

Supporting Information

Syntheses and biological evaluation of new cephalosporin-oxazolidinone conjugates

Shanshan Yan,^a Marvin J. Miller,^{a*} Timothy A. Wencewicz^a and Ute Möllmann^b

^a*Department of Chemistry and Biochemistry, University of Notre Dame, Notre Dame, Indiana 46556, USA;*

^b*Leibniz Institute for Natural Products Research and Infection Biology – Hans Knoell Institute, Jena, Germany*

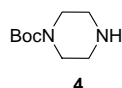
mmiller1@nd.edu

Table of Content

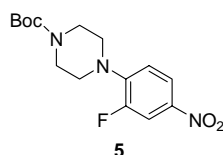
1. General Methods.....	S1
2. Experimental Details and Spectroscopic Data.....	S2
3. Description of Biological Assays.....	S9
4. Copies of ¹ H and ¹³ C NMR Spectra.....	S10

General Methods:

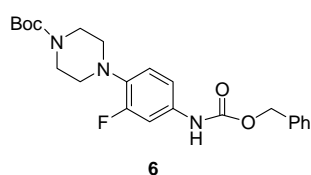
Commercial grade reagents and solvents were used without further purification except as indicated below. 7-Aminocephalosporanic acid (7-ACA) and 1,2,2,2-tetrachloroethyl chloroformate were purchased from Acros. Tetrahydrofuran (THF) was distilled from sodium and benzophenone. Reactions were carried out in oven- or flame-dried glassware under an atmosphere of dry argon only when specified in the experimental details. All reactions were magnetically stirred and monitored by analytical thin-layer chromatography using aluminum-backed 0.2 mm silica gel 60 F-254 plates. Visualization was accomplished by UV light (254 nm), and potassium permanganate. Flash chromatography was performed with silica gel 60 (230–400 mesh). ¹H NMR and ¹³C NMR spectra were recorded at ambient temperature with the residual solvent peaks as internal standards. The line positions of multiplets are given in ppm (δ) and the coupling constants (*J*) are given as absolute values in Hertz. All melting points were recorded uncorrected. High-resolution mass spectra (HRMS) data were obtained as specified. Yields refer to chromatographically and spectrographically pure compounds, unless otherwise noted.



***N*-Boc piperazine (4).** To a solution of piperazine (5.2 g, 60.37 mmol) in 100 mL of DCM at 0 °C was added a DCM (50 mL) solution of di-*tert*-butyldicarbonate (6.59 g, 30.19 mmol) dropwise. Then the mixture was stirred at 0 °C for an additional 1 h and filtered. The filtrate was concentrated under reduced pressure. Water (75 mL) was added to the resulting oil and filtered. The filtrate was saturated with potassium carbonate, and then extracted with diethyl ether (3 x 50 mL). The combined organic solvent was dried over Na₂SO₄ and filtered. The solvent was removed under reduced pressure to give **4** as a yellow solid (5.61 g, 50% yield). Spectral data is consistent with previously reported data.¹

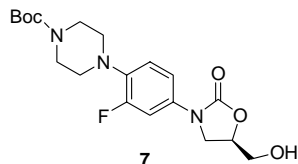


***tert*-Butyl 4-(2-fluoro-4-nitrophenyl)piperazine-1-carboxylate (5).** A solution of *N*-Boc piperazine **4** (5.18 g, 27.8 mmol), diisopropylethylamine (5.2 mL, 30.6 mmol), and 3,4-difluoronitrobenzene (3.7 mL, 33.4 mmol) in CH₃CN (100 mL) was stirred at room temperature for 2 days. Then the solvent was removed under reduced pressure. The resultant yellow solid was dissolved in 30 mL of DCM, and washed with H₂O (20 mL) and brine, dried over Na₂SO₄, filtered and concentrated by rotary evaporation to yield an orange solid. The solid was triturated with Et₂O to give **5** as an orange solid (4.65 g, 49% yield). mp: 153-155 °C; ¹H NMR (600 MHz, CDCl₃) δ 8.02-7.99 (m, 1 H), 7.93 (dd, *J* = 12.9, 2.6 Hz, 1 H), 6.93 (t, *J* = 8.7 Hz, 1 H), 3.62-3.56 (m, 4 H), 3.25-3.20 (m, 4 H), 1.49 (s, 9 H); ¹³C NMR (150 MHz, CDCl₃) δ 154.8, 154.2, 152.5, 145.7, 145.6, 141.1, 121.2, 121.1, 117.5, 117.4, 112.9, 112.7, 80.5, 49.8, 49.7, 28.6. HRMS (FAB) [*M* + Na]⁺ calcd for C₁₅H₂₀FN₃O₄Na, 348.1330, found 348.1314.

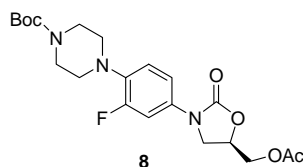


***tert*-Butyl 4-(4-(benzyloxycarbonyl)-2-fluorophenyl)piperazine-1-carboxylate (6).** A vigorously stirred solution of compound **5** (2.00 g, 6.15 mmol) in 15% aqueous THF (50 mL) previously purged with argon was charged with Pd/C (10% wt, 200 mg). The resulting mixture was stirred under an atmosphere of H₂. After 9 h, the mixture was filtered. The filtrate was cooled to 0 °C and treated with NaHCO₃ (2.06 g, 24.6 mmol), followed by benzyloxycarbonyl chloride (1.1 mL, 7.38 mmol). The mixture was stirred for an additional 1 h at 0 °C, then the solvent was removed under reduced pressure. The resultant aqueous suspension was extracted with EtOAc (3 x 15 mL). The combined organic layers were washed with brine, dried over Na₂SO₄, filtered, and concentrated by rotary evaporation. The crude product was purified by silica gel chromatography (1:10 to 1:5 EtOAc/hexanes) to afford **6** as a white solid (2.35 g, 89% yield for two steps). mp: 152-154 °C; ¹H NMR (300 MHz, CDCl₃) δ 7.42-7.35 (m, 5 H), 6.98 (dd, *J* = 8.6, 1.7 Hz, 1 H),

6.86 (t, $J = 8.8$ Hz, 1 H), 6.74 (s, 1 H), 5.19 (s, 2 H), 3.61-3.55 (m, 4 H), 2.99-2.93 (m, 4 H), 1.49 (s, 9 H); ^{13}C NMR (150 MHz, CDCl_3) δ 156.7, 155.4, 155.0, 154.9, 154.8, 153.6, 136.6, 136.1, 128.7, 128.6, 128.5, 128.4, 128.3, 128.1, 119.6, 119.5, 80.3, 80.0, 67.5, 67.1, 50.9, 28.6, 28.5; HRMS (FAB) $[\text{M} + \text{Na}]^+$ calcd for $\text{C}_{23}\text{H}_{28}\text{FN}_3\text{O}_4\text{Na}$, 452.1956, found 452.1947.

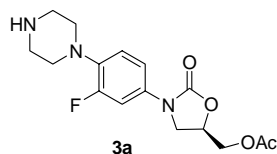


(R)-tert-Butyl-4-(2-fluoro-4-(5-(hydroxymethyl)-2-oxooxazolidin-3-yl)phenyl)piperazine-1-carboxylate (7). A stirred solution of compound **6** (1.00 g, 2.33 mmol) in anhydrous THF (50 mL) was cooled to -78 °C, and treated with *n*-BuLi (1.07 mL, 2.35 mmol, 2.19 M in THF) under Ar. After 20 min, the solution was treated with *R*-glycidyl butyrate (0.33 mL, 2.35 mmol) and then was warmed to room temperature. The mixture was stirred for an additional 3 h, then treated with saturated NH_4Cl (10 mL). The organic solvent was removed under reduced pressure. The resultant material was dissolved in 20 mL of DCM, and then separated. The organic layer was washed with water and brine, dried over Na_2SO_4 , filtered, and concentrated to yield an amber solid. The solid was purified by silica gel chromatography (1:1 to 2:1, EtOAc/hexanes) to afford **7** as an off-white solid (0.654 g, 71% yield). mp: 130-131 °C; ^1H NMR (300 MHz, CDCl_3) δ 7.44 (dd, $J = 14.2, 2.5$ Hz, 1 H), 7.11 (dt, $J = 8.7, 1.4$ Hz, 1 H), 6.91 (t, $J = 9.1$ Hz, 1 H), 4.78-4.70 (m, 1 H), 4.02-3.92 (m, 3 H), 3.74 (dd, $J = 12.8, 3.7$ Hz, 1 H), 3.61-3.55 (m, 4 H), 3.02-2.94 (m, 4 H), 1.48 (s, 9 H); ^{13}C NMR (75 MHz, CDCl_3) δ 157.1, 154.7, 153.9, 136.3, 136.2, 133.4, 133.3, 119.3, 119.2, 113.8, 107.5, 107.2, 79.9, 76.6, 72.8, 62.6, 50.6, 46.4, 29.6, 28.4; HRMS (FAB) $[\text{M}]^+$ calcd for $\text{C}_{19}\text{H}_{26}\text{FN}_3\text{O}_5$ 395.1856, found 395.1854.

