SUPPORTING INFORMATION

A Generalizable Platform for Interrogating Target- and Signal-Specific Consequences of Electrophilic Modifications in Redox-Dependent Cell Signaling

Hong-Yu Lin[†], Joseph A. Haegele[†], Michael T. Disare[†], Qishan Lin[¶], and Yimon Aye[†,‡]*

[†]Department of Chemistry and Chemical Biology, Cornell University, Ithaca, New York 14850, USA.

[‡]Department of Biochemistry, Weill Cornell Medical College, New York 10065, USA.

[†]Proteomics/Mass Spectrometry Facility, Center for Functional Genomics, University of Albany, Rensselaer, New York, 12144, USA

CONTENTS

Supporting Text

General materials and methods for **biochemical protocols** SI-3
Analysis of the rate of photoactivatable electrophile release SI-5
Analysis of the time-dependent electrophilic adduction of recombinant His_6-Keap1 SI-5
Cell culture and transient transfection SI-6
Generation of stably transfected cells SI-7
Cell lysis and western blotting SI-7
T-REX targeting in live cells SI-7
Recombinant expression and isolation of human His_6- and His_6-Halo–Keap1 in *E. coli* SI-8
Cell viability analysis SI-9
ARE-luciferase assays SI-10
LC-MS/MS determination of modified sites
With recombinant Keap1 *in vitro* SI-10
With Keap1 pulled down from live cells SI-12
Pulldown procedure for CPE **10**-modified Keap1 from intact cells SI-15
Estimating the extent of Keap1 modification under whole-cell CHE **9** flooding SI-16
General materials and methods for **synthesis protocols** SI-17
Syntheses of Compounds **1–18** (Scheme S1–S10) SI-18
^{1}H- and ^{13}C-NMR spectroscopic characterization data of Compounds **1–18** SI-43

Supporting Tables

Table S1. Release efficiency of reactive electrophiles SI-66
Table S2. Primers for constructing pET28a_His_6-(Halo)–Keap1 plasmids SI-67
Table S3. Primers for site-directed mutagenesis SI-69
Table S4. Residue-specific modifications from global CHE treatment *in vitro* SI-70
Table S5. Residue-specific modifications from T-REX CHE treatment *in vitro* SI-72
Table S6. Residue-specific modifications from global CHE treatment in cells SI-73
Table S7. Residue-specific modifications from T-REX CHE treatment in cells SI-75
Table S8. Proposed structures for the CHE-derived modifications SI-77
Table S9. Summary of antibodies SI-78

Supporting Figures

Figure S1. Release efficiency by time-dependent Cy5 signal depletion from Halo SI-79
Figure S2. T-REX delivery efficiency in cells SI-80
Figure S3. T-REX on the non-fused Halo and Keap1 proteins: DE delivery SI-81
Figure S4. T-REX on the non-fused Halo and Keap1 proteins: HNE delivery SI-82
Figure S5. Nrf2 stabilization readout: T-REX vs. whole-cell LDE treatment SI-83
Figure S6. Dose-dependent ARE upregulation by whole-cell LDE treatment SI-84
Figure S7. Dose-dependent cytotoxicity induced by whole-cell LDE treatment SI-85
Figure S8. T-REX on the non-fused Halo and Keap1 proteins: Nrf2 stabilization SI-86
Figure S9. CPE **10** modification of Keap1 subsequent to whole-cell flooding SI-87
Figure S10. CPE **10**-induced response as a function of time: T-REX vs. global SI-88
Figure S11. Functional redundancy of Keap1 Cys's in CHE **9** response SI-89
Figure S12. ARE response in native vs. cells overexpressing Keap1 and Nrf2 SI-90
Figure S13. Viability analysis on caged precursors constituting T-REX toolbox SI-91

Appendix: ^{1}H-NMR spectra of intermediate products SI-92

References SI-100

SUPPORTING TEXT

General materials and methods for biochemical protocols.

All reagents were from Sigma unless otherwise noted. Cy5-azide was from lumiprobe (B3030). All primers were from IDT. Fusion HotStartII polymerase was from Thermo and all the restriction enzymes were from NEB. phrGFP-Keap1 mammalian expression plasmid (28025) and pRK793 TEV protease (S219V mutant) bacterial expression plasmid (8827) were from Addgene. The latter was a kind gift of Dr. Jeffrey Boucher, Brandeis University, MA. pET28a empty vector and pET28a_His_6_Halo-HA plasmid[1] were kindly provided by Professor Lizbeth Hedstrom, Brandeis University, MA. pMIR-CMV_His_6_RNRα plasmid[2] was a gift from Professor JoAnne Stubbe's laboratory at MIT, MA. HEK-293 cells were from American Type Culture Collection (ATCC). 1X PBS (Dulbecco's phosphate-buffered saline), 1X TrypLE™ Express (stable trypsin-like enzyme with phenol red), 1X DMEM, AlamarBlue® cell viability reagent (DAL1100), 10mg/mL puromycin, and 100X penicillin-streptomycin were from Invitrogen. Fetal bovine serum (FBS) (100 nm-triple filtered, SH30071.03) was from Hyclone. Serum-compatible broad-spectrum transfection reagent TransIT-2020 was from Mirus Bio LLC. Polyethylenimine (PEI) was from Polyscience Inc. (23966). Antibodies used were listed in Table S9. Olympus CKX31 microscope system was used for light microscopy. In gel fluorescence analysis and imaging of the Coomassie-stained gels were performed on Bio-Rad ChemiDoc-MP imaging system. Densitometric quantitation was made by Bio-Rad Image Lab software (v 4.1). Cy5 excitation source was red epi illumination and emission filter used was 695/55 filter. Light exposure experiments were performed with the use of a hand-held UV lamp (Fisher, S45157, 365 nm, longwave 4 W tube at 0.6 mW/cm^2). His_6-Tev-S219V and His_6-Halo-HA were recombinantly expressed and purified from *E. coli* using TALON affinity chromatography (clontech). Curve fitting and data analysis were performed using GraphPad Prism (v 6.0). Luciferase reporter assay data

were collected on a BioTek™ Cytation™ 3 cell imaging multi-mode microplate reader. Mass spectrometry analyses for characterizations of the modified sites in Keap1 protein ectopically expressed in cells were performed at the Proteomics/Mass Spectrometry Facility located at the Center for Functional Genomics, University at Albany, supported by the SUNY Research Foundation. DNA sequencing and mass spectrometry analyses for characterizations of the modified sites in the recombinant human Keap1 protein in solution were performed at the Cornell University Institute of Biotechnology, Biotechnology Resource Center Genomics Facility and Proteomics and Mass Spectrometry Facility, respectively.

Analysis of the rate of photoactivatable electrophile release.

The procedure was performed in dark. The reaction mixture (13 μL) containing, in final concentrations, 1.1 μM of His_6-Halo-HA (35 μg/mL), 2.2 μM of designated HaloTag-targetable caged electrophile, and 15% v/v DMSO, was incubated at 37 °C for 20 min, prior to photoactivation (365 nm at 0.6 mW/cm^2) over an indicated time period at which point the reaction was subject to a Click coupling mixture. Briefly, in a final volume of 17 μL, the reaction mixture contained, 32 mM Hepes (pH 7.6), 1.9 mM TCEP, 5% v/v *t*BuOH, 1 mM $CuSO_4$, 0.1 mM TBTA, 20 mM Cy5azide (Lumiprobe) and the His_6-Halo-HA reaction solution above. Subsequent to 30 min incubation at 37 °C, the reaction was quenched with 5 mL of 4X Laemmli buffer that contained 6% BME and further incubated for 5 min at 37 °C. 20 μL was directly loaded into each well of 10% polyacrylamide gel. Upon completion of gel-electrophoresis, the gel was rinsed with ddH_2O (x2, 5 min) and analyzed for Cy5 signal on a Bio-Rad ChemiDoc-MP and subsequently Coomassie-stained.

Analysis of the time-dependent electrophilic adduction of recombinant His_6-Keap1.

The reaction mixture in a final volume of 225 μL contained, in final concentrations, His_6-Keap1 (1.0 μM), 0.89 mM NaCl, 89 μM DTT, 0.045% glycerol and 45 μM Tris in 50 mM Hepes (pH 7.6). (Note: NaCl, DTT, glycerol and Tris were carried over from Keap1 storage buffer). The mixture was incubated at 37 °C for 5 min and treated with 1.1 equiv of HNE(alkyne) **1**, CHE(alkyne) **9**, or dHNE(alkyne) **3**. One-shot aliquots of small-molecule stocks were prepared as 244 μM DMSO solutions such that final concentration of DMSO in the reaction is 0.4% v/v. At indicated time points, 25 μL aliquots were removed from the reaction mixture and quenched with freshly prepared 100 mM $NaBH_4$ stock solution [2.5 μL, prepared by dissolution of 3.4 mg of $NaBH_4$ powder in 1.0 mL of 50 mM Hepes (pH 7.6)

buffer]. The mixture was incubated at 37 °C for 15 min, centrifuged to remove bubbles and subsequently subject to Click coupling mixture. Briefly, in a final volume of 33 μL, the reaction mixture contained, in final concentrations, 42 mM Hepes (pH 7.6), 1.7 mM TCEP, 5% v/v *t*BuOH, 1% wt/v SDS, 1 mM $CuSO_4$, 0.1 mM TBTA, 10 mM Cy5-azide and the treated His_6-Keap1 solution above. Subsequent to 30 min incubation at 37 °C, the reaction was quenched with 5 mL of 4X Laemmli buffer that contained 6% BME and further incubated for 5 min at 37 °C. 20 μL was directly loaded into each well of 10% polyacrylamide gel. Upon completion of gel-electrophoresis, the gel was rinsed with ddH_2O (x2, 5 min) and analyzed for Cy5 signal on a Bio-Rad ChemiDoc-MP and subsequently Coomassie-stained.

Cell culture and transient transfection.

HEK-293 cells were cultured in DMEM-Glutamax (Invitrogen 41090-036) supplemented with 10% v/v FBS (100 nm-triple filtered, Hyclone SH30071.03), 5% NEAA (Invitrogen A11140-050) and 5% pyruvate (Invitrogen 11360-070) in the presence of 1X penicillin-streptomycin antibiotics (Invitrogen 15140-122). HEK-293 cells stably expressing His_6-Halo-Keap1 were cultured as in HEK-293 cells except that 1.5 μg/mL puromycin (Invitrogen A11138-03) was included. Cells were cultivated in adherent culture plates (Corning) in a humidified atmosphere of 5% CO_2 at 37 ºC and harvested by trypsinization (Invitrogen 25300-054). Transient transfection was performed in most cases at 50–60% confluency using Mirus TransIT-2020 (Mirus MIR5400) according to the manufacturer's protocol. In cases in which polyethylenimine (PEI) (Polyscience Inc, 23966) transfection was performed (e.g., Figure 7), the cells were allowed to reach a higher cell density (60–70% confluency) at the point of transfection. Briefly, 6 μL of 1 mg/mL PEI stock solution [prepared and stored as frozen aliquots in sterile filtered water (pH 7.4)] was mixed with 1.5 μg of plasmid DNA in

150 μL of serum-free media. Subsequent to 15 min-incubation period, the resulting mixture was diluted into complete cell growth media (1.5 mL). ~60–70% confluent monolayered cells in an 8 cm^2 adherent culture dish were subsequently treated with the resulting solution. Experiments were performed 24-h post transfection.

Generation of stably transfected cells.

HEK-293 cells were transfected with pMIR_DsRed-IRES-His_6-Halo-Keap1 plasmid[4] according to the Mirus protocol. TEV-protease cleavage site was encoded in the linker region between the Halo and Keap1 domains. Upon reaching full confluence, the cells were incubated with fresh media containing puromycin at 2 μg/mL, and growth was continued changing media every 4th day. Upon regaining full confluence (over ~1–2 wk period), the cells were transferred into a small flask (e.g., 25 cm^2) and cultivation was continued at the same concentration of puromycin. FACS analysis was performed subsequently to determine transfection efficiency.

Cell lysis and western blotting.

Whole cell lysates was prepared by three cycles of rapid freeze-thaw in a chilled freshly prepared lysis buffer containing 50 mM Hepes buffer (pH 7.6), 1% Nonidet P-40, and 0.3 mM TCEP. Cell extract was clarified by centrifugation at 16,000 x g for 8 min at 4 °C. Total protein concentration in lysate was determined using Bradford Assay. See Table S9 for dilutions and sources of antibodies.

T-REX targeting in live cells.

Growth media were changed to complete media that do not contain puromycin 24 h prior to treatment with HaloTag-targetable caged precursors. All steps hereafter were handled in the

dark. Cells were treated with the designated HaloTag-targetable caged precursor at a final concentration of 25 μM for 2.5 h in serum-free media. Rinsing (x3) was performed every 30 min over 1.5 h with the serum-free media. For the samples designated for light exposure, plate covers were removed and mono-layered adherent cultures were exposed to 365 nm, 0.6 mW/cm^2 lamp for 20 min at room temperature (at ~1 inch distance from the light source) and re-incubated at 37 °C for a further 5 min prior to harvest. Subsequent to centrifugation (500 x g, 8 min) and washing with 1X PBS (x2), the resultant cell pellets were flash-frozen in liq N_2 and subjected to 3 cycles of freeze-thaw in 15 μL lysis buffer that contained in final concentrations, 50 mM Hepes (pH 7.6), 0.3 mM TCEP and 1% Nonidet. All steps hereafter were performed at 4 °C. Debris was removed by centrifugation (18,000 x g, 8 min). A portion of the clarified lysate was made up to final volume of 25 μL containing, in final concentrations, 50 mM Hepes (pH 7.6), 1.0 mg/mL lysate protein (measured by Bradford assay), and 0.2 mg/mL His_6-Tev-S219V. The mixture was incubated at 37 °C for 45 min, and subsequently subjected to Click reaction. Briefly, in a final volume of 30 μL, the reaction mixture contained, in final concentrations, 42 mM Hepes (pH 7.6), 1.7 mM TCEP, 5% v/v *t*BuOH, 1% wt/v SDS, 1 mM $CuSO_4$, 0.1 mM TBTA, 10 μM Cy5 azide and Tev-protease-treated lysate above. Subsequent to 30-min incubation at 37 °C, the reaction was quenched with 5 μL of 4X Laemmli buffer that contained 6% BME and further incubated for 5 min at 37 °C. 20 μL was directly loaded into each well of 10% polyacrylamide gel. Upon completion of the gel-electrophoresis, the gel was rinsed with ddH_2O (x2, 5 min) and analyzed for Cy5 signal on a Bio-Rad ChemiDoc-MP and, where applicable, the gel was subsequently transferred to a PVDF membrane for western blot analysis.

Recombinant expression and isolation of human His_6- or His_6-Halo–Keap1 in *E. coli*. Recombinant mouse Keap1 was previously characterized to be Zinc-containing

metalloprotein.[2] Expression of human His_6- or His_6-Halo–Keap1 was thus carried out in M9 media supplemented with Zinc (12.8 g/L of $Na_2HPO_4 \cdot 7H_2O$, 3 g/L of KH_2PO_4, 0.5 g/L of NaCl, 1.0 g/L NH_4Cl, 2mM $MgSO_4$, 100 μM $CaCl_2$ 50 μM $ZnCl_2$, and 0.4% of Glucose). Overnight cultures were inoculated with 50 μg/mL of CM and 50 μg/mL KAN. Subsequent to dilution into media containing 50 μg/mL KAN, expression was induced with 250 μM IPTG at OD_{600} of 0.6–0.8 over 14 h at 19 °C. The protein was isolated by using TALON chromatography (Clontech) followed by gel filtration. The buffer conditions were 50 mM NaH_2PO_4 (pH 8.0), 10 mM Imidazole, 5 mM BME, 0.5 mM PMSF and 0.01% Triton X-100 for cell lysis, 50 mM NaH_2PO_4 (pH 8.0), 50 mM Imidazole, 500 mM NaCl, 5 mM BME and 0.01% Triton X-100 for washing x 3.5 bed volume, and 50 mM NaH_2PO_4 (pH 8.0), 125 mM Imidazole, 150 mM NaCl and 5 mM BME for elution, and 50 mM Tris (pH 8.0), 10 mM DTT, 100 mM NaCl and 5% glycerol for desalting by gel filtration (ÄKTA purification system, GE Healthcare Hiload™ 26/60 Superdex™ 200 prep grade, column ID No. 0823027) and for storage. One-shot aliquots of the purified proteins were stored in –80 °C. Typical yields were 0.20 and 0.24 mg per g of cell pellet for His_6-Keap1 and His_6-Halo-Keap1, respectively.

Cell viability analysis.

The trypan blue dye exclusion test was performed after harvesting the cells in 1X PBS buffer. Cells pelleted by centrifugation at 500 x g for 5 min were suspended in 0.2 micron-filtered trypan blue and 1X PBS (1:1) for 3 min at room temperature. Cells were then plated on a hemacytometer and counted. Colorless cells were considered viable. The AlamarBlue® cell viability analysis was performed according to the manufacturer's protocol.

ARE-luciferase assays.

Unless otherwise specified, Cignal Antioxidant Response Reporter (luc) Kit (CCS-5020L) was from Qiagen and used according to the manufacturer's protocol. In specific cases as indicated in the figure legends, a mixture of luciferase plasmids (pGL4.37, E364A from Promega and pGL4.75, E693A from Promega, used as a 20:1 pGL4.37/pGL4.75 mixture) was used instead of the plasmid mixture in the commercially available Qiagen kit. Following 18 h of treatment, cells were lysed with lysis buffer (Promega). Transcription activity was determined by the expression of firefly luciferase and was normalized to the Renilla luciferase levels by using a dual luciferase reporter assay kit (Promega). Data collection was done on a BioTek cytation3 multi-mode microplate reader.

LC-MS/MS determination of modified sites in vitro and in live cells.

All steps were handled under dim light.

Procedure for the analysis of recombinant Keap1 protein in vitro (Table S4-S5).

Recombinant His_6-Halo-Keap1 (3.0 μM) was incubated with either HaloTag-targetable caged precursors or reactive electrophile (1.1 equiv) (3.3 μM) in a final volume of 50 μL in 50 mM Hepes (pH 7.6) at 37 °C for 20 min, at which point the T-REX samples were directly exposed to 365 nm, 0.6 mW/cm^2 hand-held lamp for 20 min at 37 °C. The non-targetable samples were subjected to identical conditions except light exposure. (The light source was placed at ~0.8 inch distance from the surface of the solution). The lamp was then removed and all samples were further incubated for 20 min at 37 °C. The samples were treated with TEV protease (10 μM or 0.3 mg/mL) in a final reaction volume of 54 μL at 37 °C. Half (27 μL) of the volume in each sample was removed and analyzed by in-gel fluorescence assays as described above to confirm Keap1 modification in all cases. The remaining half (~27 μL) in each sample was further incubated for 60 min at 37 °C prior to mixing with 5.0 μL 4X

Laemmli dye (6% BME) and further incubation for 5 min at 37 °C. 30 μL was directly loaded into each well of 10% polyacrylamide gel. Upon completion of gel-electrophoresis through a Tris-Cl gel, the protein bands were visualized with Coomassie Brilliant Blue R-250 subsequent to de-staining over 24 hours. The gel was rinsed with ddH_2O for 30 min and the bands representing TEV-cleaved-Keap1 were excised. After washing and dehydrating the gel pieces, the proteins were reduced with 10 mM DTT in 100 mM NH_4HCO_3 solution for 45 min at 56 °C and alkylated with 55 mM iodoacetamide in 100 mM NH_4HCO_3 in dark for 45 min. The gel pieces were rehydrated in 6 μg/mL solution of trypsin in 50 mM NH_4HCO_3 (pH 7.8) at 30 °C overnight. Peptides were extracted sequentially with 1% formic acid, 50% acetonitrile containing 5% formic acid and 90% acetonitrile containing 5% formic acid. All supernatants were combined and dried under vacuum. LC-MS/MS analysis and data processing were performed at the Proteomics and Mass Spectrometry, Cornell University Institute of Biotechnology (Table S4–S5). Briefly, peptides were resuspended in 0.5% v/v formic acid and separated on an Ultimate 3000 (Thermo/Dionex, Inc.) nano-liquid-chromatography (LC) system coupled to a 4000 QTRAP (Applied Biosystems). Peptides were desalted onto a PepMax C18 trap column (300 μm x 5mm, Dionex, Inc.) with 98:2 H_2O:ACN (containing 0.1% v/v formic acid) at 20 μL/min. After a 3-min wash, peptides were separated on a PepMax nano-column (75 μm ×150 mm, Dionex, Inc.) using a 90-min linear gradient of 8% to 40% ACN in 0.1% formic acid at 300 nL/min. MS data acquisition was performed using Analyst 1.4.2 software (ABSciex) in positive ion mode for information dependent acquisition (IDA) analysis. The nanospray voltage was 1.85 kV used for all experiments in positive ion mode. Nitrogen was used as the curtain (value of 10) and collision gas (set to high) with heated interface at 150°C. The declustering potential was set at 50 eV and Gas1 was 20 (arbitrary unit). In IDA analysis, after each survey scan for *m/z* 375 to *m/z* 1550 and an enhanced resolution scan, the three highest intensity ions with multiple

charge states were selected for tandem MS (MS/MS) with rolling collision energy applied for detected ions based on different charge states and m/z values. The MS and MS/MS data were then used by the Mascot search engine to search the human RefSeq downloaded from NCBInr for identification of peptides and their corresponding modifications with two missed cleavage sites by trypsin allowed. The peptide tolerance was set to 1.5 Da and MS/MS tolerance was set to 0.6 Da. For instance, in the analysis of CHE(alkyne) modifications, several variable modifications were applied including methionine oxidation, cysteine carbamidomethylation along with CHE(alkyne) Michael adduct (134.07), reduced CHE(alkyne) Michael adduct (136.09), dehydrated CHE(alkyne) Michael adduct (118.08), or dehydrated CHE(alkyne) (1,2)-addition adduct or CHE(alkyne) Michael adduct in Schiff-based form (116.06) on cysteine residues (Table S8). Only significant scores for the peptides defined by Mascot probability analysis (www.matrixscience.com/help/scoring_help.html#PBM) greater than "identity" with 95% confidence were considered for the peptide identification and modification site determinations. All MS/MS spectra for the identified peptides with CHE(alkyne)-type modifications were manually inspected and validated.

Procedure for the analysis of Keap1 protein pulled down from live cells (Table S6–S7).

T-REX-targeted or global CHEylation was performed in HEK-293 cells according to the protocol described elsewhere in the SI.

For enrichment protocol under native conditions: the confluent monolayer cultures of HEK-293 cells were harvested from 4 x 21 cm^2 cultured plates, flash-frozen in liq. N_2 and subjected to 3 cycles of freeze-thaw in 100 μL lysis buffer A (50 mM Hepes, 5 mM Imidazole, pH 7.6). Debris was removed by centrifugation (18,000 x g, 8 min). The supernatant was recovered, diluted to 1.0 mg/mL with lysis buffer A, and added to 20 μL bed

volume of TALON resin (Clonetech®). The suspension was incubated in the dark at 4 °C for 1.5 h with end-over-end rotation. The resin was then washed with 360 μL wash buffer B (50 mM Hepes, 100 mM NaCl, 10 mM Imidazole, pH 7.6) and 240 μL wash buffer C (50 mM Hepes, 100 mM NaCl, 20 mM Imidazole, pH 7.6). The proteins that remained bound to the resin were eluted in 25 μL elution buffer (50 mM Hepes, 100 mM NaCl, 200 mM Imidazole, pH 7.6). The eluate obtained under native condition was then mixed with 4X reducing laemmli dye and electrophorezed through a 10% SDS-PAGE gel. The protein was visualized with Coomassie blue R-250. The gel was rinsed with ddH_2O for 30 min and the bands representing His_6-Halo-Keap1 were excised.

Sample preparation for proteomics analysis: after washing and dehydrating the gel pieces, the proteins were reduced with 5 mM TCEP in 50 mM NH_4HCO_3 solution for 45 min at 37 °C and alkylated with 20 mM iodoacetamide in 50 mM NH_4HCO_3 in dark for 45 min. After washing and drying, the gel pieces were rehydrated in 60 μL (10 μg/mL solution) of trypsin in 50 mM NH_4HCO_3 (pH 8.0) on ice for 30 min and at 37 °C overnight. Peptides were extracted sequentially with 1% formic acid, 50% acetonitrile containing 5% formic acid and 90% acetonitrile containing 5% formic acid. All supernatants were combined and dried under vacuum. LC-MS/MS analysis and data processing were performed at the Center for Functional Genomics, University at Albany, State University of New York (Table S6–S7). Briefly, peptides were re-suspended in 60 μL of 0.1% vol/vol formic acid and separated on a CapLC system (Waters Co. Milford, MA, USA) coupled to a QSTAR XL (ABSCIEX, Framingham MA). Peptides were desalted onto an Everest C18 (5 μm, 500 μm ID × 15 mm, Grace, Deerfield, IL) with solvent A (97:3 H_2O:ACN with 0.1% vol/vol formic acid and 0.01% vol/vol TFA) at 40 μL/min. After a 6-min wash, peptides were separated on a Jupiter C18 (3 μm, 100 μm ID × 150 mm, Phenomenex, Torrance, CA) using a 40-min linear gradient of 10% to 40% solvent B (85% ACN/10% isopropanol + 0.1% vol/vol formic acid +

0.0075% vol/vol TFA) at 250 nL/min. MS data acquisition was performed using Analyst QS 1.1 software (ABSciex) in positive ion mode for information dependent acquisition (IDA) analysis. The nanospray voltage was 2.1 kV used for all experiments in a positive ion mode. Nitrogen was used as the curtain (value of 20) with heated interface at 130°C. The declustering potential was set at 80 eV and Gas1 was 5 (arbitrary unit). In IDA analysis, after each survey scan from *m/z* 350 to *m/z* 1300 and the three highest intensity ions above the predefined threshold 28 cps with multiple charge states (+2 and +3) were selected for tandem MS (MS/MS) with rolling collision energy applied for detected ions based on different charge states and *m/z* values. Each MS/MS acquisition was completed and switched back to survey scan when the precursor intensity fell below a predefined threshold or after a maximum of 65 s acquisition. After data acquisition, the individual MS/MS spectra acquired for each of the precursor within a single LC run were combined, smoothened, de-isotoped using an Analyst "script" mascot.dll to create a peak list, and the peak list was saved to a file. Subsequently, the peak list file was used to query NCBI human sub-database and contaminations using the MASCOT 2.4 from Matrix Science (London, UK) with the following parameters: peptide mass tolerance, 0.3 Da; MS/MS ion mass tolerance, 0.3 Da; allow up to two missed cleavage; Several variable modifications were applied including methionine oxidation, cysteine carbamidomethylation along with CHE(alkyne) Michael adduct (134.07), reduced CHE(alkyne) Michael adduct (136.09), dehydrated CHE(alkyne) Michael adduct (118.08), or dehydrated CHE(alkyne) (1,2)-addition adduct or CHE(alkyne) Michael adduct in Schiff-based form (116.06) on cysteine residues (Table S8). Only significant scores for the peptides defined by Mascot probability analysis (www.matrixscience.com/help/scoring_help.html#PBM) greater than “identity” with 95% confidence were considered for the peptide identification and modification site determinations. All MS/MS spectra for the identified peptides with CHE-type modifications

were manually inspected and validated. To approximate the extent of modifications on a given site, the modified peptide signal (XIC) from the chromatogram was extracted and compared with XIC of the unmodified peptide (see Main Text for discussion and further details).

Pulldown procedure for CPE 10-modified Keap1 from intact cells (Figure S12).

Preparative procedure for samples from live cells: global CPE **10** treatment was performed in HEK-293 cells according to the protocol described elsewhere in the SI.

For enrichment under native conditions: the confluent monolayer cultures of HEK-293 cells were harvested from 4 x 21 cm^2 cultured plates, flash-frozen in liq. N_2 and subjected to 3 cycles of freeze-thaw in 100 µL lysis buffer B (50 mM Hepes, 5 mM Imidazole, pH = 7.6). Debris was removed by centrifugation (18,000 x g, 8 min). The supernatant was recovered, diluted to 1.0 mg/mL with lysis buffer B, and added to 20 µL bed volume of TALON resin (Clonetech®). The suspension was incubated in the dark at 4 °C for 1.5 h with end-over-end rotation. The resin was then washed with 360 µL wash buffer D (50 mM Hepes, 100 mM NaCl, 10 mM Imidazole, pH = 7.6) and 240 µL wash buffer E (50 mM Hepes, 100 mM NaCl, 20 mM Imidazole, pH = 7.6). The proteins that remained bound to the resin were eluted in 25 µL elution buffer (50 mM Hepes, 100 mM NaCl, 100 mM Imidazole, pH = 7.6). The eluate was subject to buffer exchange to storage buffer (50 mM Hepes, 0.3 mM TCEP, pH = 7.6) with a CENTRI-SEP spin column (CS900, Princeton Separations) according to manufacturer's protocol.

For coupling with Cy5 dye and visualization on the gel: see the corresponding section within "**T-REX targeting in live cells**".

Estimating the extent of Keap1 modification under whole-cell CHE 9 flooding

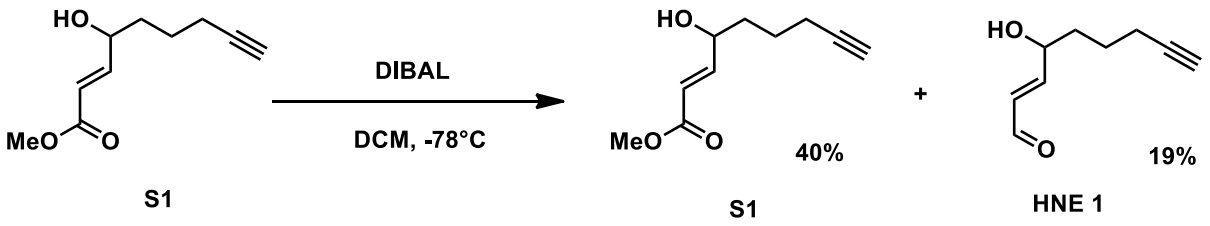
T-REX treatment without light exposure (i.e., Ht-Pre-CHE **22** treatment alone) or global CHE **9** treatment was performed in HEK-293 cells transiently transfected with Halo-Keap1 according to the protocol described in "**T-REX targeting in live cells**". Subsequent to cell harvesting, the resultant cell pellets were flash-frozen in liq N_2 and subjected to 3 cycles of freeze-thaw in 15 µL lysis buffer that contained in final concentrations, 50 mM Hepes (pH 7.6), 0.3 mM TCEP, and 1% Nonidet. All steps hereafter were performed at 4 °C and in dark. Debris was removed by centrifugation (18,000 x g, 8 min). A portion of the clarified lysate was made up to a final volume of 25 µL containing, in final concentrations, 50 mM Hepes (pH 7.6), 1.0 mg/mL lysate protein (measured by Bradford assay), and subjected to Click coupling reaction with Cy5-azide. Briefly, in a final volume of 30 µL, the Click reaction mixture contained, in final concentrations, 42 mM Hepes (pH 7.6), 1.7 mM TCEP, 5% v/v tBuOH, 1% wt/v SDS, 1 mM $CuSO_4$, 0.1 mM TBTA, 10 µM Cy5-azide and freshly prepared lysate. Subsequent to 30-min incubation at 37 °C, the reaction was quenched with 5 µL of 4X Laemmli buffer that contained 6% BME and further incubated for 5 min at 37 °C. 20 µL was directly loaded into each well of 10% polyacrylamide gel. Upon completion of the gel-electrophoresis, the gel was rinsed with ddH_2O (x2, 5 min) and analyzed for Cy5 signal on a Bio-Rad ChemiDoc-MP and, where applicable, the gel was subsequently transferred to a PVDF membrane for western blot analysis.

General Materials and Methods for Synthetic Protocols

Unless otherwise stated, all reactions were conducted in oven-dried glassware under an atmosphere of nitrogen and were stirred magnetically. The phrase "concentrated" refers to removal of solvents by means of a rotary-evaporator attached to a diaphragm pump (15-60 Torr) followed by removal of residual solvents at < 1 Torr with a vacuum pump. Flash chromatography was performed on silica gel 60 (230–400 mesh) from Silicycle Inc. Analytical thin layer chromatography (TLC) was performed using silica gel 60 F-254 pre-coated glass plates (0.25 mm). TLC Plates were analyzed by short wave UV illumination, or by permanganate stain (5 g $KMnO_4$ in 495 mL water). Tetrahydrofuran (THF) and ether were dried and purified by distillation from sodium/benzophenone. Triethylamine (Et_3N), pyridine, acetonitrile, and dichloromethane were distilled from CaH_2. ^1H and ^{13}C NMR spectra were obtained on a Varian INOVA 300, 400, or 500 MHz spectrometer in $CDCl_3$ using solvent as an internal standard ($CDCl_3$ at 7.26 ppm for ^1H and 77.0 ppm for proton-decoupled ^{13}C) unless specifically indicated. Chemical shifts are reported in δ (ppm). Coupling constants (*J*) are reported in Hz with multiplicities denoted as s (singlet), d (doublet), t (triplet), q (quartet), p (pentet), m (multiplet) and br (broad). Ozonolysis was performed on Welsbach ozonator according to the manufacturer's guidelines. Unless otherwise noted, all chemical reagents were obtained from commercial sources and used as received. Double distilled water was from Millipore water purification system. Compound **1, 3, 8–10, 11–17, 21–23** was stored in –80 °C as one-shot aliquots in DMSO.

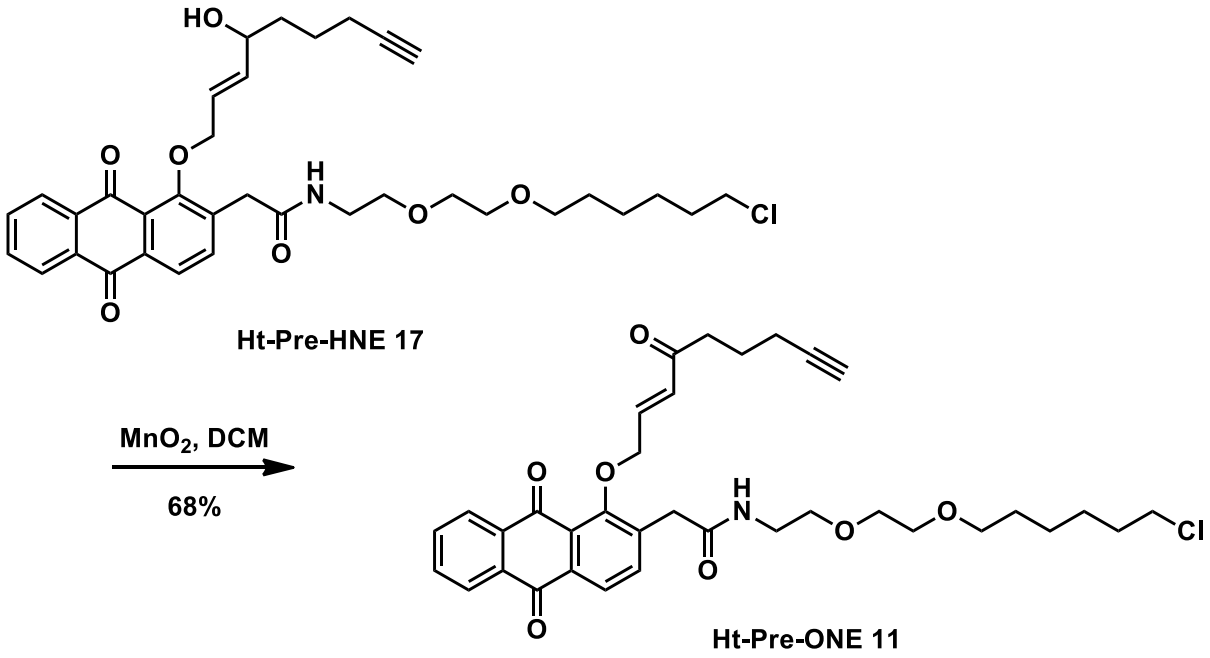
Synthesis protocols

Scheme S1. Synthesis of alkyne-functionalized HNE (HNE hereafter) 1



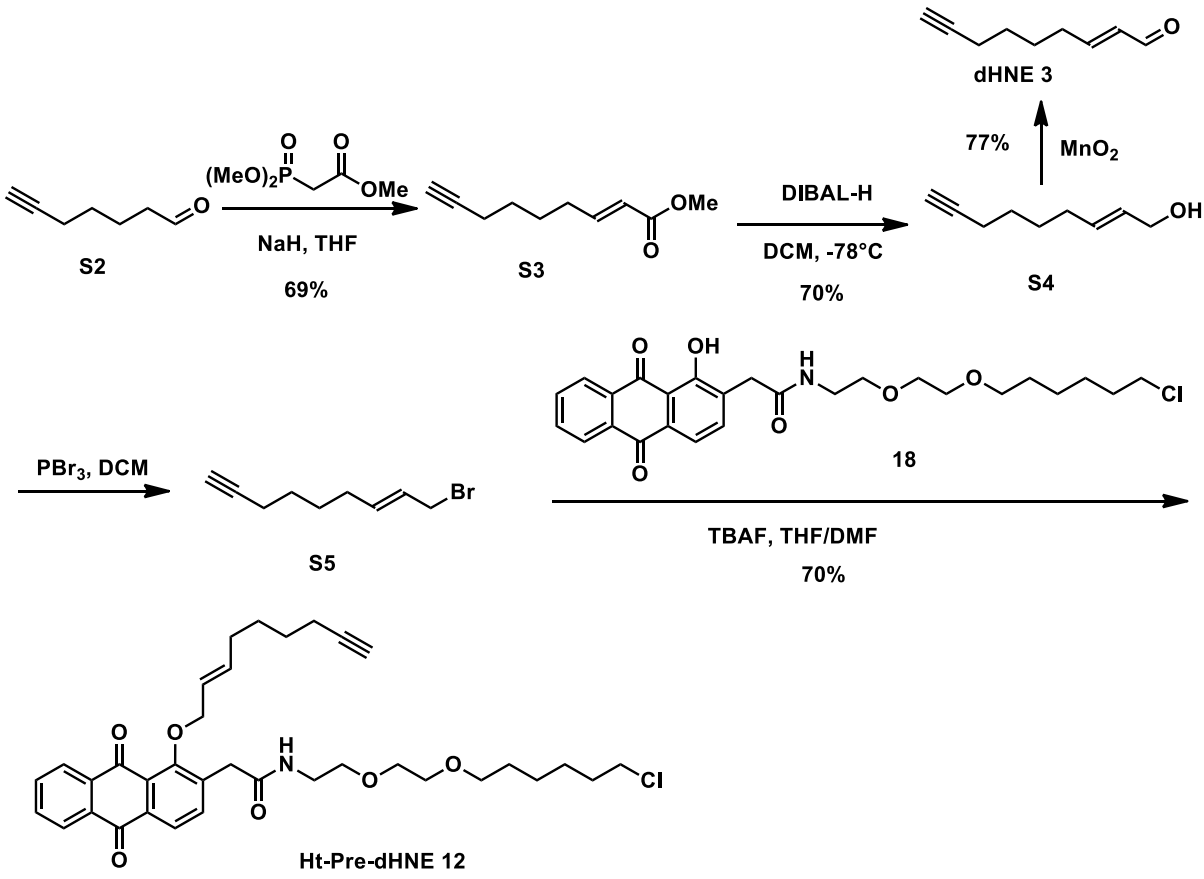
Ester **S1**[3] (362 mg, 2.0 mmol) was dissolved in CH_2Cl_2 (20 mL) and the reaction was cooled to –78 ºC. DIBAL-H (2.0 M in hexane, 2.2 mL, 2.2 mmol, 1.1 equiv) was dissolved in CH_2Cl_2 (20 mL) and added dropwise. Subsequent to 1 h stirring at –78 ºC, the reaction was diluted with Et_2O (20 mL) and quenched with 1 M HCl (24.7 mL). The mixture was stirred at 0 ºC for 20 min and extracted with Et_2O (20 mL × 2). The organic layers were combined, washed with 1 M HCl and brine, dried with $MgSO_4$, and concentrated in vacuo. Flash chromatography of the residue on silica gel (1:2 hexanes/Et_2O) gave recovered methyl ester **S1** (145 mg, 40%) and **HNE 1** (57.4 mg, 19% yield) that was homogeneous by ^{1}H NMR spectroscopy. The spectra of **HNE 1** are identical to those previously reported[3]: ^{1}H-NMR (400 MHz) 9.60 (d, 1, J = 8.0), 6.83 (dd, 1, J = 16.0, 4.8), 6.33 (ddd, 1, J = 16.0, 8.0, 1.6), 4.55-4.46 (m, 1), 2.27 (dt, 2, J = 6.0, 2.8), 1.99 (t, 1, J = 2.4), 1.88-1.60 (m, 5).

Scheme S2. Synthesis of Ht-Pre-ONE 11



To a solution of **Ht-Pre-HNE 17**[4] (30 mg, 0.048 mmol) in DCM (15 mL) was added activated MnO_2 (320 mg, 3.68 mmol). The resulting suspension was stirred at room temperature for 3 h. The mixture was filtered and the filter cake was washed with DCM (5 mL × 3). The combined filtrate was dried with Na_2SO_4, and concentrated. Flash chromatography of the residue on silica gel (1:3 hexanes/EtOAc) gave **Ht-Pre-ONE 11** (20.3 mg, 68% from **17**) as a yellow solid that was homogeneous by ^{1}H NMR spectroscopy: ^{1}H NMR (400 MHz) 8.29-8.23 (m, 2), 8.17 (d, 1, J = 8.0), 7.83-7.73 (m, 3), 7.10 (dt, 1, J = 16.0, 4.4), 6.57 (br dt, 1, J = 16.0, 1.8), 6.26 (br t, 1, J = 5.2), 4.77 (br dd, 2, J = 4.4, 1.8), 3.70 (s, 2), 3.61-3.39 (m, 12), 2.82 (t, 2, J = 7.6), 2.30 (td, 2, J = 6.8, 2.8), 2.00 (t, 1, J = 2.8), 1.94-1.85 (m, 2), 1.79-1.70 (m, 2), 1.62-1.27 (m, 6); ^{13}C NMR (100 MHz) 199.3, 182.7, 182.5, 169.4, 156.9, 140.6, 137.7, 136.9, 134.9, 134.5, 134.3, 133.8, 132.5, 129.8, 127.3, 126.8, 125.9, 124.2, 83.7, 72.8, 71.2, 70.2, 70.0, 69.5, 69.2, 45.0, 39.5, 38.9, 38.1, 32.5, 29.4, 26.6, 25.4, 22.5, 17.8; LC-MS (ESI+) calcd for $C_{35}H_{41}ClNO_7$ (MH^+) 622.26, found 622.33.

