Supporting information

Site-Specific DNA Post-Synthetic Modification via Fast Photocatalytic Allylation

Ying Huang,[‡a] Yixin Zhang,[‡a] Chenchen Hu,[a] and Yiyun Chen*[abc]

[a] State Key Laboratory of Chemical Biology, Shanghai Institute of Organic Chemistry, University of Chinese Academy of Sciences, Chinese Academy of Sciences, 345 Lingling Road, Shanghai 200032 (P. R. China).

[b] School of Physical Science and Technology, ShanghaiTech University, 100 Haike Road, Shanghai 201210 (P. R. China).

[c] School of Chemistry and Material Sciences, Hangzhou Institute for Advanced Study, University of Chinese Academy of Sciences, 1 Sub-lane Xiangshan, Hangzhou 310024 (P. R. China)

‡ These authors contributed equally

* To whom correspondence should be addressed. Email: yiyunchen@sioc.ac.cn

Table of Contents

1. Materials and General Methods

Unless otherwise noted, all reactions of substrate preparation were conducted in flame-dried glassware under a nitrogen atmosphere using an anhydrous solvent passed through an activated alumina column (Innovative Technology). Commercially available reagents were used without further purification. Thin layer chromatography (TLC) was performed using Jiangyou TLC silica gel plates HSG F_{254} and visualized using UV light, and potassium permanganate. Flash chromatography was performed on the Leisure Science EZ purification system using the Santai technologies silica gel cartridge. Photochemical reactions were conducted in an eppendorf tube and carried with 4 W blue LED (468 nm peak wavelength, 25 nm spectral half-wave width, composed of 55-65 LED units each with 60 mW, 3 V, 20 mA) obtained from Qiding Photo Electric (analyzed by Everfine PMS-50). ^{1}H NMR and ^{13}C NMR were recorded on an NMR spectrometer with $CDCl_3$ as the solvent, unless otherwise noted, on a Bruker AV-400 MHz or an Agilent 500 MHz spectrometer. Chemical shifts in ^{1}H NMR spectra were reported in parts per million (ppm) on the δ scale from an internal standard of residual $CDCl_3$ (7.26 ppm). Data for ^{1}H NMR were reported as follows: chemical shift, multiplicity (s = singlet, d = doublet, t = triplet, m = multiplet, brs = broad singlet), coupling constant in Herts (Hz), and integration. Data for ^{13}C NMR spectra were reported in terms of chemical shift in ppm from the central peak of $CDCl_3$ (77.16 ppm). IR spectra were recorded on a Thermo Scientific Nicolet 380 FT-IR spectrometer. MS experiments were performed on a Bruker maXis 4G instrument for HRMS-ESI, an Agilent 5973N instrument for EI-MS, and a Waters Micromass GCT Premier instrument for HRMS-EI.

ssDNA C6 NH_2 used in the synthesis of ssDNA-conjugated aryl-substituted allyl sulfone **DNA 1** was obtained from Sangon Biotech (Shanghai) Co., Ltd (Figure S1).

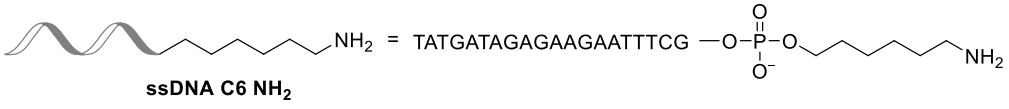
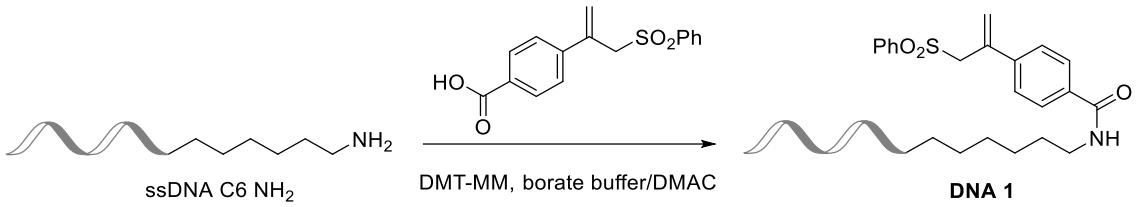


Figure S1. Sequence and structure of ssDNA C6 NH_2

The products of photoredox-catalyzed reactions on-DNA were set up on the benchtop under ambient conditions. HPLC experiments were performed on a ThermoFisher scientific/Dionex Ultimate 3000 HPLC system using a C18 column (150 x 4.6 mm internal diameter, particle size 3.5 μm). Mobile phases: 100 mM HFIP/10 mM DIPEA in HPLC grade water (A) and 100 mM HFIP/10 mM DIPEA in HPLC grade methanol (B). Eluting gradient: from 5% to 70 % of B in 15 minutes, flow rate 0.4 mL/min. Absorption was detected at 260 nm. DNA was analyzed on a Thermo LTQ XL in negative ion mode. Electrospray ionization (ESI) atomization temperature was 350 ℃, and ESI capillary was 4.5 kV. Data was analyzed based on reported peak intensities following deconvolution of the DNA charge states. % Conversion was then calculated by dividing the peak intensity of the product peak by the sum of the reported peaks for that spectra. Stain All-stained DNA bands were recorded by ChemiDoc^{TM}XRS+ gel documentation and analysis systems (Bio-Rad). DNA was quantified by Nanodrop 2000c UV/Vis spectrometer (Thermo scientific).

2. Synthesis of DNA-Conjugated Compounds

2.1 Synthesis of Substrate DNA 1



A solution of ssDNA C6 NH_2 (1 mM, dissolved in 250 mM borate buffer, pH 9.5), DMT-MM (0.97 mg, 3.5 μmol) in H_2O (17.5 μL)，4-(3-(phenylsulfonyl)prop-1-en-2-yl)benzoic acid (1.06 mg, 3.5 μmol) in DMAC (17.5 μL) oscillated at 25 ℃ for 16 h. The mixture was added 5 M NaCl solution (10 % by volume) and cold ethanol (2.5 times by volume, ethanol stored at -20 ℃). The mixture was stored in a -80 ℃ freezer for 50 minutes. Centrifuge the sample for 30 minutes at 4 ℃ in a microcentrifuge at 10000 rpm. The above supernatant was discarded and the precipitate was cooled in liquid nitrogen and then placed on a lyophilizer.

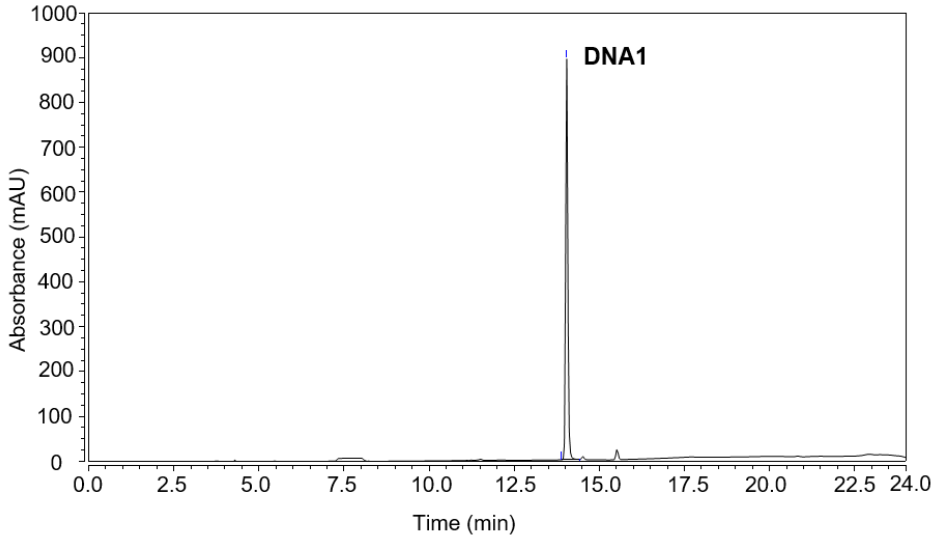
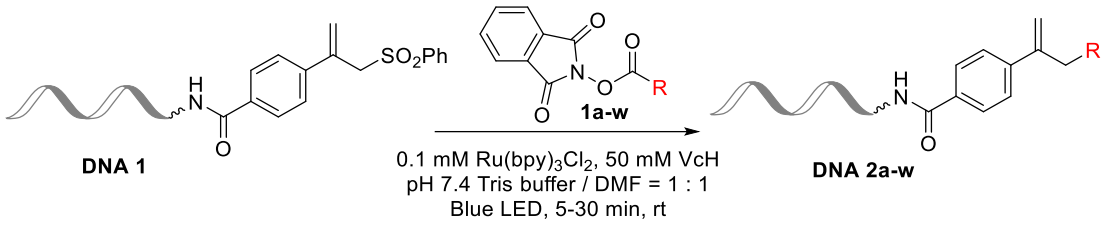


Figure S2. HPLC analysis of purified **DNA1**

2.2 General Procedures for Photocatalytic Decarboxylative Coupling Reactions



Synthesis of DNA 2a-w: To the **DNA 1** (1 nmol), was added 10 μL of **1a-w** (100-200 mM in DMF), 1 μL of $Ru(bpy)_3Cl_2$ (10 mM in H_2O) and 10 μL of ascorbic acid (500 mM in pH 7.4 tris buffer). The mixture was added 40 μL DMF and 39 μL tris buffer. The eppendorf tube was sealed and exposed to blue LEDs (two 4 W LED light bulbs 10 cm away from the vial, 468 nm peak wavelength, 25 nm spectral half-wave width, composed of 55-65 LED units each with 60 mW, 3 V, 20 mA) for 5-30 min at room temperature. The mixture was added 5 M NaCl solution (10 % by volume) and cold ethanol (2.5 times by volume, ethanol stored at -20 ℃). The mixture was stored in a -80 ℃ freezer for 50 minutes. Centrifuge the sample for 30 minutes at 4 ℃ in a microcentrifuge at 10000 rpm. The above supernatant was discarded and the precipitate was cooled in liquid nitrogen and then placed on a lyophilizer.

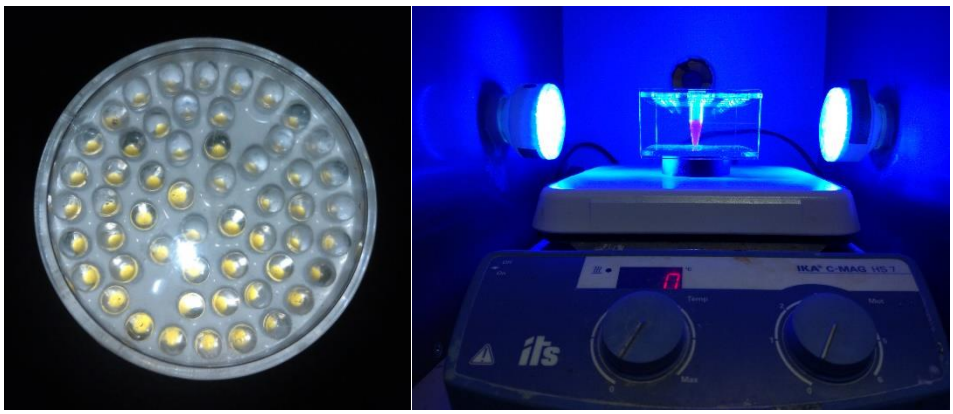


Figure S3. Blue LED light source and reaction system

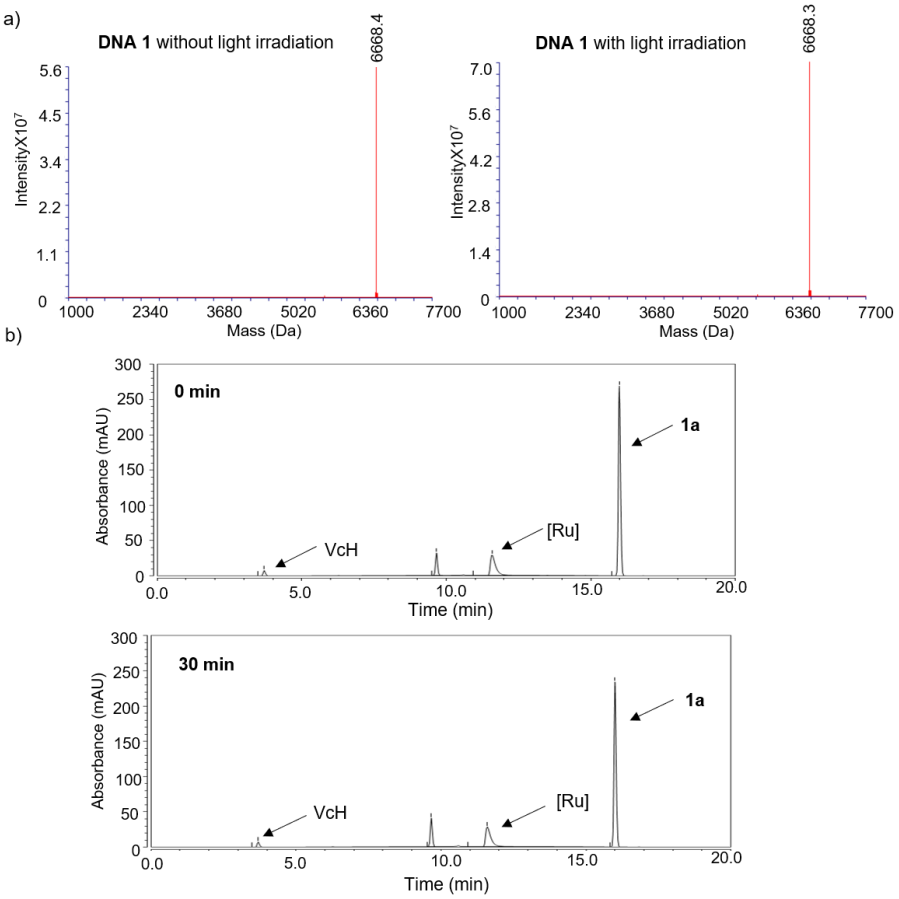


Figure S4. a) DNA integrity analysis after 30 minutes of light irradiation by LC-MS; b) **1a** stability analysis in the reaction mixture by HPLC.

Table S1. Time-dependent generation of **DNA2a** under blue LED light irradiation

Entry [a]	Reaction time	Yield [b]
1	0 min	0%
2	5 min	79%
3	30 min	80%
4	1 h	81%
5	2 h	79%

[a] Reaction conditions: **DNA 1** (1 nmol, 1 equiv.), **1a** (1 μmol, 1000 equiv.), ascorbic acid (VcH, 5 μmol, 5000 equiv.), $Ru(bpy)_3Cl_2$ (10 nmol, 10 equiv.), pH 7.4, 0.5 M Tris buffer/DMF (100 μL), and two blue LED (4 W, 468 nm) irradiation at 25 °C for 5 min.
[b] Yields were determined by LC-MS analysis.

Table S2. Photocatalysts screen

Entry[a]	Photocatalysts	Yield[b]
1	$Ru(bpy)_3Cl_2$	84%
2	$Ir(dtbbpy)(ppy)_2PF_6$	31%
3	$Fac\text{-}Ir(ppy)_3$	5%
4	Eosin Y	80%
5	4CzIPN	0%
6	$Acr\text{-}Mes^+ClO_4^-$	0%
7	$Ph\text{-}Acr\text{-}Mes^+BF_4^-$	0%

[a] Reaction conditions: **DNA 1** (1 nmol, 1 equiv.), **1a** (1 μmol, 1000 equiv.), ascorbic acid (VcH, 5 μmol, 5000 equiv.), photocatalysts (10 nmol, 10 equiv.), pH 7.4 0.5 M Tris buffer/DMF (1:1) (100 μL) with two blue LED (4 W, 468 nm) irradiation at 25 °C for 5 min. [b] Yields were determined by LC-MS analysis.

Table S3. Effects of salt ions on the on-DNA photocatalytic reactivity

Entry [a]	buffer:DMF (v:v=1:1)	Yield [b]
1	H_2O	47%
2	pH7.4 500 mM Tris-HCl buffer	53%
3	pH7.4 PBS buffer	57%
4	pH7.4 20XPB buffer	39%

[a] Reaction conditions: **DNA 1** (1 nmol, 1 equiv.), **1a** (1 μmol, 1000 equiv.), NaVc (5 μmol, 5000 equiv.), $Ru(bpy)_3Cl_2$ (10 nmol, 10 equiv.), buffer/DMF (100 μL), and two blue LED (4 W, 468 nm) irradiation at 25 °C for 5 min. [b] Yields were determined by LC-MS analysis.

Table S4. Effects of the aqueous composition ratio on the on-DNA photocatalytic reactivity

Entry [a]	pH 7.4 Tris buffer:DMF (v:v)	Yield [b]
1	1:3	31%
2	1:1	62%
3	3:1	6%

[a] Reaction conditions: **DNA 1** (1 nmol, 1 equiv.), **1g** (1 μmol, 1000 equiv.), ascorbic acid (VcH, 5 μmol, 5000 equiv.), $Ru(bpy)_3Cl_2$ (10 nmol, 10 equiv.), pH 7.4, 0.5 M Tris buffer/DMF (100 μL), and two blue LED (4 W, 468 nm) irradiation at 25 °C for 5 min. [b] Yields were determined by HPLC analysis.

Table S5. Effects of cell culture media on the on-DNA photocatalytic reactivity

Entry [a]	Cell culture buffer:DMF (v:v=1:1)	Yield [b]
1	pH7.4 500 mM Tris-HCl	79%
2	DMEM	81%
3	pH 7.5 1 M HEPES	83%

[a] Reaction conditions: **DNA 1** (1 nmol, 1 equiv.), **1a** (1 μmol, 1000 equiv.), VcH (5 μmol, 5000 equiv.), $Ru(bpy)_3Cl_2$ (10 nmol, 10 equiv.), buffer/DMF (100 μL), and two blue LED (4 W, 468 nm) irradiation at 25 °C for 5 min. [b] Yields were determined by LC-MS analysis.

Synthesis of DNA 2g with Eosin Y: To the **DNA 1** (1 nmol), was added 10 μL of **1g** (100 mM in DMF), 1 μL of Eosin Y (10 mM in DMF), and 10 μL of ascorbic acid (500 mM in pH 7.4 tris buffer). The mixture was added 39 μL DMF and 40 μL tris buffer. The eppendorf tube was sealed and exposed to blue LEDs (two 4 W LED light bulbs 10 cm away from the vial, 468 nm peak wavelength, 25 nm spectral half-wave width, composed of 55-65 LED units each with 60 mW, 3 V, 20 mA) for 5 min at room temperature. The mixture was added 5 M NaCl solution (10 % by volume) and cold ethanol (2.5 times by volume, ethanol stored at -20 ℃). The mixture was stored in a -80 ℃ freezer for 50 minutes. Centrifuge the sample for 30 minutes at 4 ℃ in a microcentrifuge at 10000 rpm. The above supernatant was discarded and the precipitate was cooled in liquid nitrogen and then placed on a lyophilizer.

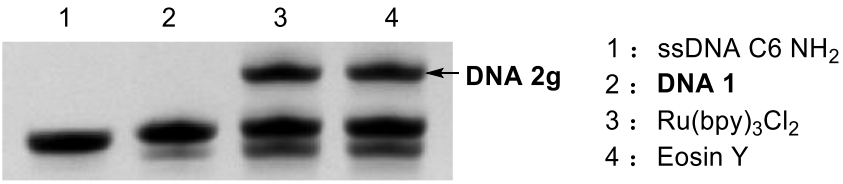
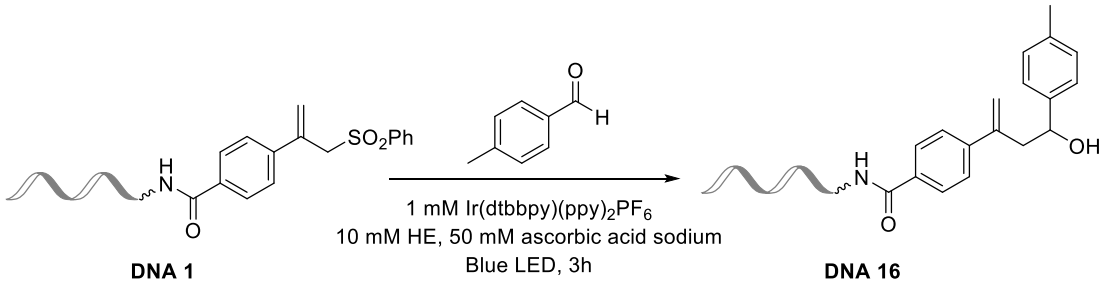


Figure S5. In-gel analysis of the photocatalytic decarboxylative coupling reactions products **DNA 2g**

2.3 Procedures for Photocatalytic Polarity Reversed Alkylation

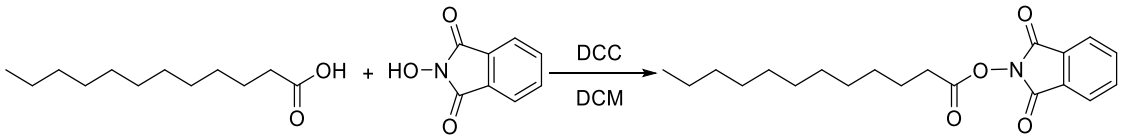


On DNA Polarity-Reversed Allylation: To the **DNA 1** solution (Obtained from the previous step, 80 μL), 1000 equiv of 4-methylbenzaldehyde (23.6 μL), 1000 equiv of HE (23.6 μL), 100 equiv of $Ir(dtbbpy)(ppy)_2PF_6$ (23.6 μL) and 5000 equiv of sodium ascorbate (VcNa, 23.6 μL) was added. The mixture was added 47.2 μL MeCN and 14.4 μL H_2O. After degassing with nitrogen for 3 min. The eppendorf tube was sealed and exposed to blue LEDs (two 4 W LED light bulbs 10 cm away from the vial, 468 nm

peak wavelength, 25 nm spectral half-wave width, composed of 55-65 LED units each with 60 mW, 3 V, 20 mA), and the reaction was allowed to proceed at room temperature for 3 h. The mixture was purified by using micro bio-spin chromatography columns after lyophilization.

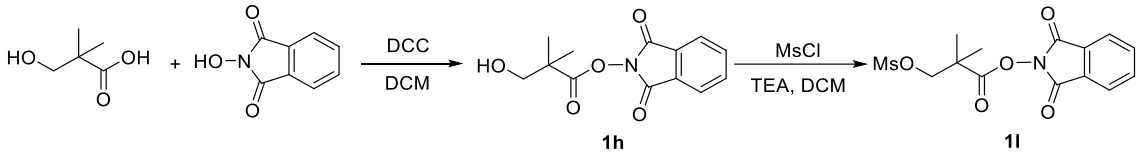
3. Synthesis of Small Molecule Substrates

The synthesis of **1a**, **1b**, **1c**, **1f**, **1i**, **1p**, **1v**, and **1w** were carried out as previously reported.[1] The synthesis of **1e**, **1h**, **1j**, **1k**, **1r**, **1s**, and **1t** were carried out as previously reported.[2]



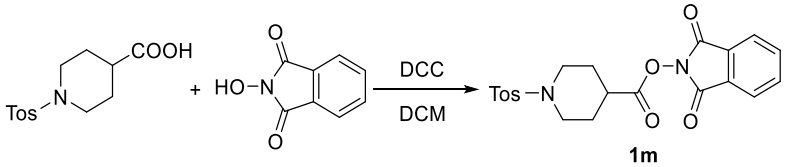
1d

Synthesis of 1d: A solution of dodecanoic acid (1.01 g, 5.40 mmol) and 2-hydroxyisoindoline-1,3-dione (0.97 g, 5.94 mmol) in DCM (50 mL) was added to DCC (1.23 g, 5.94 mmol) at 0 ºC and stirred at room temperature until TLC indicated the complete consumption of dodecanoic acid. The solid was removed by filtration, and the filtrate was concentrated in vacuo. The residue was purified by silica gel column chromatography to give **1d** (1.12 g，60%) as a white solid. TLC R_f= 0.66 (Petroleum ether/ EtOAc = 5/1); IR (thin film) 2926, 2854, 1817, 1790, 1747, 1467, 1367, 1186, 697 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) δ 7.88 (dd, J = 5.5, 3.1 Hz, 2H), 7.78 (dd, J = 5.5, 3.1 Hz, 2H), 2.66 (t, J = 7.5 Hz, 2H), 1.78 (p, J = 7.5 Hz, 2H), 1.44 (p, J = 7.3 Hz, 2H), 1.37 – 1.19 (m, 14H), 0.88 (t, J = 7.5 Hz, 3H); ^{13}C NMR (126 MHz, $CDCl_3$) δ 169.8, 162.2, 134.9, 129.1, 124.1, 32.0, 31.1, 29.7, 29.7, 29.5, 29.5, 29.3, 29.0, 24.8, 22.8, 14.3; HRMS-ESI (m/z) $[M+Na]^+$ calc'd for $C_{20}H_{27}NNaO_4$ 368.1832, found 368.1837.



1h 1l

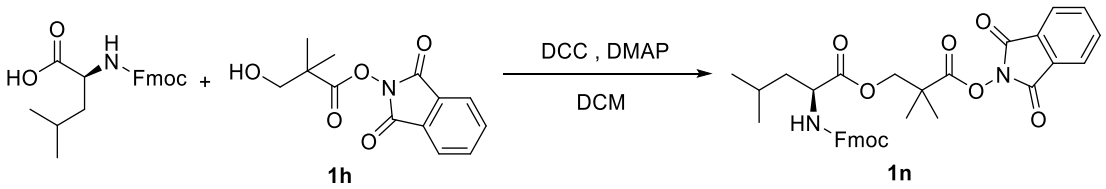
Synthesis of 1l: A solution of 3-hydroxy-2,2-dimethylpropanoic acid (1.4 g, 11.9 mmol) and 2-hydroxyisoindoline-1,3-dione (2.12 g, 13 mmol) in DCM (20 mL) was added DCC (2.67 g, 13 mmol) at 0 ºC and stirred at room temperature for 3 h until TLC indicated the complete consumption of 3-hydroxy-2,2-dimethylpropanoic acid. The solid was removed by filtration, and the filtrate was concentrated in vacuo. The residue was purified by silica gel column chromatography to give **1h** (2.16 g，69 %) as a white solid. A solution of **1h** (0.26 g, 1 mmol) and TEA (0.16 g, 1.6 mmol) in DCM (3 mL) was added to MsCl (0.14 g, 1.2 mmol) at 0 ℃ and stirred at 25 ℃ for 6 h. The mixture was diluted with DCM and washed with water (50 mL × 2), brine, dried with Na_2SO_4, concentrated under reduced pressure, and purified by column chromatography on silica gel to give **1l** (0.23 g, 67%) as a white solid. TLC R_f= 0.49 (Petroleum ether/ EtOAc = 2/1); IR (thin film) 1811, 1787, 1744, 1468, 1359, 1178, 1065, 965, 878, 697 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) δ 7.89 (dd, J = 5.4, 3.2 Hz, 2H), 7.80 (dd, J = 5.4, 3.3 Hz, 2H), 4.33 (s, 2H), 3.14 (s, 3H), 1.51 (s, 6H); ^{13}C NMR (126 MHz, $CDCl_3$) δ 171.3, 161.8, 135.0, 129.0, 124.2, 73.6, 42.8, 37.6, 22.1; HRMS-ESI (m/z) $[M+Na]^+$ calc'd for $C_{14}H_{15}NNaO_7S$ 364.0461, found 364.0468.



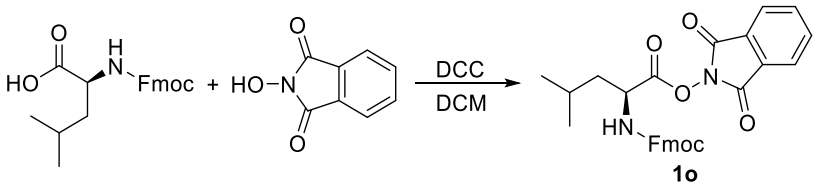
1m

Synthesis of 1m: A solution of 1-tosylpiperidine-4-carboxylic acid (7.62 g, 26.9 mmol) and 2-hydroxyisoindoline-1,3-dione (4.85 g, 30 mmol) in DCM (200 mL) was added DCC (6.16 g, 30 mmol) at 0 ºC and stirred at room temperature until TLC indicated the complete consumption of 1-tosylpiperidine-4-carboxylic acid. The solid was removed by filtration, and the filtrate was concentrated in vacuo. The residue was purified by silica gel column chromatography to give **1m** (8.2 g，71%) as a white solid. TLC R_f= 0.4 (Petroleum ether/ EtOAc = 3/1); IR (thin film) 1814, 1782, 1743, 1333, 1160, 1097,

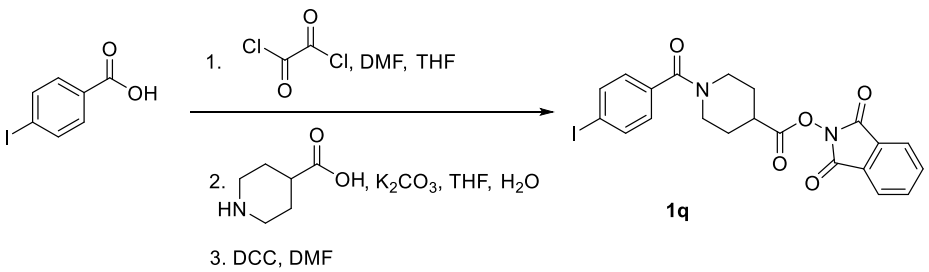
1026, 924, 878, 696 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) δ 7.86 (dd, J = 5.5, 3.2 Hz, 2H), 7.78 (dd, J = 5.6, 3.1 Hz, 2H), 7.65 (d, J = 8.3 Hz, 2H), 7.33 (d, J = 8.2 Hz, 2H), 3.64 (dt, J = 12.0, 4.6 Hz, 2H), 2.75 – 2.70 (m, 1H), 2.68 – 2.60 (m, 2H), 2.44 (s, 3H), 2.20 – 2.11 (m, 2H), 2.08 – 1.97 (m, 2H); ^{13}C NMR (126 MHz, $CDCl_3$) δ 170.2, 161.8, 143.8, 134.9, 133.1, 129.8, 128.8, 127.6, 124.0, 44.8, 37.5, 27.3, 21.5; HRMS-ESI (m/z) $[M+Na]^+$ calc'd for $C_{21}H_{20}N_2NaO_6S$ 451.0934, found 451.0938.



Synthesis of 1n: A solution of (((9H-fluoren-9-yl)methoxy)carbonyl)-L-leucine (0.39 g, 1.1 mmol) and **1h** (0.26 g, 1 mmol) in DCM (10 mL) was added DCC (0.23 g, 1.1 mmol) and DMAP (0.01 g, 0.1 mmol) at 0 ºC and stirred at room temperature until TLC indicated the complete consumption of **1h**. The solid was removed by filtration, and the filtrate was concentrated in vacuo. The residue was purified by silica gel column chromatography to give **1n** (0.55 g，93%) as a white solid. TLC R_f= 0.65 (Petroleum ether/ EtOAc = 2/1); IR (thin film) 2958, 1811, 1788, 1746, 1521, 1468, 1187, 1063, 877, 697 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) δ 7.86 (dd, J = 5.4, 3.2 Hz, 2H), 7.77 (dd, J = 5.4, 3.2 Hz, 2H), 7.73 (d, J = 7.7 Hz, 2H), 7.60 (m, 2H), 7.35 (m, 2H), 7.25 (m, 2H), 5.75 (d, J = 9.3 Hz, 1H), 4.52 (td, J = 9.4, 4.4 Hz, 1H), 4.43 – 4.37 (m, 1H), 4.36 – 4.30 (m, 2H), 4.25 (d, J = 10.8 Hz, 1H), 4.20 (t, J = 7.3 Hz, 1H), 1.85 – 1.70 (m, 2H), 1.69 – 1.57 (m, 1H), 1.45 (d, J = 6.2 Hz, 6H), 0.98 (dd, J = 6.3, 3.4 Hz, 6H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 172.4, 171.8, 162.1, 156.4, 144.0, 141.4, 135.0, 129.0, 127.7, 127.2, 125.3, 124.2, 120.0, 70.4, 67.2, 53.0, 47.3, 42.7, 41.5, 24.9, 23.0, 22.4, 22.3, 21.8. HRMS-ESI (m/z) $[M+Na]^+$ calc'd for $C_{34}H_{34}N_2NaO_8$ 621.2207, found 621.2214.

