CHEMBIOCHEM

# Supporting Information

CHEMBIOCHEM

# Supporting Information

for

# Click Chemistry as an Efficient Method of Preparing a Sensitive DNA Probe for Photochemical Ligation

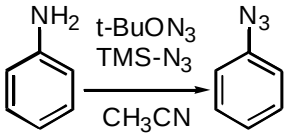
Takehiro Ami, and Kenzo Fujimoto*,

**General method and materials**

$^{1}$H NMR spectra were measured with Varian Gemini 300 (300 MHz) spectrometer. $^{13}$C NMR spectra were measured with Varian INOVA 500 (125 MHz) spectrometer. Coupling constant (*J* value) are reported in hertz. The chemical shifts are reported in δ (ppm) relative to residual $CDCl_3$ (δ = 7.24 in $^{1}$H NMR), $CD_3OD$ (δ = 3.35 in $^{1}$H NMR, δ = 49.3 in $^{13}$C NMR) and DMSO-$d_6$ (δ = 2.49 in $^{1}$H NMR, δ = 39.7 in $^{13}$C NMR) as internal standards. Mass spectra were recorded on a Voyager-DE PRO-SF, Applied Biosystems. HPLC was performed on a Chemcobond 5-ODS-H column (10 × 150 mm, 4.6 × 150 mm) or a Chemcosorb 5-ODS-H column (4.6 × 150 mm) with a JASCO PU-2080 plus, MX-2080-32, DG-2080-53 system equipped with a JASCO UV 2075 detector at 260 nm. The reagents for the DNA synthesizer such as A, G, C, T-β-cyanoethyl phosphoramidite, and CPG support were purchased from Glen Research. JASCO V-550 UV/VIS spectrophotometer was used for absorption spectra measurements. Photoirradiation was performed by UV-LED (OMURON, 366 nm) or 25 W transilluminator (FUNAKOSHI, TFL-40, 366 nm, 5,700 μW/cm$^2$).

**Microwave irradiation experiments**

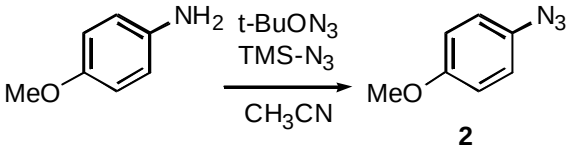
All microwave irradiation experiments were carried out in a dedicated CEM-Discover mono-mode microwave apparatus, operating at a frequency of 2.45 GHz with continuous irradiation power from 0 to 300 W utilizing the standard absorbance level of 300 W maximum power. The machine was used in the standard configuration as delivered, including proprietary software. The reactions were carried out in 10 mL glass vials sealed with an aluminum/Teflon® crimp top, which can be exposed to a maximum of 250 °C and 20 bar internal pressure. The temperature was measured with an IR sensor on the outer surface of the process vial. After the irradiation period, the reaction vessel was cooled rapidly (60-120 s) to ambient temperature by gas jet cooling.




**1**

**Scheme S1**

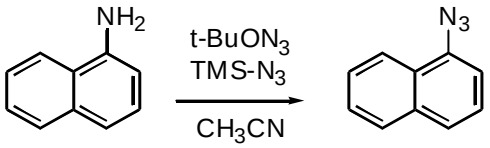
Aniline (200 mg, 2.14 mmol) was dissolved in $CH_3CN$ (4 mL) in a 25 mL round-bottomed flask and cooled to 0 °C in an ice bath. To this stirred mixture was added *t*-BuONO (331 mg, 380 µL, 3.21 mmol) followed by $TMSN_3$ (300 mg, 340 µL, 2.56 mmol) dropwise. The resulting solution was stirred at room temperature for 1 h. The reaction mixture was concentrated under vacuum and the crude product was purified by silica gel chromatography (hexane) to give the product, **1**, as a pale yellow oil (236 mg,93%). $^1$H NMR ($CDCl_3$, 300 MHz) δ: 7.34 (t, J= 7.6 Hz, 2H) , 7.12 (t, J= 7.6 Hz, 1H), 7.01 (d, J= 7.6 Hz, 2H).




**2**

**Scheme S2**

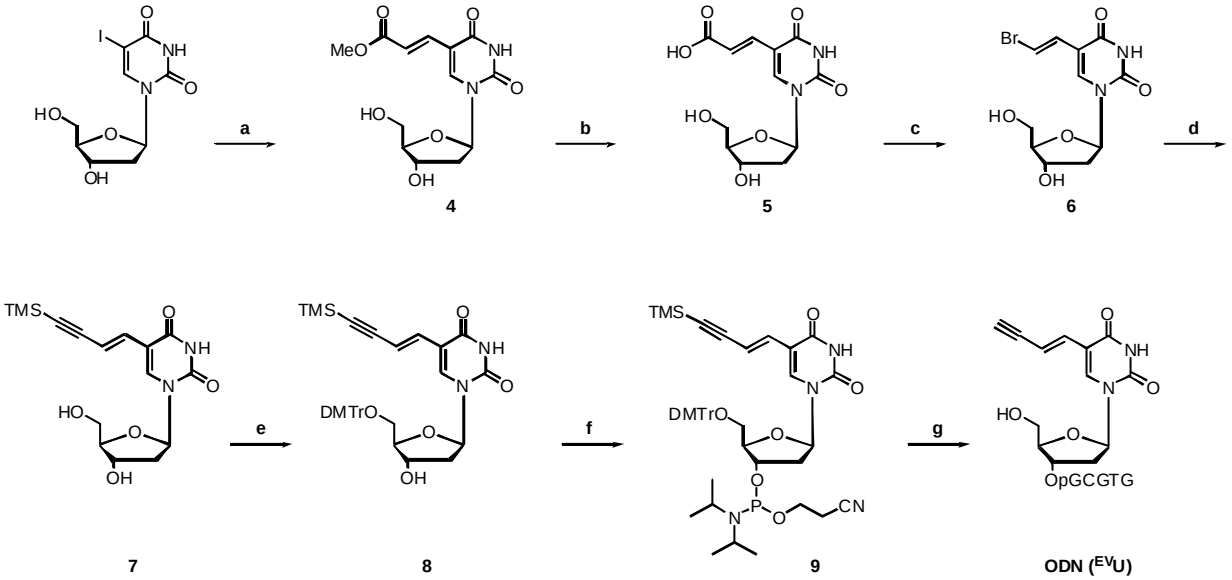
*p*-Anisidine (200 mg, 1.62 mmol) was dissolved in $CH_3CN$ (2 mL) in a 25 mL roundbottomed flask and cooled to 0 °C in an ice bath. To this stirred mixture was added *t*-BuONO (250 mg, 290 µL, 2.44 mmol) followed by $TMSN_3$ (224 mg, 260 µL, 1.95 mmol) dropwise. The resulting solution was stirred at room temperature for 2 h. The reaction mixture was concentrated under vacuum and the crude product was purified by silica gel chromatography (hexane/ethyl acetate, 8:2) to give the product, **2**, as a yellow oil (202 mg, 83%). $^1$H NMR (CDCl3, 300 MHz) δ: 6.88 (d, J = 8.8 Hz, 2H), 6.81 (d, J = 8.8 Hz, 2H), 3.70 (s, 3H).




**3**

**Scheme S3**

1-naphtylamine (0.2 g, 1.40 mmol)was dissolved in $CH_3CN$ (3 mL) in a 25 mL round-bottomed flask and cooled to 0 °C in an ice bath. To this stirred mixture was added *t*-BuONO (0.25 ml, 2.10 mmol)followed by $TMSN_3$(0.22 ml, 1.68 mmol) dropwise. The resulting solution was stirred at room temperature for 2 h. The reaction mixture was concentrated under vacuum. The resulting precipitate was washed with cold hexane, and dried under reduced pressure to give the product, **3**(0.21 g, 88%) $^1$H NMR ($CDCl_3$, 300 MHz) δ: 8.09 (d, J = 9.3, 1H), 7.79 (d, J = 9.3, 1H), 7.61 (d, J = 8.4, 1H), 7.52-7.41 (m, 3H), 7.23 (d, J = 6.9, 1H).




**Scheme S4**

**(*E*)-5-(2-Carbomethoxyvinyl)-2'-deoxyuridine (4)**

5-Iodo-deoxyuridine (1.00 g, 2.82 mmol) and $Pd(OAc)_2$ (0.06 g, 0.28 mmol) were suspended in DMF (3mL) in a 10 mL glass vial equipped with a small magnetic stirring bar. To this was added the $Bu_3N$(0.67 mL, 2.82 mmol) and methylacrylate(0.38 mL, 4.24 mmol) and the vial was tightly sealed with an aluminum/Teflon® crimp top. The mixture was then irradiated for four minutes at 100 °C, using an irradiation power of 60 W. After completion of the reaction, the vial was cooled to 50 °C by gas jet cooling before it was opened. It was then filtered and evaporated *in vacuo*. The crude was purified by column chromatography ($CHCl_3$ / MeOH= 9 / 1) to give **4** (0.84 g, 95%). $^1$H –NMR (DMSO-$d_6$, 300 MHz) δ: 11.63 (bs, 1H), 8.40 (s, 1H), 7.36 (d, 1H, *J*= 15.5 Hz), 6.84 (d, 1H, *J*= 15.5 Hz), 6.12 (t, 1H, *J*= 6.3 Hz), 5.24 (d, 1H, *J*= 4.3 Hz), 5.15 (t, 1H, *J*=5.3 Hz), 4.24 (m, 1H), 3.79 (m, 1H), 3.67 (s, 3H), 3.64-3.54 (m, 2H), 2.17 (m, 2H).

**(*E*)-5-(2-Carboxyvinyl)-2'-deoxyuridine (5)**

**4** (8.52 g, 27.29 mmol) was added to 3M NaOH (water/ ethanol= 1/ 1, 10 ml) and this mixture stirred at room temperature for 3 h. HCl was powered into this solution on cooling ice bath to give white precipitate. The precipitate was filtered and washed with hexane, and sucked dry. The product was dried under vacuum to yield **5** (7.24 g, 89%). $^1$H –NMR (DMSO-$d_6$, 300 MHz) δ? 12.16 (bs, 1H), 11.62 (s, 1H), 8.37 (s, 1H), 7.28 (d, 1H, *J*= 15.9 Hz), 6.76 (d, 1H, *J*= 15.9 Hz), 6.12 (t, 1H, *J*= 6.2 Hz), 5.25 (d, 1H, *J*= 4.1 Hz), 5.18 (t, 1H, *J*=5.1 Hz), 4.24 (m, 1H), 3.79 (m, 1H), 3.60 (m, 2H), 2.16 (m, 2H).

**(*E*)-5-(2-Bromovinyl)-2'-deoxyuridine (6)**

To a solution of **5** (5.00 g, 23.5 mmol) in DMF(100 ml) was added $K_2CO_3$(4.87 g, 35.2 mmol) at ambient temperature and stirred for 15 minutes. A solution of *N*-Bromosuccinimide(4.18 g, 23.5 mmol) in DMF(100 ml) was added dropwise over 30 min. The resulting suspension was filtered immediately under suction and the solid was washed well with DMF. The combined filtrate and washing were evapolated to dryness in vacuo to complete remove the DMF and the crude was purified by column chromatography ($CHCl_3$ / MeOH= 9 / 1) to give **6** (3.94 g, 50%) $^1$H NMR (DMSO-$d_6$, 300 MHz) δ? 11.56 (s, 1H), 8.06 (s, 1H), 7.23 (d, 1H, *J*= 13.6 Hz), 6.83 (d, 1H, *J*= 13.6 Hz), 6.11 (t, 1H, *J*= 6.6 Hz), 5.25 (d, 1H, *J*= 4.4 Hz), 5.09 (t, 1H, *J*= 5.2 Hz), 4.23 (m, 1H), 3.77 (m, 1H), 3.57 (m, 2H), 2.12 (m, 2H).

**(*E*)-5-(2-trimethylsilyl ethynyl vinyl)-2'-deoxyuridine (7)**

A mixture of **6** (3.35 g, 10.1 mmol), $Pd(PPh_3)_4$(1.17 g, 1.01 mmol), CuI(0.382 g, 2.01 mmol) and *N*,*N*-diisopropylethylamine (8.56 ml, 50.4 mmol) in DMF(50 ml) was stirred for 10 minutes. To this there was then added trimethylsilylacetylene(4.18 ml, 30.2 mmol) and the mixture was stirred for 2 h. The resulting suspension was then filtered and the crude was purified by column chromatography ($CHCl_3$ / MeOH= 9 / 1) to give **7** (2.38 g, 67%) $^1$H NMR ($CD_3OD$, 300 MHz) δ? 8.29 (s, 1H), 6.71 (d, 1H, *J*= 16.1 Hz), 6.64 (d, 1H, *J*= 16.1 Hz), 6.30 (t, 1H, *J*= 6.5 Hz), 4.45 (m, 1H), 3.97 (m, 1H), 3.84 (m, 2H), 2.32 (m, 2H), 0.21 (m, 9H); $^{13}$C NMR ($CD_3OD$, 125 MHz) ?? 164.2, 151.5, 141.0, 136.0, 112.3, 109.9, 106.5, 96.7, 89.4, 87.2, 72.1, 62.8, 42.1.

**5'-*O*-(4,4'-Dimethoxytrityl)-(*E*)-5-(2-trimethylsilyl ethynyl vinyl)-2'-deoxyuridine (8)**

To a solution of **7** (0.60 g, 1.7 mmol) in anhydrous pyridine (10 ml) was added DMTrCl(0.70g, 2.0 mmol) and DMAP(0.04 g, 0.34 mmol) at 0°C. The reaction mixture was stirred for 4 h at ambient temperature. The reaction mixture was evaporated to dryness under reduced pressure. The crude product was purified by silica gel column chromatography ($CHCl_3$/ MeOH= 97 / 3) to afford **8** (0.34 g, 31%). $^1$H NMR ($CDCl_3$, 300MHz) δ? 8.61 (bs, 1H), 8.60 (bs, 1H), 7.39-7.24 (m, 9H), 6.83 (m, 4H), 6.58 (d, 1H, *J*= 16.1 Hz), 6.30 (m, 1H), 5.98 (d, 1H, *J*= 16.1 Hz), 4.51 (m, 1H), 4.08 (m, 1H), 3.78 (s, 6H), 3.74 (s, 1H), 3.40 (d, 2H, *J*= 3.3 Hz), 2.46 (m, 1H), 2.26 (m, 1H), 0.16 (m, 9H); $^{13}$C NMR ($CD_3OD$, 125 MHz) ? 162.0, 158.4, 158.3, 149.4, 145.0, 140.4, 136.1, 135.6, 135.5, 130.0, 129.9, 128.1, 127.9, 127.0, 113.4, 110.0, 107.6, 105.7, 96.0, 85.9, 85.8, 84.7, 70.3, 64.0, 56.3, 55.2, 0.1.

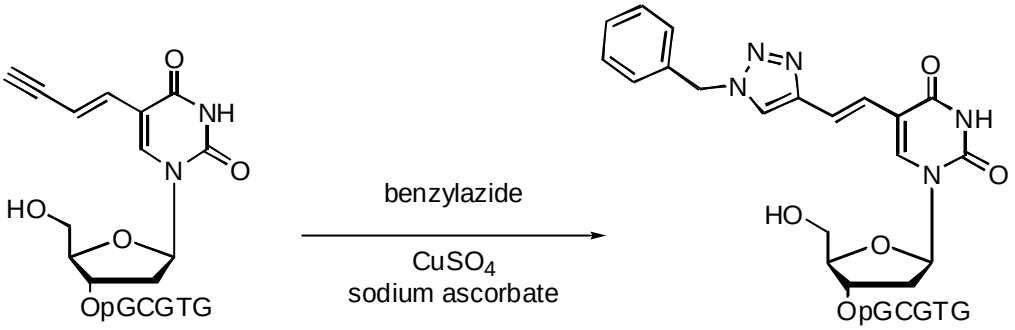
**5'-*O*-(4,4'-Dimethoxytrityl)-(*E*)-5-(2-trimethylsilyl ethynyl vinyl)-2'-deoxyuridine phosphoro-amidite (9)**

To a solution of **8** (0.20 g, 0.31 mmol) in dry $CH_3CN$ (5 ml) in a sealed bottle was added 0.45 M tetrazole in $CH_3CN$ (0.68 ml, 0.31 mmol) and 2-cyanoethyl *N,N,N',N'*-tetra-isopropyl-phosphorodiamidite (0.097 ml, 0.31 mmol) and the reaction mixture was stirred for 2 h at ambient temperature. The reaction mixture was diluted with EtOAc and organic layer was washed with a saturated aqueous solution of $NaHCO_3$. The organic layer was collected, dried over anhydrous sodium sulfate, and evaporated to dryness to yield **9**, which was directly used in an automated DNA synthesizer without further purification.

## Preparation of ODN($^{EV}$U)

ODN sequences were synthesized by the conventional phosphoramidite method by using an Applied Biosystems 3400 DNA synthesizer. The coupling efficiency was monitored with a trityl monitor. The coupling efficiency of crude mixture of $^{EV}$U was 97% yield. The coupling time of crude mixture of $^{EV}$U was 999 sec. They were deprotected by incubation with 28% ammonia for 4 h at 65 °C and were purified on a Chemcobond 5-ODS-H column (10 × 150 mm) by reverse phase HPLC; elution was with 0.05 M ammonium formate containing 3-20% $CH_3CN$, linear gradient (30 min) at a flow rate of 3.0 mL/min. Preparation of oligonucleotides was confirmed by MALDI-TOF-MS analysis.

## Synthesis of ODN ($^{BTV}$U)

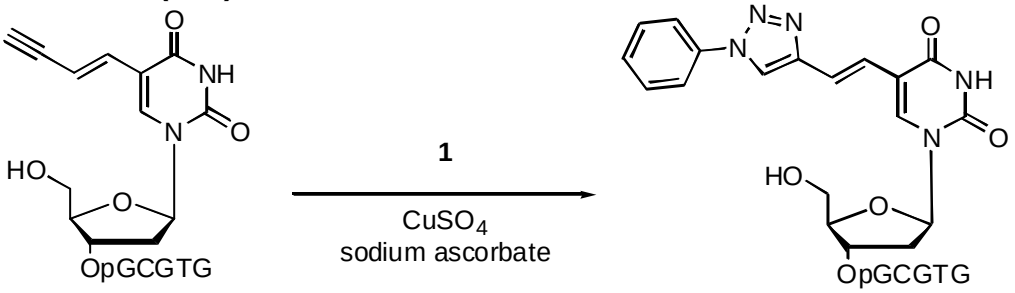



ODN ($^{EV}$U) ODN ($^{BTV}$U)

**Scheme S5**

A reaction mixture (total volume 2.0 mL) containing ODN($^{EV}$U) (50 μM strand conc.), a solution of benzylazide in EtOH (2.5 mM) , a aqueous solution of $CuSO_4$ (2 mM) and a aqueous solution of sodium ascorbate (1 mM) was shaken for 12 h. The crude product was were purified on a Chemcobond 5-ODS-H column (10 × 150 mm) by reverse phase HPLC; elution was with 0.05 M ammonium formate containing 3-20% $CH_3CN$, linear gradient (30 min) at a flow rate of 3.0 mL/ min (Figure S1).

