Supporting Information

Synthesis of skeletally diverse β-lactam haptens for the in vitro diagnosis of IgE-mediated drug allergy

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Experimental section

1. General methods

Oxygen- and moisture-sensitive reactions were carried out in flame-dried glassware under a nitrogen atmosphere. According to cited procedures, all reagents and solvents were purchased and used as received from commercial vendors or synthesized.

All yields refer to chromatographically and spectroscopically pure compounds unless otherwise stated. Reaction progress was monitored by analytical thin-layer chromatography (TLC) and ^{1}H-NMR spectroscopy. TLC analyses were performed using E. Merck silica gel 60 F254 pre-coated plates (250 μm). A handheld 254 nm UV lamp and potassium permanganate staining solution (with light heating) were used for detection. Flash column chromatography was performed using a Teledyne ISCO CombiFlash Rf+ purification system with RediSep Rf Gold Normal-Phase Silica columns.

Known compounds were characterized by, at minimum, 1H NMR spectroscopy. Novel synthetic intermediates and final compounds were characterized by, at minimum, ^{1}H-NMR, ^{13}C-NMR spectroscopy, and HRMS. Further NMR experiments were performed as needed to confirm the structural assignment.

NMR spectra were recorded on Bruker UltraShield 300 MHz and Ascend 400 MHz spectrometers. ^{1}H and ^{13}C-NMR chemical shifts (δH, δC) are reported in parts per million (ppm) relative to the appropriate solvent–$CDCl_3$: 7.26 (^{1}H), 77.16 (^{13}C) ppm. $(CD_3)_2SO$: 2.50 (^{1}H), 39.52 (^{13}C) ppm. C_6D_6: 7.16 (^{1}H), 128.06 (^{13}C) ppm. Resonances are described using the following abbreviations: s (singlet), d (doublet), t (triplet), q (quartet), quin. (quintet), m (multiplet), br (broad), dd (doublet of doublets), etc. Coupling constants (J) are given in Hz and are rounded to the nearest 0.1 Hz. All NMR data were collected at 25 °C. All deuterated solvents were purchased from Cambridge Isotope Laboratories.

Low-resolution mass spectra (LRMS) were acquired on a Waters 2795 separations module coupled to a 3100 mass detector operating in ESI+ mode.

2. Experimental procedures

(E)-2-Cyclopropylboronic acid (**3**) was purchased from Combi-Blocks and dried over 3Å molecular sieves.

General Procedure A: Trimethylsilyldiazomethane methylation.

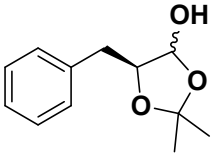
A flame-dried round-bottom flask was charged with a solution of corresponding BLC (Equiviv.) in methanol (1 mL) and DCM (0.2 M) under an N_2 atmosphere. Then, (diazomethyl)trimethylsilane (2 M, one Equiv.) was added dropwise, and the reaction was allowed to stir at room temperature until the yellow solution turned clear. The solution was concentrated by rotary evaporation and purified by flash column chromatography (ISCO) to give the desired compound.

General Procedure B: Petasis reaction.

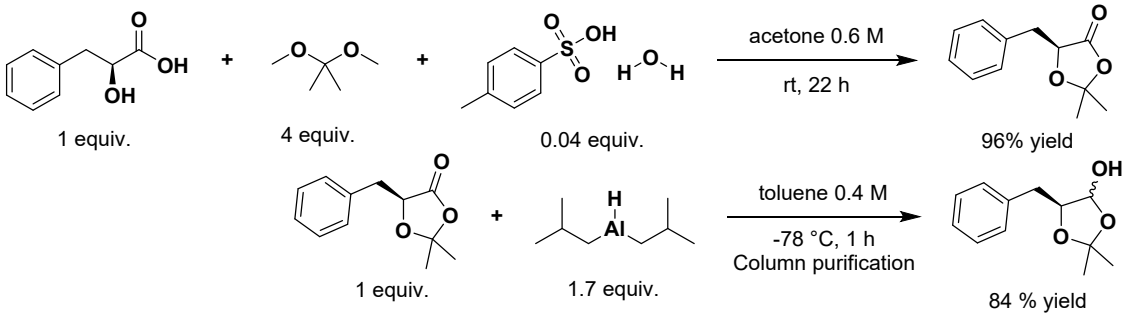
A flame-dried round-bottom flask was charged with (4R,5S)-5-benzyl-2,2-dimethyl-4-hydroxy-1,3-dioxolane **2** (1 Equiv., 0.2 M) in the solvent system of 9:1 EtOH–hexafluoroisopropanol (v/v) and 3Å molecular sieves. To this stirred solution were added the corresponding BLC methyl ester **1a-1f** (1.5 Equiv) and (*E)*-2-cyclopropylvinylboronic acid **3** (1.5 equiv), successively. After stirring for 72 h at 40 °C, the resulting mixture was concentrated by rotary evaporation, and the residue was purified by flash column chromatography (ISCO) to give the desired compound.

General Procedure C: N-alkylation of the Petasis product.

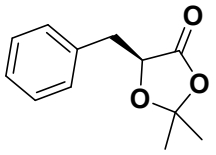
A solution of the Petasis product (1 Equiv., 0.2 M) and $NaHCO_3$ (10 Equiv.) in DMF was stirred for 30 min at 0 °C. Then, propargyl bromide (10 Equiv., 80% in toluene) was added under N_2. After stirring for 24 h at 70 °C, the reaction mixture was cooled to room temperature and quenched with NH_4Cl solution (50 mL). The resulting mixture was extracted with ether (3x50 mL), and combined organic layers were washed with brine and dried over $MgSO_4$. The solution was concentrated by rotary evaporation, and the residue was purified by flash column chromatography (ISCO) to give the desired compound.



(5S)-5-benzyl-2,2-dimethyl-1,3-dioxolan-4-ol (2)

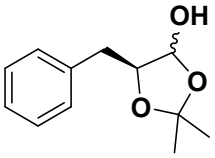


Synth. Commun. **1996**, *26,* 3453



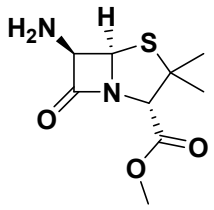
1. (S)-5-Benzyl-2,2-dimethyl-4-oxo-l,3-dioxolane

A flame-dried round-bottom flask was charged with a mixture of (S)-2-hydroxy-3-phenylpropanoic acid (10.00 g, 60.00 mmol) and p-TsOH.H_20 (500 mg) in 2,2-dimethoxypropane (30 mL) and acetone (100 mL) and was stirred at room temperature for 22 h. After concentration by rotary evaporation, the resulting residue was dissolved in ethyl acetate (150 mL) washed with sat. $NaHCO_3$ (100 mL), brine (100 mL), dried ($MgSO_4$), passed through a short pad of silica gel and concentrated to provide a white solid (11.70 g, 96% yield). ^{1}H-NMR (400 MHz, $CDC1_3$) δ: 7.28 – 7.23 (m, 5H), 4.65 (dd, 1H), 3.12 (ABq, 2H), 1.49 (s, 3H), 1.35 (s, 3H). ^{13}C NMR (100 MHz, $CDC1_3$) δ: 172.49, 135.79, 129.81, 128.38, 127.03, 110.84, 75.04, 37.65, 26.93, 26.16. Characterization data is consistent with those reported in the literature.



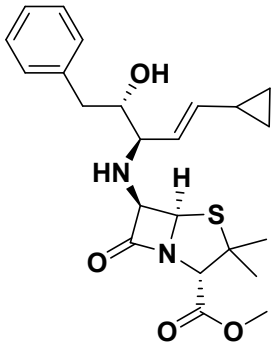
2. (4R,5S)-5-Benzy1-2,2-dimethyl-4-hydroxy-1,3-dioxolane (2)

To a stirred solution of (5S)-5-benzyl-2,2-dimethyl-1,3-dioxolan-4-one (1.83 g, 8.89 mmol) in toluene (37.5 mL) was added DIBAL-H (9.8 mL of a 1M solution in toluene, 9.78 mmol) at -78ºC. The resulting solution was stirred at the same temperature for 60 min, and to this solution was added 1N HCl (9.8 mL) very slowly. After warming to room temperature, the solution was extracted with ethyl acetate (3 x 10 mL), filtered with $MgSO_4$, and concentrated on providing a pale yellow oil **2** (1.53 g, 84% yield) after purification by flash chromatography (ISCO). ^{1}H-NMR (400 MHz, $CDC1_3$) δ: 7.36 – 7.20 (m, 5H), 5.28 – 5.21 (m. 1H), 4.31 – 4.26 (m, 1H), 3.06 (d, J = 6.8 Hz, 1H), 2.94 (qd, J =14.0, 6.6 Hz, 2H), 2.82 (br, 1H), 1.46 and 1.51 (two s, 6H, $C(CH_3)_2$ for major isomer), 1.35 and 1.57 (two s, 6H, $C(CH_3)_2$ for minor isomer); ratio of diastereomers was approximately 2: 1. Characterization data were consistent with those reported in the literature.



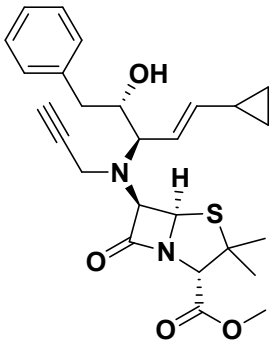
6-aminopenicillanic acid (6-APA) methyl ester (1a)

General procedure A was followed using 6-APA (2.00 g, 9.25 mmol), methanol (1 mL), and (diazomethyl)trimethylsilane (4.6 mL, 9.25 mmol) to give the desired compound **1a** as a viscous yellow oil (2.07 g, 97% yield). ^{1}H-NMR (400 MHz, $CDCl_3$) δ: 5.51 (d, *J* = 4.3 Hz, 1H), 4.57 (d, *J* = 4.3 Hz, 1H), 4.40 (s, 1H), 3.77 (s, 3H), 1.79 (br, 2H), 1.66 (s, 3H), 1.50 (s, 3H).



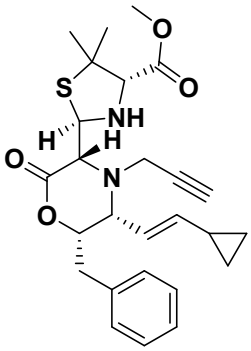
(2*S*,3*R*,*E*)-5-Cyclopropyl-2-hydroxy-3-((2*S*,5*R*,6*R*)-2-methoxycarbonyl-3,3-dimethyl-1-aza-7-oxo-4-thiabicyclo[3.2.0]heptan-6-yl)amino-1-phenyl-4-pentene (4a)

General procedure B was followed using (4R,5S)-5-benzyl-2,2-dimethyl-4-hydroxy-1,3-dioxolane **2** (759 mg, 3.64 mmol, 0.2 M) in 18.2 mL of the solvent system of 9:1 EtOH–hexafluoroisopropanol (v/v), 6-APA methyl ester **1a** (1.26 g, 5.47 mmol, 1.5 equiv.) and (*E*)-2-cyclopropylvinylboronic acid **3** (590 mg, 5.27 mmol, 1.5 equiv.) to give the desired product **4a** as a pale yellow oil (834 mg, 53% yield). ^{1}H-NMR (400 MHz, $CDCl_3$) δ: 7.31 – 7.19 (m, 5H), 5.47 (dd, *J* = 15.5, 9.0 Hz, 1H), 5.43 (d, *J* = 3.7 Hz, 1H), 5.20 (dd, *J* = 15.3, 8.6 Hz, 1H), 4.44 (d, *J* = 2.9 Hz, 1H), 4.40 (s, 1H), 4.03 – 3.95 (m, 1H), 3.77 (s, 3H), 3.16 (d, *J* = 9.1 Hz, 1H), 2.73 (d, *J* = 6.6 Hz, 2H), 2.42 (br, 2H), 1.65 (s, 3H), 1.49 (s, 3H), 1.47 – 1.42 (m, 1H), 0.77 (d, *J* = 8.0 Hz, 2H), 0.42 (s, 2H). ^{13}C-NMR (100 MHz, $CDCl_3$) δ: 177.1, 168.8, 140.4, 138.4, 129.5, 128.6, 126.5, 124.3, 74.2, 70.6, 70.5, 67.3, 64.7, 64.3, 52.4, 40.1, 31.4, 27.4, 13.8, 7.0, 6.9. LRMS (ESI^{+}) m/z calculated for $C_{23}H_{30}N_2O_4S$ ([M+H^{+}]$^{+}$): 431.19, found: 431.21.



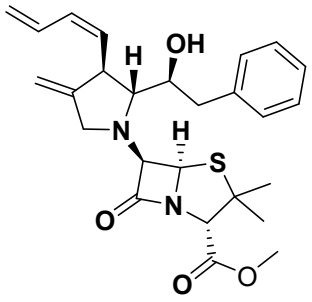
(2*S*,3*R*,*E*)-5-Cyclopropyl-2-hydroxy-3-{((2*S*,5*R*,6*R*)-2-methoxycarbonyl-3,3-dimethyl-1-aza-7-oxo-4-thiabicyclo[3.2.0]heptan-6-yl)(2-propynyl)amino}-1-phenyl-4-pentene (5a)

General procedure C was followed using **4a** (834 mg, 1.94 mmol, 0.2 M) and $NaHCO_3$ (1.63 g, 19.35 mmol, 10 equiv.) in DMF (9.7 mL) and propargyl bromide (2.2 mL, 19.35 mmol, 10 equiv., 80% in toluene) to give the desired compound **5a** (656 mg, 72% yield). ^{1}H-NMR (400 MHz, $CDCl_3$) δ: 7.33 – 7.19 (m, 5H), 5.57 (dd, *J* = 15.4, 9.8 Hz, 1H), 5.30 (d, *J* = 3.7 Hz, 1H), 5.28 (dd, *J* = 15.0, 8.0 Hz, 1H), 4.48 (d, *J* = 4.0 Hz, 1H), 4.47 (s, 1H), 4.21 (d, *J* = 18.0 Hz, 1H), 3.79 (s, 3H), 3.74 (d, *J* = 18.5 Hz, 1H), 3.34 (dd, *J* = 9.7, 2.7 Hz, 1H), 2.78 (dd, *J* = 13.9, 8.0 Hz, 1H), 2.62 (dd, *J* = 14.0, 5.4 Hz, 1H), 2.22 (s, 1H), 1.67 (s, 3H), 1.57 – 1.49 (m, 1H), 1.48 (s, 3H), 0.83 (d, *J* = 8.1 Hz, 2H), 0.53 – 0.42 (m, 2H). ^{13}C-NMR (100 MHz, $CDCl_3$) δ: 172.6, 168.7, 143.0, 138.8, 129.4, 128.5, 126.4, 121.1, 100.1, 74.3, 70.7, 70.5, 70.4, 69.3, 68.5, 63.8, 52.5, 39.9, 38.9, 31.2, 26.8, 13.9, 7.2, 7.0. LRMS (ESI^+) m/z calculated for $C_{26}H_{32}N_2O_4S$ ($[M+H^+]^+$): 469.21, found: 469.51.



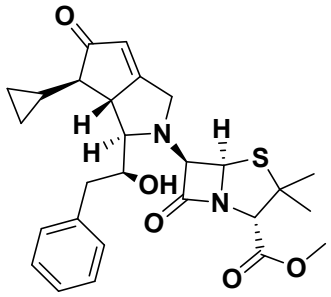
(3*R*,5*R*,6*S*)-6-Benzyl-5-((*E*)-2-cyclopropylvinyl)-3-((2*R*,4*R*)-4-methoxycarbonyl-5,5-dimethylthiazolidin- 2-yl)-4-propynylmorpholin-2-one (6a)

To a stirred solution of **5a** (47.1 mg, 0.10 mmol) in toluene (1.0 mL), was added NaH (ca 60% mineral oil dispersion, 6.2 mg, 0.15 mmol, 1.5 equiv.) at 0 °C. After stirring at room temperature for 14 h under N_2, 2 mL sat. $NaHCO_3$ aq. was added to the reaction mixture. The resulting mixture was extracted with ethyl acetate (3x2 mL), and combined organic layers were washed with brine and dried over Na_2SO_4. The solution was concentrated by rotary evaporation and the residue was purified by flash column chromatography (ISCO) to give **6a** as a colorless oil (23.4 mg, 50% yield). ^{1}H-NMR (400 MHz, C_6D_6) δ: 7.32 – 7.18 (m, 5H), 5.37 (t, 1H), 5.29 (dd, *J* = 15.2, 9.8 Hz, 1H), 5.19 (dd, *J* = 15.2, 8.5 Hz, 1H), 4.96 (d, *J* = 3.6 Hz, 1H), 4.26 (s, 1H), 3.69 – 3.58 (m, 2H), 3.54 (d, *J* = 3.5 Hz, 1H), 3.25 (d, *J* = 2.5 Hz, 1H), 3.20 (d, *J* = 2.3 Hz, 1H), 3.19 (s, 3H), 2.98 (dd, *J* = 14.2, 7.2 Hz, 1H), 2.65 (dd, *J* = 14.2, 7.4 Hz, 1H), 1.73 (s, 1H), 1.60 (s, 3H), 1.23 – 1.11 (m, 1H), 1.09 (s, 3H), 0.52 (d, *J*= 7.8 Hz, 2H), 0.28 – 0.24 (m, 2H).



(2*R*,3*R*)-3-((1*E*)-Buta-1,3-dienyl)-2-((*S*)-1-hydroxy-2-phenylethyl)-*N*-((2*S*,5*R*,6*R*)-2-methoxycarbonyl-3,3-dimethyl-1-aza-7-oxo-4-thiabicyclo[3.2.0]heptan-6-yl)-4-methylenepyrrolidine (7a)

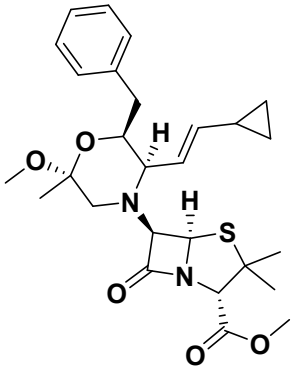
To a stirred solution of **5a** in benzene (76.3 mg, 0.16 mmol, 0.05 M), was added bis(triphenylphosphine)palladium acetate (24.0 mg, 0.03 mmol, 0.2 equiv.) at room temperature under N_2. After stirring for 1 h at 80 °C, the resulting solution was concentrated by rotary evaporation and the residue was purified by flash column chromatography (ISCO) to give **7a** as a colorless oil (31.0 mg, 39% yield, 5/1 inseparable diastereomixture). Selected spectroscopic data for major diastereomer: ^{1}H-NMR (400 MHz, $CDCl_3$) δ: 7.35 – 7.15 (m, 5H), 6.36 (dd, *J* = 11.3, 7.2 Hz, 1H), 6.18 (t, *J* = 11.6, 10.1 Hz, 1H), 5.60 (dd, *J* = 8.4, 7.5 Hz, 1H), 5.34 (d, *J* = 3.9 Hz, 1H), 5.21 (d, *J* = 16.8 Hz, 1H), 5.08 (d, *J* = 10.1 Hz, 1H), 5.00 (s, 1H), 4.90 (s, 1H), 4.51 (d, *J* = 4.0 Hz, 1H), 4.42 (s, 1H), 4.31 – 4.22 (m, 1H), 3.87 (d, *J* = 13.8 Hz, 1H), 3.78 (s, 3H), 3.55 (d, *J* = 13.1 Hz, 2H), 3.01 (d, *J* = 5.5 Hz, 1H), 2.82 (d, *J* = 7.3 Hz, 1H), 2.76 (d, *J* = 14.0 Hz, 1H), 1.69 (s, 3H), 1.48 (s, 3H). ^{13}C-NMR (100 MHz, C_6D_6) δ: 173.1, 168.1, 149.5, 139.8, 136.9, 135.8, 132.4, 129.7, 128.5, 126.2, 116.2, 107.1, 72.4, 72.3, 71.6, 69.9, 67.0, 64.4, 56.9, 51.4, 47.2, 39.6, 30.6, 26.5.



(1*R*,2*R*,5*E*,8*R*)-8-Cyclopropyl-2-((*S*)-1-hydroxy-2-phenylethyl)-3-((2*S*,5*R*,6*R*)-2-methoxycarbonyl-3,3-dimethyl-1-aza-7-oxo-4-thiabicyclo[3.2.0]heptan-6-yl)-3-azabicyclo[3.3.0]oct-5-en-6-one (8a)

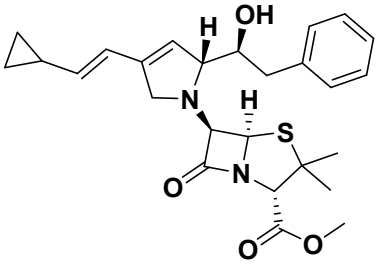
To a stirred solution of **5a** in benzene (47.8 mg, 0.10 mmol, 0.1 M), was added $Co_2(CO)_8$ (52.3 mg, 0.15 mmol, 1.5 equiv.) and 3Å molecular sieves (activated) at room temperature under N_2. After stirring for 90 min at the same temperature, trimethylamine *N*-oxide (76.6 mg, 1.02 mmol, 10 equiv.) was added at 0 °C and the resulting indigo suspension was warmed to room temperature, opened to air and stirred for 4 h at room temperature. The suspension was filtered under vacuum, concentrated by rotary evaporation and the resulting residue was purified by flash column chromatography (ISCO) to give **8a** as a colorless oil (14.4 mg, 29% yield). ^{1}H-NMR (400 MHz, C_6D_6) δ: 7.37 – 7.19 (m, 4H), 7.14 – 7.06 (m, 1H), 5.65 (s, 1H), 5.00 (d, *J* = 3.7 Hz, 1H), 4.43 (s, 1H), 4.13 (d, *J* = 9.8 Hz, 1H), 4.09 (d, *J* = 3.6 Hz, 1H), 3.81 (d, *J* = 14.0 Hz, 1H), 3.37 (d, *J* = 15.7 Hz, 1H), 3.16 (s, 3H), 2.93 (dd, *J* = 13.8, 3.6 Hz, 1H),

2.88 (s, 1H), 2.66 (dd, *J* = 14.0, 3.1 Hz, 1H), 2.59 (dd, *J* = 9.0, 2.0 Hz, 1H), 1.64 (s, 1H), 1.32 (s, 3H), 1.21 (s, 3H), 0.81 – 0.72 (m, 2H), 0.61 – 0.50 (m, 1H), 0.39 – 0.26 (m, 2H).



(2*R*,5*R*,6*S*)-6-Benzyl-5-((*E*)-2-cyclopropylvinyl)-2-methoxy-4-((2*S*,5*R*,6*R*)-2-methoxycarbonyl-3,3-dimethyl-1-aza-7-oxo-4-thiabicyclo[3.2.0]heptan-6-yl)-2-methylmorpholine (9a)

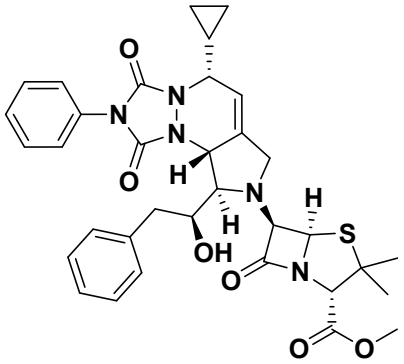
To a stirred solution of **5a** in MeOH (73.7 mg, 0.16 mmol, 0.05 M), was added $NaAuCl_4 \cdot 2H_2O$ (7.2 mg, 17.00 μmol, 0.1 equiv.) at room temperature under N_2. After stirring for 1 h at room temperature, reaction mixture was concentrated by rotary evaporation and the resulting residue was purified by flash column chromatography (ISCO) to give **9a** as a colorless oil (28.6 mg, 31% yield). ^{1}H-NMR (400 MHz, $CDCl_3$,) δ: 7.26 – 7.15 (m, 5H), 5.87 (dd, *J* = 15.4, 10.1 Hz, 1H), 5.30 (dd, *J* = 15.4, 8.8 Hz, 1H), 5.22 (d, *J* = 4.0 Hz, 1H), 4.43 (d, *J* = 4.0 Hz, 1H), 4.35 (s, 1H), 4.29 – 4.23 (m, 1H), 3.75 (s, 3H), 3.36 (dd, *J* = 10.0, 2.5 Hz, 1H), 2.92 (s, 3H), 2.80 (d, *J* = 11.5 Hz, 1H), 2.67 (dd, *J* = 13.8, 8.8 Hz, 1H), 2.61 (d, *J* = 11.5 Hz, 1H), 2.54 (dd, *J* = 13.8, 5.1 Hz, 1H), 1.70 (s, 3H), 1.54 – 1.47 (m, 1H), 1.44 (s, 3H), 1.25 (s, 3H), 0.77 (d, *J* = 8.0 Hz, 2H), 0.47 – 0.39 (m, 2H). LRMS (ESI+) calculated for $C_{27}H_{36}N_2O_5S$ $[M+H^+]^+$: 501.23, found: 501.57.



(2*R*)-4-((*E*)-2-Cyclopropylvinyl)-2-((*S*)-1-hydroxy-2-phenylethyl)-*N*-((2*S*,5*R*,6*R*)-2-methoxycarbonyl-3,3-dimethyl-1-aza-7-oxo-4-thiabicyclo[3.2.0]heptan-6-yl)-3-pyrroline (10a)

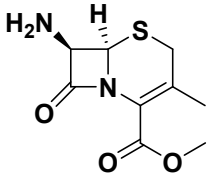
To a stirred solution of **5a** in CH_2Cl_2 (56.7 mg, 0.12 mmol, 0.05 M), was added Grubbs catalyst second generation (11.0 mg, 12.70 μmol, 0.1 equiv.) at room temperature under N_2. After stirring for 1 h under reflux, the reaction mixture was concentrated by rotary evaporation and the resulting residue was purified by flash column chromatography (ISCO) to give **10a** as a yellow oil (32.5 mg, 88% yield, *trans*/*cis* = 5.6/1, inseparable mixture). ^{1}H-NMR (400 MHz, $CDCl_3$,) δ: 7.32 – 7.27 (m, 4H), 7.23 – 7.17 (m, 1H), 6.28 (d, *J* = 15.8 Hz, 1H), 5.58 (s, 1H), 5.36 (d, *J* = 3.6 Hz, 1H), 5.03 (dd, *J* = 15.7, 8.9 Hz, 1H), 4.50 (d, *J* = 3.4

Hz, 1H), 4.43 (s, 1H), 4.20-4.07 (s, 2H), 4.15 – 4.09 (m, 1H), 3.77 (s, 3H), 3.50 (d, *J* = 10.3 Hz, 1H), 2.84 (dd, *J* = 13.8, 8.4 Hz, 1H), 2.72 (dd, *J* = 14.0, 5.1 Hz, 1H), 1.67 (s, 3H), 1.47 (s, 3H), 1.45 – 1.40 (m, 1H), 0.79 (d, *J* = 8.4 Hz, 2H), 0.45 – 0.38 (m, 2H). ^{13}C-NMR (100 MHz, $CDCl_3$) δ: 171.4, 168.7, 139.0, 137.0, 129.2, 128.4, 126.2, 121.2, 119.4, 75.08, 72.67, 72.05, 70.12, 65.6, 64.6, 59.4, 52.4, 39.4, 30.8, 26.8, 14.3, 7.6, 7.5.



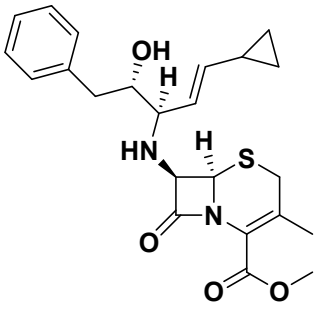
Methyl (2S,5R)-6-((5R,9aR)-5-cyclopropyl-9-((R)-1-hydroxy-2-phenylethyl)-1,3-dioxo-2-phenyl-2,3,5,7,9,9a-hexahydro-1H,8H-pyrrolo[3,4-c][1,2,4]triazolo[1,2-a]pyridazin-8-yl)-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylate (11a)

To a stirred solution of **10a** (*trans*/*cis* = 5.6/1) in CH_2Cl_2 (28.9 mg, 0.06 mmol, 0.1 M), was added 4-phenyl-1,2,4-triazolin-3,5-dione (21.6 mg, 0.12 mmol, 2 equiv.) at room temperature under N_2. After stirring for 30 min at room temperature, the reaction mixture was concentrated by rotary evaporation and the resulting residue was purified by flash column chromatography (ISCO) to give **11a** as a colorless oil (14.5 mg, 37% yield). ^{1}H-NMR (400 MHz, $CDCl_3$,) δ: 7.58 – 7.45 (m, 4H), 7.43 – 7.36 (m, 1H), 7.28 – 7.23 (m, 3H), 7.22 – 7.14 (m, 1H), 5.85 (s, 1H), 5.38 (d, *J* = 3.7 Hz, 1H), 4.55 (s, 1H), 4.52 (d, *J* = 3.7 Hz, 1H), 4.47 (s, 1H), 4.18 (dd, *J* = 8.2, 3.8 Hz, 1H), 4.10 – 4.03 (m, 1H), 3.86 – 3.79 (m, 1H), 3.78 (s, 3H), 3.68 (d, *J* = 13.9 Hz, 1H), 3.36 (d, *J* = 13.9 Hz, 1H), 3.20 (d, *J* = 14.0 Hz, 1H), 2.68 (dd, *J* = 14.0, 10.4 Hz, 1H), 1.74 (s, 3H), 1.49 (s, 3H), 1.16 – 1.07 (m, 1H), 0.98 – 0.90 (m, 1H), 0.72 – 0.56 (m, 2H), 0.41 – 0.32 (m, 1H). LRMS (ESI+) calculated for $C_{34}H_{37}N_5O_6S$ $[M+H^+]^+$: 644.25, found: 644.70.



