Electronic Supplementary Material (ESI) for ChemComm.

Supplementary Information

Chemical Control of CRISPR/Cpf1 Editing *via* Orthogonal Activation and Deactivation of Crosslinked crRNA

Cui-Lian Lin,[a,b] Wen-Da Chen,[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

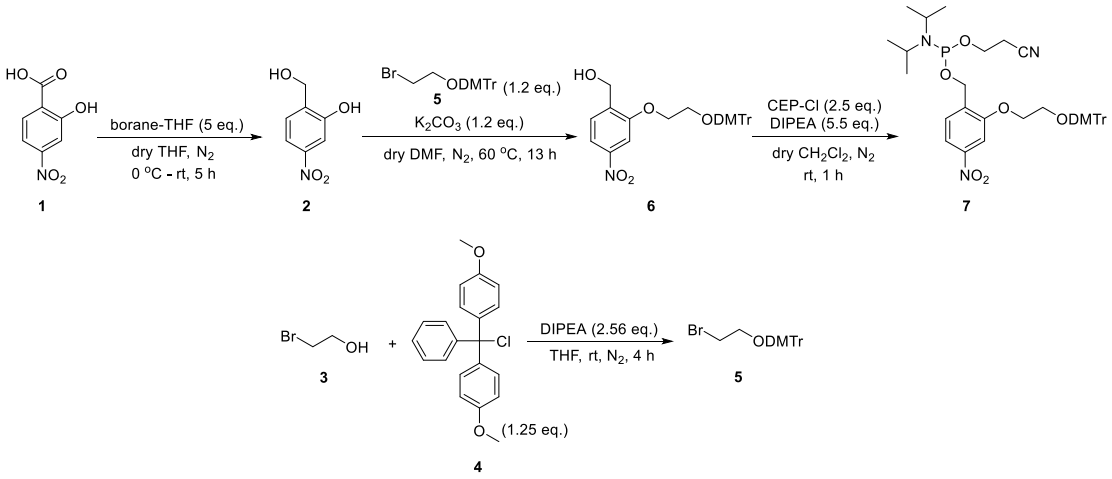
Table of contents

1. General information

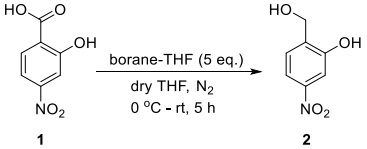
Unless otherwise noted, all reagents were obtained from commercial suppliers and required no further purification for use. The ^{1}H NMR, ^{13}C NMR and ^{31}P NMR spectra were collected by Bruker 300 MHz, 400 MHz or 500 MHz spectrometer. Chemical shifts δ are given in ppm relative to the residual proton signals of the deuterated solvent for ^{1}H NMR, ^{13}C NMR. Mass spectrometry was collected by Thermo Fisher Scientific (Exactive) and Bruker Solarix. crRNAs were purchased from GenScript (Nanjing, China) and Biosyntech (Suzhou, China). 40% Page pre-solution and and 6 × DNA Loading buffer were purchased from Solarbio. Ultra GelRed (10,000 ×) was purchased from Vazyme. Agarose was purchased from Tsingke. 5 × TBE and DNA/RNA Loading Buffer (2X, for Denaturing PAGE) were purchased from Beyotime. NEB buffer r3.1 was purchased from New England Biolabs (NEB). Proteinase K was purchased from TransGen Biotech. 50 × TAE was purchased from Sangon Biotech. 2 × PCR Mix (+Dye) was purchased from Gene-Protein Link Biotech. Cycle Pure Kit for purifying PCR product was purchased from Omega Bio-Tek. 365nm ultraviolet lamp was purchased from Zhongshan Tiandou lighting electrical appliance factory in Taobao. The concentration of RNA was quantified by Nanodrop One (Thermo Fisher Scientific). Vertical and horizontal electrophoresis system were purchased from Bio-Rad. Gel Imaging was performed with a Tanon-5200 Multi Fluorescence Imager. The plasmid of the coding sequence for AsCpf1 (Plasmid #79007) was used to express AsCpf1 in the E.coli Rosseta (DE3) cells. AsCpf1 was expressed by Dr. Y.-J Sun. See Angew. Chem. Int. Ed., 2023, 62, e202212413 for details.

2. Chemical Synthesis

2.1 Synthesis of reduction-cleavable spacer phosphoramidite.



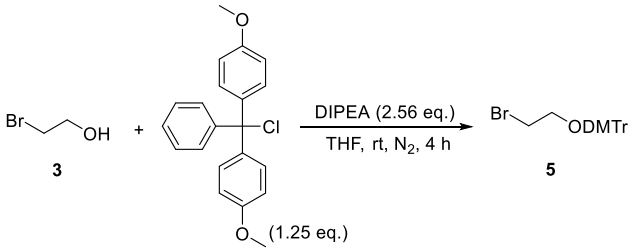
2.1.1 Synthesis of **2**[S1].



4-nitrosalicylic acid (5 mmol, 915.6 mg, 1 eq.) and dry THF (9 mL) were added to a three-necked flask equipped with a pressure-equalizing dropping funnel. Then, air was withdrawn from the three-necked flask and backfilled with N_2. Borane-THF (25 mL) was injected into pressure-equalizing dropping funnel after reaction unit was put into ice-water bath to cool down. Borane-THF dripped

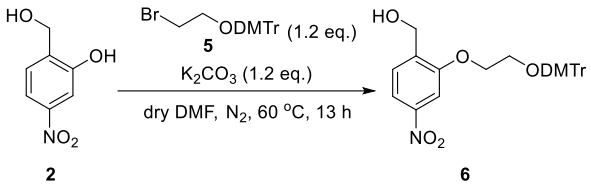
slowly to flask under the ice-water bath. When the additive of borane-THF was completed, ice-water bath was removed and reaction proceeded under room temperature for 5 h. After completion of the reaction (TLC), reaction mixture was cool by ice-water bath and MeOH was added carefully to the flask until no bubbles were generated at all. The mixture was extracted with ethyl acetate, washed by water and brine, dried over anhydrous sodium sulfate. The solvent was removed under reduced pressure, and the obtained residue was purified by flash column chromatography on silica-gel (eluent: petroleum ether/ethyl acetate = 3:1 to 2:1) to give compound **2** (601.7 mg, 71.2%) as a yellow solid. ^{1}H NMR (300 MHz, Acetone-d_6) δ 9.43 (s, 1H), 7.75 (dd, *J* = 8.4, 2.3 Hz, 1H), 7.66 (d, *J* = 2.1 Hz, 1H), 7.63 (d, *J* = 8.4 Hz, 1H), 4.81 (d, *J* = 5.5 Hz, 3H), 4.71 – 4.62 (m, 1H). ^{13}C NMR (101 MHz, Acetone-d_6) δ 155.74, 148.52, 137.23, 128.36, 115.24, 109.99, 60.33. HRMS (ESI) *m/z*: $[M-H]^-$ calcd for $C_7H_6NO_4$: 168.0302; found: 168.0302.

2.1.2 Synthesis of **5**[S2].



3 (600 μL, 8 mmol, 1 eq.) and THF (20 mL) were added to a two-necked flask. Then, air was withdrawn from double-neck flask and backfilled with N_2. **4** (2.68 g,10 mmol, 1.25 eq.) and *N*,*N*-diisopropylethylamine (3.5 mL, 20.5 mmol, 2.56 eq.) were added quickly in sequence to this flask. Reaction mixture was stirred for 4 h at room temperature. Reaction was monitored by TLC and the reaction mixture was diluted with ethyl acetate and washed with saturated sodium hydrogen carbonate and brine. Organic phase was separated, dried over Na_2SO_4, filtered, concentrated and purified by chromatography (SiO_2, eluent: petroleum ether/ethyl acetate = 24:1) to yield **5** (2.35g, 68.9%) as light-yellow oil. ^{1}H NMR (300 MHz, Acetone-d_6) δ 7.54 – 7.48 (m, 2H), 7.39 – 7.34 (m, 4H), 7.33 – 7.19 (m, 3H), 6.93 – 6.86 (m, 4H), 3.78 (s, 6H), 3.58 - 3.52 (m, 2H), 3.46 – 3.40 (m, 2H).^{13}C NMR (101 MHz, Acetone-d_6) δ 159.76, 146.14, 136.85, 130.96, 128.99, 128.69, 127.70, 114.00, 87.10, 64.59, 55.58, 32.59. HRMS (ESI) *m/z*: $[M+Na]^+$ calcd for $C_{23}H_{23}BrNaO_3$: 449.0723; found: 449.0720.

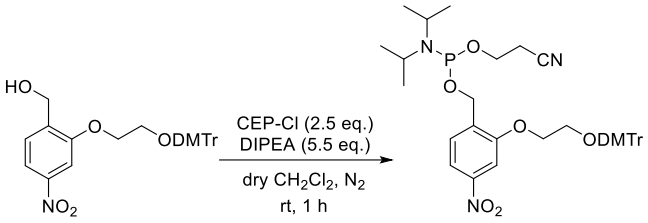
2.1.3 Synthesis of **6**[S3].



In a double-neck flask, **2** (760.7 mg, 4.5 mmol, 1 eq.), **5** (2.3 g, 5.4 mmol, 1.2 eq.) and potassium carbonate (744.7 mg, 5.4 mmol, 1.2 eq.) were dissolved in dry DMF (27 mL). Air was withdrawn from the flask and backfilled with N_2. The reaction proceeded at 60 °C for 13 h. An appropriate amount of ethyl acetate was added to reaction mixture to dilute the reaction mixture, washed the organic phase with saturated sodium chloride solution and water, separated the organic phase, dried with anhydrous sodium sulfate, filtered and obtain filtrate. Filtrate was concentrated under reduce pressure. The

crude product was purified by column chromatography (SiO_2, eluent: petroleum ether/ethyl acetate/ triethylamine = 400:100:1 to 300:100:1) to yield **6** (1.58 g, 68.1%) as yellow oil. ^{1}H NMR (300 MHz, Acetone-d_6) δ 7.90 (dd, *J* = 8.3, 2.1 Hz, 1H), 7.84 – 7.76 (m, 2H), 7.54 – 7.48 (m, 2H), 7.40 – 7.34 (m, 4H), 7.34 – 7.28 (m, 2H), 7.26 – 7.19 (m, 1H), 6.92 – 6.85 (m, 4H), 4.89 (d, *J* = 5.3 Hz, 2H), 4.46 – 4.39 (m, 2H), 3.78 (s, 6H), 3.52 – 3.45 (m, 2H). HRMS (ESI) *m/z*: $[M+Na]^+$ calcd for $C_{30}H_{29}NNaO_7$: 538.1837; found: 538.1843.

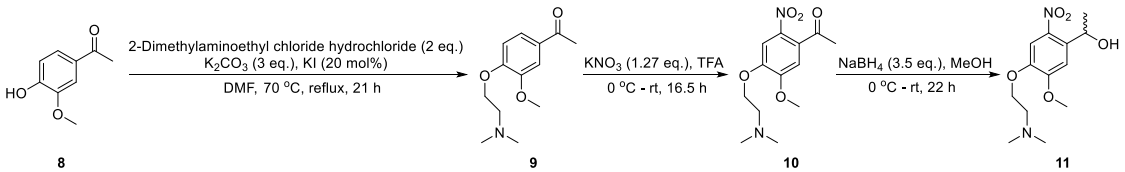
2.1.4 Synthesis of **7**[S3].



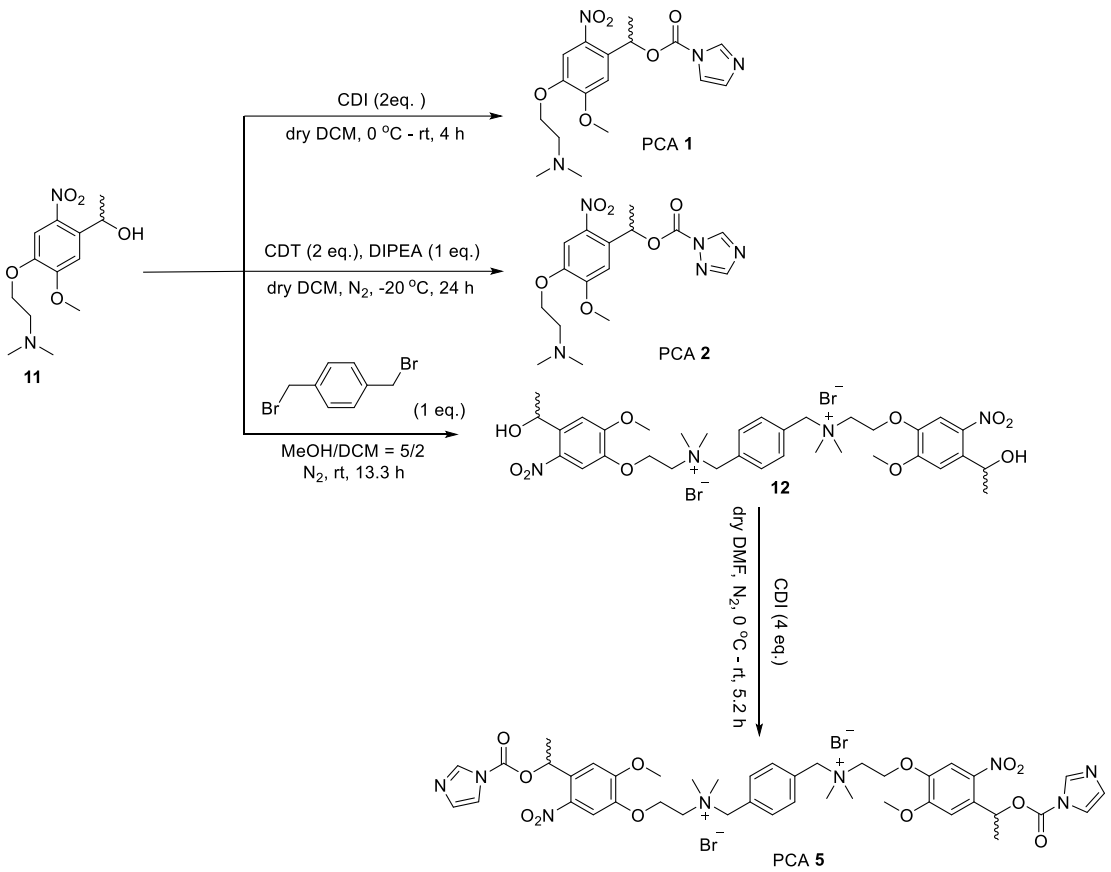
6 **7**

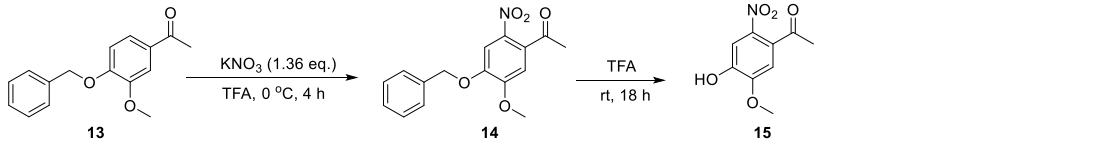
6 (360.6 mg, 0.7 mmol, 1eq.), DIEPA (699 µL, 3.85 mmol, 5.5 eq.) and dry DCM (14 mL) were added to a double-neck flask, drew the air out of the flask and filled it with nitrogen. 2-cyanoethyl-*N*,*N*-diisopropylchlorophosphoramidite (CEP-Cl, 437 µL, 1.75 mmol, 2.5 eq.) was quickly added to the reaction flask and the reaction mixture was stirred at room temperature for 33 min. To the mixture was added an appropriate amount of CH_2Cl_2 and the organic layer was washed with sat. $NaHCO_3$ aq. and brine, separated the organic phase, dried over anhydrous sodium sulfate. Organic phase was concentrated by evaporation and purified by preparative thin layer chromatography (developing solvent: petroleum ether/ethyl acetate/triethylamine = 300:100:1) to give **7** (137 mg, 27.3%) as yellowish white solid. ^{1}H NMR (400 MHz, Chloroform-*d*) δ 7.89 (dd, *J* = 8.4, 2.2 Hz, 1H), 7.72 (d, *J* = 2.1 Hz, 1H), 7.65 (d, *J* = 8.4 Hz, 1H), 7.46 (d, *J* = 7.6 Hz, 2H), 7.40 – 7.33 (m, 4H), 7.32 - 7.27 (m, 2H), 7.24 – 7.19 (m, 1H), 6.88 – 6.78 (m, 4 H), 5.00 - 4.84 (m, 2H), 4.24 (t, *J* = 4.7 Hz, 2H), 3.92 – 3.74 (m, 8H), 3.74 – 3.60 (m, 2H), 3.48 (t, *J* = 4.5 Hz, 2H), 2.52 (t, *J* = 6.5 Hz, 2H), 1.26 – 1.10 (m, 12H). ^{13}C NMR (101 MHz, Chloroform-*d*) δ 158.66, 155.74, 148.01, 144.85, 136.00, 130.15, 128.24, 127.97, 127.35, 126.95, 117.59, 116.06, 113.28, 105.79, 86.28, 68.42, 62.11, 60.82, 60.62, 58.68, 58.49, 55.35, 43.50, 43.37, 24.87, 24.79, 24.71, 20.35, 20.27. ^{31}P NMR (162 MHz, Chloroform-*d*) δ 149.42. HRMS (ESI) *m/z*: $[M+Na]^+$ calcd for $C_{39}H_{46}N_3NaO_8P$: 738.2915; found: 738.2914.

