Supporting Information

Intramolecular Substitution Uncages Fluorogenic Probes for Detection of Metallo-Carbapenemase-Expressing Bacteria

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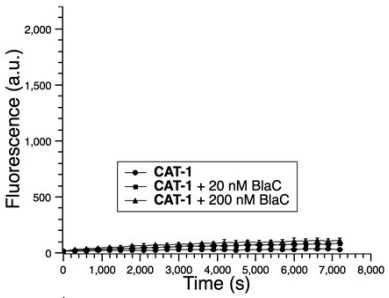
General Information

Chemicals were purchased from commercial suppliers and used without purification. The ^{1}H and ^{13}C NMR spectra were taken on Varian 400 MHz magnetic resonance spectrometer. Data for ^{1}H NMR spectra are reported as follows: chemical shifts are reported as δ in units of parts per million (ppm); multiplicities are reported as follows: s (singlet), d (doublet), t (triplet), q (quartet), dd (doublet of doublets), m (multiplet), or br (broadened); coupling constants are reported as a J value in Hertz (Hz); the number of protons (n) for a given resonance is indicated nH, and based on the spectral integration values. Analytical TLC was performed with 0.25 mm silica gel 60F plates with fluorescent indicator (254 nm or 365 nm). HPLC was performed on a Dionex HPLC System (Dionex Corporation) equipped with a GP50 gradient pump and an inline diode array UV-Vis detector. A reversed-phase C18 (Phenomenax, 5 μm, 10 x 250 mm or Dionex, 5 μm, 4.6 x 250 mm) column was used with a CH_3CN (B) / H_2O (A) gradient mobile phase containing 0.1% trifluoroacetic acid at a flow of 1 mL/min for the analysis. Fluorescence spectra were obtained on a Fluoromax-3 spectrafluorometer (Jobin Yvon). Kinetic experiments were performed in a M1000 microplate reader (TECAN, research triangle park, NC). *K. pneumonia* with SHV-18, *E. cloacae* with AmpC, *K. pneumoniae* with KPC, and *E. coli* with NDM-1 lysates were kindly provided by Dr. Niaz Banaei from the Stanford Medical Center Microbiology Lab.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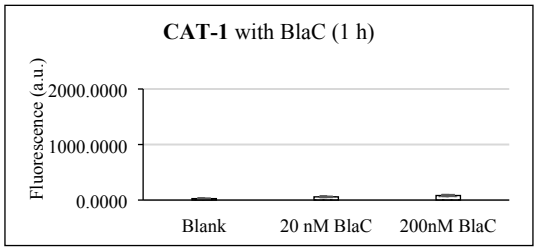
CAT-1 + 200 nM BlaC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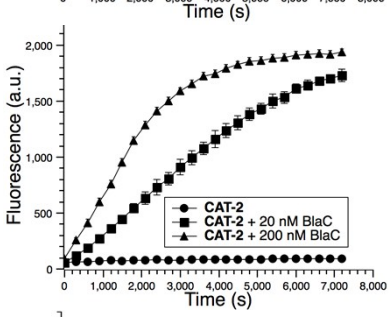
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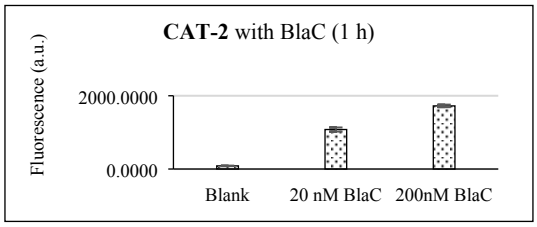
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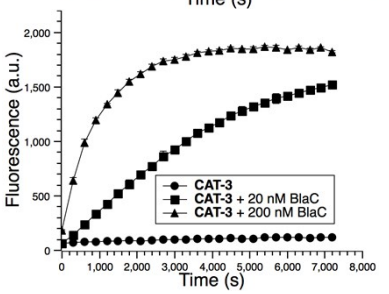
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Time (s)
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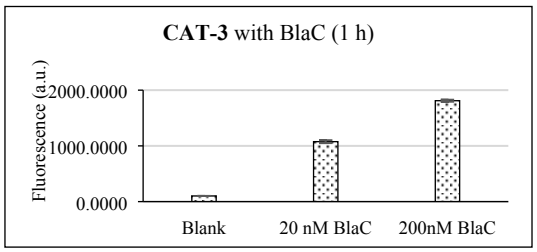
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Blank
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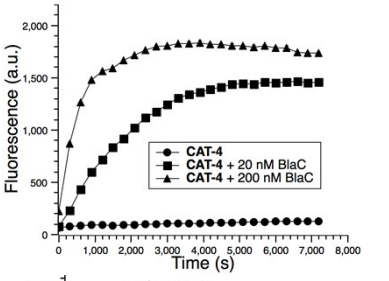
200nM BlaC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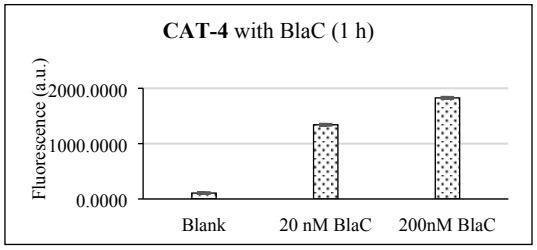
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Blank
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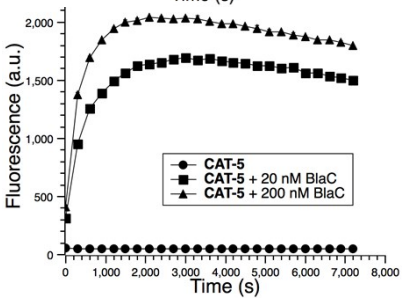
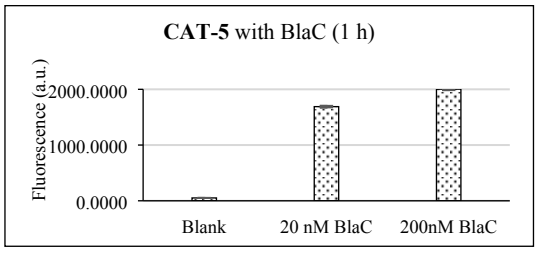
200nM BlaC


Fluorescence (a.u.)
Time (s)
CAT-5
CAT-5 + 20 nM BlaC
CAT-5 + 200 nM BlaC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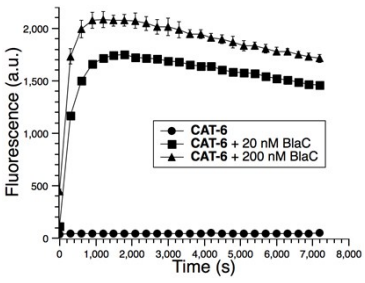
0.0000
Blank
20 nM BlaC
200nM BlaC


Fluorescence (a.u.)
Time (s)
CAT-6
CAT-6 + 20 nM BlaC
CAT-6 + 200 nM BlaC

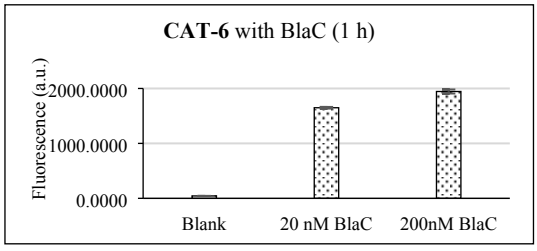
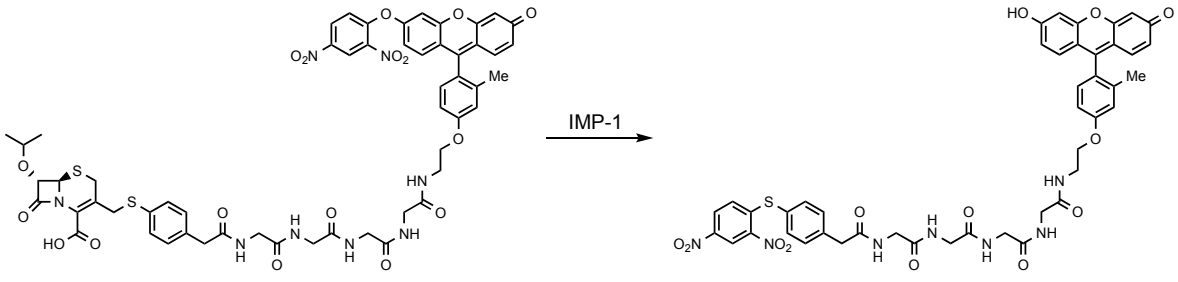

CAT-6 with BlaC (1 h)
Fluorescence (a.u.)
2000.0000
1000.0000
0.0000
Blank
20 nM BlaC
200nM BlaC

Fig. S1 Time-dependent fluorescence response of **CAT-1** to **CAT-6** (10 μM) in the absence or presence of BlaC (20 nM or 200 nM) in PBS (pH = 7.4) at 22 °C. Fluorescence data was collected with excitation at λ = 490 nm (band width 5 nm) and emission at λ = 510 nm (band width 5 nm). Error bars are ± SD.

Table S1 Kinetic parameters of fluorescent probes in the presence of BlaC[a]

Name	K_m [μM]	k_{cat} [s^{-1}]	k_{cat}/K_m [$\times 10^4$ s^{-1} M^{-1}]	k_{uncat} [$\times 10^{-8}$ s^{-1}]
CAT-1	20.4 ± 13	0.005 ± 0.1	0.029 ± 0.04	4.6 ± 0.0
CAT-2	18.3 ± 2.9	0.15 ± 0.0	0.85 ± 0.06	8.5 ± 1.3
CAT-3	6.8 ± 0.65	0.65 ± 0.58	9.5 ± 8.1	11.0 ± 2.8
CAT-4	3.1 ± 1.0	0.8 ± 0.1	27 ± 6.8	11.0 ± 1.7
CAT-5	2.1 ± 1.0	0.75 ± 0.1	39 ± 12.4	1.3 ± 0.2
CAT-6	2.6 ± 1.1	1.4 ± 0.13	58 ± 18.2	1.6 ± 0.0

[a] Kinetic data were measured in PBS buffer (100 mM, pH = 7.4) at room temperature (22°C) on plate reader with excitation at λ = 490 nm (band width 5 nm) and emission at λ = 510 nm (band width 5 nm). k_{uncat} measures the spontaneous hydrolysis rate in PBS. All data indicate averages of three replicate experiments.



