SUPPORTING INFORMATION

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General Experimental Procedures

Synthesis. All reactions were carried out under an argon atmosphere. Reagents were purchased at a high commercial quality (typically 97 % or higher) and used without further purification, unless otherwise stated. ^{1}H NMR and ^{13}C NMR spectra were recorded on Bruker spectrometers (at 400 or 500 MHz or at 100 or 125 MHz) and are reported relative to deuterated solvent signals ($CDCl_3$: ^{1}H NMR = 7.24, ^{13}C NMR = 77.0, MeOD: ^{1}H NMR = 3.30, ^{13}C NMR = 49.0, DMSO-d_6: ^{1}H NMR = 2.50, ^{13}C NMR = 39.5). The following abbreviations were used to explain the multiplicities: s = singlet, d = doublet, t = triplet, q = quartet, quint = quintet, dd = double doublet, dt = double triplet, dq = double quartet, m = multiplet, br = broad. Electrospray ionization mass spectrometry (ESI-MS) data were collected on triple-stage quadrupole instrument in a positive mode. Flash column chromatography was performed using reversed phase (100 Å, 20–40-micron particle size, RediSep® Rf Gold® Reversed-phase C18 or C18Aq) and silica on a CombiFlash® Rf 200i (Teledyne Isco, Inc.). LC/MS was performed using a Shimadzu LCMS-2020 Single Quadrupole utilizing a Kinetex 2.6 µm C18 100 Å (2.1 x 50 mm) column obtained from Phenomenex, Inc (Torrance, CA). Runs employed a gradient of 0-90% MeCN/H_2O with 0.1% formic acid over 4.5 min at a flow rate of 0.2 mL/min. Prep HPLC was performed on Waters HPLC equipped with Waters 515 HPLC Pump, Water 2998 Photodiode Array Detector, Waters 2545 Binary Gradient Module and Waters 2767 Sample Manager. The HPLC was installed with Luna 10 µm C18 column (100 x 30 mm) and each run employed 10-95% or 60-95% CH_3CN/H_2O gradient with 0.1% TFA over 10 mins. Absorbance curves were obtained on a Shimadzu UV-2550 spectrophotometer operated by UVProbe 2.32 software. Fluorescence traces were recorded on a PTI QuantaMaster steady state spectrofluorometer operated by FelixGX 4.2.2 software, with 5 nm excitation and emission slit widths, and a 0.1 s integration rate. Analytical LC analyses were collected from Agilent 1260 Infinity Quaternary LC module using Poroshell 120 EC-C18 2.7 µM (4.6 x 50 mm) column in 5-95% CH_3CN/water gradient with 0.1% formic acid over 25 minutes. Microplates were read on Synergy Mx microplate reader. All statistical analyses were carried out by Graphpad Prism version 9.0 (Graphpad Software).

Absorbance and Fluorescence Measurements. Absorbance curves were obtained on a Shimadzu UV-2550 spectrophotometer operated by UVProbe 2.32 software. Fluorescence traces were recorded on a PTI QuantaMaster steady-state spectrofluorometer operated by FelixGX 4.2.2 software, with 5 nm excitation and emission slit widths, and a 0.1 s integration rate.

Enzyme and Protein Assays. γ-Glutamyltranspeptidase from equine kidney (Type VI, 5-12 units/mg solid), leucine aminopeptidase, microsomal from porcine kidney (Type IV-S, 10-40 units/mg protein), Cathepsin B from human liver and Esterase from porcine liver (≥15 units/mg solid) were purchased from Millipore Sigma.

Cellular assay. SHIN-3 cells were generously provided by Dr. Hisataka Kobayashi. MDA-MB-468 and PC-3 were bought from ATCC. SHIN-3 cells were cultured in Roswell Park Memorial Institute media (RPMI) containing 10% FBS and 1% PS. MDA-MB-468 cells were grown in Dulbecco's Modified Eagle's medium (DMEM) containing 10% fetal bovine serum (FBS) and 1% Penicillin-Streptomycin (PS). PC-3 cells were grown in F-12K media containing 10% FBS and 1%PS. Cells were grown in a cell culture incubator at 37°C in a humidified atmosphere containing 5% CO_2. Cells were grown in T-75 or T-175 culture flask till 70% confluency before splitting into next passage.

Flow Cytometry was performed at Flow Cytometry Core (CCR) using BD LSRII. The fluorescence signal in the near infrared region was excited using 633 nm laser (18 mW) and emission detected on APC-Cy7 detectors (780/60). The flow cytometry data was processed using FlowJo software.

Confocal imaging was carried out at Optical Microscopy and Image Analysis Lab (OMAL) on Andor spinning disk confocal microscope on Leica DMi8 base. The images were taken at 63x/1.4 oil immersed objective. The images were processed using Fiji software.

SHIN-3 metastatic tumor model. Animal experiments were approved by the Institutional Animal Care and Use Committee of the NIH and conducted in compliance with the Guide for the Care and Use of Laboratory Animal Resources and the National Research Council. The tumors were implanted by

intraperitoneal injection of 2×10^6 SHIN-3-ZsGreen cells suspended in 300µl of PBS into female athymic nude mice (Athymic NCr-nu/nu, strain #553). Experiments with tumor-bearing mice were performed after 15 days of implantation of SHIN-3-ZsGreen models, when disseminated peritoneal implants grew to about 1 mm in size. 100 µM of 300 µL (30 nmol) was injected intraperitoneally, and mice were sacrificed at 1, 3 and 6 h time interval.

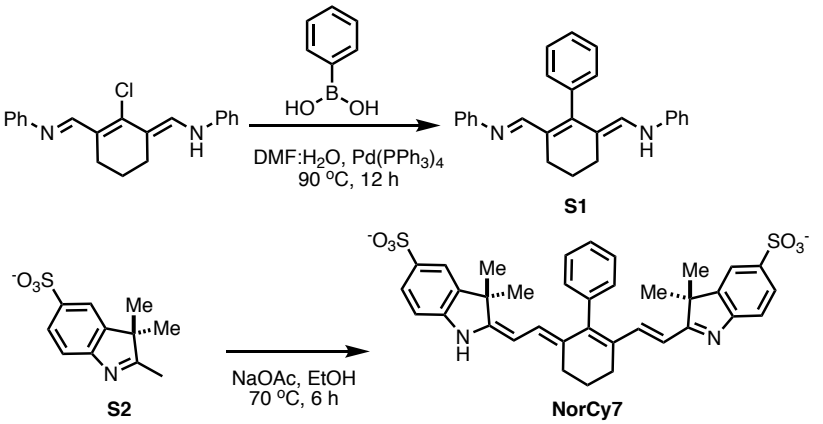
Acquisition for GFP signal (ZsGreen): Excitation: band-pass filter from 445 - 490 nm; Emission long-pass filter over 515 nm; Acquisition: 500 – 720 nm in 10 nm steps.

Acquisition for Cy7 channel signal (probe): Excitation: band-pass filter from 710 - 760 nm; Emission long-pass filter over 800 nm; Acquisition: 780 – 950 nm in 10 nm steps.

Fluorescence images were automatically acquired in 10 nm increments with constant exposure. The images, which consisted of an autofluorescence spectrum and the spectra from GFP and Cy7 channel, were reconstructed using Maestro software, based on their unique spectral patterns.

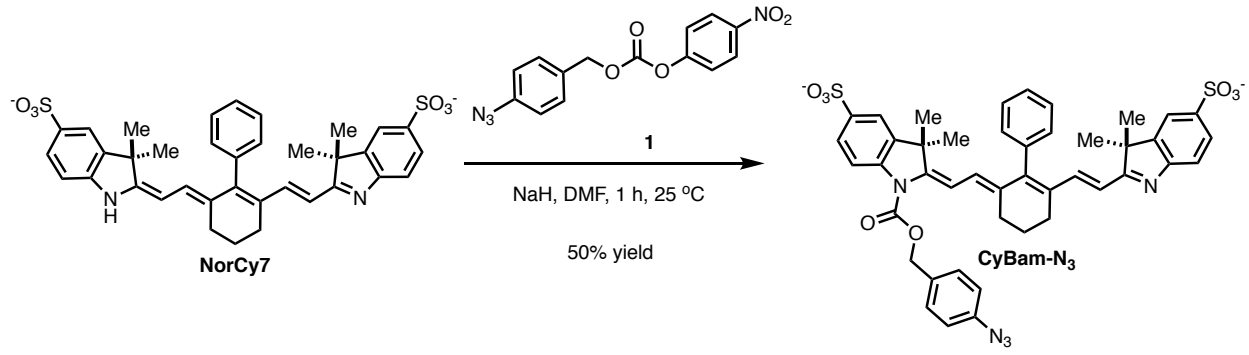
MDA-MB468 model. *In vivo* studies were performed according to Animal Care and Use committee guidelines at Frederick National Laboratory for Cancer Research (Frederick, MD). Frederick National Laboratory for Cancer Research is accredited by American Association for Accreditation of Laboratory Animal Care (AAALAC) International and follows the Public Health Service Policy for the Care and use of Laboratory Animals. Animal care was provided in accordance with the procedures outlined in the "Guide for Care and Use of Laboratory Animals" (National Research Council; 2011; National Academies Press; Washington, D.C.). Fluorescence was longitudinally monitored employing the IVIS spectrum imager (PerkinElmer Inc, Waltham, MA). Images were acquired and processed using Living Image software. Mice body temperature were maintained constant at 37 °C during the imaging procedure with a heated pad located under the anesthesia induction chamber, imaging table, and post procedure recovery cage. All mice were anesthetized in the induction chamber with 3% isoflurane with filtered (0.2 µm) air at 1 liter/minute flow rate for 3-4 minutes and then modified for imaging to 2% with O_2 as a carrier with a flow rate of 1 liter/minute. Static 2D images were acquired with the following parameters: excitation filter 745 ± 15 nm, emission filter 800 ± 10 nm, f/stop2, medium binning (8x8) and auto exposure (typically 1-60 seconds). 5-week-old female athymic nude mice were purchased from Charles River Laboratories International, Inc. (Frederick, MD). $5x10^6$ human breast cancer cells (MDA-MB-468) in 100 µL of Hanks Balanced Salt Solution (HBSS) were subcutaneously injected in the right flank of the mice. Tumors were monitored daily until they reach 4-6 mm in the longest diameter. *In vivo* studies were initiated 10 days post cell injection of the mice.

A. Synthesis



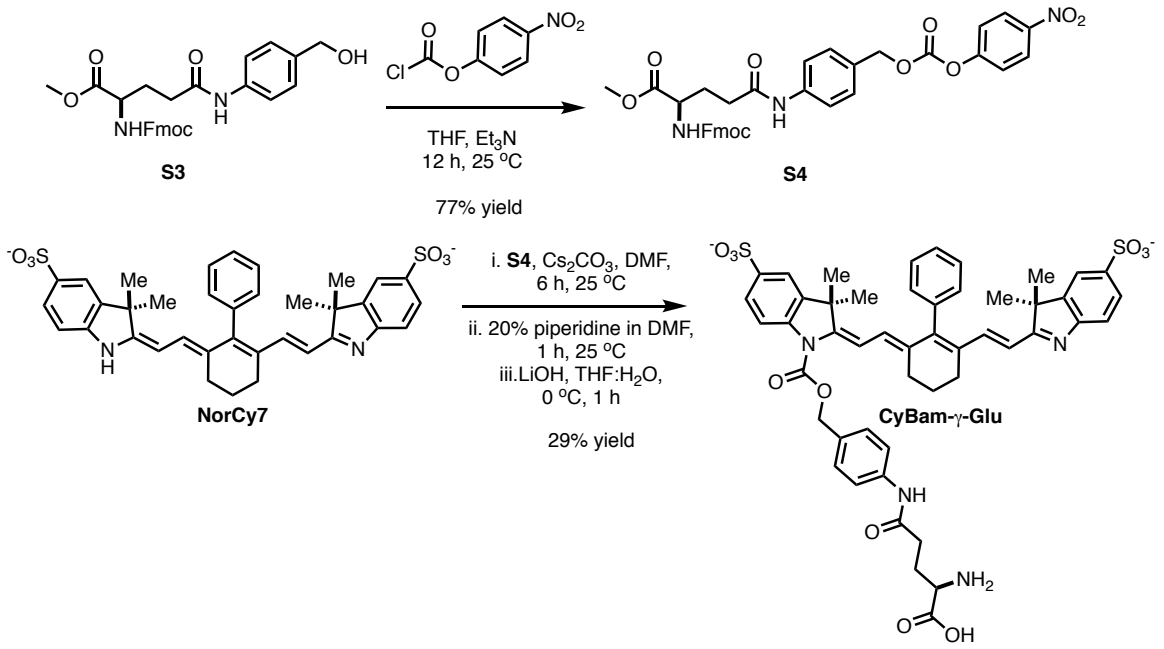
Compound S1. To a 20 mL microwave vial, Vilsmeier reagent (400 mg, 1.11 mmol), phenyl boronic acid (269.6 mg, 2.22 mmol) and K_3PO_4 (236.2 mg, 2.11 mmol) were added sequentially. $Pd(PPh_3)_4$ (45 mg, 0.038 mmol) was added in glove box and the vial was sealed. Subsequently, degassed DMF:H_2O (5:1) was added to the solution and heated at 90 °C for 18 h. The solvent was removed and filtered through celite to remove inorganic compounds. Crude mixture was taken forward to next step.

NorCy7. Compounds **S2** was synthesized according to literature procedure.[1] To a 100 mL round bottom flask, **S1** (800 mg, 2.19 mmol) **S2** (1.1 g, 4.39 mmol) and NaOAc (360 mg, 4.39 mmol) were added sequentially in 20 mL of absolute ethanol. The reaction mixture was heated at 70 °C for 6 h. The flask was cooled on ice to room temperature and solvent was removed. The crude reaction mixture was purified on reversed phase chromatography (0-50% MeCN:H_2O). (320 mg, 21%). ^{1}H NMR (500 MHz, DMSO) δ 7.62 – 7.55 (m, 5H), 7.53 (dd, J = 8.0, 1.6 Hz, 2H), 7.21 (dd, J = 6.4, 2.9 Hz, 2H), 7.07 – 6.97 (m, 4H), 6.10 (d, J = 14.1 Hz, 2H), 2.59 (t, J = 6.3 Hz, 4H), 1.92 (p, J = 6.2 Hz, 2H), 1.23 (s, 12H). ^{13}C NMR (126 MHz, DMSO) δ 160.8, 144.7, 141.7, 140.2, 138.4, 134.5, 130.7, 130.4, 130.1, 128.9, 128.7, 127.8, 126.6, 120.4, 116.7, 114.4, 111.0, 49.05, 36.25, 26.91, 25.01, 21.48. HRMS (ESI) calculated for $C_{36}H_{37}N_2O_6S_2$ $[M+H]^+$ 657.2088; observed: 657.2094.



CyBam-N_3. Compound **1** was synthesized according to a known procedure.[2] To a 5 mL microwave vial, **NorCy7** (20 mg, 0.030 mmol), NaH (1.4 mg, 0.061 mmol) were added sequentially to dry DMF (1.5 mL) and stirred for 1 h. **S3** (19 mg, 0.060 mmol) was dissolved in dry DMF (1.5 mL) and added dropwise over 10 mins. Reaction progress was monitored by LC/MS. After 2 h, the reaction was complete by LC/MS and was quenched using H_2O (1 mL). The resulting mixture was purified by reversed phase HPLC (60-95% MeCN:H_2O, 0.1% TFA) to give **CyBam- N_3** as purple amorphous solid (12.5 mg, 50%). ^{1}H NMR (500 MHz, DMSO) δ 7.79 (d, J = 1.6 Hz, 1H), 7.70 – 7.65 (m, 2H), 7.64 – 7.58 (m, 5H), 7.47 – 7.44 (m, 2H), 7.41 (d, J = 1.8 Hz, 1H), 7.30 (d, J = 13.1 Hz, 1H), 7.22 (td, J = 7.5, 7.0, 1.8 Hz, 4H), 6.70 (d, J = 15.8 Hz,

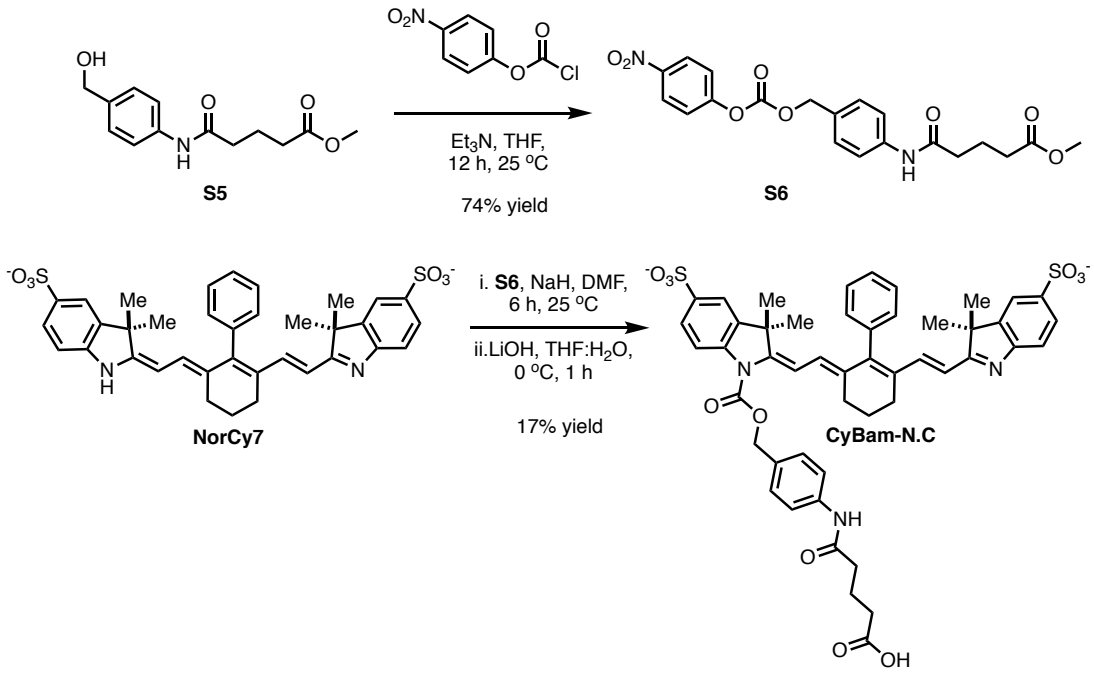
1H), 6.36 (d, J = 13.0 Hz, 1H), 5.38 (s, 2H), 2.61 (t, J = 6.2 Hz, 2H), 2.30 (t, J = 6.2 Hz, 2H), 1.86 – 1.80 (m, 2H), 1.29 (s, 6H), 1.09 (s, 6H). ^{13}C NMR (125 MHz, DMSO) δ 183.4, 153.8, 152.2, 148.1, 145.2, 143.5, 140.2, 139.5, 138.5, 138.3, 136.8, 133.8, 132.3, 131.5, 130.0, 129.1, 128.6, 128.5, 126.8, 125.9, 120.5, 119.9, 119.8, 119.6, 119.2, 116.8, 115.7, 115.0, 114.5, 108.4, 68.38, 52.01, 45.09, 29.03, 26.91, 24.90, 24.74, 24.68, 21.39. HRMS (ESI) calculated for $C_{44}H_{42}N_5O_8S_2$ $[M+H]^+$ 832.2468; observed: 832.2469.



Compound S4. Compound **S3** was prepared according to a known procedure.[3] To a 20 mL microwave vial, **S3** (100 mg, 0.20 mmol) and 4-nitrophenyl chloroformate (45 mg, 0.22 mmol) were added sequentially to dry THF (10 mL) and stirred on ice for 30 mins. Triethylamine (57 μL, 0.40 mmol) was added dropwise, and the solution was stirred for 12 h. The resulting white precipitate were filtered, and the solvent was evaporated. The product was purified by normal phase chromatography (EtOAc:hexanes; 0 – 100%, 24 g SiO_2). Product containing fractions were combined, evaporated, and triturated with ether to give compound **S4** as a white amorphous solid (100 mg, 77% yield). ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.33 – 8.20 (m, 4H), 7.81 – 7.71 (m, 3H), 7.60 (dt, J = 17.1, 8.7 Hz, 5H), 7.39 (q, J = 8.8, 7.0 Hz, 4H), 7.31 (d, J = 7.2 Hz, 2H), 5.61 (d, J = 8.1 Hz, 2H), 5.24 (s, 2H), 4.51 – 4.37 (m, 4H), 4.20 (t, J = 6.8 Hz, 2H), 3.76 (s, 3H), 3.49 (d, J = 4.2 Hz, 1H), 2.38 (dt, J = 28.9, 10.2 Hz, 4H), 2.17 (s, 2H), 1.99 – 1.88 (m, 2H). ^{13}C NMR (125 MHz, $CDCl_3$) δ 172.2, 170.3, 155.5, 152.4, 145.3, 143.7, 143.4, 141.3, 129.8, 127.8, 127.1, 127.0, 125.3, 125.0, 124.9, 121.7, 120.1, 120.0, 119.7, 70.68, 67.21, 53.14, 52.80, 47.18, 33.90, 30.01. HRMS (ESI) calculated for $C_{35}H_{32}N_3O_{10}$ $[M+H]^+$ 654.2082; observed: 654.2096.

