

Supporting Information

A Rapidly Photo-activatable Light-up Fluorescent Nucleoside and Its

Application in DNA Base Variation Sensing

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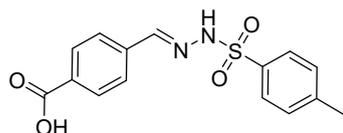
1. Materials and reagents

All chemicals were purchased from Sigma Aldrich, Alfa Aesar, Admas and Sinoreagent. The unmodified oligonucleotide was purchased from Sangon (Shanghai) co., LTD. CPG:dA and unmodified phosphoramidite monomers were purchased from AuGCT Biotech.

2. Synthesis of Compounds

Compound 1

(E)-4-((2-tosylhydrazono)methyl)benzoic acid

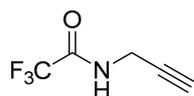


4-carboxybenzaldehyde (40.0 g, 0.267 mol) and toluenesulfonylhydrazide (49.6 g, 0.267 mol) were added into methanol (200 mL). The resulting solution was stirred at room temperature for 12 h, brought to 60 °C and, after addition of water (750 mL), was slowly cooled to room temperature. The white precipitate was collected by filtration. Water was added to the filtrate, and the resulting precipitate was collected by filtration to afford **1** (78.0 g, 92%).

¹H NMR (300 MHz, CD₃OD) δ 7.98 (d, *J* = 8.4 Hz, 2H), 7.85 (s, 1H), 7.82 (d, *J* = 8.0 Hz, 2H), 7.64 (d, *J* = 8.4 Hz, 2H), 7.36 (d, *J* = 8.0 Hz, 2H), 2.39 (s, 3H); ¹³CNMR (75 MHz, CD₃OD): δ 171.6, 149.57, 148.00, 141.99, 139.84, 135.45, 133.53, 133.21, 131.27, 130.40, 24.02; LRMS (ES) calcd for C₁₅H₁₅N₂O₄, 319.0 m/z [M+H]⁺; found 320.0.

Compound 2

2,2,2-trifluoro-N-(prop-2-yn-1-yl)acetamide ^[1]

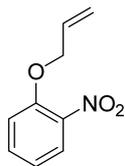


Propargylamine (2.75 g 50 mmol) and ethyl trifluoroacetate (9.24 g, 65 mmol) were dissolved in 70 mL methanol. After stirring for 10 h at room temperature, the solvent was removed under reduced pressure. Water was added to the residue and the aqueous phase was extracted with dichloromethane then washed with saturated sodium bicarbonate solution. The combined organic layers were dried over sodium sulfate, filtered and the solvent was removed under reduced pressure to afford a pale yellow oil **2** (6.65 g, 88%).

¹H NMR (300 MHz, CDCl₃) δ 2.32 (d, *J* = 2.4 Hz, 1 H), 4.13 (m, 2 H), 6.90 (brs, 1 H); ¹³CNMR (75 MHz, CDCl₃) δ 158.1, 157.6, 157.1, 156.6, 121.2, 117.3, 113.5, 109.7, 72.3, 29.3; LRMS (ES) calcd for C₅H₅F₃O, 152.0 m/z [M+H]⁺; found 152.0.

Compound 3

1-(allyloxy)-2-nitrobenzene

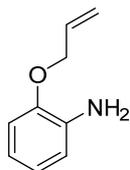


2-nitrophenol (2.78 g, 20 mmol) was dissolved in acetone (50 mL), then 3-bromoprop-1-ene (2.40 g, 20 mmol) and potassium carbonate (2.76 g, 20 mmol) were added. The suspension was refluxed for 4h. After cooled, water (200 mL) was poured into the mixture then was extracted with ethyl acetate. Organic phase washed by brine then the solvent was removed to give a pale yellow oil (3.33 g, 93%).

^1H NMR (300 MHz, CDCl_3) δ 7.82 (dd, $J = 9.9$ Hz, 1.2 Hz, 1H) 7.48 (dd, $J = 8.4$ Hz, 1.2 Hz, 1H), 7.00 (m, 2H) 5.98 (octet, 1H), 5.46(d, $J = 10.8$ Hz, 1H), 5.31(d, $J = 10.8$ Hz 1H), 4.68(d, $J = 4.8$ Hz, 2H); ^{13}C NMR (75 MHz, CDCl_3) δ 69.85, 114.78, 118.19, 120.38, 125.53, 131.63, 133.98, 139.93, 151.79; LRMS (ES) calcd for $\text{C}_9\text{H}_9\text{NO}_3$, 180.1 m/z $[\text{M}+\text{H}]^+$; found 180.0.

Compound 4

2-(allyloxy)aniline

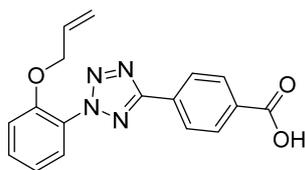


1-(allyloxy)-2-nitrobenzene (1.79 g, 10 mmol) and tin(II) chloride dihydrate (9.00 g, 40 mmol) added into ethanol (25 mL), then concentrated hydrochloric acid (5.5 mL), the mixture was heated to reflux for 6h. Water (40 mL) was added, adjusted pH to 8 with 4M sodium hydroxide solution followed with ethyl acetate (150 mL). The resulting white precipitate was removed by filtration and rinsed with ethyl acetate. Filtrate was separated with separating funnel. The combination of organic phase, the solvent was removed in *vacuo* as brown oil (1.30 g, 87%).

^1H NMR (300 MHz, CDCl_3) δ 6.76 (m, 4H), 6.09 (m, 1H), 5.42 (brd, $J = 17.1$ Hz, 1H), 5.28 (d, $J = 9.6$ Hz, 1H), 4.57 (d, $J = 5.1$ Hz, 2H), 3.68 (s, 2H). ^{13}C NMR (75 MHz, CDCl_3) δ 146.2, 136.3, 133.5, 121.3, 118.3, 117.3, 115.2, 112.0, 69.1; LRMS (ES) calcd for $\text{C}_9\text{H}_{10}\text{NO}$ 148.0 m/z $[\text{M}-\text{H}]^-$; found 148.0.

Compound 5 (TetI):

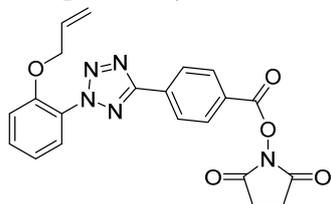
Compound 5 was synthesized according to the literature procedure.^[2]



^1H NMR (300 MHz, $\text{DMSO}-d_6$) δ 13.24 (brs, 1H) 8.28 (m, 2H), 8.16 (m, 2H), 7.74 (m, 2H), 7.30 (m, 1H), 7.25 (m, 1H), 5.94 (m, 1H), 5.23 (m, 2H), 4.70 (d, $J = 4.2$ Hz, 2H);

^{13}C NMR (75 MHz, DMSO- d_6) 165.2, 161.8, 150.7, 131.2, 128.8, 128.2, 125.8, 125.1, 124.2, 119.4, 115.7, 113.0, 67.3; LRMS (ES) calcd for $\text{C}_{17}\text{H}_{15}\text{N}_4\text{O}_3$ 323.1 $[\text{M}+\text{H}]^+$; found 323.0.

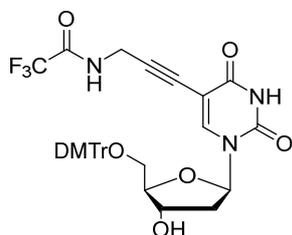
Compound 6 (TetI-succinimide activated ester 6):



4-(2-(2-(allyloxy)phenyl)-2H-tetrazol-5-yl)benzoic acid **5** (322 mg, 1.8 mmol) and N-hydroxy-succinimide (115 mg, 2.6 mmol) were dissolved in DCM (20 mL) at 0°C . EDC (155 mg, 2.6 mmol) was added in one portion. The reaction mixture was allowed to stir at the room temperature for 10 h. DMF was removed in vacuum. The residue was diluted with ethyl acetate (100 mL), washed with water and brine, dried over Na_2SO_4 , concentrated in vacuum, purified by silica gel chromatography (EA/Hexane = 1/2) to give compound **6** as a white solid (366 mg, 95%).

^1H NMR (300 MHz, CDCl_3) δ 8.38 (m, 2H), 8.26 (m, 2H), 7.60(d, $J = 7.5\text{Hz}$, 1H), 7.52(t, $J = 7.8\text{Hz}$, 1H), 7.14 (m, 2H), 5.93 (m, 1H), 5.36 (m, 1H), 5.24 (m, 1H), 4.66 (d, $J = 4.5\text{Hz}$, 2H), 2.94 (s, 4H); ^{13}C NMR (75 MHz, CDCl_3) δ 169.2, 163.4, 161.3, 152.3, 133.2, 132.1, 131.8, 131.1, 127.2, 126.8, 126.4, 120.9, 117.8, 114.2, 69.6, 25.6; HRMS (ES) calcd for $\text{C}_{21}\text{H}_{18}\text{N}_5\text{O}_5$ 420.1302 m/z $[\text{M}+\text{H}]^+$; found 420.1295.