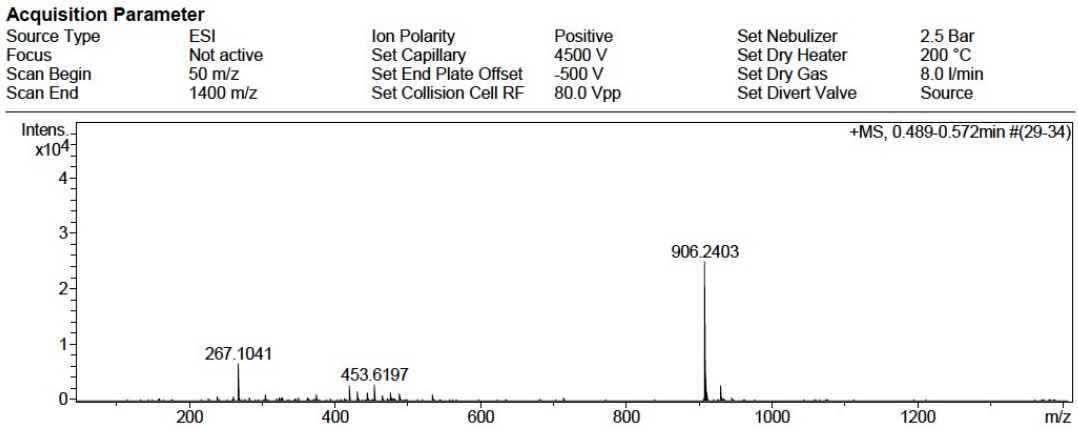


Fig. S2 High-resolution mass spectrum (HRMS) of **CAT-7-P**.

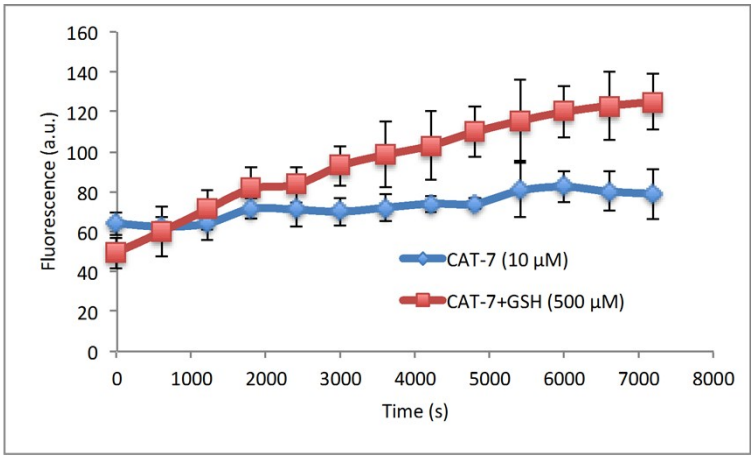


Fig. S3 Stability of **CAT-7** (10 µM) in the presence of 500 µM GSH. Fluorescence data was collected with excitation at λ = 490 nm (band width 5 nm) and emission at λ = 510 nm (band width 5 nm). Error bars are ± SD.

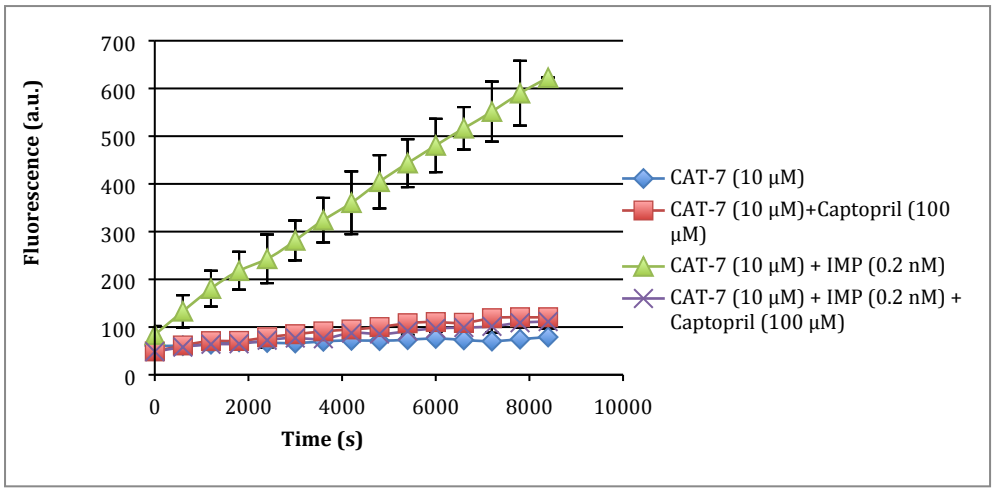


Fig. S4 Inhibition study of IMP-1 by captopril in the presence of **CAT-7**. Fluorescence intensity was measured on plate reader with excitation at λ = 490 nm (band width 5 nm) and emission at λ = 510 nm (band width 5 nm). Error bars are ± SD.

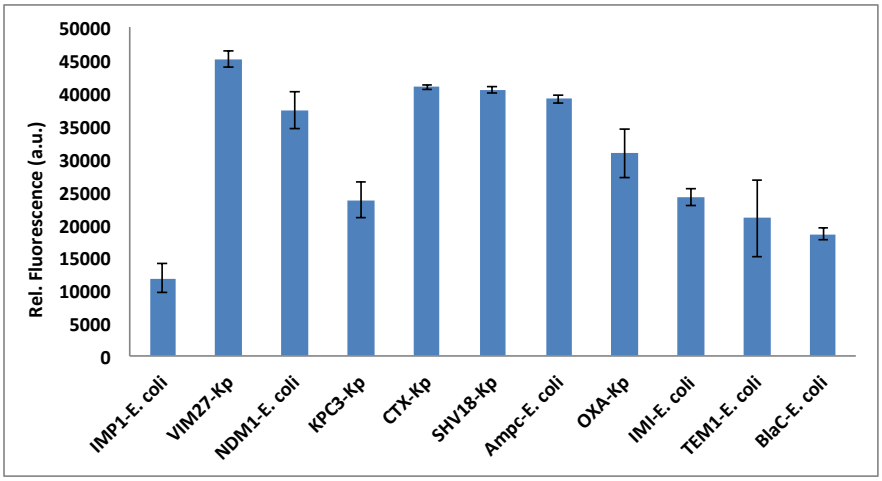
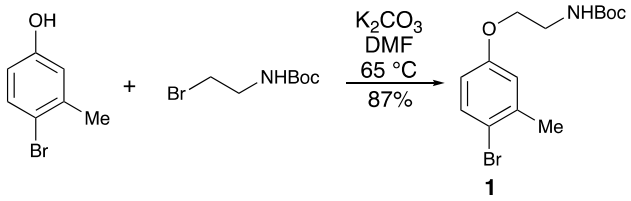


Fig. S5 Fluorescence response of Fluorocillin (10 μM) upon incubation with various bacteria lysates (4×10^5 cfu in 100 μL PBS, pH = 7.4) at 22 °C for 2 h. Fluorescence intensity was measured on plate reader with excitation at 490 nm (band width 5 nm) and emission at 520 nm (band width 5 nm). Error bars are ± SD.

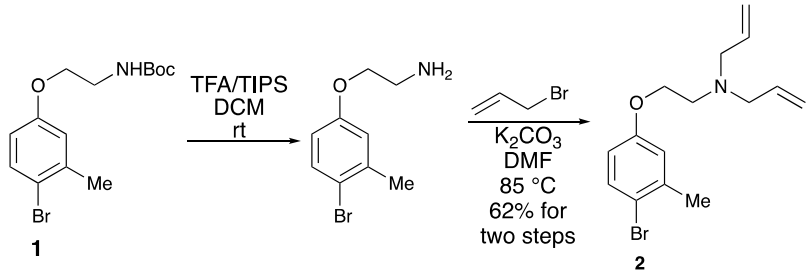
Experimental Section

Probe Synthesis



***tert*-butyl (2-(4-bromo-3-methylphenoxy)ethyl)carbamate (1)**

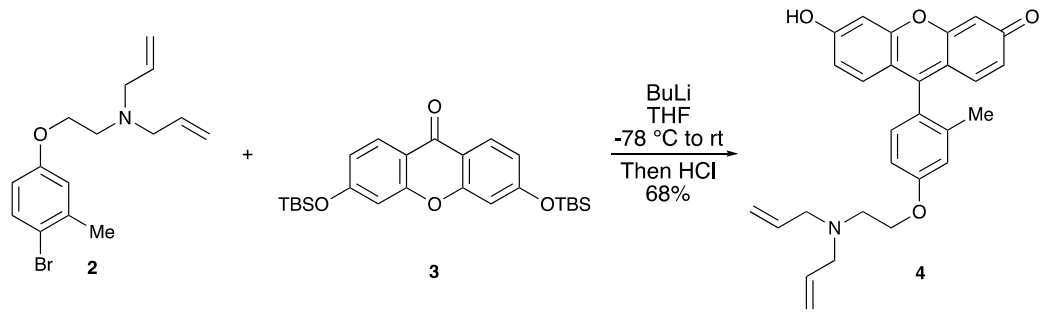
The mixture of 4-bromo-3-methylphenol (1g, 5.35 mmol) and tert-butyl (2-bromoethyl)carbamate (2.40g, 10.7 mmol) in 10 mL anhydrous DMF was purged with Ar. Then the reaction was heated to 65 °C for 15 h after addition of K_2CO_3 (1.849 g, 13.4 mmol). DMF was removed under reduced pressure when the reaction was cooled to room temperature. The crude product was diluted with 30 mL water and extracted with 20 mL ethyl acetate three times. The combined organic layer was washed with brine and dried with sodium sulfate. Then the mixture was filtrated and condensed to give the crude product which was purified by column chromatography (1.54 g, 87%). ^{1}H NMR (400 MHz, Chloroform-d) δ 7.35 (d, J = 8.7 Hz, 1H), 6.74 (dd, J = 3.1, 0.8 Hz, 1H), 6.56 (dd, J = 8.7, 3.0 Hz, 1H), 5.18 (d, J = 6.4 Hz, 1H), 3.94 (t, J = 5.2 Hz, 2H), 3.50 (q, J = 5.5 Hz, 2H), 2.32 (s, 3H), 1.45 (s, 9H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 157.70, 138.87, 132.81, 120.79, 116.96, 115.72, 113.37, 67.27, 40.01, 28.34, 27.69, 23.09. HRMS: Calculated for $C_{14}H_{19}BrNO_3Na^+$ ([M+Na]$^+$): 352.0519, Found 352.0515.



