Supporting Information

A Far-red Emissive Two-Photon Fluorescent Probe for Quantification of Uracil in Genomic DNA and Cell

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1. Materials and methods

Materials and chemicals. All chemicals were purchased from Beijing Innochem Sci. & Tech. Co. Ltd. (Beijing, China) unless mentioned otherwise. dNTP (N = A, T, C, G) were purchased from Takara Biotechnology Co., Ltd. (Dalian, China). All of the DNA oligonucleotides were synthesized and purified by GeneCreate Co., Ltd. (Wuhan, China). pH was measured with Mettler Toledo, FE20-Five Easy™ pH (Mettler Toledo, Switzerland). DNA concentration was quantified

by NanoDrop 2000c (Thermo Scientific, USA). DNA MALDITOF Mass Spectra were collected on MALDI-TOF-MS (Shimadzu, Japan) or Autoflex III MALDI-TOF L200 (Bruker Daltonics, Germany). ^{1}H NMR and ^{13}C NMR spectra were recorded on Bruker Avance 400 NMR or 300 NMR spectrometer, respectively. HRMS was acquired with Thermo Scientific™ Dionex Ultimate 3000 hybrid LTQ Orbitrap Elite Velos Pro (Thermo Scientific, USA). HPLC data was recorded on LC-6AD (Shimadzu, Japan) which equipped with an Inertsil ODS-SP column (5 μm, 250×4.6 mm) (GL Science lnc. Japan) with mobile phase A (100 mM TEAA buffer, pH=7.0) and B (CH_3CN) with a flow rate of 1 mL/min at 35°C (B conc.: 5-5-30% / 0-5-30 min) as described before. DNeasy Blood & Tissue Kit were purchased from QIAGEN (Germany). One-photo Fluorescent emission spectra were acquired with PerkinElmer LS 55 (PerkinElmer, USA). Gel Imaging was monitored with Pharos FX Molecular imager (Bio-Rad, USA). UV absorption spectra were recorded on UV-2550 (Shimadzu, Japan). The nucleic acid stains YeaRed Nucleic Acid Gel Stain (NO.: 10202ES76) was purchased from YEASEN Biotechnology Co. Ltd., (Shanghai, China). And the Super GelRed (NO.: S-2001) was bought from US Everbright Inc. (Suzhou, China). Two-photon excited fluorescence data were measured by exciting with a mode-locked Ti: sapphire femtosecond pulsed laser (Chameleon Ultra I, Coherent Inc.) with a pulse width of 140 fs and repetition rate of 80 MHz. The two-photon excited fluorescence intensity was recorded on a DCS200PC Photon Counting (Beijing Zolix Instruments Co., Ltd.) with single-photon sensitivity through an Omni-λ5008 monochromator (Beijing Zolix Instruments Co., Ltd.). Two-photon microscopy was performed on a Zeiss Axio Examiner LSM 780 multiphoton laser scanning confocal microscope (Carl Zeiss, Germany). The UNG2 overexpressed HEK293T cells were the gift form Prof. Chengqi Yi's lab in Peking University.

Measurement of Two-photon Cross Section. The two-photon cross section (δ) was determined by using femtosecond (fs) fluorescence measurement technique as described. Probe (5.0×10^{-6} M) was dissolved in water, and the two-photon induced fluorescence intensity was measured at 710-790 nm by using rhodamine B as the reference, whose two-photon property has been well characterized in the literature. The intensities of the two-photon induced fluorescence spectra of the reference and sample at the same excitation wavelength were determined. The 2P absorption cross section was calculated by using δ=δr[SsΦrϕrcr]/(SrΦsϕscs), where the subscripts s and r stand for the sample and reference molecules. The intensity of the two-photon excited fluorescence was denoted as S. Φ is the fluorescence quantum yield, and ϕ is the overall fluorescence collection efficiency of the experimental apparatus. The number density of the molecules in solution was denoted as c. δr is the 2P absorption cross section of the reference molecule.

dU excision by UNG. ODNs (100 μM, 7 μL), buffer (2 μL), UNG (1 U/μL, 1 μL) were added together into 200 μL microcentrifuge tube at 37°C for 2 h, respectively. Then the DNA was purified by ice alcohol precipitation.

ODN reaction protocol. (+) or (-) UNG ODNs (100 μM, 10 μL), reagents (10 mM in DMSO, 25 μL), HEPES buffer (1 M, pH=7.4, 5 μL) and 10 μL ddH_2O were added together into 1.5 mL microcentrifuge tube at 60°C for 12 h, respectively.

HPLC analysis of ODNs. ODNS were reacted with reagents through the ODN reaction protocol, then HPLC data was recorded on LC-6AD (Shimadzu, Japan) which equipped with an Inertsil ODS-SP column (5 μm, 250×4.6 mm) (GL Science lnc. Japan) with mobile phase A (100 mM TEAA buffer, pH=7.0) and B (CH_3CN) with a flow rate of 1 mL/min at 35°C (B conc.: 5-5-30% / 0-5-30 min).

Denaturing polyacrylamide gel electrophoresis (PAGE) analysis. The concentration of each oligonucleotide was 1 μM in 10 μL 80% deionized formamide. A 20% denaturing PAGE was prepared by using 1xTBE buffer (89 mM Boric acid, 2 mM EDTA, 89 mM Tris base) containing 7 M urea. The PAGE was carried out in 1xTBE buffer at a constant voltage of 150 V for about 1 h at room temperature. We firstly scanned the final polyacrylamide gel electrophoresis products with Pharos FX Molecular imager operated in the fluorescence mode (λ_{ex}=532 nm). Then the gel was stained with Gel Red to get other DNA bands (λ_{ex}=532 nm).

