

Supporting Information

Design, synthesis and anticancer mechanistic studies of linked azoles

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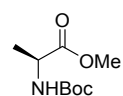
General Remarks

All reactions were carried out under an argon atmosphere with dry solvents,, unless otherwise stated. Reagents were commercially obtained (Peptides International, ChemImpex, Aldrich, and Acros) at the highest quality and used without further purification. Reactions were monitored via thin-layer chromatography (TLC) carried out on 250µm Whatman silica gel plates (4861-820) using UV light as the visualizing agent and potassium permanganate, ninhydrin and bromocresol green as developing agents. SiliCycle SiliaFlash silica gel (60, particle size 40-63µm) was used for flash chromatography. Automated column chromatography was performed on Teledyne CombiFlash Rf-200 using 12g silica flash columns and 25g solid sample cartridges. Yields refer to spectroscopically and/or chromatographically homogeneous materials.

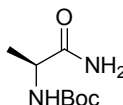
NMR spectra were obtained at 25°C on either a 600-MHz Varian NMR-S, 500-MHz INOVA, or 300-MHz Varian NMR-S using residual undeuterated solvent as an internal reference. The following abbreviations were used to explain multiplicities: s = singlet, d = doublet, t = triplet, q = quartet, p = pentet, m = multiplet, br = broad, bd = broad doublet, dd = doublet of doublet, and dq = doublet of quartet.

LC/MS was recorded on Shimadzu Prominence High performance LCMS 2010 EV system (Water Symmetry® C18 column, 3.5 µm, 4.6x75 mm) connected to connected to a Shimadzu LCMS 2010 EV mass spectrometer running in the positive electrospray ionization (ESI+) mode. The mobile phases were composed of DDI water with 0.1% (v/v) formic acid (solvent A) and HPLC grade acetonitrile with 0.1% (v/v) formic acid (solvent B). The gradient elution was as follows: flow rate 1.0 mL/min; initial 80% solvent A, 20% solvent B; at 4.5 min 10% solvent A, 90% solvent B hold 0.1 min; at 7 min 85% solvent A, 15% solvent B. HRMS data were obtained at the Bioanalytical Mass Spectrometry Facility within the Mark Wainwright Analytical Centre of the University of New South Wales. HRMS data were recorded on a Thermo LTQ FT LC/MS/MS system. Subsidised access to this facility is gratefully acknowledged.

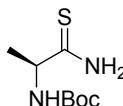
Experimental Procedures



BocHN-Ala-OMe . 2.10 g (11.1 mmol, 1.0 equiv.) of BocHN-Ala-OH was dissolved in 84.0 mL of dry MeOH and 28.0 mL of anhydrous benzene under Argon. 7.50 mL of trimethylsilyldiazomethane (TMSD)(2.0 M in diethyl ether) was added to the reaction dropwise followed by stirring for 1.0 hr. The resulting methyl ester was concentrated *in vacuo* and taken on to the next reaction without further purification (2.30 g, quantitative) as light yellow oil. R_f : 0.82 (hexane/ ethyl acetate (EtOAc) 1:1). ^1H NMR (300 MHz, DMSO): δ 1.22 (d, $J=7.36$ Hz, 3H, CH_3CH); 1.37 (s, 9H, $\text{C}(\text{CH}_3)_3$); 3.61 (s, 3H, OCH_3); 4.22-4.40 (m, 1 α H); 7.26 (d, $J=7.22$ Hz, NH). ^{13}C NMR (75MHz, DMSO) 17.35, 29.63 (3C), 49.34, 52.18, 78.60, 155.70, 174.07. ES^+MS m/z calcd for $\text{C}_8\text{H}_{15}\text{NO}_4$ ($[\text{M}+\text{Na}]$) 212.10, found 212.23



BocHN-Ala-C(O)-NH₂ (2L). BocHN-Ala-C(O)-NH₂ was synthesized utilizing 2.30 g (11.1 mmol, 1.0 equiv.) of Boc-Ala-OMe in 110 mL of ammonium hydroxide solution (25% in water) and 110 mL of methanol. The reaction was stirred overnight then concentrated *in vacuo*; the resulting amide was taken on to the next reaction without further purification (2.10 g, quantitative) as a white powder. R_f : 0.20 (hexane/ EtOAc 1:1). ^1H NMR (300 MHz, DMSO): δ 1.15-1.22 (m, $J=7.14$ Hz, 3H, CH_3CH); 1.37 (s, 9H, $\text{C}(\text{CH}_3)_3$); 3.83-3.92 (m, 1 α H); 6.68-7.27 (m, 3H, NH, NH_2). ^{13}C NMR (75MHz, DMSO) 18.78, 28.65 (3C), 49.93, 78.34, 155.45, 175.24. ES^+MS m/z calcd for $\text{C}_8\text{H}_{16}\text{N}_2\text{O}_3$ ($[\text{M}+\text{Na}]$) 211.12, found 211.17

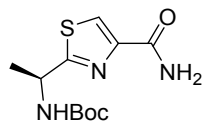


BocHN-Ala-C(S)-NH₂ (3L). BocHN-Ala-C(S)-NH₂ was synthesized utilizing 1.50g (7.97 mmol, 1.0 equiv.) of BocHN-Ala-C(O)-NH₂ and 2.42 g (7.97 mmol, 0.75 equiv.) of Lawesson's Reagent dissolved in 160 mL of THF. The reaction mixture was stirred overnight and upon completion confirmed via TLC, the crude reaction mixture was rotovapped down to dryness and purified via Flash column chromatography on silica gel (hexane/ EtOAc 1:1 to 3:7) to afford the desired thioamide (1.11 mg, 68%) as a white solid. R_f : 0.60 (hexane/EtOAc 1:1). ^1H NMR (300 MHz, DMSO): δ 1.24 (d, $J=7.20$ Hz, 1H, CHCH_3), 1.38 (s, 9H, $\text{C}(\text{CH}_3)_3$), 4.20-4.30 (m, 1 α H), 6.80 (d, $J=7.36$ Hz, NH), 9.08 (s,

1H, NH_aH_b), 9.55 (s, 1H, NH_aH_b). ¹³C NMR (75MHz, DMSO) 21.55, 28.64(3C), 56.11, 78.62, 155.02, 209.88. ES⁺MS *m/z* calcd for C₈H₁₆N₂O₂S ([M+Na]) 227.09, found 227.11.

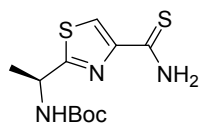


BocHN-Ala-Thiazole-OEt (4L). BocHN-Ala-Thiazole-OEt was synthesized utilizing 1.50 g (7.34 mmol, 1.0 equiv.) of BocHN-Ala-C(S)-NH₂ dissolved in 145 mL of 1,2-dimethoxyethane (DME), under Argon. 5.88 g (58.0 mmol, 8.0 equiv.) of potassium hydrogen carbonate (KHCO₃) was added to the reaction and the mixture stirred for 5 min. 2.76 mL of ethyl bromopyruvate (22.02 mmol, 3.0 equiv.) was dissolved in an additional 5 mL of DME and added dropwise (1 mL/min) to the reaction vessel. The reaction mixture stirred overnight and upon completion the desired hydroxythiazoline intermediate was concentrated *in vacuo*, re-dissolved in EtOAc, and extracted with Sat. brine solution, dried over anhydrous sodium sulphate (Na₂SO₄), filtered and concentrated *in vacuo*. The crude hydroxythiazoline intermediate was dissolved in 147 mL of DME and stirred at 0°C for 15 min. 5.30 mL (66.0 mmol, 9.0 equiv.) of pyridine was added to reaction mixture (0.1 mL/min) and stirred at 0°C for an additional 15 min. 4.15 mL (29.4 mmol, 4.0 equiv.) trifluoroacetic anhydride was added to the reaction mixture (0.1 mL/min) and stirred at 0°C for an additional 2 hours. Finally, 2.05 mL (14.70 mmol, 2.0 equiv.) triethylamine (TEA) was added to the reaction dropwise (0.1 mL/min) and ran at 0°C to room temperature overnight. The reaction was concentrated *in vacuo* and re-dissolved in 200 mL EtOAc. The crude reaction was washed with pH 1 hydrochloric acid solution (100 mL x 2), then saturated NaHCO₃ solution (100 mL x 10) and finally brine solution (100 mL x 2). The organic layer was collected, combined, dried over anhydrous Na₂SO₄, filtered and concentrated *in vacuo*. The crude product underwent column chromatographic purification on silica gel (hexanes/EtOAc 9:11 to 2:3) to afford the desired thiazole (1.64g, 74.4% over 2 steps) as a white solid. *R_f*: 0.76 (hexane/ EtOAc 1:1). ¹H NMR (300 MHz, CDCl₃): δ 1.39 (t, *J*=7.15 Hz, 3H, CH₂CH₃); 1.43 (br, 9H, C(CH₃)₃); 1.61 (d, *J*=6.80 Hz, 3H, CH₃CH); 4.40 (q, *J*=7.18 Hz, 2H, CH₂CH₃); 5.12 (br, 1αH); 5.21 (br, 1H, NH); 8.07 (s, 1H, SCHC). ¹³C NMR (75MHz, CDCl₃) δ 14.36, 21.79, 28.31(3C), 48.91, 61.43, 80.31, 127.13, 147.20, 154.85, 161.34, 174.91. ES⁺MS *m/z* calcd for C₁₃H₂₀N₂O₄S ([M+Na]) 323.11, found 322.85



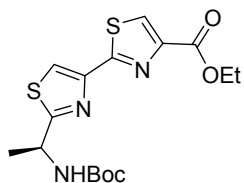
BocHN-Ala-Thiazole-C(O)-NH₂ (5L) BocHN-Ala-Thiazole-C(O)-NH₂ was synthesized

utilizing 1.50 g (5.00 mmol, 1.0 equiv.) of BocHN-Ala-Thiazole-OMe in 60 mL of ammonium hydroxide solution (25% in water) and 40 mL of EtOH. The reaction was stirred overnight and concentrated *in vacuo*; the resulting amide was taken on to the next reaction without further purification (1.49 g, quantitative) as a white powder. *R_f*: 0.26 (hexane/ EtOAc 1:1). ¹H NMR (300 MHz, CDCl₃): δ 1.46 (s, 9H, C(CH₃)₃); 1.60 (s, *J*=6.84 Hz, 3H, CH₃CH); 5.09 (br, 1αH); 5.16 (br, 1H, NH); 5.94 (br, 1H, NH₂); 7.20 (br, 1H, NH₂); 8.07 (s, 1H, SCHC). ¹³C NMR (75MHz, CDCl₃) δ 21.60, 28.32(3C), 48.69, 80.47, 124.30, 149.01, 154.89, 161.97, 174.56. ES⁺MS *m/z* calcd for C₁₁H₁₇N₃O₃S ([M+Na]) 294.10, found 293.80



BocHN-Ala-Thiazole-C(S)-NH₂ (6L). BocHN-Ala-Thiazole-C(S)-NH₂ was synthesized utilizing

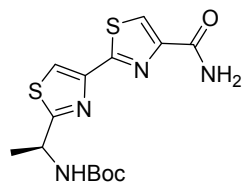
1600 mg (5.90 mmol, 1.0 equiv.) of BocHN-Ala-Thiazole-C(O)-NH₂ and 1.79 g (4.43 mmol, 0.75 equiv.) of Lawesson's Reagent dissolved in 118 mL of THF. The reaction mixture was stirred overnight and upon completion confirmed via TLC, the crude reaction mixture was rotovapped down to dryness and purified via Flash column chromatography on silica gel (hexane/ EtOAc 1:2 to 3:7) to afford the desired thioamide (1102 mg, 65%) as a yellow solid. *R_f*: 0.67 (hexanes/EtOAc 1:1). ¹H NMR (300 MHz, CDCl₃): δ 1.45 (s, 9H, C(CH₃)₃), 1.59 (d, *J*=6.70 Hz, 3H, CHCH₃), 5.07 (br, 1αH), 5.12 (br, 1H, NH), 7.70 (br, 1H, NH_aH_b), 8.36 (s, 1H, SCHC), 8.65 (br, 1H, NH_aH_b). ¹³C NMR (75MHz, CDCl₃) δ 21.49, 28.31(3C), 48.79, 80.57, 127.31, 152.74, 154.86, 174.34, 190.48. ES⁺MS *m/z* calcd for C₁₁H₁₇N₃O₂S₂ ([M+Na]) 310.08, found 309.75



BocHN-Ala-Thiazole-Thiazole-OEt (7L). BocHN-Ala-Thiazole-Thiazole-OEt was synthesized

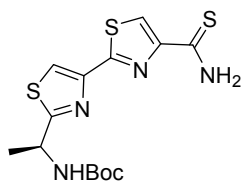
utilizing 1.05 g (3.65 mmol, 1.0 equiv.) of BocHN-Ala-Thiazole-C(S)-NH₂ dissolved in 73.0 mL of DME, under Ar. 2.92 g (29.2 mmol, 8.0 equiv.) of KHCO₃ was added to the reaction and the mixture stirred for 5 min. 1.37 mL of ethyl bromopyruvate (10.95 mmol, 3.0 equiv.) was dissolved in an additional 10 mL of 1,2-dimethoxyethane and added dropwise (1 mL/min) to the reaction vessel. The reaction mixture stirred overnight

and upon completion the desired hydroxythiazoline intermediate was concentrated *in vacuo*, redissolved in EtOAc, extracted with brine, dried over anhydrous Na₂SO₄, filtered and concentrated *in vacuo*. The crude hydroxythiazoline intermediate was dissolved in 73.0 ml DME and stirred at 0°C for 15 min. 2.65 mL (32.85 mmol, 9.0 equiv.) of pyridine was added to reaction mixture (0.1 mL/min) and stirred at 0°C for an additional 15 min. 2.06 mL (14.6 mmol, 4.0 equiv.) trifluoroacetic anhydride was added to the reaction mixture (0.1 mL/min) and stirred at 0°C for an additional 2 hours. Finally, 1018 µL (7.30 mmol, 2.0 equiv.) triethylamine was added to the reaction dropwise (0.1 mL/min) and ran at 0°C to room temperature overnight. The reaction was concentrated *in vacuo* and redissolved in 100 mL EtOAc. The crude reaction was washed with pH 1 hydrochloric acid solution (60 mL x 2), then saturated sodium bicarbonate solution (50 mL x 10) and finally brine (50 mL x 2). The organic layer was collected, combined, dried over anhydrous Na₂SO₄, filtered and concentrated *in vacuo*. The crude product underwent column chromatographic purification on silica gel (hexanes/ EtOAc 9:11 to 2:3) to afford the desired thiazole (1000 mg, 71.45%) as a white solid. *R_f*: 0.70 (hexanes/EtOAc 1:1). ¹H NMR (300 MHz, CDCl₃): δ 1.41 (t, *J*=7.05 Hz, 3H, CH₂CH₃); 1.45 (br, 9H, C(CH₃)₃); 1.63 (d, *J*=6.83 Hz, 3H, CH₃CH); 4.43 (q, *J*=7.08 Hz, 2H, CH₂CH₃); 5.10 (br, 1αH); 5.20 (br, 1H, NH); 8.06 (s, 1H, SCHC), 8.15 (s, 1H, SCHC). ¹³C NMR (75MHz, CDCl₃) δ 14.35, 21.69, 28.34(3C), 48.79, 61.45, 80.36, 117.25, 127.73, 147.83, 148.14, 154.93, 161.41, 163.38, 174.90. ES⁺MS *m/z* calcd for C₁₆H₂₁N₃O₄S₂ ([M+Na]) 406.10, found 405.80



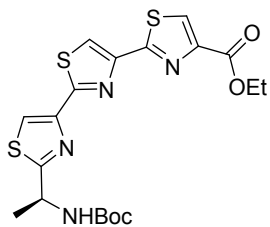
BocHN-Ala-Thiazole-Thiazole-C(O)-NH₂ (8L). BocHN-Ala-Thiazole-Thiazole-C(O)-NH₂ was synthesized utilizing 0.98 g (2.56 mmol, 1.0 equiv.) of BocHN-Ala-Thiazole-Thiazole-OEt in 30.0 mL of ammonium hydroxide solution (25% in water) and 20.0 mL of EtOH. The reaction was

stirred overnight and concentrated *in vacuo*; the resulting amide was taken on to the next reaction without further purification (0.91 g, quantitative) as a white powder. *R_f*: 0.22 (hexane/ EtOAc 1:1). ¹H NMR (300 MHz, CDCl₃): δ 1.41 (t, *J*=7.05 Hz, 3H, CH₂CH₃); 1.45 (br, 9H, C(CH₃)₃); 1.63 (d, *J*=6.83 Hz, 3H, CH₃CH); 4.43 (q, *J*=7.08 Hz, 2H, CH₂CH₃); 5.10 (br, 1αH); 5.20 (br, 1H, NH); 8.06 (s, 1H, SCHC), 8.15 (s, 1H, SCHC). ¹³C NMR (75MHz, CDCl₃) δ 21.65, 28.35(3C), 40.79, 80.37, 116.73, 124.73, 147.83, 148.16, 154.97, 162.76, 162.93, 175.21. ES⁺MS *m/z* calcd for C₁₄H₁₈N₄O₃S₂ ([M+Na]) 377.08, found 376.80



BocHN-Ala-Thiazole-Thiazole-C(S)-NH₂ (9L). BocHN-Ala-Thiazole-Thiazole-C(S)-NH₂ was synthesized utilizing 1000 mg (2.82 mmol, 1.0 equiv.) of BocNH-Ala-Thiazole-Thiazole-C(O)-NH₂ and 0.86 g (2.12 mmol, 0.75 equiv.) of Lawesson's Reagent dissolved in 56.5 mL of THF.

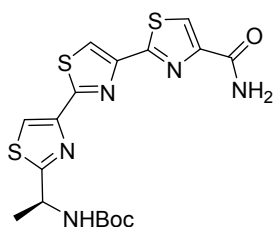
The reaction mixture was stirred overnight and upon completion confirmed via TLC, the crude reaction mixture was rotovapped down to dryness and purified via Flash column chromatography on silica gel (hexane/ EtOAc 1:1 to 5:9) to afford the desired thioamide (650 mg, 62.18%) as a white solid. *R_f*: 0.6 (hexane/EtOAc 1:1). ¹H NMR (300 MHz, CDCl₃): δ 1.47 (br, 9H, C(CH₃)₃); 1.65 (d, *J*=6.66 Hz, 3H, CH₃CH); 5.12 (br, 1αH); 5.18 (br, 1H, NH); 7.74(br, 1H, NH₂), 7.95 (br, 1H, SCHC), 8.47 (br, 1H, SCHC), 8.82 (br, 1H, NH₂). ¹³C NMR (75MHz, CDCl₃) δ 21.65, 28.34(3C), 48.84, 80.46, 117.03, 128.04, 147.91, 153.43, 154.94, 162.76, 162.28, 175.4, 190.37. ES⁺MS *m/z* calcd for C₁₄H₁₈N₄O₂S₃ ([M+Na]) 393.06, found 392.85



BocHN-Ala-Thiazole-Thiazole-Thiazole-OEt (10L). BocHN-Ala-Thiazole-Thiazole-Thiazole-OEt was synthesized utilizing 600 mg (1.62 mmol, 1.0 equiv.) of BocHN-Ala-Thiazole-Thiazole-C(S)-NH₂ dissolved in 32.5 mL of DME, under Argon. 1.30 g (12.96 mmol, 8.0 equiv.) of KHCO₃ was added to the reaction and the mixture stirred for 5 min. 0.61 mL of ethyl

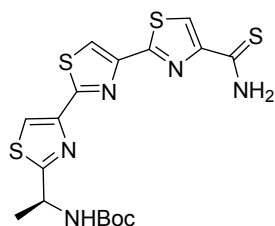
bromopyruvate (4.86 mmol, 3.0 equiv.) was dissolved in an additional 5.0 mL of DME and added dropwise (1 mL/min) to the reaction vessel. The reaction mixture stirred overnight and upon completion the desired hydroxythiazoline intermediate was concentrated *in vacuo*, re-dissolved in EtOAc, extracted with brine, all organic layer was collected, combined, dried over anhydrous Na₂SO₄, filtered and concentrated *in vacuo*. Without purification, the crude hydroxythiazoline intermediate was dissolved in 32.5 mL DME and stirred at 0°C for 15 min. 1.17 mL (14.6 mmol, 9.0 equiv.) of pyridine was added to reaction mixture (0.1 mL/min) and stirred at 0°C for an additional 15 min. 0.915 mL (6.48 mmol, 4.0 equiv.) trifluoroacetic anhydride was added to the reaction mixture (0.1 mL/min) and stirred at 0°C for an additional 2 hours. Finally, 452 μL (3.24 mmol, 2.0 equiv.) TEA was added to the reaction dropwise (0.1 mL/min) and ran at 0°C to room temperature overnight. The reaction was concentrated *in vacuo* and redissolved in 30 mL EtOAc. The crude reaction was washed with pH=1 hydrochloric acid solution (30 mL x 2), then saturated sodium bicarbonate solution

