Supporting Information

Alkyne-alkyne photo-cross-linking on the flipping-out field

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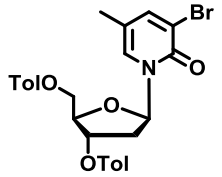
Contents

Materials and methods

The general chemicals were purchased from FUJIFILM Wako Pure Chemical, the Tokyo Chemical Industry, Kanto Chemical or Aldrich. The target DNAs and RNAs were purchased from JBioS (Japan). The ^{1}H NMR spectra (400 MHz) were recorded by a Bruker 400 spectrometer. The ^{1}H NMR spectra (600 MHz) and ^{13}C NMR spectra (150 MHz) were recorded by a Bruker AVANCE III 600 spectrometer. The high resolution electrospray mass analysis was performed by a Bruker MicrOTOFQ II. The HPLC purification was performed by a JASCO HPLC System (PU-2089Plus, UV-2075Plus, FP-2015Plus and CO-2065Plus). MALDI-TOF MS measurements were performed by a Bruker Autoflex speed instrument using a 3-hydroxypicolinic acid/diammonium hydrogen citrate matrix.

Synthesis of compound 3

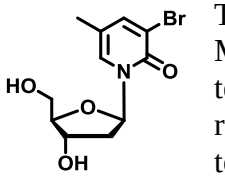


To a solution of 3-bromo-5-methyl-2-pyridone (582 mg, 3.10 mmol) in CH_3CN (2.0 mL), was added *N*,*O*-bis(trimethylsilyl)acetamide (760 µL, 3.10 mmol) and the mixture was stirred at room temperature. After 30 min, to the mixture were added CH_3CN (21.5 mL) and 2-deoxy-3,5-di-*O*-*p*-toluoyl-ribofuranosyl chloride (1.0 g, 2.58 mmol) and cooled to 0 ^{o}C. A solution of $SnCl_4$ in CH_2Cl_2 (1 M, 650 µL, 0.65 mmol) was added, and the mixture was stirred at room temperature. After 5 h, the mixture was diluted with EtOAc (230 mL). The organic phase was washed with saturated aqueous $NaHCO_3$ (90 mL×3) and brine (90 mL). The organic phase was dried over anhydrous Na_2SO_4, filtered, and concentrated under reduced pressure. The residue was purified by silica gel column chromatography (hexane/ EtOAc = 4/ 1) to give the compound **3** (β-anomer, 520 mg, 37%) as a white foam. The α-anomer (486 mg, 35%) was obtained as a white foam.

β-anomer: ^{1}H NMR ($CDCl_3$, 600 MHz) δ (ppm) 7.95 (2H, d, J = 8.4 Hz), 7.87 (2H, d, J = 8.4 Hz), 7.55 (1H, d, J = 2.4 Hz), 7.44 (1H, dd, J = 1.2, 2.4 Hz), 7.27 (2H, m), 7.22 (2H, m), 6.56 (1H, dd, J = 5.4, 8.4 Hz), 5.62 (1H, ddd, J = 1.8, 1.8, 6.6 Hz), 4.83 (1H, dd, J = 3.0, 12 Hz), 4.65 (1H, dd, J = 3.6, 12 Hz), 4.62 (1H, m), 3.02 (1H, ddd, J = 1.8, 5.4, 14.4 Hz), 2.43 (3H, s), 2.41 (3H, s), 2.24 (1H, ddd, J = 6.6, 8.4, 14.4 Hz), 1.84 (3H, s). ^{13}C NMR ($CDCl_3$, 150 MHz) δ (ppm) 166.2, 166.1, 157.4, 144.5, 144.4, 143.9, 129.5, 129.4, 129.3, 128.6, 126.5, 126.4, 115.6, 115.5, 87.4, 83.6, 75.1, 64.1, 39.3, 21.8, 21.7, 17.0. ESI-HRMS (*m*/*z*): $[M+H]^+$ calcd for $C_{27}H_{27}BrNO_6^+$, 540.1016, found 540.1019.

α-anomer: ^{1}H NMR ($CDCl_3$, 600 MHz) δ (ppm) 7.95 (2H, d, J = 8.4 Hz), 7.67 (1H, d, J = 2.4 Hz), 7.65 (2H, d, J = 7.8 Hz), 7.47 (1H,m), 7.27 (2H, d, J = 7.8 Hz), 7.17 (2H, d, J = 7.8 Hz), 6.47 (1H, dd, J = 1.2, 7.2 Hz), 5.60 (1H, d, J = 6.0 Hz), 4.95 (1H, dd, J = 4.2, 4.2 Hz), 4.55 (2H, m), 3.02 (1H, ddd, J = 6.6, 6.6, 15.6 Hz), 2.59 (1H, d, J = 15.6 Hz), 2.42 (3H, s), 2.39 (3H, s), 2.05 (3H, s). ^{13}C NMR ($CDCl_3$, 150 MHz) δ (ppm) 166.1, 165.4, 157.4, 144.4, 144.2, 143.8, 129.7, 129.5, 129.3, 129.1, 126.5, 126.3, 115.6, 114.6, 89.6, 85.8, 74.8, 64.1, 39.1, 21.7, 17.3. ESI-HRMS (*m*/*z*): $[M+H]^+$ calcd for $C_{27}H_{27}BrNO_6^+$, 540.1016, found 540.1022.

Synthesis of compound 4

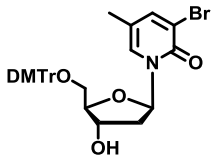


To a solution of **3** (636 mg, 1.17 mmol) in MeOH (5.8 mL), was added a solution of MeONa in MeOH (581 µL, 2.83 mmol) and the mixture was stirred at room temperature. After 1 h, the mixture was concentrated under reduced pressure. The residue was purified by silica gel column chromatography (CH_2Cl_2/ MeOH = 9/ 1) to give the compound **4** (352 mg, 98%) as a white solid.

^{1}H NMR (MeOD-d_4, 600 MHz): δ (ppm) 7.97 (1H, s), 7.82 (1H, d, J = 2.4 Hz), 6.39 (1H, t, J = 6.0 Hz), 4.36 (1H, m), 3.98 (1H, dd, J = 3.6, 7.2 Hz), 3.83 (1H, dd, J = 3.6, 12 Hz), 3.75 (1H, dd, J = 4.2,

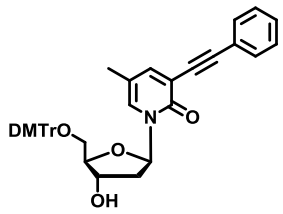
12 Hz), 2.48 (1H, ddd, *J* = 4.2, 6.0, 13.2 Hz), 2.12 (3H,s), 2.08 (1H, ddd, *J* = 6.0, 7.2, 13.2 Hz) ^{13}C NMR (MeOD-d_4, 150 MHz): δ (ppm) 159.2, 146.1, 131.8, 117.9, 115.2, 89.3, 88.4, 71.5, 62.4, 42.6, 17.0. ESI-HRMS (*m/z*): $[M+Na]^+$ calcd for $C_{11}H_{14}BrNNaO_4^+$, 325.9998, found 325.9996.

Synthesis of compound 5



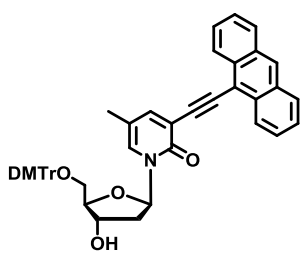
Compound **4** (245 mg, 0.81 mmol) was co-evaporated with acetonitrile and pyridine, then dissolved in pyridine (4.1 mL). To the solution was added DMTrCl (548 mg, 1.62 mmol), and the mixture was stirred at room temperature for 1 h. The reaction mixture was then diluted with EtOAc (150 mL), washed with water (90 mL) and brine (90 mL). The organic layer was dried over Na_2SO_4, then concentrated under reduced pressure. The crude was purified by silica gel column chromatography (Hexane:Ethyl acetate = 3:1→2:1→1:3) to give **5** as a white foam (463 mg, 95%).
^{1}H NMR ($CDCl_3$, 400 MHz): δ (ppm) 7.79 (1H, s), 7.60 (1H, d, *J* = 2.4 Hz), 7.40 (2H, m), 7.31-7.27 (6H, m), 7.22 (1H, m), 6.82 (4H, dd, *J* = 3.6, 14.4 Hz), 6.57 (1H, t, *J* = 6.0 Hz), 4.51 (1H, m), 4.21 (1H, dt, *J* = 3.6, 6.6 Hz), 3.79 (6H, s), 3.51 (1H, dd, *J* = 3.6, 10.2 Hz), 3.37-3.35 (2H, m), 2.79 (1H, ddd, *J* = 3.6, 6.6, 13.8 Hz), 2.24 (1H, ddd, *J* = 6.6, 7.2, 13.8 Hz), 1.75 (3H, s). ^{13}C NMR ($CDCl_3$, 150 MHz): δ (ppm) 158.6, 157.7, 144.5, 144.1, 135.6, 135.5, 130.1, 130.0, 129.8, 128.1, 127.9, 127.0, 115.6, 115.2, 113.2, 87.3, 86.8, 72.2, 63.4, 55.2, 42.3, 16.9. ESI-HRMS (*m/z*): $[M+Na]^+$ calcd for $C_{32}H_{32}BrNNaO_6^+$, 628.1305, found 628.1302.

Synthesis of compound 6-Ph



To a solution of **5** (33.7 mg, 0.056 mmol) in DMF (100 µL) were added ethynylbenzene (6.8 mg, 0.067 mmol), trimethylamine (140 µL, 1.0 mmol), $Pd(PPh_3)_4$ (13 mg, 0.011 mmol) and CuI (2.0 mg, 0.011 mmol). The reaction mixture was stirred at 80 °C for 4 h and then allowed to cool at room temperature. The reaction mixture was diluted with EtOAc and filtered through Celite. The filtrate was concentrated under reduced pressure and the residue was diluted with water (15 mL). The mixture was extract with EtOAc (15 mL×3) and the organic phases were washed with brine (15 mL), dried over Na_2SO_4, then concentrated under reduced pressure. The crude was purified by silica gel column chromatography (Hexane:Ethyl acetate = 1:1) to give **6(Ph)** as a white foam (24.9 mg, 71%).
^{1}H NMR ($CDCl_3$, 600 MHz): δ (ppm) 7.79 (1H, s), 7.56 (2H, dd, *J* = 7.8, 1.8 Hz), 7.48 (1H, d, *J* = 2.4 Hz), 7.41 (2H, m), 7.31-7.26 (9H, m), 7.21 (1H, m), 6.81 (4H, dd, *J* = 9.0, 2.4 Hz), 6.61 (1H, t, *J* = 6.0 Hz), 4.51 (1H, m), 4.23 (1H, m), 3.78 (6H, s), 3.49 (1H, dd, *J* = 10.2, 3.6 Hz), 3.33 (1H, dd, *J* = 10.2, 3.6 Hz), 2.82 (1H, ddd, *J* = 13.2, 6.0, 3.6 Hz), 2.23 (1H, ddd, *J* = 13.2, 7.2, 6.0 Hz), 1.78 (3H, s). ^{13}C NMR ($CDCl_3$, 150 MHz): δ (ppm) 160.7, 158.7, 145.0, 144.7, 135.8, 135.8, 131.9, 130.6, 130.2, 128.4, 128.3, 128.3, 128.1, 127.1, 123.3, 115.3, 115.1, 113.4, 113.2, 94.9, 86.9, 86.8, 86.7, 85.2, 72.3, 63.6, 55.4, 42.5, 17.2. ESI-HRMS (*m/z*): $[M+Na]^+$ calcd for $C_{40}H_{37}NNaO_6^+$, 650.2513, found 650.2515.

Synthesis of compound 6-An

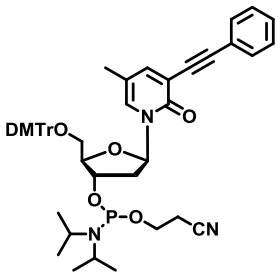


To a solution of **5** (65 mg, 0.11 mmol) in DMF (1.1 mL) were added 9-ethynylanthracene (55 mg, 0.28 mmol), trimethylamine (154 µL, 1.1 mmol), $Pd(PPh_3)_4$ (25 mg, 0.022 mmol) and CuI (4.2 mg, 0.022 mmol). The reaction mixture was stirred at 80 °C for 1.5 h and then allowed to cool at room temperature. The reaction mixture was diluted with EtOAc and filtered through Celite. The filtrate was concentrated under reduced pressure and the residue was diluted with water (45 mL). The mixture was extract with EtOAc (45 mL×3) and the organic phases were washed with brine (45 mL), dried over Na_2SO_4, then concentrated under reduced pressure. The crude was purified

by silica gel column chromatography (Hexane:Ethyl acetate = 1:1) to give **6(An)** as a red brown foam (74.8 mg, 96%).

^{1}H NMR ($CDCl_3$, 600 MHz): δ (ppm) 8.78 (2H, dd, *J* = 1.2, 9.0 Hz), 8.41 (1H, s), 7.99 (2H, d, *J* = 8.4 Hz), 7.82 (1H, m), 7.70 (1H, d, *J* = 2.4 Hz), 7.59 (2H, t, *J* = 7.2 Hz), 7.47 (2H, t, *J* = 7.2 Hz), 7.42 (2H, d, *J* = 7.2 Hz), 7.32-7.22 (7H, m), 6.82 (4H, m), 6.68 (1H, t, *J* = 6.6 Hz), 4.55 (1H, m), 4.20 (1H, dt, *J* = 3.6, 6.6 Hz), 3.77 (6H, s), 3.53 (1H, dd, *J* = 3.6, 10.8 Hz), 3.36 (1H, dd, *J* = 3.6, 10.8), 2.83 (1H, ddd, *J* = 4.2, 6.0, 13.2 Hz), 2.70 (1H, br), 2.34 (1H, ddd, *J* = 6.0, 7.2, 13.2 Hz), 1.85 (3H, s). ^{13}C NMR ($CDCl_3$, 150 MHz): δ (ppm) 160.7, 158.8, 144.7, 144.5, 135.7, 132.8, 131.3, 130.6, 130.2, 128.7, 128.3, 128.1, 128.0, 127.3, 127.2, 126.9, 125.9, 117.5, 115.8, 115.3, 113.4, 97.0, 92.1, 87.0, 86.6, 86.4, 72.3, 63.5, 55.4, 42.5, 17.3. ESI-HRMS (*m/z*): $[M+Na]^+$ calcd for $C_{48}H_{41}NNaO_6^+$, 750.2826, found 750.2822.