Scheme S3. Synthesis of alkyne-functionalized dHNE (dHNE hereafter) 3 and Ht-Pre-dHNE 12



To a suspension of NaH (60% in mineral oil, 202 mg, 5.0 mmol) in dry THF (42 mL) was added trimethyl phosphonoacetate (0.81 mL, 5.0 mmol) at 0 °C under N_2. The mixture was stirred at 0 °C for 30 min and aldehyde **S2**[4] (438 mg, 4.0 mmol) was added. The resulting mixture was warmed to room temperature and stirred for 24 h. The reaction was quenched by addition of saturated NH_4Cl (40 mL) at 0 °C. The mixture was extracted with EtOAc (50 mL × 3). The combined organic layers were washed with brine, dried with Na_2SO_4, and concentrated. Flash chromatography of the residue on silica gel (4:1 hexanes/EtOAc) gave methyl ester **S3** (456 mg, 69% from **S2**) as a pale yellow oil that was homogeneous by ^{1}H NMR spectroscopy: ^{1}H NMR (400 MHz) 6.96 (dt, 1, J = 21.2, 9.4), 5.84 (br d, 1, J = 21.2), 3.73 (s, 3), 2.29-2.10 (m, 4), 1.96 (t, 1, J = 3.4), 1.64-1.48 (m, 4).

To a solution of methyl ester **S3** (456 mg, 2.7 mmol) in DCM (15 mL) at –78 °C under N_2 was added DIBAL-H (1.0 M in hexanes, 7.0 mL, 7.0 mmol). The resulting solution was stirred at –78 °C for 4 h. The reaction was quenched by addition of 1 M HCl (30 mL). The mixture was stirred at 0 °C for 20 min and extracted with EtOAc (30 mL × 3). The combined organic layers were washed with brine, dried with Na_2SO_4, and concentrated. Flash chromatography of the residue on silica gel (3:1 hexanes/EtOAc) gave allylic alcohol **S4** (265 mg, 70% from **S3**) as a colorless oil that was homogeneous by ^{1}H NMR spectroscopy: ^{1}H NMR (400 MHz) 5.70 (dd, 1, *J* = 15.6, 5.6), 5.64 (dd, 1, *J* = 15.6, 4.8), 4.09 (d, 2, *J* = 4.8), 2.24-2.16 (m, 3), 2.12-2.02 (m, 2), 1.94 (t, 1, *J* = 2.4), 1.64-1.42 (m, 4).

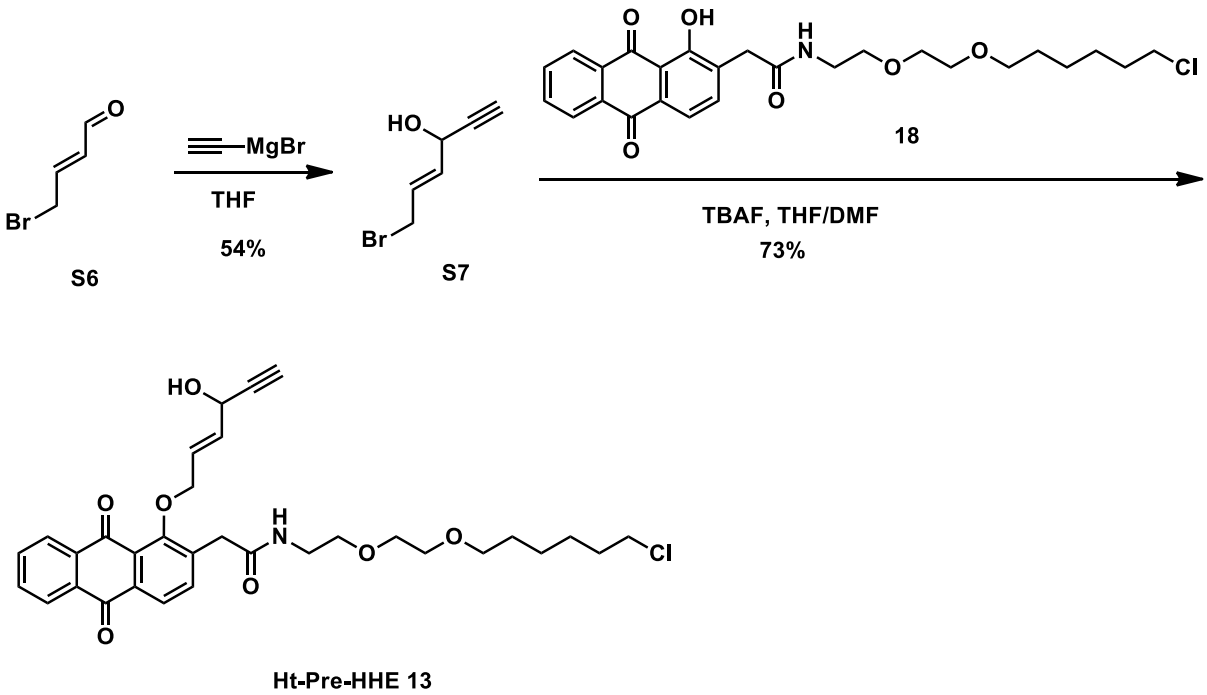
To a solution of allylic alcohol **S4** (138 mg, 1.0 mmol) in DCM (2.5 mL) was added activated MnO_2 (1.0 g, 11.6 mmol). The resulting suspension was stirred at room temperature for 3 h. The mixture was filtered through a pad of Celite®. The filtrate was concentrated. Flash chromatography of the residue on silica gel (1:7 hexanes/DCM) gave **dHNE 3** (105 mg, 77% from **S4**) as a pale yellow oil that was homogeneous by ^{1}H NMR spectroscopy: ^{1}H NMR (400 MHz) 9.52 (d, 1, *J* = 7.6), 6.85 (dt, 1, *J* = 15.6, 6.8), 6.14 (ddt, 1, *J* = 15.6, 8.0, 1.6), 2,41-2.33 (m, 2), 2.24 (td, 2, *J* = 6.8, 2.8), 1.97 (t, 1, *J* = 2.8), 1.70-1.52 (m, 4); ^{13}C NMR (125 MHz) 194.0, 158.1, 133.2, 83.8, 68.7, 32.1, 27.7, 26.8, 18.1; GC-MS (DART) calcd for $C_9H_{13}O$ (MH^+)137.0961, found 137.0964.

To a solution of alcohol **S4** (100 mg, 0.72 mmol) in DCM (15 mL) at 0 °C under N_2 was added PBr_3 (0.7 mL, 7.5 mmol). The reaction was stirred at 0 °C and quenched by addition of water (30 mL). The mixture was extracted with EtOAc (30 mL × 3). The combined organic layers were washed with brine, dried with Na_2SO_4, and concentrated to give crude allyl

bromide **S5** (60 mg) as a yellow oil, which was used in the next step without further purification.

To a solution of hydroxyanthraquinone **18**[3] (36 mg, 0.074 mmol) in DMF (1 mL) at room temperature under N_2 was added TBAF (45 mg, 0.172 mmol). To this mixture was added a solution of crude allyl bromide **S5** (60 mg) in THF (1 mL). The resulting solution was stirred at room temperature for 18 h. The reaction was quenched by addition of water (10 mL). The mixture was extracted with EtOAc (10 mL × 3). The combined organic layers were washed with brine, dried with Na_2SO_4, and concentrated. Flash chromatography of the residue on silica gel (1:3 hexanes/EtOAc) gave **Ht-Pre-dHNE 12** (31 mg, 70% from **18**) as a yellow solid that was homogeneous by ^{1}H NMR spectroscopy: ^{1}H NMR (400 MHz) 8.30-8.23 (m, 2), 8.12 (d, 1, *J* = 7.6), 7.83-7.74 (m, 3), 6.30 (br t, 1, *J* = 5.6), 5.93-5.78 (m, 2), 4.54 (d, 2, *J* = 5.6), 3.71 (s, 2), 3.59-3.37 (m, 12), 2.22-2.08 (m, 4), 1.96 (t, 1, *J* =2.6), 1.80-1.70 (m, 2), 1.60-1.28 (m, 10); ^{13}C NMR (100 MHz) 182.9, 182.5, 169.7, 157.3, 138.0, 136.49, 136.47, 134.8, 134.7, 134.2, 133.6, 132.6, 127.2, 126.7, 126.0, 125.5, 123.7, 84.3, 75.6, 71.2, 70.3, 70.0, 69.7, 68.4, 45.0, 39.5, 38.6, 32.5, 31.7, 29.4, 27.9 (2C), 26.6, 25.4, 18.2; LC-MS (ESI+) calcd for $C_{35}H_{43}ClNO_6$ (MH^+) 608.28, found 608.25.

Scheme S4. Synthesis of Ht-Pre-HHE 13

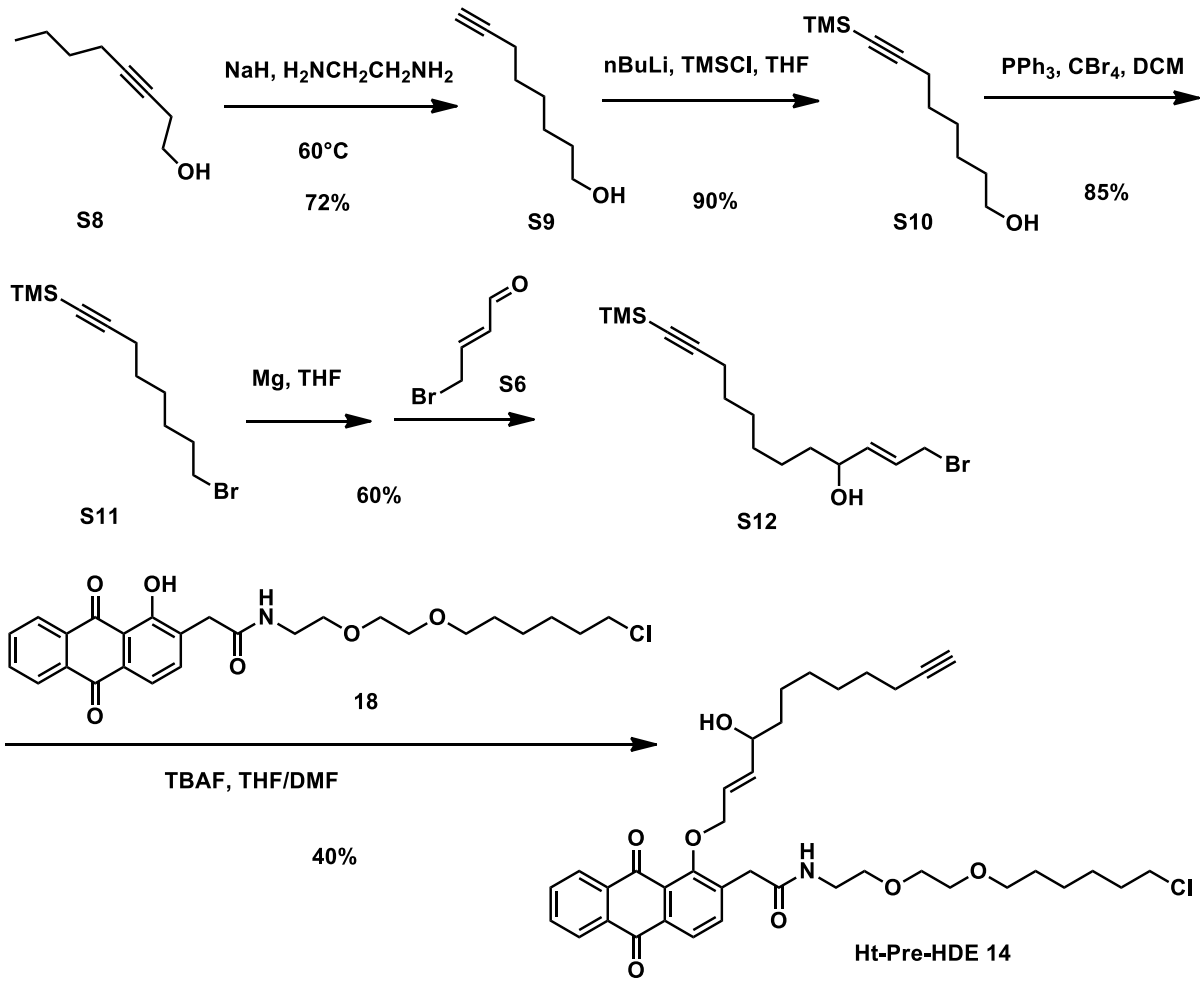


To a solution of (*E*)-4-bromobut-2-enal[3] (**S6**) (183 mg, 1.2 mmol) in THF (5 mL) at 0 °C under N_2 was added ethynylmagnesium bromide (0.5 M in THF, 5.0 mL, 2.5 mmol). The resulting solution was stirred at 0 °C for 0.5 h and at room temperature for 3 h. The reaction was quenched by addition of saturated NH_4Cl (20 mL). The mixture was extracted with EtOAc (20 mL × 3). The combined organic layers were washed with brine, dried with Na_2SO_4, and concentrated. Flash chromatography of the residue on silica gel (3:1 hexanes/EtOAc) gave allyl bromide **S7** (115 mg, 54% from **S6**) as a pale yellow oil that was homogeneous by ^{1}H NMR spectroscopy: ^{1}H NMR (400 MHz) 6.16 (br dt, 1, J = 15.2, 7.6), 5.89 (br dd, 1, J = 15.2, 5.2), 4.93 (br, 1), 3.97 (d, 2, J = 7.6), 2.61 (d, 1, J = 2.0), 1.96 (d, 1, J = 6.0).

To a solution of hydroxyanthraquinone **18**[3] (42 mg, 0.076 mmol) in DMF (1 mL) at room temperature under N_2 was added TBAF (51 mg, 0.172 mmol). To this mixture was added a solution of allyl bromide **S7** (55 mg, 0.31 mmol) in THF (1 mL). The resulting solution was

stirred at room temperature for 18 h. The reaction was quenched by addition of water (10 mL). The mixture was extracted with EtOAc (10 mL × 3). The combined organic layers were washed with brine, dried with Na_2SO_4, and concentrated. Flash chromatography of the residue on silica gel (1:4 hexanes/EtOAc) gave **Ht-Pre-HHE 13** (36 mg, 73% from **18**) as a yellow solid that was homogeneous by ^{1}H NMR spectroscopy: ^{1}H NMR (400 MHz) 8.30-8.22 (m, 2), 8.14 (d, 1, *J* = 8.0), 7.84-7.74 (m, 3), 6.59 (br, 1), 6.29 (br dt, 1, *J* = 15.6, 6.0), 6.03 (br dd, 1, *J* = 15.6, 5.4), 4.98-4.90 (br, 1), 4.68 (dd, 1, *J* = 12.4, 6.0), 4.61 (dd, 1, *J* = 12.4, 6.0), 3.76-3.40 (m, 14), 2.58 (d, 1, *J* = 2.0), 1.83-0.80 (m, 9); ^{13}C NMR (100 MHz) 182.8, 182.4, 169.8, 157.3, 137.9, 136.8, 134.8, 134.6, 134.2, 133.7, 132.8, 132.5, 127.8, 127.2, 126.7, 125.7, 123.8, 82.8, 74.2, 73.9, 71.3, 70.0, 69.9, 69.8, 61.6, 45.0, 39.5, 38.5, 32.4, 29.3, 26.6, 25.3; LC-MS (ESI+) calcd for $C_{32}H_{37}ClNO_7$ (MH^+) 582.23, found 582.33.

Scheme S5. Synthesis of Ht-Pre-HDE 14



Terminal alkyne **S9** was prepared according to a procedure previously reported.[5] **S9** was obtained in 72% yield from internal alkyne **S8** as a colorless oil that was homogeneous by ^{1}H NMR spectroscopy: ^{1}H NMR (400 MHz) 3.65 (t, 2, J = 6.8), 2.20 (td, 2, J =7.2, 2.8), 1.94 (t, 1, J =2.8), 1.63-1.36 (m, 9).

To a solution of terminal alkyne **S9** (573 mg, 4.6 mmol) in dry THF (10 mL) at –78 °C under N_2 was added *n*BuLi (4.4 mL, 2.5 M, 11.0 mmol). The resulting solution was stirred at –78 °C for 1.5 h at which point TMSCl (3.0 mL, 23.6 mmol) was added. The mixture was allowed to warm to room temperature overnight. The reaction was quenched by addition of saturated NH_4Cl solution (20 mL) and the mixture was stirred at room temperature for 1 hr before it was extracted with EtOAc (20 mL × 3). The combined organic layers were washed

with brine, dried with Na_2SO_4, and concentrated. Flash chromatography of the residue on silica gel (4:1 hexanes/EtOAc) gave alcohol **S10** (810 mg, 90% from **S9**) as a colorless oil that was homogeneous by ^{1}H NMR spectroscopy: ^{1}H NMR (400 MHz) 3.65 (t, 2, J = 6.8), 2.22 (t, 2, J =7.2), 1.63-1.32 (m, 9), 0.14 (s, 9).

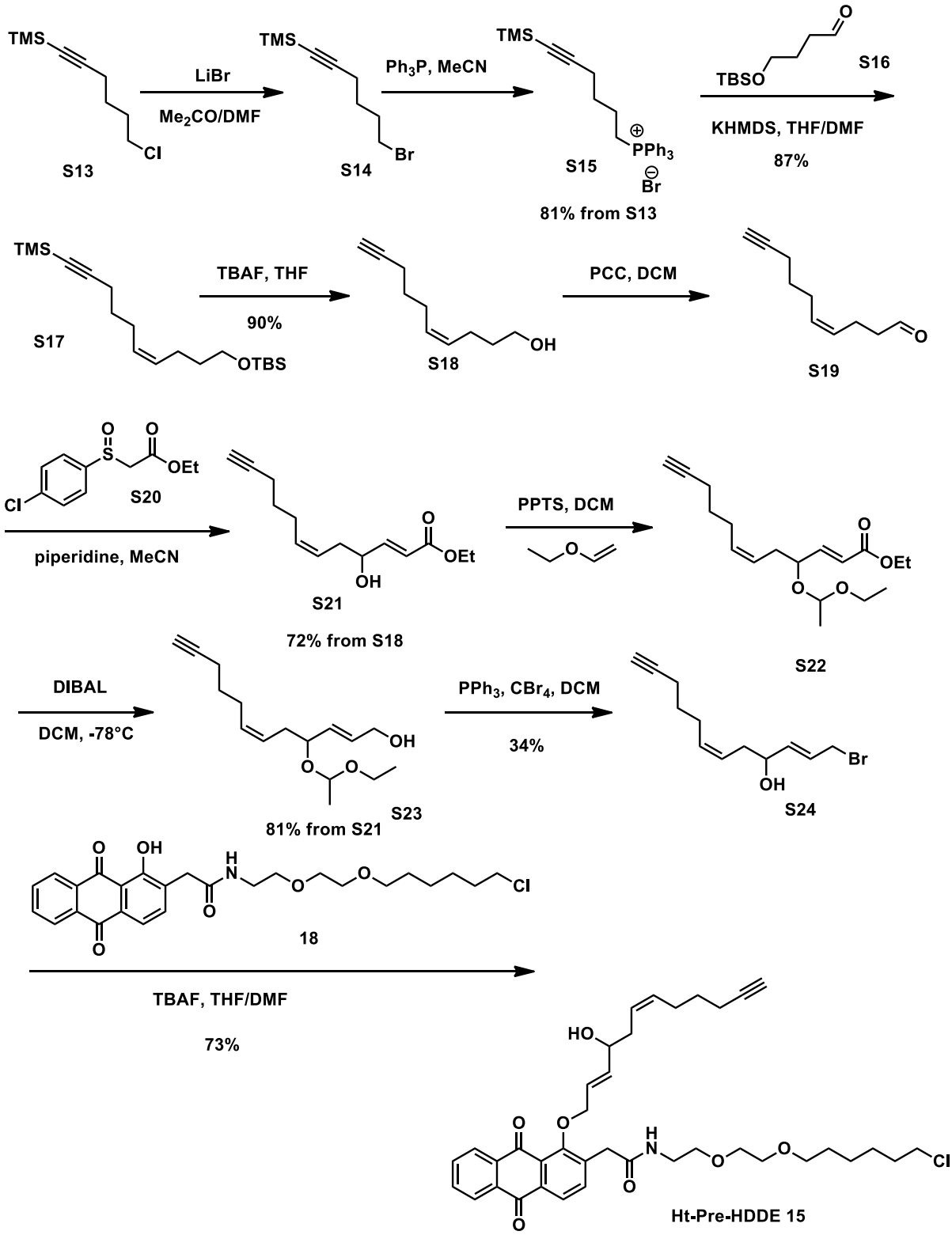
To a solution of alcohol **S10** (810 mg, 4.10 mmol) in dry DCM (18 mL) at 0 °C under N_2 was added PPh_3 (1.44 g, 5.50 mmol) and CBr_4 (1.50 g, 4.52 mmol). The mixture was stirred at 0 °C for 1 h and at room temperature for another 1 h. The reaction was quenched by addition of saturated $NaHCO_3$ (20 mL). The mixture was extracted with DCM (20 mL × 3). The combined organic layers were washed with brine, dried with Na_2SO_4, and concentrated. Flash chromatography of the residue on silica gel (50:1 hexanes/EtOAc) gave bromide **S11** (911 mg, 85% from **S10**) as a pale yellow oil that was homogeneous by ^{1}H NMR spectroscopy: ^{1}H NMR (400 MHz) 3.41 (t, 2, J = 6.8), 2.23 (t, 2, J =7.2), 1.91-1.82 (m, 2), 1.58-1.38 (m, 6), 0.15 (s, 9).

To a mixture of magnesium (181 mg, 7.5 mmol) in anhydrous THF (3 mL) under N_2 was added I_2 (10 mg, 0.039 mmol) and a solution of bromide **S11** (100 mg, 0.38 mmol) in THF (1 mL). The reaction was initiated by heating with a heat gun. A solution of bromide **S11** (811 mg, 3.1 mmol) in anhydrous THF (7.1 mL) was added dropwise. Additional heating was applied to maintain a temperature of approximately 60 °C. Upon complete addition, the mixture was refluxed for 1 h and cooled to room temperature to afford the corresponding Grignard reagent which was used directly in the following reaction. To a solution of (*E*)-4-bromobut-2-enal[3] (**S6**) (351 mg, 2.3 mmol) in THF (2 mL) at 0 °C under N_2 was added the above Grignard reagent. The resulting solution was stirred at 0 °C for 2 h. The reaction was quenched by addition of saturated NH_4Cl (20 mL). The mixture was extracted with EtOAc

(20 mL × 3). The combined organic layers were washed with brine, dried with Na_2SO_4, and concentrated. Flash chromatography of the residue on silica gel (3:1 hexanes/EtOAc) gave allyl bromide **S12** (463 mg, 60% from **S11**) as a pale yellow oil that was homogeneous by ^{1}H NMR spectroscopy: ^{1}H NMR (400 MHz) 5.90 (dtd, 1, *J* = 14.4, 7.2, 0.8), 5.79 (ddt, 1, *J* = 14.4, 6.0, 0.8), 4.18-4.10 (m, 1), 3.96 (d, 2, *J* = 7.2), 2.21 (t, 2, *J* =7.2), 1.58-1.30 (m, 10), 0.14 (s, 9).

To a solution of hydroxyanthraquinone **18**[3] (100 mg, 0.20 mmol) in DMF (2 mL) at room temperature under N_2 was added TBAF (168 mg, 0.64 mmol). To this mixture was added a solution of allyl bromide **S12** (174 mg, 0.53 mmol) in THF (2 mL). The resulting solution was stirred at room temperature for 18 h. The reaction was quenched by addition of water (10 mL). The mixture was extracted with EtOAc (15 mL × 3). The combined organic layers were washed with brine, dried with Na_2SO_4, and concentrated. Flash chromatography of the residue on silica gel (1:3 hexanes/EtOAc) gave **Ht-Pre-HDE 14** (53 mg, 40% from **18**) as a yellow solid that was homogeneous by ^{1}H NMR spectroscopy: ^{1}H NMR (400 MHz) 8.30-8.23 (m, 2), 8.13 (d, 1, *J* = 8.0), 7.83-7.74 (m, 3), 6.46 (br t, 1, *J* = 5.6), 6.04 (br dtd, 1, *J* = 15.2, 6.4, 0.8), 5.84 (br dd, 1, *J* = 15.2, 6.4), 4.65 (br dd, 1, *J* = 12.0, 6.4), 4.57 (br dd, 1, *J* = 12.0, 6.4), 4.17-4.09 (m, 1), 3.78-3.38 (m, 14), 2.68-2.60 (br, 1), 2.18 (td, 2, *J* = 7.2, 2.8), 1.94 (t, 1, *J* =2.8), 1.82-1.28 (m, 18); ^{13}C NMR (100 MHz) 182.8, 182.5, 169.8, 157.3, 138.6, 137.9, 136.6, 134.8, 134.7, 134.2, 133.7, 132.6, 127.2, 126.7, 125.8, 125.4, 123.7, 84.6, 74.9, 71.7, 71.2, 70.1, 69.9, 69.7, 68.2, 45.0, 39.4, 38.5, 36.9, 32.5, 29.4, 29.0, 28.6, 28.4, 26.6, 25.4, 25.2, 18.3; LC-MS (ESI+) calcd for $C_{38}H_{49}ClNO_7$ (MH^+) 666.32, found 666.08.

Scheme S6. Synthesis of Ht-Pre-HDDE 15



To a solution of alkyl chloride **S13**[6] (1.8 g, 9.5 mmol) in acetone/DMF (20 mL/4 mL) was added LiBr (6.5 g, 75 mmol). The mixture was refluxed at 80 °C for 24 h at which point a second portion of LiBr (6.5 g, 75 mmol) was added. The resulting mixture was further

refluxed at 80 °C for 48 h. The mixture was cooled to room temperature and concentrated. The residue was diluted with water (40 mL). The resulting mixture was extracted with EtOAc (50 mL × 3). The combined organic layers were washed with brine, dried with Na_2SO_4, and concentrated give crude alkyl bromide **S14** (2.2 g) as a yellow oil, which was used in the next step without further purification.

To a solution of crude alkyl bromide **S14** (2.2 g) in acetonitrile (25 mL) was added PPh_3 (6.6 g). The resulting solution was stirred at 90 °C for 24 h. The mixture was cooled to room temperature and concentrated. Flash chromatography of the residue on silica gel (20:1 DCM/MeOH) gave phosphonium bromide **S15** (3.8 g, 81% from **S13**) as a pale yellow solid that was homogeneous by ^{1}H NMR spectroscopy: ^{1}H NMR (400 MHz) 7.95-7.75 (m, 15), 4.05-3.90 (m, 2), 2.33 (t, 2, J = 8.6), 2.03-1.70 (m, 4), 0.03 (s, 9).

To a solution of phosphonium bromide **S15** (1.75 g, 3.5 mmol) in THF/DMF (17 mL/7 mL) at –78 °C under N_2 was added KHMDS (0.5 M in toluene, 7.5 mL, 3.8 mmol). The resulting mixture was stirred at the same temperature for 1 h. A solution of aldehyde **S16**[7] (490 mg, 2.4 mmol) in THF (4 mL) was added dropwise. The resulting mixture was stirred at –78 °C for another 2 h, warmed to room temperature, and further stirred for 1 h. The reaction was quenched by addition of saturated NH_4Cl (50 mL). The mixture was extracted with EtOAc (50 mL × 3). The combined organic layers were washed with brine, dried with Na_2SO_4, and concentrated. Flash chromatography of the residue on silica gel (12:1 hexanes/Et_2O) gave silyl ether **S17** (714 mg, 87% from **S15**) as a pale yellow oil that was homogeneous by ^{1}H NMR spectroscopy: ^{1}H NMR (400 MHz) 5.46-5.29 (m, 2), 3.61 (t, 2, J = 6.2), 2.26-2.04 (m, 6), 1.64-1.52 (m, 4), 0.90 (s, 9), 0.15 (s, 9), 0.05 (s, 6).

To a solution of silyl ether **S17** (714 mg, 2.1 mmol) in dry THF (10 mL) under N_2 was added TBAF (1.1 g, 4.2 mmol). The resulting solution was stirred at room temperature for 5 h. The reaction was quenched by addition of water (30 mL). The mixture was extracted with EtOAc (30 mL × 3). The combined organic layers were washed with brine, dried with Na_2SO_4, and concentrated. Flash chromatography of the residue on silica gel (3:1 hexanes/EtOAc) gave alcohol **S18** (289 mg, 90% from **S17**) as a pale yellow oil that was homogeneous by ^{1}H NMR spectroscopy: ^{1}H NMR (400 MHz) 5.48-5.33 (m, 2), 3.66 (apparent q, 2, J = 5.6), 2.23-2.11 (m, 6), 1.95 (t, 1, J = 2.6), 1.68-1.54 (m, 4).

To a solution of alcohol **S18** (289 mg, 1.9 mmol) in DCM (10 mL) was added Celite® (2.2 g), NaOAc (372 mg, 4.5 mmol), and PCC (650 mg, 3.0 mmol). The resulting mixture was stirred at room temperature for 1 h. The reaction was quenched by addition of Et_2O (60 mL). The resulting mixture was stirred at room temperature for 10 min and filtered through a pad of Celite®. The filtrate was washed with H_2O (40 mL × 3) and brine, dried with $MgSO_4$, and concentrated to give crude aldehyde **S19** (255 mg) as a yellow oil, which was immediately used in the next step without further purification.

To a solution of crude aldehyde **S19** (255 mg) in acetonitrile (10 mL) was added methyl 2-phenylsulfinylacetate[3] (**S20**, 295 mg, 1.5 mmol) and piperidine (0.30 mL, 3.0 mmol). The resulting solution was stirred at room temperature under N_2 for 12 h. The reaction was quenched by addition of saturated NH_4Cl (20 mL). The mixture was extracted with EtOAc (20 mL × 3). The combined organic layers were washed with brine, dried with Na_2SO_4, and concentrated. Flash chromatography of the residue on silica gel (4:1 hexanes/EtOAc) gave ethyl ester **S21** (252 mg, 72% from **S19**) as a pale yellow oil that was homogeneous by ^{1}H NMR spectroscopy: ^{1}H NMR (400 MHz) 6.96 (dd, 1, J = 20.8, 6.0), 6.07 (dd, 1, J = 20.8,

2.0), 5.64-5.38 (m, 2), 4.40-4.30 (m, 1), 4.21 (q, 2, *J* =9.6), 2.49-2.30 (m, 2), 2.25-2.12 (m, 4), 1.96 (t, 1, *J* = 3.4), 1.66-1.53 (m, 2), 1.30 (t, 3, *J* =9.6).

To a solution of ethyl ester **S21** (252 mg, 1.1 mmol) in DCM (8 mL) was added PPTS (147 mg, 0.58 mmol) and ethyl vinyl ether (1.0 mL, 10.4 mmol). The resulting solution was stirred at room temperature for 5 h. The reaction was quenched by addition of saturated $NaHCO_3$ (10 mL). The mixture was extracted with DCM (10 mL × 3). The combined organic layers were washed with brine, dried with Na_2SO_4, and concentrated to give crude ether **S22** (320 mg) as a yellow oil, which was used in the next step without further purification.

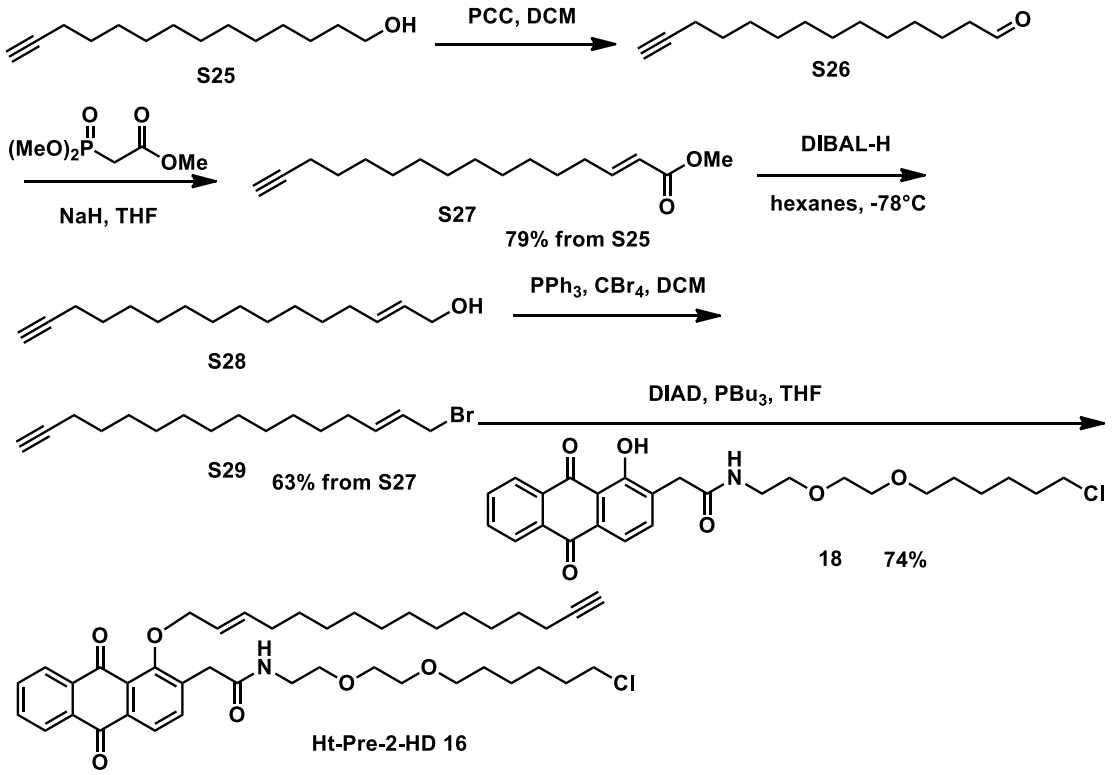
To a solution of crude ether **S22** (320 mg) in dry DCM (8 mL) at –78 °C under N_2 was added DIBAL-H (1.0 M in hexanes, 3.5 mL, 3.5 mmol). The resulting solution was stirred at –78 °C for 4 h. The reaction was quenched by addition of 3 M NaOH (12 mL). The mixture was stirred at 0 °C for 20 min and extracted with Et_2O (30 mL × 3). The combined organic layers were washed with brine, dried with $MgSO_4$, and concentrated. Flash chromatography of the residue on silica gel (1.5:1 hexanes/EtOAc) gave alcohol **S23** (230 mg, 81% from **S21**) as a colorless oil that was homogeneous by ^{1}H NMR spectroscopy: ^{1}H NMR (400 MHz) 5.87-5.53 (m, 2), 5.50-5.38 (m, 2), 4.78-4.69 (m, 1), 4.20-4.06 (m, 3), 3.70-3.40 (m, 2), 2.44-2.24 (m, 2), 2.23-2.12 (m, 4), 1.97-1.93 (m, 1), 1.64-1.54 (m, 2), 1.30 (t, 3, *J* = 4.8), 1.18 (q, 3, *J* = 5.6).

To a solution of alcohol **S23** (152 mg, 0.57 mmol) in dry DCM (12 mL) at 0 °C under N_2 was added PPh_3 (240 mg, 0.92 mmol) and CBr_4 (284 mg, 0.86 mmol). The mixture was stirred at the same temperature for 10 min. The reaction was quenched by addition of saturated $NaHCO_3$ (15 mL). The mixture was extracted with DCM (15 mL × 3). The combined organic

layers were washed with brine, dried with Na_2SO_4, and concentrated. Flash chromatography of the residue on silica gel (3:1 hexanes/EtOAc) gave allyl bromide **S24** (50 mg, 34% from **S23**) as a yellow oil that was homogeneous by ^{1}H NMR spectroscopy: ^{1}H NMR (400 MHz) 5.94 (dtd, 1, *J* = 15.2, 7.6, 0.8), 5.82 (ddt, 1, *J* = 15.2, 6.0, 0.8), 5.58-5.52 (m, 1), 5.47-5.39 (m, 1), 4.25-4.17 (m, 1), 3.96 (d, 2, *J* = 7.6), 2.39-2.30 (m, 2), 2.24-2.15 (m, 4), 1.97 (t, 1, *J* = 2.8), 1.68 (br d, 1, *J* = 4.0), 1.64-1.55 (m, 2).

To a solution of hydroxyanthraquinone **18**[3] (32 mg, 0.076 mmol) in DMF (1 mL) at room temperature under N_2 was added TBAF (43 mg, 0.16 mmol). To this mixture was added a solution of allyl bromide **S24** (50 mg, 0.19 mmol) in THF (1 mL). The resulting solution was stirred at room temperature for 18 h. The reaction was quenched by addition of water (10 mL). The mixture was extracted with EtOAc (10 mL × 3). The combined organic layers were washed with brine, dried with Na_2SO_4, and concentrated. Flash chromatography of the residue on silica gel (1:4 hexanes/EtOAc) gave **Ht-Pre-HDDE 15** (32 mg, 73% from **18**) as a yellow solid that was homogeneous by ^{1}H NMR spectroscopy: ^{1}H NMR (400 MHz) 8.29-8.23 (m, 2), 8.13 (d, 1, *J* = 8.0), 7.83-7.74 (m, 3), 6.46 (br, 1), 6.08 (br dt, 1, *J* = 15.6, 6.4), 5.87 (br dd, 1, *J* = 15.6, 6.0), 5.54-5.38 (m, 2), 4.64 (br dd, 1, *J* =12.0, 6.4), 4.56 (br dd, 1, *J* =12.0, 6.4), 4.24-4.16 (m, 1), 3.78-3.38 (m, 14), 2.78-2.68 (br, 1), 2.39-2.10 (m, 6), 1.96 (t, 1, *J* = 2.8), 1.80-1.22 (m, 10); ^{13}C NMR (100 MHz) 182.8, 182.4, 169.8, 157.3, 137.9, 137.7, 136.6, 134.8, 134.7, 134.2, 133.7, 132.6, 131.6, 127.2, 126.8, 125.81, 125.74, 125.67, 123.7, 84.3, 74.9, 71.3, 71.2, 70.1, 70.0, 69.7, 68.6, 45.0, 39.5, 38.5, 35.0, 32.5, 29.4, 28.2, 26.7, 26.3, 25.4, 17.8 ; LC-MS (ESI+) calcd for $C_{38}H_{47}ClNO_7$ (MH^+) 664.30, found 664.08.

Scheme S7. Synthesis of Ht-Pre-2-HD 16



To a solution of alcohol **S25**[8] (322 mg, 1.5 mmol) in DCM (10 mL) was added Celite® (1.7 g), NaOAc (294 mg, 3.6 mmol), and PCC (513 mg, 2.4 mmol). The resulting mixture was stirred at room temperature for 1 h. The reaction was quenched by addition of Et_2O (60 mL). The resulting mixture was stirred at room temperature for 10 min and filtered through a pad of Celite®. The filtrate was washed with H_2O (40 mL × 3) and brine, dried with $MgSO_4$, and concentrated to give crude aldehyde **S26** (260 mg) as a yellow oil, which was immediately used in the next step without further purification.

To a suspension of NaH (60% in mineral oil, 61 mg, 1.5 mmol) in dry THF (20 mL) was added trimethyl phosphonoacetate (0.24 mL, 1.5 mmol) at 0 °C under N_2. The mixture was stirred at 0 °C for 30 min and crude aldehyde **S26** (260 mg, 1.25 mmol) was added. The resulting mixture was warmed to room temperature and stirred for 24 h. The reaction was quenched by addition of saturated NH_4Cl (40 mL) at 0 °C. The mixture was extracted with

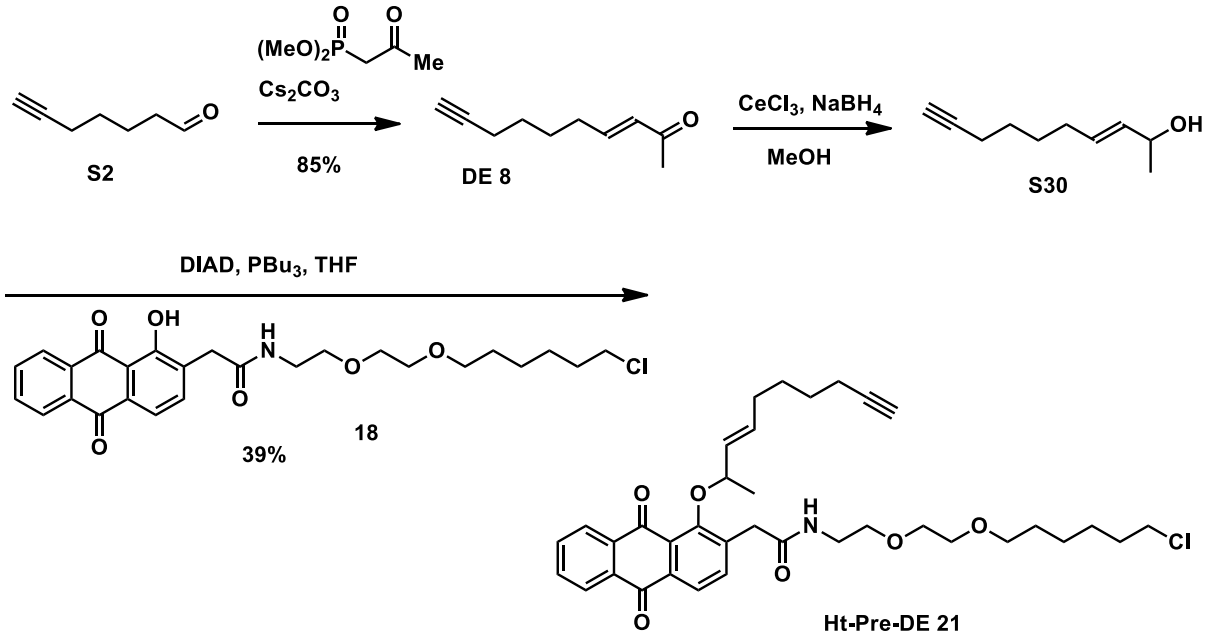
EtOAc (50 mL × 3). The combined organic layers were washed with brine, dried with Na_2SO_4, and concentrated. Flash chromatography of the residue on silica gel (4:1 hexanes/EtOAc) gave methyl ester **S27** (312 mg, 79% from **S25**) as a pale yellow oil that was homogeneous by ^{1}H NMR spectroscopy: ^{1}H NMR (400 MHz) 6.97 (ddd, 1, *J* = 15.6, 7.2, 7.2), 5.82 (ddd, 1, *J* = 15.6, 1.6, 1.6), 3.73 (s, 3), 2.23-2.15 (m, 4), 1.94 (t, 1, *J* = 2.8), 1.58-1.23 (m, 18).