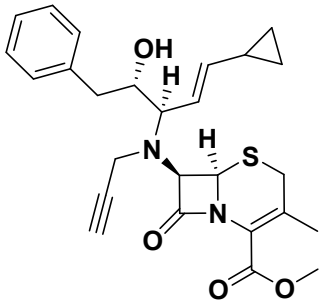
7-amino-deacetoxycephalosporanic acid (7-ACDA) methyl ester (1b)

General procedure A was followed using 7-ACDA (2.00 g, 9.34 mmol), methanol (4 mL), and (diazomethyl)trimethylsilane (4.7 mL, 9.34 mmol) to give the desired compound **1b** as a pale yellow solid (1.35 g, 64% yield). ^{1}H-NMR (400 MHz, $CDCl_3$) δ: 4.89 (s, 1H), 4.69 (s, 1H), 3.79 (s, 3H), 3.47 (d, *J* = 18.0 Hz, 1H), 3.17 (d, *J* = 17.9 Hz, 1H), 2.11 (s, 3H), 1.82 (s, 2H).



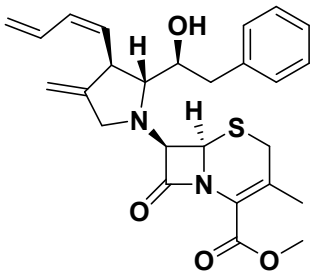
Methyl (6R,7R)-7-(((4R,E)-1-cyclopropyl-4-hydroxy-5-phenylpent-1-en-3-yl)amino)-3-methyl-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylate (4b)

General procedure B was followed using (4R,5S)-5-benzyl-2,2-dimethyl-4-hydroxy-1,3-dioxolane **2** (770 mg, 3.70 mmol, 0.2 M) in 18.5 mL of the solvent system of 9:1 EtOH–hexafluoroisopropanol (v/v), 7-ACDA methyl ester **1a** (1.27 g, 5.55 mmol, 1.5 equiv) and (*E)*-2-cyclopropylvinylboronic acid **3** (621 mg, 5.55 mmol, 1.5 equiv) to give the desired product **4b** as a pale yellow oil (1.03 g, 65% yield). ^{1}H-NMR (400 MHz, $CDCl_3$) δ: 7.34 – 7.16 (m, 5H), 5.57 (dd, *J* = 14.4, 8.0 Hz, 1H), 5.25 (dd, *J* = 15.6, 8.7 Hz, 1H), 4.85 (d, *J* = 4.1 Hz, 1H), 4.57 (d, *J* = 5.1 Hz, 1H), 4.06 – 3.93 (m, 1H), 3.83 (s, 3H), 3.49 (d, *J* = 18.1 Hz, 1H), 3.23 (s, 1H), 3.20 (d, *J* = 13.4 Hz, 1H), 2.81 – 2.66 (m, 2H), 2.44 (br, 1H), 2.12 (s, 3H), 1.54 – 1.38 (m, 1H), 0.77 (d, *J* = 6.6 Hz, 2H), 0.49 – 0.38 (m, 2H). ^{13}C-NMR (100 MHz, $CDCl_3$) δ: 166.9, 162.7, 142.1, 138.1, 129.4, 128.5, 128.5, 126.4, 122.7, 122.6, 66.4, 64.8, 60.4, 58.5, 52.3, 39.6, 20.1, 13.8, 7.0, 7.0. LRMS (ESI$^+$) m/z calculated for $C_{23}H_{28}N_2O_4S$ ([M+H$^+$]$^+$): 429.18, found: 429.53.



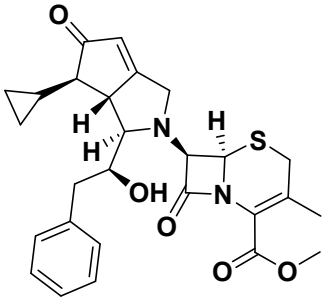
Methyl (6R,7R)-7-(((4R,E)-1-cyclopropyl-4-hydroxy-5-phenylpent-1-en-3-yl)(prop-2-yn-1-yl)amino)-3-methyl-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylate (5b)

General procedure C was followed using **4b** (897 mg, 2.09 mmol, 0.2 M) and $NaHCO_3$ (1.76 g, 20.93 mmol, 10 equiv.) in DMF (10.5 mL) and propargyl bromide (3.1 mL, 20.93 mmol, 10 equiv., 80% in toluene) to give the desired compound **5b** (640 mg, 66% yield). ^{1}H-NMR (400 MHz, $CDCl_3$) δ: 7.35 – 7.19 (m, 5H), 5.73 (dd, *J* = 15.3, 9.5 Hz, 1H), 5.33 (dd, *J* = 15.5, 8.8 Hz, 1H), 4.80 (dd, *J* = 4.4 Hz, 1H), 4.72 (dd, *J* = 4.5 Hz, 1H), 4.10 – 4.07 (m, 1H), 3.99 (d, *J* = 17.8 Hz, 1H), 3.85 (s, 3H), 3.71 (d, *J* = 17.7 Hz, 1H), 3.51 (d, *J* = 18.3 Hz, 1H), 3.39 (dd, *J* = 9.6, 3.5 Hz, 1H), 3.23 (d, *J* = 18.3 Hz, 1H), 2.75 (d, *J* = 6.6 Hz, 2H), 2.51 (br, 1H), 2.23 (s, 1H), 2.12 (s, 3H), 1.57 – 1.46 (m, 1H), 0.79 (d, *J* = 8.0 Hz, 2H), 0.52 – 0.42 (m, 2H). ^{13}C-NMR (100 MHz, $CDCl_3$) δ: 171.1, 162.9, 143.0, 138.6, 129.3, 128.4, 126.3, 122.5, 120.6, 73.6, 71.5, 69.7, 67.5, 60.4, 59.4, 52.4, 40.2, 39.9, 30.7, 20.0, 13.8, 7.1, 7.0. LRMS (ESI$^+$) m/z calculated for $C_{26}H_{30}N_2O_4S$ ([M+H$^+$]$^+$): 467.20, found: 467.53.



Methyl (6R,7R)-7-((2R,3R)-3-((Z)-buta-1,3-dien-1-yl)-2-((S)-1-hydroxy-2-phenylethyl)-4-methylenepyrrolidin-1-yl)-3-methyl-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylate (7b)

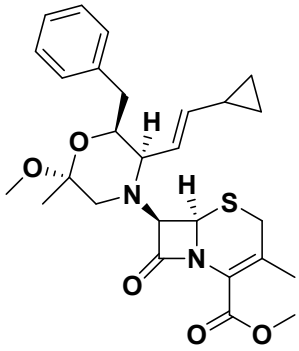
To a stirred solution of **5b** in benzene (57.8 mg, 0.12 mmol, 0.05 M), was added bis(triphenylphosphine)palladium acetate (18.4 mg, 25.00 μmol, 0.2 equiv.) at room temperature under N_2. After stirring for 1 h at 80 °C, the resulting solution was concentrated by rotary evaporation and the residue was purified by flash column chromatography (ISCO) to give **7b** as a colorless oil (32.4 mg, 56% yield). Selected spectroscopic data for major diastereomer: ^{1}H-NMR (400 MHz, C_6D_6) δ: 7.42 – 7.20 (m, 5H), 6.28 (dd, *J* = 10.8, 9.4 Hz, 1H), 6.06 (dd, *J* = 15.2, 10.4 Hz, 1H), 5.57 (dd, *J* = 15.1, 8.7 Hz, 1H), 5.07 (d, *J* = 16.9 Hz, 1H), 4.95 (d, *J* = 8.4 Hz, 1H), 4.86 (dd, *J* = 14.6, 2.2 Hz, 1H), 4.20 (d, *J* = 4.4 Hz, 1H), 4.14 (d, *J* = 4.4 Hz, 1H), 3.95 (d, *J* = 13.8 Hz, 1H), 3.72 (d, *J* = 2.1 Hz, 1H), 3.63 – 3.56 (m, 1H), 3.54 (s, 3H), 3.25 (dd, *J* = 6.0, 2.5 Hz, 1H), 2.90 (d, *J* = 8.9 Hz, 1H), 2.78 (dd, *J* = 13.9, 4.2 Hz, 1H), 2.61 (d, *J* = 18.5 Hz, 1H), 2.29 (d, *J* = 18.1 Hz, 1H), 1.67 (s, 3H), 0.43 (br, 1H). ^{13}C-NMR (100 MHz, C_6D_6) δ: 163.7, 162.3, 149.7, 139.8, 136.9, 135.5, 132.3, 129.5, 128.2, 125.8, 122.8, 115.9, 106.8, 72.6, 71.6, 71.1, 57.4, 57.2, 51.6, 47.0, 39.5, 29.4, 19.2.



Methyl (6R,7S)-7-((3S,3aS,4S)-4-cyclopropyl-3-((R)-1-hydroxy-2-phenylethyl)-5-oxo-3,3a,4,5-tetrahydrocyclopenta[c]pyrrol-2(1H)-yl)-3-methyl-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylate (8b)

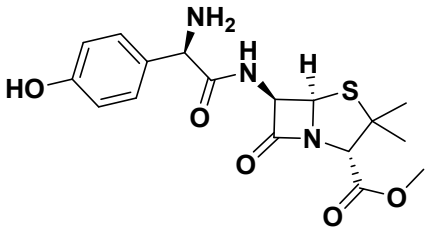
To a stirred solution of **5b** in benzene (80.0 mg, 0.17 mmol, 0.1 M), was added $Co_2(CO)_8$ (87.9 mg, 0.26 mmol, 1.5 equiv.) and 3Å molecular sieves (activated) at room temperature under N_2. After stirring for 90 min at the same temperature, trimethylamine *N*-oxide (128.78 mg, 1.71 mmol, 10 equiv.) was added at 0 °C and the resulting indigo suspension was warmed to room temperature, opened to air and stirred for 4 h at room temperature. The suspension was filtered under vacuum, concentrated by rotary evaporation and the resulting residue was purified by flash column chromatography (ISCO) to give 8a as a colorless oil (24.4 mg, 30% yield). ^{1}H-NMR (400 MHz, C_6D_6) δ: 7.32 – 7.18 (m, 4H), 7.13 – 7.08 (m, 1H), 5.72 (s, 1H), 4.43 (d, *J* = 4.2 Hz, 1H), 4.17 (d, *J* = 4.2 Hz, 1H), 3.99 (d, *J* = 10.0 Hz, 1H), 3.71 (dd, *J* =

62.8, 15.6 Hz, 2H), 3.58 (s, 3H), 3.16 (s, 1H), 2.95 – 2.88 (m, 1H), 2.90 – 2.88 (m, 1H), 2.87 (s, 1H), 2.66 (d, *J* = 18.0 Hz, 1H), 2.64 (s, 1H), 2.31 (d, *J* = 18.2 Hz, 1H), 1.69 (s, 3H), 0.81 – 0.69 (m, 1H), 0.61 – 0.52 (m, 2H), 0.37 – 0.31 (m, 1H), 0.29 – 0.23 (m, 1H). ^{13}C-NMR (100 MHz, C_6D_6) δ: 178.6, 163.3, 162.8, 129.8, 128.9, 128.8, 128.7, 126.7, 124.2, 122.9, 71.8, 71.5, 70.5, 57.5, 54.2, 52.1, 51.9, 39.0, 38.7, 30.3, 19.5, 11.9, 3.6, 3.3. LRMS (ESI$^+$) m/z calculated for $C_{27}H_{30}N_2O_5S$ ([M+H$^+$]$^+$): 495.19, found: 495.56.



Methyl (6R,7R)-7-((6R)-6-benzyl-5-((E)-2-cyclopropylvinyl)-2-methoxy-2-methylmorpholino)-3-methyl-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylate (9b)

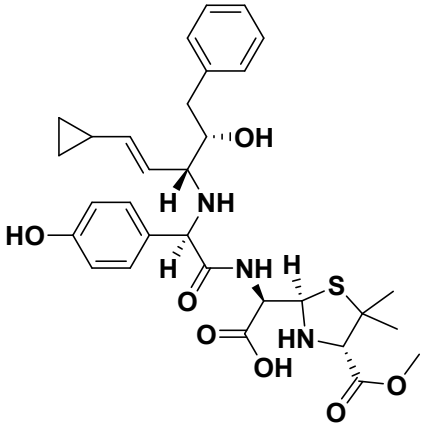
To a stirred solution of **5b** in MeOH (33.7 mg, 0.07 mmol, 0.05 M), was added $NaAuCl_4 \cdot 2H_2O$ (2.9 mg, 7.00 μmol, 0.1 equiv.) at room temperature under N_2. After stirring for 1 h at room temperature, reaction mixture was concentrated by rotary evaporation and the resulting residue was purified by flash column chromatography (ISCO) to give **9b** as a colorless oil (12.0 mg, 33% yield). ^{1}H-NMR (400 MHz, $CDCl_3$) δ: 7.28 – 7.13 (m, 5H), 5.86 (dd, *J* = 15.5, 9.9 Hz, 1H), 5.49 (dd, *J* = 15.4, 8.9 Hz, 1H), 4.78 (d, *J* = 4.4 Hz, 1H), 4.39 (d, *J* = 4.3 Hz, 1H), 4.33 – 4.27 (m, 1H), 3.85 (s, 3H), 3.61 (d, *J* = 10.6 Hz, 1H), 3.45 (d, *J* = 18.1 Hz, 1H), 3.21 (d, *J* = 18.2 Hz, 1H), 2.94 (s, 3H), 2.73 (q, *J* = 11.1 Hz, 2H), 2.66 (d, *J* = 8.4 Hz, 1H), 2.57 (d, *J* = 5.1 Hz, 1H), 2.06 (s, 3H), 1.58 – 1.48(m, 1H), 1.28 (s, 3H), 0.83 – 0.73 (m, 2H), 0.52 – 0.43 (m, 2H). ^{13}C-NMR (100 MHz, $CDCl_3$) δ: 163.1, 162.7, 143.5, 138.8, 129.3, 128.4, 126.3, 122.8, 117.0, 96.8, 72.3, 71.4, 60.0, 59.4, 57.1, 54.2, 52.3, 48.1, 38.7, 30.9, 21.9, 20.2, 14.1, 7.0, 7.0. LRMS (ESI+) calculated for $C_{27}H_{34}N_2O_5S$ [M+H$^+$]$^+$: 499.22, found: 499.55.



Amoxicillin methyl ester (1c)

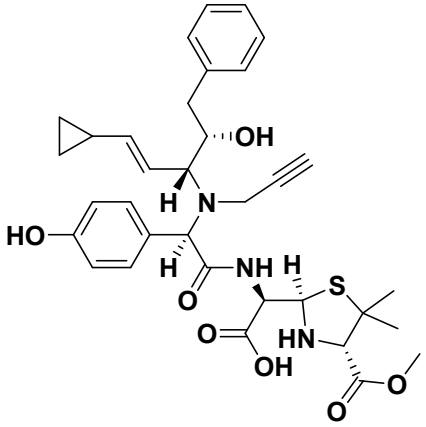
General procedure A was followed using amoxicillin (500 mg, 1.37 mmol), methanol (1 mL), and (diazomethyl)trimethylsilane (685 μL, 1.37 mmol) to give the desired compound **1c** as a pale yellow solid (383 g, 74% yield). ^{1}H-NMR (400 MHz, $CDCl_3$) δ: 8.10 (br, 1H), 7.06 (d, *J*

= 7.8 Hz, 2H), 6.61 (d, *J* = 7.7 Hz, 2H), 5.60 (s, 1H), 5.52 (s, 1H), 4.44 (s, 2H), 4.28 (br, 2H), 3.77 (s, 3H), 1.61 (s, 3H), 1.47 (s, 3H).



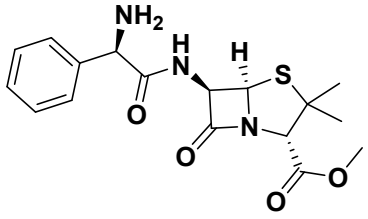
(R)-2-((R)-2-(((3R,4S,E)-1-cyclopropyl-4-hydroxy-5-phenylpent-1-en-3-yl)amino)-2-(4-hydroxyphenyl)acetamido)-2-((2R,4S)-4-(methoxycarbonyl)-5,5-dimethylthiazolidin-2-yl)acetic acid (4c)

General procedure B was followed using (4R,5S)-5-benzyl-2,2-dimethyl-4-hydroxy-1,3-dioxolane **2** (526 mg, 2.52 mmol, 0.2 M) in 12.5 mL of the solvent system of 9:1 EtOH–hexafluoroisopropanol (v/v), AMX methyl ester **1c** (1.14 g, 3.00 mmol, 1.2 equiv) and (*E*)-2-cyclopropylvinylboronic acid **3** (418 mg, 2.88 mmol, 1.5 equiv) to give the desired product **4c** as a pale yellow solid (671 mg, 46% yield). ^{1}H-NMR (400 MHz, $CDCl_3$) δ: 7.37 (d, *J* = 9.1 Hz, 2H), 7.33 – 7.21 (m, 5H), 7.17 (d, *J* = 6.9 Hz, 2H), 6.66 (d, *J* = 6.8 Hz, 2H), 5.52 (dd, *J* = 15.4, 8.7 Hz, 1H), 5.15 – 5.12 (m, 1H), 5.11 (dd, *J* = 15.4, 8.9 Hz, 1H), 4.58 (dd, *J* = 8.9, 3.3 Hz, 1H), 4.36 (s, 1H), 3.95 – 3.84 (m, 1H), 3.67 (s, 3H), 3.49 (s, 1H), 3.08 (d, *J* = 9.1 Hz, 1H), 2.76 (ddt, *J* = 3.8, 3.1 Hz, 1H), 2.70 (d, *J* = 9.3 Hz, 1H), 1.51 (s, 3H), 1.49 – 1.42 (m, 1H), 1.18 (s, 3H), 0.78 (d, *J* = 7.2 Hz, 2H), 0.42 (d, *J* = 5.1 Hz, 2H). ^{13}C-NMR (100 MHz, $CDCl_3$) δ:173.0, 169.9, 169.7, 140.6, 129.4, 129.3, 128.5, 126.5, 126.3, 123.1, 116.1, 74.2, 72.7, 65.8, 63.2, 62.6, 58.8, 57.1, 52.3, 39.5, 26.5, 26.2, 13.7, 7.1, 7.0.



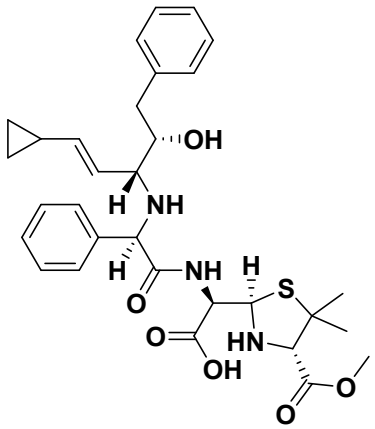
(R)-2-((R)-2-(((3R,4S,E)-1-cyclopropyl-4-hydroxy-5-phenylpent-1-en-3-yl)(prop-2-yn-1-yl)amino)-2-(4-hydroxyphenyl)acetamido)-2-((2R,4S)-4-(methoxycarbonyl)-5,5-dimethylthiazolidin-2-yl)acetic acid (5c)

General procedure C was followed using **4c** (671 mg, 1.16 mmol, 0.2 M) and $NaHCO_3$ (145 mg, 1.74 mmol, 1.5 equiv.) in DMF (5.8 mL) and propargyl bromide (155 µL, 1.39 mmol, 1.2 equiv., 80% in toluene) at room temperature to give the desired compound **5c** (404 mg, 56% yield). ^{1}H-NMR (400 MHz, $CDCl_3$) δ: 7.99 (d, *J* = 8.8 Hz, 1H), 7.32 – 7.24 (m, 3H), 7.22 – 7.15 (m, 2H), 7.05 (d, *J* = 8.1 Hz, 2H), 6.57 (d, *J* = 8.1 Hz, 2H), 5.78 (dd, *J* = 15.4, 8.9 Hz, 1H), 5.11 (dd, *J* = 15.4, 8.9 Hz, 1H), 5.06 (d, *J* = 5.9 Hz, 1H), 4.71 (s, 1H), 4.67 (d, *J* = 5.9 Hz, 2H), 4.22 – 4.10 (m, 1H), 3.79 (s, 1H), 3.72 (s, 3H), 3.54 (d, *J* = 17.6 Hz, 1H), 3.33 (s, 1H), 3.28 (d, *J* = 17.5 Hz, 1H), 2.80 (dd, *J* = 4.1, 3.4 Hz, 1H), 2.64 (dd, *J* = 13.7, 8.9 Hz, 1H), 2.15 (s, 1H), 1.54 (s, 3H), 1.52 – 1.43 (m, 1H), 1.19 (s, 3H), 0.76 (d, *J* = 8.1 Hz, 2H), 0.46 – 0.37 (m, 2H). ^{13}C-NMR (100 MHz, $CDCl_3$) δ: 173.8, 170.1, 169.8, 142.5, 131.1, 129.5, 128.5, 127.0, 126.3, 120.9, 115.8, 81.3, 73.6, 73.0, 72.6, 68.4, 66.2, 66.2, 58.9, 57.2, 52.3, 41.4, 39.2, 27.1, 26.9, 13.9, 7.1, 7.0.



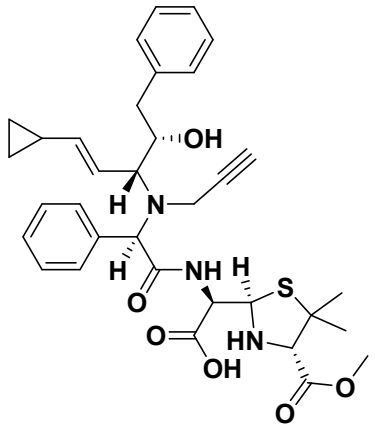
Ampicillin methyl ester (1d)

General procedure A was followed using amoxicillin (500 mg, 1.37 mmol), methanol (1 mL), and (diazomethyl)trimethylsilane (685 µL, 1.37 mmol) to give the desired compound **1d** as a pale yellow solid (383 g, 74% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ: 8.03 (d, *J* = 9.2 Hz, 1H), 7.29 – 7.42 (m, 5H), 5.68 (dd, *J* = 9.4, 4.1 Hz, 1H), 5.56 (d, *J* = 4.2 Hz, 1H), 4.60 (s, 1H), 4.47 (s, 1H), 3.79 (s, 3H), 2.62 (br, 2H), 1.66 (s, 3H), 1.51 (s, 3H).



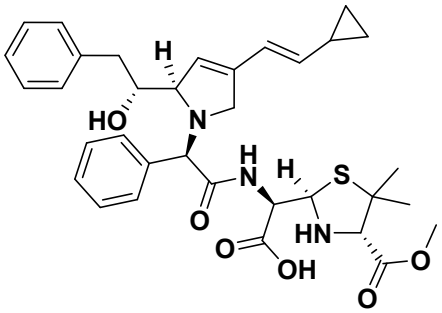
(R)-2-((R)-2-(((3R,4S,E)-1-cyclopropyl-4-hydroxy-5-phenylpent-1-en-3-yl)amino)-2-phenylacetamido)-2-((2R,4S)-4-(methoxycarbonyl)-5,5-dimethylthiazolidin-2-yl)acetic acid (4d)

General procedure B was followed using (4R,5S)-5-benzyl-2,2-dimethyl-4-hydroxy-1,3-dioxolane **2** (200 mg, 0.96 mmol, 0.2 M) in 4.8 mL of the solvent system of 9:1 EtOH–hexafluoroisopropanol (v/v), AMP methyl ester **1d** (418 mg, 1.15 mmol, 1.2 equiv) and (*E)*-2-cyclopropylvinylboronic acid **3** (161 mg, 1.44 mmol, 1.5 equiv) to give the desired product **4d** as a pale yellow solid (234 mg, 41% yield). ^{1}H-NMR (400 MHz, $CDCl_3$) δ: 7.73 (s, 1H), 7.49 – 7.08 (m, 10H), 5.53 (dd, *J* = 15.3, 8.7 Hz, 1H), 5.13 (d, *J* = 3.8 Hz, 1H), 5.08 (dd, *J* = 15.3, 8.7 Hz, 1H), 4.60 (dd, *J* = 9.1, 3.8 Hz, 1H), 4.42 (s, 1H), 3.86 – 3.70 (m, 2H), 3.68 (s, 3H), 3.46 (s, 1H), 3.01 (dd, *J* = 8.8, 3.7 Hz, 1H), 2.78 (dd, *J* = 13.9, 3.8 Hz, 1H), 2.67 (dd, *J* = 13.8, 9.2 Hz, 1H), 2.45 (s, 1H), 2.29 (br, 1H), 1.48 (s, 3H), 1.48 – 1.41 (m, 1H), 1.17 (s, 3H), 0.84 – 0.67 (m, 2H), 0.48 – 0.29 (m, 2H). ^{13}C-NMR (100 MHz, $CDCl_3$) δ: 169.8, 169.7, 164.8, 142.8, 129.3, 129.1, 128.5, 126.3, 122.3, 76.0, 72.7, 65.5, 62.8, 62.7, 58.6, 57.0, 52.2, 39.7, 26.5, 26.3, 13.7, 7.1, 7.0.



(R)-2-((R)-2-(((3R,4S,E)-1-cyclopropyl-4-hydroxy-5-phenylpent-1-en-3-yl)(prop-2-yn-1-yl)amino)-2-phenylacetamido)-2-((2R,4S)-4-(methoxycarbonyl)-5,5-dimethylthiazolidin-2-yl)acetic acid (5d)

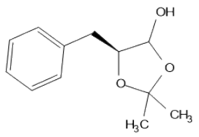
General procedure C was followed using **4d** (108 mg, 0.19 mmol, 0.2 M) and $NaHCO_3$ (24.1 mg, 0.29 mmol, 1.5 equiv.) in DMF (1.0 mL) and propargyl bromide (25 µL, 0.23 mmol, 1.2 equiv., 80% in toluene) at room temperature to give the desired compound **5d** (44.0 mg, 38% yield). ^{1}H-NMR (400 MHz, $CDCl_3$) δ: 7.73 (s, 1H), 7.36 – 7.16 (m, 10H), 5.81 (dd, *J* = 15.3, 9.5 Hz, 1H), 5.18 – 5.07 (m, 1H), 5.05 (d, *J* = 5.9 Hz, 1H), 4.84 (s, 1H), 4.71 (dd, *J* = 9.1, 6.0 Hz, 1H), 4.16 – 4.11 (m, 1H), 3.75 (s, 1H), 3.74 (s, 3H), 3.64 – 3.52 (m, 1H), 3.43 – 3.30 (m, 2H), 2.84 – 2.74 (m, 1H), 2.73 – 2.63 (m, 1H), 2.16 (br, 1H), 1.69 – 1.57 (m, 1H), 1.51 (s, 3H), 1.19 (s, 3H), 0.82 – 0.74 (m, 2H), 0.49 – 0.39 (m, 2H). ^{13}C-NMR (100 MHz, $CDCl_3$) δ: 173.4, 170.2, 169.8, 148.6, 129.8, 129.3, 128.4, 126.3, 121.5, 78.5, 73.5, 73.2, 72.4, 68.4, 66.2, 66.1, 58.7, 56.7, 52.2, 41.4, 39.4, 27.0, 26.8, 13.8, 7.0, 6.9.



