Electronic Supplementary Material (ESI) for ChemComm.

A cephalosporin-chemiluminescent conjugate increases beta-lactamase detection sensitivity by four orders of magnitude

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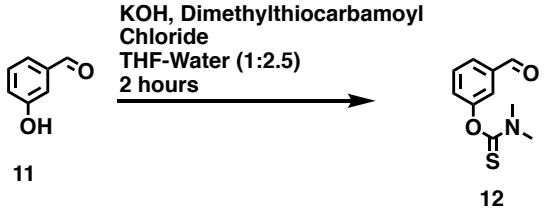
Materials: All the chemicals used in the synthesis of CCP were purchased from Sigma-Aldrich. 0.1 M phosphate buffered saline (PBS), syringes, needles, pipette tips, eppendorf tubes, and nuclear magnetic resonance (NMR) tubes were purchased from VWR. ^{1}H-NMR spectra were recorded in $CDCl_3$, Acetone-d_6 and DMSO-d_6 in a Bruker 300 MHz and 400 MHz spectrometer at 300K. TMS (δ (ppm) H = 0.00) was used as the internal reference. ^{13}C-NMR spectra were recorded in $CDCl_3$, Acetone-d_6 and DMSO-d_6 at a 100MHz on a bruker 900 MHz spectrometer, using the central resonances of $CDCl_3$ (δ (ppm) C = 77.23). Chemical shifts are reported in ppm and multiplicities are indicated by s (singlet), d (doublet), t (triplet), q (quartet), dd (doublet of doublets), and m (multiplet). Coupling constants, J, are reported in hertz (Hz). High-resolution mass spectra (HRMS) were obtained on an AB SCIEX TOF/TOF 5800 system and are reported as m/z (relative intensity). Accurate masses are reported for the molecular ion (M^+) or a suitable fragment ion. Chemicals were purchased from Aldrich or VWR and used without further purification. All solvents were purified using standard methods. Flash chromatography was carried out using silica gel (230-400 mesh). All reactions were performed under anhydrous conditions under N_2 or Argon and monitored by TLC on Kieselgel 60 F254 plates (Merck). Detection was accomplished by examination under UV light (254 nm). Luminescence was measured with Turner Designs TD 2020 TD-20/20 Luminometer.

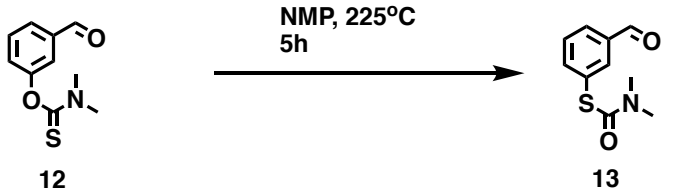
Synthesis of CPP:

Synthesis of compound 12: Compound 12 was synthesized via a published protocol with certain changes.[1]



In a flame dried round bottom flask **compound 11** (10.75 g, 0.088 mol) was suspended into water (63 mL). The suspension was cooled down to 0 °C. Potassium hydroxide (5.42 g, 0.096 mol) was added into the reaction mixture. The reaction mixture turns yellow and colorless. Dimethylthiocarbamoyl chloride (10.9 g, 0.088 mol) was dissolved into 25 mL of THF was dropwise added into the reaction mixture while maintaining the temperature between 0 °C to 8 °C. The reaction was stirred for 2 hours. The reaction mixture was quenched with 180 mL of water and the **compound 12** (15.7 g, 85%) was obtained via filtration as a white solid.

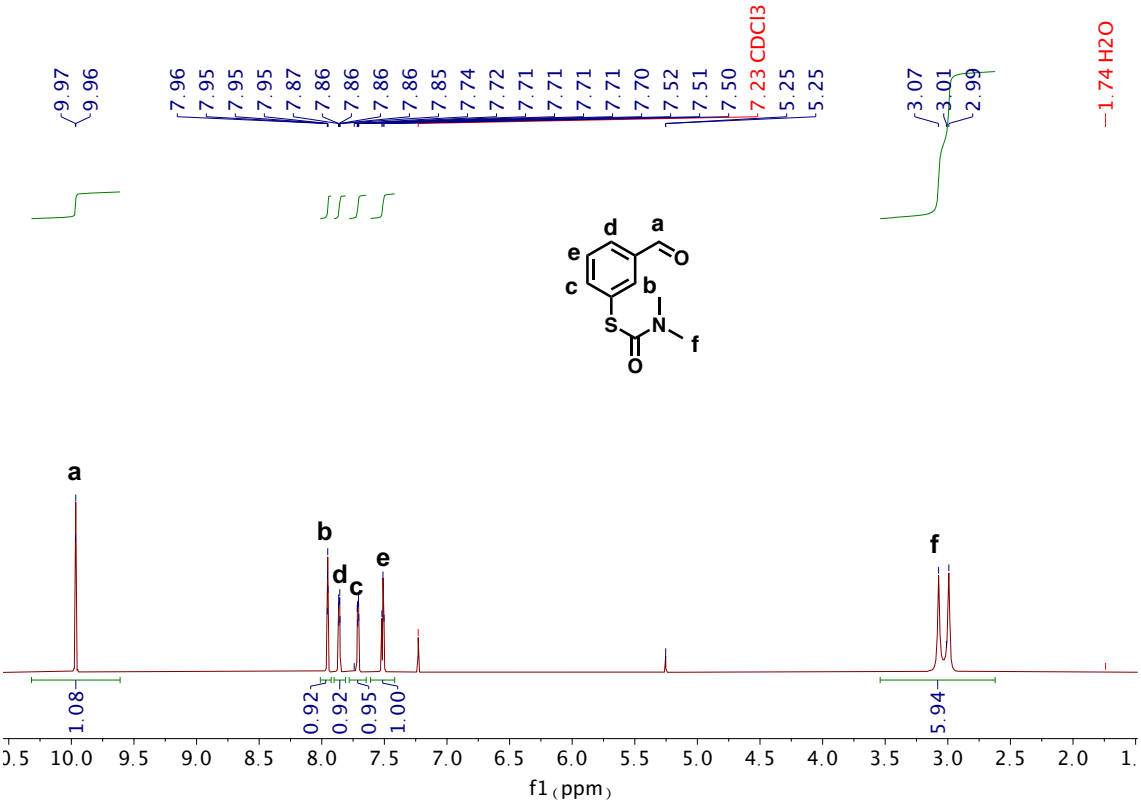
Synthesis of compound 13:

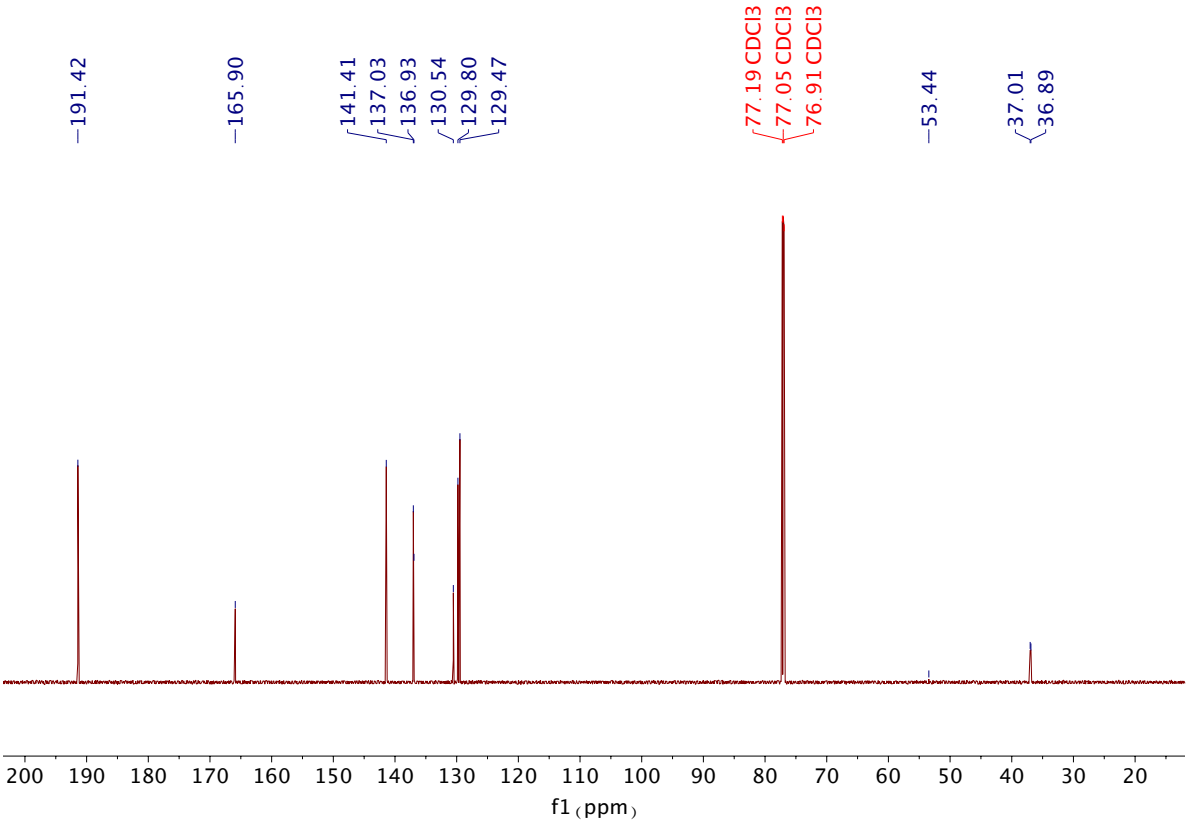


In a flame dried round bottom flask **compound 12** (6.5 g, 0.031 mol) was dissolved into 13 mL NMP. The resulting solution was heated at 225 °C for 5 hours. The reaction mixture was cooled down to RT. The crude product was purified by silica gel using 1:1 (hexane/diethyl ether) to yield **compound 13** (2.53 g, 39%).

