Electronic Supplementary Material (ESI) for Chemical Communications.

Supporting Information

Turn-on Chemiluminescence-Based Probes for Monitoring Tyrosinase Activity in Conjunction with Biological Thiols

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Supplementary Figures

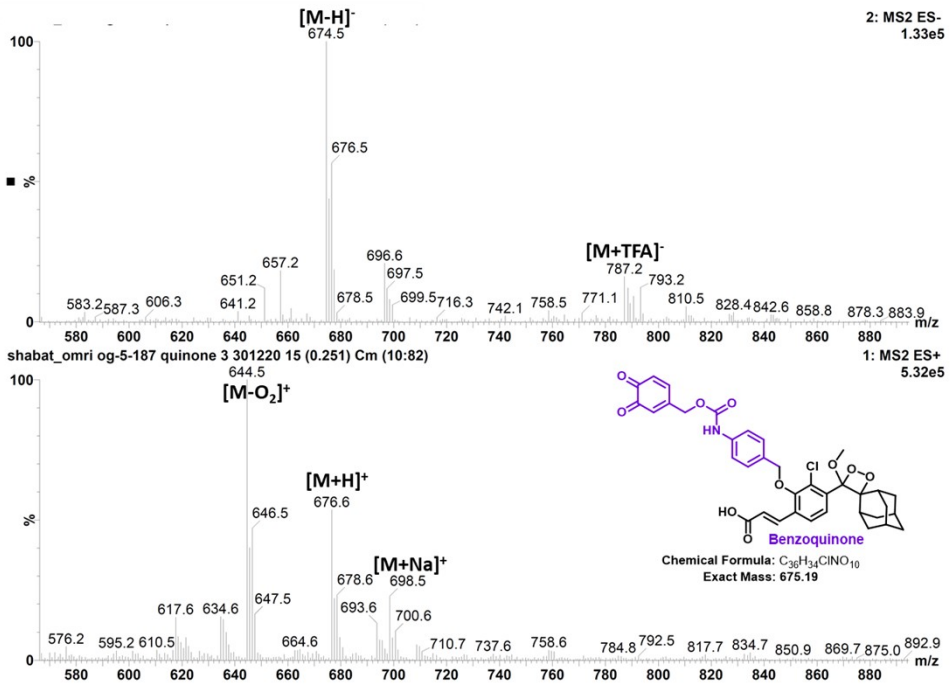


Figure S1 – MS spectra (ESI) of the *o*-benzoquinone intermediate that is formed during the reaction of **CLTP 1** [100 µM] with tyrosinase [200 U/mL] in the absence of GSH. Conditions: PBS, pH 7.4, 1% DMSO.

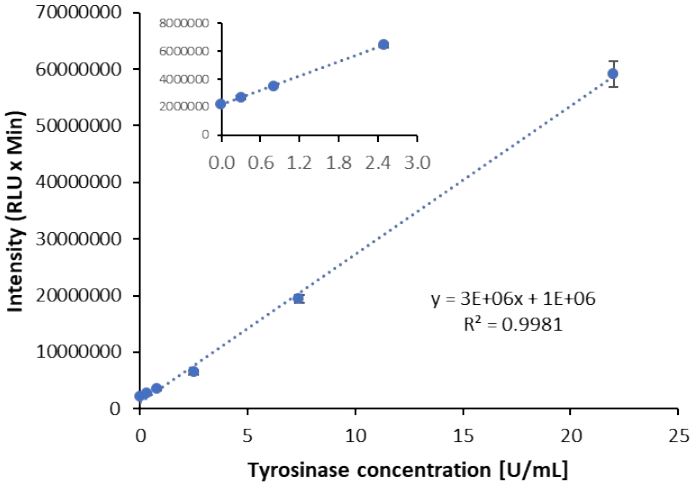


Figure S2 – Limit of detection (LOD) for **CLPT 1** [10 µM] in PBS (pH 7.4, 1% DMSO with GSH [1 mM]). LOD was defined as blank + 3SD (standard deviation). The inset shows the lower concentration data points. λ_{em} = 540 nm

HPLC-MS analysis

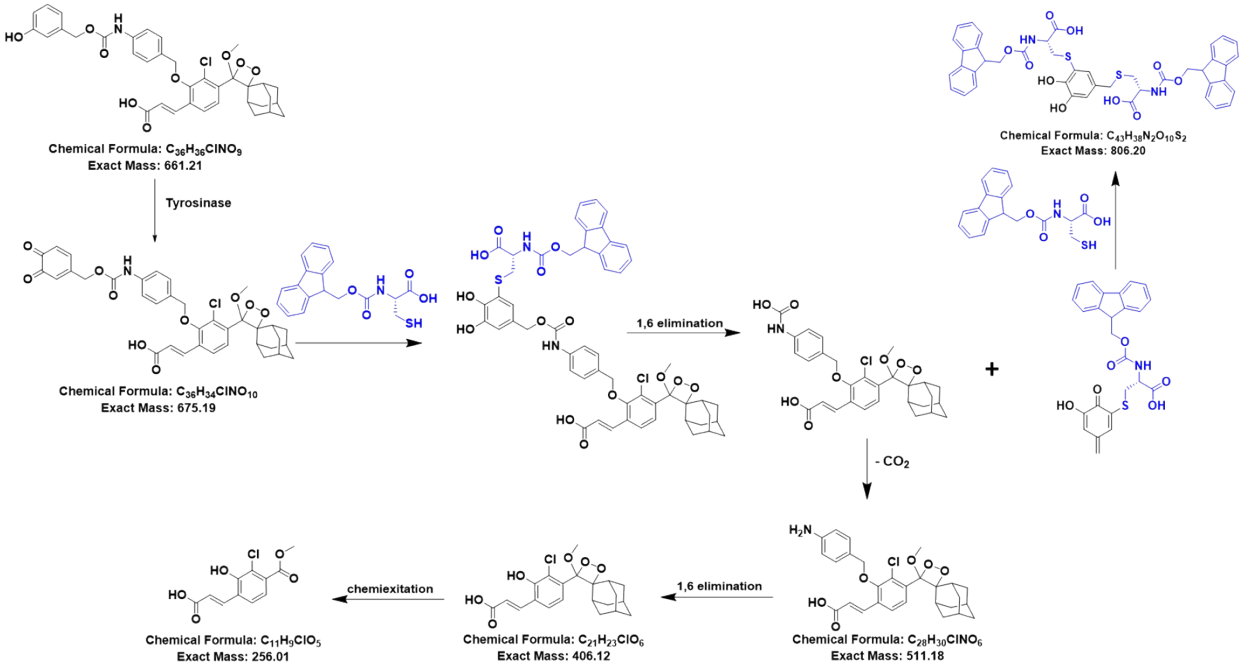


Figure S3 – Postulated mechanism for the activation of **CLPT1** with tyrosinase in the presence of Fmoc-Cys (as a thiol).

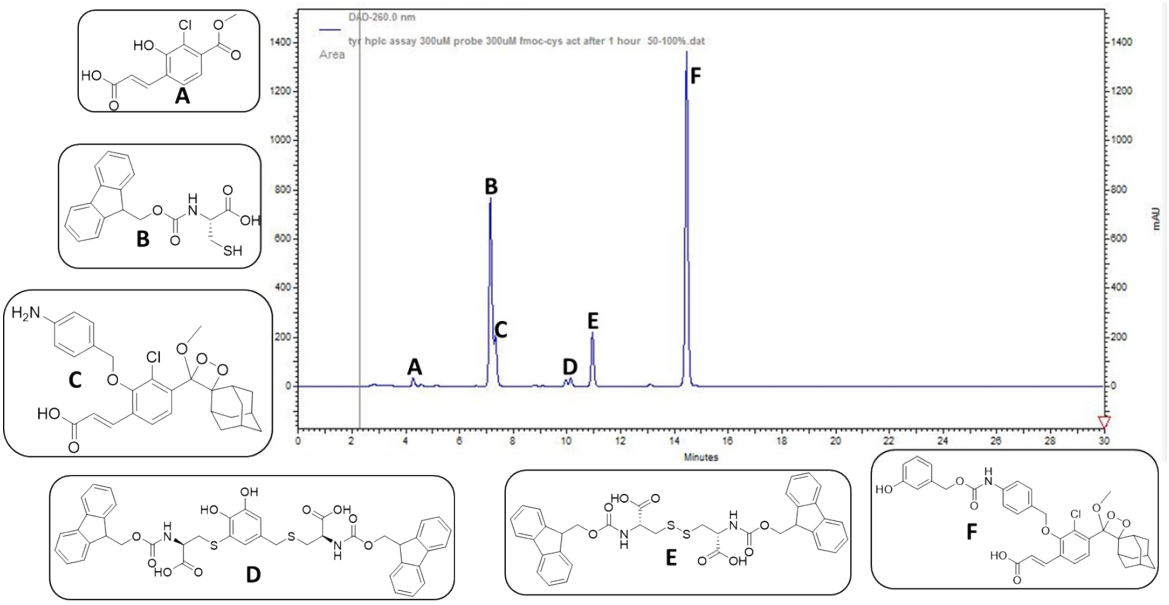


Figure S4 - HPLC analysis for the activation of **CLPT1** with tyrosinase in the presence of Fmoc-Cys (as a thiol). The identified compounds where determind by ESI-MS.

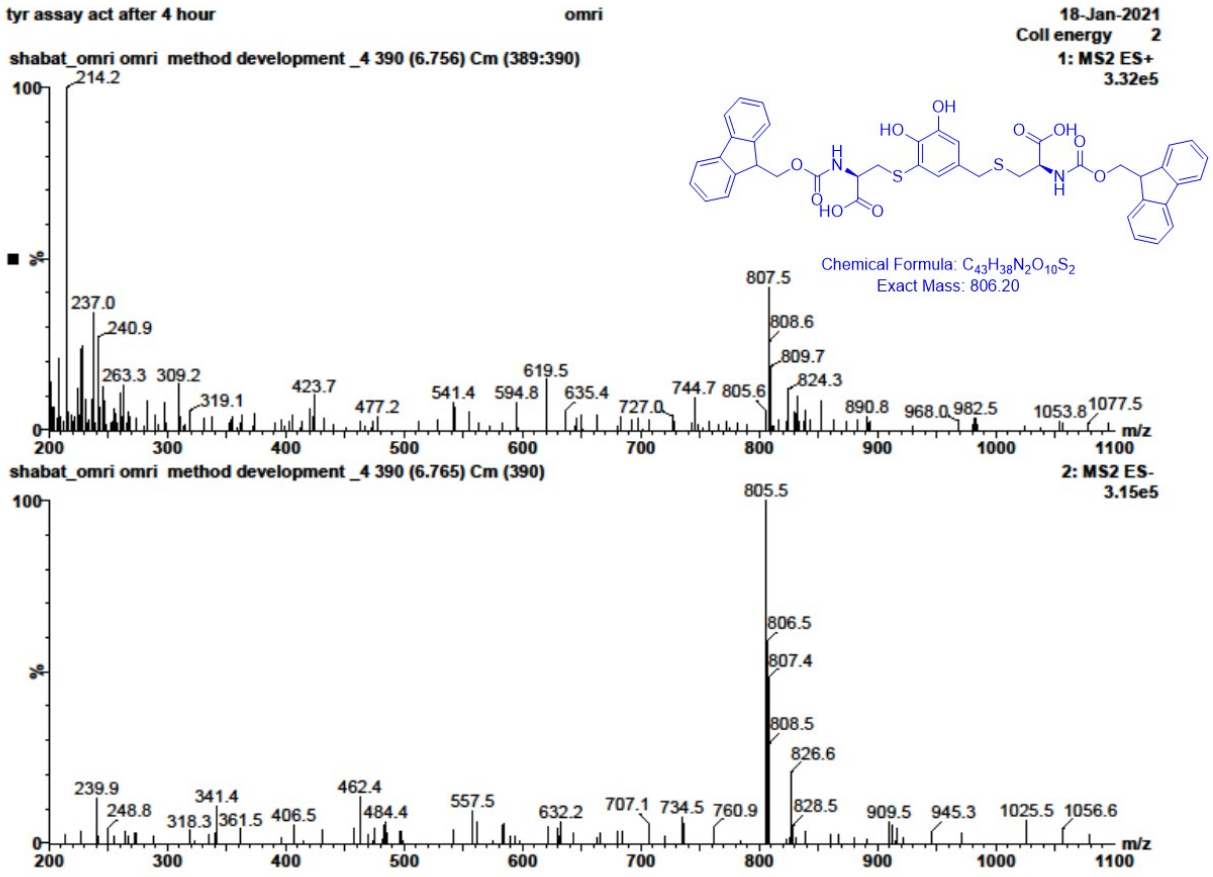


Figure S5 - MS spectra of the thiol conjugate (Fmoc-Cys) on the *o*-benzoquinone intermediate.

