Electronic Supplementary Material (ESI) for Organic & Biomolecular Chemistry.

Supporting Information

Photo-Contolled Cell-Specific Metabolic Labeling of RNA

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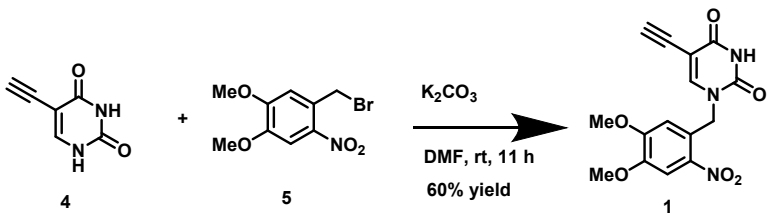
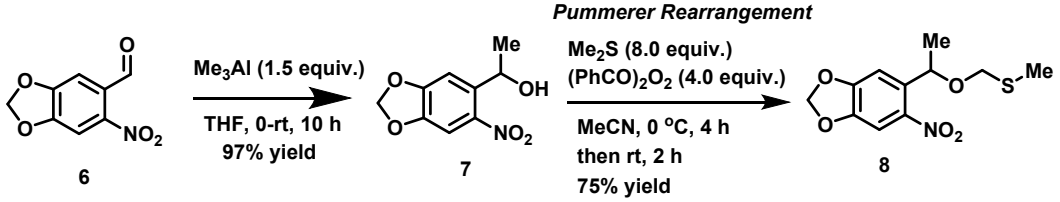
1. Synthesis



*1-(4,5-Dimethoxy-2-nitrobenzyl)-5-ethynylpyrimidine-2,4(1H,3H)-dione (**1**)*. To a solution of 5-ethynylpyrimidine-2,4(1H,3H)-dione (**4**)[1] (100 mg, 0.735 mmol) in DMF (1.5 mL) wad added potassium carbonate (101.6 mg, 0.735 mmol) at room temperature and the resulting suspension was stirred at room temperature for 10 min, after which 1-(bromomethyl)-4,5-dimethoxy-2-nitrobenzene **5** (202.9 mg, 0.735 mmol) was added and the reaction mixture was stirred at room temperature for 11 h and neutralized by concentrated HCl (aq.) until pH = 7. The resulting reaction mixture was subjected to flash chromatography on silica gel (DCM/methanol) to furnish **1** as a yellowish solid (146.4 mg, 60% yield). ^{1}H NMR (600 MHz, d_6-DMSO) δ 11.7 (brs, 1 H), 8.12 (s, 1 H), 7.67 (s, 1 H), 6.82 (s, 1 H), 5.17 (s, 1 H), 4.10 (s, 1 H), 3.87 (s, 3 H), 3.84 (s, 3 H) ppm; ^{13}C NMR (150 MHz, d_6-DMSO) δ 48.6, 56.1, 56.3, 76.3, 83.8, 97.4, 108.3, 111.2, 125.6, 140.5, 147.9, 149.5, 150.2, 153.1, 162.2 ppm. HRMS (ESI) calcd for $C_{15}H_{13}N_3O_6Na$ [$(M+Na)^+$] 354.0702, found 354.0695.

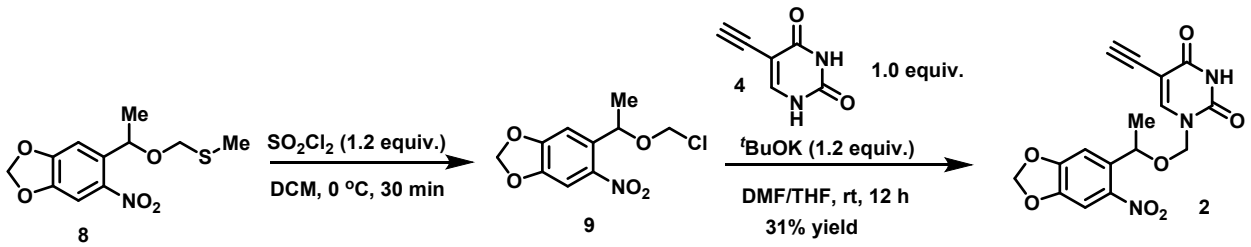


5-(1-((Methylthio)methoxy)ethyl)-6-nitrobenzo[d][1,3]dioxole (**8**).

Compounds **8** were synthesized according to a published procedure.[2] To an ice-cold solution of starting material 6-nitrobenzo[d][1,3]dioxole-5-carbaldehyde (**6**) (2.00g, 10.2 mmol) in anhydrous DCM (20 mL) was added trimethylaluminum (20.5 mL, 1.0 M in heptane, 20.5 mmol) dropwise. The reaction mixture were then stirred from 0 °C to room temperature overnight, after which the reaction mixture was cooled down to 0 °C and quenched slowly with cold water (10 mL), 1 N NaOH (10 mL). After stirring for 30 min at 0 °C, the resulting mixture was extracted with DCM, dried over $MgSO_4$, filtrated, and crystallized to furnish **7** as a yellow solid (2.09 g, 97 % yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.46 (s, 1 H), 7.27 (s, 1 H), 6.12 (d, J = 1.2 Hz, 1 H), 6.11 (d,

J = 1.2 Hz, 1 H), 5.46 (q, *J* = 6.4 Hz, 1 H), 1.54 (d, *J* = 6.4 Hz, 3 H) ppm. The ^{1}H NMR spectrum is identical to this reported.[2]

