Supporting Information

A Bioluminogenic Probe for Rapid, Ultrasensitive Detection of β-Lactam Resistant Bacteria

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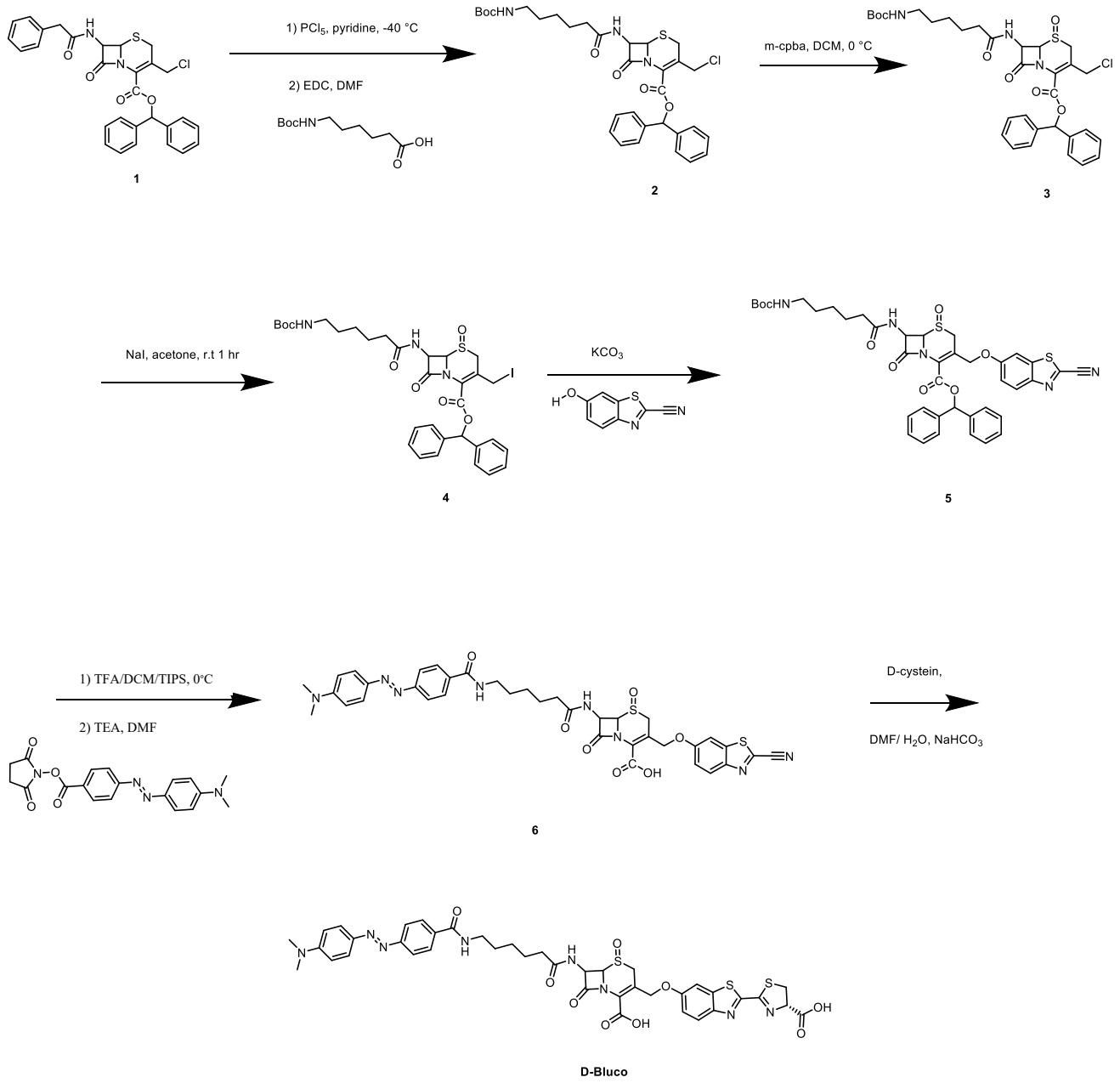
1. Experimental Procedures

1.1 General information.

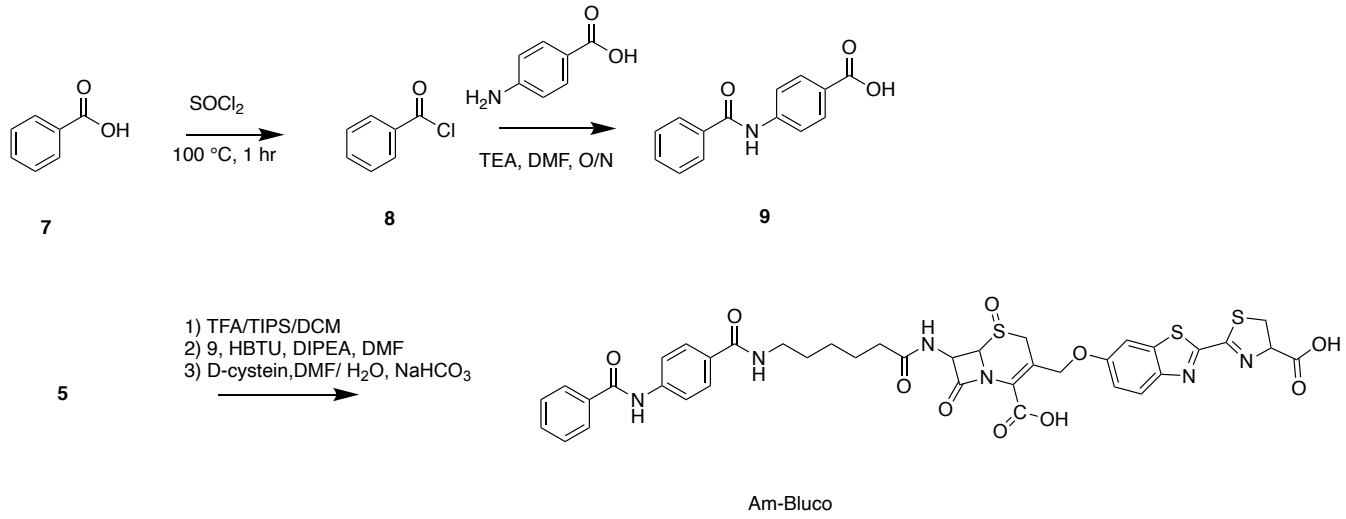
All chemicals were purchased from commercial sources and used without further purification. The clone of TEM-1 β-lactamase was described previously[1]. QuantiLum Recombinant luciferase (catalog number E1701) was purchased from Promega (Madison, WI). Reaction progress was monitored by analytical thin layer chromatography (TLC) with 0.25 mm silica gel 60F plates and visualized with fluorescent indicator (254 nm). Flash column chromatography was carried out using silica gel (SiliaFlash for flash column, 40-63 μm, 60Å). The ^{1}H and ^{13}C NMR spectra were acquired on a Varian 500 MHz or 600 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) relative to chloroform-d (δ 7.26, s); 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); and the number of protons (n) for a given resonance is indicated nH and based on the spectral integration values. High resolution mass spectra were obtained on a Thermo Exactive Orbitrap LC/MS. 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 MeCN (B) / H_2O (A) gradient mobile phase containing 0.1% trifluoroacetic acid at a flow of 1 or 3 mL/min for the analysis. Fluorescence Spectra and kinetic experiments were collected

by a SpectraMax iD3 multimode microplate reader (Molecular Device, San Jose, CA). Condition used for mass spectroscopy study: solvent A = 0.1% formic acid in water, solvent B = 0.1% formic acid in acetonitrile, C18: Zorbax Poroshell 120 SB-C18, 2.1x50 mm 2.7 µm, PN 689775-902 with Poroshell 120 SB-C18 2.1x5 mm 2.7 µm guard column, PN 821725-912, Electron spray (ESI) was used. For high resolution mass spectroscopy of D-Bluco: The samples were analyzed as provided by LC-flow injection ESI/MS on the Waters Acquity H-Class Plus UPLC and Thermo Exploris 240 BioPharma Orbitrap mass spectrometer. Methanol at a flow rate of 0.2 mL/min was used to transport the injected sample to the source directly, without chromatographic separation. The injection volume was 5 µL. Spectra were collected in full scan MS mode with polarity switching (collecting scans alternating between positive and negative ionization potentials), orbitrap resolution 120000, mass range of 100-1000 Da.

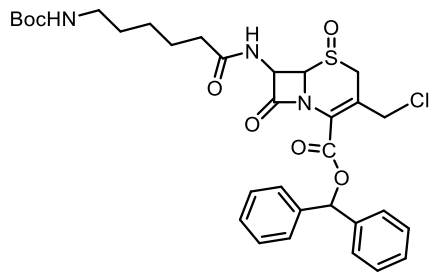
1.2. Synthetic procedures and schemes.



Scheme S1. Synthesis of D-Bluco.



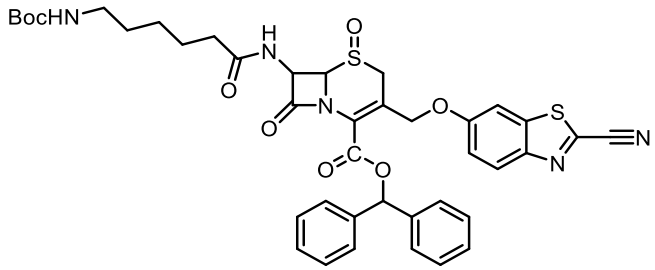
Scheme S2. Synthesis of Am-Bluco.



3

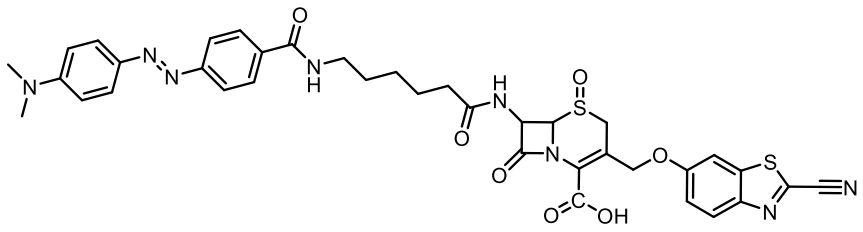
Compound 3: benzhydryl 7-(6-((tert-butoxycarbonyl)amino)hexanamido)-3-(chloromethyl)-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylate. To a stirring suspension of PCl_5 (2.3 g, 11.3 mmol) in DCM (10 mL) was added pyridine (0.89 g, 11.3 mmol) on ice. After 15 min, the solution was cooled to -40 °C, and a solution of **1** (purchased from Pharmacore, China, 1.0 g, 1.88 mmol) in DCM (10 mL) was added. The solution was subsequently stirred for 3 h before quenched by the addition of MeOH (2 mL), and stirred for another 30 min. The mixture was diluted with DCM (30 mL), washed with water, brine, dried over Na_2SO_4, filtered, and concentrated under reduced pressure to give a sticky brown solid. This solid was used for the next step without further purification. N-Boc-6-aminohexanoic Acid (278.74 mg, 1.2 mmol) in DMF (1 mL) was mixed with the crude product (500 mg, 0.113 mmol), and 1-Ethyl-3-(3-dimethylaminopropyl)-carbodiimide (EDC, 693.1 mg, 3.6 mmol). The mixture was stirred for 4 h before diluted with ethyl acetate (50 mL) and washed with water (50 mL). The organic layer was separated, dried over Na_2SO_4, filtered, and concentrated under reduced pressure to give the precursor for compound **2**. A solution of the crude compound from the previous step (60 mg, 0.096 mmol) in anhydrous DCM (5 mL) was cooled to 0 °C prior to the addition of meta-chloroperoxybenzoic acid (m-cpba, 21.9 mg, 1.0 mmol). The reaction was stirred at 0 °C for an hour. Silicon column purification afforded pure compound **3** (27.8 mg, 30.3% over 3 steps). ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.45 (d, J = 7.6 Hz, 2H), 7.41 – 7.24 (m, 9H), 6.95 (s, 1H), 6.79 (d, J = 9.8 Hz, 1H), 6.11 (dd, J = 9.8, 4.8 Hz, 1H), 4.88 (d, J = 12.3 Hz, 1H), 4.63 (s, 1H), 4.52 (d, J = 4.7 Hz, 1H), 4.21 (d, J = 12.3 Hz, 1H), 3.82 (d, J = 18.7 Hz, 1H), 3.39 (d, J = 18.6 Hz, 1H), 3.11 (d, J = 6.7 Hz, 2H), 2.29 (t, J = 7.5 Hz, 2H), 1.65 (q, J = 7.8 Hz, 2H), 1.49 (t, J = 7.8 Hz, 2H), 1.43 (s, 9H), 1.38 – 1.28 (m, 2H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 173.33, 164.49, 159.63, 156.04, 138.87, 138.78, 128.72, 128.59, 128.40, 128.31, 127.63, 127.00, 125.53, 121.32, 80.56, 79.14, 67.09, 59.00, 46.82,

43.55, 40.39, 36.08, 29.77, 28.47, 26.33, 24.94. LCMS m/z: $[M+H]^+$ calculated for $C_{32}H_{39}ClN_3O_7S$) 644.21; found 644.5.