***N*-allyl-*N*-(2-(4-bromo-3-methylphenoxy)ethyl)prop-2-en-1-amine (2)**

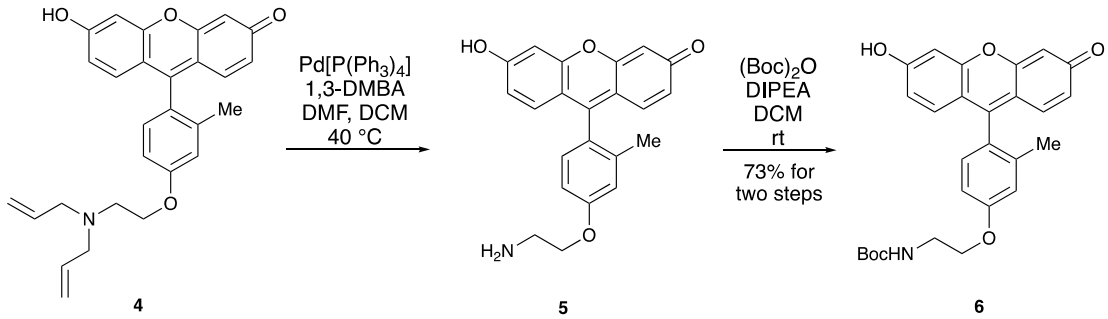
TFA (5 mL) was slowly added to a mixture of compound **1** (1.5 g, 4.54 mmol) and TIPS (0.93 mL, 4.54 mmol) in 20 mL DCM. The volatile ingredients were evaporated under reduced pressure after the completion of the reaction monitored by HPLC. Then the crude product was dissolved in 10 mL DMF followed by the addition of K_2CO_3 (3.14 g, 22.7 mmol) and allyl bromide (0.79 mL, 9.1 mmol) under Ar. The mixture was stirred overnight at 85 °C. The crude product was partitioned between water and ethyl acetate after the evaporation of DMF. The organic layer was dried with sodium sulfate and evaporated to give the crude product which was purified by column chromatography (0.873 g, 62% for two steps). ^{1}H NMR (400 MHz, Chloroform-d) δ 7.36 (d, J = 8.7 Hz, 1H), 6.77 (dd, J = 3.0, 0.8 Hz, 1H), 6.59 (dd, J = 8.7, 3.1 Hz, 1H), 5.87 (ddt, J = 17.3, 10.2, 6.5 Hz, 2H), 5.23 – 5.20 (m, 1H), 5.19 – 5.15 (m, 2H), 5.14

(dt, J = 2.2, 1.2 Hz, 1H), 3.99 (t, J = 6.1 Hz, 2H), 3.20 (t, J = 1.3 Hz, 2H), 3.18 (t, J = 1.3 Hz, 2H), 2.85 (t, J = 6.1 Hz, 2H), 2.34 (s, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 157.91, 138.60, 135.38, 132.62, 117.69, 116.99, 115.26, 113.39, 77.31, 77.00, 76.68, 66.51, 57.62, 51.72, 23.04. HRMS: Calculated for $C_{15}H_{21}BrNO^+$ ($[M+H]^+$): 310.0801, Found 310.0796.



9-(4-(2-(diallylamino)ethoxy)-2-methylphenyl)-6-hydroxy-3*H*-xanthen-3-one (4)

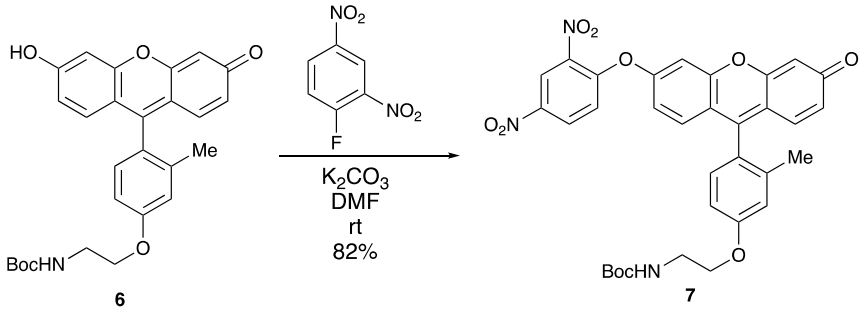
To a mixture of compound **2** (1.2 g, 3.63 mmol) in 20 mL THF was added 1.6 M BuLi (2.3 mL, 3.63 mmol) at -78 °C followed by the addition of compound **3**[1] (1.66 g, 3.63 mmol) in 10 mL THF. The reaction was gradually warmed to room temperature after stirring for 1 h. The mixture was stirred for further 30 min after slow addition of 10 mL HCl (5%). The mixture was partitioned between water and ethyl acetate after adjustment of pH to 9. The organic layer was dried with sodium sulfate and evaporated to give the crude product which was purified by column chromatography (1.089 g, 68%). ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.11 (d, J = 8.4 Hz, 1H), 7.00 (d, J = 2.5 Hz, 1H), 6.93 (dd, J = 8.4, 2.5 Hz, 1H), 6.89 (s, 1H), 6.87 (s, 1H), 6.57 (dd, J = 9.2, 2.1 Hz, 2H), 6.53 (d, J = 2.1 Hz, 2H), 5.83 (ddt, J = 16.6, 10.1, 6.3 Hz, 2H), 5.20 (dd, J = 17.2, 1.9 Hz, 2H), 5.15 – 5.09 (m, 2H), 4.10 (t, J = 6.0 Hz, 2H), 3.16 (d, J = 6.4 Hz, 4H), 2.82 (t, J = 6.0 Hz, 2H), 1.96 (s, 3H). ^{13}C NMR (101 MHz, DMSO) δ 173.91, 163.42, 159.09, 157.48, 156.33, 149.82, 137.30, 135.69, 130.31, 130.21, 127.73, 127.33, 124.24, 117.54, 116.26, 114.88, 114.00, 113.62, 112.10, 103.40, 102.09, 65.99, 56.74, 51.38, 40.15, 39.94, 39.73, 39.52, 39.31, 39.11, 38.90, 19.43. HRMS: Calculated for $C_{28}H_{28}NO_4^+$ ($[M+H]^+$): 442.2013, Found 442.2000.



tert-butyl (2-(4-(6-hydroxy-3-oxo-3H-xanthen-9-yl)-3-methylphenoxy)ethyl)carbamate (6)

The mixture of compound **4** (1.5 g, 3.40 mmol), $Pd[P(Ph_3)_4]$ (0.393 g, 0.34 mmol) and 1,3-Dimethylbarbituric acid (1.593 g, 10.20 mmol) in 20 mL of DCM: DMF=4:1 was stirred at 40 °C overnight under Ar. The mixture was diluted by water and extracted

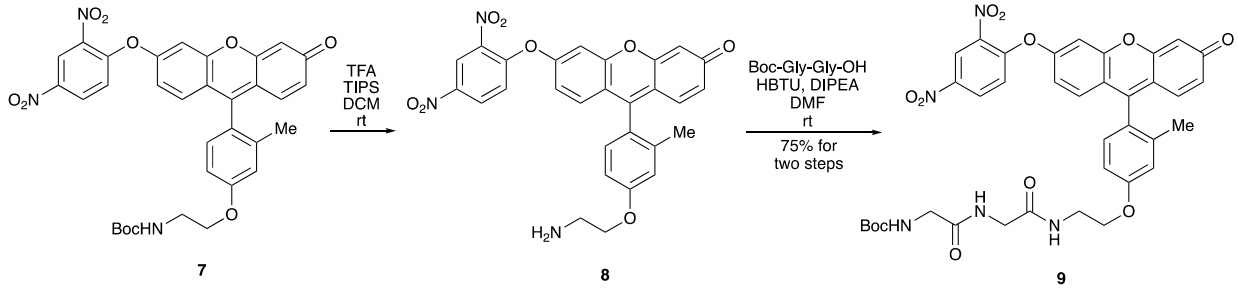
with ethyl acetate. The organic layer was dried with sodium sulfate and evaporated to give the crude product which was directly used in the next step. DIPEA (1.18 mL, 6.80 mmol) was added to the mixture of compound **5** in 20 mL DCM. The reaction was stirred for 3 h after addition of $(Boc)_2O$ (0.742 g, 3.40 mmol). The mixture was diluted by water and extracted with ethyl acetate. The organic layer was dried with sodium sulfate and evaporated to give the crude product which was purified by column chromatography (1.145 g, 73% for two steps). ^{1}H NMR (400 MHz, Chloroform-d) δ 7.10 (t, J = 9.0 Hz, 3H), 7.01 – 6.90 (m, 2H), 6.88 – 6.78 (m, 5H), 4.14 (t, J = 5.3 Hz, 2H), 3.60 (t, J = 5.3 Hz, 2H), 2.03 (s, 3H), 1.49 (s, 9H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 175.13, 159.21, 157.51, 155.95, 155.90, 154.30, 137.46, 130.77, 130.01, 128.66, 127.86, 124.41, 121.64, 116.24, 115.01, 111.68, 103.40, 79.31, 77.32, 77.00, 76.68, 66.83, 39.60, 28.10, 19.68. HRMS: Calculated for $C_{27}H_{27}NO_6^+$ ([M+H]$^+$): 462.1911, Found 462.1896.



***tert*-butyl (2-(4-(6-(2,4-dinitrophenoxy)-3-oxo-3*H*-xanthen-9-yl)-3-methylphenoxy)ethyl)carbamate (7)**

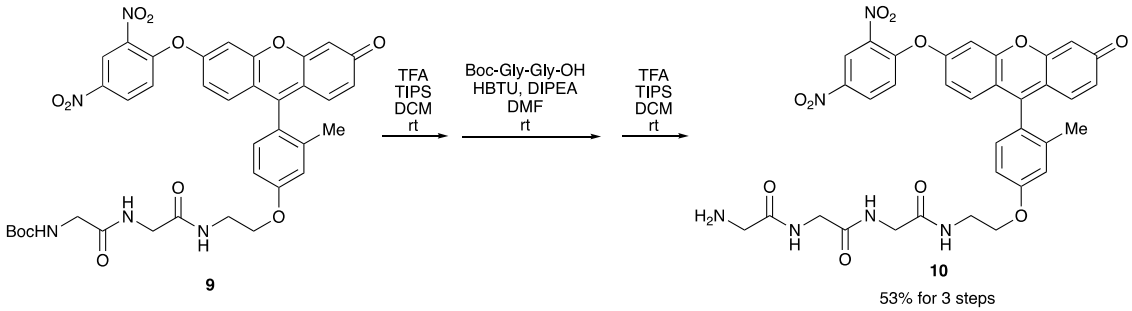
1-Fluoro-2,4-dinitrobenzene (0.65 mL, 5.20 mmol) was added to a mixture of compound **6** (1.5 g, 3.25 mmol) and K_2CO_3 (0.898 g, 6.50 mmol) in DMF. The reaction was stirred for 1 h at room temperature. The crude product was partitioned between water and ethyl acetate after the evaporation of DMF. The organic layer was dried with sodium sulfate and evaporated to give the crude product which was purified by column chromatography (1.673 g, 82%). ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.95 (d, J = 2.8 Hz, 1H), 8.56 (dd, J = 9.2, 2.8 Hz, 1H), 7.60 (d, J = 9.2 Hz, 1H), 7.54 (d, J = 2.2 Hz, 1H), 7.28 – 7.18 (m, 3H), 7.13 – 6.98 (m, 4H), 6.58 (dd, J = 9.7, 2.0 Hz, 1H), 6.37 (d, J = 1.9 Hz, 1H), 4.06 (t, J = 5.8 Hz, 2H), 3.35 (q, J = 5.8 Hz, 2H), 2.03 (s, 3H), 1.40 (s, 9H). HRMS: Calculated for $C_{33}H_{30}N_3O_{10}^+$ ([M+H]$^+$): 628.1926, Found 628.1922.

