

*Supplementary Materials*

# Indole C6 Functionalization of Tryprostatin B Using Prenyltransferase CdpNPT

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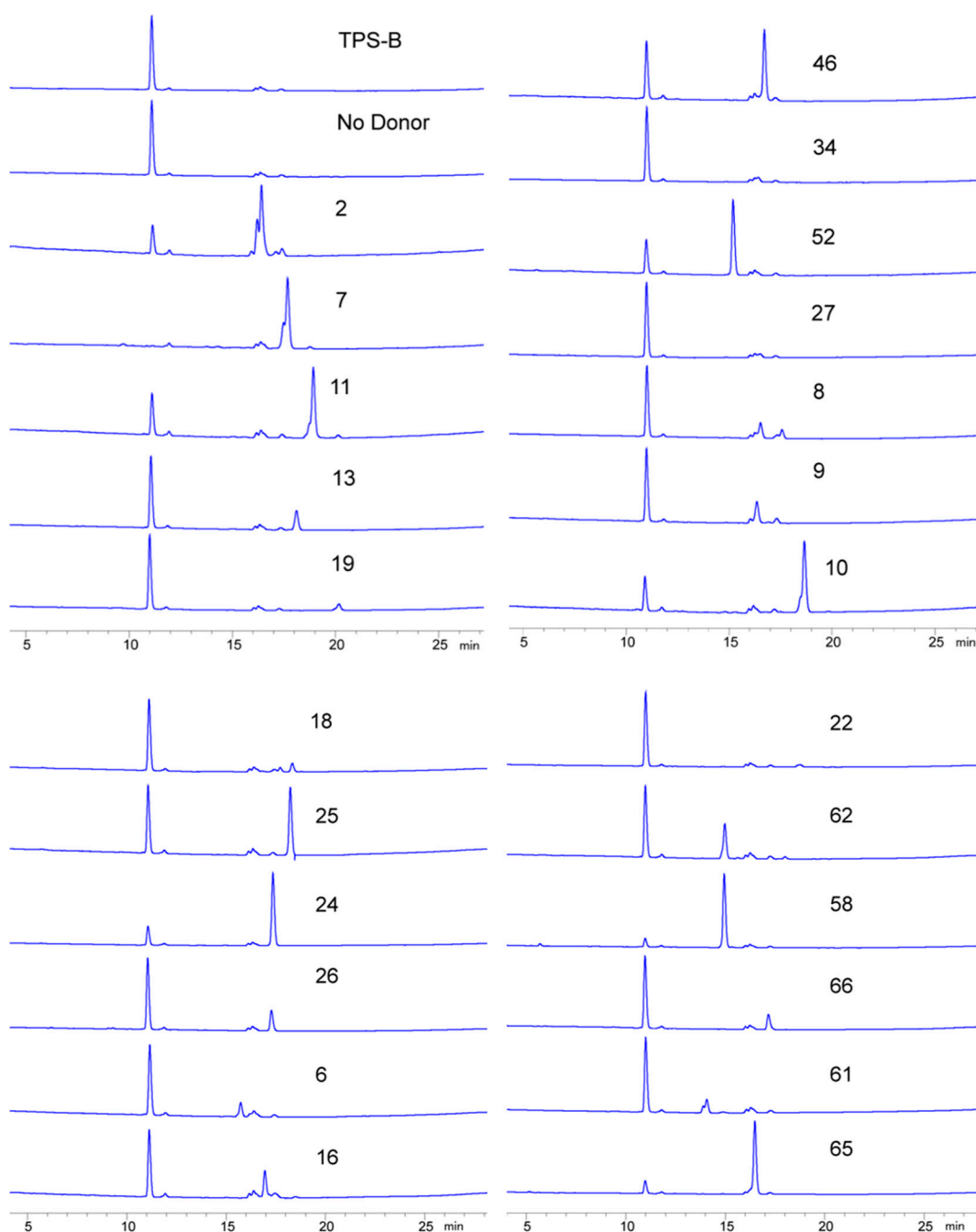
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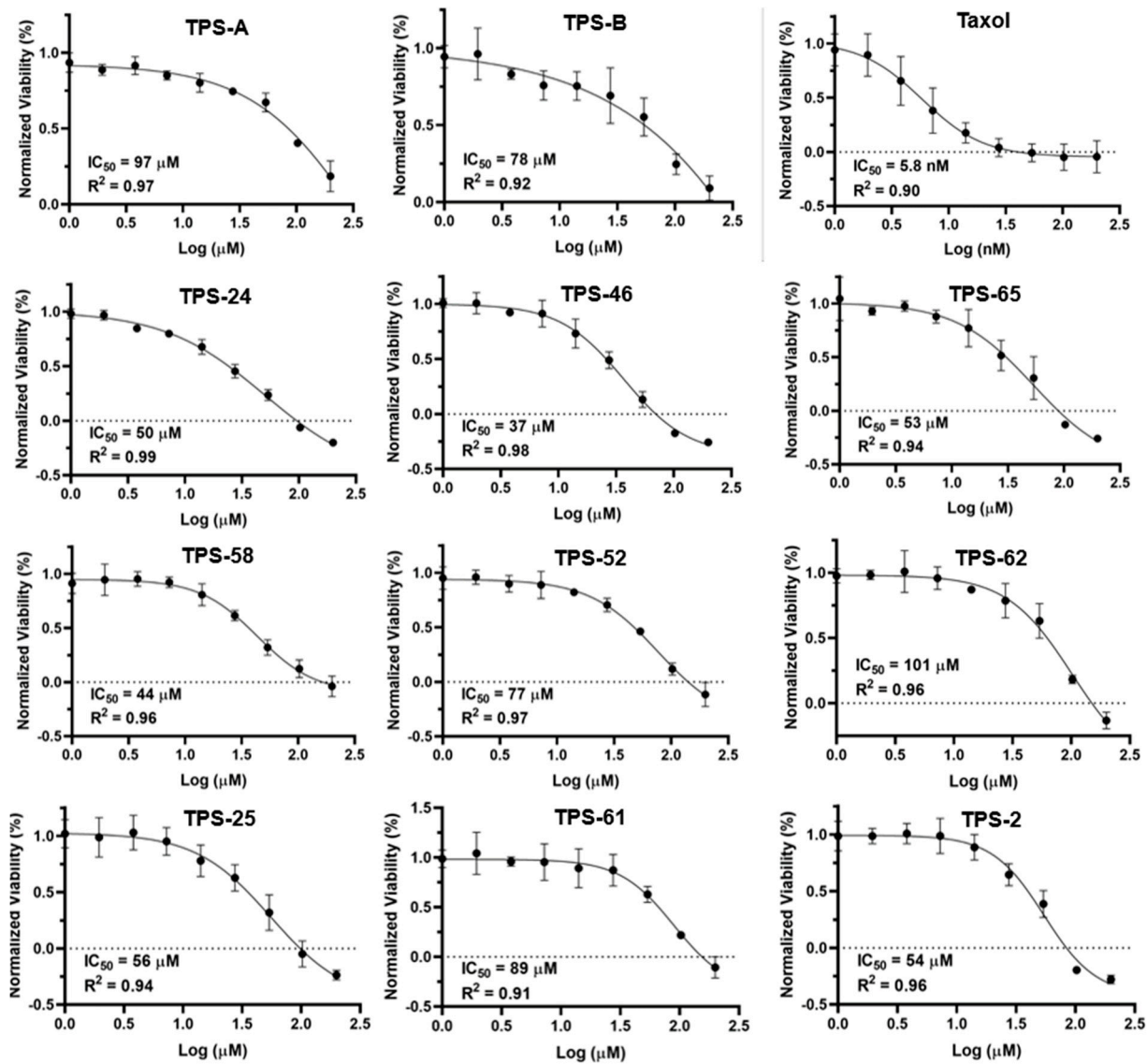
## Analytical Scale CdpNPT Screening HPLC Traces



**Figure S1.** HPLC traces of analytical scale reactions containing TPS-B and CdpNPT under standard reaction conditions. Numerical labels indicate the alkyl-PP used in that reaction. TPS-B standard and enzymatic control reaction without the donor are indicated. The TPS-B used in these screenings is ~95% pure based on HPLC.

**Table S1.** Calculated percent turnover determined by HPLC in duplicate. Percent turnover was calculated by dividing the sum of all TPS-B and product peak areas by the peak area of remaining TPS-B.

<b>OPP #</b>	<b>Trial 1 % Turnover</b>	<b>Trial 2 % Turnover</b>	<b>Mean % Turnover</b>	<b>Std Dev.</b>
2	80.93	85.40	83.17	2.23
6	18.32	21.34	19.83	1.51
7	99.15	99.95	99.55	0.40
8	30.72	32.12	31.42	0.70
9	28.13	31.64	29.88	1.76
10	74.84	76.86	75.85	1.01
11	71.88	78.25	75.06	3.19
13	25.83	30.50	28.16	2.33
16	35.52	35.28	35.40	0.12
18	12.45	13.51	12.98	0.53
19	12.63	14.44	13.54	0.90
22	5.60	6.34	5.97	0.37
24	80.40	52.77	66.59	13.81
25	52.97	58.86	55.91	2.95
26	24.63	26.63	25.63	1.00
27	7.36	8.00	7.68	0.32
34	7.45	8.79	8.12	0.67
46	57.66	64.90	61.28	3.62
52	69.94	75.40	72.67	2.73
58	89.96	92.27	91.11	1.15
61	16.45	18.73	17.59	1.14
62	36.29	38.85	37.57	1.28
65	87.13	90.40	88.76	1.64
66	20.38	24.08	22.23	1.85



**Figure S2.** Cytotoxicity assay results for TPS analogs run in triplicate against K563 leukemia cells, with TPS-A, TPS-B, and Taxol as positive controls.

**Table S2.** HRMS data of the TPS analogs produced by CdpNPT and their corresponding alkyl donor

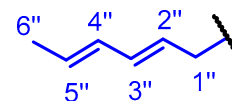
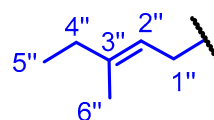
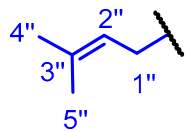
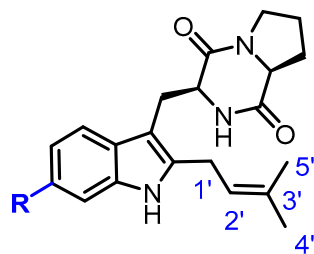
<b>TPSB analog</b>	<b>Formula</b>	<b>Calculated Mass</b>	<b>Observed Mass</b>
TPS-A	C <sub>22</sub> H <sub>27</sub> N <sub>3</sub> O <sub>3</sub> [M+H] <sup>+</sup>	382.2125	382.2133
TPS-B	C <sub>21</sub> H <sub>25</sub> N <sub>3</sub> O <sub>2</sub> [M+H] <sup>+</sup>	352.20194	352.203
TPS-2	C <sub>26</sub> H <sub>33</sub> N <sub>3</sub> O <sub>2</sub> [M+H] <sup>+</sup>	420.26453	420.2659
TPS-6	C <sub>26</sub> H <sub>31</sub> N <sub>3</sub> O <sub>2</sub> [M+H] <sup>+</sup>	418.241627	418.2498
TPS-7	C <sub>27</sub> H <sub>35</sub> N <sub>3</sub> O <sub>2</sub> [M+H] <sup>+</sup>	434.272927	434.2819
TPS-8	C <sub>27</sub> H <sub>33</sub> N <sub>3</sub> O <sub>2</sub> [M+H] <sup>+</sup>	432.257277	432.265
TPS-9	C <sub>27</sub> H <sub>33</sub> N <sub>3</sub> O <sub>2</sub> [M+H] <sup>+</sup>	432.257277	432.2652
TPS-10	C <sub>28</sub> H <sub>37</sub> N <sub>3</sub> O <sub>2</sub> [M+H] <sup>+</sup>	448.288577	448.2971
TPS-11	C <sub>28</sub> H <sub>37</sub> N <sub>3</sub> O <sub>2</sub> [M+H] <sup>+</sup>	448.288577	448.2964
TPS-13	C <sub>28</sub> H <sub>35</sub> N <sub>3</sub> O <sub>2</sub> [M+H] <sup>+</sup>	446.272927	446.2809
TPS-16	C <sub>27</sub> H <sub>33</sub> N <sub>3</sub> O <sub>2</sub> [M+H] <sup>+</sup>	432.257277	432.2653
TPS-18	C <sub>28</sub> H <sub>35</sub> N <sub>3</sub> O <sub>2</sub> [M+H] <sup>+</sup>	446.272927	446.2810
TPS-19	C <sub>29</sub> H <sub>39</sub> N <sub>3</sub> O <sub>2</sub> [M+H] <sup>+</sup>	462.304226	462.3118
TPS-22	C <sub>29</sub> H <sub>37</sub> N <sub>3</sub> O <sub>2</sub> [M+H] <sup>+</sup>	460.288577	460.2964
TPS-24	C <sub>30</sub> H <sub>33</sub> N <sub>3</sub> O <sub>2</sub> [M+H] <sup>+</sup>	468.26453	468.2651
TPS-25	C <sub>31</sub> H <sub>35</sub> N <sub>3</sub> O <sub>2</sub> [M+H] <sup>+</sup>	482.28018	482.2802
TPS-26	C <sub>31</sub> H <sub>35</sub> N <sub>3</sub> O <sub>3</sub> [M+H] <sup>+</sup>	498.267842	498.2758
TPS-27	C <sub>29</sub> H <sub>35</sub> N <sub>3</sub> O <sub>2</sub> [M+H] <sup>+</sup>	458.272927	458.2814
TPS-34	C <sub>27</sub> H <sub>34</sub> N <sub>6</sub> O <sub>2</sub> [M+H] <sup>+</sup>	475.274324	475.2823
TPS-46	C <sub>29</sub> H <sub>33</sub> N <sub>3</sub> O <sub>2</sub> [M+H] <sup>+</sup>	456.26453	456.2655
TPS-52	C <sub>29</sub> H <sub>33</sub> N <sub>3</sub> O <sub>3</sub> [M+H] <sup>+</sup>	472.25945	472.2602
TPS-58	C <sub>29</sub> H <sub>31</sub> N <sub>3</sub> O <sub>4</sub> [M+H] <sup>+</sup>	486.23871	486.2389
TPS-61-P1	C <sub>26</sub> H <sub>29</sub> N <sub>3</sub> O <sub>3</sub> [M+H] <sup>+</sup>	432.22815	432.2292
TPS-61-P2	C <sub>26</sub> H <sub>29</sub> N <sub>3</sub> O <sub>3</sub> [M+H] <sup>+</sup>	432.22815	432.2294
TPS-62	C <sub>26</sub> H <sub>29</sub> N <sub>3</sub> O <sub>2</sub> S [M+H] <sup>+</sup>	448.20531	448.2061
TPS-65	C <sub>30</sub> H <sub>31</sub> N <sub>3</sub> O <sub>3</sub> [M+H] <sup>+</sup>	482.2438	482.2442
TPS-66	C <sub>30</sub> H <sub>31</sub> N <sub>3</sub> O <sub>2</sub> S [M+H] <sup>+</sup>	498.213699	498.2216

Table S3. Tryprostatin Analog NMR Assignment Table

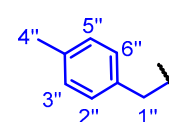
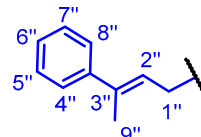
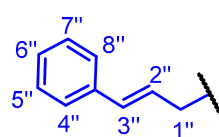
Segment	Position	$\delta$ C (in ppm), type	$\delta$ H (in ppm)
L-Trp	NH		5.62 (s)
	$\alpha$	54.5, CH	4.33 (dd)
	$\beta$	25.6, CH <sub>2</sub>	2.93 (m), 3.66 (d)
	1		7.98 (s)
	2	136.5, C	
	3	104.5, C	
	3a	126.8, C	
	4	117.9, CH	7.4 (d)
	5	121.3, CH	7.04 (d)
	6	131.0, C	
	7	111.1, CH	7.21 (s)
	7a	136.1, C	
	C=O		
	Proline	$\alpha$	59.3, CH
$\beta$		28.4, CH <sub>2</sub>	2.02 (m), 2.32 (dt)
$\gamma$		22.7, CH <sub>2</sub>	1.89 (m), 2.02 (m)
$\delta$		45.4, CH <sub>2</sub>	3.58 (m), 3.65 (m)
C=O		165.8, C	
C2-Prenyl	1'	25.1, CH <sub>2</sub>	3.44 (qd)
	2'	119.8, CH	5.28 (m)
	3'	135.5, C	
	4'	18.0, CH <sub>3</sub>	1.73 (s)
	5'	25.8, CH <sub>3</sub>	1.76 (s)
C6-Sub	1''	35.2, CH <sub>2</sub>	4.18 (s)
	2''	158.6, C	
	3''	103.3, CH	6.36 (s)
	3a''	128.9, C	
	4''	111.0, CH	7.38 (d)
	5''	123.4, CH	7.18 (td)
	6''	122.6, CH	7.15 (td)
	7''	120.5, CH	7.43 (d)
7a''	155.0, C		

**Figure S3:** Representative NMR correlations used to assign TPS analogs

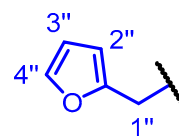
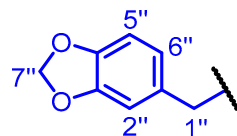
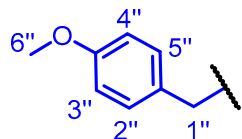




Segment	Position	TPS-2		TPS-7		TPS-16	
		$\delta C$ , type	$\delta H$	$\delta C$ , type	$\delta H$	$\delta C$ , type	$\delta H$
L-Trp	NH		5.61 (s)		5.61 (s)		5.61 (s)
	$\alpha$	54.6, CH	4.36 (m)	54.6, CH	4.34 (dd)	54.6, CH	4.35 (dd)
	$\beta$	25.5 CH <sub>2</sub>	2.93 (dd), 3.66 (m)	25.6, CH <sub>2</sub>	2.92 (dd), 3.64 (m)	25.7, CH <sub>2</sub>	2.93 (dd), 3.65 (m)
	1		7.82 (s)		7.81 (s)		
	2						
	3						
	3a						
	4	117.7, CH	7.37 (d)	117.6, CH	7.37 (d)	117.6, CH	7.38 (d)
	5	121, CH	6.94 (d)	121.0, CH	6.94 (d)	121.1, CH	6.94 (d)
	6						
	7	110.1, CH	7.12 (s)	110.1, CH	7.11 (s)	110.5, CH	7.13
7a							
C=O							
Proline	$\alpha$	59.3, CH	4.05 (t)	59.6, CH	4.05 (t)	59.3, CH	4.06 (t)
	$\beta$	28.4, CH <sub>2</sub>	2.05 (m), 2.35 (m)	28.3, CH <sub>2</sub>	2.04 (m), 2.33 (m)	28.4, CH <sub>2</sub>	2.05 (m), 2.35 (m)
	$\gamma$	22.7, CH <sub>2</sub>	1.92 (m), 2.05 (m)	22.6, CH <sub>2</sub>	1.91 (m), 2.04 (m)	22.9, CH <sub>2</sub>	1.92 (m), 2.05 (m)
	$\delta$	45.5, CH <sub>2</sub>	3.59 (m), 3.70 (m)	45.4, CH <sub>2</sub>	3.59 (m), 3.67 (m)	45.4, CH <sub>2</sub>	3.59 (m), 3.68 (m)
	C=O						
C2-Prenyl	1'	25.2, CH <sub>2</sub>	3.46 (d)	25.1, CH <sub>2</sub>	3.45 (m)	25.2, CH <sub>2</sub>	3.46 (m)
	2'	119.8, CH	5.31 (t)	119.8, CH	5.29 (t)	119.6, CH	5.31 (t)
	3'						
	4'	17.8, CH <sub>3</sub>	1.75 (s)	18.0, CH <sub>3</sub>	1.74 (s)	18.0, CH <sub>3</sub>	1.75 (s)
	5'	25.7, CH <sub>3</sub>	1.76 (s)	25.7, CH <sub>3</sub>	1.78 (s)	25.8, CH <sub>3</sub>	1.79 (s)
C6-Sub	1''	36.5, CH <sub>2</sub>	3.42 (d)	34.3, CH <sub>2</sub>	3.44 (m)	39.1, CH <sub>2</sub>	3.48 (m)
	2''	124.2, CH	5.37 (t)	122.3, CH	5.36	131.0, CH	5.72 (dt)
	3''					131.4, CH	6.06 (m)
	4''	17.8, CH <sub>3</sub>	1.75 (s)	32.4, CH <sub>2</sub>	2.05 (q)	131.4, CH	6.06 (m)
	5''	25.7, CH <sub>3</sub>	1.79 (s)	12.7, CH <sub>3</sub>	1.02 (t)	127.7, CH	5.63 (m)
	6''			16.0, CH <sub>3</sub>	1.73 (s)	18.0, CH <sub>3</sub>	1.73 (d)

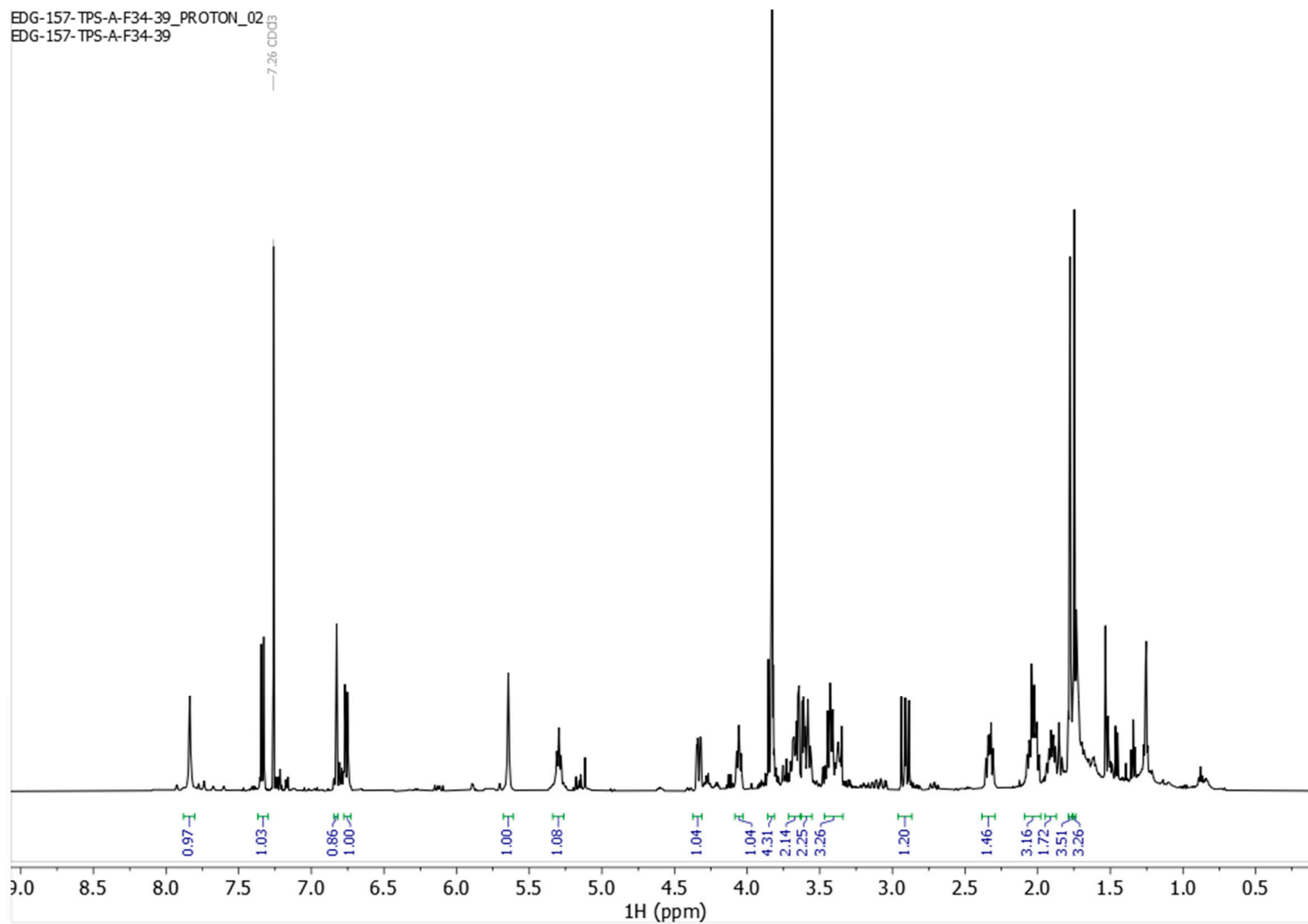


Segment	Position	TPS-24		TPS-25		TPS-46	
		$\delta C$ , type	$\delta H$	$\delta C$ , type	$\delta H$	$\delta C$ , type	$\delta H$
L-Trp	NH		5.62 (s)		5.5 (s)		5.6 (s)
	$\alpha$	54.5, CH	4.35 (dd)	54.7, CH	4.36 (dd)	54.5, CH	4.34 (dd)
	$\beta$	25.7, CH <sub>2</sub>	2.92 (m), 3.66 (m)	25.7, CH <sub>2</sub>	2.93 (dd), 3.64 (m)	25.7, CH <sub>2</sub>	2.91 (m), 3.65 (m)
	1		7.95 (s)		7.83 (s)		7.87 (s)
	2	135.9, C				136.0, C	
	3	104.4, C				104.4, C	
	3a	126.5, C				126.3, C	
	4	117.5, CH	7.40 (d)	117.8, CH	7.39 (d)	117.6, CH	7.36 (d)
	5	120.9, CH	6.99 (d)	121.1, CH	6.99 (dd)	121.3, CH	6.95 (d)
	6	134.1, C				135.7, C	
	7	110.5, CH	7.17 (s)	110.3, CH	7.17 (s)	110.9, CH	7.06 (s)
7a	135.9, C						
C=O							
Proline	$\alpha$	59.2, CH	4.04 (t)	59.3, CH	4.05 (t)	59.3, CH	4.04 (t)
	$\beta$	28.4, CH <sub>2</sub>	2.02 (m), 2.32 (dt)	28.4, CH <sub>2</sub>	2.03 (m), 2.33 (dt)	28.3, CH <sub>2</sub>	2.02 (m), 2.32 (dt)
	$\gamma$	22.8, CH <sub>2</sub>	1.89 (m), 2.02 (m)	22.7, CH <sub>2</sub>	1.91 (m), 2.03 (m)	22.6, CH <sub>2</sub>	1.90 (m), 2.02 (m)
	$\delta$	45.4, CH <sub>2</sub>	3.58 (m), 3.66 (m)	45.5, CH <sub>2</sub>	3.58 (m), 3.67 (m)	45.4, CH <sub>2</sub>	3.58 (m), 3.66 (m)
	C=O	165.9, C				165.9, C	
C2-Prenyl	1'	25.2, CH <sub>2</sub>	3.44 (qd)	25.2, CH <sub>2</sub>	3.45 (m)	25.0, CH <sub>2</sub>	3.43 (qd)
	2'	119.6, CH	5.29 (t)	119.9, CH	5.29 (t)	119.8, CH	5.26 (t)
	3'	135.4, C				135.4, C	
	4'	18.0, CH <sub>3</sub>	1.73 (s)	18.0, CH <sub>3</sub>	1.74 (s)	17.9, C	1.72 (s)
	5'	25.8, CH <sub>3</sub>	1.76 (s)	25.8, CH <sub>3</sub>	1.78 (s)	25.7	1.76 (s)
C6-Sub	1''	39.5, CH <sub>2</sub>	3.62 (d)	35.2, CH <sub>2</sub>	3.65 (d)	41.7, CH <sub>2</sub>	4.02 (s)
	2''	130.2, CH	6.44 (m)	127.4, CH	6.02 (t)	128.9, CH	7.08 (s)
	3''	129.2, CH	6.4 (m)			128.9, CH	7.08 (s)
	4''	137.7, C		125.8, CH	7.41 (m)	21.0, CH <sub>3</sub>	2.30 (s)
	5''	125.9, CH	7.35 (d)	128.2, CH	7.29 (t)	128.9, CH	7.08 (s)
	6''	128.2, CH	7.27 (t)	126.7, CH	7.21 (t)	128.9, CH	7.08 (s)
	7''	126.8, CH	7.18 (t)	128.2, CH	7.29 (t)		
	8''	128.2, CH	7.27 (t)	125.8, CH	7.41 (m)		
	9''	125.9, CH	7.35 (d)	16.0, CH <sub>3</sub>	2.16 (s)		

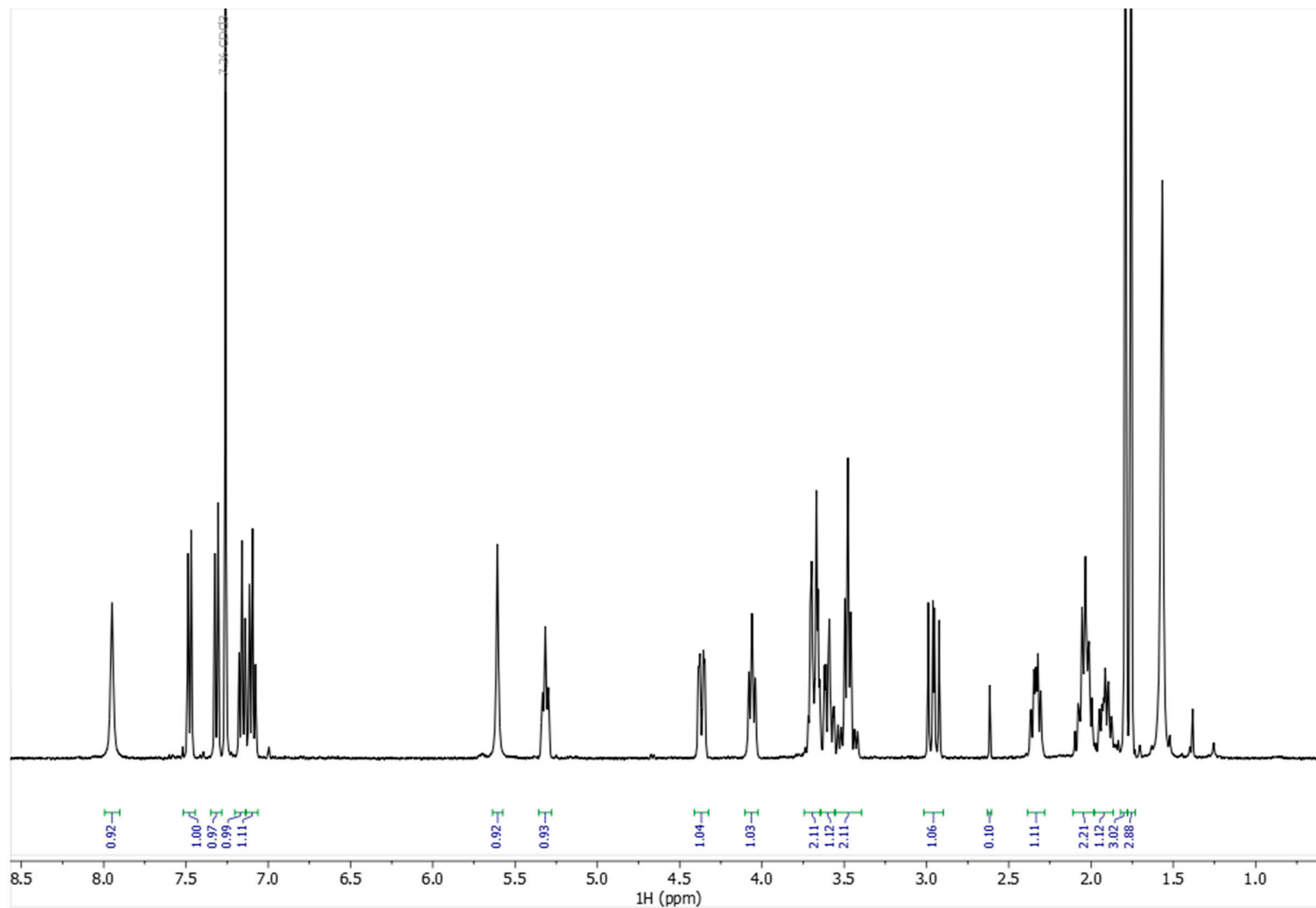


Segment	Position	TPS-52		TPS-58		TPS-61	TPS-62	$\delta C$ , type
		$\delta C$ , type	$\delta H$	$\delta C$ , type	$\delta H$	$\delta H$	$\delta H$	
L-Trp	NH		5.62 (s)			5.60 (s)	5.61 (s)	
	$\alpha$	54.5, CH	4.33 (dd)	54.5, CH	4.33 (dd)	4.35 (d)	4.36 (dd)	54.6, CH
	$\beta$	25.0, CH <sub>2</sub>	2.91 (dd), 3.64 (m)	25.7, CH <sub>2</sub>	2.91 (m), 3.63 (d)	2.93 (m), 3.66 (m)	2.93 (dd), 3.63 (m)	25.5, CH <sub>2</sub>
	1		7.82 (s)		7.91 (s)	7.86 (s)	7.85 (s)	
	2			136.0, C				
	3			104.5, C				
	3a			126.3, C				
	4	117.7, CH	7.37 (d)	117.5, CH	7.37 (d)	7.40 (d)	7.40 (d)	117.7, CH
	5	121.2, CH	6.95 (d)	121.1, CH	6.93 (dd)	7.00 (d)	7.02 (dd)	121.1, CH
	6			135.6, C				
7	110.6, CH	7.05 (s)	110.7, CH	7.07 (s)	7.16 (s)	7.16 (s)	110.7, CH	
7a			136.1, C					
	C=O							
Proline	$\alpha$	59.7, CH	4.04 (t)	59.3, CH	4.04 (t)	4.05 (s)	4.05 (t)	59.3, CH
	$\beta$	28.5, CH <sub>2</sub>	2.03 (m), 2.32 (m)	28.4, CH <sub>2</sub>	2.02 (m), 2.32 (dt)	2.04 (m), 2.33 (m)	2.03 (m), 2.31 (m)	28.5, CH <sub>2</sub>
	$\gamma$	22.6, CH <sub>2</sub>	1.90 (m), 2.03 (m)	22.7, CH <sub>2</sub>	1.89 (m), 2.02 (m)	1.90 (m), 2.33 (m)	1.89 (m), 2.01 (m)	22.8, CH <sub>2</sub>
	$\delta$	45.4, CH <sub>2</sub>	3.58 (m), 3.66 (m)	45.4, CH <sub>2</sub>	3.58 (m), 3.66 (m)	3.60 (m), 3.66 (m)	3.58 (t), 3.67 (m)	45.4, CH <sub>2</sub>
		C=O			165.9, C			
C2-Prenyl	1'	25.2, CH <sub>2</sub>	3.43 (m)	25.2, CH <sub>2</sub>	3.43 (qd)	3.45 (m)	3.44 (t)	25.0, CH <sub>2</sub>
	2'	119.5, CH	5.28 (t)	119.8, CH	5.27 (m)	5.29 (t)	5.30 (t)	119.7, CH
	3'			135.5, C				
	4'	18.1, CH <sub>3</sub>	1.73 (s)	18.1, CH <sub>3</sub>	1.73 (s)	1.74 (s)	1.74 (s)	17.7, CH <sub>3</sub>
	5'	25.7, CH <sub>3</sub>	1.76 (s)	25.9, CH <sub>3</sub>	1.76 (s)	1.78 (s)	1.78 (s)	25.8, CH <sub>3</sub>
C6-Sub	1''	41.1, CH <sub>2</sub>	4.00 (s)	41.8, CH	3.96 (s)	4.05 (s)	4.23 (s)	36.3, CH <sub>2</sub>
	2''	129.7, CH	7.11 (d)	109.2, CH	6.66 (m)	6.00 (d)	6.81 (dd)	125.1, CH
	3''	113.8, CH	6.81 (d)	145.7, C		6.28 (d)	6.92 (dd)	126.8, CH
	4''	113.8, CH	6.81 (d)	147.5, C		7.32 (s)	7.13 (dd)	123.9, CH
	5''	129.7, CH	7.11 (d)	108.0, CH	6.71 (d)			
	6''	55.2, CH <sub>3</sub>	3.77 (s)	121.5, CH	6.66 (m)			
	7''			100.8, CH <sub>2</sub>	5.88 (s)			

