# Supporting Information

**Synthesis of the natural product Marthiapeptide A**

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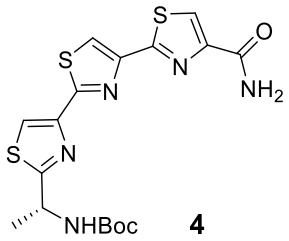
**General Remarks**

All reactions were carried out under an argon atmosphere with dry solvents under anhydrous conditions, unless otherwise stated. Reagents were commercially obtained (Peptides International, ChemImpex, Aldrich, GLbiochem 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 in water as heat and 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 30°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 variable temperature (VT) NMR spectra were taken from 318 K to 248 K. The following abbreviations were used to explain multiplicities: s = singlet, d = doublet, t = triplet, q = quartet, p = pentet, m = multiplet, b = broad, bd = broad doublet, dd = doublet of doublet, and dq = doublet of quartet.

LC/MS was recorded on an Agilent 1200 Series HPLC system (Zorbax Agilent SB-$C_{18}$ column, 3.5μm, 2.1 x 30mm) connected to an Agilent 62440A LC/MS Trap running in the positive electrospray ionization (ESI+) mode. The mobile phase was 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**



**4**

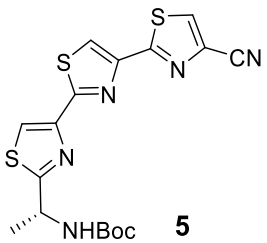
**BocHN-D-Ala-Thiazole-Thiazole-Thiazole-C(O)-$NH_2$ (4).** BocHN-D-Ala-Thiazole-Thiazole-Thiazole-C(O)-$NH_2$ was synthesized utilizing 170 mg (0.365 mmol, 1.0 equiv.) of BocHN-D-Ala-Thiazole-Thiazole-Thiazole-OEt in 6 mL of ammonium hydroxide solution (28% in water) and 6 mL of MeOH. The reaction mixture was sonicated for 24 hrs. Upon completion, The reaction was co-vaporized with MeOH 4 times followed by $CH_2Cl_2$ 2 times then further dried *in vacuo*; the resulting amine was taken on to the next reaction without further purification (171 mg, quantitative) as a white powder.

$R_f$: 0.208 (hexane/ EtOAc 1:1).

$^1$H NMR (300 MHz, DMSO): 1.423 (br, 9H, C(C$\underline{H}_3$)$_3$); 1.50 (d, *J* = 7.05Hz, 3H, C$\underline{H}_3$CH); 4.92 (dq, *J* = 7.00, 7.19 Hz, 1αH); 7.69 (br, 1H, N$\underline{H}_2$); 7.83 (br, 1H, N$\underline{H}_2$); 7.88 (d, *J* = 7.54Hz, N$\underline{H}$); 8.27 (s, 1H, SC$\underline{H}$C), 8.31 (s, 1H, SC$\underline{H}$C); 8.33 (s, 1H, SC$\underline{H}$C).

$^{13}$C NMR (75MHz, DMSO) δ 20.51, 28.19(3C), 48.59, 78.60, 117.94, 118.46, 124.63, 147.07, 148.75, 151.16, 155.11, 161.42, 162.16, 163.12, 177.75.

ES$^+$MS *m/z* calcd for $C_{17}H_{19}N_5O_3S_3$ ([M+Na]$^+$) 460.07, found 460.65



**5**

**BocHN-D-Ala-Thiazole-Thiazole-Thiazole-CN (5).** BocHN-D-Ala-Thiazole-Thiazole-Thiazole-CN was synthesized utilizing 100 mg (0.34 mmol, 1.0 equiv.) of BocHN-D-Ala-Thiazole-Thiazole-Thiazole-C(O)-$NH_2$ in 24 mL of anhydrous $CH_2Cl_2$ under nitrogen atmosphere at -20°C. While stirring, 0.15 ml (0.95 mmol, 2.8 equiv.) DIPEA was added into the reaction mixture in dropwise. After 5 min, 0.07 ml (0.38 mmol, 1.1 equiv.) TFAA was added into the reaction mixture

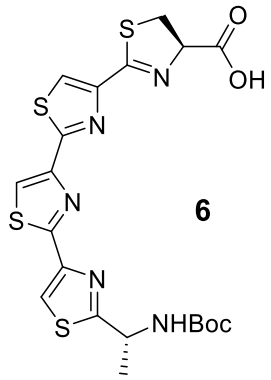
cautiously. The reaction was flushed with nitrogen to remove produced vapour. The reaction was allowed to stir overnight with gradually warm up to room temperature. Upon completion, the reaction was concentrated and re-dissolved in $CH_2Cl_2$ and directly purified over flash column chromatography on silica at gradient elute with hexane and EtOAc to give product as white solid (110mg, 65%).

$R_f$: 0.833 (hexane/ EtOAc 1:1).

$^1$H NMR (600 MHz, $CDCl_3$): 1.479 (br, 9H, C($CH_3$)$_3$); 1.664 (d, $J$ = 6.60 Hz, 3H, $CH_3$CH); 5.14 (br, 1αH); 7.99 (s, 1H, SCHC); 8.00 (s, 1H, SCHC), 8.31 (s, 1H, SCHC).

$^{13}$C NMR (150MHz, $CDCl_3$) δ 21.84, 28.49(3C), 48.95, 80.54, 114.19, 117.10, 118.81, 127.66, 130.70, 148.32, 148.45, 155.12, 163.81, 164.41, 175.18.

ES$^+$MS *m/z* calcd for $C_{17}H_{17}N_5O_2S_3$ ([M+Na]$^+$) 442.05, found 442.00




**6**

**BocHN-D-Ala-Thiazole-Thiazole-Thiazole-(R)-Thiazoline-OH (6).** BocHN-D-Ala-Thiazole-Thiazole-Thiazole-(R)-Thiazoline-OH was synthesized utilizing 110 mg (0.262 mmol, 1.0 equiv.) of BocHN-D-Ala-Thiazole-Thiazole-Thiazole-CN and 33.0 mg (0.275 mmol, 1.05 equiv.) L-Cysteine and 110 mg (1.31 mmol, 5.0 equiv.) of $NaHCO_3$ in 5.0 ml of MeOH. While stirring, 1.0 ml of pH = 5.95 phosphate buffer solution was added into the reaction mixture. The reaction mixture was then stirred at 70$^o$C for 4 hr followed by routine TLC monitoring for reaction progress. Upon completion, the reaction mixture was concentrated to remove all of the solvent then re-dissolved in EtOAc and then poured into the Sat. $NaHCO_3$ solution. The organic layer was set aside, the aqueous layer was further extract with EtOAc for 2 times. The aqueous was then acidified using pH = 1 hydrochloric acid solution in dropwise with monitoring until reach pH = 3. The aqueous layer was then extracted with EtOAc 3 times. All organic layer was then collected, combined and dried over anhydrous $Na_2SO_4$

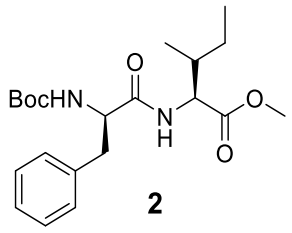
followed by filtration, concentration *in vacuo* and further dried *in silico* to give white powder (134 mg, 98%). The material was subjected for next reaction without further purification.

$R_f$: 0.4 (EtOAc/ MeOH 9:1).

$^1$H NMR (600 MHz, DMSO): 1.43 (br, 9H, C(C<u>H</u>$_3$)$_3$); 1.515 (d, *J* = 7.07 Hz, 3H, C<u>H</u>$_3$CH); 3.60 (dd, *J* = 8.21, 11.2 Hz, 1H, C<u>H</u>$_2$); 3.70 (dd, *J* = 8.21, 11.2 Hz, 1H, C<u>H</u>$_2$); ); 5.14 (br, 1αH); 5.32 (t, *J* = 8.78 Hz, 1H, C<u>H</u>CH$_2$); 7.88 (d, *J* = 7.19 Hz, 1H, N<u>H</u>); 8.283 (s, 1H, SC<u>H</u>C); 8.346 (s, 1H, SC<u>H</u>C), 8.40 (s, 1H, SC<u>H</u>C)

$^{13}$C NMR (150MHz, DMSO) δ 20.5, 28.49(3C), 34.48, 48.95, 50.17, 54.90, 61.31, 78.32, 80.04, 117.74, 118.03, 118.7, 122.75, 128.64, 147.03, 148.32, 148.87, 151.81, 155.20, 161.98, 163.11, 171.76 177.73.

ES$^+$MS *m/z* calcd for $C_{20}H_{21}N_5O_4S_4$ ([M+Na]$^+$) 546.05, found 546.24




**2**

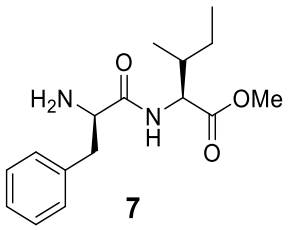
**BocHN-D-Phe-Ile-OMe (2).** BocHN-D-Phe-Ile-OMe was synthesized utilizing 2.12 g (8.0 mmol, 1.0 equiv.) of BocHN-D-Phe-OH and 2.83 g (8.8 mmol, 1.1 equiv.) TBTU dissolved in 60 mL of anhydrous $CH_2Cl_2$, 1.31 g (8.8 mmol, 1.1 equiv.) H2N-Ile-OMe dissolved in 20 mL anhydrous $CH_2Cl_2$, 4.18 mL (24.0 mmol, 3.0 equiv.) of DIPEA was added into the free acid containing reaction mixture drop wise then to the amine containing reaction mixture. After 5 min, amine reaction mixture was then taken up and injected into the free acid containing reaction mixture drop wise. Upon completion the reaction the mixture washed with acid and base and purified by flash column on silica gel elute with gradient of hexane and EtOAc to give desired product as white solid (2.88 g, 92%)

$R_f$: 0.48 (hexane/ EtOAc 1:1).

$^1$H NMR (300 MHz, $CDCl_3$): 0.75 (d, *J* = 7.6 Hz, 3H, CHC<u>H</u>$_{3(Ile)}$); 0.85 (t, *J* = 8.0 Hz, 3H, CH$_2$C<u>H</u>$_3$); 0.98 (m, 1H, C<u>H</u>$_2$CH$_3$); 1.27 (m, 1H, C<u>H</u>$_2$CH$_3$); 1.41 (br, 9H, C(C<u>H</u>$_3$)$_3$); 1.74 (m, 1H, CH$_3$C<u>H</u>); 3.06 (d, *J* = 8.0 Hz, 2H, C<u>H</u>$_{2\ (Phe)}$); 3.70 (s, 3H, OC<u>H</u>$_3$); 4.37 (m, 1H, C<u>H</u>$_{(Phe)}$); 4.50 (m, 1H, C<u>H</u>$_{(Ile}$); 5.00 (br, 1H, N<u>H</u>); 6.35(d, *J* = 9 Hz, 1H, N<u>H</u>); 7.2-7.33 (m, 5H, C<u>H</u>$_{(Phe)}$)

$^{13}$C NMR (75MHz, $CDCl_3$) δ 11.61, 15.41, 25.10, 28.38(3C), 37.91, 38.50, 52.20, 56.06, 56.56, 80.44, 127.11, 128.88, 129.38, 136.73, 155.44, 171.11, 172.11.

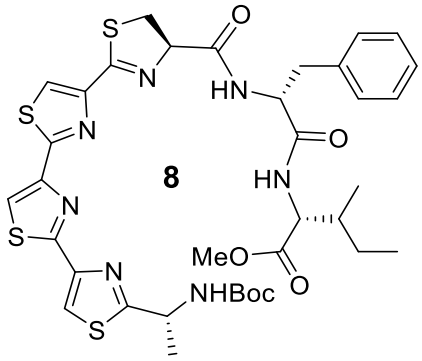
ES$^+$MS *m/z* calcd for $C_{21}H_{32}N_2O_5$ ([M+Na]$^+$) 415.23, found 415.18




**7**

**$H_2$N-D-Phe-Ile-OMe (7).** $H_2$N-D-Phe-Ile-OMe was synthesized utilizing 393 mg (1.00 mmol, 1.0 equiv.) of BocHN-D-Phe-Ile-OMe in 8.0 ml of $CH_2Cl_2$. 0.19 ml (2.0 mmol, 2.0 equiv.) of anisole was added into the reaction mixture in dropwise while stirring. 2.0 ml of TFA was added into the reaction mixture in dropwise while stirring. The reaction was allowed to stir for 4 hrs with TLC monitoring. Upon completion, the solvent was evaporated followed by co-evaporation with $CH_2Cl_2$ for 6 times. The product was then dried *in silico* and subjected to next reaction without further purification to give clear crystal (301mg, quantitative)

ES$^+$MS *m/z* calcd for $C_{16}H_{24}N_2O_3$ ([M+Na]$^+$) 315.18, found 315.00



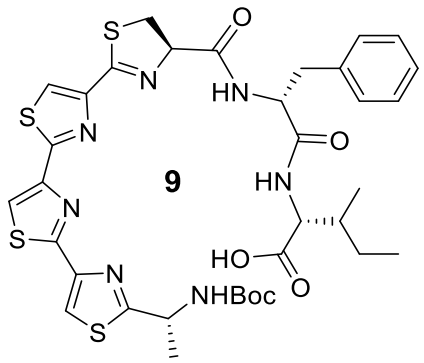

**BocHN-D-Ala-Thiazole-Thiazole-Thiazole-R-Thiazoline-D-Phe-Ile-OMe (8).** BocHN-D-Phe-Ile-OH was synthesized utilizing 0.144 g (0.275 mmol, 1 equiv.) of Boc-D-Ala-Thiazole-Thiazole-Thiazole-R-Thiazoline-OH and 0.09 g (0.30 mmol, 1.1 equiv) HATU were dissolved in 20 mL of $CH_2Cl_2$, 0.38 mL(2.20 mmol, 8.0 equiv.) DIPEA was added to the mixture and finally 87 mg (0.30 mmol, 1.1 equiv.) of NH2-D-Phe-Ile-OMe pre-dissolved in 7.5 mL of $CH_2Cl_2$ was added to the acid and coupling mixture solution in drop wise. Upon completion, the reaction mixture was purified by flash column on silica gel elute with gradient of hexane and EtOAc to give final product as white solid (0.154 g, 70%).

$R_f$: 0.45 (hexane/EtOAc 1:1).

$^{1}$H NMR (600 MHz, $CDCl_3$): δ 0.706 (m, 3H, CHC$\underline{H}_3$), 0.957 (m, 3H, $CH_2C\underline{H}_3$), 1.069 (m, 1H, C$\underline{H}_2CH_3$); 1.249 (m, 1H, C$\underline{H}_2CH_3$); 1.479 (br, 9H, C(C$\underline{H}_3$)$_3$); 1.66 (d, *J* = 6.65Hz, 3H, CHC$\underline{H}_3$); 1.70 (m, 1H, C$\underline{H}$CH$_3$); 3.141-3.171 (m, 2H, CHC$\underline{H}_2$C); 3.62 (m, 3H, OC$\underline{H}_3$); 3.67-3.78 (m, 2H, CHC$\underline{H}_2$S); 4.45 (m, 1H, C$\underline{H}_{(Ile)}$); 4.801(m, 1H, C$\underline{H}_{(Phe)}$); 5.138 (m, 1H, C$\underline{H}$); 5.22 (m, 1H, C$\underline{H}$CH$_2$S); 6.439 (br, 1H, N$\underline{H}$); 7.26 (m, 1H, C$\underline{H}$); 7.34 (m, 2H, C$\underline{H}$); 7.342 (m, 2H, C$\underline{H}$); 7.99 (m, 1H, N$\underline{H}$); 7.181(m, 2H, C$\underline{H}$), 7.235 (m, 1H, C$\underline{H}$); 7.275 (m, 2H, C$\underline{H}$); 7.31 (m, 1H, N$\underline{H}$); 7.341 (m, 1H, N$\underline{H}$); 8.02 (s, 1H, SC$\underline{H}$C), 8.04 (m, 1H, SC$\underline{H}$C); 8.144 (m, 1H, SC$\underline{H}$C).

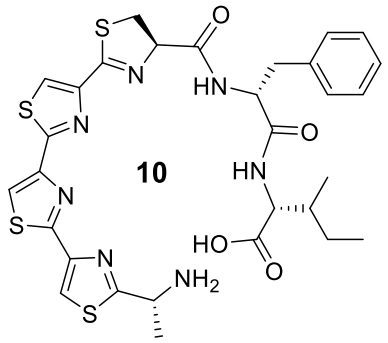
$^{13}$C NMR (150MHz, $CDCl_3$) δ 11.48, 15.31, 21.81, 25.11, 28.49(3C), 34.89, 37.78, 38.69,48.93, 52.18, 54.76, 56.67, 77.46, 80.46, 116.86, 117.63, 124.38, 127.13, 128.79, 129.47, 136.7, 148.47, 149.33, 150.10, 155.10, 161.04, 162.78, 163.43, 169.162, 170.38, 171.92, 174.9.

ES$^+$MS *m/z* calcd for $C_{36}H_{43}N_7O_4S_4$ ([M+Na]$^+$) 820.22, found 820.25




**BocHN-D-Ala-Thiazole-Thiazole-Thiazole-R-Thiazoline-D-Phe-Ile-OH (9)**. BocHN-D-Ala-Thiazole-Thiazole-Thiazole-(R)-Thiazoline-D-Phe-Ile-OH was synthesized utilizing 100 mg (0.125 mmol, 1.0 equiv.) of BocHN-D-Ala-Thiazole-Thiazole-Thiazole-R-Thiazoline-D-Phe-Ile-OMe in 5.0 ml of MeOH, 49.1 mg (2.04 mmol, 8.0 equiv.) LiOH.$H_2$Owas added into the reaction mixture in one portion while stirring. Catalytic amount of $H_2O$ was also added into the reaction mixture. The reaction was allowed to proceed at room temperature for overnight followed by ultrasound sonication for 5.0 hrs. Upon completion, the reaction mixture was washed with pH = 1 hydrochloric acid solution and extracted twice with $CH_2Cl_2$ and twice with EtOAc. The organic layer was collected, combined and dried over anhydrous $Na_2SO_4$. The product was subjected to further reaction without purification to give desired product as white solid (96 mg, 98%)

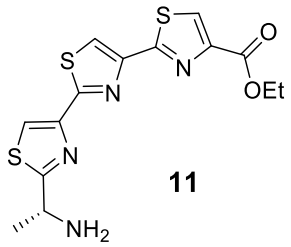
LC-MS ES$^+$MS *m/z* calcd for $C_{35}H_{41}N_7O_6S_4$ ([M+Na]$^+$) 806.20, found 805.95



**10**

**$H_2N$-D-Ala-Thiazole-Thiazole-Thiazole-R-Thiazoline-D-Phe-Ile-OH (10).** $H_2N$-D-Ala-Thiazole-Thiazole-Thiazole-R-Thiazoline-D-Phe-Ile-OMe was synthesized utilizing 90 mg (0.115 mmol, 1.0 equiv.) of BocHN-D-Ala-Thiazole-Thiazole-Thiazole-R-Thiazoline-D-Phe-Ile-OH in 3.0 ml of $CH_2Cl_2$. 25µl (0.23 mmol, 2.0 equiv.) of anisole was added into the reaction mixture in dropwise while stirring. 1.0 ml of TFA was added into the reaction mixture in dropwise while stirring. The reaction was allowed to stir for 4 hrs with TLC monitoring. Upon completion, the solvent was evaporated followed by co-evaporation with $CH_2Cl_2$ for 6 times. The product was then dried *in silico* and subjected to next reaction without further purification to give a brown oil (85mg, quantitative).

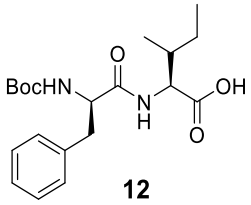
$ES^+MS$ *m/z* calcd for $C_{30}H_{33}N_7O_4S_4$ ($[M+Na]^+$) 706.15, found 705.88



**11**

**$H_2N$-D-Ala-Thiazole-Thiazole-Thiazole-OEt (11).** BocHN-D-Ala-Thiazole-Thiazole-Thiazole-OEt (400 mg, 0.857 mmol, 1.0 equiv.) was dissolved in anhydrous $CH_2Cl_2$ (9.0 mL, 0.1 M) at r.t. Anisole (0.18 mL, 1.72 mmol, 2.0 eq.) was then added into the solution. While stirring, TFA (3.0 mL) was added into the reaction in dropwise. The reaction was allowed to proceed for 3 hrs. Upon completion of reaction, confirmed via TLC, the mixture was then co-evaporate with $CH_2Cl_2$ for 6 times. The product free amine was then further dried *in vauco* to afford brown solid (300mg, quantitative)

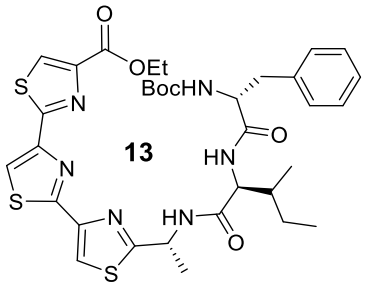
$ES^+MS$ *m/z* calcd for $C_{14}H_{14}N_4O_2S_3$ ($[M+H]^+$) 367.46, found 367.18




**12**

**BocHN-D-Phe-Ile-OH (12).** BocHN-D-Phe-Ile-OMe (370.1 mg, 0.943 mmol, 1.0 equiv.) was dissolved in MeOH (15.0 mL, 0.1 M) at r.t. While stirring, $LiOH{\cdot}H_2O$ (316.4 mg, 7.54 mmol, 8.0 equiv.) was added into the reaction in one portion. MilliQ $H_2O$ (1.0 ml) was then added into the reaction mixture. The reaction was allowed to proceed for overnight. Upon reaction completion, confirmed via TLC, the reaction mixture was concentrated followed by re-dissolving in $CH_2Cl_2$, the solution was then poured into pH = 1 hydrochloric acid solution and extracted twice with $CH_2Cl_2$ and EtOAc, the organic layers were combined and dried over anhydrous $Na_2SO_4$, filtration, and concentrated *in vauco* to give the desired carboxylic acid product as white solid (360mg, quantitative).

$ES^+MS$ *m/z* calcd for $C_{20}H_{30}N_2O_5$ ($[M+Na]^+$) 401.47, found 401.01




**13**

**BocHN-D-Phe-Ile-D-Ala-Thiazole-Thiazole-Thiazole-OEt (13).** BocHN-D-Phe-Ile-D-Ala-Thiazole-Thiazole-Thiazole-OEt was synthesized utilizing 360 mg (0.943 mmol, 1.1 equiv.) of BocHN-D-Phe-Ile-OH and 359 mg (0.943 mmol, 1.1 equiv.) of HATU in 20 mL of $CH_2Cl_2$, 300 mg (0.857 mmol, 1.0 equiv.) of $H_2N$-D-Ala-Thiazole-Thiazole-Thiazole-OEt in $CH_2Cl_2$, DIPEA (1.493 ml, 8.573mmol, 10.0 equiv.) was added into the free acid containing reaction mixture in dropwise then to the amine containing reaction mixture. After 5 min, amine reaction mixture was then taken up and injected into the free acid containing reaction mixture in dropwise. The reaction was allowed to stir for 4 hours followed by TLC monitoring. Upon completion, the reaction mixture was washed with pH = 1 hydrochloric acid solution and extracted twice with $CH_2Cl_2$ and twice with EtOAc. The organic layer was collected and combined followed by washing with Sat. $NaHCO_3$ solution and extract twice with $CH_2Cl_2$ and twice with EtOAc, the organic layer was collected, combined and dried over anhydrous

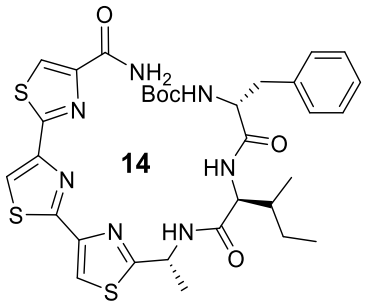
$Na_2SO_4$. The crude material was purified over flash column on silica gel elute with gradient of hexane and EtOAc to give desired product as white solid (450 mg, 72.2%)

$R_f$: 0.556 (hexane/EtOAc 1:1).

$^1$H NMR (600 MHz, $CDCl_3$): δ 0.764 (m, 3H, CHC<u>H</u>$_3$), 0.845 (m, 3H, $CH_2$C<u>H</u>$_3$), 1.382 (br, 9H, C(C<u>H</u>$_3$)$_3$); 1.46 (t, *J* = 7.56 Hz, 3H, $CH_2$C<u>H</u>$_3$); 1.54-1.70 (m, 2H, C<u>H</u>$_2$CH$_3$); 1.685 (d, *J* = 6.66Hz, 3H, CHC<u>H</u>$_3$); 1.99 (m, 1H, CHC<u>H</u>); 3.076-3.138 (m, 2H, CHC<u>H</u>$_2$C); 4.216 (m, 1H, NHC<u>H</u>CH); 4.315(m, 1H, NHC<u>H</u>CH$_2$); 4.45 (q, *J* = 7.06 Hz, 2H, C<u>H</u>$_2$CH$_3$); 5.023 (br, 1H, N<u>H</u>); 5.425 (m, 1H, N<u>H</u>);6.38 (m, 1H, N<u>H</u>); 7.176(m, 2H, CH), 7.21 (m, 1H, CH); 7.271 (m, 2H, CH); 7.30 (m, 1H, NH); 7.948 (s, 1H, SC<u>H</u>C), 8.132 (s, 1H, SC<u>H</u>C); 8.183 (s, 1H, SC<u>H</u>C).

$^{13}$C NMR (from 2D NMR) δ 11.56, 14.39, 15.60, 20.61, 24.69, 28.29(3C), 36.23, 37.85, 47.45, 56.45, 57.95, 61.54, 80.69, 116.802, 117.93, 127.21, 127.79, 128.85, 129.12, 136.07, 147.87, 147.95, 148.18, 149.12, 155.02, 161.46, 163.07, 163.37, 171.23, 171.38, 173.62.

ES$^+$MS *m/z* calcd for $C_{34}H_{42}N_6O_6S_3$ ([M+Na]$^+$) 749.22, found 748.97




**BocHN-D-Phe-Ile-D-Ala-Thiazole-Thiazole-Thiazole-C(O)-NH$_2$ (14).** BocHN-D-Phe-Ile-D-Ala-Thiazole-Thiazole-Thiazole-C(O)-$NH_2$ was synthesized utilizing 450 mg (0.619 mmol, 1.0 equiv.) of BocHN-D-Phe-Ile-D-Ala-Thiazole-Thiazole-Thiazole-OEt in 30 mL of ammonium hydroxide solution (28% w/v) and 75 mL of MeOH. The reaction mixture was sonicated for 48 hrs. Upon completion of reaction, confirmed via TLC, the reaction mixture was then concentrated *in vacuo* followed by co-evaporation with MeOH for 5 times; the resulting amide was taken on to the next reaction without further purification (350 mg, 81%) as a white powder.