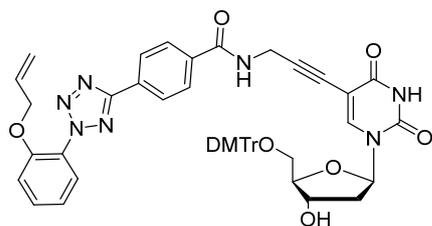
Compound 8



Compound **7**^[3] (838 mg, 1.28 mmol) was dissolved in anhydrous and degassed DMF (20 mL), and then CuI (25 mg, 0.13 mmol) was added compound **2** (550 mg, 3.84 mmol) and triethylamine (426 μL , 3.0 mmol) was introduced via syringe. Tetrakis(triphenylphosphine)palladium(0) (75 mg, 0.06 mmol) was added to the mixture. The reaction mixture was protected by argon and stirred for 12 h at 55°C . DMF was removed in vacuum. The residue was immediately purified by silica gel chromatography (EA/Hexane = 1/1) to give **8** as yellow solid (713 mg, 82%);

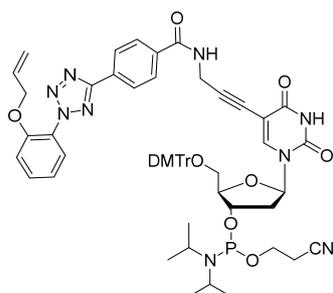
^1H NMR (300 MHz, CDCl_3) δ 2.33 (m, 1H), 2.58 (m, 1H), 3.36 (s, 2H), 3.78 (s, 6H), 3.91 (m, 2H), 4.14 (m, 1H), 4.61 (m, 1H), 6.36 (t, $J = 6.3\text{Hz}$, 1H), 6.83 (m, 4H), 6.85-7.54 (m, 9H), 8.24 (s, 1H), 9.80 (s, 1H); ^{13}C NMR (75 MHz, CDCl_3) δ = 30.3, 41.5, 55.2, 63.5, 72.2, 75.0, 86.0, 86.9, 87.0, 87.7, 98.9, 113.3, 126.9, 127.8, 128.0, 129.9, 135.3, 143.5, 144.3, 149.2, 158.5, 162.4; LRMS(ES) calcd for $\text{C}_{35}\text{H}_{33}\text{F}_3\text{N}_3\text{O}_8$: 680.2 m/z $[\text{M}+\text{H}]^+$; found 680.1.

Compound 10 (TetI-DMT-dU)



Compound **8** (490 mg, 0.72 mmol) dissolved in MeOH (10 mL) and ammonia MeOH solution (2 M, 30 mL) was added, the mixture stirred for 2 h, TLC monitored the finish of the reaction. The solvents were removed in *vacuo* to get quantitative compound **9** without further purification. Compound **9** and activated ester **6** (300 mg, 0.72 mmol) were dissolved in DCM (5 mL) was added DIPEA (93mg, 0.72 mmol), DMAP (9 mg, 0.07 mmol) at room temperature. After being stirred for 4 h, dichloromethane was removed under vacuum. The residue was purified by flash chromatography on silica gel (MeOH/DCM = 1/10) to give compound **10** (594 mg, 93%) as a pale yellow solid. $^1\text{H NMR}$ (300 MHz, CDCl_3) δ 9.01 (s, 1H), 8.26 (s, 1H), 8.21 (d, $J = 7.8$ Hz, 2H), 7.68-7.16 (m, 15H) 6.83 (d, $J = 8.7$ Hz, 4H) 6.41 (s, 1H), 6.33 (t, $J = 6.9$ Hz, 1H), 5.95 (m, 1H), 5.34 (m, 1H), 5.22 (m, 2H), 4.64 (d, $J = 3.9$ Hz, 2H), 4.54 (s, 1H), 4.16 (m, 2H), 4.13 (m, 1H), 3.72 (s, 6H), 3.34 (dd, $J = 33.6$ Hz, 9.3 Hz, 2H), 2.52 (m, 2H), 2.32 (m, 1H); $^{13}\text{C NMR}$ (75 MHz, CDCl_3) δ 166.2, 163.9, 162.5, 152.3, 149.3, 144.5, 143.2, 135.4, 135.3, 135.1, 131.9, 130.0, 129.9, 128.0, 127.8, 126.9, 126.5, 120.9, 117.8, 114.2, 112.3, 99.3, 89.4, 87.0, 86.7, 85.9, 74.3, 74.2, 69.6, 63.5, 55.2, 41.5, 30.7; HRMS (ES) calcd for $\text{C}_{50}\text{H}_{46}\text{N}_7\text{O}_9$ 888.3357 m/z $[\text{M}+\text{H}]^+$; found 888.3345.

Compound 11 (TetI-DMT-phos-dU)



To a stirred solution of compound **10** (310 mg, 0.35 mmol) in anhydrous dichloromethane (10 mL) was added tetrazole (74 mg, 1.05 mmol) under argon at 0°C , and then 2-cyanoethyl N,N,N',N' -tetra-isopropylphosphorodiamidite (316 mg, 1.05 mmol). After being stirred for 2 h, the reaction mixture was diluted with dichloromethane (100 mL) and then washed with saturated sodium bicarbonate solution. The organic layer was dried over Na_2SO_4 , and then concentrated under reduced pressure. The residue was purified by flash chromatography on silica gel, (EA/DCM = 1/4 with 3% Et_3N) to yield phosphoramidite **11** (172 mg, 45%) as a pale yellow foam. **11** was dissolved in dry acetonitrile and co-evaporated three times and was used for DNA synthesizer without further purification.^[4]

^{31}P NMR (400 MHz, CDCl_3) δ 148.84, 148.54

3. Fluorescent lifetime data^[5]

Time-resolved emission spectra and luminescence decay curves were collected on an Edinburgh FLS920 spectrophotometer equipped with a picosecond pulsed diode laser (EPL-375, wavelength 377 nm, pulse width 79.6 ps, Edinburgh Ltd.). The lifetimes (τ) was estimated by fitting the luminescence decay data to a single-exponential curve as follows:

$$y = A_0 + A_1 e^{-\frac{x}{\tau}} \quad (1)$$

y is the photon counts of the emission. x is the delay time. A_0 , A_1 are parameter values. Eq. (1) was preferred to perform fitting unless the χ^2 was great.

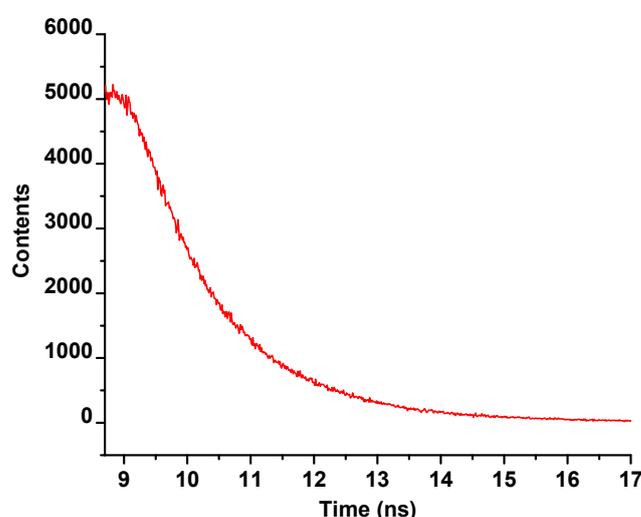


Figure S1 Luminescence decay of $\text{d}^{\text{Pr}}\text{U}$ in methanol, $[\text{d}^{\text{Pr}}\text{U}] = 10 \mu\text{M}$, $\lambda_{\text{ex}} = 377 \text{ nm}$, $\lambda_{\text{em}} = 530 \text{ nm}$. Single-exponential fitting: $\tau = 1.48 \text{ ns}$, $\chi^2 = 1.265$.

4. Oligodeoxynucleotide synthesis

Oligodeoxynucleotides were synthesised by using an Applied Biosystems Incorporated 394 automated synthesiser. CPG:dA and unmodified phosphoramidite monomers were used without further purification. **ODN(1)** were synthesised on a 200 nmol scale with standard DNA synthesis cycles on CPG:dA (trityl off mode). The efficiency of oligomers synthesis was monitored by trityl absorbance. Cleavage from the solid support and deprotection were achieved by incubation in a 28% ammonium hydroxide solution and 40% monomethylamine solution (1/1) at 60°C for 90 min. The solvents were removed in a *Speed Vac* concentrator and the pellet redissolved in water.

Semi-preparation HPLC was carried out with Phenosphere Clarity Oligo-RP C18 column (10 μm , 21.2 x 250 mm) on Agilent. UV/vis detection at 280 nm and 260 nm. Conditions: solvent A, 0.1M TEAB buffer; solvent B, acetonitrile. Started at 5% B; linear gradient to 50% B over 60 min, flow rate: 1.2 mL/min. MS was measured on MALDI-TOF.

The oligodeoxynucleotides were lyophilized and quantified by their absorbance at 260 nm on a Nanodrop ND-1000 spectrophotometer before using for next experiments.

5. Thermal denaturation assays

Circular dichroism (CD) experiments were carried out with Chariscan circular dichroism photomultiplier (Applied Photophysics Limited, UK) equipped with a Quantum Northwest TC125 temperature controller. All the CD spectra were measured from 220 nm to 320 nm in a 0.5 cm path-length cuvette with a scanning speed of 200 nm/min, 3 nm bandwidth and 2 s response time. The temperature was controlled at 4 °C. Thermal denaturation was performed by gradually increasing the temperatures from 15°C to 90°C, and the ultraviolet absorbance at 285 nm was monitored to calculate the T_m values for each sample.

Table S1 T_m values of duplexes.

