

Supporting Information

Synthetic Biology Facilitates Semi-Synthetic Development of Type V Glycopeptide Antibiotics Targeting Vancomycin Resistant *Enterococcus*

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Chemicals

All chemicals were Sigma Aldrich products.

Flash purification

Compounds were dried onto C18 resin before being packed onto a 5 g solid loading cartridge (Teledyne Isco, Inc) and loaded onto a C18 column (5 g, Teledyne Isco) for flash purification. Products were eluted with a gradient of acetonitrile in water.

HPLC analysis

Rimomycin analogs were analyzed on a Symmetry Shield RP8 Column (100 Å, 3.5 µm, 4.6 mm × 150 mm, Waters WAT094269). HPLC program used for rimomycins detection is at t = 0-1 min, 5% solvent B; t = 20-22 min, 100% solvent B; t = 23-27 min, 5% solvent B using H₂O (0.1% trifluoroacetic acid, solvent A) and acetonitrile (ACN, 0.1% TFA, solvent B) as mobile phases; 1 mL/min flow rate; 40 °C

HRMS analysis

High-resolution electrospray ionization mass spectra were acquired using an Agilent 1290 UPLC separation module and a qTOF 6550 mass detector in negative or positive ion mode. For LC separation an Agilent Eclipse XDB-C18 column (2.1 x 150 mm; 3.5 µm) or an XDB C8 column (2.1 x 150 mm; 3.5 µm) and the following method were used: from 0 to 1 min 75% A (0.1 v/v formic acid in water), from 1 to 7 min a linear gradient to 100% B (0.1 v/v formic acid in acetonitrile) at a flow rate of 0.4 mL/min.

NMR analysis

All NMR spectra were recorded on an AVIII 700 MHz NMR spectrometer, equipped with a cryoprobe. The compounds used in this study were dissolved in the corresponding deuterated solvent (Cambridge Isotope Laboratories Inc) to a concentration of approximately 1.0 to 5.0 mg/ml. Chemical shifts are reported in ppm relative to TMS using the residual solvent signal at 2.50 ppm and 39.52 ppm for dms0-d6. Chemical shifts values are expressed in ppm (δ), coupling constants (J, Hz) and peak patterns are reported as broad singlet (bs), singlet (s), doublet (d), triplet (t), quartet (q), pentet (p), and multiplet (m)

Supplementary MIC values

Supplementary Table 1. Additional MIC values against *S. aureus*, including VISA strains in $\mu\text{g/ml}$.

	Vancomycin	Corbomycin	Rimomycin	17	
<i>S. aureus</i> ATCC 29213	1	4	4	16	
<i>S. aureus</i> ATCC 33591	2	4	4	16	
<i>S. aureus</i> Mu3	2	4	4	8	hVISA
<i>S. aureus</i> Mu50	4	4	4	16	VISA
<i>E. faecium</i> ATCC 51299			8	2	
<i>E. faecalis</i> ATCC 70022			8	1	

Toxicity studies

The equation used for the 4PL curves was:

$$y = d + (a - d) / (1 + (x/c)^b),$$

where y = the sample response in relative luminescence units

x = drug concentration

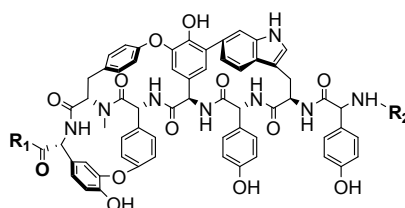
a = the maximum response for infinite standard concentration

b = -Hill slope

c = inflection point

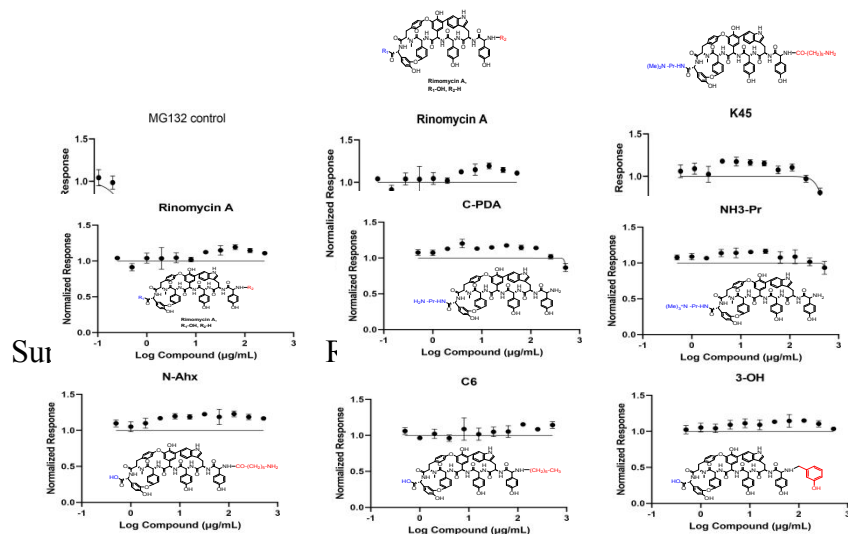
d = the response at a standard concentration of 0.

Supplementary Table 2. Toxicity studies of selected analogs, IC₅₀ values.



Compound# [Substitution]	Hill slope	IC ₅₀ (µg/ml)	R ²	95% Confidence interval	
Rimomycin A	Unstable	Unstable	-0.6694	Very wide	
R ₁	R ₂				
6. NH ₂ -Pr-NH-	H	-22.30	556.7	-0.9887	Very wide
9. ⁺ NMe ₃ -Pr-NH-	H	-2140	579.6	-1.089	Very wide
13. OH	-Hexyl	Unstable	Unstable	-0.4260	Very wide
16. OH	Ahx	Unstable	Unstable	-5.617	Very wide
17. [⁺ NMe ₃ -Pr-NH-	Ahx*	-4.052	729.0	-0.1540	Very wide
19. OH	-3-OH-Phenyl	Unstable	Unstable	-2.070	Very wide
MG132 control	-2.206	0.7938	-0.9474	0.5397 to 0.7412	

* Ahx- 6-Amino-hexanoic acid



	Concentration ($\mu\text{g/mL}$)										
	512	256	128	64	32	16	8	4	2	1	0.5
17	0.04	0.04	0.038	0.04	0.041	0.042	0.043	0.04	0.057	0.039	0.039
	0.039	0.04	0.043	0.04	0.04	0.041	0.043	0.041	0.041	0.041	0.04

Compound 17: % Hemolysis:

	Concentration ($\mu\text{g/mL}$)										
	512	256	128	64	32	16	8	4	2	1	0.5
17	0.00%	0.00%	0.00%	0.00%	0.95%	1.90%	2.84%	0.00%	16.11%	0.00%	0.00%
	0.00%	0.00%	2.84%	0.00%	0.00%	0.95%	2.84%	0.95%	0.95%	0.95%	0.00%
Average	0.00%	0.00%	1.42%	0.00%	0.47%	1.42%	2.84%	0.47%	8.53%	0.47%	0.00%

1% Triton X 100 avg absorbance: 0.1445

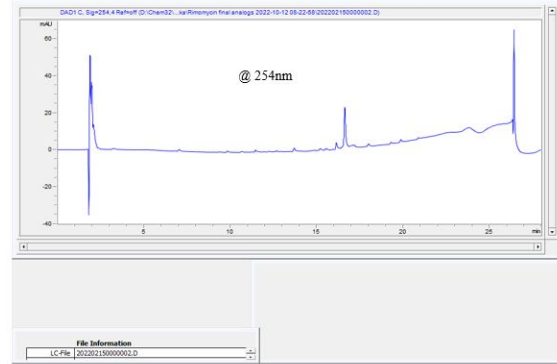
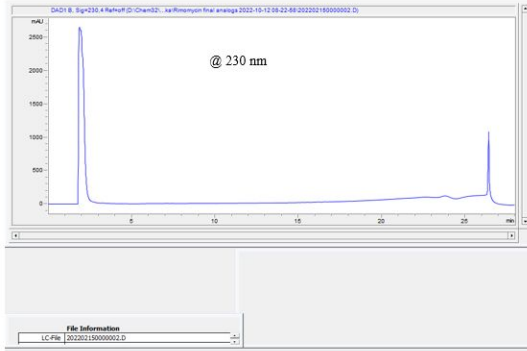
DMSO control avg absorbance: 0.039

Supplementary Table 4. Solubility assay.

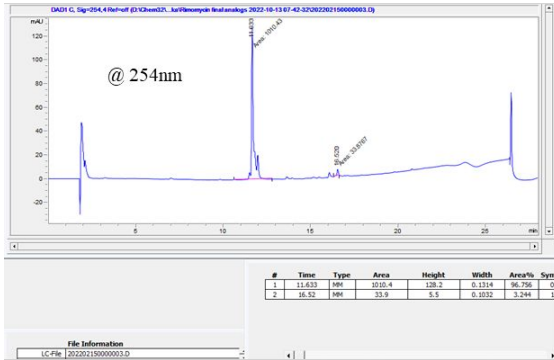
Compound	Rt, min	Transition (CE 30eV)	Solubility, $\mu\text{g/ml}$
Rimomycin A	3.402	1121.36 to 1104.35	6.1
17	3.017	659.78 to 113.10	98.6
6	2.810	589.22 to 107.05	28.4
7	2.856	589.22 to 107.05	8.3
21	3.8	1243.41 to 1243.41	16.4

Analytical Data for Rimomycin Analogs 1-32

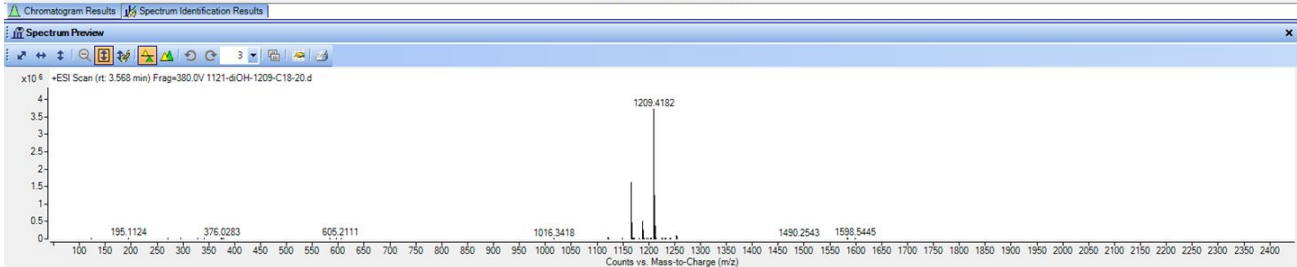
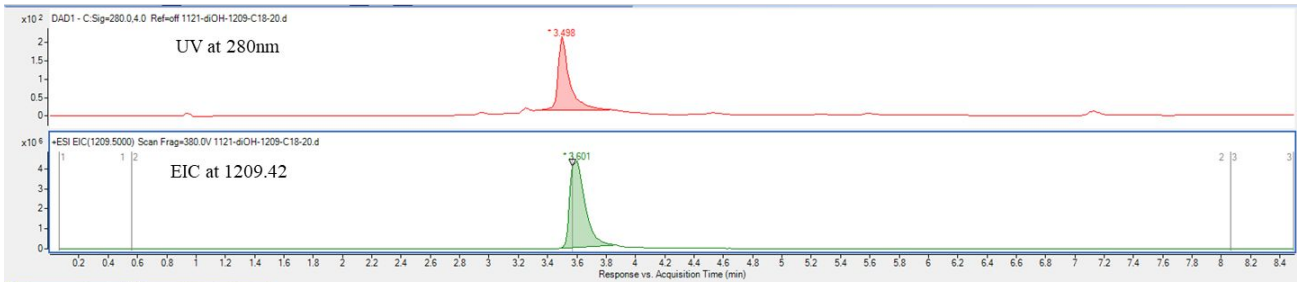
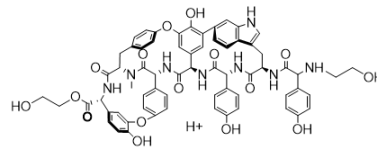
UV chromatograms of DMSO-blank