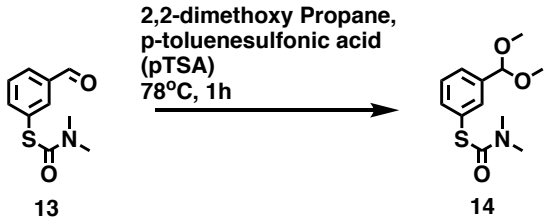
^{1}H NMR (400 MHz, $CDCl_3$): 9.96 (s, 1H), 7.95 (s, 1H), 7.86 (d, 1H, 4 Hz), 7.71 (d, 1H, 4 Hz), 7.52 (t, 1H, 4 Hz), 3.07 (s, 3H), 2.99 (s, 3H).

^{13}C NMR (125 MHz, $CDCl_3$): 191.4, 165.9, 141.4, 137.0, 130.5, 129.8, 129.4, 37.0, 36.8.


9.97
9.96
7.96
7.95
7.95
7.95
7.87
7.86
7.86
7.86
7.86
7.85
7.74
7.72
7.71
7.71
7.71
7.71
7.70
7.52
7.51
7.50
7.23 CDCl3
5.25
5.25
3.07
3.01
2.99
1.74 H2O
d
a
e
O
c
b
S
N
f
O
a
b
d
c
e
f
1.08
0.92
0.92
0.95
1.00
5.94
f1 (ppm)


191.42
165.90
141.41
137.03
136.93
130.54
129.80
129.47
77.19 CDCl3
77.05 CDCl3
76.91 CDCl3
53.44
37.01
36.89
f1 (ppm)

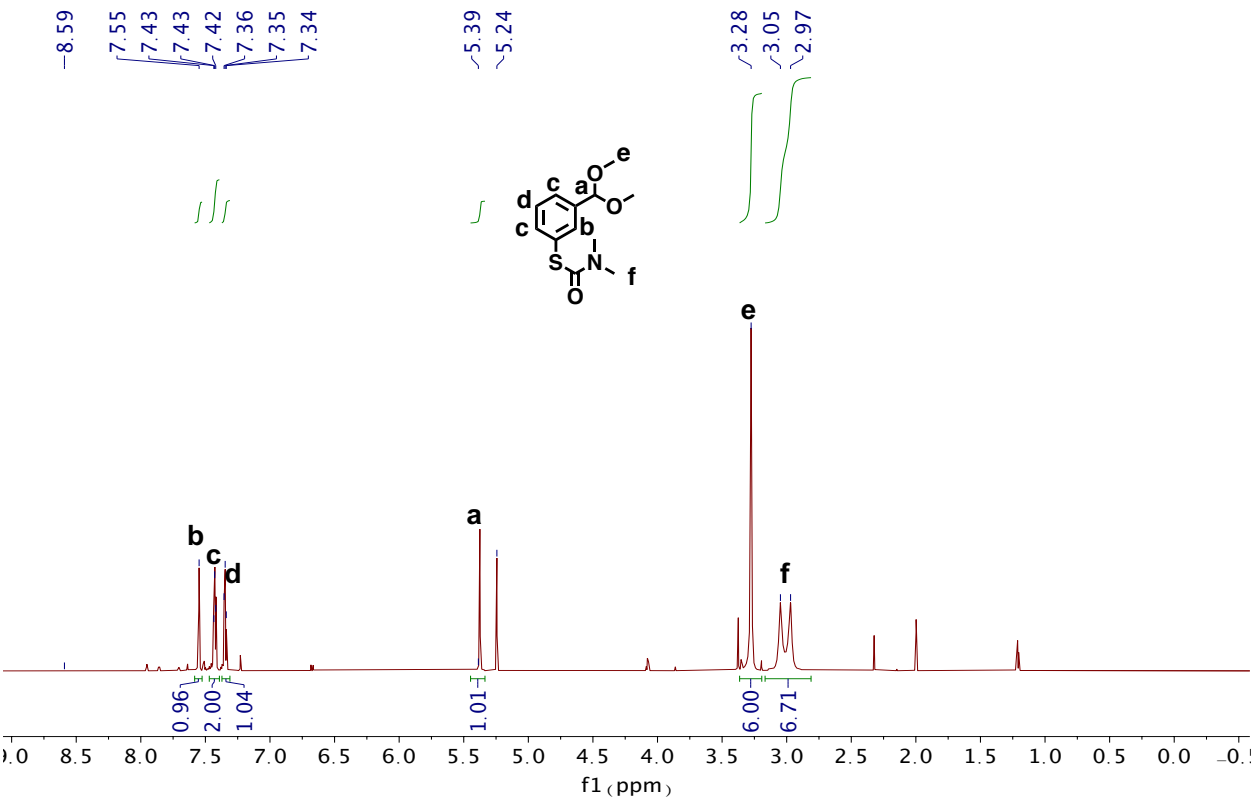
Synthesis of compound 14:

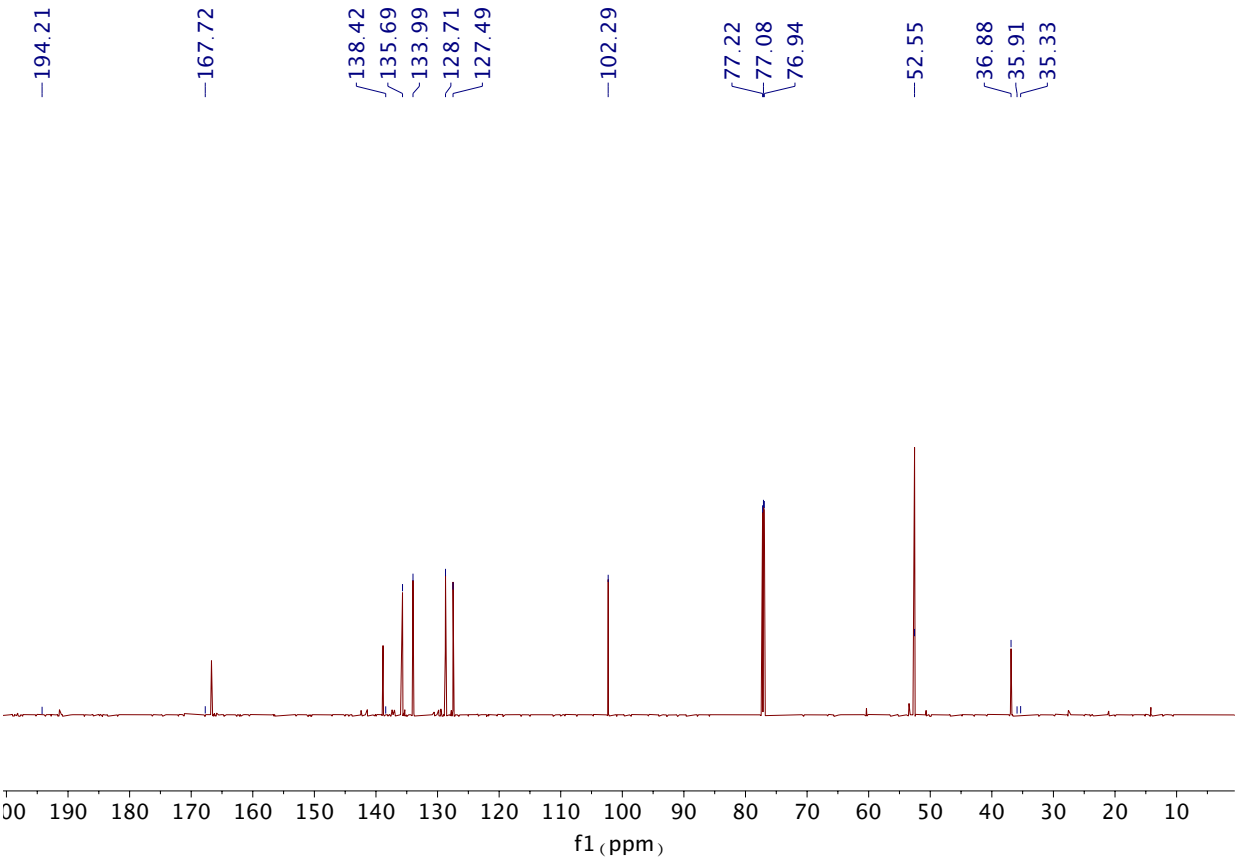


In a flame dried round bottom flask was added a suspension of **compound 13** (1.5 g, 7.16 mmol) in 2,2-dimethoxy propane (5 mL). To this resulting suspension was added p-toluenesulfonic acid (27.2 mg, 0.143 mmol). The reaction mixture was refluxed at 78 °C for 1h. A colorless suspension turns into a blackish clear viscous solution. The reaction was quenched with the addition of 20 mL triethylamine. A 200 mL of ethyl acetate was added into the reaction mixture and was extracted with 100 mL of DI water. The organic phase that contains the crude **compound 14** was dried over Na_2SO_4 and solvent was removed under high vacuum to yield a crude **compound 14**. Further, **compound 14** was purified with silica gel using 1:1 (hexane/diethyl ether) as an eluting solvent to yield as white solid (1.15 g, 63%).

