51, 112.75, 107.52, 88.04, 85.02, 70.62, 61.64, 47.03, 22.98, 18.52; MALDI-MS [MH+] calcd. for $C_{22}H_{21}N_5O_7$, 468.1519; found, 468.1515.

5'-O-(4,4'-Dimethoxytriphenylmethyl)-5-(4-methylchromen-2-one-1,2,3-triazol-1-yl)methyl-2'-deoxyuridine (4). Compound **1** (0.3 g, 0.64 mmol) was co-evaporated with anhydrous pyridine (3×5 mL) and then dissolved in pyridine (5 mL). To the solution, 4, 4'-dimethoxytriphenylmethyl chloride

(0.304 g, 0.90 mmol) was added in two portions and the reaction mixture was stirred at r. t. for 8 h. The reaction was quenched by addition of MeOH and was concentrated *in vacuo*. The crude product was purified by flash chromatography (CH_2Cl_2/MeOH/Et_3N = 96/3/1) to give **4** (0.345 g, 68%) as a off-white solid. ^{1}H NMR ($CDCl_3$, 300 MHz): δ 7.92-7.60 (m, 4H), 7.47-6.87 (m, 15H), 6.47-6.43 (t, *J* = 12 Hz, 1H), 4.83 (s, 2H), 4.43-4.38 (t, *J* = 15 Hz, 1H), 4.18-4.17 (d, *J* = 3 Hz, 1H), 4.15-4.01 (s, 3H), 3.48-4.39 (m, *J* = 27 Hz, 1H), 2.74 (s, 6H), 2.72-2.67 (d, *J* = 15 Hz, 2H), 2.44-2.38 (t, *J* = 18 Hz, 2H): ^{13}C NMR ($CDCl_3$, 300 MHz): δ 164.58, 161.45, 158.76,158.73, 153.85, 152.88, 149.92, 145.96, 144.50, 141.54, 135.47, 135.23, 134.70, 130.25, 130.15, 128.17, 128.15, 127.18, 124.99, 122.29, 121.68, 119.30, 114.49, 113.91, 113.44, 108.18, 87.08, 86.89, 85.96, 77.47, 77.25, 76.62, 72.29, 63.62, 55.27, 46.18, 46.06, 42.18, 29.70, 18.66, 10.67, 1.02; MALDI-MS [MNa+] calcd. for $C_{43}H_{39}N_5O_9$, 792.2645; found, 792.2624.

5'-O-(4,4'-Dimethoxytriphenylmethyl)-5-(4-methylchromen-2-one-1,2,3-triazol-1-yl)methyl-2'-deoxyuridine-3'-(2-cyanoethyl)-*N, N*-diisopropylphosphoramidite (5). Compound **4** (0.2 g, 0.25 mmol) was dissolved in anhydrous CH_2Cl_2 (5 mL) under Ar atmosphere. Diisopropylethylamine (96 µL, 0.55 mmol) and 2-cyanoethyl-*N,N*-diisopropylchlorophosphoramidite (96 µL, 0.43 mmol) was added. The reaction mixture was stirred at r.t. for 30 min, diluted with CH_2Cl_2 (30 mL), washed with 5% aqueous $NaHCO_3$ solution, followed by brine. The organic solution was dried over anhydrous Na_2SO_4, filtered, and concentrated. The residue was submitted to flash chromatography (EtOAc/CH_2Cl_2/Et_3N = 66/33/1) to yield product **5** (0.200 g, 80%) as a white foam. ^{31}P NMR ($CDCl_3$, 300 MHz): δ 150.0, 150.2.

Table 1: Modified ODNs and mass analysis.

Entry	**ODN Sequences**	**MALDI-MS Analysis**	
		Calculated	**Found**
ODN **7a**	5'-A GAT GGA T **1** T AGG TAC -3'	5187.4	5187.0
ODN **12a**	5'-AGA TTT TT**1** TTT GTA C-3'	5091.3	5091.0
ODN **13a**	5'-AGA TTA AA**1** AAA GTA C-3'	5147.4	5147.5
ODN **14**a	5'-AGA TTC CC**1** CCC GTA C-3'	5003.3	5003.3
ODN **15a**	5'-AGA TTG GG**1** GGG GTA C-3'	5243.4	5243.3

Interstrand cross-link formation and kinetics study. The ^{32}P-labelled ODN (0.5 μM) was annealed with 1.5 equiv of the complementary strand by heating to 65 °C for 3 min in buffer (10 mM potassium phosphate, pH 7, and 100 mM NaCl), followed by slow-cooling to room temperature overnight. The ^{32}P-labeled ODN duplex (2 μL, 0.5 μM) was mixed with 1 M NaCl (2 μL), 100 mM potassium phosphate (2 μL, pH 7.5), and appropriate amount of autoclaved distilled water to give a final volume of 20 μL. The reaction mixture was UV irradiated at 350 nm for 50 min and quenched by an equal volume of 90% formamide loading buffer, then subjected to 20% denaturing polyacrylamide gel analysis. For kinetics study, aliquots (final concentration: 50 nM ^{32}P-labeled ODN duplex, 100 mM NaCl, and 10 mM potassium phosphate) were irradiated for the prescribed times and immediately quenched by 90% formamide loading buffer and stored at -20 °C until subjecting to 20% denaturing PAGE analysis.

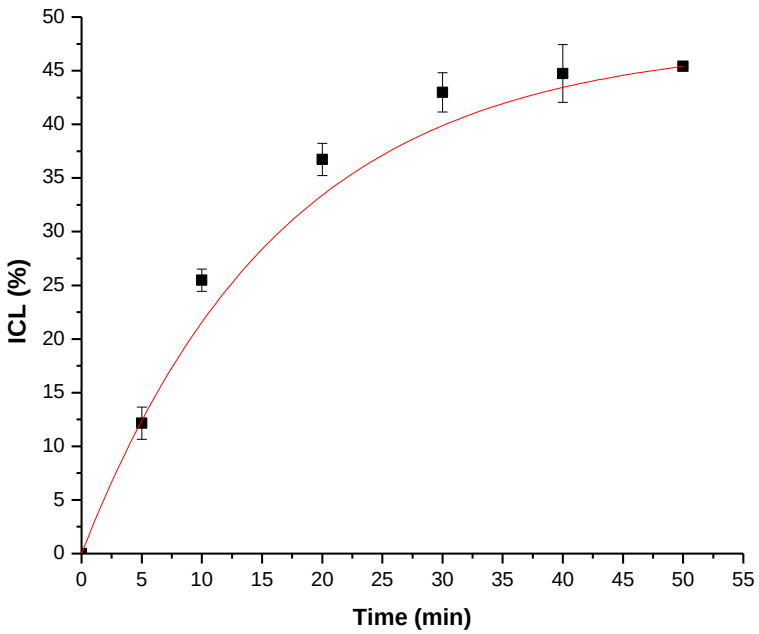


Figure S1. Rate of ICL growth of duplex **7** upon UV-irradiation at 350 nm.

Hydroxyl radical reaction (Fe·EDTA reaction). Fe(II)·EDTA cleavage reactions of ^{32}P-labelled ODN (0.1μM) were performed in a buffer containing 50 μM $(NH_4)_2Fe(SO_4)_2$, 100 μM EDTA, 5 mM sodium ascorbate, 0.5 M NaCl, 50 mM sodium phosphate (pH 7.2) and 1 mM H_2O_2 for 3 min at room temperature (total substrate volume 20 μL), then quenched with 100 mM thiourea (10 μL). Samples were lyophilized, and incubated with 1 M piperidine (20 μL) at 90 ^{o}C for 30 min. The mixture was lyophilized again, dissolved in 20 μL H_2O : 90% formamide loading buffer (1:1) and subjected to 20% denaturing PAGE analysis.

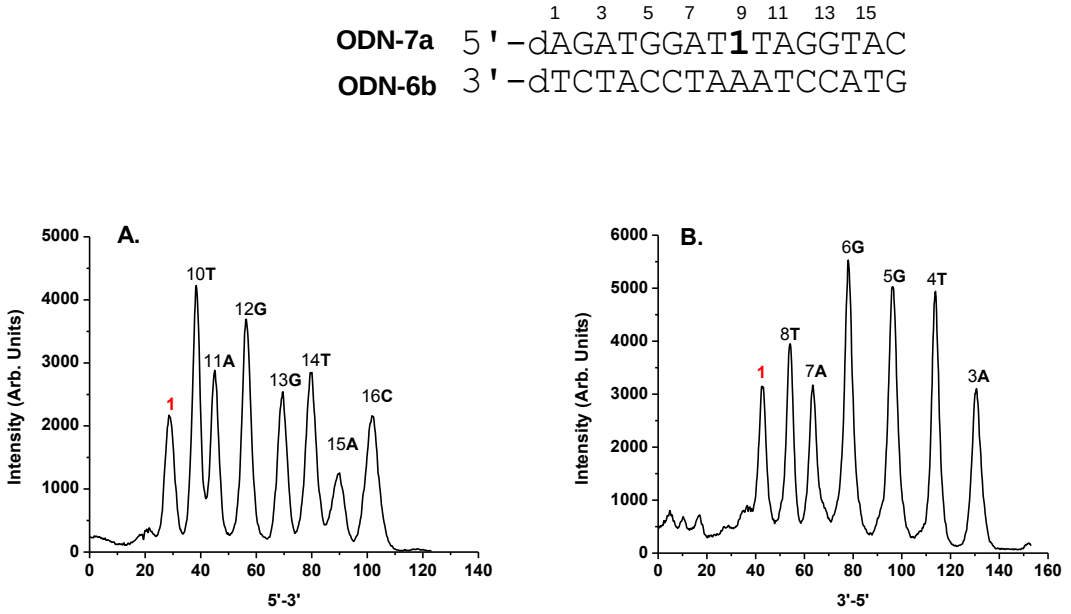


Figure S2. Determination of the interstrand cross-linking site via hydroxy radical cleavage of the ICL product isolated from UV-treated ODN duplex **7** (**7a●6b**). Histogram showing Fe·EDTA cleavage of cross-linked product formed from duplex **7**: (A) **7a** was ^{32}P-labeled at the 3'-terminus); (B) **7a** was ^{32}P-labeled at the 5'-terminus.