5

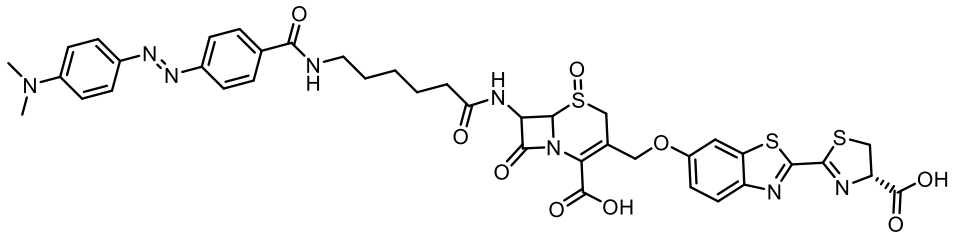
Compound 5: benzhydryl 7-(6-((tert-butoxycarbonyl)amino)hexanamido)-3-(((2-cyanobenzo[d]thiazol-6-yl)oxy)methyl)-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylate 5-oxide. A mixture of **3** (150 mg, 0.23 mmol) and sodium iodide (105 mg, 0.70 mmol) in 10 mL of acetone was stirred for 1.5 h at ambient temperature. The reaction mixture was concentrated under reduced pressure and diluted with 5 mL of water. The suspension was extracted with ethyl acetate, and the organic phase was washed with 10% $Na_2S_2O_3$, H_2O, brine and dried over Na_2SO_4. The solvent was removed to get the titled iodide **4**, which was used for the next step without further purification. A mixture of 6-hydroxybenzo[d]thiazole-2-carbonitrile (61.6 mg, 0.35 mmol), KCO_3 (48.3 mg, 0.35 mmol) and 4 in acetonitrile was stirred at 25 ºC for 9 h. DCM was added and washed with water. Flash chromatography purification on a silica gel column afforded 33.8 mg of compound **5** (55% yield over two steps). ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.93 (d, *J* = 9.1 Hz, 1H), 7.38 – 7.35 (m, 2H), 7.28 – 7.18 (m, 9H), 7.04 – 6.98 (m, 2H), 6.85 (s, 1H), 6.51 (d, *J* = 9.9 Hz, 1H), 6.06 (dd, *J* = 9.9, 4.8 Hz, 1H), 5.20 (d, *J* = 13.8 Hz, 1H), 4.73 (d, *J* = 13.8 Hz, 1H), 4.44 (d, *J* = 4.8 Hz, 1H), 4.02 – 3.96 (m, 1H), 3.29 (d, *J* = 19.0 Hz, 1H), 3.00 (d, *J* = 7.0 Hz, 2H), 2.18 (td, *J* = 7.4, 2.8 Hz, 2H), 1.59 – 1.54 (m, 2H), 1.42 – 1.33 (m, 2H), 1.31 (s, 9H), 1.26 (q, *J* = 7.7 Hz, 2H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 172.94, 164.36, 159.87, 158.07, 155.86, 147.27, 138.75, 138.68, 137.21, 134.15, 128.64, 128.55, 128.35, 128.26, 127.42, 126.85, 126.04, 124.59, 122.12, 118.34, 112.88, 103.84, 67.73, 66.81, 58.90, 45.35, 40.26, 36.07, 29.70, 29.59, 28.33, 26.21, 24.81. LCMS m/z: $[M+H]^+$ calculated for $C_{40}H_{42}N_5O_8S_2$ 784.24; found 784.92.



6

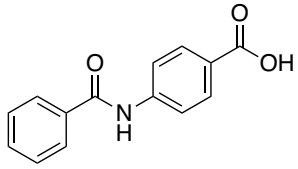
Compound 6: (E)-3-(((2-cyanobenzo[d]thiazol-6-yl)oxy)methyl)-7-(6-(4-((4-(dimethylamino) phenyl)diazenyl)benzamido)hexanamido)-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid 5-oxide. To a solution of **5** (30 mg, 0.038 mmol) in 1.5 mL of dry dichloromethane was added triisopropyl silane (TIPS) (20 µL) and trifluoroacetic acid (200 µL) at 0 °C. The mixture was stirred for 2 h at the same temperature, then the solvent was evaporated under reduced pressure. The residue was washed with ether (1 mL x 3) and this crude product was mixed with 2,5-dioxopyrrolidin-1-yl (E)-4-((4-(dimethylamino)phenyl)diazenyl)benzoate (9.8 mg, 0.027 mmol), triethylamine (TEA, 16 µL, 0.114 mmol) in DMF. The mixture was stirred for 18 h. Silicon column purification afforded pure compound **6** (25.5 mg, 0.031 mmol, 80%).^{1}H NMR (600 MHz, DMSO) δ 8.50 (d, *J* = 5.6 Hz, 1H), 8.16 (d, *J* = 8.1 Hz,

1H), 8.11 (d, *J* = 9.1 Hz, 1H), 7.94 (d, *J* = 8.5 Hz, 2H), 7.86 (d, *J* = 2.5 Hz, 1H), 7.76 (t, *J* = 9.2 Hz, 4H), 7.27 (dd, *J* = 9.1, 2.5 Hz, 1H), 6.79 (d, *J* = 9.2 Hz, 2H), 5.77 (dd, *J* = 8.1, 4.7 Hz, 1H), 5.11 (d, *J* = 12.1 Hz, 1H), 4.89 (d, *J* = 4.6 Hz, 1H), 4.83 (d, *J* = 12.0 Hz, 1H), 3.92 (d, *J* = 18.5 Hz, 1H), 3.62 (d, *J* = 18.4 Hz, 1H), 3.24 (d, *J* = 6.5 Hz, 1H), 3.03 (s, 6H), 2.33-2.14 (m, 2H), 1.64 – 1.42 (m, 4H), 1.31 (d, *J* = 9.0 Hz, 2H). LCMS m/z: $[M-H]^+$ calculated for $C_{37}H_{35}N_8O_7S_2$ 767.21; found 767.4.



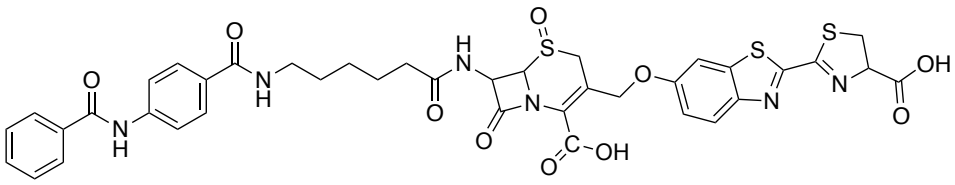
D-Bluco

Compound D-Bluco: 3-(((2-((S)-4-carboxy-4,5-dihydrothiazol-2-yl)benzo[d]thiazol-6-yl)oxy) methyl)-7-(6-(4-((E)-(4-(dimethylamino)phenyl)diazenyl)benzamido)hexanamido)-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid 5-oxide. To a solution of compound **6** (10 mg, 0.013 mmol) in 1.5 mL of DMF was added a solution of D-cysteine hydrochloride (6.9 mg, 0.04 mmol) and $NaHCO_3$ solution (1 M) was added to adjust the pH >7. The reaction mixture was stirred for 20 min. The product was purified with semi-prep HPLC to afford D-Bluco (3.4 mg, 30%). ^{1}H NMR (500 MHz, DMSO) δ 8.55 (t, *J* = 5.6 Hz, 1H), 8.23 (d, *J* = 8.1 Hz, 1H), 8.05 (d, *J* = 9.0 Hz, 1H), 7.98 (d, *J* = 8.5 Hz, 2H), 7.84 – 7.74 (m, 5H), 7.19 (dd, *J* = 9.0, 2.6 Hz, 1H), 6.81 (d, *J* = 9.1 Hz, 2H), 5.80 (dd, *J* = 8.0, 4.7 Hz, 1H), 5.42 (dd, *J* = 9.7, 8.3 Hz, 1H), 5.12 (d, *J* = 11.9 Hz, 1H), 4.93 (d, *J* = 4.4 Hz, 1H), 4.84 (d, *J* = 11.9 Hz, 1H), 3.95 (d, *J* = 18.5 Hz, 1H), 3.81 – 3.73 (m, 1H), 3.71 – 3.61 (m, 2H), 3.27 (d, *J* = 6.4 Hz, 2H), 3.06 (s, 6H), 2.38 – 2.16 (m, 2H), 1.62 – 1.47 (m, 4H), 1.34 (d, *J* = 8.9 Hz, 2H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 173.08, 164.49, 160.01, 158.21, 156.00, 147.40, 138.85, 137.34, 134.29, 128.73, 128.44, 127.56, 126.99, 126.18, 124.73, 122.25, 118.47, 113.02, 103.97, 80.59, 79.14, 67.87, 66.95, 59.04, 45.49, 40.39, 36.20, 29.83, 28.46, 26.35, 24.94. HRMS m/z: M^+ calculated for $C_{40}H_{40}N_8O_9S_3$ 872.2080; found 872.5570.



9

Compound 9: 4-benzamidobenzoic acid. The synthesis procedure was adopted from reported literature.[2] Briefly, benzoic acid (1g, 8.18 mmol) was added to thionyl chloride (1 mL) and DMF. The reaction mixture was refluxed at 100 °C for 1 h. The excess thionyl chloride and solvent was removed *in vacuo*. The acyl chloride obtained above was slowly added to the solution of 4-amino benzoic acid (1.34 g,1.2 eq) and NEt_3 (1.43 mL,1.25 eq) in DCM (1 mL) at 0 °C. The reaction mixture was stirred for 16 h. The mixture was diluted with CH_2Cl_2 and washed with 3 M HCl aq. (2 × 30 mL), water (1 × 30 mL), and brine (1 × 30 mL). The organic layer was dried over $MgSO_4$, filtered, and concentrated under reduced pressure. Purification of the crude residue by flash column chromatography on silica gel afforded the corresponding benzamide substrates (505 mg, 45%). LCMS m/z: $[M+H]^+$ calculated for $C_{14}H_{12}NO_3$ 242.25; found 242.08.



Am-Bluco

Compound Am-Bluco: 7-(6-(4-benzamidobenzamido)hexanamido)-3-(((2-(4-carboxy-4,5-dihydrothiazol-2-yl)benzo[d]thiazol-6-yl)oxy)methyl)-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid 5-oxide. The synthesis is similar to that of compound **6** and D-Bluco (30%).[1]H NMR (600 MHz, DMSO) δ 10.41 (s, 1H), 8.34 (t, *J* = 5.7 Hz, 1H), 8.18 (d, *J* = 8.2 Hz, 1H), 8.05 (d, *J* = 9.0 Hz, 1H), 7.96 – 7.94 (m, 3H), 7.87 – 7.82 (m, 3H), 7.78 (d, *J* = 2.6 Hz, 1H), 7.61 – 7.57 (m, 1H), 7.52 (d, *J* = 7.5 Hz, 2H), 7.19 (dd, *J* = 9.0, 2.6 Hz, 1H), 5.81 (dd, *J* = 8.1, 4.7 Hz, 1H), 5.42 (dd, *J* = 9.8, 8.3 Hz, 1H), 5.14 (d, *J* = 12.1 Hz, 1H), 4.94 (d, *J* = 4.6 Hz, 1H), 4.86 (d, *J* = 12.1 Hz, 1H), 3.99 (d, *J* = 18.5 Hz, 1H), 3.77 (dd, *J* = 11.2, 9.8 Hz, 1H), 3.71 – 3.65 (m, 2H), 3.24 (q, *J* = 6.6 Hz, 2H), 2.27 (ddd, *J* = 39.8, 14.4, 7.2 Hz, 2H), 1.54 (dd, *J* = 14.5, 7.2 Hz, 5H), 1.33 (q, *J* = 7.9 Hz, 3H). LSMS m/z: $[M-H]^-$ calculated for $C_{39}H_{35}N_6O_{10}S_3$ 843.93; found 843.235.

2. Supporting figures and tables.

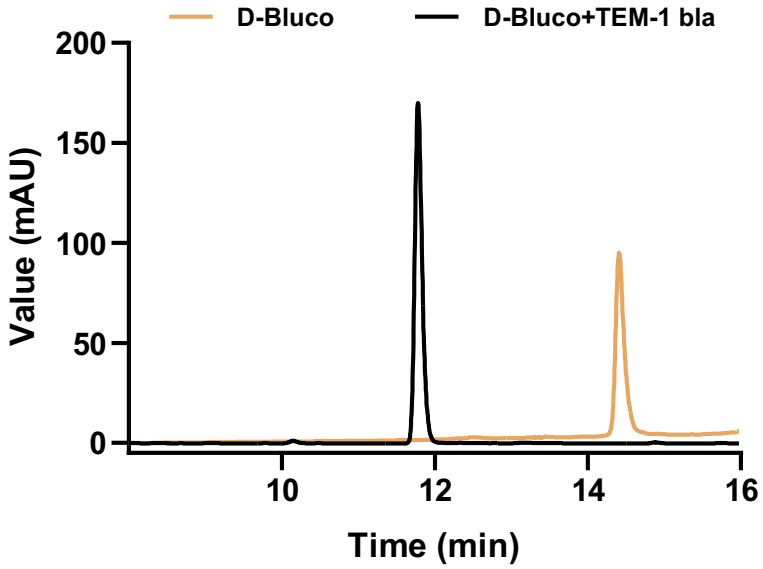


Figure S1 HPLC analysis of D-Bluco with or without TEM-1 β-lactamase. 1 μM of D-Bluco incubated with PBS or with TEM-1 (100 nM) for 60 min, then analyzed by HPLC at 320 nm.