CyBam-γ-Glu: To a 5 mL microwave vial, **NorCy7** (30 mg, 0.046 mmol) and Cs_2CO_3 (30 mg, 0.092) were added sequentially to dry DMF (2 mL) and stirred for 3 h. Compound **S4** (60 mg, 0.092 mmol) was dissolved in dry DMF (2 mL) and added dropwise over 10 mins. The reaction mixture was monitored at different intervals using LC/MS. After 3 h, the reaction was quenched using H_2O (1 mL). The crude product concentrated and then dissolved in 20% piperidine in DMF (1 mL). After 1 h, the solvent was evaporated and LiOH (1.1 mg; 0.046 mmol) was added to THF:H_2O (1:1) on ice. The reaction was monitored by LC/MS. After 1 h the reaction was complete and the crude mixture was purified by reversed phase HPLC (60-95% MeCN:H_2O, 0.1% TFA) to provide **CyBam-γ-Glu** as a purple amorphous solid (13 mg, 29%). ^{1}H NMR (500 MHz, DMSO) δ 8.29 (d, J = 5.4 Hz, 3H), 7.67 (t, J = 8.5 Hz, 3H), 7.58 – 7.54 (m, 3H), 7.50 (d, J = 8.6 Hz, 2H), 7.42 (d, J = 1.8 Hz, 1H), 7.38 (d, J = 1.8 Hz, 1H), 7.37 (s, 1H), 7.28 (s, 1H), 7.25 (s, 1H),

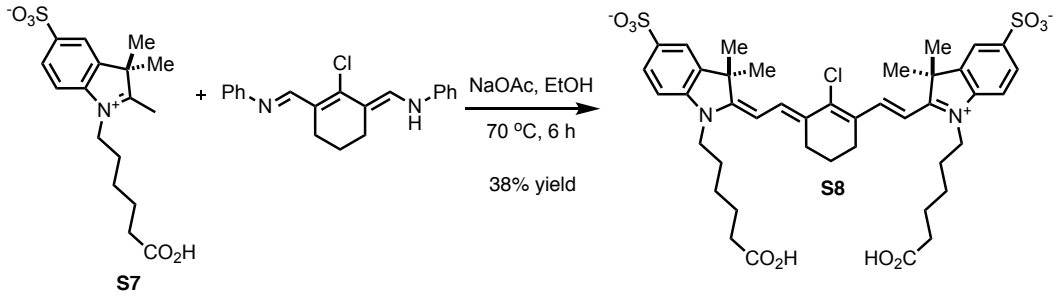
7.19 (d, J = 1.7 Hz, 1H), 7.17 (t, J = 1.5 Hz, 1H), 6.61 (d, J = 16.1 Hz, 2H), 6.14 (d, J = 12.8 Hz, 2H), 5.32 (s, 2H), 2.37 (t, J = 1.9 Hz, 1H), 2.28 (t, J = 5.9 Hz, 3H), 2.15 – 2.08 (m, 4H), 1.81 (s, 3H), 1.13 (d, J = 42.3 Hz, 12H). ^{13}C NMR (125 MHz, DMSO) δ 184.1, 171.3, 170.3, 152.3, 145.1, 139.9, 139.6, 139.1, 138.5, 136.9, 133.5, 130.3, 130.1, 130.1, 128.9, 128.1, 126.1, 120.3, 119.8, 119.5, 117.9, 115.6, 114.9, 113.2, 108.5, 68.62, 52.16, 52.00, 44.71, 31.99, 28.87, 26.08, 24.99, 24.19. HRMS (ESI) calculated for $C_{49}H_{51}N_4O_{11}S_2$ $[M+H]^+$ 935.2992; observed: 935.2990.



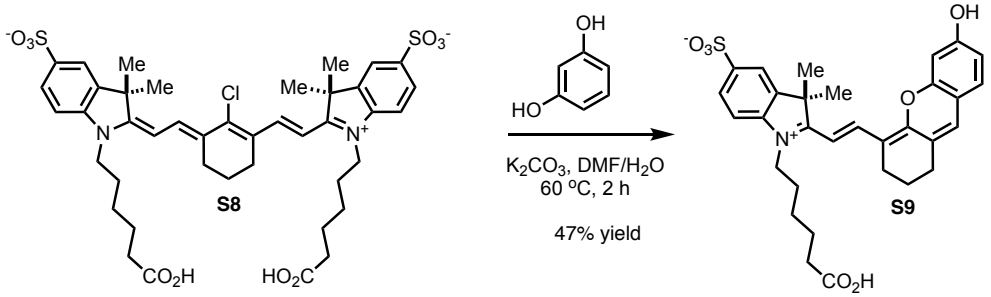
Compound S6. Compound **S5** was synthesized according to known procedure.[4] To 20 mL microwave vial, **S5** (500 mg, 1.99 mmol) and 4-nitrophenyl chloroformate (400 mg, 2.38 mmol) were added sequentially in dry THF (10 mL) and stirred on ice for 30 mins. Triethylamine (550 µL, 4.00 mmol) was added dropwise and the solution was stirred for 12 h. White precipitate were filtered, and the solvent was evaporated. The crude mixture redissolved in CH_2Cl_2 and purified by normal phase silica column (EtOAc:n-hexane; 0 – 100%, 24 g SiO_2) to give **S7** white color product (620 mg, 74% yield). ^{1}H NMR (500 MHz, DMSO) δ 10.01 (s, 1H), 8.32 (d, *J* = 8.6 Hz, 2H), 7.67 – 7.52 (m, 5H), 7.38 (dd, *J* = 24.2, 8.3 Hz, 3H), 5.24 (s, 2H), 4.71 (s, 1H), 3.60 (s, 4H), 2.51 (s, 3H), 1.84 (s, 3H).^{13}C NMR (125 MHz, DMSO) δ 173.52, 171.22, 155.76, 152.43, 145.64, 140.17, 129.93, 129.43, 125.88, 123.09, 119.49, 119.41, 70.77, 51.75, 46.70, 35.70, 33.08, 20.77. HRMS (ESI) calculated for $C_{20}H_{21}N_2O_6$ $[M+H]^+$ 417.1288; observed: 417.1292.

CyBam-N.C. To a 5 mL microwave vial, **NorCy7** (30 mg, 0.046 mmol) and NaH (2.1 mg, 0.092) were added sequentially in dry DMF (2 mL) and stirred for 30 min. Compound **S7** (38.2 mg, 0.092 mmol) was dissolved in dry DMF (2 mL) and added dropwise over 10 mins. The reaction mixture was monitored at different intervals using LC/MS. After 1 h, the reaction was quenched using H_2O (1 mL). The crude mixture was concentrated. The resulting mixture was dissolved in THF:H_2O (1:1), cooled to 4 °C, and LiOH (1.1 mg; 0.046 mmol) was added . The reaction monitored by LC/MS. After 1 h the reaction was complete. The crude mixture was purified by reversed phase HPLC (60-95% MeCN:H_2O, 0.1% TFA) to provide **CyBam-N.C** as purple amorphous solid (8.0 mg, 17%). ^{1}H NMR (500 MHz, DMSO) δ 7.62 (dd, J = 8.6, 6.4 Hz, 3H), 7.52 (dd, J = 9.5, 1.6 Hz, 2H), 7.50 – 7.43 (m, 5H), 7.43 – 7.39 (m, 2H), 7.36 (dd, J = 8.5, 1.8 Hz, 1H), 7.30 (d, J = 1.8 Hz, 1H), 7.27 (d, J = 8.1 Hz, 2H), 7.12 – 7.06 (m, 4H), 6.52 (d, J = 16.1 Hz, 3H), 6.03 (d, J = 12.8 Hz, 2H), 5.23 (s, 2H), 2.32 (t, J = 7.4 Hz, 2H), 2.23 (t, J = 7.4 Hz, 2H), 2.13 (d, J = 5.4 Hz, 2H), 1.79 – 1.74 (m, 2H), 1.70 (d, J = 6.6 Hz, 3H), 1.08 (s, 5H), 1.00 (s, 5H). ^{13}C NMR (125 MHz, DMSO) δ 184.1,

174.6, 171.3, 158.5, 152.3, 145.1, 140.2, 139.7, 139.3, 138.5, 137.0, 133.4, 130.4, 130.2, 129.8, 128.9, 128.0, 126.0, 125.8, 119.8, 119.5, 119.4, 118.4, 114.9, 108.5, 68.69, 52.19, 44.68, 35.92, 33.42, 28.86, 24.95, 24.07, 20.94. HRMS (ESI) calculated for $C_{49}H_{50}N_3O_{11}S_2$ $[M+H]^+$ 920.2809; observed: 920.2880.

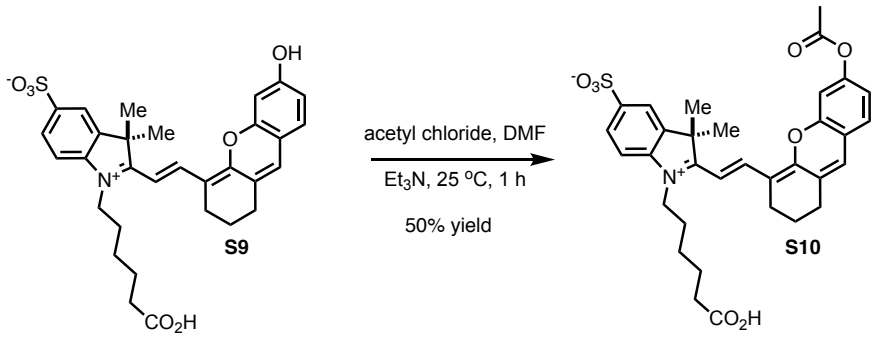


Compound S8.[1] Compound **S7** was synthesized according to known procedure.[5] To a 20 mL microwave vial **S7** (327.8 mg, 0.93 mmol) and Vilsmeier reagent (150 mg, 0.46 mmol) were added sequentially in dry ethanol (20 mL). The solution was heated at 70 °C for 6 h. The reaction was cooled, and solvent was evaporated. The crude mixture was redissolved in H_2O and purified over reversed phase chromatography (0-50% MeCN:H_2O) to provide **S8** as green amorphous solid (150 mg, 38%). ^{1}H NMR (500 MHz, DMSO) δ 8.26 (d, *J* = 13.9 Hz, 2H), 7.81 (d, *J* = 1.6 Hz, 2H), 7.66 (dd, *J* = 8.2, 1.6 Hz, 2H), 7.40 (d, *J* = 8.4 Hz, 2H), 6.34 (d, *J* = 14.1 Hz, 2H), 4.22 (t, *J* = 7.3 Hz, 4H), 2.71 (t, *J* = 6.0 Hz, 4H), 2.21 (t, *J* = 7.3 Hz, 4H), 1.90 – 1.82 (m, 2H), 1.74 (p, *J* = 7.5, 7.0 Hz, 5H), 1.68 (s, 12H), 1.56 (p, *J* = 7.4 Hz, 4H), 1.41 – 1.35 (m, 4H). ^{13}C NMR (125 MHz, DMSO) δ 174.8, 172.8, 148.4, 145.9, 143.3, 142.5, 140.9, 127.0, 126.7, 120.3, 111.1, 102.4, 49.47, 44.22, 33.92, 27.87, 27.21, 26.29, 26.08, 24.66, 20.82. LRMS: calculated for $C_{42}H_{53Cl}N_2O_{10}S_2$ $[M+H]^+$ 843.2; observed: 843.2.

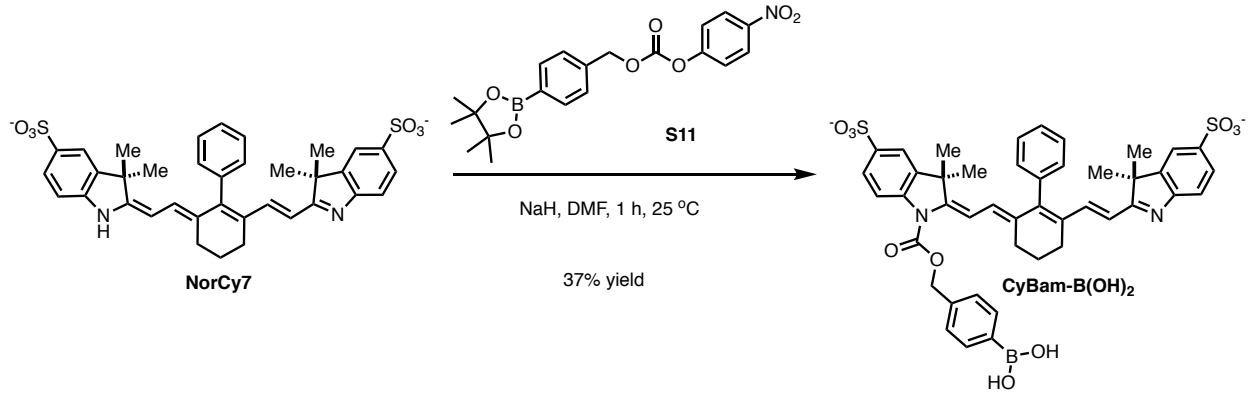


Compound S9. To a 10 mL microwave vial resorcinol (26mg, 0.24 mmol) and K_2CO_3 (49.2, 0.36 mmol) were added sequentially in dry DMF (5 mL). The solution was stirred at 25 °C for 15 mins followed by addition of **S8** (100 mg, 0.11 mmol). The solution was first stirred at 25 °C for 30 mins and then heated at 60 °C for 1.5 h. The reaction was cooled, and solvent was evaporated. The crude mixture was redissolved in H_2O and purified over reversed phase chromatography (0-50% MeCN:H_2O) to provide **S9** as blue amorphous solid (30.1 mg, 47%). ^{1}H NMR (500 MHz, DMSO) δ 8.57 (d, *J* = 14.7 Hz, 2H), 7.74 (dd, *J* = 8.3, 1.6 Hz, 1H), 7.58 (s, 2H), 7.50 (d, *J* = 8.5 Hz, 1H), 6.96 (d, *J* = 2.4 Hz, 1H), 6.86 (dd, *J* = 8.5, 2.2 Hz, 1H), 6.59 (d, *J* = 4.0 Hz, 1H), 6.49 (d, *J* = 14.8 Hz, 2H), 4.37 (t, *J* = 7.3 Hz, 2H), 2.74 – 2.68 (m, 3H), 2.68 – 2.63 (m, 2H), 2.21 (t, *J* = 7.3 Hz, 2H), 1.84 (t, *J* = 6.0 Hz, 2H), 1.75 (s, 6H), 1.59 – 1.52 (m, 2H), 1.45 – 1.35 (m, 3H). ^{13}C NMR (125 MHz, DMSO) δ 174.8, 161.7, 154.6, 147.5, 141.9, 141.7, 135.1, 129.7, 126.8, 126.3, 120.4, 114.9, 114.4, 112.6, 102.4, 50.60, 44.94, 33.86, 28.76, 27.94, 27.49, 26.00, 24.62, 20.45. HRMS (ESI) calculated for $C_{31}H_{34}NO_7S$ $[M+H]^+$ 564.2046; observed: 564.2050.

[1] The compound has been reported in *J. Mater. Chem. B*, 2017, 5, 5278-5283 and Chem. Commun., 2020, 56, 5819-5822 and *Dyes and Pigments,* 2004, 61, 103–107 but no detailed synthetic procedure could be found.

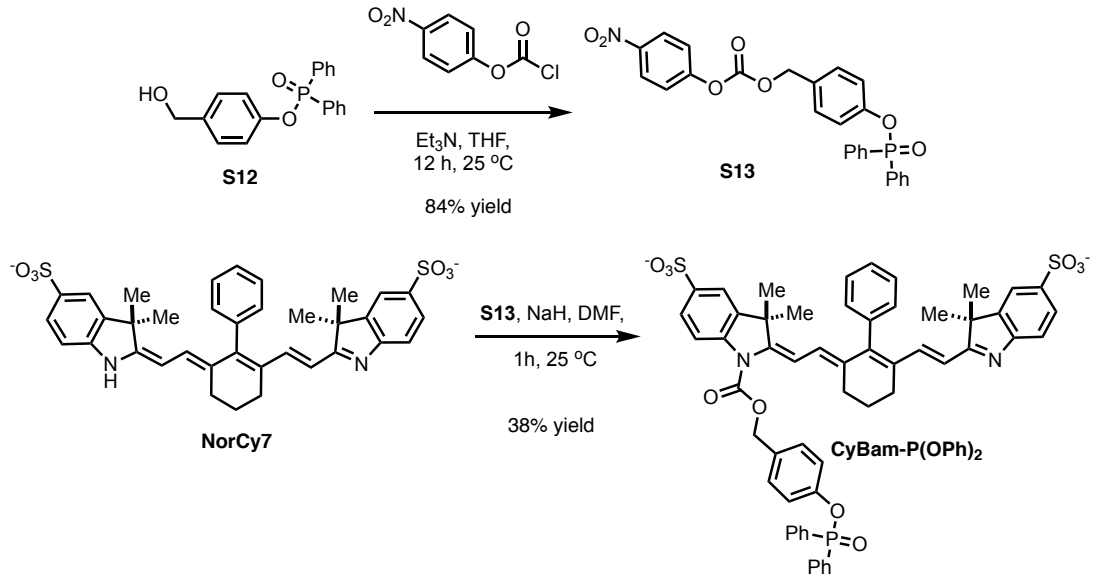


Compound S10. To a 5 mL microwave vial **S9** (10 mg, 0.021 mmol) was dissolved in dry DMF (2 mL). Acetyl chloride (2.49 µL, 0.035 mmol) and Et_3N (7.41 µL. 0.053 mmol) were added sequentially and solution stirred at 25 °C for 1 h. The crude mixture was purified by reversed phase Prep HPLC (10-95% MeCN:H_2O,0.1% TFA) to provide **S10** as blue amorphous solid (6.2 mg, 50%). ^{1}H NMR (500 MHz, MeOD) δ 8.85 (d, *J* = 15.0 Hz, 1H), 8.08 (d, *J* = 1.6 Hz, 1H), 8.02 (dd, *J* = 8.4, 1.7 Hz, 1H), 7.66 (dd, *J* = 8.4, 1.5 Hz, 1H), 7.58 (d, *J* = 8.4 Hz, 1H), 7.45 – 7.40 (m, 2H), 7.15 (dd, *J* = 8.5, 2.2 Hz, 1H), 6.63 (d, *J* = 14.9 Hz, 2H), 4.43 (t, *J* = 7.5 Hz, 2H), 2.84 (t, *J* = 5.9 Hz, 2H), 2.77 (t, *J* = 6.1 Hz, 2H), 2.37 (s, 3H), 2.01 (s, 1H), 2.00 – 1.94 (m, 4H), 1.87 (s, 6H), 1.78 – 1.71 (m, 3H), 1.58 – 1.52 (m, 3H). ^{13}C NMR (125 MHz, MeOD) δ 179.4, 175.6, 169.1, 161.2, 146.9, 144.5, 142.2, 132.4, 129.9, 128.1, 127.02, 120.2, 119.7, 119.3, 115.1, 112.6, 109.5, 104.8, 50.94, 45.02, 33.07, 28.83, 27.07, 26.73, 25.82, 24.14, 23.59, 20.08, 19.50. HRMS (ESI) calculated for $C_{33}H_{36}NO_8S$ $[M+H]^+$ 606.2155; observed: 606.2156.



CyBam-B(OH)$_2$. Compound **S11** was synthesized according to a known procedure.[6] To a 5 mL microwave vial, **NorCy7** (30 mg, 0.045 mmol), NaH (2.1 mg, 0.091 mmol) were added sequentially to dry DMF (1.5 mL) and stirred for 1 h. **S11** (36.5 mg, 0.091 mmol) was dissolved in dry DMF (1.5 mL) and added dropwise over 10 mins. Reaction progress was monitored by LC/MS. After 2 h, the reaction was complete by LC/MS and was quenched using H_2O (1 mL). The resulting mixture was purified by reversed phase HPLC (60-95% MeCN:H_2O, 0.1% TFA) to give **CyBam-B(OH)$_2$** as purple amorphous solid (13.5 mg, 37%). ^{1}H NMR (500 MHz, MeOD) δ 7.81 (dd, J = 9.9, 1.6 Hz, 2H), 7.68 (dd, J = 8.4, 3.2 Hz, 3H), 7.53 – 7.45 (m, 6H), 7.40 (d, J = 7.9 Hz, 2H), 7.35 (d, J = 8.1 Hz, 1H), 7.30 (d, J = 13.1 Hz, 1H), 7.26 – 7.23 (m, 1H), 7.20 (dd, J = 8.1, 6.5 Hz, 2H), 7.04 (dd, J = 6.6, 2.9 Hz, 2H), 6.53 (d, J = 15.6 Hz, 1H), 6.45 (d, J = 13.0 Hz, 1H), 5.29 (s, 2H), 2.45 (t, J = 6.2 Hz, 2H), 2.19 (t, J = 6.2 Hz, 2H), 1.72 (t, J = 6.2 Hz, 2H), 1.21 (s, 6H), 1.03 (s, 6H). ^{13}C NMR (125 MHz, MeOD) δ 184.5, 158.6, 155.0, 153.1, 152.1, 145.0, 142.8, 141.2, 140.9, 140.7, 139.2, 138.0, 137.0, 136.5, 136.0, 134.1, 134.0, 133.9, 133.5, 133.2, 129.5, 128.5, 128.3, 128.0, 127.1, 127.0, 126.3, 125.6, 120.4, 119.6, 115.2, 114.3, 109.6, 108.4, 68.87, 66.76, 51.60, 45.09, 38.99, 28.22,

24.41, 24.38, 24.35, 20.99. HRMS (ESI) calculated for $C_{44}H_{44}BN_2O_{10}S_2$ $[M+H]^+$ 835.2552; observed: 835.2533.



Compound S13. Compound **S12** was synthesized according to known procedure.[7] To 20 mL microwave vial, **S5** (250 mg, 0.77 mmol) and 4-nitrophenyl chloroformate (171 mg, 0.85 mmol) were added sequentially in dry THF (10 mL) and stirred on ice for 30 mins. Triethylamine (600 µL, 1.54 mmol) was added dropwise and the solution was stirred for 12 h. The solvent was removed, the crude mixture redissolved in CH_2Cl_2 and purified by normal phase silica column (EtOAc:n-hexane; 0 – 100%, 24 g SiO_2) to give **S7** white color product (320 mg, 84% yield). ^{1}H NMR (500 MHz, MeOD) δ 8.31 – 8.27 (m, 1H), 7.93 (ddt, J = 12.7, 7.1, 1.4 Hz, 4H), 7.66 – 7.62 (m, 2H), 7.56 (td, J = 7.5, 3.7 Hz, 4H), 7.46 – 7.38 (m, 4H), 7.27 (dd, J = 8.7, 1.4 Hz, 2H), 5.23 (s, 2H). ^{13}C NMR (125 MHz, MeOD) δ 155.6, 152.4, 151.0, 150.94, 145.4, 132.8, 132.8, 131.8, 131.5, 131.4, 130.3, 129.9, 129.2, 128.7, 128.7, 128.6, 128.5, 124.8, 121.8, 120.7, 120.6, 69.61. HRMS (ESI) calculated for $C_{26}H_{21}NO_7P$ $[M+H]^+$ 490.1057; observed: 490.1050.