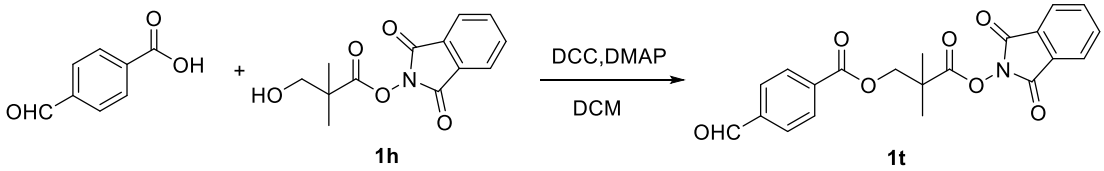


Synthesis of 1o: A solution of (((9H-fluoren-9-yl) methoxy) carbonyl)-L-leucine (0.35 g, 1 mmol) and 2-hydroxyisoindoline-1,3-dione (0.18 g, 1.1 mmol) in DCM (8 mL) was added DCC (0.23 g, 1.1 mmol) at 0 ºC and stirred at room temperature until TLC indicated the complete consumption of (((9H-fluoren-9-yl) methoxy) carbonyl)-L-leucine. The solid was removed by filtration, and the filtrate was concentrated in vacuo. The residue was purified by silica gel column chromatography to give **1o** (0.42 g，84%) as a white solid. TLC R_f= 0.56 (Petroleum ether/ EtOAc = 2/1); IR (thin film) 2959, 1818, 1789, 1746, 1522, 1450, 1081, 877, 739, 697 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) δ 7.92 – 7.84 (m, 2H), 7.82 – 7.73 (m, 4H), 7.60 (t, J = 6.2 Hz, 2H), 7.39 (t, J = 7.5 Hz, 2H), 7.31 (t, J = 7.4 Hz, 2H), 5.28 – 5.21 (m, 1H), 4.88 – 4.80 (m, 1H), 4.50 – 4.43 (m, 2H), 4.25 (t, J = 7.2 Hz, 1H), 1.96 – 1.80 (m, 2H), 1.79 – 1.70 (m, 1H), 1.05 – 0.99 (m, 6H); ^{13}C NMR (126 MHz, $CDCl_3$) δ 169.9, 161.7, 155.8, 141.5, 135.0, 129.0, 127.9, 127.2, 125.2, 124.2, 123.6, 120.1, 67.3, 51.1, 47.3, 41.8, 24.8, 22.9, 21.8; HRMS-ESI (m/z) $[M+Na]^+$ calc'd for $C_{29}H_{26}N_2NaO_6$ 521.1683, found 521.1691.



Synthesis of 1q: A solution of 4-iodobenzoic acid (4.96 g, 20 mmol) and DMF (100 μL) in THF (50 mL) was added dropwise oxalyl dichloride (2.03 mL, 24 mmol) at 0 ºC and stirred for overnight at room temperature. The resulting reaction mixture was concentrated in vacuo to afford 4-iodobenzoyl chloride which proceeded to the next step without further purification. To a solution of isonipecotic acid (2.84 g, 22 mmol) and K_2CO_3 (6.07 g, 44 mmol) in H_2O (50 mL) was added slowly with 4-iodobenzoyl chloride in THF (50 mL) at 0 ºC, and stirred for 30 min at 0 ºC. Then the reaction mixture was stirred overnight at room temperature. To the resulting reaction mixture was added H_2O (50 mL), and extracted with EtOAc. To the aqueous phase was added HCl (1 N) adjusted pH to 3, dried over anhydrous Na_2SO_4, concentrated in vacuo to

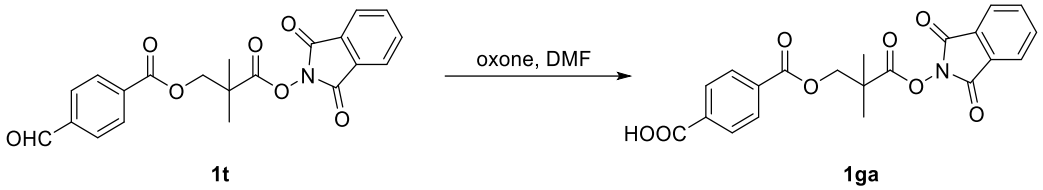
afford 1-(4-iodobenzoyl) piperidine-4-carboxylic acid which was proceeded to the next step without further purification. A solution of 1-(4-iodobenzoyl) piperidine-4-carboxylic acid obtained from the previous step and 2-hydroxyisoindoline-1,3-dione (3.16 g, 19.4 mmol) in DMF (50 mL) was added DCC (4.12 g, 20 mmol) at 0 ºC and stirred for overnight at room temperature. The solid was removed by filtration, and the filtrate was concentrated in vacuo. The residue was purified by silica gel column chromatography to give **1q** (1.5 g，15 % yield over three steps) as a light yellow solid. TLC R_f= 0.26 (Petroleum ether/ EtOAc = 3/1); IR (thin film) 1812, 1785, 1744, 1632, 1587, 1467, 1186, 997, 878, 697 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) δ 7.89 (dd, J = 5.6, 3.0 Hz, 2H), 7.80 (dd, J = 5.6, 3.0 Hz, 2H), 7.75 (d, J = 8.4 Hz, 2H), 7.17 – 7.12 (m, 2H), 4.43 (s,1H), 3.76 (s,1H), 3.36 – 3.16 (m, 2H), 3.04 (td, J = 9.8, 5.0 Hz, 1H), 2.29 – 1.81 (m, 4H); ^{13}C NMR (126 MHz, $CDCl_3$) δ 170.4, 169.8, 162.0, 137.9, 135.1, 135.0, 134.3, 128.9, 128.8, 124.2, 123.3, 96.2, 38.4. HRMS-ESI (m/z) $[M+Na]^+$ calc'd for $C_{21}H_{17}IN_2NaO_5$ 527.0074, found 527.0076.



1h **1t**

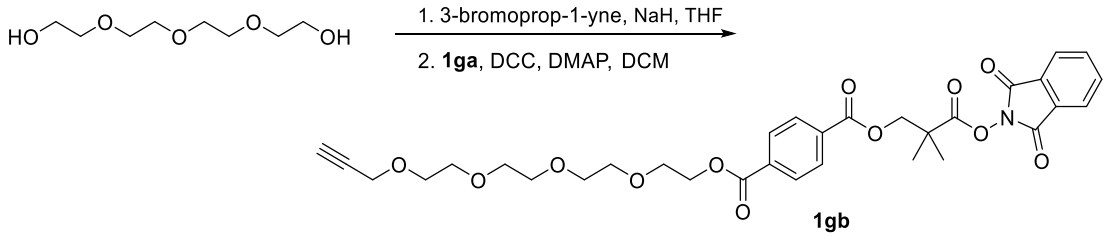
Synthesis of 1t: A solution of **1h** (2 g, 7.6 mmol), 4-formylbenzoic acid (1.25 g, 8.3 mmol) in DCM (30 mL) was added DCC (1.7 g, 8.3 mmol) and DMAP (93 mg,0.76 mmol) at 0 ºC and stirred at room temperature until TLC indicated the complete consumption of **1h**. The solid was removed by filtration, and the filtrate was concentrated in vacuo. The residue was purified by silica gel column chromatography to give **1t** (2.55 g, 85%) as a white solid. TLC R_f = 0.37 (Petroleum ether/ EtOAc = 5/1); IR (thin film) 3096, 2936, 1809, 1785, 1744, 1705, 1467, 1370, 1272, 1202 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) δ 10.11 (s, 1H), 8.29 (d, J = 8.1 Hz, 2H), 7.98 (d, J = 8.1 Hz, 2H), 7.87 (dd, J = 5.3, 3.1 Hz, 2H), 7.78 (dd, J = 5.3, 3.1 Hz, 2H), 4.54 (s, 2H), 1.54 (s, 6H); ^{13}C NMR (126 MHz, $CDCl_3$) δ 191.9, 171.9, 165.3, 161.9, 139.4, 134.9, 134.7, 130.7, 129.7, 129.0, 124.1, 70.5, 42.8, 22.5; HRMS-ESI (m/z) $[M+H]_+$ calc'd for

$C_{21}H_{17}NNaO_7$ 418.0903, found 418.0898.



1t **1ga**

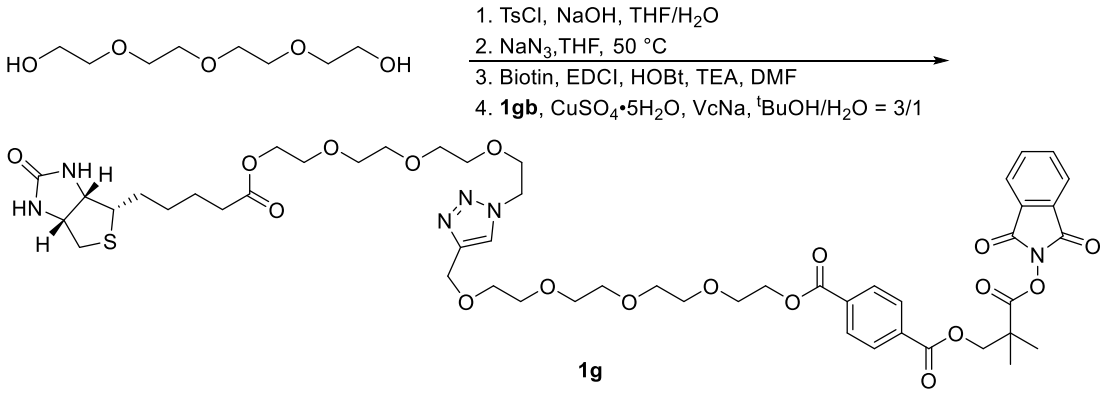
Synthesis of 1ga: A solution of **1t** (1.18 g, 3.0 mmol) in DCM (10 mL) was added to oxone (1.84 g,3.0 mmol) at 0 ºC. The temperature was allowed to rise to 25 ºC and the mixture was stirred at 25 ºC until TLC indicated the complete consumption of **1t**. The basic reaction mixture was added 1M aqueous HCl solution to dissolve insoluble solids, then extracted with ethyl acetate (3 × 50 mL), and the combined organic layers were washed with brine and dried over anhydrous Na_2SO_4, filtered, then concentrated in vacuo to give **1ga** (1.16 g, 94%) as a white solid. TLC R_f = 0.55 (EA: PE= 1:1); IR (thin film) 3105, 2985, 1809, 1786, 1745, 1697, 1467, 1368, 1270, 1061 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) δ 8.23 (d, J = 3.7 Hz, 4H), 7.89 (dd, J = 5.4, 3.1 Hz, 2H), 7.79 (dd, J = 5.5, 3.0 Hz, 2H), 4.55 (s, 2H), 1.55 (s, 6H); ^{13}C NMR (126 MHz, $CDCl_3$) δ 172.0, 170.9, 165.4, 161.9, 134.9, 134.3, 133.4, 130.4, 130.1, 129.1, 124.1, 70.4, 42.8, 22.5; HRMS-ESI (m/z) $[M+Na]^+$ calc'd 434.0852 for $C_{21}H_{17}NNaO_8$, found 434.0850.



1gb

Synthesis of 1gb: A solution of 2, 2'-((oxybis(ethane-2,1-diyl)) bis(oxy)) bis (ethan-1-ol) (10 g, 51.5 mmol) in THF (10 mL) was added NaH (0.5 g, 20.8 mmol) at 0 ºC. Dissolving the 3-bromoprop-1-yne (0.8 mL, 10.66 mmol) in 1 mL toluene and dilute it with 15 mL THF. This solution is injected into the reaction system dropwise. The temperature was allowed to rise to 25 ºC and the mixture was stirred at 25 ºC for 2 h. Then the reaction mixture was diluted with dichloromethane and washed several times with water. The organic layer was washed with brine and dried over anhydrous Na_2SO_4,

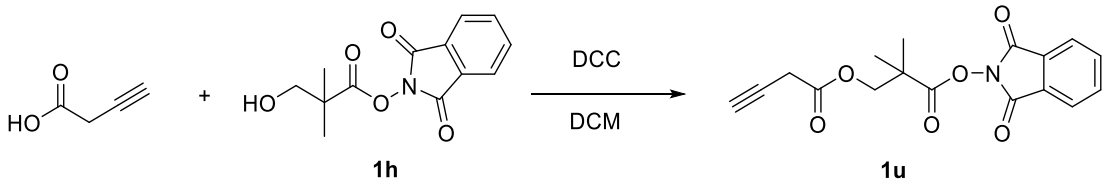
filtered, then concentrated in vacuo to give 3,6,9,12-tetraoxapentadec-14-yn-1-ol (1.39 g, 56%) as a yellow liquid. A solution of compound 3,6,9,12-tetraoxapentadec-14-yn-1-ol (0.57 g, 2.4 mmol) and compound **1ga** (0.91 g, 2.2 mmol) in DCM (10 mL) was added DCC (0.46 g, 2.2 mmol) and DMAP (27 mg, 0.22 mmol) at 0 ºC. The temperature was allowed to rise to 25 ºC and the mixture was stirred at 25 ºC until TLC indicated the complete consumption of **1ga**. The solid was removed by filtration, and the filtrate was concentrated in vacuo. The residue was purified by silica gel column chromatography to give **1gb** (0.99 g,71%) as a colorless oil. TLC R_f = 0.58 (EA: PE = 2:1); IR (thin film) 3274, 2871, 1786, 1745, 1722, 1467, 1368, 1270, 1104, 731cm^{-1}; ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.20 – 8.12 (m, 4H), 7.88 (dd, J = 5.5, 3.1 Hz, 2H), 7.78 (dd, J = 5.4, 3.1 Hz, 2H), 4.53 (s, 2H), 4.52 – 4.46 (m, 2H), 4.17 (d, J = 2.4 Hz, 2H), 3.87 – 3.79 (m, 2H), 3.71 – 3.62 (m, 12H), 2.41 (t, J = 2.4 Hz, 1H), 1.53 (s, 6H); ^{13}C NMR (126 MHz, $CDCl_3$) δ 171.9, 165.9, 165.5, 161.9, 134.9, 134.2, 133.6, 130.0, 129.9, 129.1, 124.1, 79.8, 74.7, 70.8, 70.7, 70.7, 70.5, 70.3, 69.2, 69.2, 64.6, 58.5, 42.8, 22.5; HRMS-ESI (m/z) [M+Na]$^+$ calc'd 648.2057 for $C_{32}H_{35}NNaO_{12}$, found 648.2057.



Synthesis of 1g: A solution of 2,2'-((oxybis(ethane-2,1-diyl))bis(oxy))bis(ethan-1-ol) (13.6 g, 70 mmol) in mixed solvents of 5 mL H_2O and 5 mL THF was added NaOH (465 mg, 11.6 mmol), the mixture was added dropwise a solution of TsCl (2.0 g, 10.4 mmol) in THF (30 mL) at 0 ºC. The temperature was allowed to rise to 25 ºC and the mixture was stirred at 25 ºC for 2h. The reaction mixture was poured into ice water (200 mL) and extracted with dichloromethane (100 mL × 3), and the organic phase was combined. The organic layer was washed with brine, dried over anhydrous Na_2SO_4,

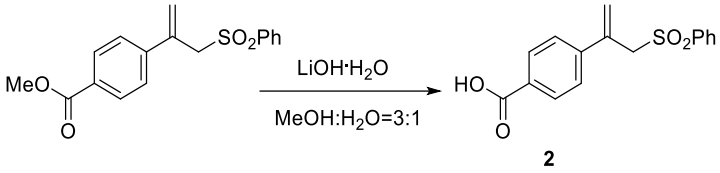
concentrated in vacuo to afford 2-(2-(2-(2-hydroxyethoxy) ethoxy) ethoxy) ethyl 4-methylbenzenesulfonate, which was proceeded to the next step without further purification. To the solution of 2-(2-(2-(2-hydroxyethoxy) ethoxy) ethoxy) ethyl 4-methylbenzenesulfonate in DMF (20 mL) was successively added NaN_3 (862 mg, 13.3 mmol). The mixture was stirred for 13 h at 50 ºC, cooled, and diluted with EtOAc (200 mL). The mixture was washed with water (50 mL × 5), brine, dried with Na_2SO_4, concentrated under reduced pressure, and purified by column chromatography on silica gel to give 2-(2-(2-(2-azidoethoxy) ethoxy) ethoxy) ethan-1-ol (1.16 g, 59%) as a colorless oil. A solution of Biotin (0.81 g, 3.33 mmol), EDCl (0.77 g, 4 mmol), HOBt (0.54 g, 4 mmol), TEA (5 mL) and 2-(2-(2-(2-azidoethoxy)ethoxy)ethoxy)ethan-1-ol (1.10 g, 5 mmol) in DMF (15 mL) was stirred at 25 ºC for 24 h, then diluted with DCM (150 mL). The mixture was washed with a saturated solution of NH_4Cl, brine, dried with Na_2SO_4, concentrated under reduced pressure and purified by column chromatography to afford 2-(2-(2-(2-azidoethoxy)ethoxy)ethoxy)ethyl 5-((3aS,4S,6aR)-2-oxohexahydro-1H-thieno[3,4-d]imidazol-4-yl)pentanoate (1.05 g, 71%) as a colorless oil. A solution of a colorless oil (408 mg, 0.92 mmol) obtained from the previous step, **1gb** (573 mg, 0.92 mmol), sodium ascorbate (36 mg, 0.18 mmol), $CuSO_4 \cdot 5H_2O$ (23 mg, 0.09 mmol) in mixed solvents of 27 mL H_2O and 9 mL tert-butanol under nitrogen atmosphere was heated to 50 ºC and stirred until TLC indicated the complete consumption of **1gb**. The reaction mixture was concentrated and purified by column chromatography to give **1g** (684 mg, 70%) as a colorless oil. TLC $R_f = 0.71$ (MeOH: DCM = 1:10); ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.23 – 8.13 (m, 4H), 7.94 – 7.86 (m, 2H), 7.82 – 7.78 (m, 2H), 7.76 (s, 1H), 5.74 (s, 1H), 5.27 (s, 1H), 4.67 (s, 2H), 4.57 – 4.52 (m, 4H), 4.52 – 4.48 (m, 3H), 4.30 (m, 1H), 4.22 (m, 2H), 3.91 – 3.81 (m, 4H), 3.74 – 3.59 (m, 22H), 3.15 (ddd, J = 8.5, 6.3, 4.5 Hz, 1H), 2.90 (dd, J = 12.8, 5.0 Hz, 1H), 2.73 (d, J = 12.8 Hz, 1H), 2.35 (t, J = 7.5 Hz, 2H), 1.68 (m, 4H), 1.55 (s, 6H), 1.51 – 1.39 (m, 2H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 173.7, 171.9, 165.9, 165.5, 163.5, 161.9, 145.0, 134.9, 134.2, 133.6, 130.0, 129.8, 129.0, 124.1, 124.0, 70.8, 70.7, 70.7, 70.6, 70.6, 70.3, 69.7, 69.6, 69.2, 69.2, 64.6, 64.6, 63.5, 62.0, 60.2, 55.5, 50.8, 50.3, 42.8, 40.6, 33.8, 28.4, 28.3, 24.8, 22.5; IR (thin film) 3369, 2923, 2863, 1786, 1745, 1724,

1467, 1271, 1104, 732cm^{-1}; HRMS-ESI (m/z) $[M+Na]^+$ calc'd 1093.4047 for $C_{50}H_{66}N_6NaO_{18}S$, found 1093.4051.



1h **1u**

Synthesis of 1u: A solution of but-3-ynoic acid (0.17 g, 2 mmol) and **1h** (0.58 g, 2.2 mmol) in DCM (8 mL) was added DCC (0.45 g, 2.2 mmol) at 0 ºC and stirred at room temperature until TLC indicated the complete consumption of but-3-ynoic acid. The solid was removed by filtration, and the filtrate was concentrated in vacuo. The residue was purified by silica gel column chromatography to give **1u** (0.14 g，21%) as a white solid. TLC Rf= 0.81 (Petroleum ether/ EtOAc = 1/1); IR (thin film) 1803, 1772, 1742, 1372, 1184, 1060, 979, 877, 751, 695 cm^{-1}; ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.88 – 7.82 (m, 2H), 7.79 – 7.73 (m, 3H), 5.73 – 5.63 (m, 1H), 5.24 (d, J = 6.5 Hz, 2H), 4.30 (s, 3H), 1.44 (s, 6H); ^{13}C NMR (126 MHz, $CDCl_3$) δ 216.4, 171.8, 165.4, 161.8, 134.8, 129.0, 124.0, 87.5, 79.6, 69.6, 42.7, 22.3; HRMS-ESI (m/z) $[M+Na]^+$ calc'd 352.0792 for $C_{17}H_{15}NNaO_6$, found 352.0794.



2

Synthesis of 2: The substrate was prepared as reported.[2] The synthesis of methyl 4-(3-(phenylsulfonyl)prop-1-en-2-yl)benzoate have been reported previously.[2] The compound (948 mg，3 mmol) was dissolved in 30 mL methanol 10 mL H_2O, and $LiOH\cdot H_2O$ (510 mg，12.15 mmol) was added. After stirring for 3 h at room temperature, the basic reaction mixture was acidified with 1M aqueous HCl solution, then extracted with ethyl acetate (3 × 50 mL). The combined organic layers were washed with brine and dried over anhydrous Na_2SO_4, filtered, then concentrated in vacuo to give **2** (766 mg，84%) as a white solid. IR (thin film) 2985, 2554, 1686, 1608, 1424, 1305, 1141, 715

cm^{-1}; ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.99 (d, J=8.4 Hz, 2H), 7.80 (d, J = 7.3 Hz, 2H), 7.58 (t, J=7.5 Hz, 1H), 7.46 (t, J=7.5 Hz, 2H), 7.38 (d, J=8.4 Hz 2H), 5.72 (s, 1H), 5.37 (s, 1H), 4.30 (s, 2H); ^{13}C NMR (126 MHz, $CDCl_3$) δ 171.3, 144.2, 138.4, 135.9, 134.0, 130.7, 130.5, 129.5, 129.2, 128.8, 126.5, 124.2, 61.9; HRMS-ESI (m/z) $[M+Na]^+$ calc'd 325.0510 for $C_{16}H_{14}NaO_4S$, found 325.0508.

4. DNA Integrity Assessment

DNA ligation and stability tests: DNA double-stranded **DNA 4** (100 pmol in H_2O, 1 equiv), DNA double-stranded **DNA 5** (150 pmol in H_2O, 1.5 equiv), 10x ligation buffer (4 μL), T4 DNA ligase (2 μL) and nuclease-free water (to total volume of 40 μL) were mixed. The reaction mixture was incubated at 16 ℃ overnight before performing gel analysis. a) Ligation reactions were monitored by gel electrophoresis on 4 % agarose gel in a TAE buffer system referenced by a 500 bp DNA ladder (Sangon Biotech). Before gel loading, the DNA samples were mixed with 0.2 volumes of the 6x gel-loading buffer (TianGen). 30 pmol of DNA sample was loaded on gel and the gel was run at 120 V for 30 min. DNA fragments were visualized and analyzed by Bio-Rad ChemiDoc XRS+ Imaging system (Bio-Rad, CA, USA). b) Ligation reactions were monitored by gel electrophoresis on 20 % denatured PAGE in TBE buffer (40 mM Tris-Cl, 45 mM boric acid, 1mM EDTA, pH 8.3) system referenced by a 500 bp DNA ladder (Sangon Biotech). Before gel loading, the DNA samples were mixed with 0.5 volumes of the urea loading buffer. 30 pmol of DNA sample was loaded on gel and the gel was run at 7 mA for 150 min. DNA fragments were visualized and analyzed by Bio-Rad ChemiDoc XRS+ Imaging system (Bio-Rad, CA, USA)

Table S6. DNA sequences used in DNA ligation (5' to 3')

DNA 3	AAATTCTTCTCTATCATA
DNA 4	TATGATAGAGAAGAATTTCG AAATTCTTCTCTATCATA
DNA 5	CTAGTACTTGTTCCTAAGACGAGCT CTCGTCTTAGGAACAAGTACTAGCG

q-PCR test (Amplifiable DNA quantification): The amplifiability of DNA after photocatalytic decarboxylative allylation by qPCR. The results indicate no signs of DNA damage as qPCR amplifiability remains high. Standard curve: The qPCR standard curve was prepared using 108bp ssDNA. The standard DNA was quantified by Nanodrop and for the standard curve samples were prepared by 10-fold gradient dilution to six points. The highest concentration evaluated was 0.3 nM (Table S6). All samples were diluted in water and were run in triplicate.

Samples analysis: The 108bp ssDNA photocatalytic products were purified by ethanol precipitation and used for qPCR. The samples were made as 10-fold gradient dilutions to 6 points. The unmodified DNA sample exposed to photoredox conditions was used as a control. All samples were run in triplicate.

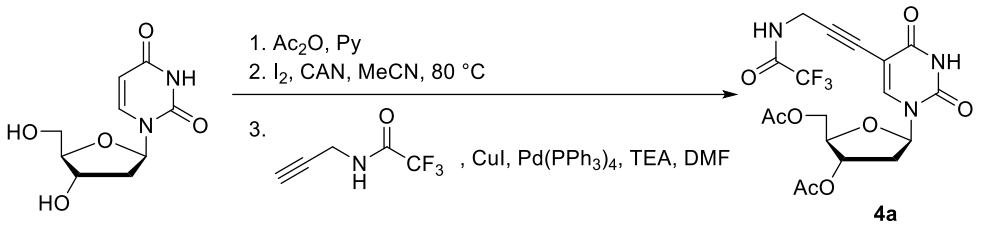
qPCR reaction system and program: The diluted samples were quantified by qPCR using a SYBR Green Master Mix kit (Vazyme) and a QuantStudio™ 12K Flex Real-Time PCR System (Applied Biosystems, USA). All samples were run following the manufacturer's guidelines in a 96-well plate and run-in duplicate in parallel. All samples and standards were subjected to the following PCR cycles: 95 °C heat activation for 5 min followed by 40 cycles of 95 °C denaturation for 15 seconds, and 60 °C annealing/extension for 15 seconds. The standard curve was used to calculate the PCR efficiency.

Table S7. DNA sequences in qPCR test (5' to 3')

Sample for standard curve	ATGCCTATTTCCACCAGCCCGAGGCTATCGA AGAGTTCCCCGTGCCGGCTCTTCACCATCCCGT TTTCCAGCAAGAGTCGTTCACCAGACAGGTGTT ATGGAAGCTGC
qPCR Forward Primer	ATGCCTATTTCCACCAGCCC
qPCR Reverse Primer	GCAGCTTCCATAACACCTG

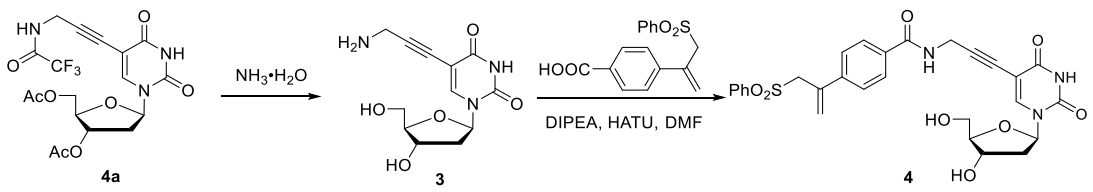
5. Site-Specific DNA Post-Synthetic Modification under Visible-light

5.1 Synthesis of Allyl Sulfone-Modified DNA Building Block 5



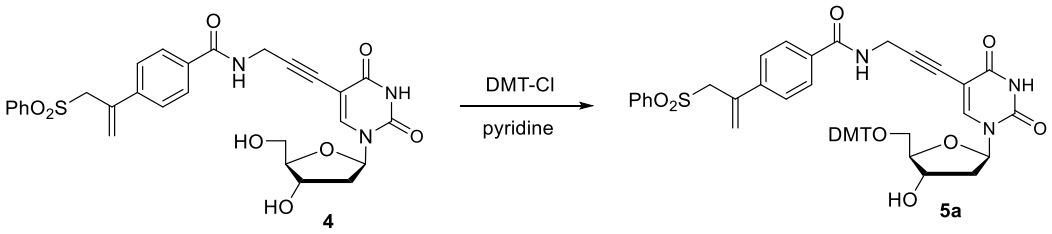
Synthesis of 4a: A solution of 2'-deoxyuridine (1 g, 4.4 mmol) in pyridine (40 mL) was added acetic anhydride (2.5 mL, 26 mmol) at room temperature and stirred until TLC indicated the complete consumption of 2'-deoxyuridine. Most of the pyridine was spun off and 1 M aqueous HCl solution was added to adjust pH to below 3, then extracted with ethyl acetate (3 × 50 mL). The combined organic layers were washed with brine and dried over anhydrous Na_2SO_4, concentrated under reduced pressure, and purified by column chromatography on silica gel to give acetyl-protected 2'-deoxyuridine (1.03 g, 75%) as a white solid. A solution of acetyl-protected 2'-deoxyuridine (0.6881 g, 2.2 mmol), I_2 (0.4551 mg, 1.8 mmol) and CAN (0.7413 g, 1.4 mmol) in acetonitrile (20 mL) was heated to 80 °C and stirred for 1 h. The mixture was cooled to room temperature, then concentrated under reduced pressure. 5% sodium sulfite solution (50 mL) and saturated brine (50 mL) were added. The mixture was extracted with EtOAc. The combined organic layers were washed with brine and dried over anhydrous Na_2SO_4, concentrated under reduced pressure, and purified by column chromatography on silica gel to give ((2R,3R,5R)-3-acetoxy-5-(5-iodo-2,4-dioxo-3,4-dihydropyrimidin-1(2H)-yl)tetrahyd-rofuran-2-yl)methyl acetate (0.72 g, 75%) as a white solid. A solution of ((2R,3R,5R)-3-acetoxy-5-(5-iodo-2,4-dioxo-3,4-dihydropyrimidin-1(2H)-yl)tetrahyd-rofuran-2-yl) methyl acetate (0.2843 g, 0.65 mmol), CuI (0.0132 g, 0.069 mmol) in DMF (10 mL) under nitrogen atmosphere was added 2,2,2-trifluoro-N-(prop-2-yn-1-yl)acetamide (0.3127, 2.07 mmol), $Pd(PPh_3)_4$ (0.0413 g, 0.0345 mmol), TEA (0.2 mL, 1.38 mmol) and stirred for 4 h. The mixture was diluted with EtOAc (100 mL) and

washed with water (50 mL × 5), brine, dried with Na_2SO_4, concentrated under reduced pressure, and purified by column chromatography on silica gel to give **4a** (0.17 g, 55%) as a light yellow solid. R_f = 0.36 (PE:EA=1:1); IR (thin film) 3232, 3083, 1718, 1460, 1236, 1103, 914, 730 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) δ 8.08 (s, 1H), 7.84 (s, 1H), 6.23 (dd, J = 7.9, 5.8 Hz, 1H), 5.21 (dt, J = 6.2, 2.4 Hz, 1H), 4.44 – 4.28 (m, 5H), 2.55 (ddd, J = 14.4, 5.8, 2.4 Hz, 1H), 2.25 (dt, J = 14.3, 7.1 Hz, 1H), 2.13 (s, 3H), 2.10 (s, 3H); ^{13}C NMR (126 MHz, $CDCl_3$) δ 170.6, 170.6, 162.5, 157.2 (d, J = 37.6 Hz), 149.3, 143.0, 115.9 (d, J = 287.5 Hz), 99.4, 88.1, 86.0, 82.9, 75.1, 73.9, 63.7, 38.3, 30.4, 20.9, 20.8; HRMS-ESI (m/z) $[M+Na]^+$ calc'd 484.0944 for $C_{18}H_{18}F_3N_3NaO_8$, found 484.0943.

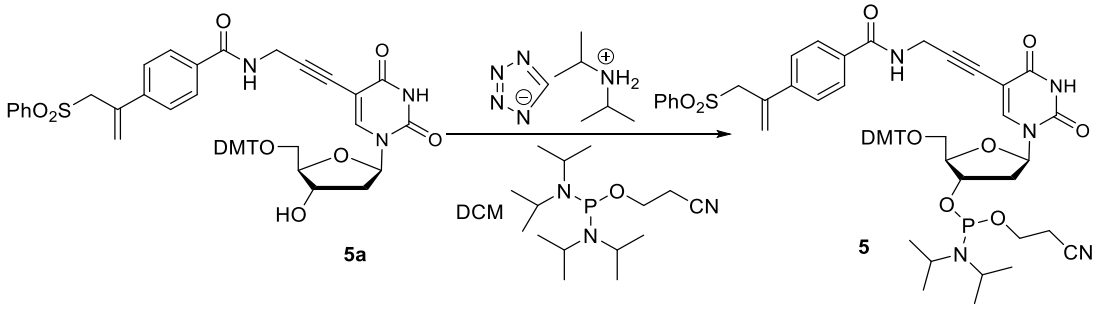


Synthesis of 4: **4a** (461 mg, 1 mmol) was dissolved in ammonia (40 mL) and stirred at room temperature for 24 h. The mixture was concentrated under reduced pressure to give **3** and put into the next step without further purification. A solution of 4-(3-(phenylsulfonyl)prop-1-en-2-yl) benzoic acid (302 mg, 1 mmol), HATU (418 mg, 1.1 mmol), DIPEA (0.52 mL, 3 mmol) in DMF (7 mL) was added dropwise the product of the previous step in DMF solution (10 mL) and stirred overnight. The mixture was diluted with EtOAc (500 mL) and washed with water, brine, dried with Na_2SO_4, concentrated under reduced pressure, and purified by column chromatography on silica gel to give **4** (250 mg, 44%) as a beige solid. R_f = 0.2 (CH_2Cl_2/MeOH = 10/1); IR (thin film) 3389, 3057, 1693, 1447, 1283, 1138, 1083, 777 cm^{-1}; ^1H NMR (500 MHz, CD_3OD) δ 8.32 (s, 1H), 7.77 (d, J = 7.2 Hz, 2H), 7.72 (d, J = 8.6 Hz, 2H), 7.61 (t, J = 7.5 Hz, 1H), 7.52 – 7.46 (m, 2H), 7.44 (d, J = 8.6 Hz, 2H), 6.23 (t, J = 6.6 Hz, 1H), 5.69 (s, 1H), 5.31 (s, 1H), 4.50 (s, 2H), 4.41 – 4.37 (m, 1H), 4.35 (s, 2H), 3.94 (q, J = 3.4 Hz, 1H), 3.84 – 3.70 (m, 2H), 2.35 – 2.17 (m, 2H); ^{13}C NMR (126 MHz, CD_3OD) δ 169.0, 164.7, 151.1, 145.4, 143.6, 139.7, 137.8, 135.0, 134.3, 130.2, 129.7, 128.5, 127.7, 124.0, 99.9,

90.1, 89.1, 87.0, 75.2, 72.0, 62.6, 62.1, 41.7, 31.0; HRMS-ESI (m/z) $[M+Na]^+$ calc'd 588.1417 for $C_{28}H_{27}N_3NaO_8S$, found 588.1414.



