

Supporting Information

Fluorogenic and Genetic Targeting of a Red-Emitting Molecular Calcium indicator

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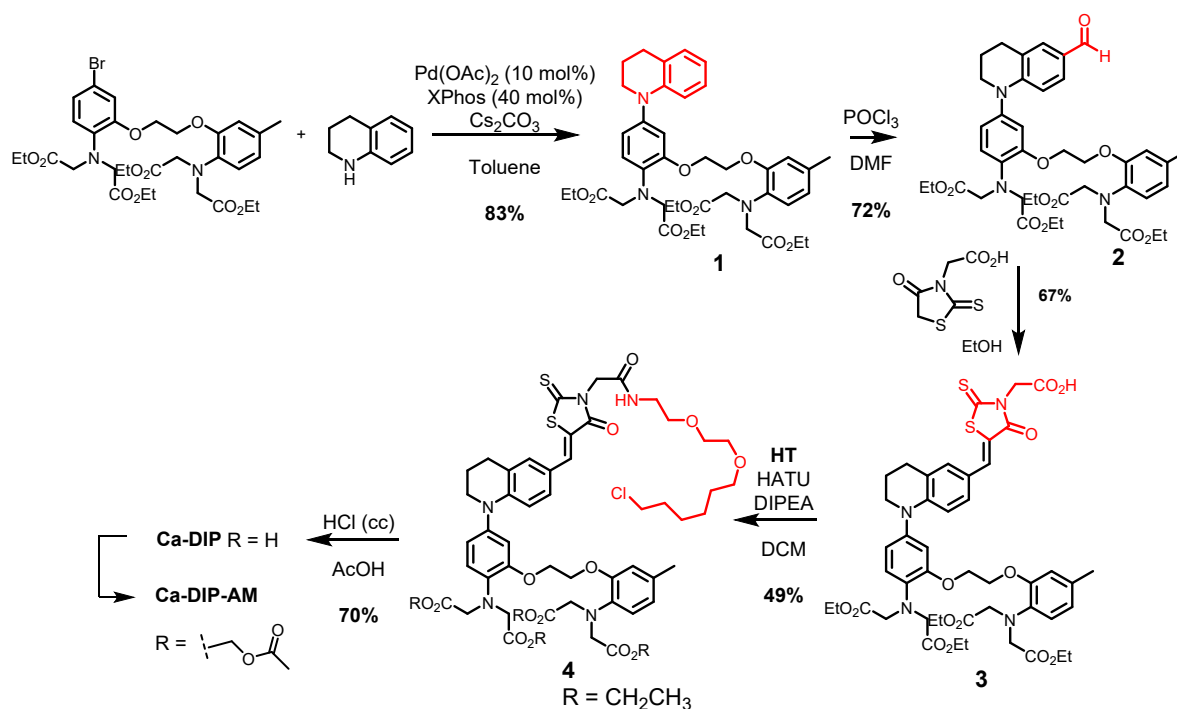
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I. Additional figure and table



Scheme S1. Synthesis of Ca-DIP and Ca-DIP-AM.

Table S1. Photophysical properties of Red-Halo2 and of CaDIP.

Conditions		λ_{abs} (nm)	ϵ ($\text{M}^{-1}\text{cm}^{-1}$)	λ_{em} (nm)	Φ_{F}	$\epsilon \cdot \Phi_{\text{F}}$	Relative fl. intensity	
Red-Halo2^a	HaloTag bound	515	21 000	592	0.17	3600	NA	
Ca-DIP	HaloTag bound	39 μM Ca^{2+}	508	31 300	580	0.009	282	68.1
	free	39 μM Ca^{2+}	498	26 800	603	-	-	2.5
	HaloTag bound	0 μM Ca^{2+}	515	26 500	575	-	-	10.6
	free	0 μM Ca^{2+}	508	33 000	606	-	-	1

^a taken from ref [1]

Table S2. Comparison of the calcium-binding properties of Ca-DIP with other reported chemogenetic calcium indicators.

	$\Delta\text{F}/\text{F}_0$ (in vitro)	$\Delta\text{F}/\text{F}_0$ (n Hela cells)	K_{D} (nM)
cpFAST ^a	3	1 - 2	57 - 99
HaloCaMP1a (labeled with JF635) ^b	5	n.d. ^c	190
HaloCaMP1b (labeled with JF635) ^b	9.2	n.d. ^c	43
Ca-DIP	5.4	4	113

^a taken from ref [2] ^b taken from ref [3] ^cHaloCaMP1a-b were not studied in Hela cells but only in neurons.

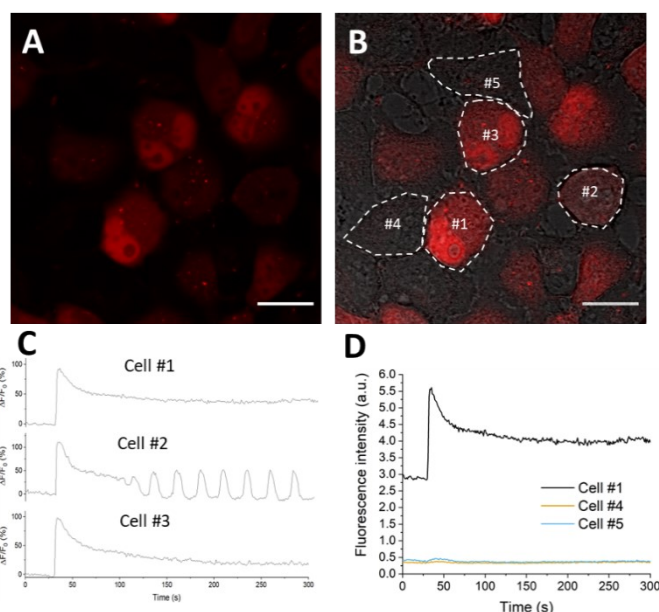


Figure S1. Live imaging of HeLa cells expressing a soluble HaloTag protein and incubated with 1 μM of Ca-DIP-AM. Time-lapse imaging over 300 s at 1 frame/s. Histamine (50 μM) was added at 30 s. (A) Average intensity projection fluorescence image. Scale bar = 20 μm (B) Overlay of fluorescence and transmission images. (C) Evolution of the average intensity in the cells numbered on panel B over the course of the experiment, plotted as a percentage of variation relative to the initial intensity $\Delta F/F_0$. (D) Average intensity over the course of the experiment in the non-transfected cells #4 and #5 compared to the transfected cell #1.

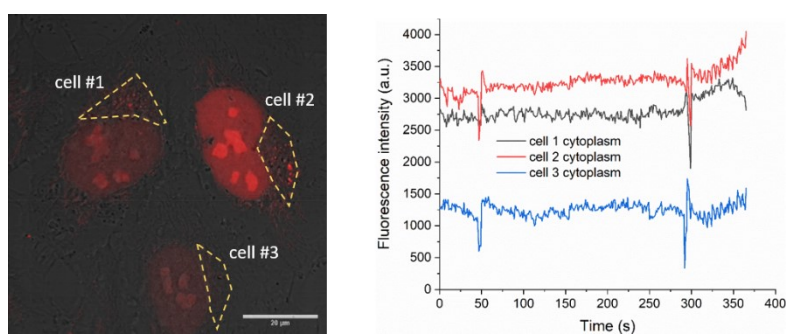


Figure S2. Evolution of the fluorescence intensity in the cytoplasm of cells 1-3 in experiments described in Figure 4.

Legend of attached movies:

Movie S1. Time-course movie of calcium imaging in HeLa Cells recorded at 1 frame/s and corresponding to the experiment described in Figure S1. The Movie is a walking average of 4 images. Ca-DIP-AM was incubated at 1 μM for 60 minutes before imaging. 50 μM Histamine was added at $t = 30$ s.

Movie S2. Time-course movie of calcium imaging in HeLa Cells recorded at 1 frame/s and corresponding to the experiment described in Figure 4 in the main text. The Movie is a walking average of 4 images. Ca-DIP-AM was incubated at 1 μM for 60 minutes before imaging. 50 μM Histamine was added at $t = 50$ s and 5 μM of ionomycin at 290 s.

II. Materials and methods

Materials. Chemical reagents and solvents were purchased from Sigma-Aldrich or TCI and were used as received. Purified GST-HaloTag protein was purchased from Promega Corp. (Madison, WI, USA). HeLa cells were obtained from the ATCC (CRL-11268). Calcium calibration buffers (0 and 39 μM free Ca^{2+}) were purchased from Interchim (Montluçon, France)

Chemical analysis. ^1H and ^{13}C NMR spectra were recorded on a Bruker Avance 300 MHz spectrometer. All chemical shifts (δ) for ^1H and ^{13}C NMR spectra are reported in parts per million (ppm) relative to the internal residual solvent signals. The abbreviations used are: singlet (s), doublet (d), doublet of doublet (dd), triplet (t), doublet of triplet (dt), multiplet (m) and coupling constants are reported in hertz (Hz). HPLC: analytical HPLC was performed on an Agilent 1200 series equipped with a quaternary pump using a Proto 200 C18 from Higgins Analytical Inc (particles size 3 μm , 100 \times 4.6 mm column). Preparative HPLC was performed on an Agilent 1260 Infinity using a Nucleodur C18 HTech column from Macherey-Nagel Inc. (particles size 5 μm , 250 \times 16 mm column) with a gradient of 30 min from 30 or 50% to 100% acetonitrile (0.1% TFA) / water (0.1% TFA) at 220 nm. ESI-MS experiments were carried out using a LTQ-Orbitrap XL from Thermo Scientific (Thermo Fisher Scientific, Courtaboeuf, France) and operated in positive or negative ionization mode, with a spray voltage at 3.6 kV. Applied voltages were 40 and 100 V for the ion transfer capillary and the tube lens, respectively. The ion transfer capillary was held at 275°C. Detection was achieved in the Orbitrap with a resolution set to 100,000 (at m/z 400) and a m/z range between 200-2000 in profile mode. Spectrum was analyzed using the acquisition software XCalibur 2.1 (Thermo Fisher Scientific, Courtaboeuf, France). The automatic gain control (AGC) allowed accumulation of up to 2.105 ions for FTMS scans, Maximum injection time was set to 300 ms and 1 μscan was acquired. 10 μL was injected using a Thermo Finnigan Surveyor HPLC system (Thermo Fisher Scientific, Courtaboeuf, France) with a continuous infusion of methanol at 100 $\mu\text{L}\cdot\text{min}^{-1}$. Thin layer chromatography (TLC) analysis was run on silica gel (Merck 60F – 254) with visualization at 254 nm.

Absorption and fluorescence spectroscopy. UV spectra were recorded on a Cary 300 spectrophotometer (Agilent technologies, Santa Clara, CA, USA). Scanning was set at 600 nm/min with a step of 1 nm. Fluorescence emission spectra were recorded on a Jasco FP8300 spectrofluorometer (Jasco Inc., Easton, MD, USA). Scanning speed was set to 500 nm/min, integration time to 0.1 s, excitation and emission slits were set to 5 nm and the PMT to medium, unless otherwise stated. The excitation wavelength was 500 nm. Measurements were performed in quartz cuvettes (1 cm pathlength, Hellma Analytics).

