Total Synthesis and Complete Structural Assignment of Thiocillin I

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Supporting Information

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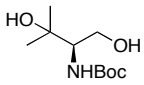
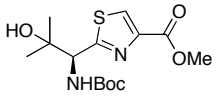
1. Experimental Protocols

Unless otherwise stated, ^{1}H and ^{13}C NMR spectra were recorded on Bruker model AVANCE II+ 300 (300 MHz for ^{1}H and 75.5 MHz for ^{13}C) spectrometer using deuteriochlorofom ($CDCl_3$) as the solvent. Chemical shifts are reported in parts per million (ppm) on the δ scale and coupling constants, *J*, are in hertz (Hz). Multiplicities are reported as "s" (singlet), "d" (doublet), "t" (triplet), "q" (quartet), "dd" (doublet of doublets), "ddd" (doublet of doublets of doublets), "m" (multiplet), "app" (apparent) and "br" (broad). Infrared (IR) spectra (cm^{-1}) were recorded on a Perkin–Elmer model 1710 Fourier transform spectrophotometer from films deposited on NaCl plates. Optical rotations were measured on a Jasco P–1010 polarimeter at the sodium D line (589 nm). Low–resolution mass spectra (m/z) were obtained in the electrospray (ESI) mode on a Waters Micromass ZQ mass spectrometer. High–resolution mass spectra (m/z) were recorded in the electrospray (ESI) mode on a Micromass LCT mass spectrometer by the UBC Mass Spectrometry laboratory. Low– and high–resolution mass spectra obtained in electron impact (EI) mode were recorded on MASPEC II System mass spectrometer by the UBC Mass Spectrometry laboratory. Melting points (uncorrected) were measured on a Mel–Temp apparatus. All reagents and solvents were commercial products and used without further purification except THF, Et_2O (both freshly distilled from Na/benzophenone under argon) and CH_2Cl_2 (freshly distilled from CaH_2 under argon). Commercial *n*-BuLi was titrated against *N*-benzylbenzamide in THF at –78°C until persistence of a light blue color. Flash chromatography was performed on Silicycle 230-400 mesh silica gel. Analytic and preparative TLC was carried out with Merck silica gel 60 plates with fluorescent indicator. Spots were visualised with UV light. All reactions were performed under dry argon in flame– or oven–dried flasks equipped with Teflon™ stirbars. All flasks were fitted with rubber septa for the introduction of substrates, reagents and solvents via syringe. Solvents, pure liquid reagents or reagents in solution, and solids were added in one portion, unless otherwise stated.

2. Synthesis and Characterization of Various Intermediates

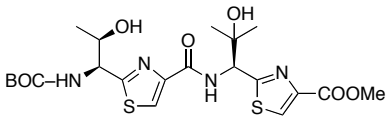


(*R*)-*tert*-Butyl 1,3-dihydroxy-3-methylbutan-2-ylcarbamate (24). (D)-Serine methyl ester hydrochloride (4.14 g, 26.6 mmol) was suspended in 15 mL of CH_2Cl_2. A solution of $NaHCO_3$ (2.23 g, 26.6 mmol) in 10 mL of H_2O was added, followed by a solution of BOC_2O (6.38 g, 29.3 mmol) in 15 mL of CH_2Cl_2. Solid NaCl (4.63 g, 79.8 mmol) was added and the mixture was heated at 50° C for 12 hours, then it was cooled to r.t. and the organic layer was separated. The aqueous phase was extracted with CH_2Cl_2 (2 x 30 mL) and the combined extracts were dried (Na_2SO_4) and concentrated under vacuum. The crude N-Boc serine was dissolved in 30 mL of CH_2Cl_2. Neat DHP (4.82 mL, 53.2 mmol) was added, followed by PPTS (0.668 g, 2.66 mmol). The mixture was stirred overnight, then it was washed with 20 mL of sat. $NaHCO_3$ sol. and concentrated to give the protected (THP) alcohol. This crude material was dissolved in 20 mL of THF and then added to a solution of 3M MeMgBr (35.4 mL, 106 mmol) in 10 mL of THF under argon at 0º C. After 5 minutes, the reaction mixture was heated to 45° C for 1 hour. Upon cooling to r.t., the reaction was quenched by the slow addition of 20 mL of sat. NH_4Cl sol., followed by 30 mL of water; then it was extracted with EtOAc (3 x 35 mL). The combined extracts were dried (Na_2SO_4) and concentrated in vacuum. The crude residue was redissolved in MeOH (40 mL) containing TsOH (0.5 g, 2.7 mmol) and the solution was stirred for 3 hours. The mixture was then evaporated and the residue redissolved in 40 mL of EtOAc. The organic layer was washed with 10 mL of saturated $NaHCO_3$ solution, dried (Na_2SO_4) and evaporated in vacuo. The residue was then purified by flash chromatography (30% EtOAc/ Hexanes) to give compound **24** (4.80 g, 82% over 4 steps) as a colorless oil, which solidified upon standing: m.p.: 61-63 °C; $[\alpha]_D$: –17.3° (*c* = 1.0, $CHCl_3$). **IR**: 3418, 1709. **^{1}H**: 5.38 (br d, 1H), 4.04 (m, 1H), 3.81 (m, 1H), 3.47 (br m, 1H), 2.61 (s, 1H), 2.53 (m, 1H), 1.46 (s, 9H), 1.36 (s, 3H), 1.26 (s, 3H). **^{13}C**: 156.5, 79.6, 73.6, 63.1, 57.8, 28.3, 27.4, 27.1. **MS**: 242.3 [M + Na^+]. **HRMS**: calcd for $C_{10}H_{21}NO_4Na^+$: 242.1368; found: 242.1366.

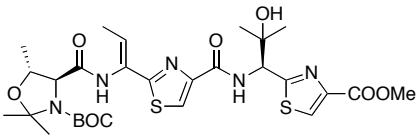


***(S)*-Methyl-2-(1-(*tert*-butoxycarbonylamino)-2-hydroxy-2-methylpropyl)thiazole-4-carboxylate (27)**. Sulfur trioxide-pyridine complex (5.30 g, 33.2 mmol) was dissolved in 20 mL of warm DMSO and added to a solution of compound **24** (2.43 g, 11.1 mmol) in CH_2Cl_2 (30 mL). The mixture was stirred for 45 minutes, then it was quenched with 30 mL of saturated solution of $NaHCO_3$. The volatiles were removed in vacuum and the product was extracted with EtOAc (3 x 20 mL). The organic layer was then washed with 15 mL of 1M $NaHSO_4$, 15 mL of brine, 15 mL of H_2O and then dried with Na_2SO_4 and concentrated to give the sensitive aldehyde **25**, which was immediately dissolved in 20 mL of MeOH and treated with a solution of L-cysteine methyl

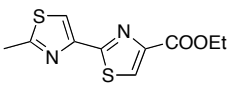
ester hydrochloride (2.85 g, 16.6 mmol) in 10 mL of H_2O. After stirring for 2 hours the volatiles were evaporated and the product was extracted with EtOAc (3 x 30 mL). The organic layer was dried with Na_2SO_4 and concentrated in vacuum to give the thiazolidine as a 1:1 mixture of diastereomers. The crude thiazolidine was dissolved in 30 mL of MeCN containing oven-dried "chemical" MnO_2 (38.6 g, 444 mmol, purchased from Wako Pure Chemicals, 99.5% purity). The reaction mixture was heated to 60° C for 24 hrs, then it was filtered over Celite® and concentrated in vacuum. The residue was purified by flash chromatography (5% acetone/ CH_2Cl_2) to give compound **27** (1.10 g, 30% over 3 steps) as a white solid, m.p.: 47-50 °C; $[\alpha]_D$: +24.5° (*c* = 0.2, $CHCl_3$). **IR**: 3386, 1708. ^{1}**H**: 5.38 (br s, 1H), 4.04 (m, 1H), 3.81 (m, 1H), 3.47 (br, 1H), 2.61 (s,1H), 1.46 (s, 9H), 1.36 (s, 3H), 1.26 (s,3H). ^{13}**C**: 156.5, 79.6, 73.6, 63.1, 57.8, 28.3, 27.4, 27.1. **MS**: 242.3 [M+Na^+]. **HRMS**: calcd for $C_{10}H_{21}NO_4Na^+$: 242.1368; found: 242.1366.



Methyl-2-((*R*)-1-(2-((1*S*,2*R*)-1-(*tert*-butoxycarbonyl-amino)-2-hydroxypropyl)thiazole-4-carboxamido)-2-hydroxy-2-methyl-propyl)thiazole-4-carboxylate (30). A solution of hydroxyvaline-derived thiazole **27** (711 mg, 2.15 mmol) in 5 mL of a 4:1 mixture of CH_2Cl_2 and TFA was stirred for 1 hr under argon. The reaction was then concentrated under vacuum. The residue was taken up with more CH_2Cl_2 (5 mL) and the solution again concentrated in vacuum to promote complete removal of TFA. The residue of TFA salt of amine **28** thus obtained (quantitative yield) was dissolved in 5 mL of CH_2Cl_2 and added to a solution of acid **29** (0.651 g, 2.15 mmol), HOBt (0.349 g, 2.58 mmol) and Et_3N (0.95 mL, 6.88 mmol) in 7 mL of CH_2Cl_2. The mixture was stirred for 2 minutes, then EDCI (0.495 g, 2.58 mmol) was added and stirring was continued overnight. The reaction was quenched with 10 mL of aq. sat. NH_4Cl sol. and the organic layer was separated and concentated under vacuum. The residue was redissolved in 30 mL of EtOAc and was sequentially washed with 10 mL of aq. sat. $NaHCO_3$ sol., 10 mL of H_2O and 10 mL of brine, then dried with Na_2SO_4 and concentrated under vacuum to give a brown solid. Purification by flash chromatography (70% EtOAc/ Hexanes) gave **30** (0.720 g, 65% over 3 steps) as a white solid, m.p.: 73-75 °C; $[\alpha]_D$: –33.0° (*c* = 0.2, $CHCl_3$). **IR**: 3387, 1731, 1695. ^{1}**H**: 8.22 (d, 1H, *J* = 9.1), 8.13 (s, 1H), 8.05 (s, 1H), 5.70 (d, 1H, *J* = 8.7), 5.34 (d, 1H, *J* = 8.7), 4.89 (d, 1H, *J* = 9.3), 4.63 (m, 1H), 3.92 (s, 3H), 1.46 (s, 9H), 1.36 (s, 3H), 1.33 (d, 3H, *J* = 6.4), 1.28 (s, 3H). ^{13}**C**: 173.2, 169.1, 161.5, 160.8, 155.9, 149.0, 146.2, 128.0, 124.5, 80.4,72.7, 68.5, 57.5, 57.3, 52.4, 28.3, 27.8, 26.4, 19.6. **MS**: 537.3 [M + Na^+]. **HRMS**: calcd for $C_{21}H_{30}N_4O_7S_2Na^+$: 537.1454; found: 537.1464.

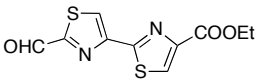


(4*S*,5*R*)-*tert*-butyl-4-((*Z*)-1-(4-((*R*)-2-hydroxy-1-(4-(methoxycarbonyl)thiazol-2-yl)-2-methylpropylcarbamoyl) thiazol-2-yl)prop-1-enylcarbamoyl)-2,2,5-trimethyloxaz-olidine-3-carboxylate (34). A solution of **30** (0.720 g, 1.49 mmol) in 10 mL of 4 : 1 CH_2Cl_2 – TFA was stirred for one hour, then it was concentrated under vacuum (repeated twice for complete removal of TFA) and the residue was redissolved in 5 mL of CH_2Cl_2. The resulting solution was then added to a solution of threonine derivative **32** (0.387g, 1.49 mmol), HOBt (0.246 g, 1.82 mmol) and Et_3N (0.923 mL, 1.82 mmol). After the mixture was allowed to stir for 2 minutes, EDCI (0.350 g, 1.82 mmol) was added and stirring was continued overnight. The reaction was quenched with 10 mL of saturated solution of NH_4Cl and the organic layers were separated. The volatiles were removed under vacuum and the residue was redissolved in 30 mL of EtOAc. The organic layer was washed once with 10 mL of saturated $NaHCO_3$, 10 mL of H_2O and 10 mL of brine. The organic layer was dried with Na_2SO_4 and concentrated under vacuum to give crude **33** (brown solid), which was redissolved in 4 mL of CH_2Cl_2 and treated with Et_3N (0.61 mL, 4.47 mmol) and MsCl (0.14 mL, 1.79 mmol). After stirring for 2 h (under argon) at rt, DBU (0.67 mL, 4.47 mmol) was added and the mixture and stirred for 4 more hours. Once the reaction was complete (TLC), it was quenched by the addition of 10 mL of saturated NH_4Cl solution. The organic layer was separated and the aqueous phase was further extracted with CH_2Cl_2 (3 x 10 mL). The combined organic phases were dried with Na_2SO_4 and concentrated under vacuum. The crude was then subjected to chromatography to give the title compound as a white solid (0.30g, 32% over 3 steps), m.p. 122-124 °C; $[\alpha]_D$: –22.4° (*c* = 1, $CHCl_3$). **IR**: 3387, 3360, br 1677. **^{1}H**: 8.19 (d, 1H, *J* = 8.6 Hz), 8.12 (s, 1H), 7.99 (s, 1H), 6.57 (br s, 1H), 5.34 (d, 1H, *J* = 9.4 Hz), 4.36 (m, 1H), 4.29 (br s, 1H), 4.02 (d, 1H, *J* = 9.4), 3.90 (s, 3H), 1.86 (d, 3H, *J* = 6.7), 1.64 (s, 3H), 1.62 (s, 3H), 1.47 (d, 3H, *J* = 6.0), 1.42 (br s, 9H), 1.35 (s, 3H), 1.26 (s, 3H). **^{13}C**: 169.2, 168.3, 167.0, 161.5, 160.6, 152.4, 149.1, 146.3, 127.9,127.6, 123.8, 95.1, 81.3, 74.3, 72.6, 67.7, 57.4, 52.3, 28.3, 27.7, 26.7, 25.7, 19.2, 14.4. **MS**: 660.3 [M+H^+]. **HRMS**: calcd for $C_{28}H_{39}N_5O_8S_2Na^+$: 660.2138; found: 660.2131.

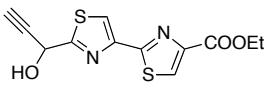


Ethyl-2'-methyl-2,4'-bithiazole-4-carboxylate (10). Ethyl 2-methylthiazole-4-carboxylate (8.7 g, 51.1 mmol) was dissolved in aq. conc. NH_4OH sol. (40 mL) and the mixture was heated to 70 °C for 2 h. The solution was cooled to rt and extracted with EtOAc (3 x 30 mL). The combined extracts were evaporated and the residue of 2-methylthiazole-4-carboxamide was directly treated with the Lawesson's reagent (10.3 g, 25.6 mmol) in refluxing toluene (40 mL) for 2 h. Upon cooling to 0 °C, the thioamide precipitated as a brown solid. The toluene was

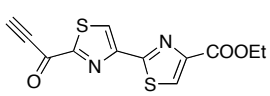
decanted and the thioamide was treated with ethyl bromopyruvate (10.0 g, 51.1 mmol) in refluxing EtOH (40 mL) for 2 hours. The cooled reaction mixture was evaporated and the residue was suspended in EtOAc (10 mL). Neutralization with aq sat $NaHCO_3$ sol (40 mL) caused the precipitation of a white solid, which was filtered and washed with small amounts of cold hexane to give 10.6 g (80% over 3 steps) of the known **10** (Huang, L.; Quada, J. C., Jr.; Lown, J. W. *Heterocyclic Commun*. **1995**, *1*, 335) as a white solid, m.p.: 95-97 °C. **IR**: 1705. **^{1}H**: 8.17 (s, 1H), 8.01 (s, 1H), 4.45 (q, 2H, *J* = 7.14), 2.78 (s, 3H), 1.43(t, 3H, *J* = 7.14). **^{13}C**: 166.8, 163.4, 161.5, 148.0, 147.9, 127.6, 117.0, 61.5, 19.2, 14.4. **MS**: 255.2 [M+H$^+$]. **HRMS**: calcd for $C_{10}H_{10}N_2S_2O_2Na^+$: 277.0081; found: 277.0075.