(30 mL x 10) and finally brine (30 mL x 2). The organic layer was collected, combined, dried over anhydrous Na₂SO₄, filtered and concentrated *in vacuo*. Crude product was underwent column chromatographic purification on silica gel (hexanes/ EtOAc 9:11 to 2:9) to afford the desired product thiazole (550 mg, 72.7 %) as a light yellow solid. *R_f*: 0.72 (hexane/ EtOAc 1:1). ¹H NMR (300 MHz, CDCl₃): δ 1.42 (t, *J*=7.11 Hz, 3H, CH₂CH₃); 1.45 (br, 9H, C(CH₃)₃); 1.63 (d, *J*=6.69 Hz, 3H, CH₃CH); 4.44 (q, *J*=7.19 Hz, 2H, CH₂CH₃); 5.11 (br, 1αH); 5.19 (br, 1H, NH); 7.96 (s, 1H, SCHC), 8.14 (s, 1H, SCHC), 8.18 (s, 1H, SCHC). ¹³C NMR (75MHz, CDCl₃) δ 14.37, 21.72, 28.35(3C), 48.80, 61.56, 80.32, 116.67, 117.97, 127.82, 147.95, 148.39, 149.13, 154.96, 161.45, 163.10, 163.36, 174.83. ES⁺MS *m/z* calcd for C₁₉H₂₂N₄O₄S₃ ([M+Na]) 489.68, found 489.90



Boc-Ala-Thiazole-Thiazole-Thiazole-C(O)-NH₂ (11L). BocHN-Ala-Thiazole-Thiazole-Thiazole-C(O)-NH₂ was synthesized utilizing 200 mg (0.43 mmol, 1.0 equiv.) of BocHN-Ala-Thiazole-Thiazole-Thiazole-OEt in 10 mL of ammonium hydroxide solution (25% in water) and 10 mL of EtOH. The reaction mixture was sonicated for 2 hr, with subjective NH₄OH (20.0 ml)

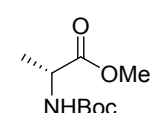
and EtOH (20.0 ml) addition. The reaction was stirred overnight and concentrated *in vacuo*; the resulting amide was taken on to the next reaction without further purification (250 mg, quantitative) as a white powder. *R_f*: 0.21 (hexane/ EtOAc 1:1). ¹H NMR (300 MHz, DMSO): 1.48 (br, 9H, C(CH₃)₃); 1.66 (d, *J*=6.24 Hz, 3H, CH₃CH); 5.13 (br, 1αH); 5.16 (br, 1H, NH); 5.72 (br, 1H, NH₂); 7.32 (br, 1H, NH₂); 8.01 (s, 1H, SCHC), 8.02 (s, 1H, SCHC); 8.19 (s, 1H, SCHC). ¹³C NMR (75MHz, DMSO) δ 20.98, 28.66(3C), 49.06, 79.07, 118.41, 118.93, 125.10, 147.55, 149.22, 151.64, 155.58, 161.89, 162.63, 163.60, 178.22. ES⁺MS *m/z* calcd for C₁₇H₁₉N₅O₃S₃ ([M+Na]) 460.07, found 460.10



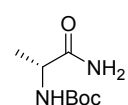
Boc-Ala-Thiazole-Thiazole-Thiazole-C(S)-NH₂ (12L). BocHN-Ala-Thiazole-Thiazole-Thiazole-C(S)-NH₂ was synthesized utilizing 250 mg (0.43 mmol, 1.0 equiv.) of BocHN-Ala-Thiazole-Thiazole-Thiazole-C(O)-NH₂ and 130 mg (0.323 mmol, 0.75 equiv.) of Lawesson's Reagent dissolved in 9.0 mL of THF. The reaction mixture was stirred overnight and upon

completion confirmed via TLC, the crude reaction mixture was rotovapped down to dryness and purified via Flash

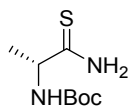
column chromatography on silica gel (hexane/ EtOAc 1:1 to 3:11) to afford the desired thioamide (160 mg, 82.1%) as a yellow solid. R_f : 0.67 (hexanes/EtOAc 1:1). ^1H NMR (300 MHz, DMSO): 1.42 (br, 9H, $\text{C}(\text{CH}_3)_3$); 1.51 (d, $J=7.02$ Hz, 3H, CH_3CH); 4.92 (m, 1 α H); 7.88 (d, $J=7.54$ Hz, NH); 8.27 (s, 1H, SCHC), 8.36 (s, 1H, SCHC); 8.49 (s, 1H, SCHC), 9.60 (s, 1H, NH_2); 10.10 (s, 1H, NH_2). ^{13}C NMR (75MHz, DMSO) δ 20.98, 28.66(3C), 49.06, 79.07, 118.43, 119.43, 128.08, 147.53, 149.20, 154.81, 155.59, 161.47, 163.66, 178.23, 189.44. ES^+MS m/z calcd for $\text{C}_{17}\text{H}_{19}\text{N}_5\text{O}_2\text{S}_4$ ($[\text{M}+\text{Na}]$) 476.04, found 476.15



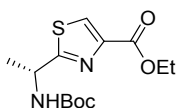
BocHN-D-Ala-OMe. BocHN-D-Ala-OMe was synthesized utilizing 2.10 g (11.1 mmol, 1.0 equiv.) of BocHN-D-Ala-OH dissolved in 84.0 mL of dry MeOH and 28.0 mL of anhydrous benzene. In total, 7.50 mL of TMSD, 2.0 M in diethyl ether was added to the reaction in dropwise followed by stirring for 1.0 hr. The resulting methyl ester was concentrated *in vacuo* and taken on to the next reaction without further purification (2.30 g, quantitative) as light yellow oil. R_f : 0.82 (hexane/ EtOAc 1:1). R_f : 0.89 (hexane/ EtOAc 1:1). ^1H NMR (300 MHz, DMSO): δ 1.23 (d, $J=7.30$ Hz, 3H, CH_3CH); 1.38 (s, 9H, $\text{C}(\text{CH}_3)_3$); 3.62 (s, 3H, OCH_3); 3.97-4.07 (m, 1 α H); 7.27 (d, $J=7.35$ Hz, NH). ^{13}C NMR (75MHz, DMSO) 17.35, 29.63 (3C), 49.34, 52.17, 78.59, 155.69, 174.06. ES^+MS m/z calcd for $\text{C}_8\text{H}_{15}\text{NO}_4$ ($[\text{M}+\text{Na}]$) 212.10, found 212.15



BocHN-D-Ala-C(O)-NH₂ (2D). BocHN-D-Ala-C(O)-NH₂ was synthesized utilizing 2.30 g (11.1 mmol, 1.0 equiv.) of BocNH-D-Ala-OMe in 110 mL of ammonium hydroxide solution (25% in water) and 110 mL of MeOH. The reaction was stirred overnight and concentrated *in vacuo*; the resulting amide was taken on to the next reaction without further purification (2.10 g, quantitative) as a white powder. R_f : 0.20 (hexane/ EtOAc 1:1). ^1H NMR (300 MHz, DMSO): δ 1.10 (d, $J=7.19$ Hz, 3H, CH_3CH); 1.31 (s, 9H, $\text{C}(\text{CH}_3)_3$); 3.77-3.87 (m, 1 α H); 6.69 (d, $J=7.41$ Hz, NH), 6.84 (br, 1H, NH_2), 7.15 (br, 1H, NH_2), ^{13}C NMR (75MHz, DMSO) 18.78, 28.66 (3C), 49.94, 78.35, 155.45, 175.25. ES^+MS m/z calcd for $\text{C}_8\text{H}_{16}\text{N}_2\text{O}_3$ ($[\text{M}+\text{Na}]$) 211.12, found 211.27

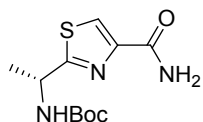


BocHN-D-Ala-C(S)-NH₂ (3D). BocHN-D-Ala-C(S)-NH₂ was synthesized utilizing 1.50g (7.97 mmol, 1.0 equiv.) of BocHN-D-Ala-C(O)-NH₂ and 2.42 g (7.97 mmol, 0.75 equiv.) of Lawesson's Reagent dissolved in 160 mL of THF. The reaction mixture was stirred overnight and upon completion confirmed via TLC, the crude reaction mixture was rotovapped down to dryness and purified via Flash column chromatography on silica gel (hexane/ EtOAc 1:1 to 3:7) to afford the desired thioamide (1.11 mg, 68%) as a white solid. *R_f*: 0.60 (hexane/EtOAc 1:1). ¹H NMR (300 MHz, DMSO): δ 1.24 (d, *J*=7.20 Hz, 1H, CHCH₃), 1.38 (s, 9H, C(CH₃)₃), 4.20-4.30 (m, 1αH), 6.80 (d, *J*=7.36 Hz, NH), 9.08 (s, 1H, NH_aH_b), 9.55 (s, 1H, NH_aH_b). ¹³C NMR (75MHz, DMSO) 21.55, 28.64(3C), 56.11, 78.62, 155.02, 209.88. ES⁺MS *m/z* calcd for C₈H₁₆N₂O₂S ([M+Na]) 227.09, found 227.25.

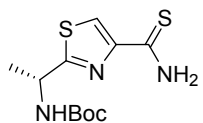


BocHN-D-Ala-Thiazole-OEt (4D). BocHN-D-Ala-Thiazole-OEt was synthesized utilizing 1.50 g (7.34 mmol, 1.0 equiv.) of BocHN-D-Ala-C(S)-NH₂ dissolved in 140 mL of DME under Argon. 5.88 g (58.0 mmol, 8.0 equiv.) of KHCO₃ was added to the reaction and the mixture stirred for 5 min. 2.76 mL of ethyl bromopyruvate (22.02 mmol, 3.0 equiv.) was dissolved in an additional 6 mL of DME and added dropwise (1 mL/min) to the reaction vessel. The reaction mixture stirred overnight and upon completion the desired hydroxythiazoline intermediate was concentrated *in vacuo*, re-dissolved in EtOAc, extracted with brine, all organic layer was collected, combined, and dried over anhydrous Na₂SO₄, filtered and concentrated *in vacuo*. The crude hydroxythiazoline intermediate was dissolved in 147 mL DME and stirred at 0°C for 15 min. 5.30 mL (66.0 mmol, 9.0 equiv.) of pyridine was added to reaction mixture (0.1 mL/min) and stirred at 0°C for an additional 15 min. 4.15 mL (29.4 mmol, 4.0 equiv.) trifluoroacetic anhydride was added to the reaction mixture (0.1 mL/min) and stirred at 0°C for an additional 2 hours. Finally, 2.05 mL (14.70 mmol, 2.0 equiv.) triethylamine (TEA) was added to the reaction dropwise (0.1 mL/min) and ran at 0°C to room temperature overnight. The reaction was concentrated *in vacuo* and re-dissolved in 150 mL EtOAc. The crude reaction was washed with Ph = 1 hydrochloric acid solution (100 mL x 2), then saturated NaHCO₃ solution (100 mL x 10) and finally brine (100 mL x 2). The organic layer was collected, combined, dried over anhydrous Na₂SO₄, filtered and concentrated *in vacuo*. The crude product underwent purification via column chromatography on silica gel (hexanes/EtOAc 9:11 to 2:3) to afford the desired thiazole (1.64g, 74.4% over 2 steps) as a white solid. *R_f*: 0.76 (hexane/

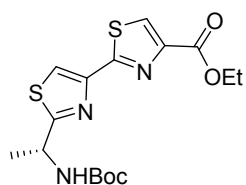
EtOAc 1:1). ^1H NMR (300 MHz, CDCl_3): δ 1.39 (t, J = 7.11 Hz, 3H, CH_2CH_3); 1.43 (br, 9H, $\text{C}(\text{CH}_3)_3$); 1.61 (d, J =6.80 Hz, 3H, CH_3CH); 4.40 (q, J =7.14 Hz, 2H, CH_2CH_3); 5.10 (br, 1 α H); 5.20 (br, 1H, NH); 8.07 (s, 1H, SCHC). ^{13}C NMR (75MHz, CDCl_3) δ 14.36, 21.79, 28.31(3C), 48.91, 61.43, 80.31, 127.13, 147.19, 154.85, 161.34, 174.90. ES⁺MS m/z calcd for $\text{C}_{13}\text{H}_{20}\text{N}_2\text{O}_4\text{S}$ ($[\text{M}+\text{Na}]$) 323.11, found 322.85



BocHN-D-Ala-Thiazole-C(O)-NH₂ (5D). BocHN-D-Ala-Thiazole-C(O)-NH₂ was synthesized utilizing 1.50 g (5.00 mmol, 1.0 equiv.) of BocHN-D-Ala-Thiazole-OMe in 60 mL of ammonium hydroxide solution (25% in water) and 40 mL of EtOH. The reaction was stirred overnight and concentrated *in vacuo*; the resulting amide was taken on to the next reaction without further purification (1.49 g, quantitative) as a white powder. R_f : 0.26 (hexane/ EtOAc 1:1). ^1H NMR (300 MHz, CDCl_3): δ 1.46 (s, 9H, $\text{C}(\text{CH}_3)_3$); 1.60 (s, J =6.84 Hz, 3H, CH_3CH); 5.09 (br, 1 α H); 5.16 (br, 1H, NH); 5.94 (br, 1H, NH_2); 7.20 (br, 1H, NH_2); 8.07 (s, 1H, SCHC). ^{13}C NMR (75MHz, CDCl_3) δ 21.60, 28.32(3C), 48.69, 80.47, 124.30, 149.01, 154.89, 161.97, 174.56. ES⁺MS m/z calcd for $\text{C}_{11}\text{H}_{17}\text{N}_3\text{O}_3\text{S}$ ($[\text{M}+\text{Na}]$) 294.10, found 293.80

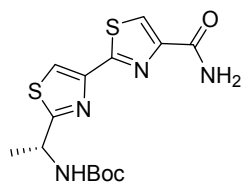


BocHN-D-Ala-Thiazole-C(S)-NH₂ (6D). BocHN-D-Ala-Thiazole-C(S)-NH₂ was synthesized utilizing 1600 mg (5.90 mmol, 1.0 equiv.) of BocHN-D-Ala-Thiazole-C(O)-NH₂ and 1.79 g (4.43 mmol, 0.75 equiv.) of Lawesson's Reagent dissolved in 118 mL of THF. The reaction mixture was stirred overnight and upon completion confirmed via TLC, the crude reaction mixture was rotovapped down to dryness and purified via Flash column chromatography on silica gel (hexane/ EtOAc 1:1 to 3:7) to afford the desired thioamide (1102 mg, 65%) as a yellow solid. R_f : 0.67 (hexanes/EtOAc 1:1). ^1H NMR (300 MHz, CDCl_3): δ 1.44 (s, 9H, $\text{C}(\text{CH}_3)_3$), 1.58 (d, J =6.93 Hz, 3H, CHCH_3), 5.05 (br, 1 α H), 5.18 (br, 1H, NH), 7.92 (br, 1H, NH_aH_b), 8.34 (s, 1H, SCHC), 8.63 (br, 1H, NH_aH_b). ^{13}C NMR (75MHz, CDCl_3) δ 21.48, 28.32(3C), 48.79, 80.54, 127.27, 152.87, 154.92, 174.27, 190.53. ES⁺MS m/z calcd for $\text{C}_{11}\text{H}_{17}\text{N}_3\text{O}_2\text{S}_2$ ($[\text{M}+\text{Na}]$) 310.08, found 309.75



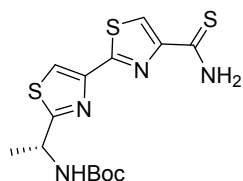
BocHN-D-Ala-Thiazole-Thiazole-OEt (7D).

BocHN-D-Ala-Thiazole-Thiazole-OEt was synthesized utilizing 1.05 g (3.65 mmol, 1.0 equiv.) of BocHN-D-Ala-Thiazole-C(S)-NH₂ dissolved in 73.0 mL of DME, under Ar. 2.92 g (29.2 mmol, 8.0 equiv.) of KHCO₃ was added to the reaction and the mixture stirred for 5 min. 1.37 mL of ethyl bromopyruvate (10.95 mmol, 3.0 equiv.) was dissolved in an additional 10 mL of DME and added dropwise (1 mL/min) to the reaction vessel. The reaction mixture stirred overnight and upon completion the desired hydroxythiazoline intermediate was concentrated *in vacuo*, re-dissolved in EtOAc, extracted with brine, all organic layer was collected, combined, and dried over anhydrous Na₂SO₄, filtered and concentrated *in vacuo*. The crude hydroxythiazoline intermediate was dissolved in 73.0 mL DME and stirred at 0°C for 15 min. 2.65 mL (32.85 mmol, 9.0 equiv.) of pyridine was added to reaction mixture (0.1 mL/min) and stirred at 0°C for an additional 15 min. 2.06 mL (14.6 mmol, 4.0 equiv.) trifluoroacetic anhydride was added to the reaction mixture (0.1 mL/min) and stirred at 0°C for an additional 2 hours. Finally, 1018 µL (7.30 mmol, 2.0 equiv.) triethylamine was added to the reaction dropwise (0.1 mL/min) and ran at 0°C to room temperature overnight. The reaction was concentrated *in vacuo* and re-dissolved in 100 mL EtOAc. The crude reaction was washed with pH = 1 hydrochloric acid solution (80 mL x 2), then saturated NaHCO₃ solution (100 mL x 10) and finally brine (100 mL x 2). The organic layer was collected, combined, dried over anhydrous Na₂SO₄, filtered and concentrated *in vacuo*. The crude product underwent column chromatographic purification on silica gel (hexanes/ EtOAc 9:11 to 2:3) to afford the desired thiazole (1000 mg, 71.45%) as a white solid. *R*_f: 0.70 (hexanes/EtOAc 1:1). ¹H NMR (300 MHz, CDCl₃): δ 1.44 (t, *J*=7.19 Hz, 3H, CH₂CH₃); 1.48 (br, 9H, C(CH₃)₃); 1.66 (d, *J*=6.71 Hz, 3H, CH₃CH); 4.46 (q, *J*=7.11 Hz, 2H, CH₂CH₃); 5.13 (br, 1αH); 5.19 (br, 1H, NH); 8.09 (s, 1H, SCHC), 8.18 (s, 1H, SCHC). ¹³C NMR (75MHz, CDCl₃) δ 14.36, 21.72, 28.34(3C), 48.78, 61.54, 80.32, 117.22, 127.73, 147.87, 148.18, 154.93, 161.43, 163.38, 174.67. ES⁺MS *m/z* calcd for C₁₆H₂₁N₃O₄S₂ ([M+Na]) 406.10, found 405.85



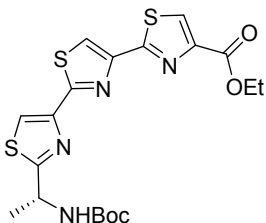
BocHN-D-Ala-Thiazole-Thiazole-C(O)-NH₂ (8D). BocHN-D-Ala-Thiazole-Thiazole-C(O)-NH₂ was synthesized utilizing 0.98 g (2.56 mmol, 1.0 equiv.) of BocHN-D-Ala-Thiazole-Thiazole-OEt in 30.0 mL of ammonium hydroxide solution (25% in water) and 20.0 mL of EtOH. The reaction

was stirred overnight and concentrated *in vacuo*; the resulting amide was taken on to the next reaction without further purification (0.91 g, quantitative) as a white powder. *R_f*: 0.22 (hexane/ EtOAc 1:1). ¹H NMR (300 MHz, CDCl₃): δ 1.47 (br, 9H, C(CH₃)₃); 1.65 (d, *J*=6.77 Hz, 3H, CH₃CH); 5.12 (br, 1αH); 5.19 (br, 1H, NH); 5.91 (br, 1H, NH₂); 7.27 (br, 1H, NH₂); 8.06 (s, 1H, SCHC), 8.15 (s, 1H, SCHC). ¹³C NMR (75MHz, CDCl₃) δ 20.99, 28.65(3C), 49.06, 79.05, 117.92, 124.84, 148.01, 151.57, 155.58, 162.18, 162.65, 178.13. ES⁺MS *m/z* calcd for C₁₄H₁₈N₄O₃S₂ ([M+Na]) 377.08, found 376.75



BocHN-D-Ala-Thiazole-Thiazole-C(S)-NH₂ (9D). BocHN-D-Ala-Thiazole-Thiazole-C(S)-NH₂ was synthesized utilizing 1000 mg (2.82 mmol, 1.0 equiv.) of BocNH-D-Ala-Thiazole-Thiazole-C(O)-NH₂ and 0.86 g (2.12 mmol, 0.75 equiv.) of Lawesson's Reagent dissolved in 56.5 mL of

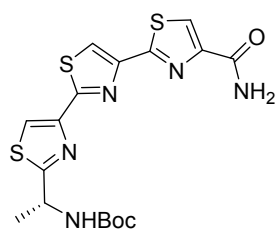
THF. The reaction mixture was stirred overnight and upon completion confirmed via TLC, the crude reaction mixture was rotovapped down to dryness and purified via Flash column chromatography on silica gel (hexane/ EtOAc 1:1 to 3:7) to afford the desired thioamide (650 mg, 62.18%) as a white solid. *R_f*: 0.6 (hexane/EtOAc 1:1). ¹H NMR (300 MHz, CDCl₃): δ 1.47 (br, 9H, C(CH₃)₃); 1.64 (d, *J*=6.72 Hz, 3H, CH₃CH); 5.11 (br, 1αH); 5.17 (br, 1H, NH); 7.74(br, 1H, NH₂), 7.86 (br, 1H, SCHC), 8.45 (br, 1H, SCHC), 8.72 (br, 1H, NH₂). ¹³C NMR (75MHz, CDCl₃) δ 21.65, 28.34(3C), 48.84, 80.46, 117.03, 128.04, 147.91, 153.43, 154.94, 162.76, 162.28, 175.4, 190.37. ES⁺MS *m/z* calcd for C₁₄H₁₈N₄O₂S₃ ([M+Na]) 393.06, found 392.75