## Synthesis of ODN ($^{PTV}$U)

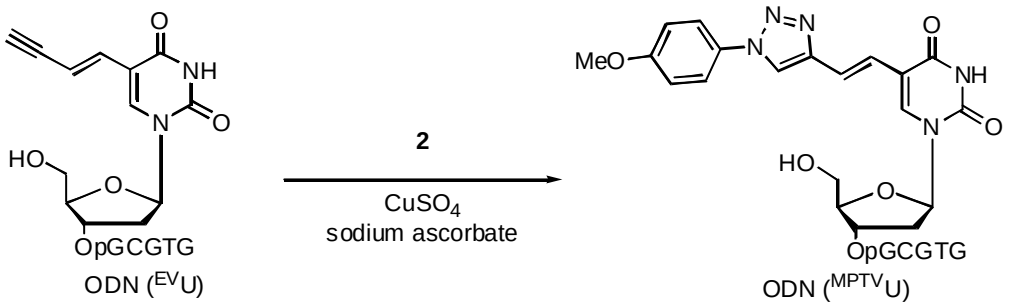



ODN ($^{EV}$U) ODN ($^{PTV}$U)

**Scheme S6**

A reaction mixture (total volume 2.0 mL) containing ODN($^{EV}$U) (50 μM strand conc.), a solution of 1 in EtOH (2.5 mM) , a aqueous solution of $CuSO_4$ (2 mM) and a aqueous solution of sodium ascorbate (1 mM) was shaken for 12 h. The crude product was were purified on a Chemcobond 5-ODS-H column (10 × 150 mm) by reverse phase HPLC; elution was with 0.05 M ammonium formate containing 3-20% $CH_3CN$, linear gradient (30 min) at a flow rate of 3.0 mL/ min (Figure S2).

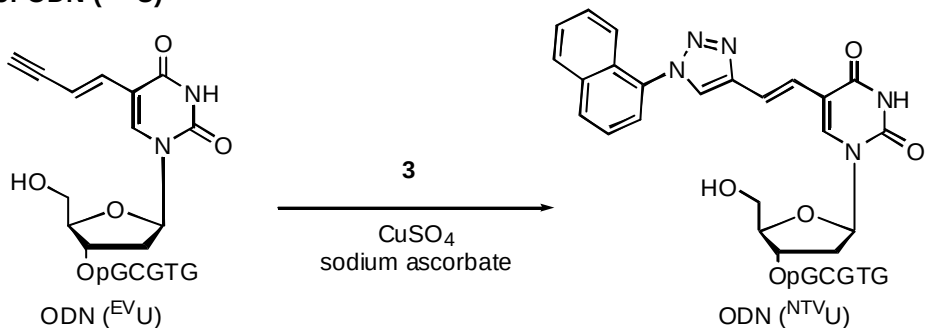
## Synthesis of ODN ($^{MPTV}$U)




**Scheme S7**

A reaction mixture (total volume 2.0 mL) containing ODN($^{EV}$U) (50 μM strand conc.), a solution of 2 in EtOH (2.5 mM) , a aqueous solution of $CuSO_4$ (2 mM) and a aqueous solution of sodium ascorbate (1 mM) was shaken for 12 h. The crude product was were purified on a Chemcobond 5-ODS-H column (10 × 150 mm) by reverse phase HPLC; elution was with 0.05 M ammonium formate containing 3-20% $CH_3CN$, linear gradient (30 min) at a flow rate of 3.0 mL/ min (Figure S3).

**Synthesis of ODN ($^{NTV}$U)**




**Scheme S8**

A reaction mixture (total volume 2.0 mL) containing ODN($^{EV}$U) (50 μM strand conc.), a solution of 3 in EtOH (2.5 mM) , a aqueous solution of $CuSO_4$ (2 mM) and a aqueous solution of sodium ascorbate (1 mM) was shaken for 12 h. The crude product was were purified on a Chemcobond 5-ODS-H column (10 × 150 mm) by reverse phase HPLC; elution was with 0.05 M ammonium formate containing 3-20% $CH_3CN$, linear gradient (30 min) at a flow rate of 3.0 mL/ min (Figure S4).

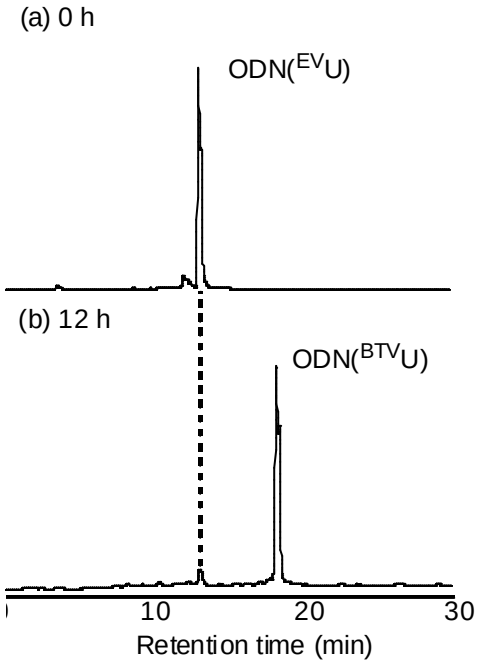



**Figure S1** HPLC analysis of a post modification with benzylazide.

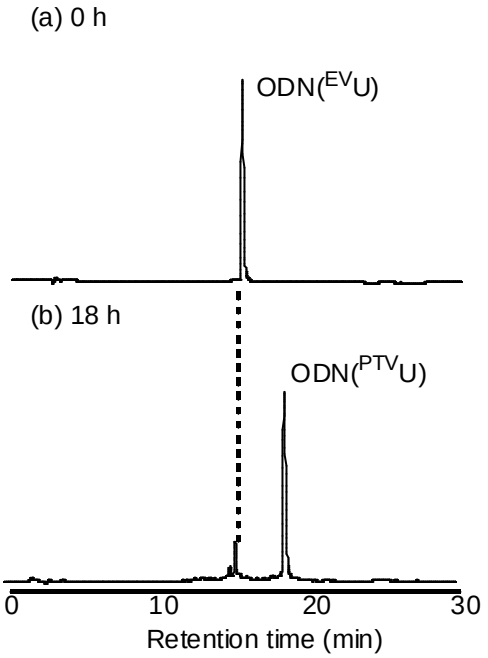



**Figure S2** HPLC analysis of a post modification with **2**.

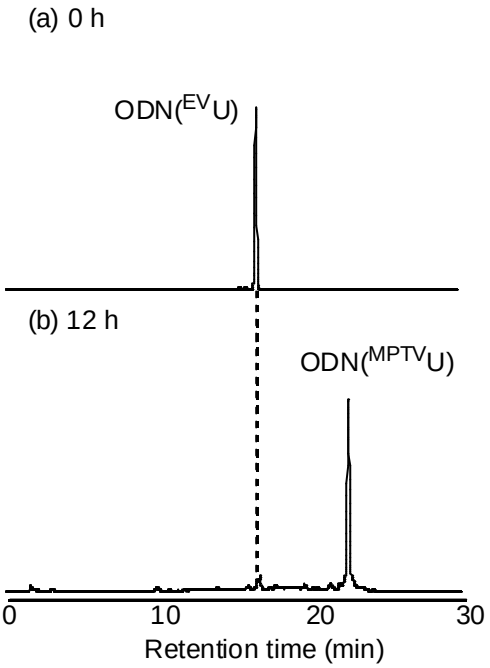



**Figure S3** HPLC analysis of a post modification with **3**.

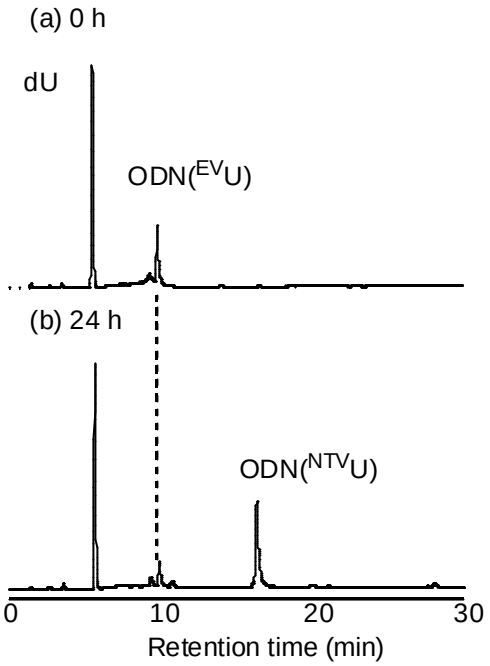
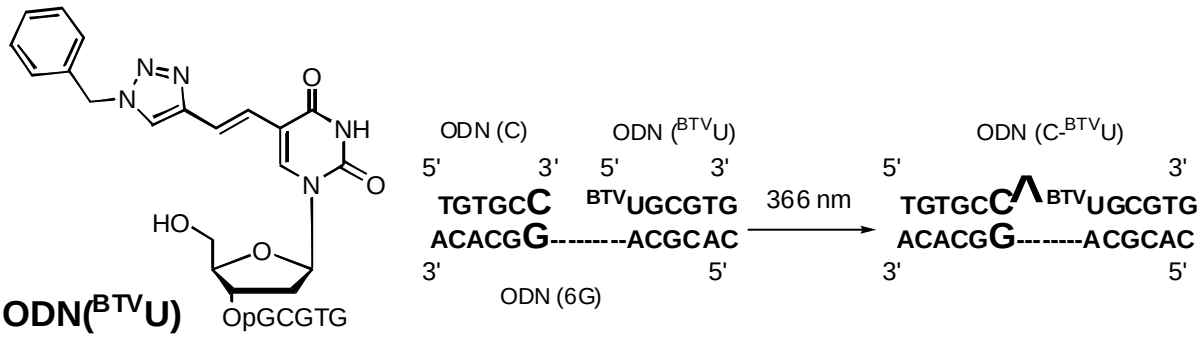
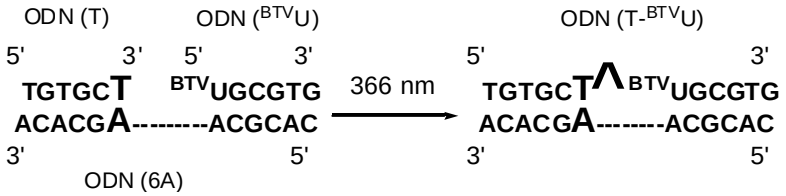



**Figure S4** HPLC analysis of a post modification with **4**.

## Template-directed photoligation of ODNs via $^{BTV}$U



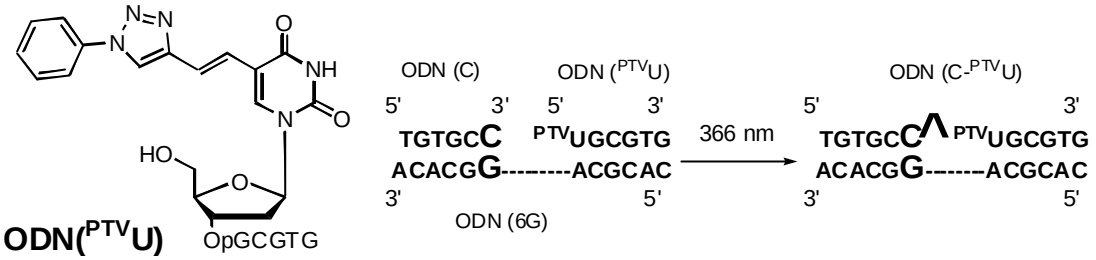

A reaction mixture (total volume 200 μl) containing ODN(C) (10 μM strand conc.), ODN($^{BTV}$U) (10 μM strand conc.) and ODN(6A) (12 μM strand conc.) in 50 mM sodium cacodylate buffer (pH 7.0) was irrasiated a 0 °C with UV-LED (366 nm). After irradiation 10 μL of the aliquot was taken up and subjected to HPLC analysis. Analysis was carried out on a Chemcobond 5-ODS-H column (4.6 ×150 mm), detected at 260 nm; elution was with 0.05 M ammonium formate containing 5–8% acetonitrile, linear gradient (20 min), then 8–30% acetonitrile, linear gradient (20 min) at a flow rate of 1.0ml/ min (Figure S5). Irradiation at 366 nm caused the ligated product, ODN(C-$^{BTV}$U). ODN(C-$^{BTV}$U) was characterized by MALDI-TOF-MS (Figure S17).



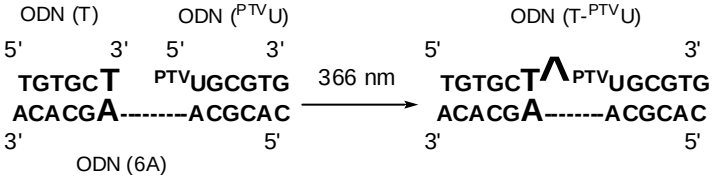

A reaction mixture (total volume 200 μl) containing ODN(T) (10 μM strand conc.), ODN($^{BTV}$U) (10 μM strand conc.) and ODN(6G) (12 μM strand conc.) in 50 mM sodium cacodylate buffer (pH 7.0) in a quarts cell was irrasiated a 0 °C with UV-LED (366 nm). After irradiation 10 μL of aliquot was taken up and subjected to HPLC analysis. Analysis was carried out on a Chemcobond 5-ODS-H column (4.6 ×150 mm), detected at 260 nm; elution was with 0.05 M ammonium formate containing 3–

7% acetonitrile, linear gradient (20 min), 7–8% acetonitrile, linear gradient (20 min), then 8–30% acetonitrile, linear gradient (20 min) at a flow rate of 1.0 ml/ min (Figure S6). Irradiation at 366 nm caused the ligated product, ODN(T-$^{BTV}$U). ODN(T-$^{BTV}$U) was characterized by MALDI-TOF-MS (Figure S18).

## Template-directed photoligation of ODNs via $^{PTV}$U

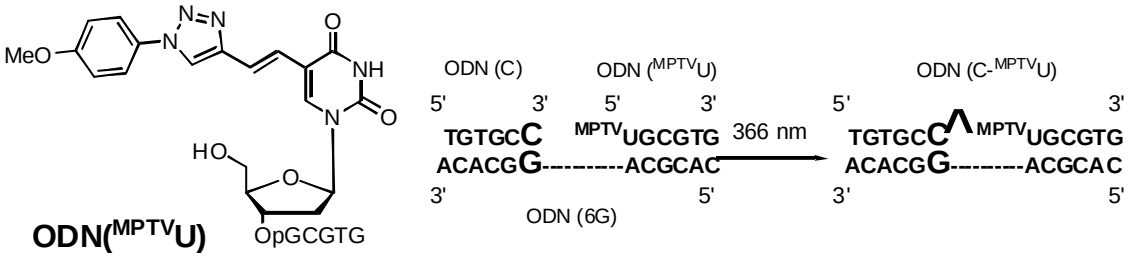



A reaction mixture (total volume 200 µl) containing ODN(C) (10 µM strand conc.), ODN($^{PTV}$U) (10 µM strand conc.) and ODN(6A) (12 µM strand conc.) in 50 mM sodium cacodylate buffer (pH 7.0) in a quarts cell was irrasiated a 0 °C with UV-LED (366 nm). After irradiation 10 µL of aliquot was taken up and subjected to HPLC analysis. Analysis was carried out on a Chemcobond 5-ODS-H column 4.6 ×150 mm), detected at 260 nm; elution was with 0.05 M ammonium formate containing 5–8% acetonitrile, linear gradient (20 min), then 8–30% acetonitrile, linear gradient (20 min) at a flow rate of 1.0 ml/ min (Figure S7). Irradiation at 366 nm caused the ligated product, ODN(C-$^{PTV}$U). ODN(C-$^{PTV}$U) was characterized by MALDI-TOF-MS (Figure S19).

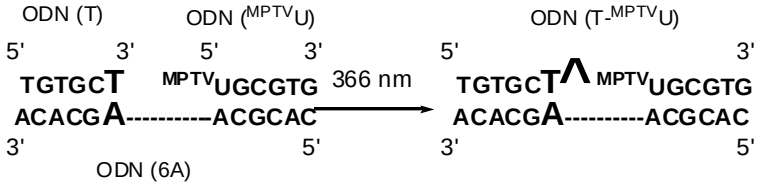



A reaction mixture (total volume 200 µl) containing ODN(T) (10 µM strand conc.), ODN($^{PTV}$U) (10 µM strand conc.) and ODN(6G) (12 µM strand conc.) in 50 mM sodium cacodylate buffer (pH 7.0) in a quarts cell was irrasiated a 0 °C with UV-LED (366 nm). After irradiation 10 µL of the aliquot was taken up and subjected to HPLC analysis. Analysis was carried out on a Chemcobond 5-ODS-H column (4.6 ×150 mm), detected at 260 nm; elution was with 0.05 M ammonium formate containing 3–7% acetonitrile, linear gradient (20 min), 7–8% acetonitrile, linear gradient (20 min), then 8–30%. acetonitrile, linear gradient (20 min) at a flow rate of 1.0 ml/ min (Figure S8). Irradiation at 366 nm caused the ligated product, ODN(T-$^{PTV}$U). ODN(T-$^{PTV}$U) was characterized by MALDI-TOF-MS (Figure S20).

## Template-directed photoligation of ODNs via $^{MPTV}$U

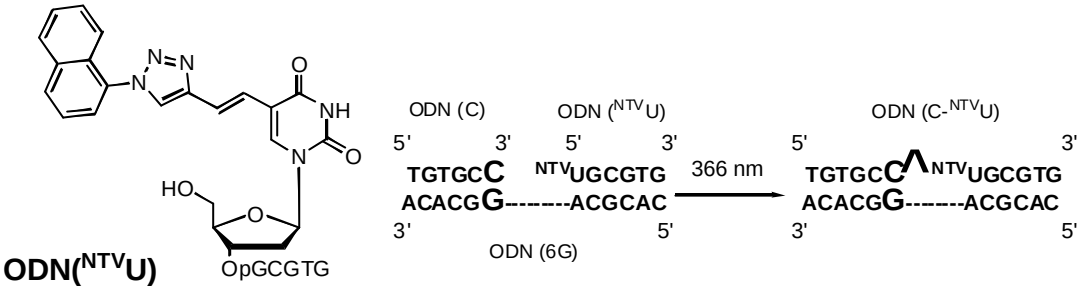



A reaction mixture (total volume 200 µl) containing ODN(C) (10 µM strand conc.), ODN($^{MPTV}$U) (10 µM strand conc.) and ODN(6A) (12 µM strand conc.) in 50 mM sodium cacodylate buffer (pH 7.0) in a quarts cell was irrasiated a 0 °C with UV-LED (366 nm). After irradiation 10 µL of the aliquot was taken up and subjected to HPLC analysis. Analysis was carried out on a Chemcobond 5-ODS-H column (4.6 ×150 mm), detected at 260 nm; elution was with 0.05 M ammonium formate containing 5–8% acetonitrile, linear gradient (20 min), then 8–30% acetonitrile, linear gradient (20 min) at a flow rate of 1.0 ml/ min (Figure S9). Irradiation at 366 nm caused the ligated product, ODN(C-$^{MPTV}$U). ODN(C-$^{MPTV}$U) was characterized by MALDI-TOF-MS (Figure S21).