Calcium titration. Fluorimetric calcium titration was performed between 0 and 39 μM by combination of pH7.2 calcium calibration buffers (10 mM MOPS, 100 mM KCl) containing either 10 mM K2EGTA (0 μM free Ca^{2+}) or 10 mM CaEGTA (39 μM free Ca^{2+}) according to the manufacturer's protocol. Titration was performed at 1 μM in Ca-DIP with 1.3 equivalents of HaloTag in a 10 μL working volume. Solutions were left to incubate for 30 minutes for the reaction of Ca-DIP with HaloTag to occur and the fluorescence was measured at 293 K on a

Jasco FP8300 spectrofluorimeter equipped with a one-drop accessory: $\lambda_{exc} = 500$ nm, excitation and emission slits = 10 and 5 nm respectively, integration time = 0.2 s and PMT = high.

HaloTag interaction. The fluorogenic dyes were dissolved in DMSO at a stock concentration of 0.5 mM. The interaction of the probes with Halotag was assessed by incubating the dye at 1 μ M (0.4 μ L of stock solution) concentration with 1.3 μ M (ca. 6 μ L of commercial stock solution) of protein in a 200 μ L working volume of pH7.4 Phosphate buffer (10 mM phosphate with 100 mM NaCl). The fluorescence spectra were then recorded at regular time intervals over the course of the reaction. After complete reaction with Halotag, the absorption spectra of the protein-bound chromophores were also recorded.

Fluorescence quantum yield measurements. Fluorescence quantum yield were calculated by relative measurement using Rhodamine 6G as reference ($\Phi_f = 0.94$ in ethanol). The absorption and fluorescence emission of solutions of Ca-DIP at 0.75 μ M, 1 μ M and 1.5 μ M with constant 1:1.3 Ca-DIP: HaloTag ratio were measured. For reference the absorbance and fluorescence of 5 solutions of Rhodamine 6G were measured. The fluorescence intensity (I) was plotted against the absorbance at the excitation wavelength (A) and the quantum yield of Ca-DIP was calculated using the following formula:

$$(1) \quad \Phi_f^i = \Phi_f^s \frac{grad_i n_i^2}{grad_s n_s^2}$$

Where Φ_f is the fluorescence quantum yield, $grad$ the slope of the curve $I = f(A)$, n the refractive index of the solvent and the superscript s and i refer to the standard and the sample respectively. Measurements were duplicated.

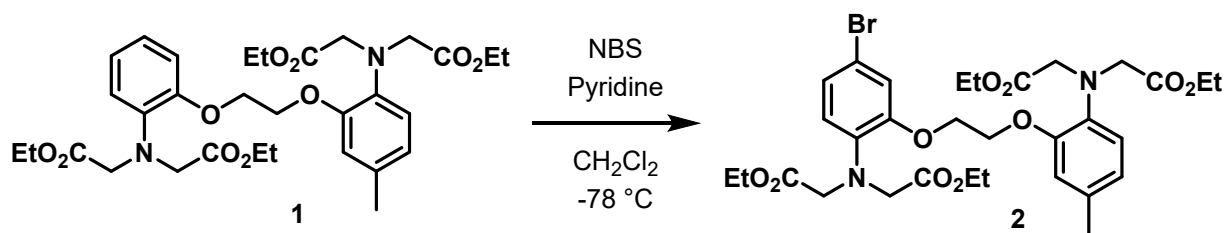
Molecular dynamics. 3D models of HaloTag coupled to the fluorogen compound were generated based on a 3D structure of Halotag (pdb entry: 4KAF) and then refined by several cycles of minimization and equilibrated by molecular dynamics simulations (2 ns runs) using the CHARMM force field. Bonds involving hydrogen atoms were constrained using the SHAKE algorithm. The Ramachandran plot of the final 3D model showed 91% (270/295) favored, 6% (17/295) allowed, and 3% (1 Glu, 1 Leu and 6 Gly) disallowed residues. A solvent box with a periodic boundary of 9 Å was added to the model. Solvation was completed with 0.145 M KCl using the solvation protocol implemented in Discovery Studio (Dassault Systèmes BIOVIA, Discovery Studio, 2019, San Diego: Dassault Systèmes, 2020).

Confocal microscopy. Hela cells were grown for 48 hrs on 8-well polymer μ slides from Ibidi (#1.5 polymer coverslip, tissue culture treated) at 30k cells/well in 300 μ L of MEM (Gibco, supplemented with 10 % fcs, sodium pyruvate and non-essential aminoacids). Cells were washed twice with DPBS and then transfected with the Halo-NLS or Halo-Soluble plasmids using Fugene 6 (Promega Corp.) according to the manufacturer's protocol. After 24 hours, the cells were washed twice with DPBS and the medium replaced with 250 μ L of HEPES-buffered Hank's balanced salt solution (HBHBSS) containing 1 or 1.5 μ M of Ca-DIP with 0.04 % of pluronic F-127. After 60 minutes incubation the cells were directly imaged live on a Zeiss LSM710 laser scanning confocal microscope

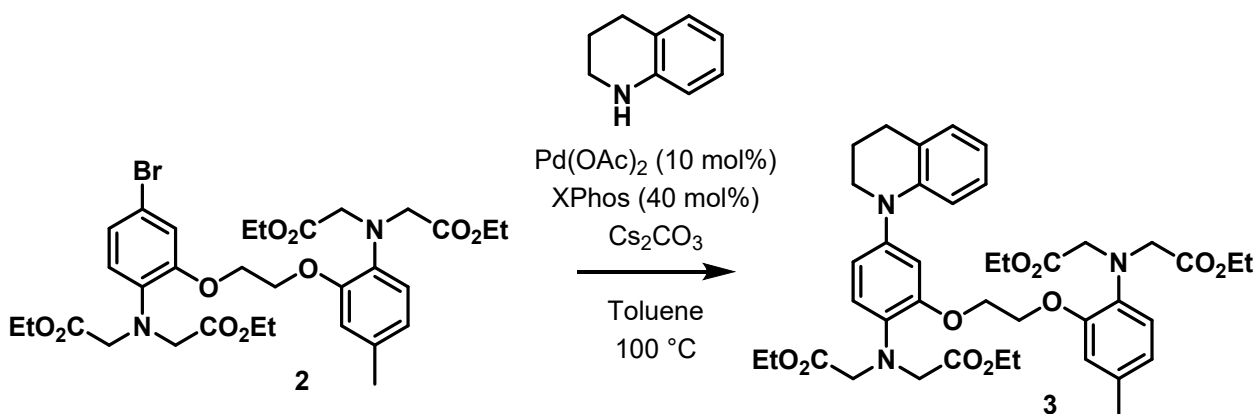
equipped with a Plan apochromat 40X/1.4 NA objective. $\lambda_{exc} = 514$ nm, collection: 520 – 797 nm. Time-series imaging were performed at 1 image per second. Calcium stimulation was achieved by adding 50 μ L of 300 μ M Histamine in the well (300 μ L total volume) during time-course imaging. Images were acquired using Zen 2009 software and then processed using ImageJ. Movies are walking average of 4 images.

III. Synthetic methods

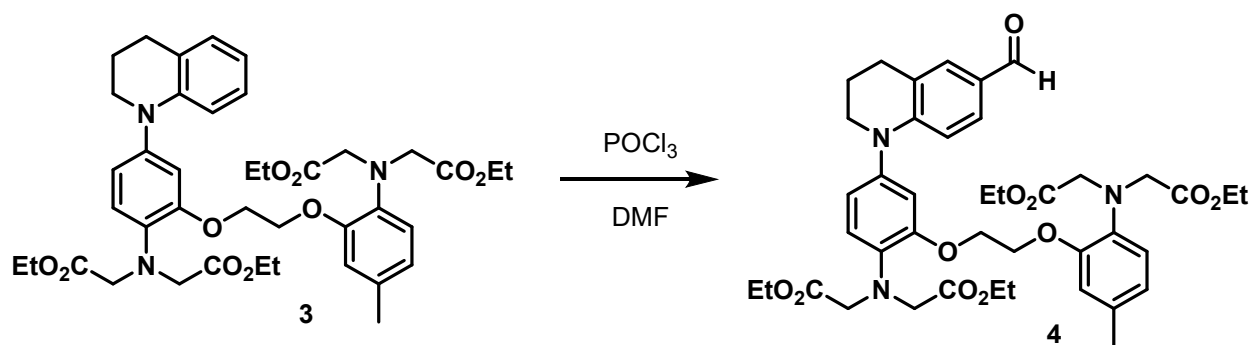
Intermediate **1**^[4] and **S1**^[5] were synthesized according to reported procedures.



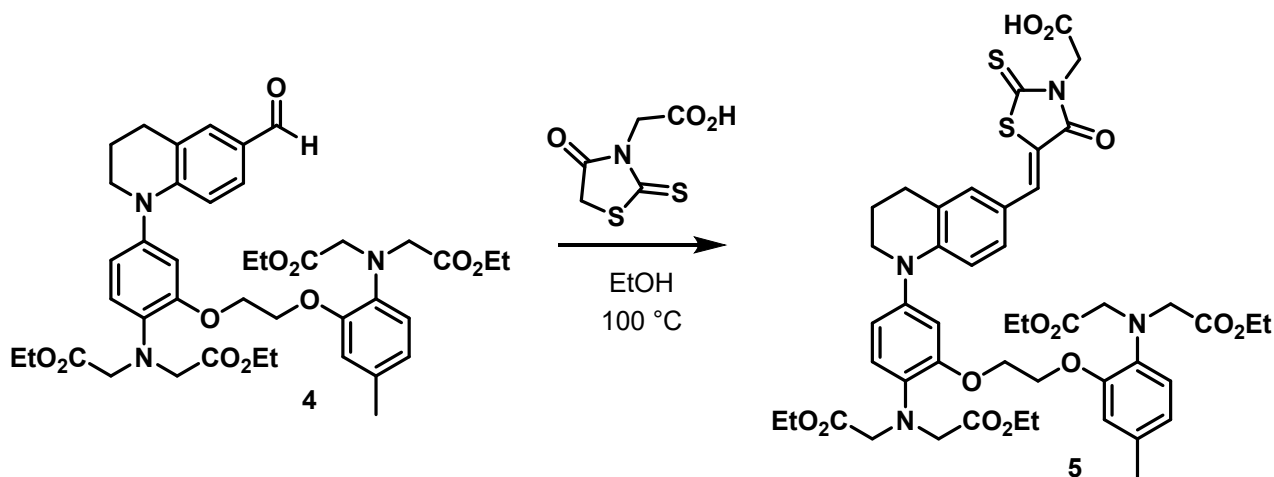
To a stirred solution of **1** (200 mg, 0.33 mmol, 1.0 eq) and pyridine (0.04 mL, 0.50 mmol, 1.5 eq) in CH₂Cl₂ (3.3 mL) at -78 °C was added N-bromosuccinimide (71 mg, 0.40 mmol, 1.2 eq) and the reaction was stirred at that temperature for 3 hours. The mixture was washed with HCl (1M) and brine and extracted with dichloromethane. The organic layer was dried over MgSO₄, filtered and evaporated to dryness to give **2** (223 mg, 99%) as a colorless solid. ¹H NMR (300 MHz, CDCl₃) δ 7.00-6.95 (m, 2H), 6.76-6.63 (m, 4H), 4.28-4.22 (m, 4H), 4.10 (s, 8H), 4.05 (qd, J = 7.3, 3.4 Hz, 8H), 2.25 (s, 3H), 1.15 (td, J = 7.3, 3.4 Hz, 12H). ¹³C NMR (75 MHz, CDCl₃) δ 171.7, 171.3, 151.2, 150.3, 138.7, 137.1, 132.2, 124.3, 122.1, 120.3, 119.4, 116.6, 114.8, 114.1, 67.7 (x2), 60.9, 60.8, 53.7, 53.6, 21.0, 14.2 (x2). HRMS calcd for C₃₁H₄₂BrN₂O₁₀: 681.2017. Found: 681.2013.