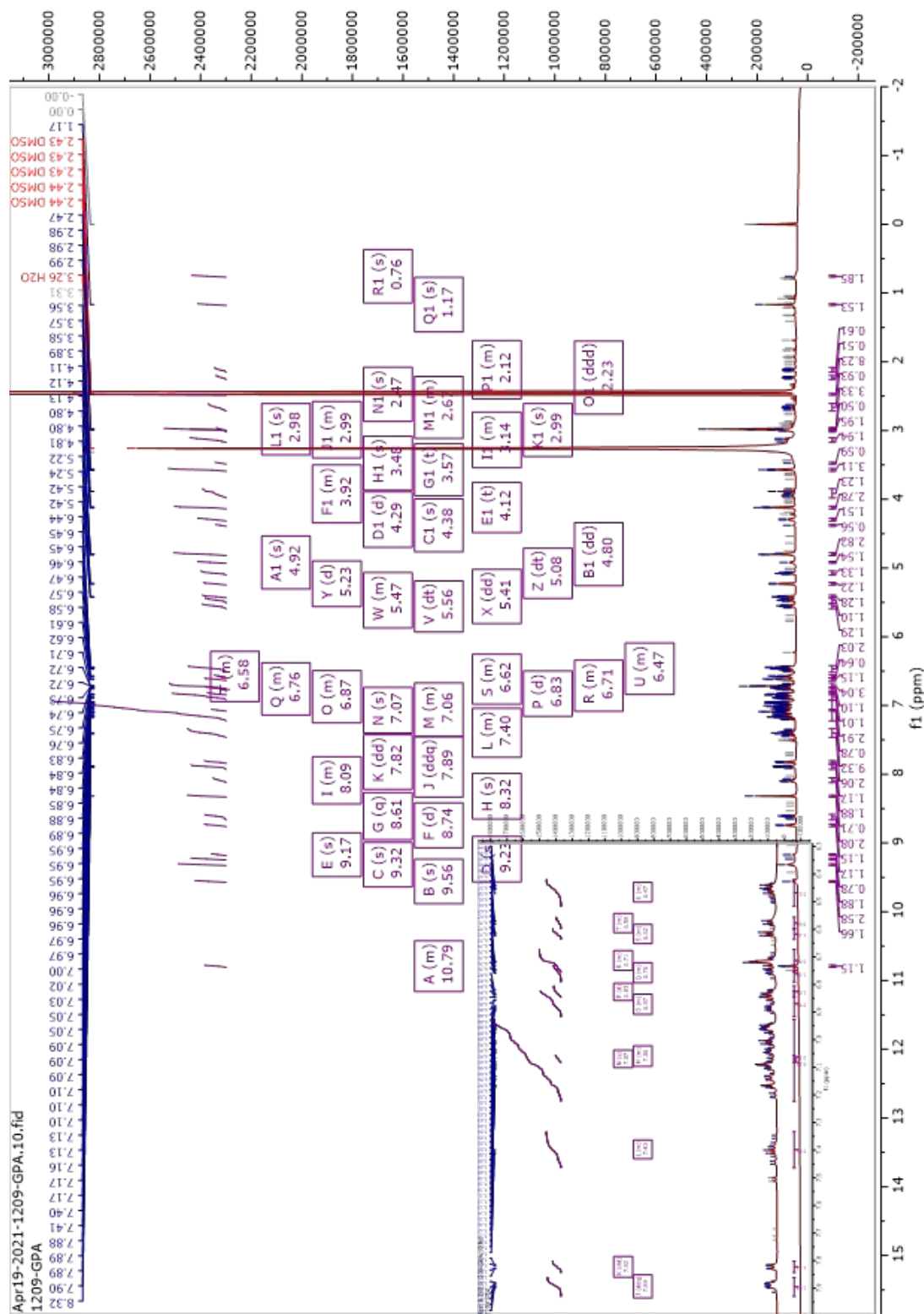
Compound 1



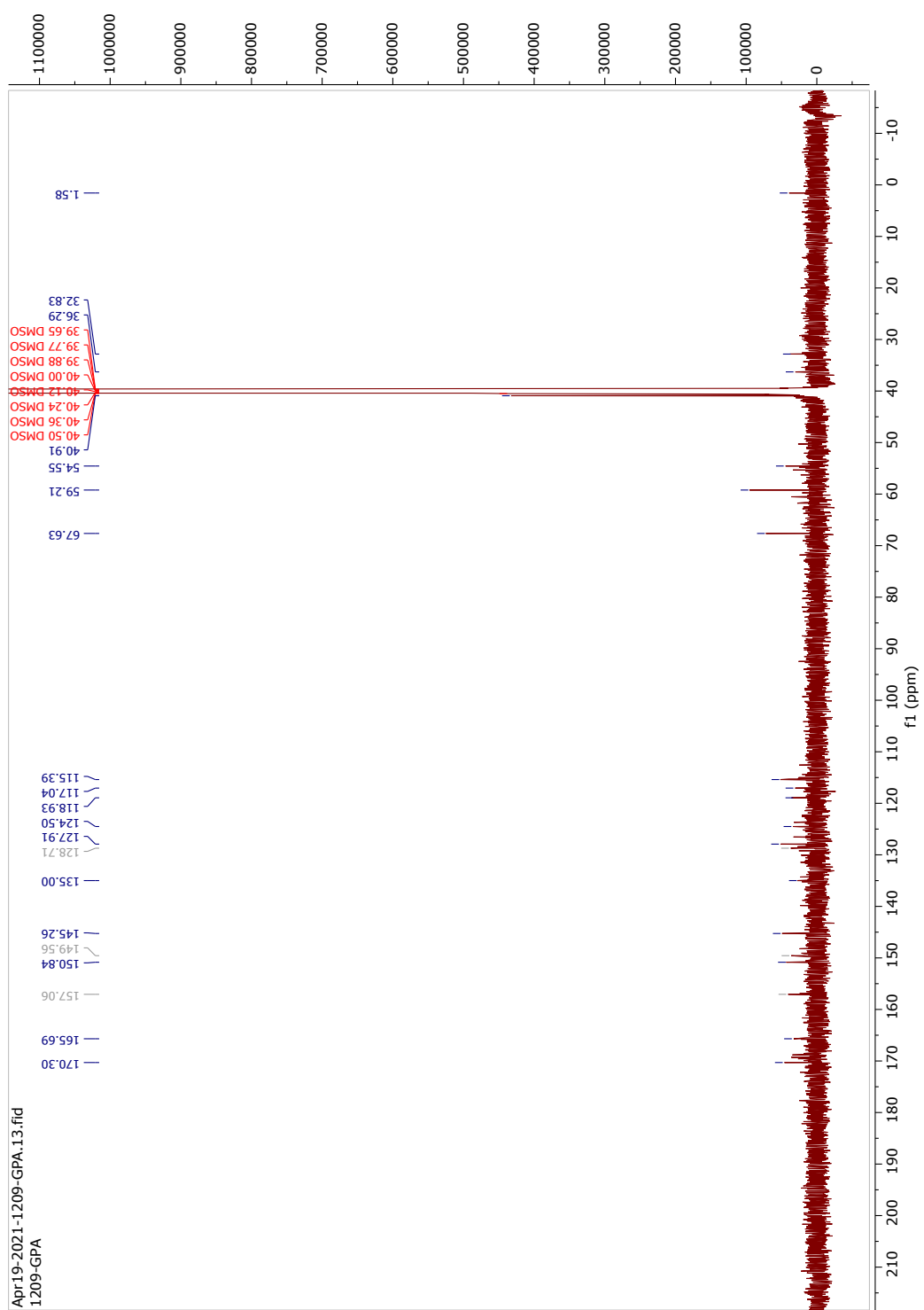
Compound 1



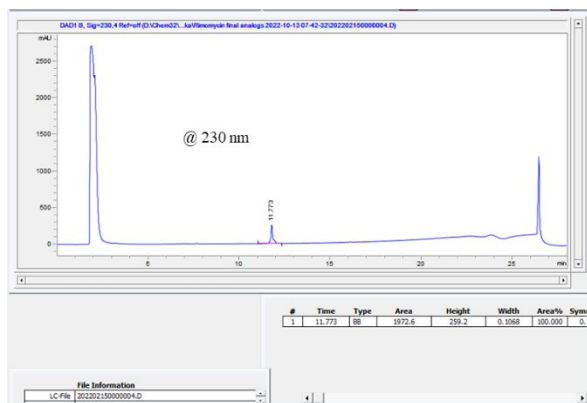
¹H-NMR of compound 1



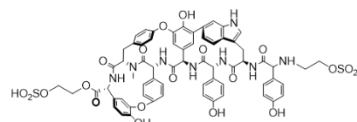
^{13}C -NMR of compound 1



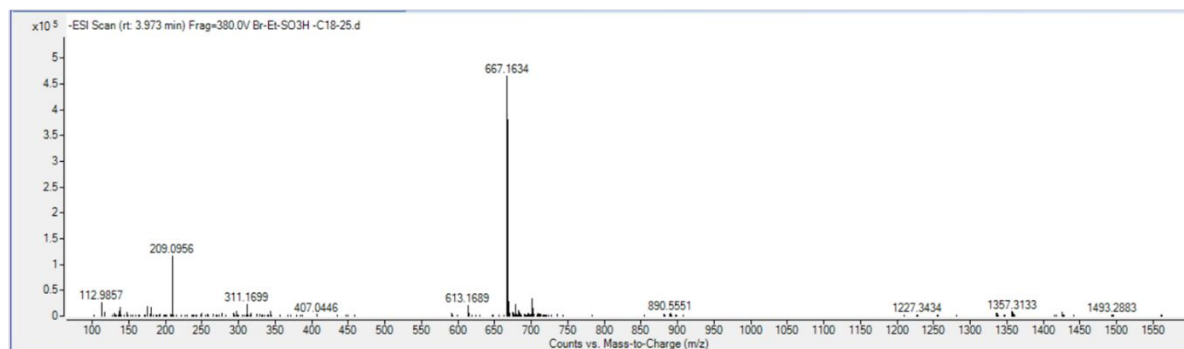
Compound 2



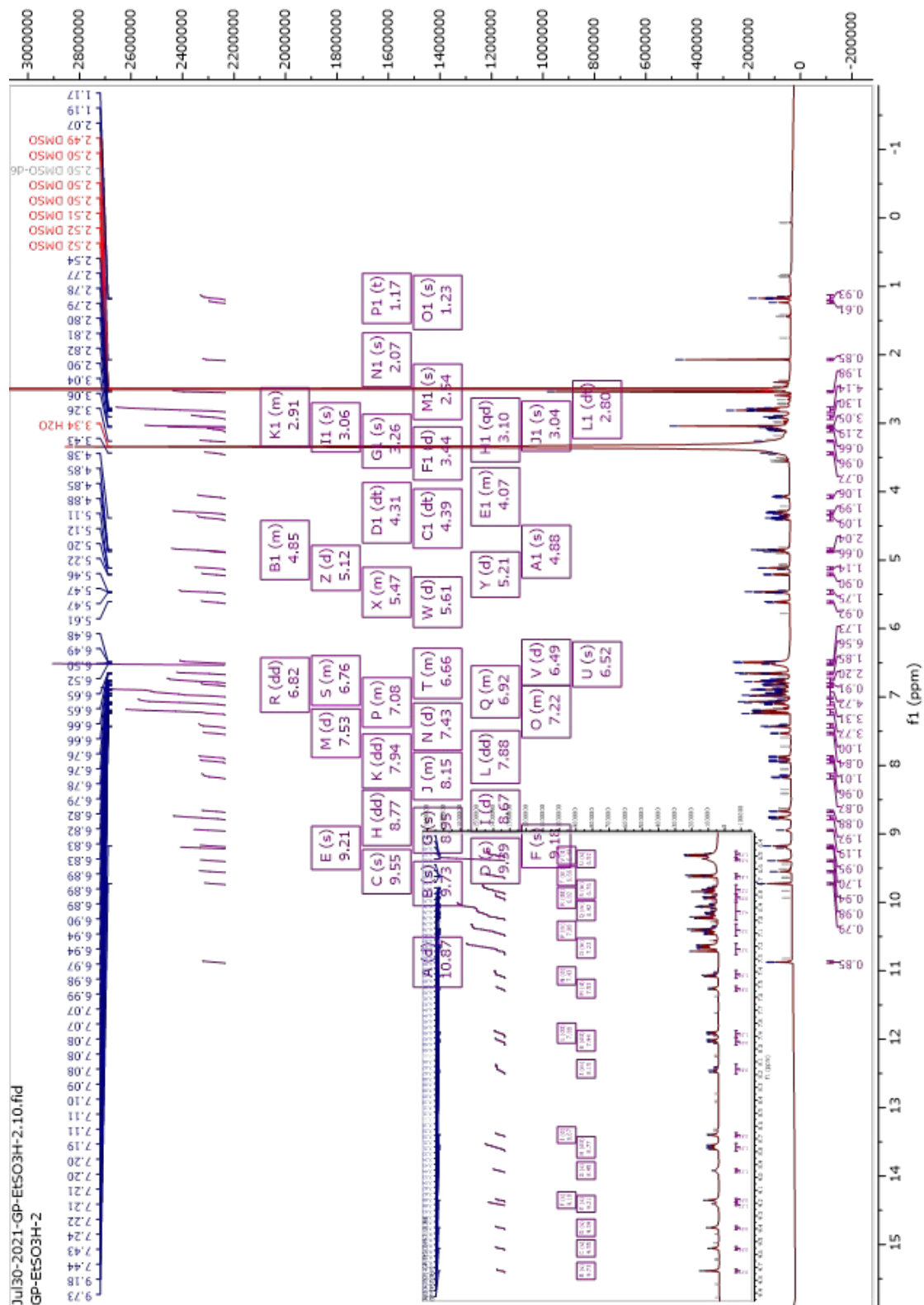
Compound 2



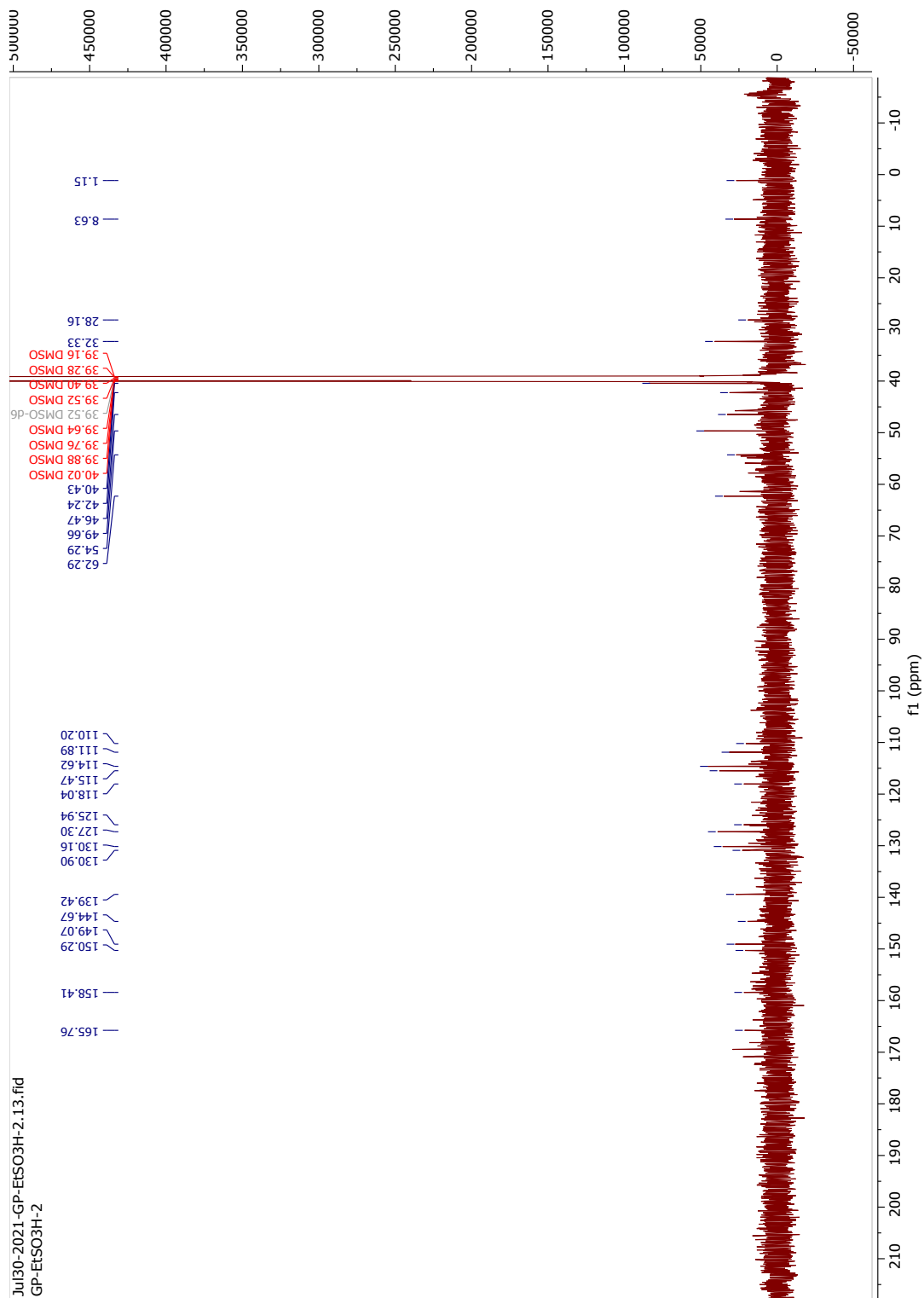
Chemical Formula: $C_{43}H_{49}N_8O_{20}S_2^-$
 Exact Mass: 1335.3293



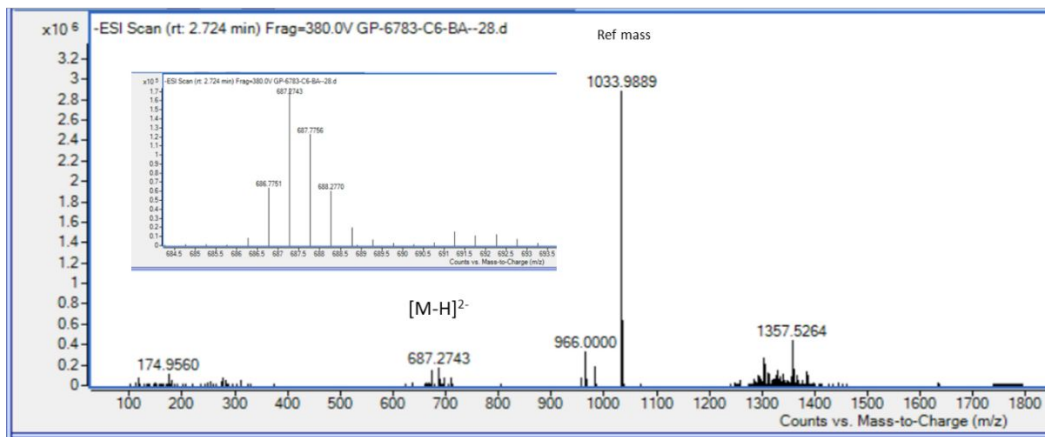
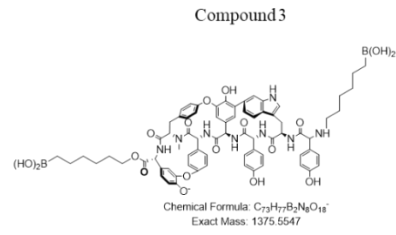
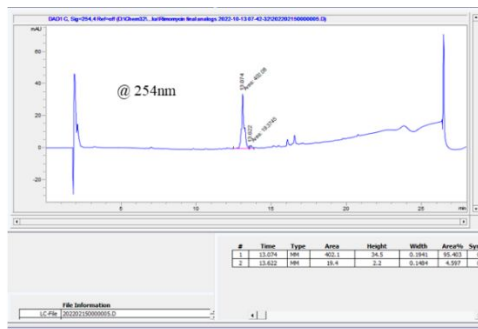
¹H-NMR of compound 2