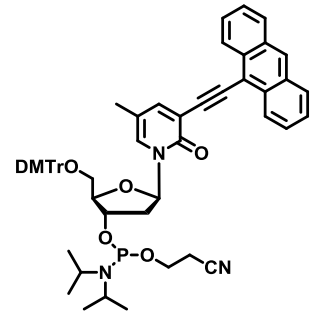
Synthesis of compound 7-Ph



Compound **6a** (24.9 mg, 0.040 mmol) was co-evaporated with acetonitrile and dissolved in CH_2Cl_2 (0.5 mL). To the solution were added DIPEA (42 μL, 0.24 mmol) and 2-cyanoethyl *N*,*N*-diisopropylchlorophosphoramidite (23 μL, 0.10 mmol), and the mixture was stirred at room temperature for 1 h. The reaction mixture was then quenched with MeOH (50 μL) and saturated aqueous $NaHCO_3$ (11 mL), and extracted three times with EtOAc (15 mL). The combined organic layers were washed with brine (10 mL), dried over Na_2SO_4, and concentrated under reduced pressure. The residue was purified by silica gel column chromatography (Hexane/Ethylacetate = 3:1) to give **7(Ph)** as a white solid (18.5 mg, 56%).

^{31}P NMR ($CDCl_3$, 162 MHz): δ (ppm) 149.2, 148.4. ESI-HRMS (*m/z*): $[M+Na]^+$ calcd for $C_{49}H_{54}N_3NaO_7P^+$, 850.3592, found 850.3590.

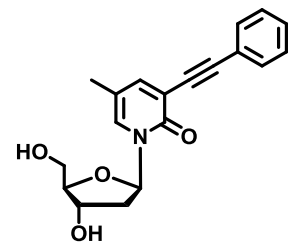
Synthesis of compound 7-An



Compound **6b** (70.6 mg, 0.096 mmol) was co-evaporated with acetonitrile and toluene, then dissolved in CH_2Cl_2 (1.2 mL). To the solution were added DIPEA (100 μL, 0.58 mmol) and 2-cyanoethyl *N*,*N*-diisopropylchlorophosphoramidite (54 μL, 0.24 mmol), and the mixture was stirred at room temperature for 1 h. The reaction mixture was then quenched with MeOH (50 μL) and saturated aqueous $NaHCO_3$ (15 mL), and extracted with EtOAc (20 mL). The combined organic layers were washed with brine (15 mL), dried over Na_2SO_4, and concentrated under reduced pressure. The residue was purified by silica gel column chromatography (Hexane/ Ethylacetate = 3:1) to give **7(An)** as a white solid (45.1 mg, 51%).

^{31}P NMR ($CDCl_3$, 162 MHz): δ (ppm) 149.1, 148.4. ESI-HRMS (*m/z*): $[M+Na]^+$ calcd for $C_{57}H_{58}N_3NaO_7P^+$, 928.4085, found 928.4065.

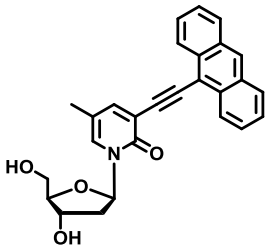
Synthesis of compound 8-Ph



To a solution of **6a** (21 mg, 0.033 mmol) in CH_2Cl_2 (550 μL), was added TFA (5.0 μL, 0.066 mmol) at 0 ^{o}C and the mixture was stirred. After 20 min, the reaction mixture was quenched with saturated aqueous $NaHCO_3$ (15 mL) and diluted with CH_2Cl_2 (100 mL). The organic phase was washed with saturated aqueous $NaHCO_3$ (30 mL×2), dried over Na_2SO_4, and concentrated under reduced pressure. The residue was purified by silica gel column chromatography (Hexane/ Ethylacetate = 1:2 → CH_2Cl_2/ MeOH = 9:1) to give **8(Ph)** as a white solid (8.2 mg, 77%).

^{1}H NMR (MeOD-d_4, 400 MHz): δ (ppm) 8.00 (1H, s), 7.67 (1H, d, J = 2.4 Hz), 7.57-7.55 (2H, m), 7.37-7.35 (3H, m), 6.46 (1H, t, J = 6.4 Hz), 4.39 (1H, ddd, J = 6.0, 4.0, 4.0 Hz), 4.01 (1H, dt, J = 7.6, 4.0 Hz), 3.86 (1H, dd, J =12.0, 4.0 Hz), 3.76 (1H, dd, J =12.0, 4.0), 2.52 (1H, ddd, J = 13.2, 6.4, 4.0 Hz), 2.15 (3H, s), 2.13 (1H, ddd, J = 13.2, 6.8, 6.4 Hz). ^{13}C NMR (MeOD-d_4, 150 MHz): δ (ppm) 162.3, 146.8, 132.7, 132.6, 129.7, 129.5, 124.4, 117.6, 115.6, 95.4, 89.3, 87.8, 85.6, 71.7, 62.5, 42.7, 17.2. ESI-HRMS (*m/z*): $[M+H]^+$ calcd for $C_{19}H_{20}NO_4^+$, 326.1387, found 326.1389.

Synthesis of compound 8-An



To a solution of **6b** (21 mg, 0.028 mmol) in CH_2Cl_2 (500 μL), was added TFA (4.3 μL, 0.056 mmol) at 0 ^{o}C and the mixture was stirred. After 1 h, the reaction mixture was quenched with saturated aqueous $NaHCO_3$ (15 mL) and diluted with CH_2Cl_2 (100 mL). The organic phase was washed with saturated aqueous $NaHCO_3$ (30 mL×2), dried over Na_2SO_4, and concentrated under reduced pressure. The residue was purified by silica gel column chromatography (Hexane/ Ethylacetate = 1:2 → CH_2Cl_2/ MeOH = 9:1) to give **8(An)** as a white solid (7.2 mg, 60%).

^{1}H NMR (MeOD-d_4, 600 MHz): δ (ppm) 8.73 (2H, dd, J = 8.4, 1.2 Hz), 8.68 (1H, s), 8.16 (2H, d, J = 8.4 Hz), 7.98 (1H, d, J = 2.4 Hz), 7.96 (1H, dd, J = 1.2, 2.4 Hz), 7.71-7.68 (2H, m), 7.63-7.60 (2H, m), 6.47 (1H, t, J = 6.6 Hz), 4.30 (1H, m), 3.91 (1H, dt, J = 7.8, 3.6 Hz), 3.70 (1H, dd, J = 3.6, 12.0 Hz), 3.63 (1H, dd, J = 4.2, 12.0 Hz), 2.37 (1H, ddd, J = 13.2, 6.6, 3.6 Hz), 2.15 (3H, s), 2.07 (1H, J = 13.2, 6.6, 6.0 Hz). ^{13}C NMR (MeOD-d_4, 150 MHz,): δ (ppm) 162.3, 146.3, 133.8, 133.1, 132.8, 132.7, 130.0, 129.8, 129.2, 128.0, 126.9, 118.0, 117.7, 116.1, 97.7, 92.5, 89.3, 87.9, 71.6, 62.5, 42.8, 17.3. ESI-HRMS (*m/z*): $[M+H]^+$ calcd for $C_{27}H_{24}NO_4^+$, 426.1700, found 426.1695.

Preparation of DNA oligonucleotides

DNA oligonucleotides were synthesized following standard protocols on a 1.0 μmol scale on an Applied Biosystems model 392 DNA/RNA synthesizer. Deprotection and cleavage from the CPG support were carried out under a mild condition with 28% ammonia solution for 4 h at room temperature. Then, the oligonucleotides were purified by HPLC (JASCO HPLC System: PU-2089Plus, UV-2075Plus, FP-2015Plus and CO-2065Plus). HPLC purification conditions: C-18 column (Nacalai tesque: COSMOSIL 5C_{18}-MS-II, 10 x 250 mm) by a linear gradient of 10%-40%/20min acetonitrile in H_2O at a flow rate of 4 mL/min at 40 ^{o}C. Peaks were monitored by UV detector (λ = 254 nm). The concentration of the ODNs was determined by UV absorption at 260 nm.

Fluorescence measurement with 2-aminopurine-containing ODN

A mixture (50 μL) of the duplex (5.0 μM) in MES buffer (50 mM, pH 7.0) containing NaCl (100 mM) was transferred to a quartz cell with a 3-mm path length. The emission spectra were obtained with an excitation wavelength at 310 nm at 25 ^{o}C. The fluorescence measurement was performed by a FP-6500 (JASCO Corporation) with a temperature controller.

Melting temperature (T_m) measurement (UV)

A mixture (325 μL) of the duplex (4.0 μM) in MES buffer (50 mM, pH 7.0) containing NaCl (100 mM) was transferred to a microquartz cell with a 1-cm path length. The melting temperature was then measured under UV absorption at 260 nm from 15 to 90 ^{o}C at the rate of 1 ^{o}C/min. The measurements were carried out three times per each sample and averaged for obtaining the final value. The melting temperature measurement was performed by a DU-800 (Beckman-coulter) with a temperature controller.

Melting temperature (T_m) measurement (CD)

A mixture (100 μL) of the duplex (4.0 μM) in phosphate buffer (20 mM, pH 7.0) containing NaCl (20 mM) was transferred to a micro quartz cell with a 1-cm path length. For the melting temperature measurement, the ellipticity at 285 nm was recorded from 20 °C to 85 °C at an interval of 1 °C, with temperature increase at a rate of 1 °C/min. The measurements were repeated three times. CD spectra were recorded on a J-720WI (JASCO Co., Hachioji, Japan) equipped with a Peltier temperature controller.

Photo-crosslinking reaction (PAGE analysis)

A mixture (10 μL) of the duplex (1.0 μM) in MES buffer (50 mM, pH 7.0) containing NaCl (100 mM) in a PCR tube (BIO-BIK) was exposed to 360 nm light (300 W Xe lamp, 4.2 mW/ cm^2, SM-25Xe, Bunkoukeiki Co.) at room temperature. The distance between the lamp and sample was 5 cm. Aliquots of the reaction mixture was collected at various point of time and quenched by addition of loading buffer (95% formamide, 50mM EDTA pH 8.0). PAGE was performed on a 20% polyacrylamide gel electrophoresis with 1X TBE and 7.5 M urea at 300 V for 40 min. The gel was stained by a SYBR gold and ODNs were visualized with FLA-5100 (Fujifilm Co., Tokyo, Japan).

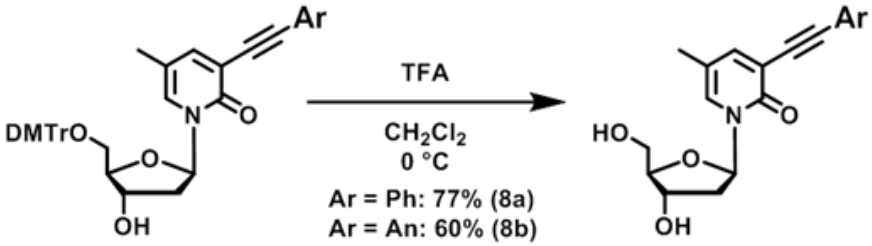
Photo-crosslinking reaction (HPLC analysis)

A mixture (100 μL) of the duplex (5.0 μM) in MES buffer (50 mM, pH 7.0) containing NaCl (100 mM) in a PCR tube (BIO-BIK) was exposed to 360 nm or 440 nm light (300 W Xe lamp, 4.2 or 8.4 mW/ cm^2, SM-25Xe, Bunkoukeiki Co.) at room temperature. The distance between the lamp and sample was 5 cm. After 1 or 10 min, the reaction mixture was analyzed by HPLC. HPLC analysis conditions: C-18 column (CAPCELL PAK C_{18} MGII, Shiseido, 4.6×250 mm) by a linear gradient of 5%-30%/20 min and 30%-100%/25 min acetonitrile in H_2O at a flow rate of 1 mL/min at 40 ^oC. Peaks were monitored by UV detector (λ = 254 nm).

Table S1. MALDI-TOF mass data of ODNs synthesized in this study

Entry	X or Z	[a]Calcd.	[b]Found
ODN1	X = Ph	3109.9	3110.1
ODN1	X = An	3209.9	3212.0
ODN3	Z = Ph	3084.6	3081.2
ODN3	Z = An	3184.6	3185.2

[a] $[M-H]^-$, [b] All data were collected in negative mode.



Scheme S1. Synthesis of diol compounds (**8**).

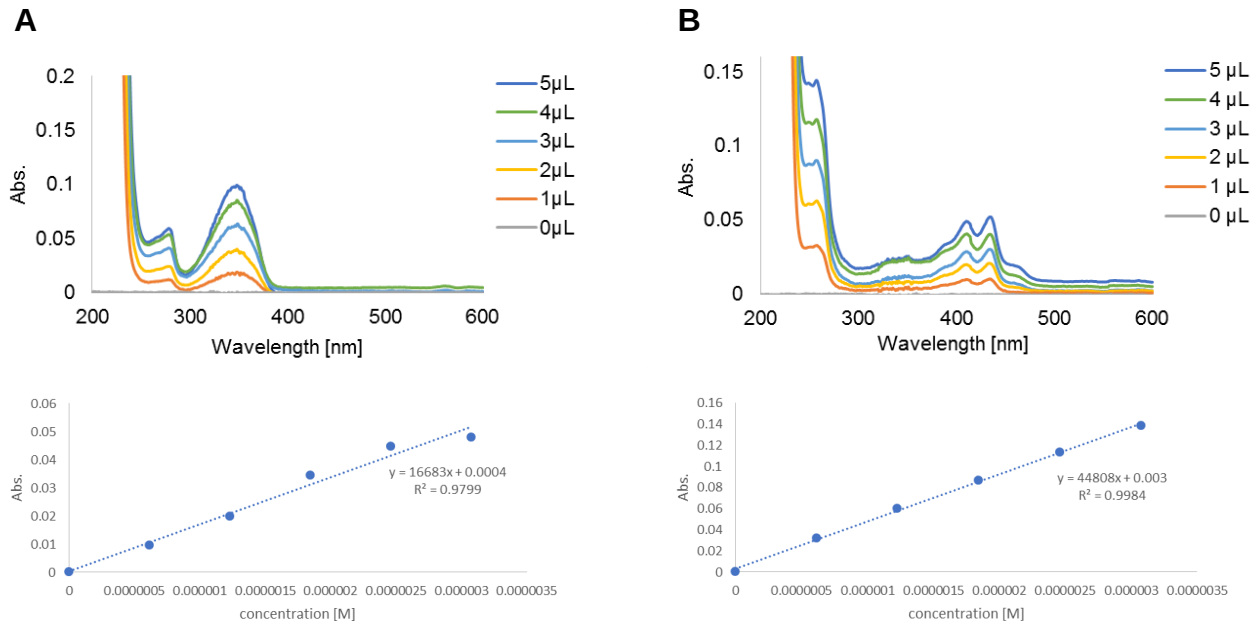


Figure S1. Calculation of the molar extinction coefficients at 260 nm (ε_{260}) of Ph and An. The UV spectra and titration graphs of (A) **8-Ph** and (B) **8-An**. The DMSO solution (200 μM) of the nucleoside was titrated to H_2O (325 μL). The molar extinction coefficients were calculated by the average of three individual titrations.