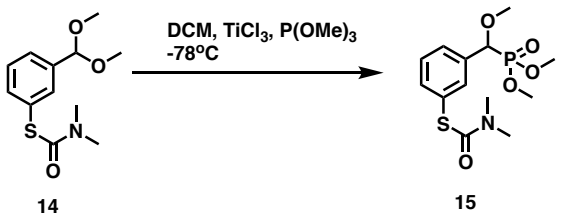
^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.55 (s, 1H), 7.42 (t, 4 Hz, 2H), 7.34 (t, 4 Hz, 1H), 5.39 (s, 1H), 3.28 (s, 6H), 3.05 (s, 3H), 2.97 (s, 3H).

^{13}C NMR (125 MHz, $CDCl_3$) δ: 167.7, 138.4, 135.6, 133.9, 128.7, 127.4, 102.2, 52.5, 35.3.


8.59
7.55
7.43
7.43
7.42
7.36
7.35
7.34
5.39
5.24
3.28
3.05
2.97
a
b
c
d
e
f
0.96
2.00
1.04
1.01
6.00
6.71
f1 (ppm)


194.21
167.72
138.42
135.69
133.99
128.71
127.49
102.29
77.22
77.08
76.94
52.55
36.88
35.91
35.33
f1 (ppm)

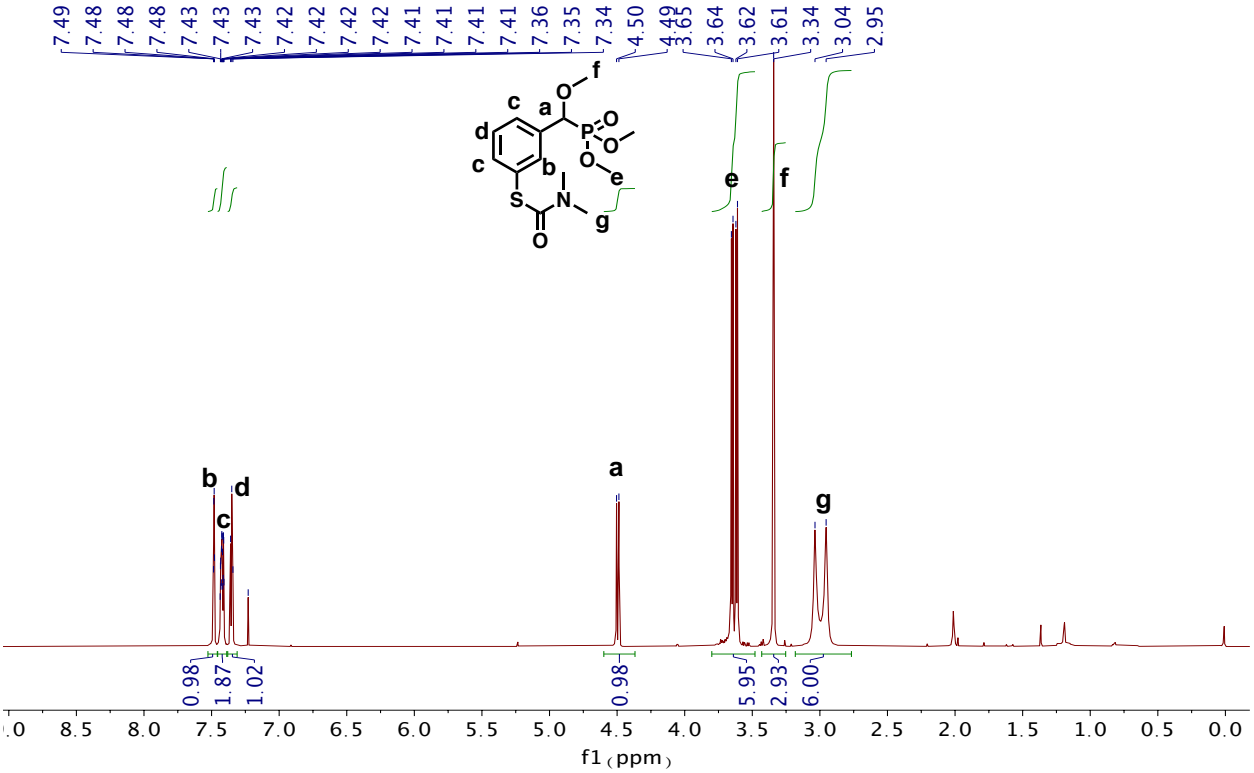
Synthesis of compound 15:

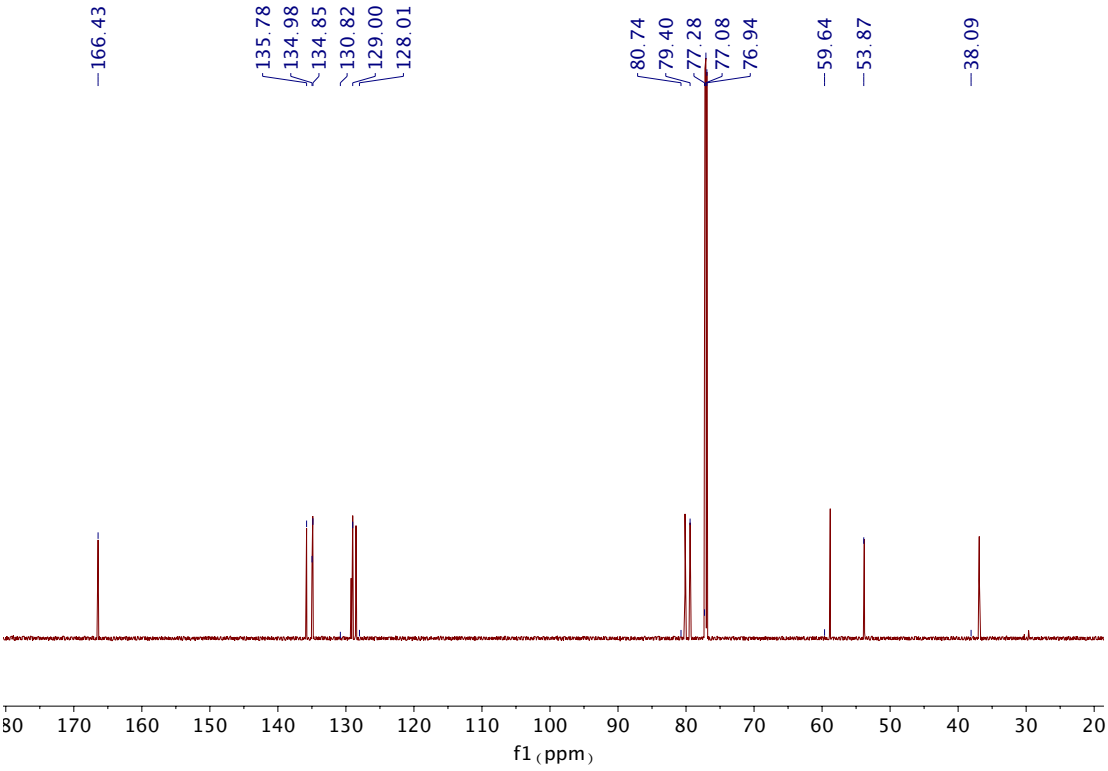


In a flame dried flask was added **compound 14** (1.14 g, 4.46 mmol) and DCM (50 mL). The resulting solution was cooled down to -78 °C. To this solution was added trimethyl phosphite (0.737 mL, 6.25 mmol). A 1M solution of Titanium tetrachloride (0.665 mL, 6.25 mmol) in DCM was added dropwise. The reaction mixture was stirred at -78 °C for 1h and the reaction mixture was quenched with 5 mL methanol. Solvent was removed under reduced pressure and diluted with ethyl acetate (50 mL) and was extracted with water (50 mL) twice. The organic layer was dried over anhydrous Na_2SO_4. The crude product was purified by silica gel 1: 9 (Hexane/ethyl acetate) to yield **compound 15** as a colorless oil (1.3 g, 87%).