To an ice-cold solution of **7** (1.26 g, 5.97 mmol) in anhydrous acetonitrile (25 mL) was added dimethyl sulfide (3.50 mL, 47.7 mmol) dropwise, followed by addition of benzoylperoxid (5.79 g, 23.9 mmol) portionwise. After stirring at 0 °C for 4 h and at room temperature for 2 h, the reaction mixture was quenched by 1 N NaOH (until pH = 9), diluted with brine, extracted with EtOAc. The organic phase were collected and dried over $MgSO_4$, concentrated to afford a residue, which as purified by flash chromatography on silica gel (Hexane/EtOAc = 90:10) to afford **8** as a yellowish solid (1.22 g, 75% yield). ^{1}H NMR (400 MHz, $CDCl_3$) *δ* 7.47 (s, 1 H), 7.17 (s, 1 H), 6.12 (s, 1 H), 6.11 (s, 1 H), 5.45 (q, *J* = 6.5 Hz, 1 H), 4.61 (d, *J* = 11.6 Hz, 1 H), 4.30 (d, *J* = 11.6 Hz, 1 H), 2.14 (s, 3 H), 1.51 (d, *J* = 6.5 Hz, 3 H) ppm. The ^{1}H NMR spectrum is identical to this reported.[2]

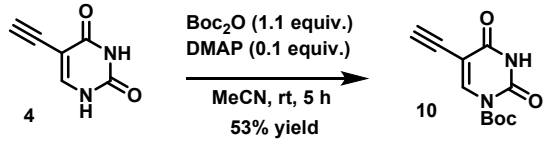


5-Ethynyl-1-((1-(6-nitrobenzo[d][1,3]dioxol-5-yl)ethoxy)methyl)pyrimidine-2,4(1H,3H)-dione (2).

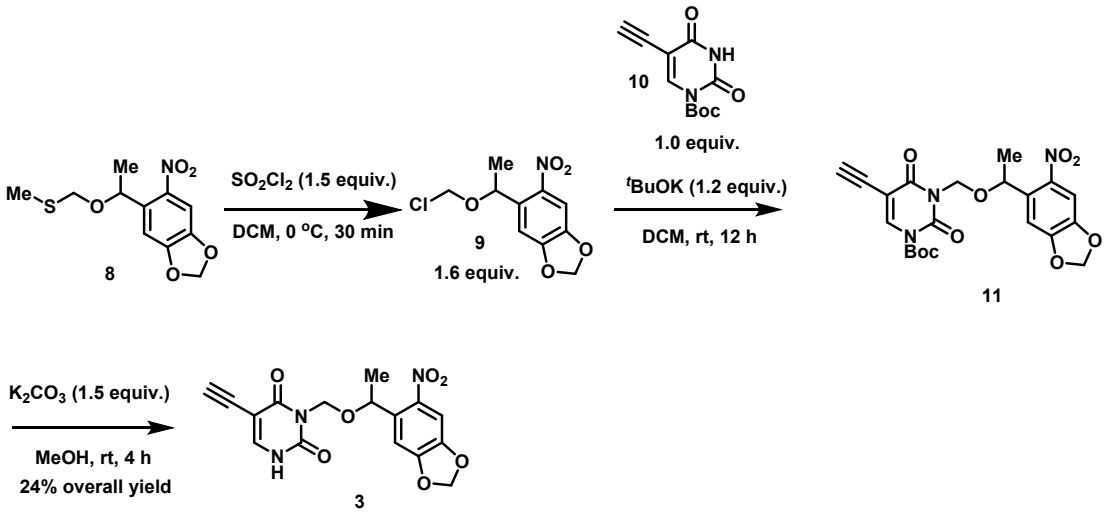
To an ice-cold solution of 5-(1-((Methylthio)methoxy)ethyl)-6-nitrobenzo[d][1,3]dioxole (**8**) (199.4 mg, 0.735 mmol) in anhydrous DCM (4 mL) was added sulfuryl chloride (70 uL, 0.882 mmol) dropwise and the resulting solution was stirred at 0 °C for 30 min, after which the solution was concentrated under reduced pressure to afford **9** as a yellow solid, which was used for next reaction without further purification.

To an ice-cold solution of 5-ethynylpyrimidine-2,4(1H,3H)-dione (**4**) (100 mg, 0.735 mmol) and *t*BuOK (99.0 mg, 0.882 mmol) in dry DMF (3 mL) was added a solution of above yellow solid **9** in dry DCM (2 mL) dropwise and the resulting reaction mixture was stirred from 0 °C to room temperature overnight and concentrated under reduced pressure to afford a residue, which was purified by flash chromatography on silica gel directly (Hexane/EtOAc = 50:50) to furnish **2** as a yellowish solid (81.9 mg, 31% yield from **4**). ^{1}H NMR (600 MHz, d_6-DMSO) *δ* 11.55 (s, 1 H), 8.04 (s, 1 H), 7.54 (s, 1 H), 7.07 (s, 1 H), 6.23 (s, 1 H), 6.12 (s, 1 H), 5.18 (q, *J* = 6.0 Hz, 1 H), 5.07 (d, *J* = 10.2 Hz, 1 H), 5.03 (d, *J* = 10.2 Hz, 1 H), 4.08 (s, 1 H), 1.44 (d, *J* = 6.0 Hz, 3 H) ppm; ^{13}C NMR (150 MHz, d_6-DMSO) *δ* 23.3, 72.7, 75.6, 75.9, 83.7, 97.5, 103.3, 104.6,

106.0, 136.3, 141.4, 146.8, 148.6, 149.9, 152.0, 161.7 ppm. HRMS (ESI) calcd for $C_{16}H_{12}N_3O_7$ [$(M-H)^-$] 358.0675, found 358.0686.