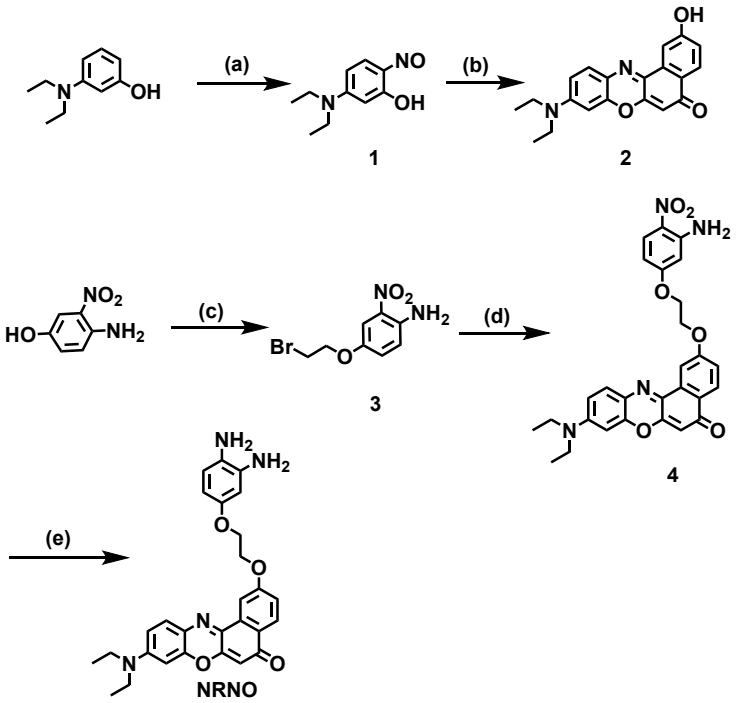
The site-specific analysis of ODN2-dU in primer-extension assays. The extension reaction was performed at 37°C for 10 min in 1x PCR buffer using 1 μM 5'-FAM-labeled primer (ODN2- Primer), 1 μM DNA template (ODN2-dU, ODN2-dU after reaction, UNG treated ODN2-dU, UNG treated ODN2-dU after reaction), 200 μM dNTP and 0.05 U/μL BST DNA polymerase (NEB). Then the primer extensions were analyzed by denaturing PAGE.

Uracil assay in genomic DNA. HEK293T cells were harvested, and genomic DNA was extracted by DNeasy Blood & Tissue Kit (QIAGEN). Genomic DNA was digested to 100–300 bp with Covaris sonicator. DNA fragments were purified by ice alcohol precipitation. (+) or (-) UNG total DNA then incubated with NRNO as described before.

Cytotoxicity Assay. The cytotoxicity was evaluated by MTT assay. HEK293T cells were cultured in Dulbecco's modified Eagle's medium (DMEM) in 96-well microplates at 37°C under 5% CO_2 for 12 h. The medium was next replaced by fresh medium containing various concentrations of NRNO (0-30 μM/mL). Each concentration was tested in three replicates. Cells were rinsed twice with phosphate buffer saline 24 h later and incubated with 0.5 mg/mL MTT reagent for 4 h at 37°C. 100 μL of DMSO was then added to dissolve formazan. The absorbance at 490 nm was measured in a microplate reader. Cell viability (%) was calculated according to following equation: Viability = (mean Abs. of treated wells/mean Abs. of control wells) ×100%.

Cell Culture and Imaging. HEK293T cells were cultured with DMEM supplemented with 10% (v/v) newborn calf serum (Gibco), 100 U mL^{-1} penicillin, and 100 μg mL^{-1} streptomycin in a humidified atmosphere with 5/95 (v/v) of CO_2/air at 37°C. The cell suspension was then transferred to confocal dishes to grow with adherence. Before probe loading, the cells were fixed by methanol and acetic acid (3:1), then incubated with 5.0 μM **NRNO** at 37°C under 5% CO_2 for 12 h. Next, the cells were washed with serum-free DMEM for three times for imaging. Two-photon excited fluorescence images were obtained by Zeiss Axio Examiner LSM 780 multiphoton laser scanning confocal microscope.

2. Synthesis.



Scheme S1. Structure and synthesis of NRNO according to previous report. [1] Reagents and conditions: (a) $NaNO_2$, 37% HCl, H_2O,0-5°C; (b) 1, 6-dihydroxynaphthalene, DMF, 140°C; (c) 1, 2-dibromoethane, K_2CO_3, CH_3CN, 85°C; (d) Compound 2, K_2CO_3, KI, TBAI, DMF, 80°C; (e) 5% Pd/C, 85% N_2H_4 •H_2O, THF, reflux.

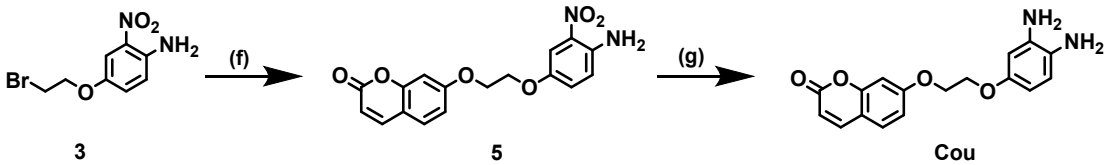
Synthesis of Compound 1. 3.36 g (20 mmol) of 3-diethylaminophenol was dissolved in 8.0 mL of concentrated HCl and 20 g of ice. A cold solution of 1.68 g (24 mmol) of $NaNO_2$ in 6 mL of deionized water was added dropwise to the above solution (during ca. 1 h) and the temperature was kept at 0-5°C. The resulting slurry was stirred for another 2 h at 0°C. The resulting precipitate was filtered and washed with 10 mL of 4 M HCl solution, dried to yield yellow powder (3.10 g, 67%). The crude product was pure enough from ^{1}H NMR analysis and used for next reaction without further purification. ^{1}H NMR (400 MHz, DMSO-*d6*) δ 7.57 (d, *J* = 10.4 Hz, 1H), 7.23 (m, 1H), 6.65 (d, *J* = 1.9 Hz, 1H), 3.83 (m, 4H), 1.28 (t, *J* = 6.9 Hz, 6H).

Synthesis of Compound 2. 0.80 g (5.0 mmol) of 1, 6-dihydroxy naphthalene was added to a solution of 2-nitrosophenol (1.15 g, 5.0 mmol) in 20 mL of DMF under argon atmosphere. The mixture was reflux at 140°C for 5 h. After the mixture cooled down to room temperature, 100 mL of water was added to the mixture and extracted with dichloromethane, dried over anhydrous Na_2SO_4. The solvent was removed and the product was purified by silica gel column chromatography (ethyl acetate: dichloromethane, 1:10) to yield dark green solid (0.42 g, 25%). ^{1}H NMR (400 MHz, DMSO-*d6*) δ 10.45 (s, 1H), 7.97 (d, *J* = 8.6 Hz, 1H), 7.88 (d, *J* = 2.4 Hz, 1H), 7.57 (d, *J* = 9.1 Hz, 1H), 7.09 (dd, *J* = 8.6, 2.5 Hz, 1H), 6.80 (d, *J* = 2.6 Hz, 1H), 6.63 (d, *J* = 2.5 Hz, 1H), 6.15 (s, 1H), 3.49 (q, *J* = 7.0 Hz, 4H), 1.16 (t, *J* = 7.0 Hz, 6H).