A stirred solution of **2** (500 mg, 0.73 mmol, 1.0 eq), 1,2,3,4-tetrahydroquinoline (0.14 mL, 1.10 mmol, 1.5 eq), palladium acetate (16 mg, 0.07 mmol, 0.1 eq), XPhos (140 mg, 0.29 mmol, 0.4 eq) and cesium carbonate (479 mg, 1.47 mmol, 2.0 eq) in toluene (7.3 mL) was stirred at 100 °C overnight. The mixture was filtered through celite and washed with ethyl acetate. The solution was evaporated to dryness and the crude product was purified by column chromatography (EtOAc:cyclohexane, 0:1 to 3:7) to give **3** (484 mg, 90%) as a white solid. ¹H NMR (300 MHz, CDCl₃) δ 7.00 (dt, J = 7.5, 1.5 Hz, 1H), 6.92-6.74 (m, 5H), 6.69-6.59 (m, 3H), 6.54 (dd, J = 8.5, 1.0 Hz, 1H), 4.31-4.18 (m, 3H), 4.15 (s, 4H), 4.12-4.00 (m, 12H), 3.58-3.48 (m, 2H), 2.84 (t, J = 6.5 Hz, 2H), 2.25 (s, 3H), 2.08-1.98 (m, 2H), 1.1-1.11 (m, 12H). ¹³C NMR (75 MHz, CDCl₃) δ 171.8, 171.7, 151.3, 150.4, 145.2, 143.0, 139.6, 137.1, 136.4, 132.2, 129.4, 126.6, 123.5, 122.0, 119.8, 119.4, 118.8, 117.6, 114.9, 114.5, 111.4, 67.3, 67.1, 60.9, 60.8, 53.8, 51.5, 27.9, 22.7, 21.1, 14.3, 14.2. HRMS calcd for C₄₀H₅₁N₃O₁₀Na: 756.3467. Found: 756.3469.

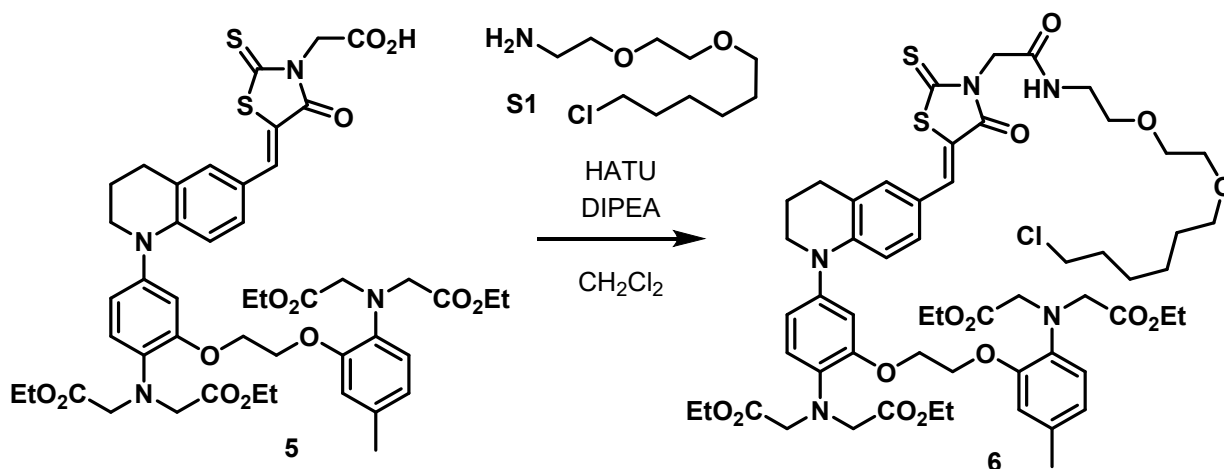


To a stirred solution of DMF (0.25 mL, 2.01 mmol, 3.3 eq) was added phosphoryl chloride (0.06 mL, 0.67 mmol, 1.1 eq) dropwise at 0 °C and the reaction was stirred for 30 min. **3** (447 mg, 0.61 mmol, 1.0 eq) in 1.0 mL of DMF was added and the reaction was stirred for 5 hours at room temperature. The reaction was quenched with water, washed with brine and extracted with ethyl acetate. The organic layer was dried over MgSO₄, filtered and evaporated to dryness and the crude product was purified by column chromatography (EtOAc:Cyclohexane, 0:1 to 1:1) to give **4** (335 mg, 72%) as a white solid. ¹H NMR (300 MHz, CDCl₃) δ 9.65 (s, 1H), 7.55-7.47 (m, 1H), 7.34 (dd, J = 8.5, 2.0 Hz, 1H), 6.87-6.81 (m, 1H), 6.80-6.71 (m, 3H), 6.71-6.63 (m, 2H), 6.41 (d, J = 8.5 Hz, 1H), 4.28-4.20 (m, 4H), 4.17 (s, 4H), 4.13-3.99 (m, 12H), 3.61 (t, J = 6.5 Hz, 2H), 2.89 (t, J = 6.5 Hz, 2H), 2.25 (s, 3H), 2.12-2.02 (m, 2H), 1.16 (td, J = 7.0, 5.0 Hz, 12H). ¹³C NMR (75 MHz, CDCl₃) δ 190.4, 171.7, 171.6, 151.3, 150.9, 150.3, 140.2, 138.1, 137.1, 132.2, 131.1, 130.2, 126.1, 122.1, 122.0, 120.0, 119.7, 119.5, 114.6, 112.9, 112.1, 67.4, 67.0, 61.0, 60.7, 53.7 (x2), 52.1, 27.8, 22.0, 21.0, 14.2 (x2). HRMS calcd for C₄₁H₅₁N₃O₁₁Na: 784.3416. Found: 784.3411.

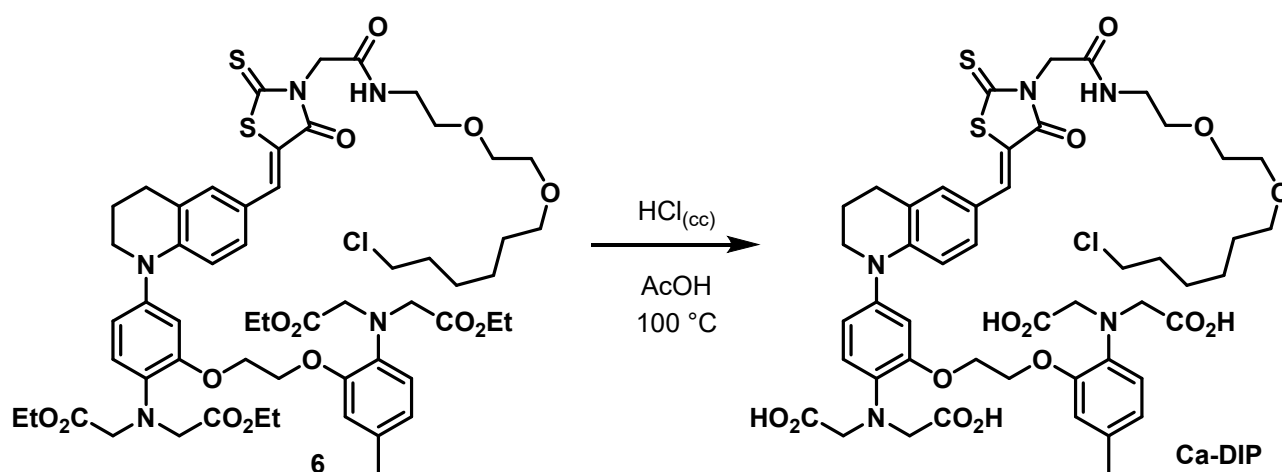


A solution of **4** (41 mg, 0.05 mmol, 1.0 eq) and 2-(4-oxo-2-thioxothiazolidin-3-yl)acetic acid (11 mg, 0.05 mmol, 1.1 eq) in ethanol (1.0 mL) was stirred at 100 °C overnight in the dark. The mixture was evaporated and purified by preparative HPLC (65% to 100% MeCN in H₂O, 0.1% TFA) to give **5** (34 mg, 67%) as an orange solid. ¹H NMR (300 MHz, CDCl₃) δ 7.58 (s, 1H), 7.12 (d, J = 2.0 Hz, 1H), 7.01 (dd, J = 9.0, 2.0 Hz, 1H), 6.85 (d, J = 8.0 Hz, 1H), 6.78-6.72 (m, 3H), 6.69-6.64 (m, 2H), 6.44 (d, J = 9.0 Hz, 1H), 4.81 (s, 2H), 4.26 (s, 4H), 4.17 (s, 4H), 4.14-4.01 (m, 12H), 3.62 (t, J = 5.5 Hz, 3H), 2.88 (t, J = 6.0 Hz, 3H), 2.25 (s, 3H), 2.15-2.04 (m, 2H), 1.16 (q, J = 7.0 Hz, 12H). ¹³C

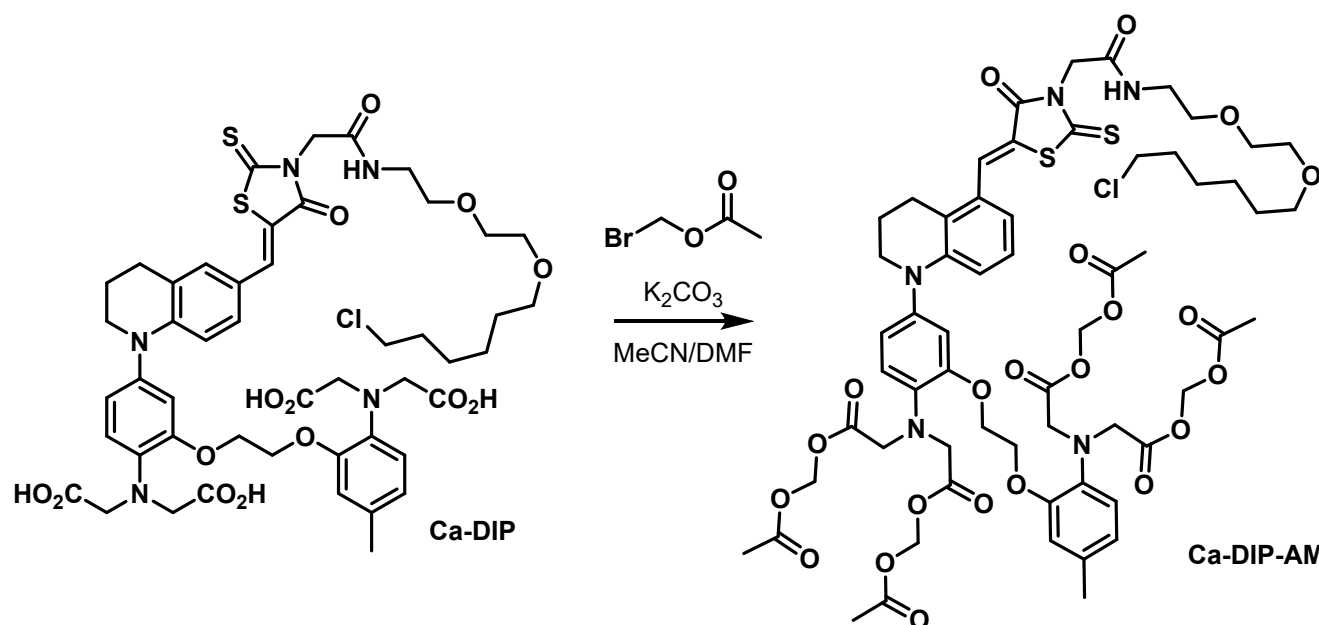
NMR (75 MHz, CDCl₃) δ 193.0, 171.8, 171.7, 171.8, 169.6, 167.3, 151.4, 150.4, 148.6, 140.0, 138.1, 136.7, 135.7, 132.8, 132.5, 131.6, 122.9, 122.0, 121.7, 119.9, 119.7, 115.0, 114.5, 114.0, 112.0, 67.4, 66.9, 61.1, 60.8, 53.8, 53.7, 52.1, 44.5, 27.9, 22.0, 21.1, 14.3, 14.2, 14.2. **HRMS** calcd for C₄₆H₅₅N₄O₁₃S₂: 935.3202. Found: 935.3203.