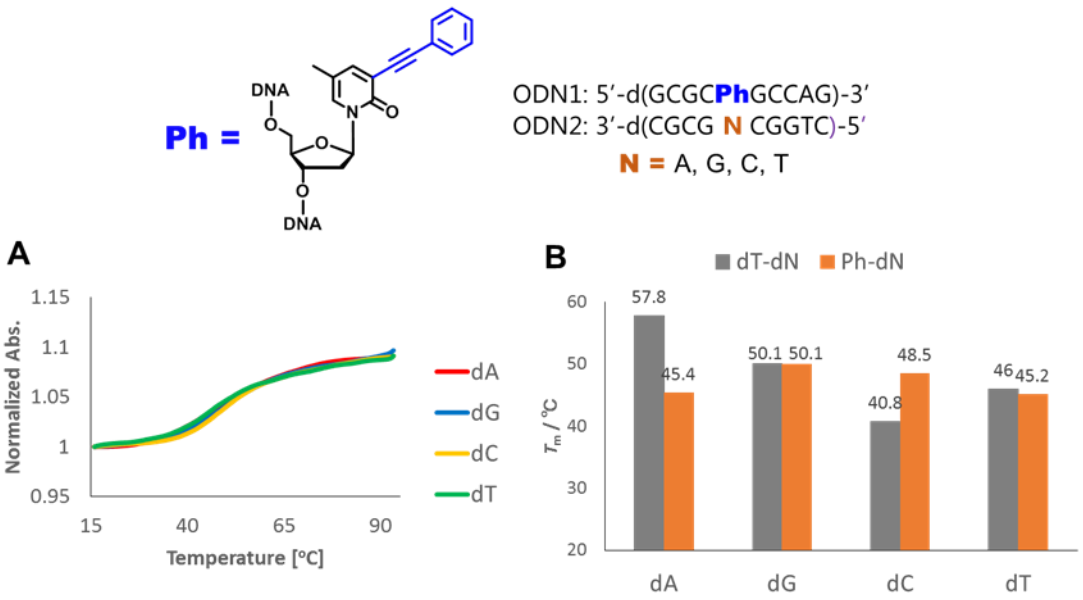


Figure S2. T_m measurement of Ph. (A) UV-melting curves of ODN**1**(X = Ph)-ODN**2** duplex. (B) T_m values of ODN**1**(X = dT or Ph)-ODN**2** duplex. The T_m values were measured using duplex (4.0 μM) in MES buffer (50 mM, pH 7.0) containing NaCl (100 mM).

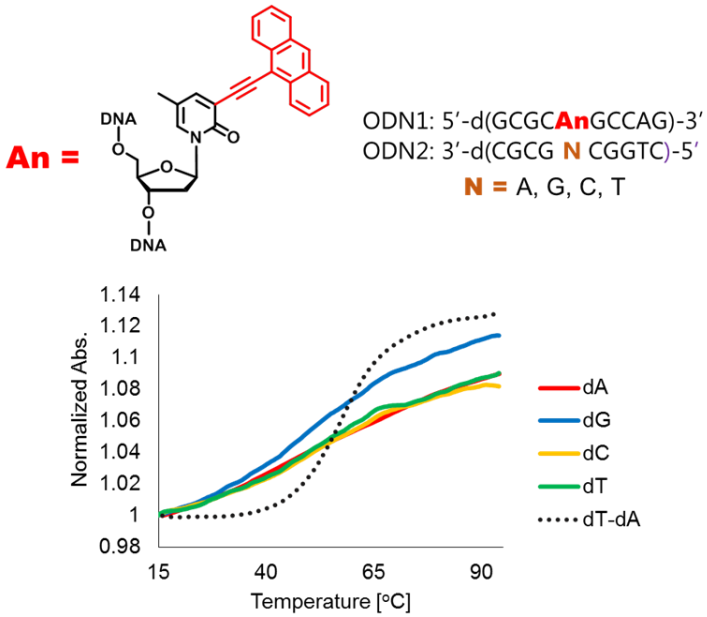


Figure S3. UV-melting curves of ODN**1**(X = An)-ODN**2** (solid line) or ODN**1**(X = dT)-ODN**2** (dotted line) duplex. The T_m measurements were performed using duplex (4.0 μM) in MES buffer (50 mM, pH 7.0) containing NaCl (100 mM).

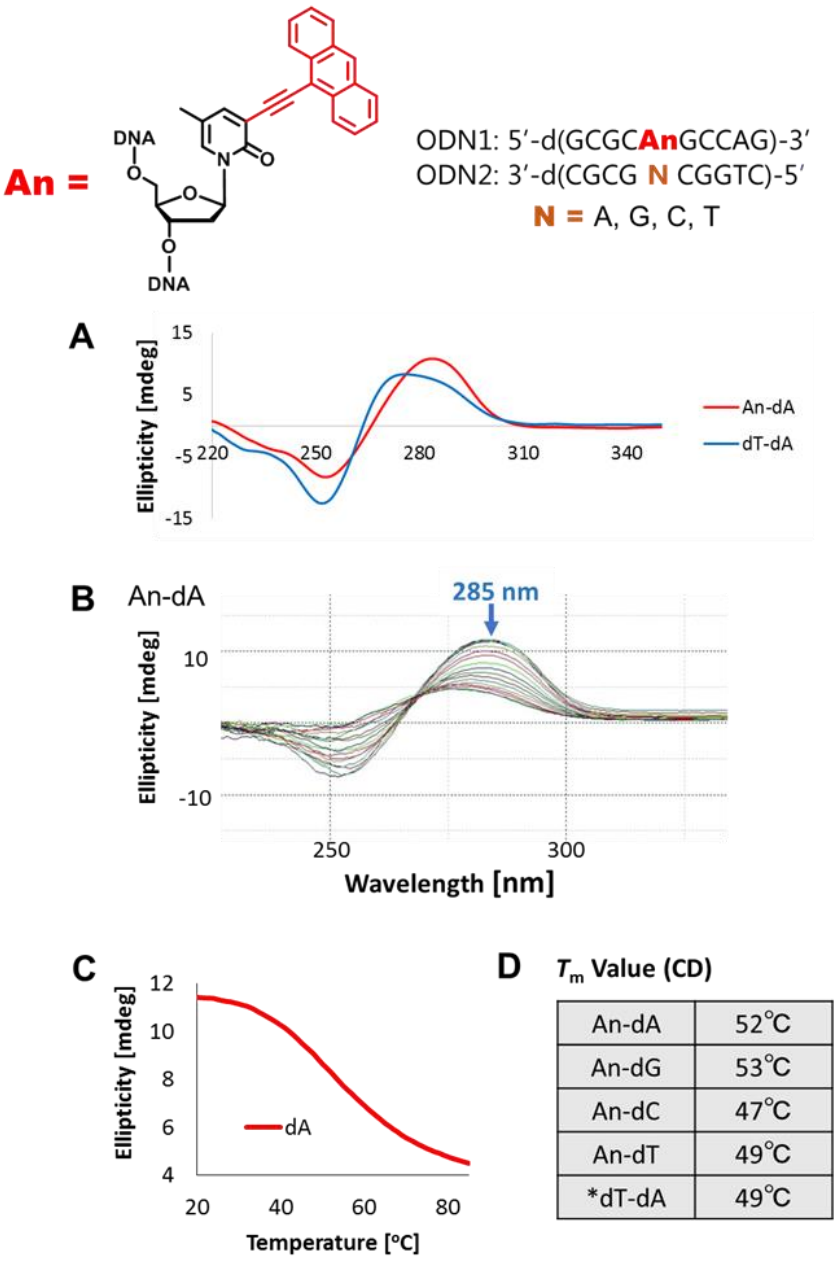


Figure S4. T_m measurement of An by CD. (A) CD spectra of ODN**1**(X = An or dT)-ODN**2** duplex. (B) CD spectra change of ODN**1**(An)-ODN**2**(dA) duplex. The CD spectra were recorded from 20°C to 80°C at an interval of 4°C. (C) CD-melting curve of ODN**1**(An)-ODN**2**(dA) duplex was recorded at 285 nm from 20°C to 85°C at an interval of 1°C, with temperature increase at a rate of 1°C/min. The T_m measurements were performed using duplex (4.0 μM) in phosphate buffer (20 mM, pH 7.0) containing NaCl (20 mM). (D) The T_m values of ODN**1**(An)-ODN**2** or ODN**1**(dT)-ODN**2**(dA) duplex. *The T_m measurement of ODN**1**(dT)-ODN**2**(dA) duplex was recorded at 270 nm.

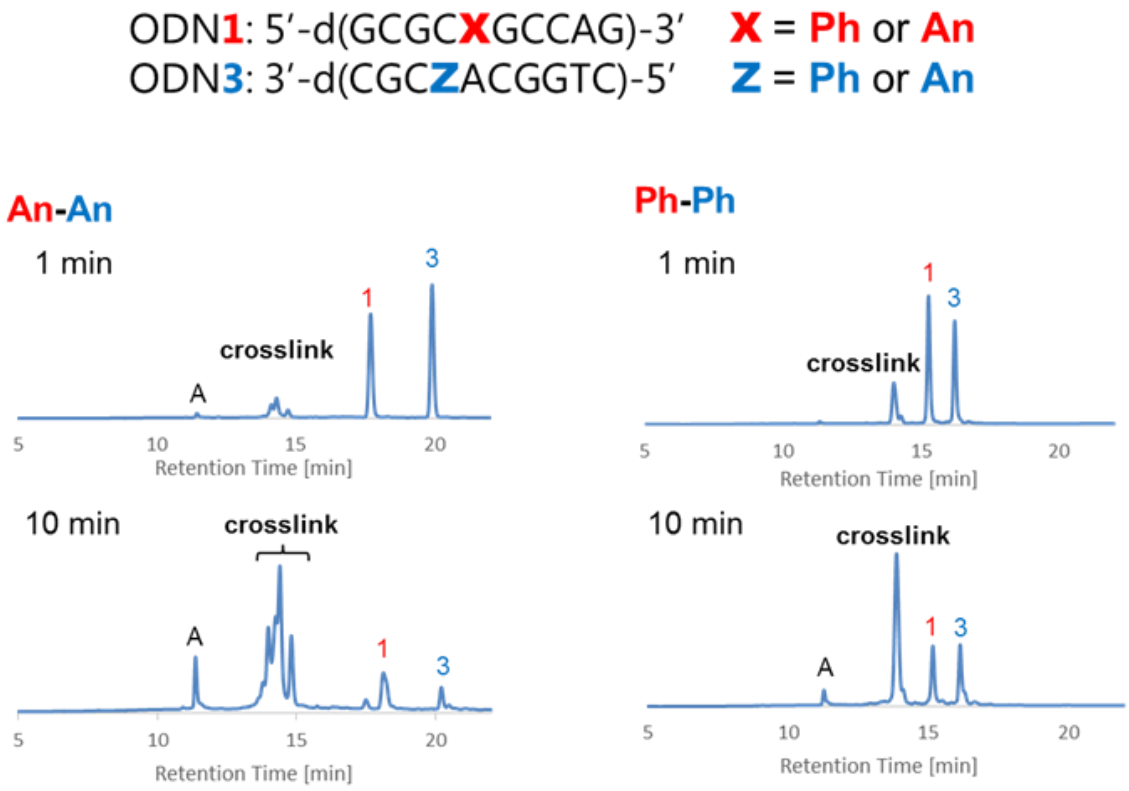


Figure S5. HPLC profiles of photo-crosslinking reaction after 1-min (top) and 10-min (bottom) photoirradiation (360 nm). (A) ODN**1**(An)-ODN**3**(An), (B) ODN**1**(Ph)-ODN**3**(Ph).

Table S2. MALDI-TOF mass data of photo-reaction products in this study

Entry	ODN	X or Z	[a]Calcd.	[b]Found
1	ODN**1**-ODN**3**	X = An, Y = An	6427.5	6428.4
2	ODN**1**-ODN**3**	X = An, Y = Ph	6327.5	6327.6
3	ODN**1**-ODN**3**	X = Ph, Y = An	6327.2	6327.2
4	ODN**1**-ODN**3**	X = Ph, Y = Ph	6227.2	6227.4
5	ODN**1**-ODN**3**	X = Ph, Y = Ph (non-O_2)	6194.2	6194.9
6	ODN**1**	X = abasic sugar	2917.8	2918.0
7	ODN**3**	Z = abasic sugar	2893.5	2893.5
8	ODN**1**	X = Ph (dione)	3141.9	3143.7
9	ODN**3**	Z = Ph (dione)	3116.6	3116.4
10	ODN**3**	Z = An (dione)	3216.6	3216.8

[a] $[M-H]^-$, [b] All data were collected in negative mode.

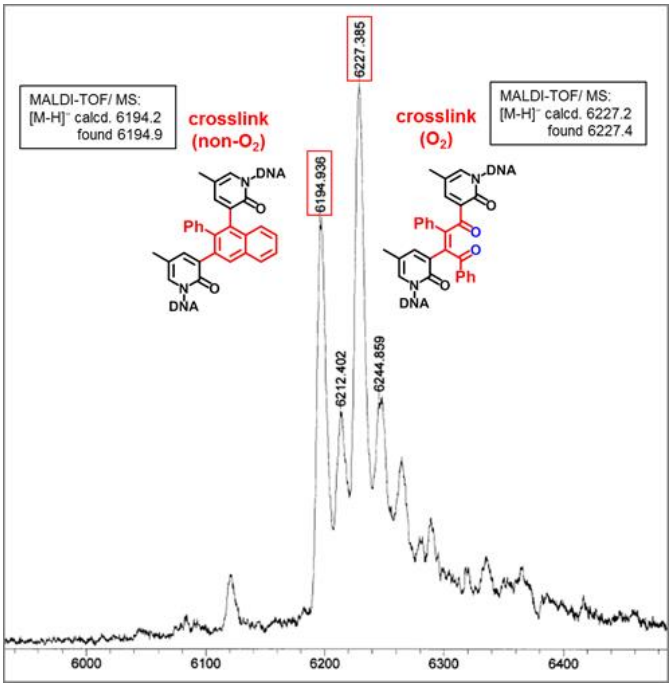


Figure S6. MALDI-TOF mass spectrum of the ODN**1**(Ph)-ODN**3**(Ph) crosslinked product.