CyBam-P(OPh)$_2$. To a 5 mL microwave vial, **NorCy7** (30 mg, 0.045 mmol), NaH (2.1 mg, 0.091 mmol) were added sequentially to dry DMF (1.5 mL) and stirred for 1 h. **S13** (45 mg, 0.091 mmol) was dissolved in dry DMF (1.5 mL) and added dropwise over 10 mins. Reaction progress was monitored by LC/MS. After 2 h, the reaction was complete by LC/MS and was quenched using H_2O (1 mL). The resulting mixture was purified by reversed phase HPLC (60-95% MeCN:H_2O, 0.1% TFA) to give **CyBam-P(OPh)$_2$** as purple amorphous solid (15.5 mg, 38%). ^{1}H NMR (500 MHz, MeOD) δ 7.85 – 7.80 (m, 6H), 7.72 – 7.67 (m, 1H), 7.60 – 7.57 (m, 2H), 7.56 – 7.51 (m, 3H), 7.50 (dd, J = 6.1, 4.2 Hz, 4H), 7.45 (td, J = 7.6, 3.6 Hz, 5H), 7.37 – 7.33 (m, 2H), 7.23 (dd, J = 8.5, 1.3 Hz, 2H), 7.12 – 7.09 (m, 2H), 6.57 (d, J = 15.6 Hz, 1H), 6.49 (d, J = 13.0 Hz, 1H), 5.24 (s, 2H), 2.51 (t, J = 6.1 Hz, 2H), 2.31 (t, J = 6.2 Hz, 2H), 1.83 – 1.76 (m, 2H), 1.26 (s, 6H), 1.05 (s, 6H). ^{13}C NMR (125 MHz, MeOD) δ 184.6, 155.2, 153.1, 151.9, 151.2, 151.1, 145.0, 142.8, 141.3, 140.8, 140.7, 139.1, 138.0, 137.0, 136.2, 134.1, 132.9, 132.9, 131.9, 131.8, 131.8, 131.5, 131.4, 130.9, 130.8, 130.7, 130.3, 129.6, 129.2, 128.7, 128.7, 128.6, 128.5, 128.3, 128.3, 128.2, 127.0, 125.6, 120.8, 120.8, 120.4, 119.6, 115.1, 114.2, 109.4, 108.3, 68.00, 51.67, 45.06, 28.17, 24.44, 24.35, 21.02. HRMS (ESI) calculated for $C_{56}H_{52}N_2O_{10}PS_{22}^-$ $[M+H]^+$ 1007.2828; observed: 1007.2796.

B. Photophysical properties

Determination of Molar Absorption Coefficients. Molar absorption coefficients (ε) were determined in PBS (pH 7.2) or acetate buffer (pH 4.5) using Beer's law, from plots of absorbance vs. concentration (1 - 10 μM). Spectra were recorded with disposable micro-UV-Cuvette holder with 10 mm path length, with absorbance at the highest concentration ≤ 0.20. Three independent readings were taken at each concentration.

Absolute Fluorescence Quantum Yields. Absolute fluorescence quantum yields (Φ_F) were measured in PBS (pH 7.2) or acetate buffer (pH 4.5) using a Horiba fluorimeter QM-8075-11-C equipped with R928 PMT point detector and an integrating sphere to determine photons absorbed and emitted by a sample. Probe was excited at 710 nm and emission was collected from 730-850 nm. Measurements were carried out a concentration with optical density of less than 0.1 in buffer and self-absorption corrections were performed using the instrument software.

Determination of pKa. Absorption and emission spectra were recorded in different buffers (pH 3.00 to pH 10.0) using a spectrophotometer and fluorometer, respectively. Following buffers were used in the study: Citrate buffers (pH 3.05 – 3.50), acetate buffers (pH 3.75 to 5.75) and PBS (pH 6.00 – 10.0).

C. Kinetic Studies

Kinetics of release by Staudinger reaction. **CyBam-N_3** (10 μM) and PPh_3 (10 eq) added in PBS: MeOH (1:1) at pH 7.4 or pH 5.2. A spectral scan reading was taken after every 5 min for 1 h.
monitored at different intervals using microplate reader (300 - 800 nm). Three independent experiments were carried out.

D. *In vitro* Procedures

For GGT probe. All the assays were conducted at 37 ºC for 30 mins unless otherwise stated. The pH was adjusted to pH 5.2 after completion of the assay as described below. The absorbance was measured using plate reader and fluorescence was measured using a fluorimeter.

1. *Kinetics of Activation.* **CyBam-γ-Glu** (20 μM) was dissolved PBS buffer (1X, pH = 7.4) at increasing concentration of GGT (0 - 800 U/L) at 37 °C. K_M was calculated after incubating **CyBam-γ-Glu** (2.5 - 50 μM) with 100 U/L.
2. *Inhibition of GGT*. 6-Diazo-5-oxo-L-norleucine (DON; 1 mM) and GGsTop (0.5 mM) were incubated with GGT (100 U/L) for 1 h prior to addition of **CyBam-γ-Glu** (20 μM).
3. *Specificity against GGT.* **CyBam-γ-Glu** (20 μM) was incubated with GGT (100 U/L), LAP (leucine amino peptidase; 800 U/L) and PLE (pig liver esterase; 800 U/L).
4. *pH and cell culture media stability.* **CyBam-γ-Glu** (20 μM) was incubated with DMEM with 10% FBS, 100% FBS and pH (4.5, 5.5, 6.5 and 7.5) for 18 h. The stability of the compound was monitored by observing the absorbance of the probe every 1 h.
5. *Activity at different pH.* Different pH (6.0, 6.5, 7.0, 7.5 and 8.0) was generated using Gibco PBS (1X). The probes were incubated for 30 mins with GGT (100 U/L).

E. Cellular Studies

Fluorescent Imaging in Live Cells: Briefly, 25,000 cells were seeded on Greiner Bio-One CELLview™ Cell Culture Slides and allowed to adhere overnight.

Subcellular Localization. **NorCy7** (20 μM) in RPMI media was incubated for 4 h followed by addition of Lysosome (Lysotracker Green DND 26; Invitrogen), Mitochondria (Mitotracker Green FM; Invitrogen) for 1 h. Afterwards, the cells were washed with PBS (pH 7.4) and nucleus was stained with NucBlue™ Live

Ready Probes (Invitrogen) for 15 mins. Following concentrations of probes were used in the study: Mitotracker Green (500 nM), Lysotracker Green (75 nM) and NucBlue (1 drop/500 μL). Live cell microscopy was carried out in DMEM phenol red free media. The images were captured using three different lasers: nucleus (405, blue channel), mitochondria and lysosome (488 nm, green channel) and **NorCy7** (640 nm, red channel).

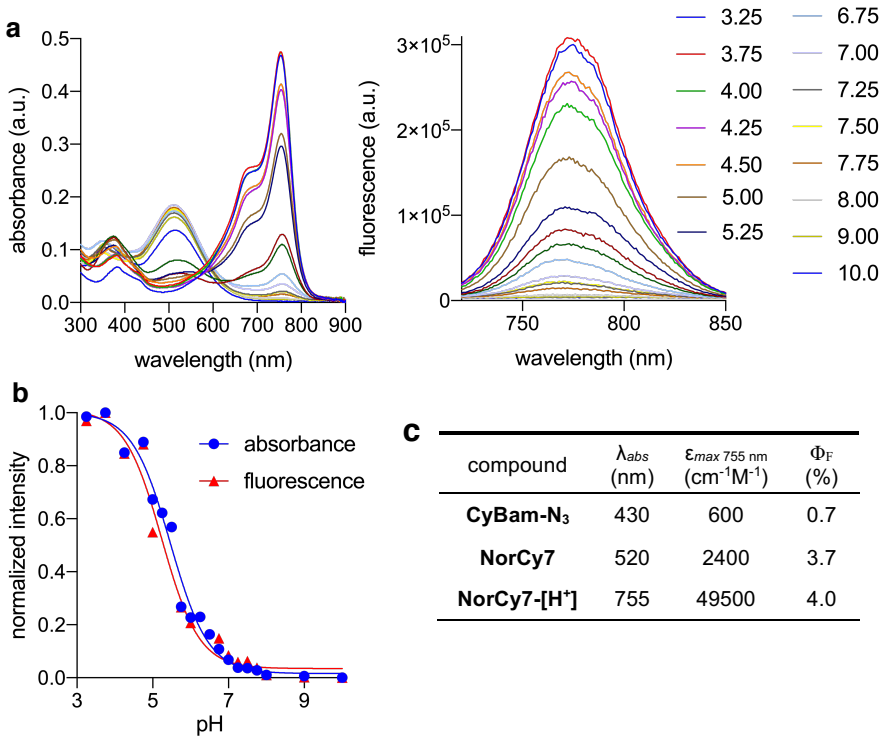
Competition experiment. The cells were divided in three groups: Group 1 contained untreated cells, Group 2 contained blocked probes and Group 3 contained nonselective and non-cleavable probe. Group 2 was treated with DON (1 mM) and GGsTop (1 mM), 1 h prior to study. **CyBam-γ-Glu** (20 μM) was incubated in Group 1 and 2, whereas **CyBam-N.C** was incubated in Group 3. After 3 h the cells were washed, and nucleus was stained as described above. The media was changed to HBSS for live cell study.

Flow Cytometry Analysis. 300,000 cells/well were seeded on 12 well plate (Corning Costar). The cells were treated with compounds, described as below, follow by cleavage using cell dissociation free buffer and spun down at 200 rcf using centrifuge. The cells were suspended in DMEM, phenol red free and live cells counting was performed on flow cytometer. Geometric mean fluorescence intensity was measured at least in three independent trials (at least 10,000 cells counted). Flow cytometry data was processed using FlowJo. **CyBam-γ-Glu**: The cells were divided in three groups: Group 1 was untreated, Group 2 contained blocked probes and Group 3 contained nonselective and non-cleavable probe. Group 2 was treated with DON (1 mM) and GGsTop (1 mM), 1 h prior to study. **CyBam-N.C** (20 μM) was incubated in Group 1 and 2, whereas CyBam-N.C was incubated in Group 3. After 3 h the cells were washed twice with PBS (pH 7.4).

Cytotoxicity Assay. 5000 SHIN-3 cells/well were seeded on 96 well plate and allowed to adhere overnight. Stock solutions of **NorCy7**and **CyBam-γ-Glu** (20 mM in DMSO) were diluted with protein-free medium (PFHM-II) to make desired final concentrations varying from 0.01 to 80 μM. The cells were incubated with the desired concentration for 72h. The cell viabilities were calculated using alamarBlue assay. Briefly, 10 μL of alamarBlue™ Cell Viability Reagent (Invitrogen) was incubated for 1 h and fluorescence was measured with excitation at 560 nm and emission wavelength at 590 nm with a Microplate Reader. The viability of each cell line in response to the treatment with tested compounds was calculated as: % dead cells = 100 − (OD treated/OD control) × 100.

ROS generation. PC-3 cells (250,000) were seeded in 12 well plate (Corning Costar) and allowed to adhere overnight. The cells were divided in five groups: Group 1 - doxorubicin (20 μM), Group 2 **CyBam-B(OH)$_2$** (20 μM), Group 3 **CyBam-P(OPh)$_2$** (20 μM), Group 4 coincubation of **CyBam-B(OH)$_2$** (20 μM) + doxorubicin (20 μM) and Group 5 coincubation of **CyBam-P(OPh)$_2$** (20 μM) + doxorubicin (20 μM). The groups were incubated with compounds for 12 hours. Afterwards, cells were washed with PBS twice, cleaved from plate using cell dissociation free buffer and spun down at 200 rcf using centrifuge. Geometric mean fluorescence intensity was measured in four independent trials (~10,000 cells counted). Flow cytometry data was processed using FlowJo.

F. Supporting Figures



c

compound	λ_{abs} (nm)	$\varepsilon_{max\ 755\ nm}$ ($cm^{-1}M^{-1}$)	Φ_F (%)
CyBam-N_3	430	600	0.7
NorCy7	520	2400	3.7
NorCy7-[H^+]	755	49500	4.0

Figure S1. a. Absorbance (300 – 800 nm) and fluorescence spectra (720 – 850 nm) with 710 excitation of **NorCy7** (10 μM) at complete range of acidic and basic pH **b.** pKa of **NorCy7** (10 μM) was measured using increase in absorbance at 755 nm and fluorescence intensity at 775 nm over different pH. pKa was determined as 5.4 (by absorbance) and 5.2 (by fluorescence). **c.** Summary of the photophysical properties of **CyBam-N_3** (pH 7.2)**, NorCy7** (pH 7.2) and **NorCy7-[H^+]** (pH 4.5)

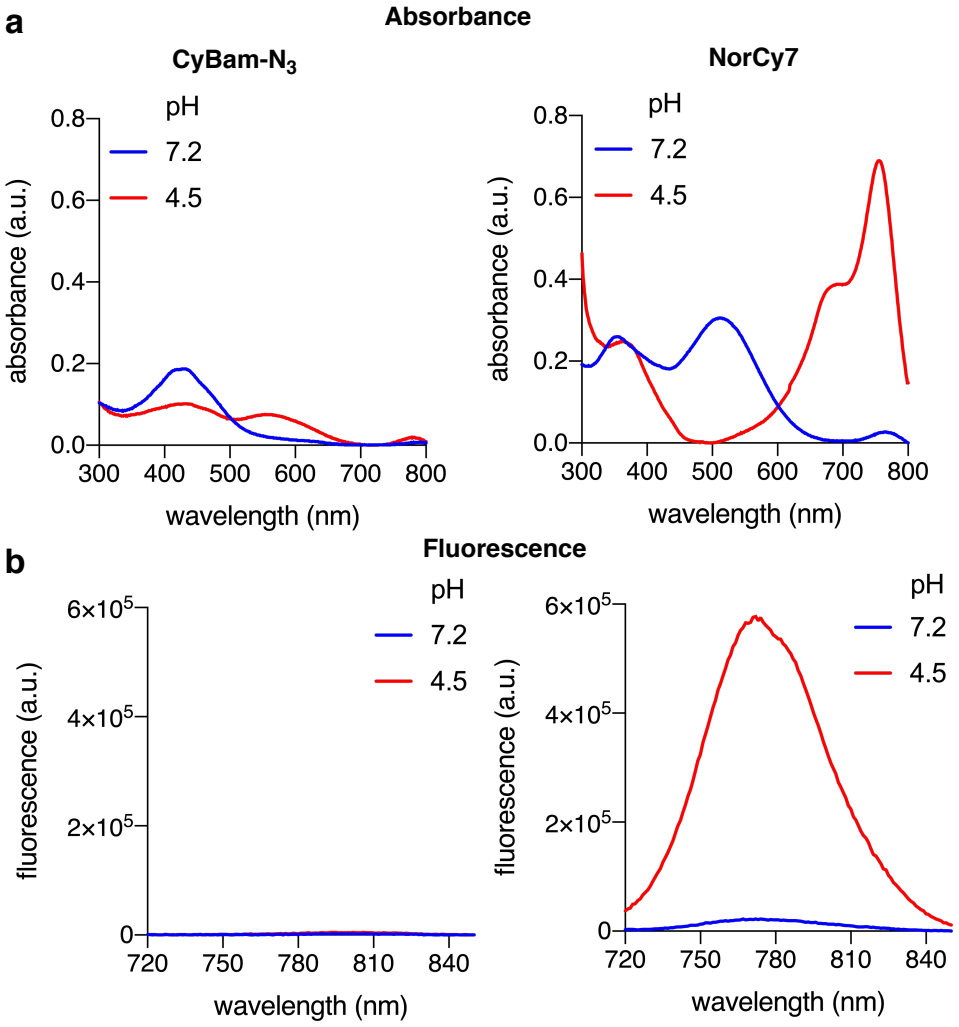


Figure S2. a. Absorbance (300 – 800 nm) and **b.** fluorescence spectra (720 – 850 nm) with 710 excitation of **CyBam-N$_3$** (10 μM) and **NorCy7** (10 μM) at pH 7.2 (PBS) and pH 4.5 (acetate buffer).

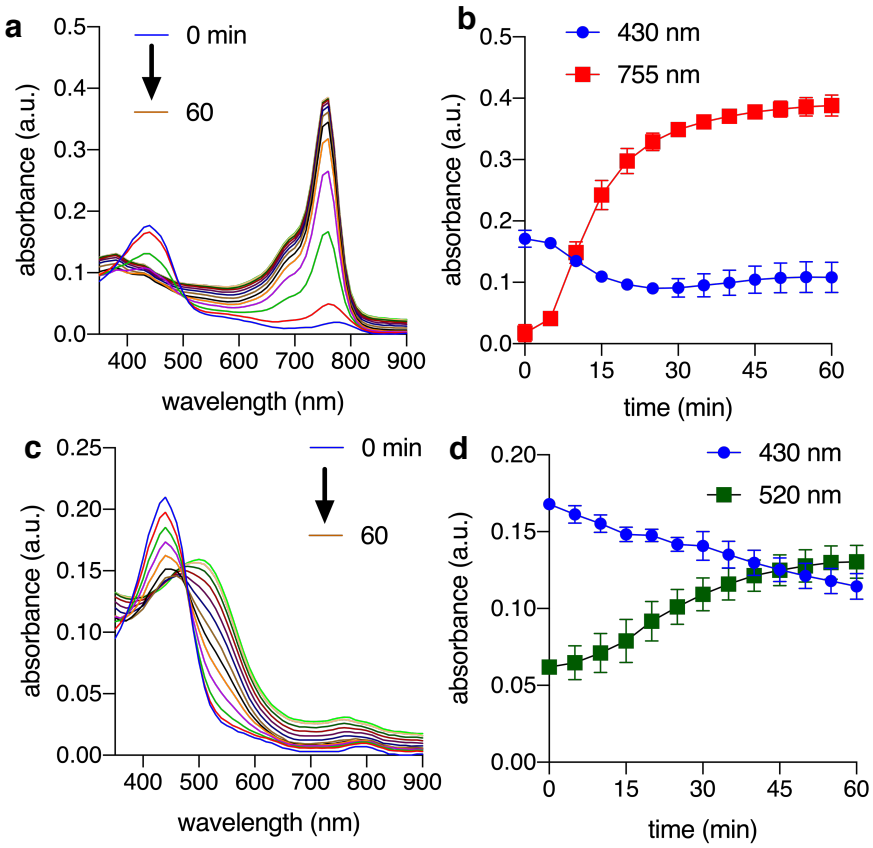


Figure S3. Staudinger release of **CyBam-N$_3$** (10 μM) and PPh$_3$(100 μM; 10 eq) in **a.** acidic (pH 5.2) and **c.** basic (pH 7.4) conditions (MeOH:PBS; 1:1) to give **NorCy7** and **NorCy7-[H$^+$]** respectively. absorbance spectra (300 - 800 nm) monitored for 60 mins at 5 min intervals. **b**. rate of increase at 755 nm (**NorCy7-[H$^+$]**) and decrease in 430 nm peak (**CyBam-N$_3$)** was plotted over 60 mins. **d**. rate of increase at 520 nm (**NorCy7**) and decrease in 430 nm peak (**CyBam-N$_3$)** was plotted over 60 mins.

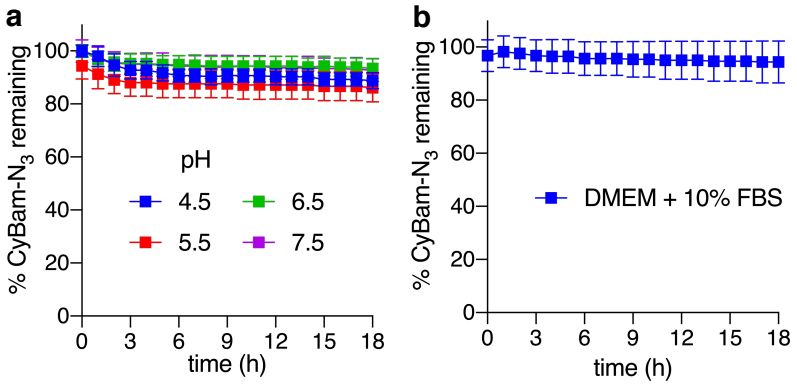


Figure S4. Stability of **CyBam-N$_3$** (20 μM) in **a.** at different pH's (pH 4.5, 5.5, 6.5, and 7.5) and **b.** commonly used cell culture conditions (DMEM + 10% FBS). Absorbance at 430 nm was measured after at 30 min interval for up to 18 h using plate reader. Mean ± SD of absorbance signal at 430 nm from three independent experiments.

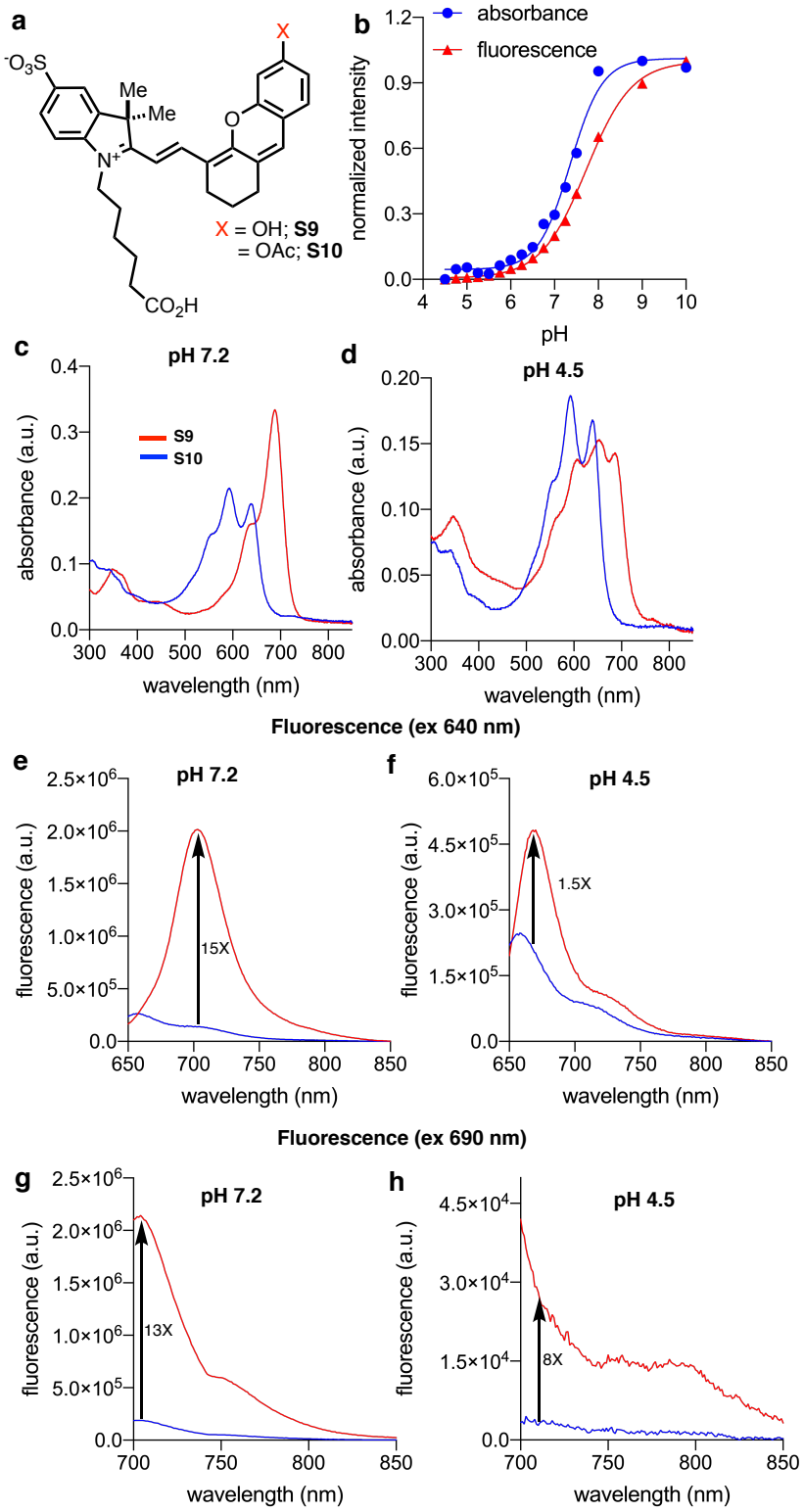


Figure S5. **a.** Structure of xanthene cyanine (**S9**) and acylated fluorogenic probe (**S10**). **b.** pKa of **S9** (10 µM) was measured as using absorbance (690 nm) and fluorescence (705 nm) intensity over different pH. pKa was determined as 7.4 (by absorbance) and 7.7 (by fluorescence). Absorbance spectra of 10 µM of **S9**

(red line) and **S10** (blue line) at **c.** pH 7.2 (PBS) and **d.** pH 4.5 (acetate buffer). Emission spectra (excitation 640 nm) of **S9** and **S10** were measured at **e.** pH 7.2 (PBS) and **f.** pH 4.5 (acetate buffer). **S9** had a 15-fold and 1.5-fold fluorescent turn-ON response over **S10** at pH 7.2 and 4.5 respectively. Emission spectra (excitation 690 nm) of **S9** and **S10** were measured at **g.** pH 7.2 (PBS) and **h.** pH 4.5 (acetate buffer). **S9** had a 13-fold and 8-fold fluorescent turn-ON response over **S10** at pH 7.2 and 4.5 respectively.