# NMR Spectra for Tryprostatin Analogs:

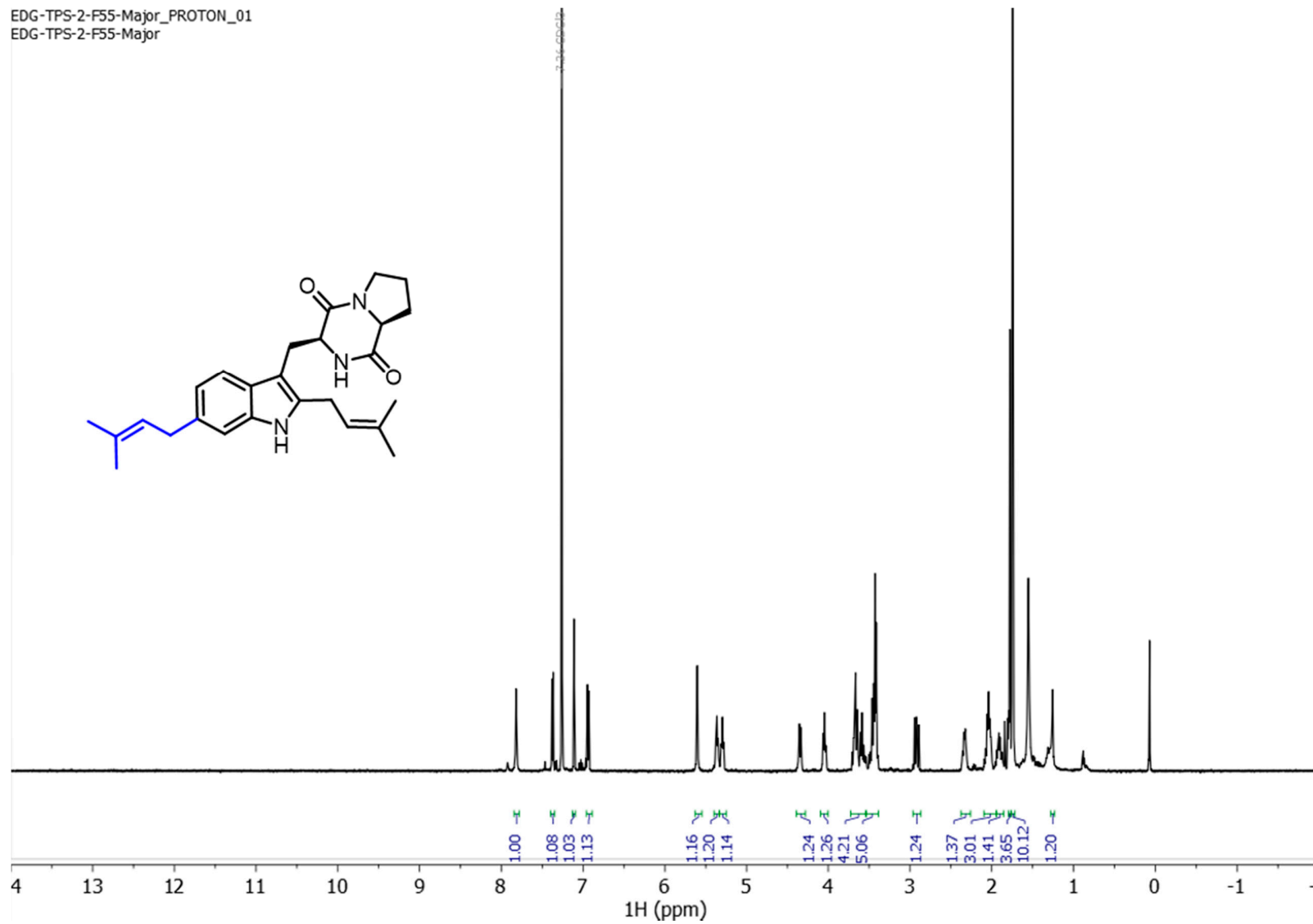


<sup>1</sup>H NMR spectrum (500 MHz) of TPS-A in CDCl<sub>3</sub>

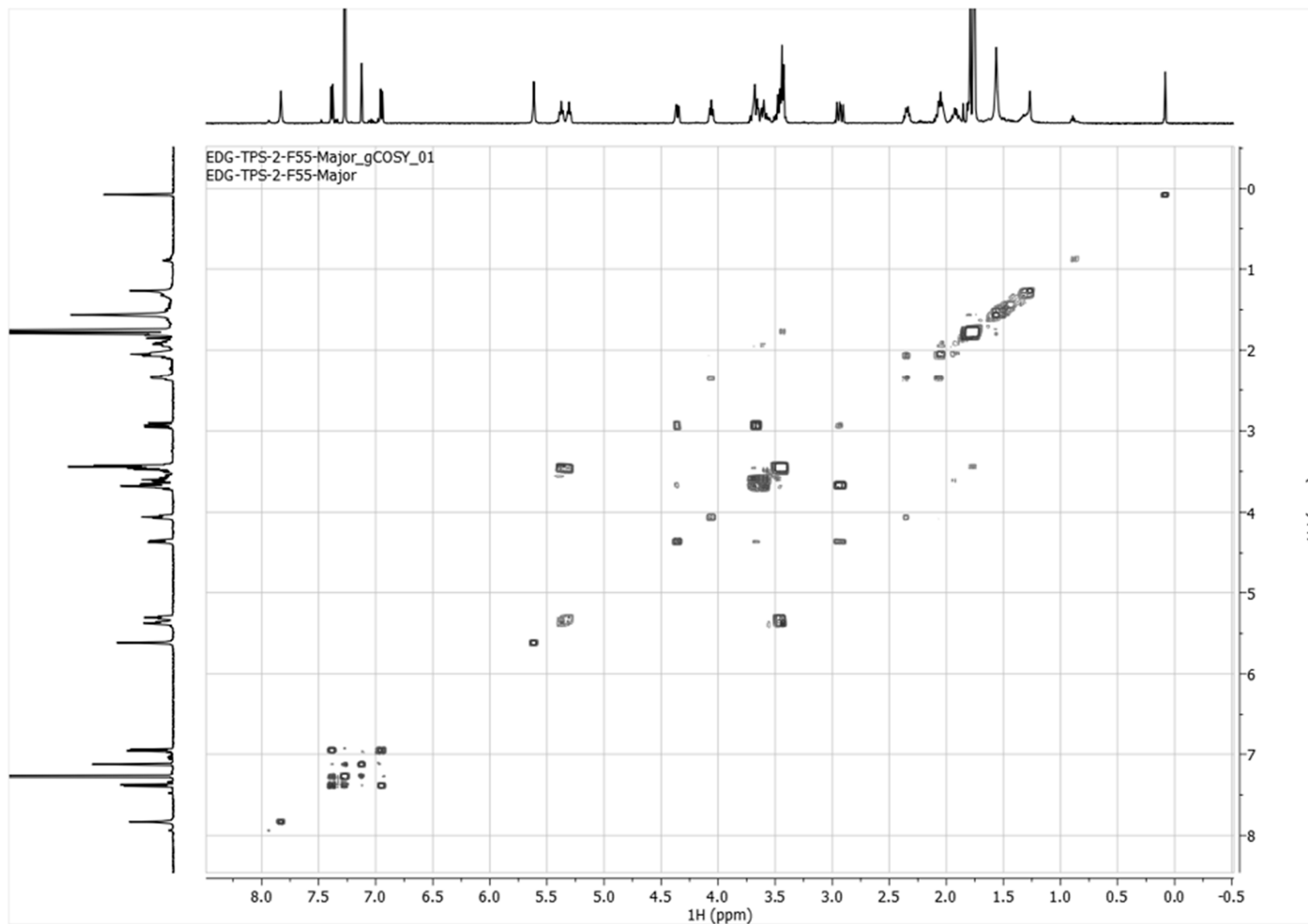


$^1\text{H}$  NMR spectrum (400 MHz) of **TPS-B** in  $\text{CDCl}_3$

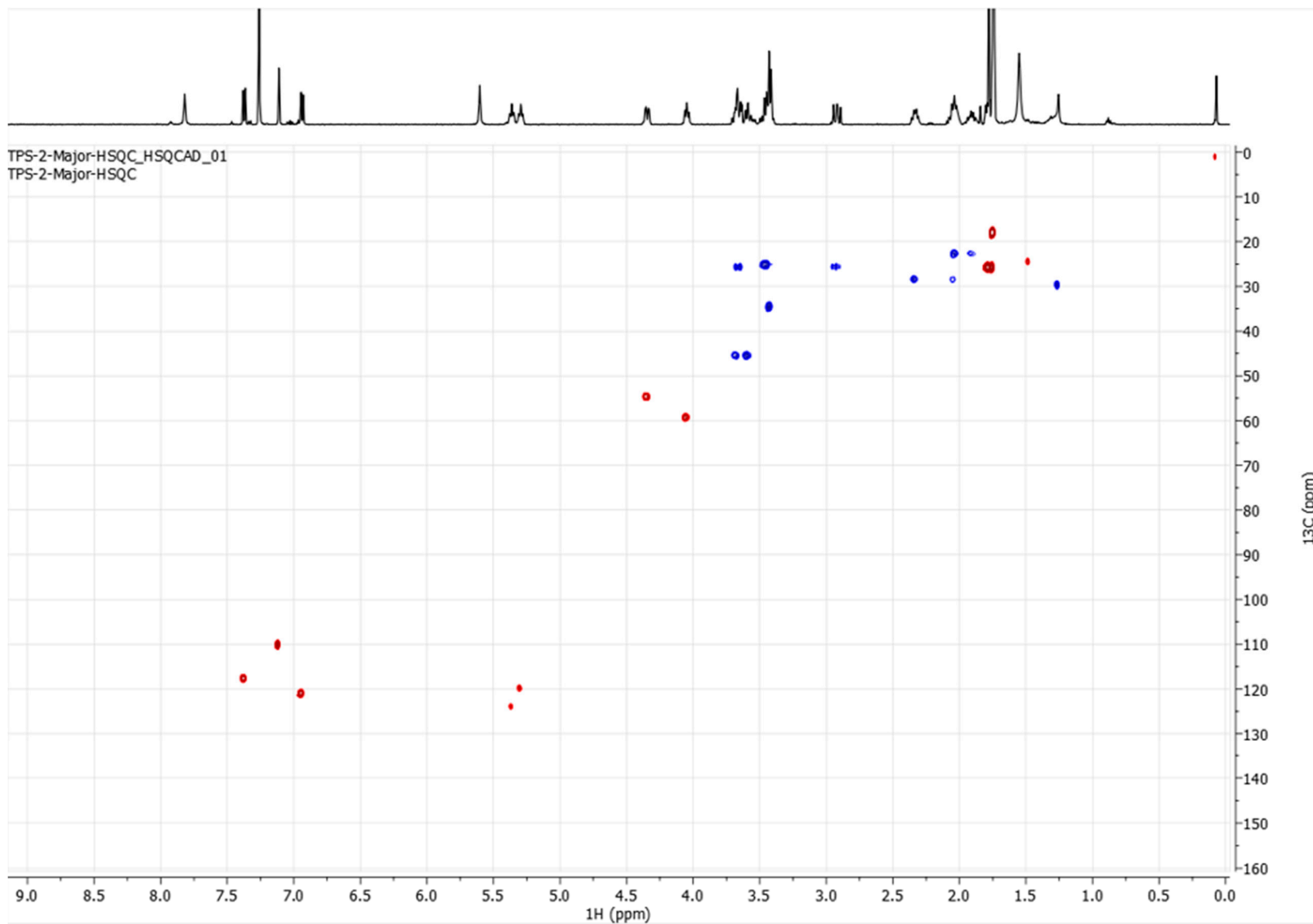
EDG-TPS-2-F55-Major\_PROTON\_01  
EDG-TPS-2-F55-Major



<sup>1</sup>H NMR spectrum (500 MHz) of TPS-2-C6 in CDCl<sub>3</sub>



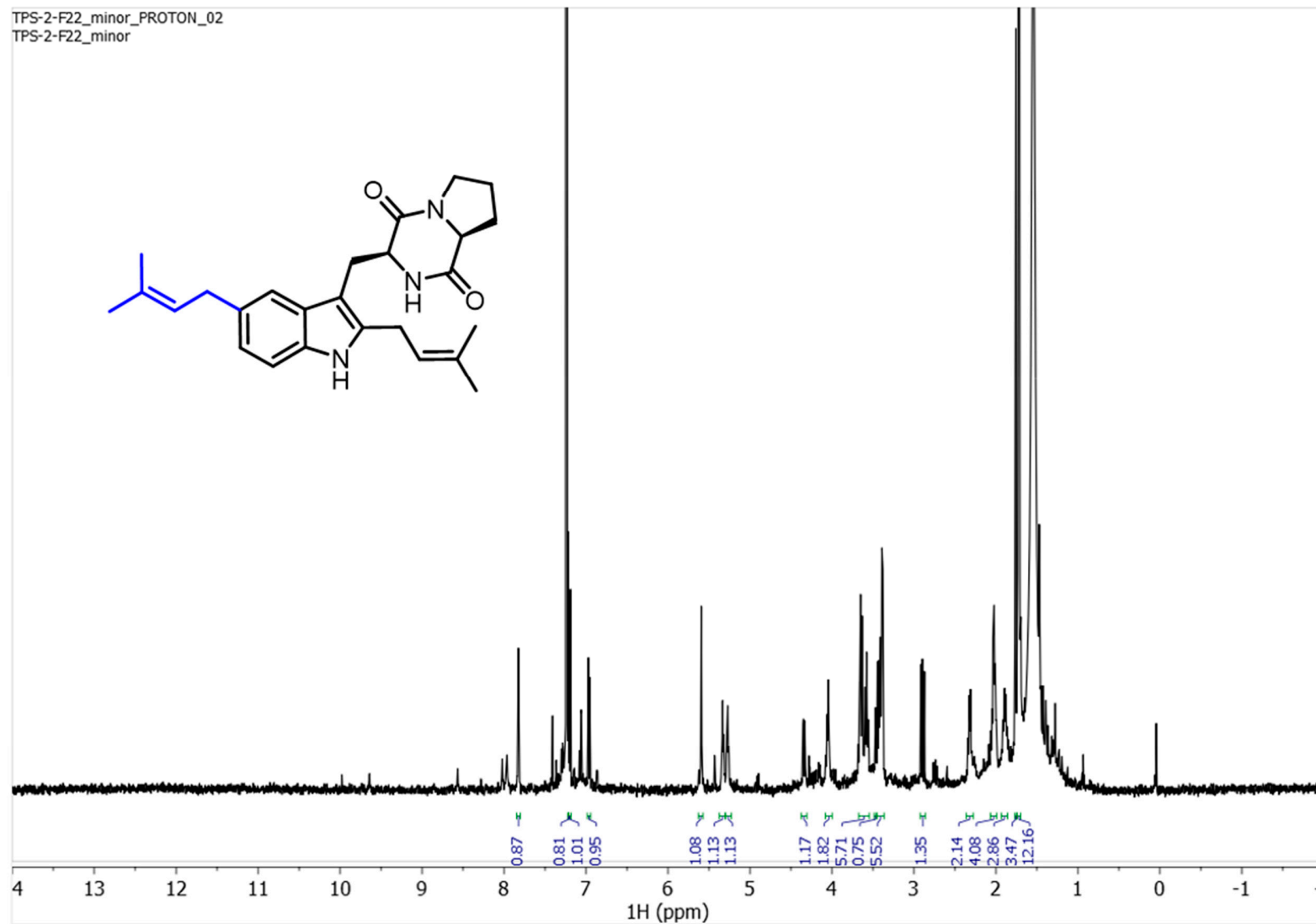
COSY NMR spectrum (500 MHz) of TPS-2-C6 in CDCl<sub>3</sub>



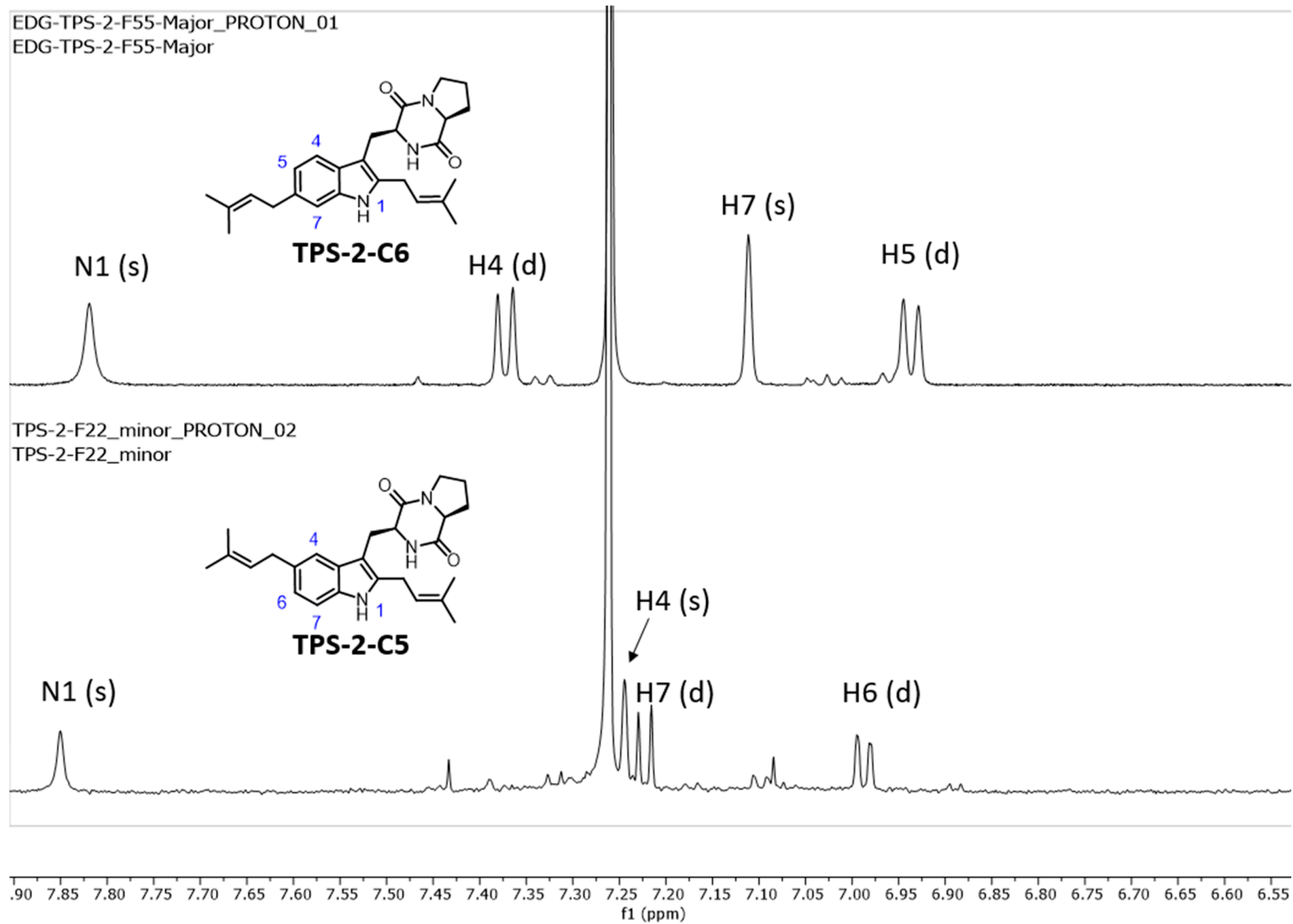
HSQC NMR spectrum (500 MHz) of TPS-2-C6 in  $\text{CDCl}_3$



TPS-2-F22\_minor\_PROTON\_02  
TPS-2-F22\_minor

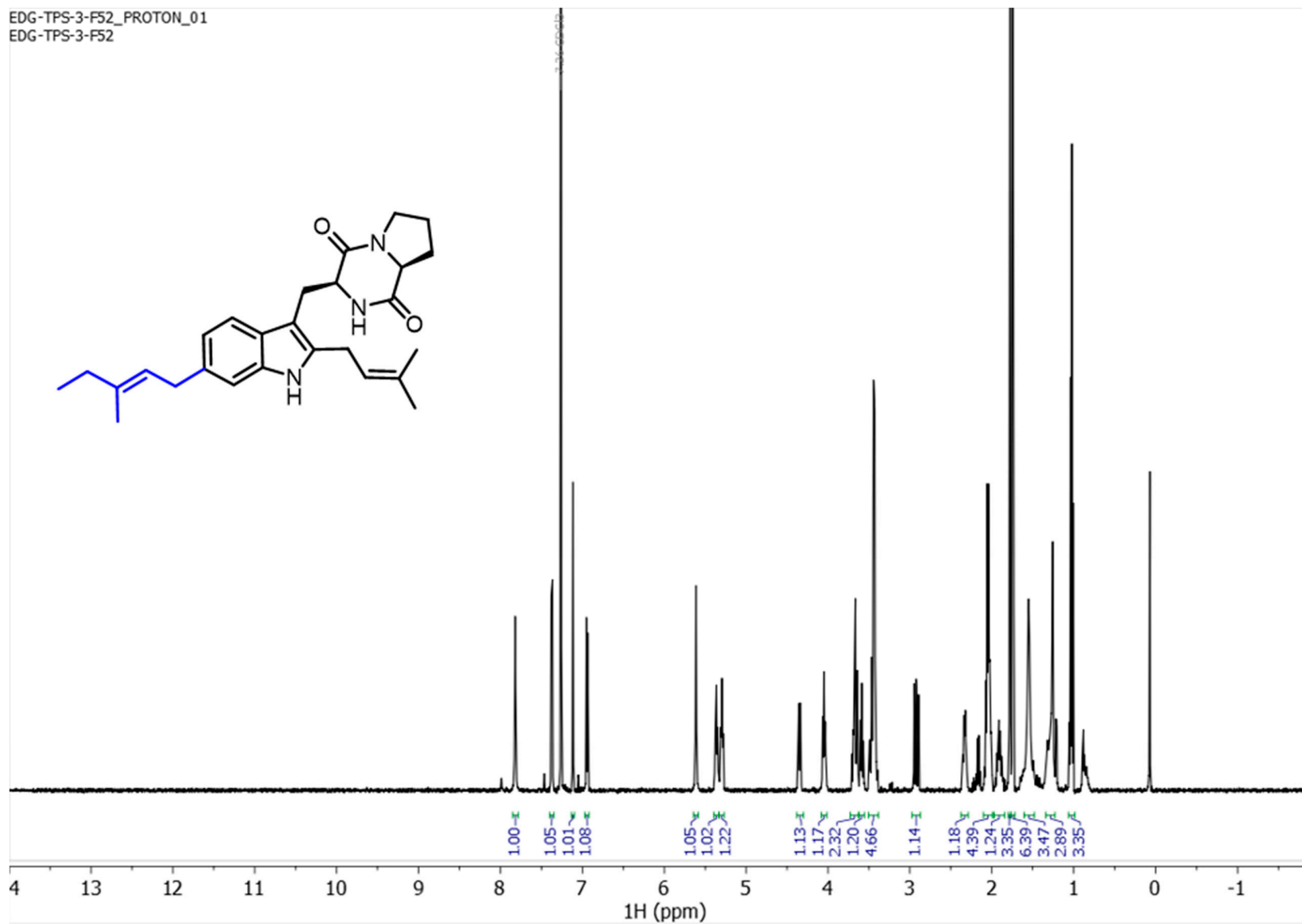


$^1\text{H}$  NMR spectrum (500 MHz) of TPS-2-C5 in  $\text{CDCl}_3$

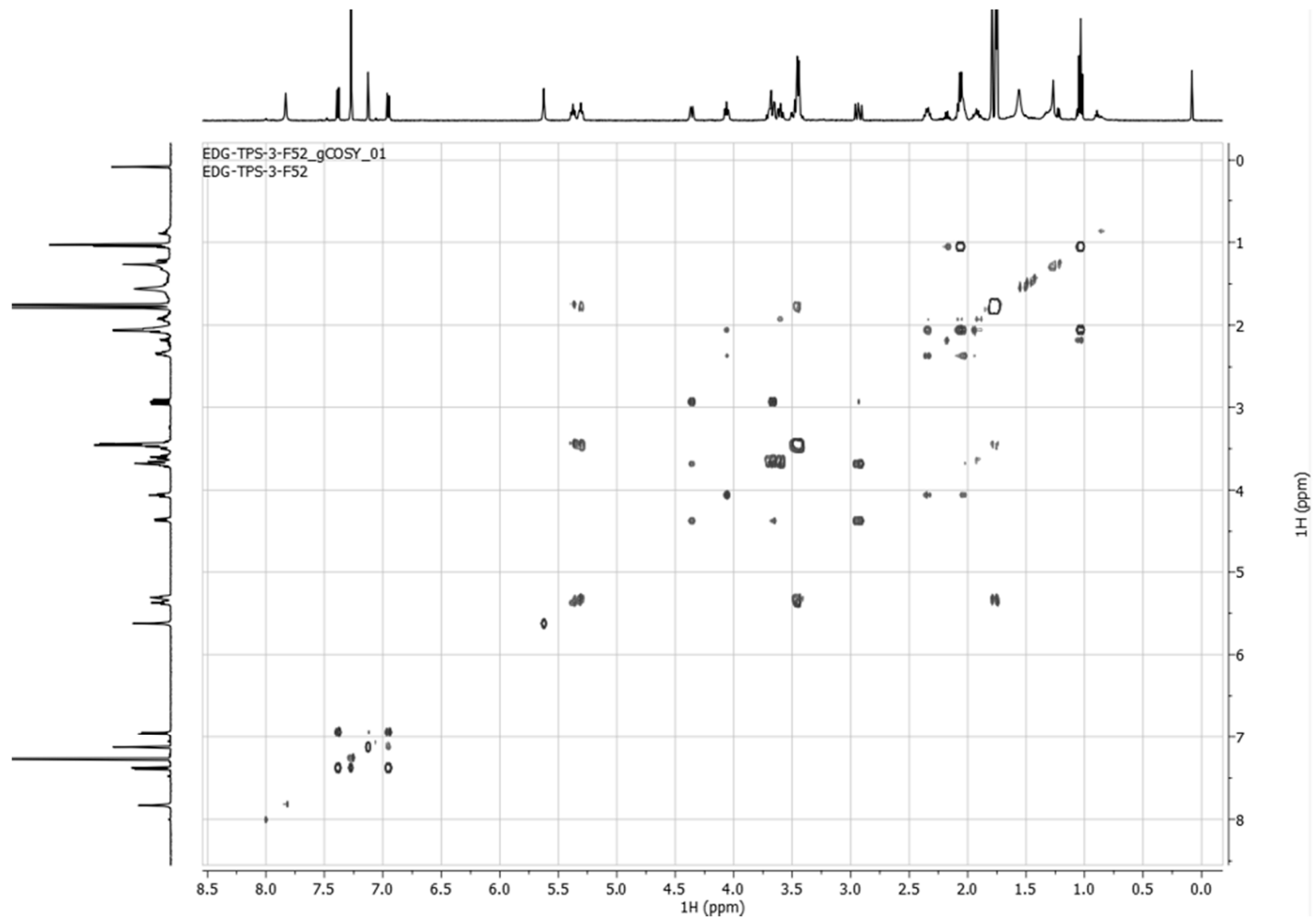


Stacked  $^1\text{H}$  NMR Spectra (500 MHz) of isolated **TPS-2** regioisomers in  $\text{CDCl}_3$

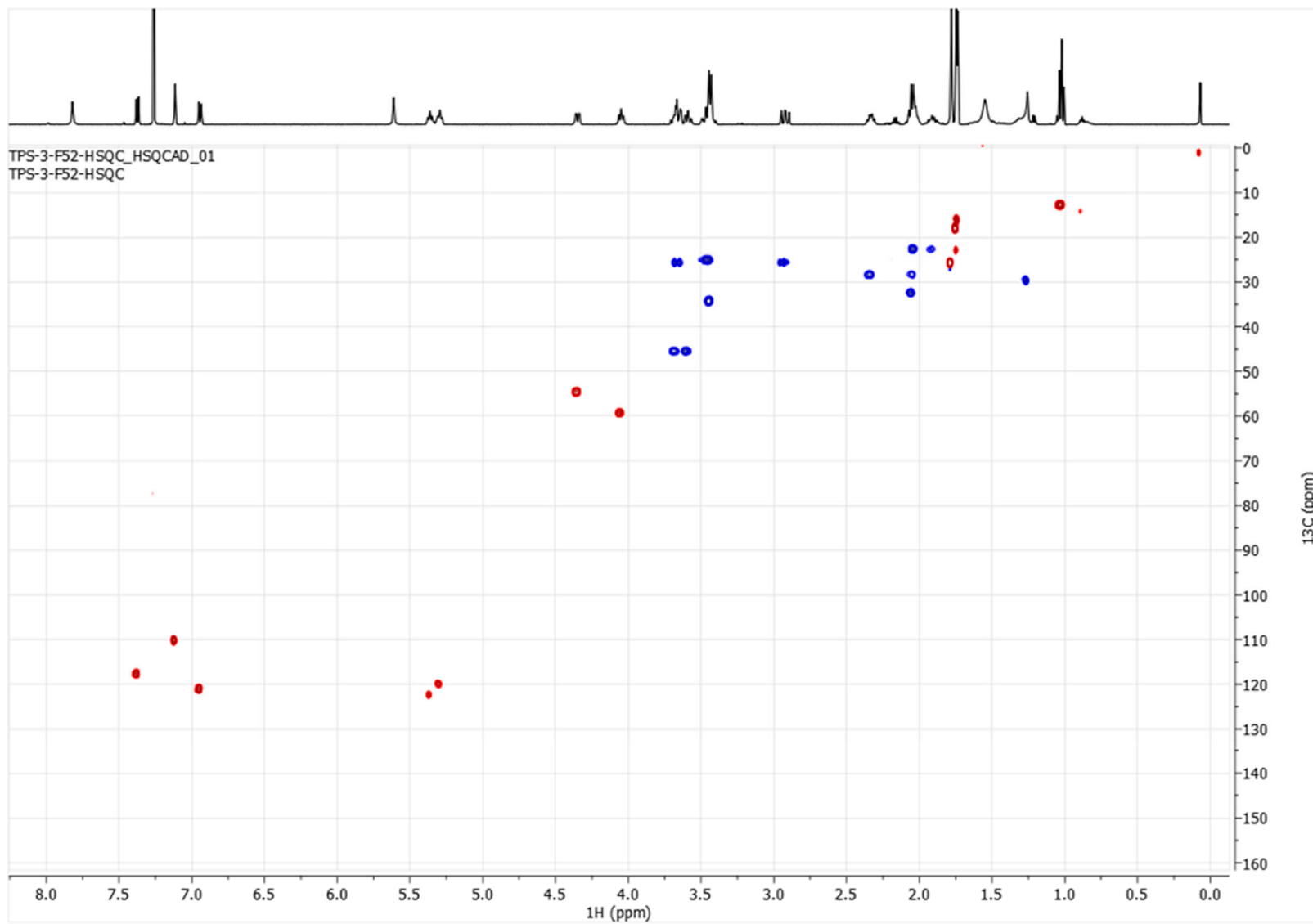
EDG-TPS-3-F52\_PROTON\_01  
EDG-TPS-3-F52



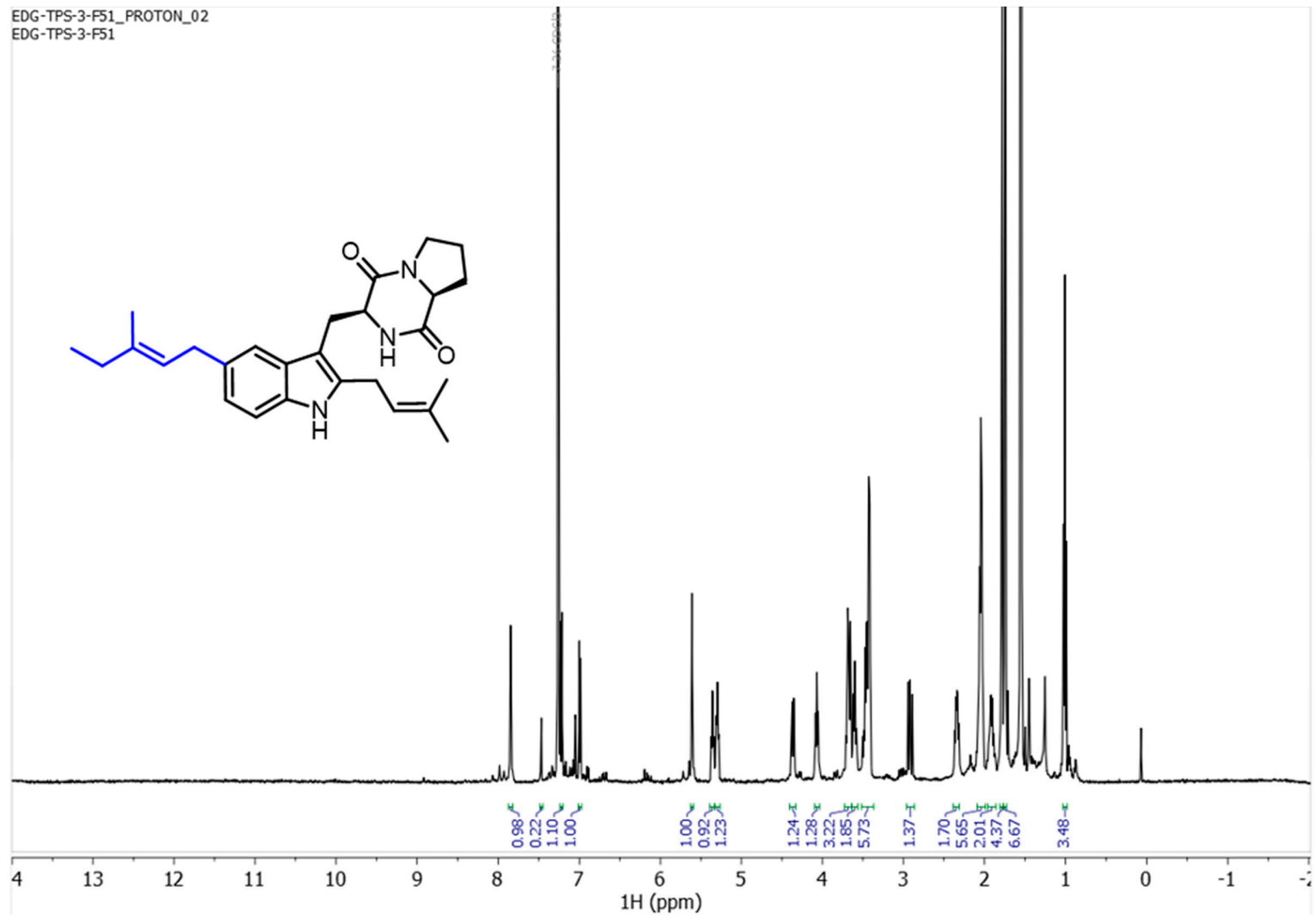
<sup>1</sup>H NMR spectrum (500 MHz) of TPS-7 in CDCl<sub>3</sub>