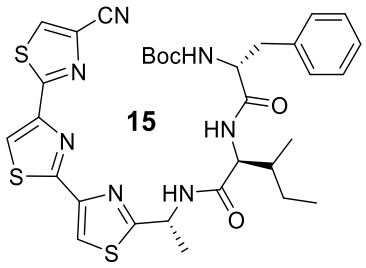
$R_f$: 0.222 (hexane/EtOAc 1:1).

$^1$H NMR (600 MHz, $CD_3OD$): δ 0.764 (m, 3H, CHC<u>H</u>$_3$), 0.845 (m, 3H, $CH_2$C<u>H</u>$_3$), 1.382 (br, 9H, C(C<u>H</u>$_3$)$_3$); 1.46 (t, *J* = 7.56 Hz, 3H, $CH_2$C<u>H</u>$_3$); 1.54-1.70 (m, 2H, C<u>H</u>$_2$CH$_3$); 1.685 (d, *J* = 6.66Hz, 3H, CHC<u>H</u>$_3$); 1.99 (m, 1H, CHC<u>H</u>); 3.076-3.138 (m, 2H, CHC<u>H</u>$_2$C); 4.216 (m, 1H, NHC<u>H</u>CH); 4.315(m, 1H, NHC<u>H</u>CH$_2$); 4.45 (q, *J* = 7.06 Hz, 2H, C<u>H</u>$_2$CH$_3$); 5.023 (br, 1H,

NH); 5.425 (m, 1H, NH); 6.38 (m, 1H, NH); 7.176(m, 2H, CH), 7.21 (m, 1H, CH); 7.271 (m, 2H, CH); 7.30 (m, 1H, NH); 7.948 (s, 1H, SCHC), 8.132 (s, 1H, SCHC); 8.183 (s, 1H, SCHC).

$^{13}$C NMR (from 2D NMR) δ 11.78, 15.68, 20.46, 24.69, 28.38(3C), 36.23, 38.39, 48.48, 56.45, 57.95, 79.28, 116.802, 118.1, 125.51, 127.45, 129.24, 130.11, 136.61, 147.87, 147.93, 148.18, 149.18, 150.25, 155.84, 161.46, 162.61, 163.56, 171.23, 171.38, 174.79.

ES$^+$MS *m/z* calcd for $C_{32}H_{39}N_7O_5S_3$ ([M+Na]$^+$) 698.23, found 697.99



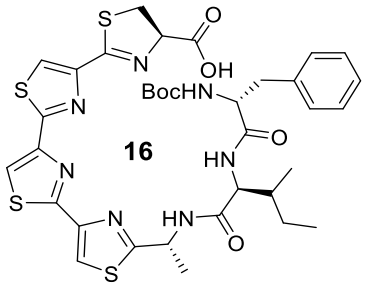

**BocHN-D-Phe-Ile-D-Ala-Thiazole-Thiazole-Thiazole-CN (15).** 350 mg (0.502 mmol, 1.0 equiv.) of BocHN-D-Phe-Ile-D-Ala-Thiazole-Thiazole-Thiazole-C(O)-$NH_2$ in 40 mL of anhydrous $CH_2Cl_2$ and pre-activated 4Å molelcular seives under nitrogen atmosphere at -20$^o$C. While stirring, DIPEA (192µl, 1.104mmol, 2.2 equiv.) was added into the reaction mixture in dropwise. After 5 min, 80 µl (0.552 mmol, 1.1 equiv.) TFAA was added into the reaction mixture cautiously. The reaction was flushed with nitrogen to remove produced vapor. The reaction was allowed to stir overnight with gradually warm up to r.t. Upon completion, the reaction was concentrated and re-dissolved in $CH_2Cl_2$ and directly purified over flash column chromatography on silica gel elute with gradient of hexane and EtOAc to give product as white solid (208mg, 61%).

$R_f$: 0.625 (hexane/EtOAc 1:1).

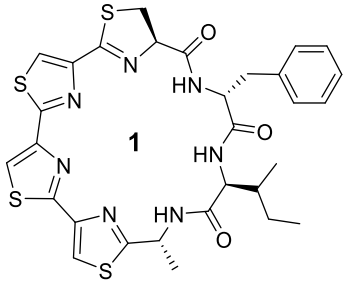
$^1$H NMR (600 MHz, $CDCl_3$): δ 0.77 (m, 3H, CHC$H_3$), 0.845-0.91 (m, 3H, $CH_2CH_3$), 1.386 (br, 9H, C($CH_3$)$_3$); 1.543 (m, 2H, $CH_2CH_3$); 1.685 (d, *J* = 6.64Hz, 3H, CHC$H_3$); 1.96 (m, 1H, CHCH); 3.045-3.122 (m, 2H, CHC$H_2$C); 4.21 (m, 1H, NHCHCH); 4.311(m, 1H, NHCH$CH_2$); 4.968 (m, 1H, NH); 7.30 (m, 1H, NH); 5.392 (m, 1H, NH); 6.29 (m, 1H, NH); 7.181(m, 2H, CH), 7.235 (m, 1H, CH); 7.275 (m, 2H, CH); 7.31 (m, 1H, NH); 7.963 (s, 1H, SCHC), 7.996 (s, 1H, SCHC); 8.061 (s, 1H, SCHC).

$^{13}$C NMR (from 2D NMR) δ 11.54, 15.61, 21.01, 24.66, 28.22(3C), 36.23, 37.71, 47.53, 56.95, 58.01, 80.07, 114.04, 117.09, 118.57, 127.55, 127.75, 128.89, 129.09, 130.55, 136.11, 147.96, 148.33, 149.12, 156.82, 163.62, 164.29, 171.38, 171.52, 173.79.

ES$^+$MS *m/z* calcd for $C_{32}H_{37}N_7O_4S_3$ ([M+Na]$^+$) 680.22, found 680.00




**BocHN-D-Phe-Ile-D-Ala-Thiazole-Thiazole-Thiazole-(R)-Thiazoline-OH (16).** 200 mg (0.295 mmol, 1.0 equiv.) of BocHN-D-Phe-Ile-D-Ala-Thiazole-Thiazole-Thiazole-CN and 43.0 mg (0.353 mmol, 1.2 equiv.) L-Cysteine and 124 mg (1.47 mmol, 5.0 equiv.) of $NaHCO_3$ in 10.0 ml of MeOH. While stirring, 5.0 ml of pH = 5.95 phosphate buffer solution was added into the reaction mixture. The reaction mixture was then stirred at 70°C for 4 hr. followed by routine TLC monitoring for reaction progress. Upon starting material was fully consumed, the reaction mixture was concentrated to remove all of the solvent then re-dissolved in EtOAc and then poured into the Sat. $NaHCO_3$ solution. The organic layer was set aside, the aqueous layer was further extract with EtOAc for 2 times. The aqueous layer was then acidified using pH = 1 hydrochloric acid solution in dropwise with pH monitoring until reach pH = 3. The aqueous layer was then extracted with EtOAc 3 times. All organic layer was then collected, combined and dried over anhydrous $Na_2SO_4$ followed by filtration, concentration *in vacuo* and further dried *in silico* to give white solid. The material was subjected for next reaction without further purification. $ES^+MS$ *m/z* calcd for $C_{35}H_{41}N_7O_6S_4$ ($[M+H]^+$) 784.21, found 784.01

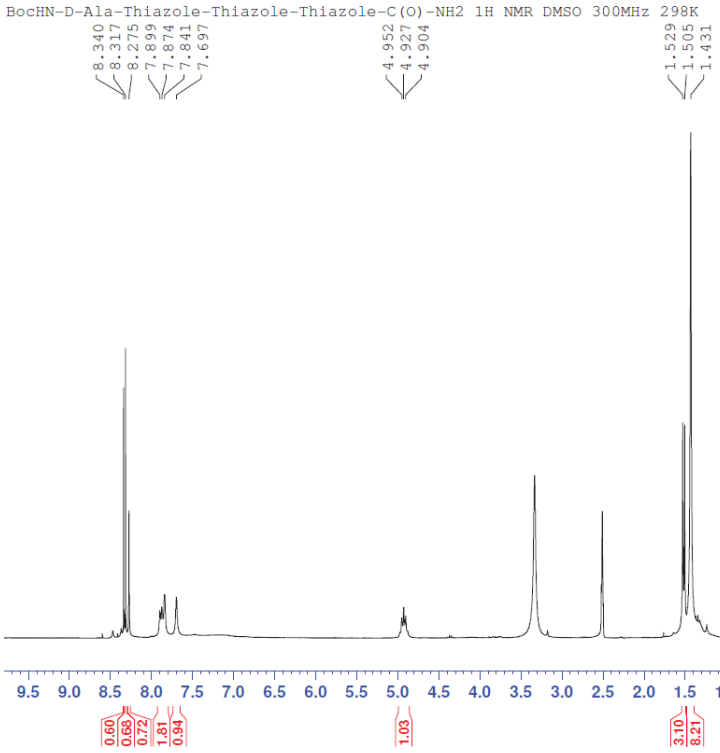



**Cyclo-D-Phe-Ile-D-Ala-Thiazole-Thiazole-Thiazole-R-Thiazoline (1).** BocHN-D-Phe-Ile-D-Ala-Thiazole-Thiazole-Thiazole-R-Thiazoline-OH (130 mg, 0.166 mmol, 1.0 equiv.) dissolved in anhydrous $CH_2Cl_2$ (4.0 ml) at r.t. Anisole (54µL, 0.498 mmol, 3.0 eq.) was then added into the solution. While stirring, TFA (1.0 mL) was added into the reaction in dropwise. The reaction was allowed to proceed for 3 hrs. Upon completion of reaction, confirmed via TLC, the mixture was then co-

evaporate with $CH_2Cl_2$ 6 times. The product free amine was then further dried *in vauco* to afford linear precursor. The coupling reagent cocktail contains 63 mg (0.166 mmol, 1.0 equiv.) HATU and 46 mg (0.166 mmol, 1.0 equiv.) DMTMM was weighted into the heat gun blow dried round bottom flask followed by immediate vacuum. 120 ml of anhydrous $CH_2Cl_2$ was then added into the coupling reagent cocktail containing flask followed by nitrogen purging. Pre-dried linear precursor (120 mg, 0.166 mmol, 1.0 equiv.) was weighted in a round bottom flask and vacuumed to remove all of the air moisture. 40 ml of anhydrous $CH_2Cl_2$ was charged into the flask followed by nitrogen purging. The 250 µl (0.166 mmol, 1.0 equiv) of HOAt in DMF was added into the coupling reagent containing cocktails in dropwise while stirring. After 1hr, 290 µl (1.66 mmol, 10.0 equiv.) of DIPEA was added into the coupling cocktail containing flask followed by linear precursor containing flask in dropwise. After 5 min, the linear precursor solution was withdraw from the flask using a 30 ml syringe and then injected into the coupling reagent containing flask at a rate of 0.25ml/min with utilization of a syringe pump. Upon completion of injecting all of the linear precursor solution, the reaction progress was monitored via LC-MS routinely every 1 hr. Upon completion of reaction, the reaction mixture was concentrate the redissolved in $CH_2Cl_2$ then poured into the pH = 1 hydrochloric acid solution, the aqueous layer was further extract twice with $CH_2Cl_2$ and EtOAc. Combined organic layer was poured into the Sat. $NaHCO_3$ solution, the aqueous layer was extracted twice with $CH_2Cl_2$ and EtOAc. All organic layer was collected, combined, dried over anhydrous $Na_2SO_4$, filtered and concentrated in vacuo. The crude material was then underwent flash column chromatographical purification on silica gel at gradient running (10%EtOAc /90% Hexane – 100%EtOAc – 30%MeOH/ 70%EtOAc). After LC-MS characterization, the product containing column fractions was combined and concentrated in vacuo then redissolved in HPLC grade MeOH and re-purified via reverse phase HPLC twice to give final product as white solid (3mg, 2.7%).

## Spectral Data

Compound **4** (**DMSO**)

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PROCNO                         3

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P1                        16.56 usec
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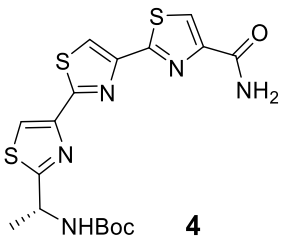


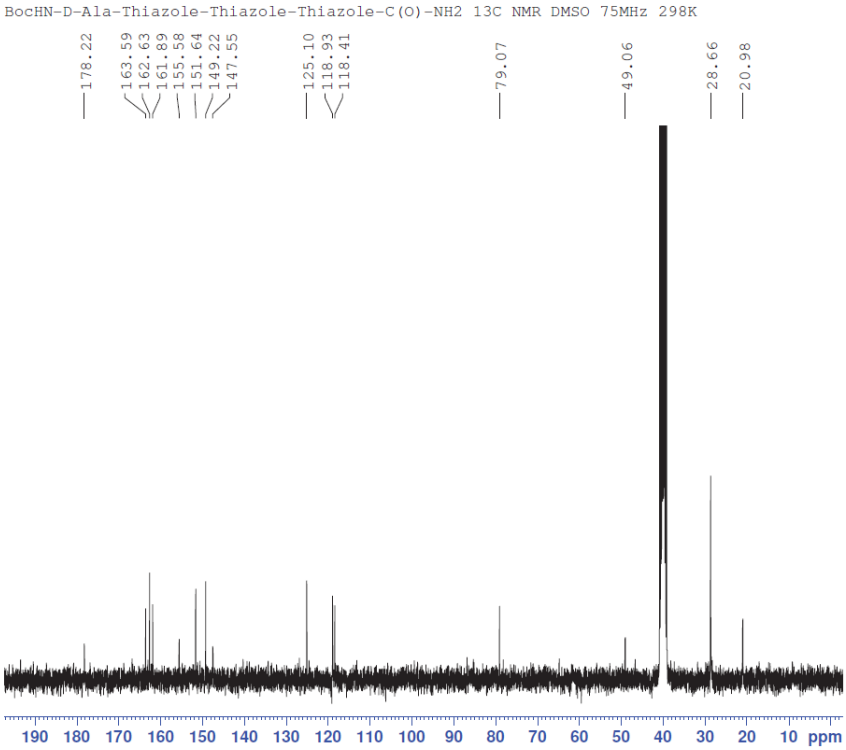


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TE                       298.0 K
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NUC2                         1H
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PLW2                 8.20349979 W
PLW12                0.27774000 W
PLW13                0.22497000 W

F2 - Processing parameters
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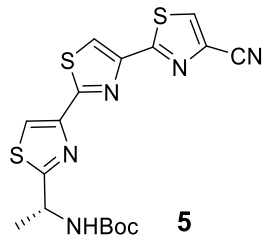
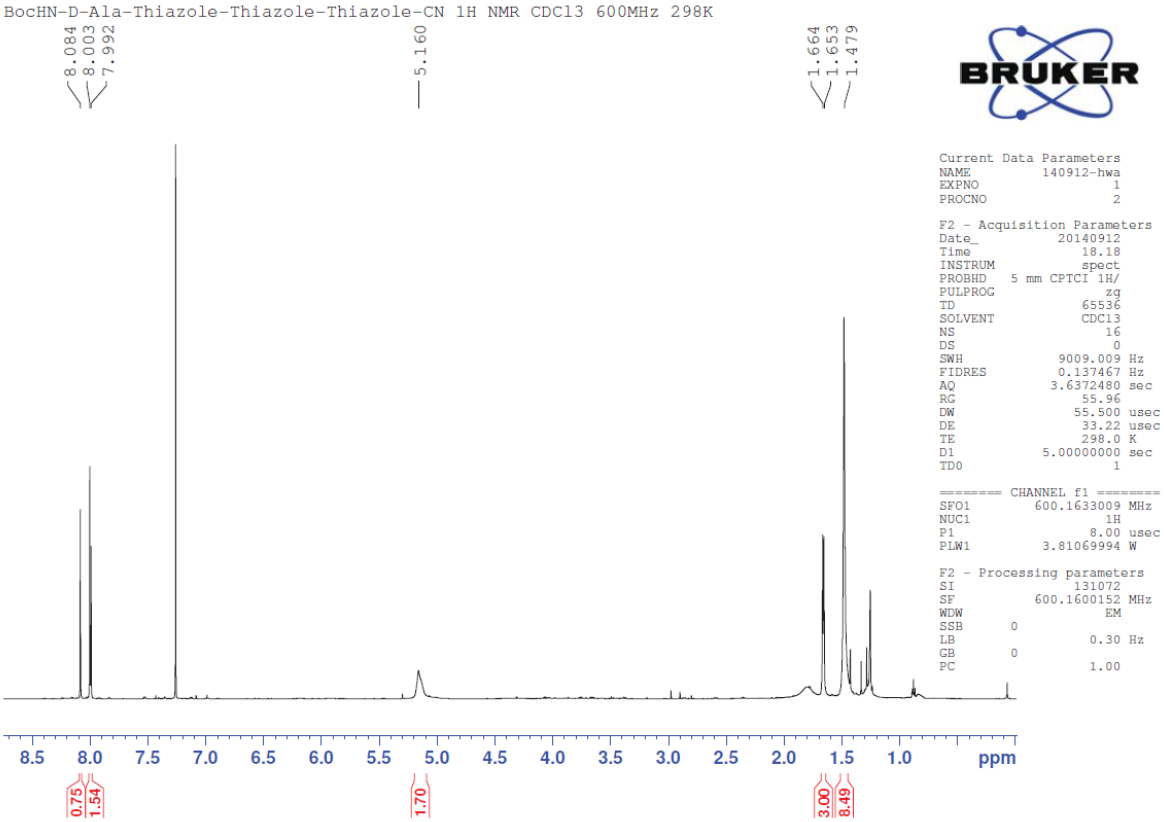
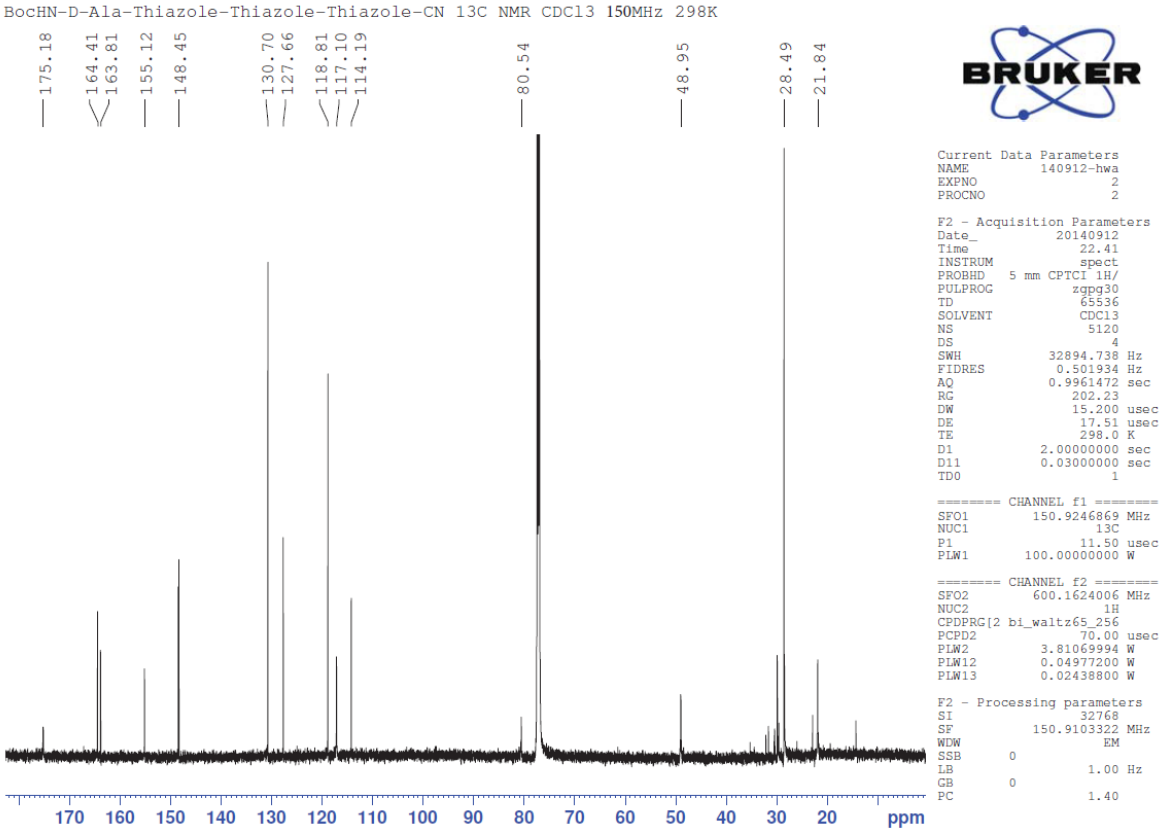



**5**



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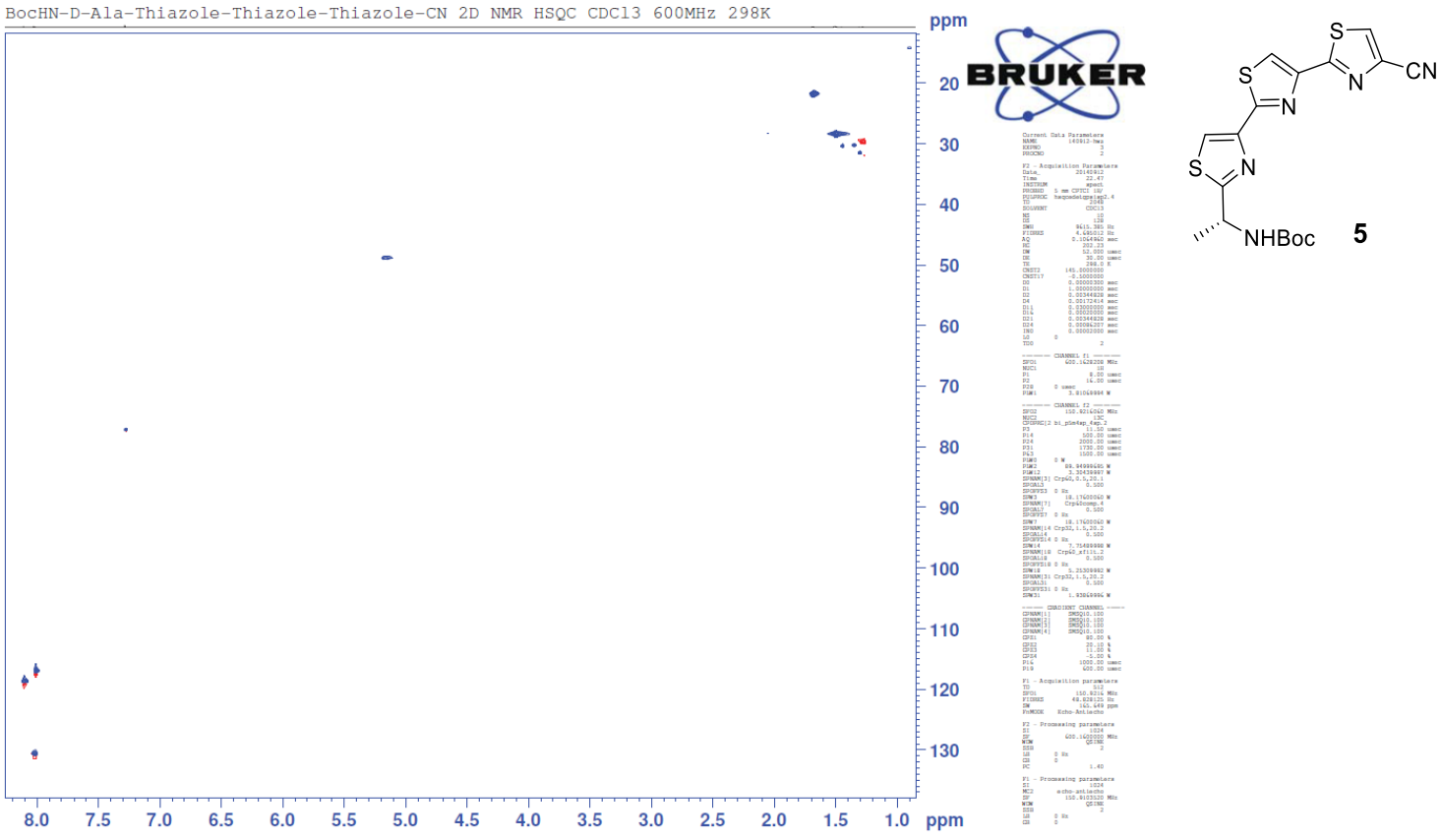