Entry	Strand	$T_m/^\circ\text{C}$	$T_m/^\circ\text{C} (+h\nu)$
1	ODN(1):ODN(AP)	45.1	43.1
2	ODN(2):ODN(AP)	45.7	
3	ODN(1):ODN(C)	46.7	43.8
4	ODN(2):ODN(C)	50.8	
5	ODN(1):ODN(T)	45.9	45.6
6	ODN(2):ODN(T)	52.0	
7	ODN(1):ODN(G)	45.9	45.4
8	ODN(2):ODN(G)	51.9	
9	ODN(1):ODN(A)	56.1	53.8
10	ODN(2):ODN(A)	60.0	

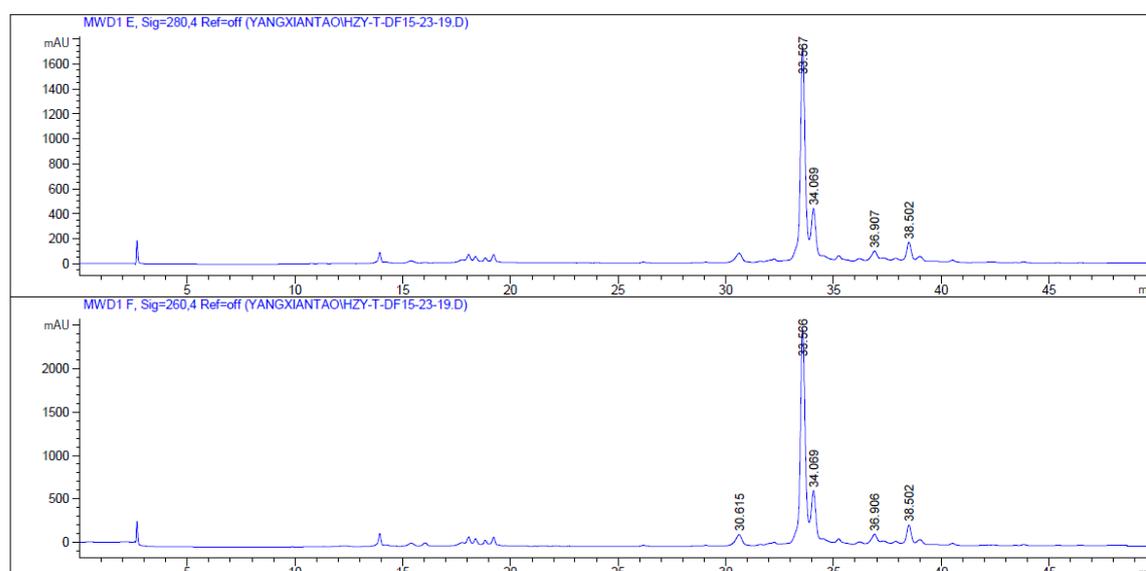


Figure S2 Semi-preparative reversed phase liquid chromatography spectra of DNA

synthesis monitoring at 280 nm (up) and 260 nm (down) absorption.

Table S1 Oligodeoxynucleotides (ODNs) Used in This Study

ODN(X): 5'-TCAGTGAA^XAAGACTGC-3'

Name	sequences
ODN(1)	5'-GCAGTCTT ^{Tet} UTTCACTGA-3'
ODN(2)	5'-GCAGTCTTTTTCACTGA-3'
ODN(AP)	5'-TCAGTGAA(AP)AAGACTGC-3'
ODN(A)	5'-TCAGTGAA ^A AAGACTGC-3'
ODN(T)	5'-TCAGTGAA ^T AAGACTGC-3'
ODN(C)	5'-TCAGTGAA ^C AAGACTGC-3'
ODN(G)	5'-TCAGTGAA ^G AAGACTGC-3'

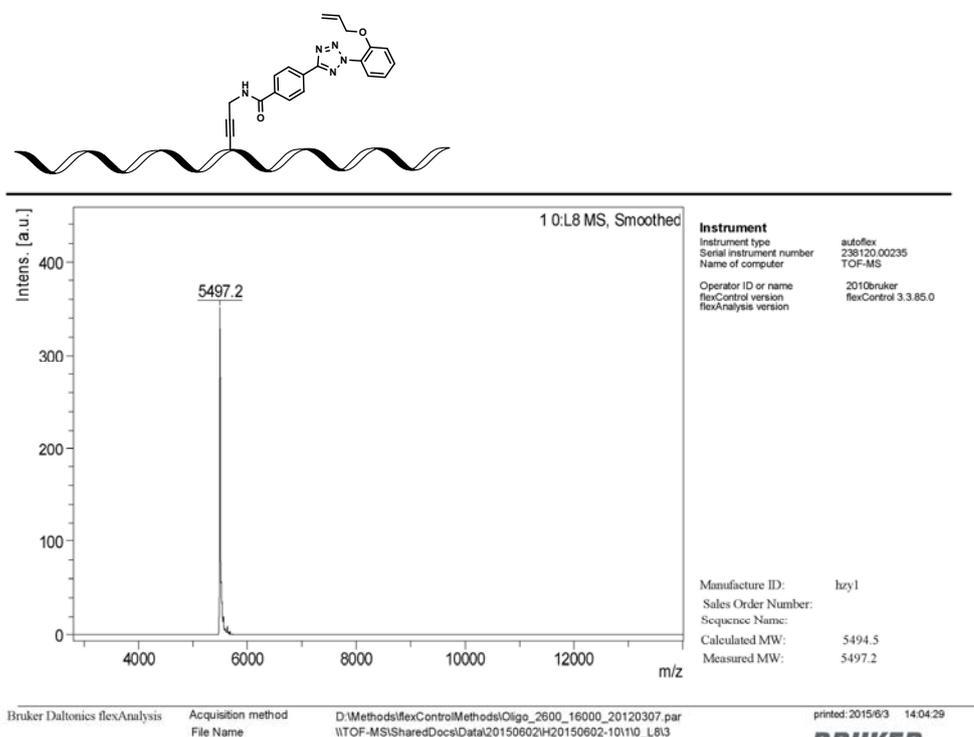


Figure S3 MALDI-mass spectrum of ODN(1), calculated mass: 5494.5 Da; found mass 5497.2 Da.

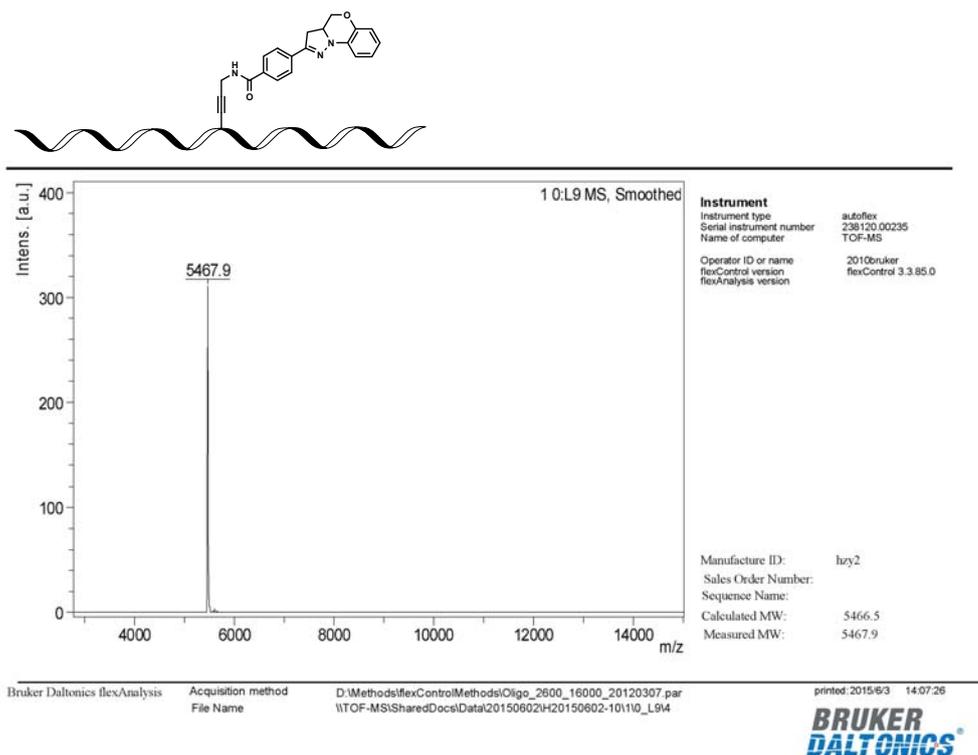


Figure S4 MALDI-mass spectrum of **ODN(1)** irradiation product, calculated mass: 5466.5 Da; found mass 5467.9 Da.

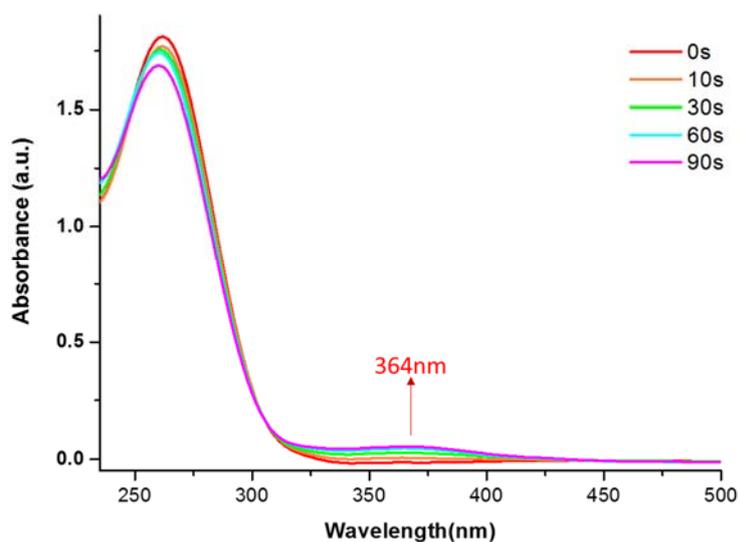


Figure S5 UV-vis absorption spectrum of the “photoclick” reaction. The reaction was performed with **ODN(1)** (10 μ M) at 25 $^{\circ}$ C for a total time of 60 s, in 10 mM PBS buffer, pH 7.4.