BocHN-D-Ala-Thiazole-Thiazole-Thiazole-OEt (10D). BocHN-D-Ala-Thiazole-Thiazole-Thiazole-OEt was synthesized utilizing 600 mg (1.62 mmol, 1.0 equiv.) of BocHN-D-Ala-Thiazole-Thiazole-C(S)-NH₂ dissolved in 32.5 mL of DME, under Argon. 1.30 g (12.96 mmol, 8.0 equiv.) of KHCO₃ was added to the reaction and the mixture stirred for 5 min. 0.61 mL of

ethyl bromopyruvate (4.86 mmol, 3.0 equiv.) was dissolved in an additional 5.0 mL of DME and added dropwise (1

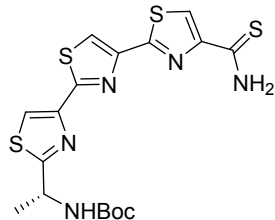
mL/min) to the reaction vessel. The reaction mixture stirred overnight and upon completion the desired hydroxythiazoline intermediate was concentrated *in vacuo*, re-dissolved in EtOAc, extracted with brine, all organic layer was collected, combined, dried over anhydrous Na₂SO₄, filtered and concentrated *in vacuo*. The crude hydroxythiazoline intermediate was dissolved in 32.5 mL DME and stirred at 0°C for 15 min. 1.17 mL (14.6 mmol, 9.0 equiv.) of pyridine was added to reaction mixture (0.1 mL/min) and stirred at 0°C for an additional 15 min. 0.915 mL (6.48 mmol, 4.0 equiv.) trifluoroacetic anhydride was added to the reaction mixture (0.1 mL/min) and stirred at 0°C for an additional 2 hours. Finally, 452 µL (3.24 mmol, 2.0 equiv.) triethylamine was added to the reaction dropwise (0.1 mL/min) and ran at 0°C to room temperature overnight. The reaction was concentrated *in vacuo* and re-dissolved in 20 mL EtOAc. The crude reaction was washed with pH = 1 hydrochloric acid solution (30 mL x 2), then saturated sodium bicarbonate solution (30 mL x 10) and finally brine (30 mL x 2). The organic layer was collected, combined, dried over anhydrous Na₂SO₄, filtered and concentrated *in vacuo*. Crude product was underwent column chromatographic purification on silica gel (hexanes/ EtOAc 9:11 to 2:9) to afford the desired product thiazole (550 mg, 72.7 % over 2 steps) as a light yellow solid. *R_f*: 0.72 (hexane/ EtOAc 1:1). ¹H NMR (300 MHz, CDCl₃): δ 1.42 (t, *J*=7.12 Hz, 3H, CH₂CH₃); 1.46 (br, 9H, C(CH₃)₃); 1.64 (d, *J*=6.85 Hz, 3H, CH₃CH); 4.44 (q, *J*=7.14 Hz, 2H, CH₂CH₃); 5.11 (br, 1αH); 5.22 (br, 1H, NH); 7.97 (s, 1H, SCHC), 8.15 (s, 1H, SCHC), 8.18 (s, 1H, SCHC). ¹³C NMR (75MHz, CDCl₃) δ 14.37, 21.73, 28.35(3C), 48.81, 61.57, 80.35, 116.66, 117.97, 127.81, 147.95, 148.39, 149.13, 154.94, 161.45, 163.10, 163.38, 174.80. ES⁺MS *m/z* calcd for C₁₉H₂₂N₄O₄S₃ ([M+Na]) 489.08, found 489.20



BocHN-D-Ala-Thiazole-Thiazole-Thiazole-C(O)-NH₂ (11D). BocHN-D-Ala-Thiazole-Thiazole-Thiazole-C(O)-NH₂ was synthesized utilizing 200 mg (0.43 mmol, 1.0 equiv.) of BocHN-D-Ala-Thiazole-Thiazole-Thiazole-OEt in 6 mL of ammonium hydroxide solution (25% in water) and 6 mL of EtOH. The reaction mixture was sonicated for 1hr, with subjective NH₄OH

(20.0 ml) and EtOH (20.0 ml) addition. The reaction was stirred overnight and concentrated *in vacuo*; the resulting amide was taken on to the next reaction without further purification (250 mg, quantitative) as a white powder. *R_f*: 0.21 (hexane/ EtOAc 1:1). ¹H NMR (300 MHz, DMSO): 1.43 (br, 9H, C(CH₃)₃); 1.52 (d, *J*=7.13Hz, 3H, CH₃CH); 4.93 (m, 1αH); 7.70 (br, 1H, NH₂); 7.84 (br, 1H, NH₂); 7.89 (d, *J*=7.43Hz, NH); 8.28 (s, 1H, SCHC), 8.32 (s, 1H, SCHC); 8.34 (s, 1H, SCHC).

^{13}C NMR (75MHz, DMSO) δ 20.98, 28.66(3C), 49.06, 79.07, 118.41, 118.93, 125.10, 147.55, 149.23, 151.60, 155.58, 161.89, 162.63, 163.59, 178.28. ES⁺MS m/z calcd for C₁₇H₁₉N₅O₃S₃ ([M+Na]) 460.07, found 460.24

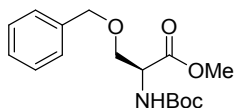


BocHN-D-Ala-Thiazole-Thiazole-Thiazole-C(S)-NH₂ (12D).

BocHN-D-Ala-Thiazole-

Thiazole-Thiazole-C(S)-NH₂ was synthesized utilizing 250 mg (0.43 mmol, 1.0 equiv.) of BocHN-D-Ala-Thiazole-Thiazole-Thiazole-C(O)-NH₂ and 130 mg (0.323 mmol, 0.75 equiv.) of Lawesson's Reagent dissolved in 9.0 mL of THF. The reaction mixture was stirred overnight

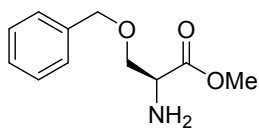
and upon completion confirmed via TLC, the crude reaction mixture was rotovapped down to dryness and purified via Flash column chromatography on silica gel (hexane/ EtOAc 1:1 to 7:11) to afford the desired thioamide (160 mg, 82.1%) as a yellow solid. R_f : 0.67 (hexane/ EtOAc 1:1). ^1H NMR (300 MHz, DMSO): 1.43 (br, 9H, C(CH₃)₃); 1.52 (d, J =6.98 Hz, 3H, CH₃CH); 4.93 (m, 1 α H); 7.89 (d, J =7.87 Hz, NH); 8.28 (s, 1H, SCHC), 8.37 (s, 1H, SCHC); 8.50 (s, 1H, SCHC), 9.60 (s, 1H, NH₂); 10.10 (s, 1H, NH₂). ^{13}C NMR (75MHz, DMSO) δ 20.99, 28.66(3C), 49.09, 79.07, 118.43, 119.43, 128.08, 147.53, 149.17, 154.81, 155.60, 161.47, 163.66, 178.23, 189.41. ES⁺MS m/z calcd for C₁₇H₁₉N₅O₂S₄ ([M+Na]) 476.04, found 476.15



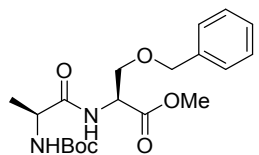
BocHN-Ser(Bn)-OMe.

Under nitrogen atmosphere, Boc-Ser(Bn)-OH **13** (1181 mg, 4.00 mmol, 1.0 equiv.) was dissolved in 40.0 mL of a mixture of benzene/ methanol (3:2). While stirring,

TMSD in 2.0 M diethyl ether was added into the reaction mixture in dropwise until a distinct color change from colorless to pale yellow was indicated with ceased gas evoking. Reaction was allowed to proceed for further 1-2 hrs. Upon reaction completion, confirmed via TLC, the solution was concentrated *in vacuo* to provide product methyl ester as yellow oil (1236.6 mg, quantitative). R_f : 0.40 (hexane/ EtOAc 2:1) ^1H NMR (300Hz, CDCl₃) δ ppm 1.48 (s, 9H, CCH₃), 3.70-3.75 (m, 1H, CHCH₂O), 3.76 (s, 3H, OCH₃), 3.87-3.91 (m, 1H, CHCH₂O), 4.45-4.59 (m, 2H, OCH₂C), 5.44-5.47 (d, J = 8.5Hz, 1H, NH), 7.28-7.35 (m, 5H_{Ar}), ^{13}C NMR (75MHz, CDCl₃) δ 28.36 (3C), 52.46, 54.04, 69.98, 73.25, 80.00, 127.63, 127.86, 128.46, 137.61, 155.54, 171.21. ES⁺MS m/z calcd. for C₁₆H₂₃NO₅ (M+Na)⁺ 332.16, found 332.42

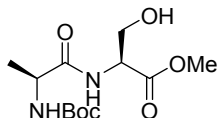


H₂N-Ser(Bn)-OMe. BocHN-Ser(Bn)-OMe (1209 mg, 4.00 mmol, 1.0 equiv.) was dissolved in anhydrous dichloromethane (DCM) (40.0 mL, 0.1 M) at r.t. Anisole (0.85 mL, 8.00 mmol, 2.0 eq.) was then added into the solution. While stirring, TFA (8.0 mL) was added into the reaction in dropwise. The reaction was allowed to proceed for 2-3 hrs. Upon completion of reaction, confirmed via TLC, the mixture was then concentrated with 3 times MeOH followed by twice with DCM. The product free amine was dried *in vacuo* to afford red brown oil (836.6 mg, 100%). *R_f*: 0.20 (hexane/ EtOAc 1:1). ¹H NMR (300Hz, CDCl₃) δ 3.75 (s, 3H, OCH₃), 3.90(s, 2H), 4.00 (s, 1H), 4.24 (s, 1H), 4.45-4.63 (m, 2H, OCH₂C), 7.26-7.38 (m, 5H_{Ar}). ¹³C NMR (75MHz, CDCl₃) δ 53.58, 66.43, 73.55, 128.05, 128.34, 128.67, 136.65, 168.00. ES⁺MS *m/z* calcd. C₁₁H₁₅NO₃ (M+Na)⁺ 232.11, found 232.37

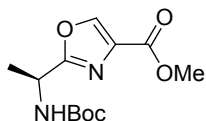


BocHN-Ala-Ser(Bn)-OMe. BocHN-Ala-OH (813 mg, 4.30 mmol, 1.1 equiv.), TBTU (1380 mg, 4.30 mmol, 1.1 eq) was dissolved in 30 mL dry DCM under Argon. H₂N-Ser(Bn)-OMe (1209 mg, 3.91mmol, 1.0 equiv.) was dissolved in 15 mL dry DCM. While stirring, hünig's base DIPEA (2.87 mL, 15.6 mmol, 4.0 equiv.) was added into the reaction mixture in dropwise. After 10 min, the free amine solution was then taken up and added into the free acid containing mixture in dropwise. The reaction was allowed to proceed for 4 hrs. Upon completion of reaction, confirmed via TLC, the reaction mixture was poured into the PH = 1 HCl solution then extracted twice with DCM and EtOAc. The organic layers were collected, combined then wash with the saturated NaHCO₃ solution followed by extraction twice with DCM and EtOAc. The collected organic layer were combined, dried over anhydrous Na₂SO₄, filtered, and concentrated *in vacuo*. The crude material was purified via flash column chromatography using a gradient of hexane/EtOAc as eluent (4:1 to 1:1) to afford BocHN-Ala-Ser(Bn)-OMe as pale yellow oil (1473.5 mg, 90.13%). *R_f*: 0.74 (hexane/EtOAc 1:1). ¹H NMR (300Hz, CDCl₃) δ ppm 1.40 (d, *J*= 7.07Hz, 3H, CHCH₃), 1.48 (s, 9H, CCH₃), 3.69-3.73 (m, 1H), 3.78 (s, 3H, OCH₃), 3.91-3.95 (m, 1H, CHCH₂O), 4.22-4.26 (m, 1H, CHCH₂O), 4.49-4.60 (m, 2H, OCH₂C), 4.73-4.77 (m, 1αH), 4.78 (s, br, 1H, NH), 6.80 (d, *J*=8.1 Hz, 1H, NH), 7.28-7.41

(m, 5H_{Ar}) ¹³C NMR (75MHz, CDCl₃) δ 18.70, 28.41 (3C), 50.17, 52.67, 69.60, 73.39, 80.41, 127.80, 128.01, 128.56, 137.57, 155.37, 172.55, 172.67 ES⁺MS *m/z* calcd. for C₁₉H₂₈N₂O₆ (M+H)⁺ 381.19, found 381.47

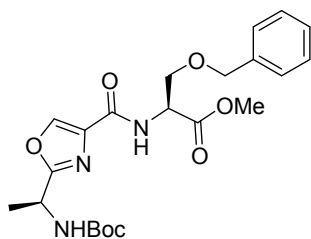


BocHN-Ala-Ser-OMe (14). EtOH (6.00 mL, 0.1 M) was added into the mixture of benzyl protected serine BocHN-Ala-Ser(Bn)-OMe (215 mg, 0.567 mmol) and Pd/C (56.6 mg, 10% w/w). Hydrogen was purged at low pressure. The reaction was allowed to stir at R.T overnight. Upon reaction completion, confirmed via TLC, the solution was filtered through Celite[®]. The organic filtrate was then concentrated *in vacuo* to afford free serine as pale yellow oil (164.43 mg, 100%). *R_f*: 0.17 (hexane/EtOAc 1:1). ¹H NMR (300Hz, CDCl₃) δ ppm 1.42 (d, *J*= 3.0 Hz, 3H, CHCH₃), 1.47 (s, 9H, CCH₃), 3.83 (s, 3H, OCH₃), 3.97-4.05 (m, 2H, CHCH₂O), 4.12-4.21 (m, 1αH), 4.65-4.70 (m, 1αH), 5.07 (br, 1H, NH), 6.95 (d, *J*= 8.1 Hz, 1H, NH) ES⁺MS *m/z* calcd. for C₁₂H₂₂N₂O₆ (M+Na)⁺ 313.15, found 313.41



BocHN-Ala-Oxazole-OMe (15). Under nitrogen, BocHN-Ala-Ser-OMe (393 mg, 1.36 mmol, 1.0 eq.) was dissolved in anhydrous DCM (13.6 mL, 0.1 M) and cooled to -78°C. While stirring, DAST (0.215 mL, 1.626 mmol, 1.1 eq.) was added to the solution in dropwise. The reaction mixture was allowed to stirred for 45 min followed by the addition of K₂CO₃ (375 mg, 2.71 mmol, 2.0 eq.) in one portion and the reaction was allowed to proceed for additional 1 hr. The reaction mixture was then warmed up to r.t and stirred for overnight. Upon completion of the reaction, confirmed via TLC, the reaction mixture was poured into the saturated NaHCO₃ solution and extracted twice with DCM and EtOAc. The collected organic layer was combined and dried over anhydrous Na₂SO₄, filtered, and concentrated *in vacuo*. Oxazoline intermediate was then re-dissolved in anhydrous DCM (13.6 mL, 0.1M) and cooled to -47°C under nitrogen. While stirring, DBU (0.405 mL, 2.71 mmol, 2.0 eq.) was added into the solution in dropwise. After 15min, BrCCl₃ (0.269 mL, 2.71 mmol, 2.0 eq.) was added into the reaction mixture in dropwise followed by stirring for an additional 2 hrs. The reaction mixture was allowed to warm to R.T and stir overnight. Upon completion, confirmed via TLC, the reaction mixture was poured into the pH = 1 hydrochloric acid solution and extracted twice with DCM and EtOAc. The collected organic layer was combined and poured into the saturated NaHCO₃ solution and

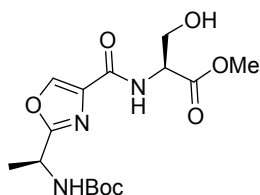
extracted twice with DCM and EtOAc. The collected organic layer was combined and dried over anhydrous Na_2SO_4 , filtered, and concentrated *in vacuo*. Obtained crude material was purified via flash column chromatography using a gradient of hexane/EA as eluent (3:1 to 1:3) to afford desired oxazole as white solid (305.6 mg, 83.22%). R_f : 0.62 (hexane/EtOAc 1:1). ^1H NMR (300MHz, CDCl_3) δ ppm 1.47 (s, 9H, CCH_3), 1.58 (d, $J=7.1$ Hz, 3H, CHCH_3), 3.95 (s, 3H, OCH_3), 5.01-5.06 (m, 1 α H), 5.23 (br, 1H, NH), 8.21 (s, 1H, OCHC). ^{13}C NMR (75MHz, CDCl_3) δ ppm 20.20, 28.30 (3C), 44.76, 52.25, 80.23, 133.21, 144.02, 154.85, 161.54, 166.0 ES⁺MS m/z calcd. for $\text{C}_{12}\text{H}_{18}\text{N}_2\text{O}_5$ ($\text{M}+\text{Na}$)⁺ 293.12, found 293.38



BocHN-Ala-Oxazole-Ser(Bn)-OMe (16). BocHN-Ala-Oxazole-OMe (119.3 mg, 0.442 mmol, 1.0 eq.) was dissolved in MeOH (5.0 mL) at R.T. While stirring, LiOH (148.2 mg, 3.532 mmol, 8.0 eq.) was added into the reaction. The reaction was allowed to proceed for overnight. Upon reaction completion, confirmed via TLC, the reaction mixture was

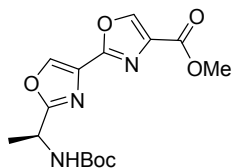
concentrated followed by re-dissolving in DCM, the solution was then poured into pH = 1 hydrochloric acid solution and extracted twice with DCM and EtOAc, the organic layers were combined and dried over anhydrous Na_2SO_4 , filtration, and concentrated *in vacuo* to give the desired carboxylic acid product as a white solid. Without further purification, BocHN-Ala-Oxazole-OH Carboxylic acid (113 mg, 0.442 mmol, 1.0 eq.) and TBTU (142 mg, 0.442 mmol, 1.1 eq.) were dissolved in anhydrous 8.0 mL DCM under nitrogen. H_2N -Ser(Bn)-OMe (84.08 mg, 0.402 mmol, 1.1 eq.) was dissolved in dry 5.0 mL DCM. While stirring, DIPEA (0.697 mL, 4.02 mmol, 10.0 eq.) was added into the reaction in dropwise. The free amine solution was then taken up and added into the free acid containing mixture in dropwise while stirring. The reaction was allowed to proceed for 4 hrs. Upon reaction completion, confirmed via TLC, the reaction mixture was poured into the pH = 1 hydrochloric acid solution then extracted twice with DCM and EtOAc. The organic layers were collected and combined then poured into the saturated NaHCO_3 solution followed by extraction twice with DCM and EtOAc. The collected organic layer were combined, dried over anhydrous Na_2SO_4 , filtered, and concentrated *in vacuo*. The crude material was purified via flash column chromatography using a gradient of hexane/EtOAc as eluent (4:1 to 1:1) to afford

product **16** as pale yellow oil (158 mg, 88% over 2 steps). R_f : 0.70 (hexane/EtOAc 1:1). ^1H NMR (300Hz, CDCl_3) δ ppm 1.46 (s, 9H, CCH_3), 1.55 (d, $J = 6.9\text{Hz}$, 3H, CHCH_3), 3.74-3.78 (m, 4H, OCH_3 , CHCH_2O), 3.95-3.99 (m, 1H, CHCH_2O), 4.50-4.61 (m, 2H, OCH_2C), 4.87-4.92(m, 1 α H), 4.98-5.11 (m, 1H, NH), 7.25-7.36 (m, 5H $_{\text{Ar}}$), 7.65 (d, $J = 8.5\text{Hz}$, 1H, NH), 8.13 (s, 1H, OCHC) ES^+MS m/z calcd. for $\text{C}_{22}\text{H}_{29}\text{N}_3\text{O}_7$ ($\text{M}+\text{Na}$) $^+$ 470.2, found 470.46



BocHN-Ala-Oxazole-Ser-OMe. EtOH (6.00 ml, 0.1 M) was added into the seal vessel that contains a mixture of BocHN-Ala-Oxazole-Ser(Bn)-OMe (215 mg, 0.567 mmol, 1.0 eq.) and Pd/C (22.0 mg, 10% w/w). While stirring, the reaction was purged with hydrogen at low pressure.