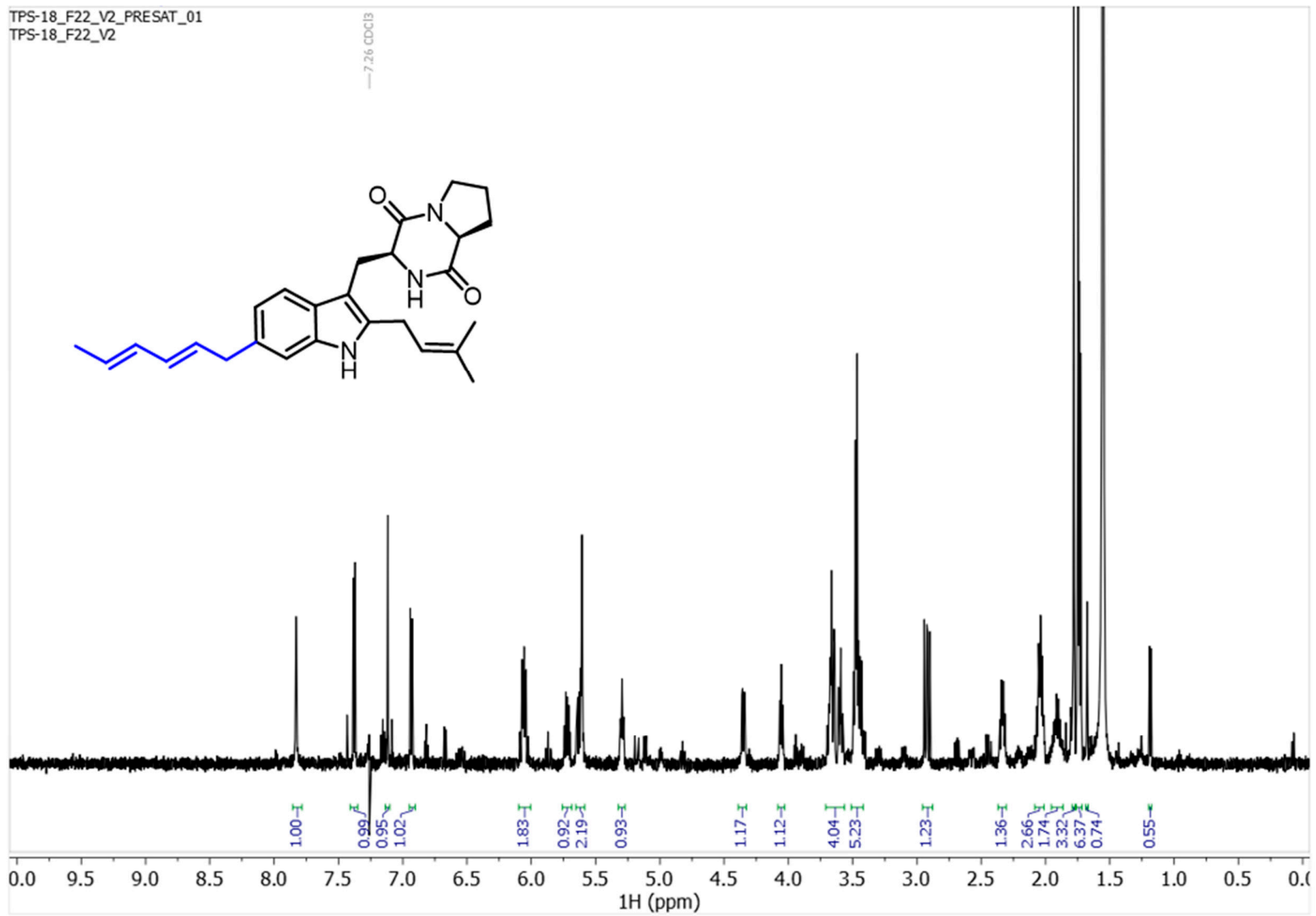
COSY spectrum (500 MHz) of **TPS-7** in  $\text{CDCl}_3$



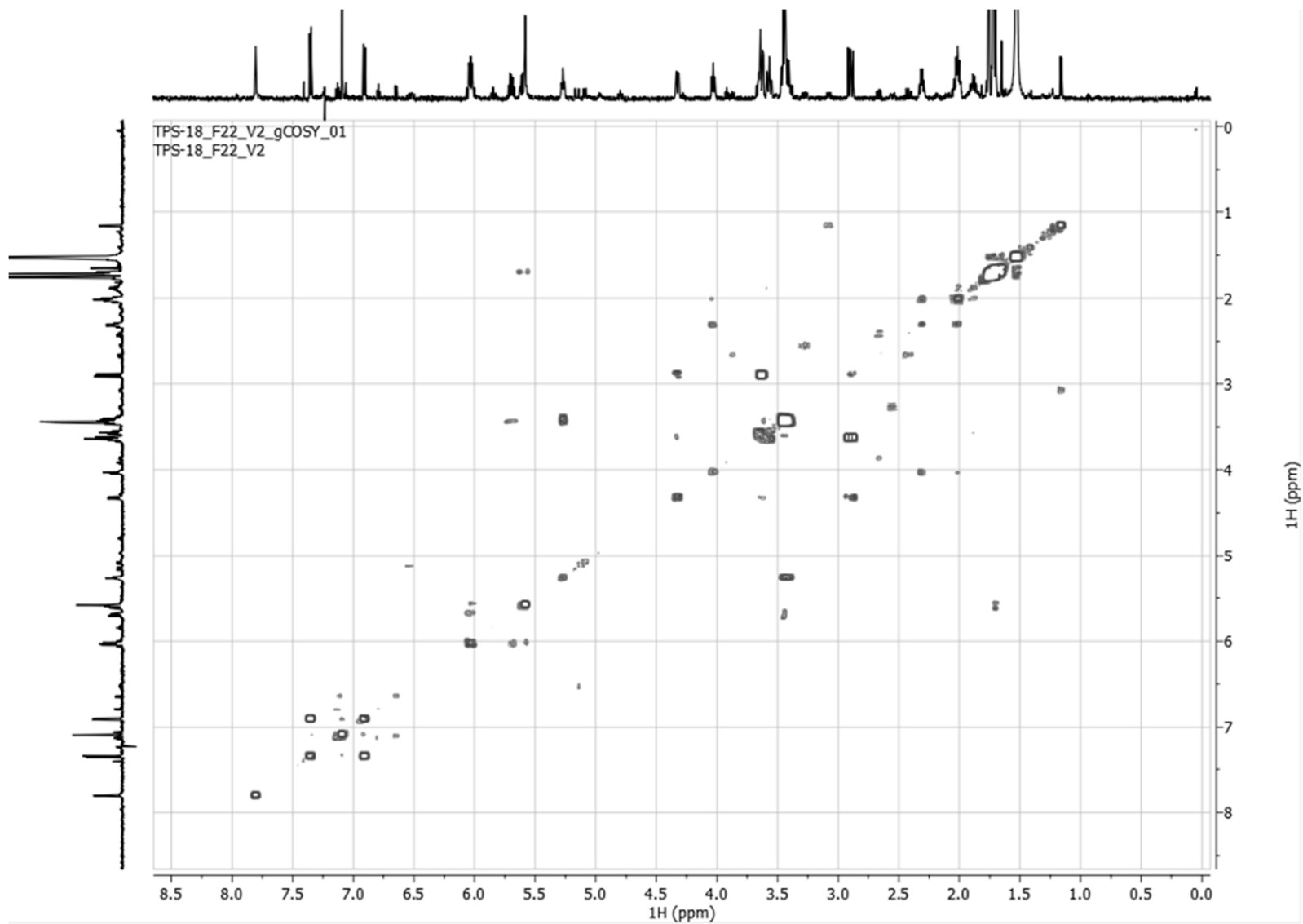
HSQC NMR spectrum (500 MHz) of **TPS-7** in  $\text{CDCl}_3$



<sup>1</sup>H NMR spectrum (500 MHz) of TPS-7-C5 (minor product) in CDCl<sub>3</sub>.

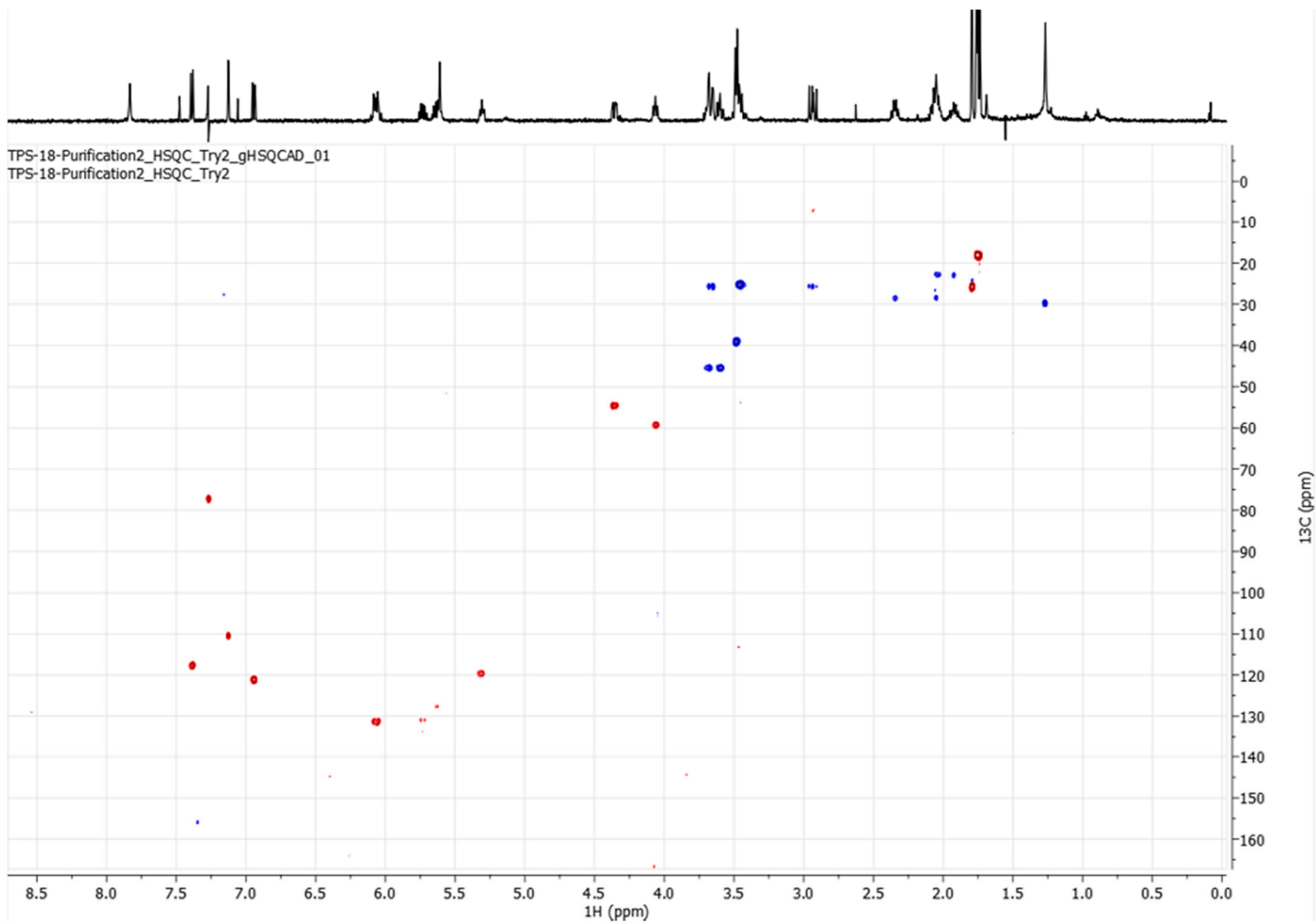


<sup>1</sup>H PRESAT NMR spectrum (500 MHz) of TPS-16 in CDCl<sub>3</sub>

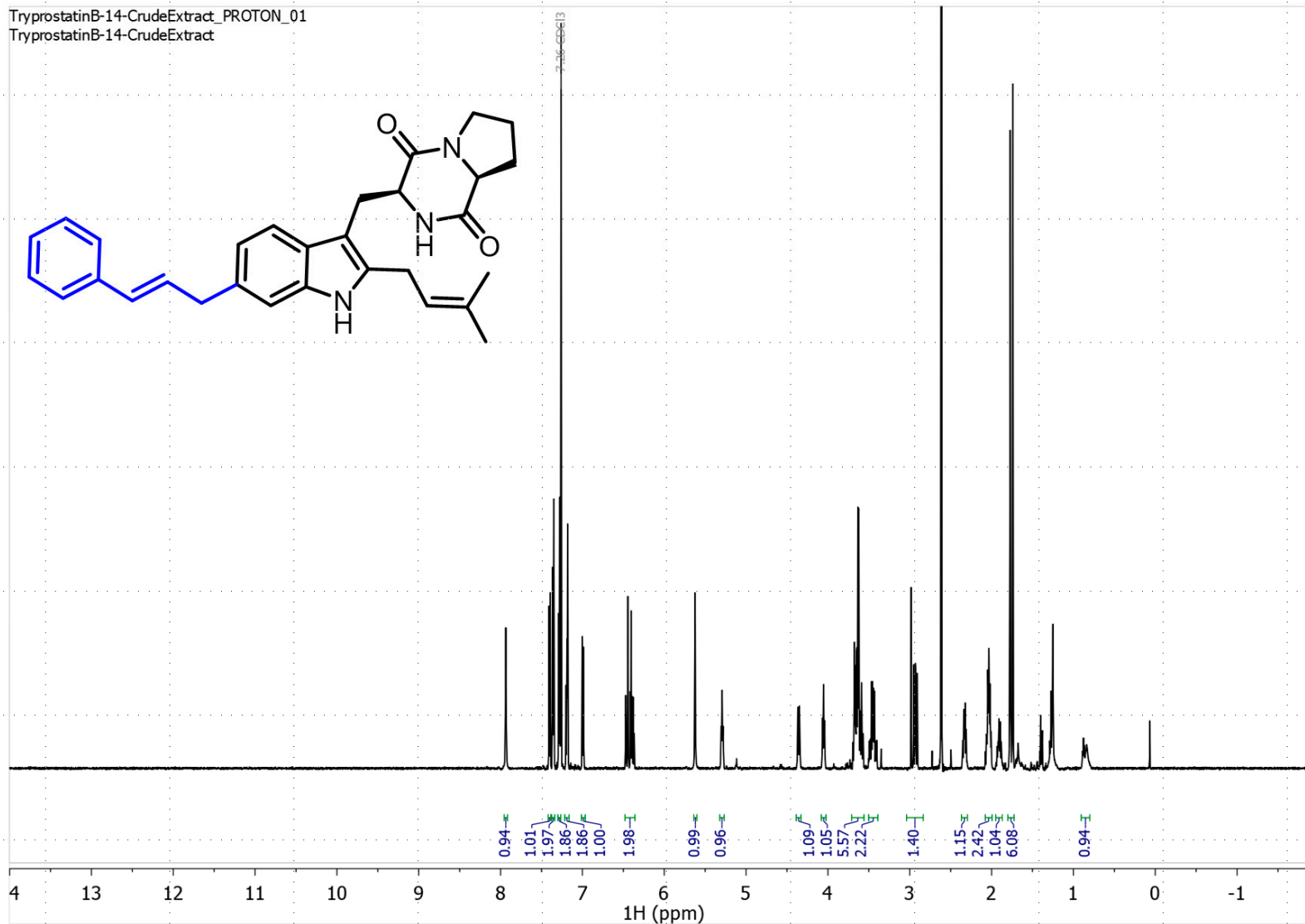


COSY NMR spectrum (500 MHz) of **TPS-16** in  $\text{CDCl}_3$

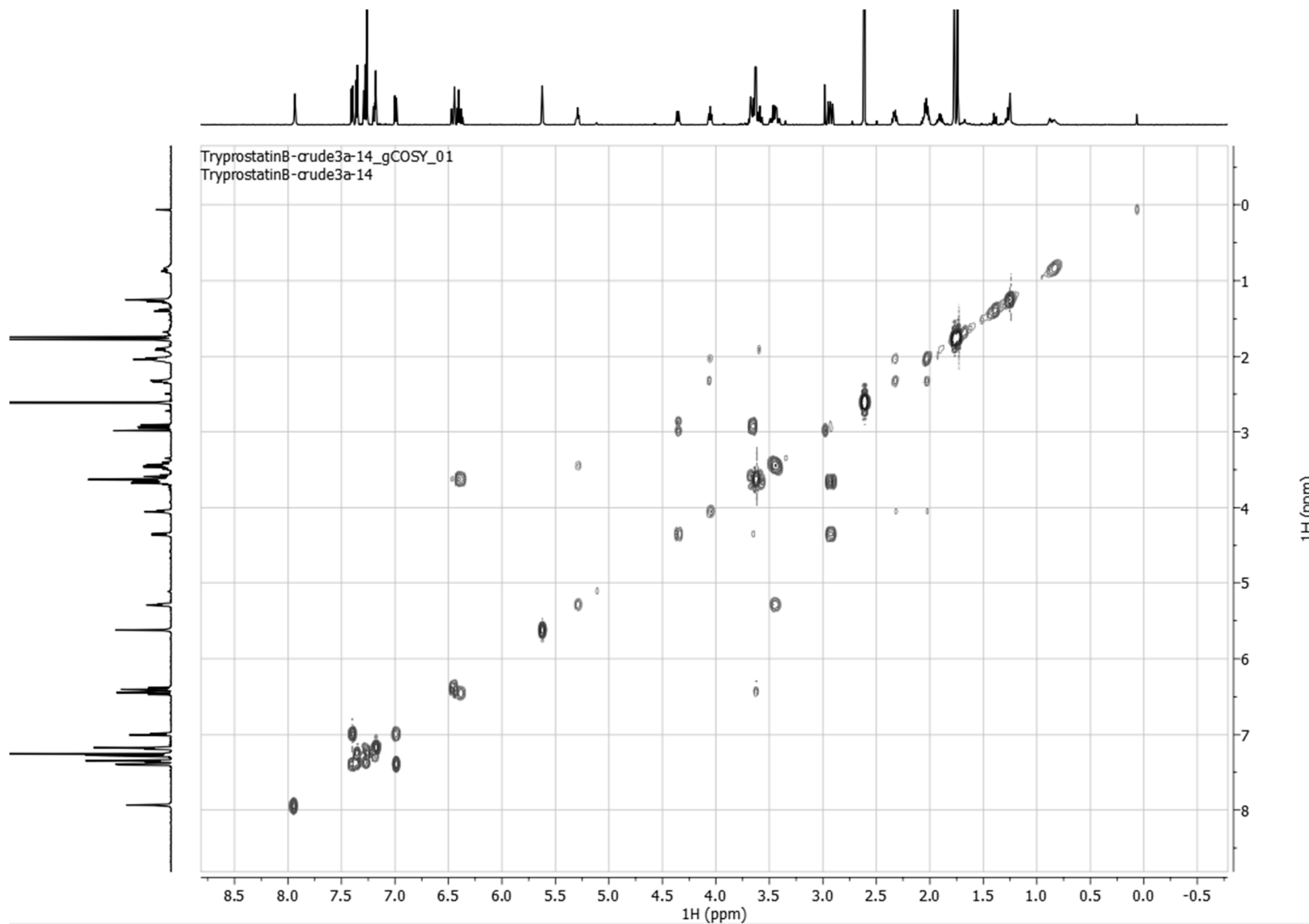




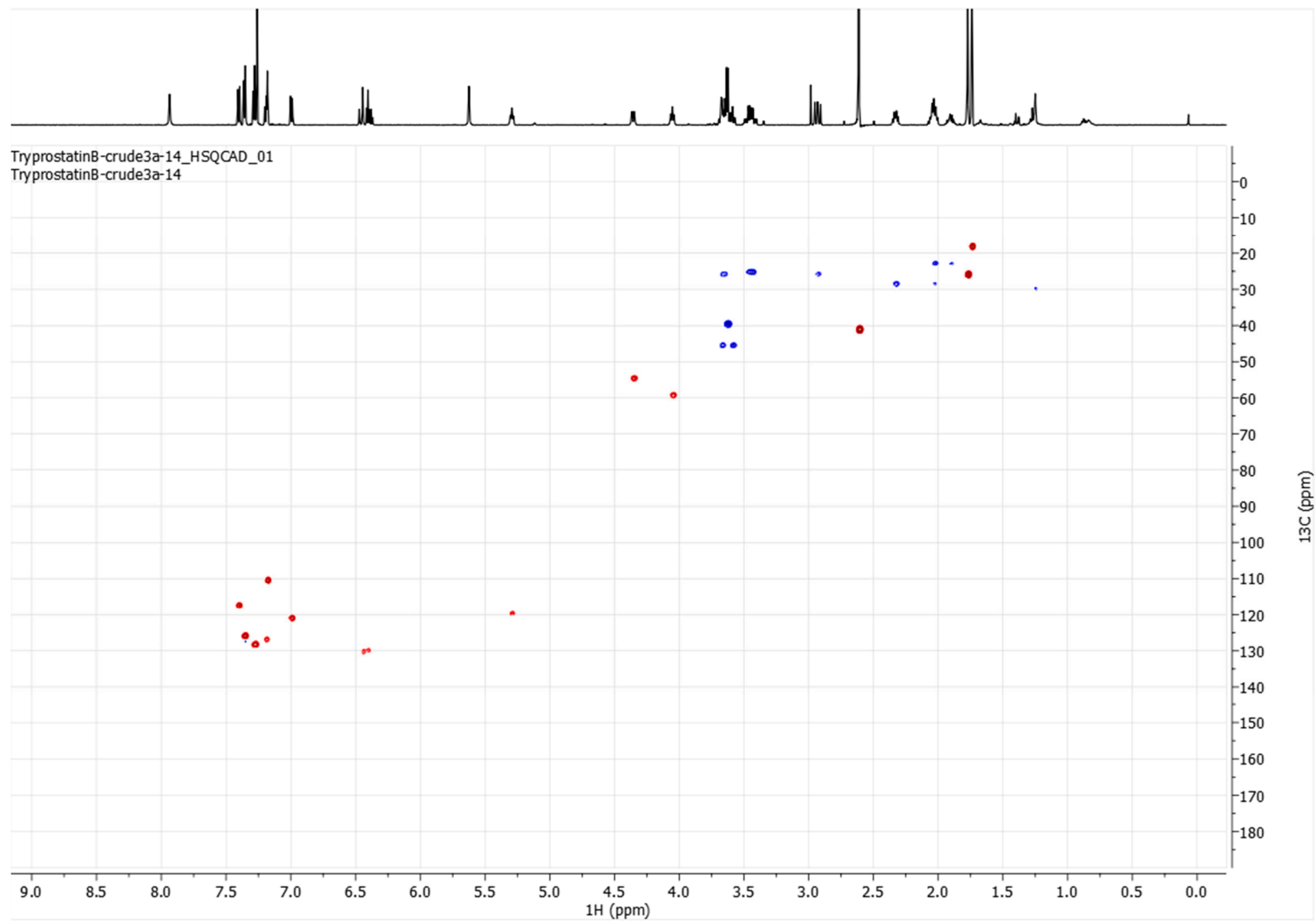
HSQC NMR spectrum (500 MHz) of **TPS-16** in  $\text{CDCl}_3$



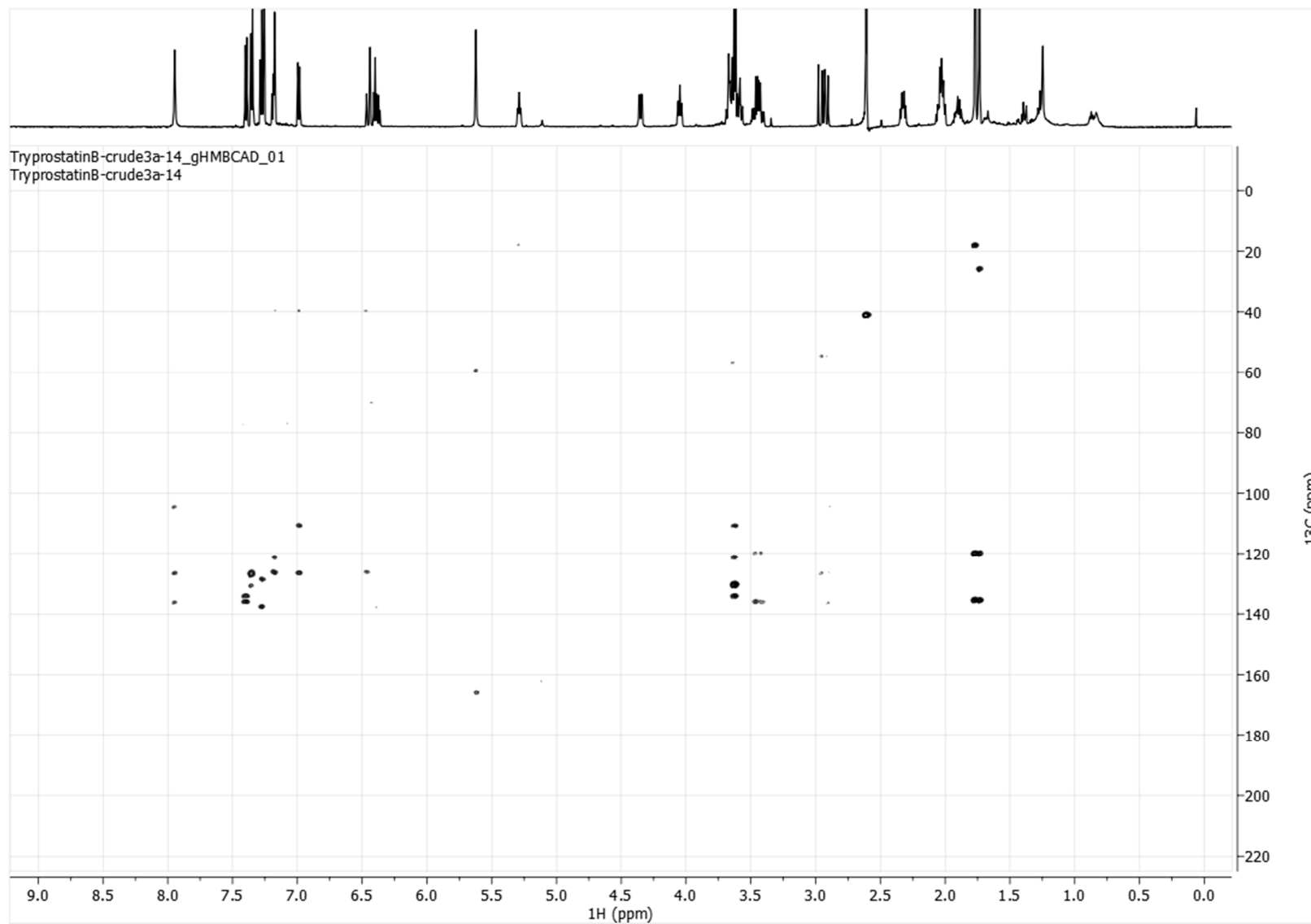
<sup>1</sup>H NMR spectrum (600 MHz) of TPS-24 in CDCl<sub>3</sub>



COSY NMR spectrum (600 MHz) of **TPS-24** in  $\text{CDCl}_3$

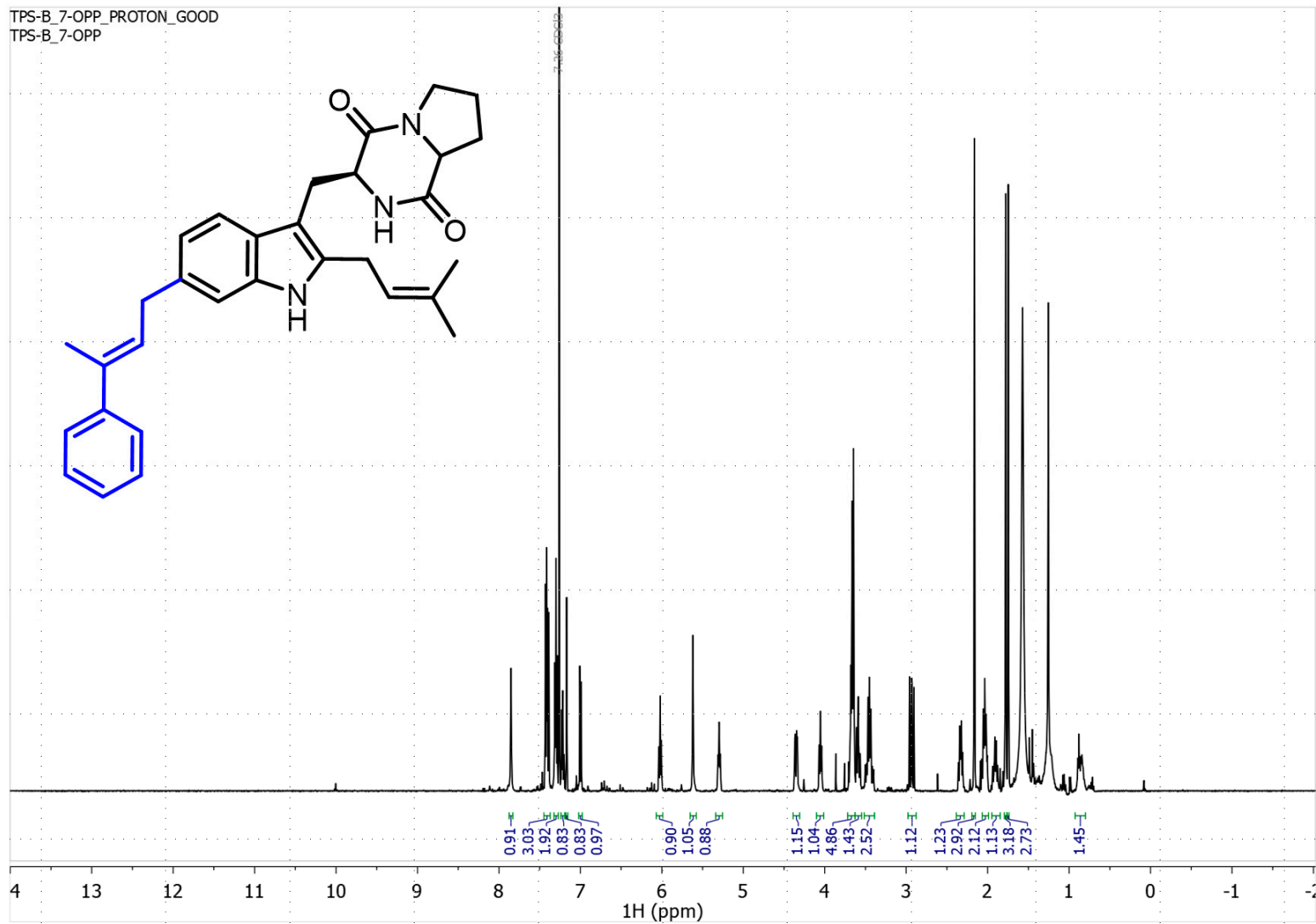


HSQC NMR spectrum (600 MHz) of **TPS-24** in CDCl<sub>3</sub>

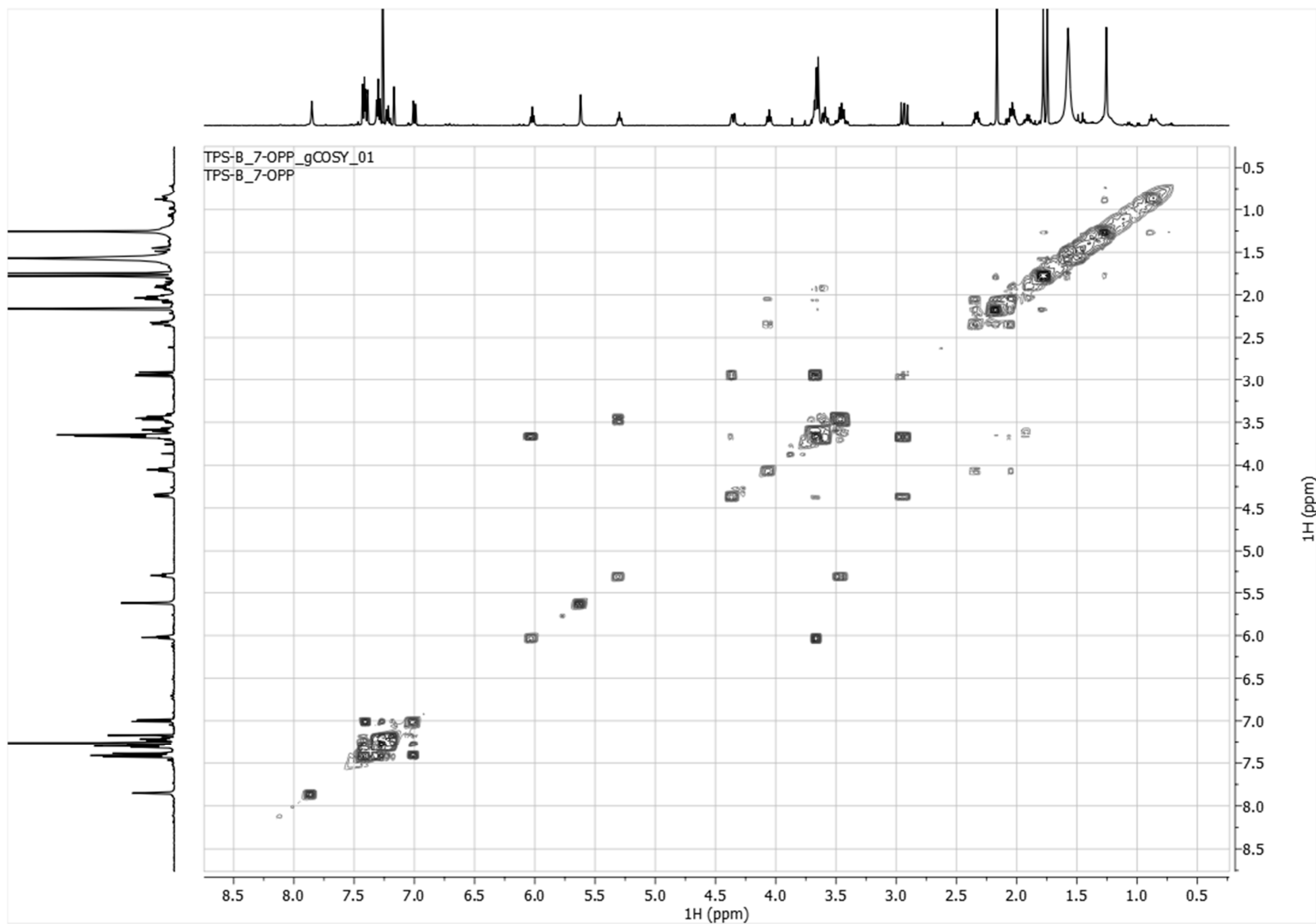


HMBC NMR spectrum (600 MHz) of **TPS-24** in  $\text{CDCl}_3$

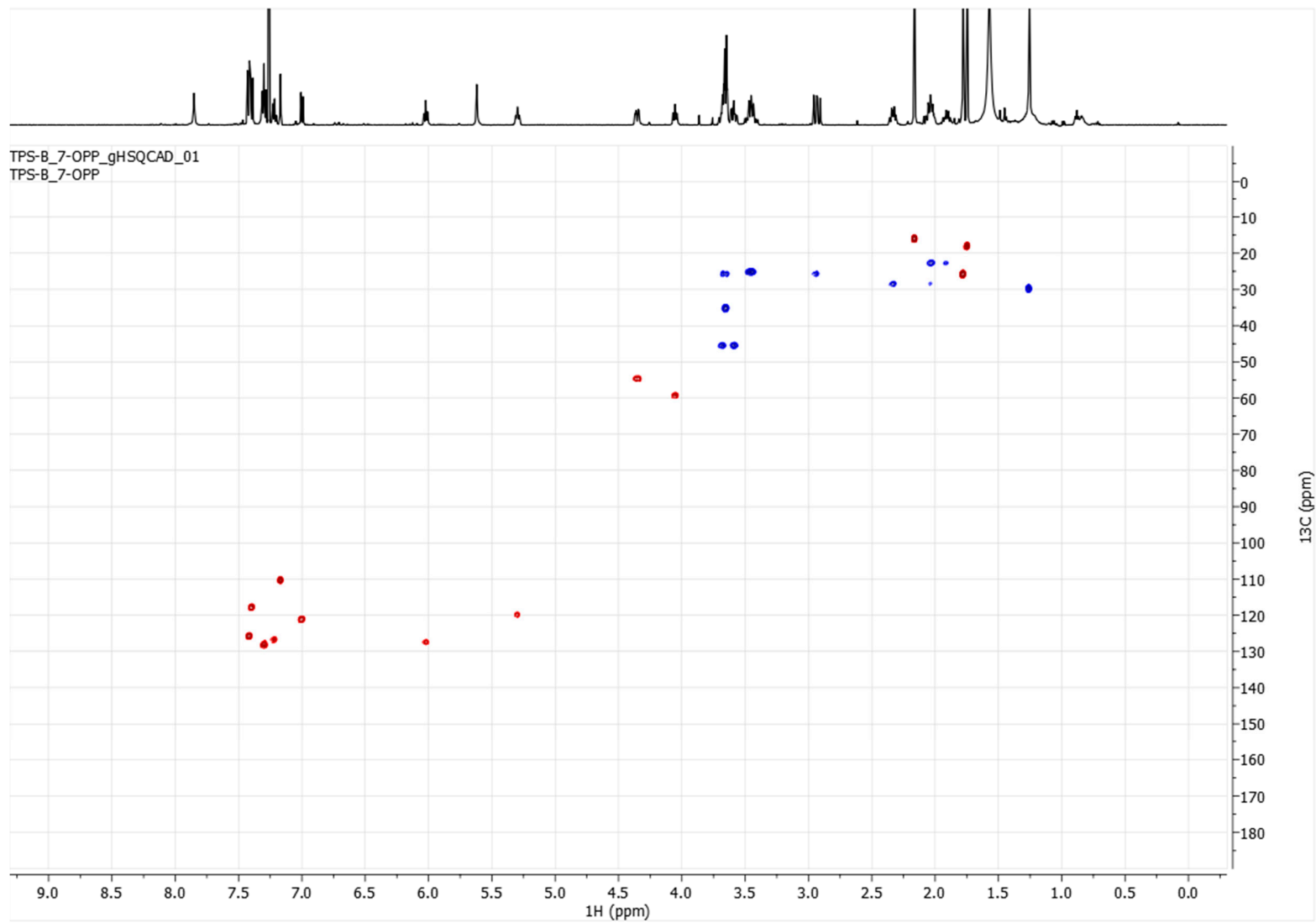
TPS-B\_7-OPP\_PROTON\_GOOD  
TPS-B\_7-OPP