General procedure for condensation of Boc-protected gly with the dye



***tert*-butyl (2-((2-((2-(4-(6-(2,4-dinitrophenoxy)-3-oxo-3H-xanthen-9-yl)-3-methylphenoxy)ethyl)amino)-2-oxoethyl)amino)-2-oxoethyl)carbamate (9)**

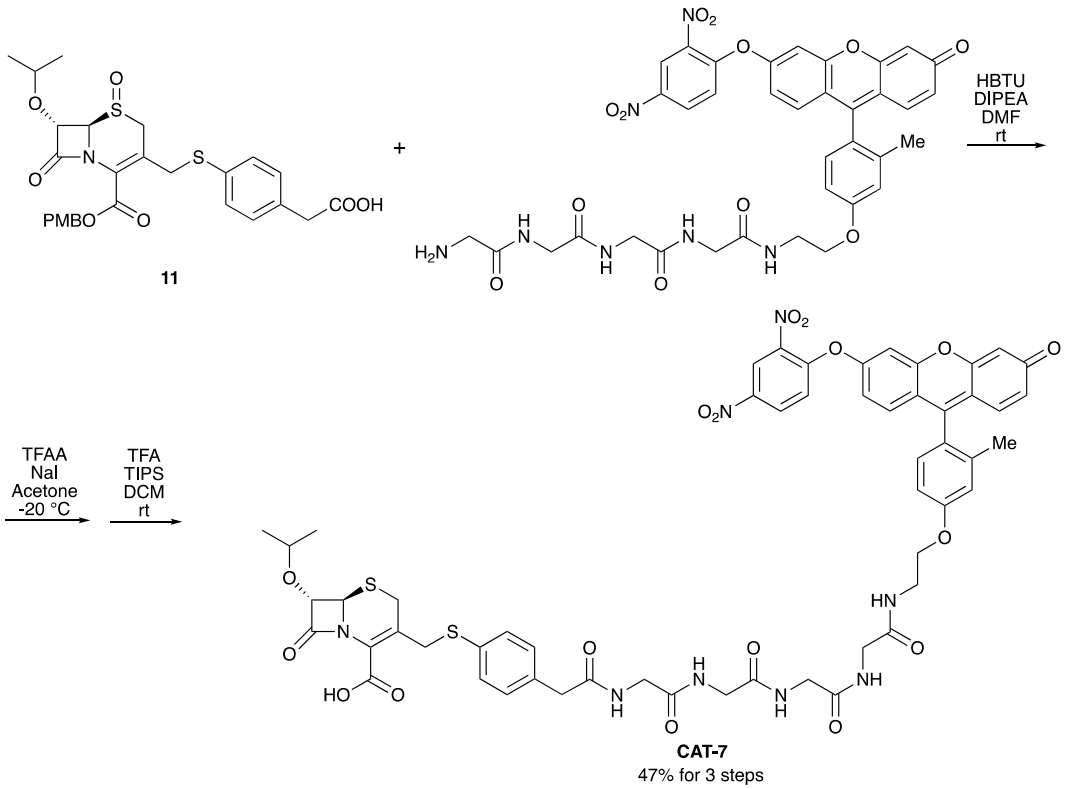
TFA (4 mL) was slowly added to a mixture of compound **7** (1.1 g, 1.75 mmol) and TIPS (0.36 mL, 1.75 mmol) in 20 mL DCM. The volatile ingredients were evaporated under reduced pressure after the completion of the reaction monitored by HPLC. The crude product was used directly in the step without further purification. To a solution of Boc-Gly-Gly-OH (0.447 g, 1.93 mmol) and HBTU (1.0 g, 2.63 mmol) in 10 mL DMF was added the mixture of compound **8** and DIPEA (0.91 mL, 5.25 mmol) in 5 mL DMF. The mixture was diluted with water and extracted with ethyl acetate. The organic layer was dried with sodium sulfate and evaporated to give the crude product which was purified by column chromatography (0.973 g, 75% for two steps). ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.96 (d, J = 2.8 Hz, 1H), 8.56 (dd, J = 9.2, 2.8 Hz, 1H), 8.10 (dt, J = 17.0, 5.7 Hz, 2H), 7.60 (d, J = 9.1 Hz, 1H), 7.54 (d, J = 2.3 Hz, 1H), 7.29 – 7.16 (m, 3H), 7.15 – 6.96 (m, 4H), 6.59 (dd, J = 9.7, 1.9 Hz, 1H), 6.38 (d, J = 1.9 Hz, 1H), 4.11 (t, J = 5.7 Hz, 2H), 3.73 (d, J = 5.7 Hz, 2H), 3.58 (d, J = 4.7 Hz, 2H), 3.51 (q, J = 5.7 Hz, 2H), 2.03 (s, 3H), 1.38 (s, 9H). HRMS: Calculated for $C_{37}H_{36}N_5O_{12}^+$ ([M+H]$^+$): 742.2355, Found 742.2335.



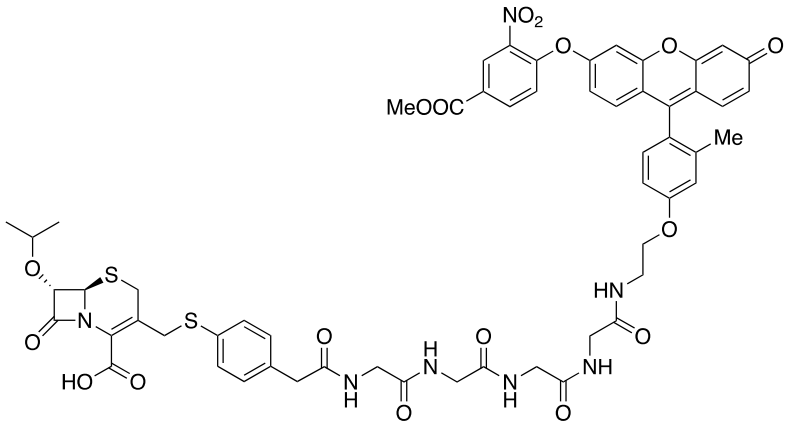
2-amino-N-(2-((2-((2-(4-(6-(2,4-dinitrophenoxy)-3-oxo-3H-xanthen-9-yl)-3-methylphenoxy)ethyl)amino)-2-oxoethyl)amino)-2-oxoethyl)acetamide (10)

Compound **10** was prepared by the same general procedure for the synthesis of compound **8**. ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.95 (d, J = 2.8 Hz, 1H), 8.65 (t, J = 5.7 Hz, 1H), 8.55 (dd, J = 9.2, 2.8 Hz, 1H), 8.31 (t, J = 5.8 Hz, 1H), 8.18 (t, J = 5.8 Hz, 2H), 8.02 (s, 2H), 7.58 (d, J = 9.2 Hz, 1H), 7.51 (d, J = 2.4 Hz, 1H), 7.27 – 7.19 (m, 2H), 7.16 (d, J = 8.8 Hz, 1H), 7.09 (d, J = 2.5 Hz, 1H), 7.02 (dd, J = 8.4, 2.5 Hz, 1H), 6.99 (d, J = 9.8 Hz, 1H), 6.53 (dd, J = 9.7, 1.9 Hz, 1H), 6.29 (d, J = 1.9 Hz, 1H), 4.10 (t, J = 5.6 Hz, 2H), 3.86 (d, J = 5.6 Hz, 2H), 3.78 (d, J = 5.7 Hz, 2H), 3.74 (d, J = 5.8 Hz, 2H), 3.62 (q, J = 5.7 Hz, 2H), 3.50 (q, J = 5.6 Hz, 2H), 2.03 (s, 3H). HRMS: Calculated for $C_{36}H_{34}N_7O_{12}^+$ ([M+H]$^+$): 756.2265, Found 756.2236.

General procedure for condensation, reduction and deprotection



Compound **11** was prepared by the oxidation and sulfa-substitution from the reported cephalosporin precusor.[2] To a solution of compound **11** (55 mg, 0.10 mmol) and HBTU (57 g, 0.15 mmol) in 2 mL DMF was added the mixture of compound **10** (76 mg, 0.10 mmol) and DIPEA (52 μL, 0.30 mmol) in 1 mL DMF. The product was purified by reverse-phase HPLC and dried by lyphilizer after the completion of the reaction monitored by HPLC. The obtained intermediate was dissolved in 5 mL acetone followed by the addition of NaI under Ar. TFAA was added to the mixture after cooling to -20 °C. The product was purified by reverse-phase HPLC and dried by lyophilizer after the completion of the reaction monitored by HPLC. The obtained intermediate was dissolved in 10 mL DCM followed by the addition of TIPS and TFA in ice bath. The product was purified by reverse-phase HPLC and dried by lyphilizer after the completion of the reaction monitored by HPLC. ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.95 (d, J = 2.8 Hz, 1H), 8.54 (dd, J = 9.2, 2.8 Hz, 1H), 8.31 (t, J = 5.7 Hz, 1H), 8.17 (ddt, J = 24.8, 8.5, 5.7 Hz, 4H), 7.57 (d, J = 9.2 Hz, 1H), 7.51 (d, J = 2.4 Hz, 1H), 7.32 – 7.27 (m, 2H), 7.24 – 7.19 (m, 3H), 7.14 (d, J = 8.9 Hz, 1H), 7.09 (d, J = 2.5 Hz, 1H), 7.02 (dd, J = 8.4, 2.5 Hz, 1H), 6.97 (d, J = 9.8 Hz, 1H), 6.51 (dd, J = 9.8, 1.9 Hz, 1H), 6.26 (d, J = 1.9 Hz, 1H), 4.78 (d, J = 1.6 Hz, 1H), 4.72 (d, J = 1.6 Hz, 1H), 4.12 – 4.07 (m, 3H), 3.81 (d, J = 6.1 Hz, 4H), 3.77 – 3.72 (m, 8H), 3.47 (d, J = 6.6 Hz, 4H), 2.03 (s, 3H), 1.14 (dd, J = 9.1, 6.1 Hz, 6H). HRMS: Calculated for $C_{55}H_{53}N_8O_{17}S_2^+$ ($[M+H]^+$): 1161.2970, Found 1161.2974.