Synthesis of 5a: A solution of **4** (1.131 g, 2 mmol), DMT-Cl (928 mg, 2.7 mmol) in ultra-dry pyridine (10 mL) under nitrogen atmosphere was stirred for 48 h. Most of the pyridine was spun off, and the mixture was purified by column chromatography on silica gel (silica gel columns need to be alkalized with TEA beforehand) to give **5a** (577 mg, 33%) as a white solid. R_f = 0.36 (CH_2Cl_2/MeOH = 10/1); IR (thin film) 3064, 2926, 1692, 1507, 1462, 1250, 1177, 1084, 727 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) δ 8.15 (s, 1H), 7.75 (d, J = 7.7 Hz, 2H), 7.61 – 7.48 (m, 3H), 7.47 – 7.35 (m, 4H), 7.24 – 7.31 (m, 9H), 6.81 (d, J = 8.3 Hz, 4H), 6.28 (s, 1H), 5.62 (s, 1H), 5.23 (s, 1H), 4.53 (s, 1H), 4.24 (s, 2H), 4.17 – 4.02 (m, 4H), 3.71 (s, 7H), 2.54 – 2.46 (m, 1H), 2.35 – 2.19 (m, 1H); ^{13}C NMR (126 MHz, $CDCl_3$) δ 166.5, 162.8, 158.7, 149.4, 144.7, 143.4, 141.9, 138.2, 135.8, 135.7, 135.6, 134.0, 133.2, 130.1, 129.2, 128.7, 128.2, 128.0, 127.5, 127.1, 126.4, 123.4, 113.5, 99.5, 89.8, 87.2, 86.8, 86.1, 74.4, 72.3, 63.7, 61.9, 55.4, 41.7, 30.7; HRMS-ESI (m/z) $[M+NH_4]^+$ calc'd 885.3169 for $C_{49}H_{49}N_4O_{10}S$, found 885.3164.



Synthesis of 5: A solution of tetrazole (156 mg, 2.22 mmol) in diisopropylamine (0.5 mL, 3.56 mmol) was stirred for 20 min and concentrated under reduced pressure and put into the next step without further purification. A solution of **5a** (434 mg, 0.5 mmol)

and the product of the previous step (85 mg, 0.5 mmol) in ultra-dry DCM (10 mL) under nitrogen atmosphere was added 3-((bis(diisopropylamino) phosphaneyl)oxy)propanenitrile (226 mg, 0.75 mmol) and stirred for 24 h. The mixture was concentrated under reduced pressure and purified by column chromatography on silica gel (silica gel columns need to be alkalized with TEA beforehand) to give **5** (316 mg, 58%) as a white solid. R_f = 0.38 (CH_2Cl_2/MeOH = 20/1); IR (thin film) 2965, 2928, 1697, 1533, 1447, 1251, 1179, 1083, 729 cm^{-1}; 1H NMR (500 MHz, $CDCl_3$) δ 8.24 (d, J = 20.8 Hz, 1H), 7.77 (d, J = 7.9 Hz, 2H), 7.58 – 7.40 (m, 7H), 7.38 – 7.24 (m, 9H), 6.83 (d, J = 8.4 Hz, 4H), 6.30 (q, J = 7.4 Hz, 1H), 5.65 (s, 1H), 5.29 (s, 1H), 4.65 – 4.59 (m, 1H), 4.26 (s, 2H), 4.23 – 3.99 (m, 4H), 3.74 (s, 7H), 3.62 – 3.51 (m, 2H), 3.49 – 3.29 (m, 2H), 2.62 (s, J = 10.0 Hz, 1H), 2.65 – 2.54 (m, 1H), 2.44 (s, J = 10.0 Hz, 1H), 2.40 – 2.30 (m, 1H), 1.19 – 1.13 (m, 9H), 1.06 (d, J = 6.8 Hz, 3H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 166.2, 162.2, 158.8, 149.2, 144.6, 143.3, 141.9, 138.3, 135.8, 135.6, 134.0, 133.2, 130.1, 129.1, 128.7, 128.2, 128.1, 127.4, 127.2, 126.3, 123.4, 117.6, 113.5, 99.6, 89.5, 87.2, 86.2, 86.0, 74.5, 73.5, 63.2, 61.9, 58.3, 55.4, 43.4, 30.7, 24.7, 20.4; HRMS-ESI (m/z) $[M+Na]^+$ calc'd 1090.3802 for $C_{58}H_{62}N_5NaO_{11}PS$, found 1090.3703.

5.2 Procedures for Post-synthetic Modification of DNA

To the DNA oligonucleotides bearing allyl-sulfone (1 nmol), was added 10 μL of **1g** (100 mM in MeCN), 1 μL of $Ru(bpy)_3Cl_2$ (10 mM in H_2O) and 10 μL of ascorbates (500 mM in H_2O). The mixture was added 40 μL MeCN and 39 μL H_2O. The eppendorf tube was sealed and exposed to blue LEDs (two 4 W LED light bulbs 10 cm away from the vial, 468 nm peak wavelength, 25 nm spectral half-wave width, composed of 55-65 LED units each with 60 mW, 3 V, 20 mA) for 5 min at room temperature. The mixture was added 5 M NaCl solution (10 % by volume) and cold ethanol (2.5 times by volume, ethanol stored at -20 ℃). The mixture was stored in a -80 ℃ freezer for 50 minutes. Centrifuge the sample for 30 minutes at 4 ℃ in a microcentrifuge at 10000 rpm. The above supernatant was discarded and the precipitate was cooled in liquid nitrogen and then placed on a lyophilizer.

Table S8. DNA sequences used in post-synthetic modification (5' to 3')

DNA 7	CTGTTdU*TTGAGTCACGA
DNA 8	dU*ATGATAGAGAAGAATTTCG
DNA 9	dU*ATGATAGAGAAGAATTTCG ATACTATCTCTTCTTAAAGC

PCR reactions include primers (10 μM each) were subjected to the following PCR cycles: 94 °C heat activation for 2 min followed by 30 cycles of 94 °C denaturation for 30 seconds, 55 °C annealing for 30 seconds and 72 °C extension for 20 seconds.

Table S9: DNA sequences in long enzymatically amplified DNA strands (5' to 3')

DNA template	CAATCCCATCATCTTCGCATCCGATGGCTTCCTGGAGCTG ACCGAGTATTCCAGAGAGGAGATCCTGGGCCGCAATGGC CGCTTTCTGCAGGGACCAGAGACAGACCAGGCCACAGT GCAGAAGATTCGCGATGCCATTAGAGATCAGCGCGAGAT TACCGTGCAGC
DNA 13	dU*CAATCCCATCATCTTCGC

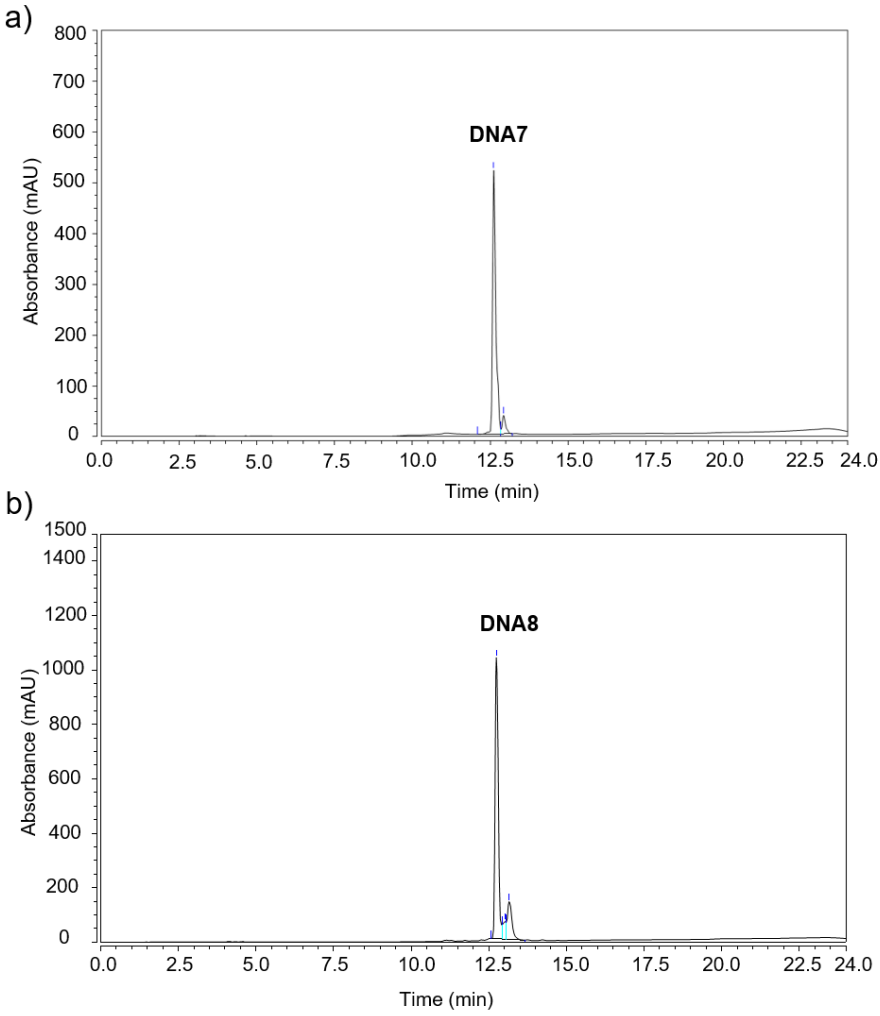


Figure S6. HPLC analysis of purified **DNA 7** and **DNA 8**

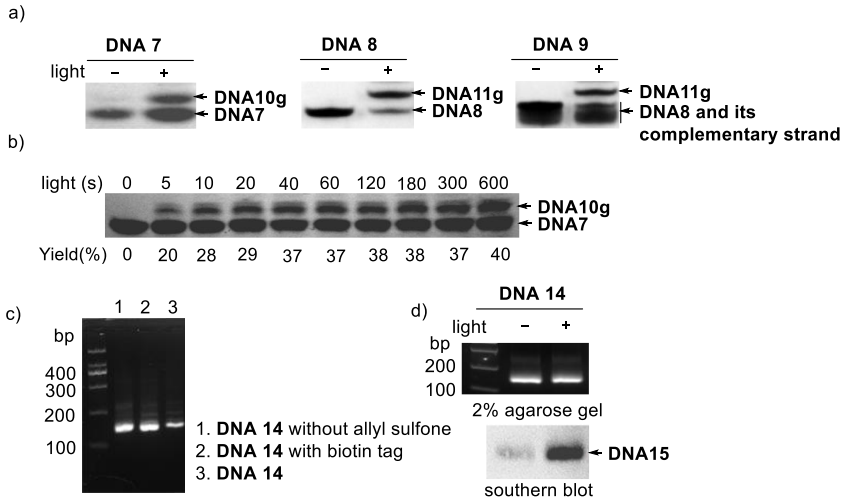


Figure S7. Characterizations of site-specific DNA post-synthetic modification. a) In-urea-PAGE-gel analysis of site-specific DNA post-synthetic modification of **DNA 7**, **8** and **9** upon light irradiation, leading to the formation of **DNA 10g**, **11g, and 12.** During urea-PAGE analysis, the modification products **DNA 12** dissociated into **DNA 11g** and its complementary strand; b) Time course of site-specific DNA post-synthetic modification of **DNA 7** under light irradiation to generate **DNA 10g** through in urea-PAGE-gel analysis. The yield was roughly calculated by the relative grayscale of the bands in-gel analysis, and the low yield was attributed to the use of NaVc instead of VcH in this photocatalytic reaction; c) In-agarose-gel analysis of enzymatically amplified long-chain DNA. **DNA14** without allyl sulfone was obtained by enzymatic amplification of the DNA template using unmodified primers; **DNA14** with biotin tag was obtained by enzymatic amplification of the DNA template using biotin-modified primers; d) In-gel analysis of site-specific DNA post-synthetic modification of **DNA 14** upon light irradiation to generate **DNA 15**.

6. Mass Spectra Characterization of On-DNA Transformation Products

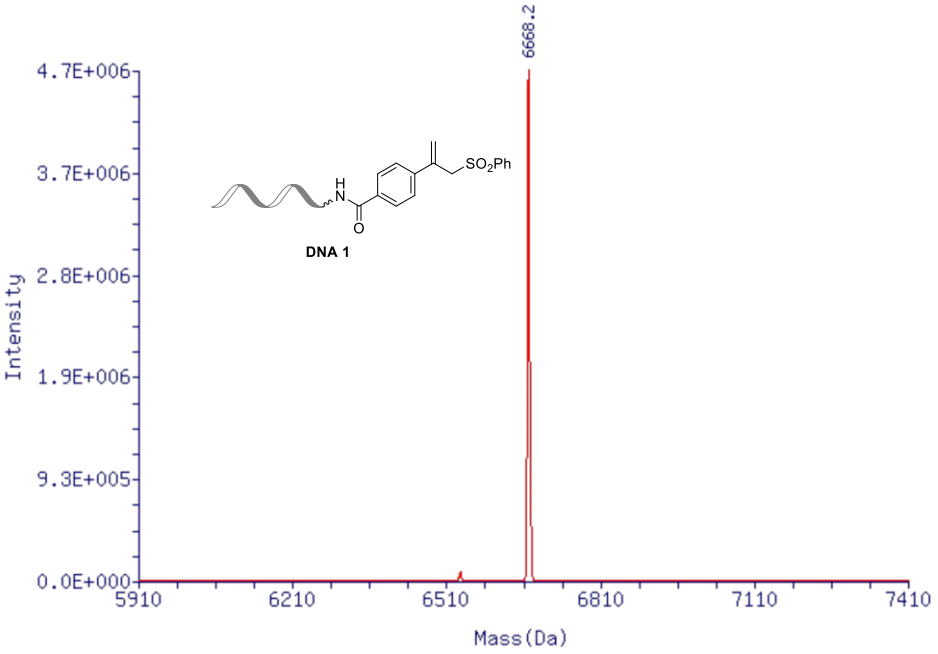


Figure S8. Deconvoluted mass spectrum of **DNA 1**, expected Mass: 6667.3; observed Mass:6668.2.

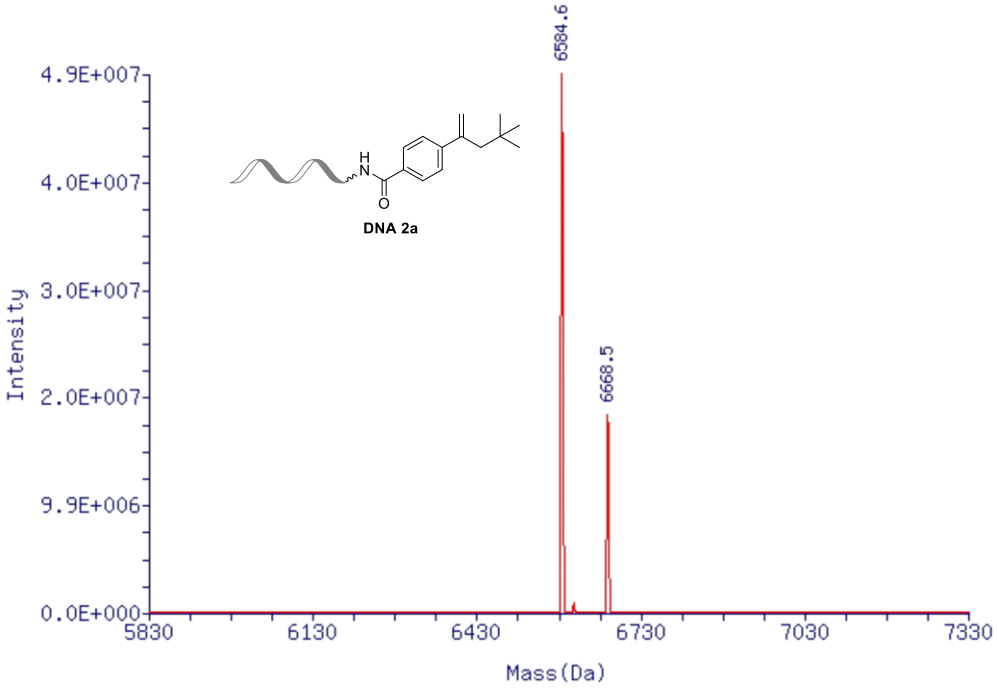


Figure S9. Deconvoluted mass spectrum of **DNA 2a**, expected Mass: 6583.4; observed Mass: 6584.6. The mass peak of 6668.5 is attributed to the presence of unreacted **DNA 1**.

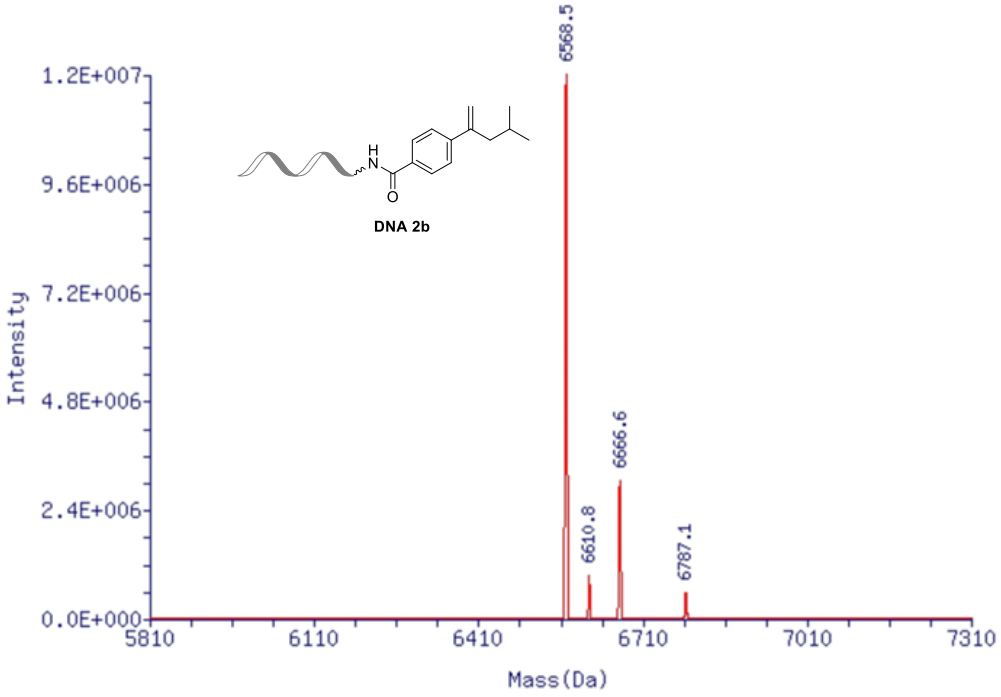


Figure S10. Deconvoluted mass spectrum of **DNA 2b**, expected Mass: 6569,4; observed Mass: 6568.5. The mass peak of 6666.6 is attributed to the presence of unreacted **DNA 1**. The mass spectral peaks of 6610.8 and 6787.1 may be attributed to the byproducts formed through transformation at the alkene sites.

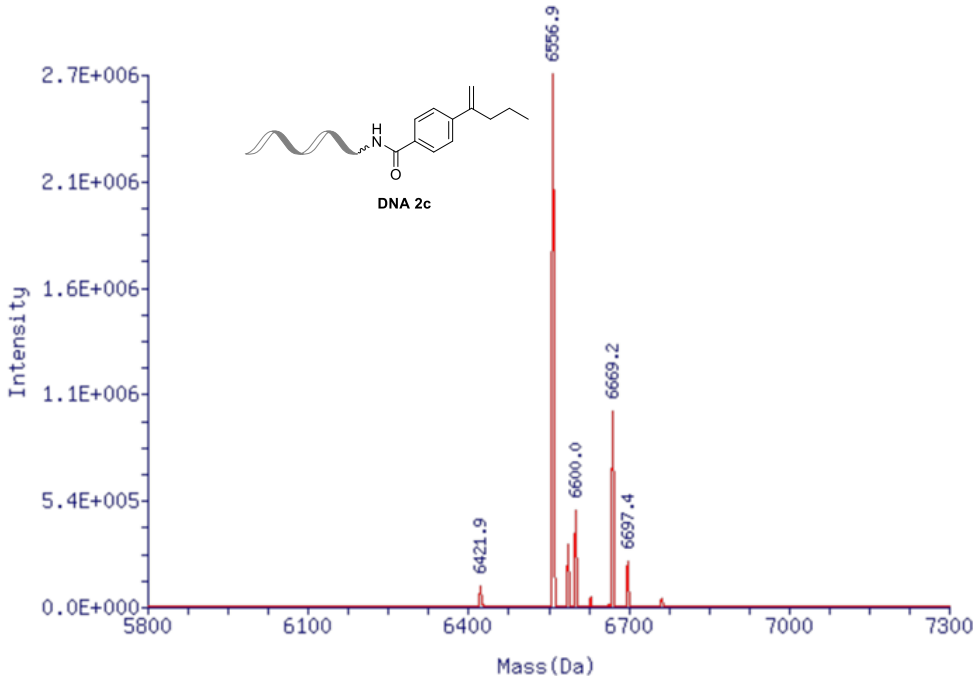


Figure S11. Deconvoluted mass spectrum of **DNA 2c**, expected Mass: 6555.3; observed Mass: 6556.9. The mass peak of 6669.2 is attributed to the presence of unreacted **DNA 1**.

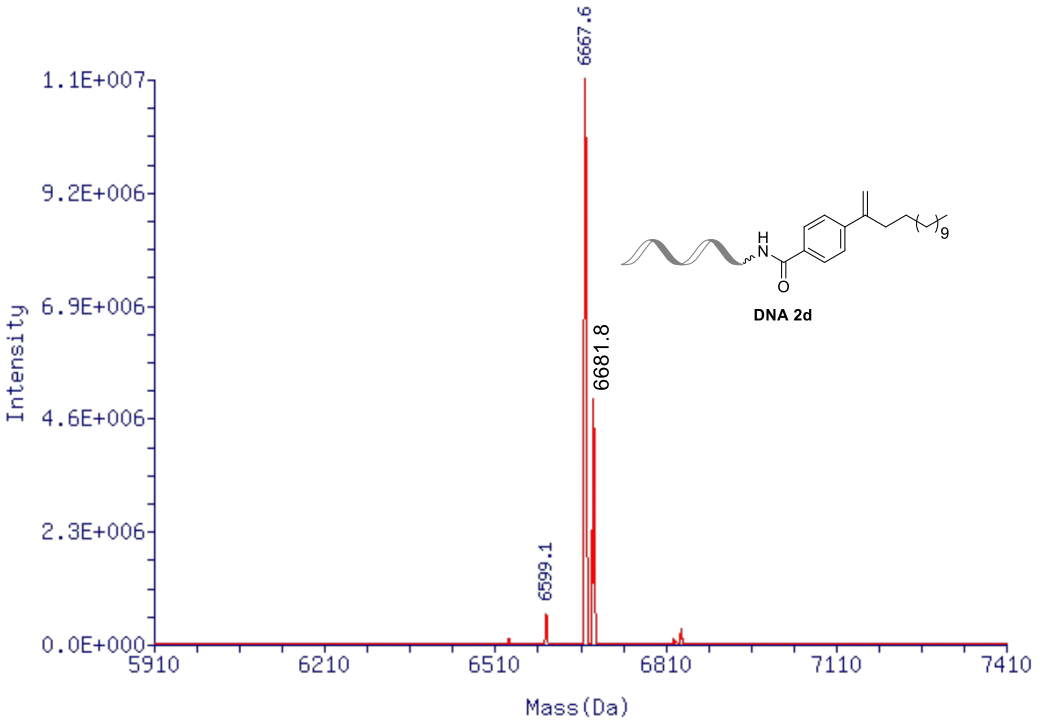


Figure S12. Deconvoluted mass spectrum of **DNA 2d**, expected Mass: 6681.5; observed Mass: 6681.8. The mass peak of 6667.6 is attributed to the presence of unreacted **DNA 1**.

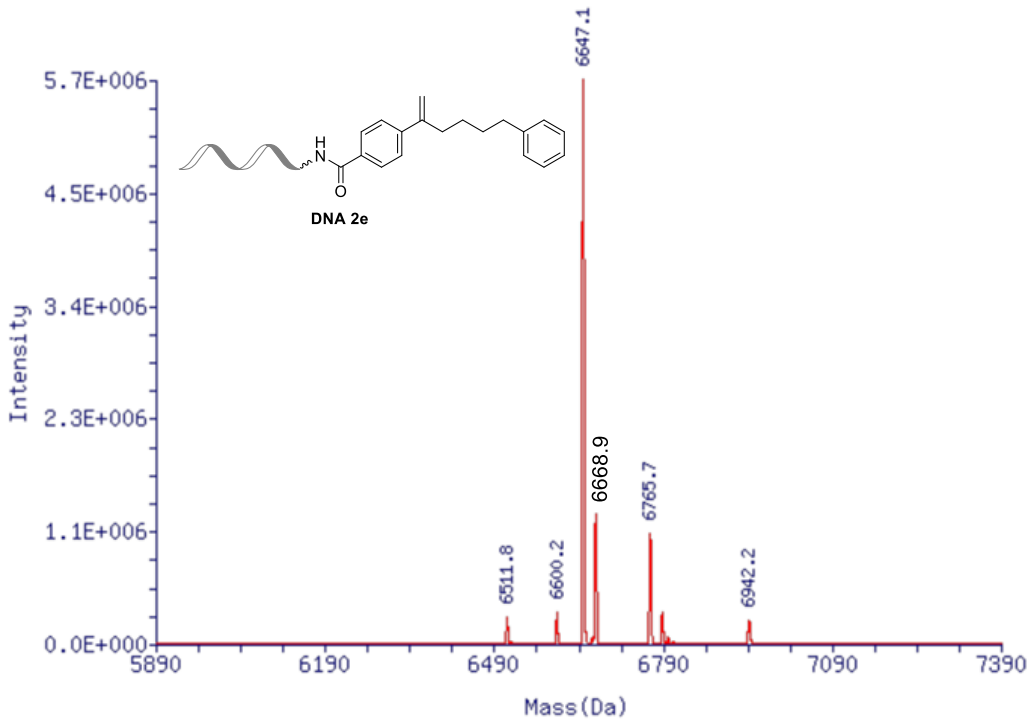


Figure S13. Deconvoluted mass spectrum of **DNA 2e**, expected Mass: 6645.4; observed Mass: 6647.1. The mass peak of 6668.9 is attributed to the presence of unreacted **DNA 1**. The mass spectral peaks of 6765.7 may be attributed to the byproducts formed through transformation at the alkene sites.

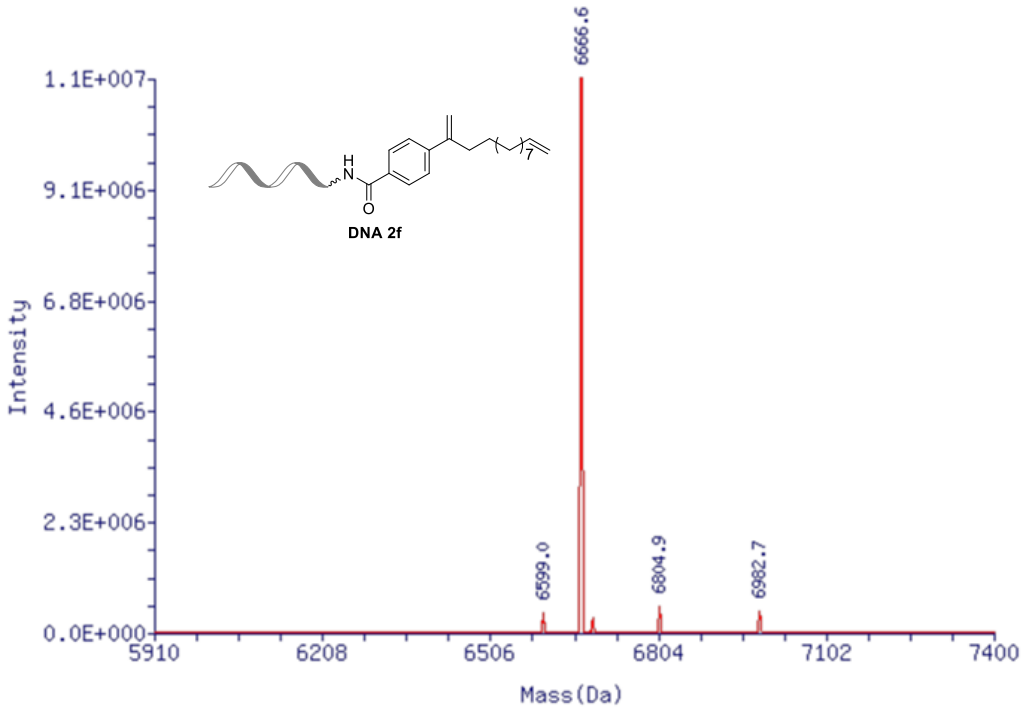


Figure S14. Deconvoluted mass spectrum of **DNA 2f**, expected Mass: 6665.4; observed Mass: 6666.6. Due to almost the same molecular weights of **DNA 1** and **DNA 2f**, the yield of **DNA 2f** was determined by HPLC analysis.

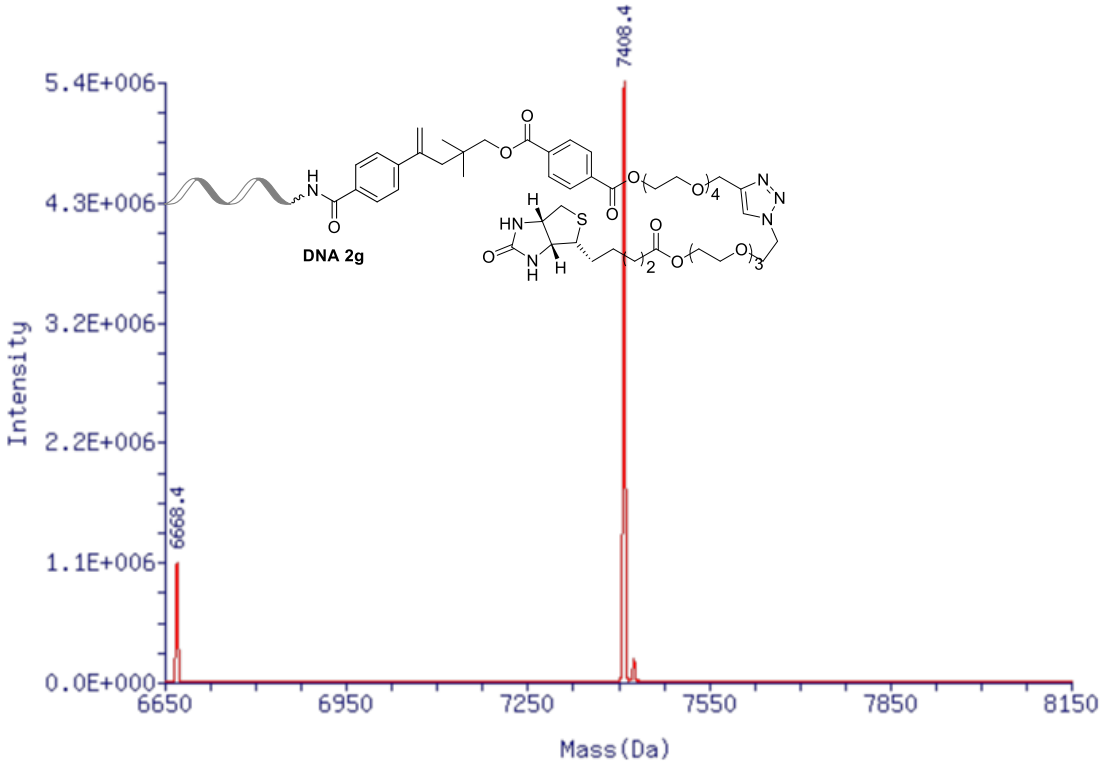


Figure S15. Deconvoluted mass spectrum of **DNA 2g**, expected Mass: 7406.7; observed Mass: 7408.4. The mass peak of 6668.4 is attributed to the presence of unreacted **DNA 1**.

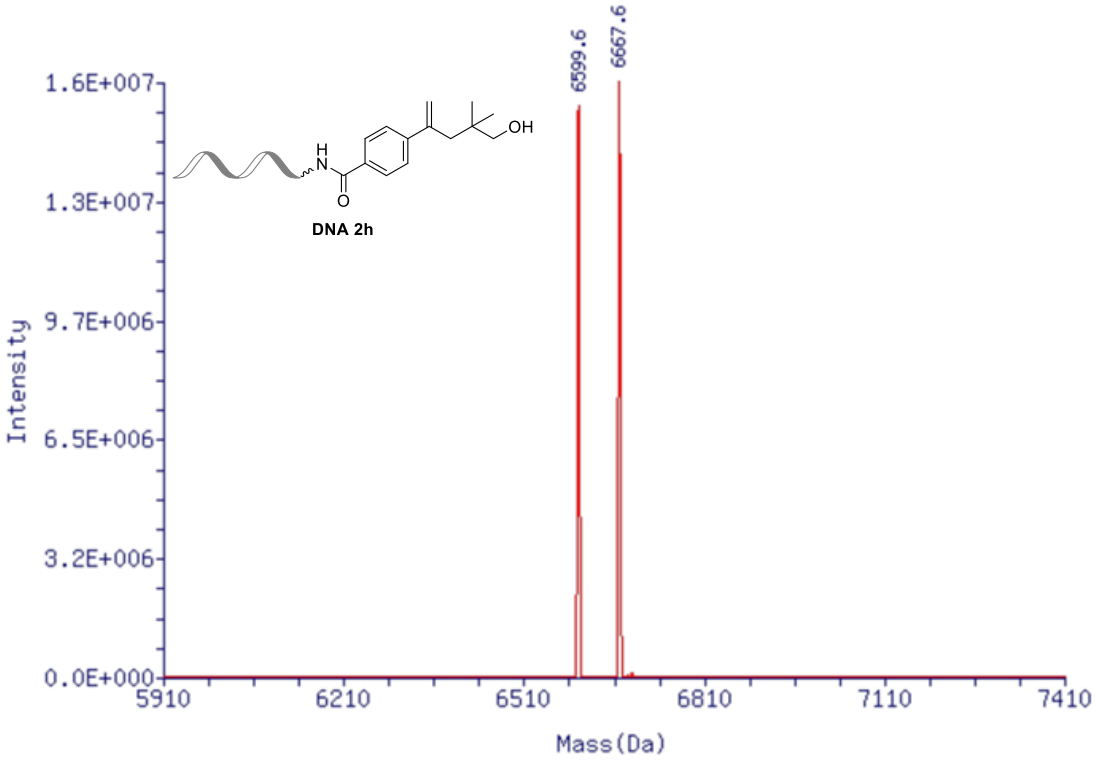


Figure S16. Deconvoluted mass spectrum of **DNA 2h**, expected Mass: 6599.4; observed Mass: 6599.6. The mass peak of 6667.6 is attributed to the presence of unreacted **DNA 1**.