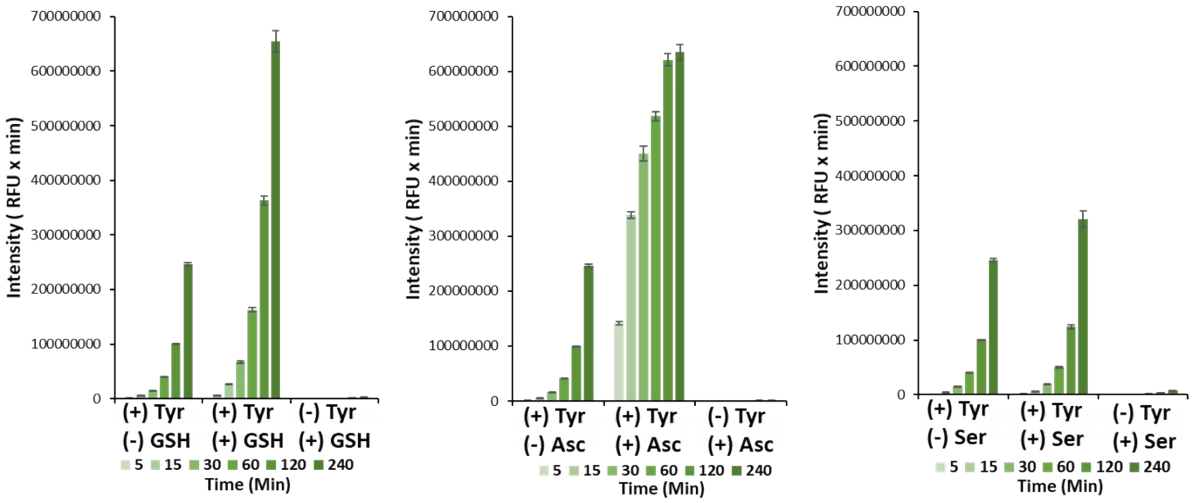


Figure S6 – Total light emission during 4 hours emitted from **CPLT1** [10 µM] in PBS, pH 7.4, 1% DMSO at 37°C with and without tyrosinase [200 U/mL] and in the presence or absence of either **G SH** [1 mM] or Serine [**Ser**, 1 mM] or sodium ascorbate [**Asc**, 1 mM]. Colors represent different time points; 5, 15, 30, 60, 120 and 240 minutes. λ_{em} = 540 nm

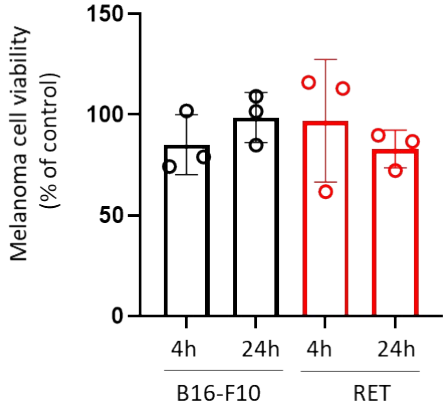


Figure S7 – CLPT3 probe biocompatibility in melanoma cells. B16-F10 and RET cell viability following incubation with **CLPT3** [10 µM] for 4 to 24 h. Mean ± SD of N=3.

Synthetic Schemes and Experimental Procedures

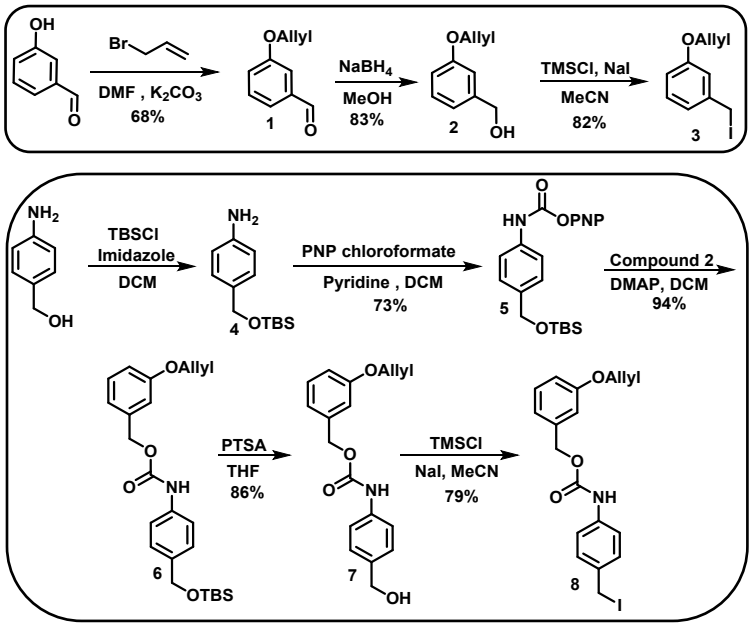
General methods

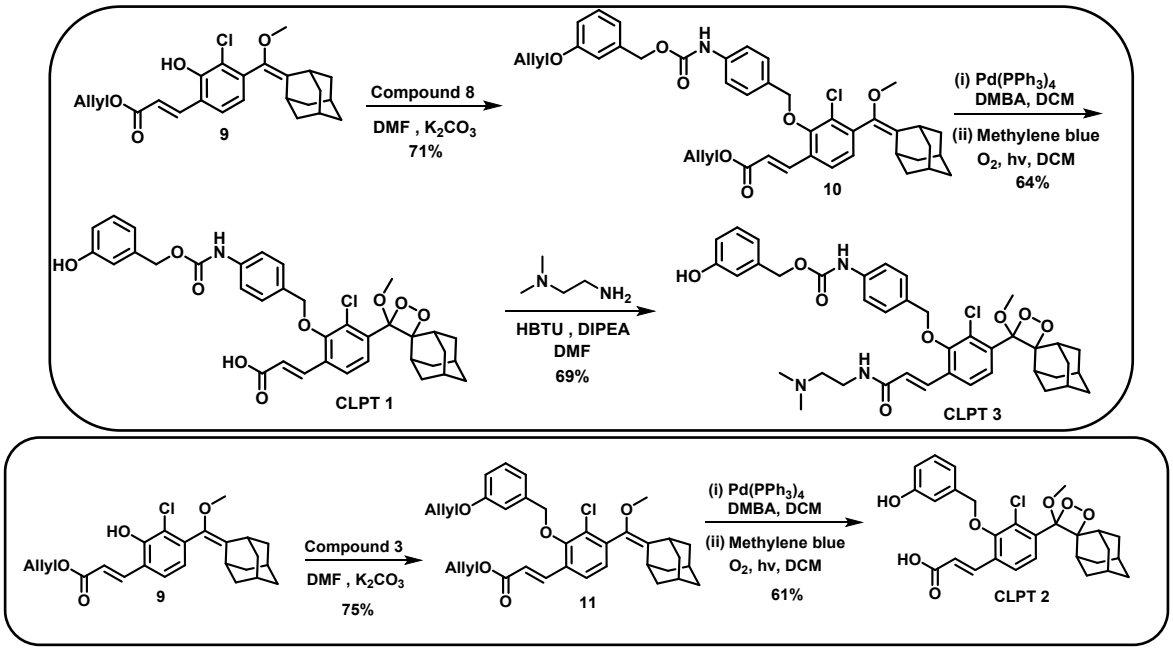
All reactions were carried out at room temperature unless stated otherwise. Chemicals and solvents were either analytical reagent (A.R.) grade or purified by standard techniques. All general reagents, including salts and solvents, were purchased from Sigma-Aldrich. Thin layer chromatography (TLC): silica gel plates Merck 60 F254; compounds visualized by irradiation with UV light. Column chromatography (FC): silica gel Merck 60 (particle size 0.040-0.063 mm), eluent given in parentheses. Reverse-phase high pressure liquid chromatography (RP-HPLC): C18 5u, 250x4.6 mm, eluent: H_2O (0.1% TFA) and MeCN. Preparative RP-HPLC: C18 5u, 250x21b mm, eluent: H_2O (0.1% TFA) and MeCN. ^1H NMR spectra were measured using a Bruker Avance spectrometer operated at 400 MHz. ^{13}C NMR spectra were measured using a Bruker Avance spectrometer operated at 100 MHz. Chemical shifts are reported in ppm on the δ scale relative to a residual solvent ($CDCl_3$: δ = 7.26 for ^1H NMR and 77.16 for ^{13}C NMR). Mass spectra were measured on a Waters Xevo TQD instrument. Chemiluminescence responses were recorded on a Molecular Devices Spectramax i3x.

Abbreviations

PNP - *p*-nitrophenol, **DCM** - dichloromethane, **DIPEA** - N,N-diisopropylethylamine, **DMAP** - 4-dimethylaminopyridine, **DMBA** - dimethylbarbituric acid, **DMEM** - Dulbecco's Modified Eagle Medium, **DMF** - *N,N'*-dimethylformamide, **HBTU** – hexafluoro phosphate benzotriazole tetramethyl uronium, **Hex**-hexanes, **PABA**- *p*-aminobenzyl alcohol, **PTSA** - *p*-toluenesulfonic acid, **TBSCl** - *tert*-butyldimethylsilyl chloride, **THF** - tetrahydrofuran, **TMSCl** - trimethylsilyl chloride.

General synthetic schemes

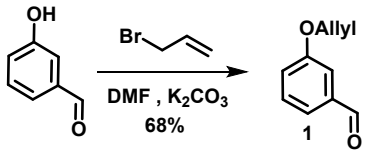



Compound 8
DMF , K2CO3
71%
(i) Pd(PPh3)4
DMBA, DCM
(ii) Methylene blue
O2, hv, DCM
64%
HBTU , DIPEA
DMF
69%
9
10
CLPT 1
CLPT 3

Compound 3
DMF , K2CO3
75%
(i) Pd(PPh3)4
DMBA, DCM
(ii) Methylene blue
O2, hv, DCM
61%
9
11
CLPT 2

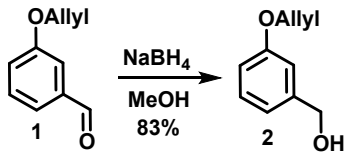
General synthetic procedures

Compound 1



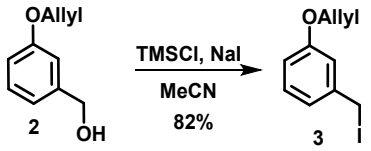
Allyl bromide (5.3 mL, 61.35 mmol) was added to a mixture of 3-hydroxybenzaldehyde (5.00 g, 40.90 mmol) and K_2CO_3 (11.29 g, 81.80 mmol) in DMF (70 mL). The reaction was left to stir for three days and H_2O (50 mL) was added. EtOAc (100 mL) was then added and the organic layer was washed with H_2O (3 x 50 mL) and brine (50 mL) before being dried ($MgSO_4$) and concentrated under reduced pressure to afford the crude product. The crude material was purified via silica gel column chromatography 5:95 (EtOAc/Hex) to afford the title compound **1** as a clear oil (4.50 g, 27.75 mmol, 68 %). ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.98 (s, 1 H), 7.48 - 7.44 (d, 2 H), 7.41 (m, 1 H), 7.21 (m, 1 H), 6.12 - 6.01 (m, 1 H), 5.45 (qd, *J* = 1.6, 17.2 Hz, 1 H), 5.33 (qd, *J* = 1.4, 10.5 Hz, 1 H), 4.61 (td, *J* = 1.5, 5.2 Hz, 2 H). Spectroscopic data was consistent with reported literature data.[1]

Compound 2



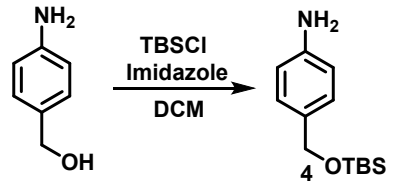
$NaBH_4$ (0.87 g, 23.12 mmol) was added to a solution of compound **1** (2.50 g, 15.41 mmol) in MeOH (10 mL) and the reaction was stirred at room temperature for 2 hours. Saturated ammonium chloride solution (50 mL) and DCM (50 mL) were added and the organic layer was washed with brine (50 mL), dried ($MgSO_4$) and concentrated under reduced pressure to afford the title compound **2**. No further purification was carried out. (2.10 g, 12.79 mmol, 83 %). ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.37 - 7.17 (m, 1 H), 6.98 - 6.93 (m, 2 H), 6.90 - 6.84 (m, 1 H), 6.13 - 6.02 (m, 1 H), 5.44 (m, 1 H), 5.31 (m, 1 H), 4.67 (s, 2 H), 4.59 - 4.53 (m, 2 H); ^{13}C NMR (126 MHz, $CDCl_3$) δ 158.9, 142.6, 133.3, 129.6, 119.3, 117.7, 114.0, 113.2, 68.8, 65.2; Mass could not be observed using ESI-MS.

Compound 3



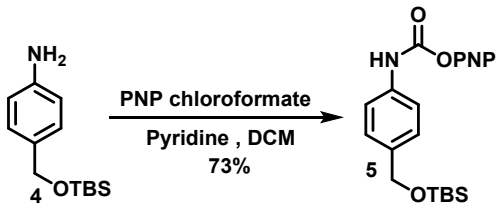
Compound **2** (200 mg, 1.22 mmol) was dissolved in 5 mL MeCN and cooled to 0°C. Then, sodium iodide (548 mg, 3.66 mmol) was added followed by the rapid addition of TMSCl (460 µL, 3.66 mmol). The reaction was allowed to warm up to room temperature and monitored by TLC (Hex:EtOAc, 90:10). When the reaction was deemed complete, the reaction mixture was diluted with EtOAc, and washed with saturated aqueous $Na_2S_2O_3$ followed by brine. The organic layer was separated, dried over Na_2SO_4, filtered and the solvent was evaporated under reduced pressure. The product was purified using column chromatography on silica gel (Hex:EtOAc 60:40) to afford compound **3** (280 mg, 82% yield) as a white solid.^{1}H NMR (400 MHz, $CDCl_3$) δ 7.26 - 7.18 (m, 1 H), 7.01 - 6.94 (m, 2 H), 6.83 (m, 1 H), 6.08 (m, 1 H), 5.45 (m, 1 H), 5.35 - 5.29 (m, 1 H), 4.55 (m, 2 H), 4.43 (s, 2 H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 158.7, 140.7, 133.2, 129.9, 121.3, 117.8, 115.2, 114.4, 68.8, 5.8; Mass could not be observed using ESI.