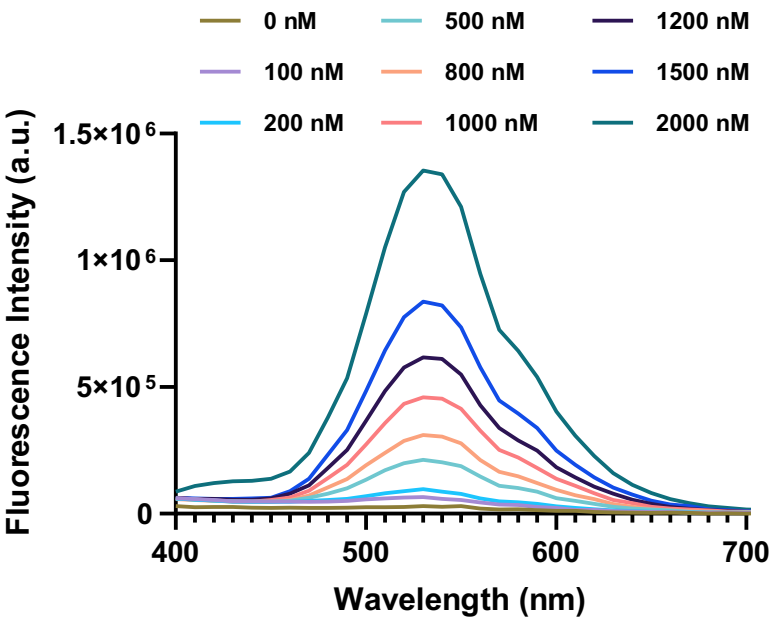


Figure S2 Fluorescence emission spectrum of D-Bluco at various concentrations incubated with TEM-1 (100 nM) at 25 ºC in PBS (pH=7.4). Data were collected on a SpectraMax iD3 multimode microplate reader with excitation at 330 nm. a.u. indicates arbitrary unit.

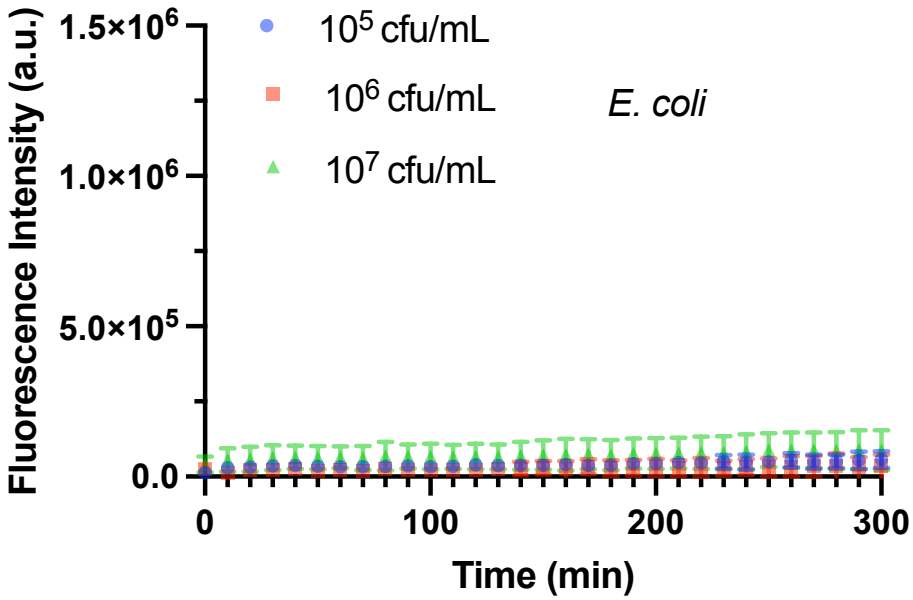


Figure S3. Longitudinal monitoring of the stability of D-Bluco (10 μM) incubated with different concentrations of *E. coli* in PBS (pH=7.4). The signal of D-Bluco in PBS was subtracted for the plot. Data were collected on a SpectraMax iD3 multimode microplate reader with excitation at 330 nm and emission at 530 nm. a.u.: arbitrary unit. Error bars indicate standard deviations (n=3).

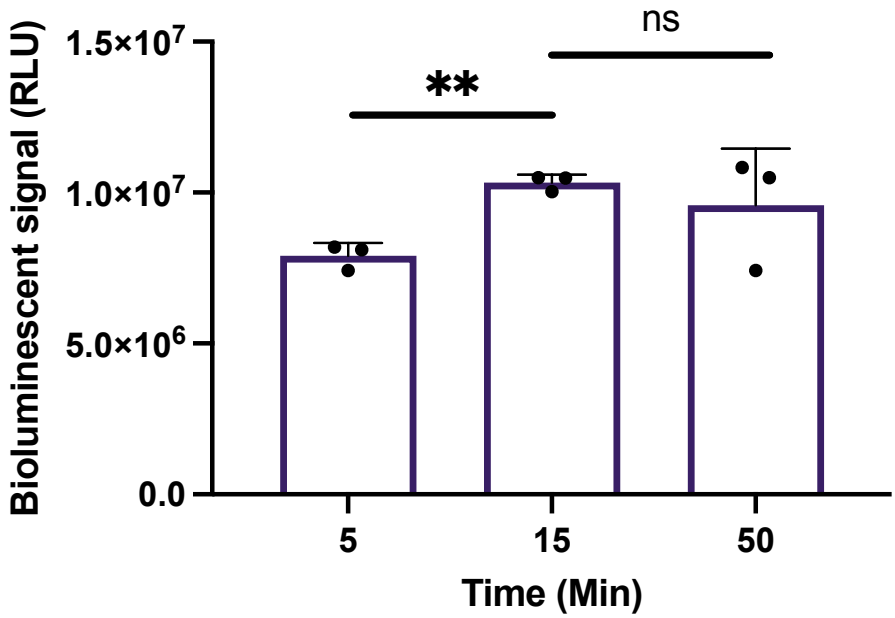


Figure S4. D-Bluco (10 μM) was incubated with TEM-1 (20 nM) at 25 ºC for 5, 15, and 45 min before adding bioluminescent assay reagents for BLI detection. Statistical significance was calculated using the unpaired two-tailed Student's t test ns: not significant). Data were collected on a SpectraMax iD3 multimode microplate reader. Error bars indicate standard deviations (n=3).

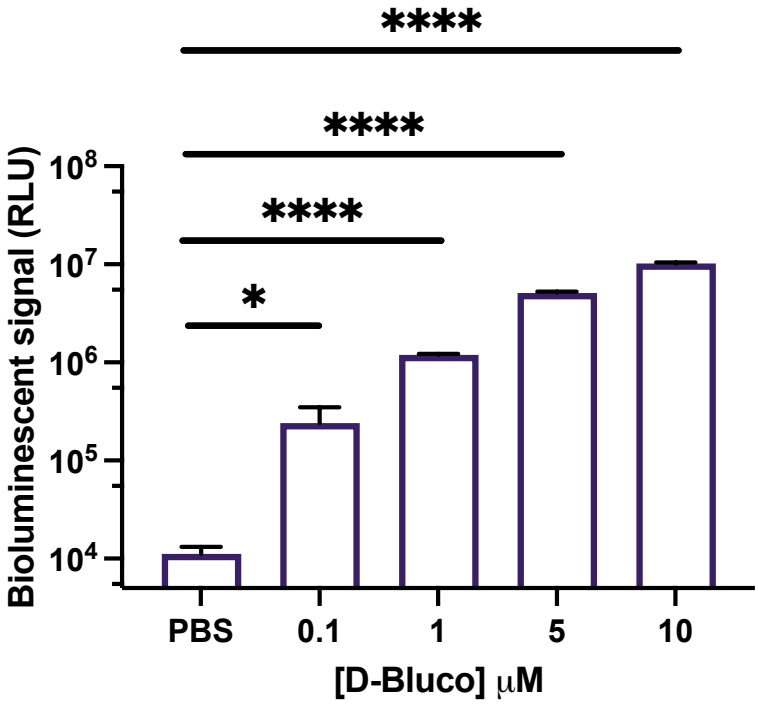


Figure S5. D-Bluco (0.1, 1, 5, 10 μM) was incubated with TEM-1 (20 nM) at 25 ºC for 15 min before the addition of bioluminescent assay reagents for BLI detection. Statistical significance was calculated using the unpaired two-tailed Student's t test (** $p < 0.0021$, *** $p < 0.0002$, ns: not significant). Data were collected on a SpectraMax iD3 multimode microplate reader. RLU: relative light units. Error bars indicate standard deviations (n=3).

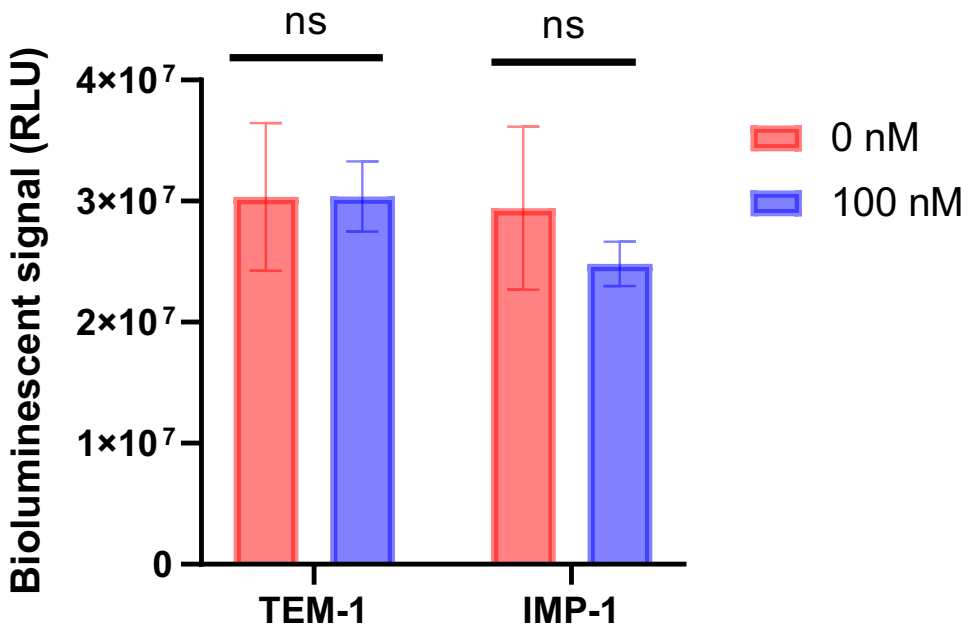


Figure S6. Effect of TEM-1 β-lactamase or IMP-1 carbapenemase on the bioluminescent signal of D-luciferin. D-luciferin (10 µM) was incubated without or with TEM-1 or IMP-1 (100 nM) in PBS (pH=7.4) for 15 min. Statistical significance was calculated using the unpaired two-tailed Student's t test (ns: not significant). Data were collected on a luminometer. Error bars indicate standard deviations (n=3).

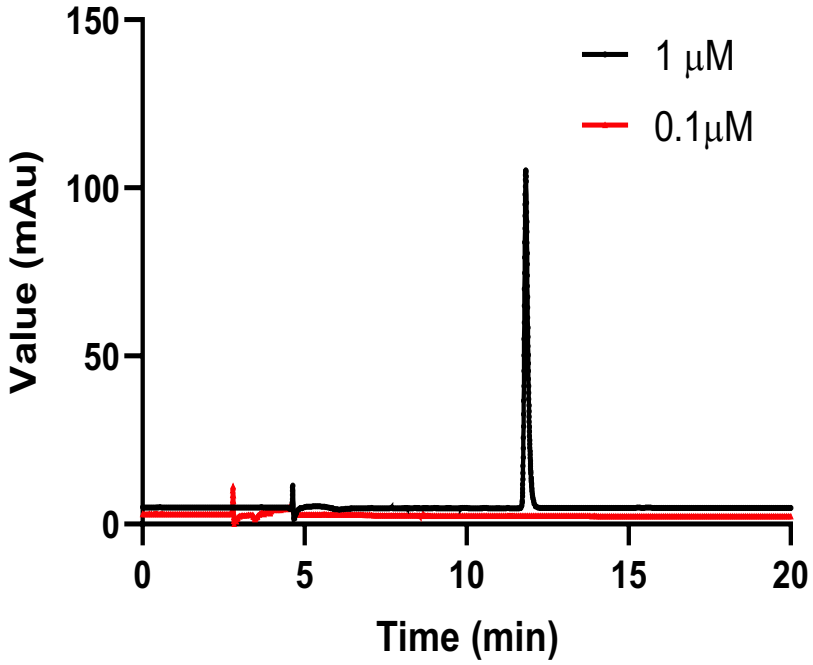


Figure S7. HPLC analysis of 0.1 and 1 μM of D-luciferin monitored at λ= 320 nm.