tert-Butyl 5-ethynyl-2,4-dioxo-3,4-dihydropyrimidine-1(2H)-carboxylate (10). To an ice-cold suspension of **4** (200 mg, 1.47 mmol) in anhydrous MeCN (5 mL) were added DMAP (18.0 mg, 0.147 mmol) and Boc_2O (352.9 mg, 1.62 mmol). The resulting reaction mixture was stirred at room temperature for 5 h and concentrated under reduced pressure to afford a residue, which was purified by flash chromatography on silica gel (DCM/EtOAc = 50:50) to afford **10** as white solid (184.7 mg, 53% yield). ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.17 (s, 1H), 8.01 (br s, 1 H), 3.22 (s, 1 H), 1.62 (s, 3 H) ppm; ^{13}C NMR (150 MHz, $CDCl_3$) δ 160.2, 147.4, 145.9, 143.4, 100.9, 88.4, 83.0, 73.7, 27.9 ppm. HRMS (ESI) calcd for $C_{11}H_{12}N_2O_4Na$ [$(M+Na)^+$] 259.0695, found 259.0692.



5-Ethynyl-3-((1-(6-nitrobenzo[d][1,3]dioxol-5-yl)ethoxy)methyl)pyrimidine-2,4(1H,3H)-dione (3).

To an ice-cold solution of 5-(1-((Methylthio)methoxy)ethyl)-6-nitrobenzo[d][1,3]dioxole (**8**) (350 mg, 1.29 mmol) in anhydrous DCM (5 mL) was added sulfuryl chloride (0.16 mL, 1.55 mmol) dropwise and the resulting solution was stirred at 0 °C for 30 min, after which the solution was concentrated under reduced pressure to afford **9** as a yellow solid, which was used for next reaction without further purification.

To an ice-cold solution of 5-ethynylpyrimidine-2,4(1H,3H)-dione (**10**) (185 mg, 0.782 mmol) and *t*BuOK (105 mg, 0.938 mmol) in dry DCM (5 mL) was added a solution of above yellow solid in dry DCM (2 mL) dropwise and the resulting reaction mixture was stirred from 0 ^{o}C to room temperature overnight and concentrated under reduced pressure to afford **11** in a residue, which was resuspended in MeOH (10 mL) at room temperature. K_2CO_3 (162 mg, 1.17 mmol) was then added and the resulting reaction mixture was stirred at room temperature for 4 h and diluted in EtOAc, washed with brine. The organic phase was dried over $MgSO_4$, concentrated under reduced pressure to generate a residue, which was purified by flash chromatography on silica gel (DCM/EtOAc = 90:10) to produce **3** as a yellowish solid (68.4 mg, 24% yield from **10**). ^{1}H NMR (400 MHz, d_6-DMSO) δ 11.55 (br s, 1 H), 7.78 (s, 1 H), 7.54 (s, 1 H), 7.11 (s, 1 H), 6.22 (d, J = 0.8 Hz, 1 H), 6.18 (d, J = 0.8 Hz, 1 H), 5.19 (d, J = 10.4 Hz, 1 H), 5.17 (q, J = 6.0 Hz, 1 H), 5.01 (d, J = 10.4 Hz, 1 H), 4.04 (s, 1 H), 1.40 (d, J = 6.0 Hz, 3 H) ppm; ^{13}C NMR (150 MHz, d_6-DMSO) δ 23.4, 68.9, 72.6, 76.2, 83.2, 95.9, 103.3, 104.5, 105.9, 137.0`, 141.4, 145.6, 146.7, 150.5, 152.0, 161.7 ppm. HRMS (ESI) calcd for $C_{16}H_{12}N_3O_7$ [(M-H)$^-$] 358.0675, found 358.0676.

3. Biochemical Methods

HPLC analysis of photodecaging.

Photocaged 5-ethynyl uracil (5EU) was dissolved in DPBS from 200 mM DMSO stocks with a final concentration of 200 μM 5EU and 1% DMSO. The solution was irradiated for 0 to 30 min with 365 nm light at room temperature and analyzed by HPLC. Different HPLC traces were overlapped to indicate the progression of UV light dependent photodecaging. The peaks corresponding to photocaged 5-ethynyl uracil were integrated and plotted over light irradiation time length.

Cell lines and culture conditions.

HeLa cell lines were cultured in DMEM supplemented with 10% FBS, 1% penicillin and streptomycin and grown at 37^{o}C, 5% CO_2. Stably transfected UPRT-HeLa (a kind gift from Dr. Sunnie Thompson) cell lines were cultured as mentioned above and supplemented with 1 mg/ml of G418 (Teknova Inc.).

Photocaged 5-ethynyl uracil labeling of cellular RNA.

UPRT-HeLa cell lines were cultured in full media and grown at 37^{o}C, 5% CO_2. Photocaged 5-ethynyl uracil was added to complete culture medium from 200 mM stocks with a final concentration of 200 μM 5EU and <1% DMSO. Cells were incubated with the analog for 30 min at 37^{o}C, 5% CO_2, irradiated for 2 to 30 min with 365 nm light at room temperature and incubated for 1 to 5 h at 37^{o}C, 5% CO_2.

RNA isolation and biotinylation via CuAAC.

After labeling, total cellular RNA was harvested using Trizol Reagent (Invitrogen) following the manufacturers instructions. Click reactions were prepared using 30 μg of total RNA, 1 mM biotin azide, and 4.6 mM THPTA to a final concentration of 1 mM, fresh 10.6 mM NaAsc to a final concentration of 1.77 mM, and 12 mM $CuSO_4$ to a final concentration of 200 μM. The reactions

were incubated with shaking at room temperature for 30 min. The reactions were purified by ethanol precipitation.