Compound 4



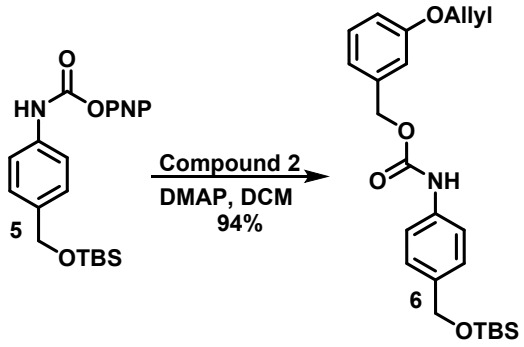
tert-Butyldimethylsilyl chloride (2.45 g, 16.23 mmol) was added portion-wise to a solution of 4-aminobenzyl alcohol (2.00 g, 16.23 mmol) and imidazole (1.10 g, 16.23 mmol) in DCM (100 mL). The reaction mixture was left to stir for 24 h before the addition of H_2O (50 mL). The organic phase was washed (3 x H_2O (50 mL)), dried ($MgSO_4$) and the volatiles were removed under reduced pressure to afford the title compound in quant. yield. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.17 - 7.06 (m, 2 H), 6.72 - 6.63 (m, 2 H), 4.63 (s, 2 H), 0.93 (s, 9 H), 0.09 (s, 6 H). Spectroscopic data was consistent with reported literature data.[2]

Compound 5



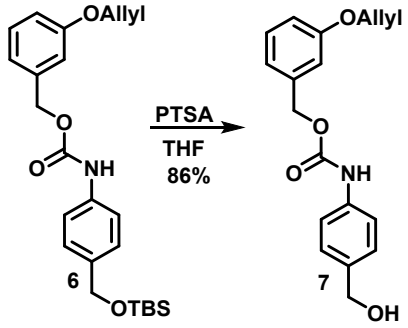
To a solution of **4** (1.22 g, 5.14 mmol) and pyridine (0.83 mL, 10.28 mmol) in DCM (50 mL) at 0 °C, 4-nitrophenylchloroformate (1.55 g, 7.71 mmol) was added portion-wise. The reaction mixture was allowed to warm to room temperature and stir for an additional 2 hours. The volatiles were removed under reduced pressure to afford a crude mixture. This crude material was purified *via* silica column chromatography 50:50 (DCM/Hex) to afford the title compound **5** as a white solid (1.50 g, 3.73 mmol, 73 %).^{1}H NMR (500 MHz, $CDCl_3$) δ 8.29 (d, *J* = 9.2 Hz, 2 H), 7.45 - 7.36 (m, 4 H), 7.33 (d, *J* = 8.2 Hz, 2 H), 7.06 (br. s., 1 H), 4.73 (s, 2 H), 0.95 (s, 10 H), 0.11 (s, 6 H); ^{13}C NMR (126 MHz ,$CDCl_3$) δ 155.4, 150.2, 145.1, 137.8, 135.4, 127.1, 126.2, 125.2, 122.2, 118.9, 115.6, 64.5, 26.0, 18.4, -5.2; MS (ES+): m/z calc. for $C_{20}H_{26}N_2O_5Si$: 402.2 found: 425.2 $[M+Na]^+$.

Compound 6



DMAP (0.681 g, 5.58 mmol) was added to a solution of **5** (1.50 g, 3.72 mmol) and **2** (0.61 g, 3.72 mmol) in DCM (2 mL). The reaction mixture was stirred at room temperature for 1 hour and the solvent was removed under reduced pressure. The crude material obtained in this way was purified *via* silica column chromatography 10:90 (EtOAc/Hex) to afford the title compound (**6**) as a clear oil (1.50 g, 3.51 mmol, 94 %). ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.26 (d, *J* = 7.6 Hz, 2 H), 7.22 - 7.15 (m, 4 H), 6.92 - 6.85 (m, 2 H), 6.80 (d, *J* = 8.2 Hz, 1 H), 6.58 (br. s., 1 H), 6.02 - 5.92 (m, 1 H), 5.35 (m, 1 H), 5.20 - 5.18 (m, 1 H), 5.08 (s, 2 H), 4.60 (s, 2 H), 4.46 (d, *J* = 5.5 Hz, 2 H), 0.84 (s, 9 H), 0.01 (s, 6 H); ^{13}C NMR (126 MHz, $CDCl_3$) δ 158.8, 153.3, 137.6, 136.7, 136.5, 133.2, 129.7, 126.9, 120.6, 118.6, 117.8, 114.6, 114.5, 68.8, 66.8, 64.6, 60.4, 53.4, 26.0, 18.4, -5.2; MS (ES+): m/z calc. for $C_{24}H_{33}NO_4Si$: 427.2 found: 450.2 $[M+Na]^+$.

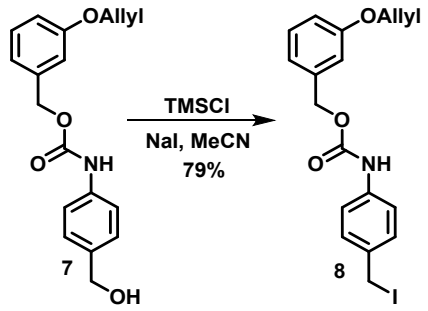
Compound 7



p-Toluenesulfonic acid monohydrate (0.68 g, 3.51 mmol) was added to a solution of **6** (1.50 g, 3.51 mmol) in THF (10 mL) and stirred at room temperature for 4 hours. Upon completion (as determined by TLC analysis), the reaction mixture was partitioned using EtOAc (50 mL) and H_2O (50 mL). The organic layer was washed with H_2O, brine, dried and concentrated under reduced pressure. The crude solid obtained in this way was triturated using Hex to afford the title compound **7** as a white solid (0 .95 g, 3.03 mmol, 86 %). ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.39 (d, *J* = 7.9 Hz, 2 H), 7.35 - 7.28 (m, 3 H), 7.03 - 6.95 (m, 2 H), 6.91

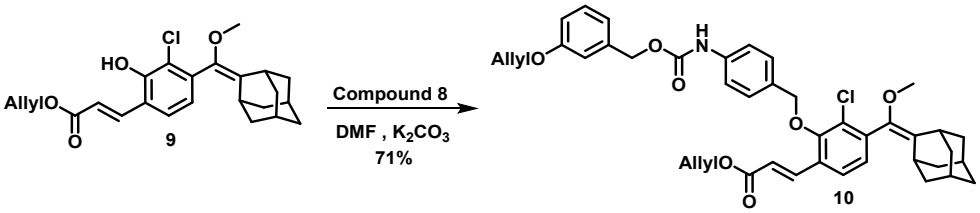
(m, 1 H), 6.72 (br. s., 1 H), 6.13 - 6.04 (m, 1 H), 5.43 (dd, *J* = 1.5, 17.4 Hz, 1 H), 5.30 (dd, *J* = 1.2, 10.4 Hz, 1 H), 5.18 (s, 2 H), 4.65 (s, 2 H), 4.56 (d, *J* = 5.2 Hz, 2 H); ^{13}C NMR (126 MHz, $CDCl_3$) δ 158.82, 153.26, 137.53, 137.27, 136.08, 133.14, 129.71, 127.99, 120.62, 118.74, 117.80, 114.64, 114.59, 77.24, 68.84, 66.91, 64.97; MS (ES+): m/z calc. for $C_{18}H_{19}NO_4$: 313.1 found: 336.1 $[M+Na]^+$.

Compound 8



Compound **7** (200 mg, 0.64 mmol) was dissolved in 5 mL MeCN and cooled to 0 ºC. Then, sodium iodide (288 mg, 1.92 mmol) was added followed by the rapid addition of TMSCl (242 µL, 1.92 mmol). The reaction was allowed to warm up to room temperature and monitored by TLC (Hex:EtOAc, 70:30). When the reaction was deemed complete, the reaction mixture was diluted with EtOAc, and washed with saturated $Na_2S_2O_3$ followed by brine. The organic layer was separated, dried over Na_2SO_4, filtered and the volatiles were evaporated under reduced pressure. The product was purified using column chromatography on silica gel (Hex:EtOAc 70:30) to afford compound **8** (213 mg, 79% yield) as a white solid.^{1}H NMR (400 MHz, $CDCl_3$) δ 7.27 (m, 4 H), 6.99 - 6.92 (m, 2 H), 6.92 - 6.83 (m, 2 H), 6.12 - 5.99 (m, 1 H), 5.46 - 5.39 (m, 1 H), 5.29 (m, 1 H), 5.16 (s, 2 H), 4.58 - 4.51 (m, 2 H), 4.44 (s, 2 H); ^{13}C NMR (126 MHz, $CDCl_3$) δ 158.81, 153.20, 137.46, 134.30, 133.14, 133.03, 129.72, 129.60, 129.48, 120.61, 120.50, 118.92, 117.80, 117.69, 114.65, 114.60, 114.54, 68.83, 68.72, 66.97, 66.86, 5.80. ; MS (ES+): m/z calc. for $C_{18}H_{18}INO_3$: 423.0 found: 424.1 $[M+H]^+$.

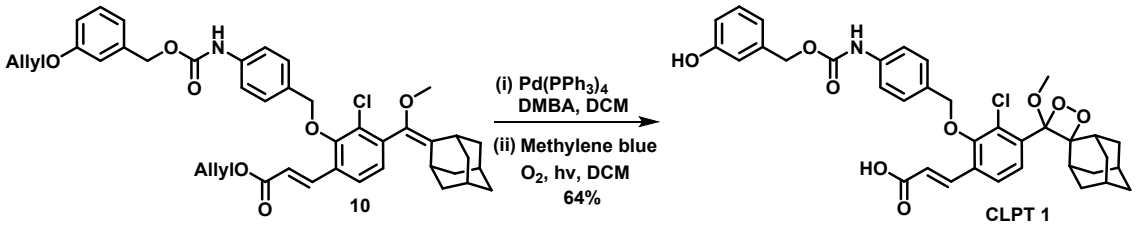
Compound 10



Compound **8** (51 mg, 0.12 mmol) was added to a solution of compound **9**[3] (50 mg, 0.12 mmol) and K_2CO_3 (25 mg, 0.18 mmol) in DMF (1 mL). The reaction was stirred at room temperature for 1 hour and monitored by TLC (Hex:EtOAc, 70:30). When the reaction was deemed complete, the mixture was diluted with EtOAc (100 mL) and washed with brine (50 mL). The organic layer was separated, dried over Na_2SO_4,

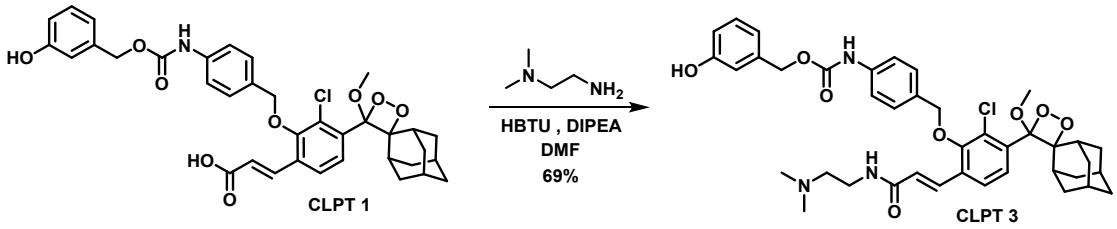
and evaporated under reduced pressure. The crude product obtained in this way was purified by column chromatography over silica gel (Hex:EtOAc, 70:30) affording compound **10** as a pale yellow solid (70 mg, 71% yield).^{1}H NMR (400 MHz, $CDCl_3$) δ 7.93 (m, 1 H), 7.48 - 7.32 (m, 4 H), 7.25 (m, 1 H), 7.07 (m, 2 H), 6.95 (m, 1 H), 6.87 (m, 1 H), 6.44 (m, 1 H), 6.13 - 5.93 (m, 1 H), 5.38 (m, 2 H), 5.27 (m, 1 H), 5.16 (s, 2 H), 4.96 (s., 2 H), 4.70 (s, 2 H), 4.53 (s, 2 H), 3.32 (s, 3 H), 3.29 (br. s., 1 H), 2.08-1.75 (m, 14 H); ^{13}C NMR (101 MHz ,$CDCl_3$-d) δ 166.3, 158.8, 153.7, 153.2, 139.5, 139.1, 138.3, 138.2, 137.6, 133.2, 132.4, 132.2, 130.8, 130.0, 129.7, 127.8, 125.1, 120.6, 119.9, 118.6, 118.3, 117.7, 114.5, 75.8, 68.8, 66.8, 65.3, 57.2, 39.2, 39.1, 38.6, 37.1, 33.0, 29.7, 28.4, 28.2; MS (ES+): m/z calc. for $C_{42}H_{44}ClNO_7$: 709.3 found: 710.2 $[M+H]^+$.