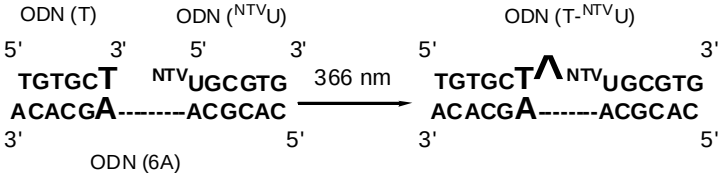



A reaction mixture (total volume 200 μl) containing ODN(T) (10 μM strand conc.), ODN($^{MPTV}$U) (10 μM strand conc.) and ODN(6G) (12 μM strand conc.) in 50 mM sodium cacodylate buffer (pH 7.0) in a quarts cell was irrasiated a 0 °C with UV-LED (366 nm). After irradiation 10 μL of the aliquot was taken up and subjected to HPLC analysis. Analysis was carried out on a Chemcobond 5-ODS-H column (4.6 ×150 mm), detected at 260 nm; elution was with 0.05 M ammonium formate containing 3–7% acetonitrile, linear gradient (20 min), 7–8% acetonitrile, linear gradient (20 min), then 8-30% acetonitrile, linear gradient (20 min) at a flow rate of 1.0 ml/ min (Figure S10). Irradiation at 366 nm caused the ligated product, ODN(T-$^{MPTV}$U). ODN(T-$^{MPTV}$U) was characterized by MALDI-TOF-MS (Figure S22).

## Template-directed photoligation of ODNs via $^{NTV}$U

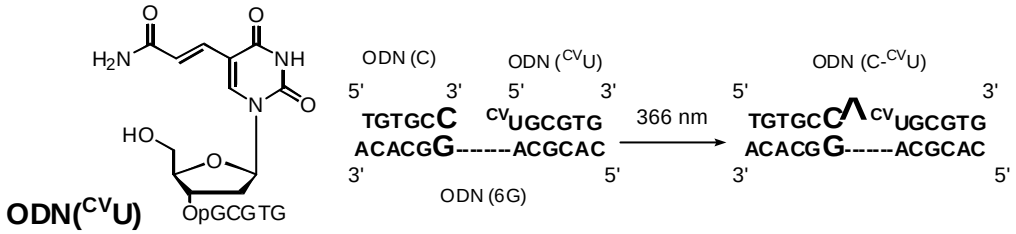



A reaction mixture (total volume 200 μl) containing ODN(C) (10 μM strand conc.), ODN($^{NTV}$U) (10 μM strand conc.) and ODN(6A) (12 μM strand conc.) in 50 mM sodium cacodylate buffer (pH 7.0) in a quarts cell was irrasiated a 0 °C with UV-LED (366 nm). After irradiation 10 μL of the aliquot was taken up and subjected to HPLC analysis. Analysis was carried out on a Chemcobond 5-ODS-H column (4.6 ×150 mm), detected at 260 nm; elution was with 0.05 M ammonium formate containing 5–8% acetonitrile, linear gradient (20 min), then 8–50% acetonitrile, linear gradient (20 min) at a flow rate of 1.0 ml/ min (Figure S11). Irradiation at 366 nm caused the ligated product, ODN(C-$^{NTV}$U). ODN(C-$^{NTV}$U) was characterized by MALDI-TOF-MS (Figure S23).

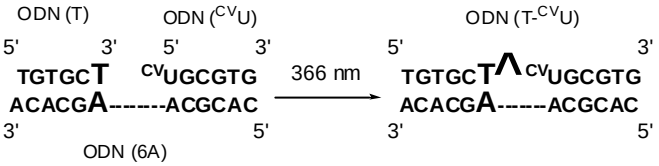



A reaction mixture (total volume 200 μl) containing ODN(T) (10 μM strand conc.), ODN($^{NTV}$U) (10 μM strand conc.) and ODN(6G) (12 μM strand conc.) in 50 mM sodium cacodylate buffer (pH 7.0) in a quarts cell was irrasiated a 0 °C with UV-LED (366 nm). After irradiation 10 μL of the aliquot was taken up and subjected to HPLC analysis. Analysis was carried out on a Chemcobond 5-ODS-H column (4.6 ×150 mm), detected at 260 nm; elution was with 0.05 M ammonium formate containing 3–7% acetonitrile, linear gradient (20 min), 7–8% acetonitrile, linear gradient (20 min), then 8-30% acetonitrile, linear gradient (20 min) at a flow rate of 1.0 ml/ min (Figure S12). Irradiation at 366 nm caused the ligated product, ODN(T-$^{NTV}$U). ODN(T-$^{NTV}$U) was characterized by MALDI-TOF-MS (Figure S24).

## Template-directed photoligation of ODNs via $^{CV}$U




A reaction mixture (total volume 200 μl) containing ODN(C) (10 μM strand conc.), ODN($^{CV}$U) (10 μM strand conc.) and ODN(6A) (12 μM strand conc.) in 50 mM sodium cacodylate buffer (pH 7.0) in a quarts cell was irrasiated a 0 °C with UV-LED (366 nm). After irradiation 10 μL of the aliquot was taken up and subjected to HPLC analysis. Analysis was carried out on a Chemcobond 5-ODS-H column (4.6 ×150 mm), detected at 260 nm; elution was with 0.05 M ammonium formate containing 5–10% acetonitrile, linear gradient (30 min), at a flow rate of 1.0 ml/ min (Figure S13). Irradiation at 366 nm caused the ligated product, ODN(C-$^{CV}$U). ODN(C-$^{CV}$U) was characterized by MALDI-TOF-MS (Figure S25).




A reaction mixture (total volume 200 μl) containing ODN(T) (10 μM strand conc.), ODN($^{NTV}$U) (10 μM strand conc.) and ODN(6G) (12 μM strand conc.) in 50 mM sodium cacodylate buffer (pH 7.0) in a quarts cell was irrasiated a 0 °C with UV-LED (366 nm). After irradiation 10 μL of the aliquot was taken up and subjected to HPLC analysis. Analysis was carried out on a Chemcobond 5-ODS-H column (4.6 ×150 mm), detected at 260 nm; elution was with 0.05 M ammonium formate containing 3–7% acetonitrile, linear gradient (20 min), 7–8% acetonitrile, linear gradient (20 min), then 8-30% acetonitrile, linear gradient (20 min) at a flow rate of 1.0 ml/ min (Figure S14). Irradiation at 366 nm caused the ligated produc,t ODN(T-$^{CV}$U). ODN(T-$^{CV}$U) was characterized by MALDI-TOF-MS (Figure S26).

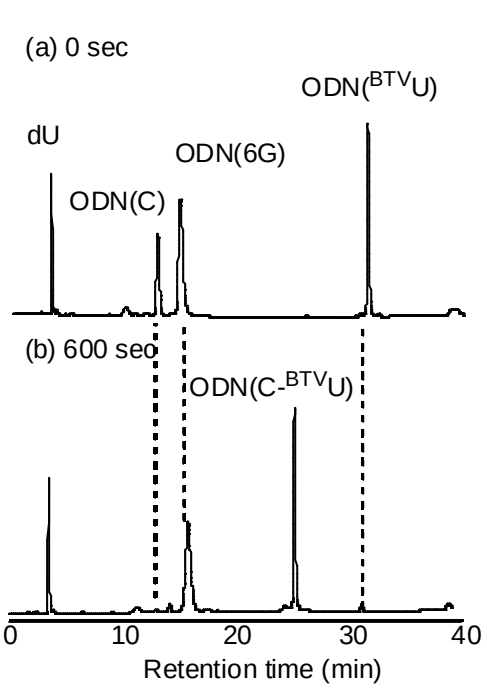



**Figure S5** HPLC analysis of photligation with ODN($^{BTV}$U) and ODN(C) in the presence of ODN(6G).

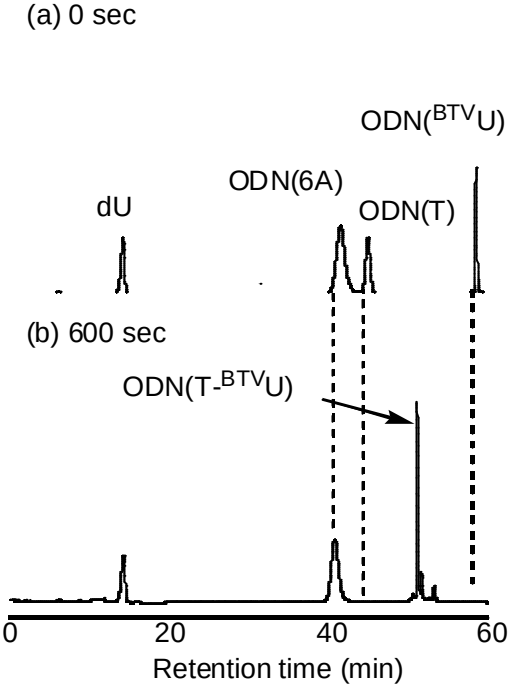



**Figure S6** HPLC analysis of photligation with ODN($^{BTV}$U) and ODN(T) in the presence of ODN(6A).

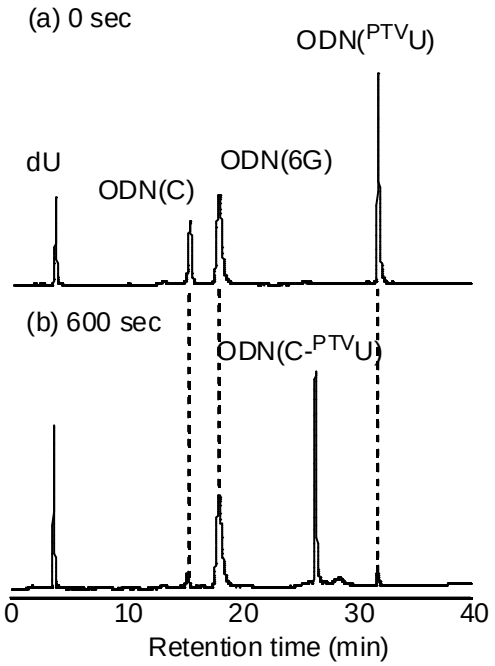



**Figure S7** HPLC analysis of photligation with ODN($^{PTV}$U) and ODN(C) in the presence of ODN(6G).

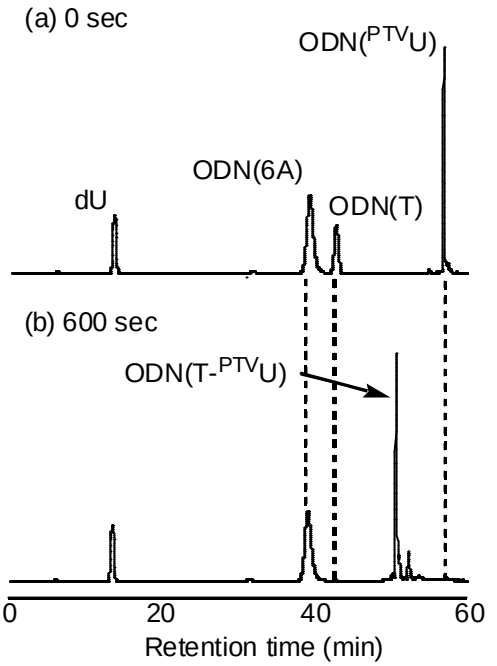



**Figure S8** HPLC analysis of photligation with ODN($^{PTV}$U).and ODN(T) in the presence of ODN(6A).

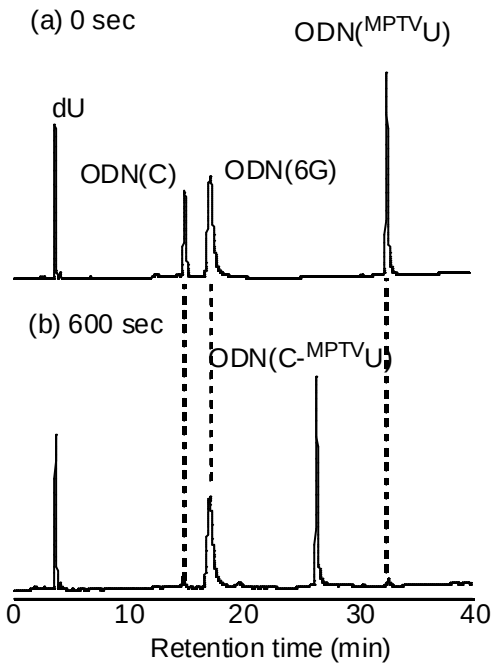



**Figure S9** HPLC analysis of photligation with ODN($^{MPTV}$U) and ODN(C) in the presence of ODN(6G).

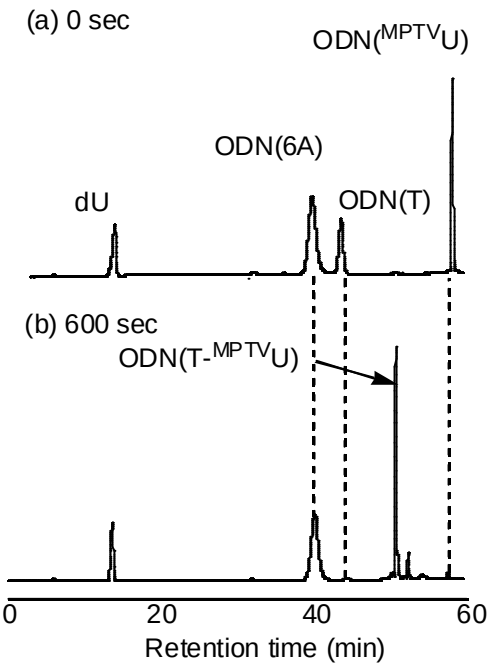



**Figure S10** HPLC analysis of photligation with ODN($^{MPTV}$U) and ODN(T) in the presence of ODN(6A).

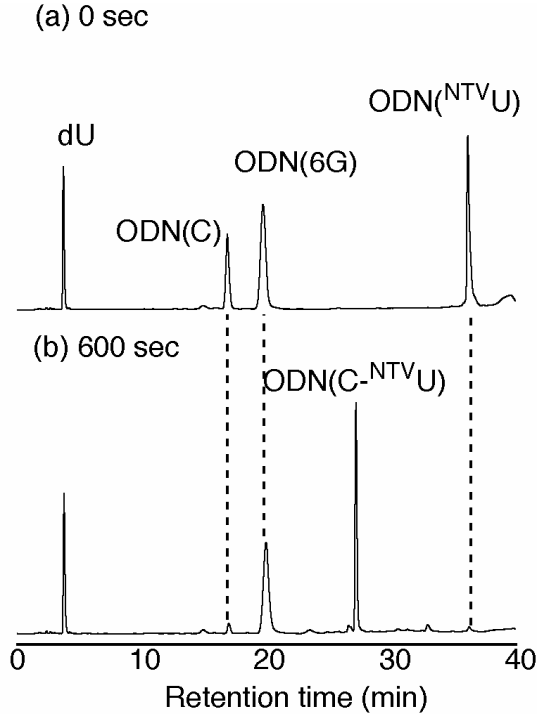



**Figure S11** HPLC analysis of photligation with ODN($^{NTV}$U) and ODN(C) in the presence of ODN(6G).

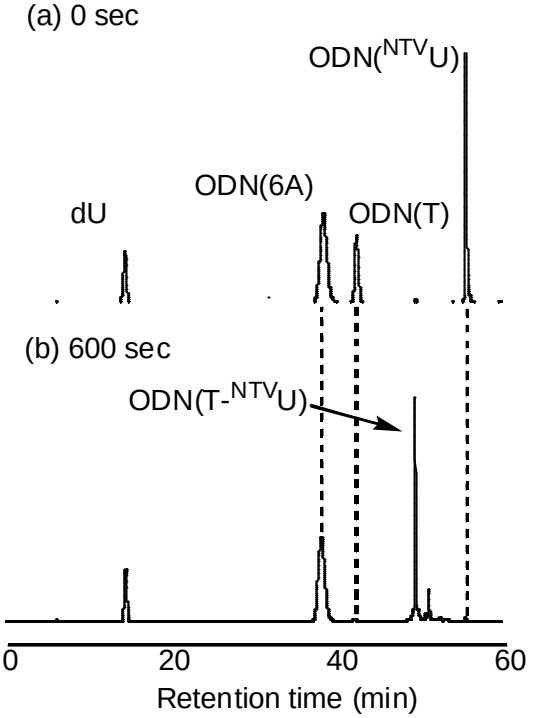



**Figure S12** HPLC analysis of photligation with ODN($^{NTV}$U).and ODN(T) in the presence of ODN(6A).

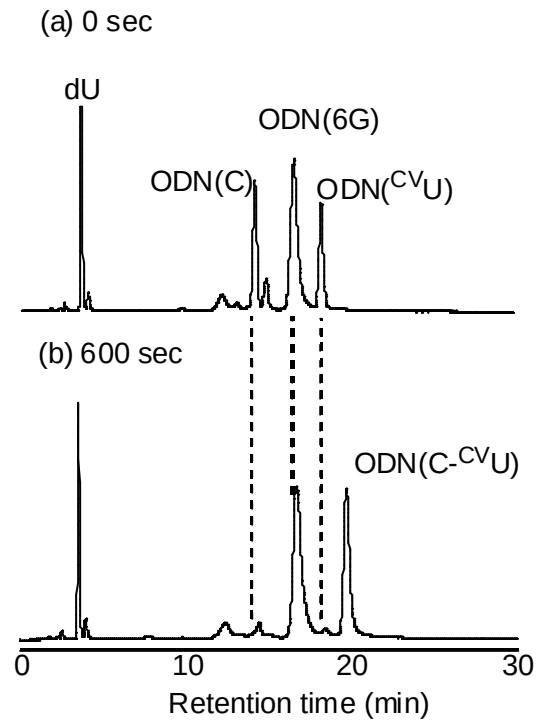



**Figure S13** HPLC analysis of photligation with ODN($^{CV}$U) and ODN(C) in the presence of ODN(6G).

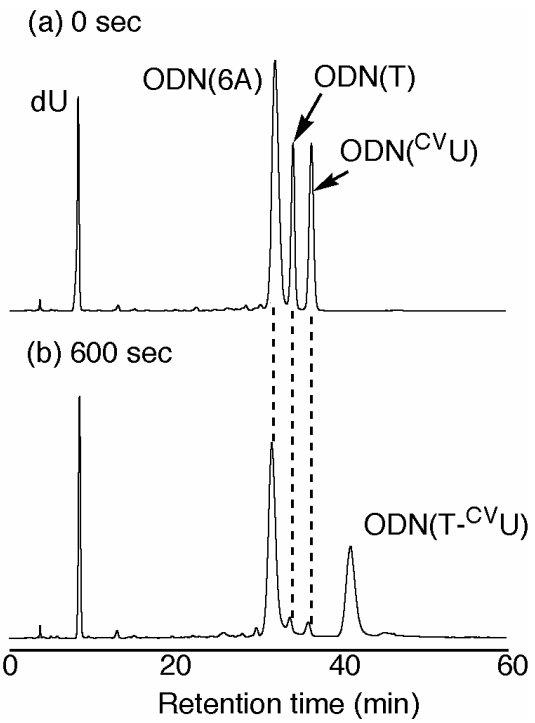



**Figure S14** HPLC analysis of photligation with ODN($^{CV}$U) and ODN(T) in the presence of ODN(6A).

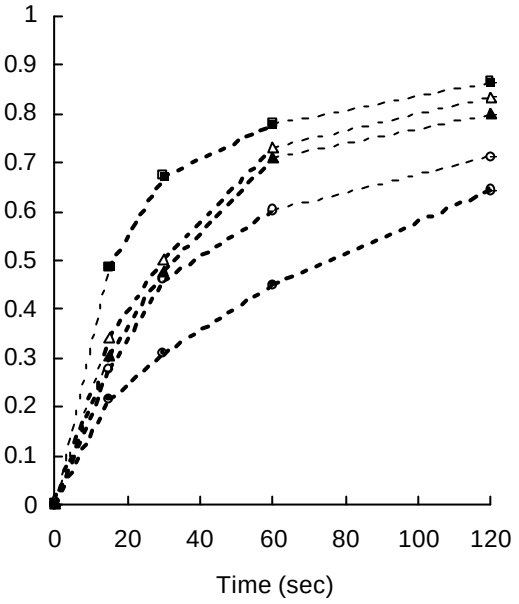



**Figure S15.** Comparison of photoligation rates with ODN($^{CV}$U) ( ) or ODN($^{BTV}$U) (○), ODN($^{PTV}$U) ( ) or ODN($^{MPTV}$U) (△) or ODN($^{NTV}$U) ( ) and ODN(C) in the presence of ODN(6G).

