Electronic Supplementary Material (ESI) for ChemComm.

Supporting Information

Photocaged Dicarbonyl Probe Provides Spatiotemporal Control Over Protein Glycation

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I. Materials.

All chemicals and reagents required for the synthesis of targetable dicarbonyl precursor (T-DiP **8**) and preparation of buffers were purchased from Sigma Aldrich unless otherwise stated. NMR solvents were obtained from Cambridge Isotope Laboratories Inc. HEK 293T cells were acquired from ATCC. Cell culture media, additives, and consumables were purchased from Corning. DAPI and anti-actin antibody were obtained from ThermoScientific. AlamarBlueHS reagent and ProlongGlass were purchased from Invitrogen. The BCA kit was bought from Pierce. Mini-protean TGX precast gels, 0.2 µm nitrocellulose and filter paper transfer stacks were obtained from BioRad. THPTA and AlexaFlour647 azide were purchased from Click Chemistry Tools. Odyssey TBS blocking buffer and secondary antibodies were purchased from Licor. TEV protease was purchased from Genscript. Anti-Halo antibody and TMR Halo-ligand were obtained from Promega. The transfection reagent TransIT-2020 was obtained from Mirus Bio LLC. A 0.2-amp Spectroline (Model ENF-280C) UV hand lamp was used as the 365 nm light source in all experiments. Plasmids were obtained from Addgene. Specifically, Halo-Cyto, Halo-Mito, and Halo-ER were gifts from Jin Wang (Addgene plasmid #124314, #124315, #124316) and pet28a-His6-Halo-Tev-Keap1 was a gift from Yimon Aye (Addgene plasmid #62456). Licor Image Studio software was used to process acquired gel images and statistical analysis was performed with GraphPad.

II. Experimental Procedures.

Photolysis of caged DiC^{yne} Monitored by HPLC:

Caged DiC^{yne} **1** samples designated for photolysis were prepared by adding 50 µL of a solution containing 150 µM **1** in PBS (pH 7.4, 0.15% DMSO) to a 0.6 mL PCR tubes. The samples were irradiated at 365 nm via a Spectroline UV hand lamp positioned 1 inch above the midpoint of the tube. At 30 s timepoints, 20 µL of the reaction were removed and analyzed by HPLC using a Luna C18 column (5 µm, 150 × 2.0 mm). The analysis method (1.0 mL/min flow rate, buffer A: H_2O, buffer B: CH_3CN) involved a linear gradient of 5-95% B (0-25 min), followed by an isocratic hold at 95% B (25-30 min). Absorbance was monitored at 254 nm and peak areas corresponding to caged DiC^{yne} **1** were integrated and normalized to the peak area at time zero to determine the half-life of **1** under 365 nm light exposure over three independent replicates.

Light Dependent DiC^{yne}-Peptide Adduct Formation:

A solution of the peptide SGFRY (100 µM) in PBS (pH 7.4) was incubated with either DMSO, 150 µM DiC^{yne} **2**, or 150 µM caged DiC^{yne} **1** in a total volume of 30 µL containing 1.6% DMSO. Samples designated for light exposure were irradiated with 365 nm light for 5 min. All samples were subsequently incubated for 18 h in the dark at 37 °C. The solutions were then prepared for MALDI-MS by desalting with Millipore C18 ziptips following the manufacturer's instructions. Desalted samples were spotted onto a MALDI plate with a saturated solution of α-cyano-4-hydroxycinnamic acid as the matrix. Spectra were acquired on an AB-Sciex 5800 MALDI/TOF-MS to monitor DiC^{yne}-peptide adduct formation over two independent replicates.

General Protocol for DiC^{yne} Light Mediated Release in Lysates:

HEK 293T cell lysates (1 mg/mL) in MPER lysis buffer with 1.0% DMSO were incubated with the indicated concentration of caged DiC^{yne} **1** for a given experiment in a total reaction volume of 50 µL and irradiated with 365 nm light via a Spectroline UV hand lamp for the specified amount of time. Following further incubation at 37 °C, a click reaction was performed with 10 µM AlexaFlour647 azide, 1.5 mM tris((1-hydroxy-propyl-1H-1,2,3-triazol-4-yl)methyl)amine (THTPA), 1 mM $CuSO_4$ and 3 mM sodium ascorbate. The reactions were agitated with an orbital shaker at 25 °C for 1 h in the dark. Proteins were then precipitated with 400 µL of cold acetone overnight at -20 °C. Following centrifugation at 21,000 g for 20 min, the acetone was carefully aspirated, and the protein pellets were dissolved into 30 µL of 1× Licor loading buffer (125 mM Tris-HCl, pH 6.8,

50% glycerol, 4% SDS, 0.2% Orange G dye) containing 10% β-mercaptoethanol and denatured for 5 min at 95 °C. Next, 13.5 µL of each sample was loaded into 4–20% gradient mini-protean TGX precast gels for SDS-PAGE. Gels were fixed overnight in an aqueous solution of 30% EtOH and 10% acetic acid to wash out excess fluorophore and imaged with a Licor odyssey CLX instrument to quantify DiC^{yne} derived AGEs. The gels were then stained with coomassie blue to confirm equivalent loading.

Expression of His6 Halo Protein:

Recombinant His6-HaloTag protein was expressed in *E. coli* BL21 cells as a His6-Halo-TEV-Keap1 fusion protein as previously described with slight modification.[1] Briefly, expression of the fusion protein was carried out in LB Broth (Miller, 25 g/L) containing 50 µg/mL of carbenicillin (LB media). Agar plates containing 50 µg/mL of carbenicillin were streaked and allowed to grow overnight. The following day, a single colony was selected and used to inoculate 50 mL of LB media and was grown overnight at 37 °C. Then 5 mL of the overnight culture was diluted in 500 mL of LB media. The flask was placed on a shaker at 37 °C at 225 RPM and allowed to grow for ~4 h (OD = ~0.45 – 0.6). Expression was induced with 250 µM IPTG over 18 h at 19 °C. The resulting suspension was pelleted at 4,500 g for 12 min at 4 °C. The pellet was suspended in lysis buffer (50 mM NaH_2PO_4 (pH 8.0), 10 mM imidazole, 5 mM β-mercaptoethanol, 0.5 mM phenylmethylsulfonyl fluoride, and 0.01% Triton X-100) and lysed by sonication at 40 W in 30 s intervals for 8 min (4 min total sonication). The cellular debris was pelleted at 38,000 g for 25 min at 4 °C and the supernatant was collected. The supernatant was purified using TALON affinity chromatography and fractions containing the fusion protein were confirmed through western blot. The desired fractions were subsequently purified via size exclusion chromatography (SEC) by injecting them on a Bio-RAD NGC SEC system equipped with a GE Superdex HiLoad 16/60 column equilibrated with SEC buffer (50 mM Tris (pH 8.0), 10 mM dithiothreitol, 100 mM NaCl and 5% glycerol) at 4 °C. The fractions containing the desired fusion protein were characterized by western blot. Cleavage of the fusion protein was achieved using TEV protease. TEV (10,000 U/mL) was incubated with the fusion protein (1.5 mg/mL) in SEC buffer for 18 h to generate His6-Halo protein. The resulting cleavage product was purified using TALON affinity chromatography and characterized through SDS-PAGE and western blot. The protein concentration was determined by A280 (calculated extinction coefficient of 59930 $M^{-1}cm^{-1}$). The protein was aliquoted and stored in SEC buffer at -80 °C until needed.

Photolysis of T-DiP-Halo Complex *in vitro*:

For photolysis experiments, 1 µg of His6-Halo protein, (66 µM in PBS, pH 7.4, 3% DMSO) was incubated with 66 µM T-DiP **8** in a total volume of 30 µL per condition. The resultant solutions were incubated in the dark at 37 °C for 30 min to allow the covalent complex to form between the Halo protein and chloroalkane ligand. Samples were then subjected to 365 nm light exposure for 0, 1, 5, or 15 min followed by a click reaction through the addition of 0.1 mM AlexaFlour647 azide, 1.5 mM THPTA, 1 mM $CuSO_4$ and 3 mM sodium ascorbate. The reaction was allowed to proceed for 1 h and quenched with 7.5 µL 4× gel loading buffer. Samples were boiled at 95 °C for 5 min, then 30 µL of each sample was loaded into 4–20% gradient mini-protean TGX precast gels for SDS-PAGE. Gels were fixed overnight in an aqueous solution of 30% EtOH and 10% acetic acid to wash out excess fluorophore and imaged with a Licor odyssey CLX instrument to quantify the T-DiP-Halo protein complex. The gels were then stained with coomassie blue to confirm equivalent loading.

Alamar Blue Cell Viability Assay:

HEK 293T cells were seeded into a 96 well plate at a density of 20,000 cells per well in 100 µL of FBS medium and incubated for 24 h. The FBS medium was removed and replaced with the indicated treatment condition in FBS-free MEM media. Specifically, following a 3 h T-DiP **8** treatment to mimic control released assay conditions, the medium was refreshed, and the cells were further incubated for 18 h, or cells were subjected to a 20 min light exposure and further incubated for 18 h. After incubation, the medium was removed and replaced with 100 µL of a 10% alamar blue solution in FBS-free MEM medium. The plates were incubated for 4 h in the dark at 37 °C and then fluorescence was measured at Ex/Em 560/590 nm on a BioTek Synergy H1 plate reader. Cell viability for a given treatment condition was calculated by normalizing the fluorescence signal to the DMSO treated control.

Confocal Microscopy:

HEK 293T cells were cultured on Neuvitro GC-25-1.5-Laminin coverslips to 70% confluency with MEM medium containing 10% FBS and Pen/Strep 100 U/mL under a 5% CO_2 atmosphere at 37 °C. Cells were transfected with 2.5 µg Halo plasmids via the serum-compatible Mirus TransIT-2020 transfection reagent following the manufacturer's instructions. After 24 h, the medium was removed and replaced with the indicated treatment condition in FBS-free medium. For competitive binding experiments, cells were treated with DMSO vehicle or 12.5 µM T-DiP **8**

for 2 h. Then the cells were washed with serum-free medium and then incubated with 5 μM HaloTag TMR for 1 h. Following the allotted incubation time, the cells were washed three times with 2 mL PBS, fixed with 2 mL of 4% PFA (molecular biology grade) in PBS for 10 min, and washed an additional three times with PBS. For in-cell click imaging experiments, the cells were treated with either DMSO, 500 μM **2** for 4 h, or 12.5 μM **8** with a 20-min 365 nm light exposure followed by a 15-min incubation prior to fixation as descried above. Following fixation, the cells were incubated in a 0.1% Triton X-100 PBS (PBT) solution containing 10 μM AlexaFlour647 azide, 1.5 mM THPTA, 1 mM CuSO4, and 50 mM sodium ascorbate for 1 h to label proteins modified by DiC^{yne} **2**. Next, three washes with PBT were performed. All cells' nuclei were stained with 300 nM of DAPI for 30 min and were further washed an additional three times with PBS. A drop of prolong glass anti-photobleaching solution was placed on the coverslips, which were mounted to glass slides and allowed to cure for 48 h. The slides were then sealed with clear nail polish and stored at 4 °C until they were imaged. Images were acquired on an Olympus FluoView FV1000 BX2 upright confocal microscope using a PLAPON 60X O NA:1.42 objective. Laser Ex/Em were set at 405:461, 557:576, and 635:668 nm for DAPI, TMR, and AlexaFlour647, respectively.

General Protocol for the Controlled Release of DiC^{yne} with T-DiP in Live Cells:

HEK 293T cells were cultured in MEM supplemented with 10% v/v FBS in the presence of 100 U/mL Pen/Strep antibiotics under a 5% CO_2 atmosphere at 37 °C. Cells were seeded into 6-well tissue treated plates at 400,000 cells per well. Cells were transfected with 2.5 μg Cyto-Halo, Mito-Halo, or ER-Halo plasmid via the serum-compatible Mirus TransIT-2020 transfection reagent (7.5 μL) following the manufacturer's instructions. After 24 h following transfection, cells were washed with PBS and subjected to the desired treatment conditions in FBS free medium. Cells treated with T-DiP **8** were incubated with 12.5 μM **8** for 2 h, then the medium was removed and replaced with fresh FBS-free medium and was further incubated for 30 min. Two additional 30-min washes were performed to ensure unbound T-DiP **8** was removed. Samples were irradiated at 365 nm with a handheld UV lamp for 20 min which was positioned 1 inch above the cell monolayer. Cells were incubated at 37 °C for the specified amount of time prior to lysis and downstream analysis.

