# Supporting Information

## Synthesis of Plantazolicin Analogues Enables Dissection of Ligand Binding Interactions of a Highly Selective Methyltransferase

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## I. General information

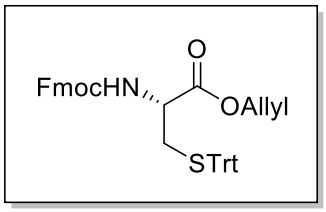
### Materials

All starting materials (Fmoc/Boc amino acids) and reagents used in chemical reactions were purchased from Sigma-Aldrich, Novabiochem, Bachem, Acros Organics, or Chem-Impex. The reagents used in methyltransferase assays were purchased from New England Biolabs, Gold Biotechnology, VWR or Santa Cruz Biotechnology. Triflic anhydride ($Tf_2O$) was distilled prior to use. Reaction solvents were supplied in anhydrous condition from a solvent delivery system using packed alumina columns as described by Pangborn and coworkers.[1] Reaction progress was monitored via thin-layer chromatography (TLC) using E. Merck silica gel 60 F254 TLC plates. The TLC plates were visualized under a UV lamp and/or by treatment with $KMnO_4$ or ninhydrin stains. Flash column chromatography was performed using standard procedures[2] or with a Teledyne-Isco CombiFlash Rf purification system using Silica gel 60 Å (230-400 or 400-632 mesh size). Chromatographic solvent systems are given as volume:volume ratios. Organic solutions were concentrated via rotary evaporation under reduced pressure with a bath temperature of 20-40 ºC. All reactions were performed in oven-dried glassware under an atmosphere of dry nitrogen/argon unless otherwise stated. The solvent (methanol) used for LC-MS (Liquid chromatography-mass spectrometric) analysis was purchased from Sigma. LC-MS analysis was performed on an Agilent 1200 series LC system that was outfitted with an Agilent G1956B single quadrupole mass analyzer. The above LC system utilized a Thermo BETASIL $C_{18}$ column (250 mm x 4.6 mm; pore size: 100 Å; particle size: 5 μm) at a flow rate of 0.85 ml/min.

### Apparatus

$^1H$ and $^{13}C$ NMR spectra were recorded on Varian Inova or Varian Unity (500 MHz, $^1H$; 126 MHz, $^{13}C$) spectrometers. The $^1H$ and $^{13}C$ chemical shifts are reported in parts per million (ppm) and referenced to residual chloroform, $H_2O$, or DMSO, as applicable. The following abbreviations are used to designate chemical shift multiplicities: s = singlet, br s = broad singlet, d = doublet, dd = double doublet, t = triplet, m = multiplet, q = quartet. All $^{13}C$ NMR spectra are proton decoupled. NMR spectra were processed using MestReNova software. High-resolution mass spectra (HRMS) were obtained at the University of Illinois Mass Spectrometry Laboratory using a Micromass Q-Tof. Low resolution electrospray ionization mass spectra (LR-ESI-MS) were obtained on Waters Quattro II or Agilent 6200 TOF LC/MS instruments. Lyophilization was performed on a Labconco instrument. Melting points were measured on a Thomas-Hoover melting point apparatus and are uncorrected. ITC experiments were conducted at 22 °C on a VP-ITC titration microcalorimeter (Microcal, Inc., Northampton, MA).

## II. Synthesis of H-Arg-Thz-OAllyl (2)



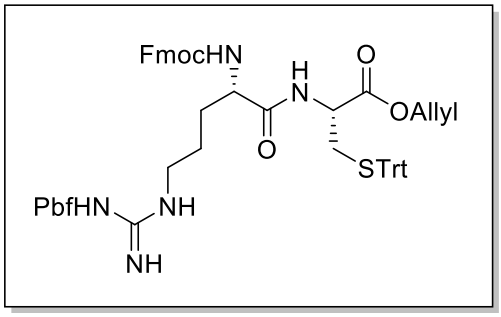

**Fmoc-Cys(Trt)-OAllyl (8):** Fmoc-Cys(Trt)-OH (4 g, 7 mmol) was dissolved in DMF/$CH_2Cl_2$ (1:1, 30 mL), and HCTU (3 g, 7 mmol) and HOBt (970 mg, 7.2 mmol) were added. After stirring the above mixture for 10 min, allyl alcohol (0.92 mL, $d$ = 0.854 g/mL at 25 °C, 14 mmol) and DIEA (*N*,*N*-diisopropylethylamine) 2.56 mL, $d$ = 0.742 g/mL at 25 °C, 14.7 mmol) were sequentially added and the solution was stirred for an additional 14 h. The organic solvents were removed *in vacuo* and the resulting residue was dissolved in EtOAc (50 mL) and washed sequentially with aq. $NH_4Cl$ (sat., 15 mL), aq. $NaHCO_3$ (sat., 15 mL), and brine (15 mL). The organic layer was dried ($Na_2SO_4$) and concentrated. The crude residue was subjected to flash column chromatography (silica gel; EtOAc/hexanes, 1:9 to 4:6) to afford **8** (3.4 g, 5.4 mmol, 76%) as a colorless foam.

$R_f$ = 0.6 (EtOAc:/hexanes, 4:6).

$^1$H NMR (500 MHz, $CDCl_3$): δ 7.75 – 7.70 (m, 2H), 7.61 – 7.55 (m, 2H), 7.39 (d, $J$ = 7.9 Hz, 6H), 7.37 – 7.33 (m, 2H), 7.29 – 7.25 (m, 2H), 7.25 – 7.22 (m, 6H), 7.19 – 7.18 (m, 1H), 7.18 – 7.17 (m, 1H), 7.17 – 7.15 (m, 1H), 5.90 – 5.78 (m, 1H), 5.33 (d, $J$ = 8.4 Hz, 1H), 5.27 (dd, $J$ = 17.1, 1.9 Hz, 1H), 5.21 (dd, $J$ = 10.5, 1.8 Hz, 1H), 4.63 – 4.54 (m, 2H), 4.40 – 4.30 (m, 3H), 4.20 (t, $J$ = 7.2 Hz, 1H), 2.73 – 2.60 (m, 2H).

$^{13}$C NMR (126 MHz, $CDCl_3$): δ 170.3, 155.6, 144.3, 143.9 & 143.8 (Fmoc rotamers),[3] 141.3, 131.4, 129.5, 128.1, 127.8, 127.1, 126.9, 125.20, 125.16, 120.0, 118.8, 67.2, & 67.1 (Fmoc rotamers),[3] 66.3, 53.0, 47.1, 34.1.

HRMS-ESI: $m/z$ $[M+Na]^+$ for $C_{40}H_{35}NNaO_4S$, calculated 648.2185; observed 648.2198.



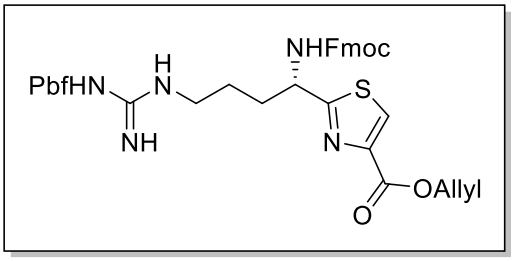

**Fmoc-Arg(Pbf)-Cys(Trt)-OAllyl (10):** To a stirred solution of Fmoc-Cys(Trt)-OAllyl (**8**, 3 g, 5 mmol) in MeCN (5 mL), diethylamine (5 mL, $d$ = 0.707 g/mL at 25 °C, 50 mmol) was added. After stirring for 1 h to ensure complete removal of the Fmoc group, the reaction mixture was concentrated *in* vacuo and further dried by azeotropic distillation with MeCN (2 × 10 mL). The resulting residue was suspended in DMF/$CH_2Cl_2$ (1:1, 30 mL), and Fmoc-Arg(Pbf)-OH (**9**, 3.7 g, 5.7 mmol), HCTU (2.8 g, 6.8 mmol), HOBt (923 mg, 6.83 mmol), and DIEA (1.2 mL, $d$ = 0.742 g/mL at 25 °C, 6.9 mmol) were sequentially added. The above mixture was stirred for 14 h; thereafter, organic solvents were removed *in vacuo*. The resulting residue was dissolved in EtOAc (50 mL) and washed with aq. $NH_4Cl$ (sat., 20 mL), aq. $NaHCO_3$ (sat., 15 mL), and brine (15 mL). The organic layer was dried ($Na_2SO_4$) and concentrated to a crude residue, which was subjected to flash column chromatography (silica gel; MeOH/$CH_2Cl_2$, 0:100 to 0.5:9.5) to afford **10** (3.4 g, 3.3 mmol, 70%) as a colorless foam.

$R_f$ = 0.3 (MeOH/$CH_2Cl_2$, 0.5:9.5).

$^1$H NMR (500 MHz, $CDCl_3$): δ 7.73 (d, $J$ = 7.6 Hz, 2H), 7.59 – 7.53 (m, 2H), 7.39 – 7.27 (m, 10H), 7.25 – 7.12 (m, 10H), 6.91 (br s, 1H), 6.17 (br s, 2H), 5.85 – 5.75 (m, 2H), 5.30 – 5.14 (m, 2H), 4.52 – 4.47 (m, 2H), 4.36 – 4.24 (m, 4H), 4.14 (t, $J$ = 7.2 Hz, 1H), 3.18 (br s, 2H), 2.94 (s, 1H), 2.91 – 2.87 (m, 2H), 2.80 – 2.76 (m, 1H), 2.57 (s, 3H), 2.50 (s, 3H), 2.06 (s, 3H), 1.87 (br s, 1H), 1.68 – 1.49 (m, 2H), 1.42 (s, 6H), 1.33 – 1.22 (m, 1H).

$^{13}$C NMR (126 MHz, $CDCl_3$): δ 170.4, 158.9, 156.3, 144.3, 141.4, 138.6, 132.5, 131.4, 129.6, 128.2, 128.1, 128.0, 127.8, 127.4, 127.2, 127.0, 125.3, 124.8, 120.1, 118.9, 117.6, 109.9, 86.5, 67.2, 66.5, 52.0, 47.2, 43.3, 33.1, 28.7, 24.7, 19.5, 18.1, 12.6.

HRMS-ESI: *m/z* $[M+H]^+$ for $C_{59}H_{64}N_5O_8S_2$, calculated 1034.4196; observed 1034.4207.



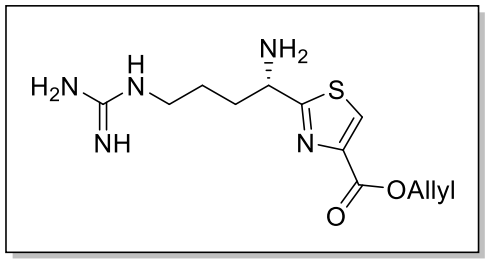

**Fmoc-Arg(Pbf)-Thz-OAllyl (5a):** To a stirred suspension of $PPh_3O$ (265 mg, 0.952 mmol) in $CH_2Cl_2$ (1.5 mL) at 0 °C was added $Tf_2O$ (245 µL, $d$ = 1.68 g/mL at 25 °C, 1.46 mmol); the mixture was allowed to stir for 20 min.[4] Next, a solution of **10** (1 g, 1 mmol) in $CH_2Cl_2$ (1.5 mL) was slowly added, and stirring was maintained for 3 h at 0 °C. The reaction was quenched by addition of aq. $NaHCO_3$ (sat., 5 mL) at 0 °C, and the aqueous layer was extracted with $CH_2Cl_2$ (3 × 15 mL). The combined organic layers were washed with brine (10 mL), dried ($Na_2SO_4$), and concentrated to give the crude thiazoline. The above residue was dissolved in $CH_2Cl_2$ (4 mL) and $MnO_2$ (1.26 g, 14.5 mmol) was added. After stirring for 24 h, the reaction mixture was filtered through a pad of Celite, concentrated, and subjected to flash column chromatography (silica gel; MeOH/$CH_2Cl_2$, 0:100 to 0.5:9.5) to afford **5a** (185 mg, 0.240 mmol, 25%) as a yellow foam.

$R_f$ = 0.4 (MeOH/$CH_2Cl_2$, 0.5:9.5).

$^1$H NMR (500 MHz, $CDCl_3$): δ 8.04 (s, 1H), 7.71 (d, $J$ = 7.6 Hz, 2H), 7.55 (d, $J$ = 7.6 Hz, 2H), 7.36 – 7.30 (m, 2H), 7.23 – 7.20 (m, 2H), 6.49 (br s, 2H), 6.39 – 6.37 (m, 1H), 6.33 (d, $J$ = 8.5 Hz, 1H), 6.02 – 5.95 (m, 1H), 5.38 (d, $J$ = 17.2 Hz, 1H), 5.28 (d, $J$ = 10.3 Hz, 1H), 5.00 – 4.96 (m, 1H), 4.79 (d, $J$ = 5.8 Hz, 2H), 4.40 (dd, $J$ = 10.6, 7.1 Hz, 1H), 4.32 (dd, $J$ = 10.7, 7.0 Hz, 1H), 4.12 (t, $J$ = 7.0 Hz, 1H), 3.32 – 3.21 (m, 2H), 2.89 (s, 2H), 2.57 (s, 3H), 2.50 (s, 3H), 2.17 – 2.10 (m, 1H), 2.05 (s, 3H), 1.99 – 1.95 (m, 1H), 1.70 (br s, 1H), 1.60 (br s, 1H), 1.41 (s, 6H).

$^{13}$C NMR (126 MHz, $CDCl_3$): δ 174.5, 161.2, 158.8, 156.5, 156.3, 146.2, 143.8, 141.3, 138.4, 133.1, 132.4, 131.7, 127.8, 127.2, 125.3, 124.7, 120.0, 119.3, 117.6, 86.4, 67.2, 66.3, 53.5, 47.2, 43.3, 40.9, 31.9, 28.72, 28.65, 25.4, 19.5, 18.1, 12.6, 12.5.

HRMS-ESI: *m/z* $[M+H]^+$ for $C_{40}H_{46}N_5O_7S_2$, calculated 772.2839; observed 772.2841.



**H-Arg-Thz-OAllyl (2):** Compound **5a** (50 mg, 0.065 mmol) was dissolved in MeCN (1 ml), and diethylamine (1 mL) was added. After stirring for 30 min to ensure complete removal of Fmoc group (monitored by TLC and ESI-MS), the reaction mixture was diluted with MeCN (4 mL), concentrated *in vacuo,* and further dried by azeotropic distillation with MeCN (2 × 5 mL). The resulting residue was washed with pentane (2 × 3 mL) before being stirred in a mixture of TFA/TIPS/$H_2O$ (94:3:3, 2 mL) at 0 °C for 1.5 h. Upon completion of the deprotection of Pbf group (monitored by TLC and ESI-MS), the reaction mixture was diluted with toluene (10 mL) and concentrated. The obtained residue was washed with $Et_2O$ (2 × 5 ml), dissolved in water (1 mL), and lyophilized to afford **5a** as a colorless foam (18 mg, 60.5 mmol, 93%).

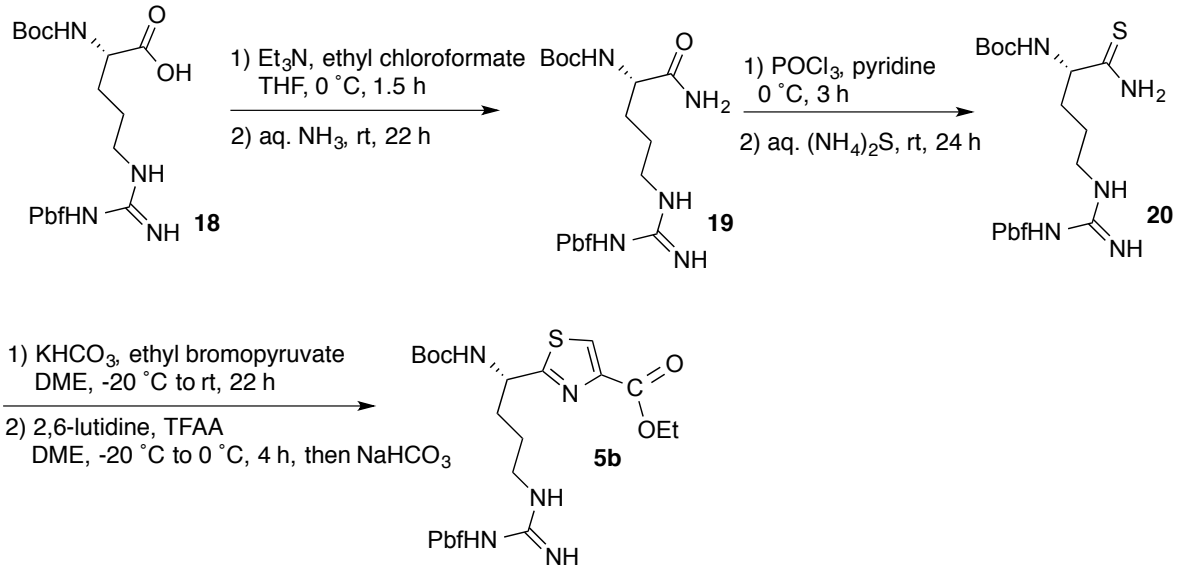
$^1$H NMR (500 MHz, $D_2O$): δ 8.55 (s, 1H), 6.09 – 6.01 (m, 1H), 5.44 – 5.39 (m, 1H), 5.35 – 5.31 (m, 1H), 4.91 – 4.87 (m, 1H), 4.88 – 4.85 (m, 2H), 3.18 (t, *J* = 6.8 Hz, 2H), 2.24– 2.12 (m, 2H), 1.74 – 1.50 (m, 2H).

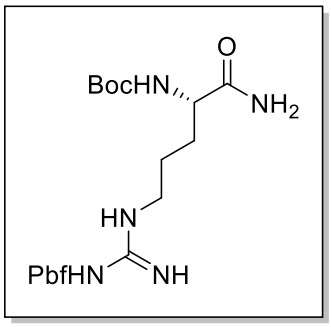
$^{13}$C NMR (126 MHz, $D_2O$): δ 166.1, 162.3, 156.9, 146.1, 131.6, 131.2, 119.2, 66.9, 51.7, 40.3, 30.5, 24.0.

HRMS-ESI: *m/z* $[M+H]^+$ for $C_{12}H_{20}N_5O_2S$, calculated 298.1338; observed 298.1341.

## III. Synthesis of Boc-Arg-Thz-OEt (5b)

**Scheme S1**





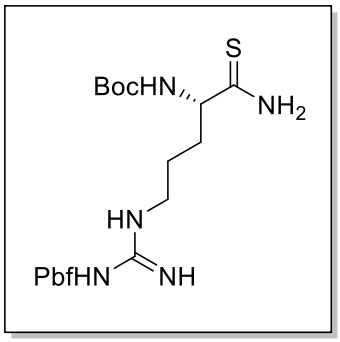

**Boc-Arg(Pbf)-CONH$_2$ (19):** To a stirred solution of Boc-Arg(Pbf)-COOH (**18**, 4.4 g, 8.4 mmol) in THF (100 mL) at 0 °C, $Et_3N$ (1.3 mL, $d$ = 0.726 g/mL at 25 °C, 9.3 mmol) and ethyl chloroformate (0.9 mL, $d$ = 1.135 g/mL at 25 °C, 9.4 mmol) were sequentially added, and the reaction mixture was stirred for 1.5 h. Then, aq. $NH_3$ (35% w/w, 35 mL) was added. The solution allowed to warm to rt and stirring was maintained for 22 h. The volatile organics were removed *in vacuo* and the resulting aqueous suspension was extracted with EtOAc (3 × 30 mL). The combined organic layers were washed with water (20 mL) and brine (10 mL), dried ($Na_2SO_4$), and concentrated to give **19** as a colorless foam (4.2 g, 8.0 mmol, 95%).

$R_f$ = 0.3 (EtOAc/hexanes, 8:2).

$^1$H NMR (500 MHz, $CDCl_3$): δ 7.03 (br s, 1H), 6.34 – 6.23 (m, 4H), 5.78 – 5.76 (m, 1H), 4.24 – 4.18 (m, 1H), 3.26 (br s, 2H), 2.95 (s, 2H), 2.56 (s, 3H), 2.49 (s, 3H), 2.09 (s, 3H), 1.85 – 1.80 (m, 2H), 1.63 – 1.61 (m, 2H), 1.46 (s, 6H), 1.40 (s, 9H).

$^{13}$C NMR (126 MHz, $CDCl_3$): δ 159.0, 156.7, 156.2, 138.5, 132.8, 132.4, 124.9, 117.8, 86.6, 80.2, 53.7, 43.4, 30.4, 29.9, 28.8, 28.5, 25.6, 19.5, 18.1, 12.6.

HRMS-ESI: $m/z$ $[M+H]^+$ for $C_{24}H_{40}N_5O_6S$, calculated 526.22699; observed 526.2683.



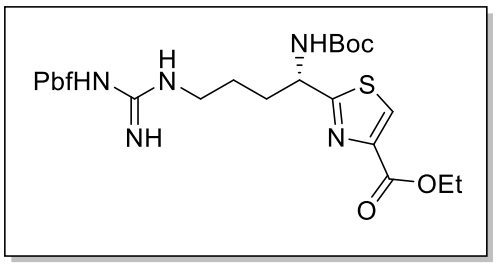

**Boc-Arg(Pbf)-CSNH$_2$ (20):** To a solution of Boc-Arg(Pbf)-CONH$_2$ (**19**, 4.2 g, 8.0 mmol) in pyridine (80 mL) at 0 °C, $POCl_3$ (2 mL, $d$ = 1.645 g/mL at 25 °C, 20 mmol)[5b,6] was added, and the reaction mixture was stirred at the same temperature for 3 h. Then, the mixture was poured over ice, diluted with water (30 mL), and extracted with $Et_2O$ (3 × 30 mL). The combined organic layers were washed sequentially with ice-cooled 2 N $KHSO_4$ (20 mL), water (20 mL), and brine (20 mL) before being concentrated. The obtained residue was dissolved in MeOH (70 mL), aq. ammonium sulfide (40 wt %, 2.8 mL, 16 mmol) was added, and the mixture was stirred for 24 h at rt. The reaction mixture was concentrated and partitioned between EtOAc (25 mL) and water (25 mL). The aqueous layer was extracted with EtOAc (3 × 30 mL) before the combined organic layers were washed with brine (20 mL), dried ($Na_2SO_4$), concentrated, and purified by flash column chromatography (silica gel; EtOAc/hexanes, 2:8 to 8:2) to afford **20** (1.1 g, 2.0 mmol, 25%) as a yellow foam.

$R_f$ = 0.5 (EtOAc/hexanes, 8:2).

$^1$H NMR (500 MHz, $CDCl_3$): δ 8.68 (br s, 1H), 6.26 (br s, 3H), 5.84 (d, $J$ = 8.2 Hz, 1H), 4.58 (br s, 1H), 3.31 (br s, 2H), 2.96 (s, 2H), 2.56 (s, 3H), 2.49 (s, 3H), 2.10 (s, 3H), 1.87 – 1.82 (m, 1H), 1.78 – 1.71 (m, 1H), 1.65 – 1.58 (m, 2H), 1.46 (s, 6H), 1.42 (s, 9H).

$^{13}$C NMR (126 MHz, $CDCl_3$): δ 159.2, 156.6, 155.9, 138.6, 132.5, 125.0, 117.9, 86.7, 80.3, 43.4, 40.8, 34.2, 29.9, 28.8, 28.6, 25.8, 19.5, 18.1, 12.6.

HRMS-ESI: *m/z* $[M+H]^+$ for $C_{24}H_{40}N_5O_5S_2$, calculated 542.2471; observed 542.2460.




**Boc-Arg(Pbf)-Thz-OEt (5b):** Boc-Arg(Pbf)-$CSNH_2$ (**20**, 1.1 g, 2.0 mmol) was dissolved in DME (20 mL). The solution was cooled to -20 °C before $KHCO_3$ (2 g, 20 mmol) and ethyl bromopyruvate (1 mL, *d* = 1.554 g/mL at 25 °C, 8.0 mmol) were sequentially added. The above mixture was allowed to warm to rt and stirred for 22 h. The reaction mixture was then concentrated, suspended in $Et_2O$ (40 mL), washed with water (5 mL) and brine (15 mL), and dried ($Na_2SO_4$). The material was concentrated and the resulting residue was dissolved in DME (20 ml). The above solution was cooled to -20 °C before 2,6-lutidine (2.2 mL, *d* = 0.92 g/mL at 25 °C, 19 mmol) and trifluoroacetic anhydride (1.2 mL, *d* = 1.511 g/mL at 20 °C, 8.6 mmol) were slowly and sequentially added. The resulting mixture was stirred at -20 °C for 10 min and then at 0 °C for 4 h. The reaction mixture was quenched by the addition of aq. $NaHCO_3$ (sat., 10 mL), diluted with water (10 mL), and extracted with EtOAc (3 × 20 mL). The combined organic layers were washed with aq. $NH_4Cl$ (sat., 15 mL) and brine (15 ml), concentrated, and subjected to flash column chromatography (silica gel; MeOH/$CHCl_3$, 0:100 to 1:9) to afford **5b** (700 mg, 1.10 mmol, 55%) as a pale yellow solid.

mp = 155-157 °C

$R_f$ = 0.5 (MeOH/$CHCl_3$, 1:9).

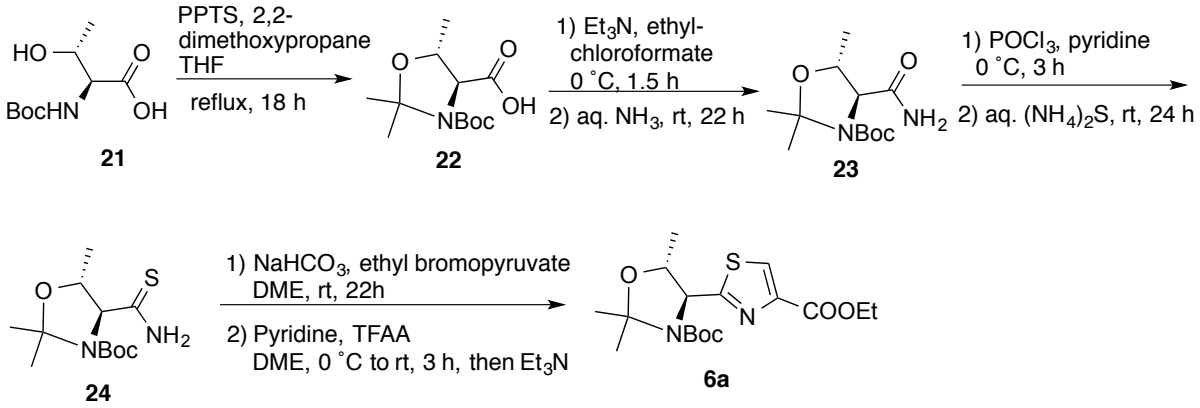
$^1$H NMR (500 MHz, $CDCl_3$): δ 8.05 (s, 1H), 6.55 – 6.35 (m, 3H), 5.63 (d, *J* = 8.3 Hz, 1H), 4.94 – 4.92 (m, 1H), 4.37 (q, *J* = 7.1 Hz, 2H), 3.33 – 3.20 (m, 2H), 2.94 (s, 2H), 2.56 (s, 3H), 2.50 (s, 3H), 2.12 – 2.09 (m, 1H), 2.07 (s, 3H), 1.95 – 1.91 (m, 1H), 1.71 – 1.57 (m, 2H), 1.44 – 1.40 (m, 15H), 1.36 (dt, *J* = 7.1 Hz, 3H).

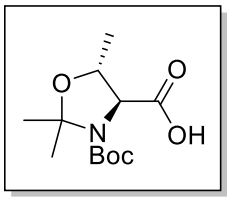
$^{13}$C NMR (126 MHz, $CDCl_3$): δ 161.7, 158.8, 156.6, 155.6, 146.6, 138.5, 133.3, 132.4, 128.1, 124.7, 117.6, 86.5, 80.6, 61.9, 52.8, 43.4, 41.0, 28.8, 28.5, 28.4, 25.5, 25.3, 19.5, 18.2, 14.5, 12.7.

HRMS-ESI: m/z $[M+H]^+$ for $C_{29}H_{44}N_5O_7S_2$, calculated 638.2682; observed 638.2676.