CLPT 1



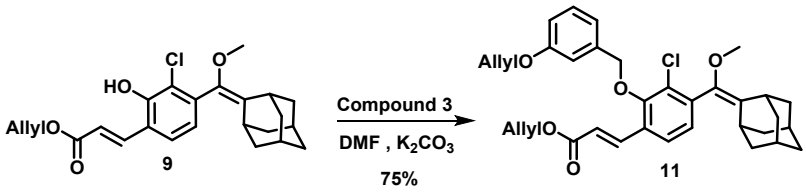
Compound **10** (20 mg, 0.028 mmol) was dissolved in DCM (2 mL), followed by the addition of DMBA (17 mg, 0.11 mmol) and $Pd(PPh_3)_4$ (3 mg, 0.003 mmol). The reaction was stirred at room temperature and monitored by RP-HPLC. Upon complete removal of allyl groups, DCM (10 mL) and a catalytic amount of methylene blue were added. Then, oxygen was bubbled through the solution while irradiating with yellow light. The reaction was monitored by RP-HPLC. When the reaction was deemed complete, the solvent was concentrated under reduced pressure and the product was purified by preparative HPLC to give **CLPT 1** as a white solid (12 mg, 64% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.91 (d, *J* = 8.4 Hz, 1 H), 7.84 (d, *J* = 16.1 Hz, 1 H), 7.52 (d, *J* = 8.4 Hz, 1 H), 7.36 (d, *J* = 7.9 Hz, 2 H), 7.29 (d, *J* = 8.5 Hz, 2 H), 7.17 (t, *J* = 8.0 Hz, 1 H), 6.89 - 6.83 (m, 2 H), 6.80 (m, 1 H), 6.35 (d, *J* = 16.1 Hz, 1 H), 5.10 (br. s., 2 H), 4.96 - 4.86 (m, 2 H), 3.24 (s, 3 H), 3.04 (br. s., 1 H), 2.34 (d, *J* = 11.8 Hz, 1 H), 1.90 - 1.35 (m, 12 H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 170.9, 156.1, 154.1, 140.4, 138.3, 137.5, 135.6, 131.6, 130.5, 130.3, 129.9, 129.0, 127.8, 125.3, 120.0, 115.5, 114.8, 111.8, 96.5, 76.2, 66.9, 49.8, 36.6, 33.9, 33.6, 32.6, 32.2, 31.6, 31.5, 26.2, 25.8; MS (ES+): m/z calc. for $C_{36}H_{36}ClNO_9$: 661.2 found: 662.2 $[M+H]^+$.

CLPT 3



A solution of **CPLT 1** (10 mg, 0.015 mmol) and 1,1-dimethylethylenediamine (6 µL, 0.05 mmol) in DMF (1mL) was added to a solution of HBTU (9 mg, 0.022 mmol) and DIPEA (5 µL, 0.03 mmol) in DMF (1 mL). The reaction was stirred at room temperature for 1 hour and monitored by RP-HPLC. Once deemed complete, the product was purified by preparative HPLC to give **CLPT 3** as a white solid (8 mg, 69% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.84 (s, 1H), 9.46 (s, 1H), 8.51 (t, J = 5.7 Hz, 1H), 7.82 (d, J = 8.4 Hz, 1H), 7.73 (d, J = 8.5 Hz, 1H), 7.69 (d, J = 15.9 Hz, 1H), 7.47 (d, J = 8.5 Hz, 2H), 7.35 (d, J = 8.5 Hz, 2H), 7.15 (t, J = 7.8 Hz, 1H), 6.80 (m, 2H), 6.71 (m, 2H), 5.05 (s, 2H), 4.83 (dd, J = 19.6, 10.6 Hz, 2H), 3.52 (t, J = 6.2 Hz, 2H), 3.20 (m, 2H), 3.10 (s, 3H), 2.86 (s, 1H), 2.82 (s, 6H), 2.48 (dd, J = 3.5, 1.7 Hz, 5H), 2.21 (d, J = 12.1 Hz, 1H), 1.89 (d, J = 4.9 Hz, 1H), 1.76 – 1.40 (m, 12H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 165.9, 158.6, 158.3, 158.1, 157.2, 153.9, 140.1, 138.5, 134.1, 133.1, 132.4, 130.2, 130.1, 129.2, 127.5, 125.9, 125.7, 119.1, 118.5, 115.5, 115.4, 111.8, 96.0, 76.0, 66.3, 56.5, 50.0, 48.1, 43.2, 36.5, 34.9, 33.9, 33.6, 32.4, 32.3, 31.7, 31.5, 26.1, 25.9, 25.8, 25.1; MS (ES+): m/z calc. for $C_{40}H_{46}ClN_3O_8$: 731.3 found: 731.4 $[M+H]^+$.

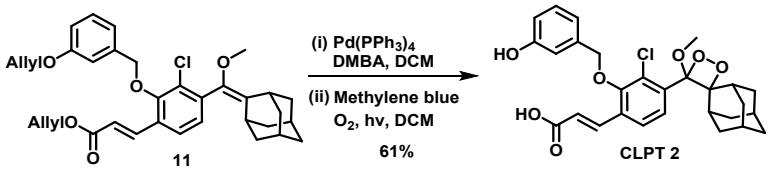
Compound 11



Compound **3** (33 mg, 0.12 mmol) was added to a solution of compound **9**[3] (50 mg, 0.12 mmol) and K_2CO_3 (25 mg, 0.18 mmol) in DMF (1 mL). The reaction was stirred at room temperature for 1 hour and monitored by TLC (Hex:EtOAc, 70:30). When the reaction was deemed complete, the mixture was diluted with EtOAc (100 mL) and washed with brine (50 mL). The organic layer was separated, dried over Na_2SO_4, and evaporated under reduced pressure. The resulting crude product was purified by column chromatography over silica gel (Hex:EtOAc, 70:30) affording compound **11** as a pale yellow solid (49 mg, 75% yield).^{1}H NMR (400 MHz, $CDCl_3$) δ 7.98 (d, *J* = 16.2 Hz, 1 H), 7.45 (d, *J* = 8.0 Hz, 1 H), 7.28 (t, *J* = 7.8 Hz, 1 H), 7.12 - 7.03 (m, 3 H), 6.94 - 6.88 (m, 1 H), 6.48 (d, *J* = 16.2 Hz, 1 H), 6.13 - 5.93 (m, 2 H), 5.46 - 5.24 (m, 4 H), 5.00 (d, *J* = 4.1 Hz, 2 H), 4.71 (m, 2 H), 4.56 (m, 2 H), 3.34 (s, 3 H), 3.30 (br. s., 1 H), 2.10 - 1.70 (m, 14 H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 166.2, 158.8, 153.7, 139.5, 139.0, 138.2, 137.4, 133.3, 132.4, 132.2, 129.8,

129.7, 129.6, 127.8, 125.1, 121.2, 120.0, 118.2, 117.6, 115.2, 114.8, 76.0, 68.8, 65.2, 57.2, 39.2, 39.1, 38.6, 37.1, 33.0, 29.7, 28.4, 28.2; MS (ES+): m/z calc. for $C_{34}H_{37}ClO_5$: 560.2 found: 583.4 $[M+Na]^+$.

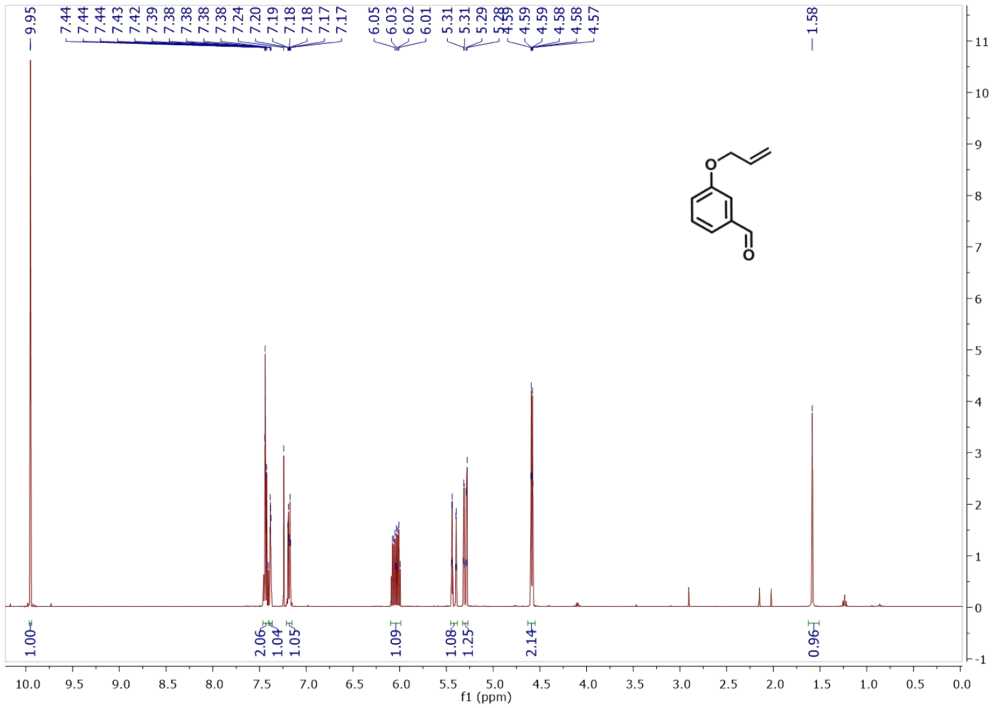
CLPT 2



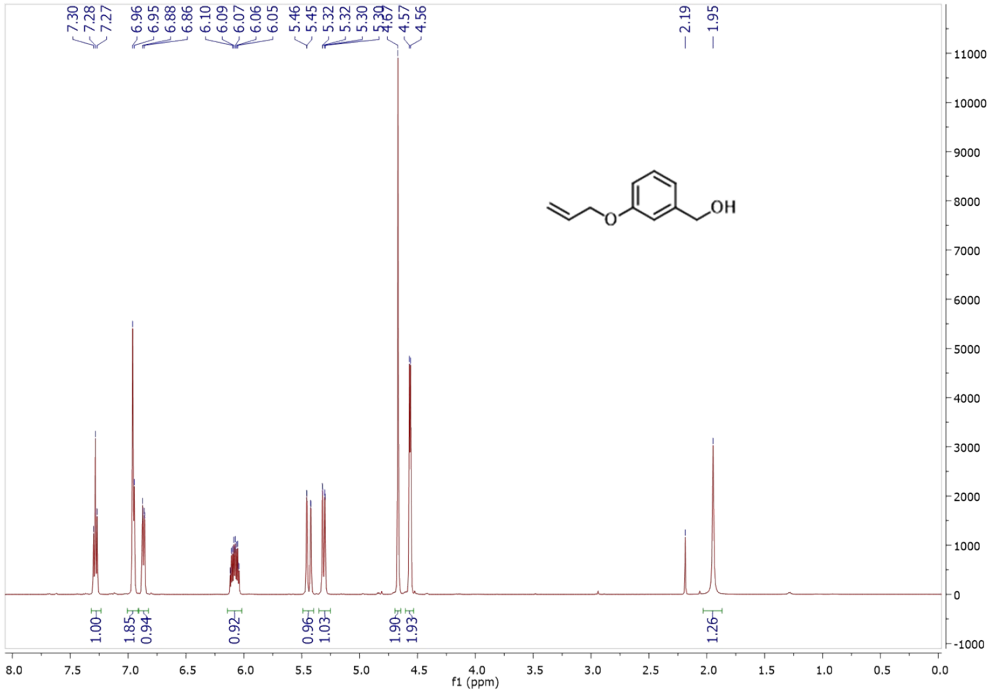
Compound **11** (20 mg, 0.035 mmol) was dissolved in DCM (2 mL), followed by the addition of DMBA (23 mg, 0.14 mmol) and $Pd(PPh_3)_4$ (4 mg, 0.004 mmol). The reaction was stirred at room temperature and monitored by RP-HPLC. Upon complete removal of the allyl groups (as inferred from TLC analysis), DCM (10 mL) and a catalytic amount of methylene blue were added. Then, oxygen was bubbled through the solution while irradiating with yellow light. The reaction was monitored by RP-HPLC. Once the reaction was deemed complete, the reaction mixture was concentrated under reduced pressure. The crude product obtained in this way was purified by preparative HPLC to give **CLPT 2** as a white solid (11 mg, 61% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.99 (d, *J* = 16.2 Hz, 1 H), 7.93 (d, *J* = 8.4 Hz, 1 H), 7.58 (d, *J* = 8.4 Hz, 1 H), 7.21 (t, *J* = 7.8 Hz, 1 H), 7.10 (s, 1 H), 6.89 (m, 1 H), 6.84 (d, *J* = 7.5 Hz, 1 H), 6.43 (d, *J* = 16.1 Hz, 1 H), 4.91 (s, 2 H), 3.25 (s, 3 H), 3.05 (br. s., 1 H), 2.34 (d, *J* = 11.7 Hz, 1 H), 1.92 - 1.68 (m, 6 H), 1.63 (br. s., 3 H), 1.49 (d, *J* = 12.0 Hz, 1 H), 1.36 (br. s., 3 H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 171.4, 156.1, 154.2, 140.8, 136.9, 135.8, 131.4, 130.0, 129.0, 127.8, 125.2, 121.5, 119.9, 116.4, 116.2, 111.8, 96.5, 76.4, 49.8, 36.6, 33.9, 33.6, 32.6, 32.2, 31.6, 31.5, 26.2, 25.8; MS (ES+): m/z calc. for $C_{28}H_{29}ClO_7$: 512.2 found: 535.3 $[M+Na]^+$.

