SUPPORTING INFORMATION

Sequence-Independent Optical Regulation of CRISPR/Cas Editing Using Star-Shaped crRNA Dendrimers

Xiao-Li Liu,[a,b] Li Liu[a,b] and Liang Cheng[a,b,*]

[a] *Beijing National Laboratory for Molecular Sciences (BNLMS), CAS Key Laboratory of Molecular Recognition and Function, CAS Research/Education Center for Excellence in Molecular Sciences, Institute of Chemistry, Chinese Academy of Sciences, Beijing 100190, China*

[b] *University of Chinese Academy of Sciences, Beijing 100049, China*

* chengl@iccas.ac.cn

1. **General information**

All chemical reagents were obtained from commercial suppliers and used without further purification. The ^{1}H, ^{13}C, and ^{31}P NMR spectra were recorded on Bruker AVANCE spectrometers operating at 400 (AVANCE III HD). Chemical shifts (δ) are reported in ppm and referenced to the residual solvent signals for ^{1}H and ^{13}C NMR. Mass spectra were acquired on a Thermo Fisher Scientific Exactive instrument. crRNAs were obtained from Biosyntech (Suzhou). Alt-R® tracrRNA and Alt-R® Genome Editing Detection Kit (T7E1) were obtained from Integrated DNA Technologies (IDT). DPBS buffer, DMEM, Opti-MEM, and Penicillin/Streptomycin were purchased from Gibco, and FBS from Biological Industries (BI). Lipofectamine 3000 was purchased from Thermo Fisher Scientific. NEB buffer 3.1 and Gel Loading Dye, Blue (6×) were purchased from New England Biolabs (NEB). 40% PAGE pre-solution, 10 ×TBE, 50×TAE, and Solar Red nucleic acid stain (10,000×) were obtained from Solarbio. 2× Taq Master Mix (Dye Plus) were purchased from Vazyme. RNA concentrations were quantified using a Nanodrop One spectrophotometer (Thermo Fisher Scientific). Gel imaging was performed on a Tanon-5200 Multi Fluorescence Imager.

2. **Chemical synthesis**

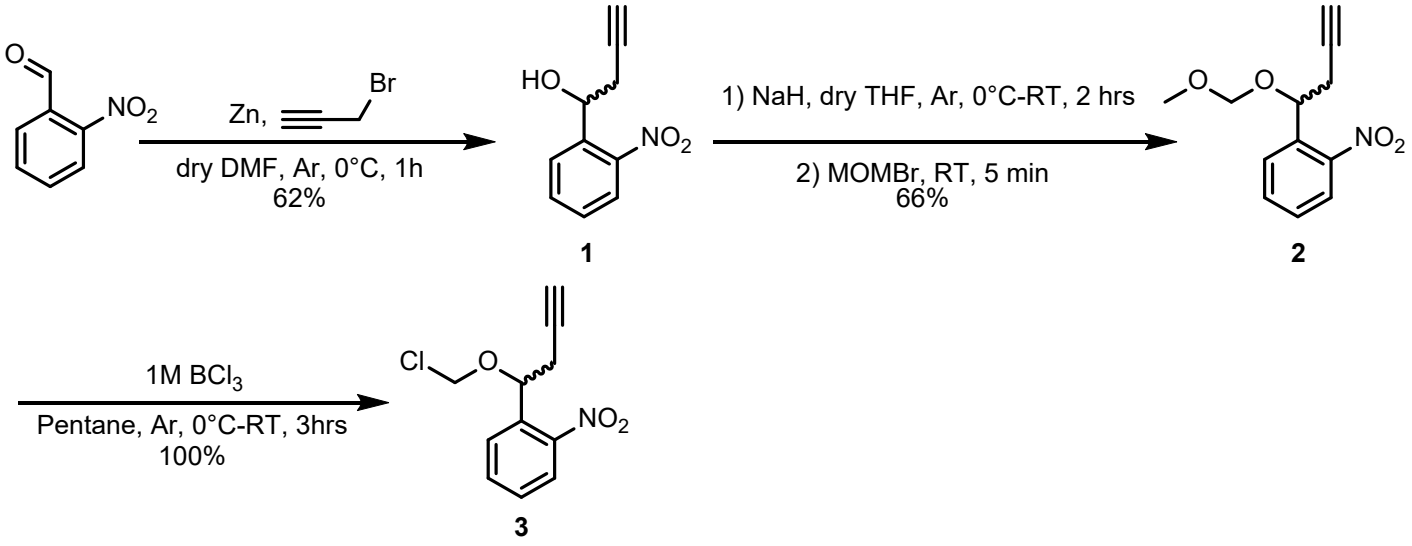


Figure S1. Synthesis of terminal alkyne photo-responsive linkage 3.

Under an argon atmosphere, activated zinc powder (8.5 g, 130 mmol) was suspended in anhydrous dimethylformamide (DMF, 150 mL) and cooled to 0°C. Propargyl bromide (8.6 mL, 150 mmol) was then added dropwise. The reaction mixture was stirred at 0°C for 1 h, after which 2-nitrobenzaldehyde (7.6 g, 50 mmol) was added, and the mixture was stirred for an additional 10 min. The reaction was quenched by the addition of saturated

ammonium chloride solution. The mixture was extracted with ethyl acetate (3×), and the combined organic layers were dried over anhydrous sodium sulfate, filtered, and concentrated to dryness under reduced pressure. The residue was purified by column chromatography (petroleum ether/ethyl acetate = 5/1) to afford intermediate 1 as a pale yellow solid. (5.9 g, yield 62%). ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.95-7.73 (d, *J* = 8.0 Hz, 1H), 7.88-7.86 (d, *J* = 8.0 Hz, 1H), 7.68-7.64 (t, *J* = 8.0 Hz, 1H), 7.47-7.43 (t, *J* = 8.0 Hz, 1H), 5.47-5.43 (m, 1H), 2.92-2.86 (m, 1H), 2.69-2.62 (m, 1H), 2.10-2.09 (t, *J* = 2.0 Hz, 1H). ^{13}C NMR (100.6 MHz, $CDCl_3$): δ 147.8, 137.8, 133.7, 128.7, 128.3, 124.6, 79.9, 71.9, 67.5, 28.6. HRMS calculated for $C_{10}H_9NO_3$ $[M-H]^-$: 190.05097, found: 190.05104.

Under an argon atmosphere, sodium hydride (2.0 g, 50 mmol, 60% dispersion in mineral oil) was suspended in anhydrous tetrahydrofuran (THF, 100 mL) and stirred. At 0 °C, a solution of compound 1 (4.8 g, 25 mmol) in THF (20 mL) was added dropwise. The mixture was stirred for 15 min at 0°C and then for an additional 2 h at room temperature. Bromomethyl methyl ether (2.6 mL, 33 mmol) was then added dropwise. After 5 min, the reaction mixture was filtered through Celite, and the filtrate was concentrated to dryness under reduced pressure. The residue was purified by column chromatography (petroleum ether/ethyl acetate = 20/1) to afford intermediate 2 as a yellow solid (3.9 g, yield 66%). ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.97-7.95 (d, *J* = 8.0 Hz, 1H), 7.86-7.84 (d, *J* = 8.0 Hz, 1H), 7.68-7.64 (t, *J* = 8.0 Hz, 1H), 7.48-7.44 (t, *J* = 8.0 Hz, 1H), 5.44-5.41 (m, 1H), 4.68-4.66 (dd, J = 26.0 Hz, 8.0 Hz, 1H), 4.55-5.53 (dd, J = 26.0 Hz, 8.0 Hz, 1H), 3.36 (s, 3H), 2.90-2.84 (m, 1H), 2.78-2.72 (m, 1H), 2.04-2.02 (t, *J* = 4.0 Hz, 1H). ^{13}C NMR (100.6 MHz, $CDCl_3$): δ 148.5, 136.6, 133.4, 129.1, 128.8, 124.6, 95.4, 80.2, 71.5, 70.9, 56.1, 27.5. HRMS calculated for $C_{12}H_{14}NO_4$ $[M+H]^+$: 236.09173, found: 236.09158.

To a suspension of intermediate 2 (1.77 g, 7.5 mmol) in pentane (30 mL) was added a solution of boron trichloride (1 M, 6 mL, in hexane) dropwise. The reaction mixture was stirred at 0°C for 15 minutes, then warmed to room temperature and stirred for an additional 3 hours. Upon completion of the reaction, as confirmed by ^1H NMR analysis, the mixture was concentrated under reduced pressure, and the resulting crude product 3 was used directly in the next step without purification.^{1}H NMR (400 MHz, $CDCl_3$): δ 8.02-8.00 (d, *J* = 8.0 Hz, 1H), 7.77-7.77 (d, *J* = 8.0 Hz, 1H), 7.70-7.66 (t, *J* = 8.0 Hz, 1H), 7.53-7.49 (t, *J* = 8.0 Hz, 1H), 5.62-5.59 (m, 2H), 5.33-5.31 (d, *J* = 8.0 Hz, 1H), 2.93-2.87 (m, 1H), 2.83-2.77 (m, 1H), 2.05-2.03 (t, *J* = 4.0 Hz, 1H). 13C NMR (100.6 MHz, $CDCl_3$): δ 148.2, 134.7, 133.5, 129.2, 129.0, 124.7, 80.5, 78.9, 74.2, 71.4, 26.9.

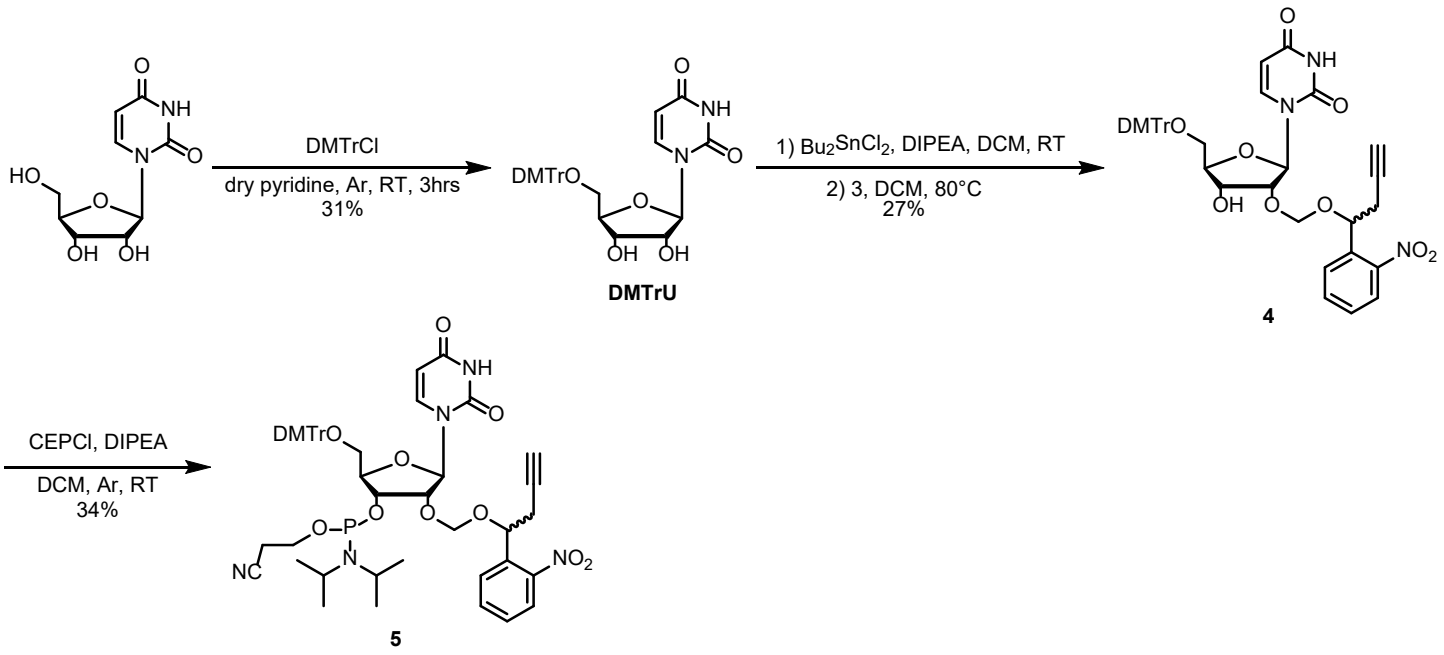


Figure S2. Synthesis of photo-responsive uridine phosphoramidite 5.

Under an argon atmosphere, 4,4'-dimethoxytrityl chloride (10.2g, 30 mmol) was added to a solution of uracil (4.9 g, 20 mmol) in anhydrous pyridine (50 mL). The reaction mixture was stirred at room temperature for 3 h and then evaporated to dryness. The residue was purified by column chromatography (dichloromethane/methanol = 50/1) to afford intermediate DMTrU as a white solid (3.3 g, yield 31%). ^{1}H NMR (400 MHz, DMSO-d_6): δ 11.36 (s, 1H), 7.73-7.71 (d, J = 8.0 Hz, 1H), 7.39-7.23 (m, 9H), 6.91-6.89 (d, J = 8.0 Hz, 4H), 5.76-5.75 (d, J = 4.0 Hz, 1H), 5.51-5.49 (d, J = 8.0 Hz, 1H), 5.32-5.30 (d, J = 8.0 Hz, 1H), 5.16-5.15 (d, J = 4 Hz, 1H), 4.10-4.08 (m, 2H), 3.97-3.94 (m, 1H), 3.74 (s, 6H), 3.29-3.20 (m, 2H). ^{13}C NMR (100.6 MHz, DMSO-d_6): δ 163.5, 158.6, 151.0, 145.2, 141.1, 135.9, 135.6, 129.4, 128.7, 127.3, 113.7, 101.9, 89.4, 86.3, 82.9, 73.9, 71.5, 70.0, 63.5, 55.5. HRMS calculated for $C_{30}H_{29}N_2O_8$[M-H]$^-$: 545.19294, found: 545.19373.

Compound DMTrU (2.7 g, 5 mmol) was dissolved in dichloromethane (50 mL), and N,N-diisopropylethylamine (DIPEA, 4.3 mL, 25 mmol) and dibutyltin dichloride (1.8 g, 6 mmol) were added successively. The reaction mixture was stirred at room temperature for 2 h, followed by slow dropwise addition of crude compound 3 (7.5 mmol, dissolved in 10 mL anhydrous dichloromethane). The mixture was then heated and stirred at 80°C for 20 min until TLC indicated the complete consumption of DMTrU. The reaction was quenched and washed three times with aqueous sodium bicarbonate. The organic phase was dried over anhydrous sodium sulfate, filtered, and concentrated to dryness under reduced pressure. The residue was purified by column chromatography

(dichloromethane/ethyl acetate = 3/1) to afford product 4 as a yellow foam (1.0 g, yield 27%). ^{1}H NMR (400 MHz, DMSO-d_6): δ 11.43-11.31 (m, 1H), 7.99-7.93 (m, 1H), 7.79-7.50 (m, 4H), 7.40-7.25 (m, 9H), 6.92-6.90 (m, 4H), 5.86-5.7 (m, 1H), 5.44-5.35 (m, 2H), 5.26-5.21 (m, 1H), 4.95-4.88 (m, 1H), 4.74-4.62 (m, 1H), 4.29-4.13 (m, 2H), 3.96-3.92 (m, 1H), 3.75 (s, 6H), 3.29-3.21 (m, 2H), 2.79-2.75 (m, 2H), 2.74-2.70 (m, 1H). HRMS calculated for $C_{30}H_{29}N_2O_8$[M+Na]$^+$: 772.24823, found: 772.24628.

Under an argon atmosphere, intermediate 4 (187.5 mg, 0.25 mmol) was dissolved in anhydrous dichloromethane (2.5 mL), and N,N-diisopropylethylamine (DIPEA, 226 μL, 1.25 mmol) was added. The reaction mixture was stirred for 5 min, followed by the addition of 2-cyanoethoxy-N,N-diisopropylaminochlorophosphine (CEPCl, 111 μL, 0.5 mmol). After stirring for 2 h, the reaction mixture was purified by column chromatography (petroleum ether/ethyl acetate = 1/2, containing 0.5% triethylamine) to afford product 15 as a yellow foam (85 mg, yield 34%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.98-7.57 (m, 4H), 7.45-7.27 (m, 10H), 6.87-6.83 (m, 4H), 6.08-5.91 (m, 1H), 5.57-5.50 (m, 1H), 5.34-4.93 (m, 3H), 4.95-4.61 (m, 2H), 4.57-4.37 (m, 2H), 4.24-4.17 (m, 1H), 3.81-3.80 (m, 6H), 3.64-3.38 (m, 5H), 2.92-2.37 (m, 5H), 1.21-0.97 (m, 12H).^{31}P NMR (161.9 MHz, $CDCl_3$): δ 150.7, 150.2. HRMS calculated for $C_{50}H_{57}N_5O_{12}P$[M+H]$^+$: 950.37359, found: 950.37170.

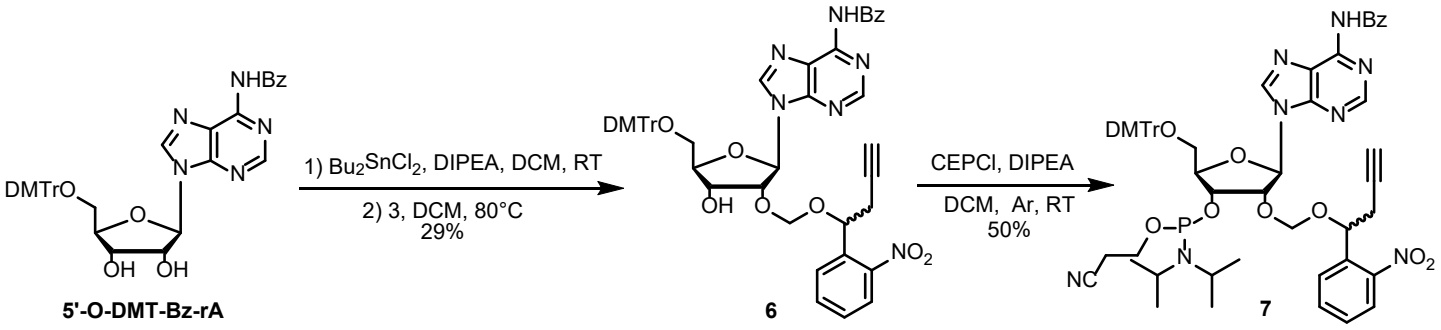


Figure S3. ynthesis of photo-responsive adenosine phosphoramidite7.