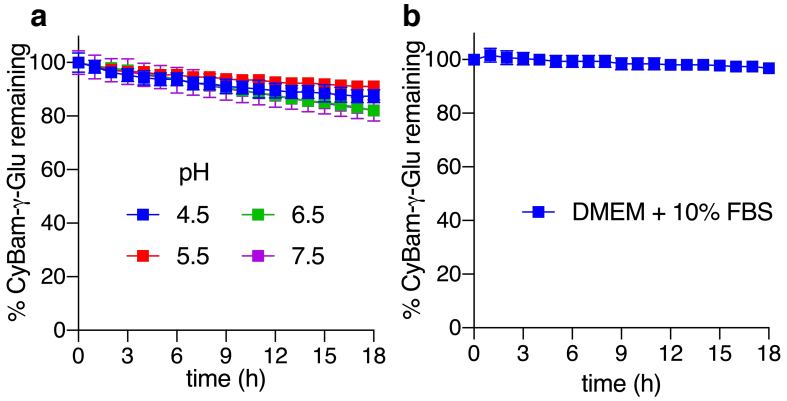


Figure S6. Stability of **CyBam-γ-Glu** (20 μM) in **a.** at different pH's (pH 4.5, 5.5, 6.5, and 7.5) and **b.** commonly used cell culture conditions (DMEM + 10% FBS). Absorbance at 430 nm was measured after at 30 min interval for up to 18 h using plate reader. Mean ± SD of absorbance signal at 430 nm from three independent experiments.

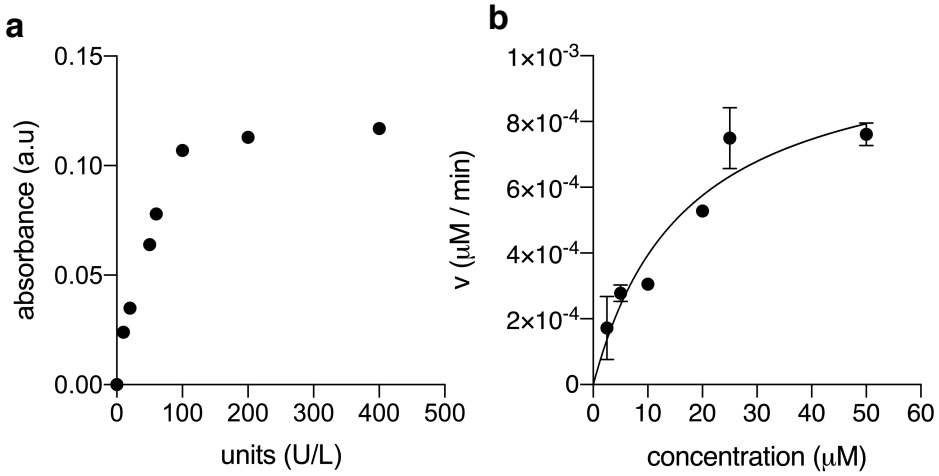


Figure S7. a. Rate of activation of **CyBam-γ-Glu** (20 μM) in the presence GGT (0 - 400 U/L) at PBS (pH 7.4) for 30 mins. **b.** Kinetics of fluorogenic probe activation at different concentrations of **CyBam-γ-Glu** (2.5 - 50 μM) in PBS (pH 7.4; 37 °C) with 100 U/L GGT. K_M was calculated using Michaelis Menten equation. Mean ± SD of fluorescent signal from three independent experiments.

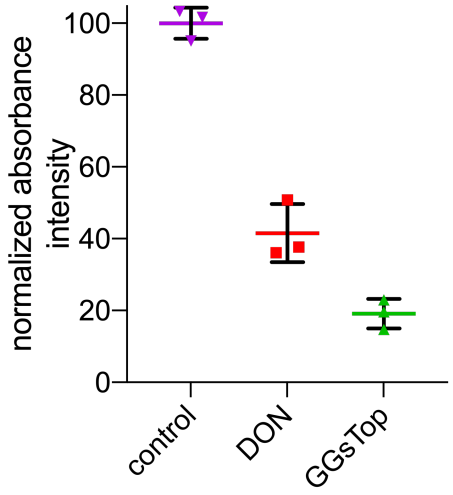


Figure S8. Inhibition of **CyBam-γ-Glu** was quantified in the presence of inhibitors. GGT (100 U/L) was pre-blocked with covalent inhibitors (DON, GGsTop; 1 mM) for 1 h in PBS (pH 7.4) followed by incubation with **CyBam-γ-Glu** (20 μM) at 37 °C. Mean ± SD of absorbance signal from at least three independent experiments.

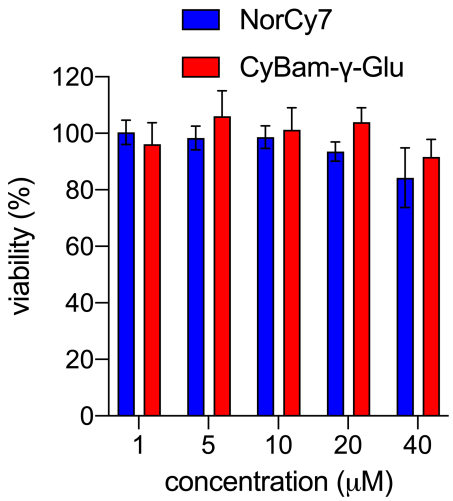


Figure S9. Toxicity of **NorCy7** and **CyBam-γ-Glu** was tested on SHIN-3 cells by incubating increasing concentration (1 - 40 μM) of compound for 72 h. Cell viability was measured using alamarBlue reagent (10 μL for 1 h). The fluorescent signal from live cells was measured using plate reader (ex 540 nm, em 560 nm). Mean ± SD of fluorescent signal from at least three independent experiments.

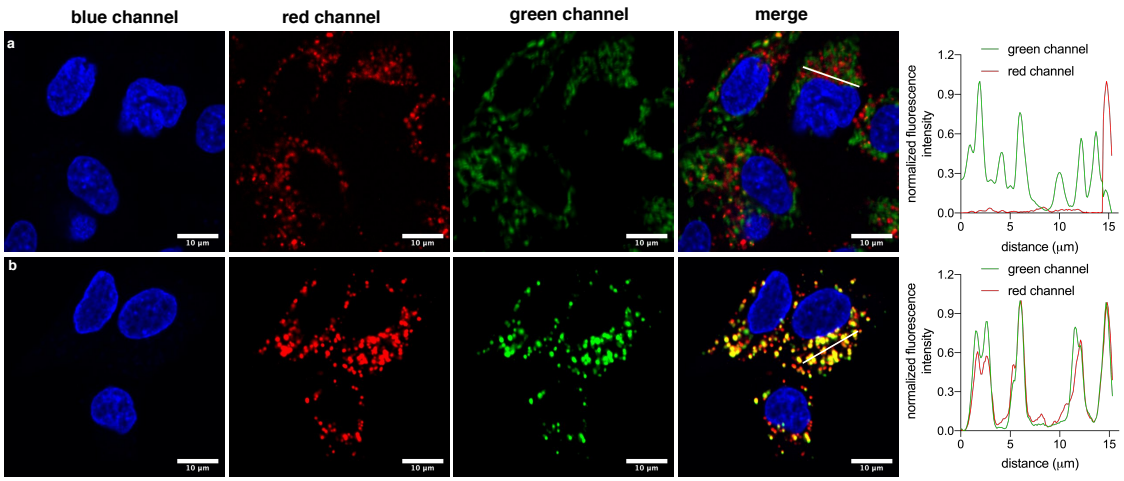


Figure S10. Cellular uptake of **NorCy7** (20 µM; red channel) in SHIN-3 cells after 4 h incubation followed by organelle staining (green channel) of **a.** mitochondria (Mitotracker Green) and **b.** lysosome (Lysotracker Green). Overlap in the green and red channel was analyzed using fluorescent line graph in the green and the red channel. Nucleus stained using NucBlue (blue channel). Confocal imaging carried out at 63X oil immersed lens with 1.4 NA. The images were processed with identical brightness/ contrast using Fiji.

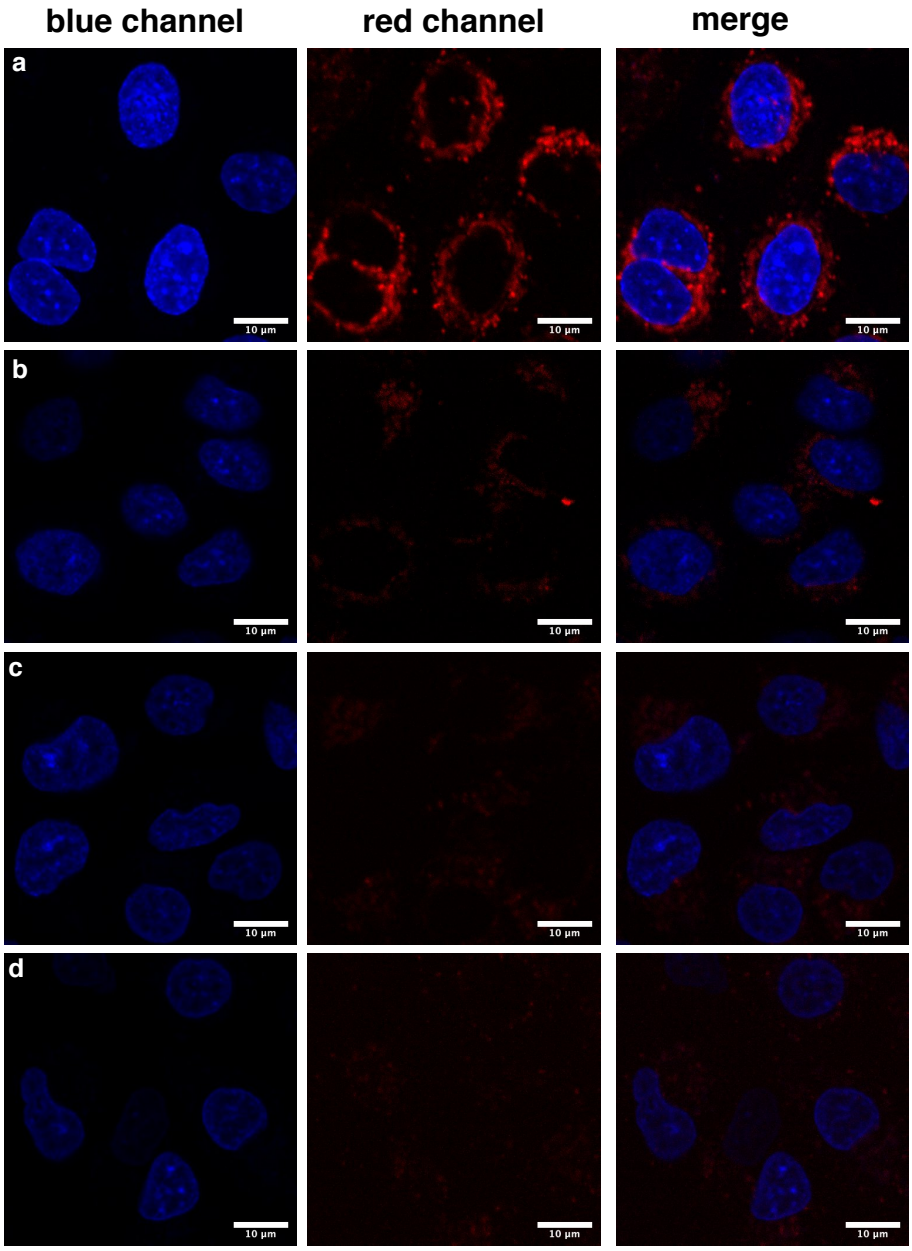


Figure S11. Confocal Imaging of SHIN-3 cells after incubation of **a. CyBam-γ-Glu** (20 µM; red channel), pretreatment of inhibitors for 2 h **b**. DON (1 mM) **c**. GGsTop (1 mM) followed by **CyBam-γ-Glu** (20 µM) for 2 h, and **d. CyBam-N.C** (20 µM) for 2 h. Nucleus stained using NucBlue (blue channel). Confocal imaging carried out at 63X oil immersed lens with 1.4 NA. The images were processed with identical brightness/ contrast using Fiji.

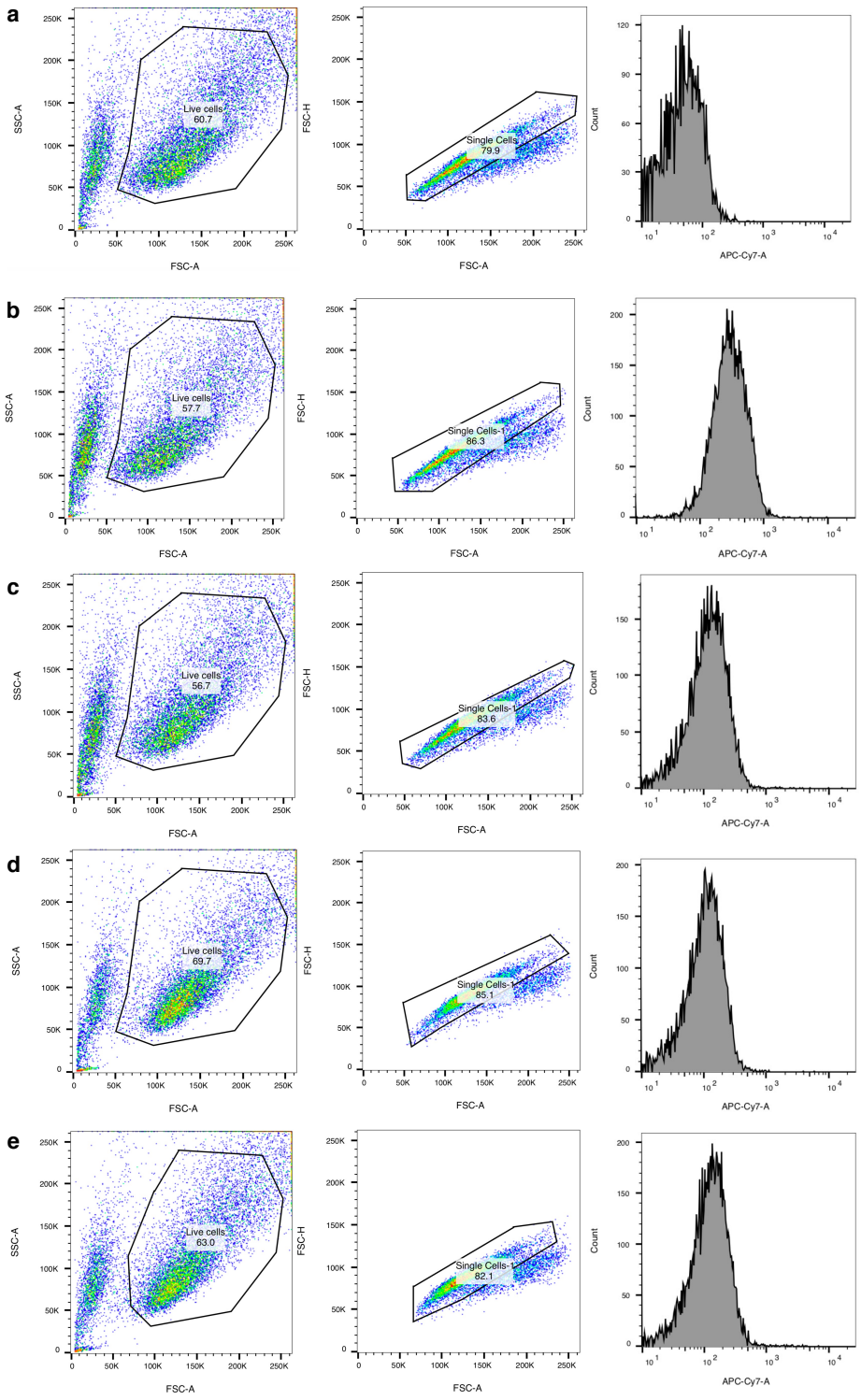


Figure S12. Flow cytometry analysis of uptake of **CyBam-γ-Glu** in SHIN-3 cells. A representative FlowJo analysis of the study provides gating used for live cells, single cells and histogram in APC-Cy7 channel of **a.** blank (cells only), **b. CyBam-γ-Glu**, **c. CyBam-N.C**, and covalent inhibition of GGT by preincubation with **d.** GGsTop, and **e.** DON.

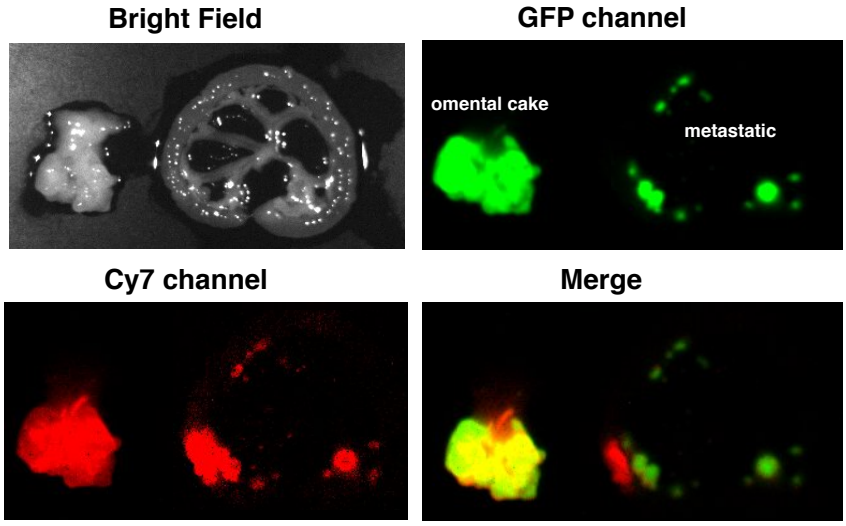


Figure S13. **CyBam-γ-Glu** (30 nmol) was injected intraperitoneally in a SHIN-3-ZsGreen metastatic tumor model. After 1 h, the mouse was euthanized, and tumors (omental cake and metastatic) were imaged. GFP ex 445 – 490 nm, em 500 – 720 nm; Cy7 ex 710 – 760 nm, em 780 – 950 nm. Green and red pseudo colors are used to represent signal from the GFP and Cy7 channels respectively.

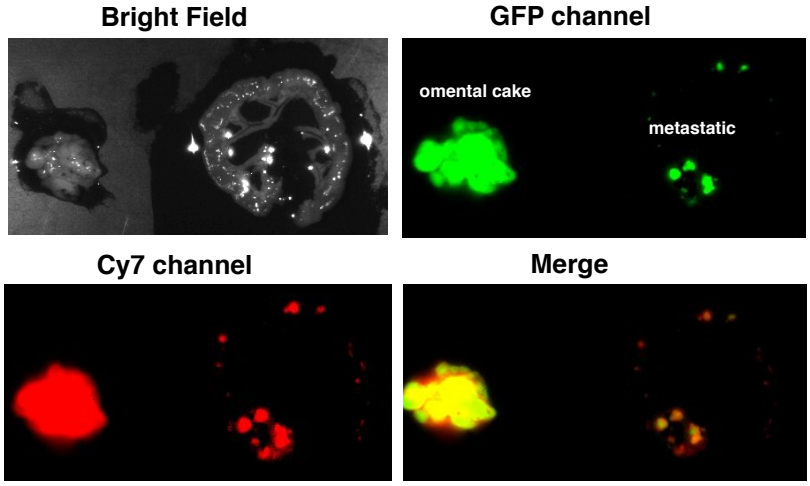


Figure S14. **CyBam-γ-Glu** (30 nmol) was injected intraperitoneally in a SHIN-3-ZsGreen metastatic tumor model. After 6 h, the mouse was euthanized, and tumors (omental cake and metastatic) were imaged. GFP ex 445 – 490 nm, em 500 – 720 nm; Cy7 ex 710 – 760 nm, em 780 – 950 nm. Green and red pseudo colors are used to represent signal from the GFP and Cy7 channels respectively.