Synthesis of Compound 3. 852 μL (10.0 mmol) of 1, 2-dibromoethane was added to a mixture of 308 mg (2.0 mmol) of 4-amino-3-nitrophenol and 544 mg (4 mmol) of K_2CO_3 in 6.0 mL of CH_3CN. The mixture was refluxed overnight at 85°C under argon atmosphere. The mixture was filtrated and the filtrate was evaporated to dryness. The crude product was purified by silica gel chromatography (ethyl acetate: petroleum ether, 1:6) to yield red powder (428 mg, 82%). ^{1}H NMR (400 MHz, DMSO-*d6*) δ 7.41 (d, *J* = 2.9 Hz, 1H), 7.32 (s, 2H), 7.22 (dd, *J* = 9.2, 2.9 Hz, 1H), 7.02 (d, *J* = 9.2 Hz, 1H), 4.35-4.24 (m, 2H), 3.84-3.74 (m, 2H). ^{13}C NMR (100 MHz, DMSO-*d6*) δ 148.05, 142.69, 129.39, 127.96, 121.29, 107.12, 68.90, 31.86.

Synthesis of Compound 4. To a solution containing 200 mg (0.60 mmol) of compound **2** and 234 mg (0.90 mmol) of compound **3** in 9 mL of DMF was added 166 mg (1.2 mmol) of K_2CO_3, 100 mg (0.60 mmol) of KI and 44 mg (0.12 mmol) of tetrabutylammonium iodide (TBAI). The mixture was stirred at 80°C overnight under argon atmosphere. After the reaction completed, 50 mL of water was added to the mixture and extracted with 200 mL of ethyl acetate for three times, dried over anhydrous Na_2SO_4. After the removal of solvent, the product was purified by silica gel column chromatography (ethyl acetate: dichloromethane, 1:10) to yield dark green solid (126 mg, 41%). ^{1}H NMR (400 MHz, DMSO-*d6*) δ 8.06 (d, *J* = 8.7 Hz, 1H), 8.00 (s, 1H), 7.62 (d, *J* = 9.0 Hz, 1H), 7.50 (d, *J* = 2.7 Hz, 1H), 7.46-7.19 (m, 4H), 7.03 (d, *J* = 9.2 Hz, 1H), 6.83 (d, *J* = 8.9 Hz, 1H), 6.66 (s, 1H), 6.20 (s, 1H), 4.51 (s, 2H), 4.38 (s, 2H), 3.51 (q, *J* = 6.7 Hz, 4H), 1.18 (t, *J* = 6.7 Hz, 6H).

Synthesis of NRNO. To a solution containing 50 mg (0.1 mmol) of compound **4** and 0.5 mL of N_2H_4•H_2O in 20 mL of THF, 50 mg of 5% Pd/C was added to the above solution and refluxed for 1.5 h. The mixture was filtrated, evaporated to dryness and re-dissolved in 100 mL of dichloromethane. Then the solution was washed with saturated NaCl solution twice and saturated $NaHCO_3$ solution once, dried over anhydrous Na_2SO_4 and evaporated to dryness to get dark green powder (25 mg, 47%). ^{1}H NMR (400 MHz, DMSO-*d6*) δ 8.05 (d, *J* = 8.6 Hz, 1H), 7.98 (s, 1H), 7.62 (d, *J* = 8.7 Hz, 1H), 7.31 (d, *J* = 8.2 Hz, 1H), 6.82 (d, *J* = 8.7 Hz, 1H), 6.65 (s, 1H), 6.45 (d, *J* = 8.1 Hz, 1H), 6.25 (s, 1H), 6.20 (s, 1H), 6.08 (d, *J* = 7.5 Hz, 1H), 4.32 (m, 8H), 3.51 (d, *J* = 6.6 Hz, 4H), 1.18 (t, *J* = 6.5 Hz, 6H). ^{13}C NMR (150 MHz, DMSO-*d6*) δ 181.87, 161.50, 152.22, 151.53, 151.30, 146.93, 138.61, 137.11, 134.04, 131.45, 129.19, 127.74, 125.48, 124.41, 118.55, 115.70, 110.57, 106.72, 104.51, 102.90, 102.48, 96.41. 67.55, 66.78, 46.02, 12.93.



Scheme S2. Structure and synthesis of Cou. Reagents and conditions: (f) coumarin, K_2CO_3, DMF, 60°C; (e) NaH_2PO_2•H_2O, 5% Pd/C, DMF, 55°C.

Synthesis of compound 5. To a solution containing 261 mg (1 mmol) of compound **3** and 243 mg (1.5 mmol) of 7-Hydroxycoumarin in 10 mL of DMF was added 276 mg (2 mmol) of K_2CO_3. The mixture was stirred at 60°C overnight under argon atmosphere. After the reaction

completed, 150 mL of water was added to the mixture and extracted with 200 mL of ethyl acetate for three times, dried over anhydrous Na_2SO_4. After the removal of solvent, the product was purified by silica gel column chromatography (ethyl acetate: petroleum ether, 1:2) to yield yellow solid (257 mg, 75%). ^{1}H NMR (400 MHz, DMSO) δ = 8.00 (d, *J*=9.5, 1H), 7.65 (t, *J*=6.8, 1H), 7.46 (d, *J*=3.0, 1H), 7.29 (s, 2H), 7.22 (dd, *J*=9.2, 3.0, 1H), 7.06 (t, *J*=5.4, 1H), 7.04 – 6.96 (m, 2H), 6.31 (d, *J*=9.5, 1H), 4.46 – 4.38 (m, 2H), 4.35 – 4.27 (m, 2H). ^{13}C NMR (101 MHz, DMSO) δ = 161.89, 160.74, 155.83, 148.50, 144.78, 142.58, 130.02, 129.48, 127.97, 121.27, 113.22, 113.10, 113.00, 106.68, 101.79, 67.54, 67.22. HRMS (ESI$^+$) calculated for $C_{17}H_{15}O_6N_2^+$ [M+H] $^+$: 343.09246. Found 343.09156.