## IV. Synthesis of Boc-Oxazolidine(Me)$_3$-Thz-OEt (6a)

**Scheme S2**



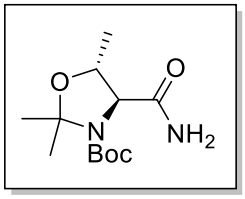
**Boc-Oxazolidine(Me)$_3$-COOH (22):** To a stirred solution of Boc-Thr-OH (**21**, 8.2 g, 37 mmol) in THF (170 mL), 2,2-dimethoxypropane (46 mL, $d$ = 0.847 g/mL at 25 °C, 370 mmol) and pyridinium *p*-toluenesulfonate (PPTS, 2.8 g, 11 mmol) were added.[5] The solution was heated at reflux for 18 h, cooled to rt, and concentrated. The residue was partitioned between water (180 mL) and EtOAc (180 mL), and the aqueous layer was further extracted with EtOAc (2 × 150 mL). The combined organic layers were washed with water (30 mL) and brine (30 mL), dried ($Na_2SO_4$), and concentrated to give **22** (9.5 g, 37 mmol, 98%) as a colorless solid whose spectral data matched those reported previously.[5]

mp = 93-95 °C

$R_f$ = 0.3 (EtOAc/hexanes, 8:2).

$^1$H NMR (500 MHz, $CDCl_3$): δ 4.30 – 4.18 (m, 1H), 4.00 (d, $J$ = 7.6 Hz, 0.4H), 3.92 (d, $J$ = 7.6 Hz, 0.58H), 1.65 (s, 1.7H), 1.60 – 1.55 (m, 4.2H), 1.48 (br s, 3.2H), 1.44 – 1.41 (m, 9H).

$^{13}$C NMR (126 MHz, $CDCl_3$) δ 176.9, 175.3, 152.5, 151.0, 95.4, 94.9, 81.5, 80.9, 77.4, 77.1, 76.9, 74.0, 73.6, 66.1, 66.0, 28.5, 28.4, 27.9, 26.6, 25.0, 24.1, 19.1.



**Boc-Oxazolidine(Me)$_3$-CONH$_2$ (23):** To a stirred solution of Boc-oxazolidine(Me)$_3$-COOH (**22**, 5.8 g 23 mmol) in THF (85 mL) at 0 °C, $Et_3N$ (3.5 mL, $d$ = 0.726 g/mL at 25 °C, 25 mmol) and ethyl chloroformate (2.4 mL, 1.135 g/mL at 25 °C, 25 mmol) were sequentially added, and the reaction mixture was stirred for 1.5 h.[5] Then, aqueous $NH_3$ (35 wt %, 4 mL, $d$ = 0.88 g/mL, 35 mmol) was added and the solution was allowed to warm to rt with stirring for 22 h. The volatile organics were removed *in vacuo* and resulting aqueous suspension was extracted with EtOAc (3 × 100 mL). The combined organic layers were washed

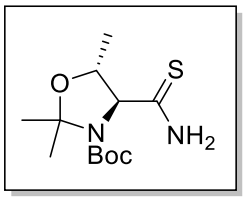
with water (40 mL) and brine (40 mL), dried ($Na_2SO_4$), and concentrated to give **23** (4.6 g, 18 mmol, 78%) as a colorless solid whose spectral data matched those reported previously.[5]

mp = 146-148°C

$R_f$ = 0.4 (EtOAc/hexanes, 8:2).

$^1$H NMR (500 MHz, $CDCl_3$): δ 6.88 – 5.93 (m, 2H), 4.16 (s, 1H), 3.88 – 3.60 (m, 1H), 1.60 – 1.53 (m, 6H), 1.41 (s, 9H), 1.35 (d, $J$ = 6.1 Hz, 3H).

$^{13}$C NMR (126 MHz, $CDCl_3$): δ 172.9, 94.9, 81.1, 74.3, 67.4, 28.4, 25.3, 18.9.



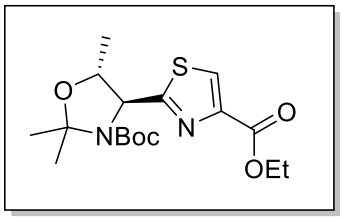

**Boc-Oxazolidine(Me)$_3$-CSNH$_2$ (24):** To a solution of Boc-oxazolidine(Me)$_3$-CONH$_2$ (**23**, 2.6 g, 10 mmol) in pyridine (50 mL) at 0 °C, $POCl_3$ (2.4 ml, $d$ = 1.645 g/mL at 25 °C, 26 mmol) was added, and the reaction mixture was stirred at this temperature for 3 h.[5b,6] Then, the mixture was poured over ice, diluted with water (120 mL), and extracted with $Et_2O$ (3 × 50 mL). The combined organic layers were washed sequentially with ice-cooled 2 N aq. $KHSO_4$ (30 mL), water (2 × 50 mL), and brine (50 mL) before being concentrated. The obtained residue was dissolved in MeOH (25 mL), aq. ammonium sulfide (20 wt %, 6 mL, 20 mmol) was added, and the mixture was stirred for 24 h at rt. The organic volatiles were removed *in vacuo* and the residue was partitioned between EtOAc (100 mL) and water (50 mL). The aqueous layer was extracted with EtOAc (2 × 100 mL). The combined organic layers were washed with brine (50 mL), dried ($Na_2SO_4$), and concentrated to give **24** (2 g, 7 mmol, 70%) as a colorless solid whose spectral data matched those reported previously.[5]

mp = 104-106 °C

$R_f$ = 0.6 (EtOAc:hexanes, 8:2).

$^1$H NMR (500 MHz, $CDCl_3$): δ 8.35 (br s, 2H), 4.13 (d, $J$ = 7.7 Hz, 1H), 4.03 (s, 1H), 1.56 – 1.47 (m, 6H), 1.39 – 1.26 (m, 12H).

$^{13}$C NMR (126 MHz, $CDCl_3$): δ 205.2, 171.1, 152.0, 94.6, 81.2, 76.6, 73.5, 28.2, 25.7, 18.7.




**Boc-Oxazolidine(Me)$_3$-Thz-COOEt (6a):** Boc-Oxazolidine(Me)$_3$-CSNH$_2$ (**24,** 2 g, 7 mmol) was dissolved in DME (40 mL). $NaHCO_3$ (5 g, 60 mmol) and ethyl bromopyruvate (2.8 mL, $d$ = 1.554 g/mL at 25 °C, 22.3 mmol) were sequentially added. The above mixture was stirred at rt for 22 h. The reaction mixture was then concentrated and the obtained residue was suspended in $Et_2O$ (100 mL), washed with water (20 mL) and brine (20 mL), and dried ($Na_2SO_4$). The organic layer was concentrated, and the resulting residue was dissolved in DME (20 mL). The above solution was cooled to 0 °C before pyridine (5.3 mL, $d$ = 0.978 g/mL at 25 °C, 66 mmol) and

trifluoroacetic anhydride (TFAA, 4.2 mL, $d$ = 1.511 g/mL at 20 °C, 30 mmol) were slowly and sequentially added. The resulting mixture was stirred at 0 °C for 3 h. Next, $Et_3N$ (2 mL, $d$ = 0.726 g/mL at 25 °C, 14 mmol) was added, and the reaction mixture allowed to warm to rt before being concentrated. The residue was dissolved in $CHCl_3$ (10 mL), washed with aq. $NH_4Cl$ (sat., 20 mL), aq. $NaHCO_3$ (sat., 20 mL), and brine (15 mL), concentrated, and subjected to flash column chromatography (silica gel; EtOAc:hexanes, 1:9 to 1:1) to afford **6a** (1.6 g, 4.3 mmol, 60%) as a colorless solid whose spectral data matched those reported previously.[5]

mp = 102-105 °C.

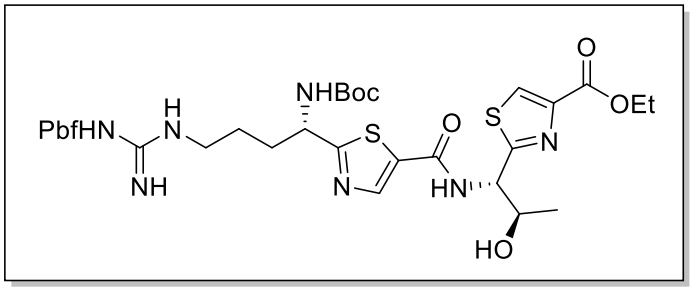
$R_f$ = 0.4 (EtOAc/hexanes, 4:6).

$^1$H NMR (500 MHz, $CDCl_3$): δ 8.22 (s, 1H), 4.86 – 4.79 (m, 1H), 4.43 (q, $J$ = 7.2 Hz, 2H), 4.23 – 4.17 (m, 1H), 1.71 (s, 6H), 1.48 – 1.39 (m, 9H), 1.20 (s, 6H).

$^{13}$C NMR (126 MHz, $CDCl_3$): δ 173.1, 160.8, 151.0, 146.5, 127.1, 94.9, 80.3, 65.7, 61.1, 27.8, 26.3, 25.6, 17.6, 14.1.

HRMS-ESI: $m/z$ $[M+H]^+$ for $C_{17}H_{27}N_2O_5S$, calculated 371.1641; observed 371.1635.

## V. Synthesis of H-Arg-Thz-Oxz(Me)-Thz-OEt (3)




**Boc-Arg(Pbf)-Thr-Thz-OEt (11):** To a stirred solution of **6a** (150 mg, 0.405 mmol) in 1,4-dioxane (2 mL), HCl (2 mL, 4 M in 1,4-dioxane, 8 mmol) was added at 0 °C, and the solution was allowed to warm to rt. After stirring for 4 h, the above mixture was diluted with toluene (10 mL) and concentrated. The residual HCl was removed by co-evaporation with $CH_2Cl_2$ (2 × 5 mL) to give the crude thiazole hydrochloride salt (**6b**), which was used as such in the next step without further purification.

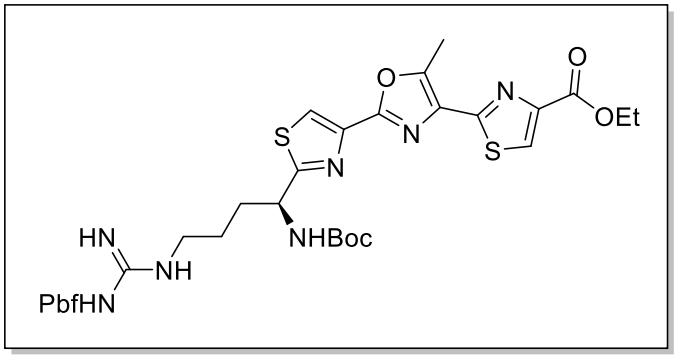
Compound **5b** (210 mg, 0.329 mmol) was dissolved in MeOH/THF (2 mL), the solution was cooled to 0 °C, and aq. LiOH (0.5 N, 1 mL, 0.5 mmol) was added. The above mixture was allowed to warm to rt and stirred for 8 h. Then, the reaction mixture was concentrated, and the obtained residue was diluted with water (3 mL) and washed with $Et_2O$ (3 mL). The aqueous layer was cooled to 0 °C and acidified with 1 N $KHSO_4$ to pH = 3. The resulting suspension was extracted with EtOAc (3 × 10 mL), and the combined organic layers were dried ($Na_2SO_4$) and concentrated to afford the crude acid **5c** (200 mg), which was used without further purification. Compound **5c** was dissolved in $CH_2Cl_2$ (4 mL), and HCTU (200 mg, 0.483 mmol) and HOBt (66 mg, 0.49 mmol) were sequentially added. After stirring the above mixture for 10 min, a solution of **6b** in $CH_2Cl_2$ (4 mL) and DIEA (0.17 mL, $d$ = 0.742 g/mL at 25 °C, 0.98 mmol) were sequentially added. The reaction mixture was stirred for 14 h before being quenched by the addition of aq. $NaHCO_3$ (sat., 10 mL) and extracted with $CH_2Cl_2$ (3 × 15 mL). The combined

organic layers were washed with aq. $NH_4Cl$ (sat., 8 mL) and brine (8 mL). The organic layer was then dried ($Na_2SO_4$), concentrated, and purified by flash column chromatography (silica gel; MeOH/$CH_2Cl_2$, 0:100 to 0.5:9.5) to afford **11** (190 mg, 0.231 mmol, 70%) as a pale yellow foam. $R_f$ = 0.4 (MeOH/$CH_2Cl_2$, 1:9).

$^1$H NMR (500 MHz, $CDCl_3$): δ 8.39 (d, *J* = 8.6 Hz, 1H), 8.05 (s, 1H), 8.02 (s, 1H), 6.35 (br s, 2H), 6.22 (br s, 1H), 5.82 (d, *J* = 8.4 Hz, 1H), 5.34 (dd, *J* = 8.6, 2.9 Hz, 1H), 4.82 (q, *J* = 7.6 Hz, 1H), 4.64 – 4.62 (m, 1H), 4.33 (q, *J* = 7.1 Hz, 2H), 3.24 – 3.19 (m, 2H), 2.91 (s, 2H), 2.52 (s, 3H), 2.45 (s, 3H), 2.15 (s, 1H), 2.05 (s, 3H), 2.03 – 1.96 (m, 1H), 1.84 – 1.79 (m, 1H), 1.65 – 1.56 (m, 2H), 1.43 – 1.39 (m, 15H), 1.33 (t, *J* = 7.1 Hz, 3H), 1.27 (d, *J* = 6.3 Hz, 3H).

$^{13}$C NMR (126 MHz, $CDCl_3$): δ 177.6, 171.2, 161.6, 161.4, 158.8, 156.4, 155.7, 148.9, 146.6, 138.4, 133.0, 132.3, 128.1, 124.7, 124.3, 117.6, 86.5, 80.4, 68.7, 61.7, 56.2, 52.7, 43.3, 40.7, 35.7, 32.2, 28.7, 28.5, 25.7, 19.9, 19.4, 18.1, 14.4, 12.6.

HRMS-ESI: *m/z* $[M+H]^+$ for $C_{36}H_{52}N_7O_9S_3$, calculated 822.2989; observed 822.2980.



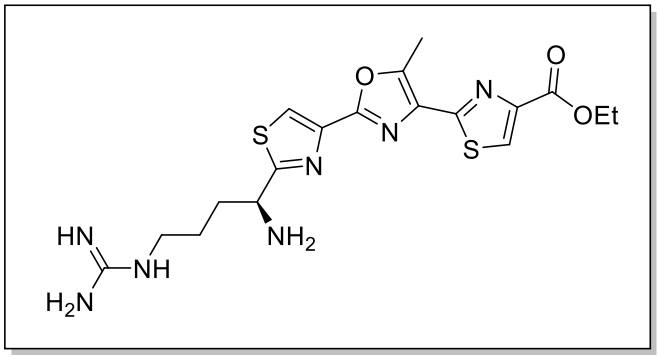

**Boc-Arg(Pbf)-Oxz(Me)-Thz-OEt (12):** To a stirred solution of **11** (190 mg, 0.231 mmol) in $CH_2Cl_2$ (4 mL) at -20 °C, Deoxo-fluor (76 µL, *d* = 1.2 g/mL at 25 °C, 0.41 mmol) was slowly added. After 3 h of stirring at rt, the reaction mixture was quenched by addition of aq. $NaHCO_3$ (sat., 10 ml) at -20 °C and extracted with $CH_2Cl_2$ (3 × 10 mL). The combined organic layers were washed with brine (5 mL), dried ($Na_2SO_4$), and concentrated to give the crude oxazoline, which was used in the next step without further purification. The above residue was dissolved in $CH_2Cl_2$ (4 mL) and the resulting solution was cooled to -10 °C. DBU (90 µL, *d* = 1.018 g/mL at 25 °C, 0.60 mmol) and $BrCCl_3$ (60 µL, *d* = 2.012 g/mL at 25 °C, 0.61 mmol) were added slowly and sequentially. The reaction mixture was stirred for 10 min at this temperature before being allowed to warm to rt and stir for an additional 48 h. The reaction was then quenched by addition of aq. $NH_4Cl$ (sat., 10 mL) at 0 °C and extracted with $CH_2Cl_2$ (3 × 15 mL). The combined organic layers were washed with aq. $NaHCO_3$ (sat., 10 mL) and brine (10 mL), dried ($Na_2SO_4$), concentrated, and subjected to flash column chromatography (silica gel; MeOH/$CH_2Cl_2$, 0:100 to 0.5:9.5) to afford **12** (130 mg, 0.162 mmol, 70%) as a yellow foam.

$R_f$ = 0.4 (MeOH/$CH_2Cl_2$, 1:9).

$^1$H NMR (500 MHz, $CDCl_3$): δ 8.11 (s, 1H), 7.85 (s, 1H), 6.46 (br s, 3H), 5.83 (d, *J* = 7.9 Hz, 1H), 4.95 (br s, 1H), 4.41 (q, *J* = 7.1 Hz, 2H), 3.32 – 3.20 (m, 2H), 2.91 (s, 2H), 2.80 (s, 3H), 2.55 (s, 3H), 2.47 (s, 3H), 2.22 (br s, 1H), 2.05 (s, 3H), 1.96 – 1.89 (m, 1H), 1.71 – 1.63 (m, 2H), 1.43 – 1.39 (m, 18H).

$^{13}$C NMR (126 MHz, $CDCl_3$): δ 161.4, 161.2, 158.7, 156.4, 155.6, 148.5, 148.0, 142.6, 138.4, 133.1, 132.3, 130.7, 127.0, 124.6, 120.5, 117.5, 86.5, 80.4, 61.6, 53.3, 43.3, 40.8, 32.5, 28.7, 28.4, 25.7, 19.4, 18.1, 14.4, 12.6, 12.2.

HRMS-ESI: *m/z* $[M+H]^+$ for $C_{36}H_{48}N_7O_8S_3$, calculated 802.2727; observed 802.2733.



**H-Arg-Thz-Oxz(Me)-Thz-OEt (3): 12** (50 mg, 0.062 mmol) was stirred in a mixture of TFA/TIPS/$H_2O$ (94:3:3, 2 mL) at 0 °C for 3 h. Upon completion of the deprotection (monitored by TLC and ESI-MS), the reaction mixture was diluted with toluene (10 mL) and concentrated. The obtained residue was washed with $Et_2O$ (2 × 5 mL), dissolved in water (1 mL), and lyophilized to afford **3** (26 mg, 0.058 mmol, 94%) as a colorless solid.

mp = 158-160 °C

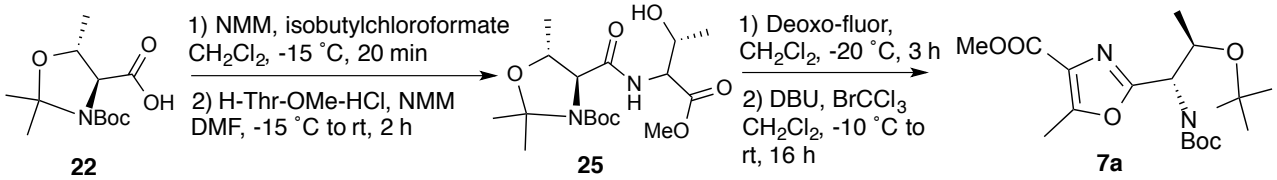
$^1$H NMR (500 MHz, $D_2O$): δ 8.43 (s, 1H), 8.31 (s, 1H), 4.95 (dd, *J* = 8.3, 5.9 Hz, 1H), 4.39 (q, *J* = 7.1 Hz, 2H), 3.23 (t, *J* = 6.8 Hz, 2H), 2.73 (s, 3H), 2.33 – 2.16 (m, 2H), 1.79 – 1.68 (m, 1H), 1.69 – 1.57 (m, 1H), 1.38 (t, *J* = 7.2 Hz, 3H).

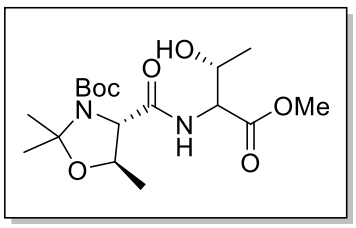
$^{13}$C NMR (126 MHz, $D_2O$): δ 166.9, 162.5, 161.1, 156.9, 155.3, 149.4, 146.5, 141.7, 129.4, 128.9, 123.7, 62.8, 51.7, 40.4, 30.7, 24.2, 13.5, 11.5.

HRMS-ESI: *m/z* $[M+H]^+$ for $C_{18}H_{24}N_7O_3S_2$, calculated 450.1382; observed 450.1377.

## VI. Synthesis of Boc-Oxazolidine(Me)$_3$-Oxz(Me)-OMe (7a)

**Scheme S3**



**Boc-Oxazolidine(Me)$_3$-Thr-OMe (25):** Boc-Oxazolidine(Me)$_3$-COOH (**22**, 3.7 g, 14 mmol) was dissolved in $CH_2Cl_2$ (20 mL) and cooled to -15 °C. *N*-methylmorpholine (NMM, 1.9 mL, *d* = 0.92

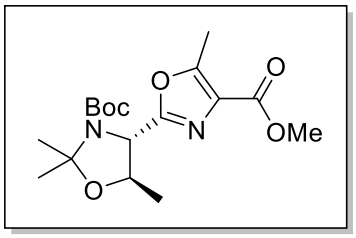
g/mL at 25 °C, 17 mmol) and isobutyl chloroformate (2.3 mL, *d* = 1.053 g/mL at 25 °C, 18 mmol) were sequentially added. The reaction mixture was stirred at this temperature for 20 min. Then, a solution of H-Thr-OMe·HCl (3.6 g, 21 mmol) and NMM (2.3 mL, 21 mmol) in 15 mL of anhydrous DMF was added, and the reaction mixture was allowed to warm to rt. After stirring for 2 h, the mixture was diluted with $H_2O$ (40 mL) and washed with $CH_2Cl_2$ (3 × 30 mL). The combined organic layers were washed sequentially with cold 2 N $KHSO_4$ (10 mL), water (20 mL), and brine (20 mL), and then dried ($Na_2SO_4$) and concentrated. The obtained residue was subjected to flash column chromatography (silica gel; EtOAc/hexanes, 1:9 to 6:4) to afford **25** (4.2 g, 11 mmol, 79%) as a pale yellow foam.

$R_f$ = 0.4 (EtOAc/hexanes, 4:6).

$^1$H NMR (500 MHz, $CDCl_3$): δ 6.90 (br s, 1H), 4.55 (br s, 1H), 4.23 (br s, 2H), 3.83 (d, *J* = 7.6 Hz, 1H), 3.71 (s, 3H), 1.55 (d, *J* = 10.2 Hz, 6H), 1.38 (d, *J* = 17.4 Hz, 12H), 1.19 (d, *J* = 6.4 Hz, 3H).

$^{13}$C NMR (126 MHz, $CDCl_3$): δ 171.2, 169.8, 152.4, 94.8, 81.3, 73.9, 68.9, 67.6, 57.7, 52.5, 28.3, 27.7, 25.1, 20.1, 18.5.

HRMS-ESI: m/z $[M+H]^+$ for $C_{17}H_{31}N_2O_7$, calculated 375.2131; observed 375.2130.




**Boc-Oxazolidine(Me)$_3$-Oxz(Me)-OMe (7a):** To a stirred solution of **25** (2.1 g, 5.6 mmol) in $CH_2Cl_2$ (20 mL) at -20 °C was slowly added Deoxo-fluor (1.7 mL, *d* = 1.2 g/mL at 25 °C, 9.2 mmol). After stirring at this temperature for 3 h, the reaction mixture was quenched by the addition of aq. $NaHCO_3$ (sat., 10 mL) at -20 °C and extracted with $CH_2Cl_2$ (3 × 20 mL). The combined organic layers were washed with brine (10 mL), dried ($Na_2SO_4$), and concentrated to give the crude oxazoline, which was used in the next step without further purification. The above residue was dissolved in $CH_2Cl_2$ (20 mL) and the resulting solution cooled to -10 °C before DBU (1.7 mL, *d* = 1.018 g/mL at 25 °C, 11 mmol) and $BrCCl_3$ (1.1 mL, 2.012 g/mL at 25 °C, 11 mmol) were added slowly and sequentially. After stirring at this temperature for 10 min, the reaction mixture was allowed to warm to rt and stirred for an additional 16 h. The reaction was quenched by the addition of saturated aqueous $NH_4Cl$ (10 mL) at 0 °C and extracted with $CH_2Cl_2$ (3 × 20 mL).The combined organic layers were washed with aq. $NaHCO_3$ (sat., 10 mL) and brine (10 mL), dried ($Na_2SO_4$), concentrated, and purified by flash column chromatography (silica gel; EtOAc/hexanes, 1:9 to 6:4) to afford **7a** (1.4 g, 4.0 mmol 71%) as a pale yellow foam.

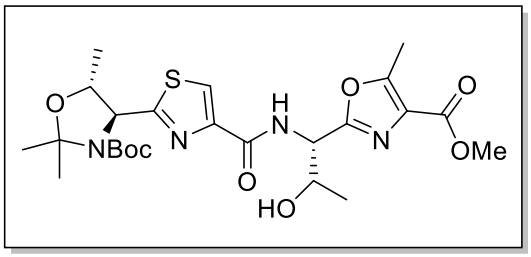
$R_f$ = 0.5 (EtOAc/hexanes, 4:6).

$^1$H NMR (500 MHz, $CDCl_3$): (mixture of rotamers) δ 4.42 (d, *J* = 7.8 Hz, 0.22H), 4.36 (d, *J* = 8.1 Hz, 0.74H), 4.18 – 4.10 (m, 1H), 3.81 (s, 2.2H), 3.77 (s, 0.8H), 2.54 (s, 2.1H), 2.50 (s, 0.8H), 1.57 (s, 5H), 1.53 (s, 1H), 1.34 (s, 3H), 1.26 (d, *J* = 6.0 Hz, 3H), 1.10 (s, 6H).

$^{13}$C NMR (126 MHz, $CDCl_3$): (mixture of rotamers) δ 162.6, 159.9, 156.4, 151.0, 127.6, 95.1, 94.6, 80.9, 80.3, 74.7, 74.5, 61.7, 52.1, 30.9, 28.3, 28.2, 28.1, 27.8, 26.4, 25.4, 24.5, 17.8, 14.4, 12.0.

HRMS-ESI: *m/z* $[M+H]^+$ for $C_{17}H_{27}N_2O_6$, calculated 355.1869; observed 355.1866.

## VII. Synthesis of H-Arg-Thz-Oxz(Me)-Thz-Oxz(Me)-Oxz(Me)-OMe (4)




**Boc-Oxazolidine(Me)$_3$-Thz-Thr-Oxz(Me)-OMe (13):** To a stirred solution of **7a** (330 mg, 0.931 mmol) in 1,4-dioxane (2 mL), HCl (2 mL, 4M in 1,4-dioxane, 8 mmol) was added at 0 °C. The solution was allowed to warm to rt and stir for 3 h before being diluted with toluene (10 mL) and concentrated. The residual HCl was removed by repeated co-evaporation with $CH_2Cl_2$ to give the crude oxazole hydrochloride salt (**7b**) as a yellow oil which was used in the next step without further purification.

Compound **6a** (320 mg, 0.93 mmol) was dissolved in MeOH/THF (2 mL). The solution cooled to 0 °C and aq. LiOH (1.4 N, 1 mL, 1.4 mmol) was added. The above mixture was allowed to warm to rt and stir for 10 h. The reaction mixture was then concentrated and the obtained residue was diluted with water (3 mL) and washed with $Et_2O$ (3 mL). The aqueous layer was cooled to 0 °C and acidified with 1 N $KHSO_4$ to pH = 3. The resulting suspension was extracted with EtOAc (3 × 10 mL), and the combined organic layers were dried ($Na_2SO_4$) and concentrated to afford the crude acid **6c** (280 mg), which was used as such without further purification.

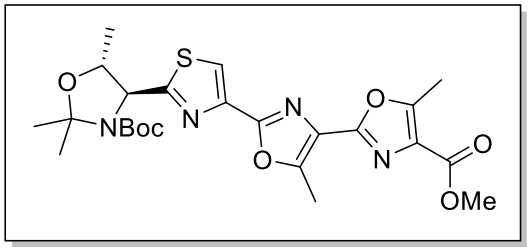
**6c** was dissolved in $CH_2Cl_2$ (4 mL) before HCTU (500 mg, 1.21 mmol) and HOBt (160 mg, 1.18 mmol) were sequentially added. After stirring the above mixture for 10 min, a solution of **7b** in $CH_2Cl_2$ (4 mL) and DIEA (0.6 mL, *d* = 0.742 g/mL at 25 °C, 3.4 mmol) were sequentially added. The reaction mixture was stirred for 14 h, quenched by the addition of aq. $NaHCO_3$ (sat., 15 mL), and extracted with $CH_2Cl_2$ (3 × 15 mL). The combined organic layer was washed with aq. $NH_4Cl$ (sat., 8 mL) and brine (8 mL). The organic layer was then dried ($Na_2SO_4$) and concentrated. The crude residue was purified by flash column chromatography (silica gel; EtOAc/hexanes, 2:8 to 9:1) to afford **13** as a colorless foam (350 mg, 0.650 mmol, 70%).

$R_f$ = 0.4 (EtOAc/hexanes, 6:4).

