Bioorthogonal Catalysis: A General Method to Evaluate Metal-catalyzed Reactions in Real Time in Living Systems Using a Cellular Luciferase Reporter System

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Supporting Information

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General information on chemical experiments

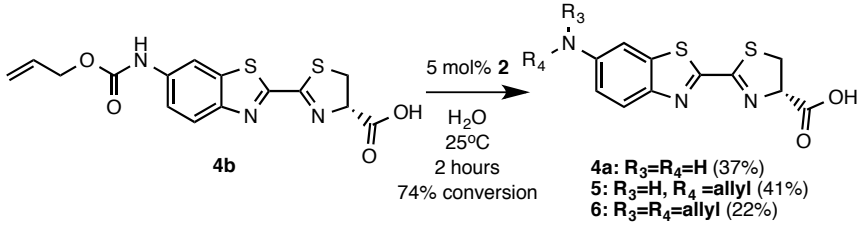
1. Chemical reactions
   All air- and moisture-sensitive reactions were carried out in glassware that was oven-dried (>130 °C) and cooled under nitrogen. Reaction vessels were sealed with rubber septa or Teflon®- coated caps and maintained in an inert environment under a positive pressure of anhydrous N_2. Stirring was accomplished via magnetic, Teflon®-coated stir bars or mechanical stirrers that were oven-dried and cooled under a nitrogen atmosphere. Vessels containing light-sensitive compounds were covered with aluminum foil. Solid reagents were measured on a Mettler Toledo AB104-S balance. Air- and moisture-sensitive liquids were transferred via syringe or cannula under an atmosphere of N_2. Reaction temperatures refer to the external or bath temperature in which the reaction vessel was partially immersed. Room temperature (r.t.) indicates an external temperature of 20-25 °C. Elevated temperatures were achieved by the use of a silicon oil bath heated by a nichrome wire under constant voltage. Temperatures of 0 °C and -78 °C were maintained with ice/water mixtures and dry ice/acetone mixtures, respectively. Temperatures in between were maintained by the periodic addition of dry ice to a bath of acetone or acetonitrile (CH_3CN). The term *in vacuo* refers to the use of a rotary evaporator with an attached vacuum membrane pump. Residual solvents were removed using vacuum held at < 1.0 Torr.
2. Reagents and Solvents
   Unless otherwise noted, all commercial solvents and reagents were used without further purification. Reagents were purchased from Sigma-Aldrich unless otherwise indicated. Dichloromethane (CH_2Cl_2) was passed through an alumina drying column (*Solvtek* Inc.) using nitrogen pressure. Petroleum ether, pentane, ethyl acetate (EtOAc), and methanol (MeOH) were obtained from Fisher Scientific. Anhydrous dimethylformamide (DMF) and acetonitrile (CH_3CN) were obtained from Acros Organics. Deuterated solvents were purchased from Cambridge Isotope Laboratories.
3. Chromatography
   Analytical TLC was performed using 0.25 mm glass-backed silica gel 60F254-coated plates from EMD Chemicals Inc. and monitored at 254 nm. Plates were visualized by treatment with solutions of *p*-anisaldehyde or potassium permanganate and gentle heating. Preparative column chromatography was performed by running solvent under a pressure of air through silica gel (230-400 mesh, 60 Å), purchased from EMD Chemical Inc. Reverse-phase high pressure liquid chromatography (RP-HPLC) was performed on a Shimadzu LC-20AP using a preparative column (Shimadzu C18, 250 x 21.2 mm) or a semi-preparative column (Restek C18, 250 x 10 mm). The products were eluted utilizing a solvent gradient (solvent A = 0.1% TFA / H_2O; solvent B = 0.1% TFA /CH_3CN). Retention times are reported for analytical runs with ramp times of 15 or 20 minutes. Water was removed via lyophilization using a Virtis Freezemobile 25EL lyophilizer.
4. Physical and Spectroscopic Measurements
   Nuclear magnetic resonance spectra were taken on a Varian Inova 500 (^1H at 500 MHz, ^{13}C at 125 MHz) or Varian Mercury 400 (^1H at 400 MHz, ^{13}C at 100 MHz) magnetic resonance spectrometer. ^1H NMR referenced to residual solvent peak ($CDCl_3$ = 7.26 ppm, CD_3OD = 3.31 ppm, or D_2O = 4.79 ppm). ^{13}C chemical shifts are reported relative to the solvent ($CDCl_3$ = 77.1 ppm, CD_3OD = 49.0 ppm). Infrared spectra were measured on a Perkin-Elmer 1600 Series Fourier transform spectrometer (FTIR), and are reported in wavenumbers (cm^{-1}). High-resolution mass spectra (HRMS) were obtained from the Vincent Coates Foundation mass spectrometry

laboratory at Stanford University.

5. HPLC Analysis of Product Distribution of the Catalytic Cleavage of **4b**
   Unless otherwise noted, **4b** (1.5-1.8mg, 0.004-0.005 mmol) was dissolved in 8.487 mL of solvent (DI water, PBS, or RPMI-1640 media) in a vial with stirring at 37°C. 1.513 mL of 130-160 μM **2** stock solution in respective solvents (DI water, PBS, or RPMI-1640 media) was added to the stirring mixture. The initial total volume of all reactions was 10 mL. The reaction was stirred at the indicated temperature, and the reaction was monitored with HPLC (Shimadzu LC-20AP, semi-prepartive column) by taking a 1.5-mL aliquot from the reaction approximately every 80 minutes with 200 μL of 5 mM 2,3,5-trimethylphenol (TMP) stock solution in MeOH as the internal standard. The products were eluted utilizing a solvent gradient (solvent A = 0.1% TFA / H_2O; solvent B = 0.1% TFA /CH_3CN, 5% - 70% B/A in 30 minutes.)
6. Caged Luciferin **7** Stability and Release Assays
   7 was dissolved in 750 μL D_2O at 55 mM. Acetone (1 equivalent) was then added to the solution and it was transferred to a dry NMR tube. A Varian Inova 500 MHz NMR spectrometer was brought to 37 °C and the prepared sample was used to lock and shim the instrument. Catalyst **2** (5 mol%) was subsequently dissolved in an additional 750 μL D_2O and added to the NMR tube and ^{1}H-NMR scans begun at once and continued at periodic time points. The reaction progress was monitored by shifts of both allylic and aromatic protons relative to the internal standard.

Experimental Procedures and Analyses

1. Catalytic cleavage of **4b** by **2**: characterization of side products **5** and **6**



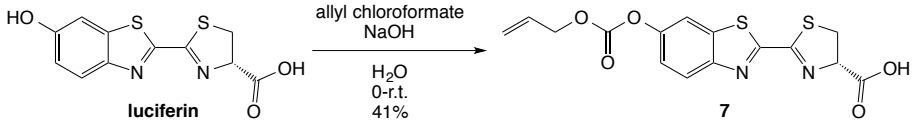
2 (0.5 mg, 0.00088 mmol) was dissolved in 1.5 mL of DI water in a vial with stirring at room temperature. **4b** (15 mg, 0.0413 mmol) was added to the stirring solution, and the reaction was stirred at room temperature for 5 hours. The reaction mixture was separated with RP-HPLC to yield **4a** (2.2 mg, 0.0079 mmol), **5** (2.8 mg, 0.0088 mmol), and **6** (1.7 mg, 0.0047 mmol).

Characterization of **5**:
^{1}H NMR (500 MHz, CD_3OD): δ 7.79 (d, *J* = 10Hz, 1H), 7.05 (d, J = 5Hz, 1H), 6.95 (dd, J = 5, 10Hz, 1H), 6.03-5.96 (m, 1H), 5.35 (t, J = 10 Hz, 1H), 5.32 (dd, J = 5, 10 Hz, 1H), 5.19 (dd, J = 5, 10Hz, 1H), 3.86 (d, J = 5Hz, 2H), 3.75 (dd, J = 2.5, 5 Hz, 2H) ppm. **^{13}C NMR** (500 MHz, CD_3OD): δ = 173.38, 167.85, 156.08, 149.00, 146.95, 139.96, 135.57, 125.51, 117.63, 117.15, 103.24, 79.15, 47.67, 35.77 ppm. **FT-IR** (thin film): ν = 3354 (s), 2356 (w), 2339 (w), 1651 (m), 1190 (m), 1132 (w), 881 (w), 800 (w), 721 (m), 667 cm^{-1}. **HRMS-ESI** (m/z): $[M + H]^+$ calc'd for $C_{14}H_{13}N_3O_2S_2$, 320.0527; found, 320.0514. **T_r** (RP-HPLC, 5% MeCN/H_2O → 70% MeCN/H_2O in 30 mins): 25 min.

Characterization of **6**:
^{1}H NMR (500 MHz, CD_3OD): δ 7.86 (d, *J* = 5Hz, 1H), 7.21 (d, J = 2.5Hz, 1H), 7.07 (dd, J = 2.5, 5Hz, 1H), 5.98-5.92 (m, 2H), 5.39 (t, J = 10 Hz, 1H), 5.22 (d, J = 10 Hz, 2H), 5.21 (dd, J

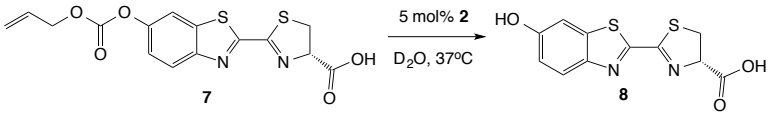
= 5, 10Hz, 2H), 4.10 (d, J = 5Hz, 4H), 3.76 (dd, J = 2.5, 5 Hz, 2H) ppm. **^{13}C NMR** (500 MHz, CD_3OD): δ = 176.71, 173.42, 167.75, 156.02, 149.89, 145.99, 139.92, 134.56, 125.27, 116.61, 115.39, 108.95, 103.27, 79.27, 54.21, 35.76, 35.66 ppm. **FT-IR** (thin film): *ν* = 3572 (s), 2932 (w), 2829 (w), 1673 (m), 1582 (m), 1489 (w), 1381 (w), 1193 (m), 1134 (w), 988 (w), 883 (w), 800 (w), 722 (w) cm^{-1}. **HRMS-ESI** (m/z): $[M + H]^+$ calculated for $C_{17}H_{17}N_3O_2S_2$, 360.0840; found, 360.0824. **T_r** (RP-HPLC, 5% MeCN/H_2O → 70% MeCN/H_2O in 30 mins): 31 min.