Synthesis of Cou. To a solution containing 103 mg (0.3 mmol) of compound **5** and 56 mg of 5% Pd/C in 15 mL of DMF, the mixture was stirred 10 min. Then 445 mg (4.2 mmol) of NaH_2PO_2•H_2O was added above solution and the mixture was stirred at 55°C for 2 h under argon atmosphere. After the reaction completed, 150 mL of acetonitrile was added to the mixture and the mixture was filtrated. the solvent was evaporated to dryness and re-dissolved in 100 mL of dichloromethane. After the removal of solvent, the product was purified by silica gel column chromatography (methanol: dichloromethane, 1:10) to yield brown solid (40 mg, 43%). ^{1}H NMR (400 MHz, DMSO) δ = 8.00 (d, J=9.5, 1H), 7.64 (d, J=8.6, 1H), 7.05 (d, J=2.4, 1H), 7.00 (dd, J=8.6, 2.4, 1H), 6.42 (d, J=8.3, 1H), 6.30 (d, J=9.5, 1H), 6.20 (d, J=2.8, 1H), 6.03 (dd, J=8.3, 2.8, 1H), 4.51 (s, 2H), 4.39 – 4.31 (m, 2H), 4.15 – 4.11 (m, 2H), 4.08 (d, J=29.6, 2H). ^{13}C NMR (101 MHz, DMSO) δ = 162.08, 160.77, 155.85, 151.46, 144.81, 137.09, 130.01, 129.30, 115.63, 113.25, 113.03, 112.92, 102.85, 102.47, 101.77, 67.87, 66.65. HRMS (ESI$^+$) calculated for $C_{17}H_{17}O_4N_2^+$ [M+H] $^+$: 313.11828. Found 313.11735.

3. Fluorescence emission spectra

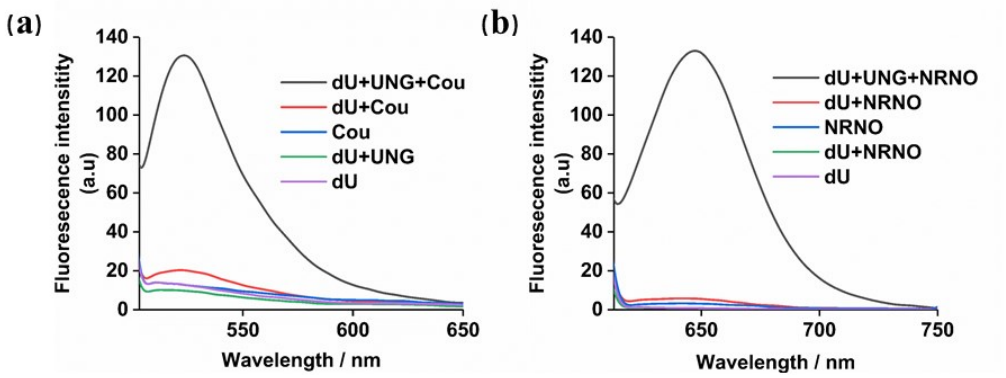


Figure S1. Fluorescence emission spectra of ODN-Cou (5.0 μM) and ODN-NRNO (5.0 μM) in water. (a) Fluorescence emission spectra of ODN1-AP after incubation with Cou (black line) in comparison with other controls such as ODN1-AP (green line), ODN1-dU after incubation with Cou (red line), ODN1-dU (purple line), Cou (blue line) under the same conditions. (b) Fluorescence emission spectra of ODN1-AP after incubation with NRNO (black line) in comparison with other controls such as ODN1-AP (green line), ODN1-dU after incubation with NRNO (red line), ODN1-dU (purple line), NRNO (blue line) under the same conditions.

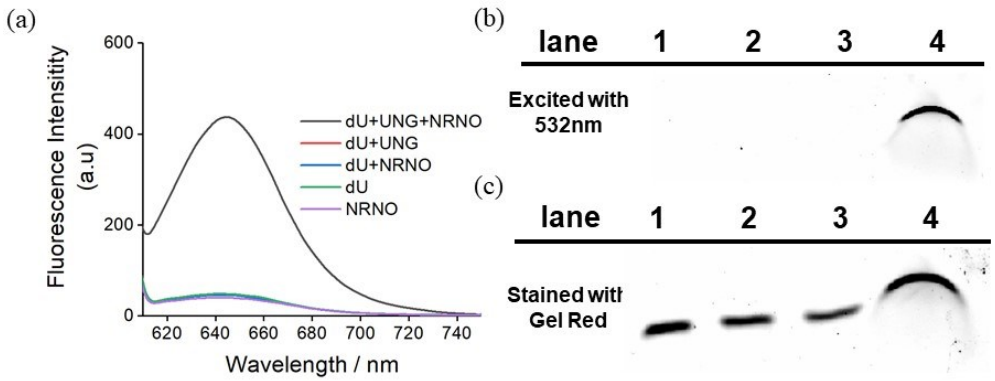


Figure S2. Fluorescence emission spectra of NRNO with 15bp dsDNA. (a) Fluorescence emission spectra of dsDNA-AP after incubation with NRNO (black line) in comparison with other controls such as dsDNA-AP (red line), dsDNA-dU after incubation with NRNO (blue line), dsDNA-dU (green line), NRNO (purple line) under the same conditions. (b, c) Denaturing PAGE analysis. Lane 1: dsDNA-dU; lane 2: dsDNA-dU after incubation with NRNO; lane 3: dsDNA-AP; lane 4: dsDNA-AP after incubation with NRNO.

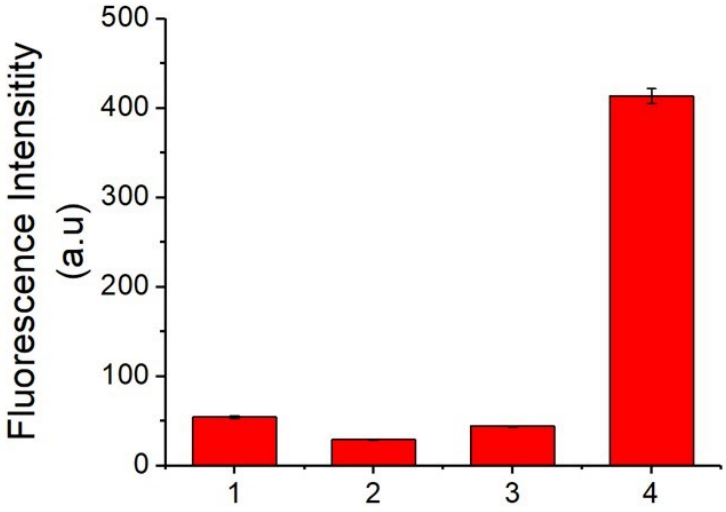


Figure S3. Fluorescence emission spectra of NRNO with 80bp dsDNA. Column 1 : 80bp dsDNA1 (no dU site) after incubation with NRNO; column 2: 80bp dsDNA2 (1 dU site) after incubation with NRNO, column 3: 80bp dsDNA3 (4 dU sites) after incubation with NRNO, column 4: 15bp dsDNA (1 dU site) treated with UNG and then incubated with NRNO. The sequences were listed in Table S2.

4. DNA MALDI-TOF Mass Spectrum

5’-GACTCAAAPAGCCGTA ⟶ 5’-GACTCAANRNOAGCCGTA
calculated 4916.0, found 4916.2.