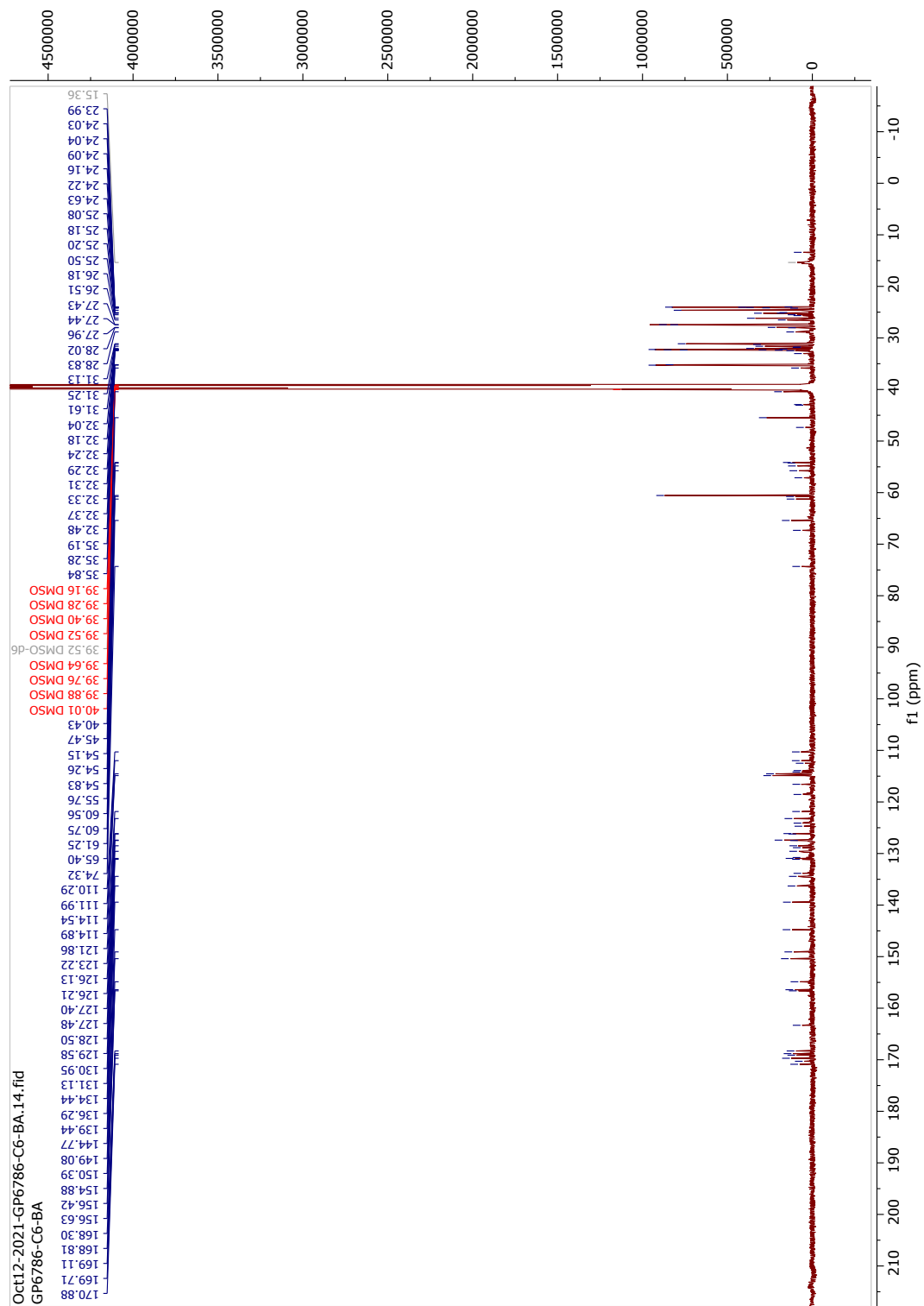
¹³C-NMR of compound 2



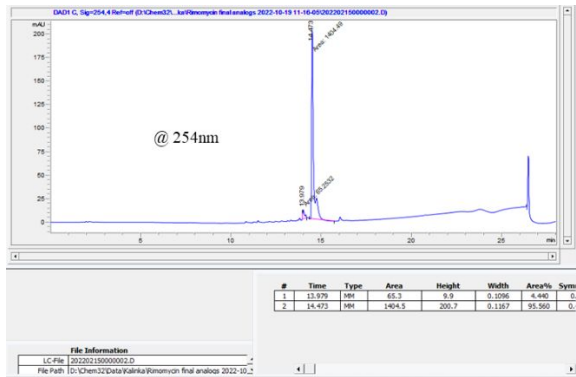
Compound 3



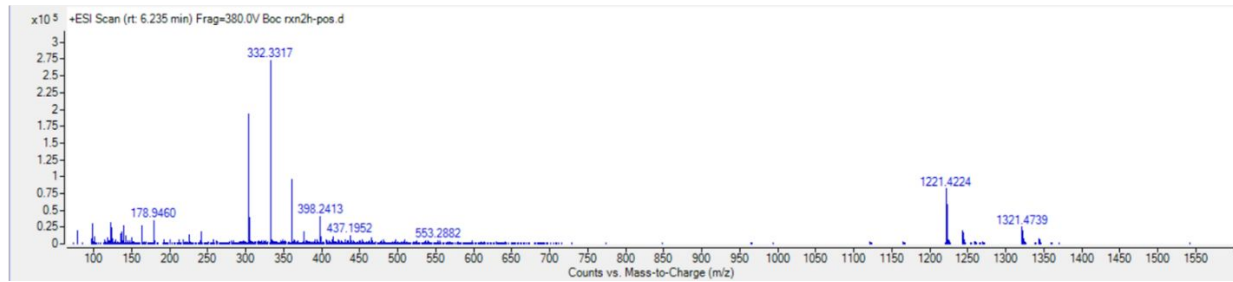
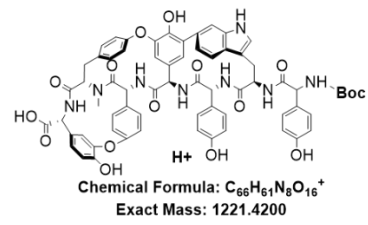
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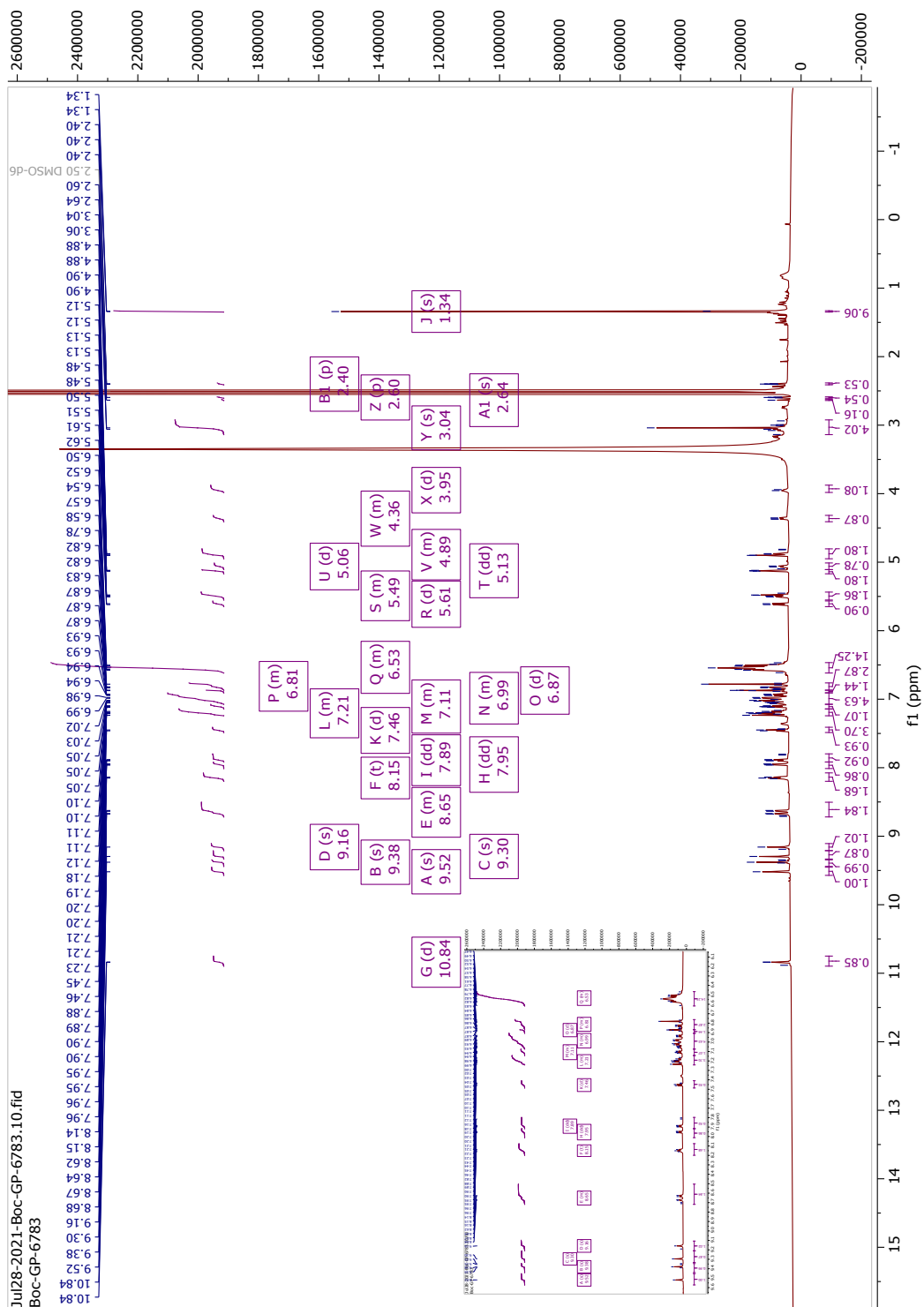
Compound 4



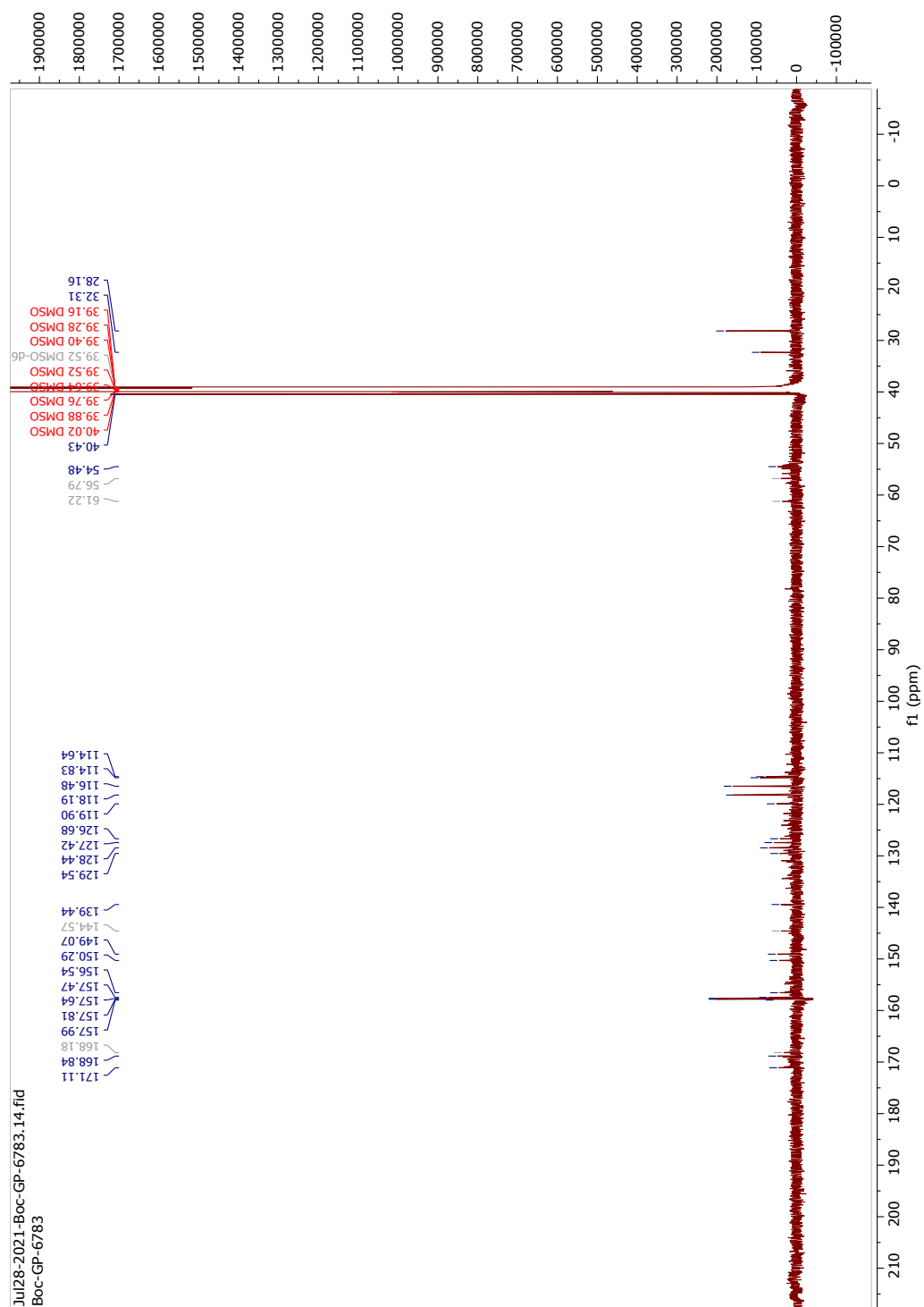
Compound 4



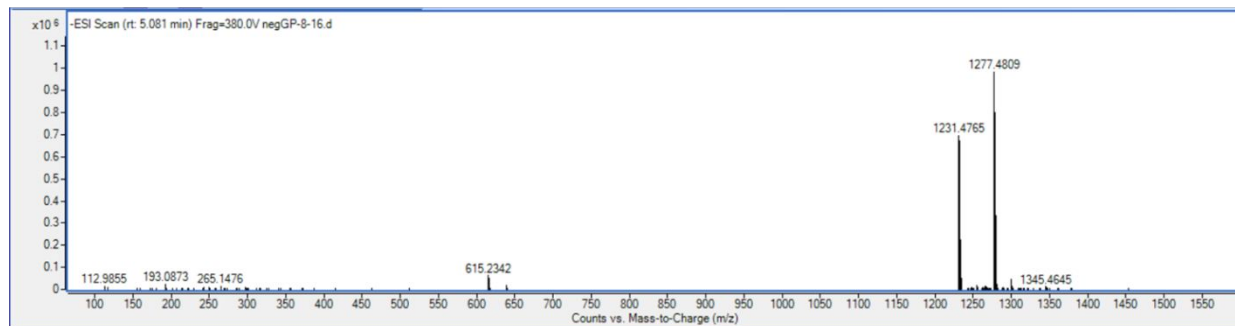
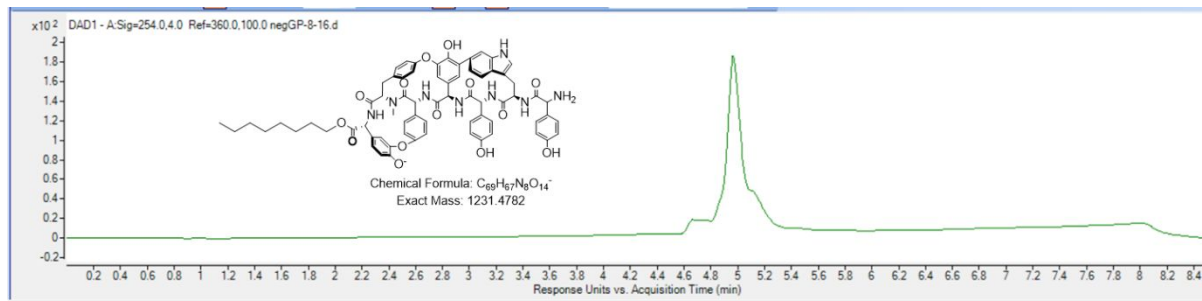
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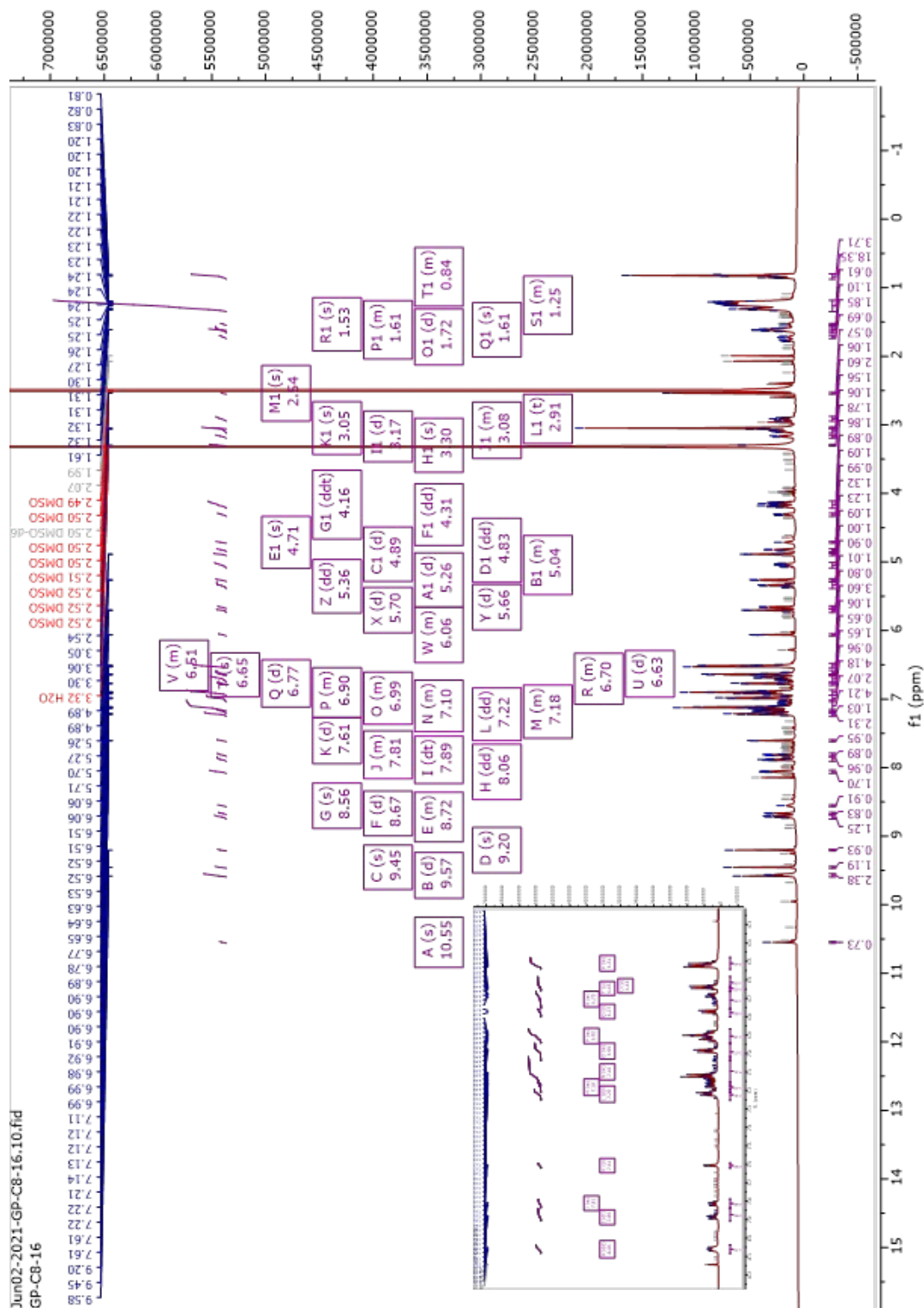
^{13}C -NMR of compound 4