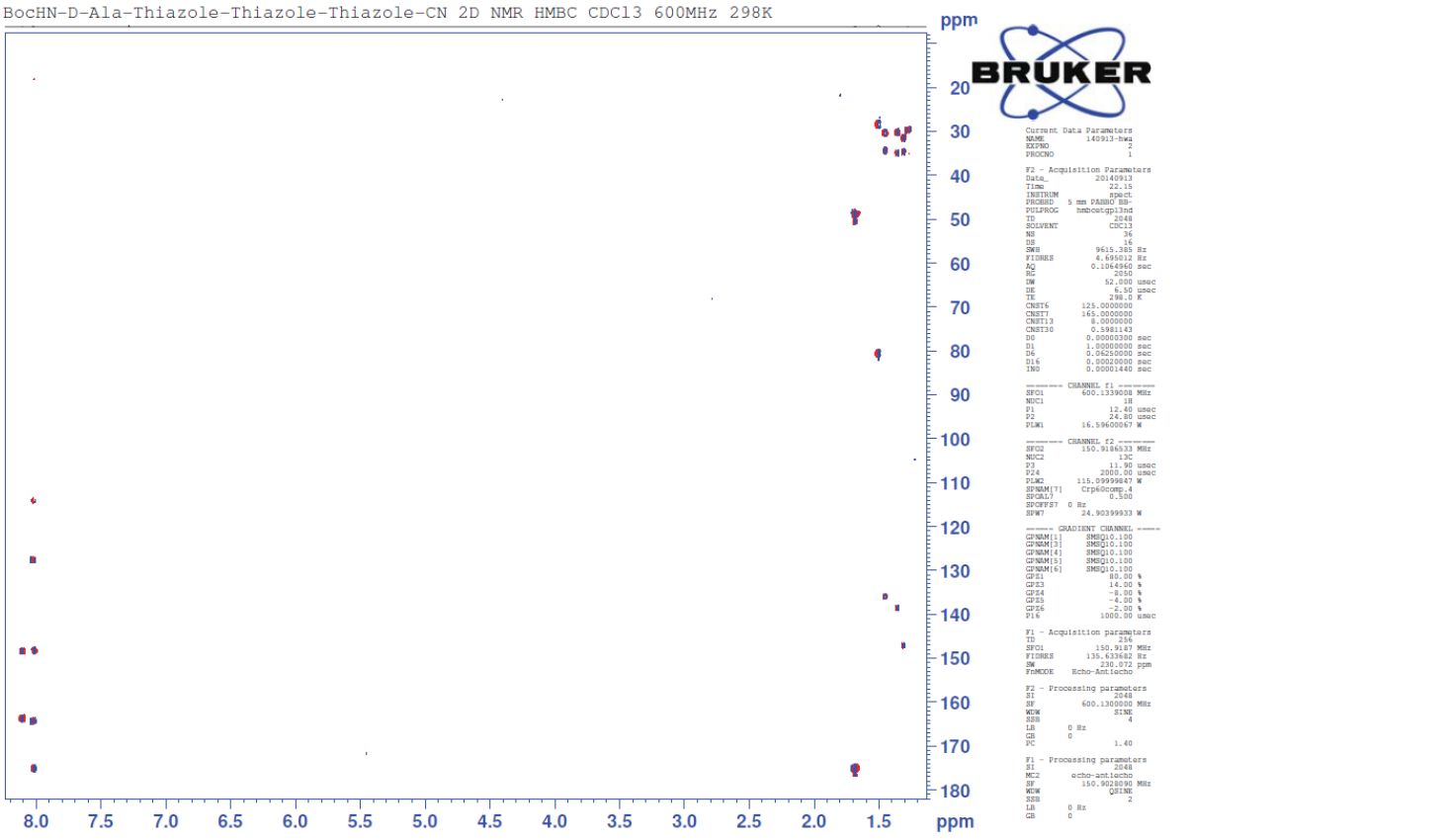

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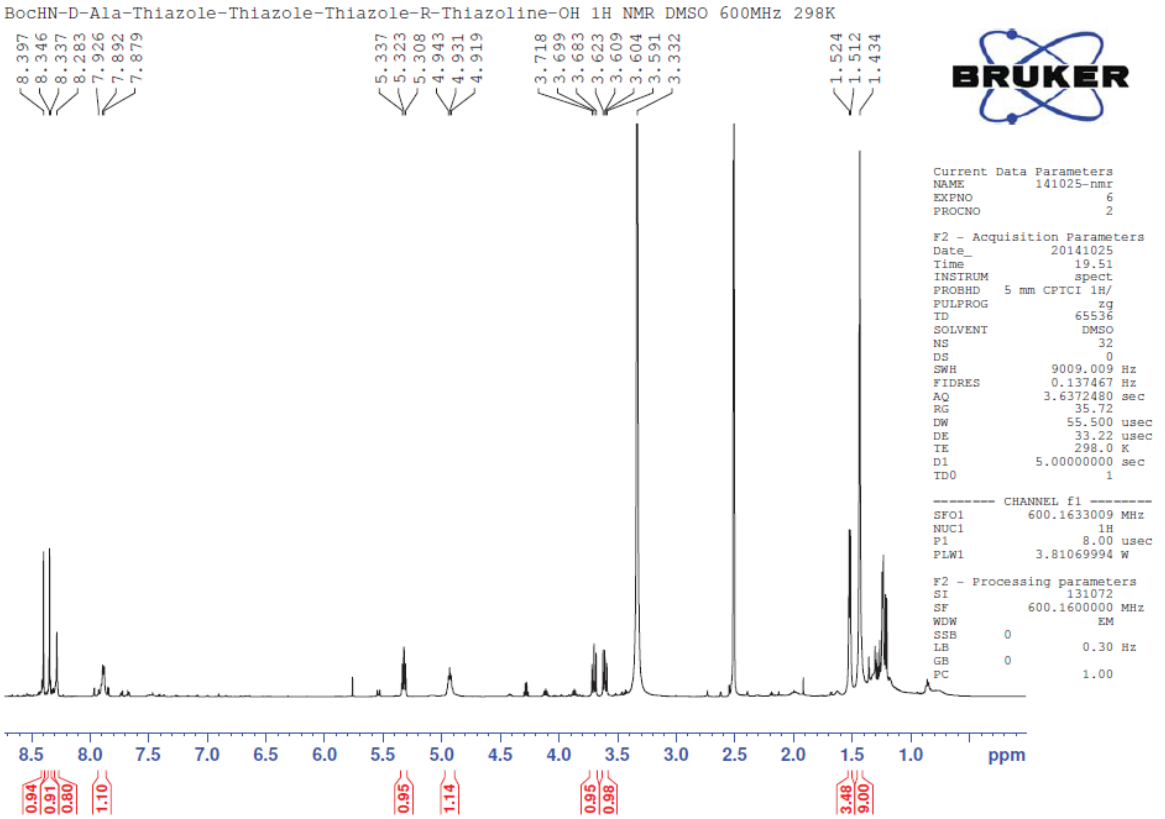


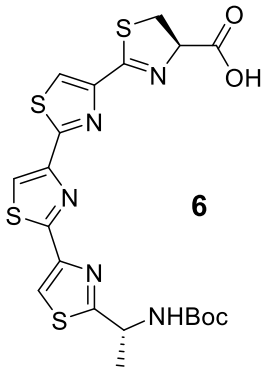


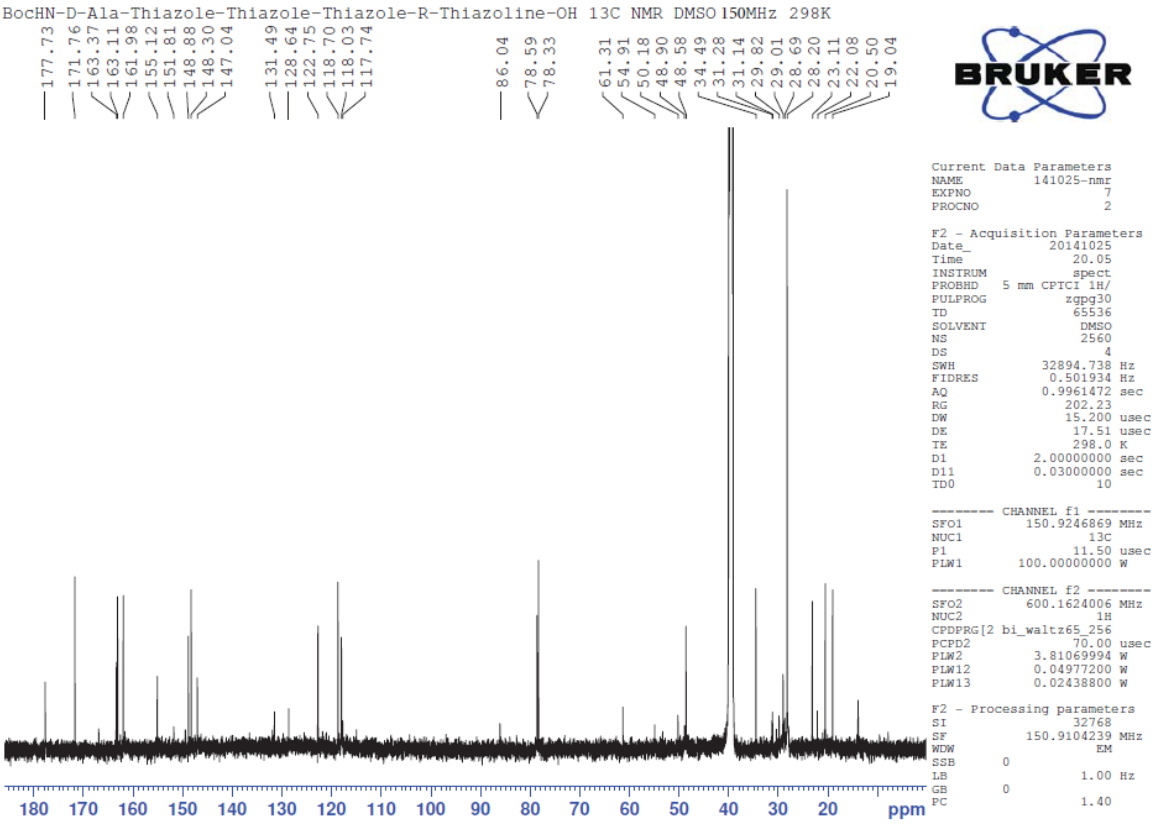

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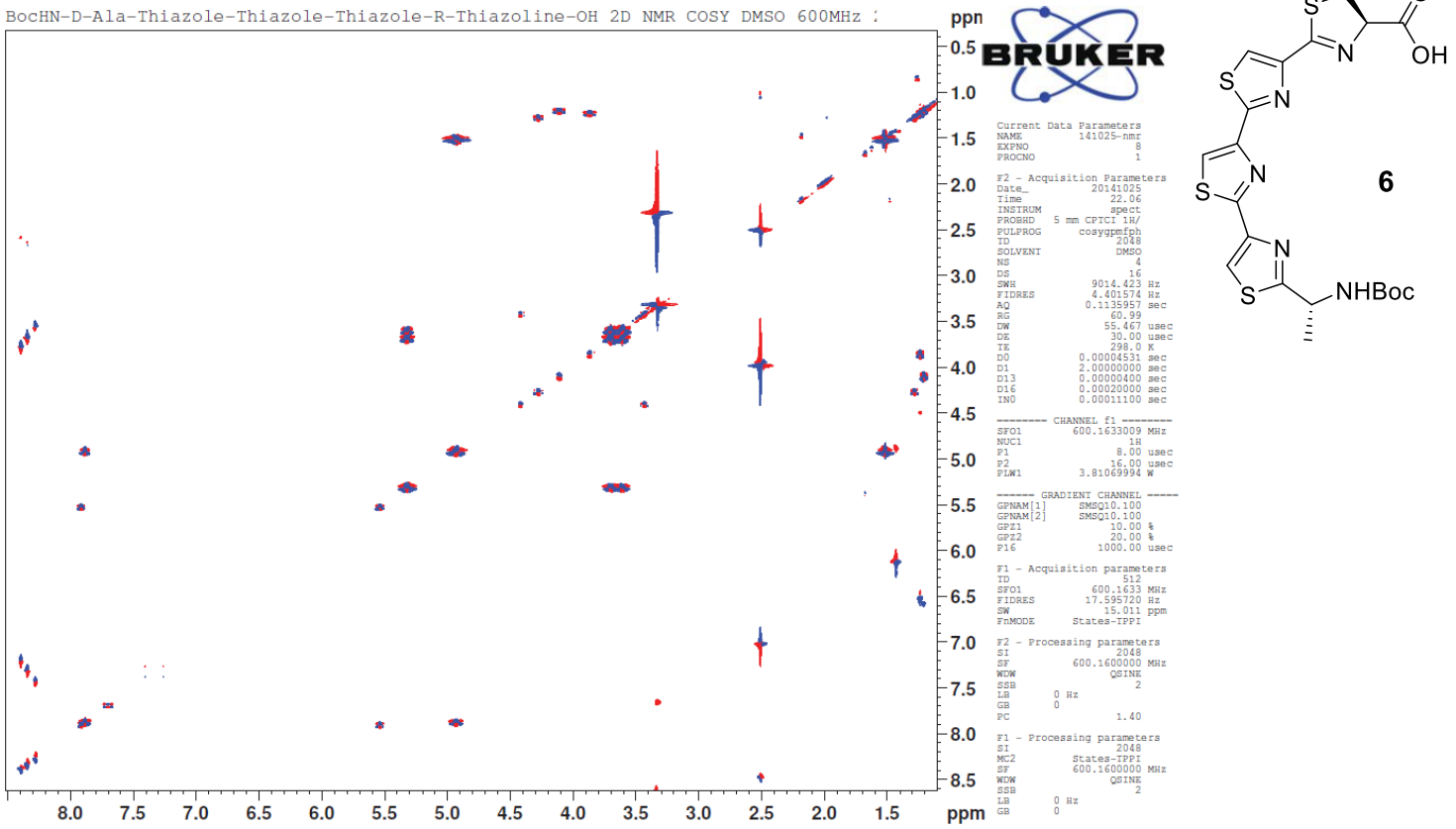


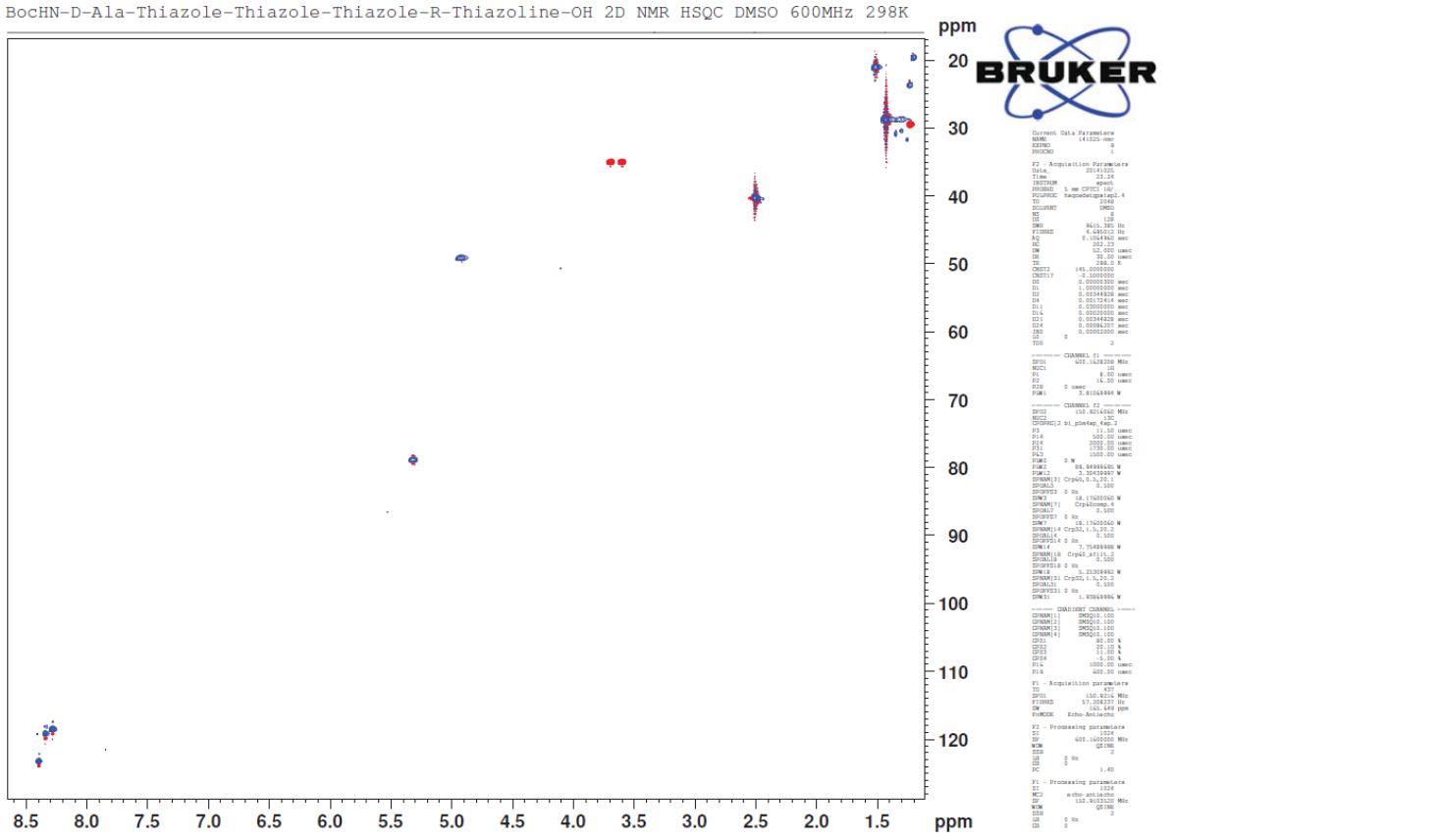

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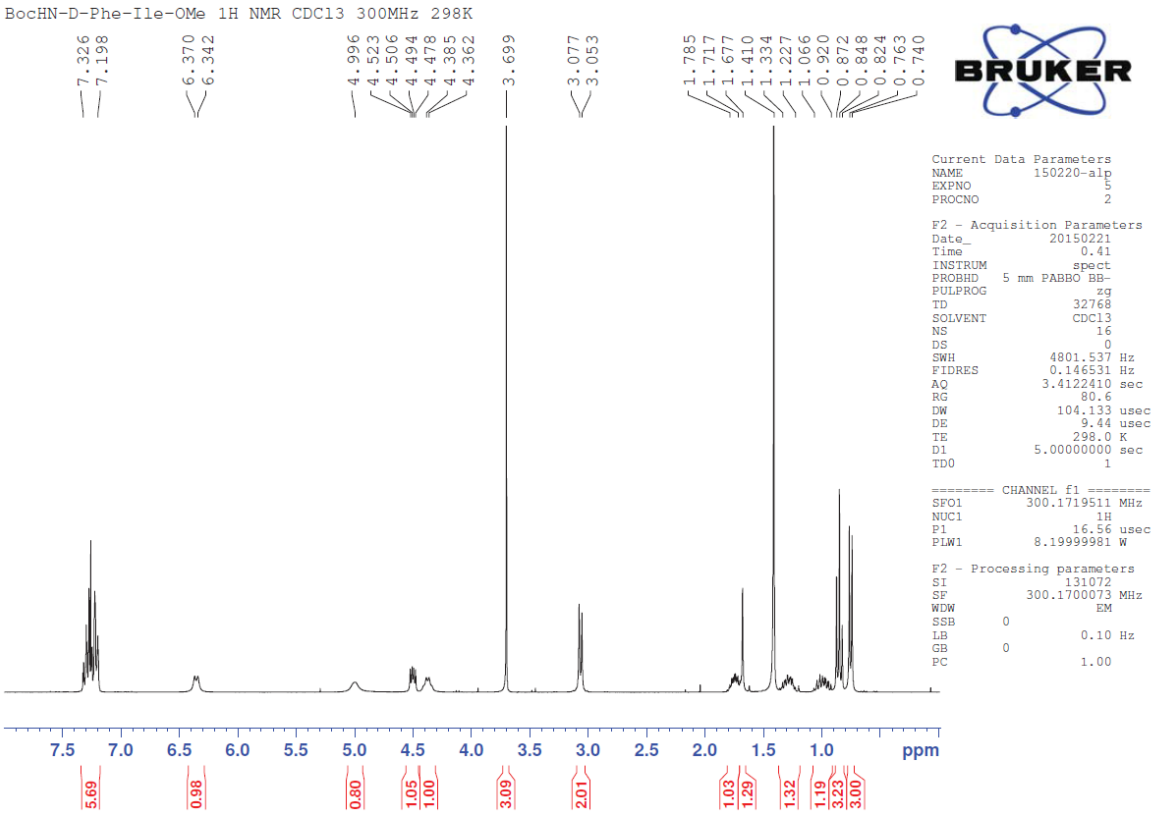