HRP-streptavidin northern blotting.

All gel reagents were from Bio-Rad. Equal amounts of purified RNA were loaded using nucleic acid loading dye and were separated on a native 1% agarose gel. RNA was transferred onto Hybond-N+ membrane (GE Healthcare) using standard vacuum transfer (Biometra, Analytic Jena Company), and UV-crosslinked to a membrane (Stratalinker UV crosslinker). Membranes were blocked followed by incubation with high sensitivity streptavidin-HRP (ThermoFisher Scientific). The membrane was washed twice in a 1:10 solution of blocking buffer and twice in Tris-saline buffer. It was then incubated in SuperSignal West Pico Chemiluminescent Substrate (ThermoFisher Scientific) and imaged on a ChemiDoc MP imaging system (Bio-Rad).

RNA fluorescence imaging via CuAAC.

Stably transfected UPRT-HeLa cells were seeded at $2.5x10^5$ and grown to ~50% confluency on glass cover slips. Cells were treated with photocaged 5-ehynyl uracil at various concentrations, irradiated for 10 min with 365 nm light at room temperature and incubated for different time periods. After labeling, cells were washed three times with DPBS, fixed and permeabilized for 30 min at room temperature with 3.7% paraformaldehyde and 0.15% Triton-X100. Cells were then washed three times (10 min each) on orbital shaker with DPBS, blocked with BSA (1 mg/mL in DPBS, 0.45% NaCl and 0.025% NaN_3) for 35 min at room temperature, washed twice with DPBS (5 min each), and incubated with 500 µL of click solution (1 mM $CuSO_4$, 2 mM THPTA ligand, 10 mM NaAsc, and 15 µM azide-Alexa 488) for 1 hour at 37 ^oC in the dark. Cells were washed three times for 5 min each on an orbital shaker: twice with DPBS-0.1% Triton-X100 and one with DPBS, and mounted using VectaShield with DAPI, 4',6-diamidino-2-phenylindole (Vector Labs). Slides were imaged via fluorescence confocal microscopy using a 40x oil immersion objective on a Leica 700 Carl Zeiss microscope.

4. Supplementary Figures

^{1}H NMR monitored decaging experiemnts shows stability of probe 3.

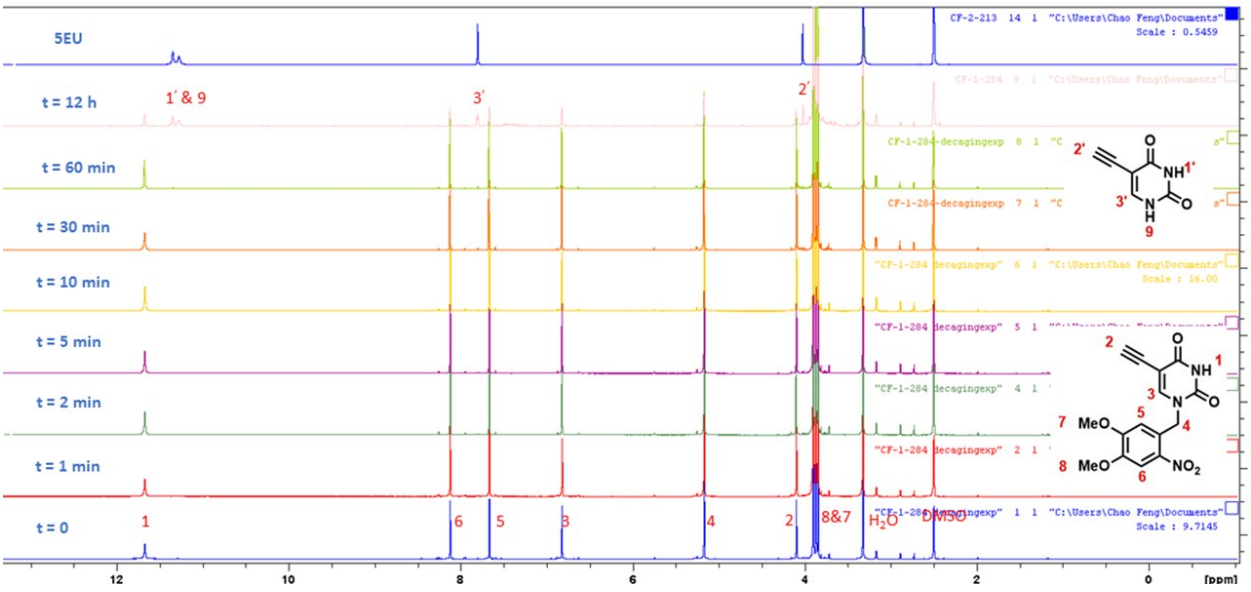


Fig. S1. ^{1}H NMR monitored decaging experiemnts shows stability of probe 3.