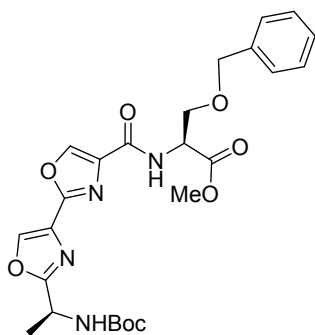
The reaction was allowed to stir at R.T for overnight. Upon reaction completion, confirmed via TLC, the solution was filtered through Celite®. The organic filtrate was then concentrated *in vacuo*. Obtained material was undergoes further column chromatography purification using a gradient of hexane/EtOAc as eluent (1:1) to afford BocHN-Ala-Oxazole-Ser-OMe as white solid (202 mg, 99.0%). R_f : 0.15 (hexane/EtOAc 1:1). ^1H NMR (300Hz, CDCl_3): δ ppm 1.46 (s, 9H, CCH_3), 1.55 (d, $J = 6.90\text{Hz}$, 3H, CHCH_3), 3.82 (s, 3H, OCH_3), 4.06 (m, 2H, CHCH_2O), 4.86 (m, 1 α H), 4.98 (br, 1 α H), 5.07 (br, 1H, NH), 7.70 (d, $J = 8.0\text{Hz}$, 1H, NH), 8.15 (s, 1H, OCHC) ES^+MS m/z calcd. for $\text{C}_{15}\text{H}_{23}\text{N}_3\text{O}_7$ ($\text{M}+\text{Na}$) $^+$ 380.15, found 380.41



BocHN-Ala-Oxazole-Oxazole-OMe (17). BocHN-Ala-Oxazole-Ser-OMe (967.3 mg, 2.707 mmol, 1.0 eq.) was dissolved in anhydrous DCM (27.1 mL, 0.1 M) and cooled to -78°C under nitrogen. While stirring, DAST (0.40 mL, 2.98 mmol, 1.1 eq.) was added to the solution in dropwise. The

reaction mixture was allowed to stirred for 45 min followed by the addition of K_2CO_3 (1364 mg, 5.41mmol, 2.0 eq.) in one portion and the reaction was allowed to proceed for additional 1 hr then warmed up to r.t for overnight. Upon completion of the reaction, confirmed via TLC, the reaction mixture was poured into the saturated NaHCO_3 solution then extracted twice with DCM and EtOAc. The collected organic layer was combined and dried over anhydrous Na_2SO_4 , filtered, and concentrated *in vacuo*. Oxazoline intermediate was then re-dissolved in anhydrous DCM (28.0 mL, 0.1 M) and cooled to -47°C under nitrogen. While stirring, DBU (0.76 mL, 5.10 mmol, 2.0 eq.) was added into the reaction

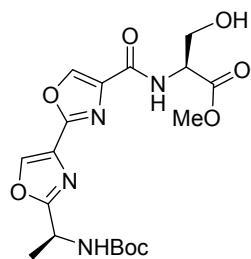
solution in dropwise. After 15min, BrCCl₃ (0.505 ml, 5.10 mmol, 2.0 eq.) was added into the reaction mixture in dropwise followed by stirring for an additional 2 hrs. The reaction mixture was allowed to warm to R.T and stir overnight. Upon completion, confirmed via TLC, the reaction mixture was poured into the pH = 1 hydrochloric acid solution and extracted twice with DCM and EtOAc. The collected organic layer was combined and poured into the saturated NaHCO₃ solution and extracted twice with DCM and EtOAc. The collected organic layer was collected, combined and dried over anhydrous Na₂SO₄, filtered, and concentrated *in vacuo*. Obtained crude oxazole was purified via flash column chromatography using a gradient of hexane/EtOAc as eluent (3:1 to 1:3) to afford desired oxazole as a white solid (730 mg, 80%). *R_f*: 0.65 (hexane/EtOAc 1:1). ¹H NMR (300Hz, CDCl₃) δ 1.44 (s, 9H, CCH₃), 1.58 (d, *J*= 7.0 Hz, 3H, CHCH₃), 3.94 (s, 3H, OCH₃), 5.04-5.06 (m, 1αH), 5.20 (br, 1H, NH), 8.29 (s, 1H, OCHC), 8.30 (s, 1H, OCHC) ¹³C NMR(75MHz, CDCl₃) δ ppm 14.13, 20.18, 22.70, 28.31(3C), 29.37, 29.71, 31.94, 44.79, 52.34, 80.4, 129.7, 134.4, 139.6, 143.7, 155.7, 161.4, 166.5 ES⁺MS *m/z* calcd. C₁₅H₁₉N₃O₆ (M+Na)⁺ 360.13, found 360.39



BocHN-Ala-Oxazole-Oxazole-Ser(Bn)-OMe. BocHN-Ala-Oxazole-Oxazole-OMe (777.3 mg, 2.305 mmol, 1.0 eq.) was dissolved in MeOH (23.0 mL, 0.1 M) at R.T. While stirring, LiOH (773.4 mg, 18.44 mmol, 8.0 eq.) was added into the reaction. The reaction was allowed to proceed for overnight. Upon reaction completion, confirmed via TLC, the reaction mixture was concentrated followed by re-dissolving in DCM, the solution was poured into pH = 1

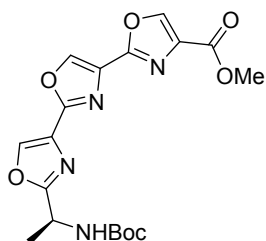
hydrochloric acid solution and extracted twice with DCM and EtOAc, the organic layers were combined and dried over anhydrous Na₂SO₄, filtration, and concentrated *in vacuo* to give the BocHN-Ala-Oxazole-Oxazole-OH carboxylic acid as white solid. Without further purification, To the mixture of Boc-Ala-Oxazole-Oxazole-OH (744 mg, 2.305 mmol, 1.0 eq.), TBTU (741 mg, 2.305 mmol, 1.1 eq.), HATU (876.4 mg, 2.305 mmol, 1.1 eq.) was dissolved in anhydrous DCM (18.0 mL) under nitrogen. H₂N-Ser(Bn)-OMe (438 mg, 2.09 mmol, 1.1 eq.) was dissolved in anhydrous DCM (10.0 mL). While stirring, DIPEA (4.01 ml, 23.0 mmol, 10.0 eq.) was added into the carboxylic acid containing mixture then to the free amine containing solution in dropwise. After 5 min, the free amine containing solution was then taken up and added into

the carboxylic acid containing mixture in dropwise while stirring. Additional anhydrous ACN (6.0 mL) was added into the reaction mixture. The reaction was allowed to proceed for 4 hrs. Upon completion of reaction, confirmed via TLC, the reaction mixture was poured into the pH = 1 hydrochloric acid solution then extracted twice with DCM and EtOAc. The organic layers were collected and combined then poured into the saturated NaHCO₃ solution followed by extraction twice with DCM and EtOAc. The collected organic layer were combined, dried over anhydrous Na₂SO₄, filtered, and concentrated *in vacuo*. The crude material was purified via flash column chromatography using a gradient of hexane/EtOAc as eluent (3:1 to 1:1) to afford product as white solid (946 mg, 88% over 2 steps). *R_f*: 0.60 (hexane/ EtOAc 1:1). ¹H NMR (300Hz, CDCl₃) δ ppm 1.45 (s, 9H, CCH₃), 1.60 (d, *J* = 6.9 Hz, 3H, CHCH₃), 3.74-3.78 (m, 4H, OCH₃, CHCH₂O), 3.95-3.99 (m, 1H, CHCH₂O), 4.56 (m, 2H, OCH₂C), 4.92-4.97 (m, 1αH), 5.07-5.09 (br, 1αH), 5.18 (br, 1H, NH), 7.25-7.36 (m, 5H_{Ar}), 7.75 (d, *J* = 8.1Hz, 1H, NH), 8.21 (s, 1H, OCHC), 8.24 (s, 1H, OCHC) ES⁺MS *m/z* calcd. for C₂₅H₃₀N₄O₈ (M+Na)⁺ 537.21, found 537.47



BocNH-Ala-Oxazole-Oxazole-Ser-OMe (18). EtOH (20.0 mL) and EA (20.0 mL) was added into the seal vessel that contains a mixture of BocHN-Ala-Oxazole-Oxazole-Ser(Bn)-OMe (1029 mg, 2.00 mmol, 1.0 eq.) and Pd/C (104.0 mg, 10% w/w). While stirring, the reaction was purged with hydrogen at low pressure. The reaction was allowed to stir at r.t for overnight. The solution

was filtered through Celite[®]. The organic filtrate was then concentrated *in vacuo*. Obtained material was undergoes further flash column purification using a gradient of hexane/EtOAc as eluent (1:1 to 1:4) to afford product **18** as white solid (823 mg, 80%). *R_f*: 0.10 (hexane/ EtOAc 1:1). ¹H NMR (300Hz, CDCl₃): δ ppm 1.48 (s, 9H, CCH₃), 1.61 (d, *J* = 6.94 Hz, 3H, CHCH₃), 3.84 (s, 3H, OCH₃), 4.61-4.78 (m, 2H, OCH₂), 4.86 (m, 1αH), 5.05-5.10 (m, 1αH), 5.25 (br, 1H, NH), 8.27 (s, 1H, OCHC), 8.34 (s, 1H, OCHC) ES⁺MS *m/z* calcd. For C₁₈H₂₄N₄O₈(M+Na)⁺ 447.16, found 447.42

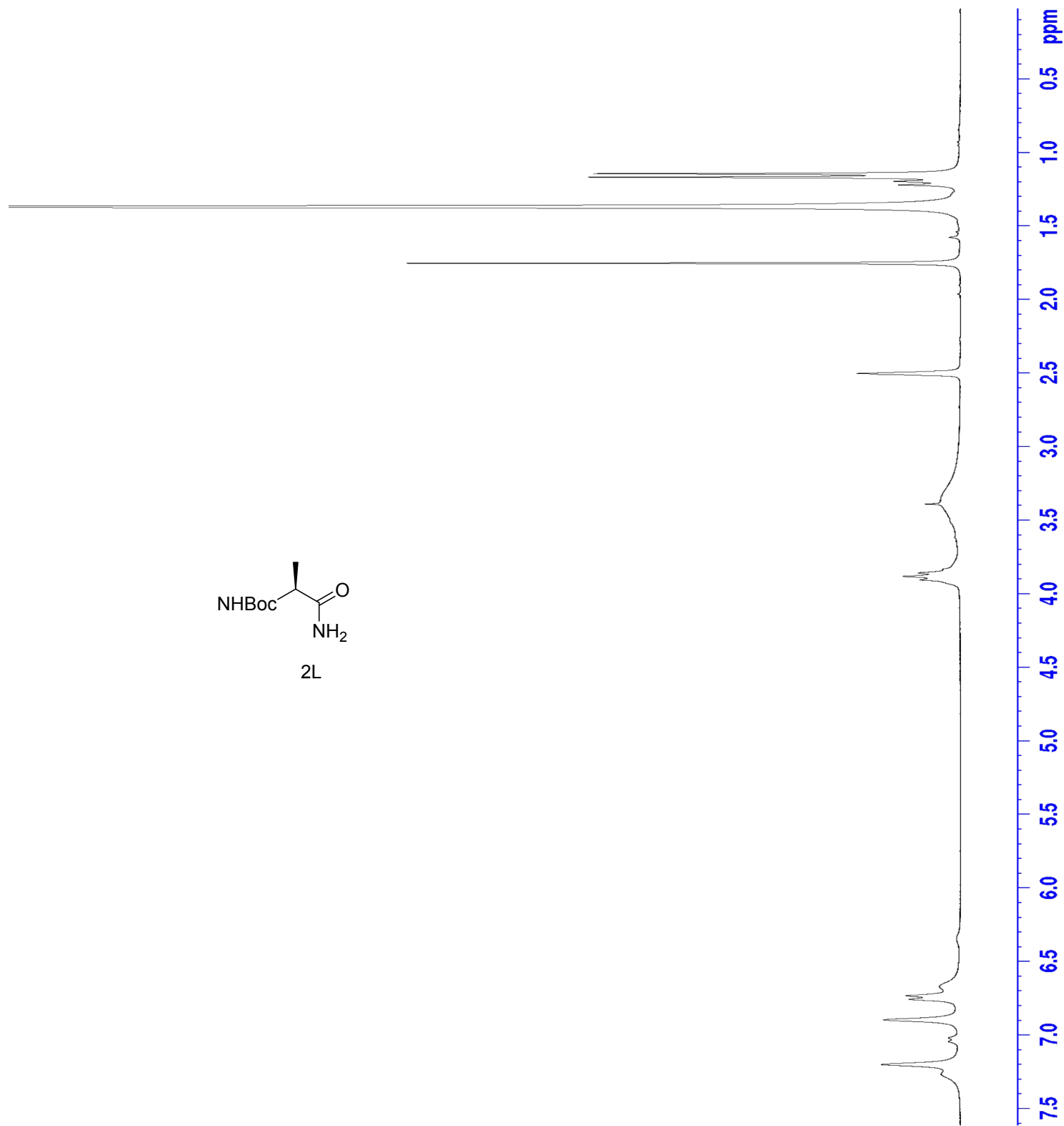
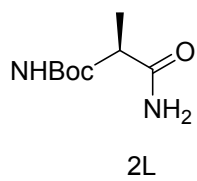


BocHN-Ala-Oxazole-Oxazole-Oxazole-OMe (19). BocHN-Ala-Oxazole-Oxazole-Ser-OMe (678.4 mg, 1.60 mmol, 1.0 eq.) was dissolved in anhydrous DCM (16.6 mL, 0.1M) and cooled to -78°C under nitrogen. While stirring, DAST (0.215 mL, 1.76mmol, 1.1 eq.) was added to the

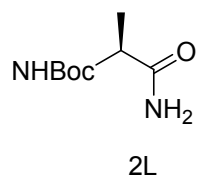
mixture in dropwise. The reaction mixture was allowed to stirred for 45 min followed by the addition of K_2CO_3 (442.3 mg, 3.20 mmol, 2.0 eq.) in one portion and the reaction was allowed to proceed for additional 1 hr. The reaction mixture was then warmed up to r.t and stirred for overnight. Upon completion of the reaction, confirmed via TLC, the reaction mixture was poured into the saturated $NaHCO_3$ solution then extracted twice with DCM and EtOAc. The collected organic layer was combined and dried over Na_2SO_4 , filtered, and concentrated *in vacuo*. Without further purification, the Oxazoline intermediate was re-dissolved in anhydrous DCM (16.6 mL, 0.10M) and cooled to $-47^\circ C$ under nitrogen. While stirring, DBU (0.479 mL, 3.20 mmol, 2.0 eq.) was added into the reaction solution in dropwise. After 15min, $BrCCl_3$ (0.315 mL, 3.20 mmol, 2.0 eq.) was added into the reaction mixture in dropwise followed by stirring for an additional 2 hrs. The reaction mixture was allowed to warm to r.t and stir overnight. Upon reaction completion, confirmed via TLC, the reaction mixture was poured into the pH = 1 hydrochloric acid solution and extracted twice with DCM and EtOAc. The collected organic layer was combined and poured into the saturated $NaHCO_3$ solution and extracted twice with DCM and EtOAc. The collected organic layer was combined and dried over anhydrous Na_2SO_4 , filtered, and concentrated *in vacuo*. Obtained crude material was purified via flash column chromatography using a gradient of hexane/EA as eluent (1:1 to 1:5) to give desired oxazole as a white solid (477 mg, 74%). R_f : 0.47 (hexane/ EtOAc 1:1). 1H NMR (300Hz, $CDCl_3$): δ ppm 1.49 (s, 9H, $C\text{CH}_3$), 1.63 (d, J = 6.96 Hz, 3H, $CH\text{CH}_3$), 3.99 (s, 3H, $O\text{CH}_3$), 5.07-5.12 (m, 1 α H), 5.25 (br, 1H, NH), 8.34 (s, 1H, $O\text{CHC}$), 8.36 (s, 1H, $O\text{CHC}$), 8.46 (s, 1H, $O\text{CHC}$) ES^+MS m/z calcd for $C_{18}H_{20}N_4O_7$ ($[M+Na]$) 427.13, found 427.39

Spectral Data

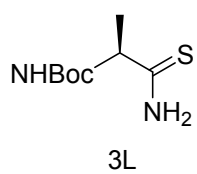
Compound 2L ^1H NMR BocHN-Ala-C(O)-NH₂



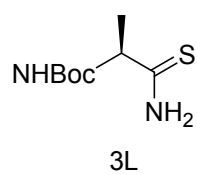
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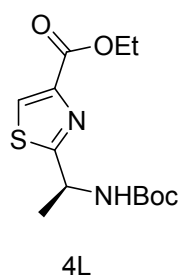
Compound 3L ^1H NMR BocHN-Ala-C(S)-NH₂



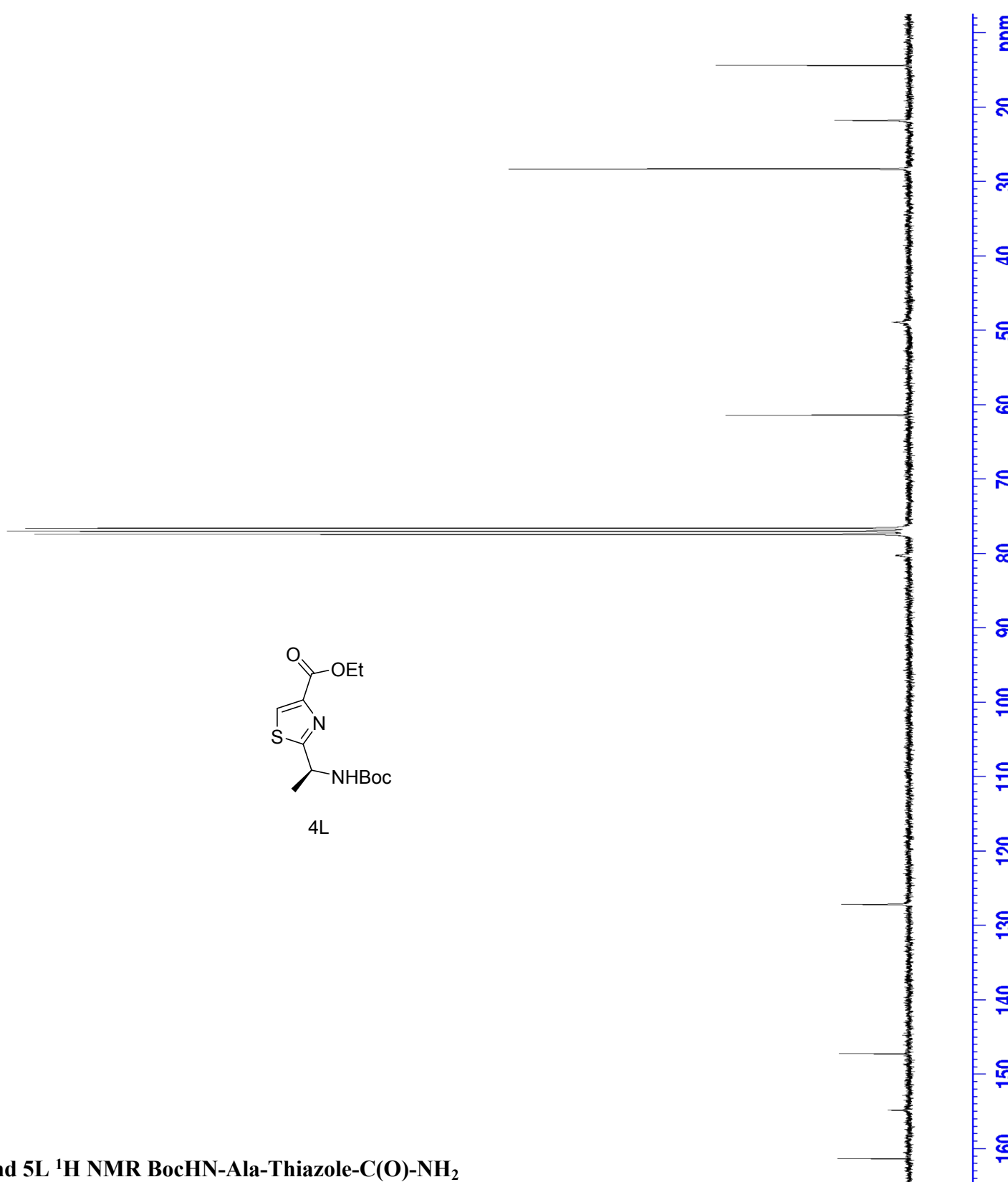
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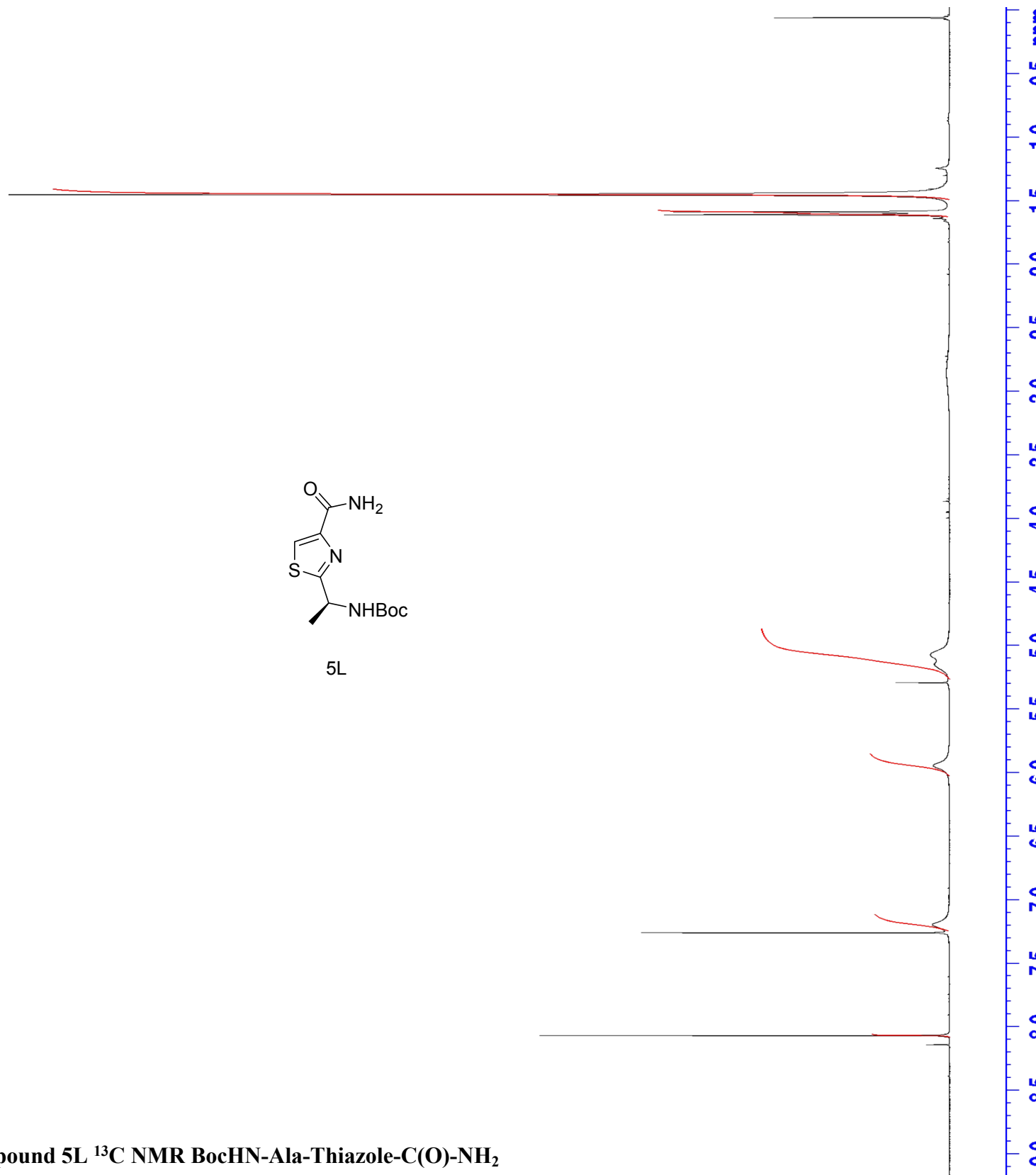