Following the allotted incubation time, the cells were collected through trypsinization, washed twice with PBS, and lysed in 200 μL of MPER lysis buffer supplemented with HALT protease inhibitors. The lysates were centrifuged at 14,000 g for 8.5 min to remove debris. The

supernatant was collected, and the protein concentrations were determined through a BCA assay ~~the~~ per manufacturer's protocol.

Click reactions were performed in a total volume of 100 µL at a protein concentration of 50 µg/mL with 10 µM AlexaFlour647 azide, 1.5 mM THPTA, 1 mM $CuSO_4$ and 3 mM sodium ascorbate. The reactions were agitated at 25 °C for 1 h in the dark. Proteins were precipitated with 400 µL cold acetone overnight at -20 °C. Following a 20 min centrifugation at 21,000 g, the acetone was carefully aspirated, and the protein pellets were dissolved into 30 µL of 1× Licor loading buffer with 10% β-mercaptoethanol and denatured for 5 min at 95 °C, then 13.5 µL of each sample was loaded into 4–20% gradient mini-protean TGX precast gels for SDS-PAGE. Gels were fixed overnight in an aqueous solution of 30% EtOH and 10% acetic acid to wash out excess fluorophore and imaged with a Licor odyssey CLX instrument to visualize and quantify DiC^{yne} **2** derived protein adducts. The gels were then stained with coomassie blue to confirm equivalent loading.

General Notes:

For all experiments involving photocaged compounds, care was taken to shield samples from stray light to prevent undesired photolysis. Experiments were performed in a dimly lit environment and samples were wrapped in foil whenever possible.

III. Supplementary Figures.

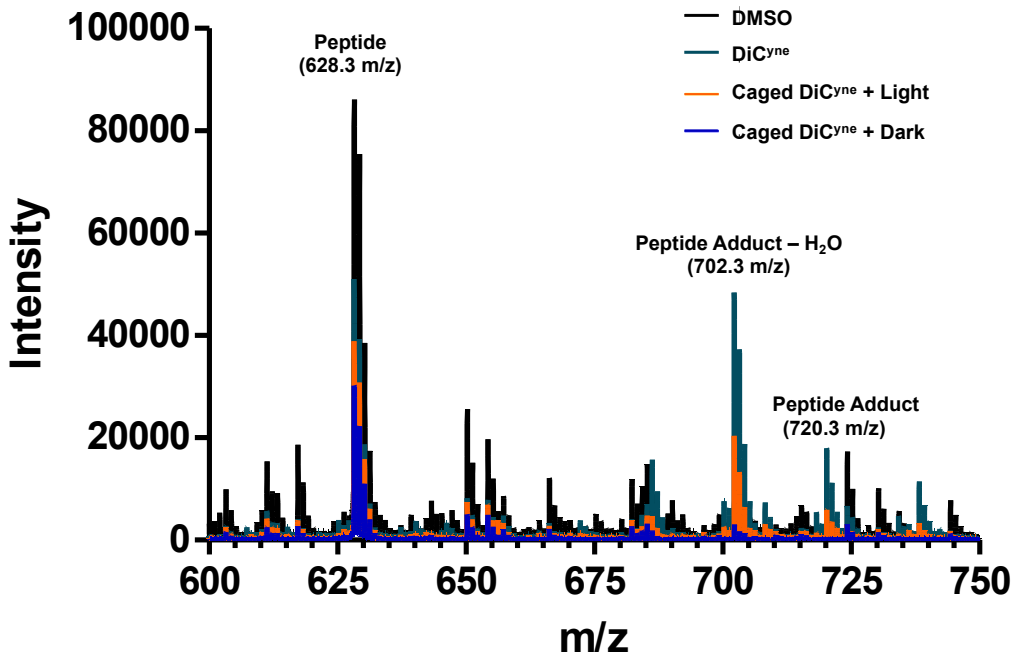


Fig S1. Light induced DiC^{yne} adduct formation of an arginine containing peptide (SGFRY, parent mass M+H$^+$ 628.3 m/z). Overlayed MALDI spectra of peptide only, peptide incubated with DiC^{yne} **2**, or peptide incubated with caged DiC^{yne} **1** with or without 5 min of 365 nm irradiation are shown. The peak at 720.3 m/z corresponds to a + 92 Da mass increase due to hydroimidazolone formation. Spectra are representative of two independent experimental replicates.

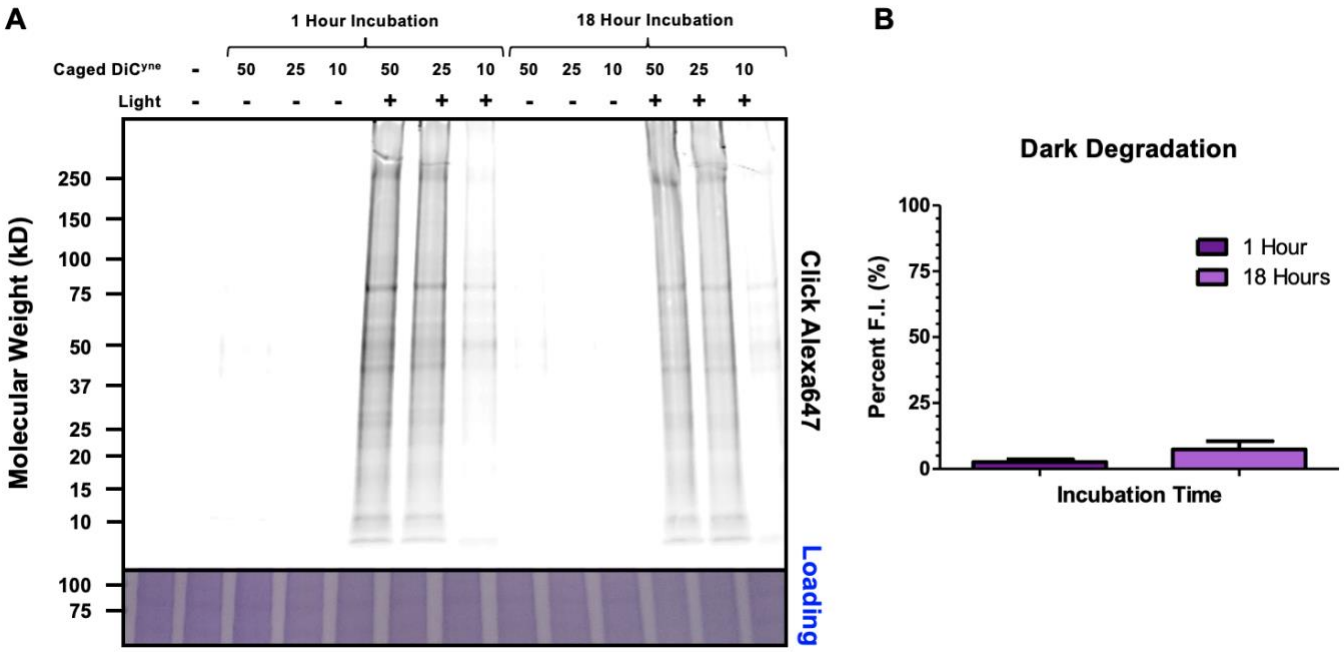
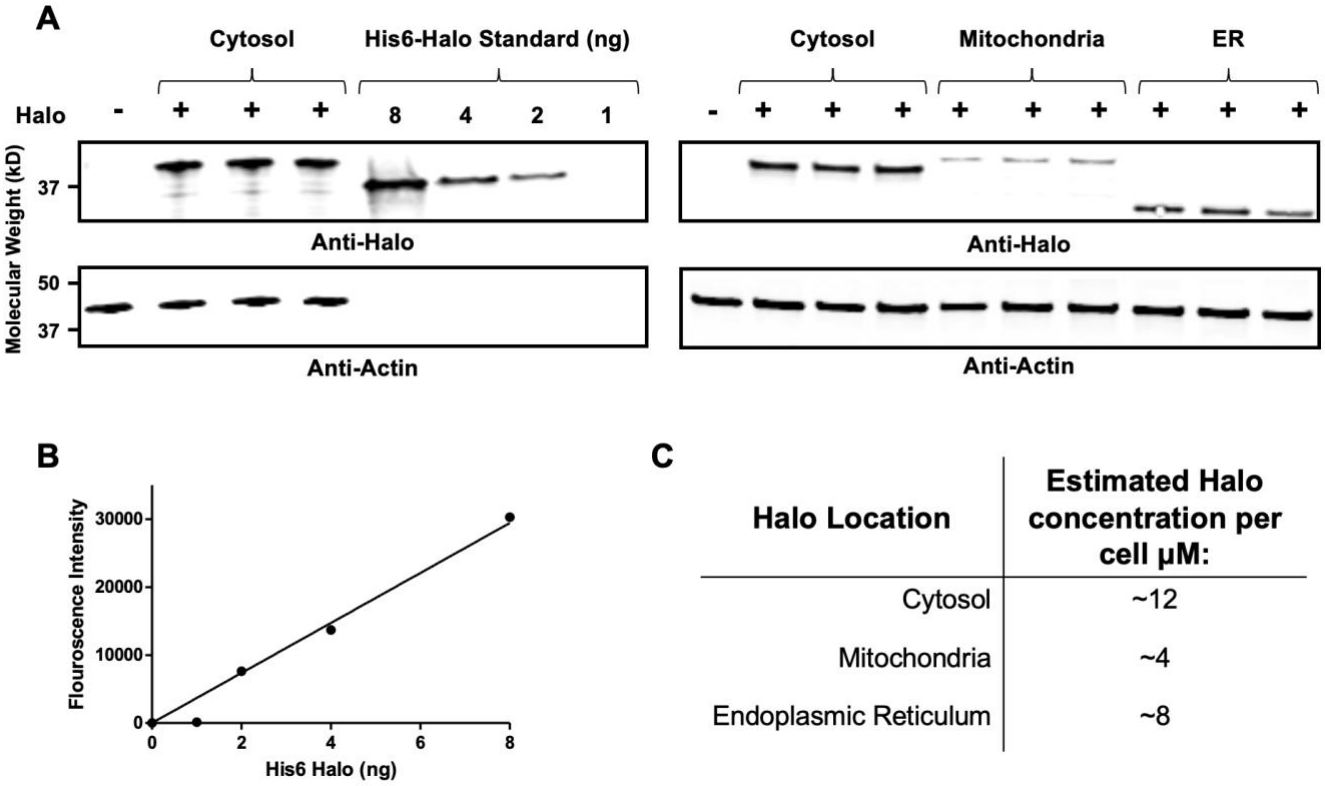


Fig S2. Stability of DiC^{yne} photocage. A) Representative gel of HEK293T lysates (1 mg/mL) incubated with 50 μM, 25 μM, or 10 μM caged DiC^{yne} **1,** which were kept in the dark or irradiated for 10 min with 365 nm light and further incubated for the allotted time at 37 °C prior to CuAAC reaction with Alexa647 azide and separation by SDS-PAGE. Top: Fluorescence imaging of Alexa647. Bottom: Coomassie stain loading control. B) Integrated fluorescent intensity (F.I.) of Alexa647 signal in samples kept in the dark normalized to their irradiated counterparts. Data are represented as mean ± SEM from 3 technical replicates.