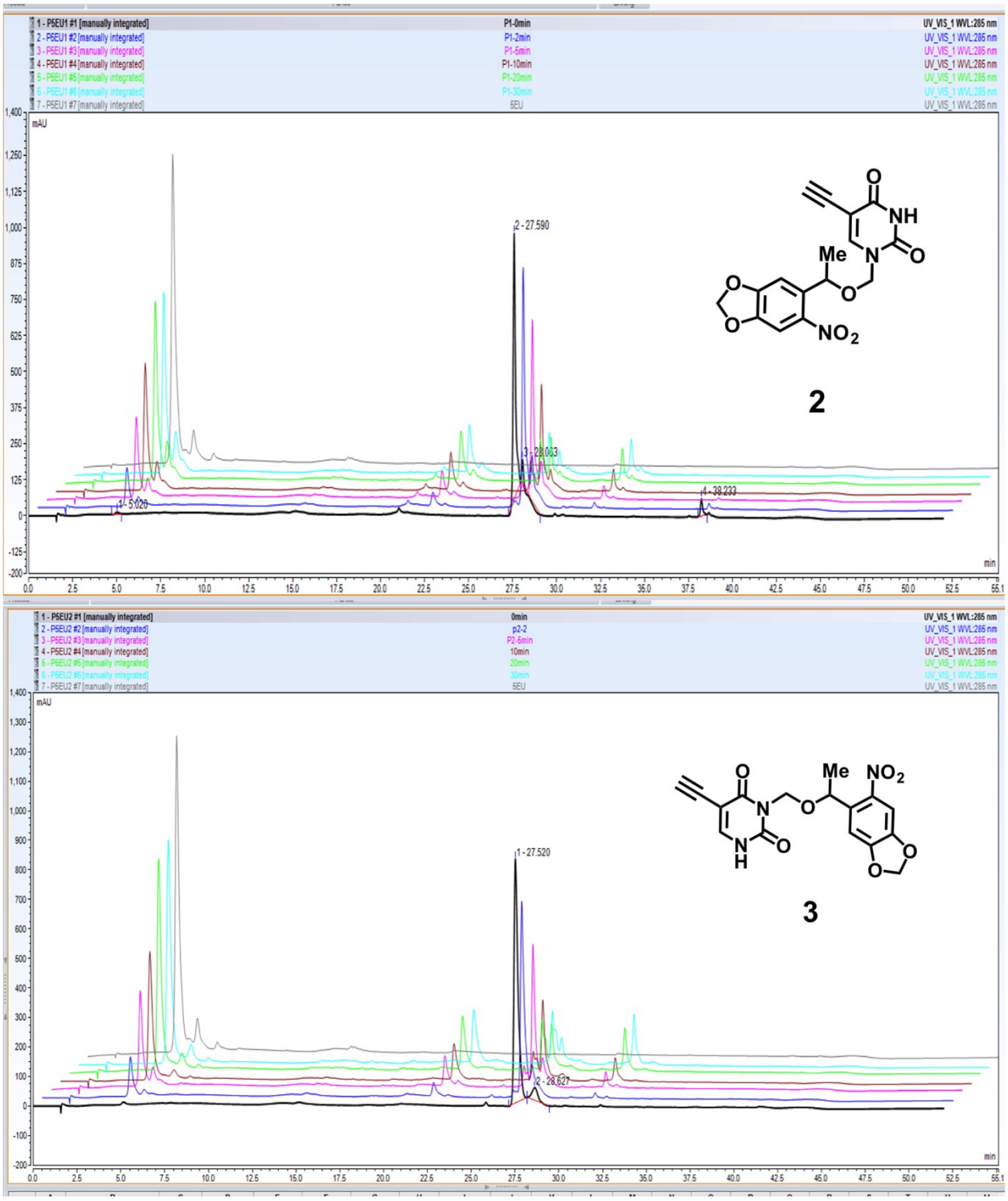


Fig. S2. HPLC traces of photouncaging.