Compound 4L ¹H NMR BocHN-Ala-Thiazole-OEt

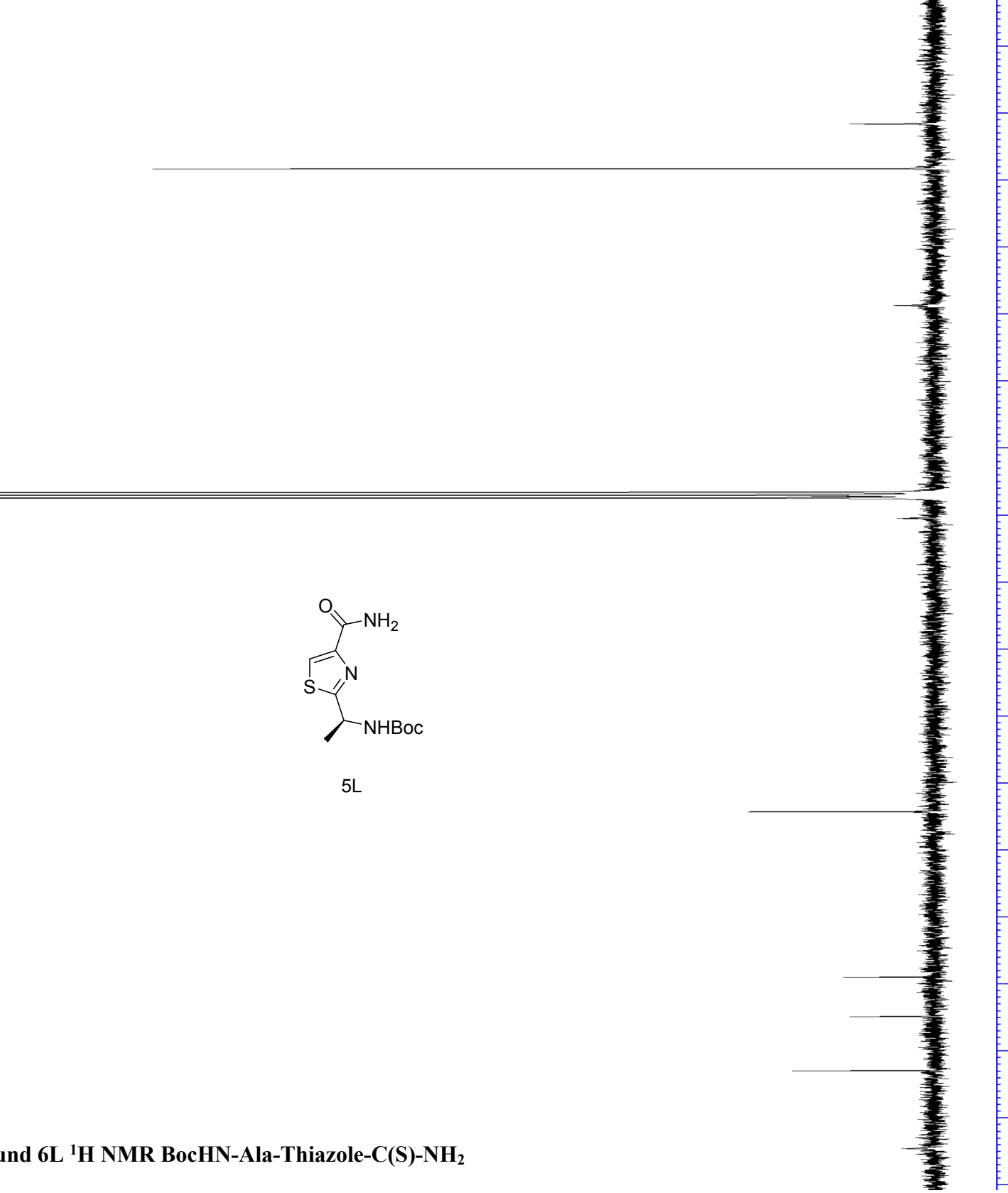


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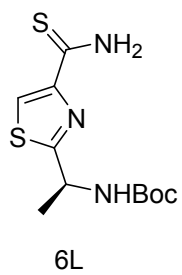




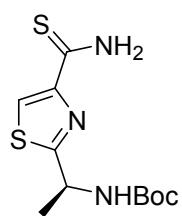
Compound 5L ¹³C NMR BocHN-Ala-Thiazole-C(O)-NH₂



Compound 6L ¹H NMR BocHN-Ala-Thiazole-C(S)-NH₂

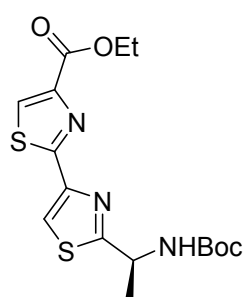


Compound 6L ^{13}C NMR BocHN-Ala-Thiazole-C(S)-NH₂



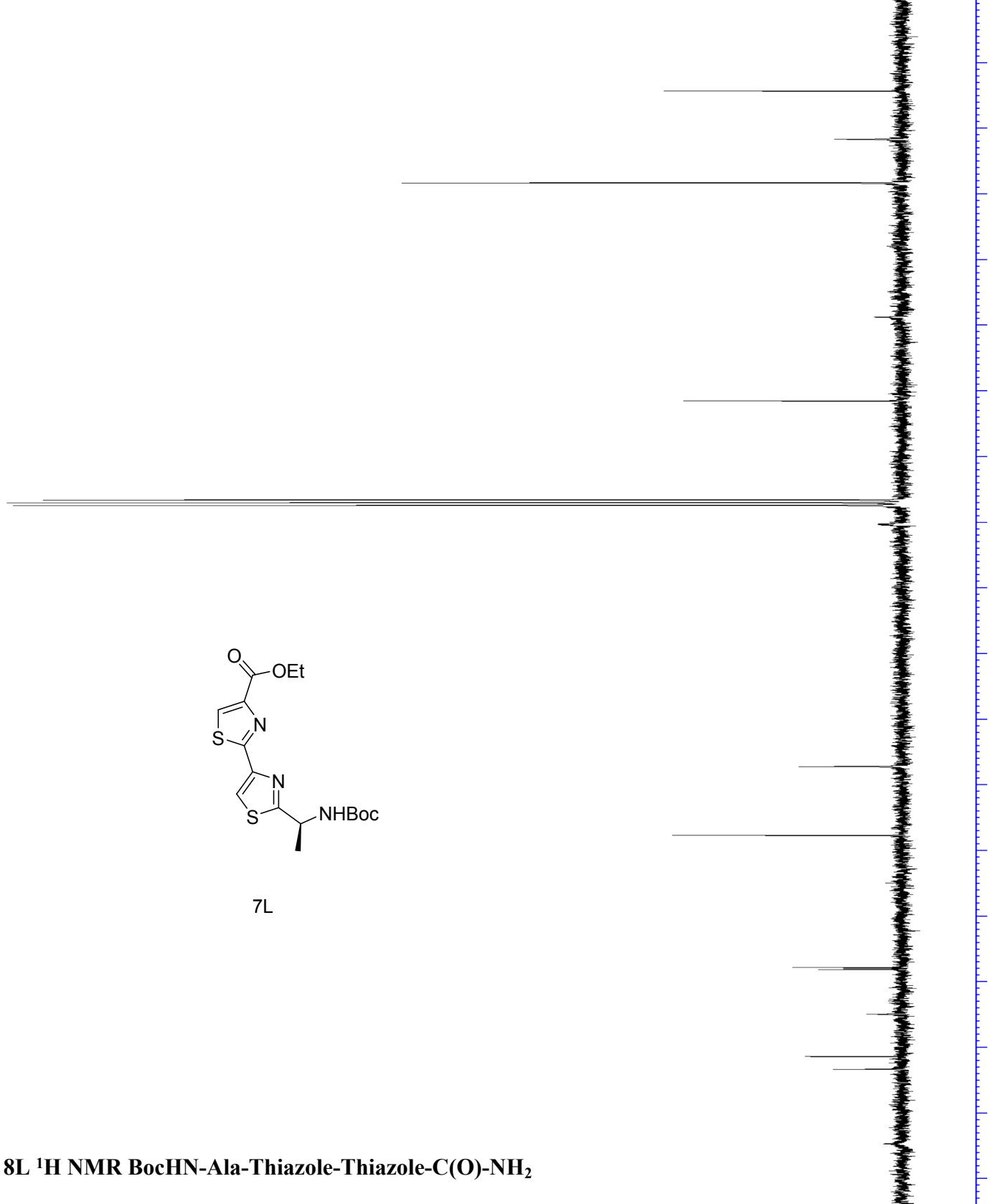
6L

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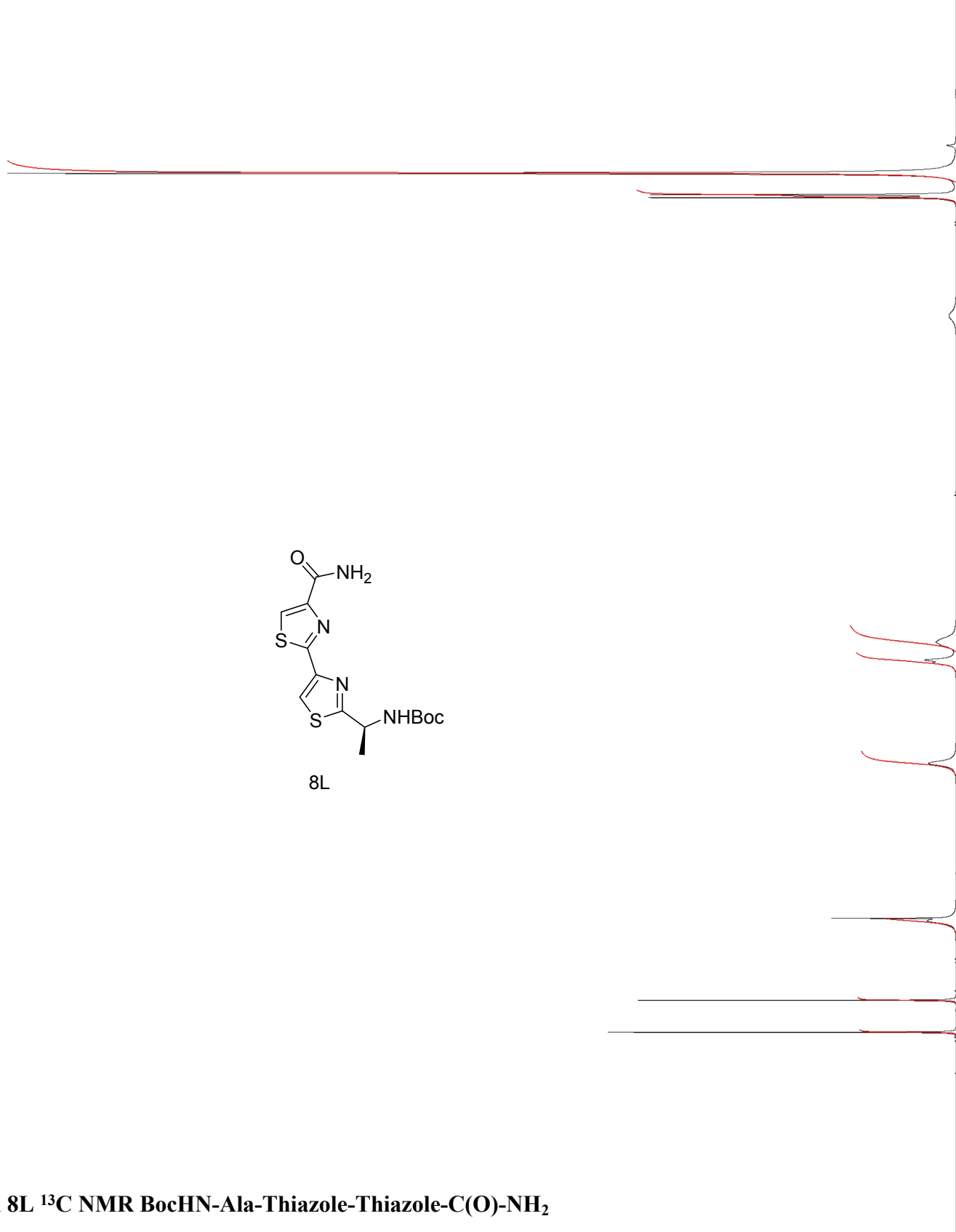


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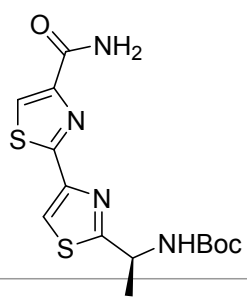
Compound 7L ^{13}C NMR BocHN-Ala-Thiazole-Thiazole-OEt



Compound 8L ¹H NMR BocHN-Ala-Thiazole-Thiazole-C(O)-NH₂

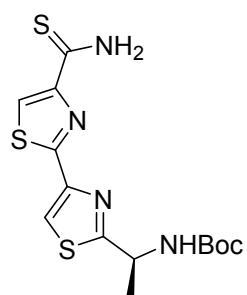


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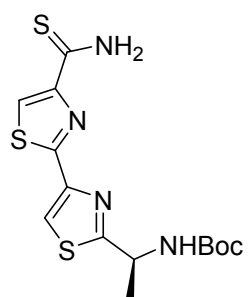
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Compound 9L ¹H NMR BocHN-Ala-Thiazole-Thiazole-C(S)-NH₂



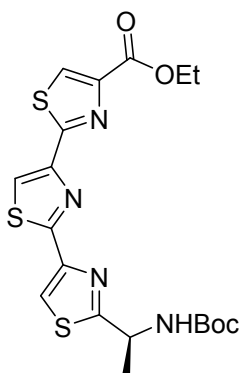
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Compound 9L ^{13}C NMR BocHN-Ala-Thiazole-Thiazole-C(S)-NH₂



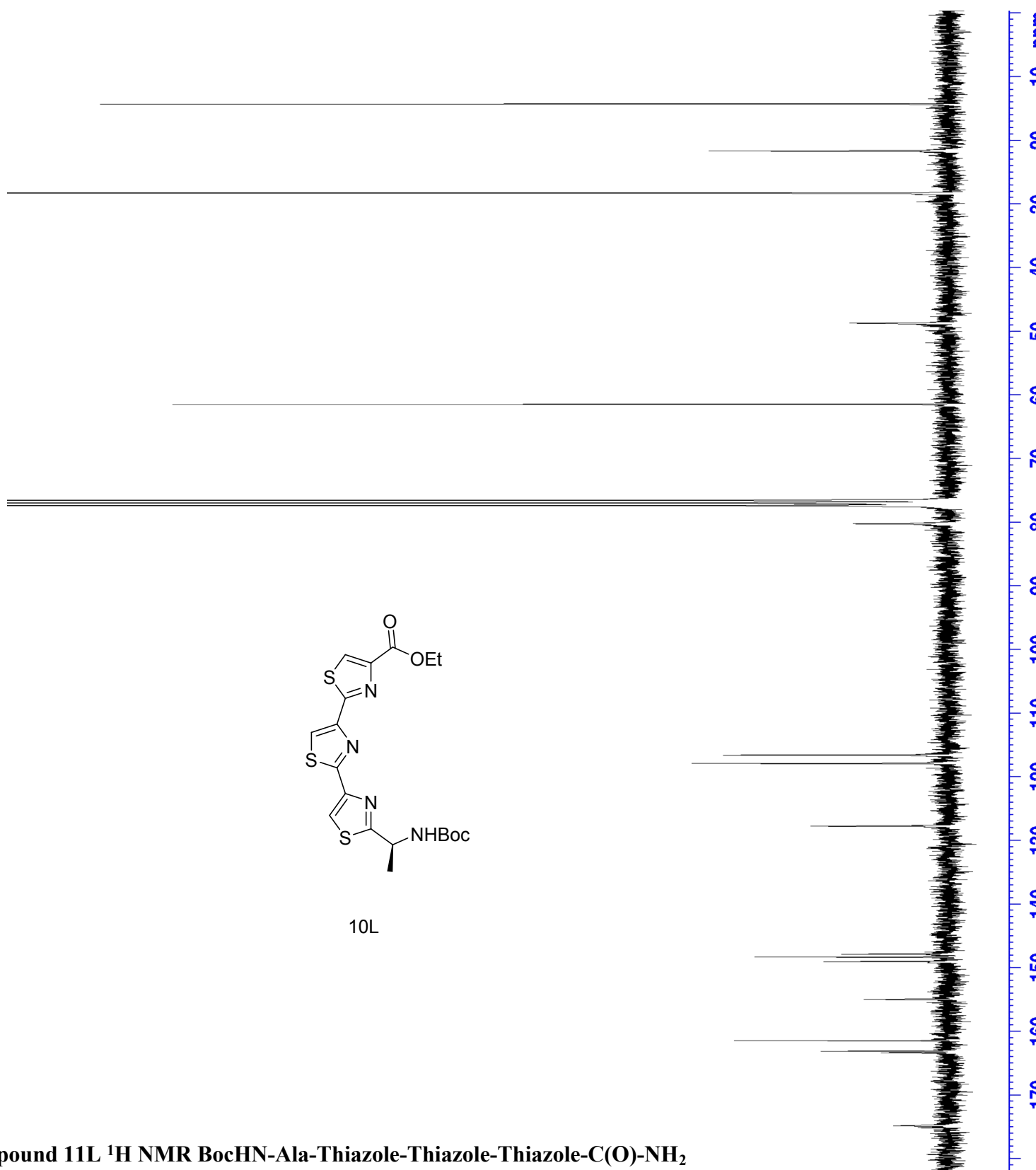
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Compound 10L ^1H NMR BocHN-Ala-Thiazole-Thiazole-Thiazole-OEt