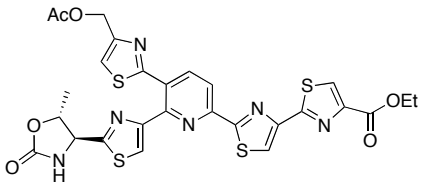
Ethyl-2'-formyl-2,4'-bithiazole-4-carboxylate (6). A solution of compound **10** (4.9 g, 19.2 mmol) and SeO_2 (6.4 g, 57.8 mmol) in AcOH (40 mL) was refluxed for 12 h. The mixture was filtered through Celite to remove a dark precipitate and the filtrate was evaporated. The residue was treated with aqueous saturated $NaHCO_3$ solution (30 mL) and extracted with EtOAc. The combined extracts were dried (Na_2SO_4) and evaporated to give 2.8 g (55%) of the known aldehyde **6** (Lefranc, D; Ciufolini, M.A. *Angew. Chem. Int. Ed.* **2009**, *48*, 4198), m.p.: 156-157 °C (recryst. from 1:1 EtOAc/Hexanes). **IR**: 1721, 1695. **^{1}H**: 10.06 (d, 1H, *J* = 1.0), 8.55 (d, 1H, *J* = 1.1), 8.26 (s, 1H), 4.47 (q, 2H, *J* = 7.1), 1.45 (t, 3H, *J* = 7.1). **^{13}C**: 183.2, 165.9, 161.9, 161.1, 151.1, 148.2, 128.5, 123.9, 61.7, 14.3. **HRMS**: calcd for $C_{10}H_8N_2O_3S_2Na^+$: 290.9874; found: 290.9865.



Ethyl-2'-(1-hydroxyprop-2-ynyl)-2,4'-bithiazole-4-carboxylate (14). Aldehyde **6** (3.5 g, 13.0 mmol) in THF (8 mL) was added dropwise to a commercial 0.5 M solution of ethynylmagnesium bromide in THF (57.6 mL, 28.8 mmol) at rt. The mixture was stirred for 30 min, then it was quenched with aqueous saturated NH_4Cl solution (30 mL). The organic layer was separated and the aqueous phase was extracted with EtOAc (2 × 30 mL). The combined extracts were dried (Na_2SO_4) and evaporated. Flash chromatographic purification of the residue (40% EtOAc/hexanes) gave **14** (2.9 g, 75%) as a white solid, m.p.: 145-148 °C. **IR**: 3247, 2116, 1709. **^{1}H** (DMSO-d_6): 8.54 (s, 1H), 8.35 (s, 1H), 7.08 (d, 1H, *J* = 6.0), 5.73 (dd, 1H, *J*= 6.0, 2.2), 4.32 (q, 2H, *J* = 7.1), 3.68 (d, 1H, *J* = 2.2), 1.31 (t, 3H, *J* = 7.1). **^{13}C** (DMSO-d_6): 174.0, 162.8, 161.1, 147.8, 147.4, 130.0, 119.4, 83.16, 77.3, 61.3, 60.8, 14.7. **MS**: 295.1 [M+H$^+$]. HRMS: calcd for $C_{12}H_{11}N_2O_3S_2^+$: 295.0211; found: 295.0191.



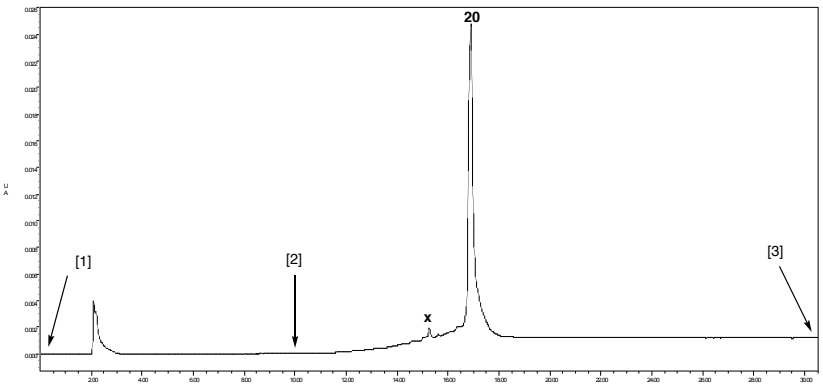
Ethyl-2'-propioloyl-2,4'-bithiazole-4-carboxylate (15). Dess−Martin reagent (735 mg, 1.8 mmol) was added in small portions to a suspension of alcohol **14** (435 mg, 1.5 mmol) in CH_2Cl_2 (5 mL) at rt and with good stirring. The solution became clear after 10 min. After 2 h of stirring, the reaction was complete (TLC), whereupon it was diluted with 10 mL each of aqueous saturated $NaHCO_3$ and aqueous saturated $Na_2S_2O_3$ solutions. The organic layer was separated and further washed with aqueous saturated $NaHCO_3$ solution (10 mL), then it was dried (Na_2SO_4) and concentrated to give **15** as an orange solid (415 mg, 96%). This reactive material was best utilized in crude form, because purification induced unacceptable loss of product. A sample purified by flash chromatography (40% EtOAc/Hexanes) had m.p. 107-109 °C. **IR**: 3214, 2098, 1725, 1640. **^{1}H**: 8.54 (s, 1H), 8.26 (s, 1H), 4.46 (q, 2H, *J* = 7.0 Hz), 3.66 (s, 1H), 1.44 (t, 3H, *J* = 7.0 Hz). **^{13}C**: 168.6, 165.7, 161.9, 161.2, 151.1, 148.2, 128.6, 124.7, 84.0, 79.1, 61.7, 14.3. **MS**: 315.1 [M+Na^+]. **HRMS**: calcd for $C_{12}H_9N_2O_3S_2^+$: 293.0055; found 293.0067.



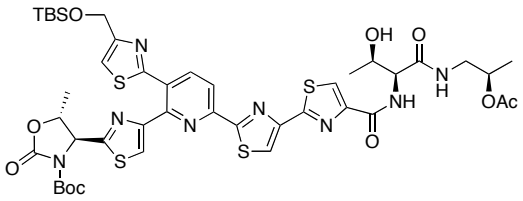
Ethyl-2'-(5-(4-(acetoxymethyl)thiazol-2-yl)-6-(2-((4*S*,5*R*)-5-methyl-2-oxooxazolidin-4-yl)thiazol-4-yl)pyridin-2-yl)-2,4'-bithiazole-4-carboxylate (23). A solution of ketone **21** (380 mg, 832 μmol), ynone **15** (248 mg, 832 μmol), and NH_4OAc (97 mg, 1.2 mmol) in AcOH (5 mL) was refluxed for 12 h, then it was concentrated, neutralized (aq. sat. $NaHCO_3$ sol.), and extracted with EtOAc (2 × 25 mL). The combined extracts were dried (Na_2SO_4) and evaporated. Purification of the residue (flash chromatography, 70% EtOAc/hexanes) afforded **23** (285 mg, 52%) as a pale yellow solid, m.p.: 210-211 °C; $[\alpha]_D$: +5.5° (*c* 0.91, $CHCl_3$). An HPLC analysis of this material indicated a purity of at least 95% (diagram on the right). **IR**: 1780, 1733, 1716. **^{1}H**: 8.34 (d, 1H, *J* = 8.0 Hz), 8.32 (s, 1H), 8.24 (s, 1H), 8.20 (d, 1H, *J* = 8.0), 8.03 (s, 1H), 7.40 (s, 1H), 5.78 (s, br, 1H), 5.23 (s, 2H), 4.65 (dd, 1H, *J* = 6.0, 1.1), 4.54 (p, 1H, *J* = 6.1), 4.47 (q, 2H, *J* = 7.2), 2.13 (s, 3H), 1.48

HPLC Trace of Compound 23

Column: Agilent ZORBAX Bonus-RP, 3.5 μm, 4.6 mm x 150 mm. **Flow**: 1.0 mL/min. **Solvent**: A = MeOH, B = H_2O, 50 mM $(NH_4)SO_4$, pH = 5.5 (1 mL/min). **Gradient**: [1] time = 0.0 min: 5% A - 95% B. [2] time = 10 min: 100% A - 0% B. [3] time = 31 min: 5% A - 95% B. **Detection**: UV, 254 nm

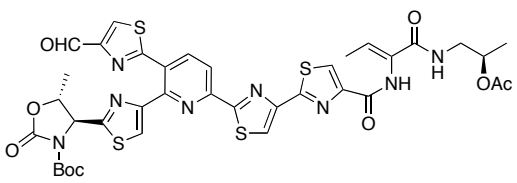


(d, 3H, *J* = 6.2), 1.45 (t, 3H, *J* = 7.2). **^{13}C** NMR: 170.8, 168.4, 168.3, 165.4, 163.1, 161.4, 157.7, 154.2, 151.6, 150.7, 150.6, 149.9, 148.1, 140.0, 129.6, 127.9, 121.5, 120.5,119.1, 118.8, 79.6, 61.8, 61.6, 60.8, 21.0, 19.9, 14.4. **MS**: 655 [M+H$^+$], 677 [M+Na$^+$]. **HRMS**:calcd for $C_{27}H_{23}N_6O_6S_4^+$: 655.0562; found: 655.0558.

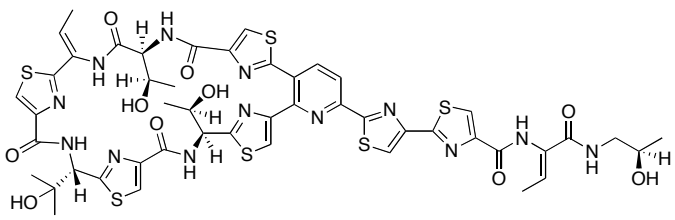


(4*S*,5*R*)-*tert*-butyl-4-(4-(6-(4-((3*R*)-1-((*R*)-2-acetoxypropyl-amino)-3-hydroxy-1-oxobutan-2-ylcar-bamoyl)-2,4'-bithia-zol-2'-yl)-3-(4-((*tert*-butyldi-methylsilyloxy)methyl)thiazol-2-yl)pyridin-2-yl) thiazol-2-yl)-5-methyl-2-oxo-oxazolidine-3-carboxylate (40). A solution of pyridine **23** (0.559 g, 0.854 mmol) in 15 mL of 1:1 CH_2Cl_2 and EtOH containing suspended K_2CO_3 (23.6 g, 171 mmol) was stirred overnight, then it was filtered and concentrated to give the corresponding free alcohol. A solution of this crude material in 5 mL of DMF was treated with TBS-Cl (0.128 g, 0.854 mmol) and imidazole (0.117g, 1.71 mmol) and stirred at r.t for 24 hrs. The mixture was then diluted with 15 mL of EtOAc, washed with aq. sat. NH_4Cl sol. (3 x 5 mL), and concentrated under vacuum. The residue was purified by flash chromatography to give silyl ether **36** (0.388 g) as a yellow solid. A solution of the latter in 10 mL of 1:1 THF - water containing LiOH·H_2O (0.067g, 1.6 mmol) was stirred for 2 hours, then it was acidified to pH = 3 and extracted with EtOAc (3 x 10 mL). The combined extracts were dried (Na_2SO_4) and concentrated to give the expected carboxylic acid **37**. A solution of this crude substance in 4 mL of CH_2Cl_2 containing Et_3N (0.15mL, 1.07 mmol), a spatula tip of DMAP, and Boc_2O (0.290 g, 1.33 mmol) was stirred under Ar for 3 hours, then it was treated with 5 mL of H_2O and acidified to pH = 3 with 1M HCl solution. The mixture was extracted with EtOAc (3 x 10 mL) and the combined extracts were dried (Na_2SO_4) and concentrated to give the BOC protected oxazolidinone **38**. A solution of this crude substance and **39** (0.133 g, 0.533 mmol) in 3 mL of MeCN containing Et_3N (0.23 mL, 1.70 mmol) and BOP-Cl (0.131g, 0.533 mmol) was stirred for 3 hours and then quenched with 3 mL of saturated NH_4Cl solution and EtOAc (10 mL) and the layers were separated. The product was extracted further with EtOAc (2 x 5 mL). The residue was purified by flash chromatography (EtOAc/Hexanes) to give the known **40** (Lefranc, D; Ciufolini, M.A. *Angew. Chem. Int. Ed.* **2009**, *48*, 4198) as a white solid (0.167 g, 20% over 5 steps). **^{1}H**: 8.34 (d, 1H, *J* = 8.3), 8.29-8.21 (m, 2H), 8.19 (s, 1H), 8.01 (s, 1H), 7.31 (s, 1H), 7.09 (t, 1H, *J* = 5.9), 5.16-4.96 (m, 2H), 4.89 (s, 1H), 4.65-4.44 (m, 3H), 3.78 (br s, 1H), 3.61-3.45 (m, 1H), 3.45-3.22 (m, 1H), 2.04 (s, 3H), 1.49 (s, 9H), 1.26 (d, 3H, *J* = 6.4), 1.23 (d, 3H, *J* = 6.4), 0.96 (s, 9H), 0.14 (s, 6H). **^{13}C**: 171.5, 170.8, 170.8, 168.8, 166.8, 164.5, 162.8, 162.2, 157.7, 153.5, 150.8, 150.4, 149.8, 149.8, 148.9, 140.0, 130.1, 124.5, 121.8, 120.3, 118.8, 75.6, 69.5, 66.3, 62.3, 62.2, 56.6, 43.7, 27.9, 25.9, 21.2, 20.2,

18.4, 18.3, 17.6, –5.3. **MS**: 1021.8 [M+Na^+]. **HRMS**: calcd for $C_{43}H_{54}N_8O_{10}S_4SiNa^+$: 1021.2513; found: 1021.2502.



(4*S*,5*R*)-*tert*-butyl-4-(4-(6-(4-((*Z*)-1-((*R*)-2-acetoxy-propyl-amino)-1-oxobut-2-en-2-ylcarbamoyl)-2,4'-bithiazol-2'-yl)-3-(4-formylthiazol-2-yl)pyridin-2-yl)thiazol-2-yl)-5-methyl-2-oxooxazolidine-3-carboxylate (43). A solution of **40** (0.167 g, 0.166 mmol), Et_3N (0.06 mL, 0.415 mmol) and MsCl (0.03 mL, 0.332 mmol) in 5 ml CH_2Cl_2 were stirred at rt for 1 h under Ar, then neat DBU (0.050 mL, 0.332 mmol) was added. The mixture was stirred for 1 more hour, then it was quenched with 3 mL of aq. sat. NH_4Cl solution. The organic layer was separated, dried (Na_2SO_4) and concentrated to give dehydroaminoacid derivative **41**. A solution of this crude substance in commercial in 0.1M TBAF in THF (0.82 mL, 0.830 mmol) was stirred for 3 h, then it was diluted with 3 mL of EtOAc and 3mL of aq. sat. NH_4Cl solution. The organic layer was separated, dried with Na_2SO_4 and concentrated. The crude alcohol **42** thus obtained was dissolved in 3 mL of CH_2Cl_2 containing Dess-Martin periodinane (0.147g, 0.349 mmol) and stirred for 2 hours. The reaction was quenched with 3 mL of aq. sat. $Na_2S_2O_4$ sol. and extracted with EtOAc (3 x 10 mL). The combined extracts were dried (Na_2SO_4) and evaporated under vacuum. The residue was purified by flash chromatography (2% MeOH in EtOAc) to give the known aldehyde **43** (Lefranc, D; Ciufolini, M.A. *Angew. Chem. Int. Ed.* **2009**, *48*, 4198) (0.120 g, 84% over 3 steps). **^{1}H**: 10.05 (s, 1H), 8.73 (br s, 1H), 8.37 (s, 1H), 8.37 (overlapping d, 1H, *J* = 8.0), 8.25 (s, 1H), 8.24 (s, 1H), 8.22 (s, 1H), 8.19 (d, 1H, *J* =8.0), 6.62 (q, 1H, *J* = 7.2), 6.57 (t, 1H, *J* = 5.2), 5.10-4.98 (m, 1H), 4.94 (d, 1H, *J* = 3.8), 4.51-4.40 (m, 1H), 3.62-3.52 (m, 1H), 3.50-3.38 (m, 1H), 2.03 (s, 3H), 1.87 (d, 3H, *J* = 7.2), 1.45 (s, 9H), 1.43 (overlapping d, 3H), 1.28 (d, 3H, *J* = 6.3). **^{13}C**: 184.2, 171.1, 168.6, 166.4, 166.2, 164.9, 162.6, 159.4, 154.7, 153.4, 151.0, 150.6, 150.2, 150.0, 148.7, 140.5, 131.0, 131.0, 129.4, 128.5, 125.0, 122.3, 120.2, 118.6, 85.0, 75.3, 69.9, 61.8, 44.5, 27.9, 21.2, 20.4, 17.7, 14.1. **MS**: 887.5 [M+Na^+]. **HRMS**: calcd for $C_{37}H_{36}N_8O_9S_4Na^+$: 887.1386; found: 887.1370.