2. Synthesis of alloc-protected luciferin **7**



To a 10 mL round bottom flask with stir bar was added a solution of luciferin (20 mg, 0.07 mmol) in H_2O (3 mL). 340µL 0.5M NaOH solution was added to the flask at 0 °C, leading to the solubilization of the luciferin. Allyl chloroformate (30µL, 0.282 mmol) was added to the mixture and the reaction stirred for 45 min, during which time the solution turned from fluorescent yellow to opaque white. The reaction mixture was carefully acidified to pH = 3 and the product was washed with H_2O and centrifuged twice to collect the solids. The product was dried on a high vacuum to give **7** (10.6 mg, 41%) as a white solid, which was chromatographically homogenous by RP-HPLC. **^{1}H NMR** (400 MHz, CD_3OD): δ = 8.11 (d, *J* = 9.2 Hz, 1 H), 7.95 (d, *J* = 2.0 Hz, 1 H), 7.43 (dd, *J* = 2.0, 9.2 Hz, 1 H), 6.09-5.99 (m, 1 H), 5.48-5.40 (m, 2 H), 5.33 (dd, *J* = 1.2, 10.4 Hz, 1 H), 4.77 (d, *J* = 5.6 Hz, 2 H), 3.79 (dd, *J* = 2.4, 8.8 Hz, 2 H) ppm. **^{13}C NMR** (100 MHz, acetone-*d6*): δ = 170.3, 165.2, 161.6, 153.1, 151.1, 150.2, 136.6, 131.7, 124.9, 121.4, 118.4, 114.9, 78.4, 69.1, 34.7 ppm. **FT-IR** (thin film): ν = 3054 (w), 2924 (s), 2854 (m), 1753 (m), 1732 (m), 1490 (w), 1446 (w), 1265(s), 1073 (w), 1031 (w), 738 (s), 705 (w) cm^{-1}. **HRMS-ESI** (m/z): $[M + H]^+$ calc'd for $C_{15}H_{13}N_2O_5S_2$, 365.0266; found, 365.0245. **T_r** (RP-HPLC, 5% MeCN/H_2O → 70% MeCN/H_2O in 30 mins): 12.3 min.

Background Hydrolysis of Alloc-protected Luciferin 7



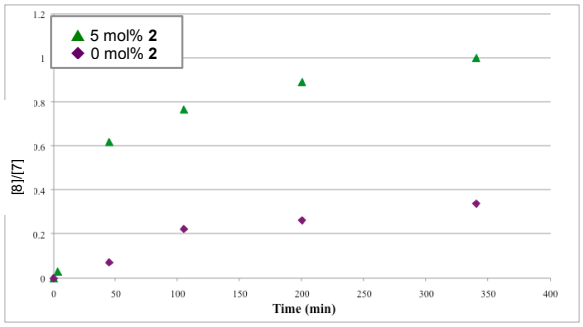


Figure S1. [**8**]/[**7**] over time determined by ^{1}H NMR. Formation of **8** was observed in the absence of **2**.

Since the background hydrolysis of **7** will complicate the bioluminescence readout, alloc-protected aminoluciferin **4b**, incorporating a hydrolytically more robust carbamate linker, was next selected for study

HPLC Analysis of Product Distribution

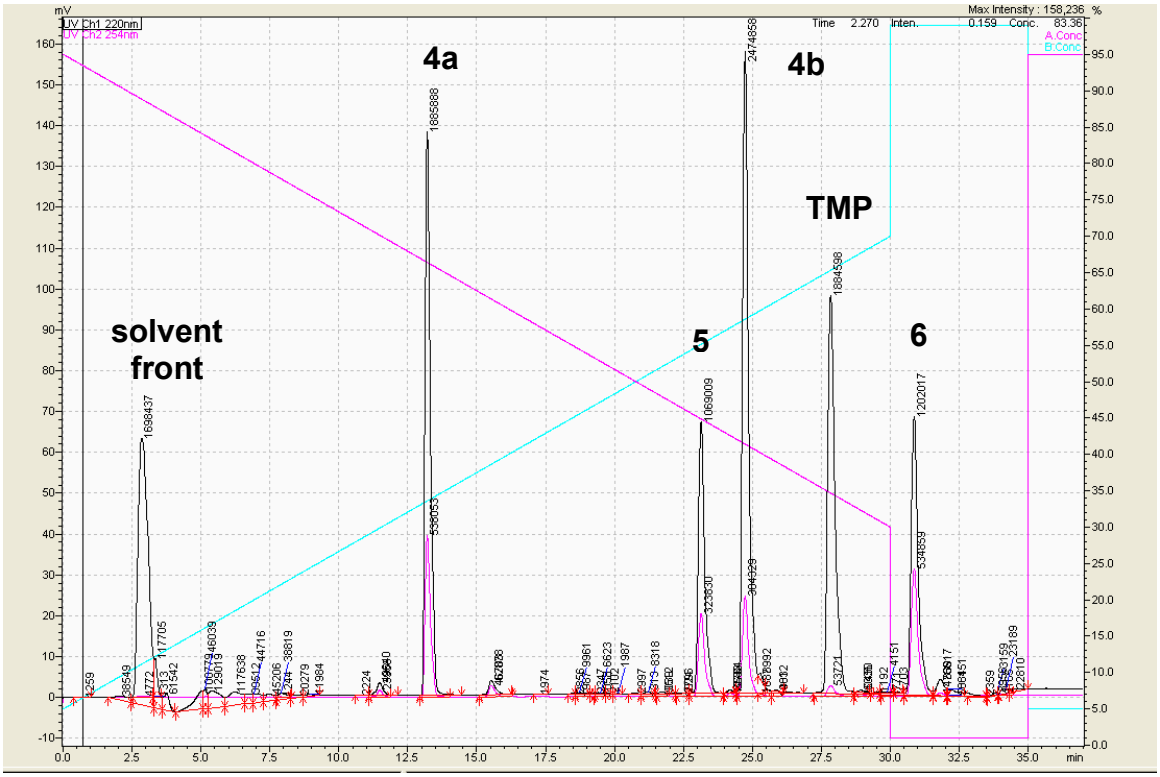


Figure S2. HPLC chromatogram of compounds **4a**, **4b**, **5**, and **6**.

Table S1. HPLC calibration of compounds **4a**, **4b**, **5**, and **6** (TMP: internal standard)

Compound	T_r (mins)	Amount/peak area (μmol)
4a	13.3	3.04E-07
5	22.3	2.87E-07
4b	25	1.91E-07
TMP	28	5.87E-07
6	31	2.27E-07

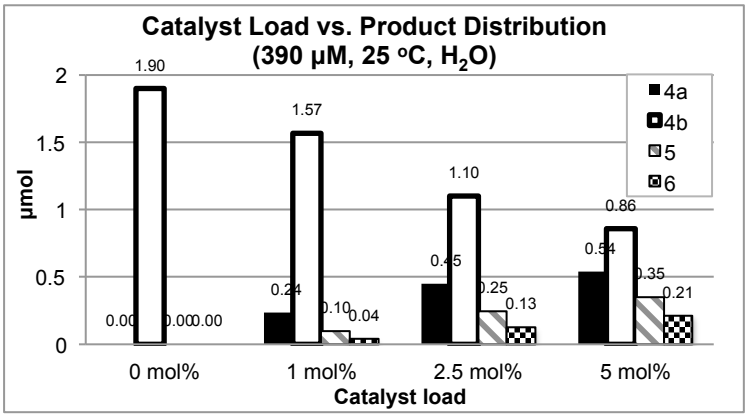
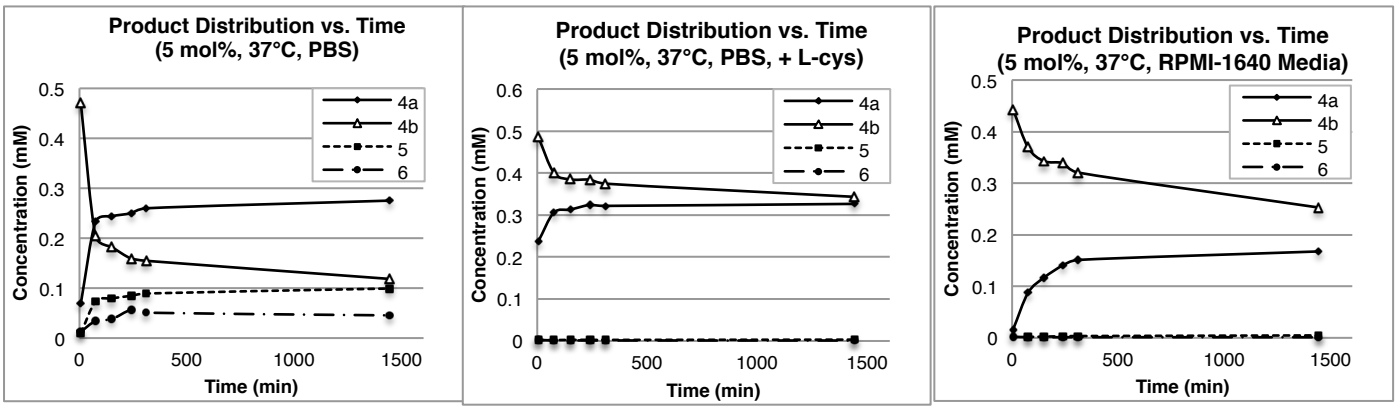


