

# Chemistry–A European Journal

Supporting Information

## **Cephalosporin Prodrug Inhibitors Overcome Metallo- $\beta$ -Lactamase Driven Antibiotic Resistance**

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## General Procedures

All reagents employed were of American Chemical Society (ACS) grade or finer and were used without further purification unless otherwise stated. Compounds **1** and **2** were prepared as previously described<sup>[1]</sup>. The final compounds were purified via preparative HPLC performed on a BESTA-Technik system with a Dr. Maisch Reprosil Gold 120 C18 column (25 × 250 mm, 10 μm) and equipped with an ECOM Flash UV detector monitoring at 214 nm. The following solvent system, at a flow rate of 12 mL/min, was used: solvent A, 0.1 % TFA in water/acetonitrile 95/5; solvent B, 0.1 % TFA in water/acetonitrile 5/95. Gradient elution was as follows: 95:5 (A/B) for 5 min, 95:5 to 0:100 (A/B) over 30 min, 0:100 (A/B) for 2 min, then reversion back to 95:5 (A/B) over 1 min, 95:5 (A/B) for 2 min.

Purity was confirmed to be ≥ 95% by LCMS analysis performed on a Shimadzu LC-20AD system with a Shimadzu Shim-Pack GISS-HP C18 column (3.0 x 150 mm, 3 μm) at 30 °C and equipped with a UV detector monitoring at 214 and 254 nm. The following solvent system, at a flow rate of 0.5 mL/min, was used: solvent A, 0.1 % formic acid in water; solvent B, acetonitrile. Gradient elution was as follows: 95:5 (A/B) for 2 min, 95:5 to 0:100 (A/B) over 13 min, 0:100 (A/B) for 2 min, then reversion back to 95:5 (A/B) over 1 min, 95:5 (A/B) for 2 min. This system was connected to a Shimadzu 8040 triple quadrupole mass spectrometer (ESI ionisation).

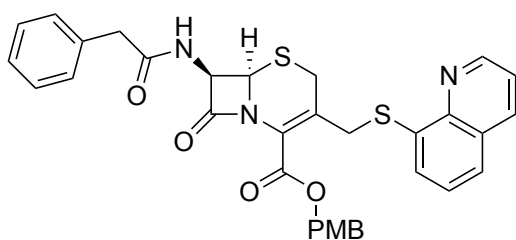
For compound characterization, <sup>1</sup>H NMR spectra were recorded at 400, 500 or 600 MHz with chemical shifts reported in parts per million (ppm) downfield relative to DMSO (δ 2.50), methanol (δ 3.31) or CHCl<sub>3</sub> (δ 7.26). <sup>1</sup>H NMR data are reported in the following order: multiplicity (s, singlet; d, doublet; t, triplet; q, quartet and m, multiplet), coupling constant (*J*) in hertz (Hz) and the number of protons. Where appropriate, the multiplicity is preceded by br, indicating that the signal was broad. <sup>13</sup>C NMR spectra were recorded at 75, 101, 126 or 151 MHz with chemical shifts reported relative to DMSO (δ 39.52) CD<sub>3</sub>OD (δ 49.00) or CDCl<sub>3</sub> (δ 77.16).

HRMS analyses on final compounds were performed on a Shimadzu Nexera X2 UHPLC system with a Waters Acquity HSS C18 column (2.1 × 100 mm, 1.8 μm) at 30 °C and equipped with a diode array detector. The following solvent system, at a flow rate of 0.5 mL/min, was used: solvent A, 0.1 % formic acid in water; solvent B, 0.1 % formic acid in acetonitrile. Gradient elution was as follows: 95:5

(A/B) for 1 min, 95:5 to 15:85 (A/B) over 6 min, 15:85 to 0:100 (A/B) over 1 min, 0:100 (A/B) for 3 min, then reversion back to 95:5 (A/B) for 3 min. This system was connected to a Shimadzu 9030 QTOF mass spectrometer (ESI ionisation) calibrated internally with Agilent's API-TOF reference mass solution kit (5.0 mM purine, 100.0 mM ammonium trifluoroacetate and 2.5 mM hexakis(1H,1H,3H-tetrafluoropropoxy)-phosphazine) diluted to achieve a mass count of 10000.

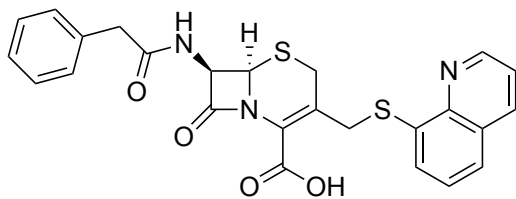
## Chemical synthesis

### 4-methoxybenzyl (6R,7R)-8-oxo-7-(2-phenylacetamido)-3-((quinolin-8-ylthio)methyl)-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylate (3)



4-methoxybenzyl (6R,7R)-3-(chloromethyl)-8-oxo-7-(2-phenylacetamido)-5-thia-1-azabicyclo-[4.2.0]oct-2-ene-2-carboxylate (GCLE, 370 mg, 0.76 mmol, 1 eq) was dissolved in DMF (5 mL) and sodium iodide (112 mg, 0.80 mmol, 1.05 eq) is added. After stirring for 1 hour at room temperature, freshly reduced 8-thioquinoline hydrochloride (198 mg, 1.2 mmol, 1.5 eq) and sodium bicarbonate (170 mg, 2.0 mmol, 2.5 eq) were added and the mixture was stirred at room temperature for 4 hours. The mixture was partitioned between DCM and water, the aqueous layer was extracted with DCM and the combined organic layers were washed with Brine, dried over sodium sulfate and concentrated. The crude product was purified by column chromatography (DCM/EtOAc 92:8 to 80:20) yielding 462 mg (99%) yellowish powder. <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>) δ 8.95 (dd, *J* = 4.2, 1.3 Hz, 1H), 8.15 (dd, *J* = 8.2, 1.5 Hz, 1H), 7.64 (d, *J* = 8.1 Hz, 1H), 7.55 – 7.42 (m, 2H), 7.42 – 7.22 (m, 7H), 6.82 (d, *J* = 8.5 Hz, 2H), 6.05 (d, *J* = 9.1 Hz, 1H), 5.74 (dd, *J* = 9.1, 4.8 Hz, 1H), 5.15 – 4.94 (m, 2H), 4.81 (d, *J* = 4.8 Hz, 1H), 4.45 (d, *J* = 12.9 Hz, 1H), 3.95 (d, *J* = 12.9 Hz, 1H), 3.77 (s, 3H), 3.72 (d, *J* = 18.3 Hz, 1H), 3.69 – 3.56 (m, 2H), 3.49 (d, *J* = 18.3 Hz, 1H). <sup>13</sup>C NMR (101 MHz, CDCl<sub>3</sub>) δ 171.18, 164.52, 161.56, 159.96, 149.88, 146.33, 136.74, 136.13, 133.74, 130.74, 129.60, 129.36, 128.64, 128.36, 128.14, 127.93, 126.96, 126.68, 126.21, 124.88, 121.92, 114.05, 67.91, 59.14, 57.70, 55.38, 43.50, 35.06, 28.58.

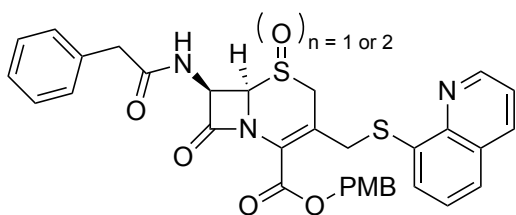
**(6R,7R)-8-oxo-7-(2-phenylacetamido)-3-((quinolin-8-ylthio)methyl)-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid (6)**



Compound 3 (462 mg, 0.75 mmol) was cooled on ice and dissolved in a mixture of TFA/anisole (5:1, 12 mL). The mixture was stirred for 30 minutes at 0°C and 30 minutes at room temperature until TLC (DCM/EtOAc

9:1) showed complete conversion. The mixture was concentrated to about 5 mL, precipitated with diethyl ether and washed with diethyl ether. The crude product was purified by preparative HPLC yielding 309 mg (84%) yellowish powder. <sup>1</sup>H NMR (400 MHz, DMSO-*d*<sub>6</sub>) δ 9.11 (d, *J* = 8.3 Hz, 1H), 8.90 (dd, *J* = 4.2, 1.6 Hz, 1H), 8.39 (dd, *J* = 8.3, 1.5 Hz, 1H), 7.78 (d, *J* = 7.9 Hz, 1H), 7.69 – 7.57 (m, 2H), 7.54 (t, *J* = 7.7 Hz, 1H), 7.36 – 7.14 (m, 5H), 5.63 (dd, *J* = 8.3, 4.7 Hz, 1H), 5.07 (d, *J* = 4.8 Hz, 1H), 4.31 (d, *J* = 12.4 Hz, 1H), 4.11 (d, *J* = 12.4 Hz, 1H), 3.80-3.45 (m, 4H). <sup>13</sup>C NMR (101 MHz, DMSO-*d*<sub>6</sub>) δ 171.00, 164.67, 163.06, 149.49, 144.85, 137.46, 136.59, 135.85, 129.05, 128.25, 127.97, 126.74, 126.51, 125.54, 125.17, 124.80, 122.16, 59.04, 57.86, 41.63, 33.07, 27.54. HRMS calculated for C<sub>25</sub>H<sub>21</sub>N<sub>3</sub>O<sub>4</sub>S<sub>2</sub> [M + H]<sup>+</sup> 492.1046, found 492.1034.

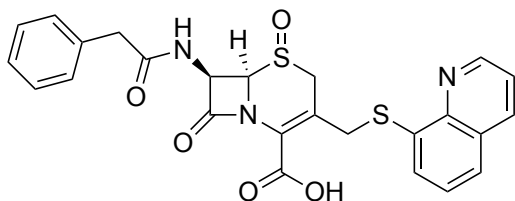
**4-methoxybenzyl (6R,7R)-8-oxo-7-(2-phenylacetamido)-3-((quinolin-8-ylthio)methyl)-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylate 5-oxide (4) and -5,5-dioxide (5)**



Oxidation of GCLE using *m*-CPBA was performed as previously described<sup>[1]</sup>, but led to a mixture of sulfoxide **1** and sulfone **2**. This mixture (400 mg, 0.8 mmol) was dissolved in DMF (5 mL) and sodium iodide (112 mg,

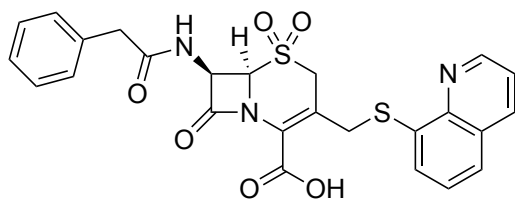
0.8 mmol, 1 eq) is added. After stirring for 1 hour at room temperature, freshly reduced 8-thioquinoline hydrochloride (198 mg, 1.2 mmol, 1.5 eq) and sodium bicarbonate (170 mg, 2.0 mmol, 2.5 eq) were added and the mixture was stirred at room temperature for 4 hours. The mixture was partitioned between DCM and water, the aqueous layer was extracted with DCM and the combined organic layers were washed with Brine, dried over sodium sulfate and concentrated. The crude mixture of compounds **4** and **5** was directly used in the next step without further purification.

**(6*R*,7*R*)-8-oxo-7-(2-phenylacetamido)-3-((quinolin-8-ylthio)methyl)-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid 5-oxide (7)**



Compounds **4** and **5** were cooled on ice and dissolved in a mixture of TFA/anisole (5:1, 12 mL). The mixture was stirred for 30 minutes at 0°C and 30 minutes at room temperature until TLC (DCM/EtOAc 9:1) showed complete conversion. The mixture was concentrated to about 5 mL, precipitated with diethyl ether and washed with diethyl ether. The crude product was purified by preparative HPLC yielding 127 mg (0.25 mmol, 32%) of compound **7** and 150 mg (0.28 mmol, 36%) of compound **8**, both as a yellowish powder. <sup>1</sup>H NMR (500 MHz, DMSO-*d*<sub>6</sub>) δ 8.90 (dd, *J* = 4.2, 1.7 Hz, 1H), 8.40 (dd, *J* = 8.3, 1.5 Hz, 2H), 7.80 (dd, *J* = 8.2, 1.3 Hz, 1H), 7.64 – 7.57 (m, 2H), 7.53 (t, *J* = 7.7 Hz, 1H), 7.34 – 7.27 (m, 4H), 7.27 – 7.19 (m, 1H), 5.76 (dd, *J* = 8.3, 4.7 Hz, 1H), 4.84 (dd, *J* = 4.8, 1.5 Hz, 1H), 4.55 (d, *J* = 12.7 Hz, 1H), 4.00 (d, *J* = 18.3 Hz, 1H), 3.94 (d, *J* = 12.7 Hz, 1H), 3.83 – 3.76 (m, 1H), 3.69 (d, *J* = 14.1 Hz, 1H), 3.53 (d, *J* = 14.0 Hz, 1H). <sup>13</sup>C NMR (75 MHz, DMSO-*d*<sub>6</sub>) δ 171.05, 164.02, 162.35, 149.47, 144.79, 136.83, 136.77, 135.83, 129.11, 128.30, 128.00, 126.76, 126.56, 125.64, 125.20, 125.02, 122.18, 120.37, 66.35, 58.03, 47.02, 41.49, 33.29. HRMS calculated for C<sub>25</sub>H<sub>21</sub>N<sub>3</sub>O<sub>5</sub>S<sub>2</sub> [M + H]<sup>+</sup> 508.0995, found 508.0982.

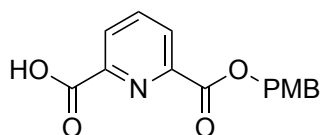
**(6*R*,7*R*)-8-oxo-7-(2-phenylacetamido)-3-((quinolin-8-ylthio)methyl)-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid 5,5-dioxide (8)**



Compounds **4** and **5** were cooled on ice and dissolved in a mixture of TFA/anisole (5:1, 12 mL). The mixture was stirred for 30 minutes at 0°C and 30 minutes at room temperature until TLC (DCM/EtOAc 9:1) showed complete conversion. The mixture was concentrated to about 5 mL, precipitated with diethyl ether and washed with diethyl ether. The crude product was purified by preparative HPLC yielding 127 mg (0.25 mmol, 32%) of compound **7** and 150 mg (0.28 mmol, 36%) of compound **8**, both as a yellowish powder. <sup>1</sup>H NMR (400 MHz, DMSO-*d*<sub>6</sub>) δ 8.95 – 8.86 (m, 2H), 8.42 (dd, *J* = 8.3, 1.7 Hz, 1H), 7.82 (dd, *J* = 8.2, 1.3 Hz, 1H), 7.68 – 7.59 (m, 2H), 7.54 (t, *J* = 7.7 Hz, 1H), 7.33 – 7.16 (m, 5H), 5.90 (dd, *J* = 8.6, 4.8 Hz, 1H), 5.36 (d, *J* = 4.1 Hz, 1H), 4.54 (d, *J* = 18.0 Hz, 1H), 4.36 (d, *J* = 12.7 Hz, 1H), 4.21 (d,

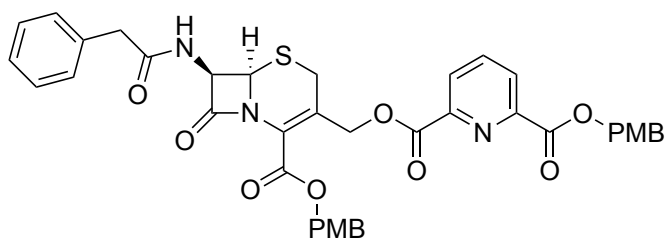
$J = 18.0$  Hz, 1H), 4.02 (d,  $J = 12.6$  Hz, 1H), 3.70 – 3.40 (m, 2H).  $^{13}\text{C}$  NMR (75 MHz,  $\text{DMSO-}d_6$ )  $\delta$  170.91, 164.11, 162.19, 149.51, 144.76, 137.14, 135.61, 129.19, 128.22, 128.11, 126.87, 126.50, 125.91, 125.60, 124.33, 122.24, 67.11, 58.06, 52.33, 41.22, 32.60. HRMS calculated for  $\text{C}_{25}\text{H}_{21}\text{N}_3\text{O}_6\text{S}_2$   $[\text{M} + \text{H}]^+$  524.0945, found 524.0931.

### 6-(((4-methoxybenzyl)oxy)carbonyl)picolinic acid (**9**)



To a suspension of dipicolinic acid (1.70 g, 10.1 mmol, 1.5 eq) in DCM (55 mL), a mixture of 4-methoxybenzyl (*E*)-*N,N'*-diisopropylcarbamimidate<sup>[2]</sup> (1.68 g, 6.4 mmol) and dimethylaminopyridine (45 mg, 5 mol%) in DCM (10 mL) was added dropwise at room temperature. The mixture is stirred overnight and partitioned between DCM and an aqueous solution of sodium bicarbonate at pH 9. The organic layer is extracted with sodium bicarbonate at pH 9 and the combined aqueous layers are washed with EtOAc (2x 100 mL), acidified to pH 3 using 2M HCl and extracted with EtOAc (2x 100 mL). The combined organic layers are washed with brine, dried over sodium sulfate and concentrated to yield **9** as a white powder (700 mg, 54% yield).  $^1\text{H}$  NMR (400 MHz, Methanol- $d_4$ )  $\delta$  8.37 – 8.25 (m, 2H), 8.14 (t,  $J = 7.8$  Hz, 1H), 7.44 (d,  $J = 8.7$  Hz, 2H), 6.93 (d,  $J = 8.7$  Hz, 2H), 5.39 (s, 2H), 3.79 (s, 3H).  $^{13}\text{C}$  NMR (101 MHz, Methanol- $d_4$ )  $\delta$  167.06, 165.88, 161.50, 149.61, 148.86, 140.48, 131.62, 129.24, 129.01, 128.84, 115.00, 68.89, 55.72.