$^1$H NMR (500 MHz, $CDCl_3$): δ 8.09 (br s, 1H), 8.02 (d, *J* = 8.2 Hz, 1H), 5.24 – 5.21 (m, 1H), 4.68 – 4.58 (m, 1H), 4.49 (br s, 1H), 4.16 – 4.11 (m, 1H), 3.82 (s, 3H), 2.73 (s, 15H), 2.53 (s, 3H), 1.40 –1.35 (m, 3H), 1.20 – 1.19 (m, 4H).

$^{13}$C NMR (126 MHz, $CDCl_3$): δ 165.7, 162.5, 161.2, 160.7, 156.7, 151.2, 148.6, 127.3, 124.1, 95.2, 80.6, 67.5, 65.8, 52.0, 38.6, 28.0, 26.5, 25.6, 19.2, 17.8, 12.1.

HRMS-ESI: *m/z* $[M+H]^+$ for $C_{24}H_{35}N_4O_8S$, calculated 539.2176; observed 539.2180.



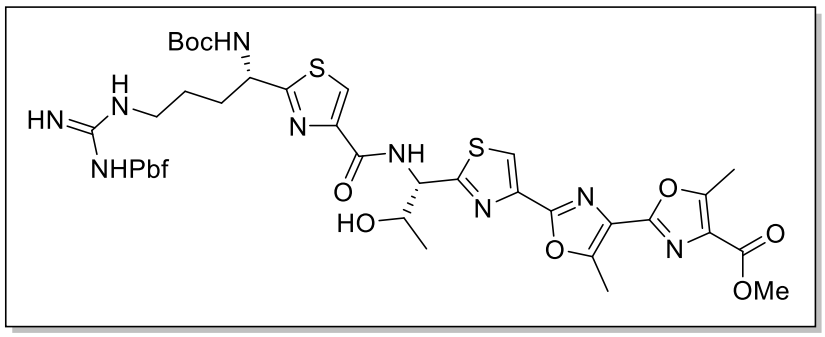

**Boc-Oxazolidine($Me_3$)-Thz-Oxz(Me)-Oxz(Me)-OMe (14, Scheme 3):** To a stirred solution of **13** (350 mg, 0.650 mmol) in $CH_2Cl_2$ (5 mL) at -20 °C, Deoxo-fluor (0.22 mL, $d$ = 1.2 g/mL at 25 °C, 1.2 mmol) was slowly added. After stirring at the same temperature for 3 h, the reaction mixture was quenched by the addition of aq. $NaHCO_3$ (sat., 5 mL) at -20 °C and extracted with $CH_2Cl_2$ (3 × 15 mL). The combined organic layers were washed with brine (8 mL), dried ($Na_2SO_4$), and concentrated to give the crude oxazoline, which was used in the next step without further purification. The crude material was dissolved in $CH_2Cl_2$ (5 mL) and cooled to -10 °C before DBU (0.5 mL, $d$ = 1.018 g/mL at 25 °C, 3 mmol) and $BrCCl_3$ (0.32 mL, $d$ = 2.012 g/mL at 25 °C, 3.2 mmol) were added slowly and sequentially. After stirring at the same temperature for 10 min, the reaction mixture was allowed to warm to rt and stir for an additional 48 h. The reaction was quenched by the addition of aq. $NH_4Cl$ (sat., 5 mL) at 0 °C and extracted with $CH_2Cl_2$ (3 × 15 mL). The combined organic layers were washed with aq. $NaHCO_3$ (sat., 5 mL) and brine (5 mL), dried ($Na_2SO_4$), and concentrated. The obtained residue was subjected to flash column chromatography (silica gel; EtOAc/hexanes, 2:8 to 9:1) to afford **14** (220 mg, 0.424 mmol, 65% (including some residual EtOAc)) as a pale yellow foam.

$R_f$ = 0.5 (EtOAc/hexanes, 6:4).

$^1$H NMR (500 MHz, $CDCl_3$): δ 8.03 (s, 1H), 4.78 (br s, 1H), 4.19 (br s, 1H), 3.89 (s, 3H), 2.75 (s, 3H), 2.67 (s, 3H), 1.67 (s, 6H), 1.40 (d, $J$ = 5.7 Hz, 6H), 1.15 (s, 6H).

$^{13}$C NMR (126 MHz, $CDCl_3$): δ 162.7, 156.1, 153.9, 150.8, 142.6, 128.5, 125.8, 120.3, 95.3, 92.4, 80.9, 77.9, 66.1, 52.0, 28.2, 26.6, 25.9, 17.99, 17.98, 12.2, 12.0.

HRMS-ESI: m/z $[M+H]^+$ for $C_{24}H_{31}N_4O_7S$, calculated 519.1913; observed 519.1907.




**Boc-Arg(Pbf)- Thz-Thr-Thz-Oxz(Me)-Oxz(Me)-OMe (15, Scheme 3):** To a stirred solution of **14** (70 mg, 0.13 mmol) in 1,4-dioxane (1.5 mL), HCl (1.5 mL, 4M in 1,4-dioxane, 6 mmol) was added at 0 °C and the solution was allowed to warm to rt. After stirring for 3 h, the above mixture was diluted with toluene (10 mL) and concentrated. The residual HCl was removed by repeated co-evaporation with $CH_2Cl_2$ to give the crude hydrochloride salt of the tetrazole amine (**14a**) as a yellow oil which was used in the next step without further purification.

Compound **5b** (100 mg, 0.15 mmol) was dissolved in MeOH/THF (2mL); the solution was cooled to 0 °C and aq. LiOH (0.23 N, 1 mL, 0.23 mmol) was added. The above mixture was

allowed to warm to rt and stir for 10 h. The reaction mixture was then concentrated and the obtained residue was redissolved in water (3 mL) and washed with $Et_2O$ (3 mL). The aqueous layer was cooled to 0 °C and acidified with 1 N $KHSO_4$ to pH = 3. The resulting suspension was extracted with EtOAc (3 × 10 mL), and the combined organic layers were dried ($Na_2SO_4$) and concentrated to afford the crude acid **5c** (90 mg), which was used without further purification.

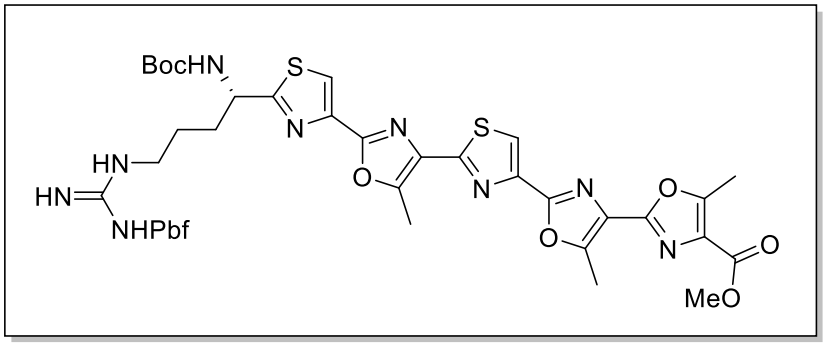
Compound **5c** was dissolved in $CH_2Cl_2$ (3 mL), and HCTU (100 mg, 0.241 mmol) and HOBt (33 mg, 0.24 mmol) were sequentially added. After stirring the above mixture for 10 min, a solution of **14a** in $CH_2Cl_2$ (2 mL) and DIEA (88 µL, $d$ = 0.742 g/mL at 25 °C, 0.5 mmol) were sequentially added. The reaction mixture was stirred for 14 h before being quenched by addition of aq. $NaHCO_3$ (sat., 8 mL) and extracted with $CH_2Cl_2$ (3 × 15 mL). The combined organic layers were washed with aq. $NH_4Cl$ (sat., 10 mL) and brine (10 mL), dried ($Na_2SO_4$), concentrated, and purified by flash column chromatography (silica gel; MeOH/$CH_2Cl_2$, 0:100 to 0.5:9.5) to afford **15** (96 mg, 0.10 mmol, 77%) as a colorless foam.

$R_f$ = 0.4 (MeOH/$CH_2Cl_2$, 0.5:9.5).

$^1$H NMR (500 MHz, $CDCl_3$): δ 8.43 (d, $J$ = 7.1 Hz, 1H), 8.02 (s, 1H), 7.94 (s, 1H), 6.45 (br s, 2H), 6.27 (br s, 1H), 5.68 (br s, 1H), 5.40 (d, $J$ = 8.2 Hz, 1H), 4.87 (br s, 1H), 4.69 (br s, 1H), 4.58 (brs, 1H), 3.90 (s, 3H), 3.26 (br s, 2H), 2.89 (s, 2H), 2.72 (s, 3H), 2.67 (s, 3H), 2.50 (s, 3H), 2.42 (s, 3H), 2.35 – 2.26 (m, 1H), 2.11 – 2.07 (m, 1H), 2.03 (br s, 3H), 1.81 (br s, 1H), 1.61 (br s, 1H), 1.44 – 1.36 (m, 15H), 1.31 (d, $J$ = 6.0 Hz, 3H).

$^{13}$C NMR (126 MHz, $CDCl_3$): δ 172.1, 162.7, 161.6, 158.7, 156.5, 156.2, 156.0, 155.6, 153.9, 150.9, 149.0, 142.2, 138.3, 133.1, 132.3, 128.4, 125.5, 124.6, 124.2, 121.3, 117.5, 86.4, 80.4, 68.9, 56.1, 55.3, 52.5, 52.1, 43.3, 32.0, 28.7, 28.4, 25.8, 20.0, 19.4, 18.1, 12.6, 12.3, 12.0.

HRMS-ESI: $m/z$ $[M+H]^+$ for $C_{43}H_{56}N_9O_{11}S_3$, calculated 970.3261; observed 970.3232.




**Boc-Arg(Pbf)-Thz-Oxz(Me)-Thz-Oxz(Me)-Oxz(Me)-OMe (16, Scheme 3):**

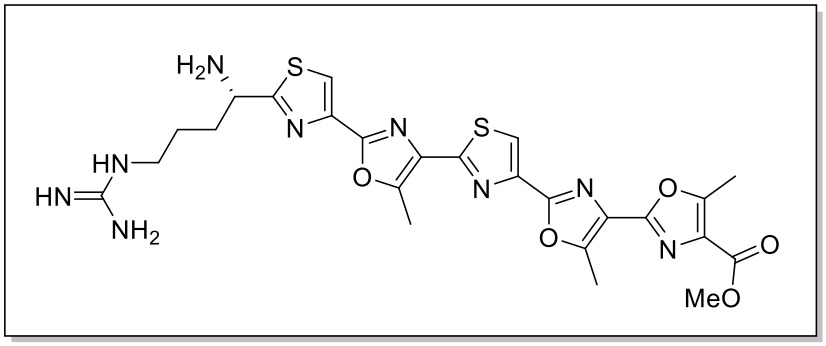
To a stirred solution of **15** (96 mg, 0.099 mmol) in $CH_2Cl_2$ (2 mL) at -20 °C, Deoxo-fluor (35 µL, $d$ = 1.2 g/mL at 25 °C, 0.19 mmol) was slowly added. After stirring at the same temperature for 3 h, the reaction mixture was quenched by the addition of aq. $NaHCO_3$ (sat., 5 mL) at -20 °C and extracted with $CH_2Cl_2$ (3 × 10 mL). The combined organic layers were washed with brine (5 mL), dried ($Na_2SO_4$), and concentrated to give the crude oxazoline, which was used in the next step without further purification. The crude material was dissolved in $CH_2Cl_2$ (2 mL), and cooled to -10 °C before DBU (80 µL, $d$ = 1.018 g/mL at 25 °C, 0.53 mmol) and $BrCCl_3$ (50 µL, $d$ = 2.012 g/mL at 25 °C, 0.51 mmol) were added slowly and sequentially. After stirring the reaction mixture at the same temperature for 10 min, it was allowed to warm to rt and further stirred for 48 h. The reaction was quenched by the addition of aq. $NH_4Cl$ (sat., 5 mL) at 0 °C and

extracted with $CH_2Cl_2$ (3 × 10 mL).The combined organic layers were washed with aq. $NaHCO_3$ (sat., 5 mL) and brine (5 mL), dried ($Na_2SO_4$), concentrated, and subjected to flash column chromatography (silica gel; MeOH/$CH_2Cl_2$, 0:100 to 0.5:9.5) to afford **15** (40 mg, 0.042 mmol, 42%) as a pale yellow foam.
$R_f$ = 0.4 (MeOH/$CH_2Cl_2$, 0.5:9.5).

$^1$H NMR (500 MHz, $CDCl_3$): δ 8.06 (s, 1H), 7.88 (s, 1H), 6.45-6.43 (br s, 3H), 5.62 (d, *J* = 7.8 Hz, 1H), 5.00 (br s, 1H), 3.93 (s, 3H), 3.40 – 3.22 (m, 2H), 2.93 (s, 2H), 2.88 (s, 3H), 2.82 (s, 3H), 2.73 (s, 3H), 2.57 (s, 3H), 2.50 (s, 3H), 2.13 (br s, 1H), 2.07 (3H, s), 1.98 (br s, 1H), 1.71 (br s, 2H), 1.44 (s, 15H).

$^{13}$C NMR (126 MHz, $CDCl_3$): δ 162.9, 158.8, 158.7, 156.32, 156.28, 156.1, 155.6, 154.1, 151.0, 148.6, 145.1, 143.9, 142.6, 138.5, 132.5, 131.0, 128.9, 128.5, 125.9, 124.6, 120.3, 117.5, 109.9, 86.5, 67.3, 52.1, 43.4, 40.8, 28.7, 28.5, 25.3, 19.4, 18.1, 12.6, 12.34, 12.28, 12.2.

HRMS-ESI: *m/z* $[M+H]^+$ for $C_{43}H_{52}N_9O_{10}S_3$, calculated 950.2999; observed 950.3001.




**H-Arg-Thz-Oxz(Me) -Thz-Oxz(Me)-Oxz(Me)-OMe (4, Scheme 3): 15** (40 mg, 0.042 mmol) was stirred in a mixture of TFA/TIPS/$H_2O$ (94:3:3, 2 mL) at 0 °C for 3 h. Upon completion of the deprotection (monitored by TLC and ESI-MS), the reaction mixture was diluted with toluene (10 mL) and concentrated. The obtained residue was washed with $Et_2O$ (2 × 3 mL), dissolved in water (1 mL), and lyophilized to afford **4** (23 mg, 0.039 mmol, 93%) as a pale yellow solid.

mp = 210-212 °C.

$^1$H NMR (500 MHz, DMSO-$d_6$): δ 8.50 (s, 1H), 8.44 (s, 1H), 7.75 (br s, 1H), 7.47 – 6.86 (br s, 3H), 4.55 (br s, 1H), 3.84 (s, 3H), 3.19 – 3.12 (m, 4H), 2.86 (s, 3H), 2.76 (s, 3H), 2.68 (s, 3H), 2.03 – 1.93 (br s, 1H), 1.89 – 1.78 (br s, 1H), 1.65 – 1.50 (br s, 2H).

$^{13}$C NMR (126 MHz, DMSO-$d_6$): δ 162.0, 161.6, 156.7, 156.0, 155.3, 155.2, 153.2, 150.8, 147.9, 142.9, 141.6, 130.0, 127.7, 125.0, 122.9, 121.8, 52.0, 51.8, 40.3, 24.8, 11.9, 11.8, 11.6.

HRMS-ESI: *m/z* $[M+H]^+$ for $C_{25}H_{28}N_9O_5S_2$, calculated 598.1655; observed 598.1676.

## VIII. Chemical biology of PZN analogues

**MBP-BamL overexpression and purification.** Wild-type MBP-BamL was transformed into chemically competent BL21(DE3)RIPL cells as previously described.[7] Transformants were selected with kanamycin (50 μg/mL) and chloramphenicol (34 μg/mL) on LB agar plates. 10 mL cultures were grown overnight and used to inoculate at a 1:1000 dilution into LB with the appropriate antibiotics. Cultures were grown at 37 ˚C until mid-log phase ($OD_{600}$ ~0.5) before addition of IPTG (isopropyl-β-D-thiogalactopyranoside, 0.4 mM final concentration) to induce protein expression. After shaking for an additional 18 h at 22 ˚C, cells were harvested by centrifugation. Cell pellets were resuspended in lysis buffer [50 mM Tris pH 7.5, 500 mM NaCl, 2.5% glycerol (v/v)] with the protease inhibitors phenylmethanesulfonyl fluoride, benzamidine, leupeptin, and E64. Cells were lysed by sonication (3 × 30 s, continuous mode, <20 W, 4 ˚C), followed by centrifugation at 40,000 × *g* for 1 h at 4 ˚C. Amylose resin columns were pre-equilibrated with 5 column volumes of lysis buffer before the cleared lysates were applied. Columns were washed with 20 column volumes of lysis buffer before the protein was eluted with elution buffer [50 mM Tris pH 7.5, 150 mM NaCl, 10 mM maltose, 2.5% glycerol (v/v)]. The eluates were concentrated using Amicon centrifugal filters (50 kDa molecular weight cut-off, Millipore) in storage buffer [50 mM Tris pH 7.5, 150 mM NaCl, 2.5% glycerol (v/v)]. Protein purity was analyzed by Coomassie-stained SDS-PAGE, and concentration was quantified by absorbance at 280 nm and Bradford analysis (Thermo Scientific). Protein was stored at -80 ˚C until use.

**Methyltransferase assays.** Overnight endpoint assays (Condition A) were run with 20 μM MBP-BamL, 10 μM Pfs SAH nucleosidase, 200 μM substrate, 3 mM SAM, and 50 mM Tris (pH 7.5) for 16 h at 37 ˚C. To increase the solubility of **4**, buffer solution with a 2.5% concentration (v/v) of DMSO was used. A more stringent test of substrate acceptance used the following conditions (Condition B): 1 μM MBP-BamL, 1 μM Pfs SAH nucleosidase, 5 μM substrate, 3 mM SAM, and 50 mM Tris (pH 7.5) for 1 h at 22 ˚C. Protein was precipitated and samples were analyzed by positive mode ESI-MS.

**LC-MS analysis of methyltransferase assays**. Methyltransferase assays for compounds **2**, **3**, and **4** were performed using the 1 h condition (condition B) described above. The supernatant from the protein precipitation was dried *in vacuo* and redissolved in 5% MeOH (LC-MS grade, Sigma) before injection onto an Agilent 1200 Series HPLC outfitted with a single quadrupole mass analyzer (G1956B). LC used a Thermo BETASIL $C_{18}$ column and a flow rate of 0.85 mL/min. The methyltransferase assays on compound **2** were injected onto a gradient of 10 – 75% MeOH (0.1% formic acid v/v) over 30 min. The methyltransferase assays on compound **3** were injected onto a gradient of 40 – 60% MeOH (0.1% formic acid v/v) over 50 min. The methyltransferase assays on compound **4** were injected onto a gradient of 5 – 95% MeOH (0.1% formic acid v/v) over 30 min.

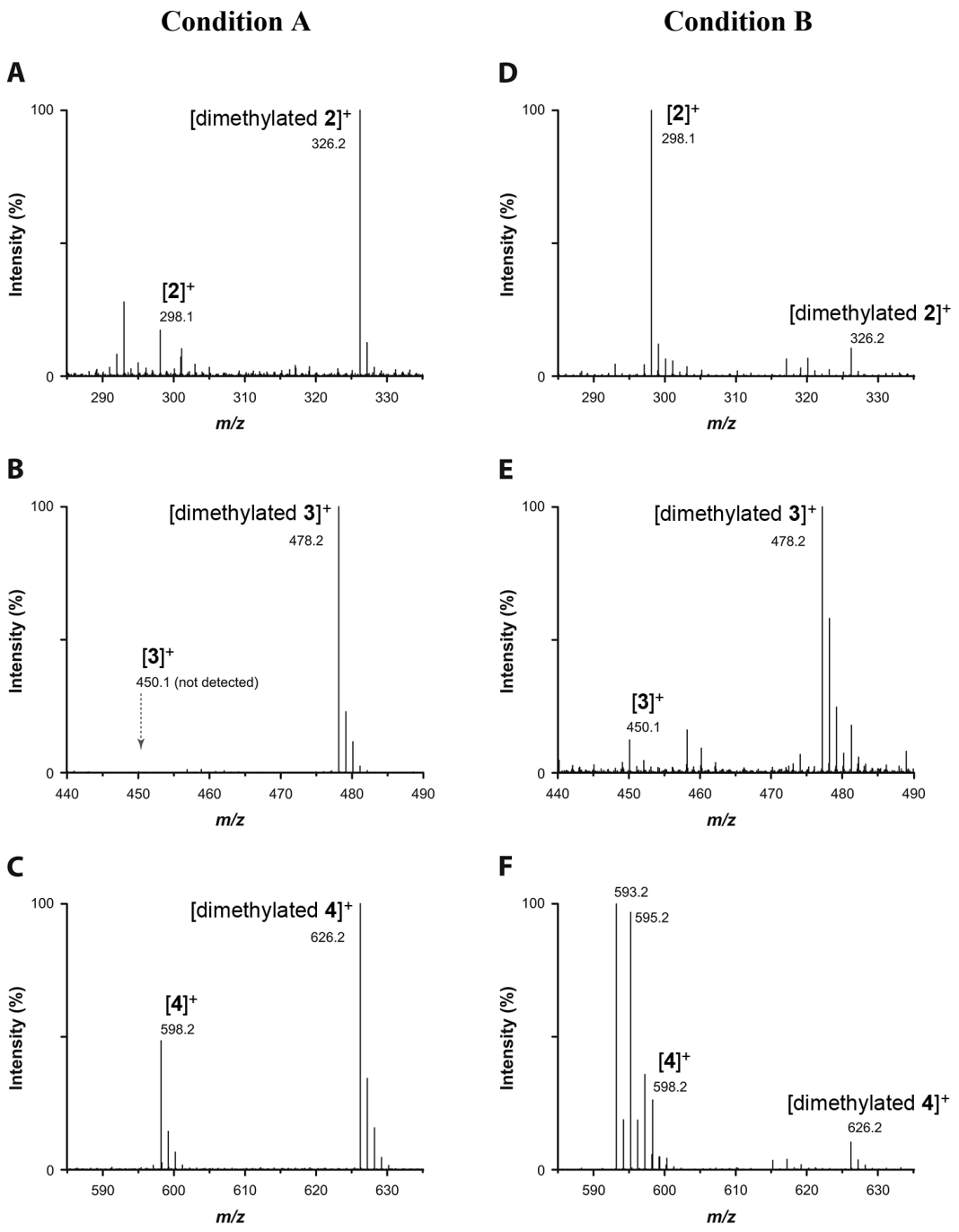



**Figure S1**. ESI-MS of methyltransferase assays. (a-c) Overnight assays (Condition A) of the monoazole **2** (a), triazole **3** (b), and pentazole **4** (c) compounds with SAM, MBP-BamL and Pfs (nucleosidase) confirmed the three compounds were substrates of MBP-BamL. (d-f) More stringent assays (Condition B) with the same compounds show decreased efficiency of processing for all compounds, as evidenced by increased relative peak intensities of the unmethylated substrates.

The dimethylated products obtained from enzymatic assays were further confirmed by ESI-HRMS (Table S2):

**Table S2.**

| Substrate | Dimethylated Formula | Calculated $[M+H]^+$ | Observed $[M+H]^+$ |
|---|---|---|---|
| **2** | $C_{14}H_{23}N_5O_2S$ | 326.1651 | 326.1652 |
| **3** | $C_{20}N_{27}N_7O_3S_2$ | 478.1695 | 478.1703 |
| **4** | $C_{27}H_{31}N_9O_5S_2$ | 626.1968 | 626.1971 |

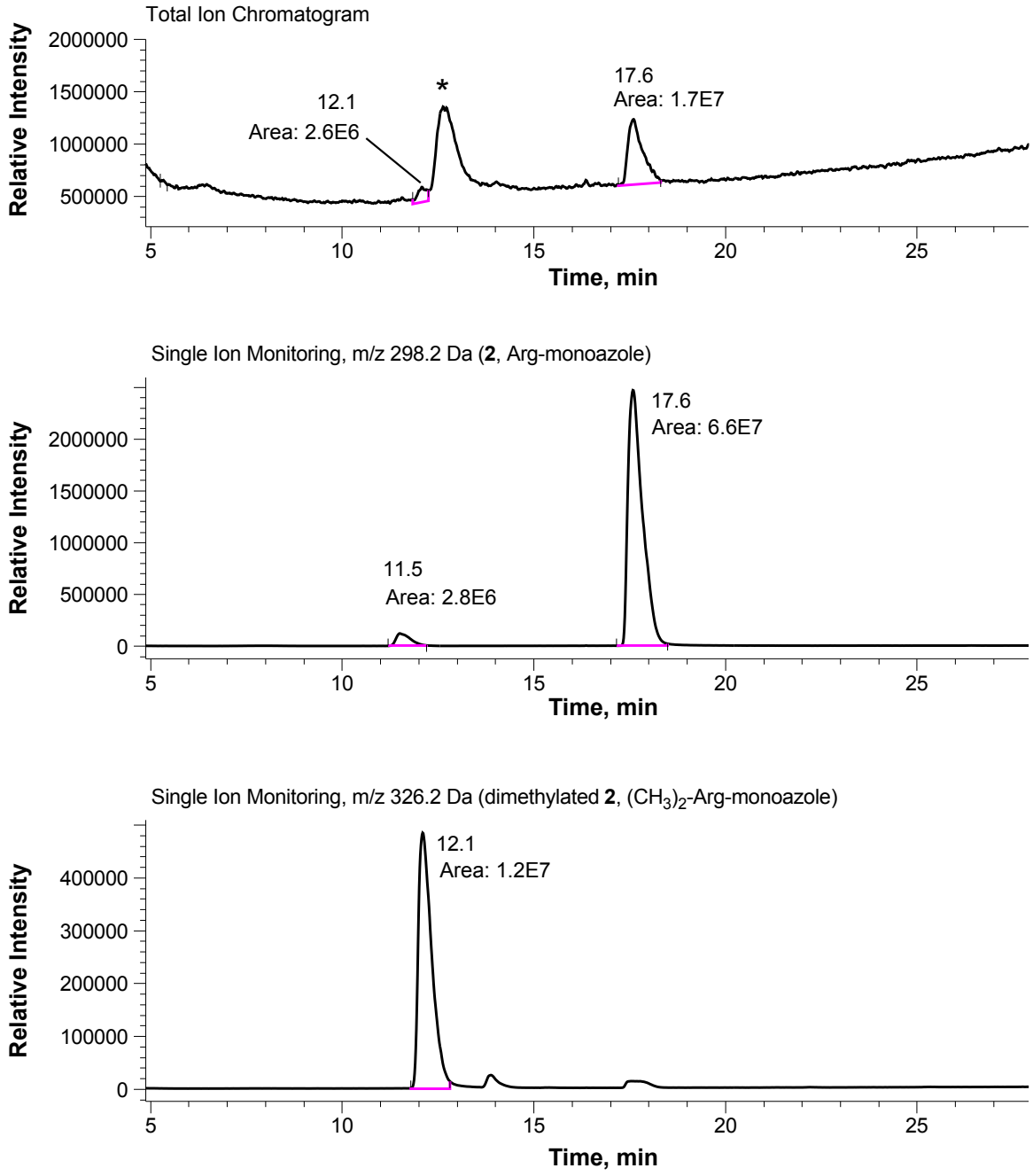



Figure S2. LC-MS analysis of 1 h methyltransferase assay with MBP-BamL and compound **2**. Top panel: Total ion chromatogram (100 Da – 1000 Da). Middle panel: Single ion monitoring for the substrate **2** (*m/z* 298.2). Bottom panel: Single ion monitoring for the product of the methyltransferase reaction, dimethylated **2** (*m/z* 326.2). "*" denotes buffer component.