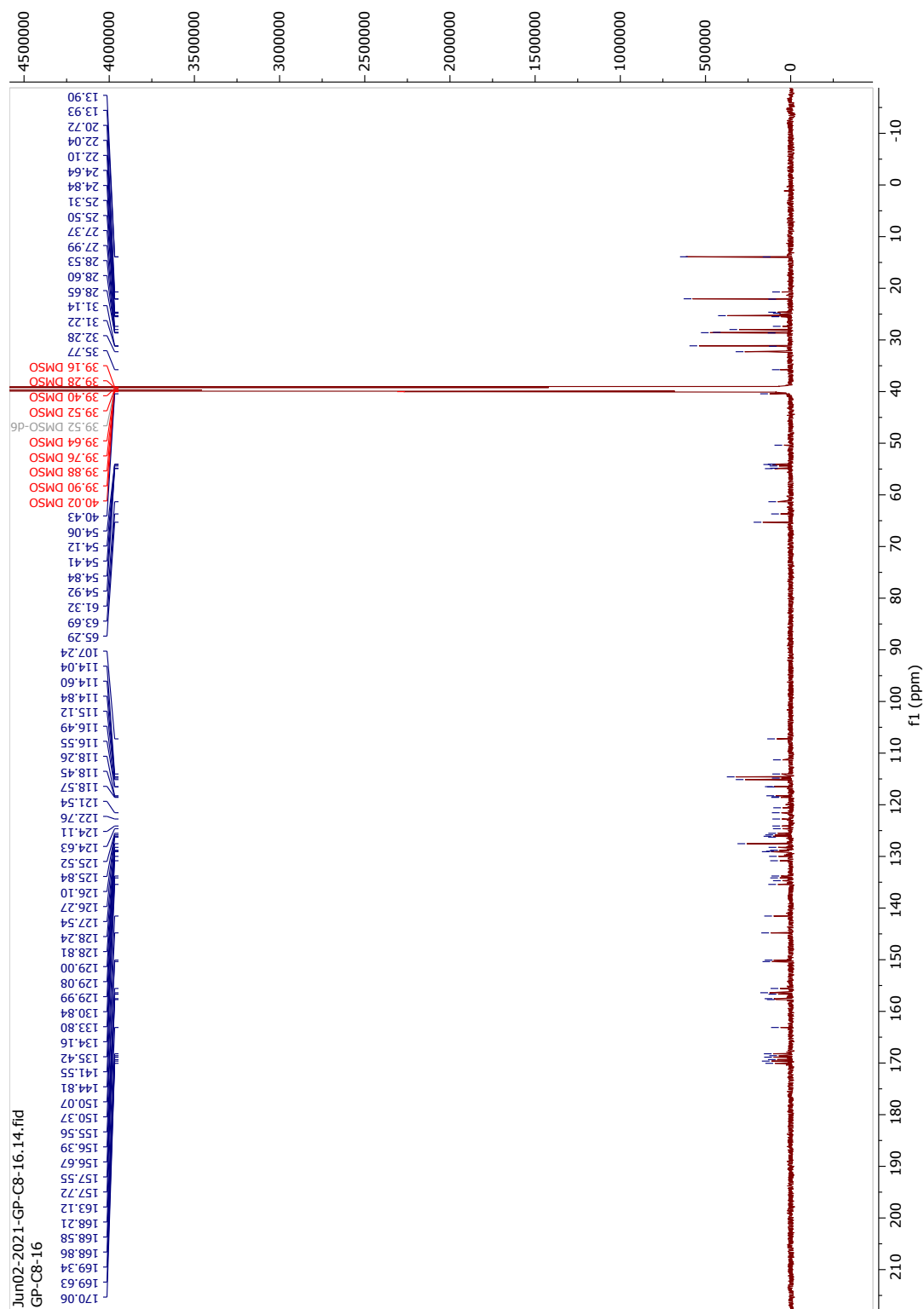
Compound 5



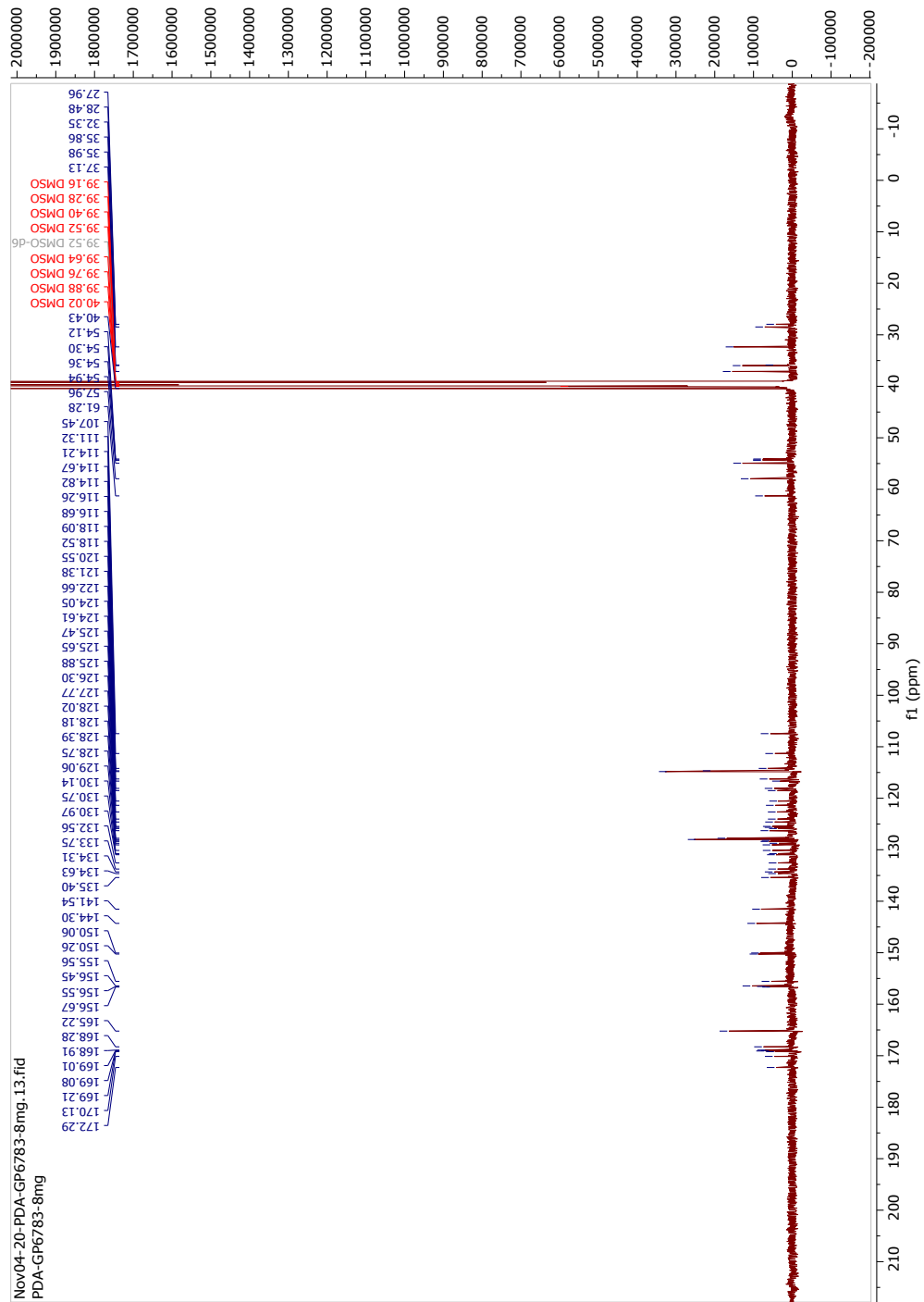
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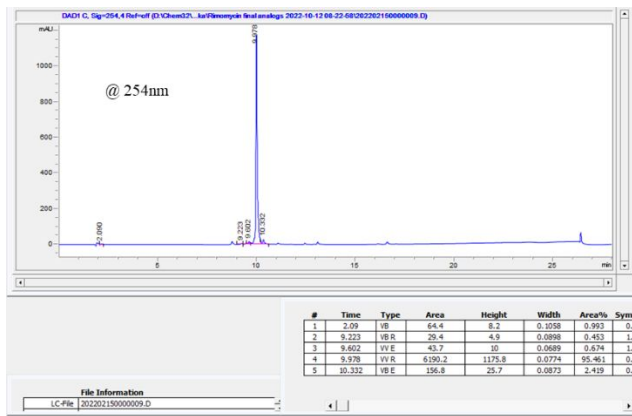
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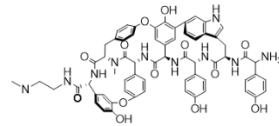
^{13}C -NMR of compound 6



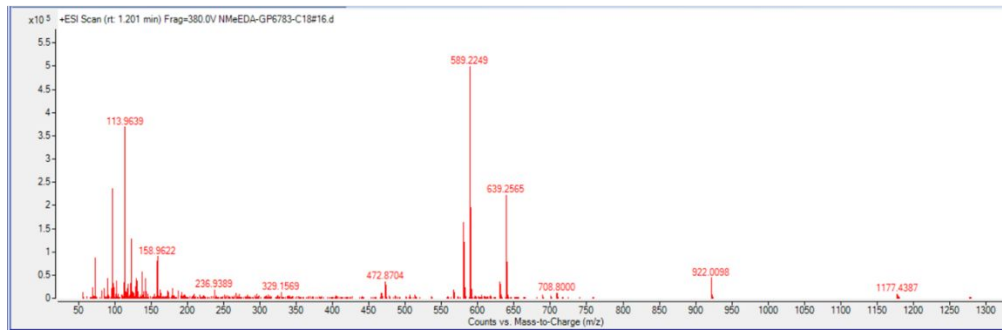
Compound 7



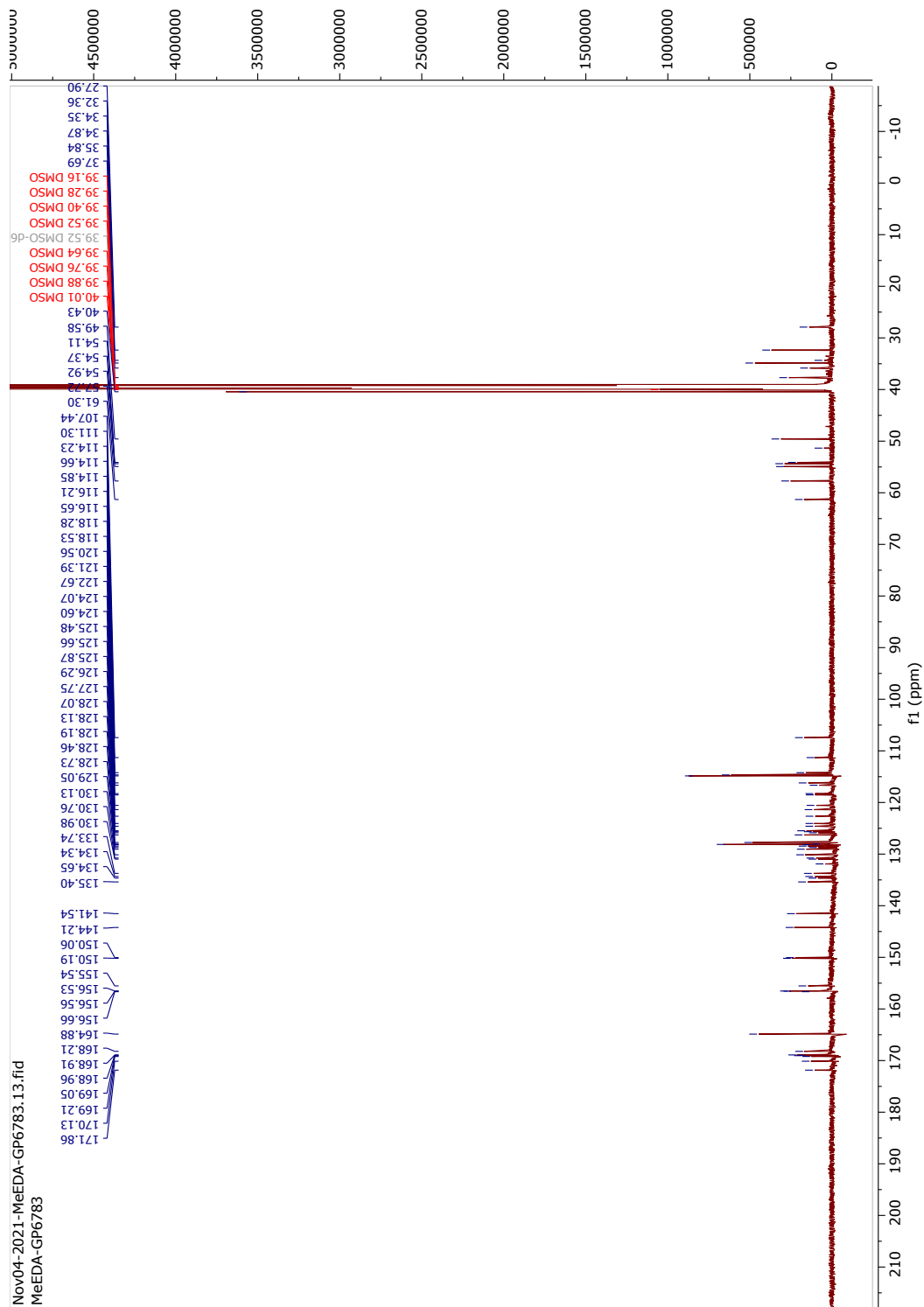
Compound 7



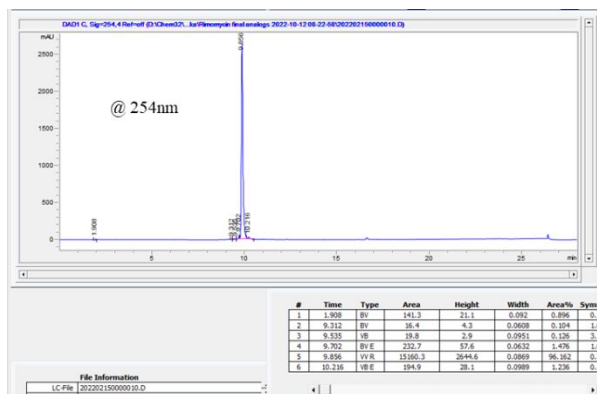
Chemical Formula: $C_{64}H_{61}N_{10}O_{13}^{2+}$
Exact Mass: 1177.4409