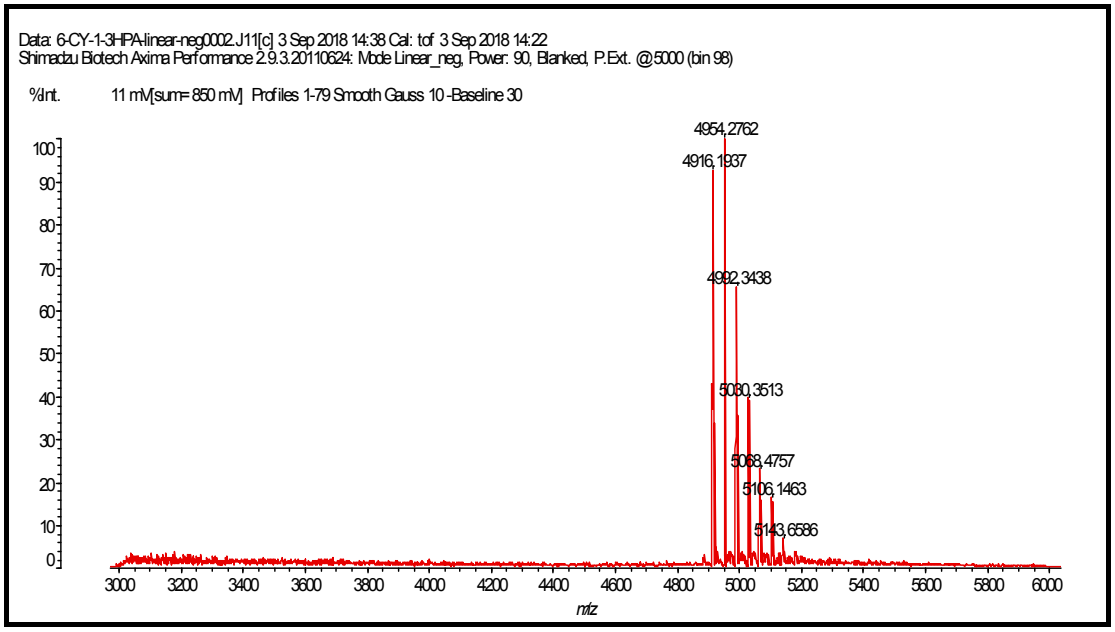


Figure S4. MALDI-TOF Mass Spectrum of NRNO labeled DNA.

5. UV-vis spectra

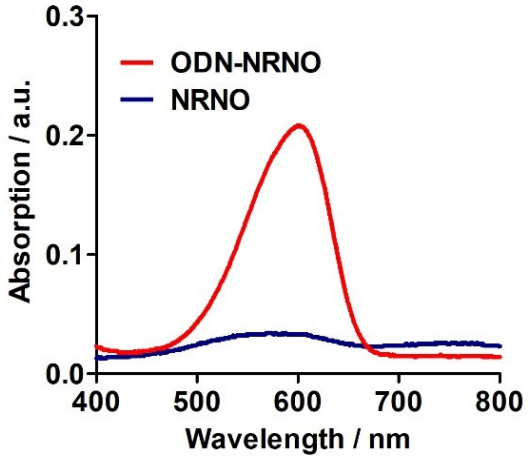
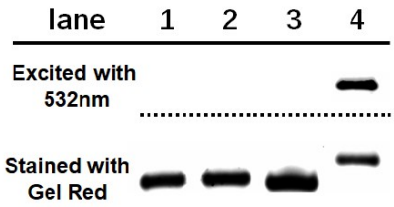


Figure S5. UV-vis spectra of probes. UV-vis spectra of ODN-NRNO (100.0 μM) and NRNO (100.0 μM) in water.

6. PAGE analysis of ODN2-dU.



ODN2-dU: AAATCAUCCTATCCTCCTTCAGGACCAACGTAC

Figure S6. Denaturing PAGE analysis. Lane 1: ODN2-dU; lane 2: ODN2-dU after incubation with NRNO; lane 3: ODN2-AP; lane 4: ODN2-AP after incubation with NRNO.

7. Spectroscopic properties of NRNO.

Table S1. Photophysical data for NRNO and NRNO labeled DNA (ODN1-NRNO) in buffer solution.

Compound[a]	λ_{max}[b]	λ_{max}[c]	Φ[d]	λ_{max}[e]	Φδ[f]
NRNO	583	648	0.03	n.d.[g]	n.d.[g]
ODN1-NRNO	600	650	0.15	710	77.4

[a] All data were measured in water. [b, c] λ_{max} of the absorption and one-photon emission spectra in nm. [d] Fluorescence quantum yield. [e] λ_{max} of the two-photon emission spectra in nm. [f]Two-photon action cross section in GM. [g] The two-photon excited fluorescence intensity was too weak to measure the two-photon action cross section accurately.

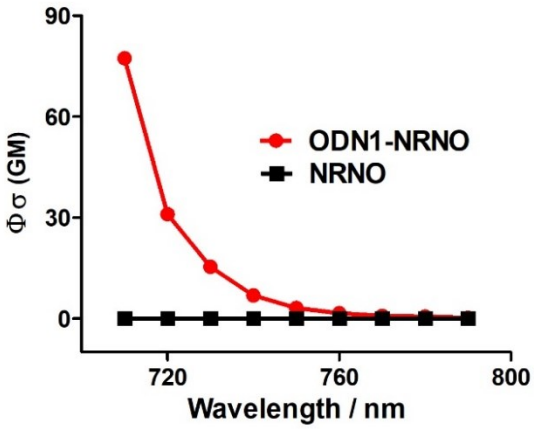


Figure S7. Two-photon excitation spectra of 5.0 μM NRNO or ODN1-NRNO in water.

8. Cytotoxicity assay

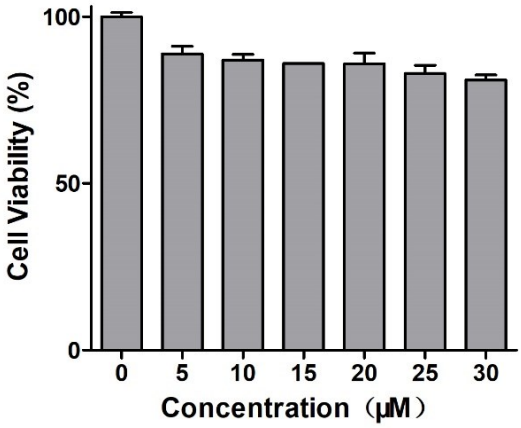


Figure S8. Viability of HEK293T cells incubated with various concentrations (0-40 μM) of NRNO for 24 h as measured by MTT assay.