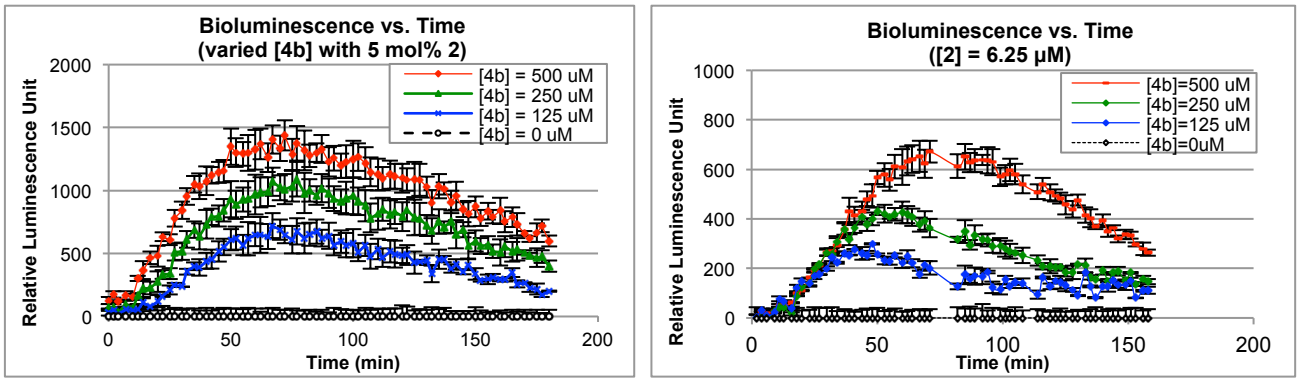
Figure S3. Product distributions of the reaction between **2** and **4b** after 24 hours with varied catalyst load. ($[\mathbf{4b}]_0$ = 390 μM. A = 500 μM **4b** stock solution in DI water; B = 100 μM **2** stock solution in DI water. 0 mol%: 3.5 mL A+0 mL B+1 mL DI water; 1 mol%: 3.5 mL A+0.175 mL B+0.825 mL DI water; 2.5 mol%: 3.5 mL A+0.4375 mL B+0.5625 mL DI water; 5 mol% = 2.5 mL A+0.875 mL B+0.125 mL DI water. Total volume of each reaction = 4.5 mL.)



(a) (b) (c)

Figure S4. Product distribution of the reaction between **2** and **4b** over time with 5 mol% catalyst load. (a) 37 °C in PBS ($[\mathbf{4b}]_0$ = 470 μM; $[\mathbf{2}]_0$ = 23.5 μM); (b) 37 °C in PBS with 1 equivalent of L-cysteine ($[\mathbf{4b}]_0$ = 490 μM; $[\mathbf{2}]_0$ = 24.5 μM; $[\text{L-cys}]_0$ = 490 μM); (c) 37°C in RPMI-1640 media ($[\mathbf{4b}]_0$ = 440 μM; $[\mathbf{2}]_0$ = 22 μM).

Figures with Error Bars



(a) (b)

Figure S5. Bioluminescence intensity over time when 4T1 cells were (a) treated with **4b** at varied concentrations immediately followed by **2** (5 mol% relative to [**4b**]). (b) Bioluminescence intensity over time when 4T1 cells were treated with varied concentrations of **4b** immediately followed by **2** ([**2**] = 6.25 μM).

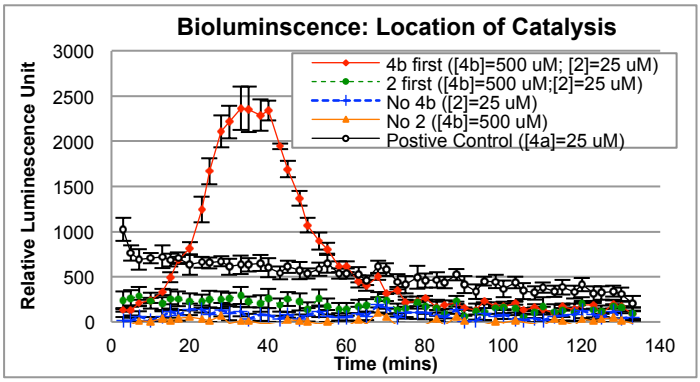


Figure S6. Treatment of 4T1 cells with **4b** and **2** in different orders. ◆: treated with **4b** at T= -15min ([**4b**] = 500 μM)→PBS wash and treated with **2** at T_0 ([**2**] = 25 μM); •: treated with **2** at T= -15 min ([**2**] = 25 μM)→PBS wash and treated with **4b** at T_0 ([**4**] = 500 μM); +: treated with **2** at T_0 ([**2**] = 25 μM); Δ: treated with **4b** at T_0 ([**4b**] = 500 μM). O: positive control – treated with **4a** at T_0 ([**4a**] = 25 μM);

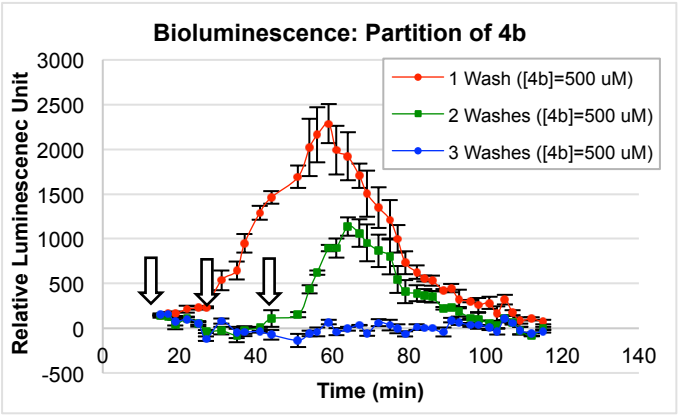


Figure S7. Bioluminescence over time when 4T1 cells were treated with **4b** and washed with PBS before the treatment of **2**. •: treated with **4b** at T_0 ([**4b**] = 500 μM)→1 PBS wash and treated with **2** ([**2**] = 25 μM); •: treated with **4b** at T_0 ([**4b**] = 500 μM)→2 PBS washes with a 15-minute interval and treated with **2** ([**2**] = 25 μM); •: treated with **4b** at T_0 ([**4b**] = 500 μM)→3 PBS washes with 2 15-minute intervals and treated with **2** ([**2**] = 25 μM).⇩: PBS wash administered.

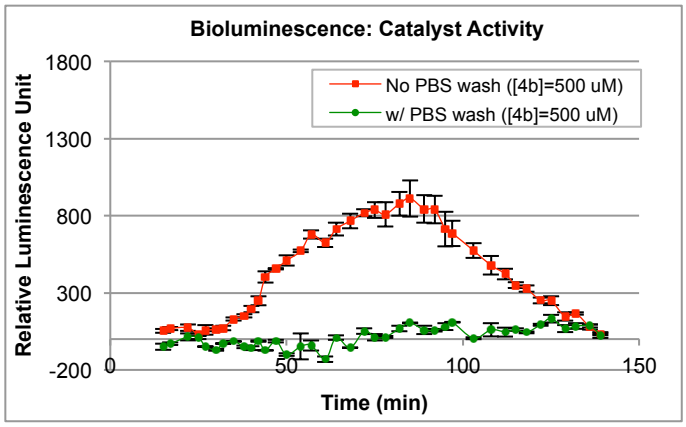


Figure S8. Treatment of 4T1 cells with **2** at T_0 followed by **4b** at T = 15 min. When a PBS wash was not performed between the treatments, bioluminescence was still observed. ■: treated with **2** ([**2**] = 25 μM)→treated with **4b** at T = 15 min; •: treated with **2** at T_0 ([**2**] = 25 μM)→PBS wash and treated with **4b** ([**4b**] = 500 μM) at T = 15 min.

Low Bioluminescence Intensity of the Positive Control

Low level of bioluminescence intensity was observed with the positive control in Figure 2. One major factor is the concentrations used for the control and the substrate: the positive control was conducted at a significantly (20-fold) lower concentration of **4a** (25 μM) relative to **4b** (500 μM with 5 mol% catalyst load). The relative signal intensity is further impacted by the timing of signal collection. We have previously reported that when luciferase-transfected PC3M cells are treated with luciferin only, a very strong initial bioluminescence signal was observed, but the signal faded away rapidly, and is close to baseline after ~3 to 4 minutes.[4] Since the first bioluminescence image taken in Figure 2 was 2 minutes after the cells were treated with **2** due to the exposure time of the CCD camera, the first 2 images only captured the end of the initial burst of bioluminescence signal of the positive control, and the low level of bioluminescence in the remaining time was the fading "tail" approaching baseline that was

observed previously. An experiment was performed to confirm that aminoluciferin turnover in luciferase-transfected 4T1 cells also follows a similar pattern (Figure S9).

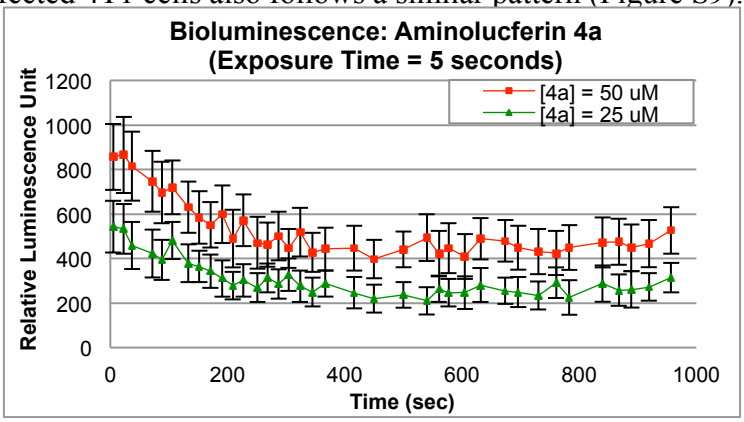
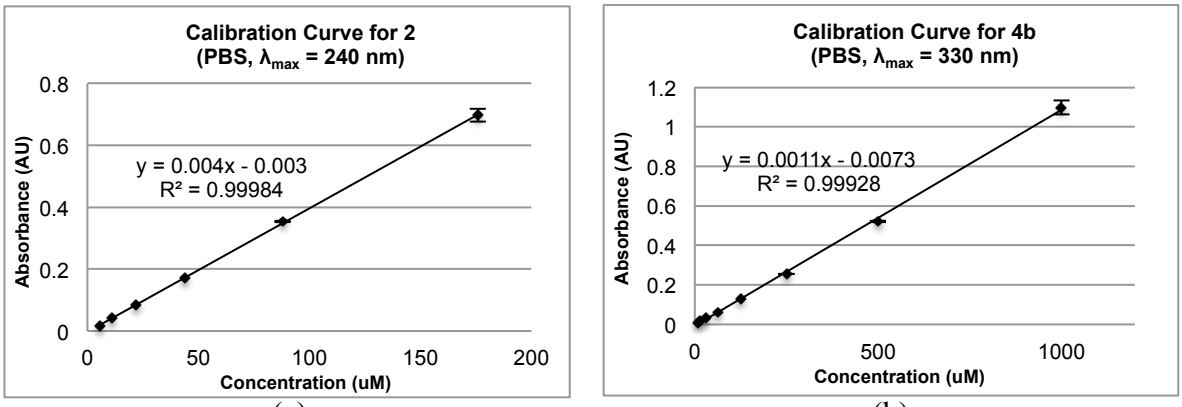


Figure S9. Bioluminescence intensity vs. time when 4T1 cells were treated with aminoluciferin **4a**. Camera was shortened from 2 minutes (Figure 2) to 5 seconds to capture the initial strong bioluminescence of the positive control.