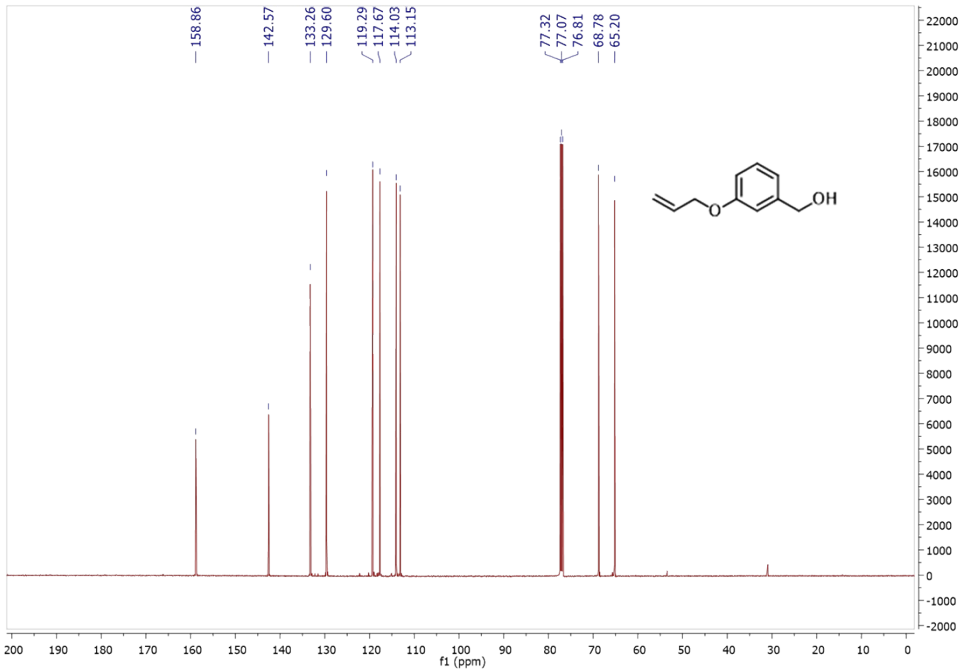
NMR Spectra (all recorded in $CDCl_3$)

Compound 1



Compound 2

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114.03
113.15
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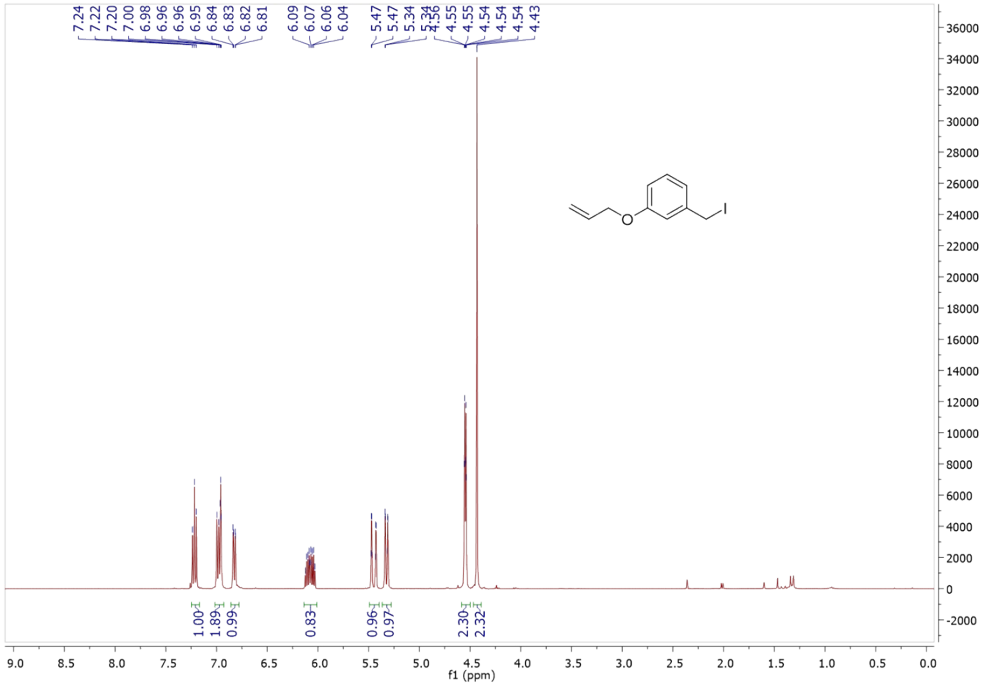
77.07
76.81
68.78
65.20
OH
f1 (ppm)

Compound 3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6.95
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6.83
6.82
6.81
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6.07
6.06
6.04
5.47
5.47
5.34
4.56
4.55
4.55
4.54
4.54
4.54
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0.96
0.97
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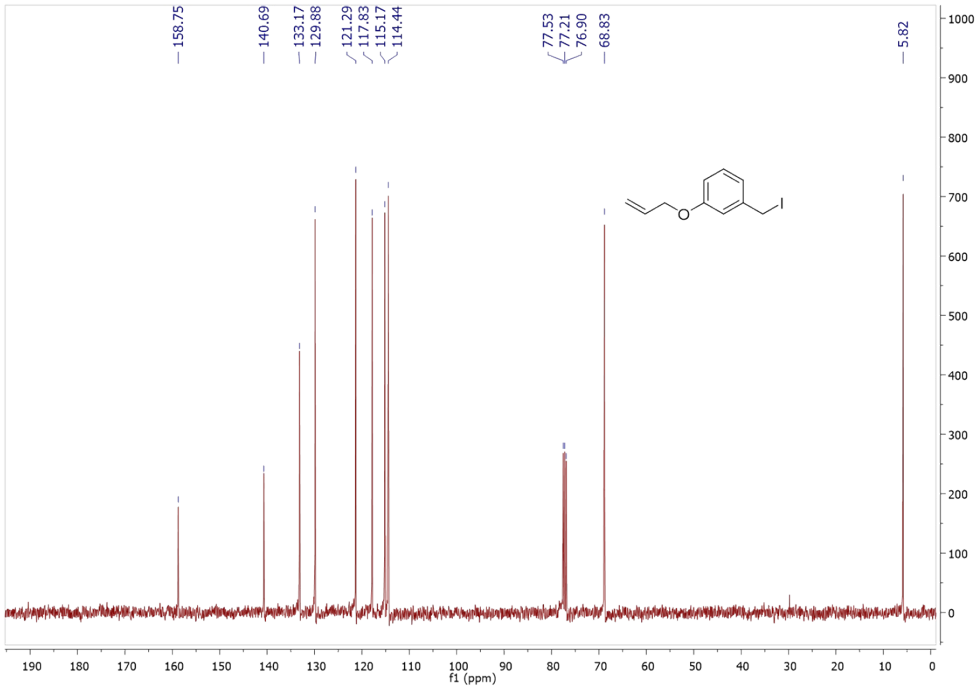
2.32
I
f1 (ppm)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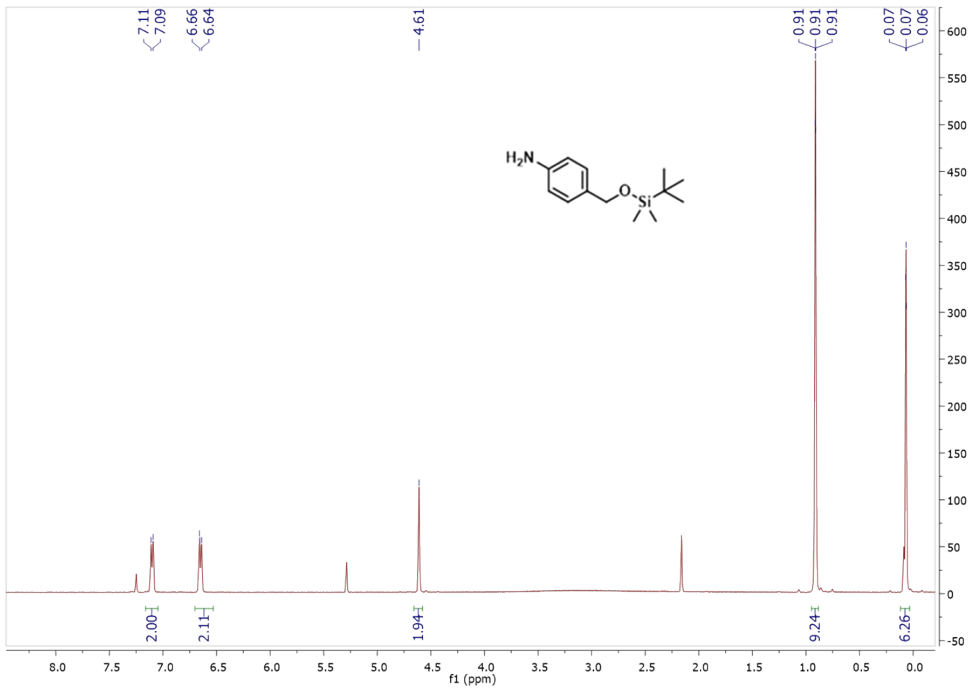
5.82
O
I
f1 (ppm)

Compound 4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0.91
0.07
0.07
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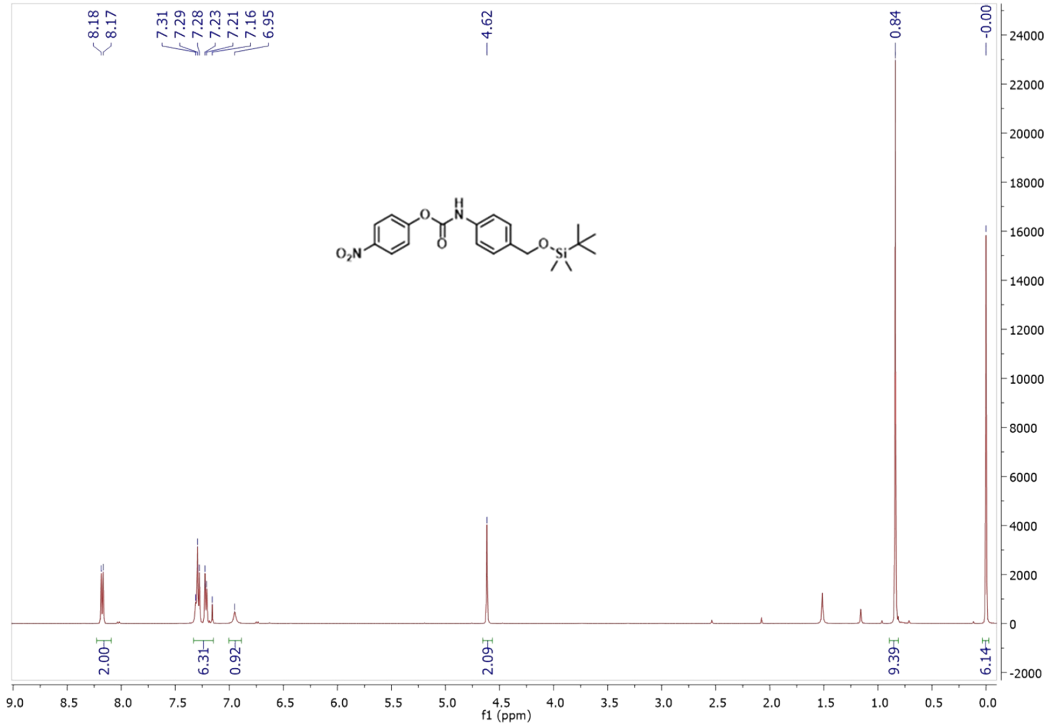
O
Si
2.00
2.11
1.94
9.24
6.26
f1 (ppm)