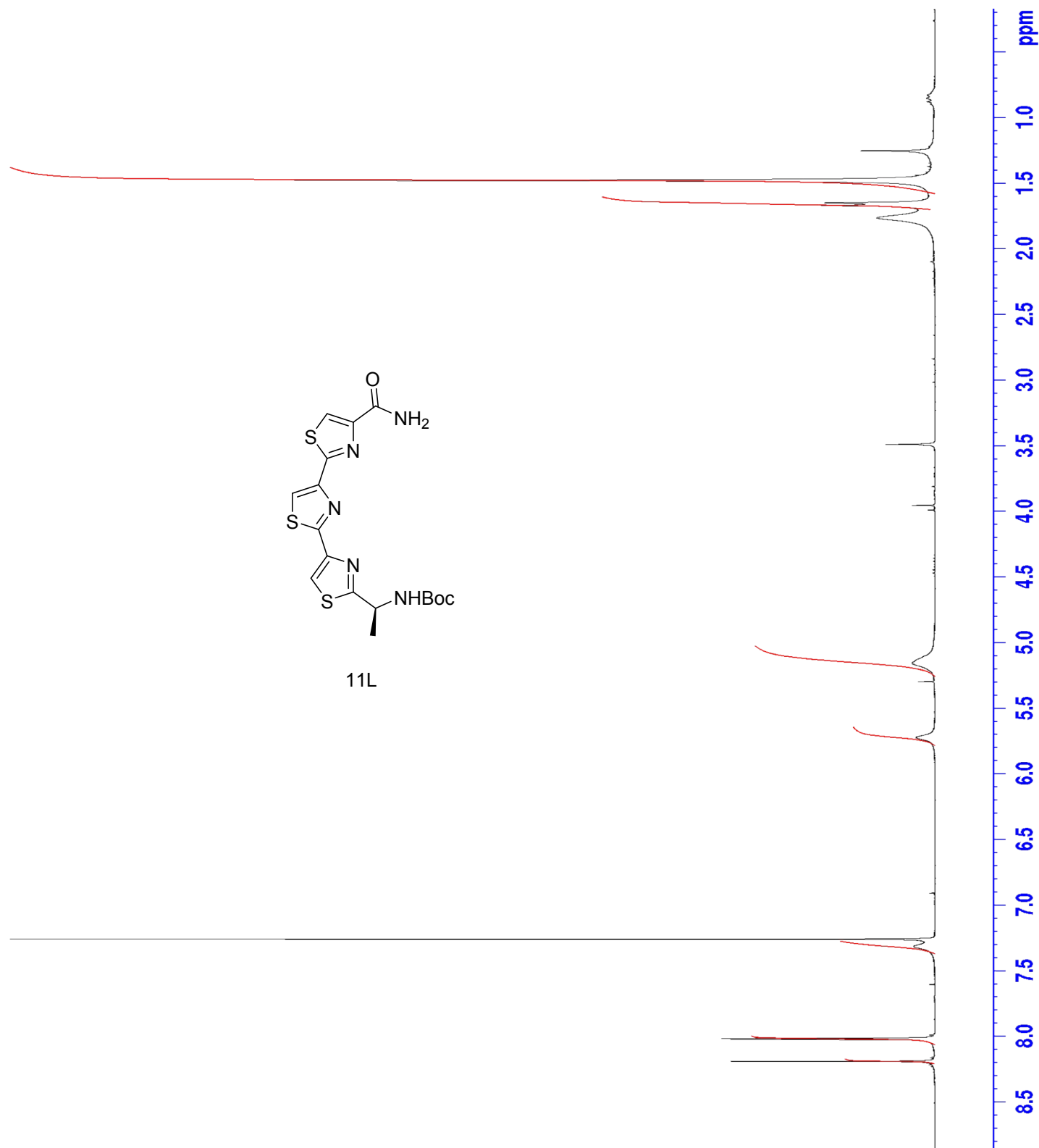


10L

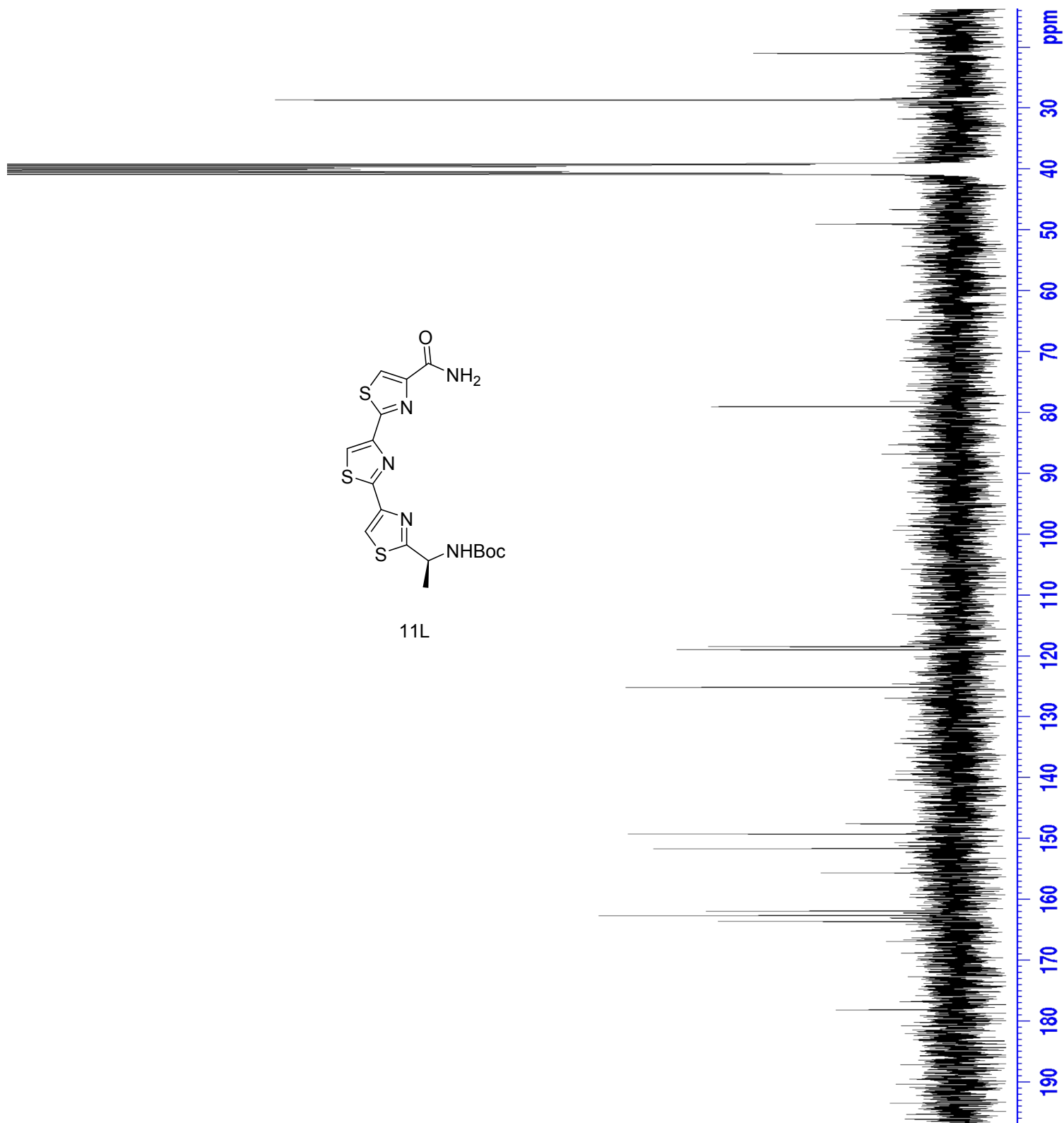
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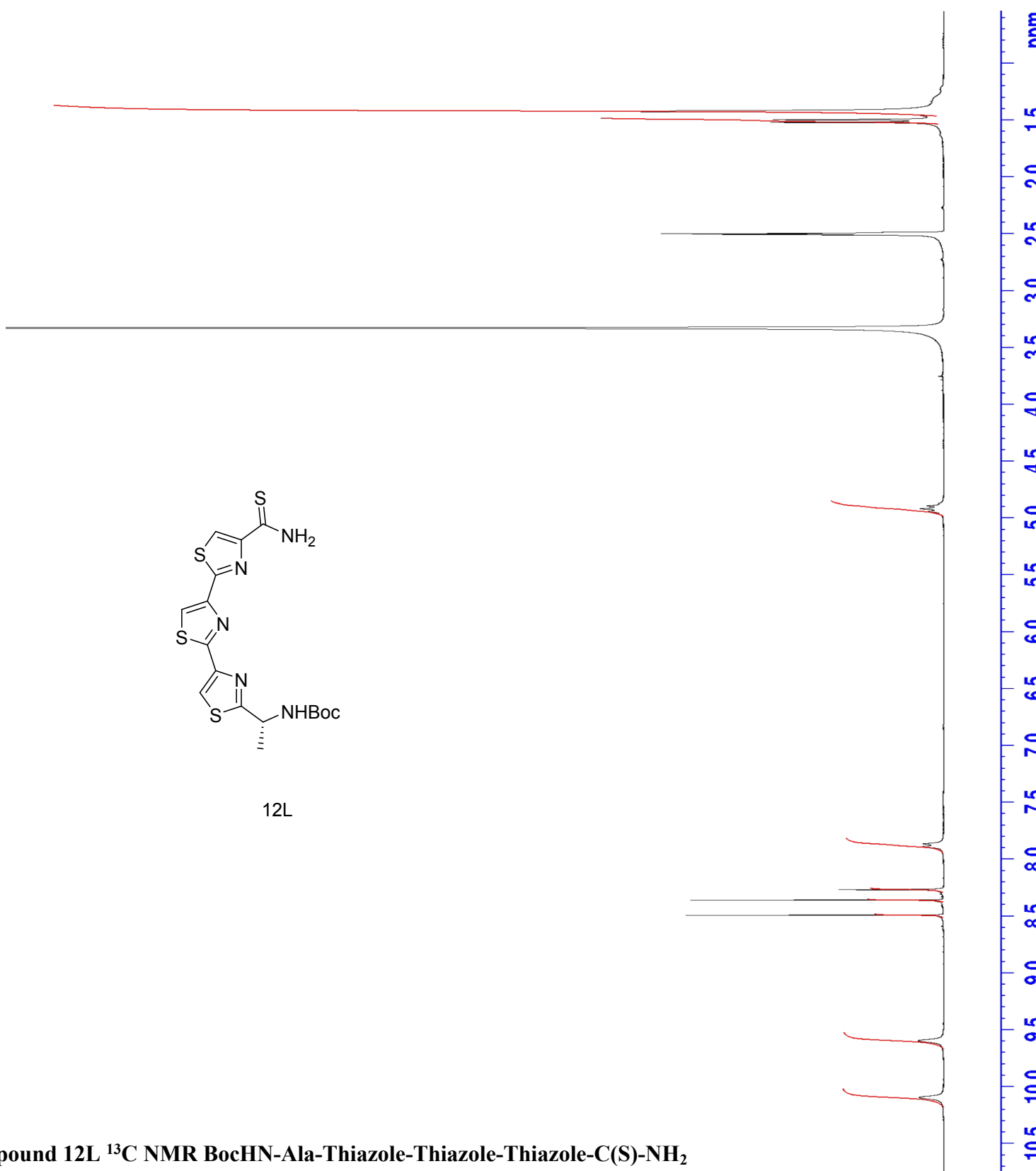
Compound 11L ¹H NMR BocHN-Ala-Thiazole-Thiazole-Thiazole-C(O)-NH₂



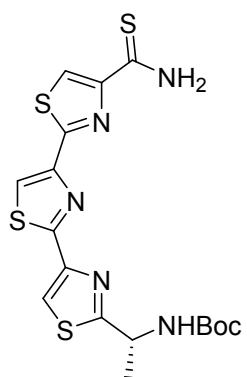
Compound 11L ¹³C NMR BocHN-Ala-Thiazole-Thiazole-Thiazole-C(O)-NH₂



Compound 12L ¹H NMR BocHN-Ala-Thiazole-Thiazole-Thiazole-C(S)-NH₂

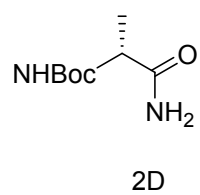


Compound 12L ¹³C NMR BocHN-Ala-Thiazole-Thiazole-Thiazole-C(S)-NH₂

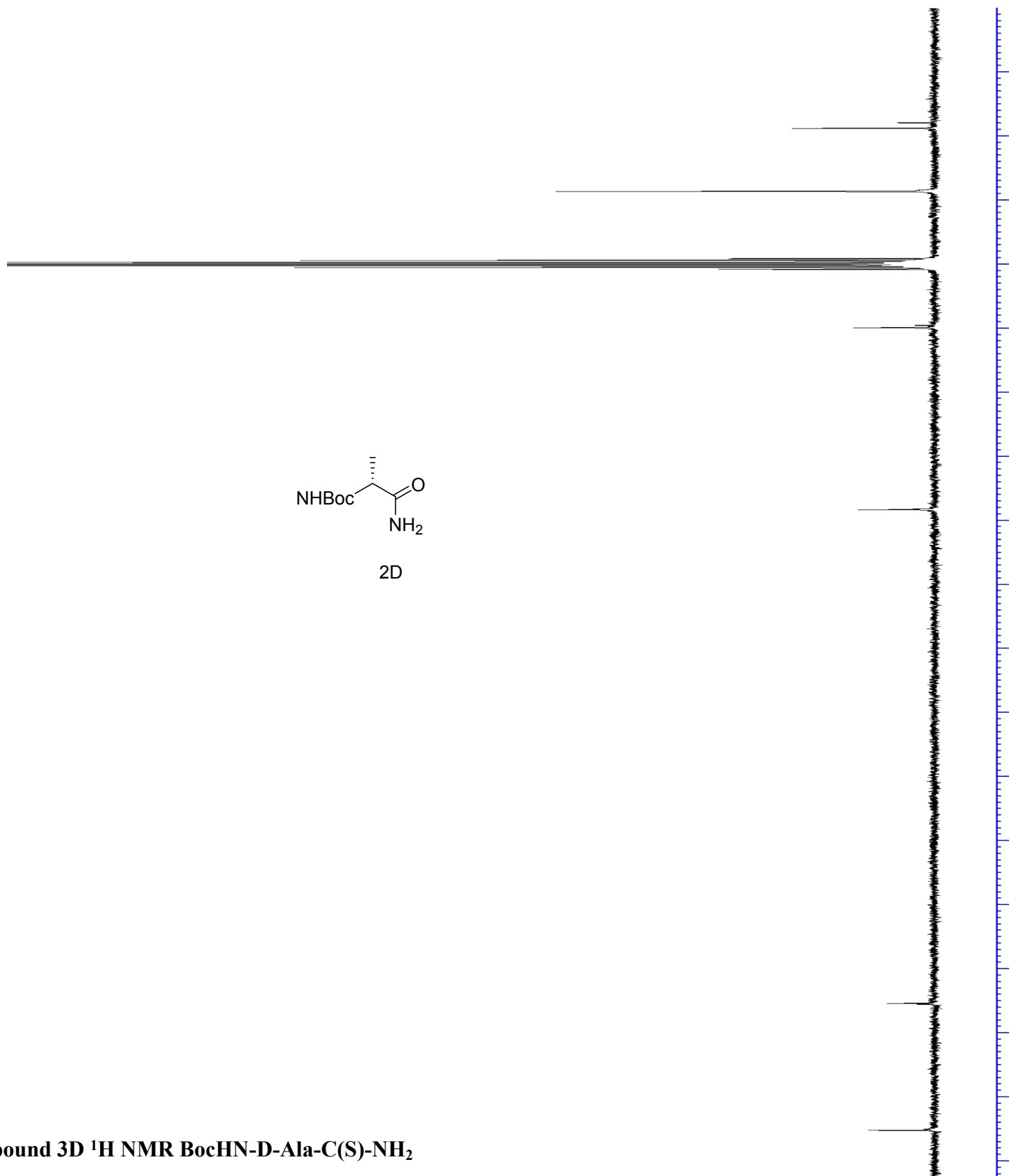


12L

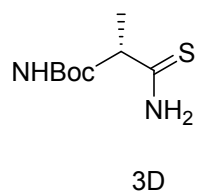
Compound 2D ¹H NMR BocHN-D-Ala-C(O)-NH₂



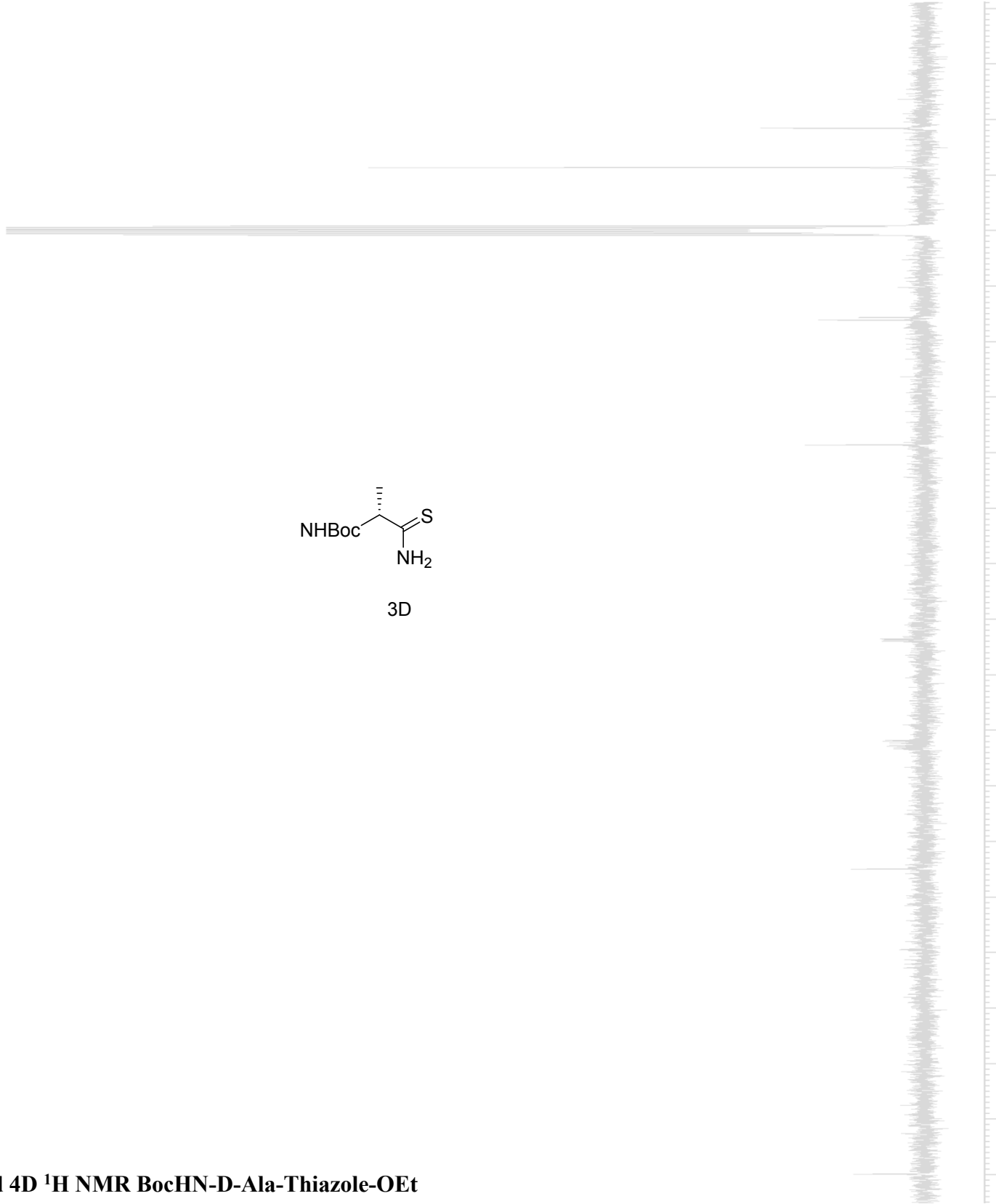
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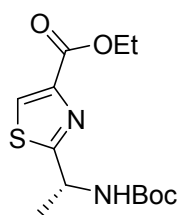
Compound 3D ¹H NMR BocHN-D-Ala-C(S)-NH₂



Compound 3D ^{13}C NMR BocHN-D-Ala-C(S)-NH₂

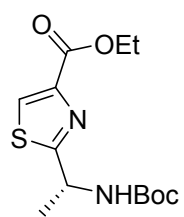


Compound 4D ¹H NMR BocHN-D-Ala-Thiazole-OEt



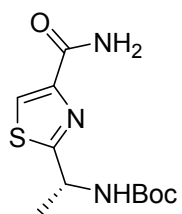
4D

Compound 4D ¹³C NMR BocHN-D-Ala-Thiazole-OEt



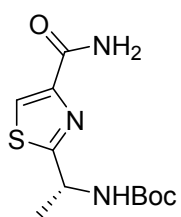
4D

Compound 5D ^1H NMR BocHN-D-Ala-Thiazole-C(O)-NH₂



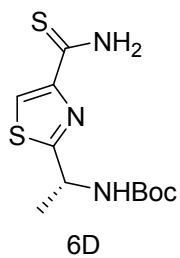
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Compound 5D ^{13}C NMR BocHN-D-Ala-Thiazole-C(O)-NH₂

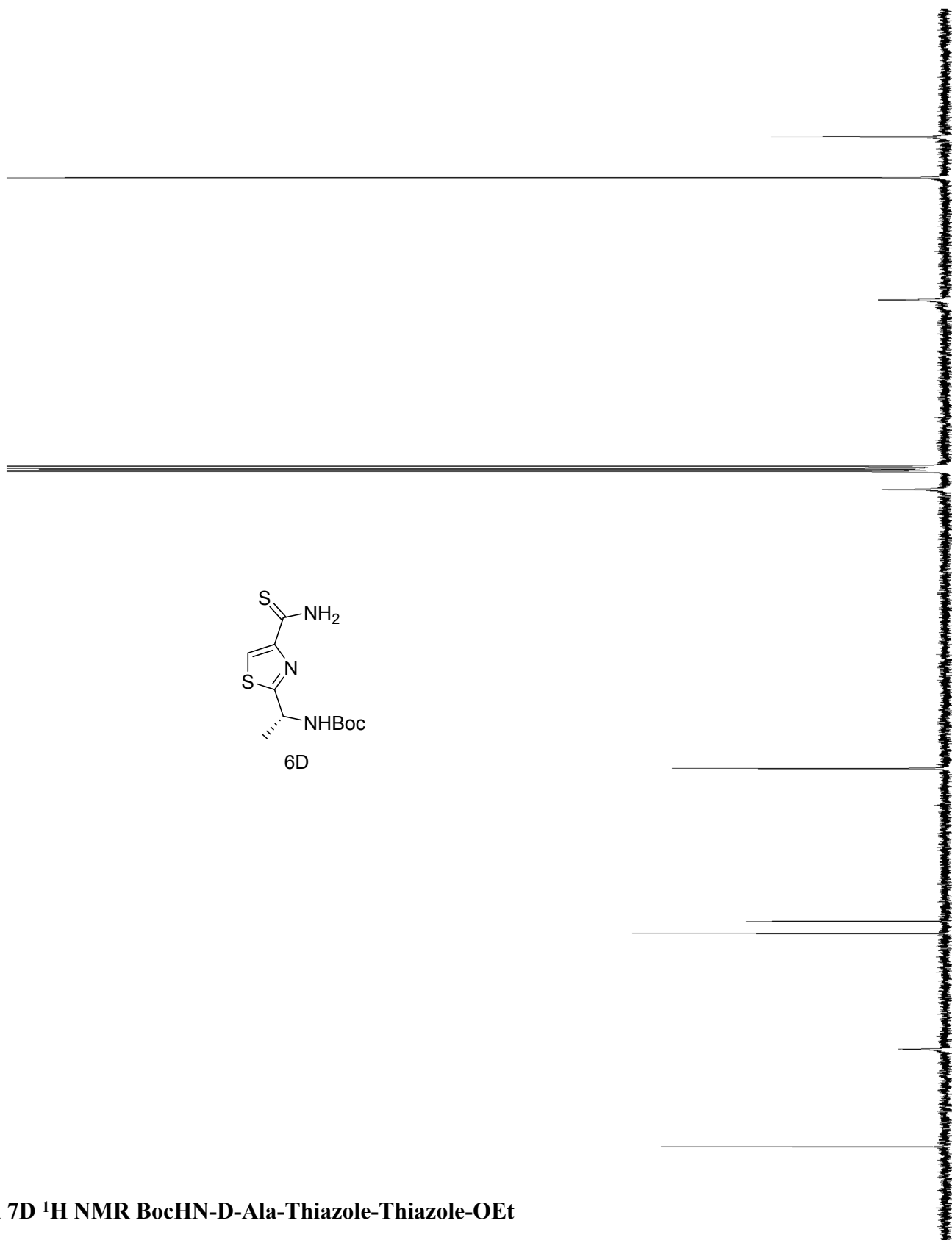


5D

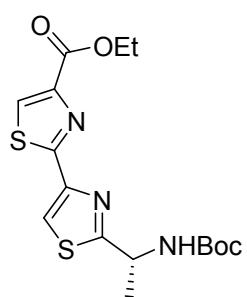
Compound 6D ¹H NMR BocHN-D-Ala-Thiazole-C(S)-NH₂