Halo Location	Estimated Halo concentration per cell μM:
Cytosol	~12
Mitochondria	~4
Endoplasmic Reticulum	~8

Fig S3. Estimation of intracellular Halo protein concentration. A) Representative western blots of HEK 293T cells 24 h post transfection with targeted Halo plasmid alongside a standard curve of titrated His6-Halo protein. Top: Anti-Halo. Bottom: Anti-Actin B) Standard curve of recombinant His6-Halo protein fluorescence intensity as measured through western blot. Data are represented as mean ± SEM from 2 technical replicates. C) Estimated cellular Halo protein concentration. Halo protein concentration was estimated based off the following calculations and assumptions:[1,2] Following cell lysis, 1% of the sample was loaded into the gel and the cytosolic Halo protein concentration in each lane was calculated to be 6 ± 2 ng by the standard curve of recombinant protein. The average number of cells collected per well across 6 biological replicates was $1 \times 10^6 \pm 2 \times 10^4$. Thus, ~$6 \times 10^{-5}$ ng (1.7×10^{-8} nmol) of Halo protein is expressed in per cell. Using data from Bionumbers (http://bionumbers.hms.harvard.edu/bionumber.aspx?id=108893) and the assumption the cells are spherical, the radius of an HEK cell is calculated to be 6.5 μm. The approximate volume of each cell (¾ π r^3) is estimated to be 1.44×10^{-12} L. Therefore, the average concentration of cytosolic Halo protein expressed in each cell is predicted to be ~12 μM. Similar calculations were performed for mitochondria and ER targeted Halo protein.

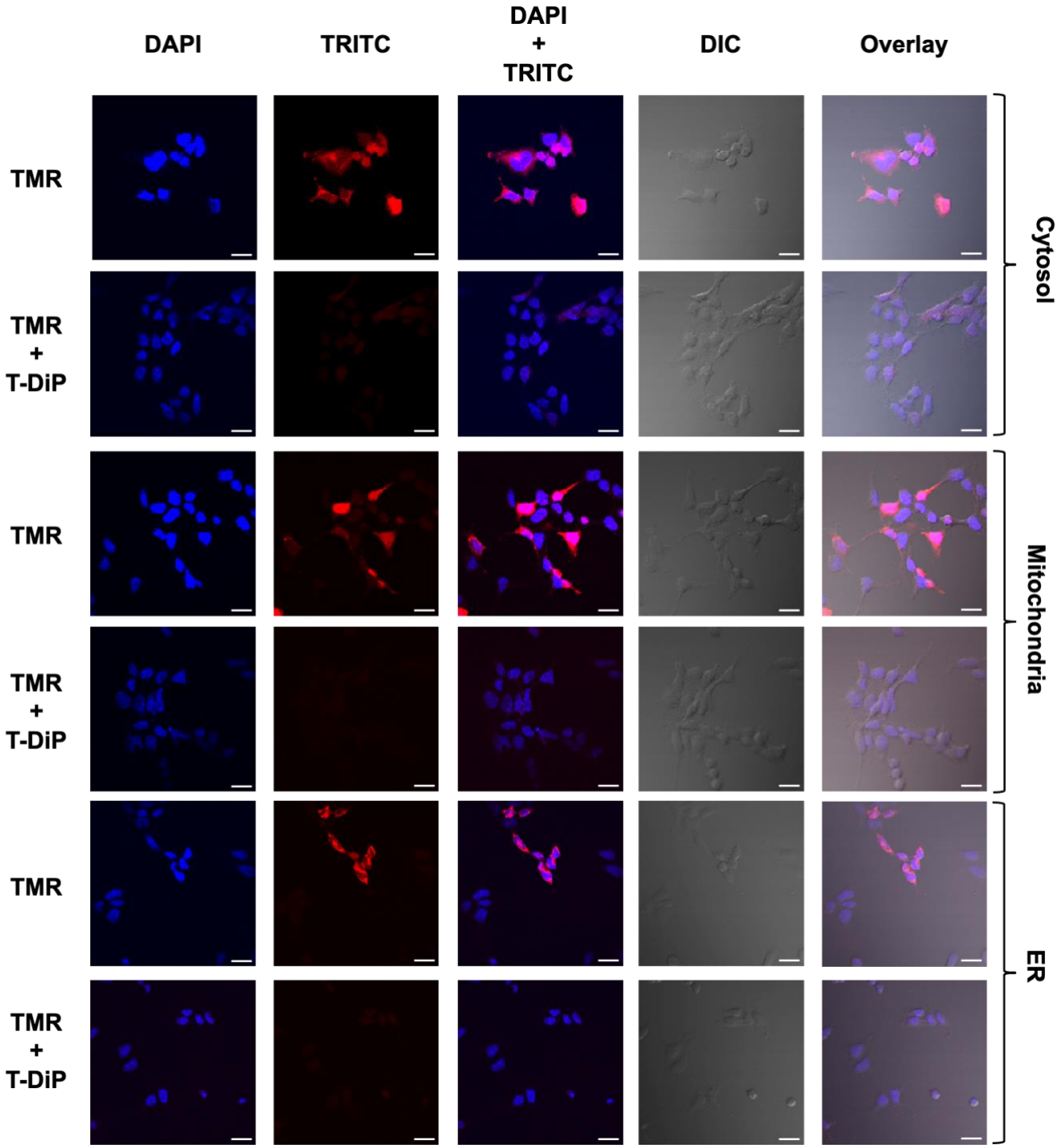


Fig S4. Confocal imaging of HEK 293T cells transfected with Halo proteins targeted the cytosol, mitochondria, and ER treated with either 5 μM TMR HaloTag only, or with 12.5 μM **8** for 1 h prior to treatment with 5 μM TMR HaloTag. Cells were fixed, washed, stained with DAPI to visualize their nuclei, and imaged at 60× magnification. Column headings signify imaging channels, left-hand row headings signify treatment conditions, and righthand row headings signify Halo protein localization. Laser Ex/Em were set at 405:461 nm and 557:576 nm for DAPI (shown in blue) and TMR (shown in red), respectively. Scale bar in bottom left denotes 20 μm.

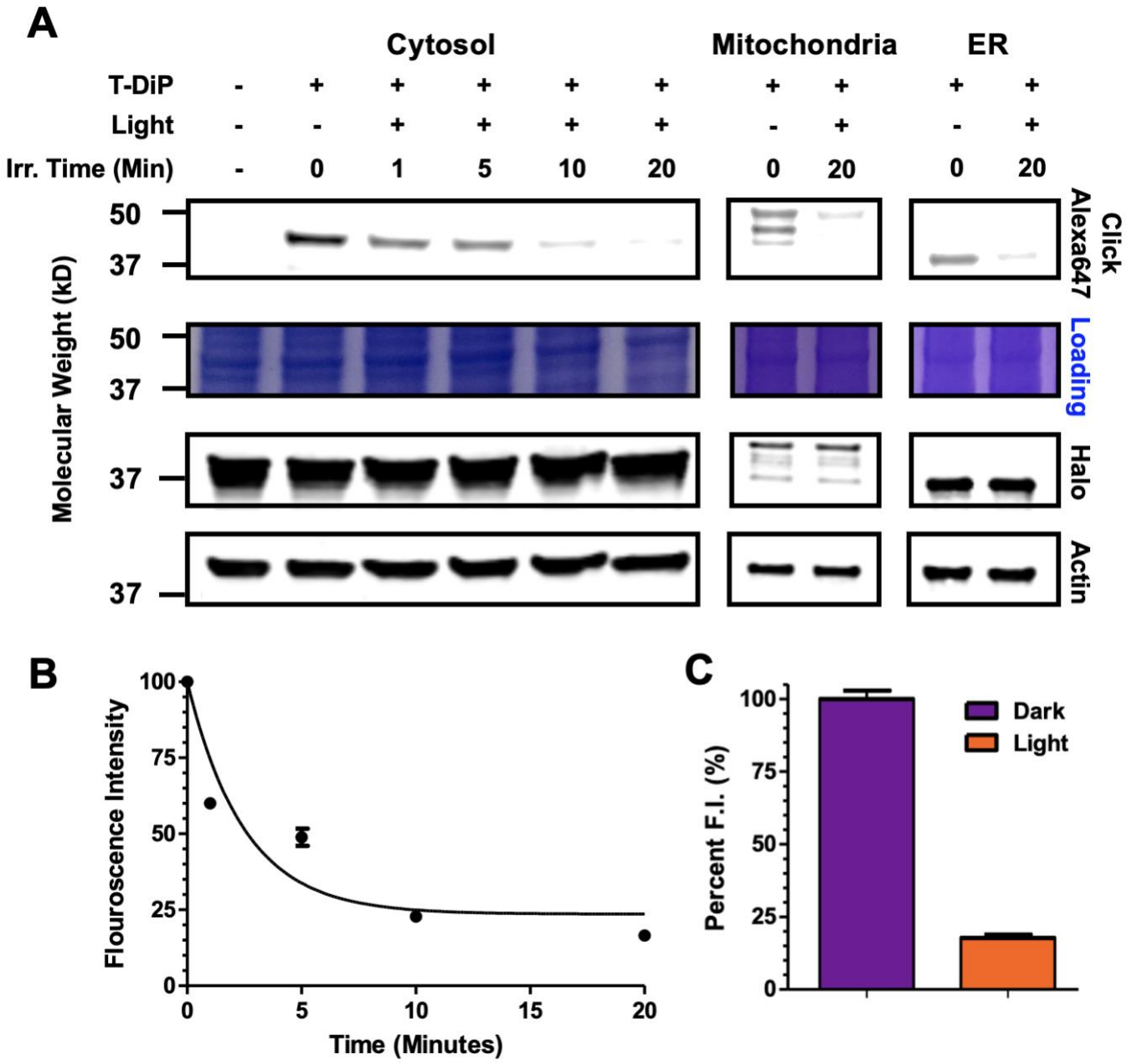


Fig S5. T-DiP controlled release system rapidly uncages DiC^{yne} in live cells. Representative gels and western blots of Halo transfected HEK 293T cells treated with 12.5 μM T-DiP **8** and irradiated with 365 nm light for the specified time. Lysates were subjected to a subsequent CuAAC reaction with Alexa647 azide and separated by SDS-PAGE. Top: Fluorescence imaging of Alexa647. Second from top: Coomassie stain loading control. Second from bottom: anti-Halo. Bottom: anti-Actin. B) Normalized fluorescence intensity of Halo:T-DiP complex following light exposure. Data are represented as mean ± SEM of two biological replicates and fit to a one phase exponential decay function, $t_{1/2}$: 1.7 ± 0.9 min. C) Normalized fluorescence intensity of Halo:T-DiP complex following 20 min light exposure. Data are represented as mean ± SEM of two biological replicates for each cytosol, mitochondria, and ER targeted Halo protein.

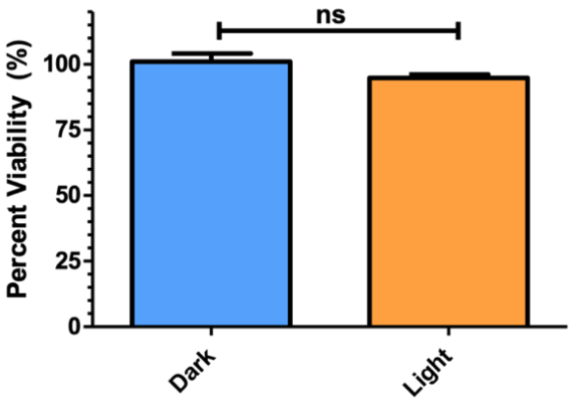


Fig S6. 20 minutes of 365 nm light exposure is nontoxic. Cell viability of HEK 293T cells following 20 min irradiation of 365 nm light as measured by alamar blue. Data are represented as mean ± SEM from 12 technical replicates and normalized to dark control. Statistical analysis was performed by a t-test (ns: $P > 0.05$).