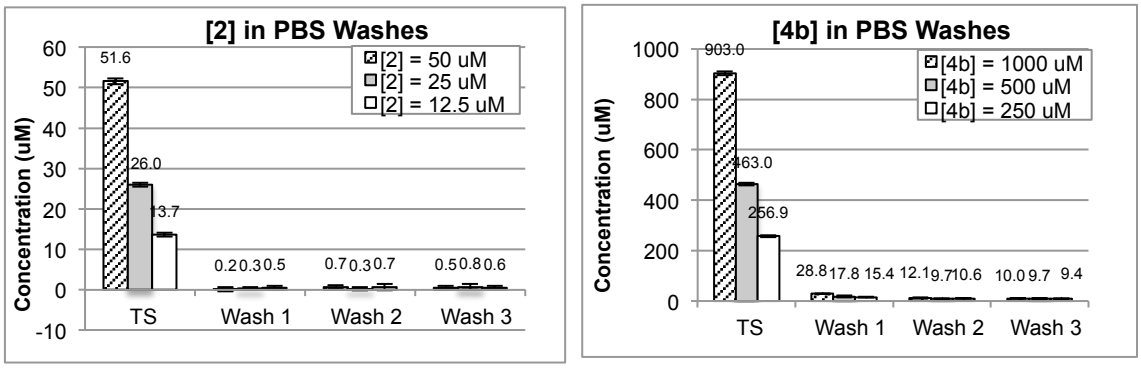
NanoDrop Calibration of 2 and 4b



(a) (b)

Figure S10. (a) Calibration curve of **2** on NanoDrop 1000 spectrophotometer in PBS: λ_{max} = 240 nm, AU = 0.004 [**2**] – 0.003, R^2 = 0.99. (b) Calibration curve of **4b** on NanoDrop 1000 spectrophotometer in PBS: λ_{max} = 330 nm, AU = 0.0011 [**4b**] – 0.0073, R^2 = 0.99.

Treatment Solutions (TS) and PBS Washes Analyses

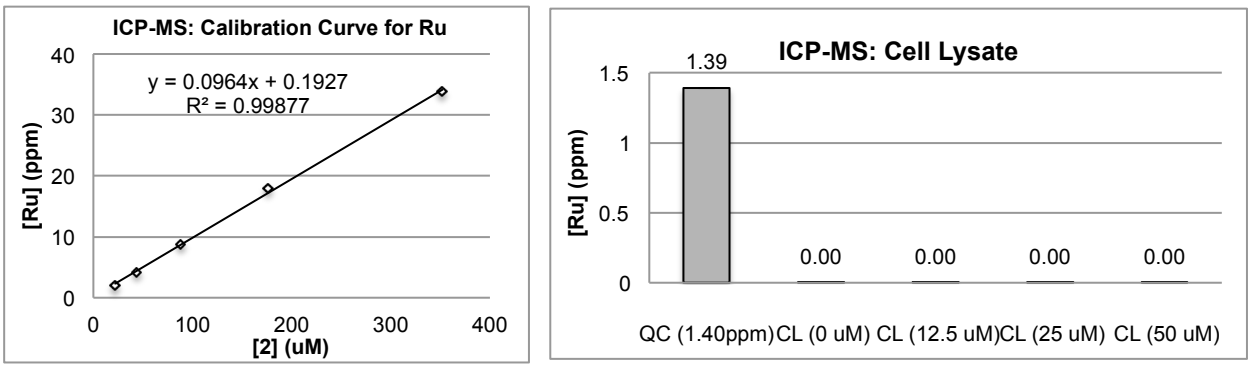


(a) (b)

Figure S11. Concentration of (a) **2** and (b) **4b** in extracellular treatment solutions (TS) and PBS washes of 4T1 cells calculated from calibration curves in Figure S10.

ICP-MS Analysis of Cell Lysates

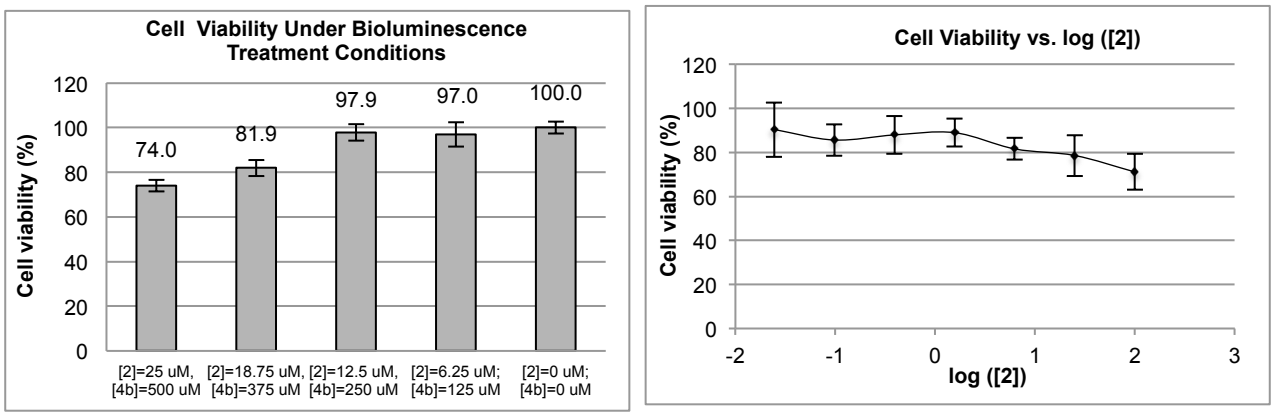
4T1 cells were treated with **2** at varied concentrations identical to previous bioluminescence experiments (50 μM, 25 μM, 12.5 μM, and 0 μM) for 15 minutes. The cells were then washed with PBS and lysed with concentrated $HCl_{(aq)}$. ICP-MS experiments were performed on the cell lysates by Stanford University Environmental Measurements Facility (Thermo Xseries II), and the result showed that no trace ruthenium was detected in the cell lysates (Figure S12). This observation further supported the hypothesis that **2** cleaves **4b** extraceullarly. In addition, it suggests that **2** might have low affinity for cell membrane because if **2** had bound to cell membrane tightly, ruthenium is likely to be released from the membrane into the aqueous phase when the cells were lysed with concentration $HCl_{(aq)}$.



(a) (b)

Figure S12. ICP-MS experiments on cell lysates. (a) Calibration curve of ruthenium. The ruthenium concentration detected by ICP-MS is consistent with the calculated ruthenium concentration from the concentration of **2**. (b) ICP-MS on cell lysates. QC = quality control ([Ru] = 1.40 ppm); CL = cell lysate. The concentration in the parenthesis is the concentration of **2** that the cells were treated with before they were lysed with concentrated $HCl_{(aq)}$.

MTT Assays for Cell Viability

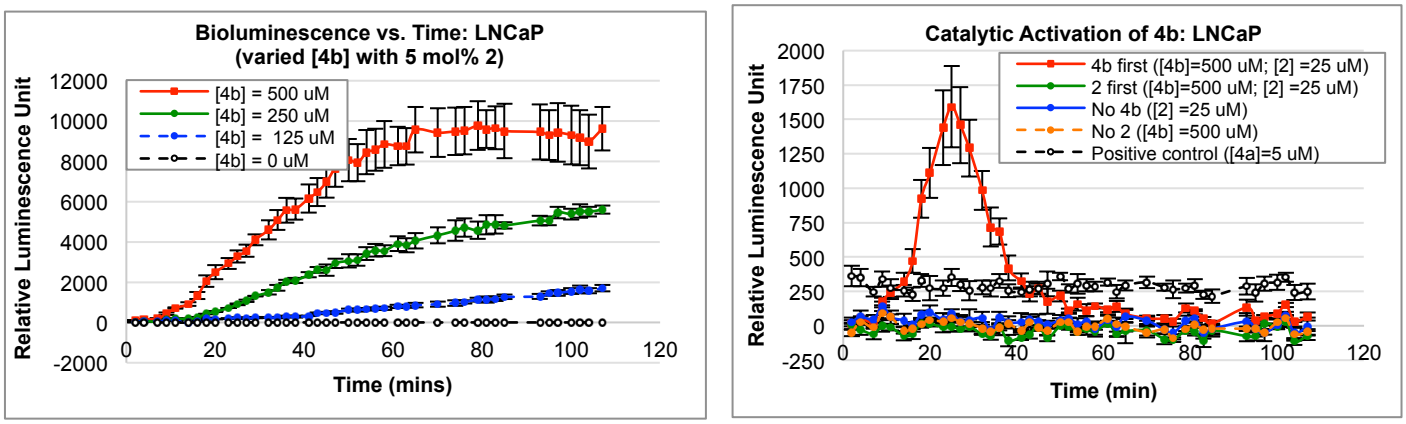


(a) (b)

Figure S13. (a) MTT assay for cell viability under bioluminescence treatment conditions. (b) MTT assay for cell viability as a function of log([**2**]).