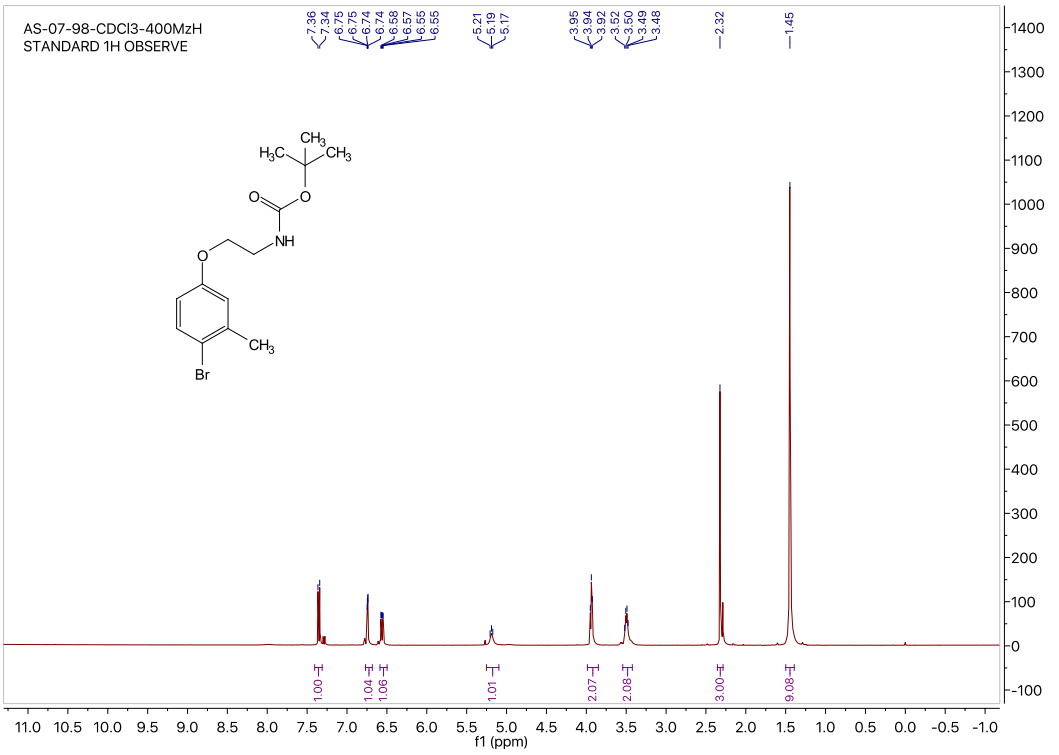
Compound **CAT-8** was prepared by the same general procedure for the synthesis of compound **CAT-7**. ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.61 (d, J = 2.2 Hz, 1H), 8.33 – 8.25 (m, 2H), 8.22 (d, J = 5.8 Hz, 1H), 8.17 (d, J = 5.8 Hz, 1H), 8.12 (dd, J = 10.1, 4.3 Hz, 2H), 7.51 (d, J = 8.6 Hz, 1H), 7.43 (d, J = 2.0 Hz, 1H), 7.31 – 7.26 (m, 2H), 7.24 – 7.19 (m, 3H), 7.16 (d, J = 2.3 Hz, 2H), 7.09 (d, J = 2.4 Hz, 1H), 7.05 – 6.95 (m, 3H), 6.53 (dd, J = 9.8, 2.0 Hz, 1H), 6.30 (d, J = 2.0 Hz, 1H), 4.78 (d, J = 1.6 Hz, 1H), 4.72 (d, J = 1.6 Hz, 1H), 3.92 (s, 7H), 3.74 (dd, J = 4.8, 3.0 Hz, 10H), 3.52 – 3.43 (m, 6H), 2.02 (s, 3H), 1.14 (dd, J = 9.1, 6.1 Hz, 6H). HRMS: Calculated for $C_{57}H_{56}N_7O_{17}S_2^+$ ([M+H]$^+$): 1174.3174, Found 1174.3170.

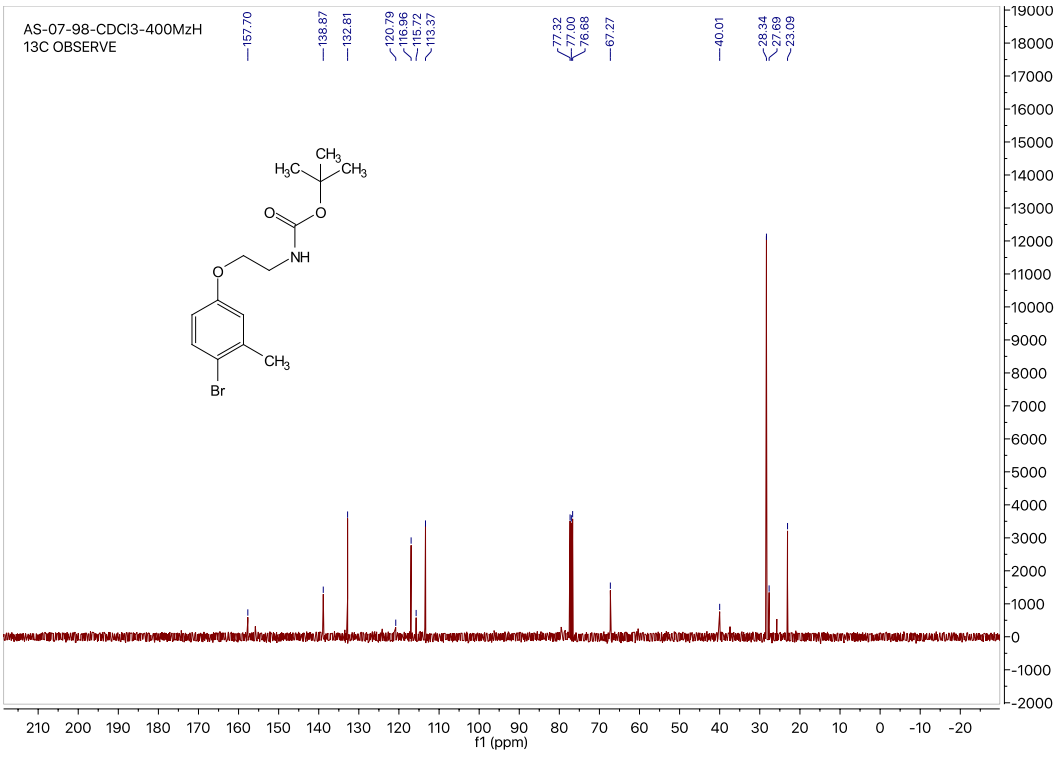
References

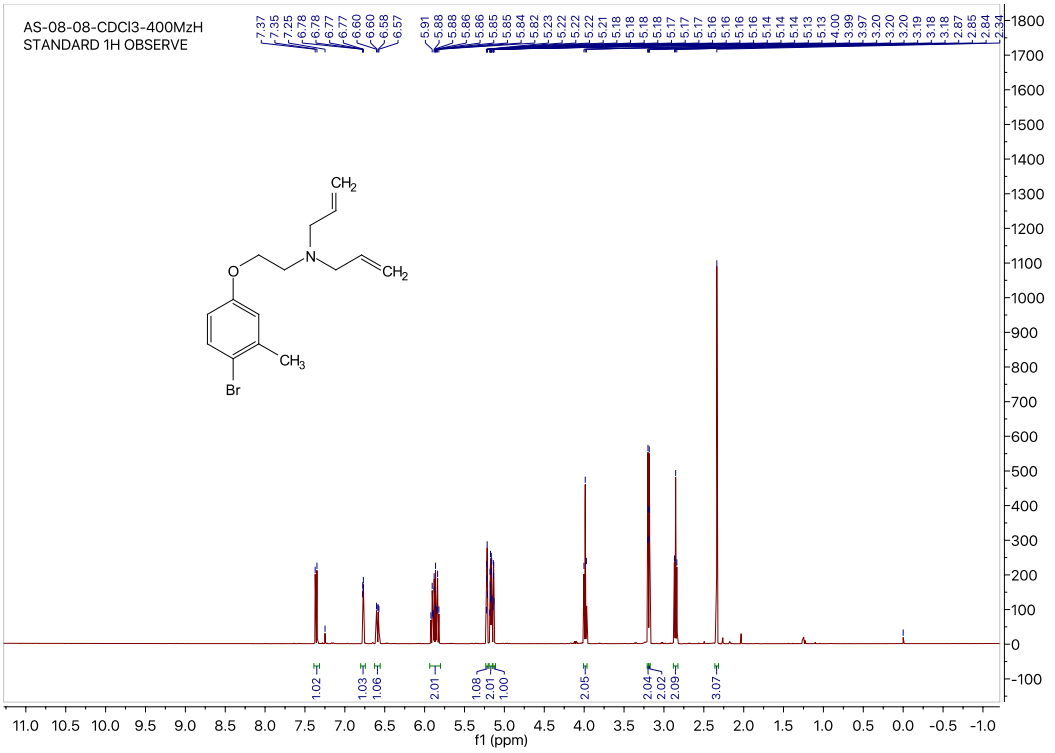
1. P. Shieh, M. J. Hangauer and C. R. Bertozzi, *J. Am. Chem. Soc.*, 2012, **134**, 17428–17431.

2. H. Shi, Y. Cheng, K. H. Lee, R. F. Luo, N. Banaei and J. Rao, *Angew. Chem. Int. Ed.*, 2014, **53**, 8113–8116; *Angew. Chem.*, 2014, **126**, 8251–8254.