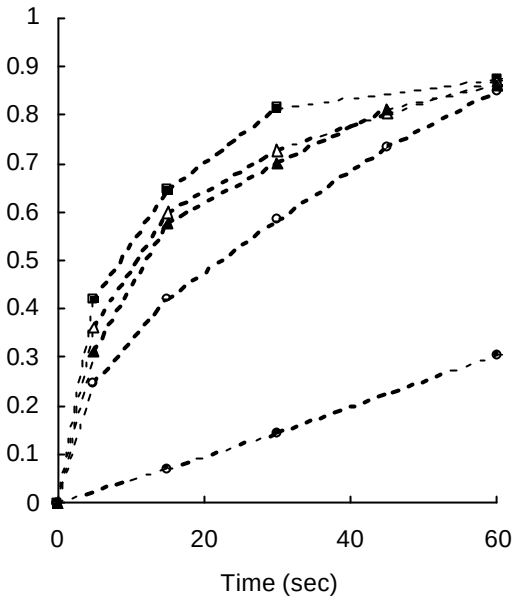



**Figure S16.** Comparison of photoligation rates with ODN($^{CV}$U) ( ) or ODN($^{BTV}$U) (○), ODN($^{PTV}$U) ( ) or ODN($^{MPTV}$U) (△) or ODN($^{NTV}$U) ( ) and ODN(T) in the presence of ODN(6A).