Thiocillin I (1). A solution of aldehyde **43** (0.120 g, 0.133 mmol), $NaClO_2$ (24.2 mg, 0.267 mmol), 2-methylbutene (0.66 mL, 1.33 mmol, 2M in THF) and NaH_2PO_4 (33 mg, 0.275 mmol) in 1 mL of a 1:1 mixture of THF and H_2O was stirred for 2 hours, then it was acidified to pH = 2 with 1M HCl solution. The aqueous phase was extracted twice with 3 mL CH_2Cl_2. The combined extracts were dried with Na_2SO_4 and concentrated under vacuum to afford

crude acid **44**, which was directly redissolved in 2 mL of acetonitrile together with compound **35** [which was prepared by exposing compound **34** above (0.170g, 0.266 mmol) to TFA:MeOH for 3 hours and then concentrating to give the TFA salt], BOP-Cl (0.070 g, 0.274 mmol), and Et_3N (0.05 mL, 0.360 mmol). The mixture was stirred under Ar overnight, then it was diluted with 5 mL of CH_2Cl_2 and successively washed with saturated solutions of NH_4Cl, $NaHCO_3$ and NaCl. The organic layer was then dried with Na_2SO_4 and concentrated under vacuum. The residue of crude **45** was then dissolved in 2 mL of 1:1 mixture of THF/H_2O, LiOH (0.033g, 7.86 mmol). The solution was stirred at r.t. for 2 hours, then it was acidified to pH = 3 with 1M HCl and extracted with CH_2Cl_2 (3 x 5 mL). The combined extracts were dried with Na_2SO_4 and concentrated under vacuum to give a crude carboxylic acid, which was dissolved in TFA/CH_2Cl_2 (1:4) and stirred at r.t. for 2 hours. Concentration under vacuum gave the TFA salt **43**. This crude material was then dissolved in 1 mL of DMF containing DPPA (0.029 mL, 0.134 mmol) and Et_3N (0.055 mL, 0.396 mmol) and stirred for 2 hours at r.t. before being diluted with 5 mL of CH_2Cl_2. The organic layer was successively washed with saturated solutions of NH_4Cl, $NaHCO_3$, NaCl and dried with Na_2SO_4 and concentrated under vacuum. The crude was then purified by flash column chromatography (1: 9 MeOH/EtOAc) to give thiocillin I **(1)** (20 mg, 12 % over 5 steps). The purity of this material was determined to be ca. 90% by ^{1}H NMR. Further purification, as required for full characterization, was achieved by HPLC. This effort was carried out at MerckFrosst Canada, Ltd., by Dr. Dan Sorensen, whom we thank warmly for his assistance in this matter. A sample of synthetic thiocillin I ultimately obtained upon HPLC purification (32% H_2O (0.1%TFA) and 67% MeCN (0.1% TFA); pressure: 171 bar; Column Temperature: 40 °C) consisted of between 50 and 80 μg (estimated by ^{1}H NMR) of **1**. A solution of this material in 200 μL of DMSO-d_6 was utilized for characterization, which was also carried out at Merck Frosst Canada, Ltd., through the courtesy of Dr. Dan Sorensen. Synthetic **1** thus purified was spectroscopically (^{1}H and ^{13}C NMR) identical to the natural product, a sample of which was kindly provided by the Shionogi Co. Table **1** provides a tabulation of ^{1}H and ^{13}C NMR chemical shifts for the two substances. It should be noted that the amount of material recovered through HPLC purification was insufficient to record a ^{13}C spectrum in the direct observation mode: the data in Table 1 were obtained through a 2D inverse detection experiment (HSQC- direct proton carbon correlation and HMBC-long range proton-carbon correlation). In a like manner, a precise optical rotation could not be measured, although an $[\alpha]_D^{25}$ value between + 100 and + 160° was obtained from the foregoing sample, which, once again, consisted of between 50 and 80 μg of purified **1** in 200 μL of DMSO-d_6 {literature: $[\alpha]_D^{24.5}$ = 97.8 ± 0.8° (c = 2.028, 90% aq. EtOH)}. **^{1}H** (DMSO-d_6, 600 MHz): 9.64 (s, 1H), 9.50 (s, 1H), 8.60 (s, 1H), 8.47 (s, 2H), 8.40 (d, 1H, J = 8.1Hz), 8.37 (s, 1H), 8.35 (br s, 1H), 8.33 (d, 1H, J = 8.1Hz),

8.32 (br s, 1H), 8.30 (br s, 1H), 8.25 (s, 1H), 7.99 (s, 1H), 7.90 (br t, 1H, J = 6.0 Hz), 7.58 (d, 1H, J = 7.9), 6.50 (2 overlapping q, 1H + 1H, J = 7.0 Hz), 5.48 (d, 1H, J = 10.1 Hz), 5.18 (br s, 1H), 5.04 (dd, 1H, J = 8.9 Hz , J = 6.1 Hz), 4.99 (br s, 1H), 4.70 (d, 1H, J = 2.1 Hz), 4.69 (d, 1H, J = 2.2 Hz), 4.50 (br m, 1H), 3.95 (br t, 1H, app J = 6.0 Hz), 3.69 (br q, 1H, app J = 6.1 Hz), 3.07 (m, 2H), 1.74 (d, 3H, J = 6.9 Hz), 1.70 (d, 3H, J = 7.0 Hz), 1.37 (d, 3H, J = 6.3 Hz), 1.24 (s, 3H), 1.22 (s, 3H), 1.01 (d, 6H, J = 6.1 Hz). **^{13}C** (DMSO-d_6, 600 MHz): 170.85, 168.80, 168.30, 167.88, 166.84, 164.72, 164.72, 161.48, 161.17, 159.86, 159.87, 159.87, 153.33, 150.91, 150.50, 149.77, 149.42, 149.09, 148.79, 148.59, 140.73, 130.47, 129.36, 128.76, 128.67, 127.98, 125.41, 125.31, 125.29, 124.43, 121.47, 120.62, 118.41, 71.26, 67.96, 66.67, 64.87, 56.97, 56.38, 56.29, 46.52, 27.22, 25.51, 20.71, 20.67, 19.91, 13.38, 13.24. **MS**: 1160.3 [M + H]. **HRMS**: calcd for $C_{48}H_{49}N_{13}O_{10}S_6Na^+$: 1182.1947; found: 1182.1974.

Comparison of ^{1}H and ^{13}C NMR data for synthetic and natural thiocillin I

A. Proton NMR Chemical Shifts[a]

Authentic thiocillin I Measured ^{1}H shifts / coupling constants (ppm / Hz)	**Synthetic thiocillin I** Measured ^{1}H shifts / coupling constants (ppm / Hz)	Chemical shift difference (ppm)	Coupling constant difference (Hz, absolute value)
9.66* (br s, 1H)	9.64* (br s, 1H)	-0.02	--
9.52* (br s, 1H)	9.50* (br s, 1H)	-0.02	--
8.58 (s, 1H)	8.60 (s, 1H)	0.02	--
8.46 (s, 2H)	8.47 (s, 2H)	0.01	--
8.40 (d, 1H, J = 8.2)	8.40 (d, 1H, J = 8.1)	0.00	0.1
8.37 (s, 1H)	8.37 (s, 1H)	0.00	--
8.36* (br s, 1H)	8.35* (br s, 1H)	-0.01	--
8.33 (d, 1H, J = 8.2)	8.33 (d, 1H, J = 8.1)	0.00	0.1
8.31* (br s, 1H)	8.30* (br s, 1H)	-0.01	--
8.25 (s, 1H)	8.25 (s, 1H)	0.00	--
7.99 (s, 1H)	7.99 (s, 1H)	0.00	--
7.91* (br t, 1H, J = 6.0)	7.90* (br t, 1H, J = 6.0)	-0.01	0.0
7.56* (br d, 1H, J = 7.8)	7.58* (br d, 1H, J = 7.9)	0.02	0.1
6.49 (2 q, 1H+1H, J = 7.8)	6.50 (2 q, 1H+1H, J = 7.0)	0.01	0.8
5.47 (d, 1H, J = 10.2)	5.48 (d, 1H, J = 10.1)	0.01	0.1
5.20* (br s, 1H)	5.18* (br s, 1H)	–0.02	--
5.04 (dd, 1H, J = 9.0, 6.1)	5.04 (dd, 1H, J = 8.9, 6.1)	0.00	0.1, 0.0
5.00* (br d, 1H, J = 3.6)	4.99* (br s, 1H)	0.01	
4.70 (d, 1H, J = 5.4)	4.70 (br d, 1H, J = 5.4)	0.00	0.0
4.64* (br d, 1H, J = 4.2)	not visible–under H_2O peak?	--	--
4.50 (br m, 1H)	4.50 (br m)	0.00	--
3.95 (br m, 1H, app J = 6.0)	3.95 (br m, 1H, app J = 6.0)	0.00	0.0
3.70 (br m, 1H, app J = 6.0)	3.69 (br m, 1H, app J = 6.1)	-0.01	0.1
3.07 (m, 2H)	3.07 (m, 2H)	0.00	--
1.73 (d, 3H, J = 6.6)	1.74 (d, 3H, J = 6.9)	0.01	0.3
1.70 (d, 3H, J = 7.2)	1.70 (d, 3H, J = 7.0)	0.00	0.2
1.37 (d, 3H, J = 6.0)	1.37 (d, 3H, J = 6.3)	0.00	0.3
1.24 (s, 3H)	1.24 (s, 3H)	0.00	--
1.22 (s, 3H)	1.22 (s, 3H)	0.00	--
1.01 (d, 6H, J = 6.0)	1.01 (d, 6H, J = 6.1)	0.00	0.1

[a]Spectra recorded at 600 MHz in DMSO-d_6. The starred chemical shifts are those of NH and OH protons.

B. Carbon-13 NMR Chemical Shifts[a]

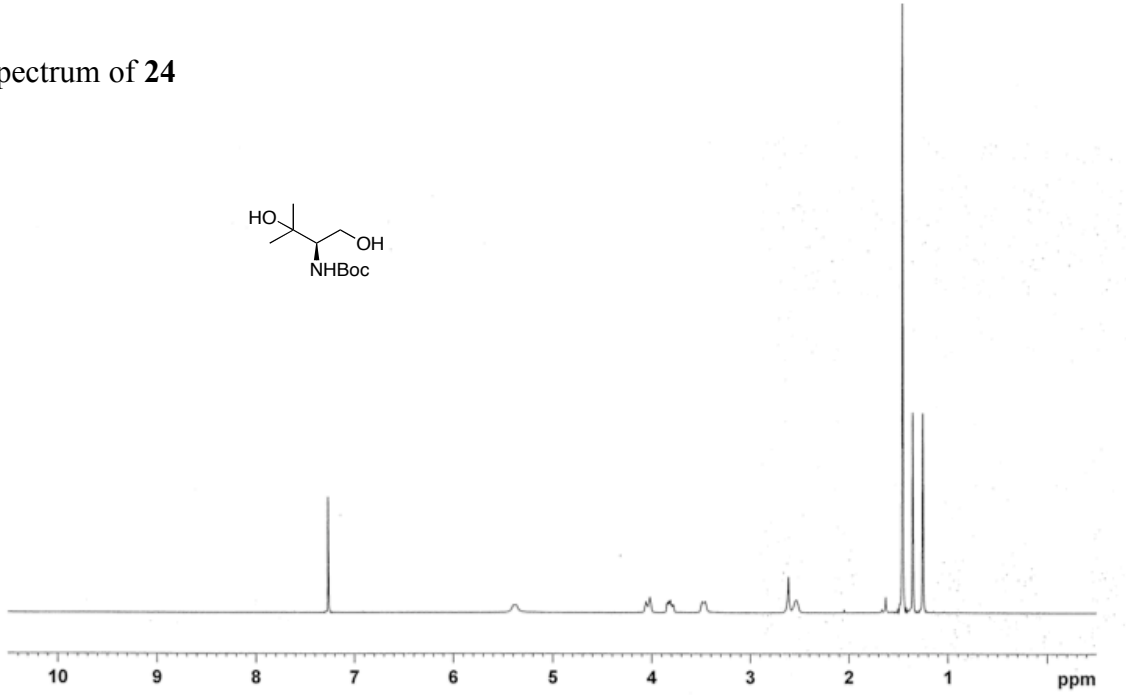
Measured ^{13}C shifts (ppm, natural thiocillin I)	Measured ^{13}C shifts (ppm, synthetic Thiocillin I)	Difference (ppm)
170.45	170.85	0.4
168.56	168.80	0.24
168.36	168.30	–0.06
167.90	167.88	–0.02
166.80	166.84	0.04
164.37	164.72	0.35
164.31	164.72	0.26
161.38	161.48	0.10
160.47	161.17	0.70
159.77	159.86	0.09
159.75	159.87	0.12
159.02	159.87	0.85
153.02	153.33	0.31
151.13	150.91	0.22
150.43	150.50	0.07
147.88	149.77	–0.11
149.73	149.42	–0.31
149.43	149.09	–0.34
148.77	148.79	0.02
148.33	148.59	0.26
140.94	140.73	–0.21
130.61	130.47	–0.14
129.34	129.36	0.02
128.89	128.76	–0.13
128.44	128.67	0.23
127.97	127.98	0.01
125.87	125.41	–0.46
125.60	125.31	–0.29
125.45	125.29	–0.16
124.72	124.43	–0.29
121.72	121.47	–0.25
120.77	120.62	–0.15
118.64	118.41	–0.23
71.37	71.26	–0.11
68.32	67.96	–0.36
67.03	66.67	–0.36
65.11	64.87	–0.24
57.21	56.97	–0.24

56.74	56.38	–0.36
56.64	56.29	-0.35
46.86	46.52	-0.34
27.48	27.22	-0.26
25.83	25.51	-0.32
21.07	20.71	-0.36
21.00	20.67	-0.33
20.25	19.91	-0.34
13.70	13.38	-0.32
13.56	13.24	-0.32

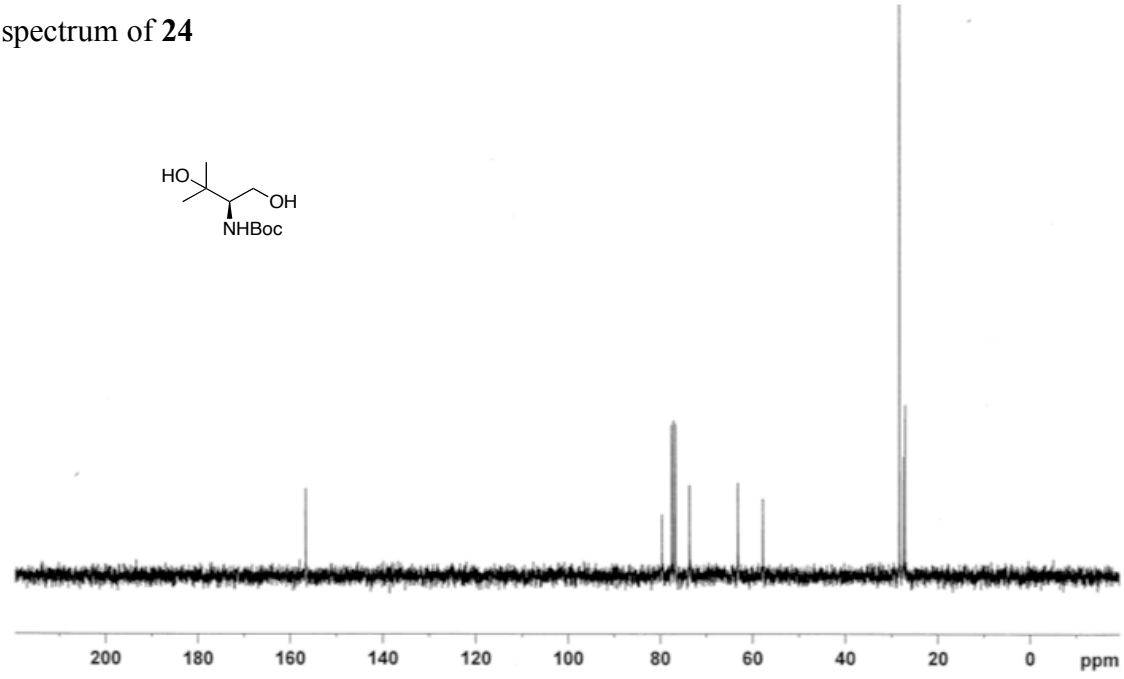
[a]Spectra recorded at 150 MHz in DMSO-d_6.