To a solution of methyl ester **S27** (312 mg, 1.2 mmol) in hexanes (6 mL) at –78 °C under N_2 was added DIBAL-H (1.0 M in hexanes, 4.2 mL, 4.2 mmol). The resulting solution was stirred at –78 °C for 1 h. The reaction was quenched by addition of 1 M HCl (30 mL). The mixture was stirred at 0 °C for 20 min and extracted with EtOAc (30 mL × 3). The combined organic layers were washed with brine, dried with Na_2SO_4, and concentrated gave crude allylic alcohol **S28** (218 mg) as a colorless oil, which was used in the next step without further purification.

To a solution of allylic alcohol **S28** (150 mg) in dry DCM (5 mL) at 0 °C under N_2 was added PPh_3 (233 mg, 0.89 mmol) and CBr_4 (253 mg, 0.76 mmol). The mixture was stirred at the same temperature for 10 min. The reaction was quenched by addition of saturated $NaHCO_3$ (15 mL). The mixture was extracted with DCM (15 mL × 3). The combined organic layers were washed with brine, dried with Na_2SO_4, and concentrated. Flash chromatography of the residue on silica gel (100:1 hexanes/EtOAc) gave allyl bromide **S29** (154 mg, 63% from **S27**) as a yellow oil that was homogeneous by ^{1}H NMR spectroscopy: ^{1}H NMR (400 MHz) 5.82-5.62 (m, 2), 3.95 (d, 3, *J* = 7.2), 2.18 (td, 2, *J* = 7.2, 2.4), 2.05 (apparent q, 2, *J* = 7.2), 1.94 (t, 1, *J* = 2.4), 1.58-1.20 (m, 18).

To a solution of hydroxyanthraquinone **18**[3] (49 mg, 0.10 mmol) in DMF (1 mL) at room temperature under N_2 was added TBAF (59 mg, 0.23 mmol). To this mixture was added a solution of allyl bromide **S29** (120 mg, 0.40 mmol) in THF (1 mL). The resulting solution was stirred at room temperature for 18 h. The reaction was quenched by addition of water (10 mL). The mixture was extracted with EtOAc (15 mL × 3). The combined organic layers were washed with brine, dried with Na_2SO_4, and concentrated. Flash chromatography of the residue on silica gel (1:4 hexanes/EtOAc) gave **Ht-Pre-2-HD 16** (52 mg, 74% from **18**) as a yellow solid that was homogeneous by ^{1}H NMR spectroscopy: ^{1}H NMR (400 MHz) 8.30-8.22 (m, 2), 8.12 (d, 1, J = 4.0), 7.83-7.71 (m, 3), 6.33 (br, 1), 5.89-5.78 (m, 2), 4.53 (d, 2, J = 4.4), 3.74-3.36 (m, 14), 2.21-2.15 (m, 2), 2.11-2.02 (m, 2), 1.94 (t, 1, J = 2.8), 1.82-1.20 (m, 26); ^{13}C NMR (125 MHz) 182.9, 182.4, 169.8, 157.3, 138.0, 137.3, 136.5, 134.8, 134.3, 133.6, 131.5, 128.5, 127.2, 127.0, 126.8, 125.0, 123.6, 84.9, 75.8, 71.3, 70.3, 70.0, 69.7, 68.1, 45.0, 39.5, 38.7, 32.5, 29.5(9C), 28.8, 28.5, 26.7, 25.4, 18.4; LC-MS (ESI+) calcd for $C_{42}H_{57}ClNO_7$ (MH^+) 706.39, found 706.50.

Scheme S8. Synthesis of alkyne-functionalized DE (DE hereafter) 8 and Ht-Pre-DE 21



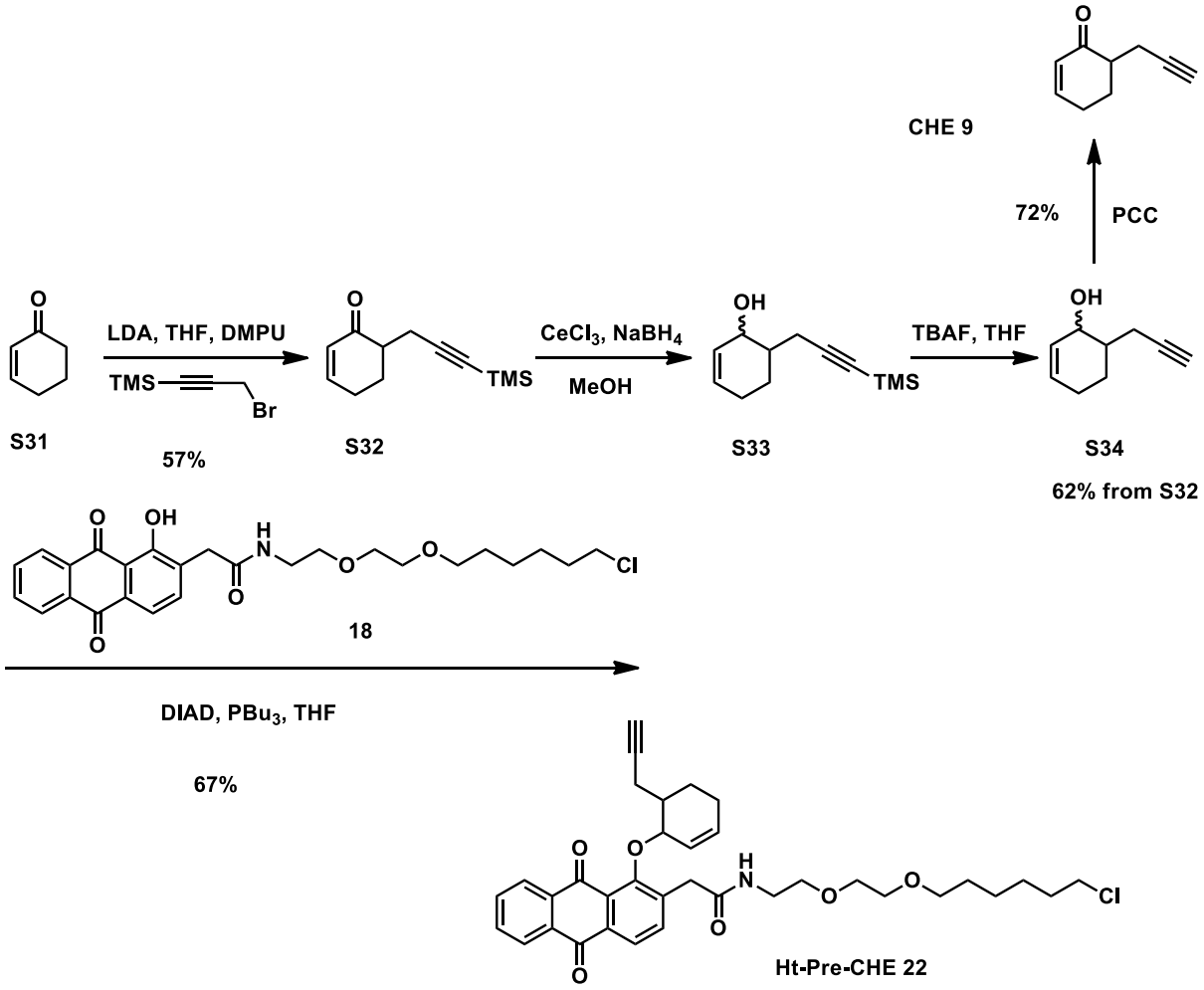
To a solution of aldehyde **S2**[3] (137 mg, 1.25 mmol), H_2O (33 μL, 1.8 mmol), and dimethyl (2-oxopropyl)phosphonate (256 mg, 1.54 mmol) in dioxane (2.0 mL) was added Cs_2CO_3 (604 mg, 1.9 mmol). The resulting mixture was stirred at room temperature for 2 h. The reaction was quenched by addition of water (10 mL). The mixture was extracted with DCM (15 mL × 3). The combined organic layers were washed with brine, dried with Na_2SO_4, and concentrated. Flash chromatography of the residue on silica gel (1:7 hexanes/DCM) gave **DE** (**8**) (159 mg, 85% from **S2**) as a pale yellow oil that was homogeneous by ^{1}H NMR spectroscopy: ^{1}H NMR (500 MHz) 6.79 (1, dt, J = 16.0, 6.8), 6.09 (1, d, J = 16.0), 2.29-2.19 (m, 4), 2.25 (s, 3), 1.96 (1, t, J = 2.5), 1.65-1.52 (m, 2); ^{13}C NMR (125 MHz) 198.6, 147.8, 131.5, 83.9, 68.6, 31.9, 27.8, 27.0, 26.9, 18.2; GC-MS (DART) calcd for $C_{10}H_{15}O$ (MH^+) 151.1117, found 151.1114.

To a solution of **DE** (**8**) (54 mg, 0.36 mmol) and $CeCl_3$•$7H_2O$ (134 mg, 0.36 mmol) in MeOH (4 mL) at 0 °C was added $NaBH_4$ (14 mg, 0.37 mmol) in small portions. The resulting mixture was stirred at the same temperature for 5 min. The reaction was quenched by

addition of water (15 mL). The mixture was extracted with EtOAc (15 mL × 3). The combined organic layers were washed with brine, dried with Na_2SO_4, and concentrated to give crude alcohol **S30** (60 mg) as a yellow oil, which was used in the next step without further purification.

To a solution of DIAD (27 μL, 0.14 mmol) in dry THF (1 mL) at –10 °C under N_2 was added PBu_3 (34 μL, 0.14 mmol). The resulting solution was stirred at the same temperature for 10 min before it was added to a solution of hydroxyanthraquinone **18**[3] (45 mg, 0.092 mmol) and crude alcohol **S30** (30 mg) in dry THF (1 mL) at –78 °C under N_2. The resulting solution was warmed to room temperature immediately, stirred for 12 h, and concentrated. Flash chromatography of the residue on silica gel (1:2 hexanes/EtOAc) gave **Ht-PreDE 21** (23 mg, 39% from **18**), which was a yellow solid that was homogeneous by ^{1}H NMR spectroscopy: ^{1}H NMR (400 MHz) 8.29- 8.23 (m, 2), 8.18 (d, 1, *J* = 8.0), 7.83-7.72 (m, 3), 6.40-6.34 (br, 1), 5.44 (dd, 1, *J* = 15.2, 8.8), 5.27 (ddd, 1, *J* = 15.2, 6.8, 6.8), 4.78-4.69 (m, 1), 3.87 (d, 1, *J* = 14.4), 3.66-3.32 (m, 13), 2.05-1.97 (m, 2), 1.91 (apparent t, 1, *J* = 2.8), 1.87-1.70 (m, 4), 1.62 (d, 3, *J* = 6.0), 1.60-1.12 (m, 10); ^{13}C NMR (125 MHz) 183.0, 182.7, 169.7, 155.9, 138.4, 136.2, 135.5, 134.7, 134.2, 133.6, 132.6, 130.2, 128.5, 127.2, 127.0, 126.7, 123.3, 82.6, 71.2, 70.3, 70.0 (2C), 69.7, 68.4, 45.0, 39.5, 39.4, 32.5, 31.3, 29.4, 27.7, 27.6, 26.7, 25.4, 21.4, 18.1; LC-MS (ESI+) calcd for $C_{36}H_{45}ClNO_6$ (MH^+) 622.29, found 622.33.

Scheme S9. Synthesis of alkyne-functionalized CHE (CHE hereafter) 9 and Ht-Pre-CHE 22



To a solution of freshly prepared LDA (2.5 mmol, prepared from 2.8 mmol of anhydrous isopropylamine and 2.5 mmol of *n*BuLi in THF at –78 °C) in THF (6 mL) at –78 °C under N_2 was added DMPU (0.6 mL, 5.0 mmol). The mixture was stirred at the same temperature for 10 min before a solution of enone **S31** (0.20 mL, 2.1 mmol) in THF (2 mL) was added dropwise. The resulting mixture was stirred at the same temperature for 45 min and subsequently added to a solution of 3-bromo-1-(trimethylsilyl)-1-propyne (0.50 mL, 3.1 mmol) in THF (3 mL). The resulting solution was allowed to warm to room temperature over 18 h. The reaction was quenched by addition of saturated NH_4Cl (20 mL). The mixture was extracted with EtOAc (20 mL × 3). The combined organic layers were washed with brine,

dried with Na_2SO_4, and concentrated. Flash chromatography of the residue on silica gel (3:1 hexanes/EtOAc) gave TMS-alkyne **S32** (247 mg, 57% from **S31**) as a pale yellow oil that was homogeneous by ^{1}H NMR spectroscopy: ^{1}H NMR (400 MHz) 6.98 (br ddd, 1, *J* = 10.2, 4.0, 4.0), 6.01 (br ddd, 1, *J* = 10.2, 2.0, 2.0), 2.86 (dd, 1, *J* = 17.2, 4.0), 2.55-2.35 (m, 4), 2.29 (dd, 1, *J* = 17.2, 9.2), 1.89-1.74 (m, 1), 0.15 (s, 9).

To a solution of TMS-alkyne **S32** (140 mg, 0.68 mmol) and $CeCl_3$•$7H_2O$ (253 mg, 0.68 mmol) in MeOH (6 mL) at 0 °C was added $NaBH_4$ (26 mg, 0.68 mmol) in small portions. The resulting mixture was stirred at the same temperature for 5 min. The reaction was quenched by addition of water (15 mL). The mixture was extracted with EtOAc (15 mL × 3). The combined organic layers were washed with brine, dried with Na_2SO_4, and concentrated to afford crude alcohol **S33** (145 mg) as a pale yellow oil, which was used in the next step without further purification.

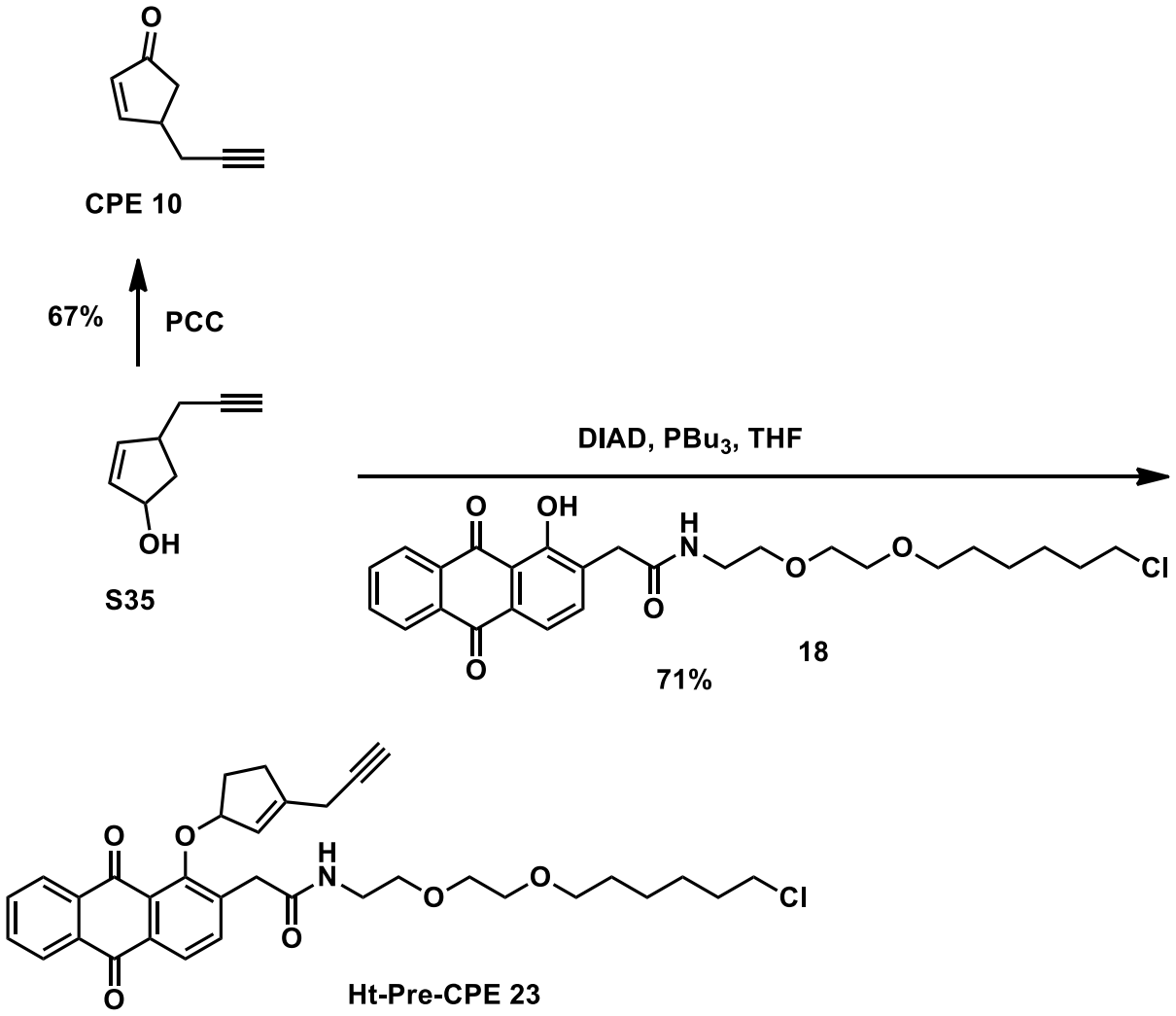
To a solution of crude alcohol **S33** (145 mg) in THF (5 mL) under N_2 was added TBAF (177 mg, 0.68 mmol). The solution was stirred at room temperature for 4 h. The reaction was quenched by addition of saturated NH_4Cl (15 mL). The mixture was extracted with EtOAc (15 mL × 3). The combined organic layers were washed with brine, dried with Na_2SO_4, and concentrated. Flash chromatography of the residue on silica gel (3:1 hexanes/EtOAc) gave alcohol **S34** (57 mg, 62% from **S32**) as a mixture of diastereomers (4:3 trans:cis), which was a pale yellow oil that was homogeneous by ^{1}H NMR spectroscopy: ^{1}H NMR (400 MHz) 5.94-5.85 (m, 1.14), 5.82-5.75 (m, 0.43), 5.65 (dddd, 0.43, *J* = 10.0, 2.4, 2.4, 2.0), 4.23-4.17 (m, 0.57), 4.08-4.01 (m, 0.43), 2.50-2.20 (m, 2), 2.20-2.03 (m, 2), 2.01-1.98 (m, 1), 1.92-1.60 (m, 2), 1.55-1.41 (m, 1.43), 1.33 (br d, 0.57, *J* = 6.8).

To a solution of alcohol **S34** (75 mg, 0.55 mmol) in DCM (6 mL) was added Celite® (200 mg), NaOAc (68 mg, 0.83 mmol), and PCC (180 mg, 0.83 mmol). The resulting mixture was stirred at room temperature for 1 h and quenched by addition of Et_2O (30 mL). The mixture was filtered through a pad of Celite®. The filtrate was washed with water and brine, dried over $MgSO_4$, and concentrated. Flash chromatography of the residue on silica gel (5:1 hexanes/EtOAc) gave **CHE 9** (53 mg, 72% from **S34**) as a pale yellow oil that was homogeneous by ^{1}H NMR spectroscopy: ^{1}H NMR (400 MHz) 6.97 (dddd, 1, *J* = 10.0, 4.0, 4.0, 1.6), 6.00 (ddd, 1, *J* = 10.0, 2.0, 2.0), 2.76 (ddd, 1, *J* = 16.8, 4, 2.8), 2.54-2.42 (m, 3), 2.38-2.26 (m, 2), 1.97 (apparent t, 1, *J* = 2.8), 1.92-1.78 (m, 1); ^{13}C NMR (100 MHz) 199.1, 150.2, 129.3, 82.1, 69.6, 45.6, 27.6, 25.7, 18.8; GC-MS (DART) calcd for $C_9H_{11}O$ (MH^+) 135.0804, found 135.0802.

To a solution of DIAD (27 μL, 0.14 mmol) in dry THF (1 mL) at –10 °C under N_2 was added PBu_3 (34 μL, 0.14 mmol). The resulting solution was stirred at the same temperature for 10 min before it was added to a solution of hydroxyanthraquinone **18**[3] (45 mg, 0.092 mmol) and alcohol **S34** (25 mg, 0.18 mmol) in dry THF (1 mL) at –78 °C° under N_2. The resulting solution was warmed to room temperature immediately, stirred for 12 h, and concentrated. Flash chromatography of the residue on silica gel (1:2 hexanes/EtOAc) gave **Ht-Pre-CHE 22** (37 mg, 67% from **18**) as a single diastereomer, which was a yellow solid that was homogeneous by ^{1}H NMR spectroscopy: ^{1}H NMR (400 MHz) 8.28- 8.22 (m, 2), 8.11 (d, 1, *J* = 8.0), 7.82-7.72 (m, 3), 6.35-6.27 (br, 1), 5.87 (br d, 1, *J* = 10.0), 5.74 (br d, 1, *J* = 10.0), 4.68-4.62 (br, 1), 3.84 (d, 1, *J* = 14.8), 3.64-3.34 (m, 13), 2.70 (br ddd, 1, *J* =14.8, 3.6, 2.8), 2.42-2.01 (m, 6), 1.95 (t, 1, *J* =2.8), 1.80-1.20 (m, 8); ^{13}C NMR (100 MHz) 182.9, 182.4, 169.6, 155.8, 138.5, 136.2, 135.0, 134.8, 134.2, 133.6, 132.5, 132.1, 127.3, 126.7, 126.0,

124.9, 123.5, 82.9, 80.7, 71.2, 70.3, 70.0, 69.7, 69.6, 45.0, 39.5, 39.4, 38.6, 32.5, 29.4, 26.6, 25.4, 24.1, 23.4, 21.0; LC-MS (ESI+) calcd for $C_{35}H_{41}ClNO_6$ (MH^+) 606.26, found 605.92.

Scheme S10. Synthesis of alkyne-functionalized CPE (CPE hereafter) 10 and Ht-Pre-CPE 23



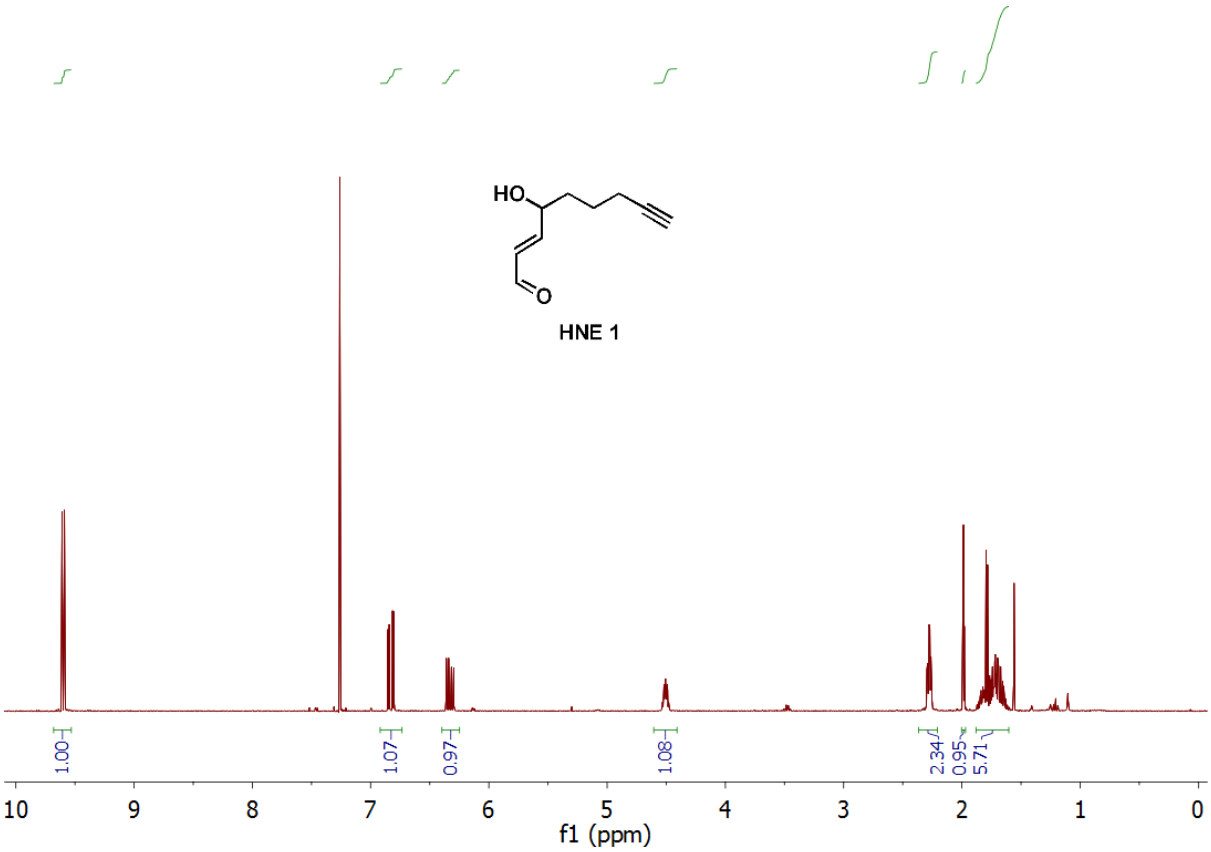
To a solution of alcohol **S35**[8] (67 mg, 0.55 mmol) in DCM (6 mL) was added Celite® (200 mg), NaOAc (68 mg, 0.83 mmol), and PCC (180 mg, 0.83 mmol). The resulting mixture was stirred at room temperature for 1 h and quenched by addition of Et_2O (30 mL). The mixture was filtered through a pad of Celite®. The filtrate was washed with water and brine, dried over $MgSO_4$, and concentrated. Flash chromatography of the residue on silica gel (3:1 hexanes/EtOAc) gave **CPE 10** (44 mg, 67% from **S35**) as a pale yellow oil that was homogeneous by ^{1}H NMR spectroscopy: ^{1}H NMR (400 MHz) 7.66 (dd, 1, J = 5.6, 2.4), 6.25 (dd, 1, J = 5.6, 2.0), 3.22-3.13 (m, 1), 2.57 (dd, 1, J = 18.8, 6.4), 2.49 (ddd, 1, J = 16.8, 6.0,

2.4), 2.41 (ddd, 1, J = 16.8, 7.2, 2.8), 2.17 (dd, 1, J = 18.8, 2.4), 1.97 (apparent t, 1, J = 2.8); ^{13}C NMR (125 MHz) 208.9, 165.9, 134.9, 80.4, 70.5, 40.0, 39.8, 23.4; GC-MS (DART) calcd for C_8H_9O (MH^+) 121.0648, found 121.0644.

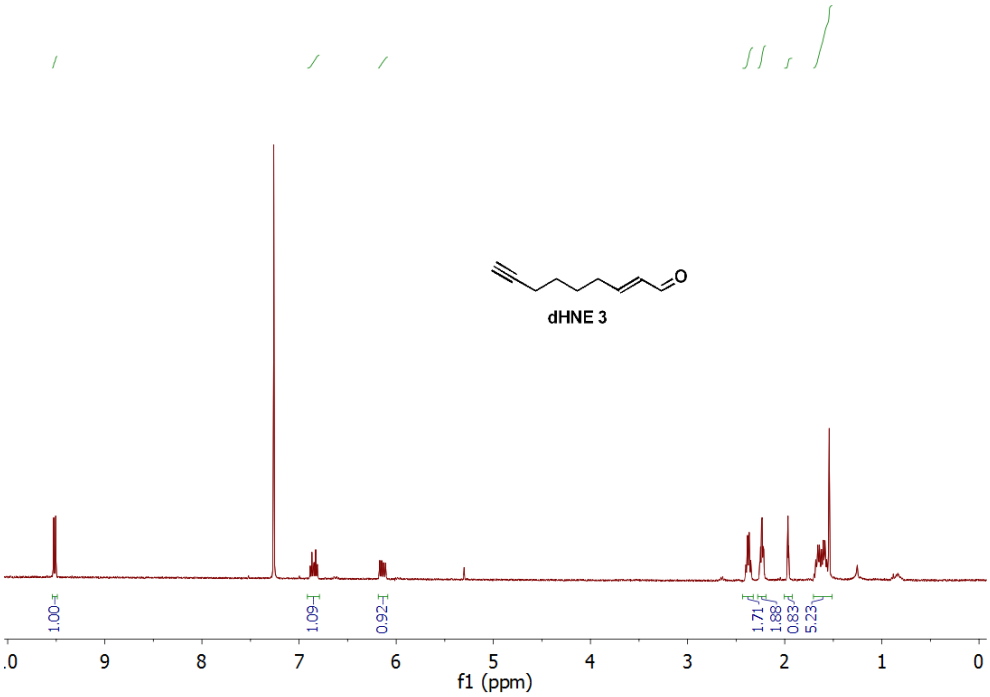
To a solution of DIAD (27 μL, 0.14 mmol) in dry THF (1 mL) at –10 °C under N_2 was added PBu_3 (34 μL, 0.14 mmol). The resulting solution was stirred at the same temperature for 10 min before it was added to a solution of hydroxyanthraquinone **18**[3] (45 mg, 0.092 mmol) and alcohol **S35**[8] (22 mg, 0.18 mmol) in dry THF (1 mL) at –78 °C under N_2. The resulting solution was warmed to room temperature immediately, stirred for 12 h, and concentrated. Flash chromatography of the residue on silica gel (1:2 hexanes/EtOAc) gave **Ht-Pre-CPE 23** (39 mg, 71% from **18**) as a single diastereomer (stereochemistry not assigned), which was a yellow solid that was homogeneous by ^{1}H NMR spectroscopy: ^{1}H NMR (400 MHz) 8.30-8.23 (m, 2), 8.11 (d, 1, J = 8.0), 7.83-7.74 (m, 3), 6.32-6.25 (br, 1), 6.14 (br d, 1, J = 5.6), 5.93 (br d, 1, J = 5.6), 5.33 (br d, 1, J = 6.8), 3.78 (d, 1, J = 14.8), 3.64-3.32 (m, 13), 2.54-2.00 (m, 5), 1.95 (t, 1, J =2.8), 1.80-1.22 (m, 8); ^{13}C NMR (100 MHz) 182.9, 182.5, 169.7, 156.6, 141.0, 138.0, 136.4, 135.1, 134.7, 134.2, 133.6, 132.5, 131.2, 127.2, 126.7, 125.6, 123.4, 90.7, 82.2, 71.2, 70.3, 70.0, 69.7, 69.4, 45.0, 43.6, 39.5, 39.0, 37.0, 32.5, 29.4, 26.7, 25.4, 24.2; LC-MS (ESI+) calcd for $C_{34}H_{39}ClNO_6$ (MH^+) 592.25, found 592.42.

^{1}H- and ^{13}C-NMR spectroscopic characterization data of Compound 1, 3, 8–10, 11–16, 21–23