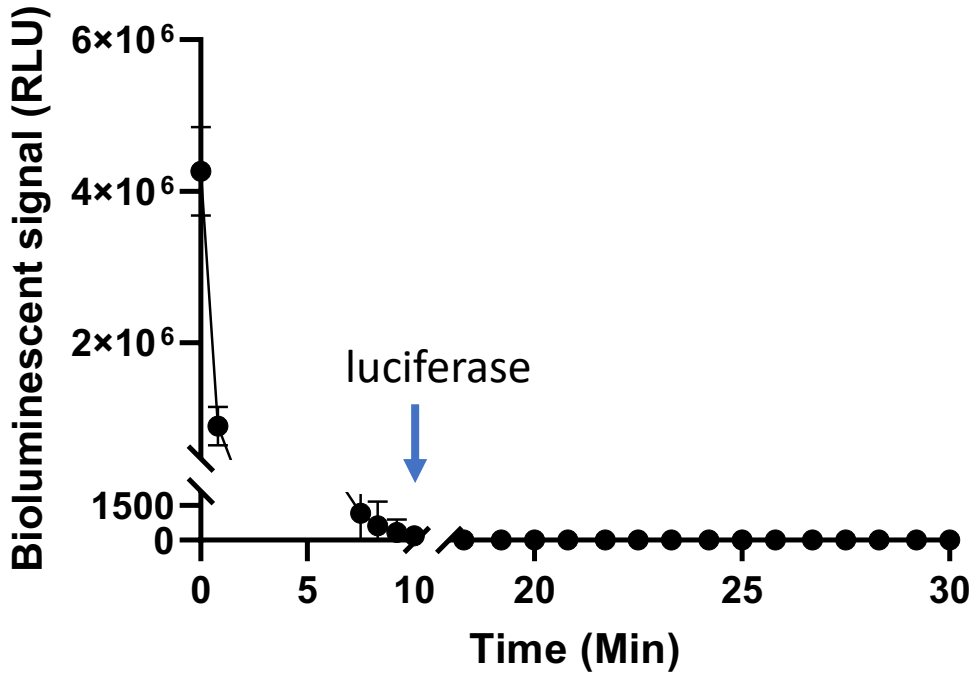
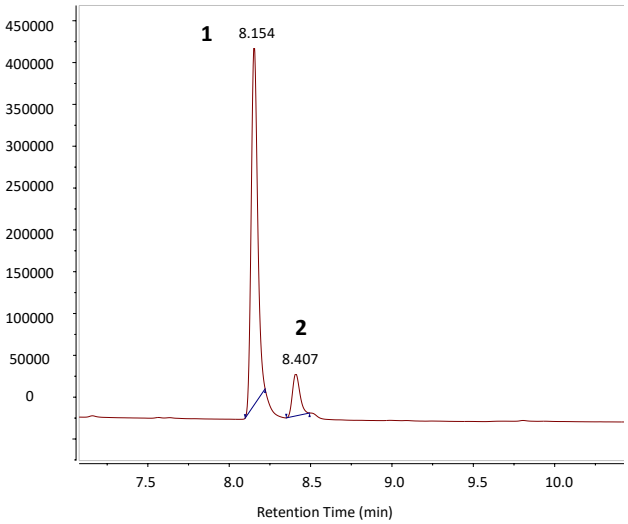


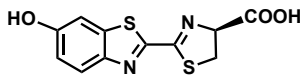
Figure S8. Bioluminescent intensity was monitored over 30 min. D-luciferin (1 μM) was incubated with 4 μM of luciferase for 10 min. A fresh batch of luciferase was added at 10 min. Data were collected on a SpectraMax iD3 multimode microplate reader. RLU indicates relative light units. Error bars indicate standard deviations (n=3).

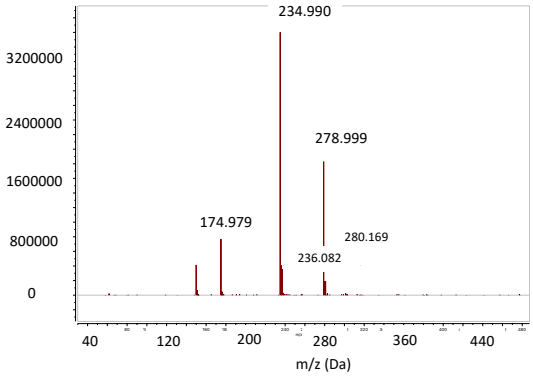
d-luciferin



Molecular Weight: 280.32

1





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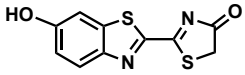
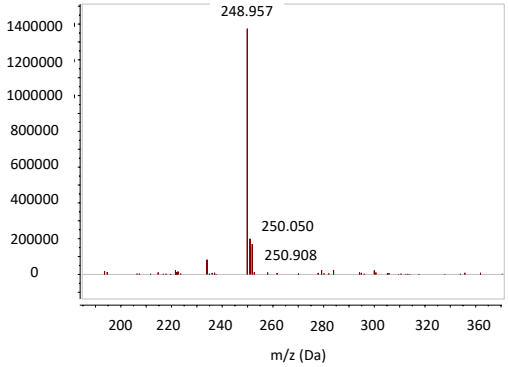
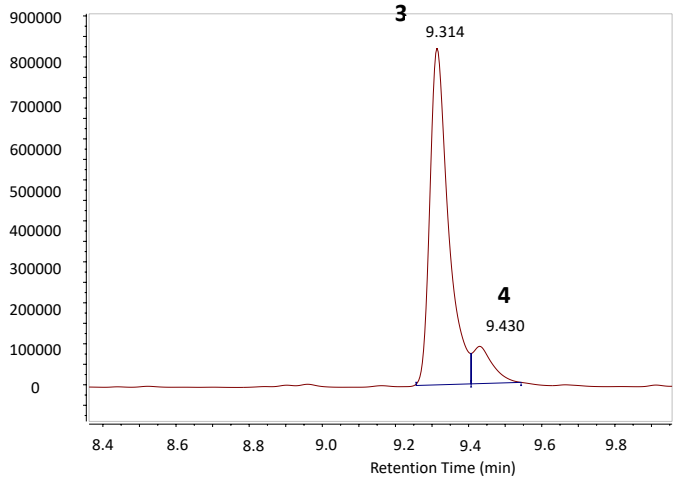




Bluco



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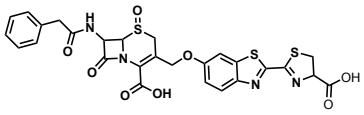

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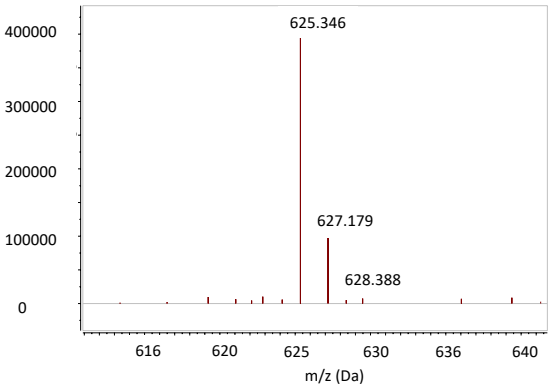

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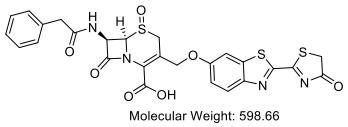

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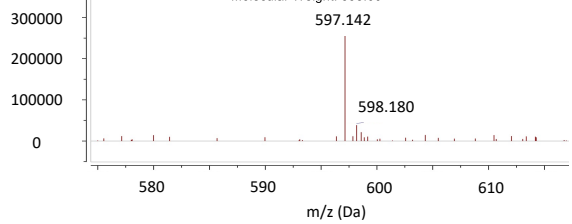

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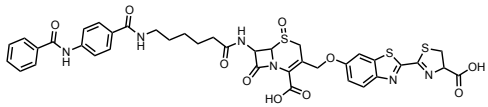
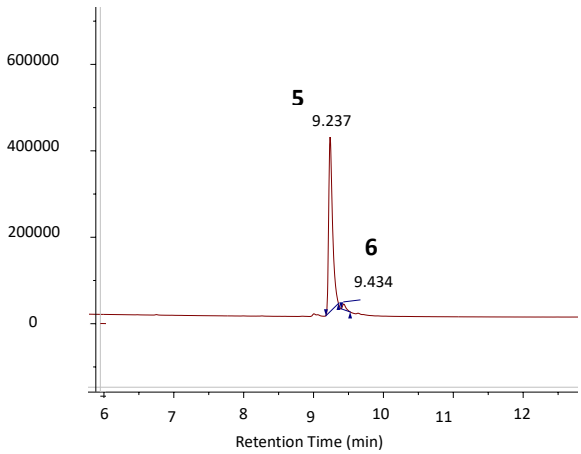
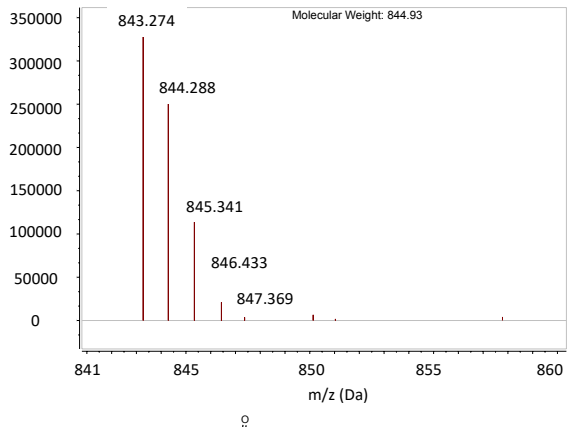


Am-Bluco





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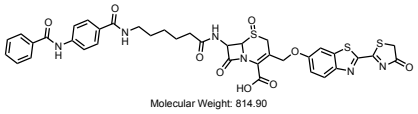

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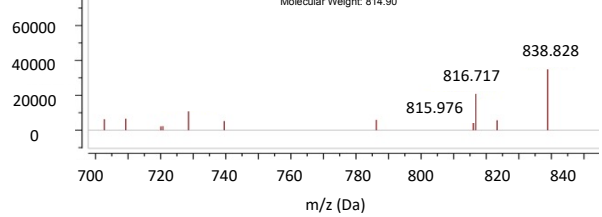


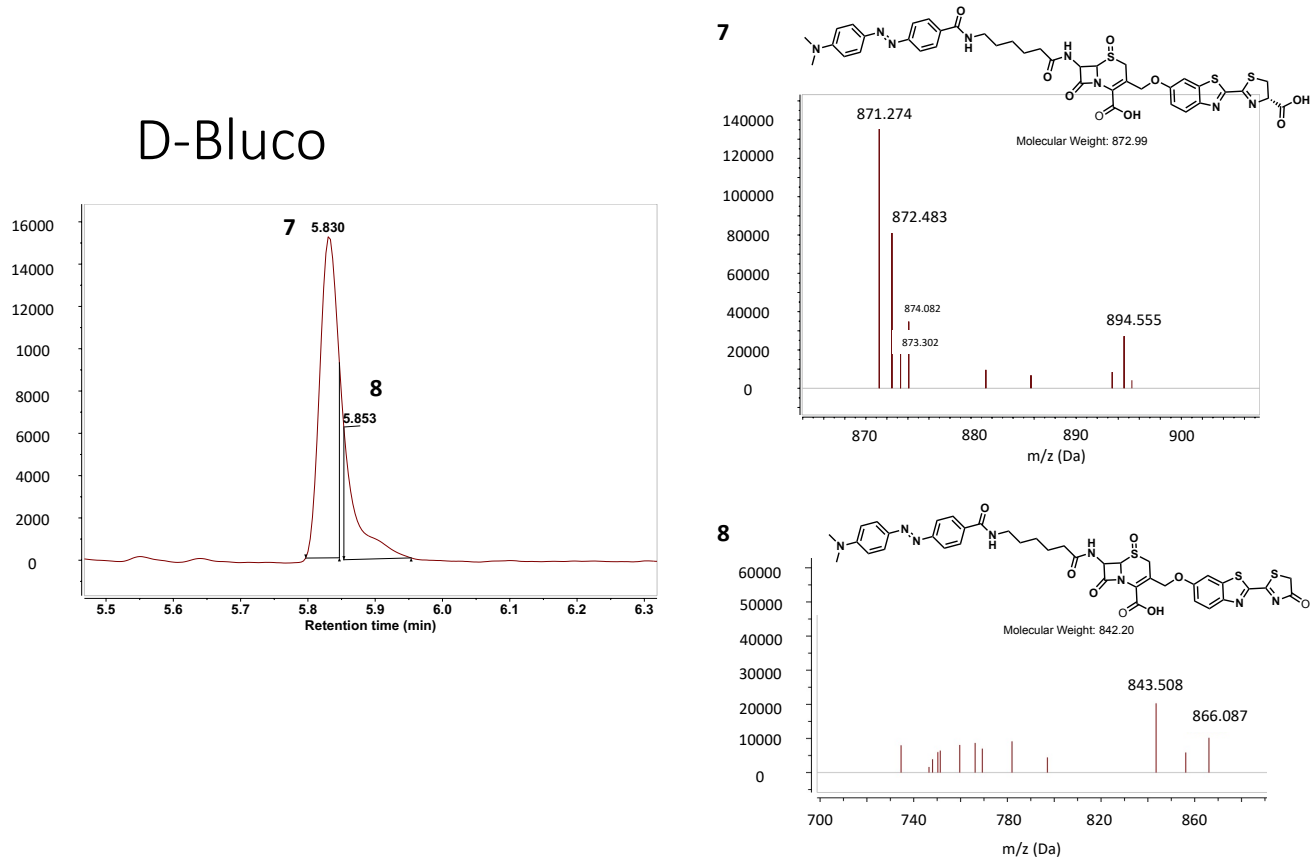


Figure S9. Mass spectrometric spectra of D-luciferin and Bluco analogues after reacting with luciferase. D-luciferin and Bluco analogues (100 μM) was incubated with luciferase (4 μM) for 4 h at 25 °C. Peak 1-5 and 7 were collected with negative mode (MS/ESI-) while 6 and 8 were collected with positive mode (MS/ESI+). D-Bluco was collected with 15 min method while the other three analogues were collected with 30 min method.