To a stirred solution of **5** (200 mg, 0.21 mmol, 1.0 eq), **S1** (57 mg, 0.26 mmol, 1.2 eq) and DIPEA (0.07 mL, 0.43 mmol, 2.0 eq) in CH₂Cl₂ (2.0 mL) was added HATU (81 mg, 0.21 mmol, 1.0 eq) and the reaction was stirred at room temperature overnight in the dark. The mixture was washed with brine and extracted with dichloromethane. The organic layer was dried over MgSO₄, filtered and evaporated to dryness and the crude product was purified by column chromatography (EtOAc:Cyclohexane, 0:1 to 1:1) to give **6** (113 mg, 46%) as a red solid. **¹H NMR** (300 MHz, DMSO-d₆) δ 8.35 (t, J = 5.5 Hz, 1H), 7.61 (s, 1H), 7.28 (d, J = 2.0 Hz, 1H), 7.17 (dd, J = 9.0, 2.0 Hz, 1H), 6.92 (d, J = 2.0 Hz, 1H), 6.83-6.73 (m, 3H), 6.66-6.56 (m, 2H), 6.40 (d, J = 9.0 Hz, 1H), 4.62 (s, 2H), 4.19-4.10 (m, 8H), 4.0-4.01 (m, 4H), 3.99-3.90 (m, 8H), 3.66-3.58 (m, 4H), 3.53-3.46 (m, 4H), 3.44-3.34 (m, 4H), 3.22 (q, J = 5.5 Hz, 2H), 2.87 (t, J = 6.0 Hz, 2H), 2.21 (s, 3H), 2.07-1.97 (m, 2H), 1.70 (dq, J = 8.0, 6.5 Hz, 2H), 1.49 (p, J = 6.5 Hz, 2H), 1.43-1.26 (m, 4H), 1.11-1.02 (m, 12H). **¹³C NMR** (75 MHz, DMSO-d₆) δ 192.8, 170.8, 170.7, 166.7, 164.6, 150.4, 149.6, 148.3, 138.7, 137.4, 136.4, 134.5, 132.6, 131.0, 130.5, 122.8, 121.2, 120.8, 119.2, 118.6, 118.1, 114.2, 114.1, 113.2, 111.9, 70.2, 69.6, 69.4, 68.9, 67.3, 66.8, 60.2, 60.0, 53.1 (x2), 51.4, 46.0, 45.4, 38.9, 32.0, 29.1, 27.1, 26.1, 24.9, 21.3, 20.5, 13.9, 13.8. **HRMS** calcd for C₅₄H₇₄ClN₅O₁₄S₂Na: 1162.4254. Found: 1162.4269.



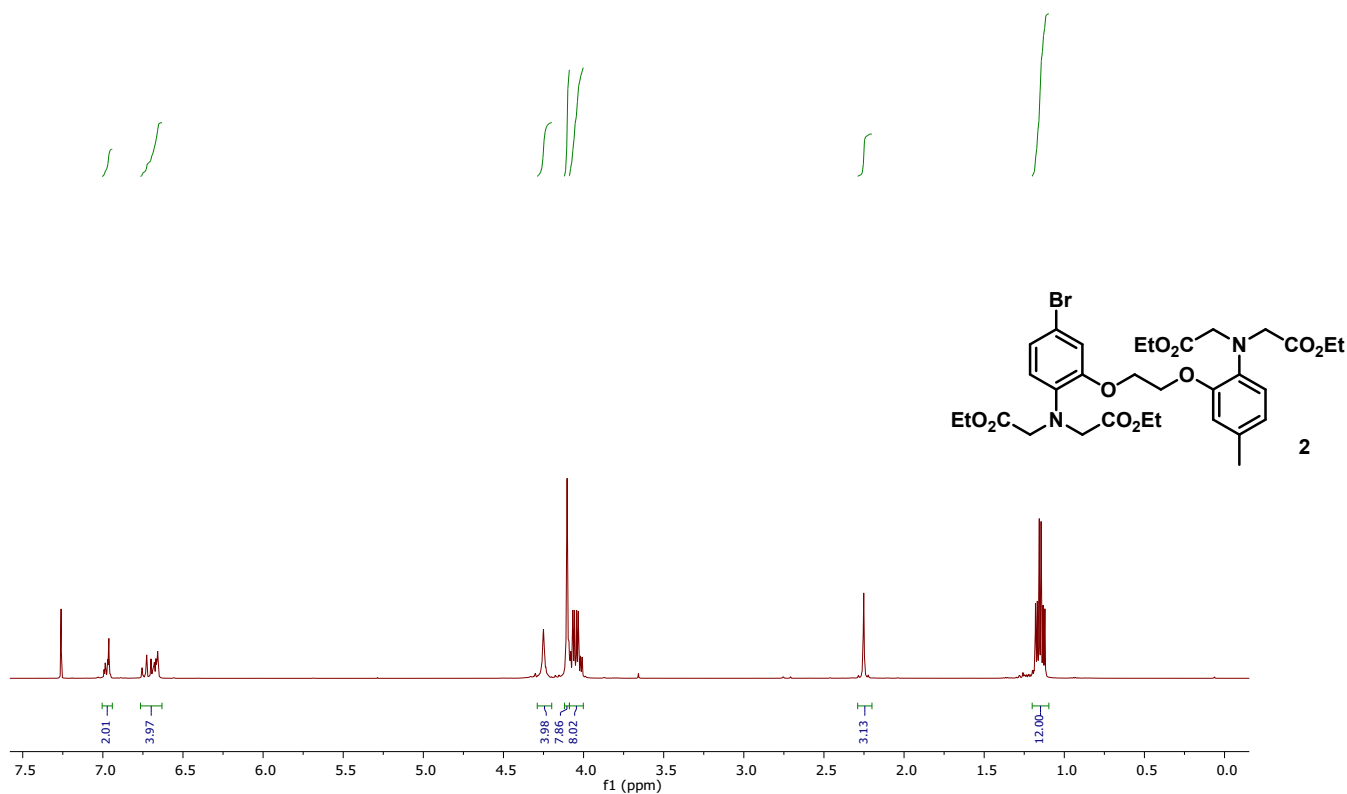
A solution of **6** (40 mg, 0.04 mmol, 1.0 eq) in acetic acid (2.5 mL) and concentrated chloridric acid (1.0 mL) was stirred at 100 °C for 3 hours. The mixture was evaporated and purified by preparative HPLC (30% to 100% MeCN in H₂O, 0.1% TFA) to give **Ca-DIP** (25 mg, 70%) as a red solid. ¹H NMR (300 MHz, DMSO-d₆) δ 12.41 (brs, 4H), 8.36 (t, J = 5.5 Hz, 1H), 7.60 (s, 1H), 7.28 (d, J = 2.0 Hz, 1H), 7.18 (dd, J = 9.0, 2.0 Hz, 1H), 6.98-6.90 (m, 1H), 6.85-6.76 (m, 3H), 6.65 (s, 2H), 6.44 (d, J = 9.0 Hz, 1H), 4.62 (s, 2H), 4.24 (s, 4H), 4.09 (s, 4H), 3.98 (s, 4H), 3.62 (t, J = 6.5 Hz, 4H), 3.52-3.46 (m, 4H), 3.45-3.32 (m, 4H), 3.23 (t, J = 5.5 Hz, 2H), 2.91-2.82 (m, 2H), 2.20 (s, 3H), 2.06-1.96 (m, 2H), 1.75-1.65 (m, 2H), 1.56-1.45 (m, 2H), 1.41-1.27 (m, 4H). HRMS calcd for C₄₈H₅₇ClN₅O₁₄S₂: 1026.3037. Found: 1026.3044. **Analytical HPLC**: t_R = 7.1 min, 99% purity (30–100% MeCN/H₂O, linear gradient, with constant 0.1% v/v TFA additive, 13 min run, 1 mL/min flow, detection at 280 nm).