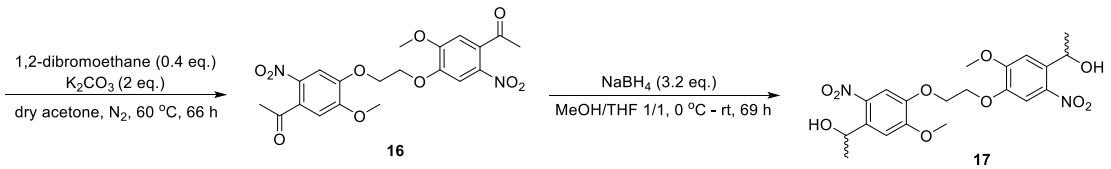
2.2 Synthesis of photocloaking agents.

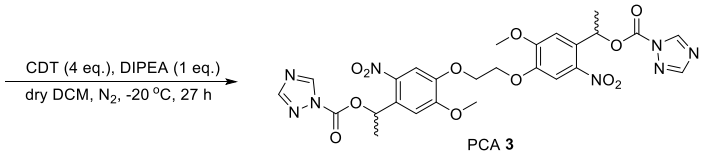


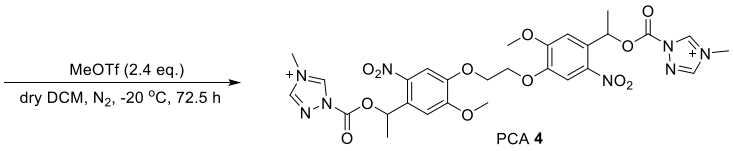
8 **9** **10** **11**


CDI (2eq.)
dry DCM, 0 °C - rt, 4 h
PCA 1
CDT (2 eq.), DIPEA (1 eq.)
dry DCM, N2, -20 °C, 24 h
PCA 2
11
(1 eq.)
MeOH/DCM = 5/2
N2, rt, 13.3 h
12
dry DMF, N2, 0 °C - rt, 5.2 h
CDI (4 eq.)
PCA 5

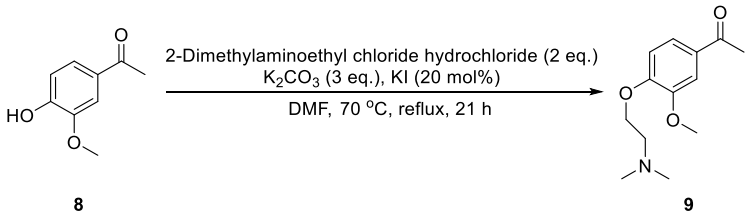

KNO3 (1.36 eq.)
TFA, 0 °C, 4 h
TFA
rt, 18 h
13
14
15


1,2-dibromoethane (0.4 eq.)
K2CO3 (2 eq.)
dry acetone, N2, 60 °C, 66 h
16
NaBH4 (3.2 eq.)
MeOH/THF 1/1, 0 °C - rt, 69 h
17


CDT (4 eq.), DIPEA (1 eq.)
dry DCM, N2, -20 °C, 27 h
PCA 3


MeOTf (2.4 eq.)
dry DCM, N2, -20 °C, 72.5 h
PCA 4

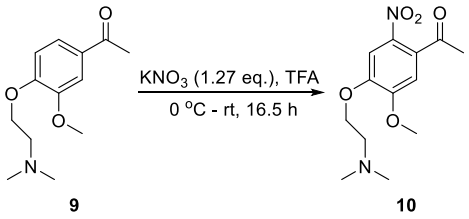
2.2.1 Synthesis of **9**[S4].



8 **9**

To Weigh acetovanillone (3.32 g, 20 mmol, 1 eq.), potassium carbonate (8.28 g, 60 mmol, 3 eq.), potassium iodide (0.664 g, 4 mmol, 20 mol%) to a double-neck flask and added DMF (160 mL) to the flask. The mixture was stirred at 70 °C and then 2-Dimethylaminoethyl chloride hydrochloride (7.2 g, 50 mmol, 2.5 eq.) was added to the reaction flask. The reaction mixture was stirred at 70 °C for 21 h. The reaction was monitored by TLC and the complete reaction of acetovanillone was observed after 21 h. Reaction mixture was diluted with EA and washed the organic phase with water and saturated sodium chloride solution. The organic phase was separated, dried with anhydrous sodium sulfate, filtered, the low boiling solvent was removed with a rotary evaporator to obtain **9** (2.49 g, 52.5%) as yellowish-brown oil. ^{1}H NMR (300 MHz, Chloroform-*d*) δ 7.50 (dd, *J* = 8.3, 2.0 Hz, 1H), 7.46 (d, *J* = 2.0 Hz, 1H), 6.85 (d, *J* = 8.3 Hz, 1H), 4.13 (t, *J* = 6.1 Hz, 2H), 3.85 (s, 3H), 2.75 (t, *J* = 6.1 Hz, 2H), 2.50 (s, 3H), 2.30 (s, 6H). HRMS (ESI) *m/z*: $[M+H]^+$ calcd for $C_{13}H_{20}NO_3$: 238.1438; found: 238.1438.

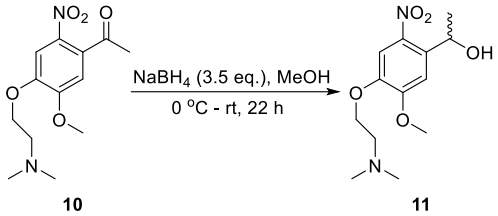
2.2.2 Synthesis of **10**[S4].



9 **10**

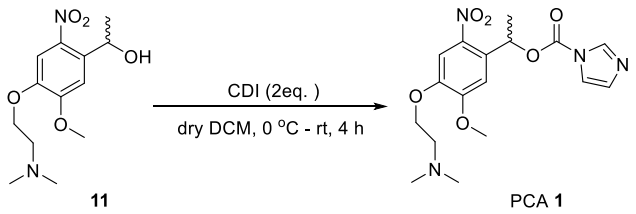
Potassium nitrate (1.35 g, 10.5 mmol, 1 27 eq.) and TFA (22 mL) were added to three-necked flask and cooled by stirring under ice water bath. **9** (2.49 g, 10.5 mmol, 1 eq.) was dissolved in TFA (22 mL) and transferred to a pressure-equalizing dropping funnel to slowly drop into the flask. After the solution was added, the ice water bath was removed and the reaction was carried out at room temperature for 6 h. Reaction mixture was concentrated by rotary evaporator and then added water. Resulting solution was basified with sat. aq. sodium bicarbonate. The aqueous solution was extracted with ethyl acetate and organic layer was then dried over Na_2SO_4, filtered, and concentrated under reduced pressure. Crude product was purified by flash column chromatography (SiO_2, eluent: petroleum ether/ethyl acetate/ triethylamine = 8:12:1 to ethyl acetate/triethylamine = 20:1) to yield **10** (2.17 g,73.2%) as yellow solid. ^{1}H NMR (300 MHz, Chloroform-*d*) δ 7.64 (s, 1H), 6.74 (s, 1H), 4.19 (t, *J* = 5.8 Hz, 2H), 3.95 (s, 3H), 2.81 (t, *J* = 5.8 Hz, 2H), 2.49 (s, 3H), 2.35 (s, 6H). HRMS (ESI) *m/z*: $[M+H]^+$ calcd for $C_{13}H_{19}N_2O_5$: 283.1288; found: 238.1290.

2.2.3 Synthesis of **11**[S4].



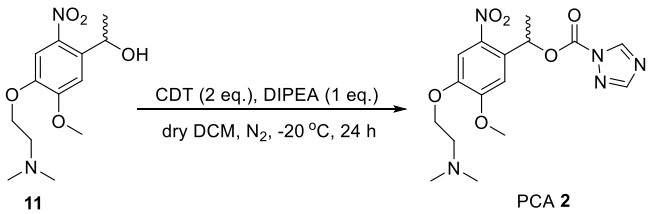
To a two-neck flask were added **10** (2.16 g, 7.65 mmol, 1eq.) and methanol (40 mL) and place the bottle under ice water bath to cool. Sodium borohydride (578.9 mg, 15.3 mmol, 2 eq.) was then slowly added to the flask in batches. Ice bath was removed, the reaction mixture was stirred at room temperature for 4.5 h and the reaction was monitored by TLC. The reaction mixture was concentrated by rotary evaporator and crude product was purified by column chromatography (SiO_2, eluent: petroleum ether/ethyl acetate/triethylamine = 100:100:5 to ethyl acetate/triethylamine = 20:1) to yield **11** (1.51 g, 69.4%) as yellow solid. ^{1}H NMR (300 MHz, Chloroform-*d*) δ 7.56 (s, 1H), 7.29 (s, 1H), 5.56 (q, *J* = 6.3 Hz, 1H), 4.12 (t, *J* = 5.8 Hz, 2H), 3.96 (s, 3H), 2.79 (t, *J* = 5.8 Hz, 2H), 2.34 (s, 6H), 1.54 (d, *J* = 6.2 Hz, 3H). HRMS (ESI) *m/z*: $[M+H]^+$ calcd for $C_{13}H_{21}N_2O_5$: 285.1445; found: 285.1444.

2.2.4 Synthesis of PCA **1**[S5].



11 (284 mg, 1mmol, 1 eq.) and dichloromethane (6 mL) were added to the two-neck flask, air was removed from the flask, then nitrogen was filled, and the reaction flask was placed in an ice water bath to cool. The 1,1'-carbonyldiimidazole (CDI, 324 mg, 2 mmol, 2eq.) was quickly added to the flask and then flask was removed from the ice bath. The resulting reaction mixture was stirred at room temperature for 4 h. Dilute the reaction solution with DCM and wash the organic phase with water. The organic phase was dried with anhydrous sodium sulfate, filtered, and the filtrate was concentrated by rotary evaporator to obtain PCA **1** (344.9 mg, 91.1%) as yellow solid. ^{1}H NMR (300 MHz, Chloroform-*d*) δ 8.15 (t, *J* = 1.1 Hz, 1H), 7.64 (s, 1H), 7.43 (t, *J* = 1.5 Hz, 1H), 7.10 – 7.07 (m, 1H), 6.98 (s, 1H), 6.72 (q, *J* = 6.4 Hz, 1H), 4.16 (t, *J* = 5.8 Hz, 2H), 3.91 (s, 3H), 2.79 (t, *J* = 5.8 Hz, 2H), 2.34 (s, 6H), 1.81 (d, *J* = 6.4 Hz, 3H). HRMS (ESI) *m/z*: $[M+H]^+$ calcd for $C_{17}H_{23}N_4O_6$: 379.1612; found: 379.1614.

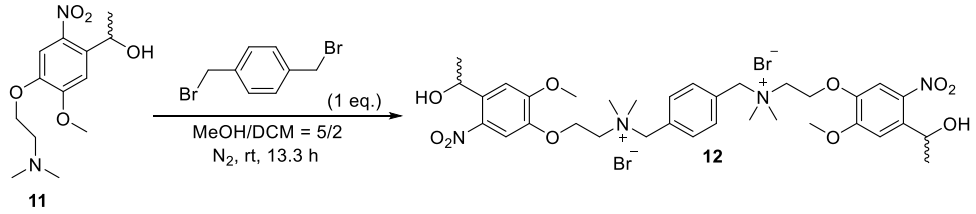
2.2.5 Synthesis of PCA **2**.



1,1'-carbonyl-di(1,2,4-triazole) (CDT, 98.5 mg, 0.6 mmol, 2 eq.) and dry dichloromethane (1 mL)

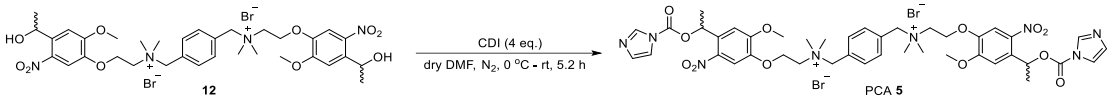
were added to a 10 mL reaction tube, air was removed from the tube, nitrogen was filled, and the reaction tube was cooled at -20 °C. **11** (85.3 mg, 0.3 mmol, 1eq.) and DIPEA (53 μL, 0.3 mmol, 1eq.) were dissolved in dry dichloromethane (1 mL+3 mL) and then the solution was slowly injected into the reaction tube . The reaction mixture was stirred at -20 °C for 24 h. Dilute the reaction solution with DCM and wash the organic phase with water and saturated sodium chloride solution. The organic phase was dried with anhydrous sodium sulfate, filtered, and the filtrate was concentrated by rotary evaporator to give PCA **2** (79.1 mg, 69.5%) as yellow solid. ^{1}H NMR (300 MHz, Chloroform-*d*) δ 8.83 (s, 1H), 8.07 (s, 1H), 7.67 (s, 1H), 7.18 (s, 1H), 6.84 (q, *J* = 6.4 Hz, 1H), 4.17 (t, *J* = 5.9 Hz, 2H), 3.93 (s, 3H), 2.79 (t, *J* = 5.8 Hz, 2H), 2.35 (s, 6H), 1.87 (d, *J* = 6.4 Hz, 3H). HRMS (ESI) *m/z*: $[M+H]^+$ calcd for $C_{16}H_{22}N_5O_6$: 380.1565; found: 380.1567.

2.2.6 Synthesis of **12**.



11 (500 mg, 1.76 mmol,2.4 eq.) and 1,4-dibromomethylbenzene (193.5 mg, 0.733 mmol, 1 eq.) were added to the two-neck flask, air was removed from the flask, then nitrogen was filled. To the flask were added methanol (10 mL) and dichloromethane (4 mL) and the reaction mixture was stirred at room temperature for 13.3 h. Reaction mixture was concentrated under reduce pressure to give yellow solid which was then washed by DCM. To collect yellow solid that was then dried by rotary evaporator (552.6 mg, 90%). ^{1}H NMR (500 MHz, DMSO-d_6) δ 7.78 (s, 4H), 7.73 (s, 2H), 7.43 (s, 2H), 5.54 (d, *J* = 4.4 Hz, 2H), 5.32 – 5.26 (m, 2H), 4.78 (s, 4H), 4.66 (t, *J* = 4.7 Hz, 4H), 3.92 (s, 6H), 3.86 (t, *J* = 4.9 Hz, 4H), 3.14 (s, 12H), 1.38 (d, *J* = 6.2 Hz, 6H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 153.38, 145.14, 138.98, 138.84, 133.56, 130.02, 109.50, 109.36, 66.40, 63.91, 62.88, 62.30, 56.33, 49.94, 25.25. HRMS (ESI) *m/z*: $[M]^{2+}$ calcd for $C_{34}H_{48}N_4O_{10}^{2+}$: 336.1680; found: 336.1679.

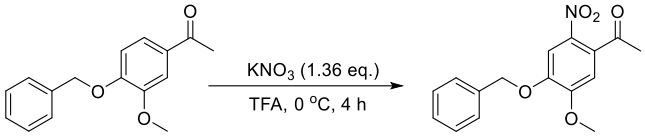
2.2.7 Synthesis of PCA **5**.



12 (50 mg, 0.06 mmol, 1 eq.) and dry DMF (4 mL) were added to a two-neck flask, air was withdrawn from the flask, then nitrogen was filled back, and the reaction flask was placed in an ice water bath to cool. 1,1'-carbonyldiimidazole (CDI, 38.95 mg, 0.24 mmol, 4eq.) was dissolved in dry DMF (2 mL), and the resulting solution was slowly injected into flask. Then flask was removed from the ice bath, reaction mixture was stirred at room temperature for 5.2 h. Reaction mixture was transferred to 50 mL round-bottom flask to freeze drying by lyophilizer for 17 h. Add acetone to the flask and put the flask into ultrasound machine, there was yellow solid to precipitate out. Yellow solid was washed twice by acetone, dissolved in CH_3CN and then concentrated by rotary evaporator at room temperature to give PCA **5** (35.2 mg, 57.4%) as yellow solid. ^{1}H NMR (500 MHz, DMSO-d_6) δ 8.39 (s, 2H), 7.79 (s, 2H), 7.75 (s, 4H), 7.68 (d, *J* = 1.5 Hz, 2H), 7.40 (s, 2H), 7.09 (s, 2H), 6.49 (q, *J* = 6.4 Hz, 2H), 4.75 (s, 4H), 4.70 (t, *J* = 4.7 Hz, 4H), 3.93 (s, 6H), 3.90 – 3.84 (m, 4H), 3.12 (s, 12H), 1.79 (d, *J* = 6.5

Hz, 6H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 153.68, 147.58, 146.29, 139.82, 137.46, 133.54, 131.12, 130.44, 129.99, 117.60, 109.49, 109.46, 71.86, 66.33, 62.92, 62.28, 56.81, 49.92, 21.22. HRMS (ESI) *m/z*: $[M]^{2+}$ calcd for $C_{42}H_{52}N_8O_{12}^{2+}$: 430.1847; found: 430.1846.

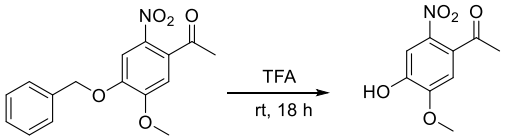
2.2.8 Synthesis of **14**.