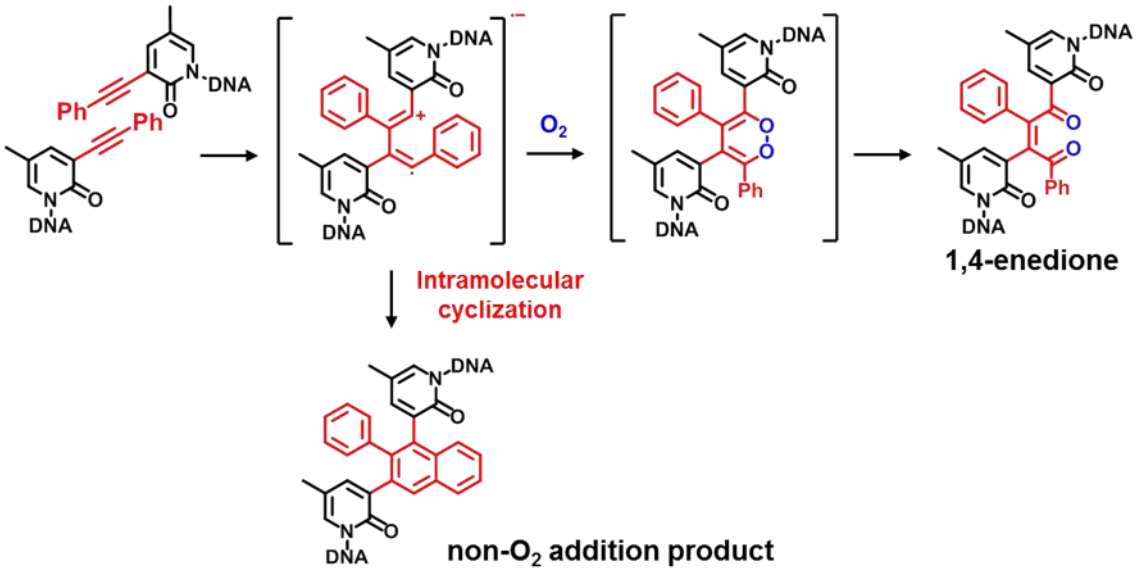


Figure S7. The proposed structure and mechanism of the non-O_2 addition crosslinked product.

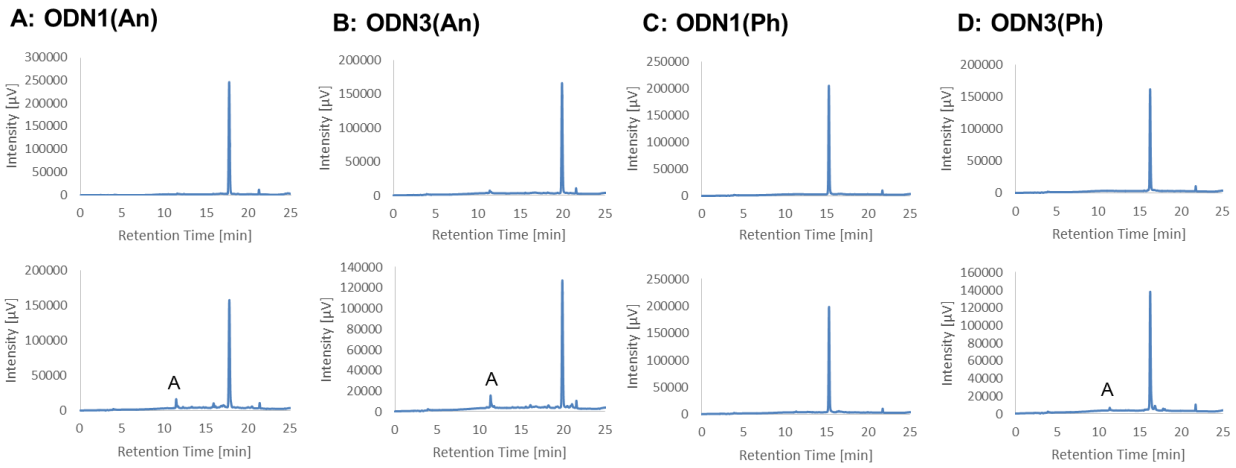


Figure S8. HPLC profiles of the single strand ODN**1** or ODN**3** before (top) and after (bottom) photoirradiation (360 nm, 10 min). The photo irradiation was carried out with ODN (1.0 μM) in MES buffer (50 mM, pH 7.0) containing NaCl (100 mM). (A) ODN**1**(An), (B) ODN**3**(An), (C) ODN**1**(Ph), (D) ODN**3**(Ph).

Figure S9. The proposed mechanism of the abasic sugar production.

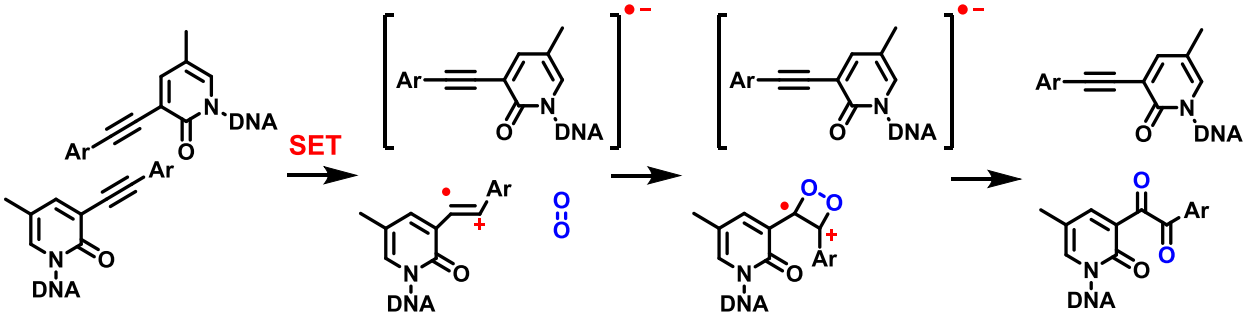


Figure S10. The proposed mechanism of 1,2-dione by-product production.

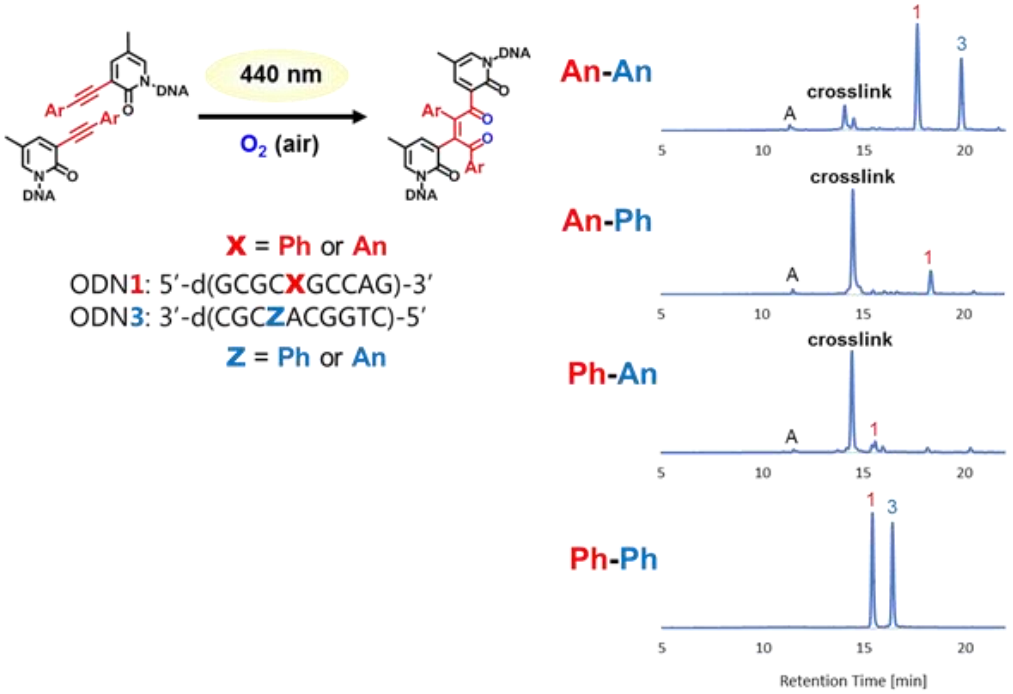


Figure S11. Photo-crosslinking reaction at 440 nm. The reaction was carried out with ODN**1**(**X** = **Ph** or **An**) (1.0 μM) and ODN**3**(**Z** = **Ph** or **An**) (1.0 μM) in MES buffer (50 mM, pH 7.0) containing NaCl (100 mM) under 440 nm photoirradiation (300 W Xenon lamp, 8.4 mW/ cm^2) for 1 min.

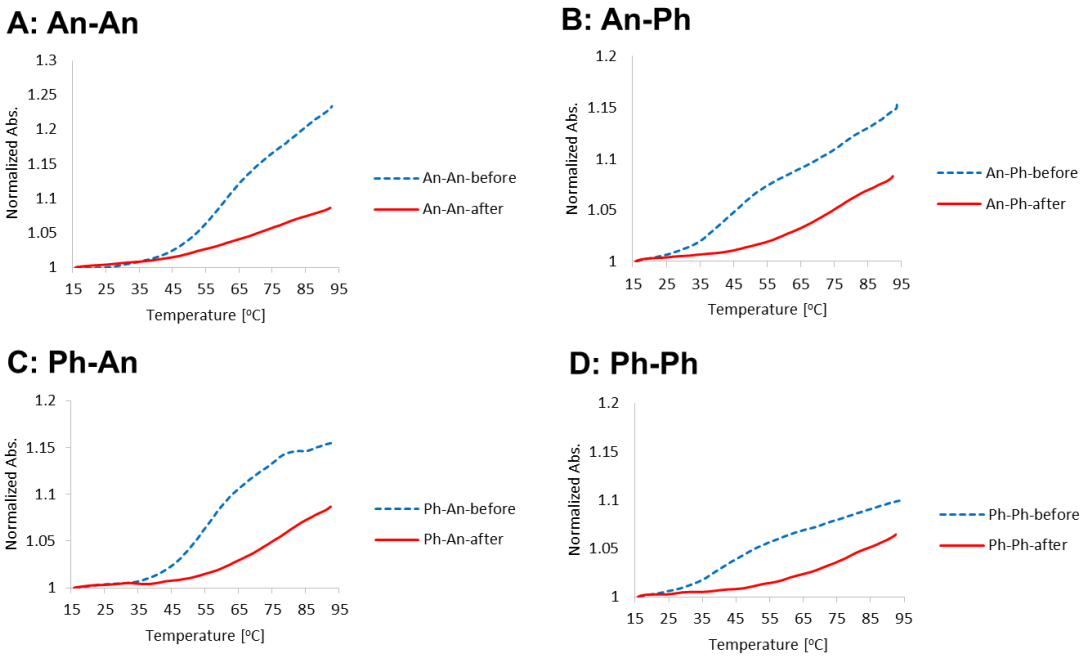
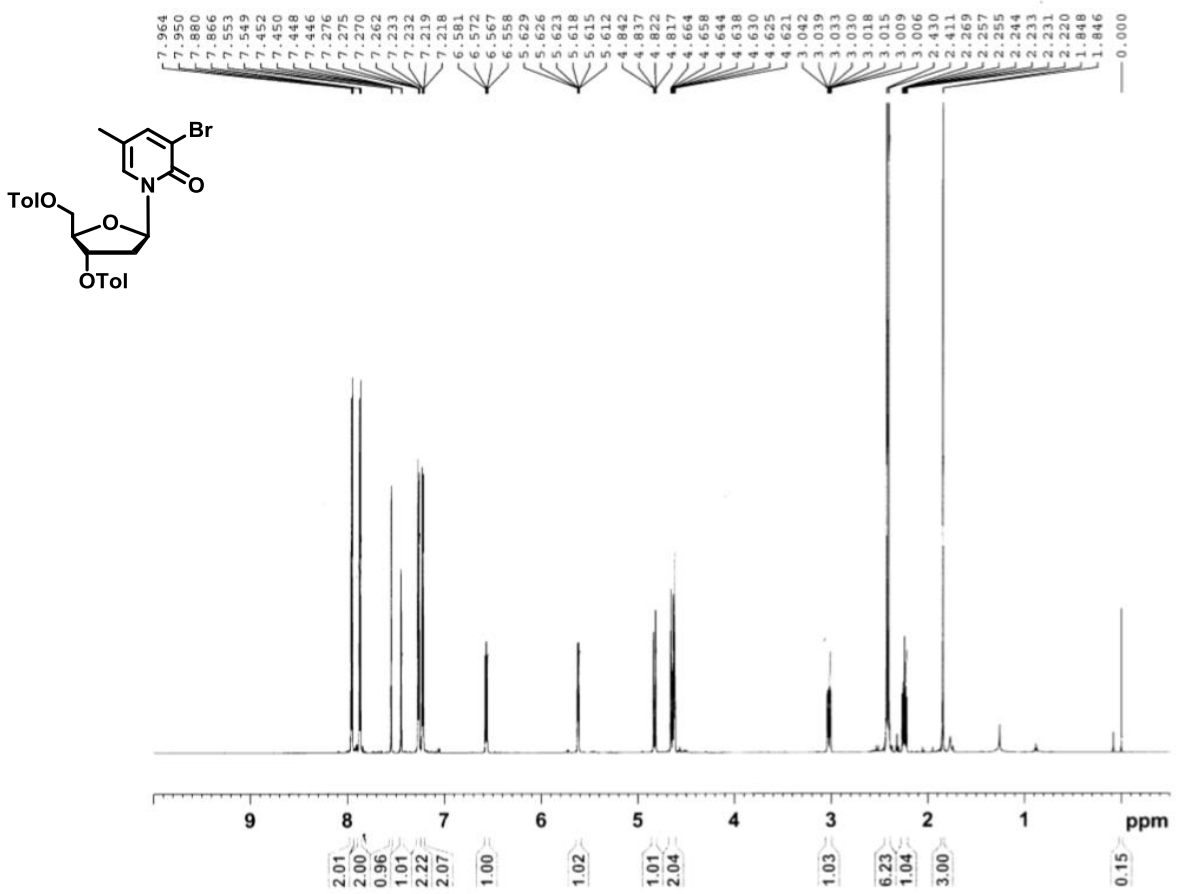