<sup>1</sup>H NMR spectrum (500 MHz) of TPS-25 in CDCl<sub>3</sub>

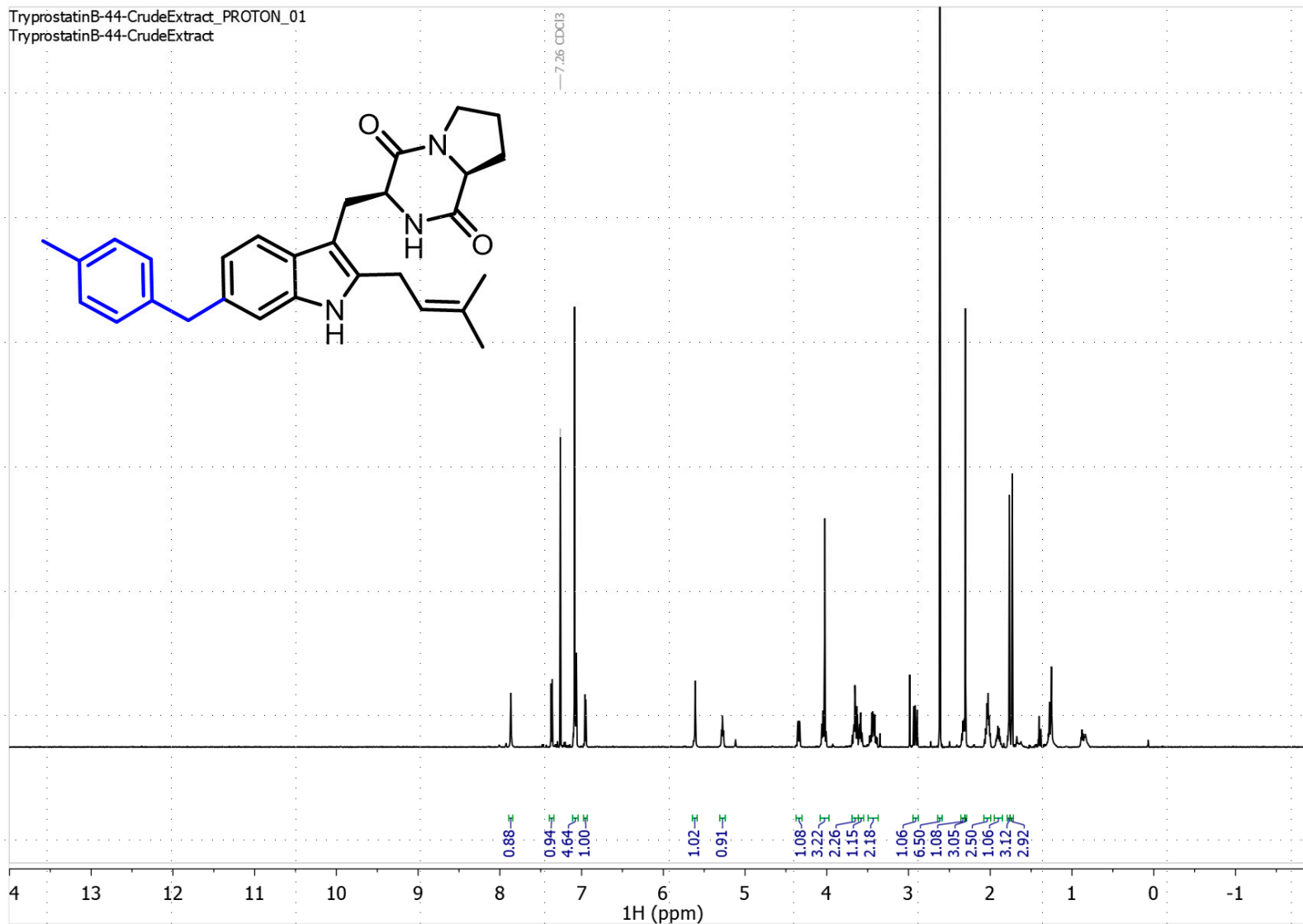


COSY NMR spectrum (500 MHz) of **TPS-25** in  $\text{CDCl}_3$

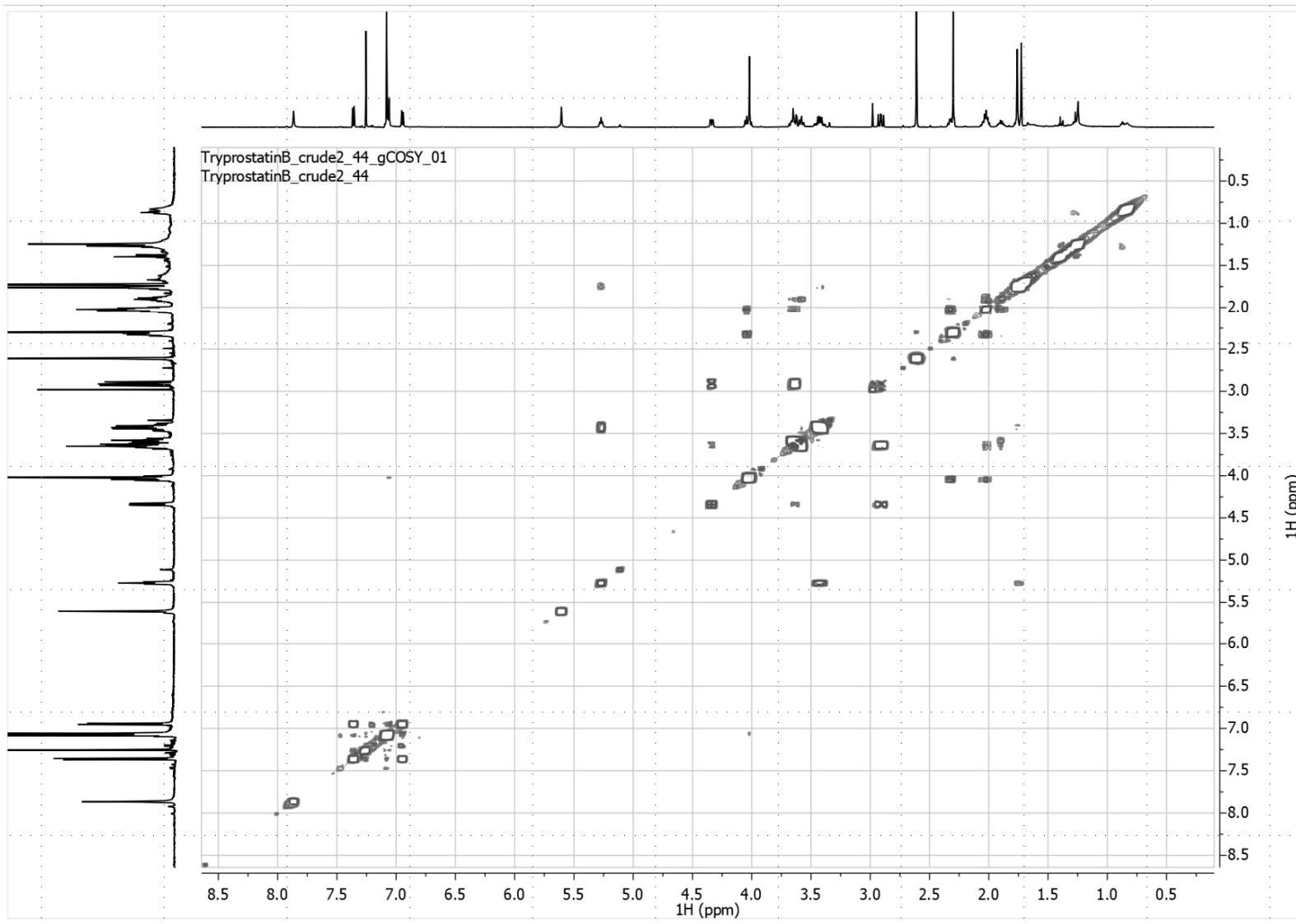


HSQC NMR spectrum (500 MHz) of **TPS-25** in  $\text{CDCl}_3$

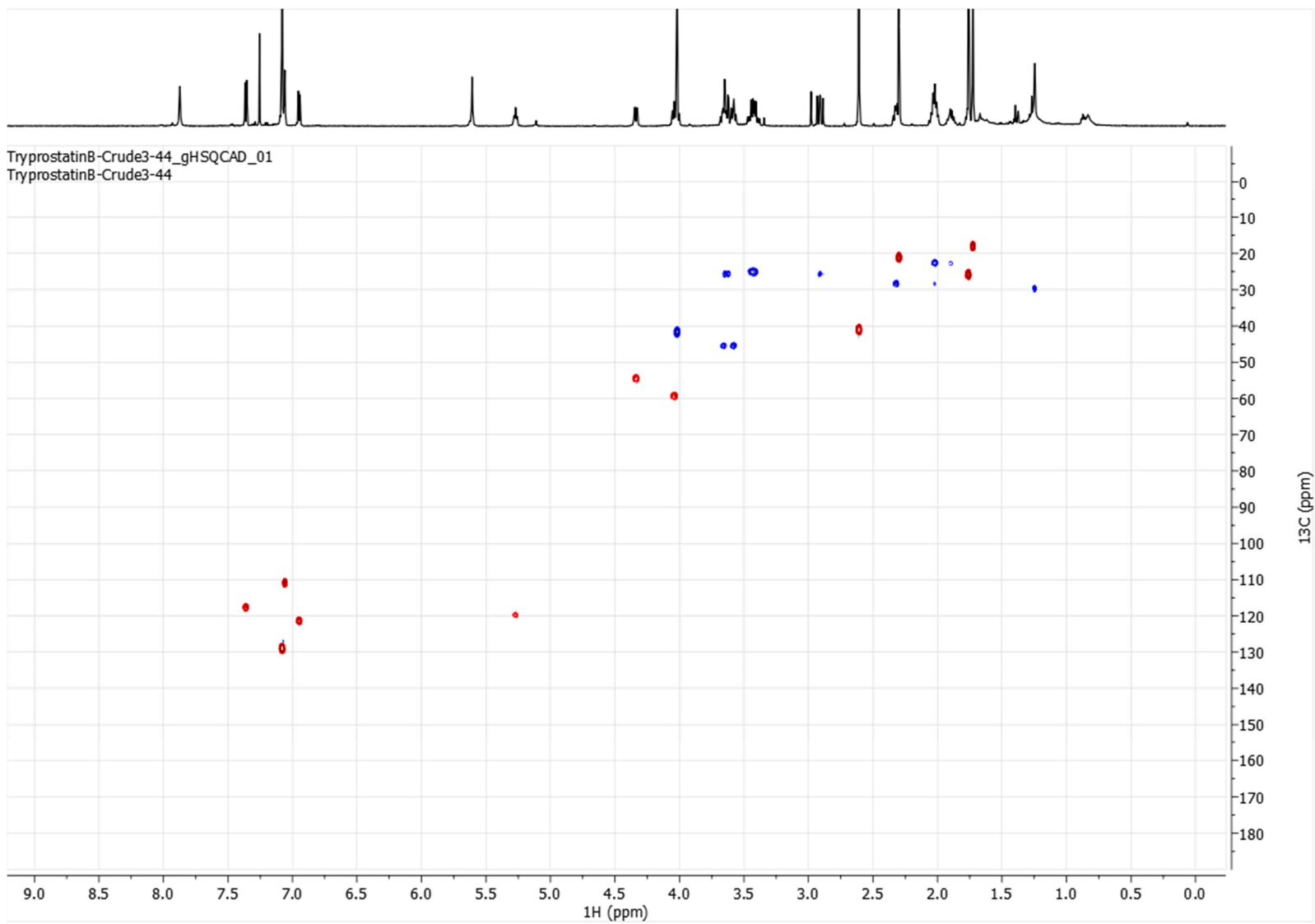




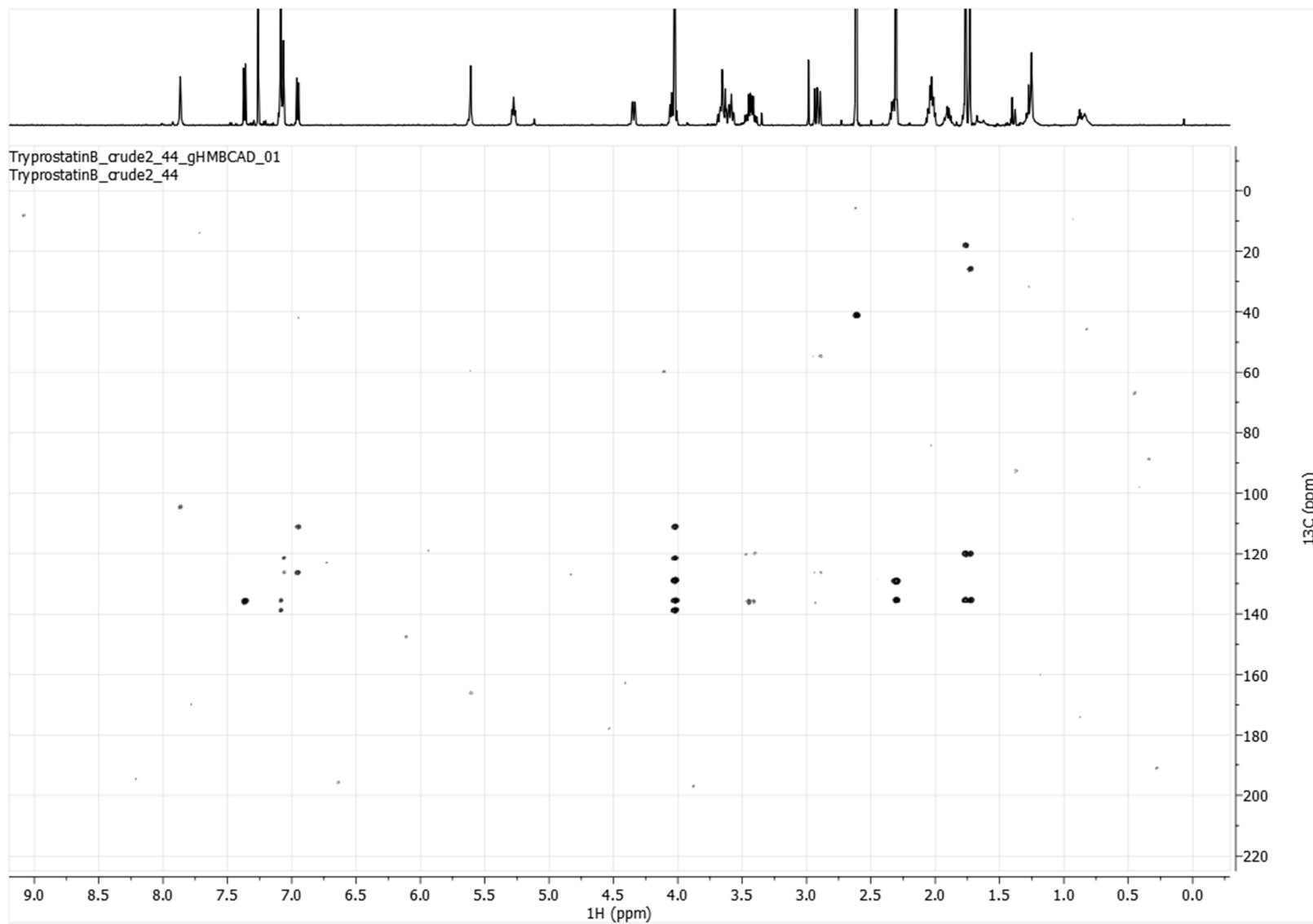
$^1\text{H}$  NMR spectrum (600 MHz) of TPS-46 in  $\text{CDCl}_3$



COSY NMR spectrum (600 MHz) of TPS-46 in CDCl<sub>3</sub>

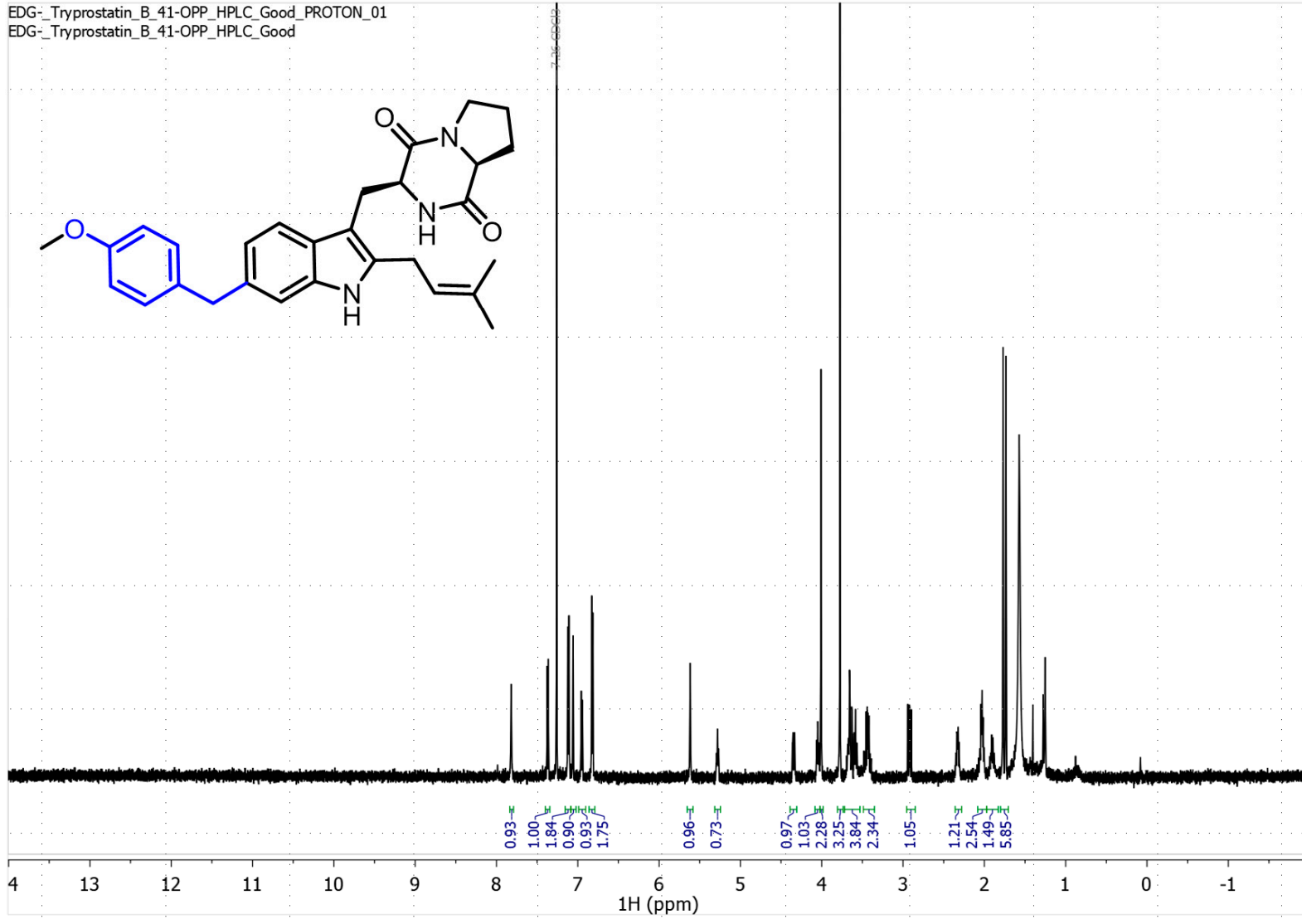


HSQC NMR spectrum (600 MHz) of **TPS-46** in CDCl<sub>3</sub>

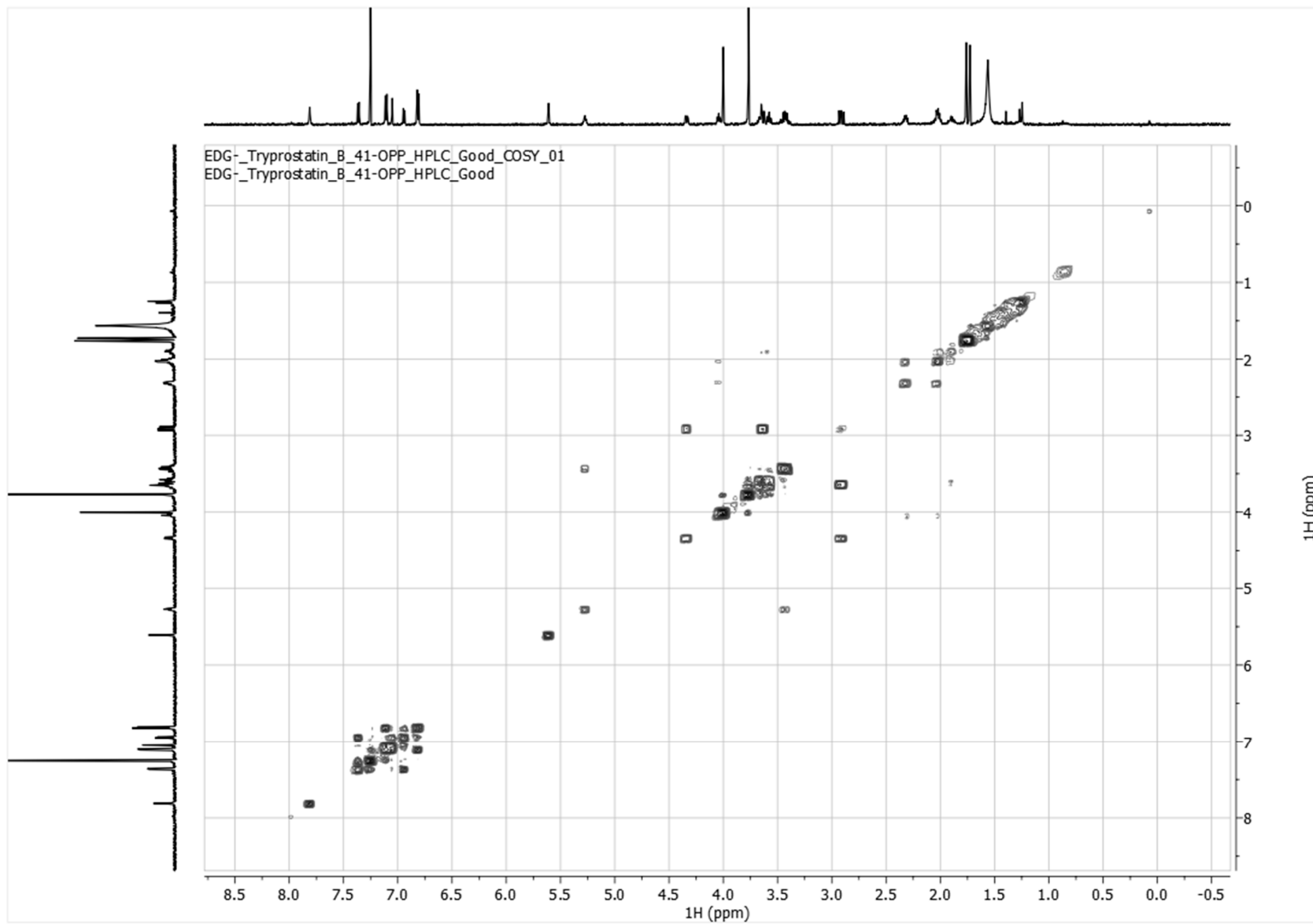


HMBC NMR spectrum (600 MHz) of **TPS-46** in  $\text{CDCl}_3$

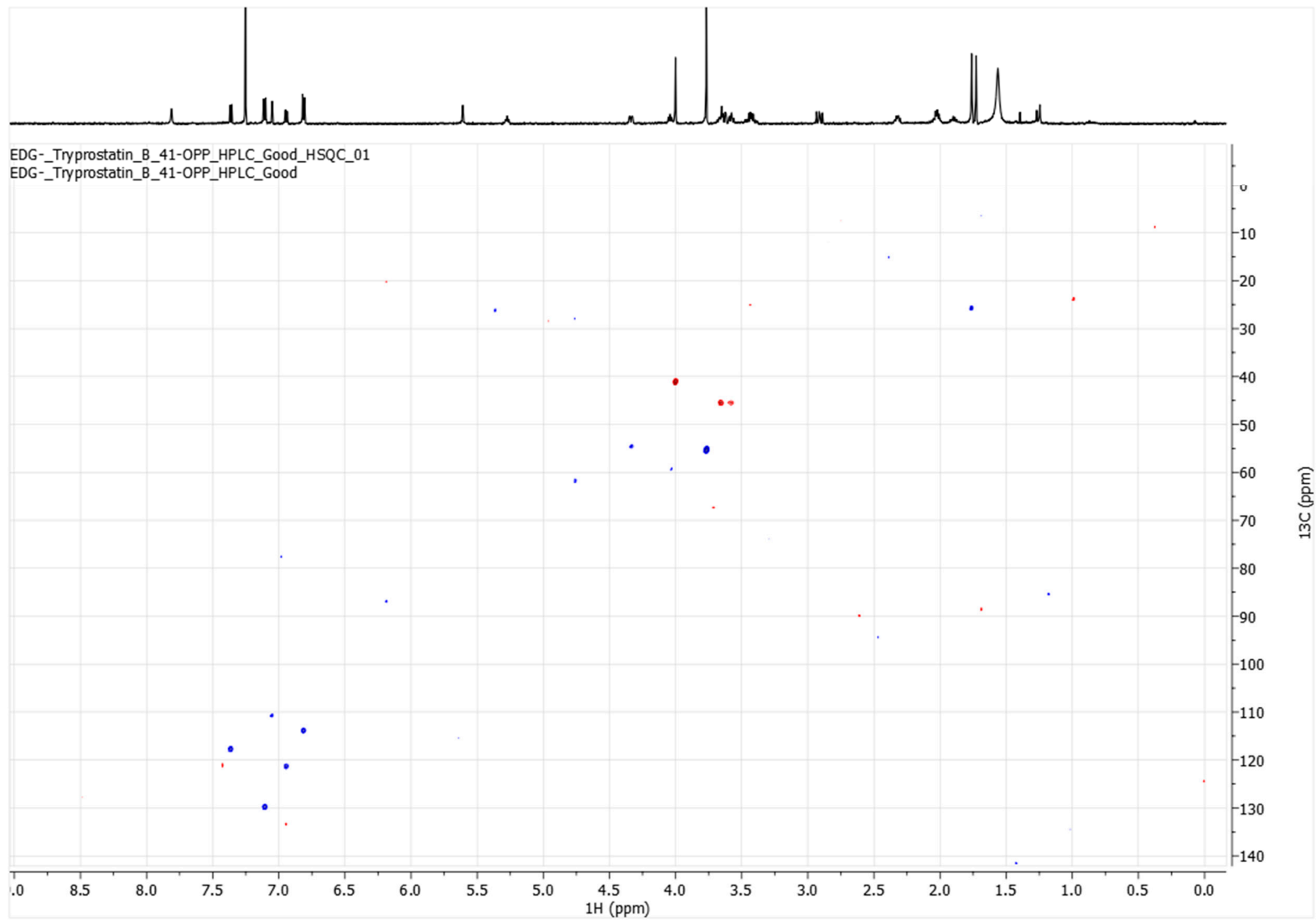
EDG\_Tryprostatin\_B\_41-OPP\_HPLC\_Good\_PROTON\_01  
EDG\_Tryprostatin\_B\_41-OPP\_HPLC\_Good



$^1\text{H}$  NMR spectrum (600 MHz) of TPS-52 in  $\text{CDCl}_3$

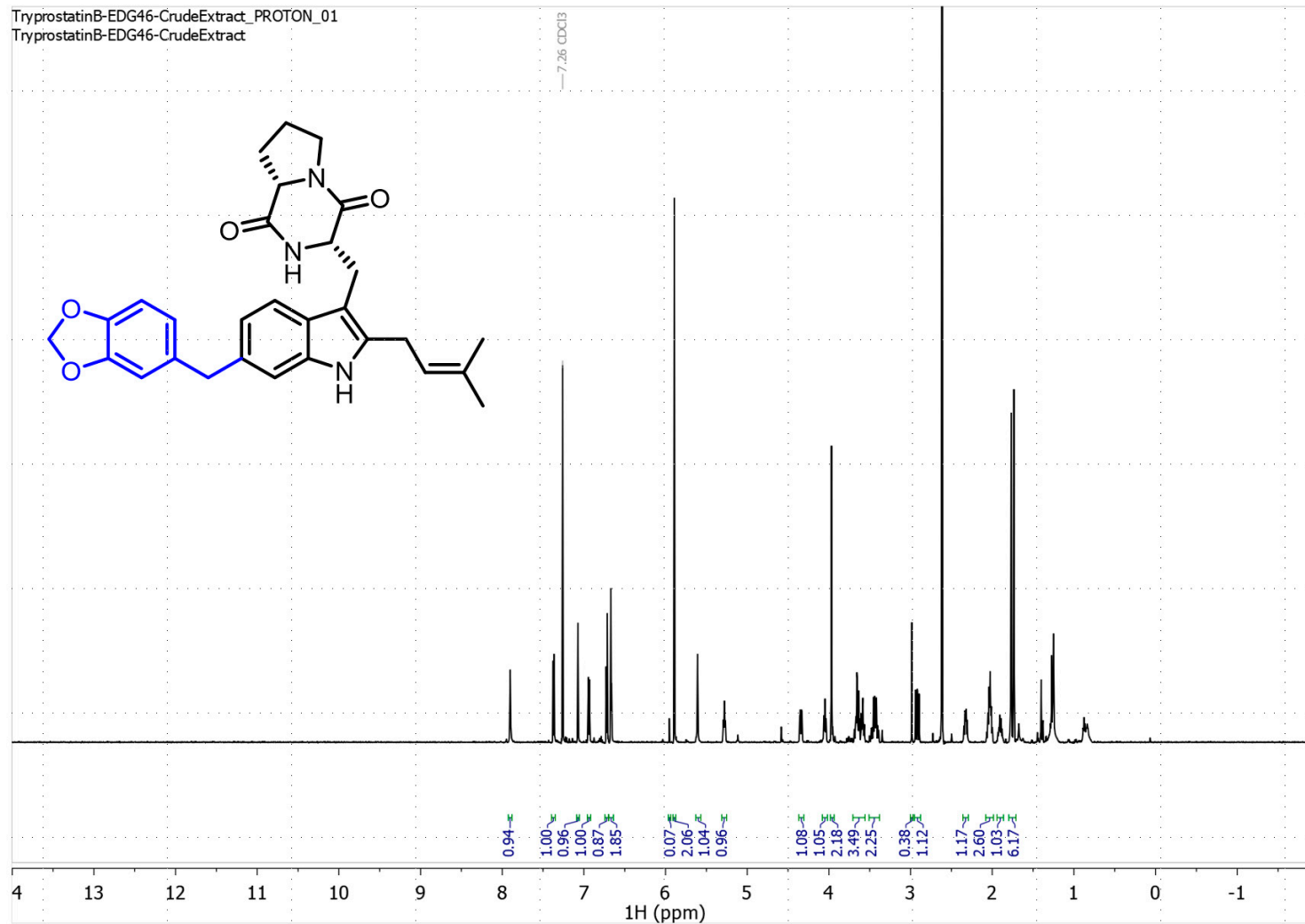


COSY NMR spectrum (600 MHz) of TPS-52 in CDCl<sub>3</sub>



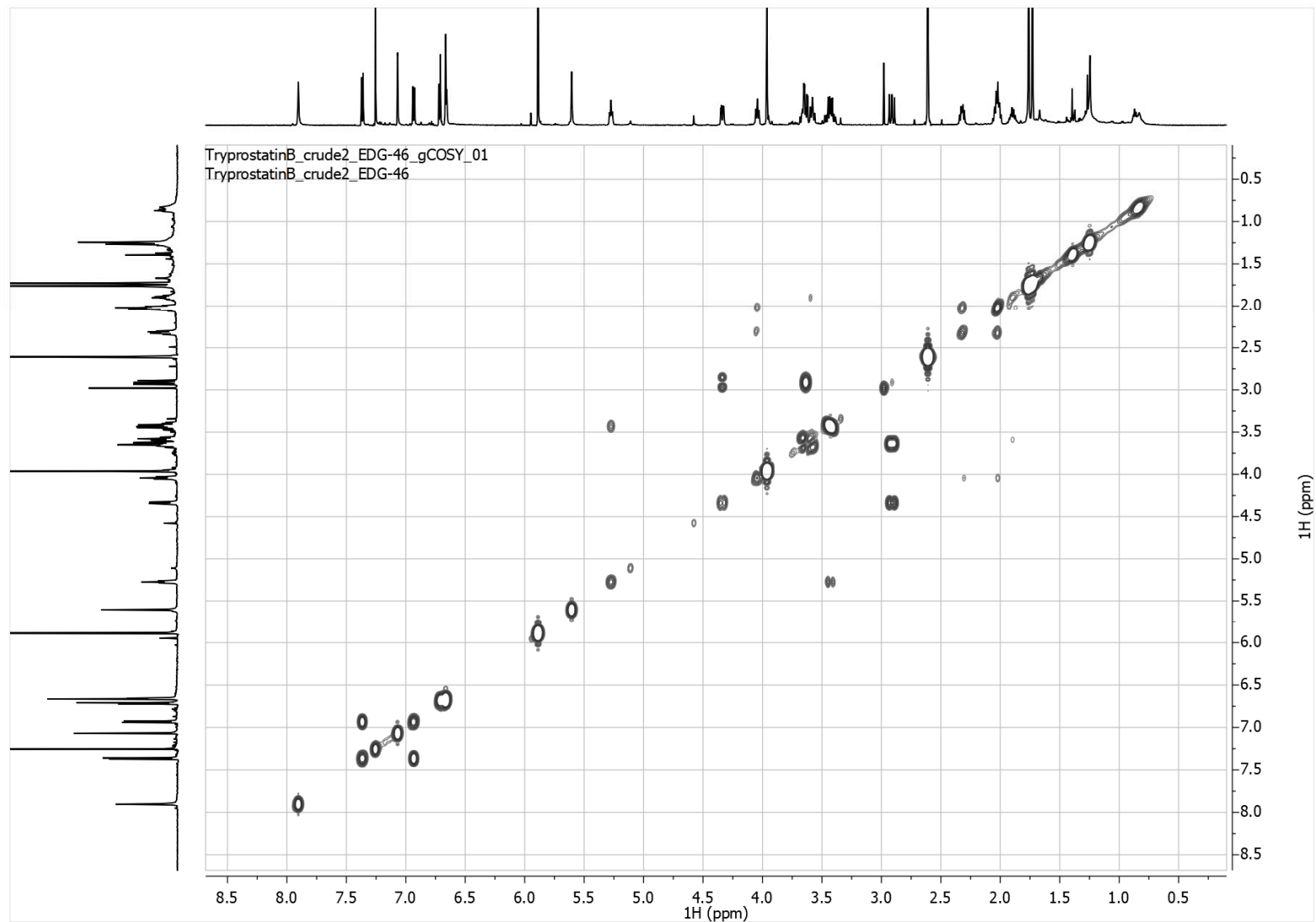
HSQC NMR spectrum (600 MHz) of **TPS-52** in CDCl<sub>3</sub>