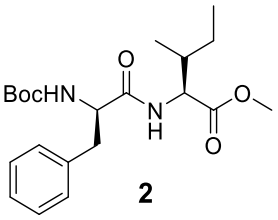


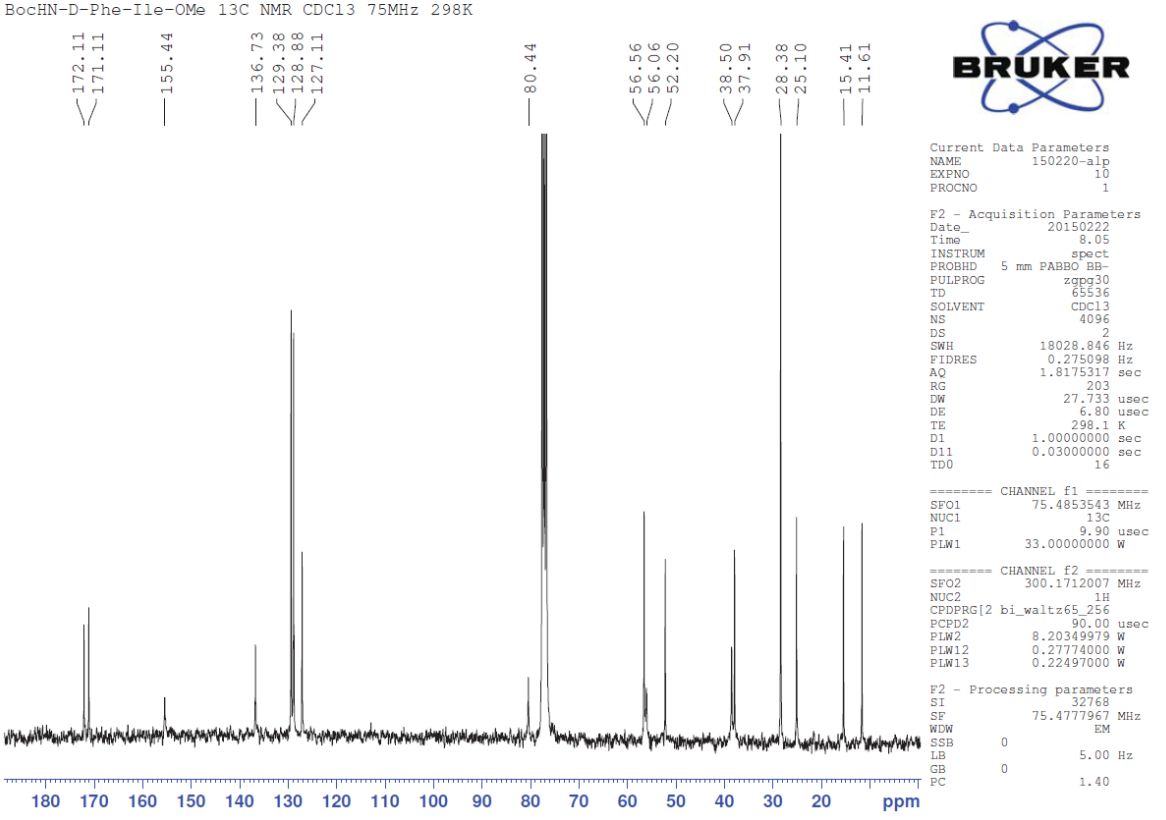

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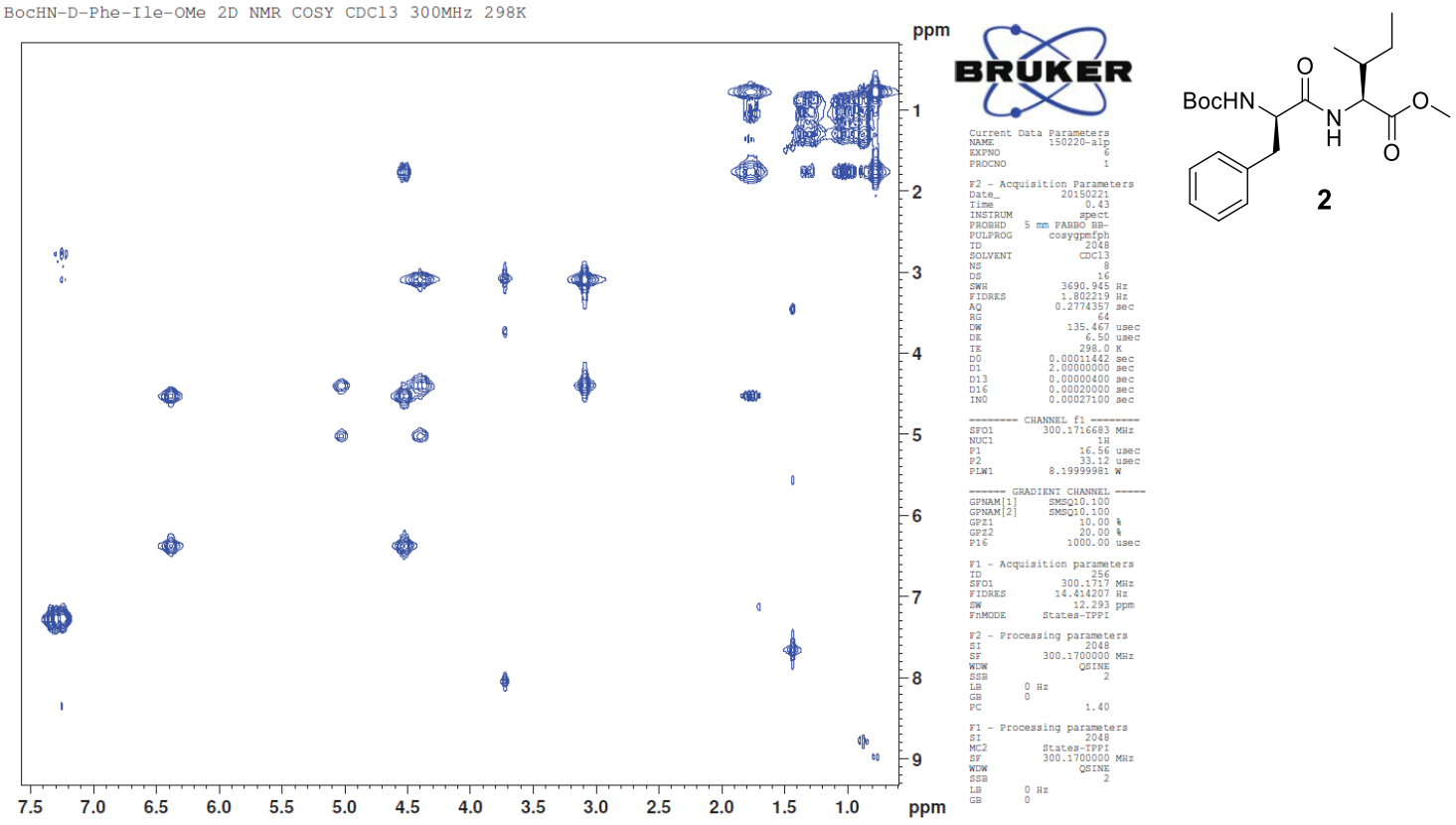
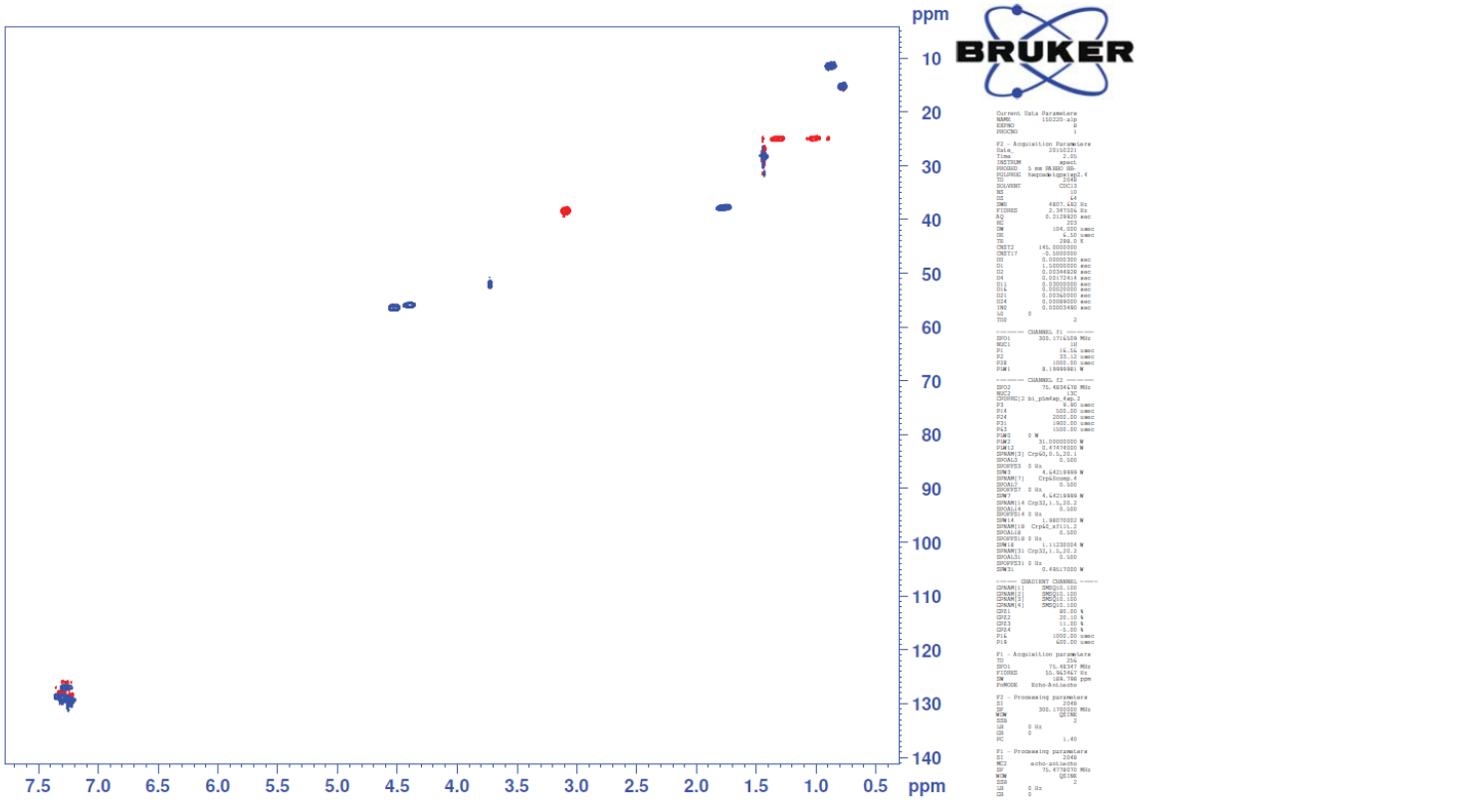
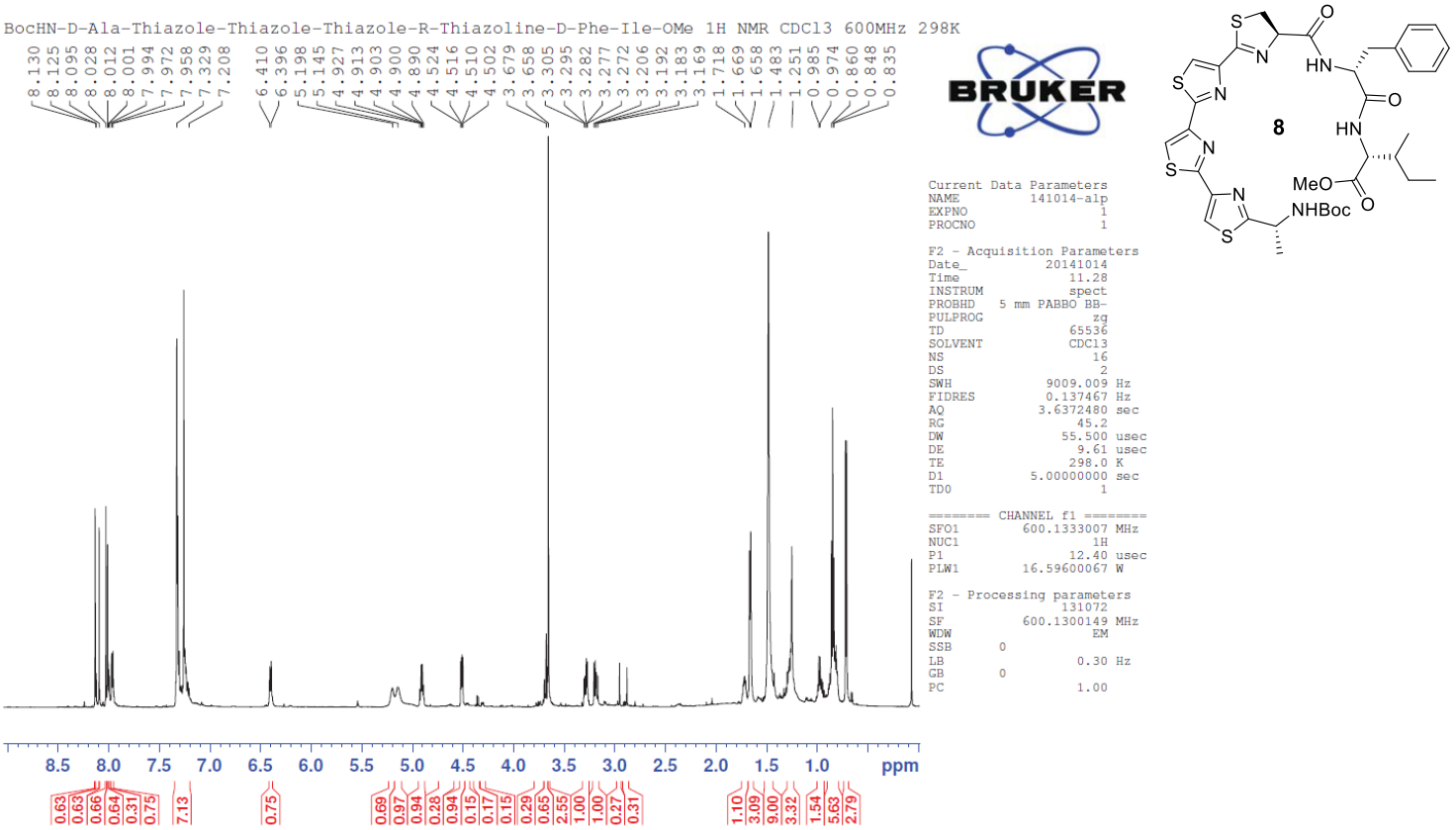


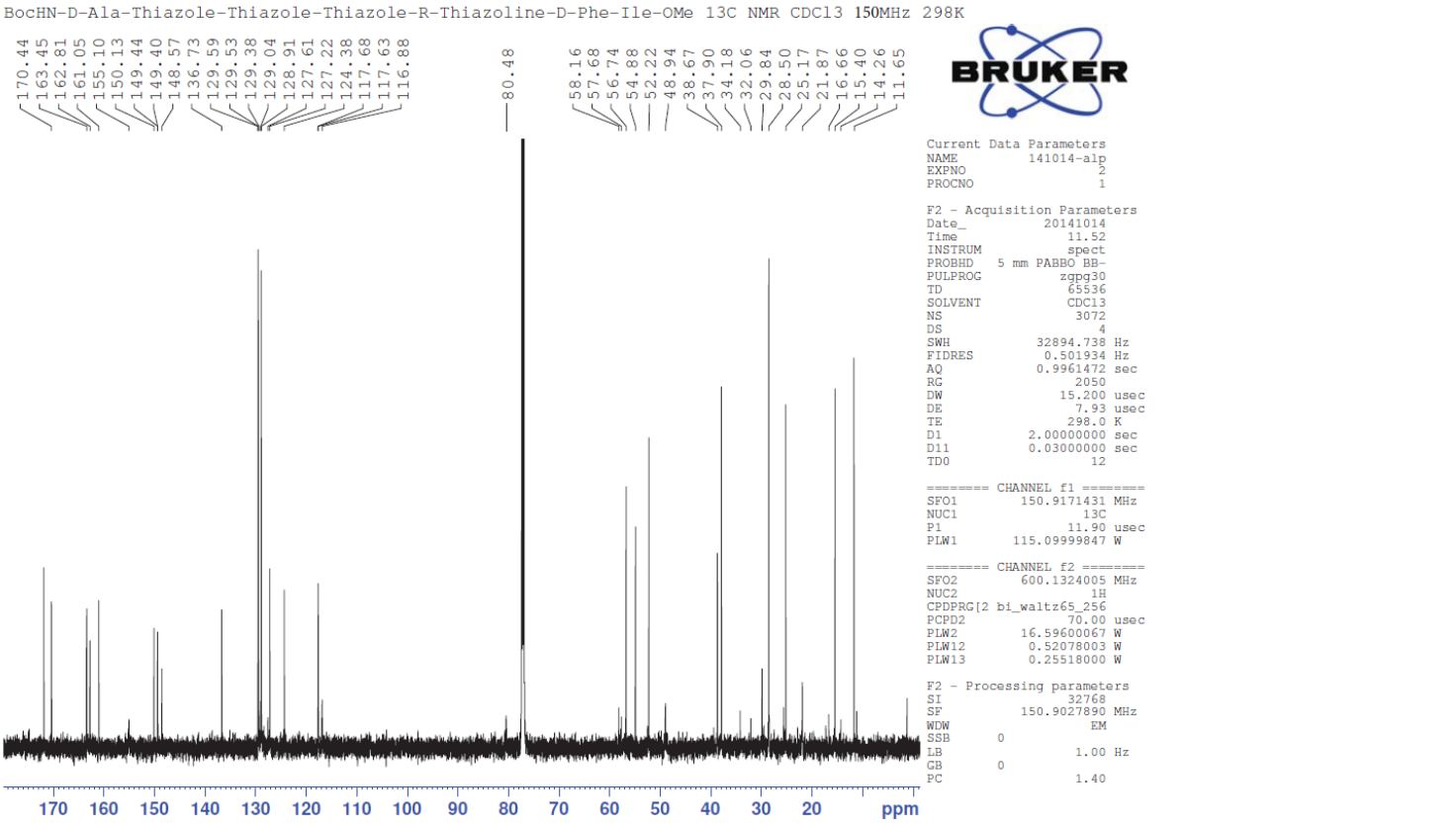



Compound **8 ($CDCl_3$)**









Compound **8** ($CDCl_3$)

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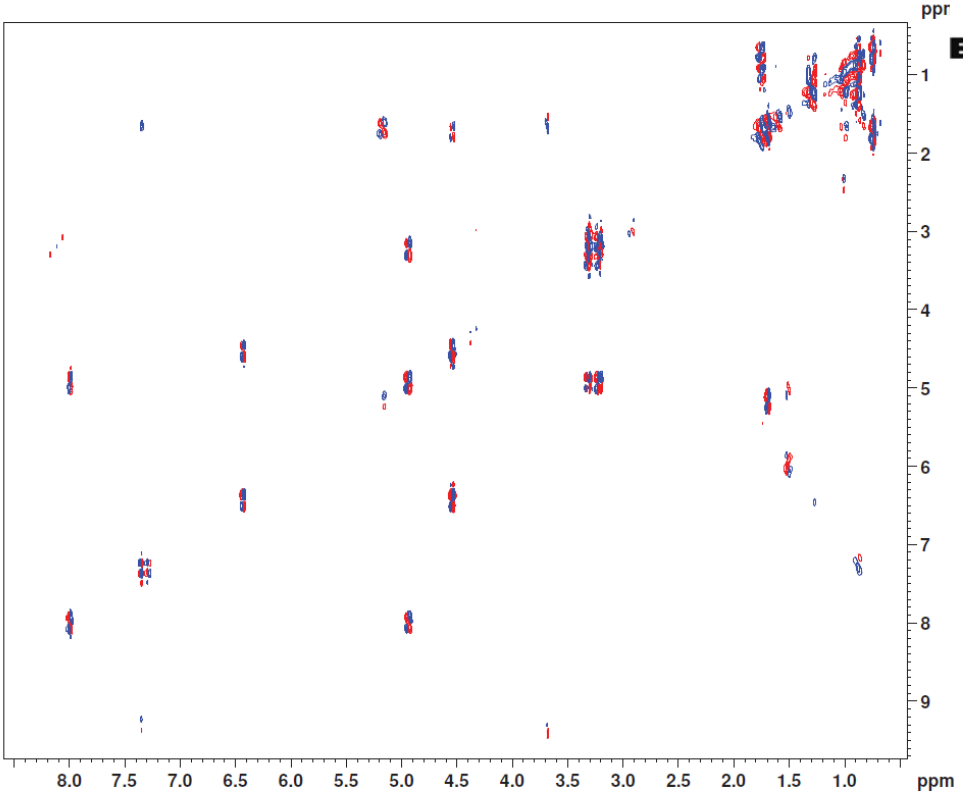
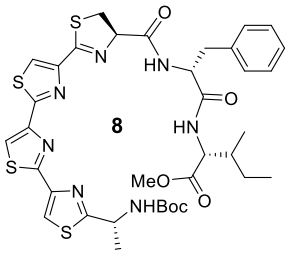
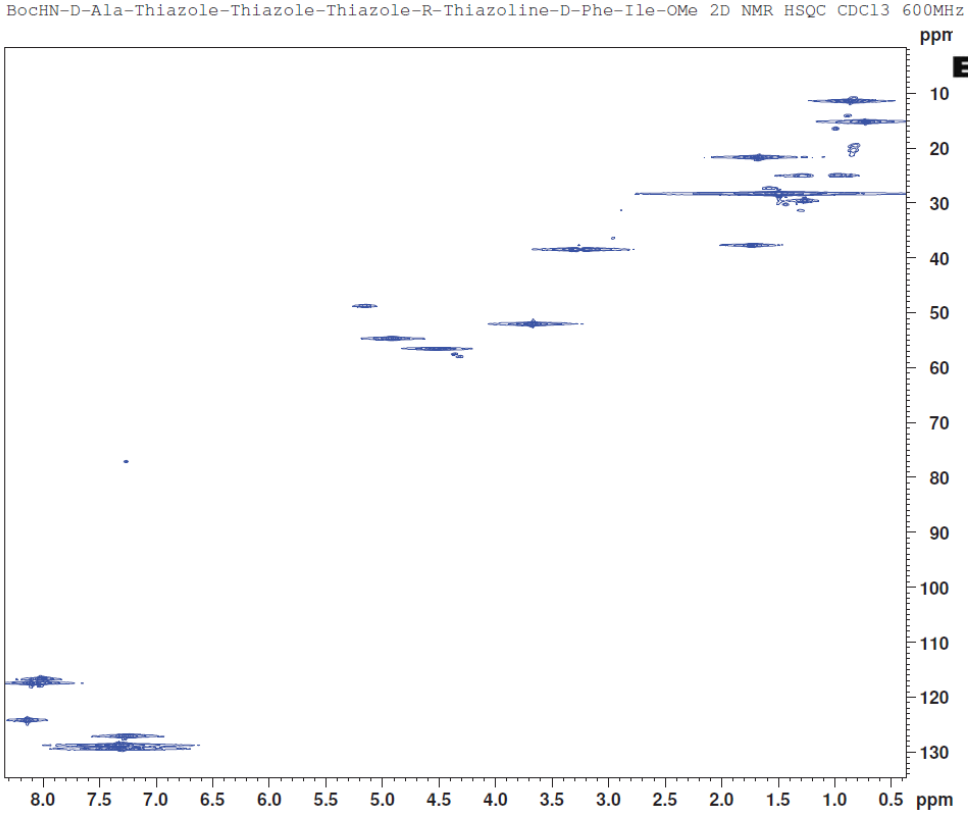
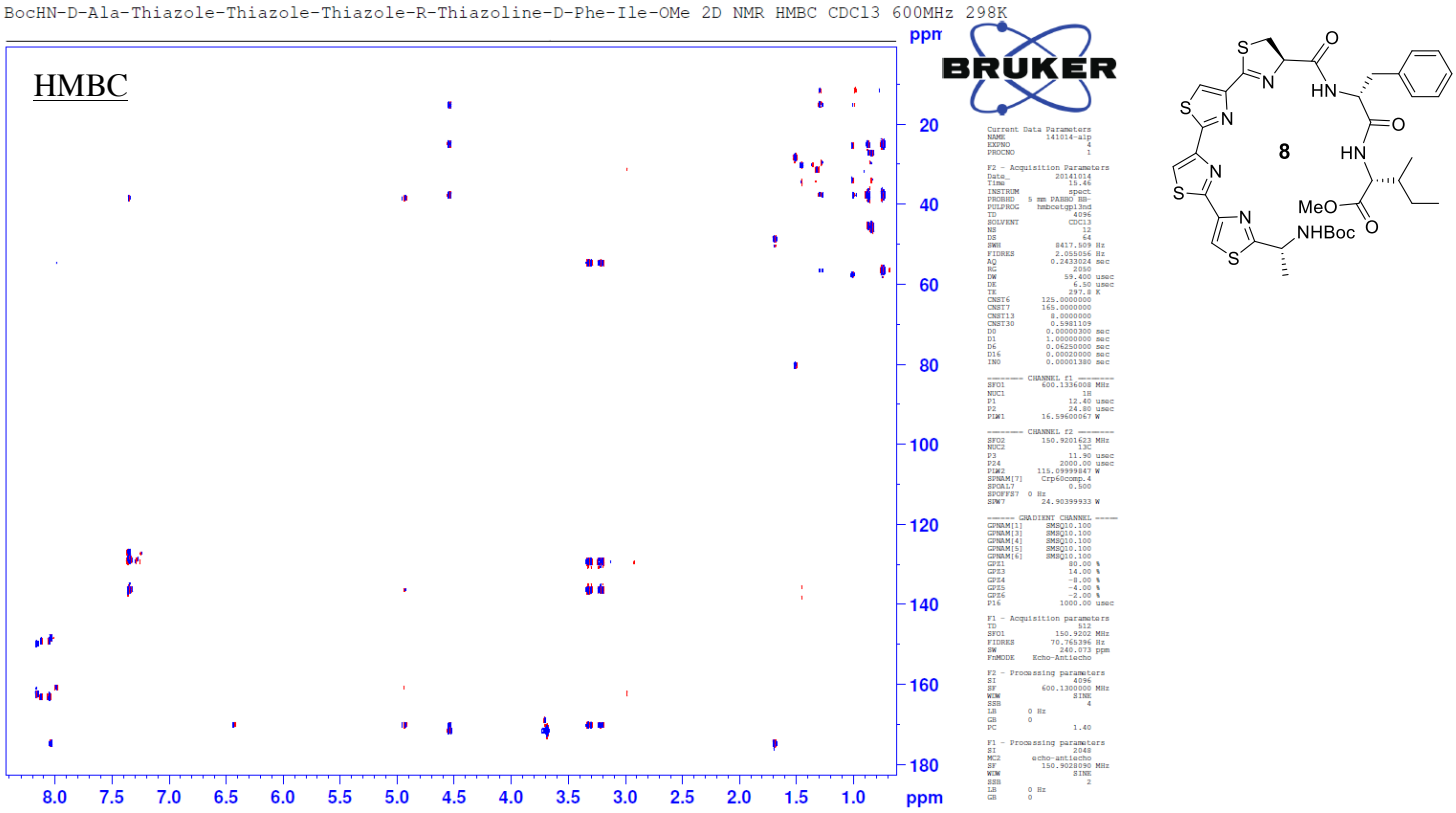


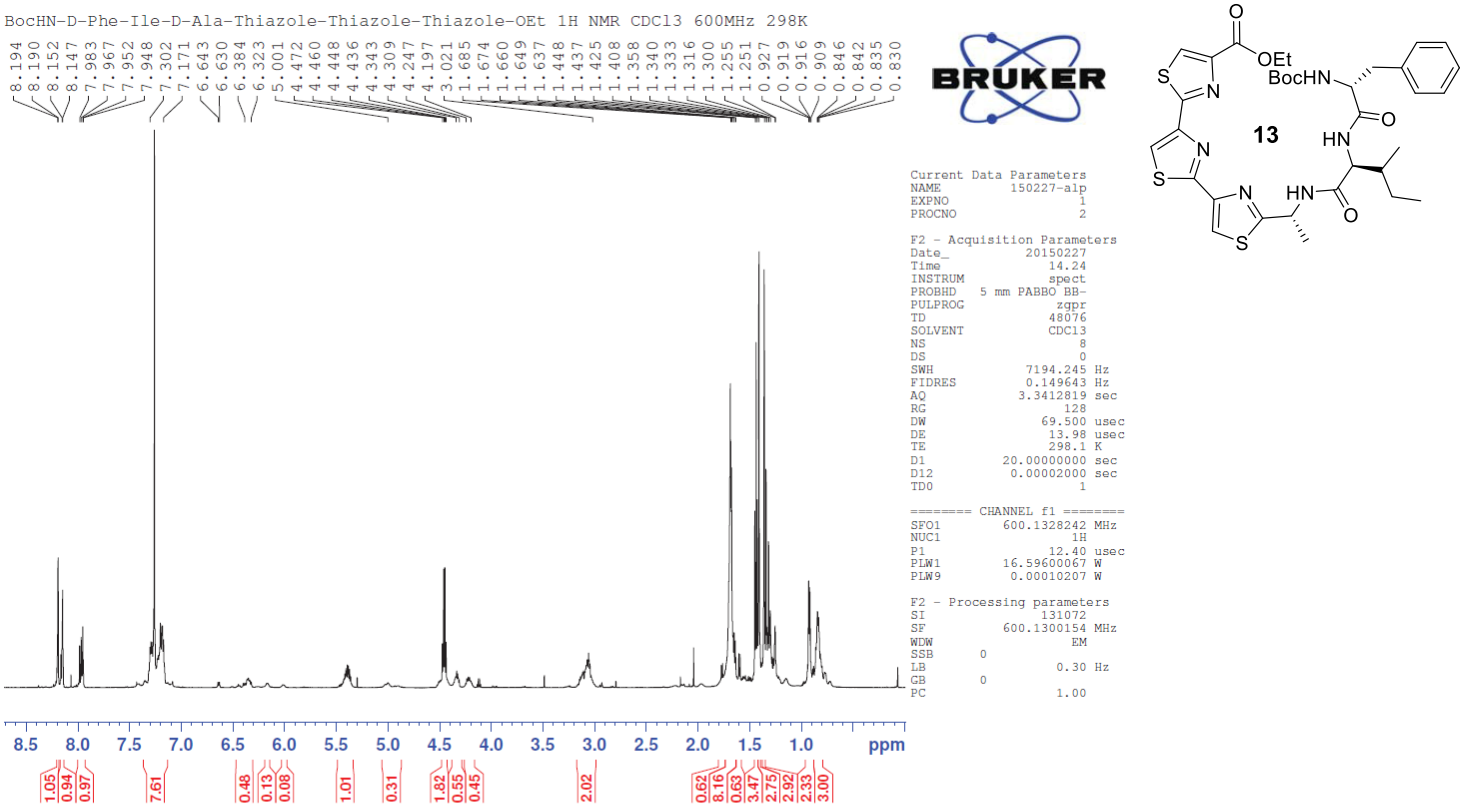

BocHN-D-Ala-Thiazole-Thiazole-Thiazole-R-Thiazoline-D-Phe-Ile-OMe 2D NMR HSQC CDCl3 600MHz 298K