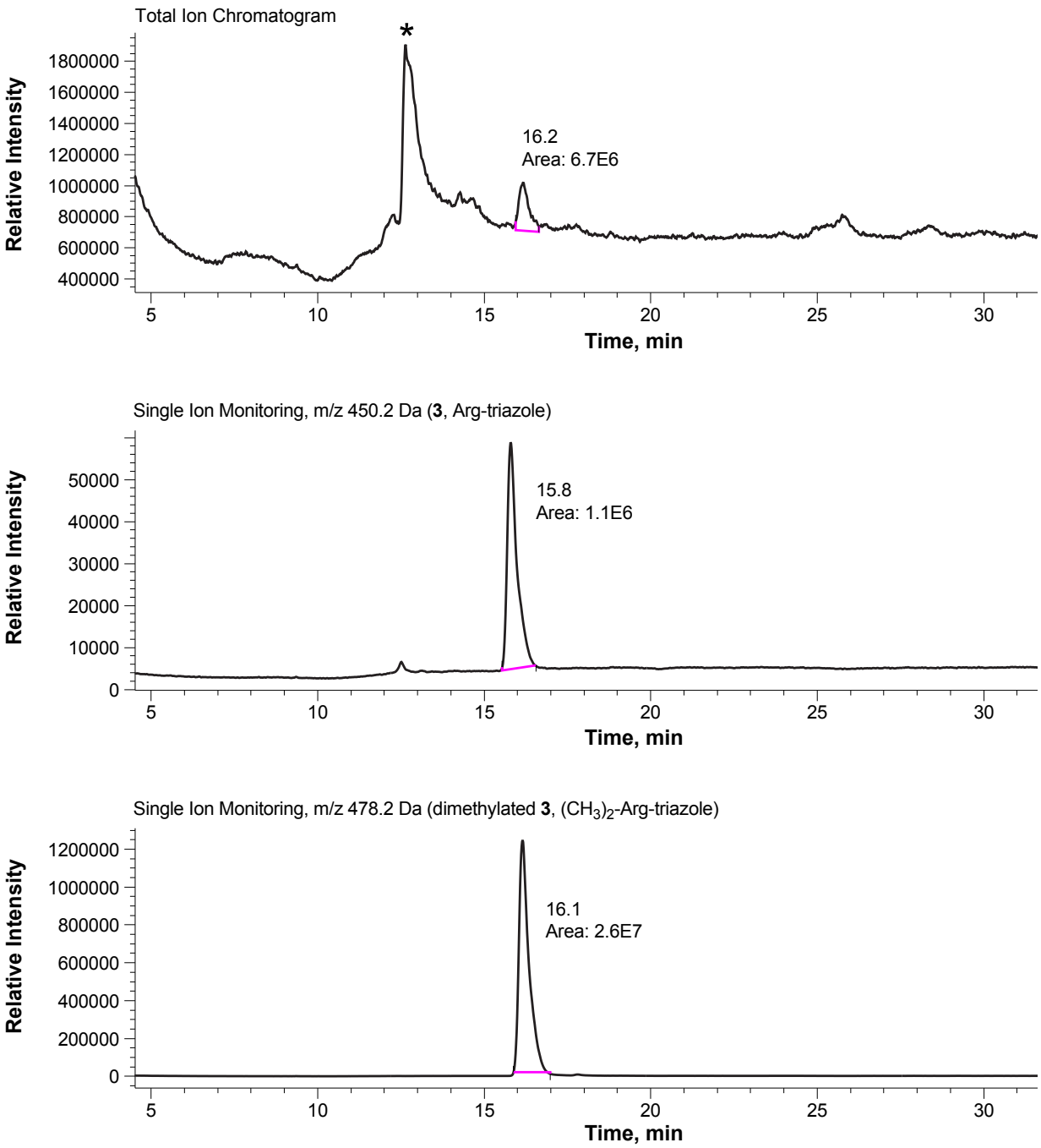



Figure S3. LC-MS analysis of 1 h methyltransferase assay with MBP-BamL and compound **3**. Top panel: Total ion chromatogram (100 Da – 1000 Da). Middle panel: Single ion monitoring for the substrate **3** (*m/z* 450.2). Bottom panel: Single ion monitoring for the product of the methyltransferase reaction, dimethylated **3** (*m/z* 478.2). "*" denotes buffer component.

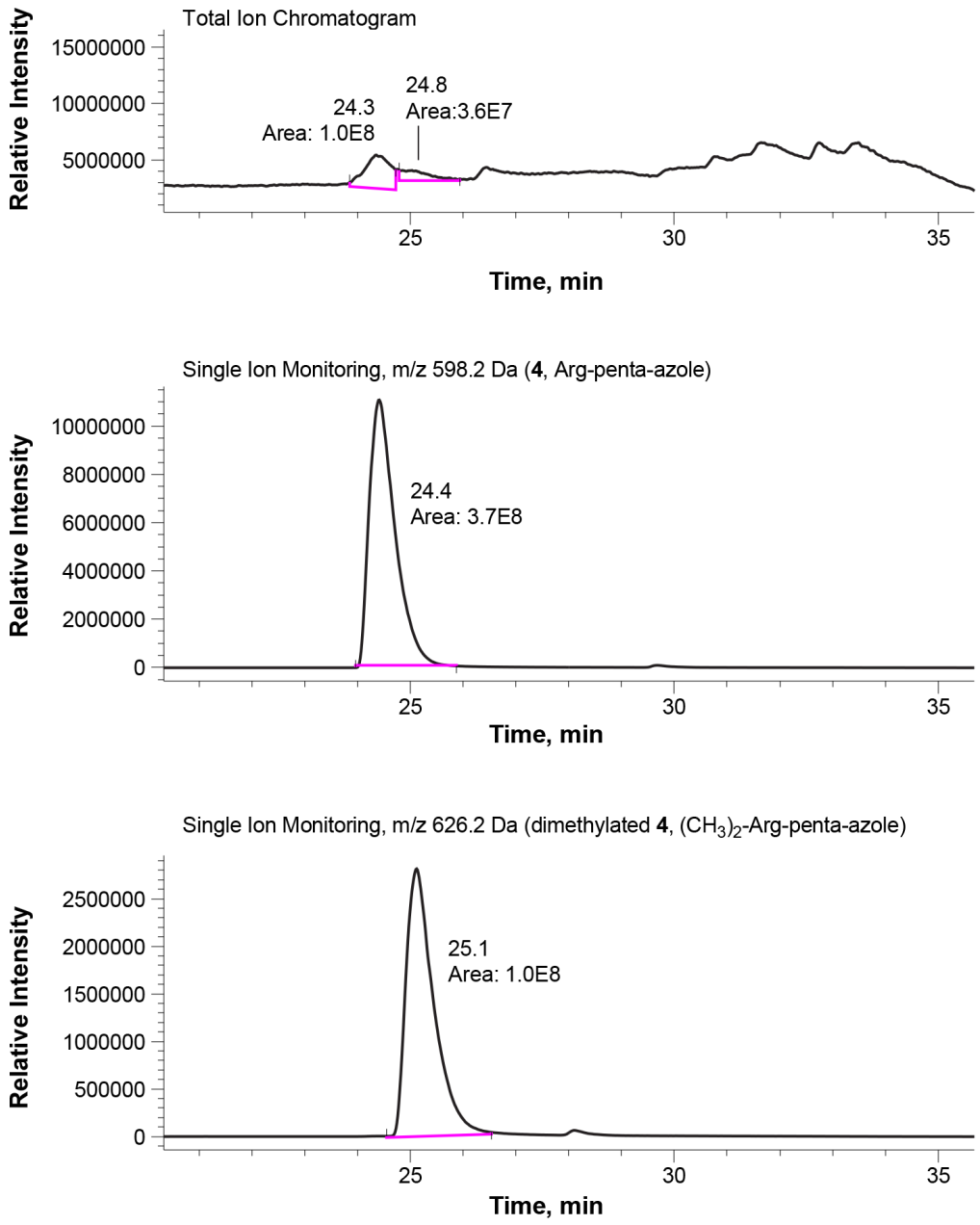



Figure S4. LC-MS analysis of 1 h methyltransferase assay with MBP-BamL and compound **4**. Top panel: Total ion chromatogram (100 Da – 1000 Da). Middle panel: Single ion monitoring for the substrate **4** (*m/z* 598.2). Bottom panel: Single ion monitoring for the product of the methyltransferase reaction, dimethylated **4** (*m/z* 626.2). "*" denotes buffer component.

**Isothermal titration calorimetry.** Calorimetry experiments were conducted at 22 ˚C on a VP-ITC titration microcalorimeter. The reference cell was filled with Milli-Q filtered water. MBP-BamL was diluted to 50 μM in ITC buffer [50 mM HEPES pH 7.5, 150 mM NaCl, 2.5% (v/v) glycerol]. The sample cell (effective volume = 1.45 mL) was filled with protein and stirred continuously at 270 rpm during the titration. The protein was titrated with 36 aliquots (8 μL) each of either 350 μM (**2**, **3**) or 700 μM (**17**, Arg-amide) ligand in the same buffer with a 300 s equilibration between titrations. To increase the solubility of **4**, buffer solution with a 2% concentration (v/v) of DMSO was used. The heat of dilution of the ligand into buffer was subtracted from the titration data. Integration of the area under each peak in the graph of heat change over time was used to determine the heat produced per injection. The MicroCal version of Origin was used to integrate, baseline correct, and normalize raw data as described elsewhere.[8]

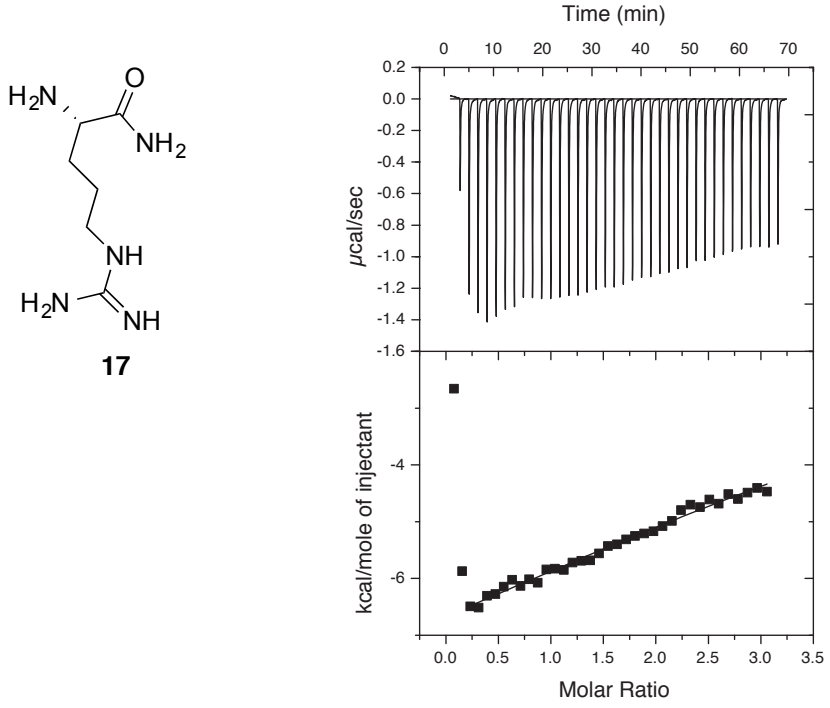



**Figure S5**. ITC data and fitting curves for the binding of **17** (Arg-$NH_2$) to BamL.

## IX. References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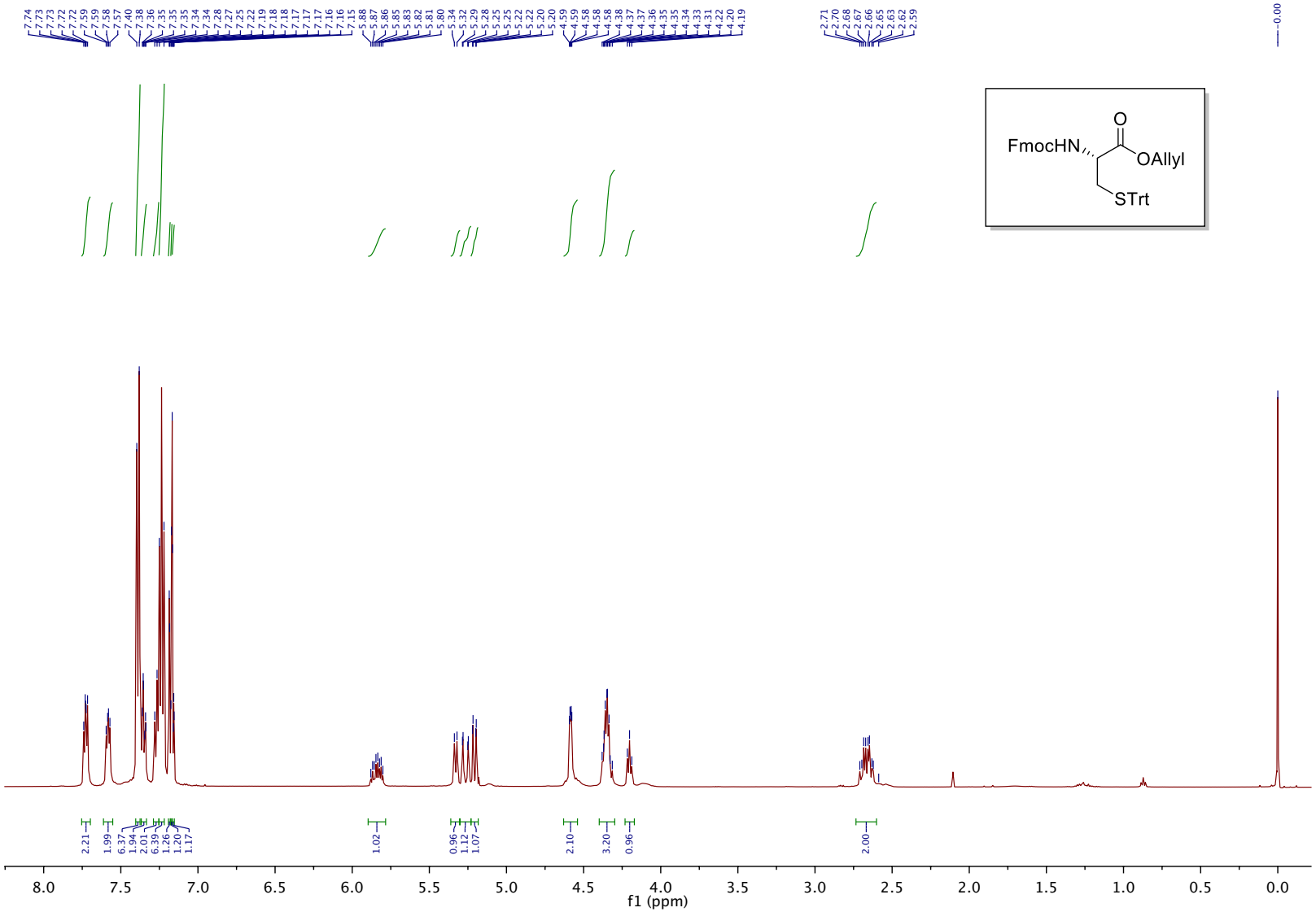
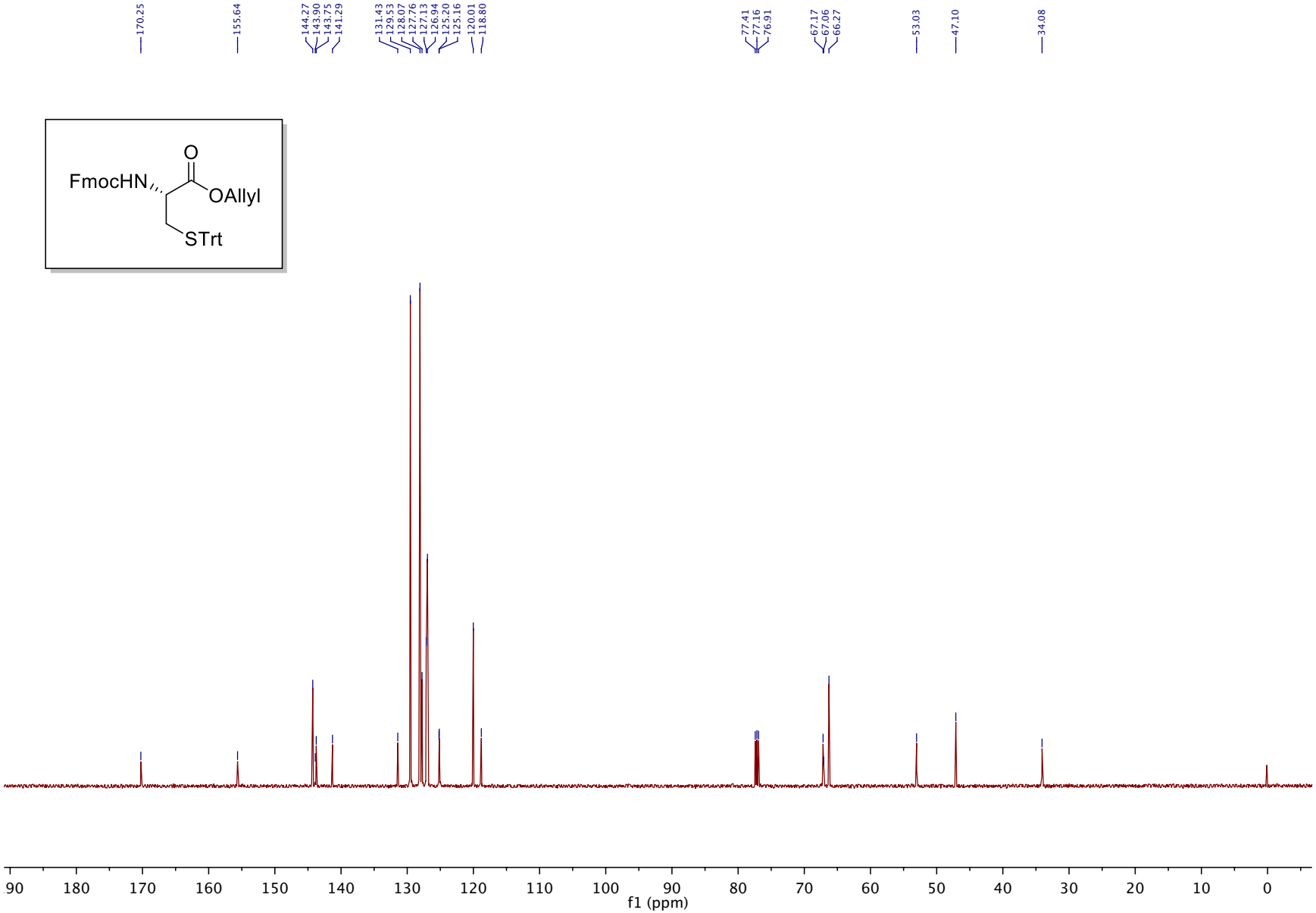
## X. NMR spectra

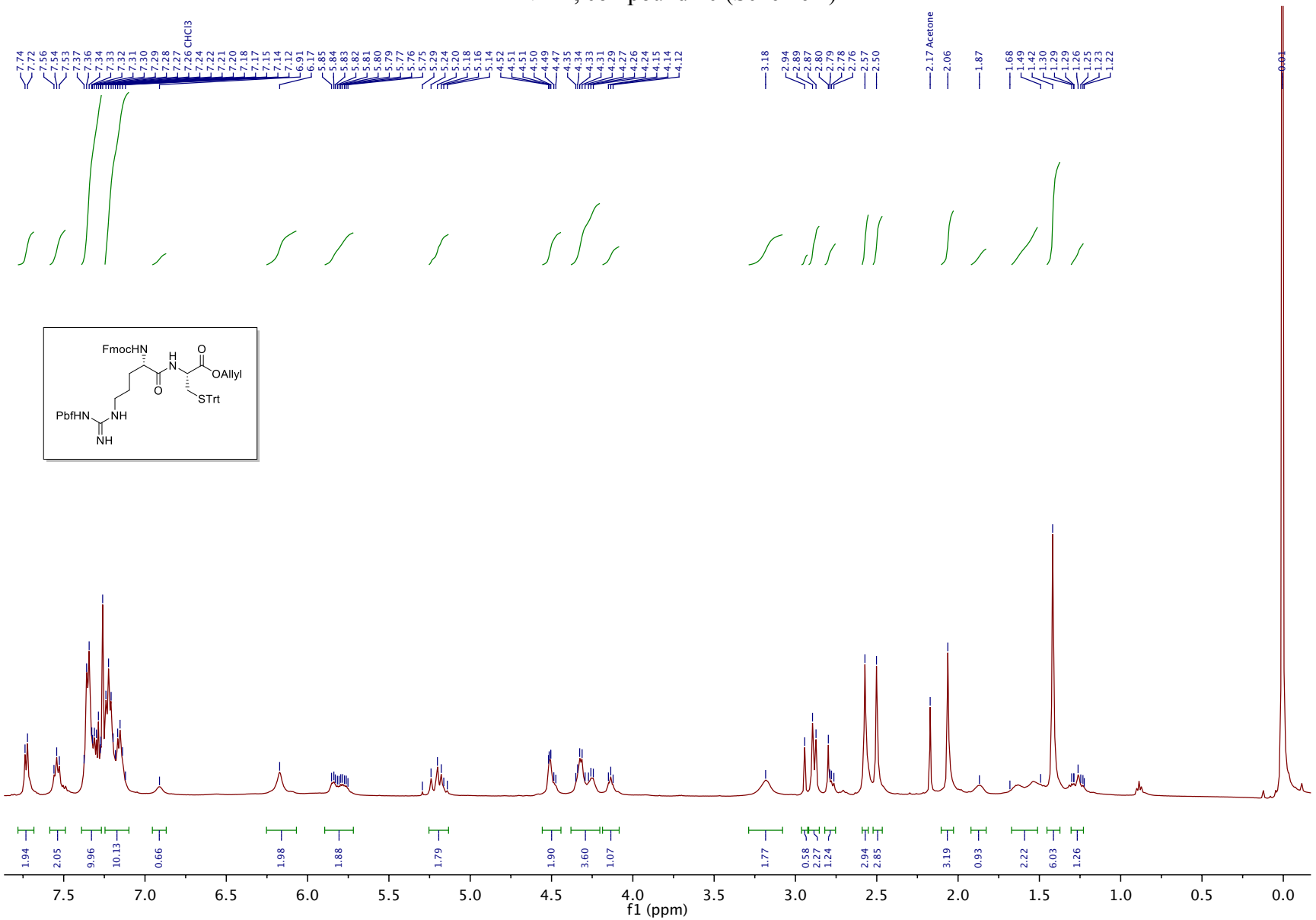
$^{1}$H NMR, compound **8** (Scheme 1)



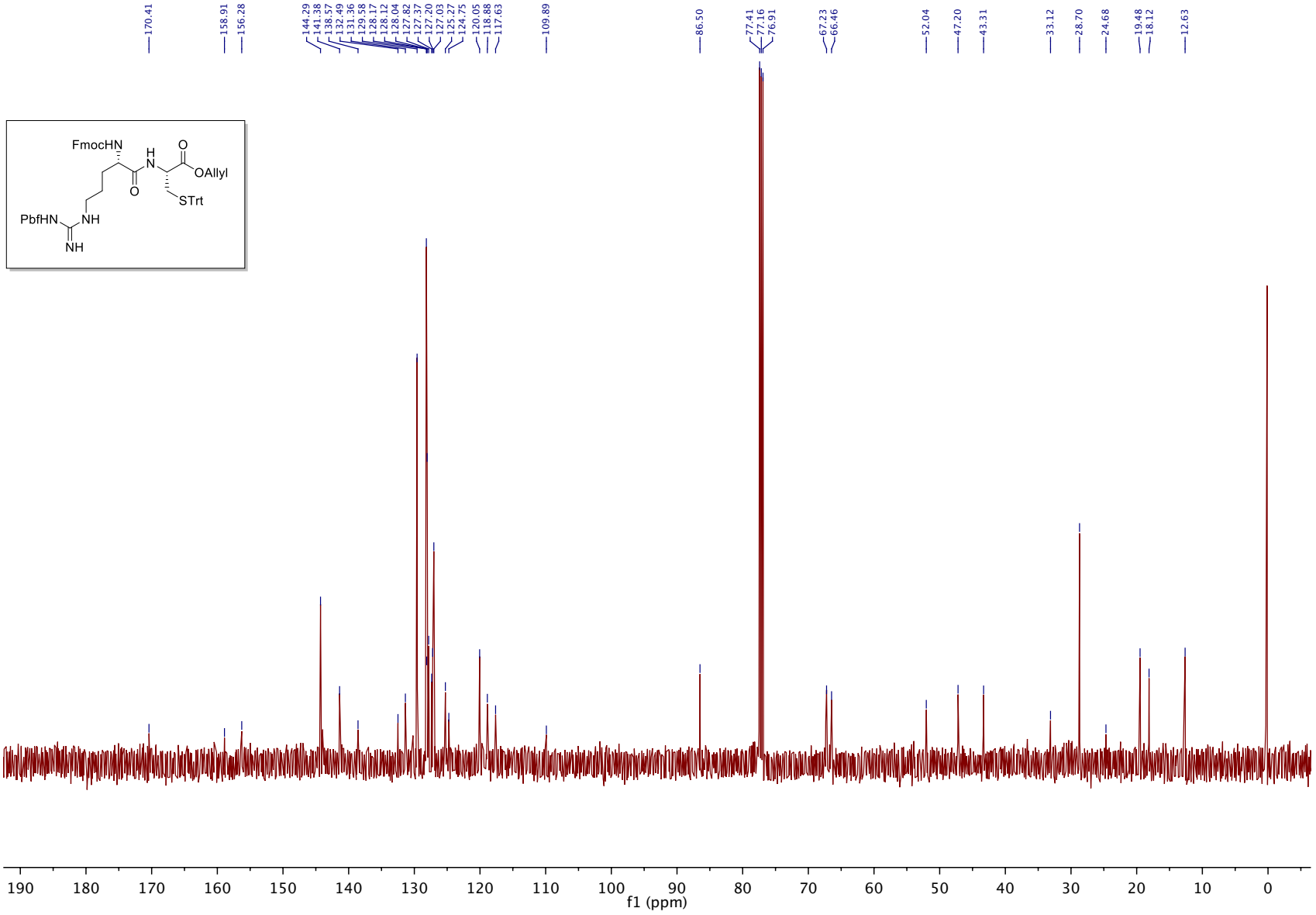
$^{3}$C NMR, compound **8** (Scheme 1)



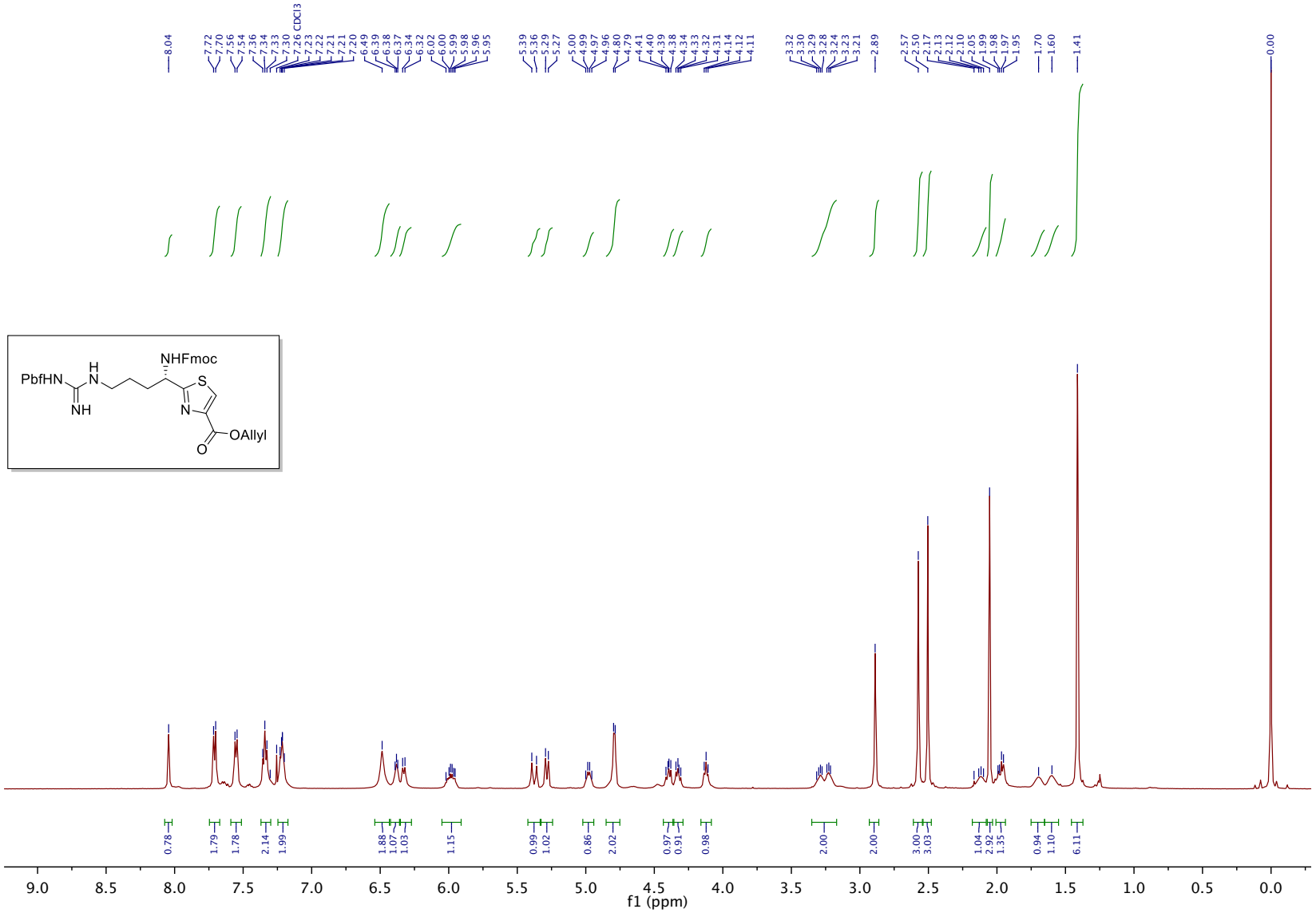
$^{1}$H NMR, compound **10** (Scheme 1)