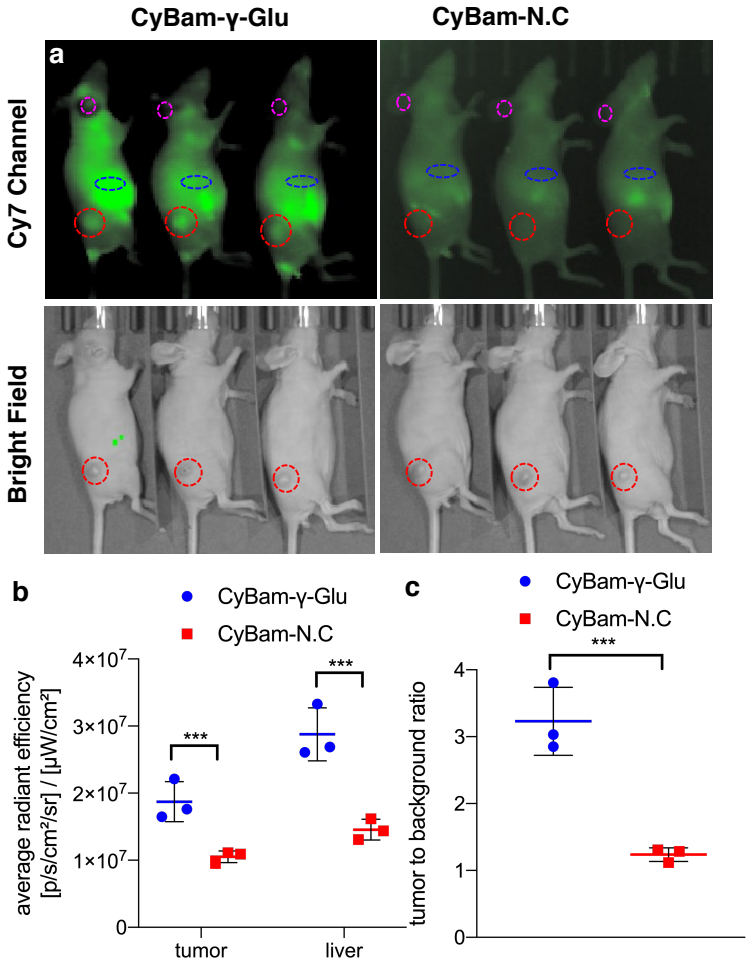


Figure S15. **a. CyBam-γ-Glu** and **CyBam-N.C** (25 nmol) were injected (in sterile 1X PBS pH 7.4) intravenously in mice containing MDA-MB-468 xenograft tumors. Mice were imaged after 4 h in bright field and Cy7 channel (ex/em filter 745/800 nm). Quantification of **b.** fluorescent signal emitting from tumors and **c.** tumor to background ratio in mice by drawing ROIs. Signal from tumors, liver and background in mice are shown in red, blue and magenta dotted circles. Green pseudo colors are used to represent fluorescent signal from and Cy7 channel. *p*-values were evaluated by student t-test (*** *p*-value ≤ 0.001).

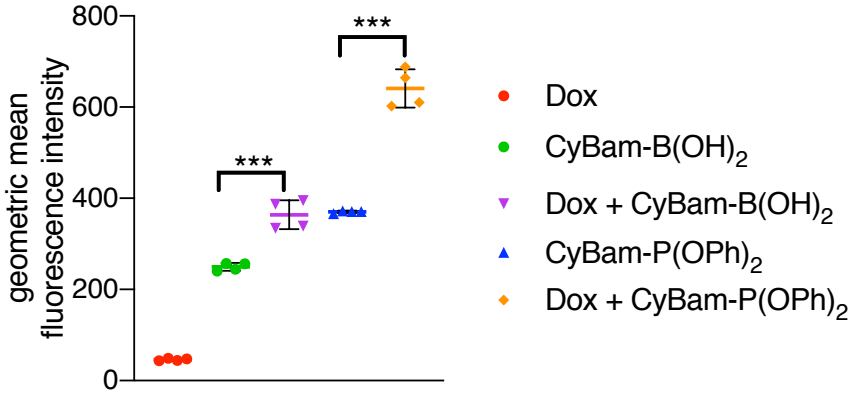
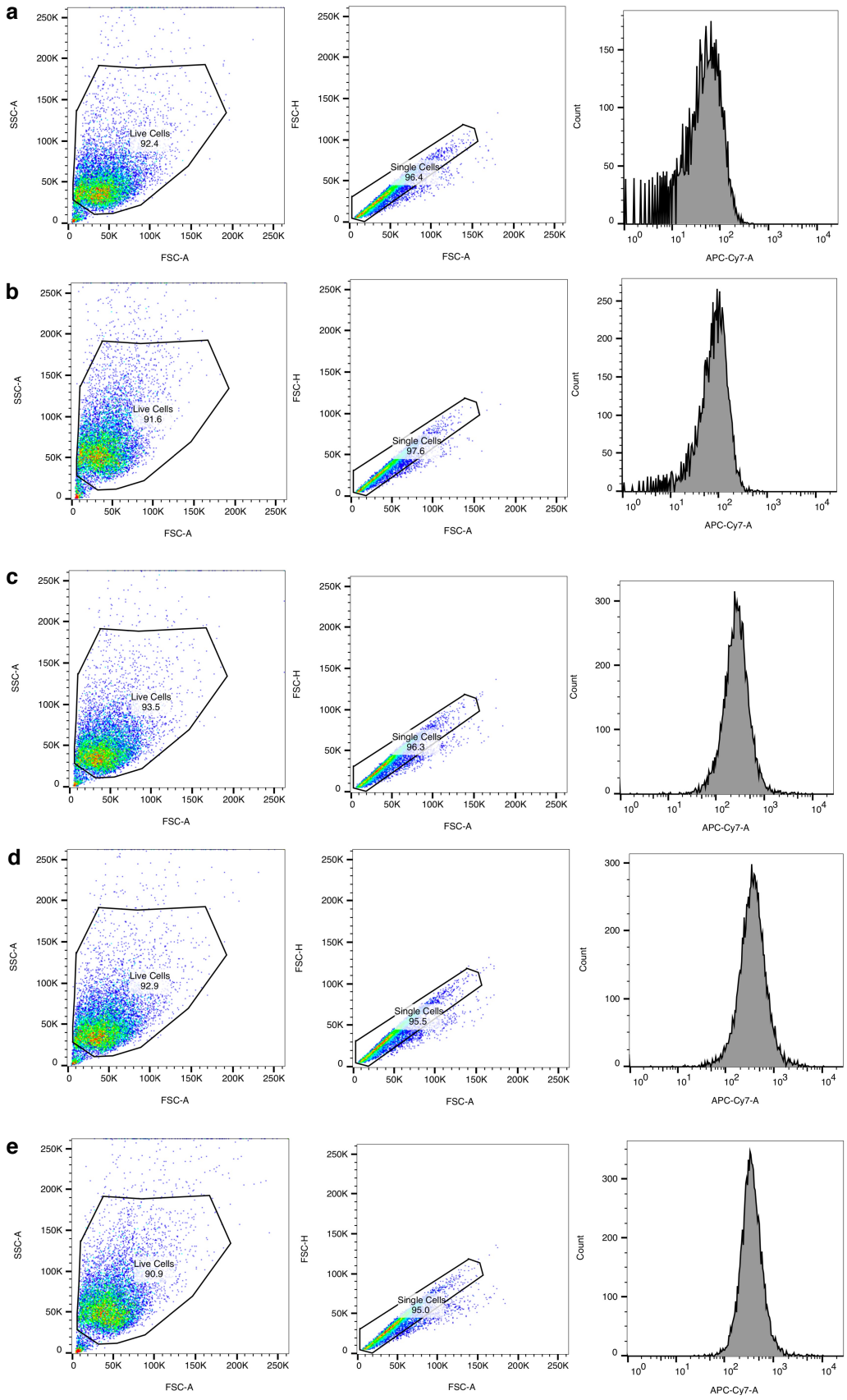


Figure S16. Flow Cytometric quantification of generation of reactive oxygen species (ROS) in PC-3 (prostate cancer cells) after co-incubation of doxorubicin (20 μM) with **CyBam-B(OH)$_2$** and **CyBam-P(OPh)$_2$** (20 μM) for 12 h. Geometric mean fluorescent intensity (± SD) of fluorescent signal in the cells is shown (n = 4 independent experiments) and *p*-values were evaluated by student t-test (*** *p*-value ≤ 0.001).


a
SSC-A
FSC-A
Live Cells
92.4
FSC-H
Single Cells
96.4
Count
APC-Cy7-A
b
Live Cells
91.6
Single Cells
97.6
c
Live Cells
93.5
Single Cells
96.3
d
Live Cells
92.9
Single Cells
95.5
e
Live Cells
90.9
Single Cells
95.0

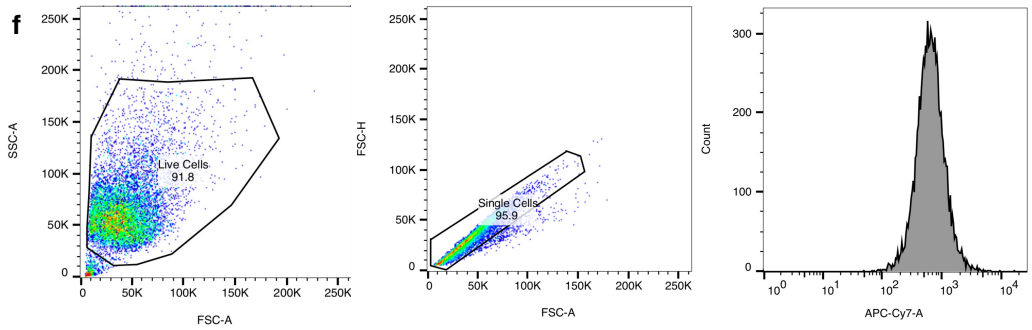
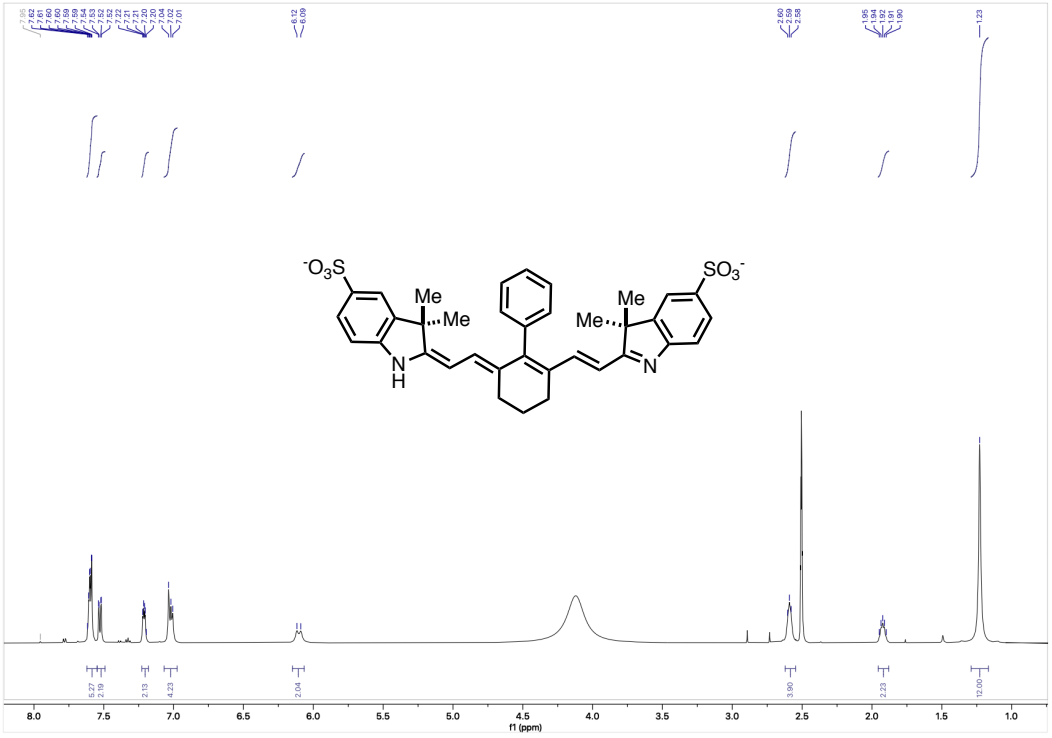
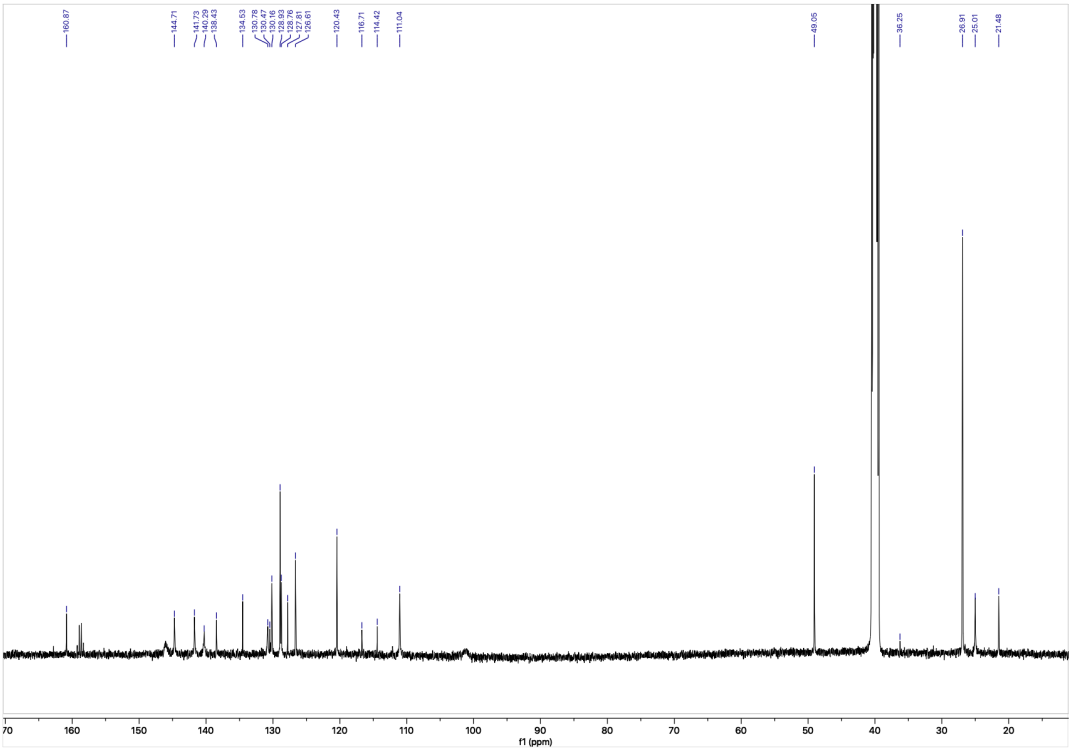


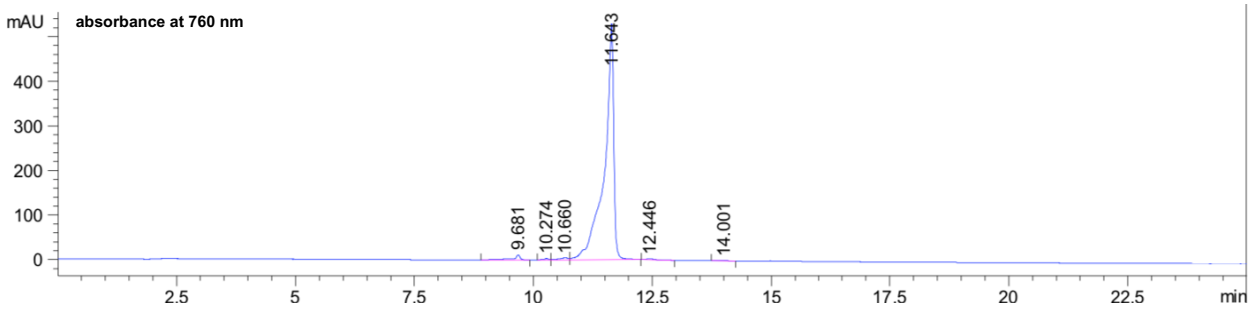
Figure S17. Flow cytometry analysis of generation of reactive oxygen species in PC-3 cells. A representative FlowJo analysis of the study provides gating used for live cells, single cells and histogram in APC-Cy7 channel of **a.** blank (cells only), **b.** Dox, **c. CyBam-B(OH)$_2$**, **d. CyBam-P(OPh)$_2$**, **e. Dox + CyBam-B(OH)$_2$**, **f. Dox + CyBam-P(OPh)$_2$**.

G. NMR and HPLC spectra

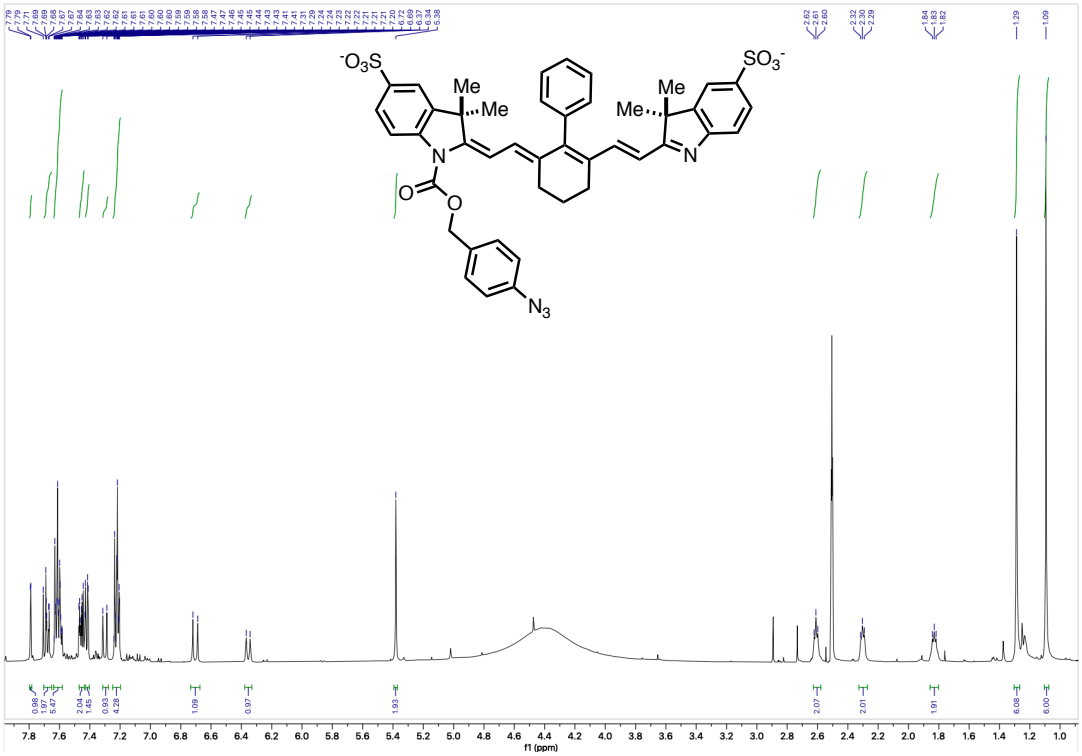
NorCy7.

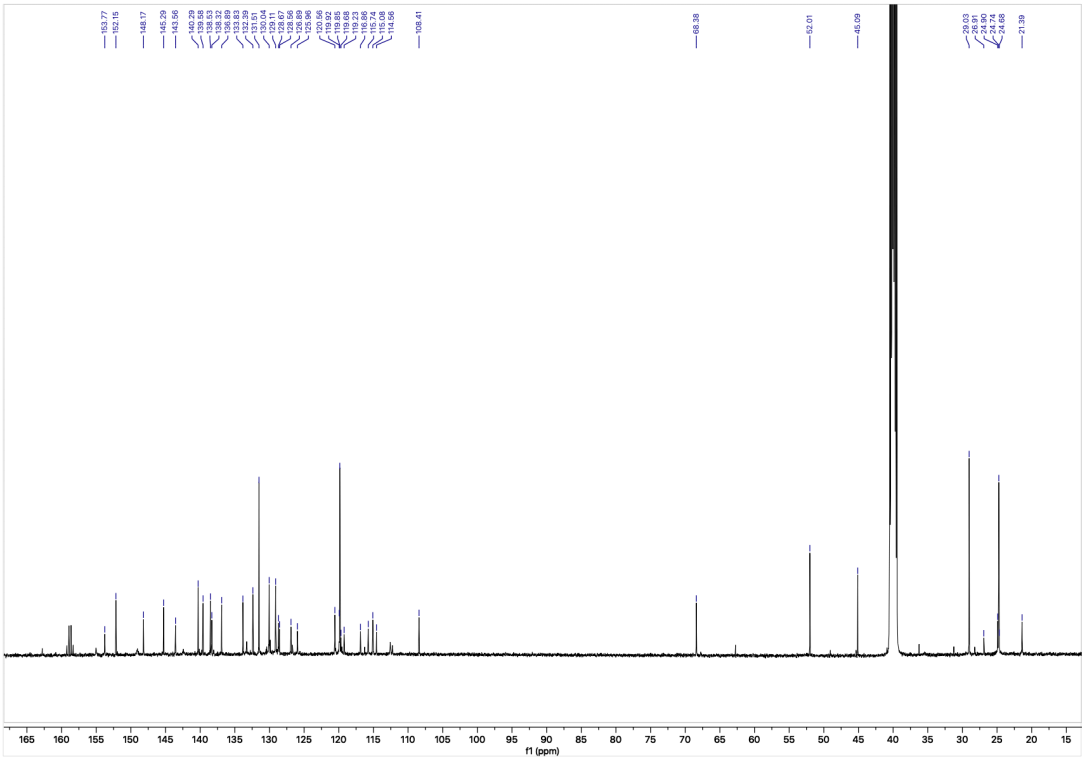


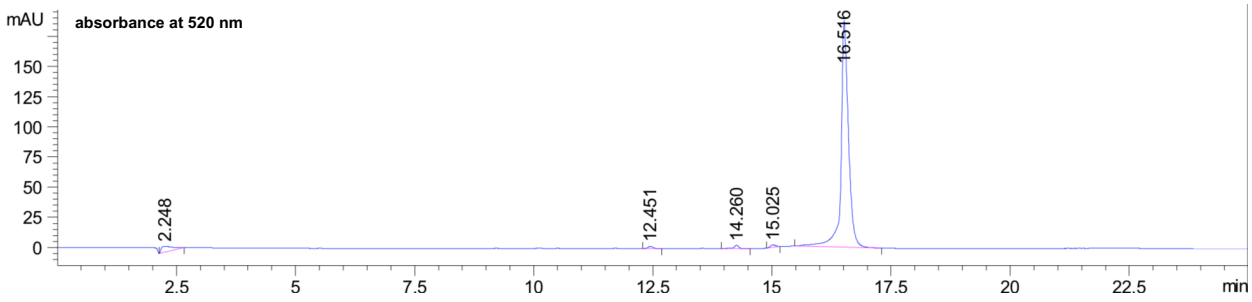



mAU
absorbance at 760 nm
400
300
200
100
0
11.643
9.681
10.274
10.660
12.446
14.001
2.5
5
7.5
10
12.5
15
17.5
20
22.5
min

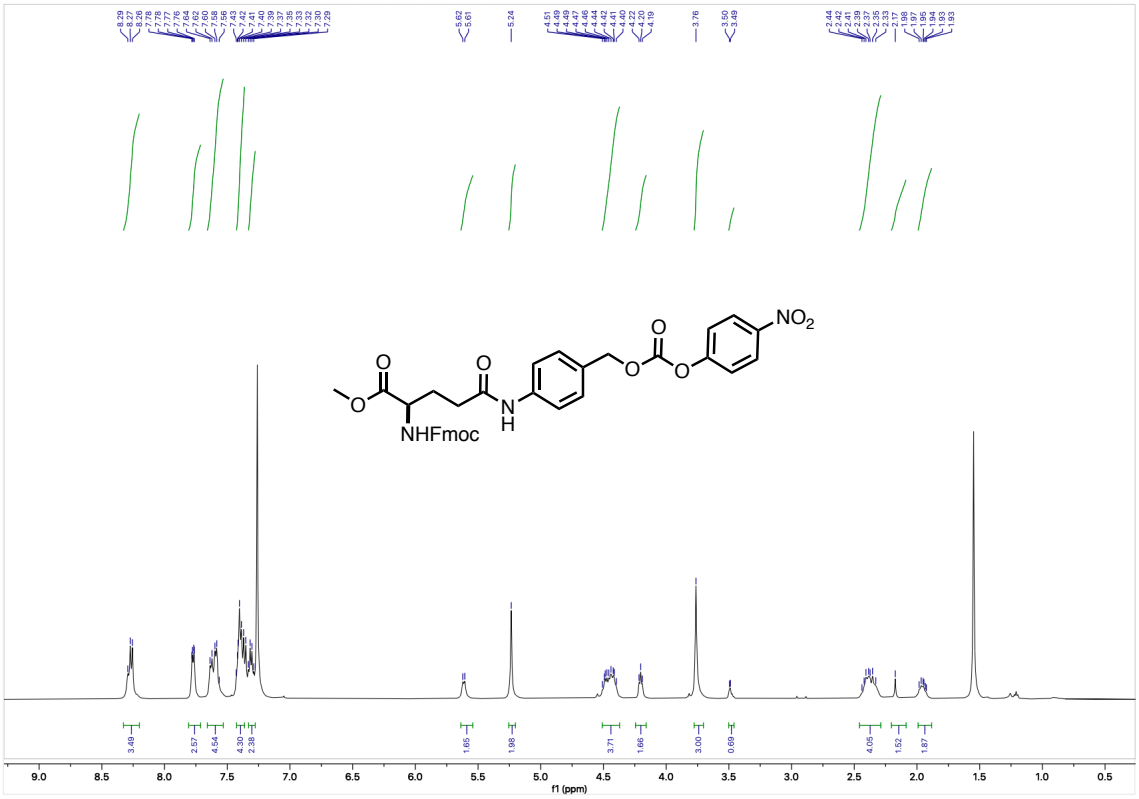
CyBam-N_3.