LC/MS/MS analysis. The isolated ICL products (50 pmol) were digested with nuclease P1 (0.5 unit) and phosphodiesterase 2 (0.005 unit) in a buffer (pH 5.6) containing 30 mM sodium acetate, 1.0 mM zinc acetate, and 1 mM *erythro*-9-(2-hydroxy-3-nonyl)adenine (EHNA) at 37°C for 48 h. Alkaline phosphatase (0.5 unit) and phosphodiesterase 1 (0.001 unit) were subsequently added to the digestion mixture and the reaction continued for 2 h in a 50-mM Tris-HCl buffer (pH 8.9). The enzymes were removed by chloroform extraction at the end of enzymatic digestion. The digestion products were then analyzed by LC-MS/MS/MS with an Agilent 1200 capillary HPLC pump (Agilent Technologies, Santa Clara, CA) and an LTQ linear ion-trap mass spectrometer (Thermo Fisher Scientific). A 0.5 × 250 mm Zorbax SB-C18 column (5 μm in particle size, Agilent Technologies) was used. A solution of 0.1% (v/v) formic acid in water (solution A) and a solution of 0.1% (v/v) formic acid in methanol (solution B) were employed as mobile phase at a flow rate of 8.0 μL/min. A gradient of 5 min 0-20% B and 25 min 20-70% B were used for the separation. The instrument was set up for monitoring the fragmentation of the $[M+H]^+$ ions of putative ICL products in the positive-ion mode.

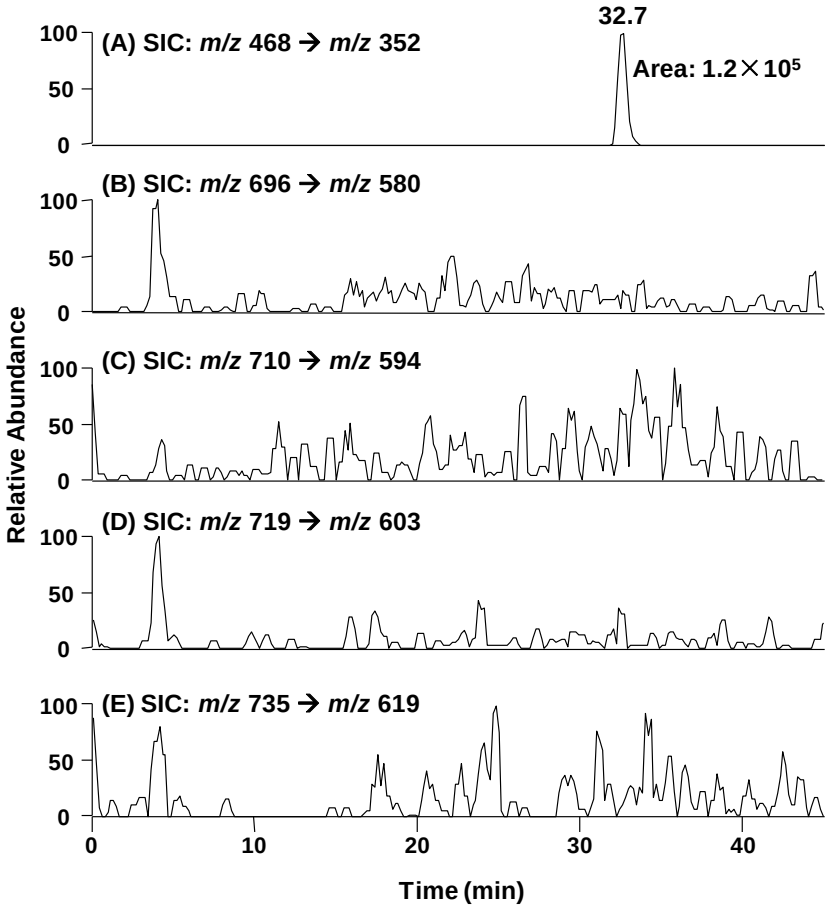


Figure S3. Selected-ion chromatograms (SICs) for monitoring the indicated transitions for coumarin-modified dT (A), dU-**1** cross-link (B), dT-**1** cross-link (C), dA-**1** cross-link (D), and dG-**1** cross-link (E) in the digestion mixture of hybridized duplex **7** (**7a•6b**).

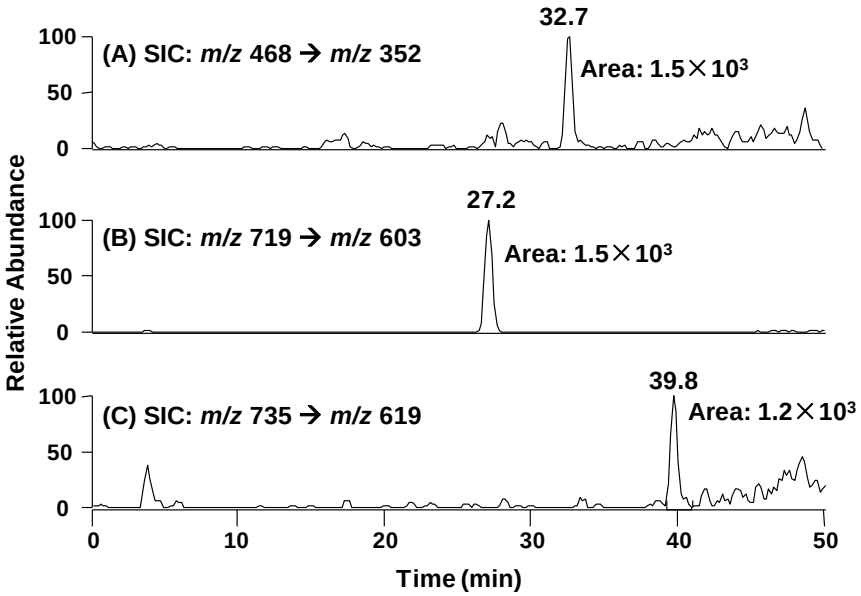


Figure S4. SICs for monitoring the indicated transitions for coumarin-modified dT (A), dA-**1** cross-link (B), and dG-**1** cross-link (C) in the digestion mixture of ICL products generated from duplex **7** (**7a•6b**).

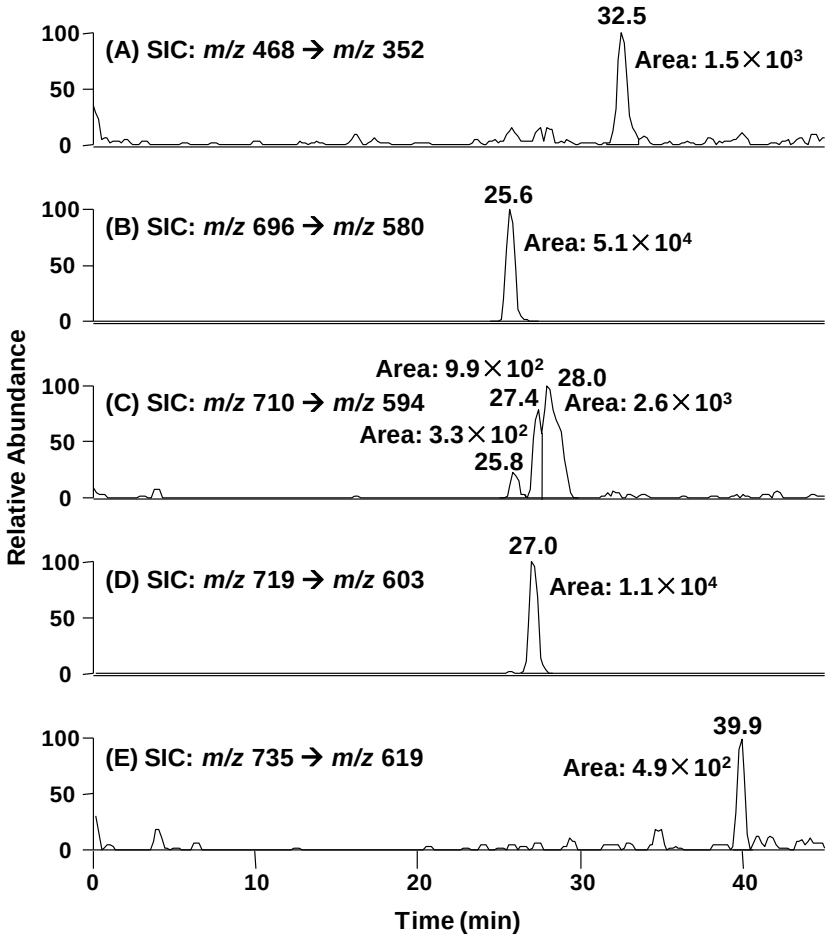


Figure S5. SICs for monitoring the indicated transitions for coumarin-modified dT (A), dU-**1** cross-link (B), dT-**1** cross-link (C), dA-**1** cross-link (D), and dG-**1** cross-link (E) in the digestion mixture of ICL products generated from duplex **9** (**7a•9b**).