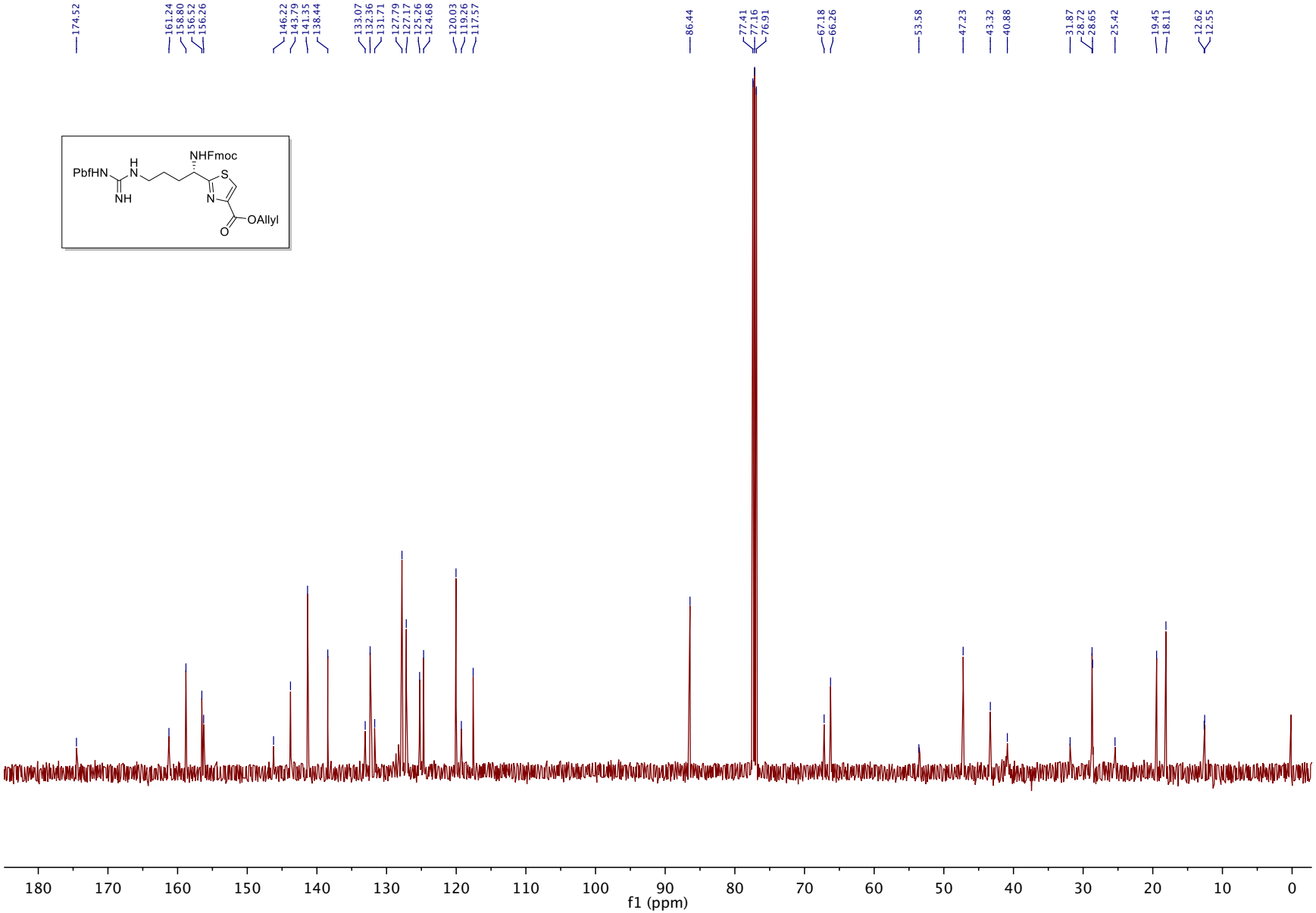
$^{13}$C NMR, compound **10** (Scheme 1)



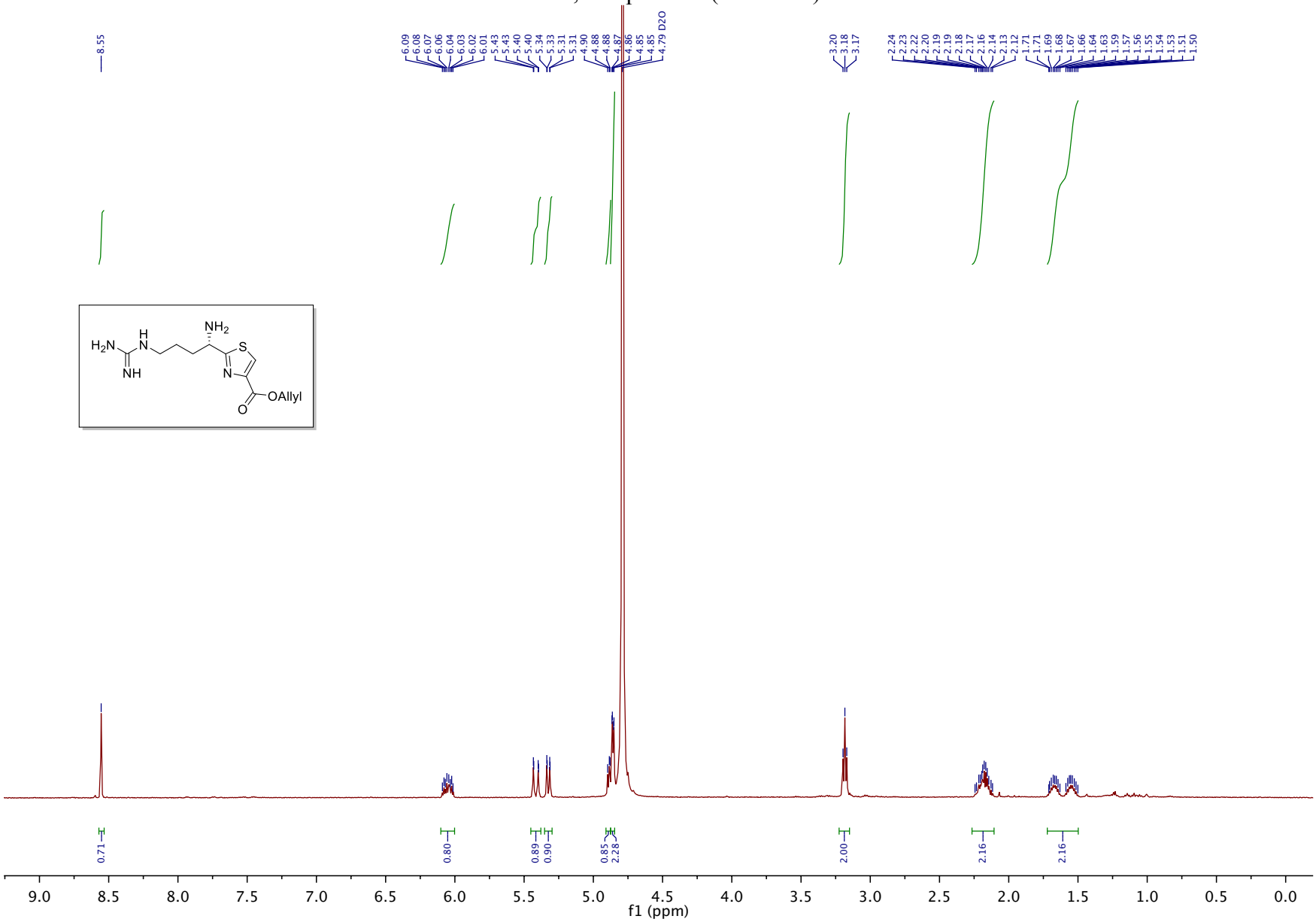
$^{1}$H NMR, compound **5a** (Scheme 1)



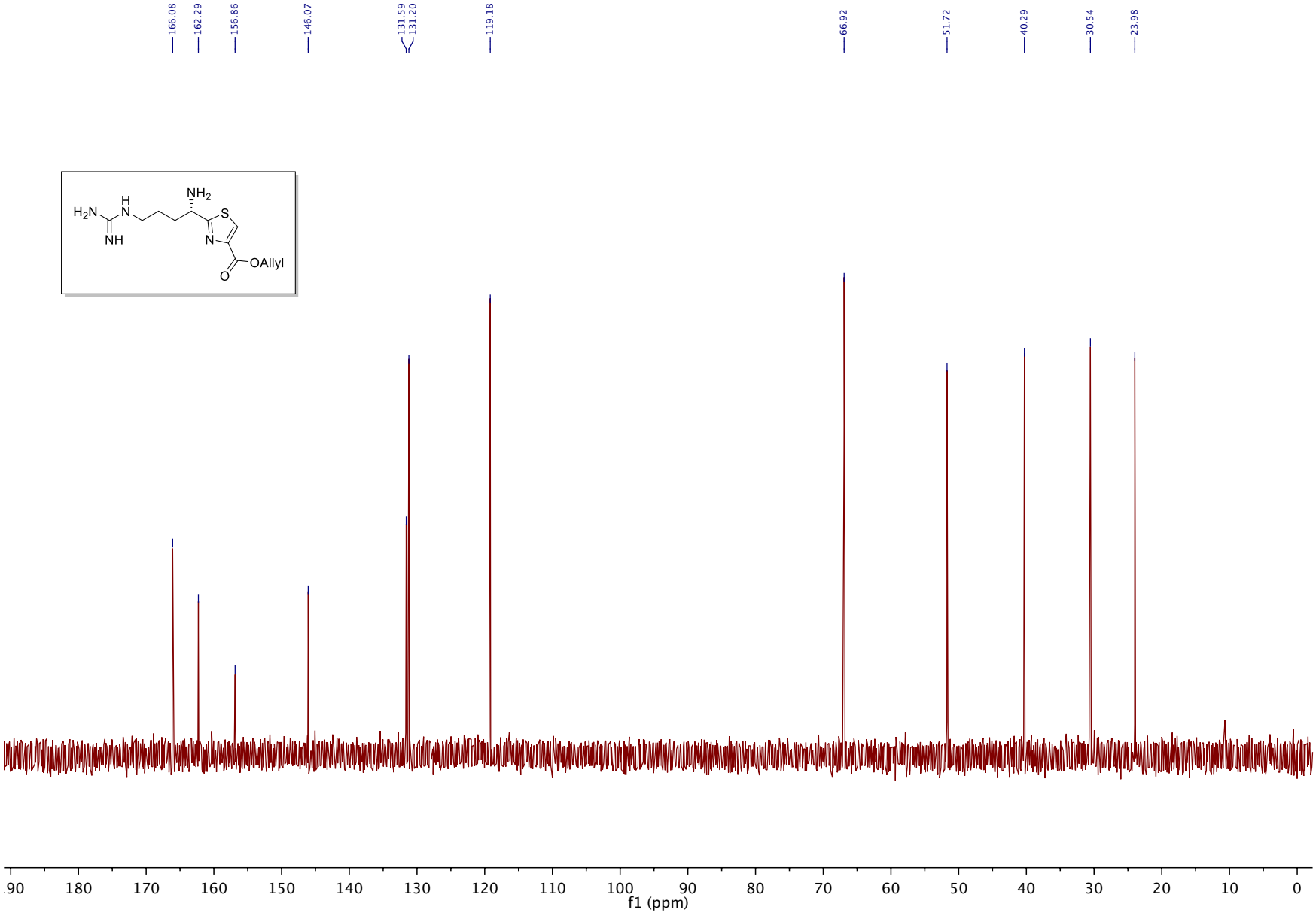
$^{13}$C NMR, compound **5a** (Scheme 1)



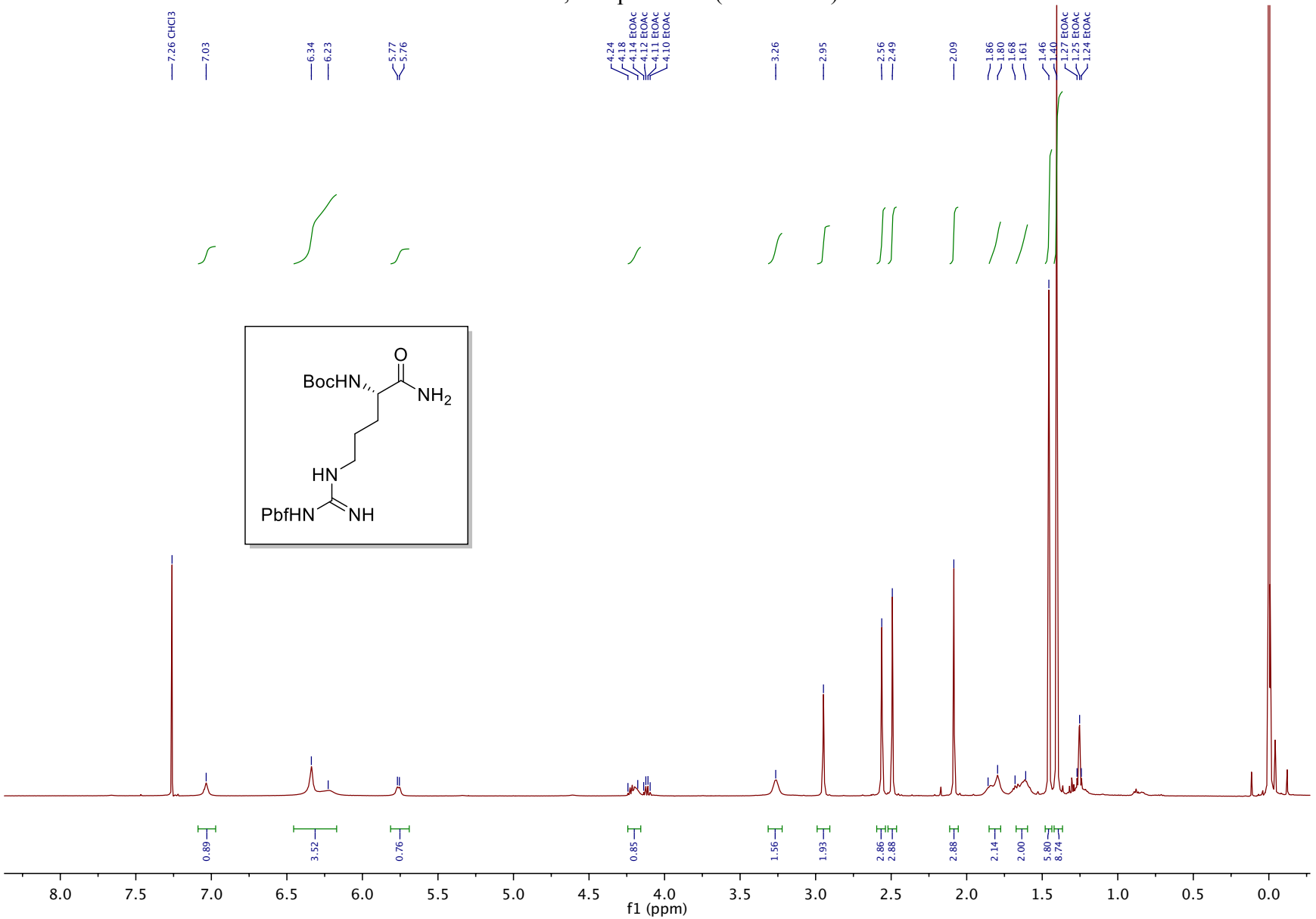
$^{1}$H NMR, compound **2** (Scheme 1)



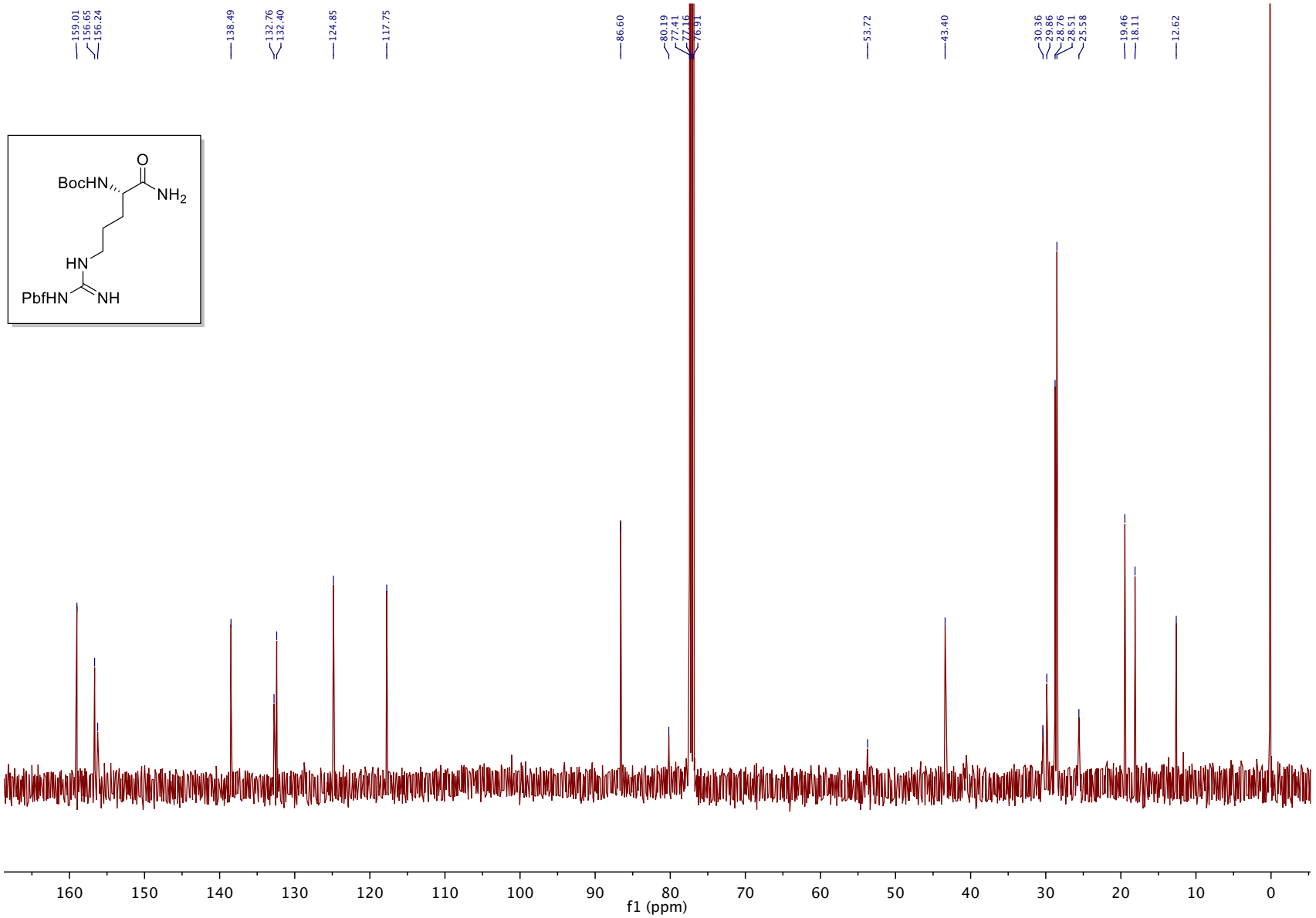
$^{13}$C NMR, compound **2** (Scheme 1)



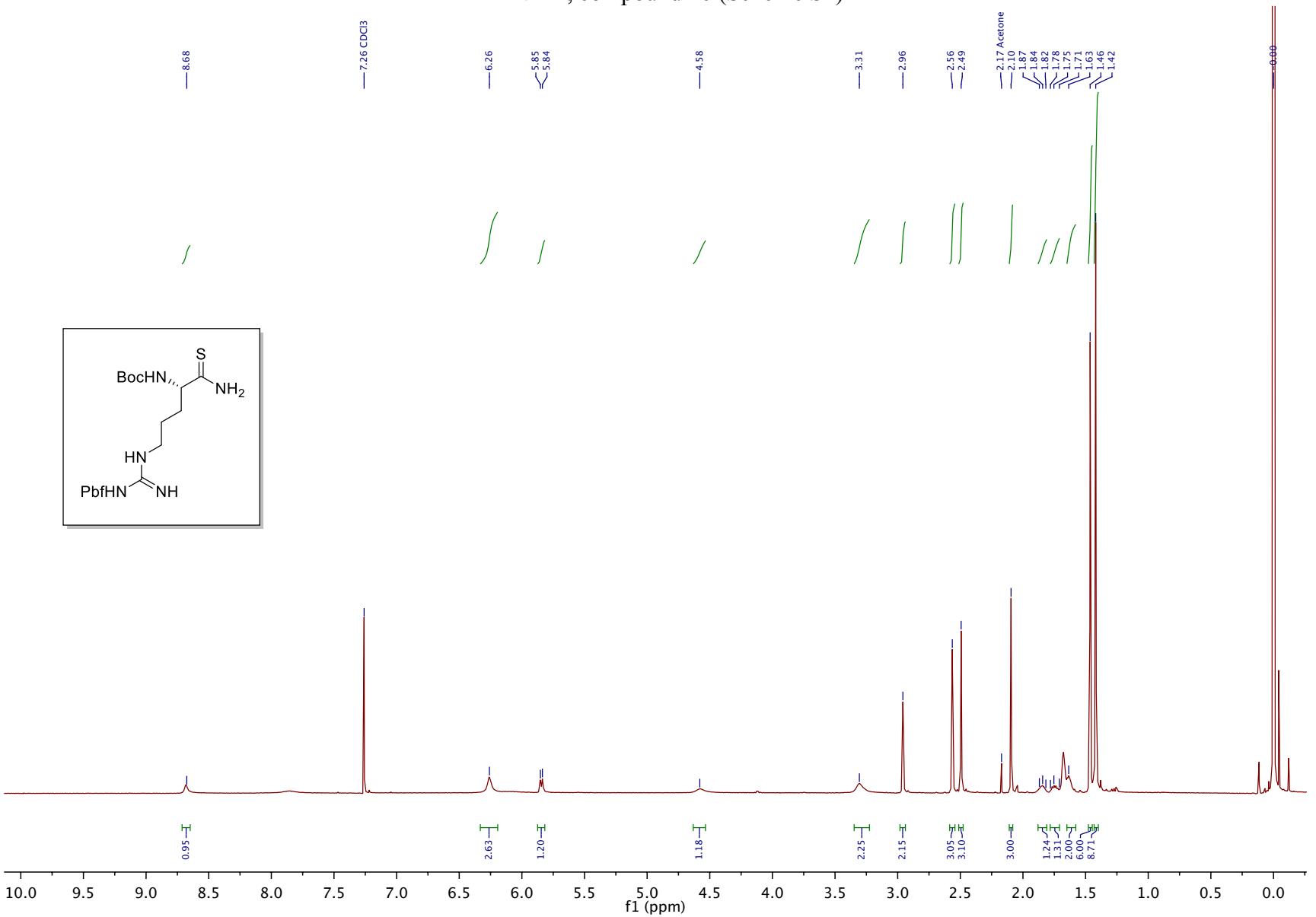
$^{1}$H NMR, compound **19** (Scheme S1)



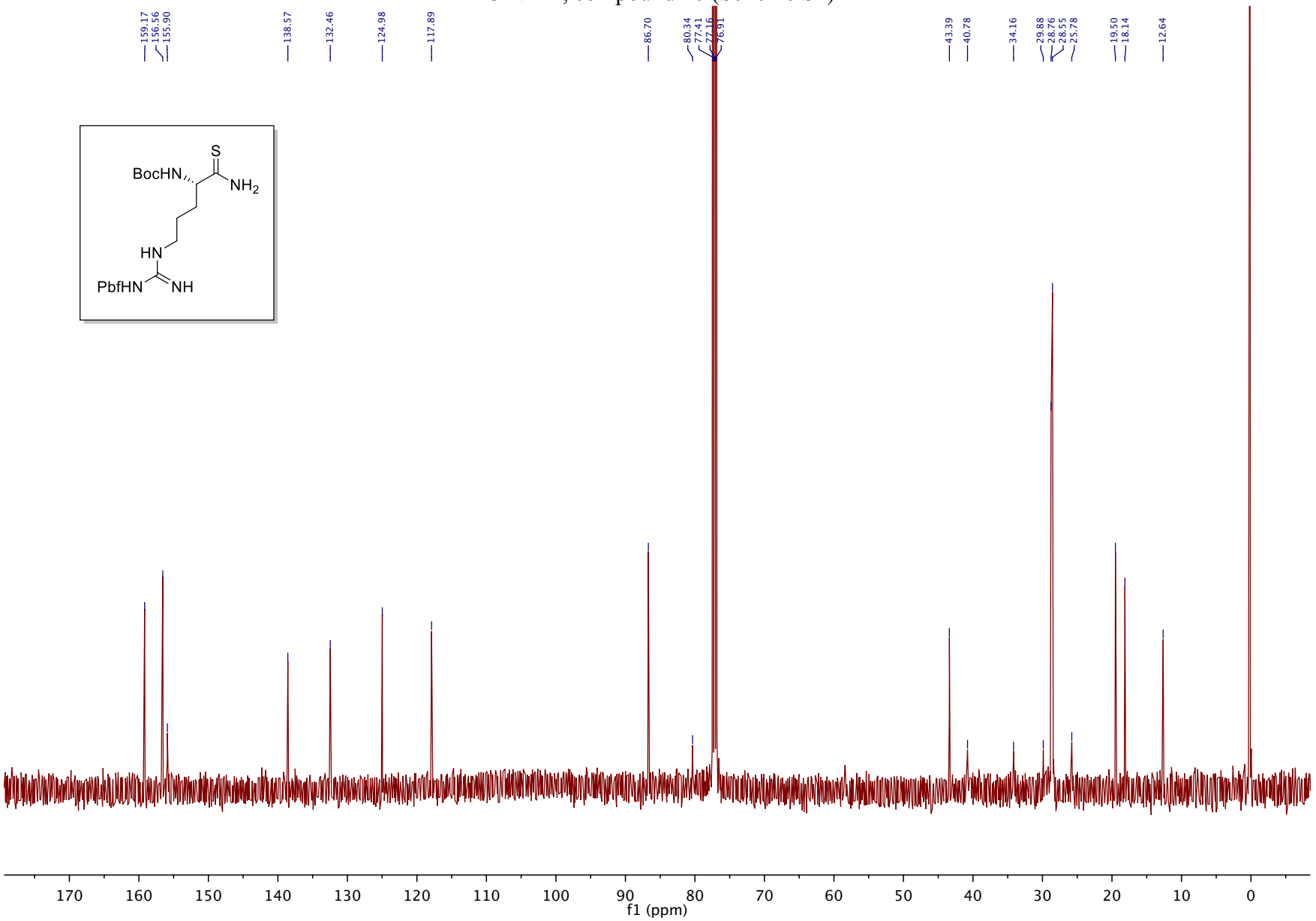
$^{13}$C NMR, compound **19** (Scheme S1)