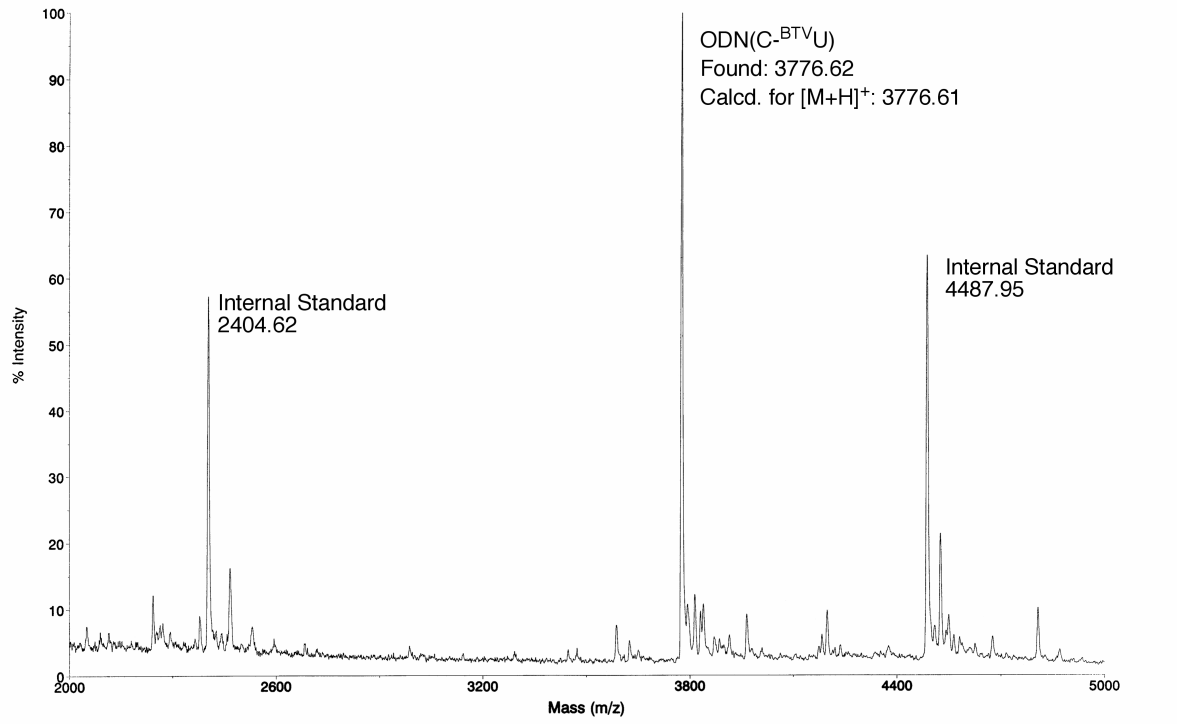



**Figure S17.** MALDI-TOF spectrum of ODN(C-$^{BTV}$U) using a 3-hydroxypicolinic acid matrix.

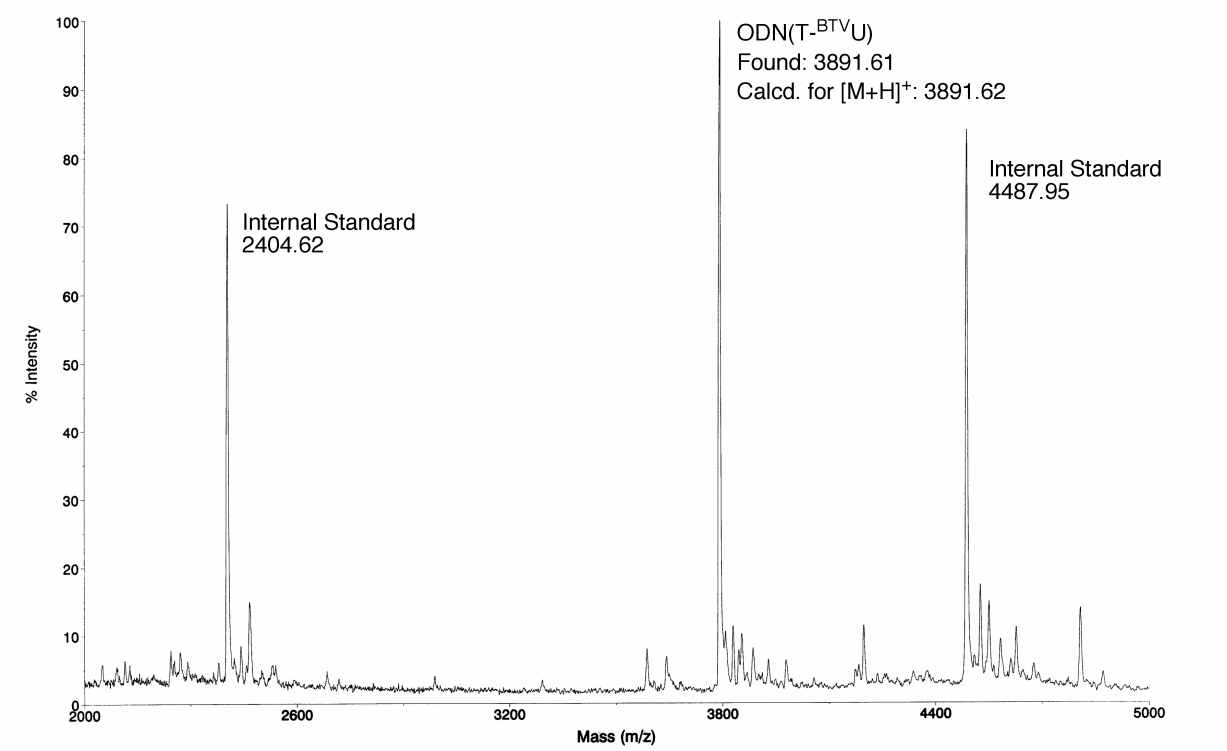



**Figure S18.** MALDI-TOF spectrum of ODN(T-$^{BTV}$U) using a 3-hydroxypicolinic acid matrix.

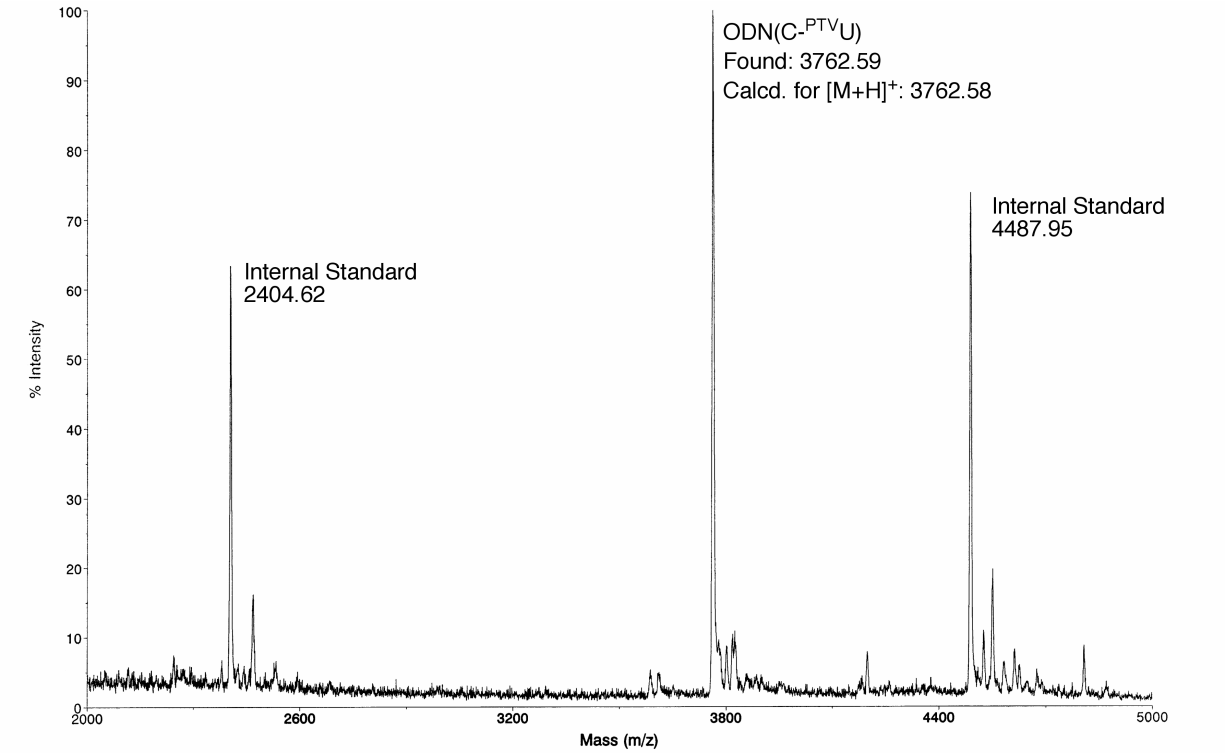



**Figure S19.** MALDI-TOF spectrum of ODN(C-$^{PTV}$U) using a 3-hydroxypicolinic acid matrix.

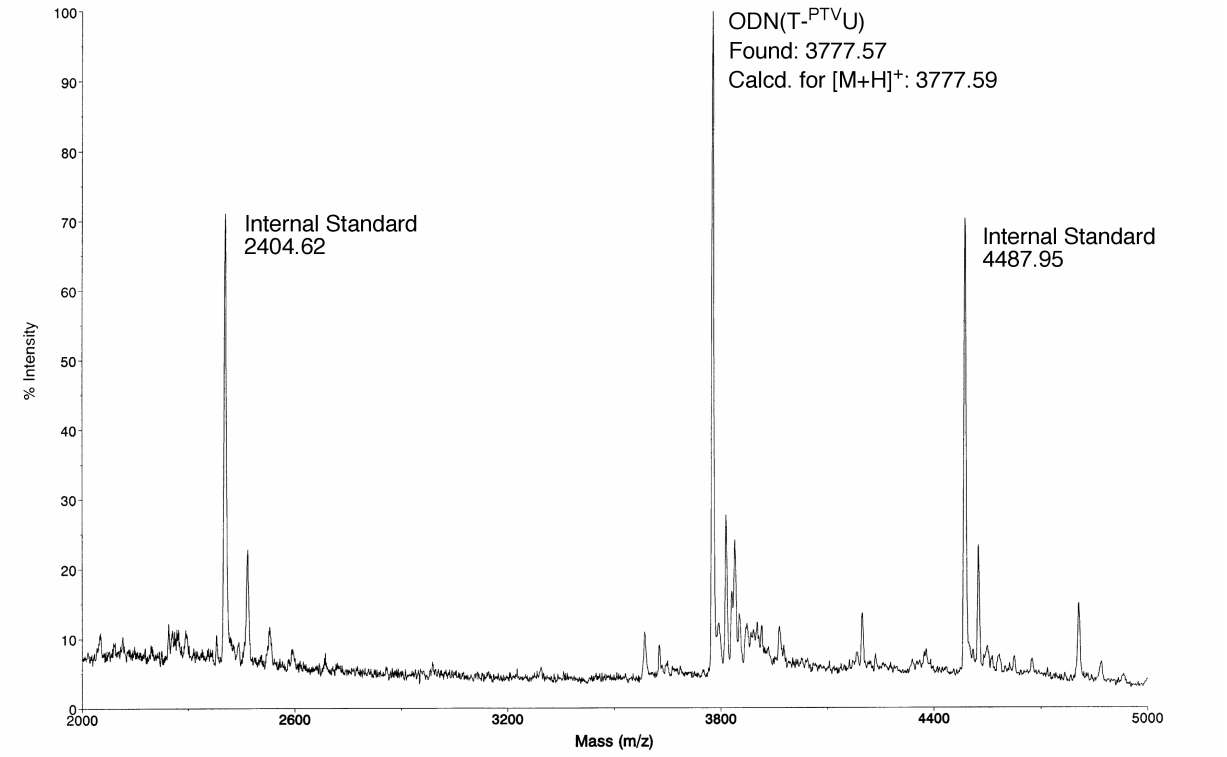



**Figure S20.** MALDI-TOF spectrum of ODN(T-$^{PTV}$U) using a 3-hydroxypicolinic acid matrix.

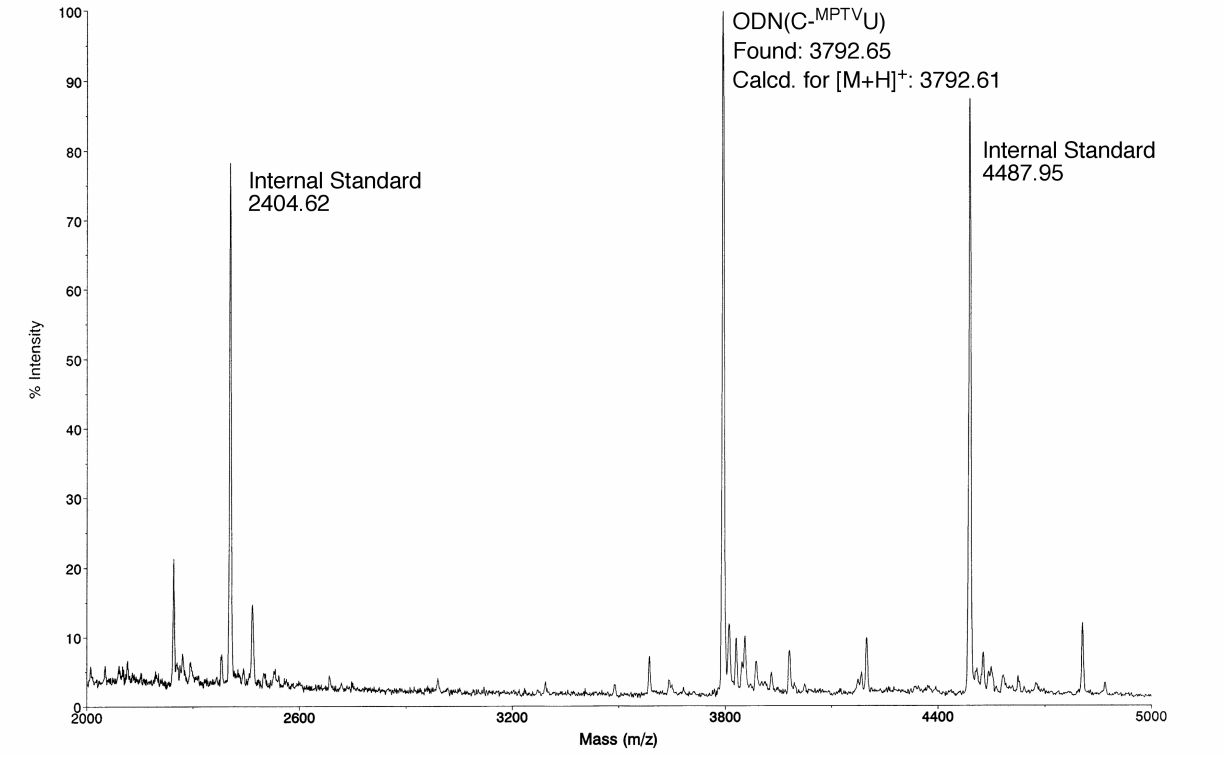



**Figure S21.** MALDI-TOF spectrum of ODN(C-$^{MPTV}$U) using a 3-hydroxypicolinic acid matrix.

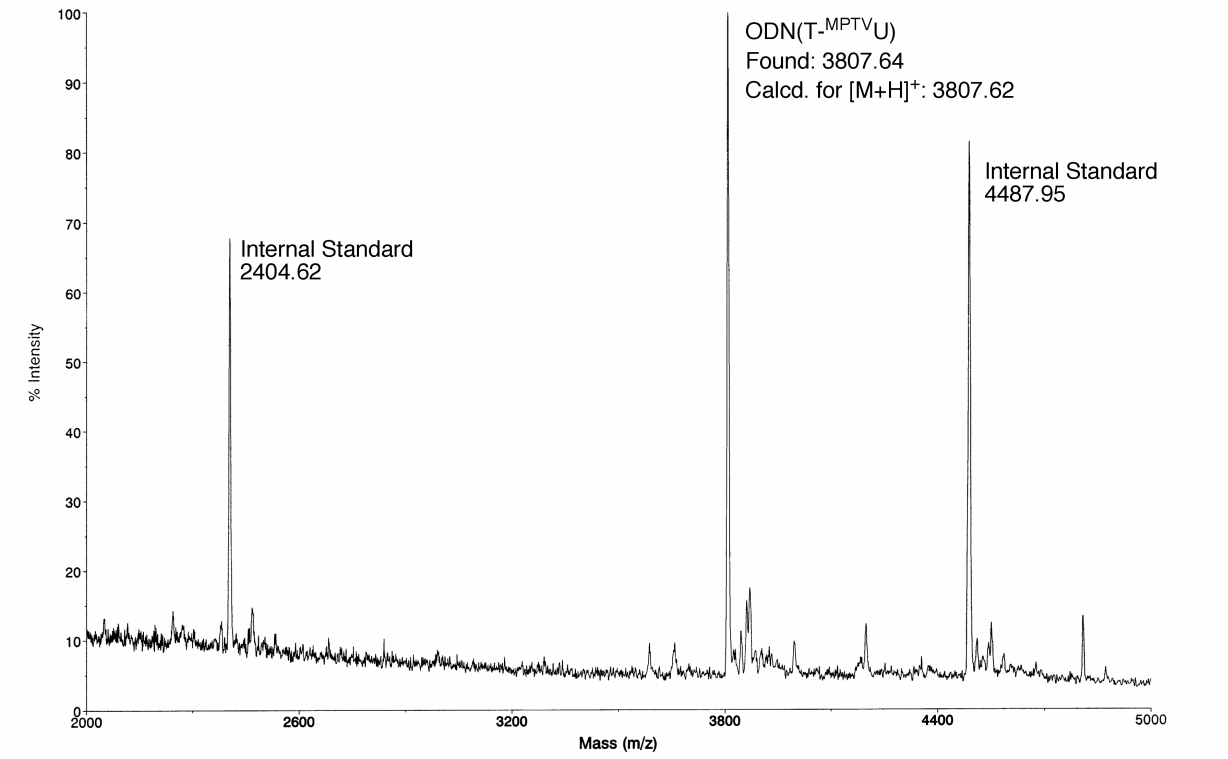



**Figure S22.** MALDI-TOF spectrum of ODN(T-$^{MPTV}$U) using a 3-hydroxypicolinic acid matrix.

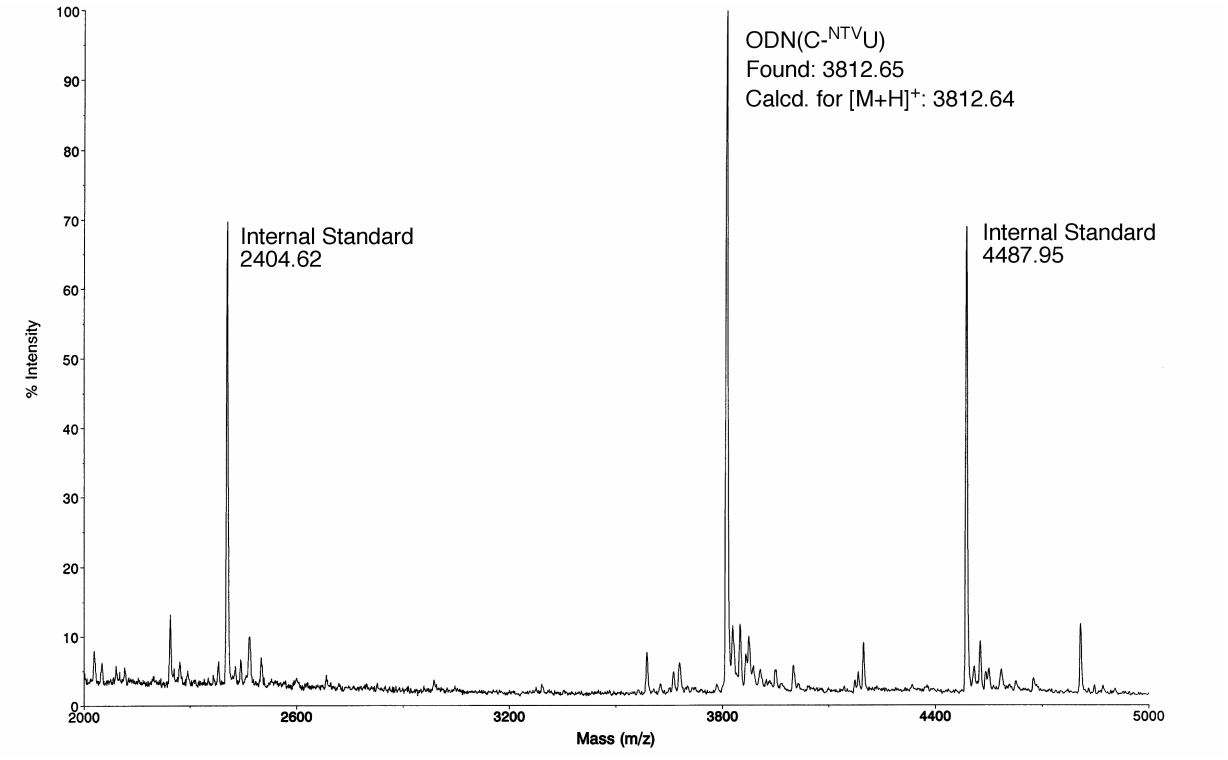



**Figure S23.** MALDI-TOF spectrum of ODN(C-$^{NTV}$U) using a 3-hydroxypicolinic acid matrix.

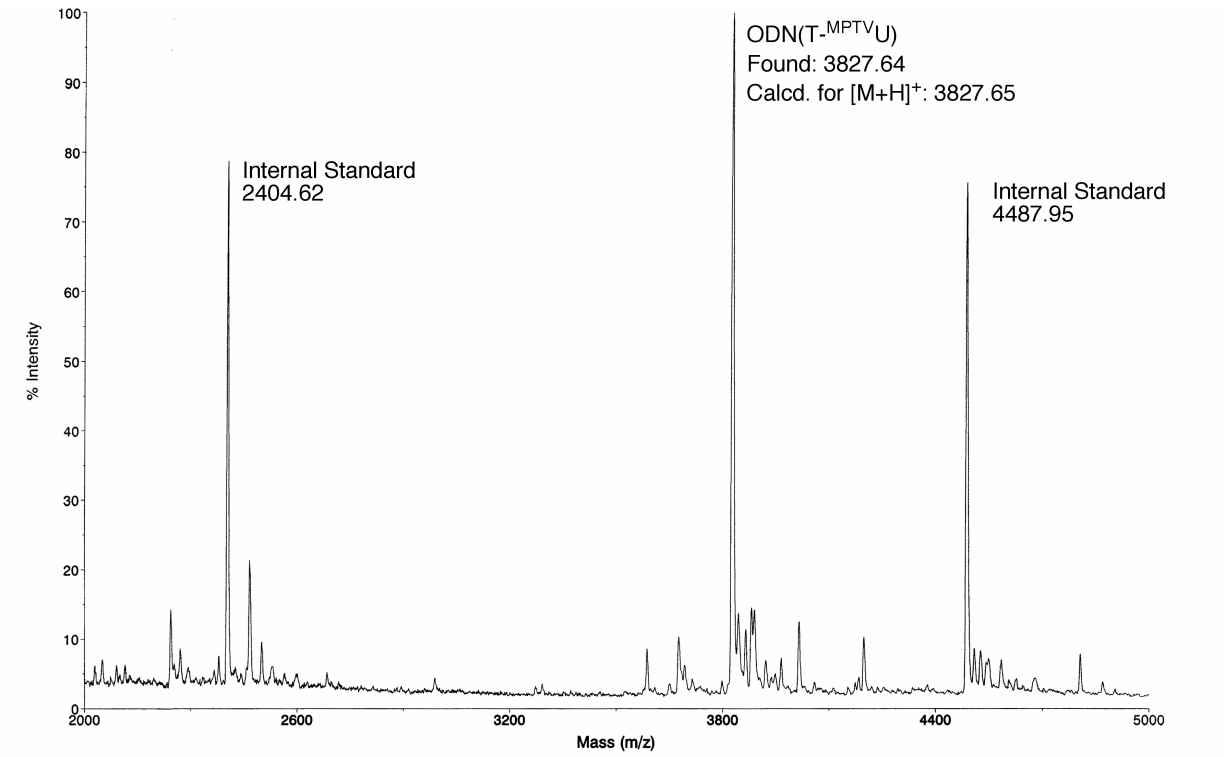



**Figure S24.** MALDI-TOF spectrum of ODN(T-$^{NTV}$U) using a 3-hydroxypicolinic acid matrix.

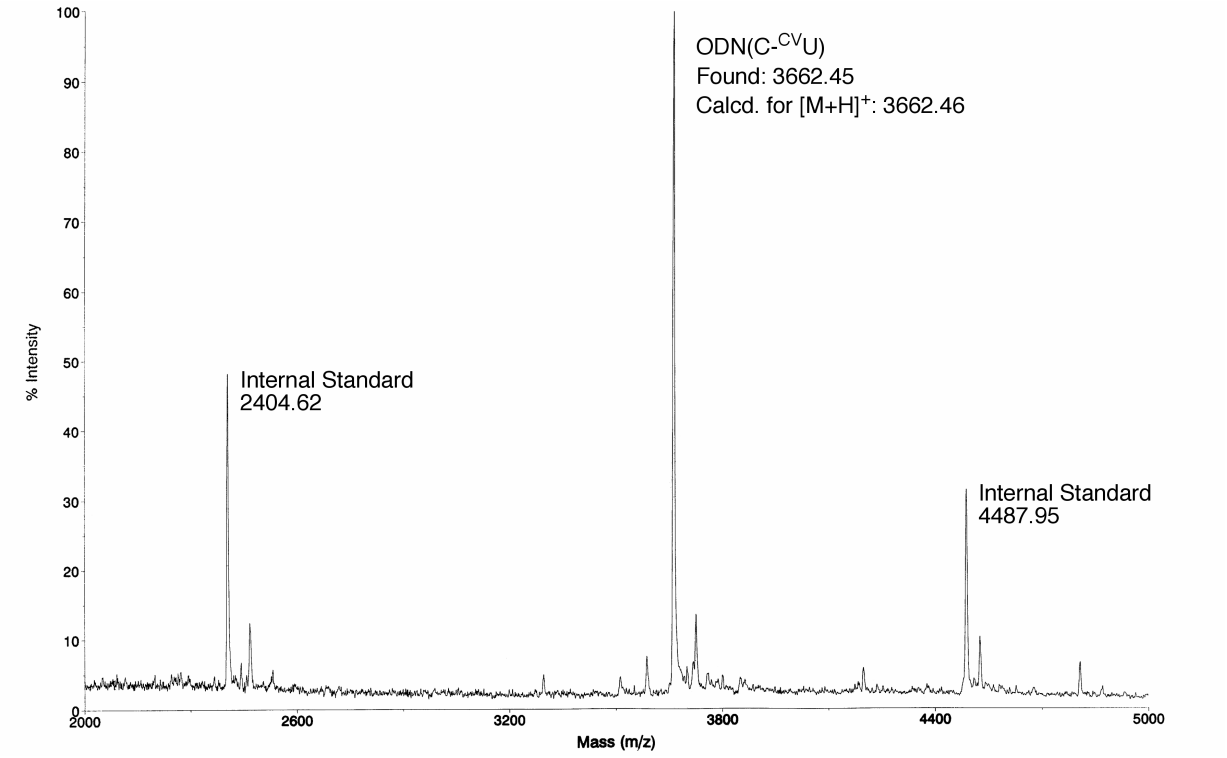



**Figure S25.** MALDI-TOF spectrum of ODN(C-$^{CV}$U) using a 3-hydroxypicolinic acid matrix.

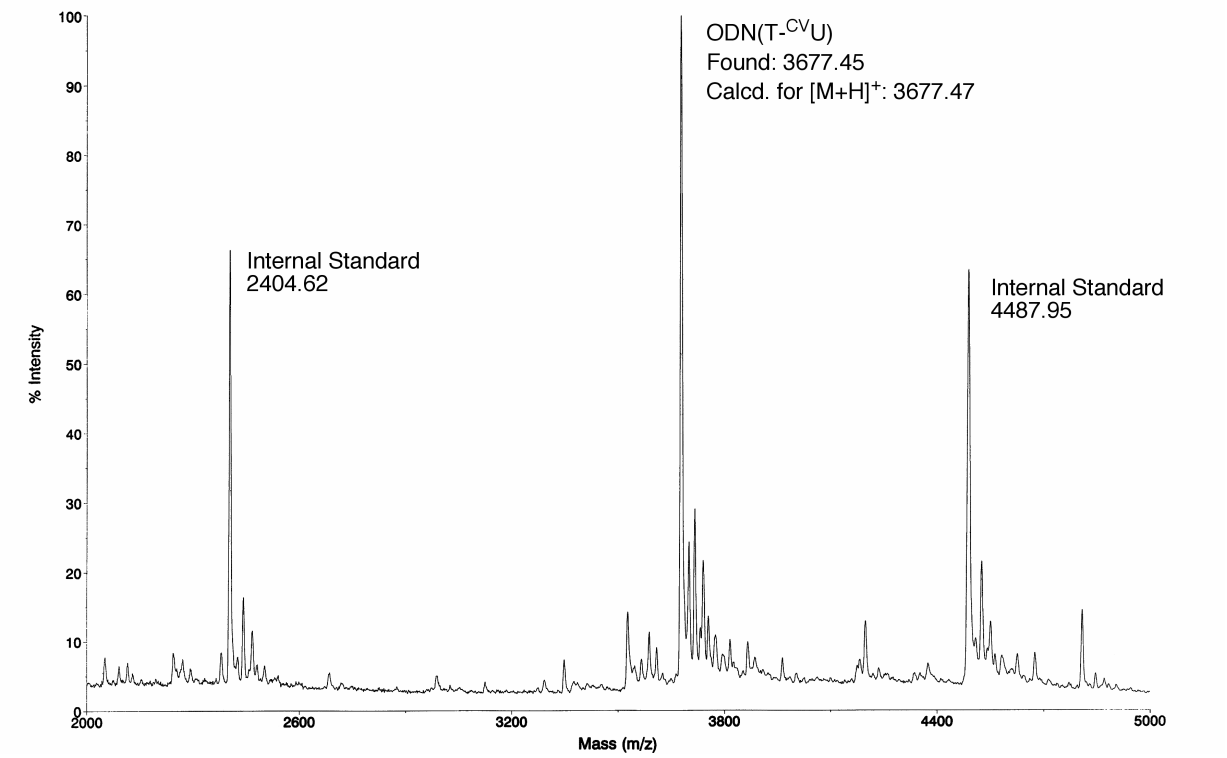
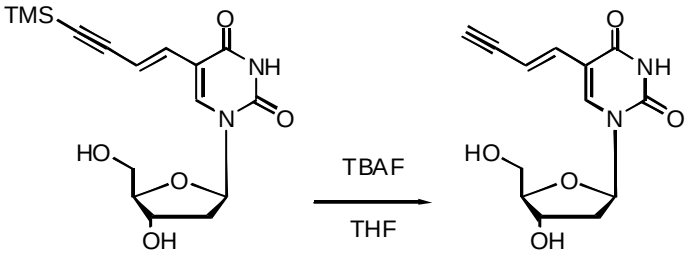



**Figure S26.** MALDI-TOF spectrum of ODN(T-$^{CV}$U) using a 3-hydroxypicolinic acid matrix.

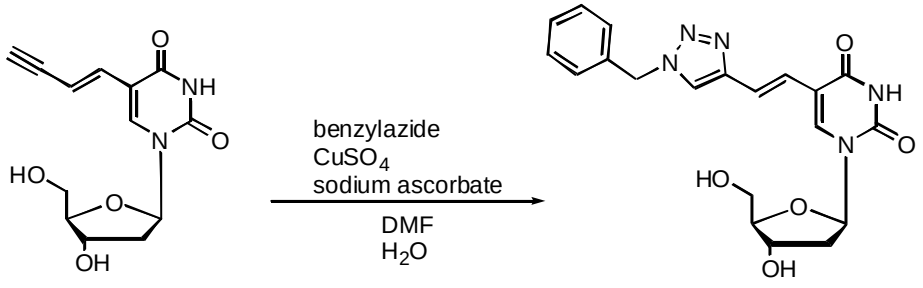
## (*E*)-5-(2-ethynyl vinyl)-2'-deoxyuridine (10)




**7** **10**

To a solution of **7** (0.23 g, 0.66 mmol) in THF(3 ml) was added 1 M TBAF in THF (1 ml) at 0 °C in an ice bath and stirred for 3 hours. The crude was purified by column chromatography ($CHCl_3$ / MeOH= 9 / 1) to give **10** (0.138 g, 75%) $^1$H NMR ($CD_3OD$, 300MHz) δ? 8.29 (s, 1H), 6.74 (d, 1H, *J*= 16.2 Hz), 6.62 (dd, 1H, *J*= 16.2, 1.9 Hz), 6.31 (t, 1H, *J*= 6.5 Hz), 4.46 (m, 1H), 3.98 (m, 1H), 3.84 (m, 2H), 3.40 (m, 1H), 2.32 (m, 2H); $^{13}$C NMR ($CD_3OD$, 125 MHz) δ: 164.3, 151.5, 141.0, 136.2, 112.2, 109.3, 89.4, 87.1, 84.4, 80.6, 72.1, 62.8, 42.1.

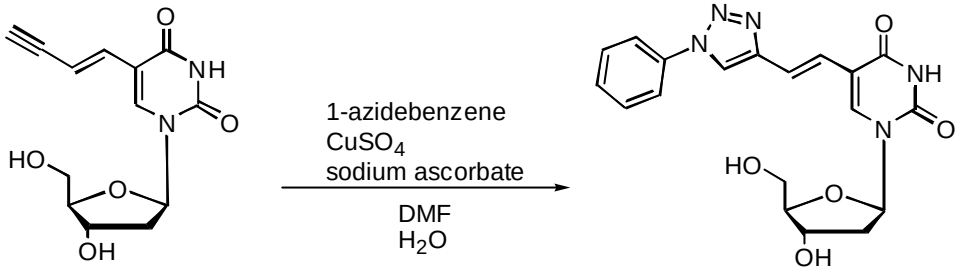
## (*E*)-5-[2-(1-phenzyl-1*H*-[1,2,3]triazol-4-yl)-vinyl] -2'-deoxyuridine (11)




**10** **11**

**10** (0.10 g 0.36 mmol), $CuSO_4$(90 mg, 0.36 mmol), sodium ascorbate (71 mg, 0.36 mmol) and benzylazide(48 mg, 0.36 mmol) in a mixture of DMF(0.5 mL) and $H_2O$(0.5 mL) was stirred for 24 hours. The crude was purified by column chromatography ($CHCl_3$ / MeOH= 9 / 1) to give **11** (0.126 g, 88%). $^1$H NMR (DMSO, 300MHz) δ? 11.48 (s, 1H), 8.21 (s, 1H), 8.14 (s, 1H), 7.42-7.25 (m, 5H), 7.40 (d, 1H, *J*= 16.3 Hz), 6.96 (d, 1H, *J*= 16.3 Hz), 6.16 (t, 1H, *J*= 6.6 Hz), 5.57 (s, 2H), 5.26 (d, 1H, *J*= 4.1Hz), 5.15 (t, 1H, *J*= 4.9 Hz), 4.26 (m, 1H), 3.79 (m, 1H), 3.61 (m, 2H), 2.15 (m, 2H); $^{13}$C NMR (DMSO-$d_6$, 125 MHz) δ: 162.3, 149.6, 145.9, 138.6, 136.2, 129.00, 128.3, 128.1, 122.4, 122.1, 117.0, 110.6, 87.7, 84.6, 70.3, 61.3, 53.0.