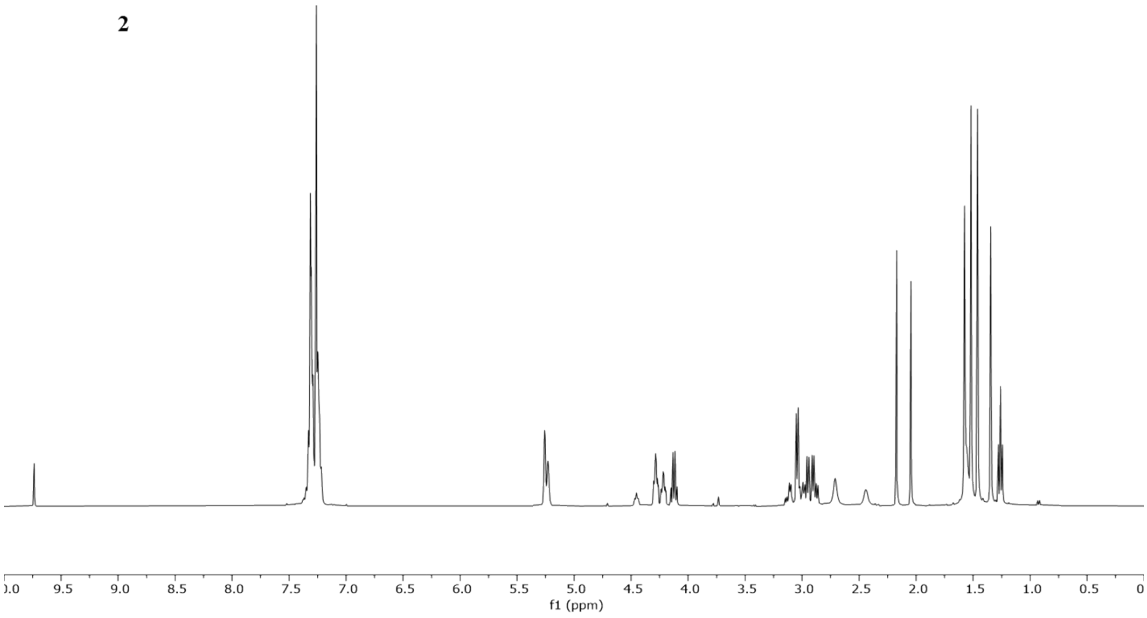
(2R)-2-((2R)-2-(4-((E)-2-cyclopropylvinyl)-2-((R)-1-hydroxy-2-phenylethyl)-2,5-dihydro-1H-pyrrol-1-yl)-2-phenylacetamido)-2-((2R,4S)-4-(methoxycarbonyl)-5,5-dimethylthiazolidin-2-yl)acetic acid (10d)

To a stirred solution of **5d** in CH_2Cl_2 (20.0 mg, 0.03 mmol, 0.05 M), was added Grubbs catalyst second generation (2.8 mg, 3.30 μmol, 0.1 equiv.) at room temperature under N_2. After stirring for 1 h under reflux, the reaction mixture was concentrated by rotary evaporation and the resulting residue was purified by flash column chromatography (ISCO) to give **10a** as a yellow oil (11.9 mg, 60% yield). ^{1}H-NMR (400 MHz, $CDCl_3$) δ: 7.47 – 7.15 (m, 10H), 7.04 – 6.91 (m, 1H), 6.29 (d, *J* = 15.8 Hz, 1H), 5.60 (d, *J* = 51.8 Hz, 1H), 5.22 – 5.13 (m, 1H), 5.10 – 5.00 (m, 1H), 4.83 (s, 1H), 4.70 (dd, *J* = 8.9, 5.4 Hz, 1H), 4.20 – 4.07 (m, 2H), 4.15 – 4.09 (m, 1H), 3.77 (s, 3H),, 3.58 – 3.48 (m, 1H), 3.37 (s, 1H), 2.71 – 2.62 (m, 1H), 2.52 (dd, *J* = 13.7, 5.3 Hz, 1H), 1.51 (s, 3H), 1.50 – 1.38 (m, 1H), 1.22 (s, 3H), 0.82 – 0.74 (m, 2H), 0.47 – 0.38 (m, 2H).

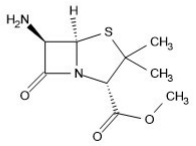
^{1}H NMR ($CDCl_3$, 400 MHz)



2

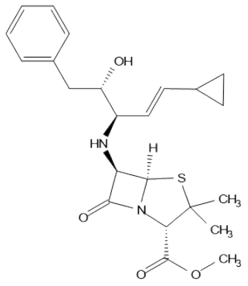


^{1}H NMR ($CDCl_3$, 400 MHz)



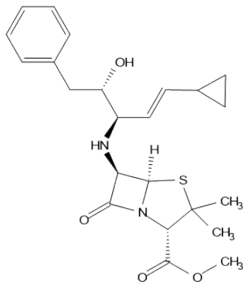
1a

^{1}H NMR ($CDCl_3$, 400 MHz)



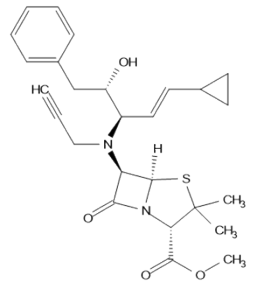
4a

^{13}C NMR ($CDCl_3$, 100 MHz)



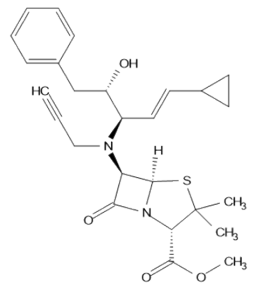
4a

^{1}H NMR ($CDCl_3$, 400 MHz)



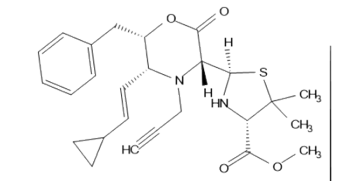
5a

^{13}C NMR ($CDCl_3$, 100 MHz)



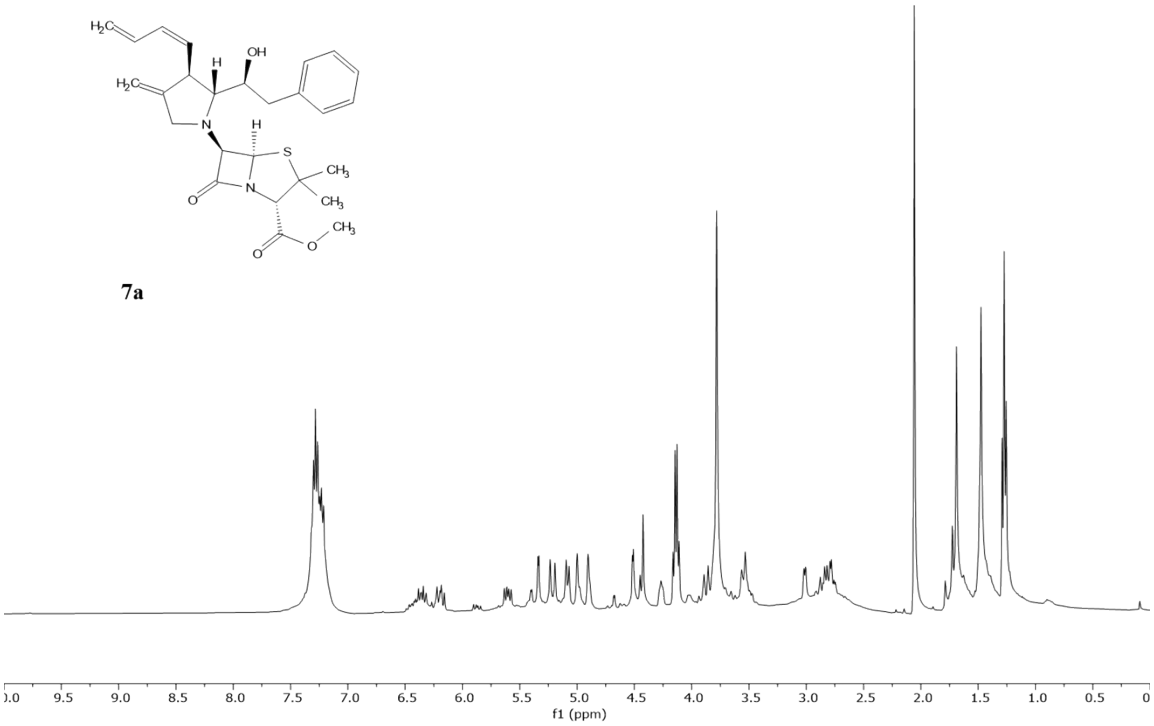
5a

^{1}H NMR (C_6D_6, 400 MHz)

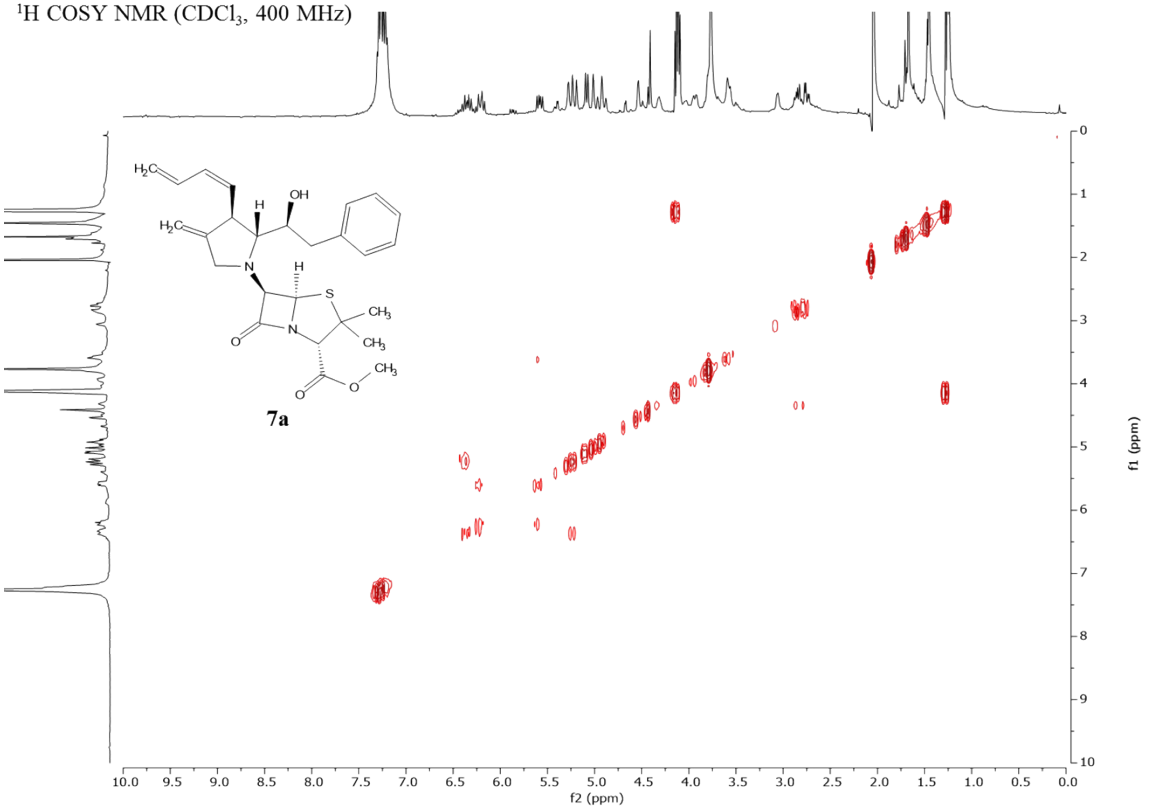


6a

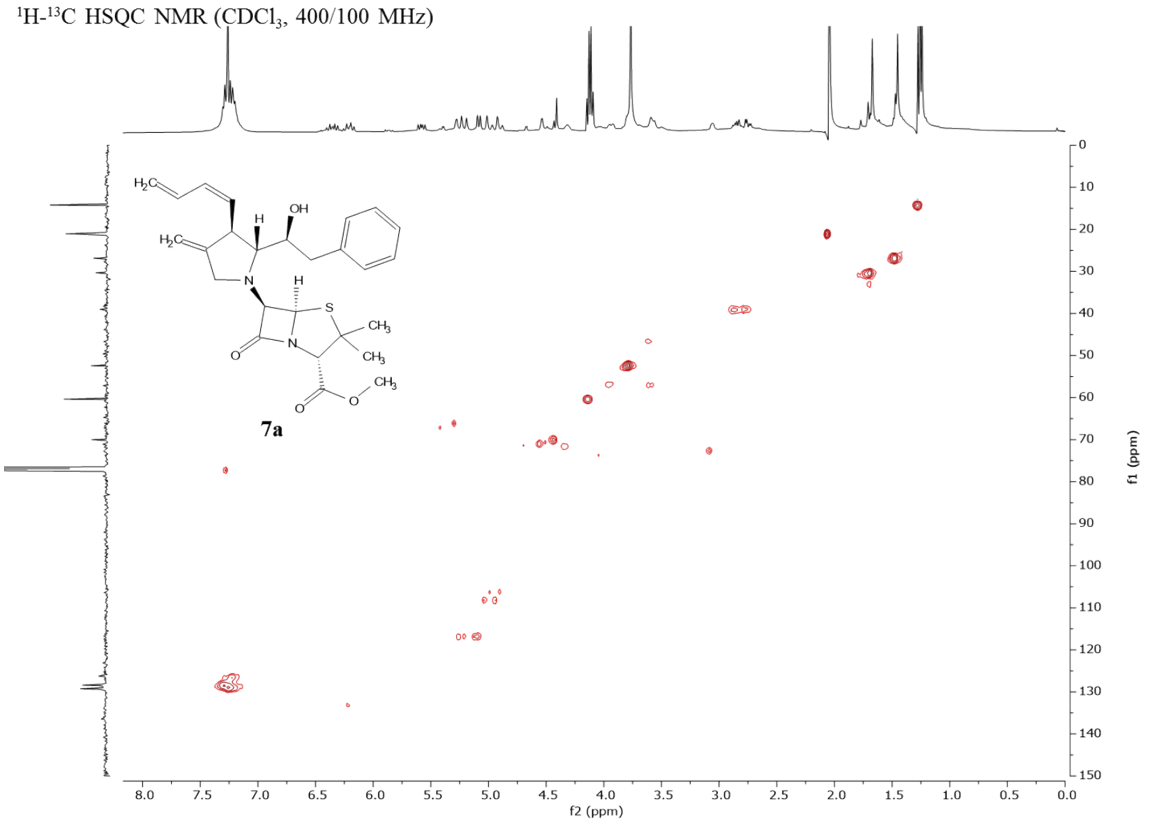
^{1}H NMR ($CDCl_3$, 400 MHz)



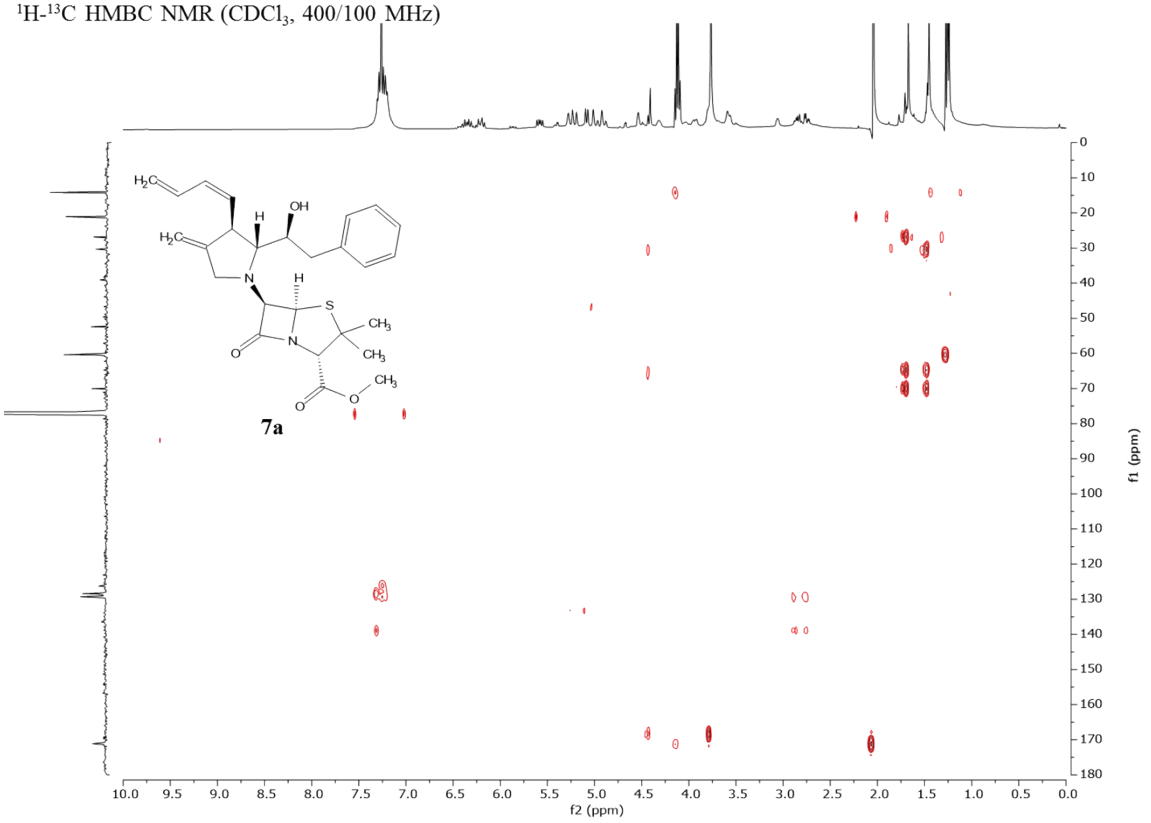
^{1}H COSY NMR ($CDCl_3$, 400 MHz)



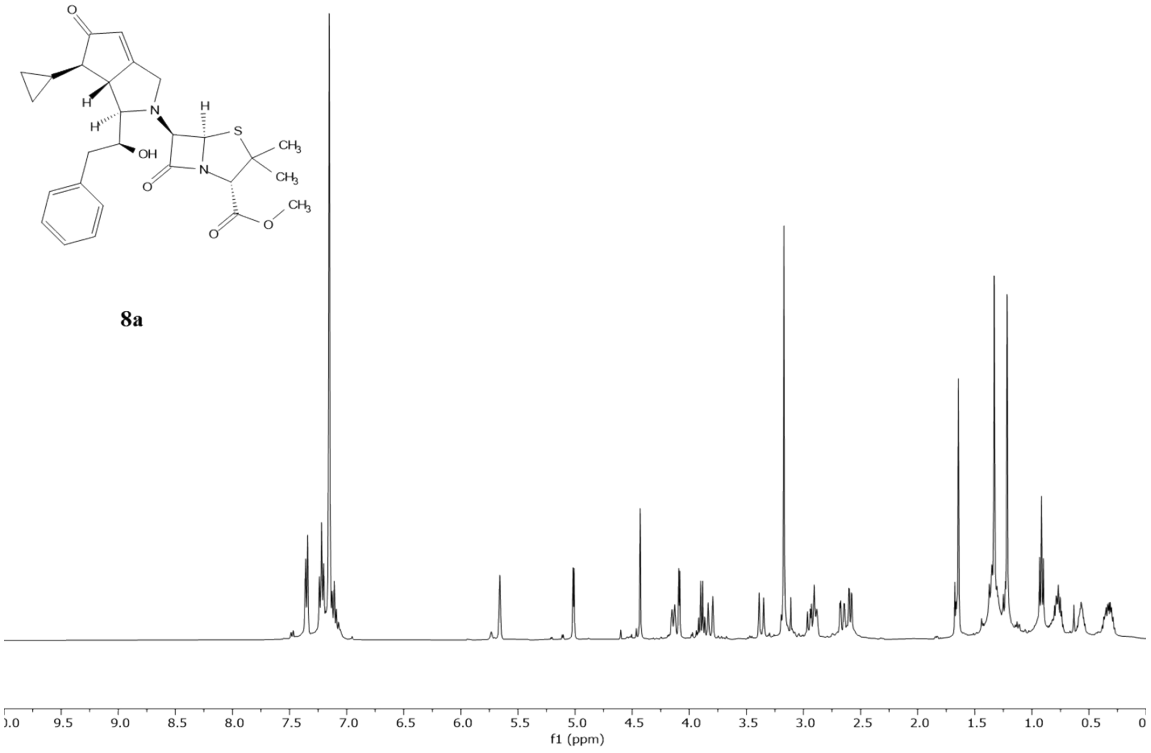
^{1}H-^{13}C HSQC NMR ($CDCl_3$, 400/100 MHz)



^{1}H-^{13}C HMBC NMR ($CDCl_3$, 400/100 MHz)

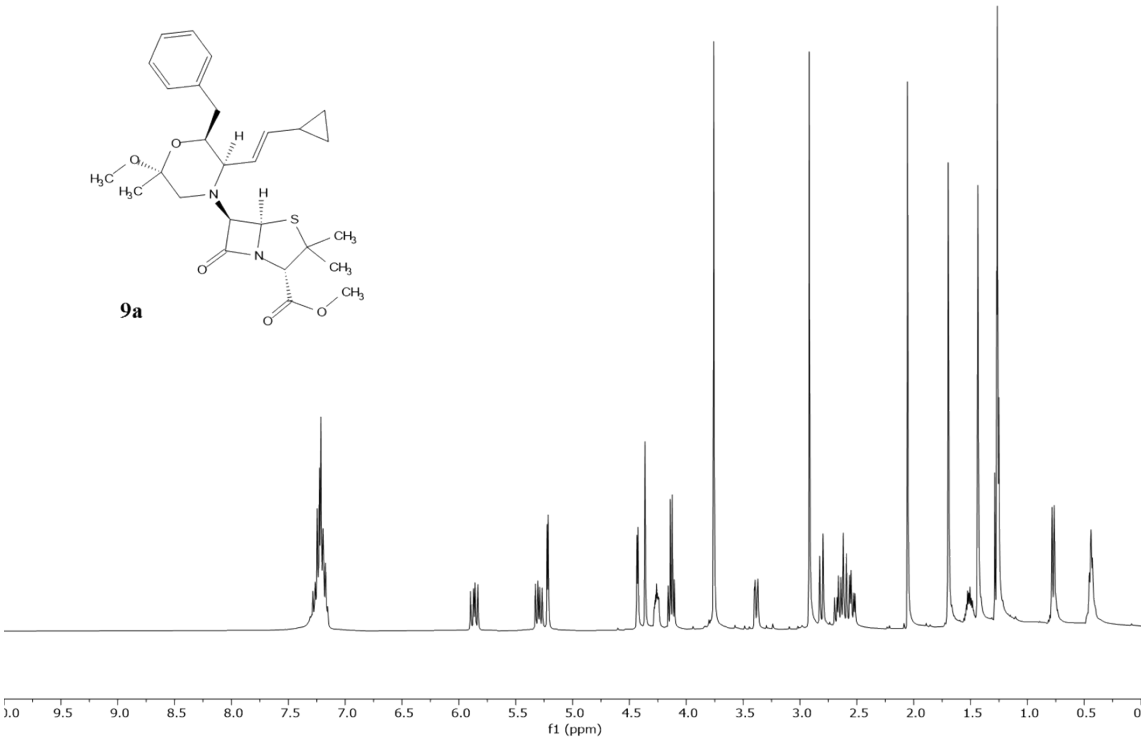


^{1}H NMR (C_6D_6, 400 MHz)

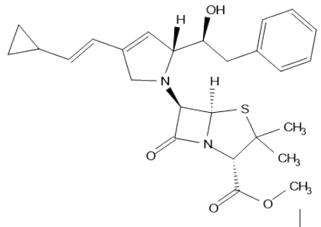


8a

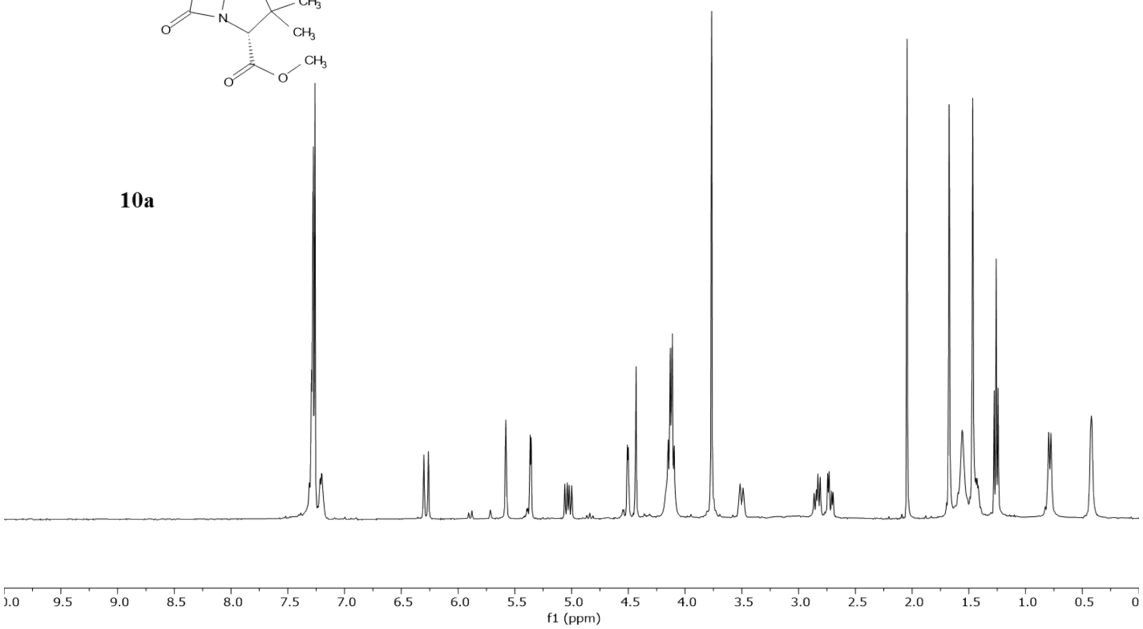
^{1}H NMR ($CDCl_3$, 400 MHz)



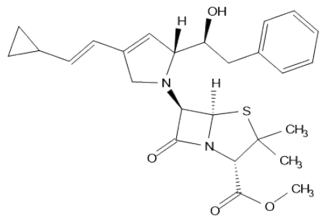
^{1}H NMR ($CDCl_3$, 400 MHz)



10a

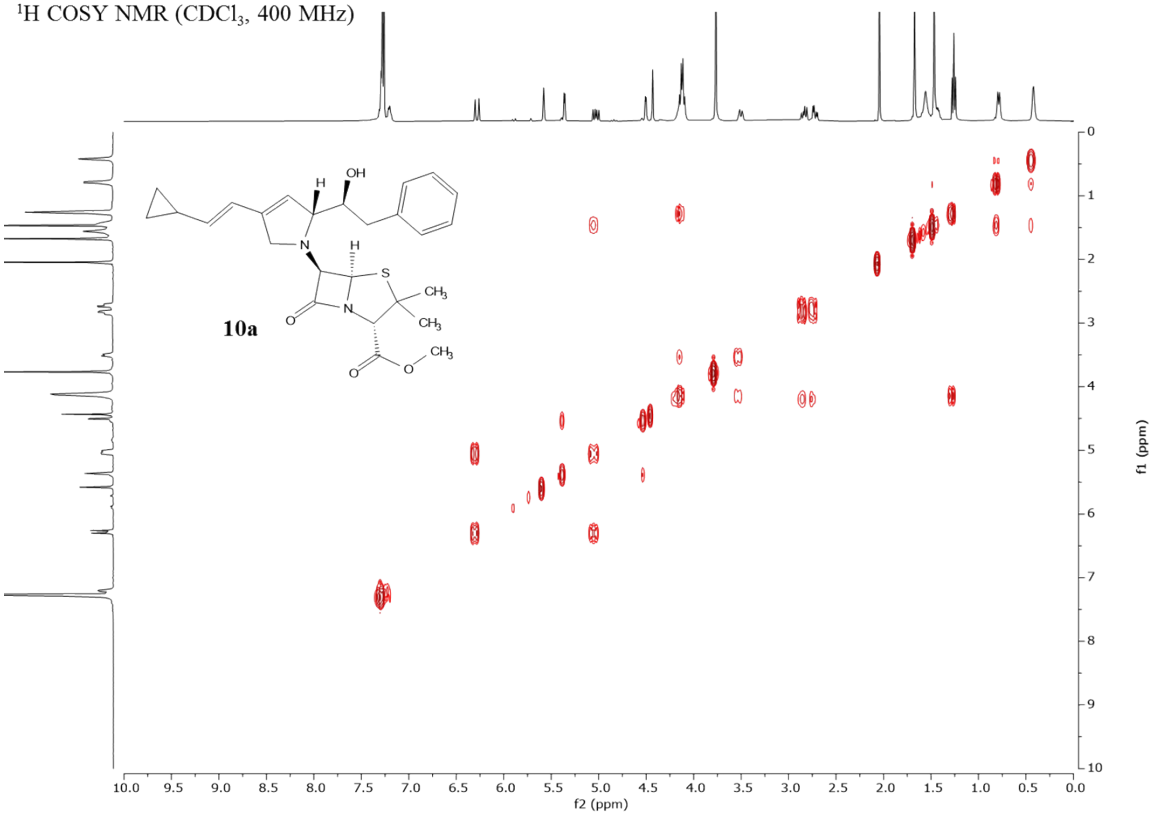


^{13}C NMR ($CDCl_3$, 100 MHz)