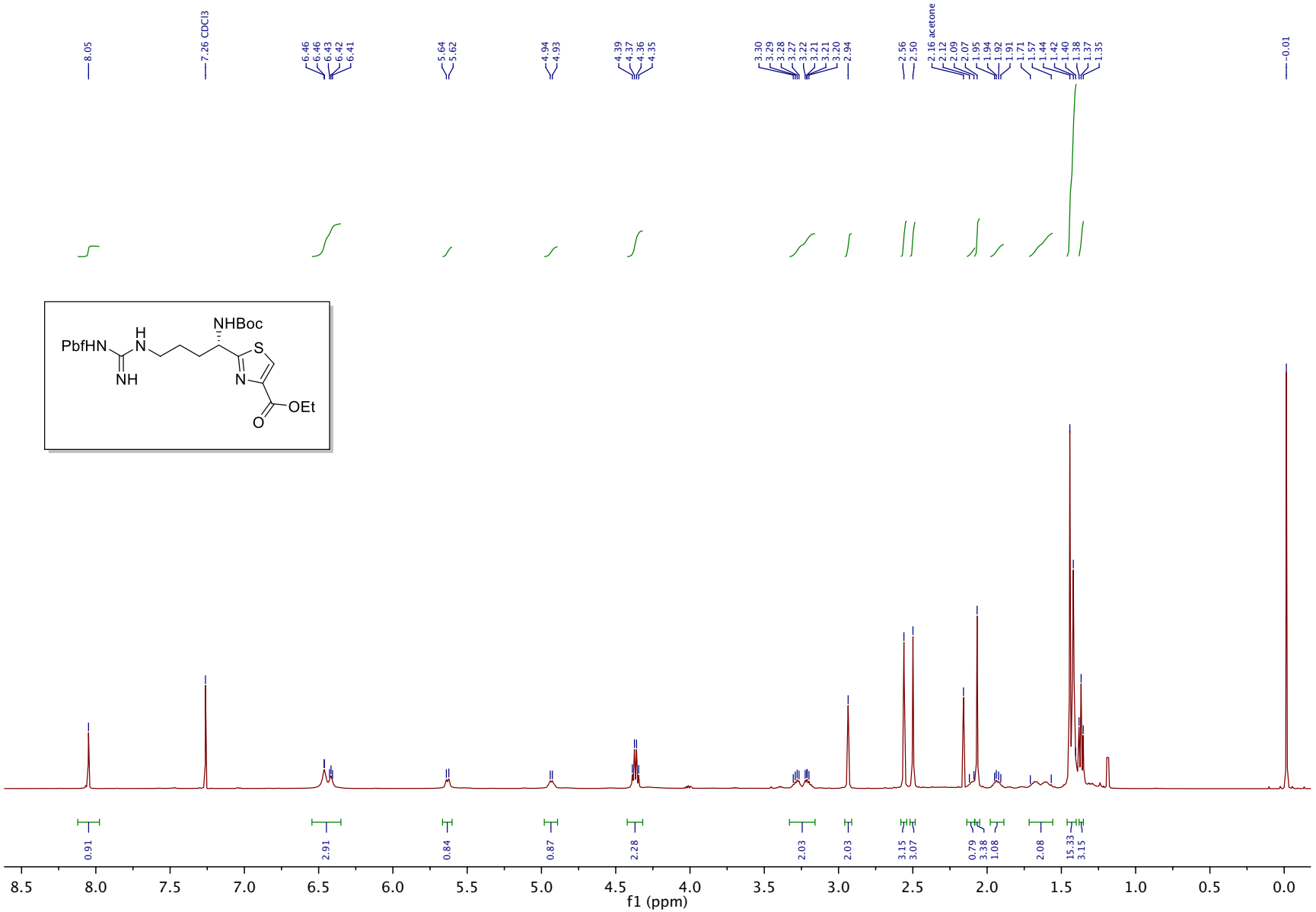
$^{1}$H NMR, compound **20** (Scheme S1)



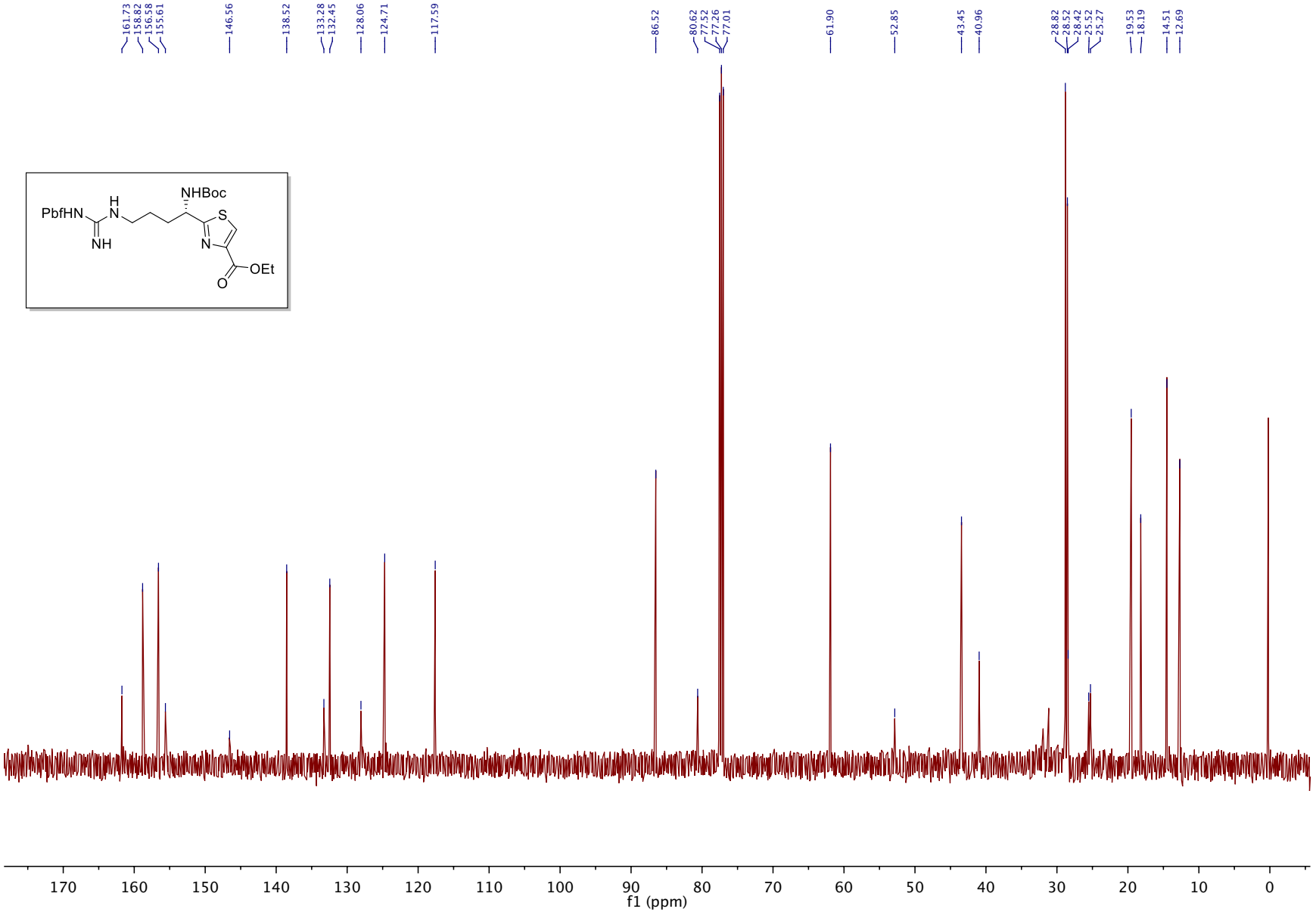
$^{13}$C NMR, compound **20** (Scheme S1)



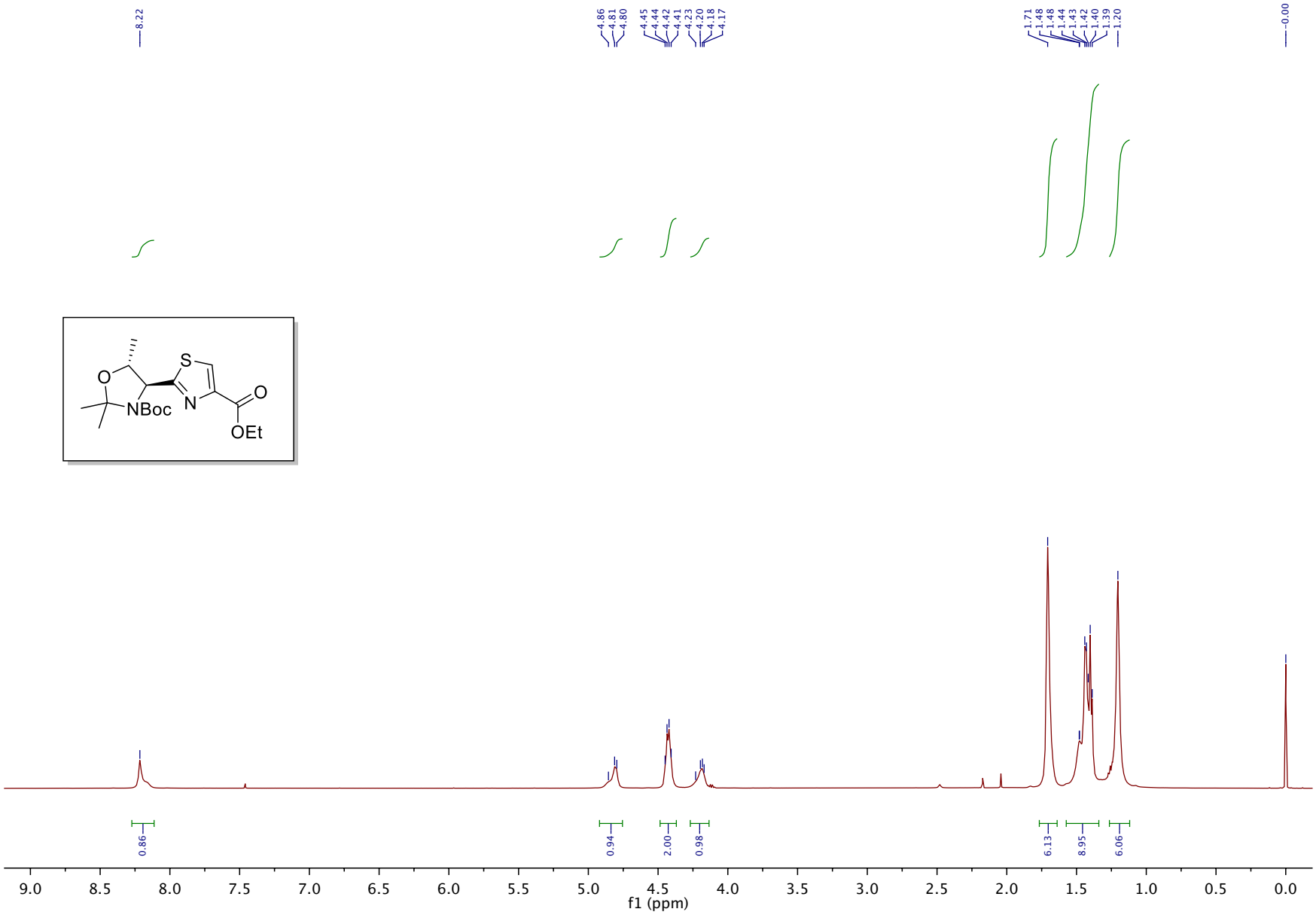
$^{1}$H NMR, compound **5b** (Schemes 2 & S1)



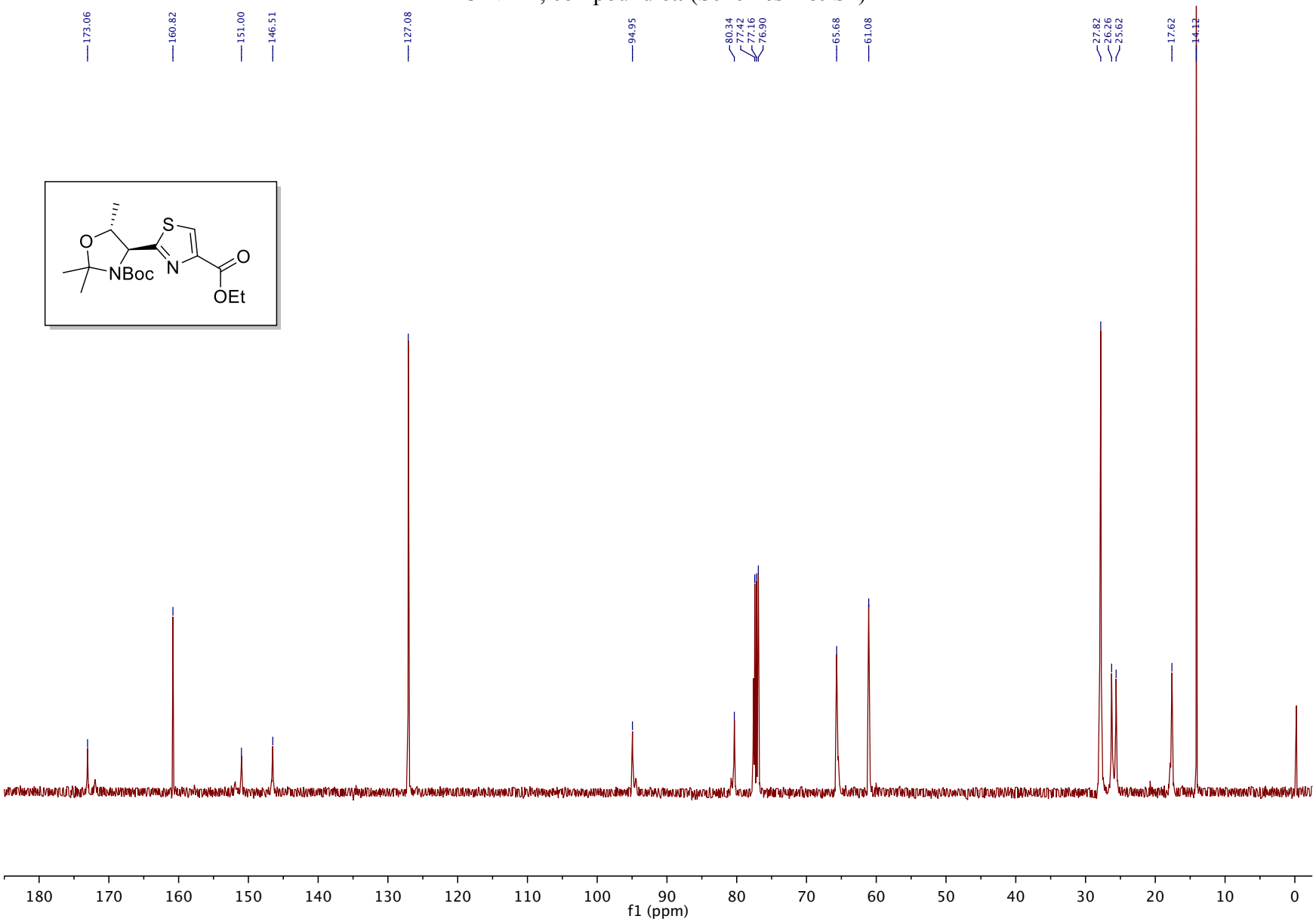
$^{13}$C NMR, compound **5b** (Schemes 2 & S1)



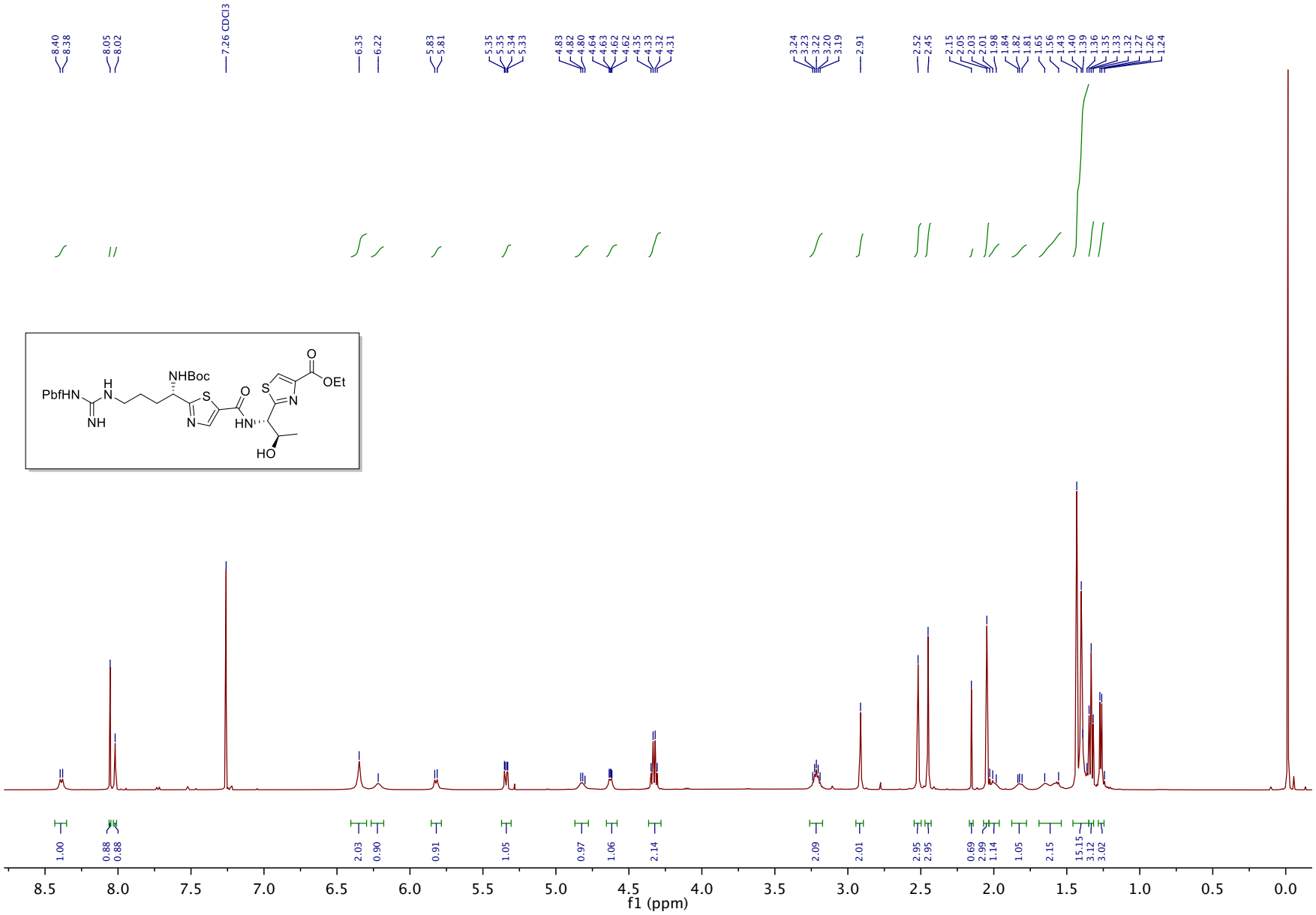
$^{1}$H NMR, compound **6a** (Schemes 2 & S2)



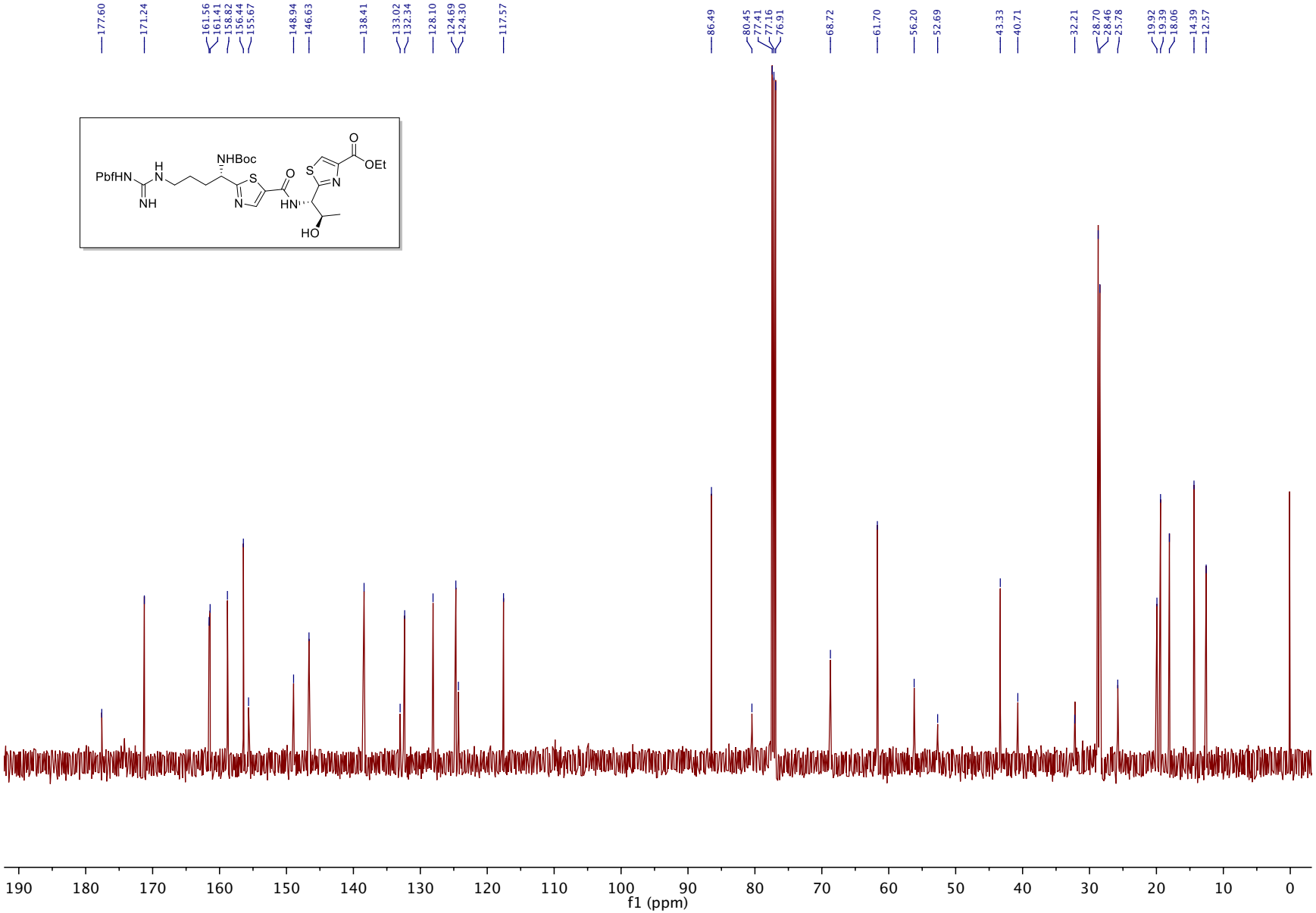
$^{13}$C NMR, compound **6a** (Schemes 2 & S2)



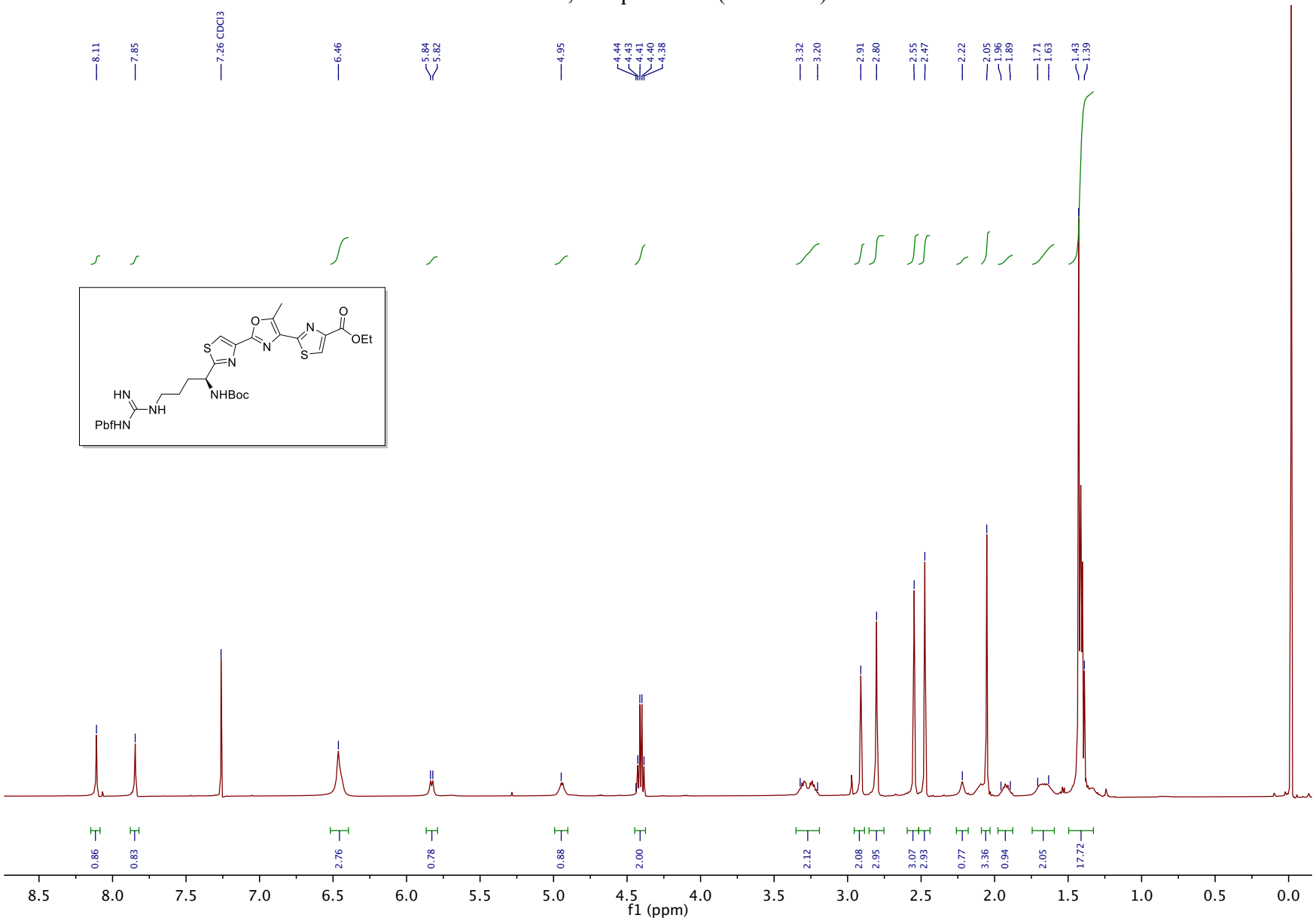
$^{1}$H NMR, compound **11** (Scheme 2)



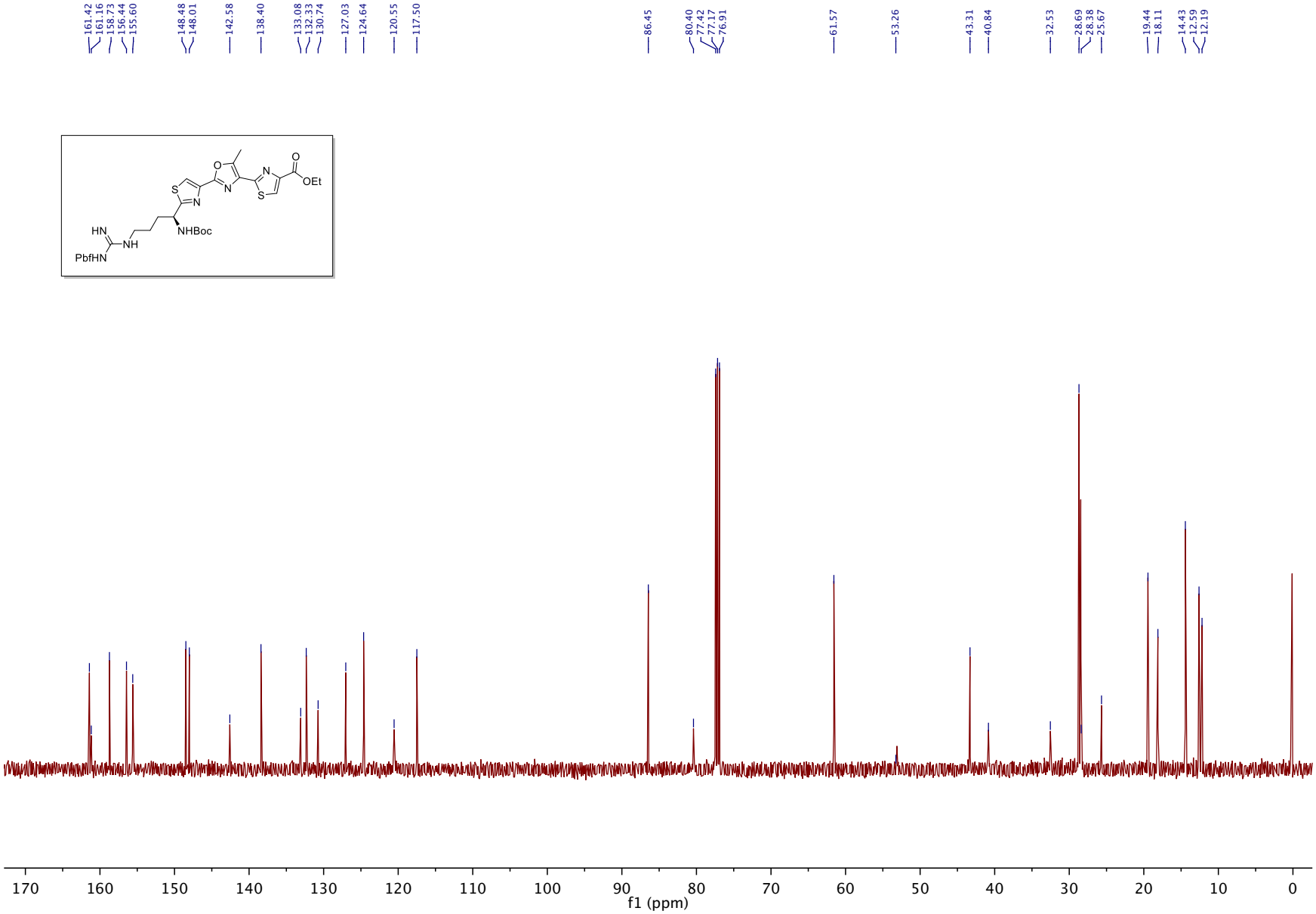
$^{13}$C NMR, compound **11** (Scheme 2)