3. ^{1}H and ^{13}C NMR Spectra

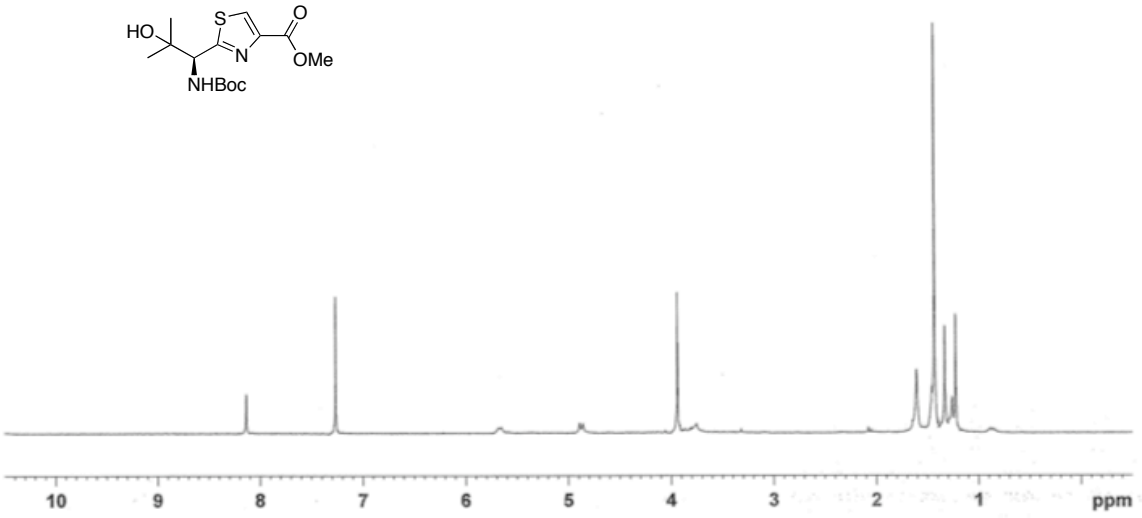
^{1}H NMR spectrum of **24**



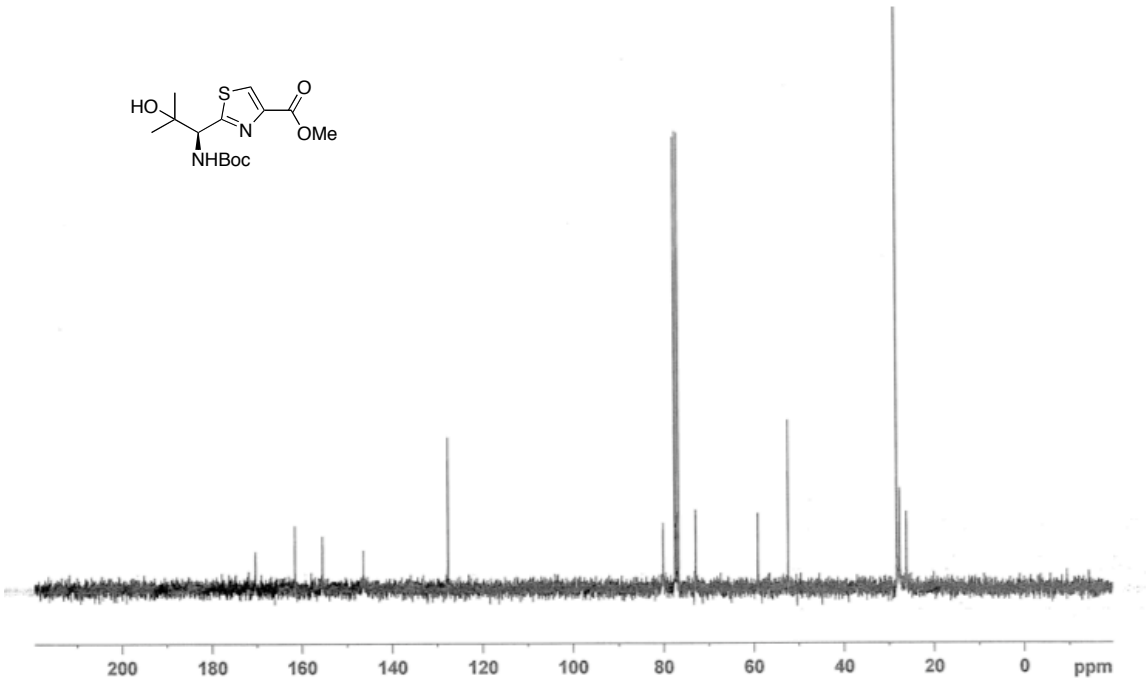
^{13}C NMR spectrum of **24**



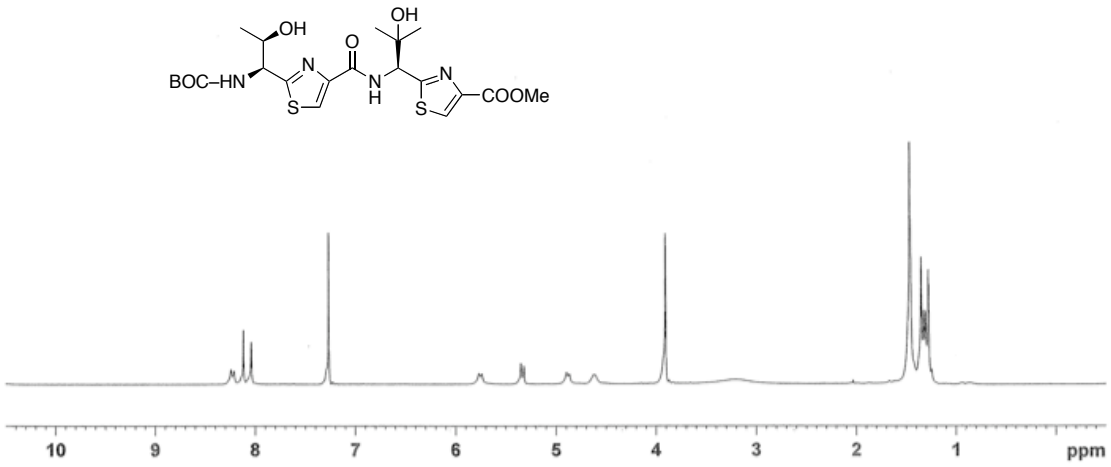
^{1}H NMR spectrum of **27**



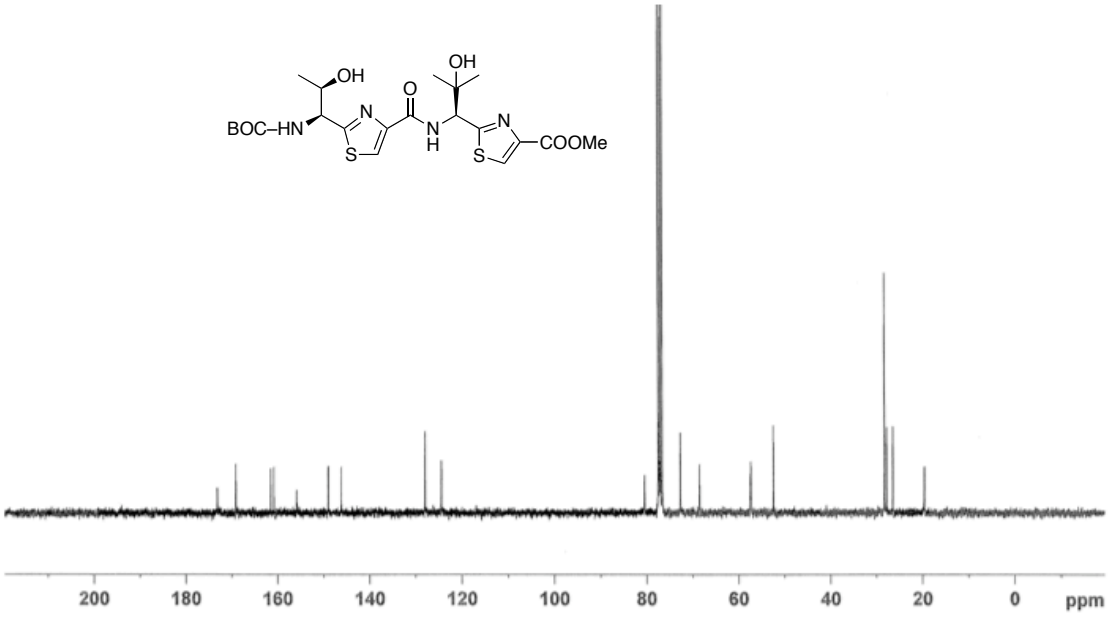
^{13}C NMR spectrum of **27**



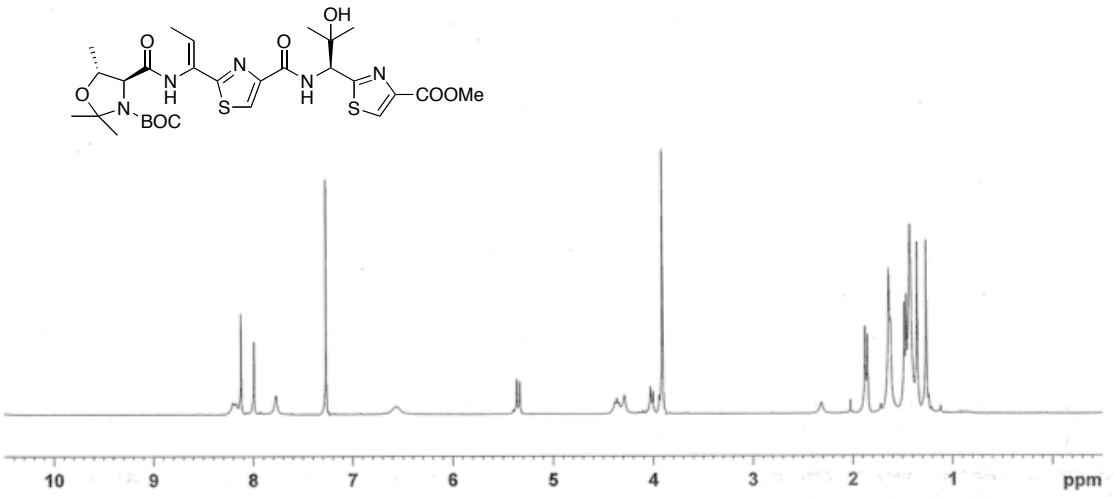
^{1}H NMR spectrum of **30**



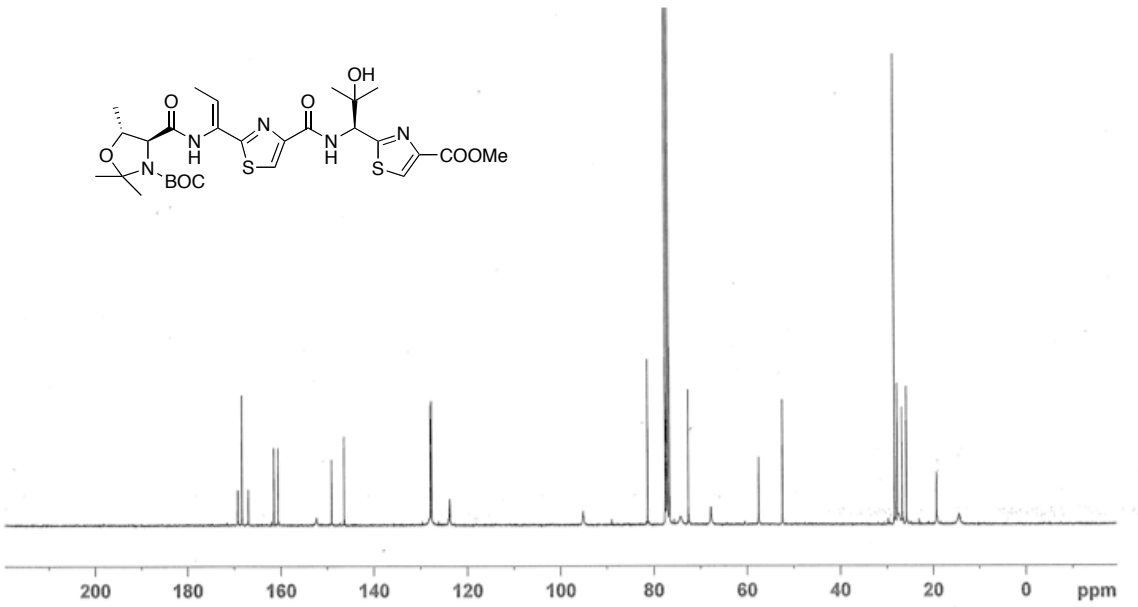
^{13}C NMR spectrum of **30**



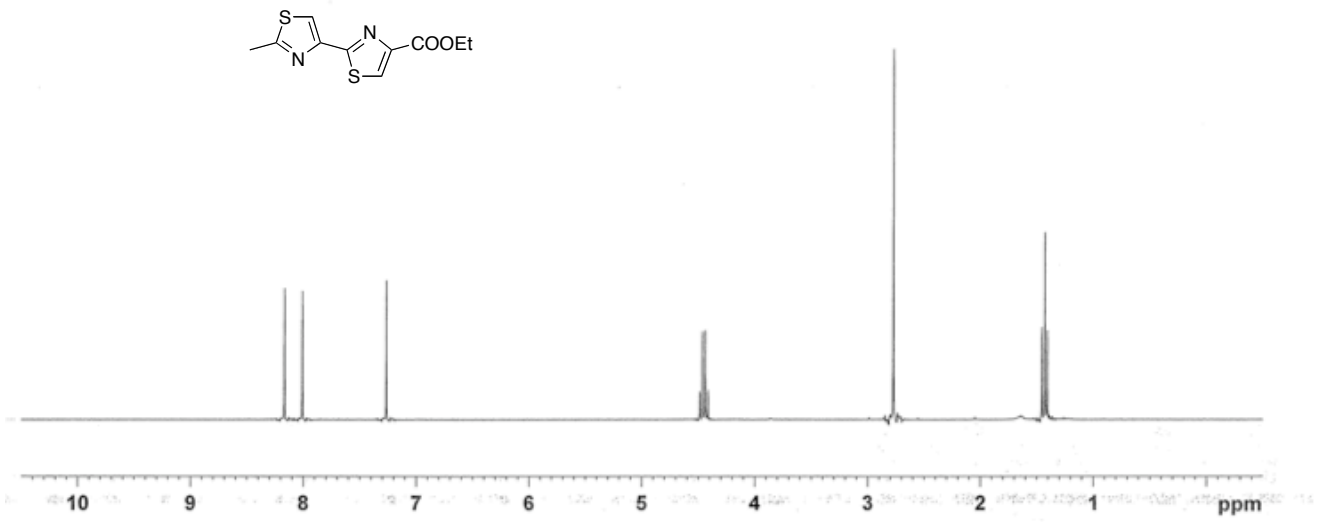