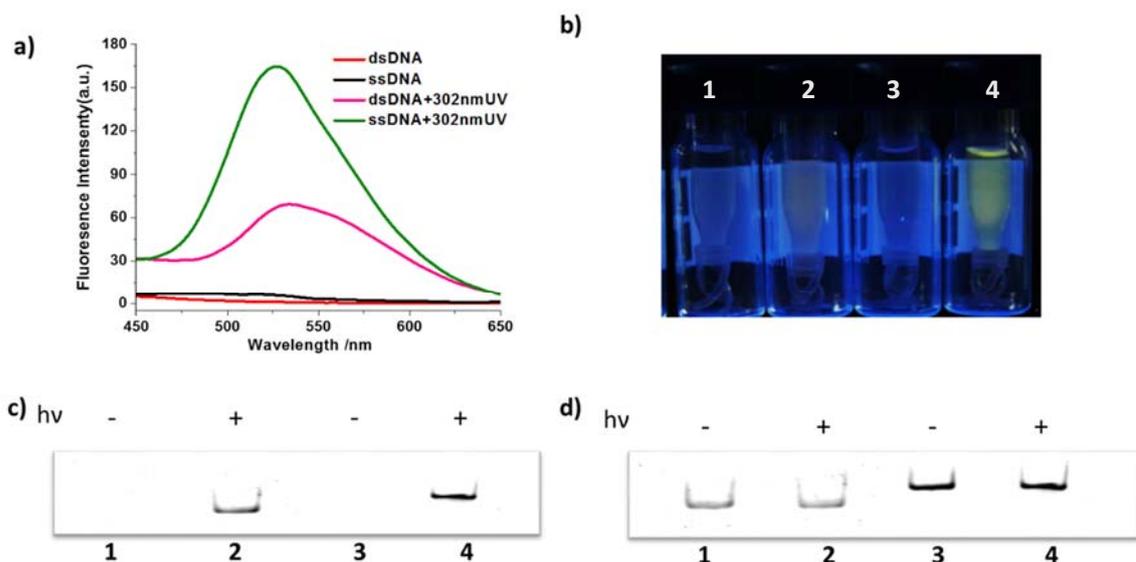


Figure S6 a) Fluorescence spectrum of single and double strands; b) Photo of single and double strand DNA solutions DNA under 365nm lamp illumination, from left to right: dsDNA, dsDNA with UV irradiation, ssDNA, ssDNA with UV irradiation; c) 20% native PAGE analysis of single and double strand, lane 1: ssDNA, lane 2: ssDNA with UV irradiation, lane 3: dsDNA, lane 4: dsDNA with UV irradiation; d) 20% native PAGE analysis of single and double strand stained with EB (10 $\mu\text{g}/\text{mL}$). ssDNA = **OND(1)** (10 μM), dsDNA= **OND(1):OND(A)** (10 μM). All irradiation experiments was taken at 25 $^{\circ}\text{C}$ for a total irradiation time of 60 s, in 10 mM PBS buffer, pH 7.4.



Figure S7 Photo of single and double strands DNA solutions under 365 nm lamp illumination. From left to right: **OND(1)**, **OND(1):OND(A)**, **OND(1):OND(T)**, **OND(1):OND(C)**, **OND(1):OND(G)**, **OND(1):OND(AP)** after irradiation. Concentration of each ODN was 10 μM . All irradiation experiments was taken at 25 $^{\circ}\text{C}$ for a total irradiation time of 60 s, in 10 mM PBS buffer, pH 7.4.

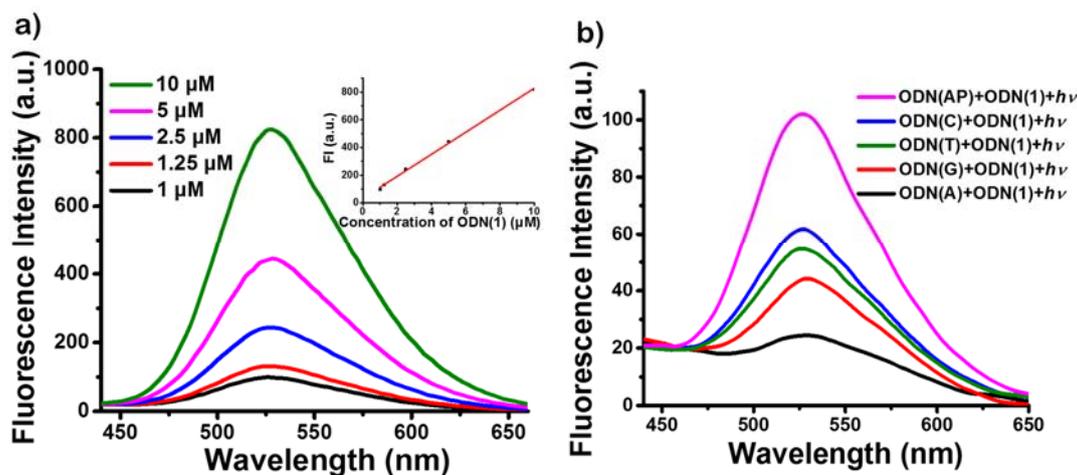


Figure S8 Fluorescent spectra of oligodeoxynucleotides containing $d^{Pr}U$ at gradient concentration, linear relationship between intensity of fluorescence emission and concentration, $R^2=0.9992$ **a)** and the corresponding duplexes fluorescent response at the concentration of $1 \mu M$ **b)**. All irradiation experiments for $d^{Tet}U$ containing single strand were taken at $25^\circ C$ for a total irradiation time of 1 min, in 10mM PBS buffer, pH 7.4, then incubated with the complementary strand. For fluorescence, $\lambda_{ex}=330$ nm.

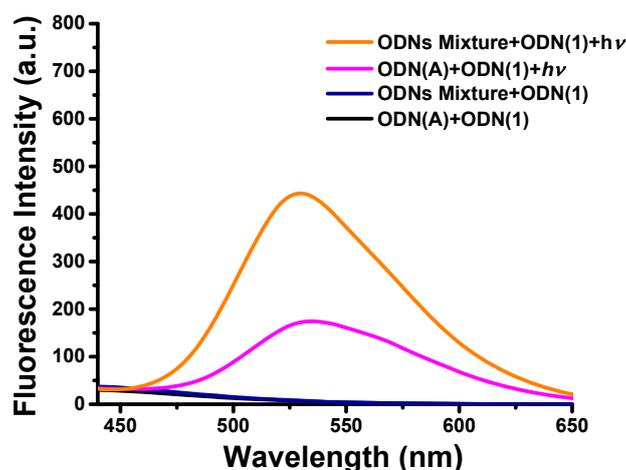


Figure S9 Fluorescence spectra of one base variation duplex vs full matched duplex. ODNs mixture (five in one: **ODN(AP)+OND(C)+OND(T)+ODN(G)+ODN(A)**, each of $2.5 \mu M$), concentration of other **ODN(1)** was $10 \mu M$. $\lambda_{ex}=330$ nm. All irradiation experiments were taken at $25^\circ C$ for a total irradiation time of 1 min, in 10mM PBS buffer, pH 7.4.