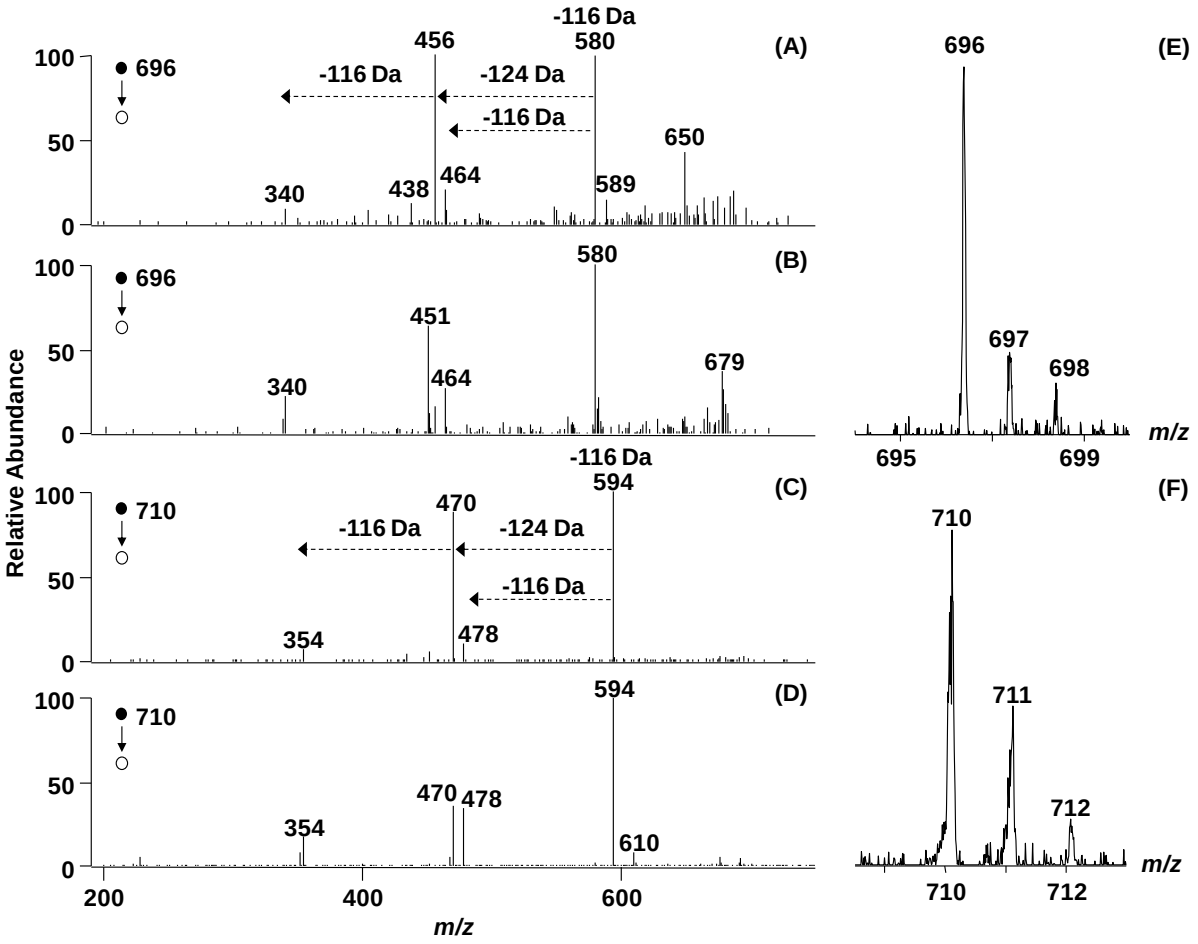


Figure S6. Positive-ion MS^2 spectra for dU-**1** cross-links shown at 25.6 min (A) and 26.9 min (B) in Figure 3A and dT-**1** cross-links shown at 26.0 min (C) and 27.4 min (D) in Figure 3B. Higher-resolution ultra-zoom-scan ESI-MS results of ions at *m/z* 696 (E) and *m/z* 710 (F).

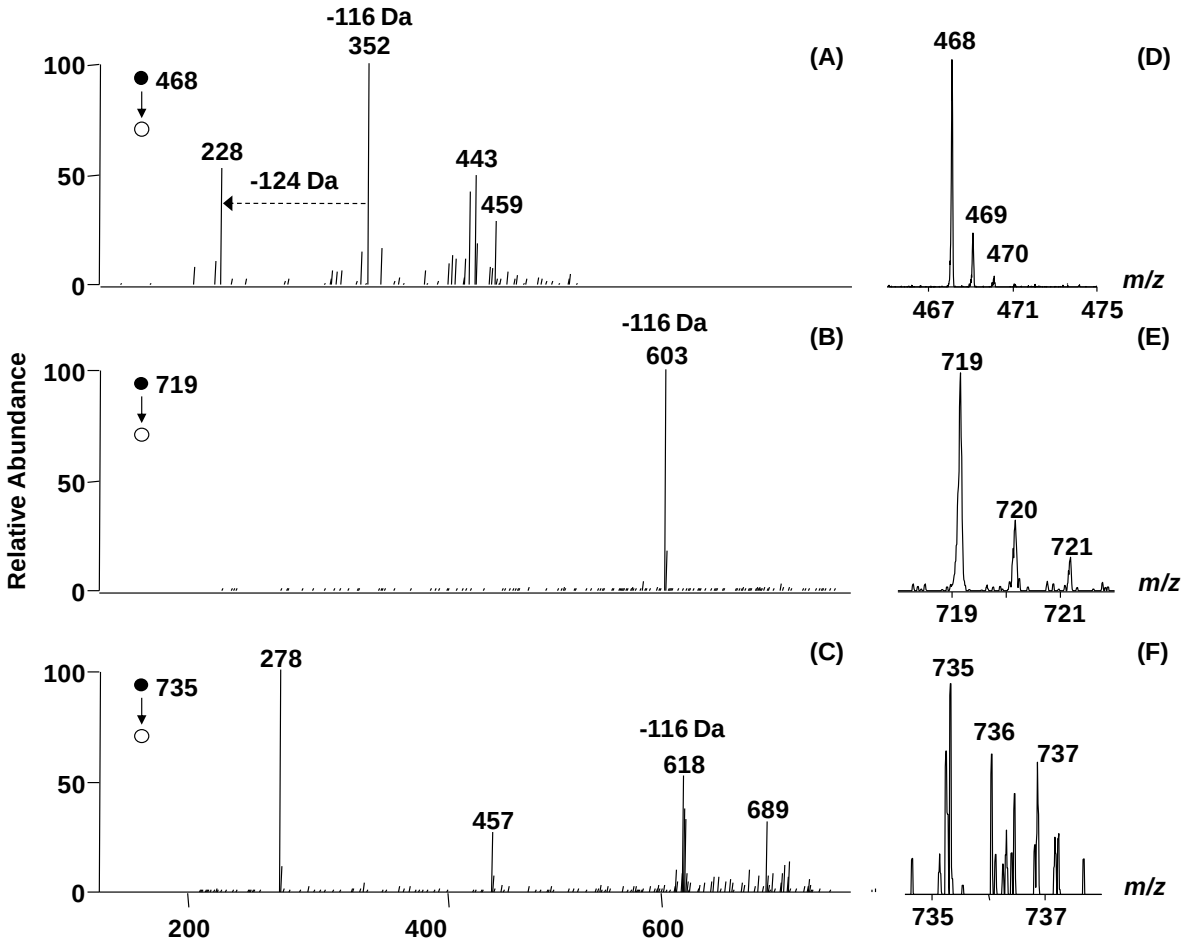


Figure S7. Positive-ion MS/MS for coumarin-modified dT shown at 32.7 min (A) in Sup Figure S4A, dA-**1** cross-link shown at 27.2 min (B) in Sup Figure S4B, and dG-**1** cross-link shown at 39.8 min (C) in Sup Figure S4C. Higher-resolution ultra-zoom-scan ESI-MS results of ions at *m/z* 468 (D), *m/z* 719 (E) and *m/z* 735 (F).

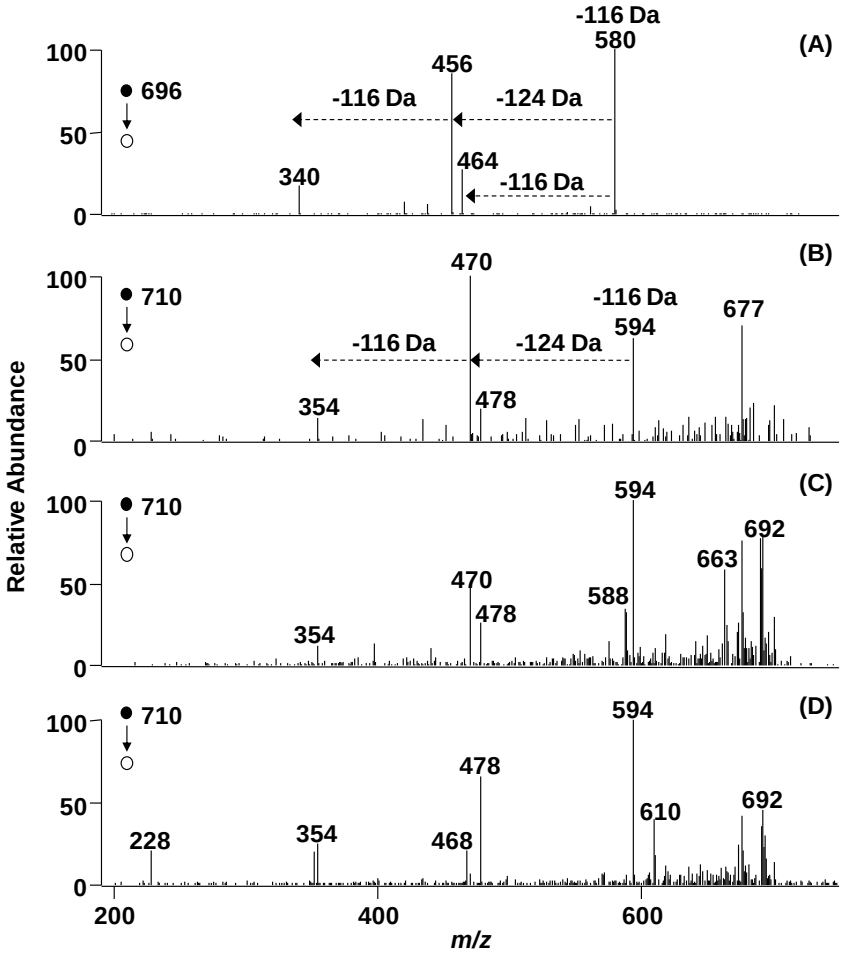


Figure S8. Positive-ion MS/MS for dU-**1** cross-link shown at 25.6 min (A) in Sup Figure S5B and for dT-**1** cross-links shown at 25.8 min (B), 27.4 min (C), and 28.0 min (D) in Sup Figure S5C.

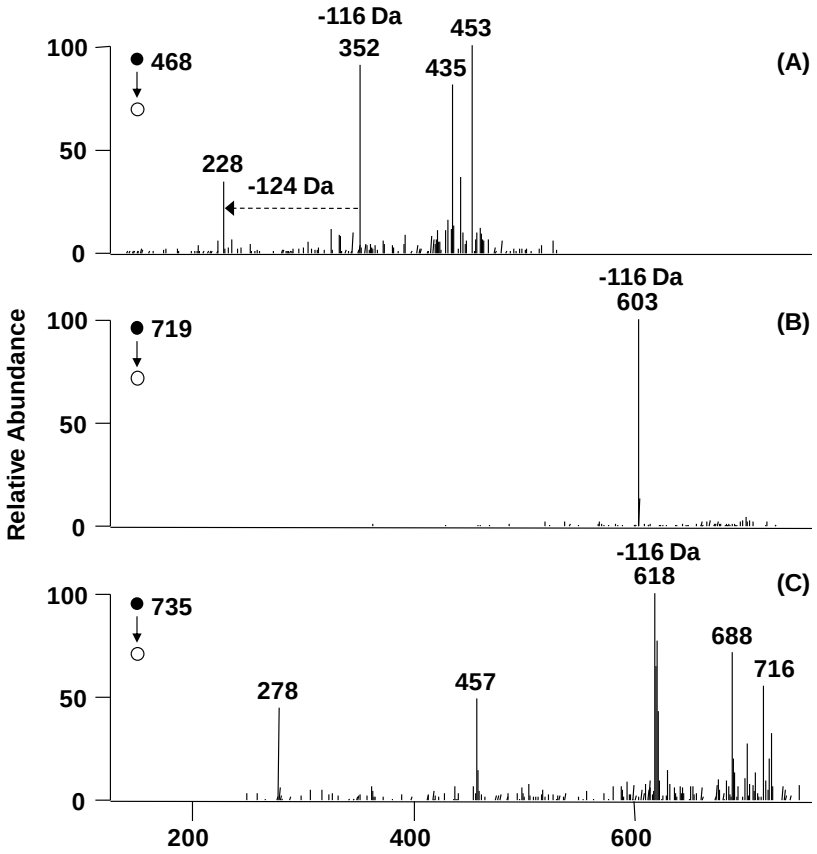


Figure S9. Positive-ion MS/MS for coumarin-modified dT shown at 32.5 min (A) in Sup Figure 5A, dA-**1** cross-link shown at 27.0 min (B) in Sup Figure 5D, and dG-**1** cross-link shown at 39.9 min (C) in Sup Figure 5E.