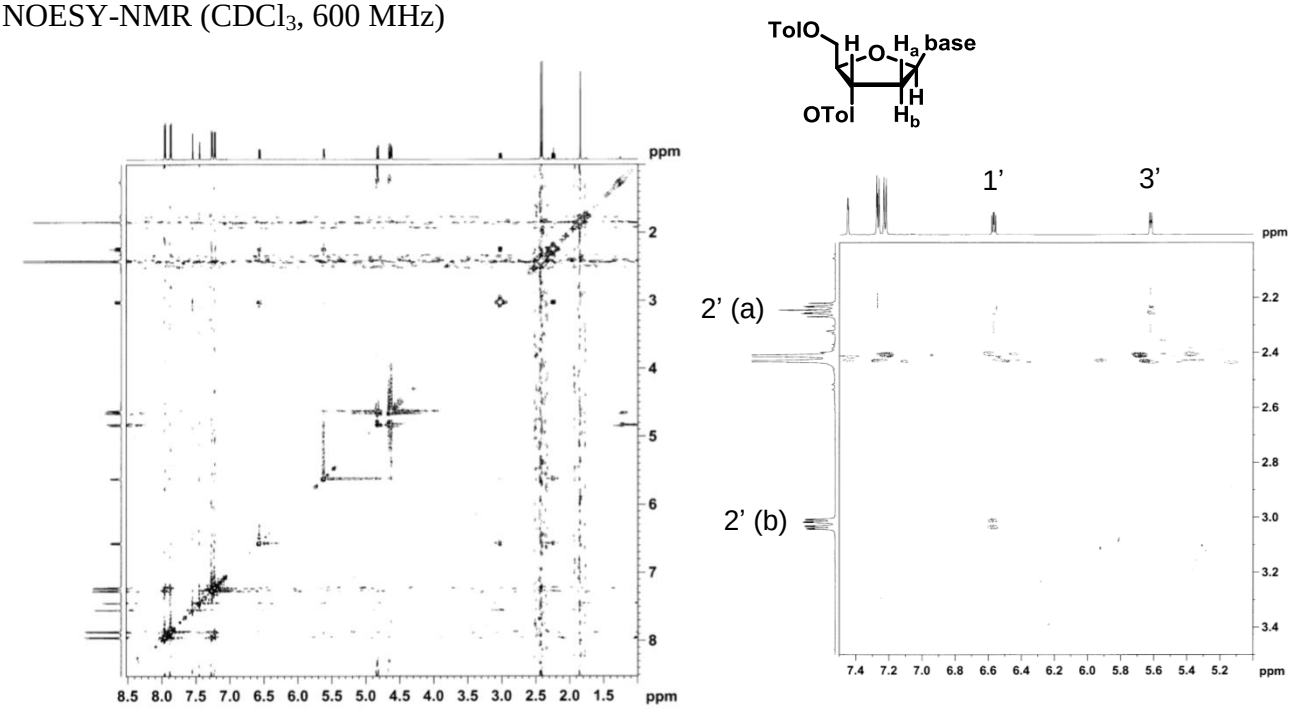
Figure S12. UV-melting curves of ODN**1**-ODN**3** duplex before (dashed line) and after (solid line) photoirradiation [365 nm, 6 W-SLUV-6 (AS ONE), 10 min]. The T_m measurements were performed using duplex (4.0 μM) in MES buffer (50 mM, pH 7.0) containing NaCl (100 mM). (A) ODN**1**(An)-ODN**3**(An), (B) ODN**1**(An)-ODN**3**(Ph), (C) ODN**1**(Ph)-ODN**3**(An), (D) ODN**1**(Ph)-ODN**3**(Ph).

NMR Data

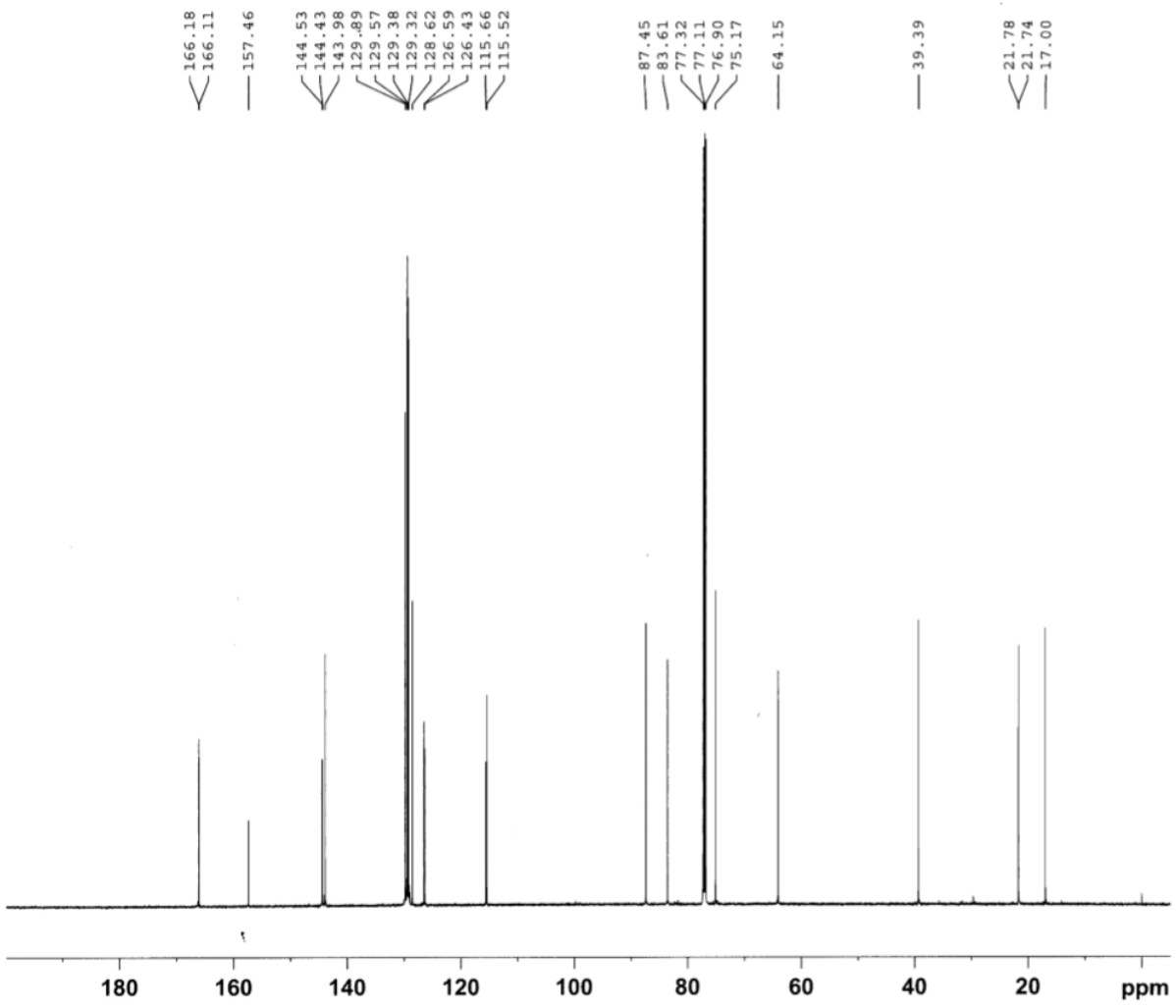
Compound **3**(β-anomer)
^{1}H NMR ($CDCl_3$, 600 MHz)



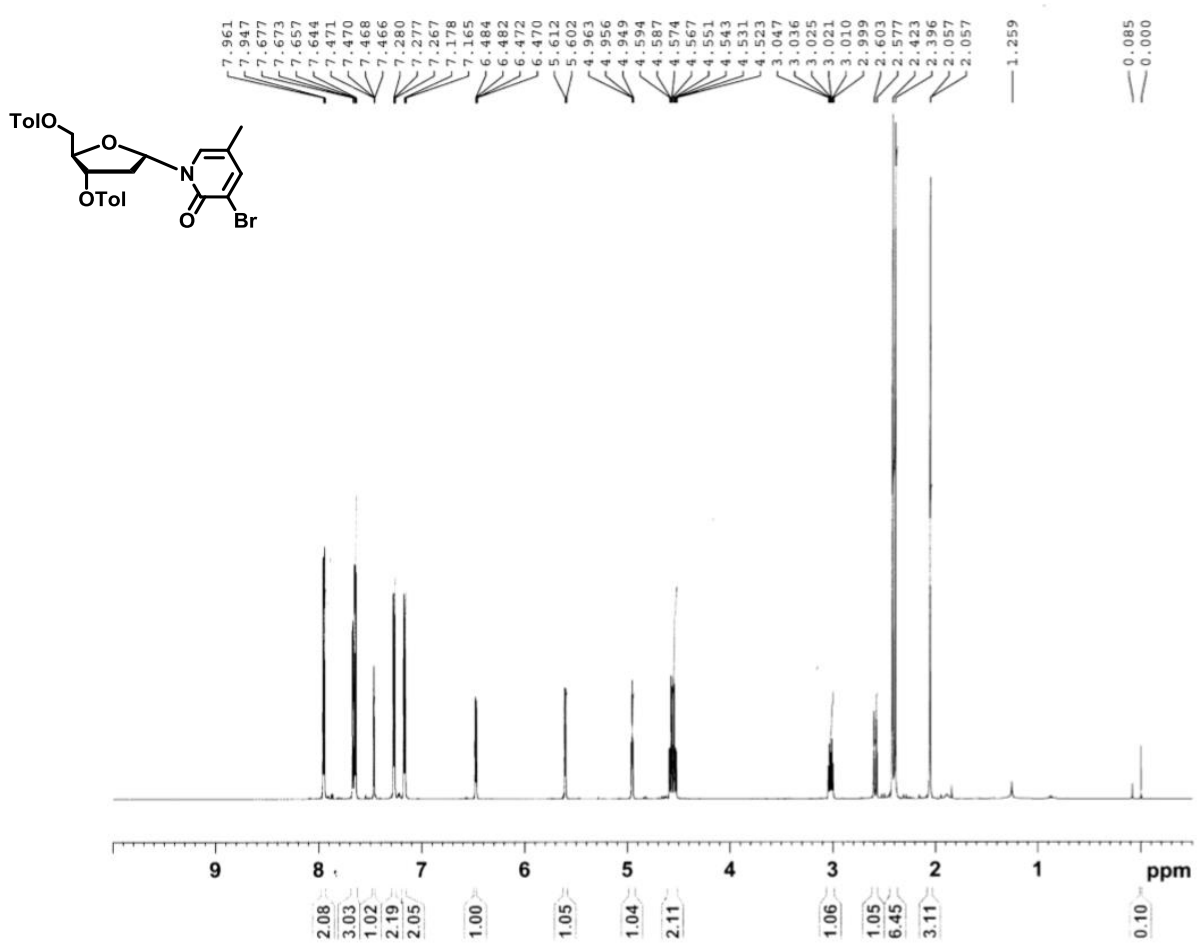
NOESY-NMR ($CDCl_3$, 600 MHz)



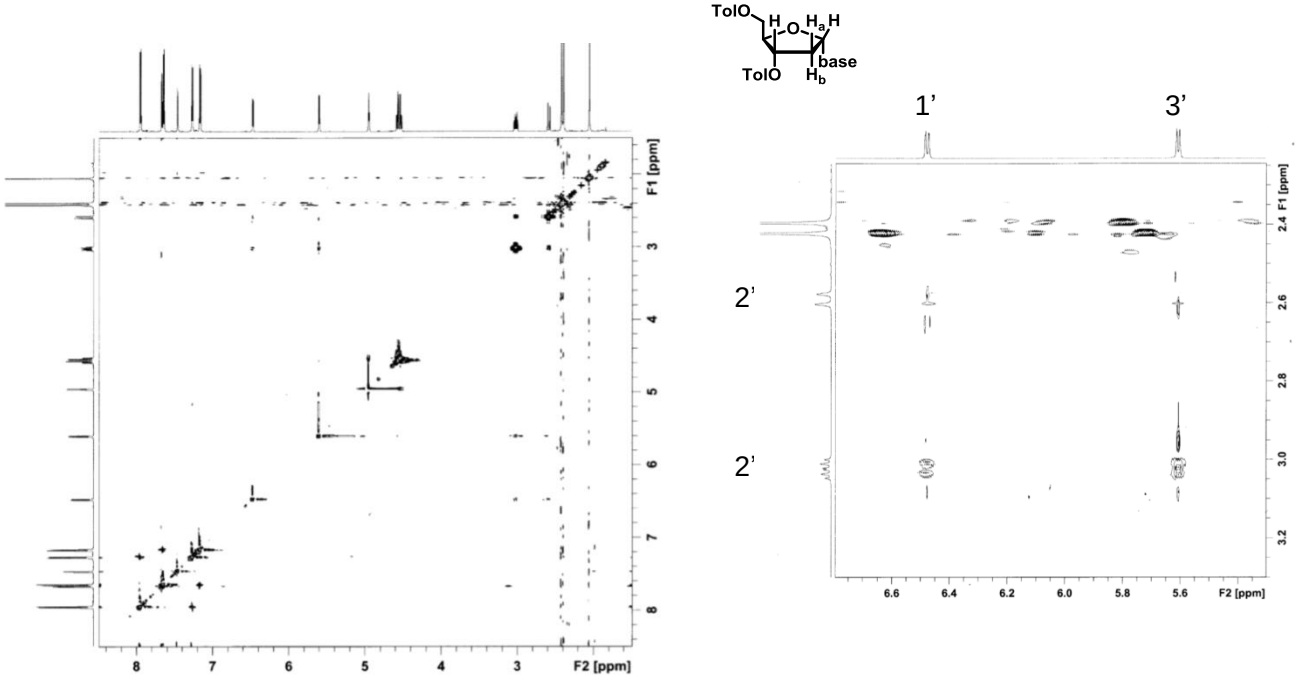
^{13}C NMR ($CDCl_3$, 150 MHz)