Catalytic Cleavage of 4b by 2 in LNCaP Cells

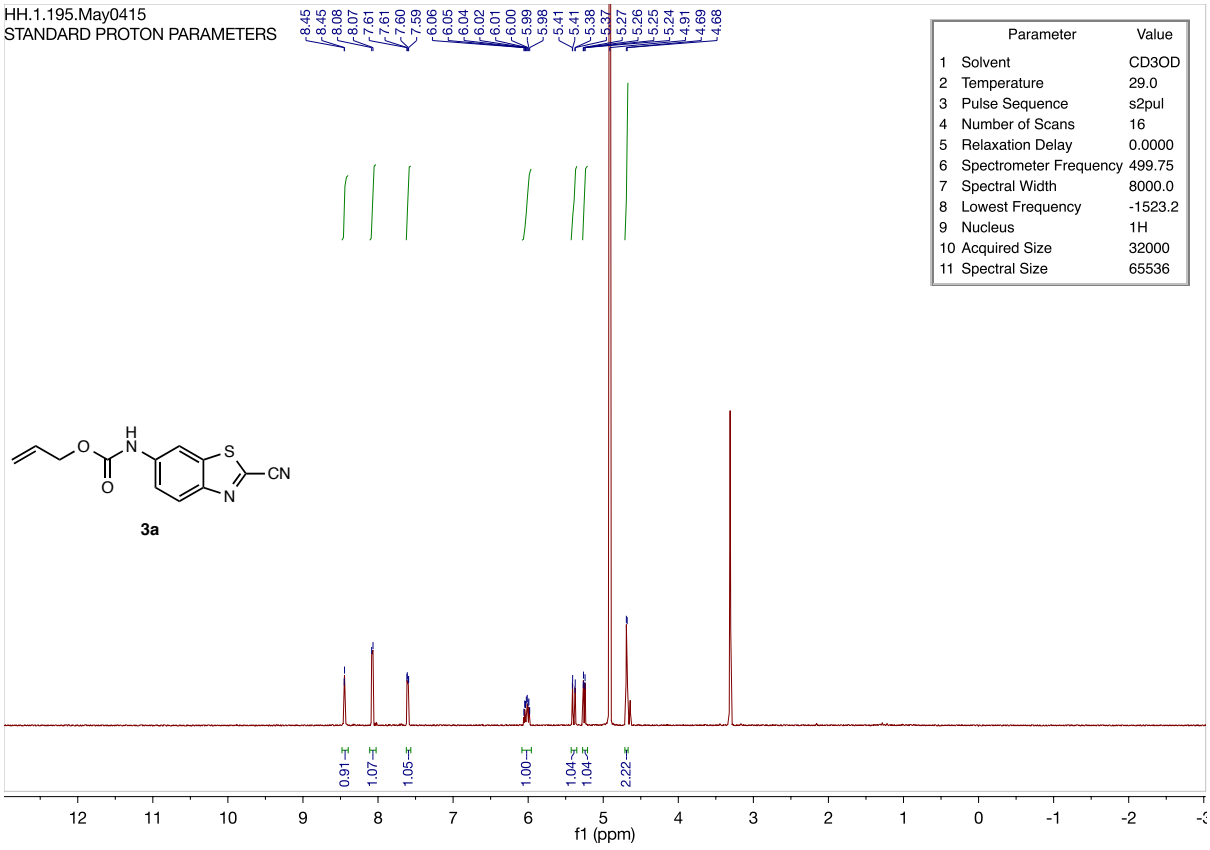
To explore whether the catalytic activated bioluminescence can be extended to a different cell type, luciferase-transfected LNCaP cells (a human prostate adenocarcinoma cell line) were treated with **2** and **4b**. When the cells were treated with **4b** immediately followed by **2**, dose-dependent photon emission was observed. When the cells were treated with pre-catalyst **2** for 15 minutes, followed by a PBS wash to remove extracellular catalyst, and then treated with alloc-protected aminoluciferin **4b**, no bioluminescence was observed. When the treatment order was reversed, bioluminescence was observed and peaked at about 25 minutes after treatment with **2**. When either **2** or **4b** was absent, no bioluminescence was observed (Figure S14). This is analogous to what was observed in the luciferase-transfected 4T1 cells, indicating that this system is robust across different cell lines.

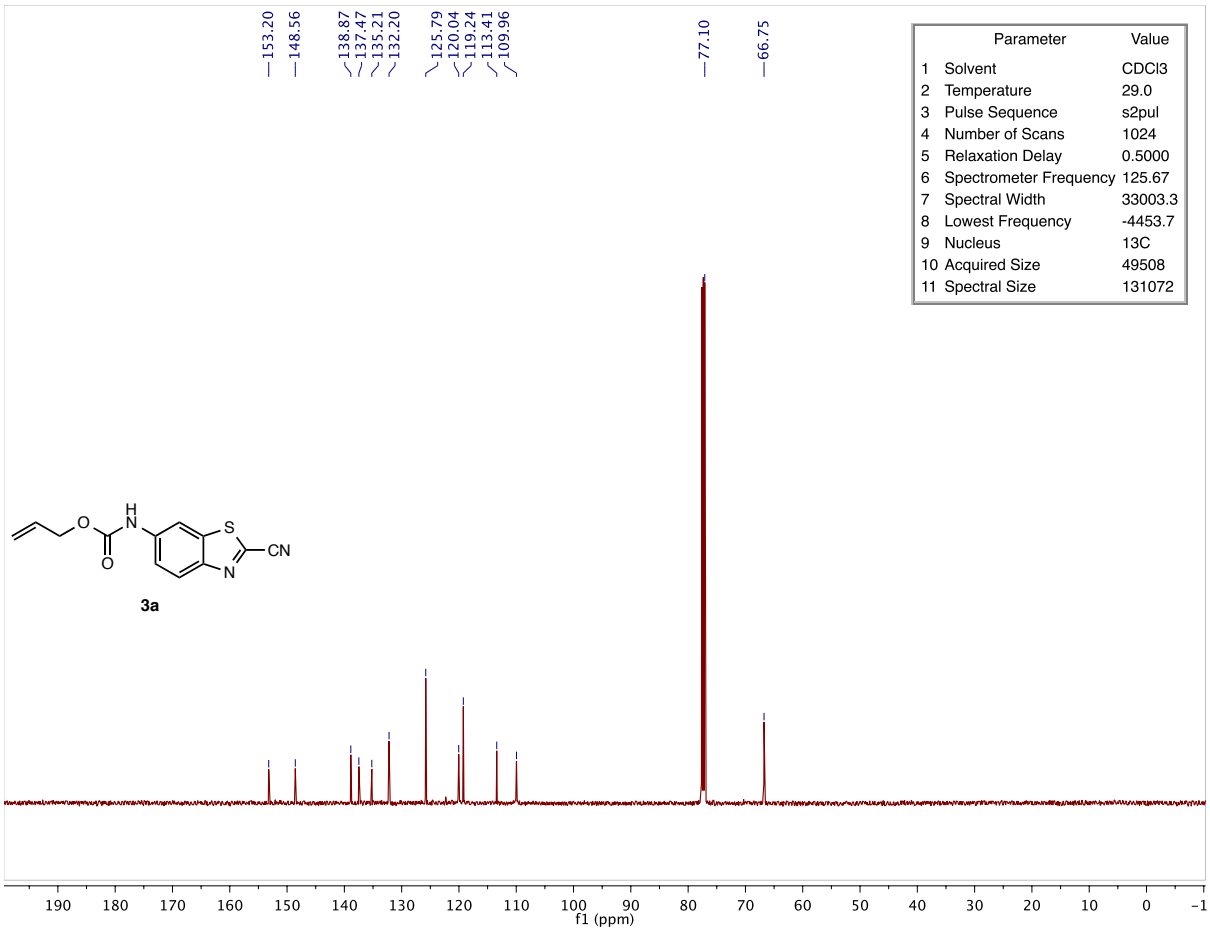


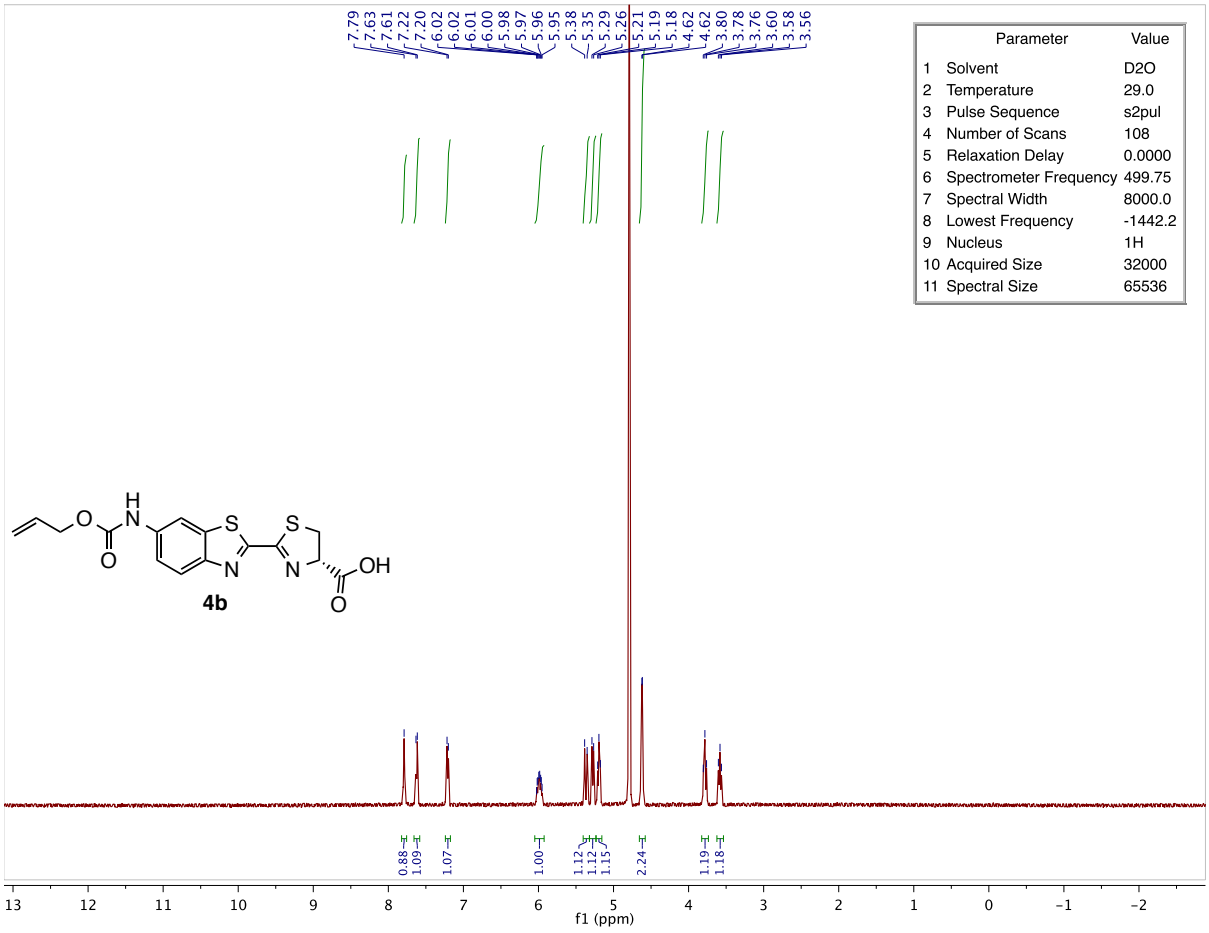
(a) (b)