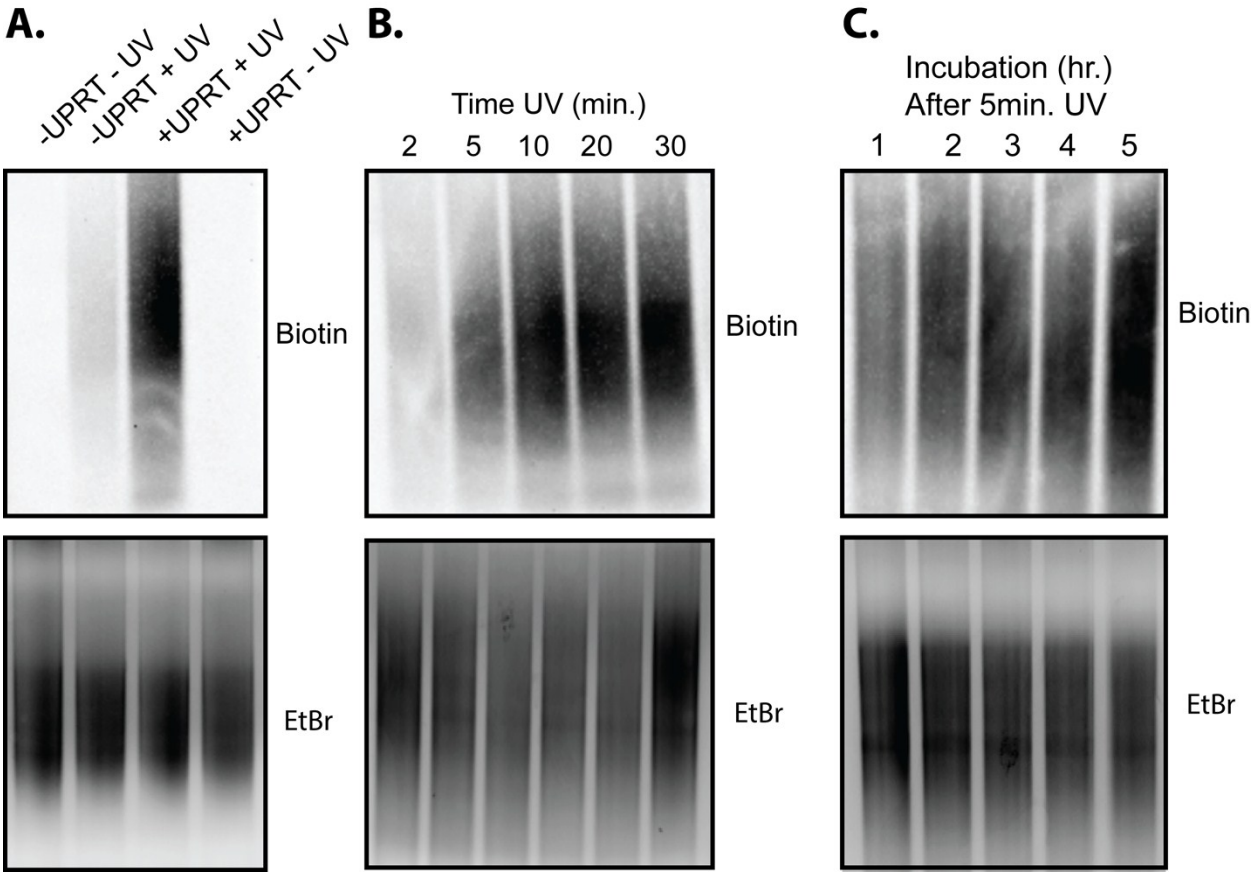
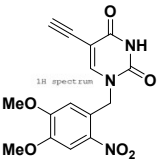
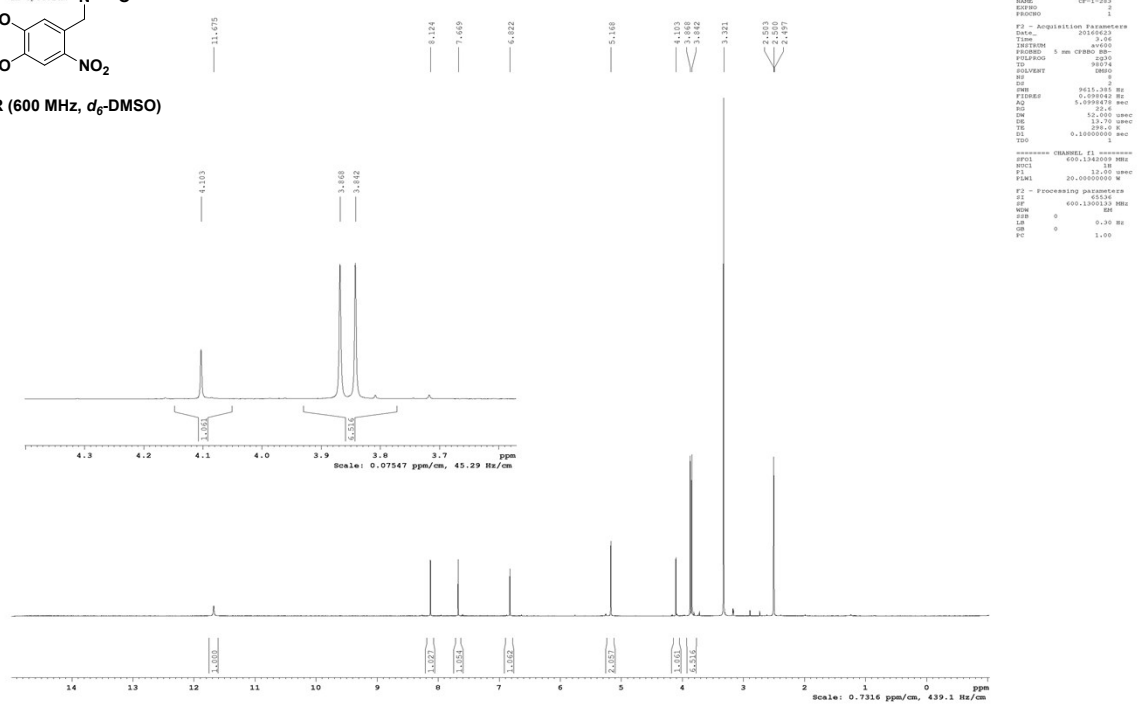


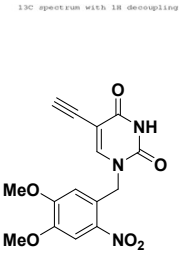
Fig. S3. Full Nothern blots from main text Figure 3. (A) Nothern blot demonstrating the specificity of uncacing and UPRT-5EU. (B) Northen blot showing 5 hours of RNA incorporation of 5EU after uncaging. (C) Northern blot showing time of incorporation of 5EU after 5 minutes of UV uncaging.

5. Spectra.

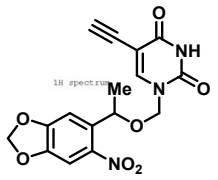


^{1}H NMR (600 MHz, d_6-DMSO)