Compound 6D ^{13}C NMR BocHN-D-Ala-Thiazole-C(S)-NH₂

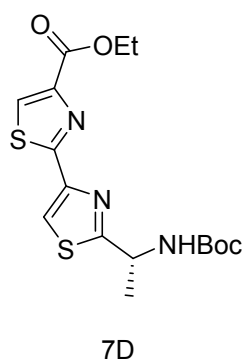


Compound 7D ^1H NMR BocHN-D-Ala-Thiazole-Thiazole-OEt

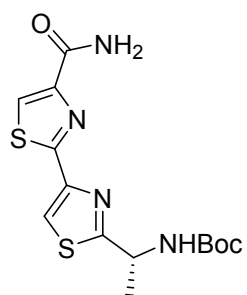


7D

Compound 7D ^{13}C NMR BocHN-D-Ala-Thiazole-Thiazole-OEt

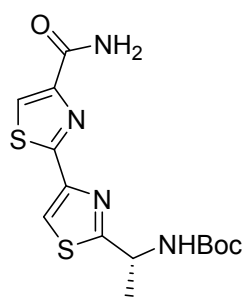


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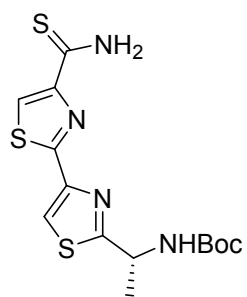
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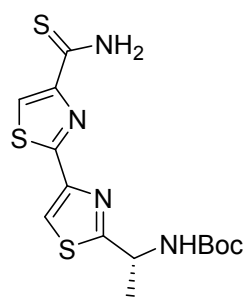
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Compound 9D ^1H NMR BocHN-D-Ala-Thiazole-Thiazole-C(S)-NH₂



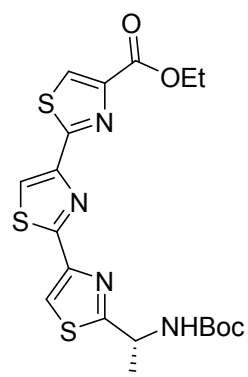
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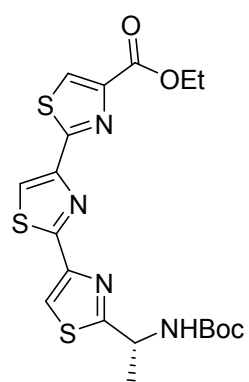
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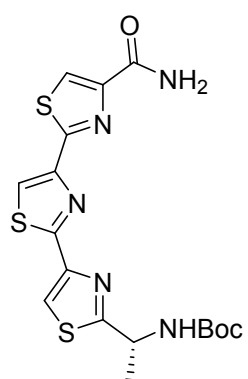
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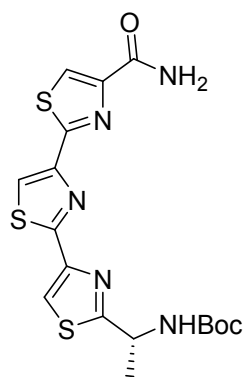
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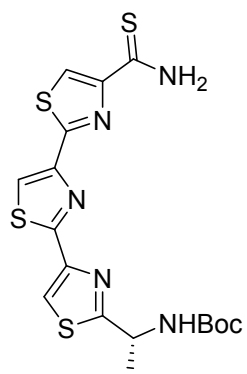
11D

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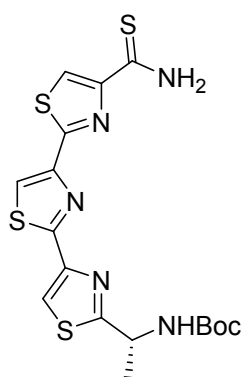
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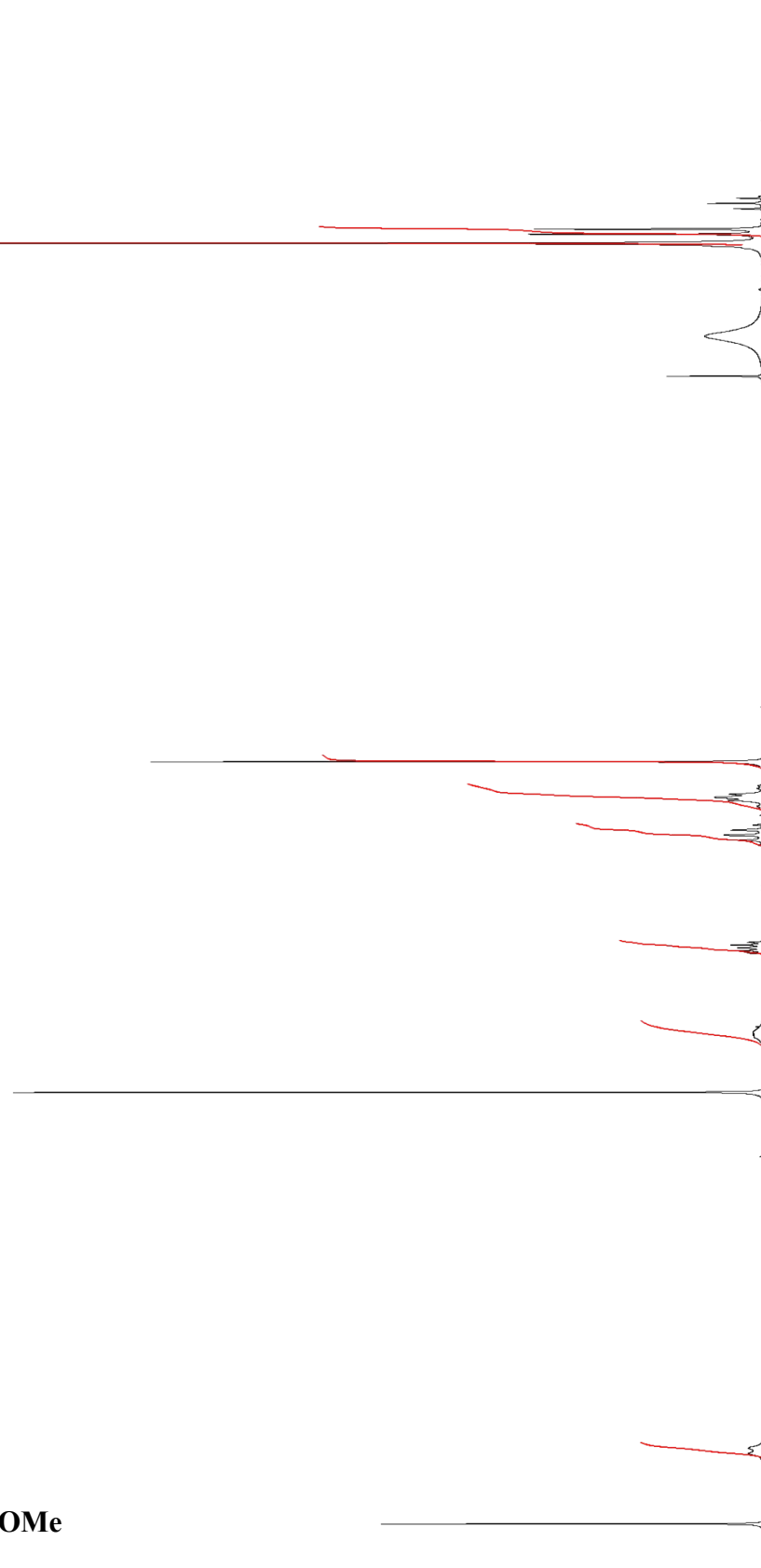
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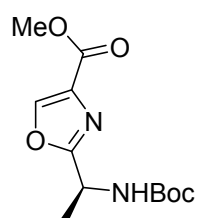
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12D

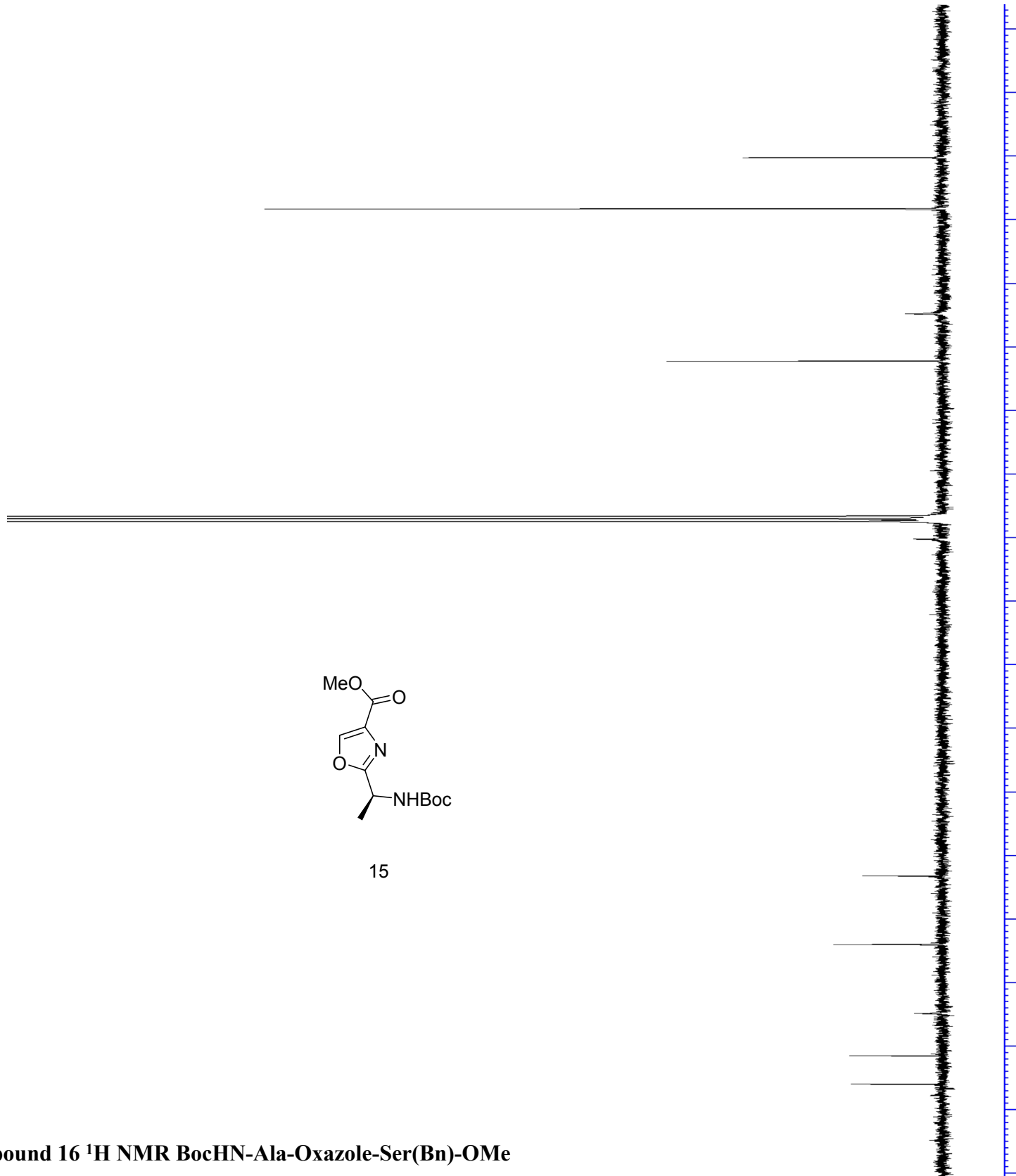
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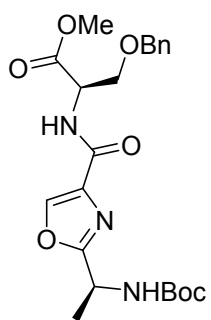


15

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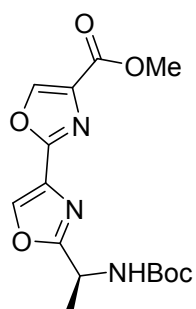


Compound 16 ¹H NMR BocHN-Ala-Oxazole-Ser(Bn)-OMe



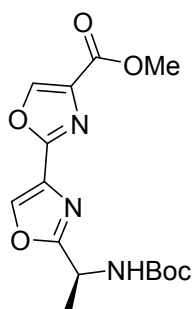
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Compound 17 ¹H NMR BocHN-Ala-Oxazole-Oxazole-OMe



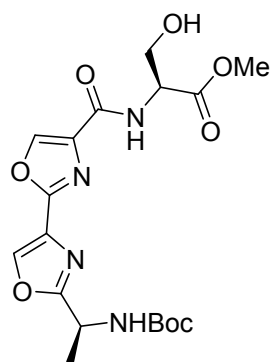
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Compound 17 ^{13}C NMR BocHN-Ala-Oxazole-Oxazole-OMe



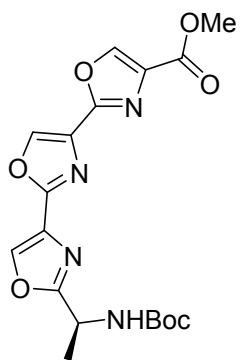
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Compound 18 ^1H NMR BocHN-Ala-Oxazole-Oxazole-Ser-OMe



18

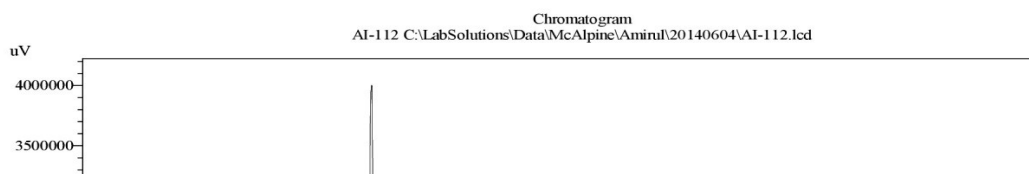
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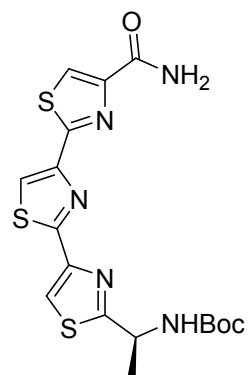


19

Compound 11L LC-MS BocHN-Ala-Thiazole-Thiazole-Thiazole-C(O)-NH₂

==== Shimadzu LCMSsolution Analysis Report ====

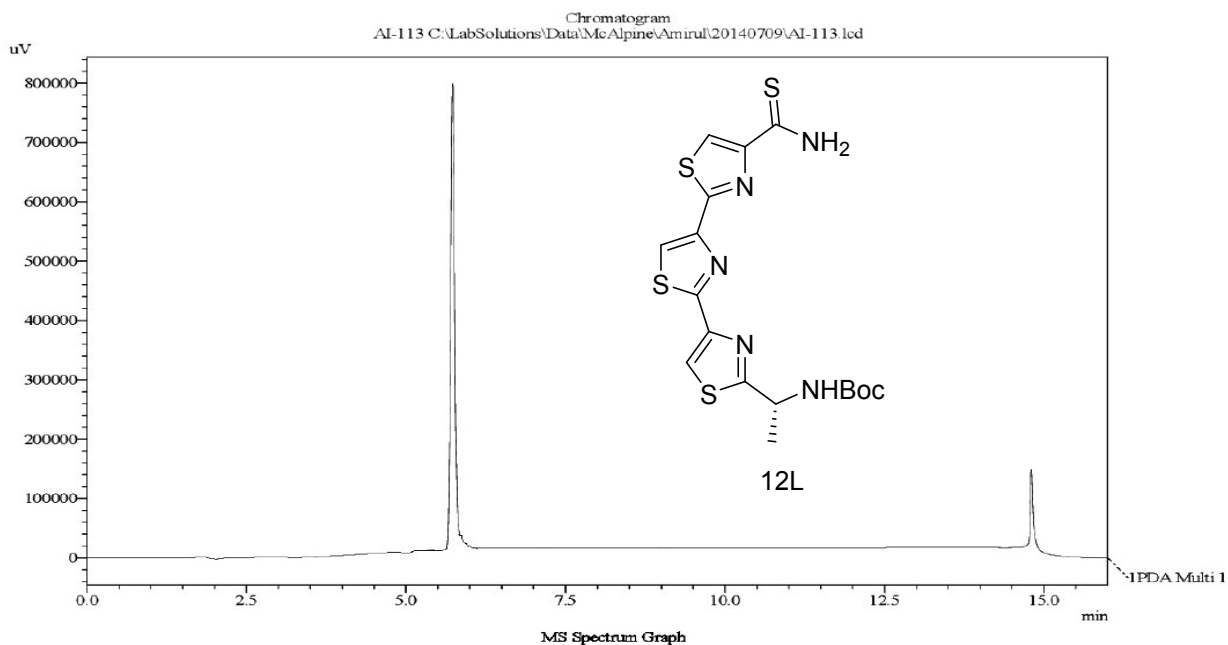




11L

Compound 12L LC-MS BocHN-Ala-Thiazole-Thiazole-Thiazole-C(S)-NH₂

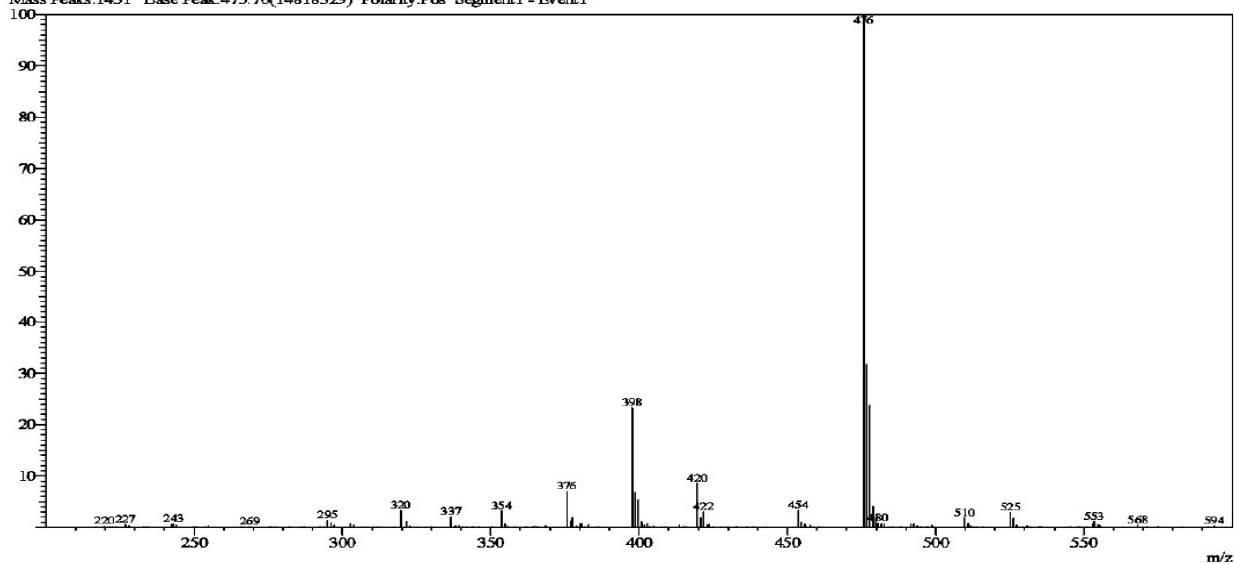
==== Shimadzu LCMSSolution Analysis Report ====