Compound 5 -O-DMT-Bz-rA (2.7 g, 4 mmol) was dissolved in dichloromethane (40 mL), and N,N-diisopropylethylamine (DIPEA, 3.5 mL, 20 mmol) and dibutyltin dichloride (1.5 g, 4.8 mmol) were added successively. The reaction mixture was stirred at room temperature for 2 h, followed by slow dropwise addition of crude compound 3 (6 mmol, dissolved in 20 mL anhydrous dichloromethane). The mixture was then heated and stirred at 80°C for 20 min until TLC analysis showed complete consumption of 5 -O-DMT-Bz-rA. The reaction was quenched and washed three times with aqueous sodium bicarbonate. The organic phase was dried over anhydrous

sodium sulfate, filtered, and concentrated to dryness under reduced pressure. The residue was purified by column chromatography (dichloromethane/ethyl acetate = 5/1) to afford compound 6 as a yellow foam (1.0 g, yield 29%).^{1}H NMR (400 MHz, $CDCl_3$): δ 9.31-9.29 (m, 1H), 8.76-8.68 (m, 1H), 8.26-8.13 (m, 1H), 8.07-7.91(m, 3H), 7.73-7.29 (m, 14H), 6.84-6.82 (m, 4H), 6.30-6.10 (m, 1H), 5.50-5.38 (m, 1H), 5.08-4.90 (m, 2H), 4.81-4.78 (m, 1H), 4.66-4.41 (m, 1H), 4.29-4.20 (m, 1H), 3.80 (s, 6H), 3.54-3.38 (m, 2H), 2.84-2.60 (m, 3H). ^{13}C NMR (100.6 MHz, $CDCl_3$): δ 158.6, 152.9, 152.7, 151.5, 151.3, 149.7, 149.5, 148.2, 148.0, 144.4, 142.0, 141.9, 135.6, 135.2, 133.7, 133.5,132.8, 130.1, 129.1, 128.8, 128.7, 128.2, 127.9, 127.0, 124.5 113.2, 87.4, 86.7, 84.0, 80.1, 72.5,72.6, 71.4, 71.3, 70.7, 70.5, 63.1, 55.3, 27.2. 14.2. HRMS calculated for $C_{49}H_{45}N_6O_{10}[M+H]^+$: 877.31917, found: 877.31659.

Under an argon atmosphere, intermediate 6 (350 mg, 0.4 mmol) was dissolved in anhydrous dichloromethane (5 mL), and N,N-diisopropylethylamine (DIPEA, 362 μL, 2.08 mmol) was added. The reaction mixture was stirred for 5 min, followed by the addition of 2-cyanoethoxy-N,N-diisopropylaminochlorophosphine (CEPCl, 180 μL, 0.8 mmol). After stirring for 2 h, the mixture was purified by column chromatography (dichloromethane/ethyl acetate = 4/1, containing 1% triethylamine) to afford product 7 as a yellow foam (200 mg, yield 50%).^{31}P NMR (161.9 MHz, $CDCl_3$): δ 150.6, 150.5.

1,3,5-tris(azidomethyl)benzene (Tris-azido-linker) was synthesized by Yingjie-Sun[1] in our group.

3. **Preparation of oligonucleotides**

Modified crRNAs were purchased from Biosyntech (Suzhou) and tracrRNA (Alt-R® tracrRNA) was purchased from Integrated DNA Technologies (IDT, Coralville, IA).

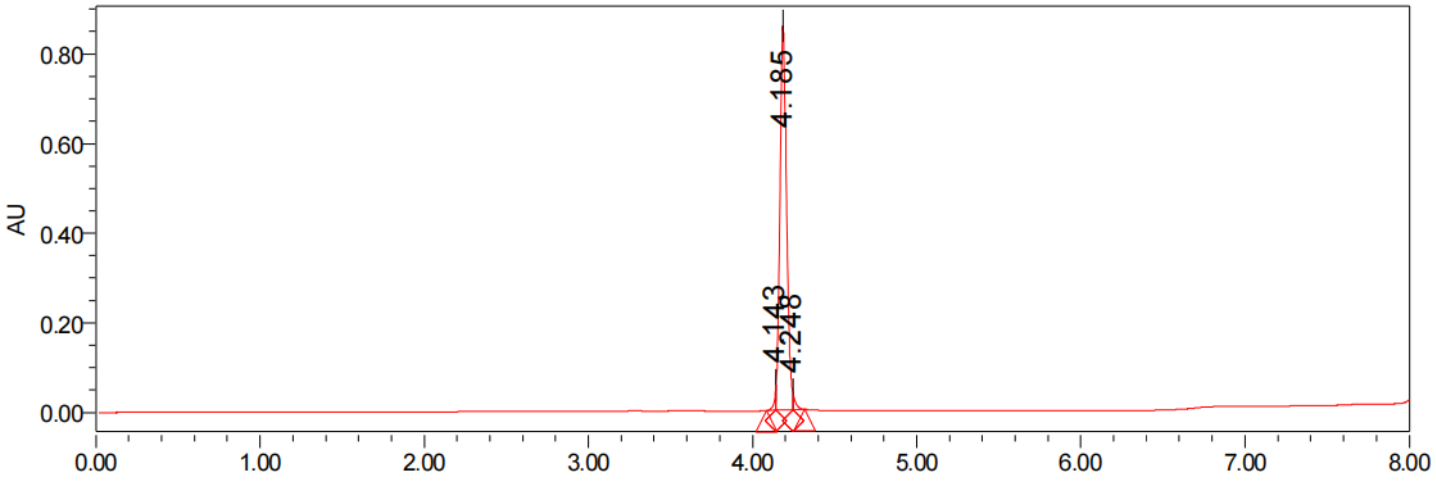


Figure S4. HPLC chromatogram of the Cas9-crRNA-GFP-U22.

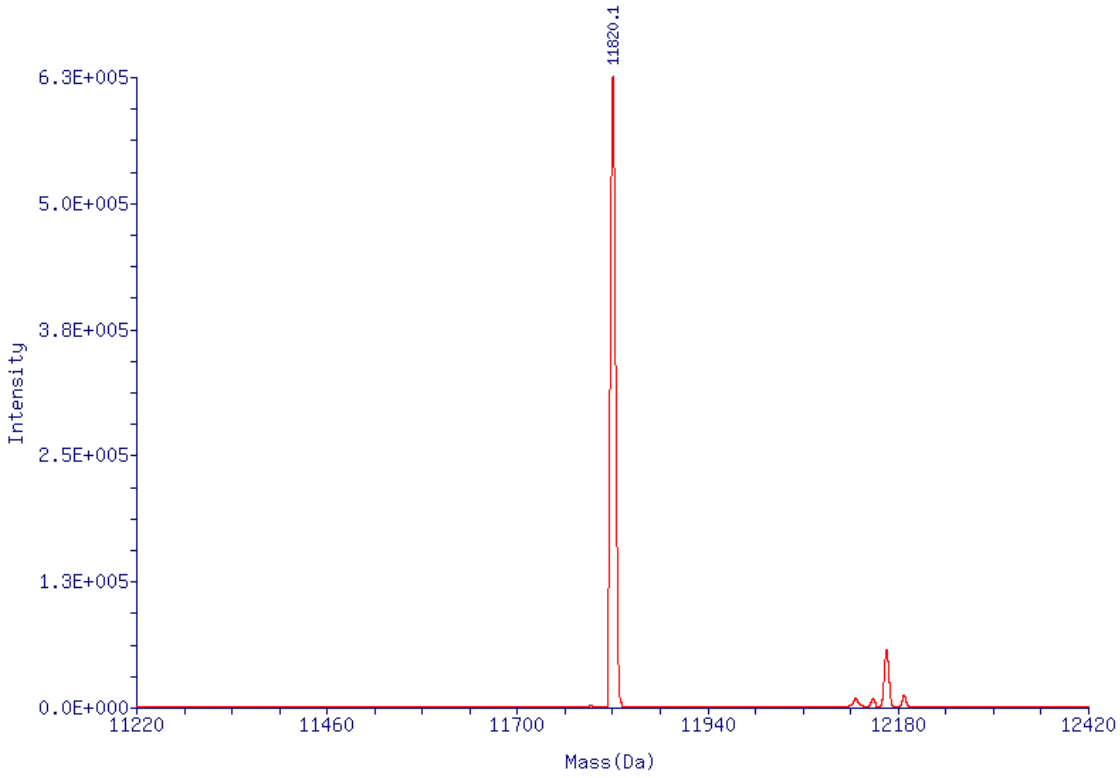


Figure S5. ESI-MS analysis of the Cas9-crRNA-GFP-U22.

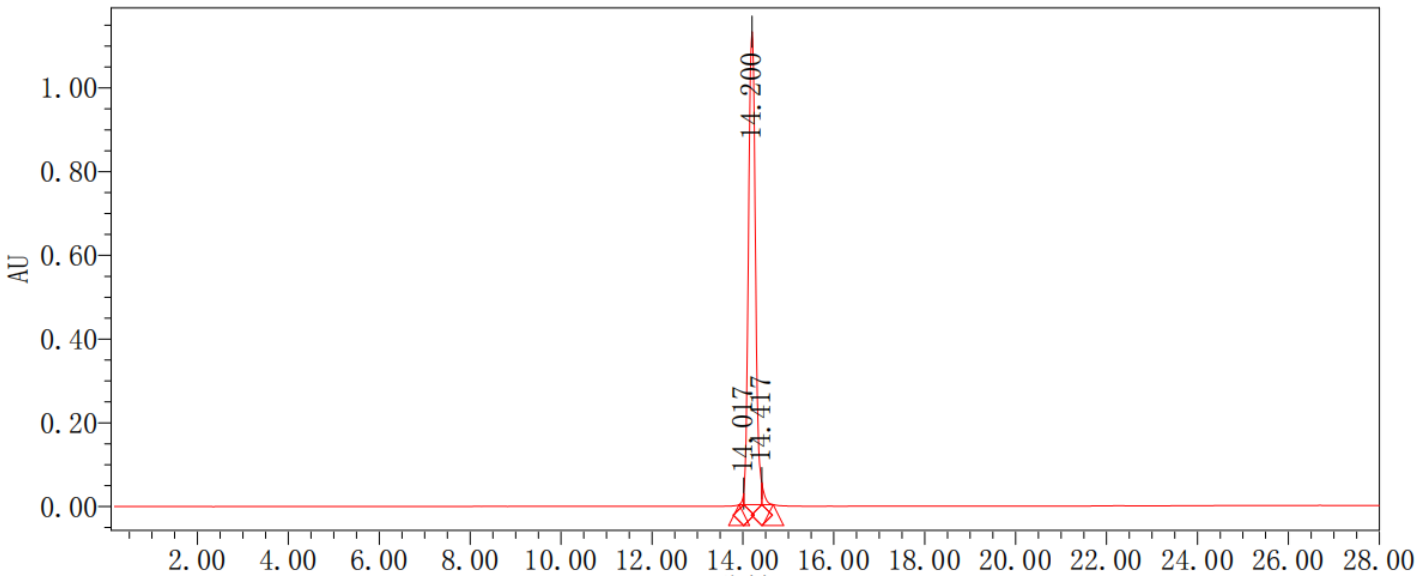


Figure S6. HPLC chromatogram of the Cas9-crRNA-EMX1-U22.

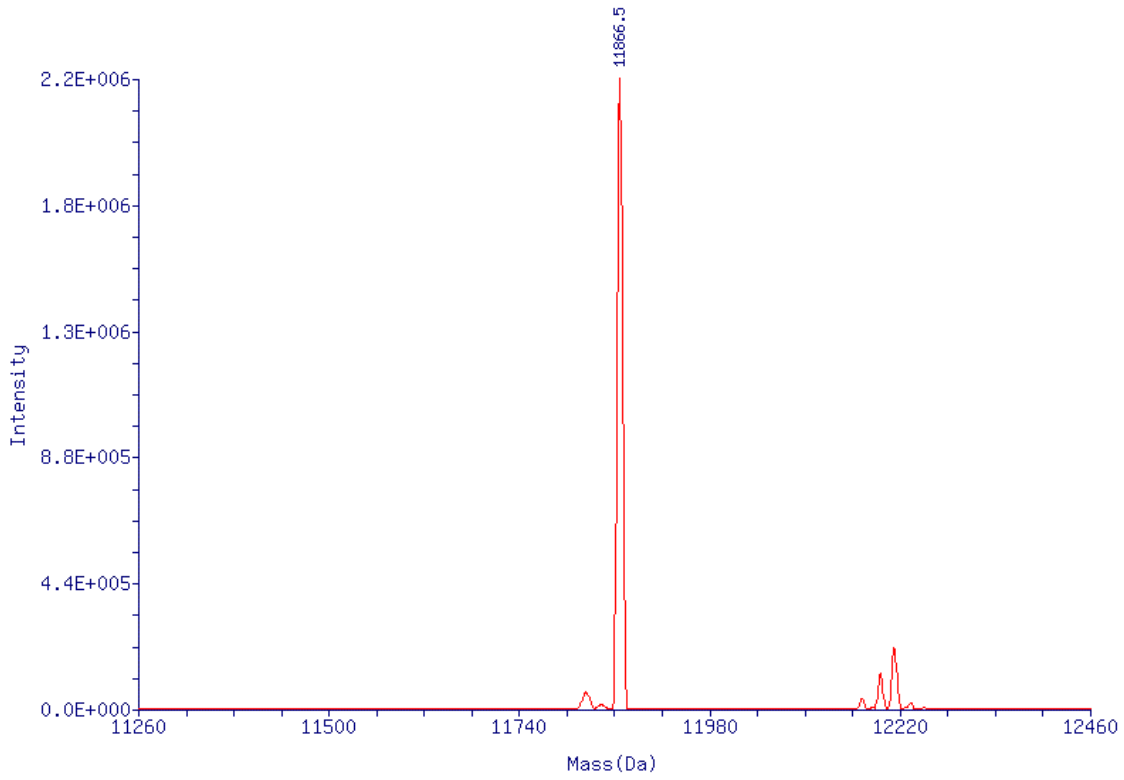


Figure S7. ESI-MS analysis of the Cas9-crRNA-GFP-U22.

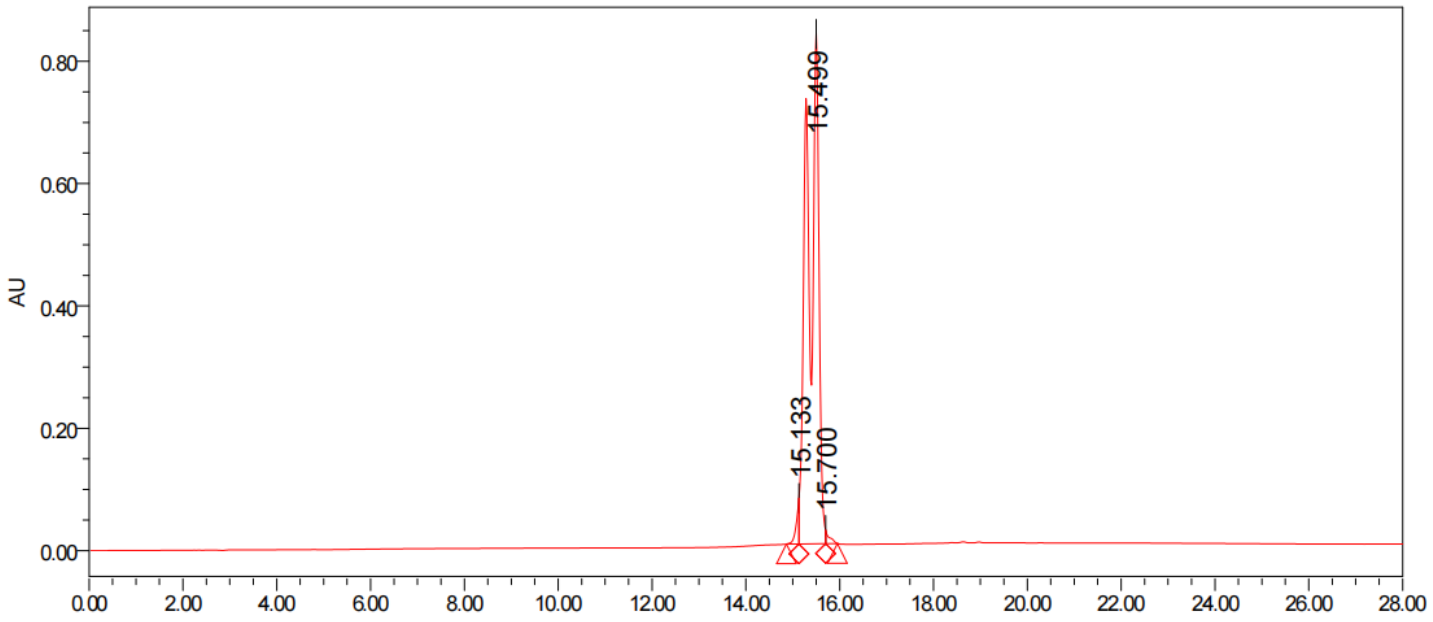


Figure S8. HPLC chromatogram of the Cas9-crRNA-VEGFA-A37.

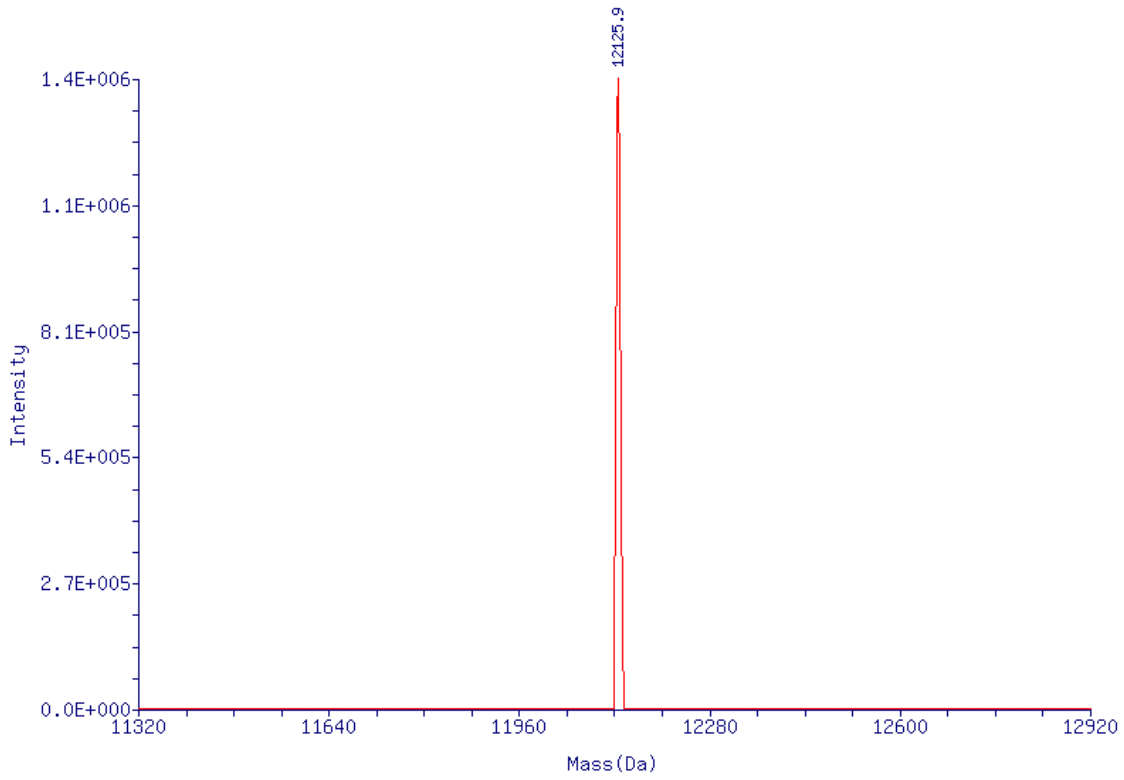


Figure S9. ESI-MS analysis of the Cas9-crRNA-VEGFA-A37.