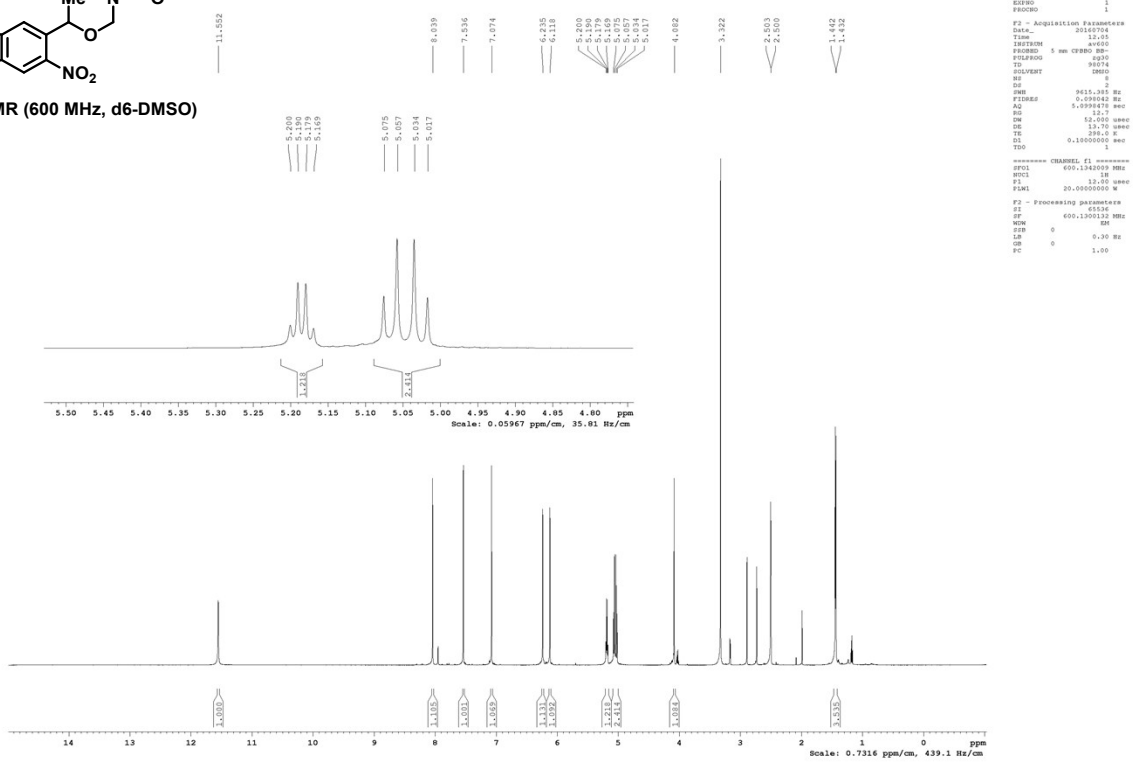


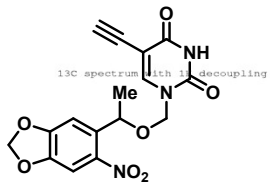


^{13}C NMR (150 MHz, d_6-DMSO)

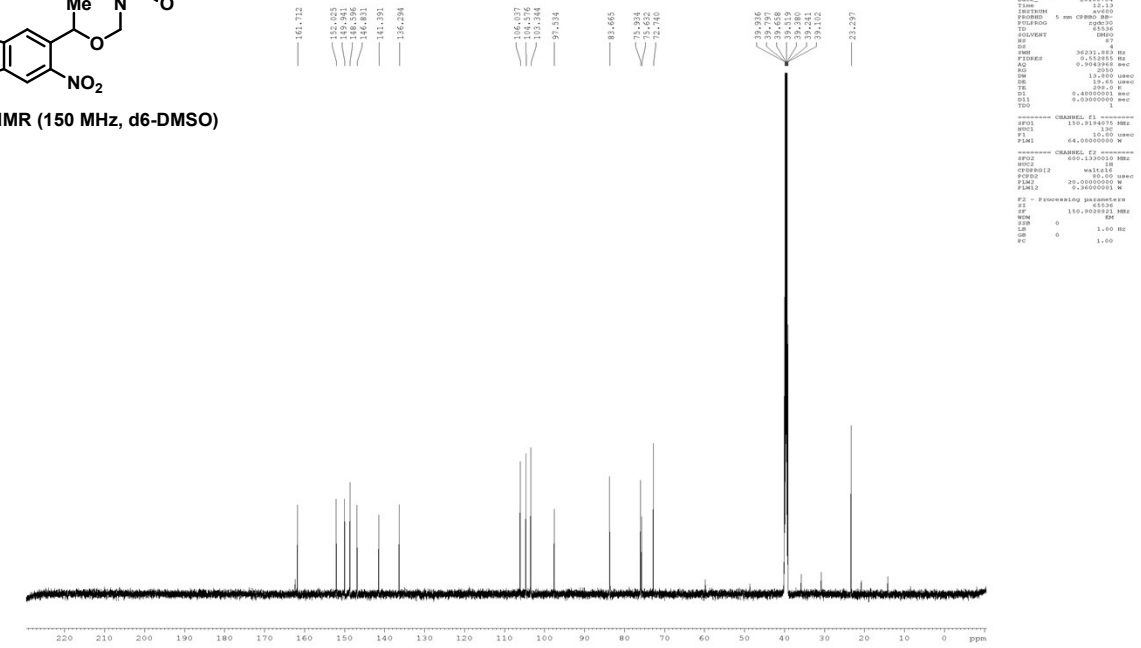


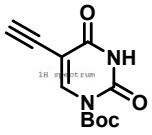
1H NMR (600 MHz, d6-DMSO)