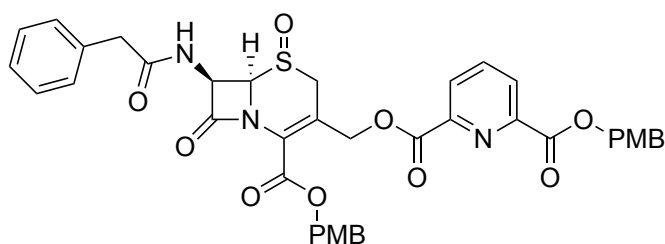
### 2-(4-methoxybenzyl)-6-(((6R,7R)-2-(((4-methoxybenzyl)oxy)carbonyl)-8-oxo-7-(2-phenylacetamido)-5-thia-1-azabicyclo[4.2.0]oct-2-en-3-yl)methyl)pyridine-2,6-dicarboxylate (**10**)



4-methoxybenzyl(6R,7R)-3-(chloromethyl) 8-oxo-7-(2-phenylacetamido)-5-thia-1-azabicyclo-[4.2.0]oct-2-ene-2-carboxylate (GCLE, 650 mg, 1.3 mmol, 1 eq) was dissolved in DMF (10 mL) and sodium iodide (220 mg, 1.45 mmol, 1.1 eq) was added. After stirring for 1 hour at room temperature, a premixed suspension of compound **9** (400 mg, 1.3 mmol, 1 eq) and sodium bicarbonate (340 mg, 4 mmol, 3 eq) in DMF (5 mL) was added and the mixture was stirred overnight at room temperature. The mixture was concentrated, extracted from water with EtOAc and the combined organic phases washed with Brine, dried over sodium sulfate and concentrated. The

crude product was purified by column chromatography (petroleum ether/EtOAc 1:1 to 1:3) yielding 545 mg white powder (57%). <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>) δ 8.26 (d, *J* = 8.0 Hz, 1H), 8.19 (d, *J* = 7.9 Hz, 1H), 7.96 (t, *J* = 7.9 Hz, 1H), 7.40 (d, *J* = 8.9 Hz, 2H), 7.36 – 7.25 (m, 7H), 6.93 – 6.82 (m, 4H), 6.21 (d, *J* = 9.3 Hz, 1H), 5.82 (dd, *J* = 8.9, 4.7 Hz, 1H), 5.45 (d, *J* = 13.6 Hz, 1H), 5.38 (s, 2H), 5.20 (s, 2H), 5.10 (d, *J* = 13.9 Hz, 1H), 4.90 (d, *J* = 4.9 Hz, 1H), 3.80 (s, 3H), 3.78 (s, 3H), 3.67 – 3.60 (m, 3H), 3.43 (d, *J* = 18.7 Hz, 1H). <sup>13</sup>C NMR (101 MHz, CDCl<sub>3</sub>) δ 171.23, 164.80, 164.37, 164.33, 161.41, 160.02, 148.71, 147.92, 138.38, 133.81, 130.85, 130.62, 129.57, 129.30, 128.34, 128.11, 127.85, 127.57, 126.84, 126.02, 125.26, 114.13, 114.09, 68.31, 67.79, 64.68, 59.37, 57.46, 55.44, 55.37, 43.43, 26.56.

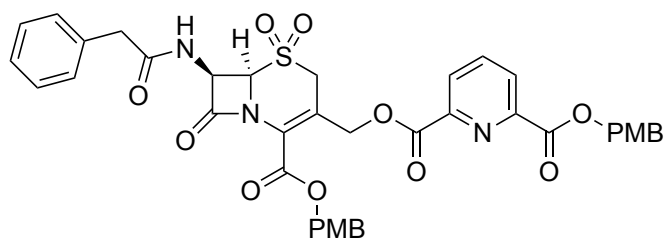
**2-(4-methoxybenzyl)-6-(((6*R*,7*R*)-2-(((4-methoxybenzyl)oxy)carbonyl)-5-oxido-8-oxo-7-(2-phenylacetamido)-5-thia-1-azabicyclo[4.2.0]oct-2-en-3-yl)methyl)pyridine-2,6-dicarboxylate (11)**



Compound **10** (220 mg, 0.30 mmol) was dissolved in dry DCM (10 mL) and cooled to 0°C. mCPBA (77%, 69 mg, 0.30 mmol) was added and the mixture was stirred for 3 hours. LCMS shows

complete conversion without overoxidation to the sulfone. The mixture was concentrated and purified by column chromatography (methanol in DCM from 1% to 3%) yielding 175 mg (78%) yellowish powder. <sup>1</sup>H NMR (500 MHz, CDCl<sub>3</sub>) δ 8.27 (d, *J* = 7.8 Hz, 1H), 8.21 (d, *J* = 7.8 Hz, 1H), 7.98 (t, *J* = 7.8 Hz, 1H), 7.40 – 7.27 (m, 9H), 6.92 – 6.82 (m, 4H), 6.77 (d, *J* = 10.0 Hz, 1H), 6.06 (dd, *J* = 10.0, 4.8 Hz, 1H), 5.70 (d, *J* = 14.1 Hz, 1H), 5.43 – 5.29 (m, 2H), 5.24 (s, 2H), 5.00 (d, *J* = 14.1 Hz, 1H), 4.37 (d, *J* = 4.4 Hz, 1H), 3.87 (d, *J* = 19.1 Hz, 1H), 3.79 (s, 3H), 3.78 (s, 3H), 3.68 – 3.59 (m, 2H), 3.29 (d, *J* = 18.8 Hz, 1H).

**2-(4-methoxybenzyl)-6-(((6*R*,7*R*)-2-(((4-methoxybenzyl)oxy)carbonyl)-5,5-dioxido-8-oxo-7-(2-phenylacetamido)-5-thia-1-azabicyclo[4.2.0]oct-2-en-3-yl)methyl)pyridine-2,6-dicarboxylate (12)**



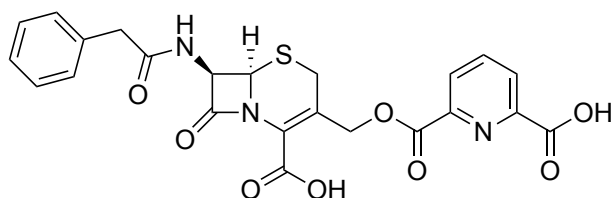
Compound **10** (58 mg, 0.08 mmol) was dissolved in dry DCM (6 mL) and cooled to 0°C. mCPBA (77%, 40 mg, 0.18 mmol) was added and the mixture was stirred

overnight allowing the mixture to reach room temperature. TLC in 5% MeOH/DCM shows full



consumption of starting material and LCMS shows formation of the sulfoxide and sulfone products in a ratio of 1:3. The mixture was concentrated and purified by column chromatography (methanol in DCM from 1% to 3%) yielding 60 mg (97%) yellowish powder.  $^1\text{H}$  NMR (500 MHz,  $\text{CD}_3\text{OD}$ )  $\delta$  8.26 (d,  $J = 7.8$  Hz, 1H), 8.19 (d,  $J = 7.8$  Hz, 1H), 8.02 (t,  $J = 7.9$  Hz, 1H), 7.41 – 7.33 (m, 4H), 7.33 – 7.26 (m, 5H), 6.88 (d,  $J = 8.7$  Hz, 2H), 6.83 (d,  $J = 8.7$  Hz, 2H), 5.99 (d,  $J = 4.8$  Hz, 1H), 5.47 (d,  $J = 14.0$  Hz, 1H), 5.35 (s, 2H), 5.20 (s, 2H), 5.04 (d,  $J = 13.9$  Hz, 1H), 4.98 (d,  $J = 4.8$  Hz, 1H), 4.32 (d,  $J = 18.7$  Hz, 1H), 3.94 (d,  $J = 18.6$  Hz, 1H), 3.65 – 3.56 (m, 2H).

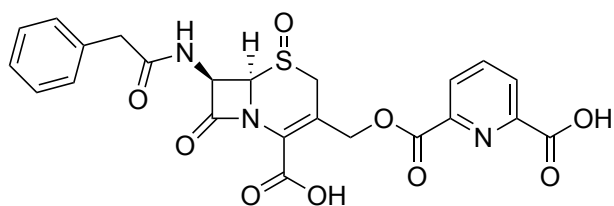
**(6*R*,7*R*)-3-(((6-carboxypicolinoyl)oxy)methyl)-8-oxo-7-(2-phenylacetamido)-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid (13)**



Compound **10** (170 mg, 0.23 mmol) was dissolved in DCM (6 mL). Anisole (0.40 mL) was added and the mixture was cooled to 0°C. Trifluoroacetic acid (0.95 mL) was added

dropwise over a period of 10 minutes and the mixture was stirred for 2 hours at 0°C. The mixture was concentrated, washed with cold diethyl ether and filtered. The crude compound was purified by preparative HPLC yielding 50 mg white powder.  $^1\text{H}$  NMR (600 MHz,  $\text{DMSO}-d_6$ )  $\delta$  9.16 (d,  $J = 8.3$  Hz, 1H), 8.26 (d,  $J = 7.7$  Hz, 2H), 8.19 (t,  $J = 7.6$  Hz, 1H), 7.40 – 7.17 (m, 5H), 5.82 – 5.62 (m, 1H), 5.33 (d,  $J = 12.8$  Hz, 1H), 5.14 (d,  $J = 4.6$  Hz, 1H), 5.06 (d,  $J = 12.7$  Hz, 1H), 3.75 (d,  $J = 18.2$  Hz, 1H), 3.67 (d,  $J = 18.1$  Hz, 2H), 3.58 (d,  $J = 13.9$  Hz, 2H), 3.50 (d,  $J = 13.9$  Hz, 4H).  $^{13}\text{C}$  NMR (151 MHz, DMSO)  $\delta$  171.00, 165.63, 164.91, 163.85, 162.89, 148.92, 147.40, 139.12, 135.82, 129.81, 129.05, 128.26, 128.02, 127.96, 126.84, 126.54, 122.87, 64.27, 59.15, 57.41, 41.61, 25.65. HRMS calculated for  $\text{C}_{23}\text{H}_{19}\text{N}_3\text{O}_8\text{S}$   $[\text{M} + \text{H}]^+$  498.0966, found 498.0963.

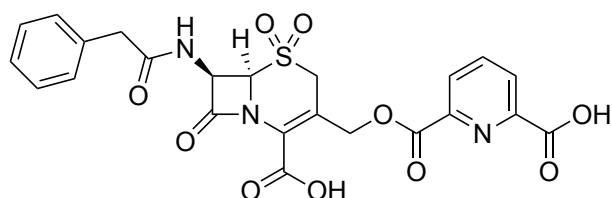
**(6*R*,7*R*)-3-(((6-carboxypicolinoyl)oxy)methyl)-8-oxo-7-(2-phenylacetamido)-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid 5-oxide (14)**



Compound **14** (175 mg, 0.23 mmol) was prepared as described for compound **13** yielding 50 mg white powder.  $^1\text{H}$  NMR (500

MHz, DMSO- $d_6$ )  $\delta$  8.42 (d,  $J$  = 8.3 Hz, 1H), 8.28 – 8.21 (m, 2H), 8.18 (t,  $J$  = 7.7 Hz, 1H), 7.34 – 7.27 (m, 4H), 7.27 – 7.20 (m, 1H), 5.82 (dd,  $J$  = 8.3, 4.8 Hz, 1H), 5.58 (d,  $J$  = 13.1 Hz, 1H), 4.94 (d,  $J$  = 13.1 Hz, 1H), 4.89 (dd,  $J$  = 4.8, 1.6 Hz, 1H), 4.04 (d,  $J$  = 18.4 Hz, 1H), 3.69 (d,  $J$  = 14.1 Hz, 1H), 3.68 (d,  $J$  = 18.3 Hz, 1H), 3.55 (d,  $J$  = 14.0 Hz, 1H).  $^{13}\text{C}$  NMR (126 MHz, DMSO)  $\delta$  171.21, 165.69, 164.38, 163.84, 162.25, 148.99, 147.41, 139.19, 135.85, 129.21, 128.40, 128.11, 128.03, 126.68, 126.40, 118.35, 66.38, 64.66, 58.32, 45.46, 41.55. HRMS calculated for  $\text{C}_{23}\text{H}_{19}\text{N}_3\text{O}_9\text{S}$   $[\text{M} + \text{H}]^+$  514.0915, found 514.0918.

**(6*R*,7*R*)-3-(((6-carboxypicolinoyl)oxy)methyl)-8-oxo-7-(2-phenylacetamido)-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid 5,5-dioxide (15)**



Compound **15** (60 mg, 0.08 mmol) was prepared as described for compound **13** yielding 20 mg white powder.  $^1\text{H}$  NMR (500 MHz, DMSO- $d_6$ )  $\delta$  8.89 (d,  $J$  = 8.7 Hz, 1H), 8.29

– 8.23 (m, 2H), 8.19 (t,  $J$  = 7.7 Hz, 1H), 7.36 – 7.14 (m, 5H), 5.97 (dd,  $J$  = 8.7, 4.8 Hz, 1H), 5.46 (d,  $J$  = 13.2 Hz, 1H), 5.37 (d,  $J$  = 4.7 Hz, 1H), 4.96 (d,  $J$  = 13.2 Hz, 1H), 4.47 (d,  $J$  = 18.1 Hz, 1H), 4.31 (d,  $J$  = 18.1 Hz, 1H), 3.66 – 3.53 (m, 2H).  $^{13}\text{C}$  NMR (126 MHz, DMSO- $d_6$ )  $\delta$  170.79, 165.80, 163.99, 163.89, 146.96, 138.99, 135.61, 129.14, 129.02, 128.21, 128.19, 127.85, 126.47, 120.80, 115.44, 65.88, 65.48, 58.69, 51.14, 41.28. HRMS calculated for  $\text{C}_{23}\text{H}_{19}\text{N}_3\text{O}_{10}\text{S}$   $[\text{M} + \text{H}]^+$  530.0864, found 530.0868.

## Enzyme Production and Purification

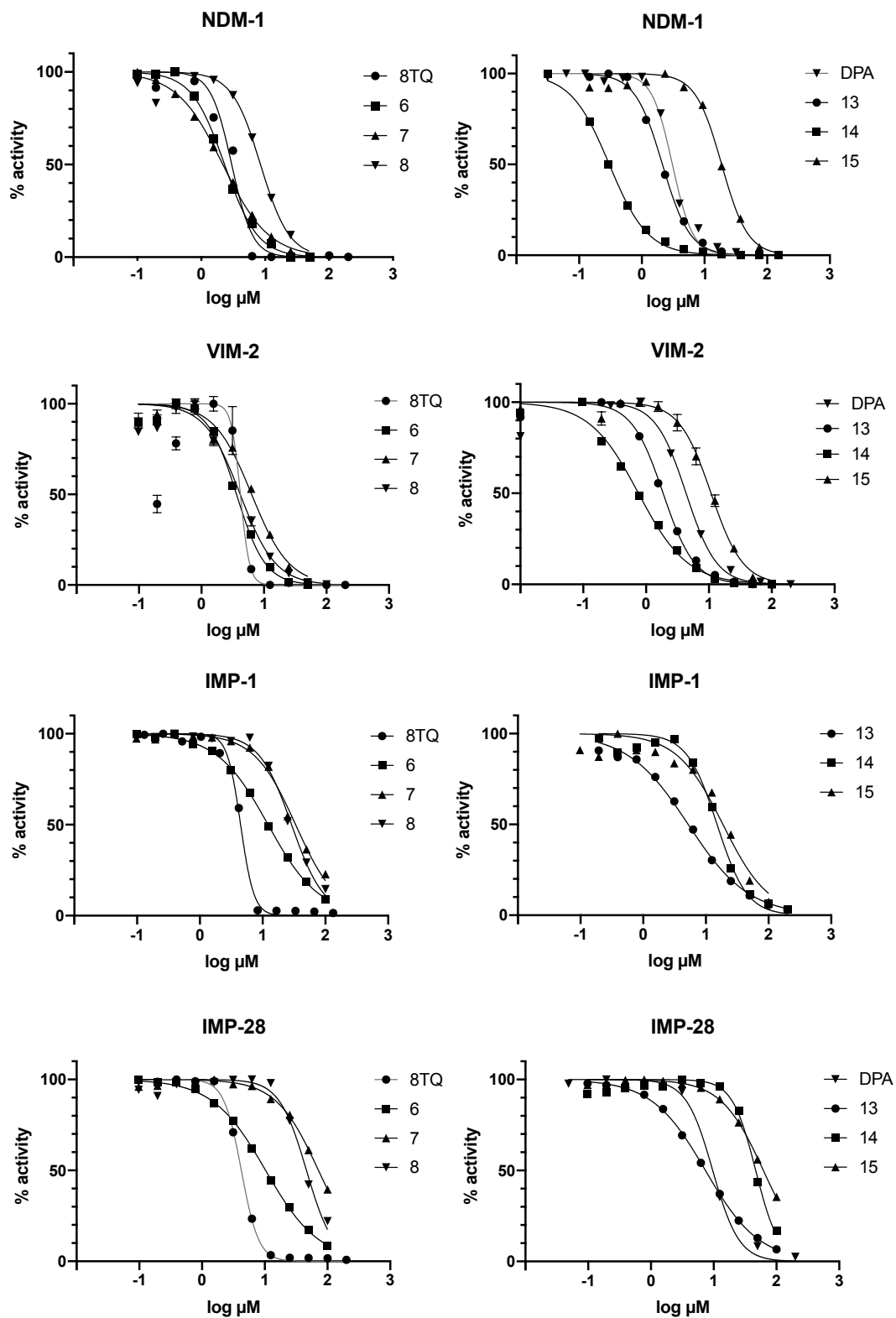
The plasmid constructs of NDM-1, VIM-2 and IMP-1 were a kind gift from Prof. Christopher J. Schofield (Oxford University). The IMP-28 construct was designed in the pET28b vector with a C-terminal His-tag. NDM-1, VIM-2 and IMP-28 were produced and purified as previously described.<sup>[3]</sup>

To obtain IMP-1 protein<sup>[4]</sup>, *Escherichia coli* BL21 (DE3) pLysS transformed with the plasmid was grown at 37°C in 2 L of Luria-Bertani (LB) medium supplemented with kanamycin (50  $\mu\text{g}/\text{mL}$ ) and chloramphenicol (37  $\mu\text{g}/\text{mL}$ ) to ensure selective growth. When the culture reached an optical density ( $\text{OD}_{600 \text{ nm}}$ ) of 0.6, protein production was induced using 0.5 mM isopropyl- $\beta$ -D-thiogalactopyranoside (IPTG). The cultures were grown for another 4 hours before the cells were harvested by centrifugation

(4000 g) for 20 min and resuspended in 50 mL of 50 mM HEPES pH 7.0, 250 mM NaCl. The cells were lysed using sonication and centrifuged for 30 min at 30000 g. The supernatant was diluted with Buffer A (50 mM HEPES pH 7.0, 100  $\mu$ M ZnSO<sub>4</sub>) before being loaded onto a 1 mL HiTrap SP FF column (GE Healthcare) and subsequently eluted using a gradient of Buffer B (50 mM HEPES pH 7.0, 100  $\mu$ M ZnSO<sub>4</sub>, 1 M NaCl). The fractions were run on an SDS-PAGE gel and those containing IMP-1 were combined, concentrated using spin filter columns (MilliPore) and loaded onto a size-exchange column (Superdex75 16/60; GE Healthcare). The fractions were once again assessed by an SDS-PAGE gel and the pure fractions were combined and concentrated. The concentration of the protein was determined using a NanoDrop spectrometer.