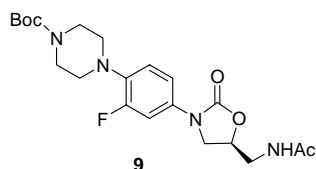


(R)-tert-Butyl-4-(4-(5-(acetoxymethyl)-2-oxooxazolidin-3-yl)-2-fluorophenyl)piperazine-1-carboxylate (8). To a solution of compound **7** (80 mg, 0.202 mmol) in 3 mL of anhydrous DCM was added pyridine (3 mL), DMAP (5 mg, 0.04 mmol) and Ac_2O (0.023 mL, 0.243 mmol) in order. The reaction mixture was stirred at room temperature for 4 h until TLC analysis indicated that compound **7** was consumed. The reaction mixture was concentrated to a slurry under reduced pressure and then EtOAc (5 mL) and 10% citric acid (5 mL) were added. The mixture was separated and the organic layer was washed with 10% citric acid (3 x 5 mL), water, and brine. The organic layer was dried over Na_2SO_4 , filtered, and concentrated to a white solid. The crude material was purified by silica gel chromatography (1:1, EtOAc/hexanes) to provide **8** as a white solid (84 mg, 95% yield). mp: 120-122 °C; ^1H NMR (600 MHz, CDCl_3) δ 7.45 (dd, $J = 14.1, 2.6$ Hz, 1 H), 7.13 (dd, $J = 8.8, 1.8$ Hz, 1 H), 6.94 (t, $J = 9.1$ Hz, 1 H), 4.91-4.83 (m, 1 H), 4.37 (dd, $J = 12.3, 3.8$ Hz, 1 H), 4.31 (dd, $J = 12.2, 5.1$ Hz, 1 H), 4.09 (t, $J = 9.0$ Hz, 1 H), 3.79 (dd, $J = 9.0, 6.3$ Hz, 1 H), 3.64-3.55 (m, 4 H), 3.04-2.95 (m, 4 H), 2.11 (s, 3 H), 1.49 (s, 9 H); ^{13}C NMR (150

MHz, CDCl₃) δ 170.8, 156.6, 155.0, 154.9, 154.2, 136.8, 136.7, 133.4, 133.3, 119.6, 119.5, 114.1, 114.0, 107.7, 107.5, 80.1, 70.1, 64.2, 50.9, 47.3, 28.6, 20.9; HRMS (FAB) [M + Na]⁺ calcd for C₂₁H₂₈FN₃O₆Na 460.1854, found 460.1862.

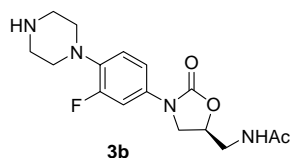


(R)-3-(3-Fluoro-4-(piperazin-1-yl)phenyl)-2-oxooxazolidin-5-ylmethyl-acetate (3a). To a DCM solution (anhydrous, 5 mL) of compound **8** (50 mg, 0.114 mmol) in a round-bottomed flask was added TFA (0.08 mL, 1.14 mmol) at once at room temperature. After that, the reaction mixture was stirred at room temperature for an additional 1h and 40 min until TLC analysis indicated that compound **8** was consumed. Then the reaction was diluted with CH₂Cl₂ (10 mL) and washed with saturated Na₂CO₃ solution until the pH value of the aqueous layer was 9. Then the aqueous layer was extracted with DCM (3 x 10 mL) and the combined organic solvent was washed with brine, dried over Na₂SO₄, filtered, and concentrated under reduced pressure to afford compound **3a** as a light yellow glass (35.5 mg, 92%). ¹H NMR (600 MHz, CDCl₃) δ 7.43 (dd, *J* = 14.2, 2.5, Hz, 1 H), 7.12 (dd, *J* = 8.8, 1.8 Hz, 1 H), 6.95 (t, *J* = 9.1 Hz, 1 H), 4.92-4.79 (m, 1 H), 4.37 (dd, *J* = 12.3, 3.8 Hz, 1 H), 4.30 (dd, *J* = 12.2, 5.1 Hz, 1 H), 4.09 (t, *J* = 9.0 Hz, 1 H), 3.79 (dd, *J* = 8.8, 6.2 Hz, 1 H), 3.08-3.05 (m, 4 H), 3.05-3.02 (m, 4 H), 2.11 (s, 3 H); ¹³C NMR (150 MHz, CDCl₃) δ 170.8, 156.6, 154.9, 154.2, 137.4, 137.3, 133.0, 132.9, 119.3, 119.2, 114.1, 114.0, 107.7, 107.5, 70.1, 64.3, 52.1, 52.0, 47.4, 46.3, 29.9, 20.9; HRMS (FAB) [M + H]⁺ calcd. for C₁₆H₂₁FN₃O₄ 338.1511, found 338.1529.



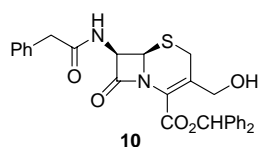
(S)-tert-Butyl 4-(4-(5-(acetamidomethyl)-2-oxooxazolidin-3-yl)-2-fluorophenyl)piperazine-1-carboxylate (9). A solution of compound **7** (100 mg, 0.253 mmol) in dry CH₂Cl₂ (15 mL) was cooled to 0 °C. The solution was treated with Et₃N (0.039 mL, 0.278 mmol) followed by methanesulfonyl chloride (0.022 mL, 0.278 mmol). After 0.5 h, the solution was washed with 10 mL of distilled H₂O followed by washing with brine. The organic layer was dried over Na₂SO₄, filtered, and was concentrated under reduced pressure to give a yellow solid (119 mg, 1.93 mmol). A mixture of the obtained solid (119 mg, 1.93 mmol) in 1:1:1 THF/2-propanol/14 M aqueous ammonium hydroxide solution (12 mL) was heated in a heavy walled sealed tube to 95 °C for 16 h. After this time the solvent was removed under reduced pressure. The remaining crude solid was dissolved in CH₂Cl₂ (40 mL), treated with pyridine (0.043 mL, 0.531 mmol) followed by Ac₂O (0.0263 mL, 0.278 mmol), and allowed to stir at 25 °C for 0.5 h. Then the reaction solution was washed with H₂O followed by brine, dried over Na₂SO₄, filtered, and concentrated under reduced pressure. Purification by silica gel chromatography (1.5-3.5% MeOH/CHCl₃) gave compound **9** as an off-white solid (68 mg, 61%). mp: 150-154 °C; ¹H NMR (600 MHz, CDCl₃) δ 7.43 (dd, *J* =

14.1, 2.6 Hz, 1 H), 7.09-7.03 (m, 1 H), 6.91 (t, $J = 9.1$ Hz, 1 H), 6.41-6.33 (m, 1 H), 4.77 (tt, $J = 9.0, 3.2$ Hz, 1 H), 4.02 (t, $J = 9.0$ Hz, 1 H), 3.75 (dd, $J = 9.1, 6.7$ Hz, 1 H), 3.71-3.65 (m, 1 H), 3.65-3.60 (m, 1 H), 3.60-3.56 (m, 4 H), 3.02-2.95 (m, 4 H), 2.02 (s, 3 H), 1.48 (s, 9 H); ^{13}C NMR (150 MHz, CDCl_3) δ 171.4, 156.5, 154.91, 154.89, 154.6, 136.8, 136.7, 133.3, 133.2, 119.5, 119.4, 114.08, 114.06, 107.8, 107.6, 80.13, 80.12, 72.1, 50.8, 47.8, 42.1, 28.6, 23.3; HRMS (FAB) $[\text{M} + \text{Na}]^+$ calcd for $\text{C}_{21}\text{H}_{29}\text{FN}_4\text{O}_5\text{Na}$ 459.2014, found 459.2032.



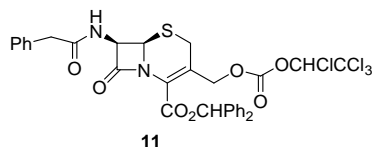
(S)-N-((3-(3-Fluoro-4-(piperazin-1-yl)phenyl)-2-oxooxazolidin-5-yl)methyl)acetamide (3b).

To a solution of compound **9** (0.058 g, 0.132 mmol) in anhydrous DCM (5 mL) was added TFA (0.099 mL, 1.33 mmol) at once at room temperature. After that, the reaction mixture was stirred at room temperature for an additional 1 h and 40 min until the TLC monitoring indicated that **9** was consumed. The reaction mixture was diluted with CH_2Cl_2 (10 mL) and washed with saturated Na_2CO_3 solution until the pH value of aqueous layer was 9. The water was extracted with DCM (3 x 10 mL) and the combined organic solvent was washed with brine, dried over Na_2SO_4 , filtered, and concentrated to afford compound **3b** as a light yellow glass (42 mg, 90%). ^1H NMR (600 MHz, CDCl_3) δ 7.42 (dd, $J = 14.4, 2.6$ Hz, 1 H), 7.06 (dd, $J = 8.8, 1.8$ Hz, 1 H), 6.98-6.86 (m, 1 H), 6.33-6.23 (m, 1 H), 4.83-4.69 (m, 1 H), 4.02 (t, $J = 9.0$ Hz, 1 H), 3.75 (dd, $J = 8.8, 6.7$ Hz, 1 H), 3.70 (ddd, $J = 14.7, 5.9, 2.9$ Hz, 1 H), 3.61 (dt, $J = 14.6, 6.1$ Hz, 1 H), 3.09-3.05 (m, 4 H), 3.04-3.01 (m, 4 H), 2.02 (s, 3 H); ^{13}C NMR (150 MHz, CDCl_3) δ 171.3, 156.5, 154.9, 154.6, 137.3, 132.9, 132.8, 119.3, 119.2, 114.1, 114.0, 107.8, 107.6, 72.1, 52.0, 47.9, 46.3, 42.2, 23.3; HRMS (FAB) $[\text{M} + \text{H}]^+$ calcd. for $\text{C}_{16}\text{H}_{21}\text{FN}_4\text{O}_3$ 337.1676, found 337.1670.