13 **14**

Potassium nitrate (3.073 g, 30.4 mmol, 1 36 eq.) and TFA (50 mL) were added to a two neck flask and cooled by stirring under 0 °C. **13** (5.72 g, 22.3 mmol, 1 eq.) was dissolved in TFA (50 mL) and transferred to a pressure-equalizing dropping funnel to slowly drop into the flask. The reaction underwent at 0 °C for 4 h. Reaction mixture was concentrated by rotary evaporator and then added water, basified with sat. aq. sodium bicarbonate. The aqueous solution was extracted with ethyl acetate and organic layer was then dried over Na_2SO_4, filtered, and concentrated under reduced pressure. The crude product was added petroleum ether/ethyl acetate = 6:1 to wash, filtered, filter residue was dried by rotary evaporator to give **14** (4.056 g, 60.4%) as yellow solid. ^{1}H NMR (300 MHz, Chloroform-*d*) δ 7.67 (s, 1H), 7.49 – 7.32 (m, 5H), 6.77 (s, 1H), 5.22 (s, 2H), 3.98 (s, 3H), 2.49 (s, 3H). ^{13}C NMR (101 MHz, Chloroform-*d*) δ 200.20, 154.71, 148.74, 138.43, 135.37, 133.24, 128.99, 128.72, 127.71, 109.01, 71.59, 56.82, 30.52. HRMS (ESI) *m/z*: $[M+H]^+$ calcd for $C_{16}H_{15}NNaO_5$: 324.0842; found: 384.0845

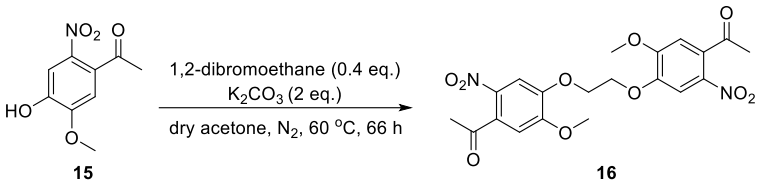
2.2.9 Synthesis of **15**[S6].



14 **15**

In a round-bottomed flask to dissolve **14** (4.04 g, 13.4 mmol, 1eq.) in TFA (60 mL) and stir at room temperature for 18 h. The reaction was monitored by TLC. Reaction mixture was concentrated by rotary evaporator, added water and sat. aq. sodium bicarbonate to basified. The aqueous solution was extracted with ethyl acetate and organic layer was then dried over Na_2SO_4, filtered, and concentrated under reduced pressure. The crude product was added petroleum ether and ethyl acetate to wash, filtered, filter residue was dried by rotary evaporator to give **15** (1.388 g, 49.1%) as yellow solid. ^{1}H NMR (400 MHz, Chloroform-d) δ 7.67 (s, 1H), 6.80 (s, 1H), 4.02 (s, 3H), 2.48 (s, 3H). ^{13}C NMR (101 MHz, Chloroform-d) δ 200.19, 151.44, 147.10, 132.38, 111.21, 109.09, 57.22, 30.75. HRMS (ESI) *m/z*: $[M-H]^-$ calcd for $C_9H_8NO_5$: 210.0408; found: 210.0410.

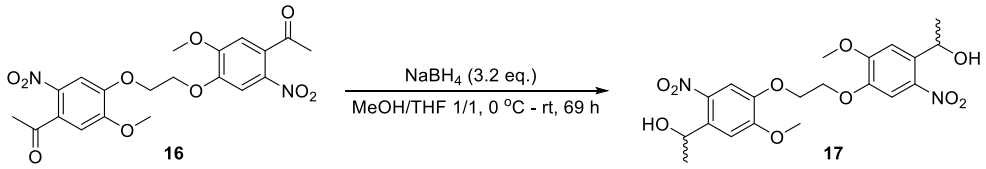
2.2.10 Synthesis of **16**.



15 **16**

15 (528 mg, 2.5 mmol, 1 eq.), potassium carbonate (691 mg, 5 mmol, 2 eq.) and dry acetone (50 mL) were added to a two-neck flask, air was withdrawn from the flask, then nitrogen was filled back. The mixture was heated to 60 °C and then 1,2-dibromoethane (86.2 μL, 1 mmol, 0.4 eq.) was injected to the flask using microinjector. Reaction underwent at 60 °C for 66 h. Add water to the flask and put the flask into ultrasound machine, there was yellow solid to precipitate out. Yellow solid was washed twice by MeOH and then dried by rotary evaporator (342.3 mg, 76.3%). ^{1}H NMR (500 MHz, DMSO-d_6) δ 7.76 (s, 2H), 7.24 (s, 2H), 4.52 (s, 4H), 3.90 (s, 6H), 2.52 (s, 6H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 199.32, 153.29, 148.22, 138.26, 131.58, 109.94, 108.76, 67.83, 56.63, 30.05. HRMS (APCI) *m/z*: $[M+H]^+$ calcd for $C_{20}H_{21}N_2O_{10}$: 449.1191; found: 449.1180.

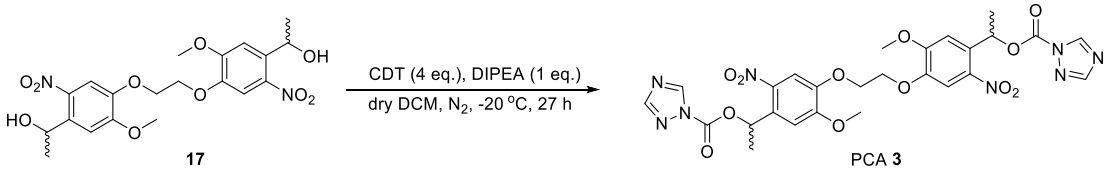
2.2.11 Synthesis of **17**.



16 **17**

In a round-bottomed flask to dissolve **16** (733 mg, 1.635 mmol, 1eq.) in THF (35 mL) and MeOH (35 mL), and the reaction flask was placed in an ice water bath to cool. Sodium borohydride (136.2 mg, 3.6 mmol, 3.2 eq.) was then slowly added to the flask in batches. Ice bath was removed, the reaction mixture was stirred at room temperature for 69 h and the reaction was monitored by TLC. The reaction mixture was concentrated by rotary evaporator, added MeOH to wash, filtered and then collect filtrate. The crude product was purified by flash column chromatography on silica-gel (eluent: petroleum ether/ethyl acetate = 3:1 to 1:1) to yield 17 (109.8 mg, 14.8%) as yellow solid. ^{1}H NMR (400 MHz, Chloroform-*d*) δ 7.71 (s, 2H), 7.30 (d, *J* = 3.8 Hz, 2H), 5.56 (q, *J* = 6.3 Hz, 2H), 4.49 (s, 4H), 3.99 – 3.94 (m, 6H), 1.55 (d, *J* = 6.2 Hz, 6H). ^{13}C NMR (101 MHz, Chloroform-*d*) δ 154.49, 146.81, 146.78, 139.64, 137.75, 110.49, 109.08, 68.34, 65.92, 56.50, 24.47. HRMS (ESI) *m/z*: $[M+Cl]^-$ calcd for $C_{20}H_{24}ClN_2O_{10}$: 487.1125; found: 487.1127.

2.2.12 Synthesis of PCA **3**.



17 PCA **3**

1,1'-carbonyl-di(1,2,4-triazole) (CDT, 65.6 mg, 0.4 mmol, 4 eq.) and dry dichloromethane (2 mL) were added to a 10 mL reaction tube, air was removed from the tube, nitrogen was filled, and the reaction tube was cooled at -20 °C. **17** (45.2 mg, 0.1 mmol, 1eq.) and DIPEA (53 μL, 0.3 mmol, 1eq.) were dissolved in dry dichloromethane (5 mL) and then the solution was slowly injected into the

reaction tube. The reaction mixture was stirred at -20 °C for 27 h. Dilute the reaction solution with DCM and wash the organic phase with water. The organic phase was dried with anhydrous sodium sulfate, filtered, and the filtrate was concentrated by rotary evaporator to obtain PCA **3** (47.1 mg, 73.3%) as yellow solid. ^{1}H NMR (400 MHz, Chloroform-*d*) δ 8.83 (s, 2H), 8.07 (s, 2H), 7.78 (s, 2H), 7.19 (s, 2H), 6.82 (q, *J* = 6.3 Hz, 2H), 4.51 (s, 4H), 3.92 (s, 6H), 1.87 (d, *J* = 6.4 Hz, 6H). ^{13}C NMR (101 MHz, Chloroform-*d*) δ 154.63, 153.96, 147.70, 146.98, 146.03, 139.85, 131.57, 110.32, 108.57, 74.19, 68.29, 56.64, 22.00. HRMS (ESI) *m/z*: $[M+Na]^+$ calcd for $C_{26}H_{26}N_8NaO_{12}$: 665.1562; found: 665.1565.

PCA **4** was synthesized by PCA **3** and MeOTf in DCM. HRMS (ESI) *m/z*: $[M]^{2+}$ calcd for $C_{28}H_{32}N_8O_{12}$: 336.1064; found: 336.1067.

3. Preparation of oligonucleotides

Unmodified crRNA (GFP-crRNA) was purchased from Biosyntech (Suzhou, China). Modified crRNAs (GFP-crRNA-M1, GFP-crRNA-M2 and GFP-crRNA-M3) were purchased from GenScript (Nanjing, China), in which the specially modified phosphoramidite monomer was provided by us.

4. Detection for activity of modified crRNAs

Activity of modified crRNAs (GFP-crRNA-M1, GFP-crRNA-M2 and GFP-crRNA-M3) were detected by Cpf1 cleavage assay. Unmodified crRNA (100 ng) or each of modified crRNA (100 ng) was mixed with target DNA (100 ng), and subjected to an enzymatic digest with Cpf1 (10 pmol) at 37 °C for 2 h. Reaction was quenched by adding 1 μL proteinase K and the mixture was incubated at 55 °C for 10 min followed by 95 °C for 5 min. Subsequently, 6 × DNA loading buffer was added to the mixture and resulting solution was loaded onto agarose gel containing 1 × Ultra GelRed for visualization.

5. General procedure for cloaking and uncloaking

5.1 Modified crRNA was cloaked by (PCA) 1.

Cloaked crRNA: 1 μL 100 μM modified crRNA and 8 μL nuclease-free water were added to a centrifuge tube and then heated to 95 °C for 2 minutes followed by 4 °C for 2 minutes of cooling. Next, 1 μL 1 M PCA **1** (in dry DMSO) was added to the centrifuge tube. The mixture was incubated at 37 °C for 3 h. After 3 h 36 μL ethanol, 1 μL NaOAc (3 M) and 1 μL glycogen (10 mg/mL) were added to quench the reaction and purify RNA. The mixture was placed at −20 °C for 4 h. The resulting suspension was centrifuged at 14800 rpm for 30 min to remove the supernatant, then washed the precipitate with 0.5 mL 75% ethanol twice, and centrifuged at 14800 rpm for 5 min to remove the supernatant. Dry the centrifuge tube in air for about 10 minutes, then redissolve the RNA with 10 μL of nuclease-free water to give cloaked crRNA. The concentration of RNA was quantified by Nanodrop One.

GFP-crRNA-M2-**1** (150 ng) obtained from the above method was analyzed by denaturing PAGE gel. Activity of GFP-crRNA-M2-**1** was detected by Cpf1 cleavage assay: Cloaked GFP-crRNA-M2-**1** (150 ng) was mixed with target DNA (100 ng), and subjected to an enzymatic digest with Cpf1 (10 pmol) 37 °C for 16 h. Reaction was quenched by adding 1 μL proteinase K and the mixture was incubated at 55 °C for 10 min followed by 95 °C for 5 min. Subsequently, 6 × DNA loading buffer was added to the mixture and resulting solution was loaded onto agarose gel containing 1 × Ultra GelRed for visualization.

5.2 Modified crRNA was cloaked by (PCA) 2.

Cloaked crRNA: 1 μL 100 μM modified crRNA and 6 μL nuclease-free water were added to a centrifuge tube and then heated to 95 °C for 2 minutes followed by 4 °C for 2 minutes of cooling. Next, 3 μL 1 M PCA **2** (in dry DMSO) was added to the centrifuge tube. The mixture was incubated at 37 °C for 12 h. After 12 h, 36 μL ethanol, 1 μL NaOAc (3 M) and 1 μL glycogen (10 mg/mL) were added to quench the reaction and purify RNA. The mixture was placed at −80°C for 4 h. The resulting suspension was centrifuged at 15000 rpm for 30 min to remove the supernatant, then washed the precipitate with 0.5 mL 75% ethanol twice, and centrifuged at 15000 rpm for 5 min to remove the supernatant. Dry the centrifuge tube in air for about 10 minutes, then redissolve the RNA with 10 μL of nuclease-free water to give cloaked crRNA. The concentration of RNA was quantified by Nanodrop One.

GFP-crRNA-M1-**2** (100 ng) obtained from the above method was analyzed by denaturing PAGE gel.

Activity of GFP-crRNA-M1-**2** was detected by Cpf1 cleavage assay: Cloaked GFP-crRNA-M1-**2** (100 ng) was mixed with target DNA (100 ng), and subjected to an enzymatic digest with Cpf1 (10 pmol) 37 °C for 2 h. Reaction was quenched by adding 1 μL proteinase K and the mixture was incubated at 55 °C for 10 min followed by 95 °C for 5 min. Subsequently, 6 × DNA loading buffer was added to the mixture and resulting solution was loaded onto agarose gel containing 1 × Ultra GelRed for visualization.

5.3 Modified crRNA was cloaked by (PCA) 3.

Cloaked crRNA: 1 μL 100 μM modified crRNA and 5 μL SHAPE buffer (200 mM HEPES, 200 mM NaCl, 12 mM $MgCl_2$, pH 7.5) were added to a centrifuge tube and then heated to 95 °C for 2 minutes followed by 4 °C for 2 minutes of cooling. Next, 1 μL 1 M DMAP (in dry DMSO) and 3 μL 3.3 mM PCA **3** (in dry DMSO) were added to the centrifuge tube. The mixture was incubated at 37 °C for 12 h. After 12 h, 90 μL ethanol, 3 μL NaOAc (3 M) and 1 μL glycogen (10 mg/mL) were added to quench the reaction and purify RNA. The mixture was placed at −80°C for 4 h. The resulting suspension was centrifuged at 15000 rpm for 30 min to remove the supernatant, then washed the precipitate with 0.5 mL 75% ethanol twice, and centrifuged at 15000 rpm for 5 min to remove the supernatant. Dry the centrifuge tube in air for about 10 minutes, then redissolve the RNA with 10 μL of nuclease-free water to give cloaked crRNA. The concentration of RNA was quantified by Nanodrop One.

GFP-crRNA-M1-**3** (100 ng) obtained from the above method was analyzed by denaturing PAGE gel.

5.4 Modified crRNA was cloaked by (PCA) 4.

Cloaked crRNA: 1 μL 100 μM modified crRNA and 5 μL SHAPE buffer (200 mM HEPES, 200 mM NaCl, 12 mM $MgCl_2$, pH 7.5) were added to a centrifuge tube and then heated to 95 °C for 2 minutes followed by 4 °C for 2 minutes of cooling. Next, 1 μL 1 M DMAP (in dry DMSO) and 3 μL 0.5 M PCA **4** (in dry DMSO) were added to the centrifuge tube. The mixture was incubated at 37 °C for 6 h. After 6 h, 100 μL ethanol, 3 μL NaOAc (3 M) and 1 μL glycogen (10 mg/mL) were added to quench the reaction and purify RNA. The mixture was placed at −80°C for 12 h. The resulting suspension was centrifuged at 15000 rpm for 30 min to remove the supernatant, then washed the precipitate with 0.5 mL 75% ethanol twice, and centrifuged at 15000 rpm for 5 min to remove the supernatant. Dry the centrifuge tube in air for about 10 minutes, then redissolve the RNA with 10 μL of nuclease-free water to give cloaked crRNA. The concentration of RNA was quantified by Nanodrop One.

GFP-crRNA-M1-**4** (30 ng) obtained from the above method was analyzed by denaturing PAGE gel.

5.5 Modified crRNA was cloaked by (PCA) 5 and then was uncloaked by 365nm ultraviolet lamp.

Cloaked crRNA: 1 μL 100 μM modified crRNA, 5 μL SHAPE buffer (200 mM HEPES, 200 mM NaCl, 12 mM $MgCl_2$, pH 7.5) and 1 μL nuclease-free water were added to a centrifuge tube and then heated to 95 °C for 2 minutes followed by 4 °C for 2 minutes of cooling. Next, 1 μL 1 M DMAP (in dry DMSO) and 2 μL 0.5 M PCA **5** (in dry DMSO) were added to the centrifuge tube. The mixture was incubated at 37 °C for different time period. After corresponding time, 36 μL ethanol, 1 μL NaOAc (3 M) and 1 μL glycogen (10 mg/mL) were added to quench the reaction and purify RNA. The mixture was placed at −80°C for 2 h. The resulting suspension was centrifuged at 15000 rpm for 30 min to remove the supernatant, then washed the precipitate with 0.5 mL 75% ethanol twice, and centrifuged at 15000 rpm for 5 min to remove the supernatant. Dry the centrifuge tube in air for about 10 minutes, then redissolve the RNA with 10 μL of nuclease-free water to give cloaked crRNA. The concentration of RNA was quantified by Nanodrop One.

GFP-crRNA-M2-**5** (50 ng) obtained from the above method was analyzed by denaturing PAGE gel.

Activity of GFP-crRNA-M2-**5** was detected by Cpf1 cleavage assay: GFP-crRNA-M2-**5** (50 ng) was mixed with target DNA (100 ng), and subjected to an enzymatic digest with Cpf1 (10 pmol) 37 °C for 2 h. Reaction was quenched by adding 1 μL proteinase K and the mixture was incubated at 55 °C for 10 min followed by 95 °C for 5 min. Subsequently, 6 × DNA loading buffer was added to the mixture and resulting solution was loaded onto agarose gel containing 1 × Ultra GelRed for visualization. GFP-crRNA-M3-**5** (150 ng) obtained from the above method was analyzed by denaturing PAGE gel. Activity of GFP-crRNA-M3-**5** was detected by Cpf1 cleavage assay: GFP-crRNA-M3-**5** (100 ng) was mixed with target DNA (100 ng), and subjected to an enzymatic digest with Cpf1 (10 pmol) at 37 °C for 4 h. Reaction was quenched by adding 1 μL proteinase K and the mixture was incubated at 55 °C for 10 min followed by 95 °C for 5 min. Subsequently, 6 × DNA loading buffer was added to the mixture and resulting solution was loaded onto agarose gel containing 1 × Ultra GelRed for visualization.