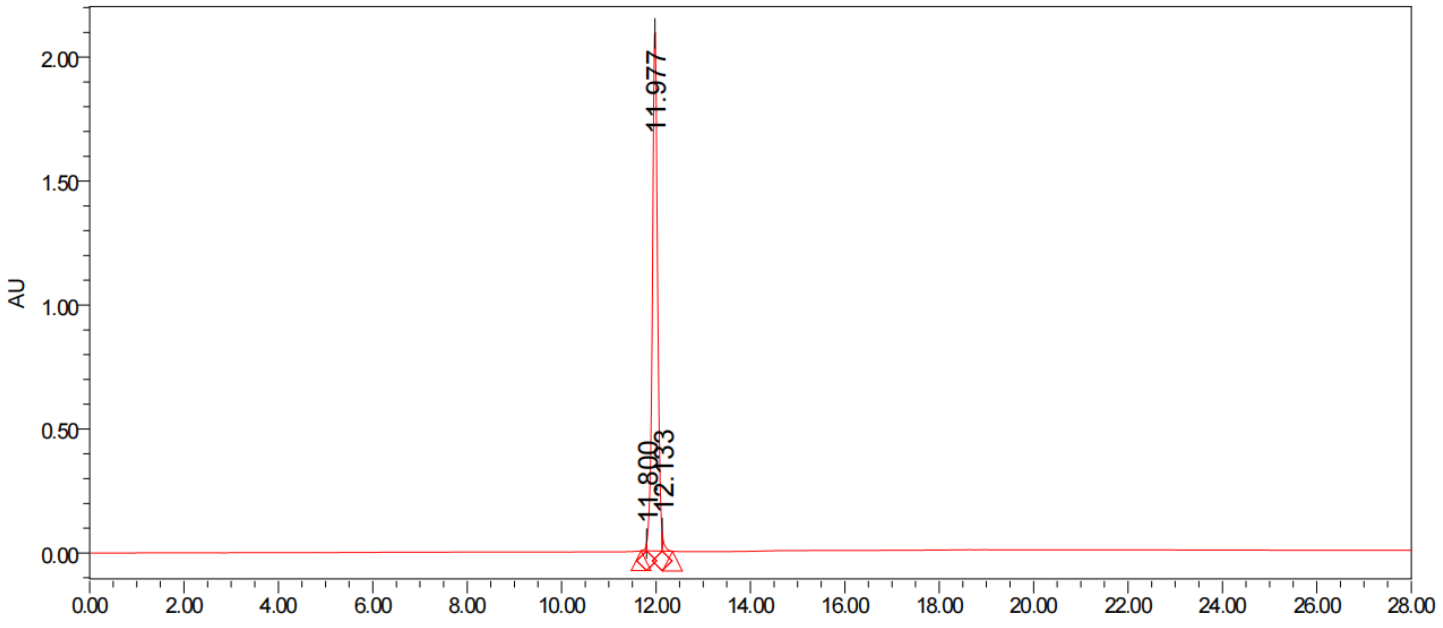


Figure S10. HPLC chromatogram of the Cas9-crRNA-VEGFA-A18.

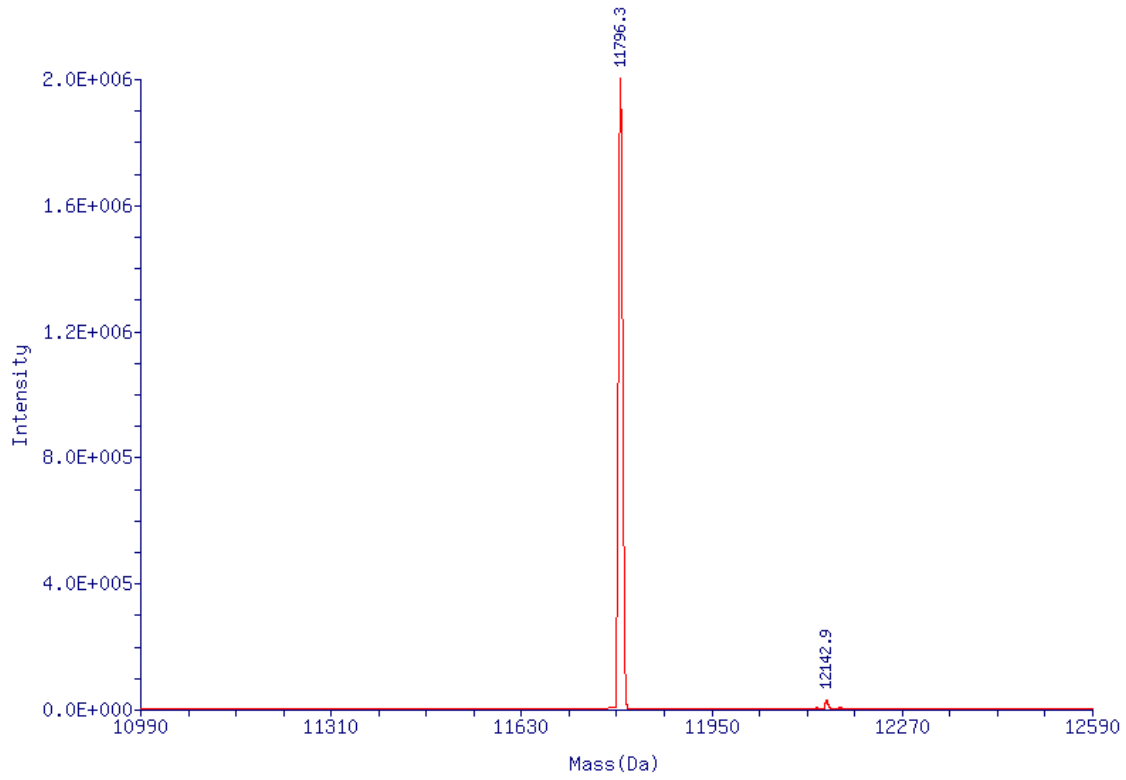


Figure S11. ESI-MS analysis of the Cas9-crRNA-VEGFA-A18.

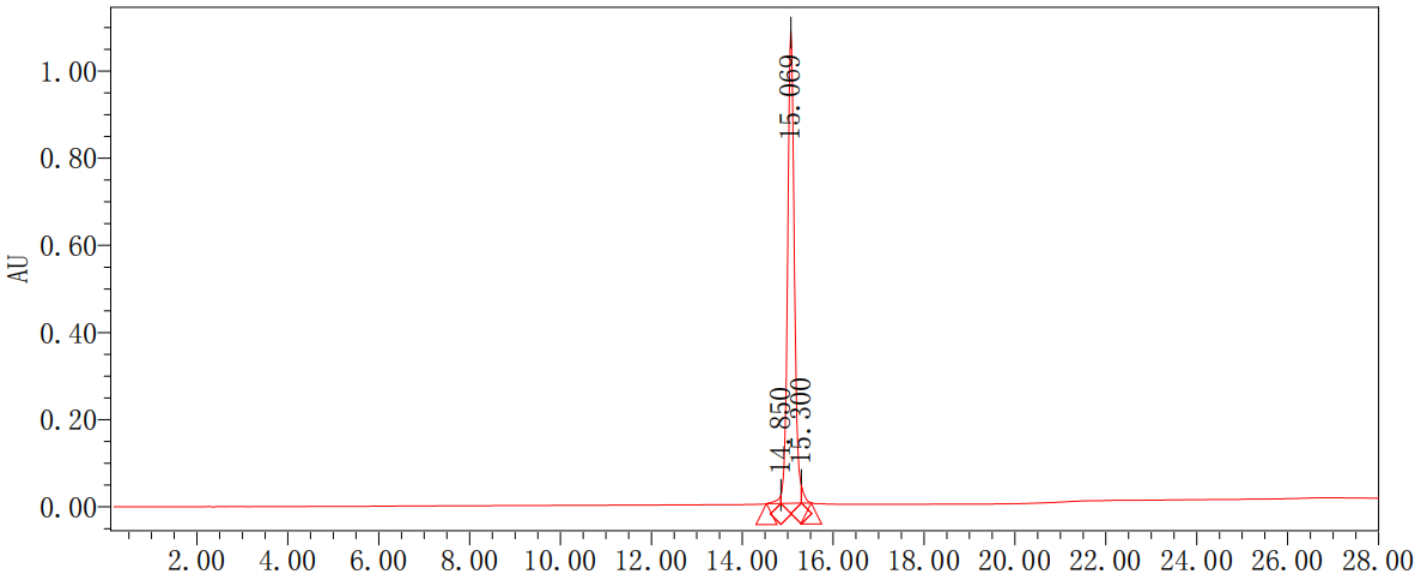


Figure S12. HPLC chromatogram of the Cas12a-crRNA-GFP-A16.

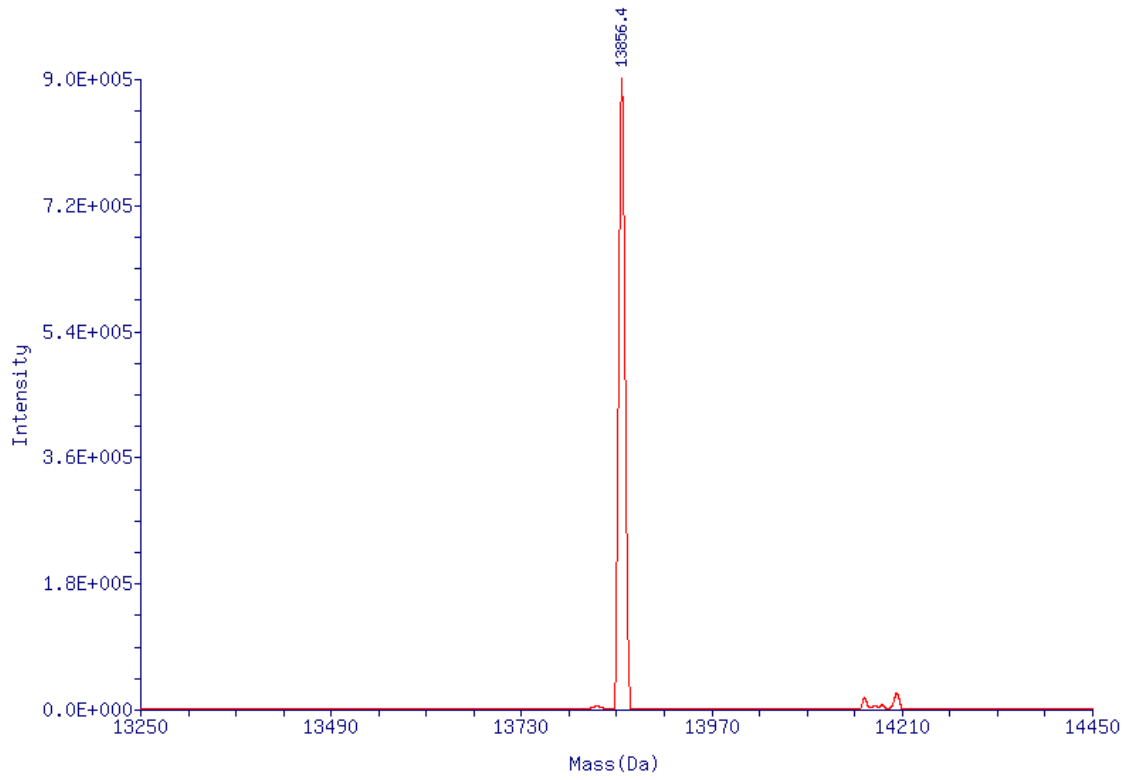


Figure S13. ESI-MS analysis of the Cas12a-crRNA-GFP-A16.

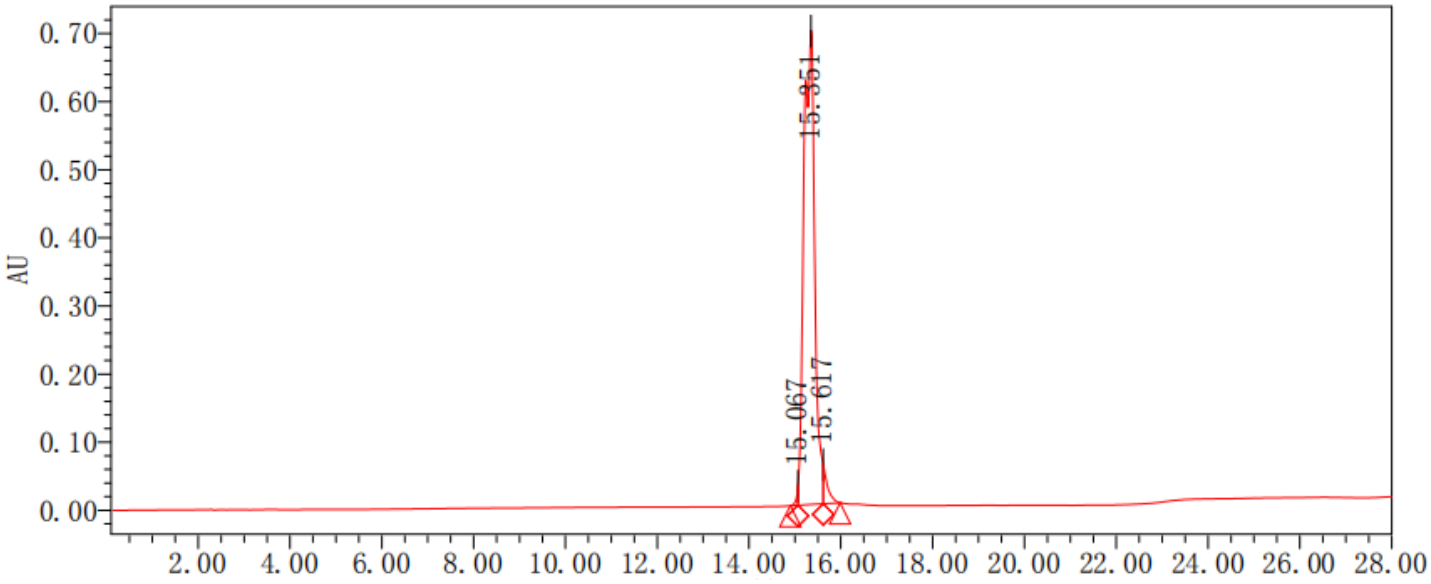


Figure S14. HPLC chromatogram of the Cas12a-crRNA-GFP-A18.

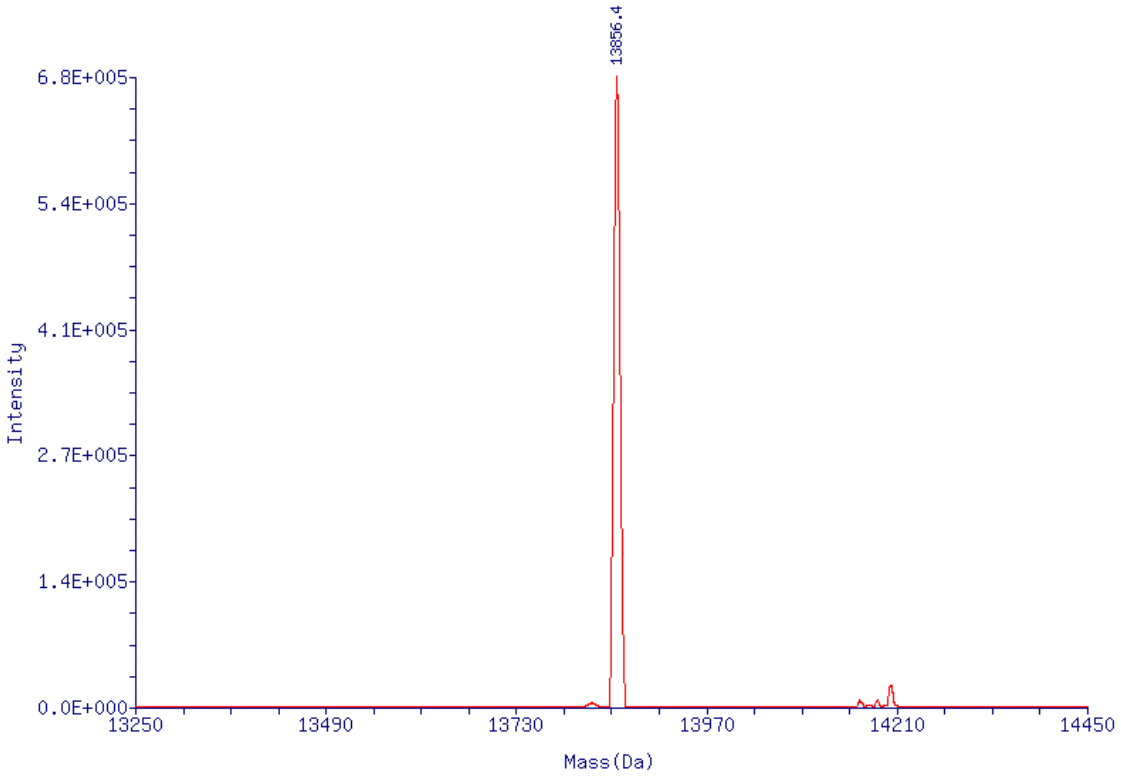


Figure S15. ESI-MS analysis of the Cas12a-crRNA-GFP-A18.

4. **General procedure for oligonucleotide trimerization**

In a reaction tube, a solution of modified crRNA (3 nmol) was sequentially added with the following reagents: Tris-azido linker (0.70 mM in water/dimethyl sulfoxide/tert-butyl alcohol = 4/3/1, 1 μL), triethylammonium acetate buffer (2 M, 4 μL, pH 7), magnesium chloride (100 mM, 2 μL), and freshly prepared ascorbic acid (125 mM, 4 μL). Dimethyl sulfoxide was added to adjust the final volume fraction to 55%, and finally a solution of copper sulfate-tris(3-hydroxypropyltriazolylmethyl)amine (250 mM in dimethyl sulfoxide/water, 2 μL, 55% v/v) was added. The reaction mixture was allowed to stand at room temperature for 4 h and analyzed by 10% denaturing polyacrylamide gel electrophoresis (PAGE). The reaction solution could then be subjected to ethanol precipitation to remove small molecules, followed by gel extraction from 10% denaturing PAGE to obtain the target product, which was further analyzed by 10% denaturing PAGE.