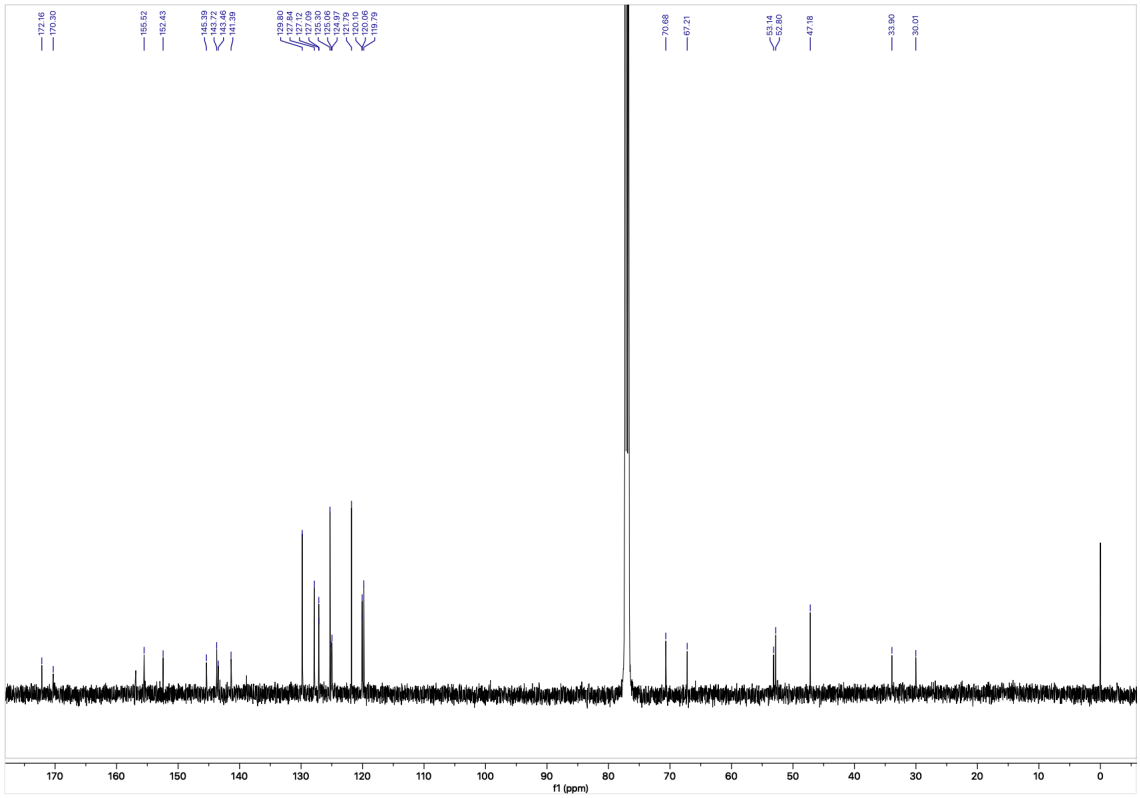




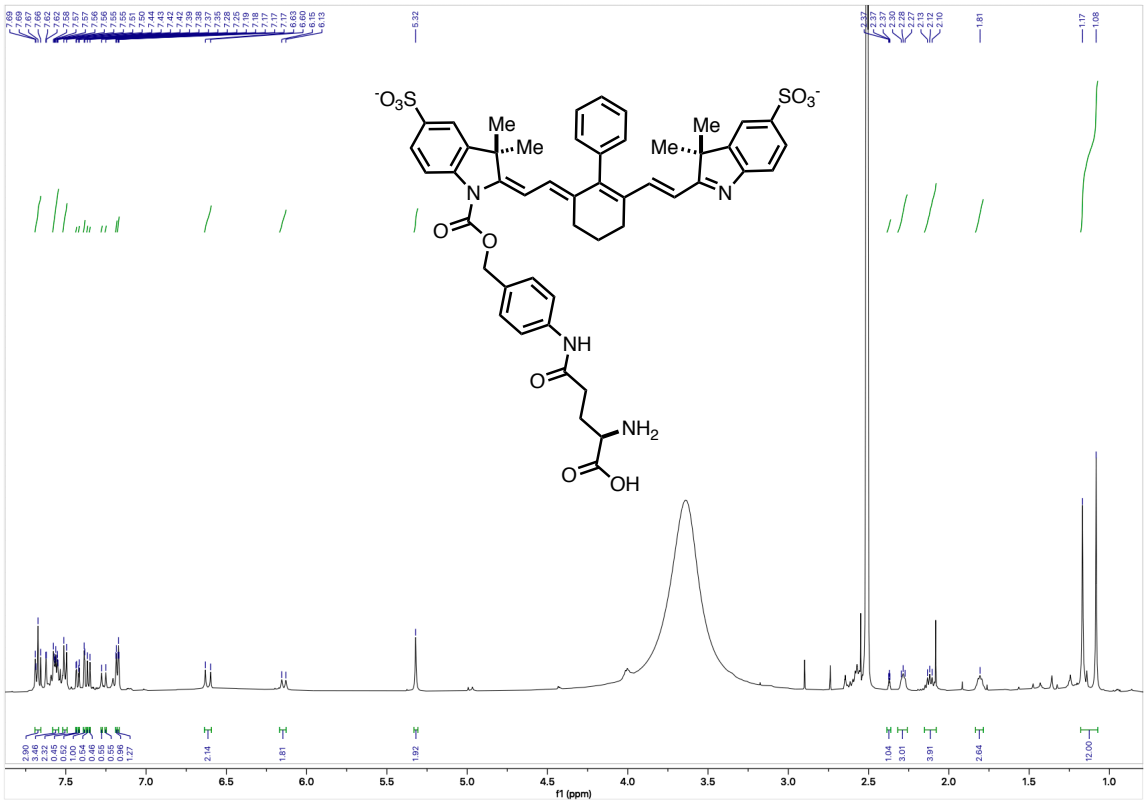

mAU
absorbance at 520 nm
150
125
100
75
50
25
0
2.248
12.451
14.260
15.025
16.516
2.5
5
7.5
10
12.5
15
17.5
20
22.5
min

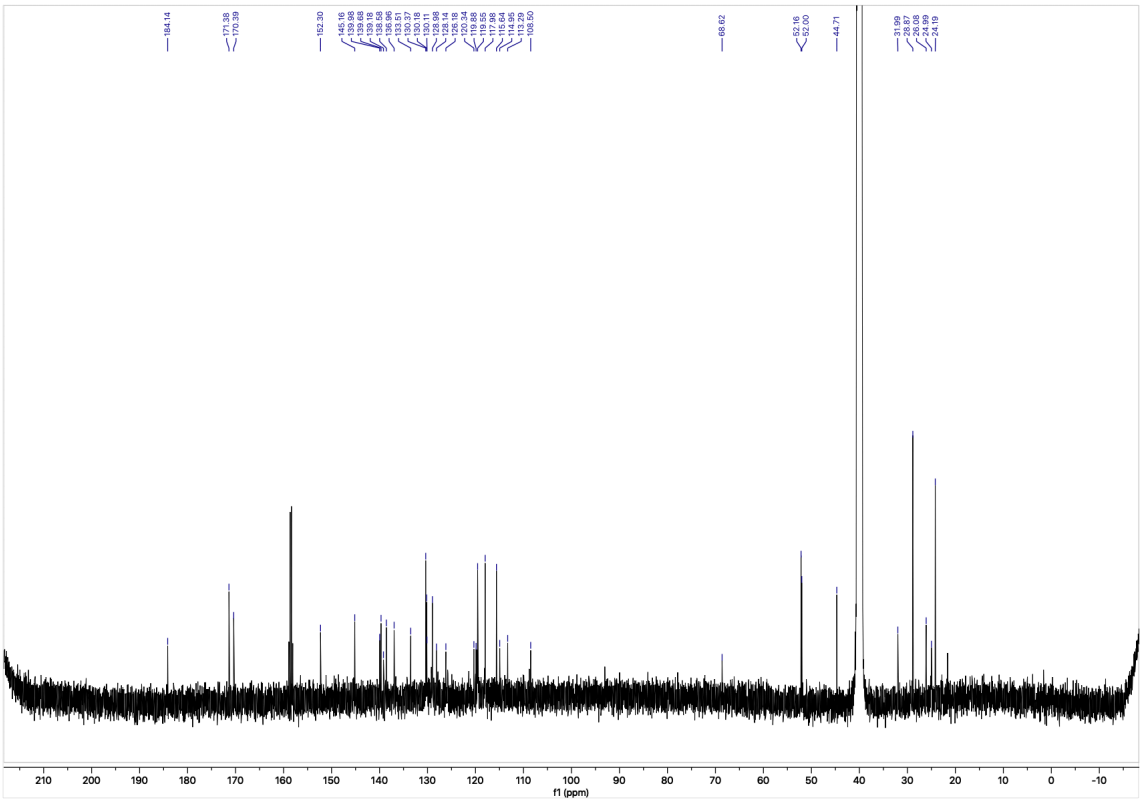
Compound S4.

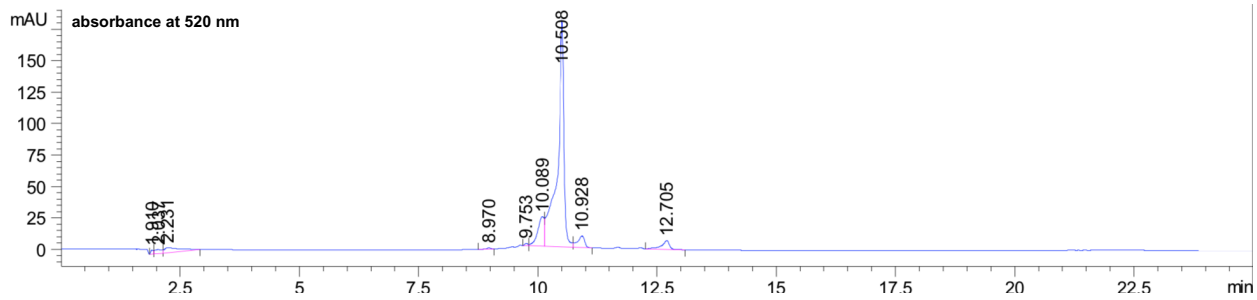




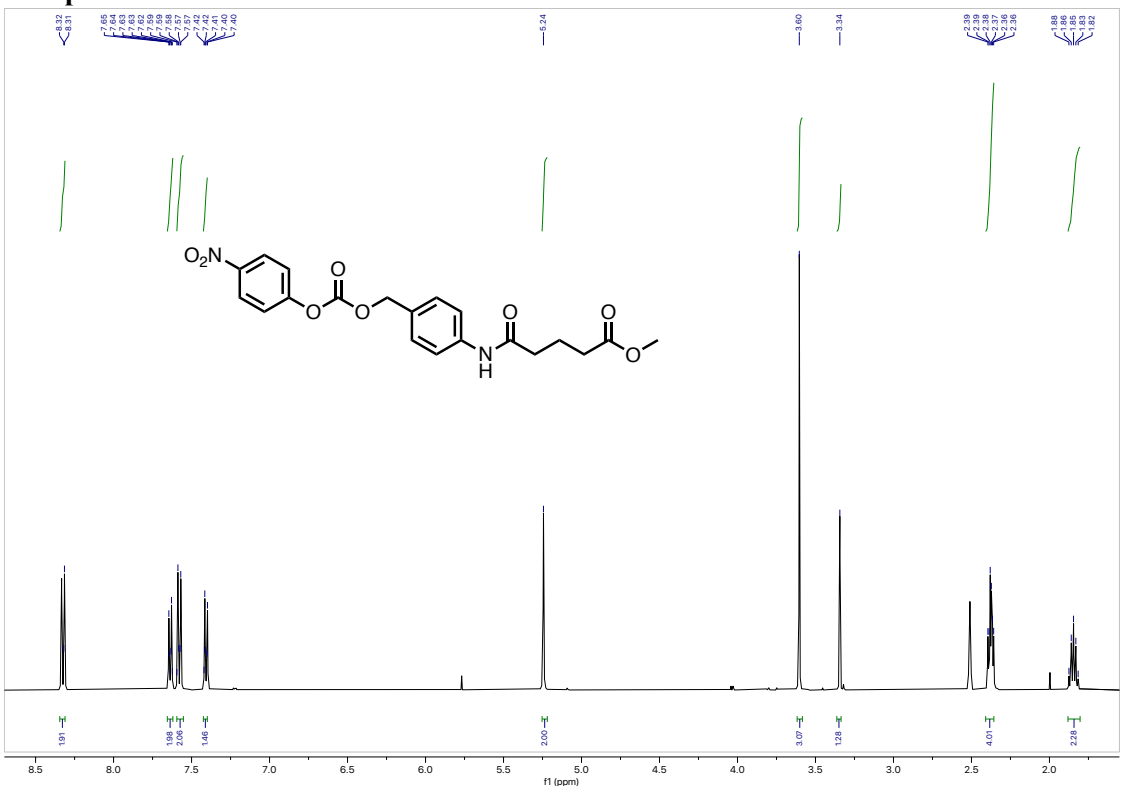
CyBam-γ-Glu.

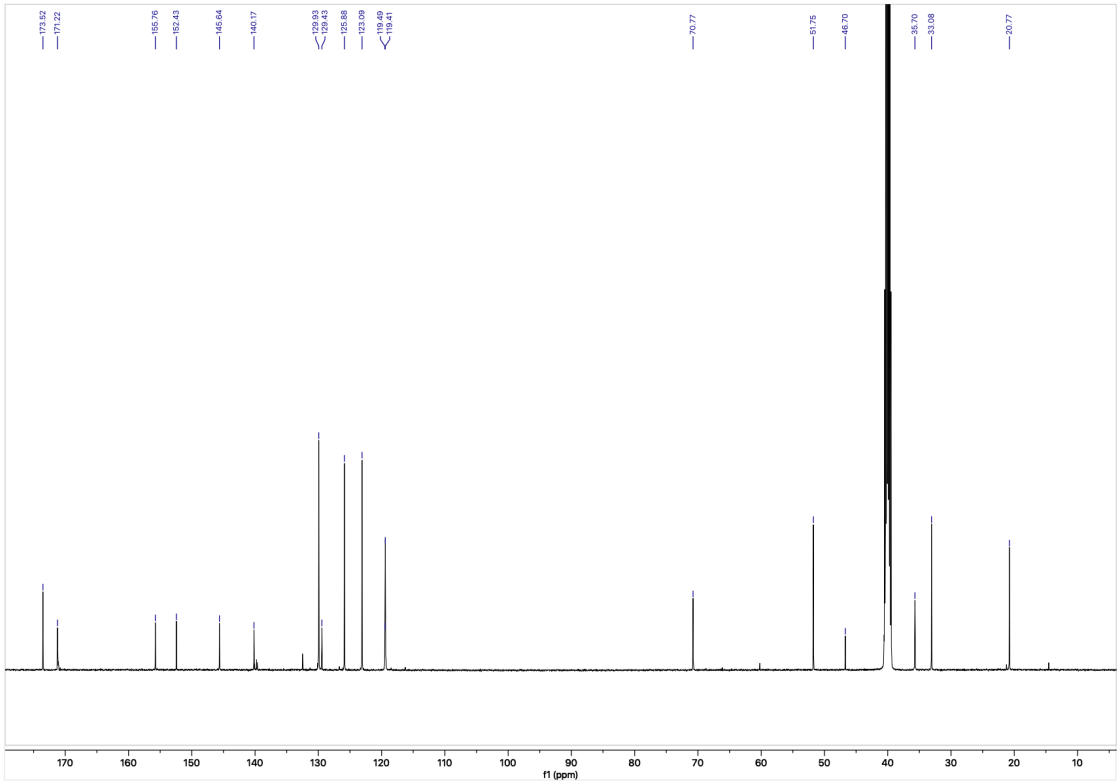




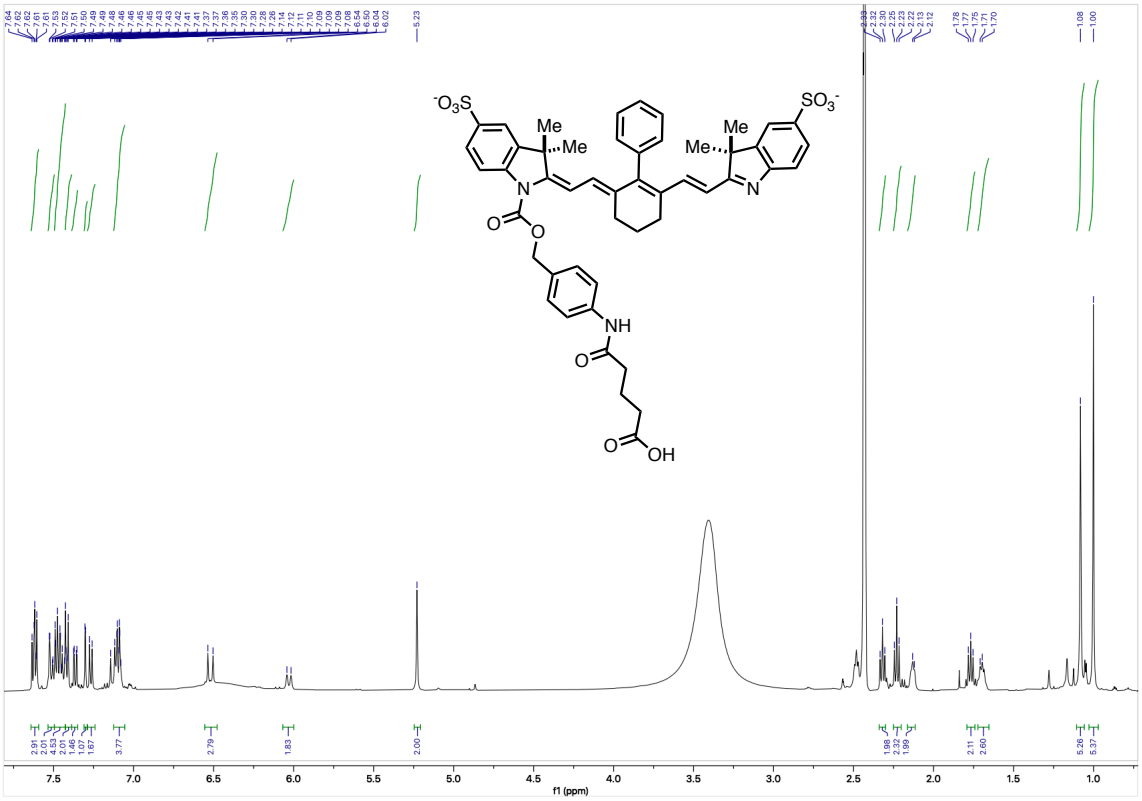

mAU
absorbance at 520 nm
150
125
100
75
50
25
0
2.231
8.970
9.753
10.089
10.508
10.928
12.705
2.5
5
7.5
10
12.5
15
17.5
20
22.5
min

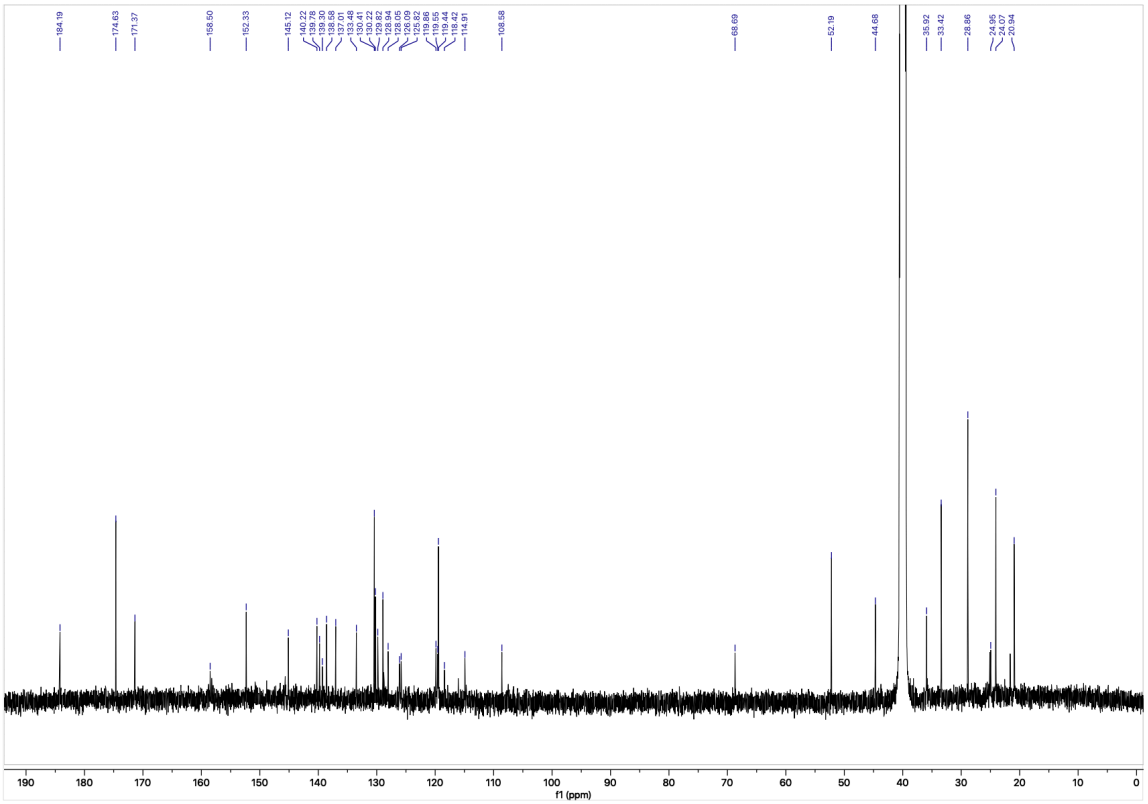
Compound S6.