Uncloaked crRNA: Cloaked crRNA obtained from the above method was added to the glass sample vial of HPLC, and the vial was placed on the top of 365nm ultraviolet lamp with the light intensity of 50 mW/cm^2 for different time period to give uncloaked crRNA.

Uncloaked GFP-crRNA-M3-**5** (150 ng) obtained from the above method was analyzed by denaturing PAGE gel. Recovery of activity of uncloaked GFP-crRNA-M3-**5** was detected by Cpf1 cleavage assay: Uncloaked GFP-crRNA-M3-**5** (100 ng) was mixed with target DNA (100 ng), and subjected to an enzymatic digest with Cpf1 (10 pmol) at 37 °C for 4 h. Reaction was quenched by adding 1 μL proteinase K and the mixture was incubated at 55 °C for 10 min followed by 95 °C for 5 min. Subsequently, 6 × DNA loading buffer was added to the mixture a and resulting solution was loaded onto agarose gel containing 1 × Ultra GelRed for visualization.

6. Reduction-responsive cleavage of modified crRNAs

Unmodified crRNA (25 ng) (GFP-crRNA) or each of modified crRNA (25 ng) (GFP-crRNA-M1, GFP-crRNA-M2 and GFP-crRNA-M3) and 1 μL of 1 M $Na_2S_2O_4$ were incubated at 37 °C for 10 min in 10 mM Tris-HCl buffer (contained 25 mM $Mg(OAc)_2$, 1 mM sodium edetate at pH 8.0). The total reaction volume was 6 μL. $Na_2S_2O_4$ was dissolved in 10 mM Tris-HCl buffer (contained 25 mM $Mg(OAc)_2$, 1 mM sodium edetate, pH 8.0) resulting a concentration of 1 M and used immediately. The cleavage of modified crRNAs was analyzed by denaturing PAGE gel.

Observe DNA cleavage to assess the responsiveness of GFP-crRNA-M to reduction in genomic editing: GFP-crRNA (25 ng) or cleaved GFP-crRNA-M (25 ng) (the reaction mixture of above method) was mixed with target DNA (100 ng), and subjected to an enzymatic digest with Cpf1 (10 pmol) at 37 °C for 2 h. Reaction was quenched by adding 1 μL proteinase K and the mixture was incubated at 55 °C for 10 min followed by 95 °C for 5 min. Subsequently, 6 × DNA loading buffer was added to the mixture and resulting solution was loaded onto agarose gel containing 1 × Ultra GelRed for visualization.

7. Dual-stimuli-responsive CRISPR/Cpf1 editing

25 ng GFP-crRNA-M3-**5** (30 min) (prepared by general method, see 5.5 (page S13) for details) or 25 ng uncloaked (10 min) GFP-crRNA-M3-**5** (prepared by GFP-crRNA-M3-**5** (30 min), see 5.5 (page S13) for details) or 25 ng cleaved uncloaked (10 min) GFP-crRNA-M3-**5** (prepared from uncloaked (10 min) GFP-crRNA-M3-**5**, see 6 (page S13) for details) was mixed with target DNA (100 ng), and subjected to an enzymatic digest with Cpf1 (10 pmol) at 37 °C for 4 h. Reaction was quenched by adding 1 μL proteinase K and the mixture was incubated at 55 °C for 10 min followed by 95 °C for 5 min.

Subsequently, 6 × DNA loading buffer was added to the mixture and resulting solution was loaded onto agarose gel containing 1 × Ultra GelRed for visualization.

8. Denaturing polyacrylamide gel electrophoresis

15% denaturing urea (7 M) polyacrylamide gel electrophoresis (19 : 1 monomer to bis ratio) was used to characterize the RNAs in vertical electrophoresis system (Bio-Rad). Unless otherwise noted, they were run in 1 × TBE at 180 V for 65 min and then were stained with 3 × Ultra GelRed and imaged with Tanon-5200 Multi Fluorescence Imager.

9. In vitro DNA cleavage assay and agarose gel electrophoresis

crRNAs were incubated with Cpf1 and target DNA in 1 × NEB buffer r3.1 (NEB) in RNase-free water and the total reaction volume was 10 μL. Cpf1 protein was degraded by incubating with proteinase K at 55 °C for 10 minutes, and protease K was inactivated by heating at 95 °C for 5 minutes finally. Subsequently, 2.2 μL of 6 × DNA loading buffer was added to the mixture and resulting solution was loaded onto agarose gel. DNA cleavage product was analyzed by 2% agarose gel containing 1 × Ultra GelRed in 1 × TAE at 170 V for 35 min. Electrophoresis was used horizontal electrophoresis system (Bio-Rad) to run. Agarose gel was imaged with Tanon-5200 Multi Fluorescence Imager.

10. Amplify target DNA by polymerase chain reaction

Target DNA was amplified by polymerase chain reaction from pEGFP-N1 vector using the following primer pairs: EGFP-F: 5’-CGGTTTGACTCACGGGGATT-3’, EGFP-R: 5’-CTCGATGTTGTGGCGGATCT-3’ and then purified using Cycle Pure Kit (Omega Bio-Tek).

11. Supplementary tables and figures

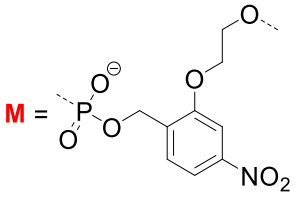
Oligonucleotides	Sequence (from 5’to 3’)
GFP-crRNA	AAUUUCUACUCUUGUAGAUCGUCGCCGUCCAGCUCGACCAGGA
GFP-crRNA-M1	AAUUUCUACU**M**UUGUAGAUCGUCGCCGUCCAGCUCGACCAGGA **M** = 
GFP-crRNA-M2	AAUUUCUACUC**M**UGUAGAUCGUCGCCGUCCAGCUCGACCAGGA
GFP-crRNA-M3	AAUUUCUACU**M**UGUAGAUCGUCGCCGUCCAGCUCGACCAGGA
Primer: EGFP-F	CGGTTTGACTCACGGGGATT
Primer: EGFP-R	CTCGATGTTGTGGCGGATCT

Table S1 DNA and RNA sequences used in the study.

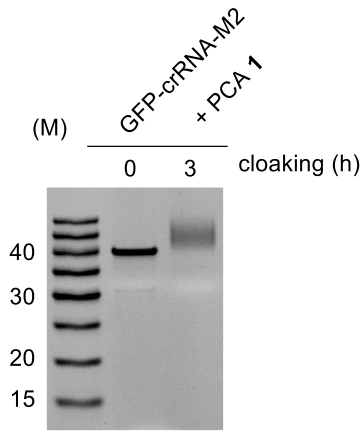


Figure S1. Analysis of GFP-crRNA-M2-**1** by denaturing PAGE gel.

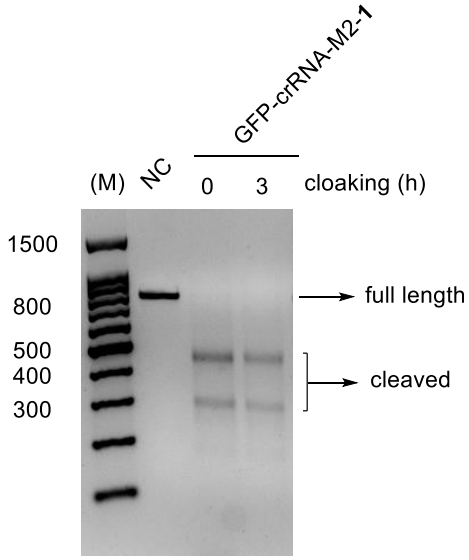


Figure S2. Detection for activity of GFP-crRNA-M2-**1** by Cpf1 cleavage assay.

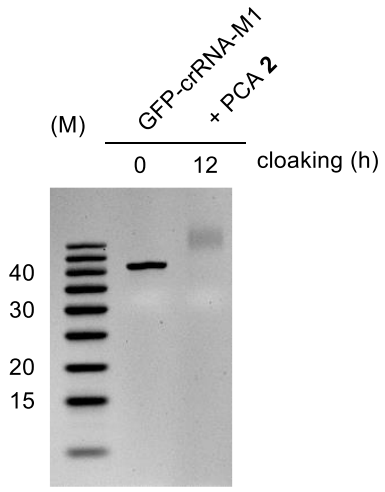


Figure S3. Analysis of GFP-crRNA-M1-**2** by denaturing PAGE gel.

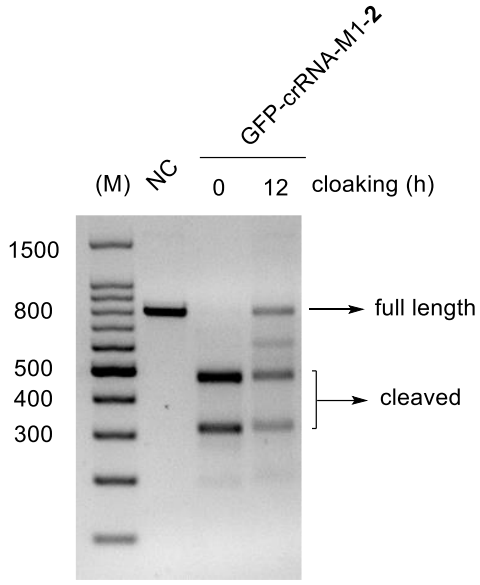


Figure S4. Detection for activity of GFP-crRNA-M1-**2** by Cpf1 cleavage assay.

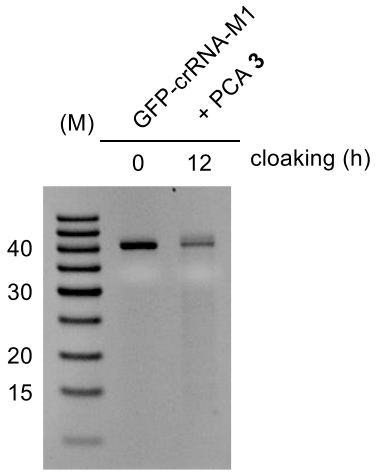


Figure S5. Analysis of GFP-crRNA-M1-**3** by denaturing PAGE gel.

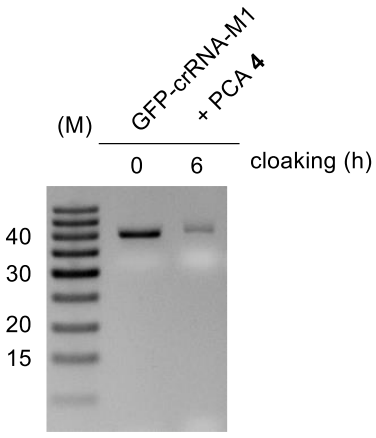
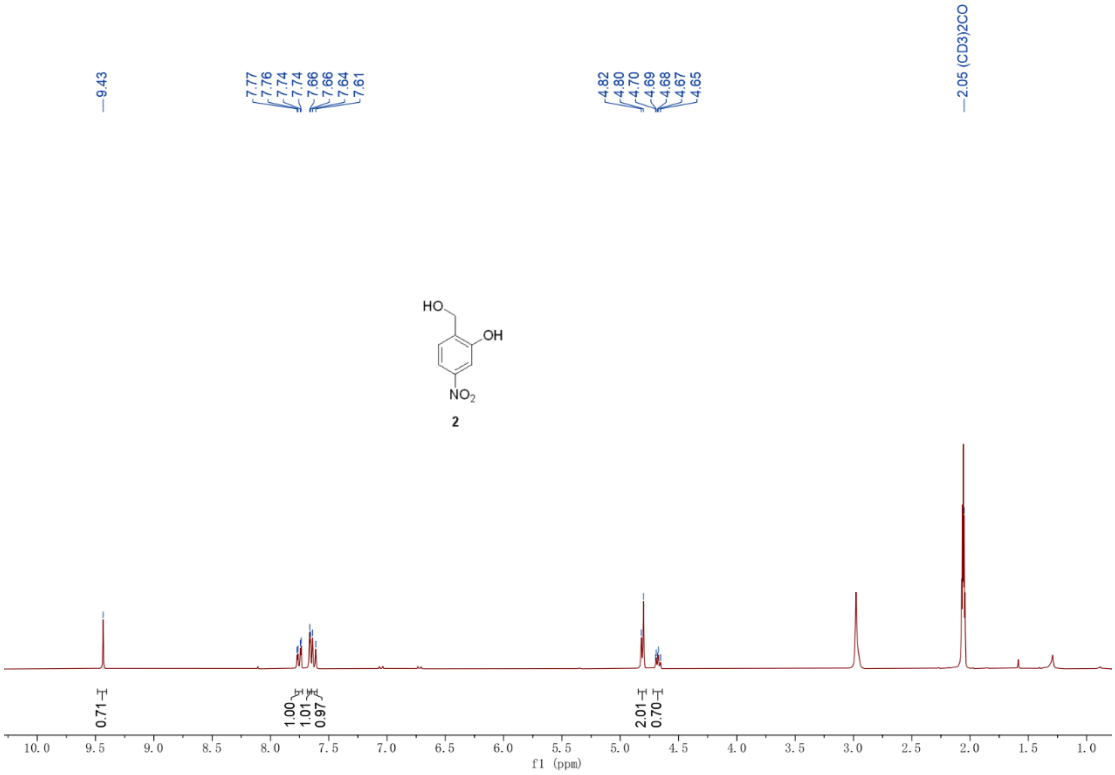


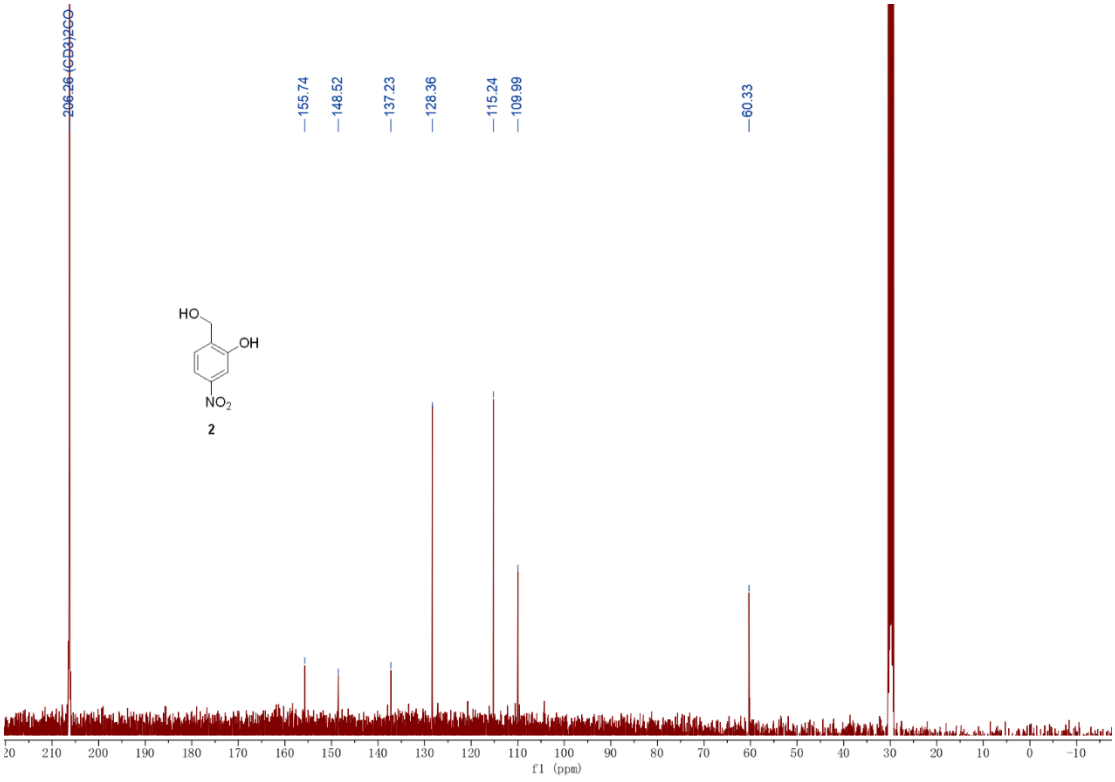
Figure S6. Analysis of GFP-crRNA-M1-**4** by denaturing PAGE gel.

