Table of Contents

Instrumentation

Stopped-flow experiments were performed on a BioLogic Science Instruments stopped-flow fluorometer consisting of a Haribo Jobin Yvon H10-61 monochromator, BioLogic ALX 250 arc lamp power supply, BioLogic PMS 250 photomultiplier tube and control unit, BioLogic MPS 70/4 syringe pump microprocessor, and a BioLogic SFM 400 stopped-flow module with 3 × 10 mL, 1 × 2 mL syringes. Data were collected using the BioLogic Bio-Kine software.

Fluorescence and absorbance kinetic and endpoint readings were acquired on a Molecular Devices SpectraMax M3 96-well plate reader.

Flow cytometry experiments were performed at the Flow Cytometry Facility at UC Berkeley's Cancer Research Laboratories using a BD Biosciences LSR Fortessa flow cytometer outfitted with five lasers: 355 nm (UV), 405 nm (violet), 488 nm (blue), 561 nm (yellow-green), and 640 nm (red). Hoechst 33342 was analyzed with the 355 nm laser and a 450/50 filter; Syto 41 was analyzed with the 405 nm laser and a 450/50 filter; Alexa Fluor 488 was analyzed with the 488 nm laser and a 525/50 filter; TAMRA was analyzed with the 561 nm laser and a 585/15 filter; Cy5 was analyzed with the 640 laser and a 670/14 filter; and Cy7 was analyzed with the 640 laser and a 780/60 filter. The sample data were analyzed using FlowJo software.

Confocal microscopy experiments were performed at the Molecular Imaging Center at UC Berkeley's Cancer Research Laboratories using a Zeiss LSM 710 AxioObserver inverted laser scanning microscope. Images were acquired with a Plan-Apochromat 40×/1.4 oil DIC M27 objective at a digital zoom of 1.0. The images were acquired at 512 × 512 px with 4, 8-bit channels. The pinhole sizes were minimized to 1 airy unit to give a resolution of 29 μm in the Hoechst 33342 channel, 32 μm in the GFP channel, and 38 μm in the tetramethylrhodamine (TAMRA) channel. Hoechst 33342 was imaged with a 405 nm laser and a 481/71 filter and false-colored blue; GFP was imaged with a 488 nm laser and a 521/29 filter and false-colored green; and TAMRA was imaged with a 561 nm laser and a 626/60 filter and false-colored red. All images presented in a single panel were imaged with the same master gain and laser power and displayed with the same contrast and brightness settings. Images were processed with Zeiss Zen 2 Lite (blue edition) software.

Fluorescence spectra were recorded on a Photon Technology International Quanta Master 4 L-format scanning spectrofluorometer equipped with an LPS-220B 75-W xenon lamp and power supply, A-1010B lamp housing with an integrated igniter, switchable 814 photon counting/analog photomultiplier detection unit, and MD5020 motor driver.

In-gel fluorescence imaging was performed on an Amersham Molecular Dynamics Typhoon 9410 variable mode molecular imager equipped with a blue laser module. TAMRA fluorescence was measured with a 532 nm laser and a 580/30 filter and false-colored red. GFP fluorescence was measured with a 488 nm laser and a 520/40 filter and false-colored green. Images were processed with ImageJ software.

UV/vis absorbance measurements for protein A_{280} determination were acquired on a Varian Cary 100 Bio spectrophotometer.

General Biological Materials and Methods

All solvents and reagents were purchased from commercial suppliers and used as received. Milli-Q water (18.2 MΩ, 0.22 μm filter) was used to prepare all buffers and aqueous solutions. Short oligonucleotide primers (<80 bp) were synthesized by Elim Biopharm (Hayward, CA) while gene blocks (>80 bp) were synthesized by Integrated DNA Technologies (Coralville, IA). Oligonucleotides were used as received without further desalting. All polymerase chain reactions (PCR) were performed on a Bio-Rad Laboratories C1000 thermal cycler. Chemically competent *E. coli* DH5α and DH10β cells were purchased from New England Biolabs while chemically competent *E. coli* BL21(DE3) cells were purchased from Life Technologies. All plasmid isolations were performed with a miniprep kit from Zymo Research or a maxiprep kit from Qiagen. DNA clean and concentrator and DNA gel purification kits were purchased from Zymo Research. All enzymes used for standard restriction enzyme cloning (Q5 Hot Start DNA polymerase, restriction enzymes, T4 DNA ligase, and Antarctic phosphatase), the NEBuilder HiFi DNA Assembly master mix for Gibson assembly cloning, and the Q5 mutagenesis kit used to perform all site-directed mutagenesis reactions were purchased from New England Biolabs. DNA sequencing service was performed by the UC Berkeley DNA Sequencing facility. The pHTC CMV-neo vector was purchased from Promega. TransIT-293 transfection reagent was purchased from Mirus Bio. DIBAC-Cy5 and tetrazine-Cy7 were purchased from Click Chemistry Tools and aminooxy-Alexa Fluor 488 was purchased from Life Technologies.

B_2pin_2 Concentration-Dependent Intracellular* N-*Oxide Activation

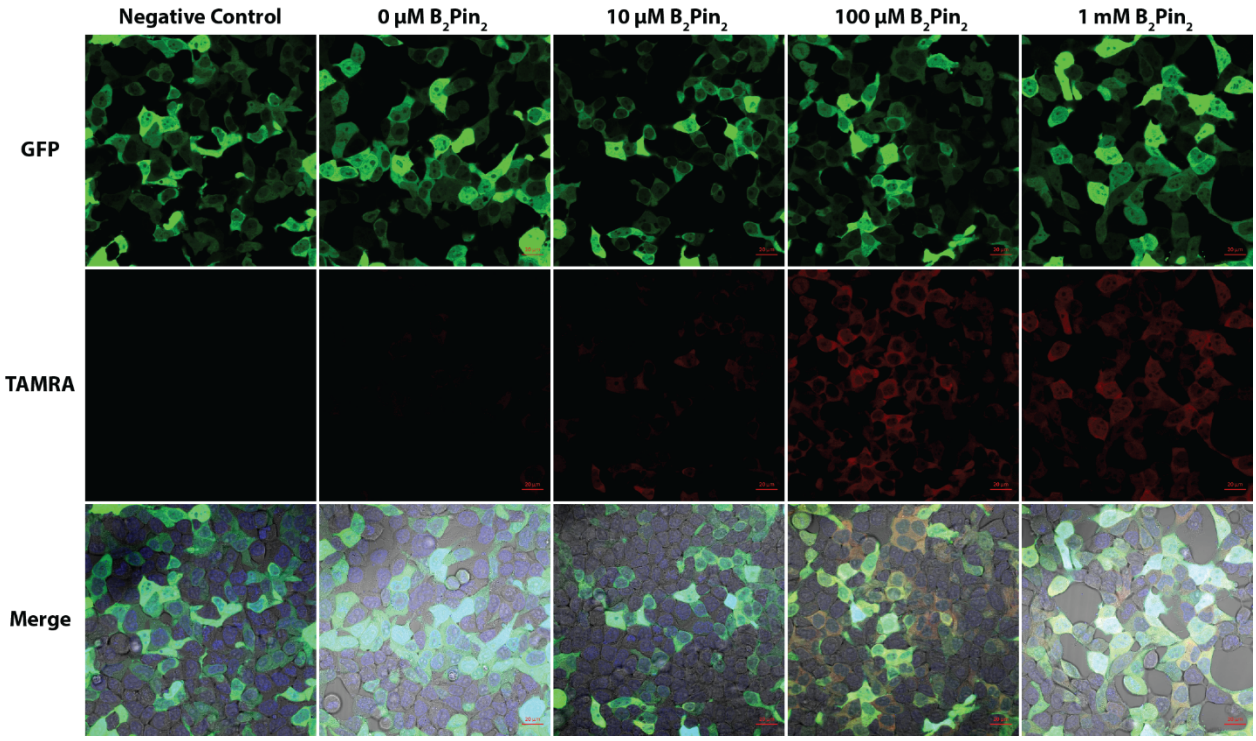


Figure S1. B_2pin_2 activates *N*-oxides on intracellular proteins in mammalian cells. HEK293T cells transiently transfected with GFP-HaloTag protein were incubated with 100 μM profluorophore **10**, washed, then treated with 0 μM, 10 μM, 100 μM, or 1 mM B_2pin_2. Confocal microscope images were acquired after 45 min. Green, red, and blue (not shown) channels represent GPF, TAMRA, and Hoechst 33342 fluorescence, respectively. The merged image is a composite of all three with a phase contrast image. While higher levels of *N*-oxide activation are observed for 100 μM and 1 mM B_2pin_2 than for 10 μM B_2pin_2, the difference is minimal between the higher two concentrations.

Kinetics of Diboron Hydrolysis

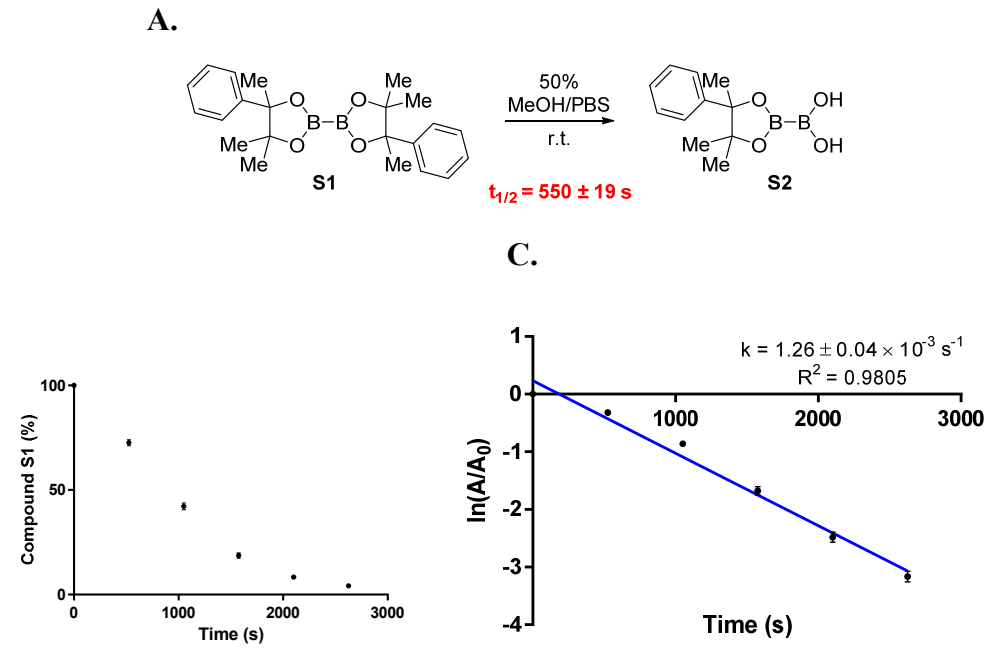


Figure S2. Ligand hydrolysis from bis(pinacolato)diboron analog diboron **S1**. (A) The rate of hydrolysis was measured using compound **S1** in 50% MeOH/PBS at room temperature. (B) Time-dependent absorbance was measured at 210 nm for compound **S1** by HPLC analysis. Peak integrals for **S1** were normalized against that of an internal standard. Each point represents the mean of three measurements. Error bars represent a standard deviation. (C) The first-order rate constant for the hydrolysis of **S1** was calculated by plotting $\ln(A/A_0)$ versus time where A represents the normalized peak integral of compound **S1**. Each point represents the mean of three measurements. Error bars represent a standard deviation.

A solution of diboron **S1** (17.5 mM in MeOH, 50.0 µL, 875 nmol) was added to MeOH (450 µL). A solution of $BnPh_3PCl$ (100 mM in PBS, 1.00 µL, 100 nmol) was then added. PBS (500 µL) was then introduced, and the resulting solution was quickly filtered through a 0.45 µm PTFE syringe filter. The solution (75 µL) was immediately analyzed by HPLC on a C_{18} analytical column (4.6 × 50 mm, 0.7 mL/min, time (min), % MeCN in H_2O + 0.1% TFA: 0, 0; 2.5, 54; 3.5, 80; 6, 100; 6.01, 50; 7.2, 50). The solution was sampled every 8 min 45 s (525 s). Peak integrals for diboron **S1** were normalized against that of the $BnPh_3PCl$ internal standard.

Intracellular Activation of N*-oxides with $B_2(OH)_4$*

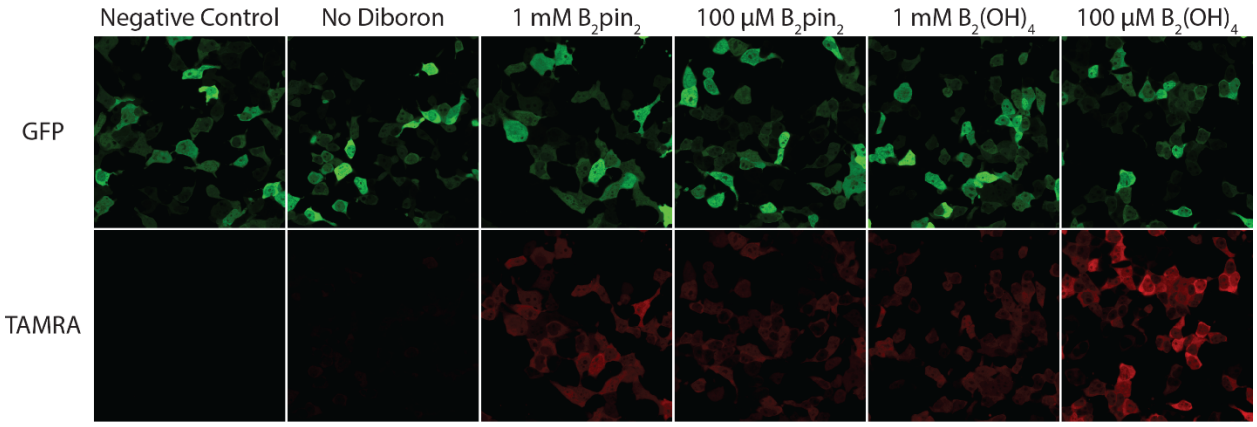


Figure S3. $B_2(OH)_4$, the hydrolysis product of B_2pin_2, activates *N*-oxides *in cellulo*. Transiently transfected HEK293T cells expressing GFP-HaloTag protein were incubated with 0 µM (only negative control) or 100 µM **10** then treated with no diboron, 1 mM B_2pin_2, 100 µM B_2pin_2, 1 mM $B_2(OH)_4$, or 100 µM $B_2(OH)_4$. GFP is represented by green fluorescence and the activated profluorophore **10** is represented by red fluorescence. The red fluorescence signal in the $B_2(OH)_4$-treated cells demonstrate the cell permeability of $B_2(OH)_4$ and its viability in reducing *N*-oxides *in cellulo*.

Compatibility of $B_2(OH)_4$ with Mammalian Cells

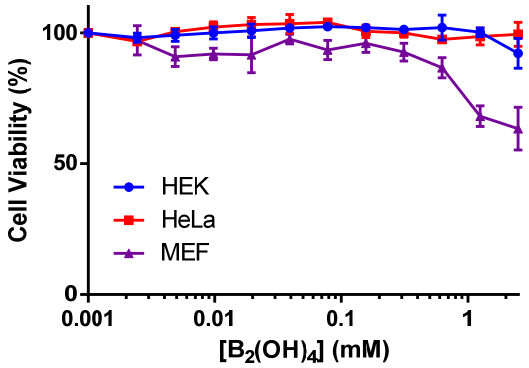


Figure S4. $B_2(OH)_4$ does not adversely affect cell viability in human cell cultures (HEK293T and HeLa) up to 2.5 mM concentrations. While MEF cells show greater sensitivity, the IC_{50} for $B_2(OH)_4$ is > 2.5 mM. Cell viability versus concentration of $B_2(OH)_4$ is plotted on a log scale of concentrations. All data are normalized to the negative control consisting of cells treated with vehicle. The negative control is plotted at a $B_2(OH)_4$ concentration of 0.001 mM. All data were baselined to a negative control consisting of no cells. Each data point represents the mean of three measurements. Error bars represent a standard deviation.

Diboron Does not Cause Aberrations in Cell Morphology

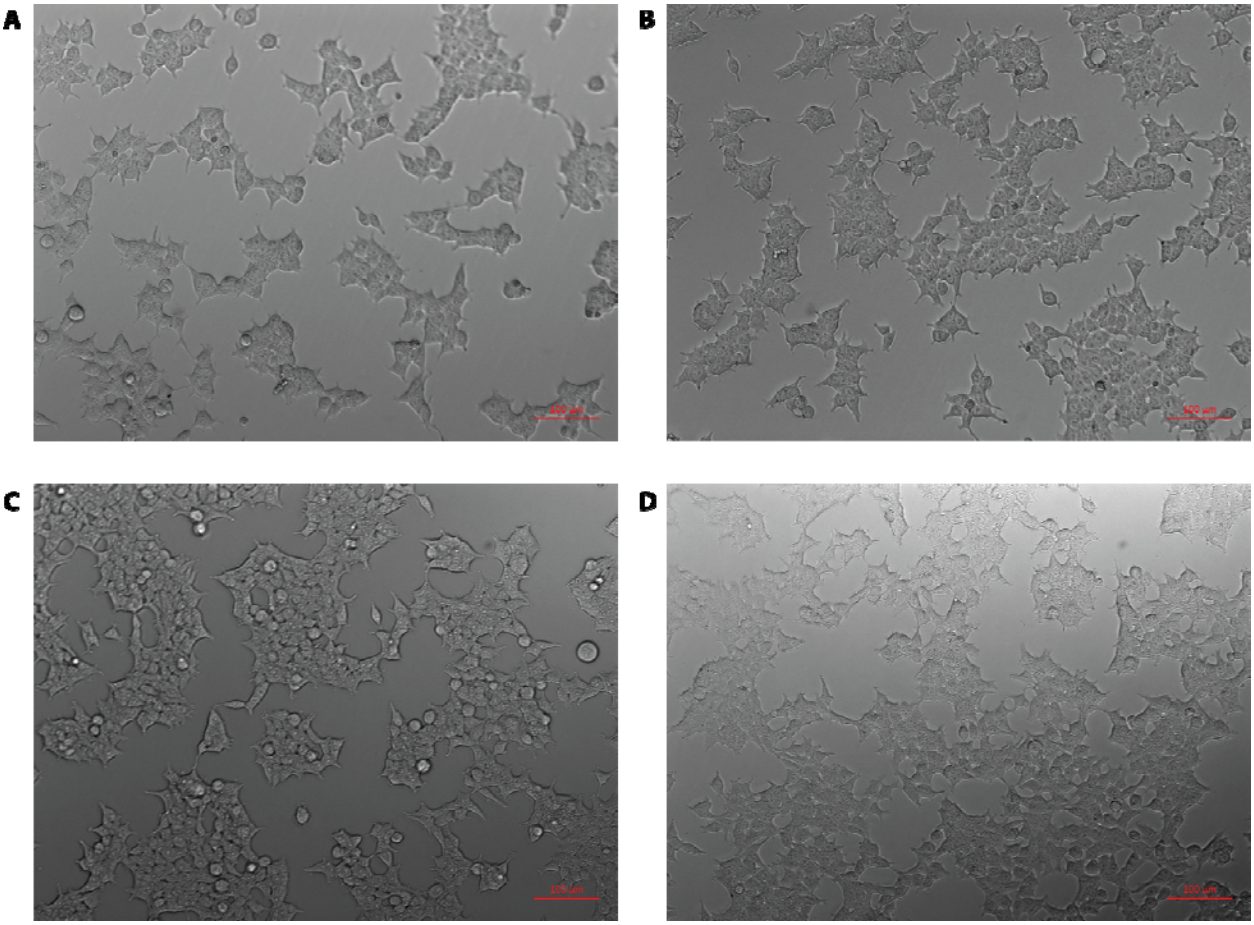


Figure S5. HEK293T cells treated with 1 mM diboron reagent do not display aberrations in cell morphology after 24 h. Brightfield images were taken at 10× magnification on an inverted light microscope. (A) B_2pin_2: Cells were treated with 1 mM B_2pin_2 in 0.5% DMSO/DMEM media for 24 h. (B) $B_2(OH)_4$: Cells were treated with 1 mM $B_2(OH)_4$ in 0.5% DMSO/DMEM media for 24 h. (C) Vehicle: Cells were grown in 0.5% DMSO/DMEM media for 24 h. (D) Control: Cells were grown in DMEM media for 24 h.