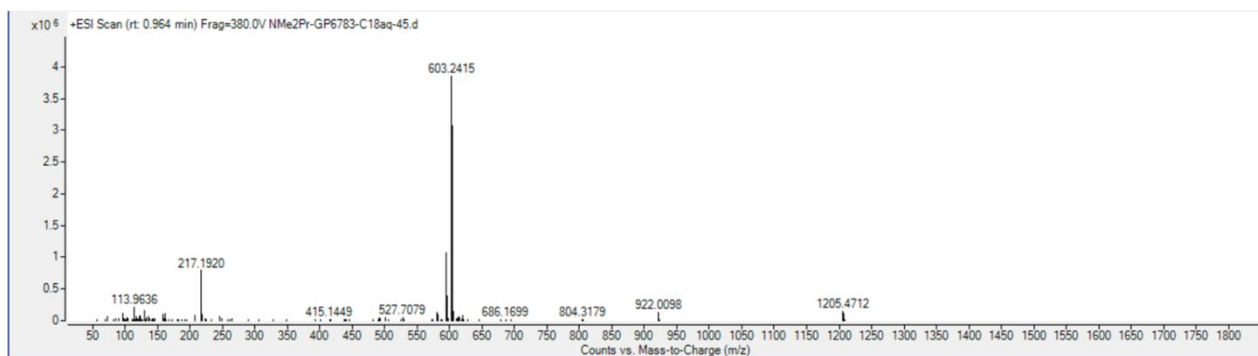
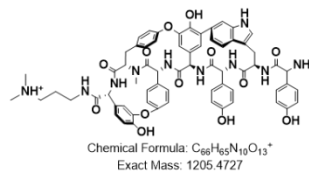
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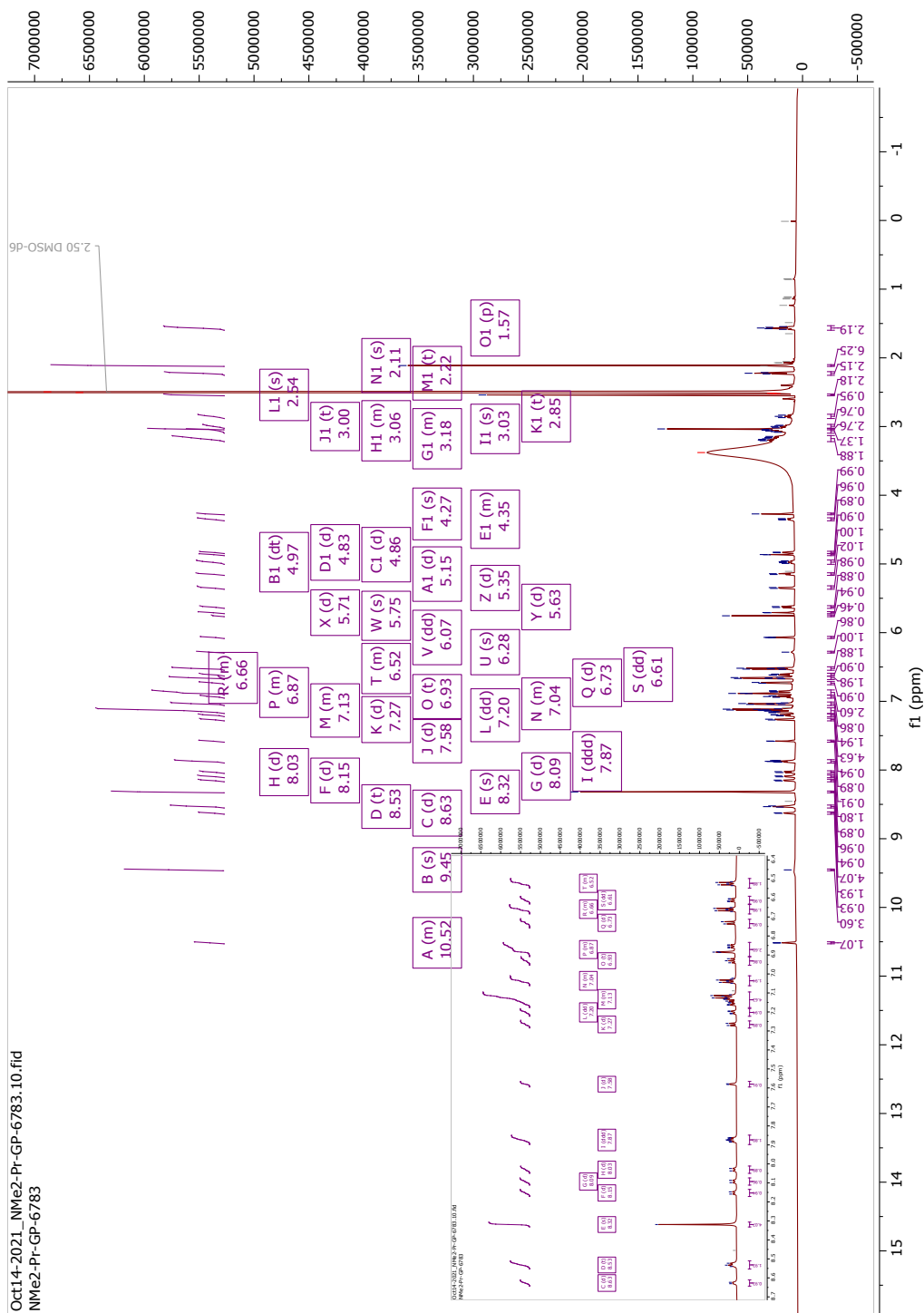
Compound 8



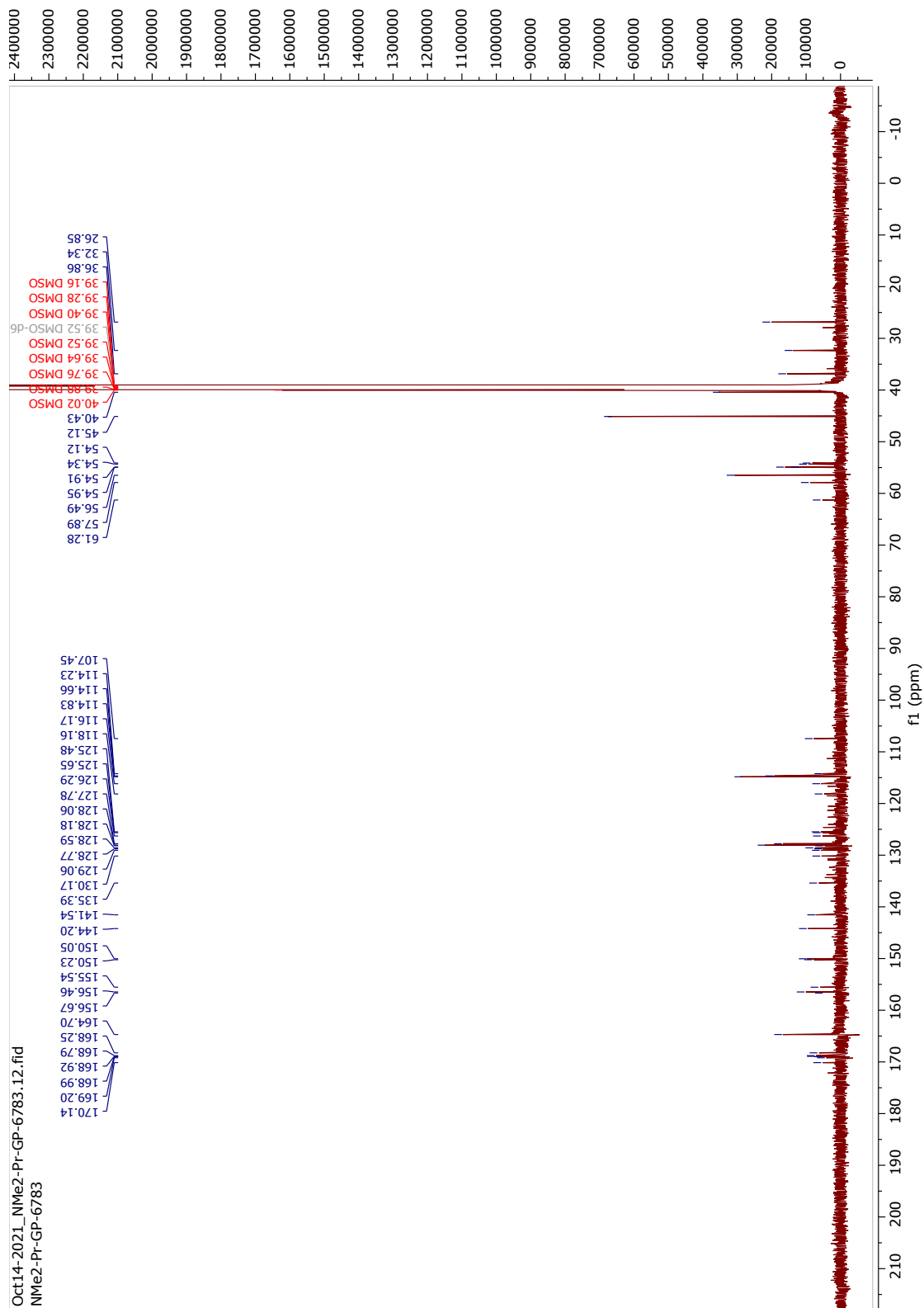
Compound 8



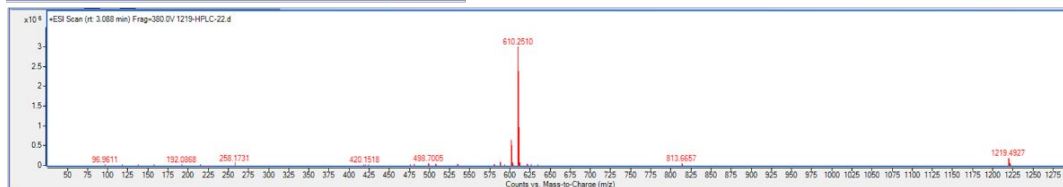
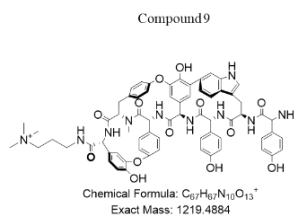
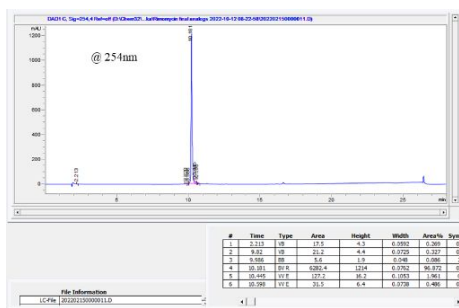
¹H-NMR of compound **8**



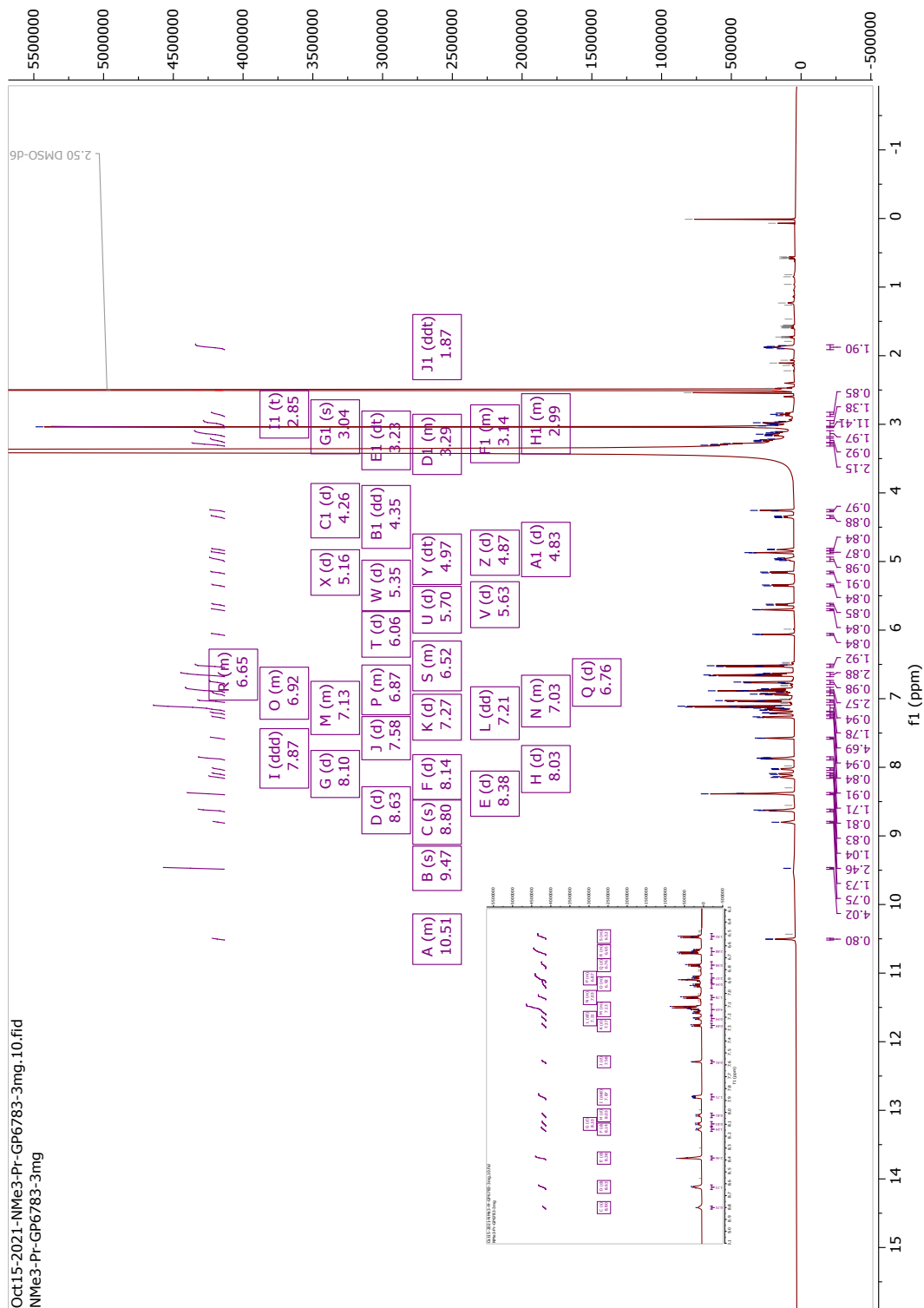
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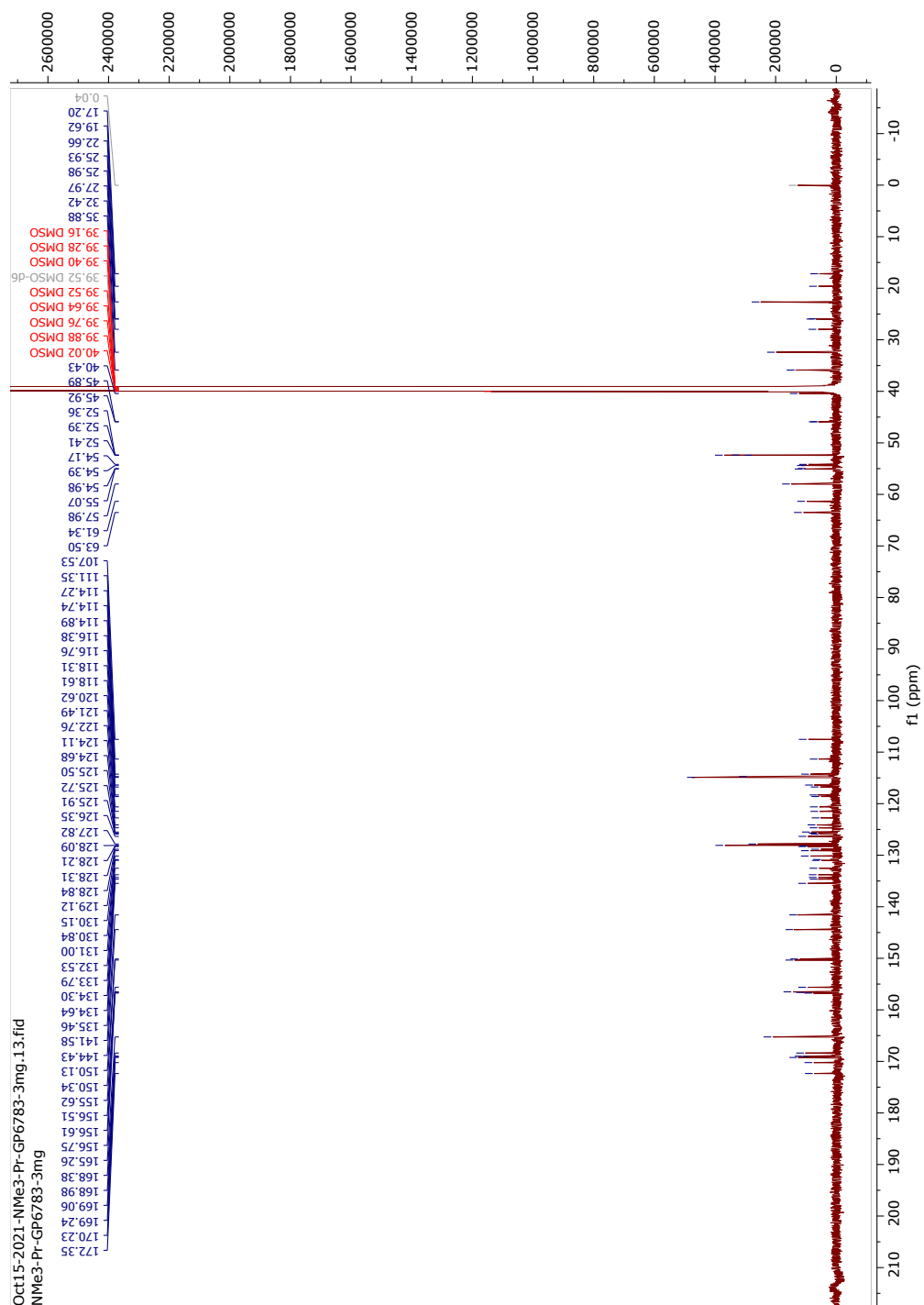
Compound 9