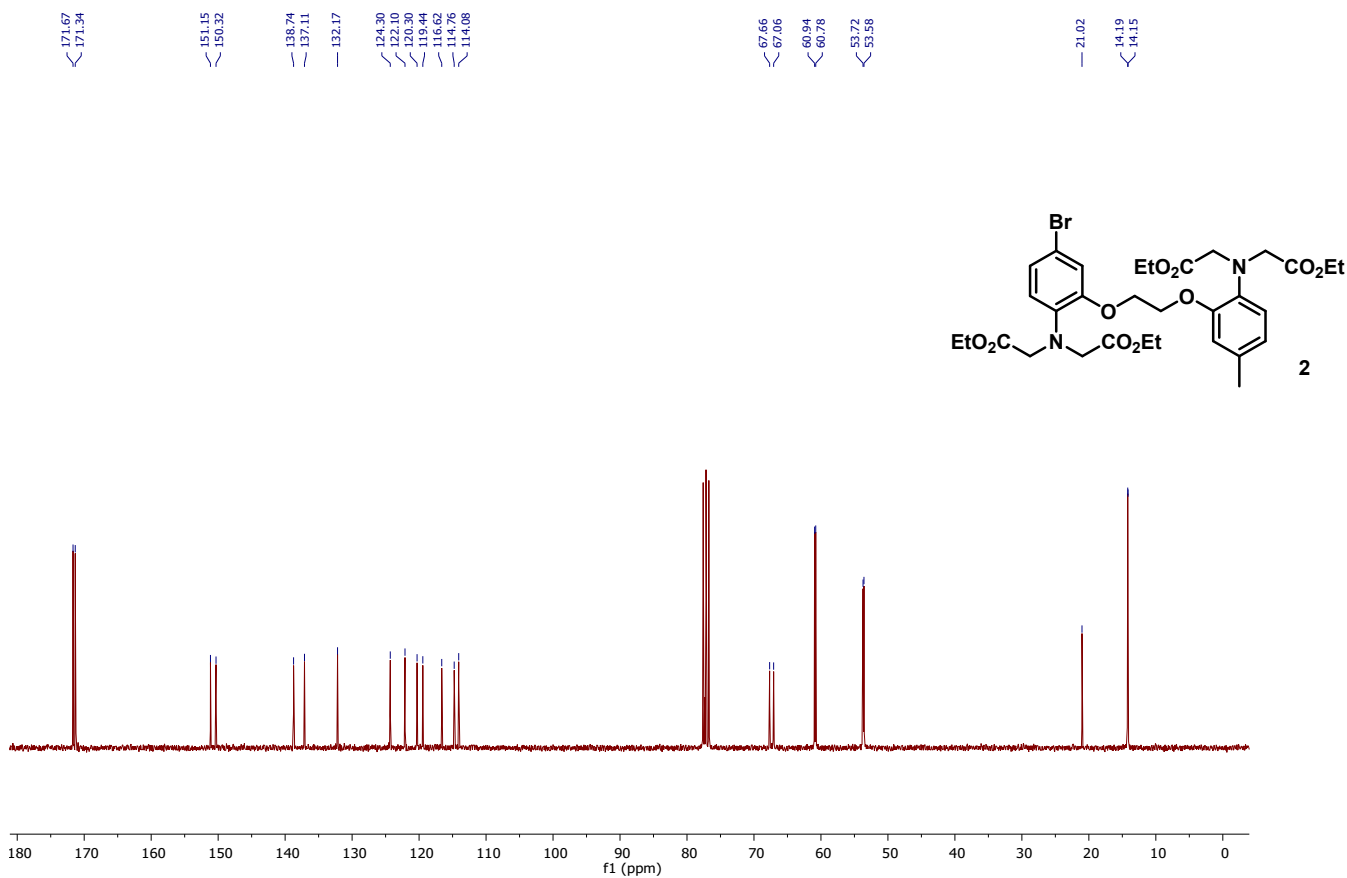
To a stirred suspension of **Ca-DIP** (21 mg, 0.02 mmol, 1.0 eq) and potassium carbonate (28 mg, 0.20 mmol, 10.0 eq) in acetonitrile (1.0 mL) and DMF (0.1 mL) was added bromomethyl acetate (0.01 mL, 0.12 mmol, 6.0 eq) and the reaction was stirred at room temperature for 5 hours. The reaction was quenched with a solution of HCl (1.0 M, 0.4 mL), evaporated to dryness and the crude product was purified by preparative HPLC (50% to 100%

MeCN in H₂O, 0.1% TFA) to give **Ca-DIP-AM** (4.2 mg, 16%) as a red solid. ¹H NMR (300 MHz, CDCl₃) δ 7.64 (s, 1H), 7.17 (d, J = 2.0 Hz, 1H), 7.05 (dd, J = 9.0, 2.0 Hz, 1H), 6.95-6.85 (m, 1H), 6.85-6.75 (m, 2H), 6.7-6.65 (m, 2H), 6.50 (d, J = 8.5 Hz, 1H), 6.39-6.32 (m, 1H), 5.64 (d, J = 2.0 Hz, 4H), 4.77 (s, 2H), 4.37-4.11 (m, 8H), 3.70-3.40 (m, 14H), 2.94-2.85 (m, 2H), 2.27 (s, 3H), 2.09 (s, 6H), 2.06 (s, 6H), 2.03-1.90 (m, 2H), 1.82-1.72 (m, 2H), 1.66-1.55 (m, 2H), 1.53-1.32 (m, 4H). **HRMS** calcd for C₆₀H₇₄ClN₅O₂₂S₂Na: 1338.3848. Found: 1338.3847. **Analytical HPLC**: t_R = 7.7 min, 84% purity (50–100% MeCN/H₂O, linear gradient, with constant 0.1% v/v TFA additive, 13 min run, 1 mL/min flow, detection at 280 nm).

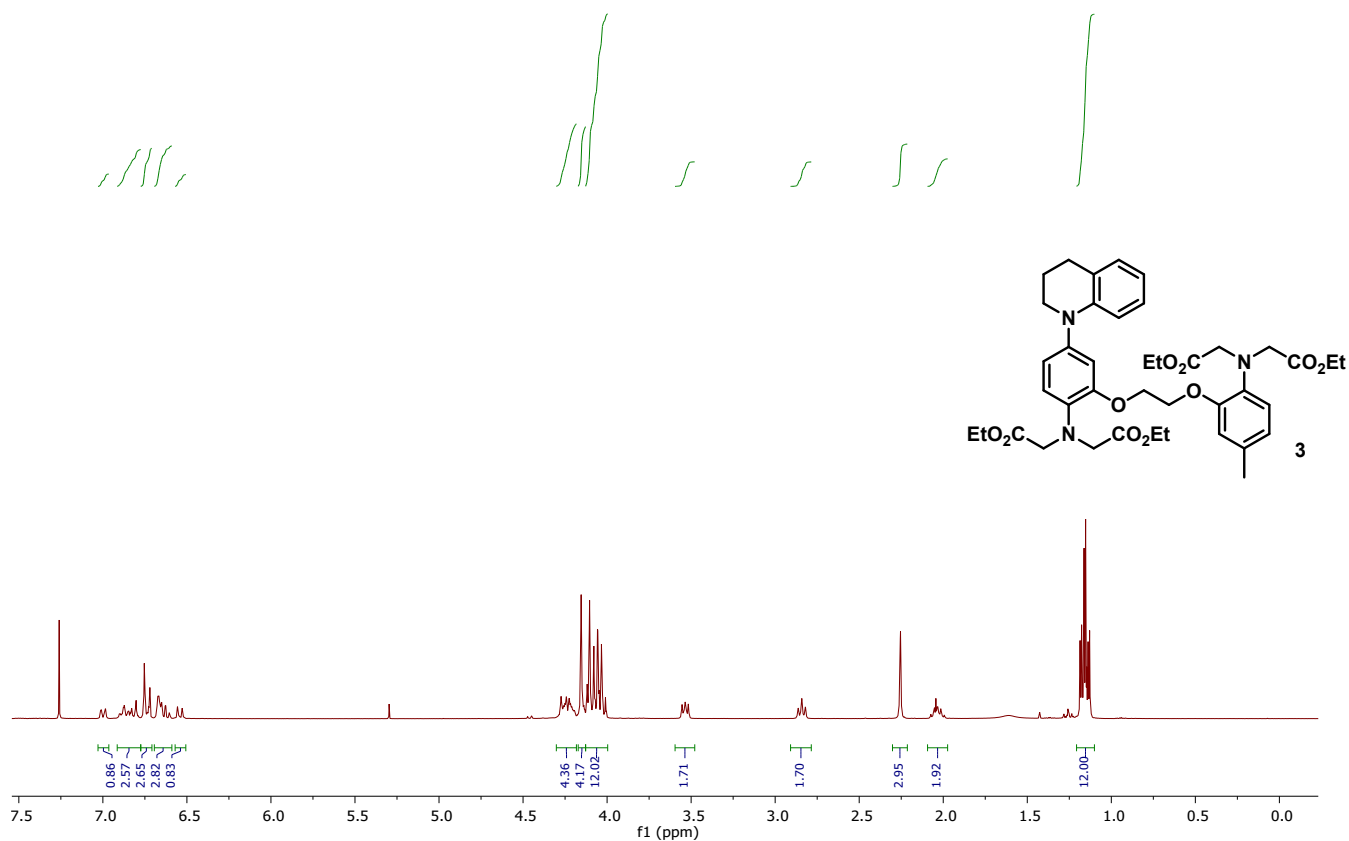
IV. Chemical analyses: NMR spectra and HPLC traces



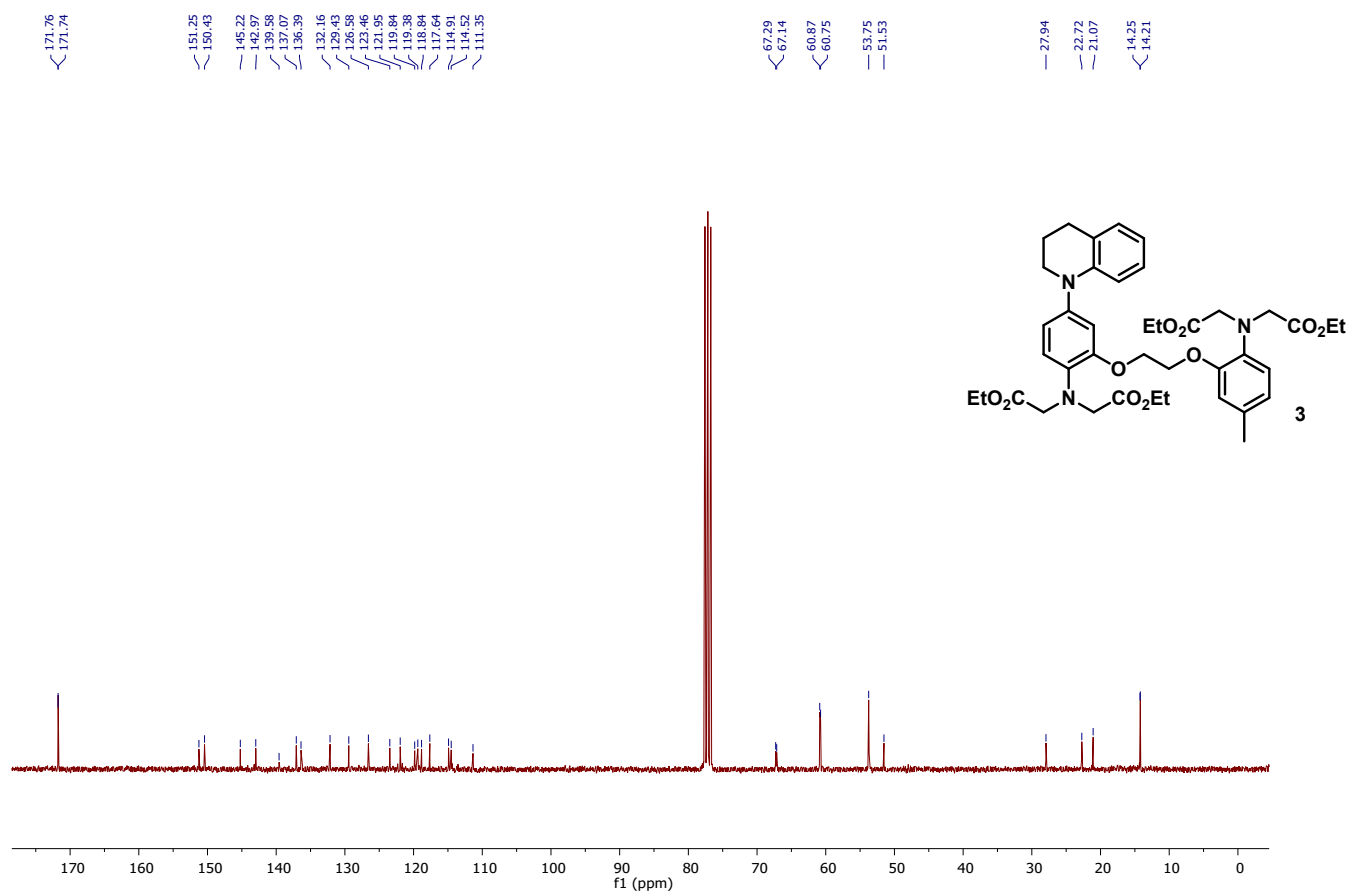
Spectrum S1. ¹H NMR (300 MHz, CDCl₃) spectrum of compound 2.