^{1}H NMR and ^{13}C NMR



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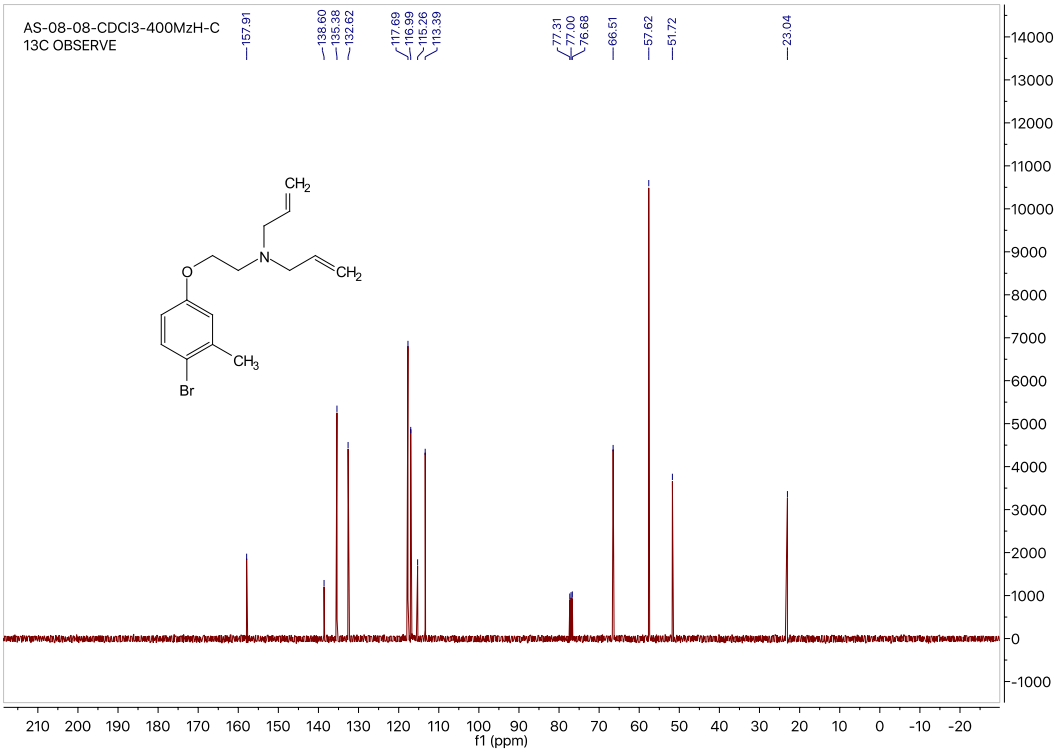
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13C OBSERVE
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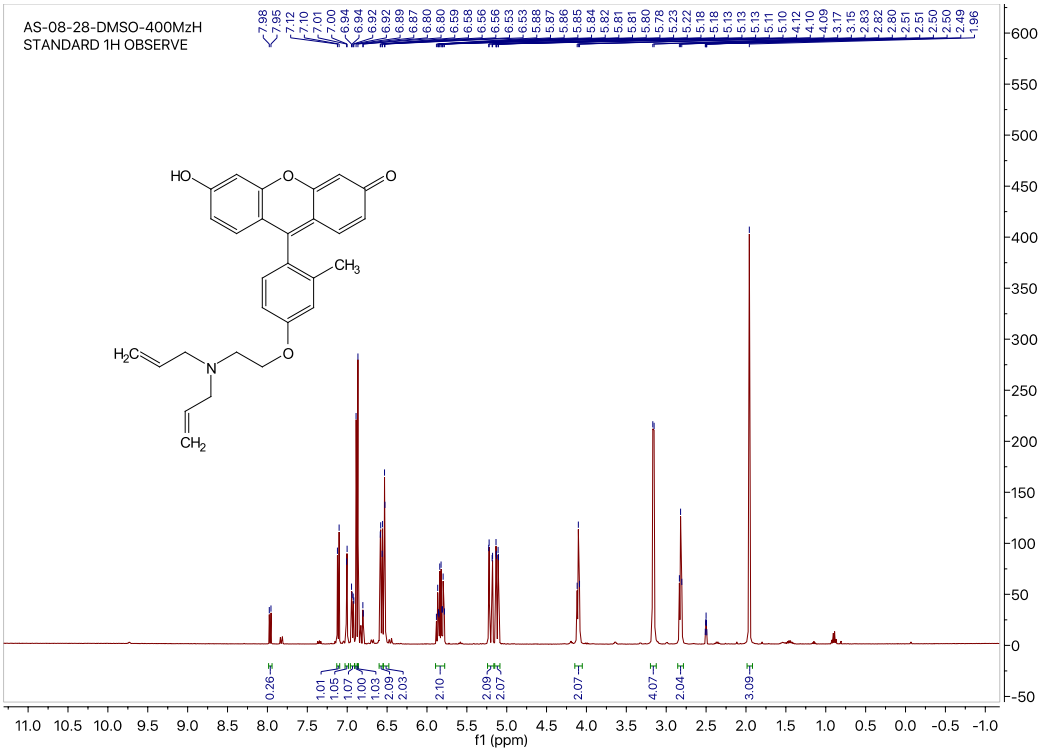
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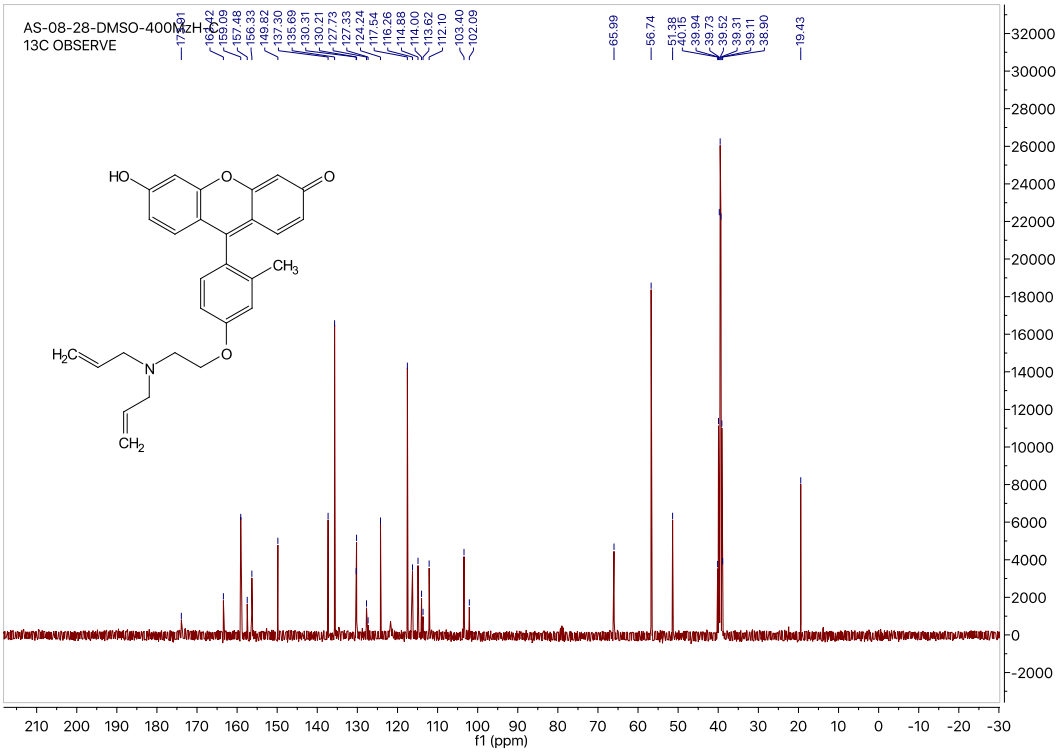
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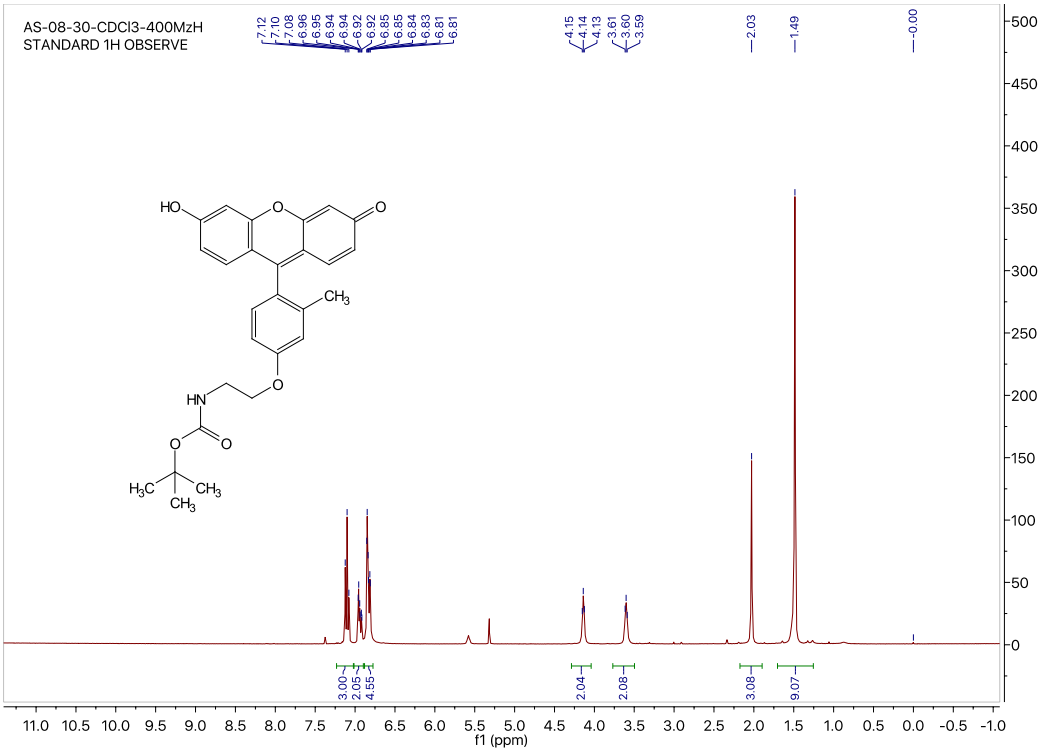
f1 (ppm)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7.08
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6.95
6.94
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6.81
4.15
4.14
4.13
3.61
3.60
3.59
2.03
1.49
0.00
HO
O
O
CH3
HN
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CH3