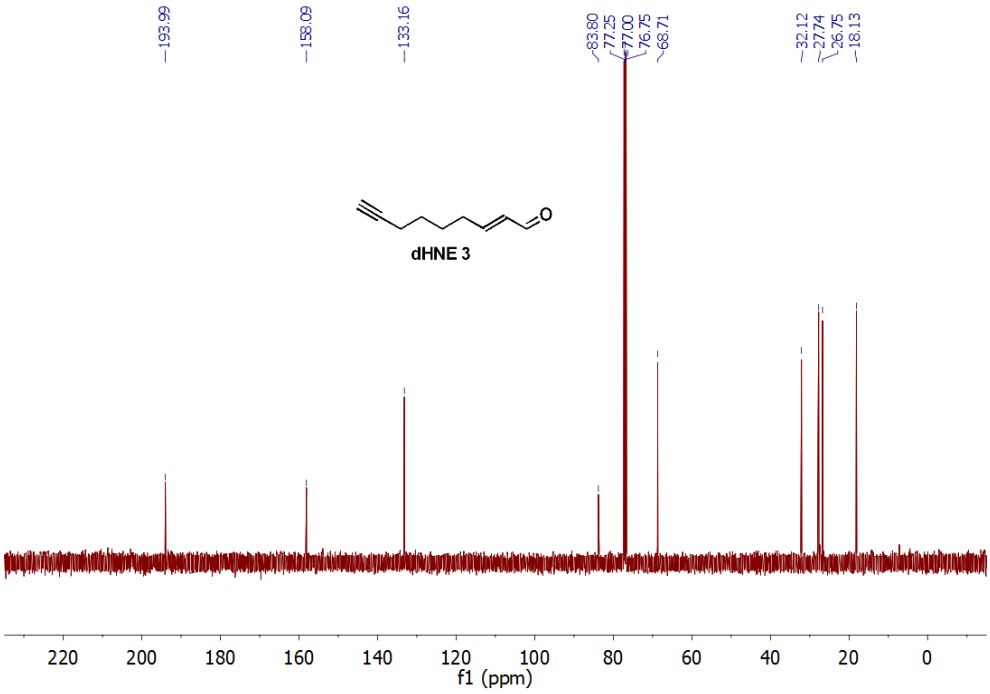
^{1}H-NMR spectrum of HNE 1



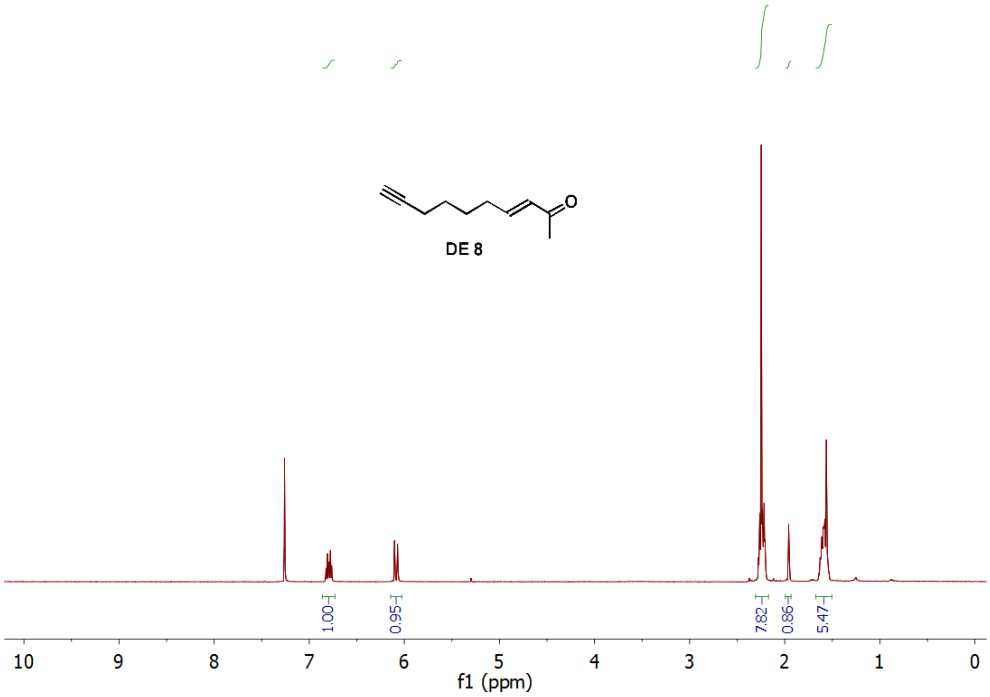
^{1}H-NMR spectrum of dHNE 3



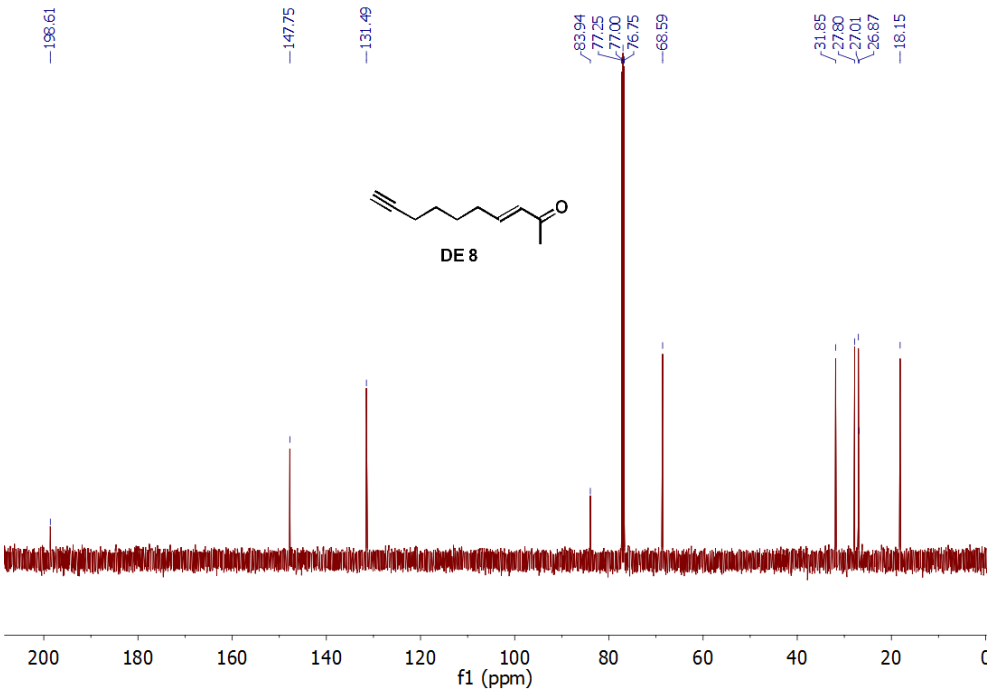
^{13}C-NMR spectrum of dHNE 3



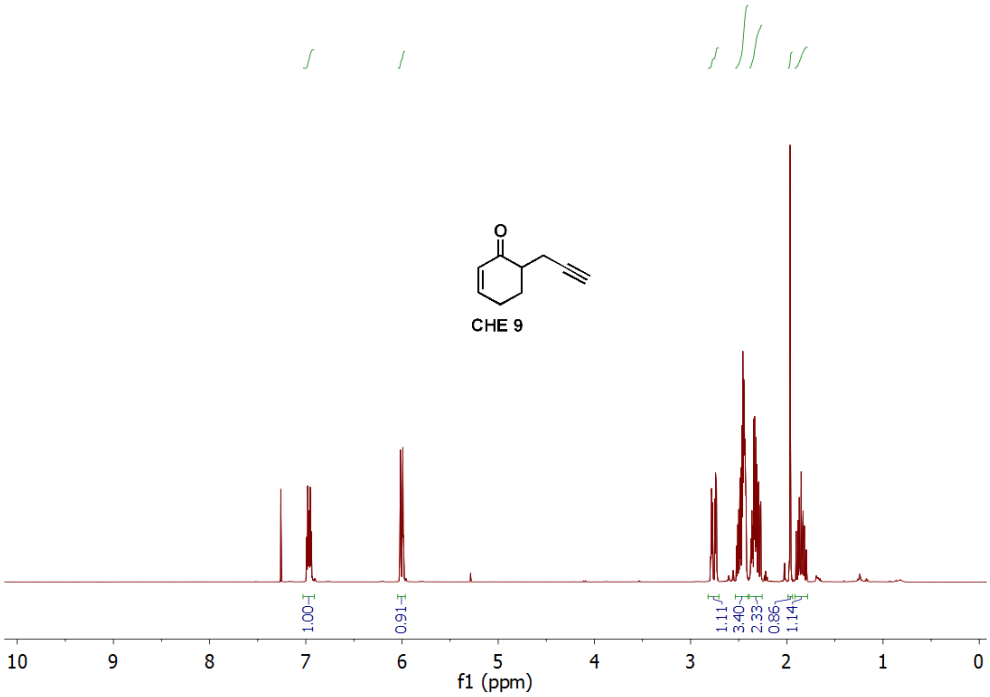
^{1}H-NMR spectrum of DE 8



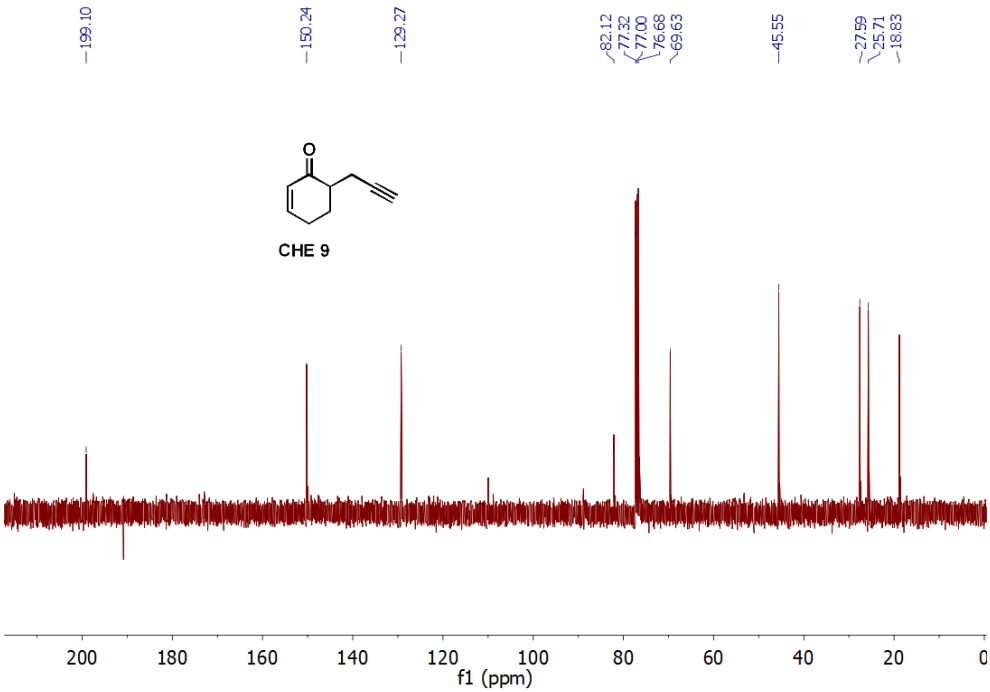
^{13}C-NMR spectrum of DE 8



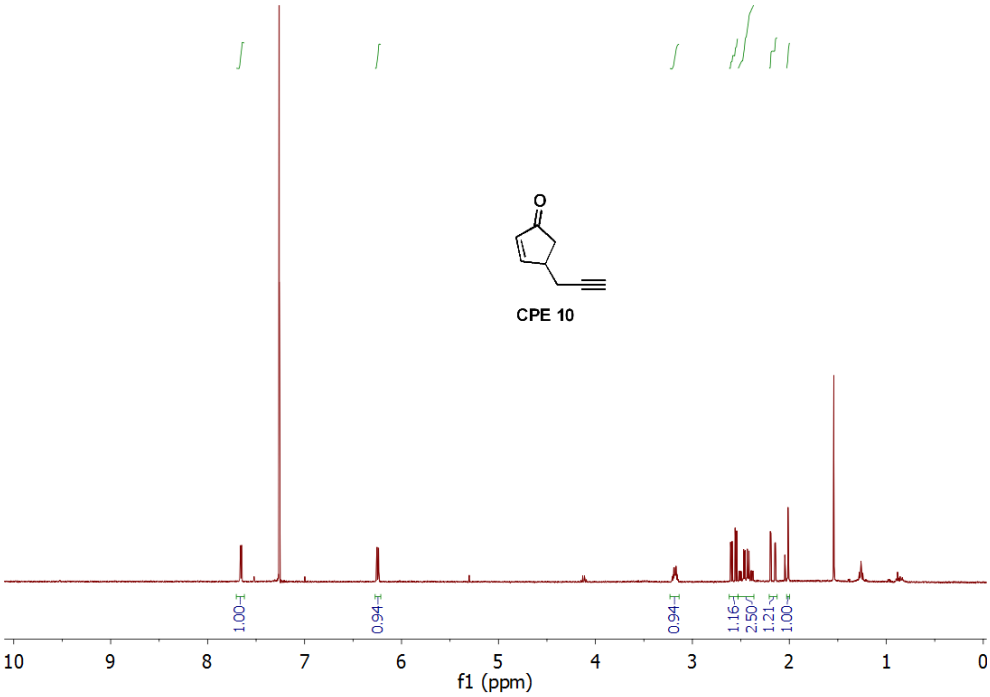
^{1}H-NMR spectrum of CHE 9



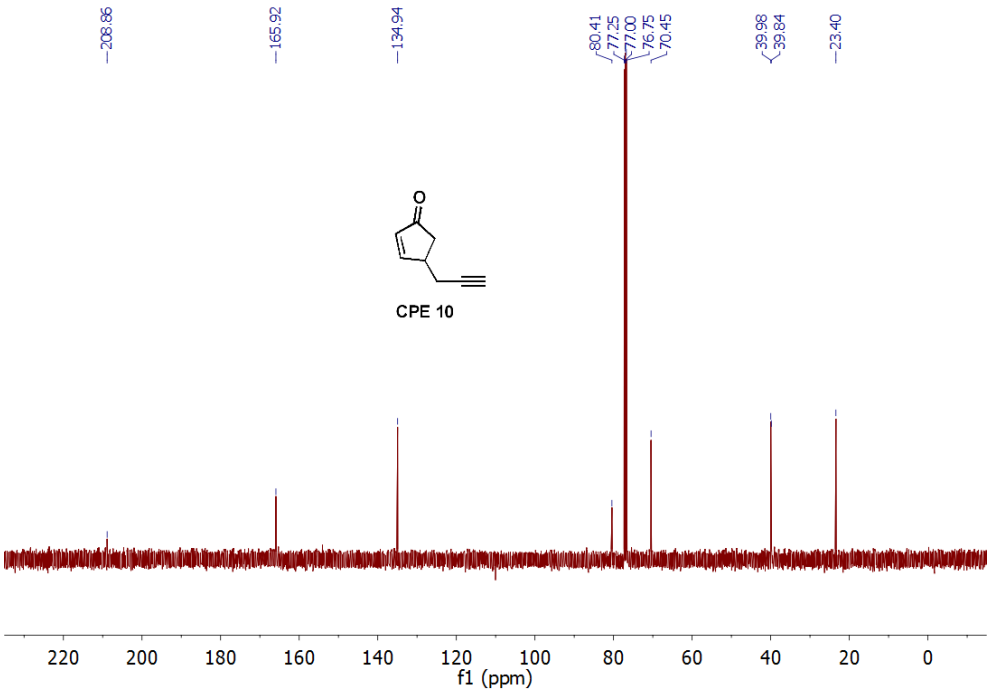
^{13}C-NMR spectrum of CHE 9



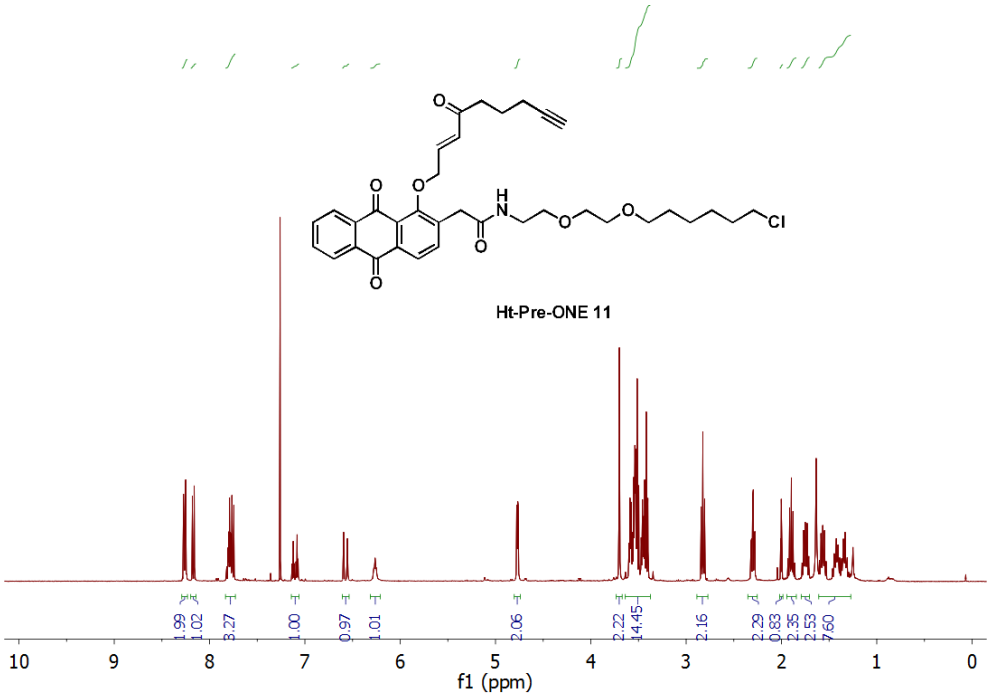
^{1}H-NMR spectrum of CPE 10



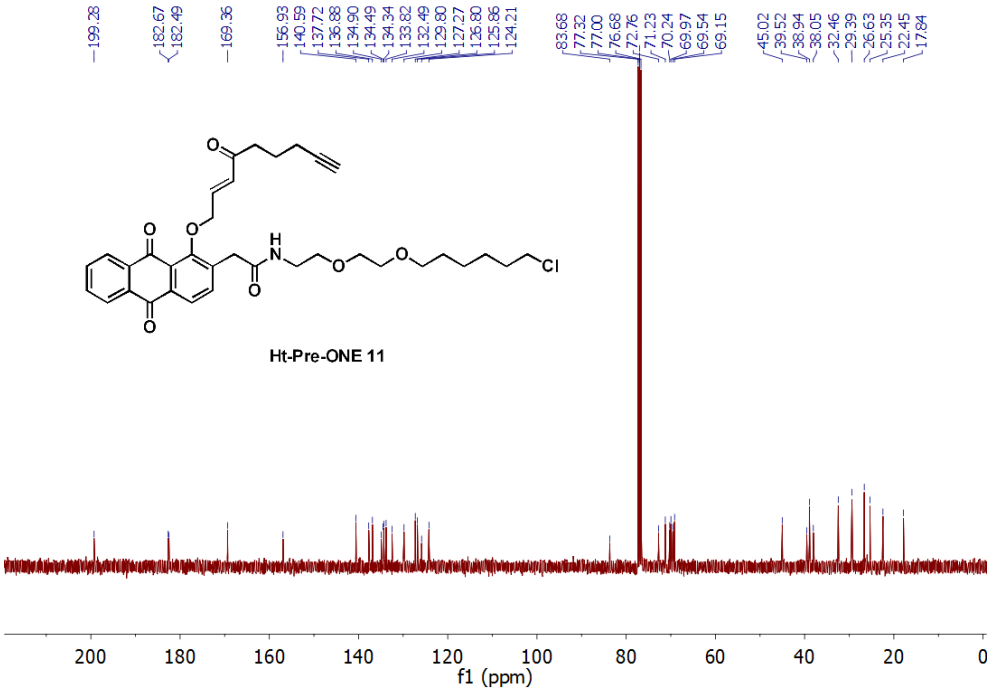
^{13}C-NMR spectrum of CPE 10



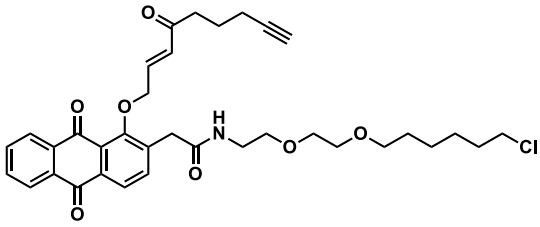
^{1}H-NMR spectrum of Ht-Pre-ONE 11



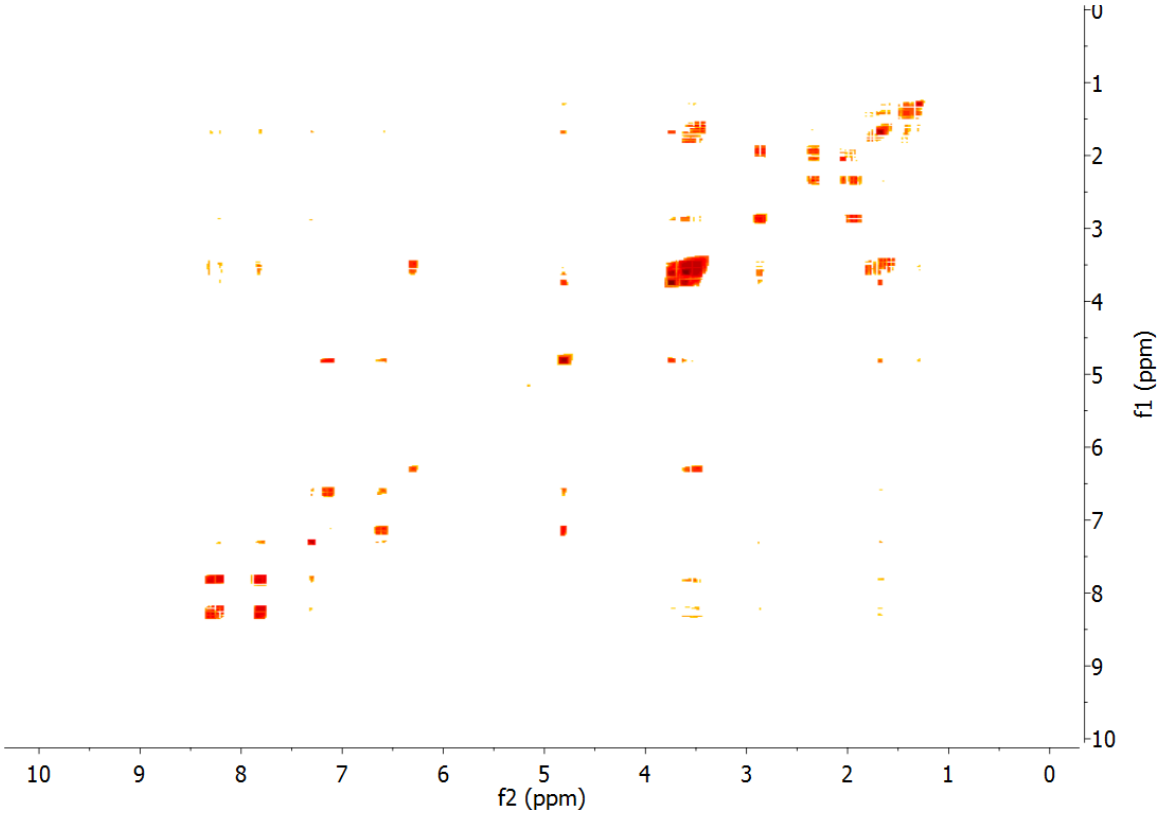
^{13}C-NMR spectrum of Ht-Pre-ONE 11



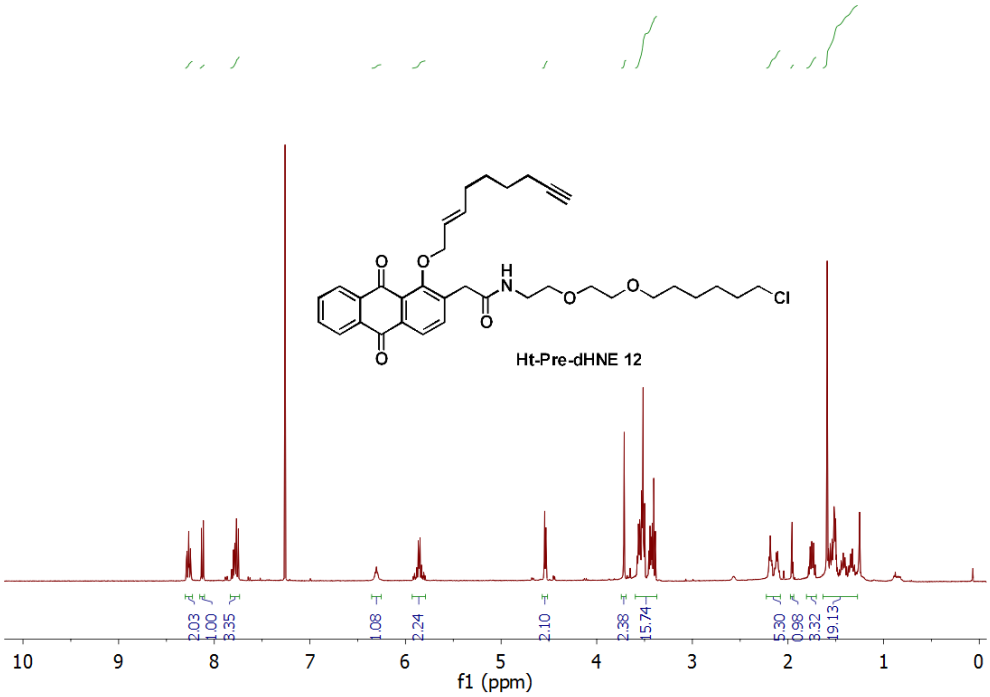
^{1}H-^{1}H-COSY-NMR spectrum of Ht-Pre-ONE 11