### **MBL inhibition Assay and IC<sub>50</sub> curves**

All the test compounds were dissolved in DMSO at a concentration to ensure a maximum final concentration of 1% DMSO. The compounds were serially diluted in 50 mM HEPES, pH 7.2, supplemented with 0.01% triton X-100 and 1  $\mu$ M ZnSO<sub>4</sub>. The MBL enzymes (60 pM NDM-1, 100 pM VIM-2, 100 pM IMP-1 and 60 pM IMP-28) were then added to the wells and incubated at 25 °C for 10 min. Next, the fluorescent cephalosporin substrate FC5<sup>[1]</sup> (0.5  $\mu$ M for NDM-1 and VIM-2, 20  $\mu$ M for IMP-1 and 16  $\mu$ M for IMP-28) was added to the wells, and the fluorescence was monitored immediately over 25 scanning cycles ( $\lambda_{\text{ex}}$  380 nm,  $\lambda_{\text{em}}$  460 nm) on a Tecan Spark plate reader. Using the initial velocity data plotted against inhibitor concentration, the half-maximal inhibitory concentrations were calculated by an IC<sub>50</sub> curve-fitting model in GraphPad Prism 8 software. IC50 curves are presented in figure S1.



**Figure S1.**  $IC_{50}$  curves for 8-TQ and DPA and corresponding cephalosporin pro-drugs 6-8 and 13-15 against NDM-1, VIM-2, IMP-1 and IMP-28.

Time dependence assays were performed with different preincubation times of the conjugate with NDM-1. Compounds **6** and **14** were preincubated with NDM-1 for 0, 10, 20, 30, 45 or 60 minutes at 25 °C before addition of the fluorescent substrate. The results are presented in figure S2 below.

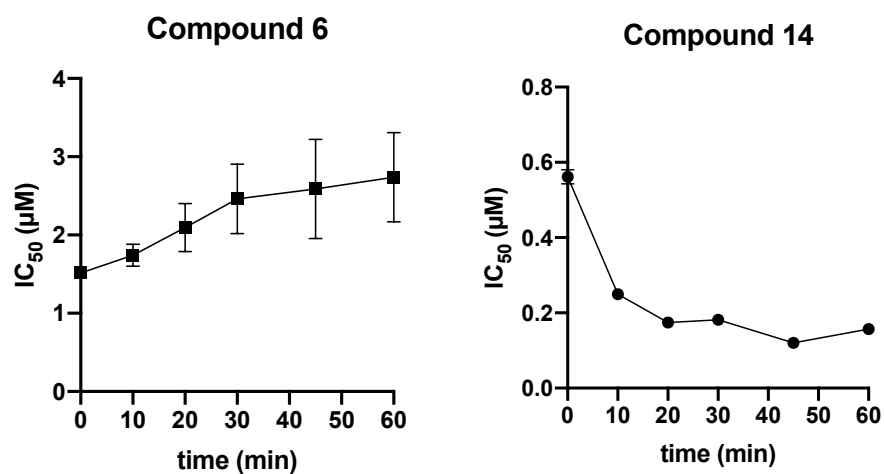
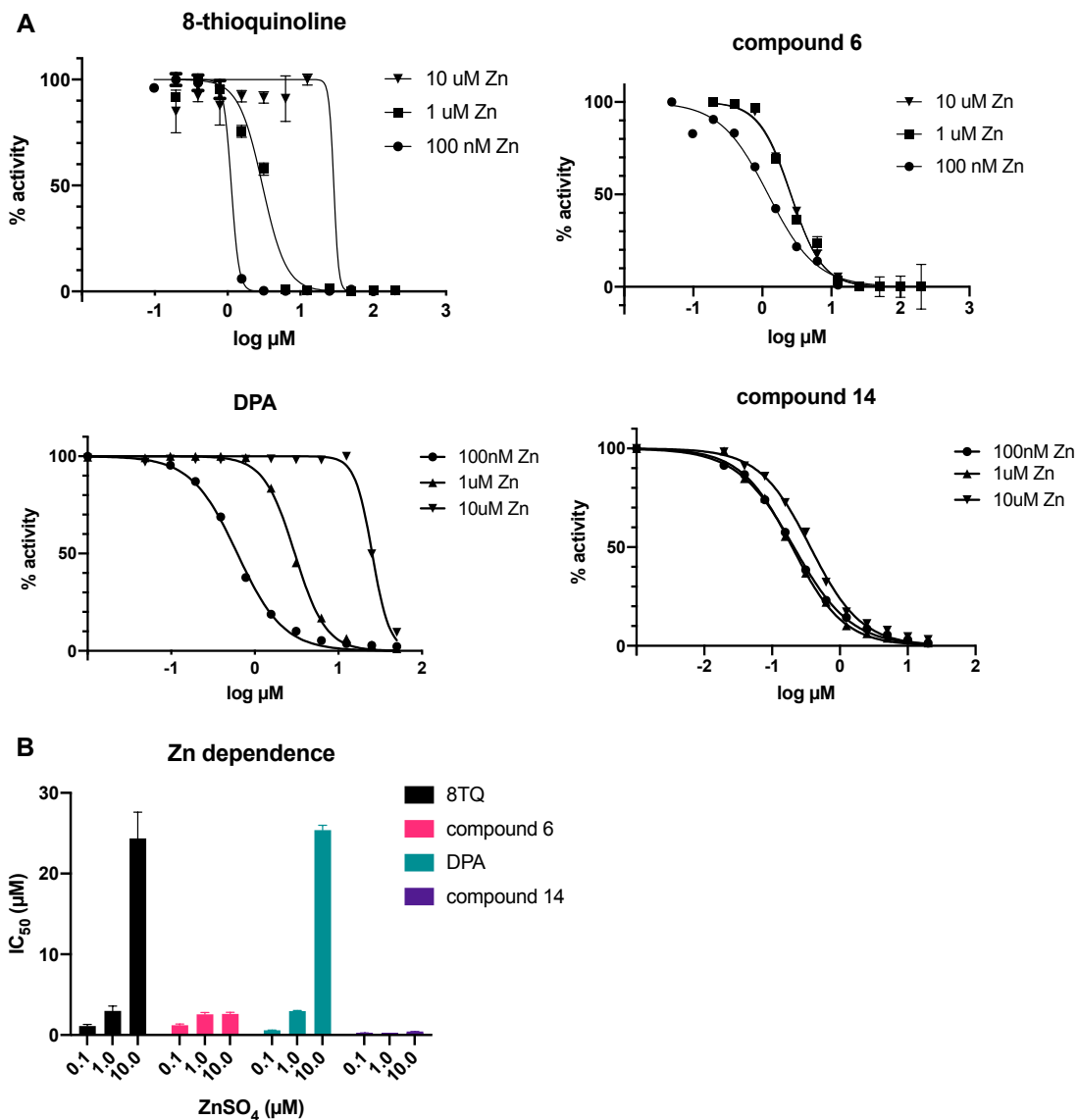


Figure S2. Time-dependent IC<sub>50</sub> values for compounds **6** and **14**.

The IC<sub>50</sub> values of 8TQ, DPA and compounds **6** and **14** were also evaluated in the presence of different concentrations of zinc sulfate (0.1, 1 and 10 μM) against NDM-1, following the procedure described above. The results are depicted in figure S3 below



**Figure S3. A)** Zinc dependent IC<sub>50</sub> curves for 8-TQ, DPA and compounds **6** and **14**. **B)** Bar graph summarizing the results of zinc dependence.

## Isothermal Titration Calorimetry

All binding experiments are performed using a MicroCal PEAQ-ITC Automated microcalorimeter (Malvern). The samples are equilibrated to 25°C prior to the measurement. The solution of ZnSO<sub>4</sub> in 20 mM Tris HCl, pH 7.0 is titrated into a solution of compound in the same buffer, except for 8TQ which is titrated into an equilibrated solution of ZnSO<sub>4</sub> and DPA. The titrations are conducted at 25°C under constant stirring at 750 rpm. Each binding experiment consisted of an initial injection of 0.4 μL followed by 18 separate injections of 2.0 μL into the sample cell of 200 μL. The time between each injection is 150 seconds, the measurements are performed with the reference power set at 10 μcal s<sup>-1</sup> and the feedback mode set at “high”. For the titration of compound **14** a modified protocol was employed wherein the time between injections is 180 seconds and the reference power set at 5 μcal s<sup>-1</sup>. The concentrations used for zinc sulfate and the ligand are provided below in Table S1.

**Table S1.** Concentrations used for zinc sulfate and ligands in the ITC experiments

Experiment (metal + ligand)	Zinc sulfate concentration (mM)	Ligand concentration (mM)
ZnSO <sub>4</sub> + DPA	0.5	0.05
ZnSO <sub>4</sub> + <b>14</b>	5.0	0.05
ZnSO <sub>4</sub> /DPA + 8TQ	0.05/0.1	1.0
ZnSO <sub>4</sub> + <b>6</b>	2.0	0.2

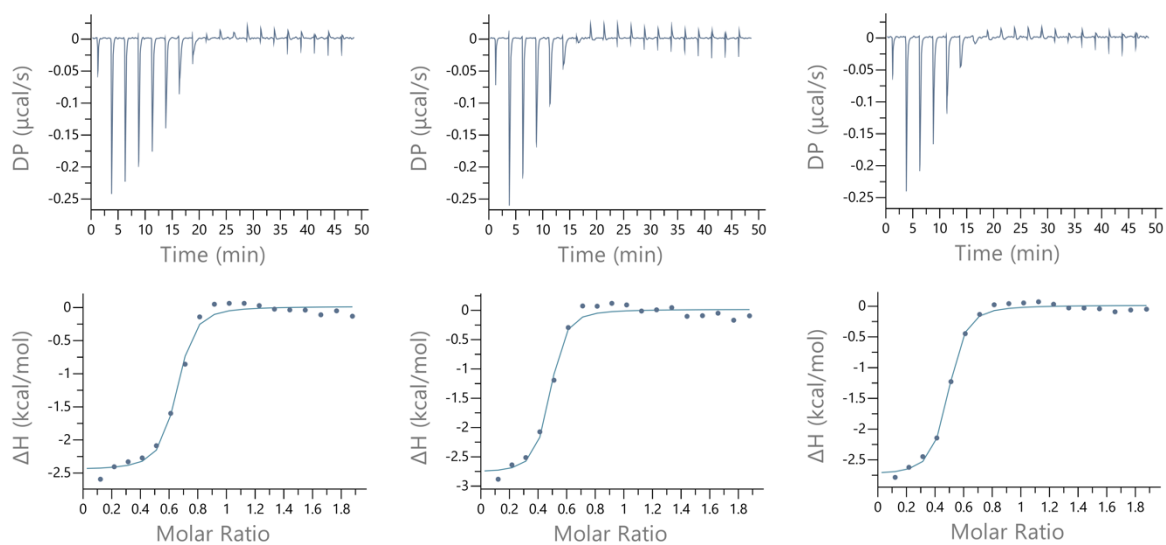
The calorimetric data obtained is analyzed using MicroCal PEAQ-ITC Analysis Software Version 1.20. ITC data fitting is made based on the “One set of sites” fitting model of the software. The best fit is defined by chi-square minimization. The thermodynamic parameters generated by the displacement titration ZnSO<sub>4</sub>/DPA + 8TQ are used in a competitive binding model.<sup>[5]</sup> All thermodynamic parameters are reported as the average of three independent experiments (Table S2). Thermograms of the measurements are presented in figure S4.

**Table S2.** Thermodynamic parameters with standard deviations. All experiments were performed in triplicate

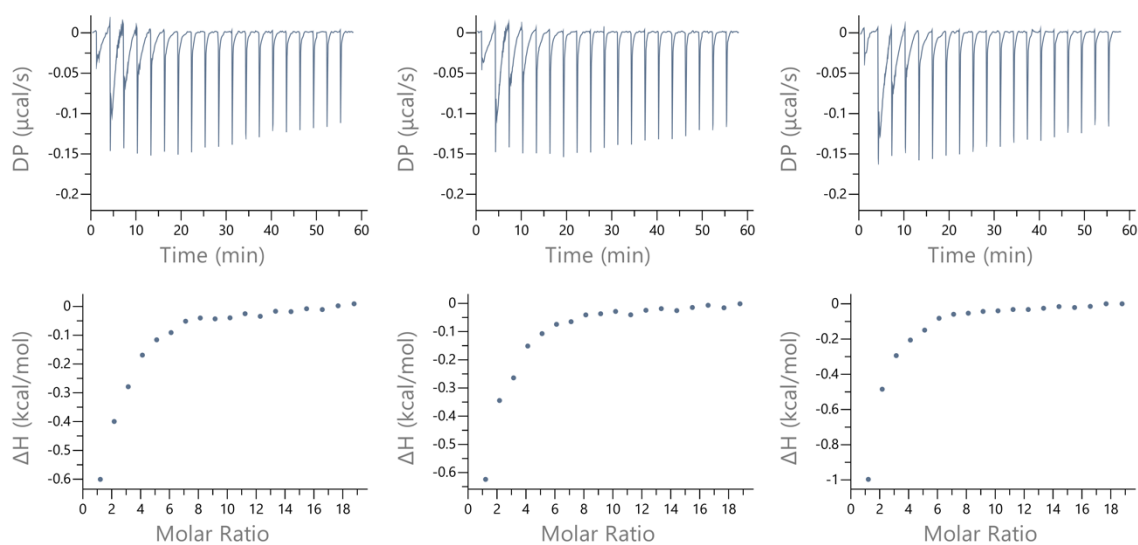
Compound	ligand	Stoichiometry	Kd (nM)	ΔH (kcal/mol)	-TΔS (kcal/mol)	ΔG (kcal/mol)
DPA	Zn <sup>2+</sup>	2.06 ± 0.22	398 ± 45	-2.68 ± 0.12	-6.06 ± 0.57	-8.74 ± 0.69
compound <b>14</b>	Zn <sup>2+</sup>	n/a	85270 ± 14744	n/a	n/a	n/a
8TQ	Zn <sup>2+</sup>	2.04 ± 0.22	1.752 ± 0.287	-12.18 ± 0.49	0.22 ± 0.41	-11.96 ± 0.097
compound <b>6</b>	Zn <sup>2+</sup>	n/a	n/a	n/a	n/a	n/a