TryprostatinB-EDG46-CrudeExtract\_PROTON\_01  
TryprostatinB-EDG46-CrudeExtract

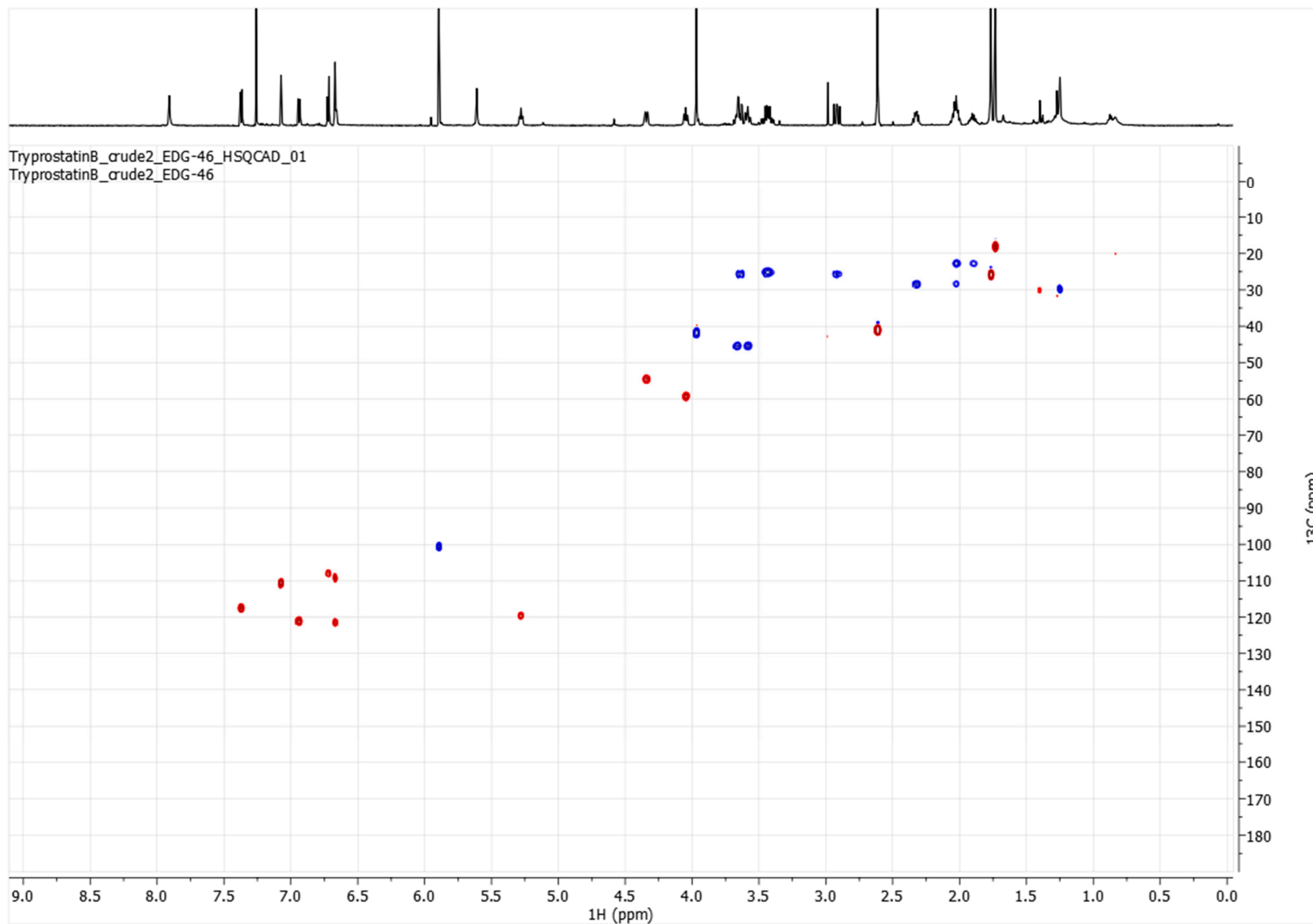


$^1\text{H}$  NMR Spectrum (600 MHz) of TPS-58 in  $\text{CDCl}_3$

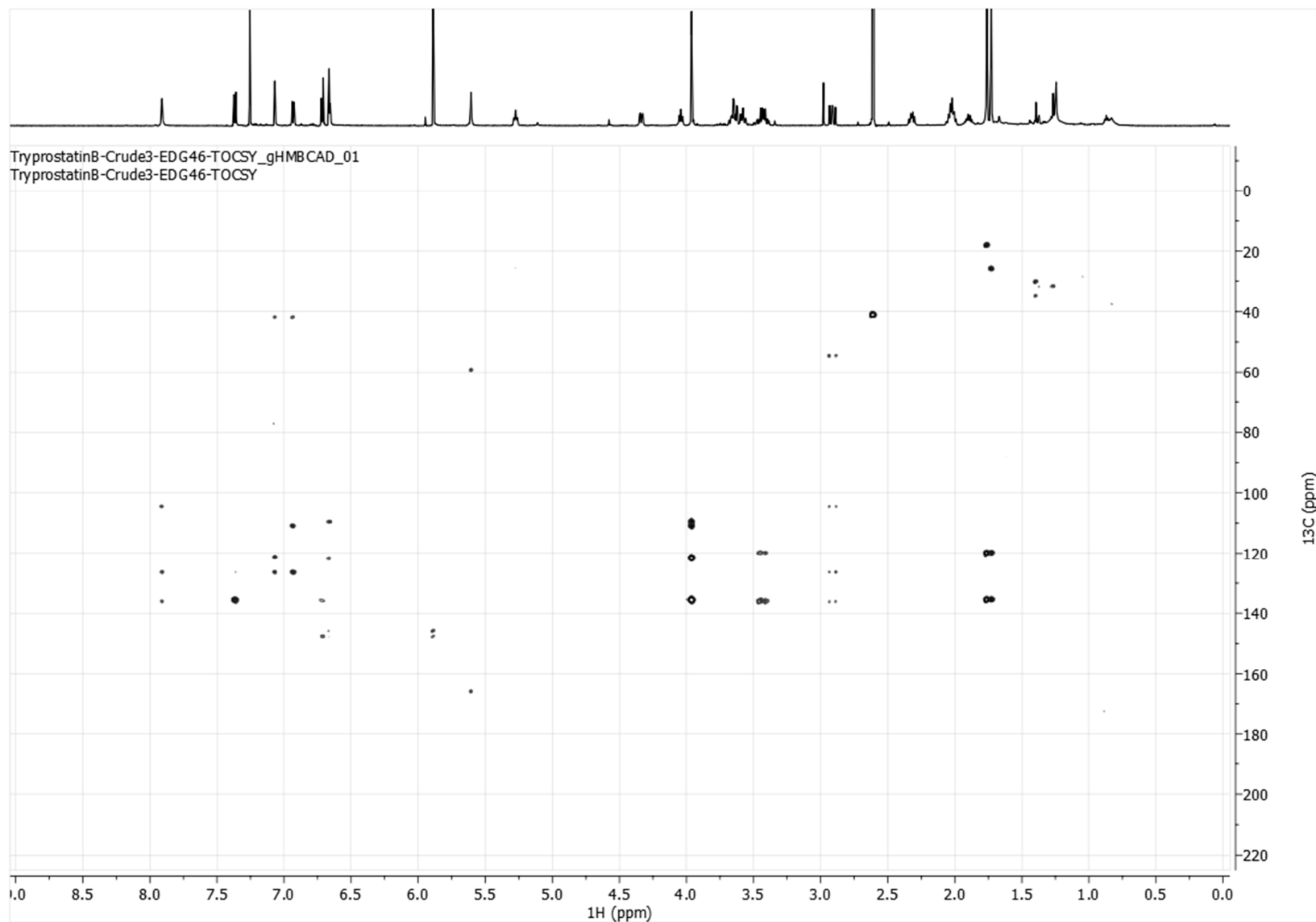




COSY NMR spectrum (600 MHz) of TPS-58 in CDCl<sub>3</sub>

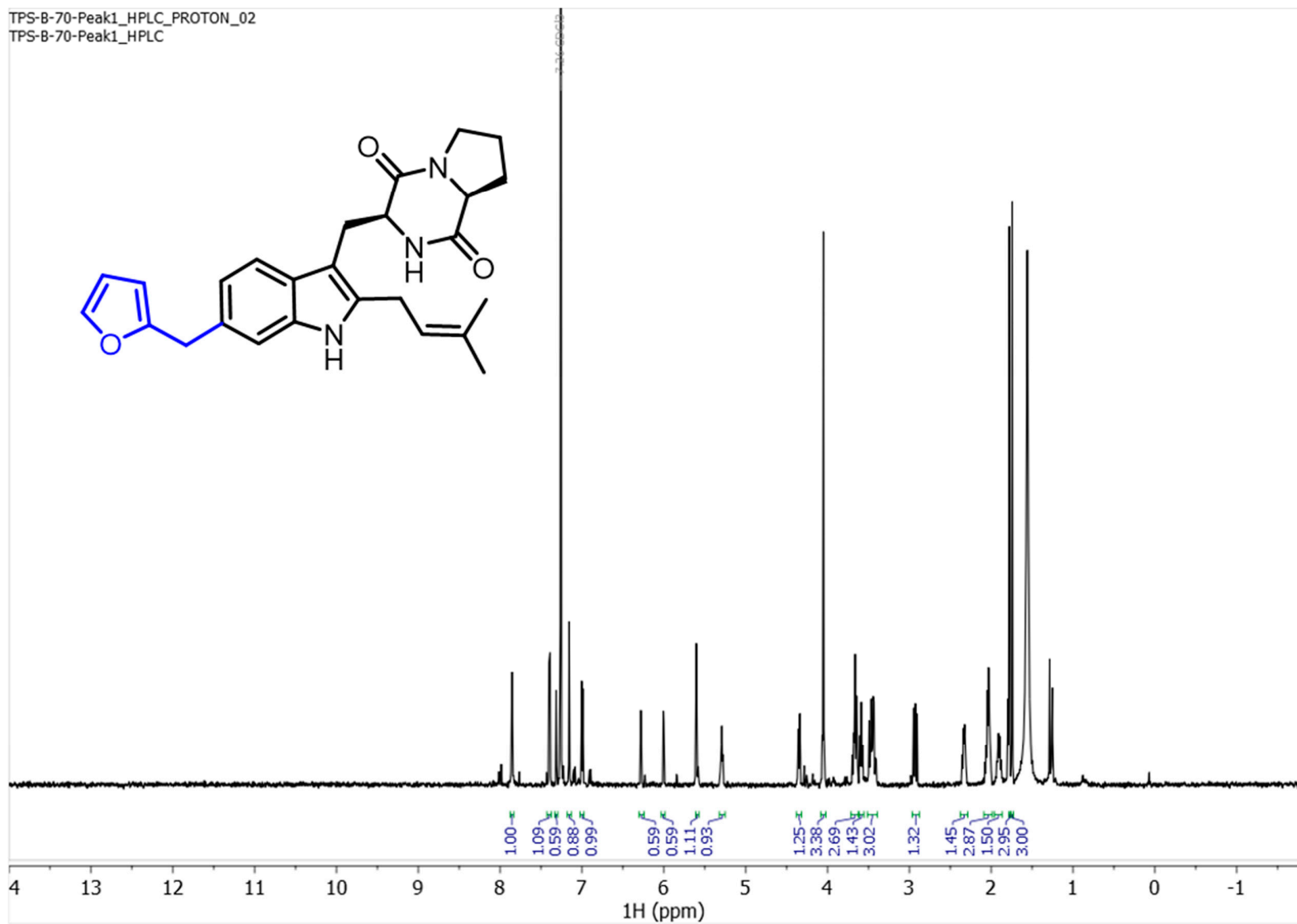


HSQC NMR spectrum (600 MHz) of **TPS-58** in  $\text{CDCl}_3$

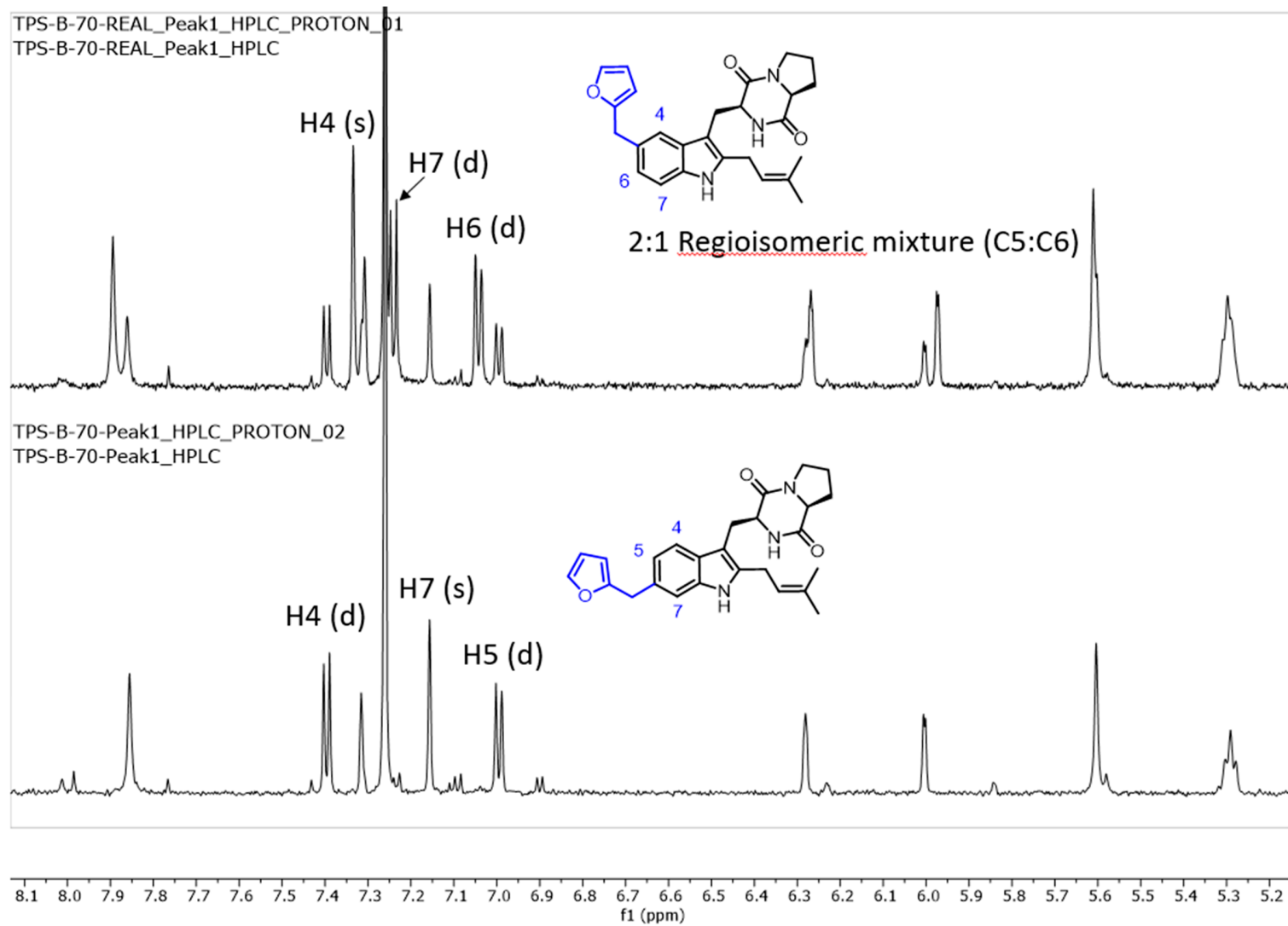


HMBC NMR spectrum (600 MHz) of **TPS-58** in  $\text{CDCl}_3$

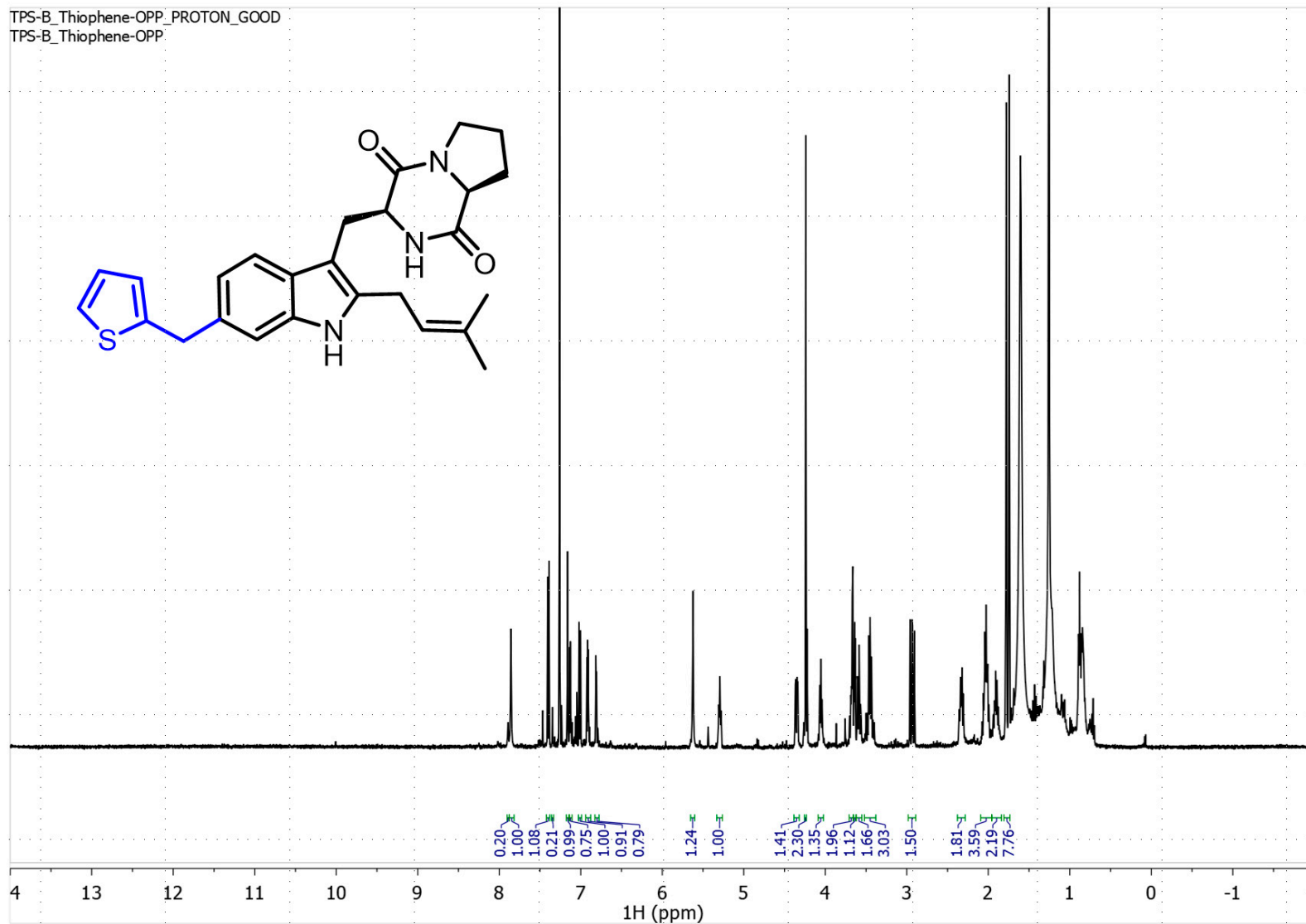
TPS-B-70-Peak1\_HPLC\_PROTON\_02  
TPS-B-70-Peak1\_HPLC



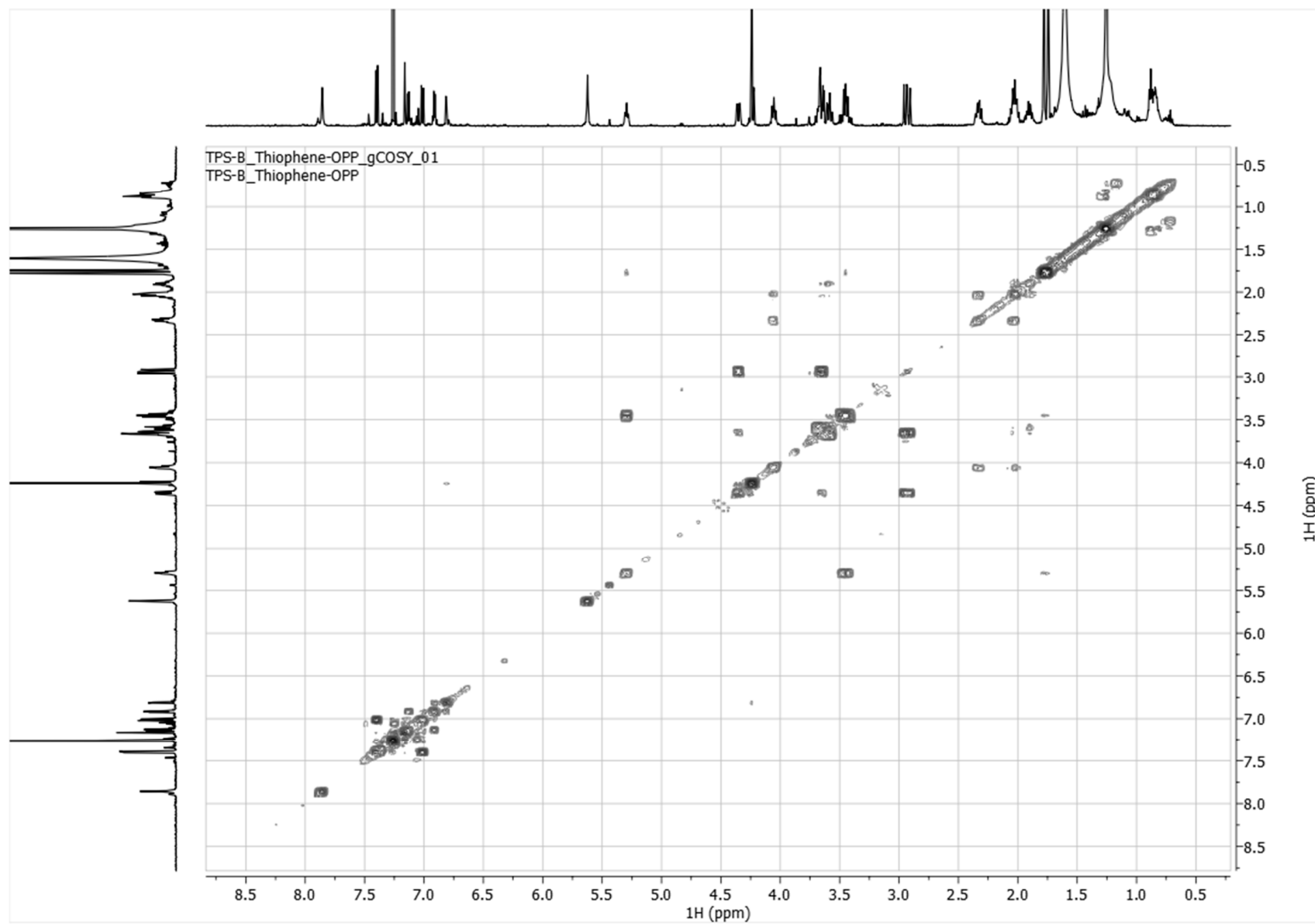
<sup>1</sup>H NMR spectrum (500 MHz) of **TPS-61** in CDCl<sub>3</sub>



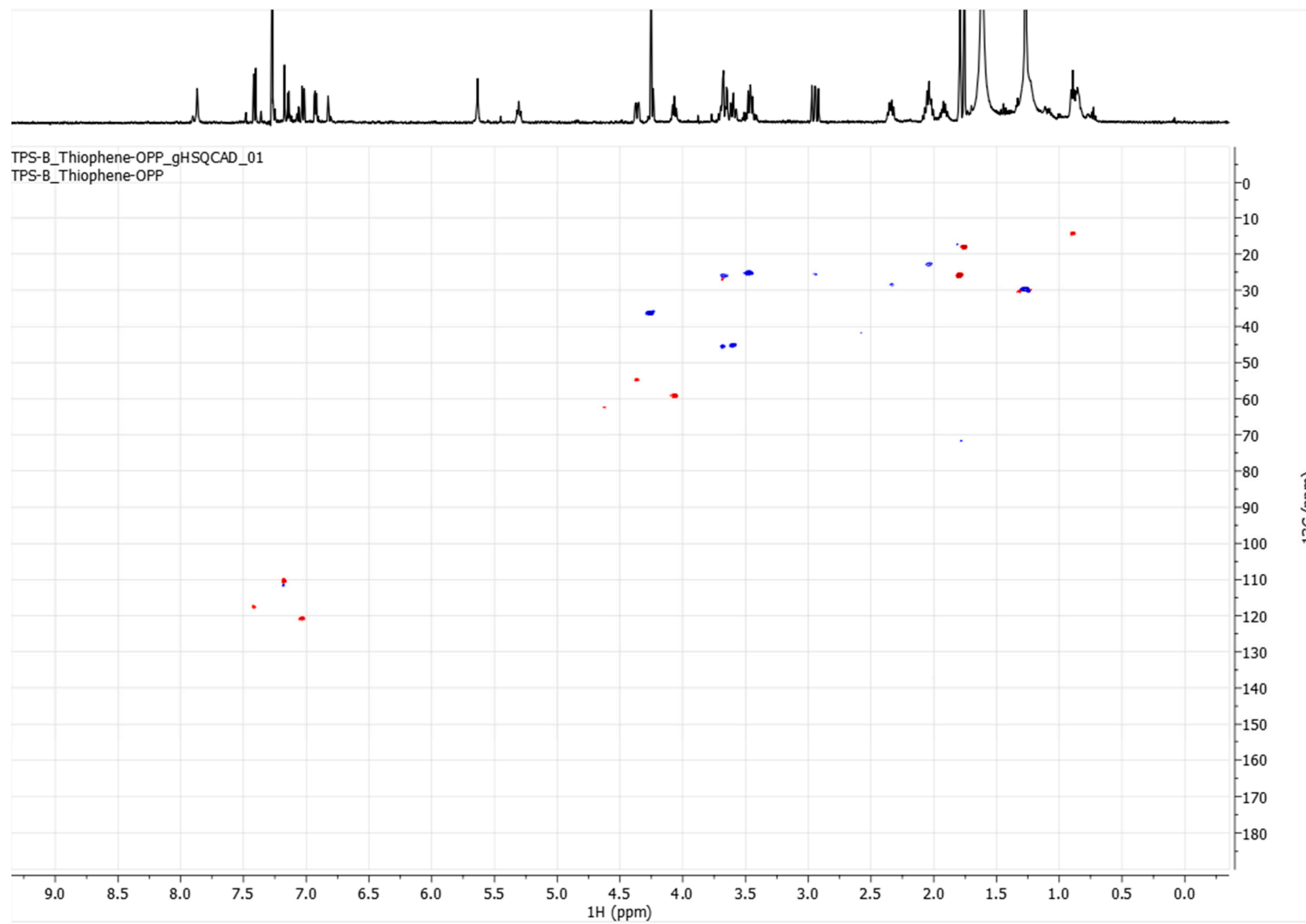
Stacked  $^1\text{H}$  spectra of **TPS-61** fractions after partial purification. The top spectrum displays the H7 doublet and H4 singlet indicative of C-5 alkylation as a minor reaction product. The bottom spectrum is the purified C6 alkylated major product.



$^1\text{H}$  NMR spectrum (500 MHz) of TPS-62 in  $\text{CDCl}_3$ . The minor C5 alkylated product is 20% of the products based on integrations.



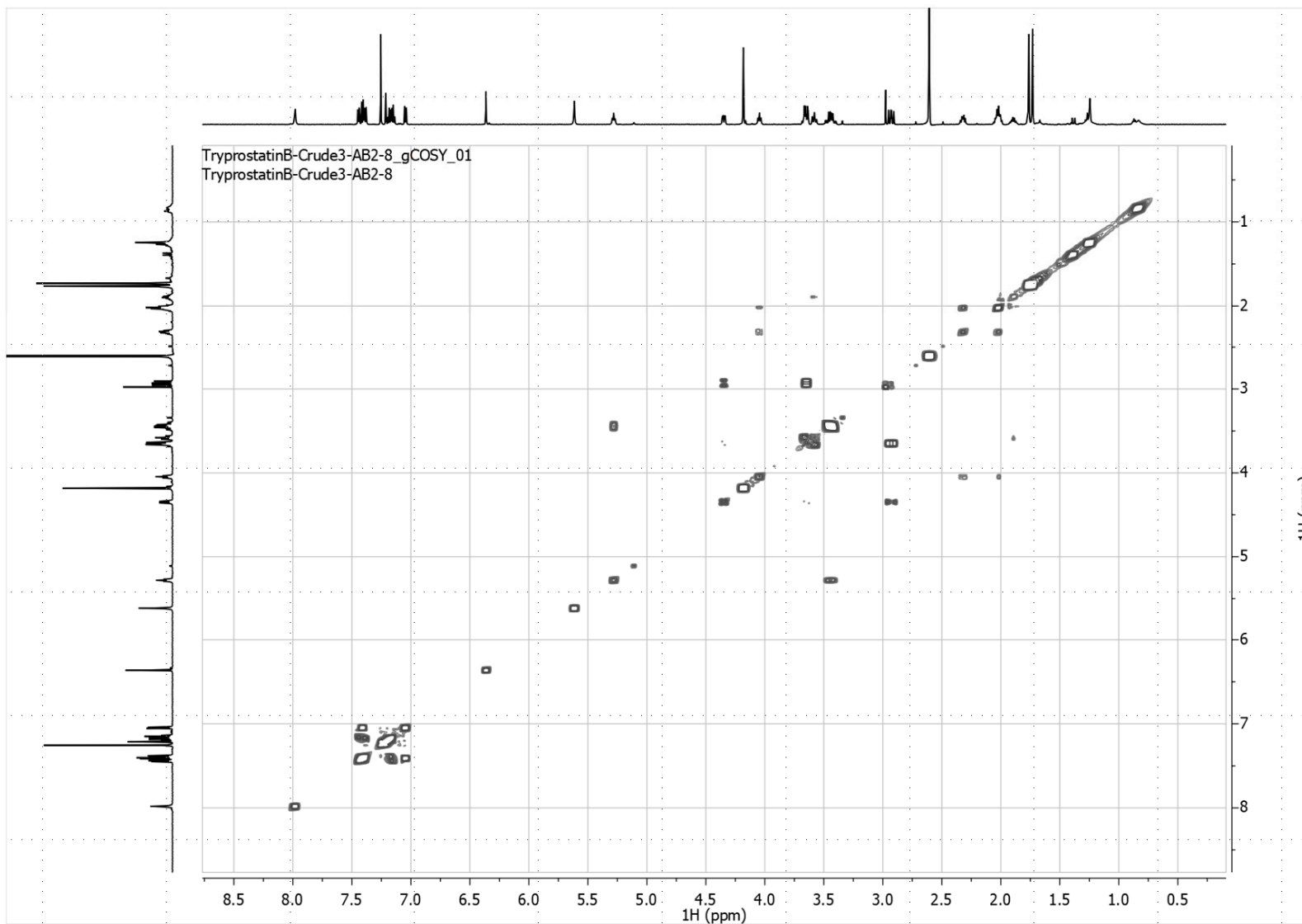
COSY NMR spectrum (500 MHz) of **TPS-62** in  $\text{CDCl}_3$



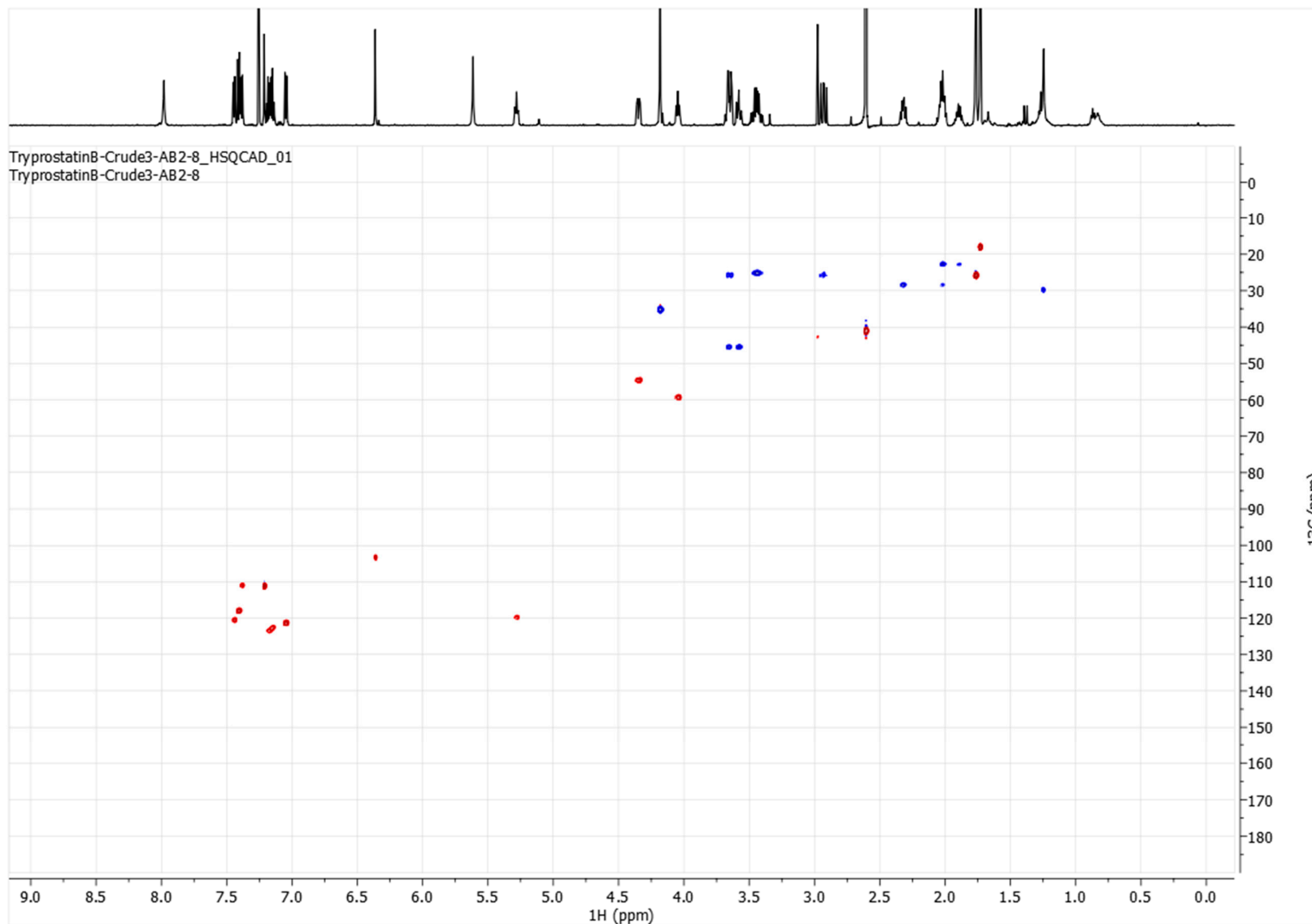
HSQC NMR spectrum (500 MHz) of **TPS-62** in  $\text{CDCl}_3$