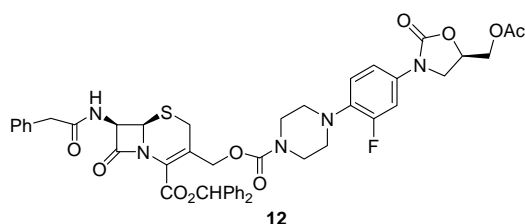


3-Hydroxymethylcephalosporin (10). To a stirred suspension of 7-aminocephalosporanic Acid (1.0 g, 3.67 mmol) in H_2O (5 mL) was added a 20% NaOH solution (0.5 mL) at 2-5 °C over 10 min. The reaction solution was adjusted to pH 8.5 with AcOH, and then diluted with acetone (3 mL). To this solution was added dropwise a solution of phenylacetyl chloride (0.6 mL, 4.4 mmol) in acetone (1 mL) at 0-5 °C at pH 7.5-8.5 adjusted by Et_3N . After stirring for one hour at 0-5 °C, the organic solvent was removed under reduced pressure. To the resulting aqueous solution was added ethyl acetate (5 mL), and the aqueous portion was acidified to pH 3.0 with 1M HCl while stirring. Then the organic layer was separated and the aqueous layer was further extracted with ethyl acetate (3 x 3 mL). The combined organic layers were washed with brine, dried over anhydrous MgSO_4 , and filtered. The filtrate was charged with a solution of freshly prepared diphenyldiazomethane¹ (0.72 g, 3.7 mmol) in ethyl acetate under stirring until the color of diphenyldiazomethane persisted. The reaction solution was concentrated and cooled in a refrigerator. The precipitate was collected by filtration and washed with ethyl acetate to give

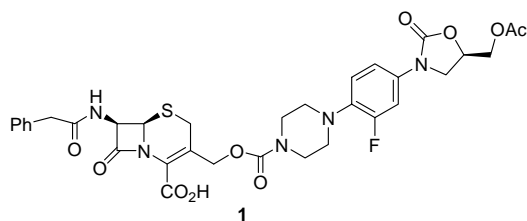
compound **10** as a white solid (550 mg, 29%). Spectral data was consistent with previously reported data.²



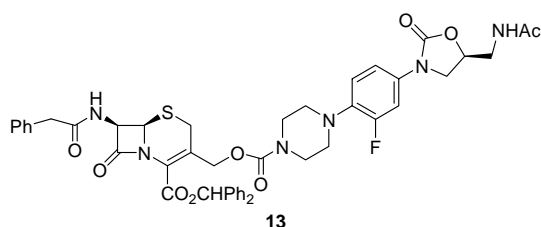
(6R,7R)-Benzhydryl-8-oxo-7-(2-phenylacetamido)-3-(((1,2,2,2-tetrachloroethoxy)carbonyloxy)methyl)-5-thia-1-aza-bicyclo[4.2.0]oct-2-ene-2-carboxylate (11). To a stirred suspension of compound **10** (150 mg, 0.292 mmol) in CH₂Cl₂ (15 mL) was added 1,2,2,2-tetrachloroethyl chloroformate (0.045 mL, 0.292 mmol) followed by the dropwise addition of pyridine (0.031 mL, 0.379 mmol) at 0 °C. The reaction mixture was stirred for 35 min and then diluted with CH₂Cl₂ (30 mL) and washed with ice cold 0.5 N HCl (10 mL) and cold water (10 mL). The organic layer was dried over anhydrous MgSO₄, filtered and concentrated. The residue was purified by flash column chromatography (1:2, EtOAc/hexanes) to give compound **11** as a white solid (184 mg, 87%). Spectral data was consistent with previously reported data.²



(6R,7R)-Benzhydryl-3-((1-(4-((R)-5-(acetoxymethyl)-2-oxooxazolidin-3-yl)-2-fluorophenyl)pi perazine-4-carboxyloxy)methyl)-8-oxo-7-(2-phenylacetamido)-5-thia-1-aza-bicyclo[4.2.0]oct-2-ene-2-carboxylate (12). To a solution of compound **11** (80 mg, 0.11 mmol) in anhydrous dichloromethane (5 mL) were added amine **3a** (62 mg, 0.166 mmol) of a solution in DCM (2 mL) and the mixture was stirred at rt for 4.5 h until TLC analysis indicated the consumption of **11**. Dichloromethane (15 mL) was added, and the organic layer was washed with brine and distilled water, separated, dried over anhydrous MgSO₄, filtered, and concentrated under reduced pressure to give an off white solid (45 mg). Purification by silica gel chromatography (1:2, 1:1, EtOAc/haxanes) to give compound **12** as a white solid (58 mg, 60%). mp: 116-117 °C; ¹H NMR (300 MHz, CDCl₃) δ 7.51-7.17 (m, 15 H), 7.05 (d, *J* = 8.0 Hz, 1 H), 6.94- 6.71 (m, 2 H), 6.22 (d, *J* = 8.8 Hz, 1 H), 5.80 (dd, *J* = 9.1, 5.0 Hz, 1 H), 5.14-4.97 (m, 1 H), 4.89 (d, *J* = 5.0 Hz, 1 H), 4.83-4.69 (m, 2 H), 4.26 (qd, *J* = 12.1, 4.4 Hz, 2 H), 4.12-3.92 (m, 1 H), 3.80-3.63 (m, 1 H), 3.57 (d, *J* = 4.1 Hz, 4 H), 3.47 (d, *J* = 18.8 Hz, 4 H), 3.40-3.21 (m, 1 H), 3.01-2.80 (m, 4 H), 2.15-1.95 (m, 3 H); ¹³C NMR (75 MHz, CDCl₃) δ 171.4, 170.7, 165.0, 160.9, 157.4, 154.8, 154.2, 139.4, 139.2, 136.3, 133.9, 133.7, 133.5, 129.7, 129.3, 129.0, 128.9, 128.7, 128.6, 128.4, 128.3, 128.0, 127.9, 127.8, 127.2, 126.9, 125.4, 119.7, 119.6, 114.0, 107.8, 107.4, 79.9, 70.2, 64.2, 64.1, 59.3, 57.5, 50.7, 47.2, 43.5, 29.9, 26.6, 20.8; ESI-MS: *m/z* = 878.2 [M + H]⁺.

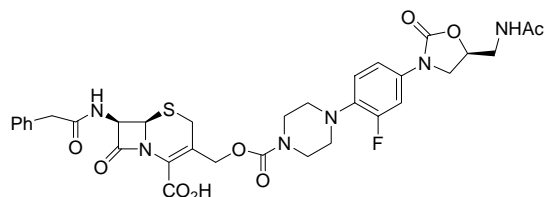


(6R,7R)-3-((1-(4-((R)-5-(Acetoxymethyl)-2-oxooxazolidin-3-yl)-2-fluorophenyl)piperazine-4-carboxyloxy)methyl)-8-oxo-7-(2-phenylacetamido)-5-thia-1-aza-bicyclo[4.2.0]oct-2-ene-2-carboxylic acid (1). To a suspension of **12** (40 mg, 0.046 mmol) in CH₂Cl₂ (1 mL) and anisole (0.1 mL) at 0 °C, was added TFA (0.04 mL, 0.54 mmol) dropwise. The resulting mixture was stirred at 0 °C for 0.5 h. Then the solvent was removed under reduced pressure. The residue was treated with CH₂Cl₂ (3 mL) and EtOAc (3 mL) separately and the resulting precipitate was removed by filtration. The filtrate was concentrated and the residue was treated with EtOAc (3 mL) and ether (3 mL) separately. The precipitate was filtered to give the compound **45** as an off white solid (9.2 mg, 28%). mp: > 136 °C (dec); ¹H NMR (500 MHz, DMSO-*d*₆) δ 9.11 (d, *J* = 8.4 Hz, 1 H), 7.50 (dd, *J* = 14.8, 2.4, Hz, 1 H), 7.32-7.25 (m, 5 H), 7.25-7.18 (m, 2 H), 7.08 (t, *J* = 9.4 Hz, 1 H), 5.69 (dd, *J* = 8.4, 4.8 Hz, 1 H), 5.10 (d, *J* = 5.0 Hz, 1 H), 5.04 (d, *J* = 13.0 Hz, 1 H), 4.96-4.86 (m, 1 H), 4.71 (d, *J* = 13.0 Hz, 1 H), 4.28 (d, *J* = 3.0 Hz, 1 H), 4.25 (d, *J* = 5.6 Hz, 1 H), 4.12 (t, *J* = 9.2 Hz, 1 H), 3.80 (dd, *J* = 9.2, 6.6 Hz, 1 H), 3.68-3.60 (m, 1 H), 3.60-3.55 (m, 1 H), 3.51 (s, 4 H), 3.48 (s, 1 H), 2.99 (s, 1 H), 2.95 (t, *J* = 4.7 Hz, 4 H), 2.04 (s, 3 H); ¹³C NMR (125 MHz, DMSO-*d*₆) δ 171.0, 170.2, 164.9, 162.9, 155.6, 154.1, 153.9, 153.7, 135.8, 135.5, 135.4, 133.6, 129.0, 128.2, 126.5, 125.9, 124.0, 119.9, 119.8, 114.2, 106.8, 70.3, 64.2, 63.8, 59.1, 57.4, 50.2, 46.3, 43.4, 42.1, 41.6, 25.4, 20.5; ESI-MS: *m/z* = 712.2 [M + H]⁺.

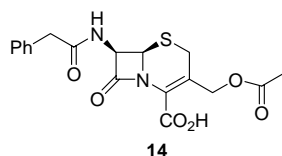


(6R,7R)-Benzhydryl-3-((1-(4-((S)-5-(acetamidomethyl)-2-oxooxazolidin-3-yl)-2-fluorophenyl)piperazine-4-carboxyloxy)methyl)-8-oxo-7-(2-phenylacetamido)-5-thia-1-aza-bicyclo[4.2.0]oct-2-ene-2-carboxylate (13). To a solution of compound **11** (80 mg, 0.11 mmol) in anhydrous dichloromethane (5 mL) was added compound **3b** (62 mg, 0.166 mmol) of a solution in DCM (2 mL) and the mixture was stirred for 4.5 h. Dichloromethane (10 mL) was added, and the organic layer was washed with brine and distilled water, separated, dried over anhydrous MgSO₄, filtered, and concentrated under reduced pressure. Purification by silica gel chromatography (30:1, DCM/MeOH) gave the desired product **13** as a white solid (49 mg, 51%). ¹H NMR (600 MHz, CDCl₃) mp: > 147 °C (dec); δ 7.40-7.26 (m, 15 H), 7.14-7.04 (m, 1 H), 6.94 (s, 1 H), 6.89 (t, *J* = 9.1 Hz, 1 H), 6.20 (d, *J* = 9.1 Hz, 1 H), 6.12 (t, *J* = 6.3 Hz, 1 H), 5.88 (dd, *J* = 5.0, 9.1 Hz, 1 H), 5.14-5.05 (m, 1 H), 4.97 (d, *J* = 4.7 Hz, 1 H), 4.84 (d, *J* = 13.8 Hz, 1 H), 4.80-4.71 (m, 1 H), 4.01 (t, *J* = 9.1 Hz, 1 H), 3.75 (dd, *J* = 9.1, 6.7 Hz, 1 H), 3.72-3.66 (m, 2 H), 3.65-3.57 (m, 4 H), 3.55-3.50 (m, 4 H), 3.43-3.33 (m, 1 H), 3.02-2.85 (m, 4 H), 2.01 (s, 3 H); ¹³C NMR (150 MHz, CDCl₃) δ 171.3, 171.2, 165.0, 160.9, 154.9, 154.4, 139.4, 139.2, 133.7, 129.7, 129.5, 128.8, 128.7,

128.4, 128.3, 128.0, 127.9, 127.8, 127.3, 125.5, 119.6, 114.0, 79.9, 72.0, 59.3, 57.4, 47.8, 43.6, 42.2, 29.9, 26.6, 23.4; ESI-MS: $m/z = 877.3 [M + H]^+$.