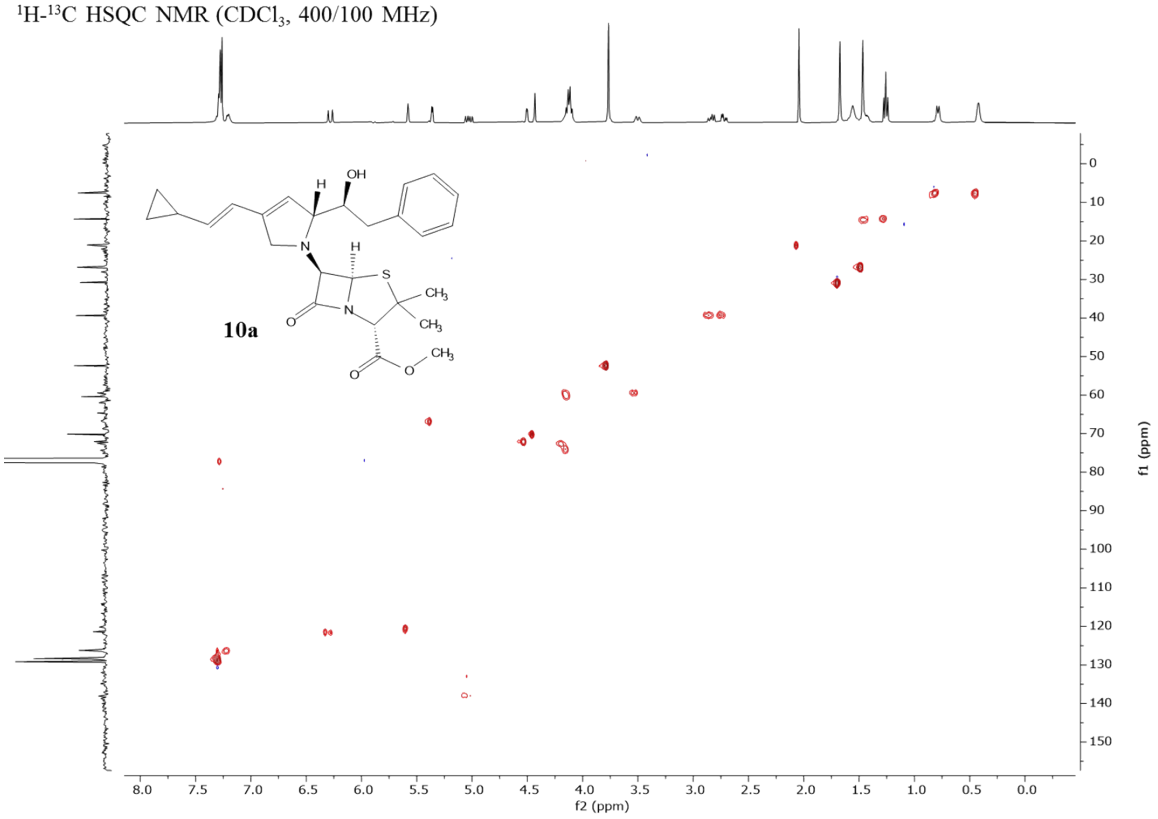


10a

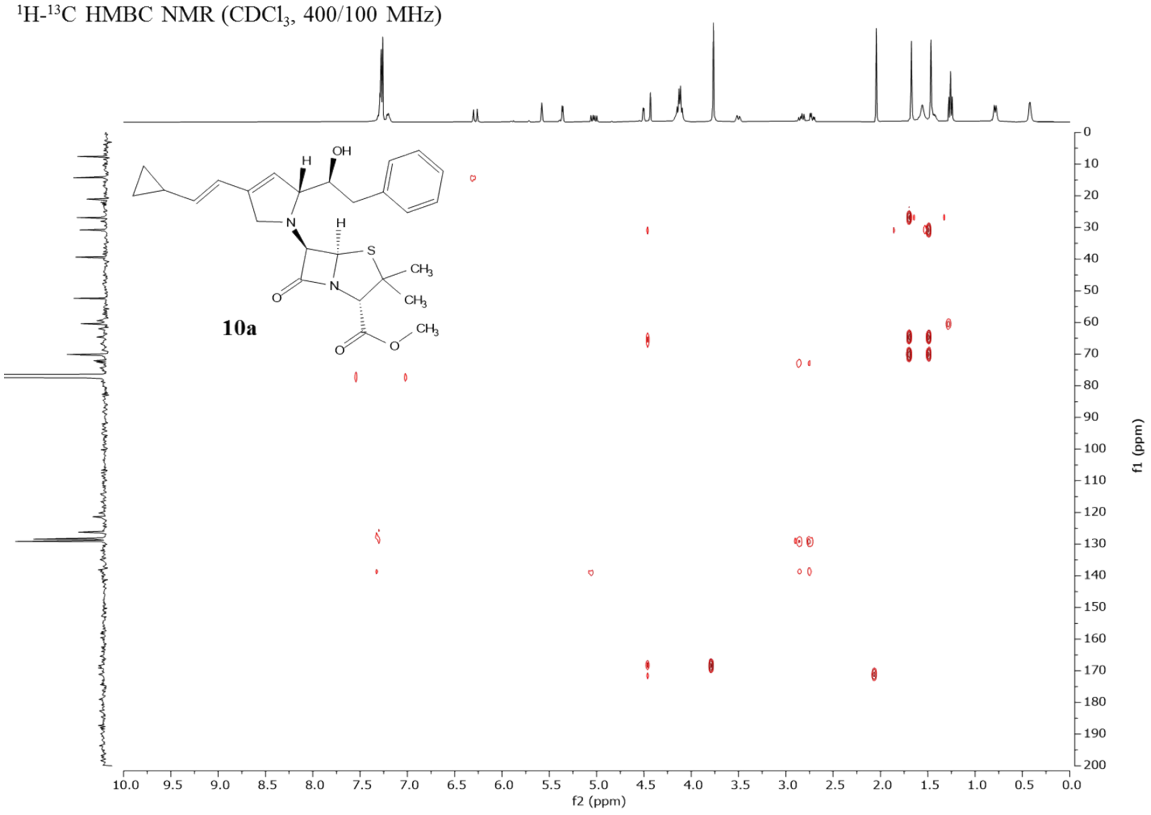
^{1}H COSY NMR ($CDCl_3$, 400 MHz)



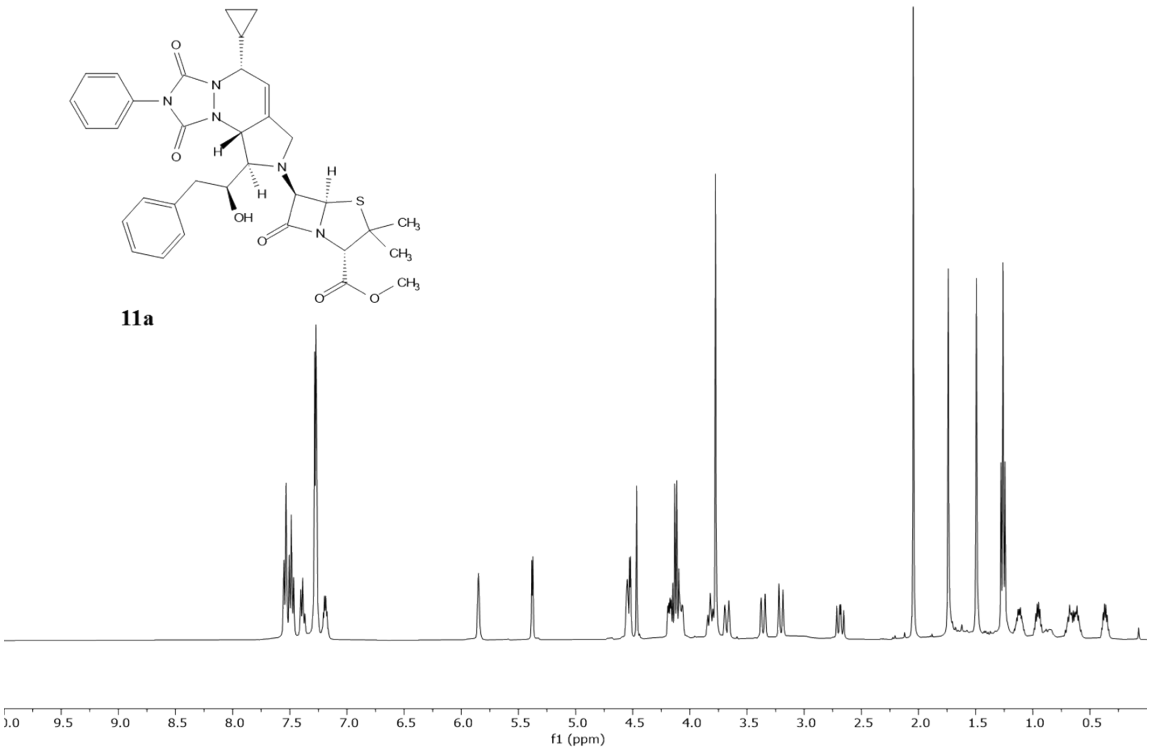
^{1}H-^{13}C HSQC NMR ($CDCl_3$, 400/100 MHz)



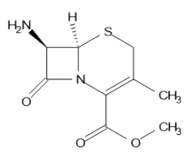
^{1}H-^{13}C HMBC NMR ($CDCl_3$, 400/100 MHz)



^{1}H NMR ($CDCl_3$, 400 MHz)

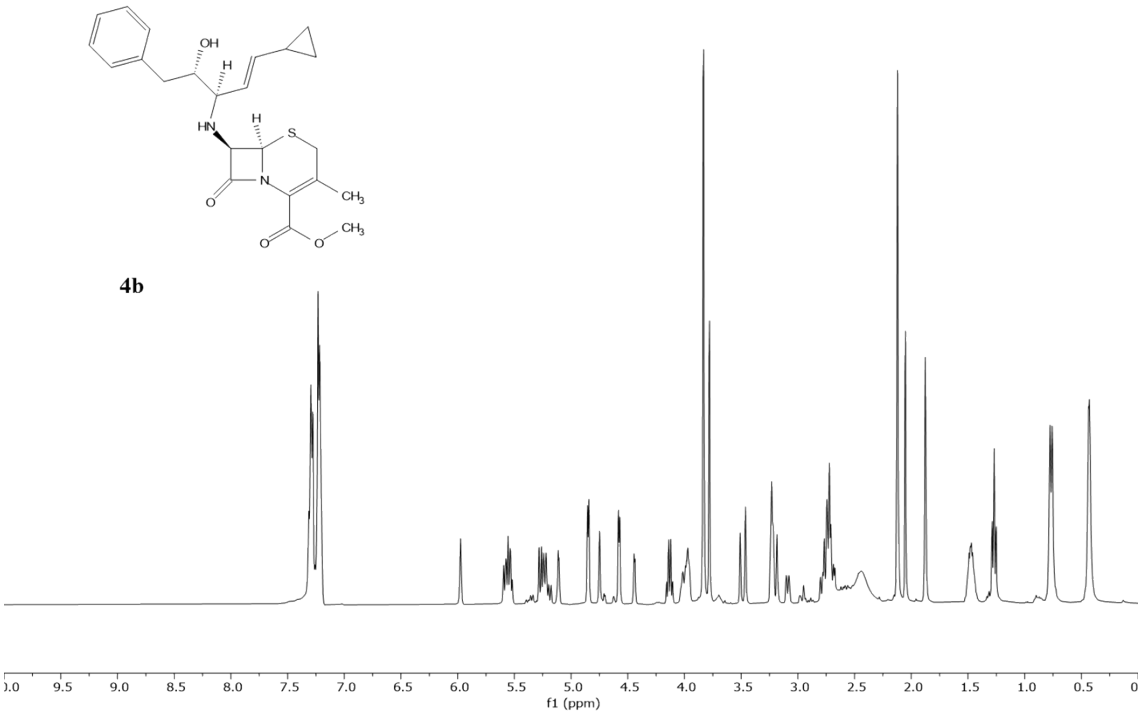


^{1}H NMR ($CDCl_3$, 400 MHz)



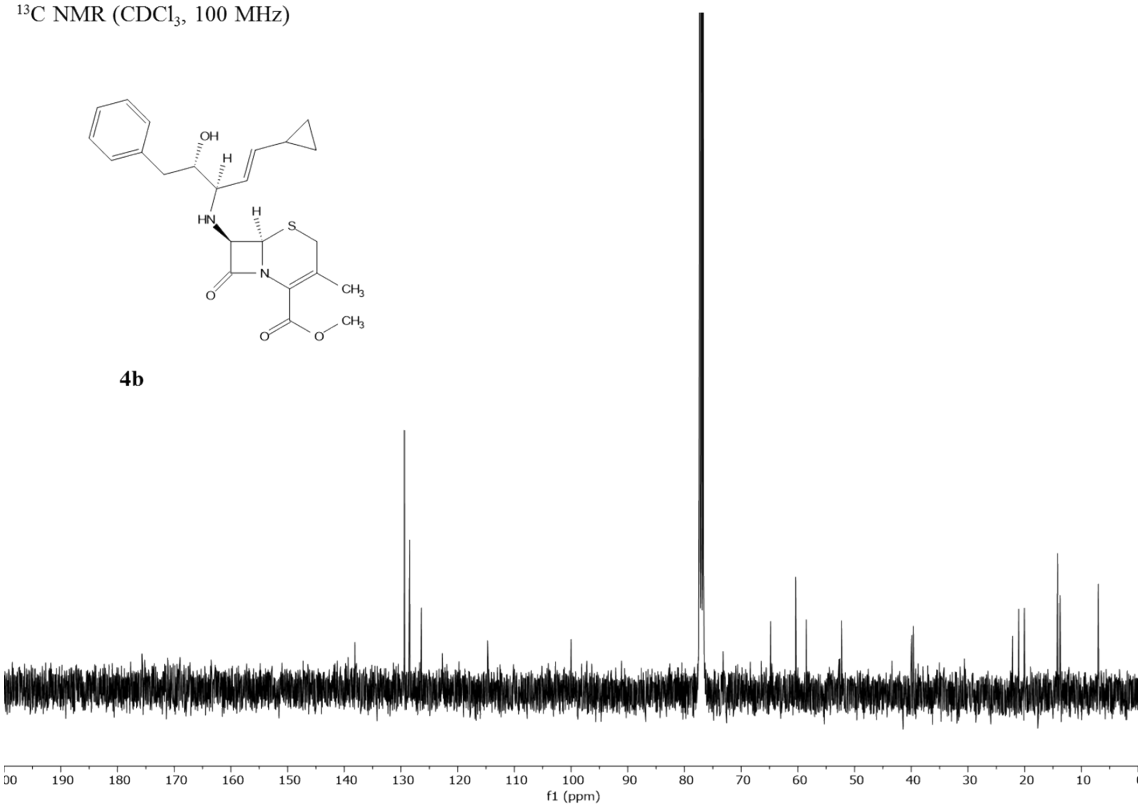
1b

^{1}H NMR ($CDCl_3$, 400 MHz)



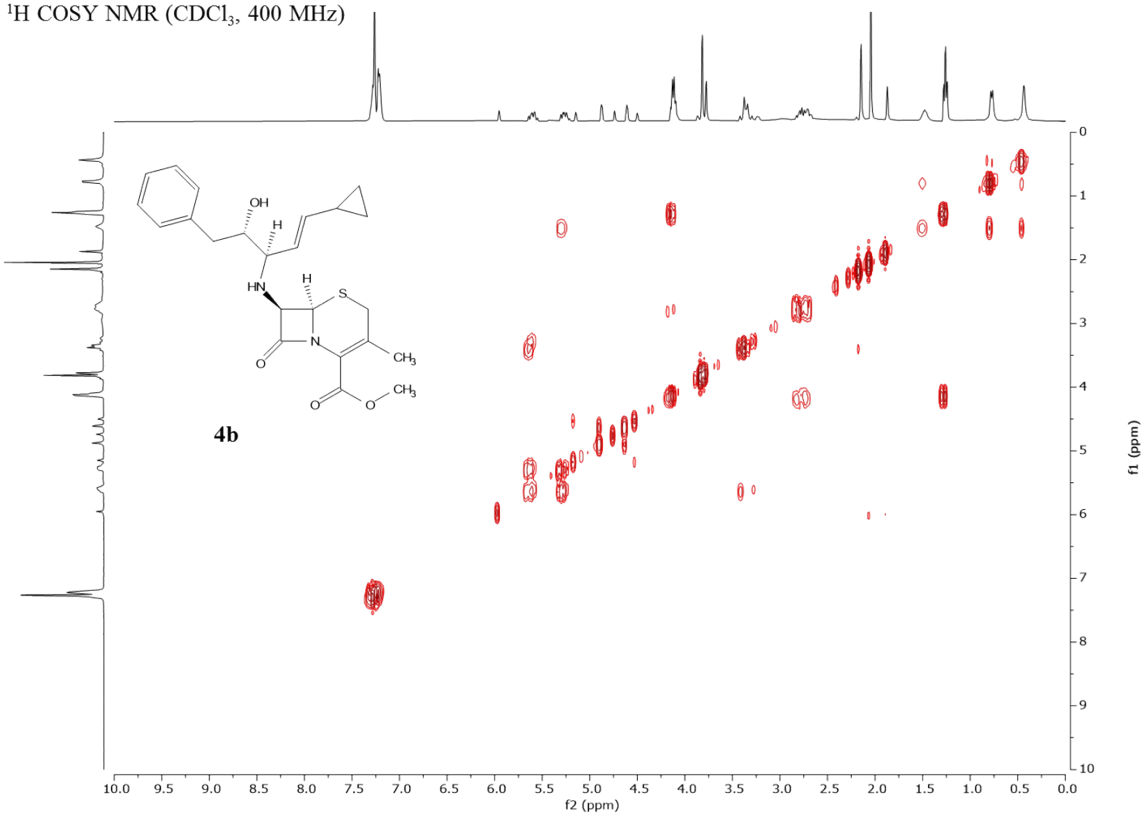
4b

^{13}C NMR ($CDCl_3$, 100 MHz)

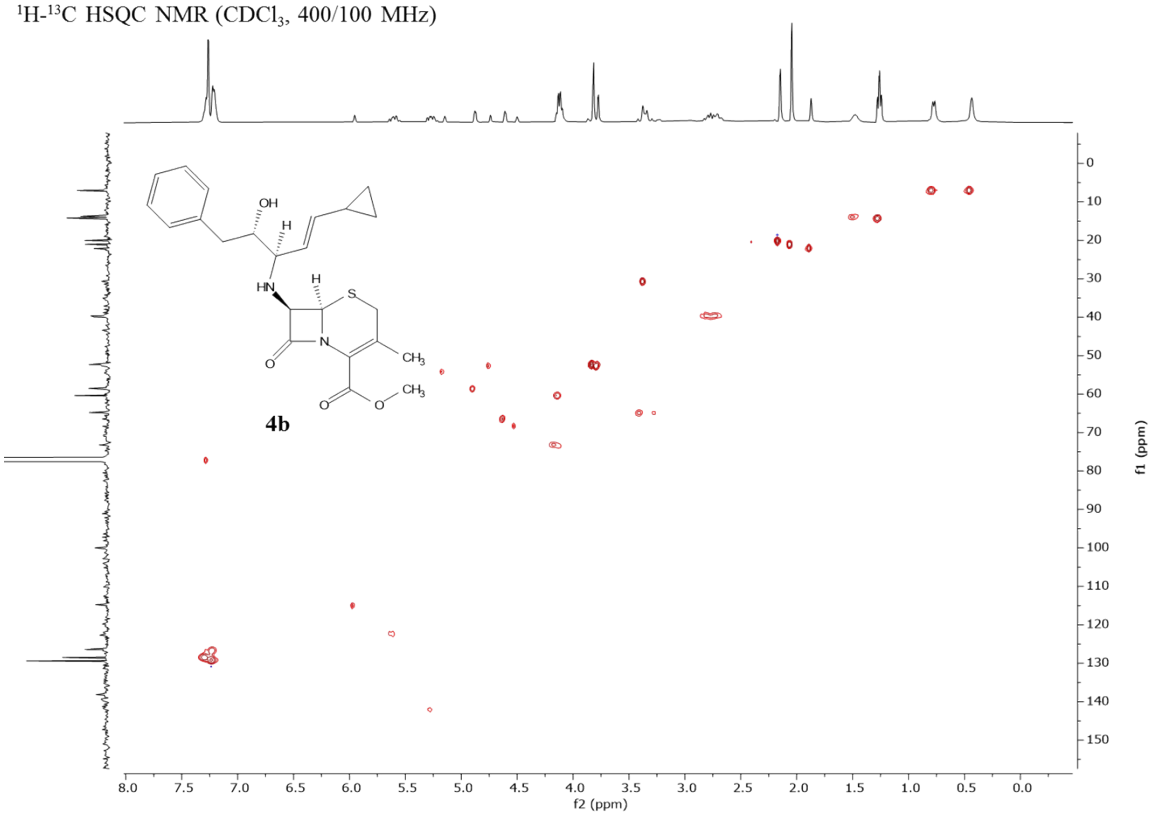


4b

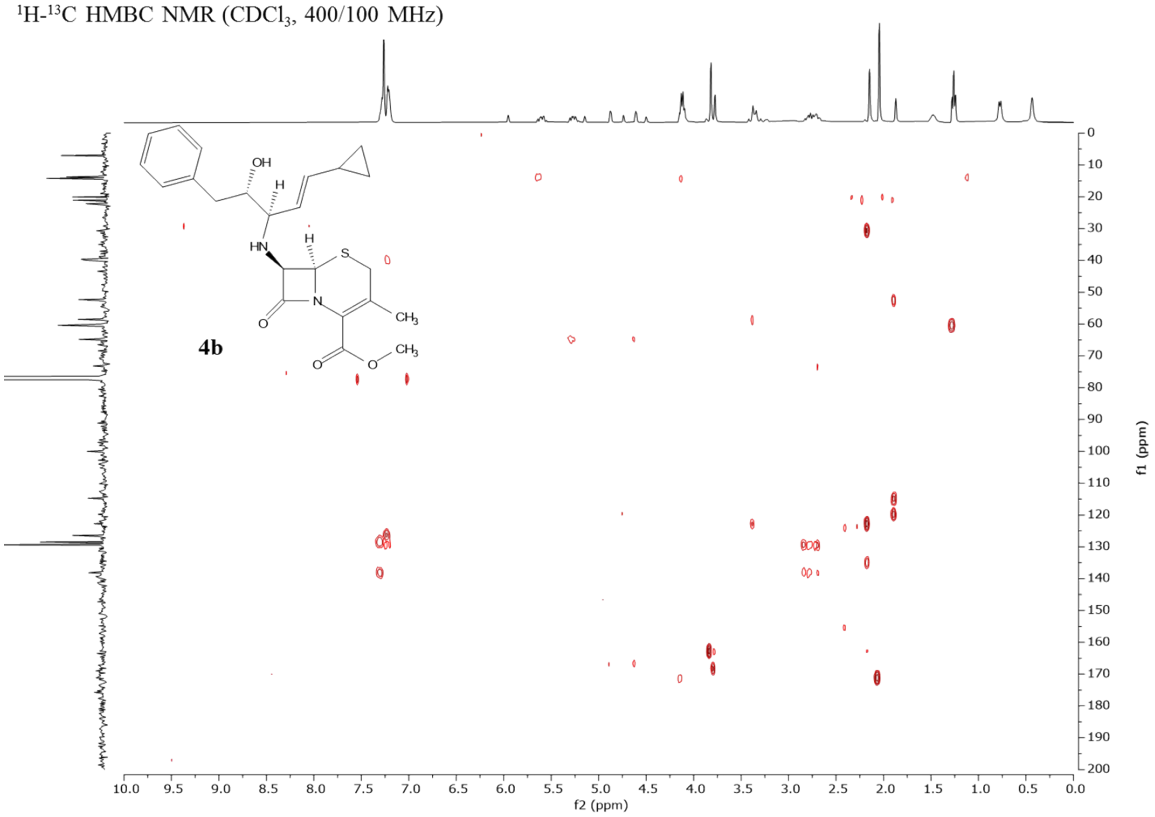
^{1}H COSY NMR ($CDCl_3$, 400 MHz)



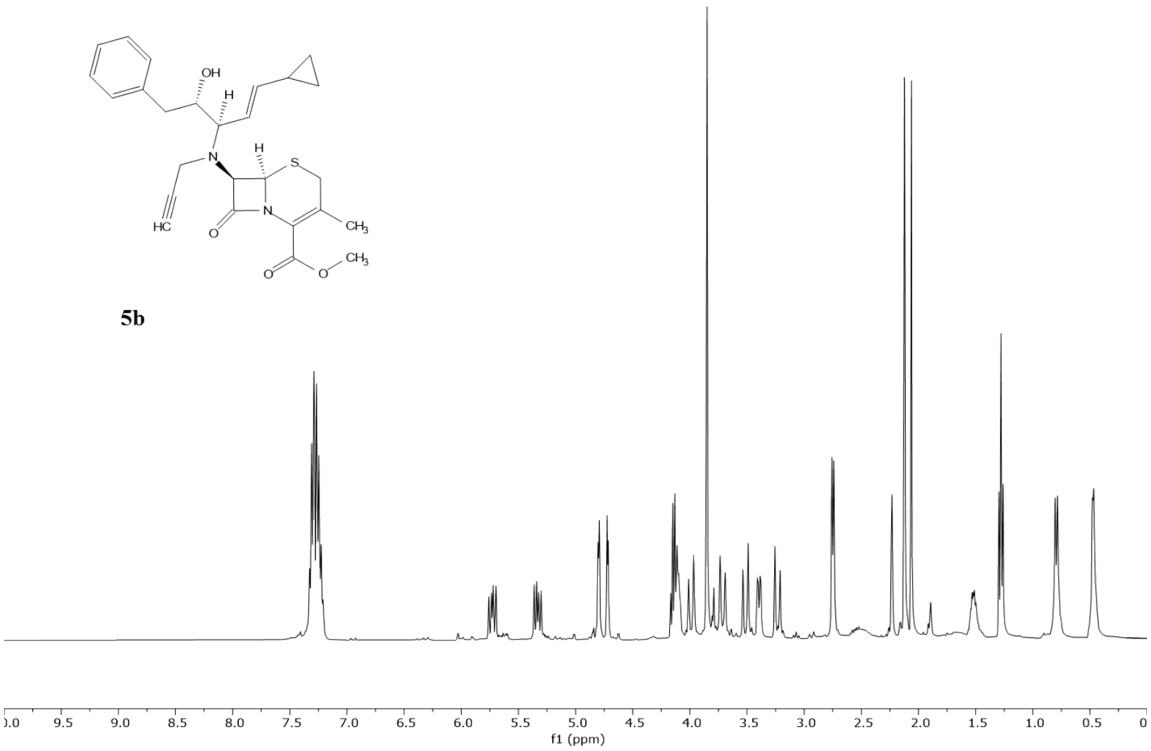
^{1}H-^{13}C HSQC NMR ($CDCl_3$, 400/100 MHz)



^{1}H-^{13}C HMBC NMR ($CDCl_3$, 400/100 MHz)

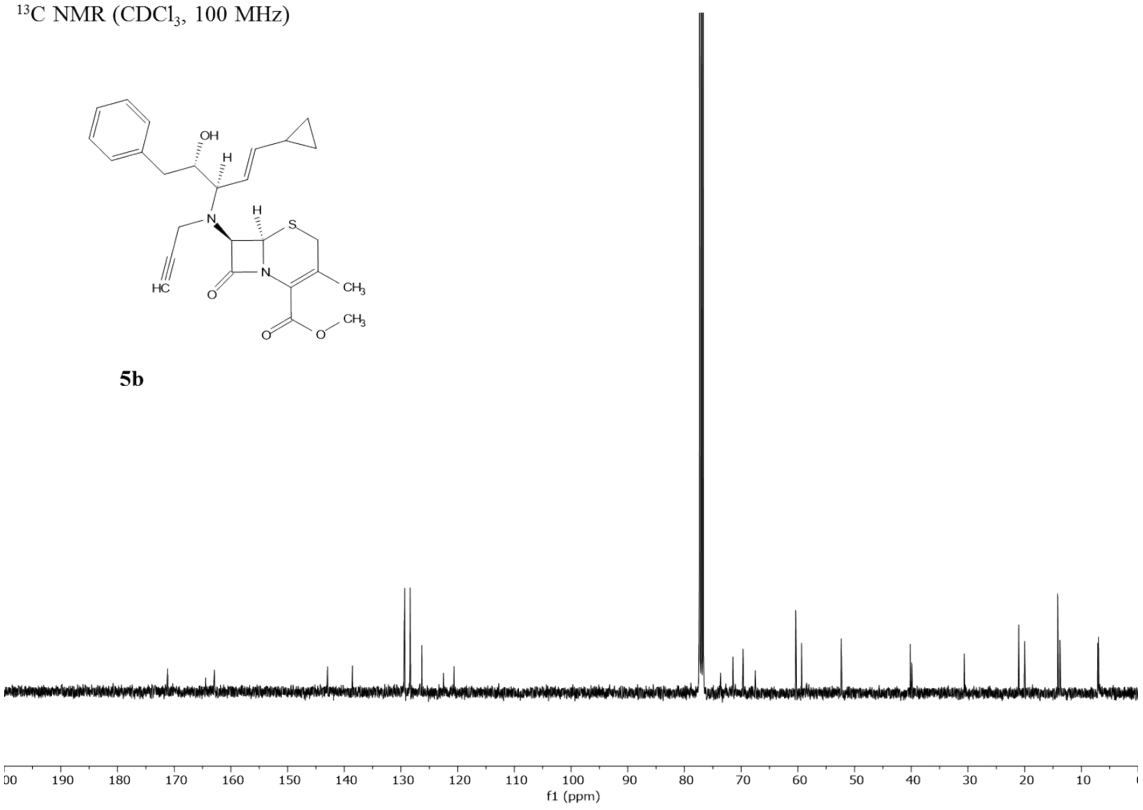


^{1}H NMR ($CDCl_3$, 400 MHz)