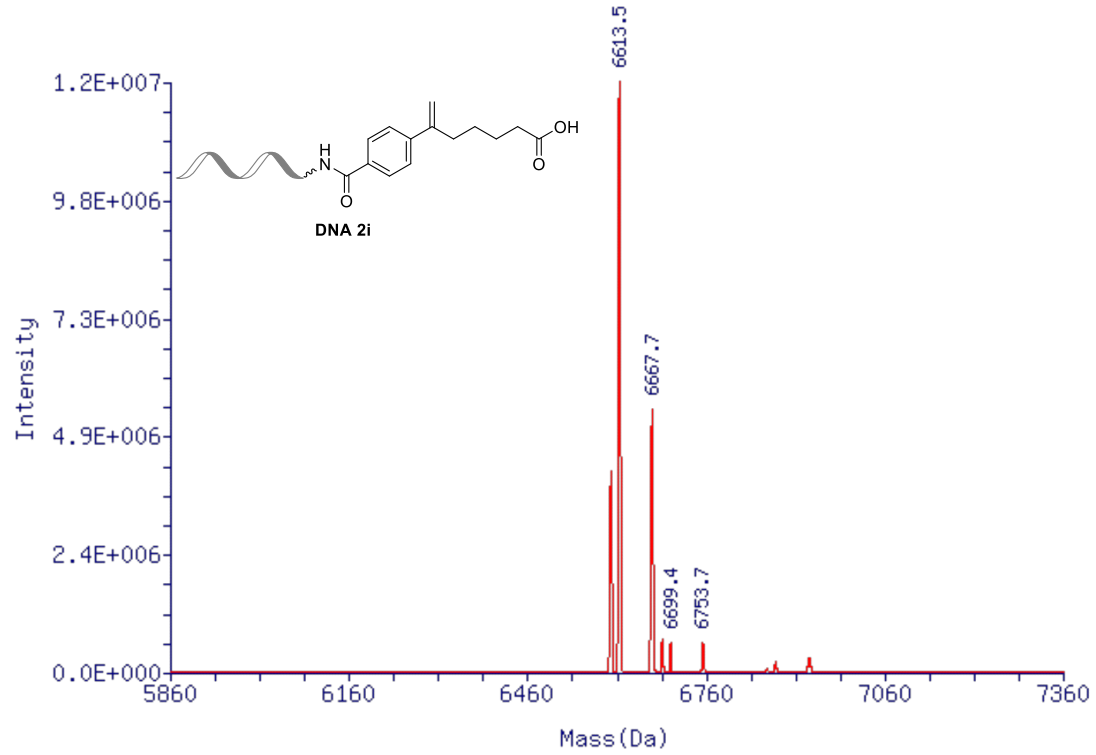


Figure S17. Deconvoluted mass spectrum of **DNA 2i**, expected Mass: 6613.3; observed Mass: 6613.5. The mass peak of 6667.7 is attributed to the presence of unreacted **DNA 1**.

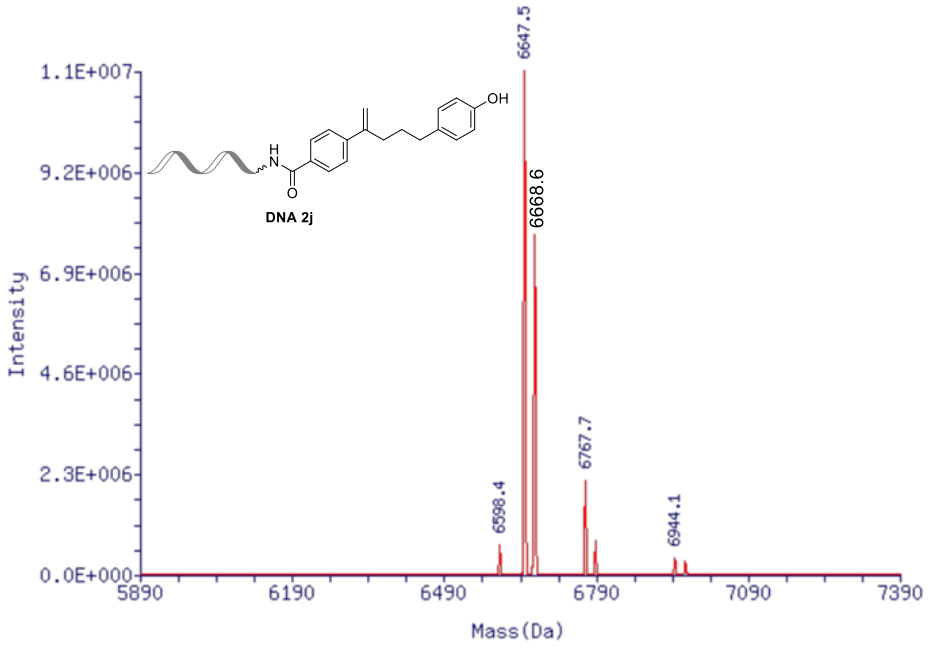


Figure S18. Deconvoluted mass spectrum of **DNA 2j**, expected Mass: 6647.4; observed Mass: 6647.5. The mass peak of 6668.6 is attributed to the presence of unreacted **DNA 1**. The mass spectral peaks of 6767.7 may be attributed to the byproducts formed through transformation at the alkene sites.

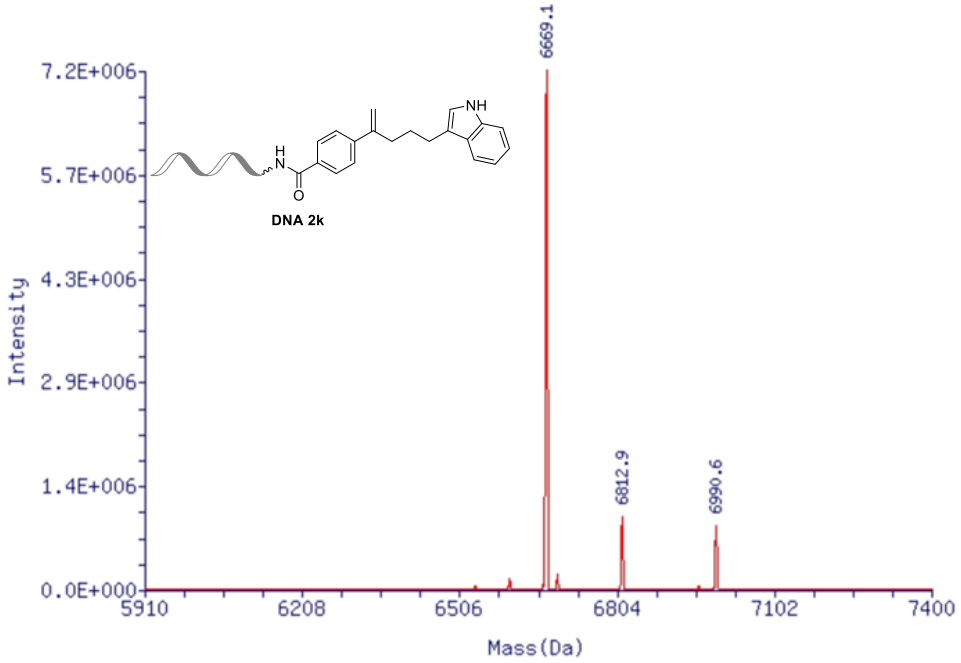


Figure S19. Deconvoluted mass spectrum of **DNA 2k**, expected Mass: 6670.4; observed Mass: 6669.1. Due to almost the same molecular weights of **DNA 1** and **DNA 2k**, the yield of **DNA 2k** was determined by HPLC analysis. The mass spectral peaks of 6812.9 may be attributed to the byproducts formed through transformation at the alkene sites.

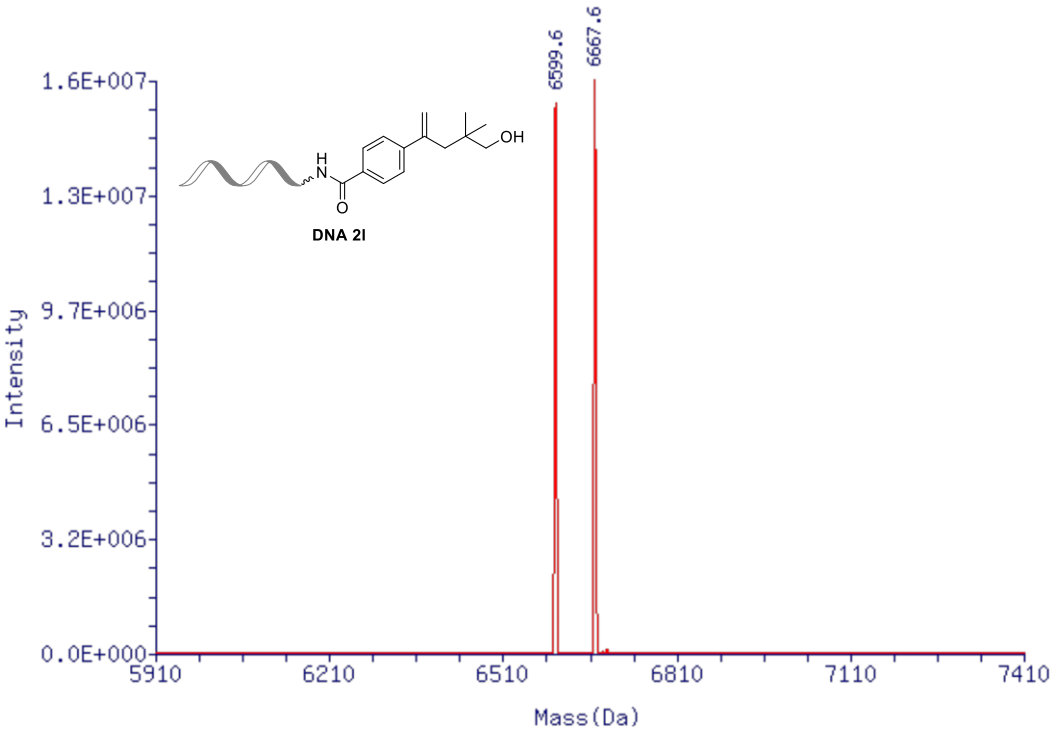


Figure S20. Deconvoluted mass spectrum of **DNA 2l**, expected Mass: 6677.3; observed Mass: 6599.6. The mass peak of 6667.6 is attributed to the presence of unreacted **DNA 1**.

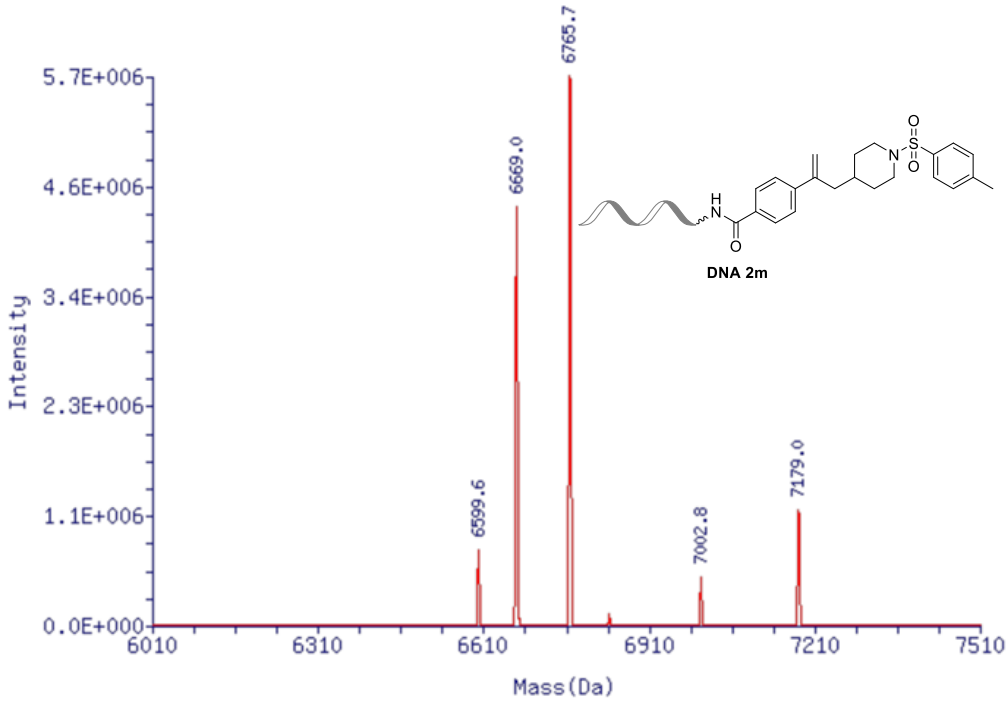


Figure S21. Deconvoluted mass spectrum of **DNA 2m**, expected Mass: 6764.4; observed Mass: 6765.7. The mass peak of 6669.0 is attributed to the presence of unreacted **DNA 1**. The mass spectral peaks of 7002.8 may be attributed to the byproducts formed through a transformation at the alkene sites.

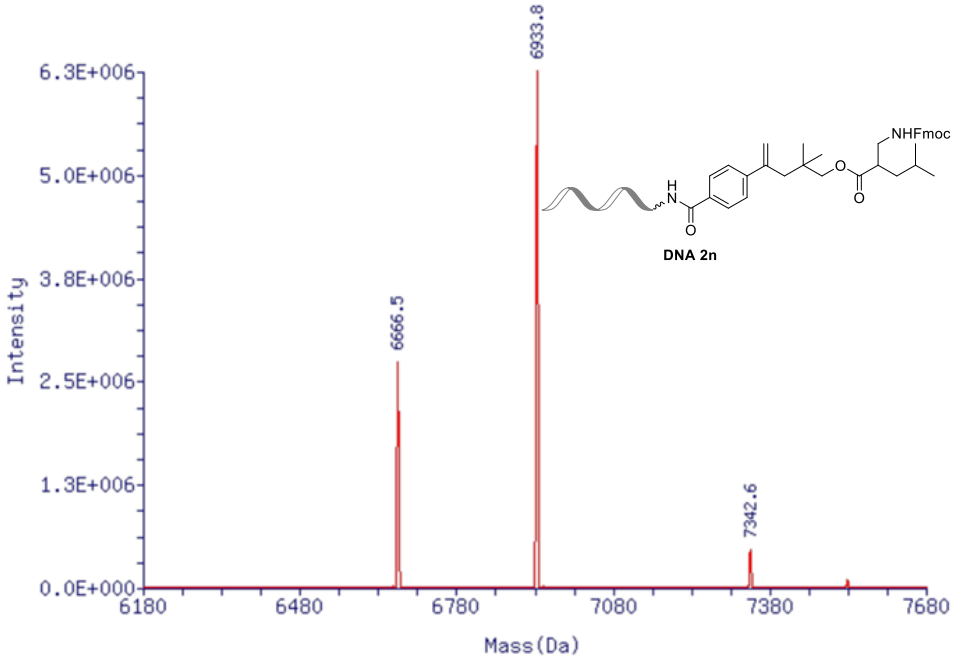


Figure S22. Deconvoluted mass spectrum of **DNA 2n**, expected Mass: 6934.5; observed Mass: 6933.8. The mass peak of 6666.5 is attributed to the presence of unreacted **DNA 1**. The mass spectral peaks of 7342.6 may be attributed to the byproducts formed through transformation at the alkene sites.

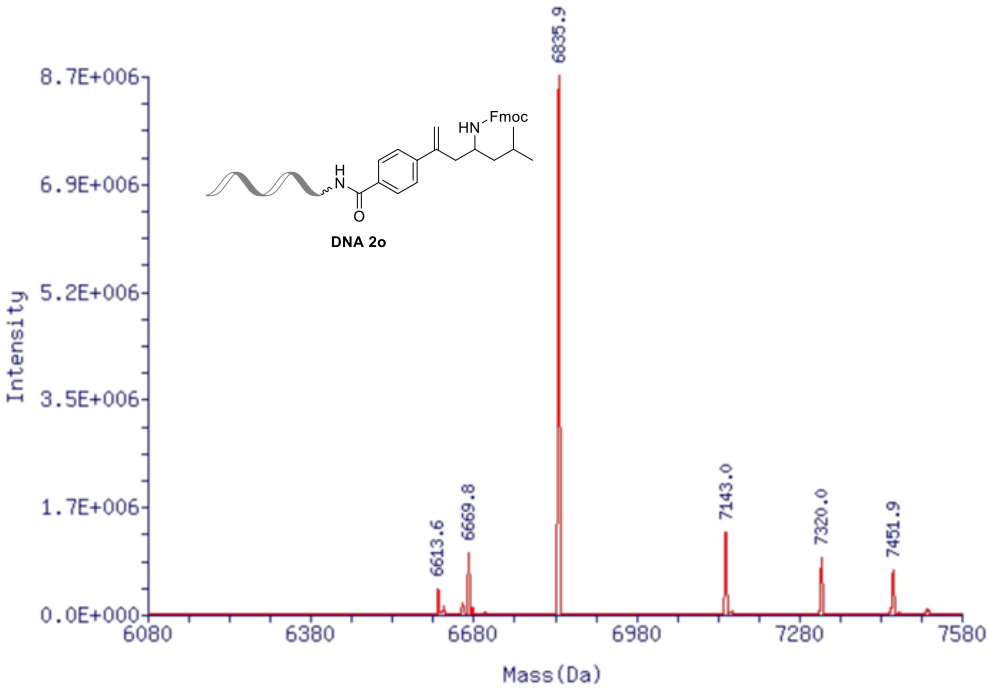


Figure S23. Deconvoluted mass spectrum of **DNA 2o**, expected Mass: 6834.5; observed Mass: 6835.9. The mass spectral peaks of 7143.0 and 7451.9 may be attributed to the byproducts formed through transformation at the alkene sites.

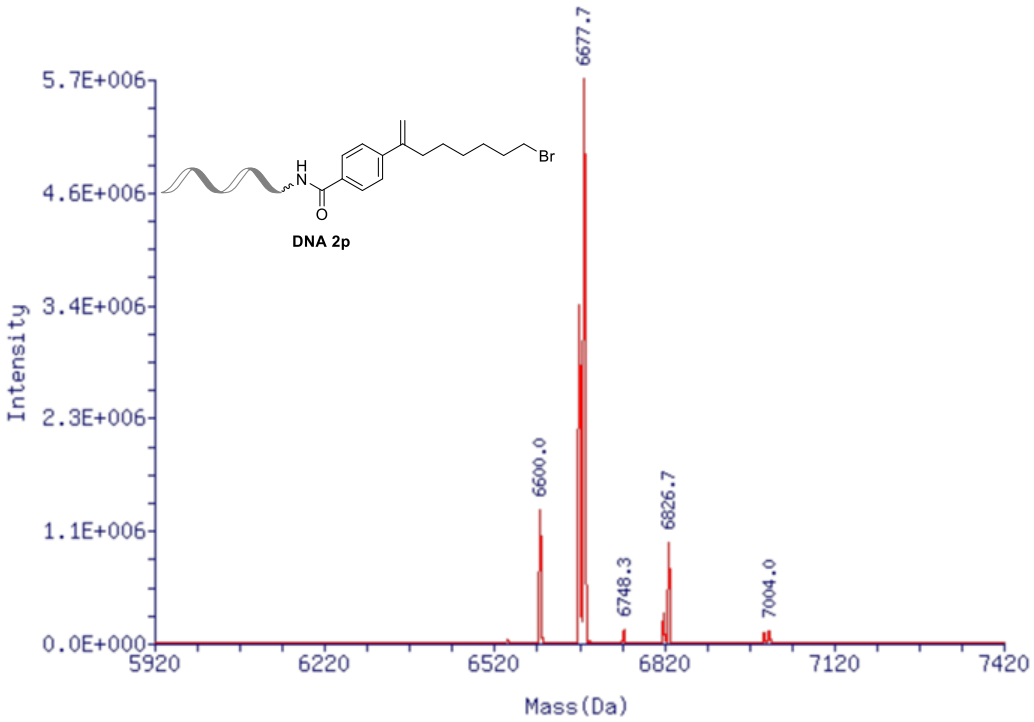


Figure S24. Deconvoluted mass spectrum of **DNA 2p**, expected Mass: 6675.3; observed Mass: 6677.7. The mass spectral peaks of 6826.7 may be attributed to the byproducts formed through transformation at the alkene sites.

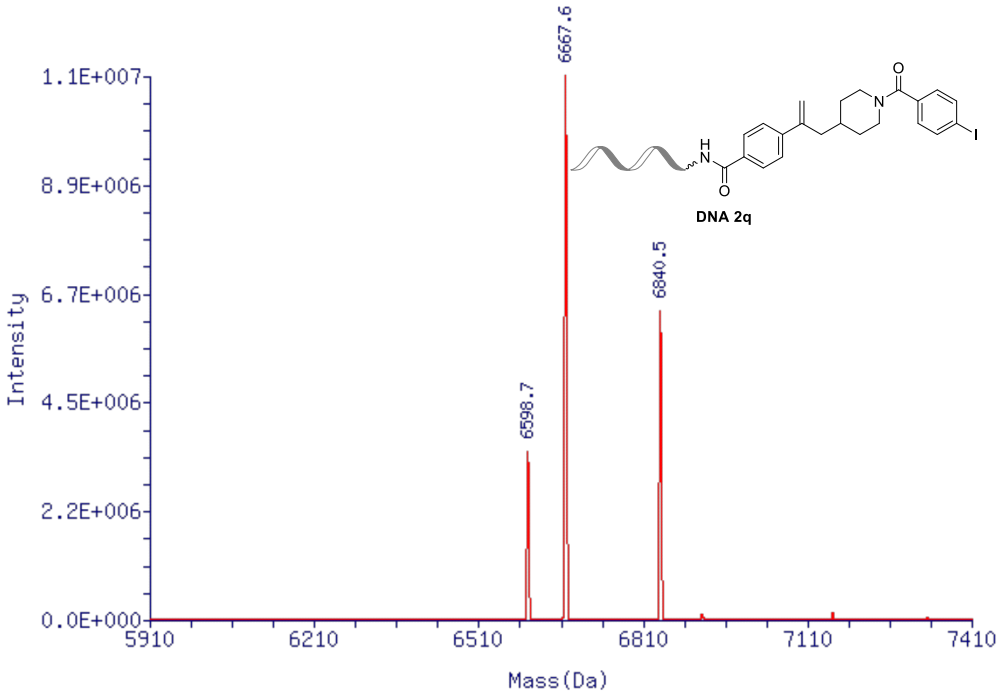


Figure S25. Deconvoluted mass spectrum of **DNA 2q**, expected Mass: 6840.3; observed Mass: 6840.5. The mass peak of 6667.6 is attributed to the presence of unreacted **DNA 1**.

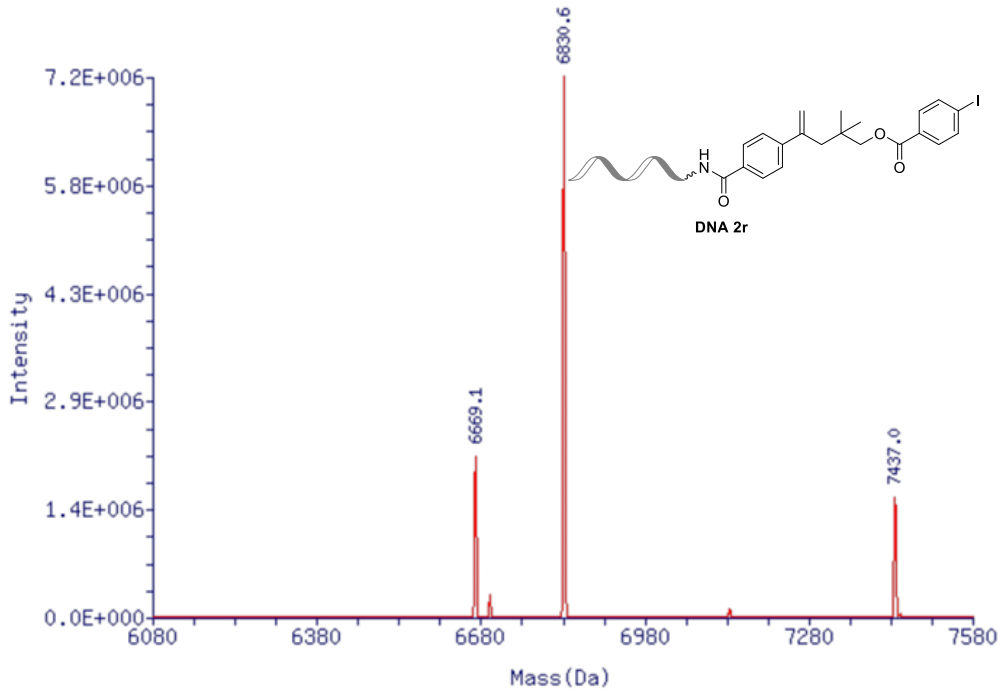


Figure S26. Deconvoluted mass spectrum of **DNA 2r**, expected Mass: 6829.3; observed Mass: 6830.6. The mass spectral peaks of 7437.0 may be attributed to the byproducts formed through transformation at the alkene sites.

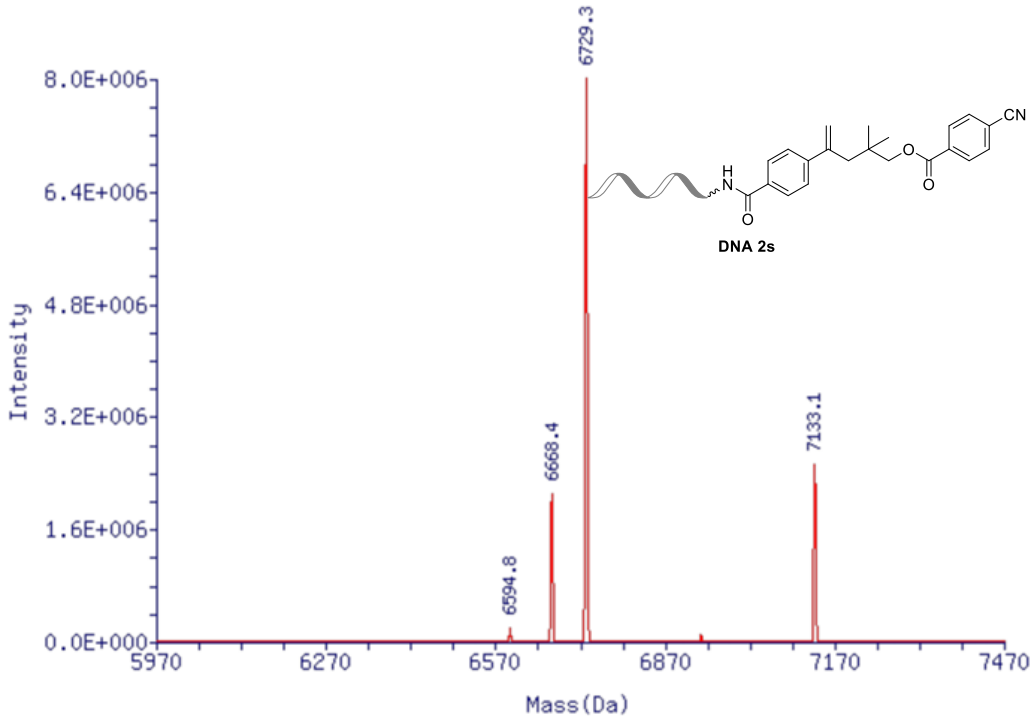


Figure S27. Deconvoluted mass spectrum of **DNA 2s**, expected Mass: 6728.4; observed Mass: 6729.3. The mass spectral peaks of 7133.1 may be attributed to the byproducts formed through transformation at the alkene sites.

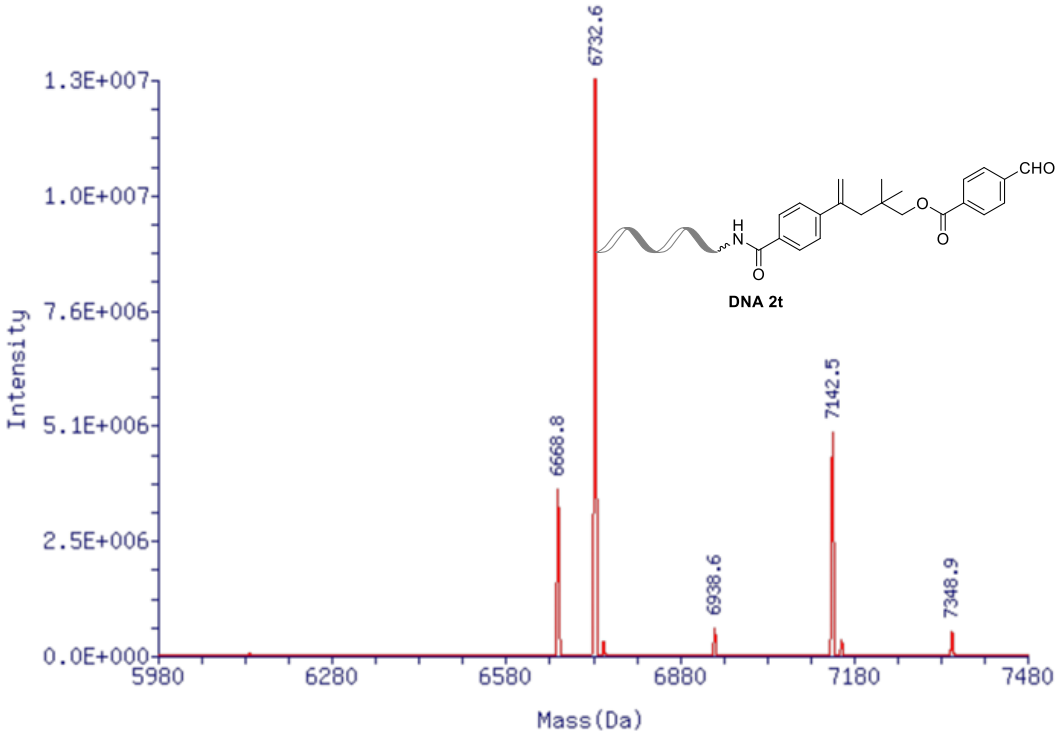


Figure S28. Deconvoluted mass spectrum of **DNA 2t**, expected Mass: 6731.4; observed Mass: 6732.6. The mass spectral peaks of 6938.6, 7142.5, and 7348.9 may be attributed to the byproducts formed through transformation at the alkene sites.

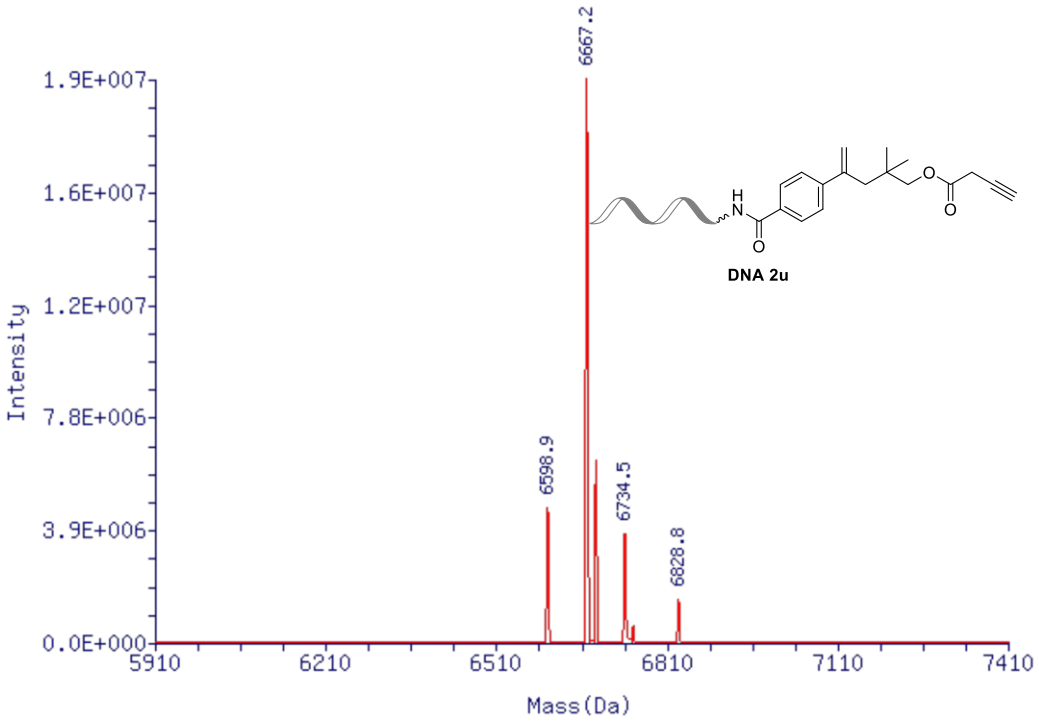


Figure S29. Deconvoluted mass spectrum of **DNA 2u**, expected Mass: 6665.4; observed Mass: 6667.2. The mass peak of 6667.2 is attributed to the presence of unreacted **DNA 1** and **DNA 2u**.