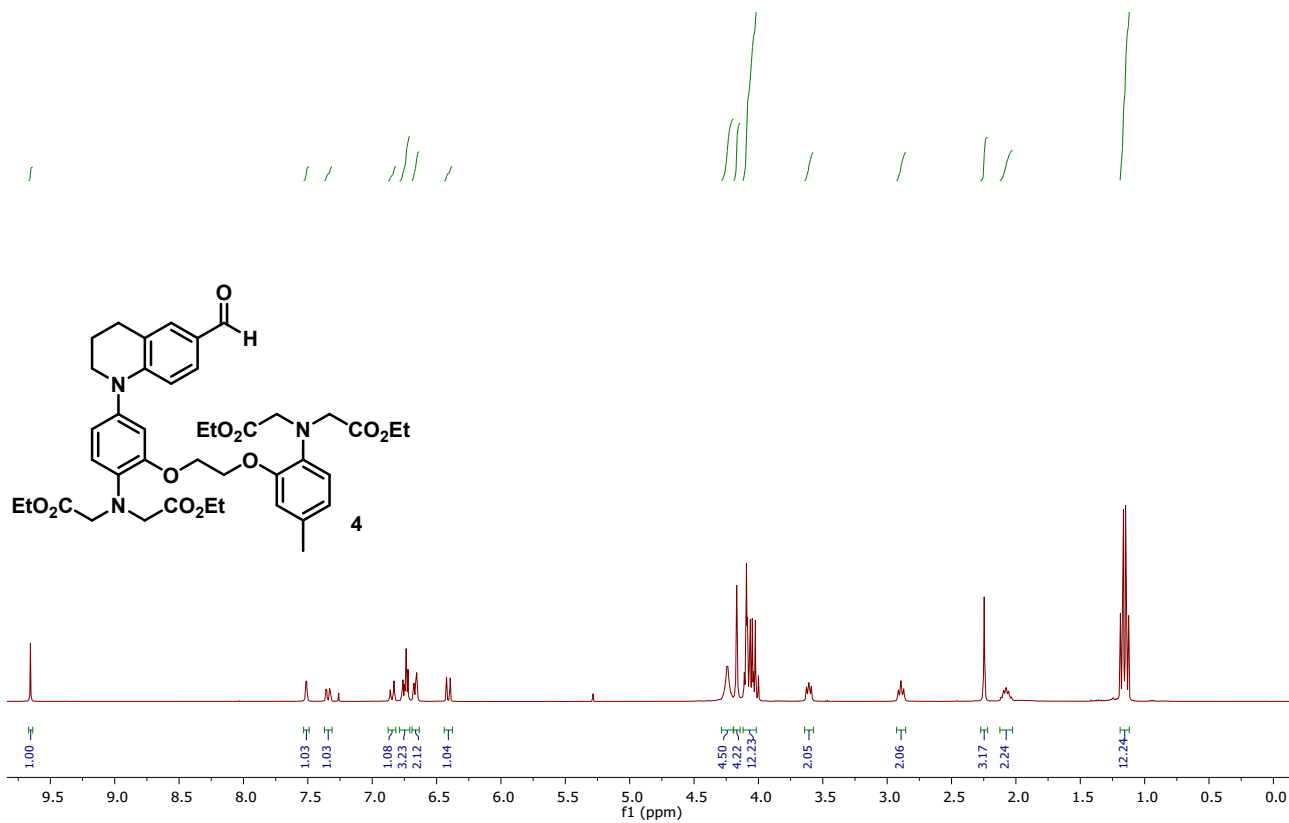
Spectrum S2. ¹³C NMR (75 MHz, CDCl₃) of compound 2.



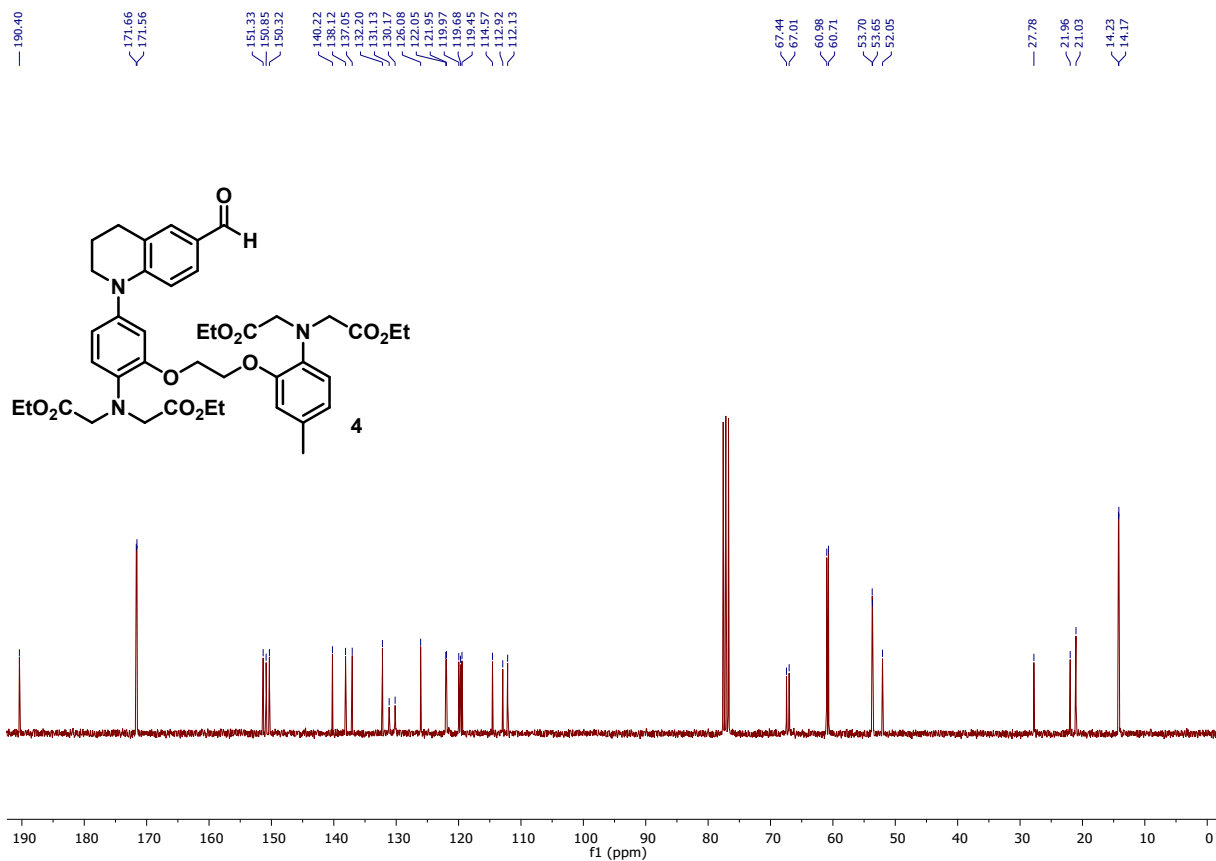
Spectrum S3. ¹H NMR (300 MHz, CDCl₃) spectrum of compound 3.



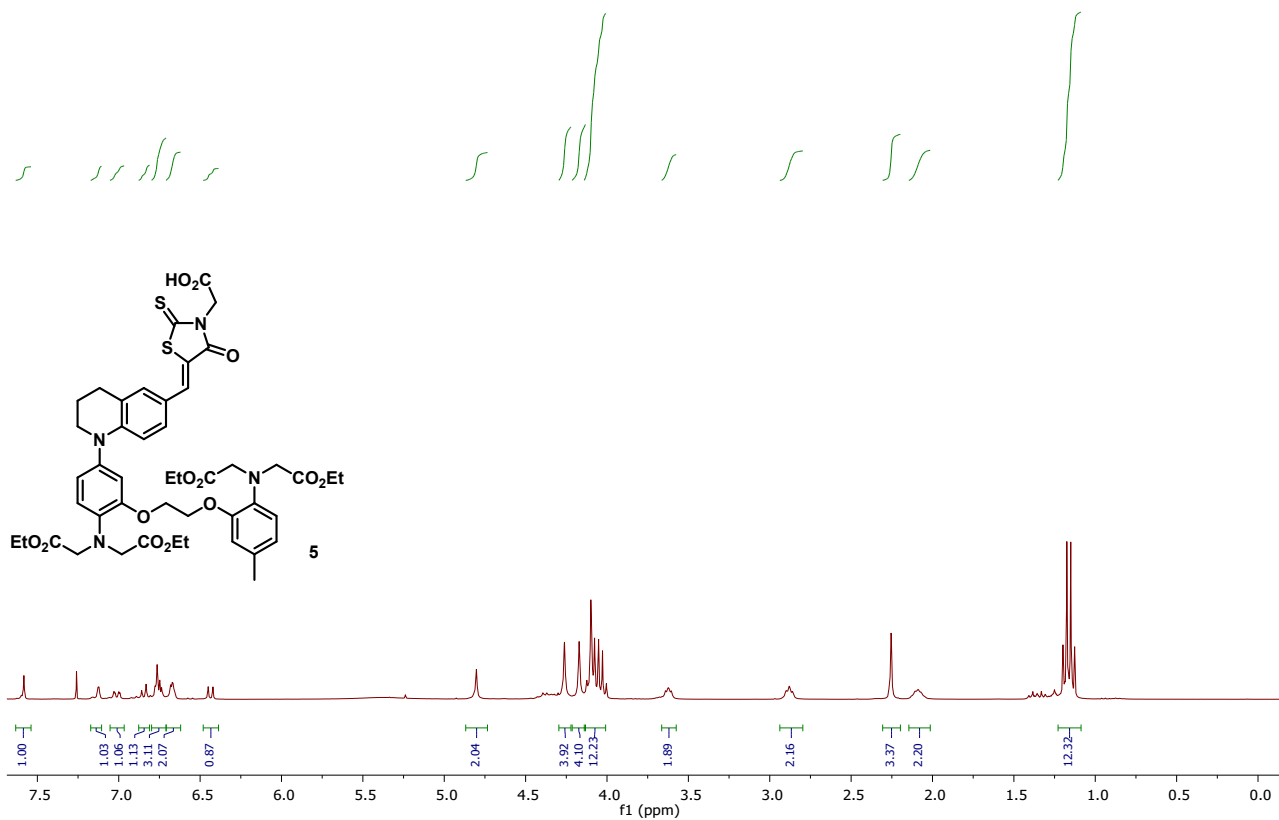
Spectrum S4. ¹³C NMR (75 MHz, CDCl₃) of compound 3.