5. **Decaging assay of trimeric crRNA**

A sample of trimeric crRNA (10 pmol) was irradiated with UV light (365 nm, 10 $mW \cdot cm^{-2}$) for 5 min, and then analyzed by 10% denaturing polyacrylamide gel electrophoresis (PAGE) at 200 V for 45 min.

6. ***In vitro* DNA cleavage assay**

Target DNA was amplified by polymerase chain reaction (PCR) from the pEGFP-N1 vector using the primer pair GFP-FW/GFP-RV, and the product was purified with the Cycle Pure Kit (Omega BIO-TEK). The prepared crRNA/tracrRNA duplex (10 pmol each) was incubated with 10 pmol spCas9 protein and 100 ng target DNA in RNase-free water containing 1×NEB buffer 3.1 (NEB) at 37°C for 120 min. SpCas9 protein was degraded by incubation with proteinase K (TransGen Biotech) at 55°C for 10 min, and proteinase K was subsequently inactivated by heating at 95°C for 5 min. DNA cleavage products were analyzed by 2% agarose gel electrophoresis. For the light activation assay, the trimeric crRNA/tracrRNA duplex was irradiated with UV light (365 nm, 10 $mW \cdot cm^{-2}$) for 5 min prior to incubation with spCas9.

The related in vitro DNA cleavage assay for crRNA (Cas12a-GFP-crRNA) was carried out in a similar manner. The crRNA or trimeric crRNA (10 pmol each) was incubated with 10 pmol AsCas12a protein and 100 ng target DNA in RNase-free water containing 1×NEB buffer 3.1 (NEB) at 37°C for 120 min. AsCas12a protein was degraded by incubation with proteinase K at 55°C for 10 min, and proteinase K was subsequently inactivated by heating at 95°C for 5 min. DNA cleavage products were also analyzed by 2% agarose gel electrophoresis.

7. Light-activated endogenous VEGFA gene editing in cells

293T-Cas9 cells stably expressing Cas9 protein (Ubigene) were seeded in 48-well plates at a density of 80,000 cells per well and cultured in DMEM supplemented with 10% FBS for 12 h prior to transfection. Unmodified crRNA and trimeric-crRNA were premixed with tracrRNA at ratios of 1:1 and 1:3, respectively, in Nuclease Free Duplex Buffer (Integrated DNA Technologies). For transfection, crRNA/tracrRNA duplexes (40 pmol per well) were combined with Lipofectamine 3000 in 25 μL Opti-MEM (Gibco) and incubated for 15 min at room temperature before addition to the cells. After 6 h of incubation, the medium was replaced with complete DMEM, and the cells were further cultured for an additional 42 h at 37 °C.Genomic DNA was extracted using the TIANamp Genomic DNA Kit (Vazyme), and the target VEGFA locus was amplified by PCR. The primer sequences were as follows: VEGFA-FW, 5 -CCTCTCTGCTCTTATGGTGCC-3; VEGFA -RV, 5 -TCCGGGCTCGGTGATTTAG-3. PCR product purity was confirmed by 2% agarose gel electrophoresis. T7E1 assays were performed according to the manufacturer's protocol, and the digested products were analyzed using 2% agarose gels.For light-activated genome editing, 293T-Cas9 cells were transfected with 40 pmol trimeric-crRNA/tracrRNA duplexes as described above. Six hours after

transfection, cells were irradiated with 365 nm UV light (10 mW·cm^{-2}) for 5 min using a UV lamp, followed by replacement with fresh culture medium.

8. Supplementary tables and figures

Table S1. DNA and RNA sequences used in the study(The red bold letters indicate the modification sites).

Names	Sequences (5'-3')	calcd MW	found MW
Cas9-crRNA-GFP-U22	GGGCACGGGCAGCUUGCCGGG**U**UUUAGAGCUAUGCU		
Cas9-crRNA-EMX1-U22	GAGUCCGAGCAGAAGAAGAAG**U**UUUAGAGCUAUGCU		
Cas9-crRNA-VEGFA-A37	UCAGAUGUGACAAGCCGAGGGUUUUAGAGCUAUGCU**A**		
Cas9- crRNA-VEGFA-A18	UCAGAUGUGACAAGCCG**A**GGGUUUUAGAGCUAUGCU		
Cas12a-crRNA-GFP-A16	AAUUUCUACUCUUGU**A**GAUCGUCGCCGUCCAGCUCGACCAGGA		
Cas12a-crRNA-GFP-A18	AAUUUCUACUCUUGUAG**A**UCGUCGCCGUCCAGCUCGACCAGGA		
Cas9-crRNA-GFP-U22$^{3/3}$		35694.8	36820.9
Cas9-crRNA-EMX1-U22$^{3/3}$		35839.1	37290.9
Cas9-crRNA-VEGFA-A37$^{3/3}$		36612.5	37773.9
Cas9-crRNA-VEGFA-A18$^{3/3}$		35624.9	36408.2
Cas12a-crRNA-GFP-A16$^{3/3}$		41800.4	42988.6
Cas12a-crRNA-GFP-A18$^{3/3}$		41800.4	43351.9
GFP-FW	CGGTTTGACTCACGGGGATT		
GFP-RV	CTCGATGTTGTGGCGGATCT		

EMX1-FW	CATCCACTCTGTGAAGAAGCG
EMX1-RV	CAAAAGGGAGATTGGAGACACG
VEGFA-FW	CCTCTCTGCTCTTATGGTGCC
VEGFA-RV	TCCGGGCTCGGTGATTTAG

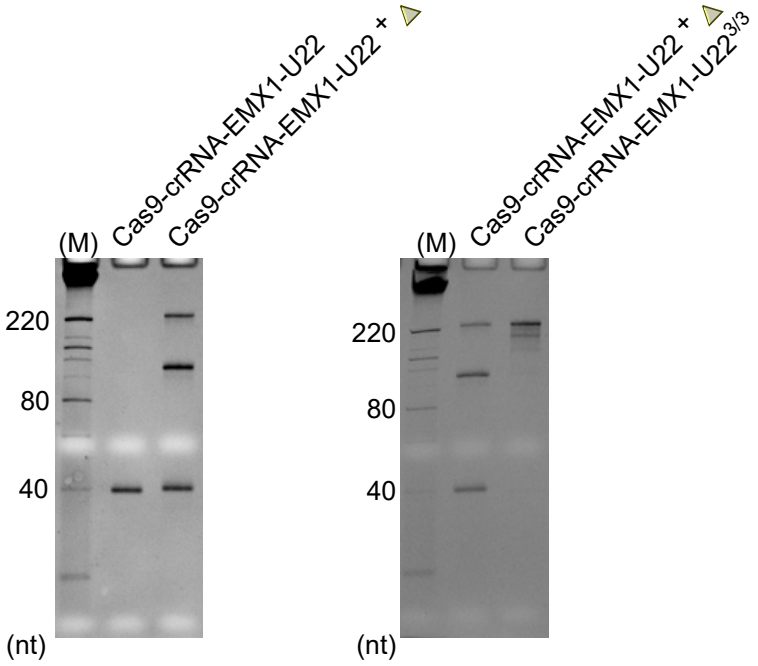


Figure S16. Left: PAGE analysis of Cas9-crRNA-EMX1-U22 reacted with tri-azide benzene, generating multivalent Cas9-crRNA-EMX1-U22$^{n/3}$. Right: Purification of the reaction mixture of Cas9-crRNA-EMX1-U22 with tri-azide benzene to afford Cas9-crRNA-GFP-U22$^{3/3}$.

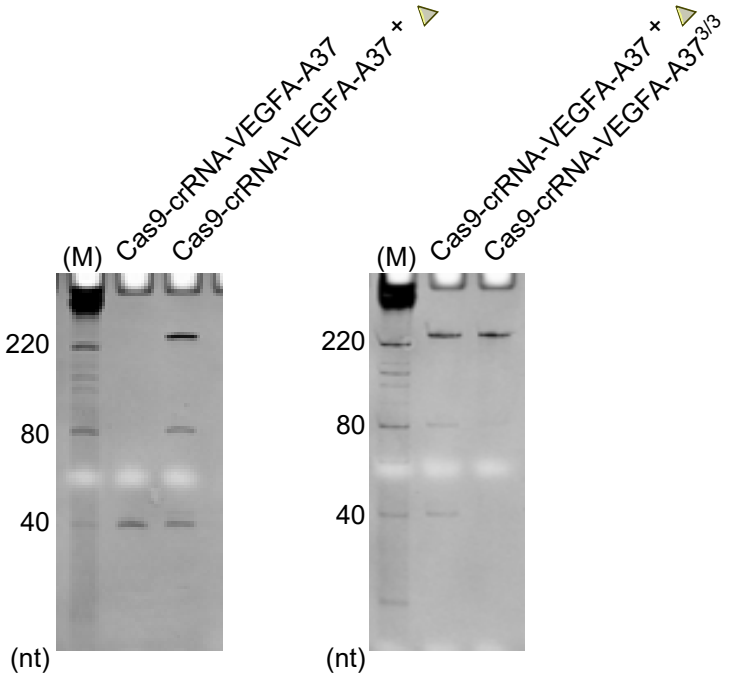


Figure S17. Left: PAGE analysis of Cas9-crRNA-VEGFA-A37 reacted with tri-azide benzene, generating multivalent Cas9-crRNA-VEGFA-A37$^{n/3}$. Right: Purification of the reaction mixture of Cas9-crRNA-VEGFA-A37 with tri-azide benzene to afford Cas9-crRNA-VEGFA-A37$^{3/3}$.

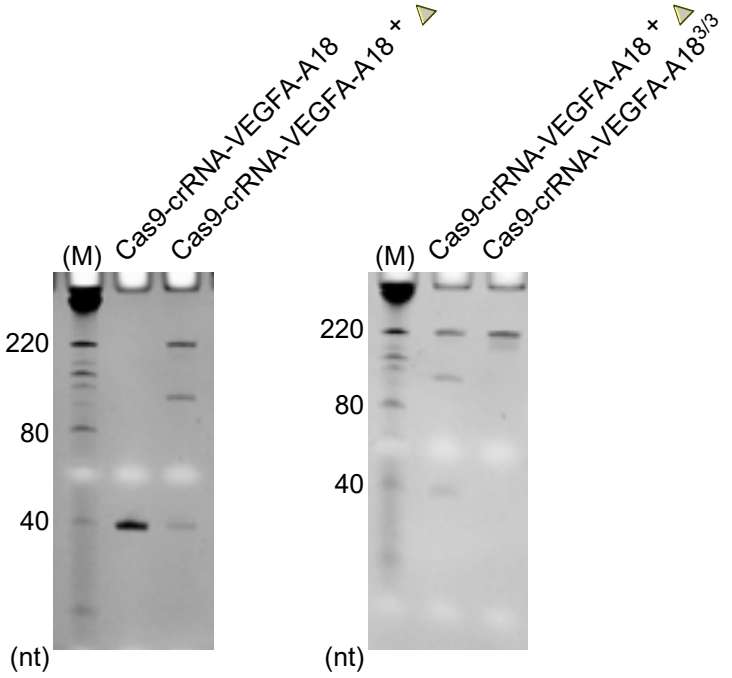


Figure S18. Left: PAGE analysis of Cas9-crRNA-VEGFA-A18 reacted with tri-azide benzene, generating multivalent Cas9-crRNA-VEGFA-A18$^{n/3}$. Right: Purification of the reaction mixture of Cas9-crRNA-VEGFA-A18 with tri-azide benzene to afford Cas9-crRNA-VEGFA-A18$^{3/3}$.

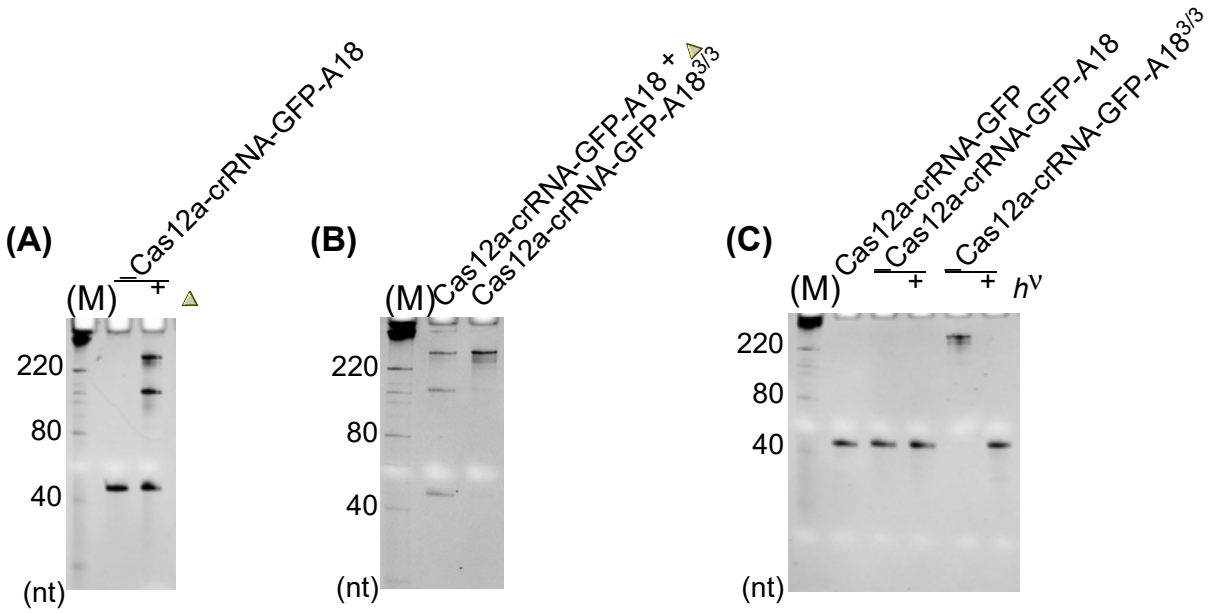


Figure S19. A: PAGE analysis of Cas12a-crRNA-GFP-A18 reacted with tri-azide benzene, generating multivalent Cas12a-crRNA-GFP-A18$^{n/3}$. B: Purification of the reaction mixture of Cas9-crRNA-VEGFA-A18 with tri-azide benzene to afford Cas9-crRNA-VEGFA-A18$^{3/3}$. C:PAGE analysis of the photo-cleavage of Cas12a-crRNA-GFP-A18$^{3/3}$ under 365 nm irradiation (10 mW·cm^{-2}, 300 s). Monomeric crRNA-GFP-A18 was used as the reference.

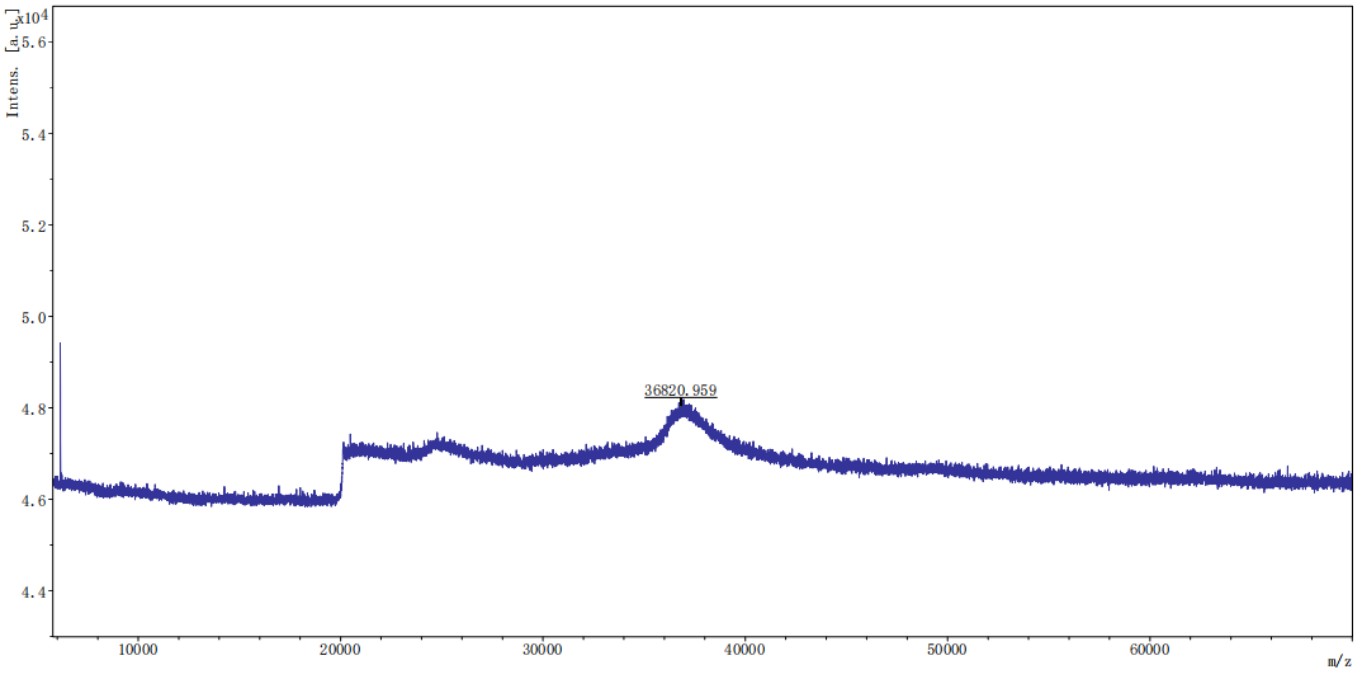


Figure S20. MALDI-TOF of Cas9-crRNA-GFP-U22$^{3/3}$.