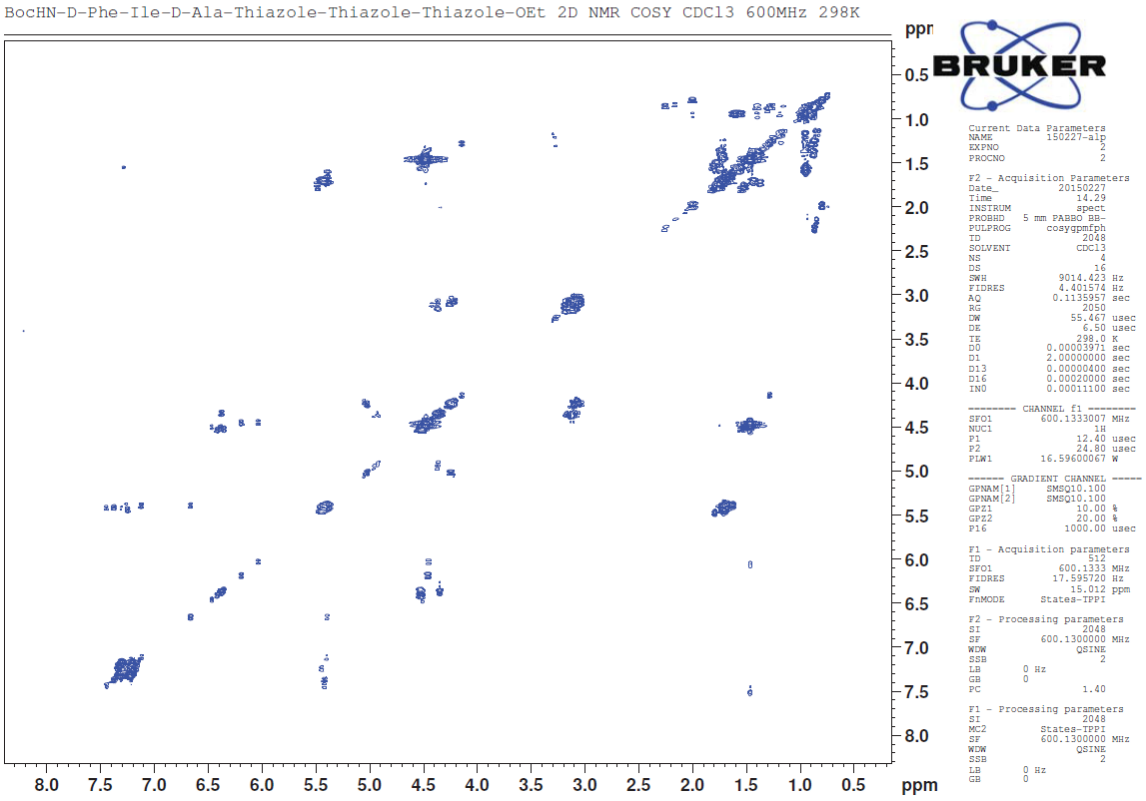


Compound **8 ($CDCl_3$)**

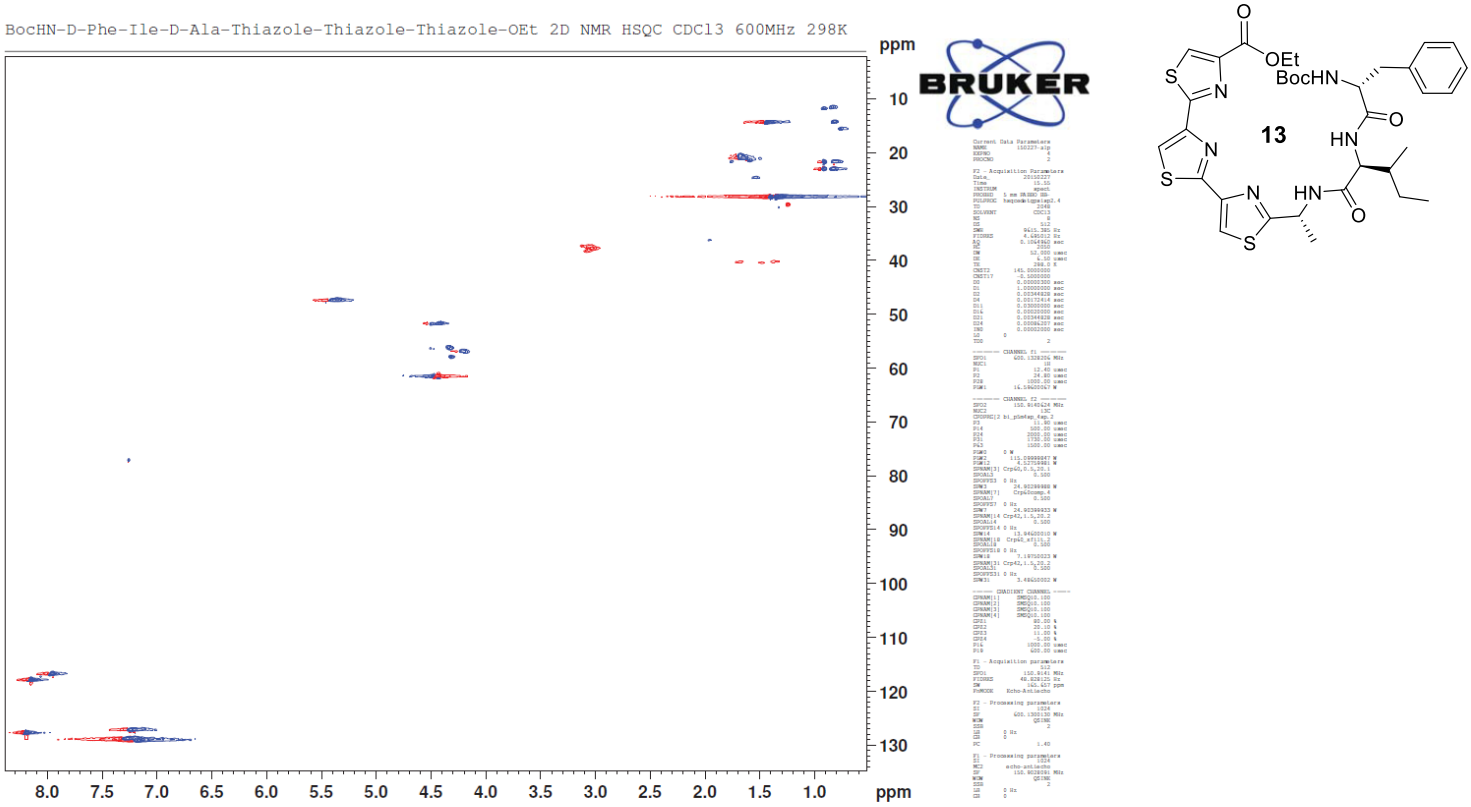


Compound **13 ($CDCl_3$)**



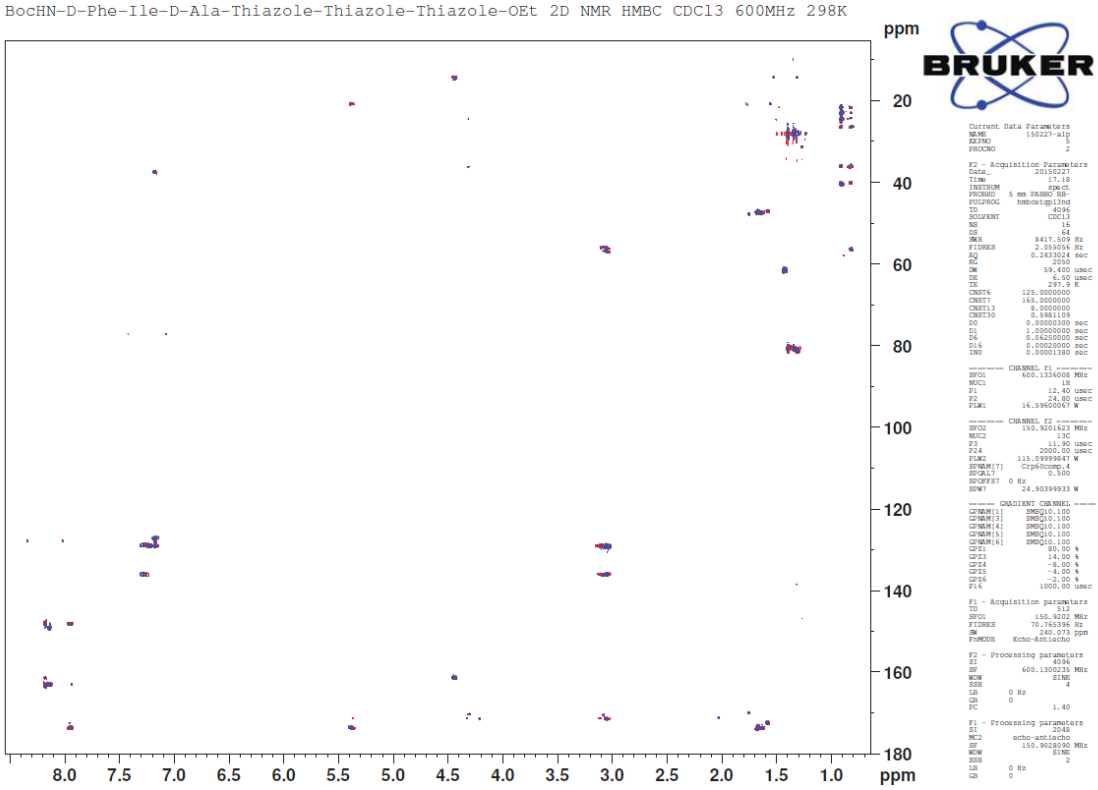



Compound **13 ($CDCl_3$)**

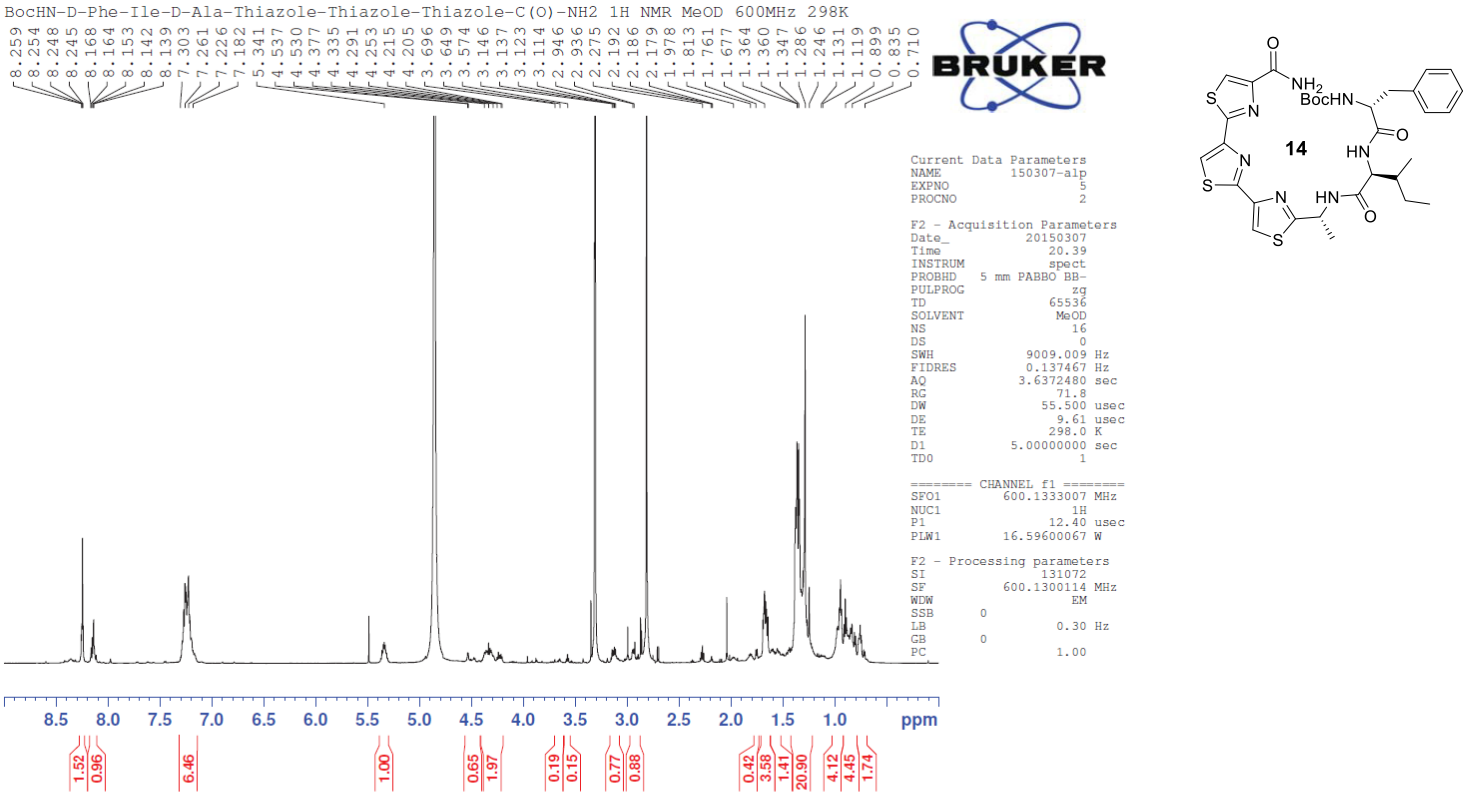


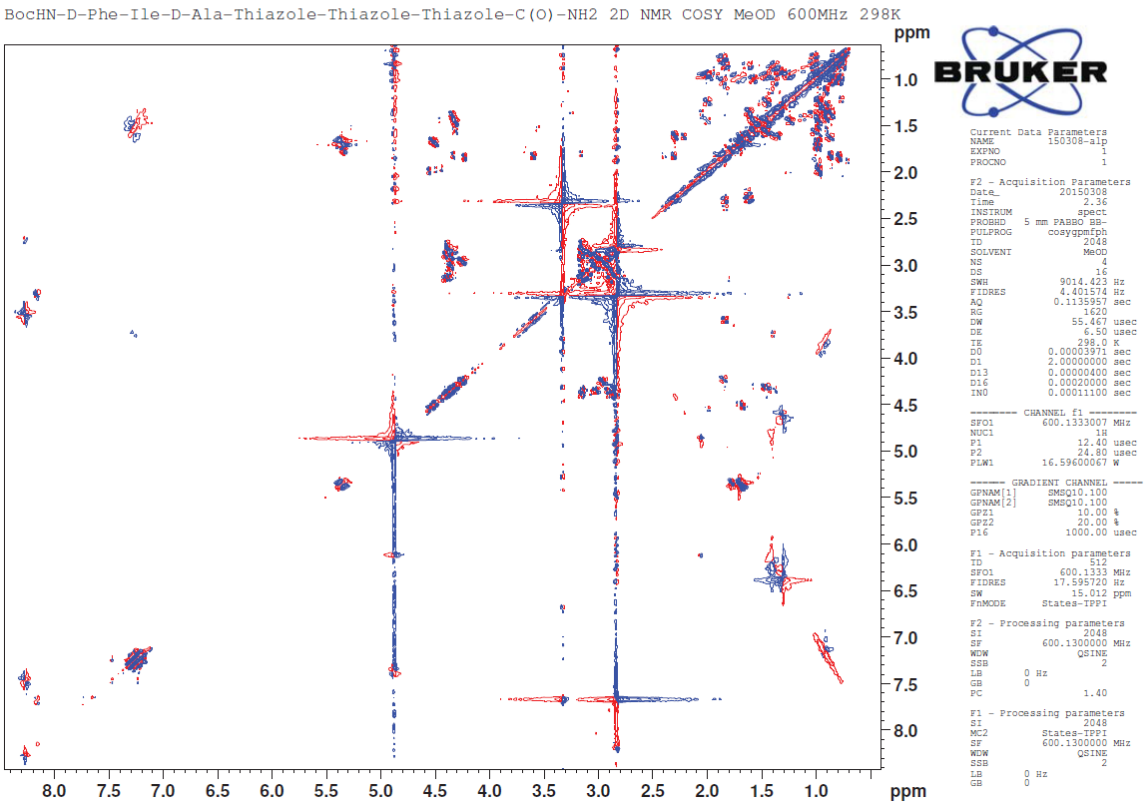






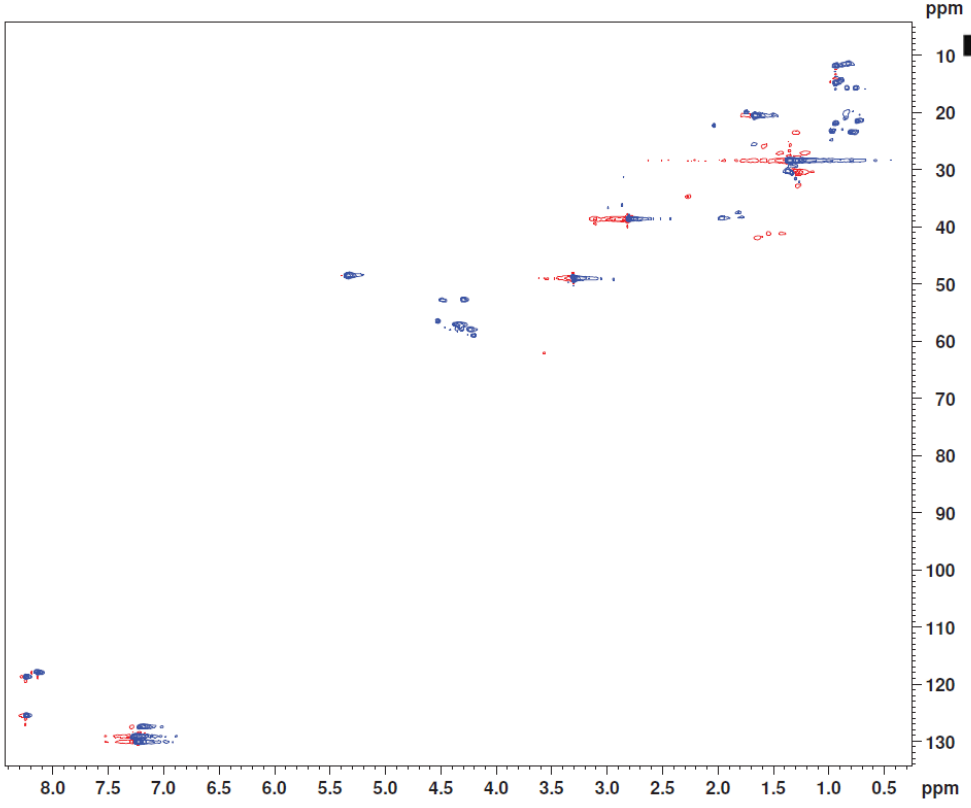
Compound **14 ($CD_3OD$)**

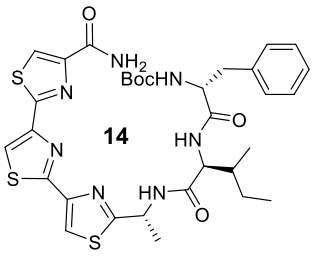


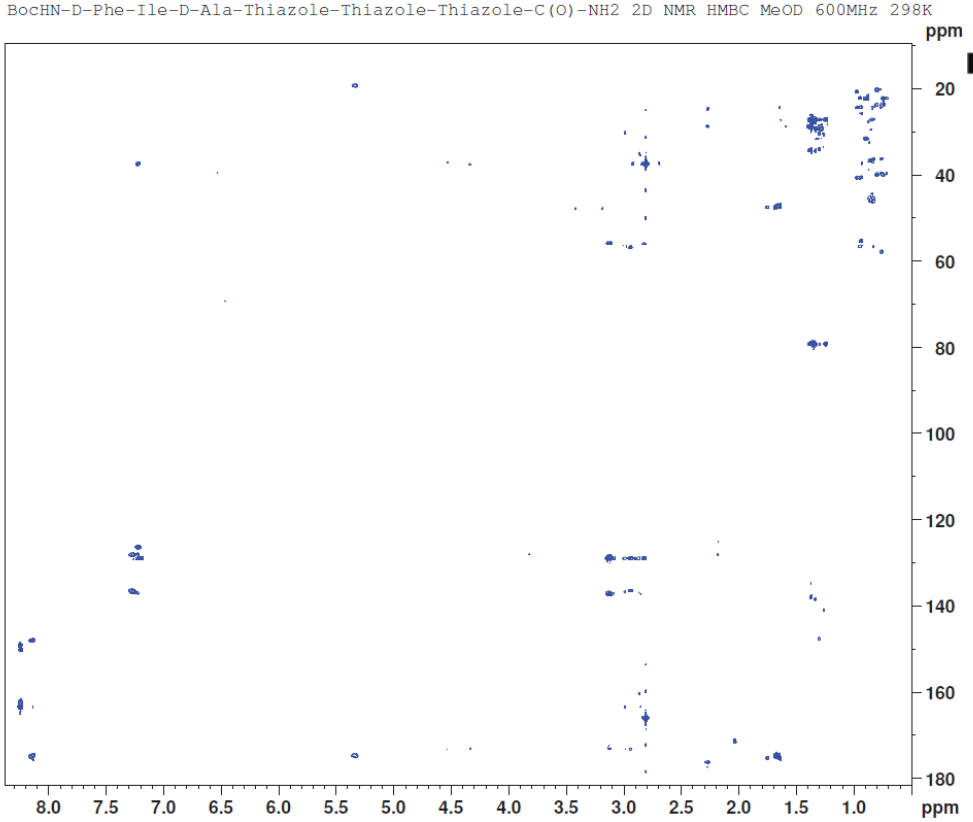
Compound **14 ($CD_3OD$)**

BocHN-D-Phe-Ile-D-Ala-Thiazole-Thiazole-Thiazole-C(O)-NH2 2D NMR HSQC MeOD 600MHz 298K

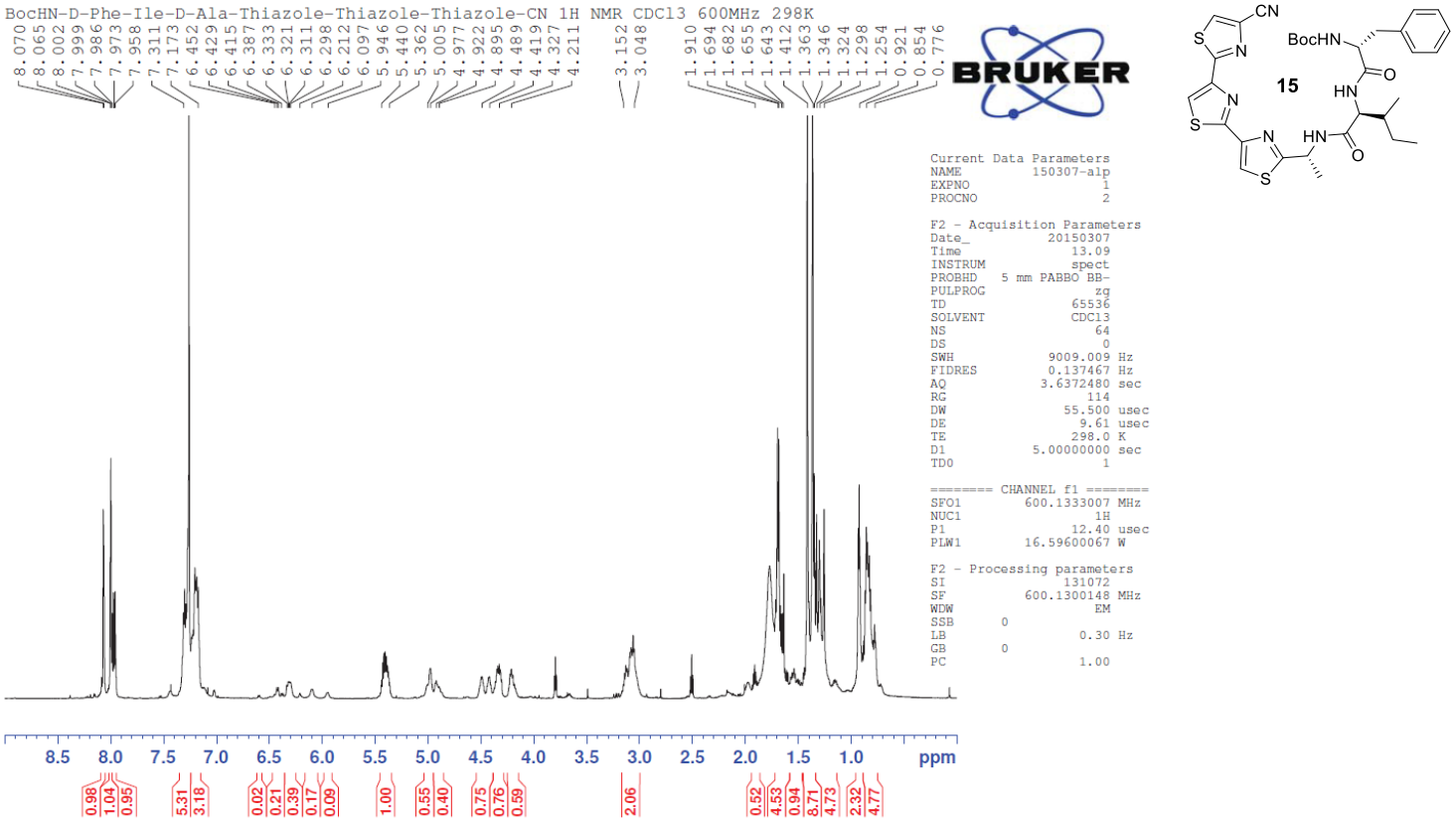



BocHN-D-Phe-Ile-D-Ala-Thiazole-Thiazole-Thiazole-C(O)-NH2 2D NMR HMBC MeOD 600MHz 298K

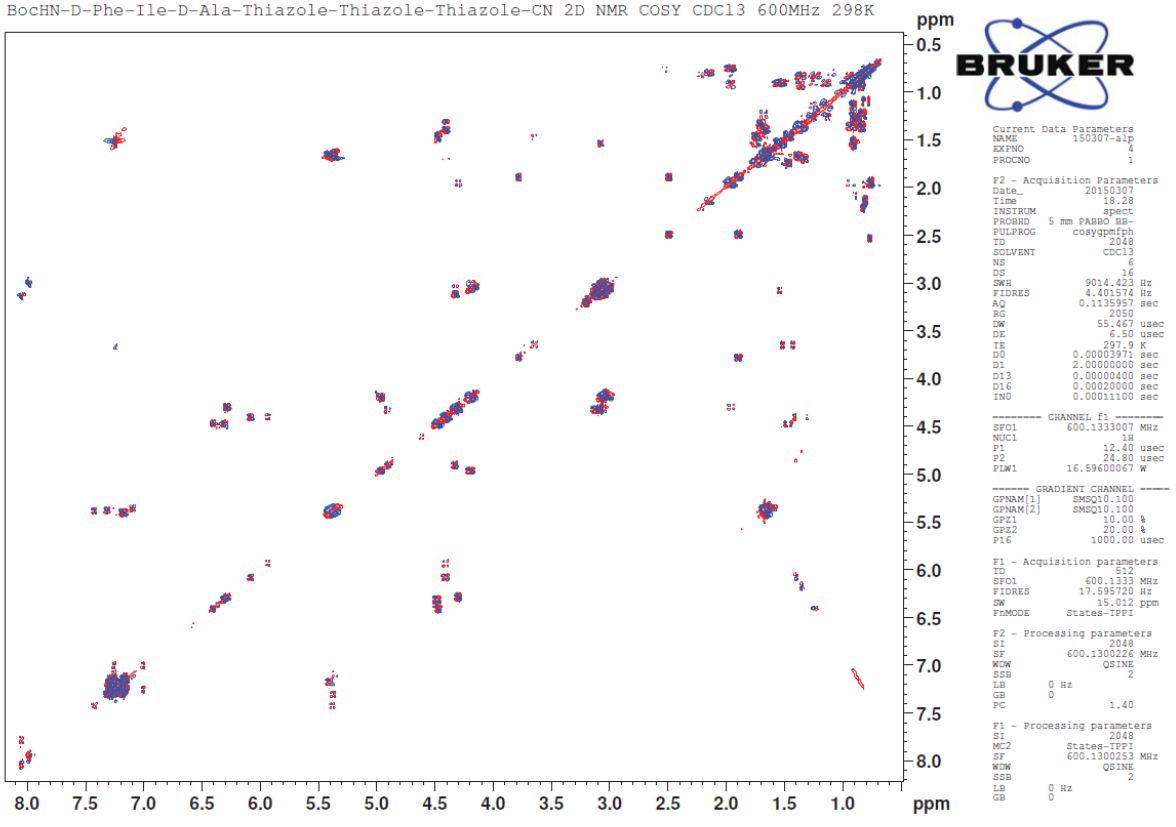


Compound **15 (CDCl3)**



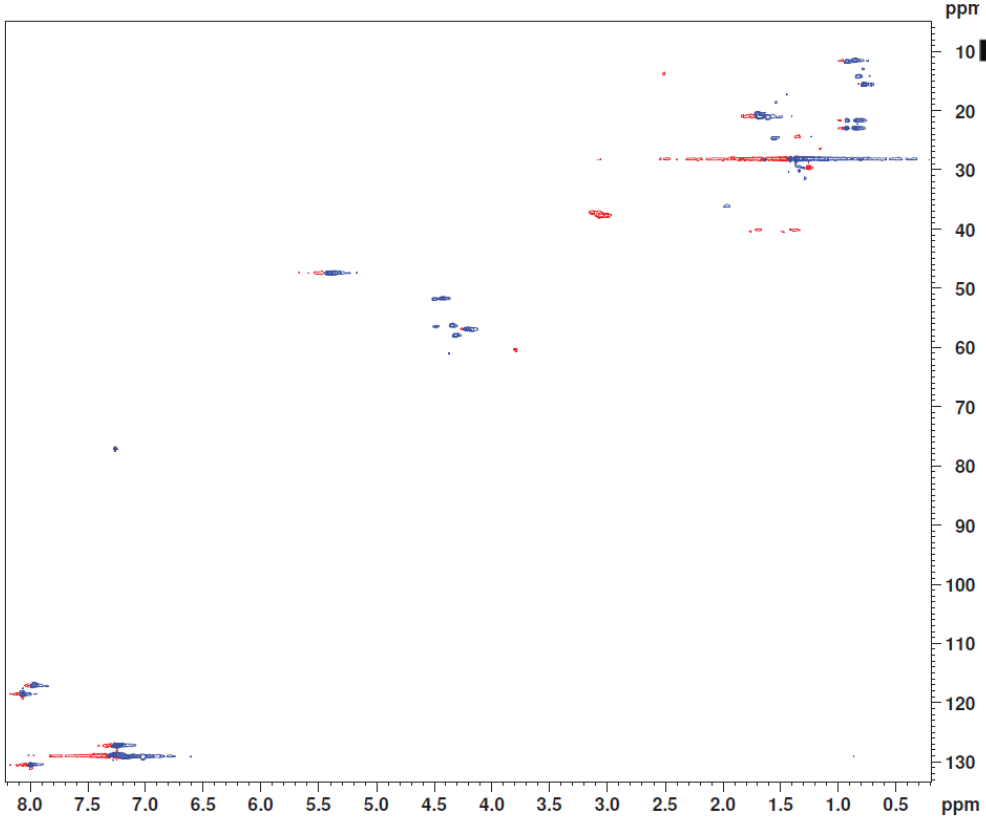


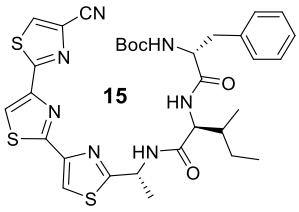




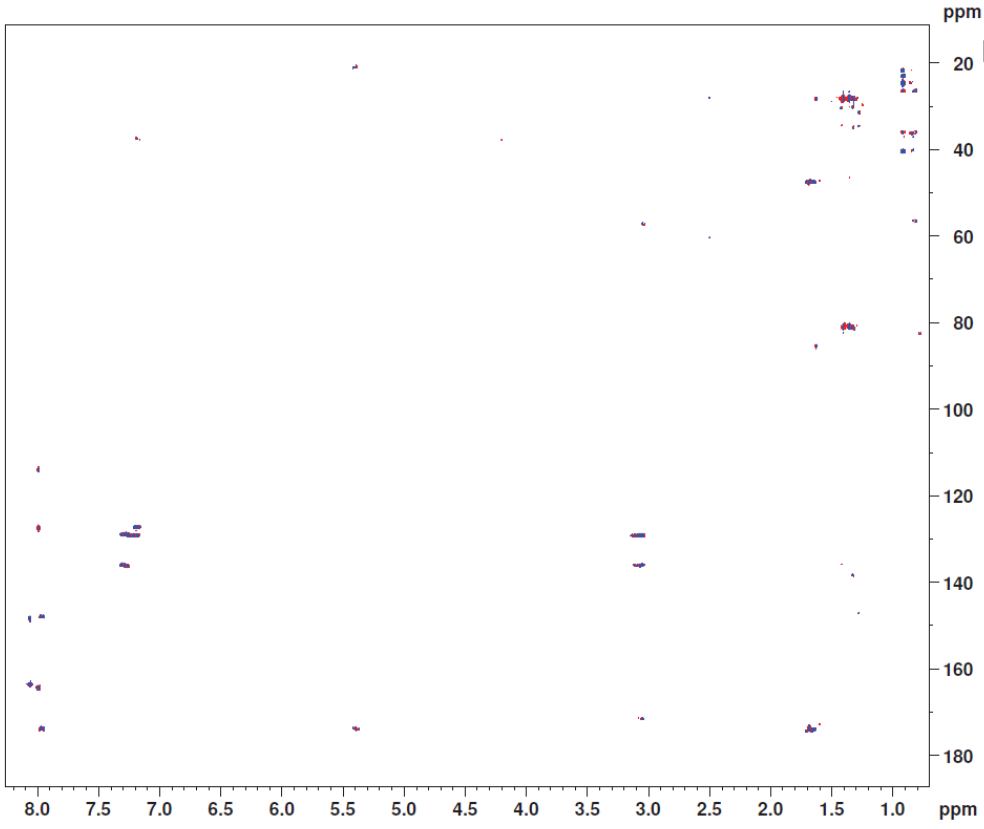
Compound **15 (CDCl3)**

BocHN-D-Phe-Ile-D-Ala-Thiazole-Thiazole-Thiazole-CN 2D NMR HSQC CDCl3 600MHz 298K