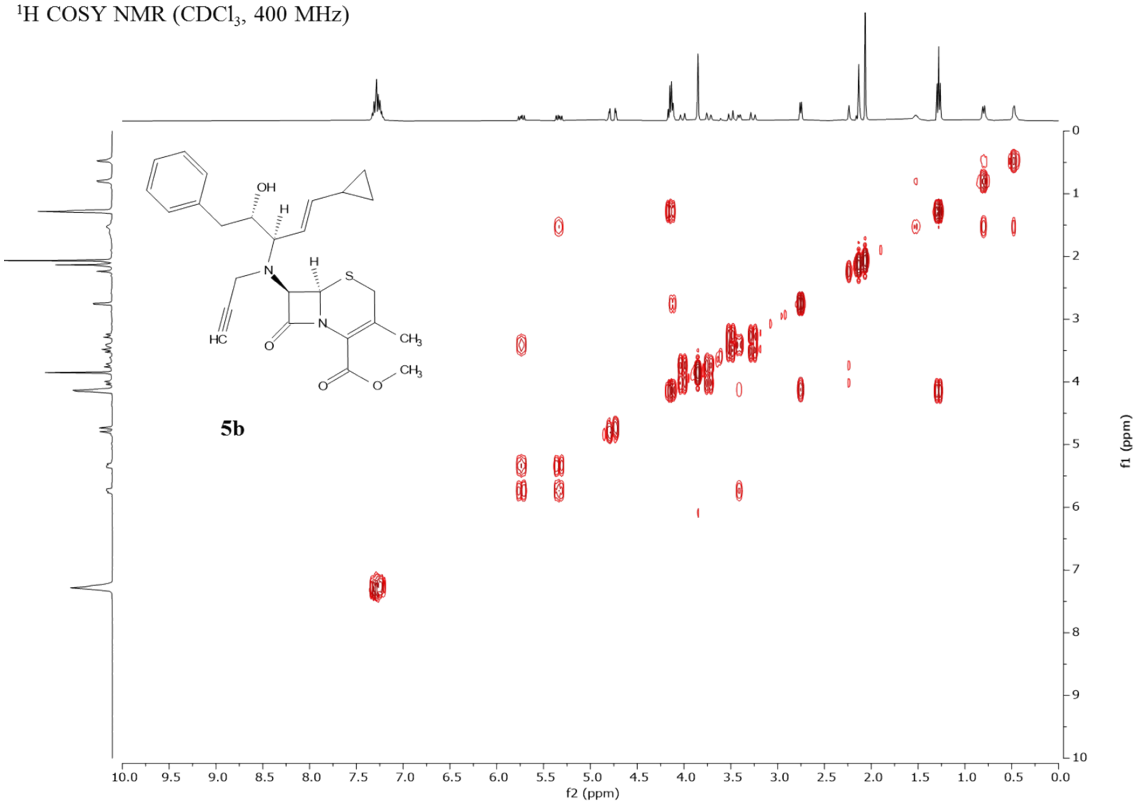
5b

^{13}C NMR ($CDCl_3$, 100 MHz)

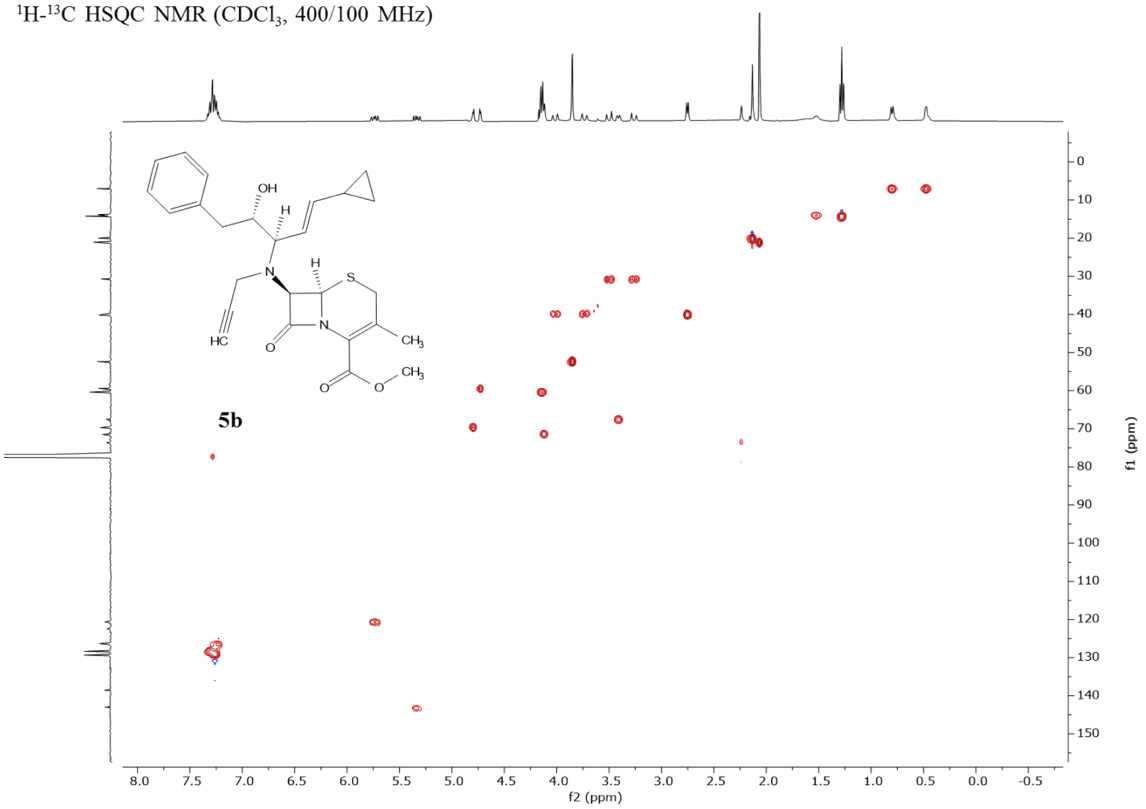


5b

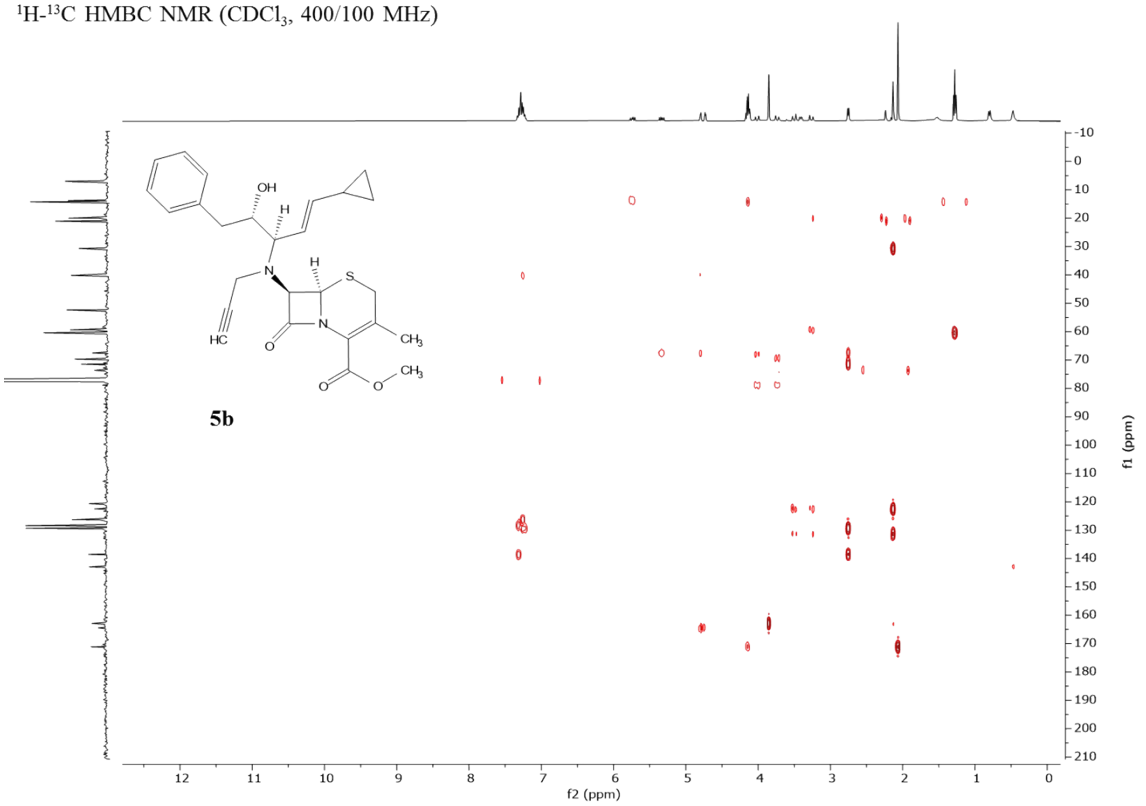
^{1}H COSY NMR ($CDCl_3$, 400 MHz)



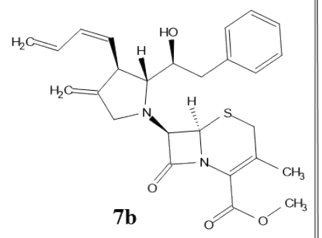
^{1}H-^{13}C HSQC NMR ($CDCl_3$, 400/100 MHz)



^{1}H-^{13}C HMBC NMR ($CDCl_3$, 400/100 MHz)

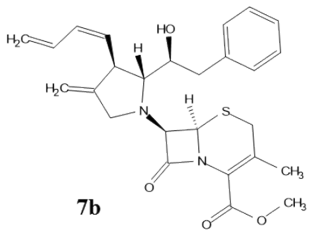


^{1}H NMR (C_6D_6, 400 MHz)



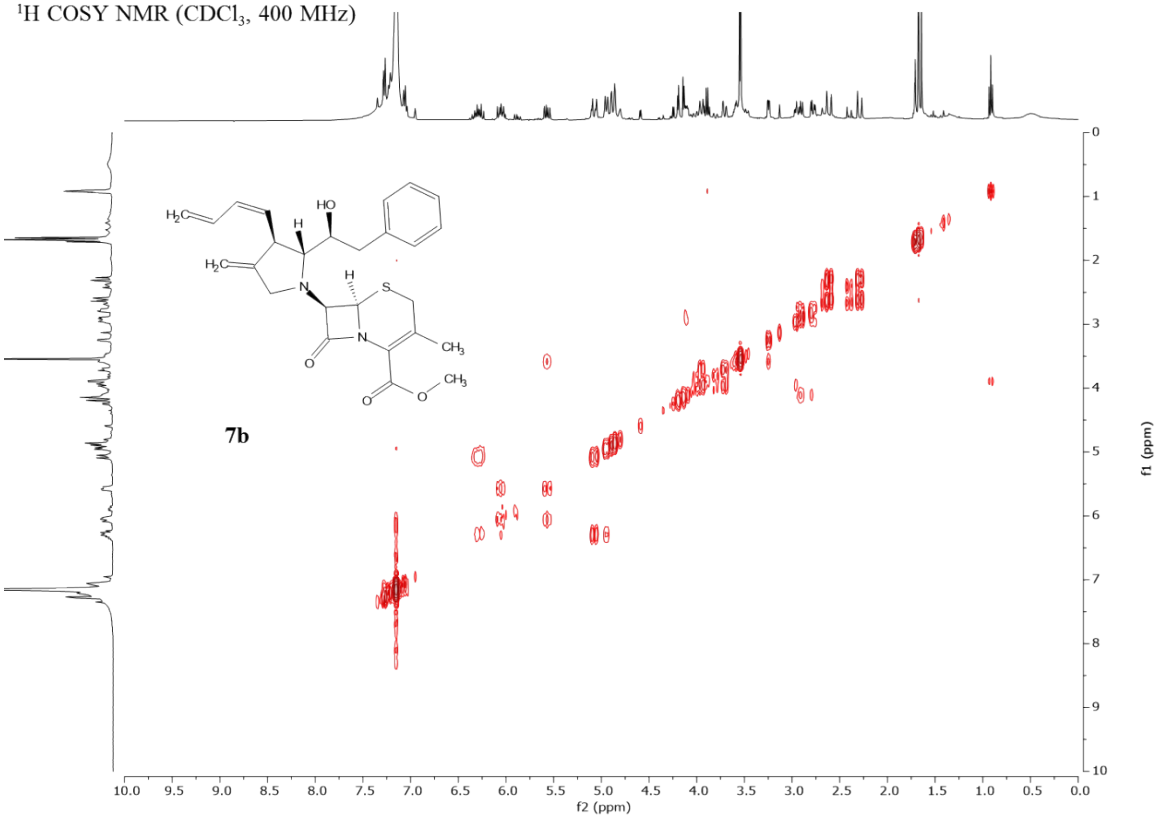
7b

^{13}C NMR (C_6D_6, 100 MHz)

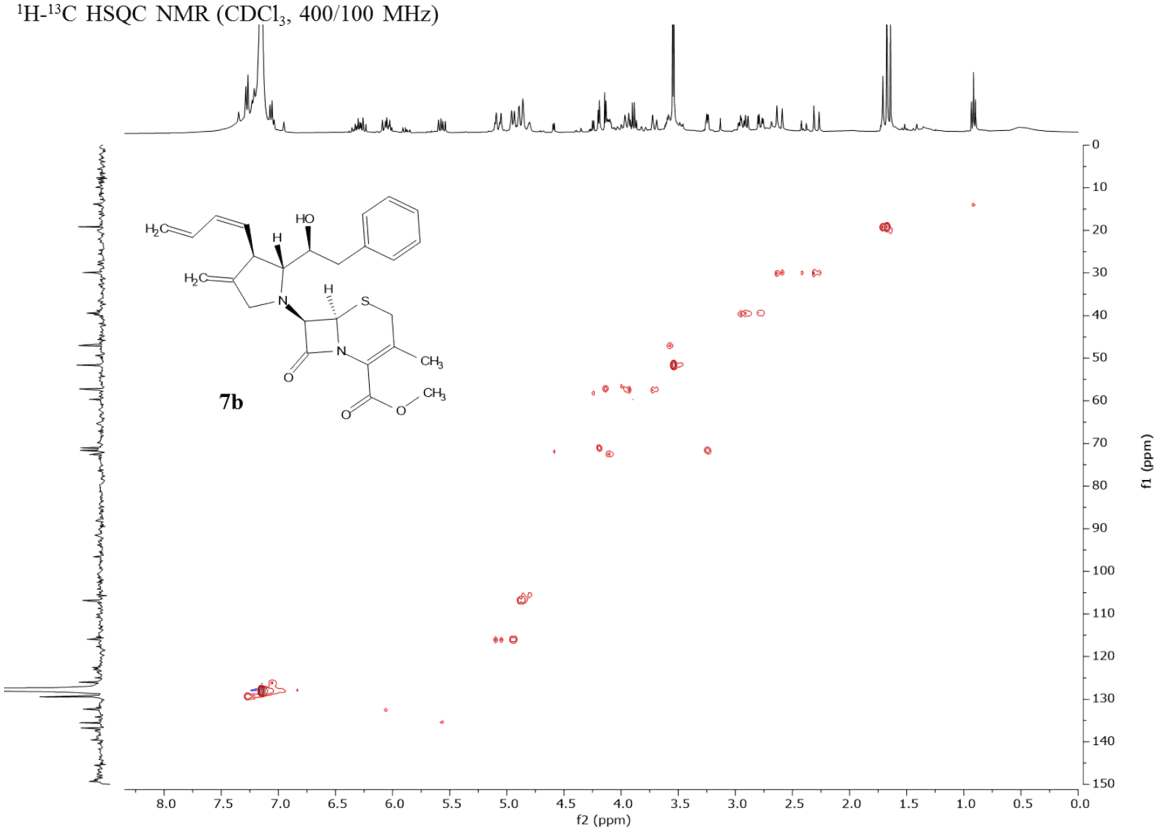


7b

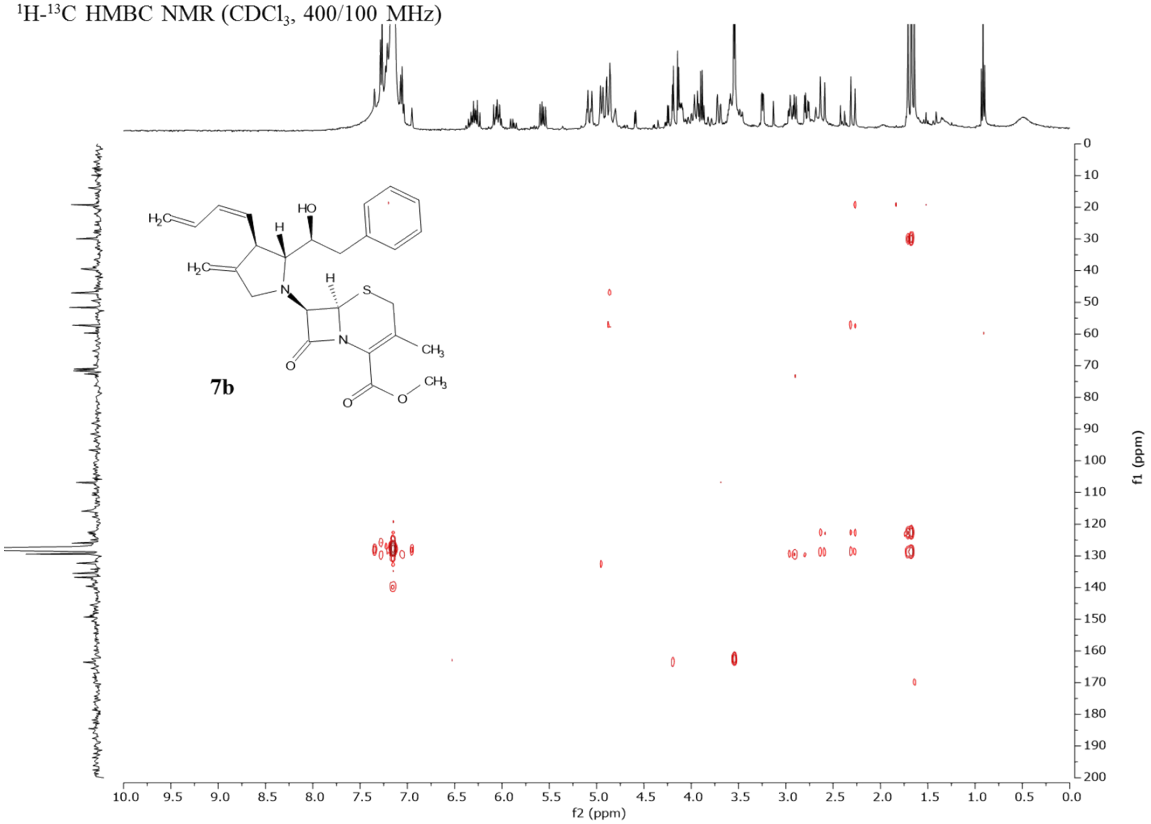
^{1}H COSY NMR ($CDCl_3$, 400 MHz)



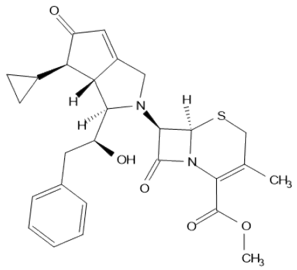
^{1}H-^{13}C HSQC NMR ($CDCl_3$, 400/100 MHz)



^{1}H-^{13}C HMBC NMR ($CDCl_3$, 400/100 MHz)

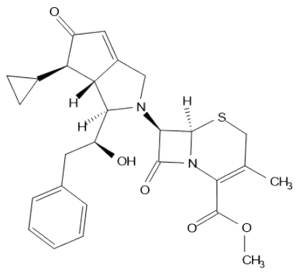


^{1}H NMR (C_6D_6, 400 MHz)



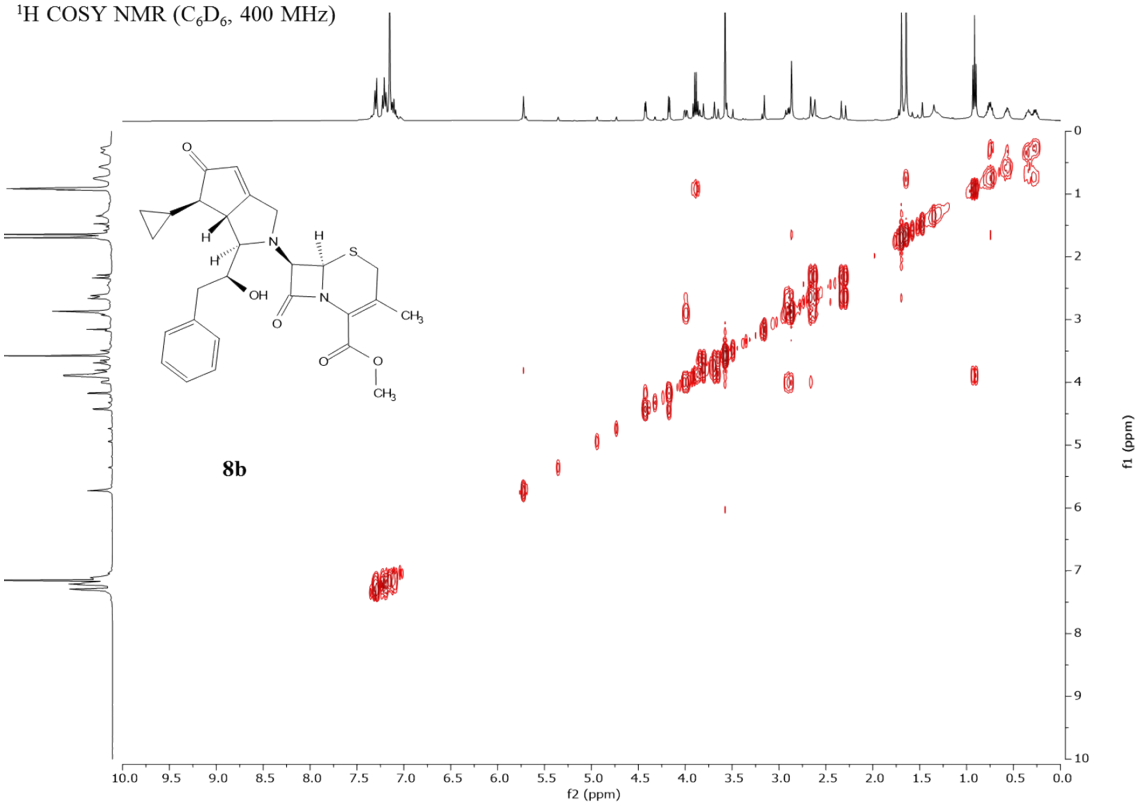
8b

^{13}C NMR (C_6D_6, 100 MHz)

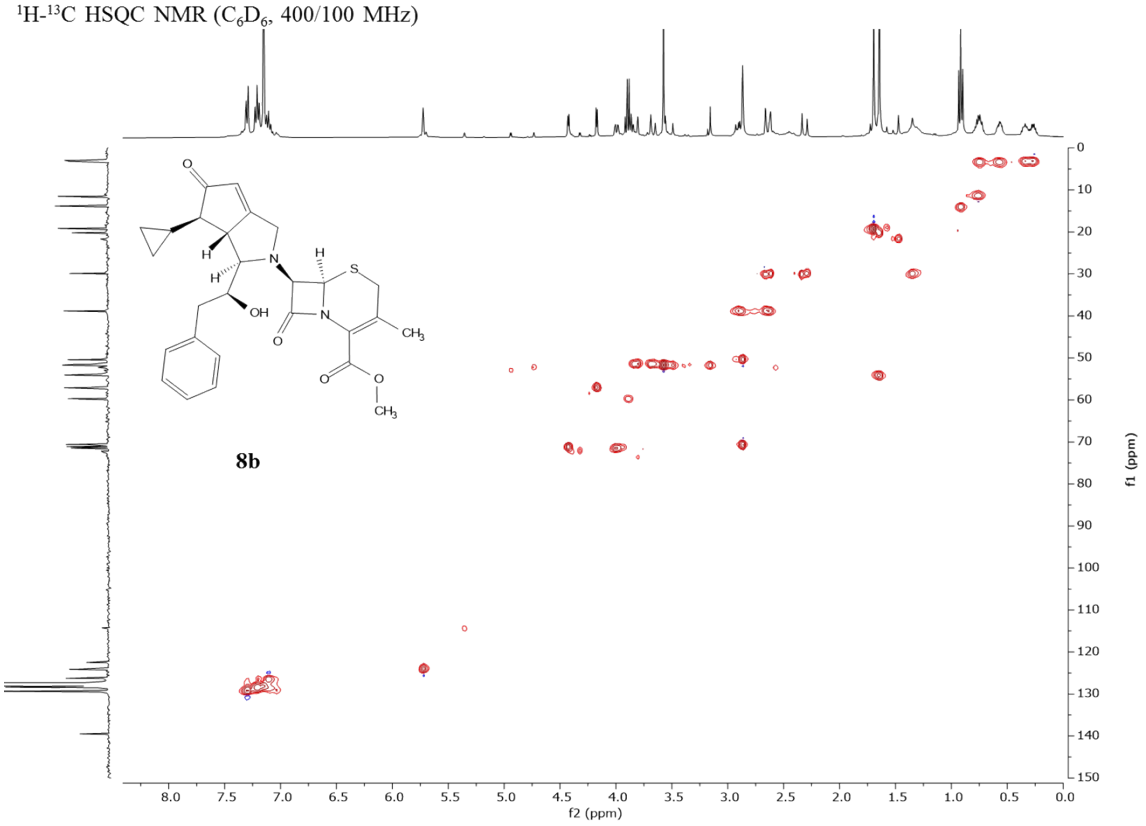


8b

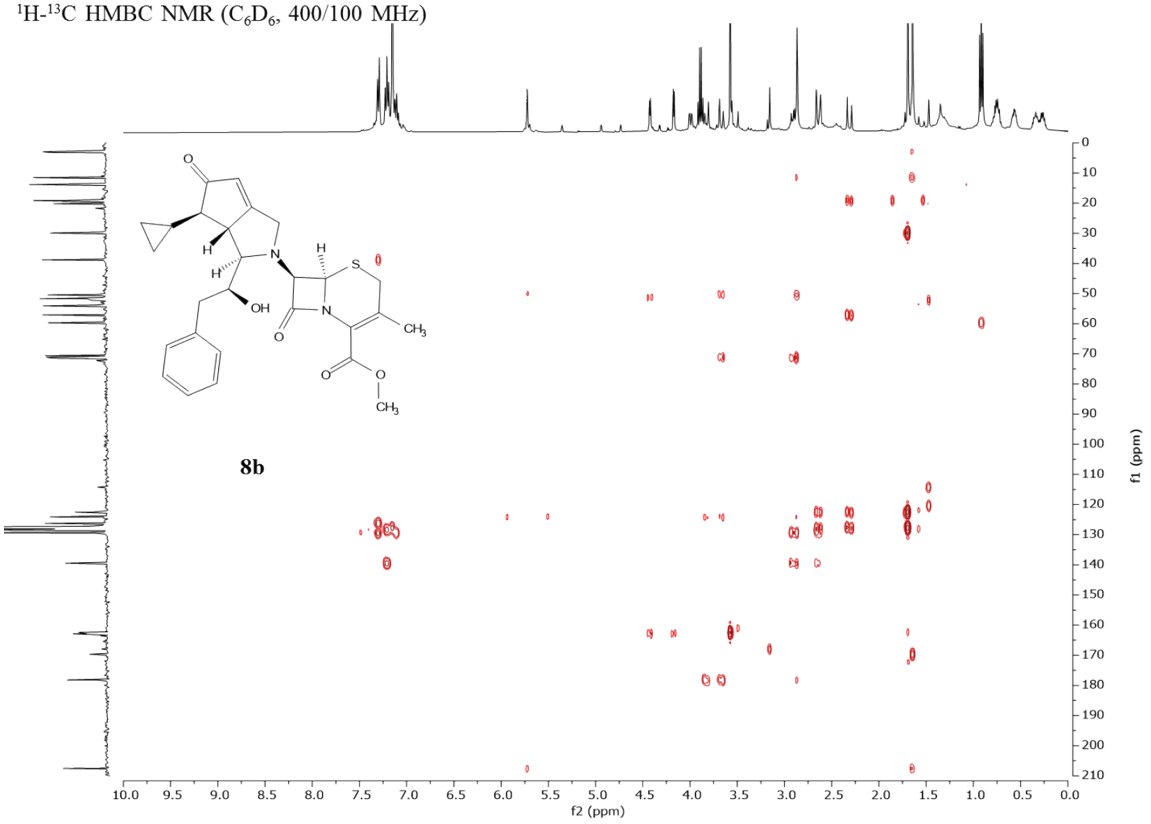
^{1}H COSY NMR (C_6D_6, 400 MHz)