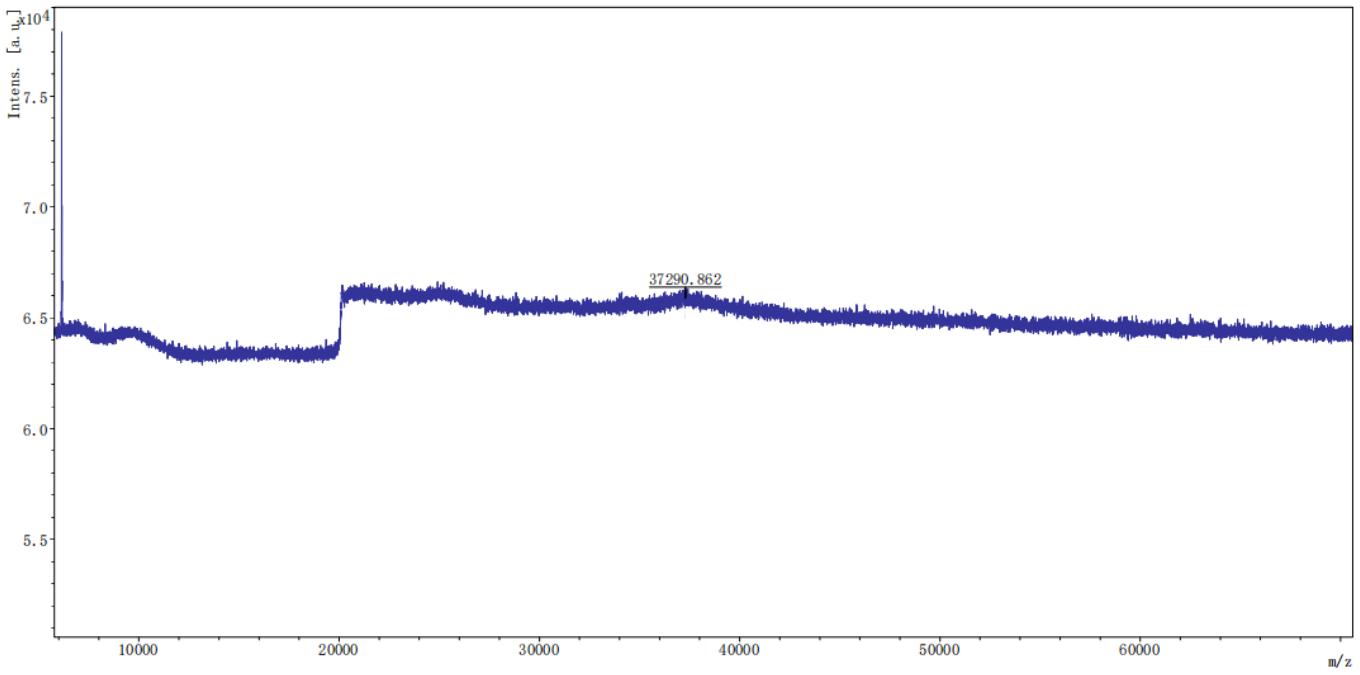


Figure S21. MALDI-TOF of Cas9-crRNA-EMX1-U22$^{3/3}$.

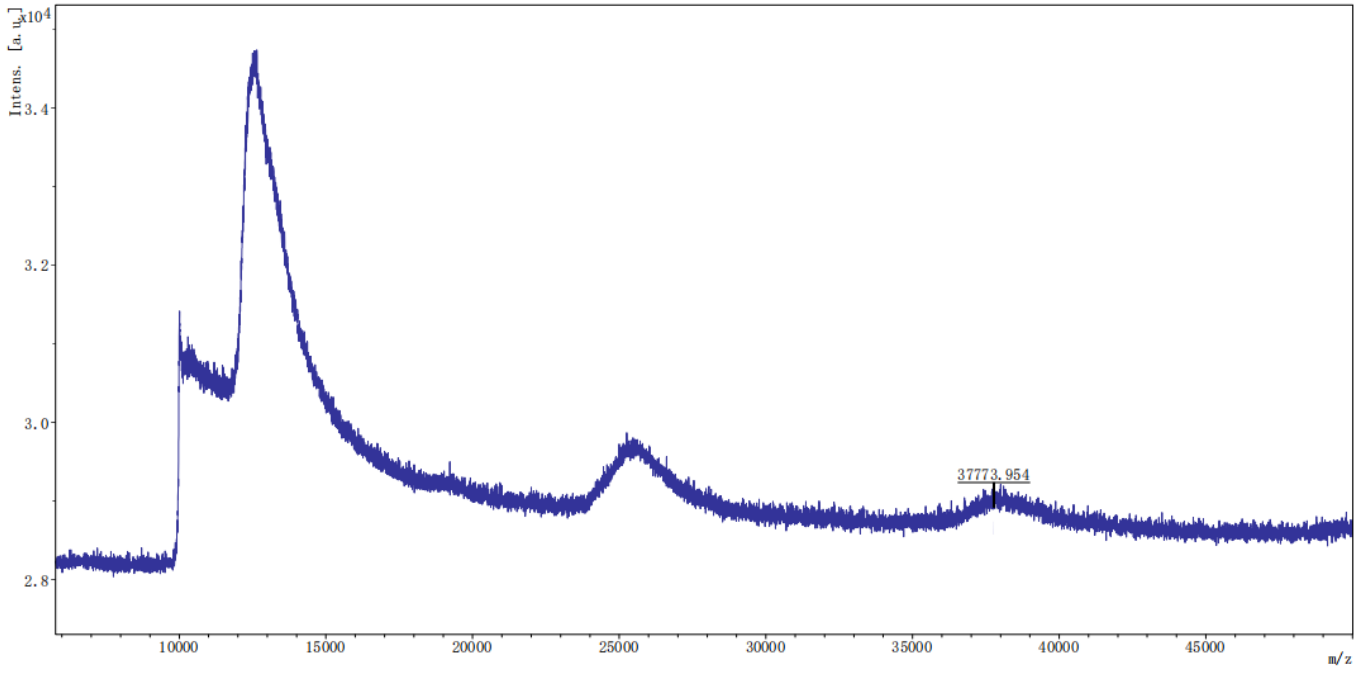


Figure S22. MALDI-TOF of Cas9-crRNA-VEGFA-A37$^{3/3}$.

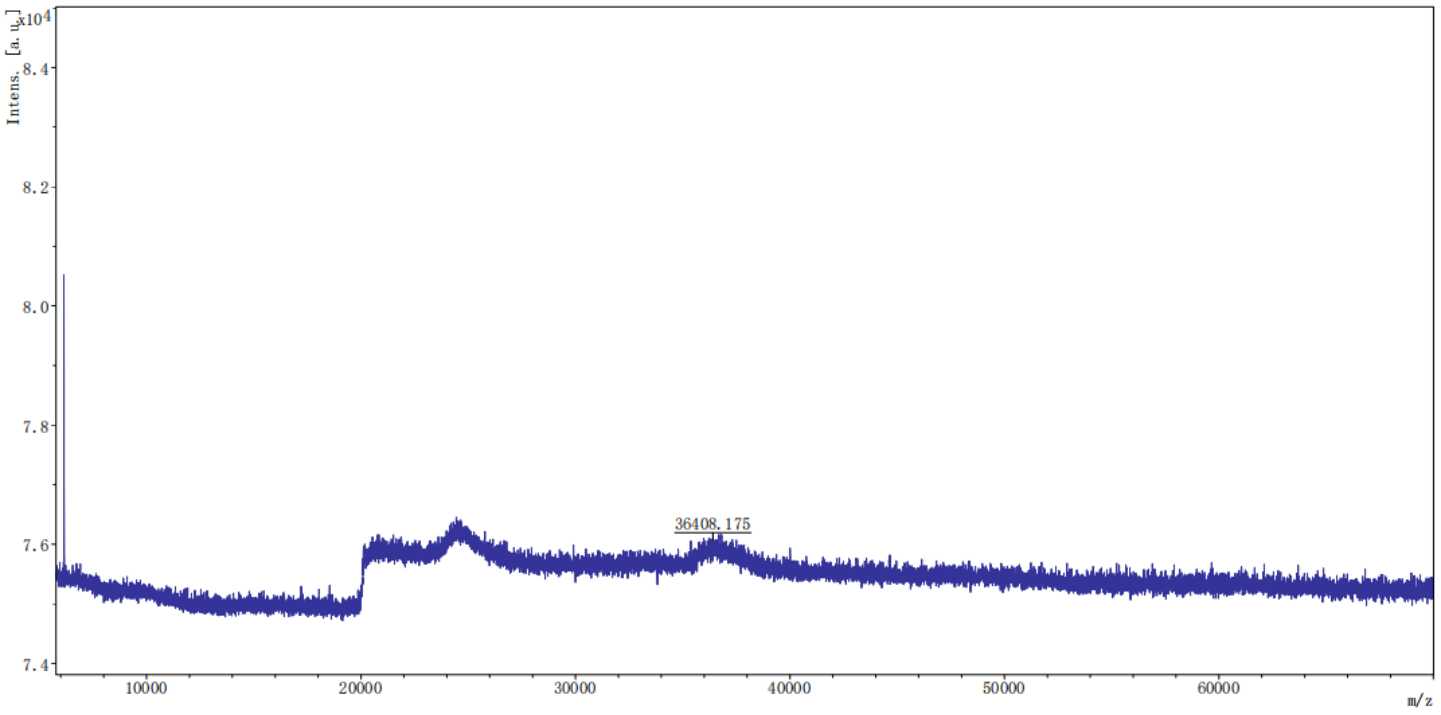


Figure S23. MALDI-TOF of Cas9-crRNA-VEGFA-A18$^{3/3}$.

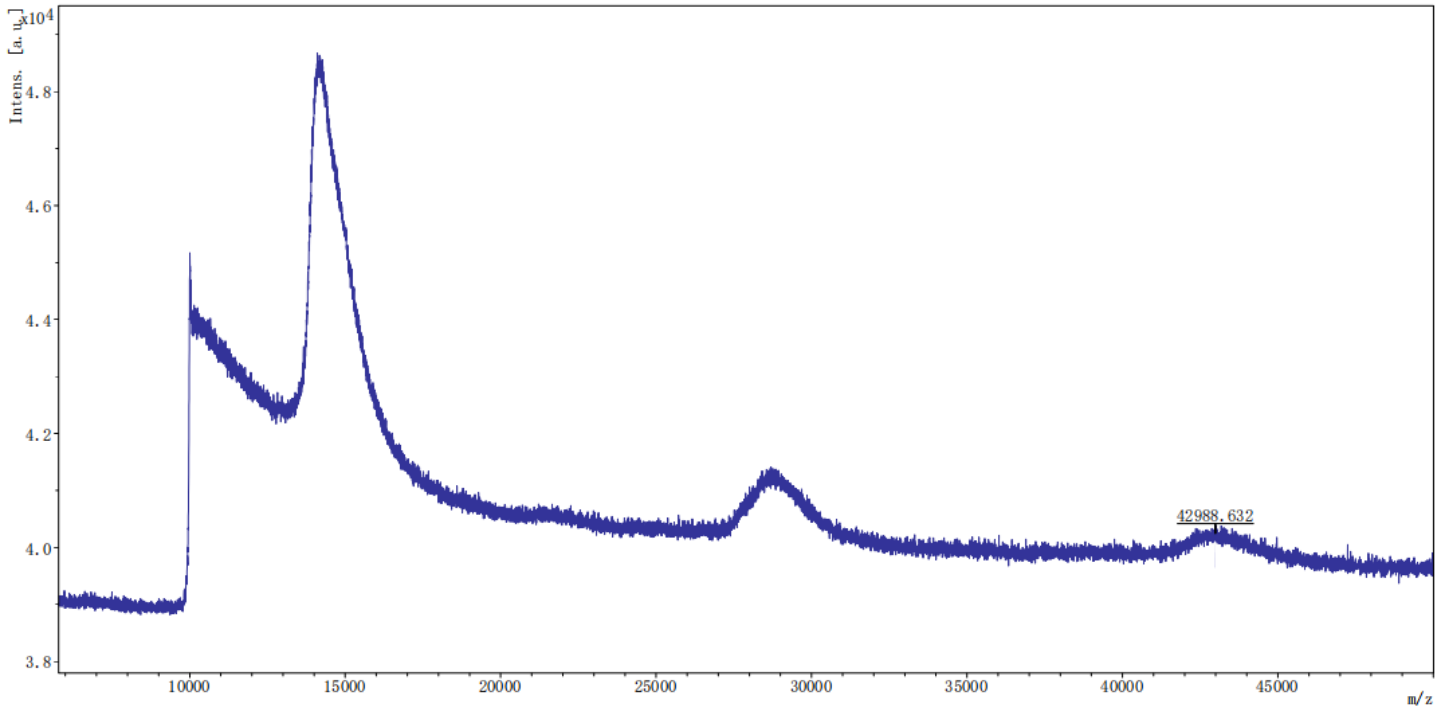


Figure S24. MALDI-TOF of Cas12a-crRNA-GFP-A16$^{3/3}$.

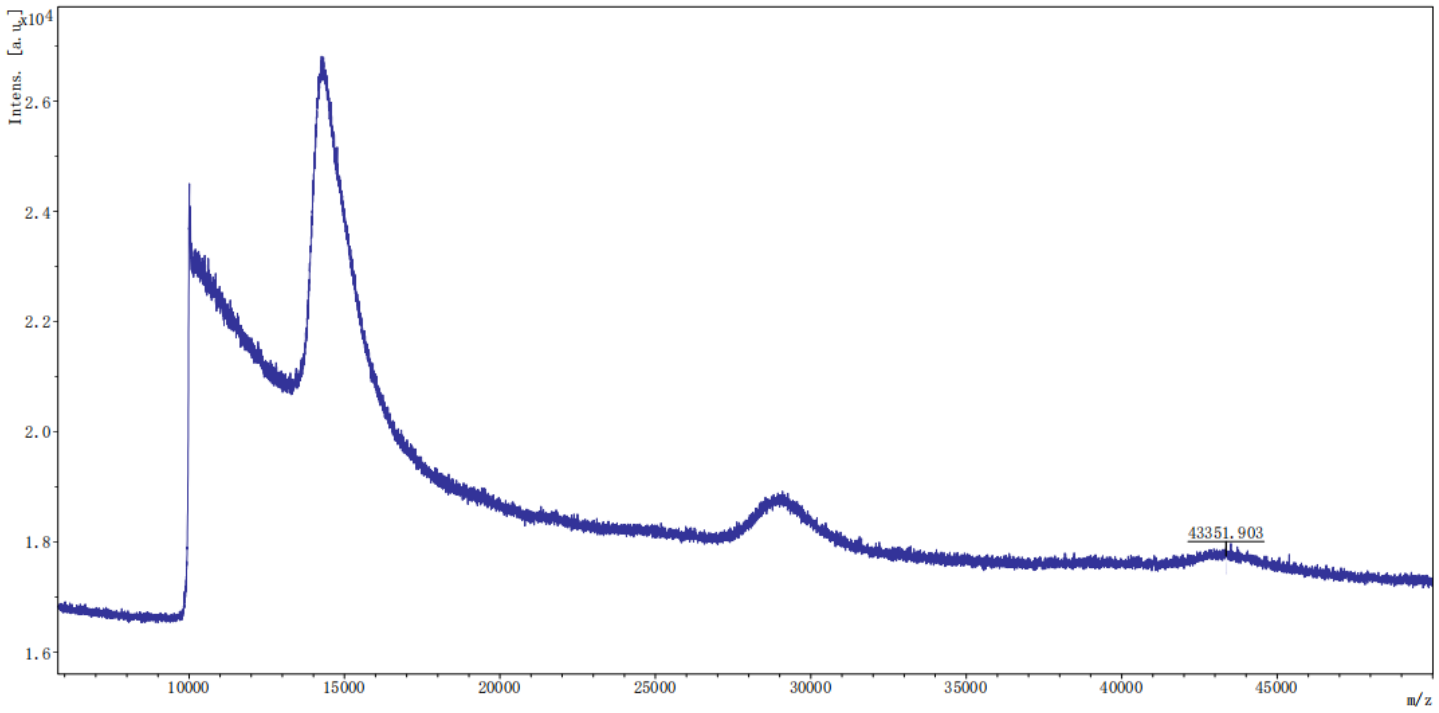
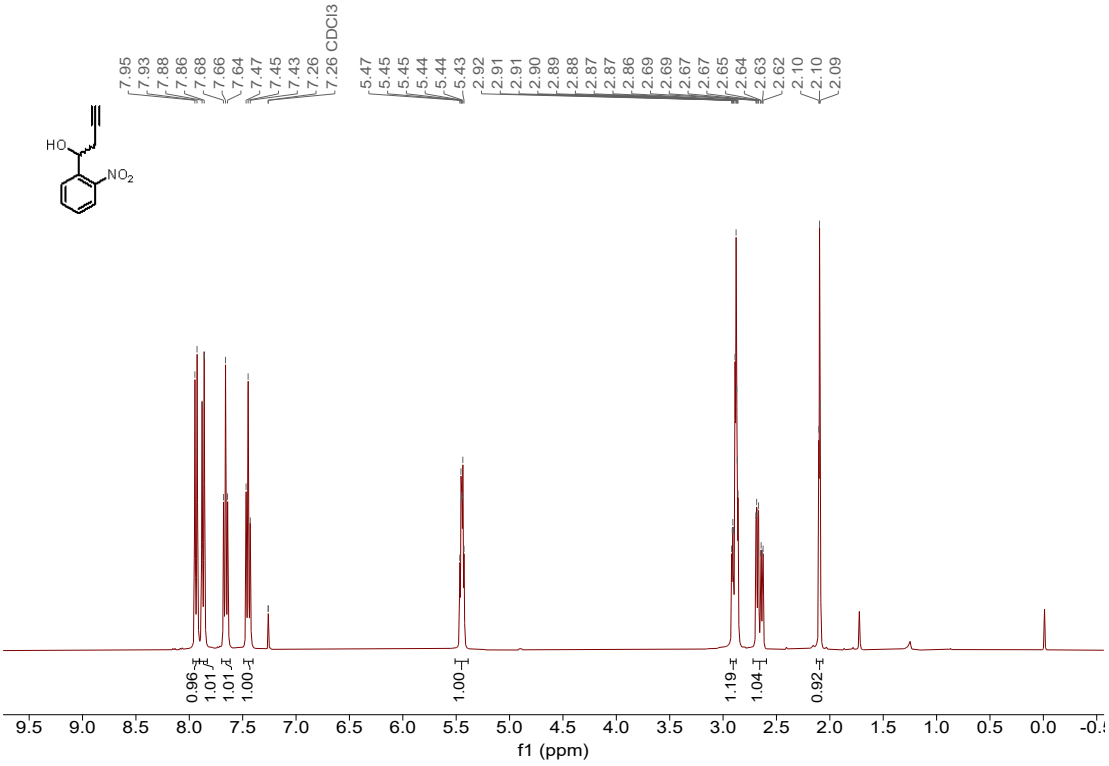
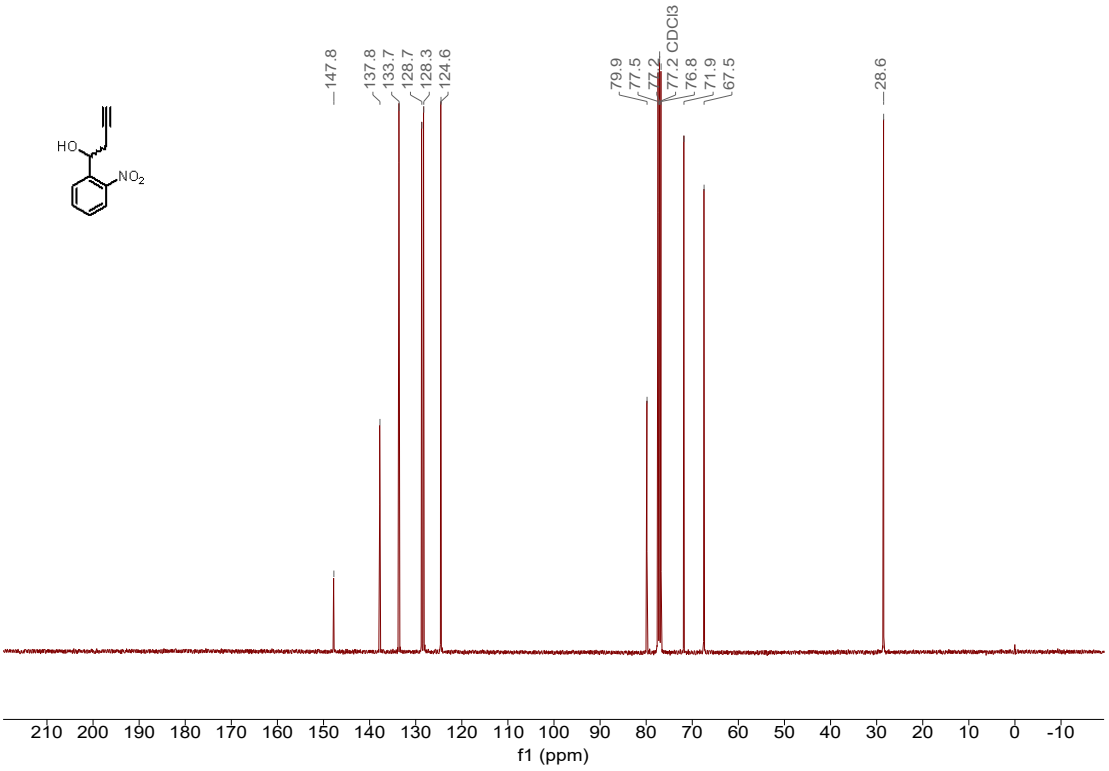


Figure S25. MALDI-TOF of Cas12a-GFP-crRNA-A18$^{3/3}$.