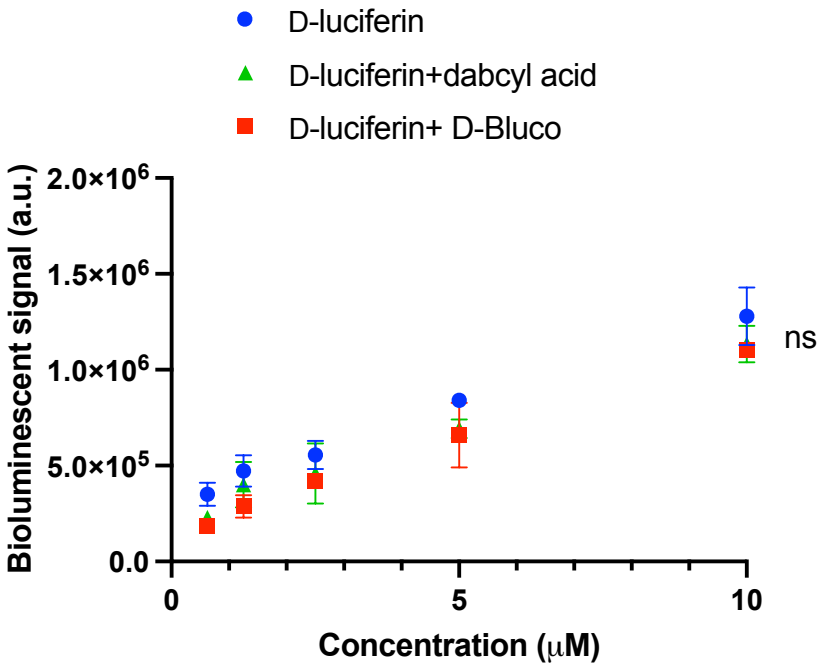


Figure S10. Investigation of intermolecular quenching by D-Bluco. Bioluminescent signal of D-luciferin, D-luciferin mixed with dabcyl acid or D-Bluco (10 μM) in PBS (pH=7.4). Experiments were conducted at 25 °C. Statistical significance was calculated using the unpaired two-tailed Student's t test (ns: not significant). Error bars indicate standard deviations (n=3).

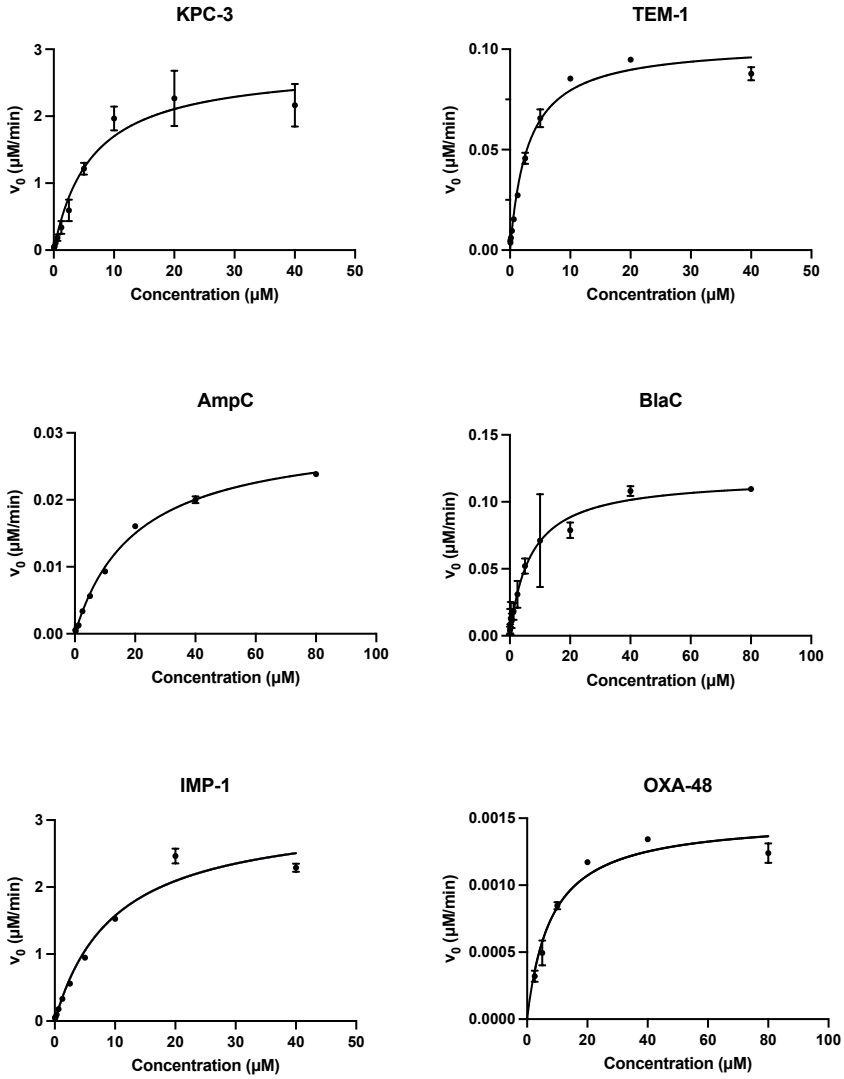


Figure S11. Michaelis–Menten kinetic plots of KPC-3, TEM-1, AmpC, IMP-1, BlaC and OXA-48 towards D-Bluco. Error bars indicate standard deviations (n=3).

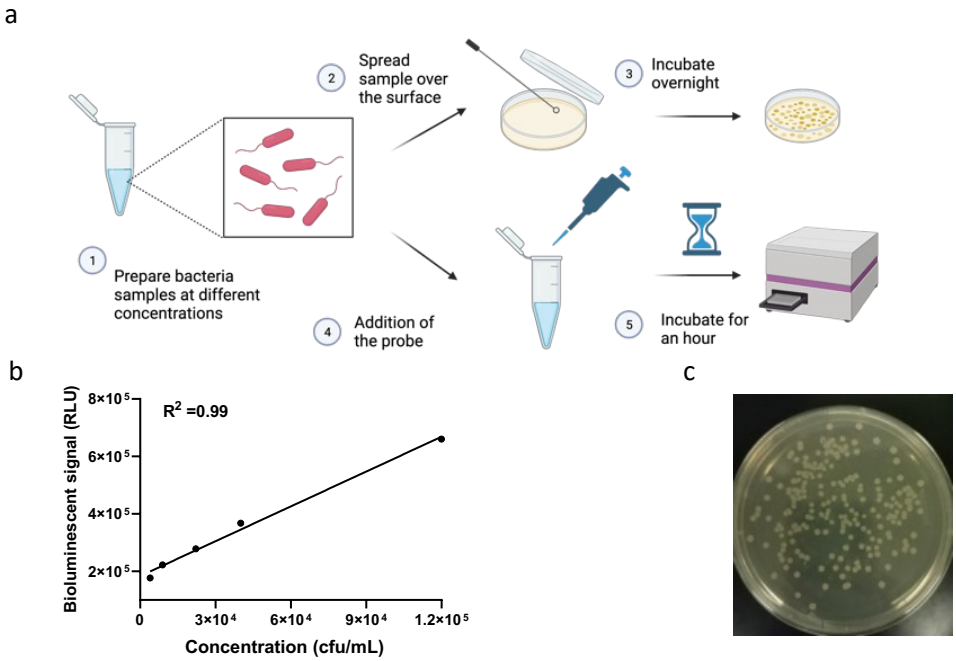


Figure S12. Calibration of bacteria concentrations with the plate assay. a) The workflow for the validation process. b) Bioluminescence intensity of D-Bluco incubated with *E. coli*/TEM-1 at different concentrations. The signal of D-Bluco incubated with 10^5 cfu/mL *E. coli* was subtracted to obtain the absolute values for plotting. Data were collected on a SpectraMax iD3 multimode microplate reader. c) Representative image of the plate to validate the cfu count in the assay. The working concentration of D-Bluco was 10 μM. Part of the figure was created with http://BioRender.com

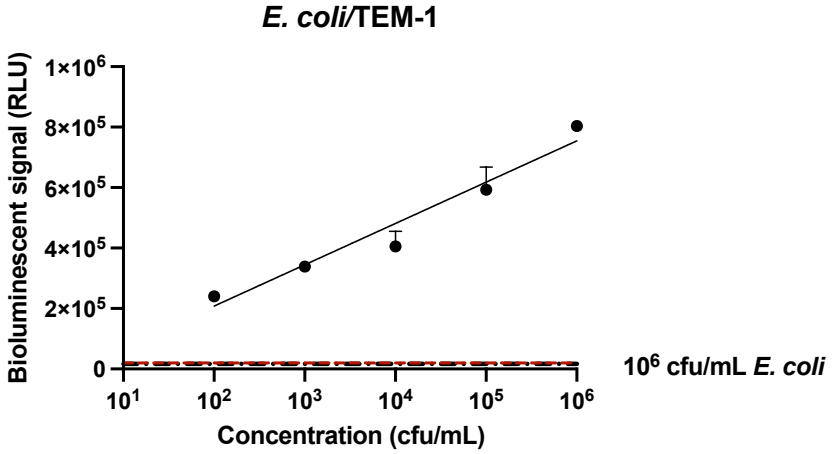


Figure S13. D-Bluco was incubated with different concentrations of *E. coli* expressing TEM-1 (*E. coli*/TEM-1 in PBS (pH=7.4) at 25 °C. Then bioluminescent reagent was added and BLI signal was recorded. The black dashed line is the 10^6 cfu/mL of *E. coli* negative control while the maroon dashed line is the 3 times the standard deviation above the *E. coli* negative control. The working concentration of D-Bluco was 10 μM. The signal of D-Bluco in PBS was subtracted for the plot. Error bars indicate standard deviations (n=3).

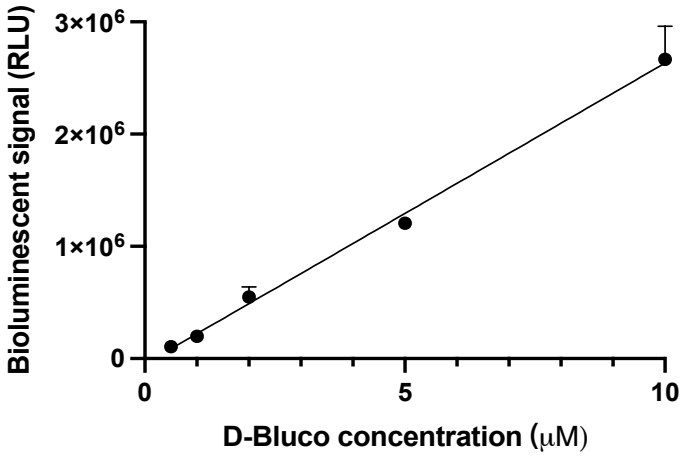


Figure S14. 10^8 cfu/mL of *E. coli*/TEM was incubated with different concentrations of D-Bluco for 1 h in PBS (pH=7.4). The signal of D-Bluco in PBS (pH=7.4) was subtracted before plotting. The working concentration of D-Bluco was 10 μM. Data was collected on a TurnerBiosystem luminometer. Error bars indicate standard deviations (n=3).