Compatibility of Diboron and Disulfides

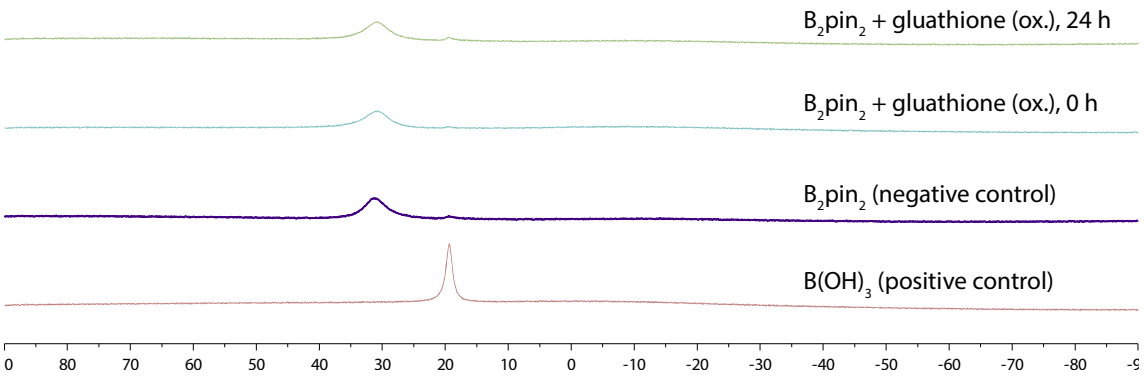


Figure S6. ^{11}B NMR shows no reaction between 50 mM B_2pin_2 and 50 mM oxidized glutathione in 50% CD_3OD/*d*-PBS over 24 h.

Reaction Between* N*-oxides and Diboron is Clean and Quantitative

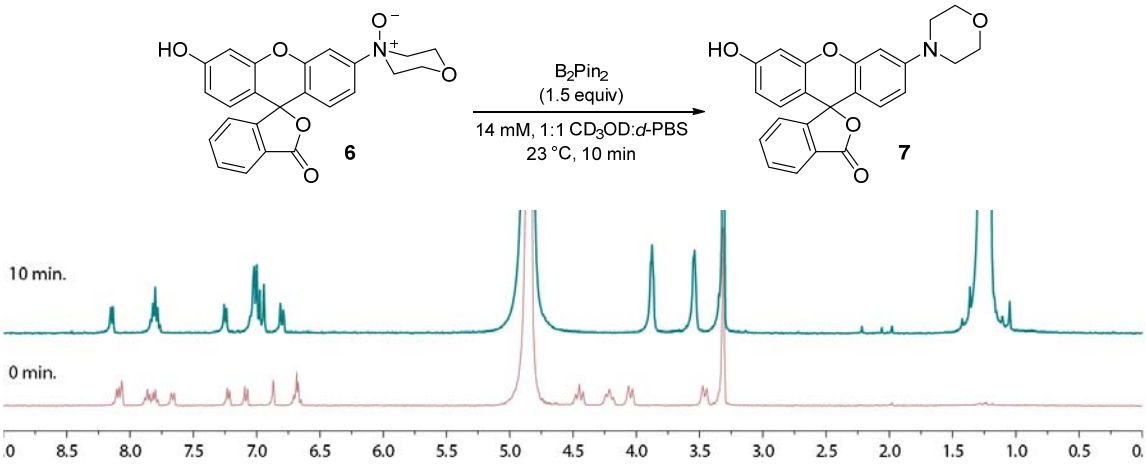


Figure S7. *In situ* monitoring of the reaction between *N*-oxide **6** and B_2pin_2 by ^{1}H NMR demonstrates its clean and quantitative progress in PBS at room temperature within 10 minutes.

Primers

sfGFP-His6-F	5'-GCAGCCGCTAGC**ATGGTTAGCAAAGGTGAAGAACT**-3'
sfGFP-His6-R	5'-ATAGCGCTCGAG**ATGGTGATGATGATGGTGGCTGC**-3'
sfGFP-HaloTag-Gibson-F	5'-GTTTAACTTTAAGAAGGAGATATACC**ATGGTTAGCAAAGGTGAAGAACTG**-3'
sfGFP-HaloTag-Gibson-R	5'-ATCTCAGTGGTGGTGGTGGTGGTGCTC**ACCGGAAATCTCCAGAGTAGAC**-3'
pHTC-SignalSeq-Mut-F	5'-GGACGGCCGGGGCAGCGCTCCTGGCGCTGCTGGCTGCGCTCTGCCCGGCGAGTCG GGCT**GGATCCGAAATCGGTACTGGC**-3'
pHTC-SignalSeq-Mut-R	5'-CGGAGGGTCGCAT**GCTAGCCCTATAGTGAGTCGTATTAATACTCTAGC**-3'
pHTC-vector-F	5'-GAATTTATTGGAGCA**TAATAGAATTGGCATGCAAGCTGATCCGGC**-3'
pHTC-vector-R	5'-TCCTCCAGAGTGGTTGG**ACCGGAAATCTCCAGAGTAGACAG**-3'
EGFR-Gibson-F	5'-TTTCCGGTCCAACCACT**CTGGAGGAAAAGAAAGTTTGCCAAG**-3'
EGFR-Gibson-R	5'-CCAATTCTATTA**TGCTCCAATAAATTCACTGCTTTGTG**-3'
EGFR-Mut-F	5'-AAT**AAAACCGGACTGAAGGAGCTGCCCATG**-3'
EGFR-Mut-R	5'-**TGCATCATAGTTAGATAAGACTGCTAAGGCATAGG**-3'

*Sequences overlapping with template DNA are bolded. Restriction sites are underlined.

Synthetic Genes and Plasmids

sfGFP-His6 gene	ATGGTTAGCAAAGGTGAAGAACTGTTTACCGGCGTTGTGCCGATTCTGGTGGAACTGG ATGGTGATGTGAATGGCCATAAATTTAGCGTTCGTGGCGAAGGCGAAGGTGATGCGA CCAACGGTAAACTGACCCTGAAATTTATTTGCACCACCGGTAAACTGCCGGTTCCGTGG CCGACCCTGGTGACCACCCTGACCTATGGCGTTCAGTGCTTTAGCCGCTATCCGGATCA TATGAAACGCCATGATTTCTTTAAAAGCGCGATGCCGGAAGGCTATGTGCAGGAACGT ACCATTAGCTTCAAAGATGATGGCACCTATAAAACCCGTGCGGAAGTTAAATTTGAAG GCGATACCCTGGTGAACCGCATTGAACTGAAAGGTATTGATTTTAAAGAAGATGGCAA CATTCTGGGTCATAAACTGGAATATAATTTCAACAGCCATGCGGTGTATATTACCGCCG ATAAACAGAAAAATGGCATCAAAGCGAACTTTAAAATCCGTCACAACGTGGAAGATGG TAGCGTGCAGCTGGCGGATCATTATCAGCAGAATACCCCGATTGGTGATGGCCCGGTG CTGCTGCCGGATAATCATTATCTGAGCACCCAGAGCGTTCTGAGCAAAGATCCGAATG AAAAACGTGATCATATGGTGCTGCTGGAATTTGTTACCGCCGCGGGCATTACCCACGG TATGGATGAACTGTATAAAGGCAGCCACCATCATCATCACCAT
pHTC-sfGFP-HaloTag	Mammalian cell expression vector for sfGFP-HaloTag fusion protein.
pET28b(+)-sfGFP-HaloTag	Bacterial cell expression vector for sfGFP-HaloTag fusion protein.
pHTC-SignalSeq	Mammalian cell expression vector with an EGFR N-terminal signal sequence ahead of HaloTag protein.
pHTC-SignalSeq-HaloTag-EGFR(128TAG)	Mammalian cell expression vector with an EGFR N-terminal signal sequence ahead of a HaloTag-EGFR(128TAG) fusion protein.
pHTC-SignalSeq-HaloTag-EGFR	Mammalian cell expression vector with an EGFR N-terminal signal sequence ahead of a HaloTag-EGFR fusion protein.

Cloning

pHTC-sfGFP-HaloTag: sfGFP-His6 was PCR amplified with primers sfGFP-His6-F and sfGFP-His6-R. The PCR product was then gel purified, restriction site digested with *NheI* and *XhoI* (1 h, 37 °C), then purified with a DNA clean and concentrator kit. The pHTC CMV-neo plasmid was restriction digested with *NheI* and *XhoI*, treated with Antarctic phosphatase, then gel purified. The digested vector (100 ng) was combined with digested insert in a 1:3 molar ratio and ligated with T4 DNA ligase (30 min, room temperature). The ligation mixture (5 μL) was transformed into chemically competent DH5α cells and selected on ampicillin LB/agar plates. Several of the resulting colonies were selected and used to inoculate 5 mL of LB media with 100 μg/mL ampicillin. Plasmids were isolated using a miniprep kit and sequence verified. Plasmids for use in mammalian cell transfections were prepared with a maxiprep kit.

pET28b(+)-sfGFP-HaloTag: The sfGFP-HaloTag gene was PCR amplified from pHTC-sfGFP-HaloTag with primers sfGFP-HaloTag-Gibson-F and sfGFP-HaloTag-Gibson-R and gel purified. Plasmid pET28b(+) (Novagen) was linearized by restriction site digestion with *NcoI*-HF and *XhoI* (1 h, 37 °C) then gel purified. The vector (100 ng) was combined with the sfGFP-HaloTag PCR product in a 1:2 molar ratio and assembled by Gibson assembly using the NEB HiFi DNA Assembly master mix (15 min, 50 °C). The assembly mixture (2 μL) was transformed into chemically competent DH5α cells and selected on kanamycin LB/agar plates. Several of the resulting colonies were selected and used to inoculate 5 mL of LB media with 50 μg/mL kanamycin. Plasmids were isolated using a miniprep kit and sequence verified.

pHTC-SignalSeq: The N-terminal signal sequence of EGFR was added to the N-terminus of HaloTag by PCR mutagenesis. pHTC CMV-neo vector was PCR amplified with primers pHTC-SignalSeq-Mut-F and pHTC-SignalSeq-Mut-R. The PCR product was then added to a KLD mix (kinase, ligase, and *DpnI* mixture) from New England Biolabs for blunt end ligation and template digestion. The ligation mixture (5 μL) was transformed into chemically competent DH5α cells and selected on ampicillin LB/agar plates. Several of the resulting colonies were selected and used to inoculate 5 mL of LB media with 100 μg/mL ampicillin. Plasmids were isolated using a miniprep kit and sequence verified.

pHTC-SignalSeq-HaloTag-EGFR(128TAG): The EGFR gene with an amber stop codon mutation at position 128 was PCR amplified from pMmPyls-EGFR(128TAG)-GFP-HA (Chin lab)[1] with primers EGFR-Gibson-F and EGFR-Gibson-R and gel purified. Plasmid pHTC-SignalSeq was linearized by PCR amplified with primers pHTC-vector-F and pHTC-vector-R. The PCR mixture (8 μL) was digested with *DpnI* (30 min, 50 °C). The *DpnI* was then heat inactivated (20 min, 80 °C). The linearized vector (100 ng) was combined with the purified EGFR PCR product in a 1:2 molar ratio and assembled by Gibson assembly using the NEB HiFi DNA Assembly master mix (15 min, 50 °C). The assembly mixture (2 μL) was transformed into chemically competent DH10β cells and selected on carbenicillin LB/agar plates. Several of the resulting colonies were selected and used to inoculate 5 mL of LB media with 50 μg/mL carbenicillin. Plasmids were isolated using a miniprep kit and sequence verified.

pHTC-SignalSeq-HaloTag-EGFR: The amber stop codon at position 128 of EGFR in pHTC-SignalSeq-HaloTag-EGFR(128TAG) was replaced with the native asparagine residue by site-directed mutagenesis. pHTC-SignalSeq-HaloTag-EGFR(128TAG) was PCR amplified with primers EGFR-Mut-F and EGFR-Mut-R. The PCR product was then added to a KLD mix (kinase, ligase, and *DpnI* mixture) from New England Biolabs for blunt end ligation and template digestion. The ligation mixture (5 µL) was transformed into chemically competent DH10β cells and selected on carbenicillin LB/agar plates. Several of the resulting colonies were selected and used to inoculate 5 mL of LB media with 100 µg/mL carbenicillin. Plasmids were isolated using a miniprep kit and sequence verified. Plasmids for use in mammalian cell transfections were prepared with a maxiprep kit.

Protein Purification

sfGFP-HaloTag: pET28b(+)-sfGFP-HaloTag (20 ng) was transformed into chemically competent DH5α cells and selected on kanamycin LB/agar plates. A single colony was selected and used to inoculate LB media (100 mL) containing 50 µg/mL kanamycin. The starter culture was grown to saturation overnight. 2 × 1 L LB broth each supplemented with D-glucose (5 mL, 40% w/v) and 50 µg/mL kanamycin were inoculated with the starter culture (50 mL) and grown to OD ~0.5. Protein expression was induced with 1 mM IPTG for 6 h. The cells were pelleted by centrifugation (20 min, 5000×g), and the supernatant was discarded. Lysis buffer (20 mL, pH 8.0, 50 mM NaH_2PO_4, 300 mM NaCl, 10 mM imidazole, 1 mg/mL lysozyme, Roche cOmplete protease inhibitor cocktail tablet) and benzonase (25 U/mL) were added to the cell pellet at 0 °C, and the cells were resuspended with a Dounce homogenizer. The cells were then lysed with a homogenizer (15000 psi) and clarified by centrifugation (30 min, 15000×g). The clarified lysate was loaded onto a His Tag affinity column (GE HisTrap HP, 5 mL), washed with wash buffer (50 mL, pH 8.0, 50 mM NaH_2PO_4, 300 mM NaCl, 10 mM imidazole), and eluted with elution buffer (100 mL, pH 8.0, 50 mM NaH_2PO_4, 300 mM NaCl, 10→250 mM imidazole). Fractions containing protein were collected then concentrated with a 30 kDa molecular weight cutoff filter (Amicon) to a concentration of 10 mg/mL. The protein was then purified by size exclusion column chromatography (HiLoad 16/60 Superdex 75, GE Healthcare) on an FPLC with elution buffer (pH 7.5, 50 mM NaH_2PO_4, 300 mM NaCl). Pure fractions were collected and concentrated with a 30 kDa molecular weight cutoff filter to ~10 mg/mL. Glycerol was added to a final volume of 10% v/v, and the protein concentration was determined by A_{280} measurements in denaturing buffer (6 M guanidinium, 30 mM MOPS, pH 7.0) on a spectrophotometer. The protein solution was aliquoted, flash frozen in liquid nitrogen, and stored at –80 °C.

HaloTag Conjugation

Pure lyophilized HaloTag protein (4.10 mg) was dissolved in phosphate buffer (1 mL, pH 7.5, 50 mM NaH_2PO_4, 300 mM NaCl) and reacted with a HaloTag linker-bound profluorophore **8** (30.4 µL, 10 mM in 1:1 DMF:PBS, 304 nmol, 2.5 equiv) at room temperature. After 1 h, the solution was concentrated to a volume of 250 µL with a 10 kDa molecular weight cutoff filter (Amicon). The conjugated protein was purified by size exclusion chromatography (HiLoad 16/60 Superdex 75, GE Healthcare) on an FPLC with elution buffer (pH 7.5, 50 mM NaH_2PO_4, 300 mM NaCl). Column fractions containing the protein were concentrated to a concentration of ~10 mg/mL with a 10 kDa molecular weight cutoff filter (Amicon). Protein concentration was determined by A_{280} measurements in denaturant (6 M guanidinium, 30 mM MOPS, pH 7.0) on a spectrophotometer.

Stopped-Flow Kinetics

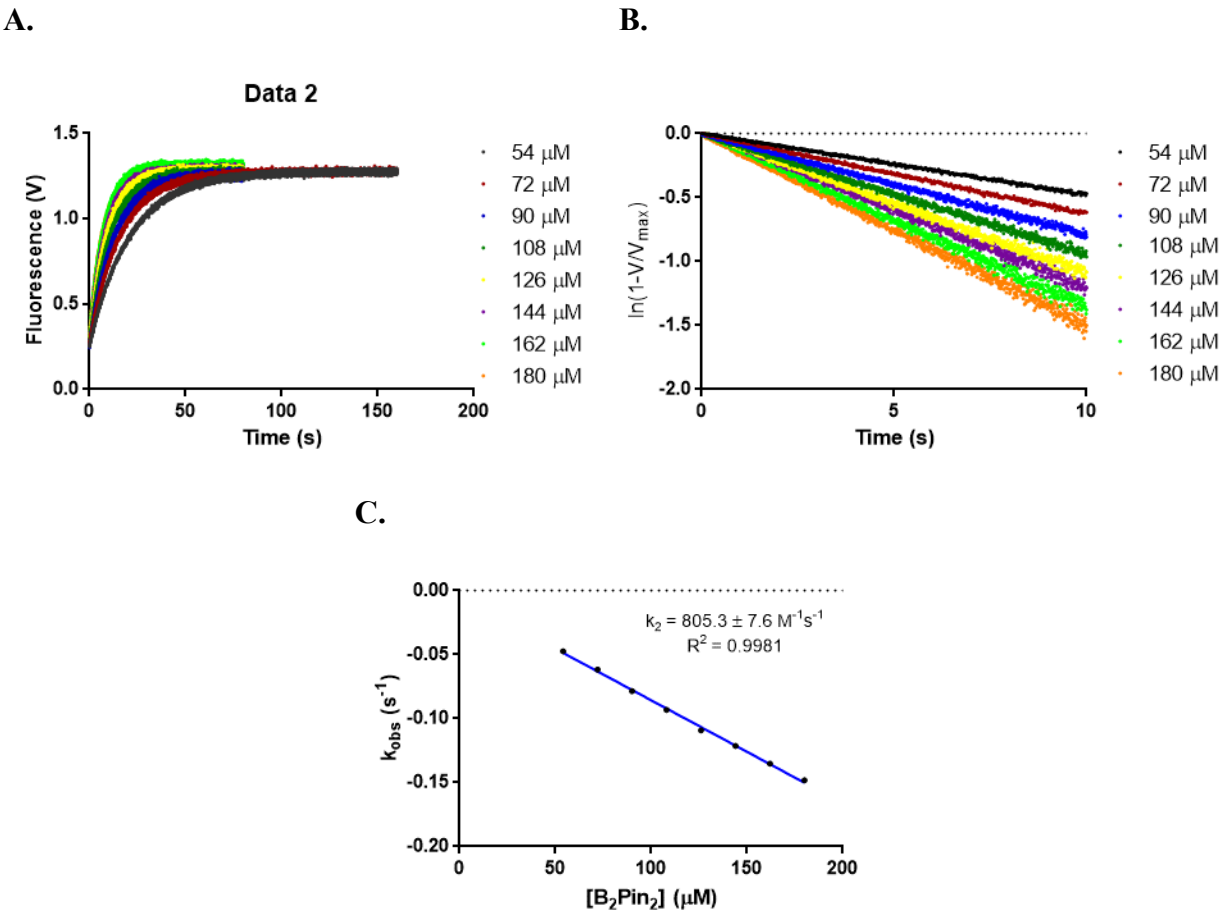


Figure S8. Second-order rate constant determination for the reaction between profluorophore **6** and B_2pin_2 using pseudo-first-order kinetics. (A) Time-dependent fluorescence was recorded at multiple concentrations of B_2pin_2 upon reaction with 2 µM **6**. Each point represents an average of three measurements. (B) The observed pseudo-first-order rate constant for the reaction was determined from the slope of the plot $\ln(1–V/V_{max})$ versus time. Each point represents the mean of three measurements. (C) The second-order rate constant was determined from the slope of the plot of the observed rate constant versus the concentration of the saturating component B_2pin_2. Each point represents the mean of three measurements for the pseudo-first-order rate constant. Error bars, not visible on the displayed scale, are present for each value and represent a standard deviation. The blue line is a best fit line of the mean values.