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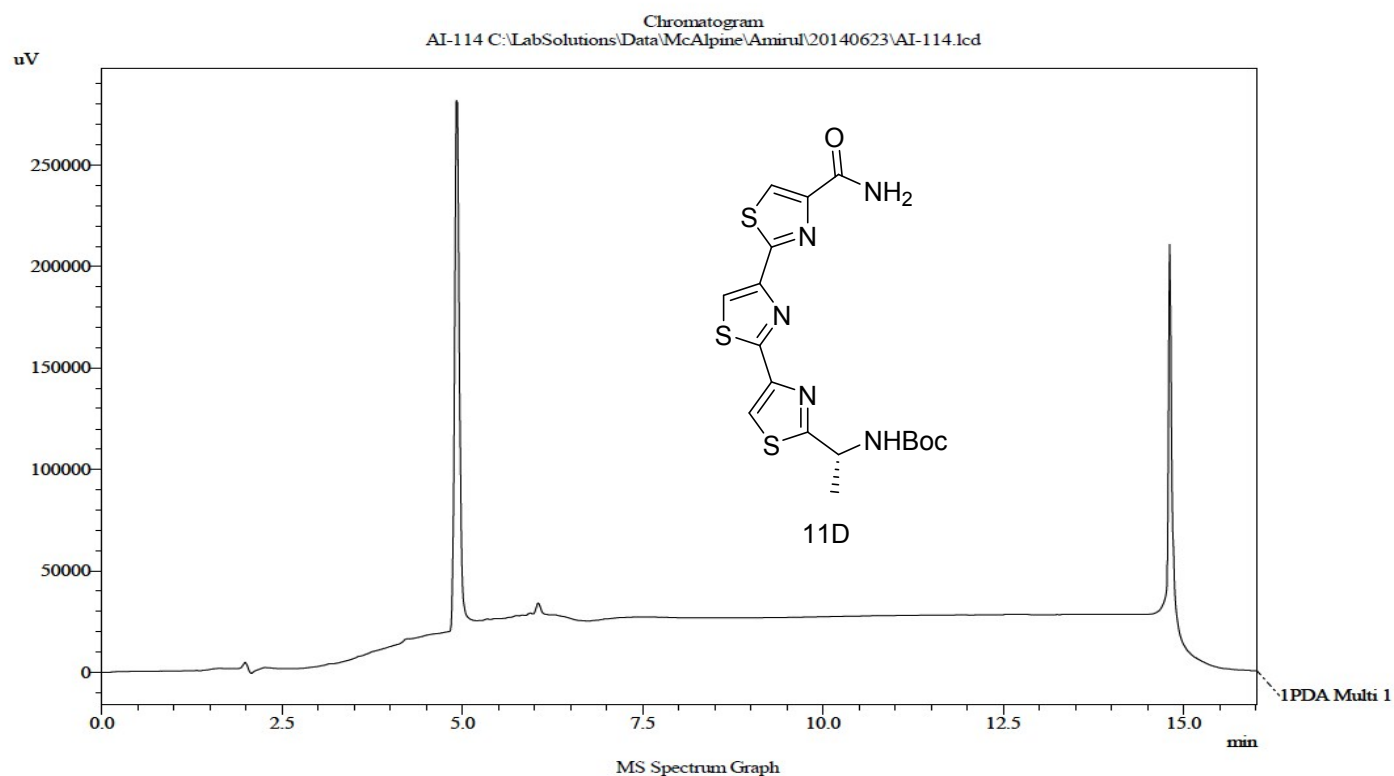
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Mass Peaks: 1451 Base Peak: 475.70 (14818329) Polarity: Pos Segment: 1 - Event: 1



Compound 11D LC-MS BocHN-D-Ala-Thiazole-Thiazole-Thiazole-C(O)-NH₂

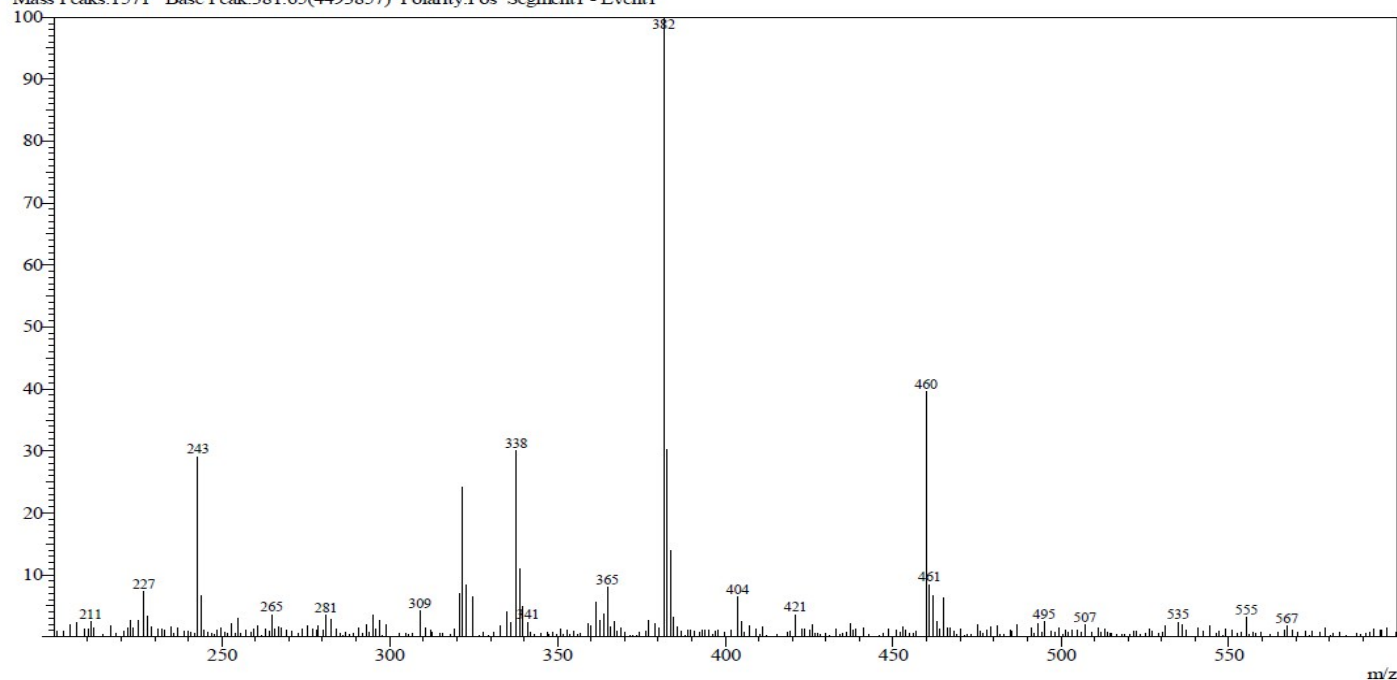
==== Shimadzu LCMSsolution Analysis Report ====



Ret Time: 4.933(Scan#:291)

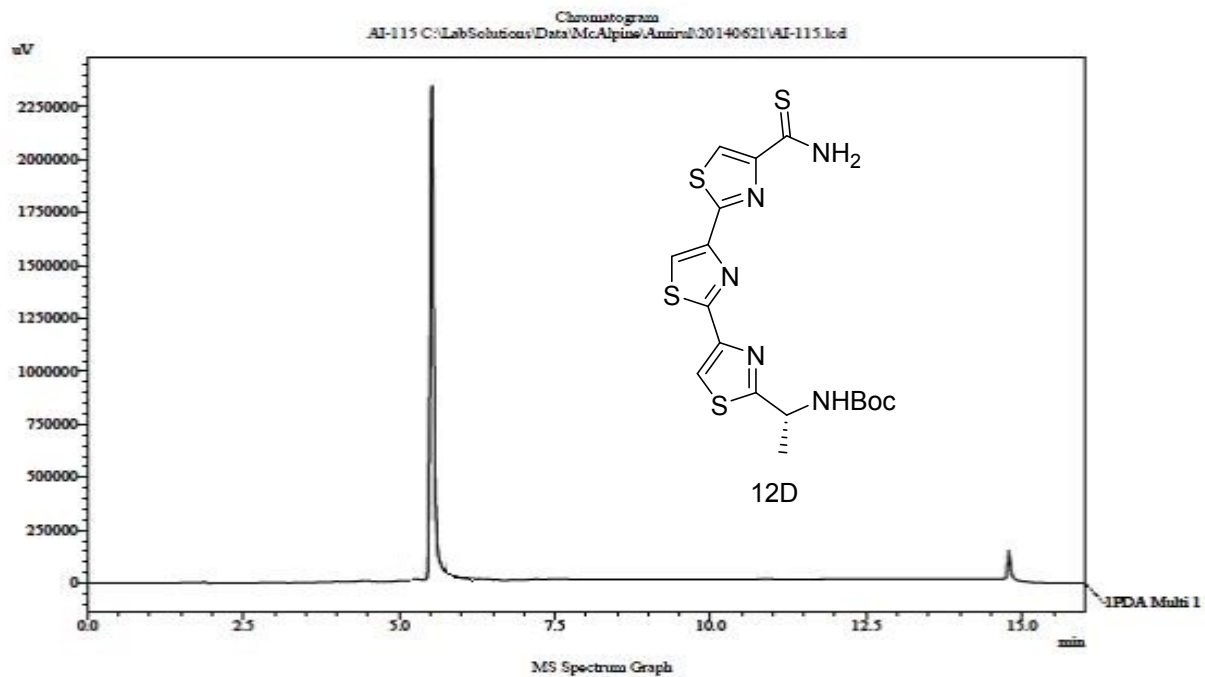
BG Mode:?

Mass Peaks:1571 Base Peak:381.65(4493857) Polarity:Pos Segment1 - Event1

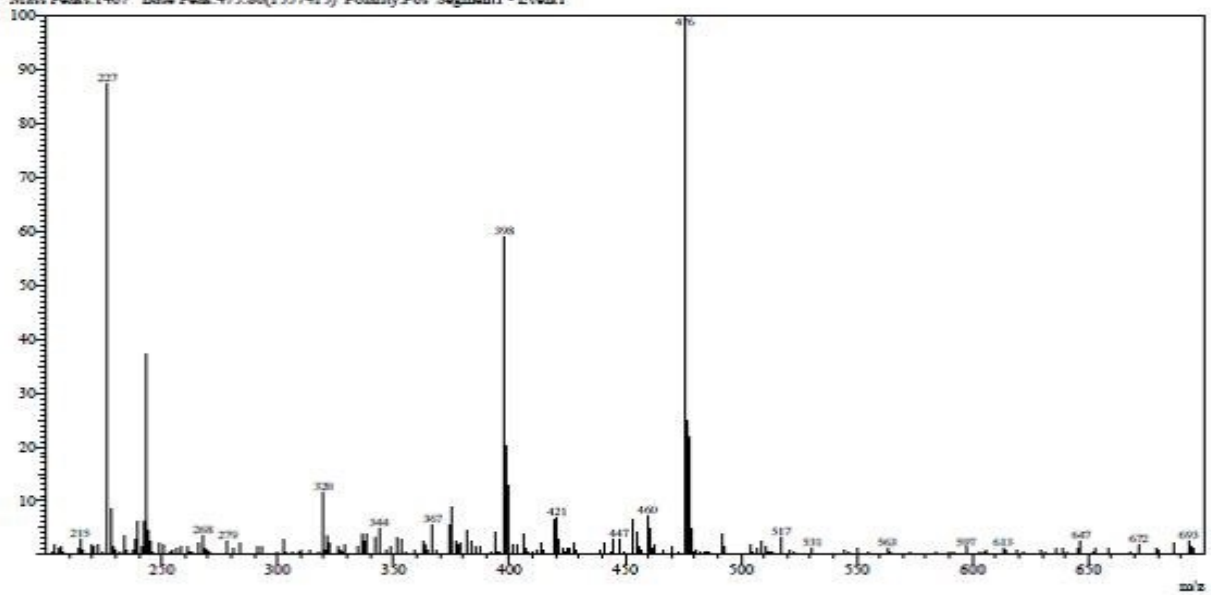


Compound 12D LC-MS BocHN-D-Ala-Thiazole-Thiazole-Thiazole-C(S)-NH₂

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Ret Time: 5.500 (Scan# 315)
BG Mode: None
Mass Peak: 1467 Base Peak: 475.80 (1557415) Polarity: Pos Segment1 - Event1

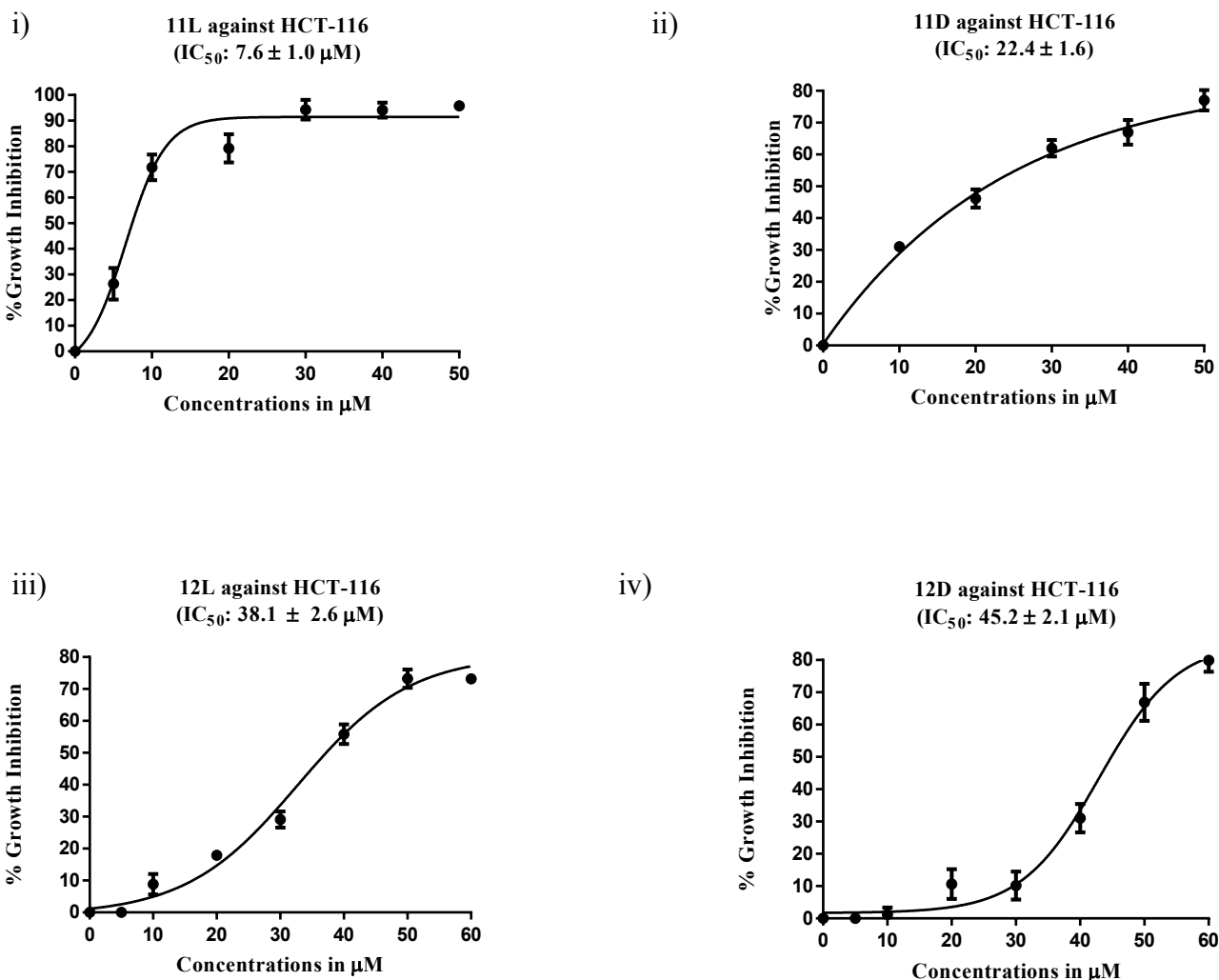


Cytotoxicity assay

Cytotoxicity of the compounds against human colon cancer cell line HCT-116 cells was determined using Cell Counting Kit-8 [2-(2-methoxy-4-nitrophenyl)-3-(4-nitrophenyl)-5-(2,4-disulfophenyl)-2H-tetrazolium, monosodium salt] reduction assay. The cells were maintained in Dulbecco's Modified Eagle Medium (DMEM), supplemented with 10% fetal bovine serum (FBS) and 1% penicillin/streptomycin (Invitrogen/Life Technologies). HCT-116 cells were seeded in 96-well dishes (2×10^3 cells per well) and allowed to adhere to the dish for 24 hours. For each assay (100 μ l reaction volume), cells were incubated in the presence of control compounds or test compounds in complete growth medium (DMEM with 10% FBS). To determine the IC_{50} values of linked thiazole, a range of concentrations of each compound were tested (1- 60 μ M). Controls for the assay were media alone (no cells; background control), DMSO (1%; negative control) or the 17-AAG (17-(allylamino)-17-demethoxygeldanamycin; 100 nM; positive control; Sigma Aldrich). Cells were incubated in the presence of compound for 72 hours at 37°C with 5% CO₂.

Proliferation of HCT-116 cells was measured using a Cell Counting Kit-8 assay (CCK-8; Dojindo Molecular Technologies, Rockville, Maryland, USA), following the manufacturer's instructions. Reduction of the formazan dye was measured using a ChroMate 4300 microplate reader (450 nm; Awareness Technology Inc.). The absorbance values for compound-treated samples were compared to the DMSO control, and the average percent growth inhibition was determined for each compound tested. IC_{50} values were determined by plotting the percent growth inhibition versus the concentration of compound, and analysis was performed using GraphPad Prism software (GraphPad Software, Inc., La Jolla, California, USA). Assays were performed in at least triplicate with each data point performed in quadruplicates. An IC_{50} values is an average of three independent cytotoxicity assays with standard error of mean represent error.

IC₅₀ curves against HCT-116 cell line



Supplemental figure 1: IC₅₀ curves for 11L, 11D, 12L and 12D against HCT-116. The average IC₅₀ values were calculated from at least three independent experiments with standard error of mean.

Apoptosis and cell-cycle analysis

HCT-116 cells were seeded in 6-well plates with a density of 1×10^5 cells per well, incubated at 37 °C for 24 h, and then treated with indicated drugs or DMSO for another 20 or 24 h. Treated cells were harvested by trypsinization, collected and washed with phosphate buffered saline (PBS; Sigma Aldrich) for one time, and then separated equally for apoptosis and cell-cycle analyses. Cells for apoptosis analysis were stained with Annexin V-FITC (Biolegend) and 7AAD (Biolegend)

in Annexin-V binding buffer (Biolegend) for 15 min, and then analyzed by using BD LSRFortessa flow cytometer immediately. Data was quantified by CellQuest software (BD Biosciences). Cells separated for cell-cycle analysis were fixed with -20 °C cold 75% ethanol (in PBS) overnight. Fixed cells were washed once with PBS and stained at 37 °C for 30 min with propidium iodide (PI; Life Technologies) in the presence of ribonuclease A (RNase A; Sigma Aldrich) in PBS. Cell cycle distribution was analyzed by BD LSRFortessa flow cytometer. Data was quantified by CellQuest software (BD Biosciences).

Detection of G2/M DNA damage checkpoint activation

HCT-116 cells were seeded in 6-well plates with a density of 1×10^5 cells per well, incubated at 37 °C for 24 h, and then treated with indicated drugs or DMSO for another 20 or 24 h. Treated cells were collected, washed with PBS once and then fixed with -20 °C cold 75% ethanol (in PBS) overnight. Fixed cells were washed once with PBS, and further permeabilized with 0.1% Tween 20 in 2% BSA-PBS for 30 min at room temperature. Cells were then incubated with anti-Histone H3 (phospho, Ser-10) rabbit polyclonal antibody (Abcam, 1:1000 dilution in 2% BSA-PBS with 0.1% Tween 20) at 37 °C for 1 h. After washing with 0.1% PBST once, cells were incubated with a secondary antibody, goat anti-rabbit IgG (FITC) (Biolegend, 1:150 dilution in 2% BSA-PBS with 0.1% Tween 20), at 37 °C for 30 min in dark. Cells were then washed with 0.1% PBST once and stained with PI at 37 °C for 30 min in the presence of RNase A in PBS. Cell cycle distribution and the percentage of mitotic cells were analyzed by BD LSRFortessa flow cytometer. Data was quantified by CellQuest software (BD Biosciences).

Quantification of the DNA damage marker γ -H2AX by flow cytometry

HCT-116 cells were seeded in 6-well plates with a density of 1×10^5 cells per well, incubated at 37 °C for 24 h, and then treated with indicated drugs or DMSO for another 20 or 24 h. Treated cells were collected, washed with PBS once, and then fixed with -20 °C cold 75% ethanol (in PBS) overnight. Fixed cells were washed once with PBS, and further permeabilized with 0.1% Tween 20 in 2% BSA-PBS for 30 min at room temperature. Cells were then incubated with anti- γ -H2AX rabbit polyclonal antibody (Abcam, 1:1000 dilution in 2% BSA-PBS with 0.1% Tween 20) at 37 °C for 1 h. After washing with 0.1% PBST once, cells were incubated with a secondary antibody, goat anti-rabbit IgG (FITC)

(Biolegend, 1:150 dilution in 2% BSA-PBS with 0.1% Tween 20), at 37 °C for 30 min in dark. Cells were then washed with 0.1% PBST once and stained with PI at 37 °C for 30 min in the presence of RNase A in PBS. Fluorescence intensities were determined with a BD LSRFortessa flow cytometer. Data was quantified by CellQuest software (BD Biosciences).