12. NMR spectra

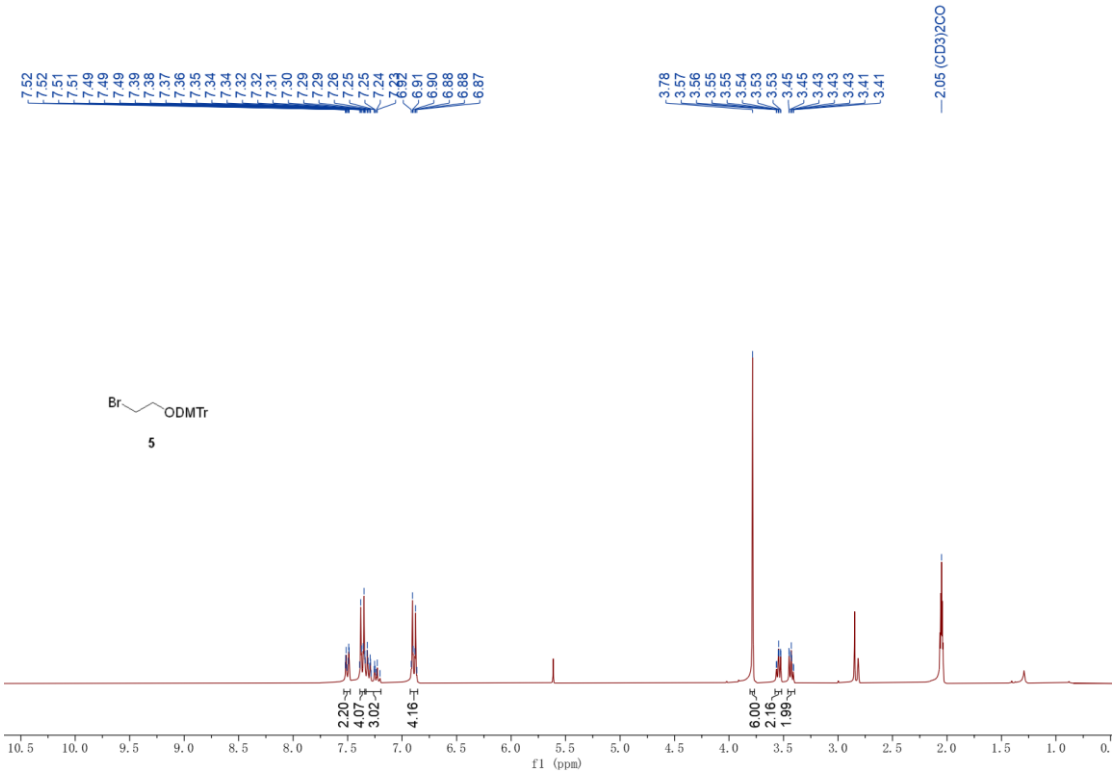
^{1}H NMR (300 MHz, Acetone-d_6) spectrum of **2**



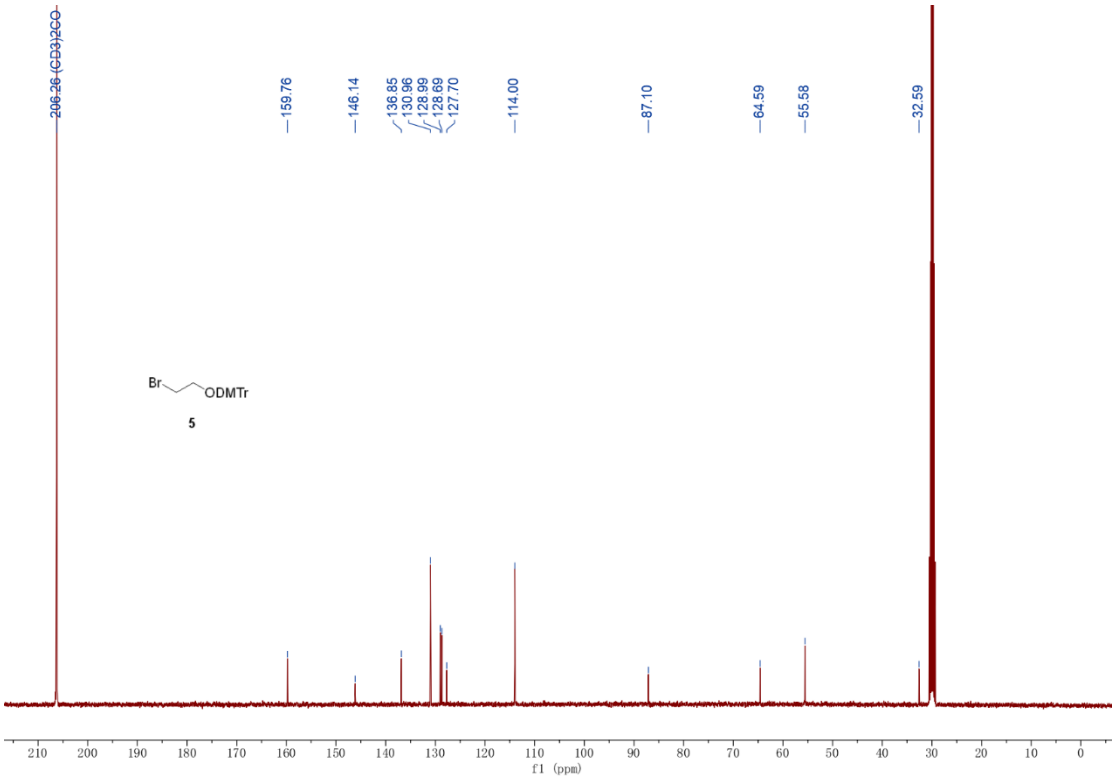
^{13}C NMR (101 MHz, Acetone-d_6) spectrum of **2**



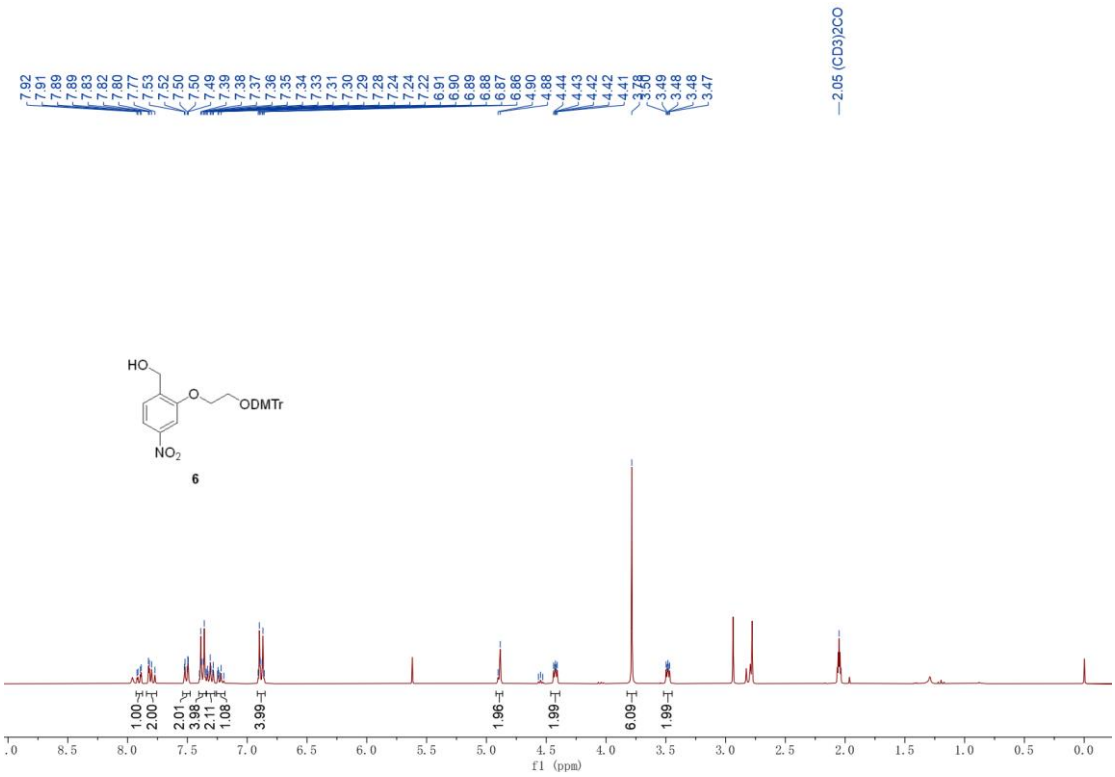
^{1}H NMR (300 MHz, Acetone-d_6) spectrum of **5**



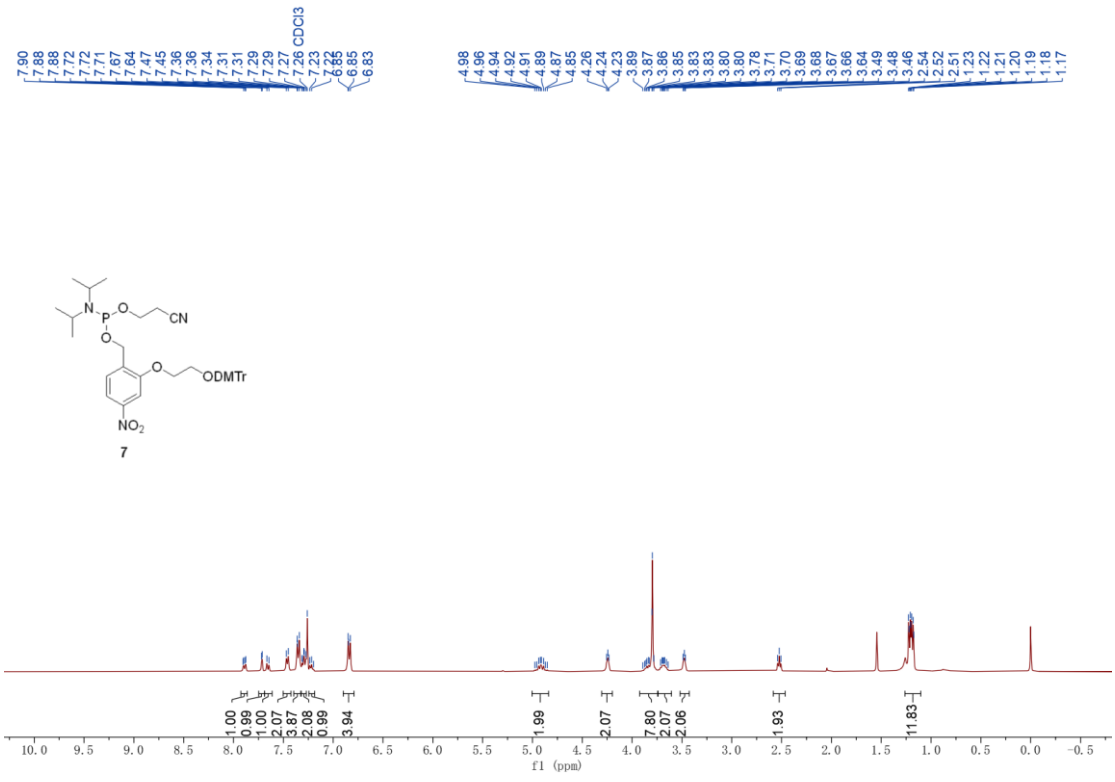
^{13}C NMR (101 MHz, Acetone-d_6) spectrum of **5**



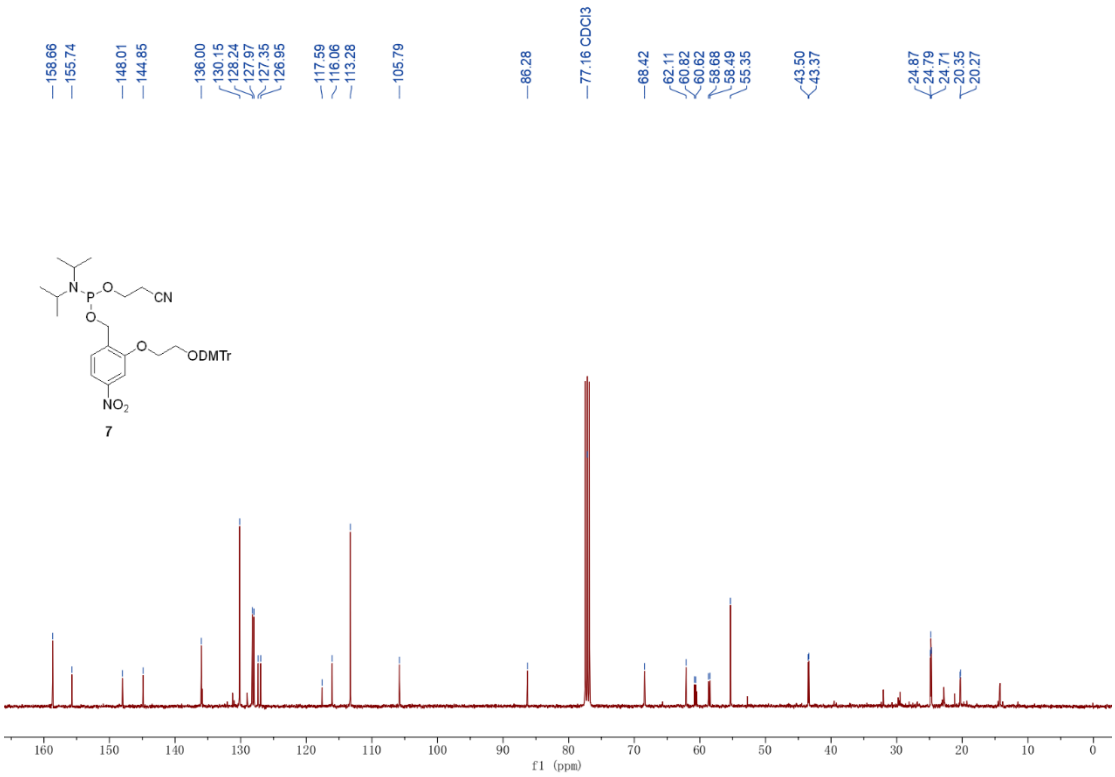
^{1}H NMR (300 MHz, Acetone-d_6) spectrum of **6**



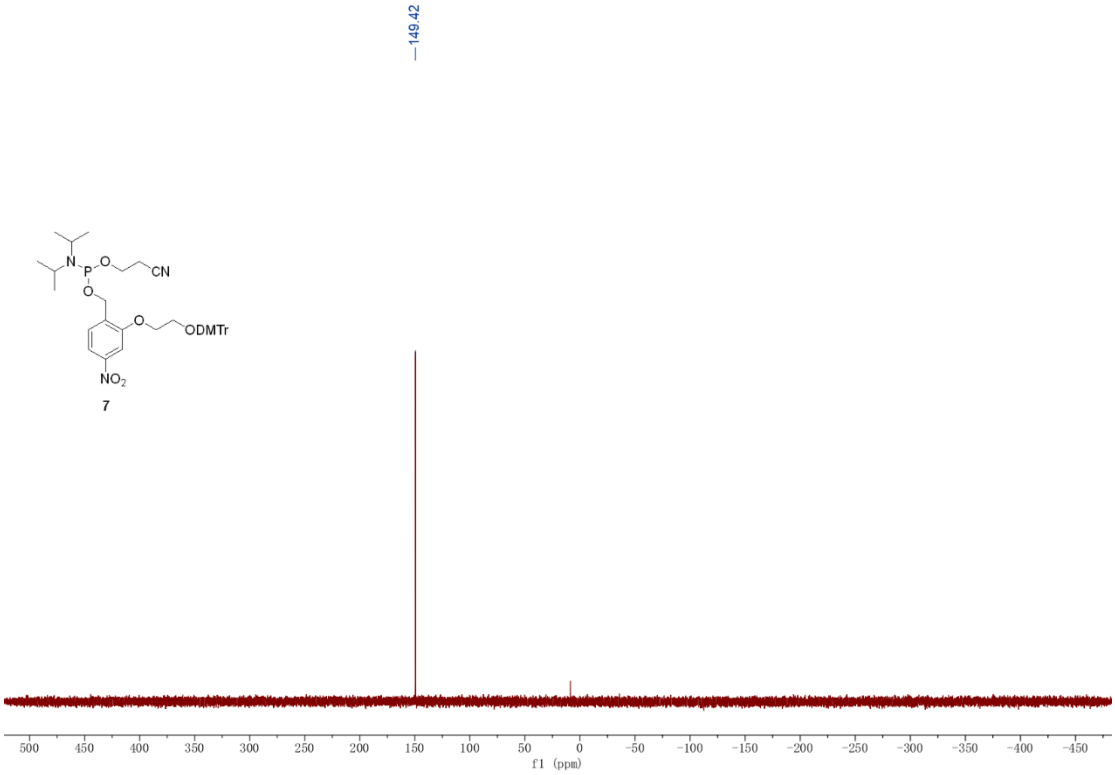
^{1}H NMR (400 MHz, Chloroform-*d*) spectrum of **7**



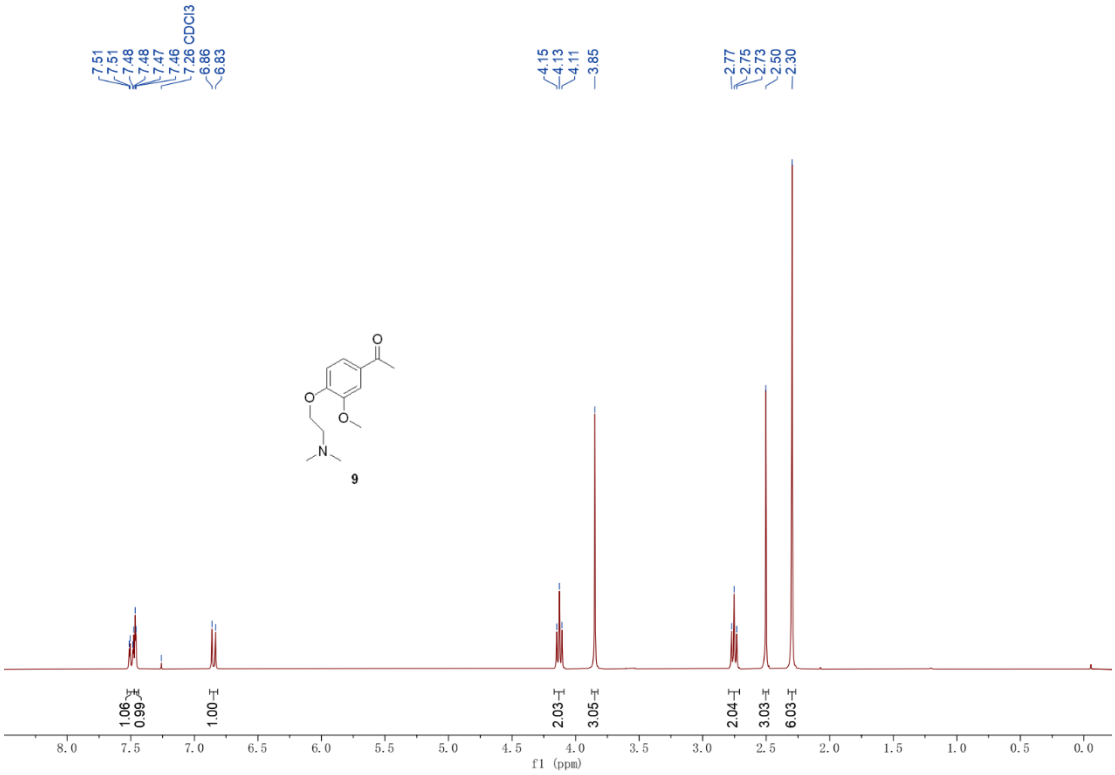
^{13}C NMR (101 MHz, Chloroform-*d*) spectrum of **7**