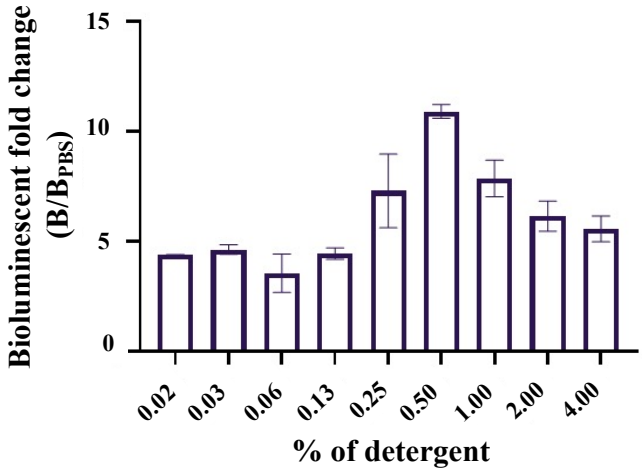


Figure S15. Bioluminescent enhancement of 10^6 cfu/mL of clinic isolates *E. coli*/TEM by incubation with different concentrations of CHAPS for 15 min. D-Bluco was subsequently added for another 15 min incubation before bioluminescent measurement. The working concentration of D-Bluco was 10 μM. The Data was collected on a TurnerBiosystem luminometer. Error bars indicate standard deviations (n=3).

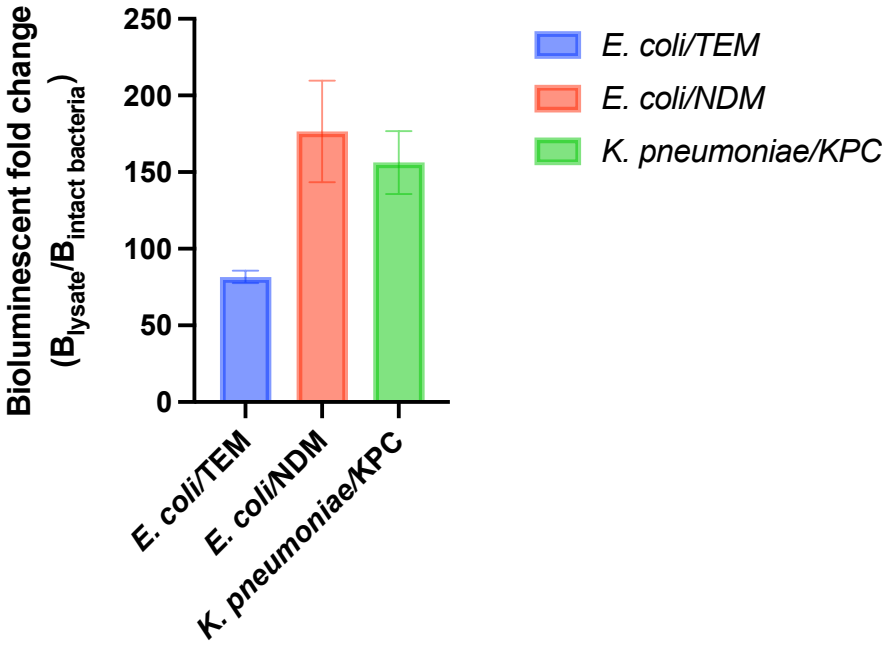


Figure S16. Bioluminescent intensity of 10^8 cfu/mL *E. coli*/TEM, *E. coli*/NDM, or *K. pneumoniae*/KPC. These isolates were incubated with CHAPS (lysate bacteria) or without CHAPS (intact bacteria) for 15 min before the addition of D-Bluco for an hour. The working concentration of D-Bluco was 10 μM. Data was collected on a TurnerBiosystem luminometer. Error bars indicate standard deviations (n=3).

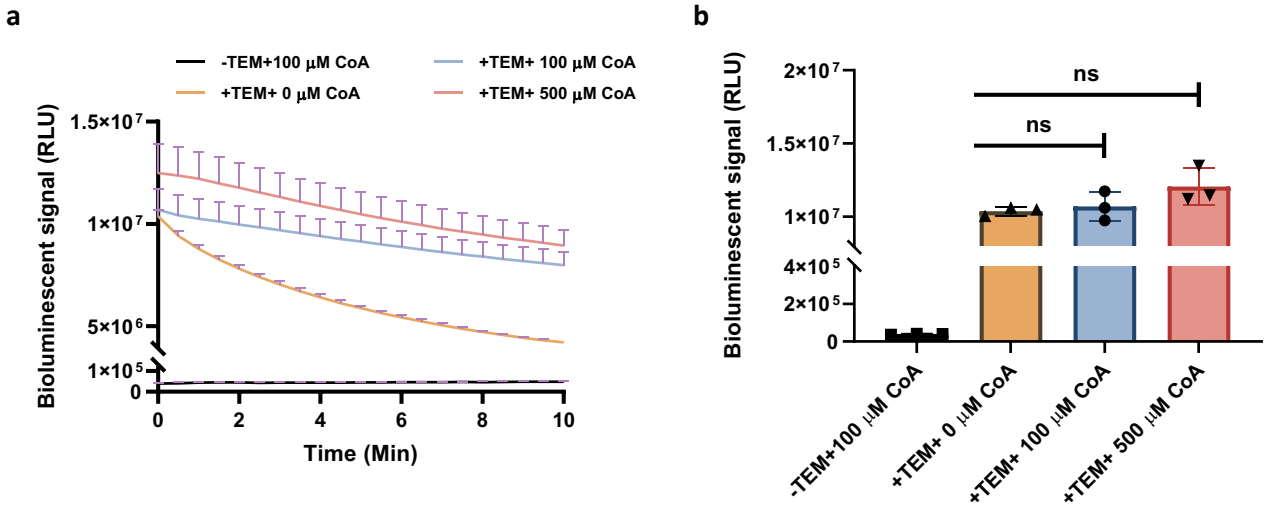


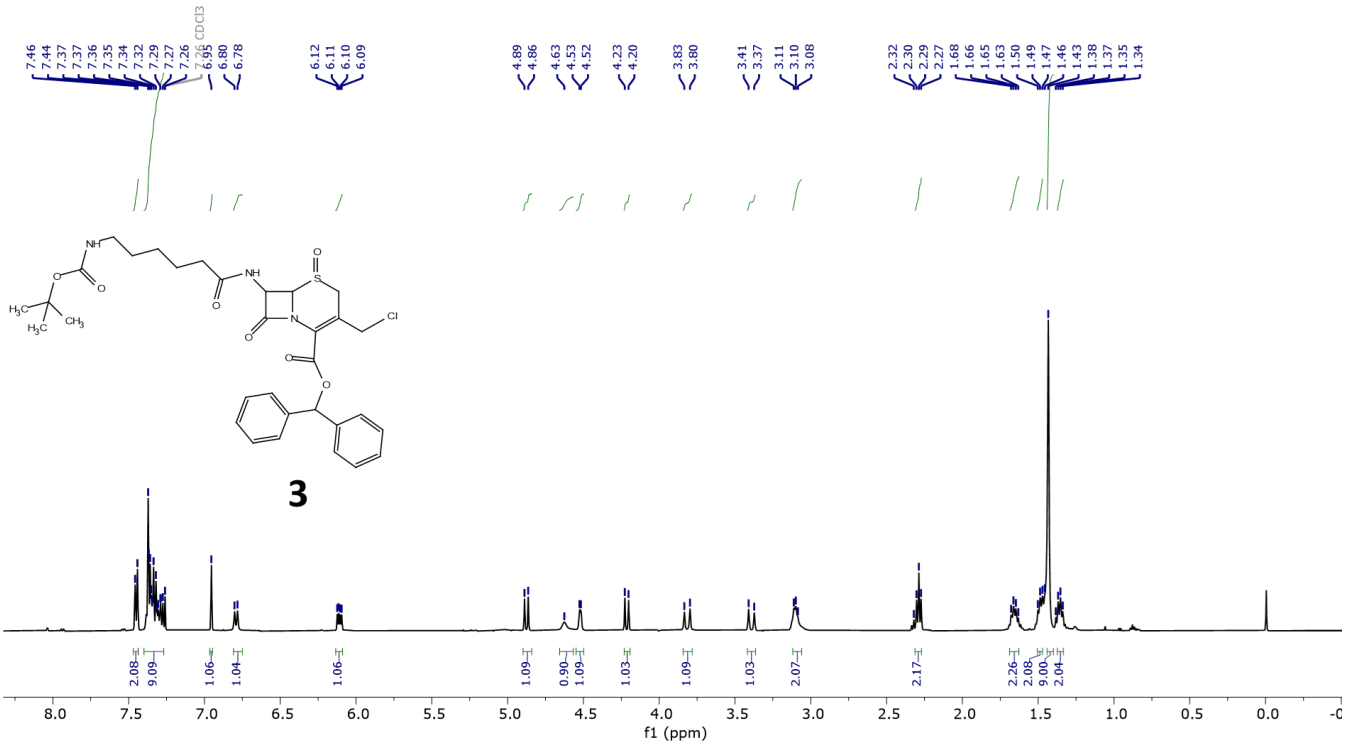
Figure S17. D-Bluco was incubated with or without 20 nM TEM-1 for 15 min before adding different concentrations of Enzyme CoA (0, 100, 500 μM) and BLI assay reagents for bioluminescent detection. a) Bioluminescent intensity was monitored over 10 min after the addition of bioluminescent assay reagents. b) The bioluminescent intensity at 0 min (immediate BLI detection after the addition of bioluminescent reagent). Statistical significance was calculated using the unpaired two-tailed Student's t test (ns: not significant). The working concentration of D-Bluco was 10 μM. Data were collected on a SpectraMax iD3 multimode microplate reader. RLU indicates relative light units. Error bars indicate standard deviation (n=3).

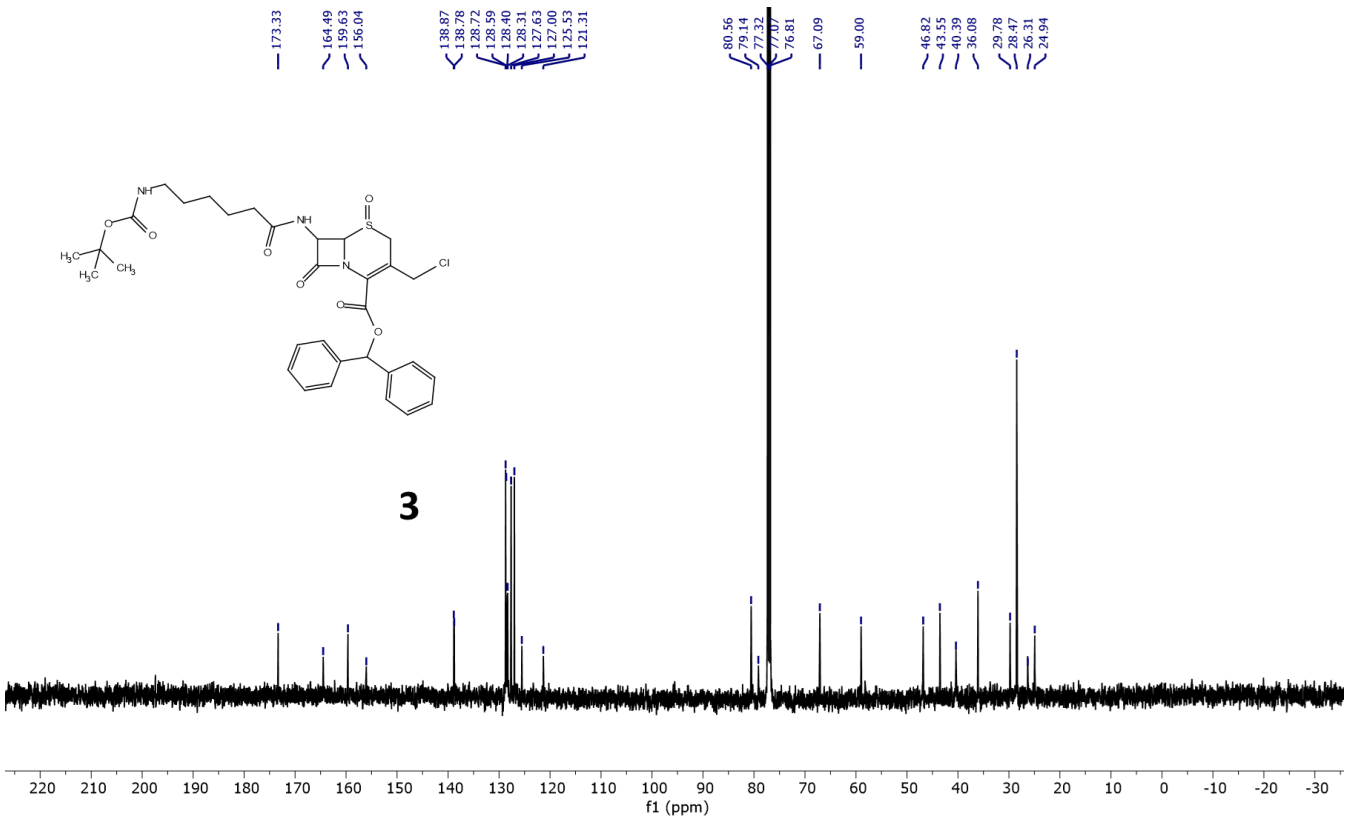
Table S1 Characterization of Bluco analogues

	Bluco	Am-Bluco	D-Bluco
k_{uncat} (s^{-1})	$3.35\pm0.17\times10^{-5}$	$4.21\pm0.17\times10^{-6}$	$1.79\pm0.1\times10^{-6}$
D-luciferin formed rate for 1 μM Bluco analog (μM/s)	2.13×10^{-5}	4.10×10^{-6}	1.51×10^{-6}
Bioluminescence signal for 1 μM Bluco analog (AU/s) *	67	13	5

*The amount of bioluminescent BLI produced by hydrolyzed luciferin was estimated via a standard calibration curve.