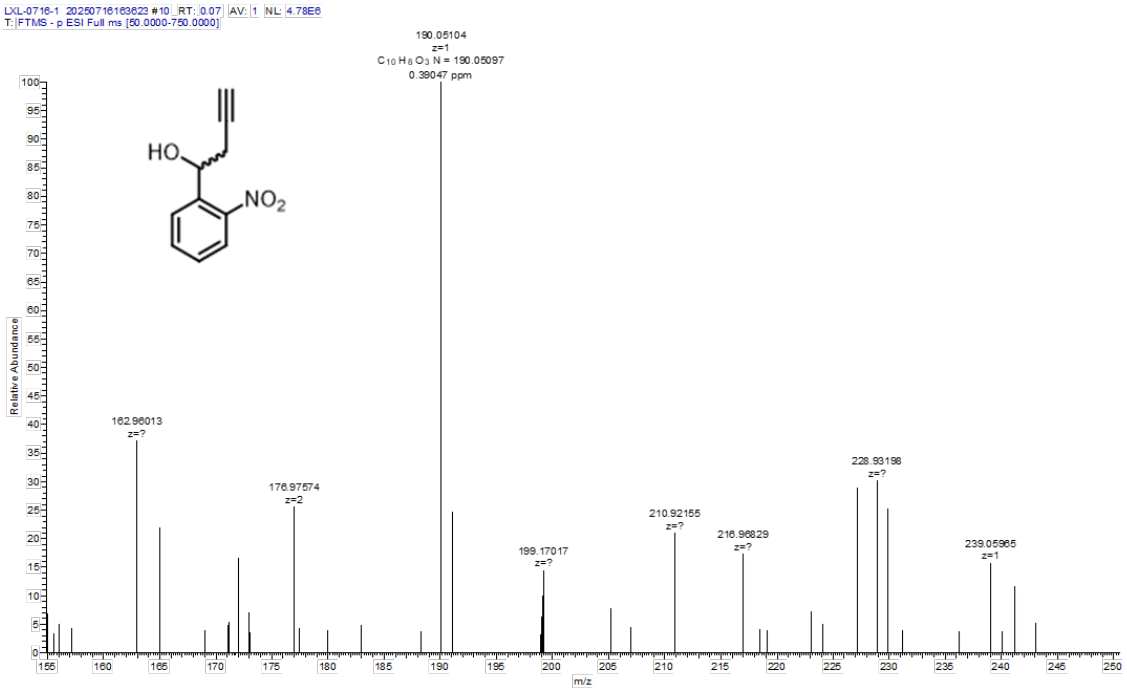
9. NMR and mass spectra



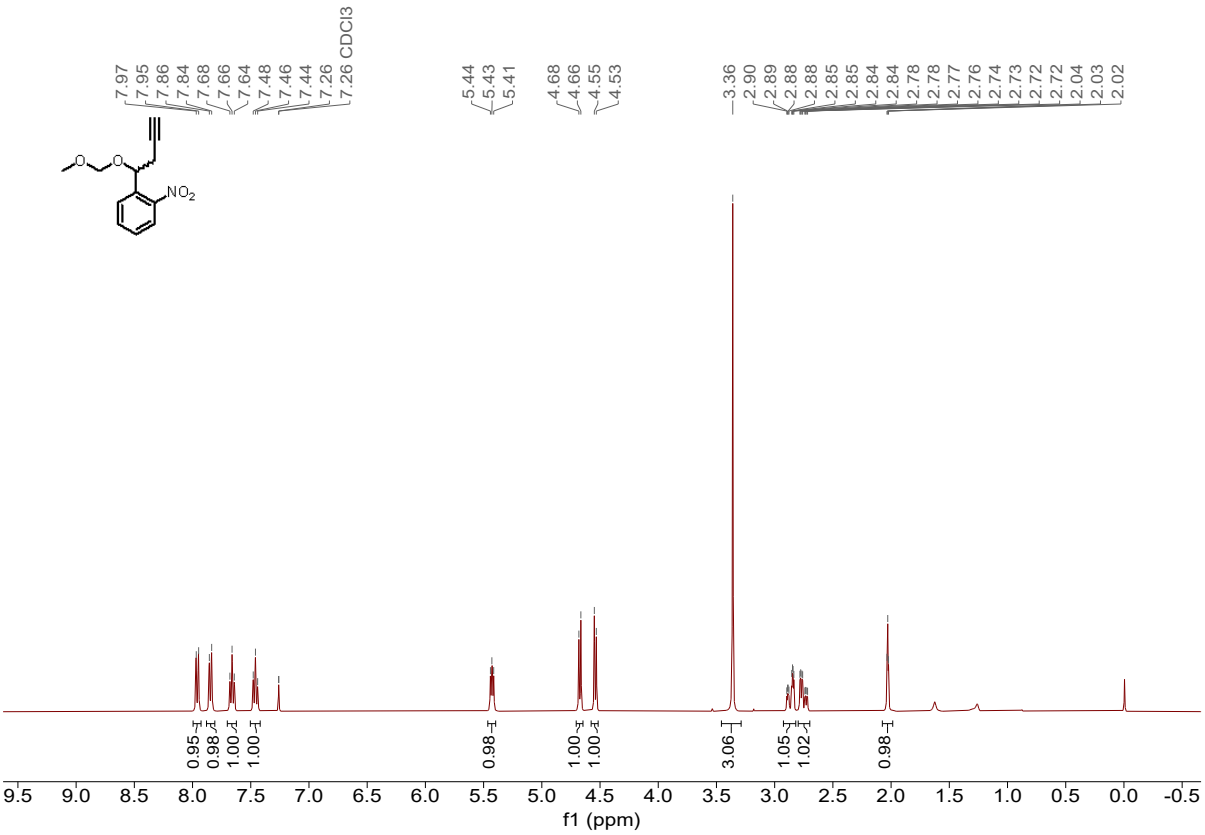
^{1}H NMR of compound 1



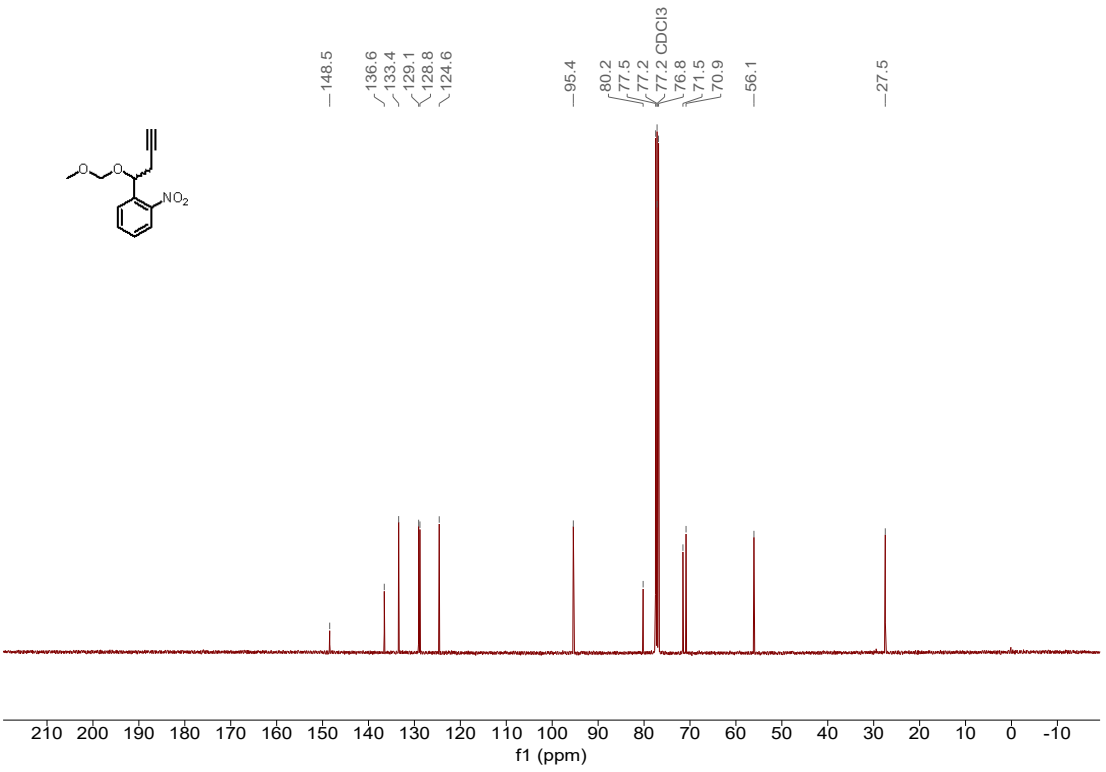
^{13}C NMR of compound **1**



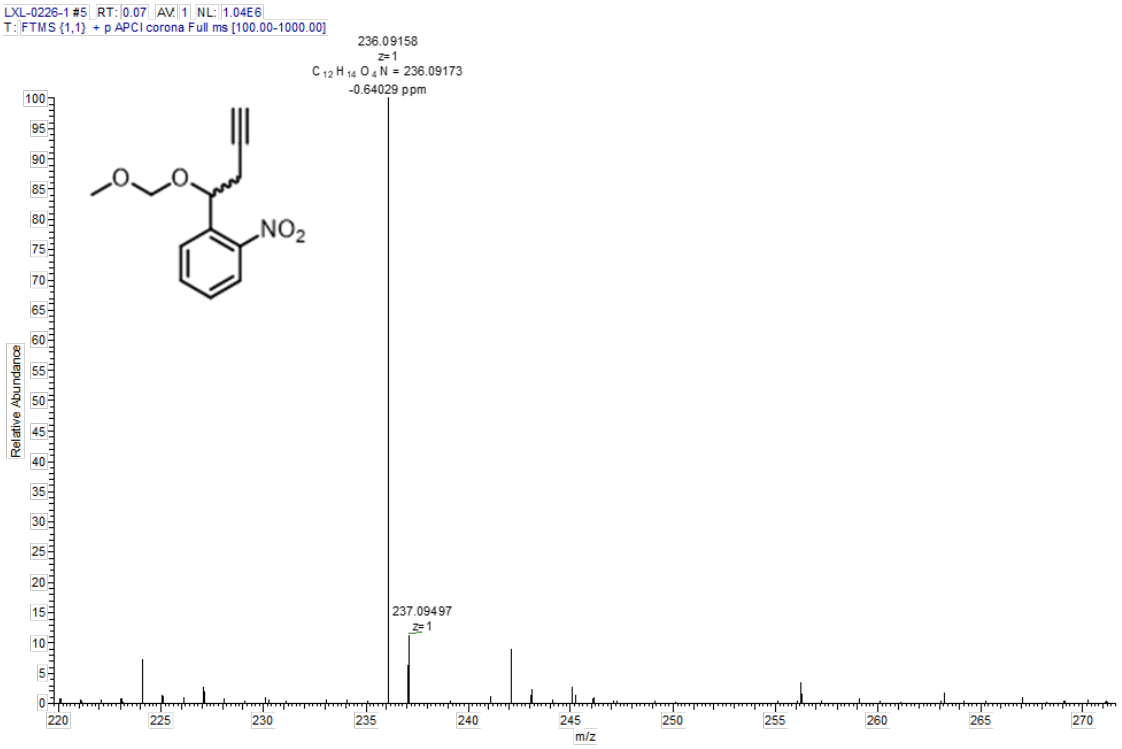
MS spectrum of compound **1**



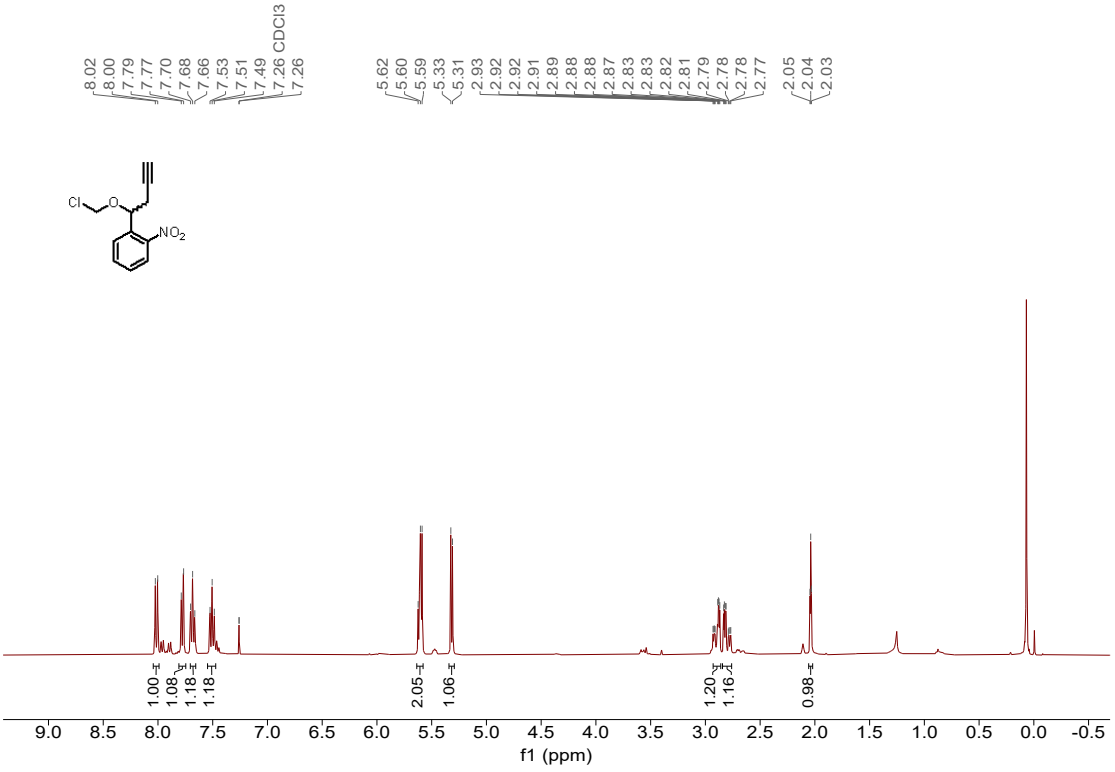
^{1}H NMR of compound **2**



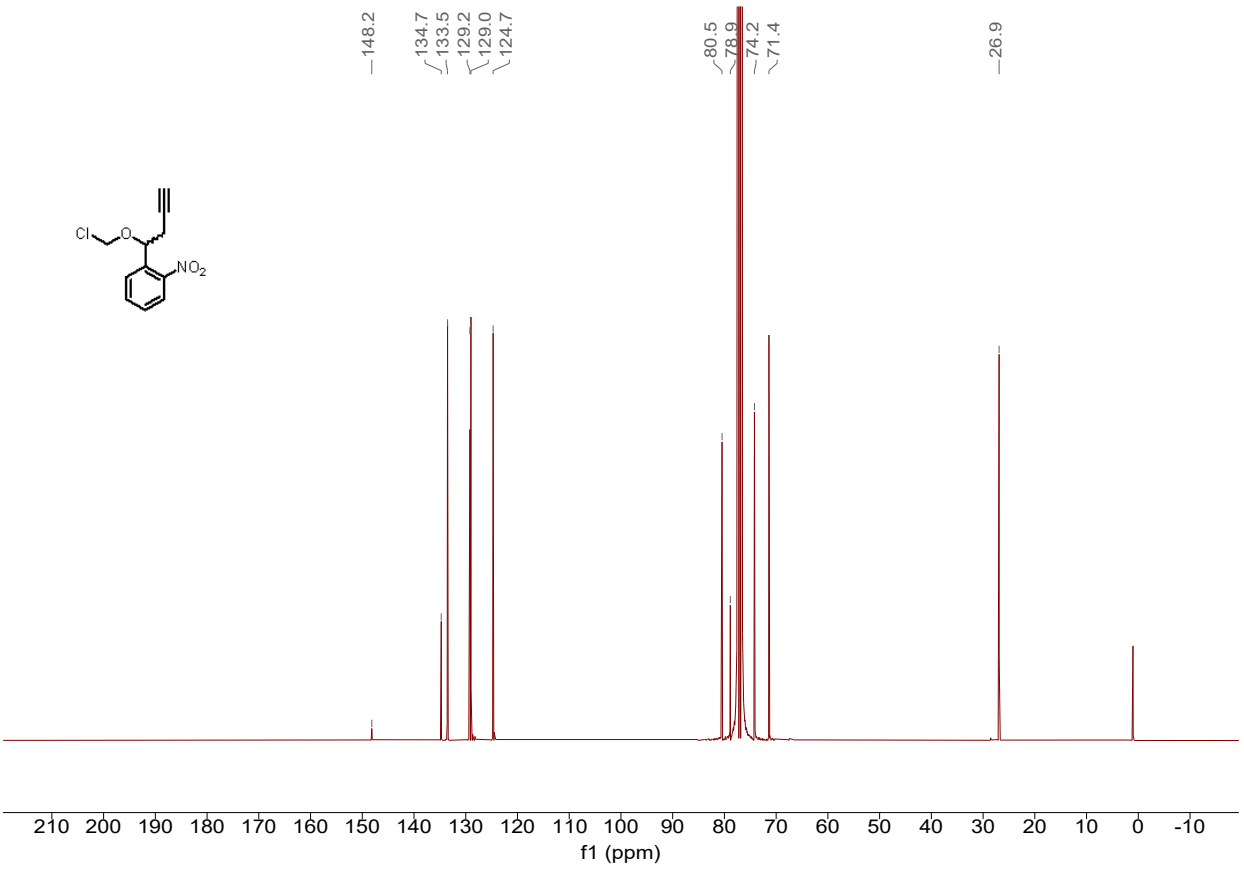
^{13}C NMR of compound **2**



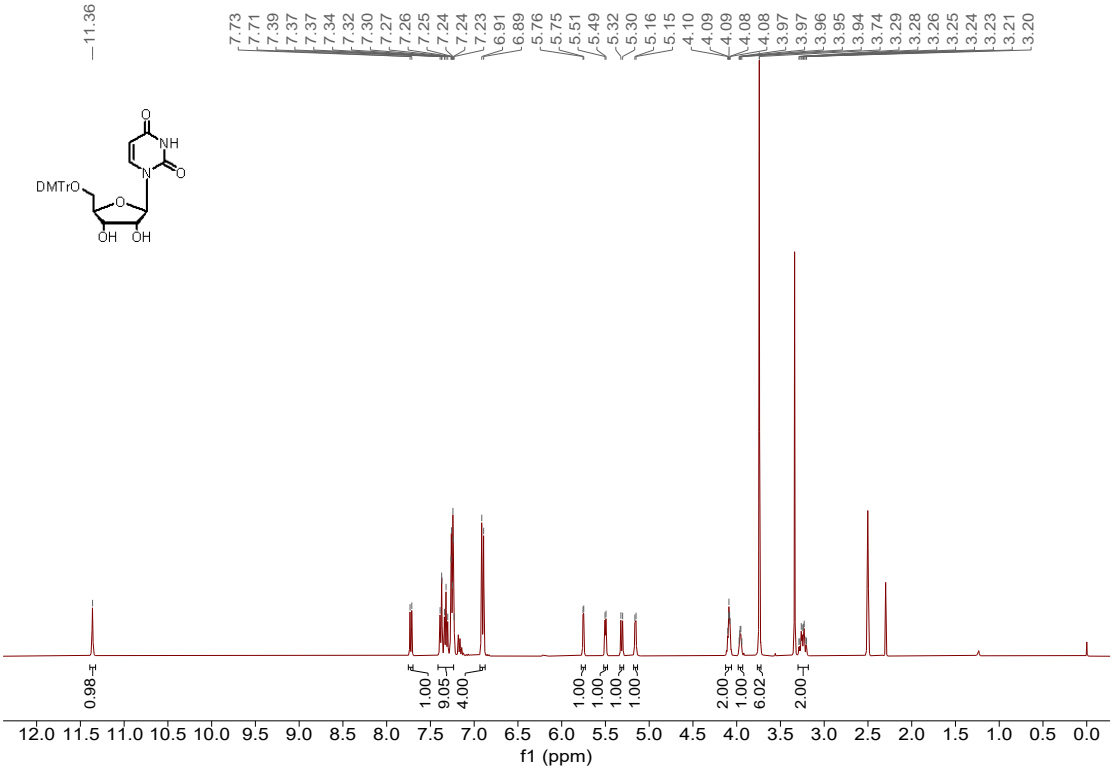
MS spectrum of compound **2**



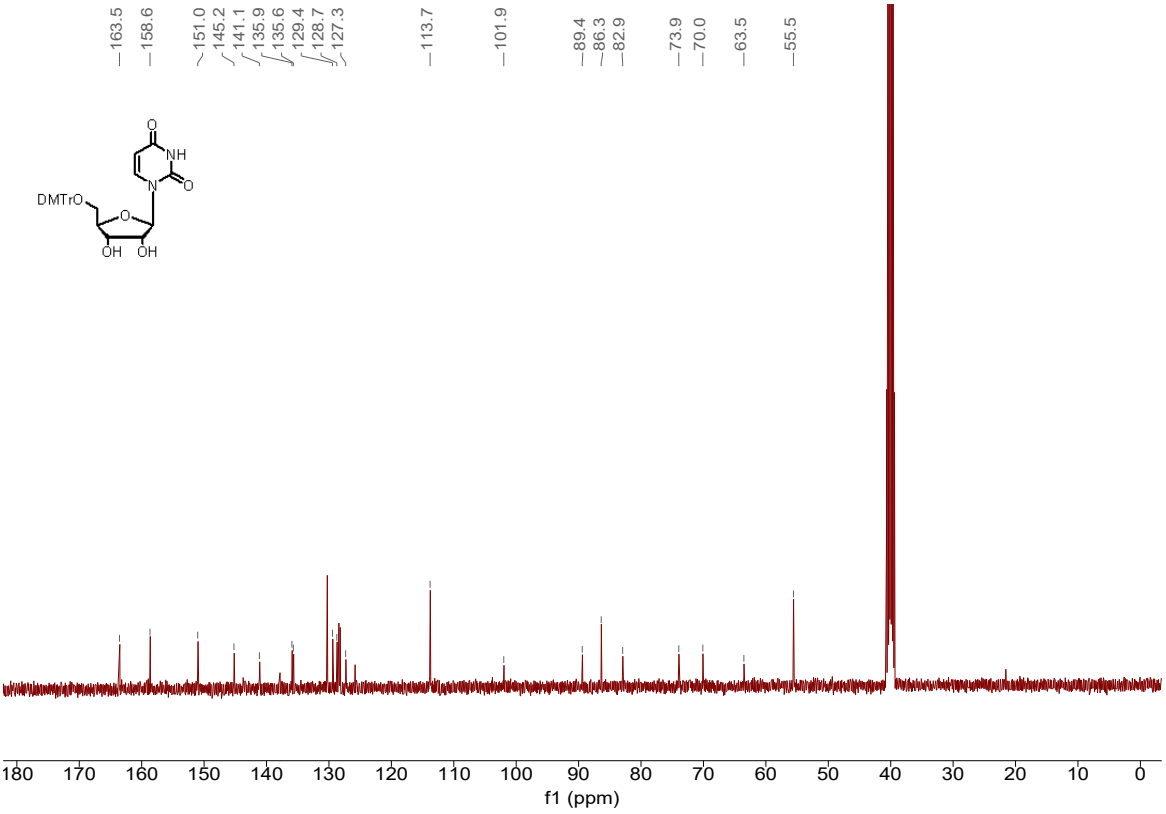