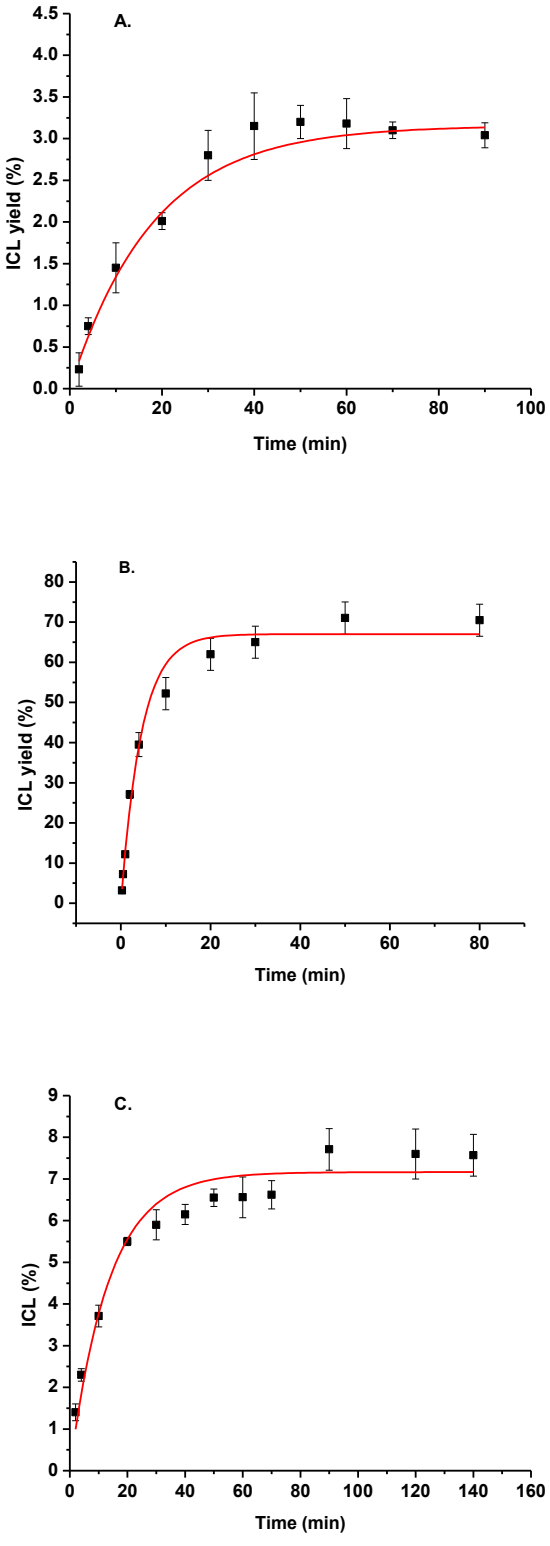


Figure S10. Rate of ICL growth of duplexes **12** (**A**), **13** (**B**), and **15** (**C**) upon UV-irradiation at 350 nm.

Stability study of ICL products formed with 1: After UV-photolysis (350 nm, 50 min), the cross-linked DNA was purified by 20% denaturing PAGE. The band containing cross-linked product were cut, crushed, and eluted with 200 mM NaCl, 20 mM EDTA (2.0 mL). The crude product was further purified by C18 column eluting with H_2O (3 × 10.0 mL) followed by MeOH:H_2O (3:2, 1.0 mL). The dried DNA fragments were dissolved in 1.0 M piperidine (20 μL) and incubated at 90 ^oC for 30 min. The samples were subjected to electrophoresis on a 20% denaturing polyacrylamide gel.

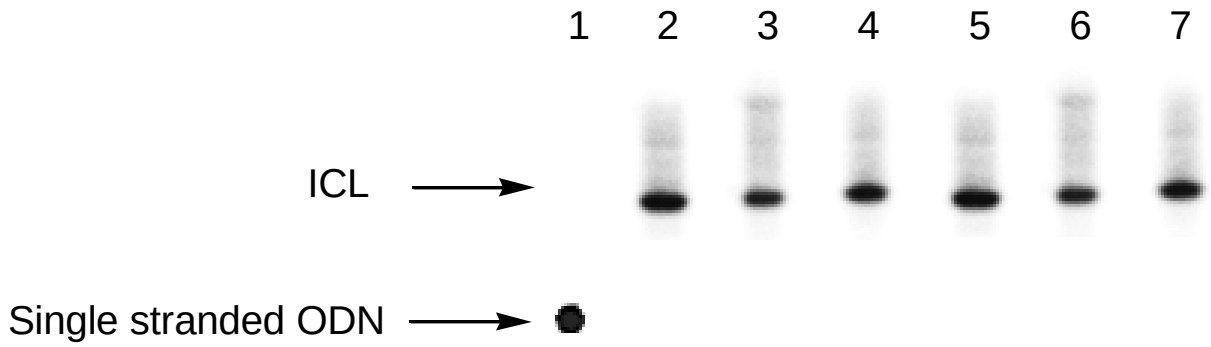


Figure S11. The heating stability of the ICL products formed from duplexes **7-12**. Phosphorimage autoradiogram of 20% denaturing PAGE analysis of the ICL product upon heating in phosphate buffer or piperidine. Lane 1, Single stranded ODN **7a**; Lanes 2-6, piperidine treatment of ICL products at 90 ^oC for 30 min (lane 2: ICL product formed from duplex **7**; lane 3: ICL product formed from duplex **9**; lane 4: ICL product formed from duplex **10**; lane 5: ICL product formed from duplex **11**; lane 6: ICL product formed from duplex **12**); Lane 7, the cross-linked products formed from duplex **7** were heated in phosphate buffer (pH 8) at 90 ^oC for 30 min.

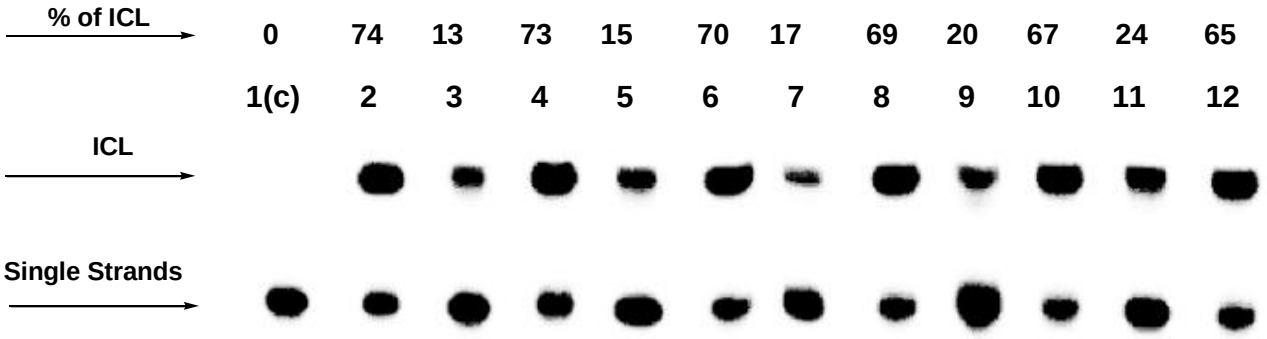


Figure S12. Phosphorimage autoradiogram of 20% denaturing PAGE analysis of photoreversible DNA cross-linking with duplex **10** upon UV irradiation at 350 nm (50 min) and 254 nm (6 min). Lane 1, single strand ODN **7a**; Lane 2, UV irradiation of **10** at 350 nm for 50 min to form ICL; Lane 3, UV irradiation at 254 nm for 6 min to cleave the ICL product leading to single strand ODN; Lane 4, UV irradiation at 350 nm for 50 min to form ICL; Lane 5, UV irradiation at 254 nm for 6 min to form reversed single strand ODN. Lane 6; UV irradiation at 350 nm for 50 min to form ICL; Lane 7, UV irradiation at 254 nm for 6 min to form reversed single strand ODN; Lane 8, UV irradiation at 350 nm for 50 min to form ICL; Lane 9, UV irradiation at 254 nm for 6 min to form reversed single strand ODN; Lane 10, UV irradiation at 350 nm for 50 min to form ICL; Lane 11, UV irradiation at 254 nm for 6 min to form reversed single strand ODN; Lane 12, UV irradiation at 350 nm for 50 min to form ICL.

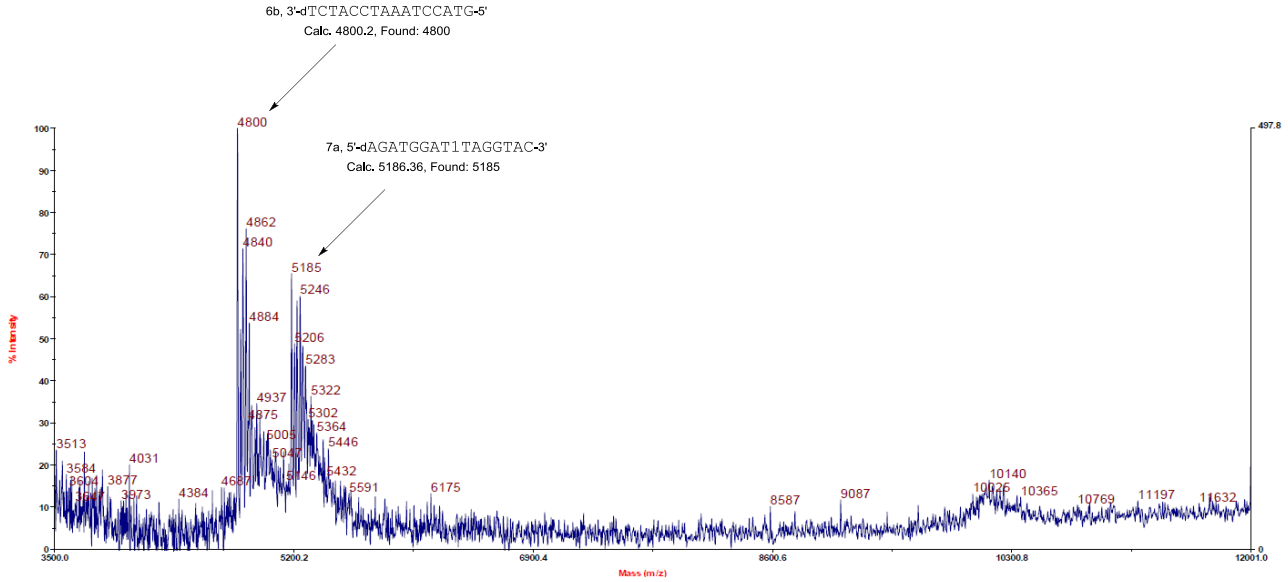


Figure S13. MALDI-TOF MS spectra of the isolated ICL product formed from duplex **7** (**7a●6b**) after UV-irradiation at 254 nm for 6 min.