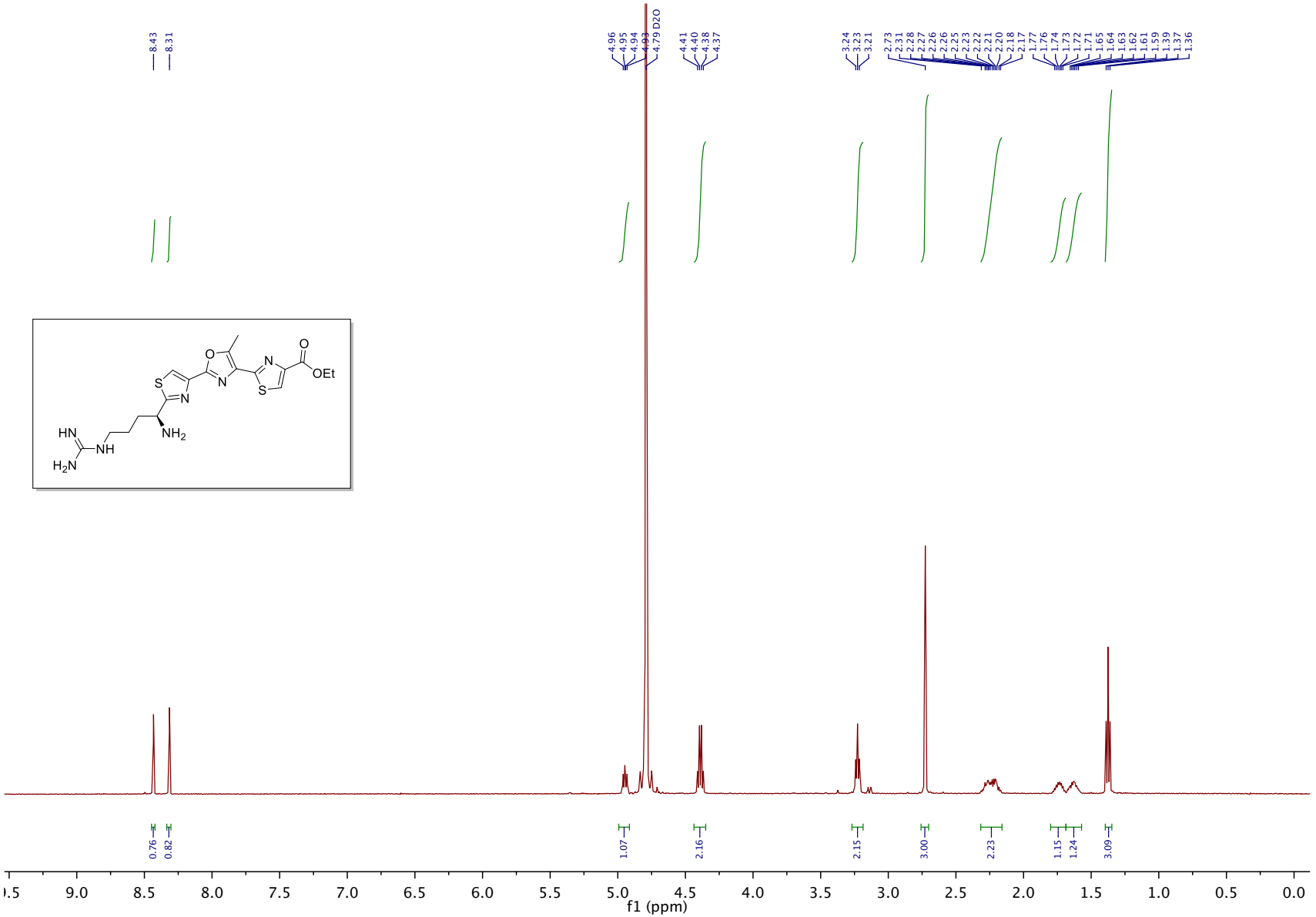
$^{1}$H NMR, compound **12** (Scheme 2)



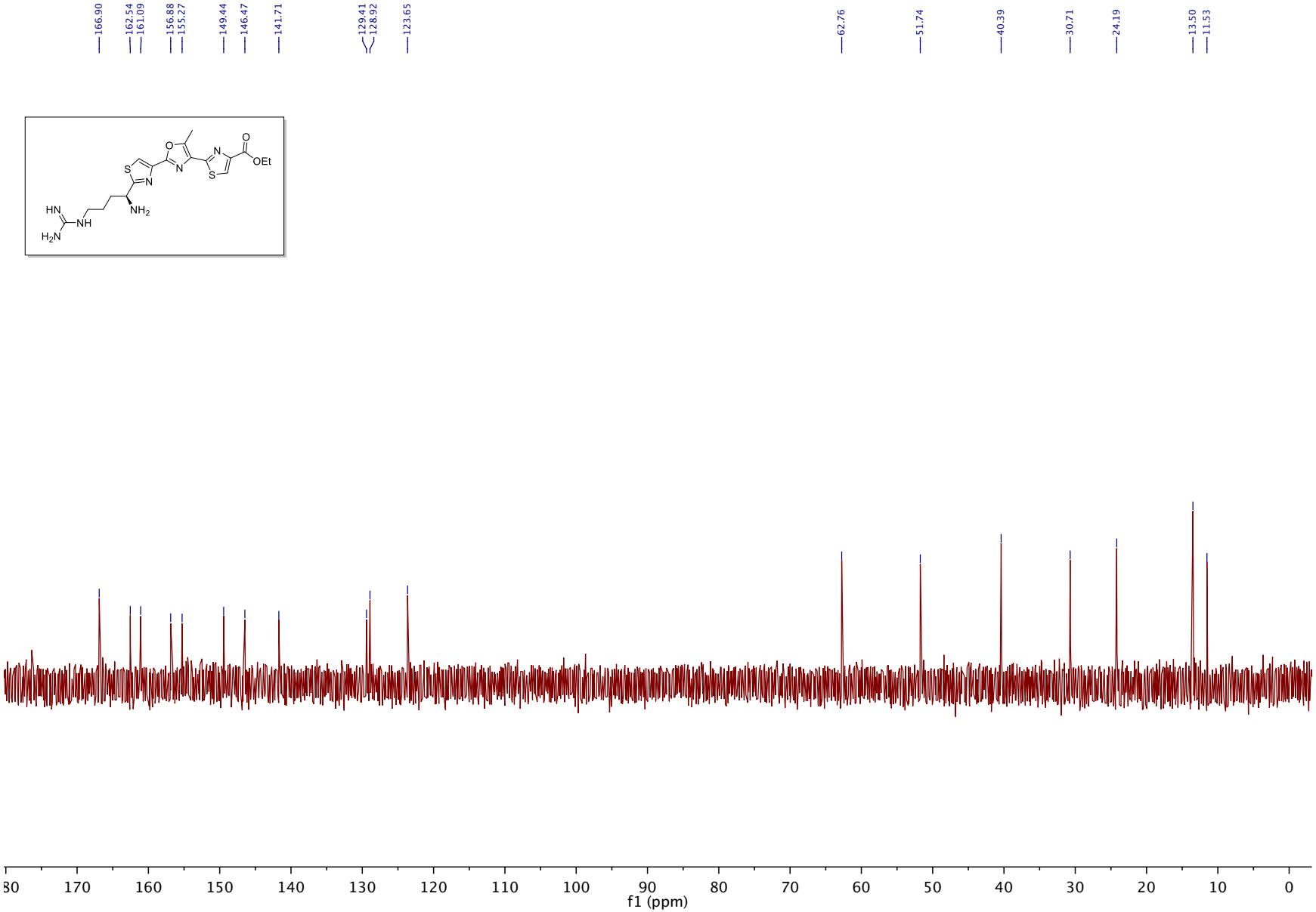
$^{13}$C NMR, compound **12** (Scheme 2)



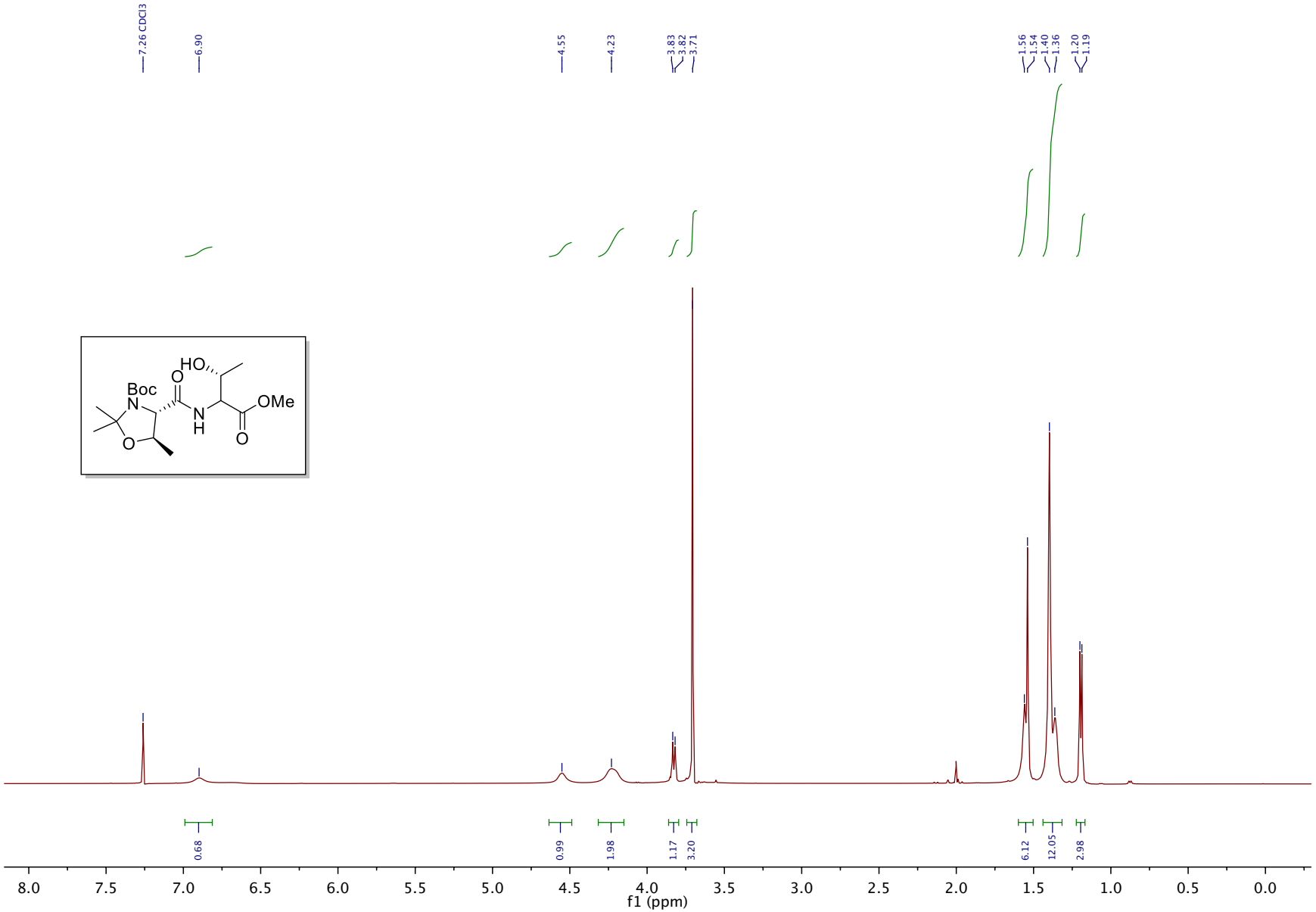
$^{1}$H NMR, compound **3** (Scheme 2)



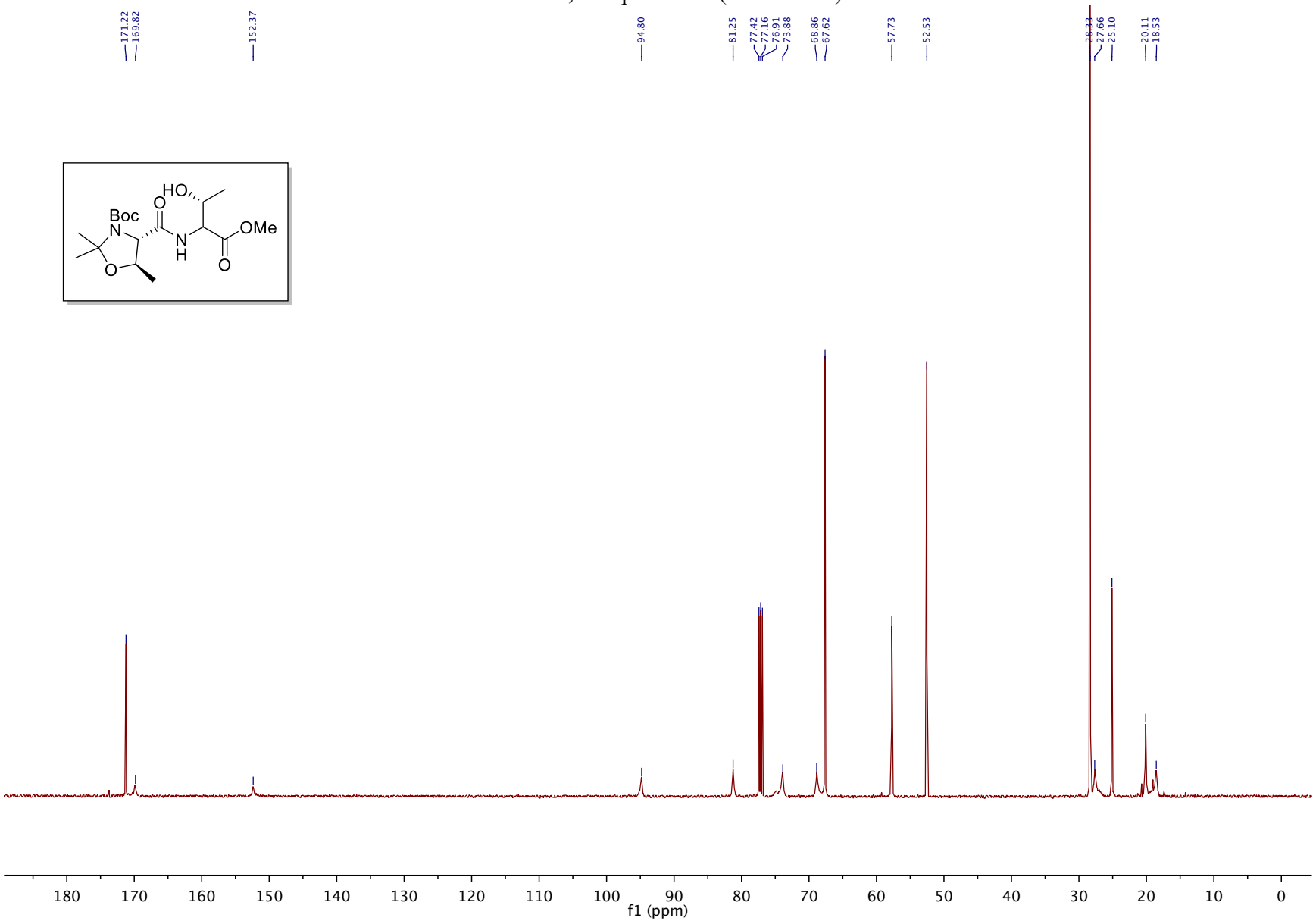
$^{13}$C NMR, compound **3** (Scheme 2)



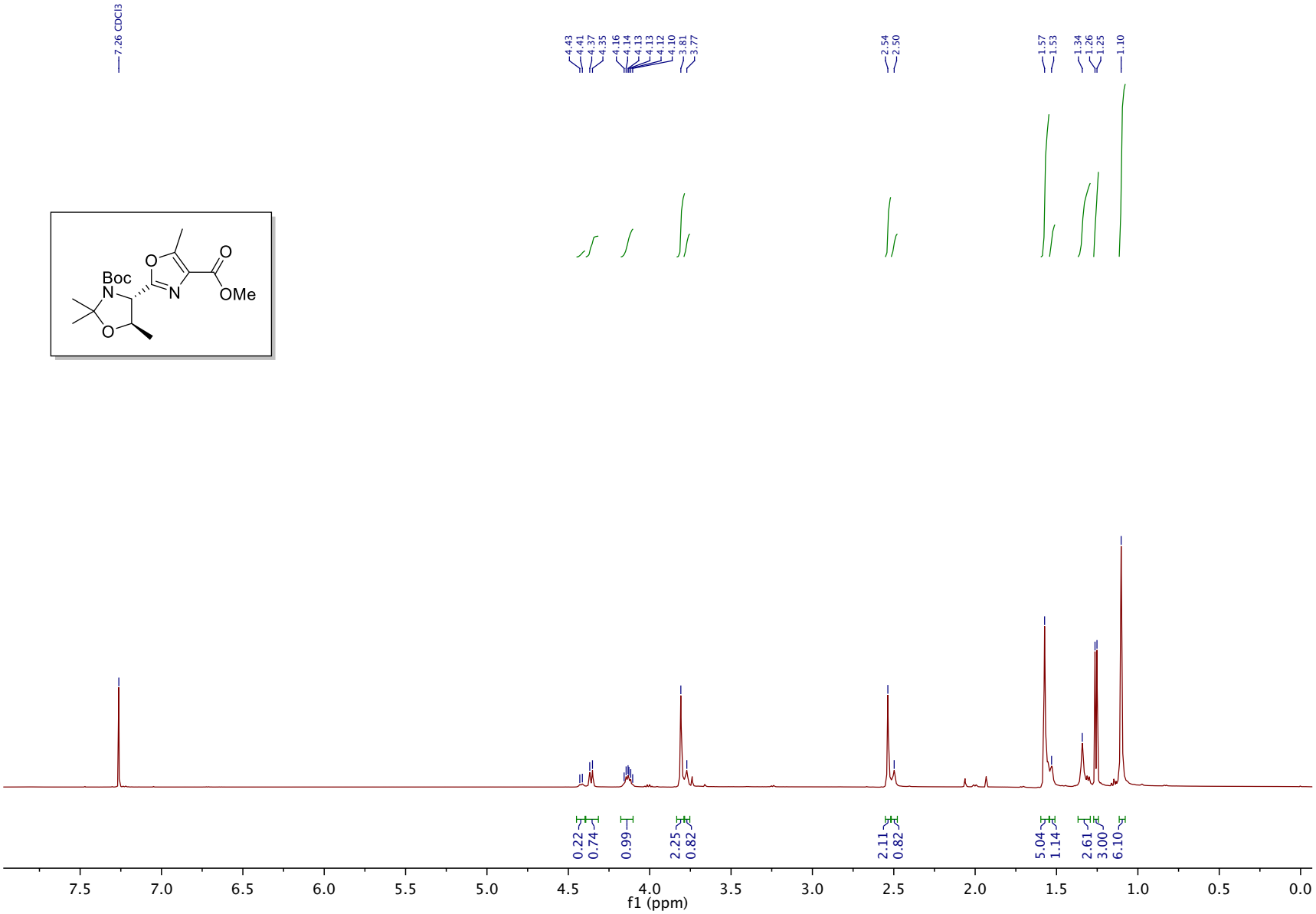
$^{1}$H NMR, compound **25** (Scheme S3)



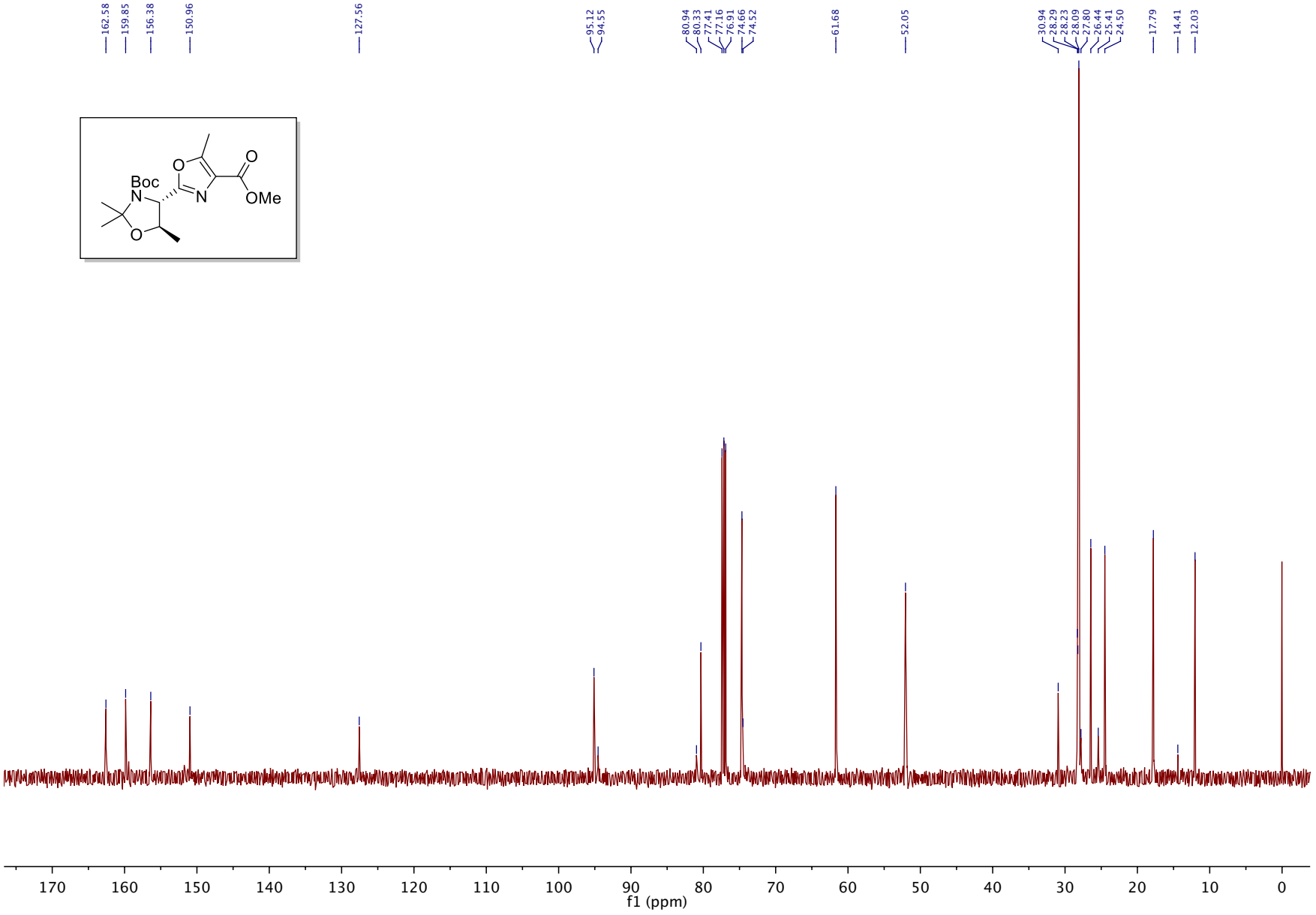
$^{13}$C NMR, compound **25** (Scheme S3)



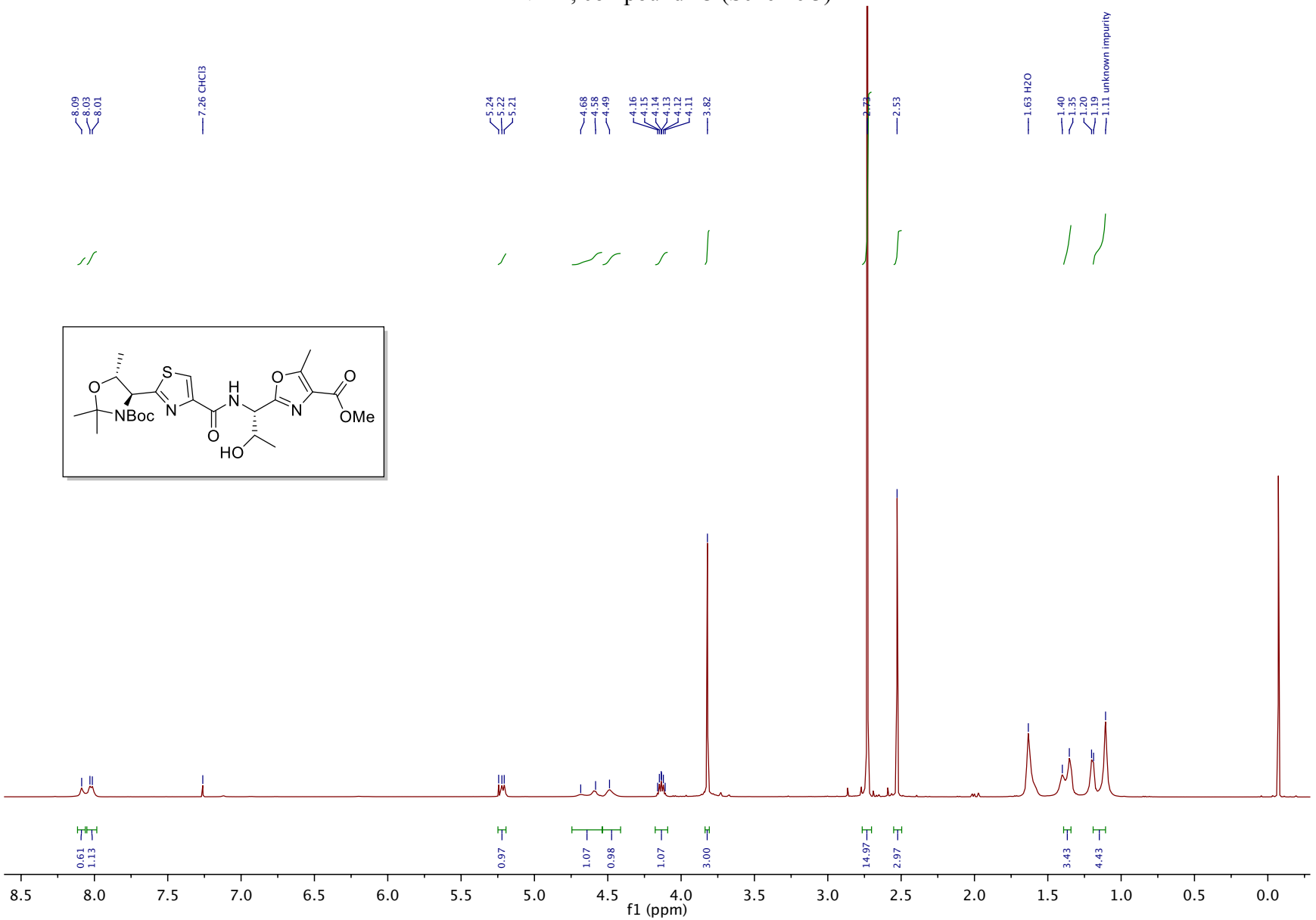
$^{1}$H NMR, compound **7a** (Schemes 3 & S3)



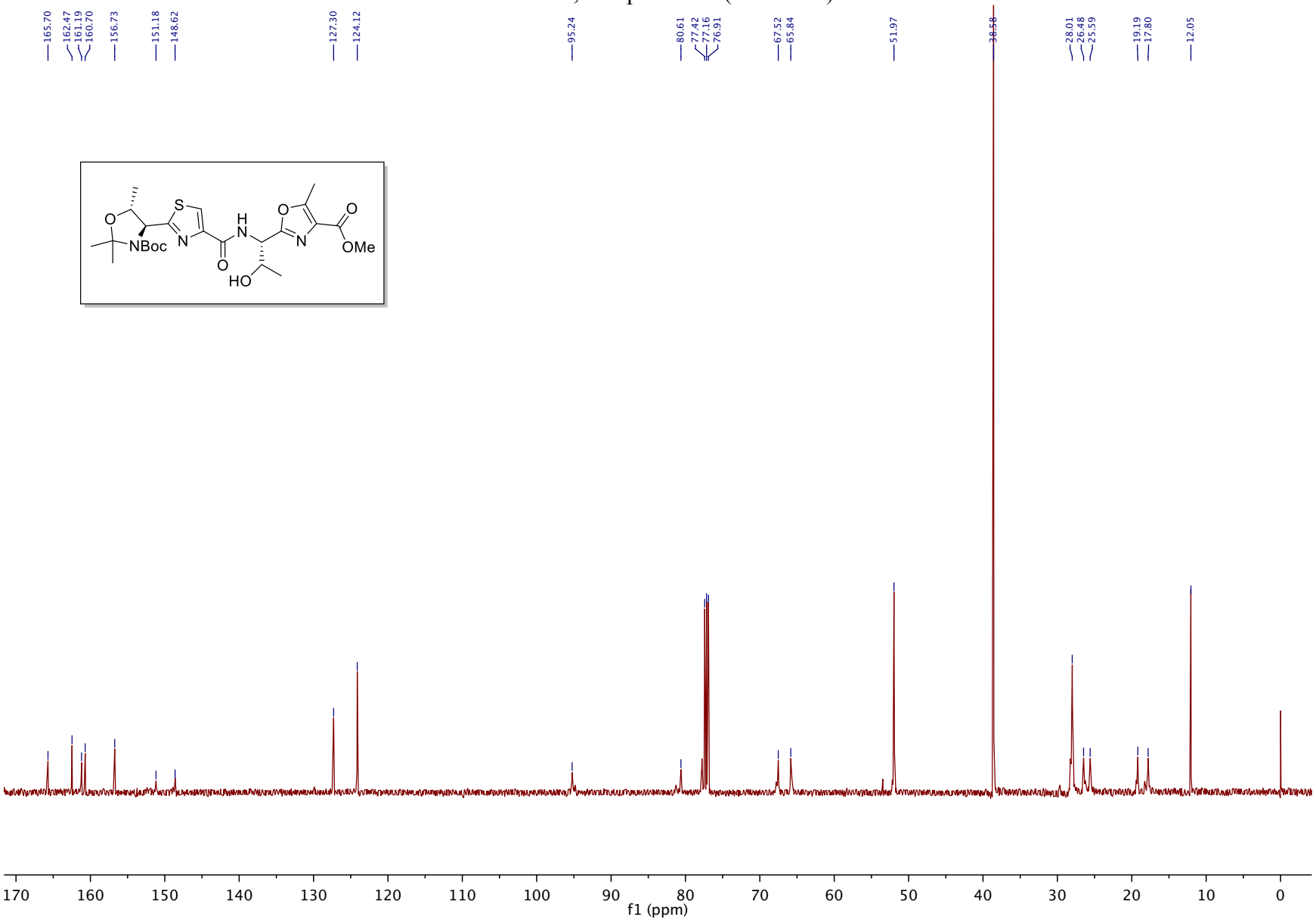
$^{13}$C NMR, compound **7a** (Schemes 3 & S3)