^{1}H NMR of compound **3**



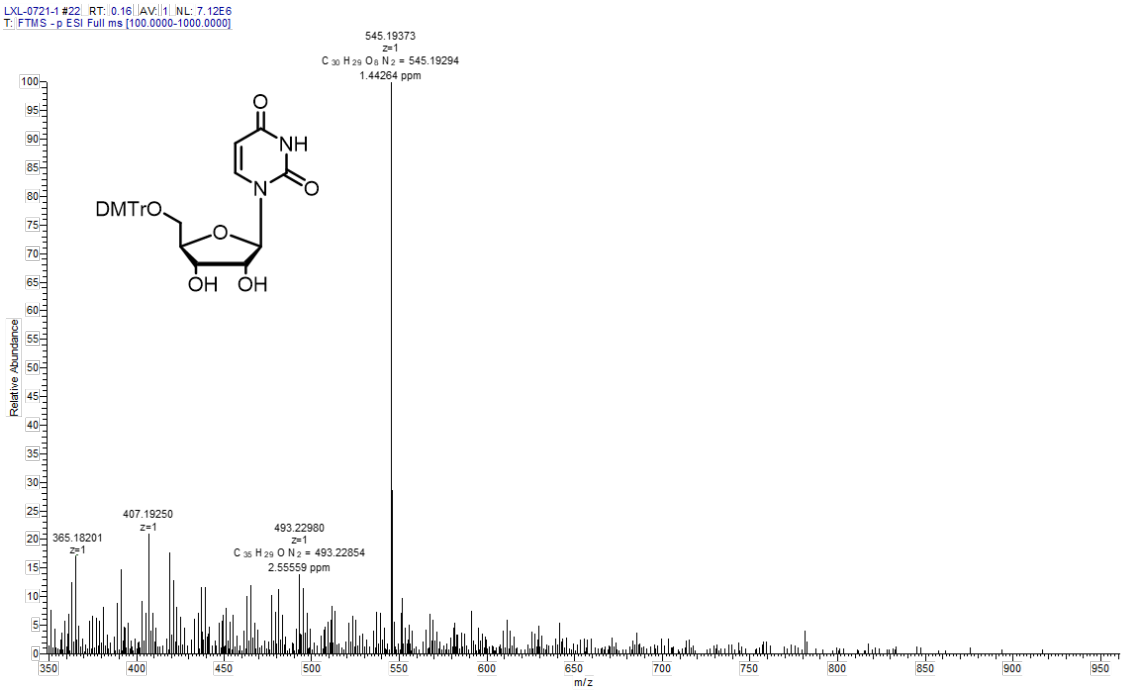
^{13}C NMR of compound **3**



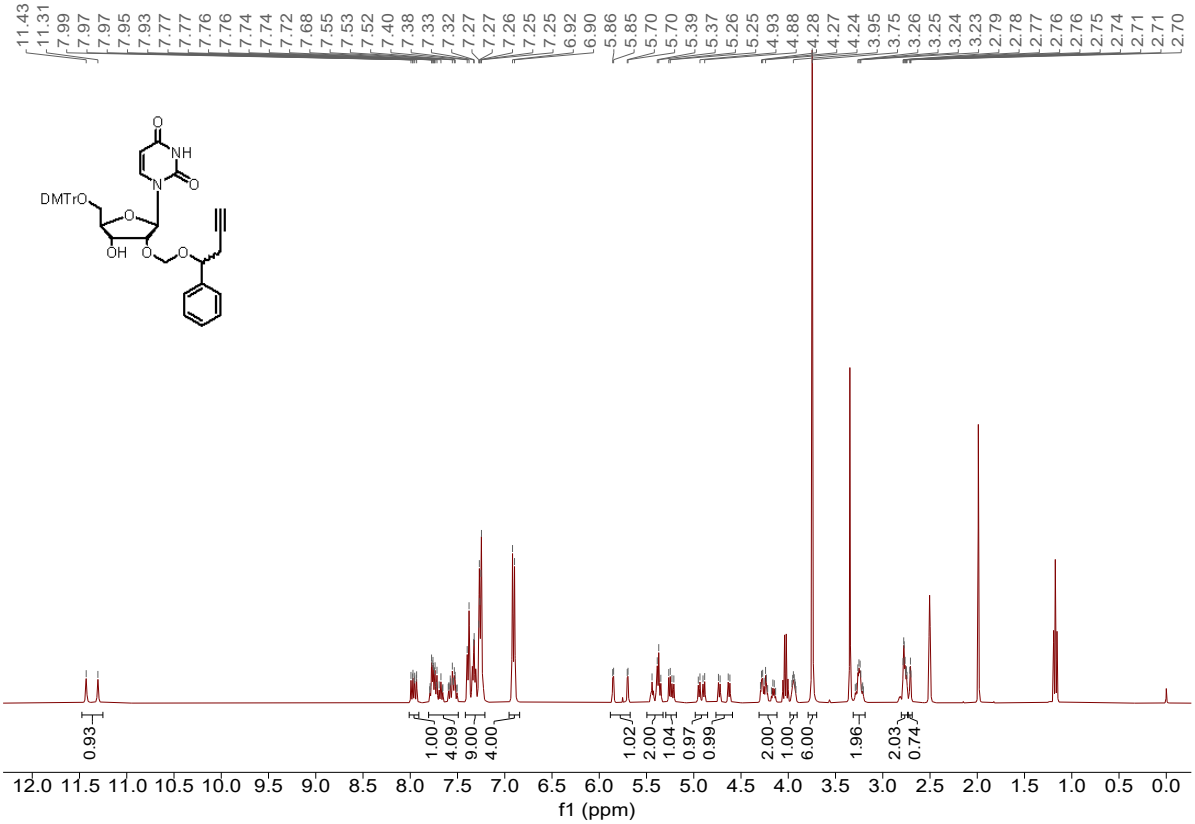
^{1}H NMR of compound **DMTrU**



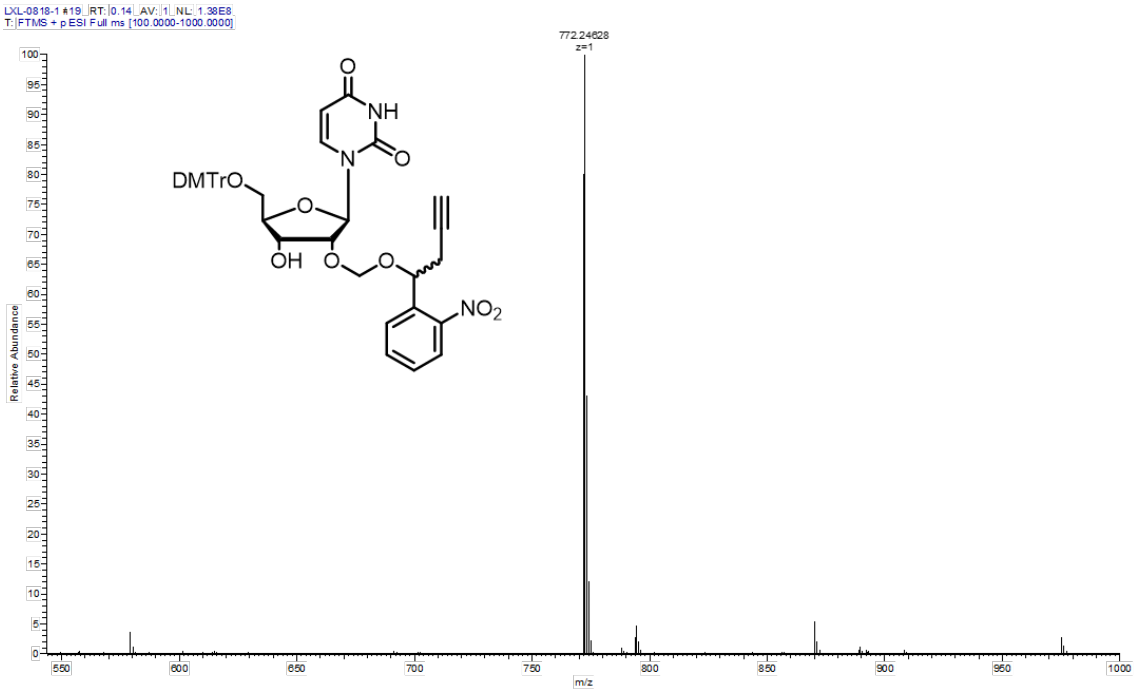
^{13}C NMR of compound **DMTrU**



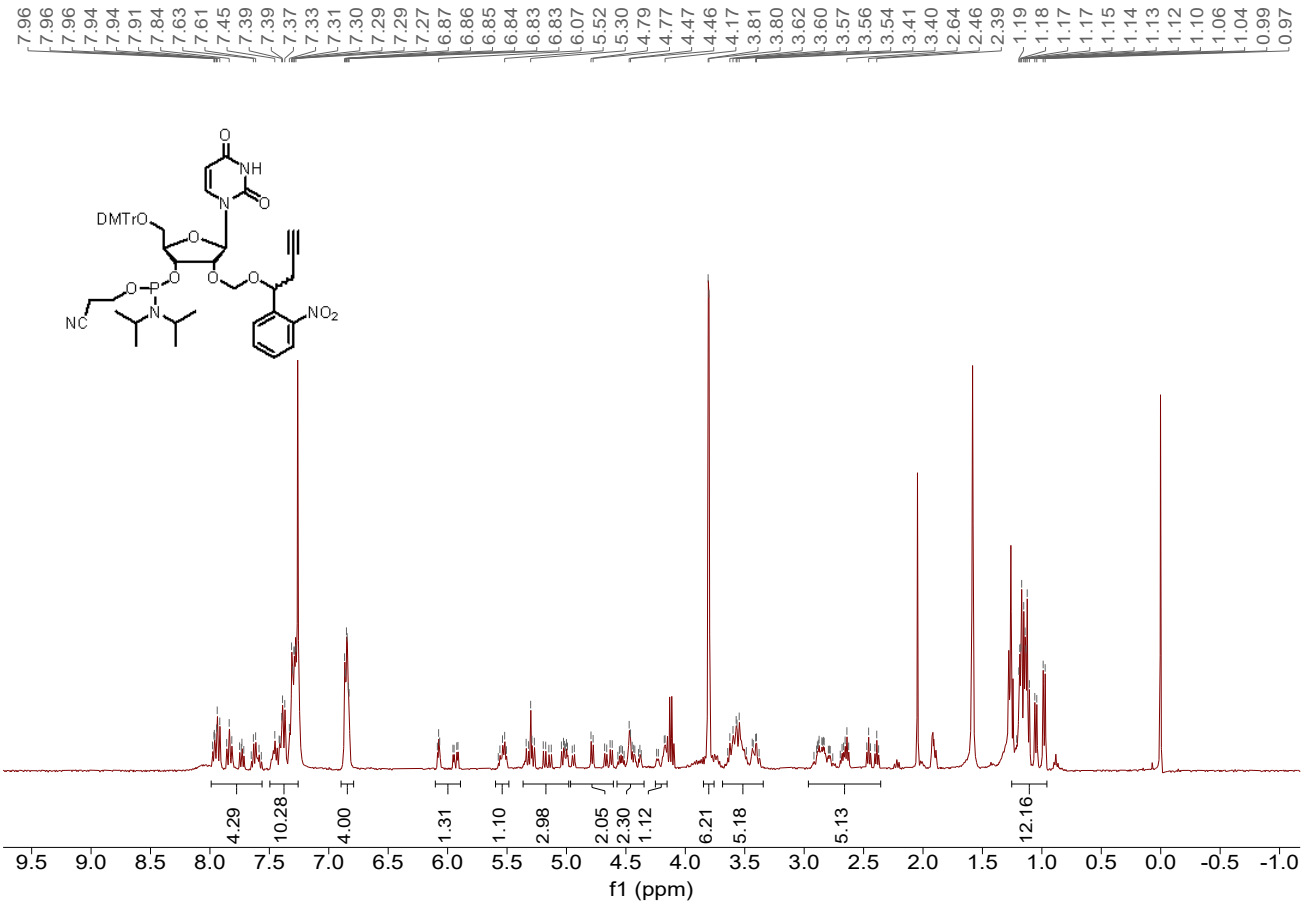
MS spectrum of compound **DMTrU**



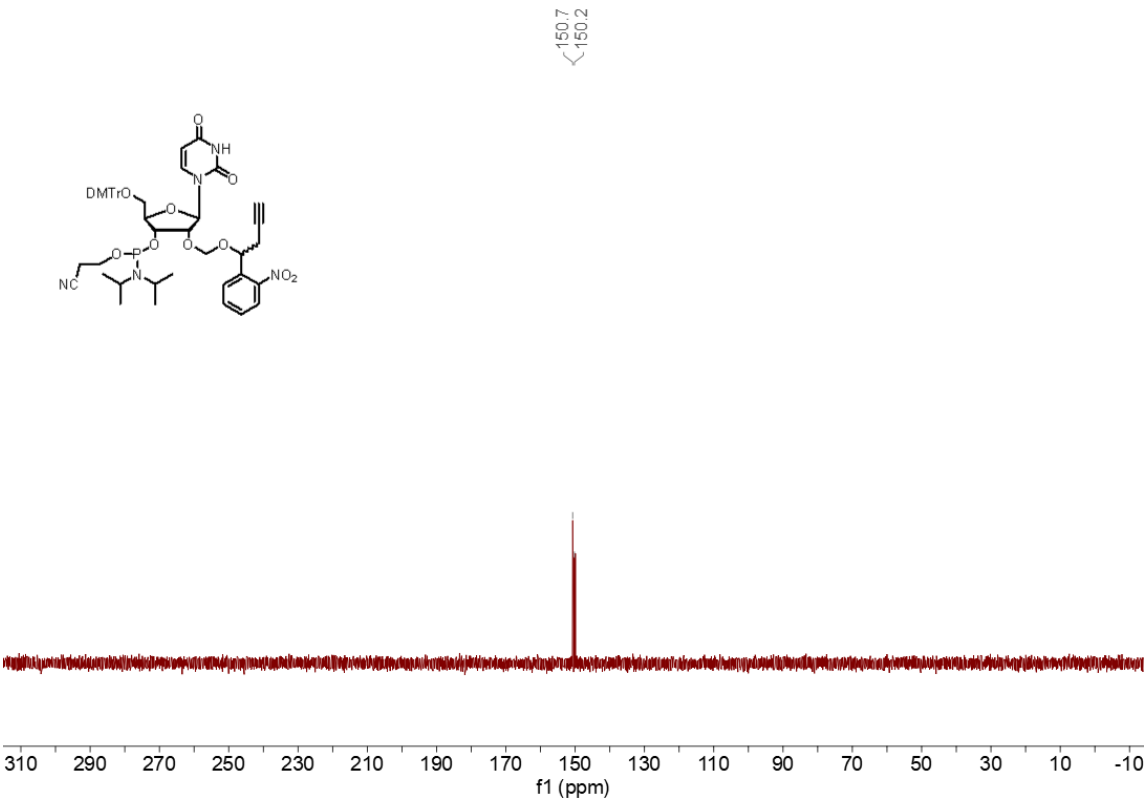
^{1}H NMR of compound **4**



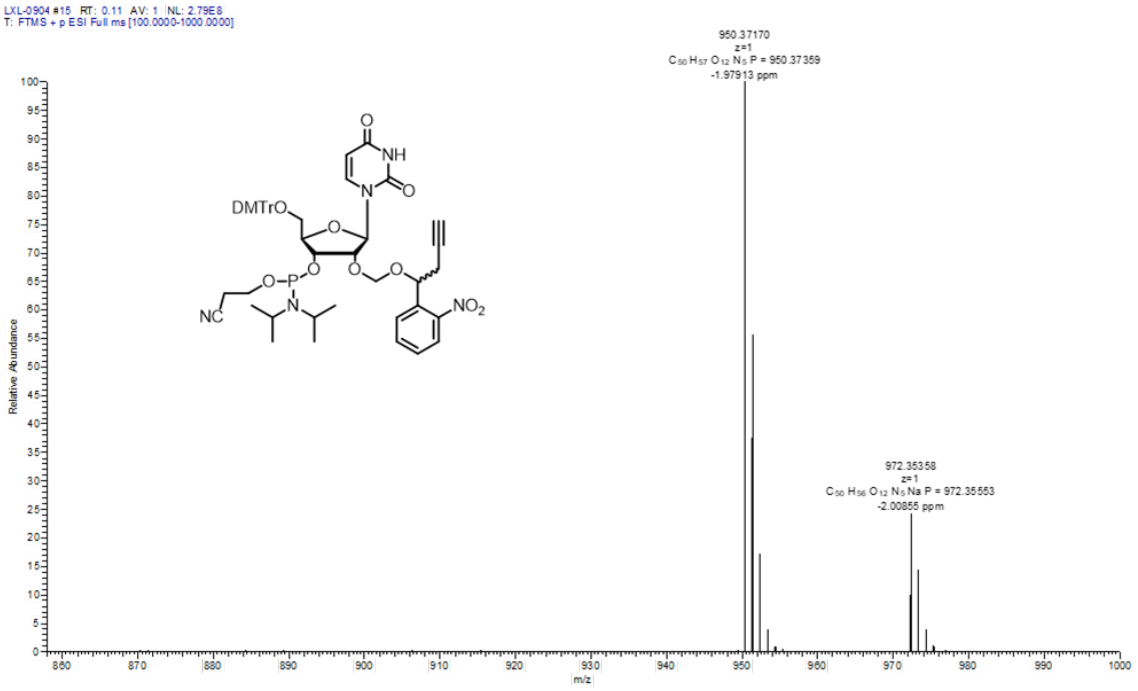
MS spectrum of compound **4**



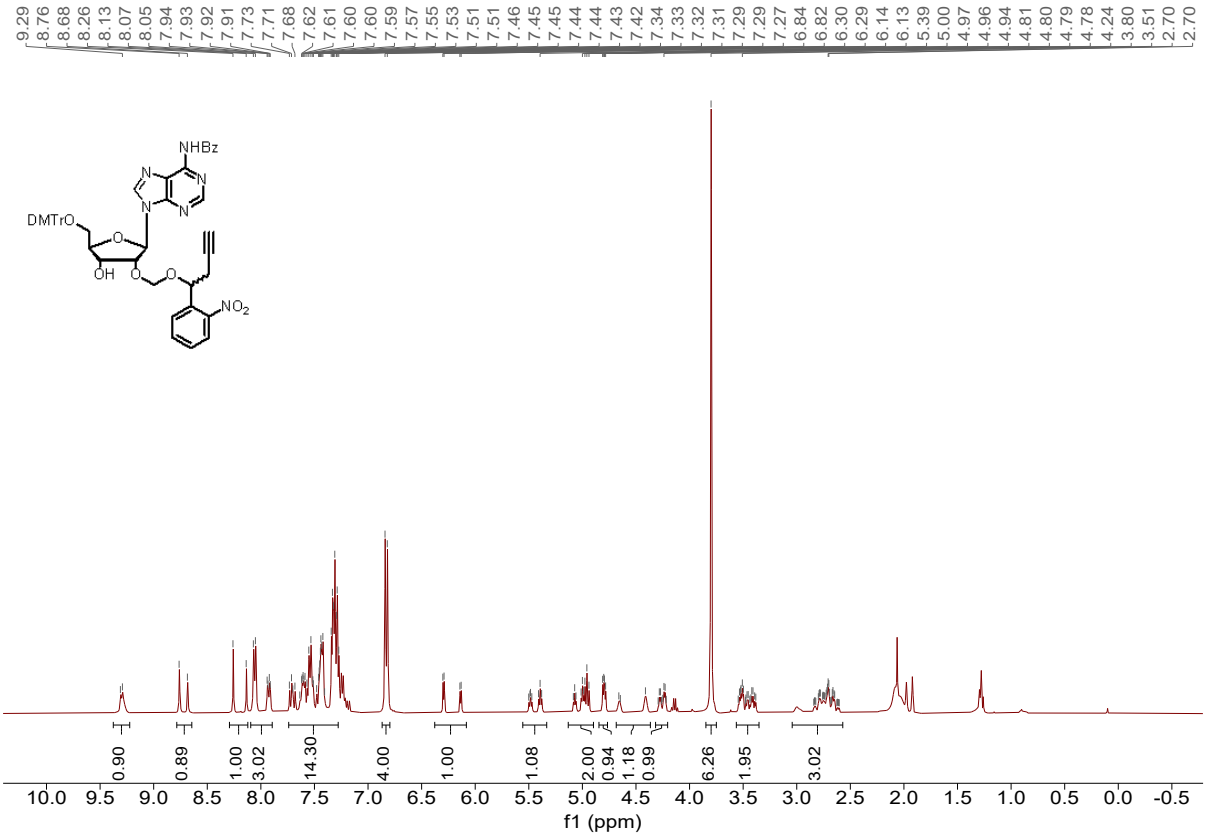