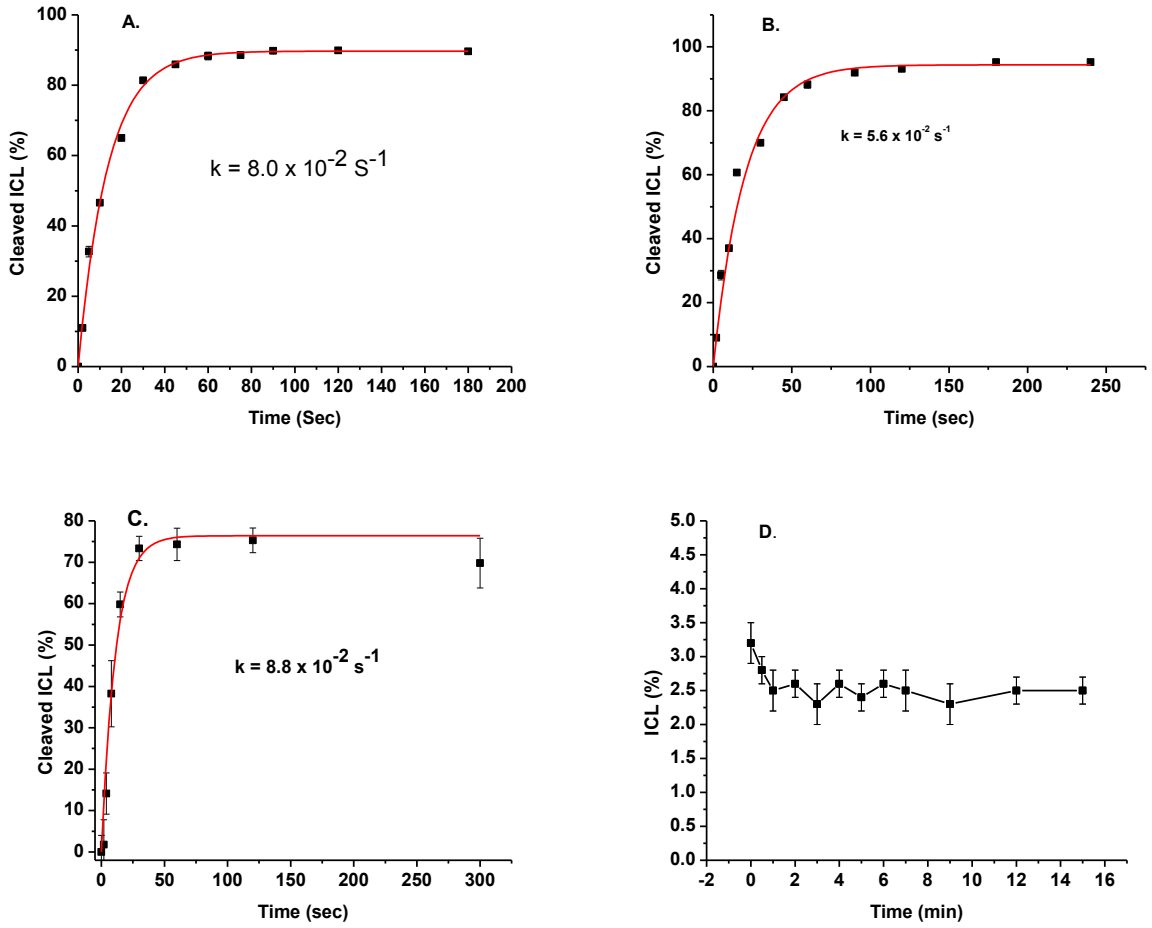


Figure S14. The cleavage rate of the ICLs formed from duplexes **10** (**A**), **13** (**B**), and **15** (**C**) upon UV-irradiation at 254 nm (the rate constant was calculated based on disappearance of the interstrand cross-link (ICL) prodructs); (**D**) the ICLs formed from duplex **12** were irradiated by 254 nm UV light for 0, 0.5, 1, 2, 3, 4, 5, 6, 7, 9, 12, and 15 mins.