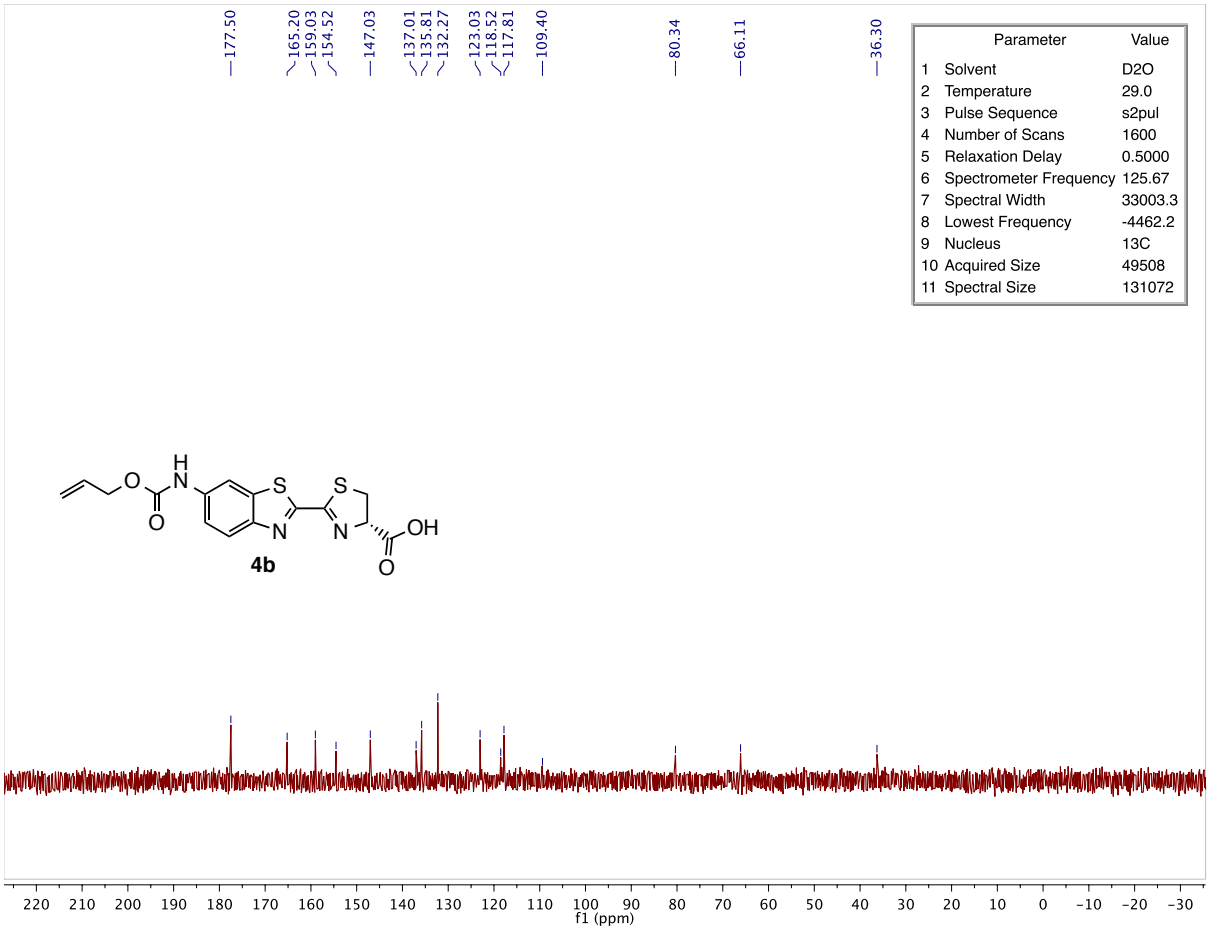
Figure S14. (a) Bioluminescence intensity over time when LNCaP cells were treated with varied concentrations of **4b** immediately followed by **2** (5 mol% with relative to [**4b**]). (b) Bioluminescence intensity over time when LNCaP cells were treated with **4b** and **2** in different orders. •: treated with **4b** at T= -15min ([**4b**] = 500 μM)→PBS wash and treated with **2** at T_0 ([**2**] = 25 μM); •: treated with **2** at T= -15 min ([**2**] = 25 μM)→PBS wash and treated with **4b** at T_0 ([**4b**] = 500 μM); • : treated only with **2** at T_0 ([**2**] = 25 μM); •: treated only with **4b** at T_0 ([**4b**] = 500 μM); O: positive control – treated with **4a** at T_0 ([**4a**] = 5 μM).