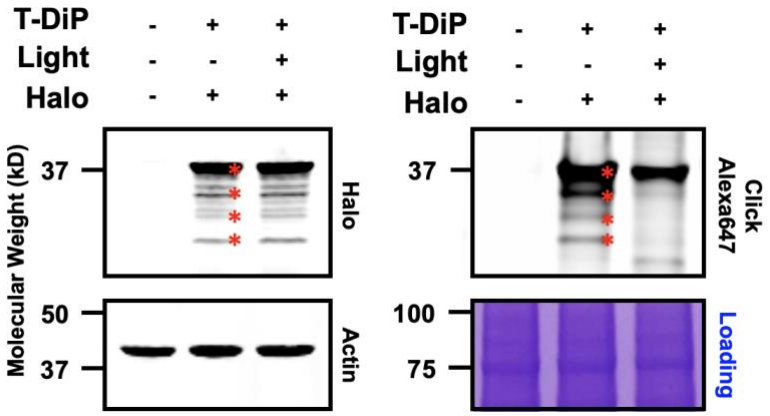


Fig S1. T-DiP binds to truncated Halo protein. Representative gels and western blots of HEK 293T with or without transfection of cytosolic Halo treated with DMSO or 12.5 μM T-DiP **8**, with or without 20 min exposure to 365 nm light. Lysates were subjected to a subsequent CuAAC reaction with Alexa647 azide and separated by SDS-PAGE. Left: western blots probed for Halo protein (top) and actin (bottom). Right: Fluorescence imaging of Alexa647 (top) and Coomassie stain loading control (bottom). Red stars (*) denote overlap of Halo protein western bands and fluorescent bands observed at the same molecular weight, signifying binding of T-DiP **8** to these truncation products.

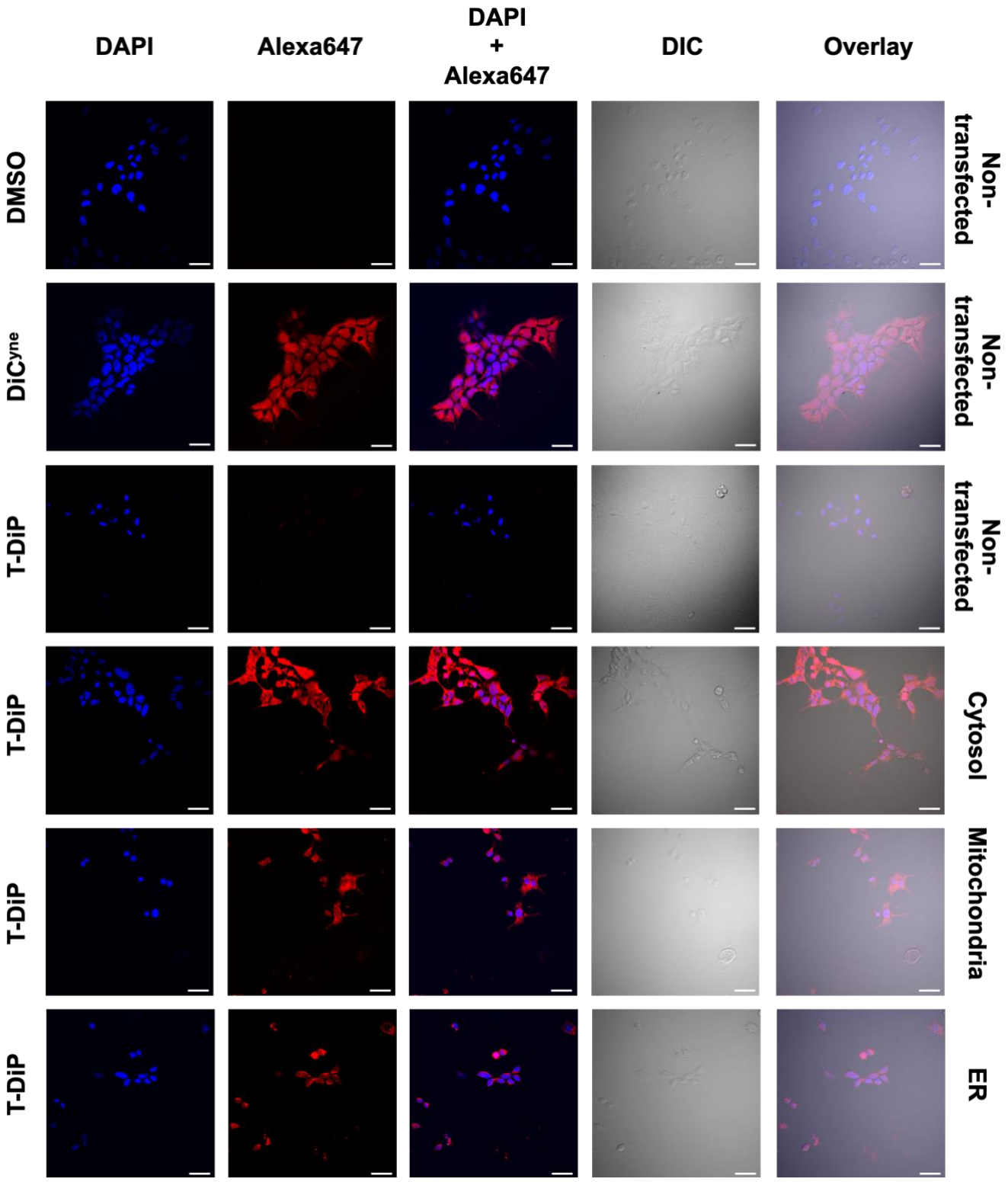
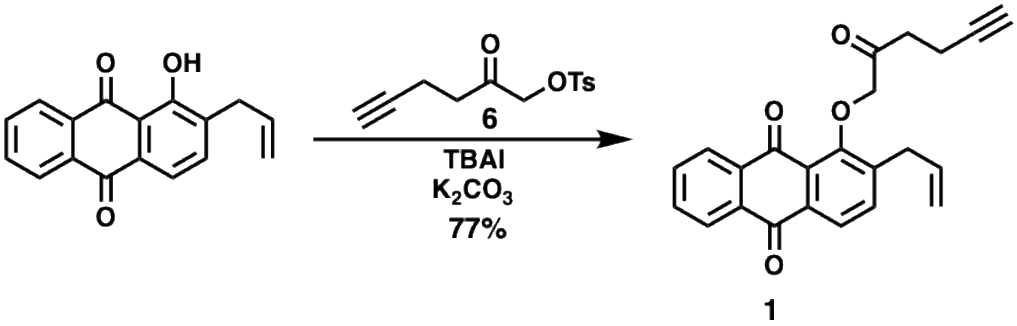


Fig S8. Confocal imaging of non- or Halo- transfected cells and treatment with either DMSO, 500 μM **2** for 4 h, or 12.5 μM **8** with a 20-min 365 nm light exposure followed by a 15-min incubation prior to fixation. Fixed cells were subjected to a CuAAC, stained with DAPI, and imaged at 60× magnification. Column headings signify imaging channels, left-hand row headings signify treatment conditions, and righthand row headings signify Halo protein localization. Laser Ex/Em were 405:461 nm for DAPI (shown in blue) and 635:668 nm for Alexa647 (shown in red). Scale bar in bottom left denotes 20 μm.

IV. General Reaction Conditions.

Chemical reactions were conducted with oven-dried glassware under argon atmosphere unless otherwise stated. Anhydrous solvents were dried with activated 4 Å molecular sieves. Thin-layer chromatography (TLC) was performed with Analtech silica uniplates and visualized under 254 nm UV light or with $KMnO_4$ staining. Column chromatography was conducted with 60 mesh silica gel. NMR spectra were acquired on a 400 MHz Bruker spectrometer at room temperature. HRMS spectra were obtained on a Bruker ESI-BioTOF II instrument and internally calibrated with PEG 300.

V. Synthetic Procedures.

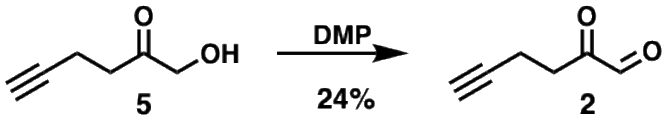


2-Allyl-1-[(2-oxohex-5-yn-1-yl)oxy]anthracene-9,10-dione (**1**, caged DiC^{yne}): To a solution of 2-Allyl-1-hydroxyanthra-9,10-quinone (75 mg, 0.33 mmol, 3.0 equiv), K_2CO_3 (45 mg, 0.33 mmol, 3.0 equiv), and TBAI (20 mg, 0.050 mmol, 0.50 equiv) in THF:DMF (1:1 v/v, 2 mL) was added a solution of **6** (30 mg, 0.11 mmol, 1.0 equiv) in THF (1 mL). The reaction was stirred for 18 h at 25 °C, then diluted with EtOAc (20 mL) and washed successively with 0.1 N aqueous HCl (2 × 20 mL), brine (20 mL), dried over anhydrous Na_2SO_4, and concentrated *in vacuo*. Purification by silica gel flash chromatography (gradient of 0 to 10% EtOAc in hexanes) afforded the title compound **1** (30 mg, 77%) as an orange solid: R_f = 4.5 (9:1 hexanes:EtOAc).

^{1}H NMR (400 MHz, $CDCl_3$) δ 2.00 (t, J = 2.4 Hz, 1H), 2.64 (td, J = 7.6 Hz, J = 2.8 Hz, 2H), 3.11 (t, J = 7.6 Hz, 2H), 3.60 (d, J = 6.4 Hz, 2H), 4.58 (s, 2H), 5.09 (dd, J = 16.8 Hz, J = 1.6 Hz, 1H), 5.17 (dd, J = 10.2 Hz, J = 1.2 Hz, 1H) 5.94–6.04 (m, 1H), 7.66 (d, J = 8.0 Hz, 1H), 7.76–7.79 (m, 2H), 8.15 (d, J = 8.0 Hz, 1H), 8.22–8.26 (m, 2H).

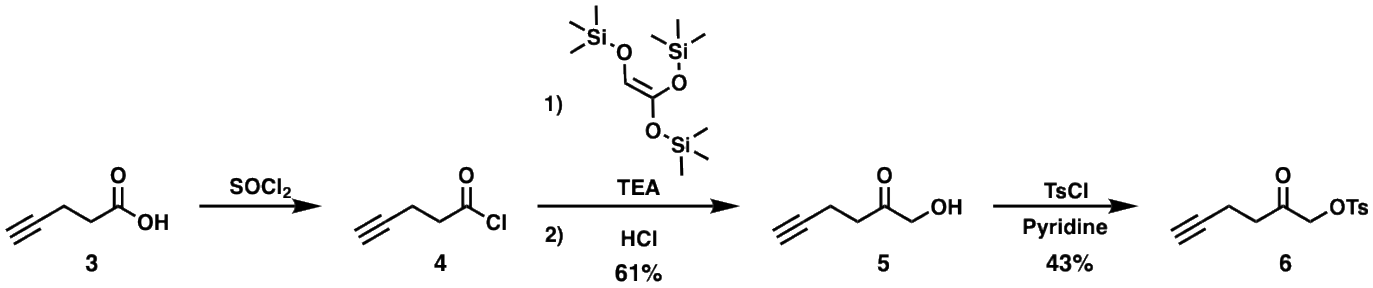
^{13}C NMR (101 MHz, $CDCl_3$) δ 12.5, 34.3, 38.2, 68.8, 78.0, 83.1, 117.4, 124.2, 125.3, 126.8, 127.3, 132.6, 133.8, 134.2, 134.3, 134.5, 135.5, 136.2, 142.3, 156.3, 182.6, 182.8, 205.4.

HRMS (ESI-TOF) *m/z* $[M + Na]^+$ calculated for $C_{23}H_{18}O_4Na^+$ 381.1103, found 381.1117.



2-Oxohex-5-ynal (**2**, DiC^{yne}): Compound **2** was prepared with slight modification of a previously reported protocol.[3] Briefly, compound **5** (47 mg, 0.41 mmol, 1.00 equiv) was dissolved into 10 mL of anhydrous CH_2Cl_2. Dess-Martin periodinane (181 mg, 0.42 mmol, 1.02 equiv) was added and the reaction mixture was stirred for 18 h at 25 °C. The reaction was then cooled to 0 °C for 10 min and filtered over celite to remove precipitate. Next, 10 mL of aqueous 2.5% $Na_2S_2O_3$ in saturated $NaHCO_3$ was added to the filtrate and the resultant solution was for vigorously stirred for 1 h. The reaction was extracted with CH_2Cl_2 (3 × 20 mL), washed with brine (60 mL), dried over anhydrous Na_2SO_4, and concentrated *in vacuo*. The title compound was purified via silica gel flash chromatography (gradient of 0 to 100% CH_2Cl_2 in hexanes) to afford DiC^{yne} **2** (11 mg, 24%) as a pale oil: R_f = 0.8 (3:2 hexanes:EtOAc).