3. NMR and HRMS spectra.

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127.63
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125.53
121.31
80.56
79.14
77.32
77.07
76.81
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59.00
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29.78
28.47
26.31
24.94
3
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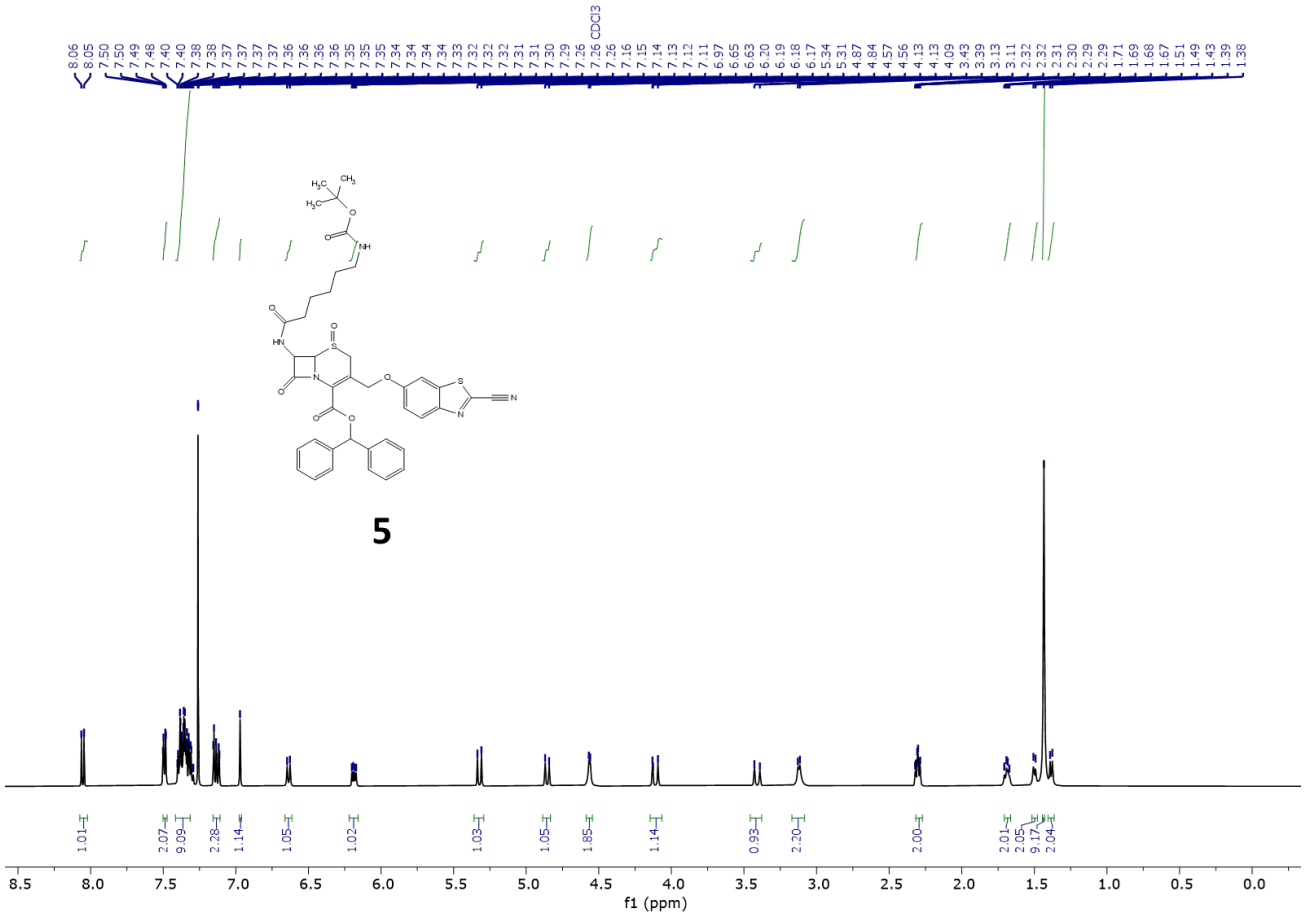
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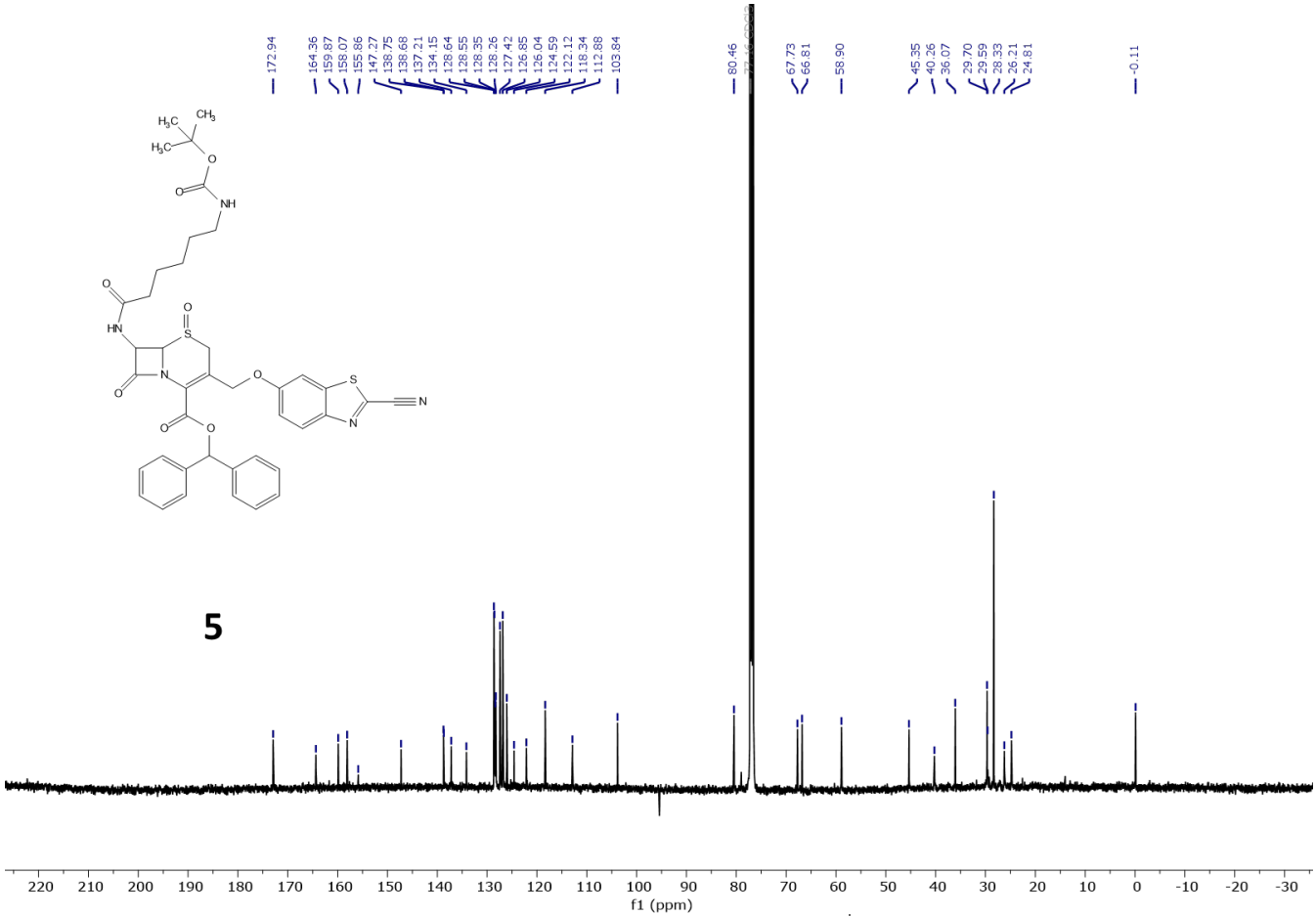
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66.81
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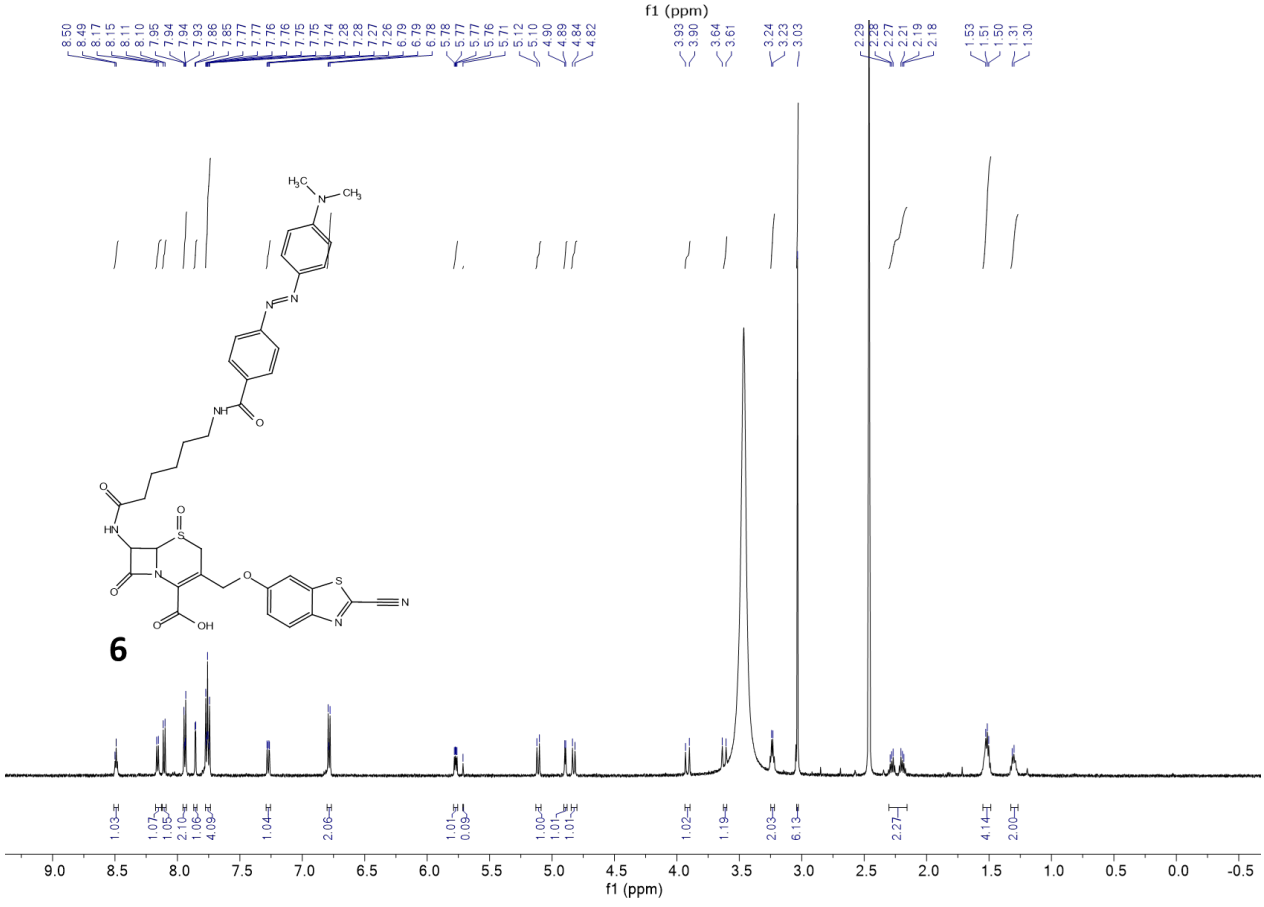
f1 (ppm)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7.76
7.76
7.75
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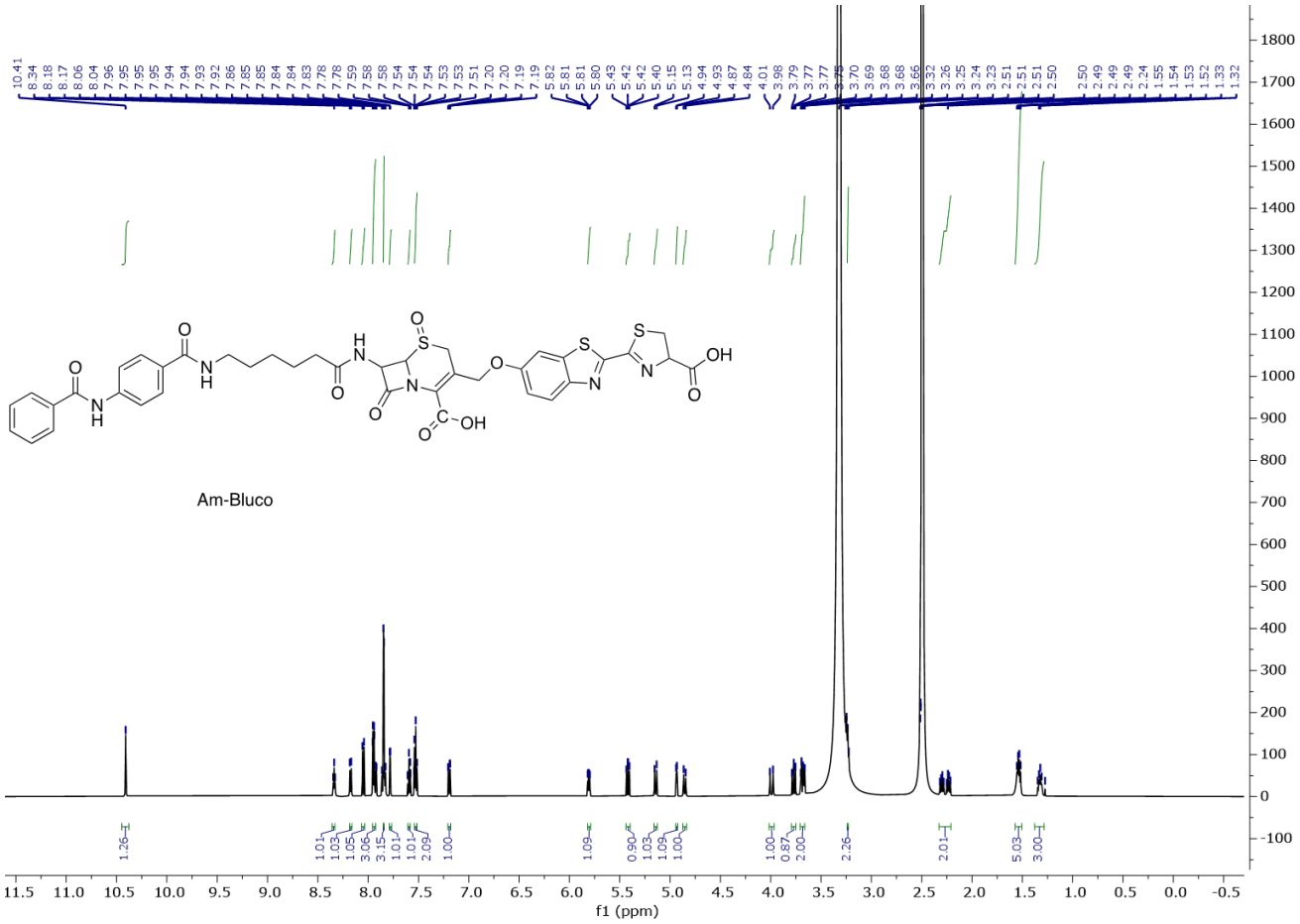