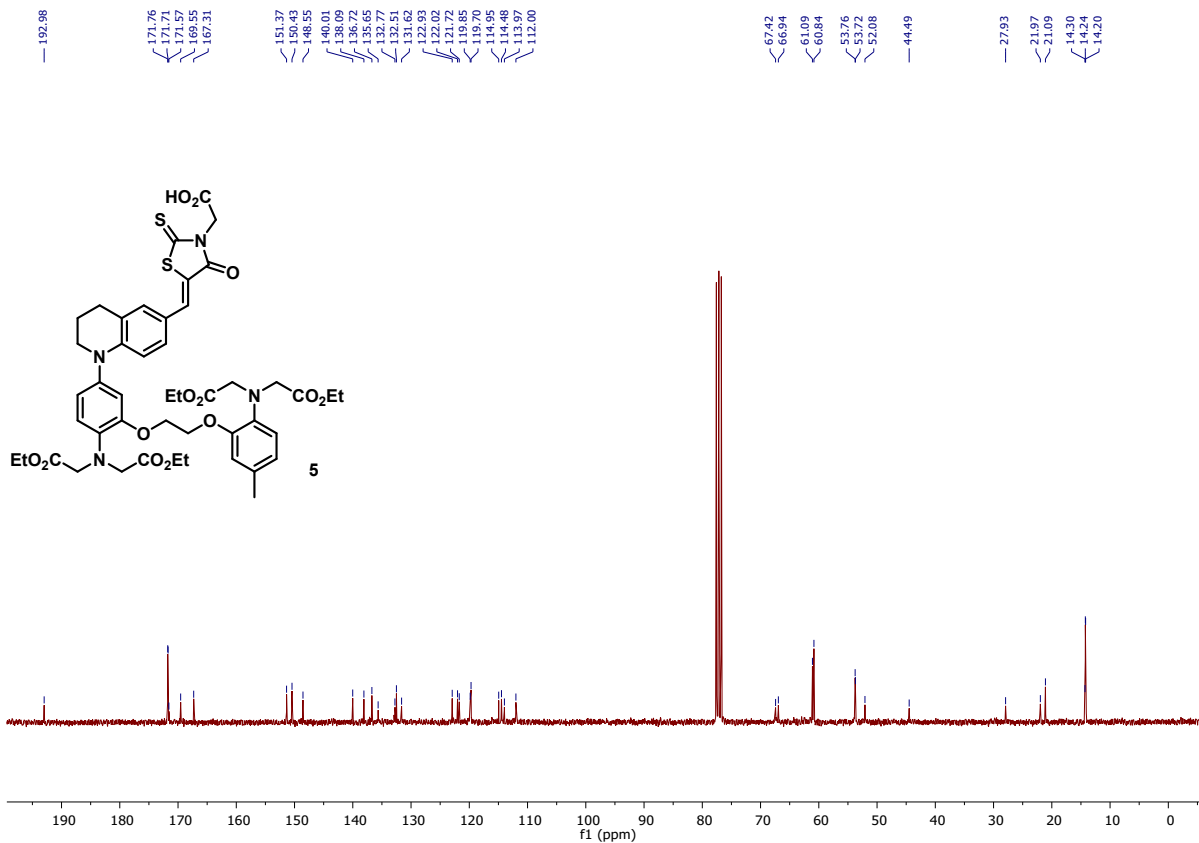
Spectrum S5. ¹H NMR (300 MHz, CDCl₃) spectrum of compound 4.



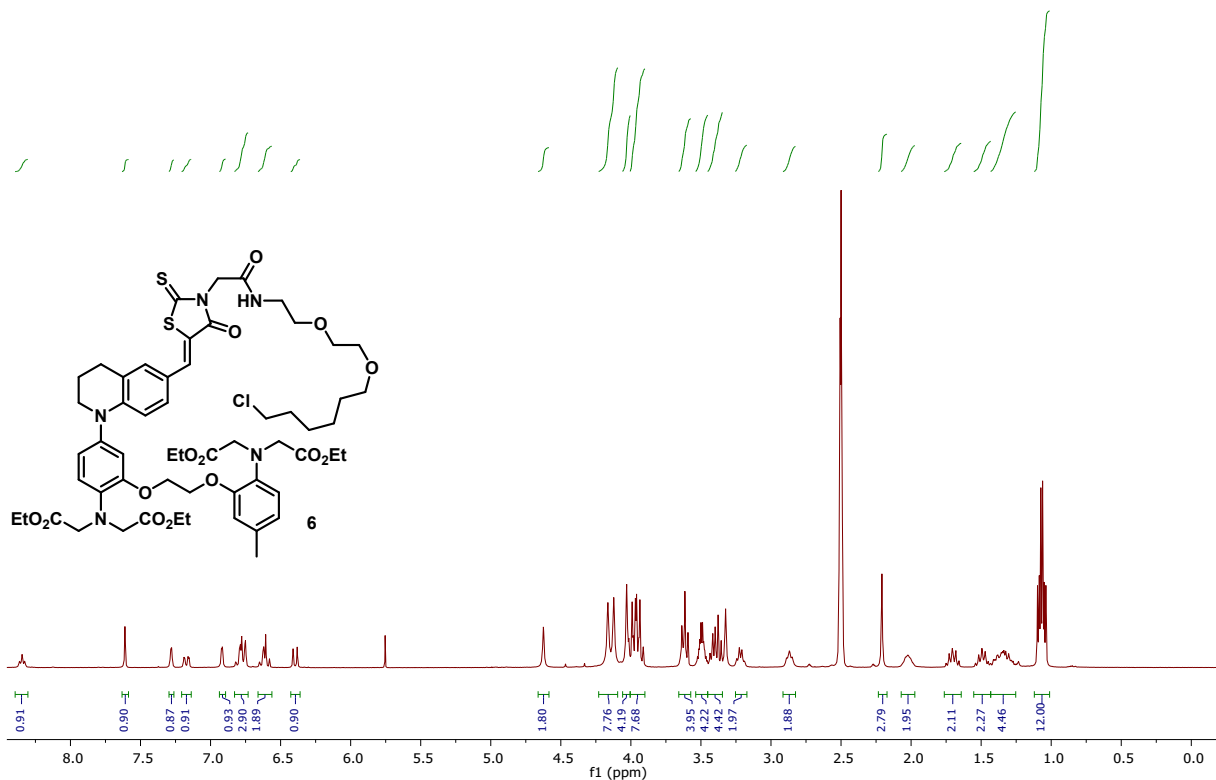
Spectrum S6. ¹³C NMR (75 MHz, CDCl₃) of compound 4.



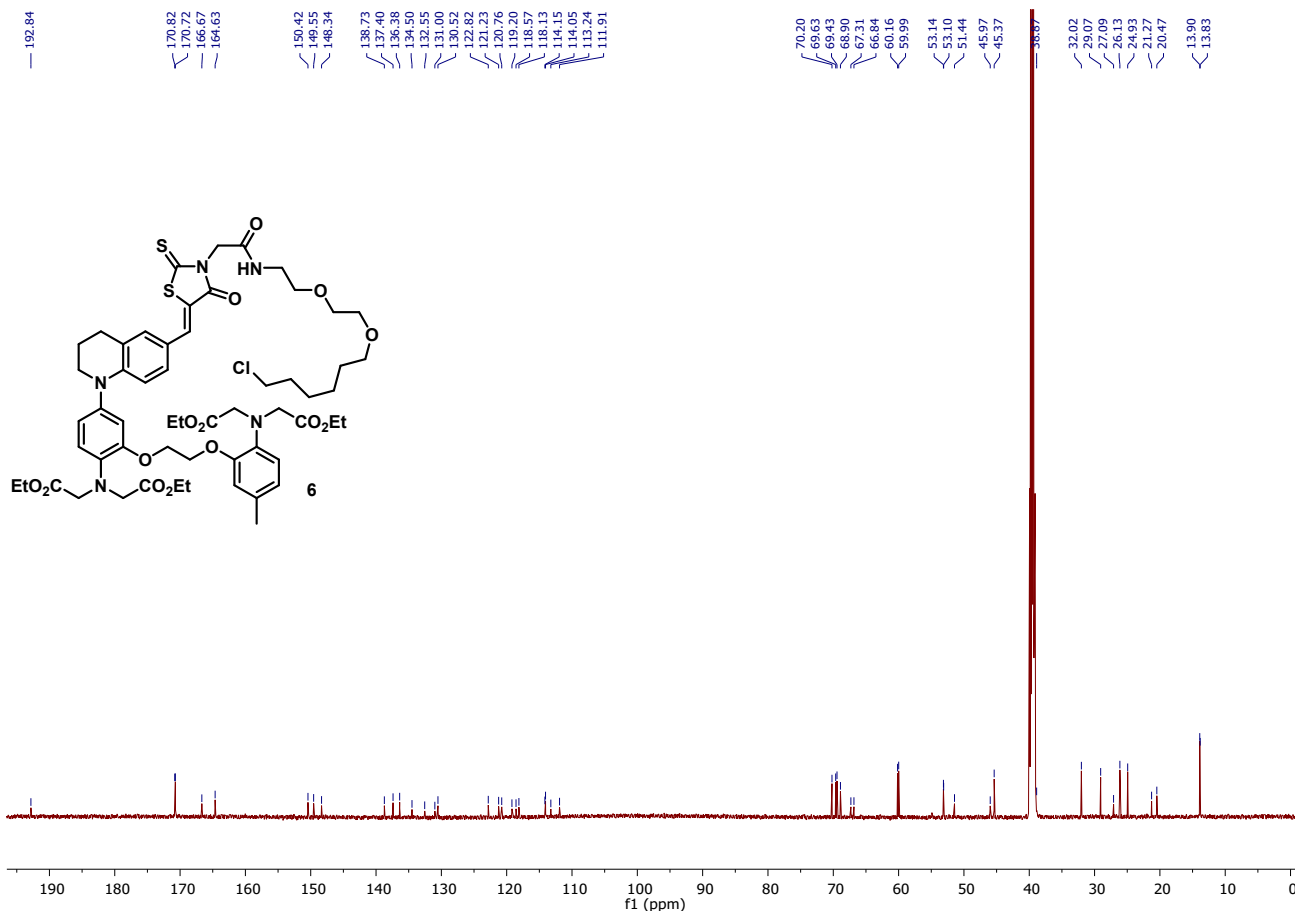
Spectrum S7. ¹H NMR (300 MHz, CDCl₃) spectrum of compound 5