^{1}H NMR spectrum of **34**



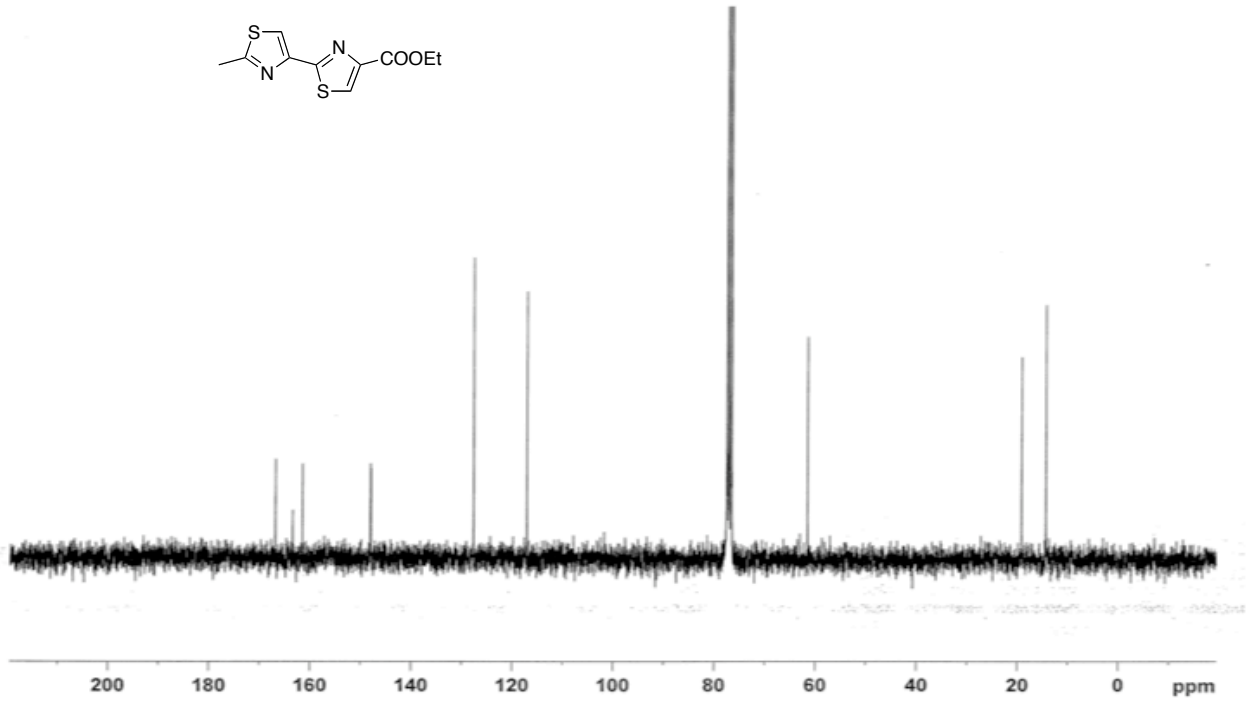
^{13}C NMR spectrum of **34**



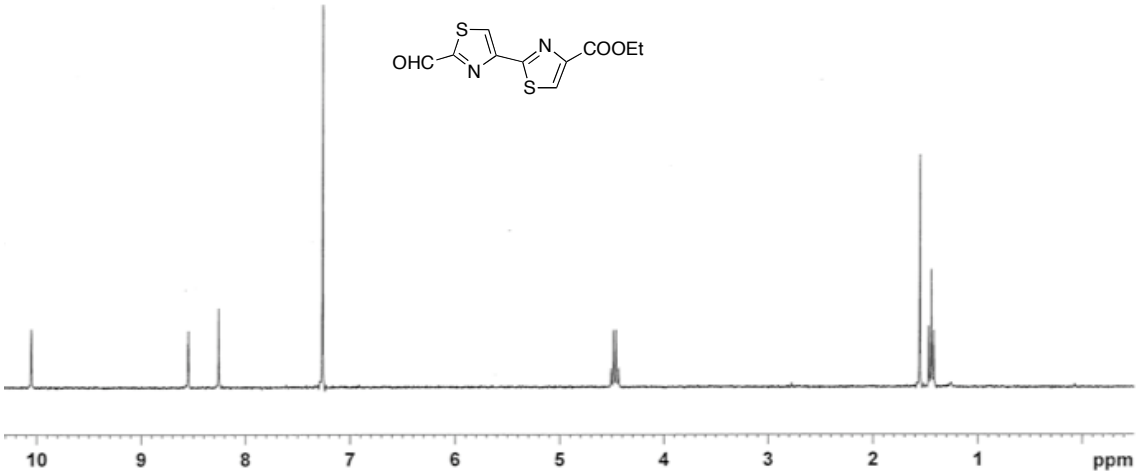
^{1}H NMR spectrum of **10**



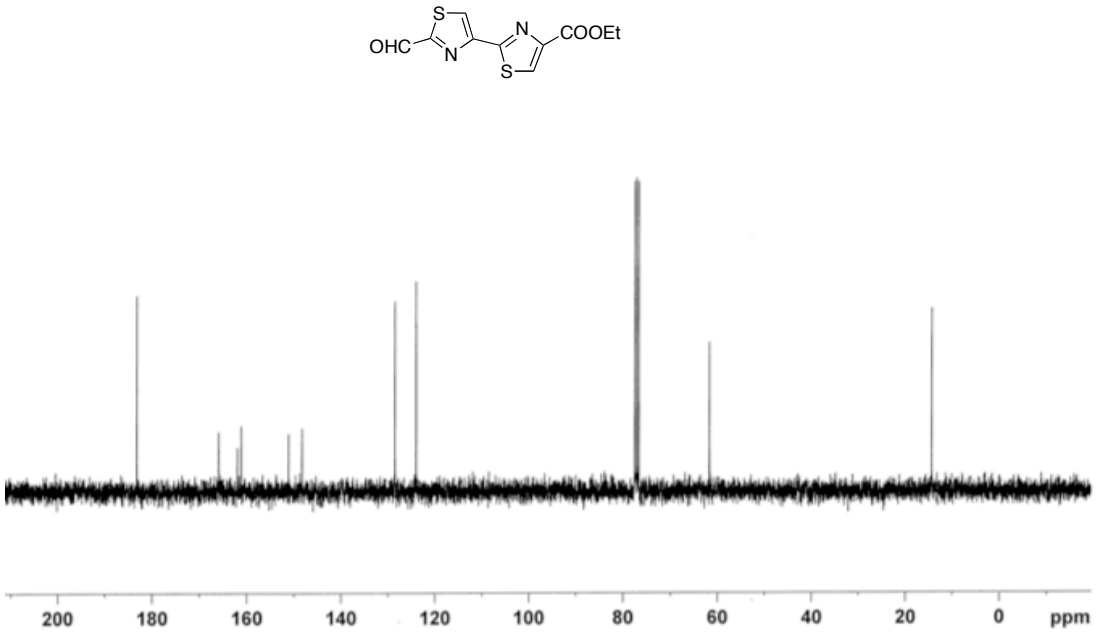
^{13}C NMR spectrum of **10**



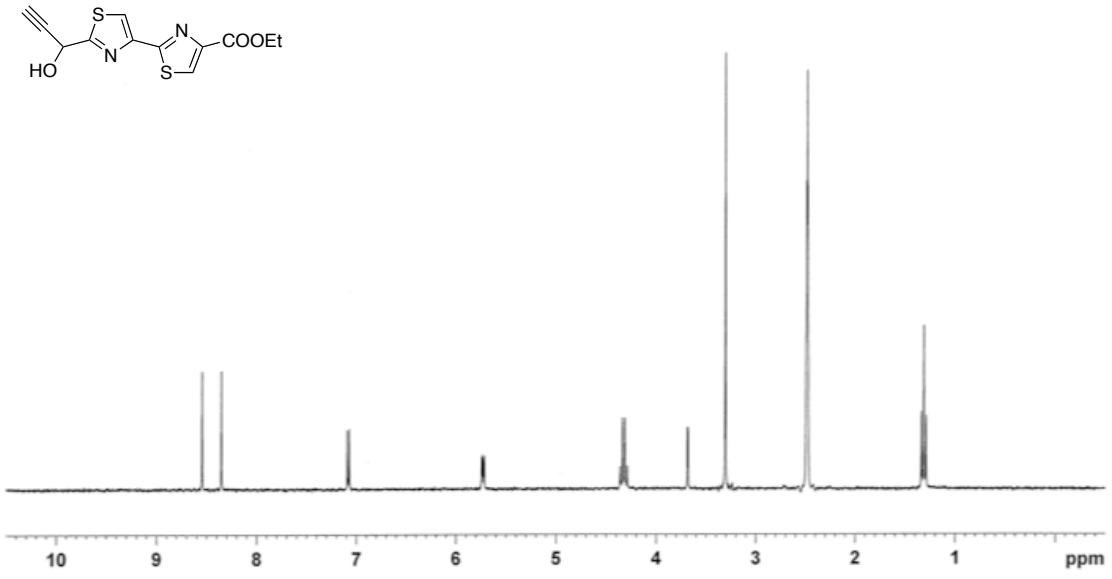
^{1}H NMR spectrum of **6**



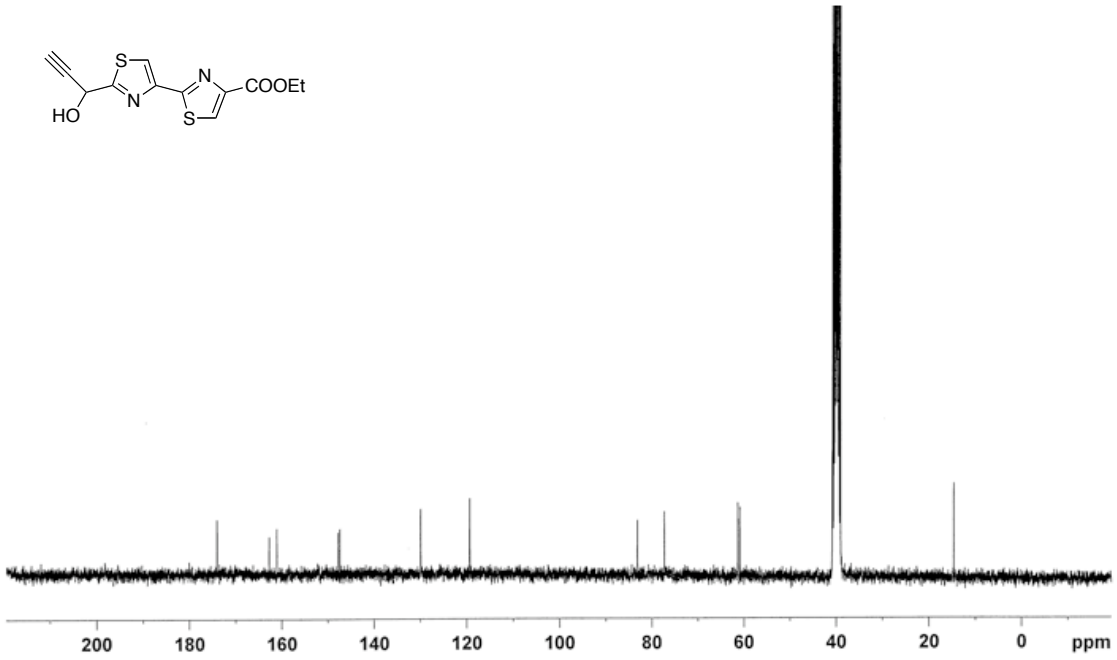
^{13}C NMR spectrum of **6**



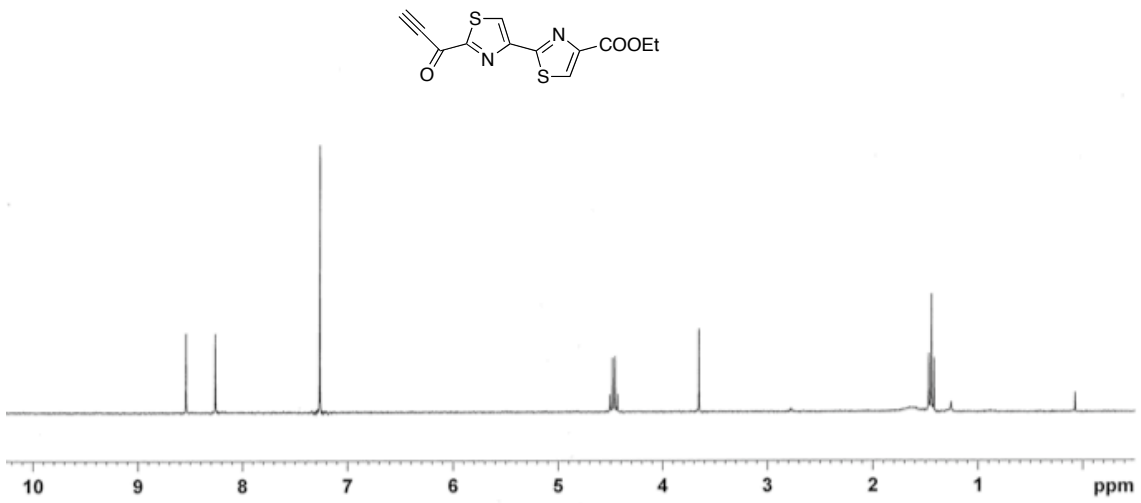
^{1}H NMR spectrum of **14**



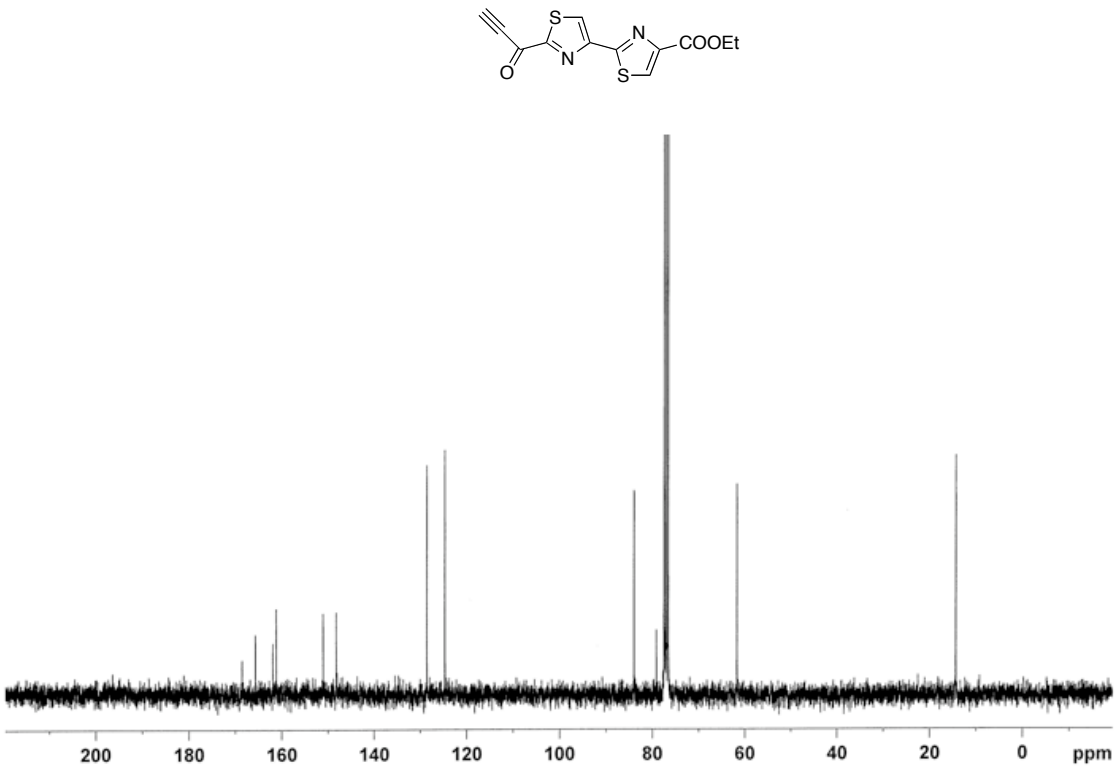
^{13}C NMR spectrum of **14**