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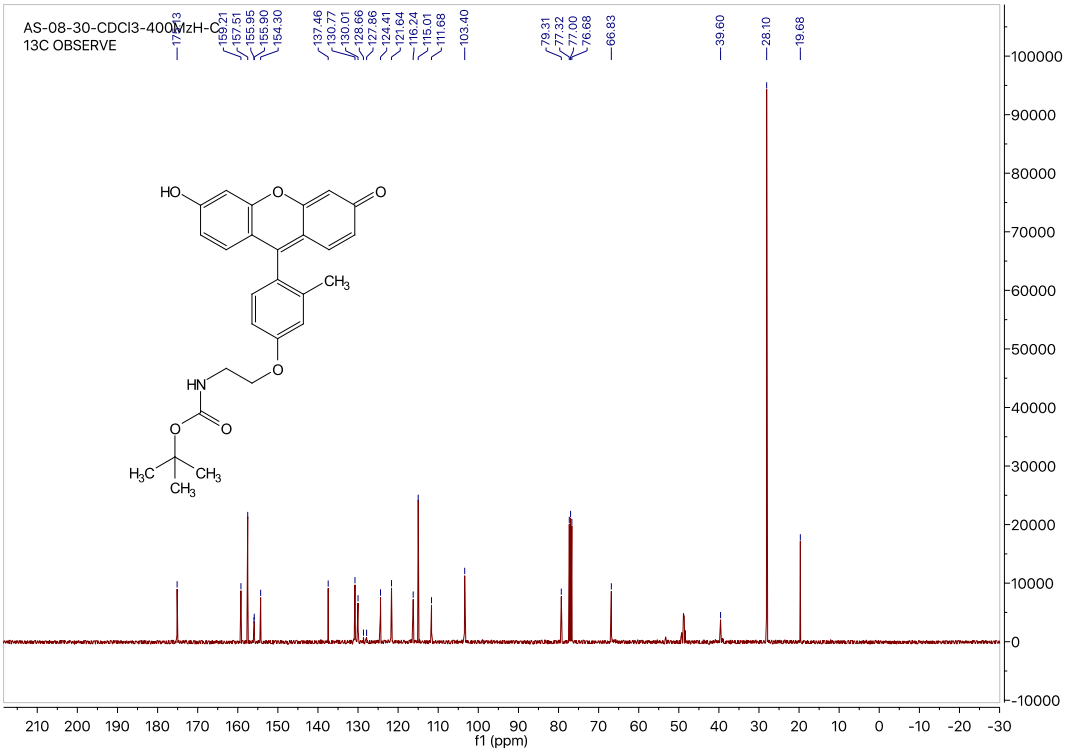
3.08
9.07
f1 (ppm)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115.01
111.68
103.40
79.31
77.32
77.00
76.68
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O
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O
O
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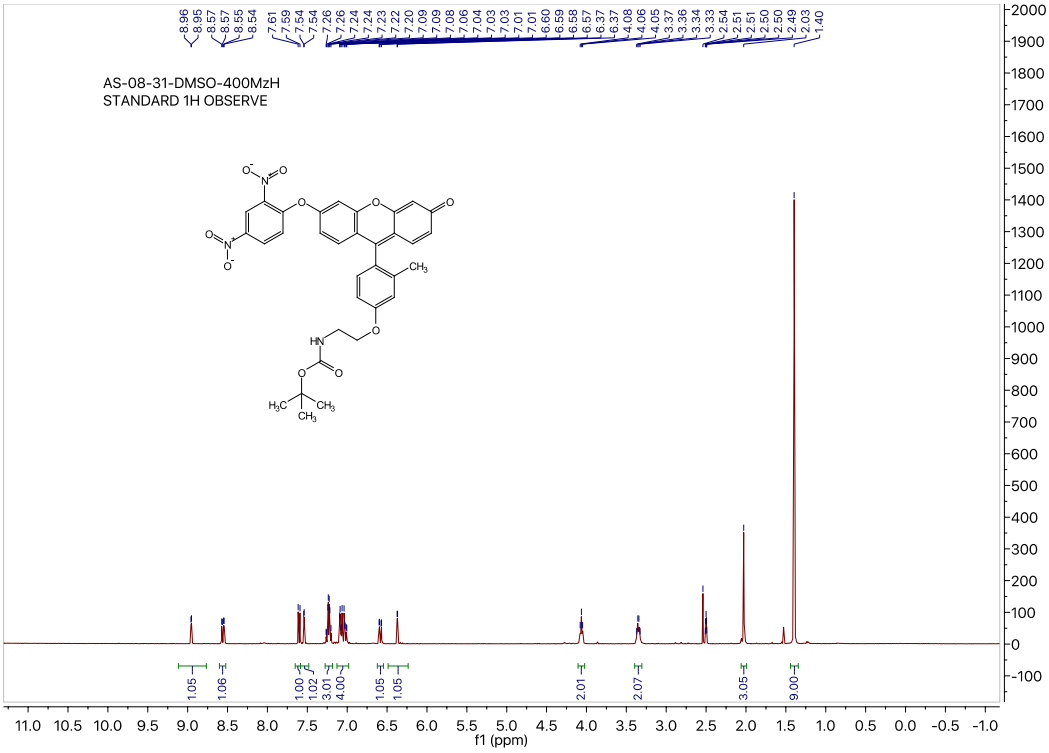
CH3
CH3
f1 (ppm)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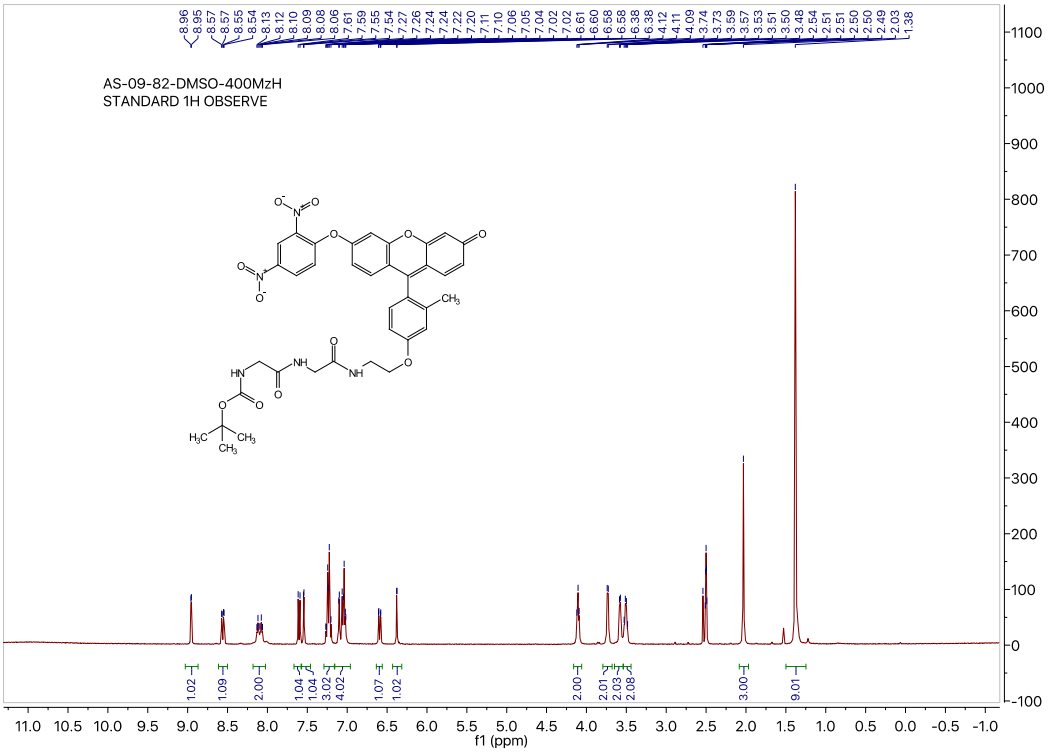
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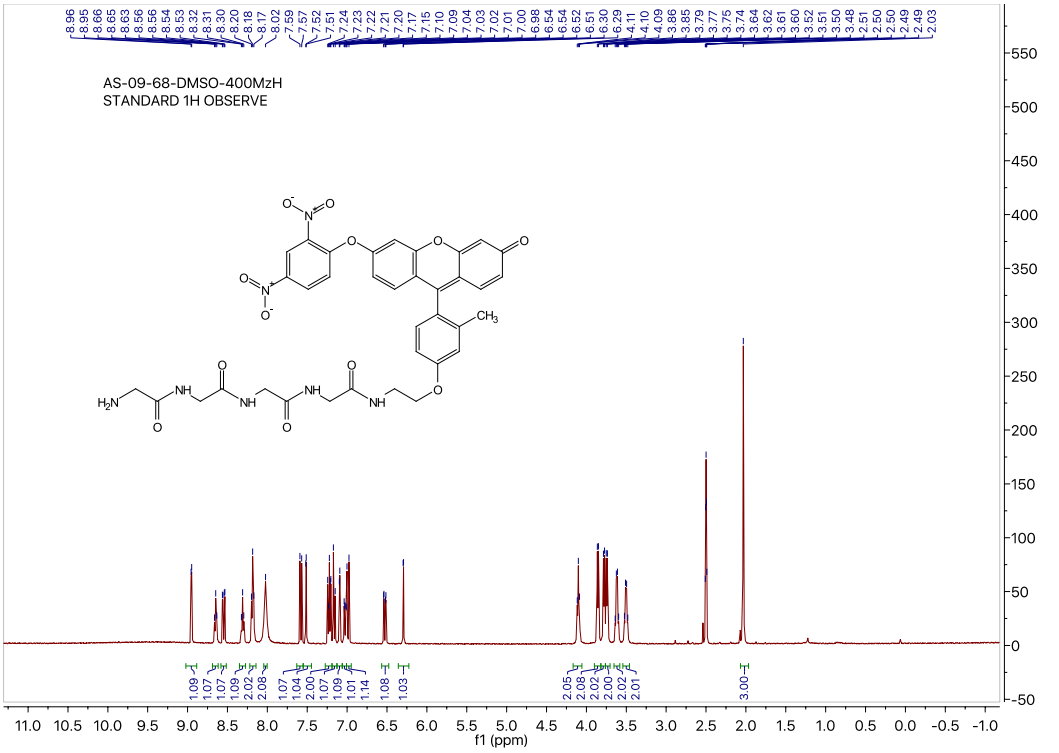
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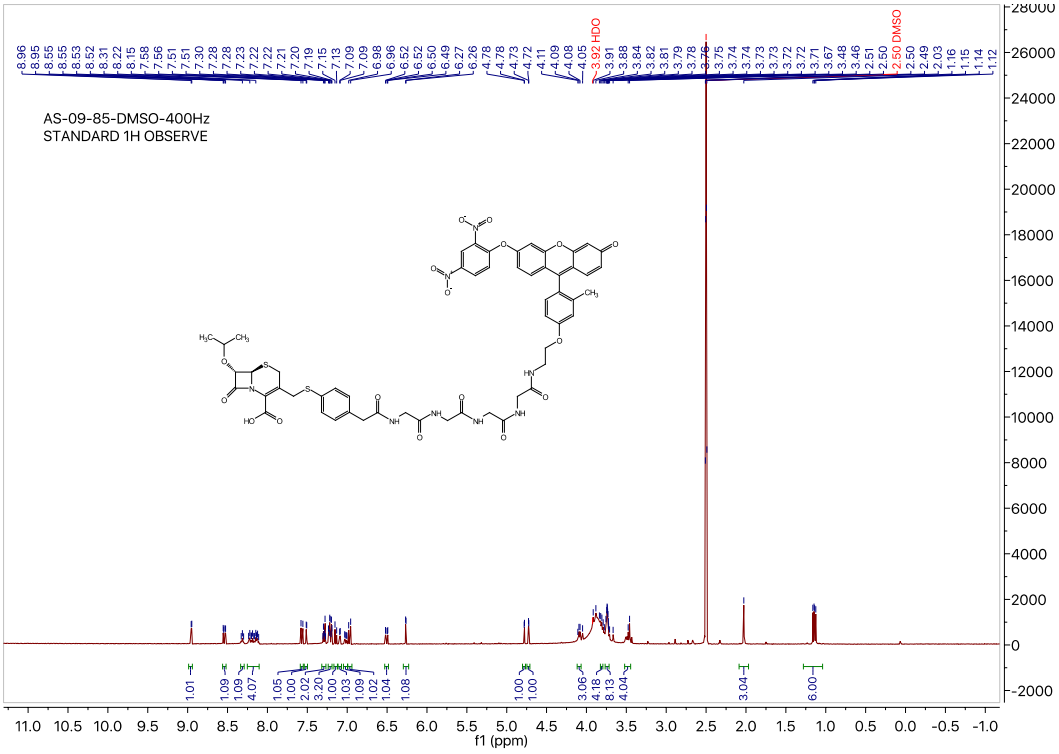
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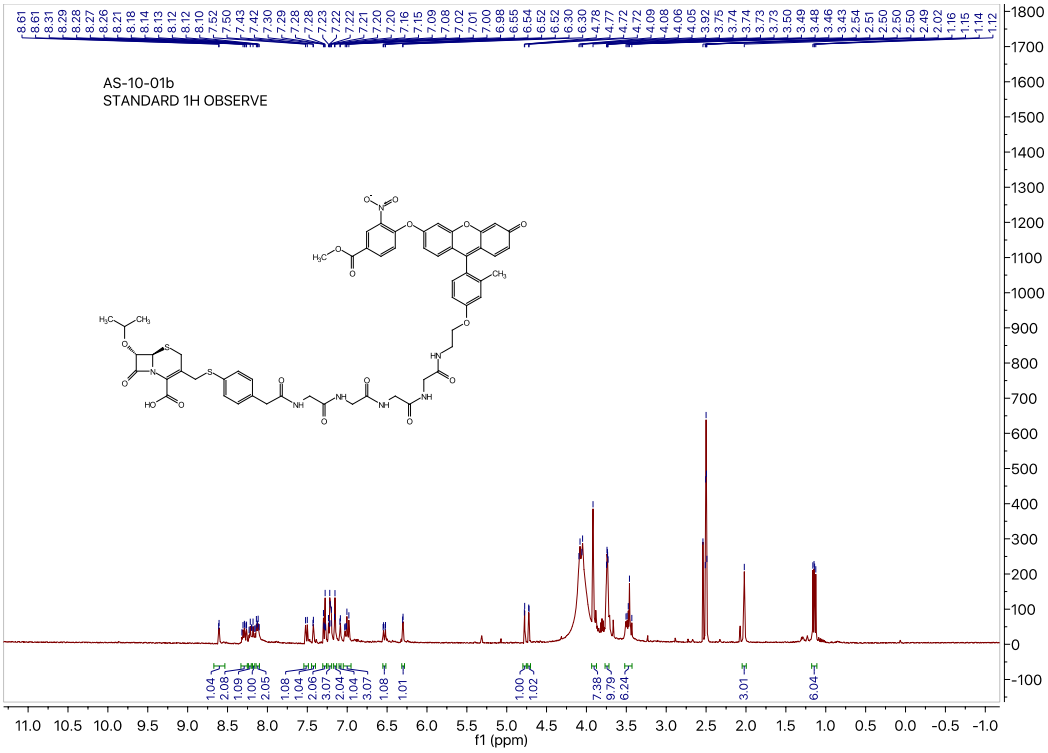
2.50 DMSO
f1 (ppm)