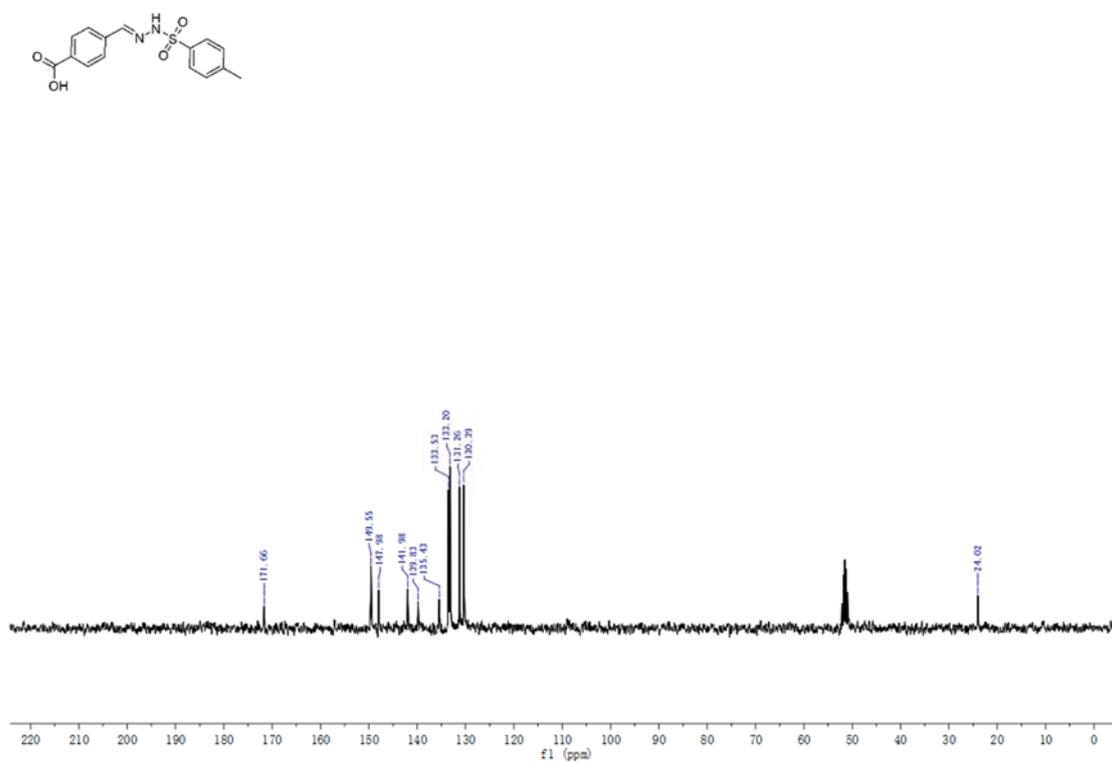
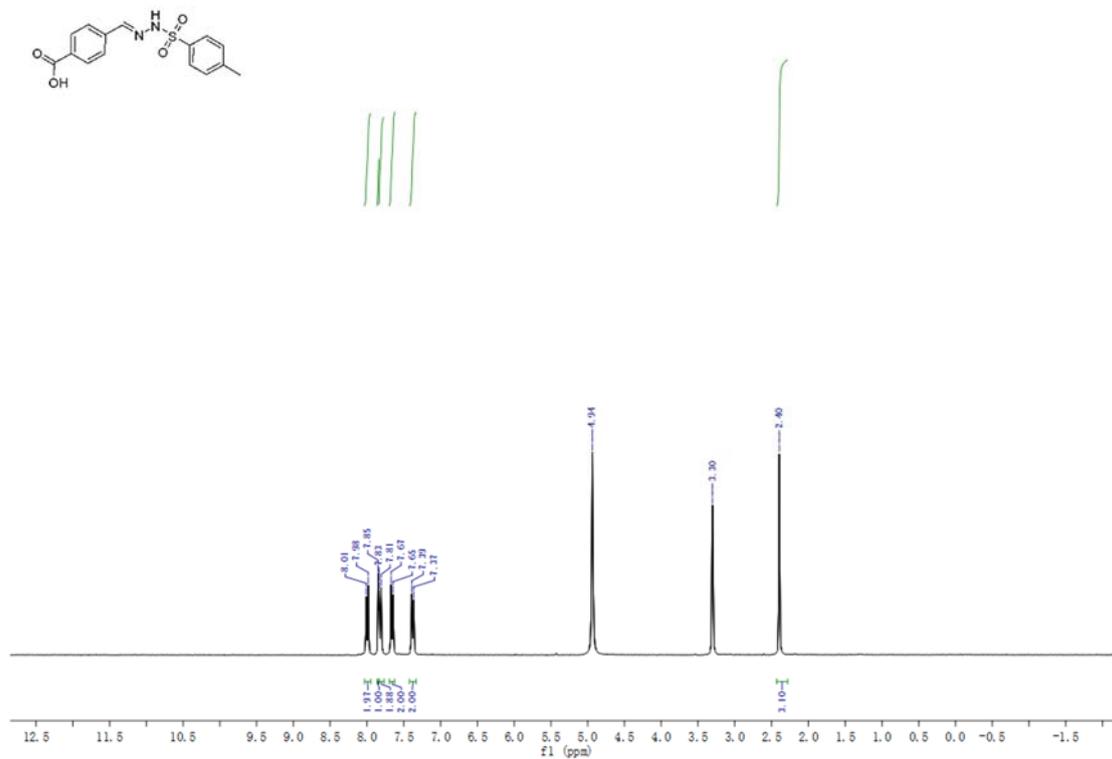
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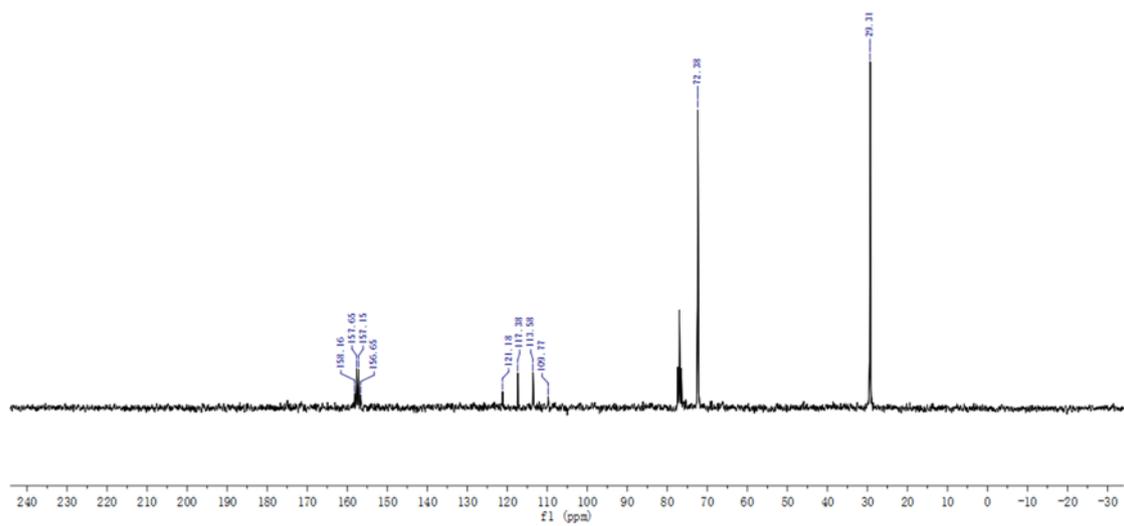
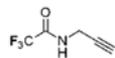
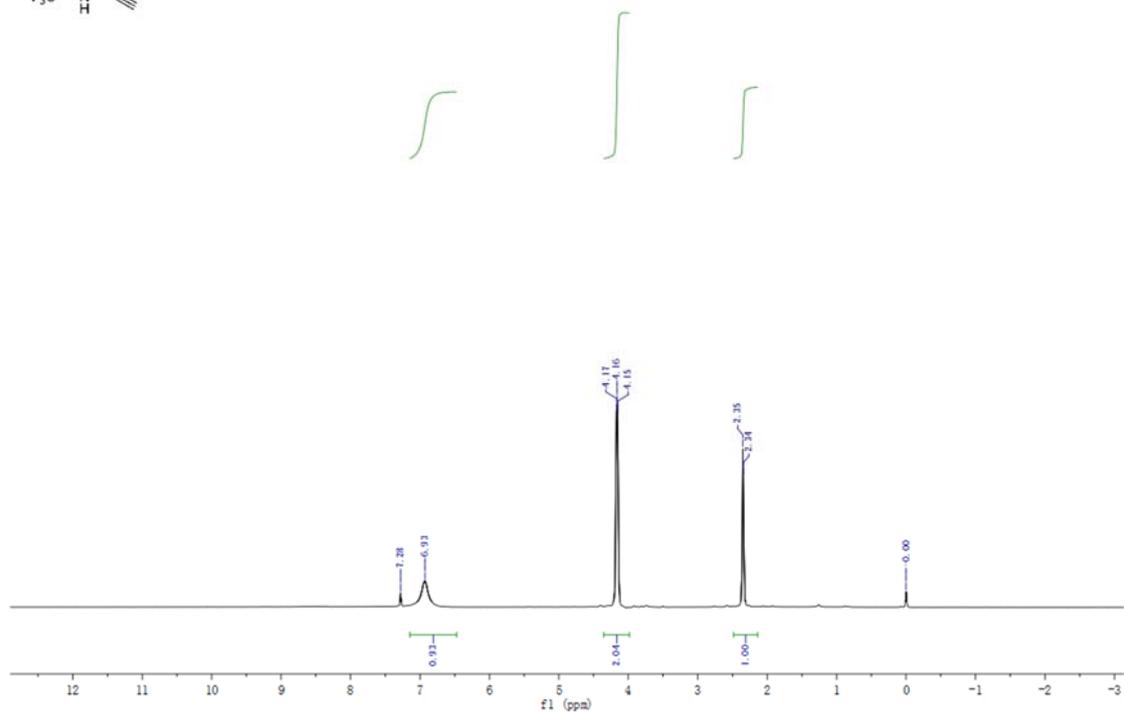
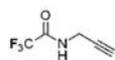
- [4] A. Suzuki, T. Yanaba, and I. Saito, Y. Saito, *Chem. Bio. Chem.*, 2014, **15**, 1638-1644.
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7. NMR spectra