Compound 5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7.31
7.29
7.28
7.23
7.21
7.16
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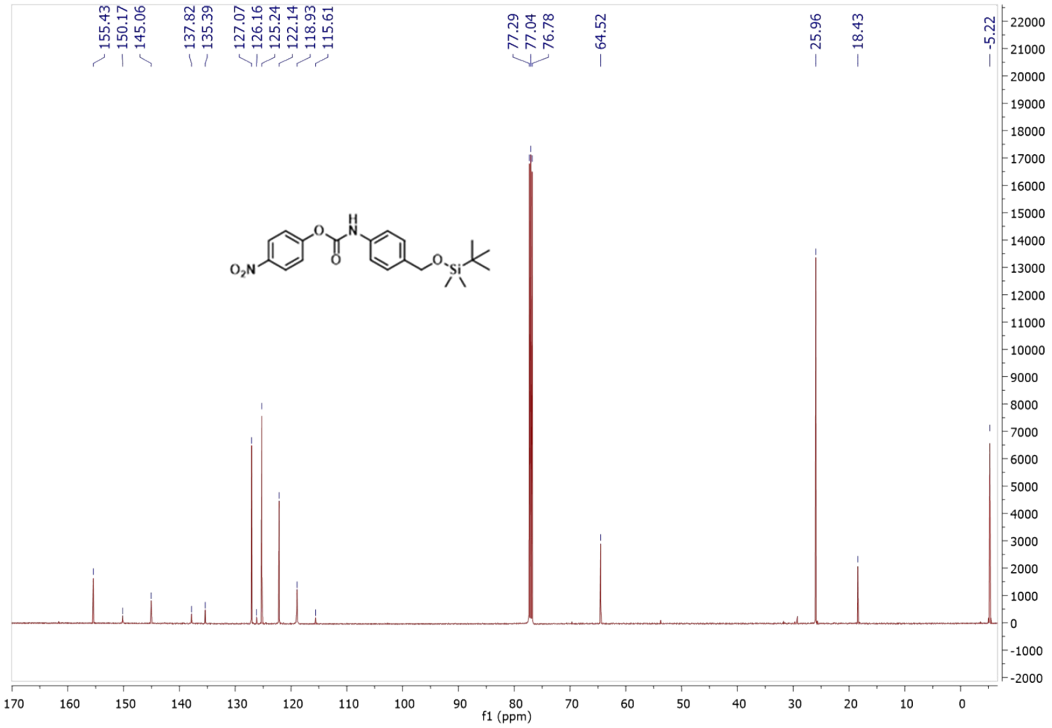
-0.00
2.00
6.31
0.92
2.09
9.39
6.14
f1 (ppm)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127.07
126.16
125.24
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77.04
76.78
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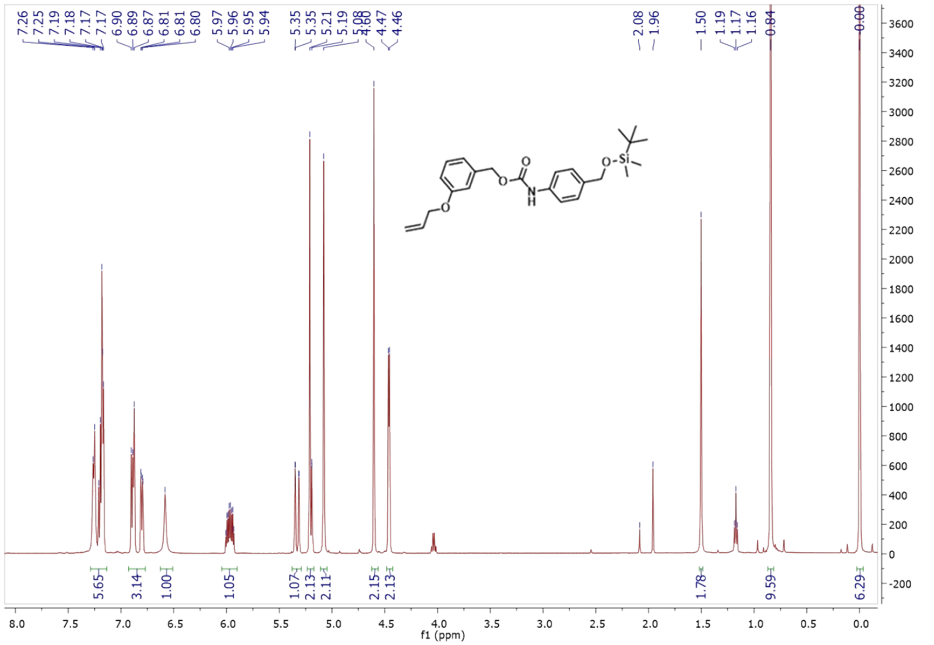
25.96
18.43
-5.22
f1 (ppm)

Compound 6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5.19
5.08
4.60
4.47
4.46
2.08
1.96
1.50
1.19
1.17
1.16
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5.65
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1.07
2.13
2.11
2.15
2.13
1.78
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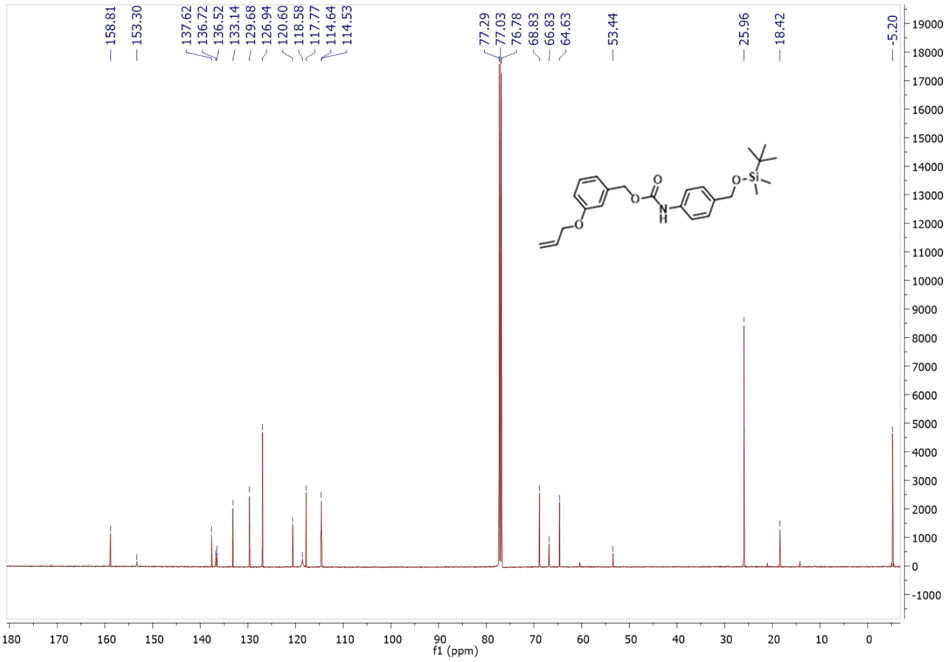
6.29
f1 (ppm)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118.58
117.77
114.64
114.53
77.29
77.03
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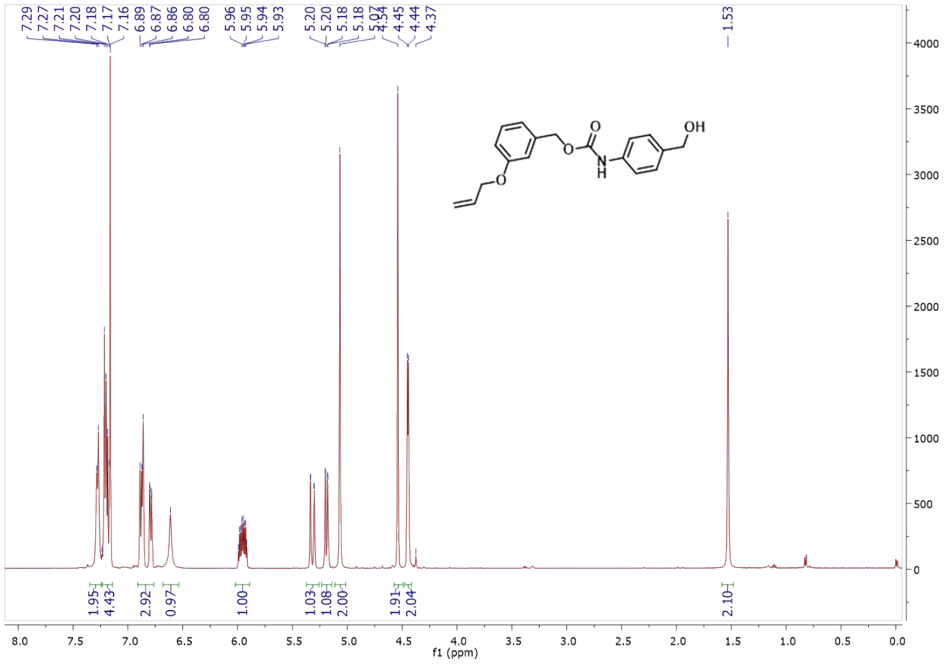
68.83
66.83
64.63
53.44
25.96
18.42
-5.20
f1 (ppm)

Compound 7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6.86
6.80
6.80
5.96
5.95
5.94
5.93
5.20
5.20
5.18
5.18
5.04
4.45
4.44
4.37
1.53
OH
O
N
H
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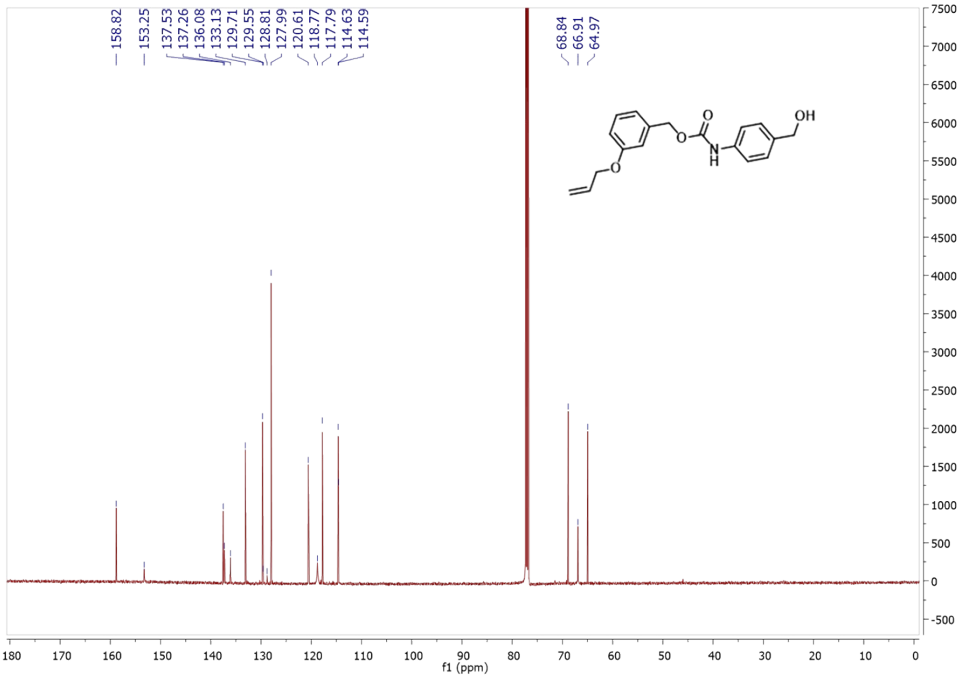
2.10
f1 (ppm)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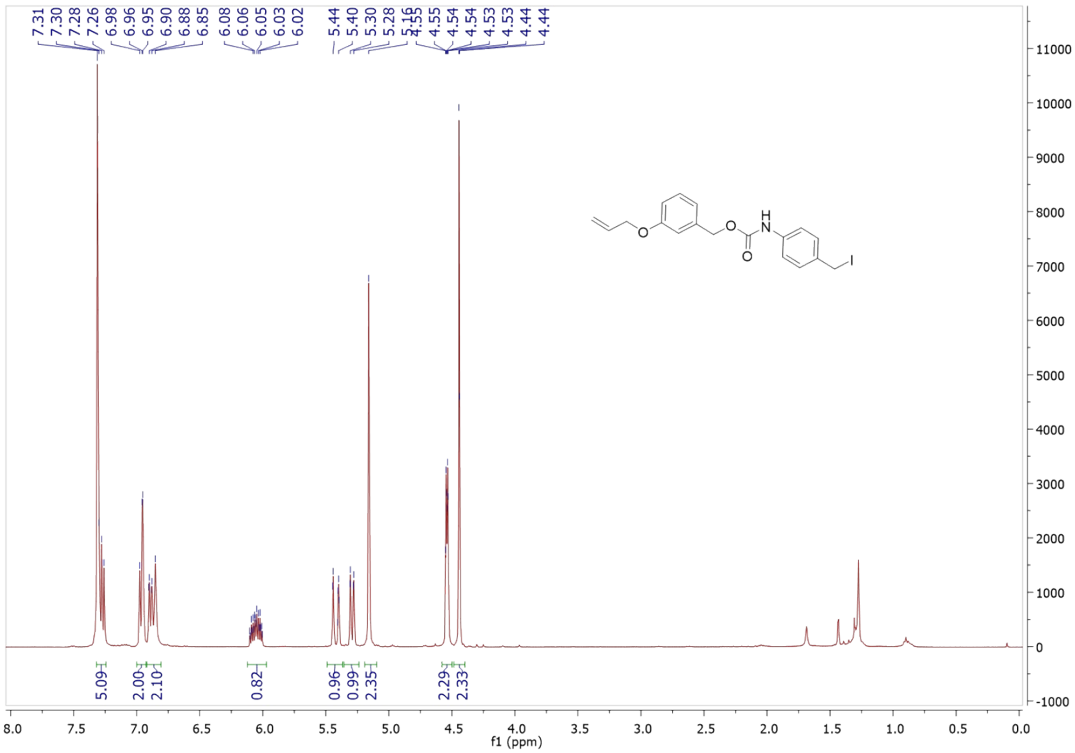
OH
O
N
H
f1 (ppm)