(6R,7R)-3-((1-(4-((S)-5-(acetamidomethyl)-2-oxooxazolidin-3-yl)-2-fluorophenyl)piperazine-4-carboxyloxy)methyl)-8-oxo-7-(2-phenylacetamido)-5-thia-1-aza-bicyclo[4.2.0]oct-2-ene-2-carboxylic acid (2). To a suspension of **13** (40 mg, 0.046 mmol) in CH_2Cl_2 (1 mL) and anisole (0.1 mL) at 0 °C, was added TFA (0.04 mL, 0.54 mmol) dropwise. The resulting mixture was stirred at 0 °C for 0.5 h. Then the solvent was removed under reduced pressure. The residue was treated with CH_2Cl_2 (3 mL) and EtOAc (3 mL) separately and the resulting precipitate was removed by filtration. The filtrate was concentrated and the residue was treated with EtOAc (3 mL) and ether (3 mL). The precipitate was filtered to give compound **2** as a light yellow solid (10 mg, 31%). mp: > 148 °C (dec); ^1H NMR (600 MHz, $\text{DMSO}-d_6$) δ 13.66 (s, 1 H), 9.12 (d, $J = 8.3$ Hz, 1 H), 8.24 (t, $J = 5.8$ Hz, 1 H), 7.49 (dd, $J = 14.8, 2.3$ Hz, 1 H), 7.32-7.25 (m, 3 H), 7.24-7.20 (m, 1 H), 7.18 (dd, $J = 8.8, 1.9$ Hz, 1 H), 7.08 (t, $J = 9.3$ Hz, 1 H), 5.69 (dd, $J = 8.3, 4.7$ Hz, 1 H), 5.10 (d, $J = 4.7$ Hz, 1 H), 5.04 (d, $J = 13.0$ Hz, 1 H), 4.74-4.67 (m, 2 H), 4.08 (t, $J = 9.0$ Hz, 1 H), 3.72-3.62 (m, 2 H), 3.59-3.46 (m, 7 H), 3.40 (t, $J = 5.5$ Hz, 2 H), 3.02-2.93 (m, 5 H), 1.83 (s, 3 H); ^{13}C NMR (150 MHz, $\text{DMSO}-d_6$) δ 170.9, 170.0, 164.8, 162.9, 155.4, 154.1, 154.0, 153.8, 135.8, 135.4, 135.3, 133.8, 133.7, 129.0, 128.2, 126.5, 125.9, 124.0, 119.9, 119.8, 114.1, 114.0, 106.6, 106.5, 71.6, 63.8, 59.1, 57.4, 50.2, 50.1, 47.3, 41.6, 41.4, 25.4, 22.4; ESI-MS: $m/z = 711.3 [M + H]^+$.



(7R,7aR)-3-[(Acetyloxy)methyl]-6-oxo-7-[(2-phenylacetyl)amino]-7,7a-dihydro-2H,6H-azeto[2,1-b][1,3]thiazine-4-carboxylic Acid (14). To a solution of 7-ACA (120 mg, 0.44 mmol) in saturated aqueous NaHCO_3 (15 mL) and acetone (5 mL) was added phenylacetyl chloride (ca. 2 equivalents, ca. 0.06 mL) in two portions. After stirring for 17 h, the reaction mixture was acidified with concentrated HCl to pH 1.5 and extracted with CH_2Cl_2 (2 x 10 mL) and the combined organic layers were washed with brine, dried over MgSO_4 , filtered and concentrated under reduced pressure. The residue was suspended in diethyl ether and stirred for 17 h to remove phenylacetic acid (by-product). The suspension was filtered and the obtained crude product was washed with diethyl ether and dried in vacuo to afford compound **14** as an off-white solid (142 mg, 83%). Spectral data was consistent with previously reported data.³

Antimicrobial assay by the agar diffusion method

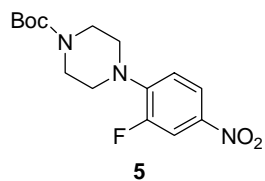
Antibacterial activity was determined by agar diffusion tests according to the literature.^{4,5}

Test organisms (0.1 mL of a McFarland 0.5 Standard in 0.9% NaCl) were suspended in the melted agar medium (34 mL, Standard I Nutrient Agar, Serva or Mueller Hinton II Agar, Becton, Dickinson and Company) and poured into petri dishes. Holes 9 mm in diameter were cut in the agar and filled with 50 μ L of a 2 mM solution of the compounds in DMSO: MeOH (1: 9). Inhibition zones were read after overnight incubation at 37 °C. The details of the method are described also in: S. Afonin, R. W. Glaser, M. Berditchevskaja, P. Wadhvani, K.-H. Gührs, U. Möllmann, A. Perner, and A. S. Ulrich. 4-Fluoro-phenylglycine as a label for ¹⁹F-NMR structure analysis of membrane associated peptides. *ChemBioChem* **2003**, *4*, 1151–1163.

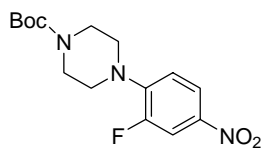
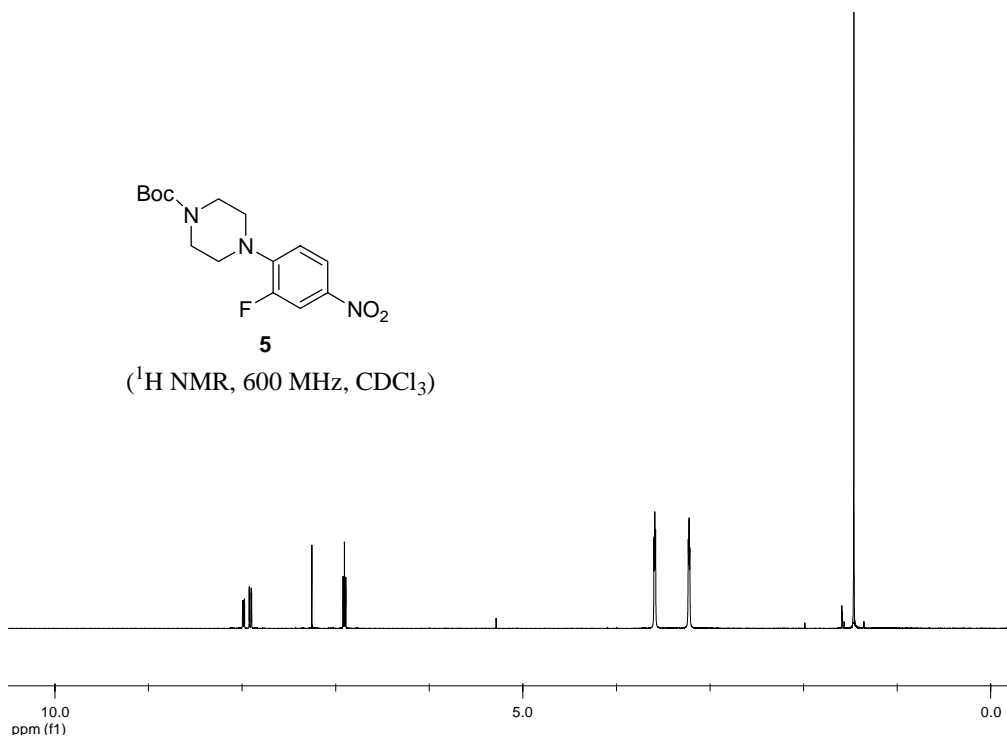
The minimal inhibitory concentration (MIC) of cephalosporin-oxazolidinone **1** against several organisms was determined by broth microdilution method, following NCCLS guidelines.⁶

References:

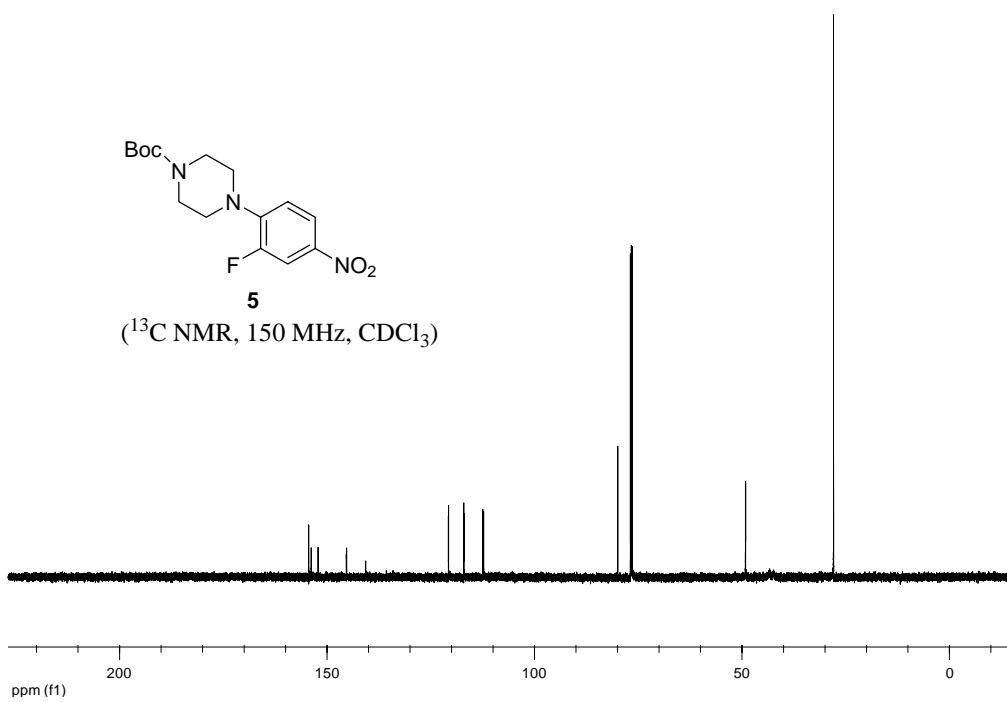
- (1) S. Kumart and R. W. Murray, *J. Am. Chem. Soc.*, 1984, **106**, 1040.
- (2) G. Y. Zhao, M. J. Miller, S. Franzblau, B. J. Wan and U. Möllmann, *Bioorg. Med. Chem. Lett.*, 2006, **16**, 5534.
- (3) R. Keltjens, S. K. Vadivel, E. D. Vroom, A. J. H. Klunder and B. Zwanenburg, *Eur. J. Org. Chem.*, 2001, **13**, 2529.
- (4) B. Glauner, *Biotec.*, 1991, **5**, 58.
- (5) H. M. Dahse, B. Schlegel and U. Gräfe, *Pharmazie*, 2001, **56**, 489.
- (6) National Committee for Clinical Laboratory Standards. Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria That Grow Aerobically-Sixth Edition: Approved Standard M7-A6. NCCLS, Wayne, PA, USA, 2003.

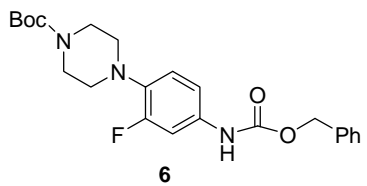


(¹H NMR, 600 MHz, CDCl₃)

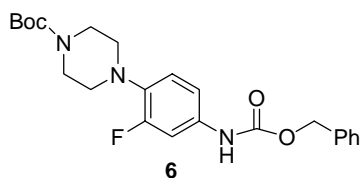
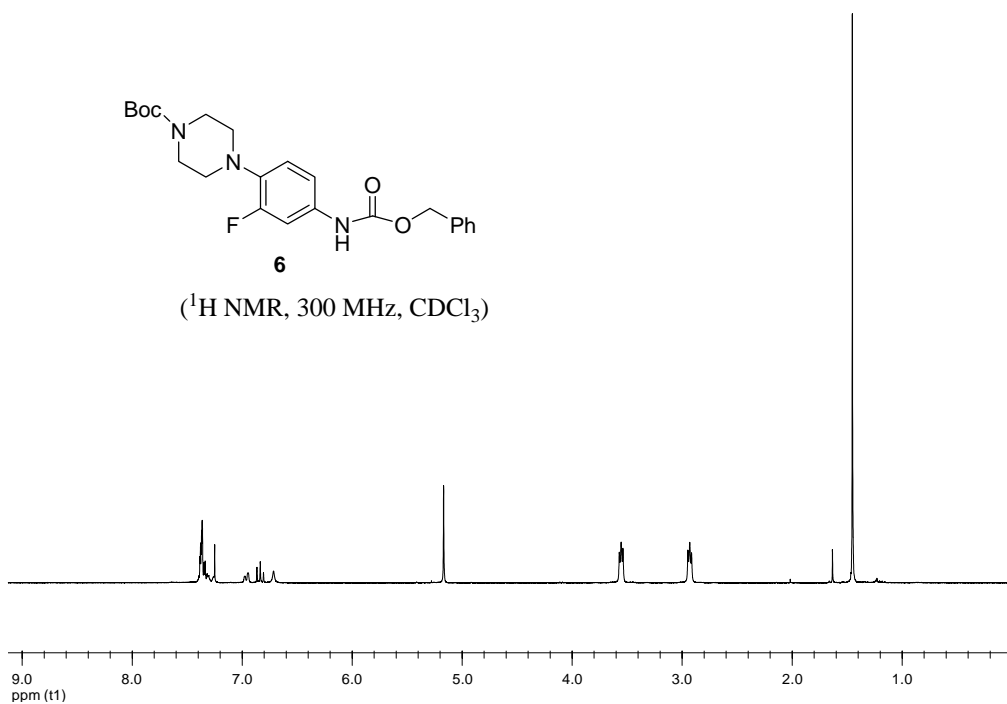


(¹³C NMR, 150 MHz, CDCl₃)

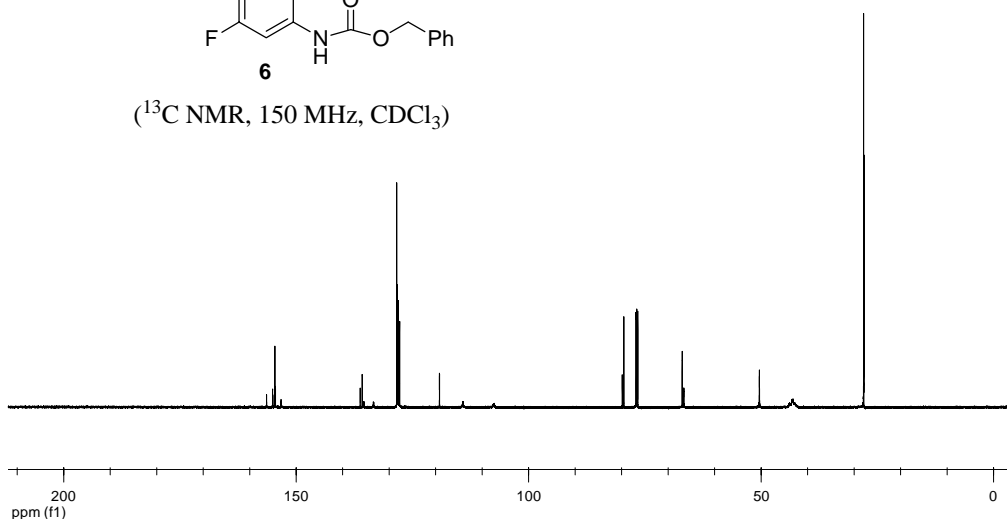


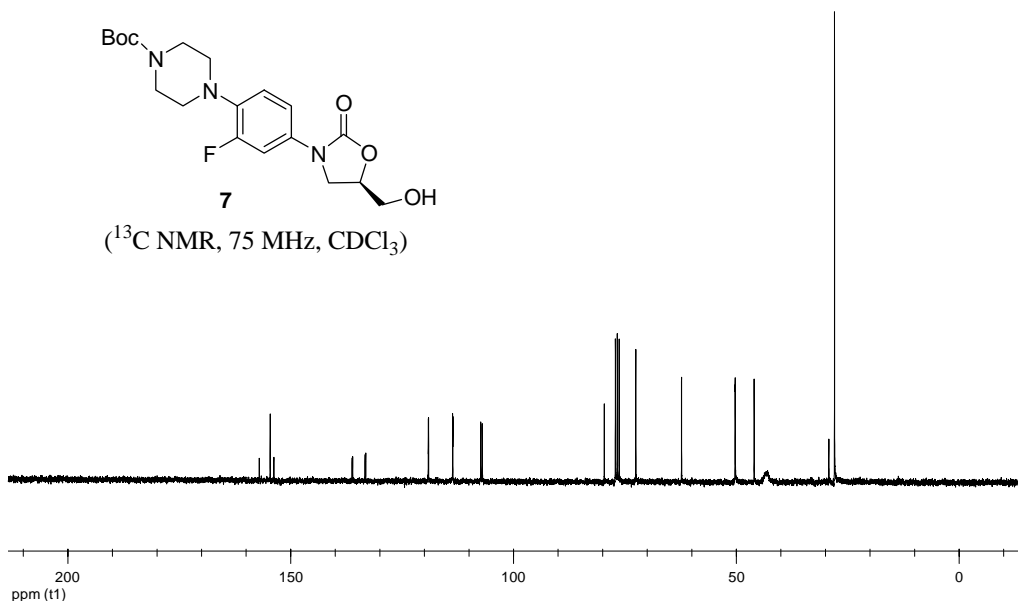
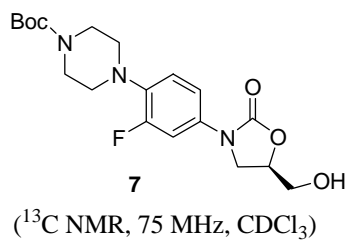
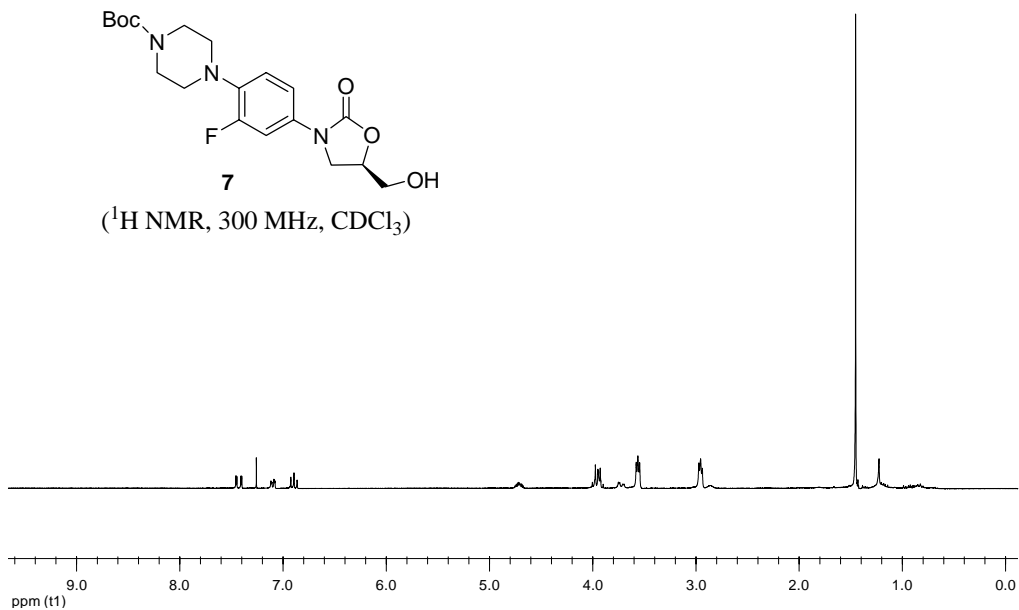
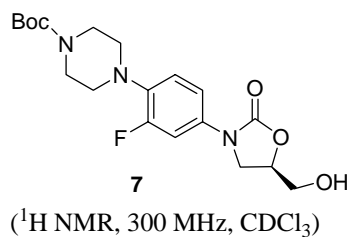