Profluorophore **6**: A 20 µM solution of profluorophore **6** was prepared in PBS (5 mL) from a 1 mM stock solution in PBS. 200, 180, 160, 140, 120, 100, 80, and 60 µM solutions of freshly crystallized B_2pin_2 were prepared in PBS (10 mL) from a 100 mM stock solution in DMF. The profluorophore and B_2pin_2 solutions were drawn into separate syringes of the stopped flow instrument. A total of six injections were made for each concentration of B_2pin_2. The first three injections from each set were required to ensure that the system was cleared of solution from the previous set, so only data from the last three injections were used. The total volume per injection

was 250 µL; the solution volume ratio of B_2pin_2:**6** was 9:1; the flow rate was 3 mL/min, and the dead time was 12.2 ms. The excitation wavelength was 522 nm, and an emission cutoff filter of 530 nm was used. Fluorescence intensities were measured every 10 ms for B_2pin_2 concentrations ≥ 100 µM and every 20 ms for B_2pin_2 concentrations of 60 and 80 µM. 8000 data points were collected for each run.

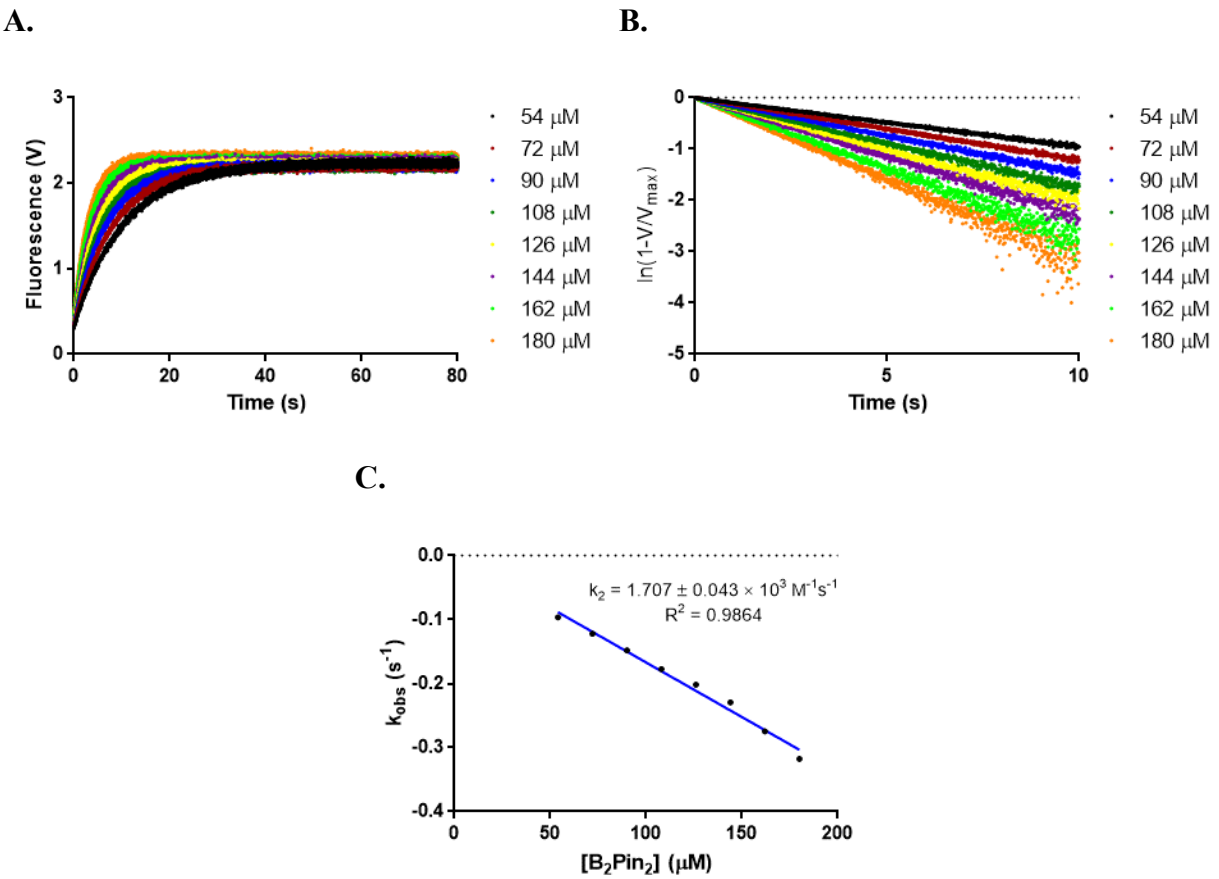


Figure S9. Second-order rate constant determination for the reaction between profluorophore **8** and B_2pin_2 using pseudo-first-order kinetics. (A) Time-dependent fluorescence was recorded at multiple concentrations of B_2pin_2 upon reaction with 2 µM **8**. Each point represents an average of three measurements. (B) The observed pseudo-first-order rate constant for the reaction was determined from the slope of the plot $\ln(1–V/V_{max})$ versus time. Each point represents the mean of three measurements. (C) The second-order rate constant was determined from the slope of the plot of the observed rate constant versus the concentration of the saturating component B_2pin_2. Each point represents the mean of three measurements for the pseudo-first-order rate constant. Error bars, not visible on the displayed scale, are present for each value and represent a standard deviation. The blue line represents a best fit line of the mean values.

Profluorophore **8**: A 20 µM solution of profluorophore **8** was prepared in PBS (5 mL) from a 10 mM stock solution in 1:1 DMF:PBS. 200, 180, 160, 140, 120, 100, 80, and 60 µM solutions of freshly crystallized B_2pin_2 were prepared in PBS (10 mL) from a 100 mM stock solution in DMF. The profluorophore and B_2pin_2 solutions were drawn into separate syringes of the stopped flow

instrument. A total of six injections were made for each concentration of B_2pin_2. The first three injections from each set were required to ensure that the system was cleared of the solutions from the previous set, so only data from the last three injections were used. The total volume per injection was 250 µL; the injection volume ratio of B_2pin_2:**8** solutions was 9:1; the flow rate was 3 mL/min, and the dead time was 12.2 ms. The excitation wavelength was 508 nm, and an emission cutoff filter of 515 nm was used. Fluorescence intensities were measured every 10 ms. 8000 data points were collected for each run.

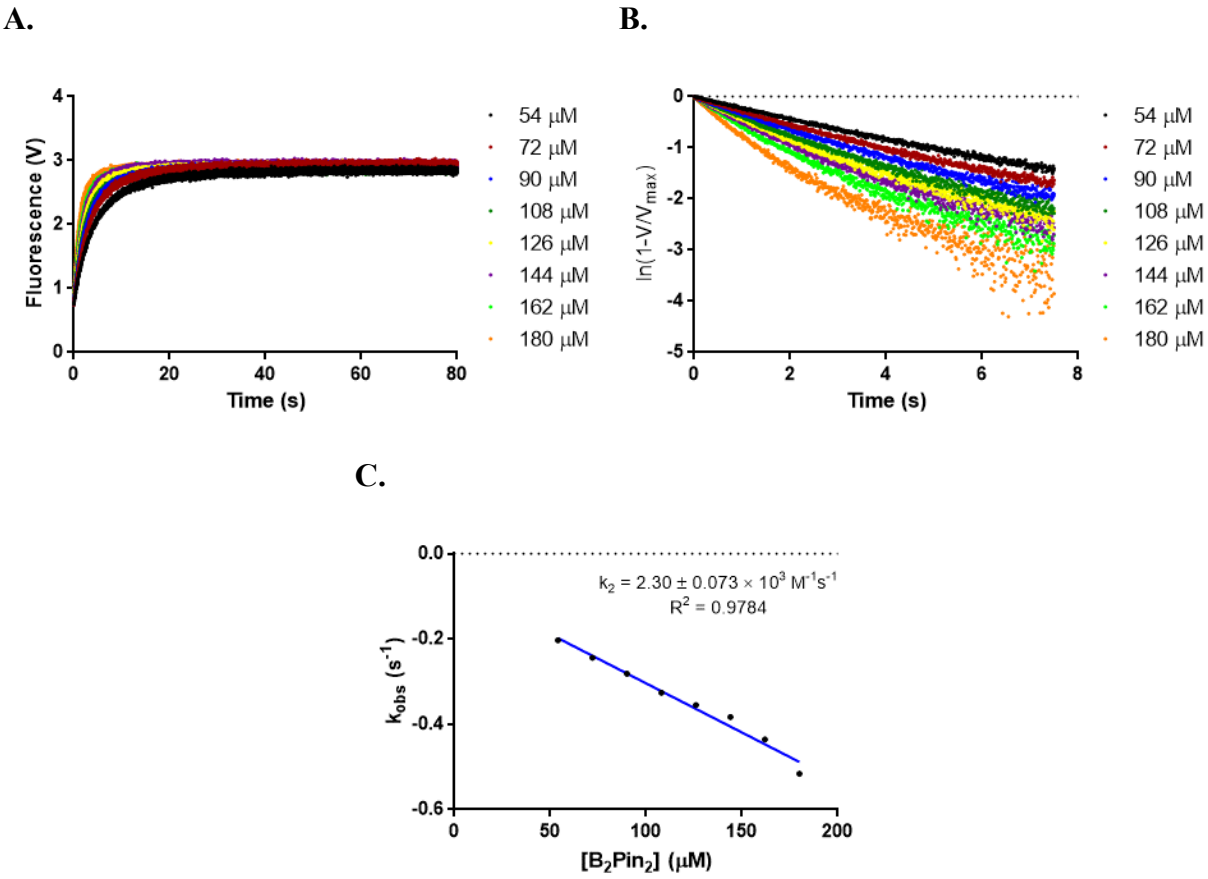


Figure S10. Second-order rate constant determination for the reaction between HaloTag-profluorophore **9** conjugate and B_2pin_2 using pseudo-first-order kinetics. (A) Time-dependent fluorescence was recorded at multiple concentrations of B_2pin_2 upon reaction with 2 µM protein-profluorophore **9** conjugate. Each point represents an average of three measurements. (B) The observed pseudo-first-order rate constant for the reaction was determined from the slope of the plot $\ln(1–V/V_{max})$ versus time. Each point represents the mean of three measurements. (C) The second-order rate constant was determined from the slope of the plot of the observed rate constant versus the concentration of the saturating component B_2pin_2. Each point represents the mean of three measurements for the pseudo-first-order rate constant. Error bars, not visible on the displayed scale, are present for each value and represent a standard deviation. The blue line represents a best fit line of the mean values.

HaloTag-profluorophore **9** conjugate: A 20 μM solution of HaloTag-conjugated profluorophore **9** was prepared in PBS (3 mL) from a 162 μM stock solution in phosphate buffer (pH 7.5, 50 mM NaH_2PO_4, 300 mM NaCl). 200, 180, 160, 140, 120, 100, 80, and 60 μM solutions of freshly crystallized B_2pin_2 were prepared in PBS (10 mL) from a 100 mM stock solution in DMF. The HaloTag-profluorophore **9** conjugate and B_2pin_2 solutions were drawn into separate syringes of the stopped flow instrument. A total of six injections were made for each concentration of B_2pin_2. The first three injections from each set were required to ensure that the system was cleared of the solutions from the previous set, so only data from the last three injections were used. The total volume per injection was 250 μL; the solution volume ratio of B_2pin_2:HaloTag-profluorophore **9** conjugate was 9:1; the flow rate was 3 mL/min, and the dead time was 12.2 ms. The excitation wavelength was 508 nm, and an emission cutoff filter of 515 nm was used. Fluorescence intensities were measured every 10 ms. 8000 data points were collected for each run.

Cell Lysate Preparation

Mammalian cell lysate: Jurkat cells were grown to a density of 850,000 cell/mL in RPMI medium (2 × 200 mL) then pelleted by centrifugation. The cells were resuspended in PBS (25 mL), pelleted, then resuspended in PBS (5 mL). The cells were lysed on ice with a tip sonicator (12 × 10 sec on, 1 min off, power level 4). The solution was centrifuged (10 min, 3700×g), and the clarified lysate was separated. The protein concentration was quantified by BCA assay.

Bacterial cell lysate: *E. coli* DH5α were grown to saturation in LB medium (250 mL) then pelleted by centrifugation. The cells were resuspended in PBS (10 mL), pelleted, then resuspended in PBS (5 mL). The cells were lysed on ice with a tip sonicator (12 × 10 sec on, 1 min off, power level 4). The solution was centrifuged (10 min, 3700×g), and the clarified lysate was separated. The protein concentration was quantified by BCA assay.

Reactions in Cell Lysate

Reaction viability in cell lysate: Six reaction conditions were set up in triplicate for each of three media: PBS, Jurkat cell lysate (1 mg/mL final protein concentration), *E. coli* cell lysate (1 mg/mL final protein concentration). 2 μM 2× solution of activated fluorophore was created by adding 100 mM B_2pin_2 in DMSO (5 μL) to 2 μM profluorophore **6** in PBS (5 mL). Condition 1: 2× medium (100 μL) + PBS (100 μL); Condition 2: 2× medium (100 μL) + 2 μM 2× solution of activated fluorophore **7** in PBS (100 μL); Condition 3: 2× medium (100 μL) + 2 μM 2× solution of profluorophore **6** in PBS (100 μL); Condition 4: 500 μM B_2pin_2 in 2× medium (100 μL) + 2 μM 2× solution of profluorophore **6** in PBS (100 μL); Condition 5: 50 μM B_2pin_2 in 2× medium (100 μL) + 2 μM 2× solution of profluorophore **6** in PBS (100 μL); Condition 6: 5 μM B_2pin_2 in 2× medium (100 μL) + 2 μM 2× solution of profluorophore **6** in PBS (100 μL). The reactions were allowed to proceed in the dark. After 30 min, the fluorescence intensity was recorded on a plate reader with an excitation wavelength of 522 nm and an emission wavelength of 553 nm. Measurements for a given medium were baselined against the value for condition 1 then normalized against the value for condition 2.

N-*oxide stability in cell lysate*: Three conditions were set up in triplicate for each of three media: PBS, Jurkat cell lysate (1 mg/mL final protein concentration), *E. coli* cell lysate (1 mg/mL final

protein concentration). 2 μM 2× solution of activated fluorophore was created by adding 100 mM B_2pin_2 in DMSO (5 μL) to 2 μM profluorophore **6** in PBS (5 mL). Condition 1: 2× medium (100 μL) + PBS (100 μL); Condition 3: 2× medium (100 μL) + 2 μM 2× solution of profluorophore **6** in PBS (100 μL); Condition 2: 2× medium (100 μL) + 2 μM 2× solution of activated fluorophore **7** in PBS (100 μL). The fluorescence intensity was recorded every 15 sec on a plate reader with an excitation wavelength of 522 nm, emission wavelength of 553 nm, and an emission cutoff of 550 nm. Measurements for a given medium were baselined against the value for condition 1 then normalized against the value for condition 3.

Plate Reader Kinetics

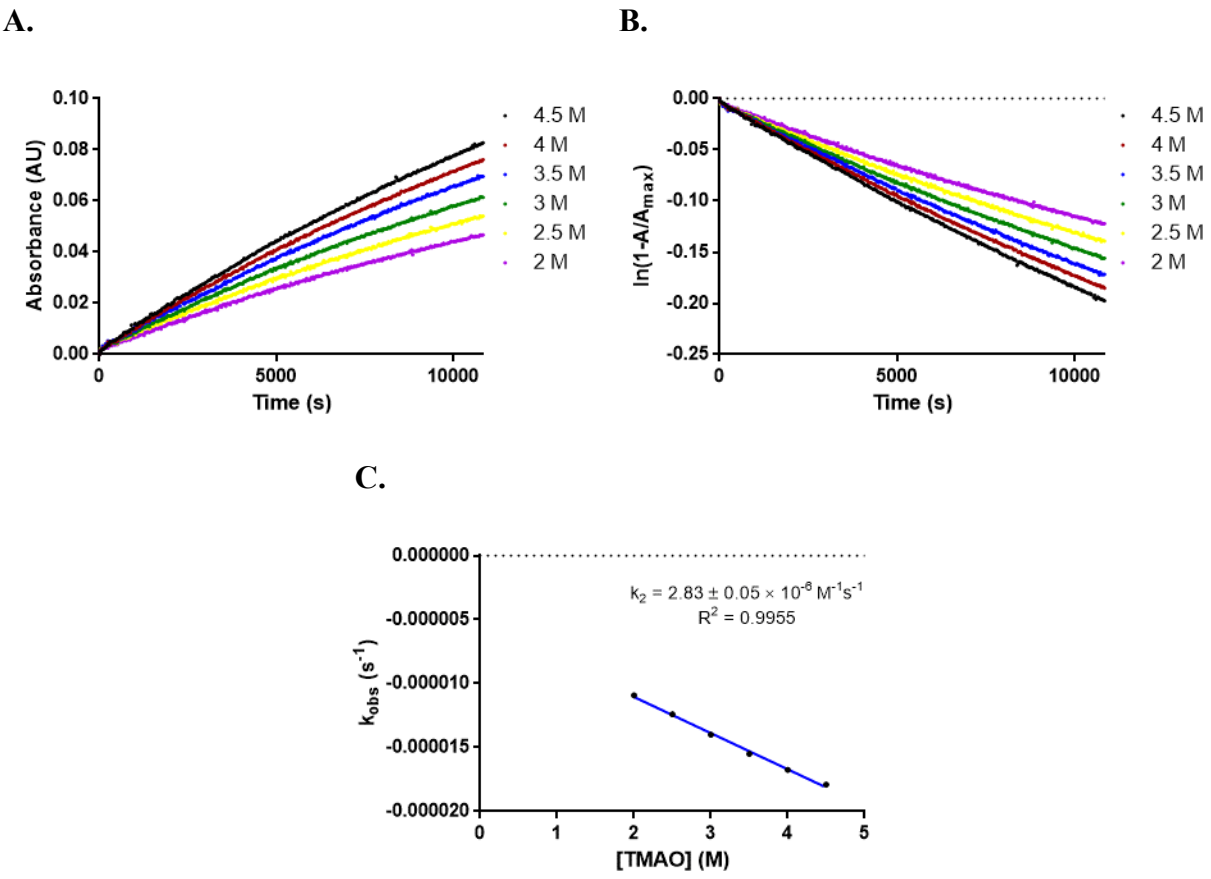


Figure S11. Second-order rate constant determination for the reaction between *p*-nitrophenylboronic acid (**2**) and TMAO using pseudo-first-order kinetics. (A) Time-dependent absorbance was recorded at multiple concentrations of TMAO upon reaction with 2 μM **2**. Each point represents an average of three measurements. (B) The observed pseudo-first-order rate constant for the reaction was determined from the slope of the plot $\ln(1-A/A_{max})$ versus time. Each point represents the mean of three measurements. (C) The second-order rate constant was determined from the slope of the plot of the observed rate constant versus the concentration of the saturating component B_2pin_2. Each point represents the mean of three measurements for the pseudo-first-order rate constant. Error bars, not visible on the displayed scale, are present for each value and represent a standard deviation. The blue line represents a best fit line of the mean values.