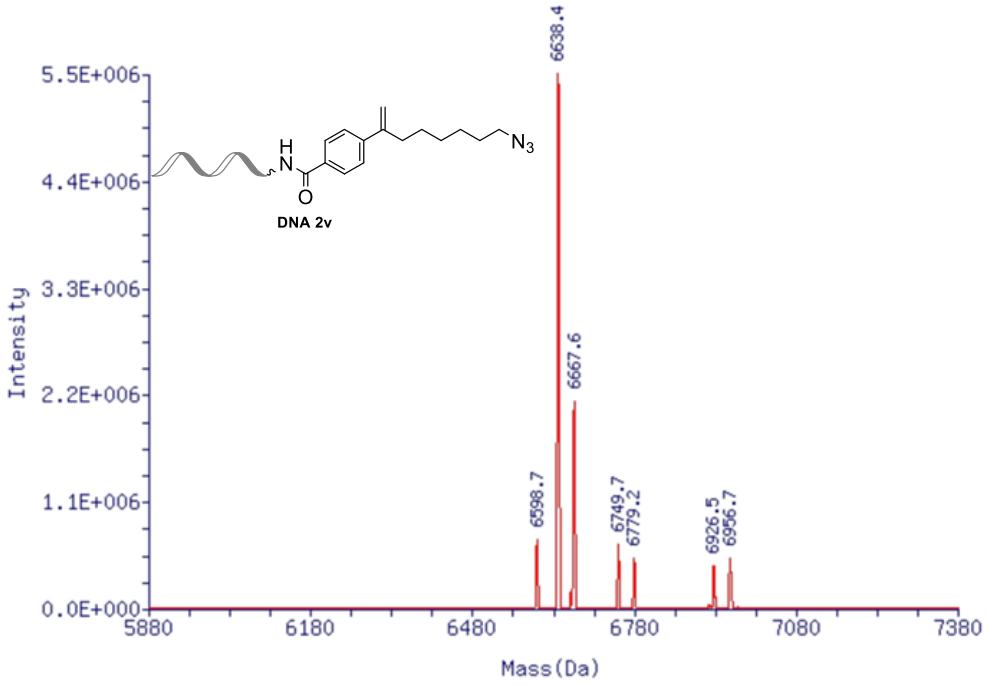


Figure S30. Deconvoluted mass spectrum of **DNA 2v**, expected Mass: 6638.4; observed Mass: 6638.4. The mass peak of 6667.6 is attributed to the presence of unreacted **DNA 1**. The mass spectral peaks of 6749.7 may be attributed to the byproducts formed through transformation at the alkene sites.

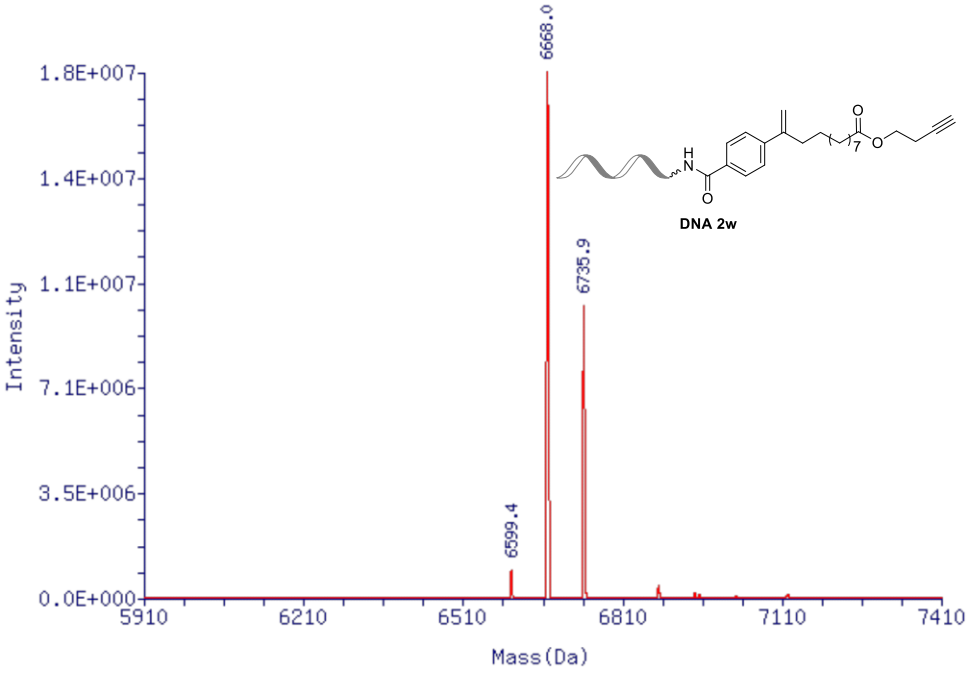


Figure S31. Deconvoluted mass spectrum of **DNA 2w**, expected Mass: 6735.5; observed Mass: 6735.9. The mass peak of 6668.0 is attributed to the presence of unreacted **DNA 1**.

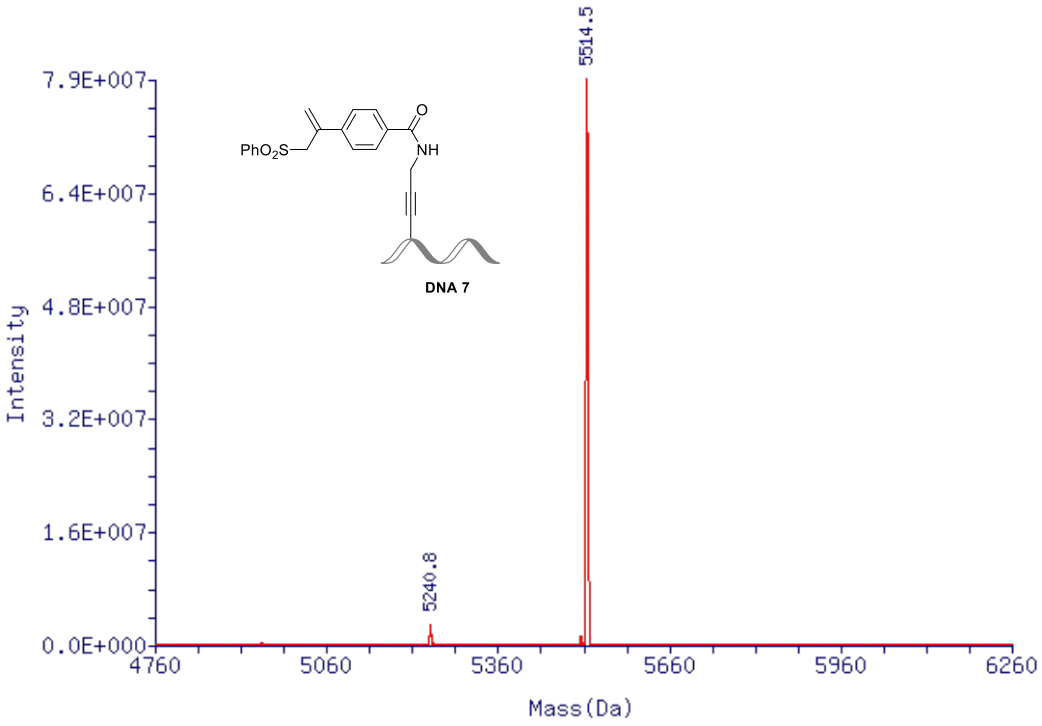


Figure S32. Deconvoluted mass spectrum of **DNA 7**, expected Mass: 5514.2; observed Mass: 5514.5.

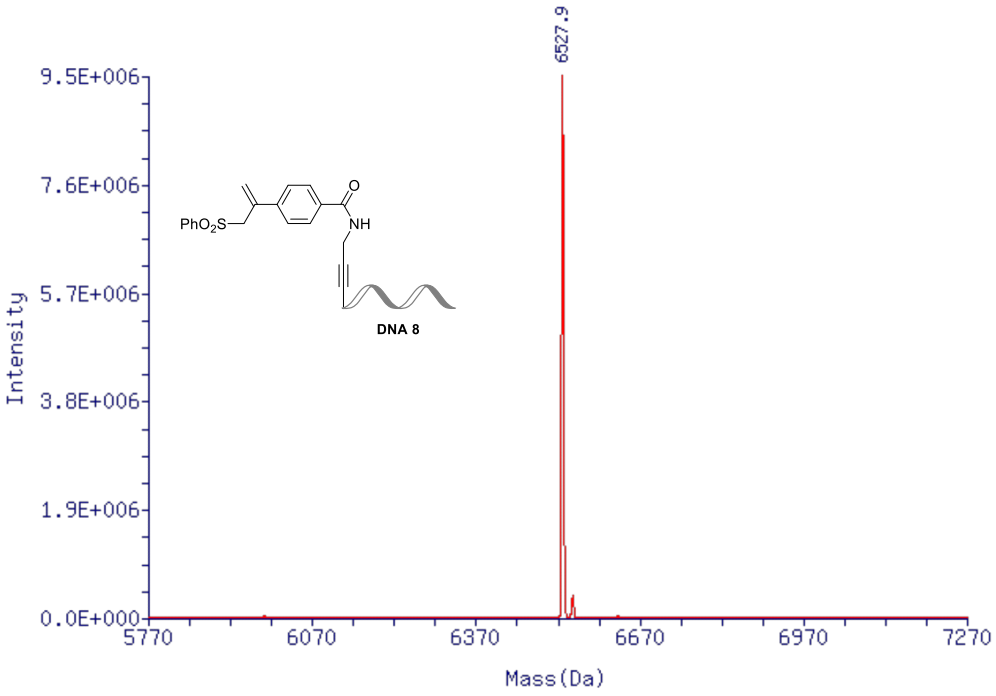


Figure S33. Deconvoluted mass spectrum of **DNA 8**, expected Mass: 6528.1; observed Mass: 6527.9.

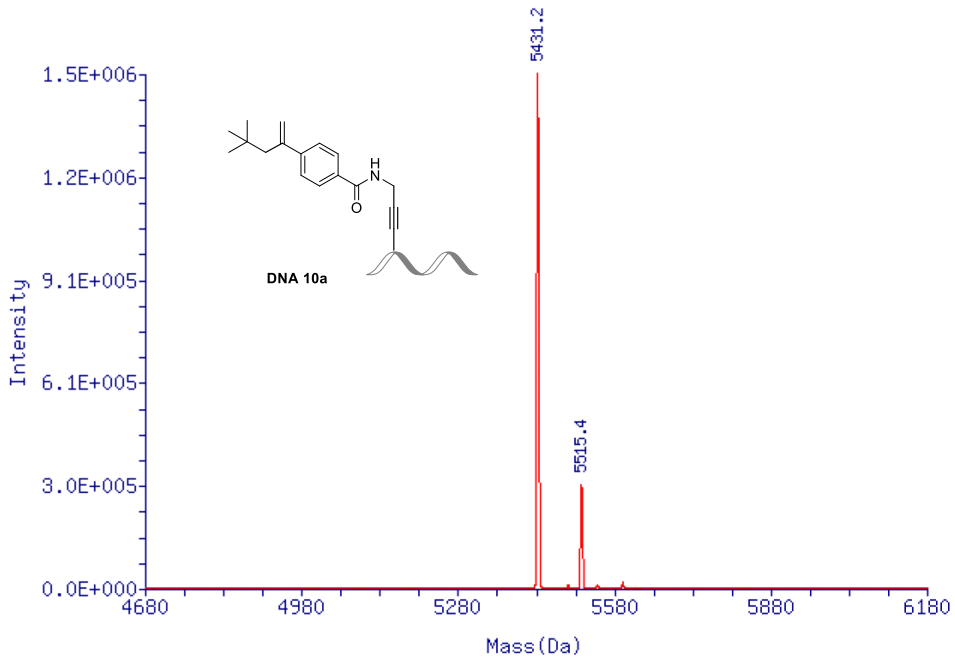


Figure S34. Deconvoluted mass spectrum of **DNA 10a**, expected Mass: 5432.4; observed Mass: 5431.2.

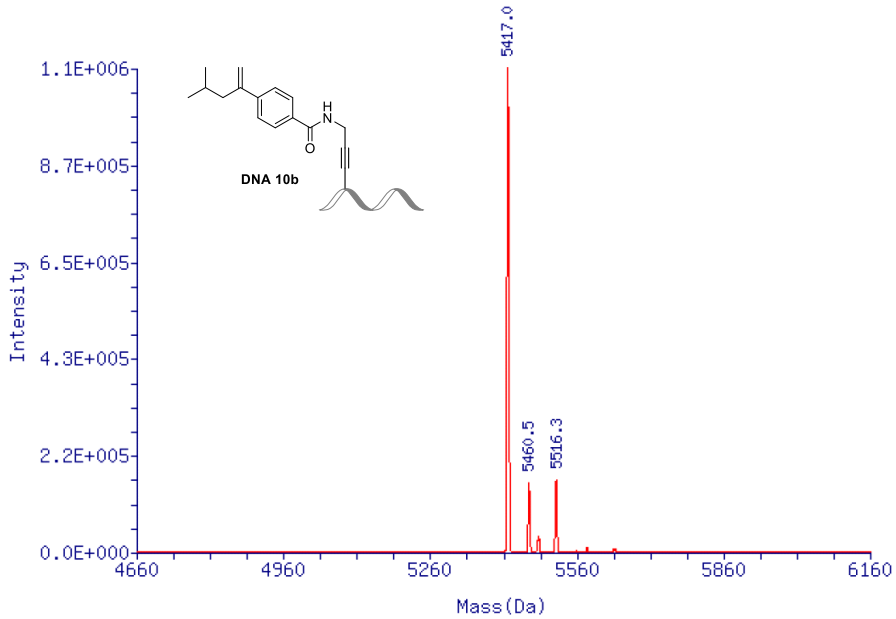


Figure S35. Deconvoluted mass spectrum of **DNA 10b**, expected Mass: 5418.4; observed Mass: 5417.0

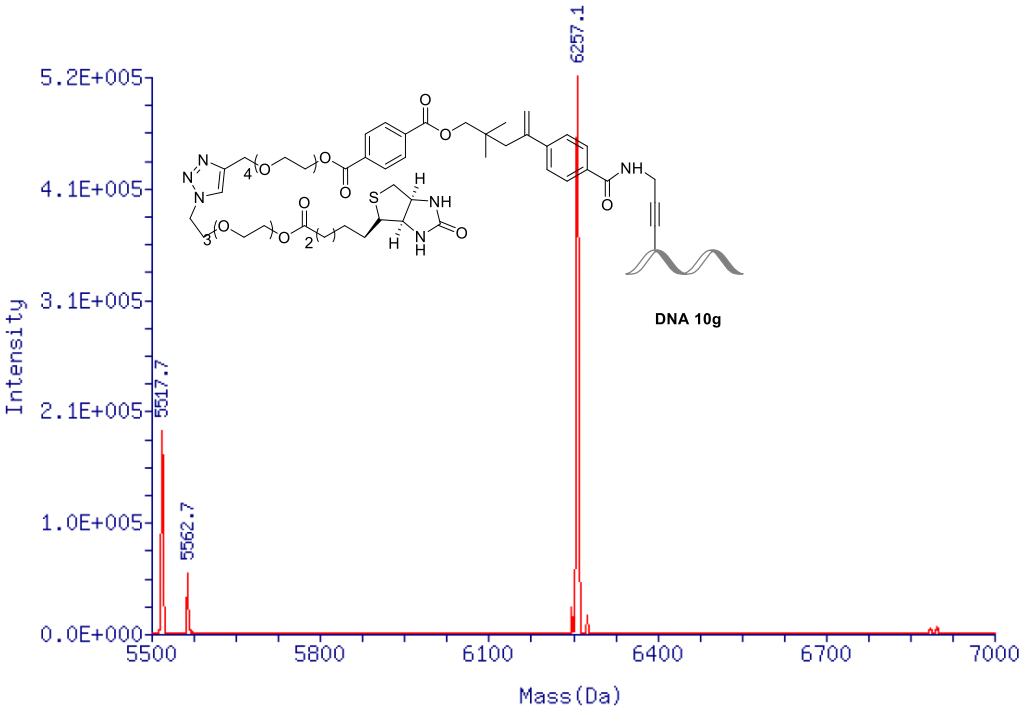


Figure S36. Deconvoluted mass spectrum of **DNA 10g**, expected Mass:6255.7; observed Mass: 6257.1.

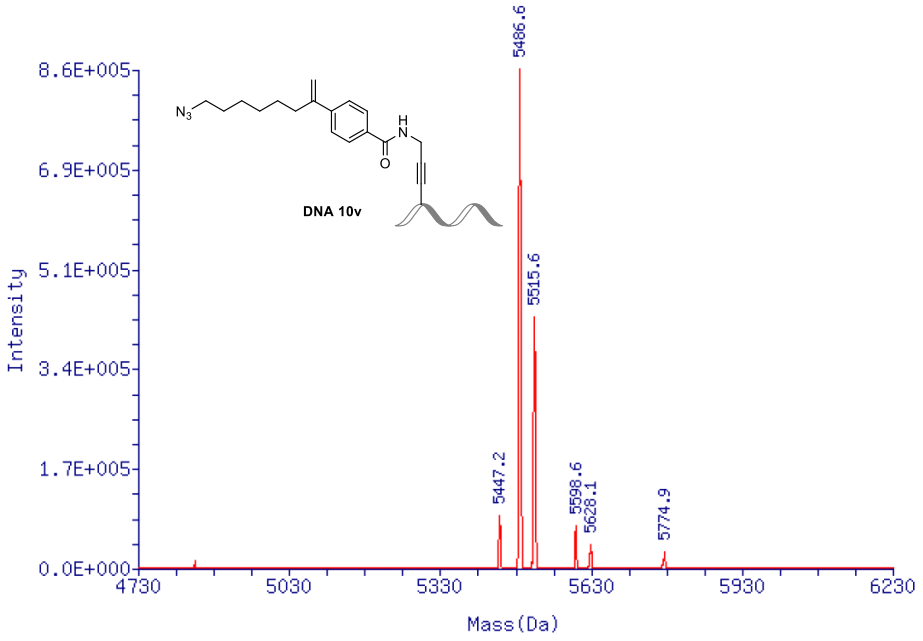


Figure S37. Deconvoluted mass spectrum of **DNA 10v**, expected Mass:5487.4; observed Mass: 5486.6.

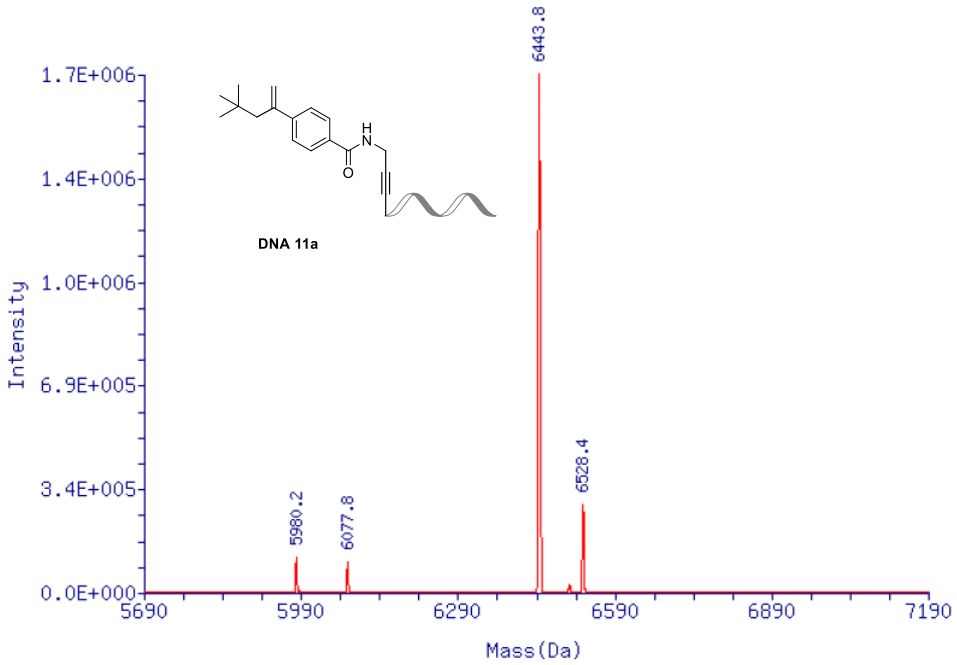


Figure S38. Deconvoluted mass spectrum of **DNA 11a**, expected Mass: 6443.2; observed Mass: 6443.8.

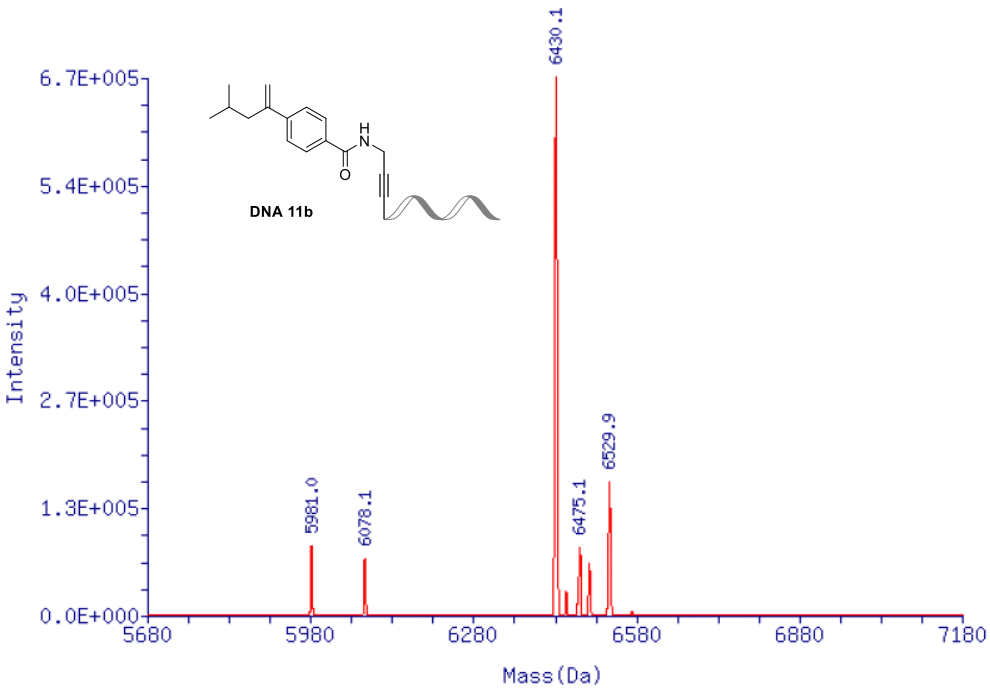


Figure S39. Deconvoluted mass spectrum of **DNA 11b**, expected Mass: 6430.2; observed Mass: 6430.1

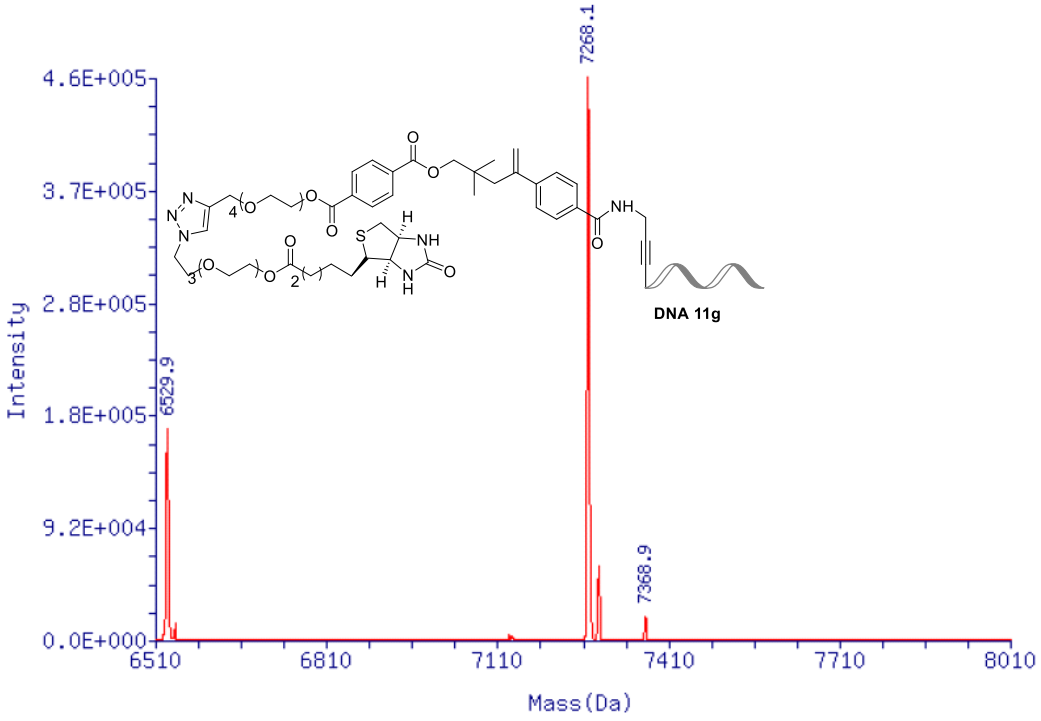


Figure S40. Deconvoluted mass spectrum of **DNA 11g**, expected Mass:7267.5; observed Mass: 7268.1.

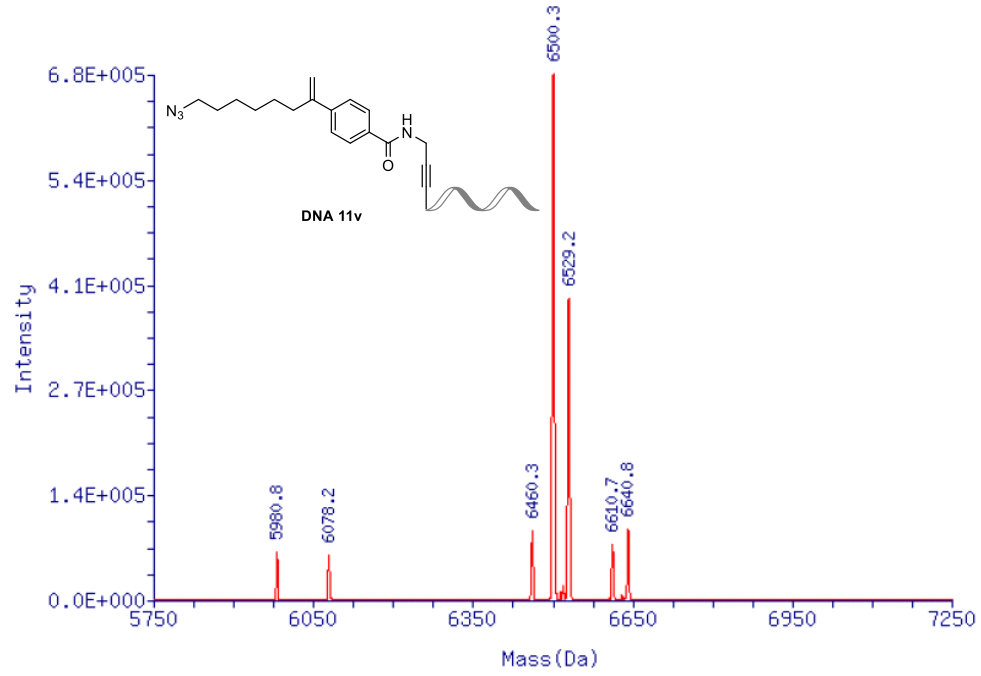


Figure S41. Deconvoluted mass spectrum of **DNA 11v**, expected Mass: 6499.2; observed Mass: 6550.3.

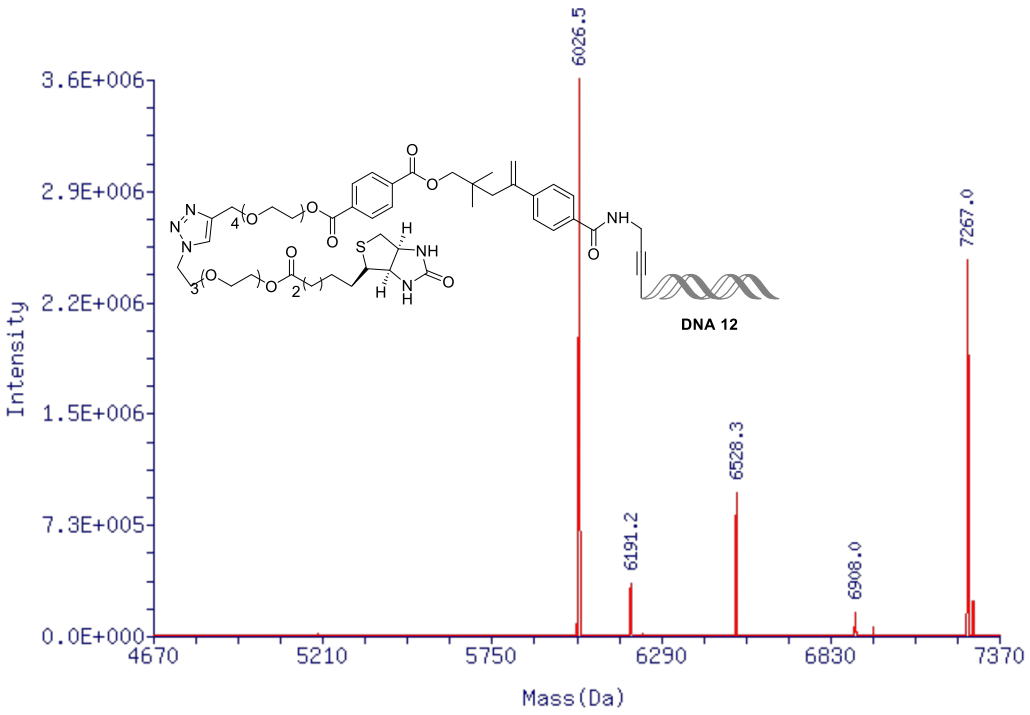


Figure S42. Deconvoluted mass spectrum of **DNA 12**, expected Mass: 7267.5 and 6026.0; observed Mass: 7267.0 and 6026.5.

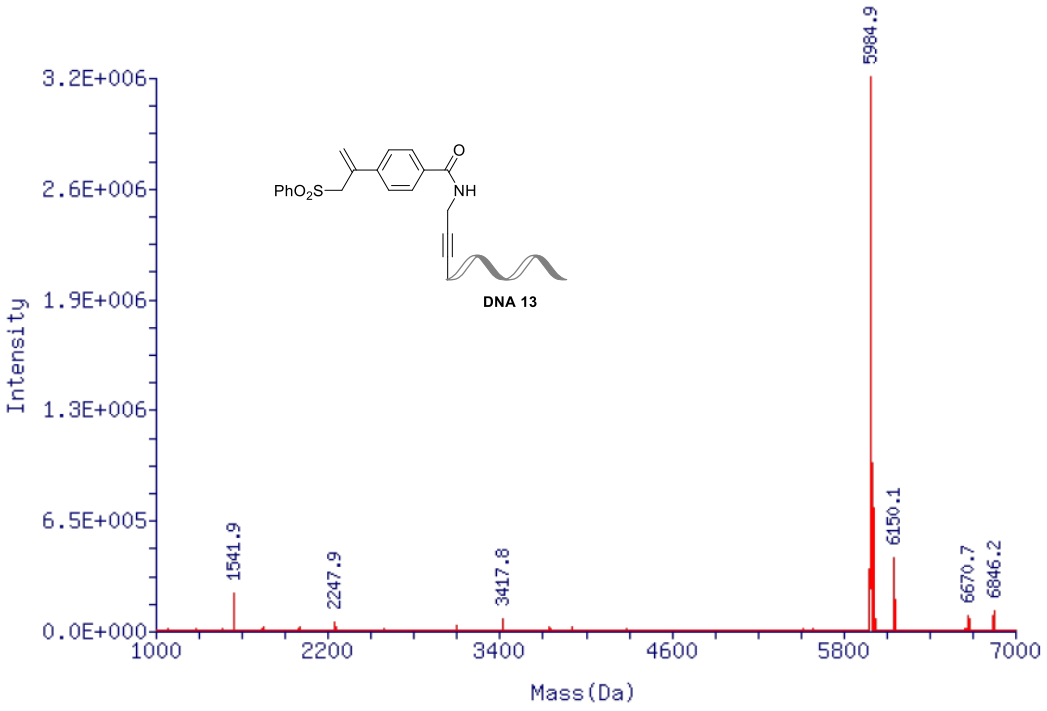


Figure S43. Deconvoluted mass spectrum of **DNA 13**, expected Mass: 5981.4; observed Mass: 5984.9.