n/a: Parameters unable to fit in a binding model

## ITC thermograms

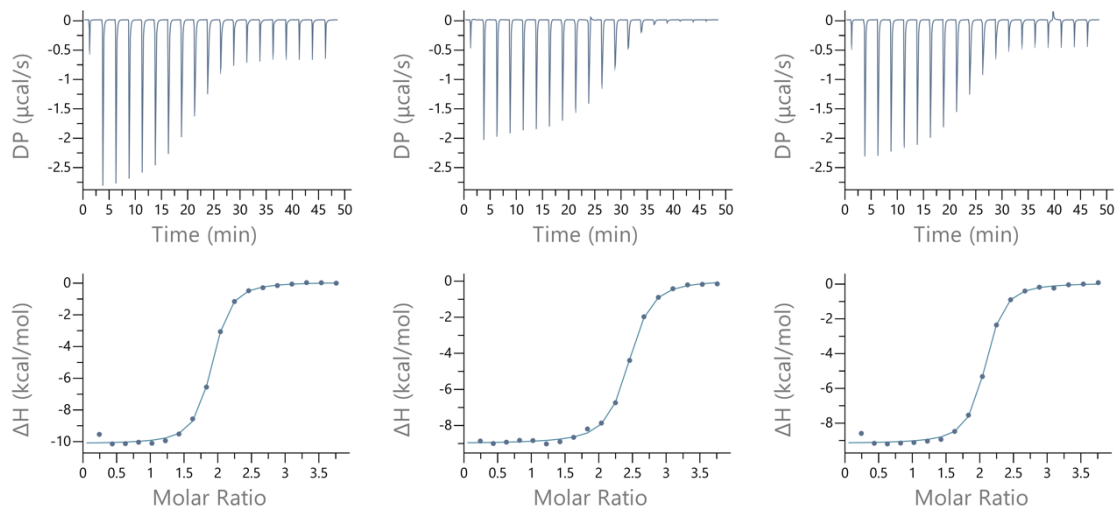


**Figure S4(A).** ITC thermograms of 0.5 mM ZnSO<sub>4</sub> into 0.05 mM DPA in triplicate

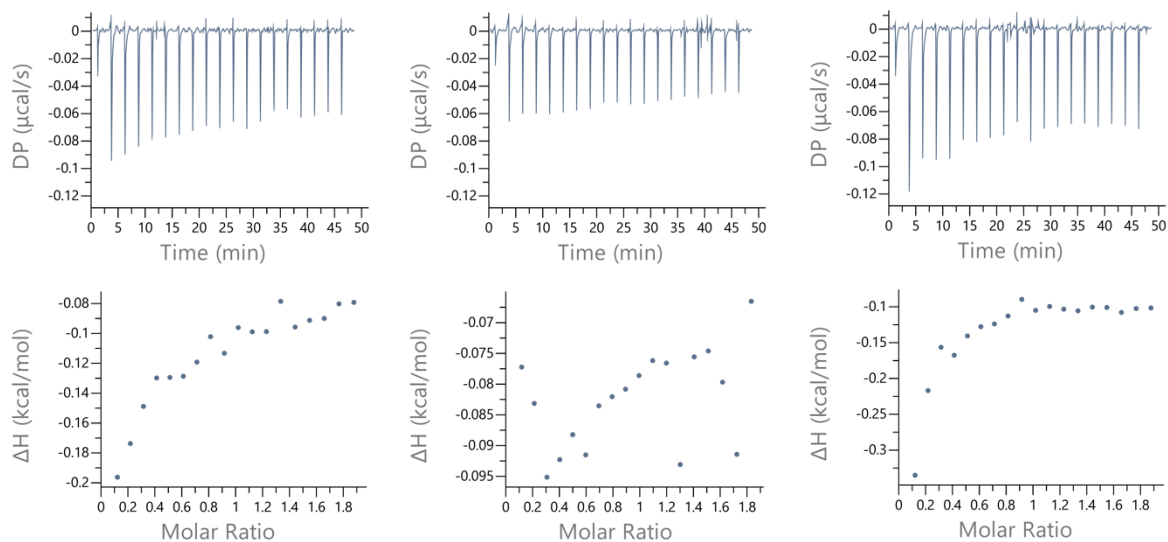


**Figure S4(B).** ITC thermograms of ZnSO<sub>4</sub> 5 mM into 14 0.05 mM in triplicate

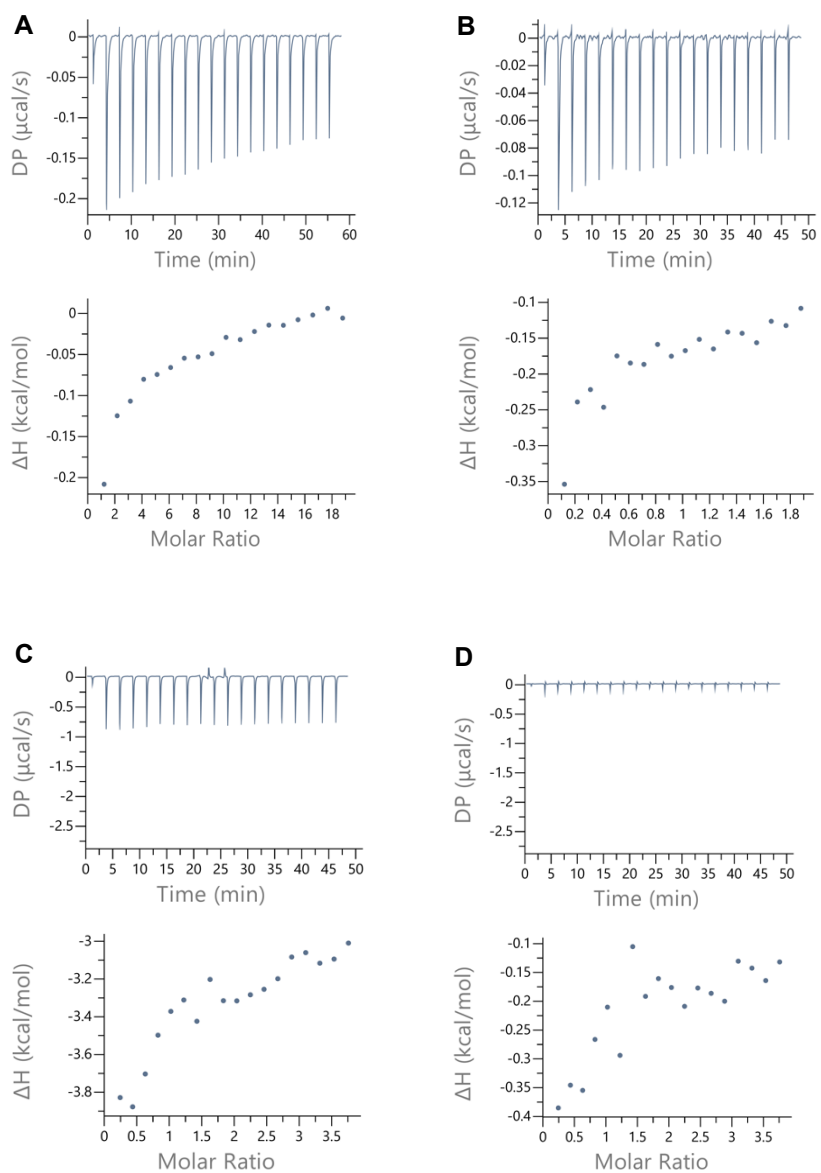




**Figure S4(C).** ITC thermograms of 8TQ 1 mM into ZnSO<sub>4</sub>/DPA 0.05 mM /0.1 mM in triplicate



**Figure S4(D).** ITC thermograms of 2 mM ZnSO<sub>4</sub> into 0.2 mM compound 6 in triplicate



**Figure S4(E).** ITC thermograms of control titrations. A. 5 mM  $\text{ZnSO}_4$  into 20 mM Tris buffer, B. 2 mM  $\text{ZnSO}_4$  into 20 mM Tris buffer, C. 5 mM 8-TQ into 0.1 mM DPA, D. 20 mM Tris buffer into  $\text{ZnSO}_4/\text{DPA}$  0.05 mM / 0.1 mM.

## Stability testing

Conjugates **6-8** and **13-15** were dissolved in DMSO to a concentration of 20 mg/mL. The DMSO stock solutions were diluted 100-fold in Mueller-Hinton Broth (MHB) and incubated at 37°C. After 0, 2, 4, 6 and 24 hours, 100µL of MHB was added to 200µL acetonitrile to deproteinate the solution. After 10 minutes on ice, the samples were centrifuged for 10 minutes at 12,000 rpm and the supernatant analyzed by LC-MS using the general procedure. The normalized results are presented in figure S5 below.

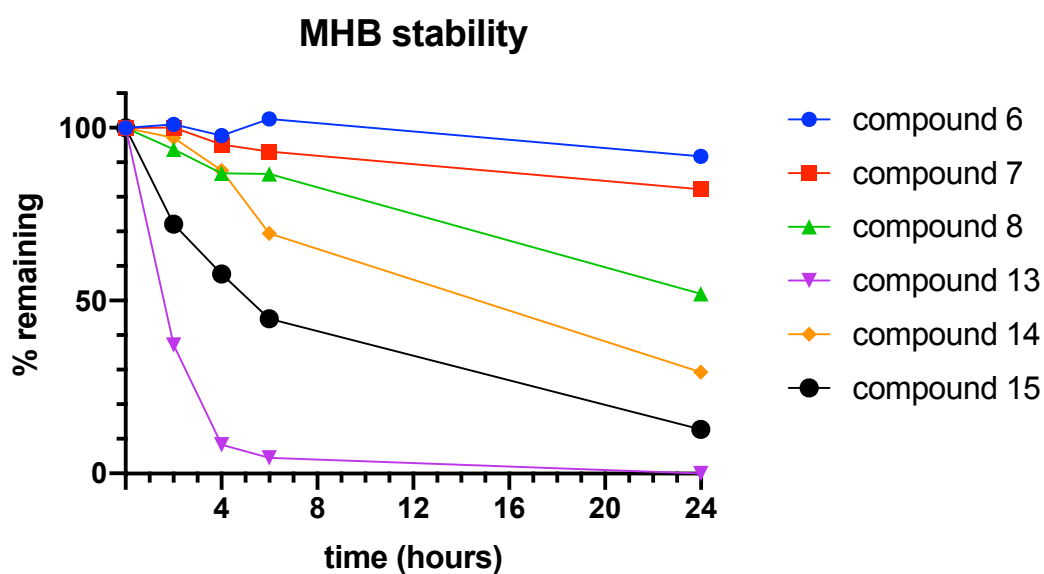
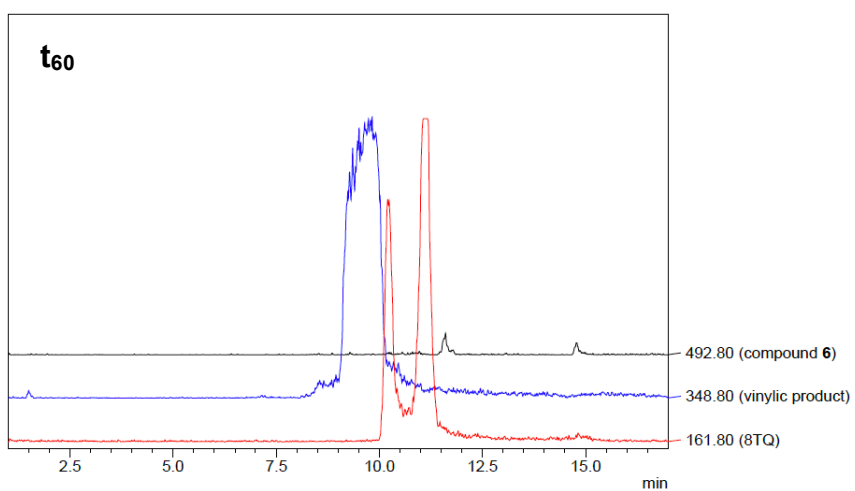
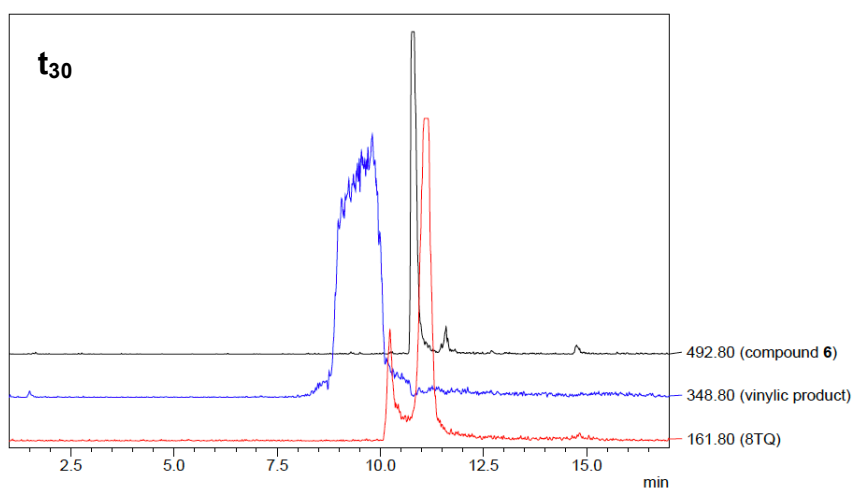
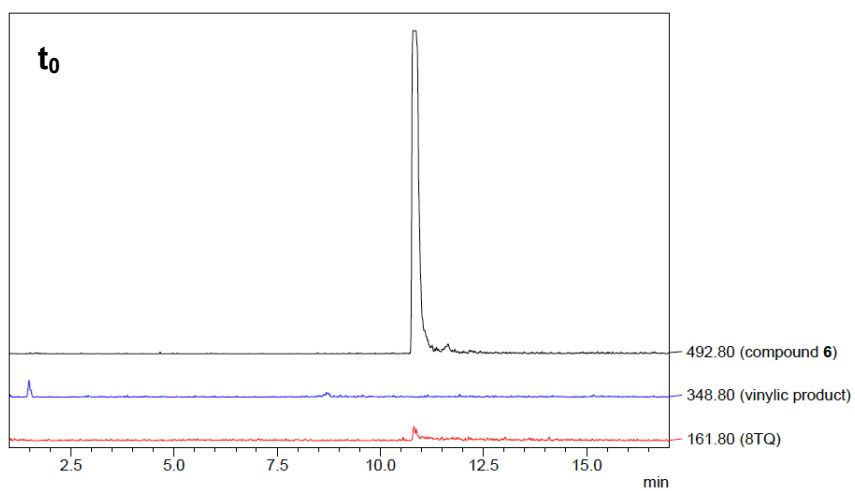


Figure S5. Stability data of compounds 6-8 and 13-15 in Mueller-Hinton Broth at 37°C over 24 hours.

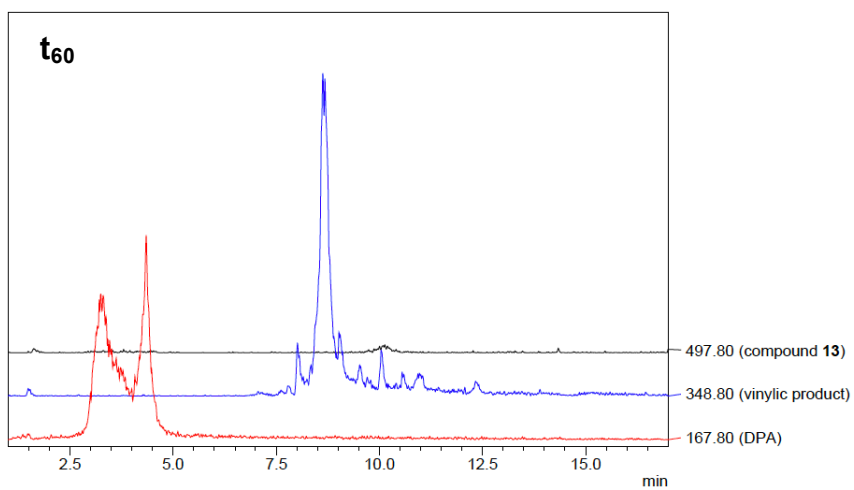
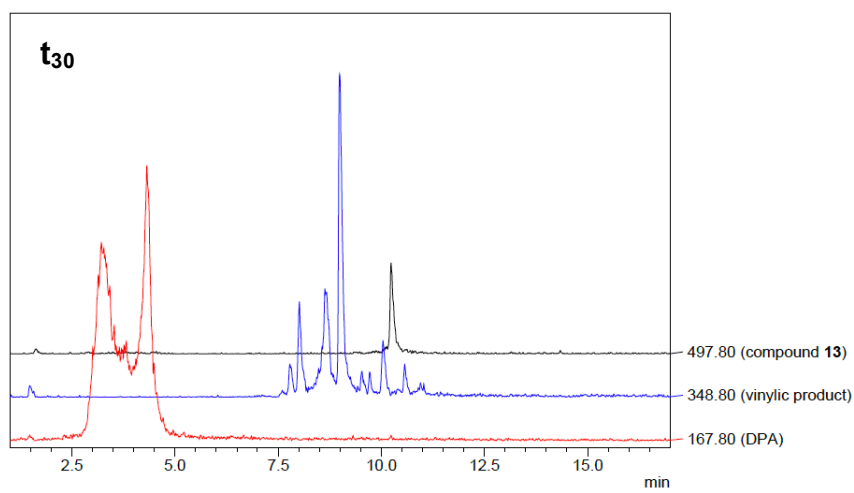
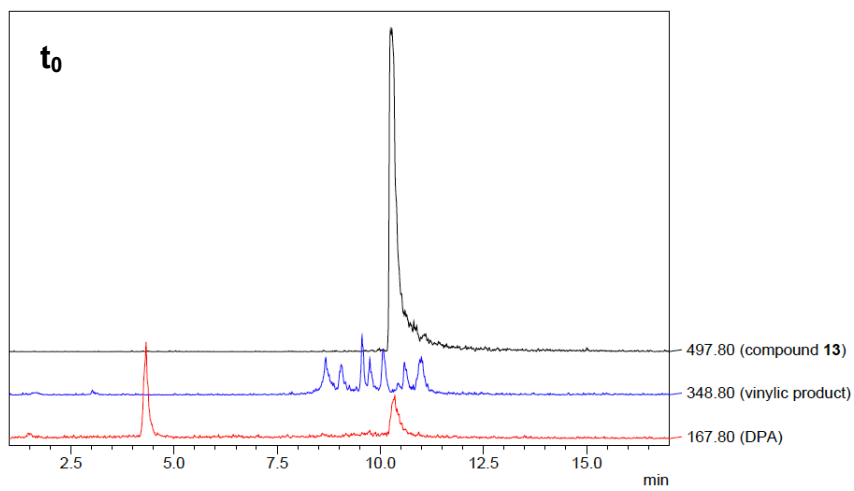
## Hydrolysis experiments

The hydrolysis of the conjugates was evaluated using  $^1\text{H}$  NMR and LC-MS. For the NMR experiment, the mixture consisted of 582  $\mu\text{L}$  HEPES buffer (20 mM in  $\text{D}_2\text{O}$ ), 6  $\mu\text{L}$   $\text{ZnSO}_4$  (50 mM in  $\text{D}_2\text{O}$ ) and 6  $\mu\text{L}$  compound **6** or **13** (100 mg/mL in  $\text{DMSO-d}_6$ ). The reaction was initiated by the addition of 6  $\mu\text{L}$  NDM-1 (37.4  $\mu\text{M}$ ). Using a 500 MHz NMR, a  $^1\text{H}$  NMR spectrum was recorded every 5 minutes for 2 hours or until full hydrolysis was observed. Using presat settings, the peak for residual  $\text{H}_2\text{O}/\text{HDO}$  was suppressed to obtain a clearer spectrum.

The experiment was repeated in a similar fashion for LC-MS analysis using non-deuterated solvents. At different time-points samples were taken from the reaction mixture and diluted in acetonitrile (1:2 v/v) to quench the reaction by precipitation of the enzyme. After centrifugation for 4 minutes at 10,000 rpm, the supernatant was analyzed following the general procedure for LC-MS. Figures S1 and S2 show the conversion of the conjugates to the vinylic product and the release of 8TQ and DPA over time (Fig. S6 and S7).



**Figure S6.** LCMS traces of the hydrolysis of compound **6** by NDM-1 showing the conversion of the conjugate to its vinyl product and the release of 8TQ.



**Figure S7.** LCMS traces of the hydrolysis of compound **13** by NDM-1 showing the conversion of the conjugate to its vinylic product and the release of DPA.

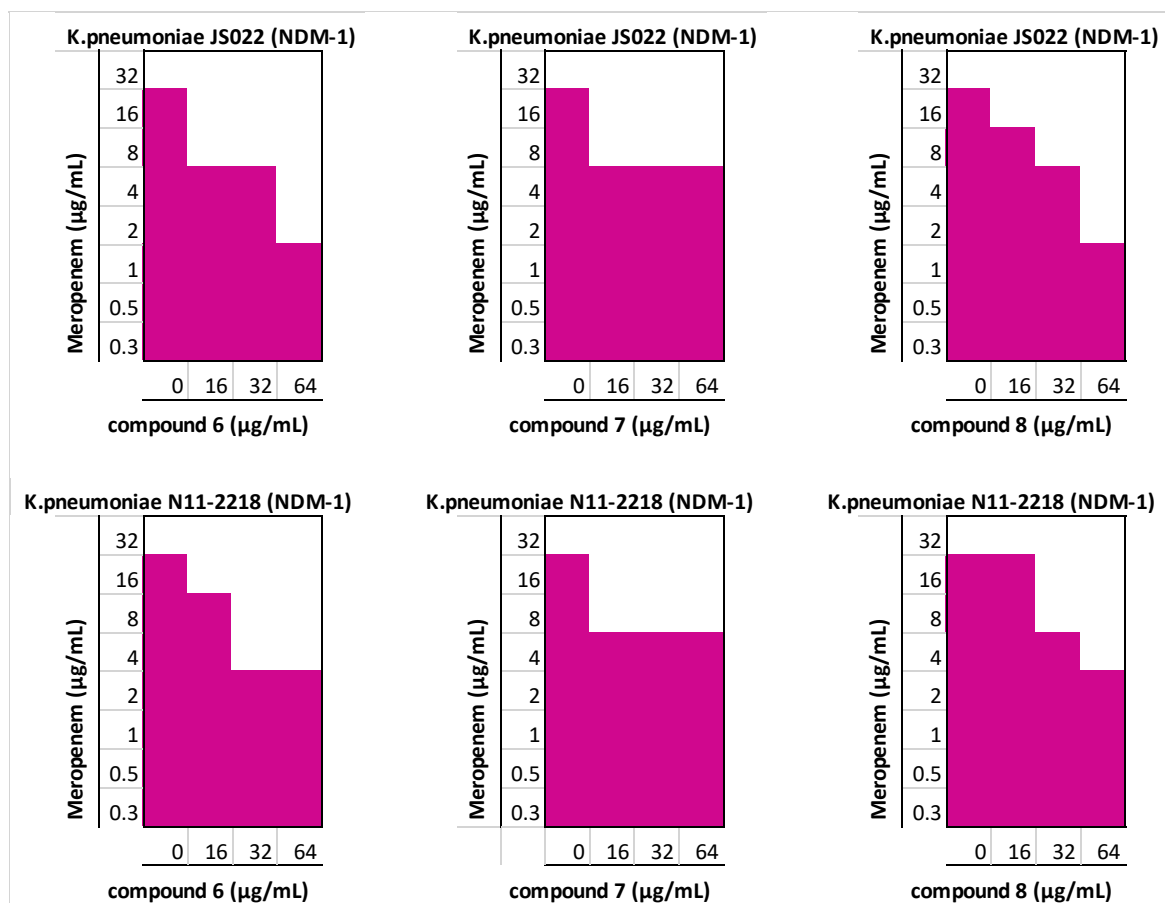
## Bacterial growth inhibition assays

The antibacterial assays were performed according to the guidelines published by the clinical and laboratory standards institute (CLSI). On a polypropylene 96-well plate, test compounds were serially diluted in Mueller-Hinton broth (MHB). In the same day, 2-5 colonies of the bacterial strain of interest were suspended in tryptic soy broth (TSB) and incubated with shaking at 37 °C until an OD<sub>600</sub> of 0.5. The bacterial suspension was diluted in MHB to reach 10<sup>6</sup> CFU/mL and added to the microplate containing the test compounds. After incubation with shaking at 37°C for 15-20 h, the microplates were inspected for bacterial growth, either visually or spectrophotometrically (absorption at 600 nm). The MIC was defined as the lowest concentration of the compound that prevented the visible growth of the bacteria. The MIC determination was performed in triplicate.