Kinetics of reaction of TMAO and p-nitrophenylboronic acid: 5.00, 4.44, 3.89, 3.33, 2.78, 2.22, and 0 M solutions of TMAO in H_2O (2 mL) were prepared from a stock solution of 5.22 M TMAO in H_2O (10 mL). Each solution (180 µL) was added to a 96-well flat clear-bottom plate in sextuplicate. A negative control of H_2O (200 µL) was also added. A 20 µM 10× solution of *p*-nitrophenylboronic acid (**2**) in H_2O (20 µL), prepared from 100 mM **2** in DMSO, was added in triplicate to each of the conditions. A 20 µM 10× solution of *p*-nitrophenol (**3**) in H_2O (20 µL), prepared from 100 mM **3** in DMSO, was added in triplicate to each of the conditions. The absorbance of the solutions at 405 nm was measured on a plate reader. Readings were taken every 30 sec for 3 h. Absorbance readings were baselined against the initial time point and the absorbance for the *p*-nitrophenol (**3**) positive control for each condition was used as the maximum absorbance for each condition.

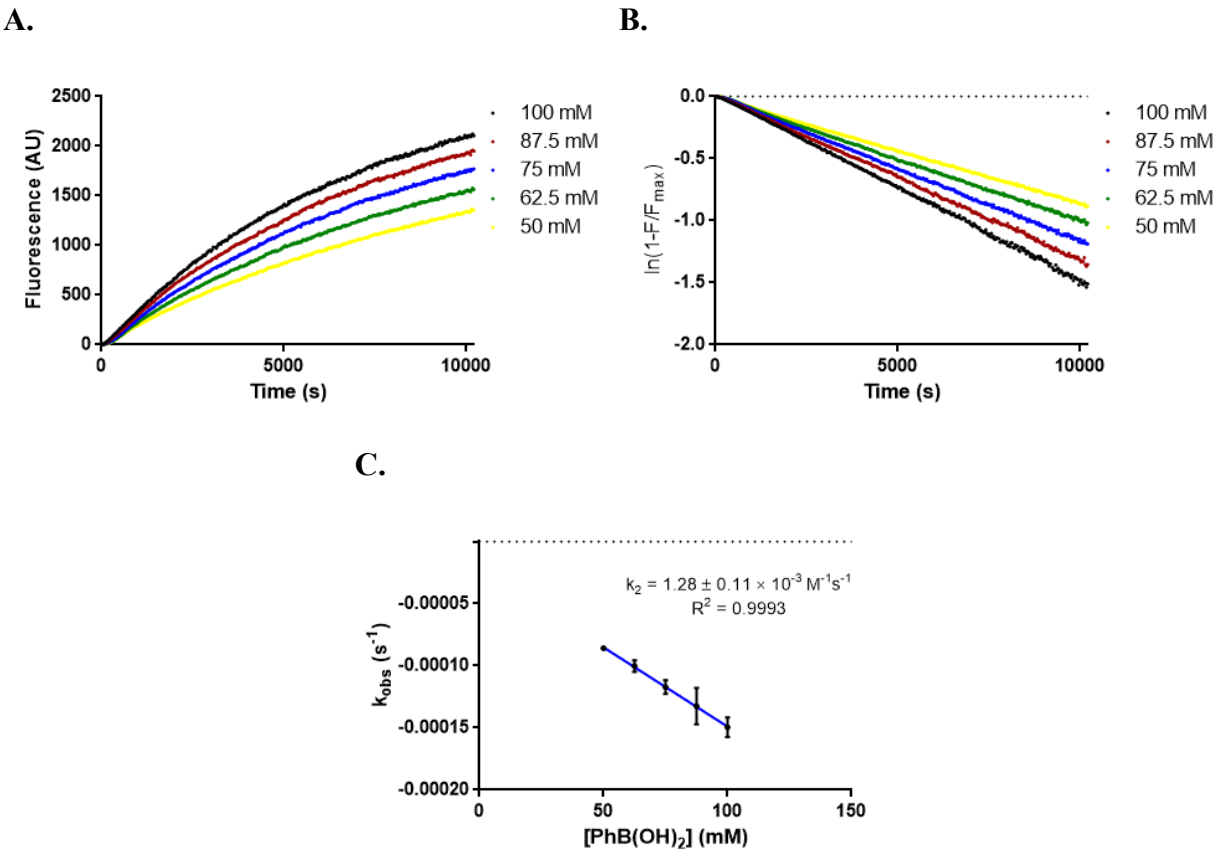


Figure S12. Second-order rate constant determination for the reaction between profluorophore **6** and $PhB(OH)_2$ using pseudo-first-order kinetics. (A) Time-dependent fluorescence was recorded at multiple concentrations of $PhB(OH)_2$ upon reaction with 2 µM profluorophore **6**. Each point represents an average of three measurements. (B) The observed pseudo-first-order rate constant for the reaction was determined from the slope of the plot $\ln(1–F/F_{max})$ versus time. Each point represents the mean of three measurements. (C) The second-order rate constant was determined from the slope of the plot of the observed rate constant versus the concentration of the saturating component $PhB(OH)_2$. Each point represents the mean of three measurements for the pseudo-first-

order rate constant. Error bars, some not visible on this scale, represent a standard deviation. The blue line represents a best fit line of the mean values.

*Kinetics of reaction of profluorophore **6** and phenylboronic acid*: 275, 250, 225, 200, 175, 150, 125, 100 and 0 mM 2× solutions of phenylboronic acid in PBS (1 mL) were prepared from a stock solution of 275 mM phenylboronic acid in PBS (10 mL). Each solution (100 µL) was added to a 96-well black round-bottom plate in sextuplicate. A negative control of PBS (200 µL) was also added. A 4 µM 2× solution of profluorophore **6** in PBS (100 µL) was added in triplicate to each of the conditions. A 4 µM 2× solution of activated fluorophore **7** in PBS (100 µL), prepared from the addition of a 100 mM 1000× solution of B_2pin_2 in DMSO (5 µL) to a 4 µM 2× solution of profluorophore **6** in PBS (5 mL), was added in triplicate to each of the conditions. The fluorescence intensity was recorded every 15 sec on a plate reader with an excitation wavelength of 522 nm, emission wavelength of 553 nm, and an emission cutoff of 550 nm. Fluorescence intensity readings were baselined against the initial time point, and the fluorescence intensity of the activated fluorophore positive control for each condition was used as the maximum fluorescence intensity for each condition.

MTT Cell Viability Assay

A 5 mM $B_2(OH)_4$ solution in 1% DMSO/DMEM medium without phenol red was made from a 500 mM $B_2(OH)_4$ solution in DMSO. Starting with this solution, 10 2× serial dilutions were performed in a flat-bottom 96-well plate with 1% DMSO/DMEM medium without phenol red to generate a total of eleven 50 µL solutions of $B_2(OH)_4$ in 1% DMSO/DMEM without phenol red with concentrations ranging from 4.88 µM–5 mM. A solution of 1% DMSO/DMEM without phenol red with no $B_2(OH)_4$ served as a negative control. A confluent plate of HEK293T cells were trypsinized, diluted with DMEM containing fetal bovine serum and pelleted by centrifugation. The cells were washed with 10 mL DMEM media without phenol red then resuspended in the same medium to a concentration of 300,000 cells/mL. 50 µL of cell suspension was added to each well. A no cell control well containing 100 µL of 0.5% DMSO/DMEM without phenol red was added. The plate was then placed in an incubator at 37 °C with 5% CO_2. After 24 h, 10 µL 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT, 5 mg/mL in PBS) was added to each well. After 2 h, 100 µL solubilizing solution (10% v/v TritonX-100 and 0.1% v/v conc. HCl in isopropanol) was added to each well. The plates were covered in foil and stirred on a shaker. After 2 h, absorbance at 570 nm was recorded on a plate reader. Data were baselined to the no cell control and normalized against the no diboron control. Validity of the no cell control was independently verified using a positive control of cells treated with 0.1% v/v Triton X-100 in PBS. All data were prepared in triplicate. The experiment was performed analogously with 2 mM B_2pin_2, and all of the experiments were repeated with HeLa and MEF cell lines.

In-Gel Fluorescence Imaging

10 mM Halotag linker-bound TAMRA **S3** in DMSO (3.12 μL, 2.00 equiv) was added to a solution of GFP-HaloTag (1 mg, 1.00 equiv) in phosphate buffer (163.1 μL, 95.69 μM, pH 7.5, 50 mM NaH_2PO_4, 300 mM NaCl). 10 mM HaloTag linker-bound TAMRA bis-*N*-oxide **10** in DMSO (3.12 μL, 2.00 equiv) was added to a solution of GFP-HaloTag (1 mg, 1.00 equiv) in phosphate buffer (163.1 μL, 95.69 μM, pH 7.5, 50 mM NaH_2PO_4, 300 mM NaCl). Each conjugate was purified by FPLC on a size exclusion column (Superdex 200 10/300 GL, GE Healthcare) with phosphate elution buffer (pH 7.5, 50 mM NaH_2PO_4, 300 mM NaCl). The fractions containing desired protein conjugates were collected and concentrated using a 30 kDa molecular weight cutoff filter. The protein concentration was determined by A_{280} measurements in denaturing buffer (6 M guanidinium hydrochloride, 30 mM MOPS, pH 7.0) on a spectrophotometer.

1 μM 2× GFP-HaloTag fluorophore and bis-*N*-oxide profluorophore (**S3** and **10**) conjugates in phosphate buffer (pH 7.5, 50 mM NaH_2PO_4, 300 mM NaCl) were prepared. 2, 5, 10, and 50 μM 2× B_2pin_2 in phosphate buffer were also prepared. The 2× GFP-HaloTag profluorophore conjugate solution was aliquoted (6.75 μL) into four sets of four PCR tubes. Each 2× B_2pin_2 solution was added in quadruplicate to the aliquoted enzymes. After 5, 15, 30, and 60 min, one of each distinct reaction conditions was quenched with 4× SDS sample loading buffer containing 400 mM TMAO (4.5 μL). For a positive control, phosphate buffer (6.75 μL) was added to 2× GFP-HaloTag fluorophore conjugate (6.75 μL) in phosphate buffer. For a second positive control, 100 μM 2× B_2pin_2 in phosphate buffer (6.75 μL) was added to a 2× solution of GFP-HaloTag profluorophore conjugate (6.75 μL). For a negative control, phosphate buffer (6.75 μL) was added to 2× GFP-HaloTag fluorophore conjugate (6.75 μL) in phosphate buffer. After 30 min, all three controls were quenched with 4× SDS sample loading buffer containing 400 mM TMAO (4.5 μL). Each solution (16 μL) was loaded onto a 26-well XT Criterion Bis-Tris gel. The gel was run at 0 °C and at 175 V for 1 h using XT MES as a running buffer. The gel was imaged on a Typhoon molecular imager.

Flow Cytometry

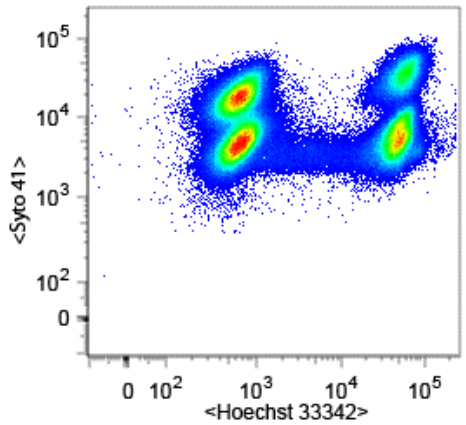


Figure S13. Four populations of cells displaying distinct cell surface modifications (HaloTag-EGFR/*N*-oxide, aldehyde, cyclopropene, and azide) were each labeled with a unique combination of Hoechst 33342 and Syto 41 nuclear stains in a binary fashion. The cell types were differentiated using flow cytometry and analyzed as separate populations.

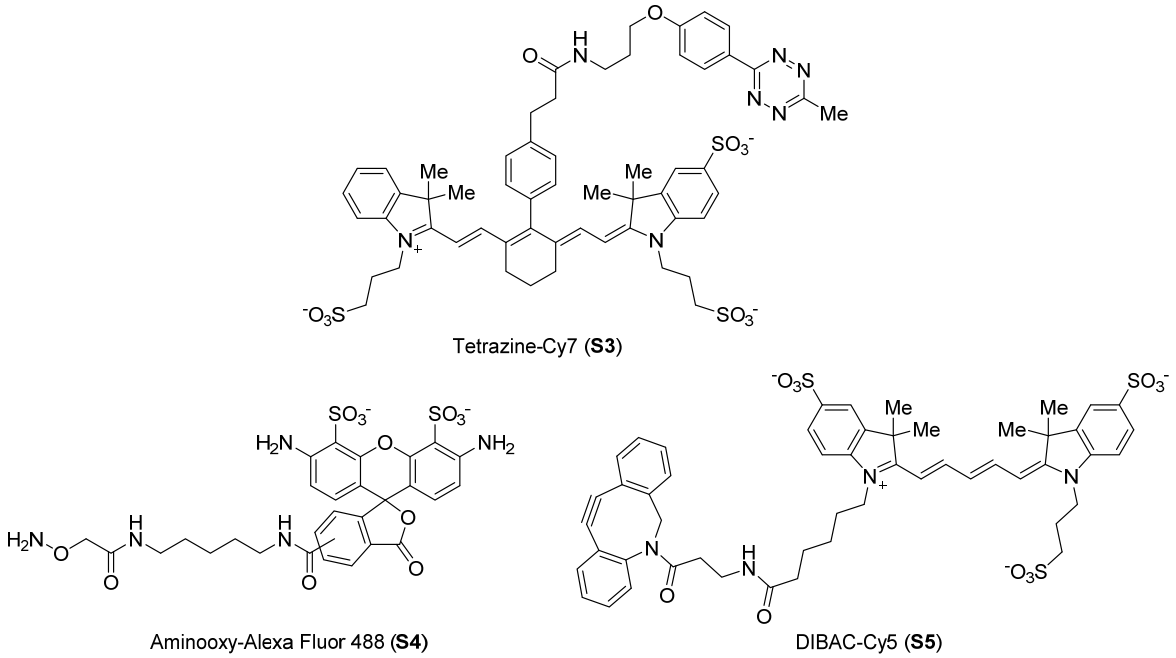


Figure S14. Reagents used for cell surface labeling.

HEK293T cells were maintained in culture in Dulbecco's Modified Eagle Medium (DMEM) high glucose media in a 37 °C incubator with 5% CO_2. A confluent plate of HEK293T cells was trypsinized and split at a ratio of 1:6 into 5×10-cm plates containing DMEM (10 mL). 50 mM Ac_4ManNAz in DMSO (10 µL) was added to one plate. 50 mM Ac_4ManNCp in DMSO (10 µL) was added to a second plate. After 36 h, a third plate was supplemented with DMEM (5 mL) and transfected with Mirus Bio TransIT-293 transfection reagent (45 µL) and plasmid pHTC-SignalSeq-HaloTag-EGFR (15 µg) in OptiMEM I reduced serum medium (1.5 mL). After 24 h,

the five cell populations were either unmodified or designated for modification with azide, cyclopropene, aldehyde, or HaloTag-EGFR/*N*-oxide. Each were trypsinized, centrifuged (233×g, 3 min), and washed with PBS (10 mL). The aldehyde population was resuspended in 1 mM sodium periodate in PBS (5 mL) and incubated at 0 °C. The transfected cells were resuspended in PBS (2 mL) and 10 mM profluorophore **10** (2 μL) was added. They were then split in half, and 5 mM Syto 41 (1 μL) and 10 mM Hoechst 33342 (1 μL) were added to one of the new populations. The azide modified cells were resuspended in PBS (2 mL) and split in half. 5 mM Syto 41 (1 μL) was added to one of the new populations. The cyclopropene modified cells were resuspended in PBS (2 mL) and split in half. 10 mM Hoechst 33342 (1 μL) was added to one of the new populations. The unmodified cells were resuspended in PBS (1 mL) and an aliquot (200 μL) was removed. This aliquot was diluted to 1 mL, and 10 mM profluorophore **10** (1 μL) was added. All of these cells were incubated at room temperature. After 30 min, the sodium periodate solution for the aldehyde population was quenched by addition of 10 mM glycerol (5 mL). After 5 min, all of the cell populations were centrifuged (233×g, 3 min) and washed with PBS (10 mL). They were then resuspended in PBS (10 mL) and incubated at room temperature. After 15 min, all of the cell populations were pelleted and the supernatants were aspirated. The unmodified population was resuspended in pH 6.7 PBS containing 10 mM aniline (1 mL) and split into four populations. 10 mM aminooxy-Alexa Fluor 488 in PBS (2.5 μL) was added to one aliquot. The other aliquots were diluted to 1 mL in the same buffer. 10 mM tetrazine-Cy7 in DMSO (1 μL), 10 mM DIBAC-Cy5 in DMSO (1 μL), and 100 mM B_2pin_2 in DMSO (1 μL) were each added to a separate aliquot. The unmodified cells incubated with profluorophore **10** were resuspended in pH 6.7 PBS containing 10 mM aniline (2 mL) and split into two aliquots. 100 mM B_2pin_2 in DMSO (1 μL) was added to one of the aliquots. The transfected cells without nuclear stains were resuspended in pH 6.7 PBS containing 10 mM aniline (750 μL) and split into three aliquots. A cocktail of reagents was prepared by adding 10 mM tetrazine-Cy7 in DMSO (0.25 μL) and 10 mM DIBAC-Cy5 in DMSO (0.25 μL) to 10 mM aminooxy-Alexa Fluor 488 in PBS (2.5 μL). This cocktail was added to one aliquot. The other two aliquots were diluted to 1 mL with pH 6.7 PBS containing 10 mM aniline. 10 mM B_2pin_2 in DMSO (1 μL) was added to one of these aliquots. The cyclopropene-modified cells were resuspended in pH 6.7 PBS with 10 mM aniline (1 mL) and 10 mM tetrazine-Cy7 in DMSO (1 μL) was added. The azide-modified cells were resuspended in pH 6.7 PBS with 10 mM aniline (1 mL) and 10 mM DIBAC-Cy5 in DMSO (1 μL) was added. Aldehyde-modified cells were resuspended in pH 6.7 PBS with 10 mM aniline (500 μL) and split into two aliquots. 10 mM aminooxy-Alexa Fluor 488 in PBS (2.5 μL) was added to one aliquot. The other aliquot was transferred to an Eppendorf tube. Azide-labeled cells with nuclear staining was resuspended in pH 6.7 PBS with 10 mM aniline (500 μL). An aliquot of this suspension (250 μL) was added to the aforementioned tube. Cyclopropene-labeled cells with nuclear staining was resuspended in pH 6.7 PBS with 10 mM aniline (500 μL). An aliquot of this suspension (250 μL) was added to the aforementioned tube. Transfected and *N*-oxide labeled cells with nuclear staining was resuspended in pH 6.7 PBS with 10 mM aniline (500 μL). An aliquot of this suspension (250 μL) was added to the aforementioned tube. The contents of the tube were split into two aliquots. A cocktail of reagents was prepared by adding 10 mM tetrazine-Cy7 in DMSO (0.25 μL), 10 mM B_2pin_2 in DMSO (0.25 μL), and 10 mM DIBAC-Cy5 in DMSO (0.25 μL) to 10 mM aminooxy-Alexa Fluor 488 in PBS (2.5 μL). This cocktail was added to one of the aliquots containing the mélange of