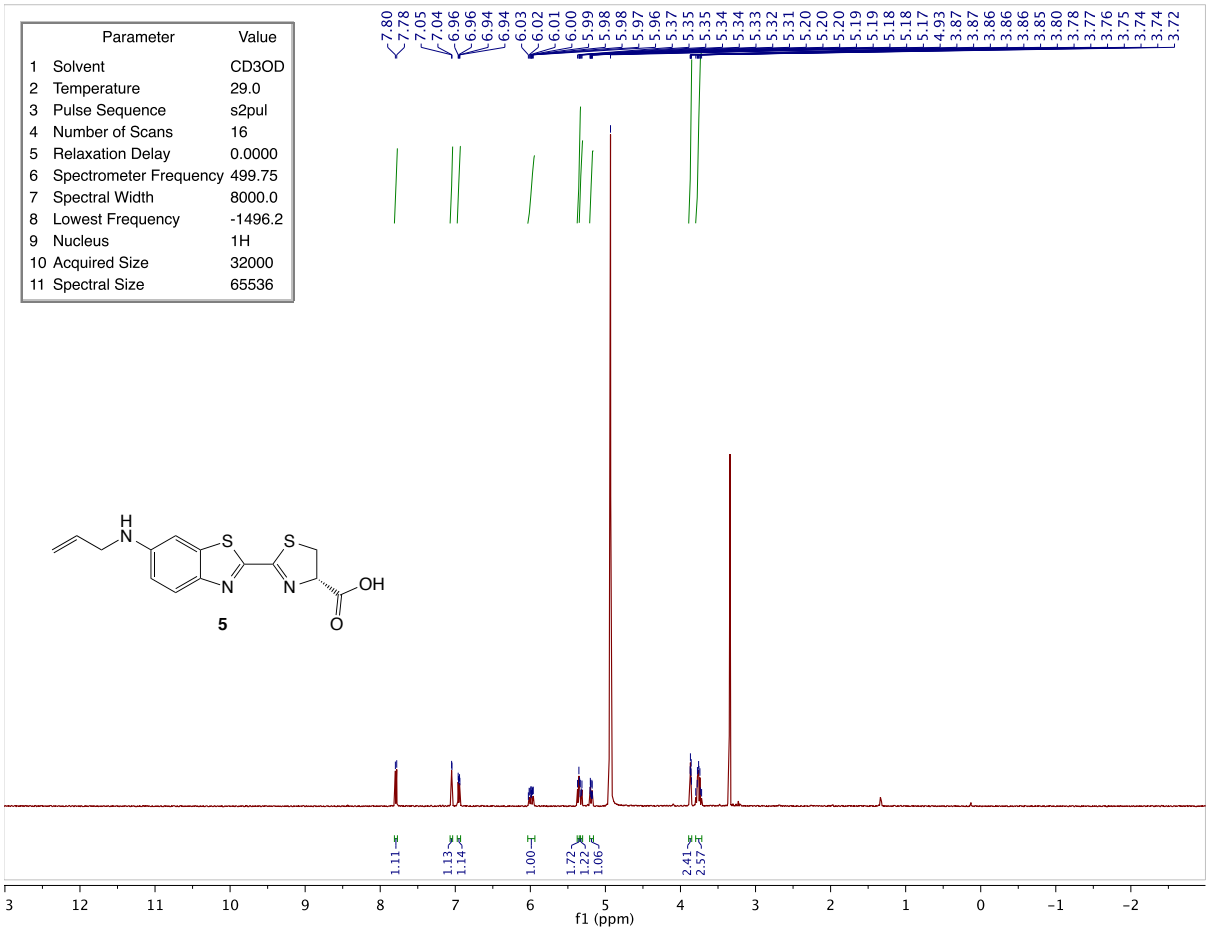
$^{1}H/^{13}C$ NMR Spectra



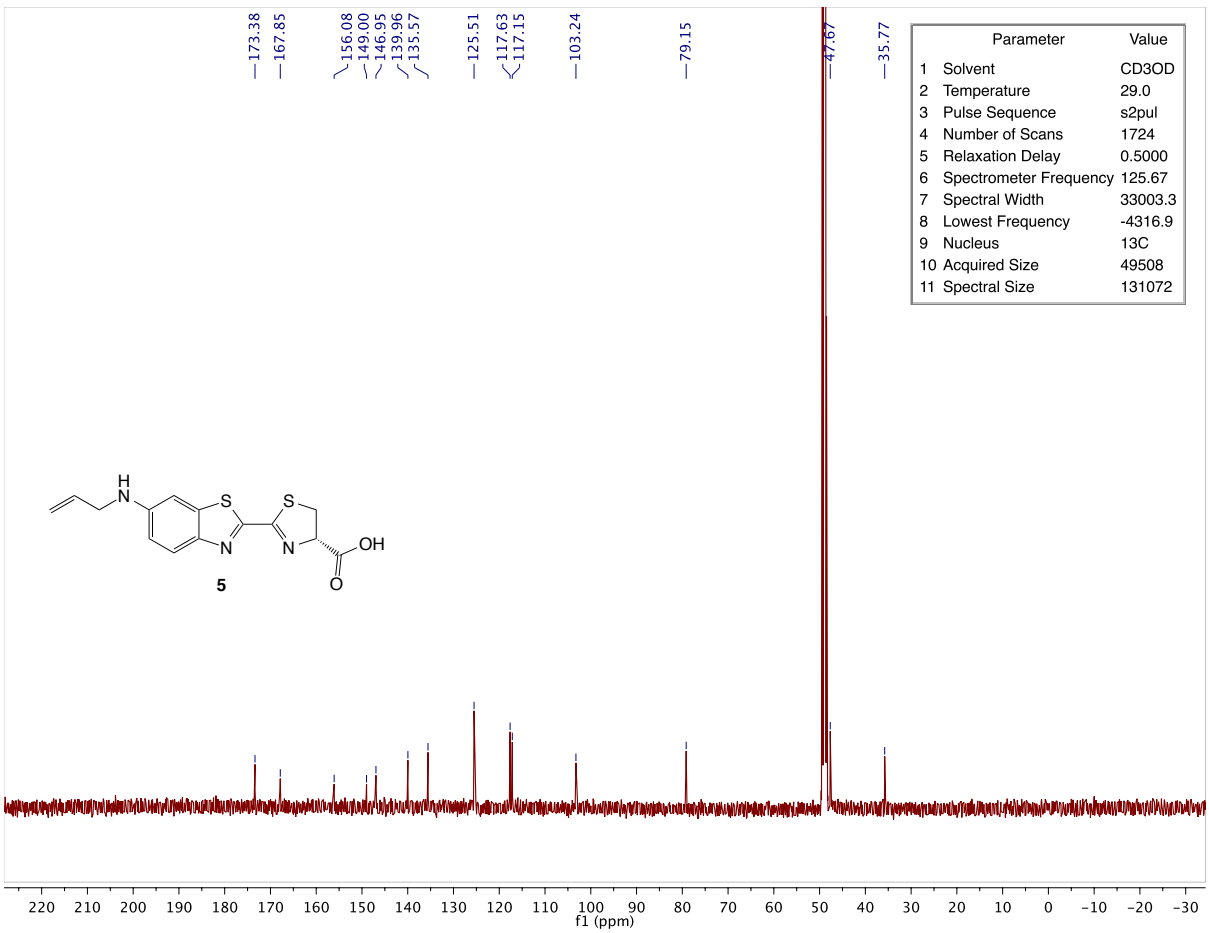



7.79
7.63
7.61
7.22
7.20
6.02
6.02
6.01
6.00
5.98
5.97
5.96
5.95
5.38
5.35
5.29
5.26
5.21
5.19
5.18
4.62
4.62
3.80
3.78
3.76
3.60
3.58
3.56
Parameter Value
1 Solvent D2O
2 Temperature 29.0
3 Pulse Sequence s2pul
4 Number of Scans 108
5 Relaxation Delay 0.0000
6 Spectrometer Frequency 499.75
7 Spectral Width 8000.0
8 Lowest Frequency -1442.2
9 Nucleus 1H
10 Acquired Size 32000
11 Spectral Size 65536
4b
0.88
1.09
1.07
1.00
1.12
1.12
1.15
2.24
1.19
1.18
f1 (ppm)


177.50
165.20
159.03
154.52
147.03
137.01
135.81
132.27
123.03
118.52
117.81
109.40
80.34
66.11
36.30
Parameter Value
1 Solvent D2O
2 Temperature 29.0
3 Pulse Sequence s2pul
4 Number of Scans 1600
5 Relaxation Delay 0.5000
6 Spectrometer Frequency 125.67
7 Spectral Width 33003.3
8 Lowest Frequency -4462.2
9 Nucleus 13C
10 Acquired Size 49508
11 Spectral Size 131072
4b
f1 (ppm)


Parameter Value
1 Solvent CD3OD
2 Temperature 29.0
3 Pulse Sequence s2pul
4 Number of Scans 16
5 Relaxation Delay 0.0000
6 Spectrometer Frequency 499.75
7 Spectral Width 8000.0
8 Lowest Frequency -1496.2
9 Nucleus 1H
10 Acquired Size 32000
11 Spectral Size 65536
7.80 7.78 7.05 7.04 6.96 6.96 6.94 6.94 6.03 6.02 6.01 6.00 5.99 5.98 5.98 5.97 5.96 5.37 5.35 5.35 5.34 5.34 5.33 5.32 5.31 5.20 5.20 5.19 5.19 5.18 5.17 4.93 3.87 3.87 3.86 3.86 3.85 3.80 3.78 3.77 3.76 3.75 3.74 3.74 3.72
1.11 1.13 1.14 1.00 1.72 1.22 1.06 2.41 2.57
f1 (ppm)

5


Parameter Value
1 Solvent CD3OD
2 Temperature 29.0
3 Pulse Sequence s2pul
4 Number of Scans 1724
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6 Spectrometer Frequency 125.67
7 Spectral Width 33003.3
8 Lowest Frequency -4316.9
9 Nucleus 13C
10 Acquired Size 49508
11 Spectral Size 131072
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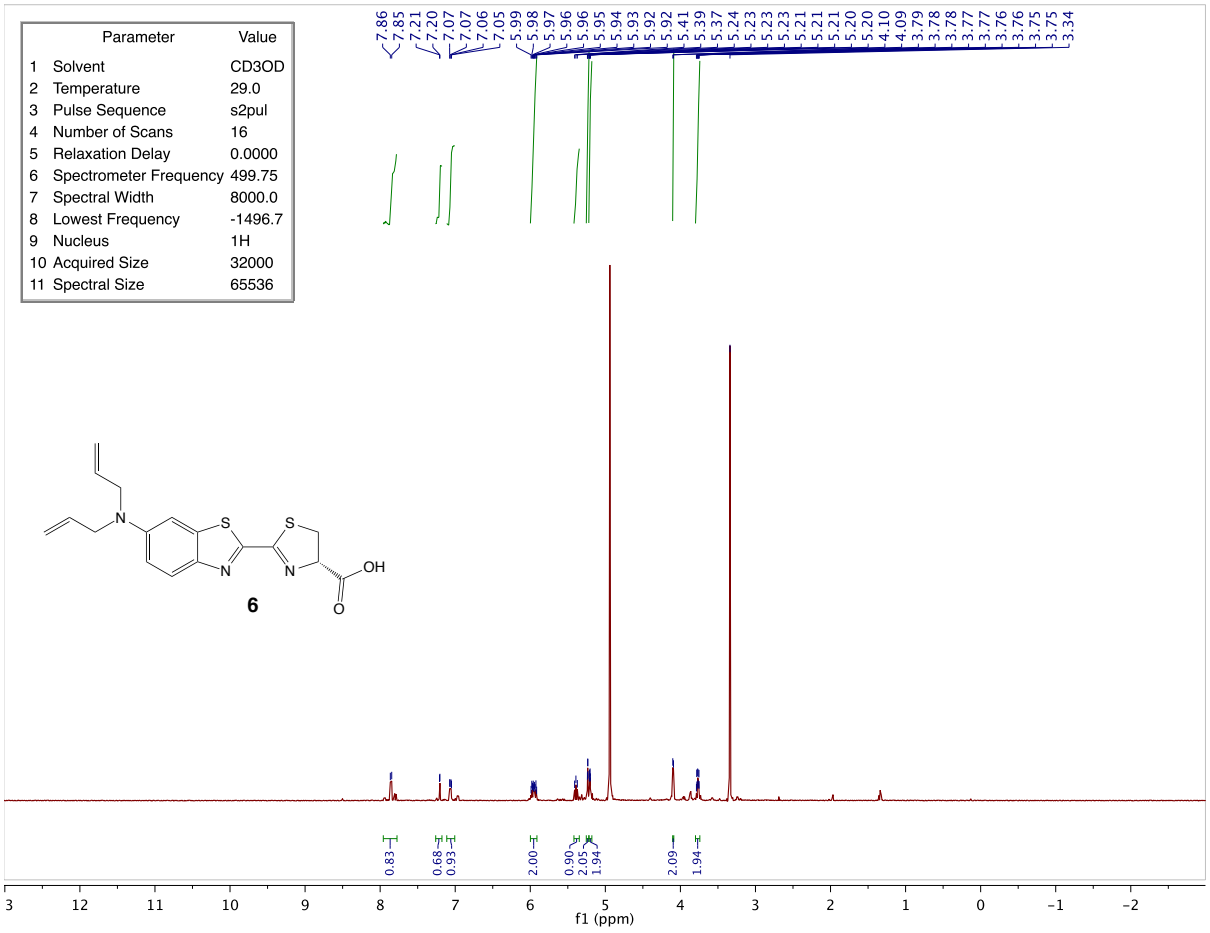
f1 (ppm)