^{1}H-^{13}C HSQC NMR (C_6D_6, 400/100 MHz)

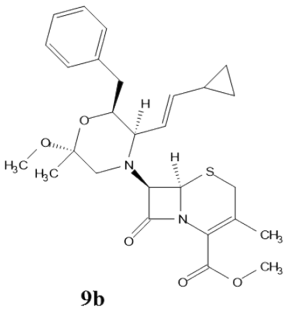


^{1}H-^{13}C HMBC NMR (C_6D_6, 400/100 MHz)



8b

^{1}H NMR ($CDCl_3$, 400 MHz)



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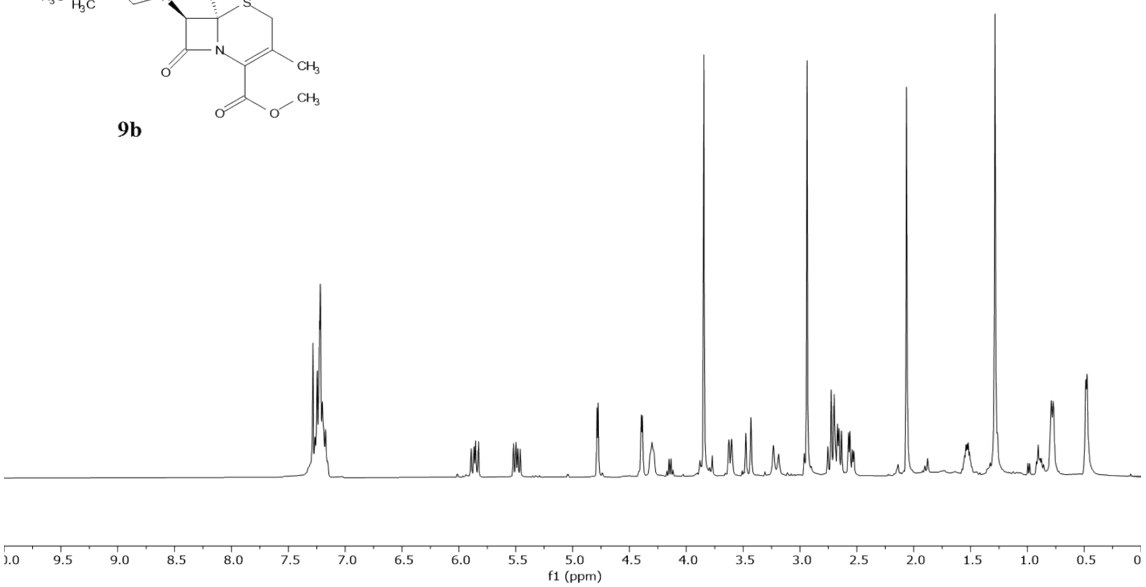

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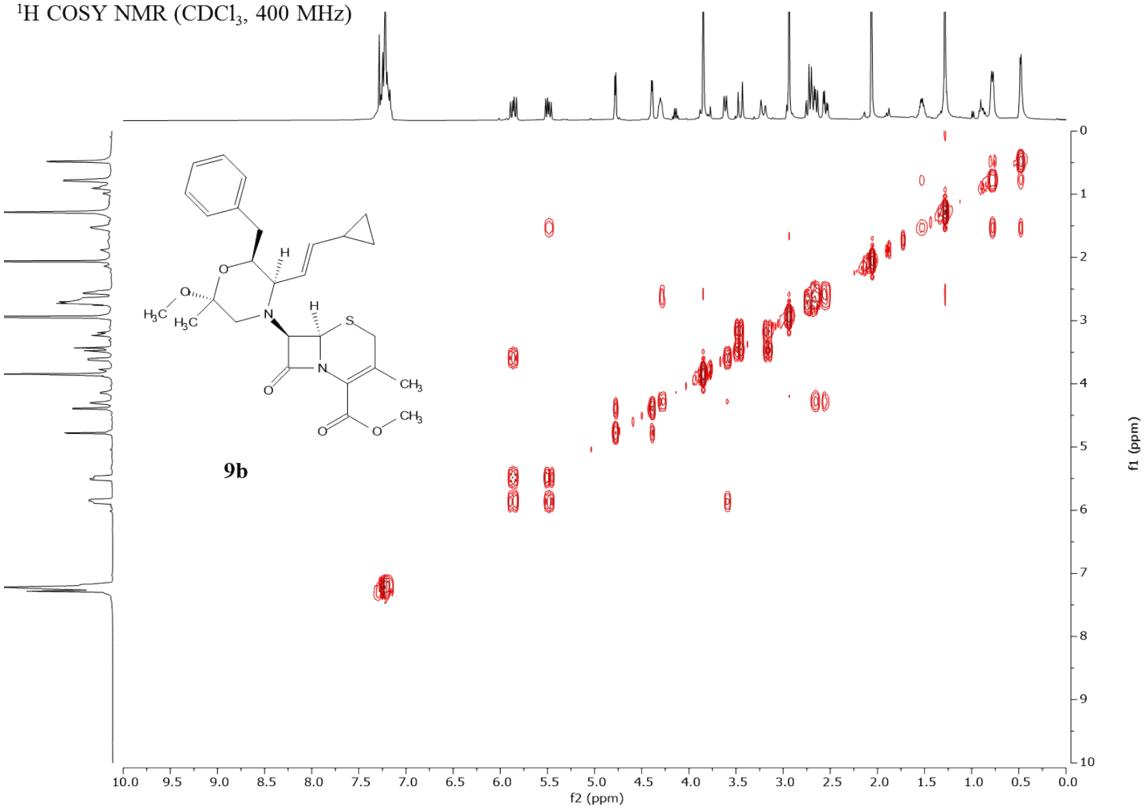

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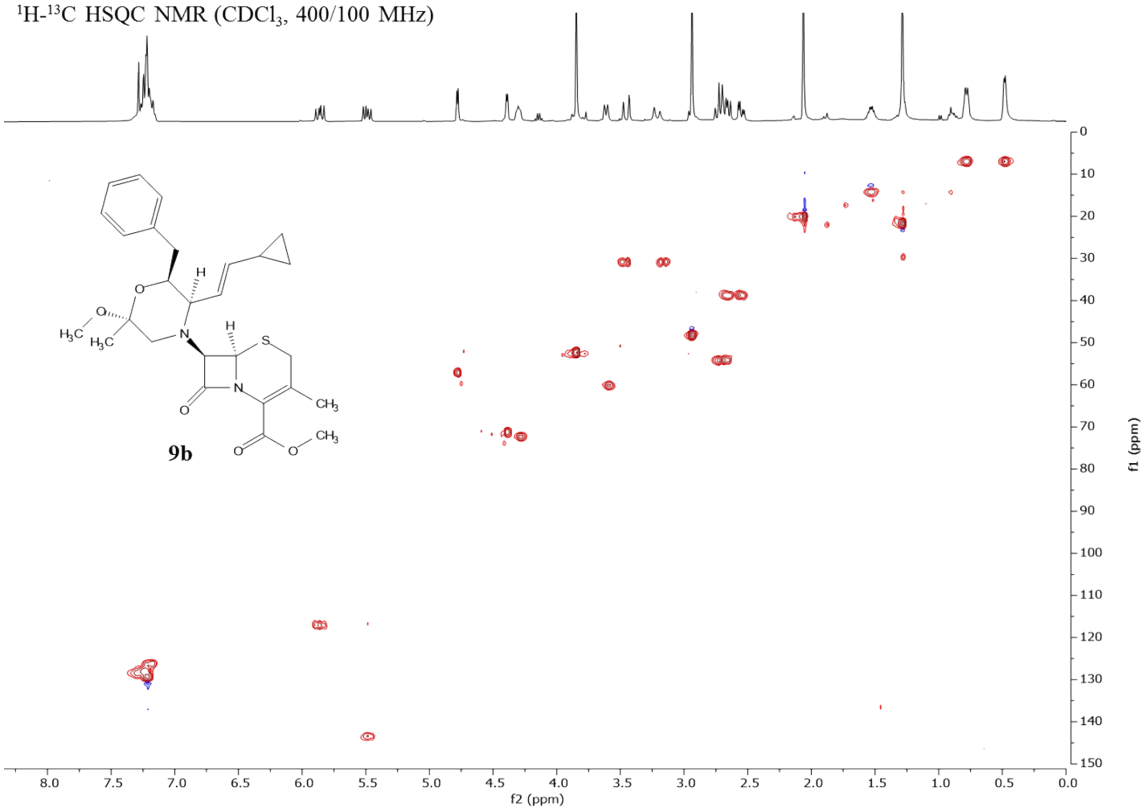
^{1}H-^{13}C HSQC NMR ($CDCl_3$, 400/100 MHz)



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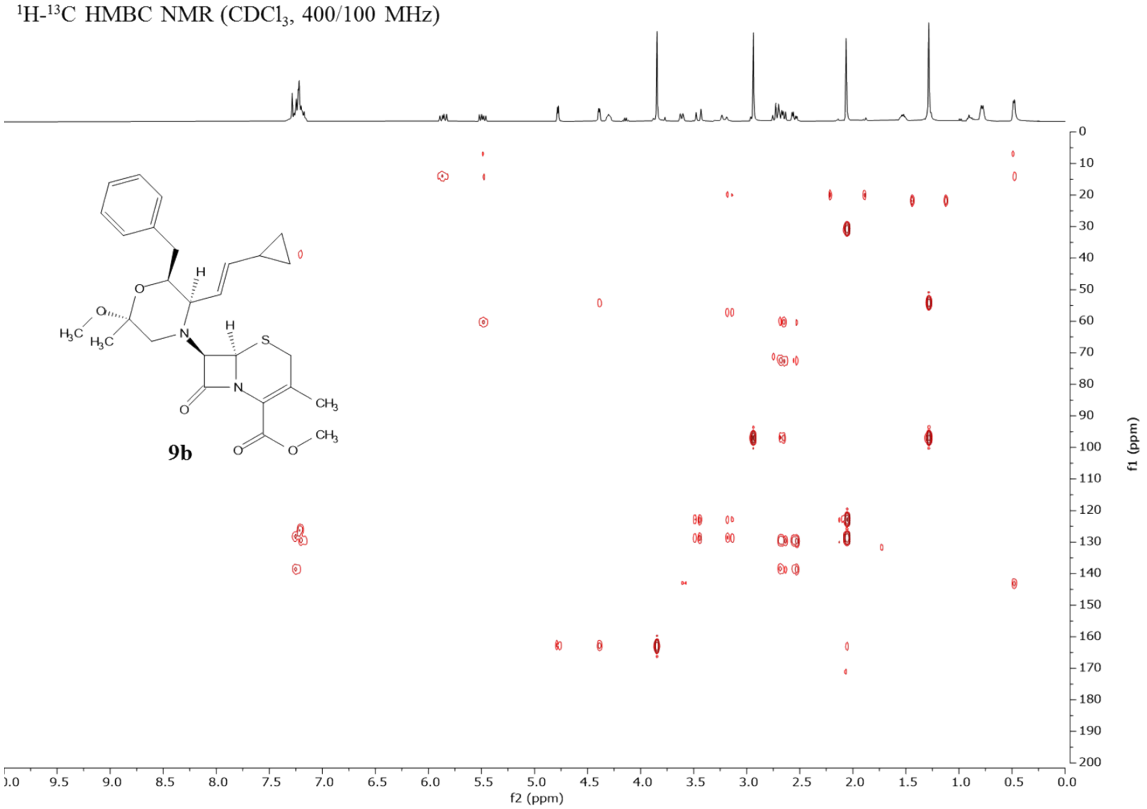
^{1}H-^{13}C HMBC NMR ($CDCl_3$, 400/100 MHz)



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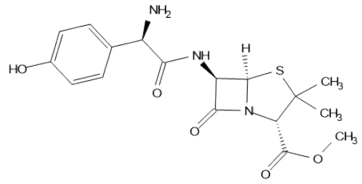
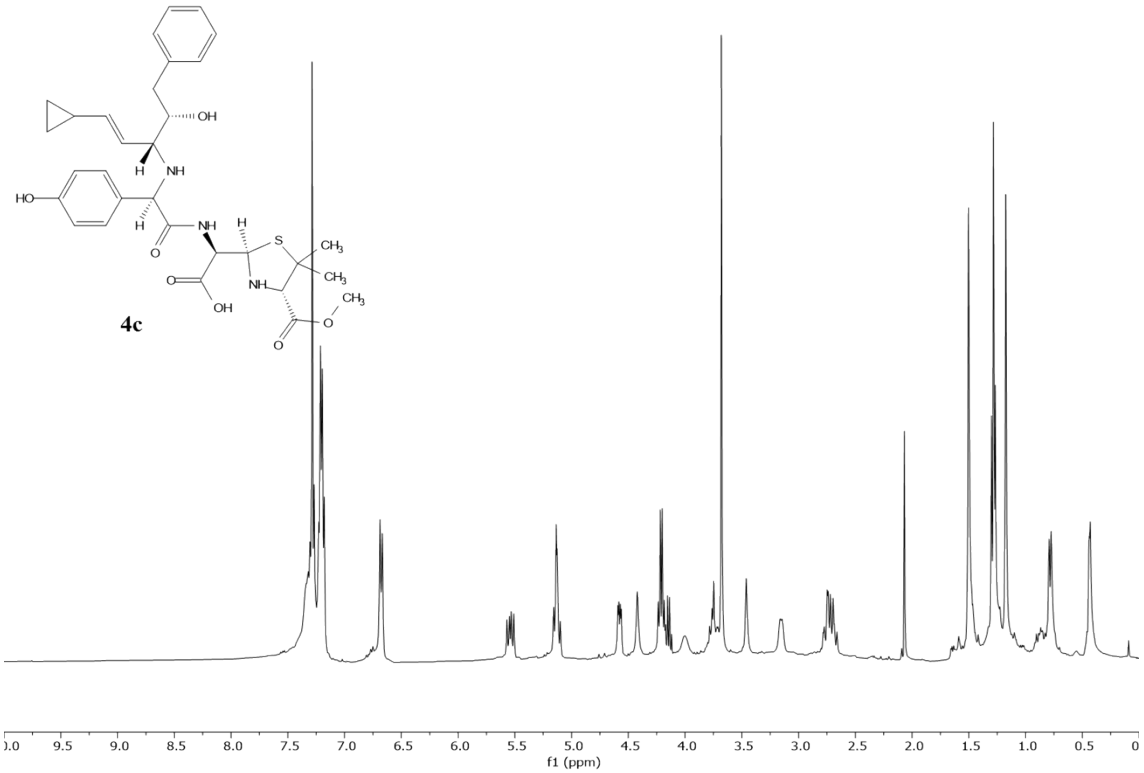
^{1}H NMR ($CDCl_3$, 400 MHz)

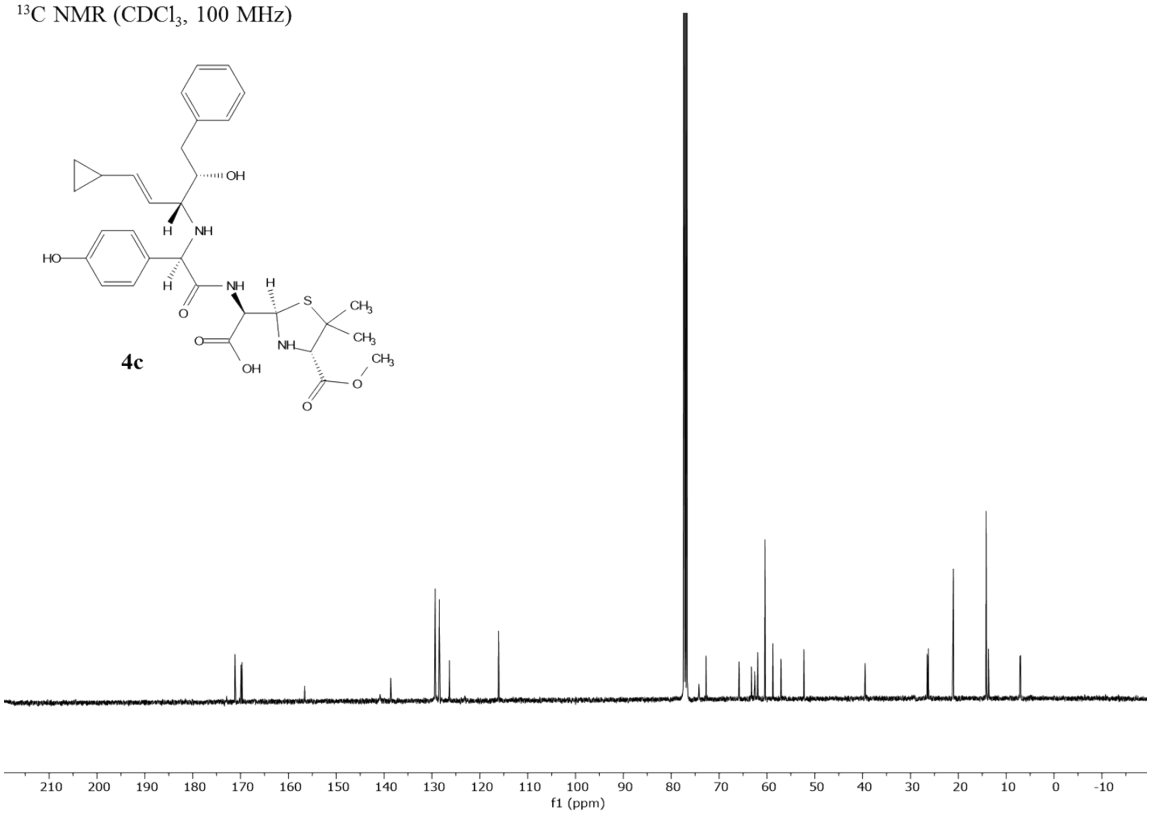


1c

^{1}H NMR ($CDCl_3$, 400 MHz)



^{13}C NMR ($CDCl_3$, 100 MHz)



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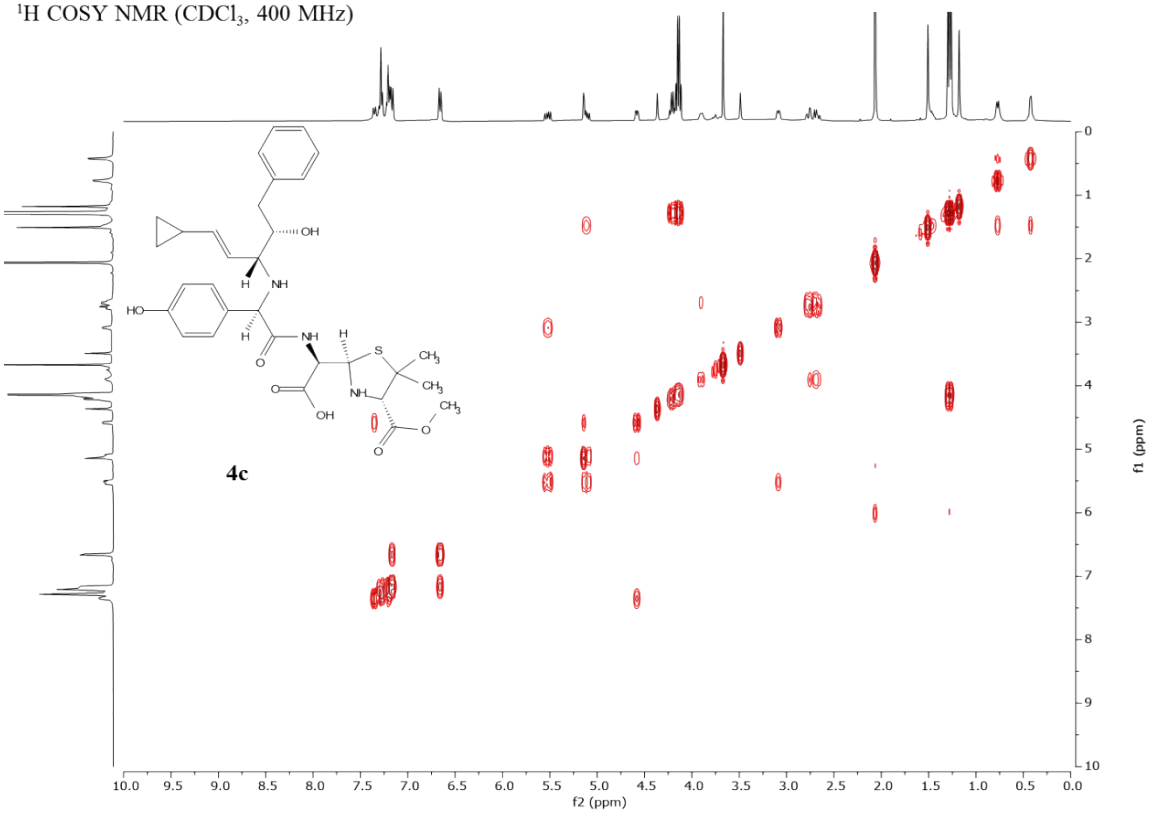
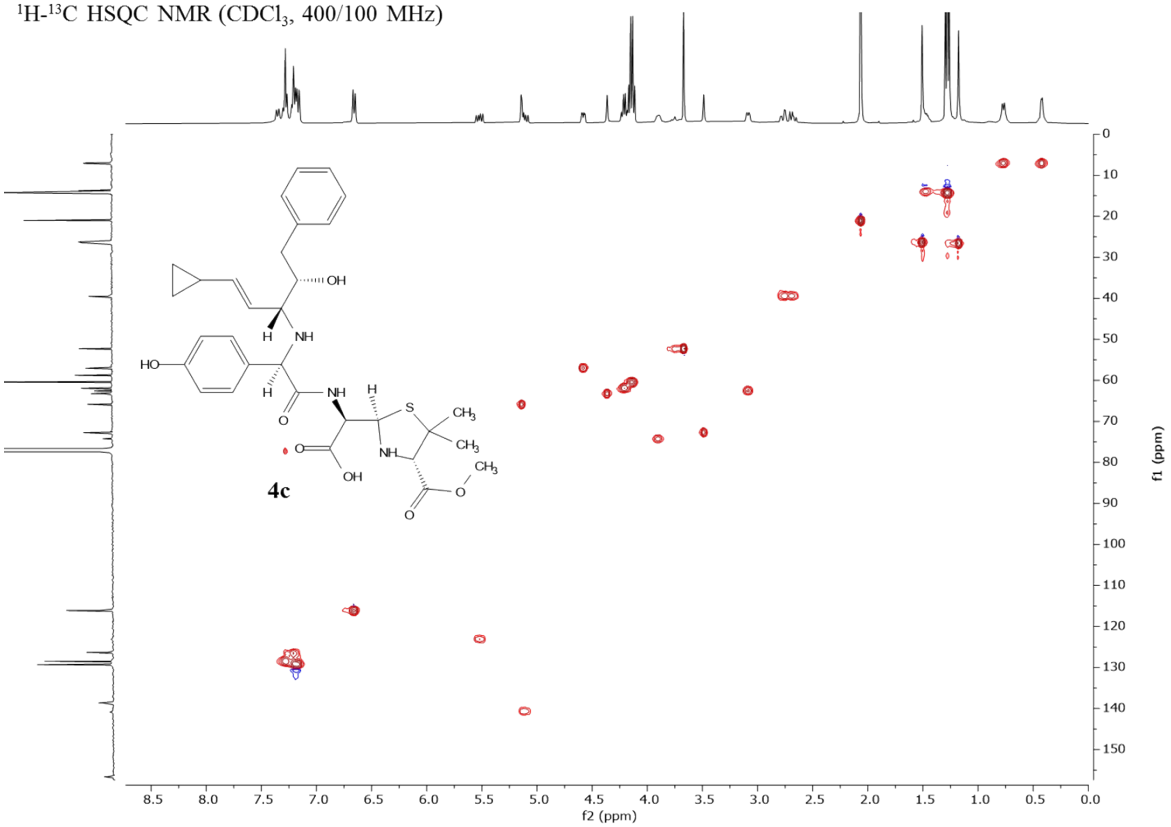


^{1}H-^{13}C HSQC NMR ($CDCl_3$, 400/100 MHz)



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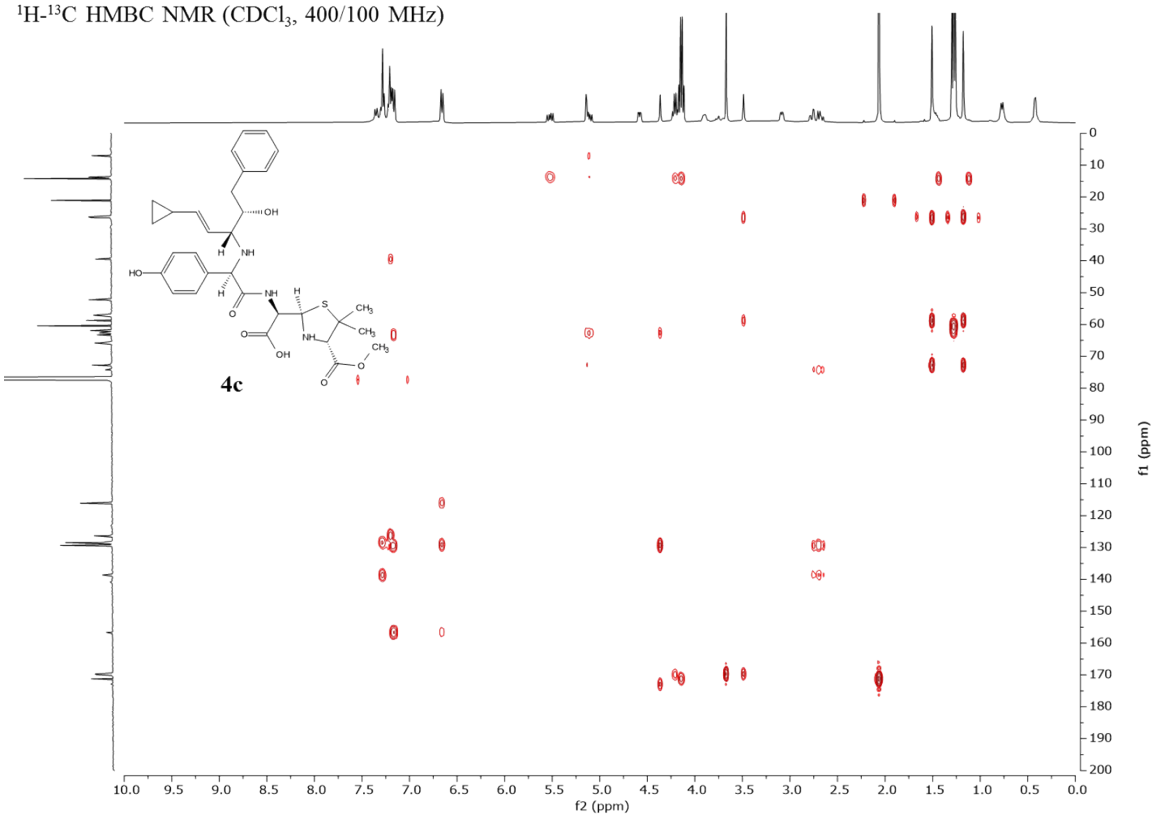
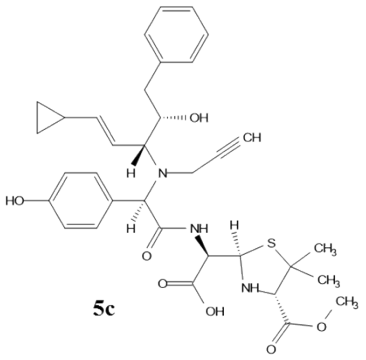
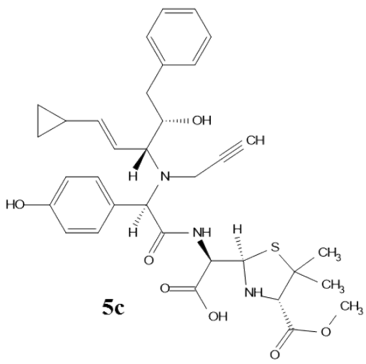