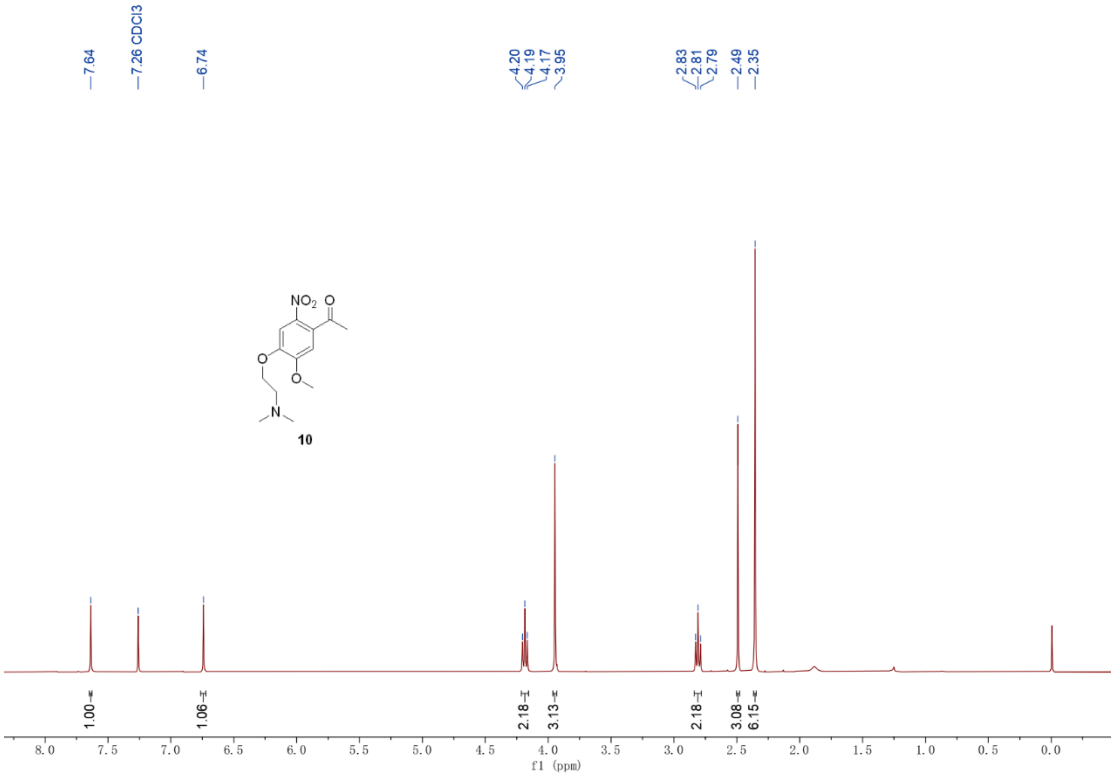
^{31}P NMR (162 MHz, Chloroform-*d*) spectrum of **7**



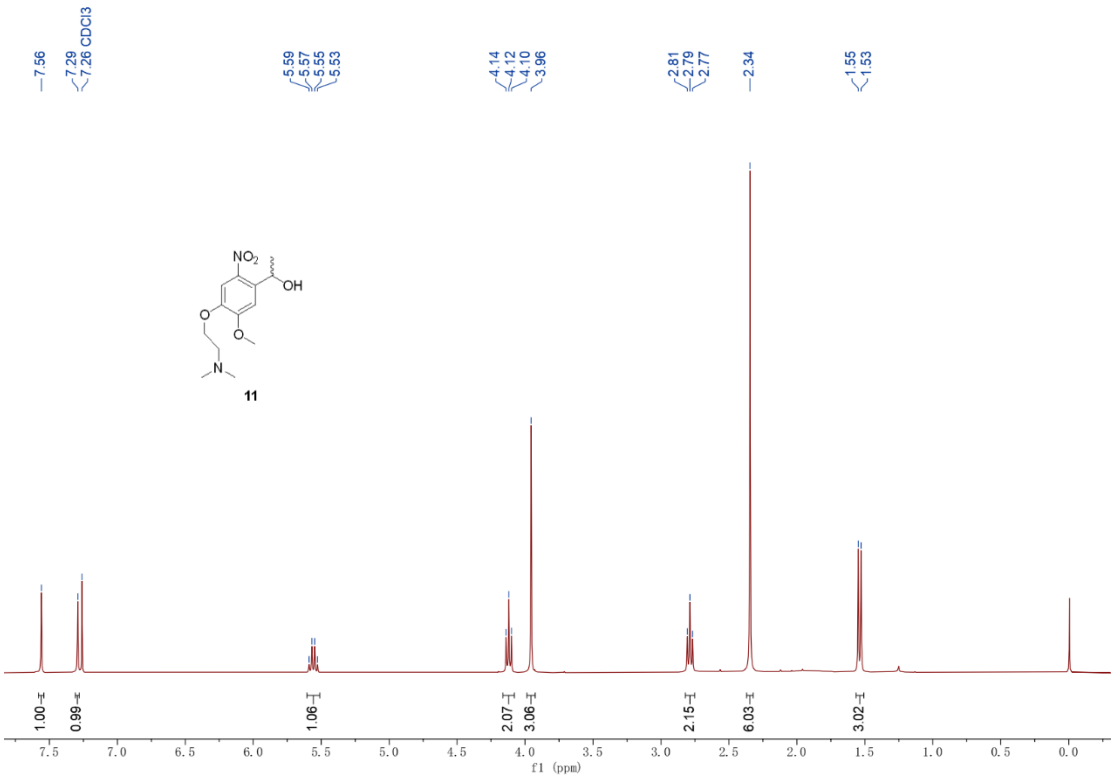
^{1}H NMR (300 MHz, Chloroform-*d*) spectrum of **9**



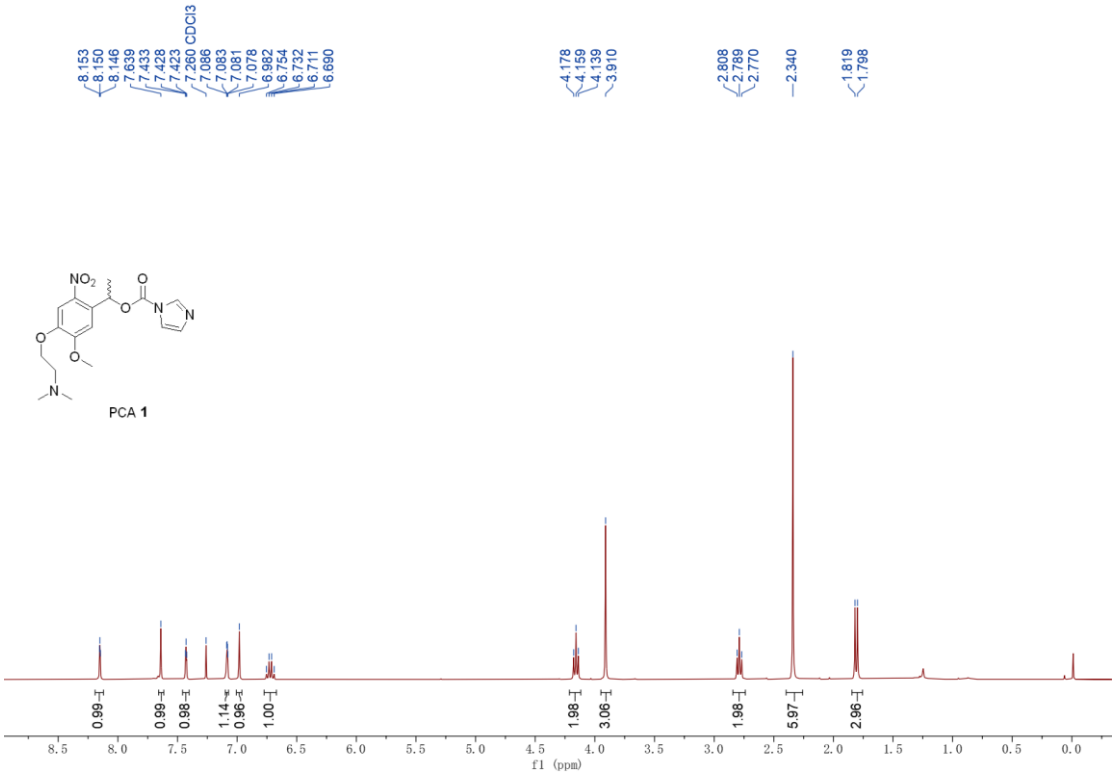
^{1}H NMR (300 MHz, Chloroform-*d*) spectrum of **10**



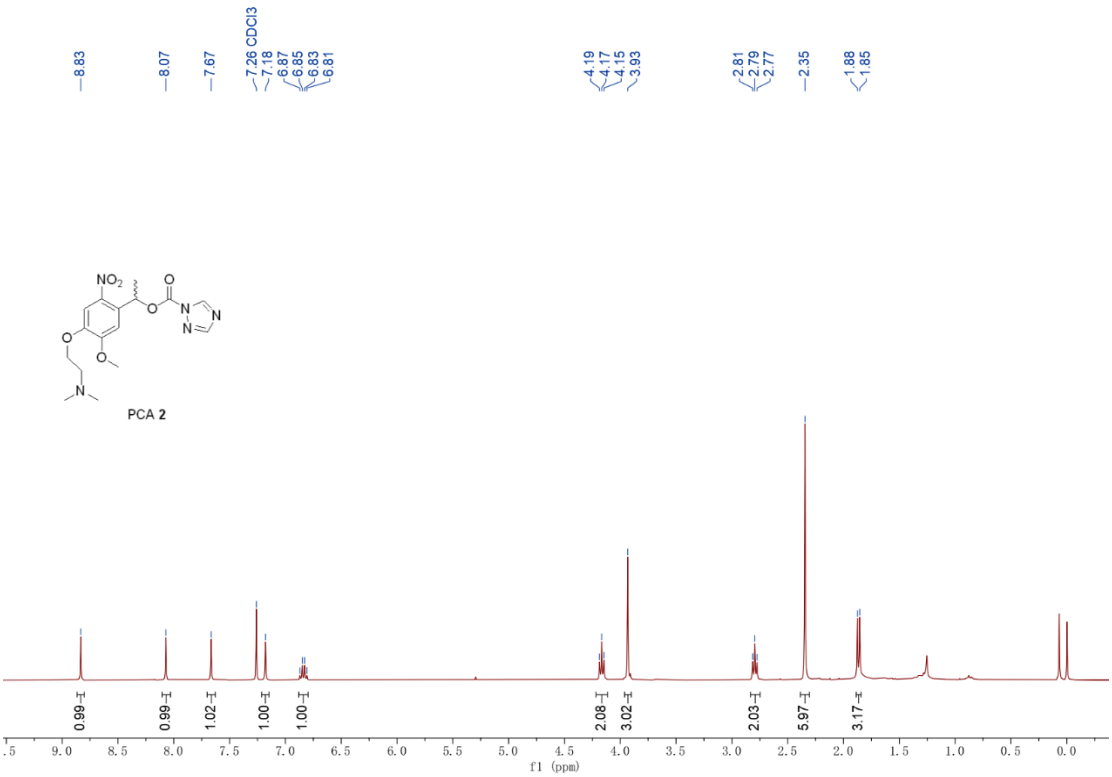
^{1}H NMR (300 MHz, Chloroform-*d*) spectrum of **11**



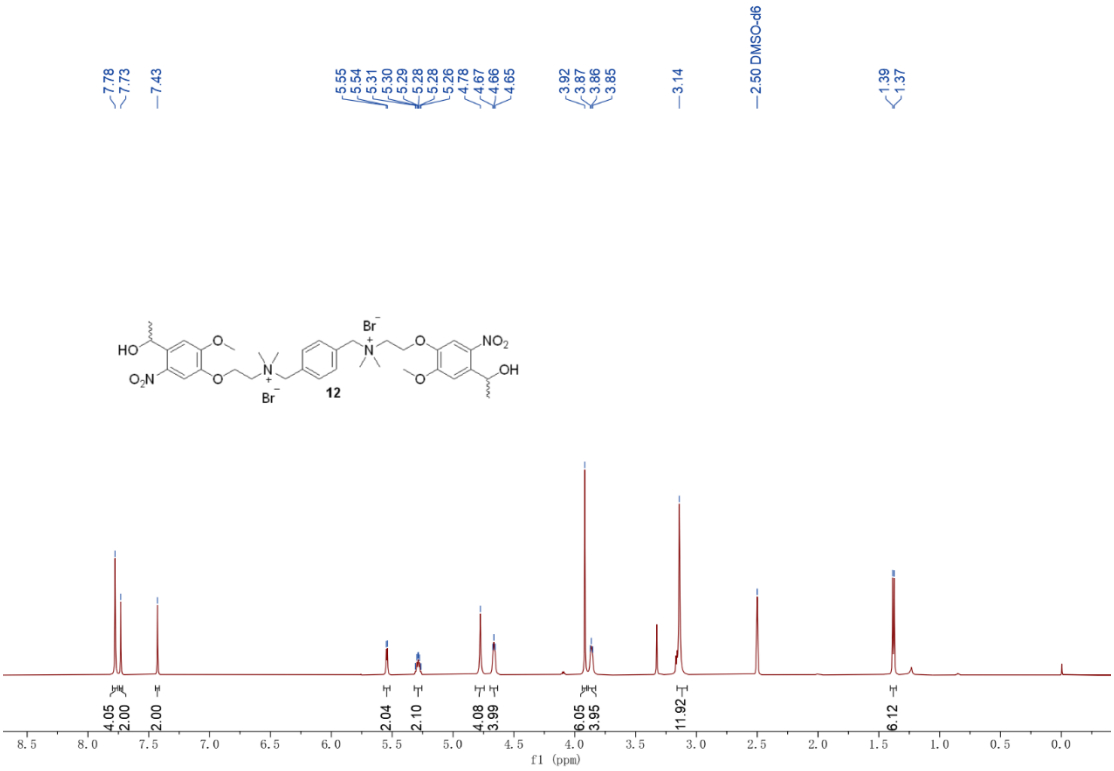
^{1}H NMR (300 MHz, Chloroform-*d*) spectrum of PCA **1**



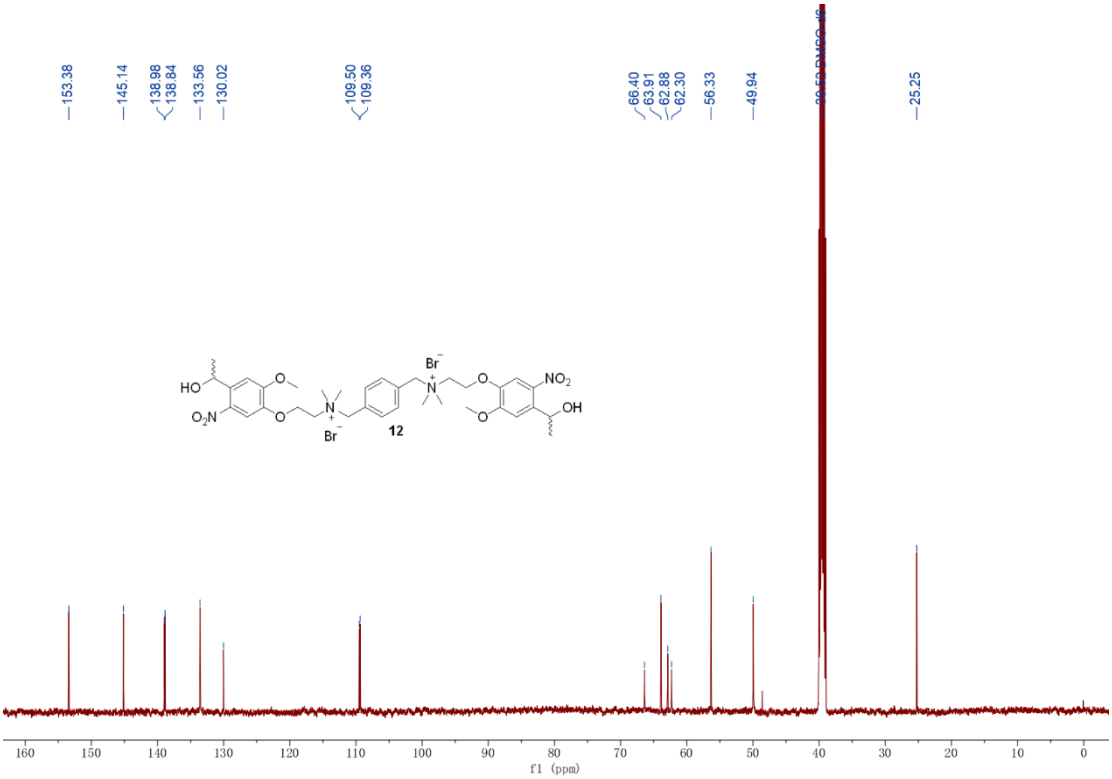
^{1}H NMR (300 MHz, Chloroform-*d*) spectrum of PCA **2**