Spectral characterization of this compound was in agreement with previous reports.[3,4]



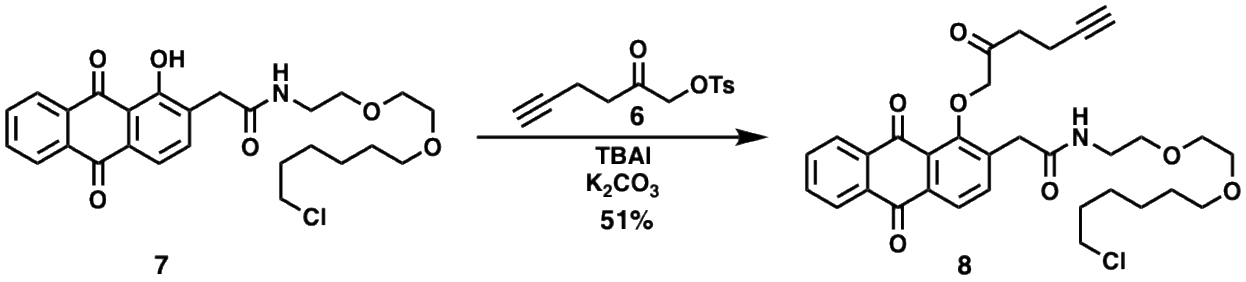
Supplementary Scheme 1. Synthesis of intermediate **6**.

2-Oxohex-5-yn-1-yl 4-methylbenzenesulfonate (**6**): Intermediates **4** and **5** were synthesized as previously reported.[5] Compound **5** (170 mg, 1.5 mmol, 1.0 equiv) was dissolved into 3 mL of anhydrous CH_2Cl_2 and cooled to 0 °C. Pyridine (180 µL, 2.3 mmol, 1.5 equiv) and TsCl (430 mg, 2.3 mmol, 1.5 equiv) were added and the reaction was allowed to warm to room temperature and stirred for 1 h. The reaction was diluted with CH_2Cl_2 (20 mL) and washed with 1 N aqueous HCl (20 mL), brine (mL), dried over anhydrous Na_2SO_4, and concentrated *in vacuo*. Purification by silica gel flash chromatography (gradient of 0 to 50% EtOAc in hexanes) afforded the title compound (172 mg, 43%) as a clear oil: R_f = 0.4 (4:1 hexanes:EtOAc).

^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 1.94 (t, J = 2.8 Hz, 2H), 2.39–2.52 (m, 4 H, m), 2.78 (t, J = 7.6 Hz, 1H), 4.52 (s, 2H), 7.37 (d, J = 8.0 Hz, 2H), 7.82 (d, J = 8.0 Hz, 2H).

^{13}C NMR (101 MHz, $CDCl_3$) δ ppm 12.3, 21.7, 38.0, 69.2, 71.8, 82.1, 128.1, 130.1, 132.2, 145.6, 201.4.

HRMS (ESI-TOF) *m/z* $[M + Na]^+$ calculated for $C_{13}H_{14}O_4SNa^+$ 289.0510, found 289.0523.



***N*-(2-(2-((6-Chlorohexyl)oxy)ethoxy)ethyl)-2-(9,10-dioxo-1-((2-oxohex-5-yn-1-yl)oxy)-9,10-dihydroanthracen-2-yl)acetamide** (**8**, T-DiP): Compound **7** was synthesized according to previous reports.[6] To a solution of **7** (61 mg, 0.125 mmol, 1 equiv), K_2CO_3 (51 mg, 0.375 mmol, 3 equiv), and TBAI (14 mg, 0.038 mmol, 0.3 equiv) in THF:DMF (1:1 v/v, 2 mL) was added a solution of **6** (100 mg, 0.375 mmol, 3 equiv) in THF (1 mL). The reaction was stirred for 18 h at 25 °C, then diluted with EtOAc (20 mL) and washed successively with 0.1 N aqueous HCl (2 × 20 mL), brine (20 mL), dried over anhydrous Na_2SO_4, and concentrated *in vacuo*. The crude material was purified by silica gel flash chromatography (gradient of 50 to 100 % EtOAc in hexanes) to afford T-DiP **8** (37 mg, 51%) as an orange solid: R_f = 0.5 (9:1 hexanes:EtOAc). T-DiP **8** was subjected to an additional RP-HPLC purification step on a Sunfire C18 column (5 µm, 19 × 250 mm) prior to cellular studies (buffer A: H_2O, buffer B: CH_3CN, flow rate; 10 mL/min, gradient: 5% B to 70% B from 0 to 12.5 min, 70% B to 95% B from 12.5 to 27 min, 95% B until 35 min; **8** eluted at ~ 90% B).

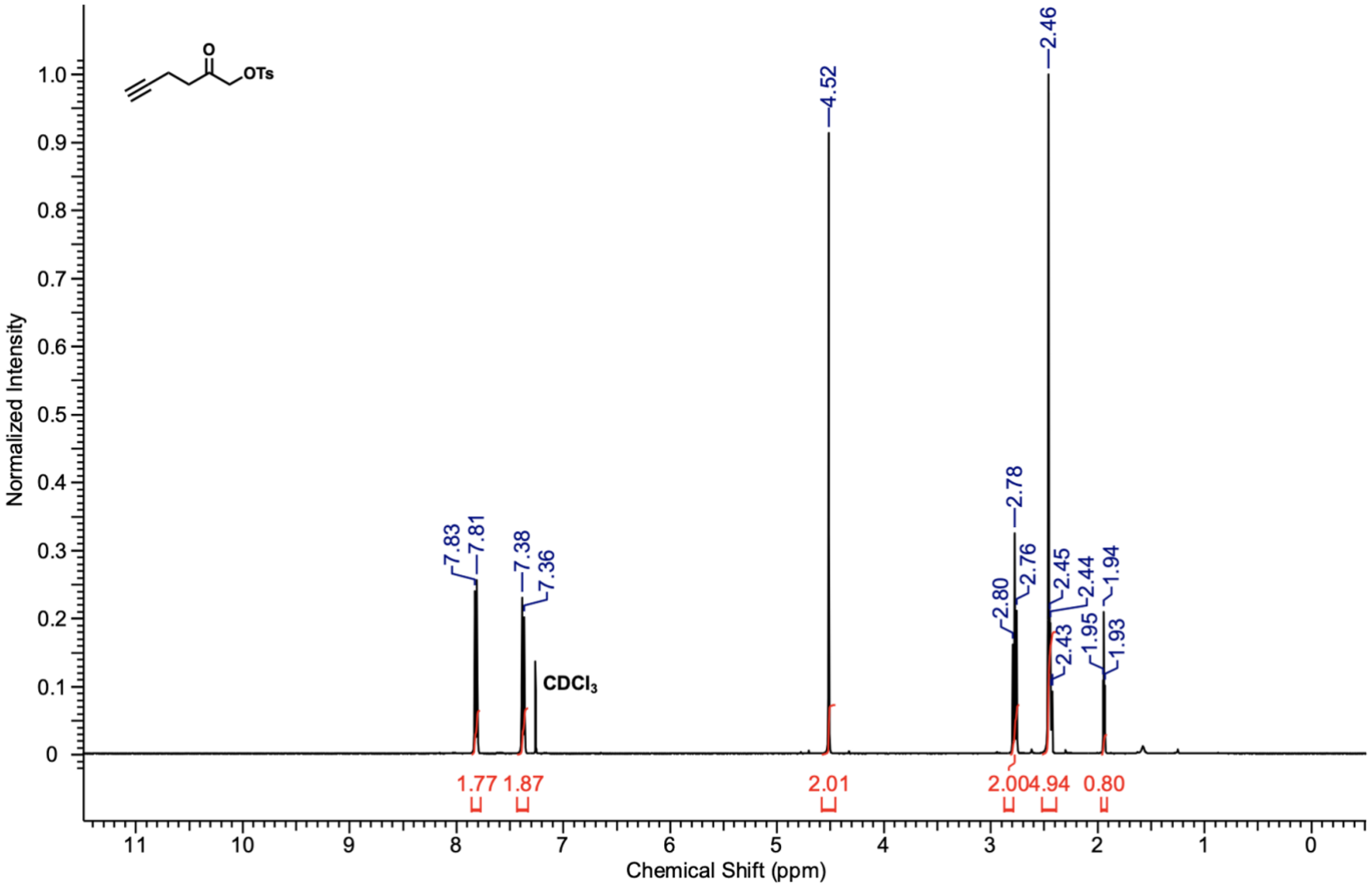
^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 1.31–1.39 (m, 3H), 1.39–1.48 (m, 3H), 1.53–1.63 (m, 5H), 1.71–1.81 (m, 3H), 2.00 (t, *J* = 2.4 Hz, 1H), 2.60 (td, *J* = 7.2 Hz, *J* = 2.4 Hz, 2H), 2.90 (t, *J* = 7.2 Hz, 2H), 3.41–3.47 (m, 4H), 3.49–3.63 (m, 10H), 3.74 (s, 2H), 4.79 (s, 2H), 6.73 (m, 1H), 7.74–7.84 (m, 3H), 8.16 (d, *J* = 7.6 Hz, 1H), 8.25 (m, 2H).

^{13}C NMR (101 MHz, $CDCl_3$) δ ppm 12.7, 25.4, 26.7, 29.4, 32.5, 37.7, 38.6, 39.5, 45.0, 69.12, 69.6, 70.0, 70.3, 71.2, 82.7, 124.4, 126.8, 127.3, 133.9, 134.3, 134.4, 134.8, 137.1, 156.7, 169.4, 182.55, 204.6.

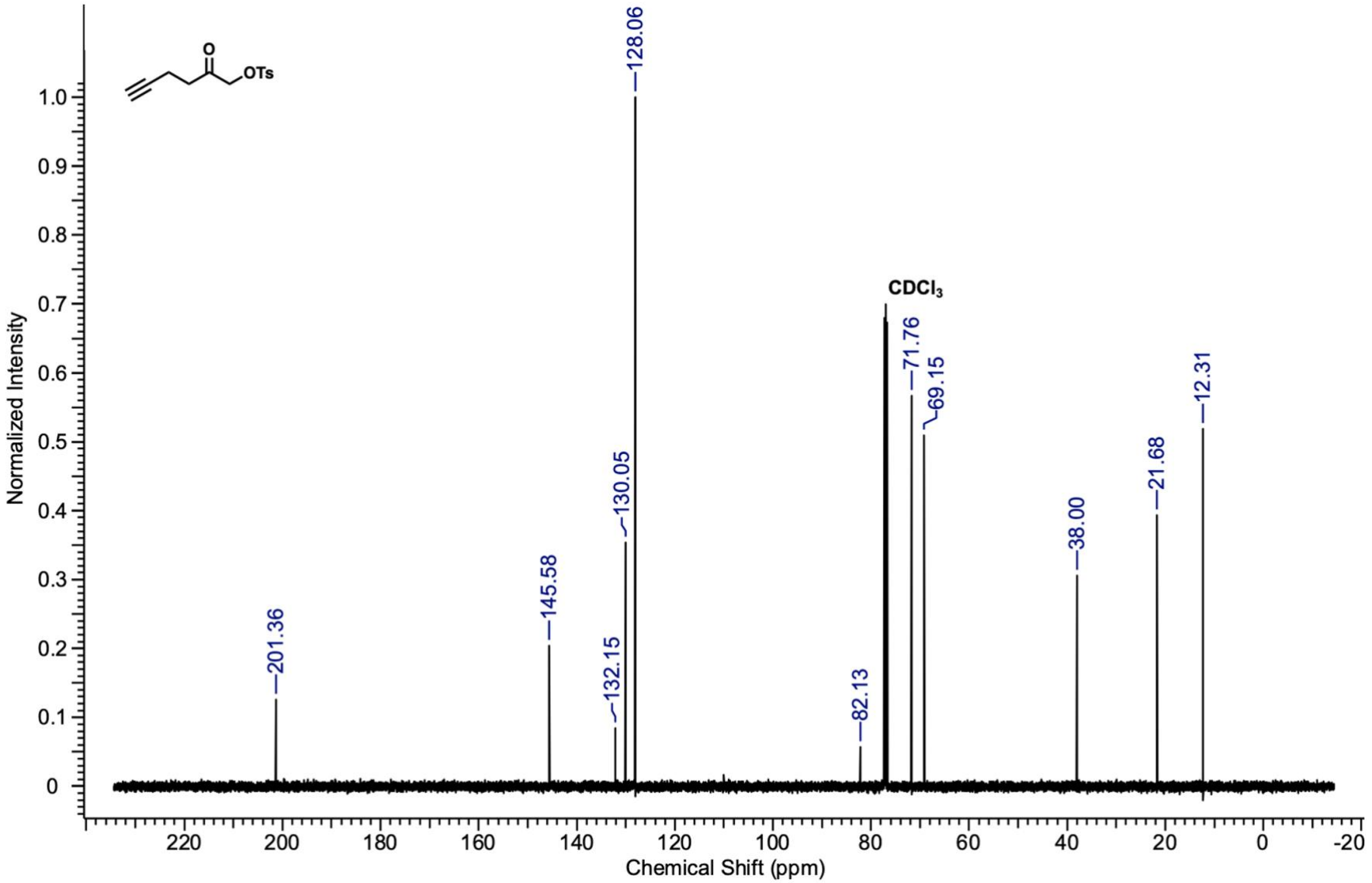
HRMS (ESI-TOF) *m/z* $[M + H]^+$ calculated for $C_{32}H_{37}NO_7Cl^+$ 582.2253, found 582.2265.