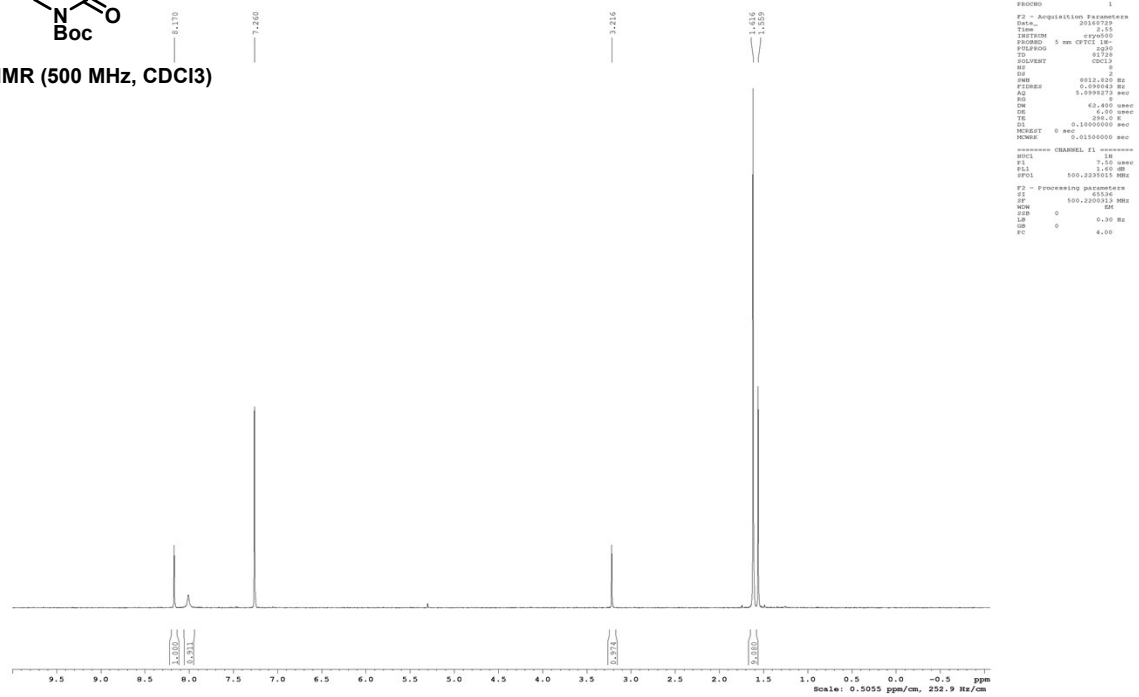


13C NMR (150 MHz, d6-DMSO)

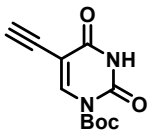




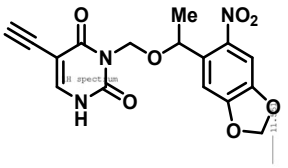
1H NMR (500 MHz, CDCl3)



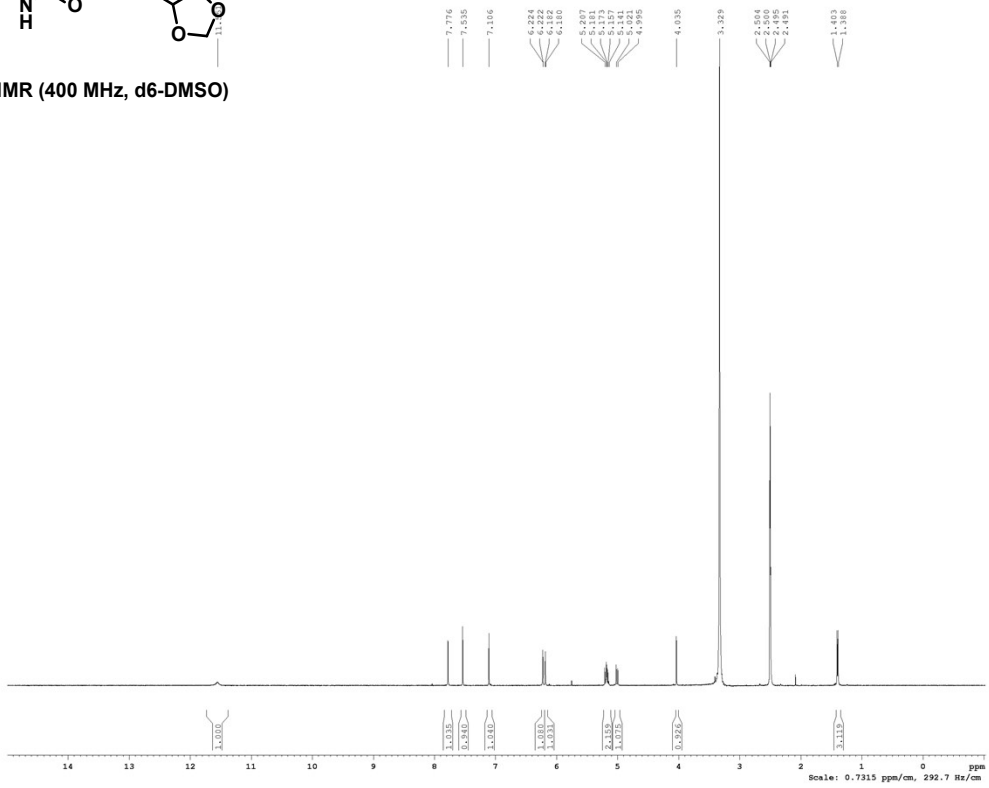
13C spectrum with 1H decoupling

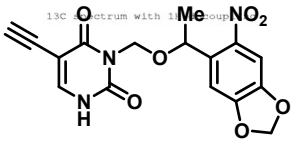


13C NMR (150 MHz, CDCl3)



1H NMR (400 MHz, d6-DMSO)





13C NMR (150 MHz, d6-DMSO)

References.

1. Janeba, Z. *et al. Can. J. Chem*. **2006**, *84*, 580.
2. Lusic, H. *et al. Org. Lett.*, **2010**, *12*, 916.