AS-10-01b
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f1 (ppm)

HRMS

Acquisition Parameter

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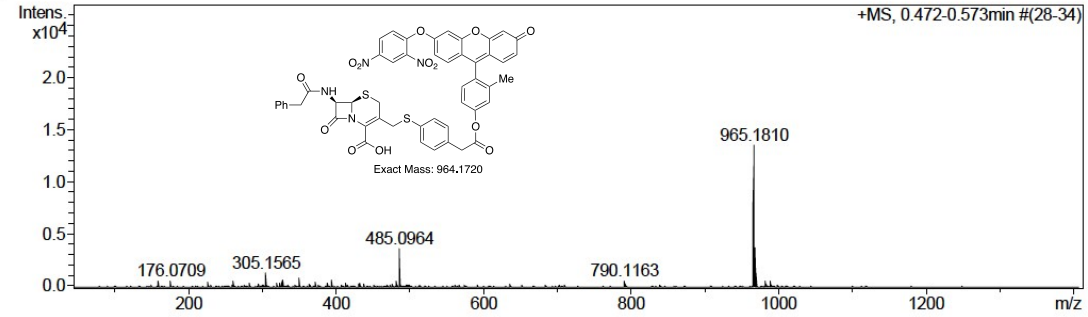

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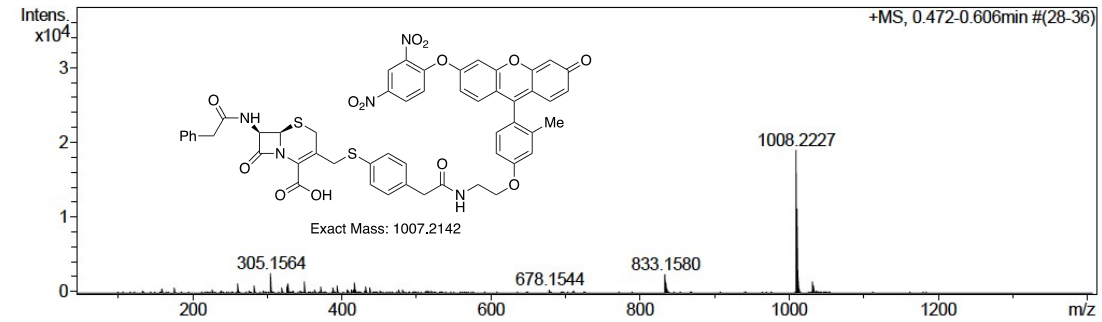

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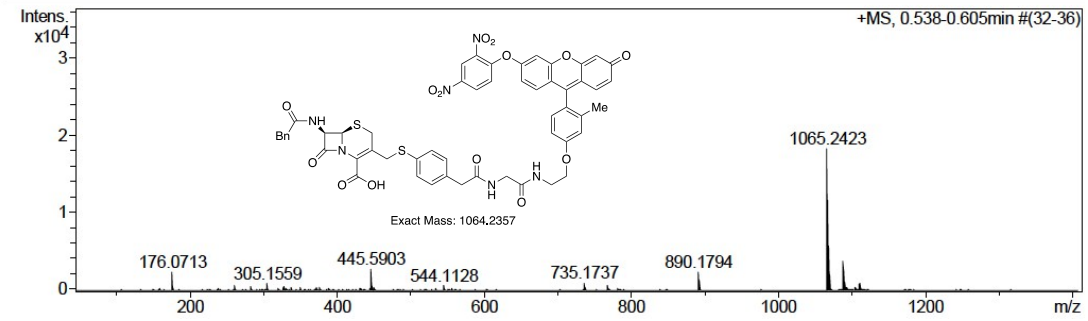

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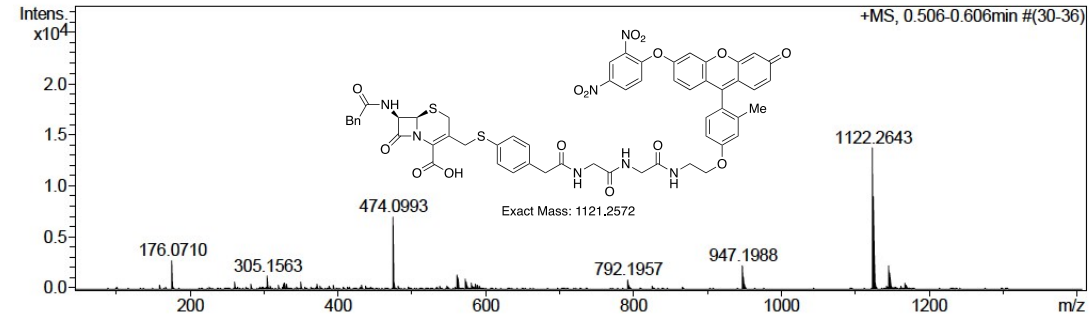

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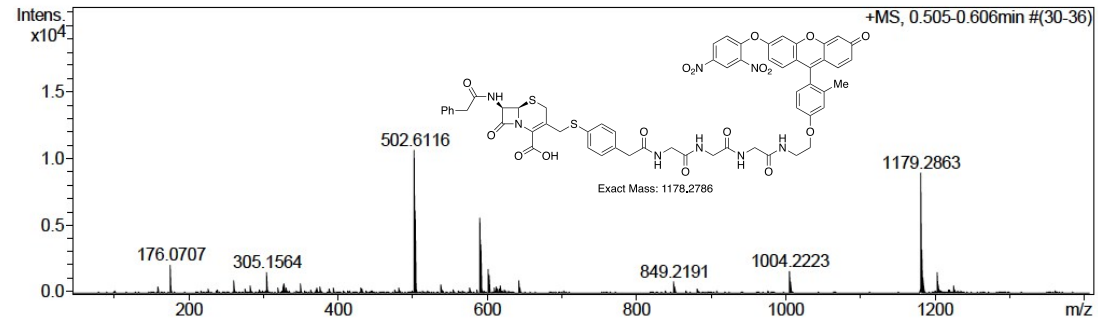

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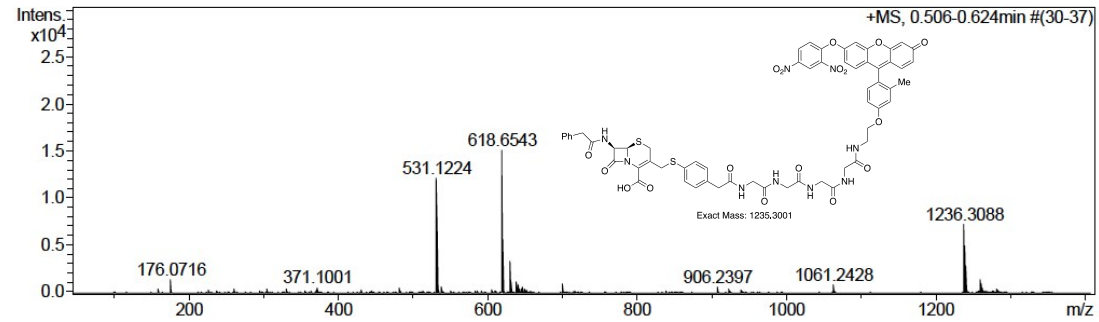

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