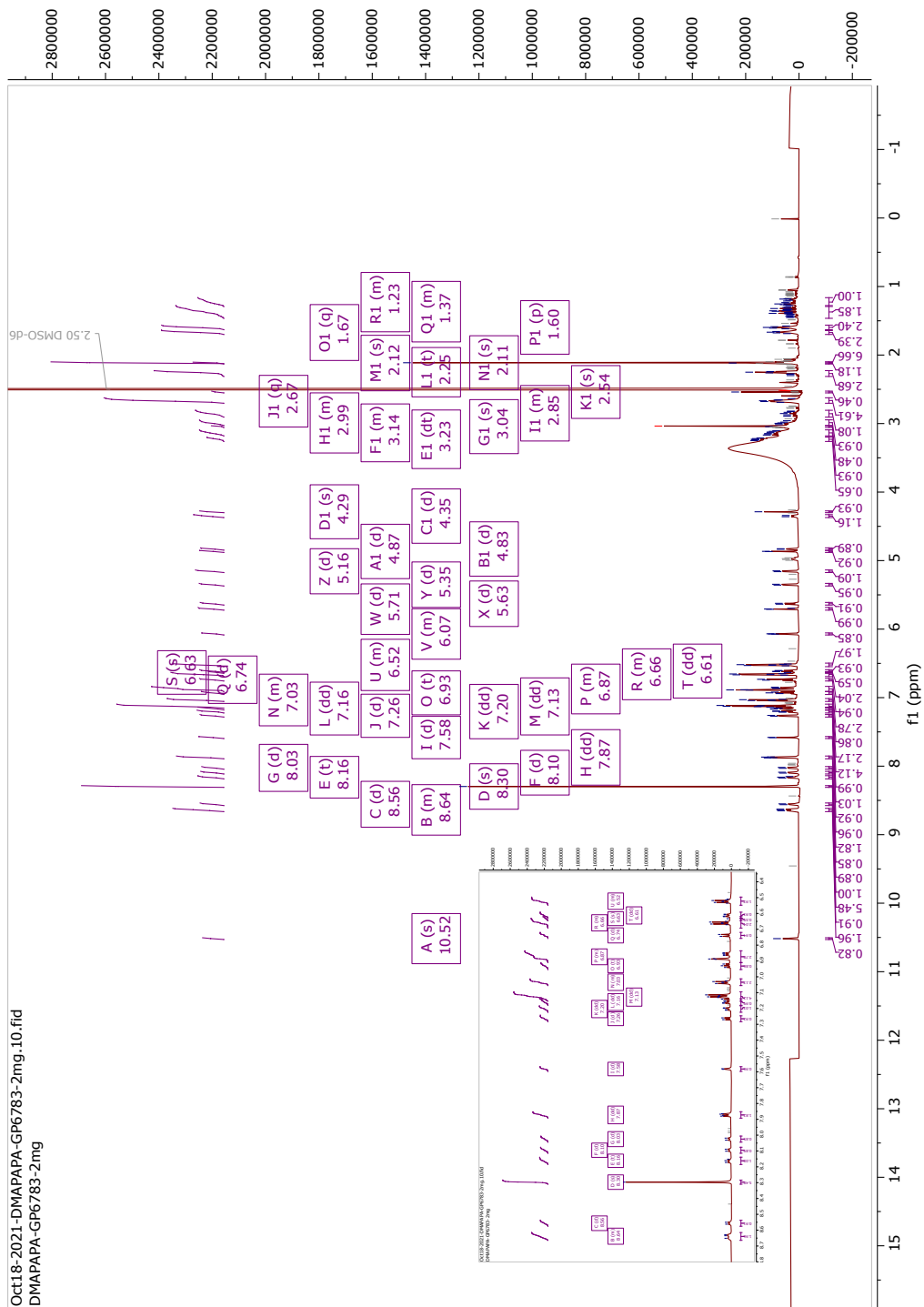
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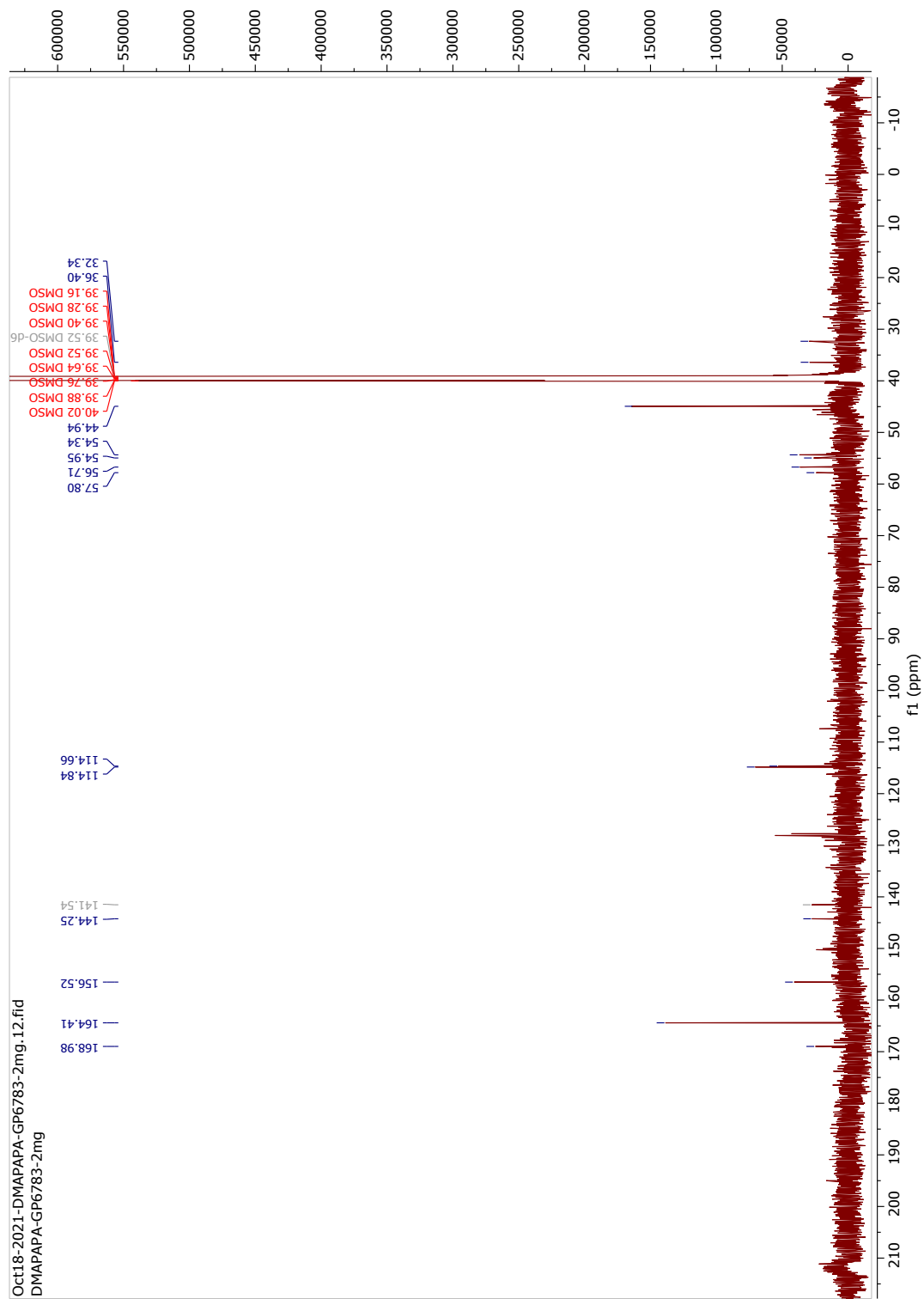
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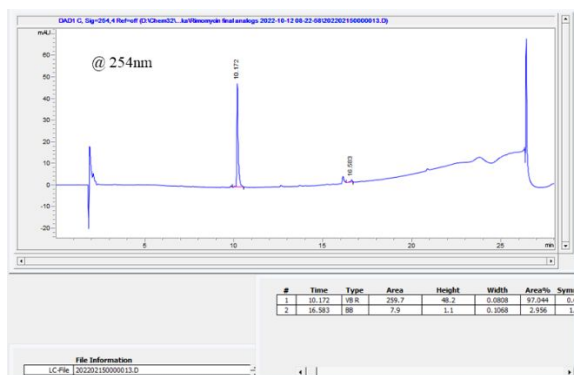
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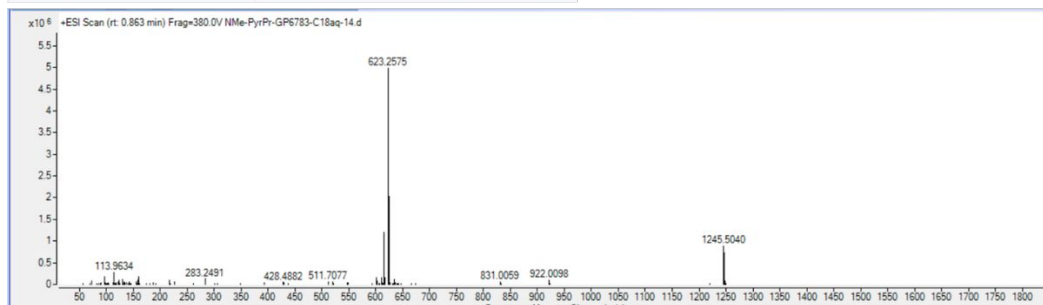
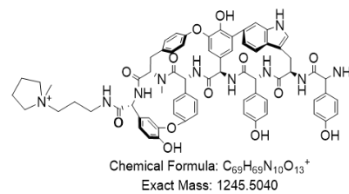
¹³C-NMR of compound **10**