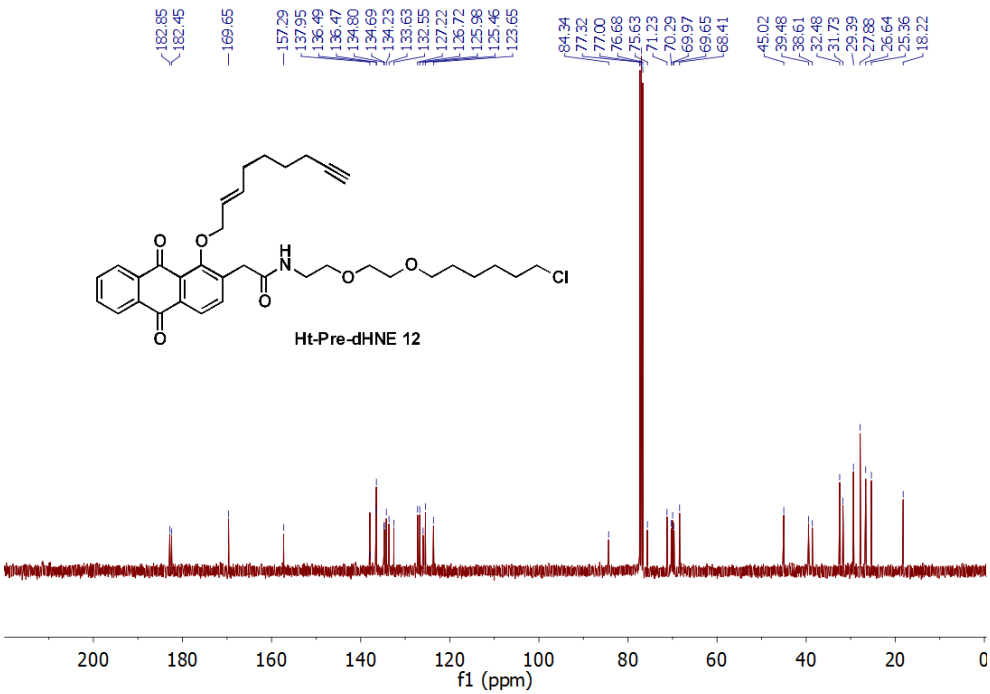
Ht-Pre-ONE 11



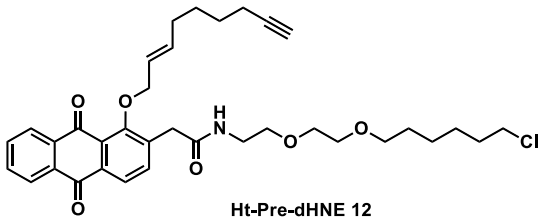
^{1}H-NMR spectrum of Ht-Pre-dHNE 12

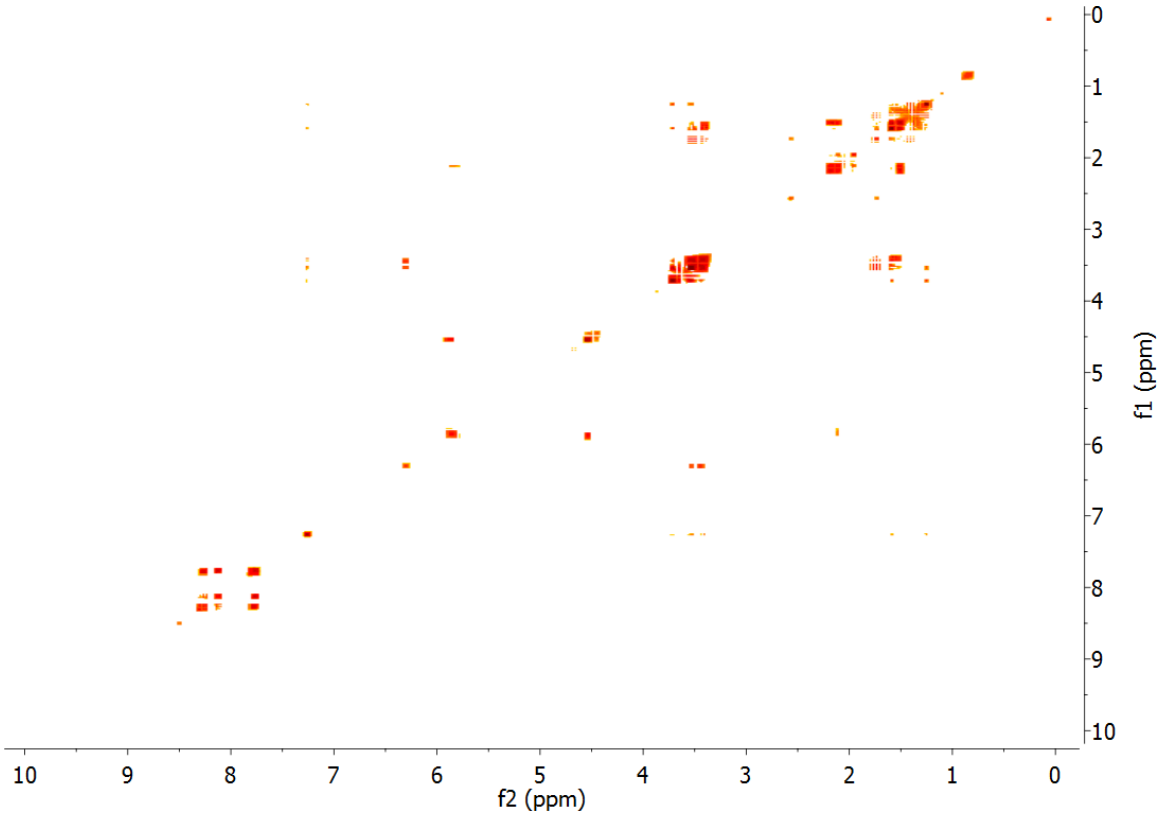


^{13}C-NMR spectrum of Ht-Pre-dHNE 12

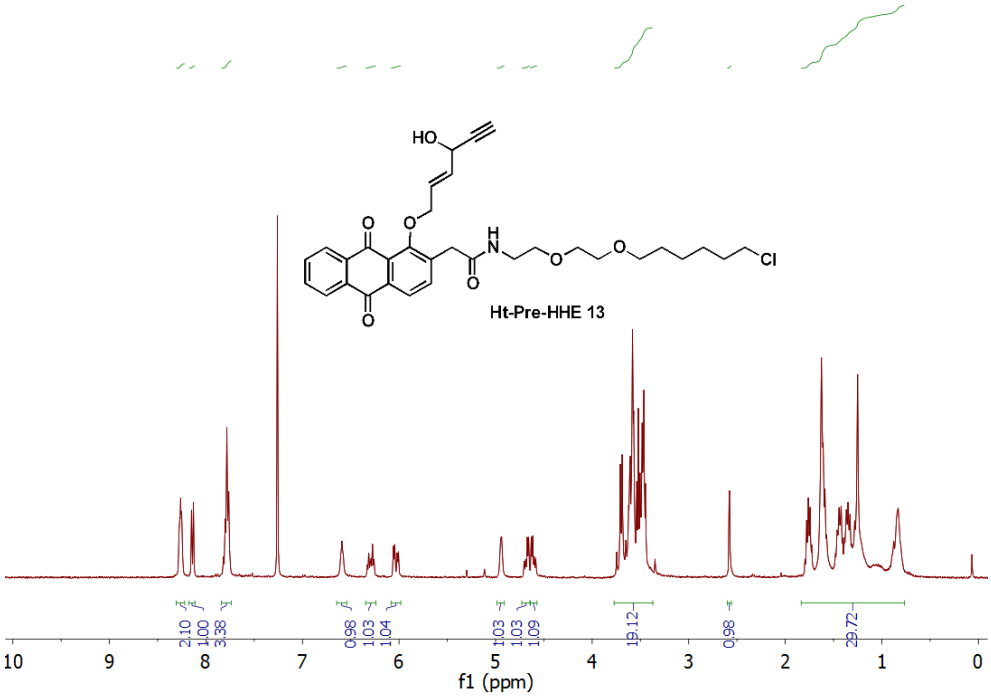


^{1}H-^{1}H-COSY-NMR spectrum of Ht-Pre-dHNE 12

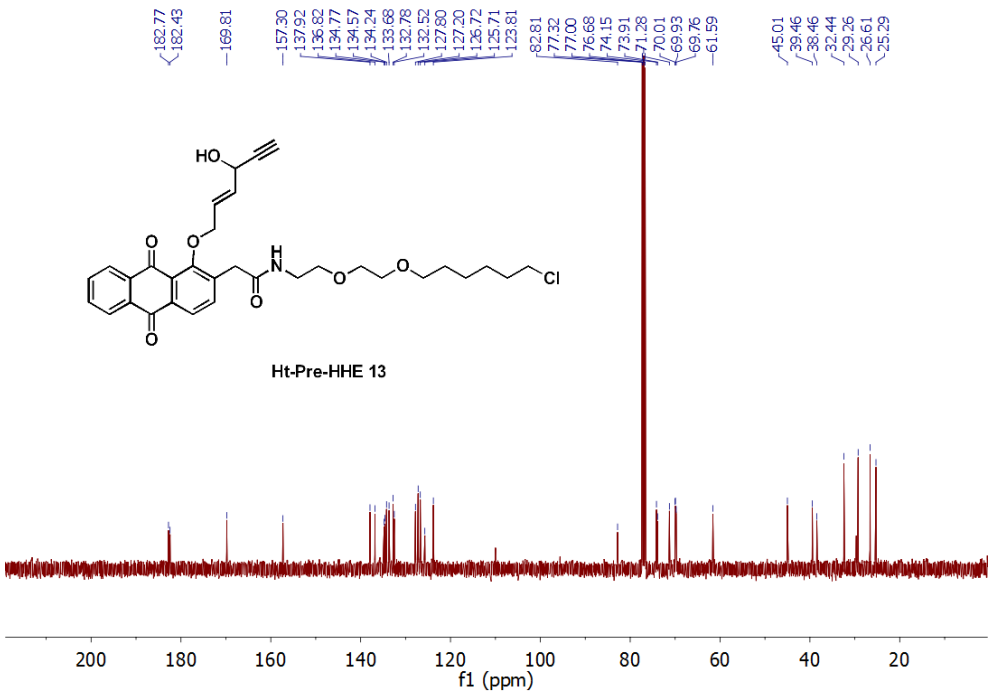




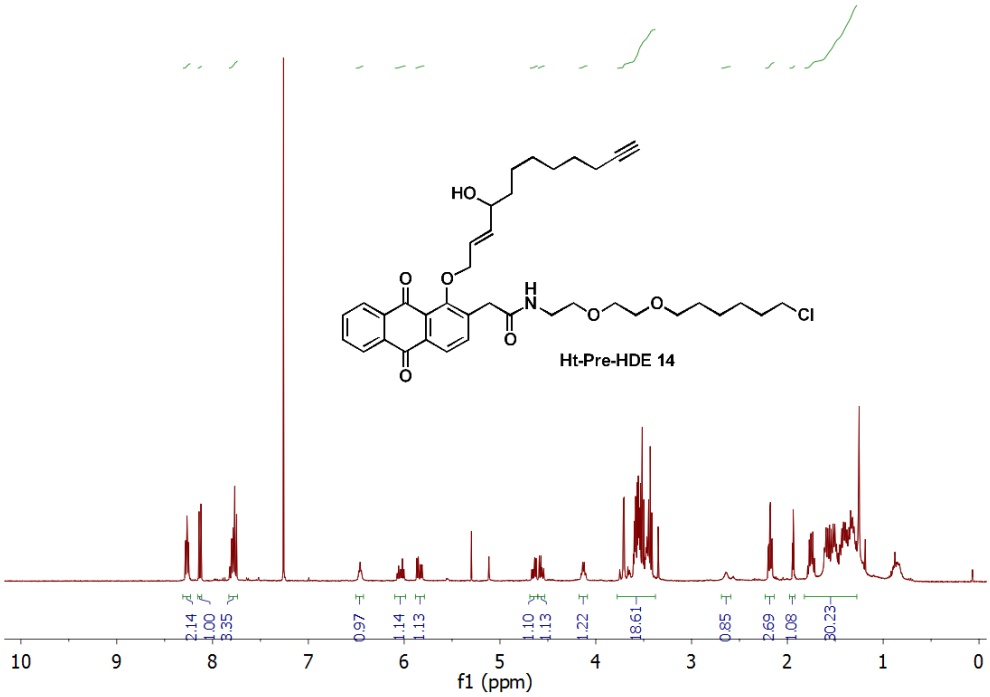
^{1}H-NMR spectrum of Ht-Pre-HHE 13



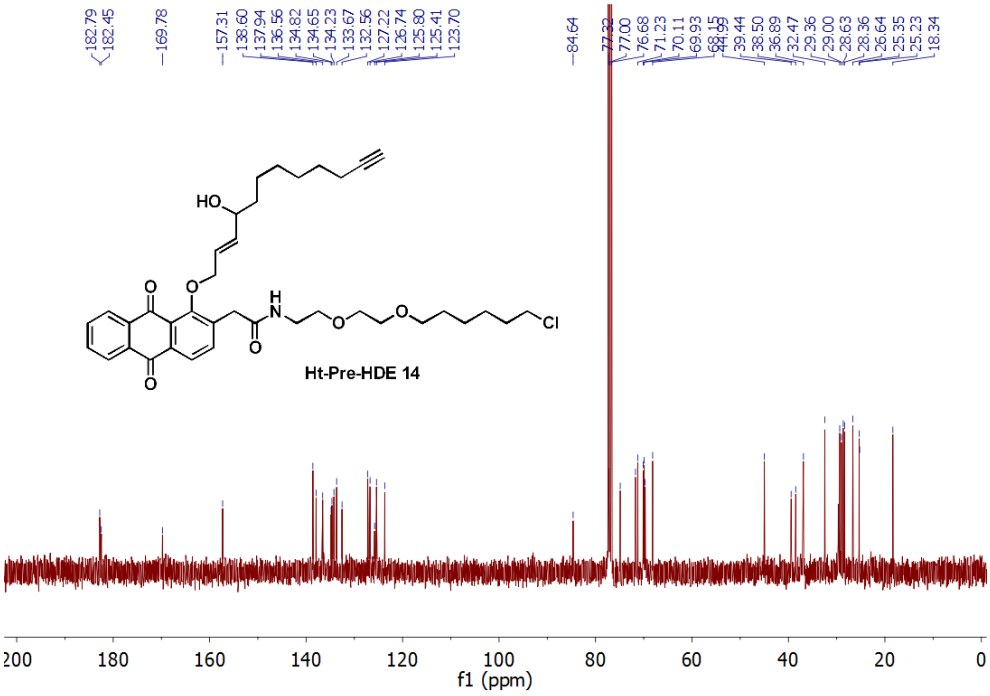
^{13}C-NMR spectrum of Ht-Pre-HHE 13



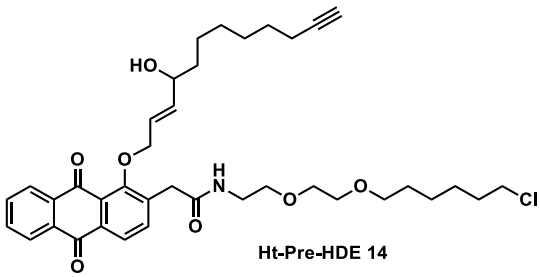
^{1}H-NMR spectrum of Ht-Pre-HDE 14

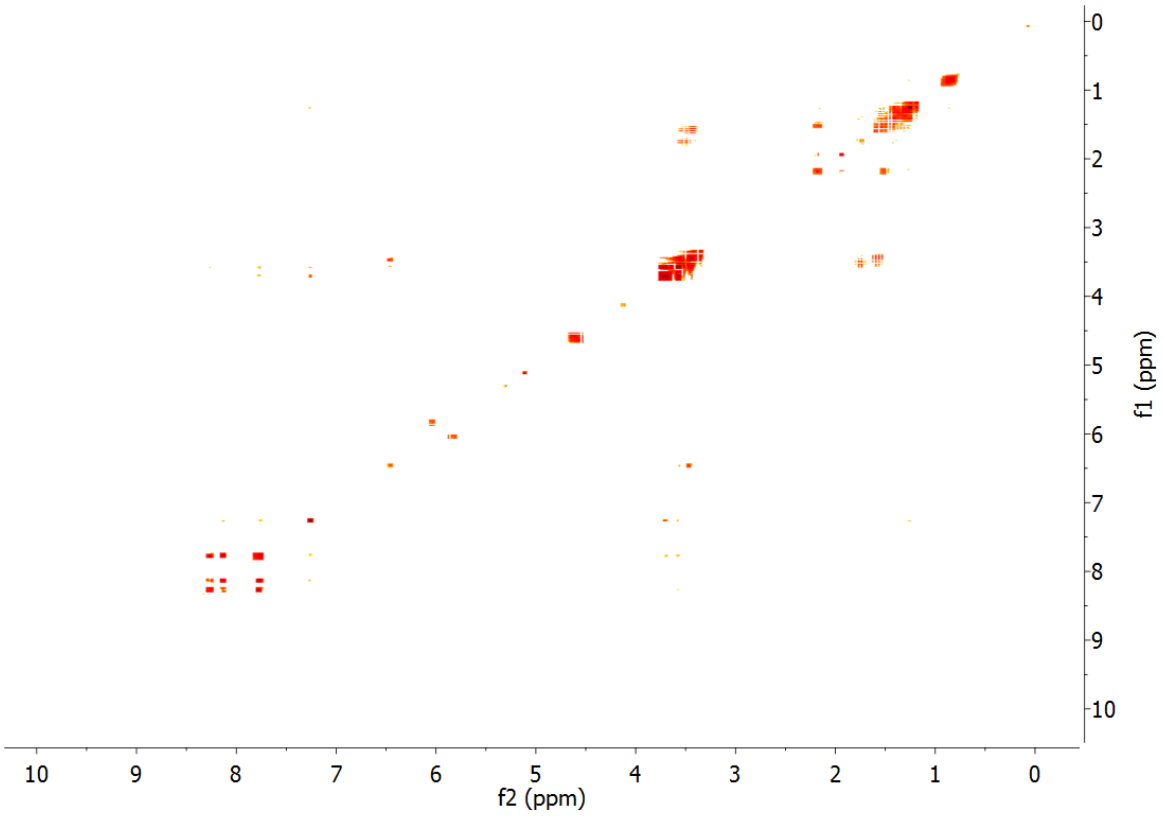


^{13}C-NMR spectrum of Ht-Pre-HDE 14

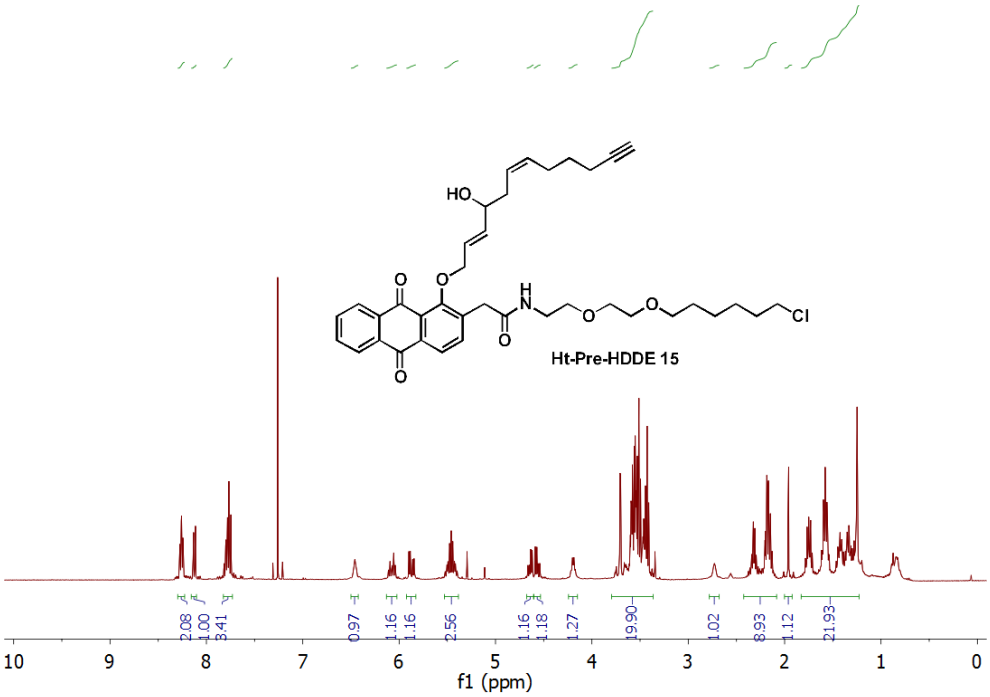


^{1}H-^{1}H-COSY-NMR spectrum of Ht-Pre-HDE 14

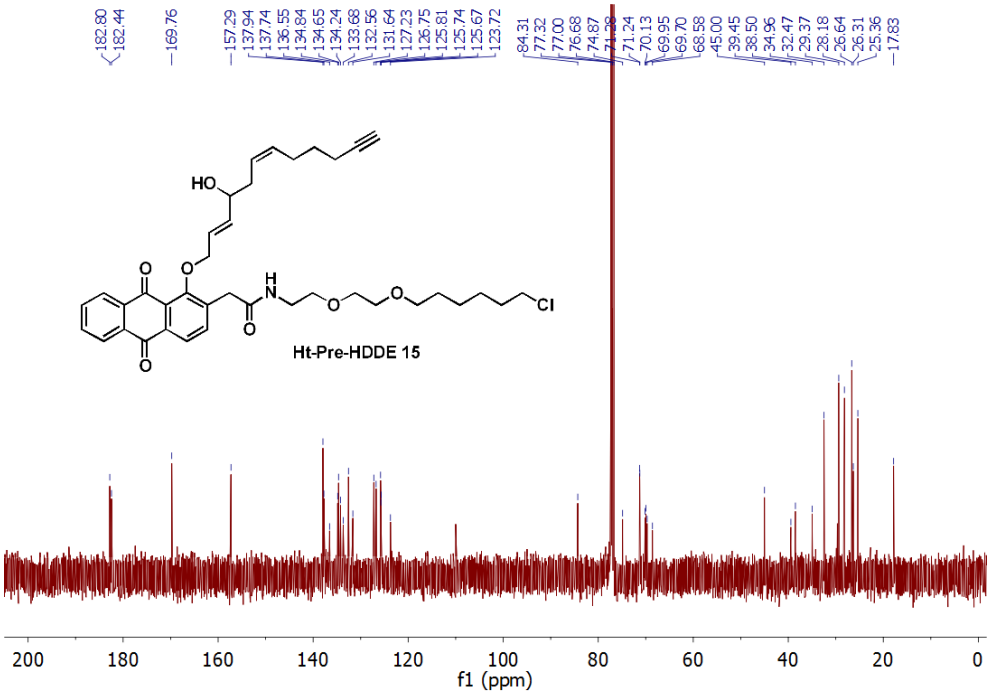




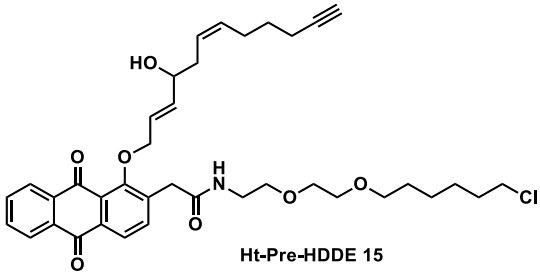
^{1}H-NMR spectrum of Ht-Pre-HDDE 15

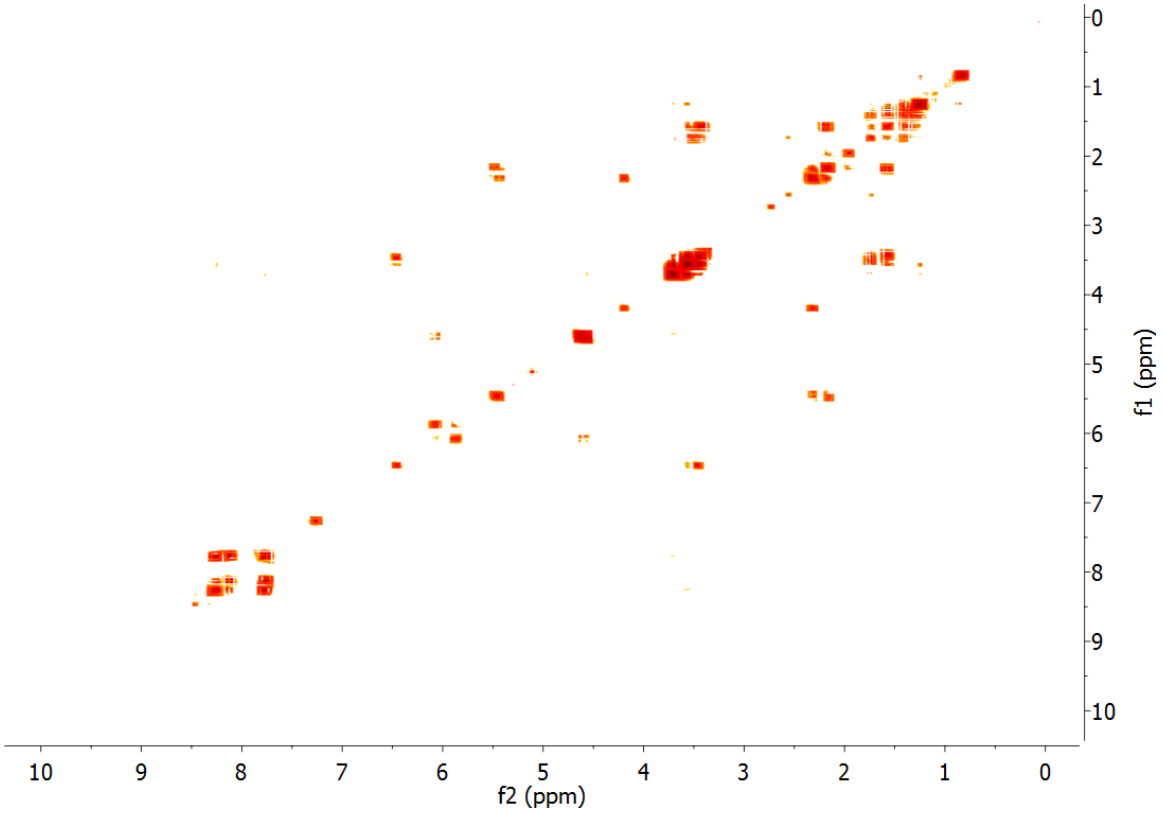


^{13}C-NMR spectrum of Ht-Pre-HDDE 15

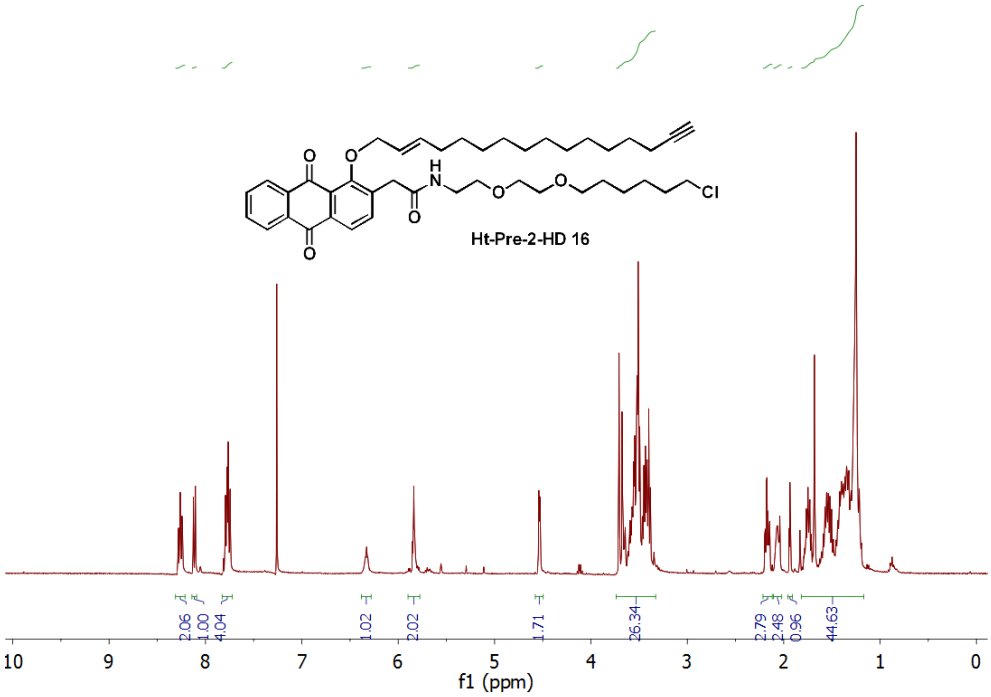


^{1}H-^{1}H-COSY-NMR spectrum of Ht-Pre-HDDE 15

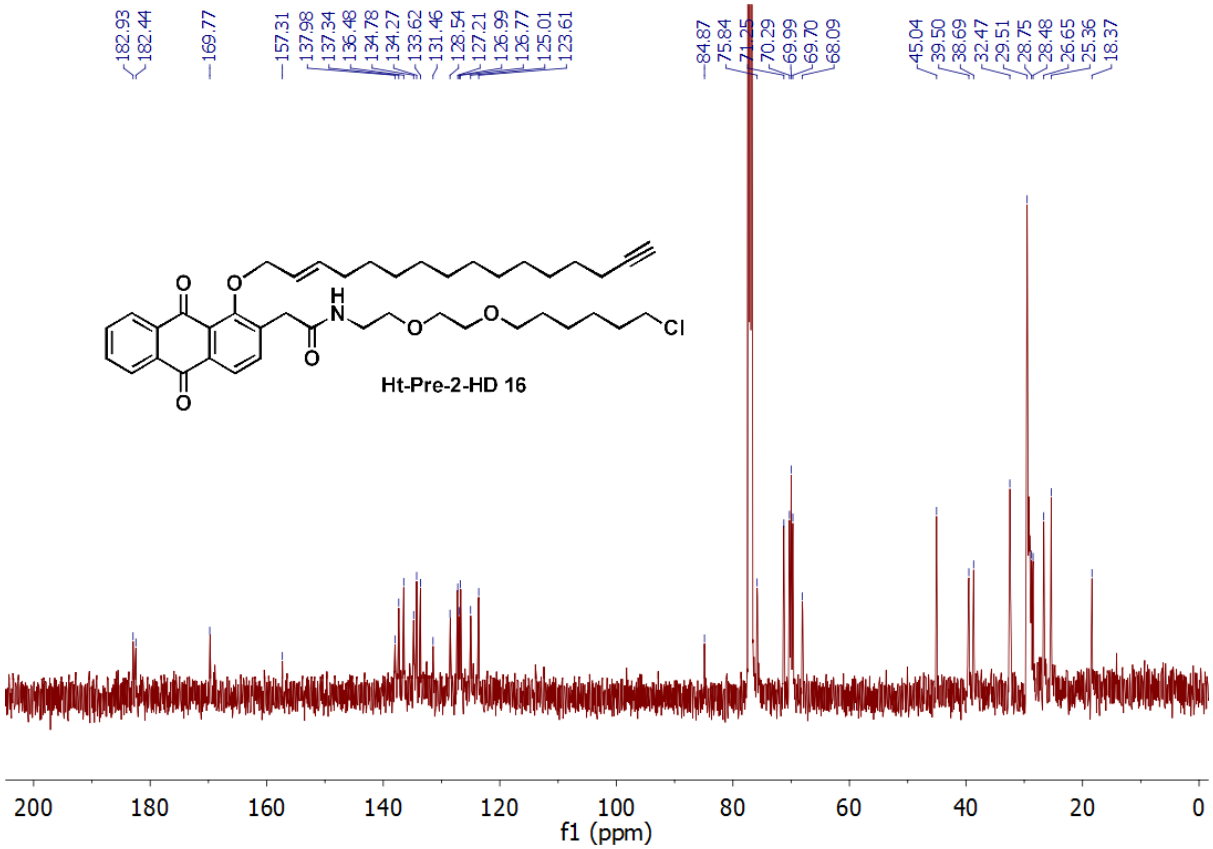




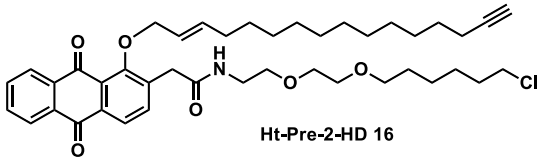
^{1}H-NMR spectrum of Ht-Pre-2-HD 16

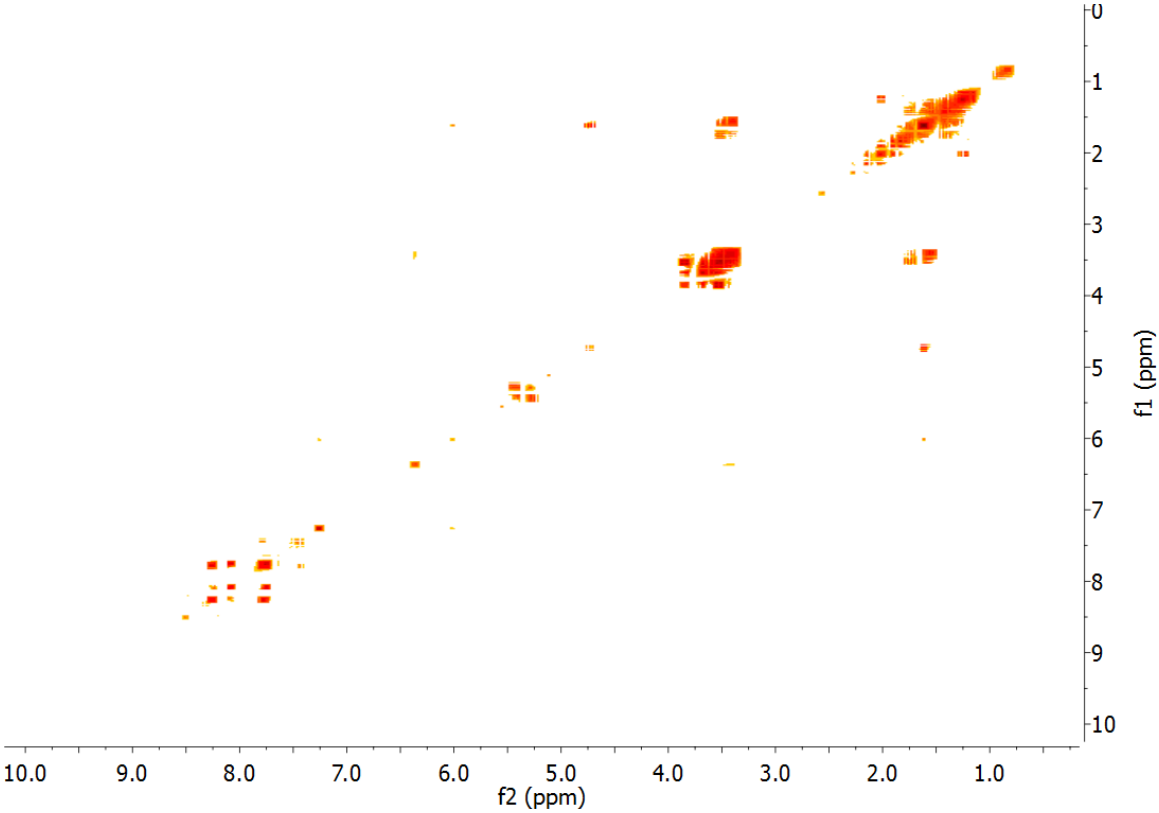


^{13}C-NMR spectrum of Ht-Pre-2-HD 16

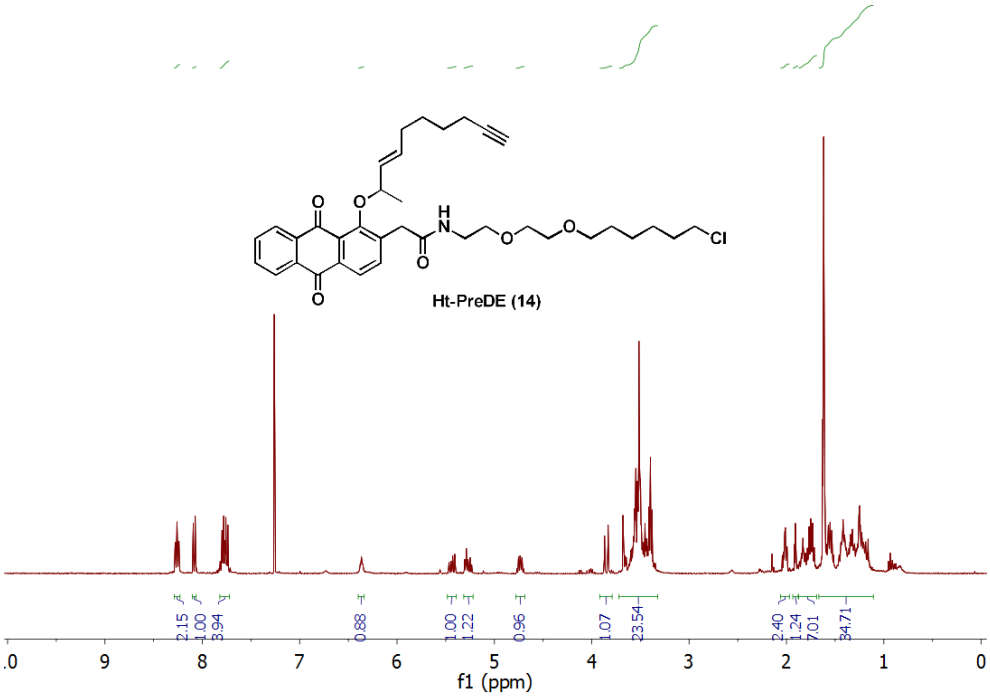


^{1}H-^{1}H-COSY-NMR spectrum of Ht-Pre-2-HD 16

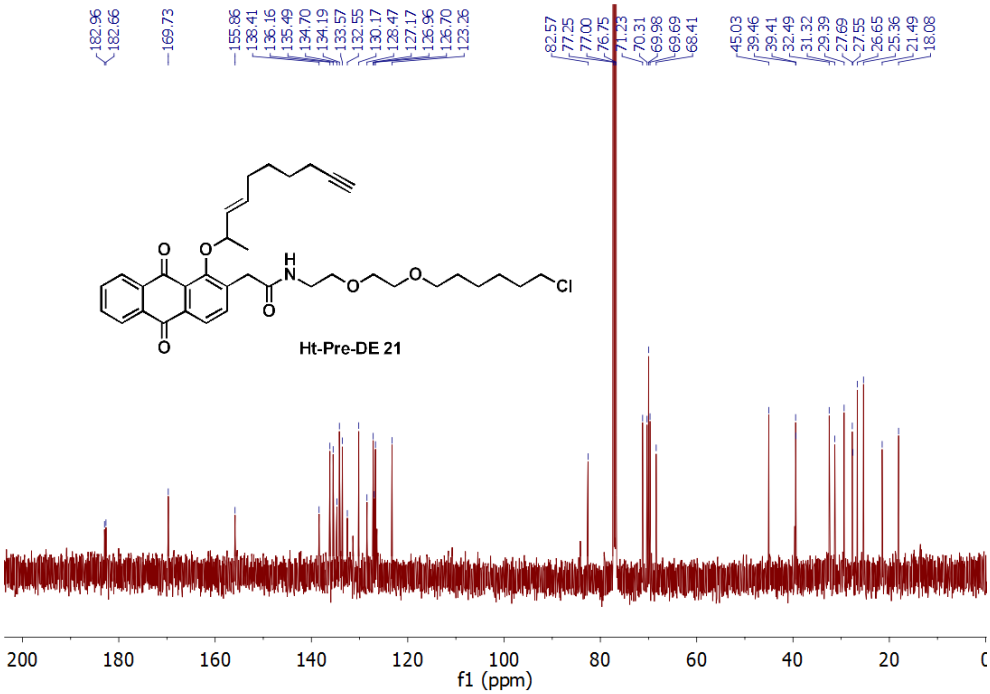




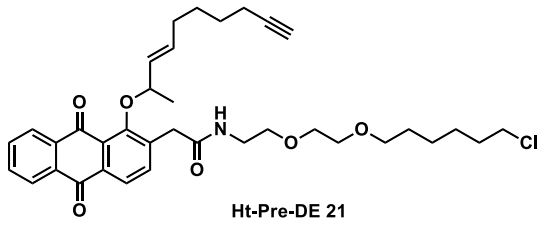
^{1}H-NMR spectrum of Ht-Pre-DE 21

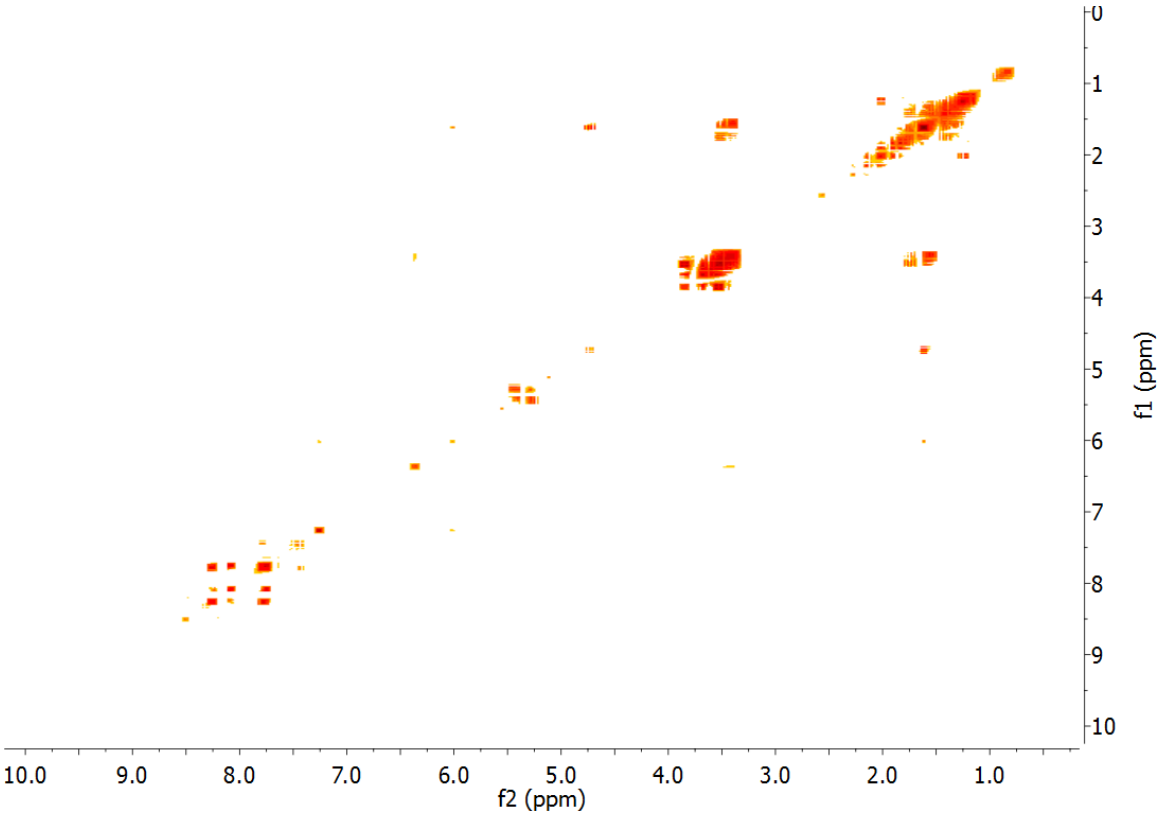


^{13}C-NMR spectrum of Ht-Pre-DE 21

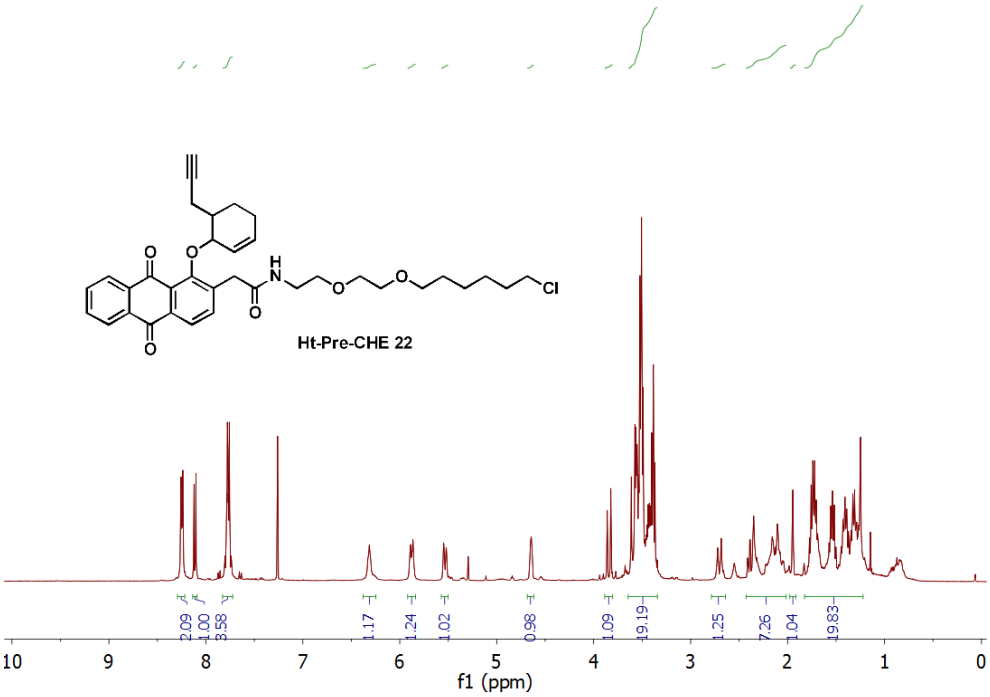


^{1}H-^{1}H-COSY-NMR spectrum of Ht-Pre-DE 21

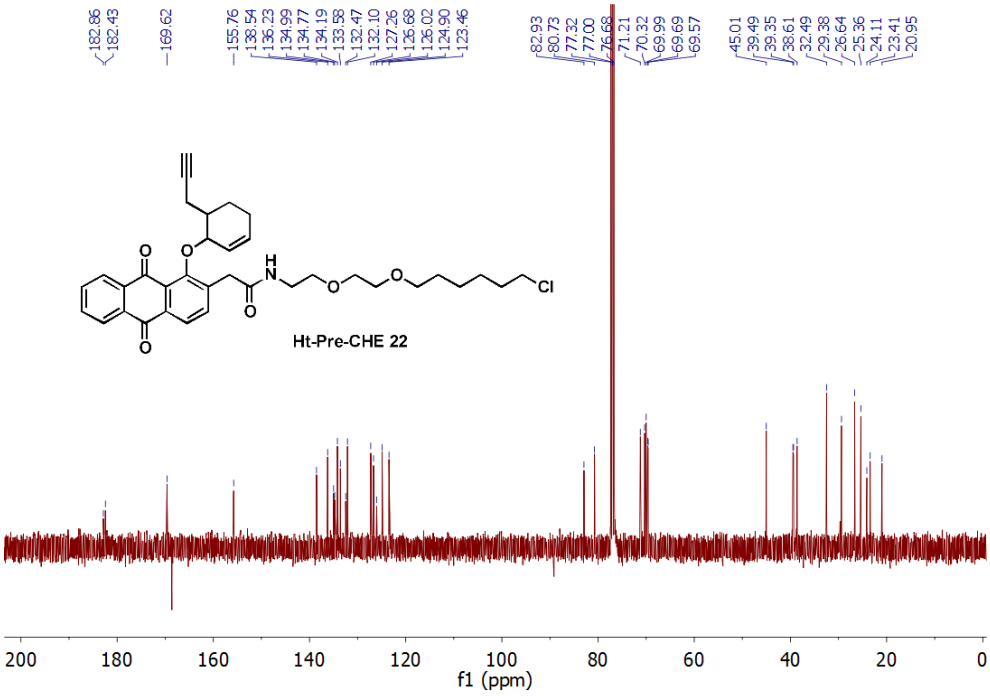




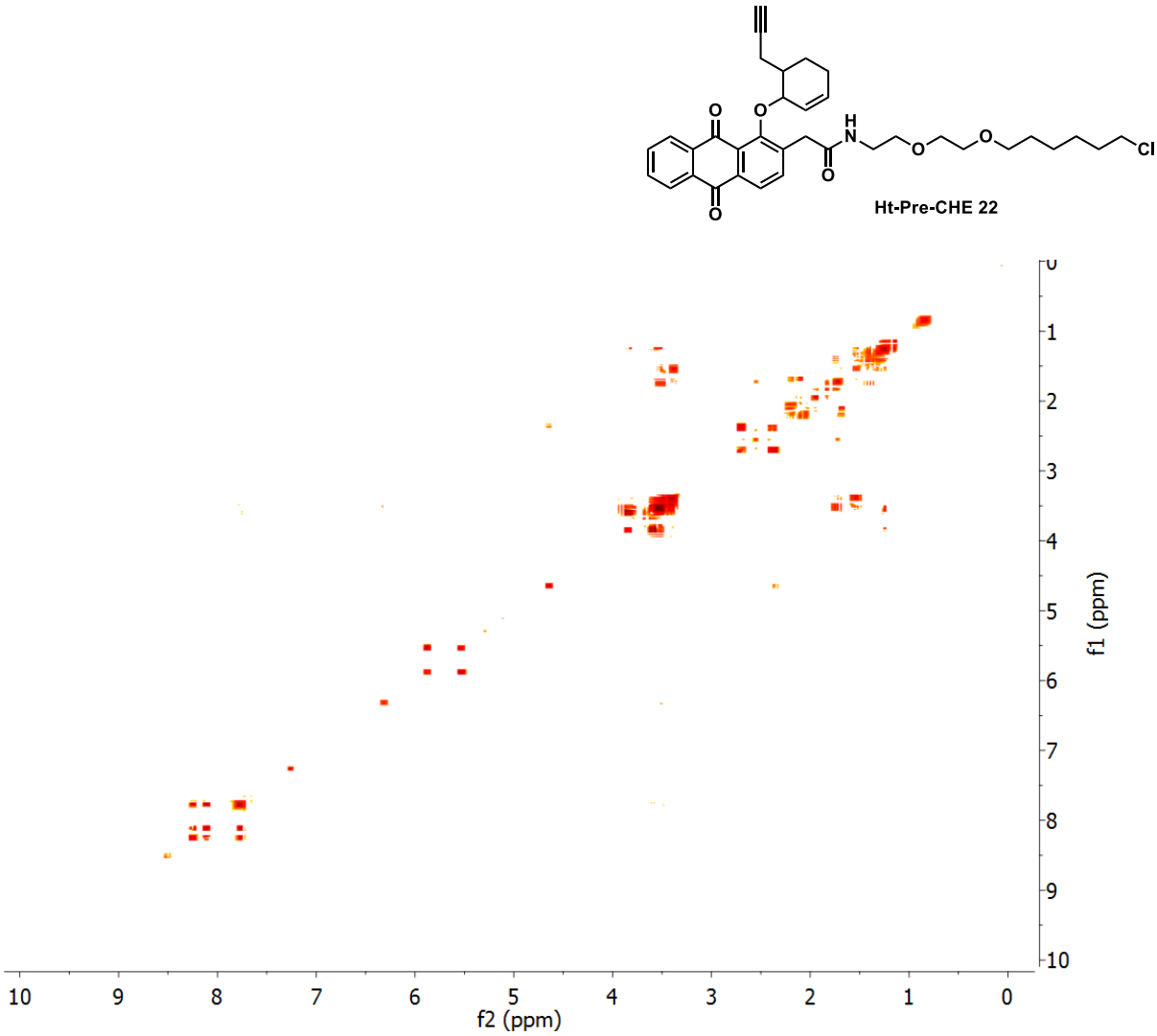
^{1}H-NMR spectrum of Ht-Pre-CHE 22



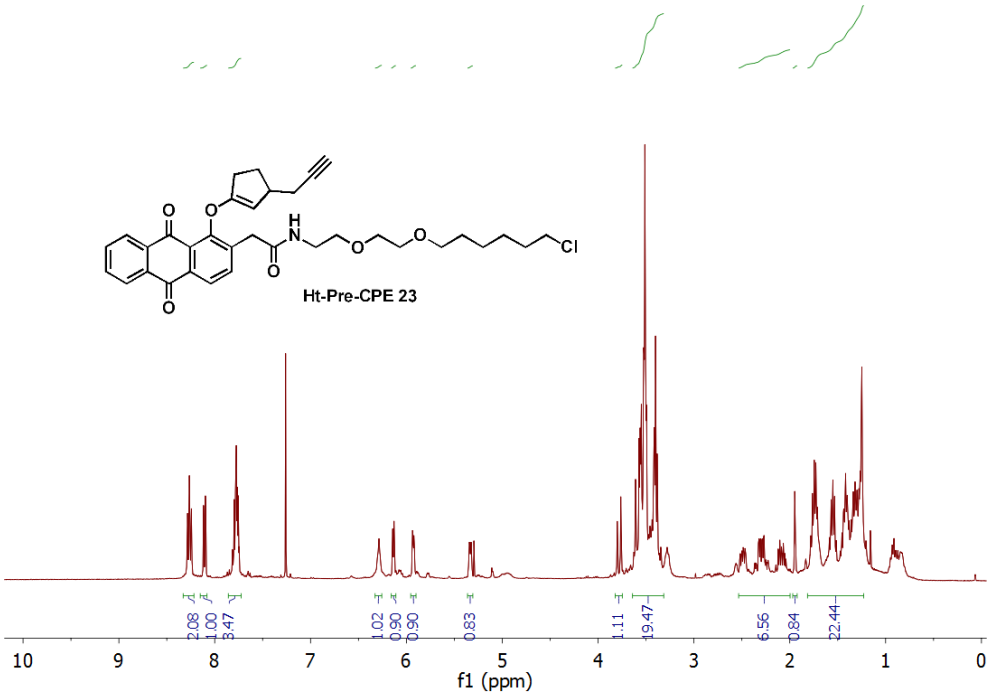
^{13}C-NMR spectrum of Ht-Pre-CHE 22



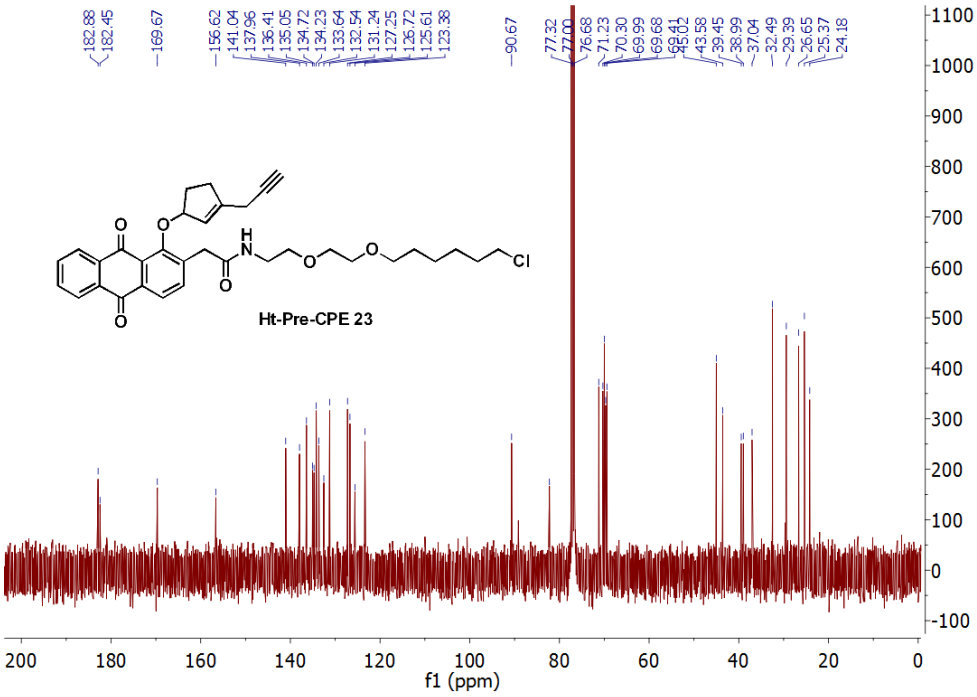
^{1}H-^{1}H-COSY-NMR spectrum of Ht-Pre-CHE 22



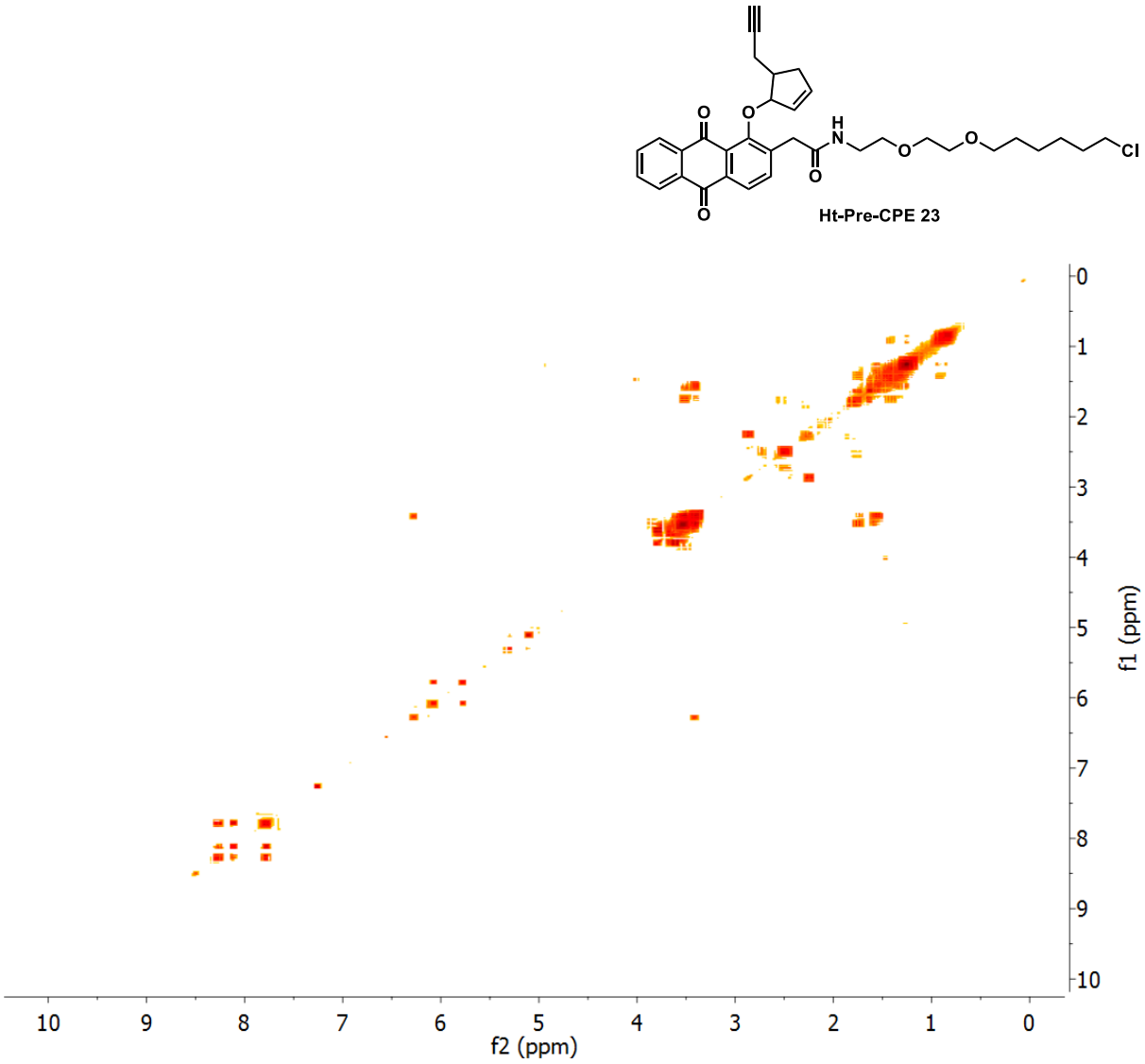
^{1}H-NMR spectrum of Ht-Pre-CPE 23



^{13}C-NMR spectrum of Ht-Pre-CPE 23

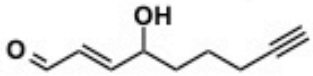
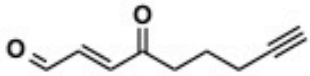
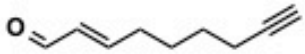
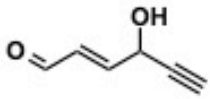
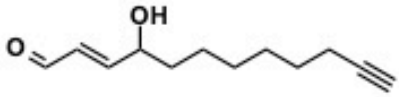
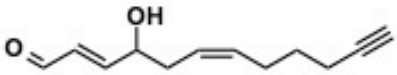
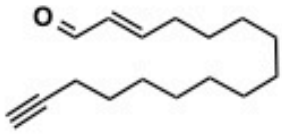
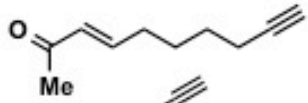
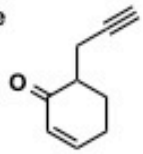
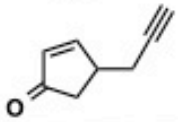


^{1}H-^{1}H-COSY-NMR spectrum of Ht-Pre-CPE 23



SUPPORTING TABLES

Table S1. Release efficiency of reactive electrophiles.

Entry	HaloTag-targetable caged precursor	Liberated reactive electrophiles (alkyne-functionalized)[a]		Release efficiency $t_{1/2}$ (min)[b]
1	Ht-Pre-HNE **17**		**1**	0.93 ± 0.11
2	Ht-Pre-ONE **11**		**2**	21± 11
3	Ht-Pre-dHNE **12**		**3**	0.92 ± 0.33
4	Ht-Pre-HHE **13**		**4**	0.44 ± 0.11
5	Ht-Pre-HDE **14**		**5**	0.41 ± 0.04
6	Ht-Pre-HDDE **15**		**6**	0.28 ± 0.06
7	Ht-Pre-2-HD[c] **16**		**7**	0.60 ± 0.14[c]
8	Ht-Pre-DE **21**		**8**	0.27 ± 0.03
9	Ht-Pre-CHE **22**		**9**	0.43 ± 0.11
10	Ht-Pre-CPE **23**		**10**	0.28 ± 0.3

[a]Racemic where applicable. [b]See Figure S1. [c]See discussion in Main Text.

Table S2. Primers for constructing (a) pET28a_His$_6$-Keap1, (b) pET28a_His$_6$-Halo–Keap1, and (c) pMIR-CMV_DsRed-(IRES)-His$_6$-Keap1 plasmids.

(a) Human Keap1 gene (longest isoform, isoform 1) from the commercially available phrGFP-Keap1 plasmid (Addgene 28025) was cloned into an empty pET28a vector.

Fwd-1:
5´-TGGTGCCTCGTGGTAGCCATATGCAGCCAGATCCCAGGC-3´

Rev-1:
5´-CTCAGCTTCCTTTCGGGCTTTGTTATCAACAGGTACAGTT
CTGCTGGTC-3´

Fwd-2:
5´-ATGGGCAGCAGCCATCATCATCATCATCACAGCAGC
GGCCTGGTGCCTCGTGGTAGCCAT-3´

Rev-2:
5´-TATGCTAGTTATTGCTCAGCGGTGGCAGCAGCCAACTCA
GCTTCCTTTCGGGCTTTGTTA-3´

(b) Human Keap1 gene from phrGFP-Keap1 plasmid (Addgene 28025) was cloned into pET28a_His$_6$_Halo-HA plasmid[1a].

Fwd-1:
5´-TCGAGATTTCCGGCTCCGGAGAAAACTTGTATTTCCAGGG
CTCAGGGATGCAGCCAGATC-3´

Rev-1:
5´-CTCAGCTTCCTTTCGGGCTTTGTTATCAACAGGTACAGTTCT
GCTGGTC-3´

Fwd-2:
5´-GGACCTGATCGGCAGCGAGATCGCGCGCTGGCTGTCGACGCTC
GAGATTTCCGGCTCCGG-3´

Rev-2:
5´-TATGCTAGTTATTGCTCAGCGGTGGCAGCAGCCAACTCAGCTTCC
TTTCGGGCTTTGTTA-3´

(c) Sub-cloning was performed using the plasmid in (a) bearing His_6-Keap1 gene. pMIR vector encoding DsRed-(IRES)-His_6-RNRα was used as a template[1b].

Fwd-1:
5´-TTTTCCTTGAAAAACACGATGATAATATGGCCACAACCATGGGCAGCAGC CATCATC-3´

Rev-1:
5´-TTTAGTACTCTTGAGTCTGGACTTTCTGATCAACAGGTACAGTTCTGCTGGTC-3´

Fwd-2:
5´-ACGTCTAGGCCCCCCGAACCACGGGGACGTGGTTTTCCTTTGAAAAACACG ATGATAATA-3´

Rev-2:
5´-AGTTTTAAGGAAAATCCATTATTATTAAAAGTTTAGTACTCTTGAGTCTGG ACTTTCTGA-3´

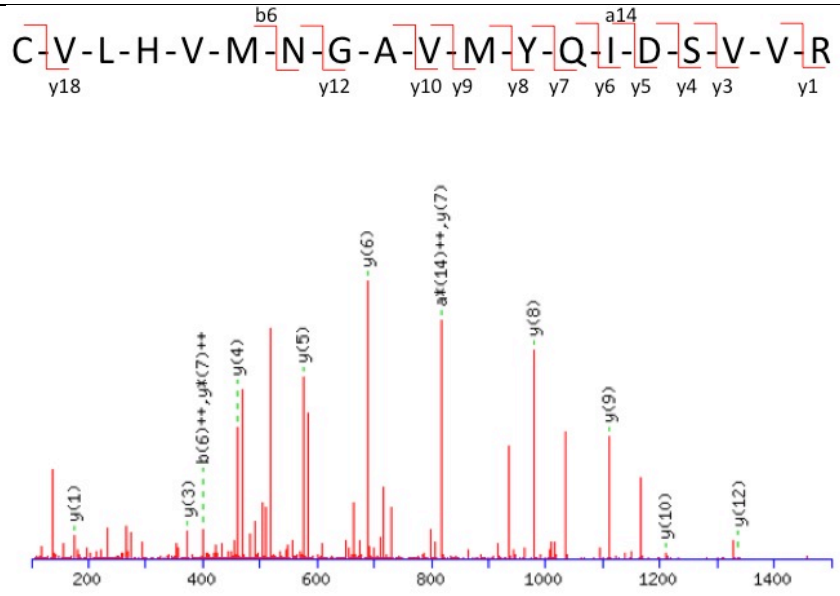
Table S3. Primers for site-directed mutagenesis. Sites of mutations are underlined.

Entry	Plasmid	Primer Sequence
1)	**pMIR-DsRed-(IRES)-His$_6$-Halo-C489S-Keap1**	**pMIR vector encoding DsRed-(IRES)-His$_6$-Halo-Keap1 was used as a template for the site-directed mutagenesis.** **Fwd:** 5'-GCCTTAATTCAGCTGAG**A**GTTACTACCCAGAGAGG-3' **Rev:** Rev: 5'-CCTCTCTGGGTAGTAACTCTCAGCTGAATTAAGGC-3'
2)	**pMIR-DsRed-(IRES)-His$_6$-Halo-C613S-Keap1**	**pMIR vector encoding DsRed-(IRES)-His$_6$-Halo-Keap1 was used as a template for the site-directed mutagenesis.** **Fwd:** 5'-CACCATGGAGCCC**A**GCCGGAAGCAGAT-3' **Rev:** 5'-ATCTGCTTCCGGCTGGGCTCCATGGTG-3'
3)	**pMIR-DsRed-(IRES)-His$_6$-Halo-C489S-C613S-Keap1**	**pMIR vector encoding DsRed-(IRES)-His$_6$-Halo-C613S-Keap1 was used as a template for the site-directed mutagenesis.** **Fwd:** 5'-GCCTTAATTCAGCTGAG**A**GTTACTACCCAGAGAGG-3' **Rev:** Rev: 5'-CCTCTCTGGGTAGTAACTCTCAGCTGAATTAAGGC-3'

Table S4. LC-MS/MS identification of CHE modifications on Keap1 from direct CHE 9 treatment *in vitro*. See also Table S5–S8.

Human Keap1 (100%), 70.0 kDa, Mascot Score 3644, 49 unique peptides with different modifications, 2 distinct tryptic peptides with added mass of 116.06 Da or 134.07 Da for possible modifications of Schiff base CHE(alkyne) Michael adduct (structure **B**) or CHE(alkyne) Michael adduct (structure **A**) (Table S8). Both of the modified peptides were found present in corresponding unmodified forms of the native peptides with Cys being alkylated by carbamidomethylation. 581/624 amino acids (93% coverage). Matched peptide with CHE related modifications shown in green, other matched peptide shown in red.