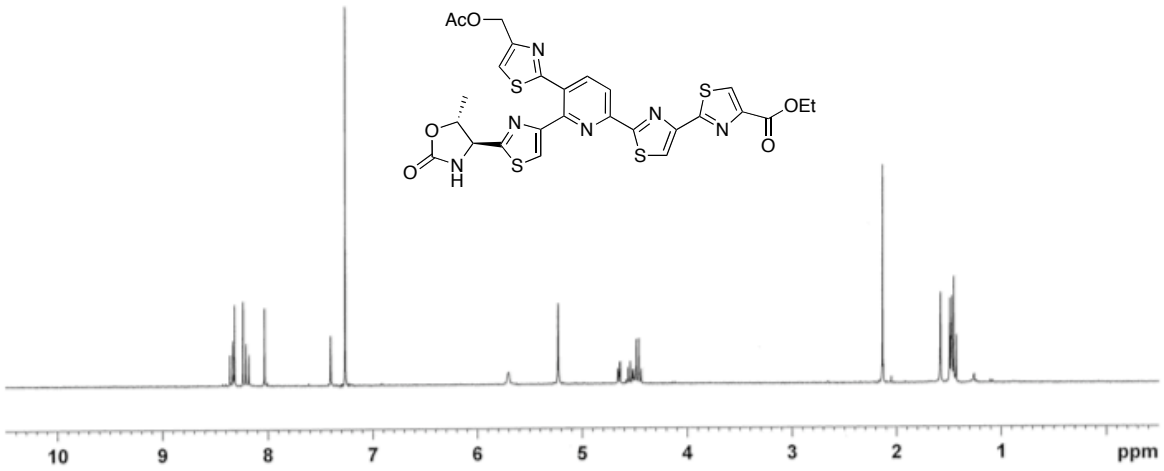
^{1}H NMR spectrum of **15**



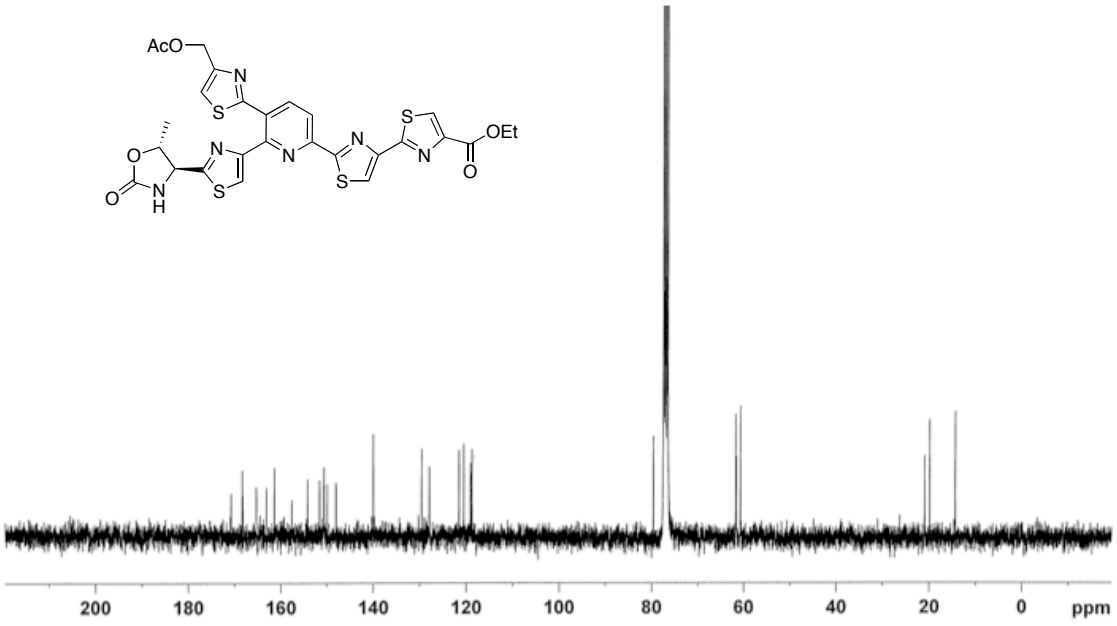
^{13}C NMR spectrum of **15**



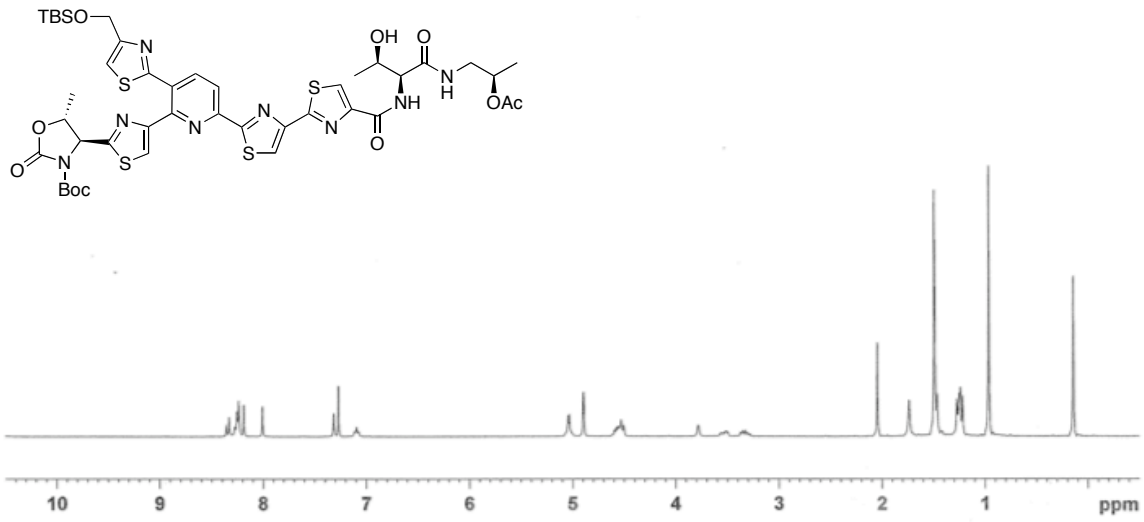
^{1}H NMR spectrum of **23**



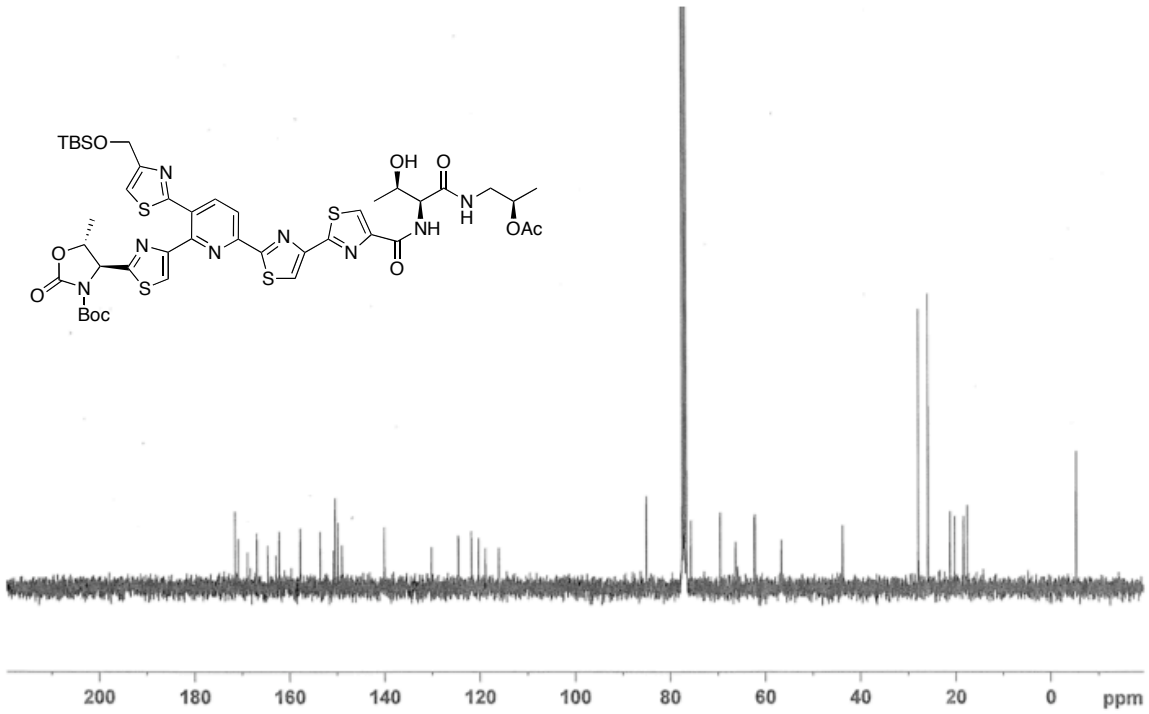
^{13}C NMR spectrum of **23**



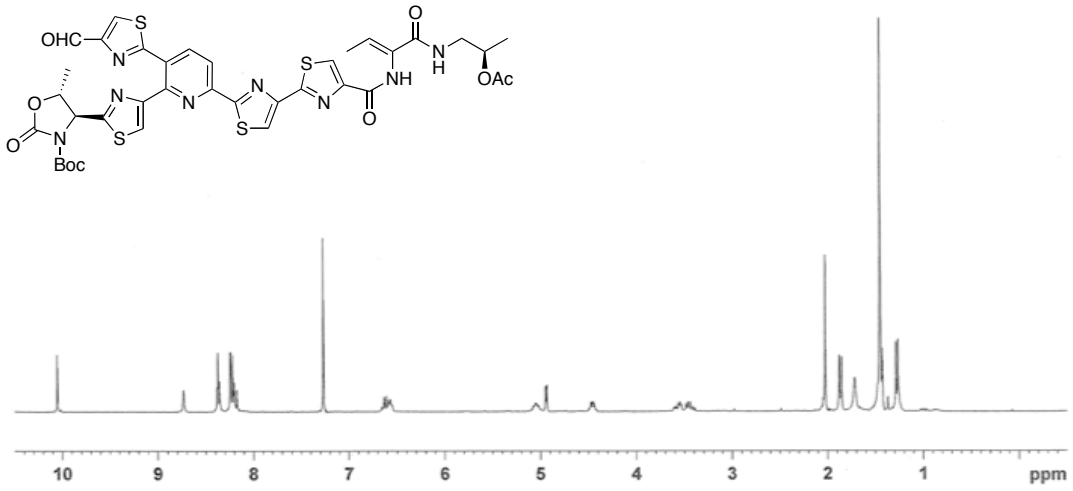
^{1}H NMR spectrum of **40**



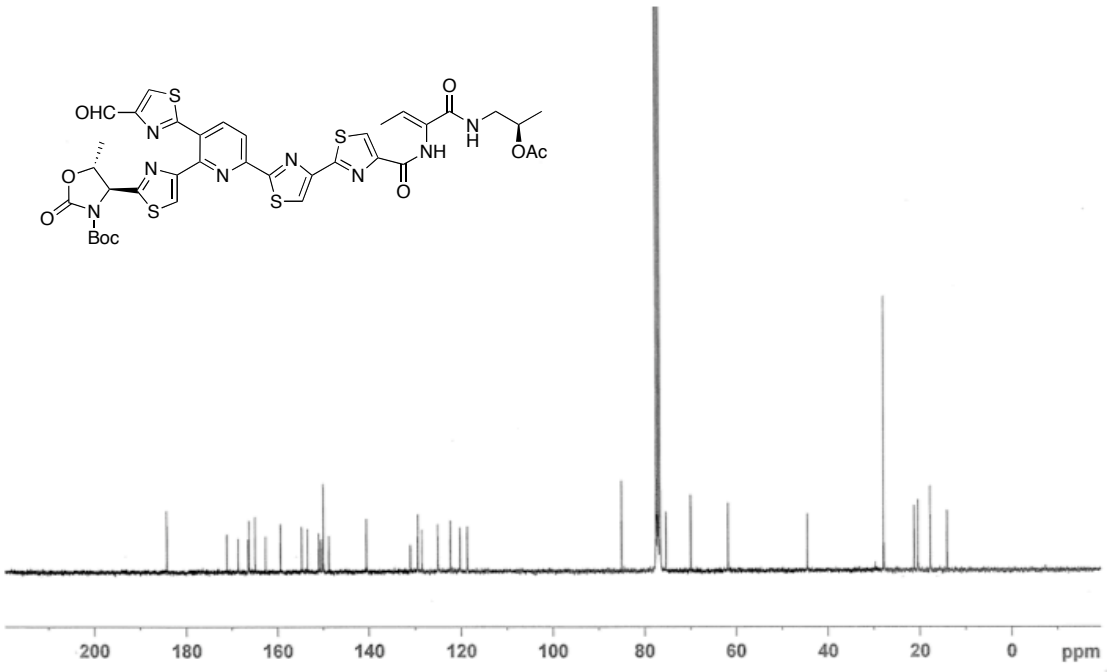
^{13}C NMR spectrum of **40**

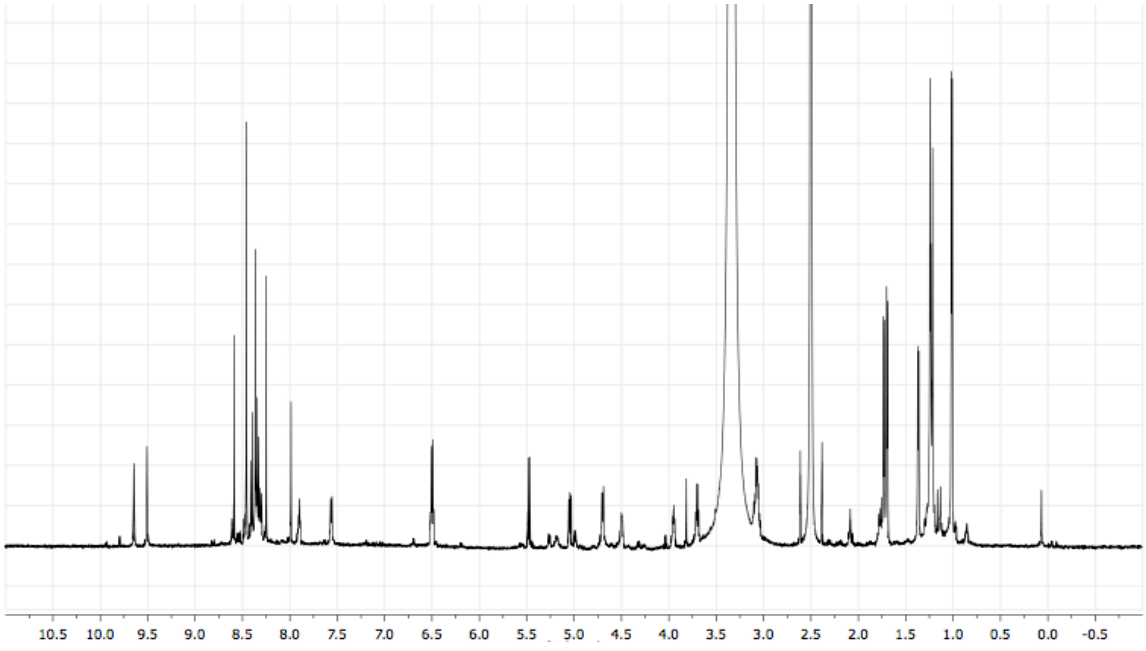


^{1}H NMR spectrum of **43**