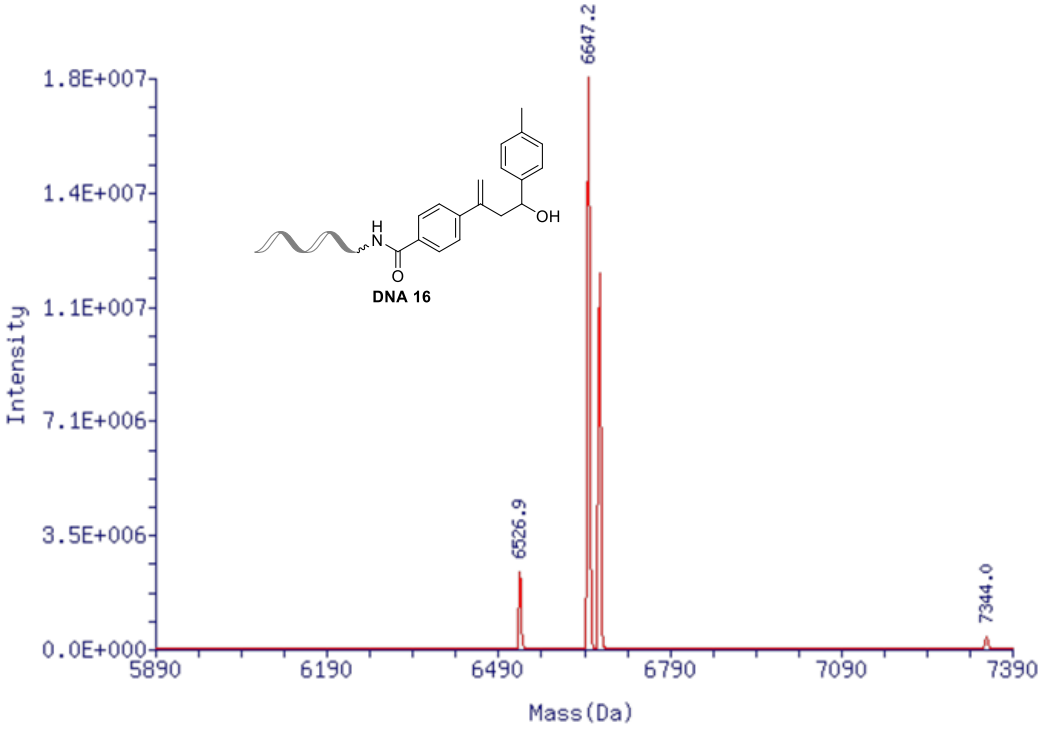


Figure S44. Deconvoluted mass spectrum of **DNA 16**, expected Mass: 6647.4; observed Mass: 6647.2.

7. HPLC Chromatograms of DNA Post-Modification

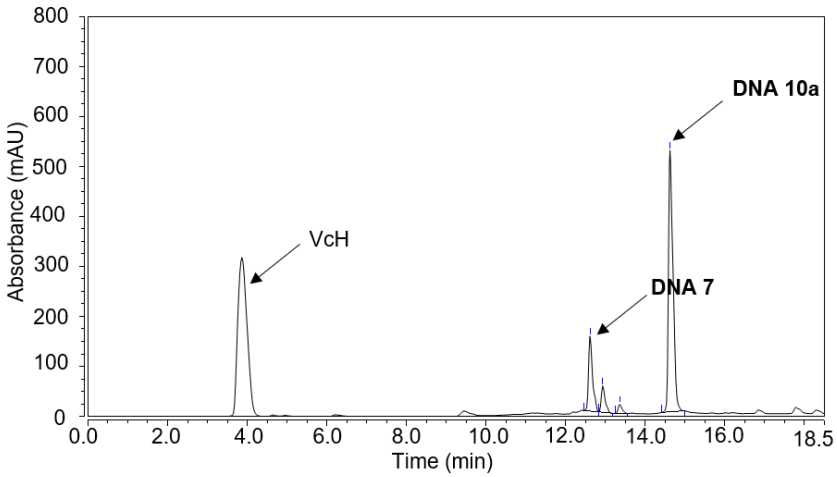


Figure S45. HPLC chromatogram analysis for DNA post-modification to produce **DNA 10a**

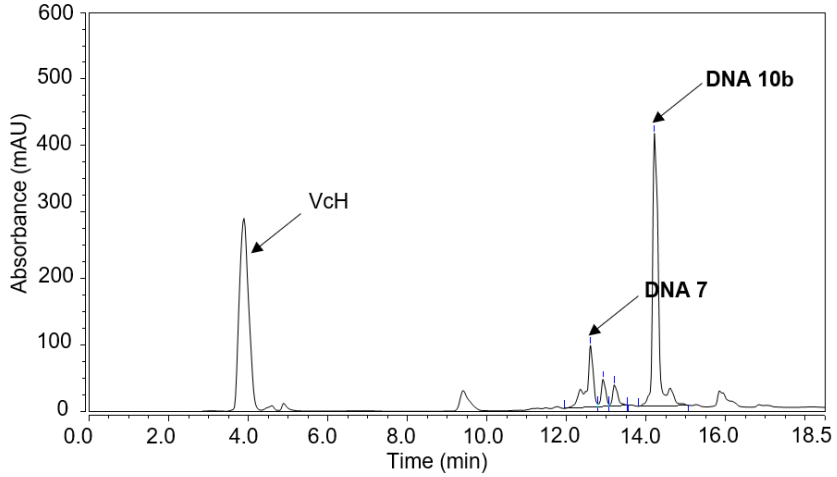


Figure S46. HPLC chromatogram analysis for DNA post-modification to produce **DNA 10b**

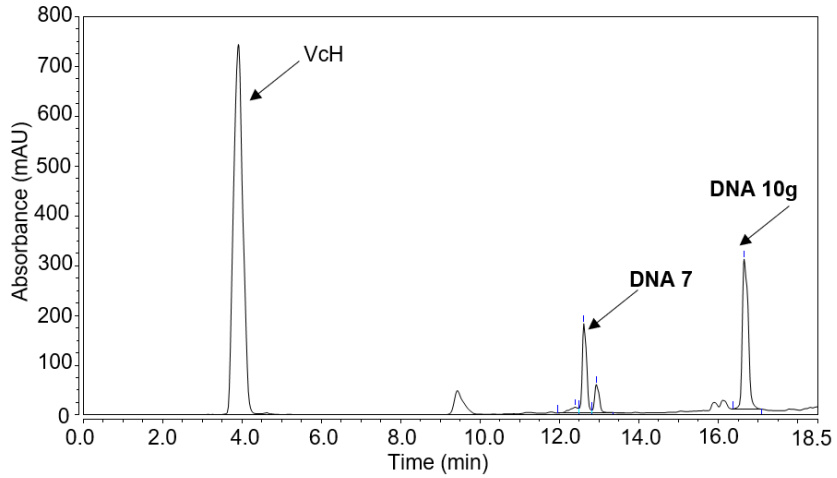


Figure S47. HPLC chromatogram analysis for DNA post-modification to produce **DNA 10g**

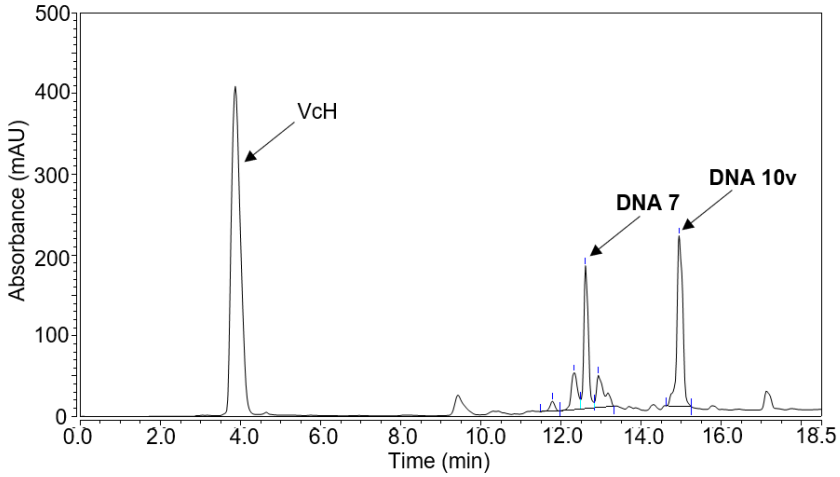


Figure S48. HPLC chromatogram analysis for DNA post-modification to produce **DNA 10v**

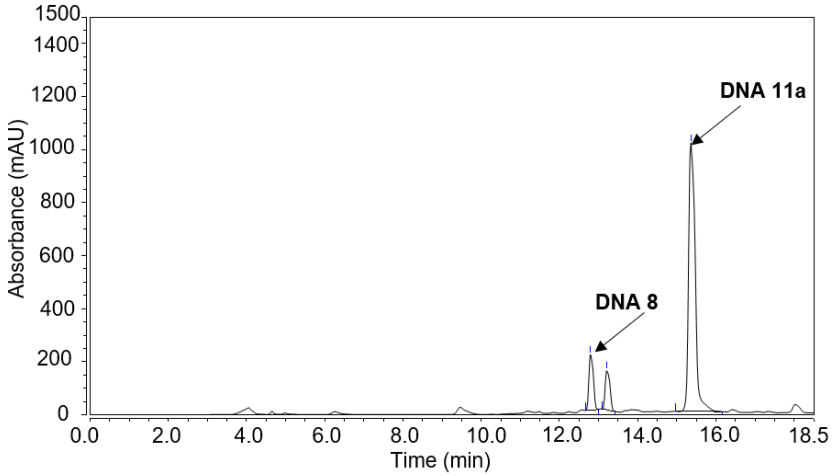


Figure S49. HPLC chromatogram analysis for DNA post-modification to produce **DNA 11a**

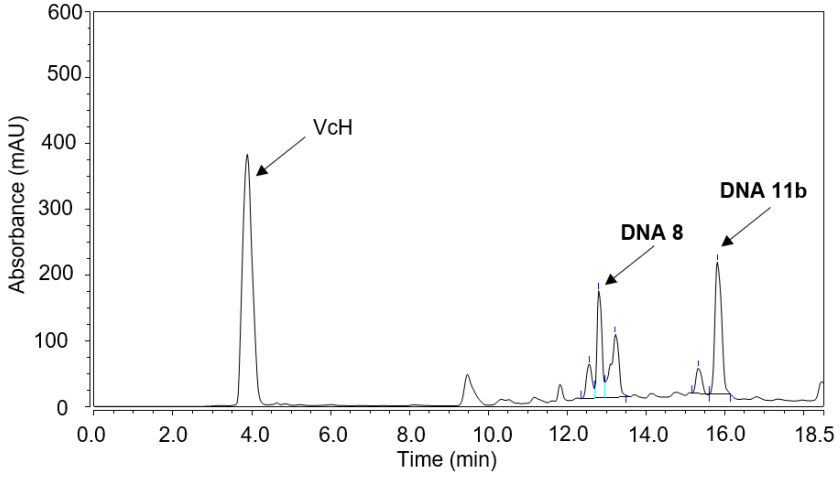


Figure S50. HPLC chromatogram analysis for DNA post-modification to produce **DNA 11b**

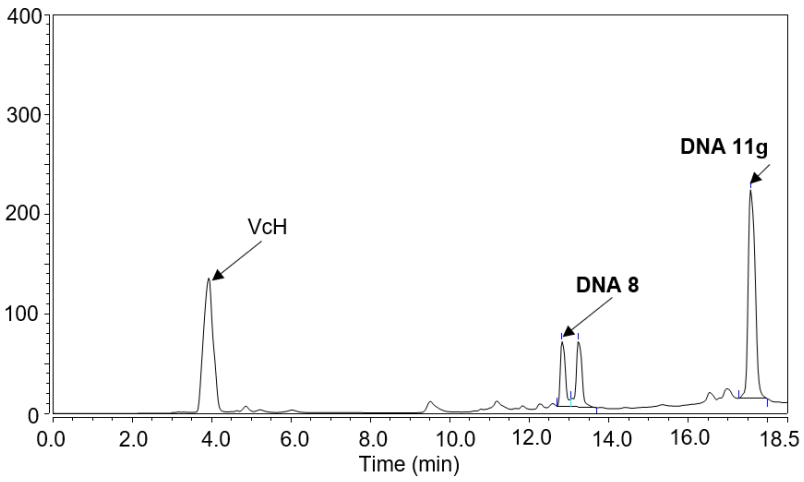


Figure S51. HPLC chromatogram analysis for DNA post-modification to produce **DNA 11g**

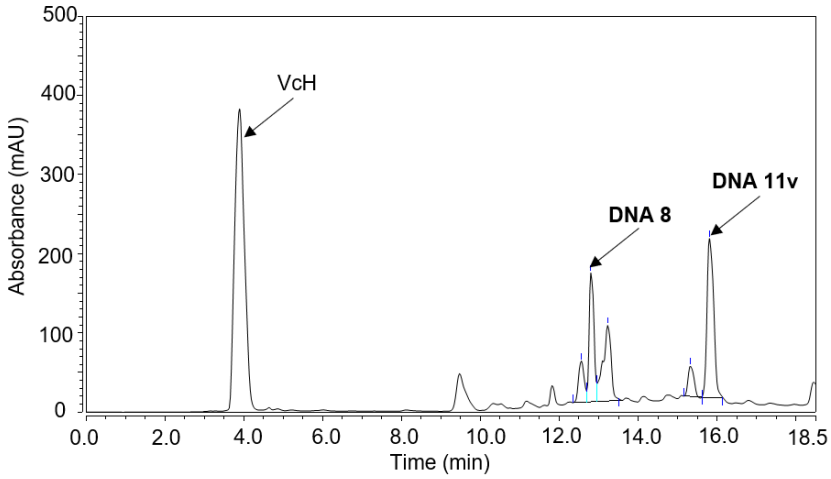


Figure S52. HPLC chromatogram analysis for DNA post-modification to produce **DNA 11v**