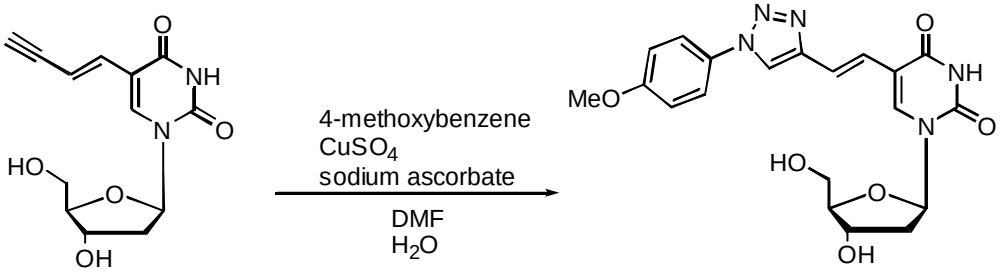
## (*E*)-5-[2-(1-phenyl-1*H*-[1,2,3]triazol-4-yl)-vinyl] -2'-deoxyuridine (12)




**10** **12**

**10** (0.10 g 0.36 mmol), $CuSO_4$(90 mg, 0.36 mmol), sodium ascorbate (71 mg, 0.36 mmol) and 1-azidebenzene(43 mg, 0.36 mmol) in a mixture of DMF(0.5 mL) and $H_2O$(0.5 mL) was stirred for 24 hours. The crude was purified by column chromatography ($CHCl_3$ / MeOH= 9 / 1) to give **12** (0.114 g, 80%). $^1$H NMR (DMSO, 300MHz) δ: 11.54 (s, 1H), 8.88 (s, 1H), 8.21 (s, 1H), 7.90 (m, 2H), 7.62-7.45 (m, 3H), 7.53 (d, 1H, *J*= 16.2 Hz), 7.10 (d, 1H, *J*= 16.2 Hz), 6.19 (t, 1H, *J*= 6.6 Hz), 5.28 (d, 1H, *J*= 4.4Hz), 5.19 (t, 1H, *J*= 5.2 Hz), 4.28 (m, 1H), 3.81 (m, 1H), 3.63 (m, 2H), 2.18 (m, 2H); $^{13}$C NMR (125 MHz, DMSO-$d_6$) δ: 162.4, 149.6, 146.6, 139.1, 136.8, 130.1, 128.8, 123.3, 120.0, 116.5, 110.5, 87.7, 84.6, 70.3, 61.3.

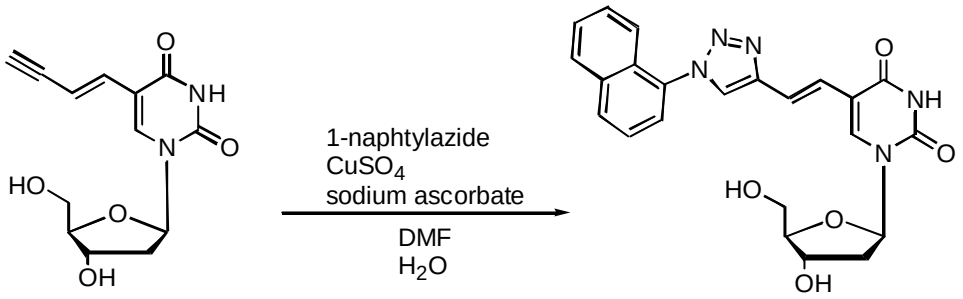
## (*E*)-5-[2-(1-(4-Methoxy-phenyl)-1*H*-[1,2,3]triazol-4-yl)-vinyl] -2'-deoxyuridine (13)




**10** **13**

**10** (0.10 g 0.36 mmol), $CuSO_4$(90 mg, 0.36 mmol), sodium ascorbate (71 mg, 0.36 mmol) and 4-methoxybenzene(54 mg, 0.36 mmol) in a mixture of DMF(0.5 mL) and $H_2O$(0.5 mL) was stirred for 24 hours. The crude was purified by column chromatography ($CHCl_3$ / MeOH= 9 / 1) to give **13** (0.118 g, 77%). $^1$H NMR (DMSO, 300MHz) δ: 11.53 (bs, 1H), 8.77 (s, 1H), 8.19 (s, 1H), 7.79 (d, 1H, *J*= 9.1 Hz), 7.51 (d, 1H, *J*= 16.4 Hz), 7.13 (d, 1H, *J*= 9.1 Hz), 7.07 (d, 1H, *J*= 16.4 Hz), 6.18 (t, 1H, *J*= 6.7 Hz), 5.27 (m, 1H), 5.20 (m, 1H), 4.28 (m, 1H), 3.82 (s, 3H), 3.80 (m, 1H), 3.63 (m, 2H), 2.17 (m, 2H); $^{13}$C NMR (125 MHz, DMSO-$d_6$) δ: 162.5, 159.4, 149.7, 146.5, 138.9, 130.2, 123.1, 121.7, 120.0, 116.6, 115.1, 110.5, 87.7, 84.6, 70.3, 61.3, 55.8.

**(*E*)-5-[2-(1-Naphthalen-1-yl-1*H*-[1,2,3]triazol-4-yl)-vinyl] -2'-deoxyuridine (14)**




**10** **14**

**10** (0.10 g 0.36 mmol), $CuSO_4$(90 mg, 0.36 mmol), sodium ascorbate (71 mg, 0.36 mmol) and 1-naphtylazide(92 mg, 0.36 mmol) in a mixture of DMF(0.5 mL) and $H_2O$(0.5 mL) was stirred for 24 hours. The crude was purified by column chromatography ($CHCl_3$ / MeOH= 9 / 1) to give **14**. $^1$H NMR (DMSO, 300 MHz) δ: 11.54 (s, 1H), 8.70 (s, 1H), 8.23 (s, 1H), 8.20-8.11 (m, 2H), 7.62-7.52 (m, 5H), 7.59(d, 1H, *J*= 17.1 Hz), 7.16 (d, 1H, *J*= 17.1 Hz), 6.20 (t, 1H, *J*= 6.6 Hz), 5.27 (d, 1H, *J*= 4.1Hz), 5.18 (t, 1H, *J*= 5.1 Hz), 4.29 (m, 1H), 3.81 (m, 1H), 2.20 (m, 2H); $^{13}$C NMR (DMSO-$d_6$, 125 MHz) δ: 162.4, 149.6, 145.9, 139.1, 133.9, 133.5, 130.4, 128.6, 128.2, 128.1, 127.4, 125.7, 124.8, 124.0, 123.3, 122.3, 116.6, 110.6, 87.7, 84.7, 70.3, 61.3.