cells. 17 total cell populations were created: 1. Unmodified, unstained. 2. Azide-modified, Syto 41-stained. 3. Cyclopropene-modified, Hoechst 33342-stained. 4. HaloTag-EGFR/*N*-oxide-modified, Syto41 and Hoechst 33342-stained. 5. Unmodified, unstained, tetrazine-Cy7-treated. 6. Unmodified, unstained, DIBAC-Cy5-treated. 7. Unmodified, unstained, aminooxy-Alexa Fluor 488-treated. 8. HaloTag-EGFR/*N*-oxide-modified, unstained. 9. HaloTag-EGFR/*N*-oxide-modified, unstained, B_2pin_2-treated. 10. Cyclopropene-modified, unstained, tetrazine-Cy7-treated. 11. Aldehyde-modified, unstained, aminooxy-Alexa Fluor 488-treated. 12. Azide-modified, unstained, DIBAC-Cy5-treated. 13. Mixture of populations 2, 3, 4, and aldehyde-modified, unstained cells. 14. Population 13 treated with aminooxy-Alexa Fluor 488, DIBAC-Cy5, tetrazine-Cy7, and B_2pin_2. 15. Unmodified, unstained, *N*-oxide-treated. 16. Unmodified, unstained, *N*-oxide and B_2pin_2-treated. 17. HaloTag-EGFR/*N*-oxide-modified, aminooxy-Alexa Fluor 488, DIBAC-Cy5, and tetrazine-Cy7-treated. Each population was analyzed by flow cytometry. For cells transfected with HaloTag-EGFR and treated with *N*-oxide, a bimodal distribution of cells was observed in the TAMRA channel likely due to variation in the copy number of the gene acquired during transfection. Populations statistics were taken for the more highly expressing subpopulation during analysis.

Confocal Microscopy

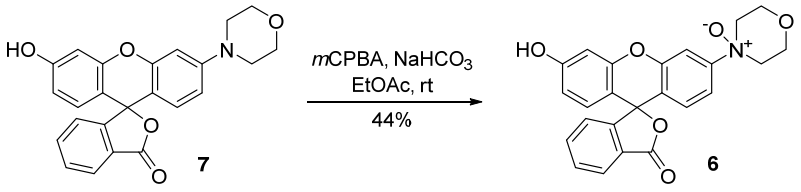
HEK293T cells were maintained in culture in Dulbecco's Modified Eagle Medium (DMEM) high glucose media in a 37 °C incubator with 5% CO_2. A confluent plate of HEK293T cells was trypsinized and split at a ratio of 1:6. Six wells of 8-well Lab-Tek chambered slides, precoated with fibronectin, were seeded with the cell suspension (375 µL) and cultured at 37 °C. The cells, which reached ~60% confluency after 24 h, were transfected using Mirus Bio TransIT-293 transfection reagent and plasmid pHTC-sfGFP-HaloTag in OptiMEM I reduced serum medium (37.5 µL). After 24 h, the media were replaced with the following: Well 1: DMEM medium (400 µL). Well 2: 10 µM HaloTag linker-bound TAMRA **S3** in DMEM (400 µL). Wells 3–6: 100 µM HaloTag linker-bound bis-*N*-oxide TAMRA **10** in DMEM (400 µL). The chambered slides were placed in the incubator at 37 °C. After 30 min, each well was washed twice with DMEM medium without phenol red (400 µL). DMEM medium without phenol red (400 µL) was then added for a third wash and incubated at room temperature. After 15 min, wells 1–3 were replaced with DMEM medium without phenol red containing 200 ng/mL Hoechst 33342 (400 µL). Well 4 was replaced with DMEM medium without phenol red containing 200 ng/mL Hoechst 33342 and 1 mM B_2pin_2 (400 µL). Well 4 was replaced with DMEM medium without phenol red containing 200 ng/mL Hoechst 33342 and 100 µM B_2pin_2 (400 µL). Well 6 was replaced with DMEM medium without phenol red containing 200 ng/mL Hoechst 33342 and 10 µM B_2pin_2 (400 µL). The chambered slide was placed in the incubator at 37 °C. After 45 min, the cells were imaged using a confocal microscope.

General Synthetic Methods

All reagents were obtained from Sigma–Aldrich, Acros, TCI, or Chem-Impex and used without further purification. Bis(pinacolato)diboron was purchased from Frontier Scientific and purified by recrystallization from pentane. Commercial *m*-chloroperbenzoic acid purchased from Sigma–Aldrich was washed with a pH 7.5 phosphate buffer and dried under reduced pressure.[2] Anhydrous solvents were dried and deoxygenated by purification through columns of alumina and Q-5[3] with the exception of *N,N*-dimethylformamide, which was purchased in a sealed bottle and stored over molecular sieves. Deuterated solvents were purchased from Cambridge Isotope Laboratories. Solvents were removed on a Buchi Rotavapor R-114 equipped with a Welch 2026 self-cleaning dry vacuum pump or with an Edwards RV3 vacuum pump.

Analytical thin layer chromatography (TLC) was performed with Silicycle 60 Å silica gel plates and analyzed by UV illumination or CAM staining. Flash chromatography was performed as described by Still et al.[4] on silica gel (60 Å pore size, 40–63 μm, 230-400 mesh silica gel, Silicycle). High performance liquid chromatography (HPLC) was performed on a Varian ProStar instrument with a UV absorption detector operating at 210, 220 and 254 nm. Preparative-scale HPLC was performed on a 100 Å C_{18} reverse phase column (250 x 21.4 mm) with a solvent flow rate of 20 mL/min.

Proton nuclear magnetic resonance (^{1}H NMR) spectra were recorded with a Bruker DRX-500 NMR spectrometer, are reported in parts per million on the δ scale, and are referenced from the residual protium in the NMR solvent ($CDCl_3$: δ 7.26 ($CHCl_3$) or CD_3OD: δ 3.31 (CD_2HOD)).[5] Data are reported as follows: chemical shift [multiplicity (s = singlet, d = doublet, t = triplet, m = multiplet), coupling constant(s) in Hertz, integration]. Carbon-13 nuclear magnetic resonance (^{13}C NMR) spectra were recorded with a Bruker DRX-500 NMR Spectrometer, are reported in parts per million on the δ scale, and are referenced from the carbon resonances of the solvent (CDCl3: δ 77.23 or CD_3OD: δ 49.00). Infrared data (IR) were obtained with a Bruker ALPHA Platinum ATR FT-IR and are reported as follows: frequency of absorption (cm^{-1}), intensity of absorption (s = strong, m = medium, w = weak, br = broad). NMR spectra were processed using MestReNova (Mestrelab Research S.L.). High-resolution ESI mass spectra of small molecules were obtained at the UC Berkeley Mass Spectrometery Facility on a Thermo LTQ Orbitrap mass spectrometer.



Rhodol *N*-Oxide (6):

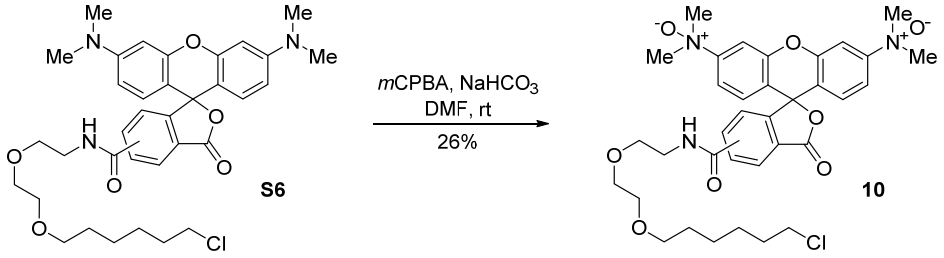
Trifluoroacetic acid (1 mL) was introduced via syringe to a solution of rhodol **7**[6] (14.3 mg, 32.1 µmol, 1 equiv) in dichloromethane (1 mL) at room temperature. After 1 h, the solution was concentrated to dryness under reduced pressure. The residue was dissolved in toluene (10 mL), then concentrated under reduced pressure. The resulting residue was dissolved in ethyl acetate (3 mL). Sodium bicarbonate (8.1 mg, 96.3 µmol, 3.00 equiv) and *m*-chloroperbenzoic acid (5.5 mg, 32.1 µmol, 1.00 equiv) were successively added to the solution at room temperature. After 1 h, the solution was concentrated to dryness. The pink residue was dissolved in methanol (4 mL), filtered, and purified by preparative HPLC on a C_{18} column (20 mL/min, time (min), % MeCN in H_2O + 0.1% TFA: 0, 0; 60, 100) to afford rhodol *N*-oxide **6** (5.9 mg, 14.1 µmol, 44%) as a pink film.

^{1}H NMR (500 MHz, CD_3OD) δ 8.11–8.04 (m, 2H), 7.78 (dt, *J* = 24.5, 7.4 Hz, 2H), 7.70 (dd, *J* = 9.0, 2.8 Hz, 1H), 7.23 (d, *J* = 7.6 Hz, 1H), 7.16 (d, *J* = 8.9 Hz, 1H), 7.23 (d, *J* = 7.6 Hz, 1H), 7.16 (d, *J* = 8.9 Hz, 1H), 6.78 (d, *J* = 2.4 Hz, 1H), 6.68 (d, *J* = 8.7 Hz, 1H), 6.63 (dd, *J* = 8.7, 2.5 Hz, 1H), 4.46–4.33 (m, 4H), 4.12 (d, *J* = 10.5 Hz, 2H), 3.98 (d, *J* = 10.8 Hz, 2H).

^{13}C NMR (126 MHz, CD_3OD) δ 170.7, 161.7, 153.9, 153.4, 153.2, 151.4, 137.0, 131.8, 131.7, 130.2, 127.5, 126.2, 125.1, 124.3, 116.3, 114.6, 111.2, 110.3, 103.6, 83.0, 67.0, 62.4.

FTIR (thin film, cm^{-1}) 1765 (s), 1671 (m), 1612 (s), 1465 (w), 1429 (s), 1248 (w), 1193 (s), 1122 (s).

HRMS (ESI, m/z) calculated for $C_{24}H_{20}NO_6$ $[M+H]^+$: 418.1285; found: 418.1276.



5(6)-HaloTag Tetramethylrhodamine Bis-*N*-Oxide (10)

Sodium bicarbonate (139.9 mg, 1.66 mmol, 30.0 equiv) and *m*-chloroperbenzoic acid (191.6 mg, 1.11 mmol, 20.0 equiv) were added sequentially to a solution of 5(6)-HaloTag tetramethylrhodamine **S6** (35.3 mg, 55.5 µmol, 1 equiv) in *N*,*N*-dimethylformamide (1.50 mL) at room temperature. After 30 m, the solution was cooled to –78 °C, and *N*,*N*-diisopropylethylamine (241.6 µL, 1.39 mmol, 25.0 equiv) was added. The reaction mixture was then diluted with ice-water (10 mL), and immediately allowed to warm to room temperature over 30 min. The solution

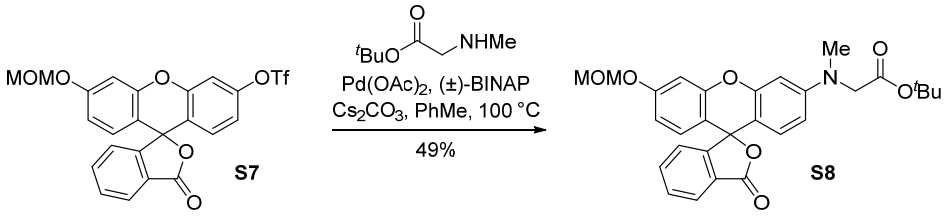
was purified by automated flash column chromatography on a C_{18} column (Biotage SNAP KP-C18-HS, 30 g, 50 mL/min, time (min), % MeCN in H_2O: 0, 0; 4.5, 0; 18, 100). Column fractions containing tetramethylrhodamine bis-*N*-oxides **10** were collected and concentrated under reduced pressure. This mixture was purified by preparative HPLC on a C_{18} column (20 mL/min, time (min), % MeCN in H_2O + 0.1% TFA: 0, 0; 60, 100). The clean column fractions were collected and neutralized by the addition of sodium bicarbonate (200 mg) and concentrated under reduced pressure. The residue was dissolved in H_2O (10 mL) and desalted on a C_{18} column (Biotage SNAP KP-C18-HS, 30 g, 50 mL/min; time (min), % MeCN in H_2O: 0, 0; 9, 100) to afford a 1.5:1 regioisomeric mixture of tetramethylrhodamine bis-*N*-oxides **10** (9.7 mg, 14.5 µmol, 26%) as a slightly pink film.

^{1}H NMR (500 MHz, CD_3OD) δ 8.56 (s, 0.6H), 8.27 (d, *J* = 8.1 Hz, 0.6H), 8.23–8.12 (m, 2.8H), 7.77 (d, *J* = 8.5 Hz, 2H), 7.68 (s, 0.4H), 7.39 (d, *J* = 8.1 Hz, 0.6H), 7.35–7.23 (m, 2H), 4.00 (s, 12H), 3.75–3.40 (m, 12H), 1.81–1.66 (m, 2H), 1.64–1.49 (m, 2H), 1.49–1.31 (m, 4H).

^{13}C NMR (126 MHz, CD_3OD) δ 169.1, 169.1, 167.8, 167.8, 155.6, 153.9, 152.4, 152.3, 152.1, 152.1, 143.1, 138.9, 136.4, 131.8, 131.7, 131.0, 128.7, 127.2, 127.0, 125.6, 125.3, 124.1, 122.9, 122.8, 117.3, 117.3, 111.1, 111.1, 80.8, 80.8, 72.2, 72.1, 71.3, 71.2, 71.2, 71.1, 70.4, 70.3, 61.0, 61.0, 45.7, 45.7, 41.2, 41.1, 33.7, 30.5, 30.5, 27.7, 27.7, 26.5, 26.4.

FTIR (thin film, cm^{-1}) 1775 (m), 1656 (m), 1424 (m), 1191 (s), 1137 (s), 720 (w).

HRMS (ESI, m/z) calculated for $C_{35}H_{43}ClN_3O_8$ $[M+H]^+$: 668.2733; found: 668.2720.



Rhodol *tert*-Butyl Ester (S5):

A 10 mL pressure vessel was sequentially charged with freshly free-based sarcosine *tert*-butyl ester (295.8 mg, 2.04 mmol, 1.50 equiv), rhodol triflate **S7** (690.0 mg, 1.36 mmol, 1 equiv), cesium carbonate (663.7 mg, 2.04 mmol, 1.50 equiv), (±)-2,2'-bis(diphenylphosphino)-1,1'-binaphthyl (BINAP, 254.1 mg, 408 µmol, 0.30 equiv), and palladium(II) acetate (61.1 mg, 272 µmol, 0.20 equiv). Toluene (6.90 mL) was added to the reaction mixture and sparged with nitrogen. After 5 min, the vessel was quickly sealed and the reaction was stirred at room temperature. After 30 min, the reaction was heated to 100 °C. After 4 h, the reaction was cooled to room temperature and purified directly by flash column chromatography on silica gel (eluent: 25% ethyl acetate in hexanes) to afford rhodol *tert*-butyl ester **S8** (337 mg, 670 µmol, 49%) as a white foam.

^{1}H NMR (500 MHz, $CDCl_3$) δ 8.00 (d, *J* = 7.6 Hz, 1H), 7.64 (t, *J* = 7.5 Hz, 1H), 7.59 (t, *J* = 7.4 Hz, 1H), 7.16 (d, *J* = 7.6 Hz, 1H), 6.94 (s, 1H), 6.67 (s, 2H), 6.60 (d, *J* = 8.8 Hz, 1H), 6.47 (d, *J* =

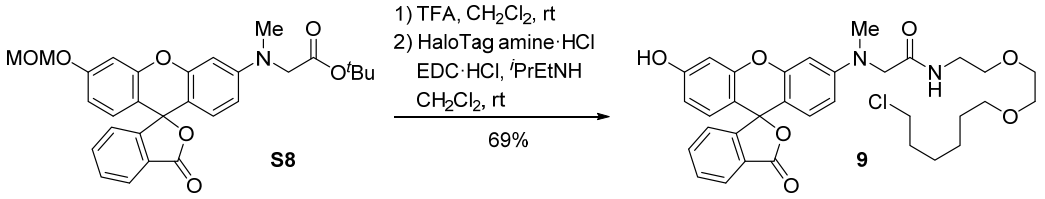
2.7 Hz, 1H), 6.34 (dd, *J* = 8.9, 2.7 Hz, 1H), 5.18 (s, 2H), 3.95 (s, 2H), 3.47 (s, 3H), 3.07 (s, 3H), 1.43 (s, 9H).

^{13}C NMR (126 MHz, $CDCl_3$) δ 169.8, 169.7, 158.8, 153.3, 152.8, 152.7, 151.0, 134.9, 129.6, 129.2, 129.0, 127.2, 125.0, 124.2, 112.9, 112.7, 108.8, 107.2, 103.7, 98.9, 94.4, 84.0, 82.1, 56.3, 55.1, 39.8, 28.2.

FTIR (thin film, cm^{-1}) 2975 (br, m), 2929 (br, m), 1762 (s), 1614 (s), 1520 (w), 1503 (w), 1426 (m), 1250 (m), 1154 (s), 1107 (s), 1004 (m).