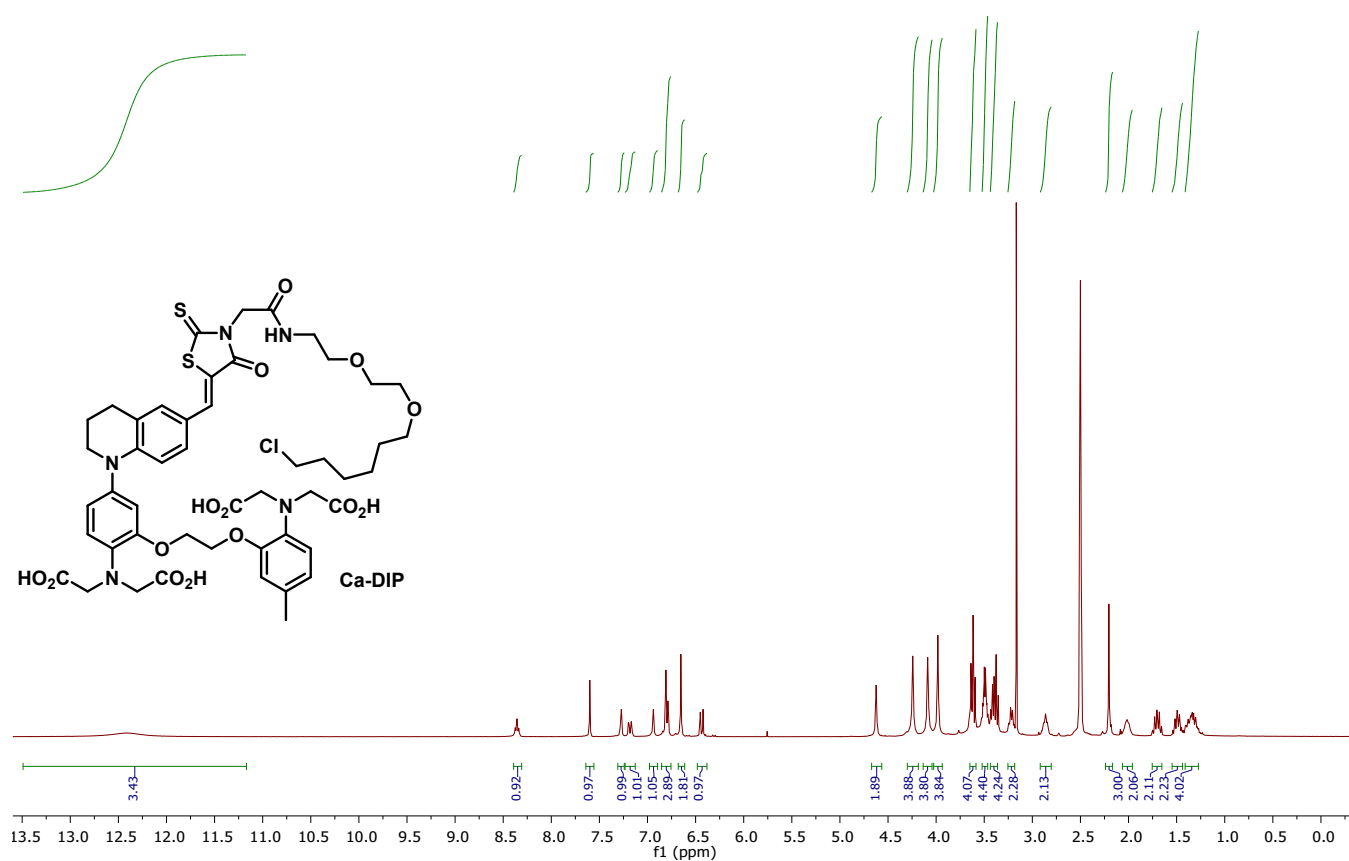
Spectrum S8. ¹³C NMR (75 MHz, CDCl₃) of compound 5



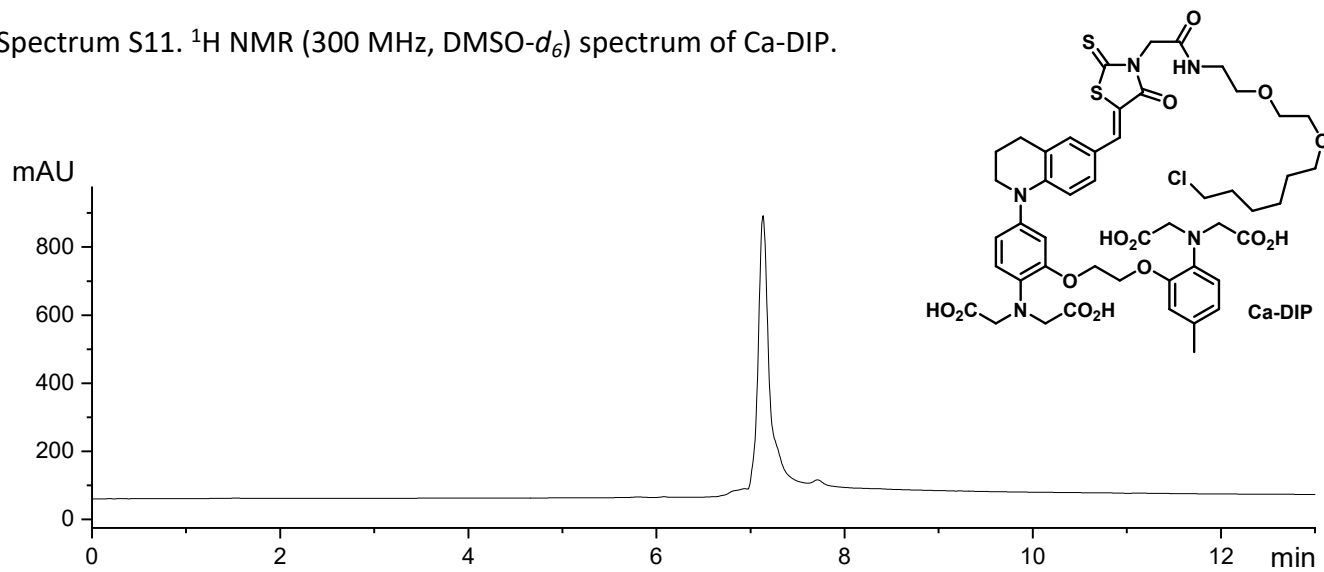
Spectrum S9. ¹H NMR (300 MHz, DMSO-*d*₆) spectrum of compound 6



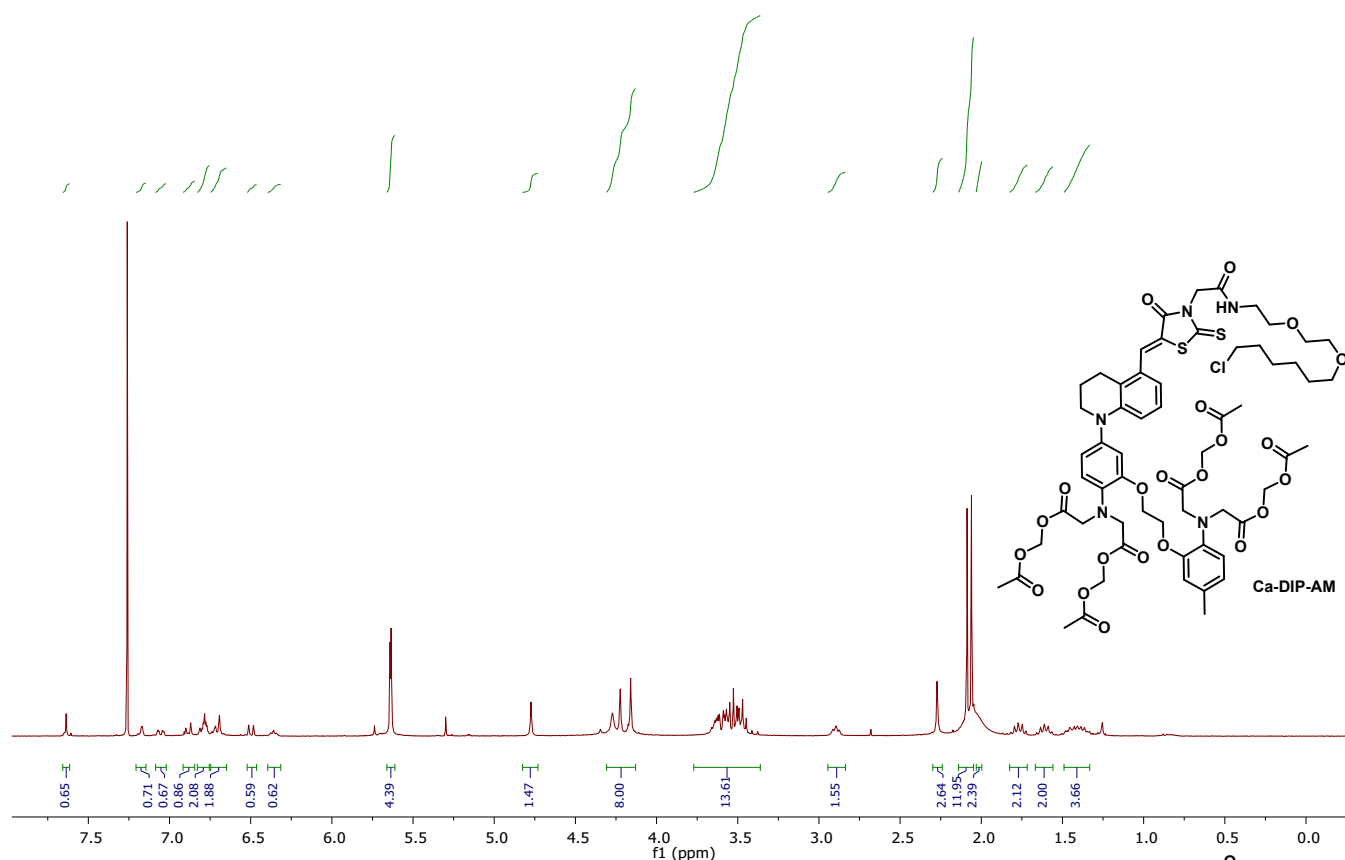
Spectrum S10. ¹³C NMR (75 MHz, DMSO-*d*₆) of compound 6



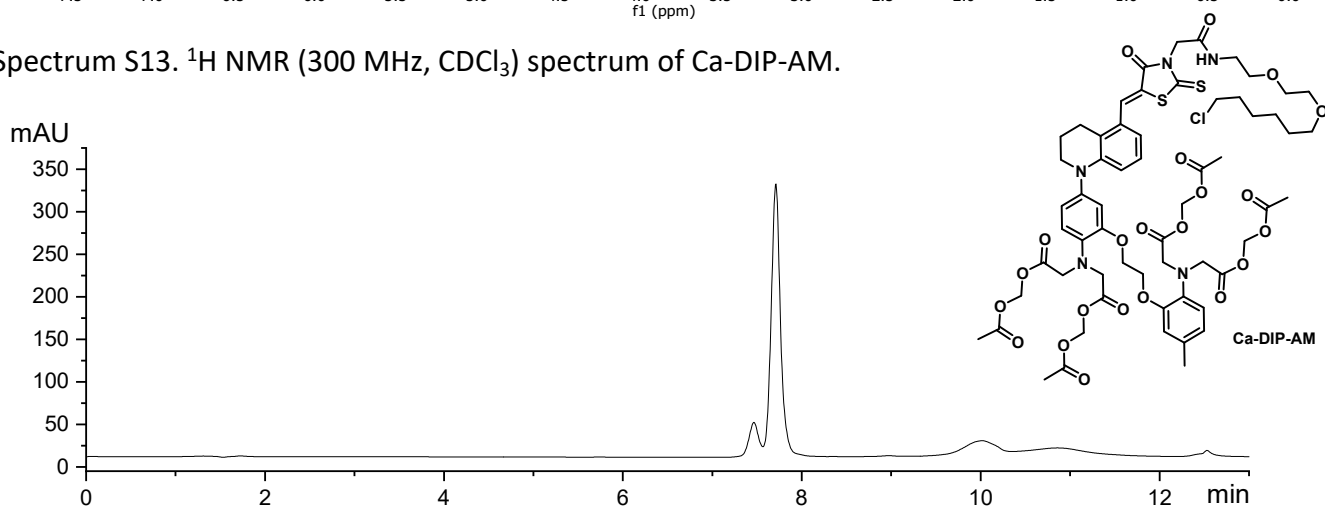
Spectrum S11. ^1H NMR (300 MHz, $\text{DMSO-}d_6$) spectrum of Ca-DIP.



Spectrum S12. HPLC chromatogram of Ca-DIP.



Spectrum S13. ^1H NMR (300 MHz, CDCl_3) spectrum of Ca-DIP-AM.



Spectra S14. HPLC chromatogram of Ca-DIP-AM.

V. References

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