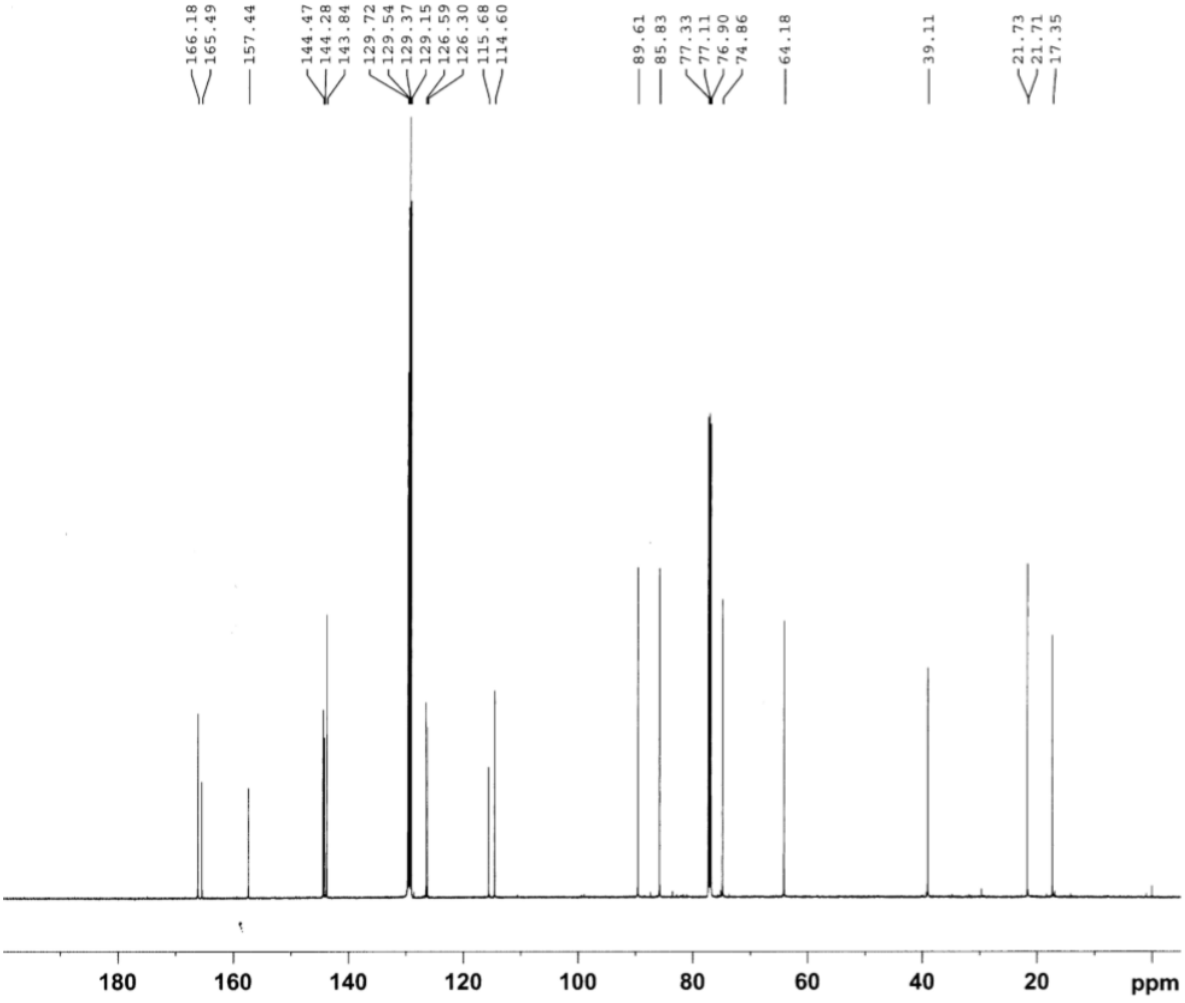
Compound **3**(α-anomer)
^{1}H NMR ($CDCl_3$, 600 MHz)



NOESY-NMR ($CDCl_3$, 600 MHz)

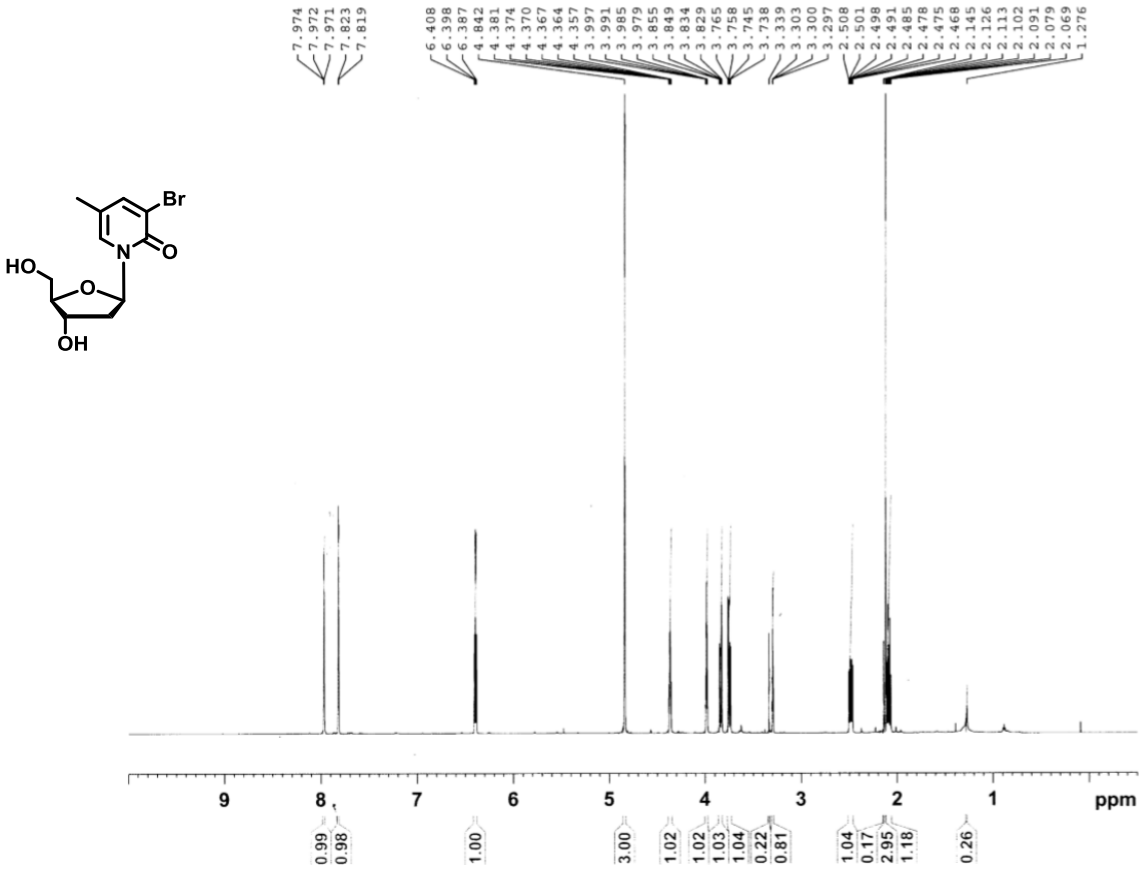


^{13}C NMR ($CDCl_3$, 150 MHz)

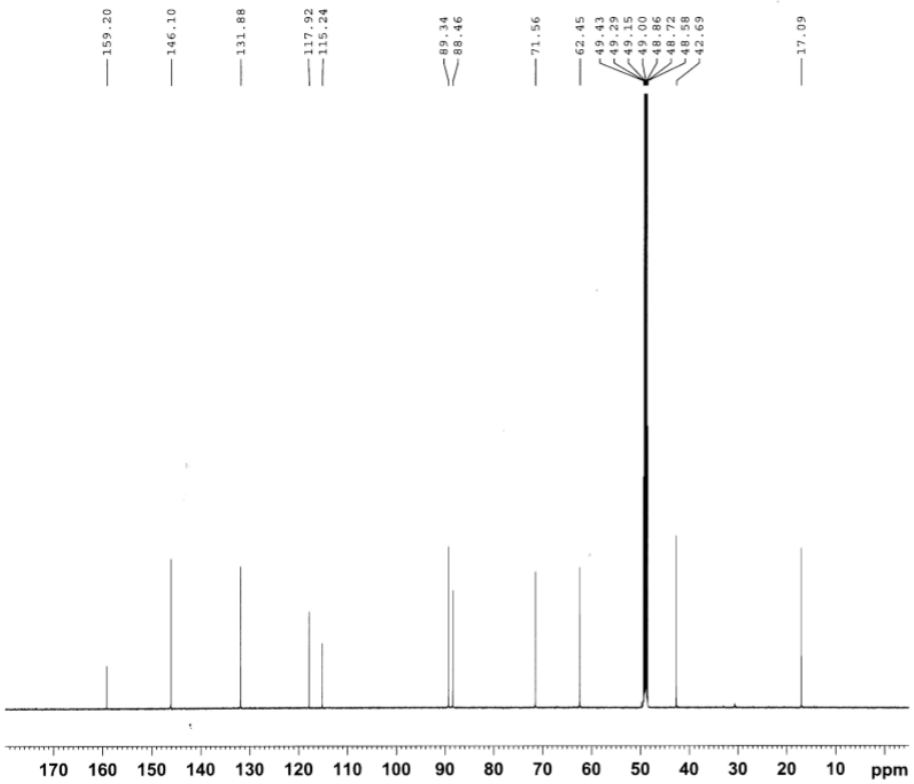


Compound **4**

^{1}H NMR (MeOD-d_4, 600 MHz)

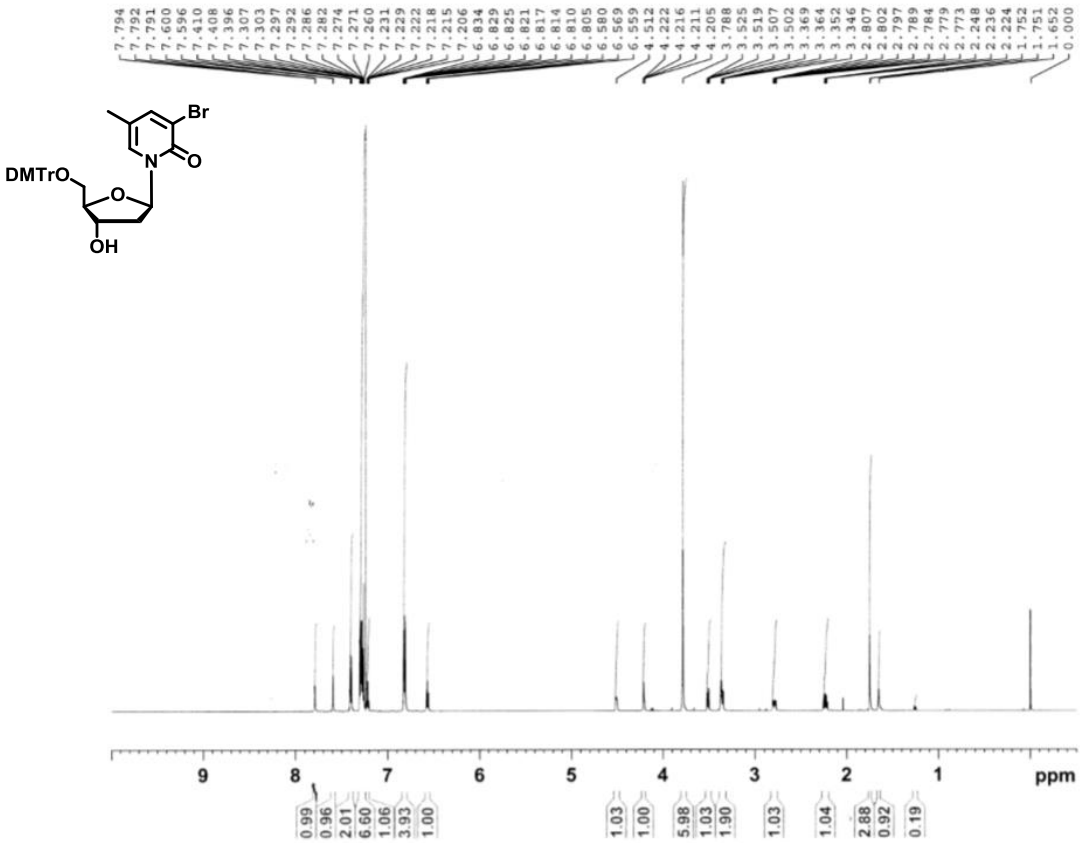


^{13}C NMR (MeOD-d_4, 150 MHz)

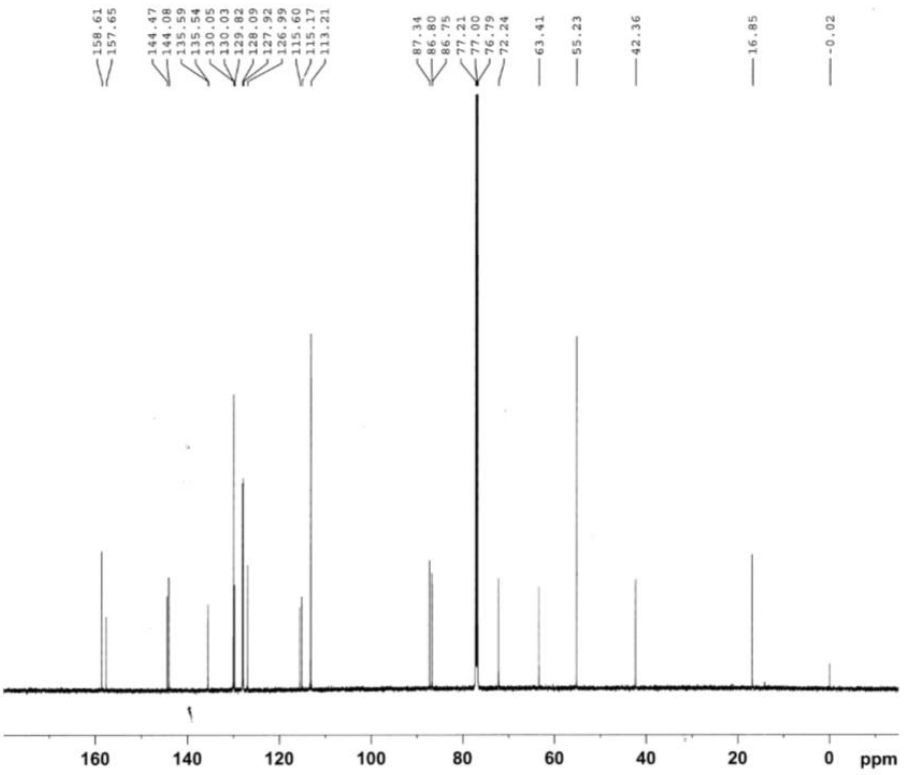


Compound **5**

^{1}H NMR ($CDCl_3$, 400 MHz)