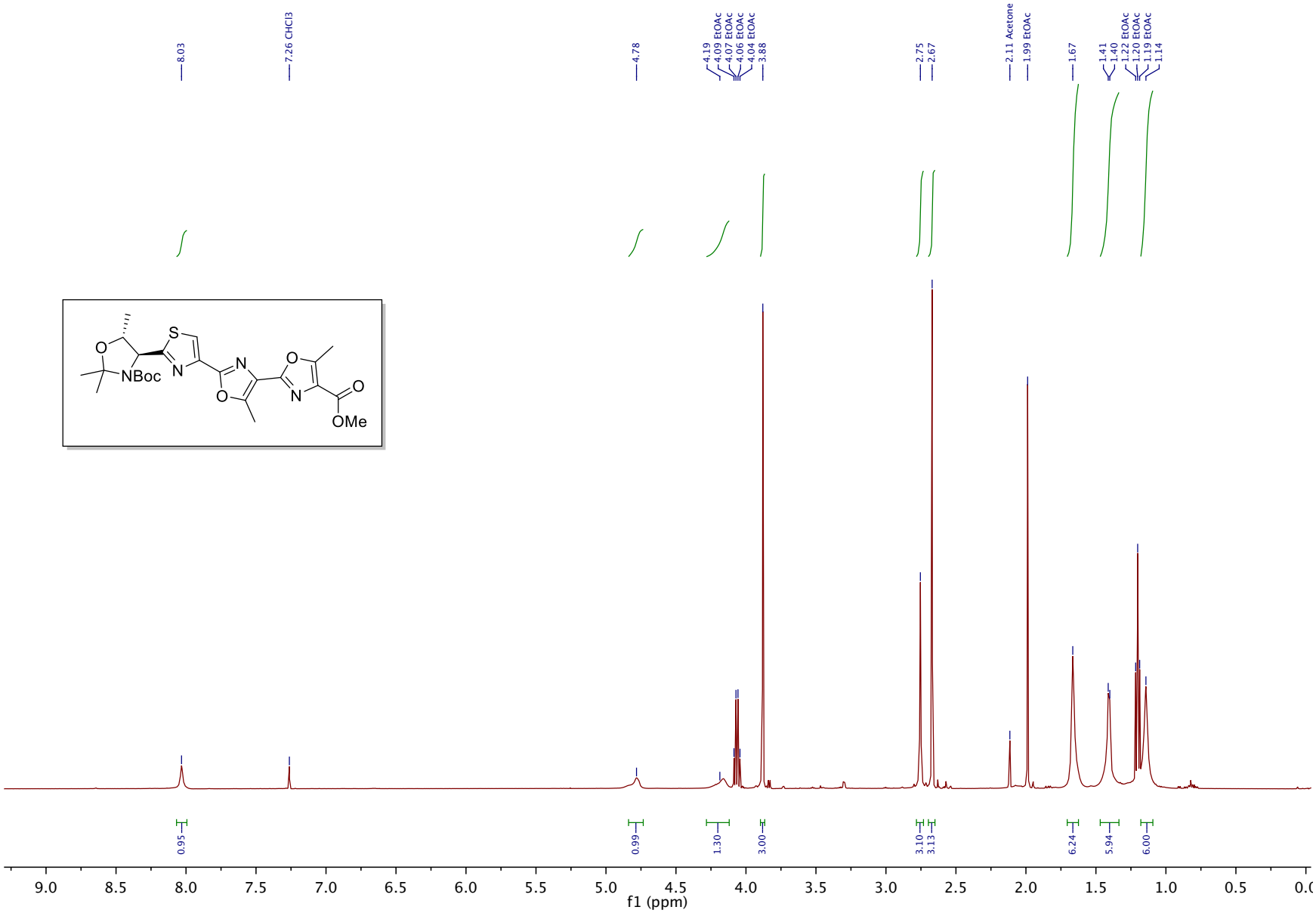
$^{1}$H NMR, compound **13** (Scheme 3)



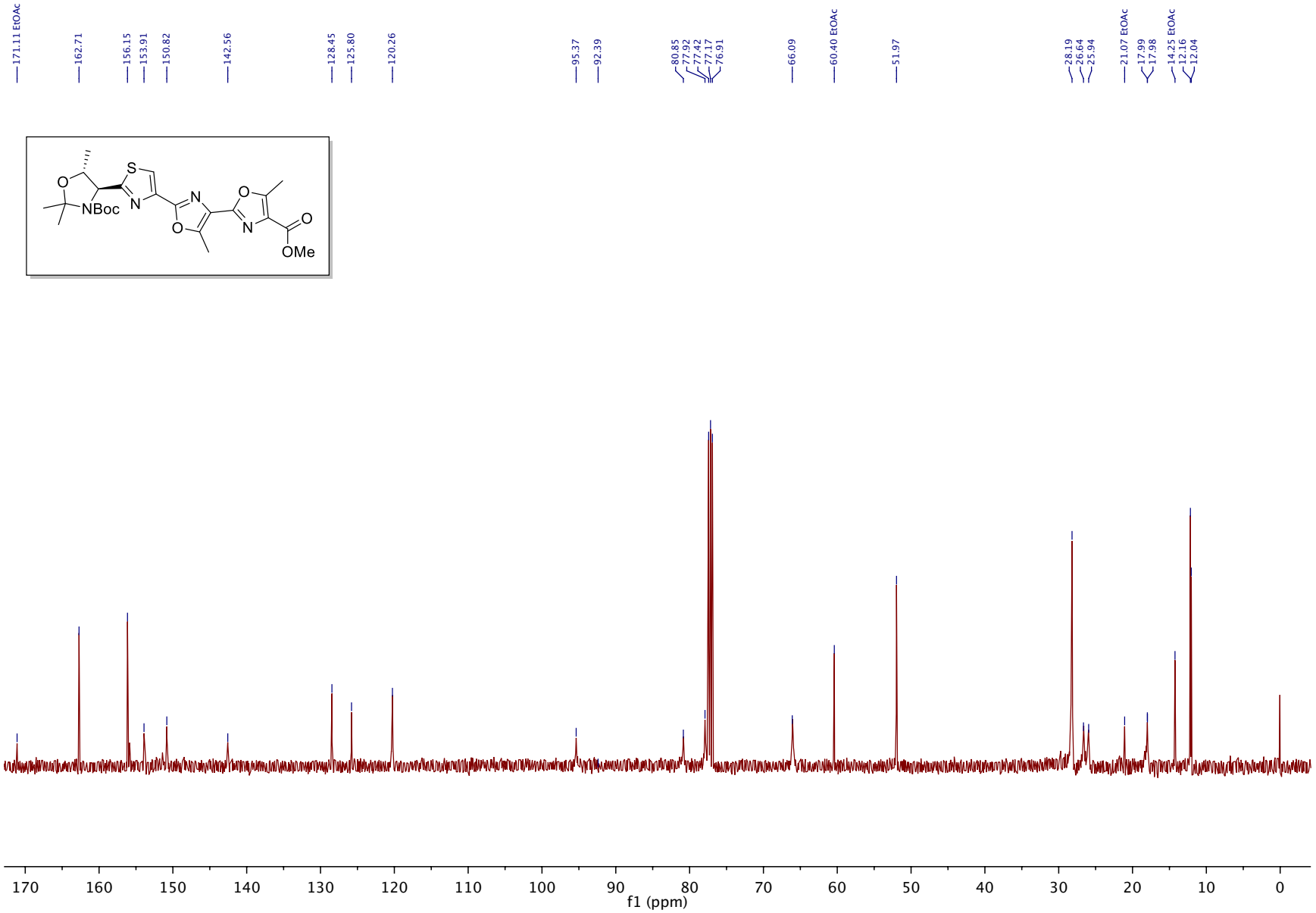
$^{13}$C NMR, compound **13** (Scheme 3)



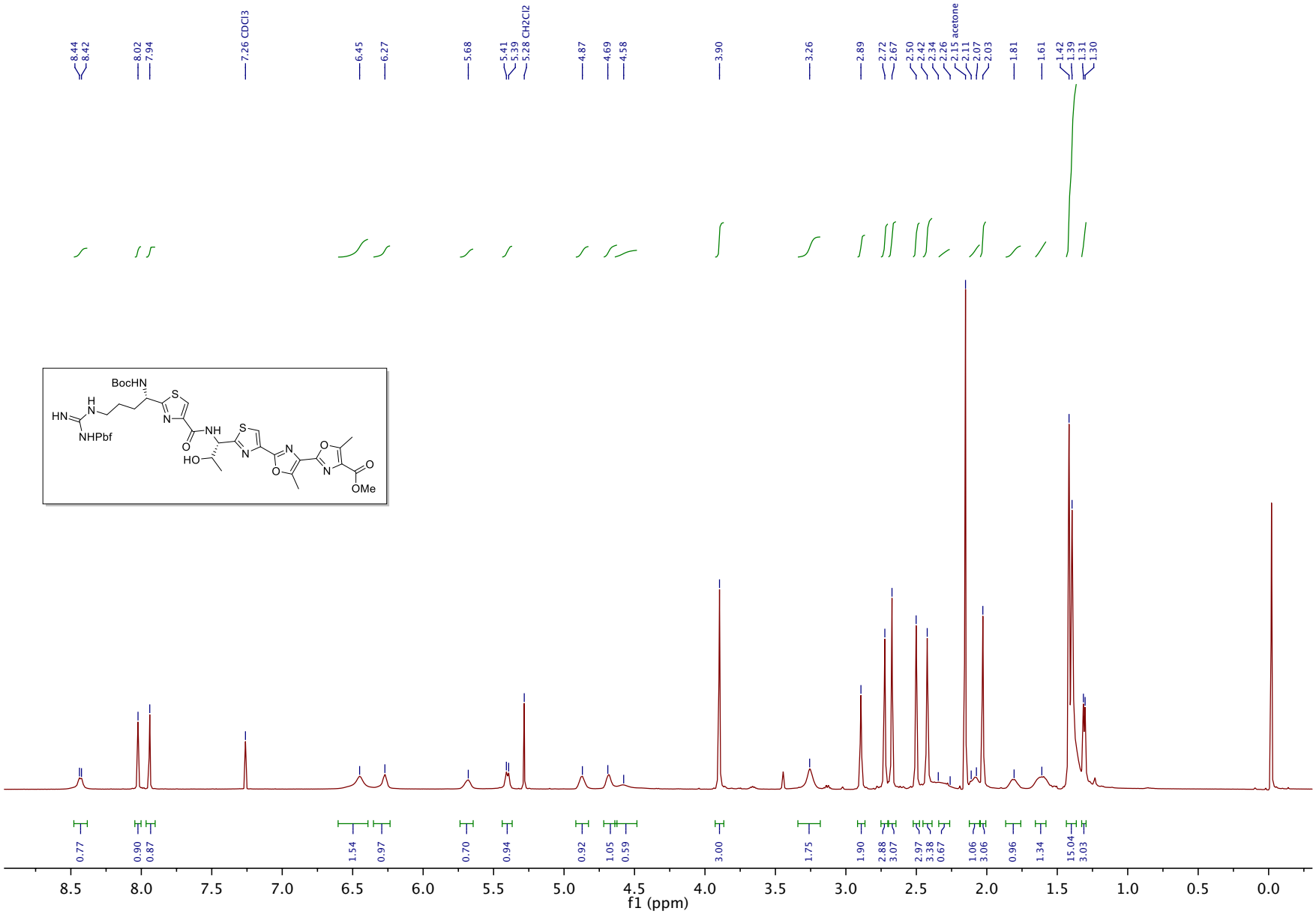
$^{1}$H NMR, compound **14** (Scheme 3)



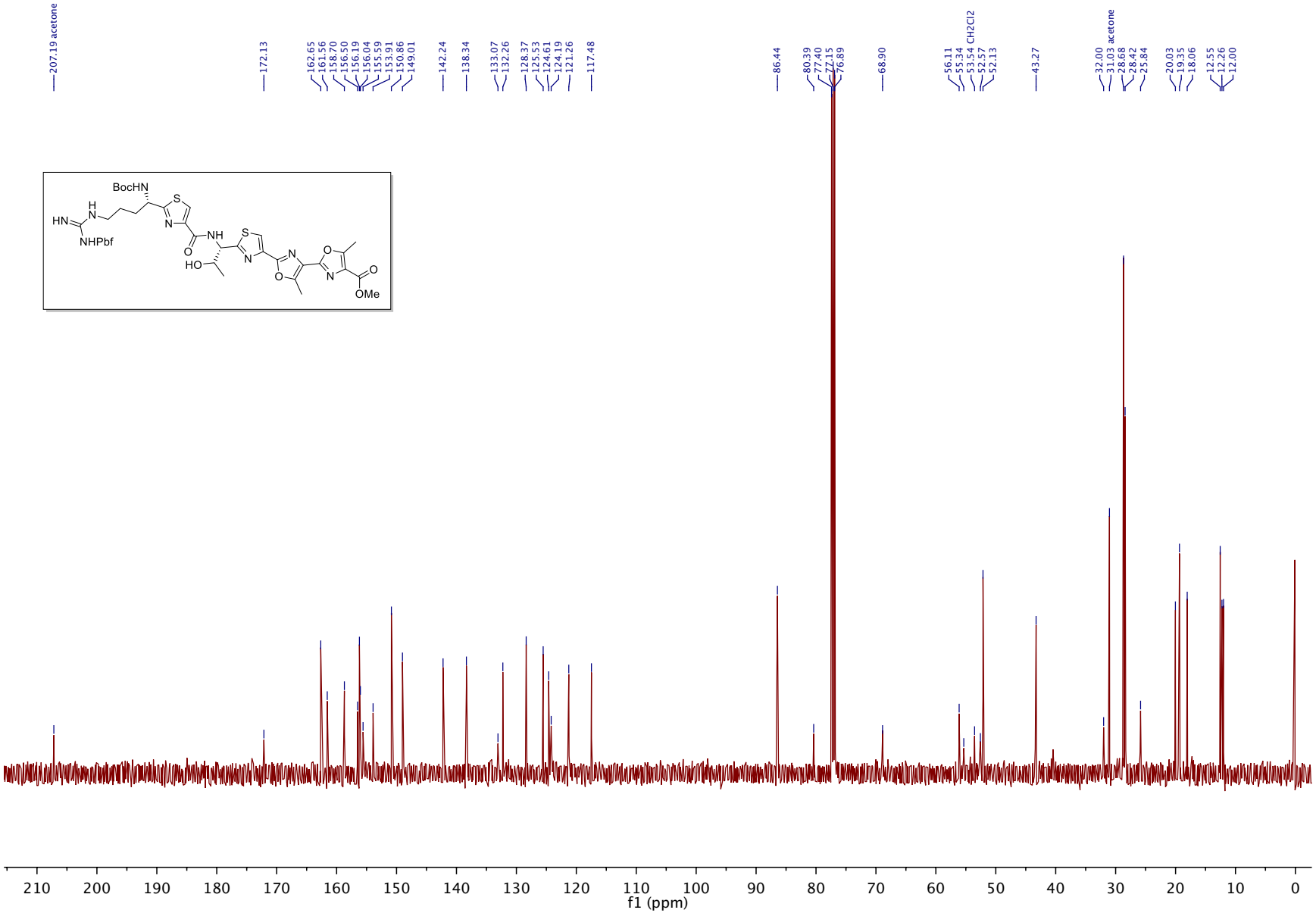
$^{13}$C NMR, compound **14** (Scheme 3)



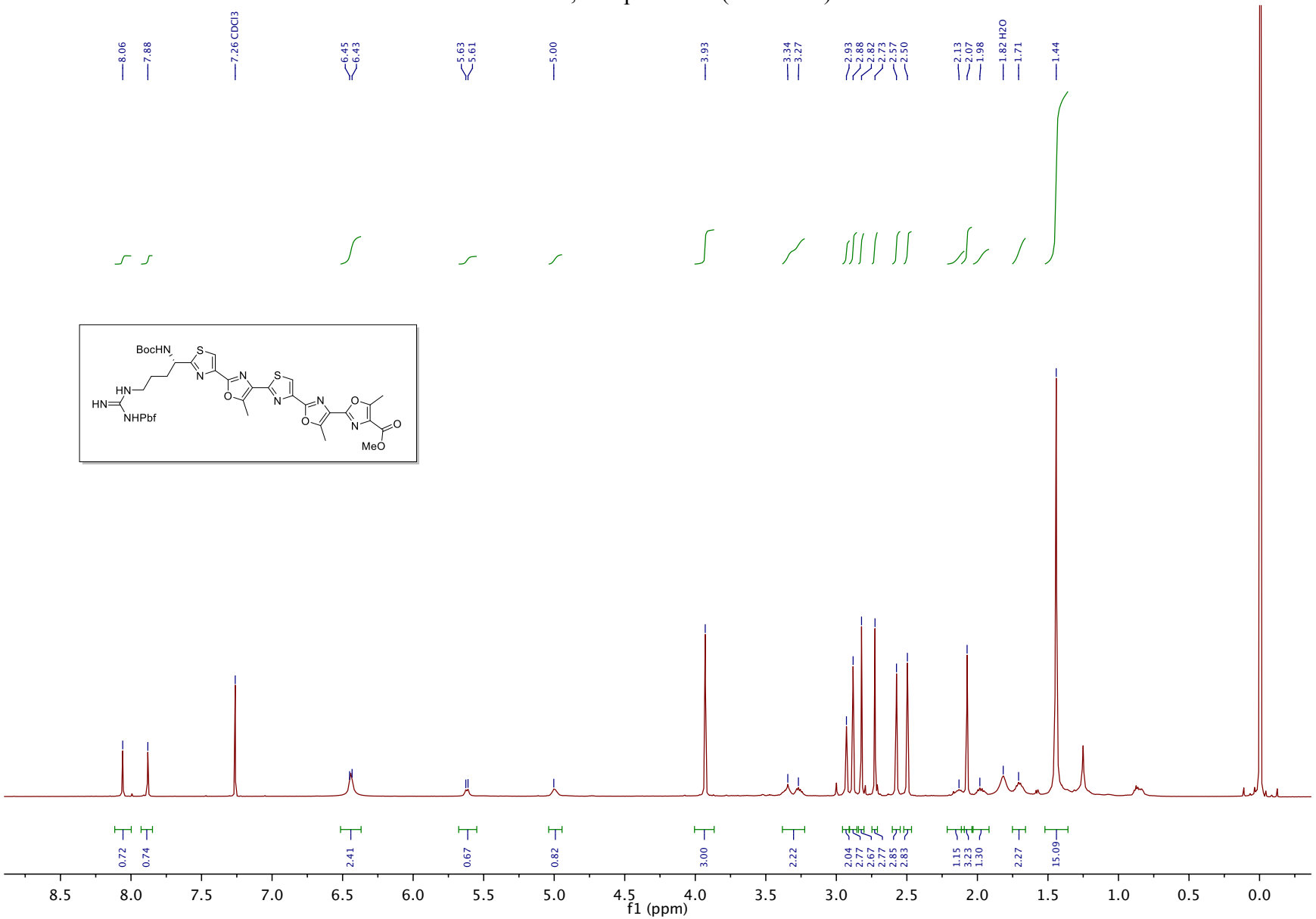
$^{1}$H NMR, compound **15** (Scheme 3)



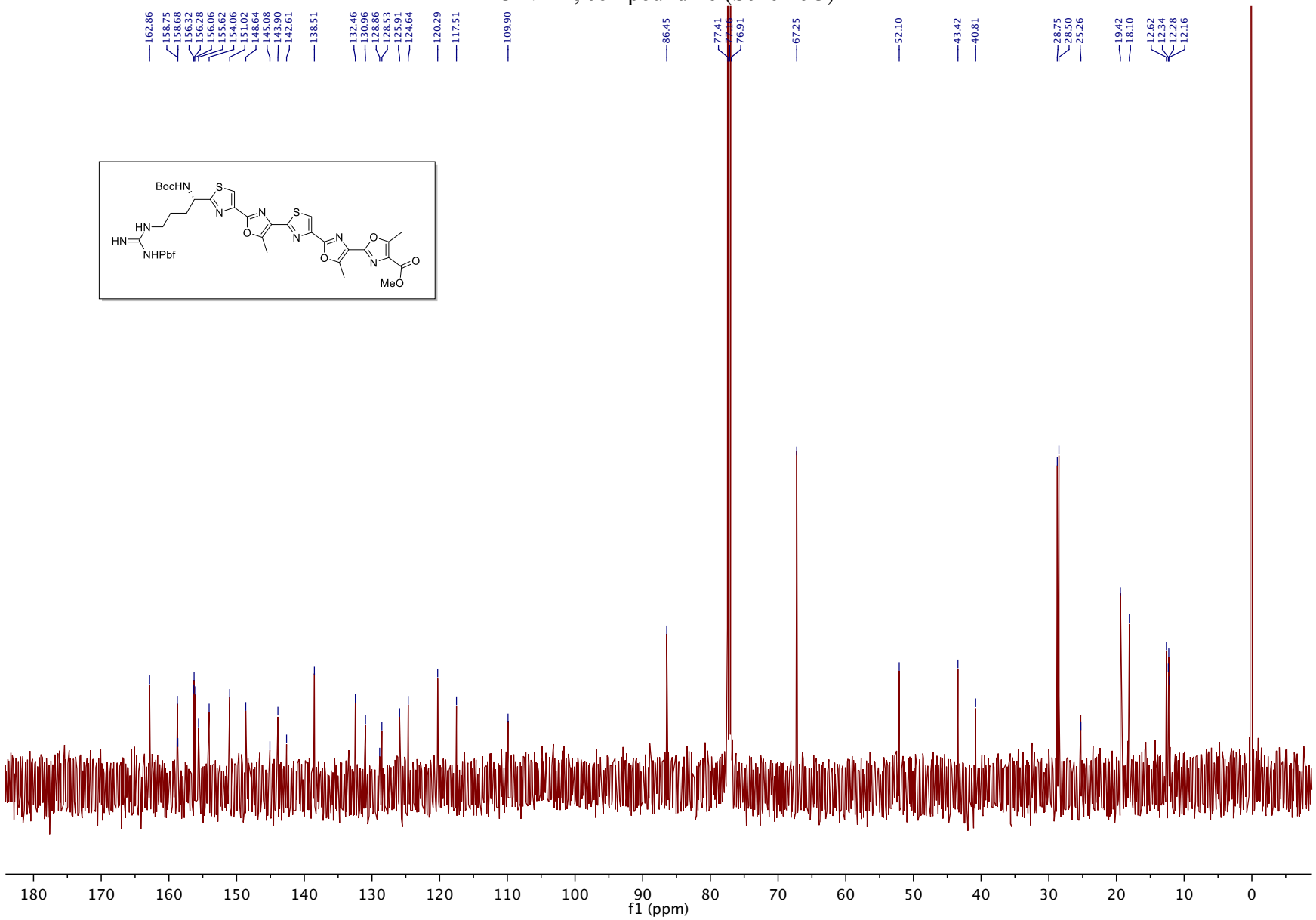
$^{13}$C NMR, compound **15** (Scheme 3)



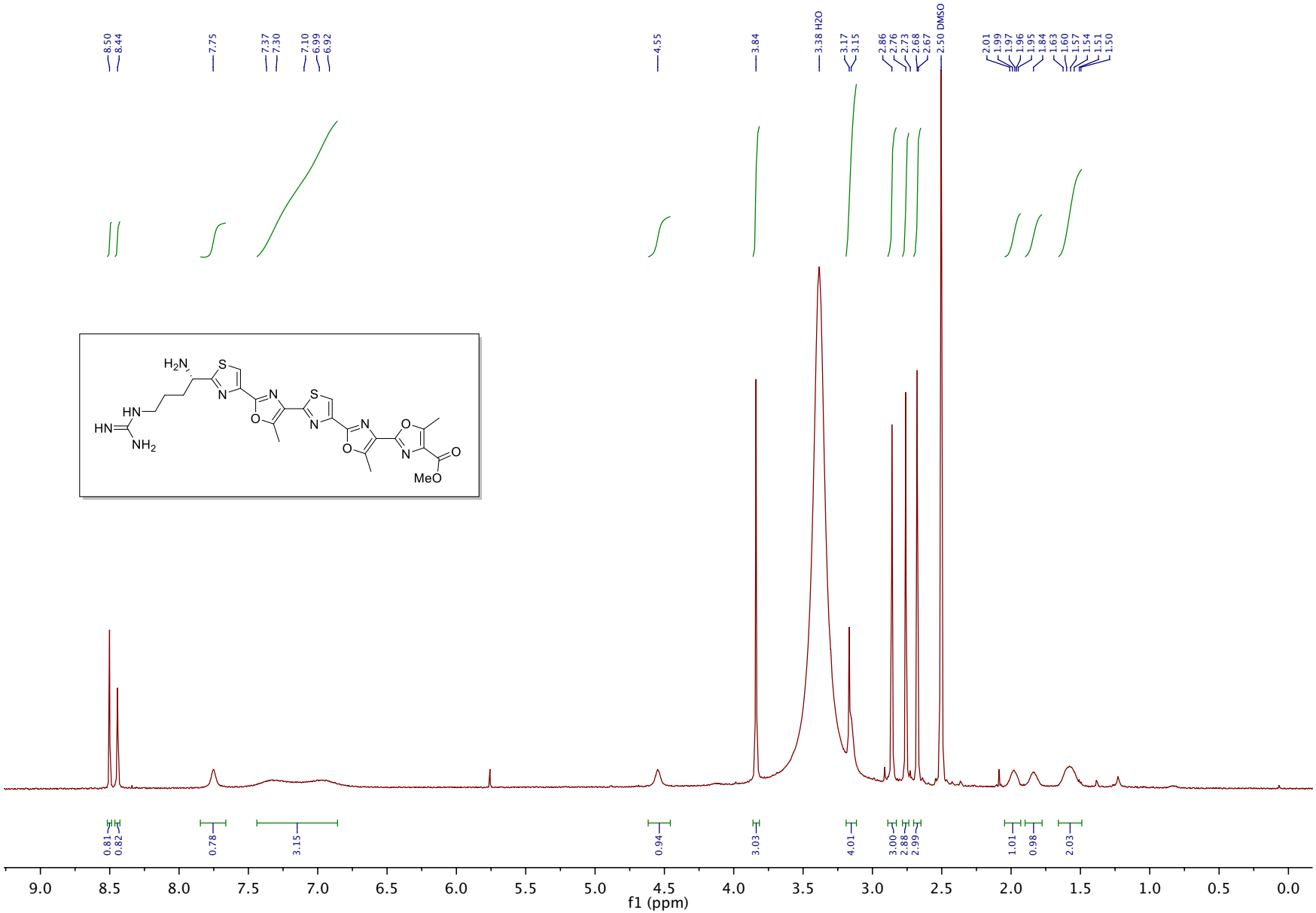
$^{1}$H NMR, compound **16** (Scheme 3)



$^{13}$C NMR, compound **16** (Scheme 3)



$^{1}$H NMR, compound **4** (Scheme 3)



$^{13}$C NMR, compound **4** (Scheme 3)