HRMS (ESI, m/z) calculated for $C_{24}H_{20}NO_6$ $[M+H]^+$: 504.2017; found: 504.2007.

TLC (30% ethyl acetate in hexanes), R*f*: 0.31 (UV).



HaloTag Rhodol (9):

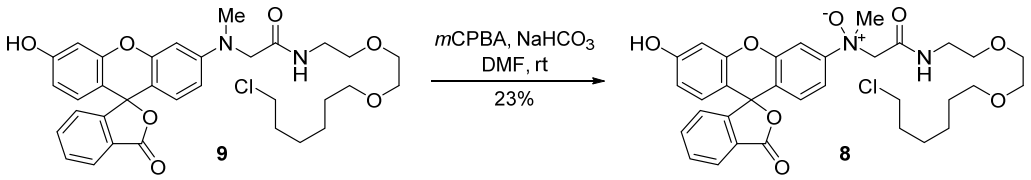
Trifluoroacetic acid (2 mL) was added in one portion to a solution of *tert*-butyl ester **S8** (43.2 mg, 85.8 μmol, 1 equiv) in dichloromethane (2 mL) at room temperature. After 1 h, the reaction mixture was concentrated under reduced pressure to afford a red oil. HaloTag amine hydrochloride (67.0 mg, 257 μmol, 3.00 equiv), *N*-(3-dimethylaminopropyl)-*N*'-ethylcarbodiimide hydrochloride (EDC·HCl, 164.5 mg, 858 μmol, 10.0 equiv), and dichloromethane (5 mL) were sequentially added to the oil. *N*,*N*-diisopropylethylamine (299 μL, 1.72 mmol, 20.0 equiv) was then added to the solution at room temperature. After 2 h, the reaction was concentrated to dryness under reduced pressure and purified by automated flash column chromatography on a C_{18} column (Biotage, KP-C18-HS, 30 g, 50 mL/min; time (min), % MeCN in H_2O + 0.1% TFA: 0, 0; 100, 13.5). The column fractions containing HaloTag rhodol **9** were concentrated under reduced pressure then purified by preparative HPLC on a C_{18} column (20 mL/min, time(min), % MeCN in H_2O + 0.1% TFA: 0, 0; 60, 100) to afford pure HaloTag rhodol **9** (36.3 mg, 59.6 μmol, 69%) as a red oil.

^{1}H NMR (500 MHz, CD_3OD) δ 8.34 (d, *J* = 7.4 Hz, 1H), 7.86 (dt, *J* = 7.4, 1.5 Hz, 1H), 7.82 (dt, *J* = 7.6, 1.4 Hz, 1H), 7.41 (d, *J* = 7.4 Hz, 1H), 7.23 (dd, *J* = 12.4, 9.8 Hz, 2H), 7.12 (dd, *J* = 11.5, 2.0 Hz, 2H), 7.04 (d, *J* = 2.5 Hz, 1H), 6.98 (dd, *J* = 8.8, 2.4 Hz, 1H), 4.42 (s, 2H), 3.64–3.50 (m, 8H), 3.48–3.39 (m, 4H), 3.36 (s, 3H), 3.35 (s, 1H), 1.80–1.66 (m, 2H), 1.61–1.50 (m, 2H), 1.49–1.39 (m, 2H), 1.39–1.30 (m, 2H).

^{13}C NMR (126 MHz, CD_3OD) δ 169.5, 168.9, 168.1, 160.2, 159.6, 158.4, 136.6, 134.2, 132.7, 132.6, 132.0, 131.8, 131.8, 130.8, 118.5, 117.2, 116.5, 116.3, 103.3, 98.3, 72.2, 71.2, 71.2, 70.3, 56.7, 49.8, 45.7, 41.3, 40.5, 33.7, 30.5, 27.7, 26.4.

FTIR (thin film, cm^{-1}) 2939 (br, m), 2866 (br, m), 1592 (s), 1498 (m), 1406 (m), 1323 (w), 1278 (w), 1186 (s), 1131 (s).

HRMS (ESI, m/z) calculated for $C_{33}H_{38}ClN_2O_7$ $[M+H]^+$: 609.2362; found: 609.2352.



HaloTag Rhodol *N*-Oxide (8):

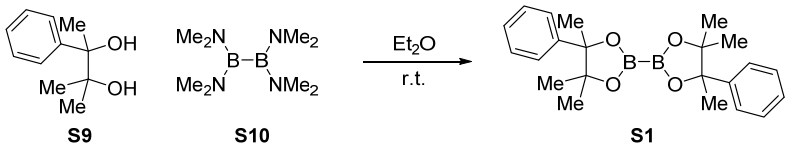
Sodium bicarbonate (57.9 mg, 690 µmol, 10.0 equiv) and *m*-chloroperbenzoic acid (39.5 mg, 345 µmol, 5.0 equiv) were added sequentially to a solution of HaloTag rhodol **9** (42.0 mg, 69.0 µmol, 1 equiv) in *N*,*N*-dimethylformamide (1 mL) at room temperature. After 30 m, the solution was cooled to –78 °C, and *N*,*N*-diisopropylethylamine (120.1 µL, 690 µmol, 10.0 equiv) was added. The reaction mixture was then diluted with ice-water (10 mL), and immediately allowed to warm to room temperature over 30 min. The solution was purified by automated flash column chromatography on a C_{18} column (Biotage SNAP KP-C18-HS, 30 g, 50 mL/min, time (min), % MeCN in H_2O: 0, 0; 4.5, 0; 18, 100). Column fractions containing HaloTag rhodol *N*-oxides **8** were collected and concentrated under reduced pressure. This mixture was purified by preparative HPLC on a C_{18} column (20 mL/min, time (min), % MeCN in H_2O + 0.1% TFA: 0, 0; 60, 100) to afford an inseparable diastereomeric mixture of HaloTag rhodol *N*-oxides **8** (9.8 mg, 15.7 µmol, 23%) as a light pink film.

^{1}H NMR (500 MHz, CD_3OD) δ 8.12–8.01 (m, 2H), 7.83–7.69 (m, 2H), 7.69–7.56 (m, 1H), 7.28–7.17 (m, 1H), 7.03–6.92 (m, 1H), 6.78–6.70 (m, 1H), 6.69–6.62 (m, 1H), 6.62–6.54 (m, 1H), 4.53 (dd, J = 14.4, 2.9 Hz, 1H), 4.42 (d, J = 14.5 Hz, 1H), 3.72–3.62 (m, 3H), 3.58–3.45 (m, 6H), 3.45–3.37 (m, 4H), 3.21–3.11 (m, 2H), 1.78–1.68 (m, 2H), 1.58–1.48 (m, 2H), 1.48–1.38 (m, 2H), 1.38–1.30 (m, 2H).

^{13}C NMR (126 MHz, CD_3OD) δ 170.9, 165.7, 161.6, 154.9, 154.2, 153.5, 152.8, 152.7, 136.8, 131.5, 131.5, 130.5, 130.4, 130.2, 127.7, 126.1, 125.2, 122.0, 121.9, 117.3, 117.1, 114.4, 111.8, 111.5, 110.6, 103.6, 73.5, 72.2, 72.2, 71.4, 71.3, 71.2, 71.1, 70.4, 70.4, 63.3, 63.2, 45.7, 39.9, 33.7, 33.7, 30.5, 27.7, 26.5.

FTIR (thin film, cm^{-1}) 2934 (br, s), 2863 (br, s), 1766 (s), 1674 (m), 1609 (m), 1425 (m), 1284 (w), 1107 (s), 762 (w).

HRMS (ESI, m/z) calculated for $C_{33}H_{38}ClN_2O_8$ $[M+H]^+$: 625.2311; found: 625.2300.



4,4′,4′,5,5′,5′-Hexamethyl-4,5-diphenyl-2,2′-bi-1,3,2-dioxaborolane (S1):

Tetrakis(dimethylamino)diboron (**S10**, 29.7 µL, 139 µmol, 0.500 equiv) was added to pinacol derivative (**S9**, 50.0 mg, 277 µmol, 1 equiv) in diethyl ether (1 mL) at room temperature. After 2 h 40 min, the reaction was concentrated to dryness by passing a stream of nitrogen gas over the solution. The residue was dissolved in minimal DMF and purified by preparative HPLC on a C_{18} column (20 mL/min, time (min), % MeCN in H_2O + 0.1% TFA: 0, 0; 5, 0; 35, 100). Only the pure fractions were collected and concentrated on a rotary evaporator to yield the major diastereomer of diboron **S1** (9.4 mg, 24.9 µmol, 18%) as a white solid. The pure fractions of the minor diastereomer of diboron **S1** (5.4 mg, 14.3 µmol, 10%) was also isolated as a white solid. The major diastereomer was fully characterized and used in further kinetics studies.

^{1}H NMR (500 MHz, $CDCl_3$) δ 7.51–7.39 (m, 2H), 7.39–7.31 (m, 2H), 7.31–7.26 (m, 1H), 1.63 (s, 3H), 1.57 (s, 3H), 0.84 (s, 3H).

^{13}C NMR (126 MHz, $CDCl_3$) δ 143.1, 128.2, 127.2, 125.0, 87.8 (d, J = 5.2 Hz), 84.9 (d, J = 5.6 Hz), 28.5, 27.0, 24.5.

^{11}B NMR (160 MHz, $CDCl_3$) δ 31.1.

FTIR (thin film, cm^{-1}) 2978 (w), 2926 (w), 1456 (w), 1372 (w), 1283 (m), 1264 (m), 1169 (m), 1139 (m), 701 (m).

HRMS (ESI, m/z) calculated for $C_{22}H_{28}B_2NaO_4$ $[M+Na]^+$: 401.2066; found: 401.2062.