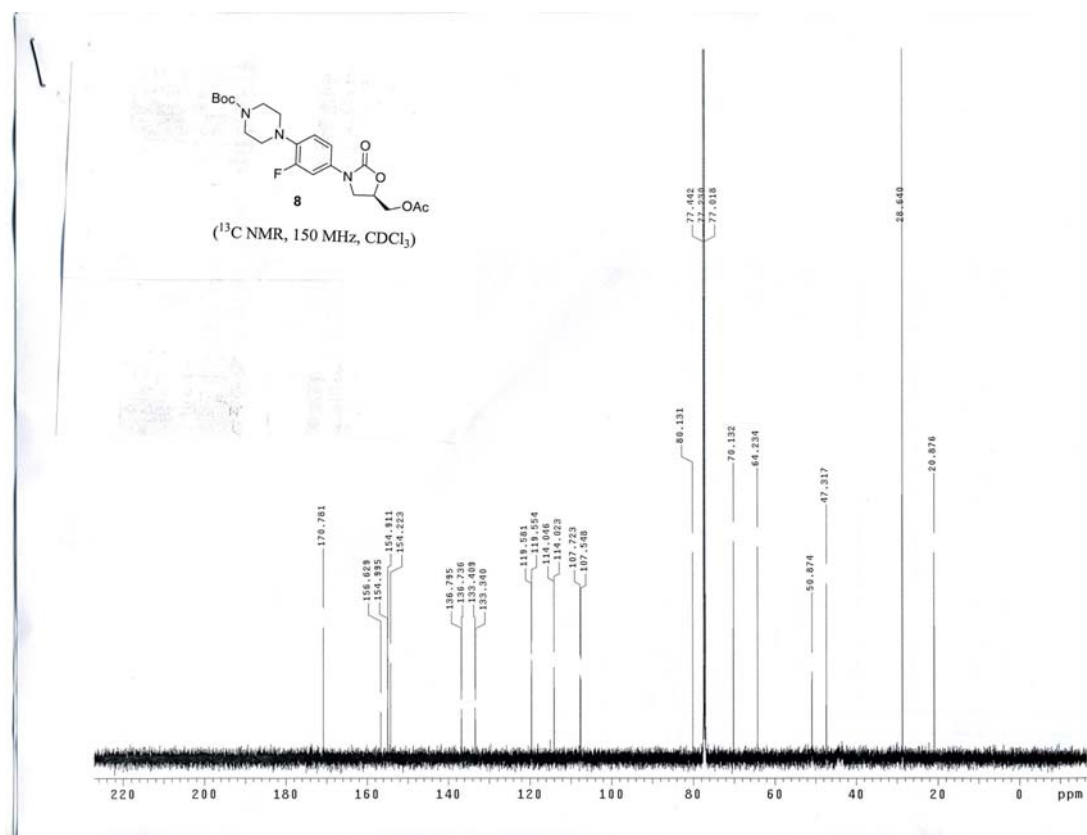
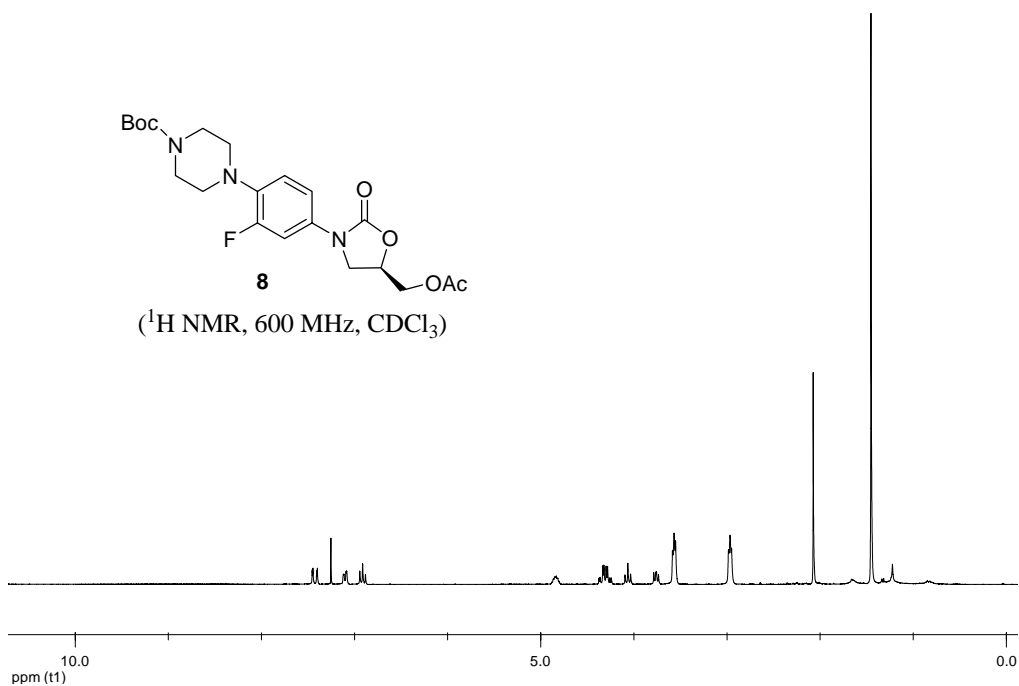
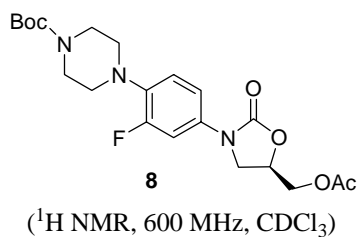
(^1H NMR, 300 MHz, CDCl_3)

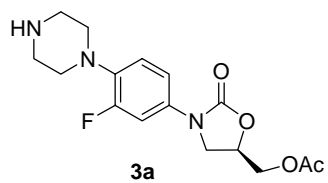


(^{13}C NMR, 150 MHz, CDCl_3)

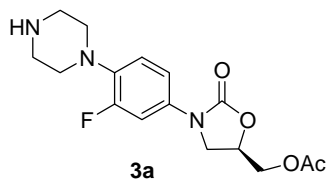
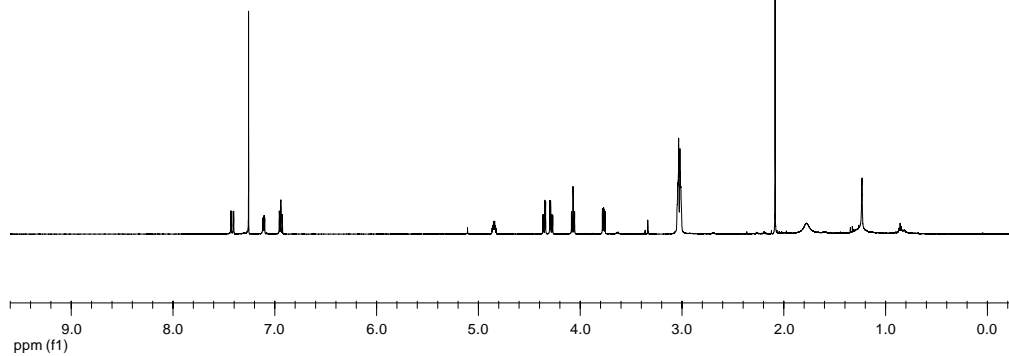