VI. NMR Spectroscopic Data of Novel Compounds.

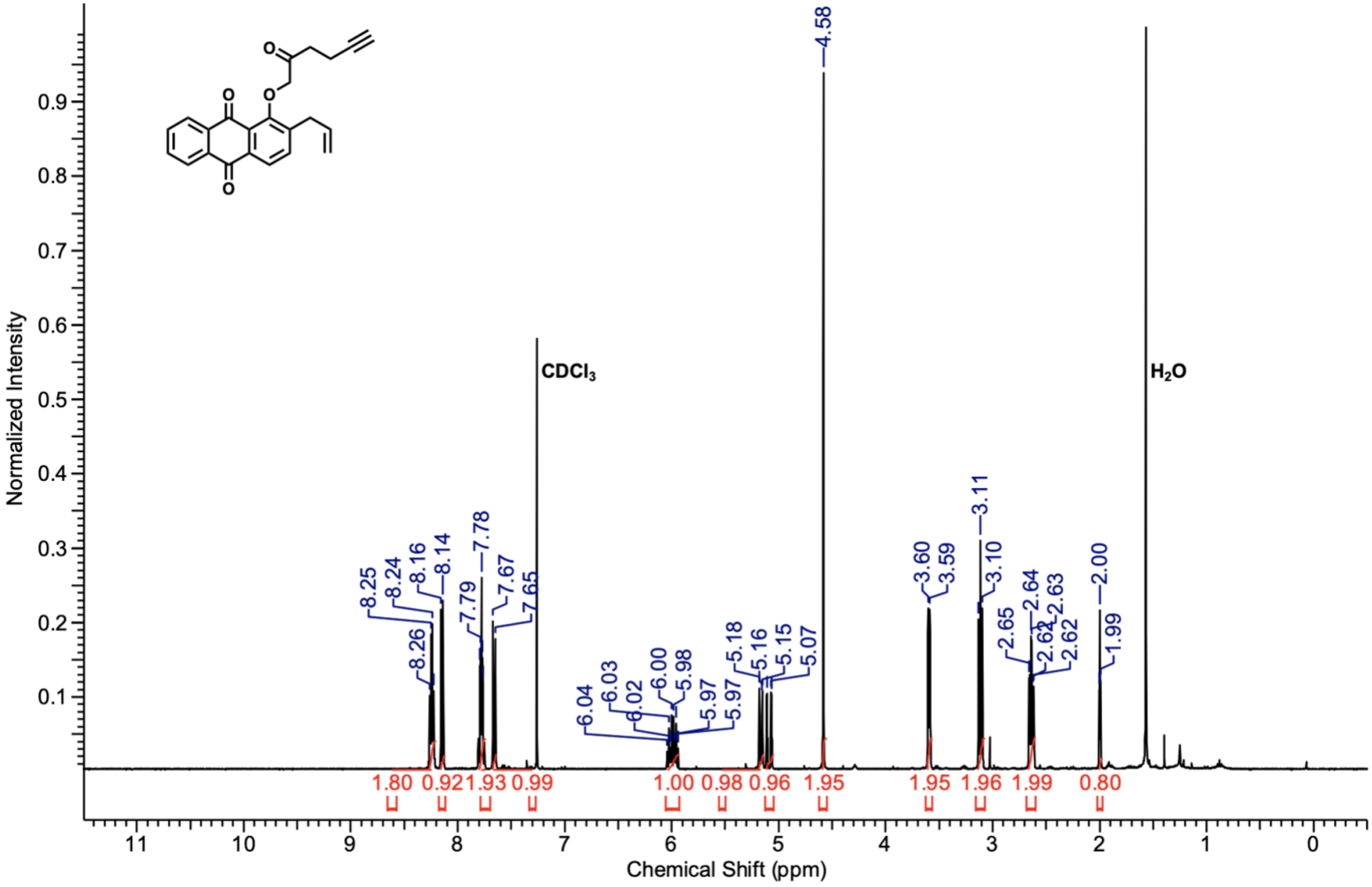
2-Oxohex-5-yn-1-yl 4-methylbenzenesulfonate (6)



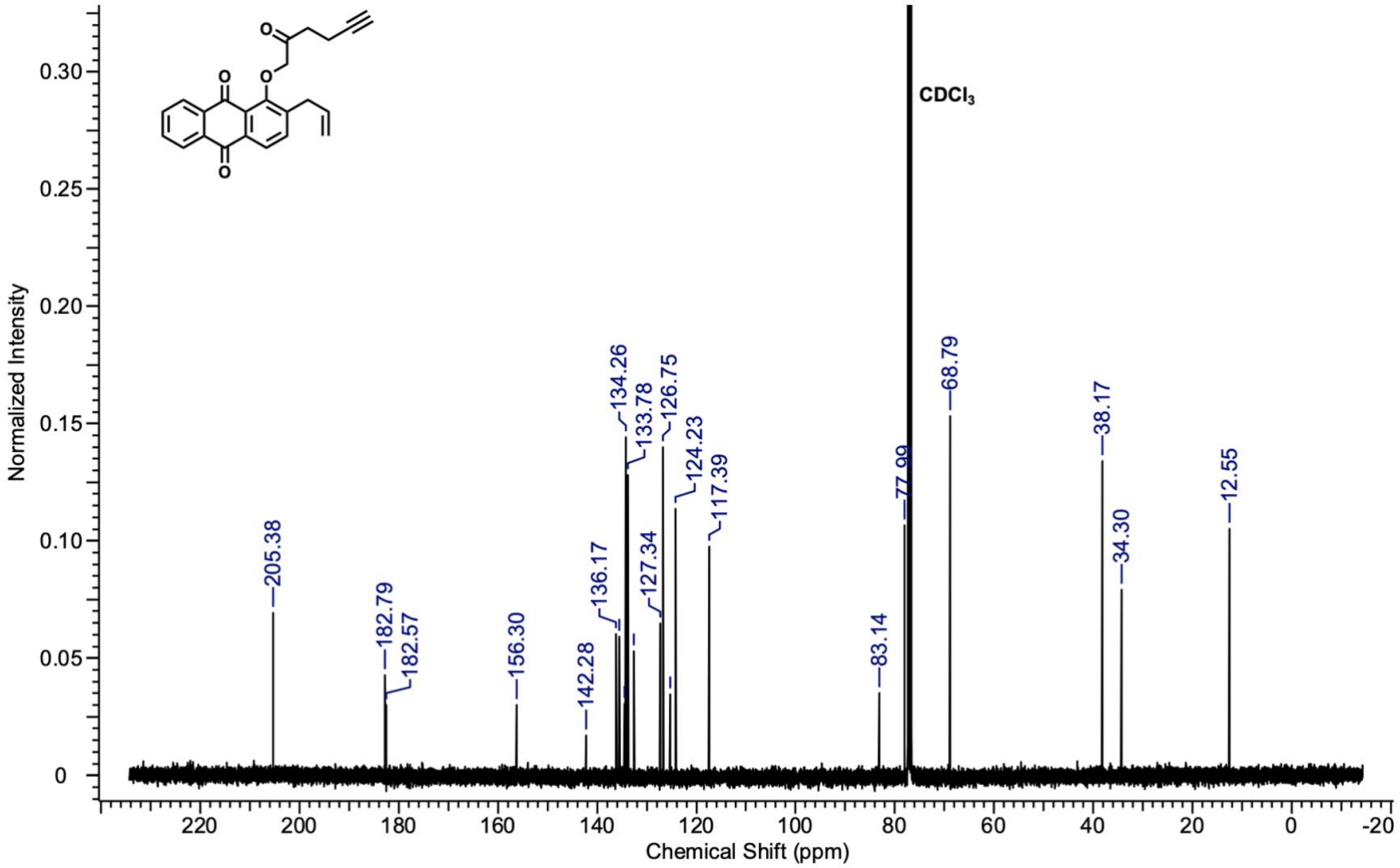
2-Oxohex-5-yn-1-yl 4-methylbenzenesulfonate (**6**)



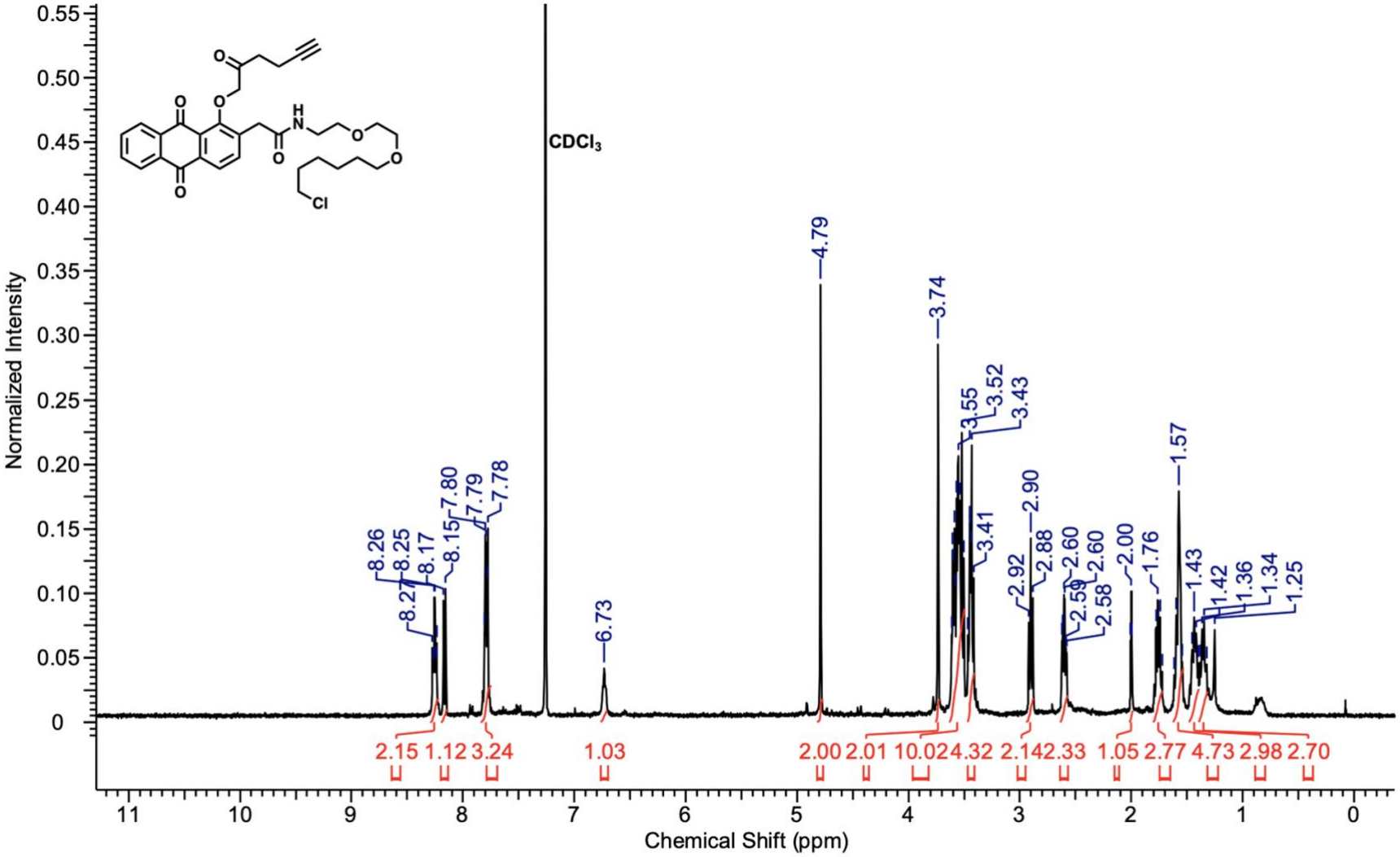
2-Allyl-1-[(2-oxohex-5-yn-1-yl)oxy]anthracene-9,10-dione (**1**, caged DiC^{yne})