8. NMR Spectra of New Compounds

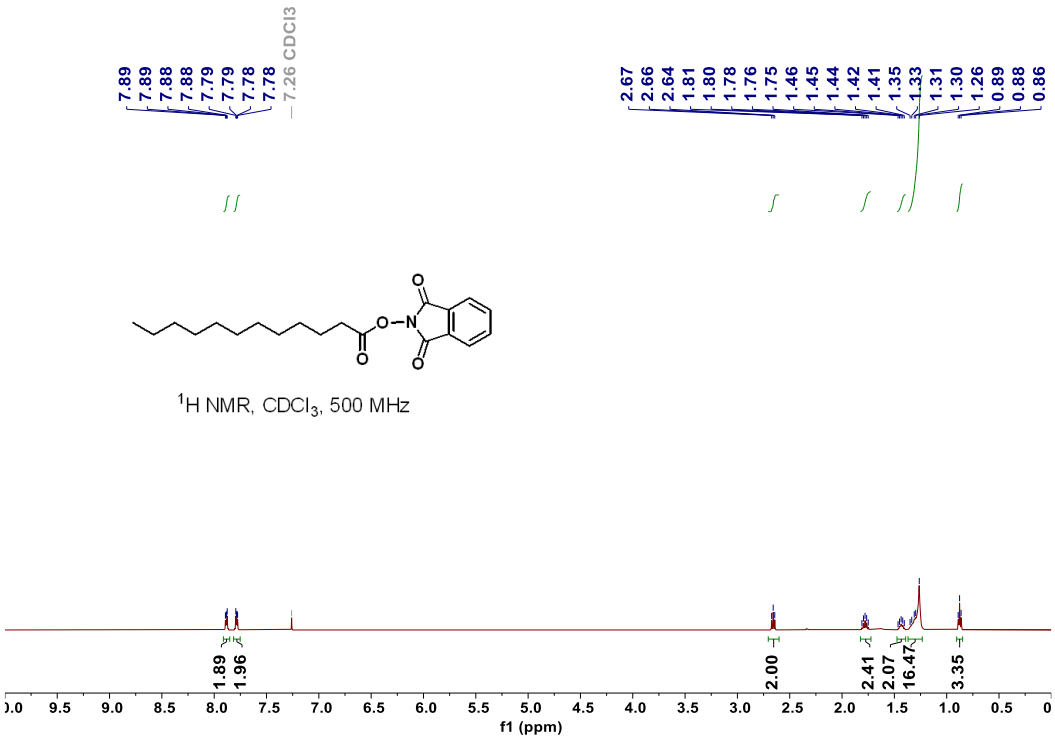


Figure S53. ^{1}H NMR spectrum of **1d**

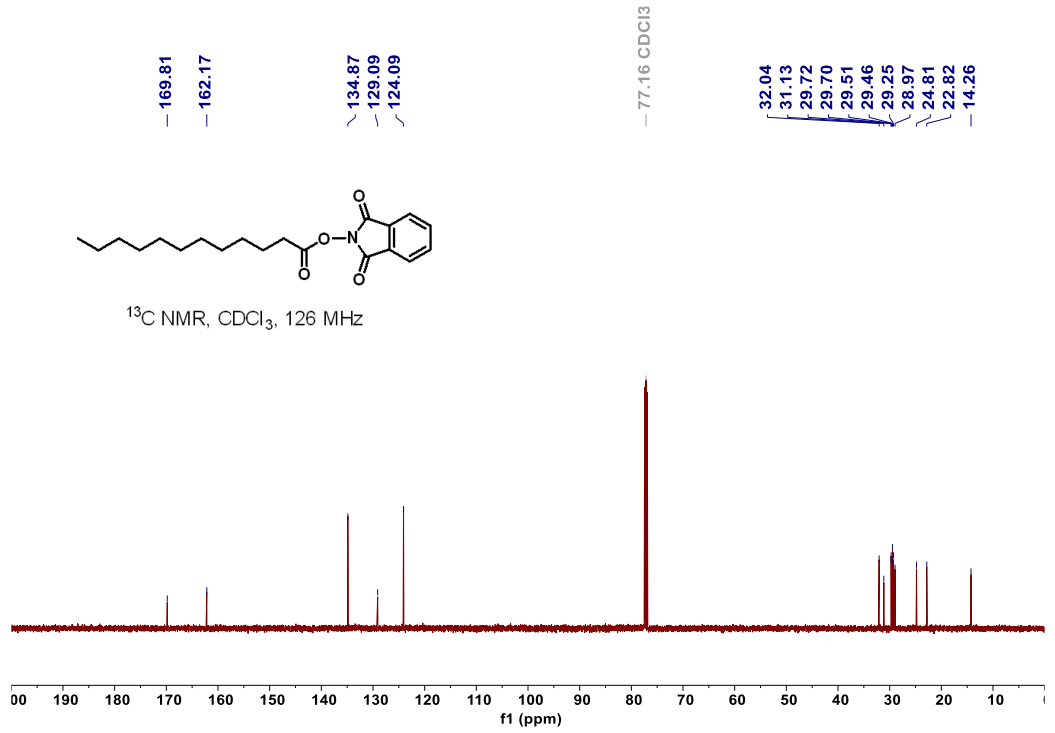


Figure S54. ^{13}C NMR spectrum of **1d**

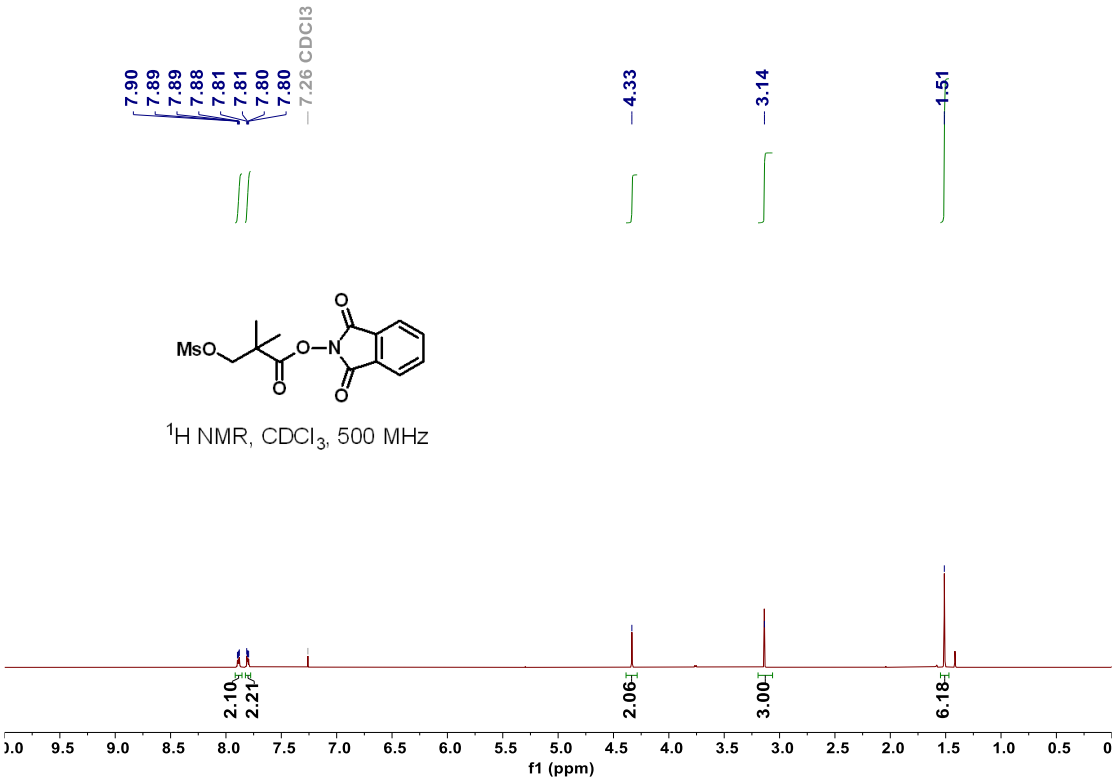


Figure S55. ^{1}H NMR spectrum of **1l**

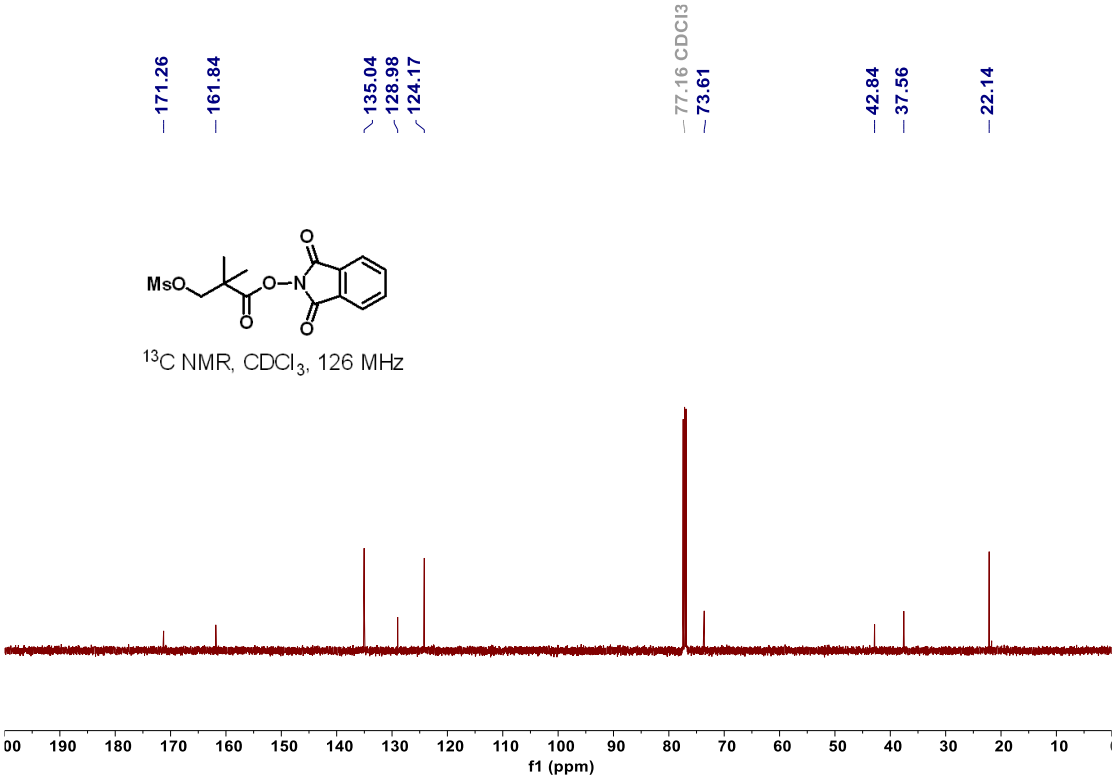


Figure S56. ^{13}C NMR spectrum of **1l**

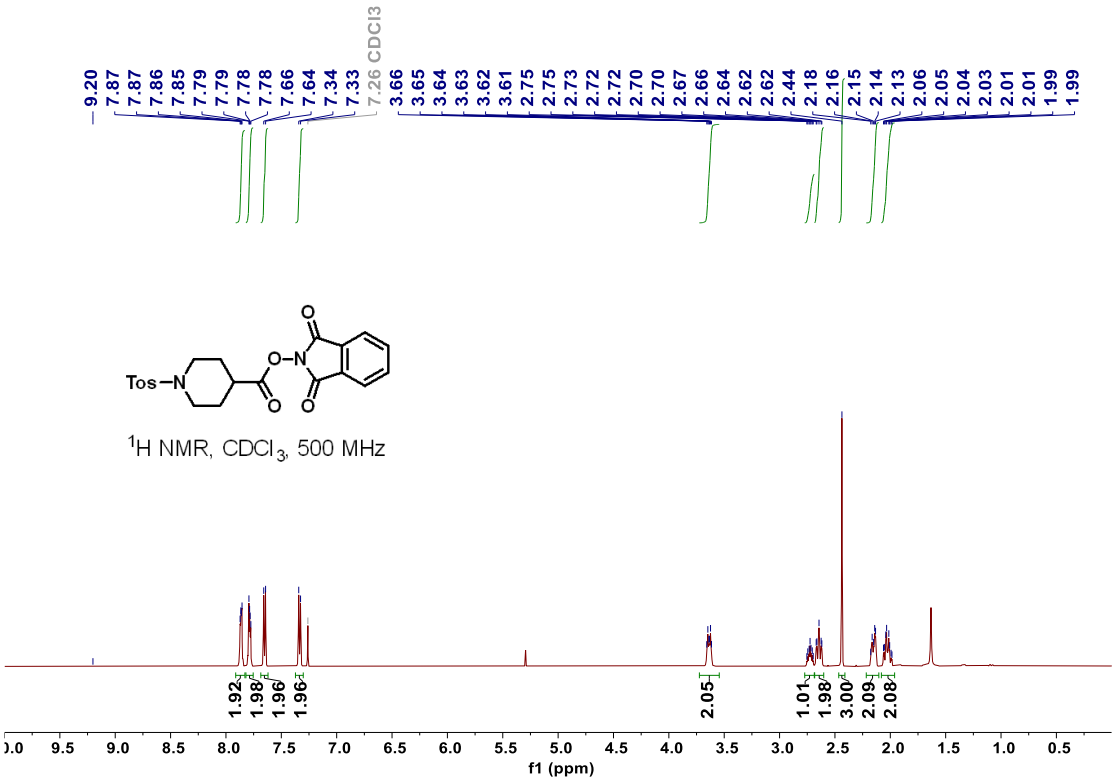


Figure S57. ^{1}H NMR spectrum of **1m**

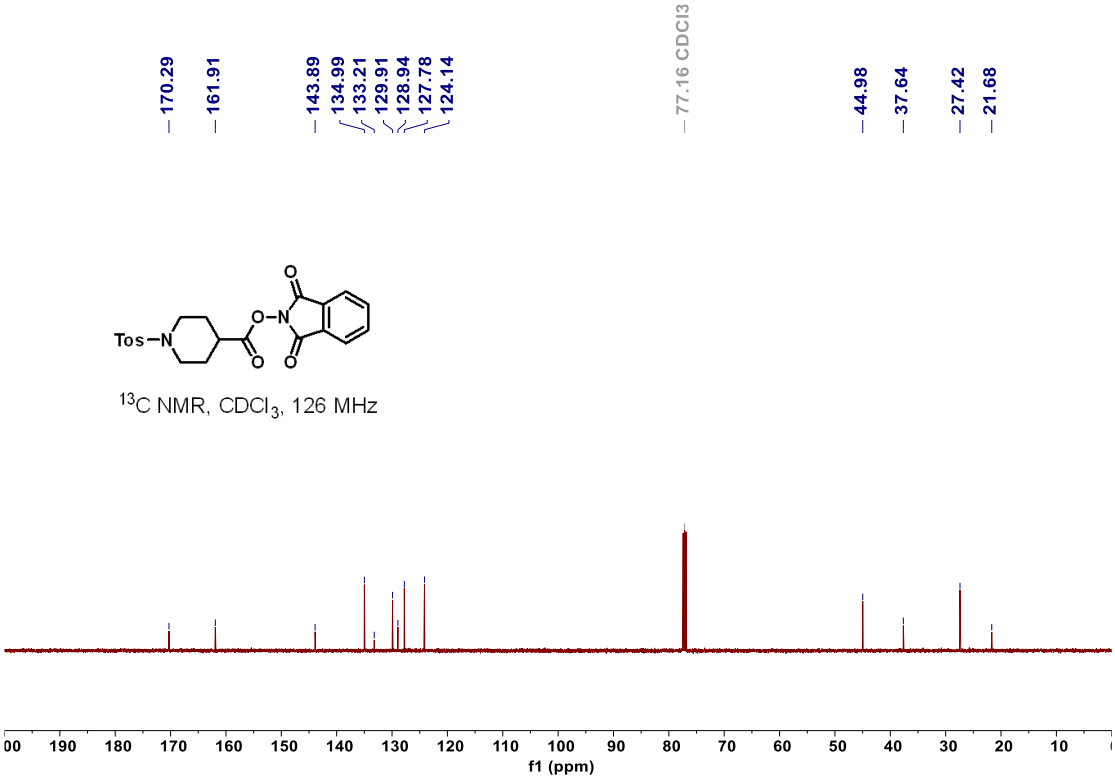


Figure S58. ^{13}C NMR spectrum of **1m**

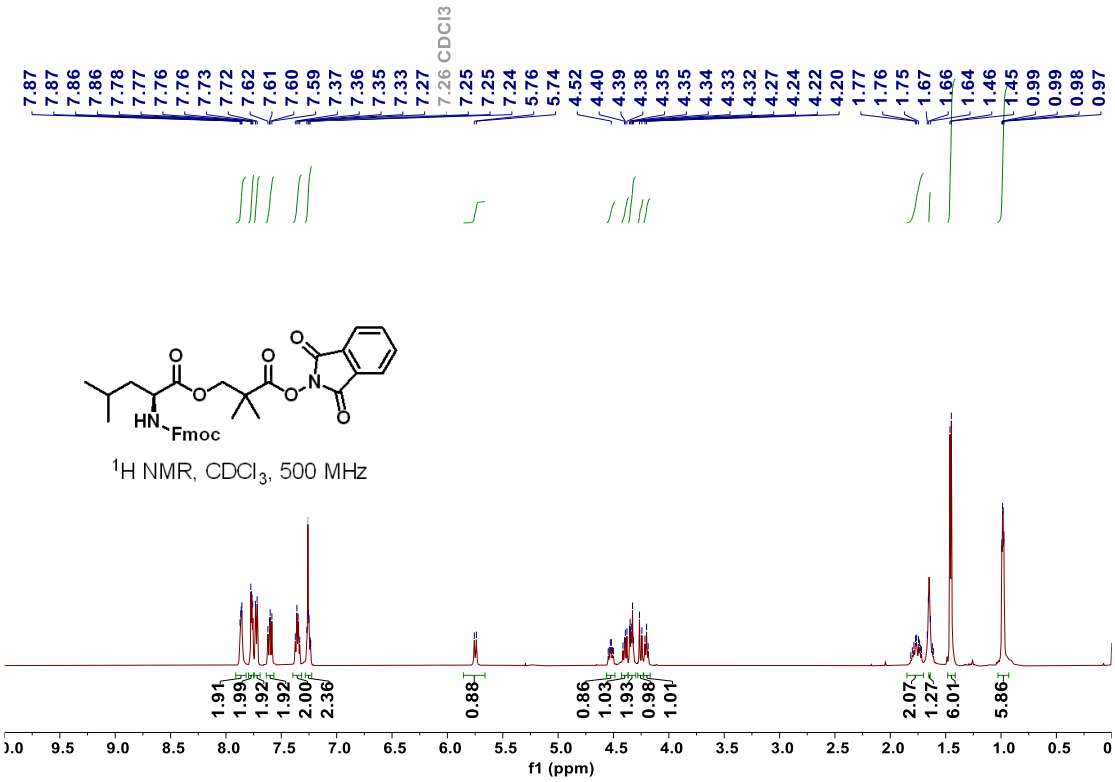


Figure S59. ^{1}H NMR spectrum of **1n**

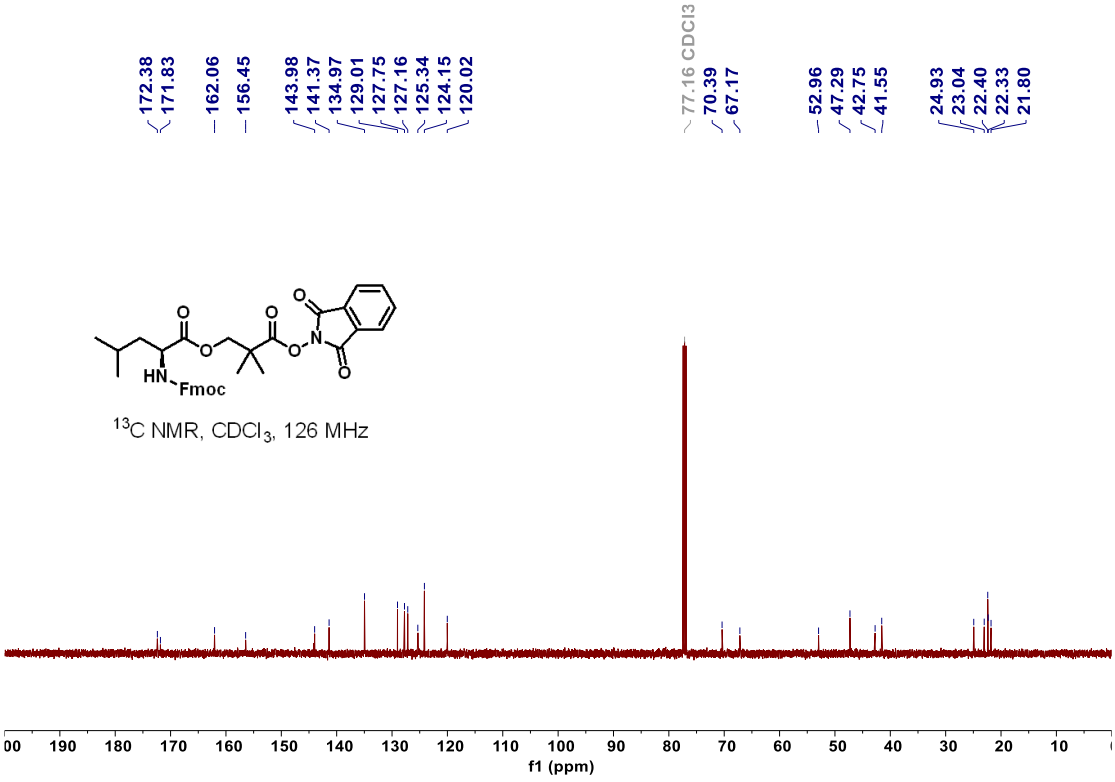


Figure S60. ^{13}C NMR spectrum of **1n**

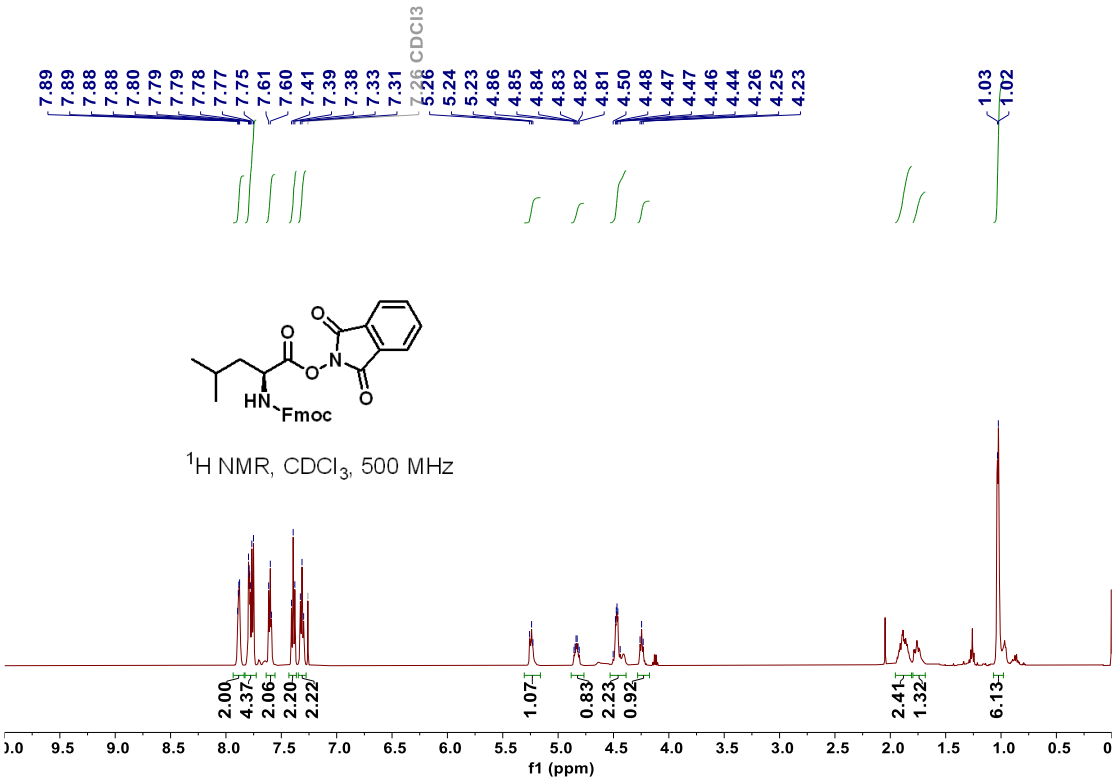


Figure S61. ^{1}H NMR spectrum of **1o**

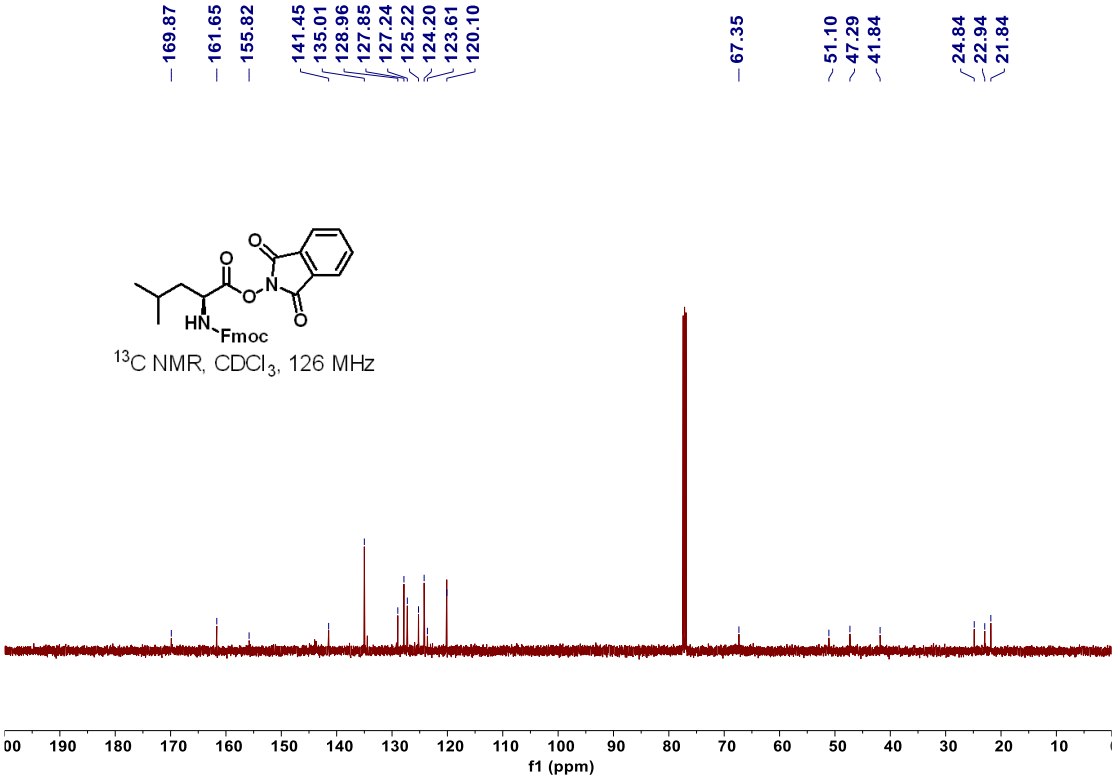


Figure S62. ^{13}C NMR spectrum of **1o**

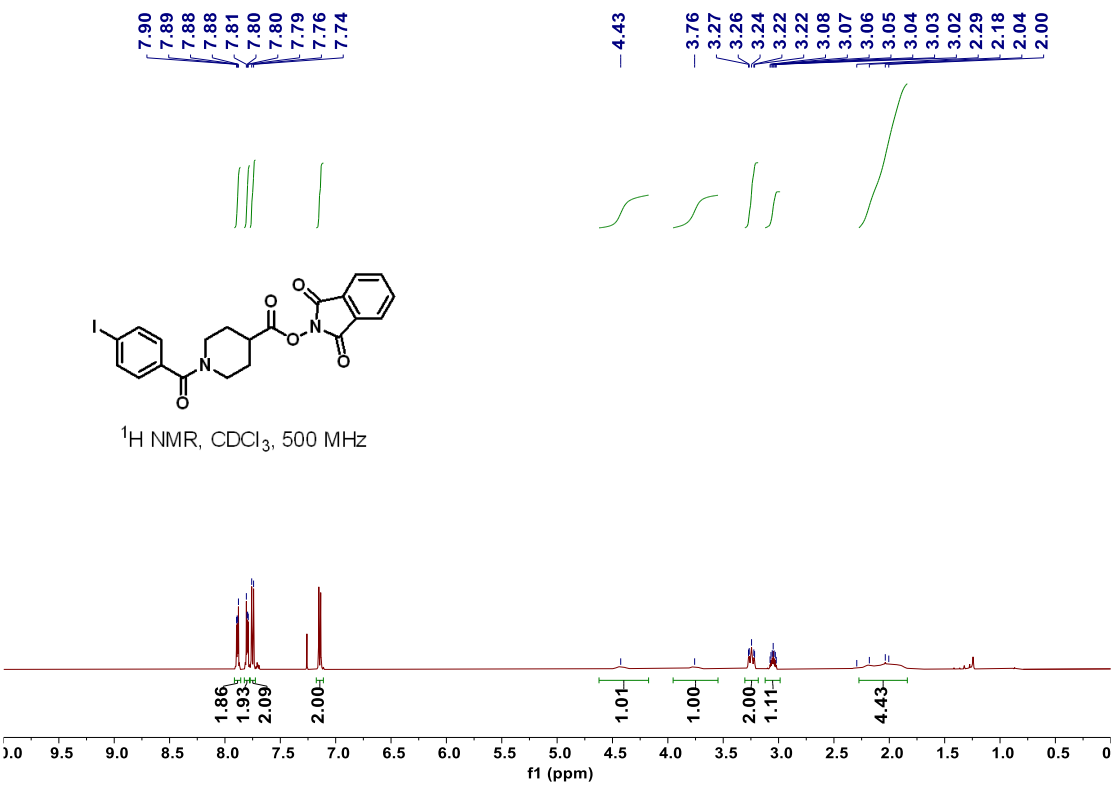


Figure S63. ^{1}H NMR spectrum of **1q**

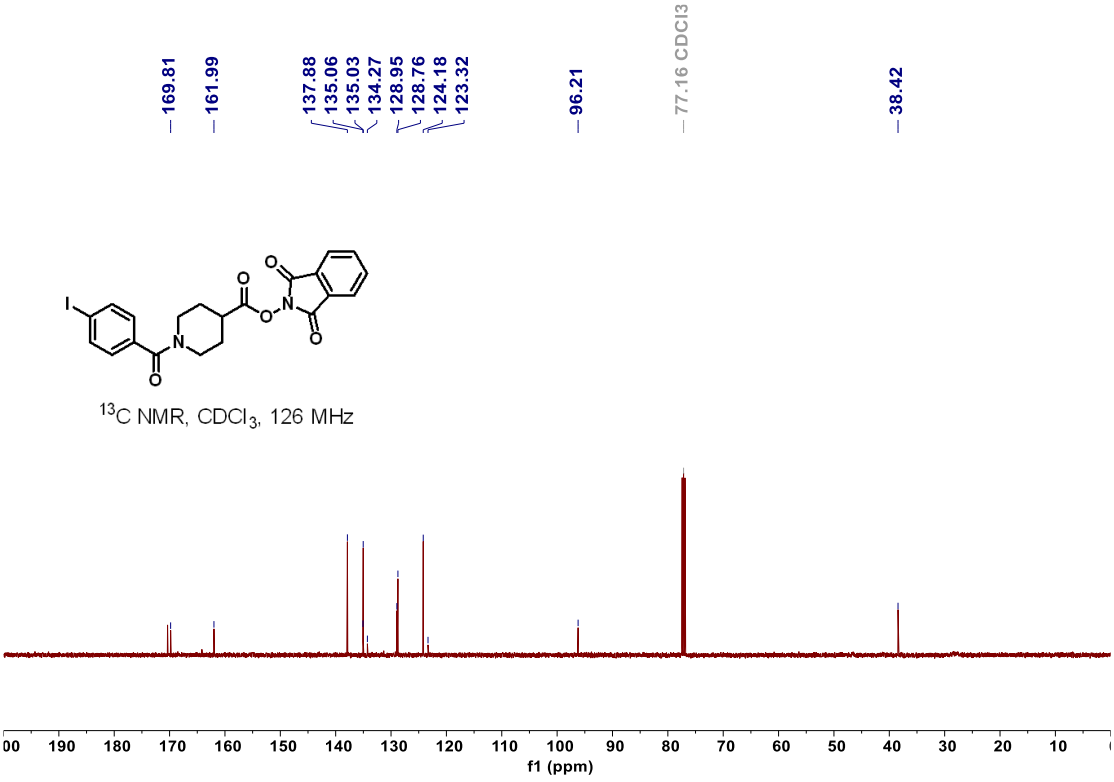


Figure S64. ^{13}C NMR spectrum of **1q**

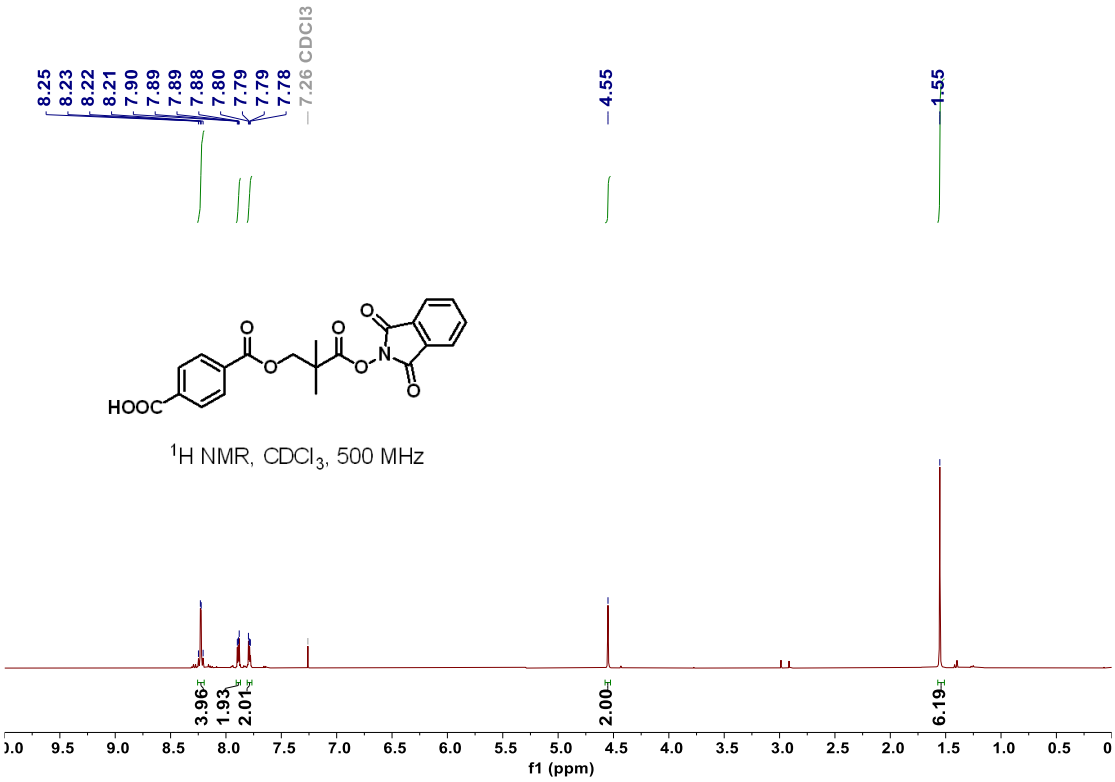


Figure S65. ^{1}H NMR spectrum of **1ga**

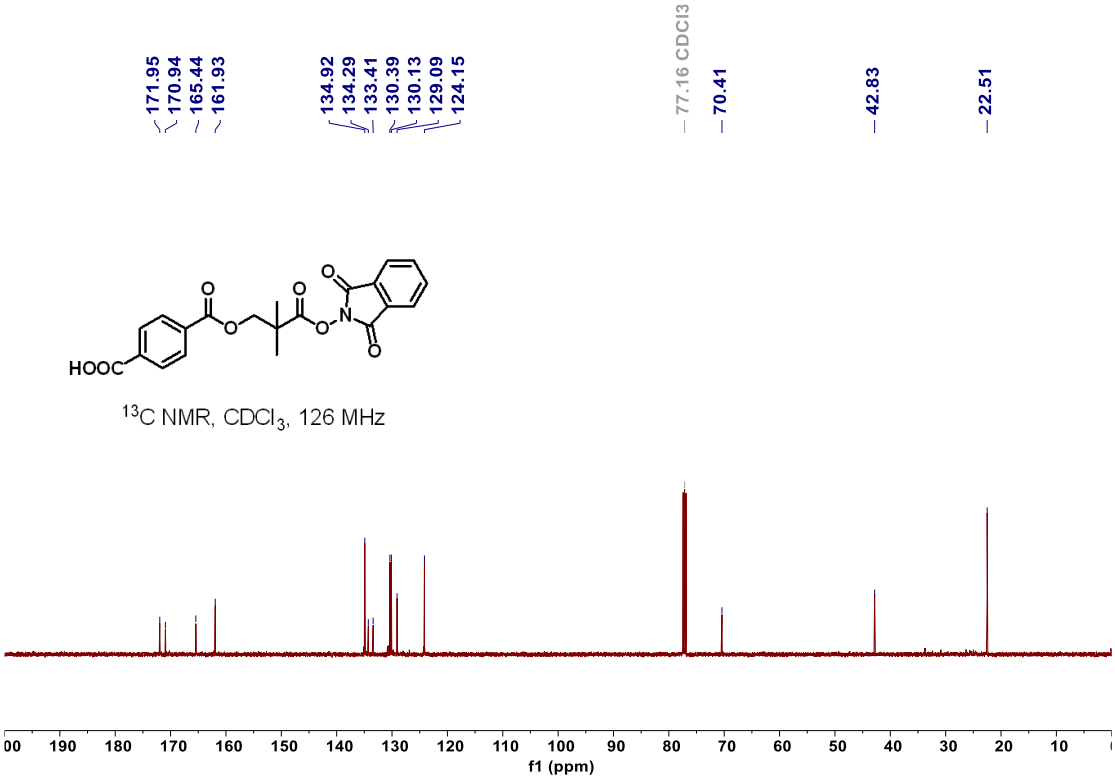


Figure S66. ^{13}C NMR spectrum of **1ga**

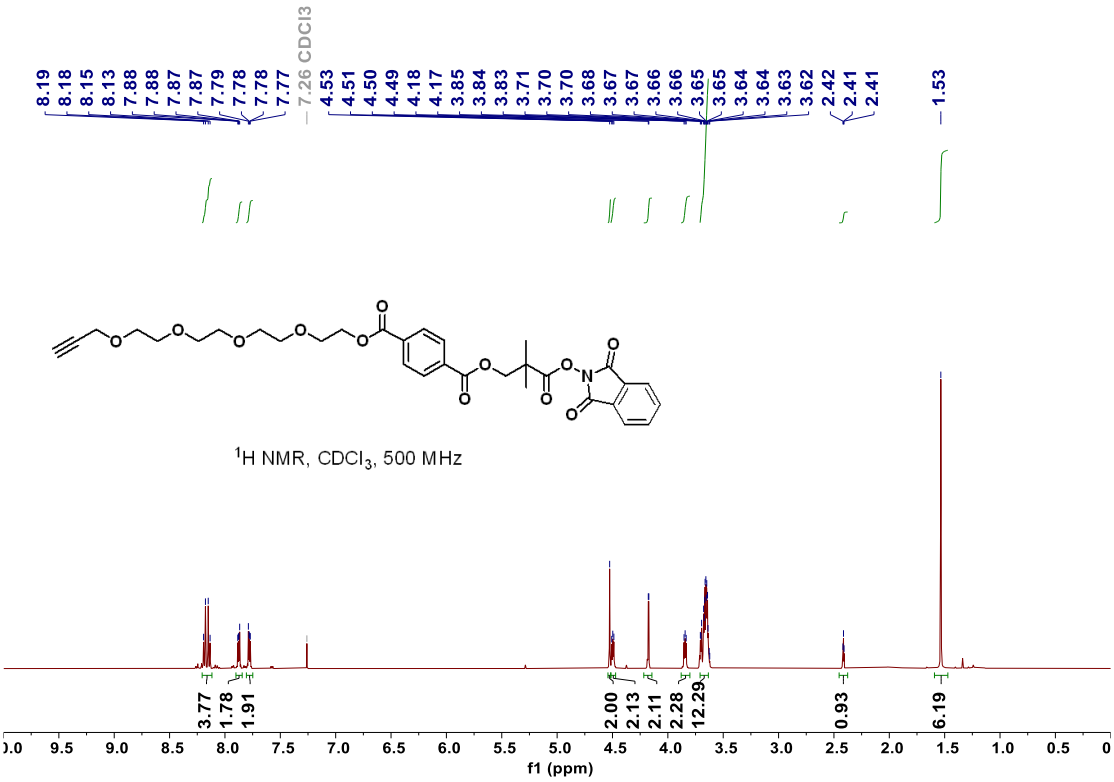


Figure S67. ^{1}H NMR spectrum of **1gb**

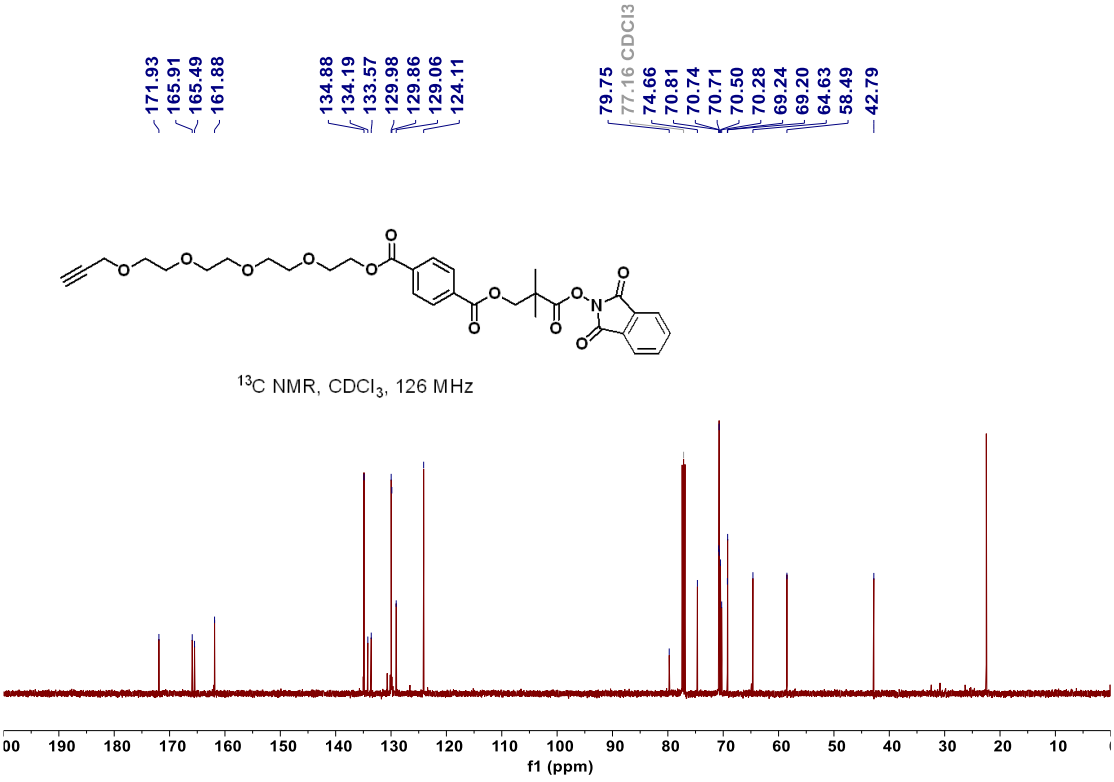


Figure S68. ^{13}C NMR spectrum of **1gb**

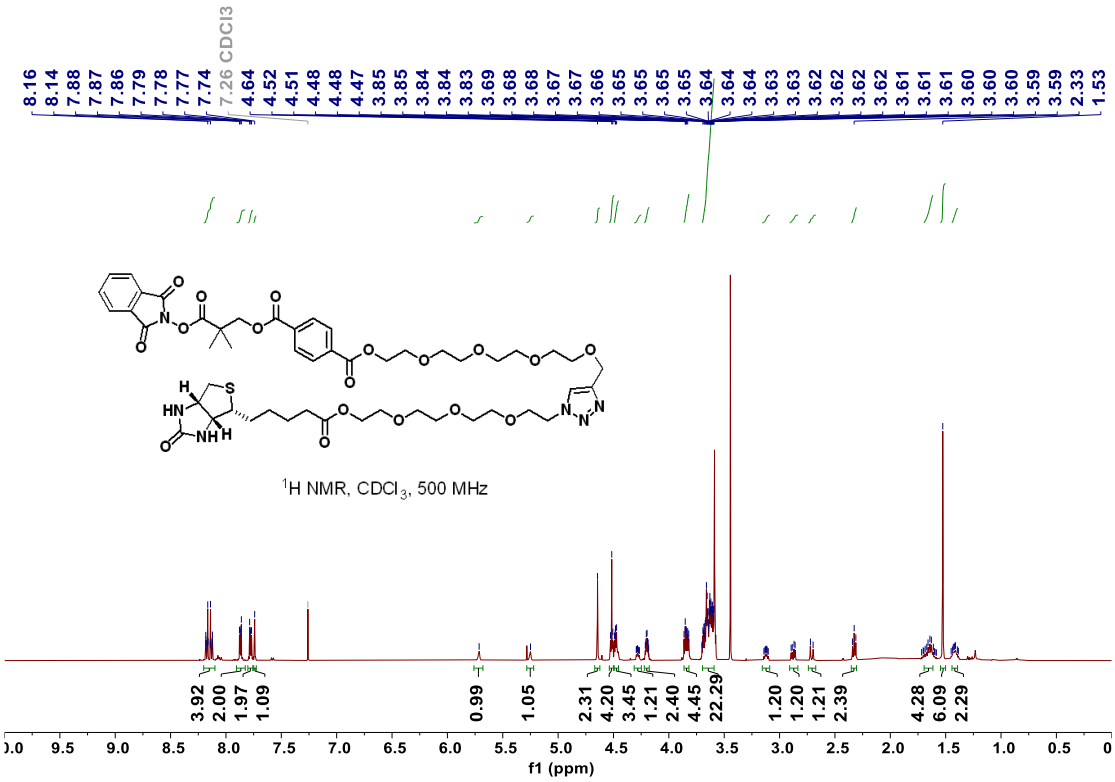


Figure S69. ^{1}H NMR spectrum of **1g**

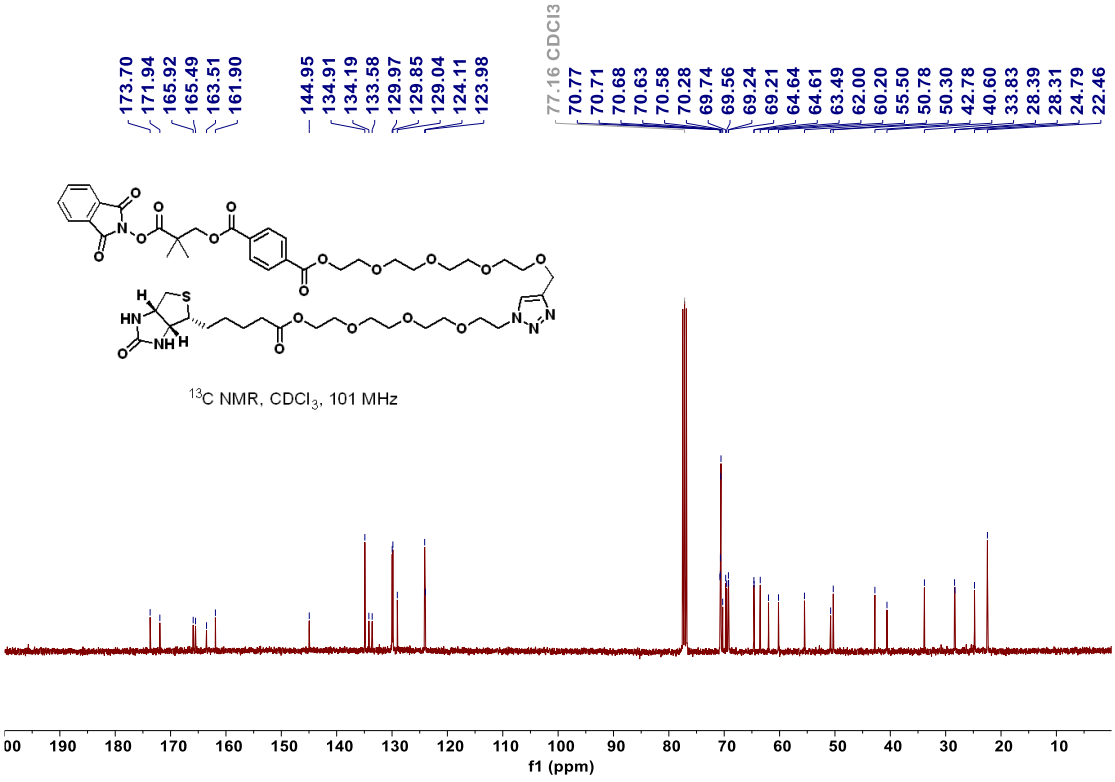


Figure S70. ^{13}C NMR spectrum of **1g**

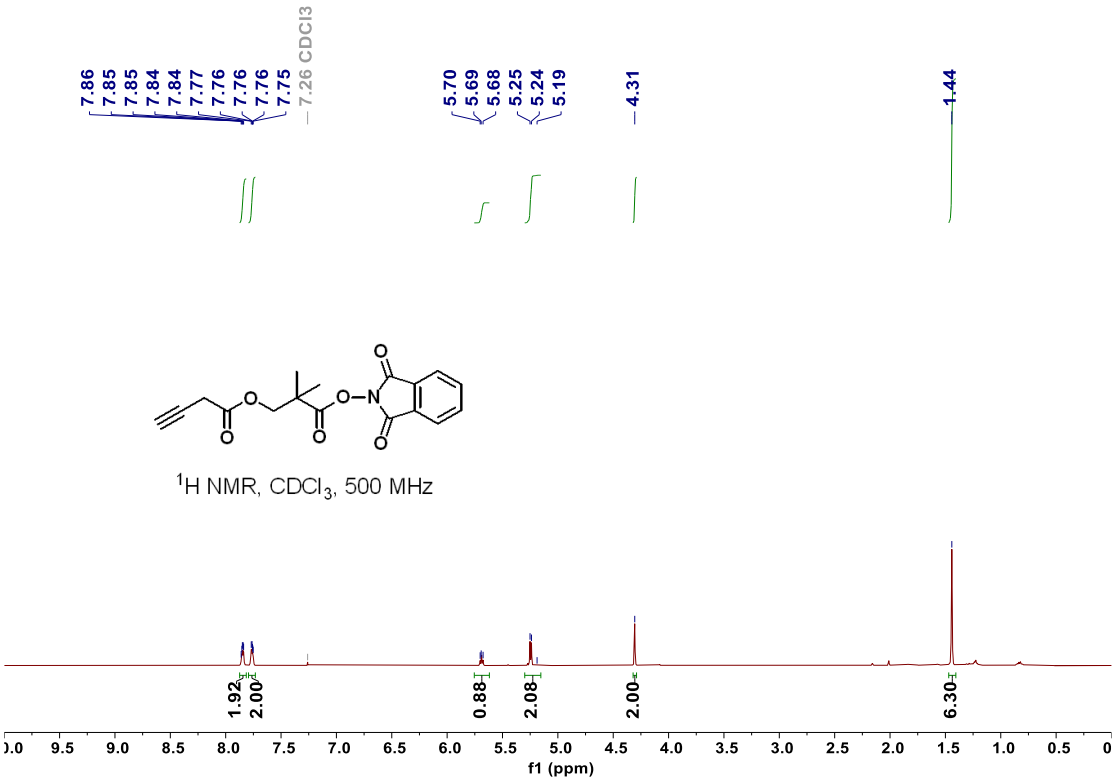