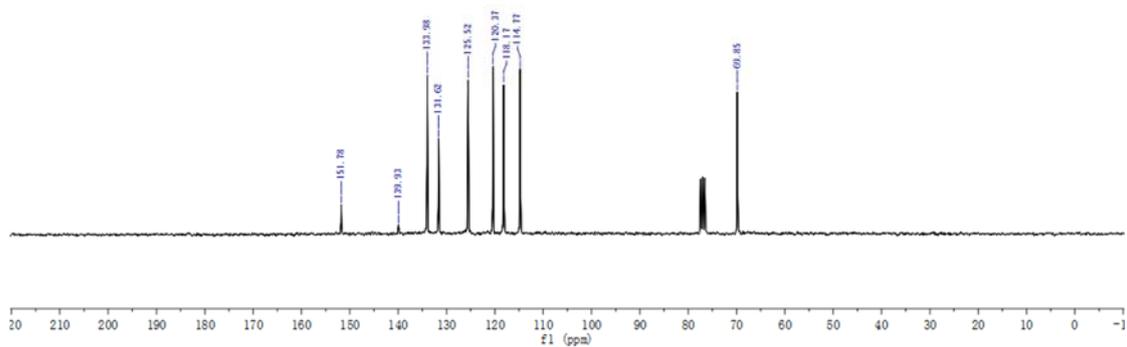
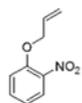
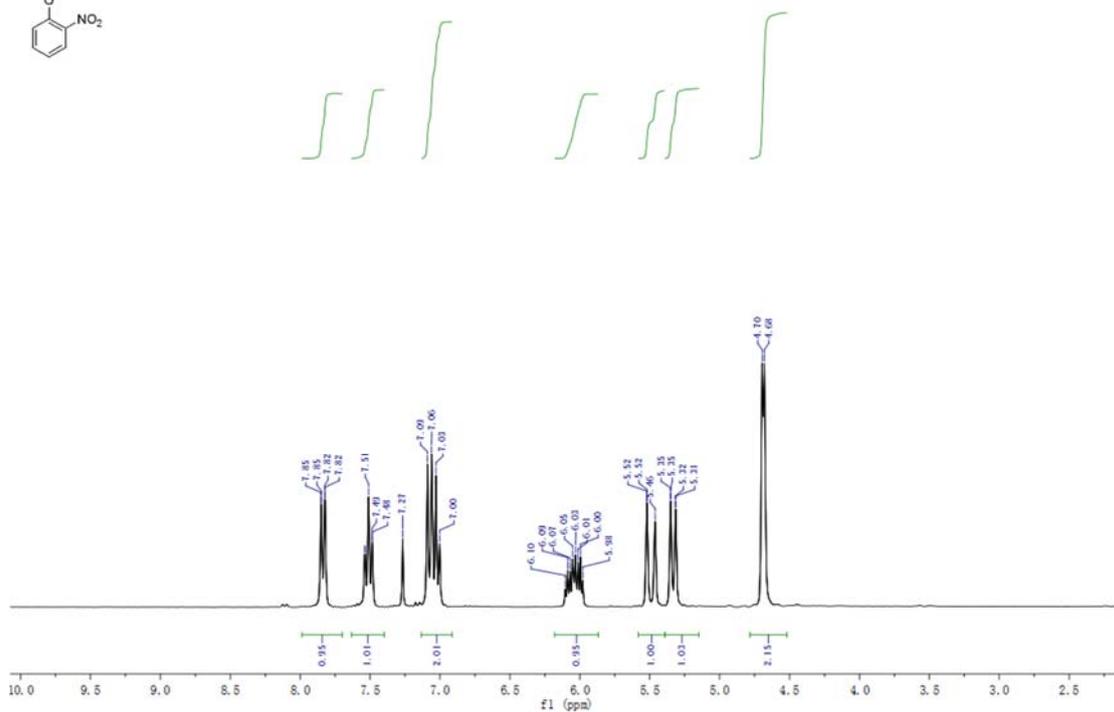
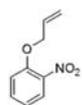
^1H NMR and ^{13}C NMR spectra of compound 1



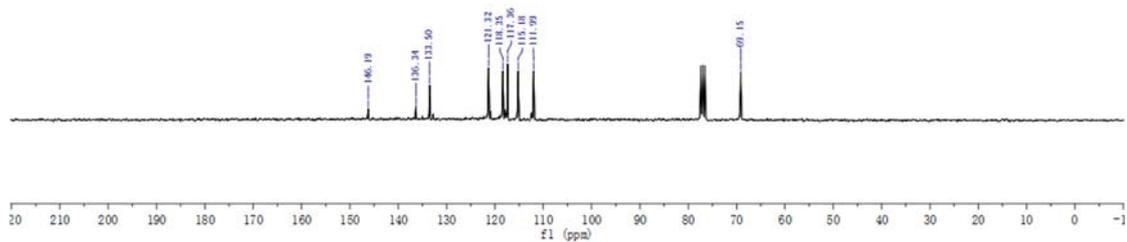
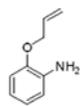
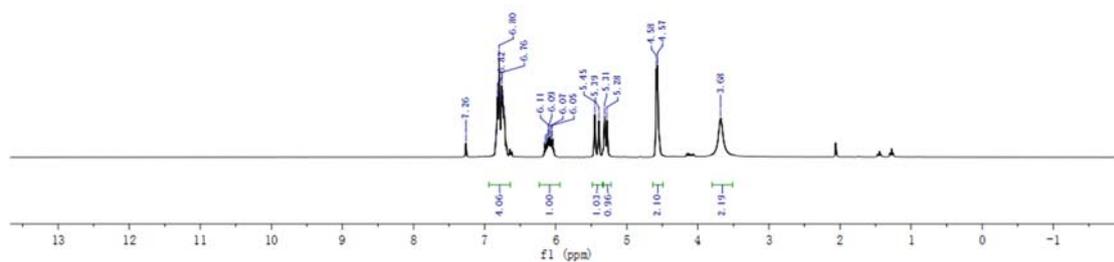
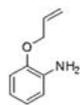
¹H NMR and ¹³C NMR spectra of compound 2



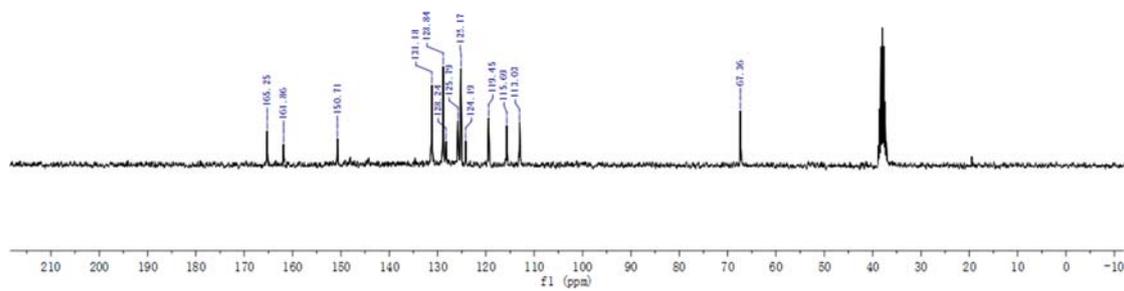
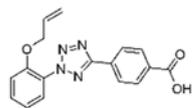
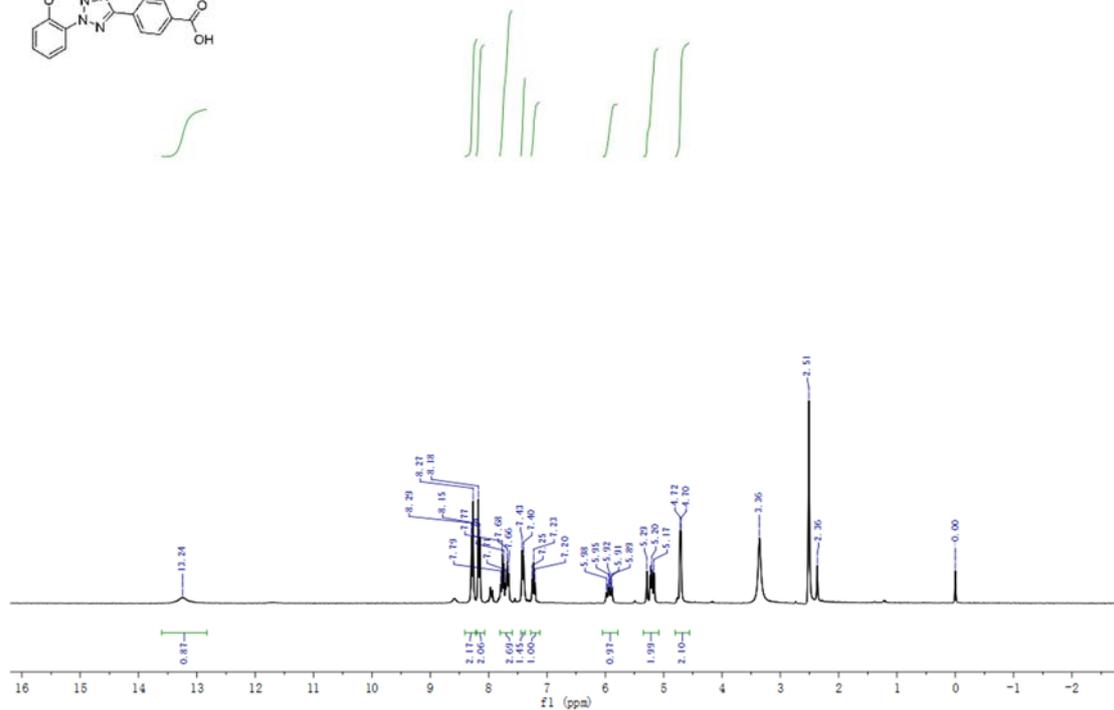
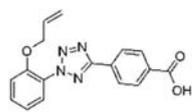
¹H NMR and ¹³C NMR spectra of compound 3