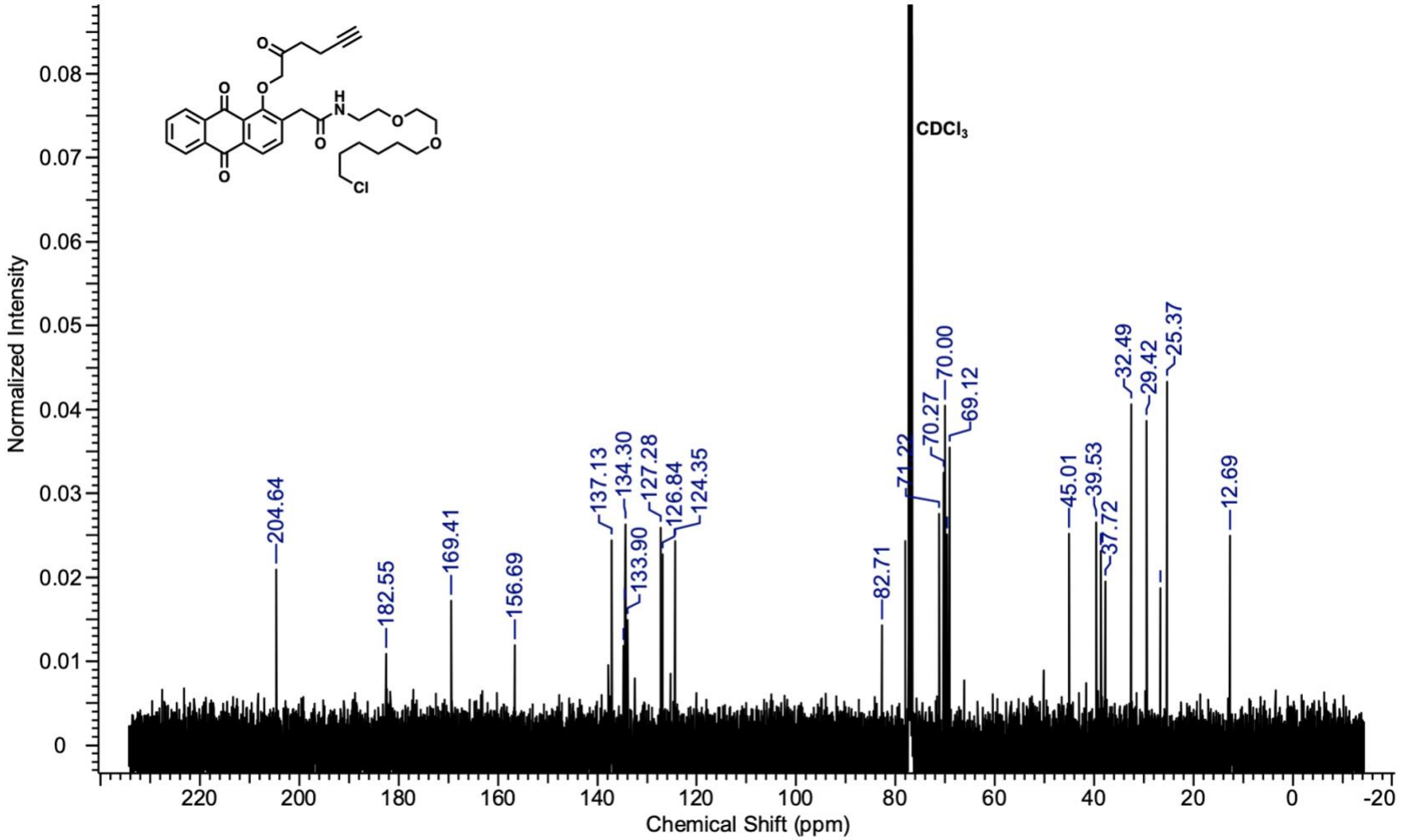
2-Allyl-1-[(2-oxohex-5-yn-1-yl)oxy]anthracene-9,10-dione (**1**, caged DiC^{yne})



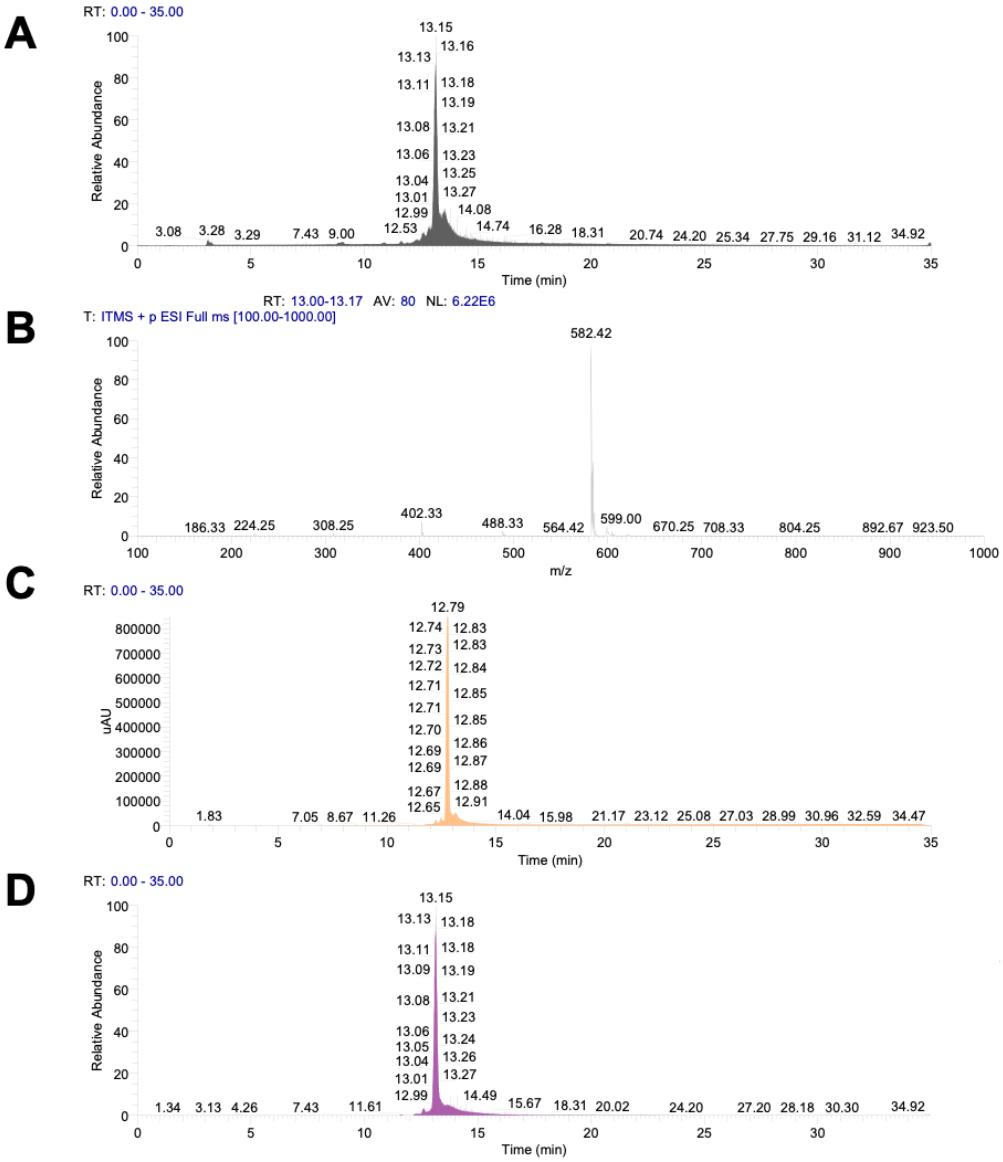
***N*-(2-(2-((6-Chlorohexyl)oxy)ethoxy)ethyl)-2-(9,10-dioxo-1-((2-oxohex-5-yn-1-yl)oxy)-9,10-dihydroanthracen-2-yl)acetamide** (**8**, T-DiP)



***N*-(2-(2-((6-Chlorohexyl)oxy)ethoxy)ethyl)-2-(9,10-dioxo-1-((2-oxohex-5-yn-1-yl)oxy)-9,10-dihydroanthracen-2-yl)acetamide** (**8**, T-DiP)

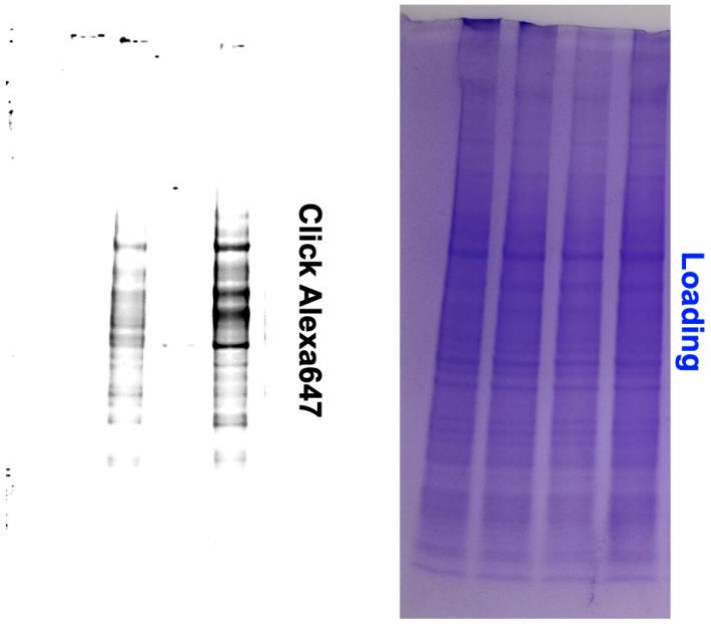


VII. LC-MS Analysis of T-DiP.

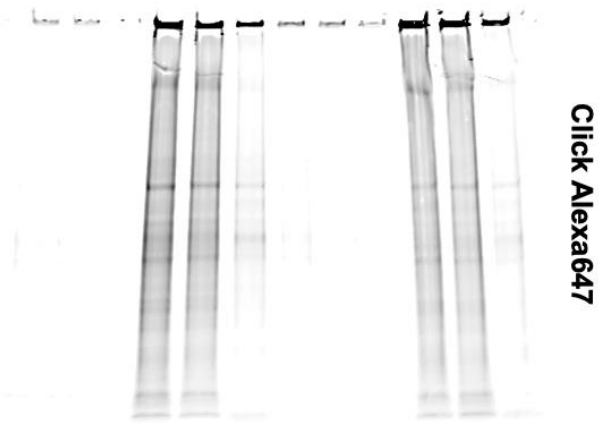


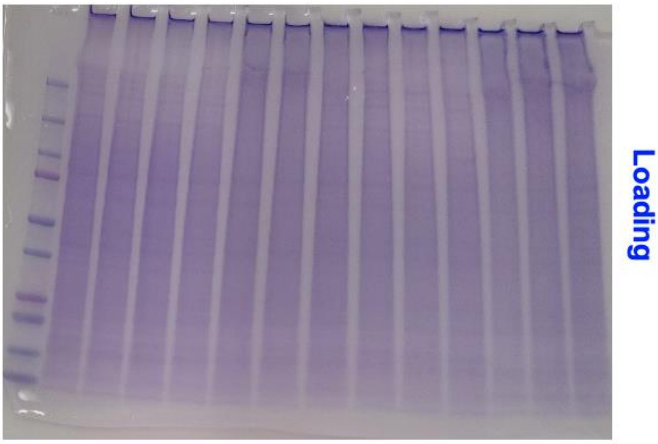
LC-MS analysis of T-DiP **8**. Chromatograms of T-DiP **8** separated over a Zorbax 150 × 0.5 mm 5 μm C18 column with a flow rate of 300 μL/min in line with a Thermo LTQ XL linear ion trap mass spectrometer. The LC used gradient was the same as the preparative method described above. A) Total ion count (TIC) chromatogram. B) Full spectral scan at 13.15 minutes. C) UV chromatogram monitored at 254 nm. Integration of this peak reveals T-DiP to be > 95% pure. D) Extracted ion chromatogram over the mass range 581.9 – 582.9.