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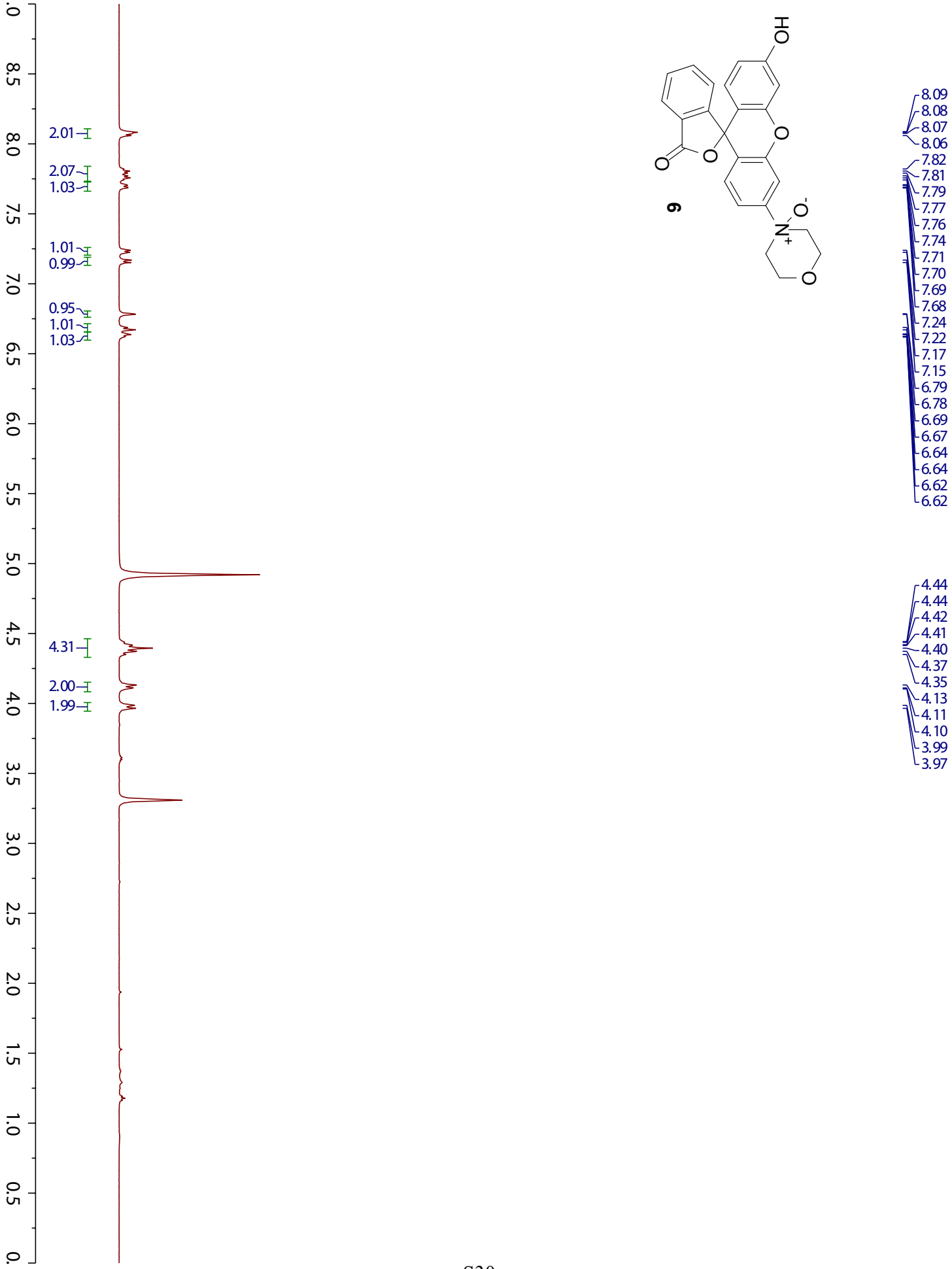
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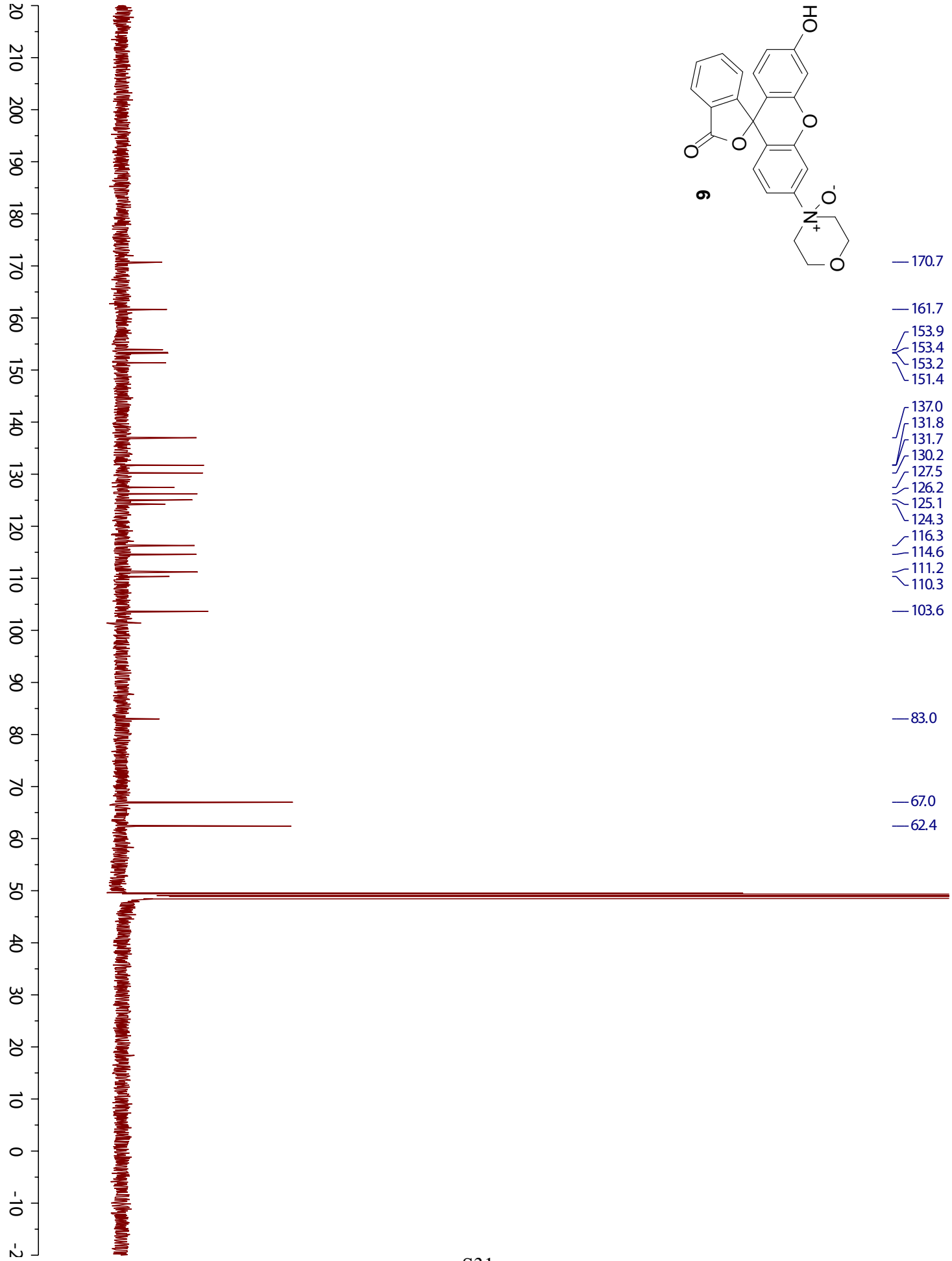
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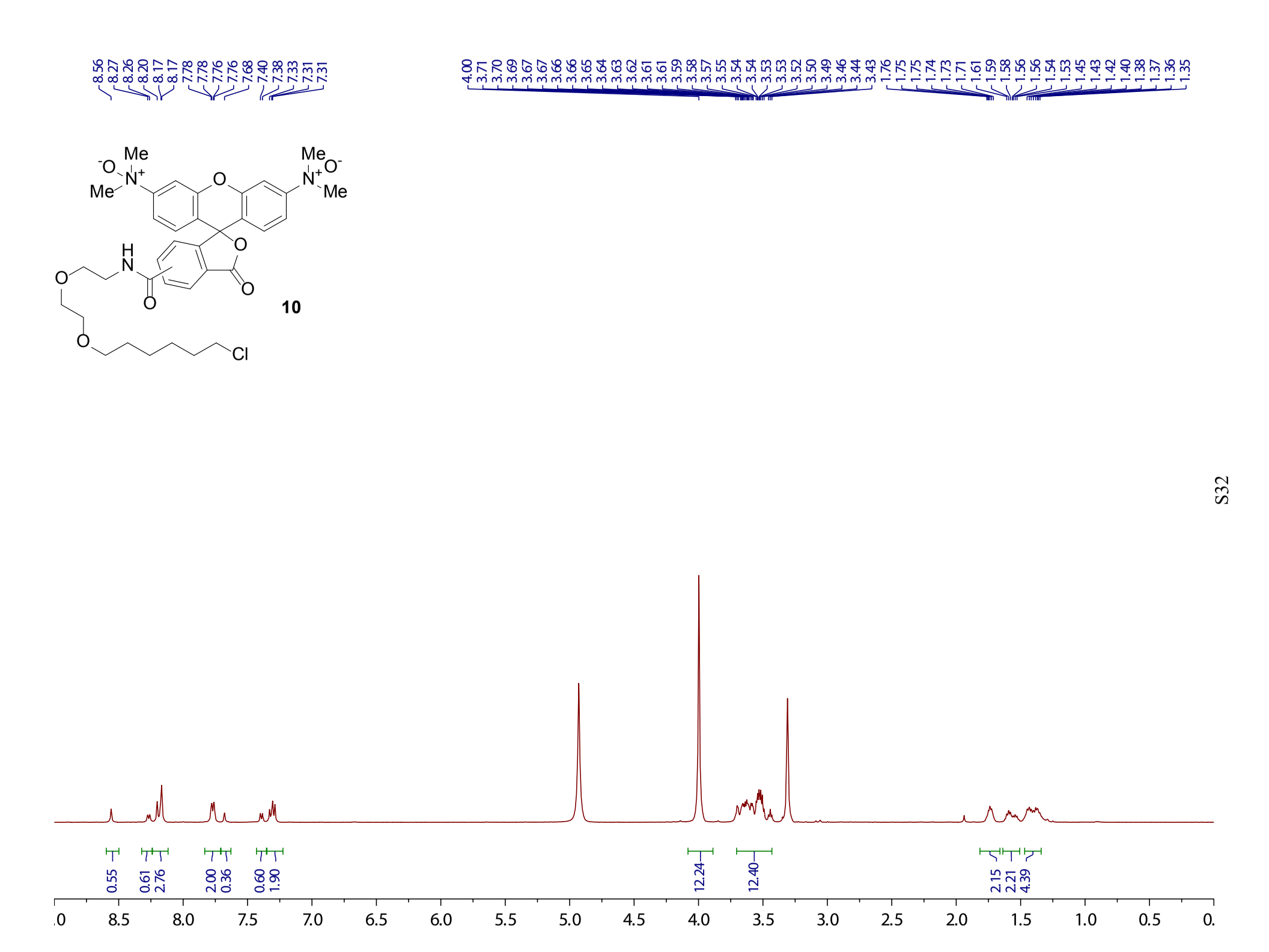
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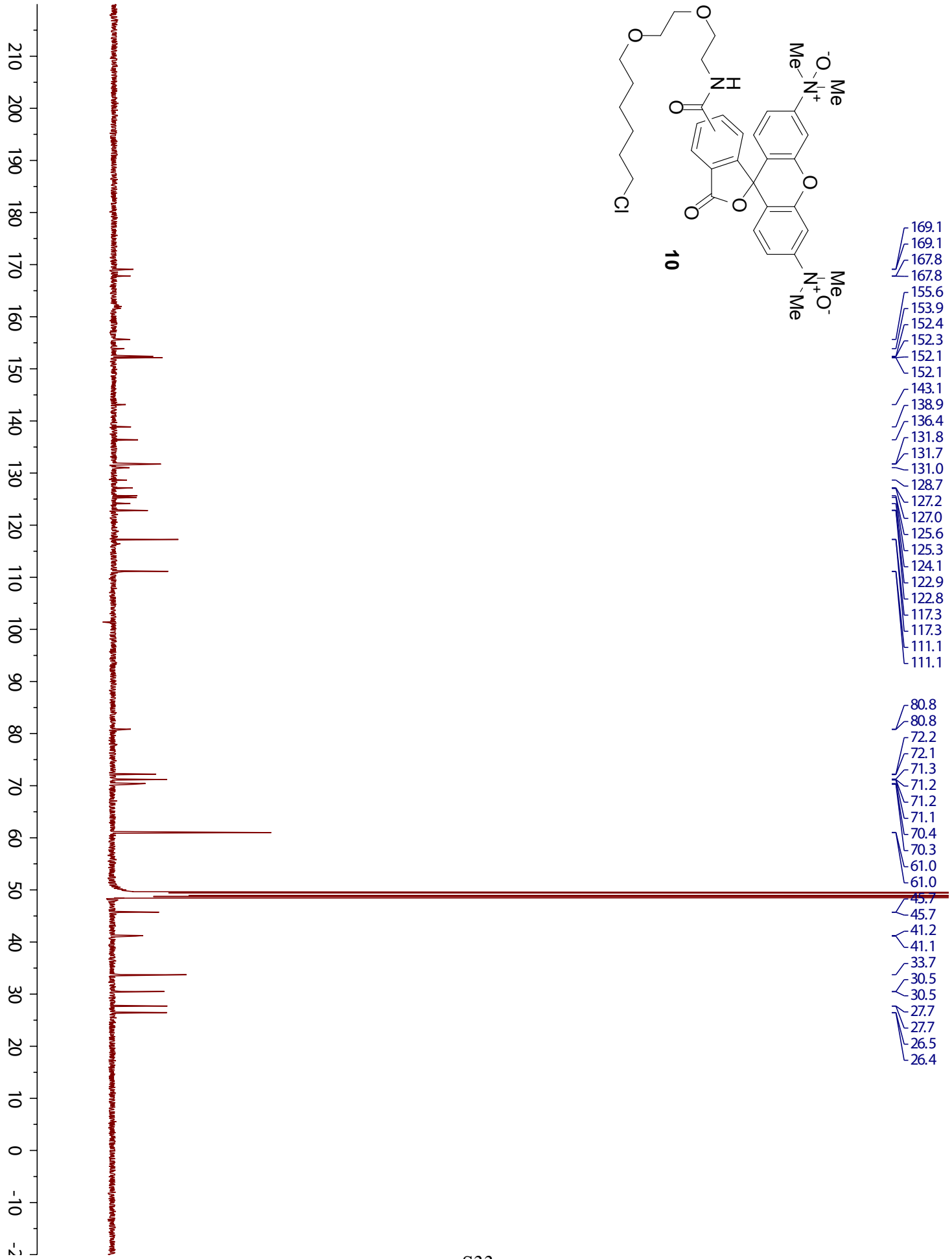
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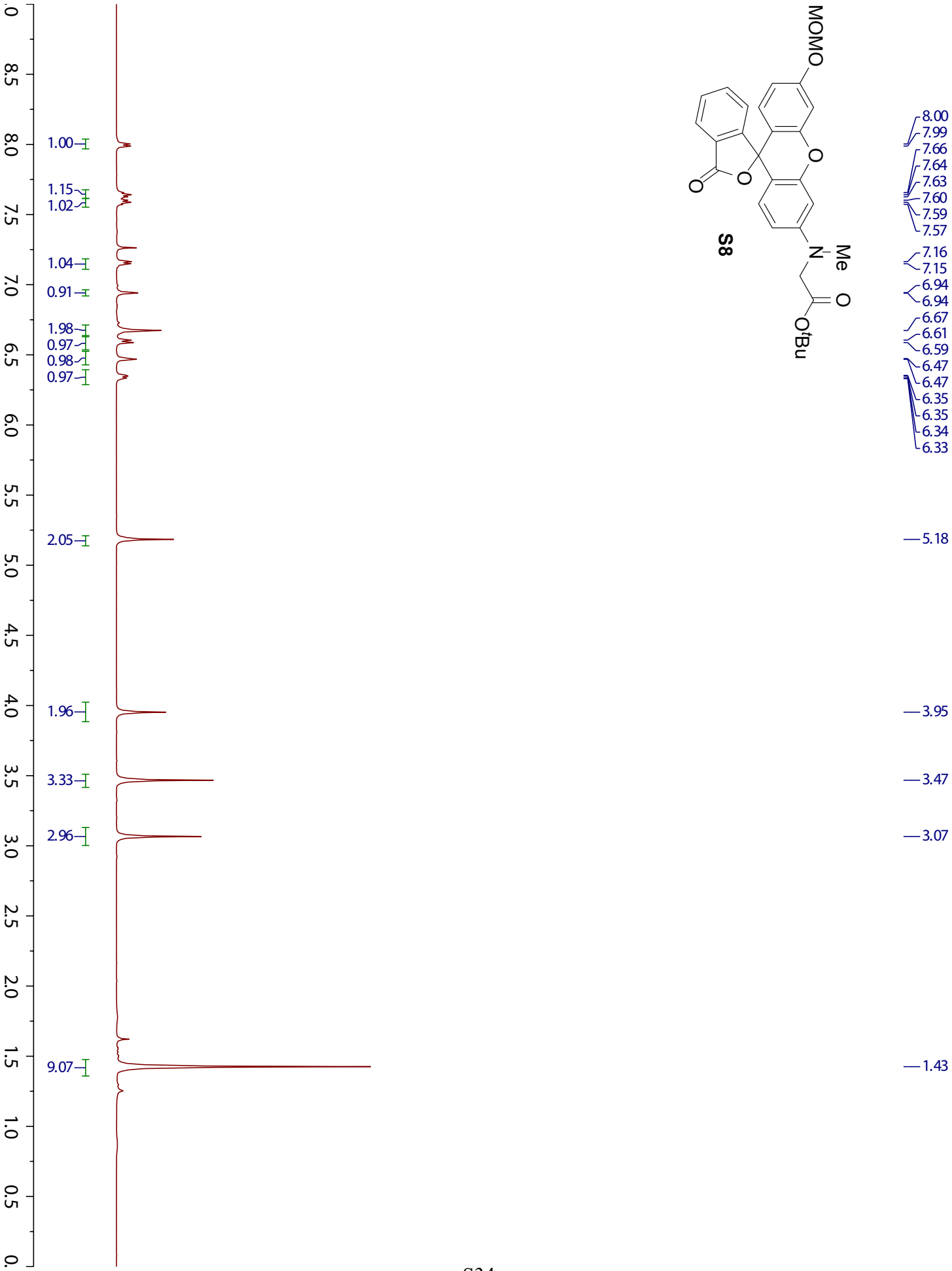
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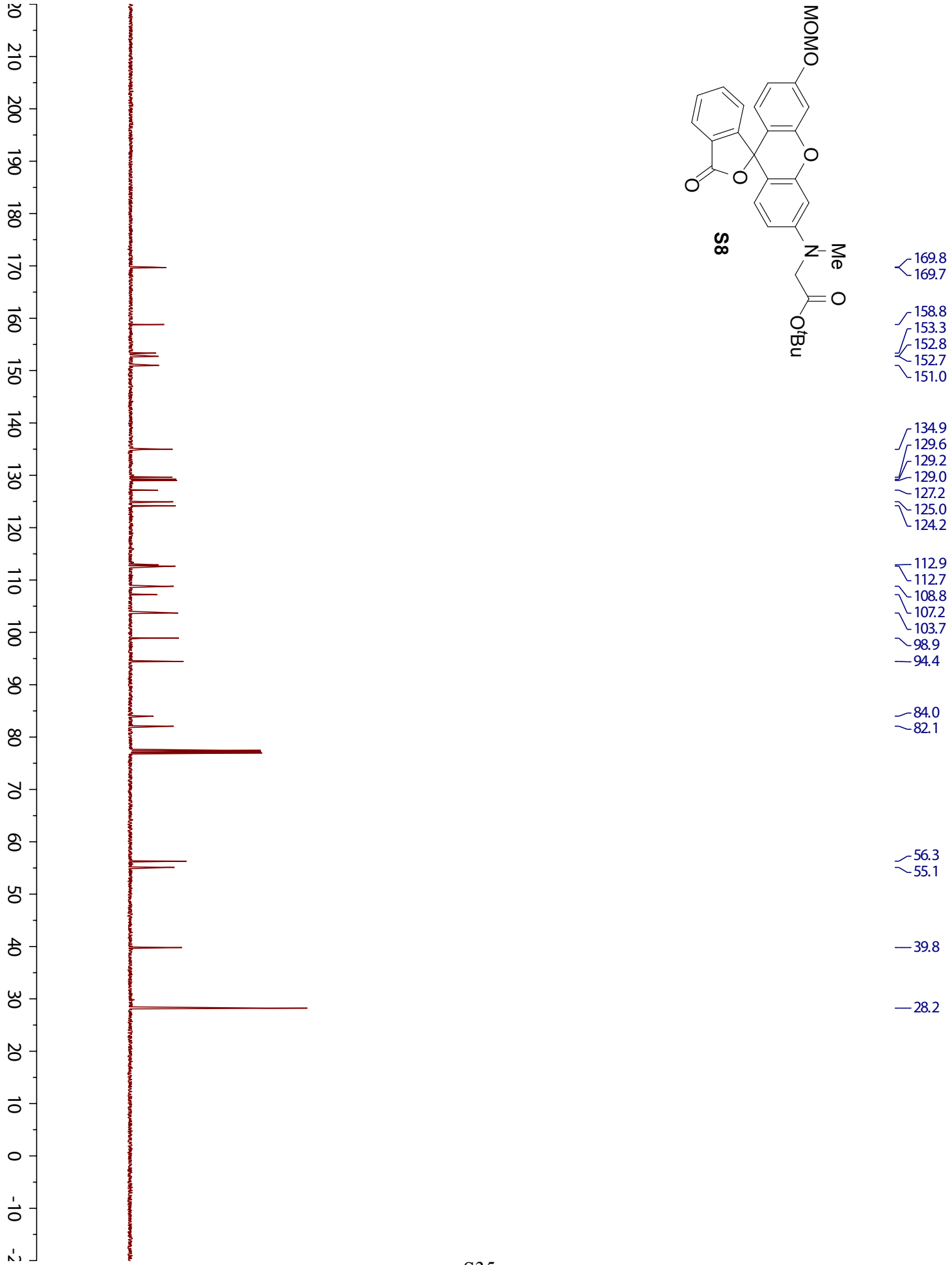
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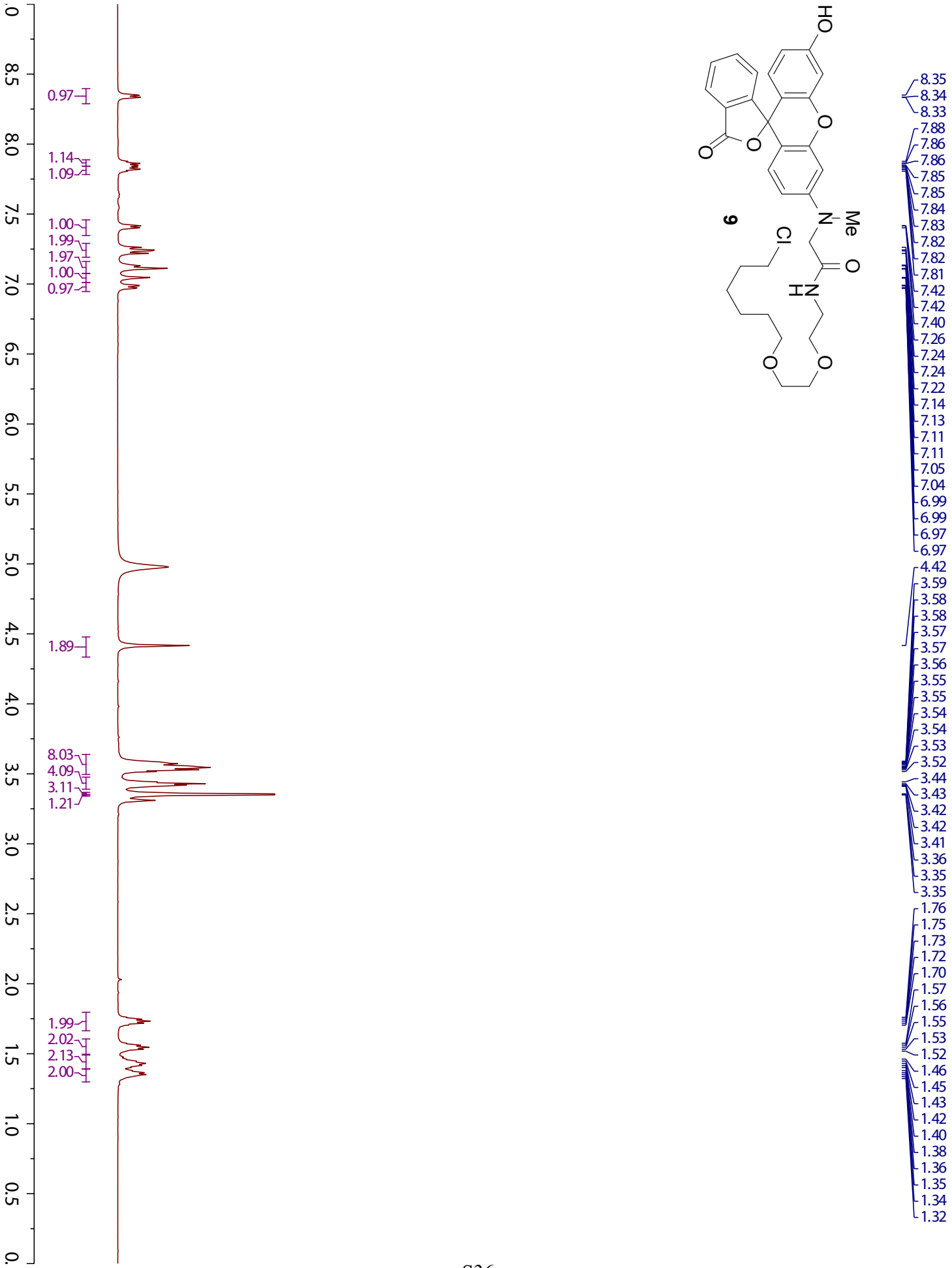
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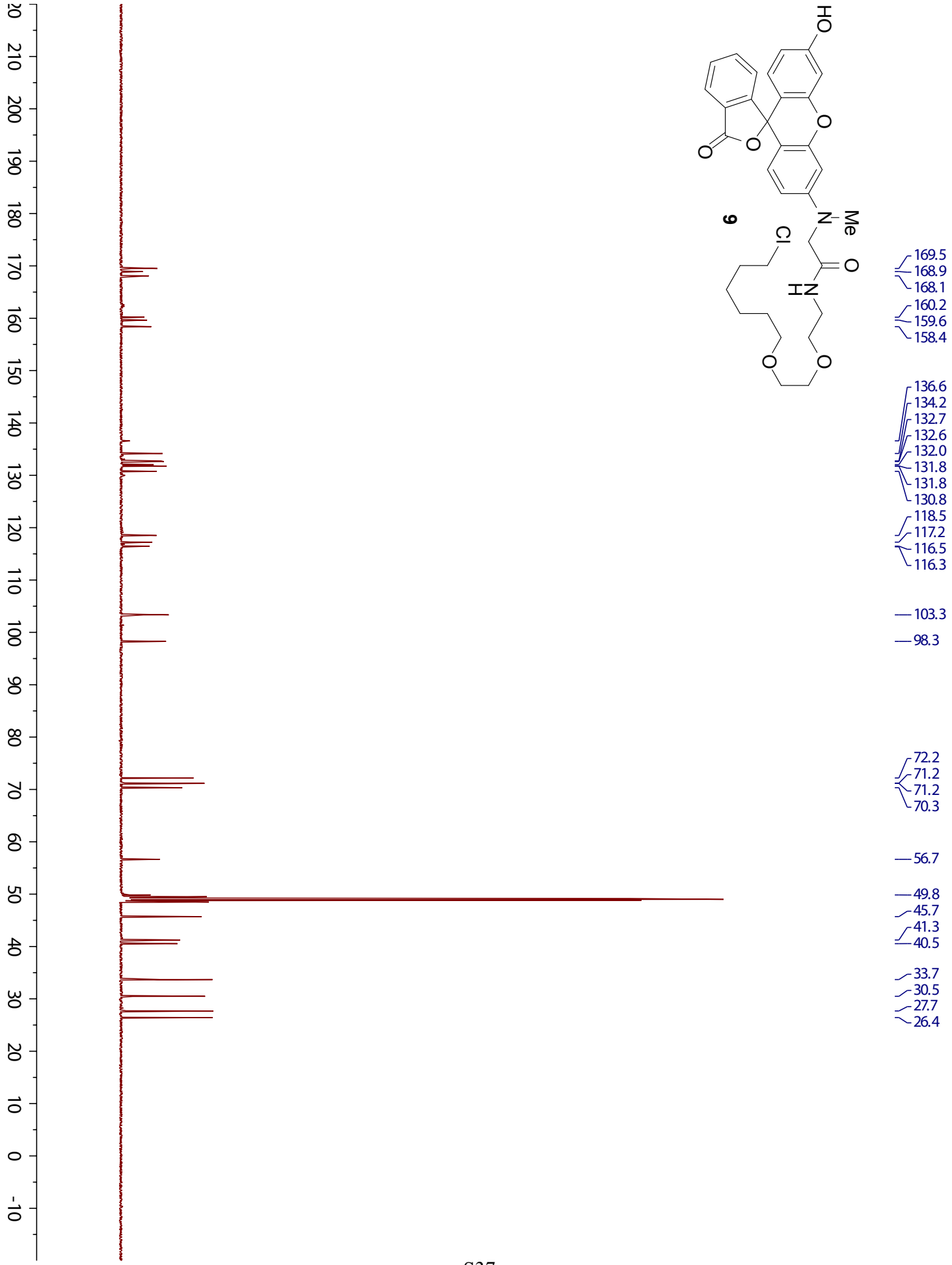
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159.6
158.4
136.6
134.2
132.7
132.6
132.0
131.8
131.8
130.8
118.5
117.2
116.5
116.3
103.3
98.3
72.2
71.2
71.2
70.3
56.7
49.8
45.7
41.3
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26.4
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200
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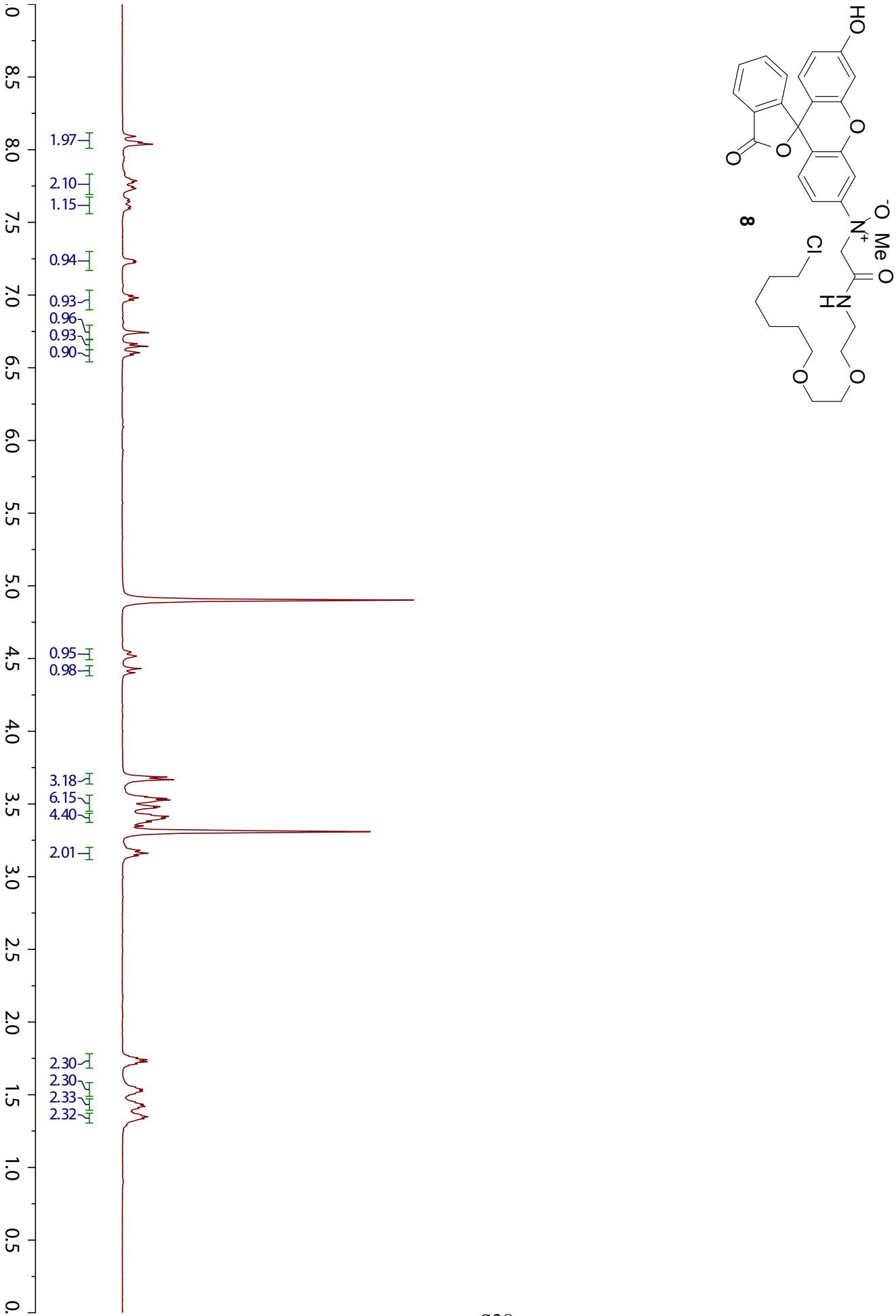
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2.30