^{1}H NMR of compound **5**



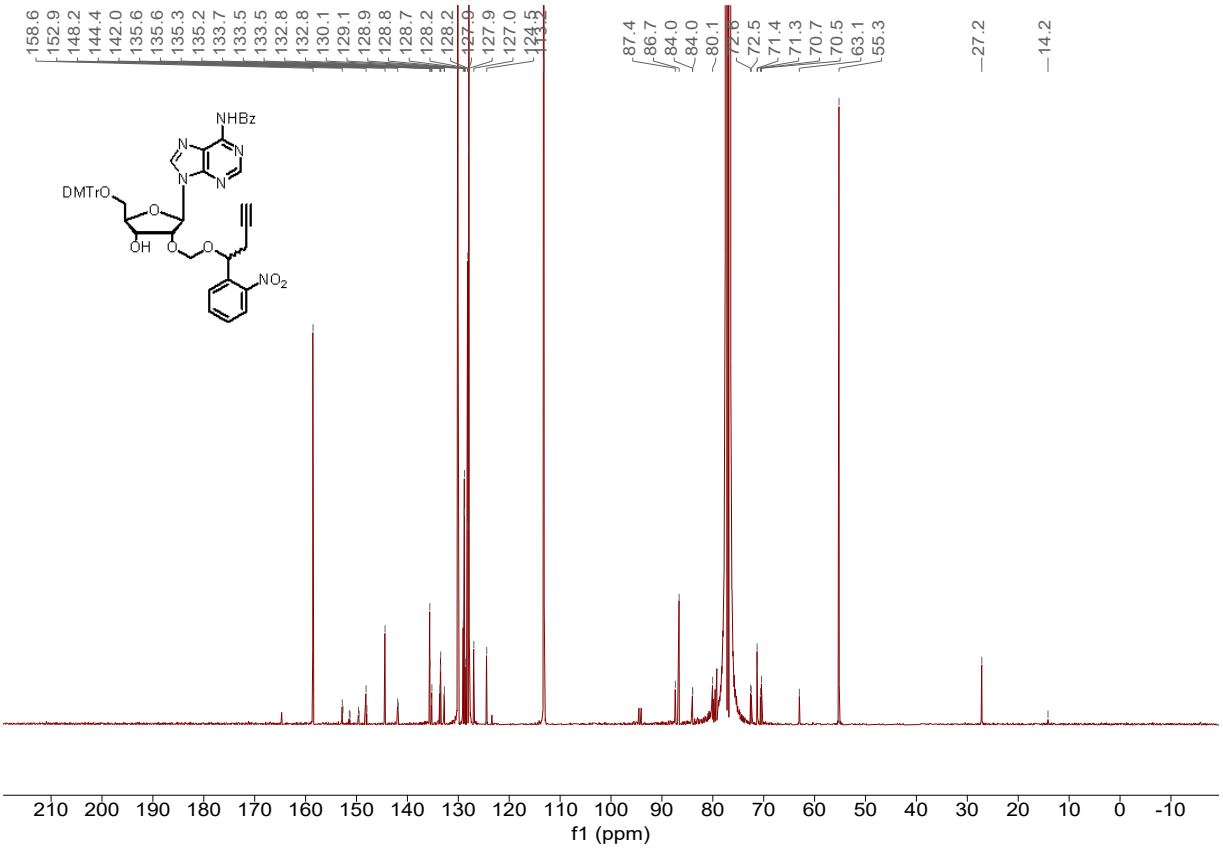
^{31}P NMR of compound **5**



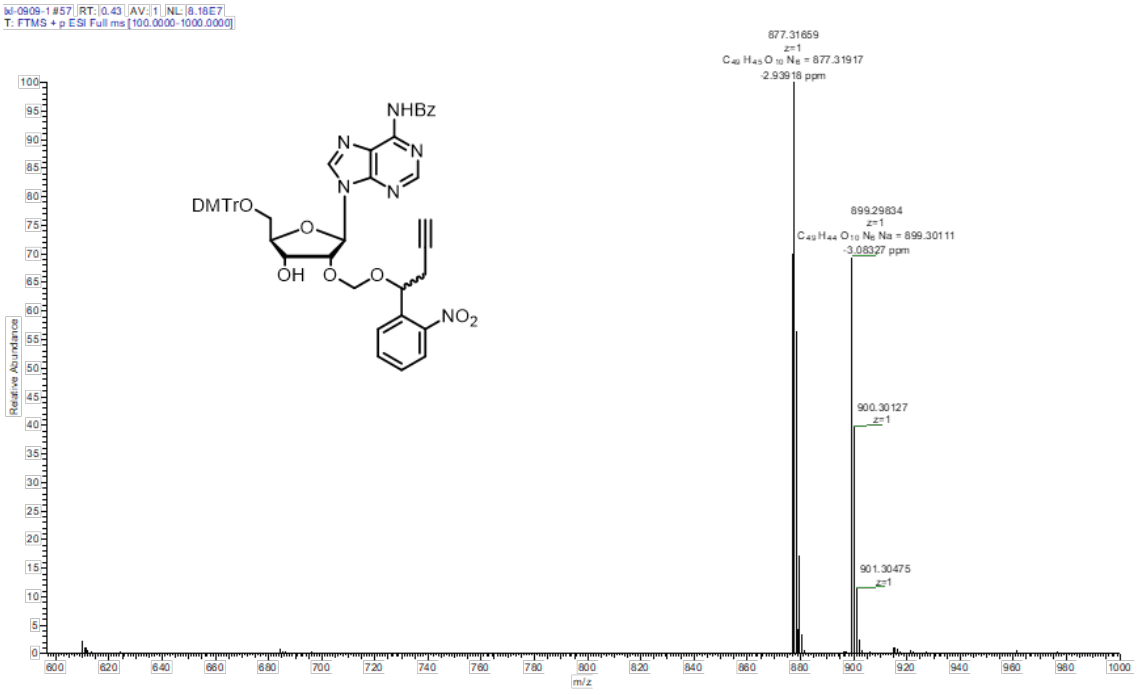
MS spectrum of compound **5**



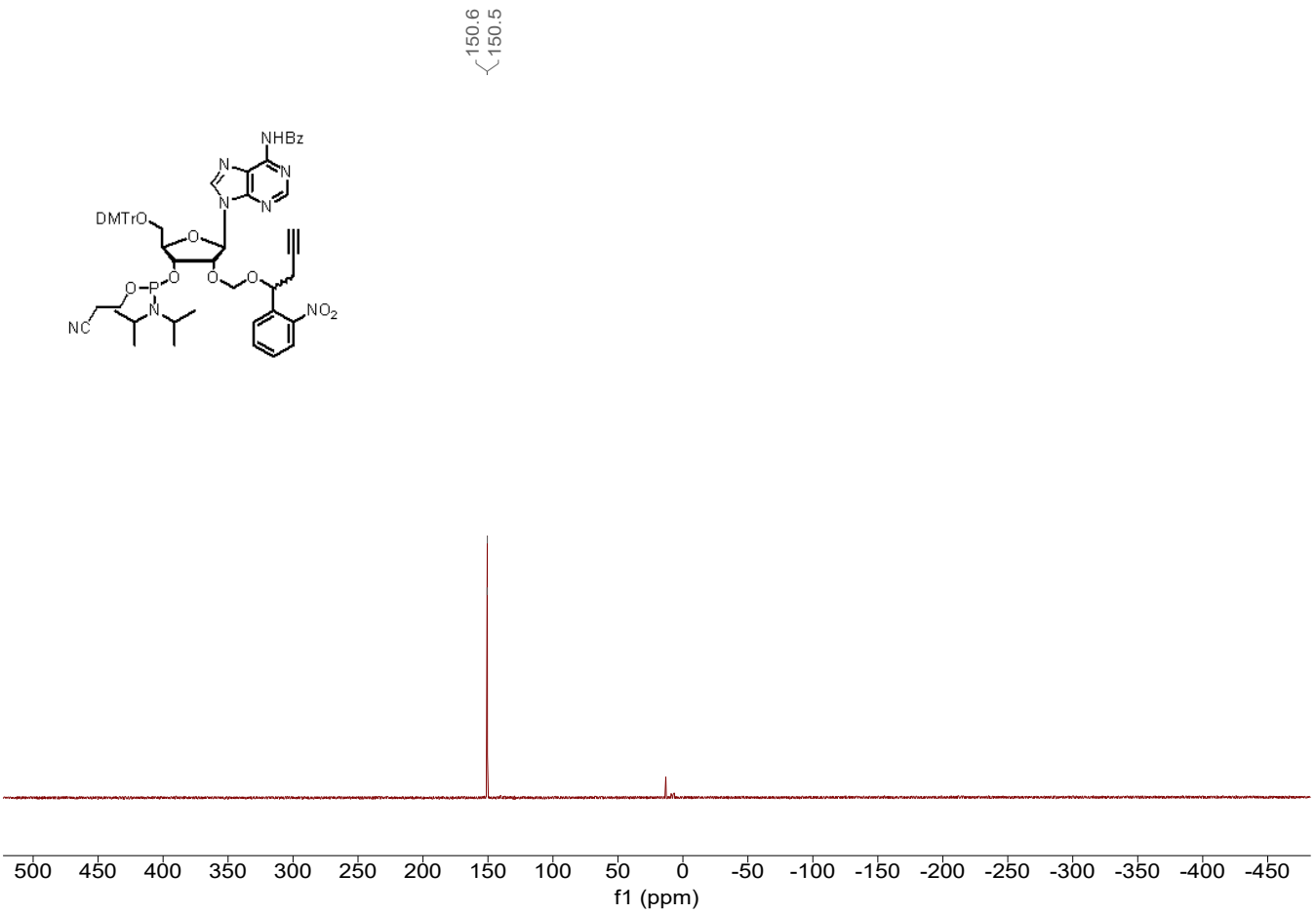
^{1}H NMR of compound **6**



^{13}C NMR of compound **6**



MS spectrum of compound **6**



^{31}P NMR of compound **7**

10. References

[1] Y.-J. Sun, J. Liu, J.-J. Li, Y. Zhang, W.-D. Chen, W.-Q. Cai, L. Liu, X.-J. Tang, J. Hou, M. Wang, L. Cheng, Angew. Chem. Int. Ed. 2023, 62, e202212413.