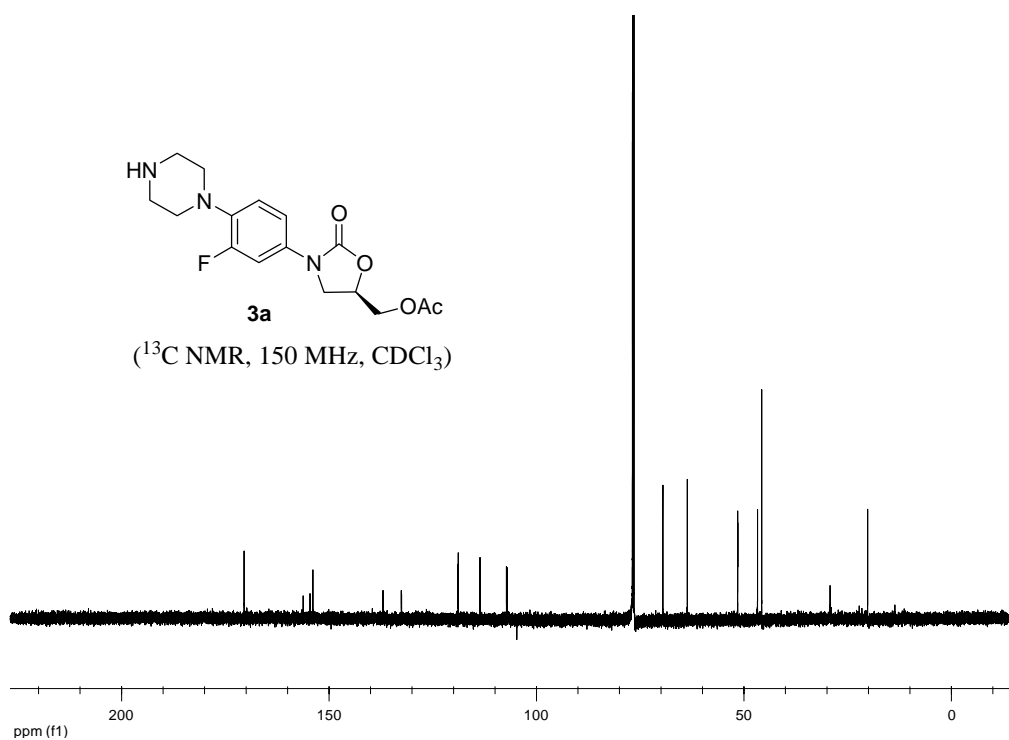


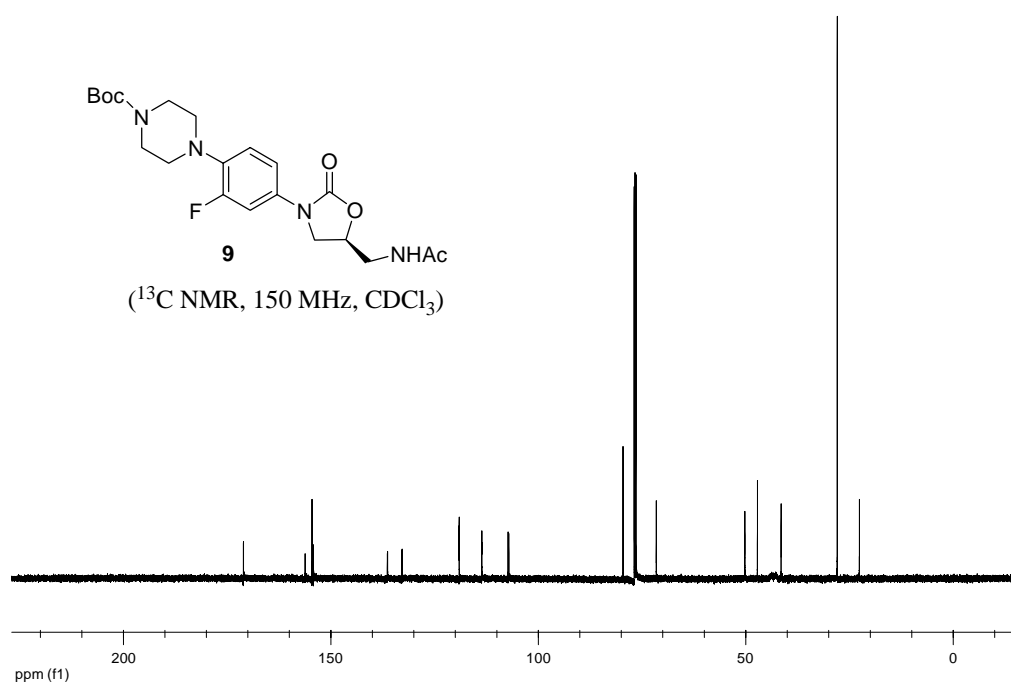
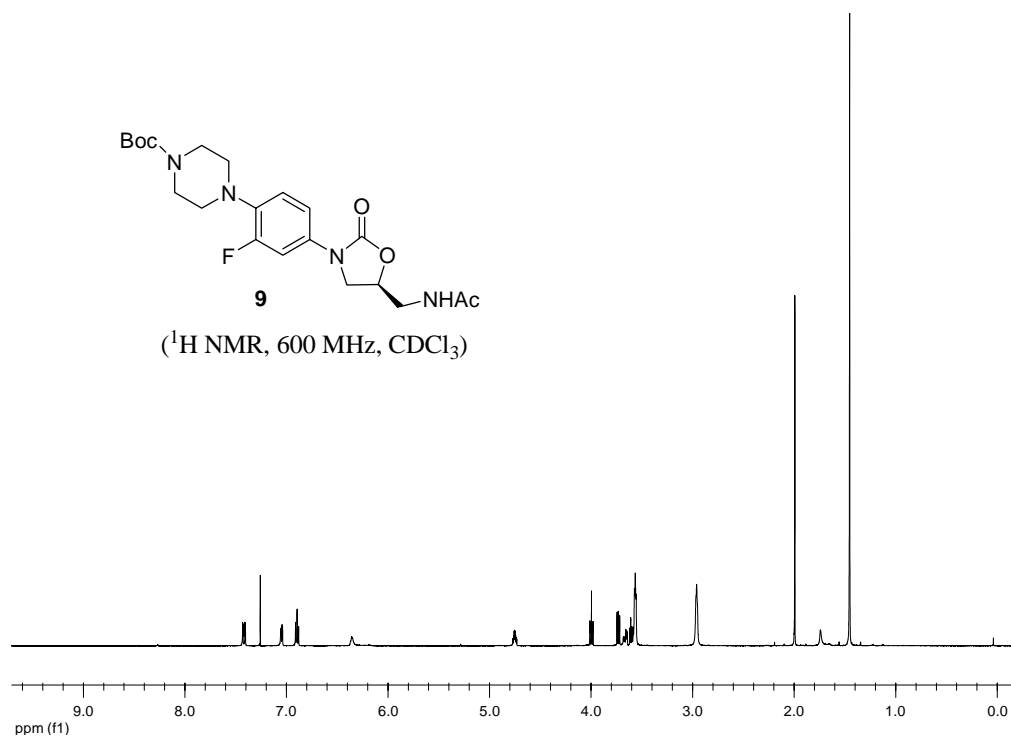


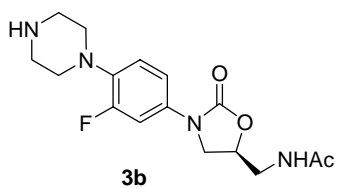
(¹H NMR, 600 MHz, CDCl₃)



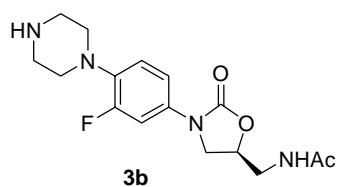
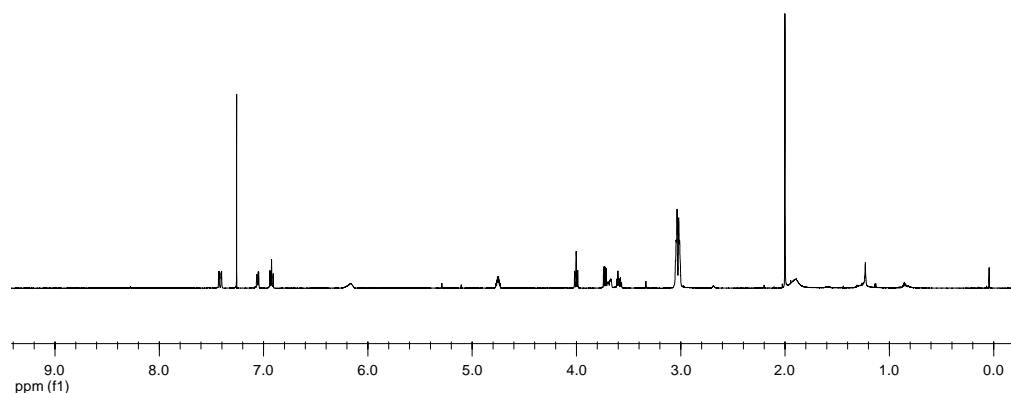
(¹³C NMR, 150 MHz, CDCl₃)



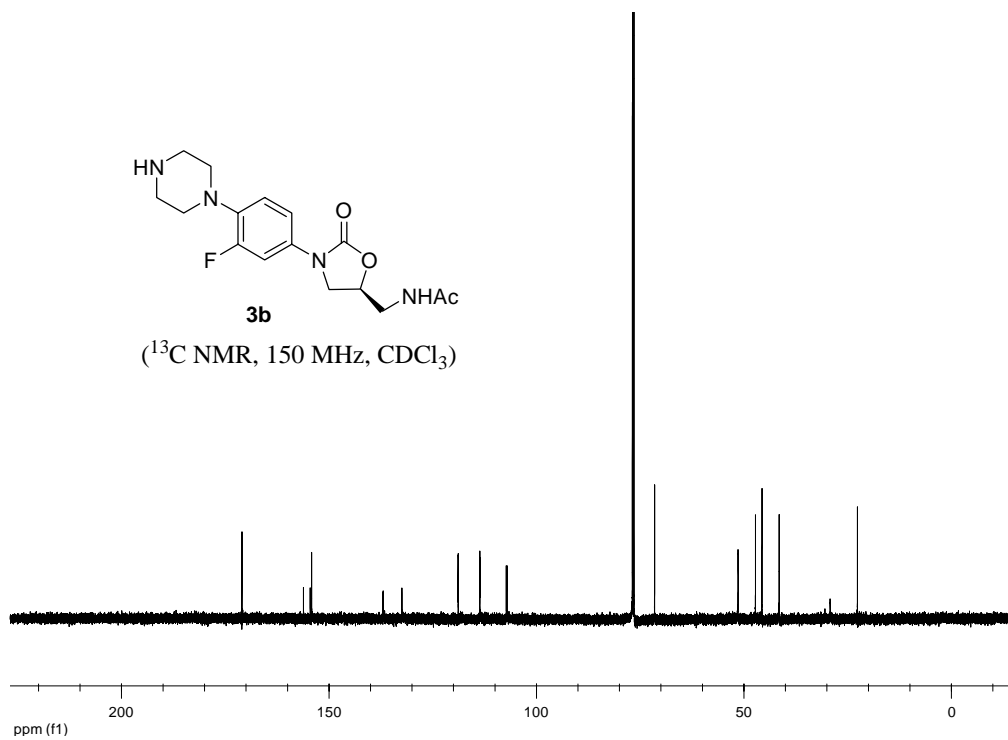


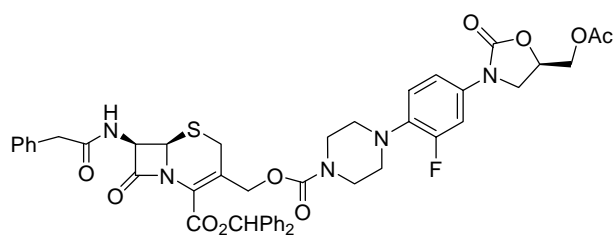


(¹H NMR, 600 MHz, CDCl₃)