First, the MICs of meropenem in combination with compounds **6-8** were determined against two representative NDM-1 producing clinical isolates of *K. pneumoniae*. Meropenem was serially diluted in Mueller-Hinton broth (MHB) and the compounds were screened in 2-fold dilution steps at final concentrations of 0, 16, 32 and 64 µg/mL in MHB. The results are presented in figure S8 below. As no significant difference in activity was observed for compounds **6-8**, compound **6** was selected for further evaluation against clinical isolates of *K. pneumoniae*, *E. coli* and *P. aeruginosa* expressing either NDM, VIM or IMP-type MBLs. Compound **6** was initially tested at a fixed concentration of 64 µg/mL and for the strains for which synergy was established (FIC <0.5), representative strains for each type of MBL were selected and compound **6** was screened against those strains in different concentrations. The results are presented in figure S9.

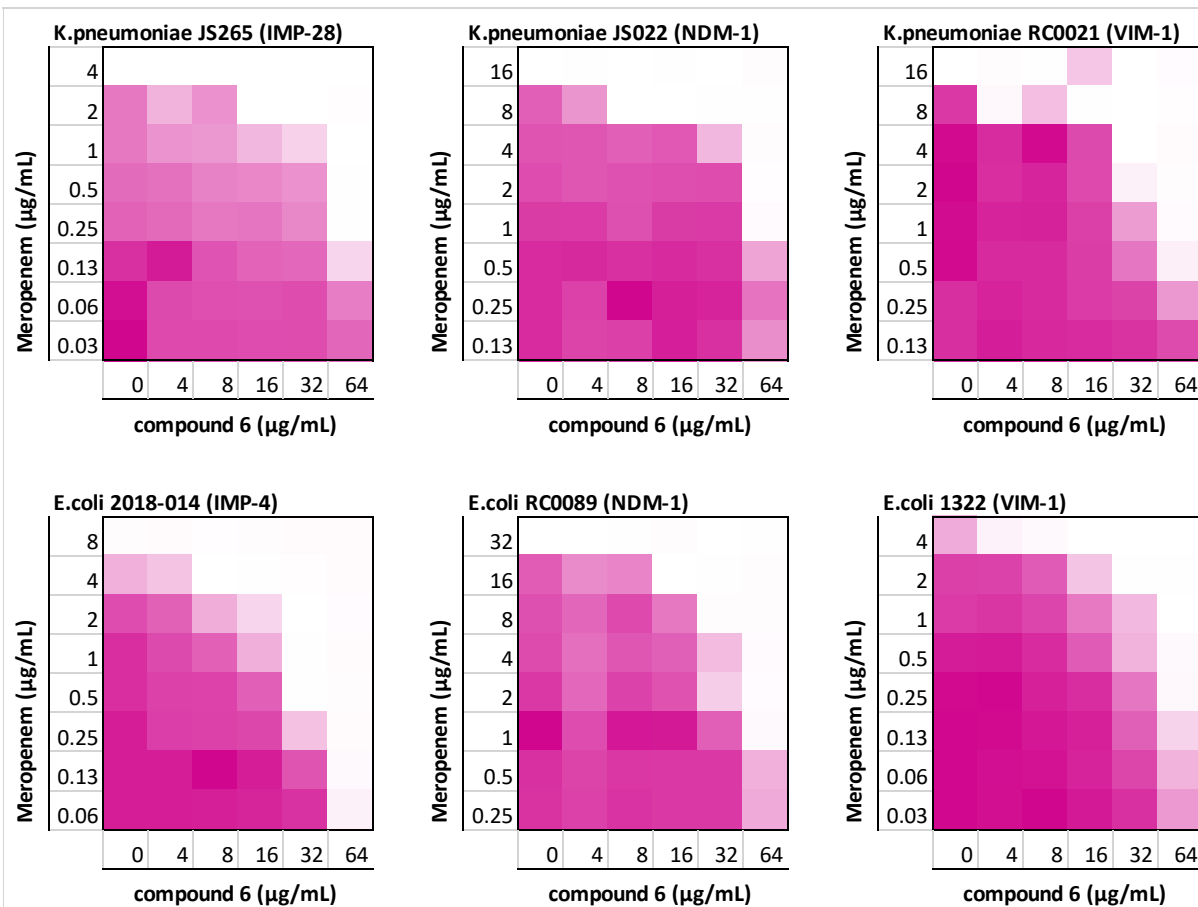
**Table S3.** Activity of compounds **6-8** against meropenem sensitive strains and NDM-1 positive isolates

	MIC (µg/mL) Meropenem sensitive strains			MIC (µg/mL) NDM-1 positive clinical isolates			
	<i>E. coli</i> (25922)	<i>K. pneumoniae</i> (11228)	<i>P. aeruginosa</i> (27853)	<i>E. coli</i> (2018-015)	<i>K. pneumoniae</i> (JS022)	<i>K. pneumoniae</i> (N11-2218)	<i>P. aeruginosa</i> (NRZ08418)
meropenem	<2	<2	<2	16	32	32	>128
<b>6</b>	128	32	>128	>128	>128	>128	>128
<b>7</b>	>128	16	>128	>128	>128	>128	>128
<b>8</b>	128	32	>128	>128	>128	>128	>128



**Figure S8.** Results of synergy assays of compounds 6-8 against NDM1-producing clinical isolates of *K.Pneumoniae*. Bacterial growth by visual inspection is indicated in magenta, no growth is indicated in white.

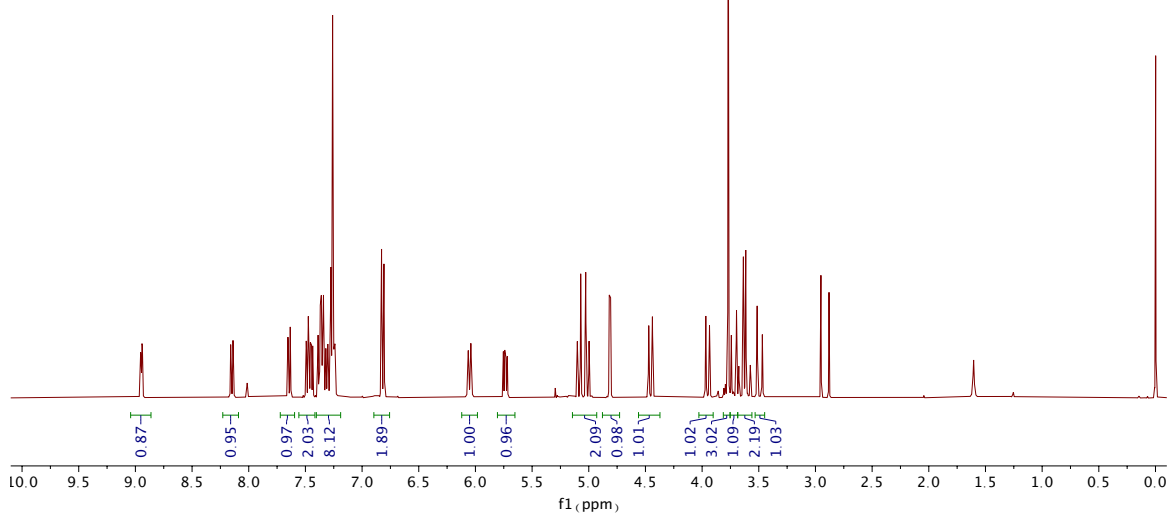
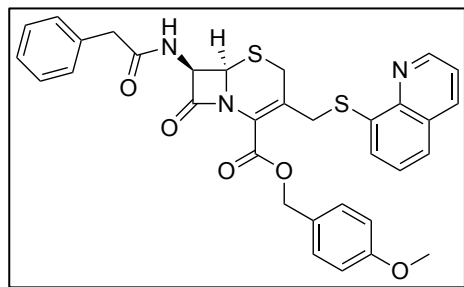




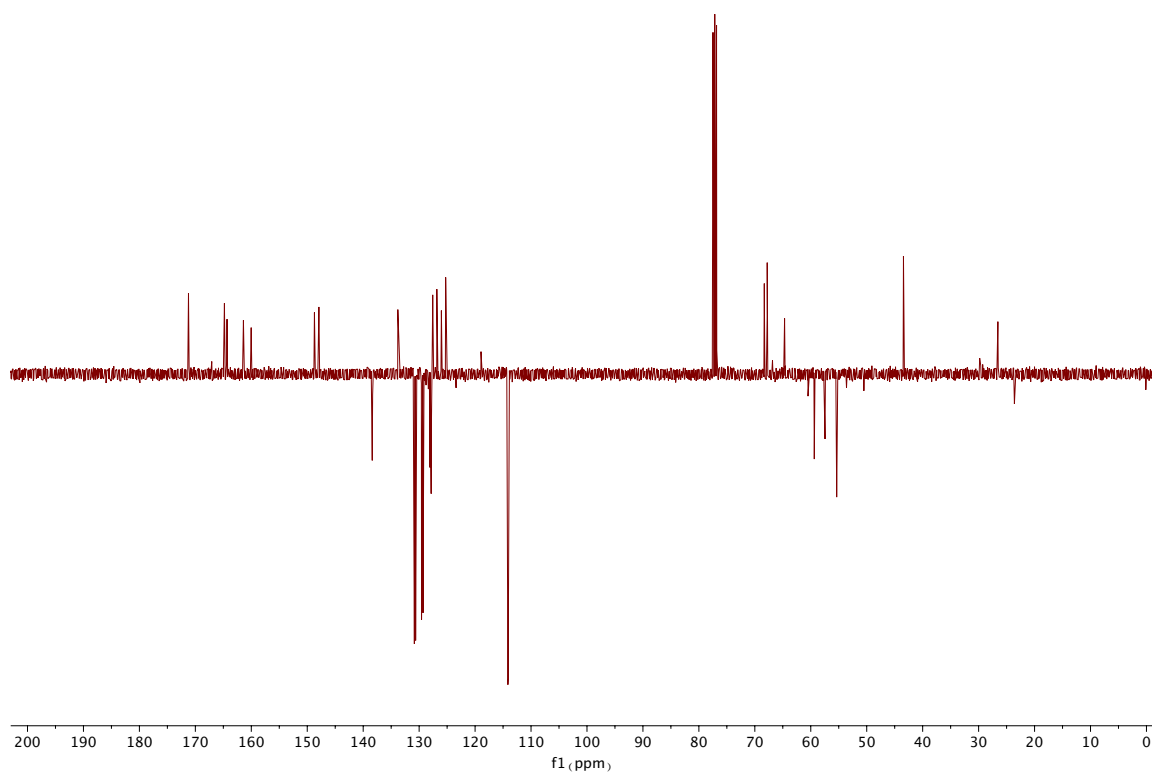
**Figure S9.** Results of synergy assays of compound 6 against IMP, NDM and VIM-producing clinical isolates of *K. Pneumoniae* and *E. Coli*. Bacterial growth was determined spectrophotometrically (absorption at 600 nm). Growth is indicated in magenta; no growth is indicated in white.

## NMR spectra

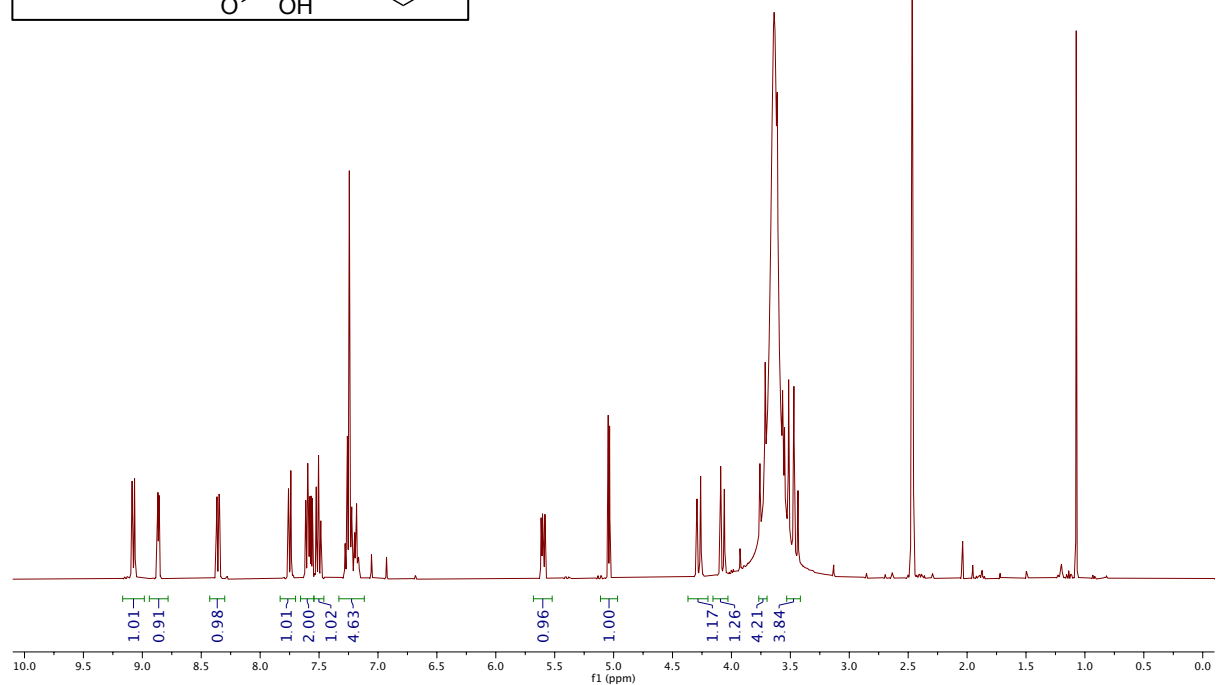
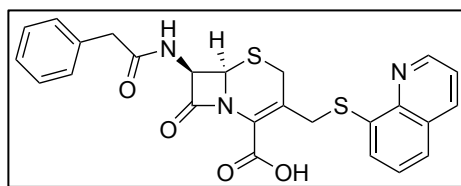
Compound **3** –  $^1\text{H}$  NMR  $\text{CDCl}_3$  (400 MHz)



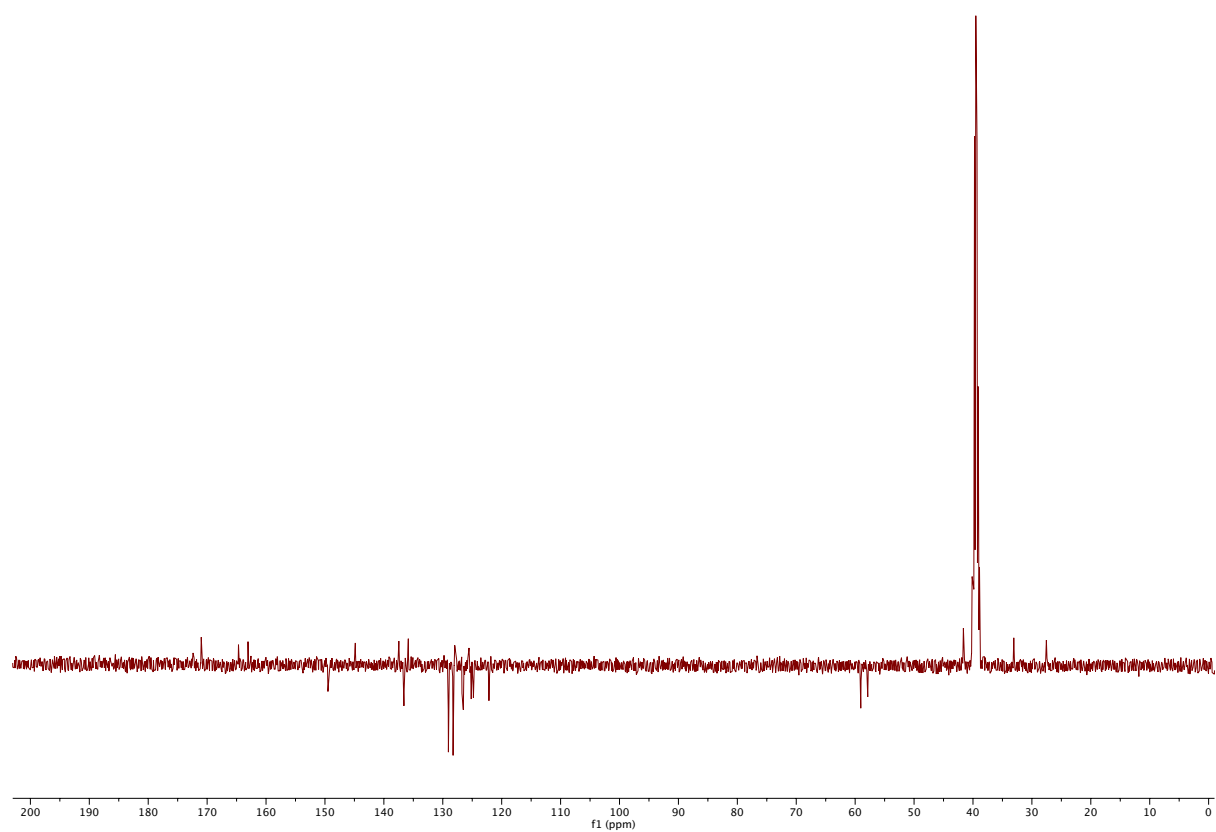
Compound **3** –  $^{13}\text{C}$  NMR  $\text{CDCl}_3$  (101 MHz)