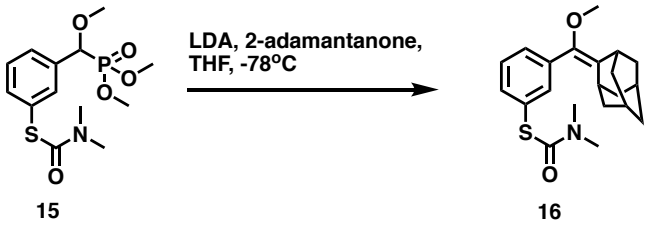
1H NMR (400 MHz, $CDCl_3$) δ: 7.48 (s, 1H), 7.44-7.41 (m, 2H), 7.35 (t, 1H, 4 Hz), 4.50 (d, 1H, 4 Hz), 3.65-3.61 (dd, 6H, 4 Hz, 12 Hz), 3.34 (s, 1H), 3.04 (s, 3H), 2.95 (s, 3H).

13C NMR (125 MHz, $CDCl_3$) δ: 166.4, 135.7, 134.9, 134.8, 130.8, 129.0, 128.0, 80.7, 79.4, 59.6, 53.8, 38.0.


7.49 7.48 7.48 7.48 7.43 7.43 7.43 7.42 7.42 7.42 7.42 7.41 7.41 7.41 7.41 7.36 7.35 7.34 4.50 4.49 3.65 3.64 3.62 3.61 3.34 3.04 2.95
f
c
a
d
c
b
O
P
O
O
e
S
N
g
O
e
f
b
c
d
a
g
0.98
1.87
1.02
0.98
5.95
2.93
6.00
f1 (ppm)


166.43
135.78
134.98
134.85
130.82
129.00
128.01
80.74
79.40
77.28
77.08
76.94
59.64
53.87
38.09
f1 (ppm)

Synthesis of compound 16:

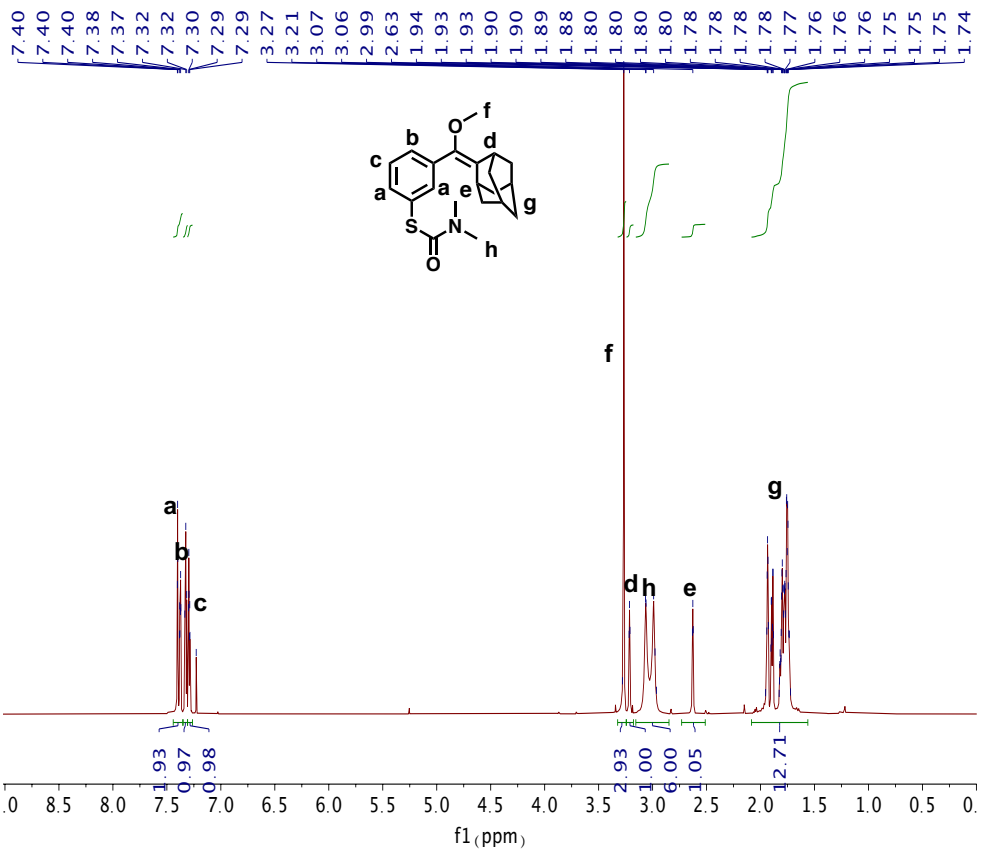


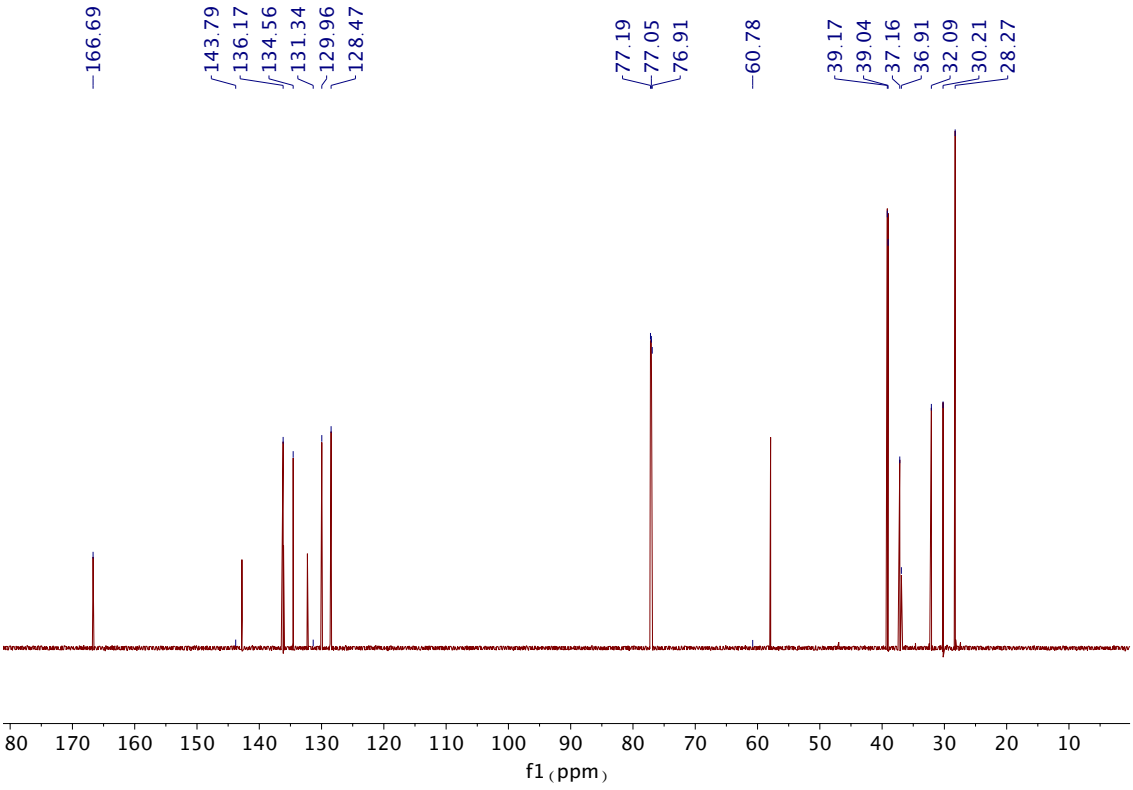
15 16

In a flame dried round bottom flask was added **compound 15** (500 mg, 1.49 mmol). The compound was dissolved into THF (5 mL). The solution was cooled down to -78 °C and was added 1M LDA solution (25 mL, 4.49 mmol). The resulting reaction mixture was stirred at -78 °C for 1 h. Subsequently, 2-adamantanone (201 mg, 1.34 mmol) was added to this reaction mixture and was stirred overnight -78 °C to rt. Reaction mixture was quenched with PBS (10 mL). The reaction mixture was diluted with 50 mL ethyl acetate and was extracted with water (50 mL) twice. The organic layer was dried over anhydrous Na_2SO_4. The crude product was purified by silica gel (49/1 to 9/1 hexane/ethyl acetate) to yield **compound 16** (280 mg, 58%) as a white solid.

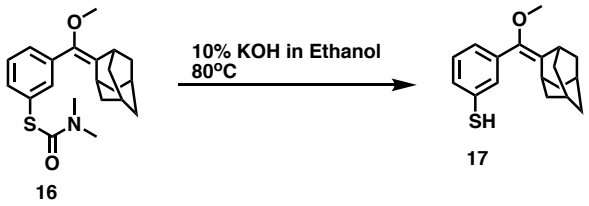
1H NMR (400 MHz, CDCl3): 7.40-7.37 (m, 2H), 7.32 (t, 1H, 4 Hz), 7.30-7.29 (m, 1H), 3.27 (s, 3H), 3.21 (t, 1H, 4 Hz), 3.07 (s, 3H), 2.98 (s, 3H), 2.62 (t, 1H, 4 Hz), 1.94-1.73 (m, 12H).

13C NMR (125 MHz, CDCl3): 166.6, 143.7, 136.1, 134.5, 131.3, 129.9, 128.4, 60.7, 39.1, 39.0, 37.1, 36.9, 32.0, 30.2, 28.2.