MQPDPRPSGA GACCRFLPLQ SQCPEGAGDA VMYASTECKA EVTPSQHGNR
TFSYTLEDHT KQAFGIMNEL RLSQQLCDVT LQVKYQDAPA AQFMAHKVVL
ASSSPVFKAM FTNGLREQGM EVVSIEGIHP KVMERLIEFA YTASISMGEK
CVLHVMNGAVMYQIDSVVRA CSDFLVQQLD PSNAIGIANF AEQIGCVELH
QRAREYIYMH FGEVAKQEEF FNLSHCQLVT LISRDDLNVR CESEVFHACI
NWVKYDCEQR RFYVQALLRA VRCHSLTPNF LQMQLQKCEI LQSDSRCKDY
LVKIFEELTL HKPTQVMPCR APKVGRLIYT AGGYFRQSLS YLEAYNPSDG
TWLRLADLQV PRSGLAGCVV GGLLYAVGGR NNSPDGNTDS SALDCYNPMT
NQWSPCAPMS VPRNRIGVGV IDGHIYAVGG SHGCIHHNSV ERYEPERDEW
HLVAPMLTRR IGVGVAVLNR LLYAVGGFDG TNRLNSAECY YPERNEWRMI
TAMNTIRSGA GVCVLHNCIY AAGGYDGQDQ LNSVERYDVE TETWTFVAPM
KHRRSALGIT VHQGRIYVLG GYDGHTFLDS VECYDPDTDT WSEVTRMTSG
RSGVGVAVTM EPCRKQIDQQ NCTC

Unique peptide with CHE-alkyne modification (asterisk suggests modification site)	**MS spectra**
C151 **C*VLHVMNGAV MYQIDSVVR** P-value: 0.00002 Mascot Ion Score: 48 Modification: structure **B** (Table S8) Expectation Values: 0.027	

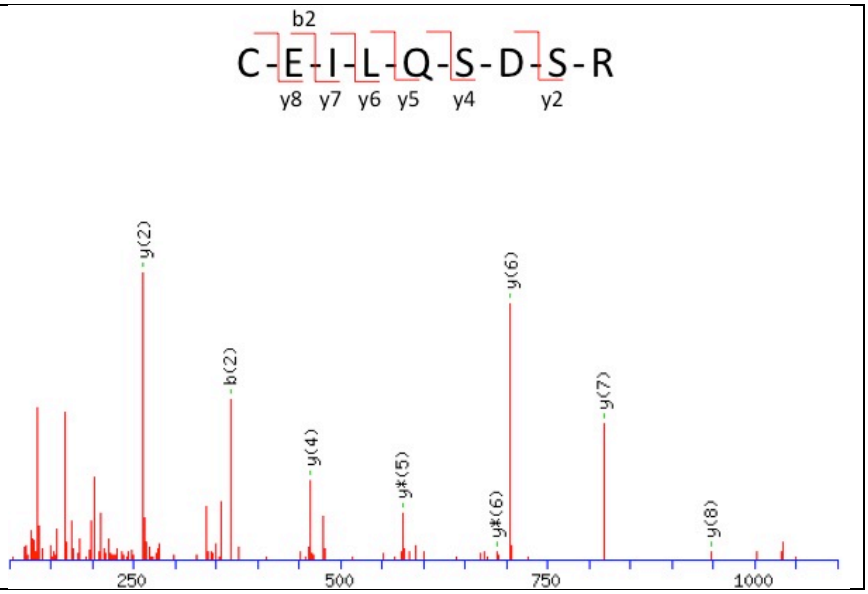
<u>C288</u> **C*EILQSDSR** p-value:0.0003 Mascot Ion Score: 36 Modification: structure **A** (Table S8) Expectation Values: 0.022	

Table S5. LC-MS/MS identification of CHE modifications on Keap1 from targeted CHEylation by using Ht-Pre-CHE 22 *in vitro*. See also Table S4, S6–S8.

Human Keap1 (100%), 70.0 kDa, Mascot Score 2674, 42 unique peptides with different modifications, 1 distinct tryptic peptides with added mass of 116.06 Da for possible modifications of Schiff base CHE(alkyne) Michael adduct (Table S8, structure **B**). The modified peptide was found present in corresponding unmodified forms of the native peptides with Cys being alkylated by carbamidomethylation. 571/624 amino acids (91% coverage). Matched peptide with CHE related modifications shown in green, other matched peptide shown in red.

MQPDPRPSGA GACCRFLPLQ SQCPEGAGDA VMYASTECKA EVTPSQHGNR
TFSYTLEDHT KQAFGIMNEL RLSQQLCDVT LQVKYQDAPA AQFMAHKVVL
ASSSPVFKAM FTNGLREQGM EVVSIEGIHP KVMERLIEFA YTASISMGEK
CVLHVMNGAVMYQIDSVVRA CSDFLVQQLD PSNAIGIANF AEQIGCVELH
QRAREYIYMH FGEVAKQEEF FNLSHCQLVT LISRDDLNVR CESEVFHACI
NWVKYDCEQRRFYVQALLRA VRCHSLTPNF LQMQLQKCEI LQSDSRCKDY
LVKIFEELTL HKPTQVMPCR APKVGRLIYT AGGYFRQSLS YLEAYNPSDG
TWLRLADLQV PRSGLAGCVV GGLLYAVGGR NNSPDGNTDS SALDCYNPMT
NQWSPCAPMS VPRNRIGVGV IDGHIYAVGG SHGCIHHNSV ERYEPERDEW
HLVAPMLTRR IGVGVAVLNR LLYAVGGFDG TNRLNSAECY YPERNEWRMI
TAMNTIRSGA GVCVLHNCIY AAGGYDGQDQ LNSVERYDVE TETWTFVAPM
KHRRSALGIT VHQGRIYVLG GYDGHTFLDS VECYDPDTDT WSEVTRMTSG
RSGVGVAVTM EPCRKQIDQQ NCTC

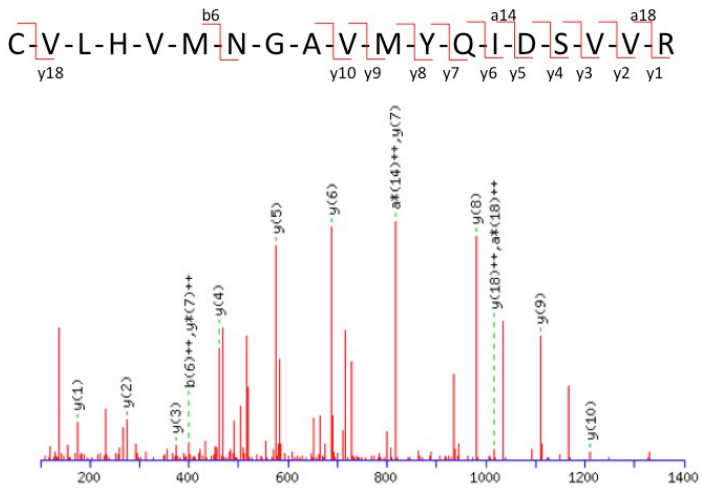
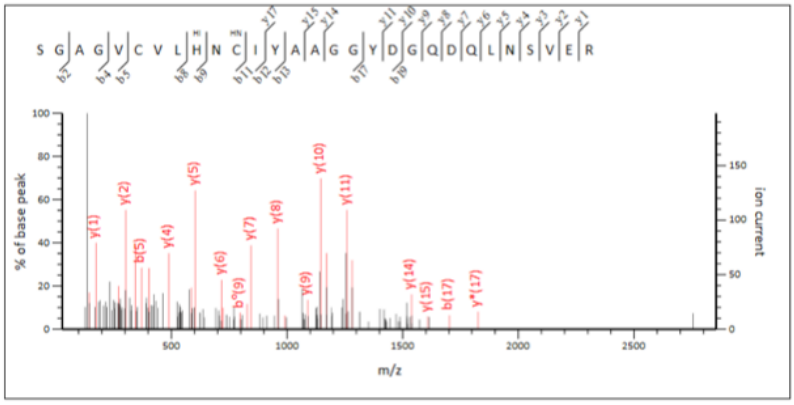
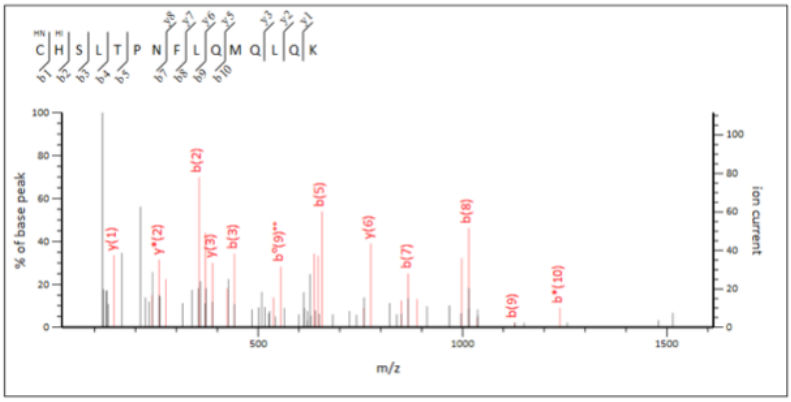
Unique peptide with CHE-alkyne modification (asterisk suggests modification site)	MS spectra
C151 **C*VLHVMNGAV MYQIDSVVR** p-value:0.0001 Mascot Ion Score: 40 Modification: structure **B** (Table S8) Expectation Values: 0.2	

Table S6. LC-MS/MS identification of CHE modifications on Keap1 from direct CHE 9 treatment in live cells ectopically expressing His$_6$-Halo–Keap1. See also Table S4–S5, and S7–S8.

Human Keap1 (100%), 70.0 kDa, Mascot Score 1396, 168 unique peptides with different modifications, 2 distinct tryptic peptides with added mass of 136 Da or 272 Da (2 CHEylation) for possible modifications of reduced CHE(alkyne) Michael adduct (Table S8, structure **E**). Both of the modified peptides were found present in corresponding unmodified forms of the native peptides with Cys being alkylated by carbamidomethylation. 549/624 amino acids (87% coverage). Matched peptide with CHE related modifications shown in green, other matched peptide shown in red.

MQPDPRPSGA GACCRFLPLQ SQCPEGAGDA VMYASTECKA EVTPSQHGNR
TFSYTLEDHT KQAFGIMNEL RLSQQLCDVT LQVKYQDAPA AQFMAHKVVL
ASSSPVFKAM FTNGLREQGM EVVSIEGIHP KVMERLIEFA YTASISMGEK
CVLHVMNGAV MYQIDSVVRA CSDFLVQQLD PSNAIGIANF AEQIGCVELH
QRAREYIYMH FGEVAKQEEF FNLSHCQLVT LISRDDLNVR CESEVFHACI
NWVKYDCEQR RFYVQALLRA VRCHSLTPNF LQMQLQKCEI LQSDSRCKDY
LVKIFEELTL HKPTQVMPCR APKVGRLIYT AGGYFRQSLS YLEAYNPSDG
TWLRLADLQV PRSGLAGCVV GGLLYAVGGR NNSPDGNTDS SALDCYNPMT
NQWSPCAPMS VPRNRIGVGV IDGHIYAVGG SHGCIHHNSV ERYEPERDEW
HLVAPMLTRR IGVGVAVLNR LLYAVGGFDG TNRLNSAECY YPERNEWRMI
TAMNTIRSGA GVCVLHNCIY AAGGYDGQDQ LNSVERYDVE TETWTFVAPM
KHRRSALGIT VHQGRIYVLG GYDGHTFLDS VECYDPDTDT WSEVTRMTSG
RSGVGVAVTM EPCRKQIDQQ NCTC

Unique peptide with CHE-alkyne-derived modification (asterisk suggests modification site)	**MS spectra**
C513¶ and C518¶ **SGAGVC*VLHNC*IYAAGGYDGQDQLNSVER** p-value:0.000000032 Mascot Ion Score: 75 Modification: structure **E** (Table S8) Expectation Values: 0.000000032	

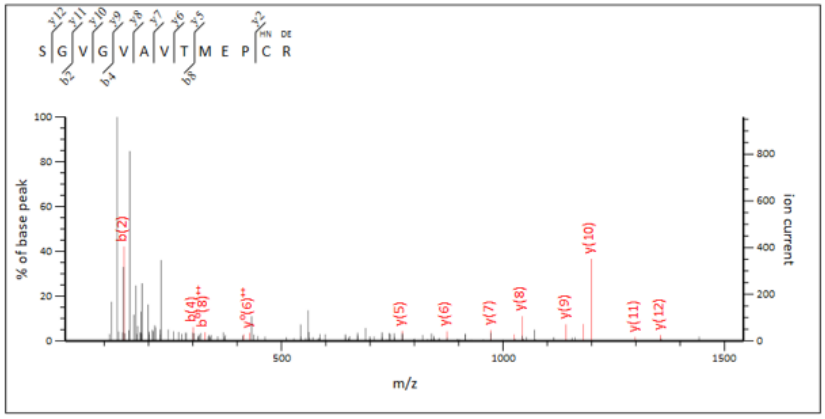
C273‡ **C*HSLTPNFLQMQLQK** p-value: 0.000006 Mascot Ion Score: 52 Modification: structure **E** (Table S8) Expectation Values: 0.000006	

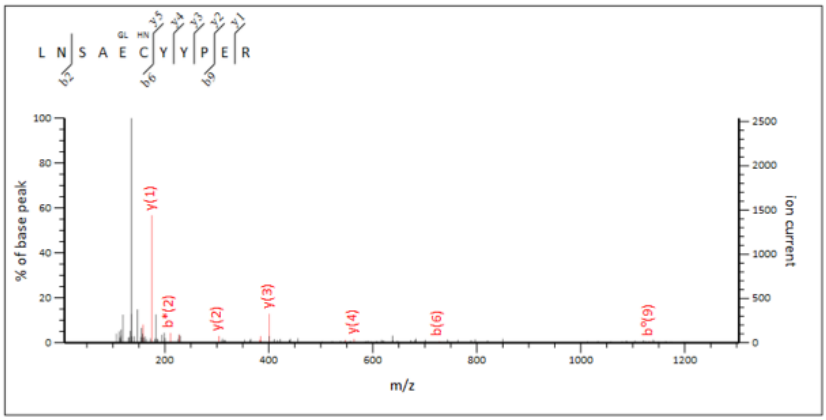
¶Peak integration method showed 2.7% of modified peptide over total peptides of given mass. See Main Text for discussion. Either C513 or C518 was modified. The chances of modification on C513 and C518 were 28.47% and 71.53%, respectively, based on the site analysis results of Mascot database search. ‡Peak integration method showed 1.6% of modified peptide over total peptides of given mass. See Main Text for discussion.

Table S7. LC-MS/MS identification of CHE modifications on Keap1 from targeted CHEylation by using Ht-Pre-CHE 22 in live cells ectopically expressing His$_6$-Halo–Keap1. See also Table S4–S6 and S8.

Human Keap1 (100%), 70.0 kDa, Mascot Score 1159, 68 unique peptides with different modifications, 2 distinct tryptic peptides with added mass of 136.09 Da for possible modifications of reduced CHE(alkyne) Michael adduct (Table S8, structure **E**). Both of the modified peptides were found present in corresponding unmodified forms of the native peptides with Cys being alkylated by carbamidomethylation. 381/624 amino acids (61% coverage). Matched peptide with CHE related modifications shown in green, other matched peptide shown in red.

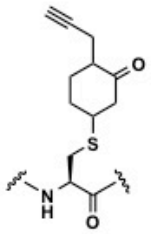
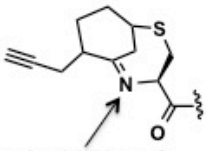
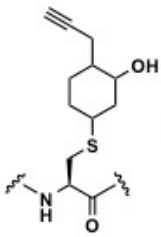
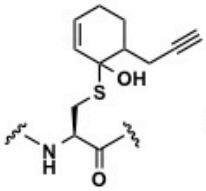
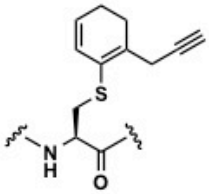
MQPDPRPSGA GACCRFLPLQ SQCPEGAGDA VMYASTECKA EVTPSQHGNR
TFSYTLEDHT KQAFGIMNEL RLSQQLCDVT LQVKYQDAPA AQFMAHKVVL
ASSSPVFKAM FTNGLREQGM EVVSIEGIHP KVMERLIEFA YTASISMGEK
CVLHVMNGAVMYQIDSVVRA CSDFLVQQLD PSNAIGIANF AEQIGCVELH
QRAREYIYMH FGEVAKQEEF FNLSHCQLVT LISRDDLNVR CESEVFHACI
NWVKYDCEQRRFYVQALLRA VRCHSLTPNF LQMQLQKCEI LQSDSRCKDY
LVKIFEELTL HKPTQVMPCR APKVGRLIYT AGGYFRQSLS YLEAYNPSDG
TWLRLADLQV PRSGLAGCVV GGLLYAVGGR NNSPDGNTDS SALDCYNPMT
NQWSPCAPMS VPRNRIGVGV IDGHIYAVGG SHGCIHHNSV ERYEPERDEW
HLVAPMLTRR IGVGVAVLNR LLYAVGGFDG TNRLNSAECY YPERNEWRMI
TAMNTIRSGA GVCVLHNCIY AAGGYDGQDQ LNSVERYDVE TETWTFVAPM
KHRRSALGIT VHQGRIYVLG GYDGHTFLDS VECYDPDTDT WSEVTRMTSG
RSGVGVAVTM EPCRKQIDQQ NCTC

Unique peptide with CHE-alkyne-derived modification (asterisk suggests modification site)	**MS spectra**
C613¶ **SGVGVAVTMEPC*R** p-value:0.005 Mascot Ion Score: 23 Modification: structure **E** (Table S8) Expectation Values: 0.005	

C489‡ **LNSAEC*YYPER** p-value:0.05 Mascot Ion Score: 13 Modification: structure **E** (Table S8) Expectation Values: 0.05	

¶ Peak integration method showed 14.7% of modified peptide over total peptides of given mass. See Main Manuscript for discussion. ‡ Note: Low-confidence modification site (p value = 0.05). See also Main Manuscript for further discussion.

Table S8. Proposed structures for the CHE-derived modifications. Structures resulting from (1,2)-addition on the keto function, **C** and **D**, are less likely due to lability of thio-hemiacetals, compared to (1,4)-addition Michael adducts **A** and **B**. Imine formation in **B** is proposed to occur upon disclosure of free N-terminal amino group post trypsin digest. (For representative examples of analogous bridge-head imines, see: *Liebigs Ann. Chem.* **1982**, 1553–1565; and *Chem. Commun.* **1970**, 1597–1598). Reduced CHE (i.e., adduction by cyclohexenol, instead of cyclohexenone) (proposed structure **E**) was reminiscent of previously characterized reduced HNE-derived modifications of human and mouse Keap1 characterized by us[9] and by McMahon et al,[10] respectively. See also Table S4–S7.

MW + 134.07[a]	MW + 116.06[b]	MW + 136.09[c]
Michael adduct  A C_{288}EILQSDSR	B  Michael adduct (subsequent Schiff base formation) Terminal NH_2 at C_{151} C_{151}VLHVMNGAVMYQIDSVVR	 E Reduced Michael adduct SGVGVAVTMEPC_{613}R LNSAEC_{489}YYPER
(1,2)-addition  C	D  (1,2)-addition followed by dehydration	

[a]Found from global treatment in vitro. [b]Found from both global treatment and T-REX method in vitro. [c]Found from T-REX method in cells. C489 is a low-confidence modification. See Table S7 and also discussion in the Main Manuscript.

Table S9. Summary of antibodies used for the western blot analysis.

Antibody	**Catalog No.; Supplier**	**Dilution**
Mouse monoclonal anti-Keap1 primary	Ab119403 (clone 1B4); Abcam	1:2000
Mouse monoclonal anti-c-myc primary	MMS-150P (clone 9E10); Covance MMS-150P (clone 9E10); BioLegend	1:2000 1:500
Rabbit polyclonal anti-Nrf2 primary	Ab137550; Abcam	1:1000
Rabbit monoclonal anti-Tubullin primary	2125S (clone 11H10); Cell Signaling	1:5000
Rabbit polyclonal anti-HaloTag® primary	9281; Promega	1:1000
Rabbit polyclonal Anti-GFP primary	sc-8334; Santa Cruz Biotechnology	1:1000
Monoclonal anti-gapdh-peroxidase	G9295; Sigma	1:30000
Secondary antibody to mouse	Ab6789; Abcam	1:7000
Secondary antibody to rabbit	Ab97051; Abcam	1:7000

SUPPORTING FIGURES

Figure S1.

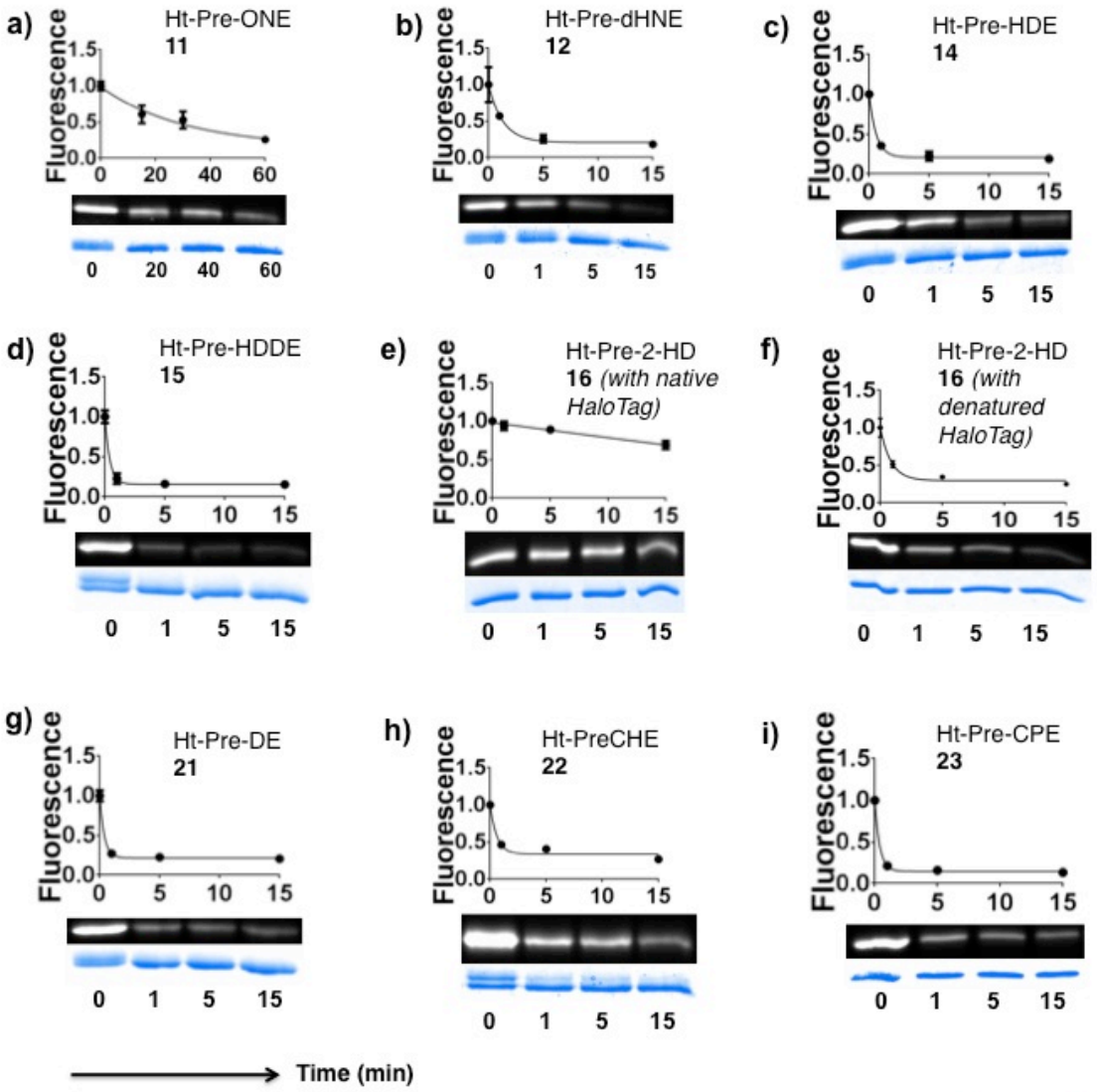


Figure S1. Release efficiency of HaloTag-targetable caged precursors in Table S1 determined by time-dependent depletion of Cy5 signal from HaloTag in solution. See also Figure 4 and Table S1. In-gel fluorescence and Coomassie-stained gel data are shown underneath each release curve. Time (X-axis) indicates time of illumination in min. Fluorescence (Y-axis) designates normalized Cy5 intensity with respect to protein loading for each time point. The value at time zero is set as 1.0. $t_{1/2}$ of electrophile release is estimated by fitting the data to one-phase exponential decay equation (Prism v. 6.0).

Figure S2.

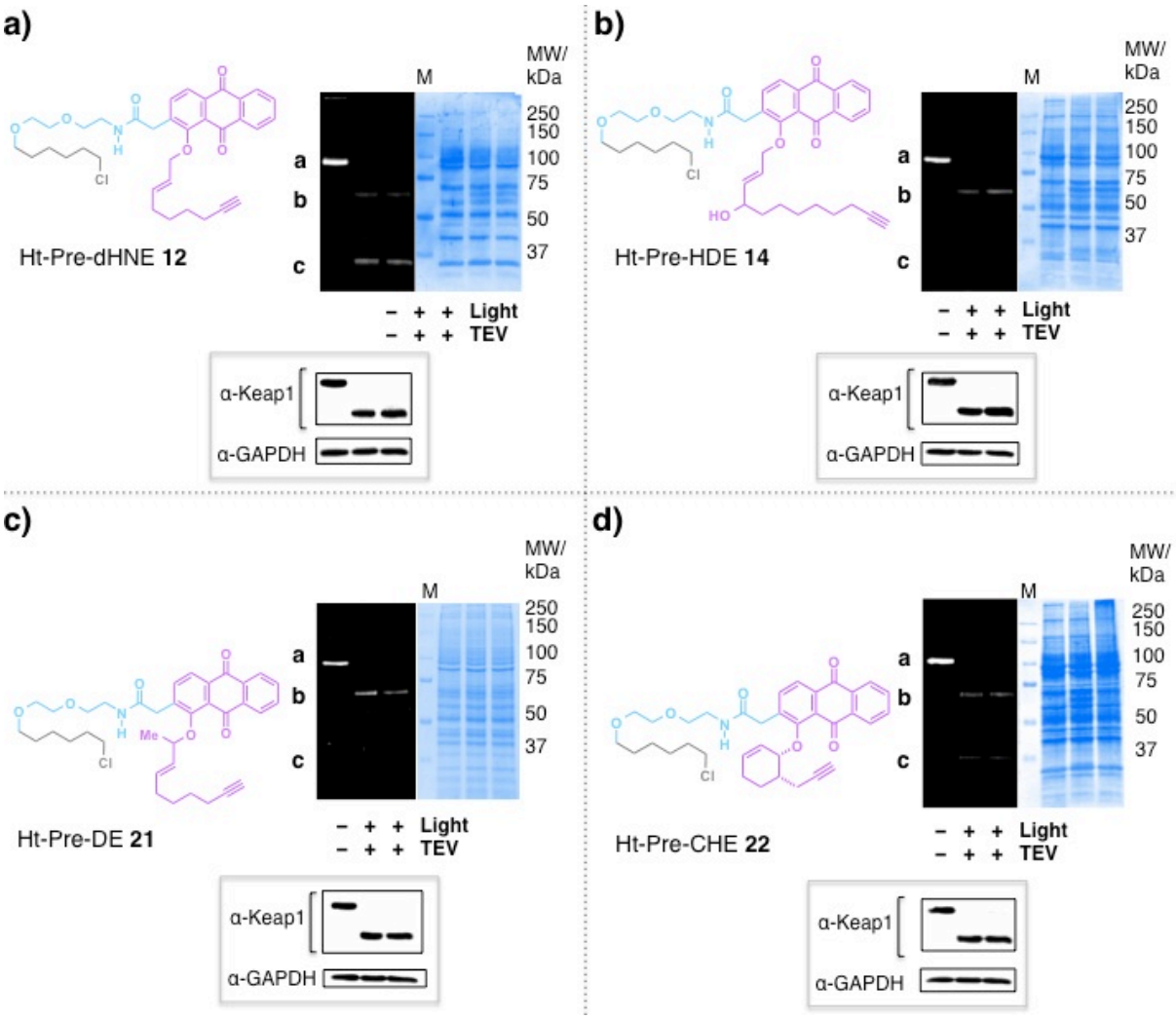


Figure S2. Representative in-gel fluorescence data for T-REX targeted delivery of reactive electrophiles dHNE **3**, HDE **5**, DE **8**, and CHE **9** to Keap1 in HEK-293 cells. Live cells stably expressing Halo–Keap1 were treated with respective HaloTag-targetable caged precursors **12**, **14**, **21** and **22** and exposed to 20-min light activation (365 nm at 600 μW/cm^2). See also Figure 6 and Table 1. In each gel, the lanes corresponding to both light and TEV-protease treatment originate from two independent biological samples. **a**, **b**, and **c** designate Halo-Keap1 (103 kDa), Keap1 (70 kDa, post TEV-cleavage), and Halo (33 kDa), respectively. Inset in each case shows western blot probed with antibodies to Keap1 (Ab119403) and GAPDH (Sigma G9295). "M" designates ladder lanes. % Delivery efficiency was calculated as shown in Eq 1 (Main Manuscript).

Figure S3.

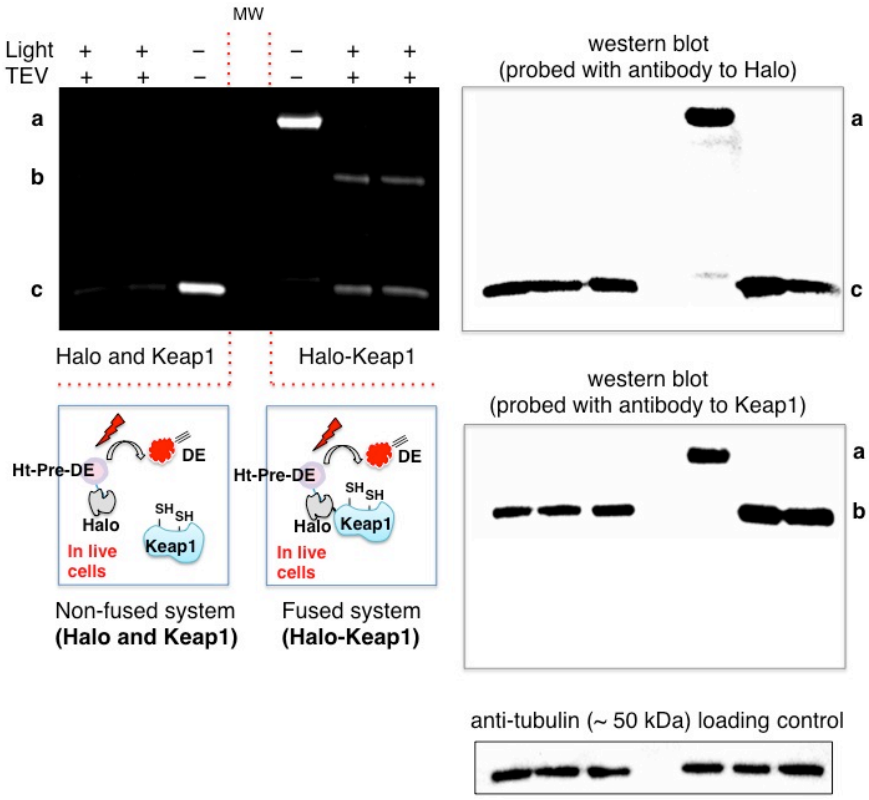


Figure S3. Analysis of T-REX-assisted DE 8 delivery on the non-fused Halo and Keap1 proteins compared with the results from Halo-Keap1 fusion protein system using Ht-Pre-DE 21. The left-hand data set of the fluorescence gel originates from executing T-REX method in HEK-293 cells transiently overexpressing Halo protein and Keap1 protein separately. The right-hand data set results from transient overexpression of Halo-Keap1 fusion protein under otherwise identical experimental conditions. The two panels below the fluorescence gel illustrate the respective setups. Independent duplicates are presented in each case for samples treated with both light and TEV protease. Corresponding western blot data sets are also shown. MW designates the gel lane comprising molecular weight markers. **a**, **b**, and **c** designate the bands corresponding to Halo–Keap1 (104 kDa), Keap1 (70 kDa, post TEV cleavage), and Halo (33 kDa), respectively. Tubulin (50 kDa) is used as loading control. See also Figure 7 in Main Text. See Table S9 for primary antibodies and dilutions.

Figure S4.

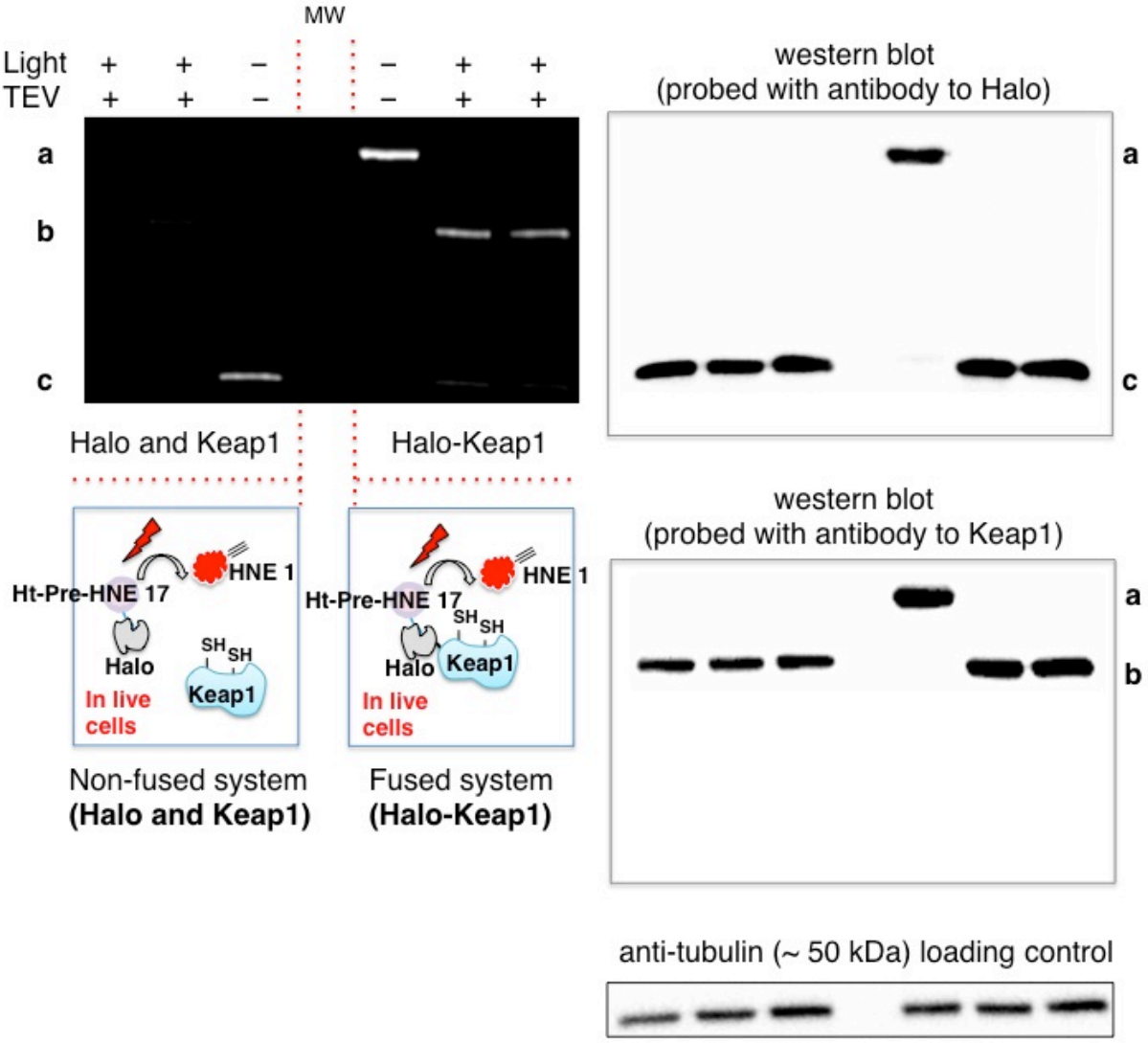


Figure S4. Analysis of T-REX-assisted HNE 1 delivery on the non-fused Halo and Keap1 proteins compared with the results from Halo-Keap1 fusion protein system using Ht-Pre-HNE 17. The left-hand data set of the fluorescence gel originates from executing T-REX method in HEK-293 cells transiently overexpressing Halo protein and Keap1 protein separately. The right-hand data set results from transient overexpression of Halo-Keap1 fusion protein under otherwise identical experimental conditions. The two panels below the fluorescence gel illustrate the respective setups. Independent duplicates are presented in each case for samples treated with both light and TEV protease. Corresponding western blot data sets are also shown. MW designates the gel lane comprising molecular weight markers. **a**, **b**, and **c** designate the bands corresponding to Halo–Keap1 (104 kDa), Keap1 (70 kDa, post TEV cleavage), and Halo (33 kDa), respectively. Tubulin (50 kDa) is used as loading control. See Table S9 for primary antibodies and dilutions.

Figure S5.

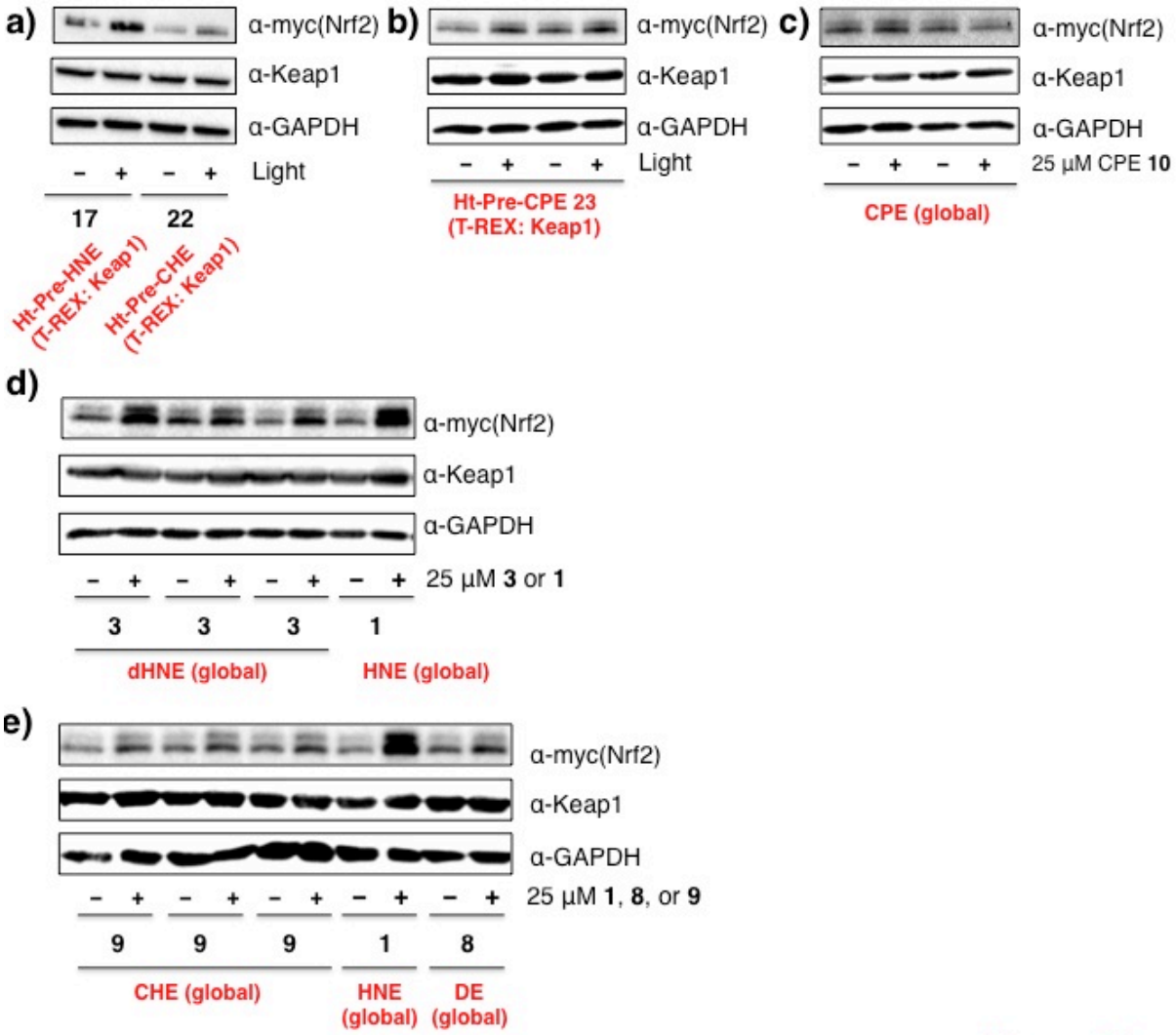


Figure S5. Nrf2 stabilization readout. See also Figure 9. (a, b) Representative results from T-REX experiments targeted to Keap1 only in cells. (c–e) Representative results from global (whole-cell) electrophile (25 μM) treatment. In all cases HEK-293 cells stably expressing Halo-Keap1 were transiently transfected with myc-Nrf2. Time of incubation is 18 h in all cases (either post illumination in TREX method, or post whole-cell direct LDE treatment). . GAPDH is used as loading control. See Table S9 for primary antibodies and dilutions.

Figure S6.

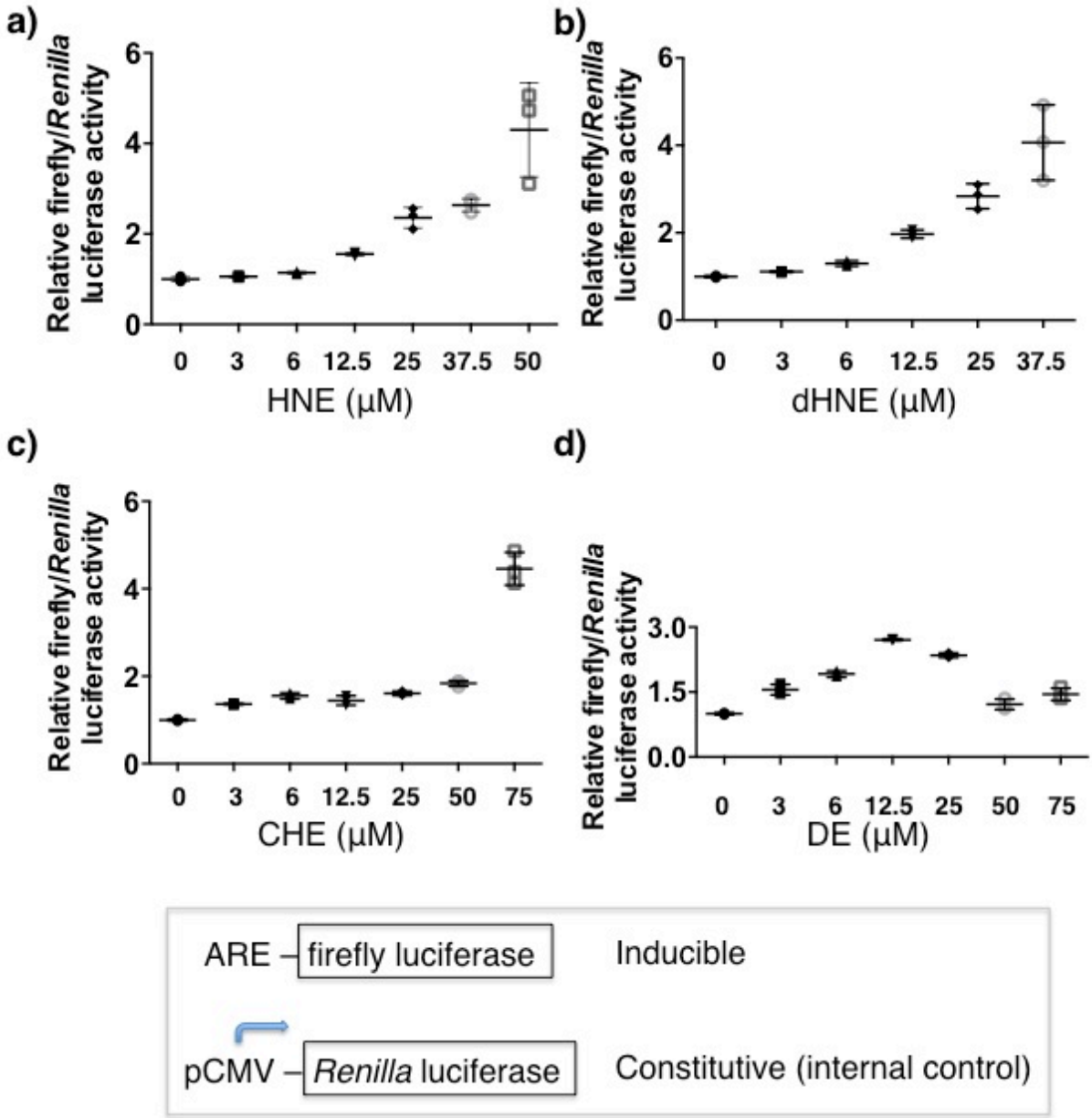


Figure S6. Representative data for dose-dependent ARE upregulation upon global LDE stimulation. HEK-293 cells stably expressing Halo-Keap1 and transiently expressing myc-Nrf2 along with ARE-inducible firefly luciferase and constitutive *Renilla* luciferase were treated with indicated LDEs. The ratios of firefly to *Renilla* signals were measured post 18 h LDE treatment. Error bars indicate S.D. over 3 independent biological replicates. Inset shows inducible firefly luciferase reporter and constitutively expressed *Renilla* luciferase reporter (internal control) used in these experiments. The relative firefly to *Renilla* signal intensity for the DMSO-alone-treated samples was set at 1.0 in each case.