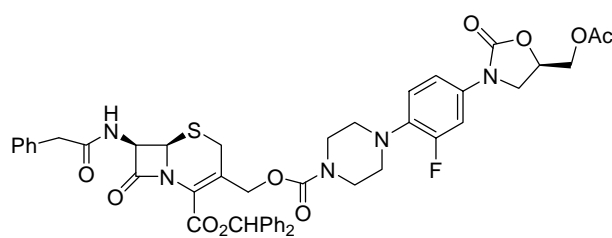
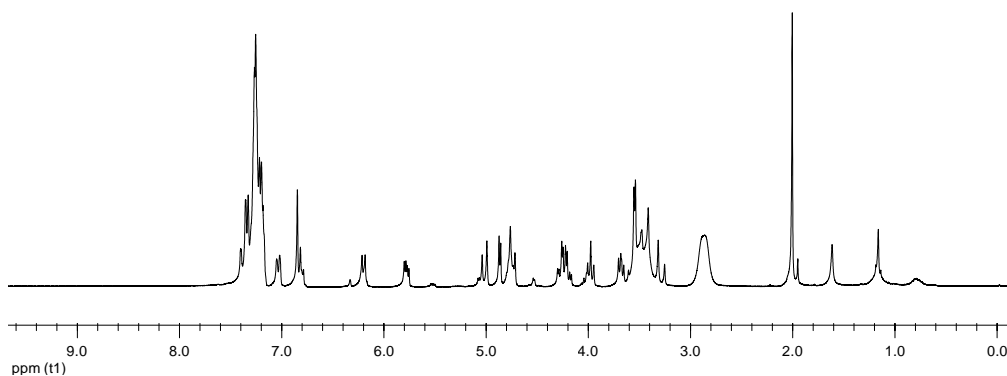
(¹³C NMR, 150 MHz, CDCl₃)





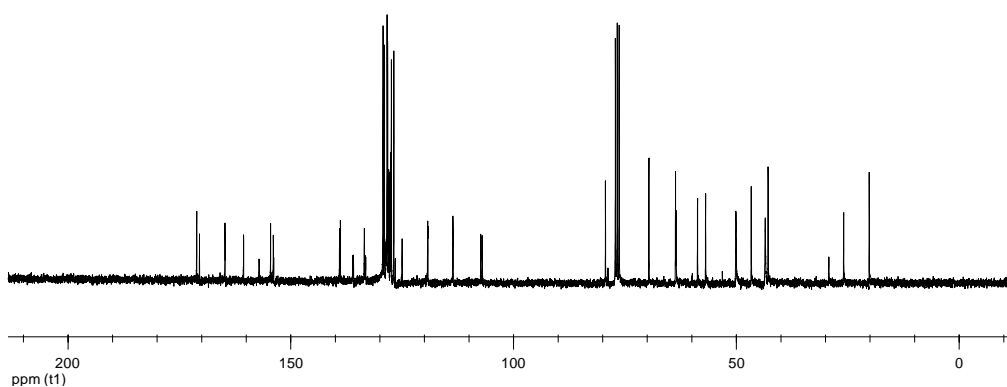
12

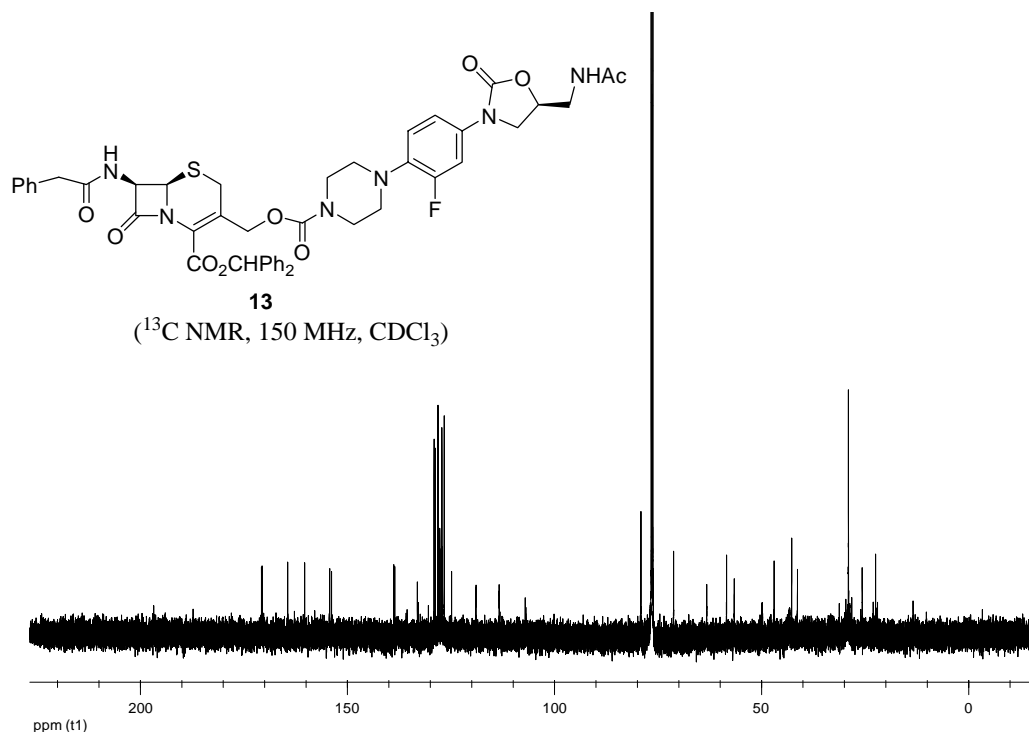
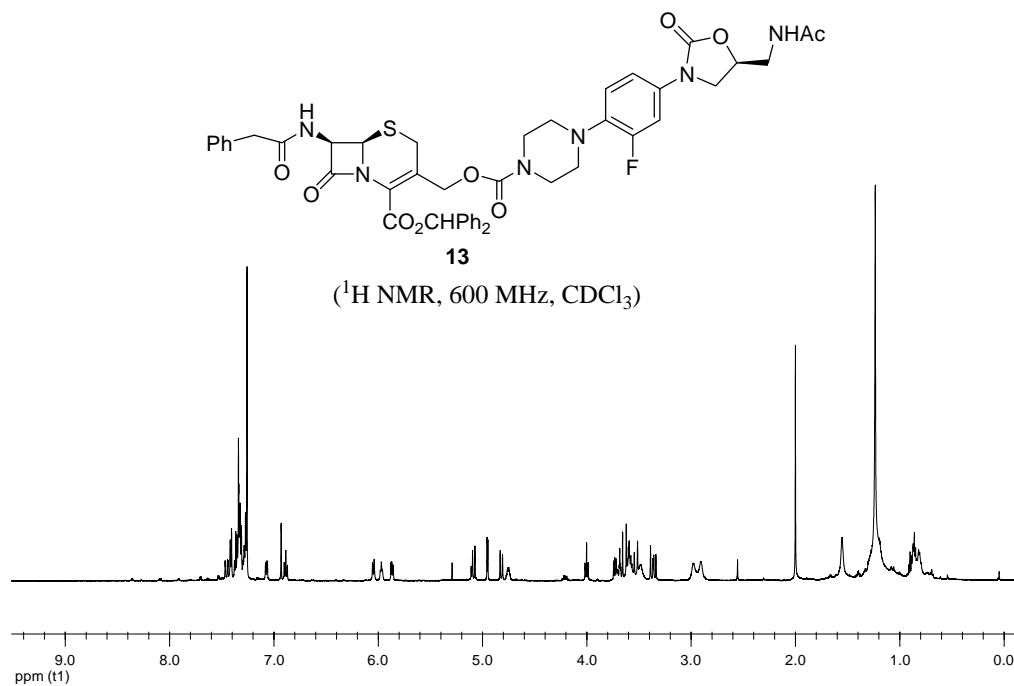
(¹H NMR, 300 MHz, CDCl₃)

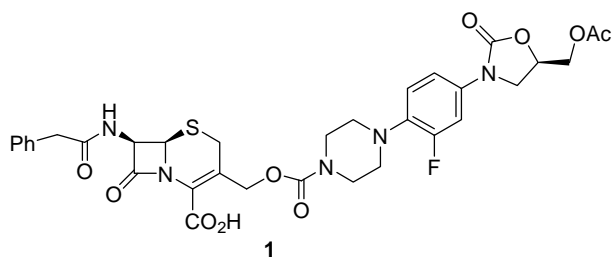


12

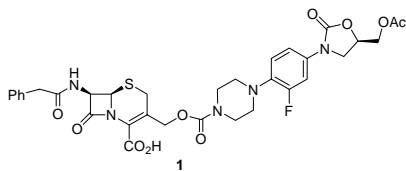
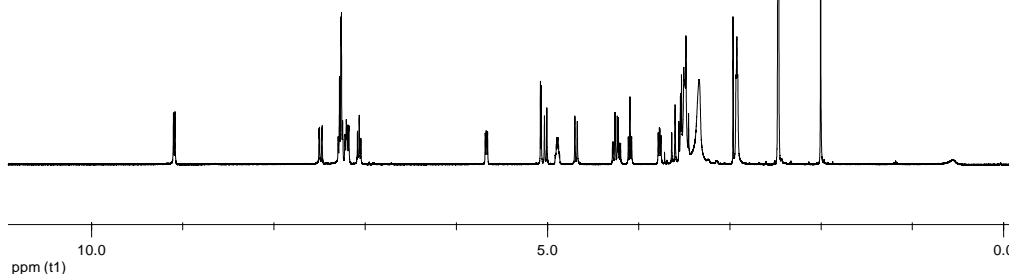
(¹³C NMR, 75 MHz, CDCl₃)







1
(¹H NMR, 500 MHz, DMSO-*d*₆)



1
(¹³C NMR, 125 MHz, DMSO-*d*₆)