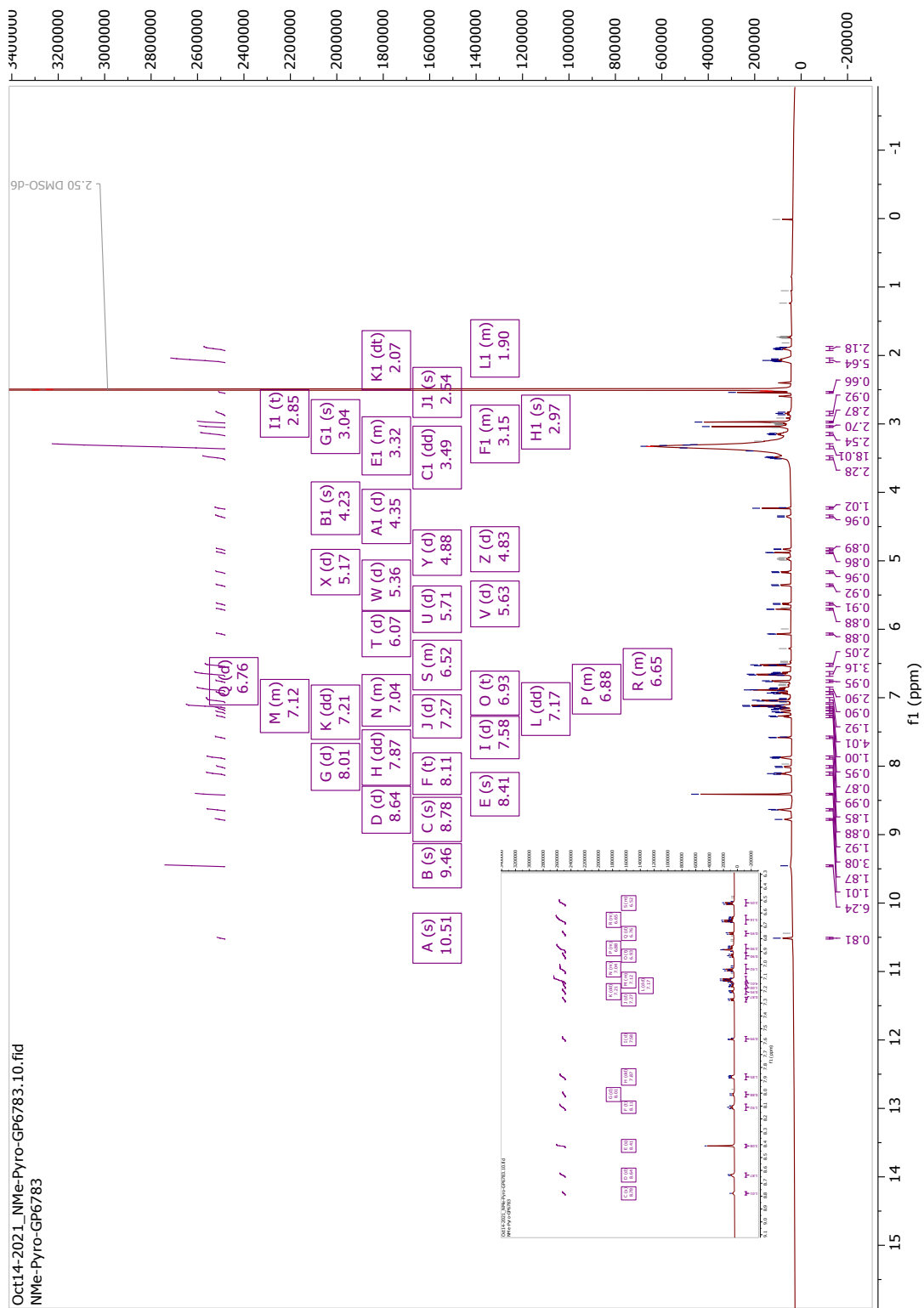
Compound 11



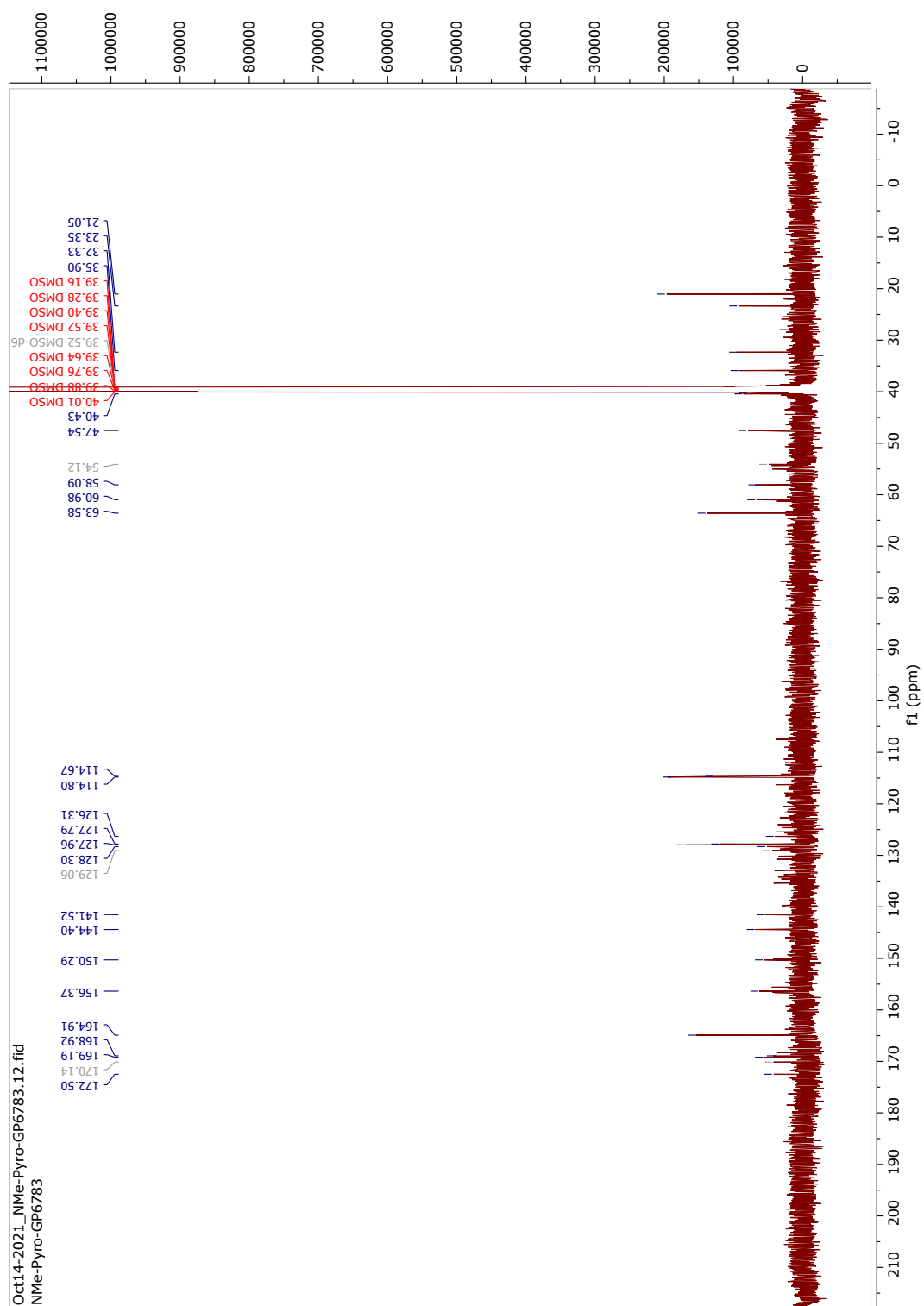
Compound 11



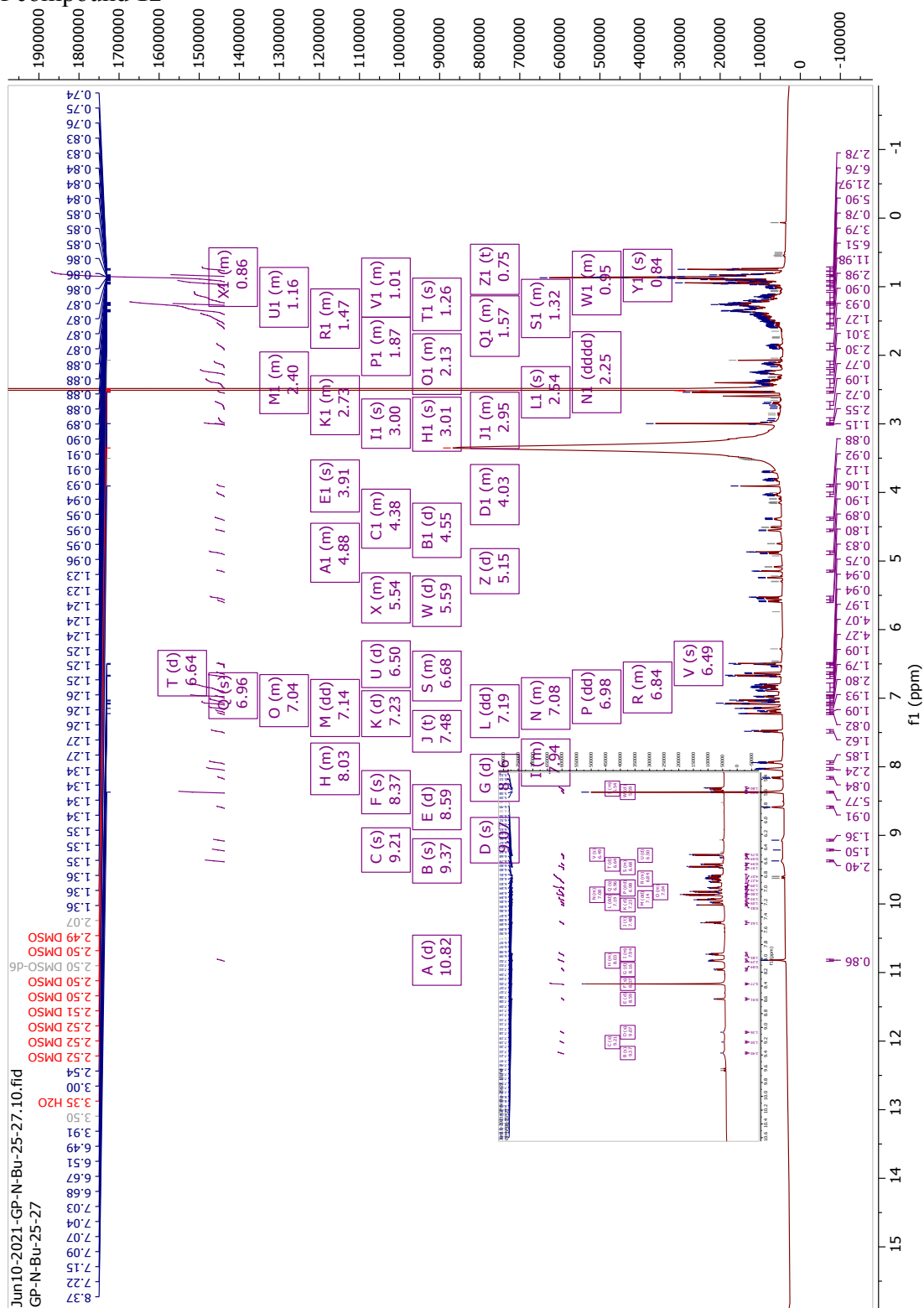
¹H-NMR of compound 11



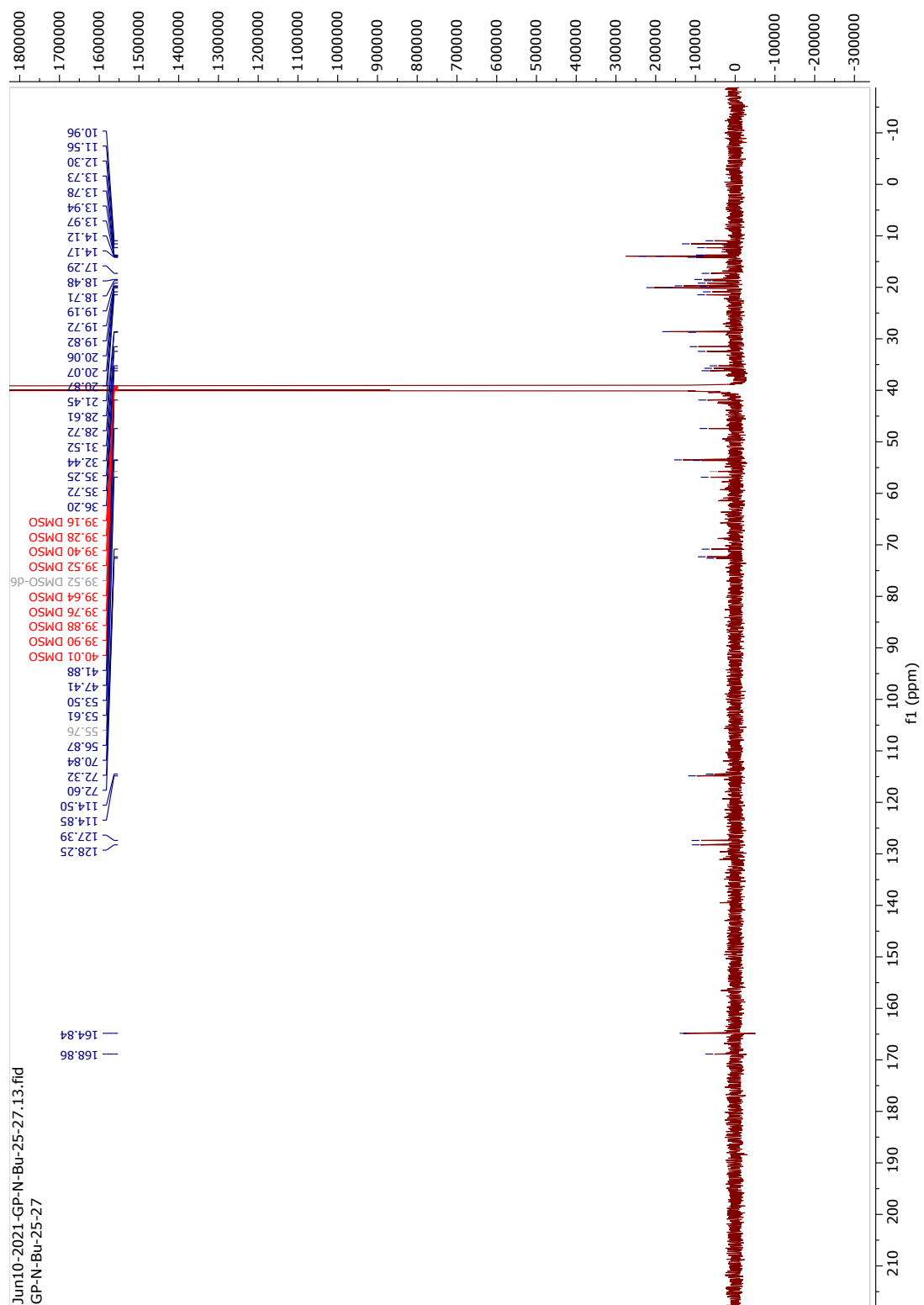
^{13}C -NMR of compound **11**



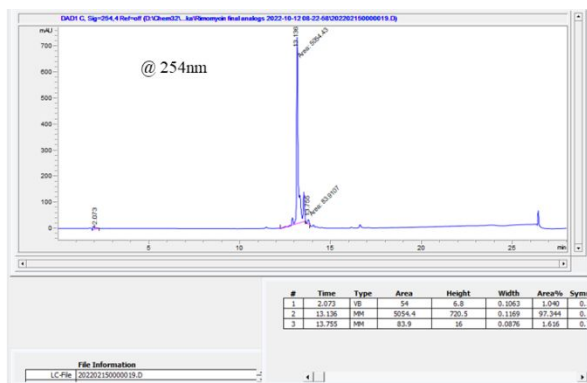
¹H-NMR of compound 12



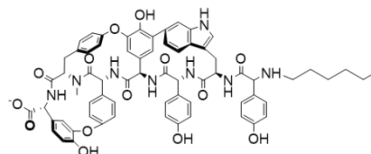
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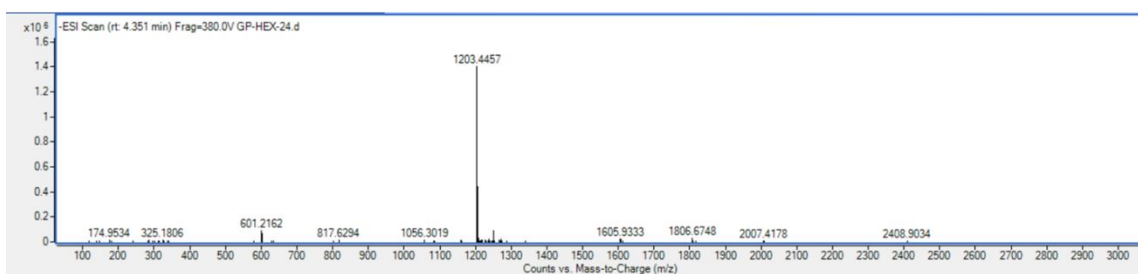
Compound 13



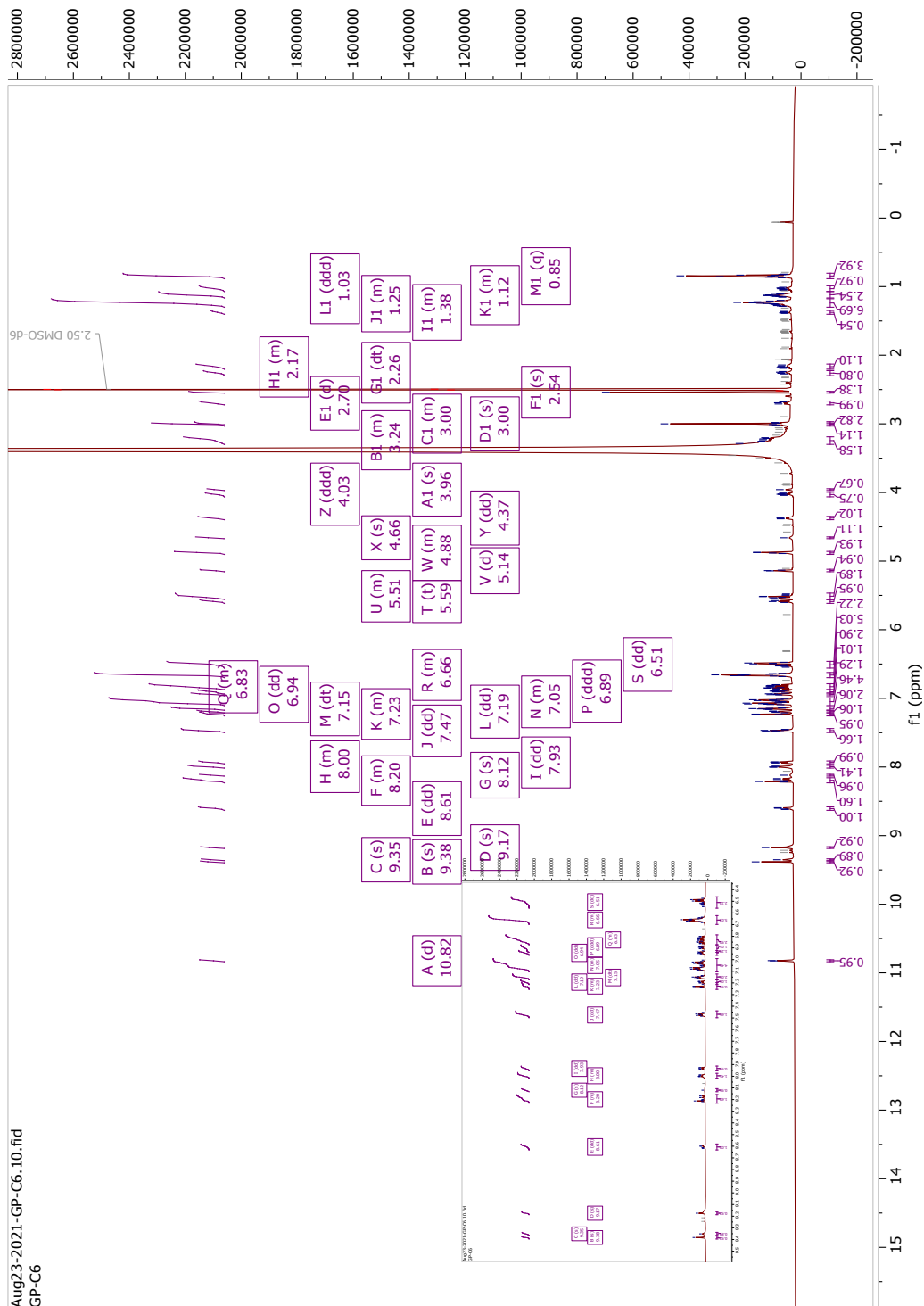
Compound 13



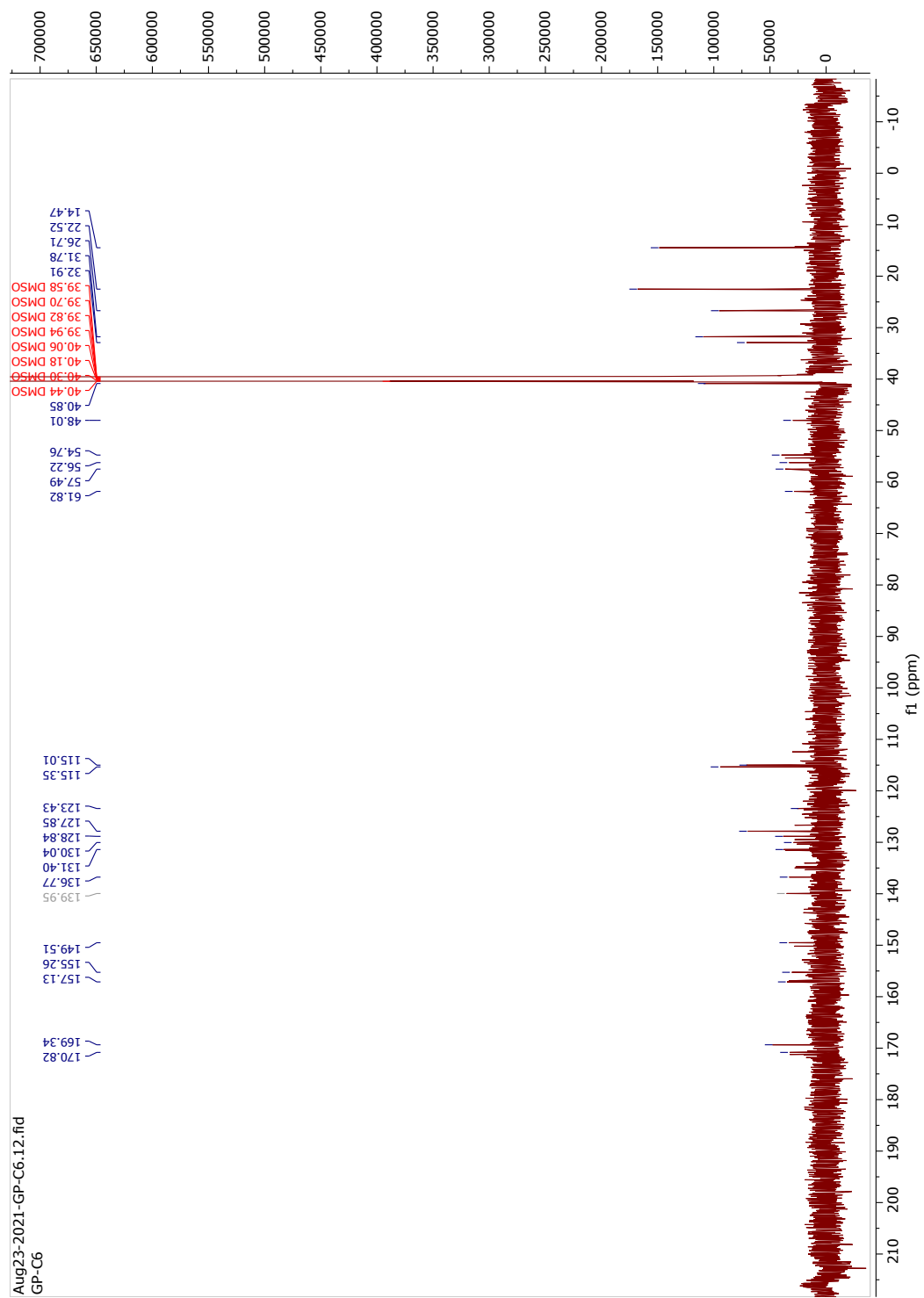
Chemical Formula: $C_{67}H_{63}N_8O_{14}$
Exact Mass: 1203.4469