Figure S7.

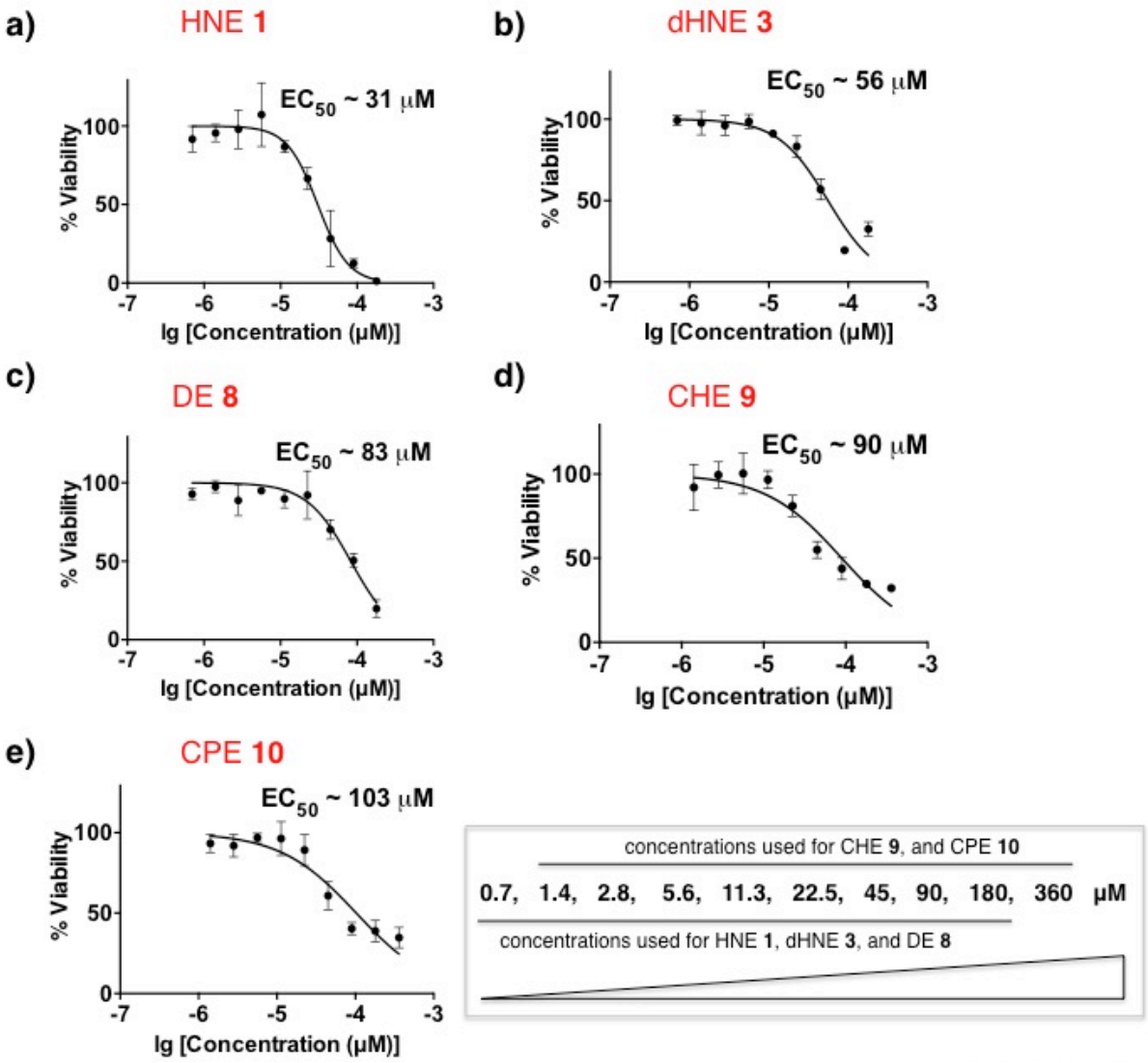


Figure S7. Representative data for dose-dependent cell viability analysis assessed by AlamarBlue® reagent (Invitrogen DAL1100)**.** HEK-293 cells stably expressing Halo-Keap1 were treated with indicated LDEs for 18 h prior to analysis. Error bars indicate S.D. over 3 independent biological replicates. Inset shows the concentrations evaluated. EC_{50} (concentration of LDE that induces 50% drop in cell viability) is estimated by the non-linear regression analysis fitting the data to "log(LDE) vs. normalized response with variable slope" model (Prism v6.0).

Figure S8.

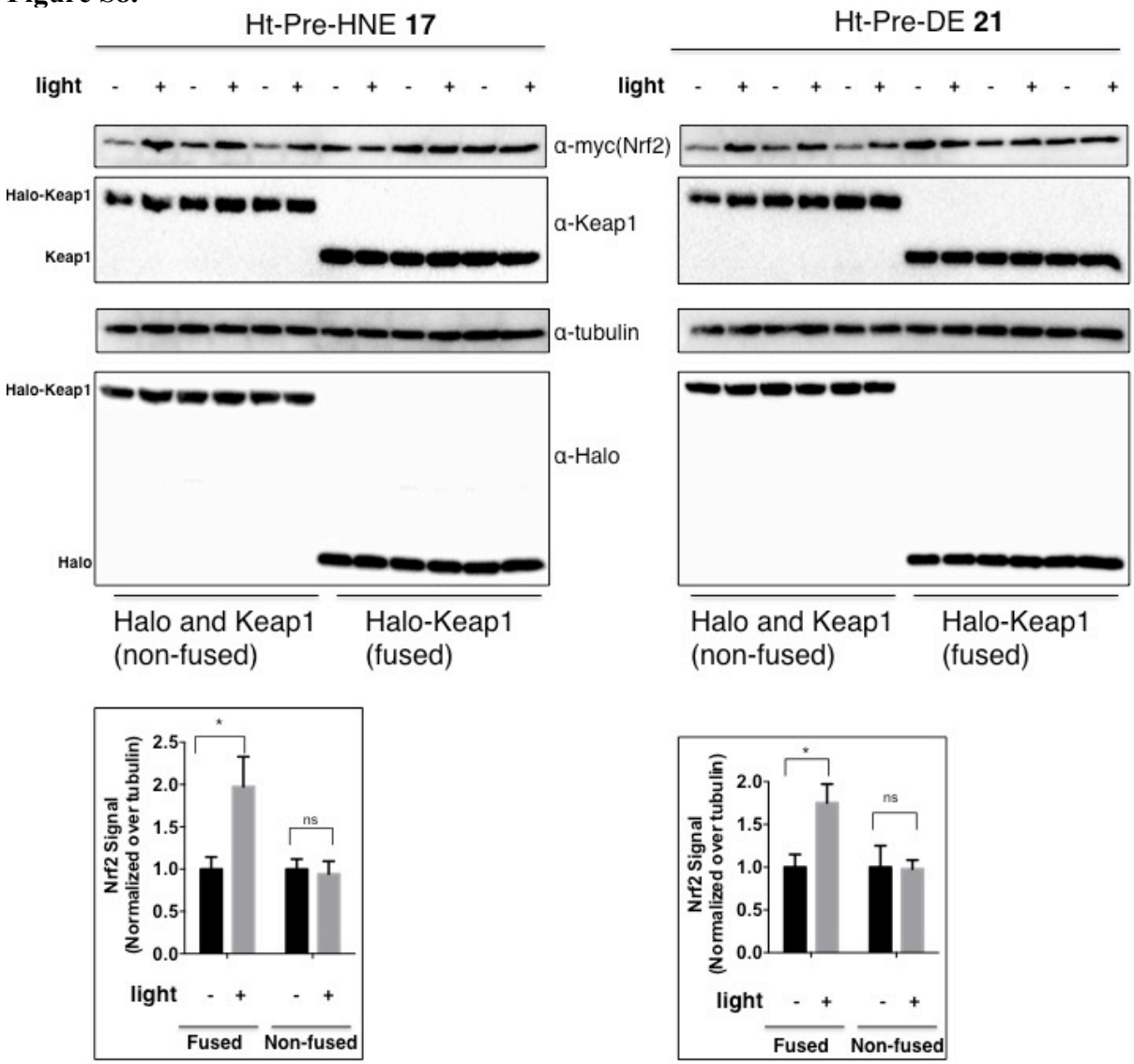


Figure S8. T-REX on the non-fused Halo and Keap1 proteins: Nrf2 stabilization. Representative data sets shown for Nrf2 stabilization readouts of the targeted delivery of HNE **1** using Ht-Pre-HNE **17** (left panel) and DE **8** using Ht-Pre-DE **21** (right panel) in the Halo-Keap1 fusion protein system and in the non-fused HaloTag and Keap1 protein system. For the Halo-Keap1 fusion protein system, T-REX was executed in HEK-293 cells transiently expressing myc-Nrf2 and Halo-Keap1. For the non-fused Halo and Keap1 protein system, T-REX was executed under otherwise identical conditions in HEK-293 cells transiently expressing myc-Nrf2, Halo, and Keap1. Time of incubation is 18 h post illumination. Tubulin is used as a loading control. The two insets below show respective quantitation of the Nrf2 band intensity from the corresponding blots. The intensity from samples not exposed to light is set to 1.0 in each case. Error bars indicate S.D. over 3 independent biological replicates. See also Figure S3 and Figure S4. See Table S9 for primary antibodies and dilutions.

Figure S9.

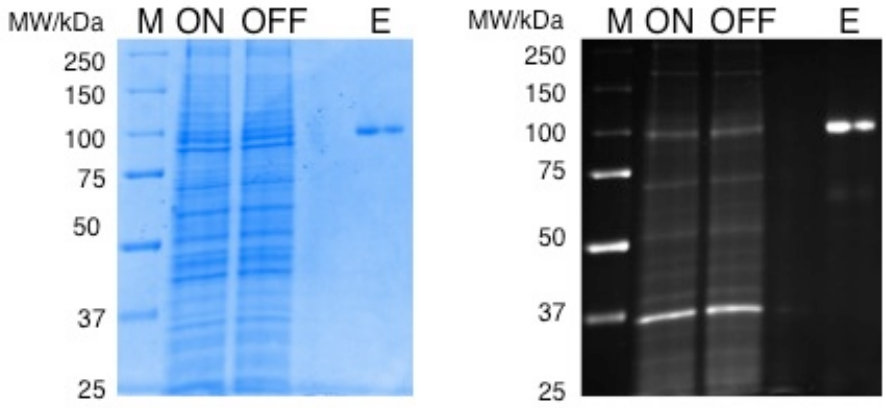


Figure S9. CPE 10 modification of Keap1 subsequent to whole-cell flooding. HEK-293 cells transiently expressing His_6-Halo-Keap1 were directly treated with 25 μM CPE **10** for 1 h and then harvested. Alkylation of Halo-Keap1 (~104 kDa) by CPE 10 was verified by in-gel fluorescence analysis subsequent to TALON beads enrichment of the protein from globally CPE **10**-treated cells. M, ON, OFF, and E respectively designate: molecular weight marker, 0.5% of input, 0.5% of flow-through, and 100% of elution.

Figure S10.

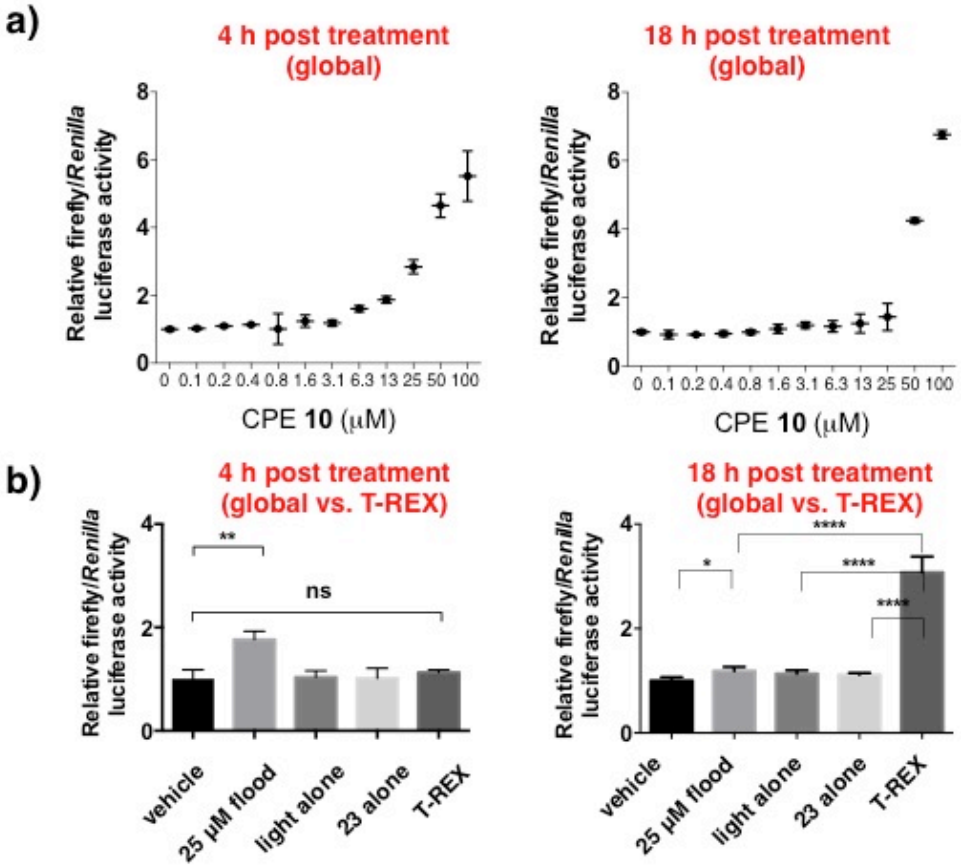


Figure S10. CPE 10-induced response as a function of time: T-REX vs. whole-cell treatment. (a) Global treatment with CPE **10** was executed in HEK 293 cells transiently expressing Halo-Keap1, myc-Nrf2, and luciferases (transfected with Promega plasmid mixture as outlined in **ARE-luciferase assays**). (b) Targeted delivery of CPE **10** using Ht-Pre-CPE **23** and global treatment with CPE **10** (25 μM flood) were executed in HEK 293 cells transiently expressing Halo-Keap1, myc-Nrf2, and luciferases (transfected with Promega plasmid mixure as outlined in **ARE-luciferase assays**). Error bars designate S.D. over 3 independent biological replicates in both (a) and (b). The relative firefly to *Renilla* signal intensity for the DMSO-alone-treated samples was set at 1.0 in each case.

Figure S11.

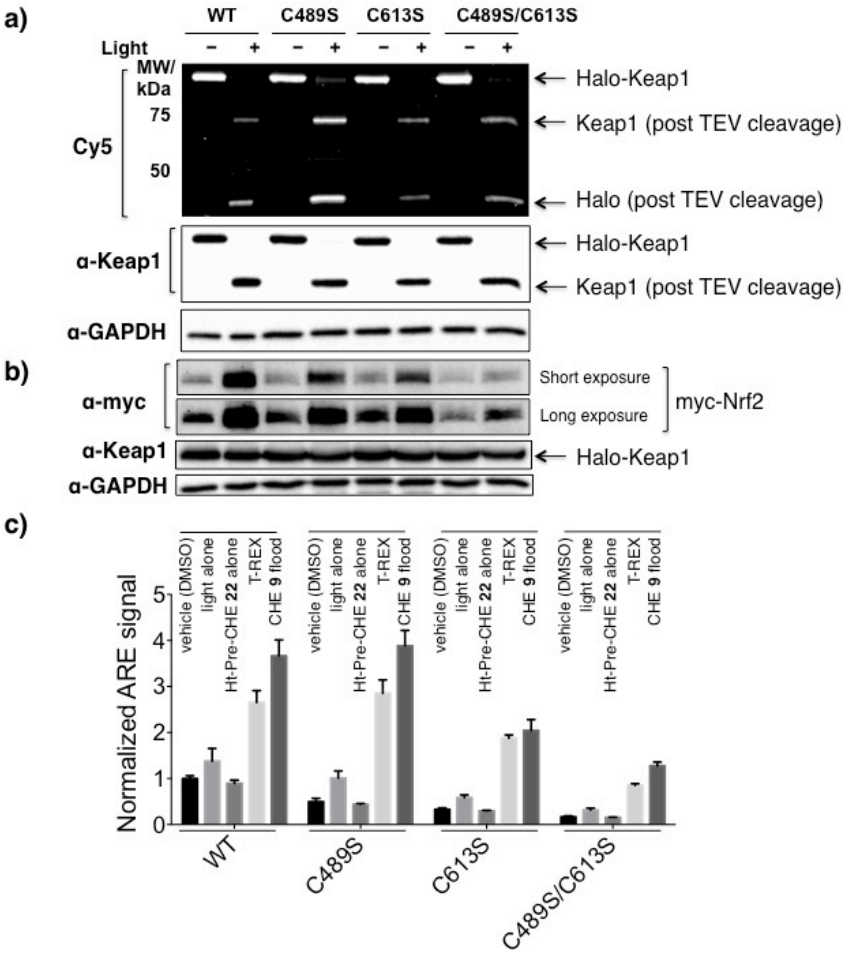


Figure S11. Functional redundancy of Keap1 Cys's in CHE 9 response. Targeted delivery of CHE **9** was executed using Ht-Pre-CHE **22** in HEK 293 cells transiently expressing Halo-Keap1 [wild type (WT) and C489S, C613S, and C489S/C613S mutants (Table S3)] and myc-Nrf2. (a) A representative data set analyzed by in-gel fluorescence, reporting successful targeted CHEylation on Keap1 WT or mutants (top band: Ht-Pre-CHE **22** covalently bound to HaloTag within Halo-Keap1; middle, CHE-modified Keap1 post TEV cleavage; bottom, Ht-Pre-CHE **22** covalently bound to HaloTag post TEV cleavage). Corresponding western blot is shown below. (b) A representative data set probing for Nrf2 stabilization. The same labels of the lanes apply as in (a). (c) A representative data set of ARE upregulation resulting from T-REX-assisted targeted CHEylation and global treatment of cells expressing Keap1 WT and mutants. Error bars designate S.D. over 3 independent biological replicates. Time of incubation is 18 h in all cases (either post illumination in TREX, or post whole-cell CHE **9** treatment). Each bar represents the firefly signal divided by *Renilla* signal (internal control) normalized over the WT vehicle-alone (DMSO) treatment. Although LC-MS/MS analysis identifies C489 and C613 as residues CHEylated under T-REX conditions in live cells (Table S7), and that C613 plays a role in sensing Zn^{2+} ion[10], H_2O_2 and nitrosative agents[11], and H_2S[12], our data show that these mutant proteins are still able to sense CHE **9** and block Nrf2 degradation. These mutants also affect unstimulated Nrf2 levels, suggesting their functional importance. See Main Manuscript for further discussion. GAPDH is used as loading control in (a) and (b). In (a) and (b), the lanes representing WT samples were only loaded with 80% and 70% of total lysate proteins with respect to the amounts loaded for the lanes representing single and double mutant samples.

Figure S12.

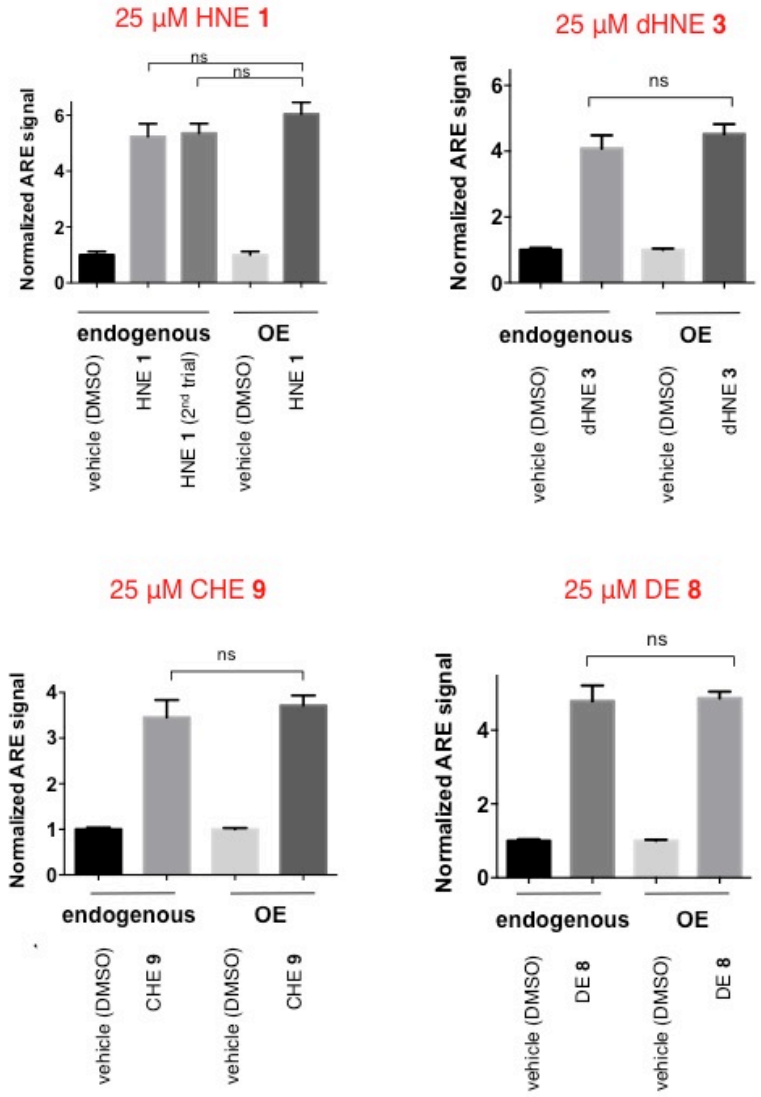


Figure S12. ARE response in native cells vs. cells overexpressing Keap1 and Nrf2. Representative data sets shown for the ARE response resulting from direct LDE treatment (25 µM) of native cells ("**endogenous"**) and cells overexpressing Keap1 and Nrf2 ("**OE"**). For the "endogenous" conditions, HEK-293 cells transfected with Promega luciferase plasmid mixture as described in the ARE-luciferase assays protocol were treated with the indicated LDE for 18 h. In the "OE" conditions, the HEK-293 cells transfected with Promega luciferase plasmid mixture were additionally transfected with plasmids encoding Halo-Keap1, and myc-Nrf2. The cells were treated with indicated LDE for 18 h. Error bars indicate S.D. over 3 independent biological replicates. Firefly signal intensity with respect to *Renilla* intensity (internal control) is measured in each case. The magnitude of ARE fold-upregulation from whole-cell LDE treatment is normalized to vehicle-alone-treated samples in each case. The latter is arbitrarily set at 1.0 across all samples.

Figure S13.

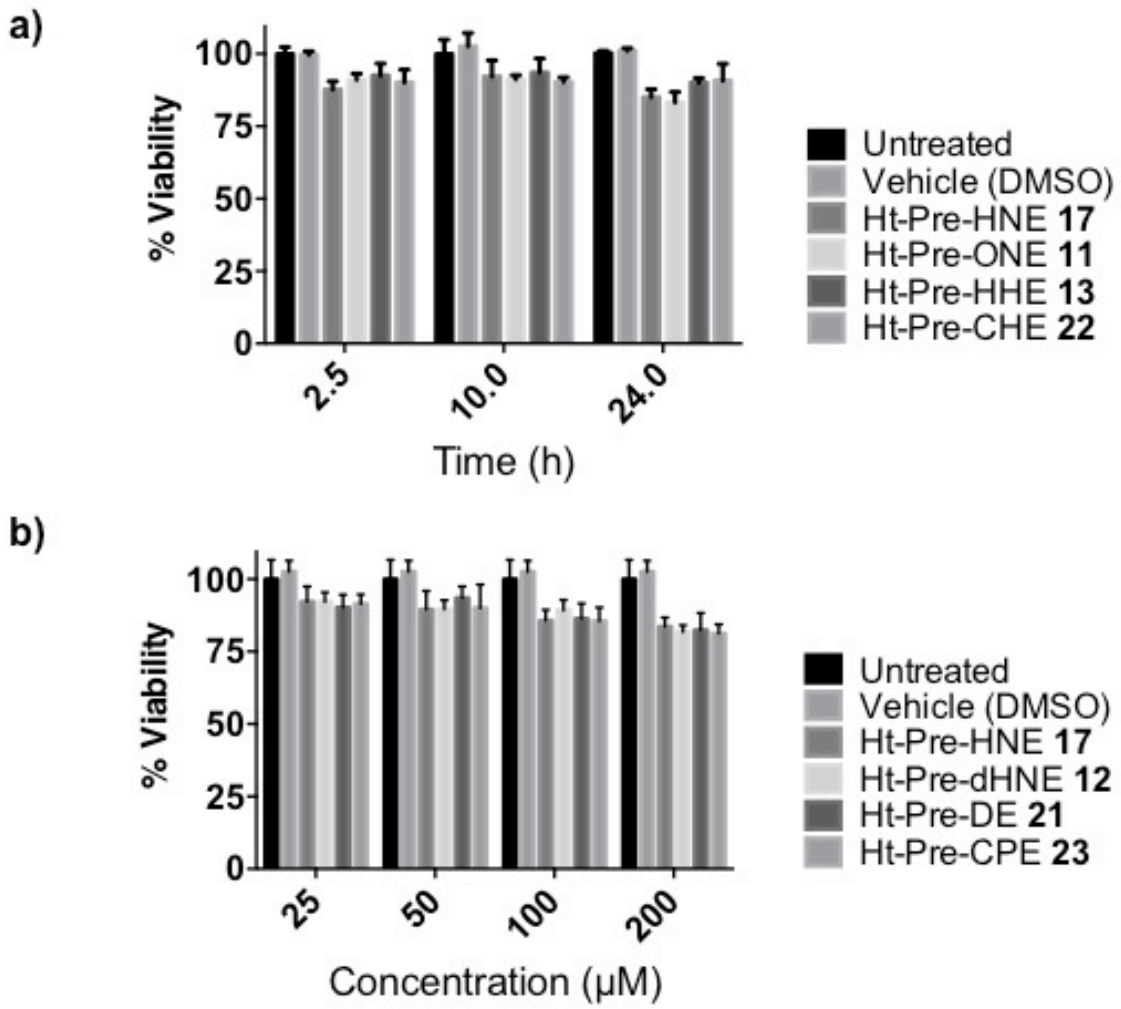
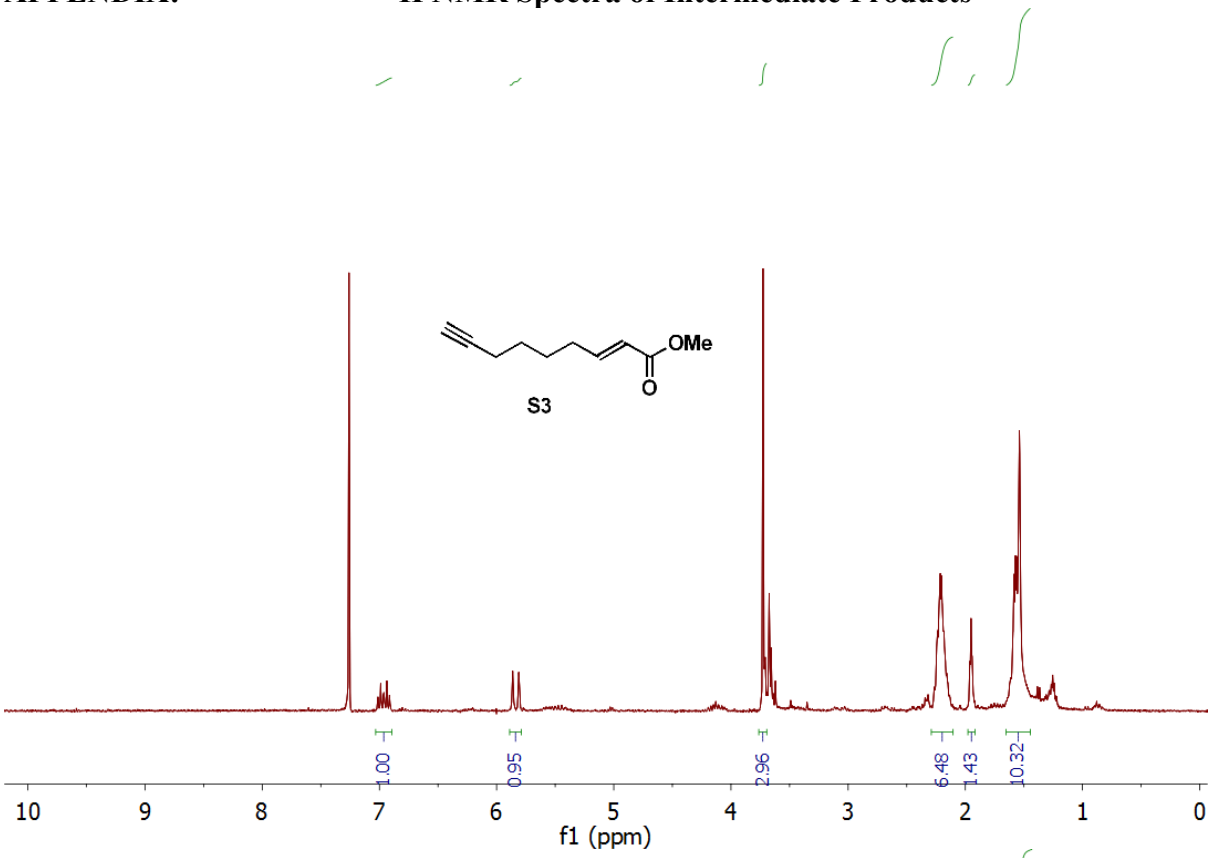
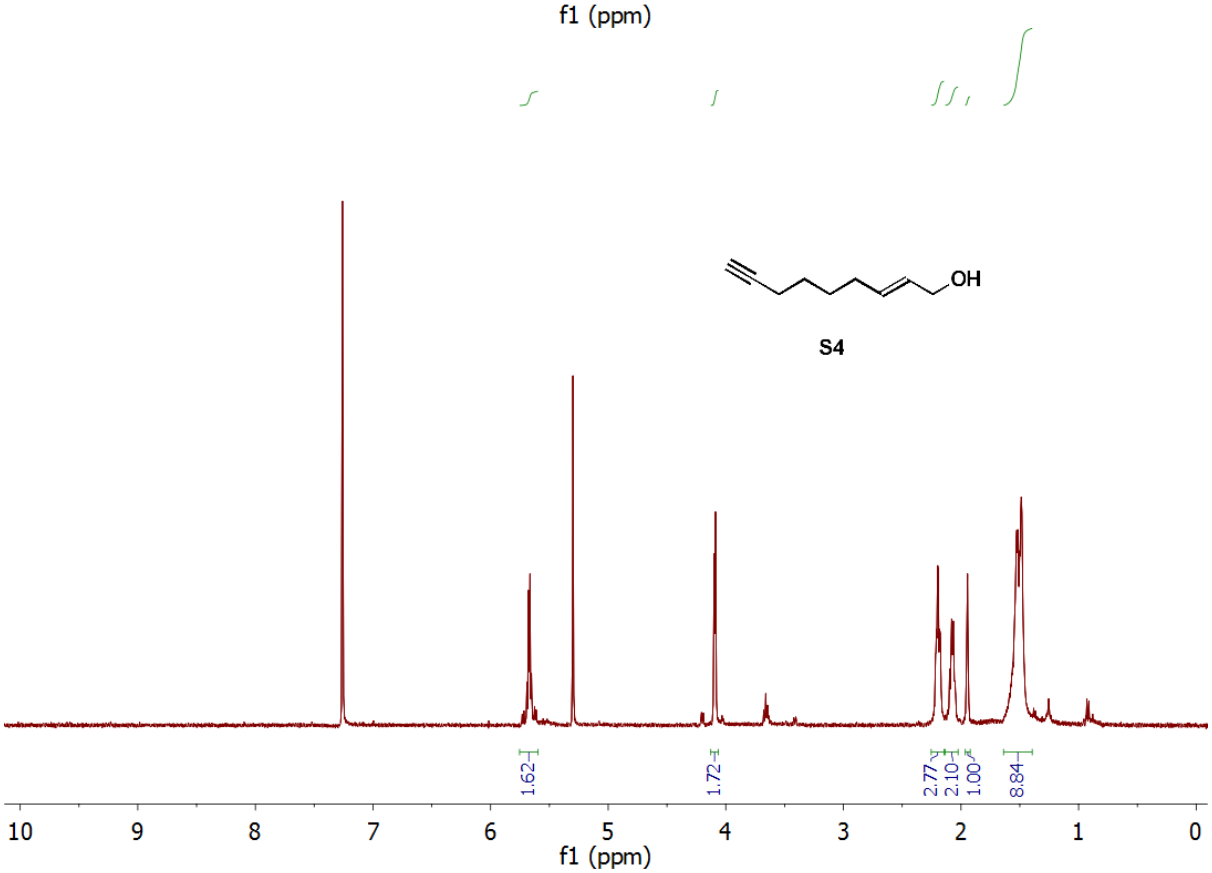
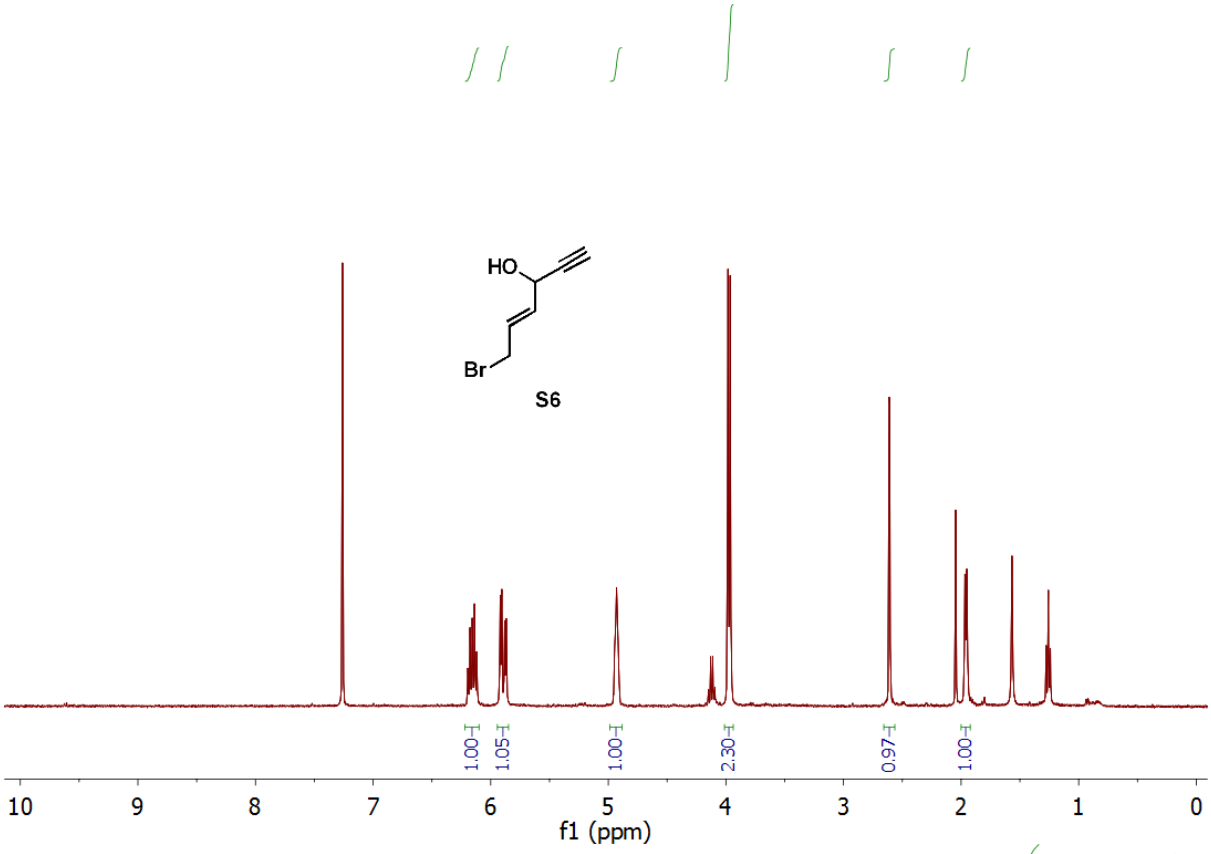


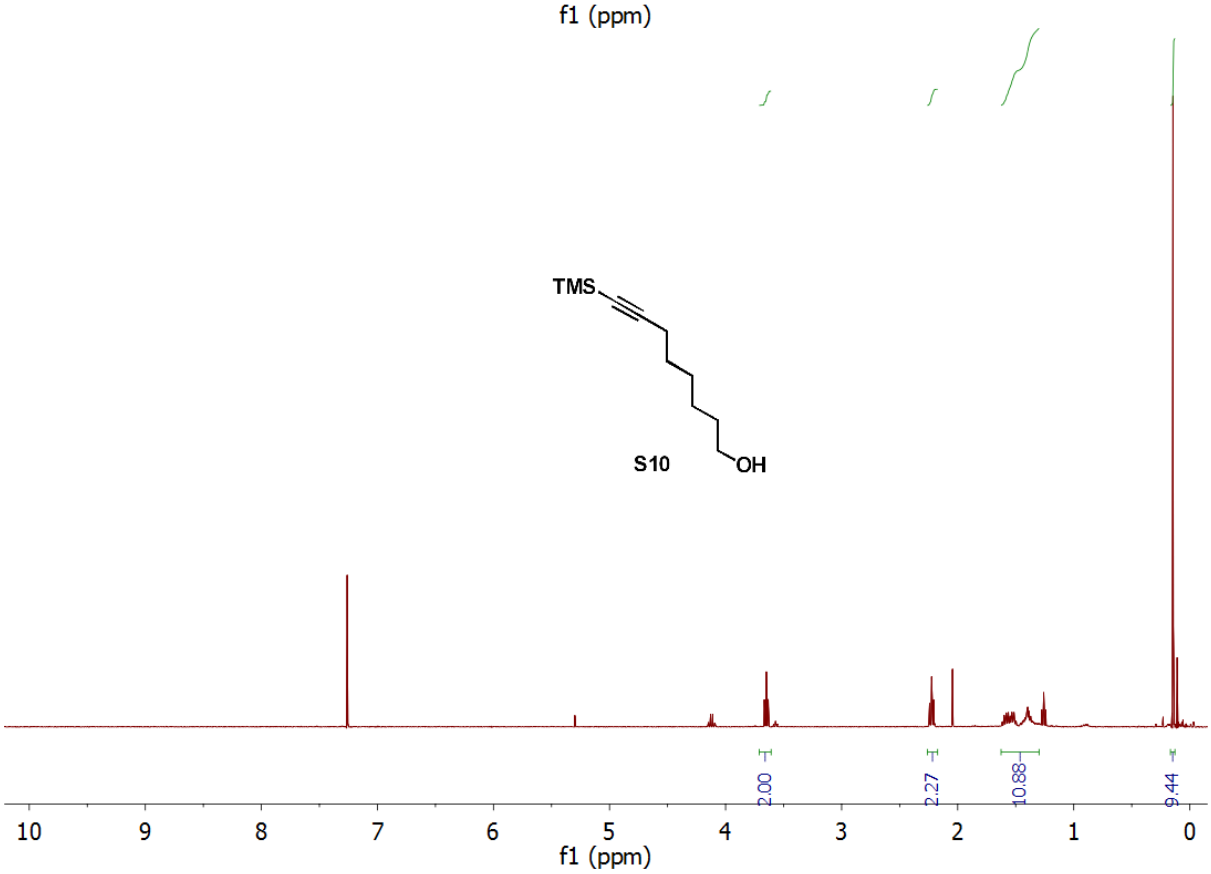
Figure S13. Time (a) and dose (b) -dependent effects on cell viability from treatment with representative HaloTag-targetable caged precursors used in T-REX method. Viability was assessed by alamarBlue® test. In (a), 25 μM caged precursors were applied for indicated periods. In (b), incubation time was fixed for 2.5 h. Error bars designate S.D. over 3 independent biological replicates.