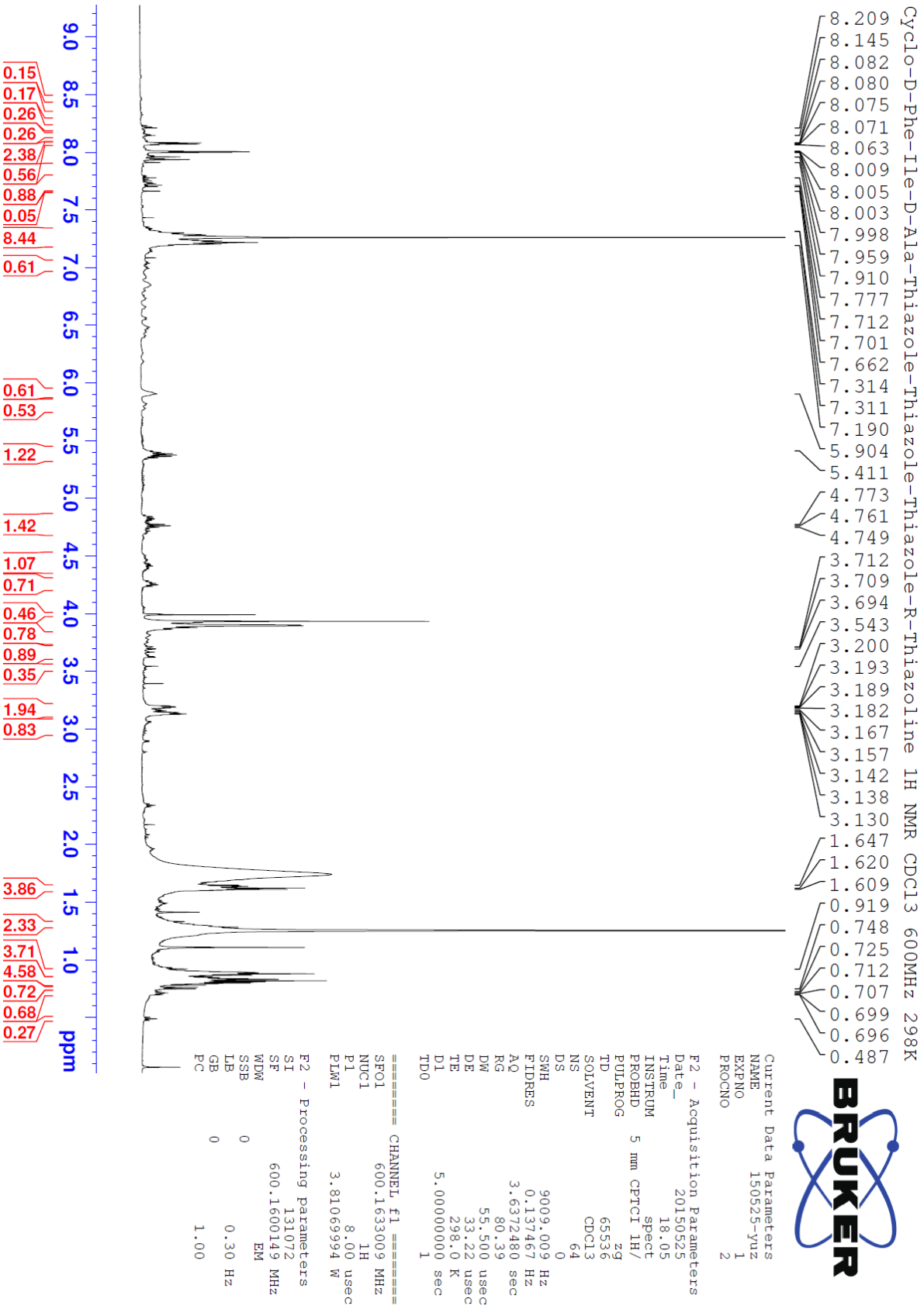


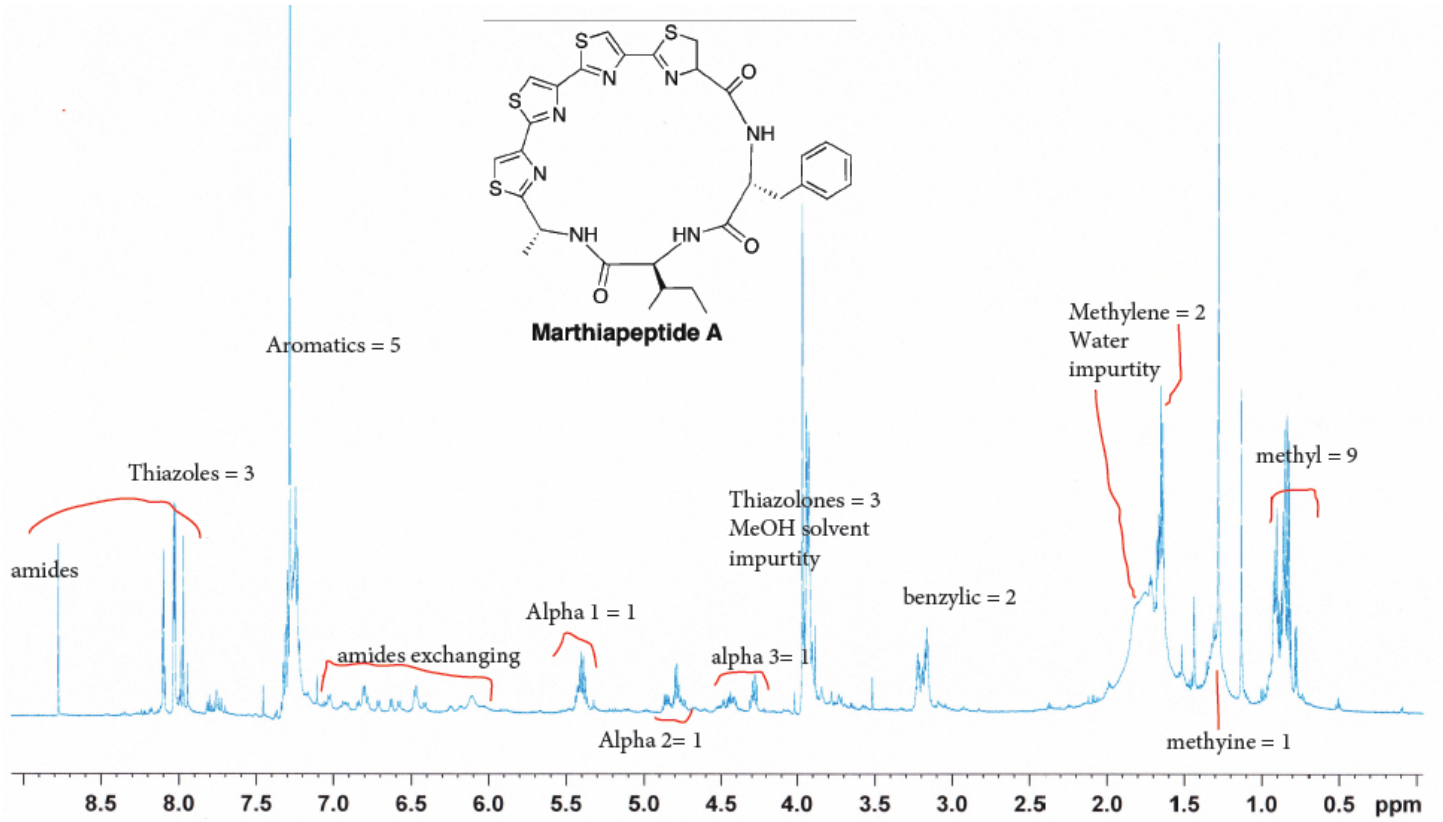

BocHN-D-Phe-Ile-D-Ala-Thiazole-Thiazole-Thiazole-CN 2D NMR HMBC CDCl3 600MHz 298K