Compound 8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5.16
4.55
4.54
4.54
4.53
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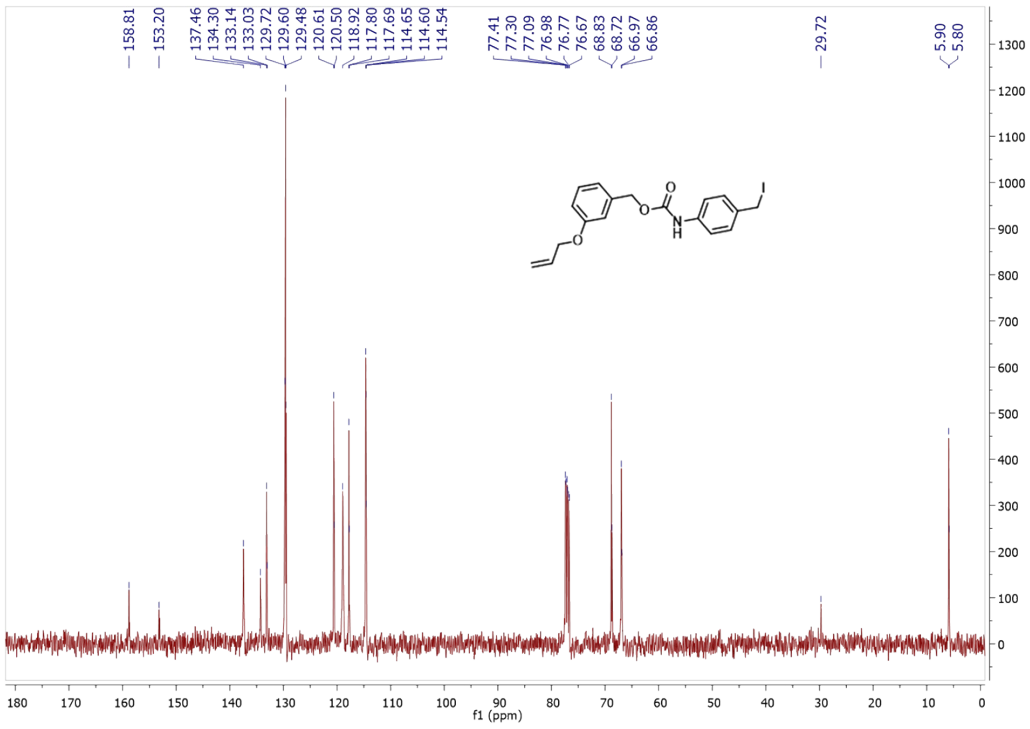
2.33
f1 (ppm)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114.54
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77.09
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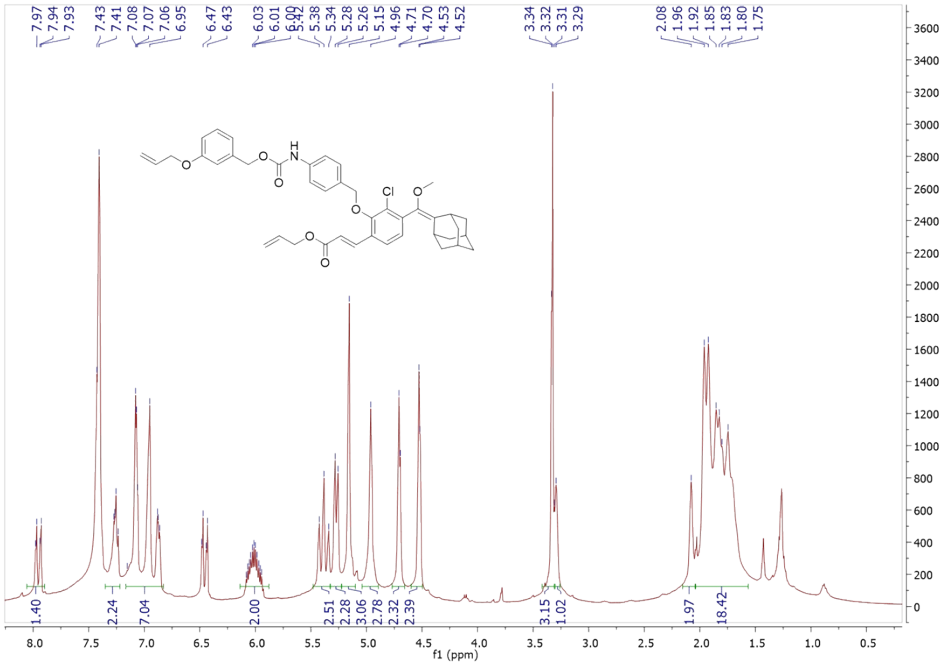
f1 (ppm)

Compound 10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5.38
5.34
5.28
5.26
5.15
4.96
4.71
4.70
4.53
4.52
3.34
3.32
3.31
3.29
2.08
1.96
1.92
1.85
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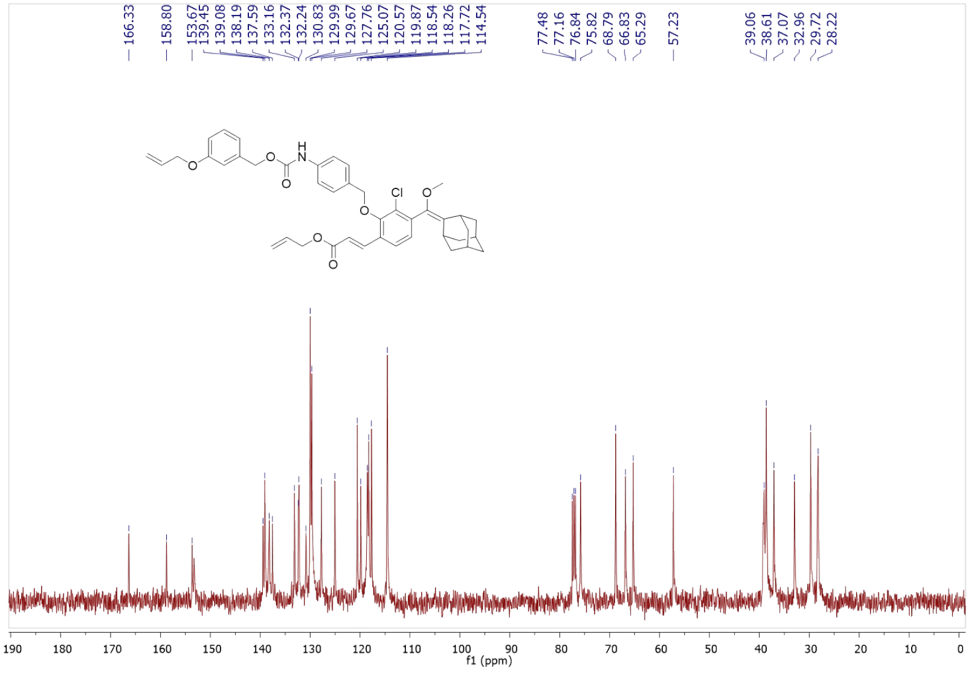
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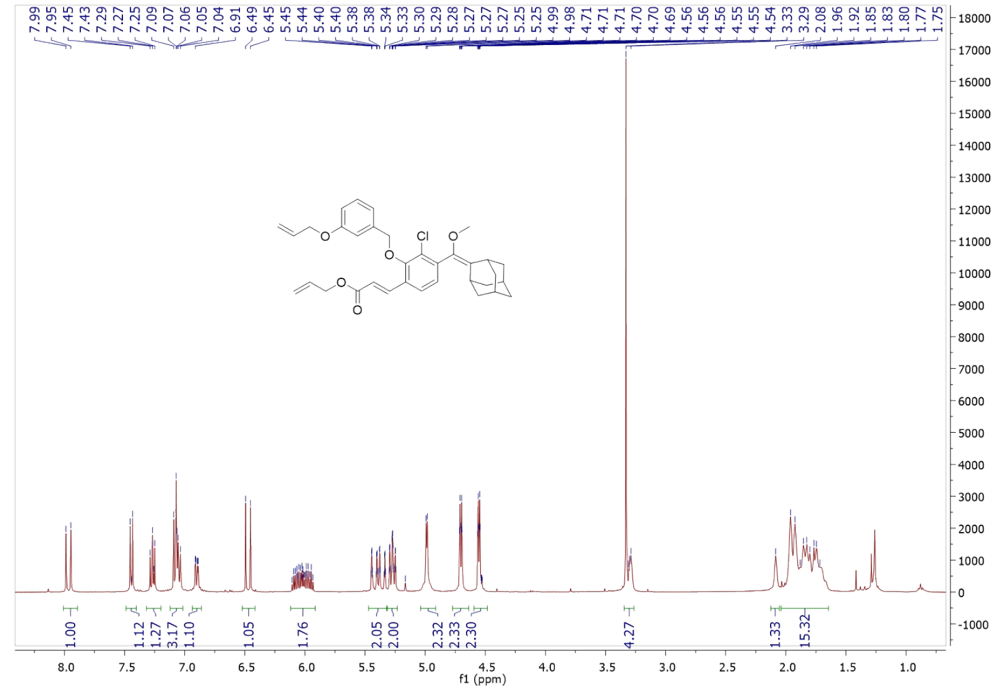
Cl
f1 (ppm)

Compound 11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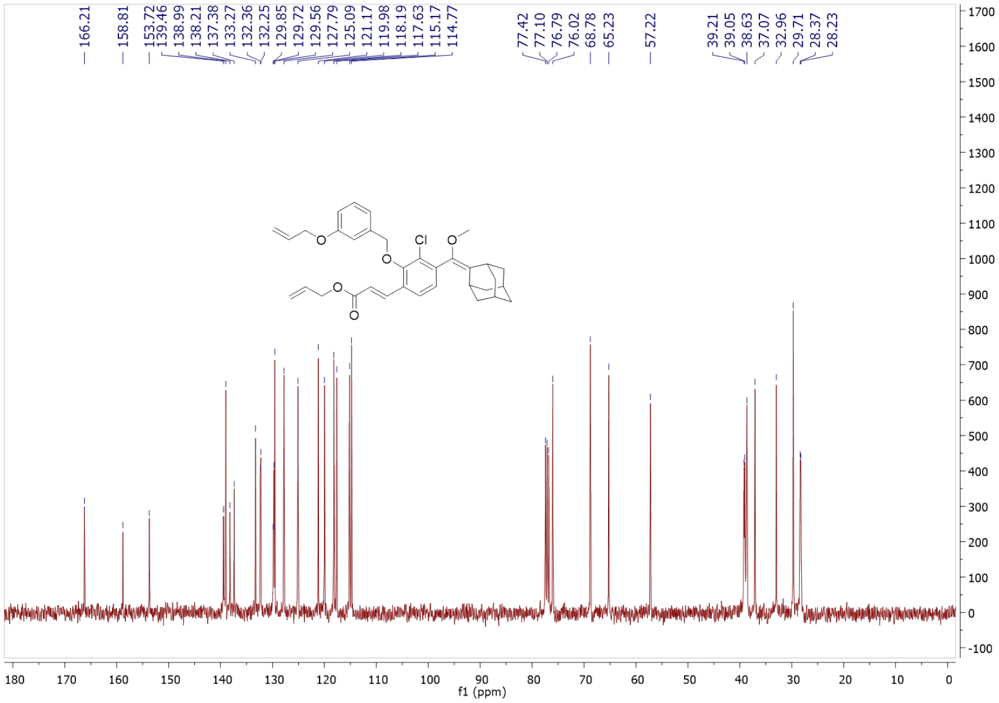
f1 (ppm)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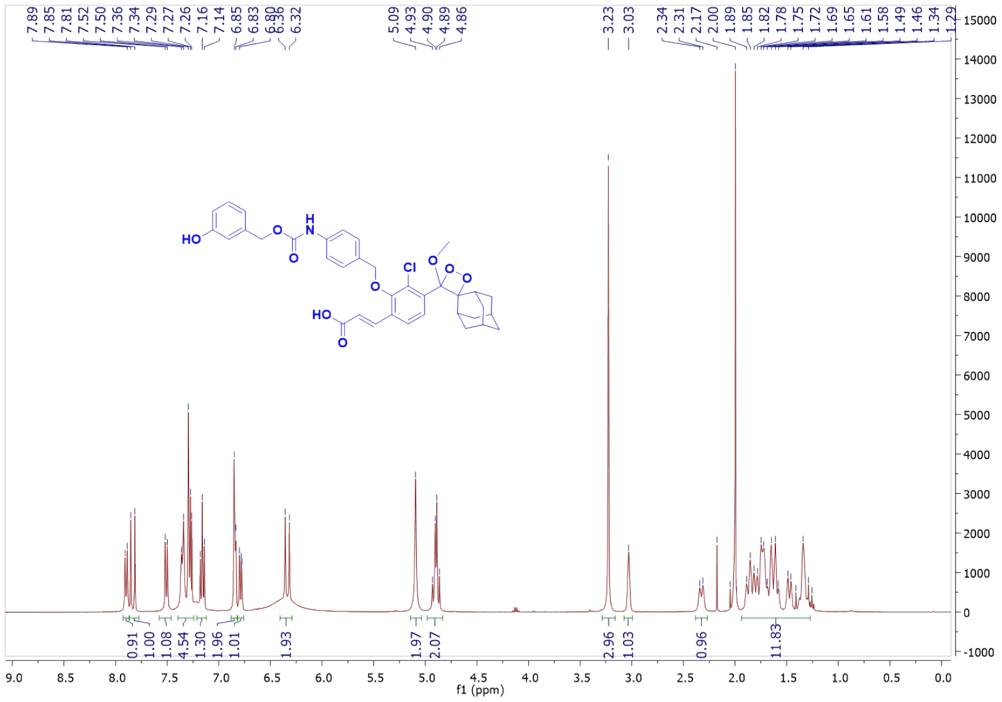
f1 (ppm)