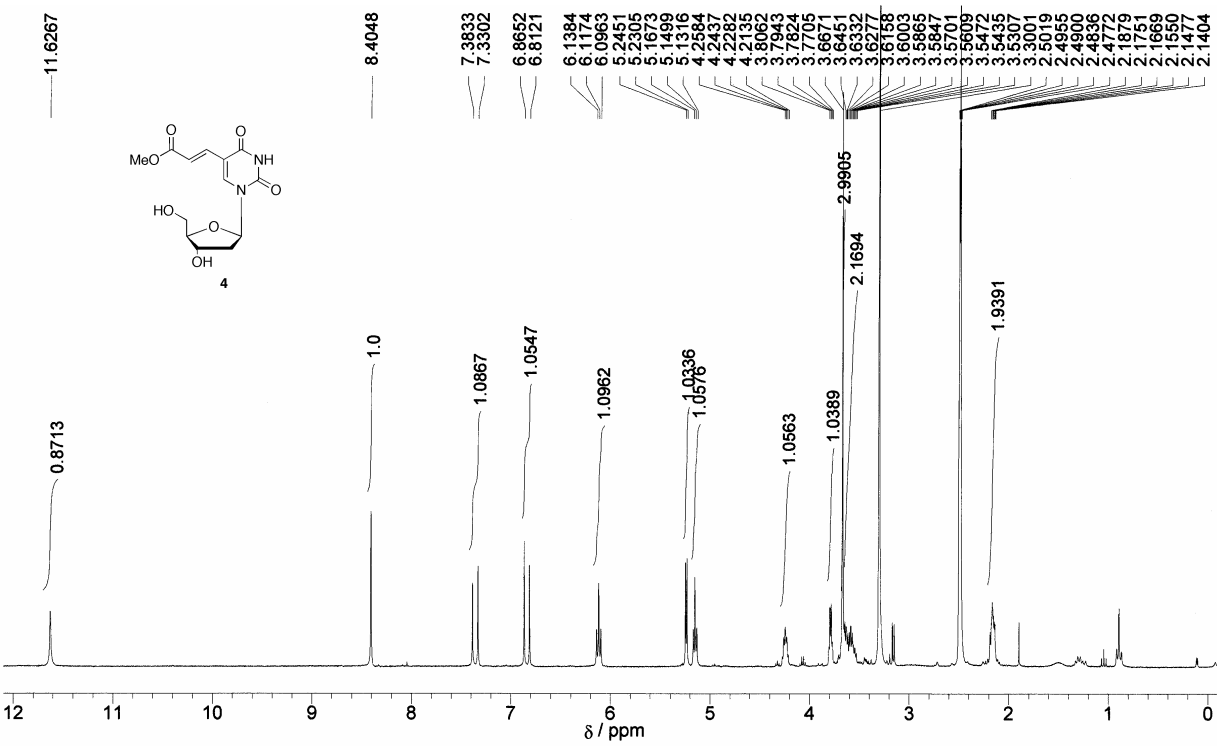



**Figure S27.** $^1$H NMR of compound **4**.

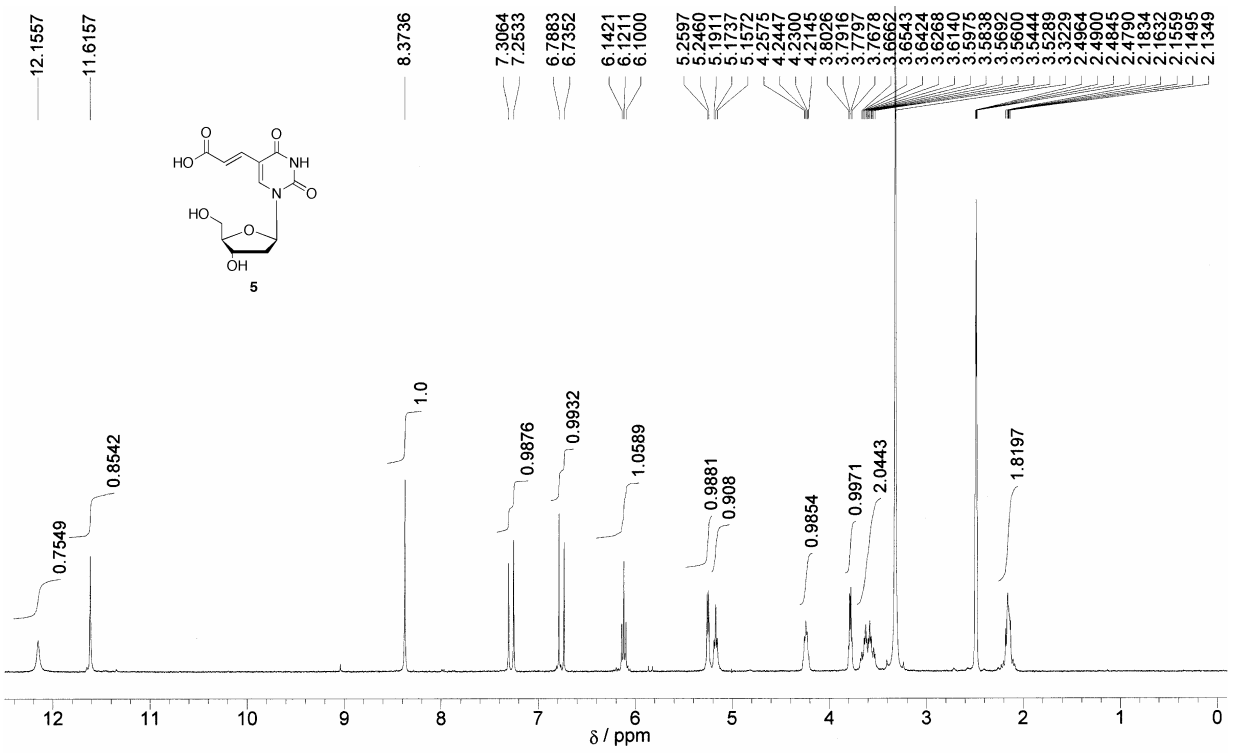



**Figure S28.** $^{1}$H NMR of compound **5**.

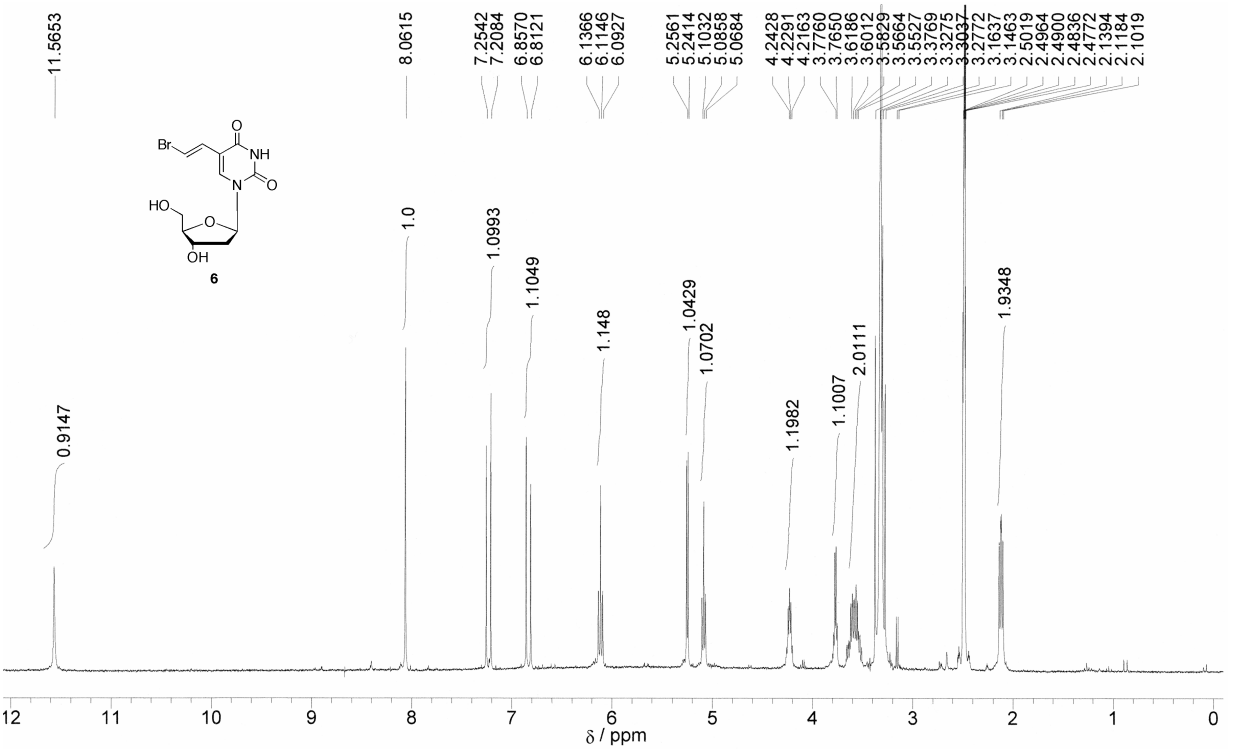



**Figure S29.** $^{1}$H NMR of compound **6**.

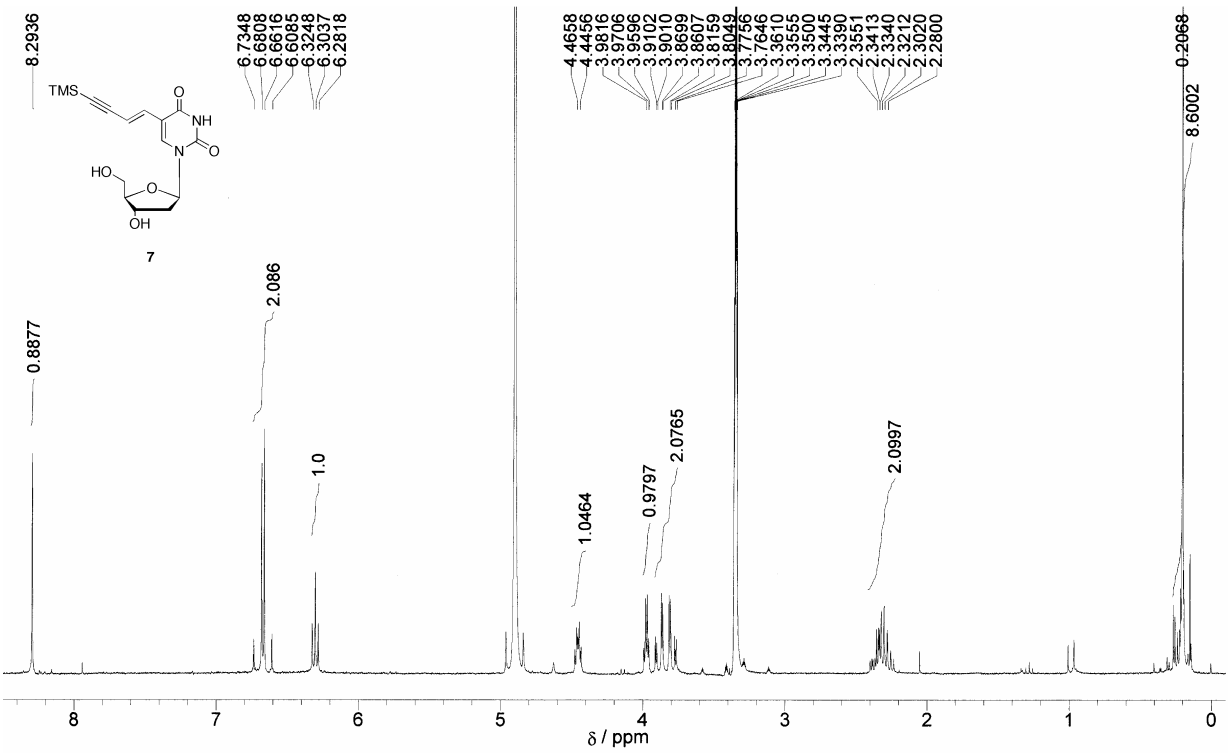



**Figure S30.** $^{1}$H NMR of compound **7**.

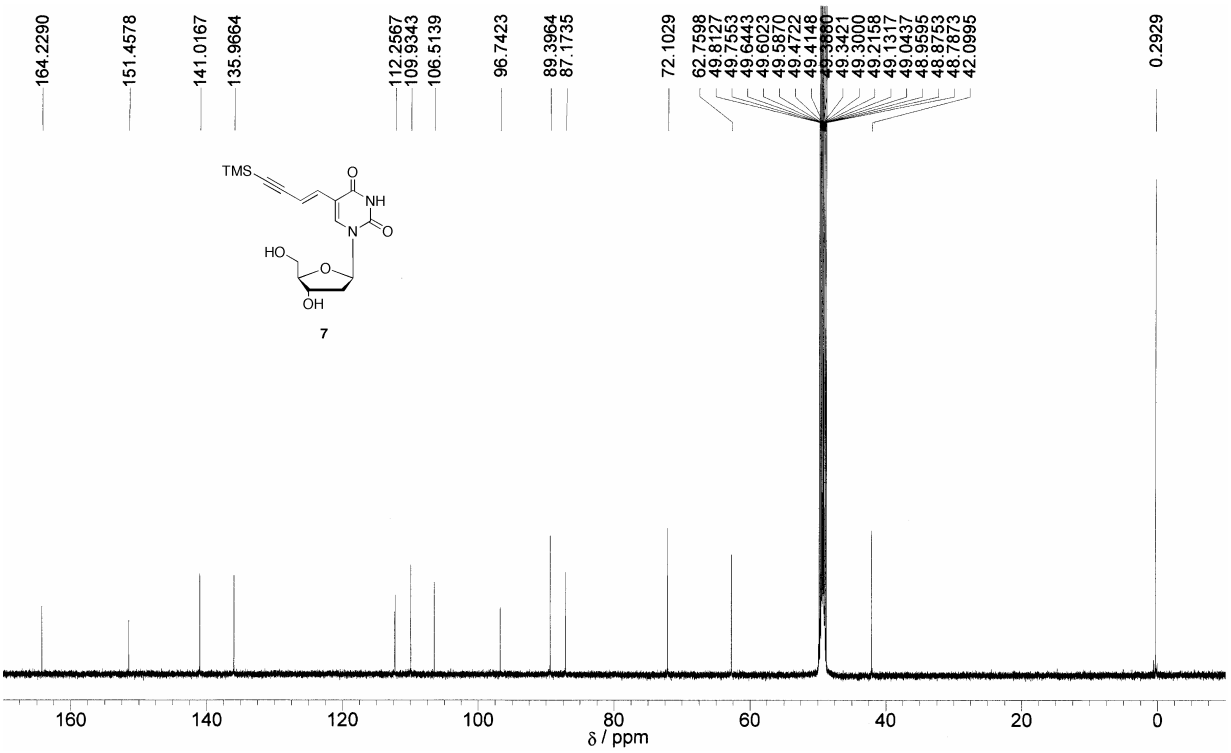



**Figure S31.** $^{13}$C NMR of compound **7**.

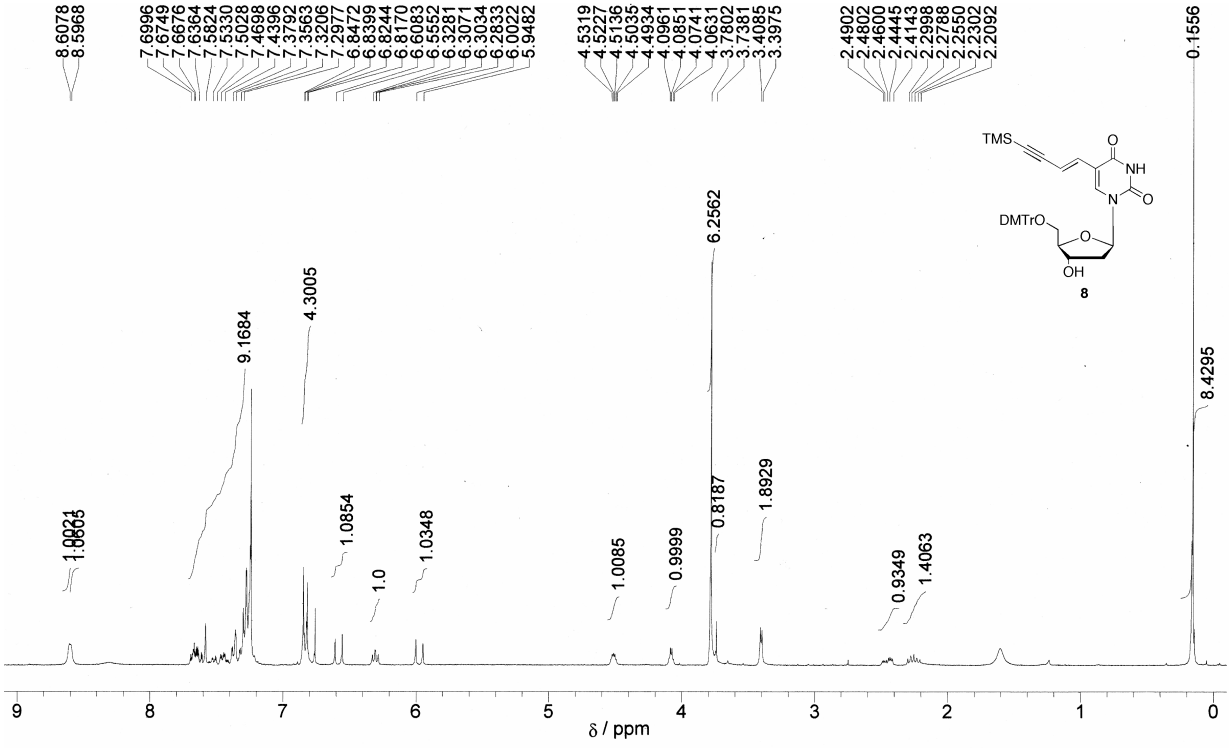



**Figure S32.** $^{1}$H NMR of compound **8**.

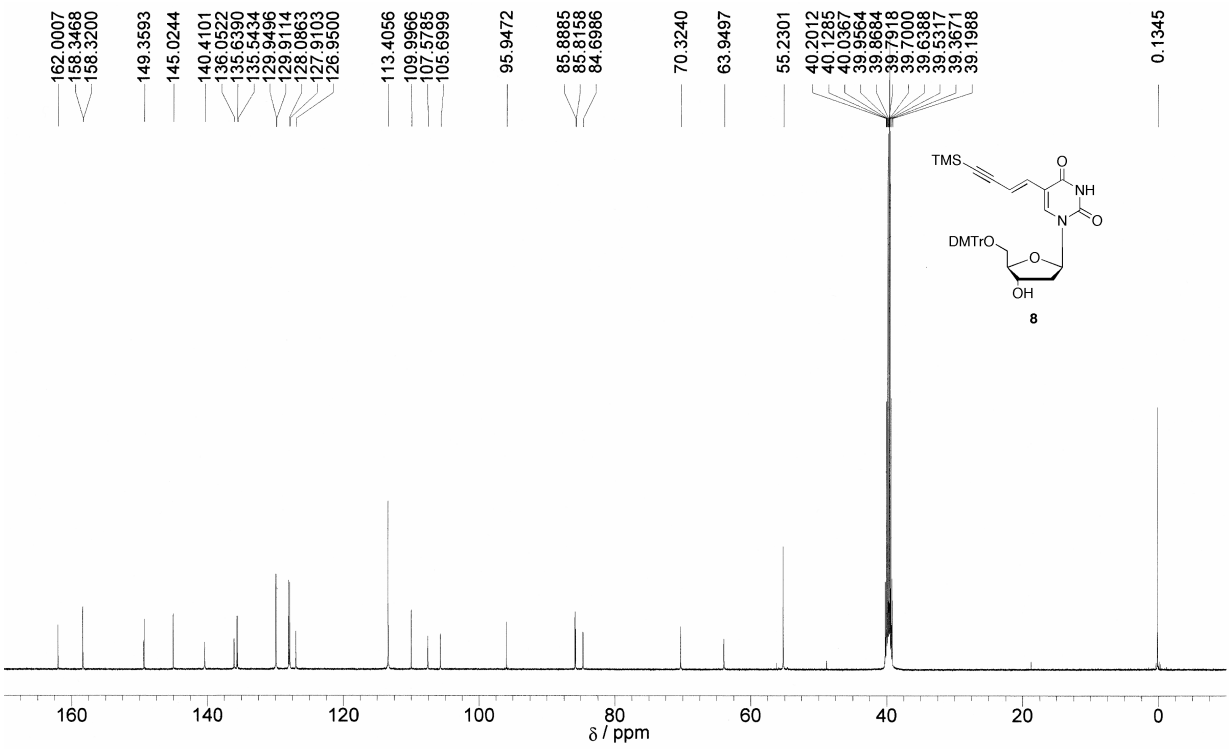



**Figure S33.** $^{13}$C NMR of compound **8**.

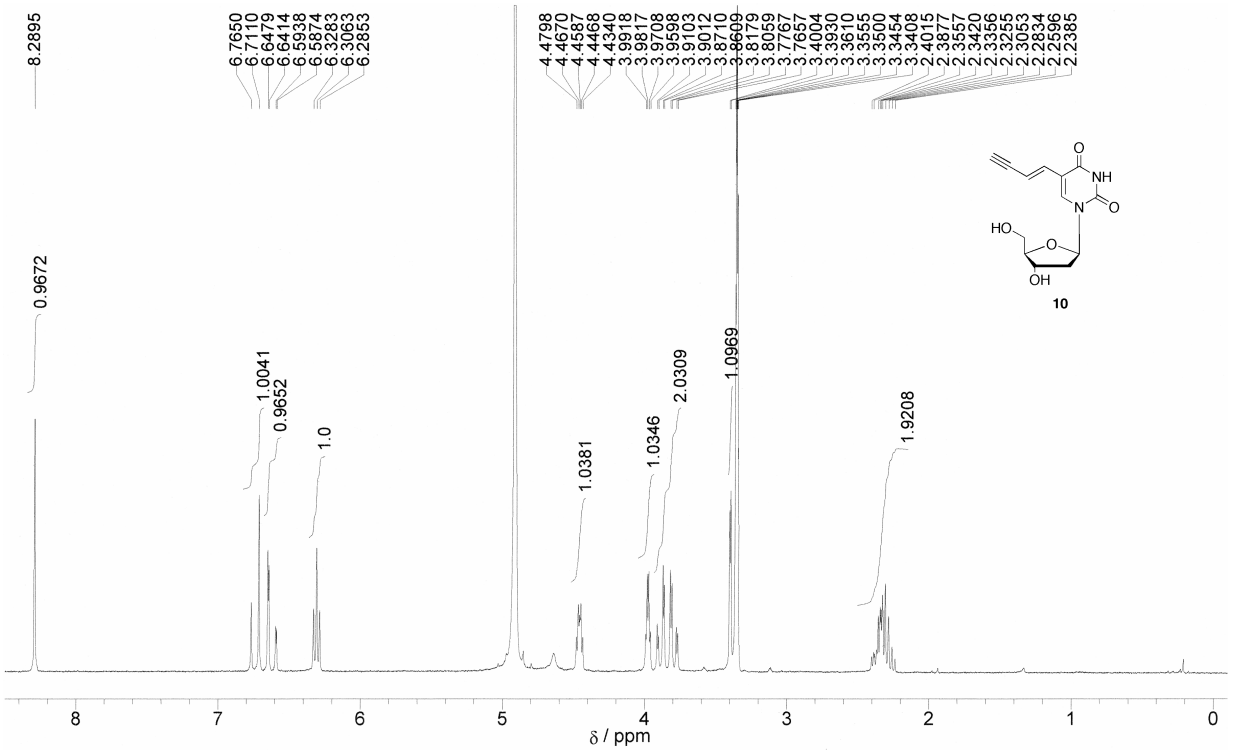



**Figure S34.** $^{1}$H NMR of compound **10**.

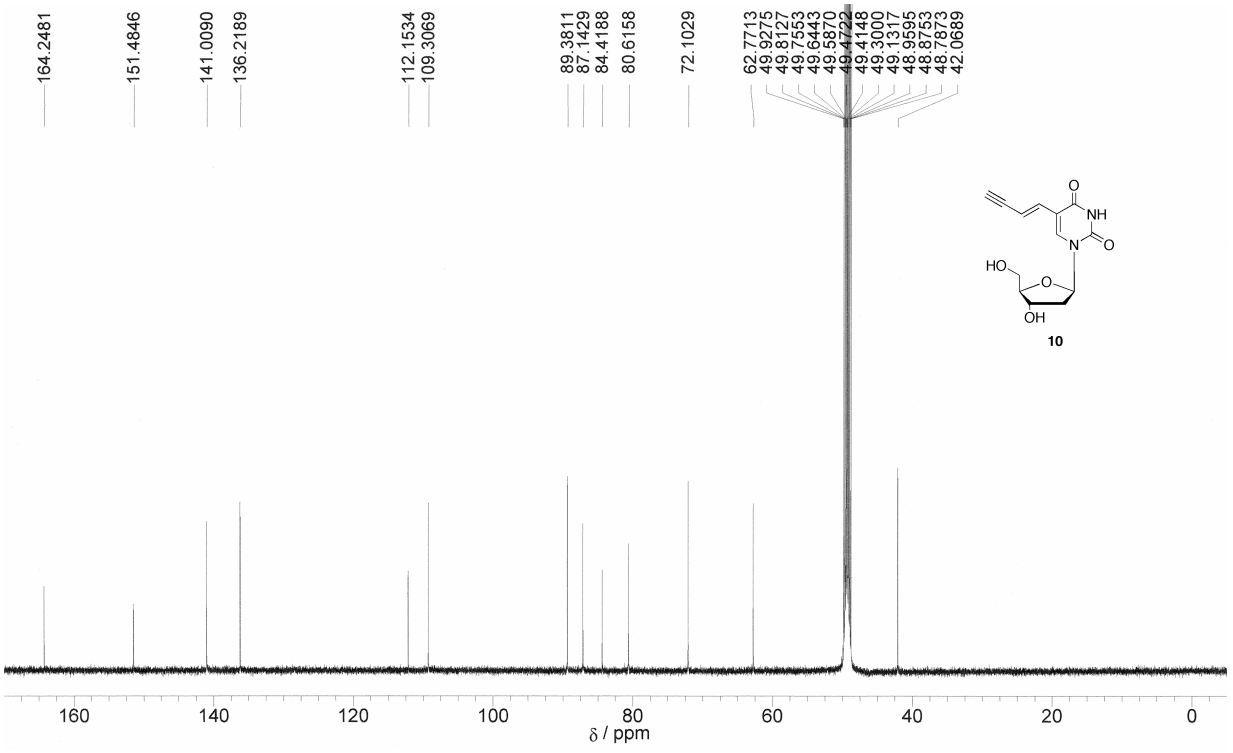