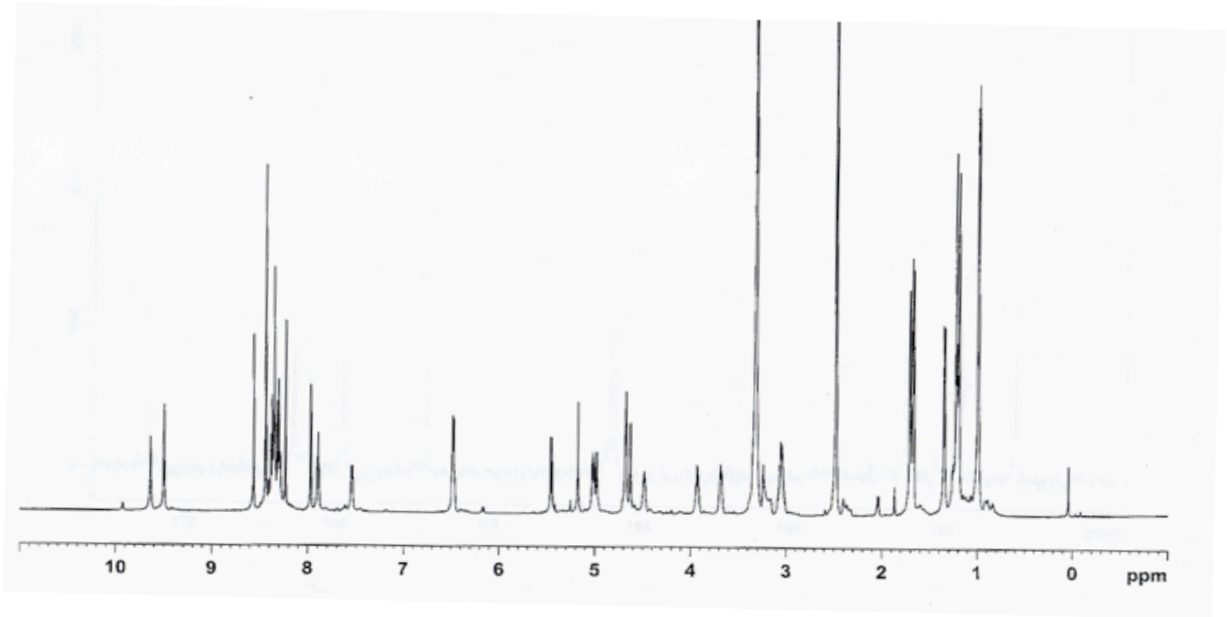


^{13}C NMR spectrum of **43**

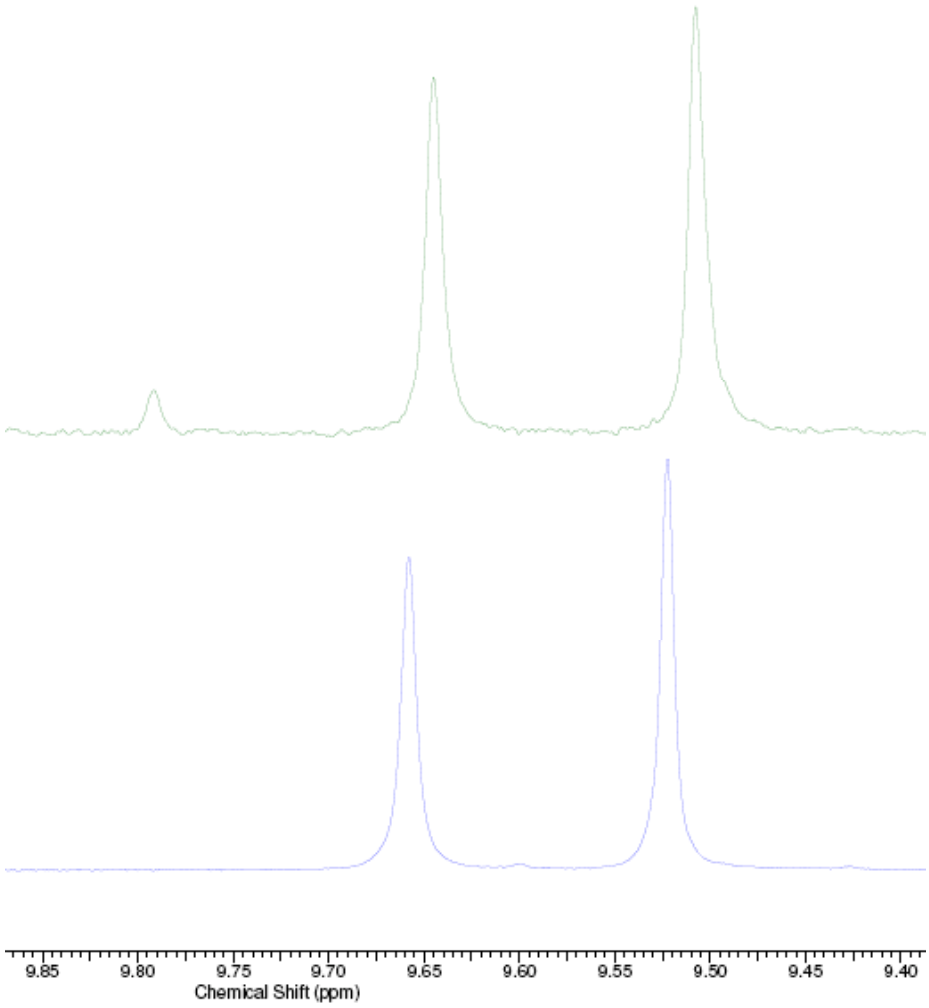




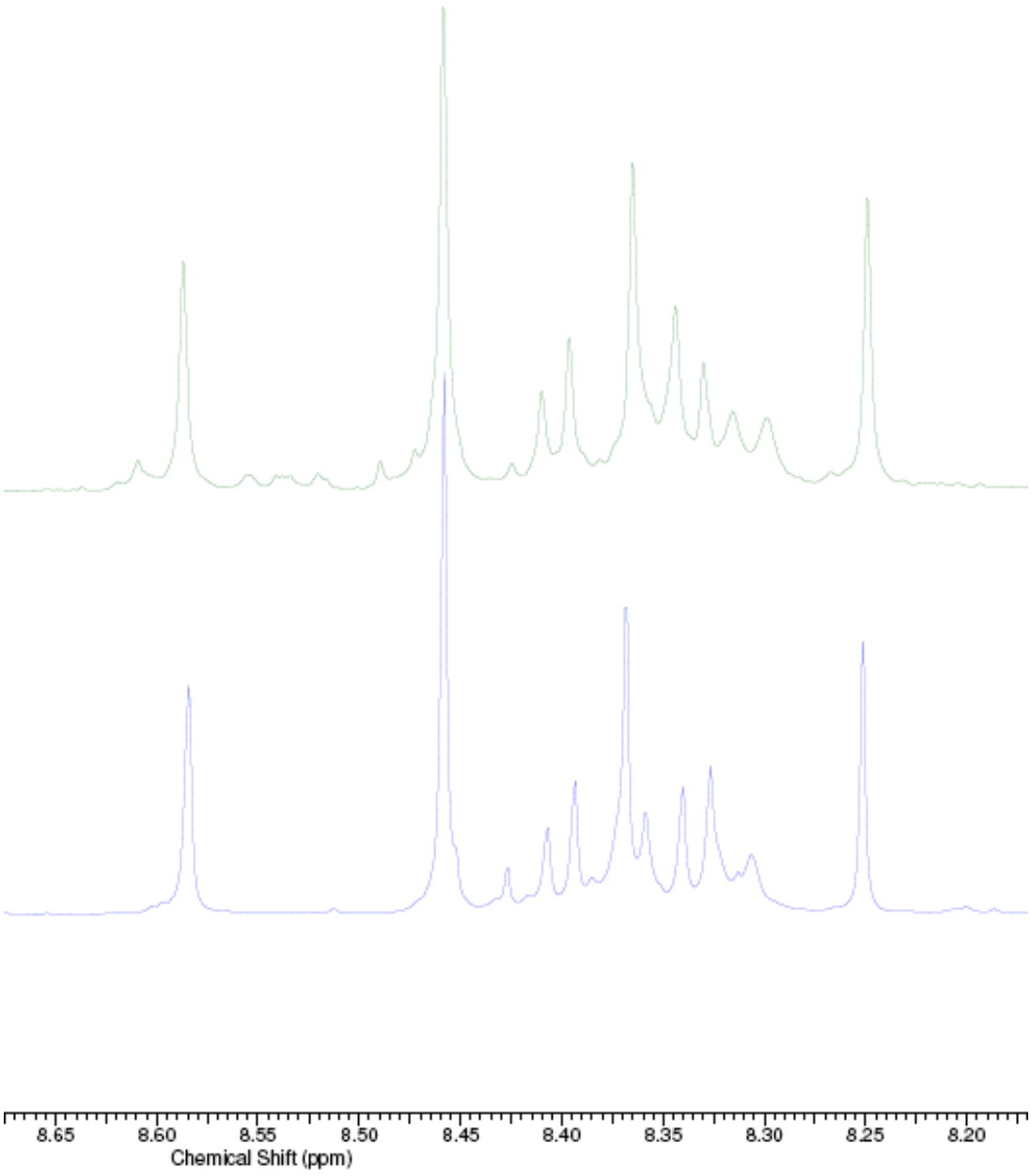
^{1}H NMR spectrum of synthetic thiocillin I (600 MHz, DMSO-d_6)



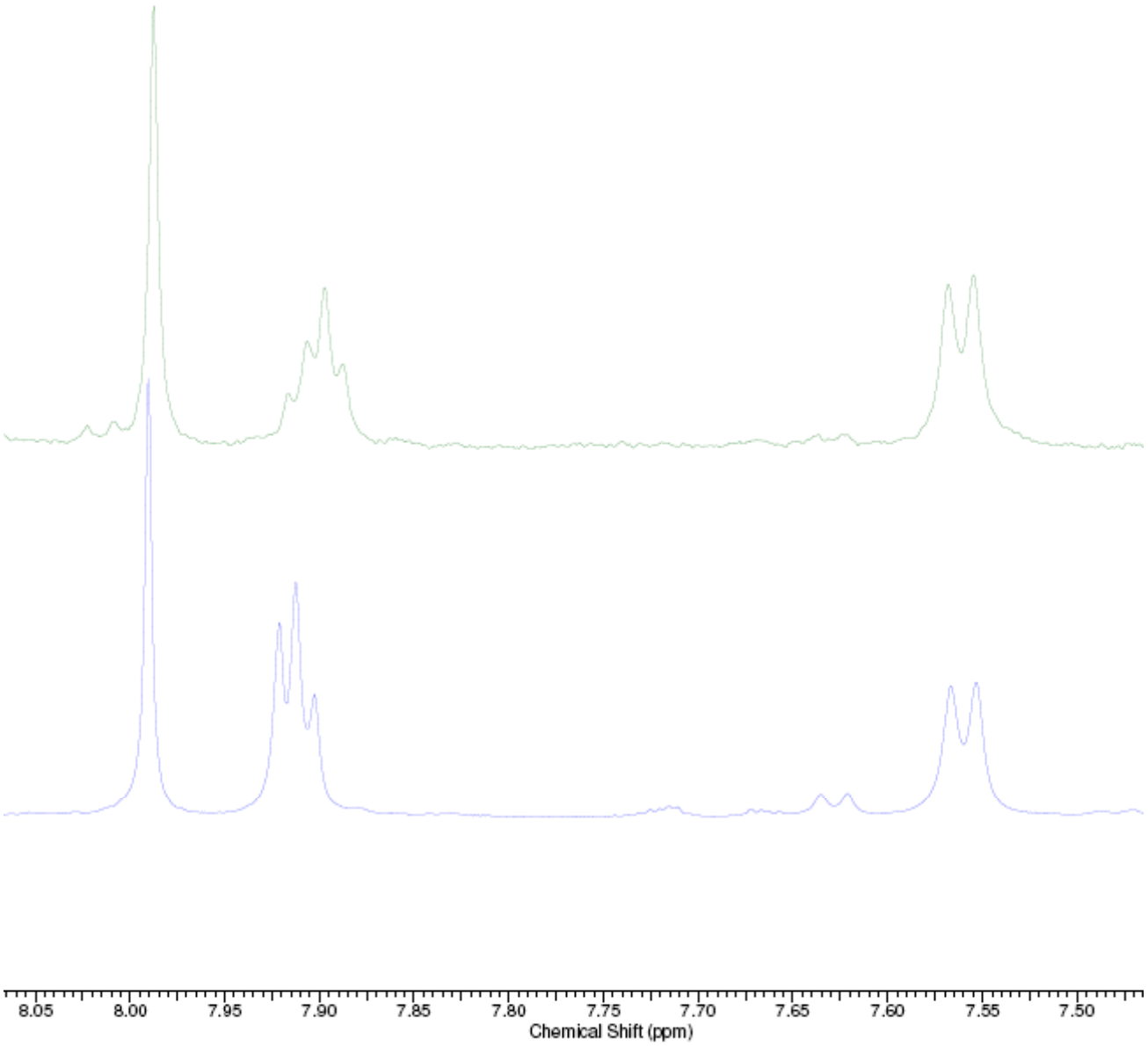
^{1}H NMR spectrum of natural thiocillin I (600 MHz, DMSO-d_6)



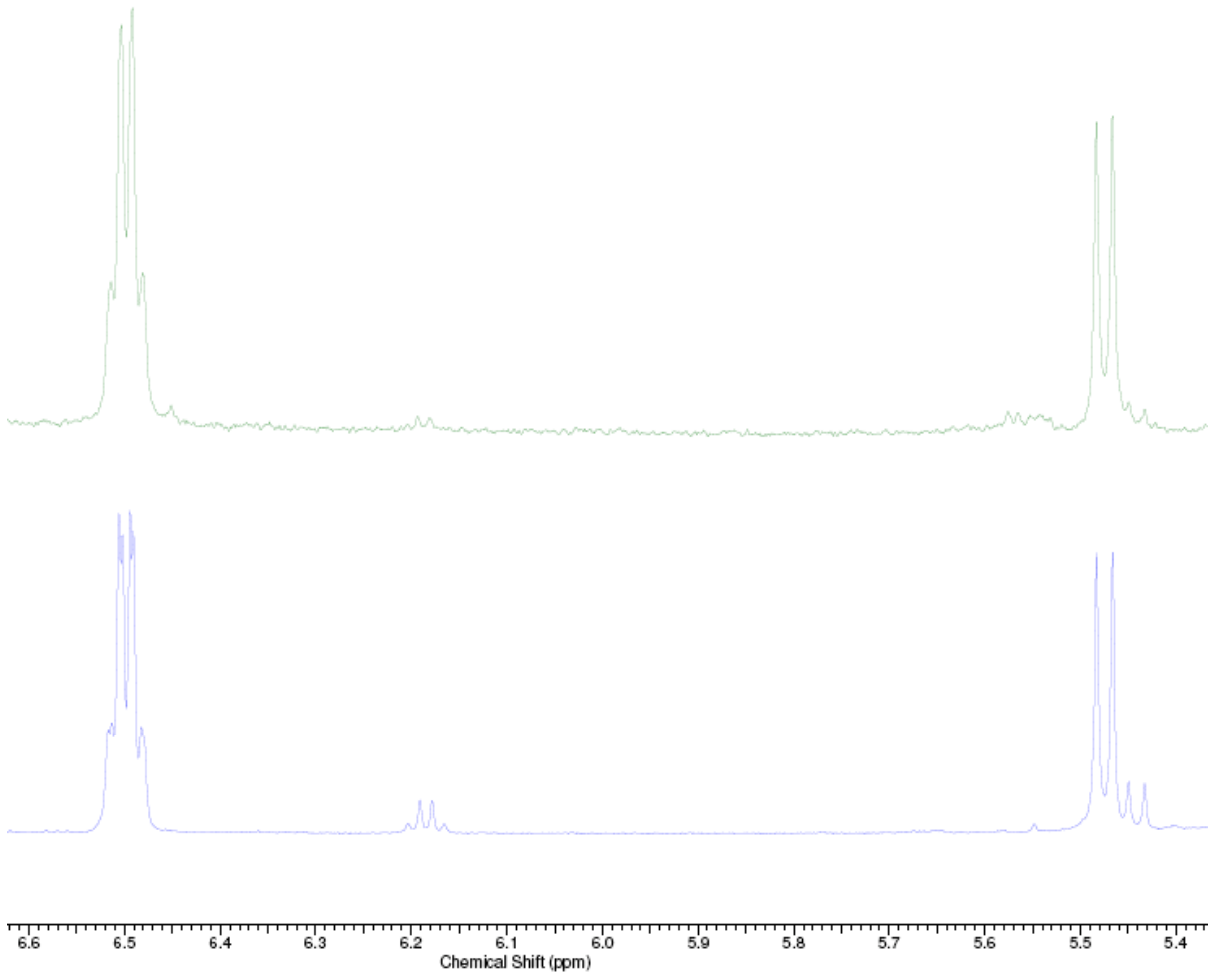
Stacked plot of ^{1}H NMR spectra of synthetic (top, green) vs. authentic (bottom, blue) thiocillin I (600 MHz, DMSO-d_6 , 9.85-9.40 ppm)