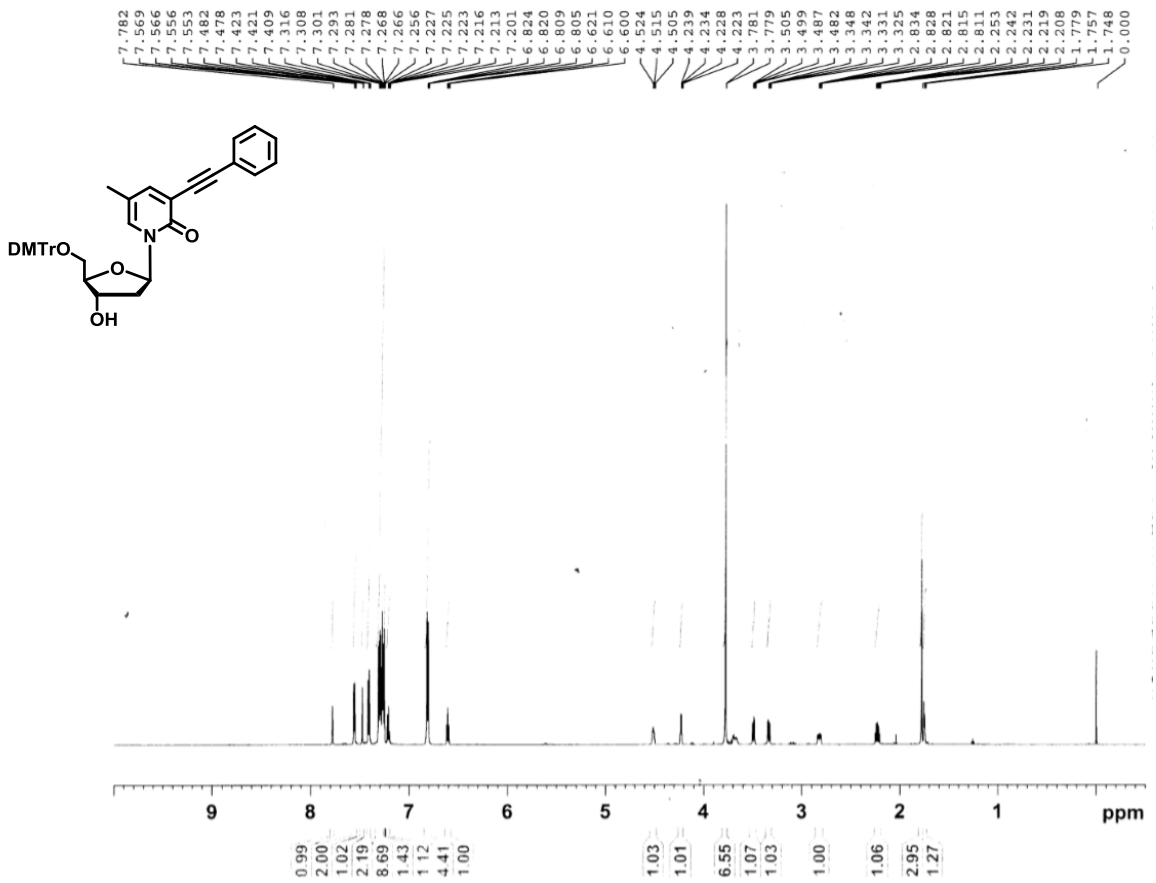


^{13}C NMR ($CDCl_3$, 150 MHz)

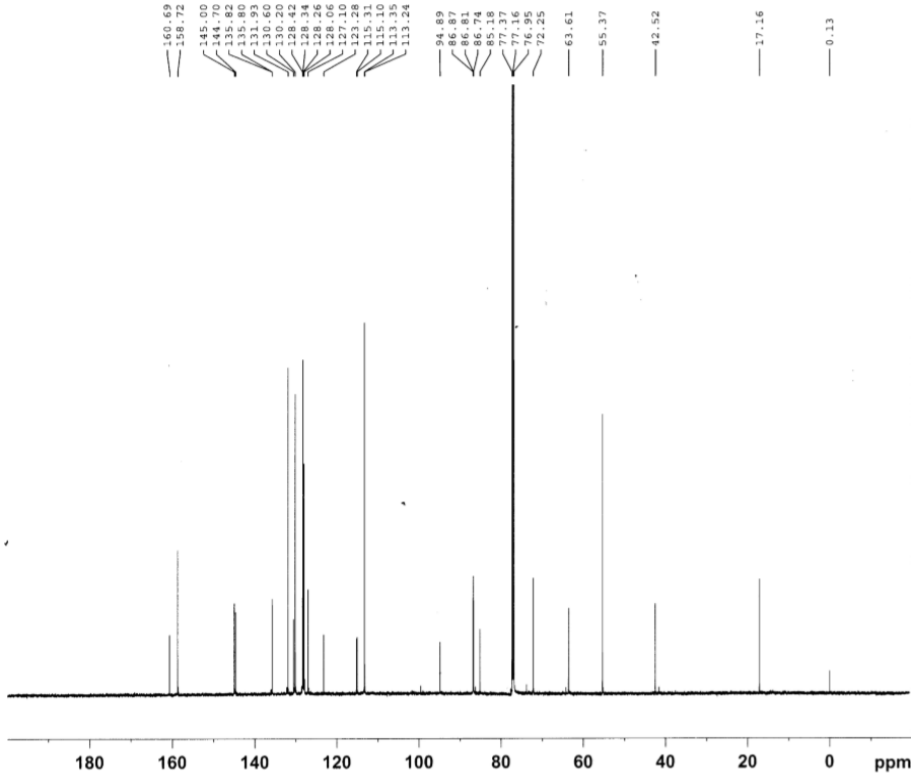


Compound **6(Ph)**

^{1}H NMR ($CDCl_3$, 600 MHz)

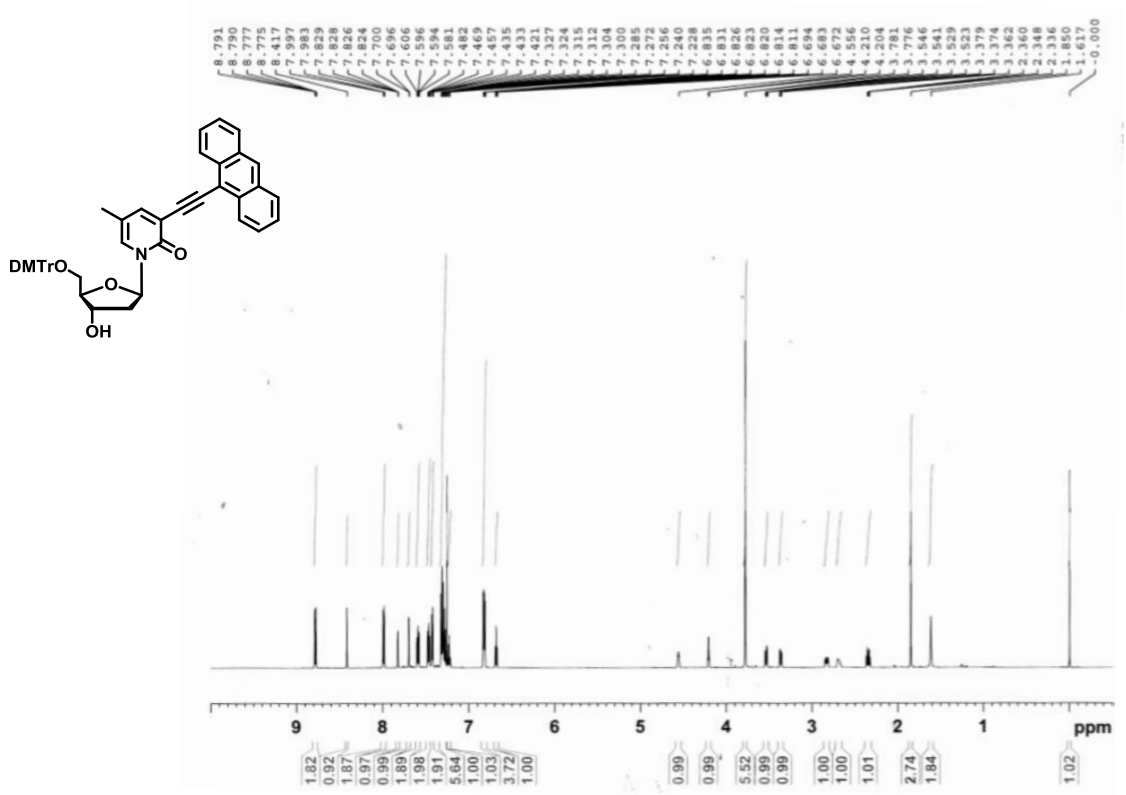


^{13}C NMR ($CDCl_3$, 150 MHz)

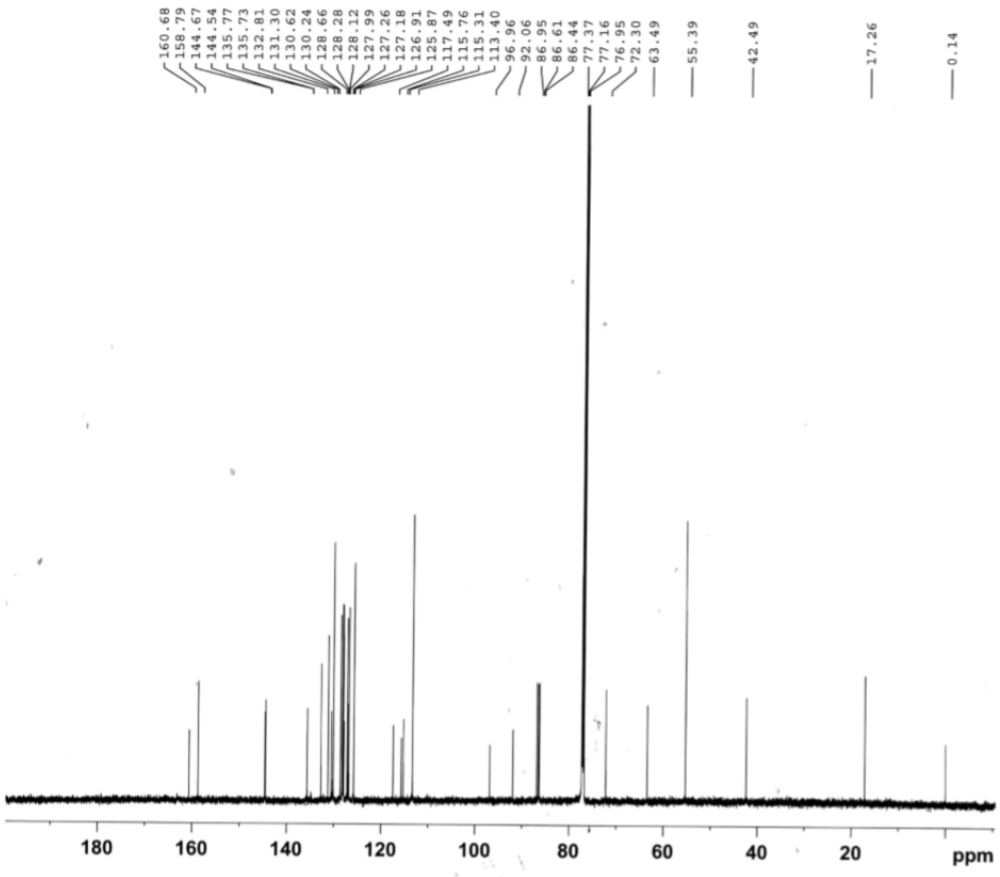


Compound **6(An)**

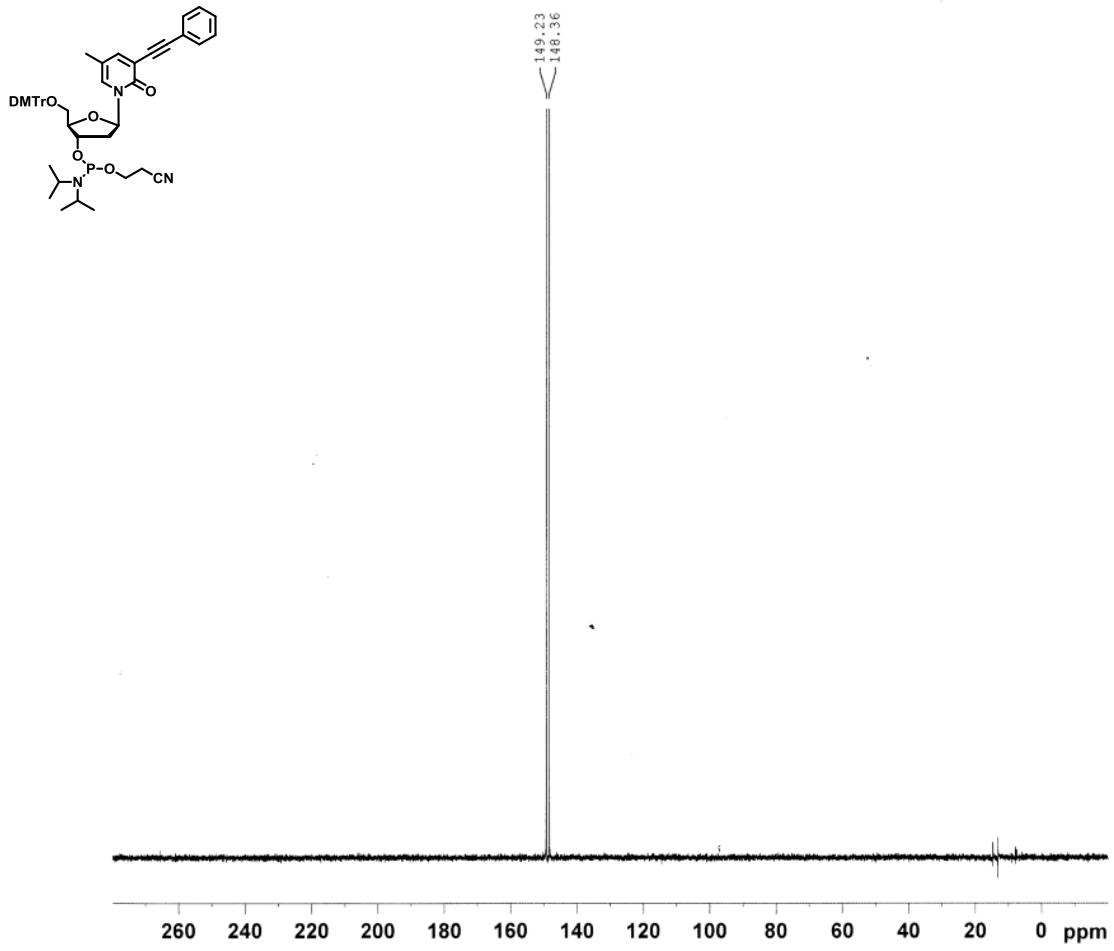
^{1}H NMR ($CDCl_3$, 600 MHz)