**Figure S35.** $^{13}$C NMR of compound **10**.

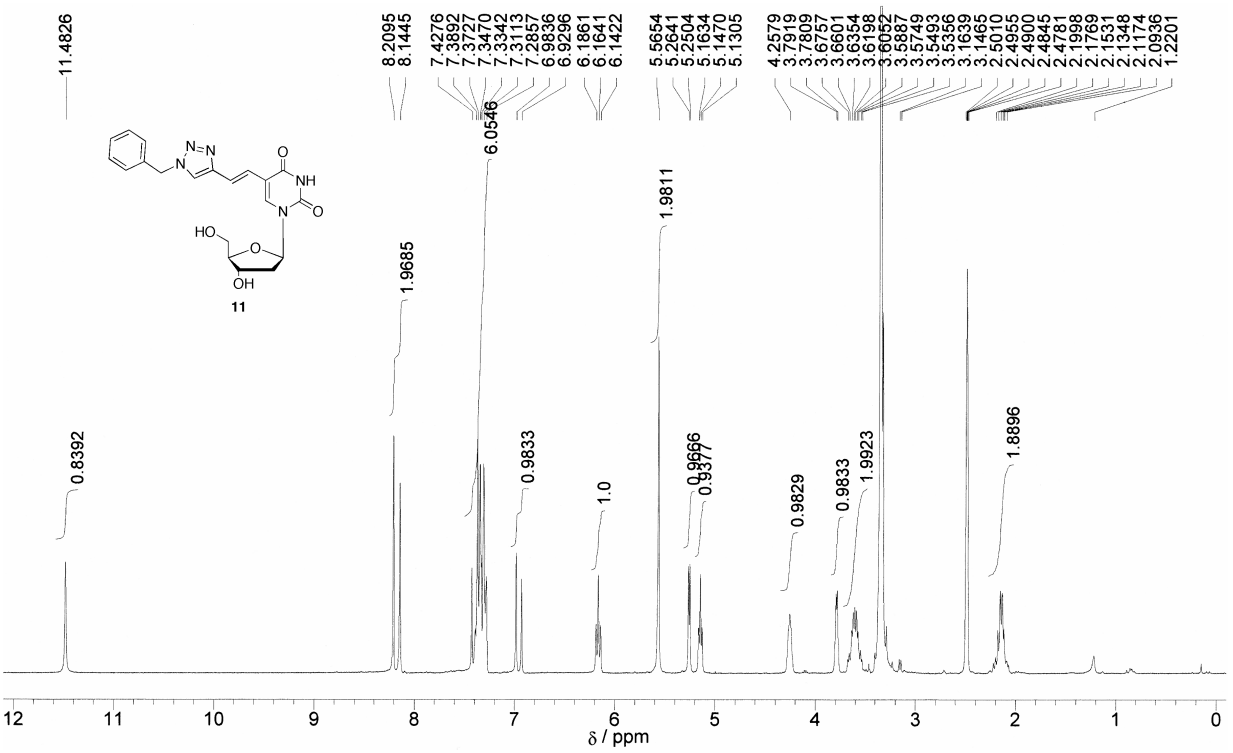



**Figure S36.** $^{1}$H NMR of compound **11**.

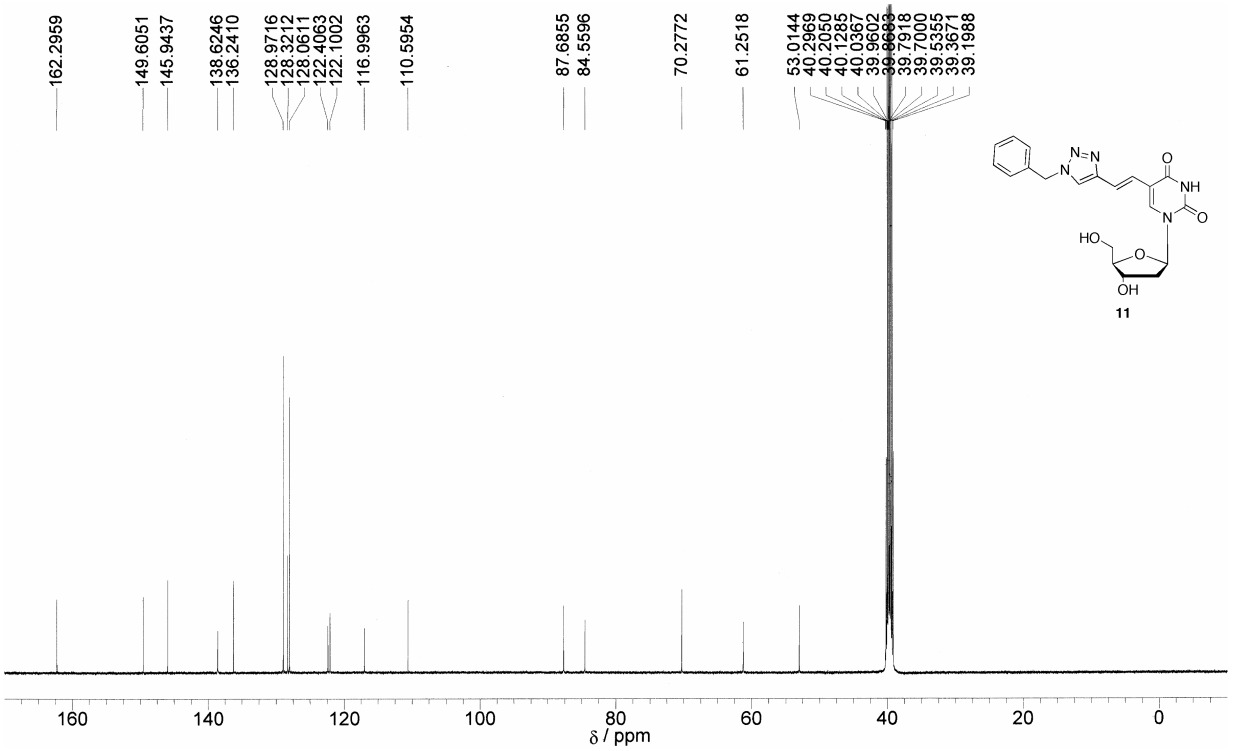



**Figure S37.** $^{13}$C NMR of compound **11**.

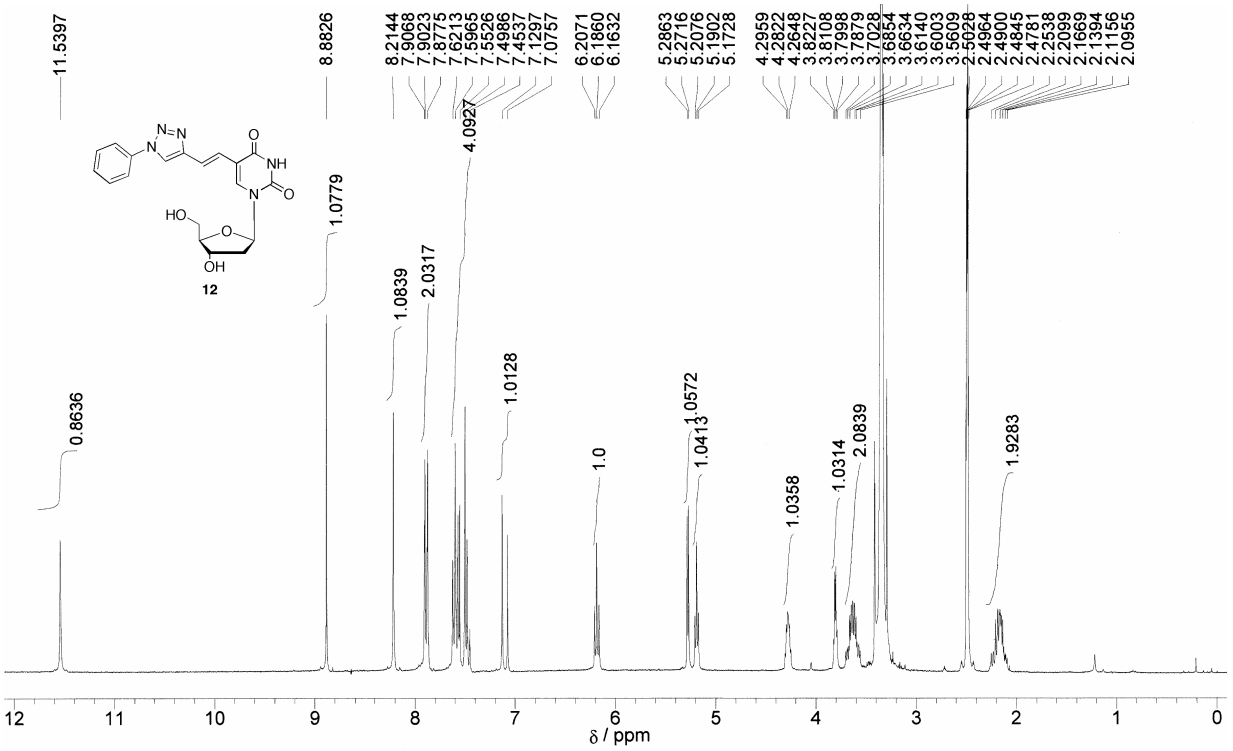



**Figure S38.** $^{1}$H NMR of compound **12**.

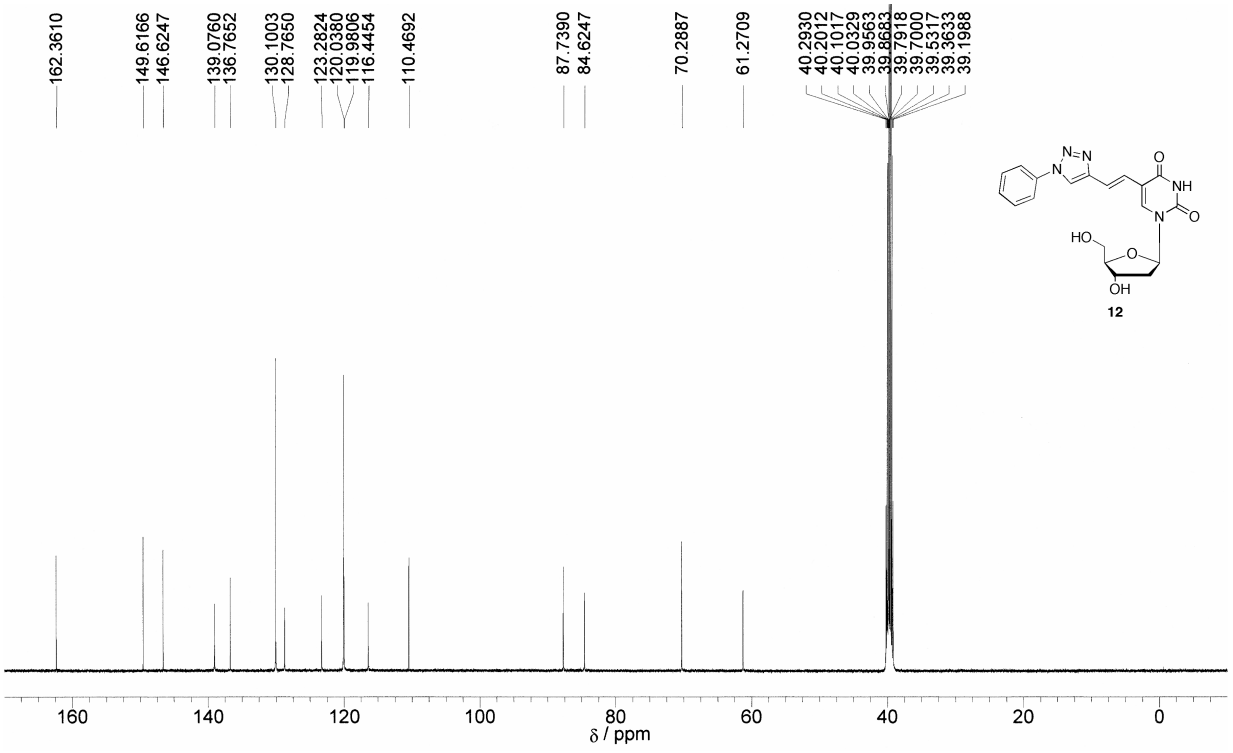



**Figure S39.** $^{13}$C NMR of compound **12**.

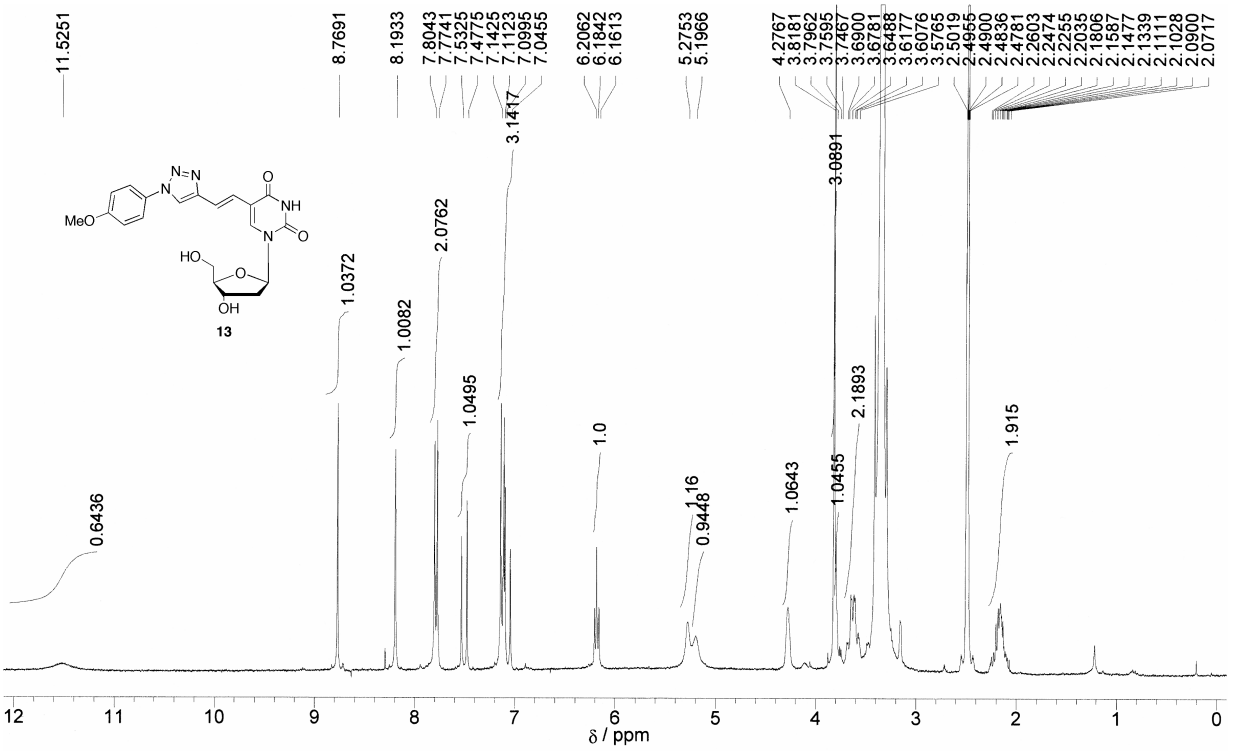



**Figure S40.** $^{1}$H NMR of compound **13**.

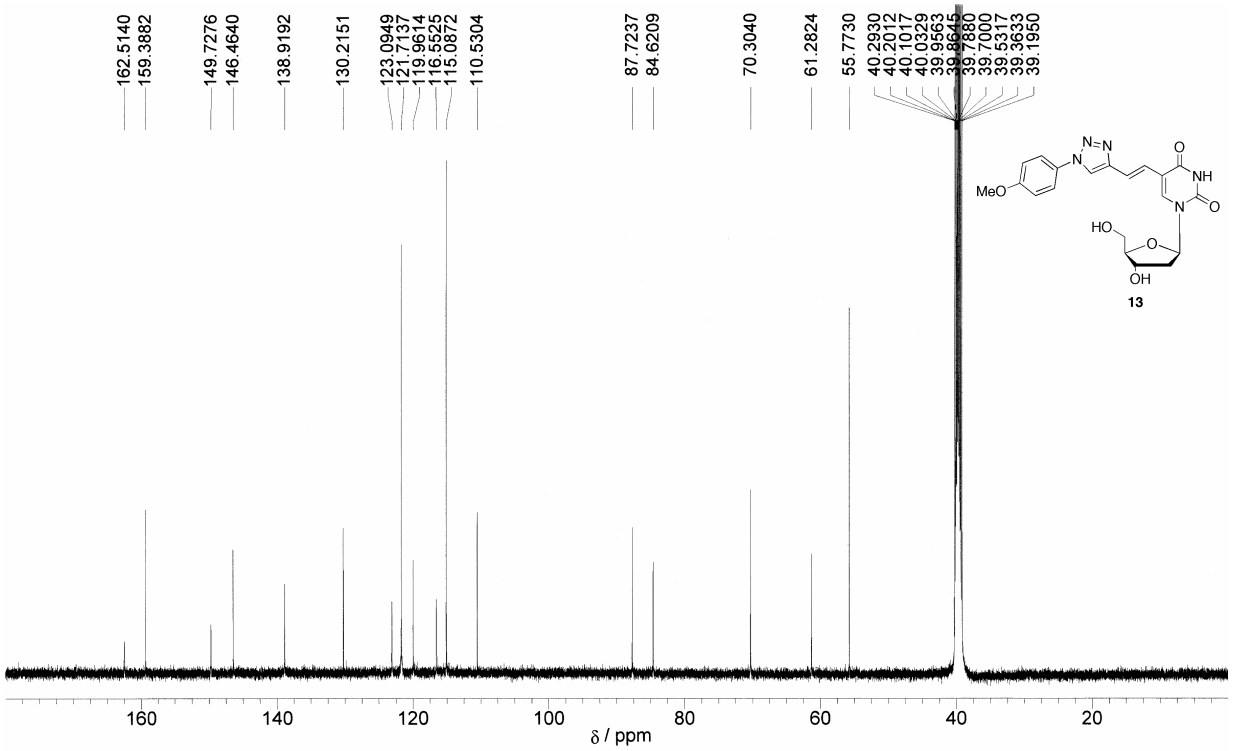



**Figure S41.** $^{13}$C NMR of compound **13**.

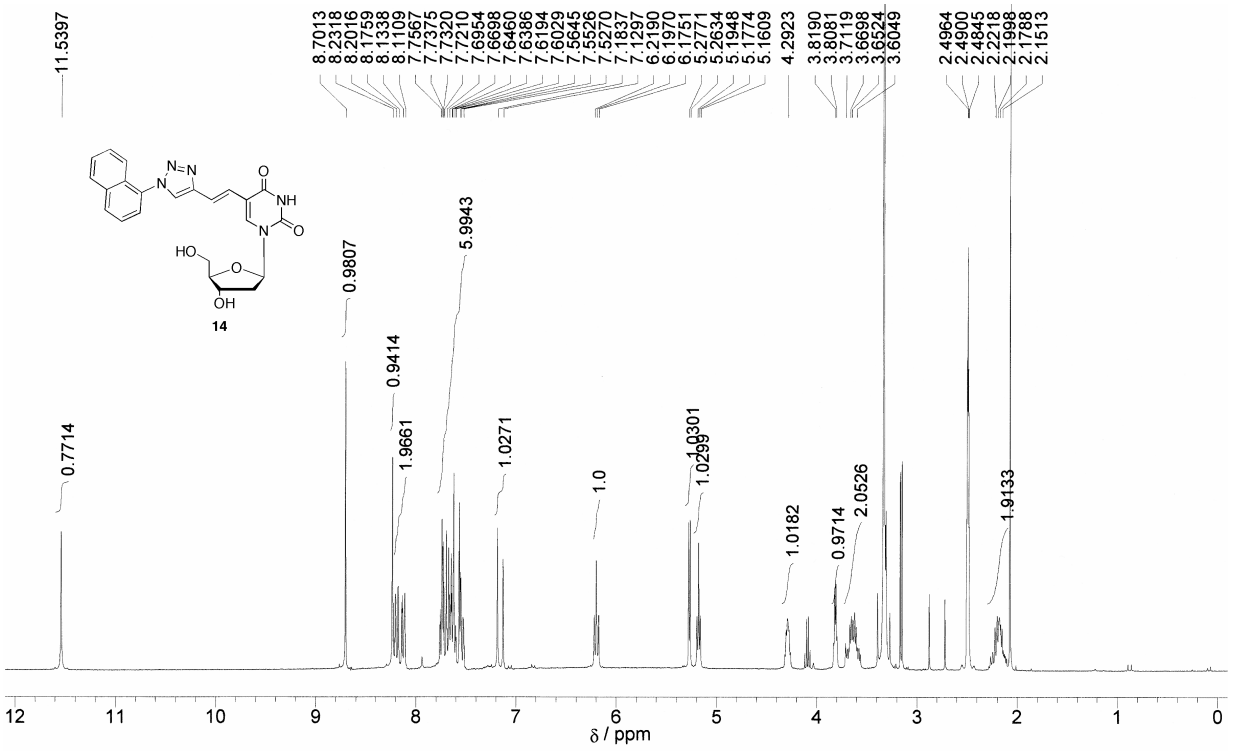



**Figure S42.** $^{1}$H NMR of compound **14**.

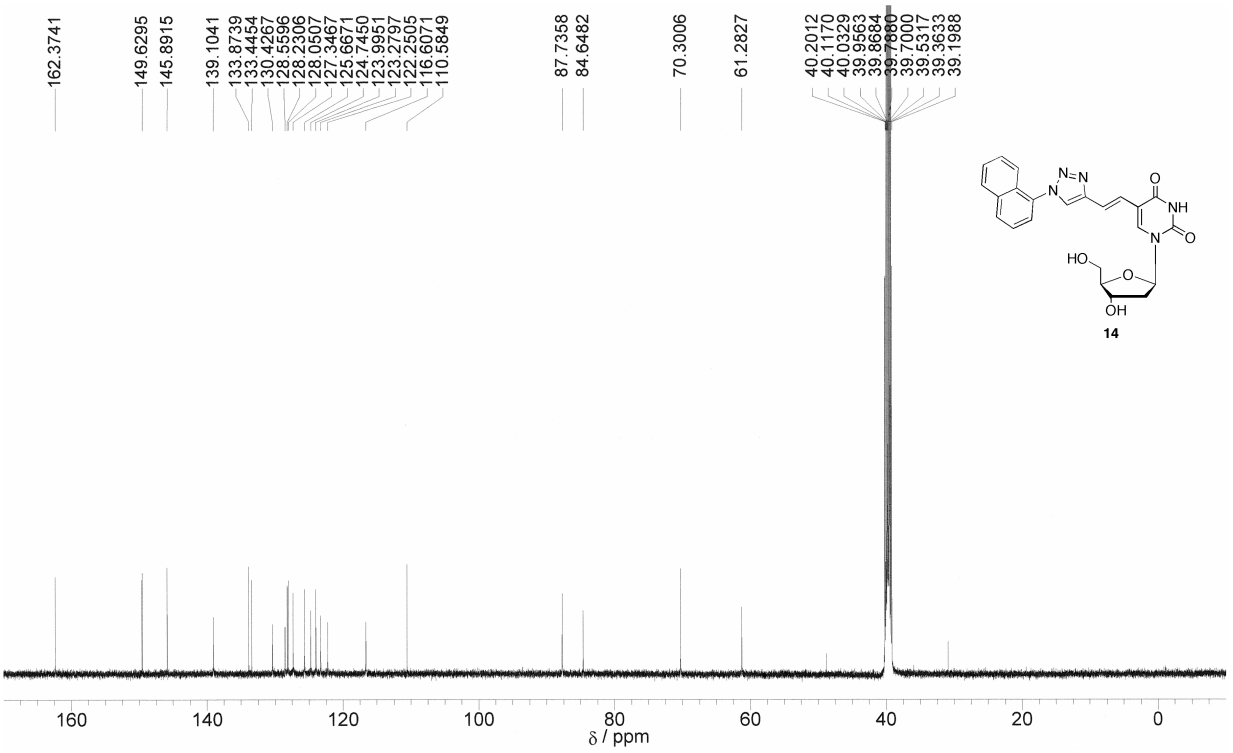



**Figure S43.** $^{13}$C NMR of compound **14**.