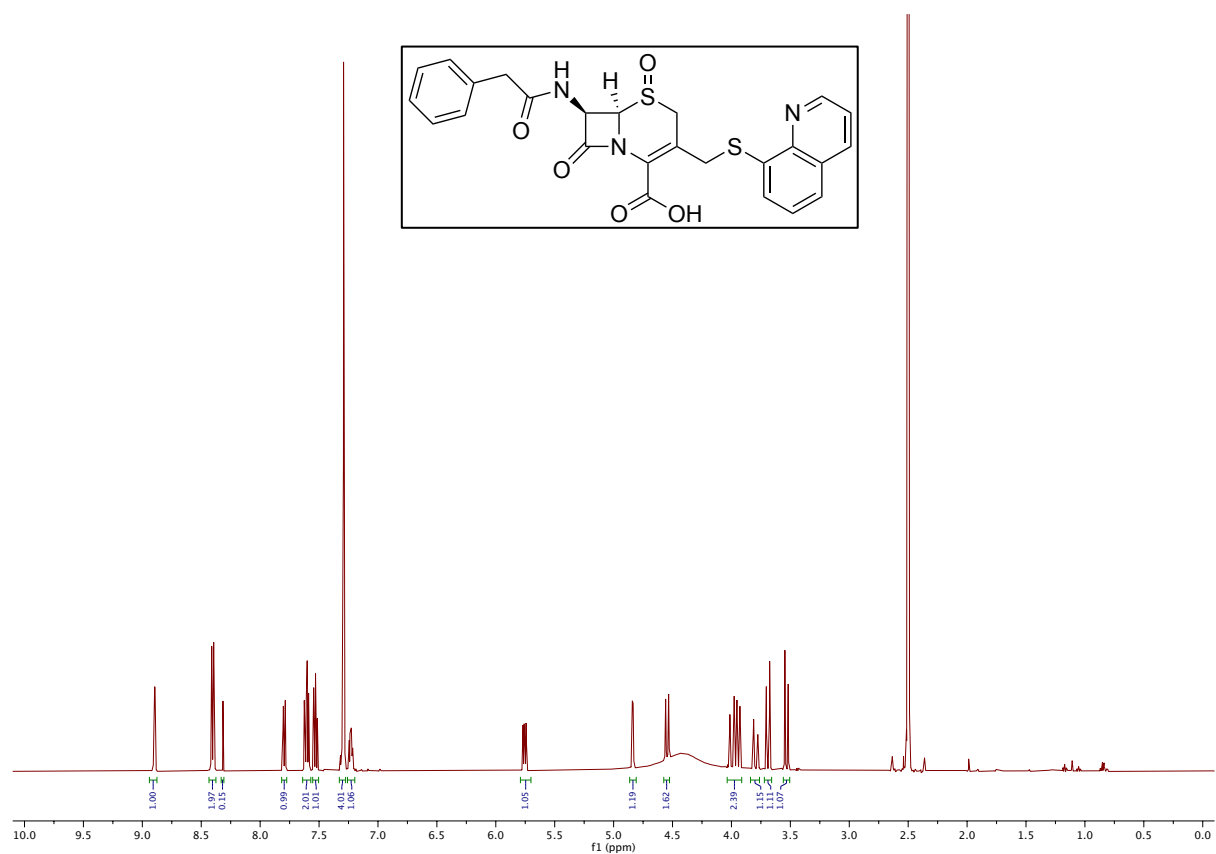
Compound **6** –  $^1\text{H}$  NMR DMSO- $d_6$  (400 MHz)



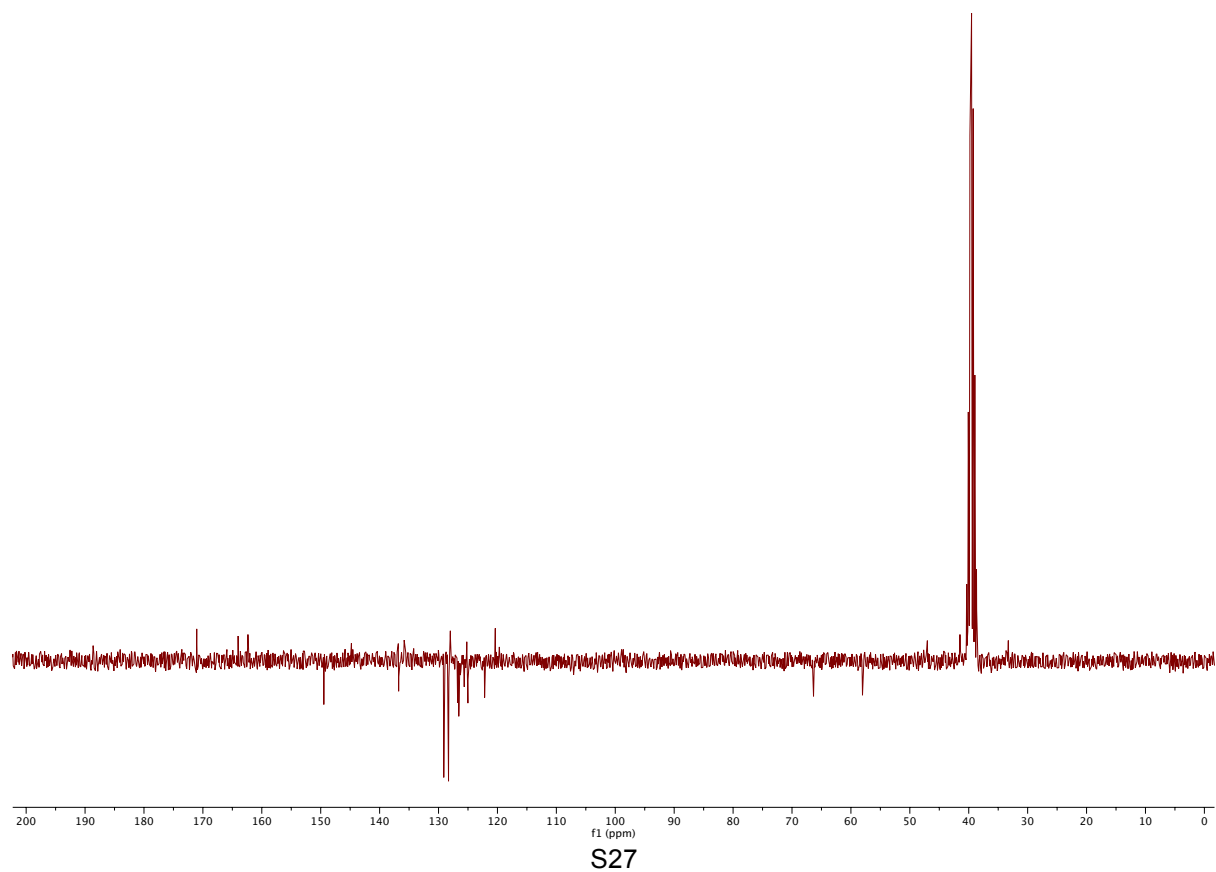
Compound **6** –  $^{13}\text{C}$  NMR DMSO- $d_6$  (101 MHz)



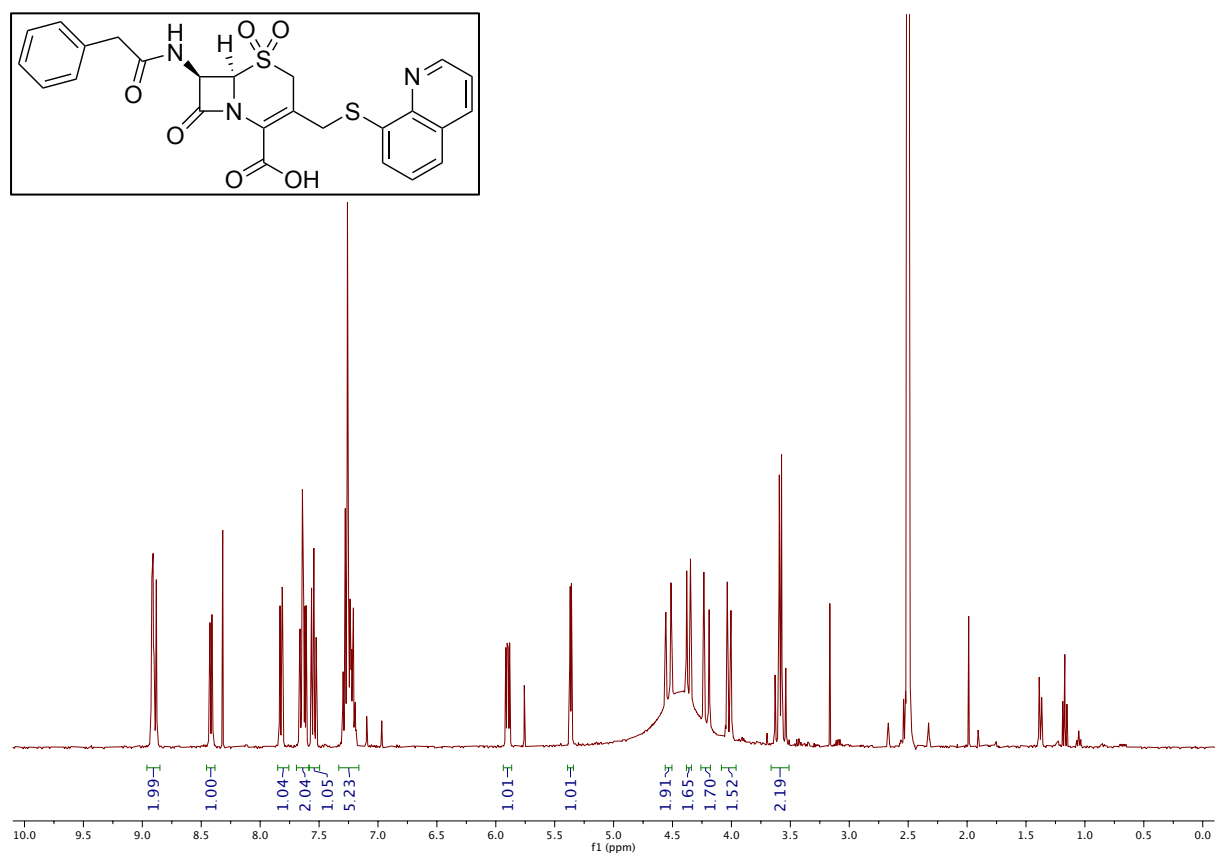
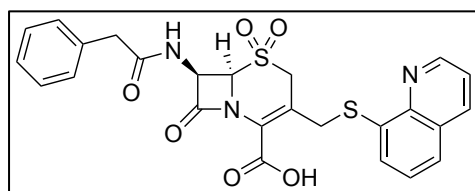
Compound 7 –  $^1\text{H}$  NMR DMSO- $d_6$  (500 MHz)



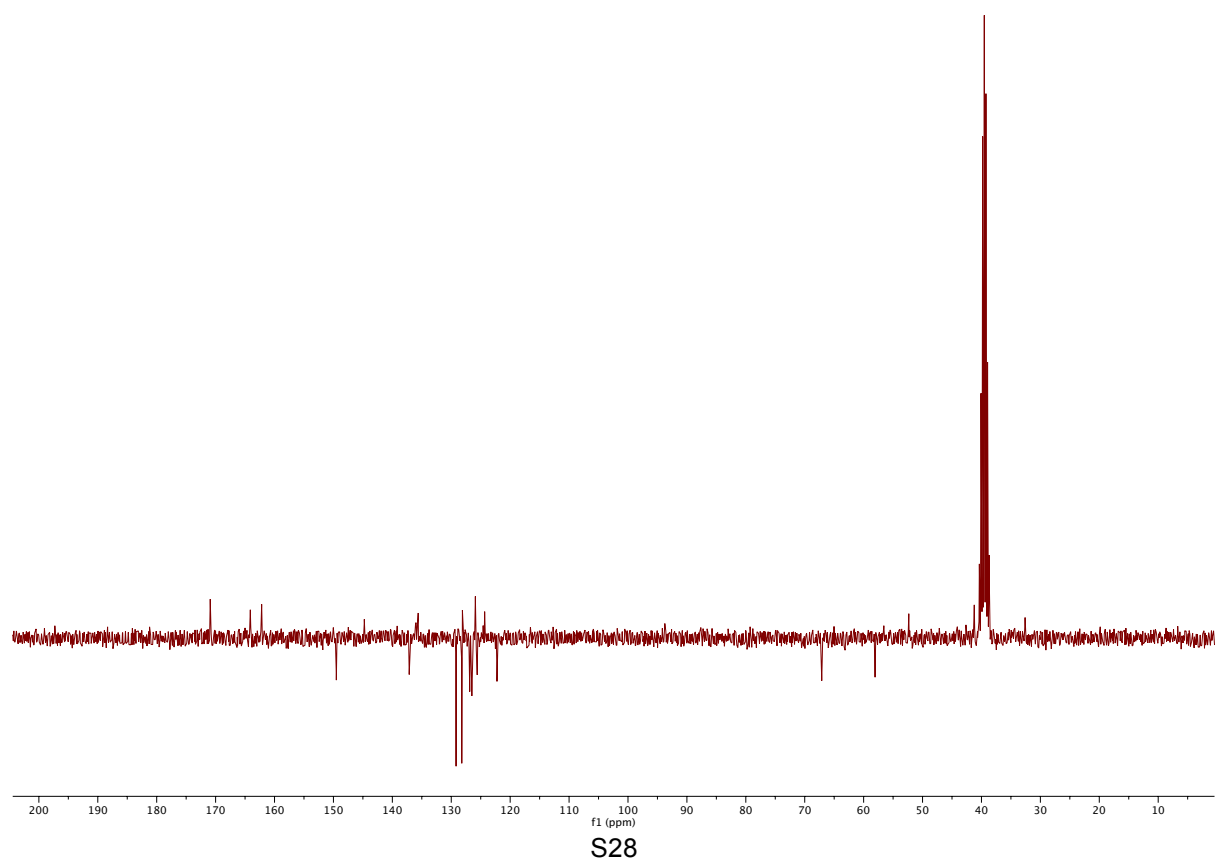
Compound 7 –  $^{13}\text{C}$  NMR DMSO- $d_6$  (75 MHz)



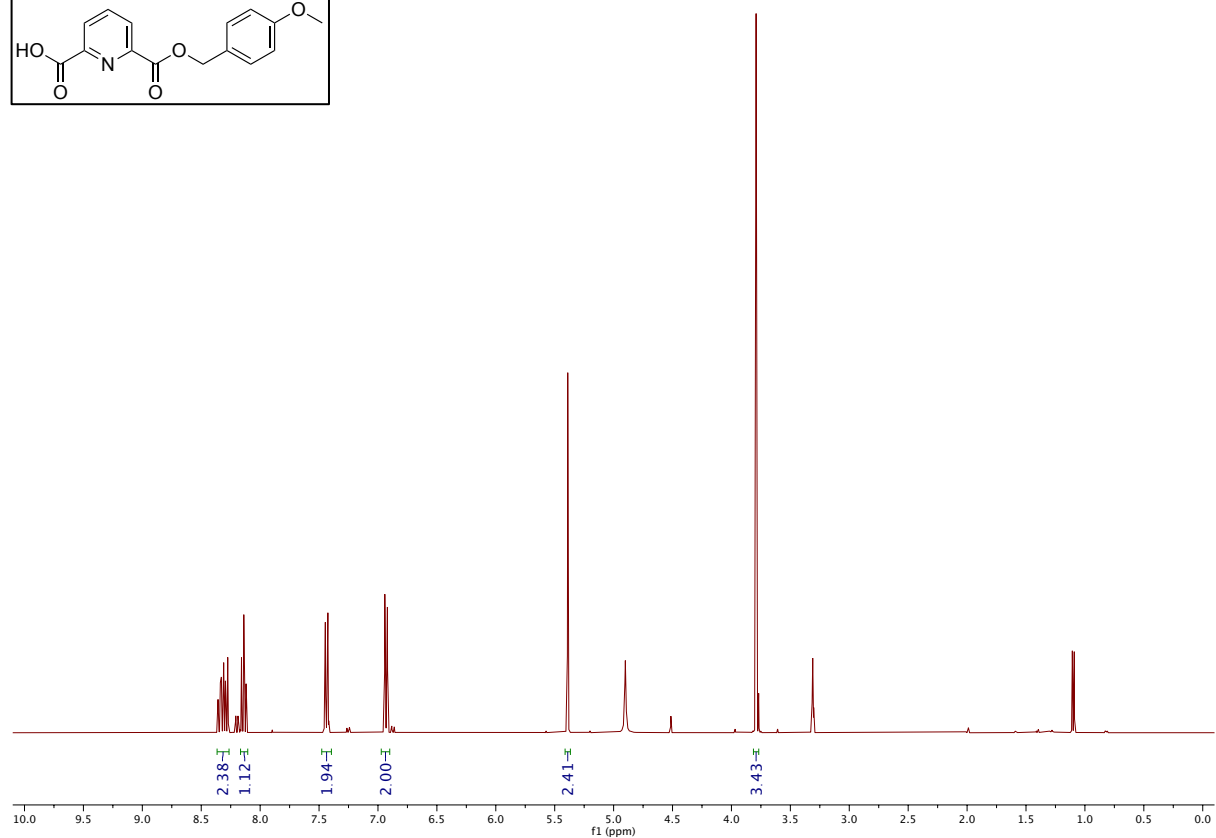
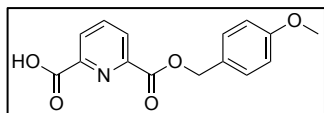
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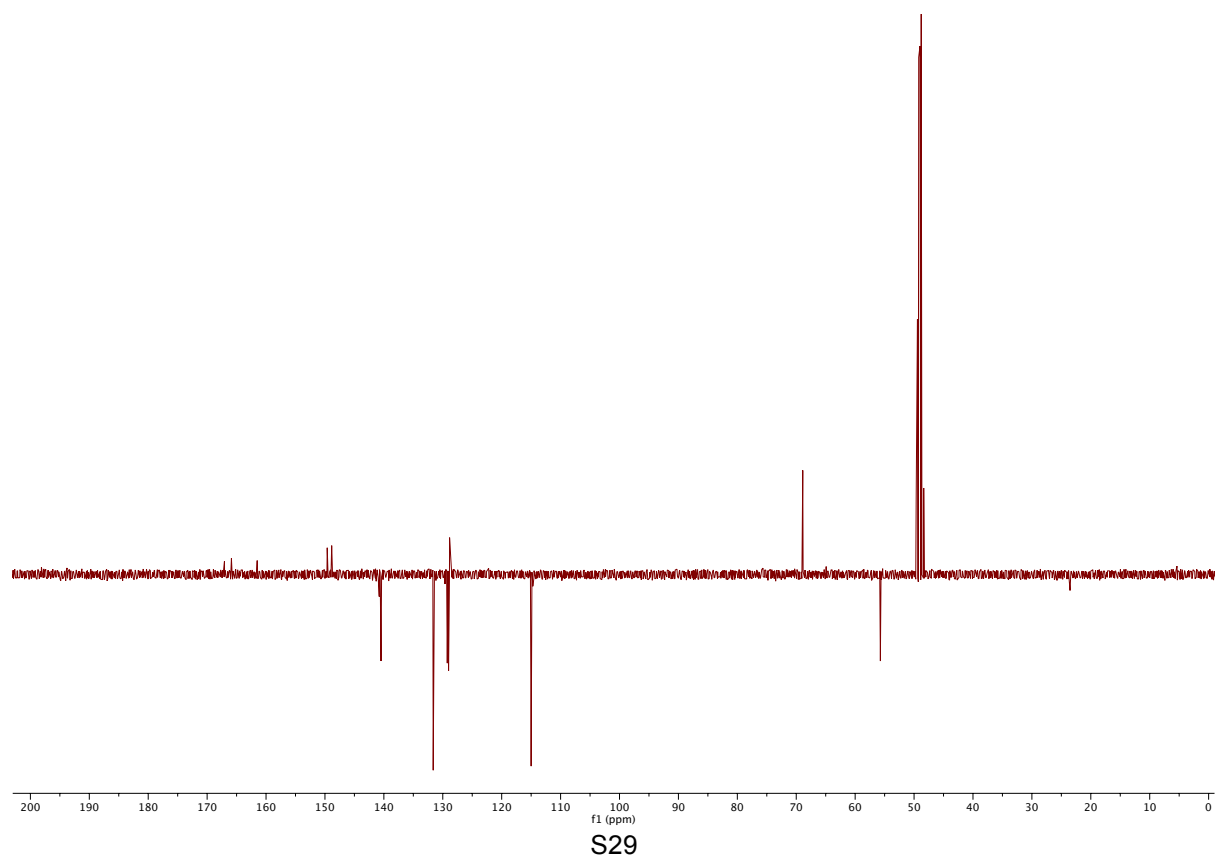
Compound **8** –  $^{13}\text{C}$  NMR DMSO- $d_6$  (101 MHz)