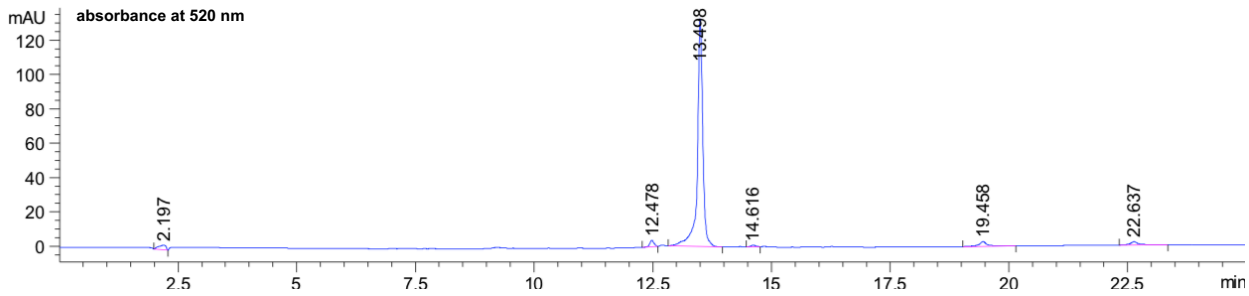

8.32
8.31
7.65
7.64
7.63
7.62
7.59
7.58
7.57
7.42
7.41
7.40
5.24
3.60
3.34
2.39
2.38
2.37
2.36
1.88
1.86
1.85
1.83
1.82
O2N
O
O
O
O
N
H
O
O
1.91
1.98
2.06
1.46
2.00
3.07
1.28
4.01
2.29
f1 (ppm)


173.52
171.22
155.76
152.43
145.64
140.17
129.93
129.43
125.88
123.09
119.49
119.41
70.77
51.75
46.70
35.70
33.08
20.77
f1 (ppm)

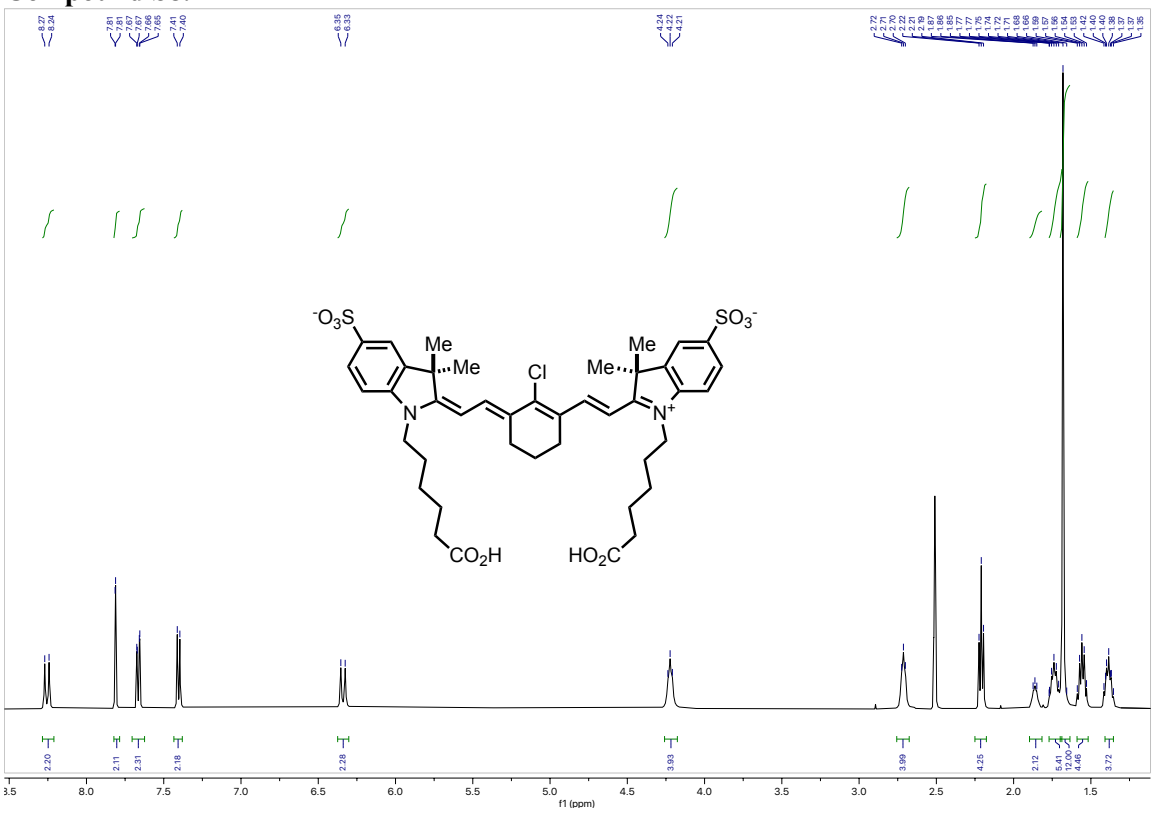
CyBam-N.C.

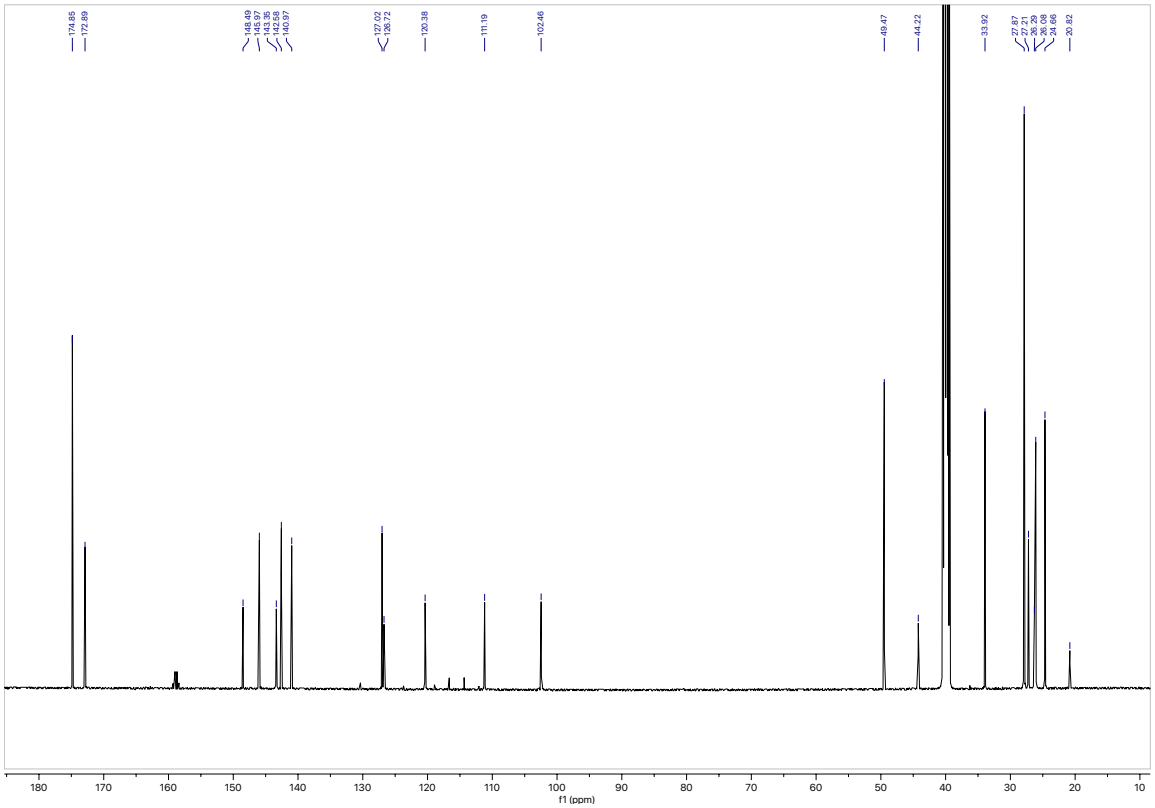


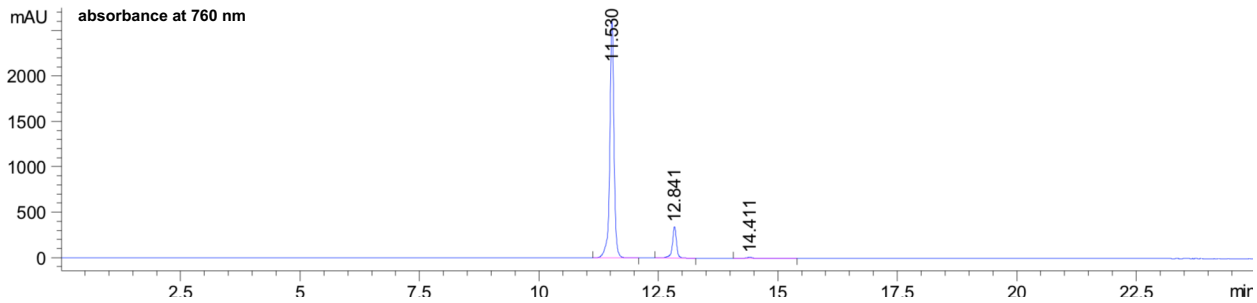



absorbance at 520 nm
mAU
120
100
80
60
40
20
0
2.197
12.478
13.498
14.616
19.458
22.637
2.5
5
7.5
10
12.5
15
17.5
20
22.5
min

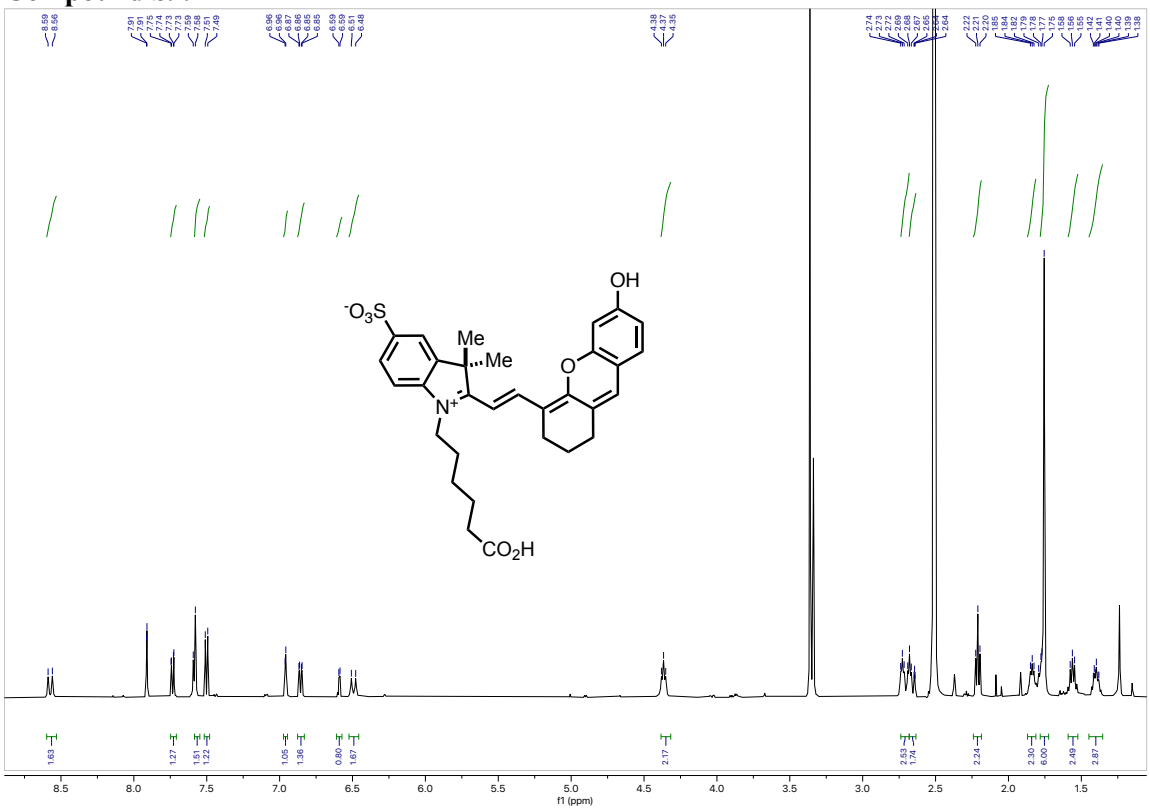
Compound S8.

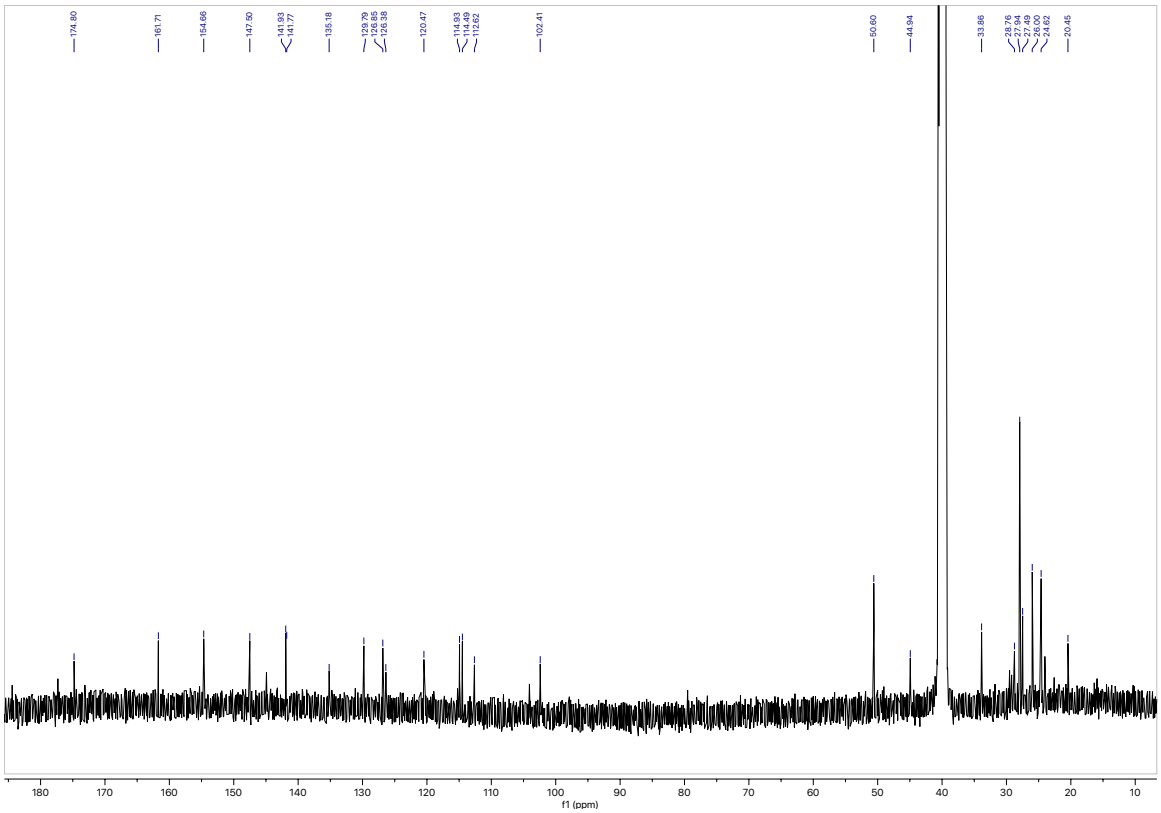


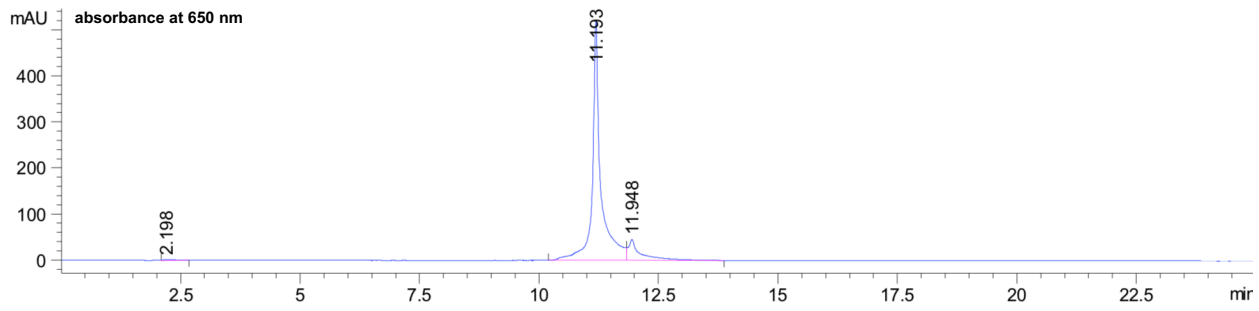



mAU
absorbance at 760 nm
2000
1500
1000
500
0
11.530
12.841
14.411
2.5
5
7.5
10
12.5
15
17.5
20
22.5
min

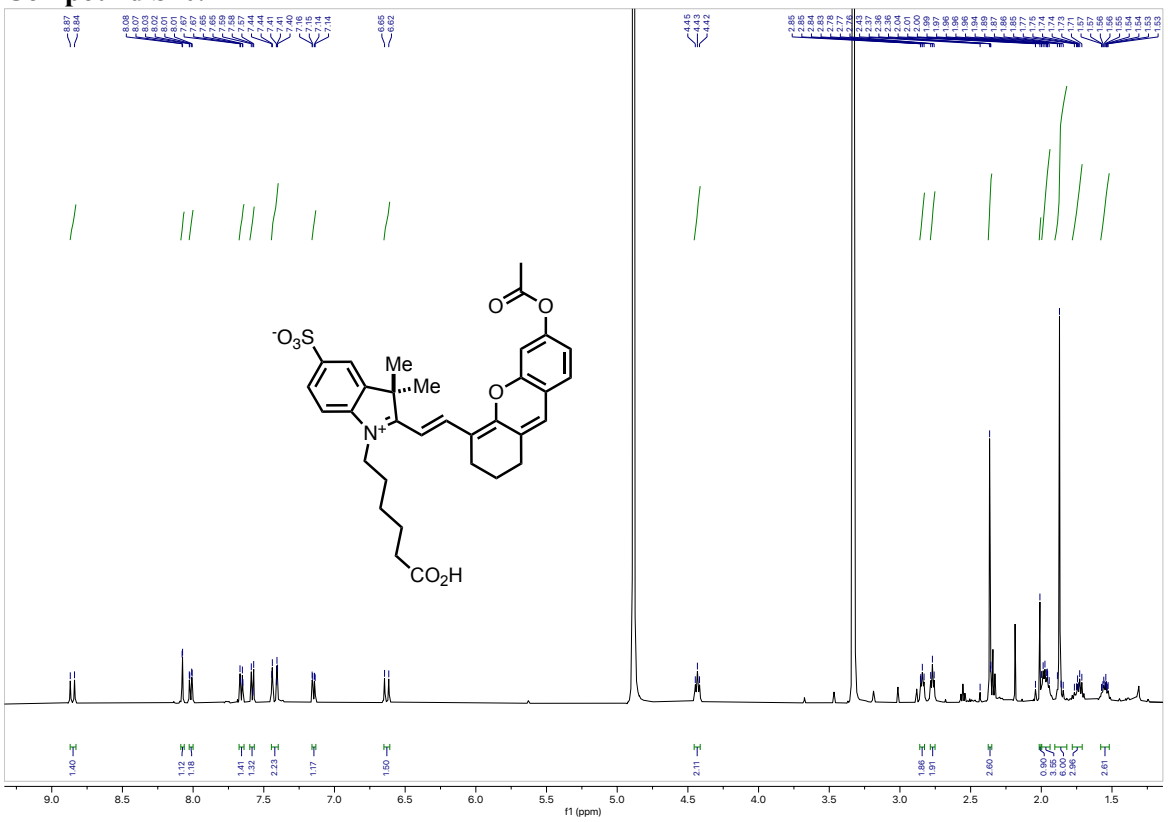
Compound S9.