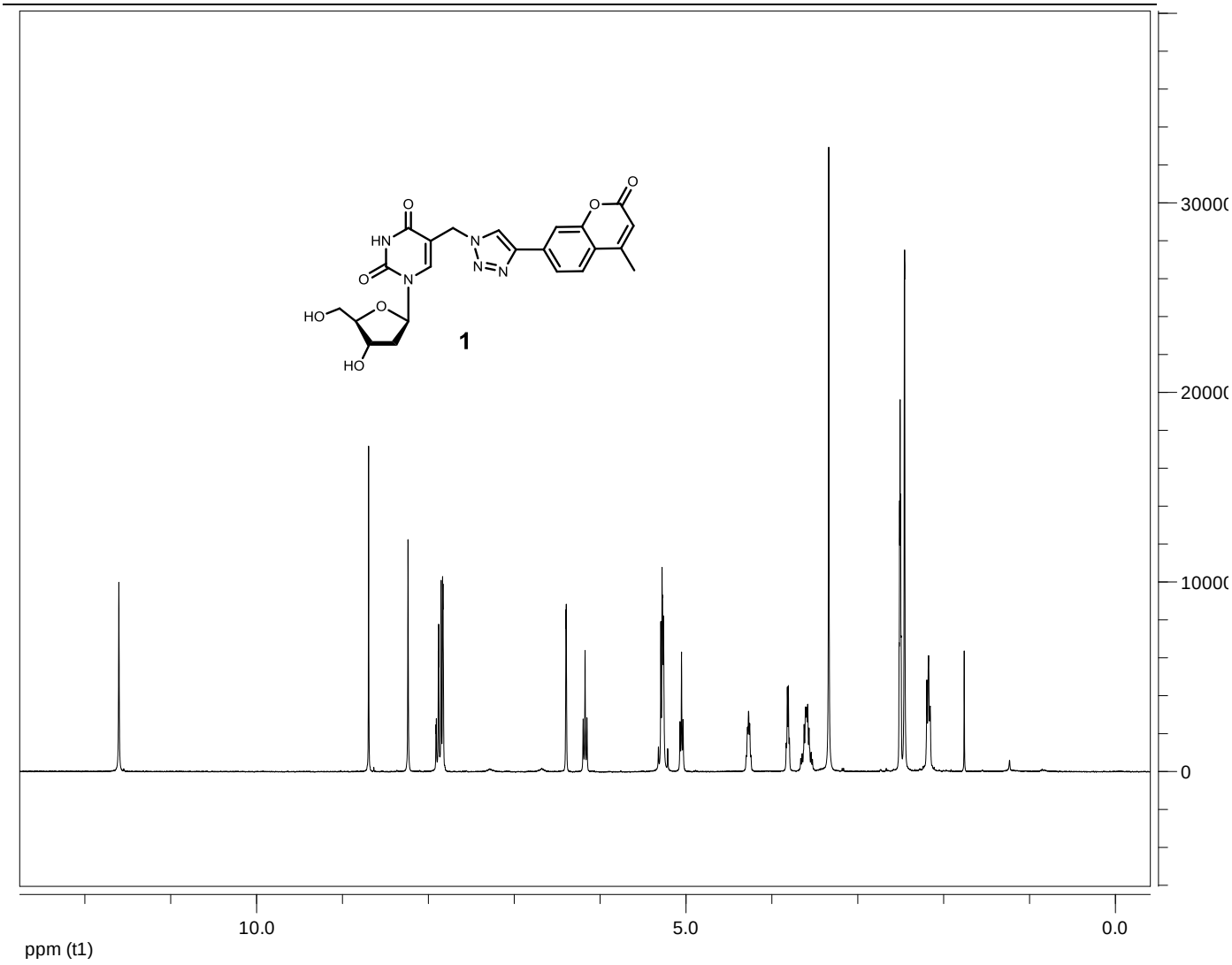


Figure S15. ^{1}H NMR (DMSO-d_6, 300 MHz) of nucleoside **1**

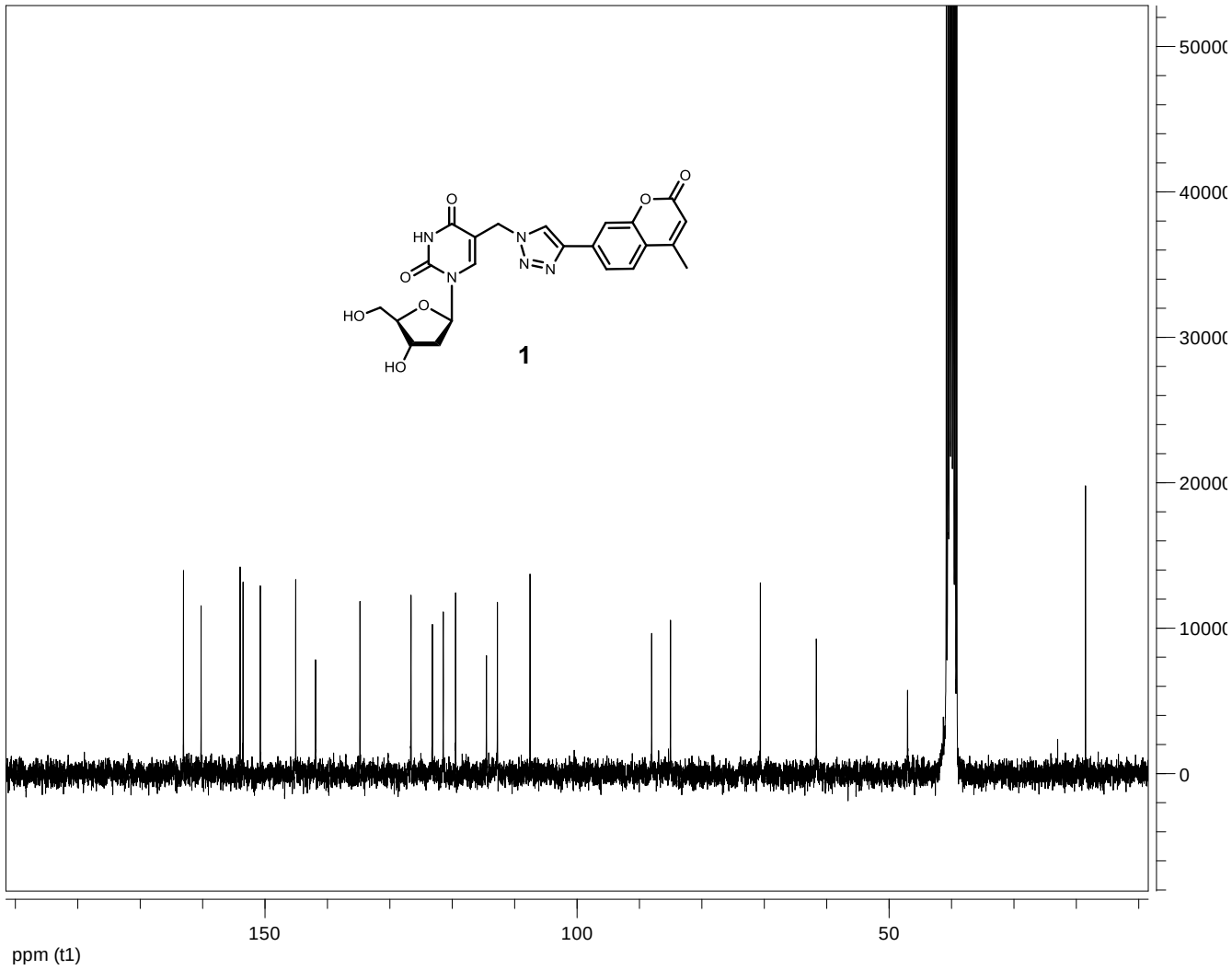


Figure S16. ^{13}C NMR (DMSO-d_6, 300 MHz) of nucleoside **1**

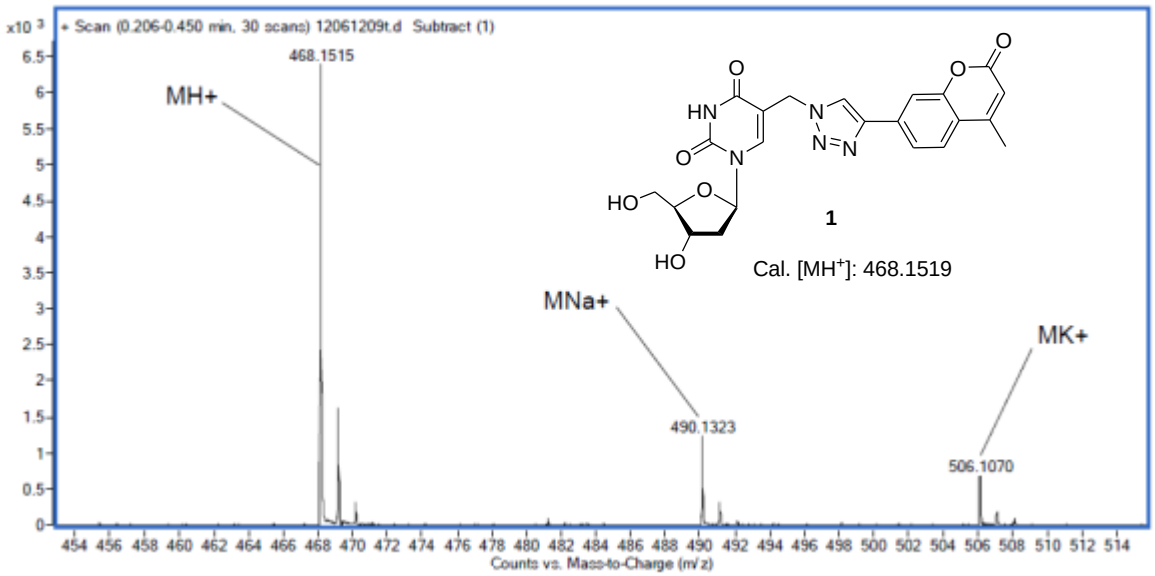


Figure S17. MS spectrum of nucleoside **1**

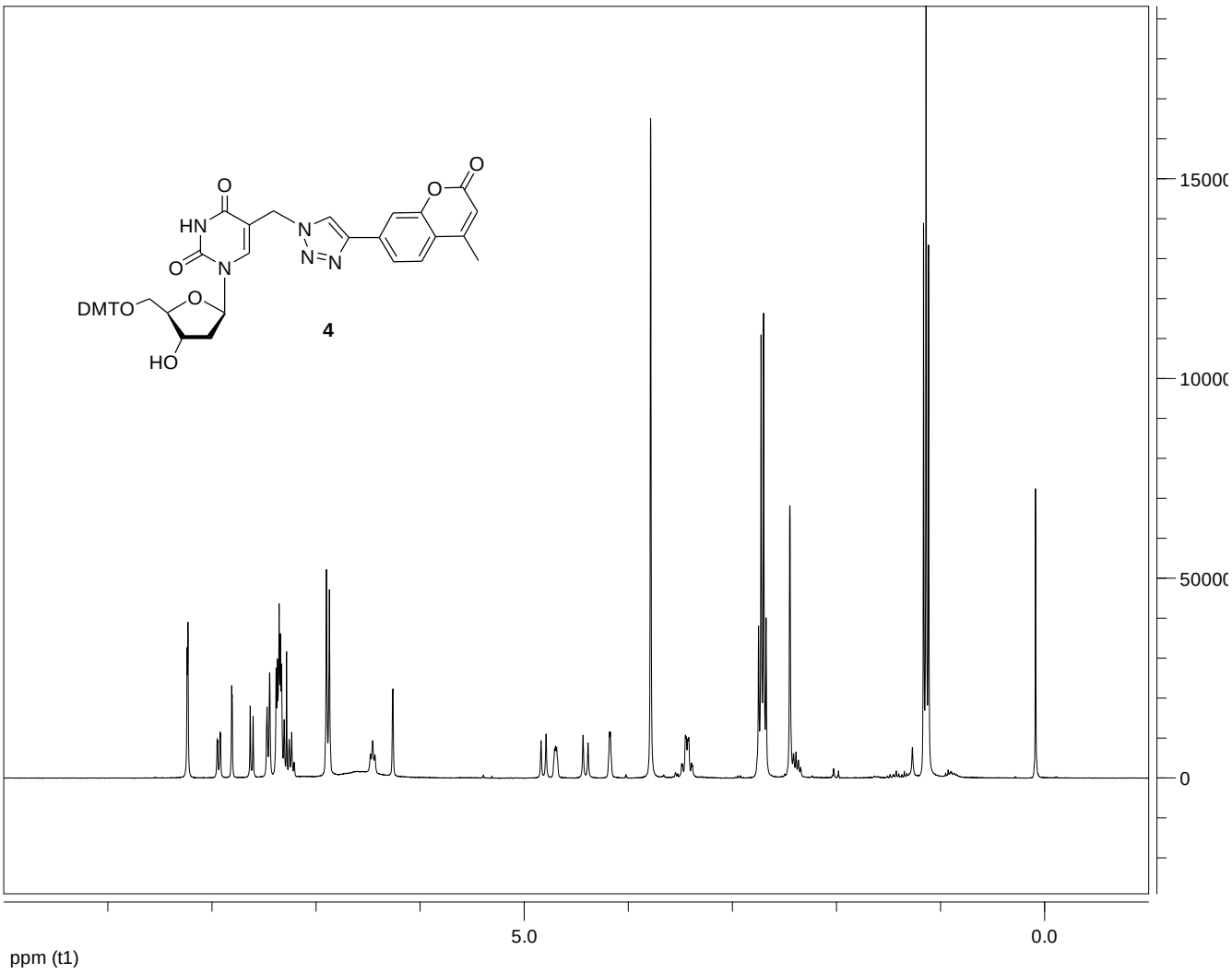


Figure S18. ^{1}H-NMR ($CDCl_3$, 300 MHz) of the DMT-protected nucleoside **4**.

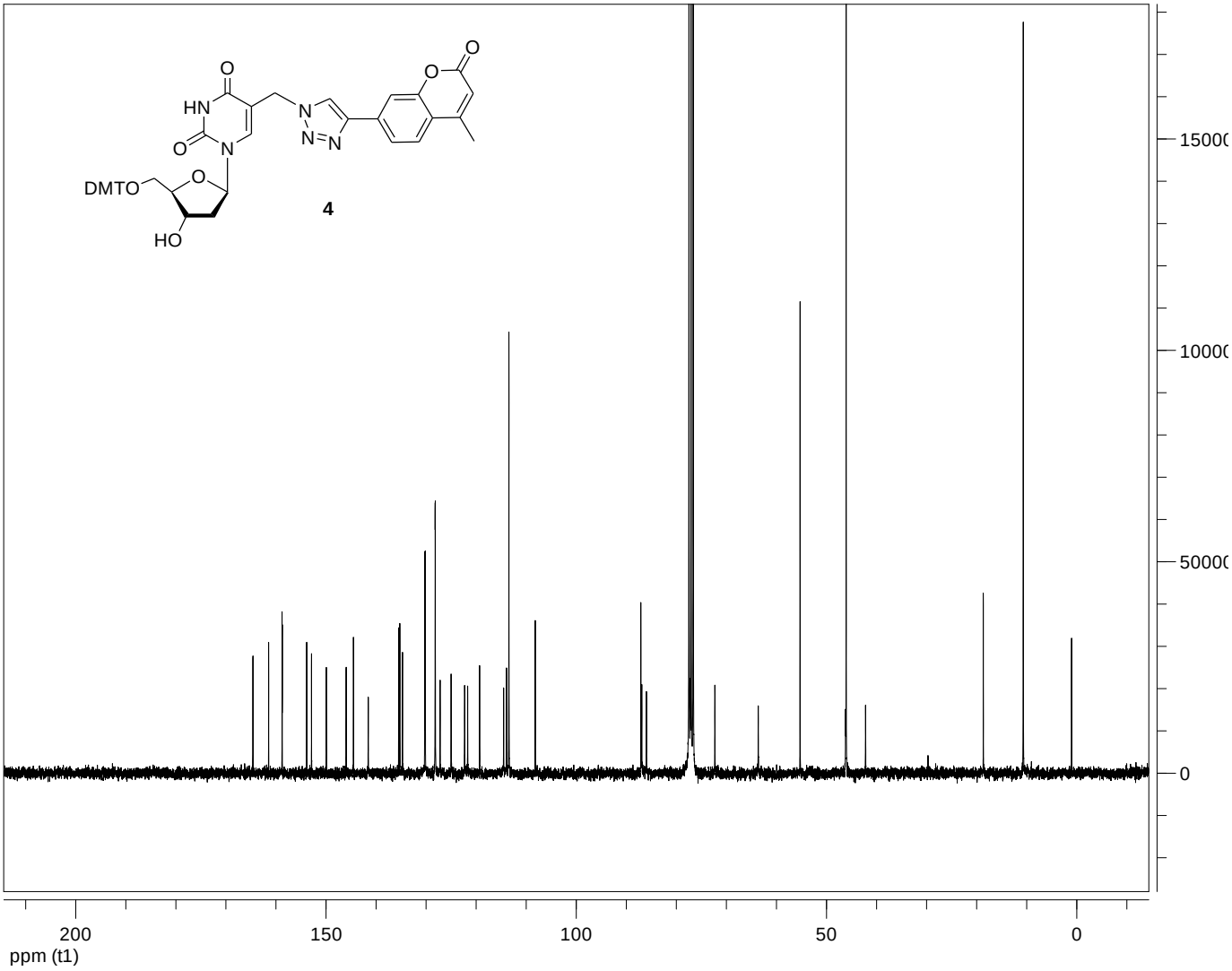
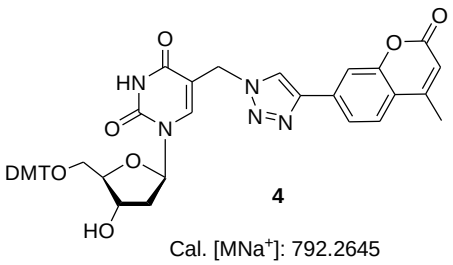