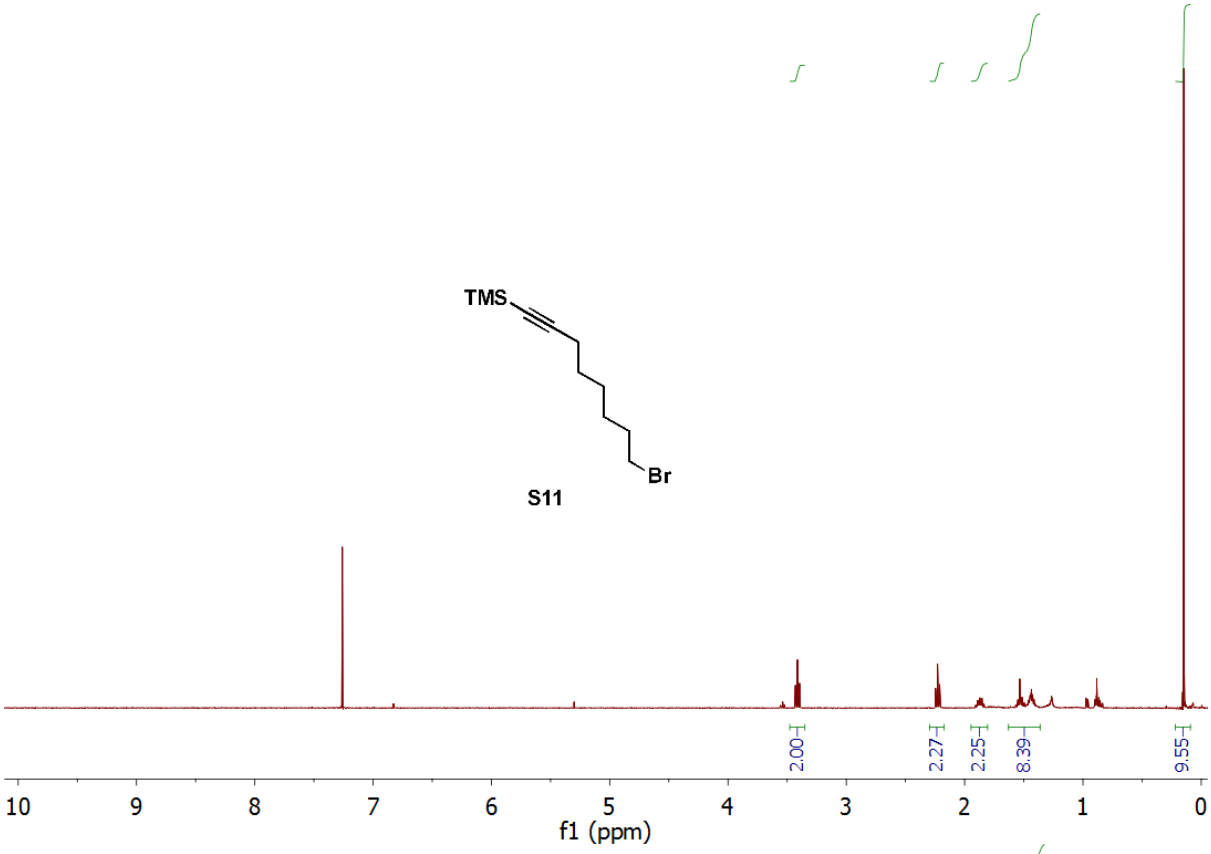
APPENDIX: **^{1}H NMR Spectra of Intermediate Products**

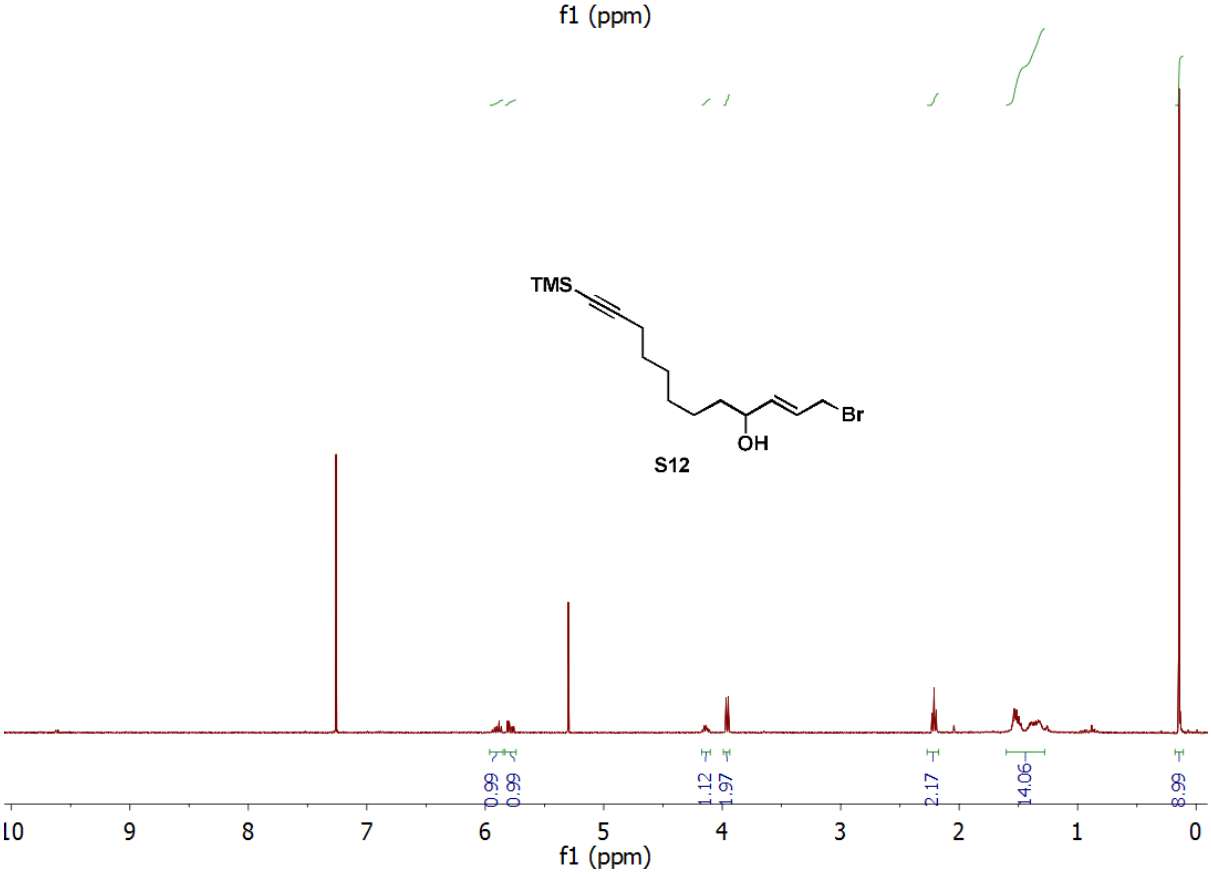


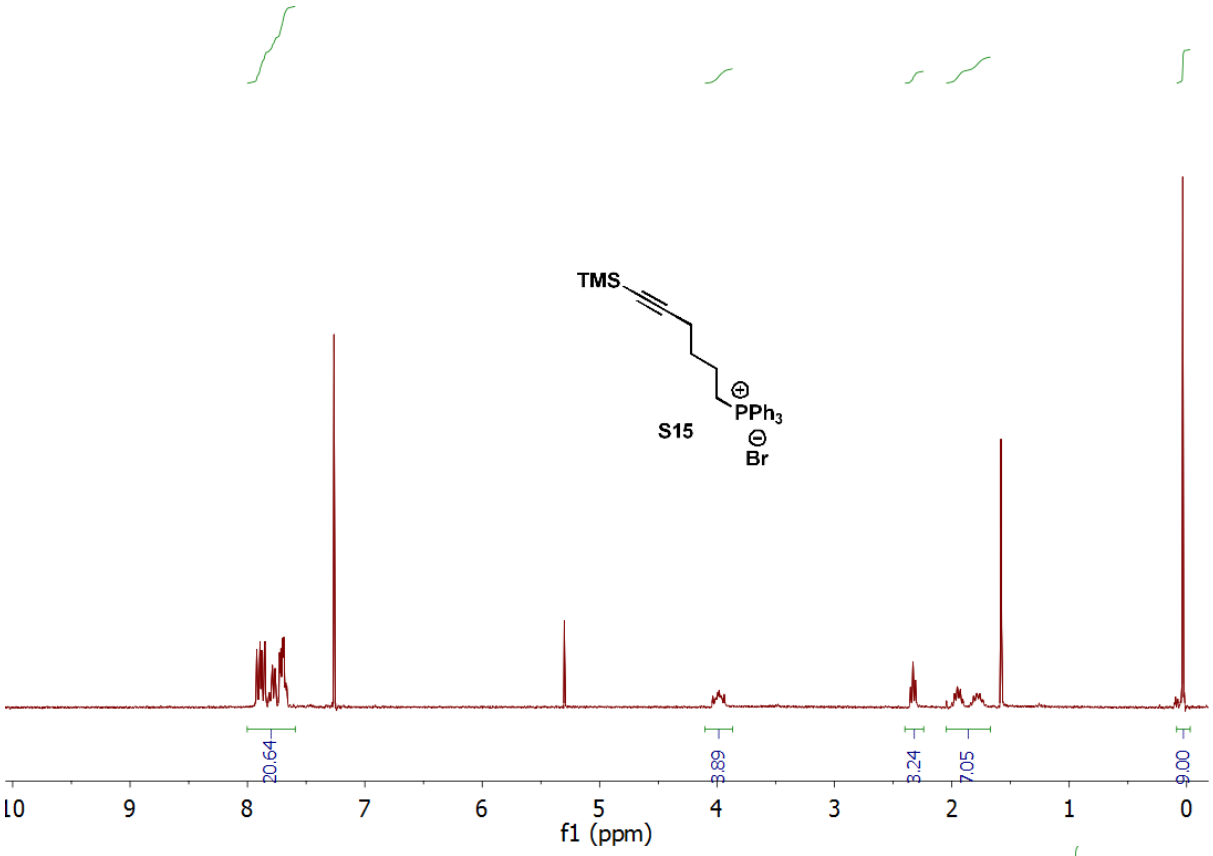


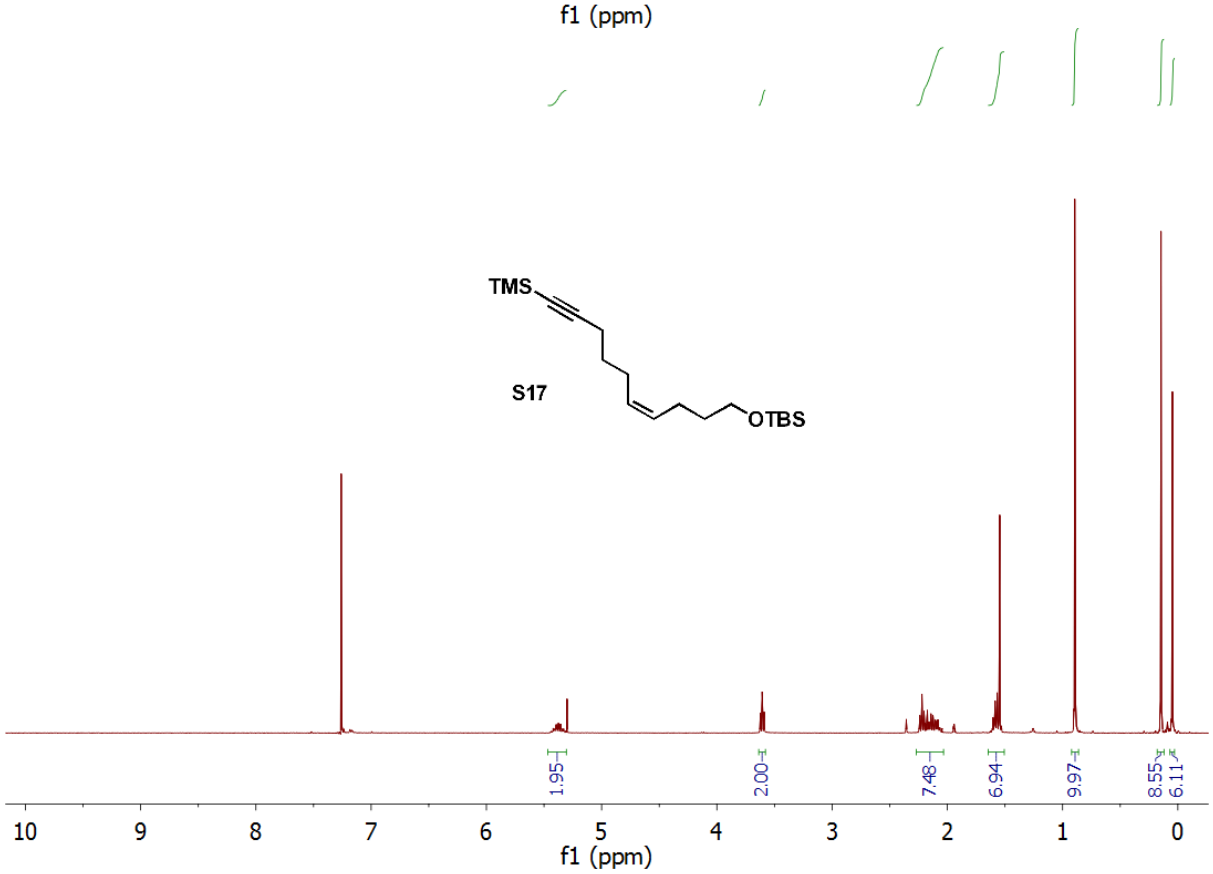

HO
Br
S6
1.00
1.05
1.00
2.30
0.97
1.00
10
9
8
7
6
5
4
3
2
1
0
f1 (ppm)

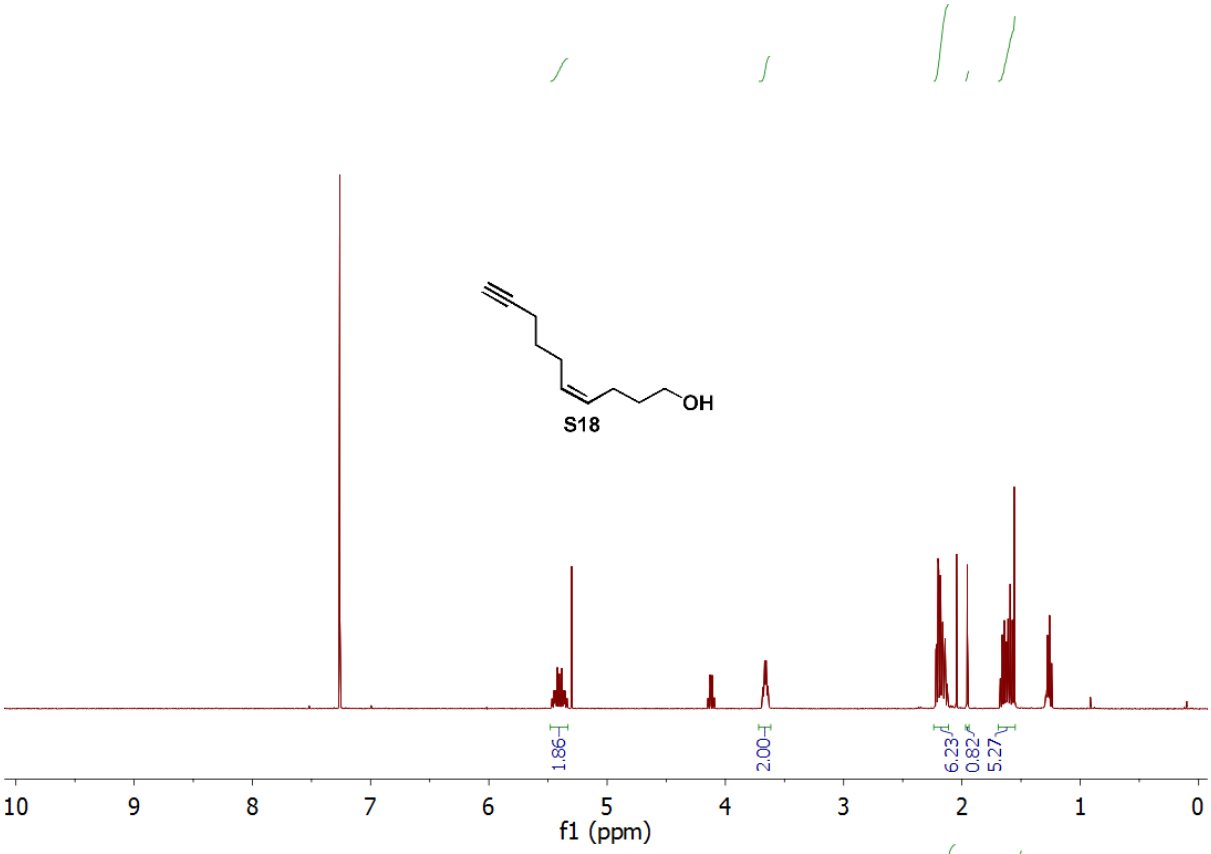

TMS
S10
OH
2.00
2.27
10.88
9.44
10
9
8
7
6
5
4
3
2
1
0
f1 (ppm)

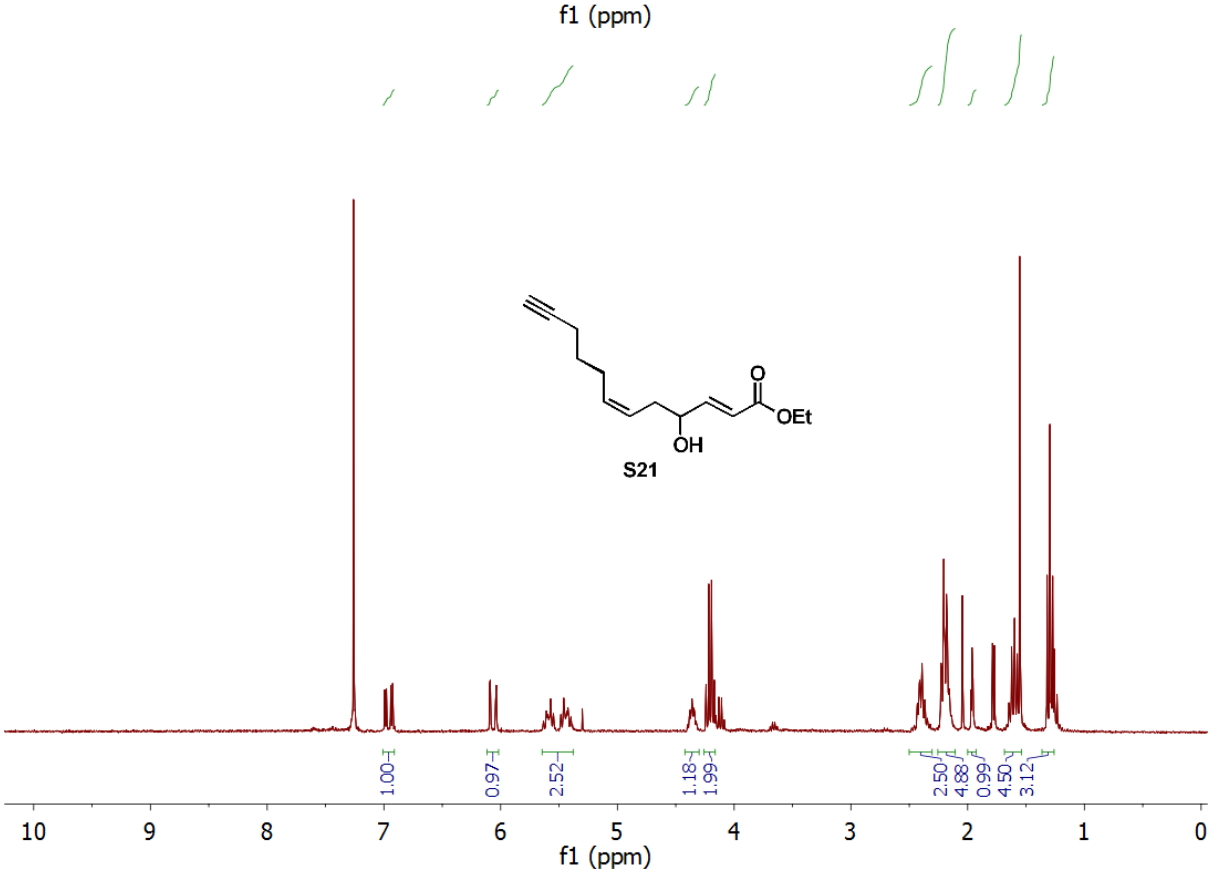

TMS
Br
S11
2.00
2.27
2.25
8.39
9.55
10
9
8
7
6
5
4
3
2
1
0
f1 (ppm)

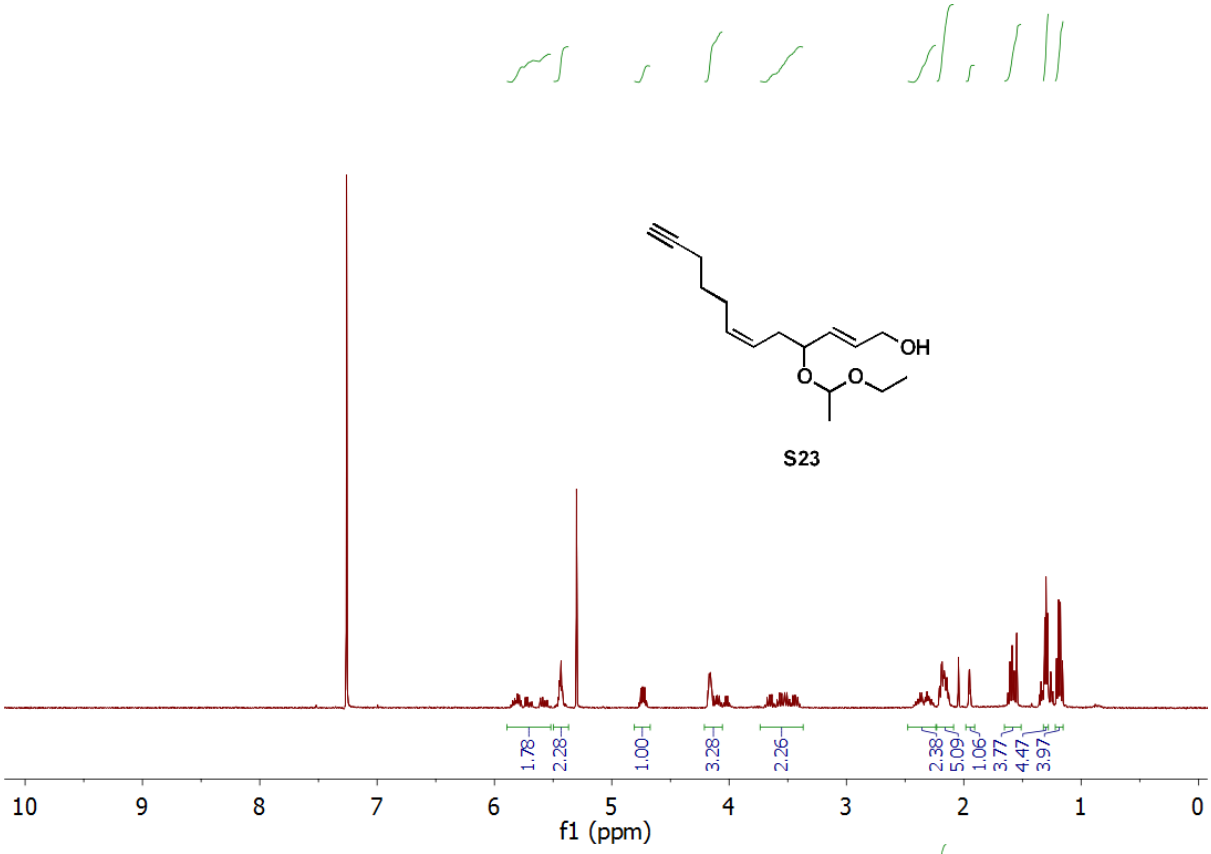

TMS
Br
OH
S12
0.99
0.99
1.12
1.97
2.17
14.06
8.99
10
9
8
7
6
5
4
3
2
1
0
f1 (ppm)

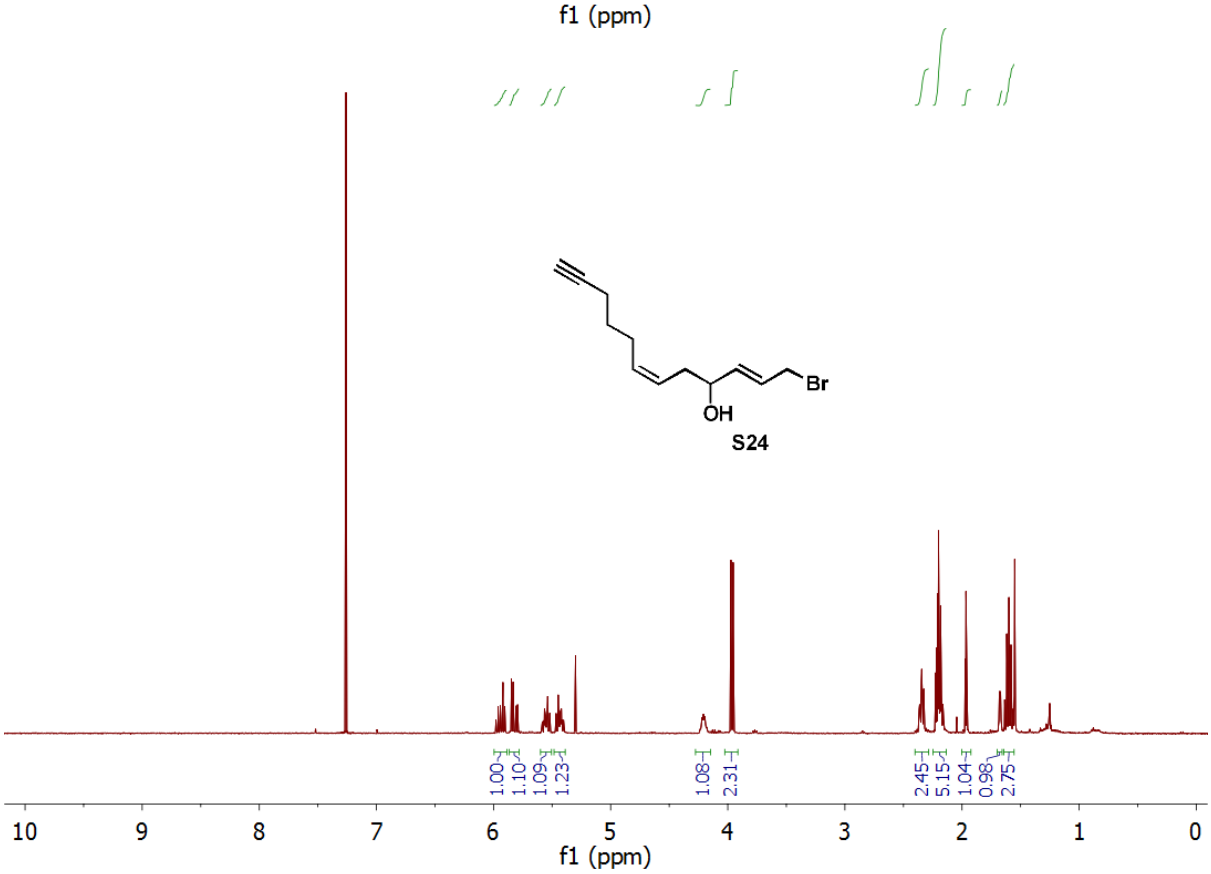

TMS
PPh3
Br
S15
20.64
3.89
3.24
7.05
9.00
10
9
8
7
6
5
4
3
2
1
0
f1 (ppm)

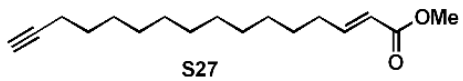

TMS
S17
OTBS
1.95
2.00
7.48
6.94
9.97
8.55
6.11
10
9
8
7
6
5
4
3
2
1
0
f1 (ppm)


OH
S18
1.86
2.00
6.23
0.82
5.27
10
9
8
7
6
5
4
3
2
1
0
f1 (ppm)

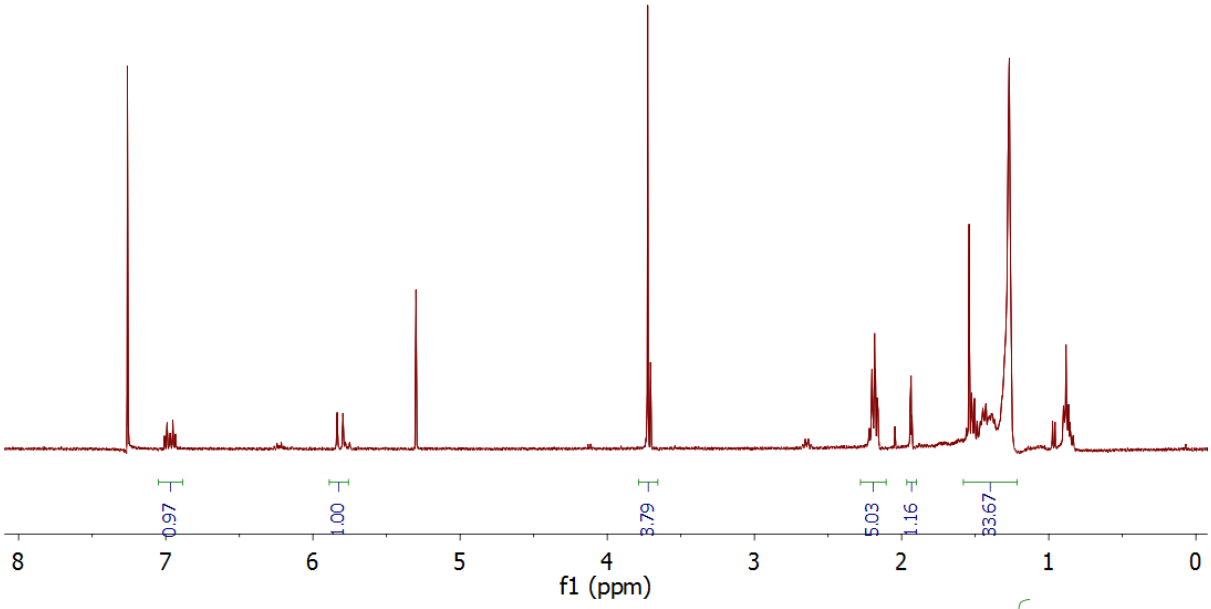

O
OEt
OH
S21
1.00
0.97
2.52
1.18
1.99
2.50
4.88
0.99
4.50
3.12
10
9
8
7
6
5
4
3
2
1
0
f1 (ppm)

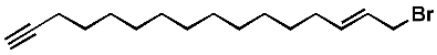

OH
O
O
S23
1.78
2.28
1.00
3.28
2.26
2.38
5.09
1.06
3.77
4.47
3.97
10
9
8
7
6
5
4
3
2
1
0
f1 (ppm)


Br
OH
S24
1.00
1.10
1.09
1.23
1.08
2.31
2.45
5.15
1.04
0.98
2.75
10
9
8
7
6
5
4
3
2
1
0
f1 (ppm)

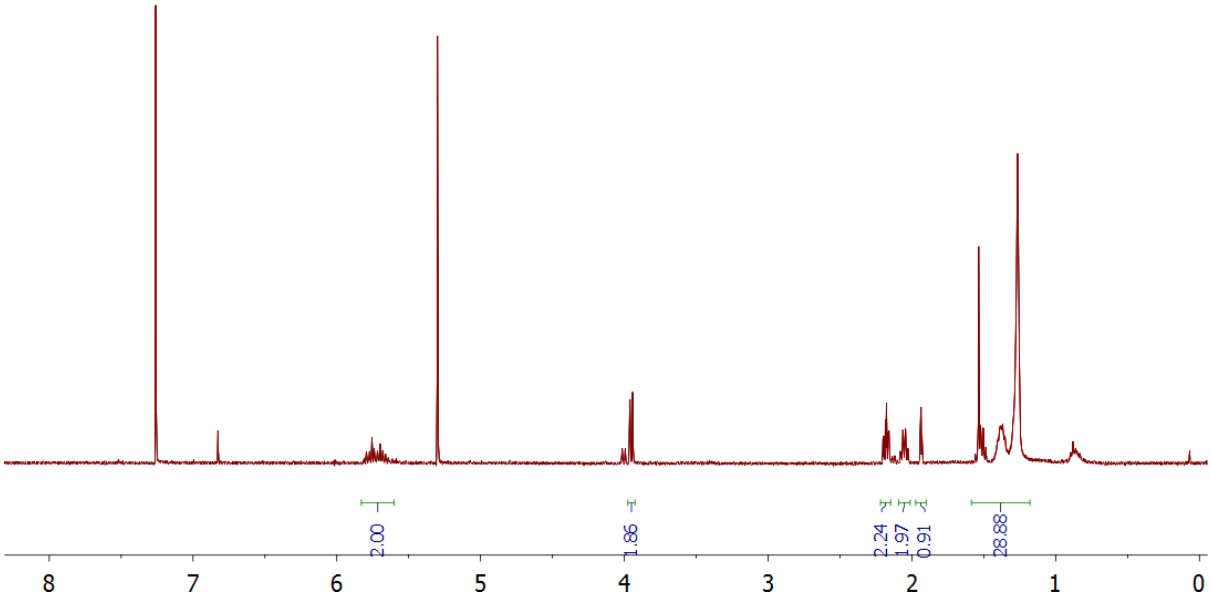

OMe
O

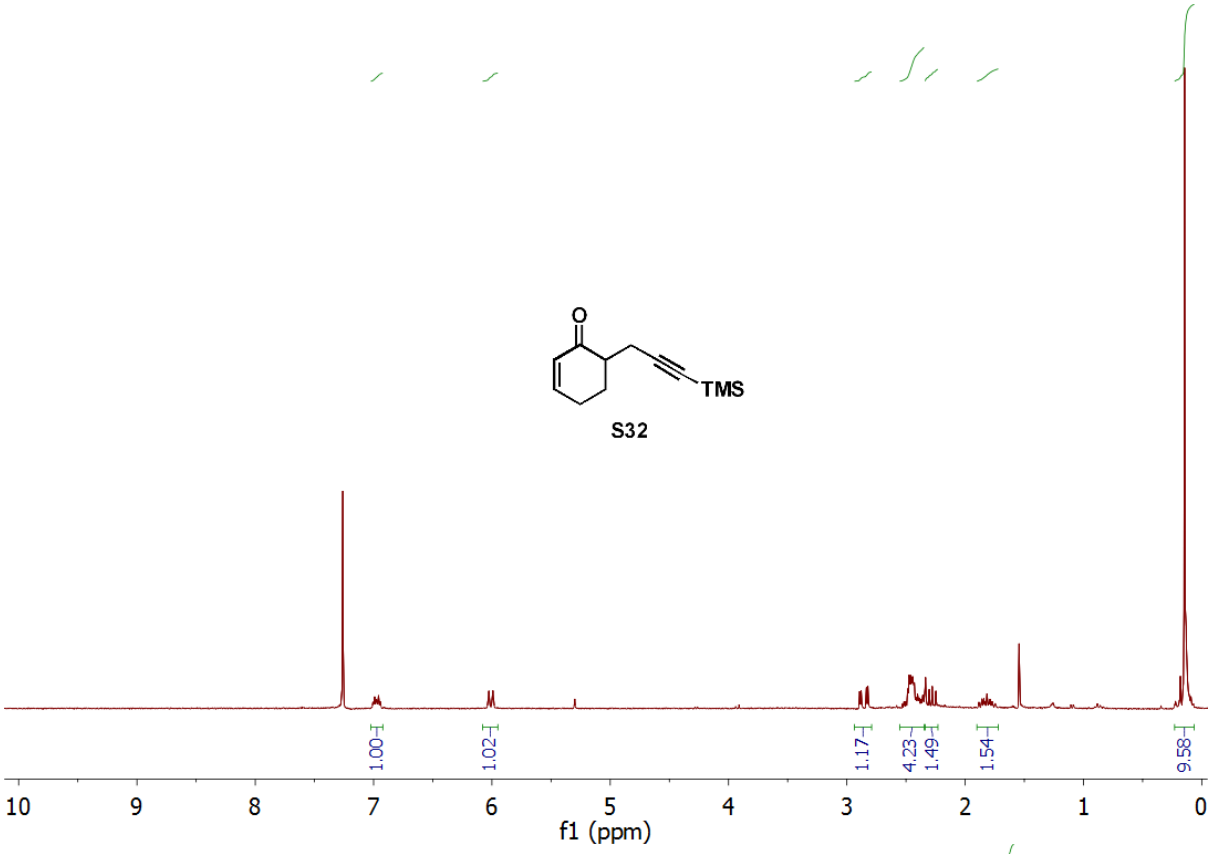
S27

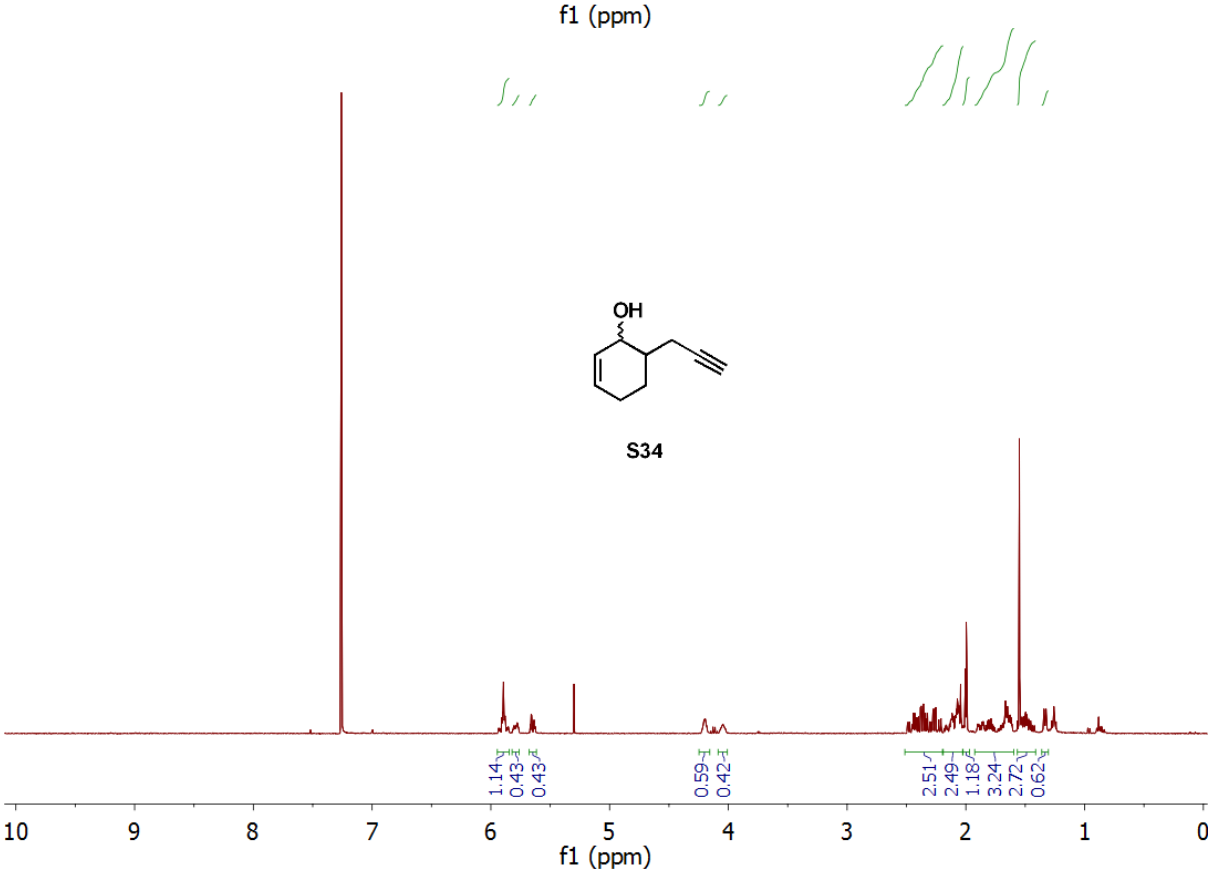

0.97
1.00
3.79
5.03
1.16
33.67
8
7
6
5
4
3
2
1
0
f1 (ppm)


Br

S29


2.00
1.86
2.24
1.97
0.91
28.88
8
7
6
5
4
3
2
1
0


O
TMS
S32
1.00
1.02
1.17
4.23
1.49
1.54
9.58
10
9
8
7
6
5
4
3
2
1
0
f1 (ppm)


OH
S34
1.14
0.43
0.43
0.59
0.42
2.51
2.49
1.18
3.24
2.72
0.62
10
9
8
7
6
5
4
3
2
1
0
f1 (ppm)

References:

[1] Long, M. J. C.; Hedstrom, L. *ChemBioChem*, **2012**, *13*, 1818.

[2] Aye, Y.; Brignole, E. J.; Long, M. J. C.; Chittuluru, J.; Drennan, C. L.; Asturias, F. J.; Stubbe, J. *Chem. Biol.* **2012**, *19*, 799.

[3] Fang, X.; Fu, Y.; Long, M. J. C.; Haegele, J. A.; Ge, E. J.; Parvez, S.; Aye, Y. *J. Am. Chem. Soc.*, **2013**, *135*, 14496.

[4] Sui, B.; Yeh, E. Y.-H.; Curran, D. P. *J. Org. Chem.* **2010**, *75*, 2942.

[5] Heinrich, T.; Katzer, M. PCT Int. Appl. 2009, WO 2009112139 A1; *Chem. Abstr.* **2009**, *151*, 358575.

[6] Satyalakshmi, G.; Suneel, K.; Shinde, D. B.; Das, B. *Tetrahedron: Asymmetry* **2011**, *22*, 1000.

[7] Pujari, S. P.; van Andel, E.; Yaffe, O.; Cahen, D.; Weidner, T.; van Rijn, C. J. M.; Zuilhof, H. *Langmuir* **2013**, *29*, 570.

[8] Tanaka, H.; Hasegawa, T.; Kita, N.; Nakahara, H.; Shibata, T.; Oe, S.; Ojika, M.; Uchida, K.; Takahashi, T. *Chem. - Asian J.* **2006**, *1*, 669.

[9] Parvez, S.; Fu, Y.; Li, J.; Long, M. J. C.; Lin, H-Y.; Lee, D. K.; Hu, G. S.; Aye, Y. *J. Am. Chem. Soc.* **2015**, *137*, 10.

[10] McMahon, M.; Lamont, D. J.; Beattie, K. A.; Hayes, J. C. *Proc. Natl. Acad. Sci. USA.* **2010**, *107*, 18838.

[11] Fourquet, S.; Guerois, R.; Biard, D.; Toledano, M. *J. Biol. Chem.* **2010**, *285*, 8463-8471.

[12] Hourihan, J. M.; Kenna, J. G.; Hayes, J. D. *Antioxid. Redox Signaling*, **2013**, *19*, 465-481.