CLPT 1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1.03
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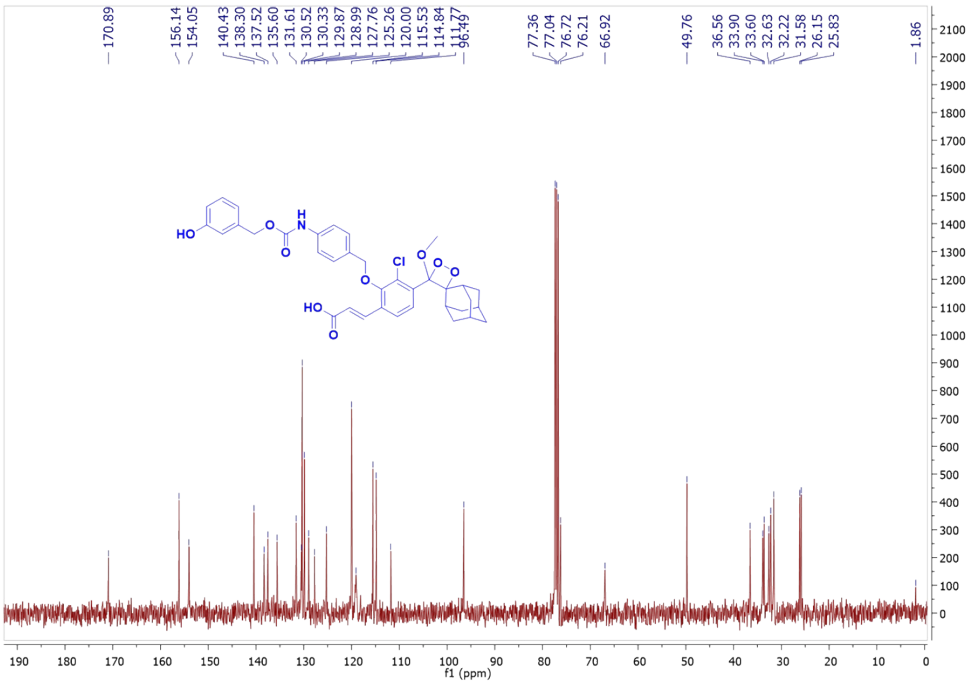
f1 (ppm)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25.83
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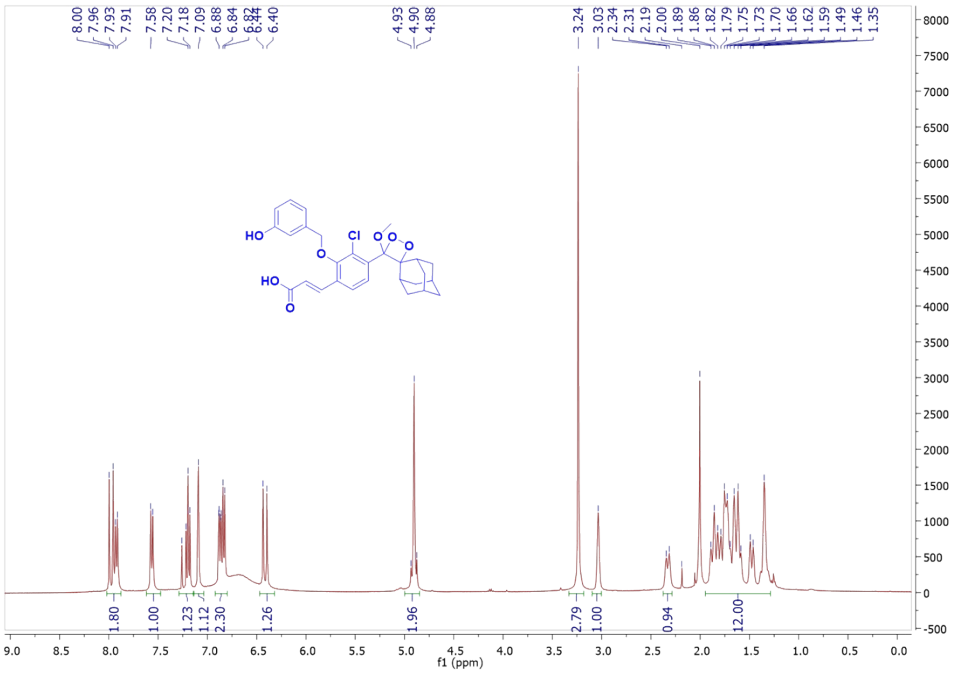
f1 (ppm)

CLPT 2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Cl
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HO
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1.12
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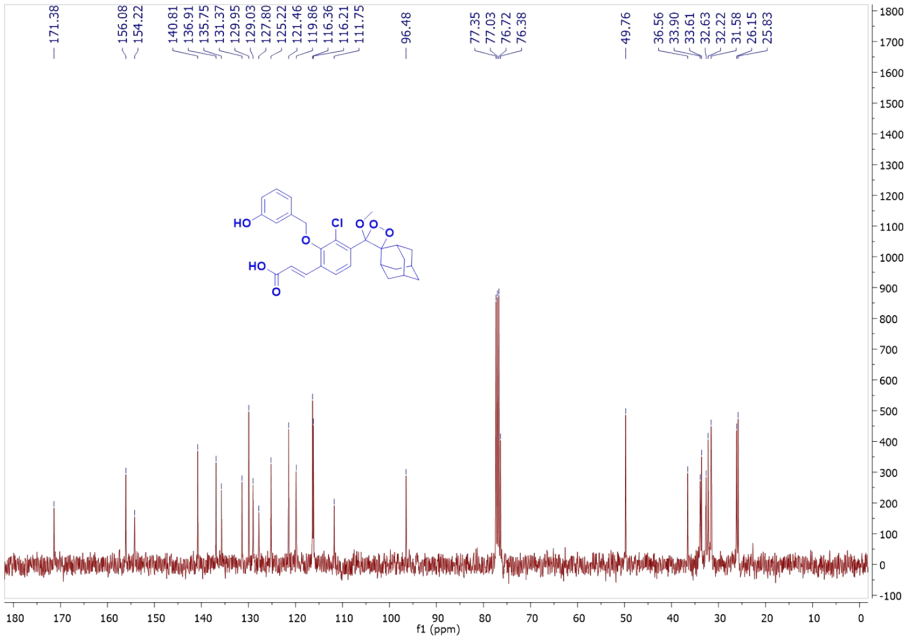
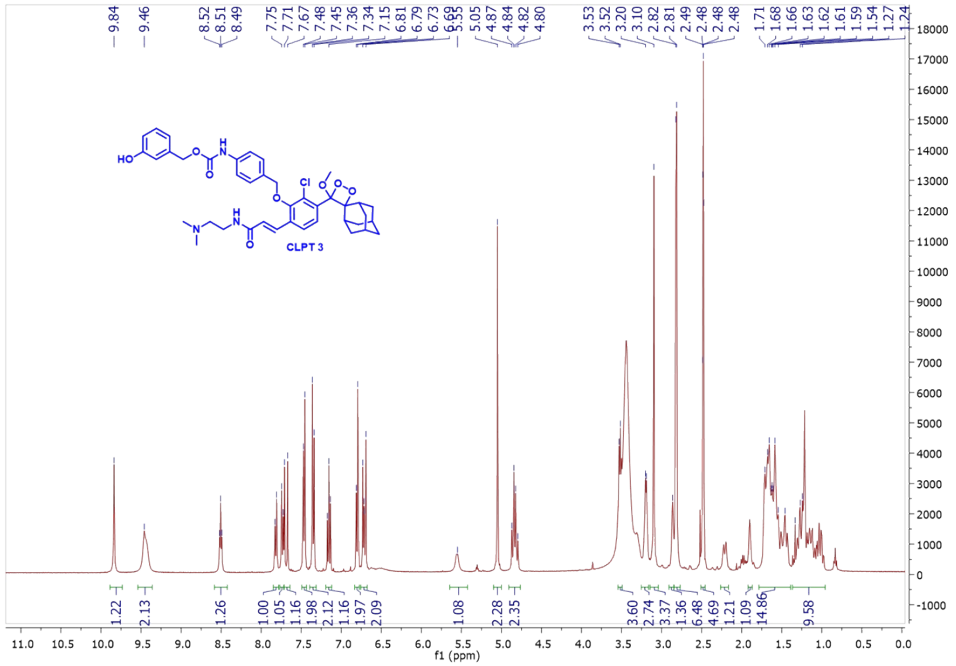
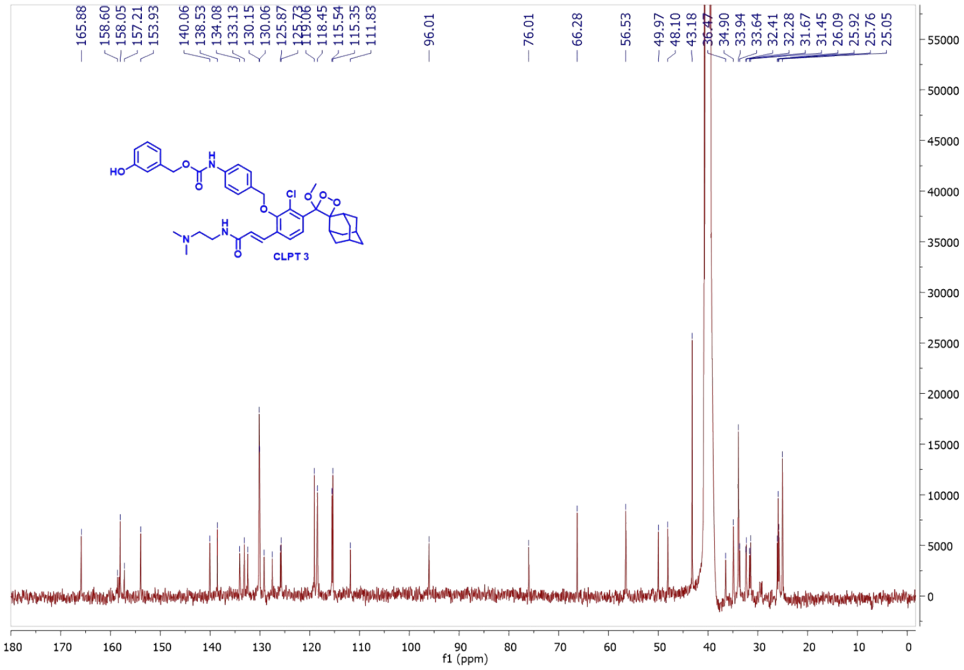
1.26
1.96
2.79
1.00
0.94
12.00
f1 (ppm)


171.38
156.08
154.22
140.81
136.91
135.75
131.37
129.95
129.03
127.80
125.22
121.46
119.86
116.36
116.21
111.75
96.48
77.35
77.03
76.72
76.38
49.76
36.56
33.90
33.61
32.63
32.22
31.58
26.15
25.83
HO
Cl
O
O
O
HO
O
f1 (ppm)

CLPT 3





In vitro Experiments

Cell culture

B16-F10, EMT6 and MC38 cells were purchased from the American Type Culture Collection (ATCC Manassas, VA, USA). Cell lines were cultured in Dulbecco's Modified Eagle Medium DMEM growth media supplemented with 10% FBS, 100 µg/mL streptomycin, 100 units/mL penicillin, 12.5 units/mL nystatin and 2 mM L-glutamine. Cells were grown at 37°C; 5% CO_2.

Chemiluminescent probe activation *in vitro*

For testing the activity of probe **CLTP 3**, B16-F10 melanoma, EMT6 breast cancer, and MC38 colon-rectal cancer cells were seeded in 96 well clear bottom plates at a concentration of 80,000 cells/well. Twenty-four hours after seeding, the cell media were removed, the monolayer of cells was washed twice with PBS, and treated with probe **CLPT 3** [10 µM, 0.1% DMSO]. The chemiluminescence signal was recorded using a Molecular Devices Spectramax i3x as noted above.

Tumor cell isolation

In order to evaluate the specificity and sensitivity of probe **CLTP 3** in melanin expressing tumor cells, B16-F10 cells were isolated from tumors arising in s.c.-injected 8 week-old male C57BL/6 mice (Envigo CRS, Israel). MC-38 cells were isolated from s.c. tumors and used as a negative control, melanin non-expressing cells. Briefly, mice were anesthetized with ketamine (100 mg/kg) and xylazine (12 mg/kg), s.c. tumors were resected, chopped and incubated in rotation with Collagenase IV\Dispase solution (Wothington Biochemical, USA) for 3 hours at 37°C. Cells were passed using a 70 µm strainer and red blood-cells lysis (BioLegend, USA) was carried out. Isolated cells containing mainly tumor cells were plated, and grown in DMEM growing media.

Cell viability assay

In order to evaluate **CLPT 3** probe biocompatibility, murine melanoma cells (B16-F10 and RET – $8x10^4$ cell/ml) were seeded in 24 well plate and immediately treated with 10 mM CLPT 3. Four to 24 h following treatment, adherent cells were detached with trypsin and counted using cell coulter counter (Z1 Coulter® Particle Counter, Beckman Coulter™, Brea, California, USA).

Supplementary References

1. Garcia-Lacuna, G. Dominguez, J. Blanco-Urgoiti and J. Perez-Castells, *Organic & Biomolecular Chemistry*, **2019**, 17, 9489-9501.

2. U. P. Dahal, C. Joswig-Jones and J. P. Jones, *Journal of Medicinal Chemistry*, **2012**, 55, 280-290.

3. N. Hananya, J. P. Reid, O. Green, M. S. Sigman and D. Shabat, *Chemical Science*, **2019**, 10, 1380-1385.