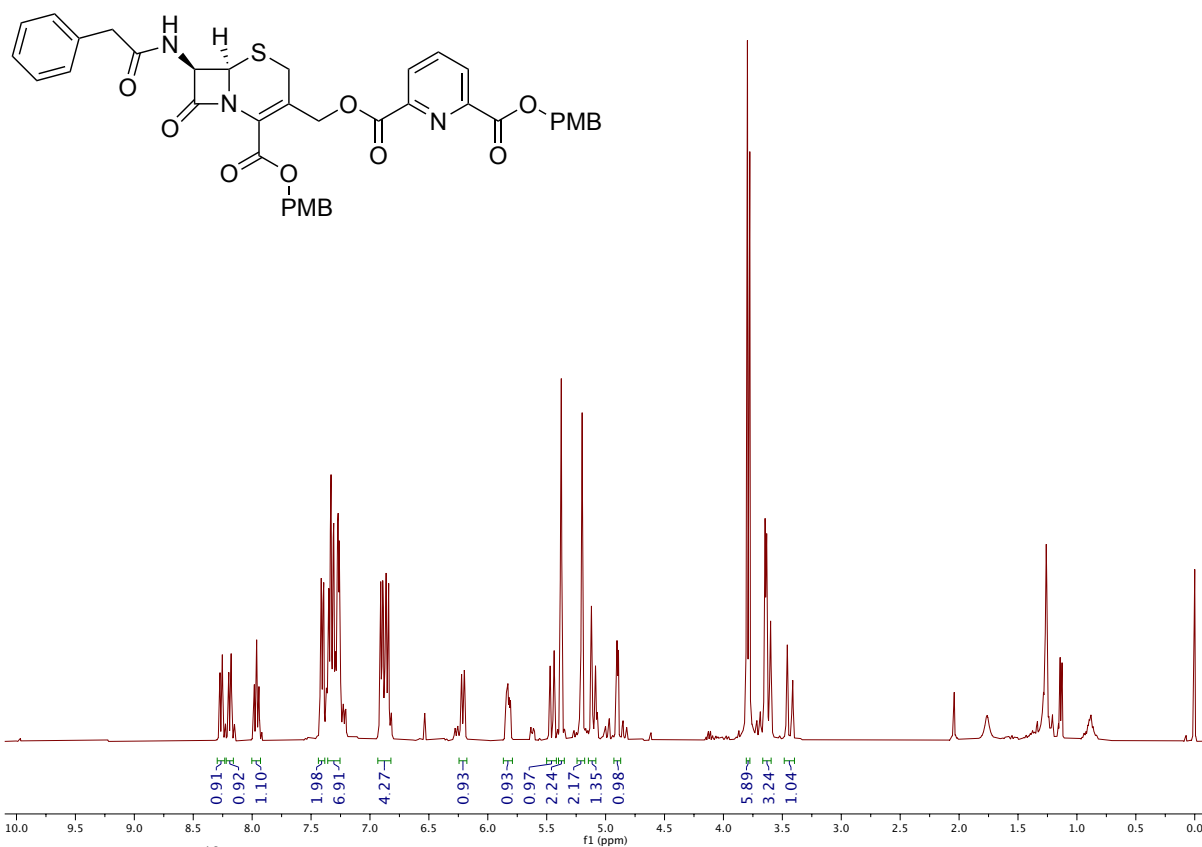
Compound **9** –  $^1\text{H}$  NMR  $\text{CD}_3\text{OD}$  (400 MHz)



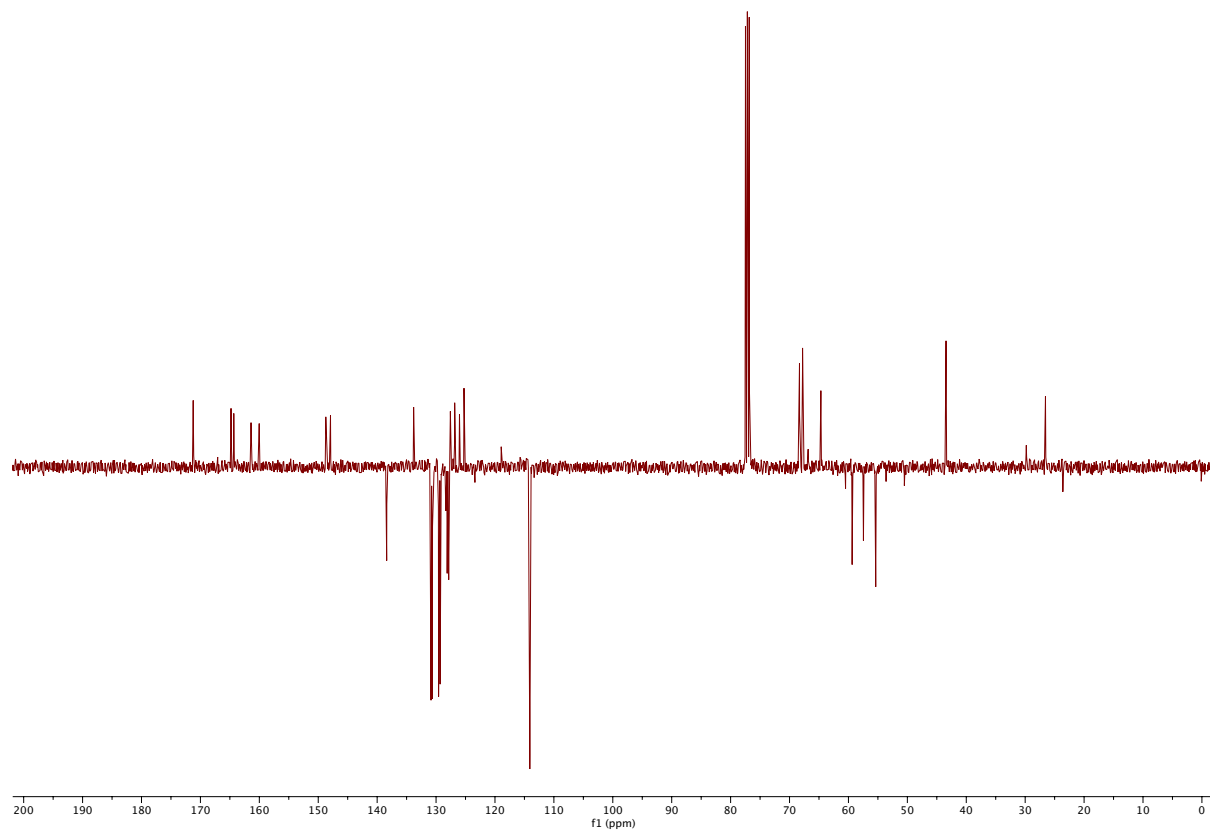
Compound **9** –  $^{13}\text{C}$  NMR  $\text{CD}_3\text{OD}$  (101 MHz)



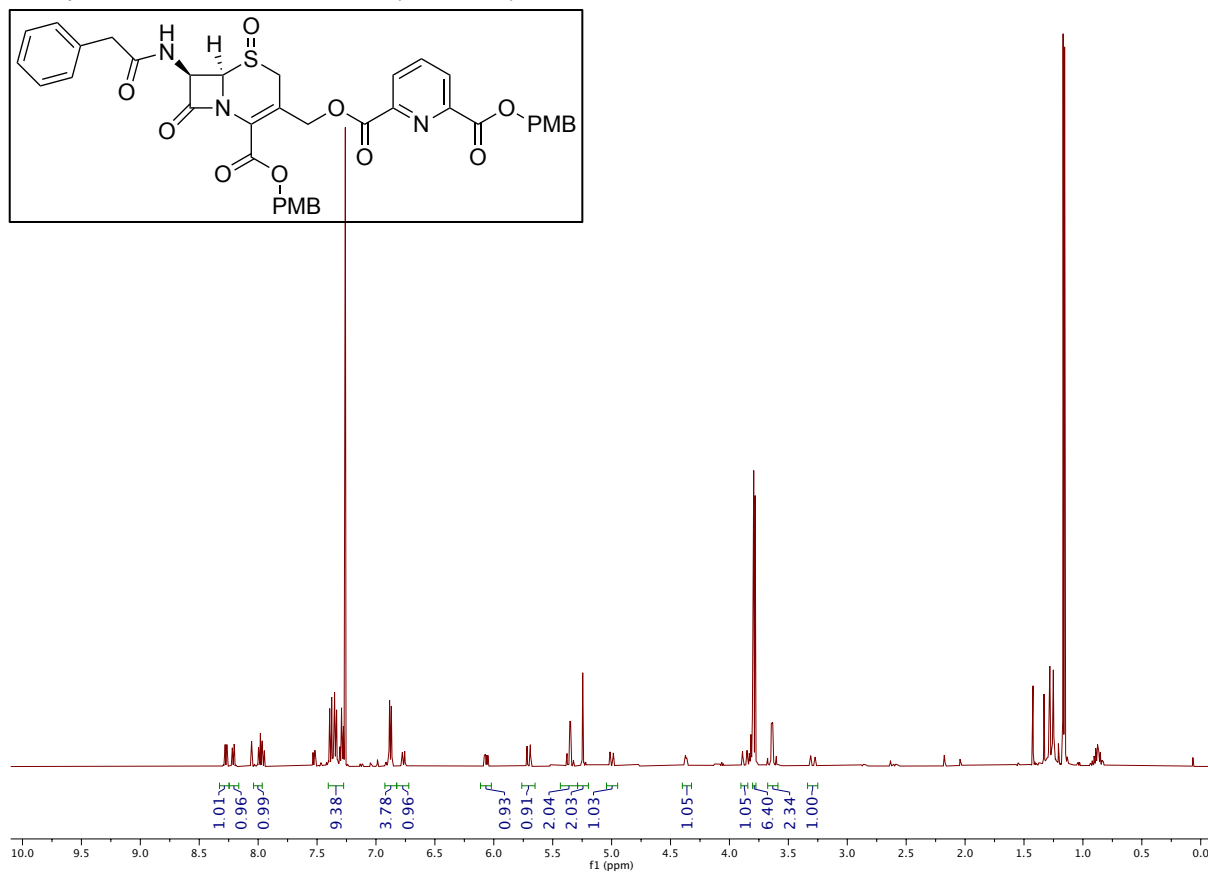
Compound **10** –  $^1\text{H}$  NMR  $\text{CDCl}_3$  (400 MHz)



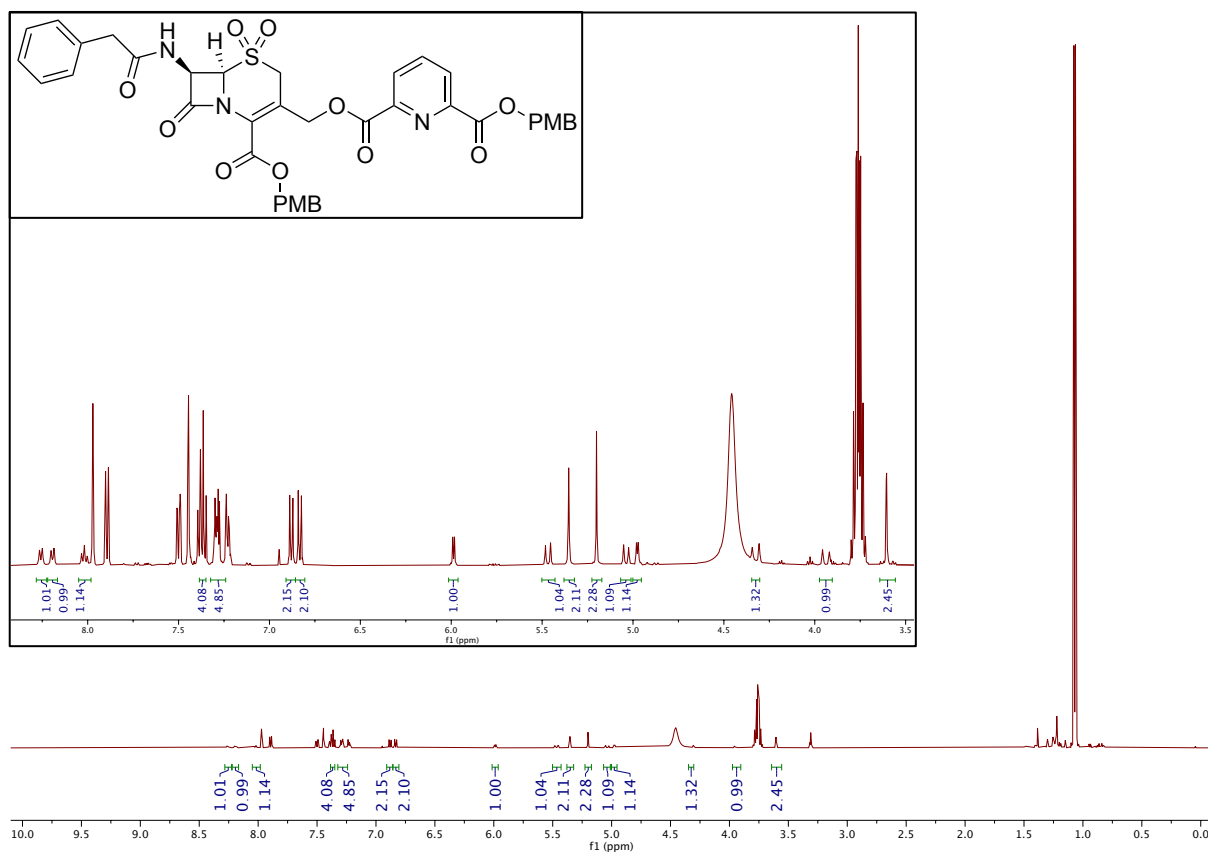
Compound **10** –  $^{13}\text{C}$  NMR  $\text{CDCl}_3$  (101 MHz)



Compound 11 – <sup>1</sup>H NMR CDCl<sub>3</sub> (400 MHz)

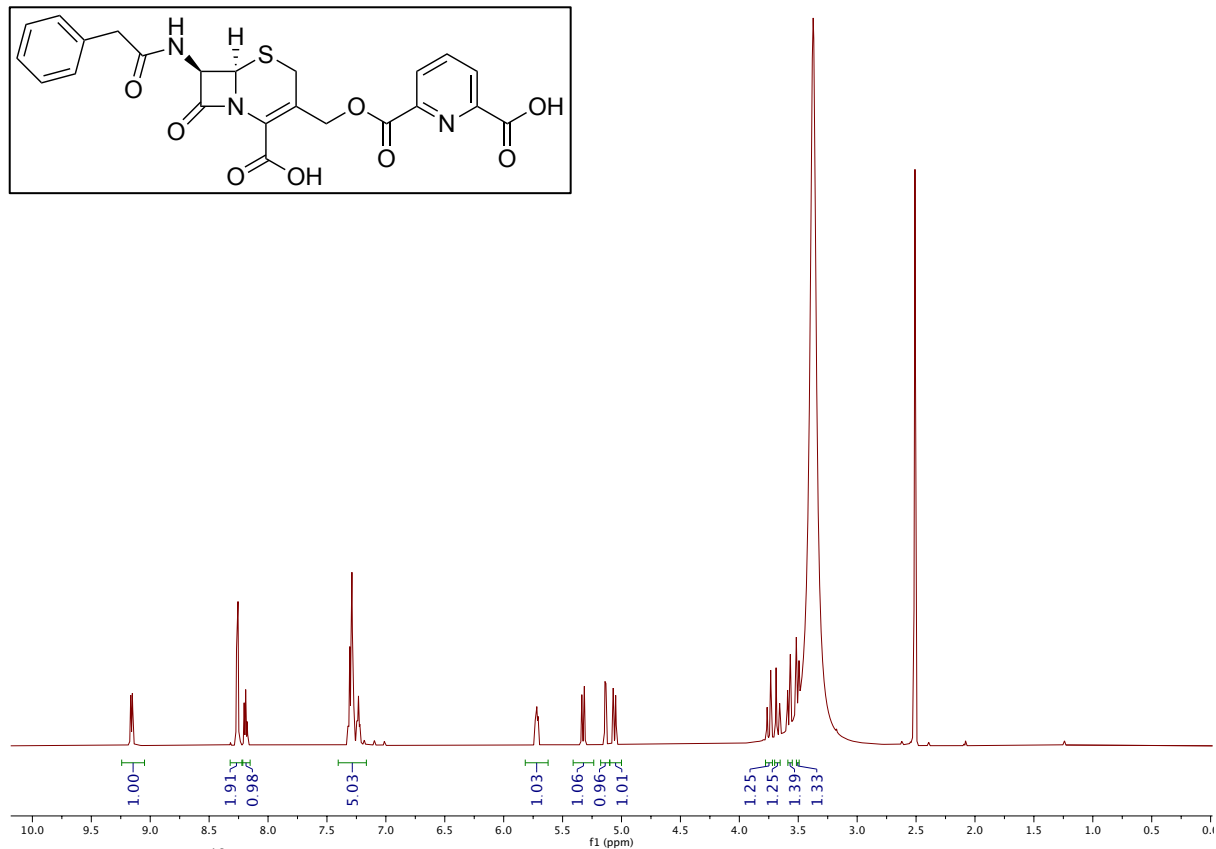
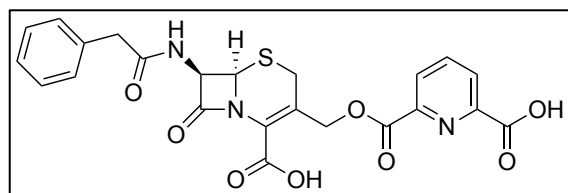