Figure S71. ^{1}H NMR spectrum of **1u**

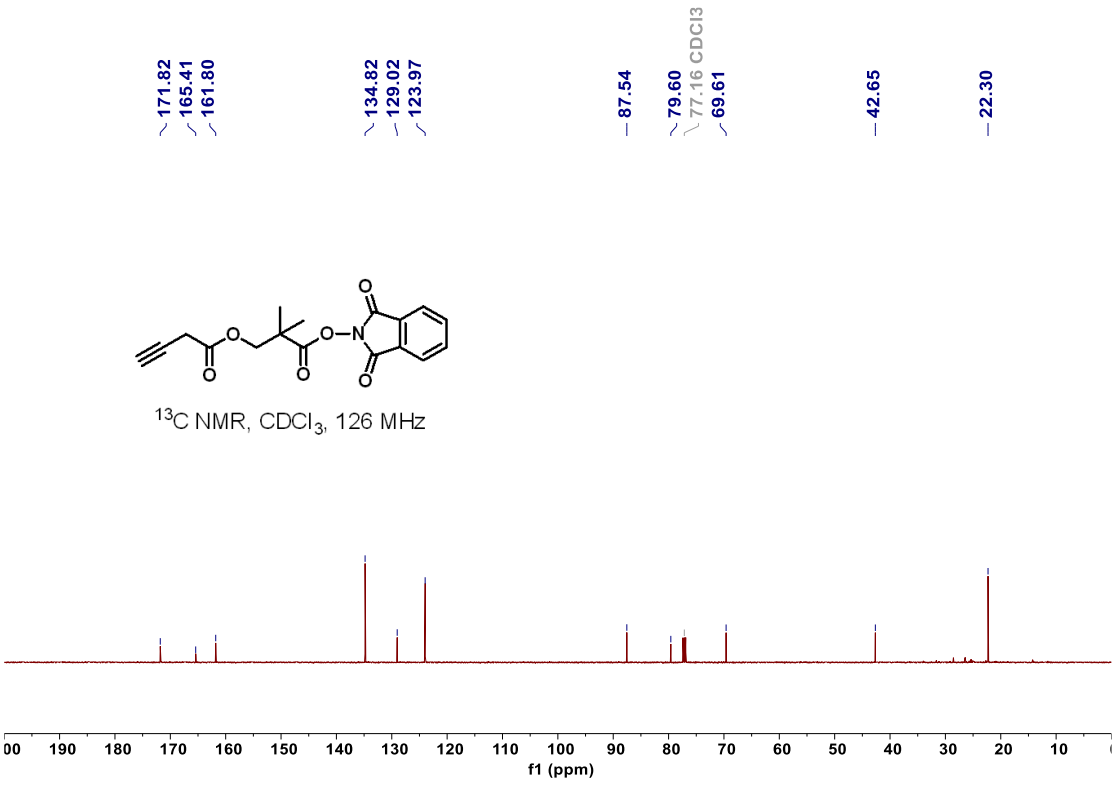


Figure S72. ^{13}C NMR spectrum of **1u**

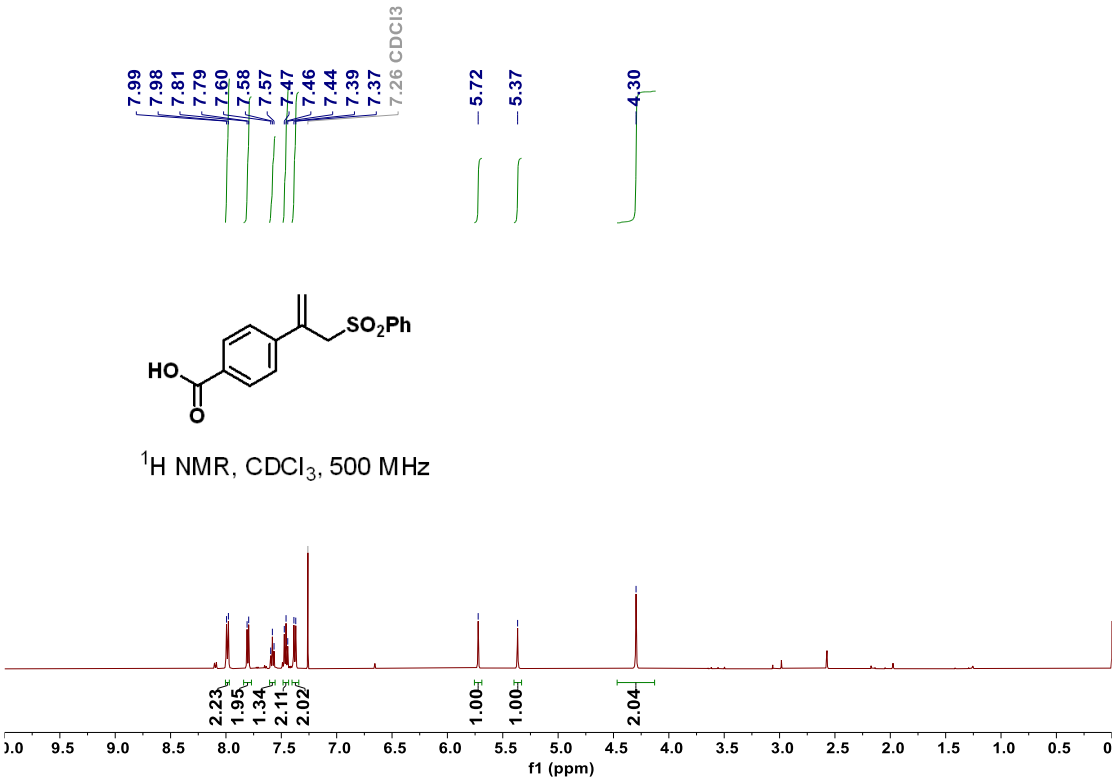


Figure S73. ^{1}H NMR spectrum of **2**

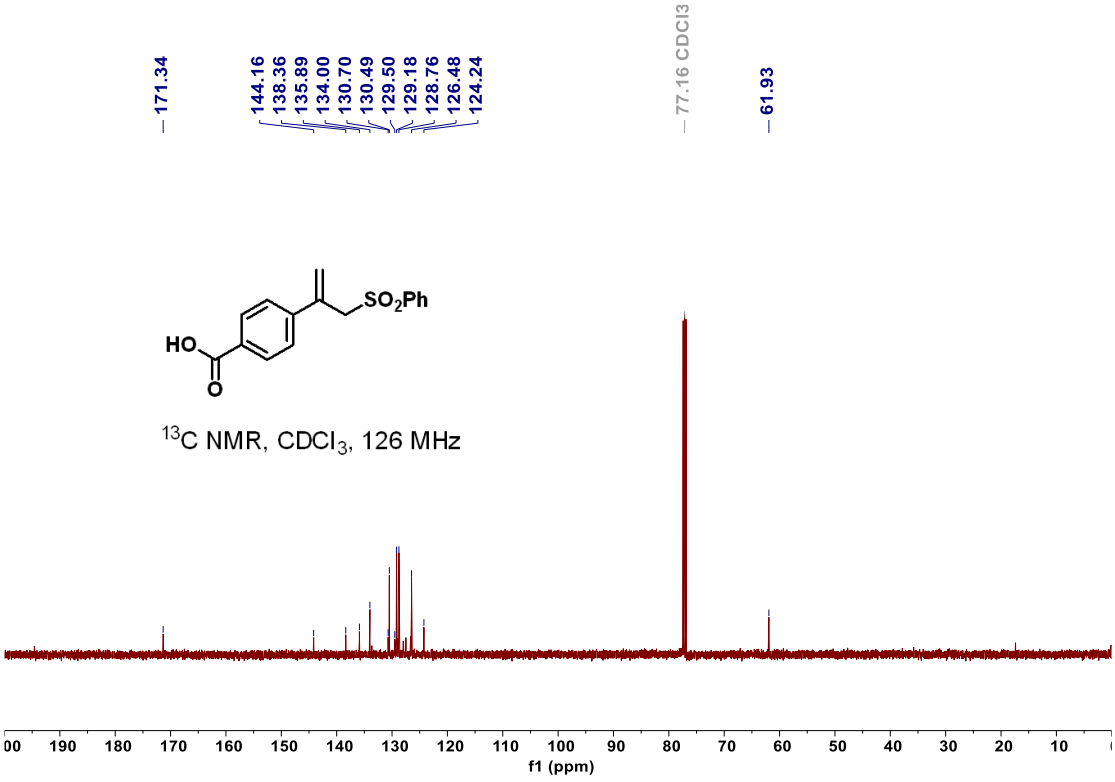


Figure S74. ^{13}C NMR spectrum of **2**

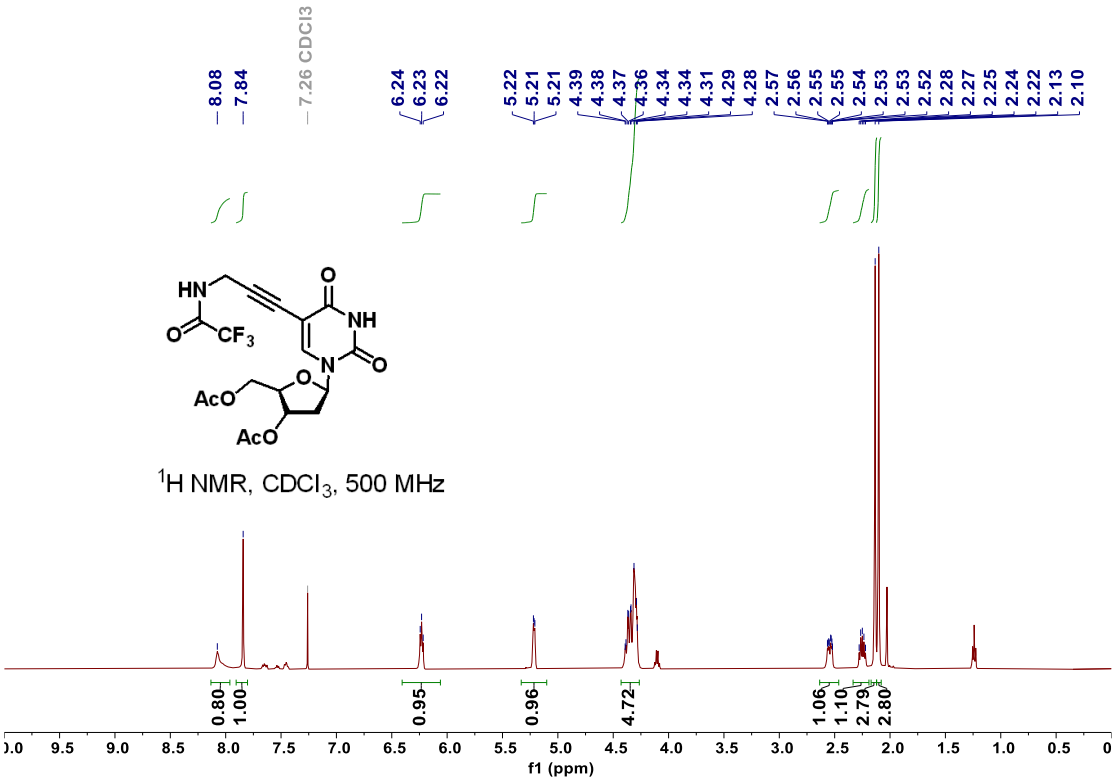


Figure S75. ^{1}H NMR spectrum of **4a**

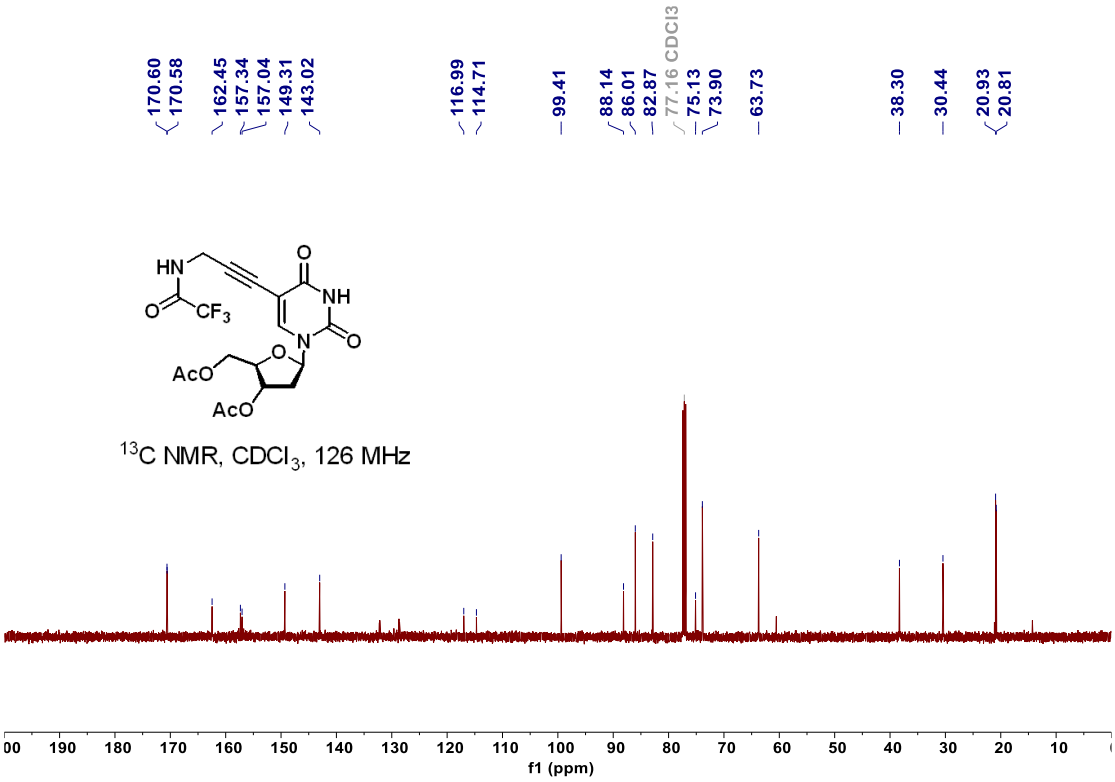


Figure S76. ^{13}C NMR spectrum of **4a**

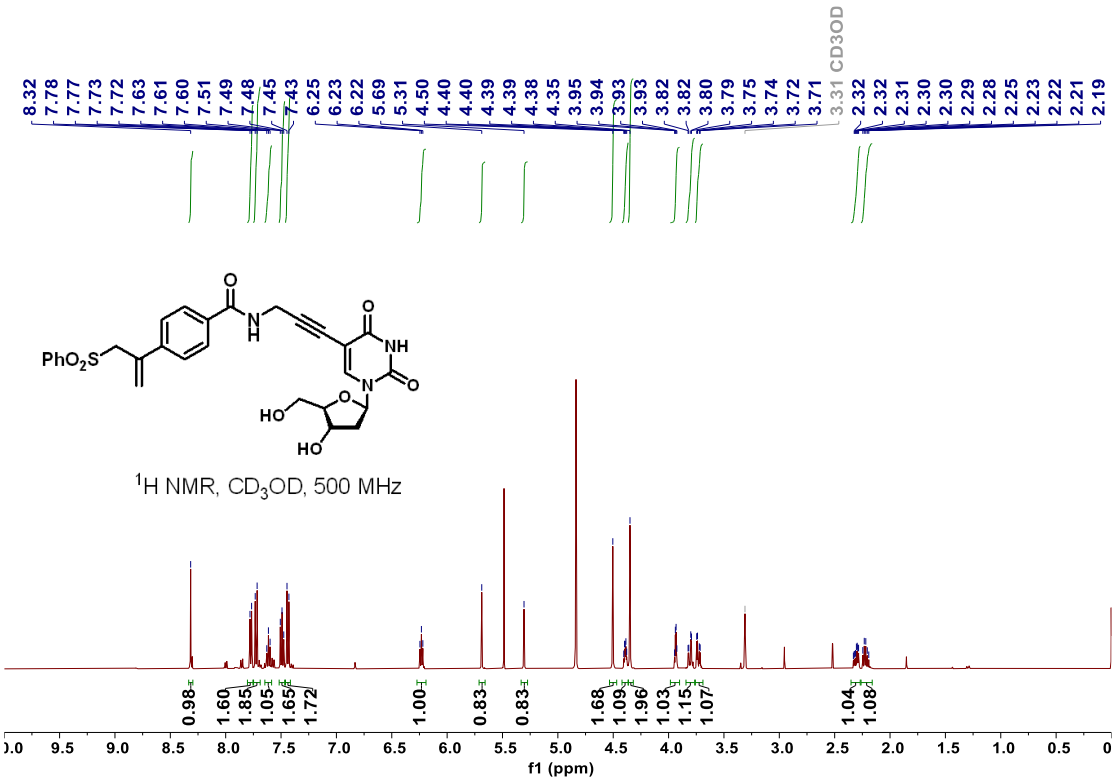


Figure S77. ^{1}H NMR spectrum of **4**

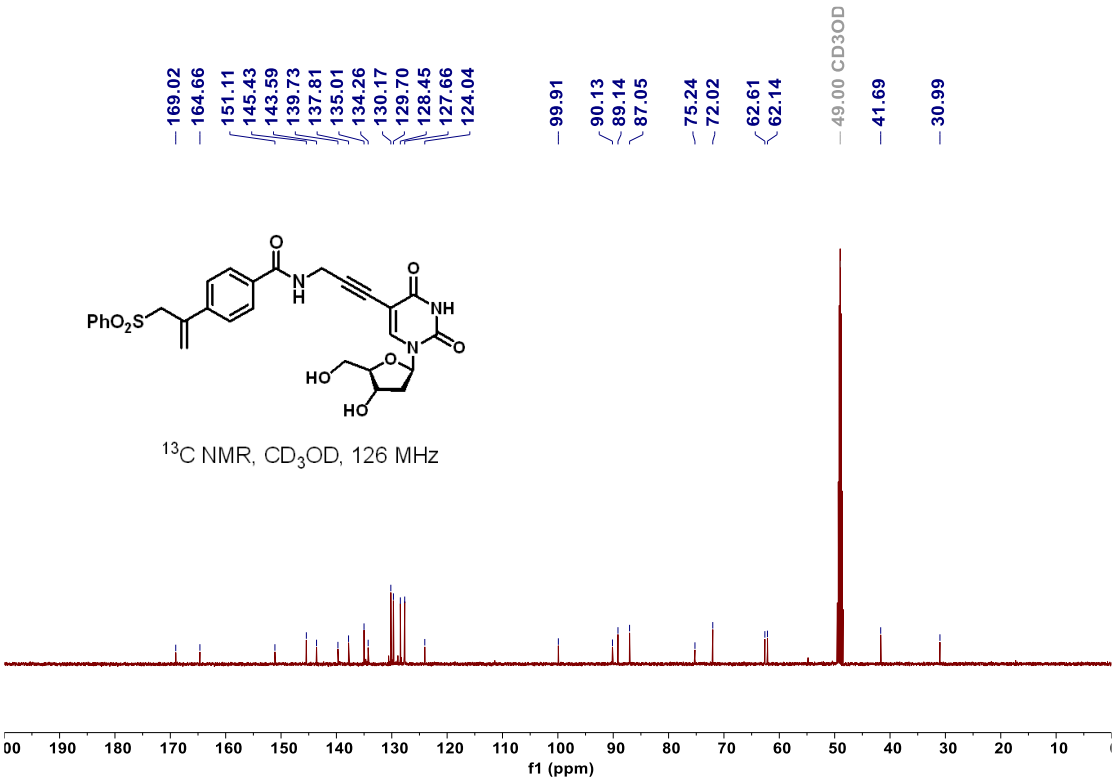


Figure S78. ^{13}C NMR spectrum of **4**

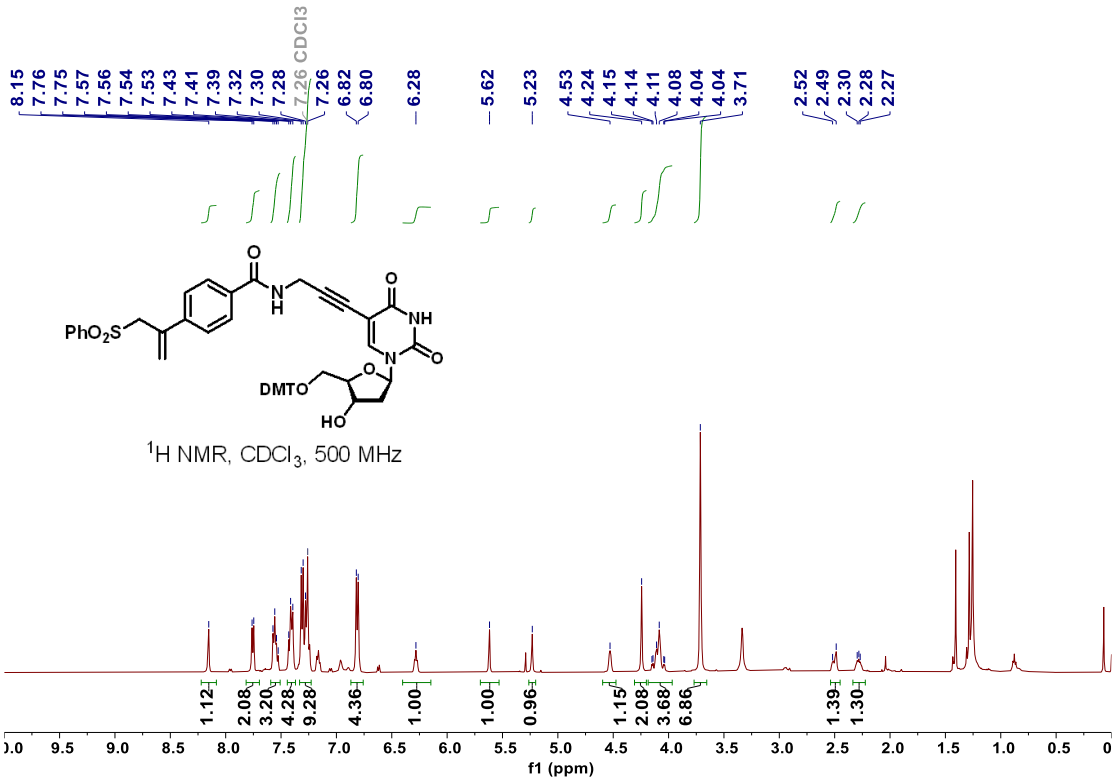


Figure S79. ^{1}H NMR spectrum of **5a**

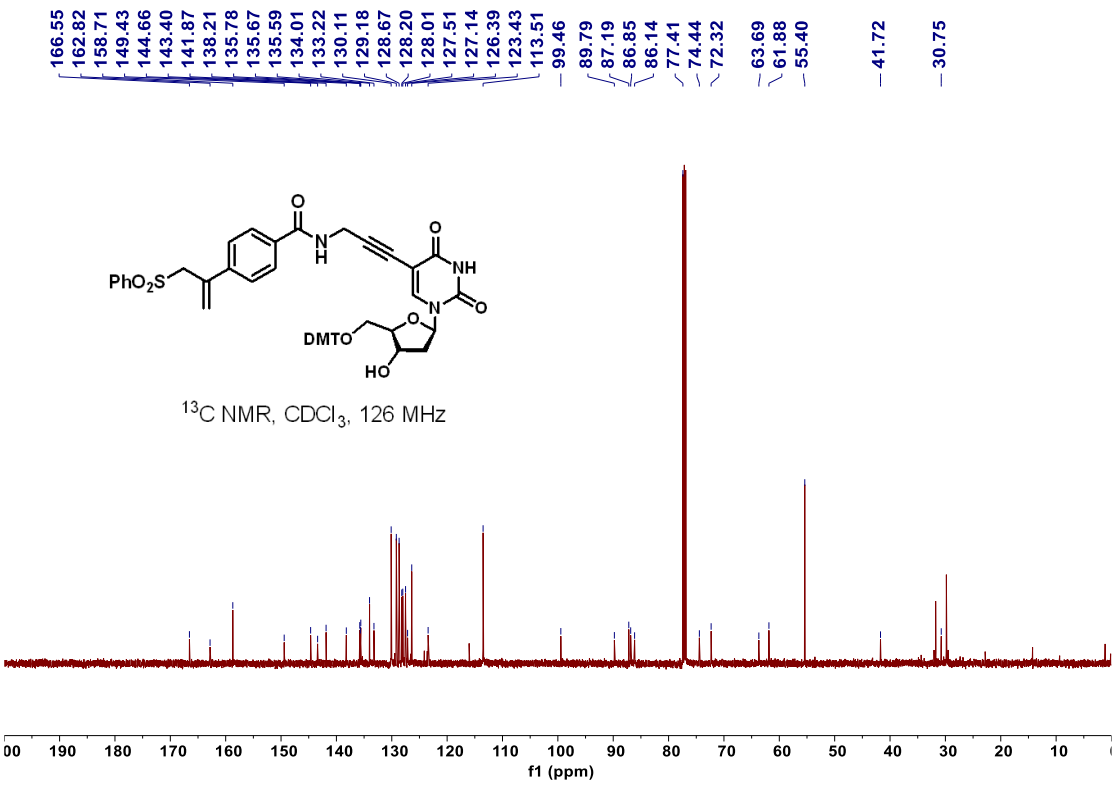


Figure S80. ^{13}C NMR spectrum of **5a**

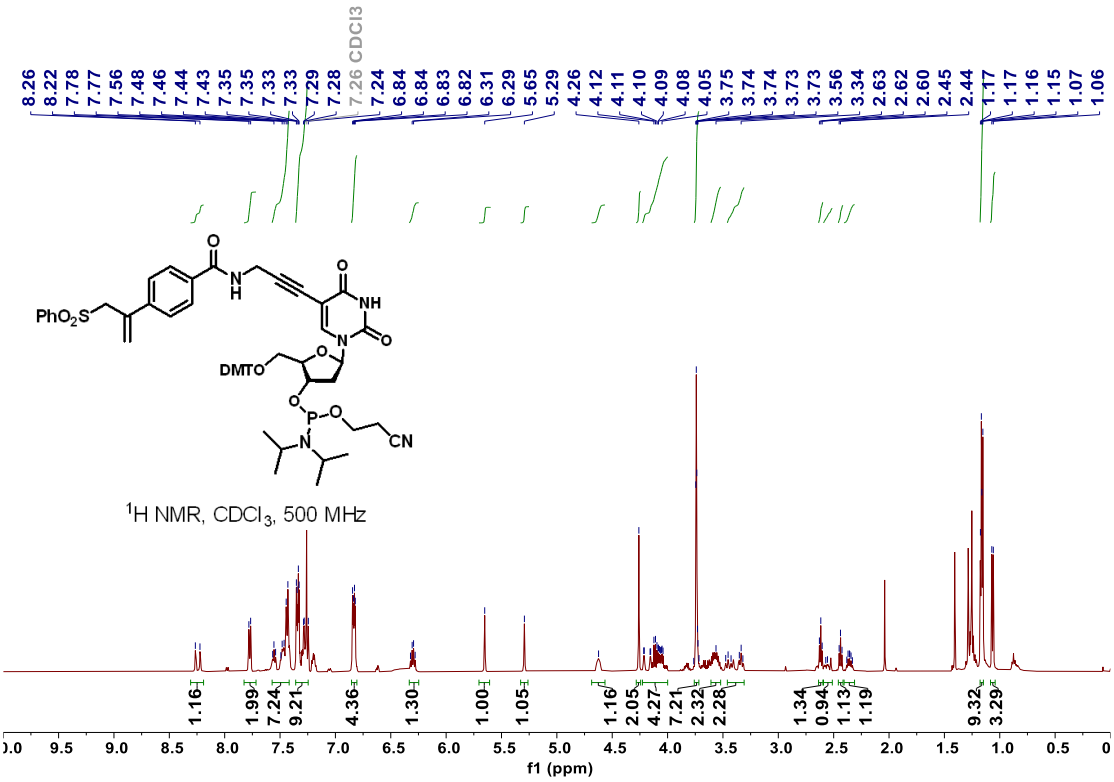


Figure S81. ^{1}H NMR spectrum of **5**

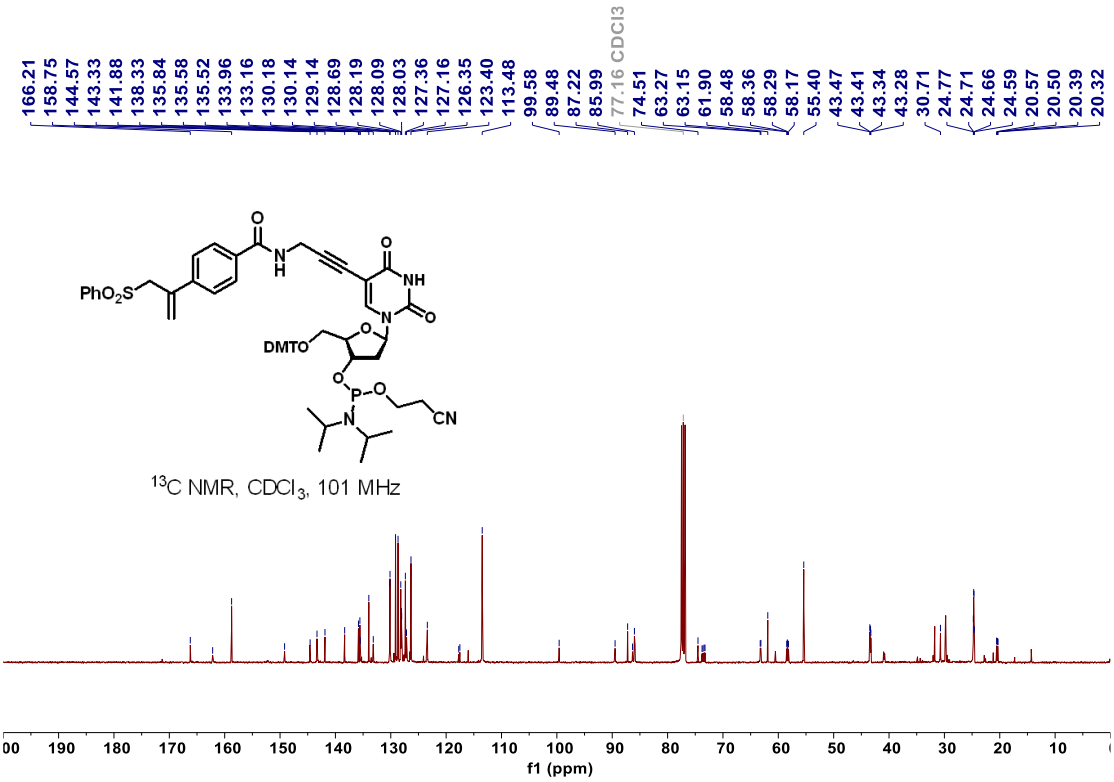


Figure S82. ^{13}C NMR spectrum of **5**

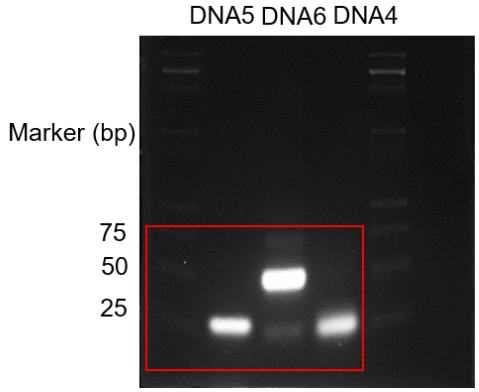
9. References

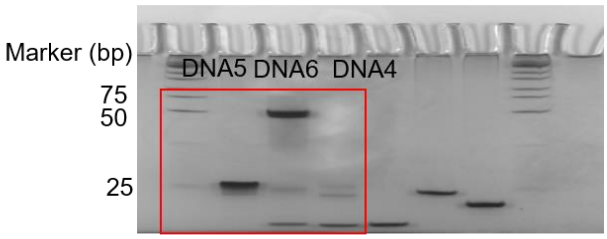
1 J. Yang, J. Zhang, L. Qi, C. Hu and Y. Chen, *Chem. Commun.*, 2015, **51**, 5275.

2 C. Hu and Y. Chen, *Org. Chem. Front.*, 2015, **2**, 1352.

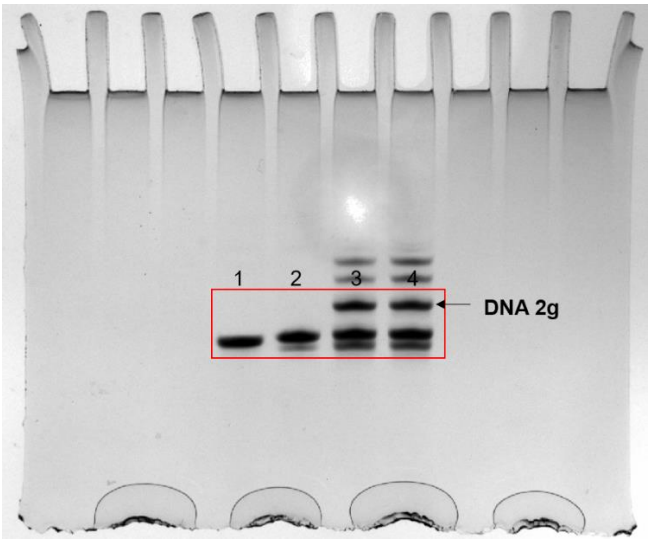
10. Raw Gel Images

Raw gel data for Scheme 3c

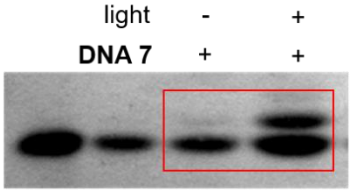


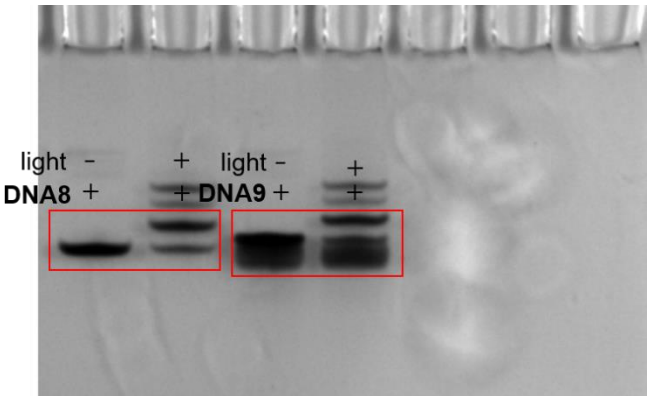


Raw gel data for Figure S5

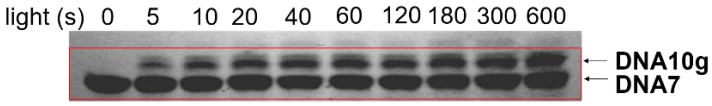


Raw gel data for Figure S7a

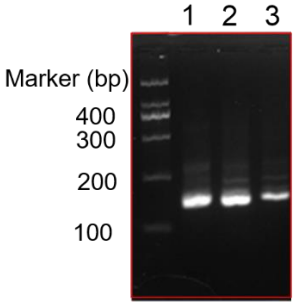




Raw gel data for Figure S7b



Raw gel data for Figure S7c



Raw gel data for Figure S7d