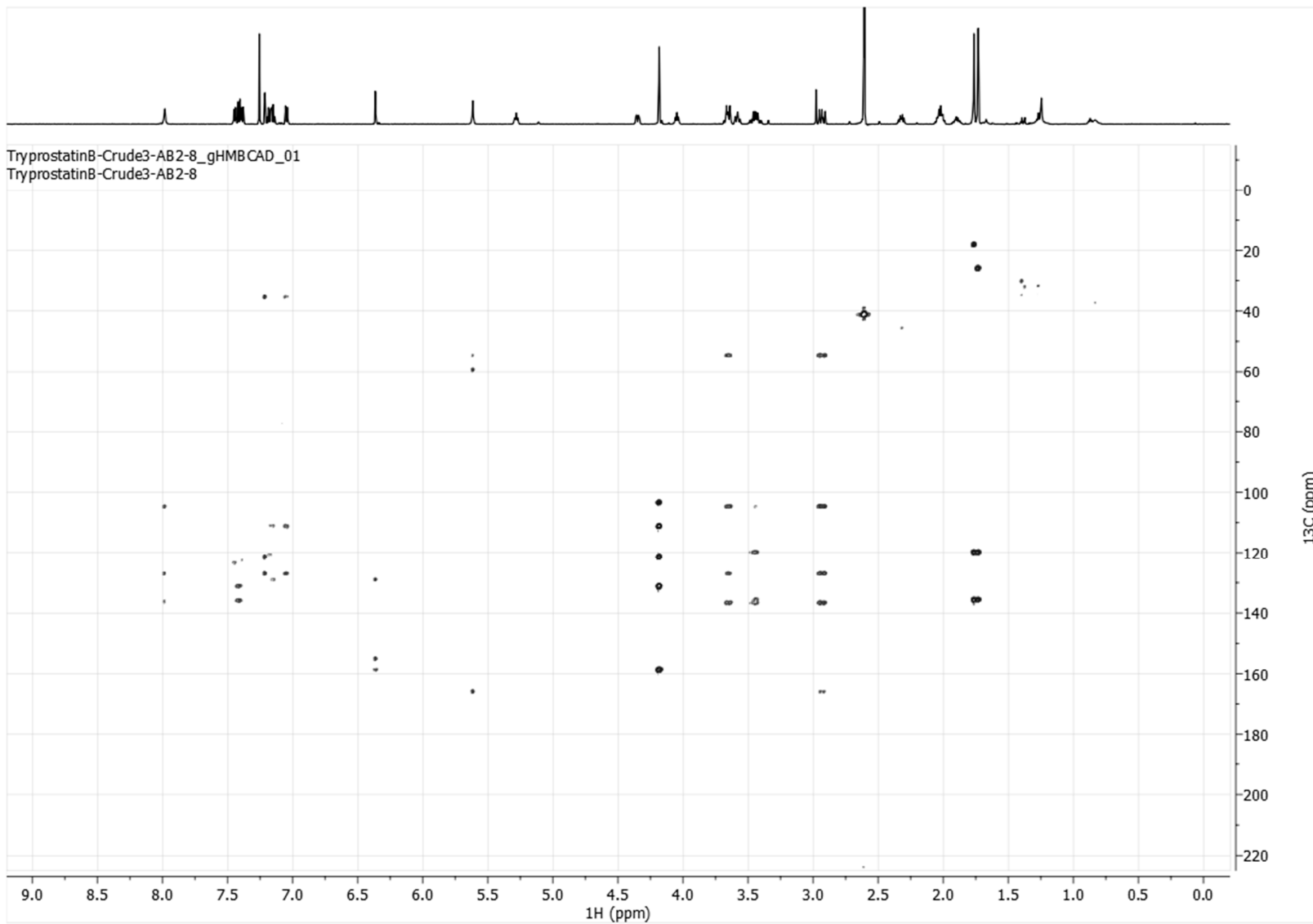




COSY NMR spectrum (600 MHz) of TPS-65 in CDCl<sub>3</sub>



HSQC NMR spectrum (600 MHz) of **TPS-65** in CDCl<sub>3</sub>



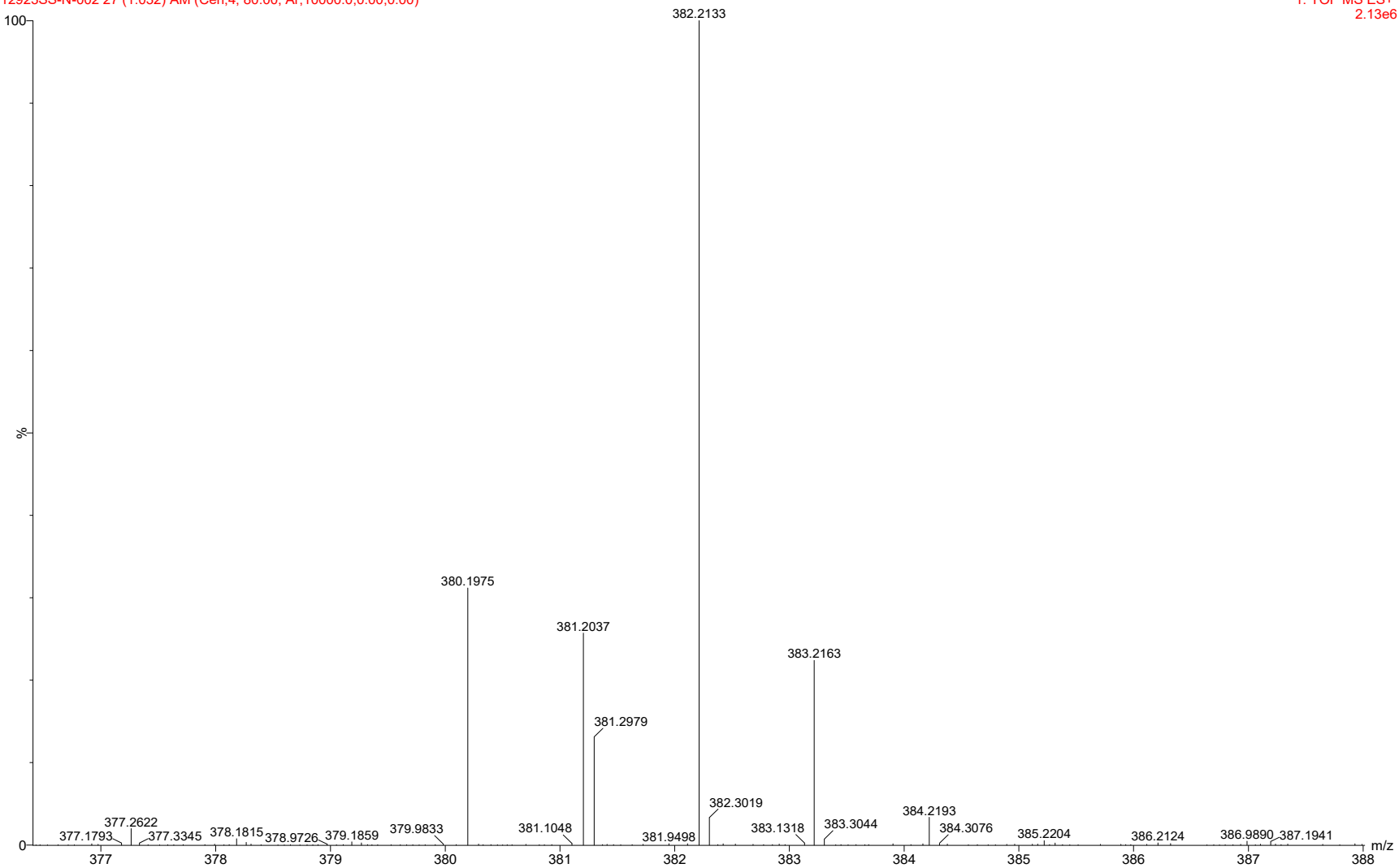
HMBC NMR spectrum (600 MHz) of **TPS-65** in CDCl<sub>3</sub>

TPS-A  
Gardner  
12923SS-N-002 27 (1.032) AM (Cen,4, 80.00, Ar,10000.0,0.00,0.00)

SYNAPT-G2-Si#UGA589

22-Aug-2019 13:10:16

1: TOF MS ES+  
2.13e6



HRMS of TPS-A

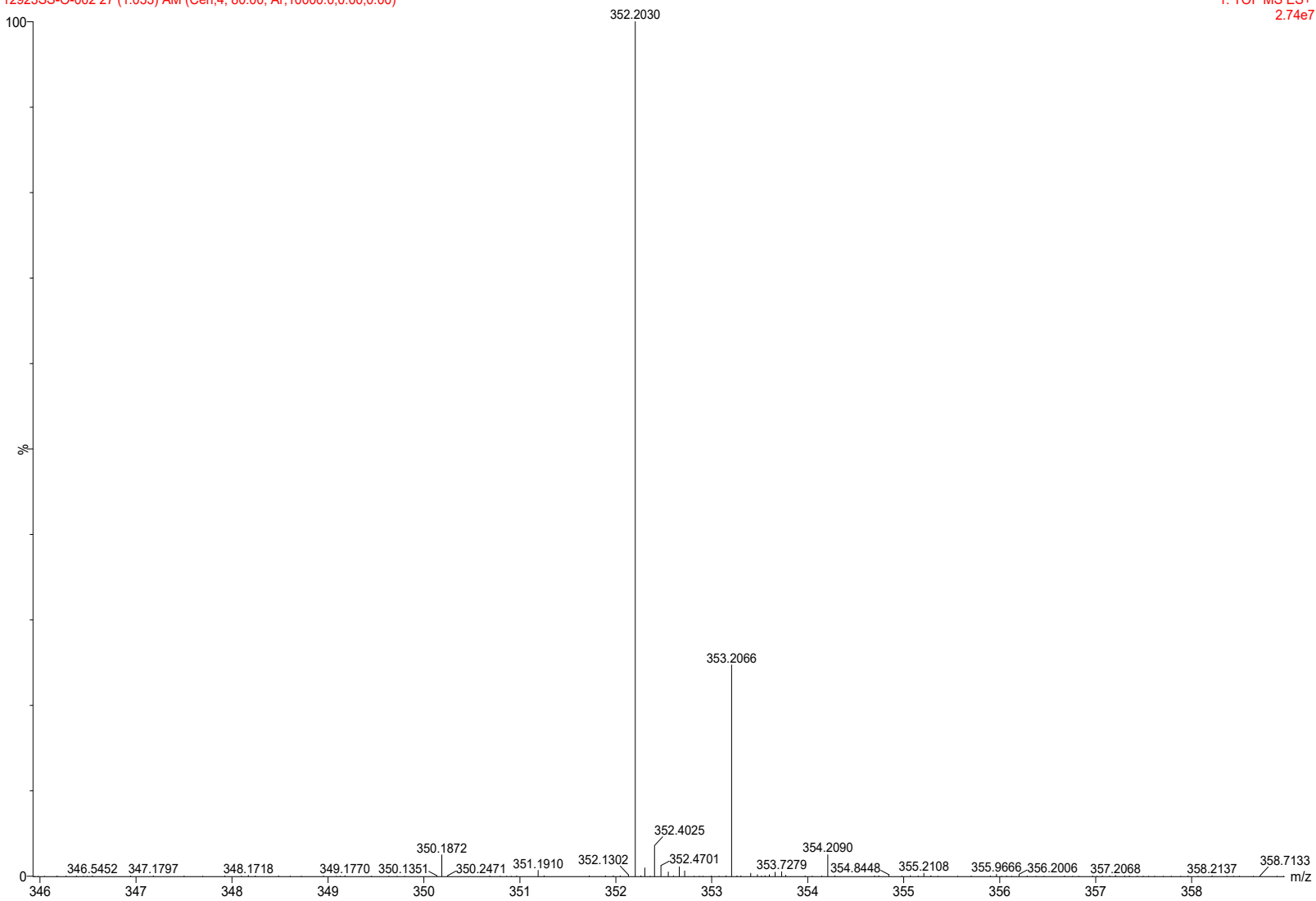
TPS-B  
Gardner

12923SS-O-002 27 (1.033) AM (Cen,4, 80.00, Ar,10000.0,0.00,0.00)

SYNAPT G2-Si#UGA589

22-Aug-2019 13:21:31

1: TOF MS ES+  
2.74e7



HRMS of TPS-B

S54

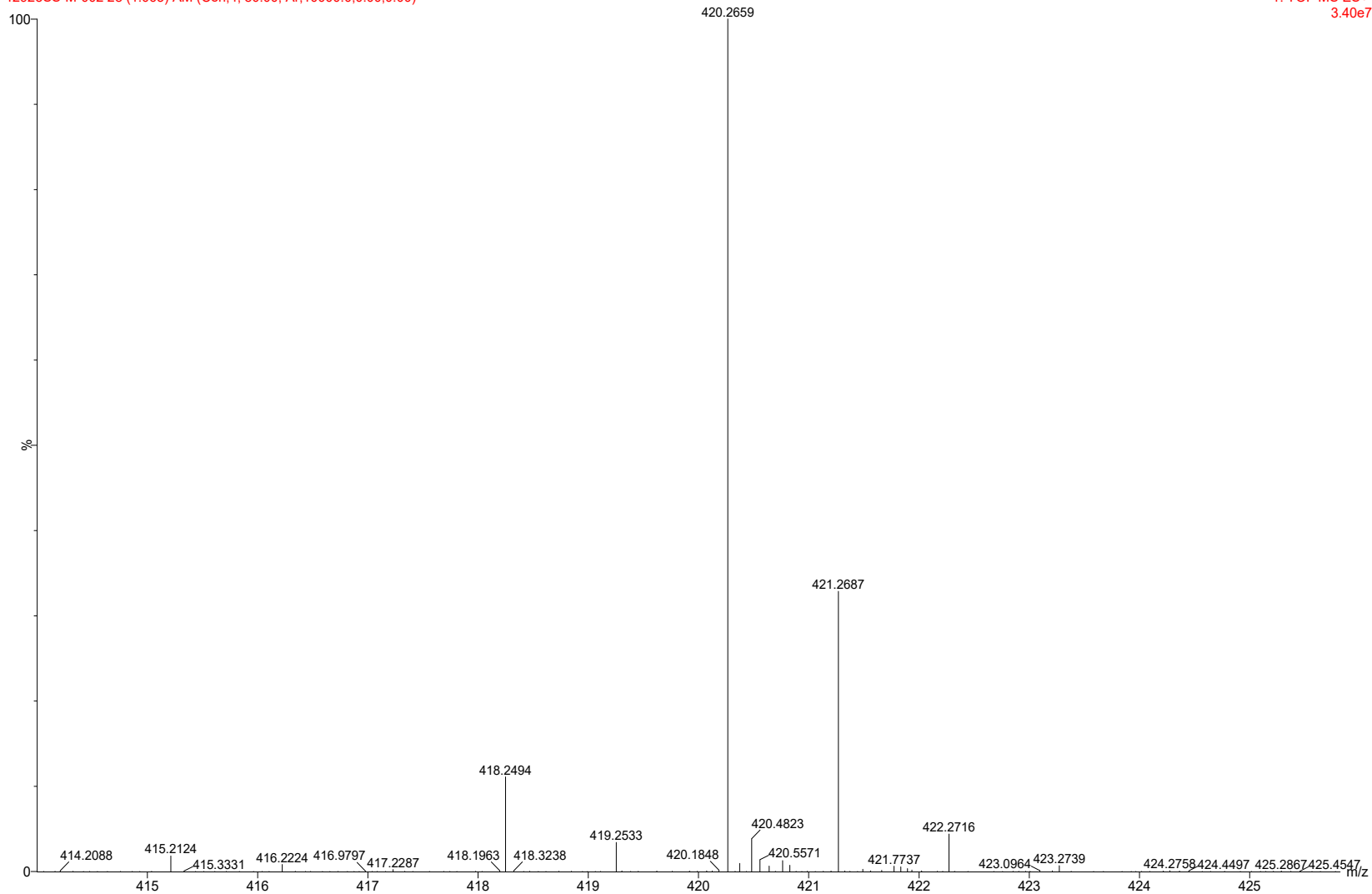
TPS-2  
Gardner

SYNAPT G2-Si#UGA589

22-Aug-2019 12:59:28

12923SS-M-002 28 (1.065) AM (Cen,4, 80.00, Ar,10000.0,0.00,0.00)

1: TOF MS ES+  
3.40e7



HRMS of TPS-2

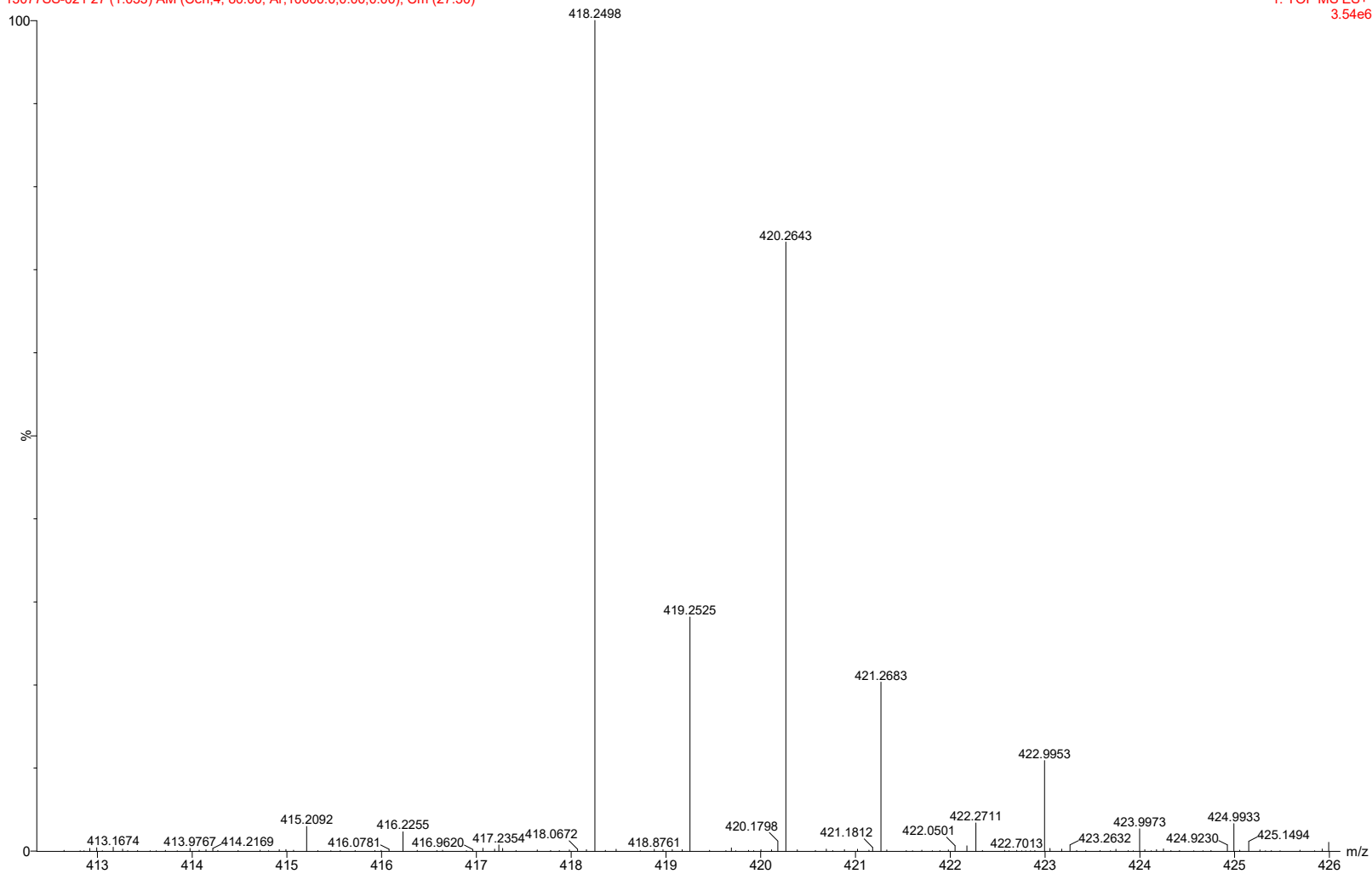
TPS-17  
Gardner

13077SS-021 27 (1.033) AM (Cen,4, 80.00, Ar,10000.0,0.00,0.00); Cm (27:30)

SYNAPT G2-Si#UGA589

24-Jul-2020 14:18:06

1: TOF MS ES+  
3.54e6



HRMS of TPS-6



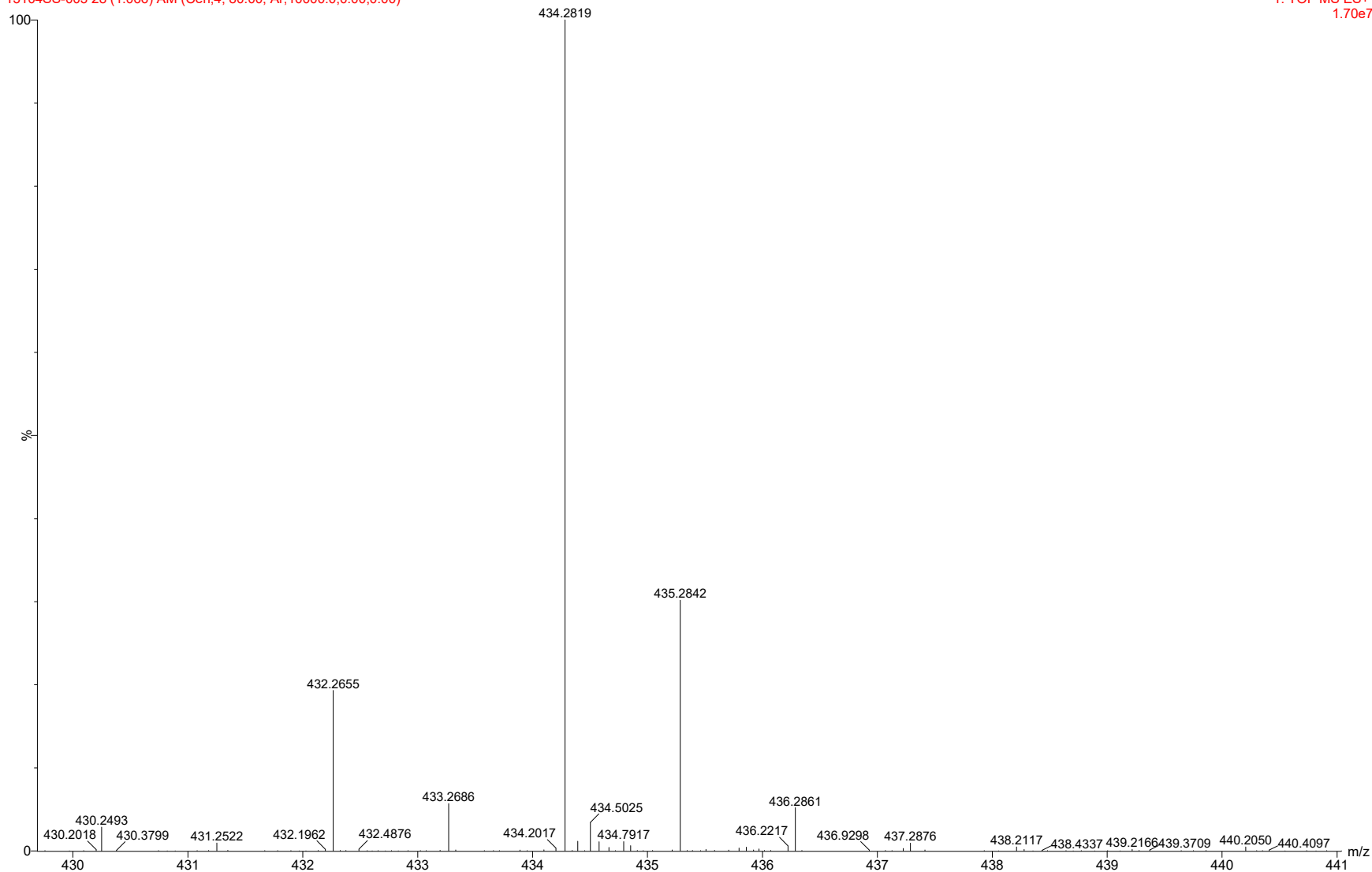
V-3  
Gardner

SYNAPTG2-Si#UGA589

21-Aug-2020 13:00:18

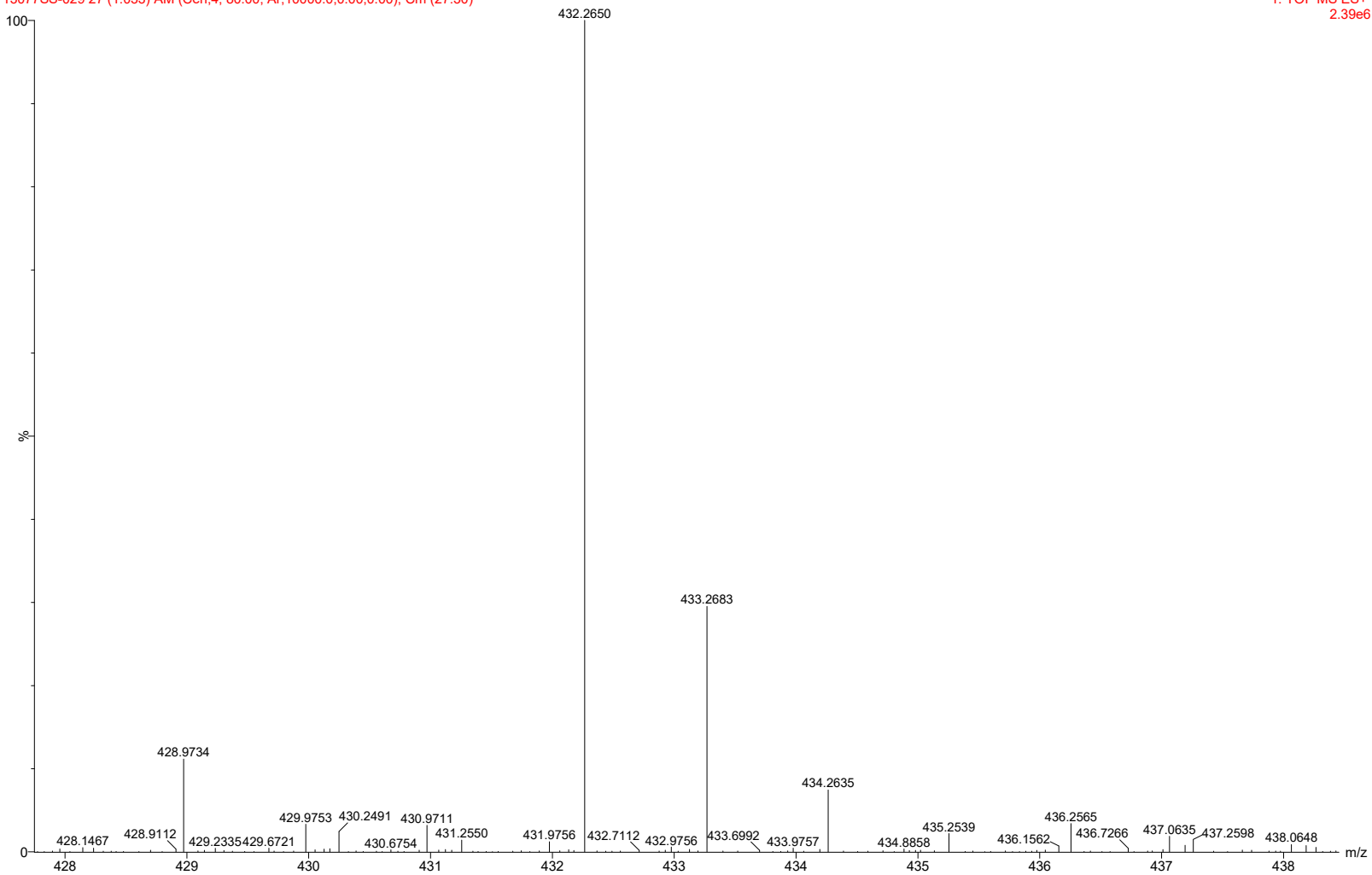
13104SS-003 28 (1.066) AM (Cen,4, 80.00, Ar,10000.0,0.00,0.00)

1: TOF MS ES+  
1.70e7



HRMS of TPS-7

S57



HRMS of TPS-8

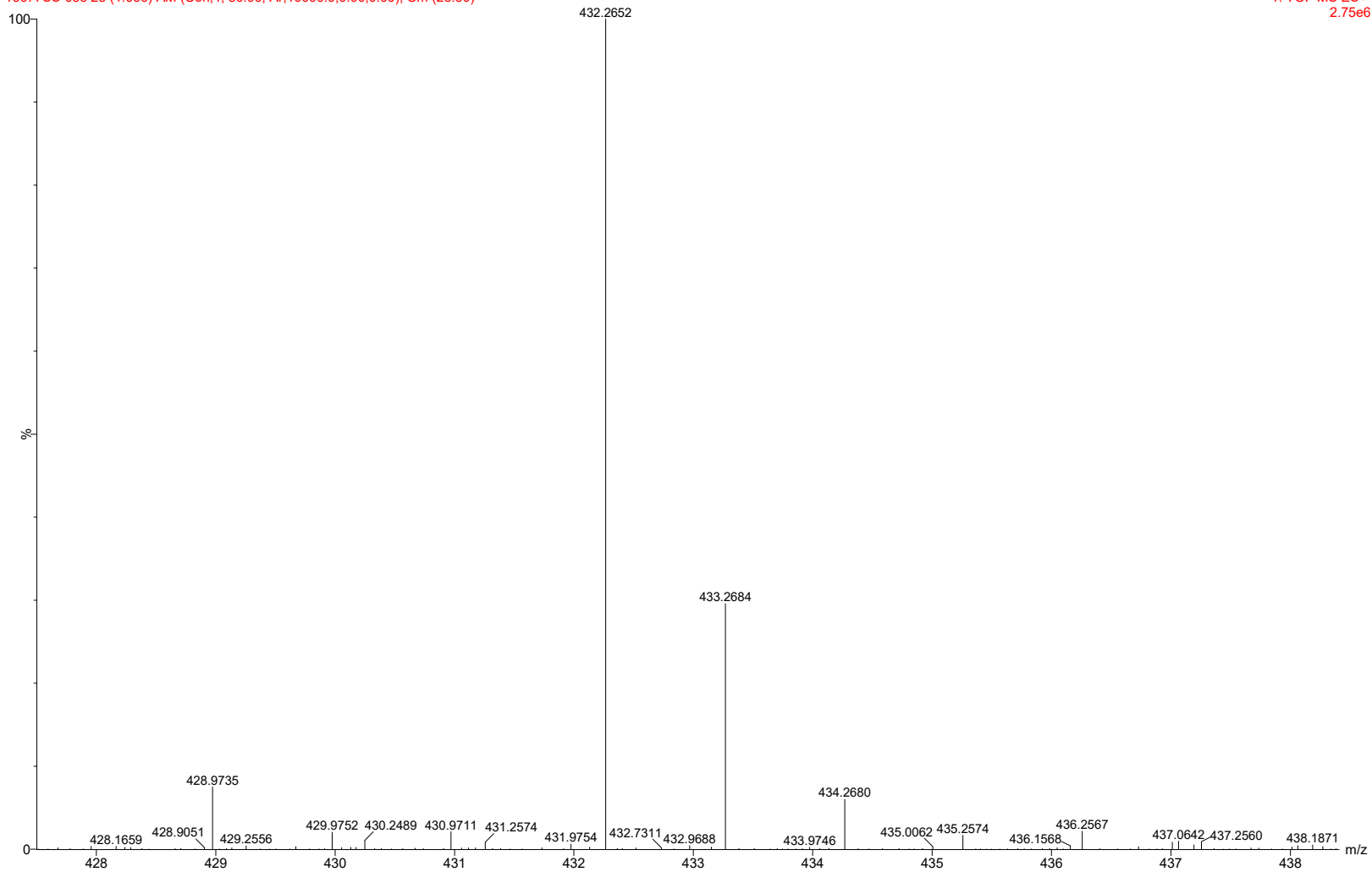
TPS-22  
Gardner

13077SS-033 28 (1.066) AM (Cen,4, 80.00, Ar,10000.0,0.00,0.00); Cm (28:30)