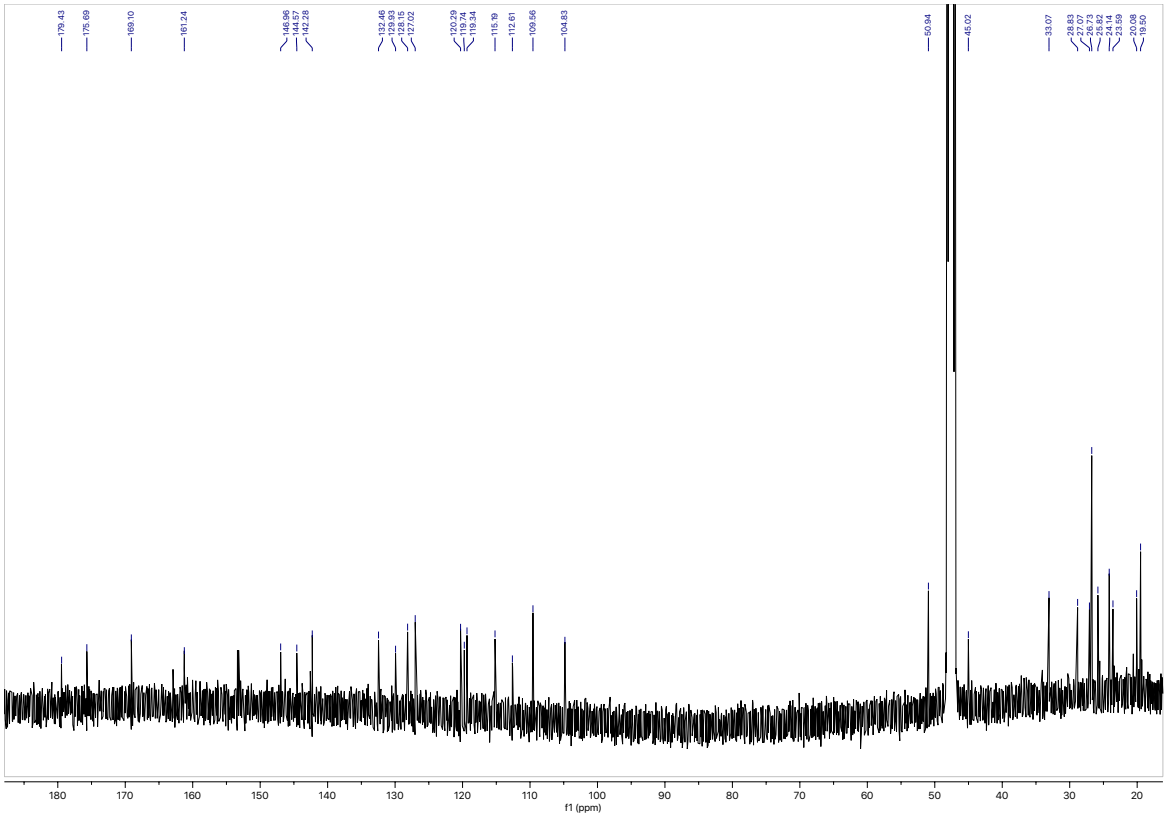


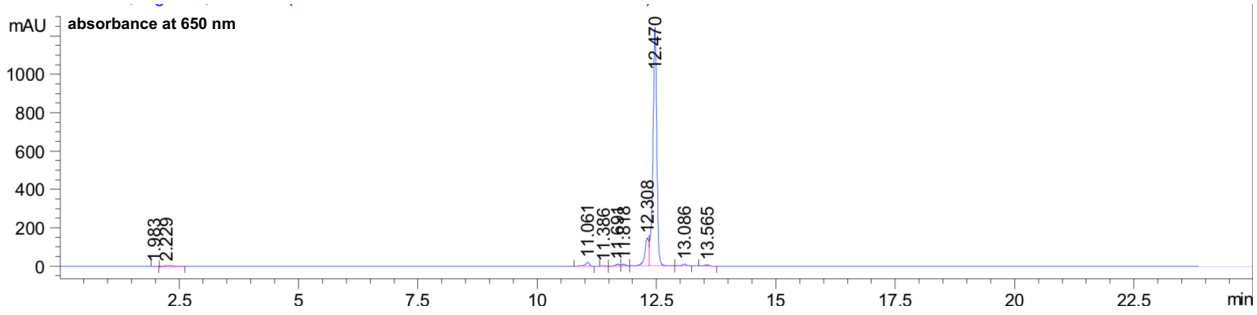



mAU
absorbance at 650 nm
400
300
200
100
0
11.193
11.948
2.198
2.5
5
7.5
10
12.5
15
17.5
20
22.5
min

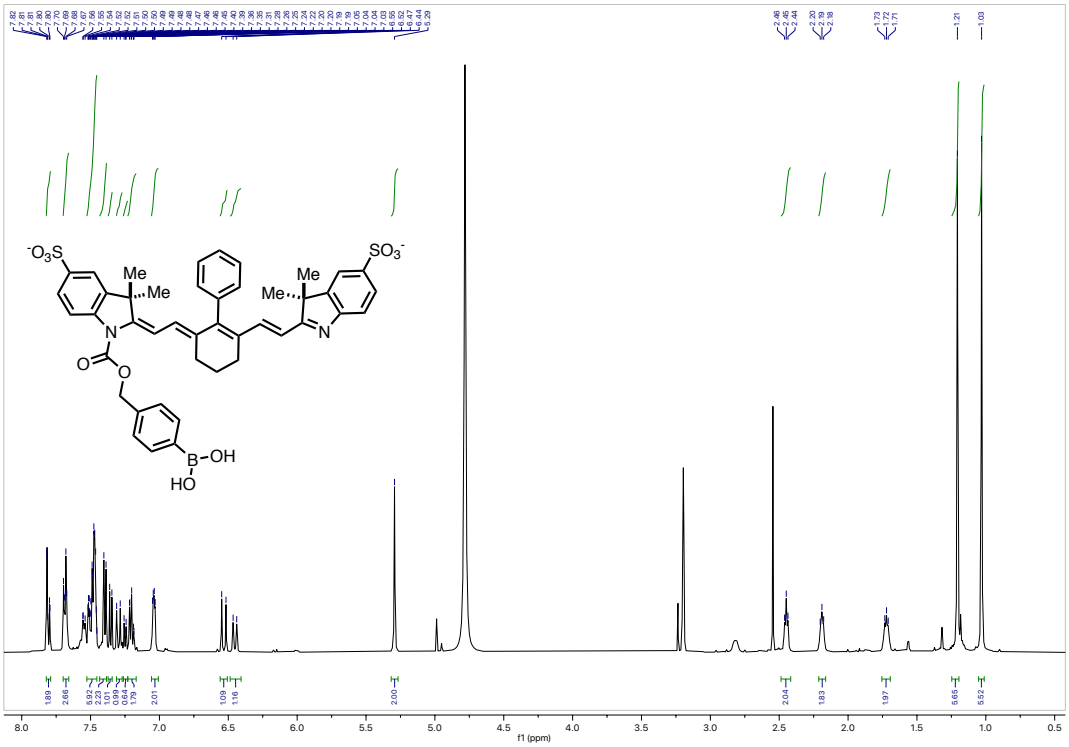
Compound S10.

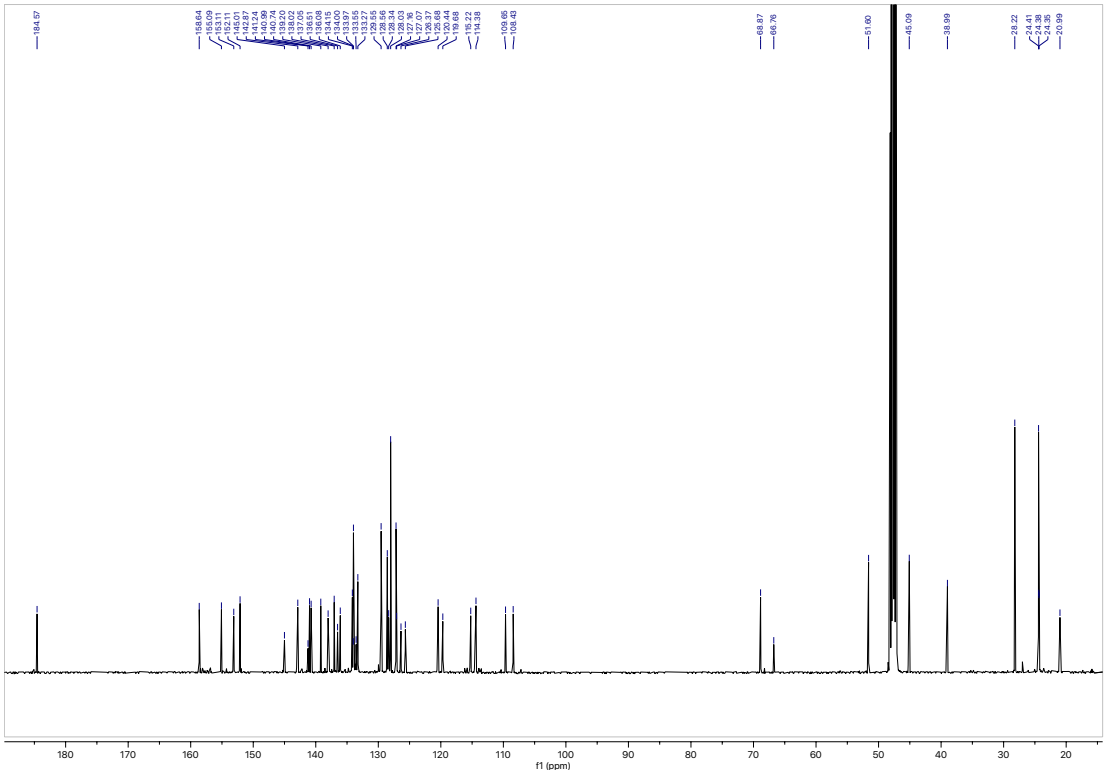


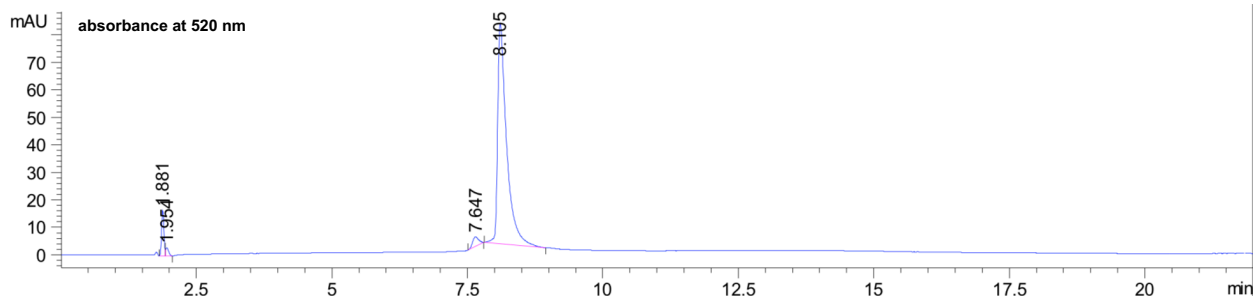



mAU
absorbance at 650 nm
1000
800
600
400
200
0
1.983
2.229
11.061
11.386
11.614
11.694
12.308
12.470
13.086
13.565
2.5
5
7.5
10
12.5
15
17.5
20
22.5
min

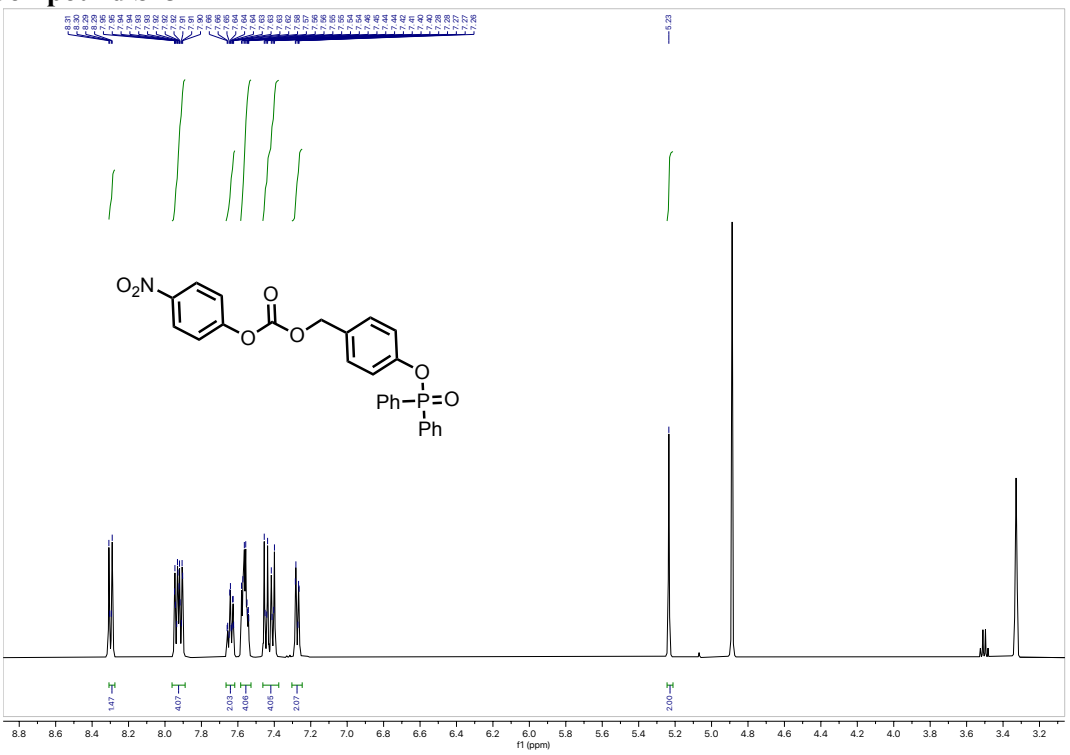
CyBam-(BOH)$_2$

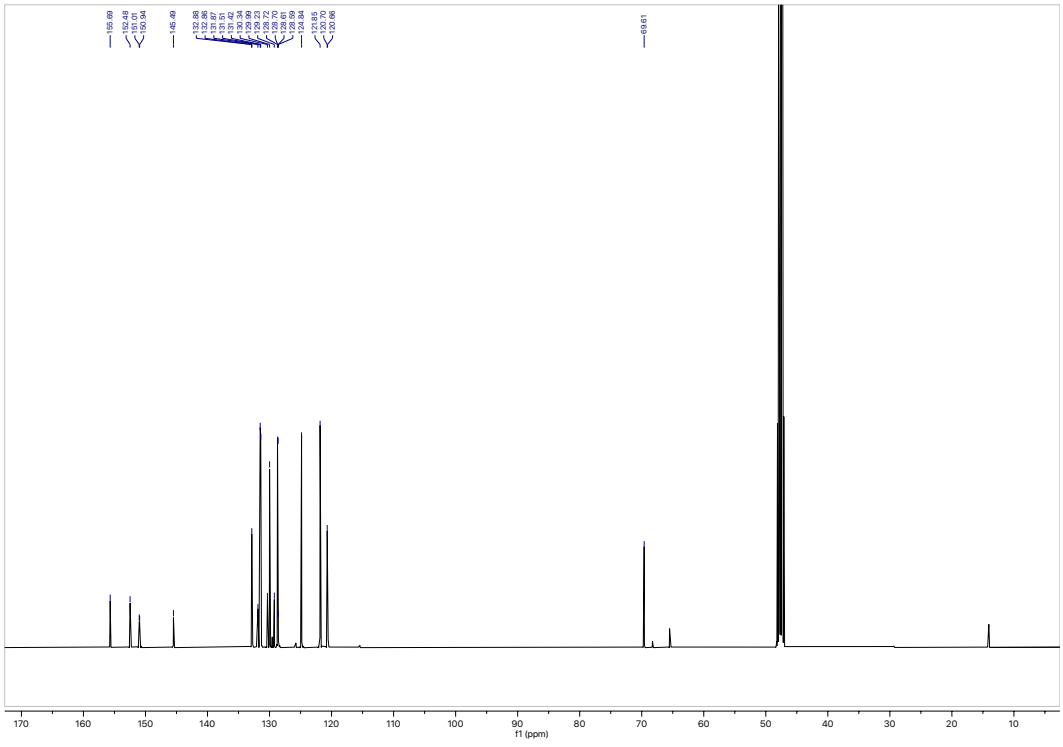




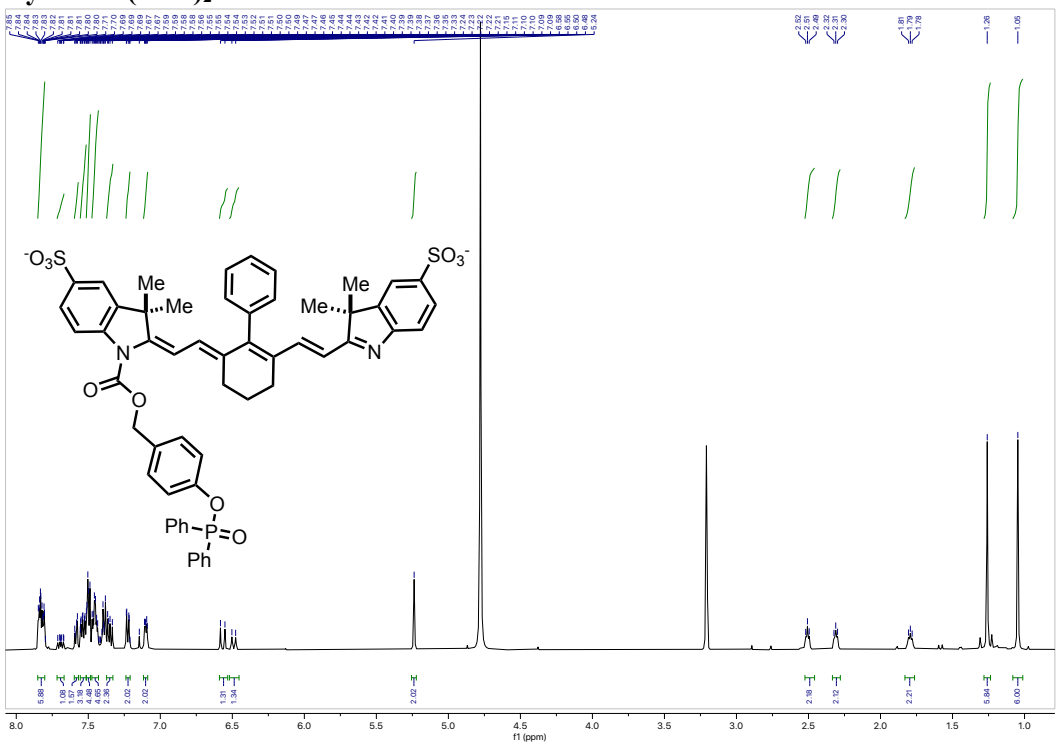

mAU
absorbance at 520 nm
70
60
50
40
30
20
10
0
1.954
1.881
7.647
8.105
2.5
5
7.5
10
12.5
15
17.5
20
min

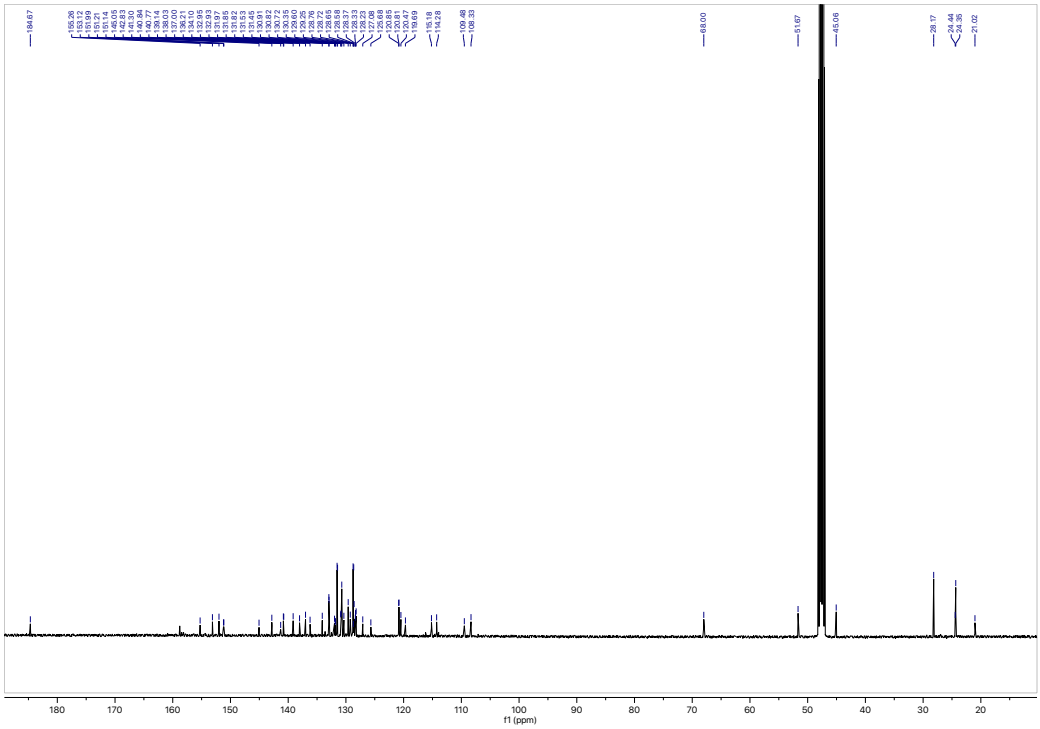
Compound S13

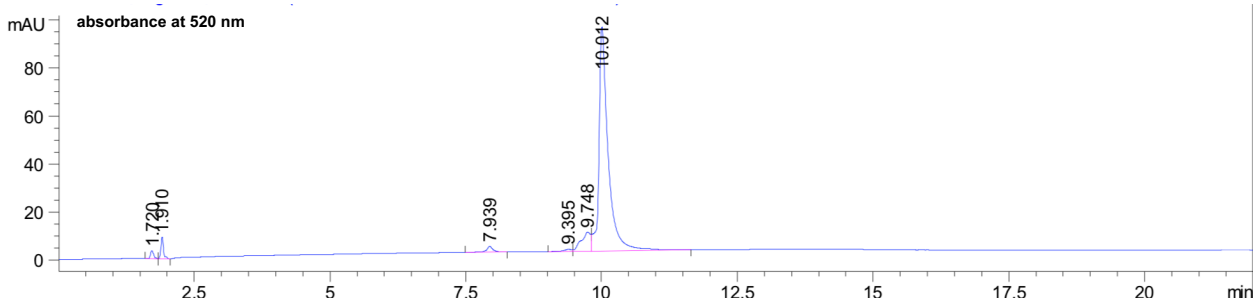




CyBam-P(OPh)$_2$






absorbance at 520 nm
mAU
80
60
40
20
0
1.720
1.910
7.939
9.395
9.748
10.012
2.5
5
7.5
10
12.5
15
17.5
20
min

H. References

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