2.30
2.33
2.32
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8.09
8.05
8.04
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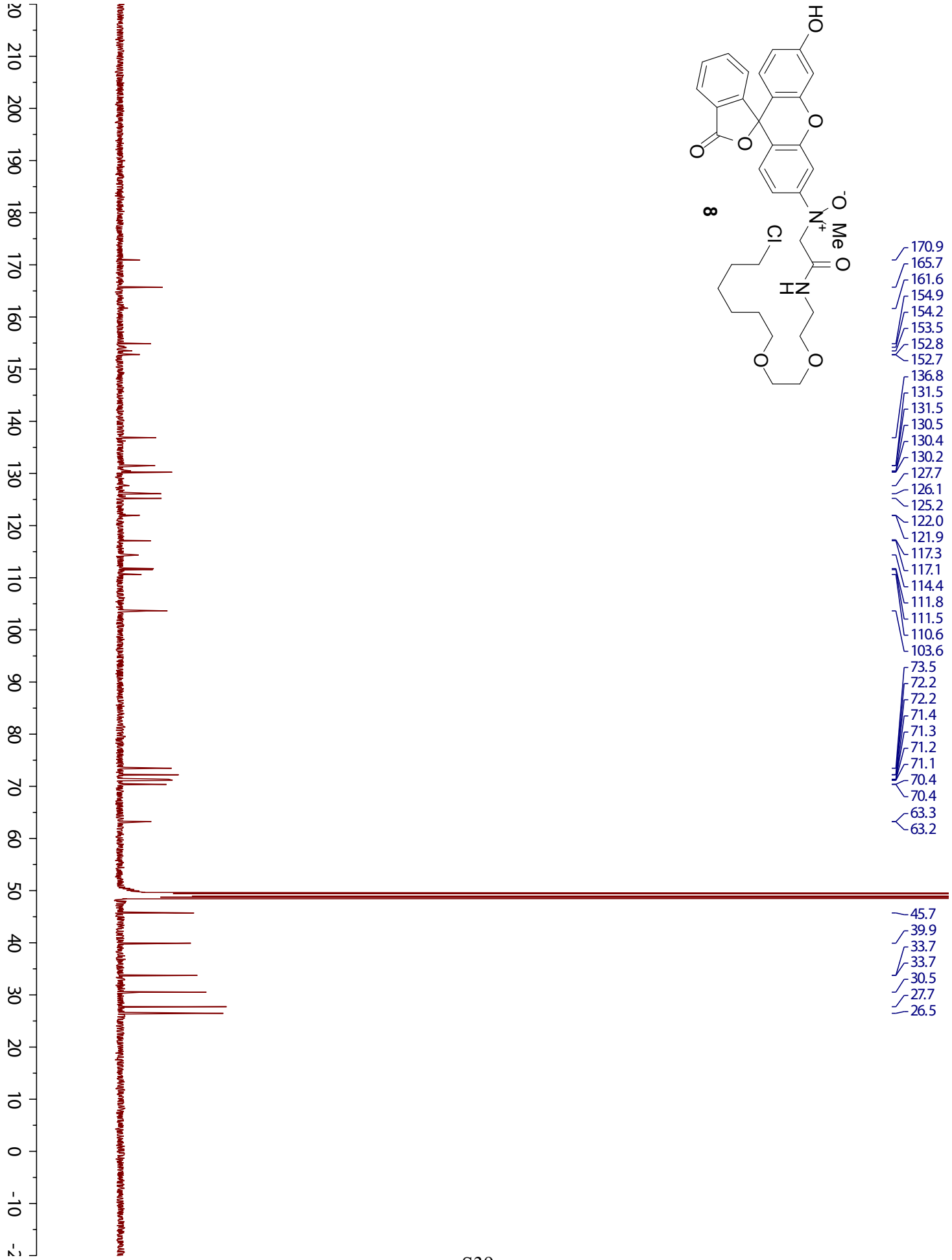
1.34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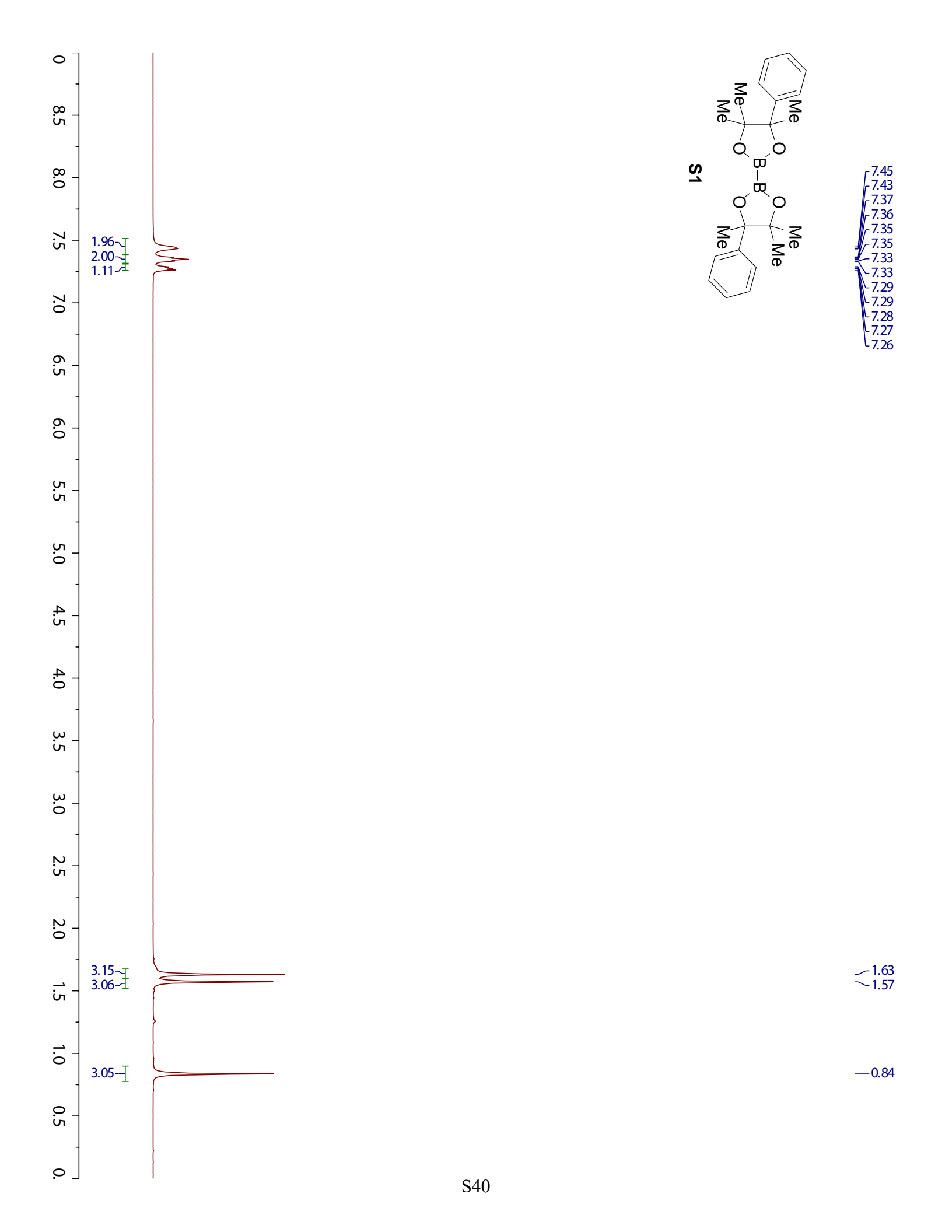
S1
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7.36
7.35
7.35
7.33
7.33
7.29
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7.28
7.27
7.26
1.96
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1.11
3.15
3.06
3.05
1.63
1.57
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8.5
8.0
7.5
7.0
6.5
6.0
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2.0
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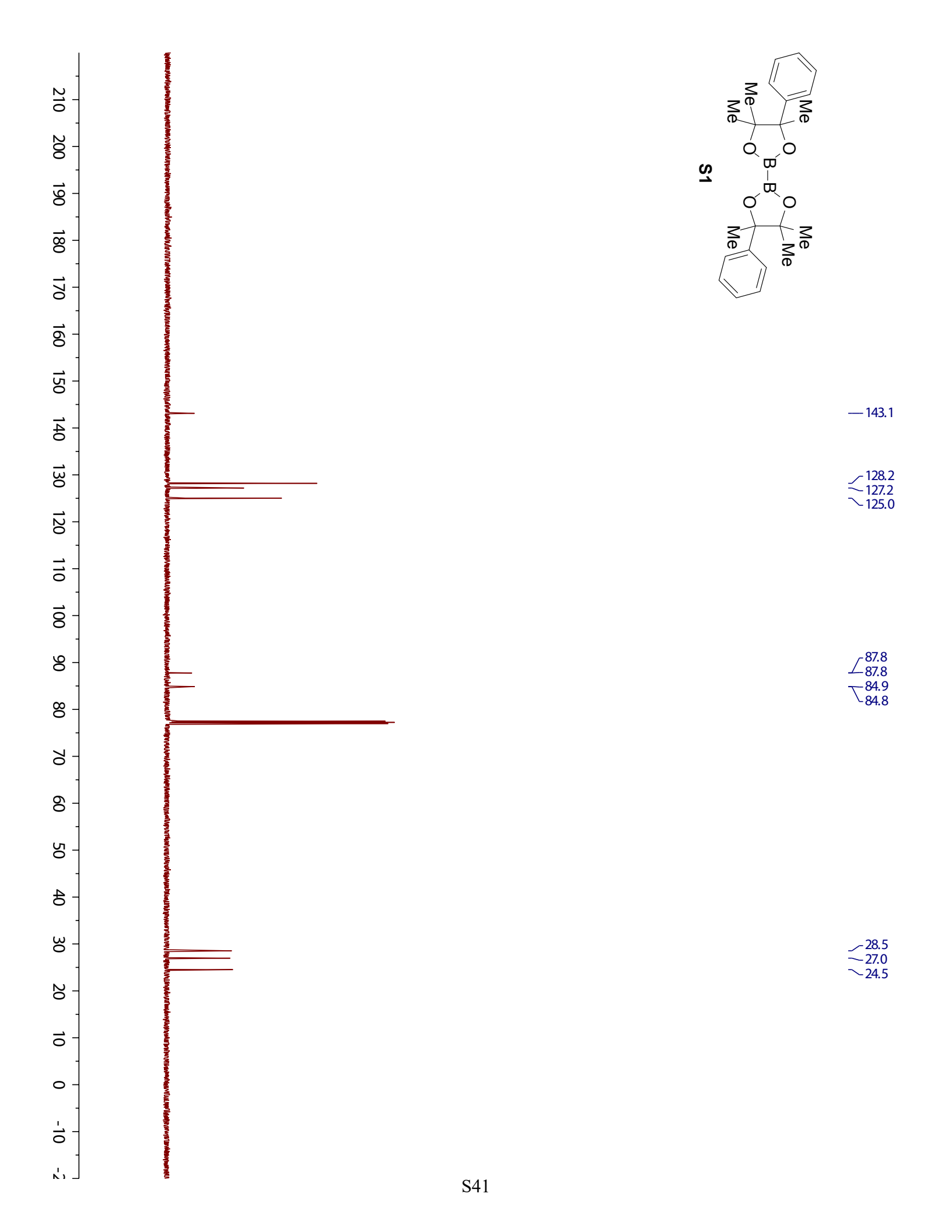
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B
O
O
Me
Me
Me
Me
S1
143.1
128.2
127.2
125.0
87.8
87.8
84.9
84.8
28.5
27.0
24.5
210
200
190
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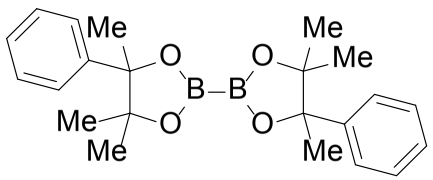
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Me
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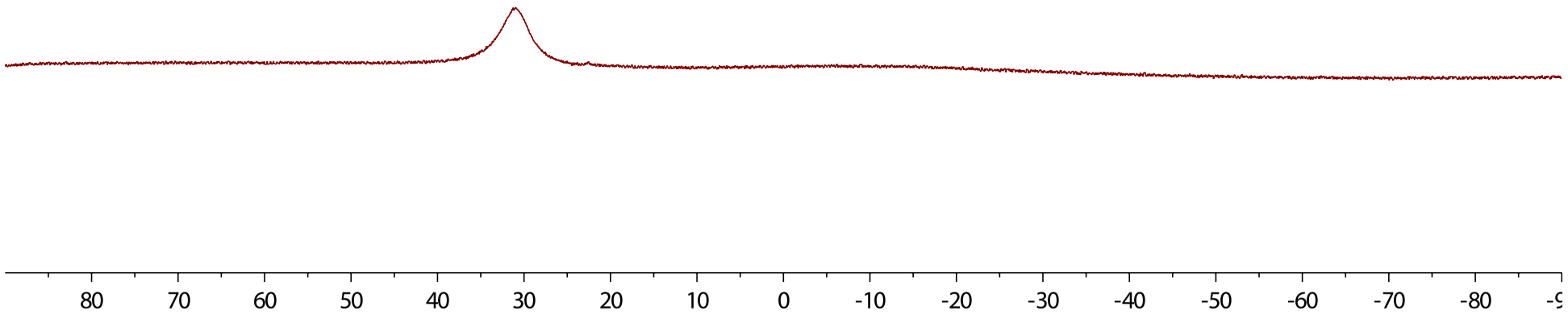
Me

S1

31.1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