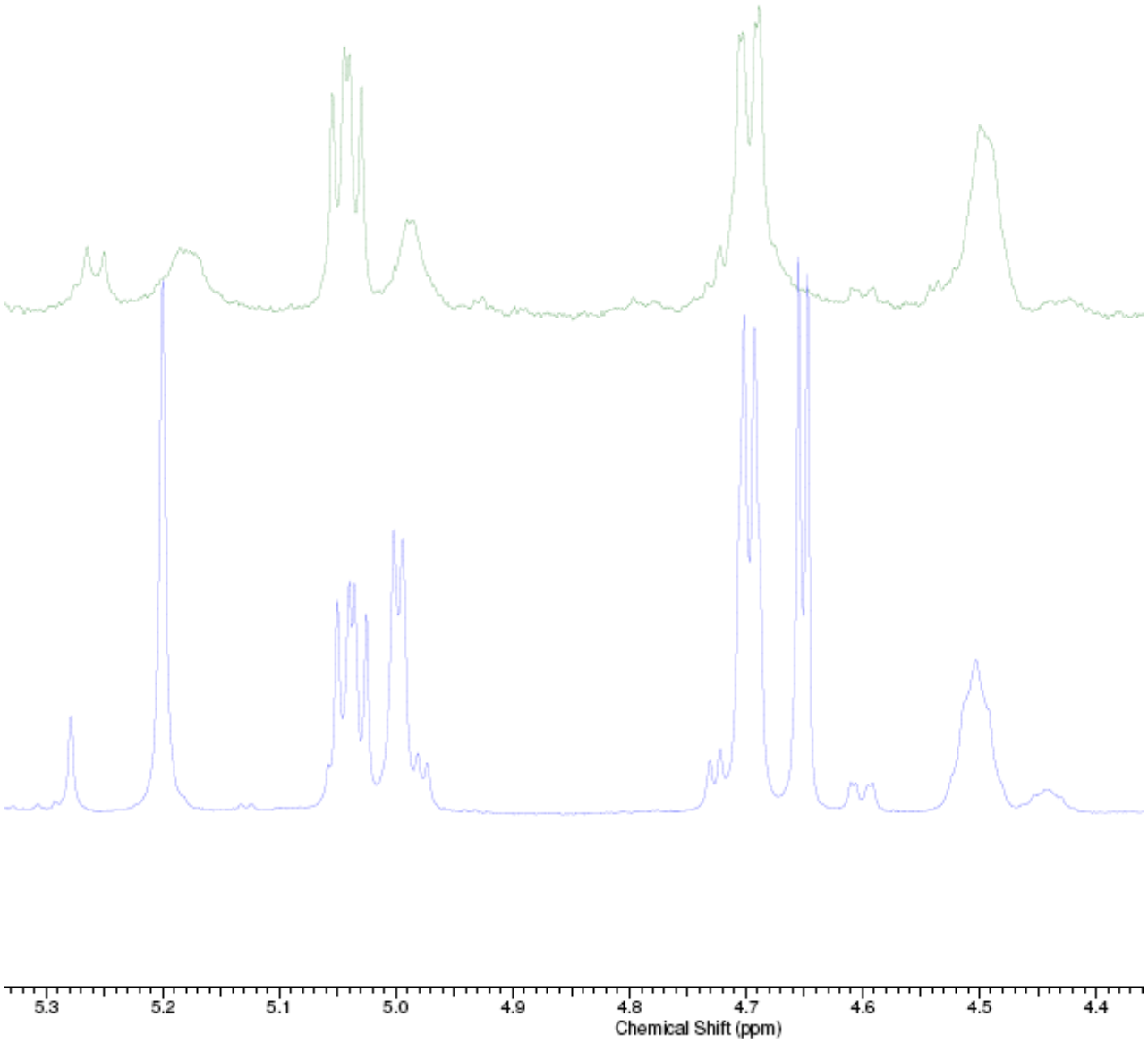
Stacked plot of ^{1}H NMR spectra of synthetic (top, green) vs. authentic (bottom, blue) thiocillin I (600 MHz, DMSO-d_6 , 8.65-8.20 ppm)



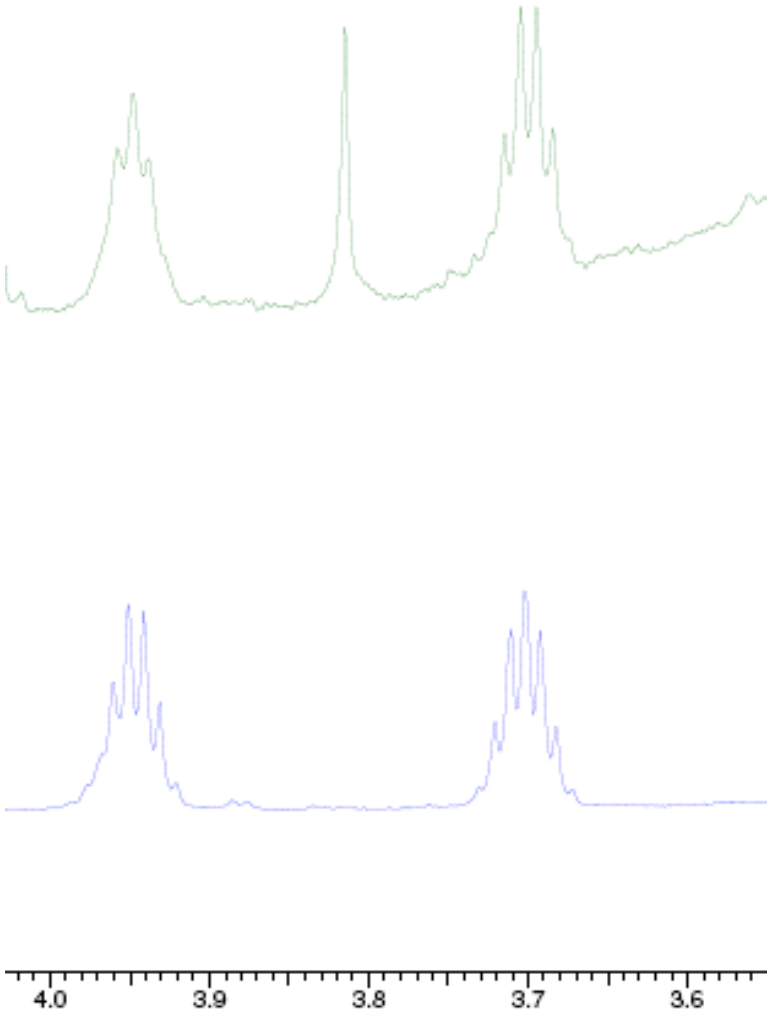
Stacked plot of ^{1}H NMR spectra of synthetic (top, green) vs. authentic (bottom, blue) thiocillin I (600 MHz, DMSO-d_6 , 8.05-7.50 ppm)



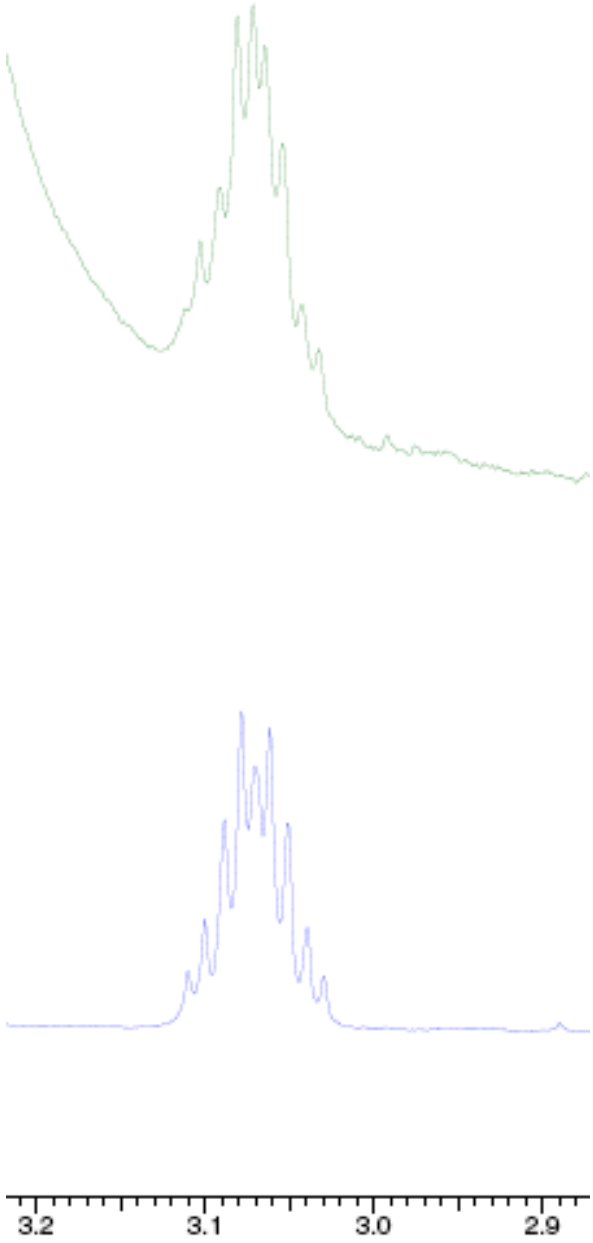
Stacked plot of ^{1}H NMR spectra of synthetic (top, green) vs. authentic (bottom, blue) thiocillin I (600 MHz, DMSO-d_6 , 6.60-5.40 ppm)



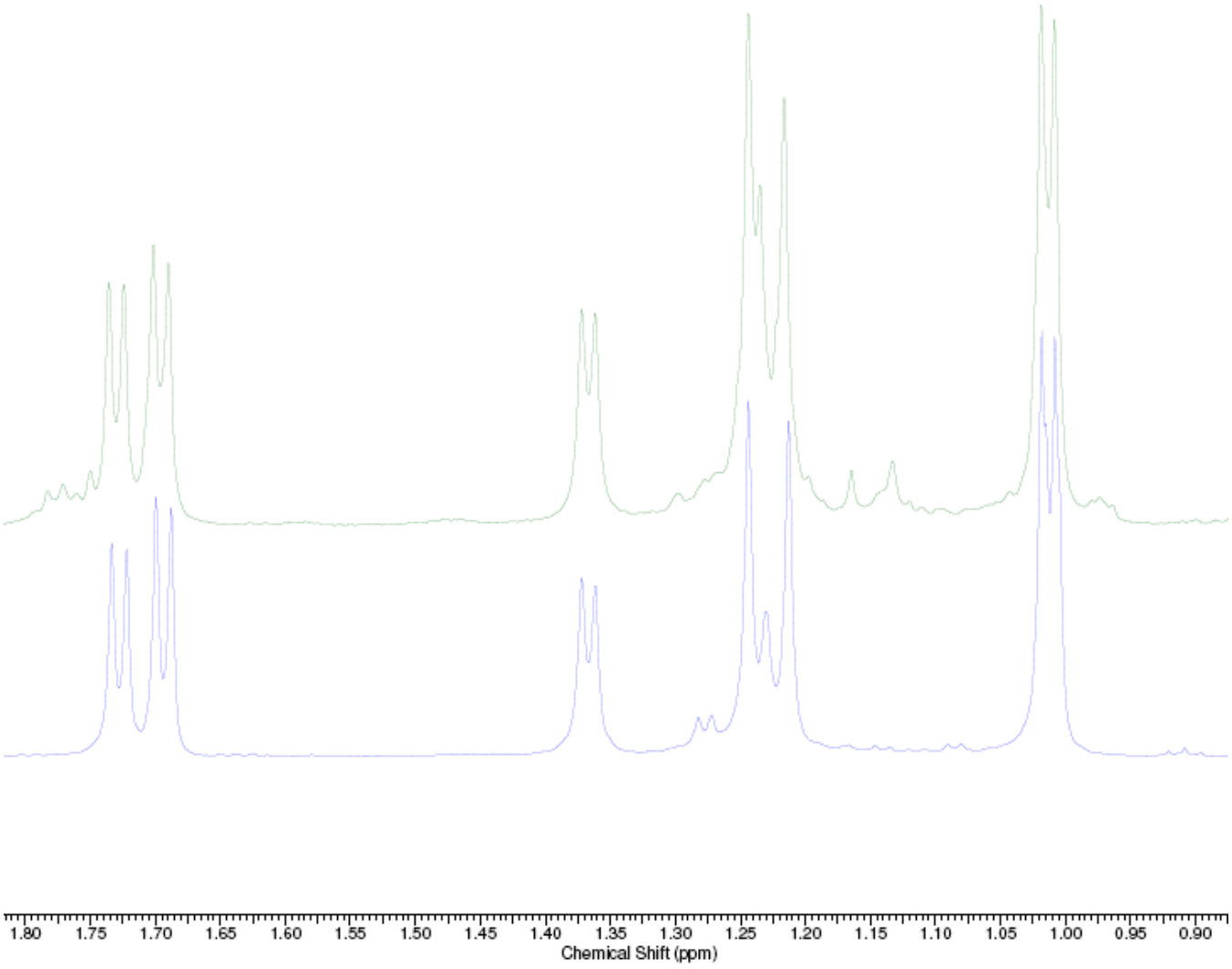
Stacked plot of ^{1}H NMR spectra of synthetic (top, green) vs. authentic (bottom, blue) thiocillin I (600 MHz, DMSO-d_6 , 5.30-4.40 ppm)



Stacked plot of ^{1}H NMR spectra of synthetic (top, green) vs. authentic (bottom, blue) thiocillin I (600 MHz, DMSO-d_6 , 4.00-3.60 ppm)

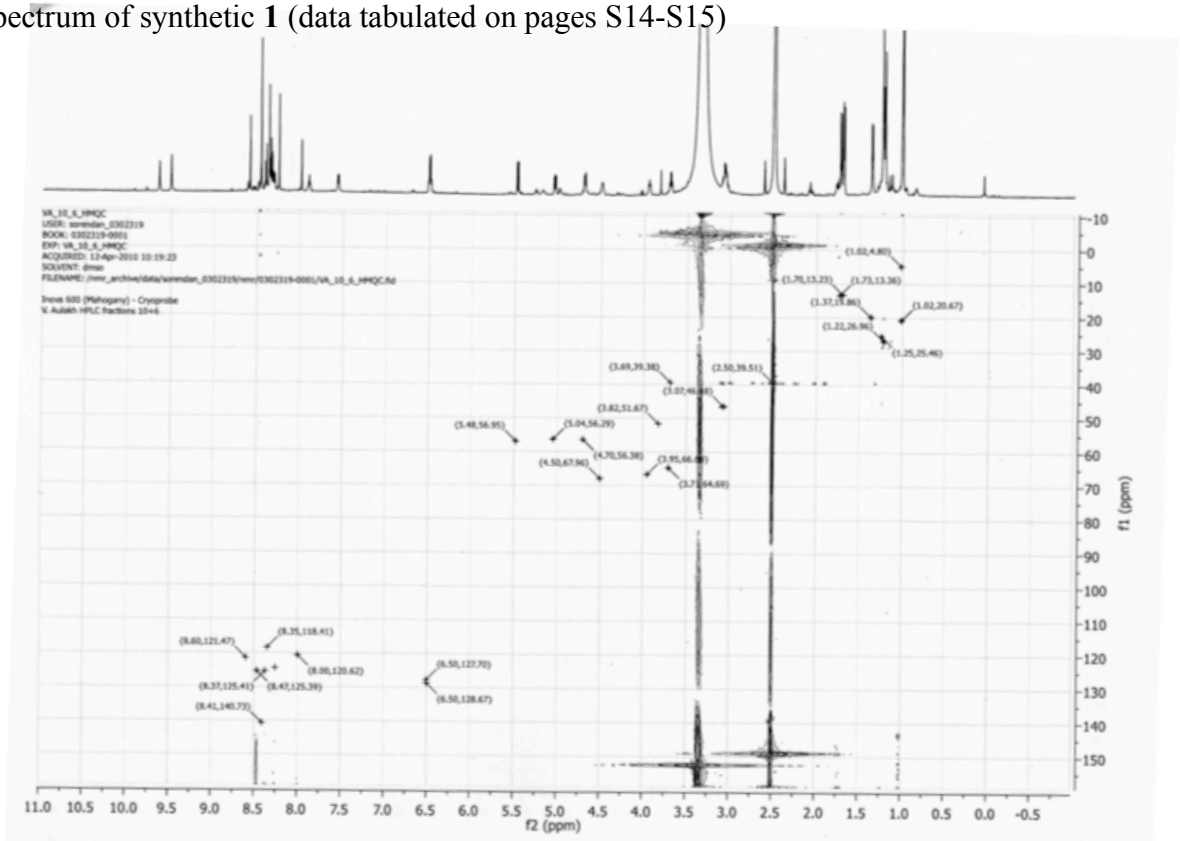


Stacked plot of ^{1}H NMR spectra of synthetic (top, green) vs. authentic (bottom, blue) thiocillin I (600 MHz, DMSO-d_6 , 3.20-2.90 ppm)



Stacked plot of ^{1}H NMR spectra of synthetic (top, green) vs. authentic (bottom, blue) thiocillin I (600 MHz, DMSO-d_6 , 1.80-0.90 ppm)

HMQC spectrum of synthetic **1** (data tabulated on pages S14-S15)



HBMC spectrum of synthetic **1** (data tabulated on page S14-S15)