7.40 7.40 7.40 7.38 7.37 7.32 7.32 7.30 7.29 7.29 3.27 3.21 3.07 3.06 2.99 2.63 1.94 1.93 1.93 1.90 1.90 1.89 1.88 1.80 1.80 1.80 1.80 1.78 1.78 1.78 1.78 1.77 1.76 1.76 1.76 1.75 1.75 1.75 1.74
a
b
c
d
e
f
g
h
1.93
0.97
0.98
2.93
1.00
6.00
1.05
12.71
f1 (ppm)


166.69
143.79
136.17
134.56
131.34
129.96
128.47
77.19
77.05
76.91
60.78
39.17
39.04
37.16
36.91
32.09
30.21
28.27
f1 (ppm)

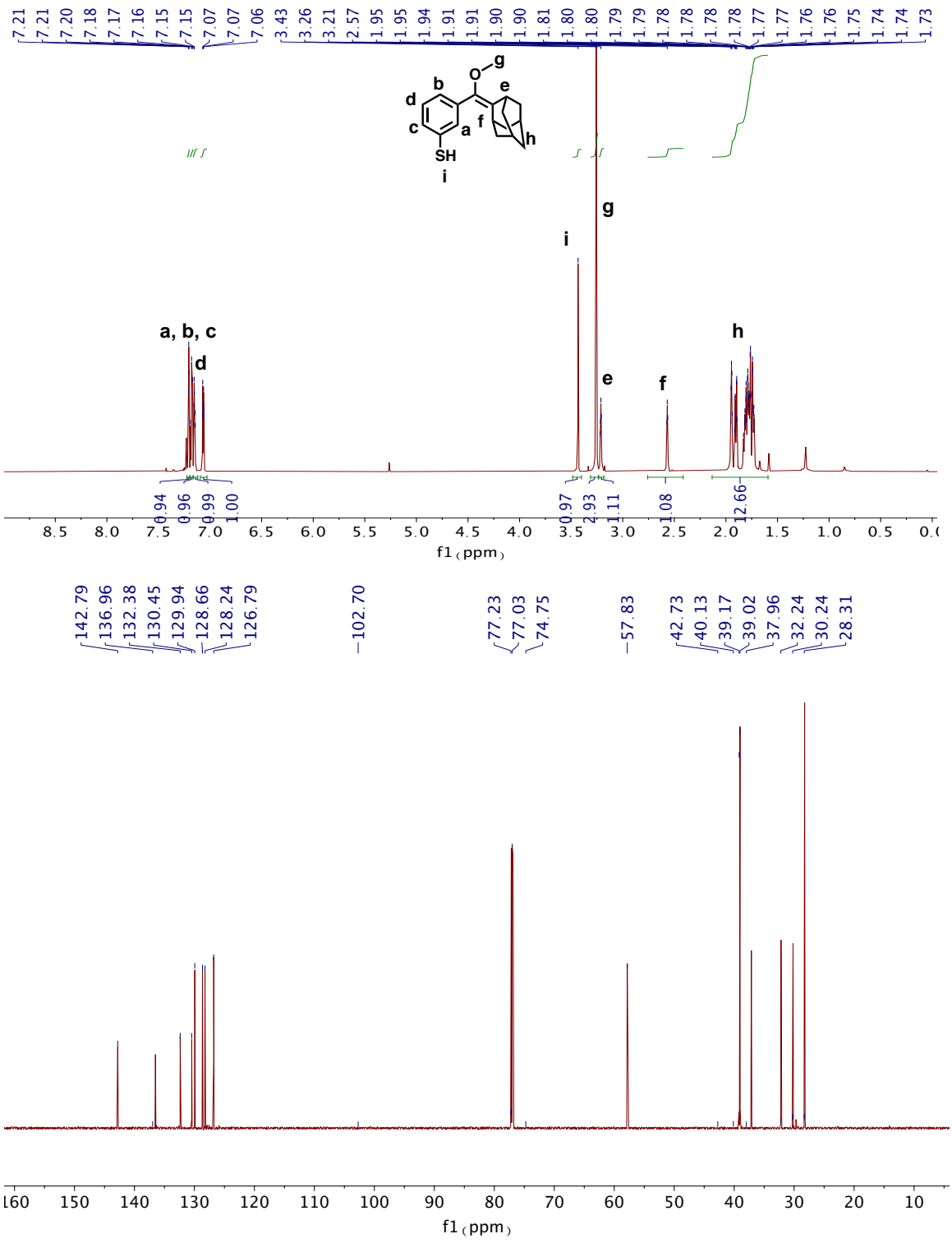
Synthesis of compound 17:



In a flame dried round bottom flask was added **compound 16** (250 mg, 0.7 mmol) and 10% KOH in ethanol (4 mL). The solution was heated at 80 °C for 40 min. The reaction was quenched with PBS (10 mL). The reaction mixture was diluted with ethyl acetate (50 mL) and was extracted with water (50 mL) twice. The organic layer was dried over anhydrous Na_2SO_4. The crude product was purified by silica gel (95/5 hexane/ethyl acetate) to yield **compound 17** (188 mg, 94%).

1H NMR (400 MHz, CDCl3): 7.21 (t, 1H, 4 Hz), 7.18 (t, 1H, 4 Hz), 7.15 (m, 1H), 7.06 (m, 1H), 3.43 (s, 1H), 3.25 (s, 3H), 3.21 (bs, 1H), 2.57 (bs, 1H), 1.94-1.73 (m, 12H).

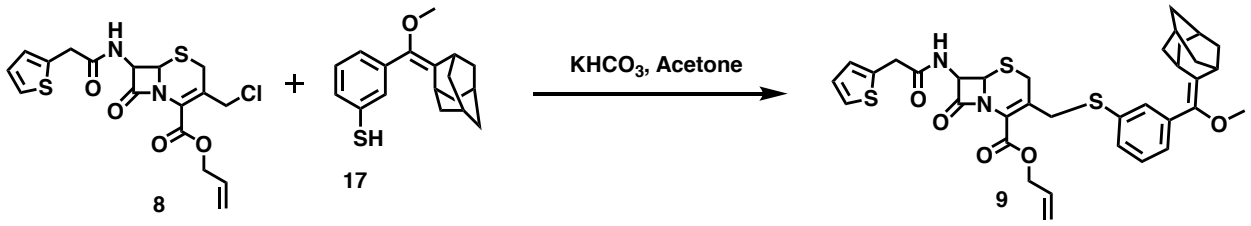
13C NMR (125 MHz, CDCl3): 142.7, 136.9, 132.3, 130.4, 129.9, 128.6, 128.2, 126.7, 57.8, 39.0, 39.0, 37.9, 32.2, 30.2, 28.3.



Synthesis of compound 7: Compound 7 was synthesized following literature procedures.[2]

Synthesis of compound 8: Compound 8 was was synthesized following literature procedures.[3]

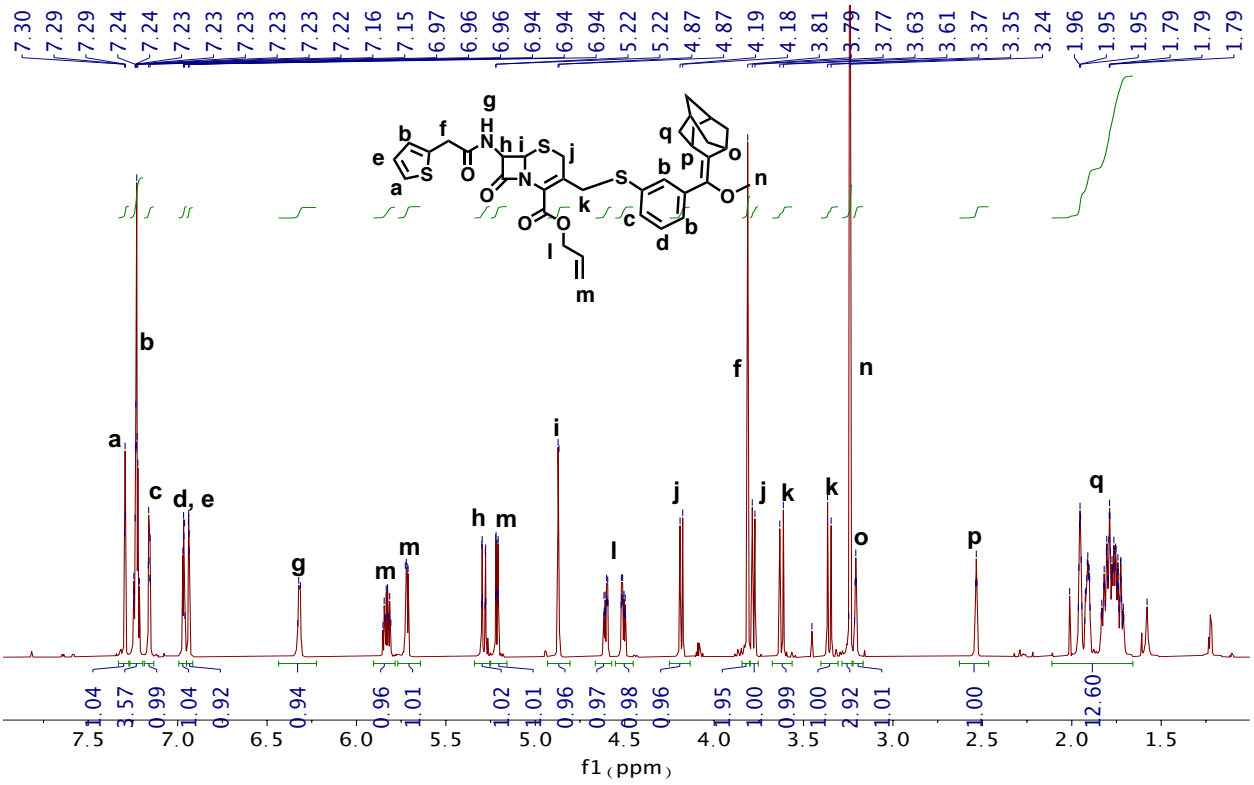
Synthesis of compound 9:

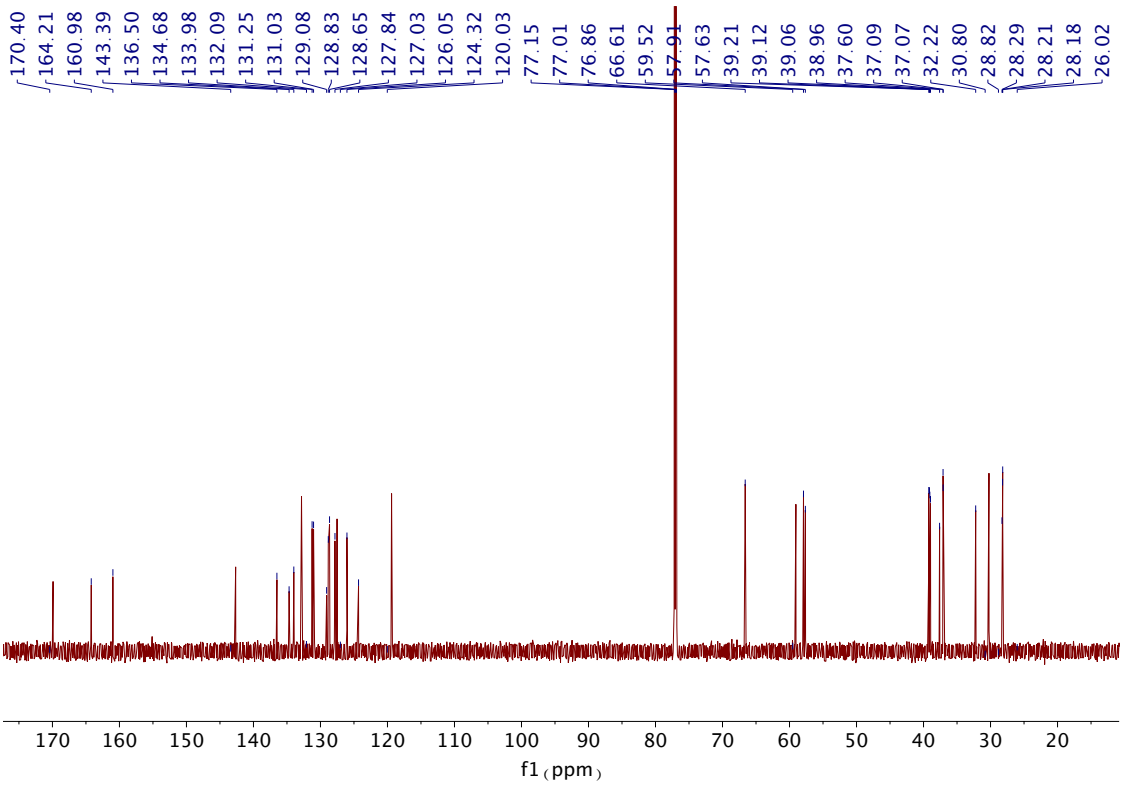


In a flame dried round bottom flask was added **compound 8** (773 mg, 1.87 mmol) and 20 mL of Acetone. Then a solution of **compound 17** (536 mg, 1.87 mmol) in 20 mL of acetone was added. To this resulting reaction mixture 4 mL water and subsequently $KHCO_3$ (224.64 mg, 2.24 mmol) were added. The reaction mixture was stirred at RT for 1h 15 min. The reaction mixture was quenched with 50 mL ice-cold water and extracted with ethyl acetate (50 ml) twice. The combined organic phase was dried over anhydrous Na_2SO_4. The organic solvent was removed under reduced pressure to yield crude **compound 9**, which was purified by silica gel (3:1 Hexane/ethyl acetate) to yield pure **compound 9** (792 mg, 64%) as an off-white solid.

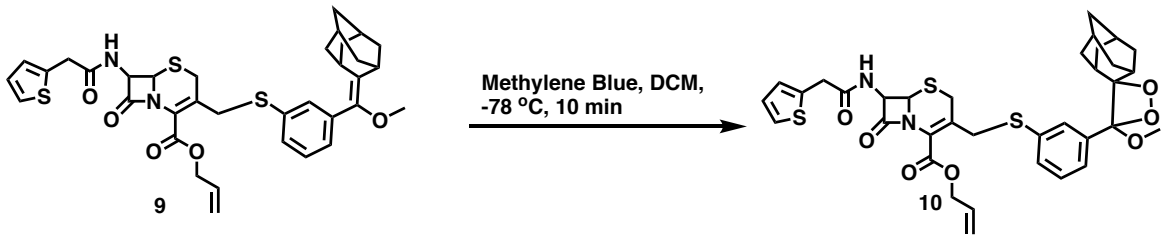
1H NMR (400 MHz, $CDCl_3$): 7.29 (S, 1H), 7.25-7.21 (m, 3H), 7.17-7.14 (m, 1H), 6.96 (t, 1H, 4 Hz), 6.94 (dd, 1H, 4 Hz, 4 Hz), 6.31 (d, 1H, 4 Hz), 5.83 (m, 1H), 5.71 (d, 1H, 8 Hz), 5.29 (d, 1H, 8 Hz), 5.21 (d, 1H, 8 Hz), 4.86 (s, 1H), 4.60 (dd, 1H, 4 Hz, 8 Hz), 4.50 (dd, 1H, 4 Hz, 8 Hz), 4.16 (d, 1H, 8 Hz), 3.81 (s, 2H), 3.76 (d, 1H, 8 Hz), 3.62 (d, 1H, 8 Hz), 3.35 (d, 1H, 8 Hz), 3.24 (s, 3H), 3.20 (s, 1H), 2.53 (s, 1H), 1.96-1.73 (m, 12H).

13C NMR (125 MHz, $CDCl_3$) 170.4, 164.2, 160.9, 143.9, 136.5, 134.6, 133.9, 132.0, 131.2, 131.0, 129.0, 128.8, 128.6, 127.8, 127.0, 126.0, 124.3, 120.0, 66.6, 59.5, 57.6, 39.2, 37.6, 37.0, 32.2, 30.8, 28.8, 28.2.


7.30 7.29 7.29 7.24 7.24 7.23 7.23 7.23 7.23 7.23 7.22 7.16 7.15 6.97 6.96 6.96 6.94 6.94 6.94 5.22 5.22 4.87 4.87 4.19 4.18 3.81 3.79 3.77 3.63 3.61 3.37 3.35 3.24 1.96 1.95 1.95 1.79 1.79 1.79
a b c d, e g m m h m i l j f j k k n o p q
1.04 3.57 0.99 1.04 0.92 0.94 0.96 1.01 1.02 1.01 0.96 0.97 0.98 0.96 1.95 1.00 0.99 1.00 2.92 1.01 1.00 12.60
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
f1 (ppm)

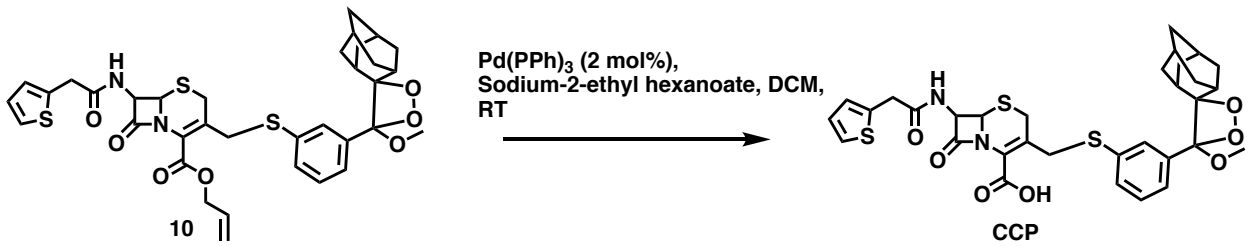

170.40 164.21 160.98 143.39 136.50 134.68 133.98 132.09 131.25 131.03 129.08 128.83 128.65 127.84 127.03 126.05 124.32 120.03
77.15 77.01 76.86 66.61 59.52 57.91 57.63 39.21 39.12 39.06 38.96 37.60 37.09 37.07 32.22 30.80 28.82 28.29 28.21 28.18 26.02
170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20
f1 (ppm)

Synthesis of Compound 10:



In a flame dried round bottom flask was added **compound 9** (230 mg, 0.347 mmol) and 50 mL DCM. To this solution was added 2.21 mg of methylene blue. The resulting reaction mixture was cooled down to -78 °C . Oxygen was bubbled through the solution for 10 min in the presence of light. After 10 min the light was switched-off and oxygen was bubbled further for 5 min. Then 50 mL ice-cold water was added to the reaction mixture and the reaction temperature was further raised to RT. An additional 50 mL DCM was added into reaction mixture and was extracted with water. The organic phase was dried over anhydrous Na_2SO_4. The crude product was purified by silica gel (3:1 hexane/ethyl acetate) to yield an in-separable mixture of **compound 9** and **compound 10** (210 mg). The mixture of compounds was proceeded to the next step.