5


Parameter Value
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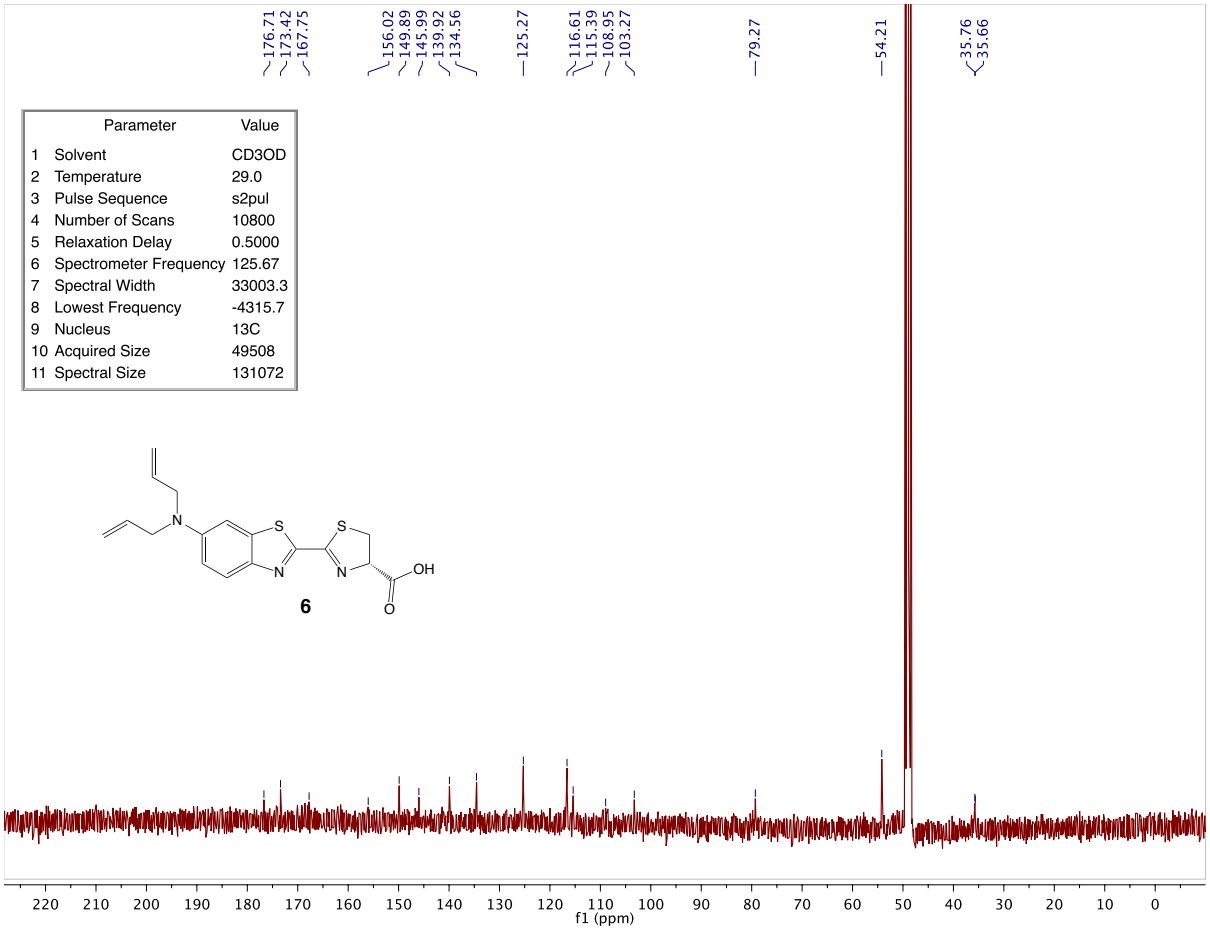
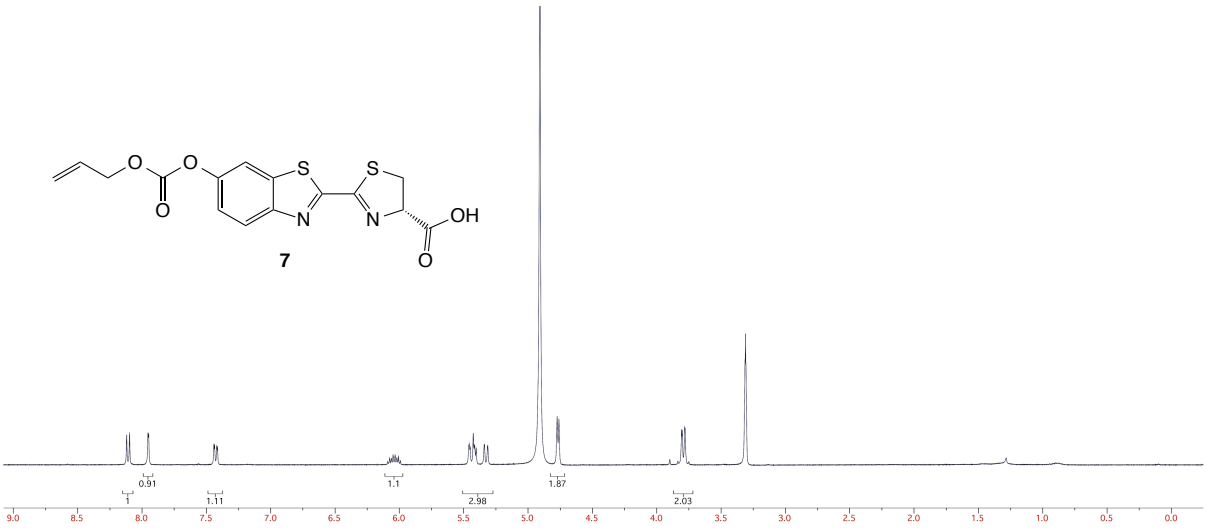
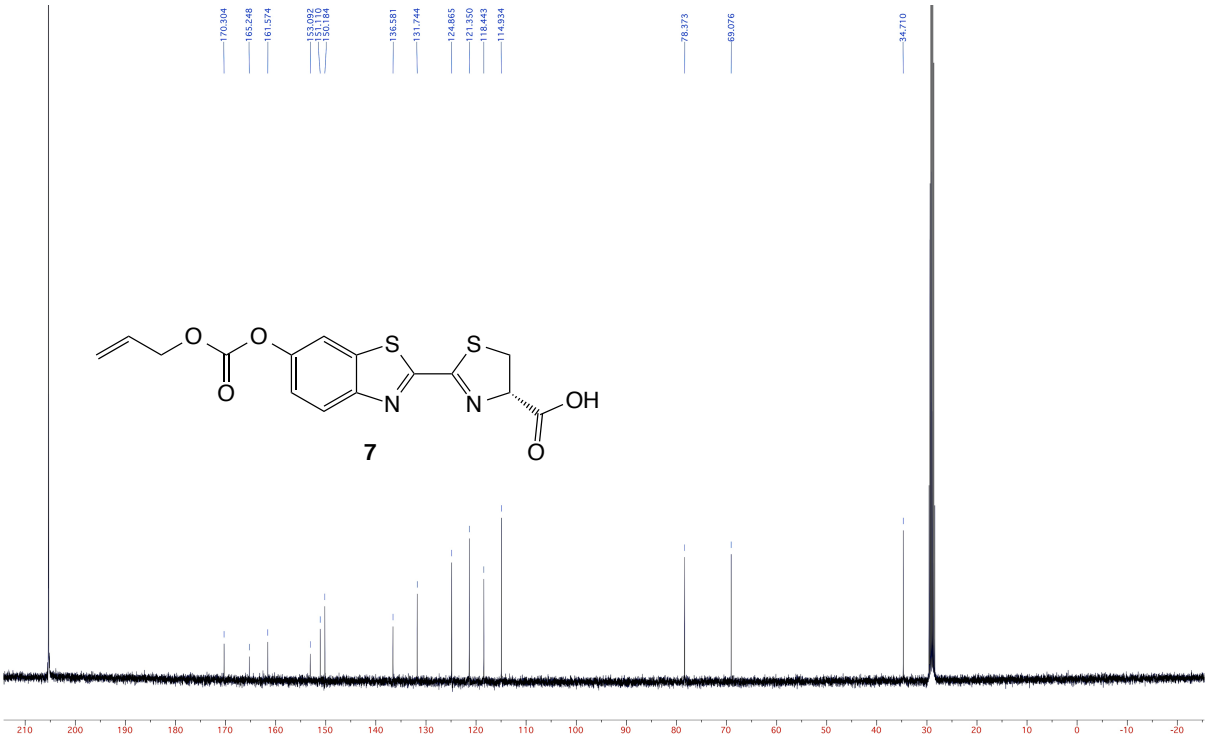
2 Temperature 29.0
3 Pulse Sequence s2pul
4 Number of Scans 16
5 Relaxation Delay 0.0000
6 Spectrometer Frequency 499.75
7 Spectral Width 8000.0
8 Lowest Frequency -1496.7
9 Nucleus 1H
10 Acquired Size 32000
11 Spectral Size 65536
7.86 7.85 7.21 7.20 7.07 7.07 7.06 7.05 5.99 5.98 5.97 5.96 5.96 5.95 5.94 5.93 5.92 5.92 5.41 5.39 5.37 5.24 5.23 5.23 5.21 5.21 5.20 4.10 4.09 3.79 3.78 3.78 3.77 3.77 3.76 3.76 3.75 3.75 3.34
0.83 0.68 0.93 2.00 0.90 2.05 1.94 2.09 1.94
f1 (ppm)

6


Parameter Value
1 Solvent CD3OD
2 Temperature 29.0
3 Pulse Sequence s2pul
4 Number of Scans 10800
5 Relaxation Delay 0.5000
6 Spectrometer Frequency 125.67
7 Spectral Width 33003.3
8 Lowest Frequency -4315.7
9 Nucleus 13C
10 Acquired Size 49508
11 Spectral Size 131072
176.71 173.42 167.75 156.02 149.89 145.99 139.92 134.56 125.27 116.61 115.39 108.95 103.27 79.27 54.21 35.76 35.66
f1 (ppm)

6


O
O
O
S
S
N
N
OH
O
7
0.91
1
1.11
1.1
2.98
1.87
2.03
9.0
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0


170.304
165.248
161.574
153.092
151.110
150.184
136.581
131.744
124.865
121.350
118.443
114.934
78.373
69.076
34.710
O
O
O
S
S
N
N
OH
O
7
210
200
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
-10
-20

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1. Kiesewetter, M. K., Waymouth, R. M. (2010) Kinetics of an Air- and Water-Stable Ruthenium(IV) Catalyst for the Deprotection of Allyl Alcohol in Water. *Organometallics* **29,** 6051–6056.
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4. Jones, L. R., Goun, E. A., Shinde, R., Rothbard, J. B., Contag, C. H. (2006) Releasable Luciferin−Transporter Conjugates: Tools for the Real-Time Analysis of Cellular Uptake and Release. *J. Am. Chem. Soc.* **128,** 6526–6527.