Compound 12 – <sup>1</sup>H NMR CDCl<sub>3</sub> (400 MHz)

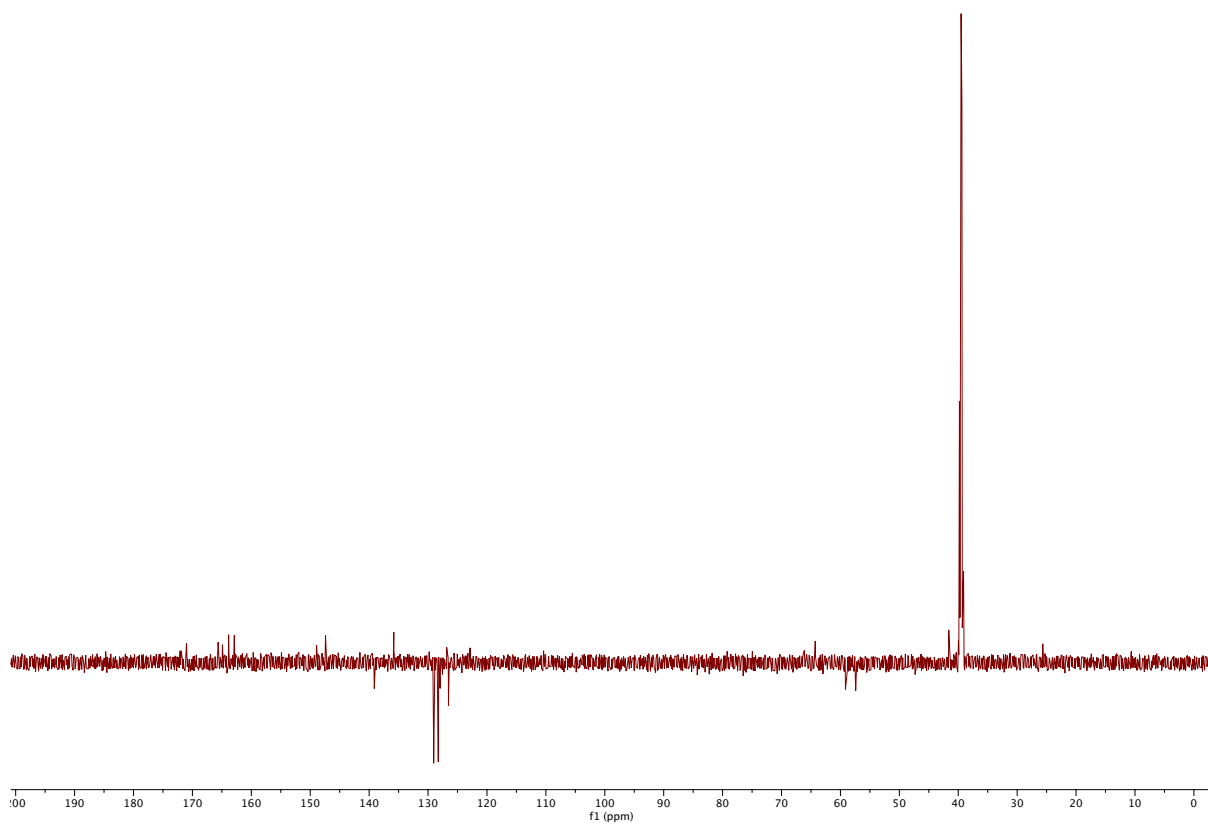




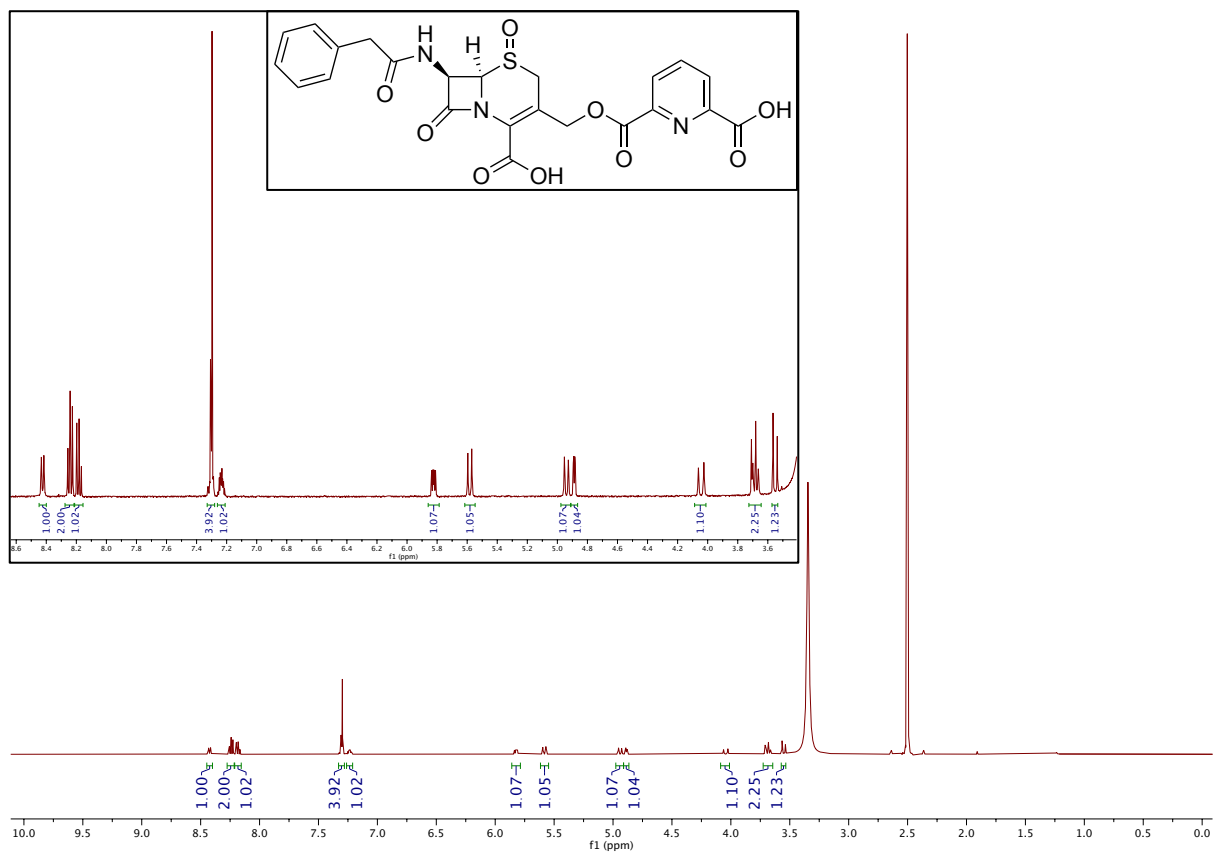
Compound **13** –  $^1\text{H}$  NMR  $\text{CDCl}_3$  (600 MHz)



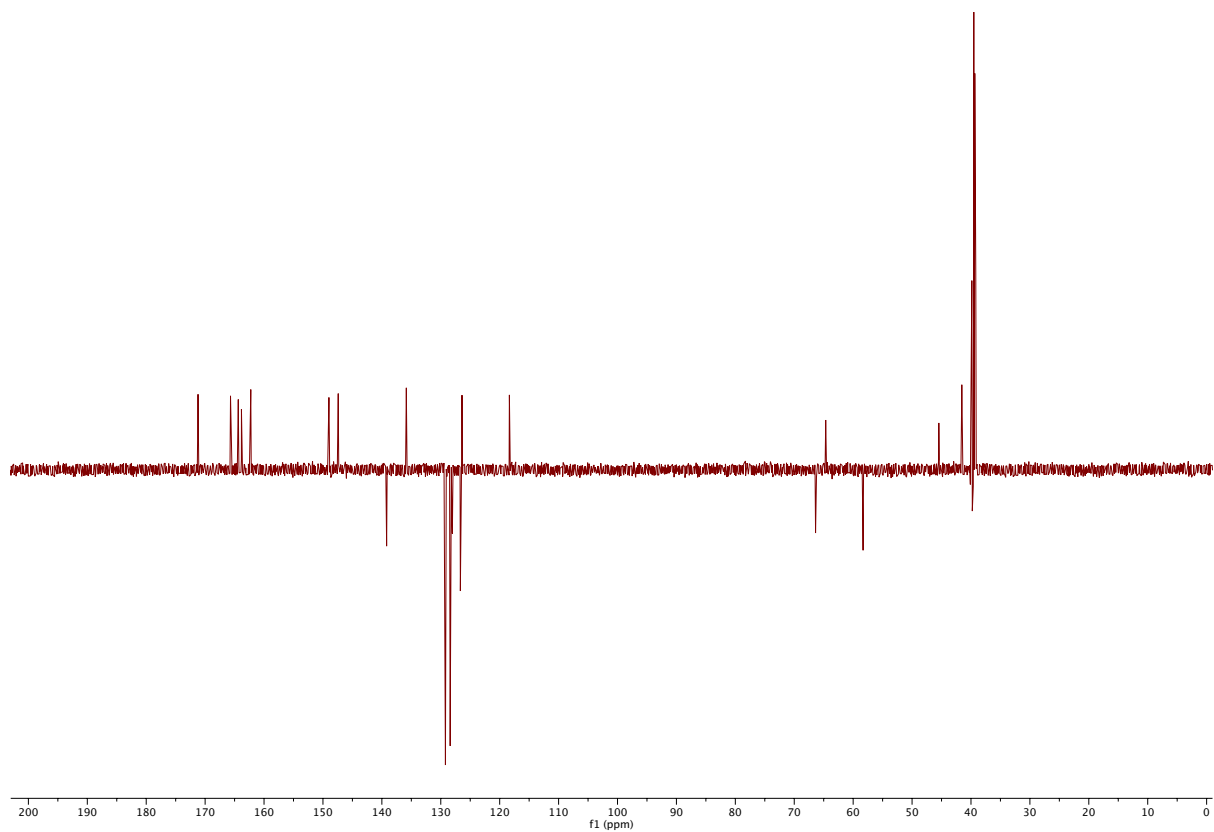
Compound **13** –  $^{13}\text{C}$  NMR  $\text{CDCl}_3$  (151 MHz)



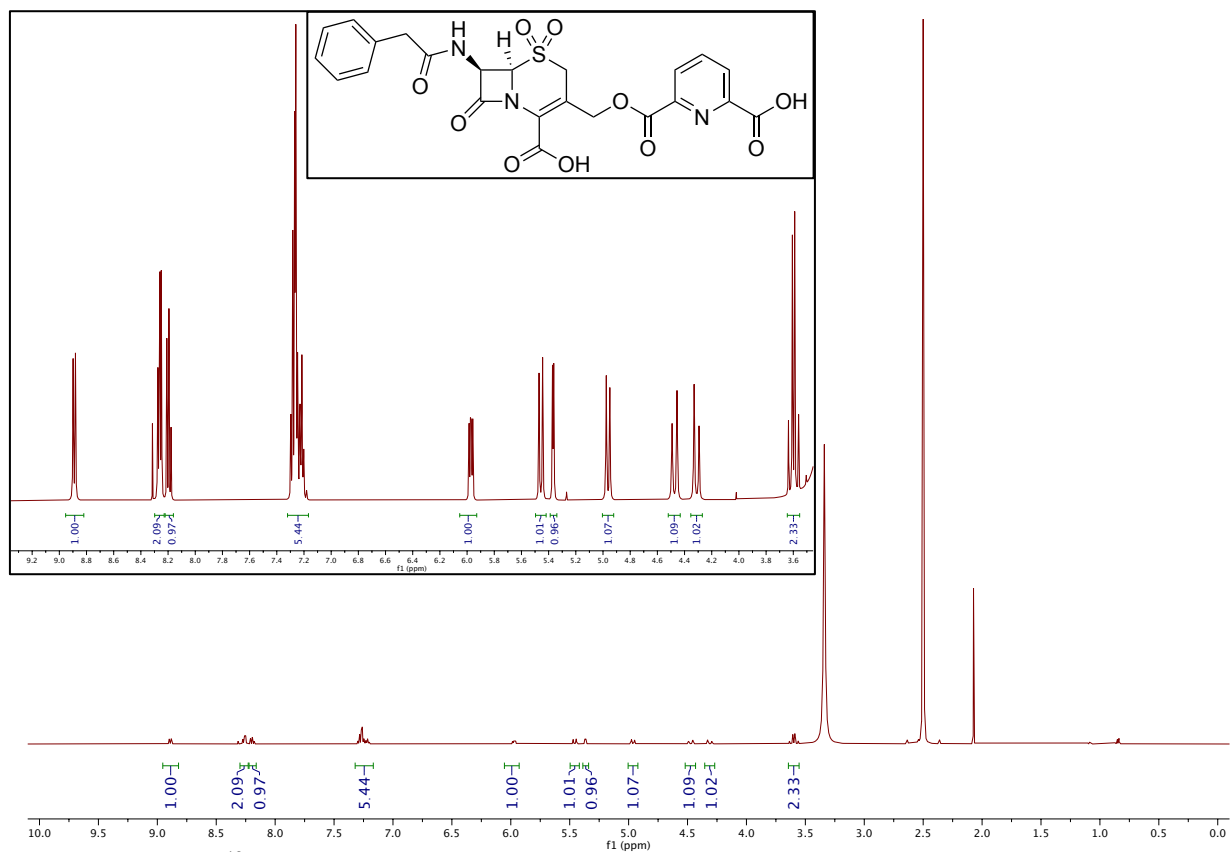
Compound **14** –  $^1\text{H}$  NMR DMSO- $d_6$  (500 MHz)



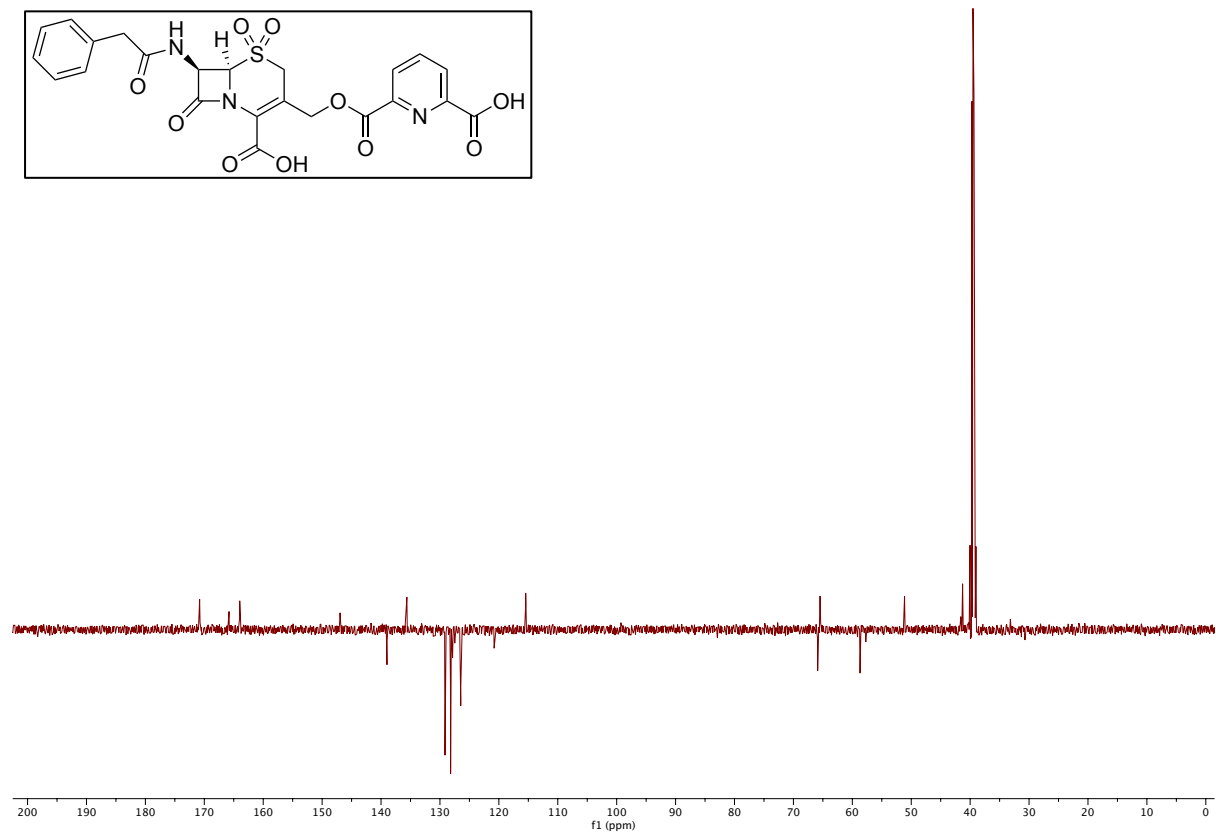
Compound **14** –  $^{13}\text{C}$  NMR DMSO- $d_6$  (126 MHz)



Compound **15** –  $^1\text{H}$  NMR DMSO- $d_6$  (500 MHz)



Compound **15** –  $^{13}\text{C}$  NMR DMSO- $d_6$  (126 MHz)



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