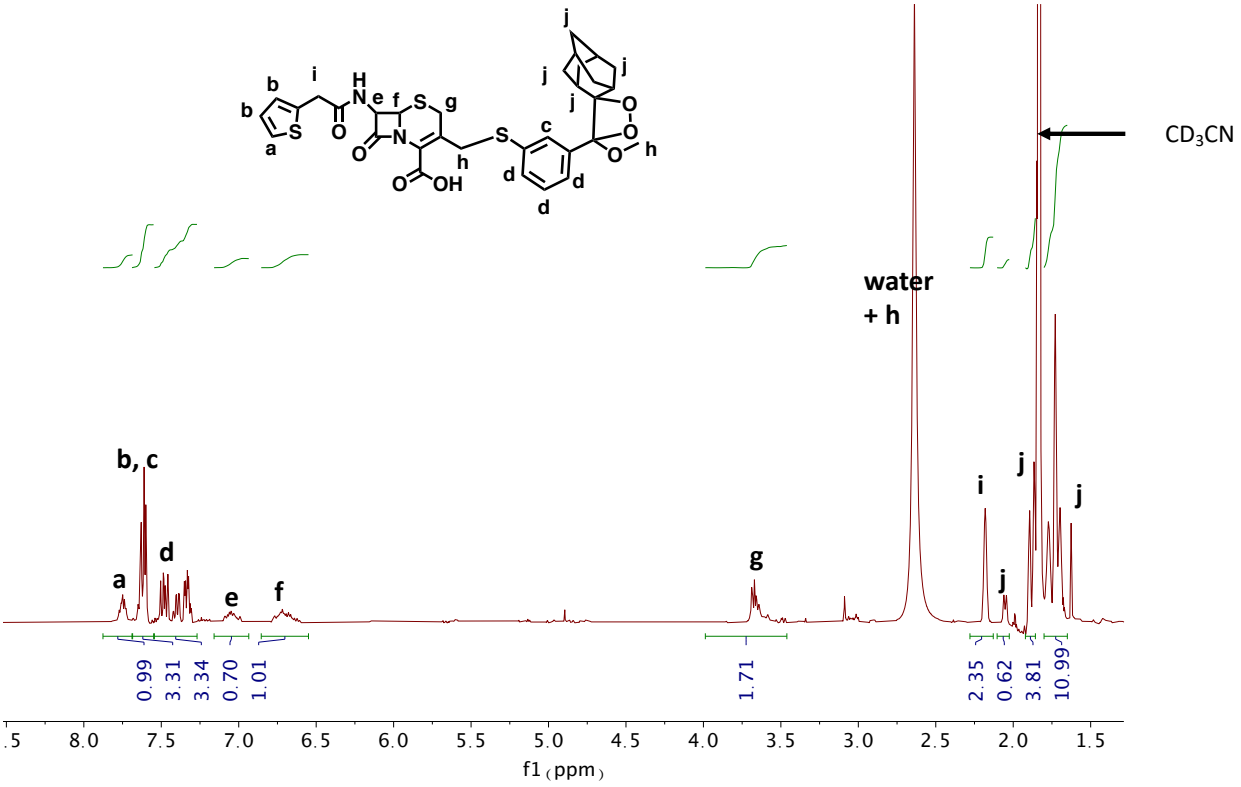
Synthesis of CCP:



In a flame dried round bottom flask was added **compound 10** (50 mg, 72 μmol) and 10 mL DCM. To this solution was added sodium 2-hexanoate (14.3 mg, 79.2 μmol) dissolved in 1 mL ethyl acetate. The reaction mixture was stirred for 5 min. $Pd(PPh_3)_4$ (16.6 mg, 2 mol%) was added into the reaction mixture and the reaction was stirred for 1.5h at RT.

DCM was removed under reduced pressure and the crude product was purified by preparative TLC using (9:1 Acetonitrile/water) to yield CCP (8 mg, 17%) as a white solid. 1H NMR (400 MHz, CD_3CN-D_2O): 7.78 (d, 1H, 4 Hz), 7.66-7.57 (m, 3H), 7.54-7.27 (m, 3H), 7.05 (m, 1H), 6.72 (m, 1H), 3.66 (m, 2H), 2.63 (m, 5H), 2.17 (s, 2H), 2.06 (d, 1H, 4 Hz), 1.93-1.67 (m, 13H).

HRMS (m/z) found: 653.1678, calculated for 653.1528 $C_{32}H_{33}N_2O_7S_3$ $[M-H]^-$

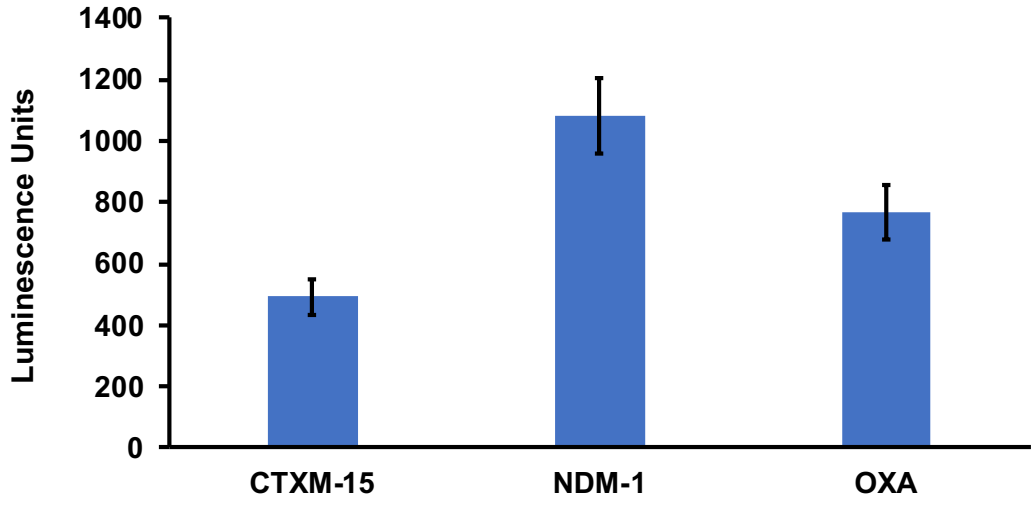


B. Characterization of CCP as a beta-lactamase substrate

B1. Preparation of stock solution: To prepare the stock solution, 8 mg of CCP was dissolved into 245 μL of acetone to make a 50 mM final concentration solution. The stock solution was diluted 1:5 fold to yield a 10 mM solution, and this solution was used for further experiments.

B2. Characterization of CCP as a TEM-1, CTXM-15, OXA-1 and NDM-1 beta-lactamase substrate: In a sterile polystyrene (Corning Catalog #352058) tube was added 83 μL of PBS, 2 μL of 10 mM stock solution of CCP, and 5 μL of TEM-1, CTXM-15, OXA-1 or NDM-1 to make final concentration of 1 nM and 10 μL of sapphire-II enhancer (Thermo Fisher Scientific; Catalog no. T2113). The reaction mixture was incubated at RT for 60 min and every 5 min the chemiluminescence was measured with a Turner Designs TD 2020 TD-20/20 Luminometer over a period of 1 min.

As a control, in a sterile polystyrene (Corning Catalog #352058) tube was added 88 uL of PBS, 2 μL of 10 mM stock solution of CCP and 10 μL of sapphire-II enhancer (Thermo Fisher Scientific; Catalog no. T2113). The reaction mixture was incubated at RT for 60 min and every 5 min the chemiluminescence was measured with a Turner Designs TD 2020 TD-20/20 Luminometer over a period of 1 min.



Supporting Figure 1: CCP is a substrate for TEM-1 (Figure 4), CTXM-15, NDM-1 and OXA-1.

B3. Determination of limit of detection of chemiluminescent based method using CCP probe: In a sterile polystyrene (Corning Catalog #352058) tube was added 83 μL of PBS, 2 μL of 10 mM stock solution of CCP, 10 μL of sapphire-II enhancer (Thermo Fisher Scientific; Catalog no. T2113) and 5 μL of TEM-1 to make final concentration of 200 fM, 300 fM, 400 fM, 500 fM, 800 fM and 1 pM. The reaction mixture was incubated at RT for 30 min and the chemiluminescence was measured with a Turner Designs TD 2020 TD-20/20 Luminometer over a period of 1 min.