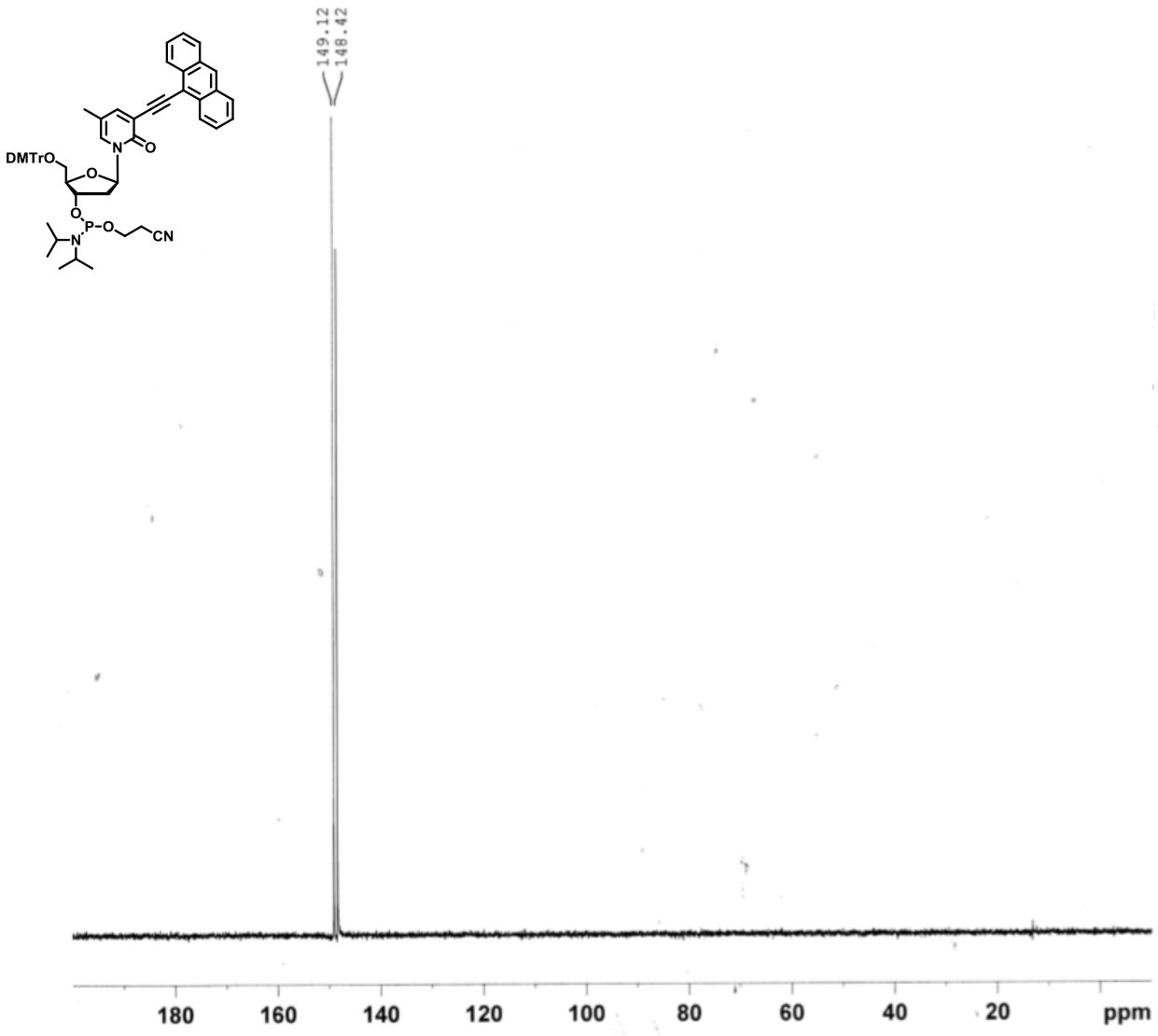
^{13}C NMR ($CDCl_3$, 150 MHz)



Compound **7(Ph)**
^{31}P NMR ($CDCl_3$, 162 MHz)

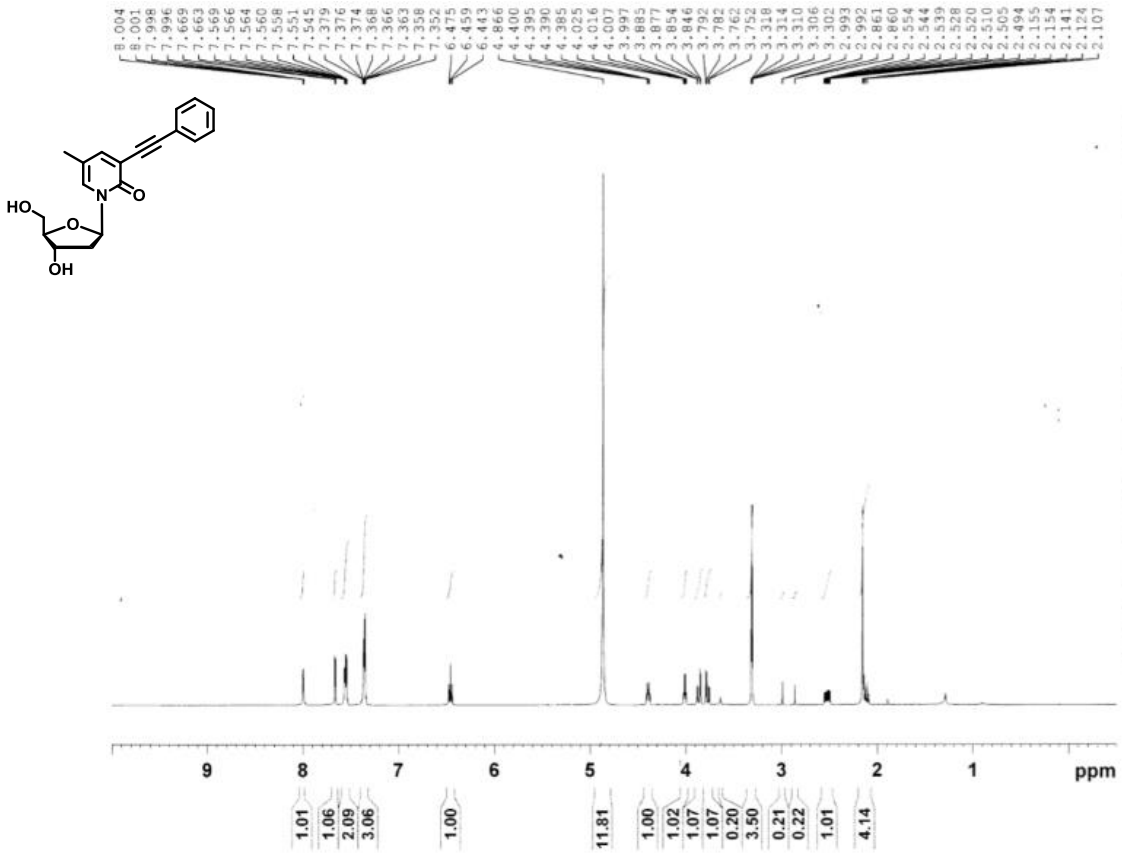


Compound **7(An)**
^{31}P NMR ($CDCl_3$, 162 MHz)

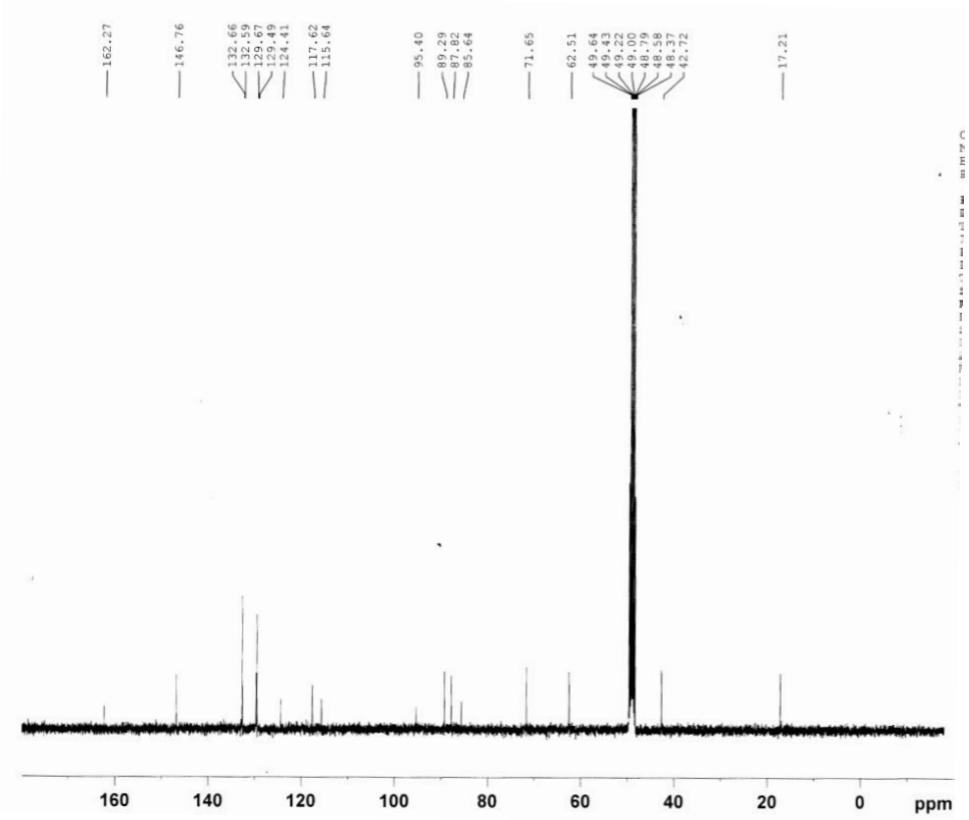


Compound **8(Ph)**

^{1}H NMR (MeOD-d_4, 400 MHz)

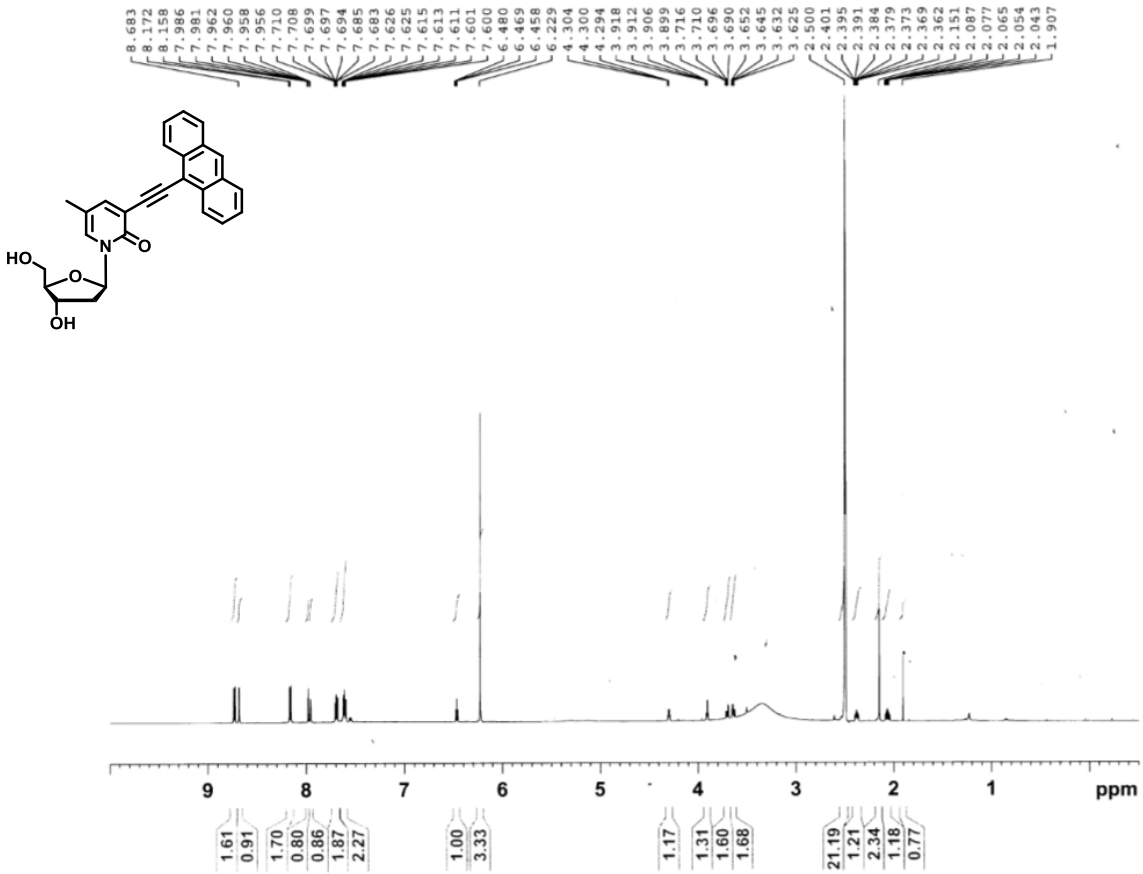


^{13}C NMR (MeOD-d_4,150 MHz)



Compound **8(An)**

^{1}H NMR (MeOD-d_4, 400 MHz)



^{13}C NMR (MeOD-d_4,150 MHz)