^{1}H NMR ($CDCl_3$, 400 MHz)



5c

^{13}C NMR ($CDCl_3$, 100 MHz)



5c

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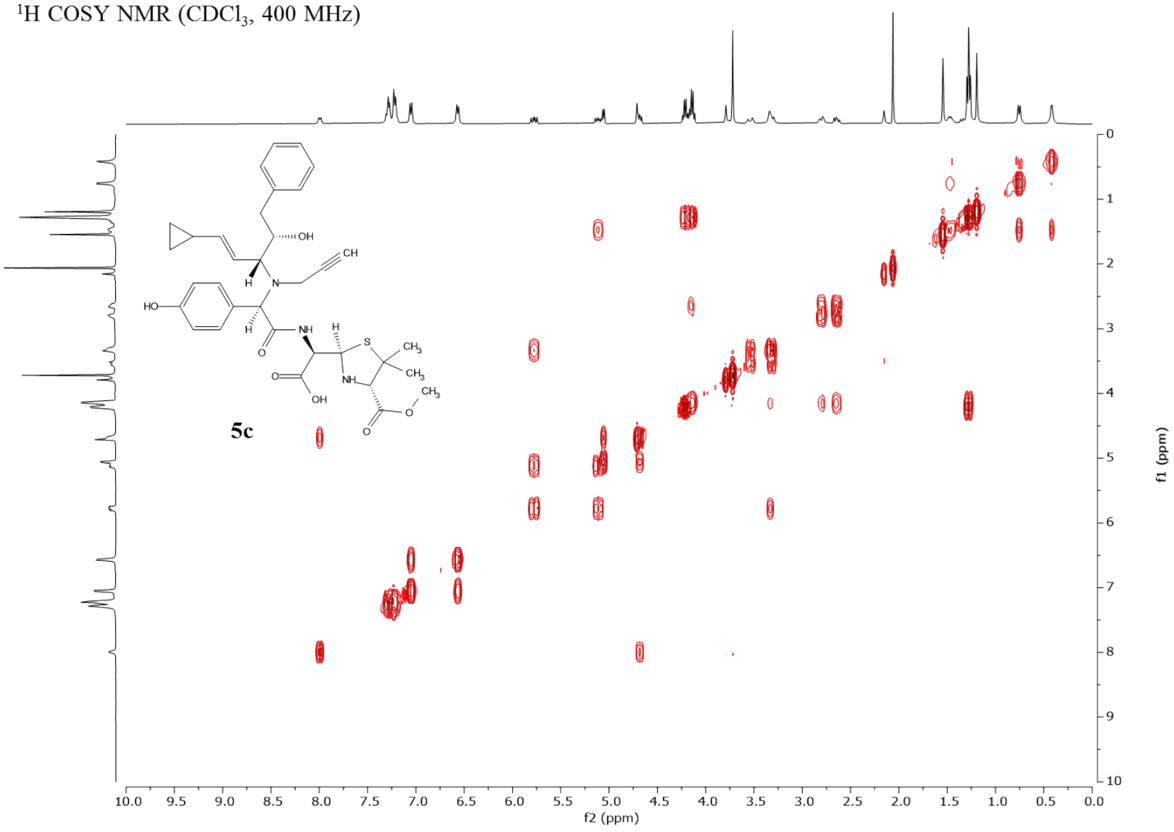
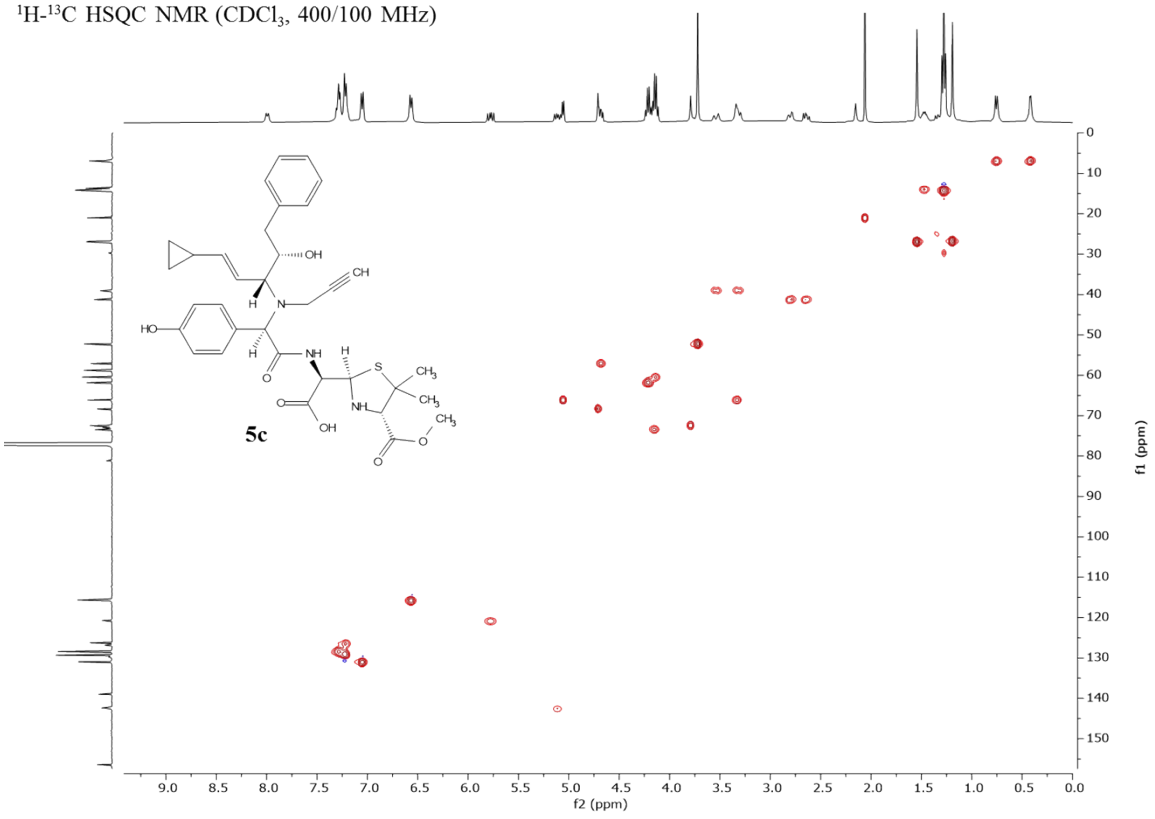
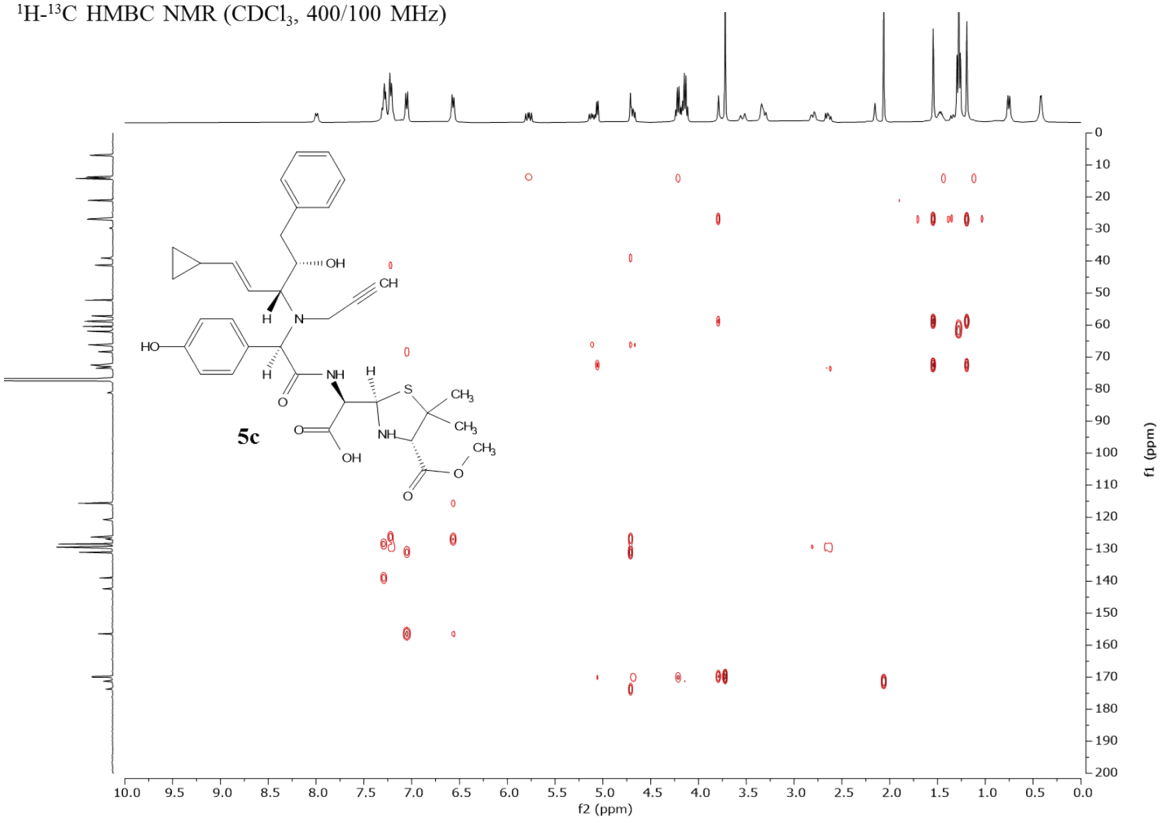


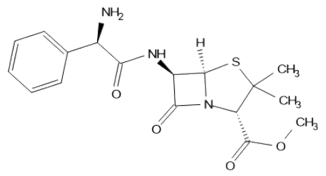
^{1}H-^{13}C HSQC NMR ($CDCl_3$, 400/100 MHz)



^{1}H-^{13}C HMBC NMR ($CDCl_3$, 400/100 MHz)

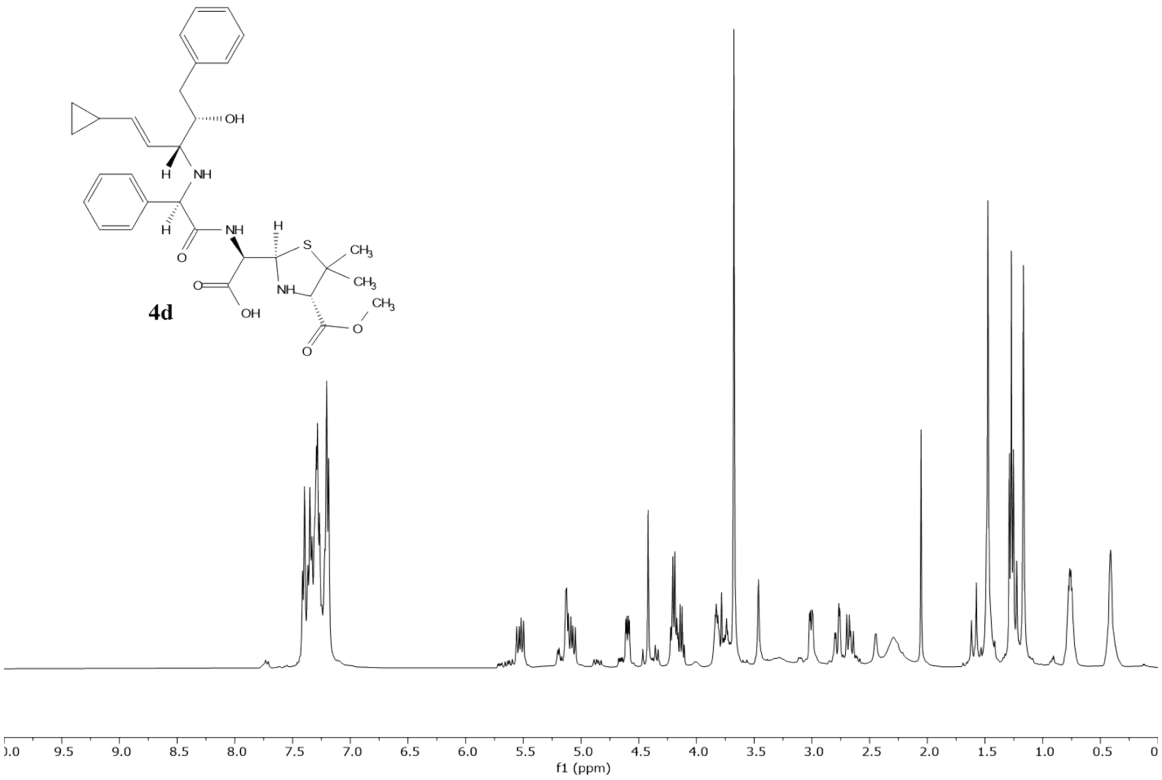


^{1}H NMR ($CDCl_3$, 400 MHz)

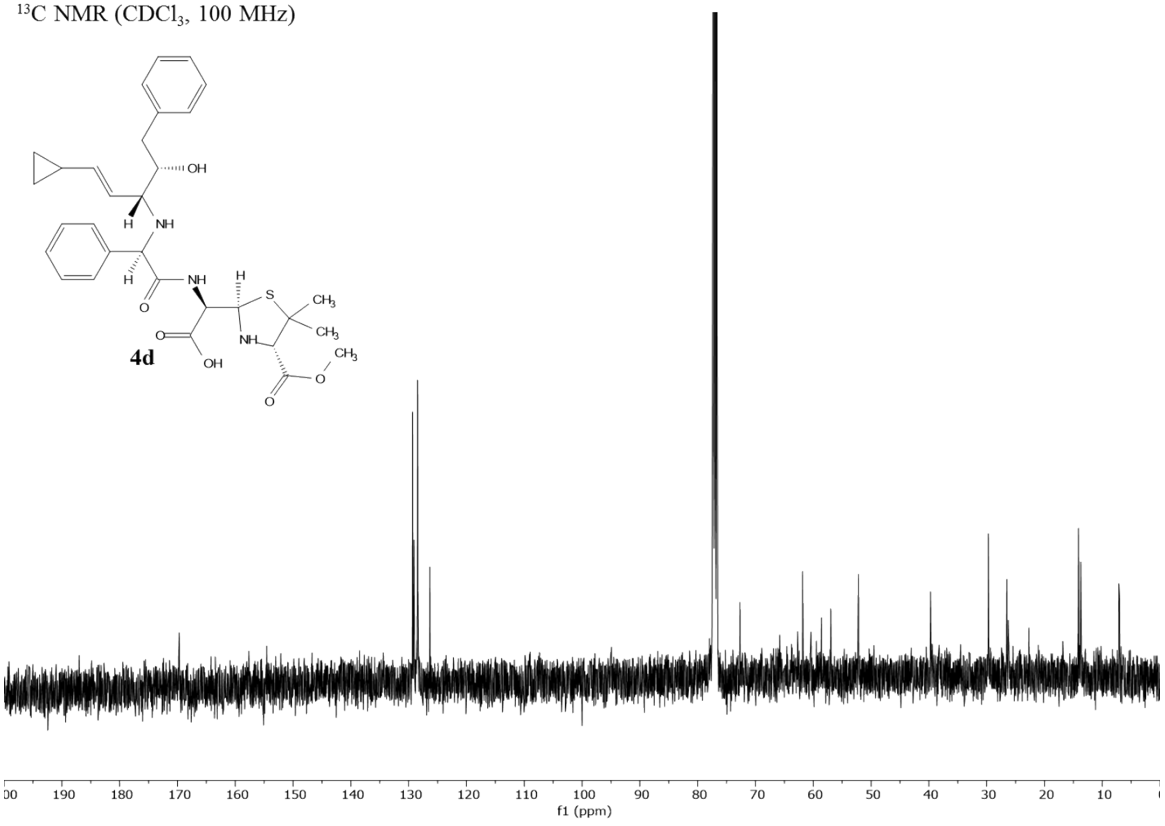


1d

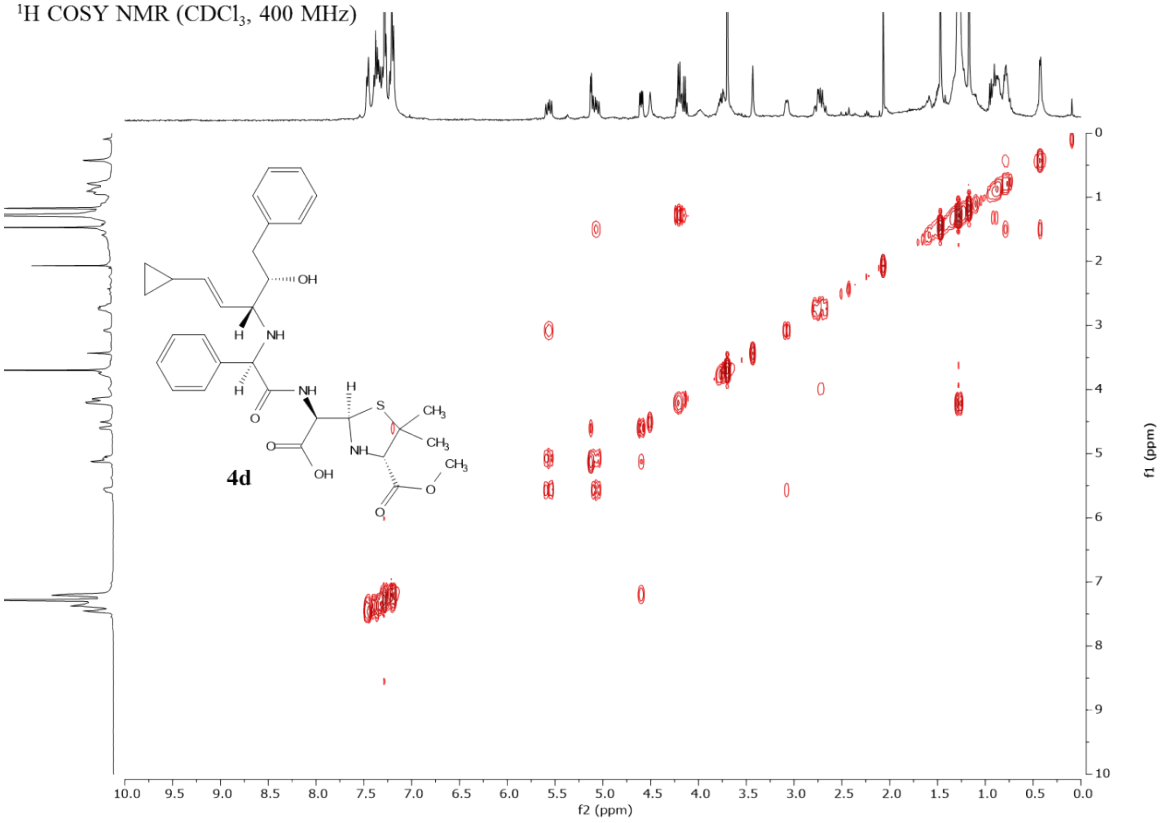
^{1}H NMR ($CDCl_3$, 400 MHz)



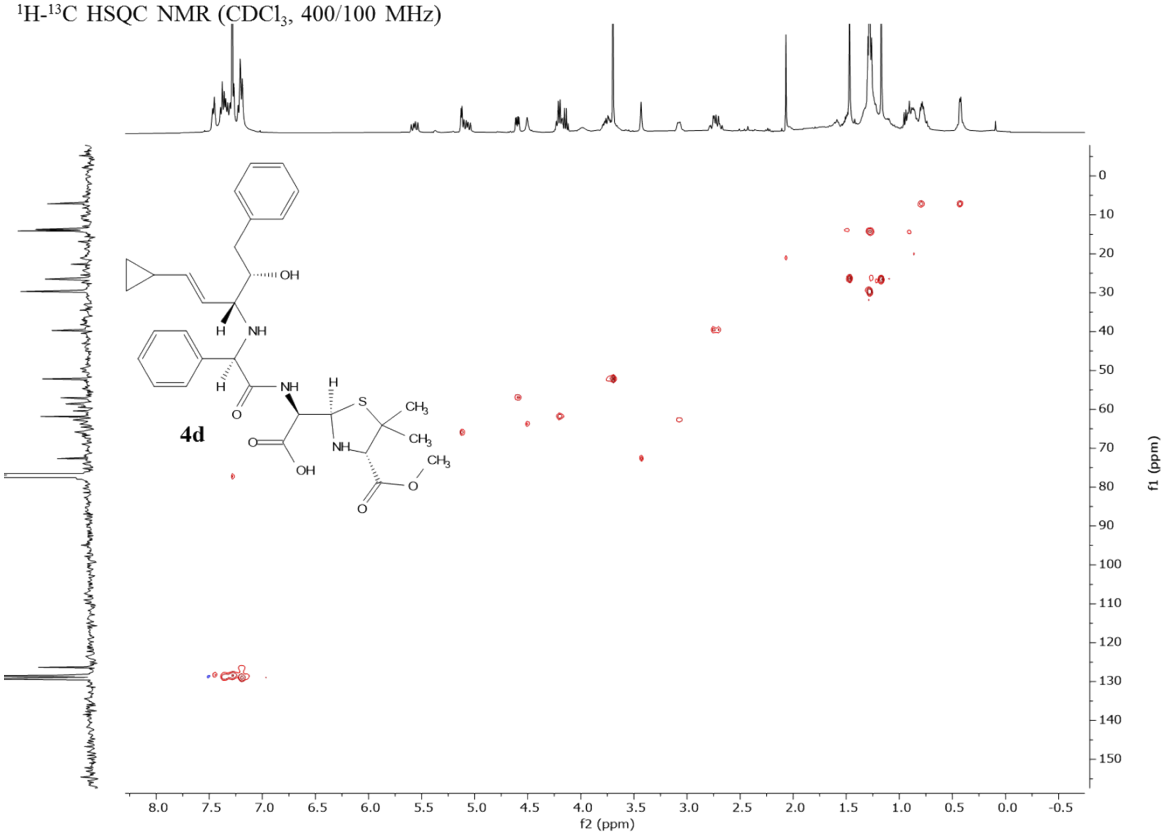
^{13}C NMR ($CDCl_3$, 100 MHz)



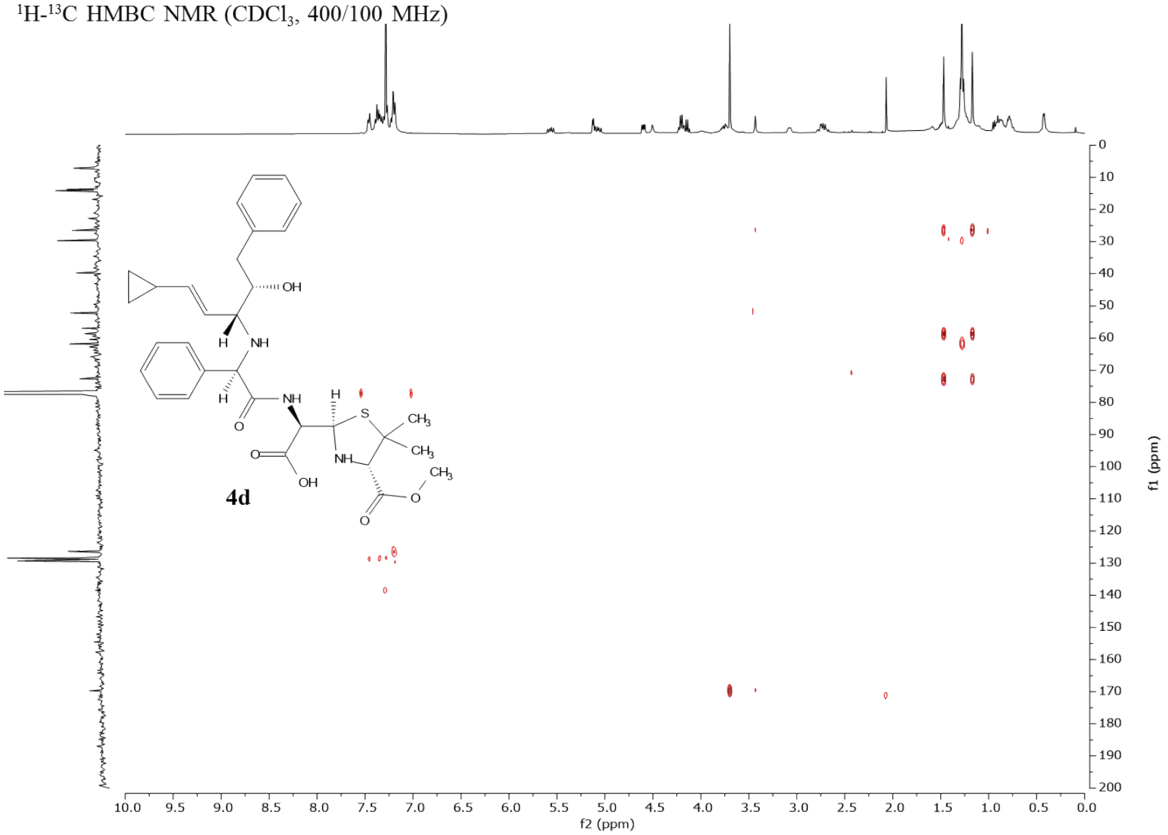
^{1}H COSY NMR ($CDCl_3$, 400 MHz)



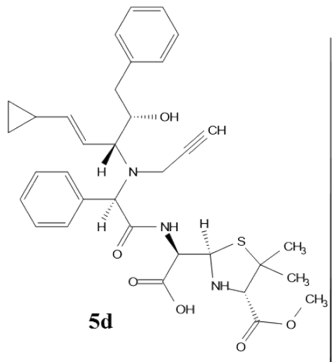
^{1}H-^{13}C HSQC NMR ($CDCl_3$, 400/100 MHz)



^{1}H-^{13}C HMBC NMR ($CDCl_3$, 400/100 MHz)

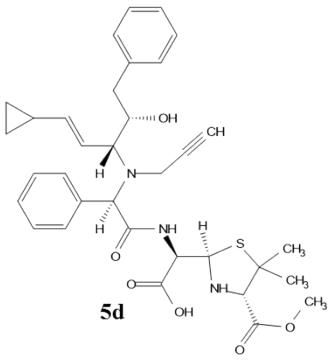


^{1}H NMR ($CDCl_3$, 400 MHz)



5d

^{13}C NMR ($CDCl_3$, 100 MHz)