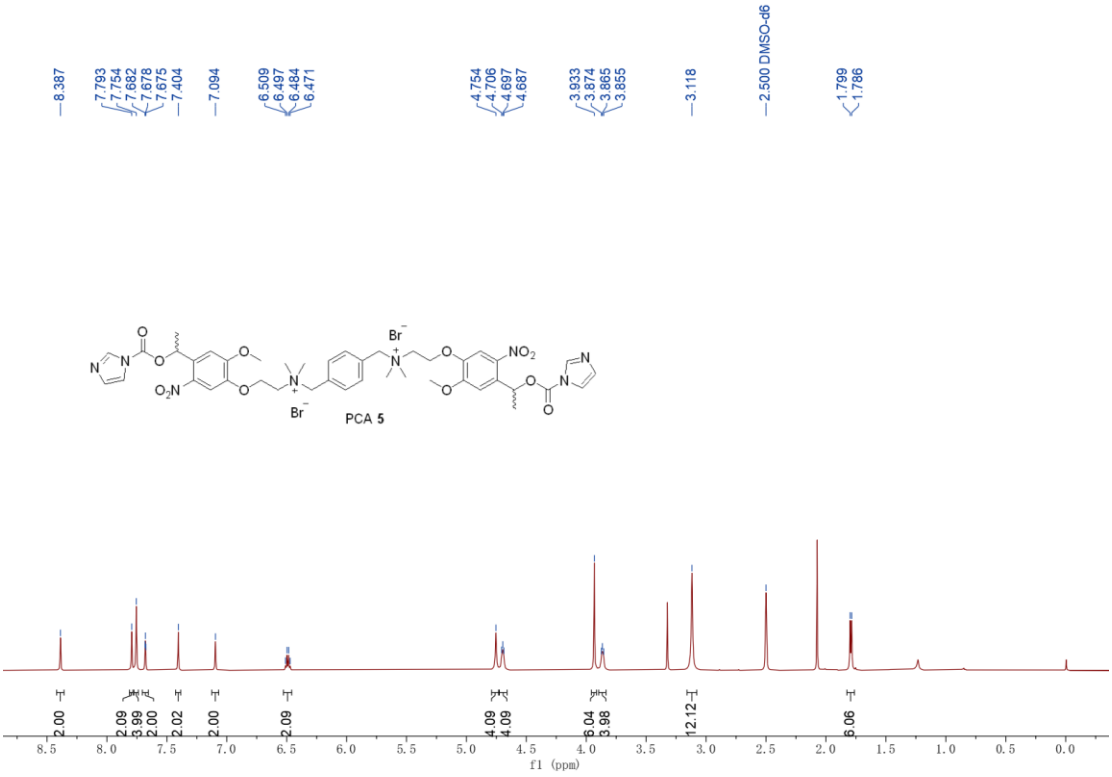
^{1}H NMR (500 MHz, DMSO-d_6) spectrum of **12**



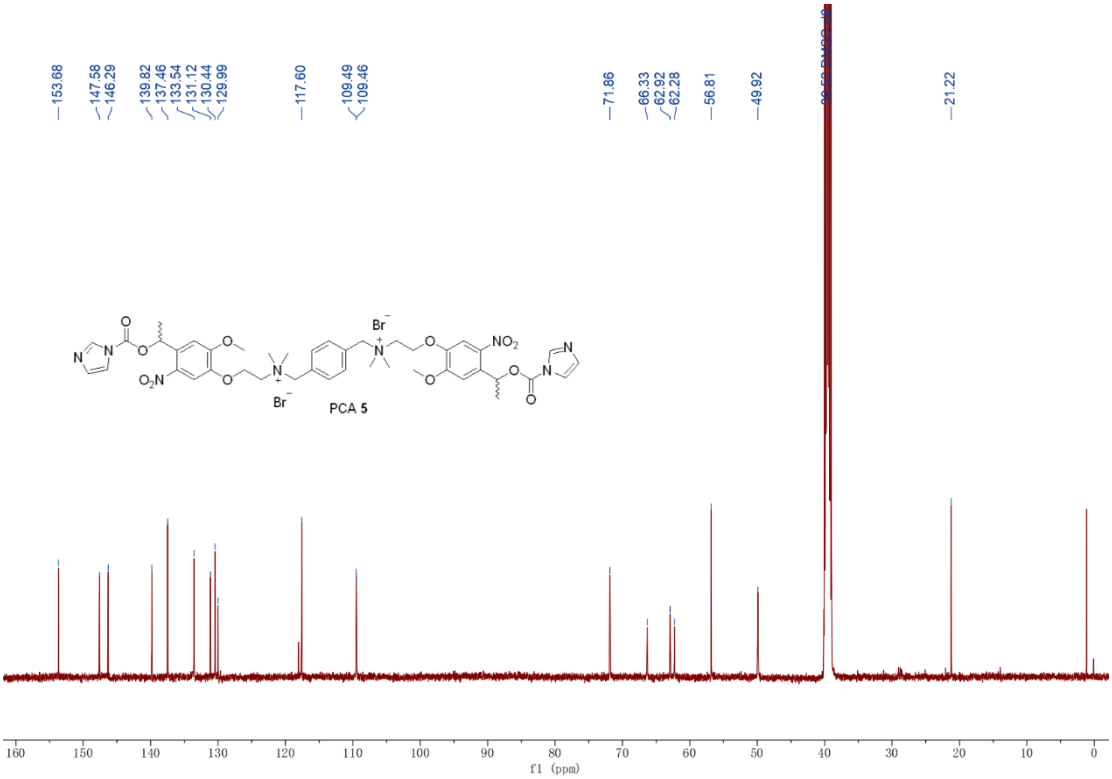
^{13}C NMR (126 MHz, DMSO-d_6) spectrum of **12**



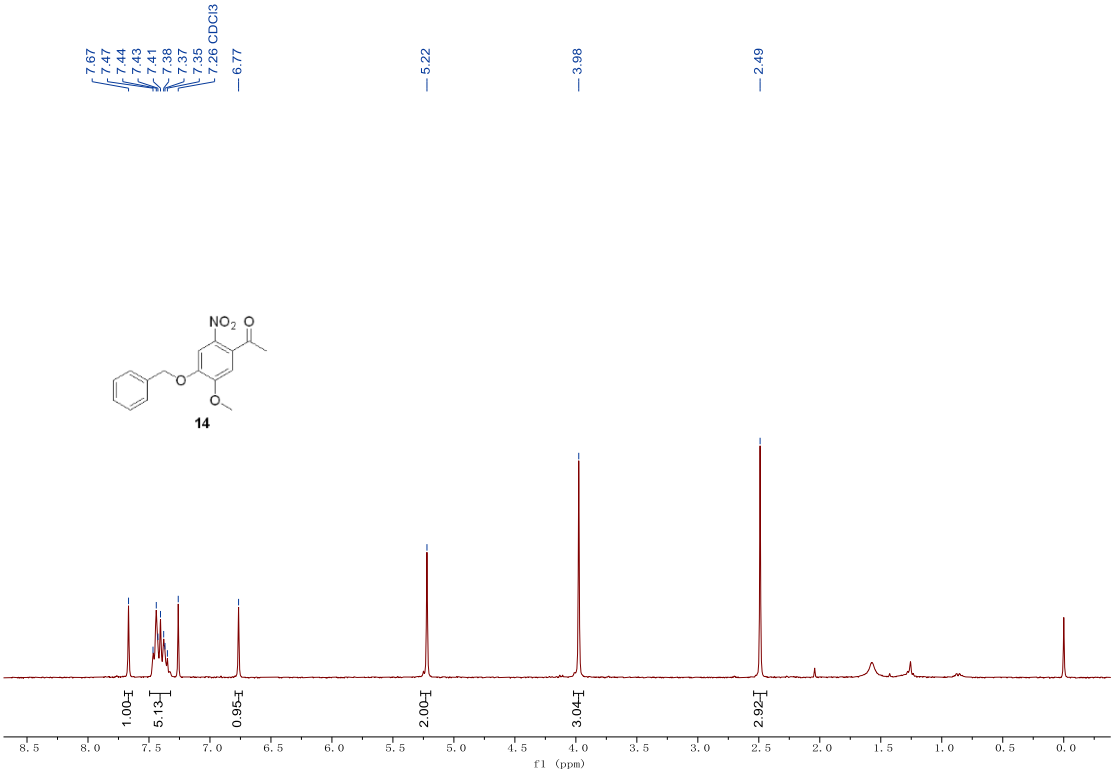
^{1}H NMR (500 MHz, DMSO-d_6) spectrum of PCA **5**



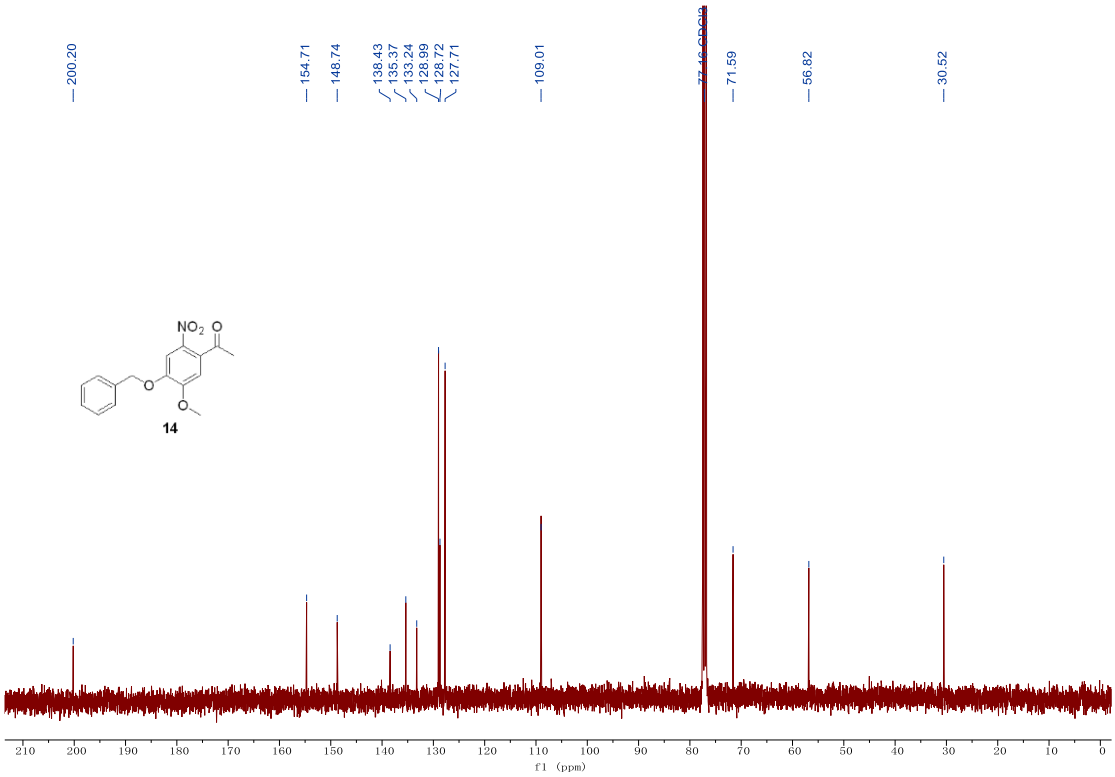
^{13}C NMR (126 MHz, DMSO-d_6) spectrum of PCA **5**



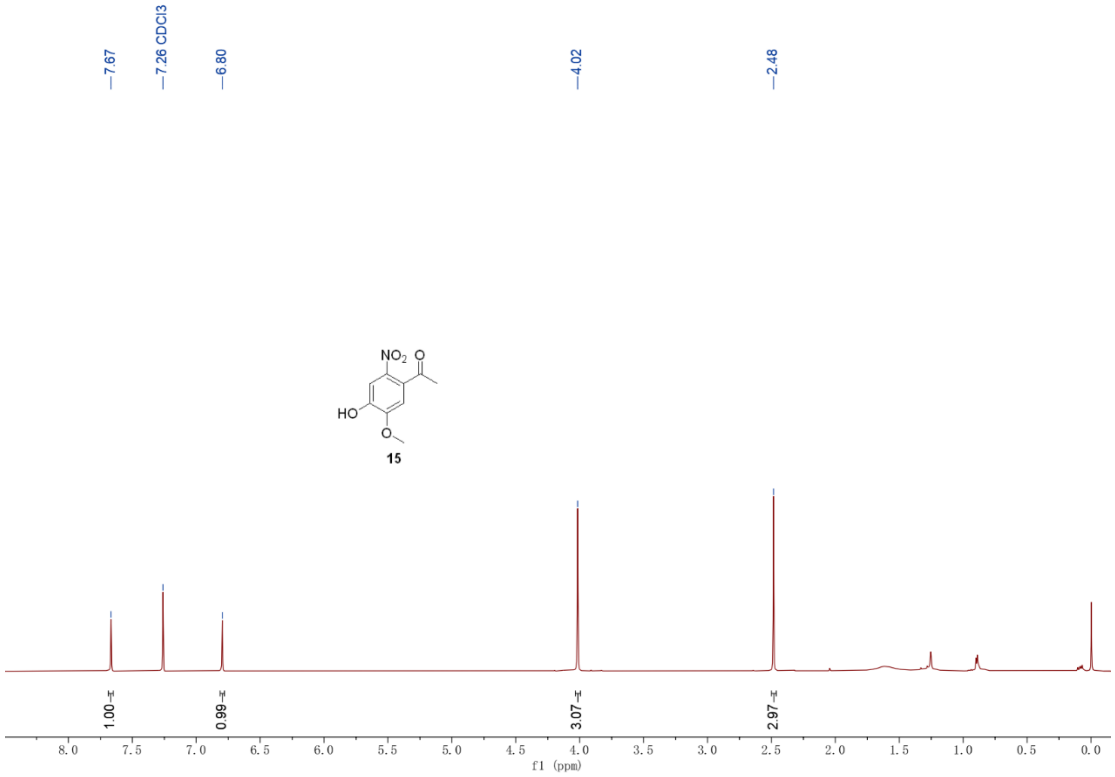
^{1}H NMR (300 MHz, Chloroform-*d*) spectrum of **14**



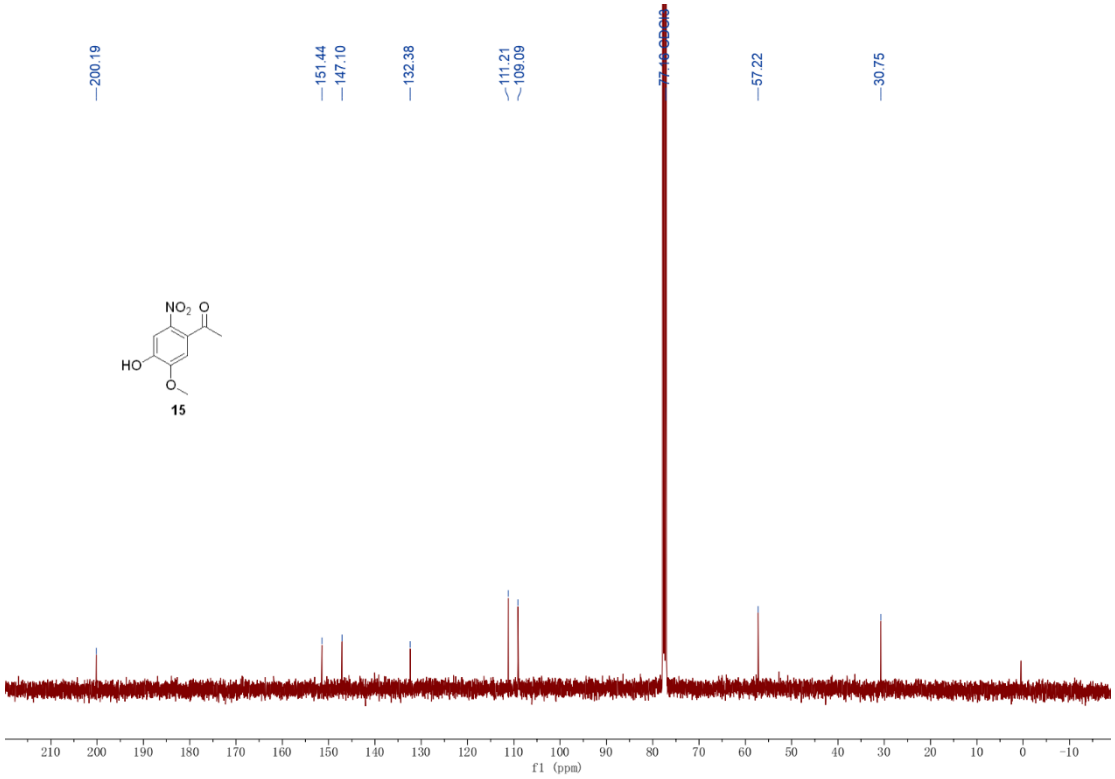
^{13}C NMR (101 MHz, Chloroform-*d*) spectrum of **14**