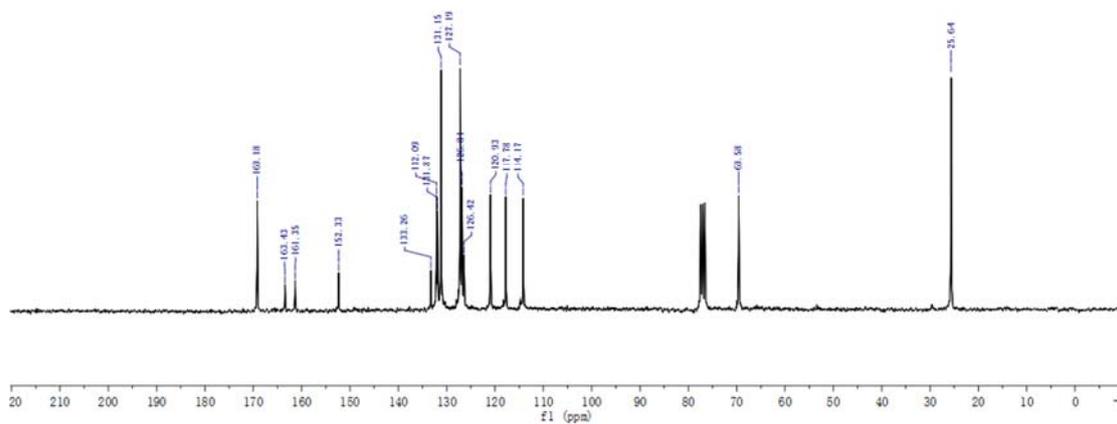
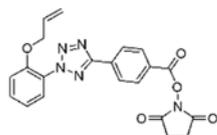
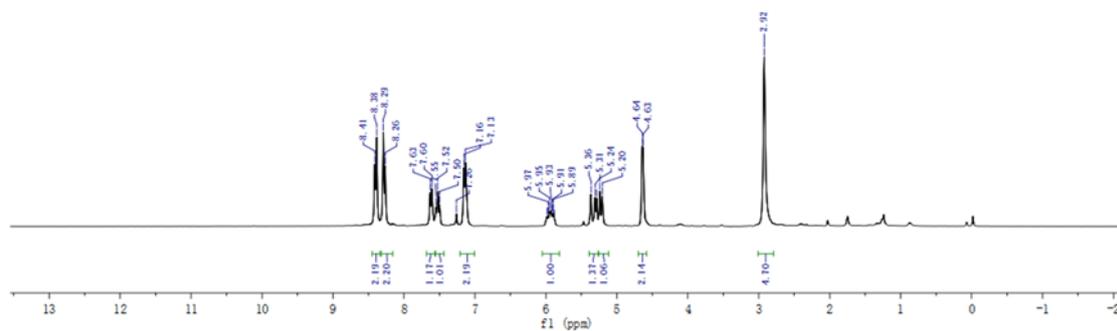
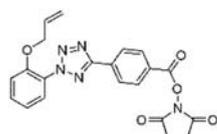
¹HNMR and ¹³CNMR spectra of compound 4



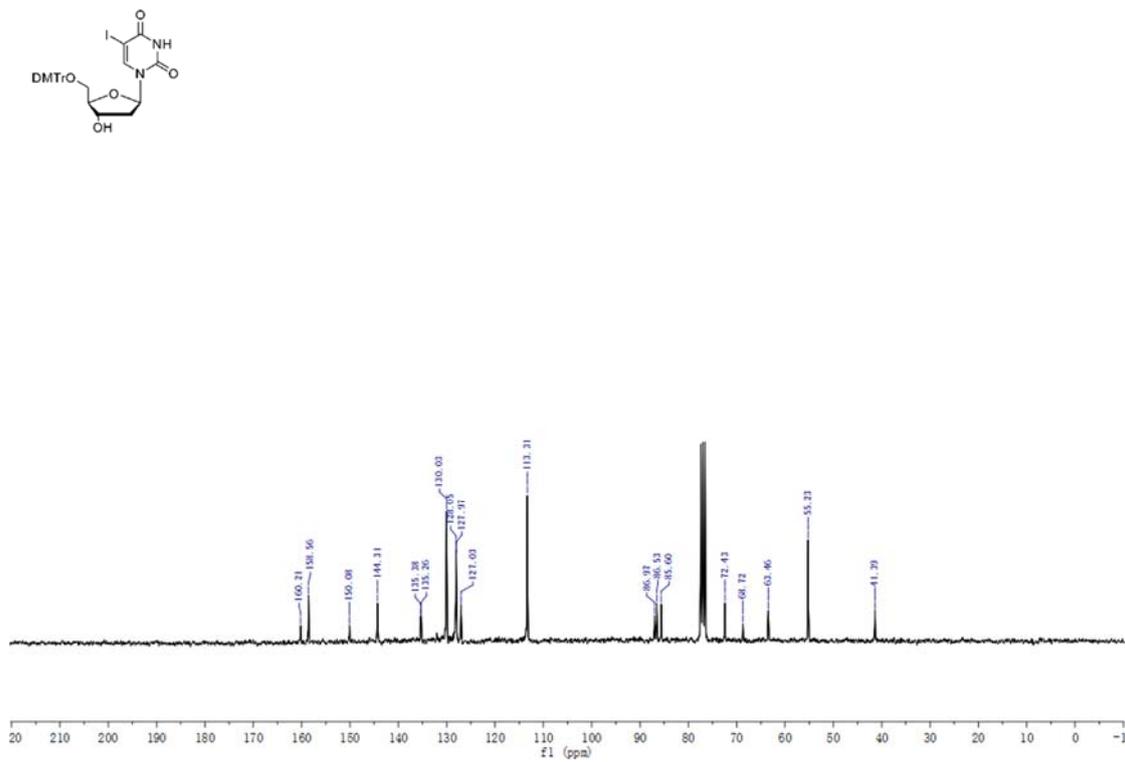
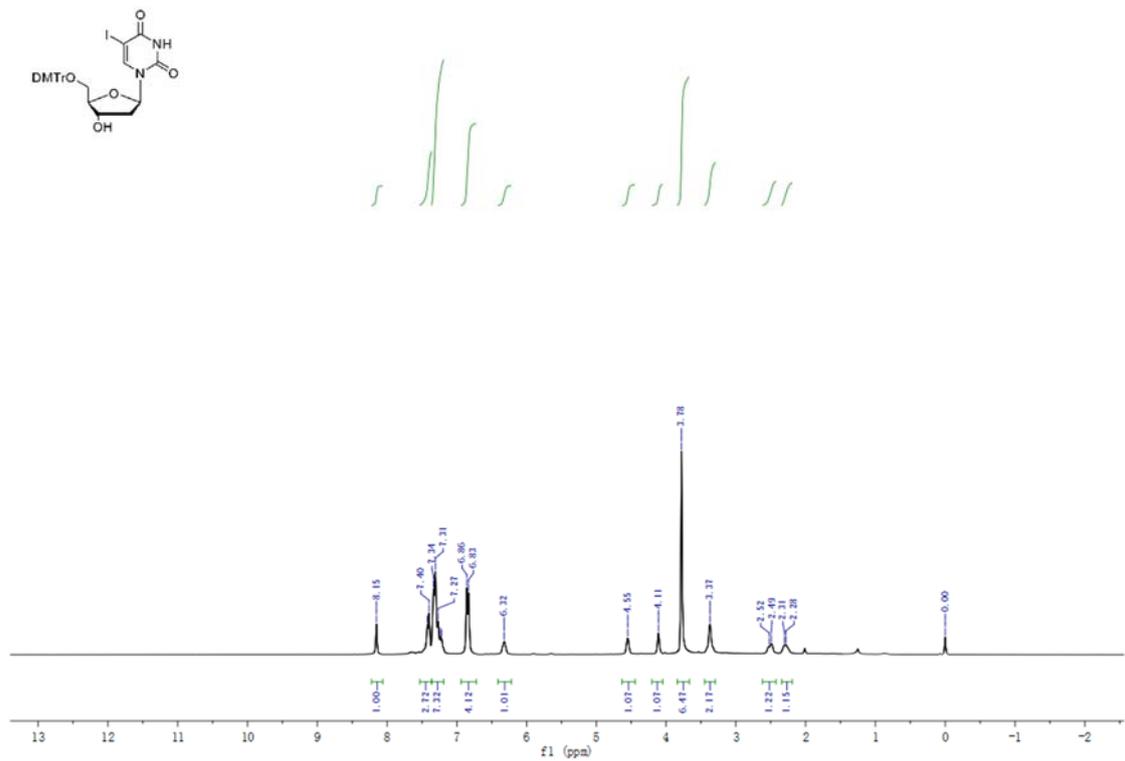
¹HNMR and ¹³CNMR spectra of compound 5