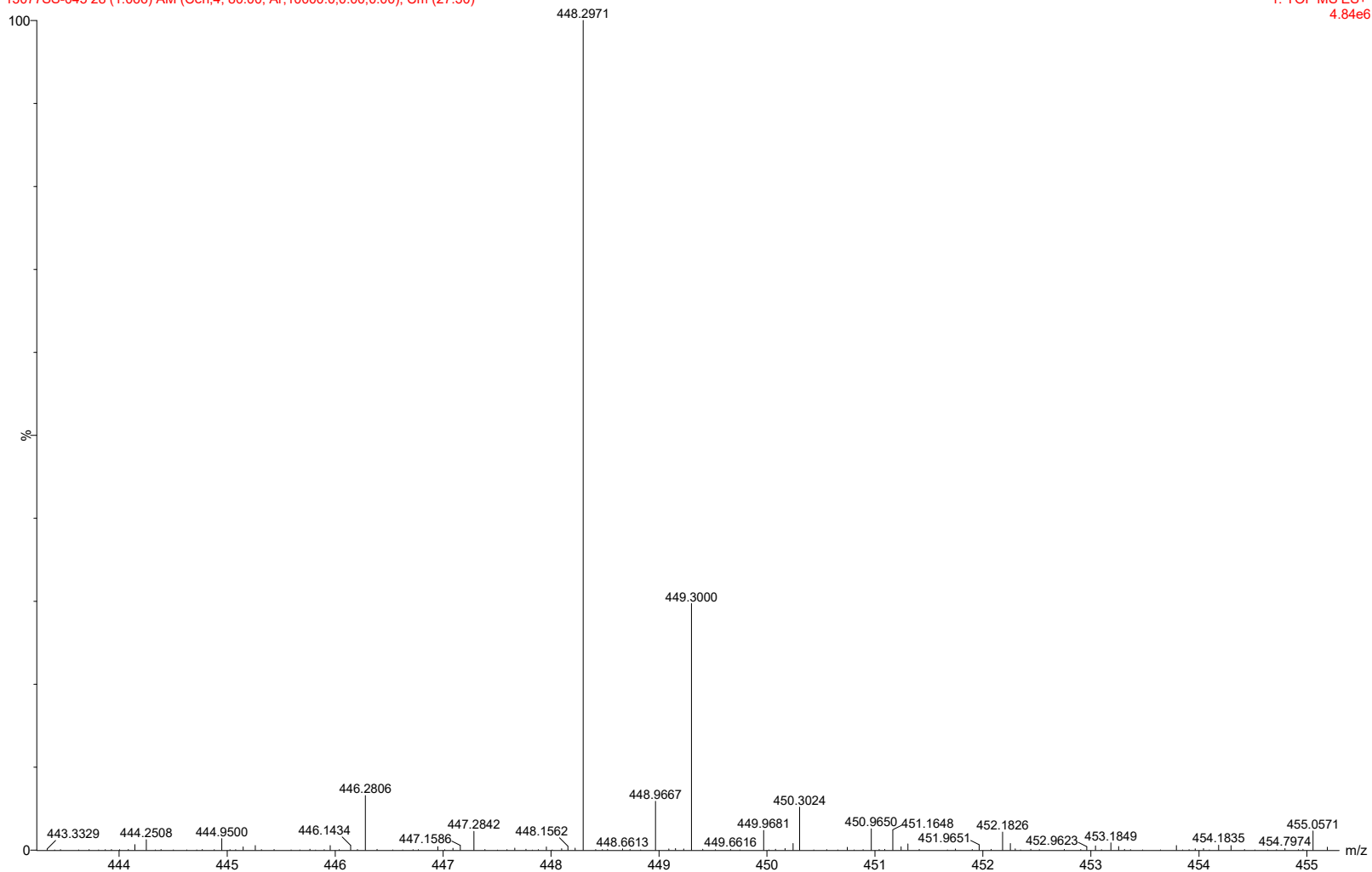
SYNAPT G2-Si#UGA589

24-Jul-2020 15:01:35

1: TOF MS ES+  
2.75e6



HRMS of TPS-9



HRMS of TPS-10

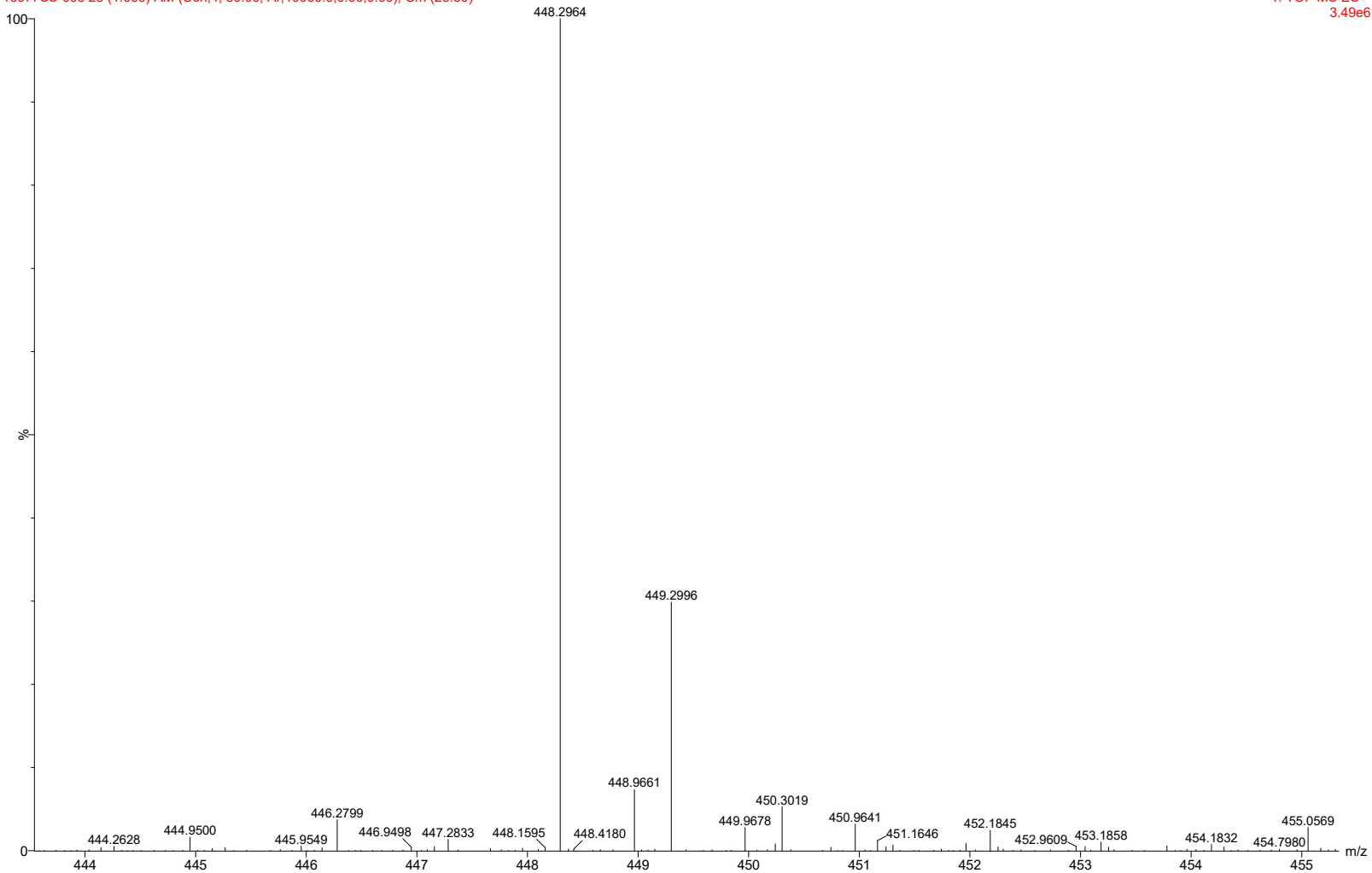
TPS-4  
Gardner

SYNAPTG2-Si#UGA589

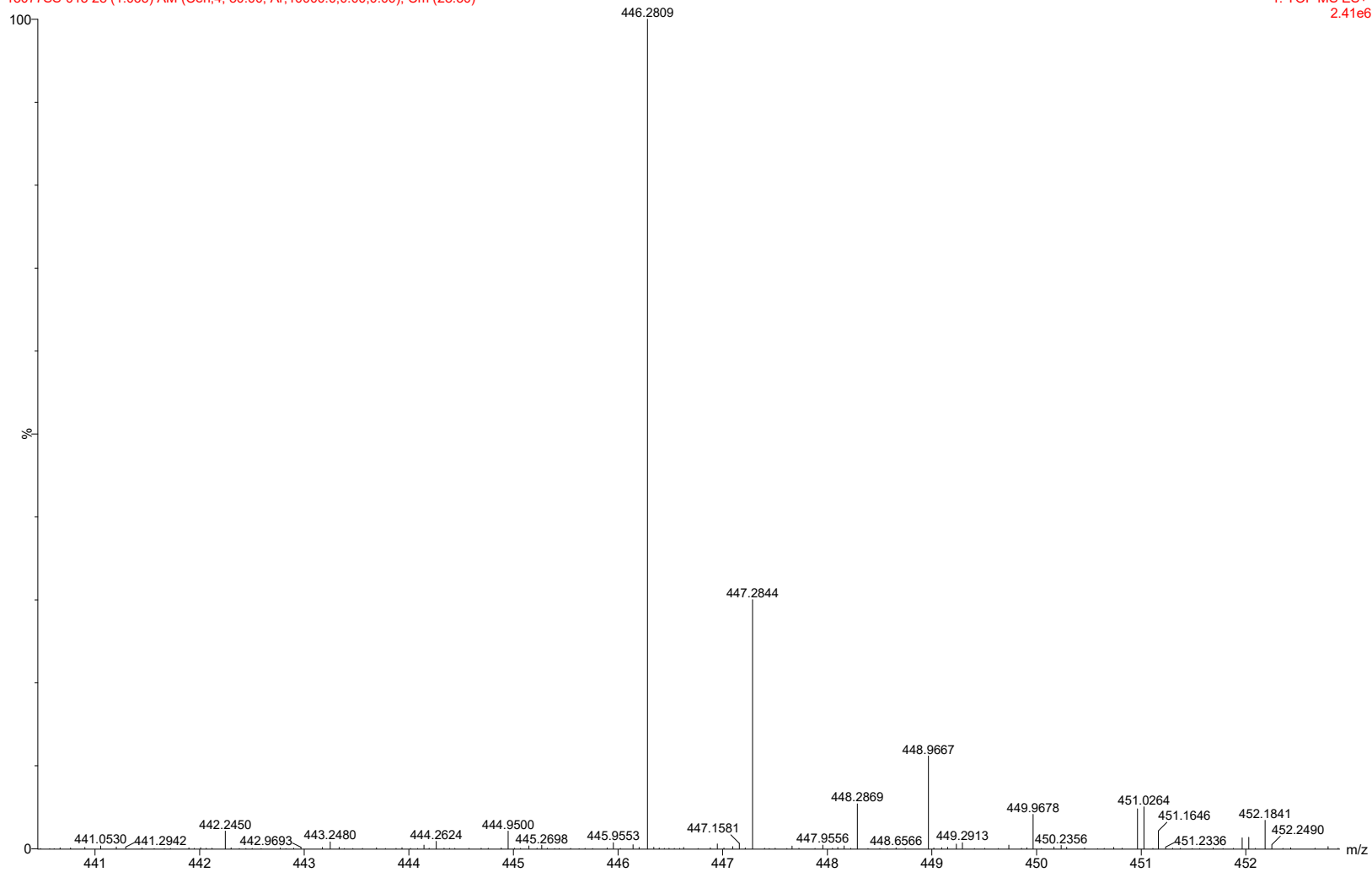
24-Jul-2020 12:55:54

13077SS-008 28 (1.066) AM (Cen,4, 80.00, Ar,10000.0,0.00,0.00); Cm (28:30)

1: TOF MS ES+  
3.49e6



HRMS of TPS-11



HRMS of TPS-13

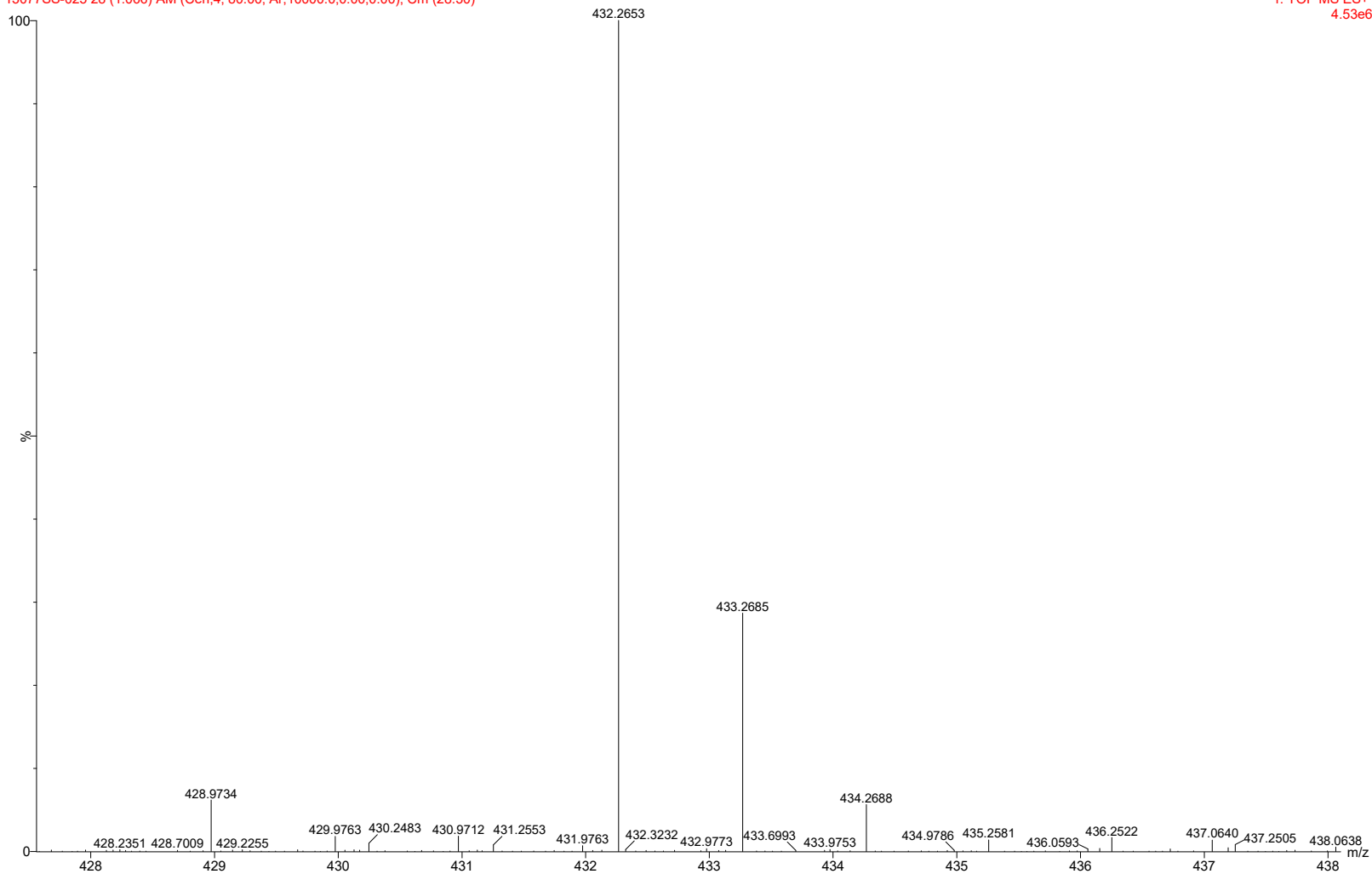
TPS-18  
Gardner

13077SS-025 28 (1.066) AM (Cen,4, 80.00, Ar,10000.0,0.00,0.00); Cm (28:30)

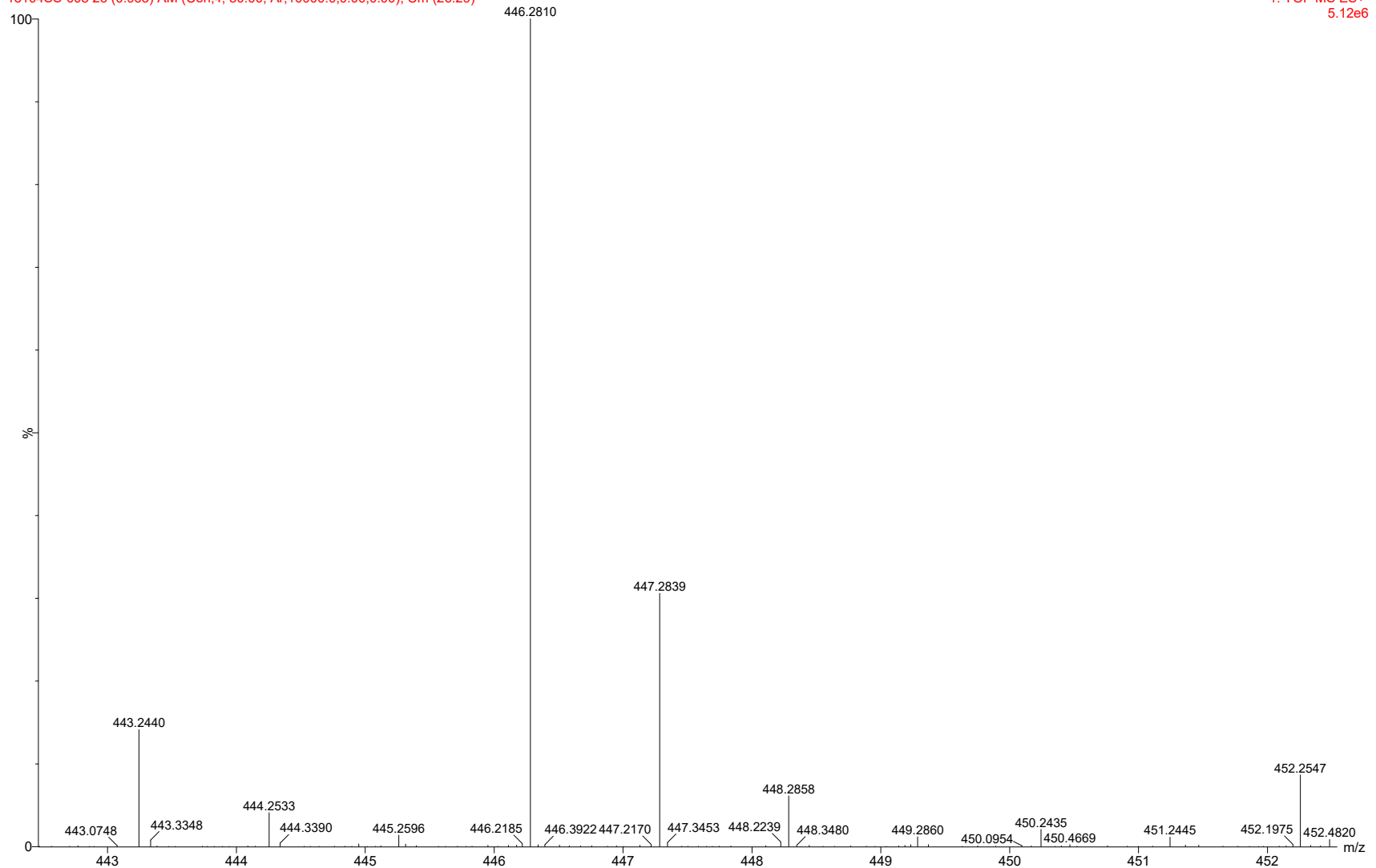
SYNAPTG2-Si#UGA589

24-Jul-2020 14:32:30

1: TOF MS ES+  
4.53e6

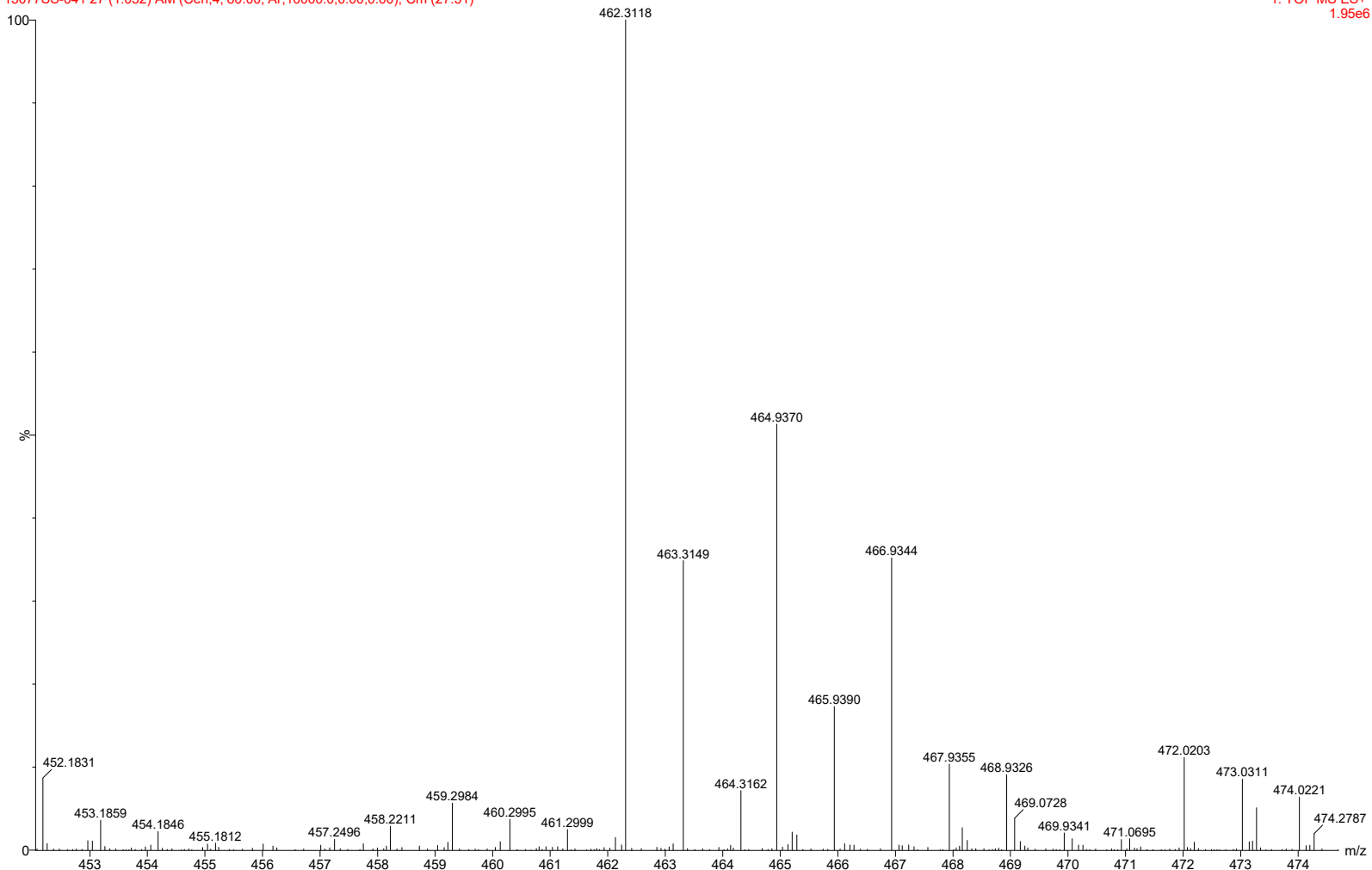


HRMS of TPS-16



HRMS of TPS-18





HRMS of TPS-19

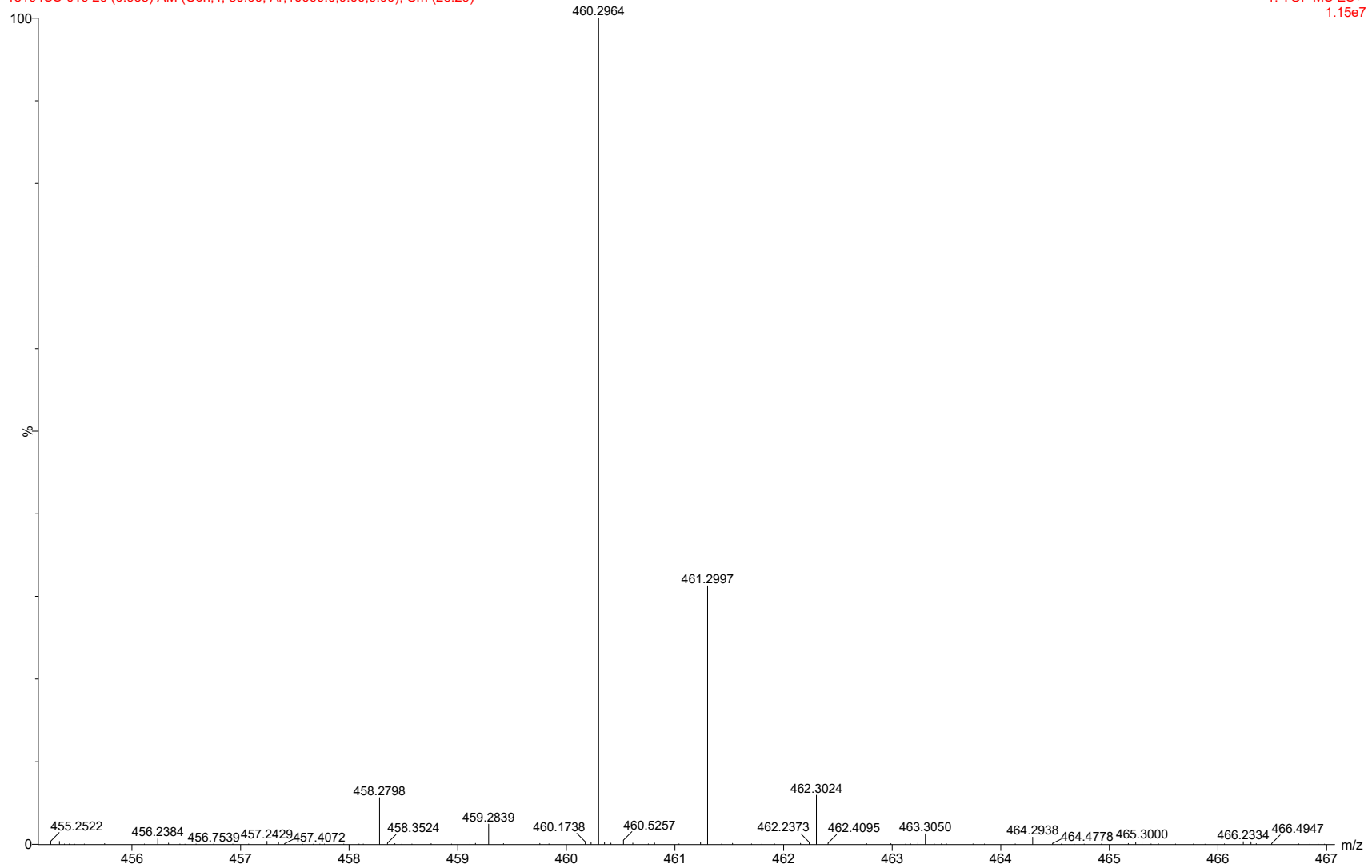
V-59  
Gardner

SYNAPT G2-Si#UGA589

21-Aug-2020 14:10:52

13104SS-010 26 (0.963) AM (Cen,4, 80.00, Ar,10000.0,0.00,0.00); Cm (25:29)

1: TOF MS ES+  
1.15e7



HRMS of TPS-22

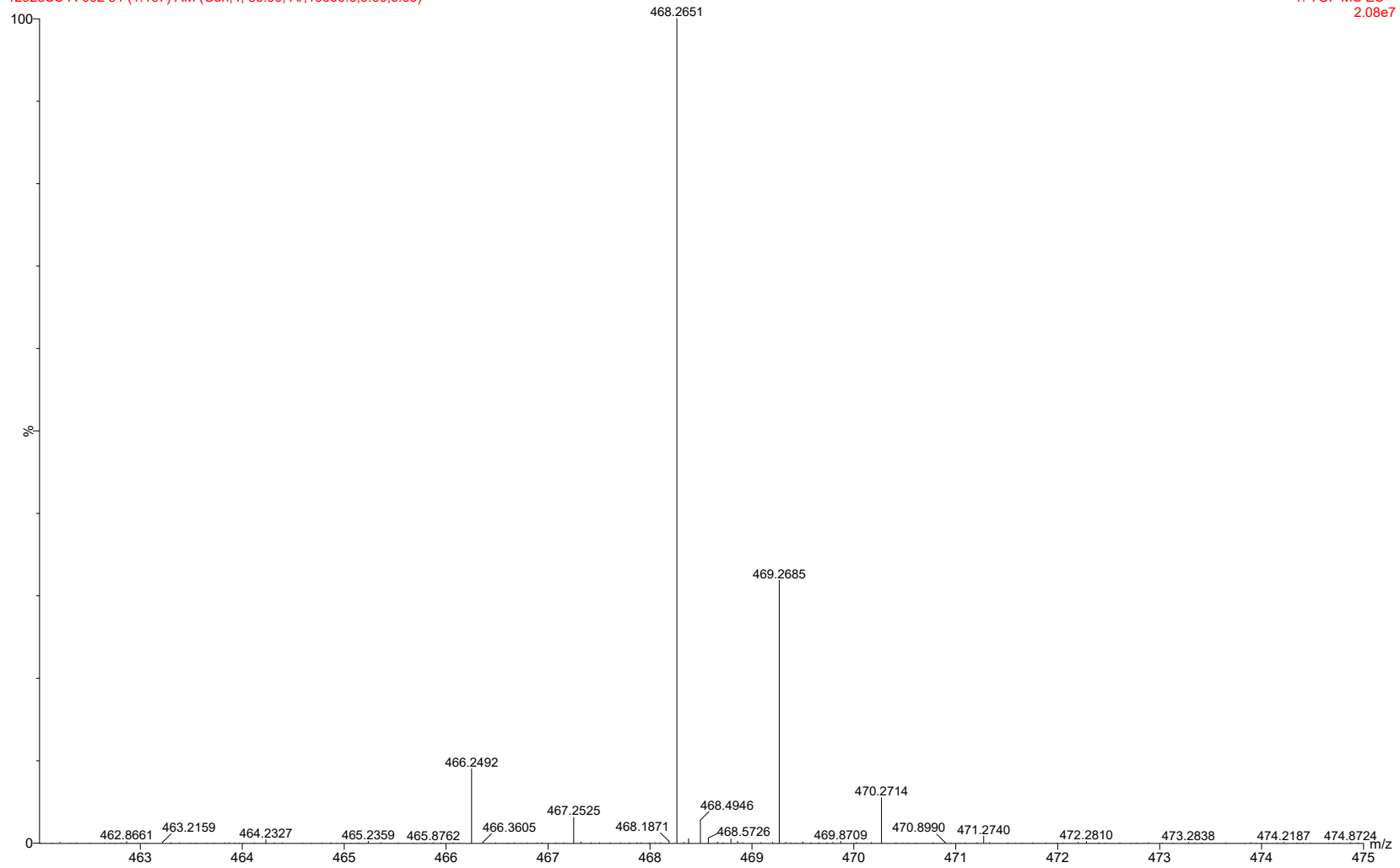
TPS-14  
Gardner

12923SS-A-002 31 (1.167) AM (Cen,4, 80.00, Ar,10000.0,0.00,0.00)

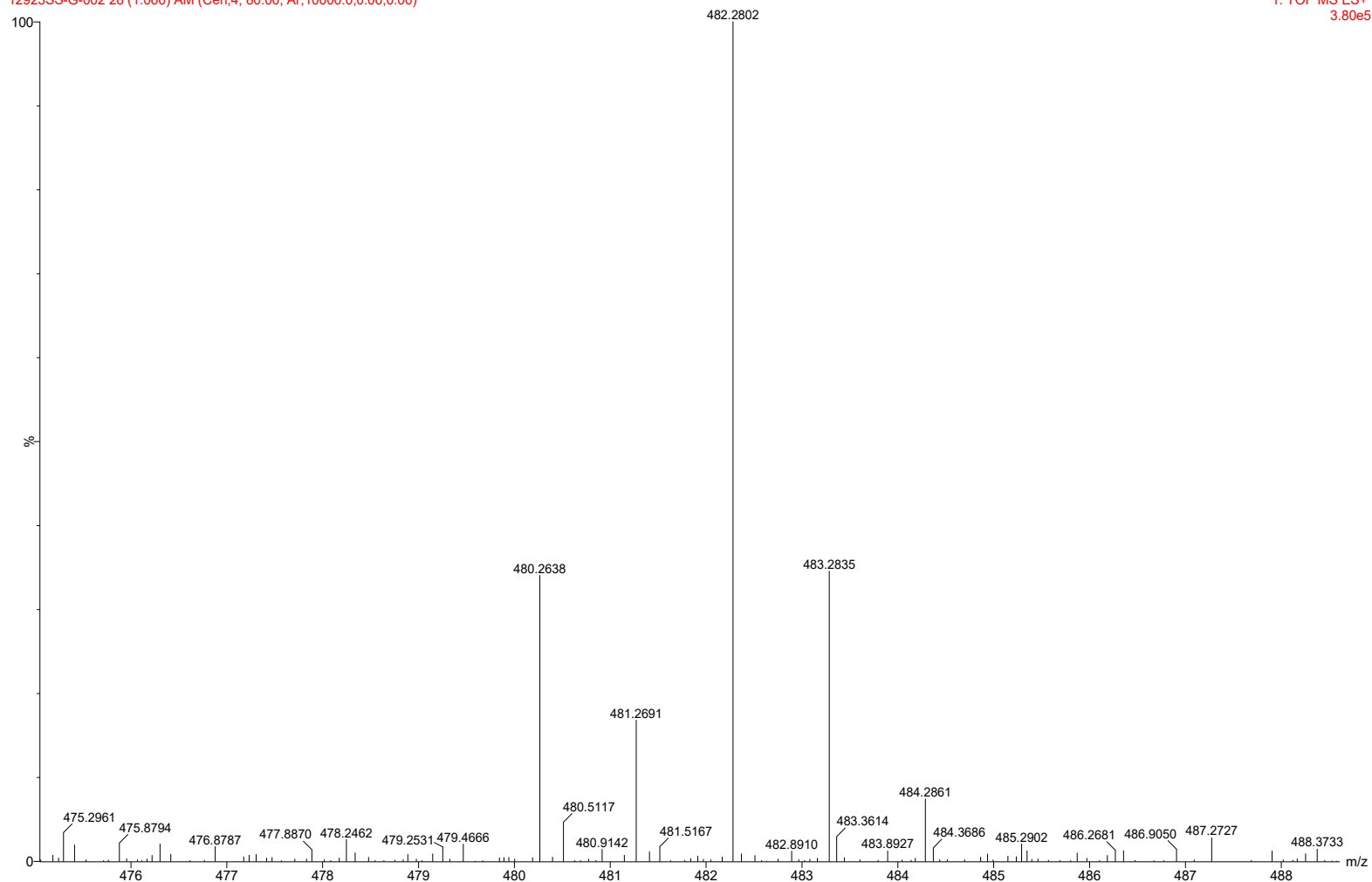
SYNAPT G2-Si#UGA589

22-Aug-2019 10:49:07

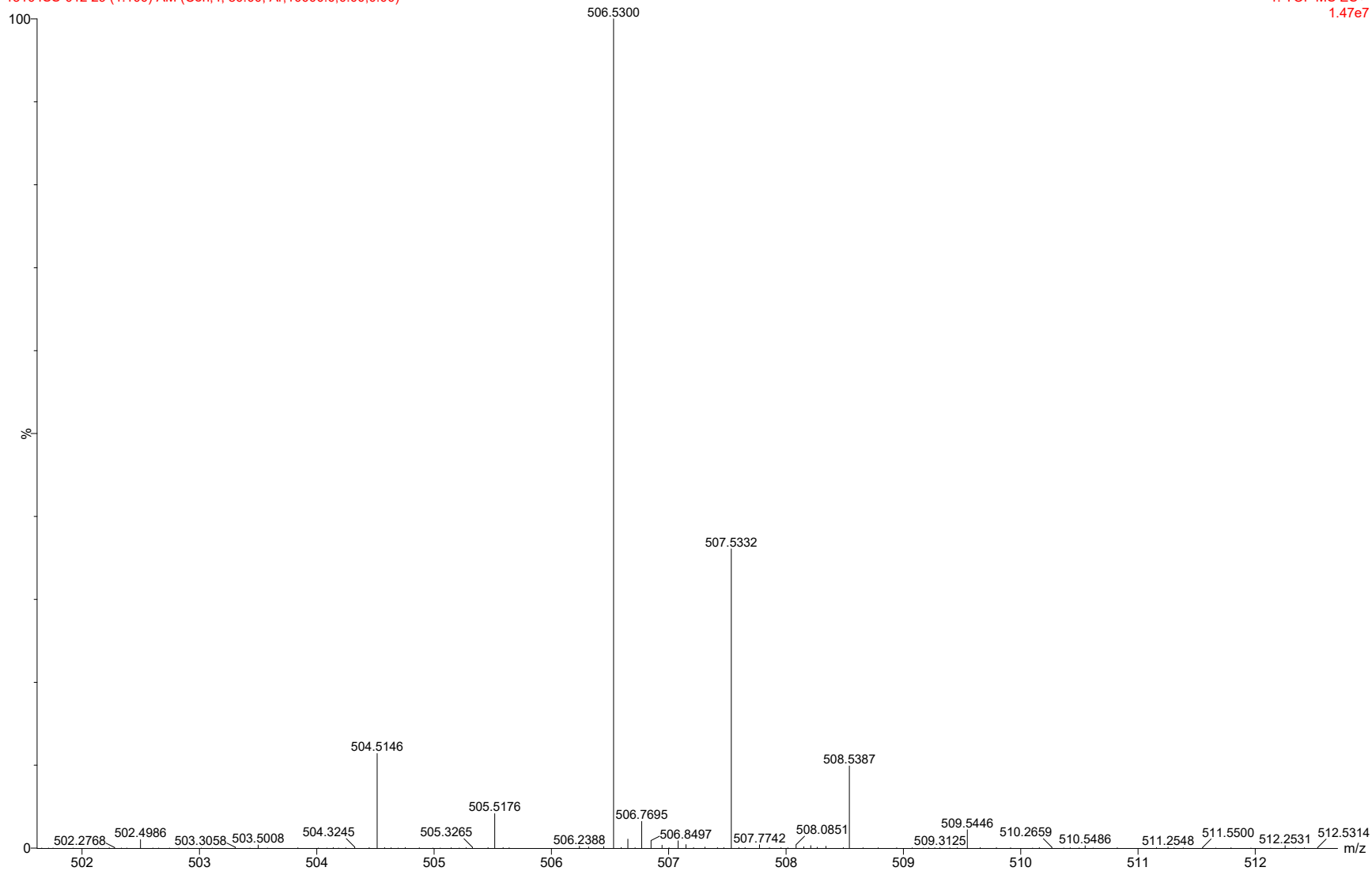
1: TOF MS ES+  
2.08e7



HRMS of TPS-24



HRMS of TPS-25



HRMS of TPS-26

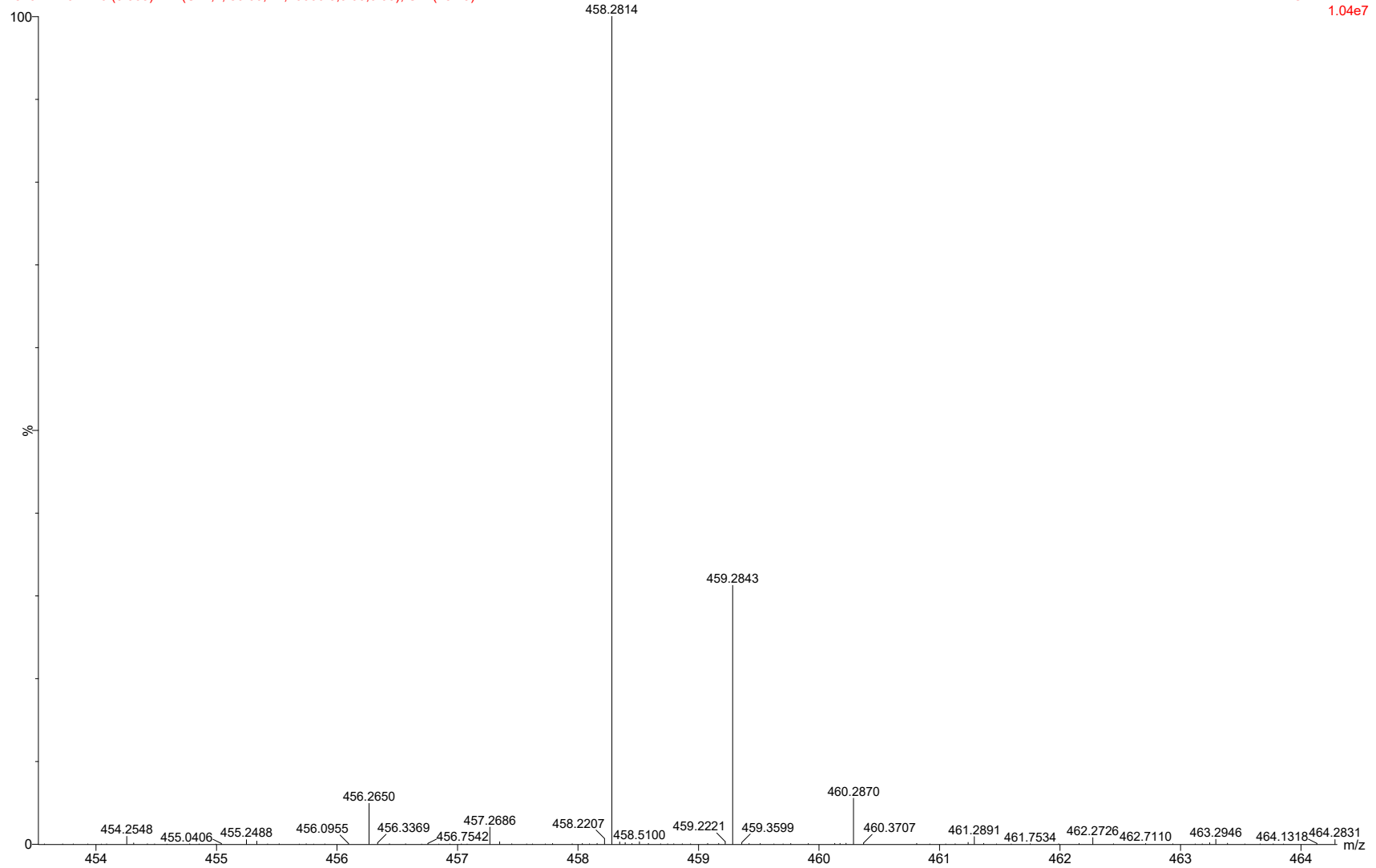
V-32  
Gardner

SYNAPT G2-Si#UGA589

21-Aug-2020 14:27:53

13104SS-014 26 (0.963) AM (Cen,4, 80.00, Ar,10000.0,0.00,0.00); Cm (25:29)