9. Cell imaging by probe NRNO

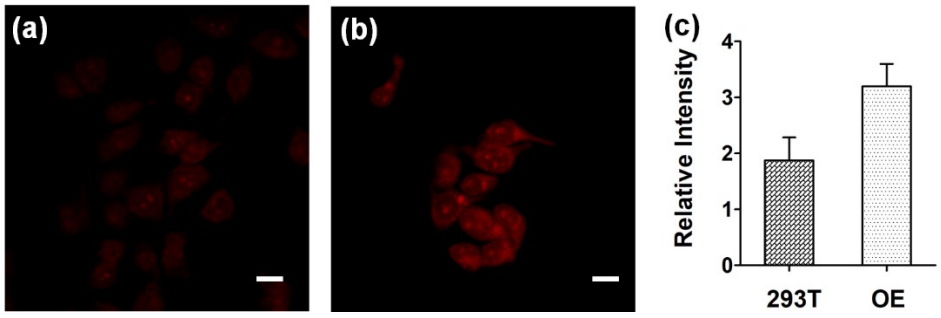


Figure S9. (a) TP image of 5.0 μM NRNO-loaded wild type HEK293T cells. (b) TP image of UNG2 over expression HEK293T cells loaded with 5.0 μM NRNO. (c) Relative TP fluorescence intensity in (a) and (b). The excitation wavelength for two-photon imaging were 710 nm, and the emissions were collected at 620-700 nm for NRNO. Scale bars: 10 μm.

10. Relative intensity of total DNA from wild type and UNG2 over-expressed HEK292T cells

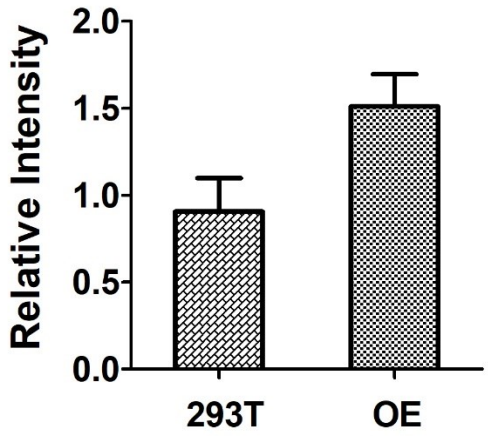


Figure S10. Relative intensity of total DNA extracted from wild type and UNG2 over-expressed HEK293T cells after incubated with 50 μM NRNO.

11. DNA sequences used in this study.

Table S2.

Name	Sequence
ODN1-dU	5'-GACTCAAUAGCCGTA-3'
ODN2-dU	5'-AAATCAUCCTATCCTCCTTCAGGACCAACGTAC-3'
ODN2 Primer	5'-**FAM**-GTACGTTGGTCATGAAGGAGGAT-3'
15nt dsDNA	5'-CGATCGAdUTCAGTCT-3' 5'-AGACTGAATCGATCG-3'
80bp dsDNA1	5'- TCCTCGGCGGTGTTGCTCTCTGTTGTGCCTCCGCCCGGCCGGCAG CGGGCAGGACAAGGACGCAGAGCCACAGCCAAGAA-3' 5'- TTCTTGGCTGTGGCTCTGCGTCCTTGTCCTGCCCGCCGCCGGCCG GGCGGAGGCACAACAGAGAGCAACACCGCCGAGGA-3'
80bp dsDNA2	5'- TCCTCGGCGGTGTTGCTCTCTGTTGTGCCTCCGCCCGGCCGGCAG CGGGCAGGACAAGGACGCAGAGCCACAGCCAAGAA-3' 5'- TTCTTGGCTGTGGCTCTGCGTCCTTGTCCTGCCCGCdUGCCGGCC GGGCGGAGGCACAACAGAGAGCAACACCGCCGAGGA-3'
80bp dsDNA3	5'- TCCTCGGCGGTGTTGCTCTCTGTTGTGCCTCCGCCCGdUCAGGCA GdUGGGCAGGACAAGGACGCAGAGCCACAGCCAAGAA-3' 5'- TTCTTGGCTGTGGCTCTGCGTCCTTGTCCTGCCCACdUGCCdU

	GACGGGCGGAGGCACAACAGAGAGCAACACCGCCGAGGA-3’

Reference

[1] Z. Mao, W. Feng, Z. Li, L. Zeng, W. Lv and Z. Liu, *Chemical Science*, **2016**, *7*, 5230-5235.

12. Supplementary note

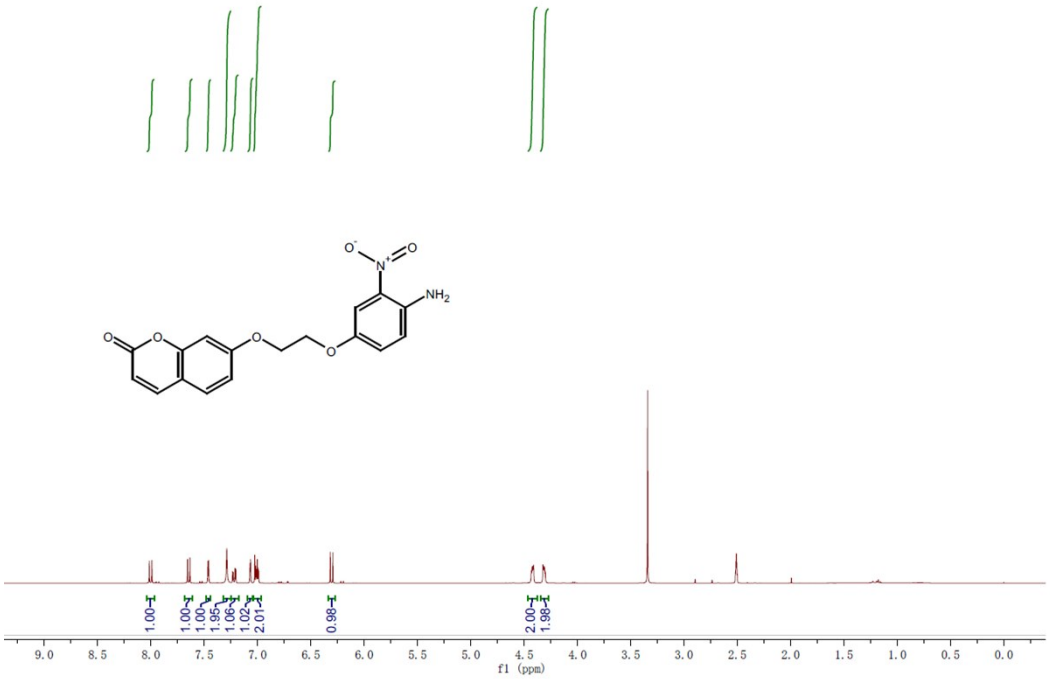


Figure S11. ^{1}H NMR spectra of compound 5 (DMSO, 400 MHz).