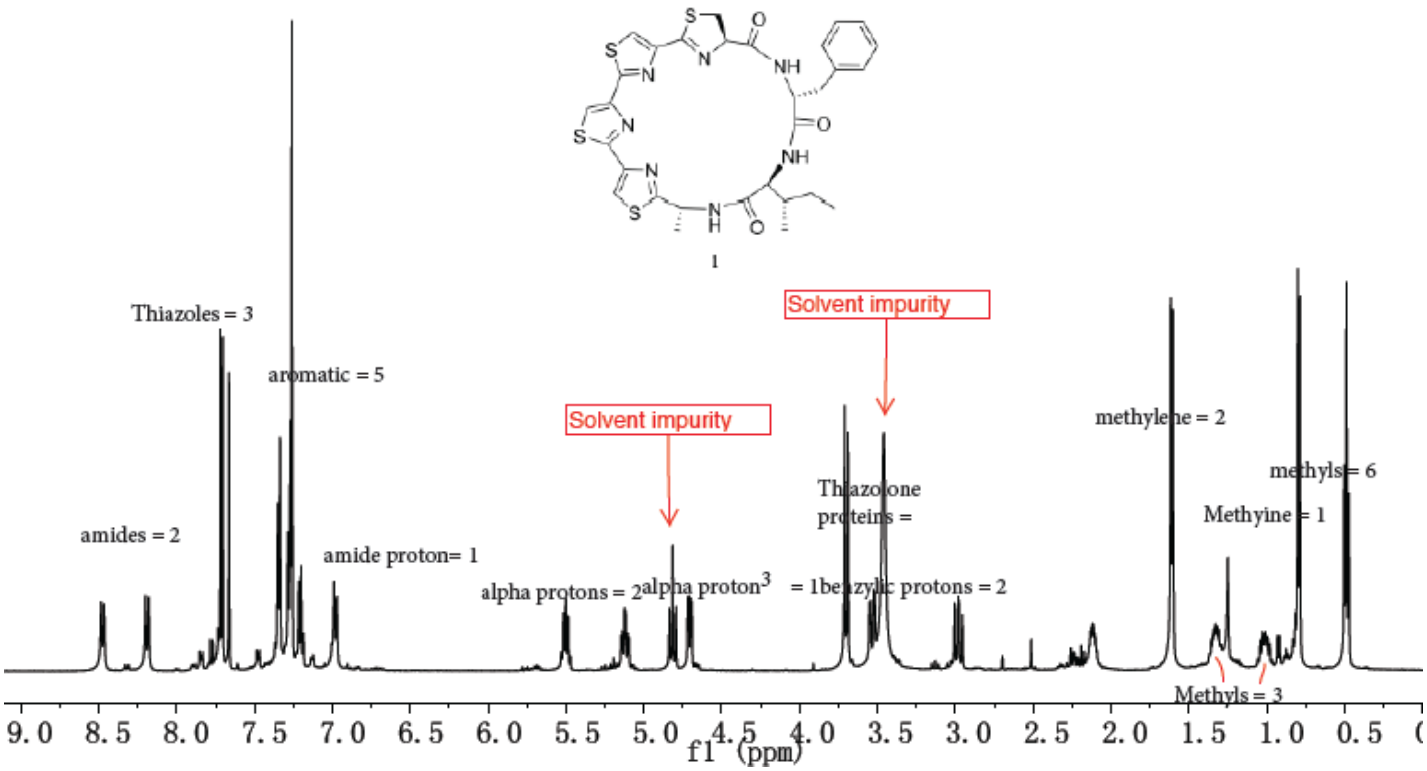


Compound **1** $^{1}$H NMR in $CDCl_3$

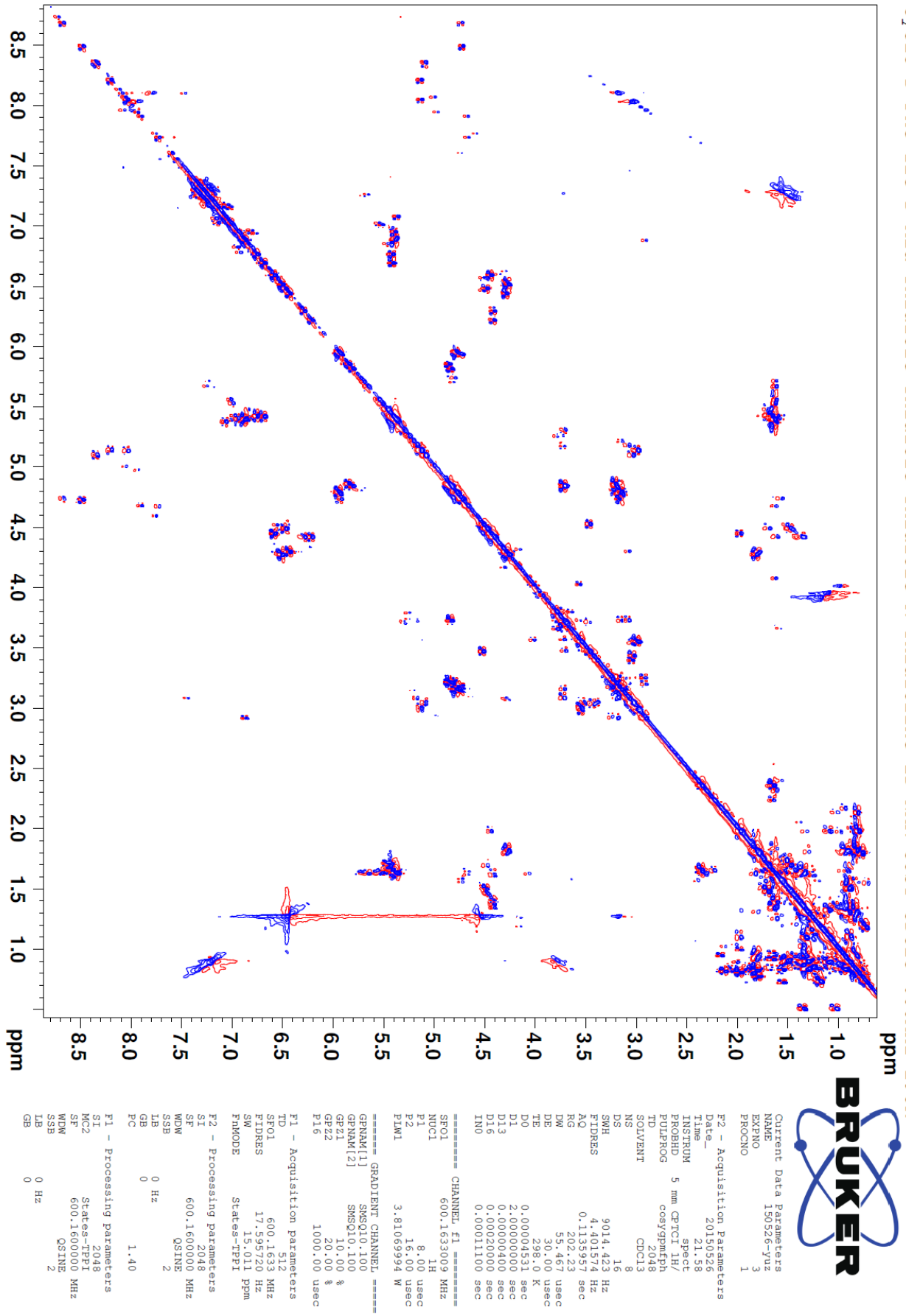


Compound **1** and the **natural product** NMR in $CDCl_3$

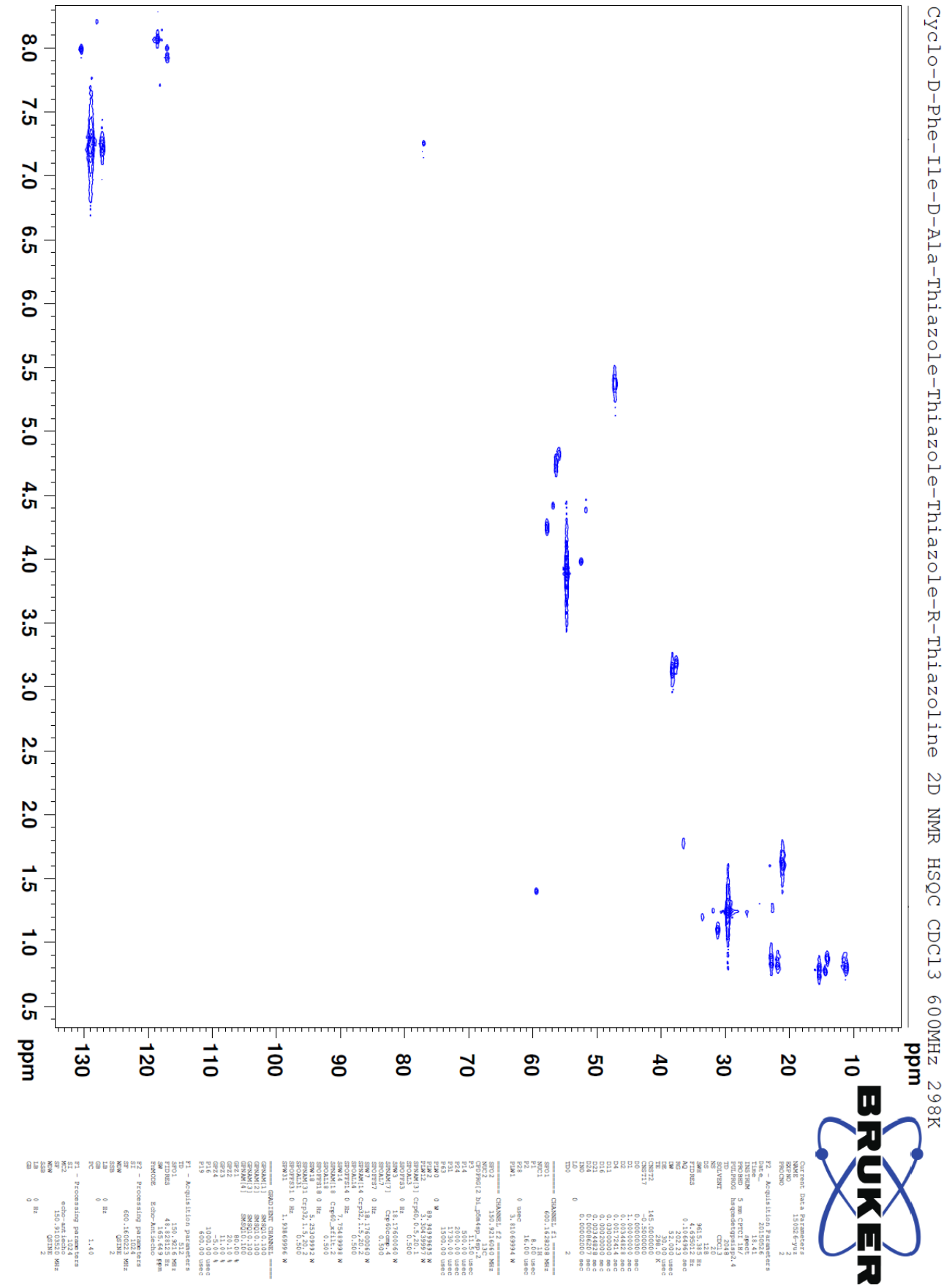


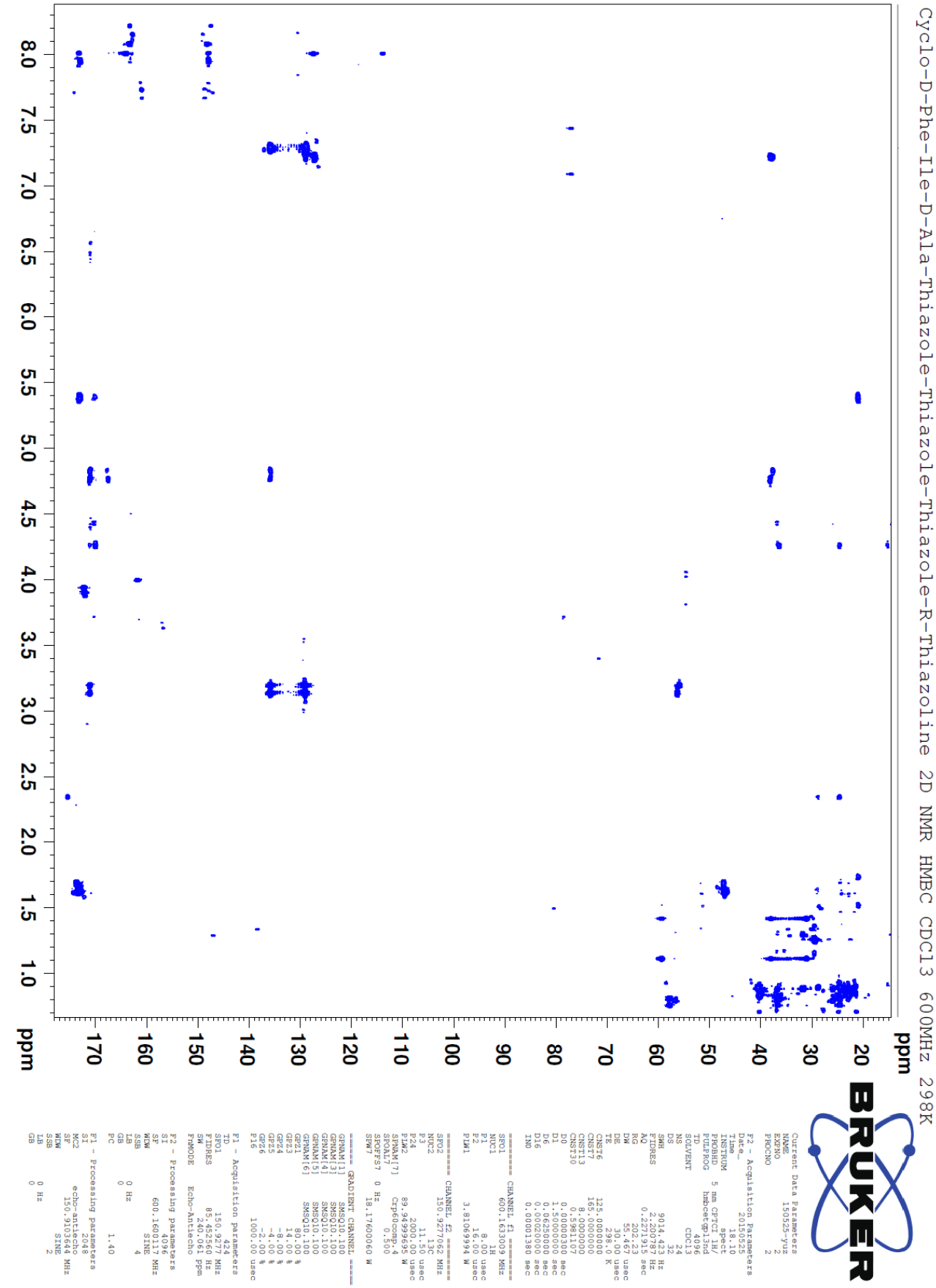
Compound **1** 2D NMR COSY in $CDCl_3$



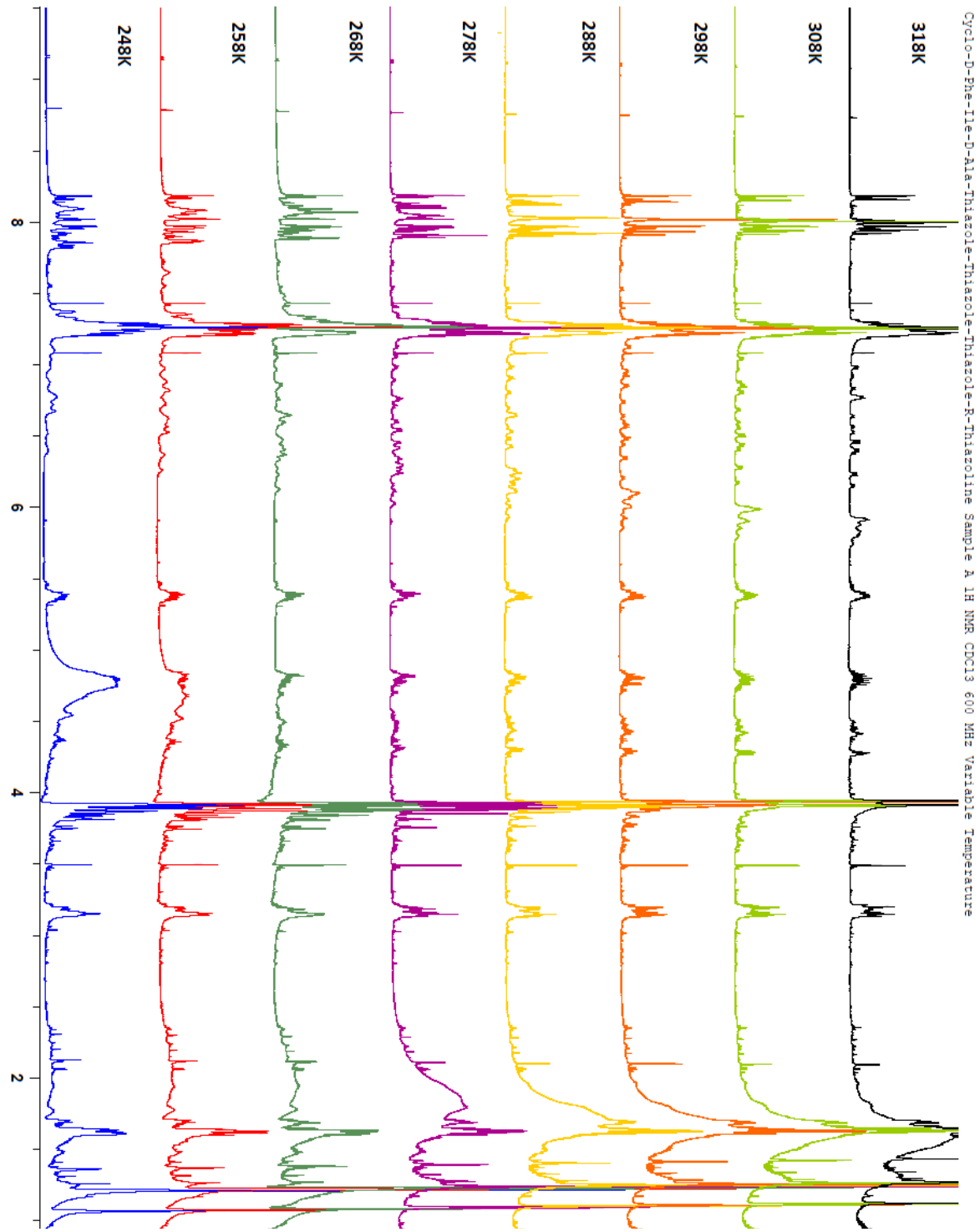
Compound **1** 2D NMR HSQC in $CDCl_3$



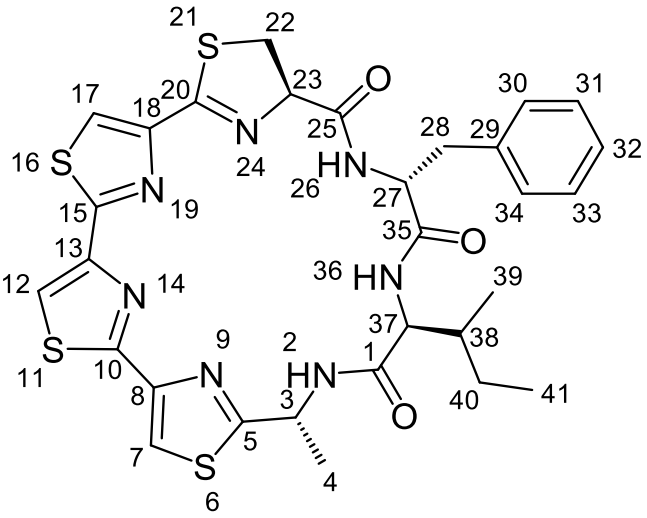
Compound **1** 2D NMR HMBC in $CDCl_3$



Compound **1** variable temperature NMR



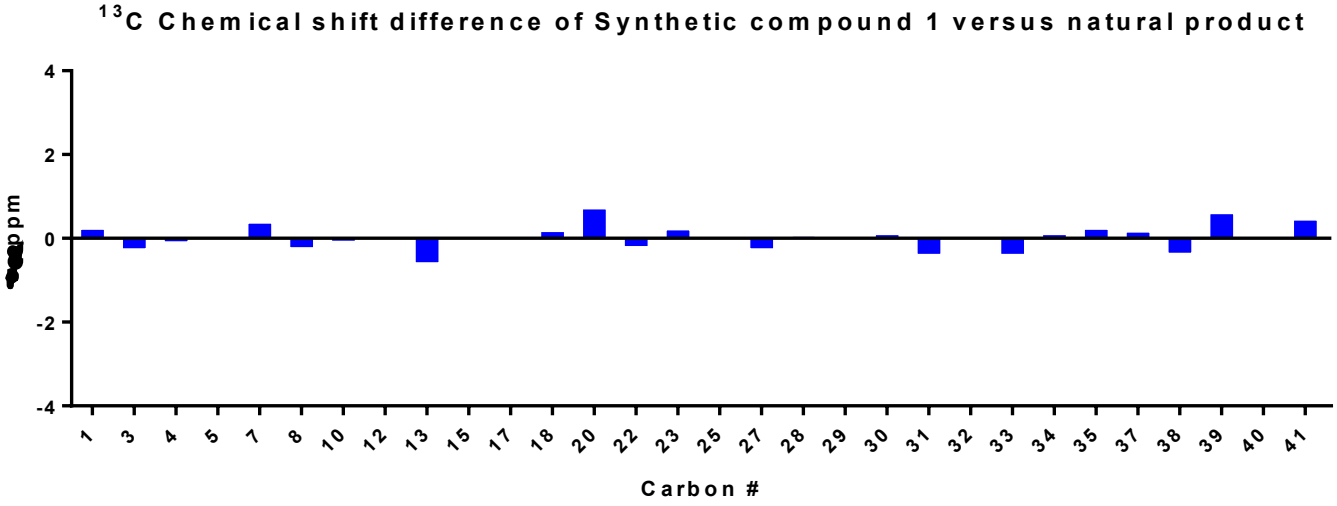
## Marthiapeptide A Natural product vs Synthetic Compound 1



| | $\delta_H$, multi. (*J* in Hz) | | $\delta_C$ | |
|---|---|---|---|---|
| **Position #** | ***Natural product**** | **Synthetic* Compound 1** | ***Natural product*** | **Synthetic Compound 1** |
| **1** | | | 171.30 | 171.39 |
| **2** | 6.99, d (8.1) | 7.00, m | | |
| **3** | 5.50, m | 5.51, m | 47.1 | 47.07 |
| **4** | 1.61, d (7.1) | 1.615, m | 23.0 | 22.96 |
| **5** | | | 174.3 | 174.21 |
| **7** | 7.74, s | 7.74, s | 118.2 | 118.6 |
| **8** | | | 147.2 | 147.11 |
| **10** | | | 161.1 | 161.07 |
| **12** | 7.72, s | 7.721, s | 118.3 | 118.42 |
| **13** | | | 148.1 | 148.9 |
| **15** | | | 161.0 | 161.13 |
| **17** | 7.66, s | 7.68, s | 122.3 | 122.8 |
| **18** | | | 148.9 | 148.87 |
| **20** | | | 161.7 | 161.05 |
| **22** | 3.70, d (10.5) | 3.703, m) | 36.3 | 36.24 |
| **23** | 4.81, t (10.5) | 4.819, m | 78.8 | 78.70 |
| **25** | | | 170.3 | 170.3 |
| **26** | 8.19, d (10.2) | 8.20, d (10.0) | | |
| **27** | 5.12, td (10.2, 3.8) | 5.12, m | 56.4 | 56.36 |
| **28** | 2.98, dd (12.6, 10.2); 3.53, dd (13.6, 3.8) | 2.98, m 3.536, m | 40.3 | 40.3 |
| **29** | | | 137.1 | 137.08 |
| **30/34** | 7.34, d (7.4) | 7.34, m | 129.4 | 129.38 |
| **31/33** | 7.27, t (7.4) | 7.29, m | 128.5 | 128.82 |
| **32** | 7.20, t (7.4) | 7.208, m | 126.9 | 126.6 |
| **35** | | | 172.0 | 172.0 |
| **36** | 8.48, d (9.6) | 8.49, d (9.34) | | |
| **37** | 4.70, dd (9.6, 3.7) | 4.66, m | 58.0 | 58.68 |
| **38** | 2.12, m | 2.14, m | 36.6 | 36.8 |

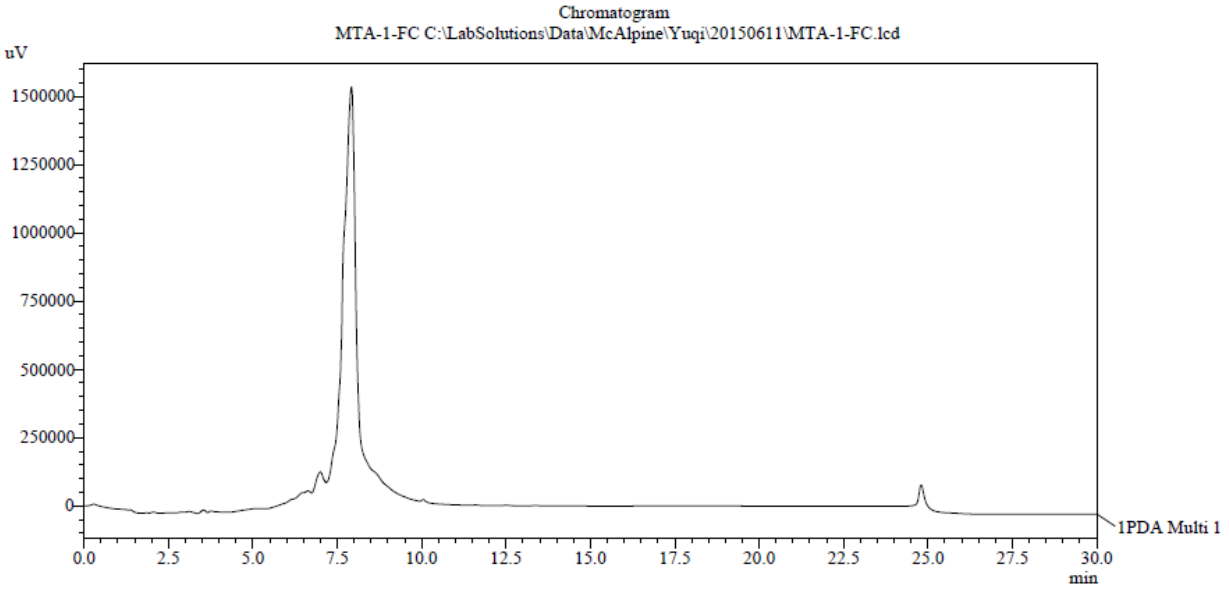
| | | | | |
|---|---|---|---|---|
| **39** | 0.79, d (7.1) | 0.816, d (7.00) | 16.0 | 16.0 |
| **40** | 1.02, m; 1.34, m | 1.034, m; 1.32, m | 24.8 | 24.6 |
| **41** | 0.49, t (7.4) | 0.485, (7.36) | 11.8 | 11.8 |

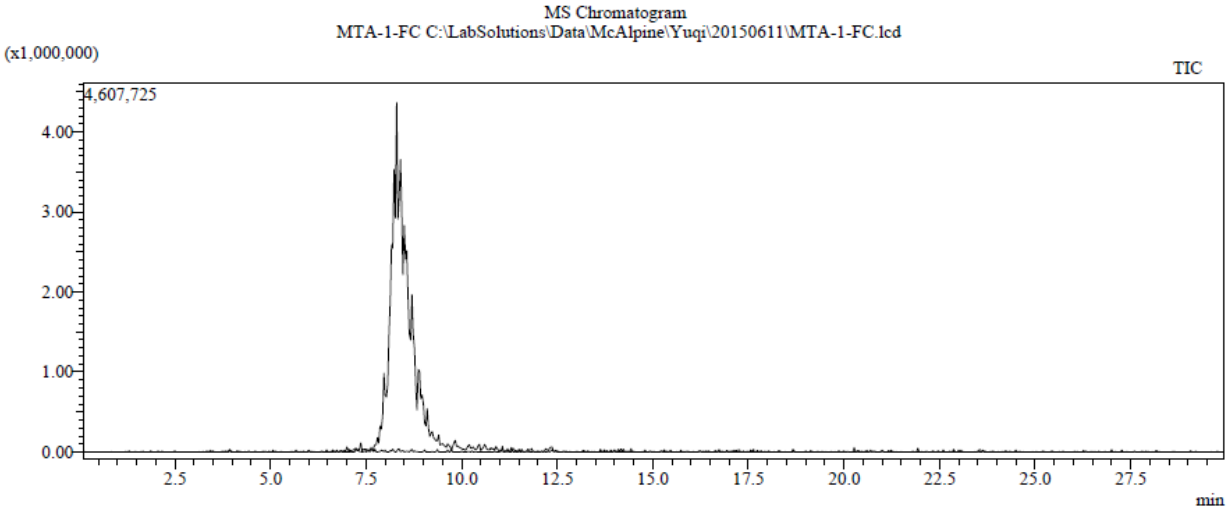
***Note:** Both **natural product** and synthetic compound **1** NMR experiment was performed using $CDCl_3$ as solvent

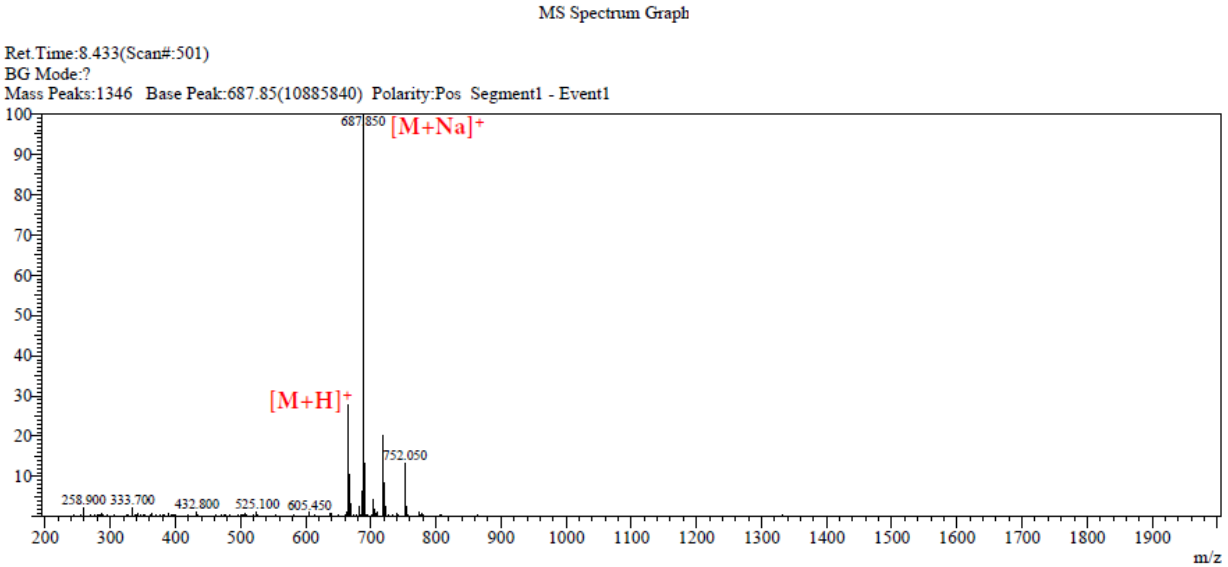


Compound **1** Shimadzu LC-MS analysis

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



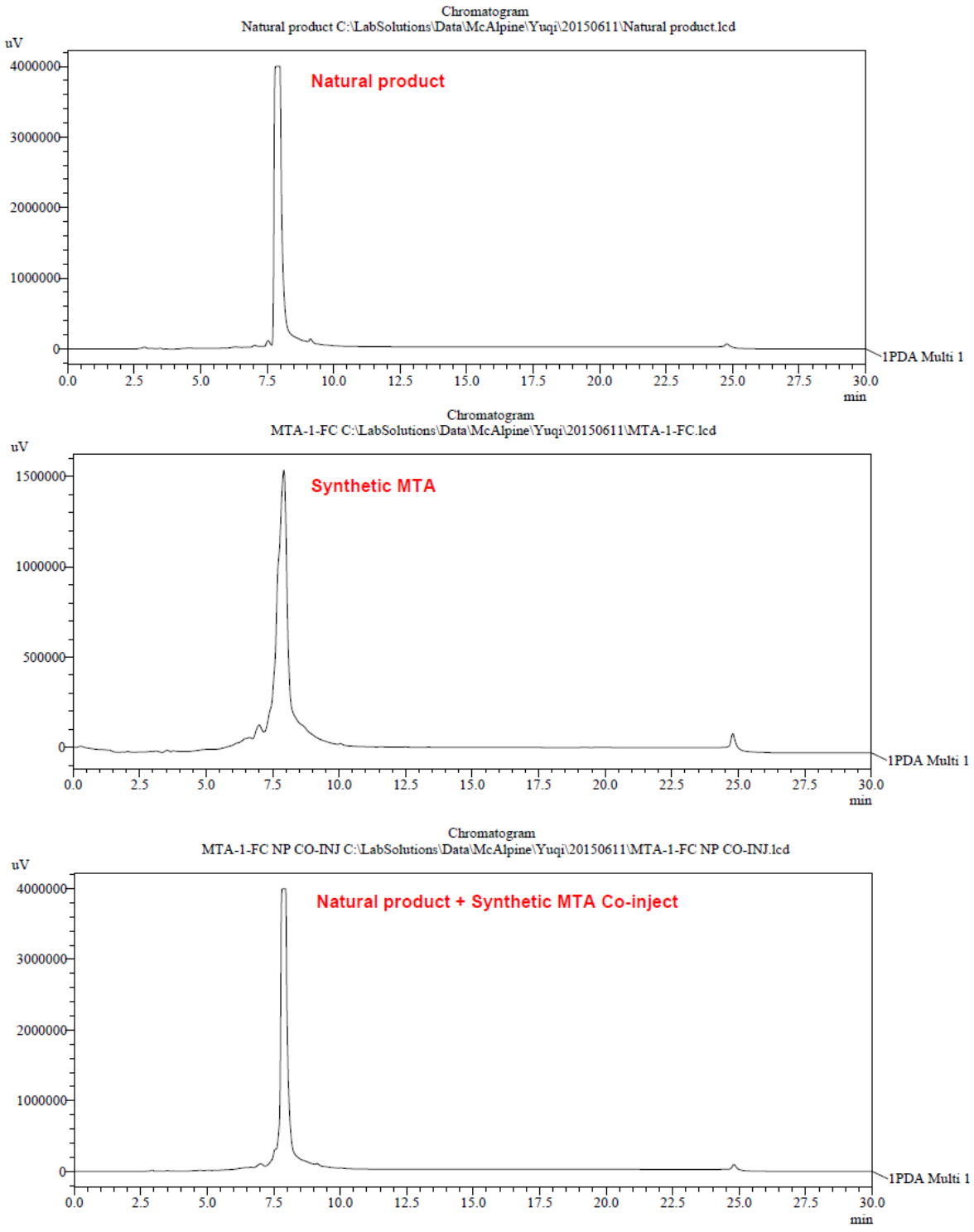



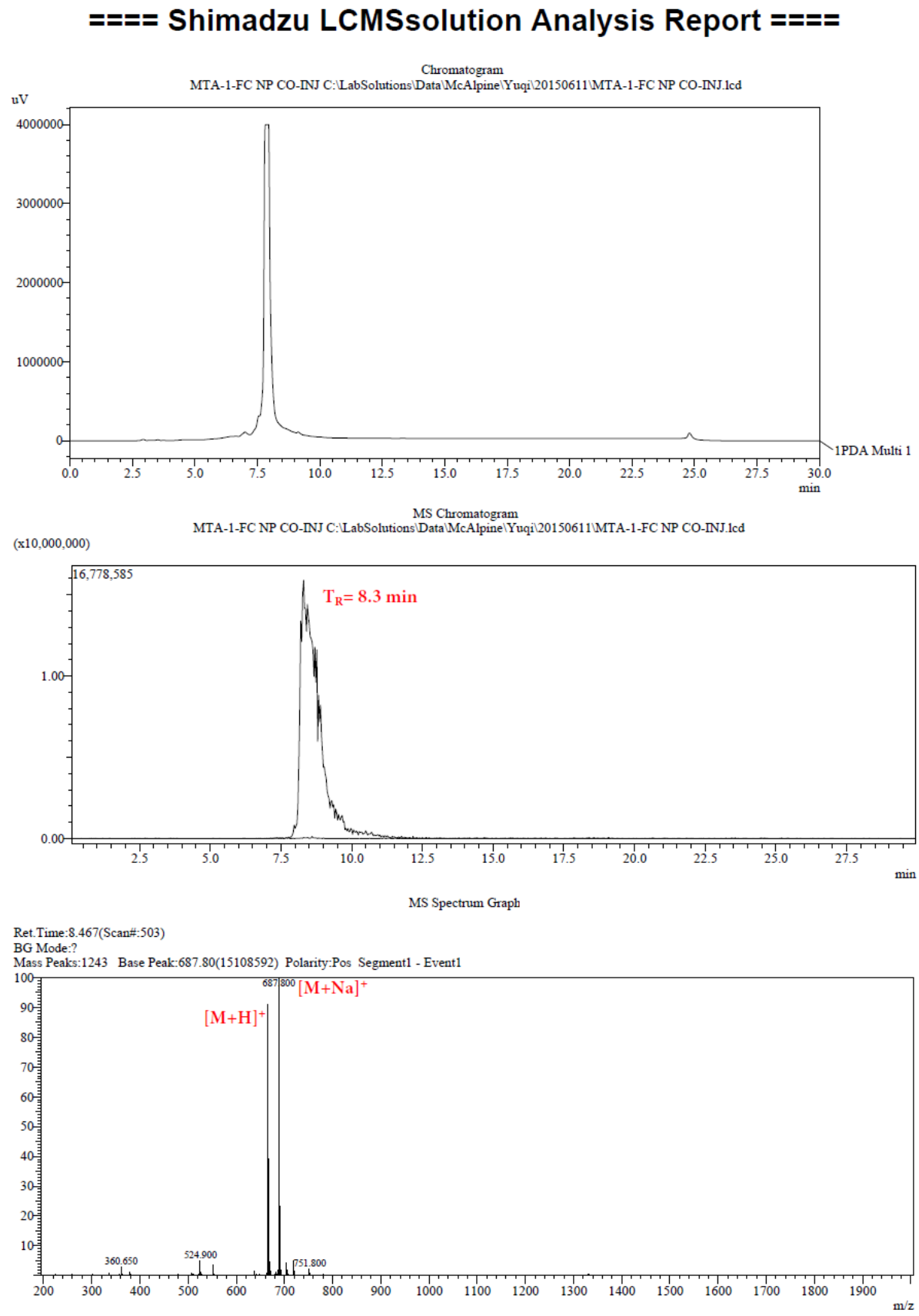
Natural product**,** Compound **1,** Natural product + compound **1** co-inject Shimadzu LC-MS PDA

16/06/2015 15:01:48 1 / 1

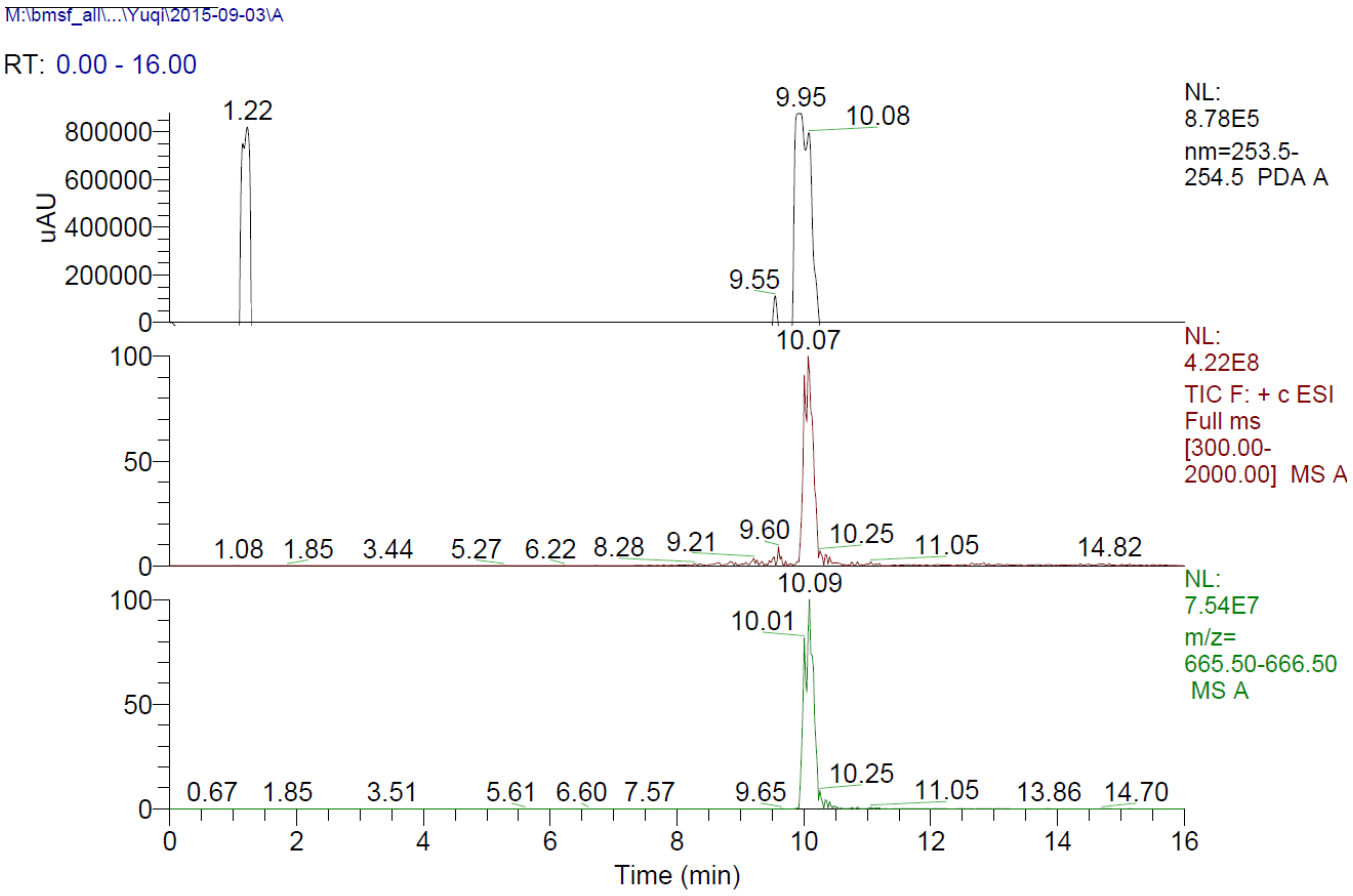
**==== Shimadzu LCMSsolution Analysis Report ====**