1: TOF MS ES+  
1.04e7



HRMS of TPS-27

S70

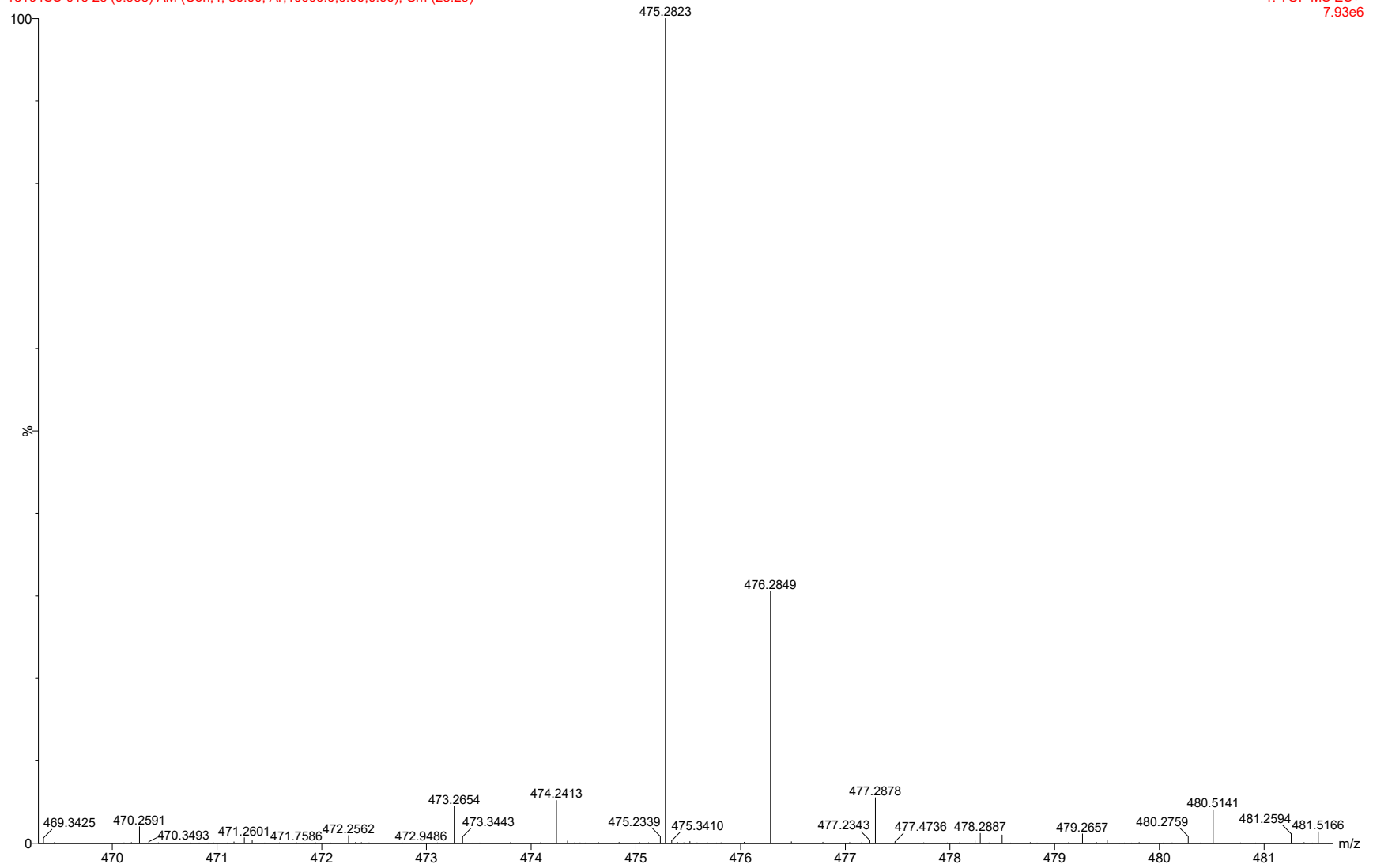
V-36  
Gardner

SYNAPT2-Si#UGA589

21-Aug-2020 14:35:10

13104SS-016 26 (0.963) AM (Cen.4, 80.00, Ar,10000.0,0.00,0.00); Cm (25:29)

1: TOF MS ES+  
7.93e6



HRMS of TPS-34

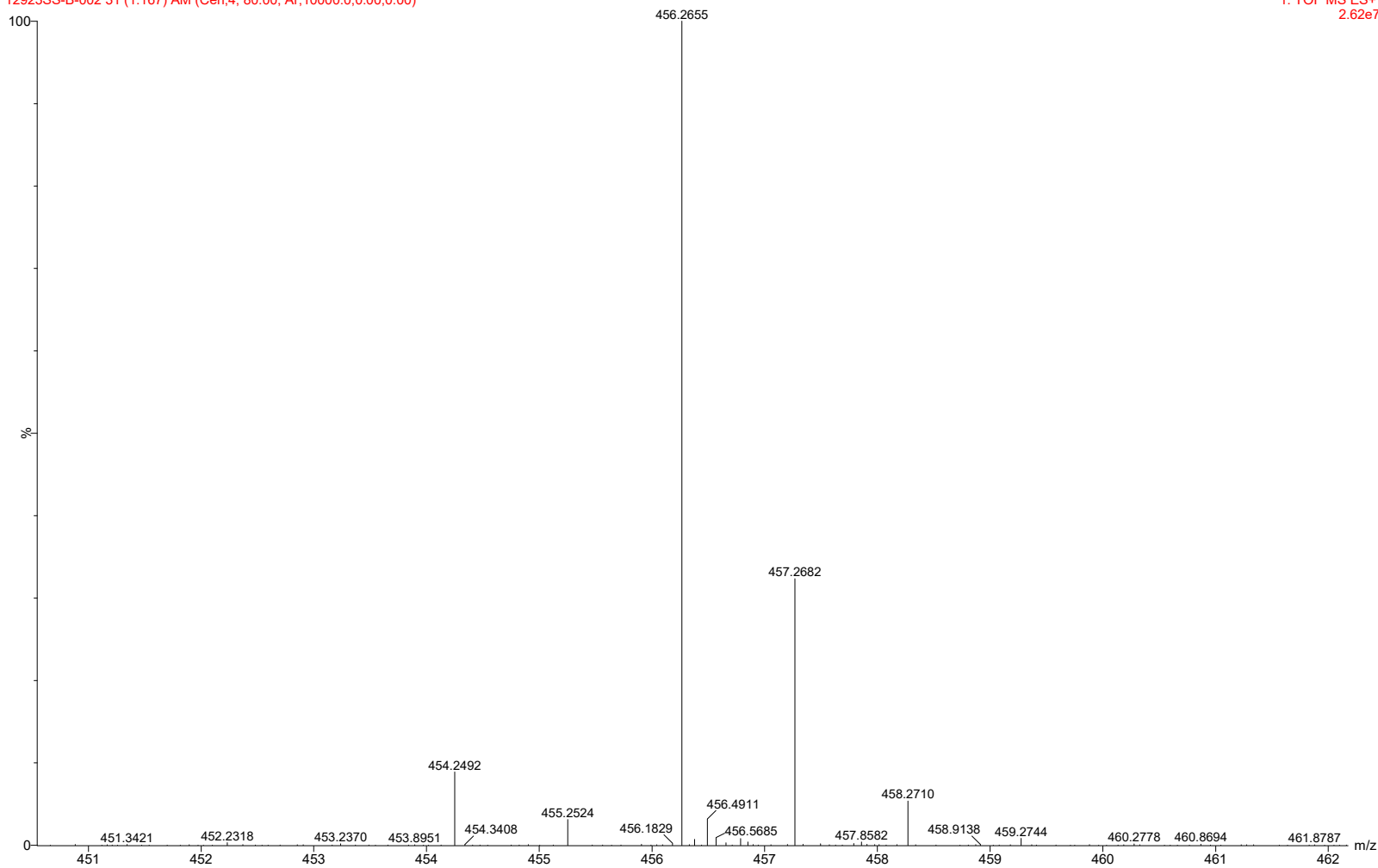
TPS-44  
Gardner

12923SS-B-002 31 (1.167) AM (Cen,4, 80.00, Ar,10000.0,0.00,0.00)

SYNAPT G2-Si#UGA589

22-Aug-2019 11:00:00

1: TOF MS ES+  
2.62e7



HRMS of TPS-46

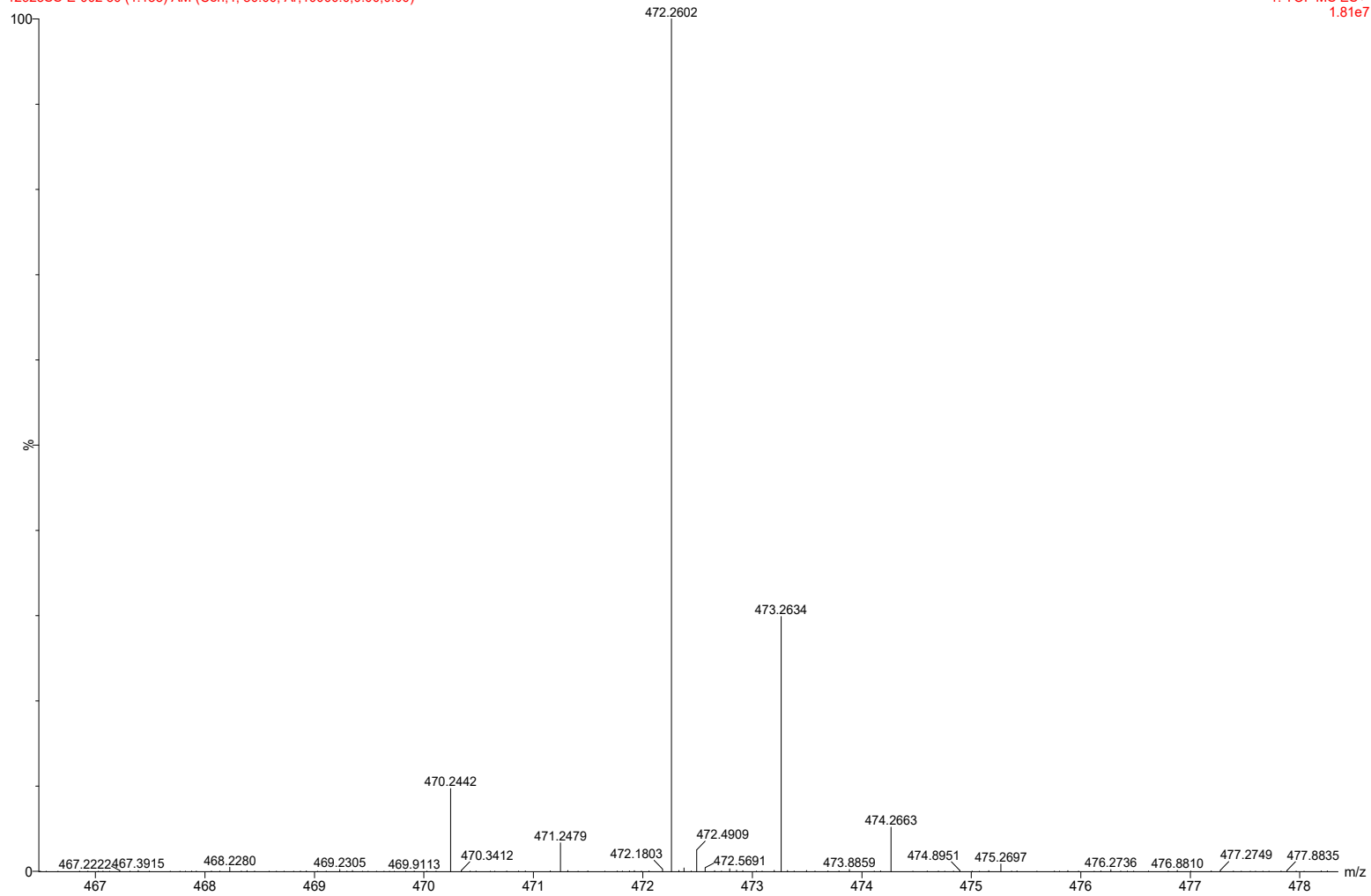


TPS-41  
Gardner  
12923SS-E-002 30 (1.133) AM (Cen,4, 80.00, Ar,10000.0,0.00,0.00)

SYNAPT G2-Si#UGA589

22-Aug-2019 11:32:24

1: TOF MS ES+  
1.81e7



HRMS of TPS-52

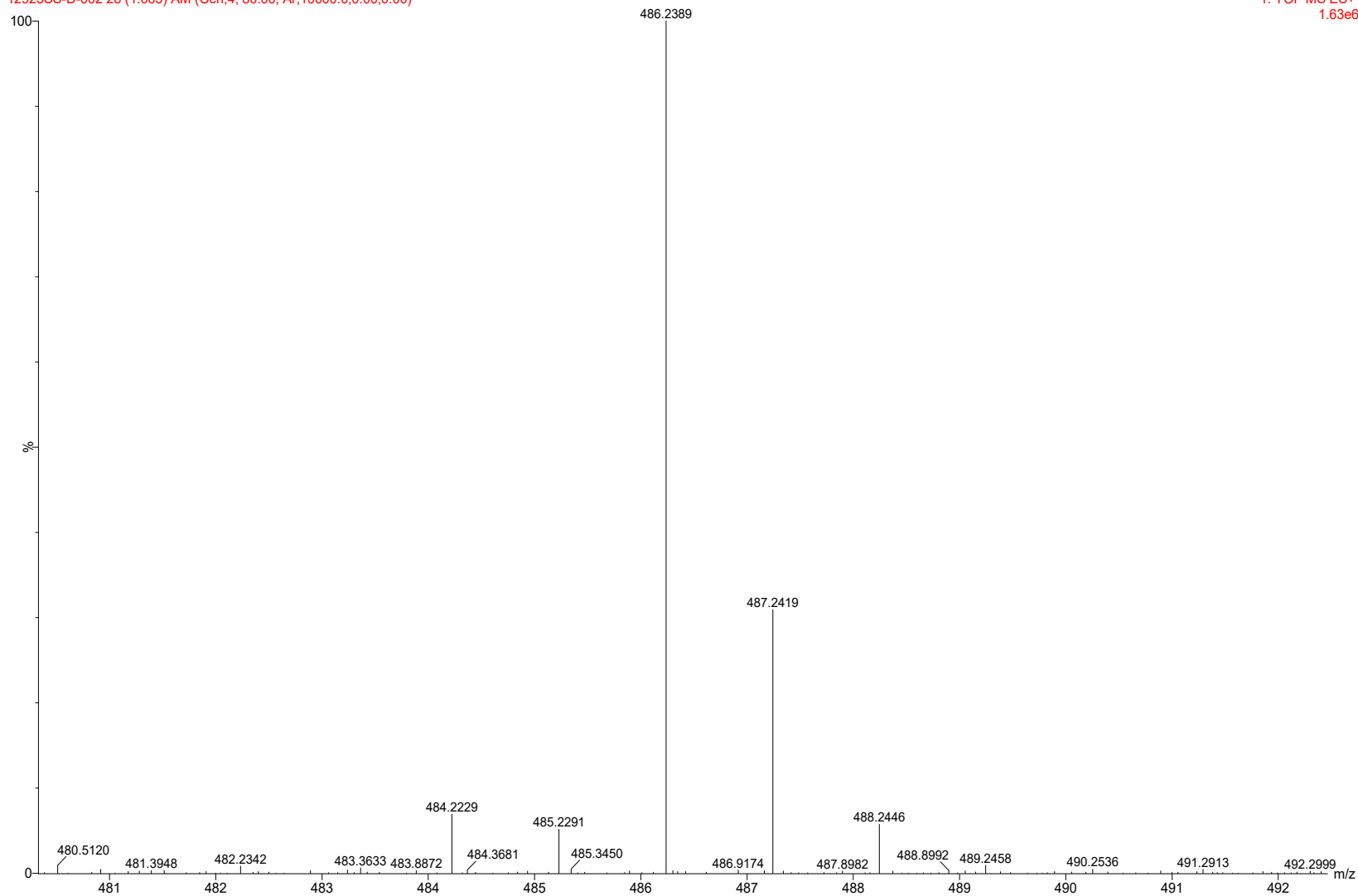
EDG-46  
Gardner

12923SS-D-002 28 (1.065) AM (Cen,4, 80.00, Ar,10000.0,0.00,0.00)

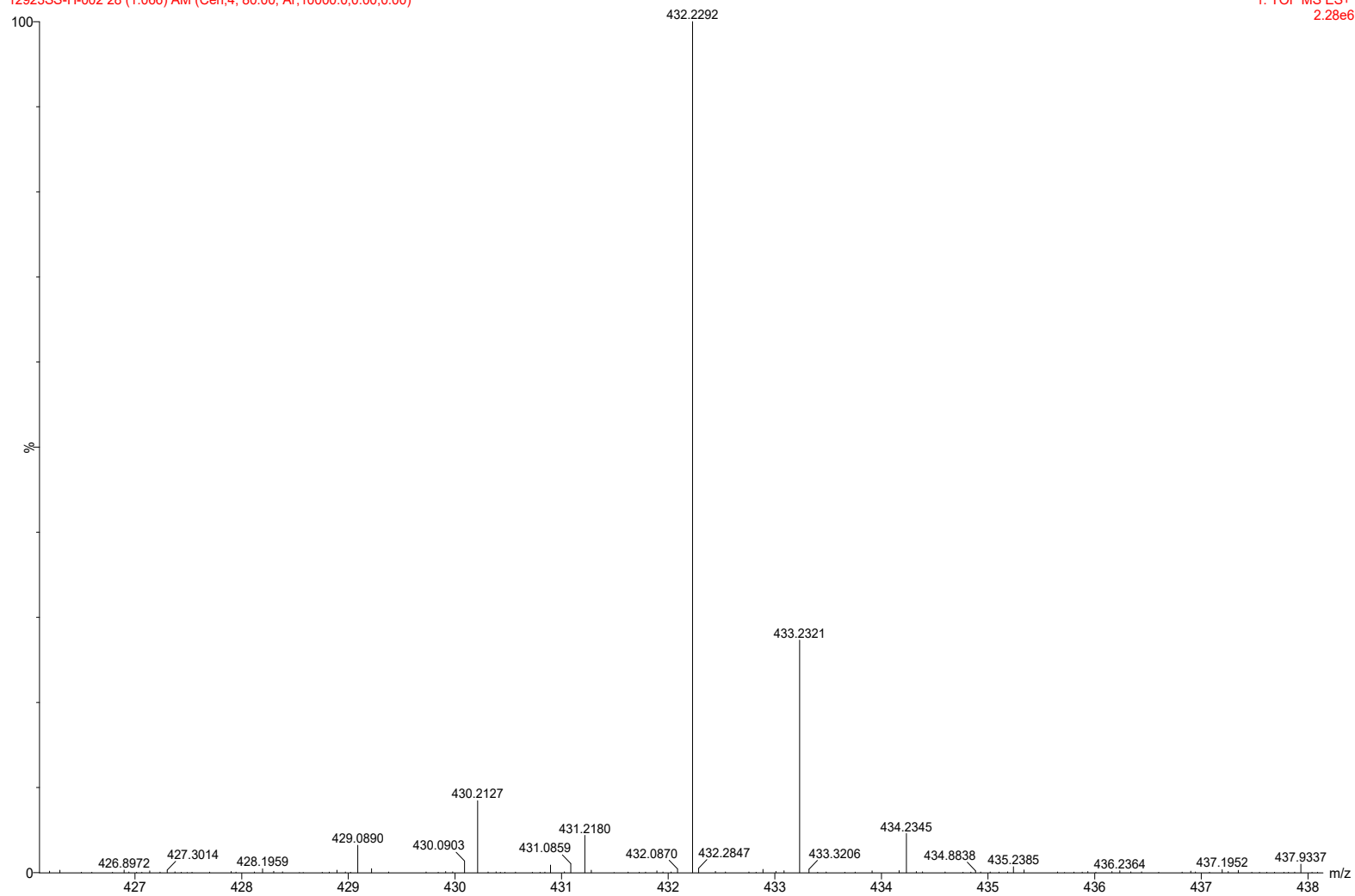
SYNAPTG2-Si#UGA589

22-Aug-2019 11:21:38

1: TOF MS ES+  
1.63e6



HRMS of TPS-58



HRMS of TPS-61-P1

TPS-70-P2

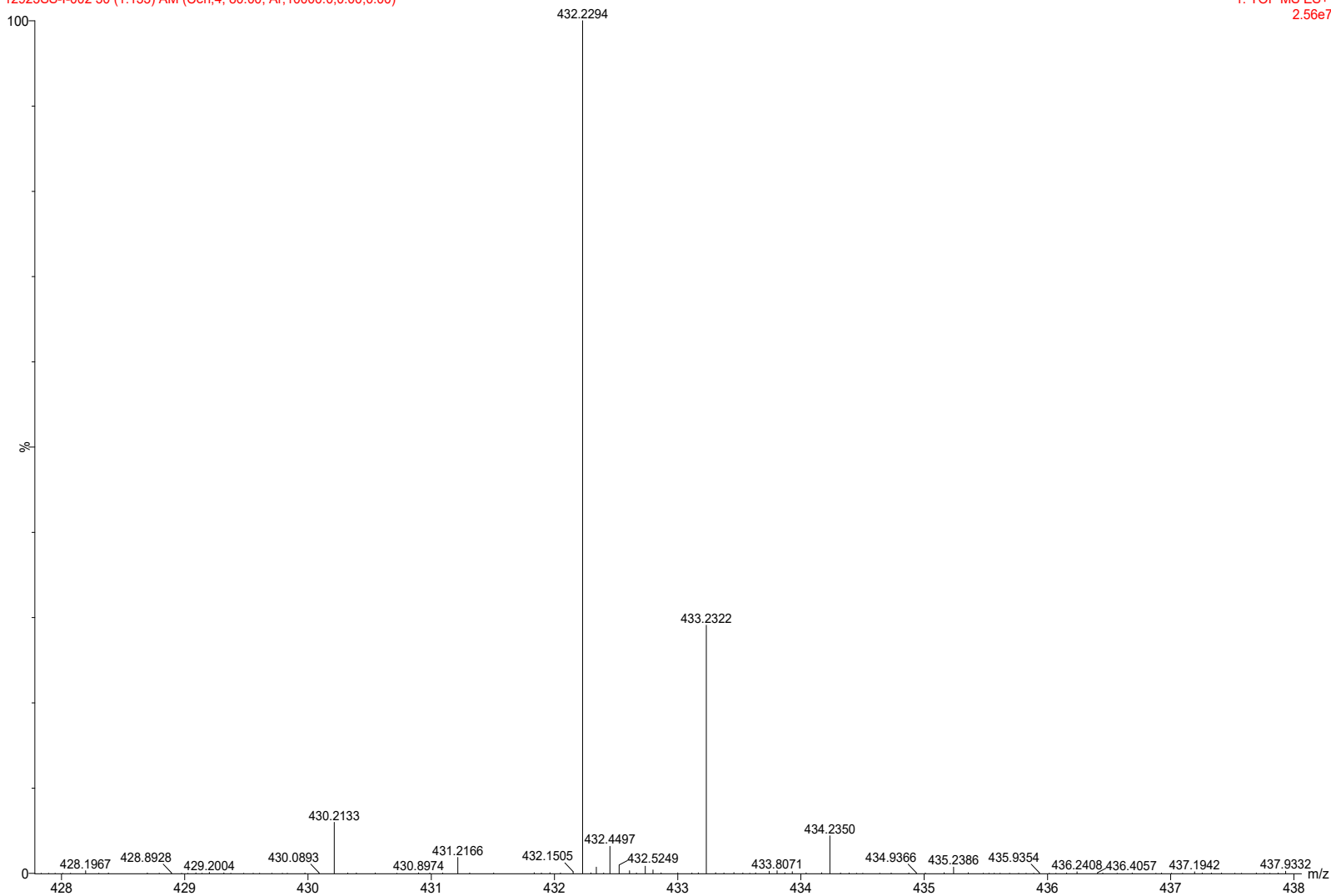
Gardner

12923SS-I-002 30 (1.133) AM (Cen,4, 80.00, Ar,10000.0,0.00,0.00)

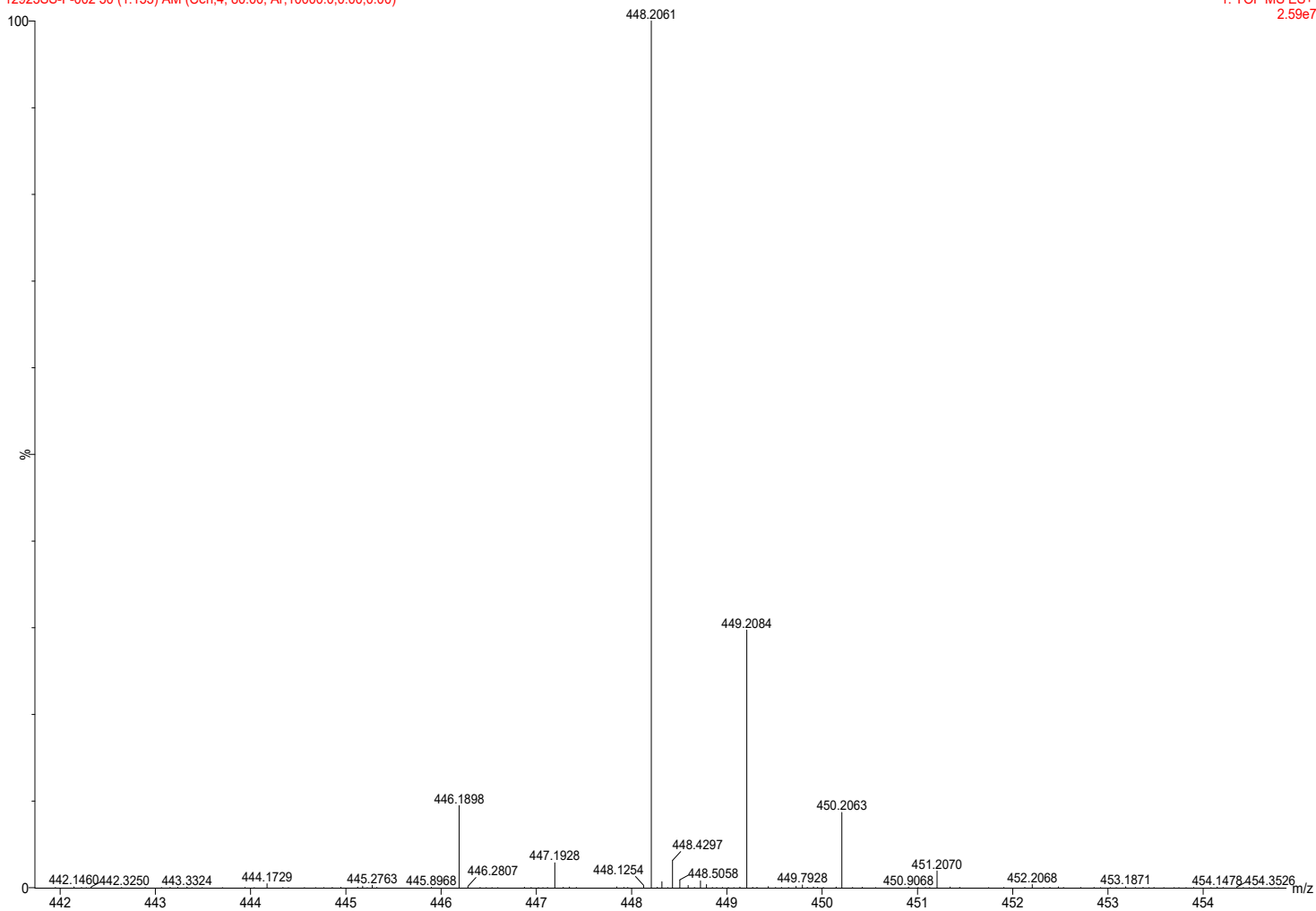
SYNAPT G2-Si#UGA589

22-Aug-2019 12:15:57

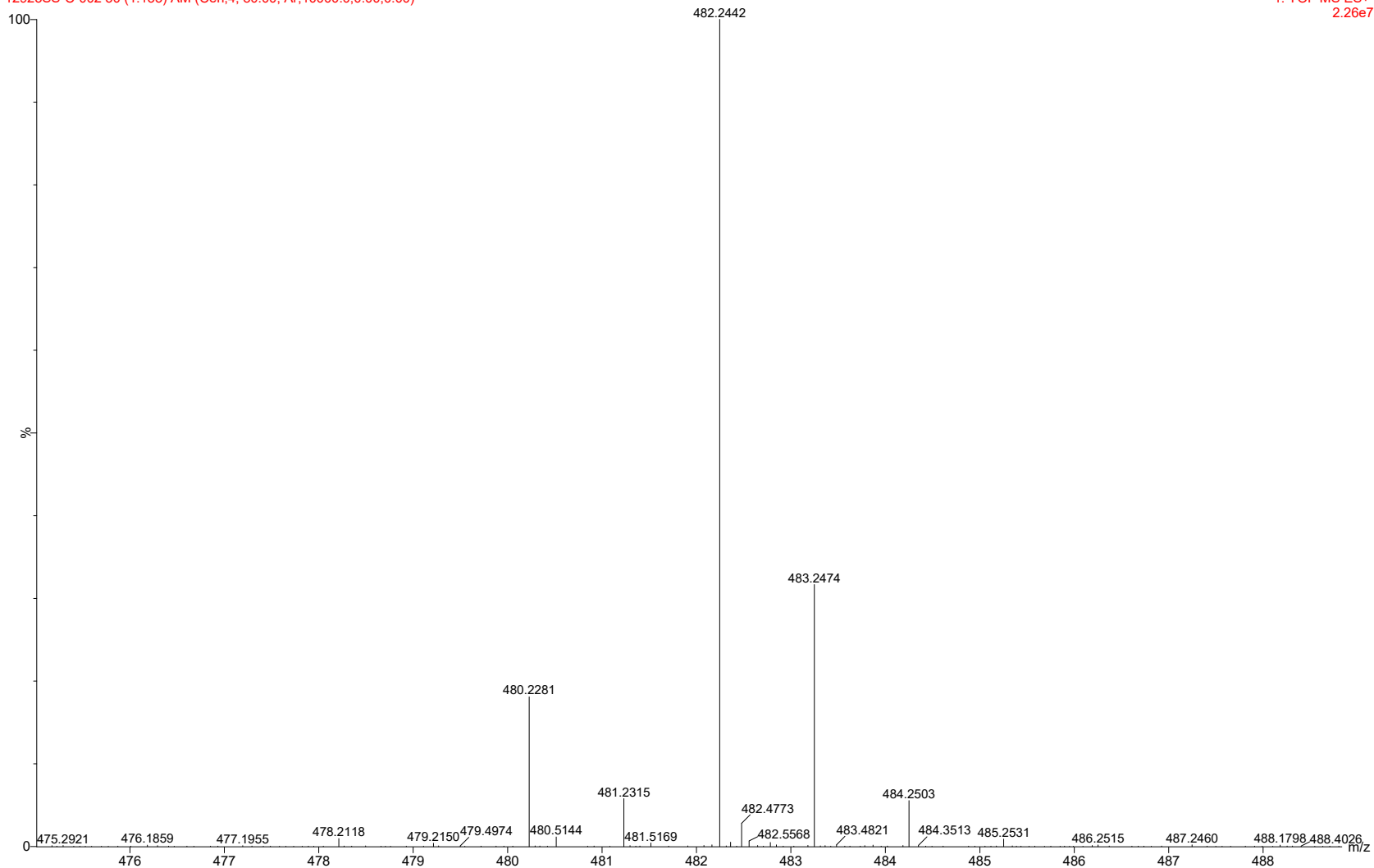
1: TOF MS ES+  
2.56e7



HRMS of TPS-61-P2



HRMS of TPS-62



HRMS of TPS-65

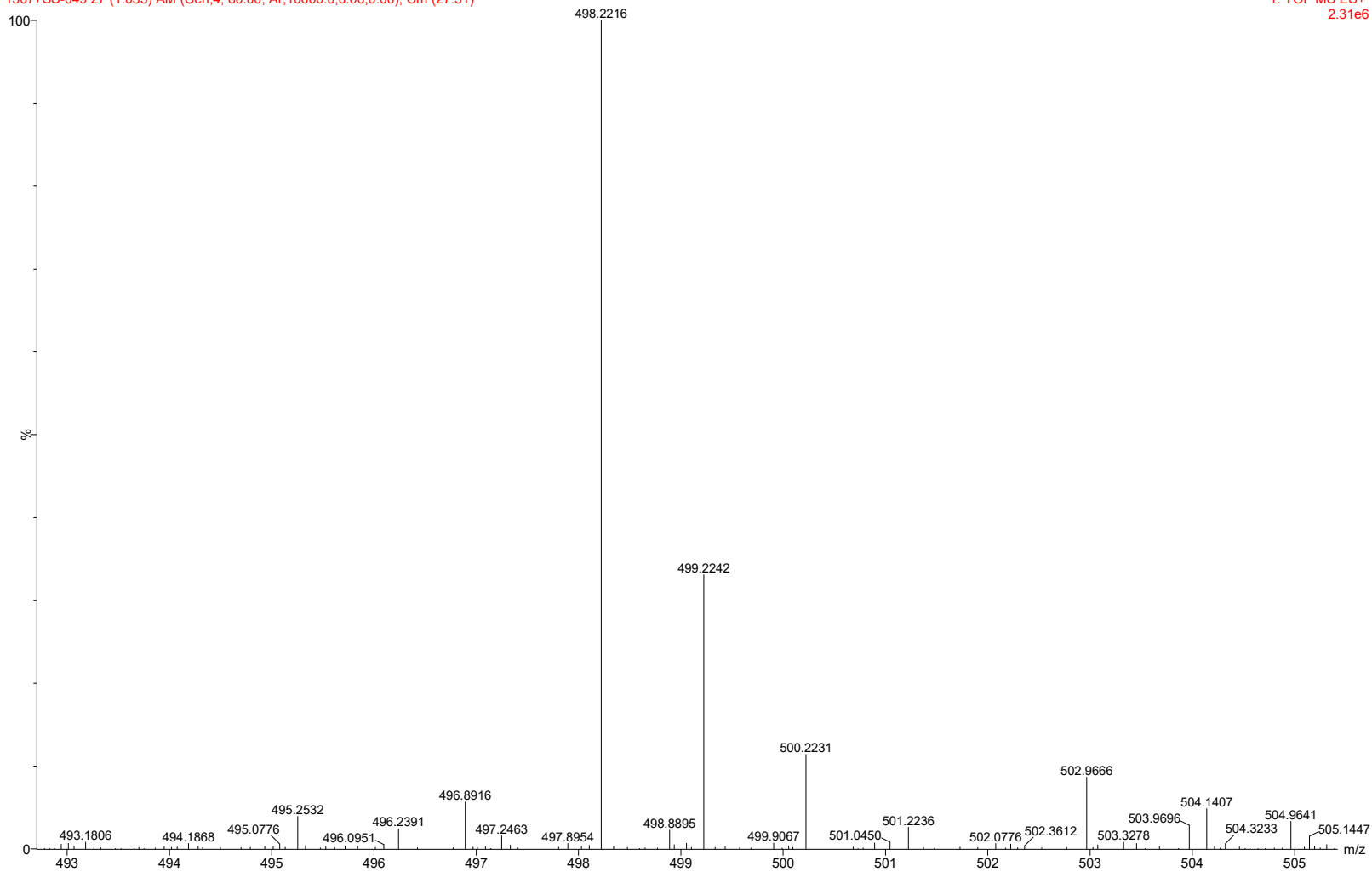
TPS-67  
Gardner

SYNAPT G2-Si#UGA589

24-Jul-2020 15:59:30

13077SS-049 27 (1.033) AM (Cen,4, 80.00, Ar,10000.0,0.00,0.00); Cm (27:31)

1: TOF MS ES+  
2.31e6



HRMS of TPS-66