Limit of detection (LOD) was calculated using following equation: [Blank + (3 x Standard Deviation$_{\text{lowest concentration}}$)]

LOD = [27.32 + (3 x 8.51)] = 52.85 Luminescence units

Hence, LOD of CPP in detecting TEM-1 = 200 fM.

B4. Determination of limit of detection of fluorescence based method using fluorocilin green: In a 96 well plate was added 95.5 μL of PBS, 2 μL of 10 mM stock solution of fluorocilin green (Thermo fisher Scientific) and 2.5 μL of TEM-1 beta-lactamase to make final concentration of 1 nM, 5 nM, 10 nM, 15 nM, 20 nM and 50 nM. The reaction mixture was incubated at RT for 30 min and the resulting fluorescence was measured with a Tecan Infinite F200 plate reader.

Limit of detection (LOD) was calculated using following equation: [Blank + (3 x Standard Deviation$_{\text{lowest concentration}}$)]

LOD = [3459 + (3 x 1209)] = 7086 Fluorescence units

Hence, LOD of fluorescent based method in detecting TEM-1 = 5 nM.

C. Characterization of CCP with beta-lactamase expressing *E.coli*

C1. Characterization of CCP with beta-lactamase expressing bacteria: Beta lactamase expressing (AmpC resistant), and non-expressing (AmpC susceptible) *E.coli* lysates were prepared at a 10^9 CFU/mL concentration. This solution was diluted to make a solution of 10^6 CFU/mL. In a sterile polystyrene (Corning Catalog #352058) tube was added 88 μL of bacterial lysate (AmpC resistant), 2 μL of 10 mM stock solution of CCP and 10 μL of sapphire-II enhancer (Thermo Fisher Scientific; Catalog no. T2113). The

reaction mixture was incubated at RT for 30 min and the chemiluminescence was measured with a Turner Designs TD 2020 TD-20/20 Luminometer over a period of 1 min.

As a control, in a sterile polystyrene (Corning Catalog #352058) tube was added 88 μL of bacterial lysate (AmpC susceptible), 2 μL of 10 mM stock solution of CCP and 10 μL of sapphire-II enhancer (Thermo Fisher Scientific; Catalog no. T2113). The reaction mixture was incubated at RT for 30 min and the chemiluminescence was measured with a Turner Designs TD 2020 TD-20/20 Luminometer over a period of 1 min.

In addition, in a sterile polystyrene (Corning Catalog #352058) tube was added 88 ml of PBS, 2 μl of 10 mM stock solution of CCP and 10 μl of sapphire-II enhancer (Thermo Fisher Scientific; Catalog no. T2113). The reaction mixture was incubated at RT for 30 min and the chemiluminescence was measured with a Turner Designs TD 2020 TD-20/20 Luminometer over a period of 1 min to determine spontaneous hydrolysis of CCP.

C2. Determination of limit of detection of beta-lactamase expressing bacteria using CCP probe (chemiluminescence based method): Beta lactamase expressing (AmpC resistant) *E.coli* lysates were prepared at a 10^9 CFU/mL concentration. This solution was diluted to make a range of solution of concentrations varying 1 x 10^5 CFU/mL to 1 x 10^6 CFU/mL. In a sterile polystyrene (Corning Catalog #352058) tube was added 88 μL of various concentrations of bacterial lysate (AmpC resistant), 2 μL of 10 mM stock solution of CCP and 10 μL of sapphire-II enhancer (Thermo Fisher Scientific; Catalog no. T2113). The reaction mixture was incubated at RT for 30 min and the chemiluminescence was measured with a Turner Designs TD 2020 TD-20/20 Luminometer over a period of 1 min.

Limit of detection (LOD) was determined by the following equation: [Blank + (3 x Standard Deviation$_{\text{lowest concentration}}$)]

LOD = [32.33 + (3 x 9.12)] = 59.69 Luminescence units

Hence, LOD of CPP in detecting beta-lactamase expressing enzyme = 1 x 10^5 CFU/mL

C2. Determination of Limit of detection of beta-lactamase expressing bacteria using fluorocilin green (fluorescence based method): Beta lactamase expressing

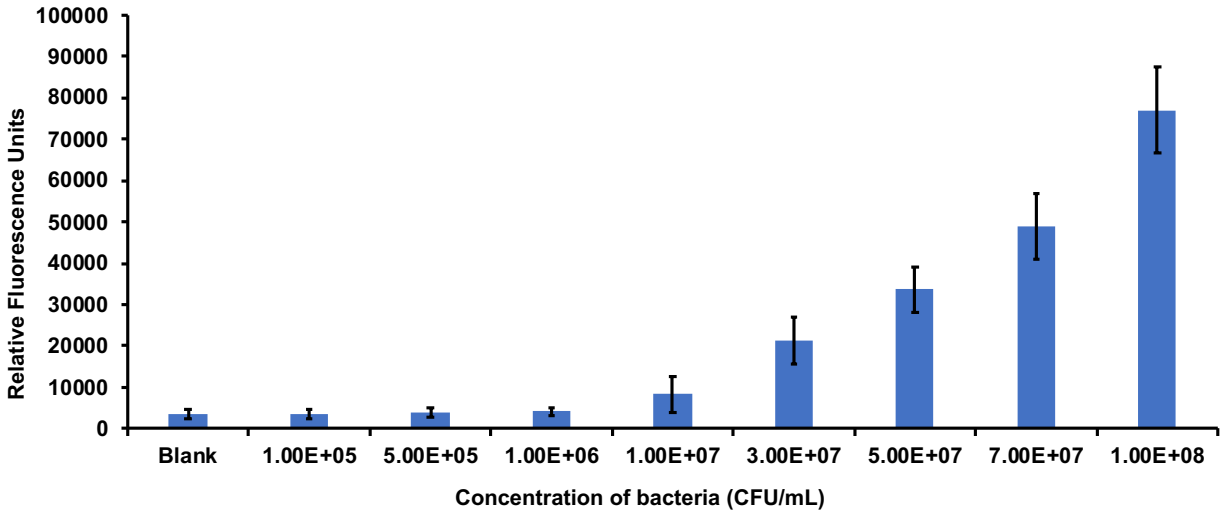
(AmpC resistant) *E.coli* lysates were prepared at a 10^9 CFU/mL concentration. This solution was diluted to make a range of solution of concentrations varying 1 x 10^5 CFU/mL to 1 x 10^8 CFU/mL.

In a 96 well plate was added 88 μL of beta-lactamase expressing bacterial lysate, 2 μL of 10 mM stock solution of fluorocilin green (Thermo fisher Scientific) and 10 μL of TEM-1 beta-lactamase and the reaction mixture was incubated at RT for 30 min and the resulting fluorescence was measured with a Tecan Infinite F200 plate reader (excitation wavelength/emission wavelength = 495 nm/525 nm).

Limit of detection (LOD) was determined by the following equation: [Blank + (3 x Standard $\text{Deviation}_{\text{lowest concentration}}$)]

LOD = [3550 + (3 x 1036)] = 6658 Fluorescence units

Hence, LOD of beta-lactamase expressing bacteria using fluorescence-based method = 1 x 10^7 CFU/mL.



Supporting Figure 2: Fluorocilin green can detect 1 x 10^7 CFU/mL of bacteria that expresses beta-lactamases.

D. Isolation of clinical isolates and their genotyping: Isolation of clinical isolates, their culture and genotyping of their beta-lactamase profile via sequencing was performed following a published procedure.[4]

Table 1: AmpC susceptible and resistant *E.coli* clinical isolates

Name of the clinical isolates	$CTXM_{pos}$	TEM_{pos}	SHV_{pos}	Oxa_{pos}
E.coli 1	No	No	No	No
E.coli 2	No	No	No	No
E.coli 3	No	No	No	No
E.coli 4	No	No	No	No
E.coli 5	No	No	No	No
E.coli 6	No	No	No	No
E.coli 7	Yes	Yes	No	No
E.coli 8	No	Yes	No	No
E.coli 9	No	Yes	No	No
E.coli 10	Yes	No	No	Yes
E.coli 11	Yes	No	No	Yes
E.coli 12	Yes	Yes	No	Yes

E. Detection of beta-lactamases in clinical samples: Ampicillin resistant and susceptible strains were cultured to the concentration of 10^8 CFU/mL and their lysates were prepared for further analysis. Bacterial lysates were diluted to the concentration of 5 x 10^5 CFU/mL. In a polystyrene (Corning Catalog #352058) tube was added 88 μL of bacterial lysate of clinical samples, 2 μL of 10 mM stock solution of CCP and 10 μL of sapphire-II enhancer (Thermo Fisher Scientific; Catalog no. T2113). The reaction mixture was incubated at RT for 30 min and the chemiluminescence was measured with a Turner Designs TD 2020 TD-20/20 Luminometer over a period of 1 min.

F. References:

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