VIII. Uncropped Gels.

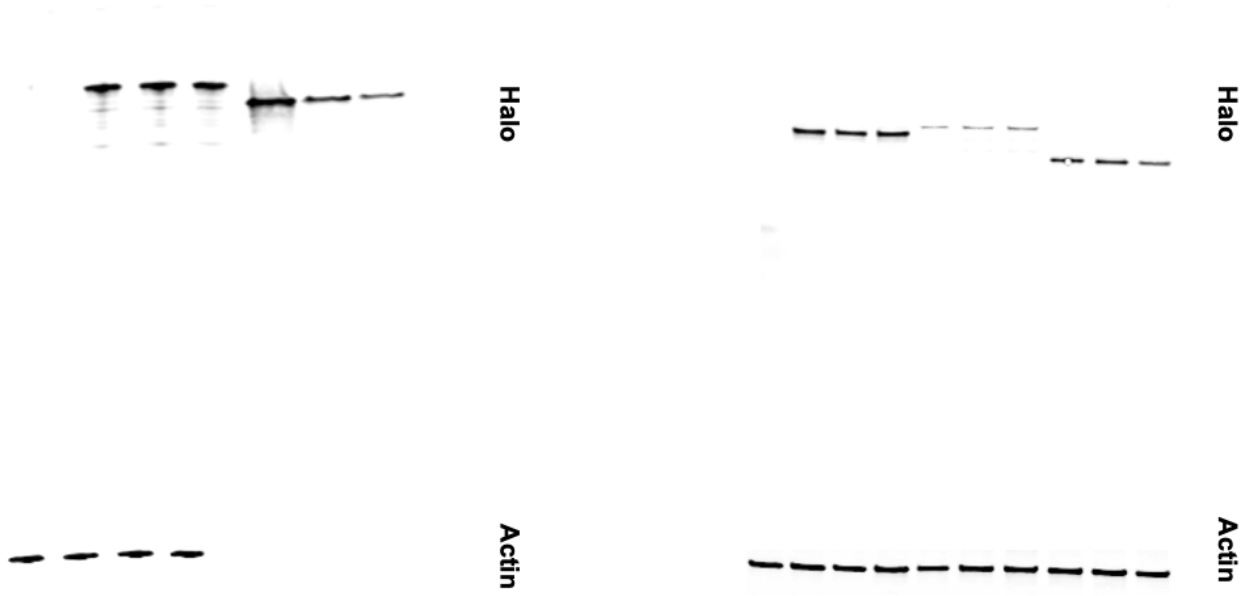


Uncropped Gels from **Fig 3.**

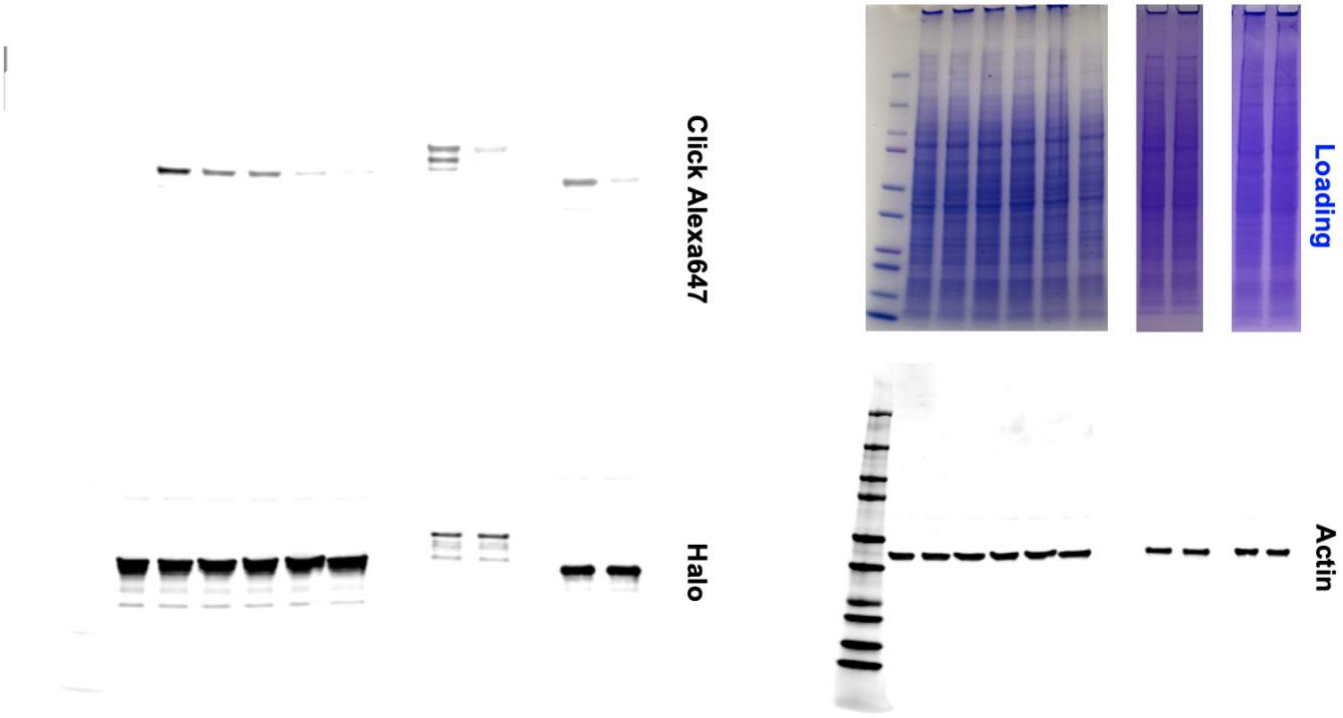




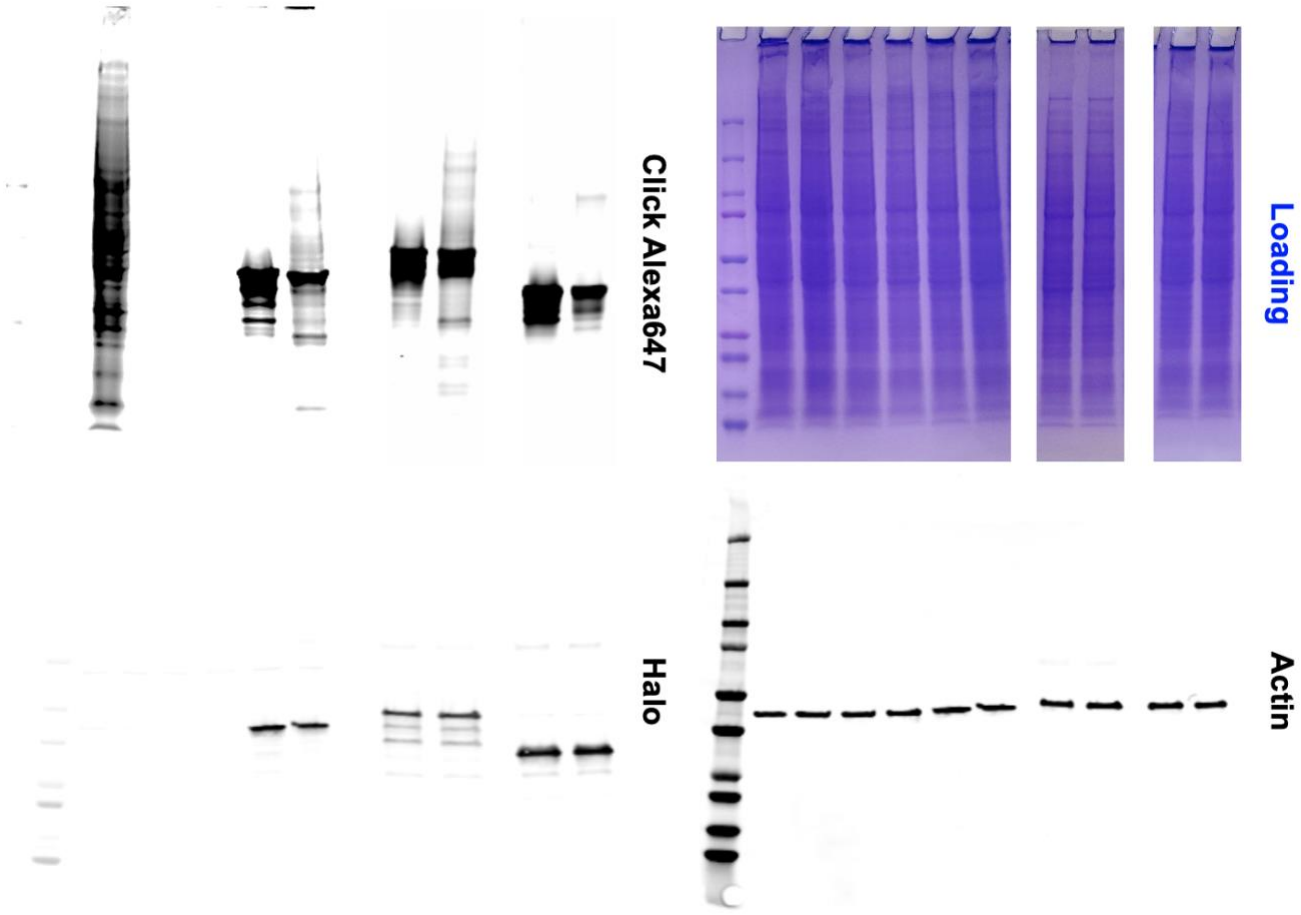
Uncropped Gels from **Fig S2.**



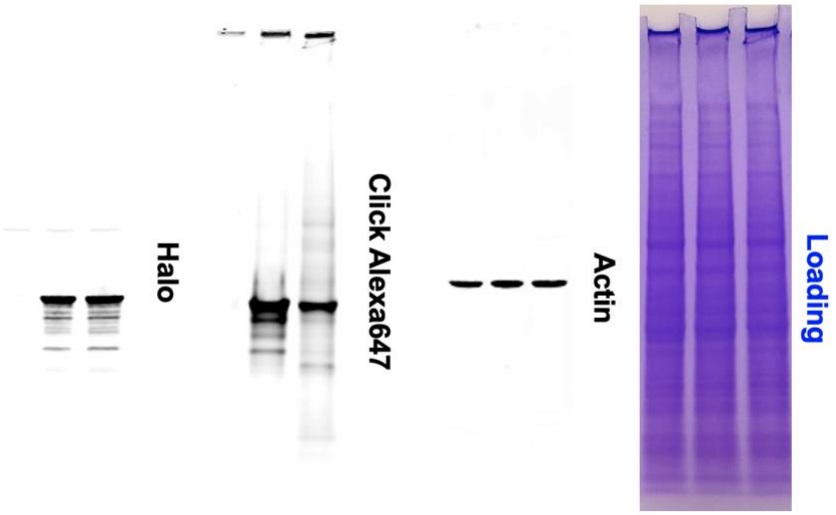
Uncropped Blots from **Fig S3.**



Uncropped Gels and Blots from **Fig S5.**



Uncropped Gels and Blots from **Fig 6.**



Uncropped Gels and Blots from **Fig S7.**

IX. References.

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