Figure S19. ^{13}C-NMR ($CDCl_3$, 300 MHz) of the DMT-protected nucleoside **4**.



4

Cal. [MNa^+]: 792.2645

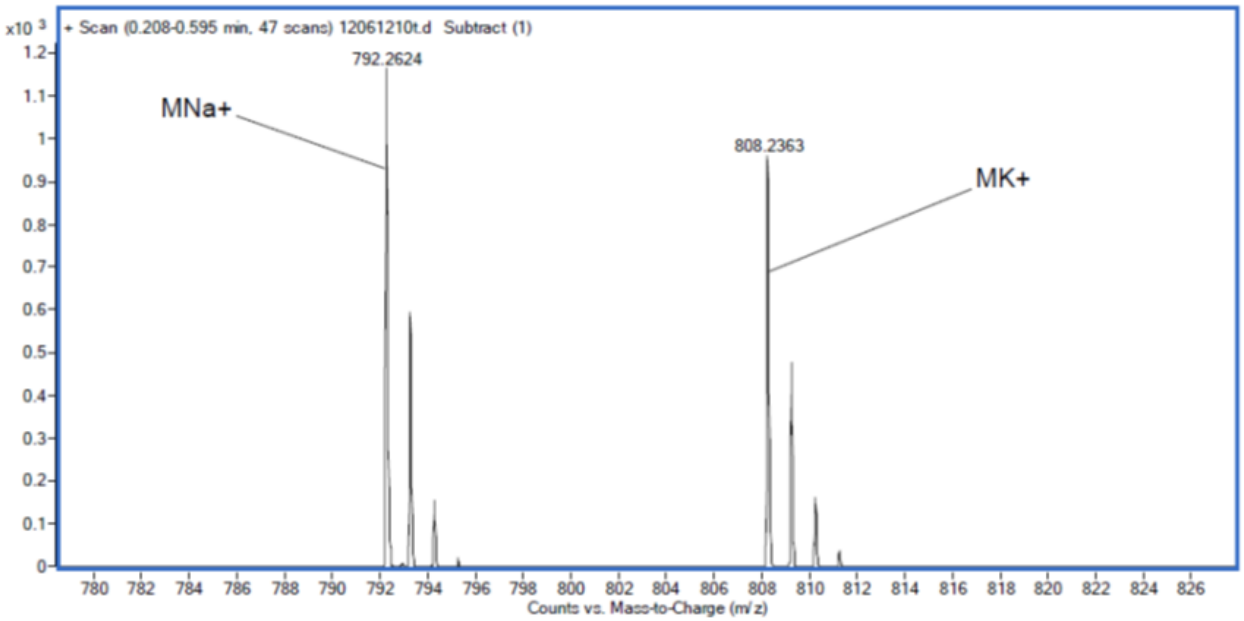


Figure S20. MS spectrum of compound **4**

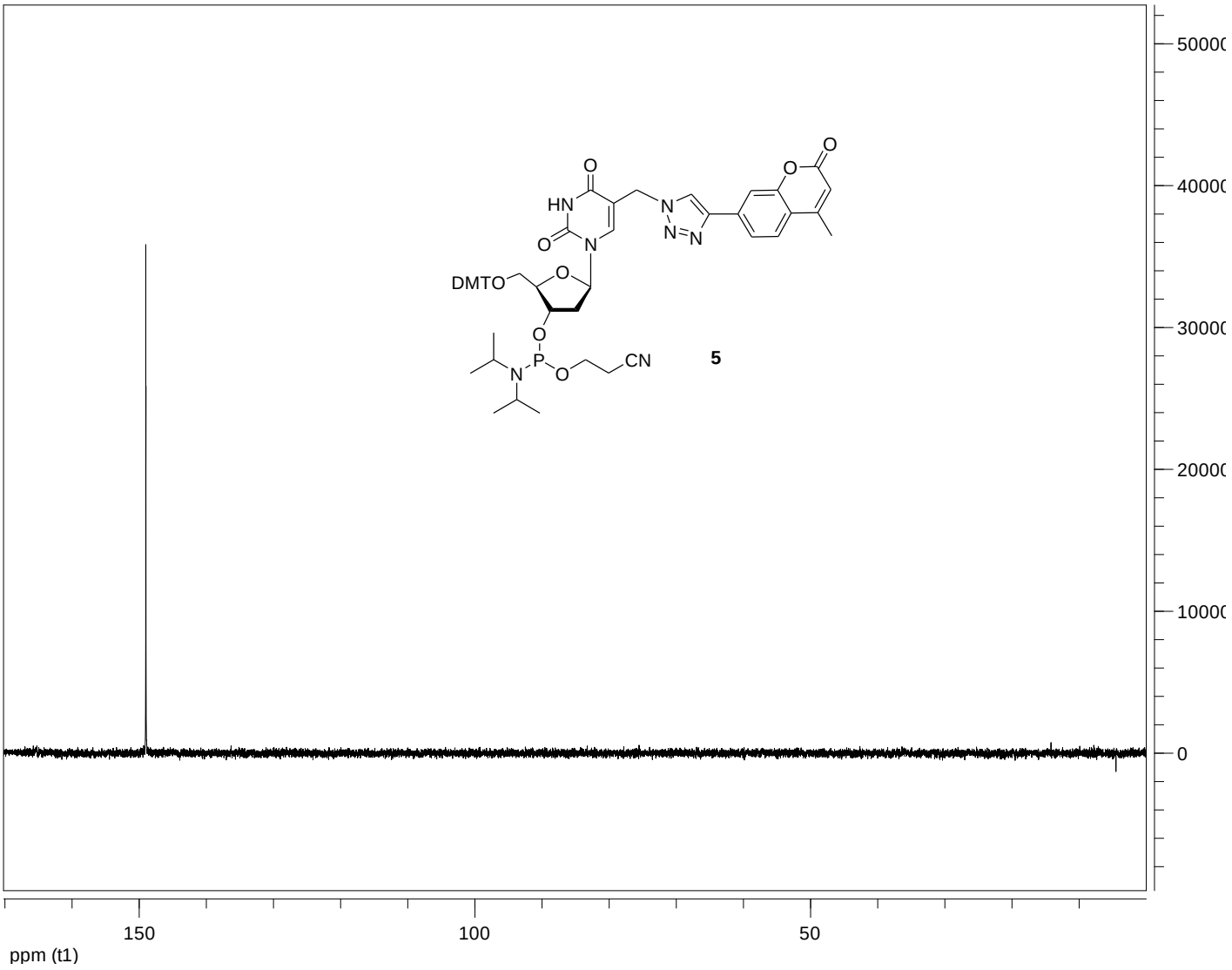


Figure S21. ^{31}P-NMR ($CDCl_3$ 300 MHz) of nucleoside **5**.

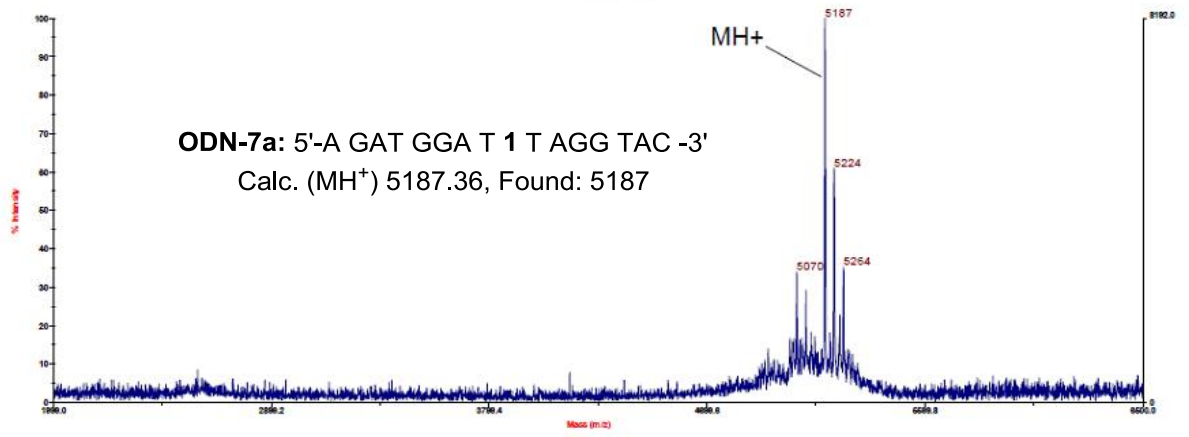


Figure S22. MS spectrum of ODN-**7a**.