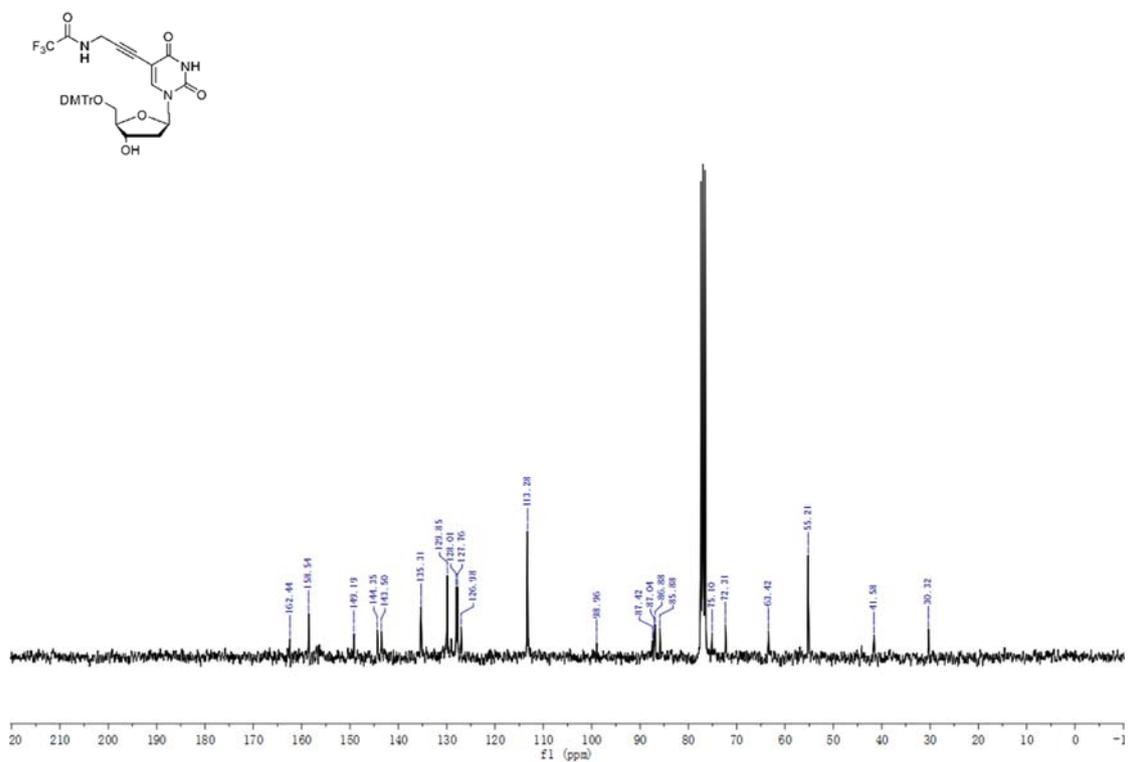
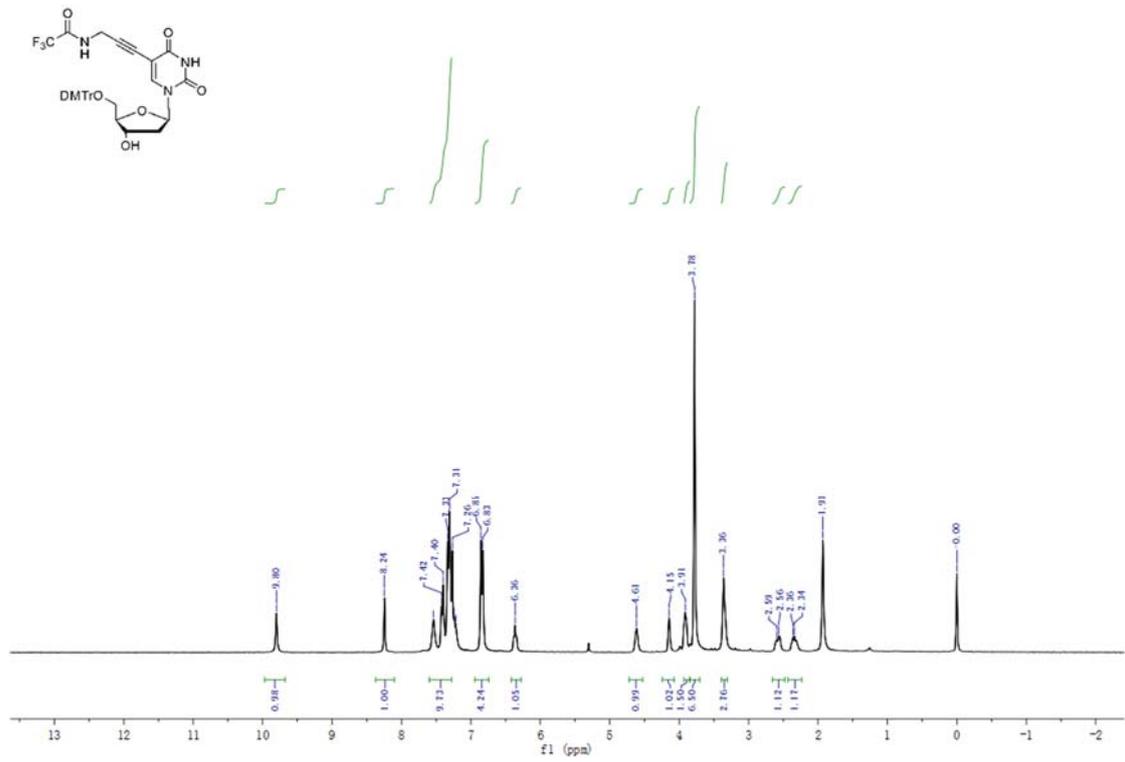
¹HNMR and ¹³CNMR spectra of compound 6



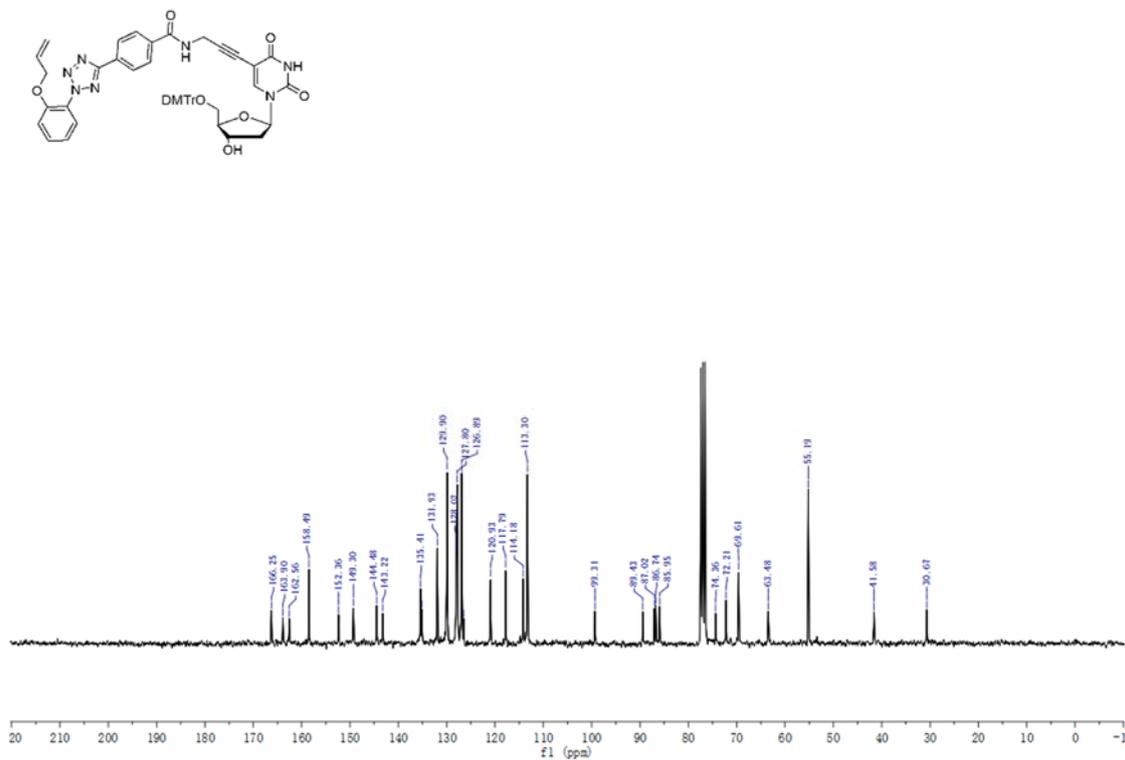
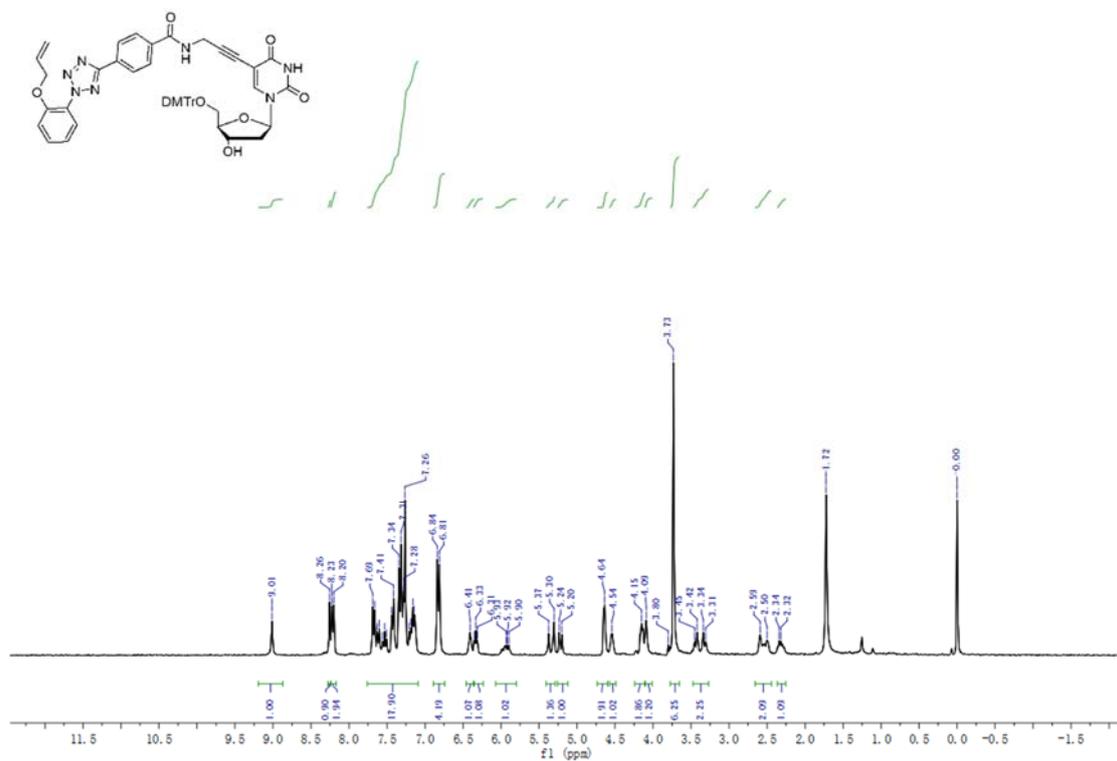
¹HNMR and ¹³CNMR spectra of compound 7



¹HNMR and ¹³CNMR spectra of compound 8



¹HNMR and ¹³CNMR spectra of compound 10



³¹P NMR spectra of compound 11