5d

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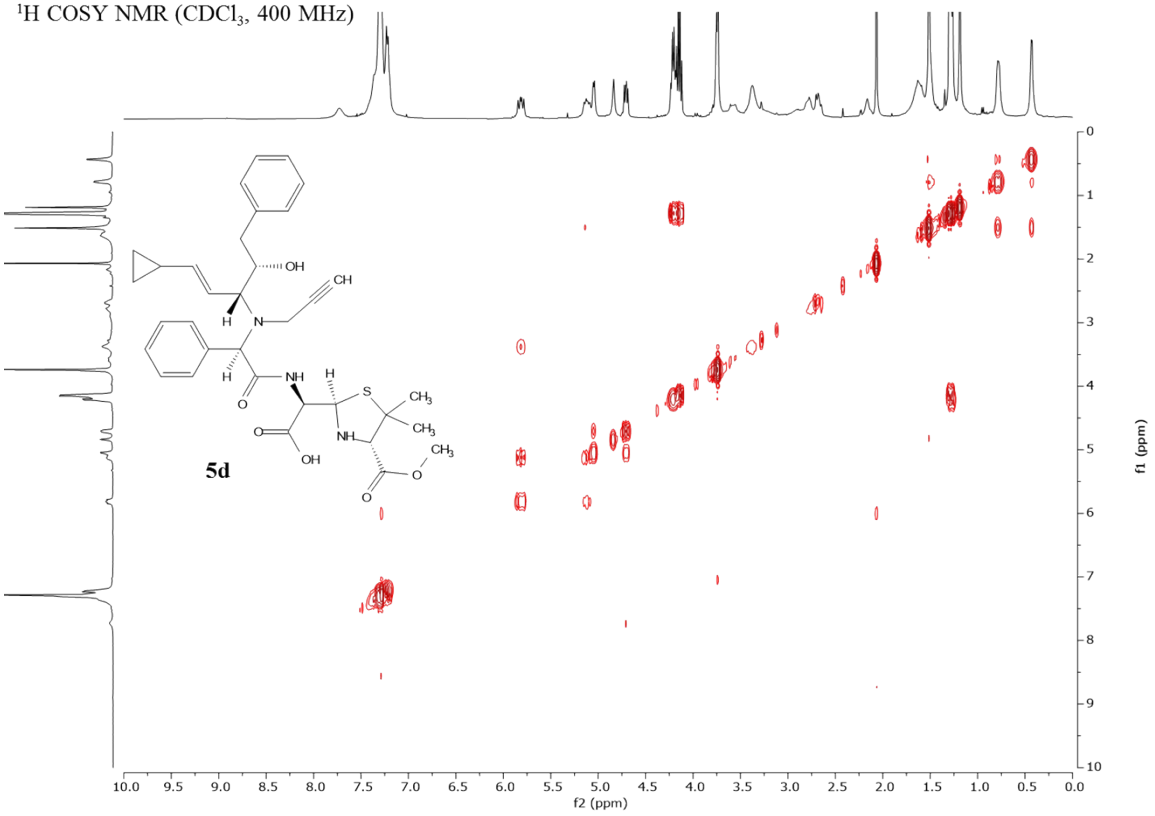
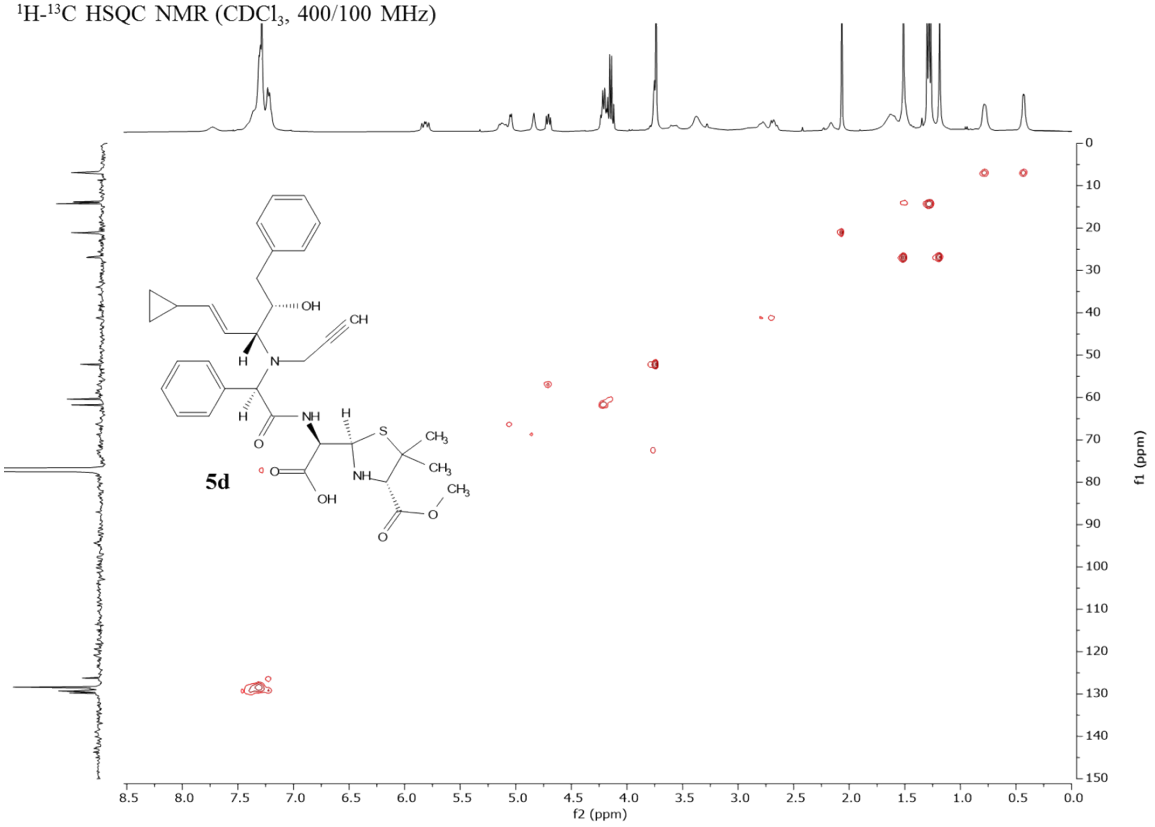
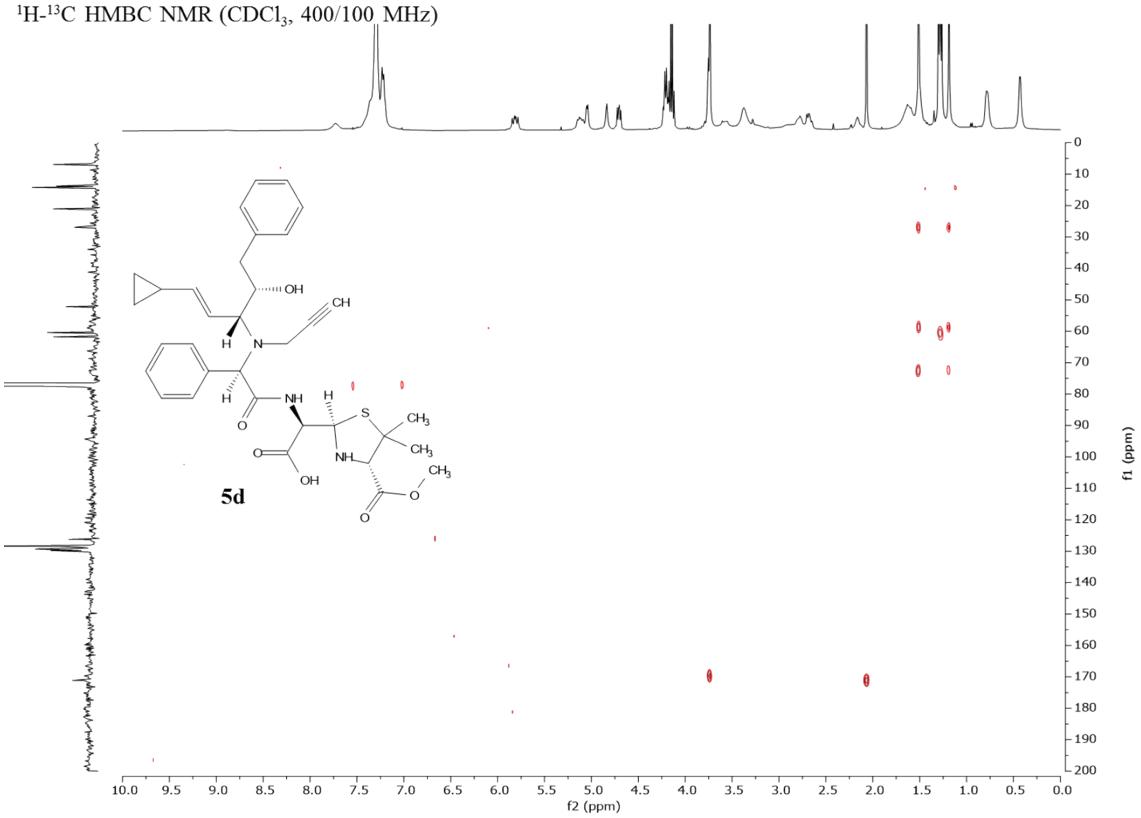


^{1}H-^{13}C HSQC NMR ($CDCl_3$, 400/100 MHz)



^{1}H-^{13}C HMBC NMR ($CDCl_3$, 400/100 MHz)



^{1}H NMR ($CDCl_3$, 400 MHz)

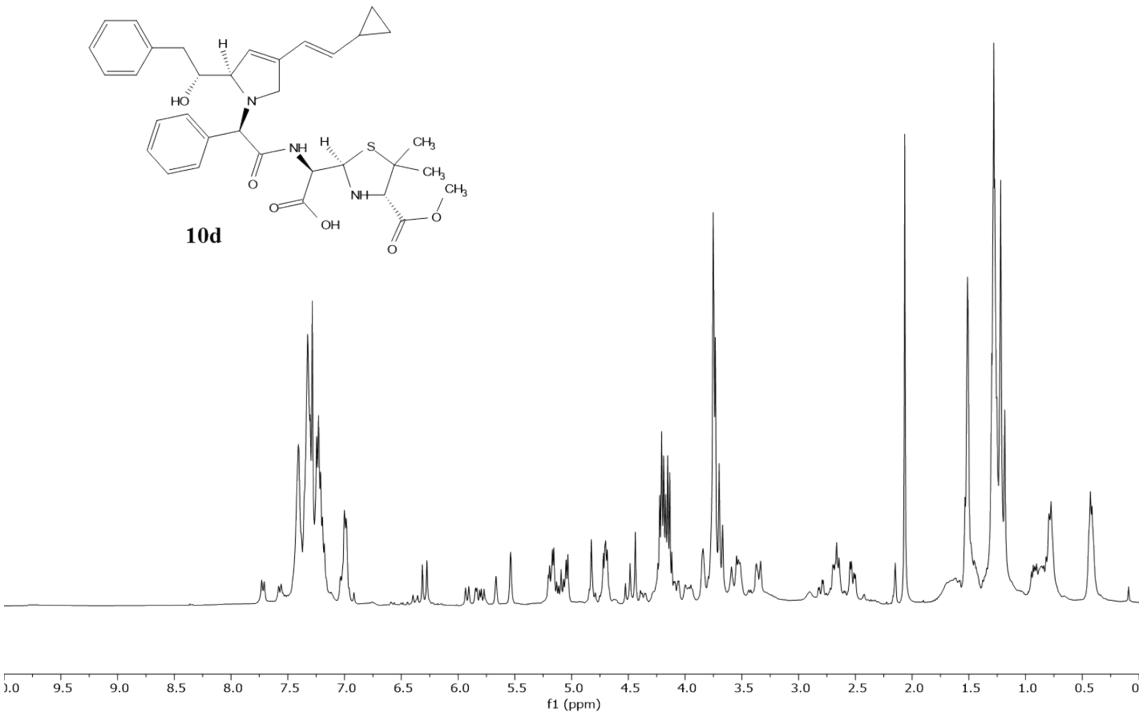


Table S1. Optimization conditions for the Petasis reaction

Entry	Solvent	Conditions	Molarity (M)	Equivalents 2:1a-f:3	Yield (%)
1	EtOH/HFIP 9:1	40 °C, 72 h	0,20	1:1:1	30
2	EtOH/HFIP 9:1	0 °C, 72 h	0,20	1:1:1	-
3	EtOH	rt, 72 h	0,25	1:1:1	-
4	DCM	rt, 72 h	0,25	1:1:1	-
5	DCM	rt, 24 h, 3Å molecular sieves	0,20	1:1:1	-
6	EtOH/HFIP 9:1	40 °C, 72 h, 3Å molecular sieves	0,20	1:1.5:1.5	53
7	EtOH/HFIP 9:1	40 °C, 72 h, 3Å molecular sieves, glycerol	0,20	1:1.5:1.5	23
8	EtOH/HFIP 9:1	rt, 24 h, (E)-2-Cyclopropylvinylboronic acid pinacol ester	0,20	1:1:1	-
9	EtOH/HFIP 9:1	40 °C, 72 h, 3Å molecular sieves, (E)-2-Cyclopropylvinylboronic acid pinacol ester	0,20	1:1.5:1.5	9

3. Biological methods

Antigen preparation

All synthesized compounds (7.0 μmol, 1,000 and 240 Equiv. for HSA and H1, respectively), dissolved in potassium carbonate 0.5 M, pH 11.0 in 10 % DMF, were mixed with the carrier protein (0.5 mg) and stirred overnight at room temperature. Reference antigens for PG, AMX, CFT, AZT and MRP were prepared following the major configuration. As commercially supplied from Sigma (240 Equiv), the corresponding BLC, dissolved in potassium carbonate 0.5 M, pH 11.0, was reacted with the carrier protein (2.0 mg) and stirred overnight at room temperature. Prepared antigens were purified by gel filtration chromatography on Amicon Ultra 0.5 pre-concentrated 10 K filters using PBS 1X, pH 7.4, as elution buffer. Carrier proteins used were HSA and H1.

All the synthesized haptens which lacks a beta-lactam ring (**5c**, **4d**, **5d**, and **10d**) were conjugated to the carrier proteins, following the the carbodiimide chemistry as previously described[1].

New Zealand white rabbits were immunized to raise specific IgG for PG, CFT, AZT, and MRP. To prepare the immunogenic antigens, the same protocol as for the preparation of the reference antigens was followed, but KLH (1,000 Equiv.) was used as the immunogenic protein. Immunogens were purified by size exclusion chromatography on dextran desalting columns using PBS 1X, pH 7.4, as the elution buffer.

All determinants were diluted to 1.0 mg/mL and stored at -20 ºC until used. Antigen concentrations were determined by the Bradford protein assay[2]**,** and the protein-hapten molar ratio was established by MS-MALDI-TOF.[3]

Protein Digestion and LC-ESI-MS/MS Analysis

Protein digestion of HSA into smaller peptides was accomplished using trypsin and nanoscale liquid chromatography coupled to tandem mass spectrometry (nano LC-MS/MS) as the analytical technique. First, 5 μg of the antigen in PBS 1X (5 μL) were taken and set to 20 μL of 50 mM ammonium bicarbonate. Then, it was summited to the following digestion protocol: i) 2 mM dithiothreitol in 50 mM NH_4HCO_3, V_f= 25 μL, 20 min (60 °C); ii) 5.5 mM iodoacetamide in 50mM NH_4HCO_3, V_f=25 μL, 30 min (room temperature, darkness); iii) 10 mM dithiothreitol in 50mM NH_4HCO_3, V_f=50 μL, 30 min (37 °C); and iv) 200 ng trypsin, V_f=52 μL, overnight (60 °C). Finally, digestion was stopped with 6 μL 10% trifluoroacetic acid. The final tryptic peptides solution was at 0.086 μg/μL.

Next, 5 μl of the digested sample was loaded onto a trap column (NanoLC Column, 3μ C18-CL, 350 um x 0.5 mm; Eksigent) and desalted with 0.1% TFA at 3μl/min during 5 min. The peptides were then loaded onto an analytical column (LC Column, 3 μ C18-CL, 75 um x 12 cm, Nikkyo) equilibrated in 5% acetonitrile 0.1% formic acid. Elution was carried out with a linear gradient of 5 to 35% B in A for 30 min. (A: 0.1% formic acid; B: acetonytrile, 0.1% formic acid) at a flow rate of 300 nl/min. Peptides were analyzed in a mass spectrometer nanoESI qQTOF (5600 TripleTOF, ABSCIEX). Eluted peptides were ionized applying 2.8 kV to the spray emitter, and analysis was carried out in a data-dependent mode. TOF-MS scanned for 250 ms from 350 to 1250 m/z and 25 ms from 100 to 1500 m/z on the 25 most intense 2-5 charged peaks. Dynamic exclusion was set to 15 s.

ProteinPilot v4.5 (ABSciex) default parameters were used to generate peak list directly from 5600 TripleTOF tiff files. The peak lists were saved as Mascot generic files (mgf) searched with MASCOT v 2.3.02 (Matrix Science).

Database search was performed on the Swissprot database with the taxonomy fixed to humans. Searches were done with tryptic specificity allowing two missed cleavages and tolerance on the mass measurement of 25 ppm in MS mode and 0.6 Da for MS/MS ions. Artificial modifications on peptides such as carbamidomethylation on Cys, oxidation on Met, and deamidation on Asn and Gln, were excluded to ensure reliable quantification.

Multiplexed Compact disc-based test protocol

Reagents printed were (I) the prepared and reference antigens and (II) controls. HSA and rabbit IgG (or human IgE) were used as negative and positive controls of the assay. Reference antigens were used to test the specificity of each serum. Antigens were prepared in sodium carbonate/bicarbonate buffer 0.1 M, pH 9.6, and controls in PBS 1X, pH 7.4.

Reagents (25 nL, 40 μg/mL) were spotted in microarray format (20 arrays per disk of 5 × 4 spots), using a noncontact printing device (AD 1500 BioDot, Inc., Irvine, CA) on a regular DVD (Digital versatile Disc) (CD Rohling-up GmbH, Saarbrücken, Germany). After printing, the DVDs were incubated overnight at 37 ºC.

To detect specific IgG against PG, CFT, MRP, and AZT, different dilutions (1/250-1/16,000) of rabbit sera and control (PBS-T) (25 μL per sample) were added to each array and incubated for 15 min. Then, the washing step consisted of washing the DVD with PBS-T and water. 25 μL of goat anti-rabbit antibody labeled with horseradish peroxidase (Abcam, Cambridge, UK) in PBS-T (dilution 1/400) was added for 15 min followed by the washing step.

The histogram depicting the ability of all antigens to elicit activity in the immunoassay is shown in Figure S2A and comprises assay data for compounds against sera raised against PG (S1) and CFT (S2).

To detect specific IgE from allergic patients and controls, 25 μL of the sample was added to each array and incubated for 30 min. Then, the DVD was washed, and 25 μL of α-human IgE monoclonal antibody (Ingenasa, Madrid, Spain) in PBS-T buffer (1 μg/mL) was added and incubated for 15 min. After washing as before, 25 μL of a 1/100 dilution of goat anti-mouse antibody labeled with horseradish peroxidase (Abcam, Cambridge, UK) was added for 15 min.

Figure S1. A) Histogram of the 'activity' of the antigens. B) Heatmaps for the cohort of the HSA- and H1-derived antigens representing the signals obtained in the immunoassays for each immunised sera (S1 and S2). 1) 6-APA, 2) 7-ACDA, 3) AMX, and 4) AMP-derived antigens.

The immunoreaction was developed by homogeneously dispensing 1.0 mL of TMB (SDT GmbH, Baesweiler, Germany) along the whole disc surface. The reaction was stopped by washing the disk with water after 8 min. A modified DVD drive read signals, and data were analyzed as previously described.[4] All experiments were repeated three times.

10 20 30 40 50
MKWVTFISLL FLFSSAYSRG VFRRDAHKSE VAHRFKDLGE ENFKALVLIA
60 70 80 90 100
FAQYLQQCPF EDHVKLVNEV TEFAKTCVAD ESAENCDKSL HTLFGDKLCT
110 120 130 140 150
VATLRETYGE MADCCAKQEP ERNECFLQHK DDNPNLPRLV RPEVDVMCTA
160 170 180 190 200
FHDNEETFLK KYLYEIARRH PYFYAPELLF FAKRYKAAFT ECCQAADKAA
210 220 230 240 250
CLLPKLDELR DEGKASSAKQ RLKCASLQKF GERAFKAWAV ARLSQRFPKA
260 270 280 290 300
EFAEVSKLVT DLTKVHTECC HGDLLECADD RADLAKYICE NQDSISSKLK
310 320 330 340 350
ECCEKPLLEK SHCIAEVEND EMPADLPSLA ADFVESKDVC KNYAEAKDVF
360 370 380 390 400
LGMFLYEYAR RHPDYSVVLL LRLAKTYETT LEKCCAAADP HECYAKVFDE
410 420 430 440 450
FKPLVEEPQN LIKQNCELFE QLGEYKFQNA LLVRYTKKVP QVSTPTLVEV
460 470 480 490 500
SRNLGKVGSK CCKHPEAKRM PCAEDYLSVV LNQLCVLHEK TPVSDRVTKC
510 520 530 540 550
CTESLVNRRP CFSALEVDET YVPKEFNAET FTFHADICTL SEKERQIKKQ
560 570 580 590 600
TALVELVKHK PKATKEQLKA VMDDFAAFVE KCCKADDKET CFAEEGKKLV

AASQAALGL

Figure S2. The amino acid sequence was obtained for HSA after protein modification with antigen **1c**.

HSA is a single chain consisting of 609 amino acids with a molecular weight of 66.5 kDa, including a signal peptide (1–18), a pro-peptide (19–24), and the active albumin (585 amino acids).[5] Both the signal peptide and pro-peptide amino acids are blue, the modified peptide is red, and the altered amino acid residue is yellow-highlighted.

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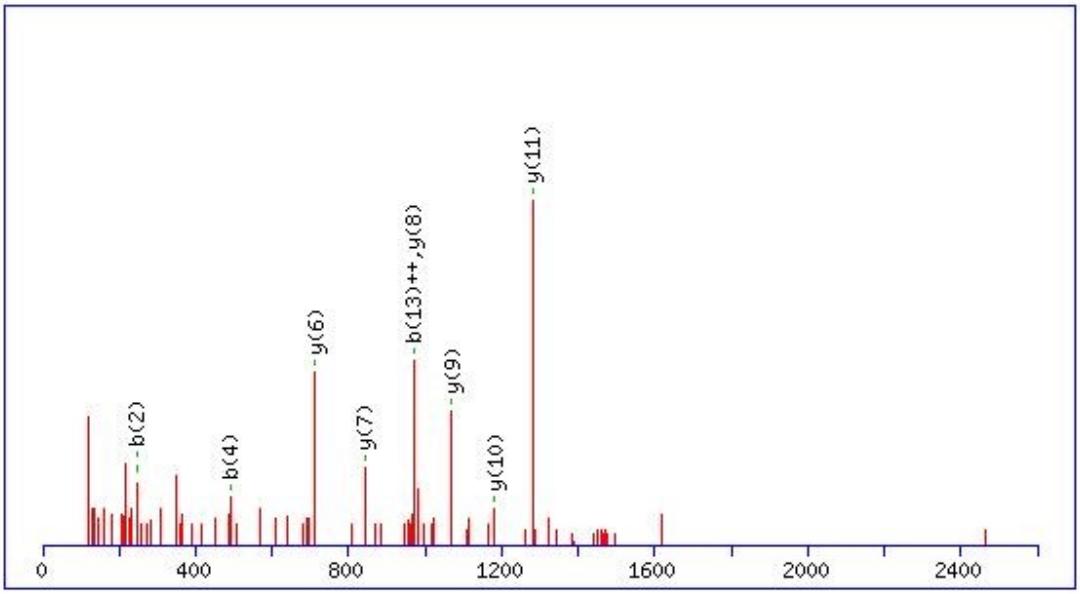

#	b	b^{++}	b*	b^{*++}	b^{0}	b^{0++}	Seq.	y	y^{++}	y*	y^{*++}	y^{0}	y^{0++}	#
1	100.0757	50.5415					V							17
2	**247.1441**	124.0757					F	2326.1550	1163.5811	2309.1284	1155.0678	2308.1444	1154.5758	16
3	362.1710	181.5892			344.1605	172.5839	D	2179.0866	1090.0469	2162.0600	1081.5336	2161.0760	1081.0416	15
4	**491.2136**	246.1105			473.2031	237.1052	E	2064.0596	1032.5334	2047.0331	1024.0202	2046.0491	1023.5282	14
5	638.2821	319.6447			620.2715	310.6394	F	1935.0170	968.0121	1917.9905	959.4989	1917.0065	959.0069	13
6	1146.5050	573.7562	1129.4785	565.2429	1128.4945	564.7509	K	1787.9486	894.4779	1770.9221	885.9647	1769.9380	885.4727	12
7	1243.5578	622.2825	1226.5312	613.7693	1225.5472	613.2773	P	***1279.7256***	640.3665	1262.6991	631.8532	1261.7151	631.3612	11
8	1356.6419	678.8246	1339.6153	670.3113	1338.6313	669.8193	L	***1182.6729***	591.8401	1165.6463	583.3268	1164.6623	582.8348	10
9	1455.7103	728.3588	1438.6837	719.8455	1437.6997	719.3535	V	***1069.5888***	535.2980	1052.5623	526.7848	1051.5782	526.2928	9
10	1584.7529	792.8801	1567.7263	784.3668	1566.7423	783.8748	E	***970.5204***	485.7638	953.4938	477.2506	952.5098	476.7585	8
11	1713.7955	857.4014	1696.7689	848.8881	1695.7849	848.3961	E	***841.4778***	421.2425	824.4512	412.7293	823.4672	412.2373	7
12	1810.8482	905.9278	1793.8217	897.4145	1792.8377	896.9225	P	***712.4352***	356.7212	695.4087	348.2080			6
13	1938.9068	969.9570	1921.8803	961.4438	1920.8962	960.9518	Q	615.3824	308.1949	598.3559	299.6816			5
14	2052.9497	1026.9785	2035.9232	1018.4652	2034.9392	1017.9732	N	487.3239	244.1656	470.2973	235.6523			4
15	2166.0338	1083.5205	2149.0072	1075.0073	2148.0232	1074.5153	L	373.2809	187.1441	356.2544	178.6308			3
16	2279.1179	1140.0626	2262.0913	1131.5493	2261.1073	1131.0573	I	260.1969	130.6021	243.1703	122.0888			2
17							K	147.1128	74.0600	130.0863	65.5468			1

Figure S3. Modified peptide and ESI-MS/MS spectra fragmentation pathway of (K) VFDEFKPLVEEPQNLIK(Q)

Calibrators used: 0, 0.35, 0.70, 3.50, 17.5 and 100. IU/mL. The standard data points (signal vs. semi-log concentration) were the mean of 5 curves performed on different days and different disks. A four-parameter logistic (4PL) curve was fitted through the points, using SigmaPlot 11 software. Sensitivity (IC_{50}= 0.92 ± 0.03 IU/mL), slope= 1.24, r^2= 0.9998. The limit of quantification (≥0.06 IU/mL) is equal to the signal of the blank plus ten times the SD (mean + 10xSD), with a relative standard deviation (RSD) ranging from 3 to 15%.

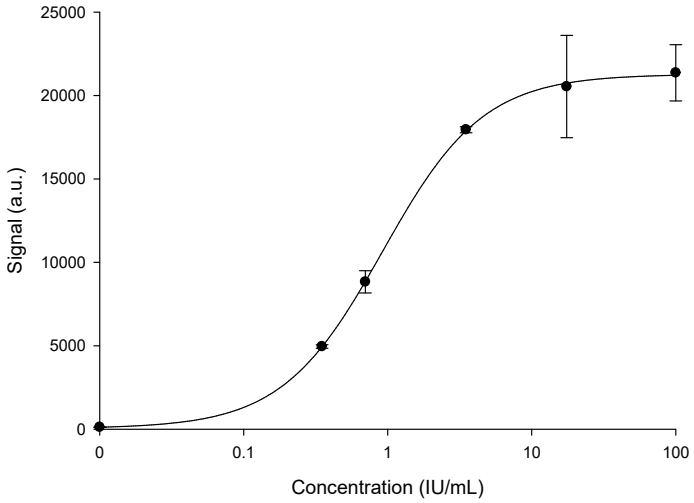

Figure S4. Calibration curve for IgE using IgE human serum (3rd WHO International Standard) and the capture antibody Omalizumab.

4. Samples from allergic patients and controls

All samples from allergic patients and controls were provided by the Hospital Universitari i Politènic La Fe (Valencia, Spain), and informed consent for the diagnostic procedures was obtained from all patients. All participants were enrolled after giving written informed consent according to protocols approved by the ethics review board at La Fe University Hospital (registry no. COBIOPHAD). The procedures followed were in accordance with the Helsinki Declaration of 1975 as revised in 2008. The patients were diagnosed following the process described in the European Network of Drug Allergy (ENDA) protocol based on skin testing, in vitro tests, or drug provocation tests, whenever necessary.

Table S2. Clinical characteristics of the cohort of allergic patients

Patient Number	Sex[a]	Age (years)	Culprit drug	Clinical manifestation	Route	Time
1	M	68	Augmentin	Anaphylaxis	Parenteral	Immediate
2	F	36	Amoxicillin	Anaphylaxis	Oral	Immediate
3	F	42	Augmentin	Anaphylaxis	Oral	Immediate
4	M	49	Augmentin	Cutaneous	Oral	Delayed
5	M	80	Augmentin	Anaphylaxis	Parenteral	Immediate
6	M	44	Augmentin	Cutaneous	Oral	Delayed
7	F	32	Augmentin	Anaphylaxis	Oral	Immediate
8	F	47	Amoxicillin	Anaphylaxis	Oral	Immediate
9	M	56	Augmentin	Cutaneous	Oral	Immediate
10	F	32	Cefuroxime	Cutaneous	Oral	Delayed
11	F	19	Unspecific	Cutaneous	Manipulation	Immediate
12	M	56	Amoxicillin	Cutaneous	Oral	Immediate

[a]Sex: F=Female, M=Male.

5. References

[1] E. Peña-Mendizabal, S. Morais, A. Maquieira. *Chem. Commun*. **2020**, *56*, 11973-11976.

[2] M. M. Bradford, *Anal. Biochem.* **1976**, *72*, 248–254.

[3] Y. Wang, C. H. He, H. Zheng, H. Bin Zhang, *Int. J. Mol. Sci.* **2012**, *13*, 84–96.

[4] P. Dobosz, S. Morais, E. Bonet, R. Puchades, Á. Maquieira, *Anal. Chem.* **2015**, *87*, 9817–9824.

[5] S. Wang, S. Liu, Y. Zhang, J. He, D. H. Coy, L. Sun, *Heal. Sci. J.* **2020**, *14*, 1–8.