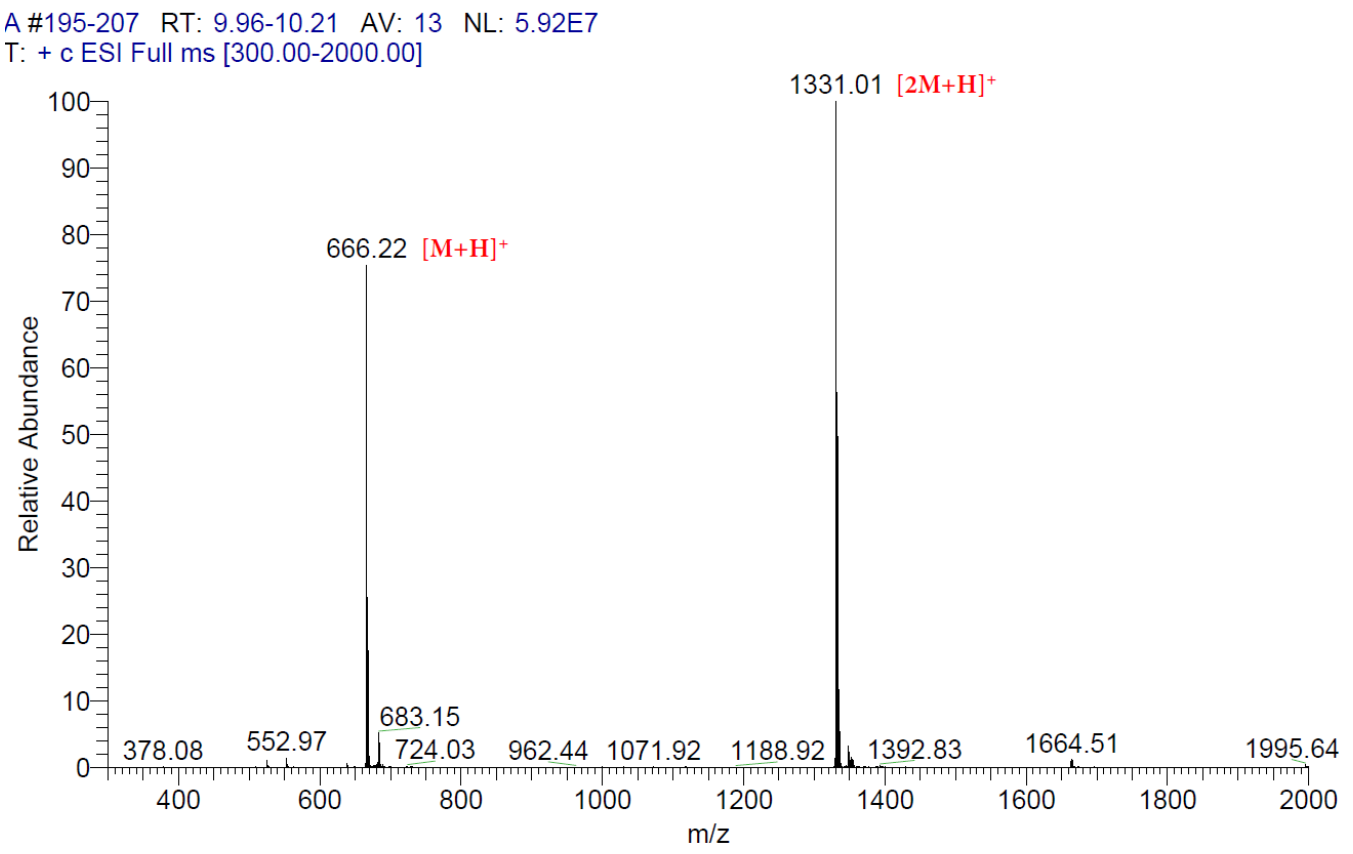


Natural product + compound **1** co-inject Shimadzu LC-MS analysis

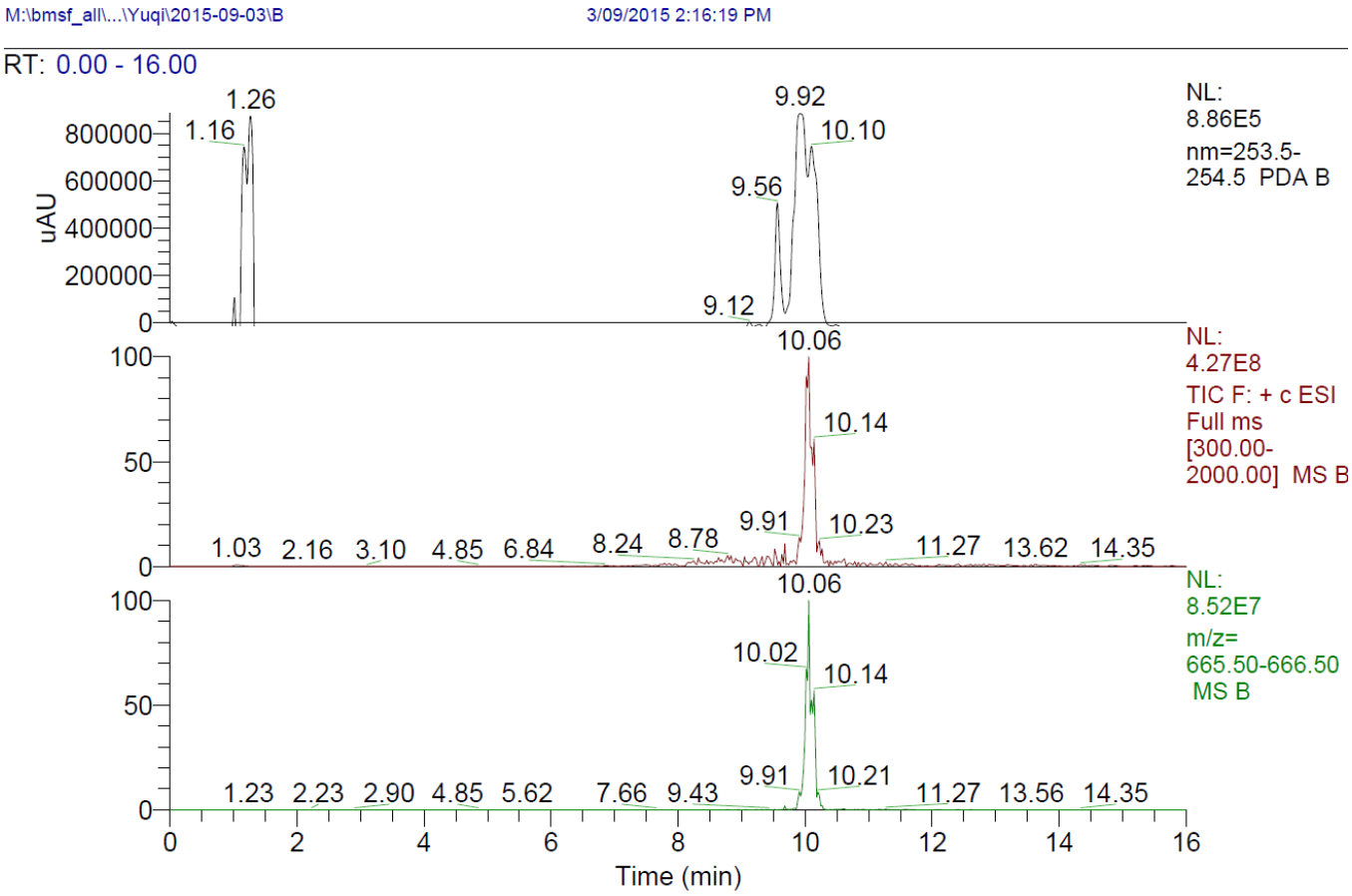


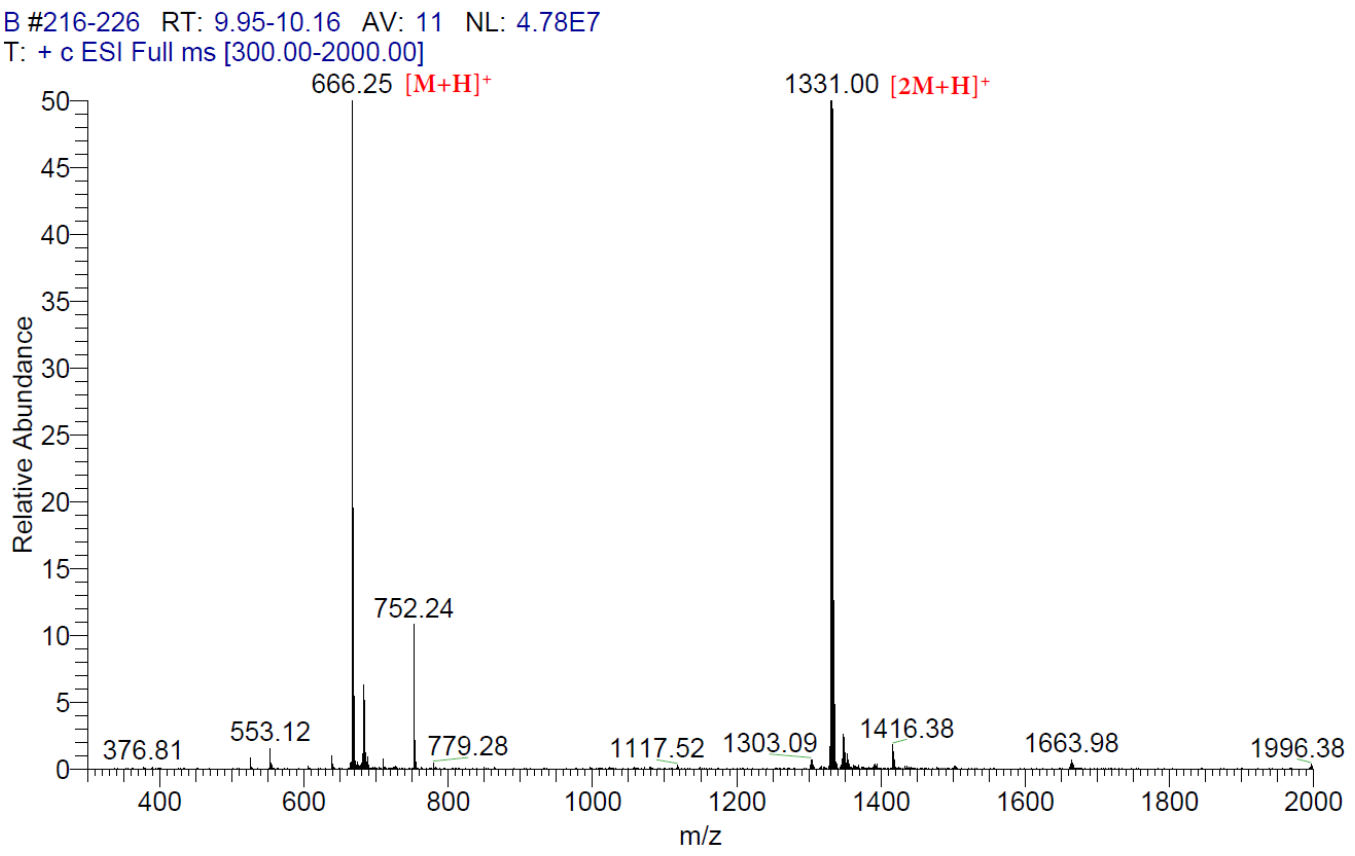
BMSF LC-MS analysis of Natural product injection



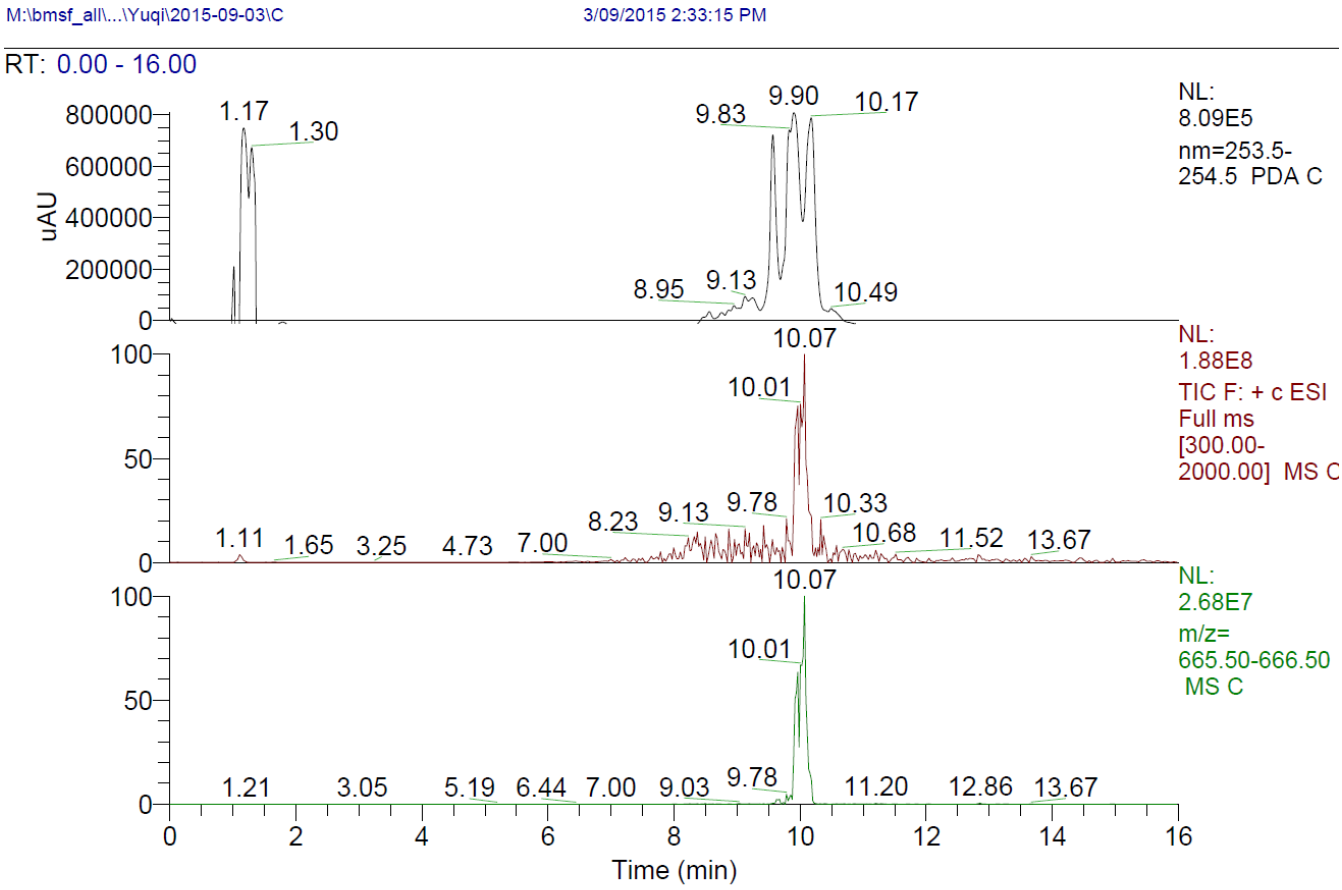



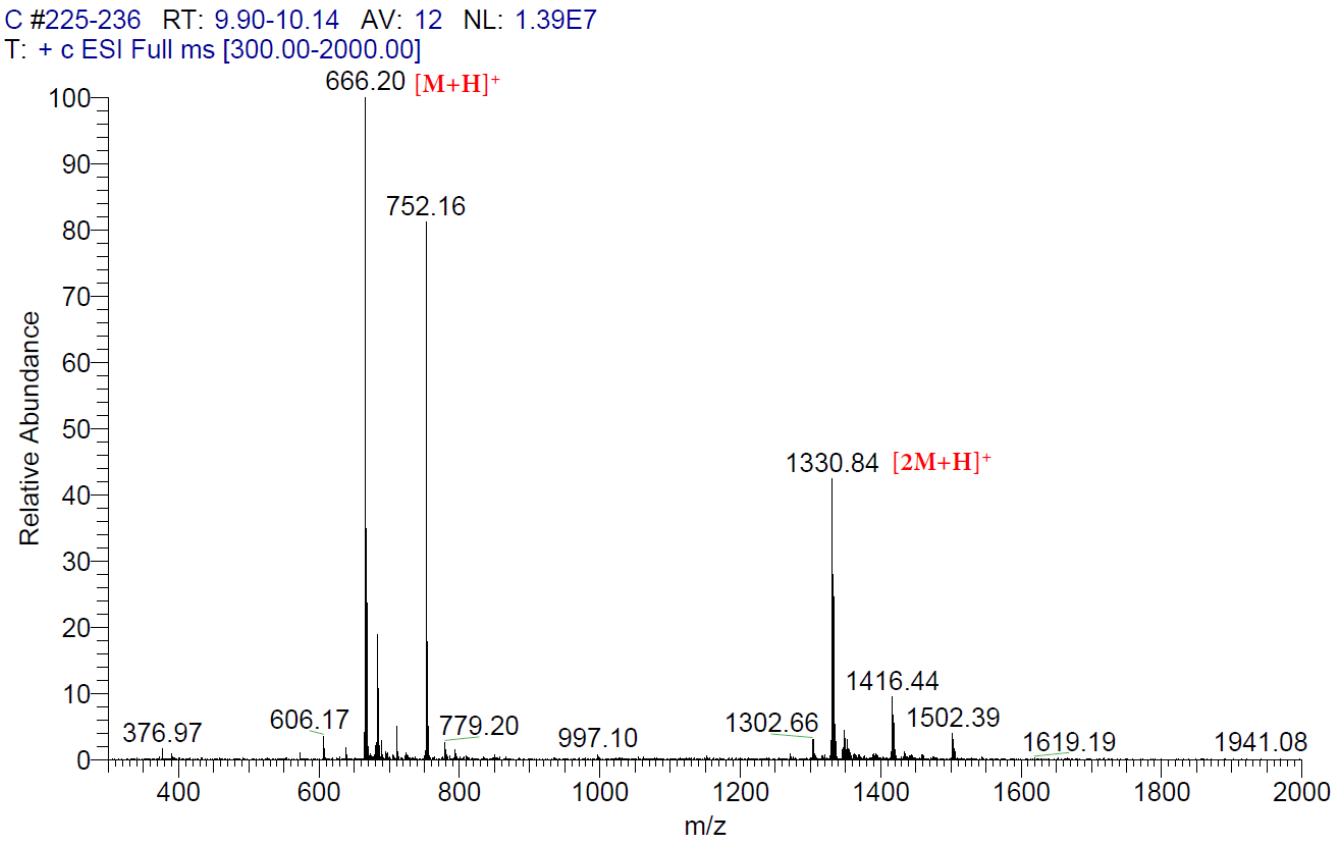
BMSF LC-MS analysis of Natural product + compound **1**



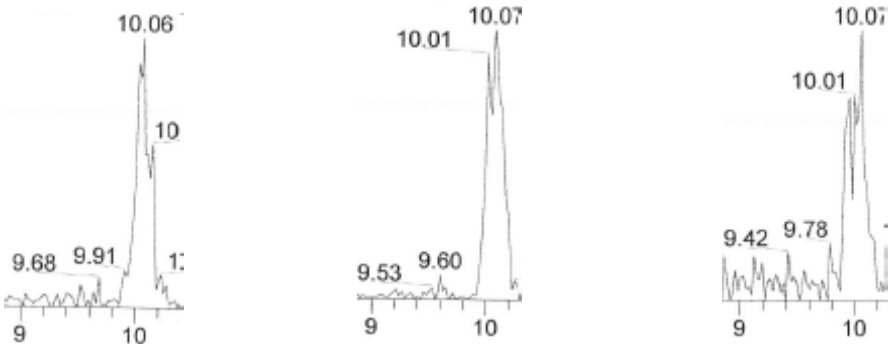
BMSF LC-MS Compound **1**



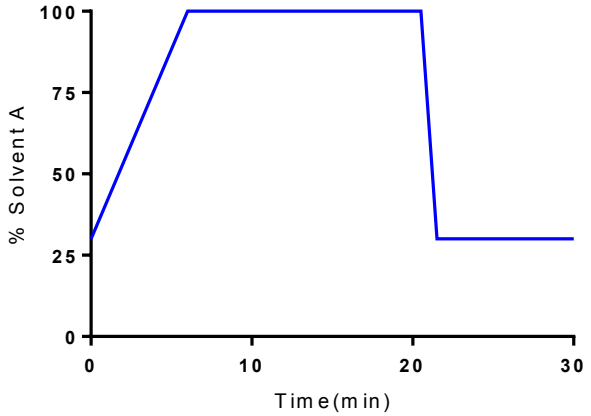



**Synthetic compound 1 and Natural product (NP) BMSF LC-MS TIC analysis**

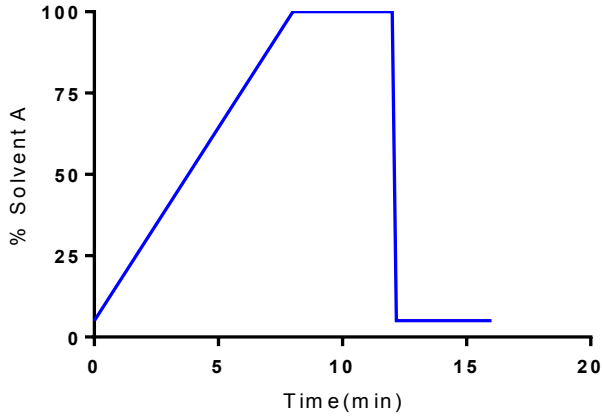
**NP** **NP + Compund 1 co-inject** **Compound 1**



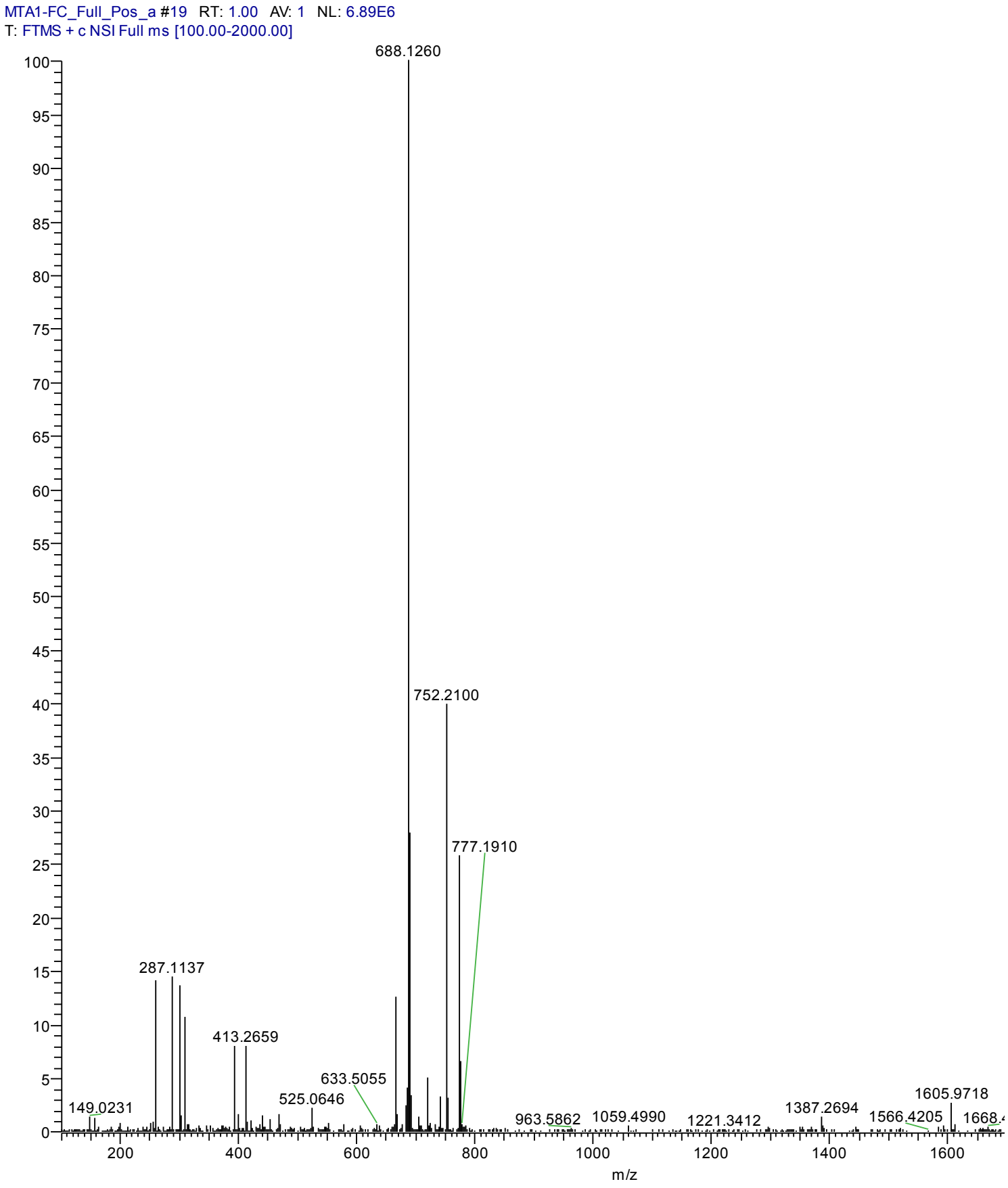

**Shimadzu LC-MS gradient of Solvent A**




**BMSF LC-MS gradient of Solvent A**



Synthetic Compound **1** HRMS analysis (Calculated $[M+Na]^+$:688.1269, found 688.1260) (HRMS from Orbitrap)



**Zoomed spectrum:**