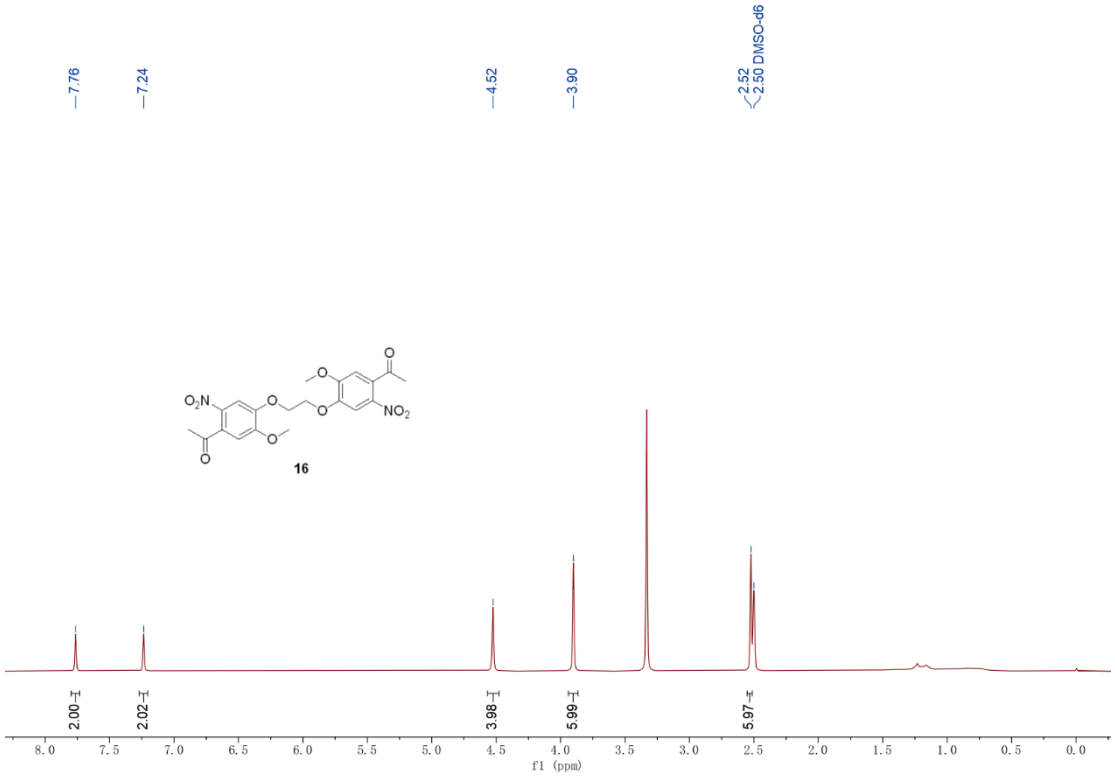
^{1}H NMR (400 MHz, Chloroform-*d*) spectrum of **15**



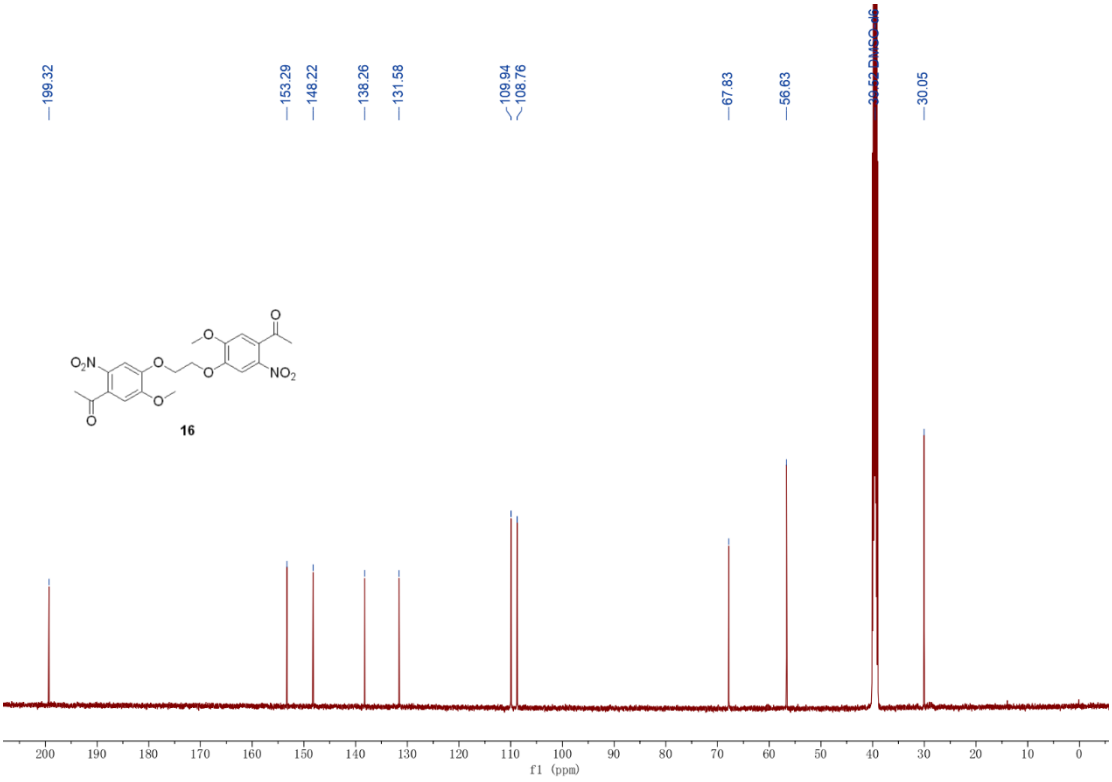
^{13}C NMR (101 MHz, Chloroform-*d*) spectrum of **15**



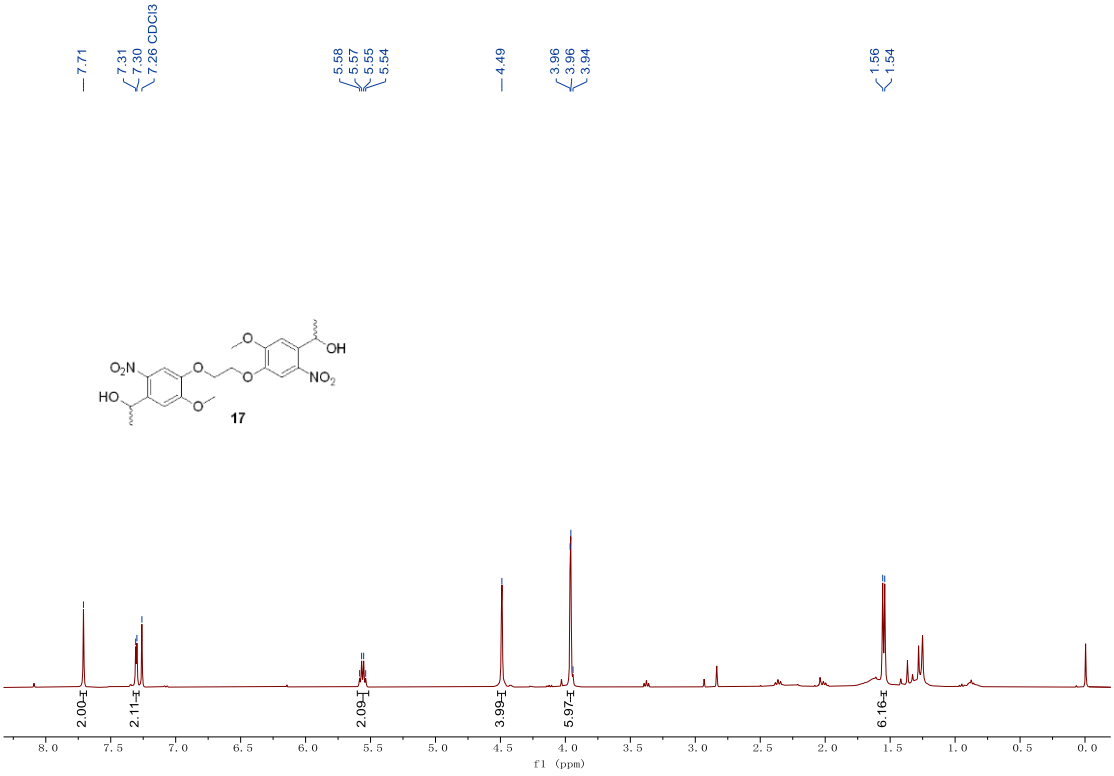
^{1}H NMR (500 MHz, DMSO-d_6) spectrum of **16**



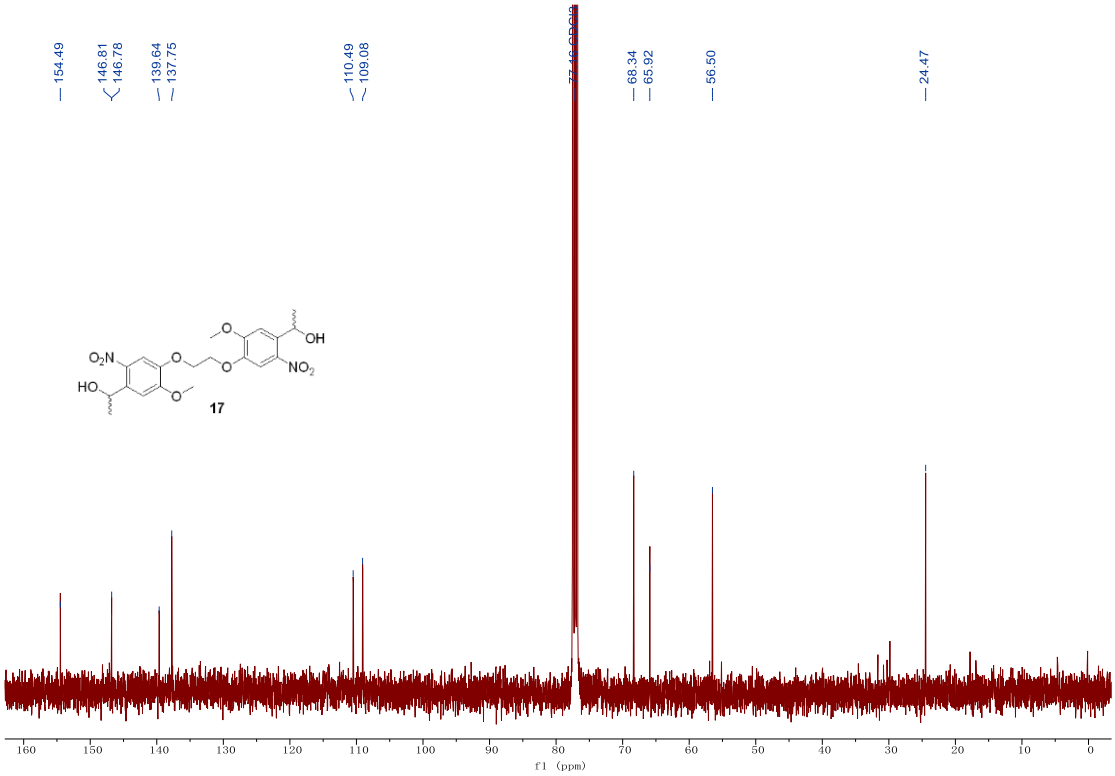
^{13}C NMR (126 MHz, DMSO-d_6) spectrum of **16**



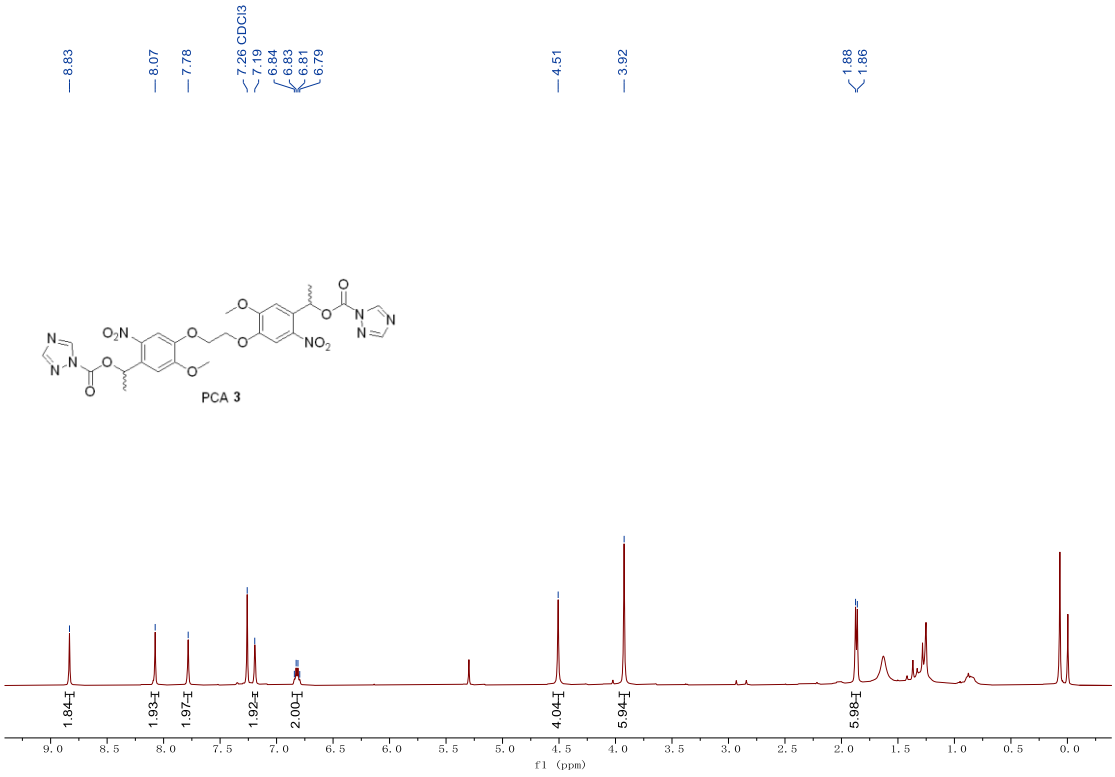
^{1}H NMR (400 MHz, Chloroform-*d*) spectrum of **17**



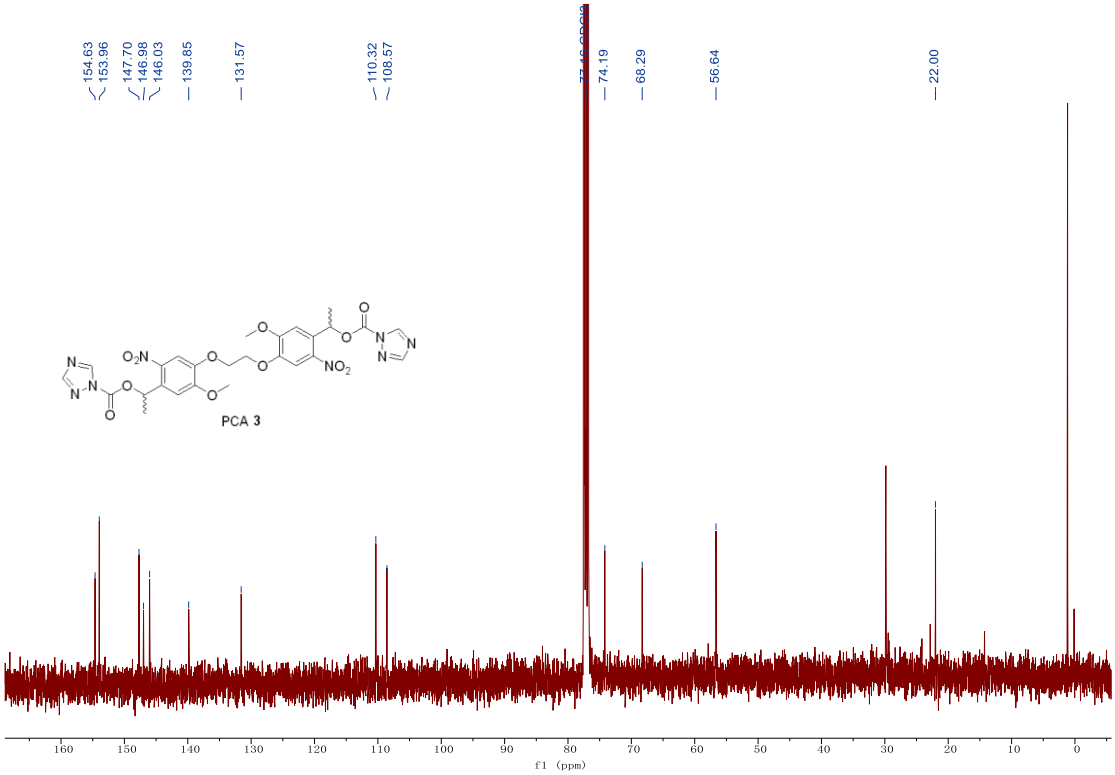
^{13}C NMR (101 MHz, Chloroform-*d*) spectrum of **17**



^{1}H NMR (400 MHz, Chloroform-*d*) spectrum of PCA **3**



^{13}C NMR (101 MHz, Chloroform-*d*) spectrum of PCA **3**



13. References

[S1] K. S. MacMillan, T. Nguyen, I. Hwang, D. L. Boger, *J. Am. Chem. Soc*., **2009**, *131*, 1187– 1194.

[S2] T. Ihara, H. Ohura, C. Shirahama, T. Furuzono, H. Shimada, H. Matsuura, Y. Kitamura, *Nat. Commun*., **2015**, *6*, 6640.

[S3] S. L. Higashi, A. Isogami, J. Takahashi, A. Shibata, K. M. Hirosawa, K. G. N. Suzuki, S. Sawada, S. Tsukiji, K. Matsuura, M. Ikeda, *Chem. Asian J*., **2022**, *17*, e202200142.

[S4] W. A. Velema, A. M. Kietrys, E. T. Kool, *J. Am. Chem. Soc.*, **2018**, *140*, 3491-3496.

[S5] S. Wang, L. Wei, J.-Q. Wang, H. Ji, W. Xiong, J. Liu, P. Yin, T. Tian, X. Zhou, *ACS Chem. Biol*., **2020**, *15*, 1455–1463.

[S6] G. Zong, Z. Hu, S. O'Keefe, D. Tranter, M. J. Iannotti, L. Baron, B. Hall, K. Corfield, A. O. Paatero, M. J. Henderson, P. Roboti, J. Zhou, X. Sun, M. Govindarajan, J. M. Rohde, N. Blanchard, R. Simmonds, J. Inglese, Y. Du, C. Demangel, S. High, V. O. Paavilainen, W. Q. Shi, *J. Am. Chem. Soc.*, **2019**, *141*, 8450–8461.