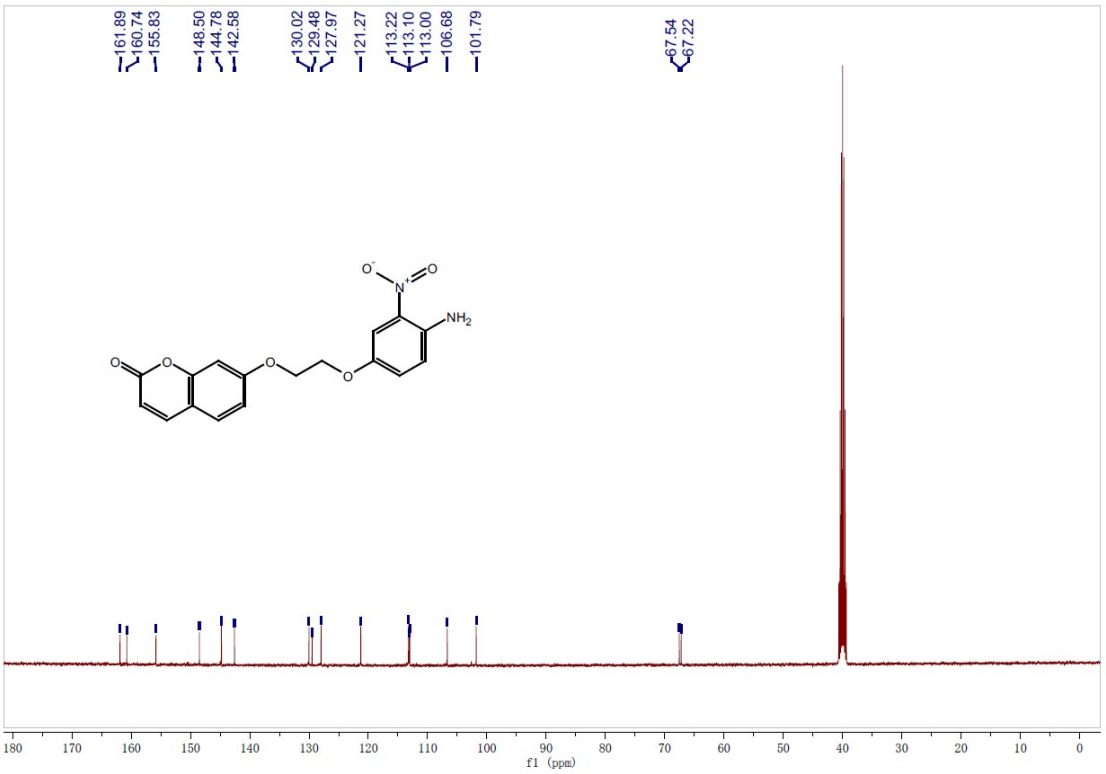


Figure S12. ^{13}C NMR spectra of compound 5 (DMSO, 101 MHz).

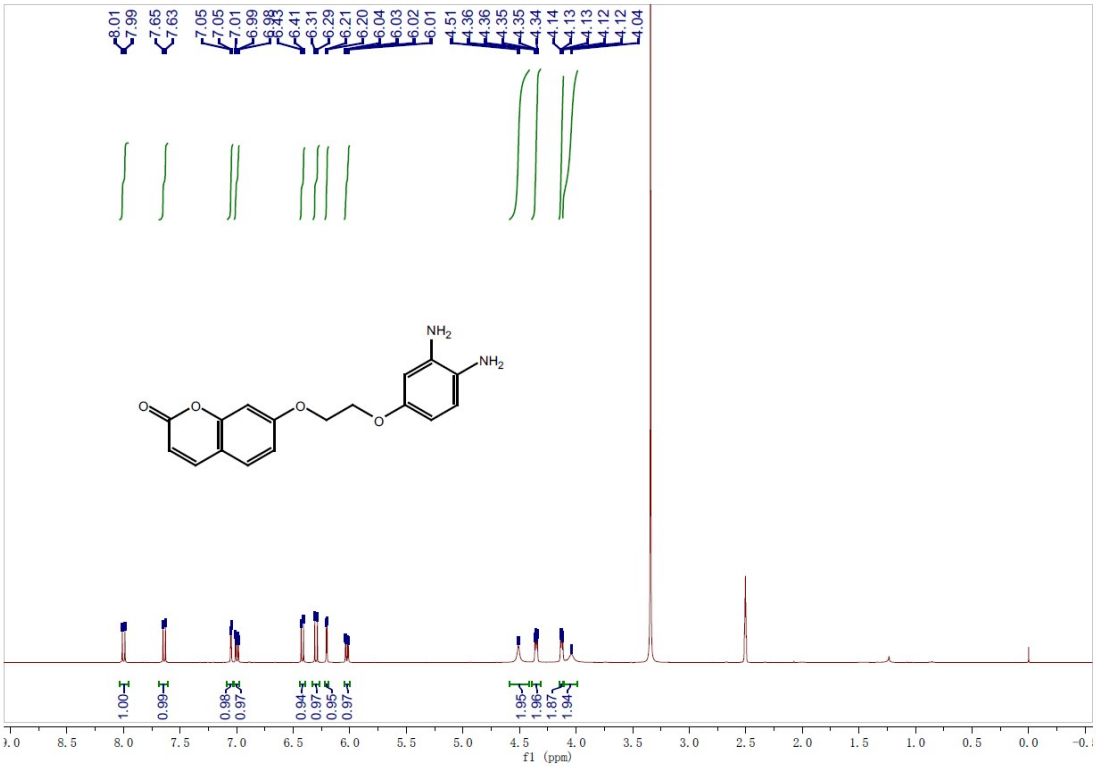


Figure S13. ^{1}H NMR spectra of Cou (DMSO, 400 MHz).