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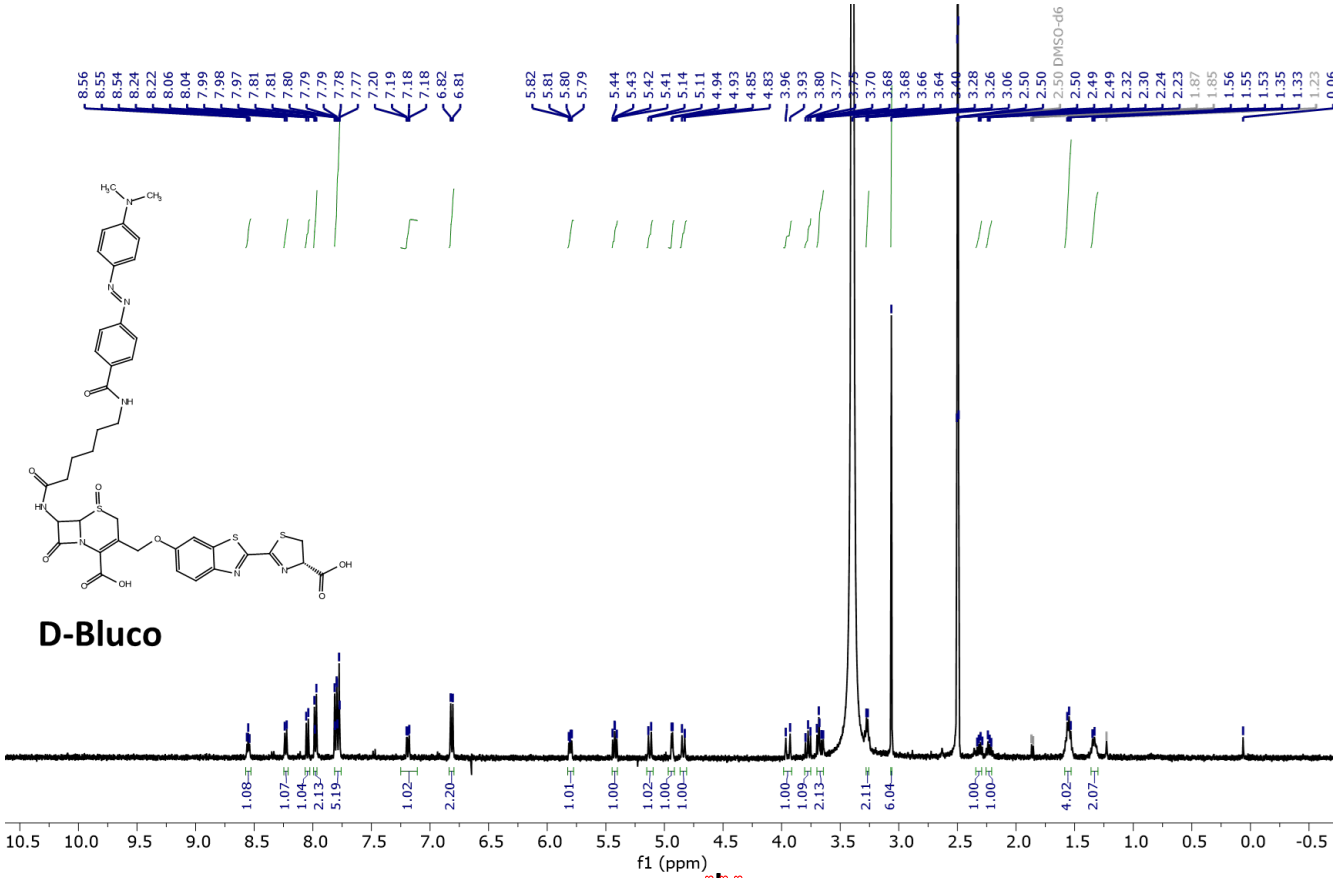
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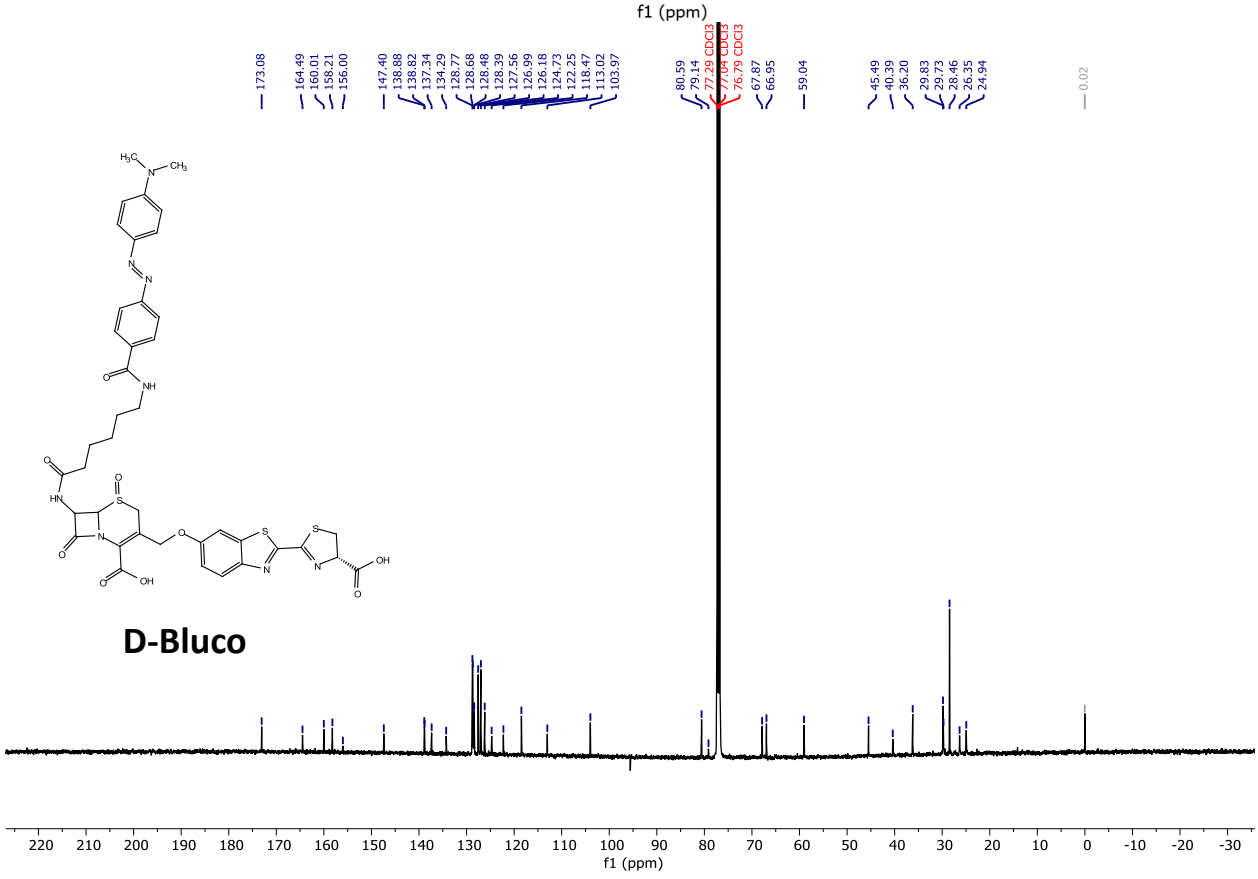
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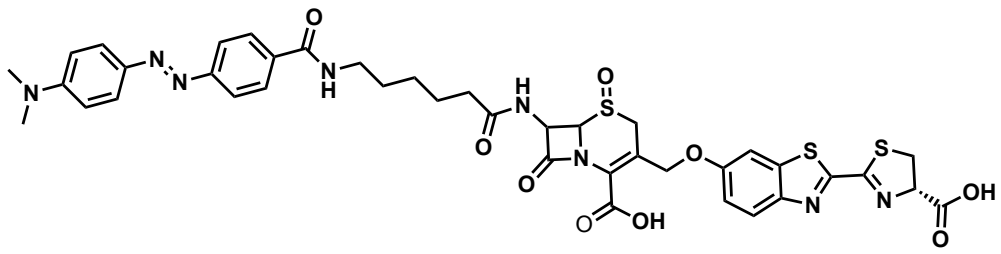
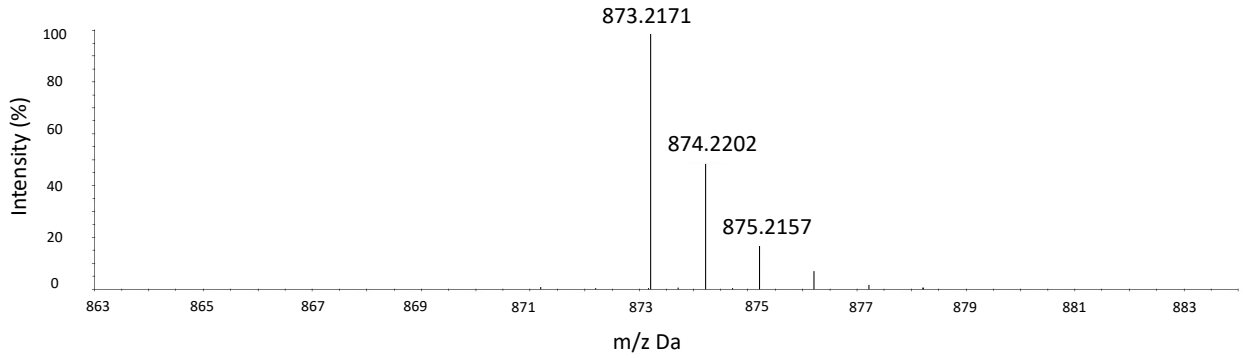


D-Bluco

Chemical Formula: $C_{40}H_{40}N_8O_9S_3$
Exact Mass: 872.21
Molecular Weight: 872.99



HRMS of D-Bluco

4. References

1. Xie, H. *et al.* Rapid point-of-care detection of the tuberculosis pathogen using a BlaC-specific fluorogenic probe. *Nat. Chem.* **4**, 802–809 (2012).
2. Tezuka, N. *et al.* Lipshutz-type bis(amido)argentates for directed ortho argentation. *Chem. Sci.* **11**, 1855–1861 (2020).