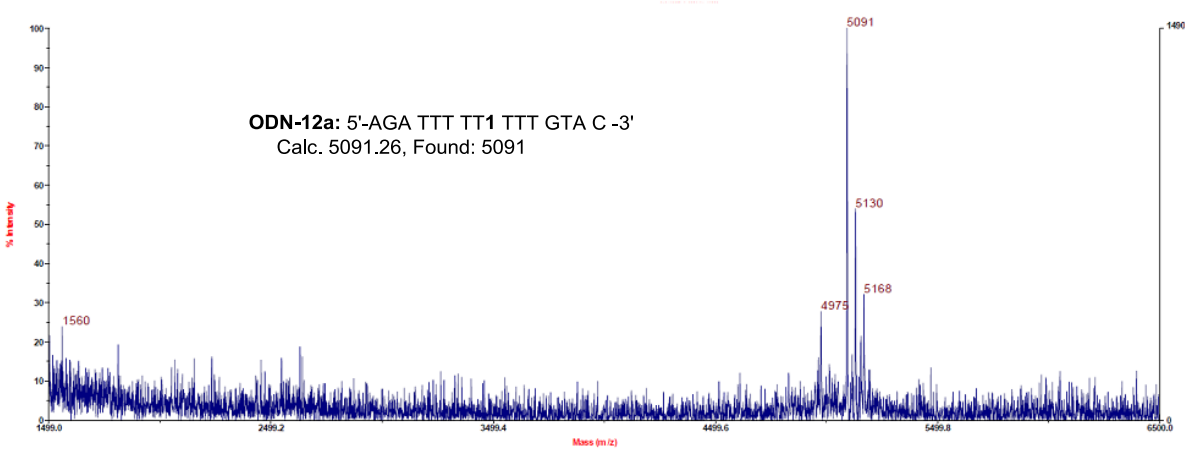


Figure S23. MS spectrum of ODN-**12a**.

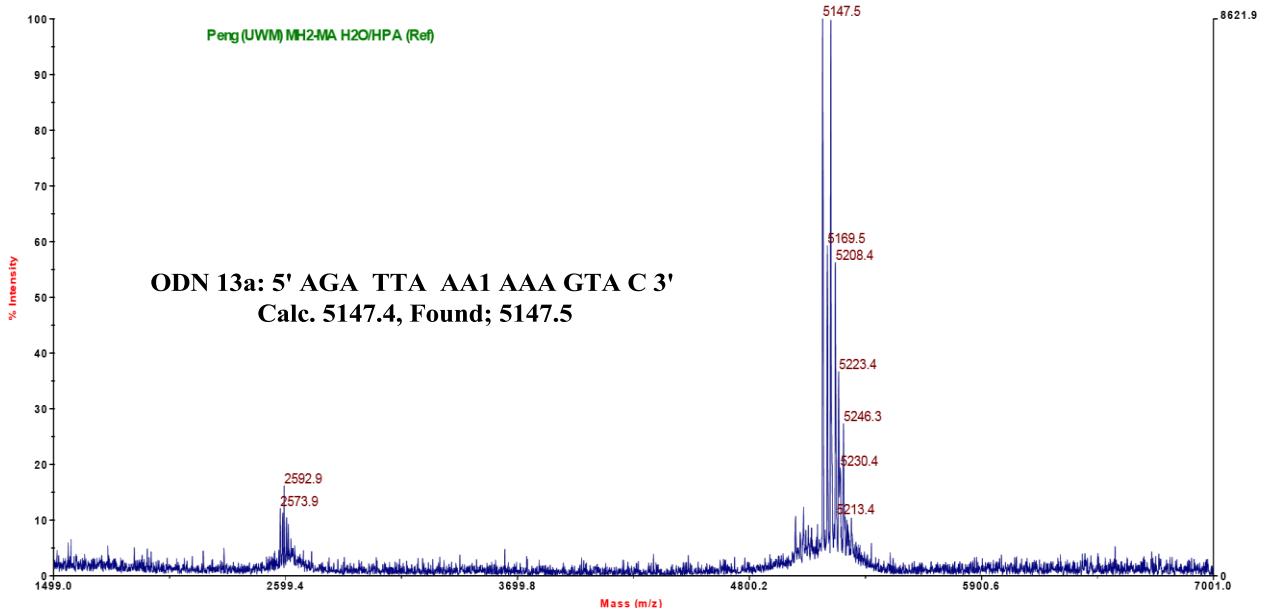


Figure S24. MS spectrum of ODN-**13a**.

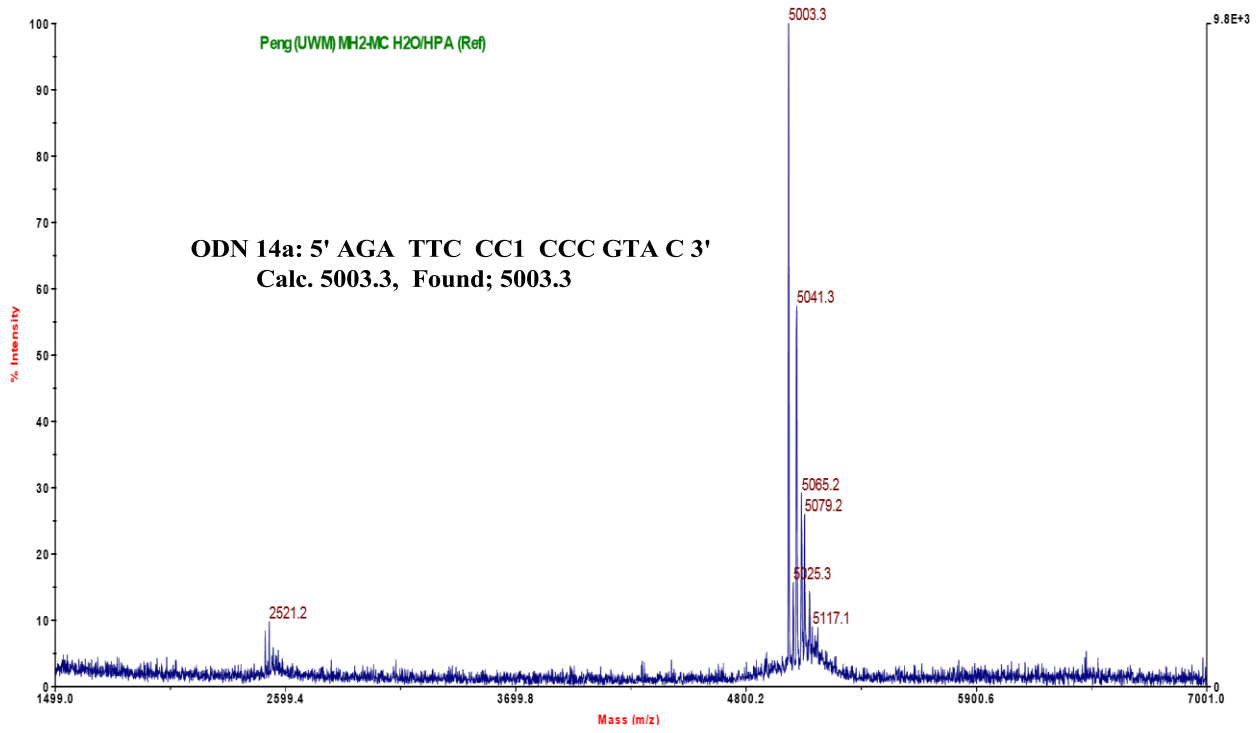


Figure S25. MS spectrum of ODN-**14a**.

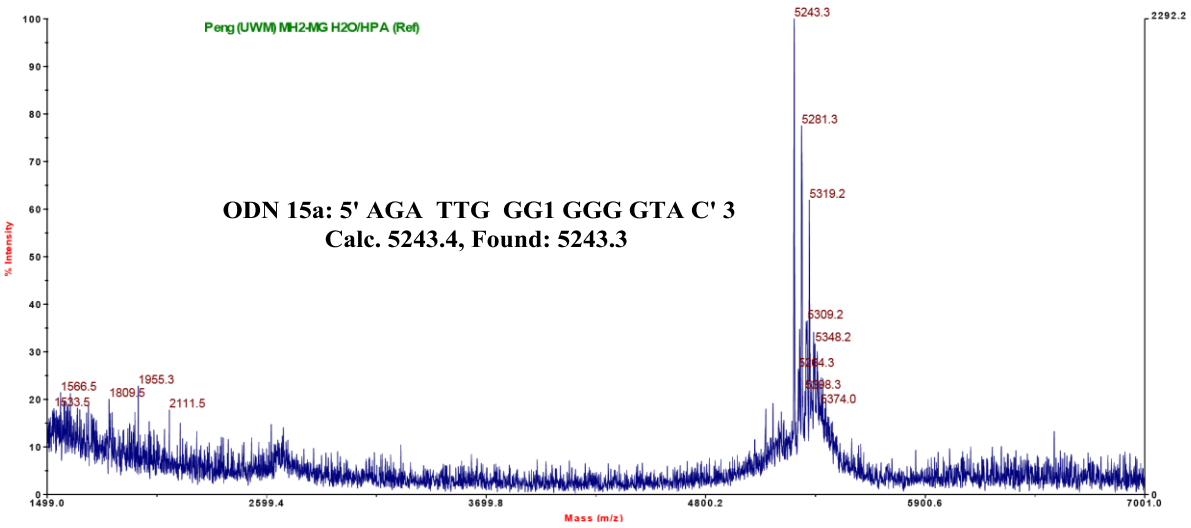


Figure S26. MS spectrum of ODN-**15a**.

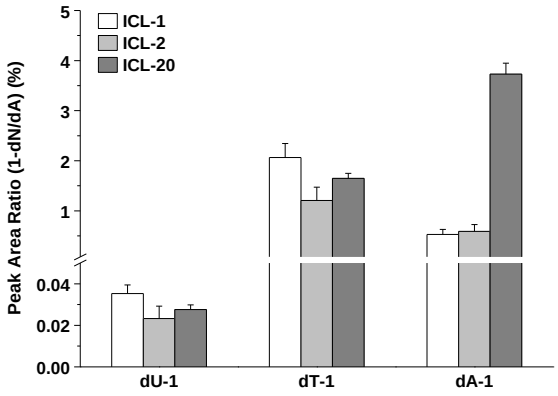


Figure S27. The ratio of area of peaks shown in SICs for monitoring the loss of a 2-deoxyribose from the $[M+H]^+$ ions of dU-**1**, dT-**1**, and dA-**1** cross-links to that for the $[M+H]^+$ ion of dA from a coumarin-modified ODN duplex **10** over one cycle of 350 nm irradiation (ICL-1), one cycle of 350 nm/254 nm irradiation (ICL-2), and ten cycles of 350 nm/254 nm UV irradiation (ICL-20).