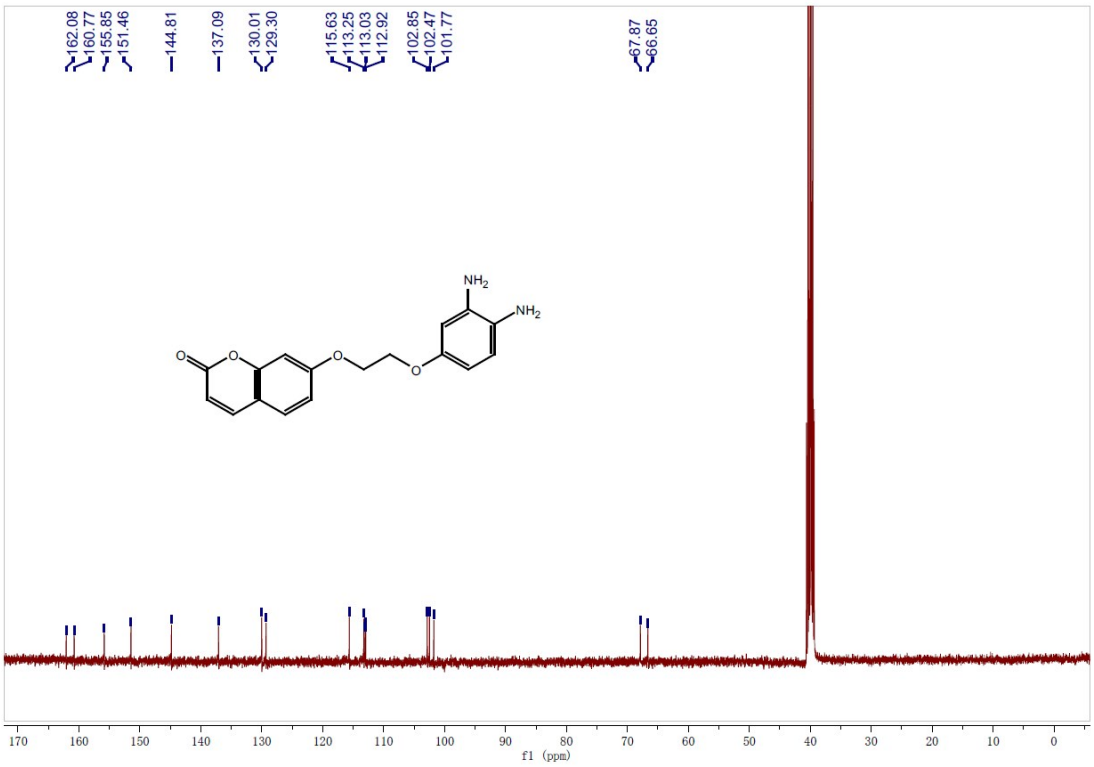


Figure S14. ^{13}C NMR spectra of Cou (DMSO, 101 MHz).

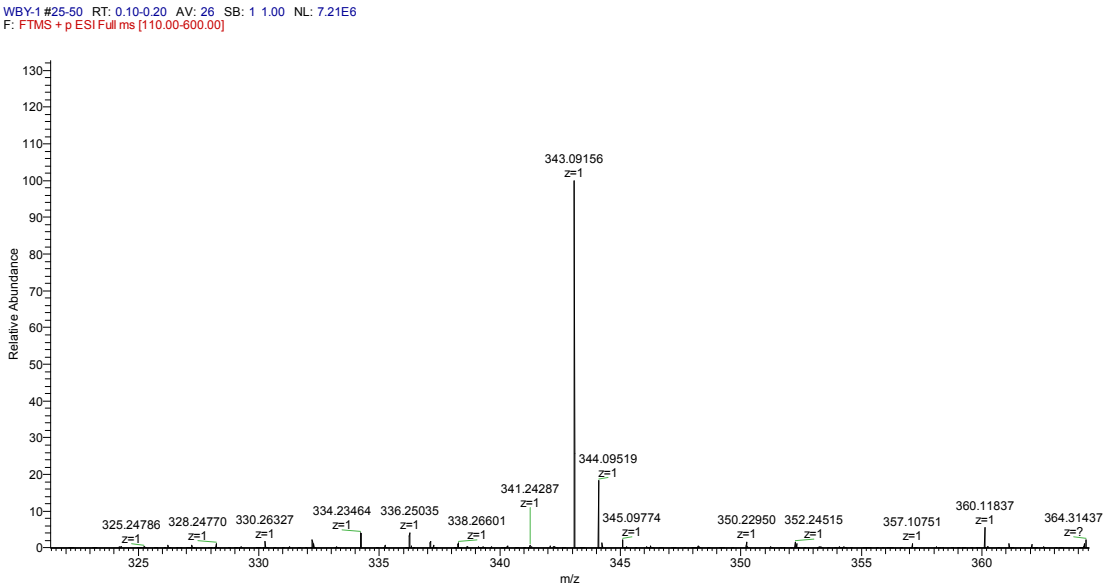


Figure S15. ESI mass spectrum of compound 5.

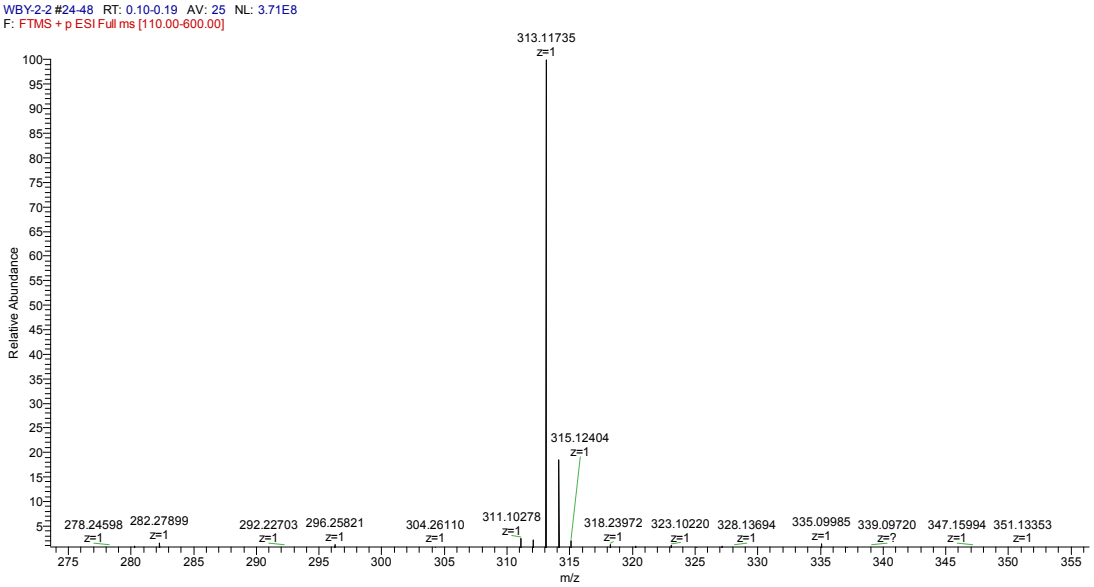


Figure S16. ESI mass spectrum of Cou.