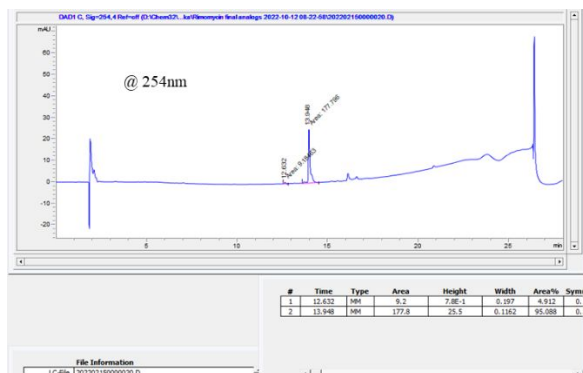
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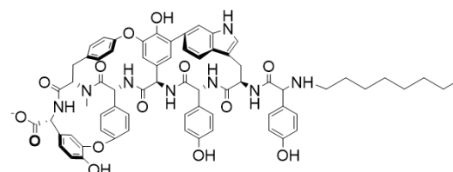
¹³C-NMR of compound 13



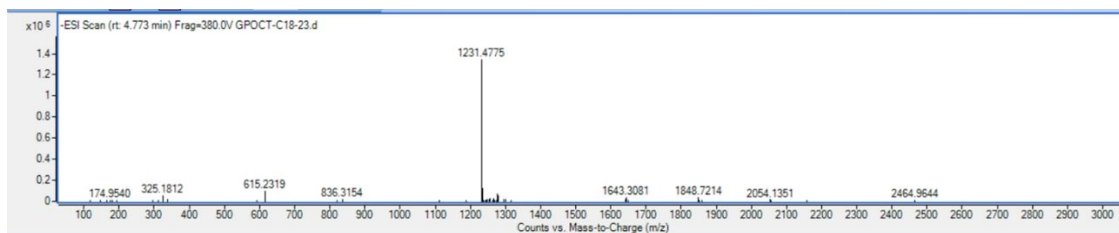
Compound 14



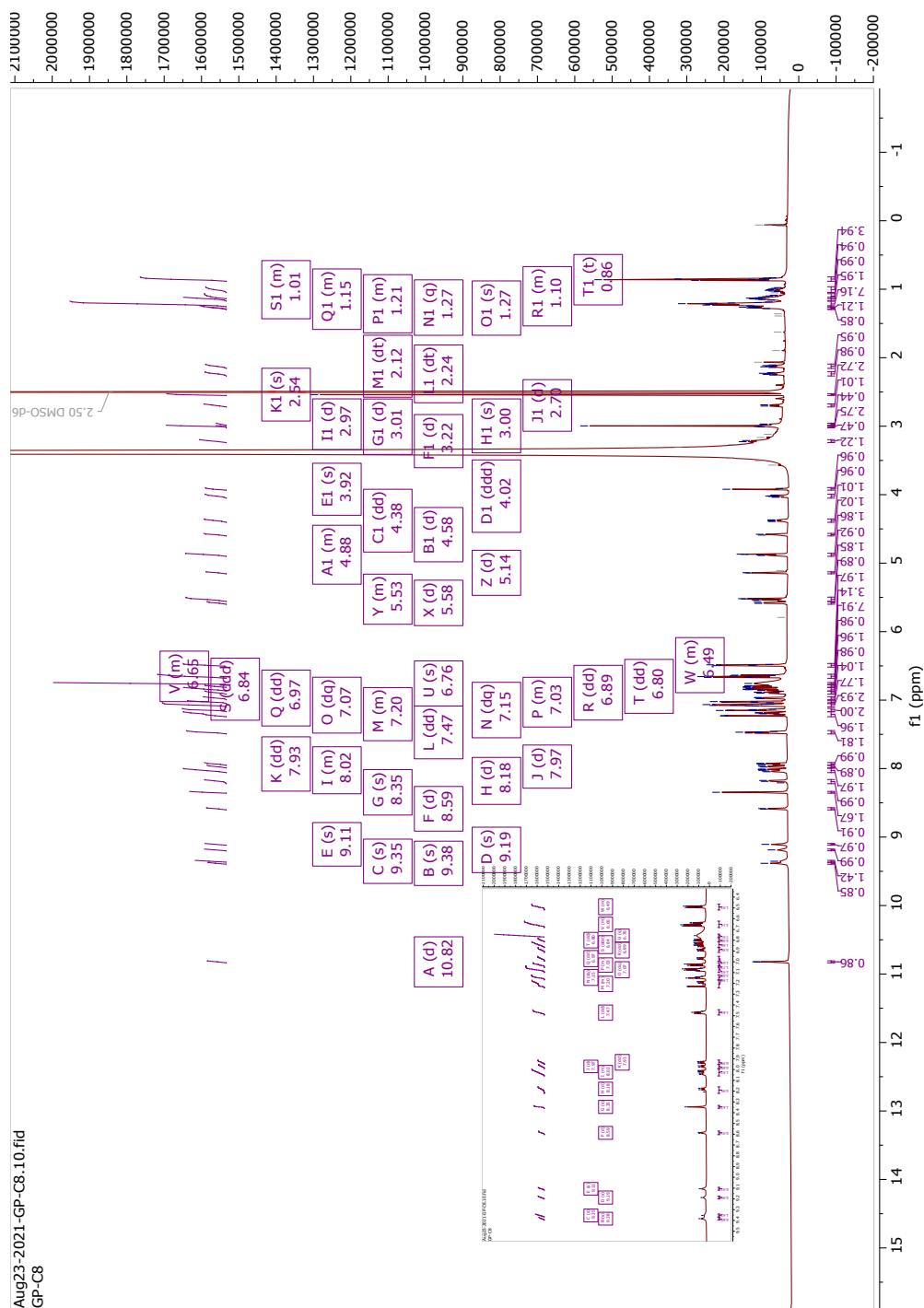
Compound 14



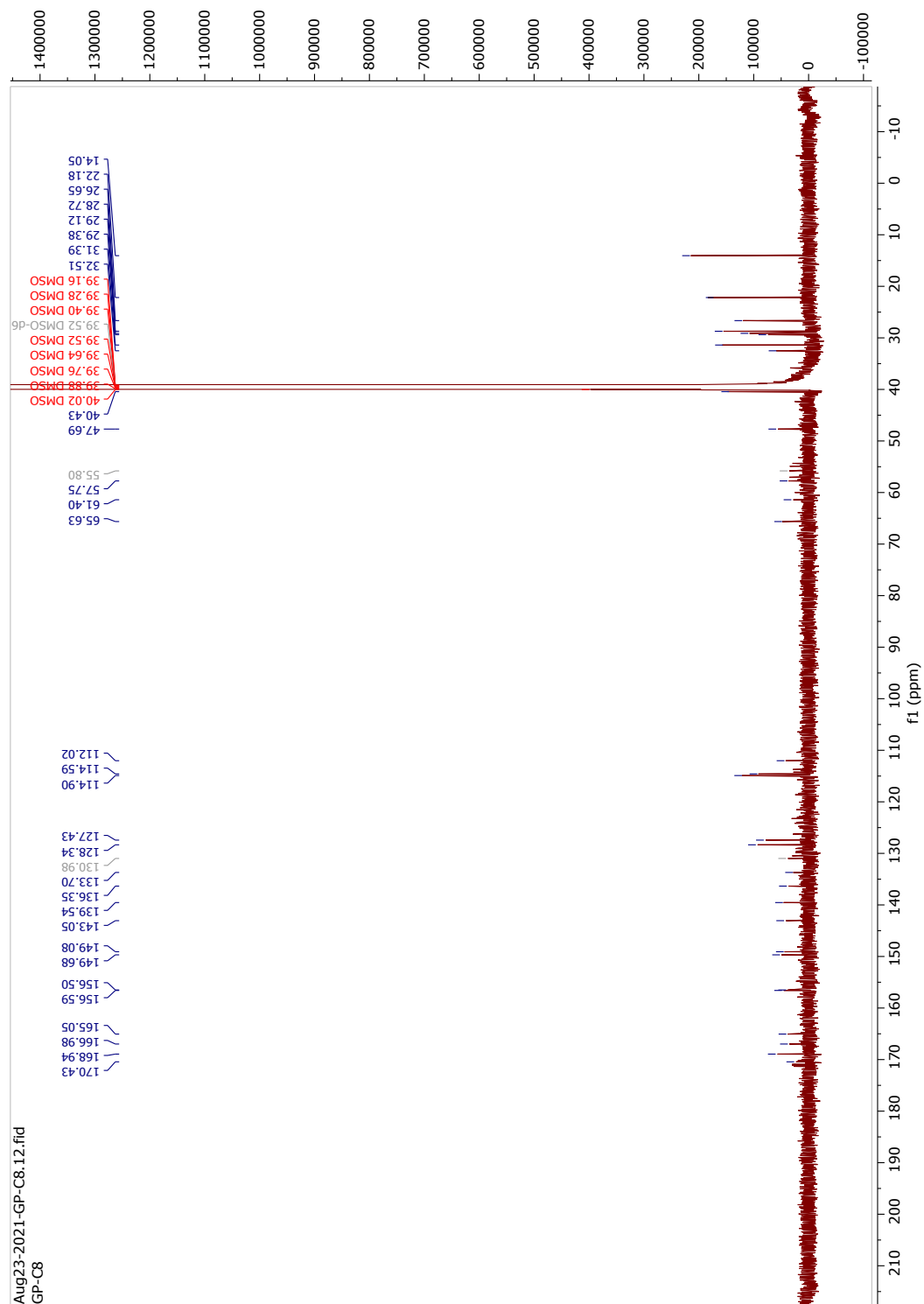
Chemical Formula: $C_{60}H_{67}N_9O_{14}$
Exact Mass: 1231.4782



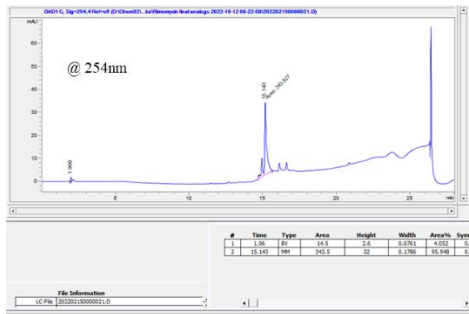
¹H-NMR of compound 14



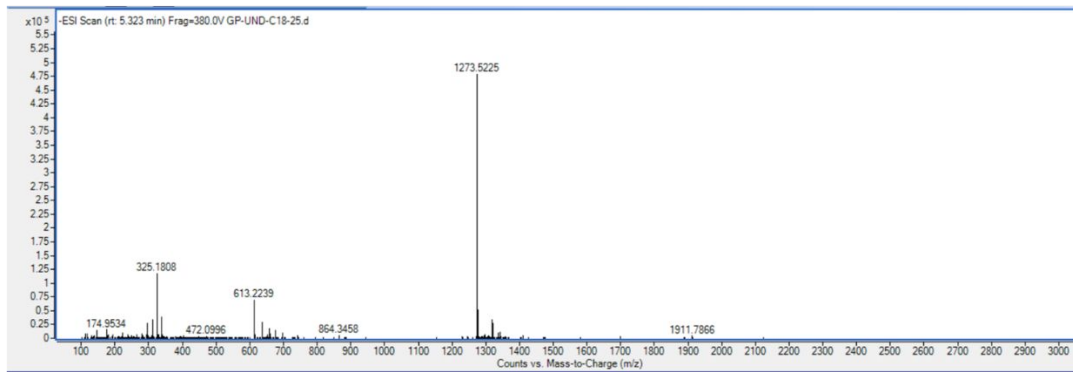
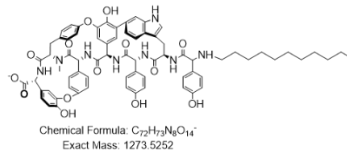
^{13}C -NMR of compound 14



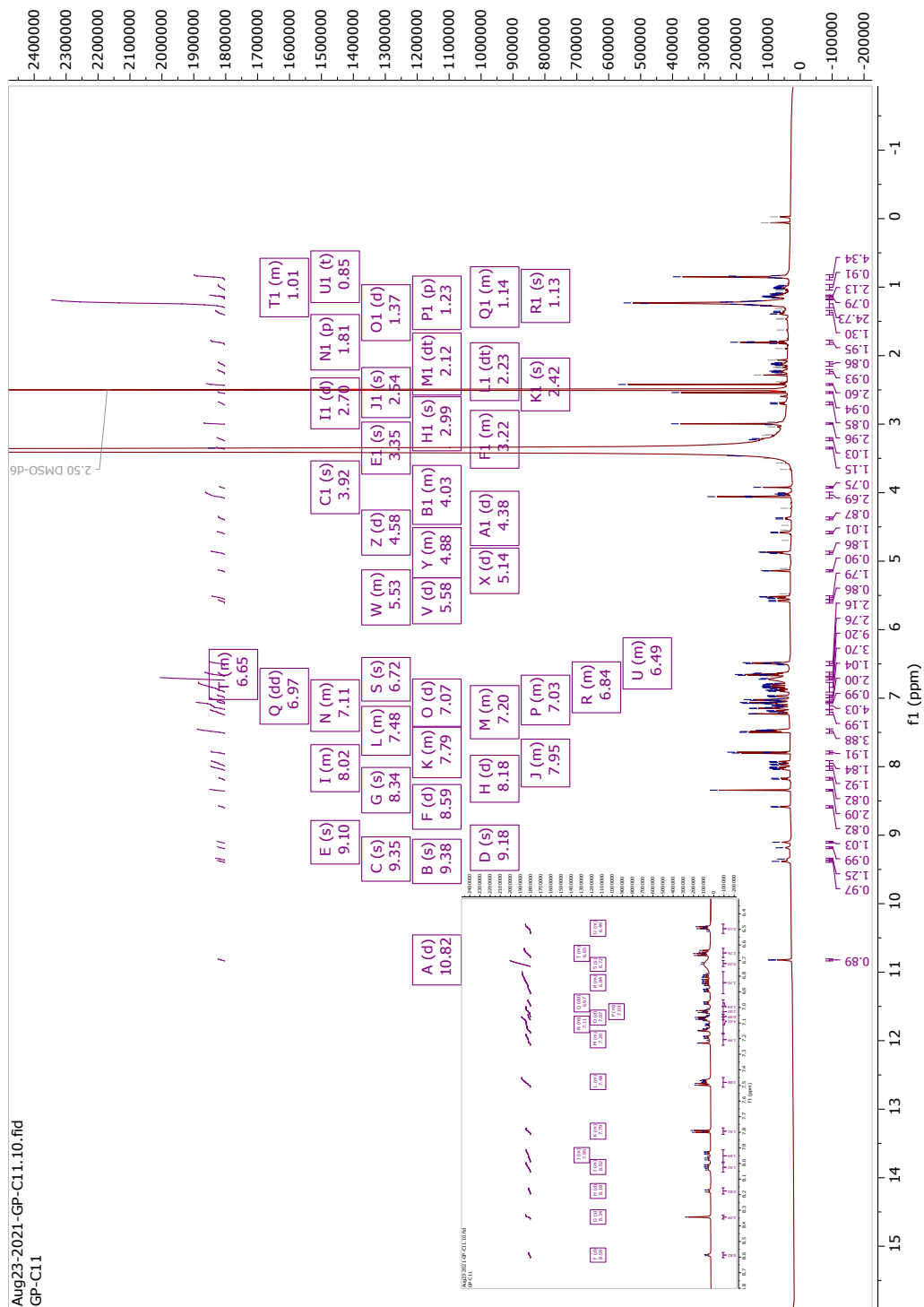
Compound 15



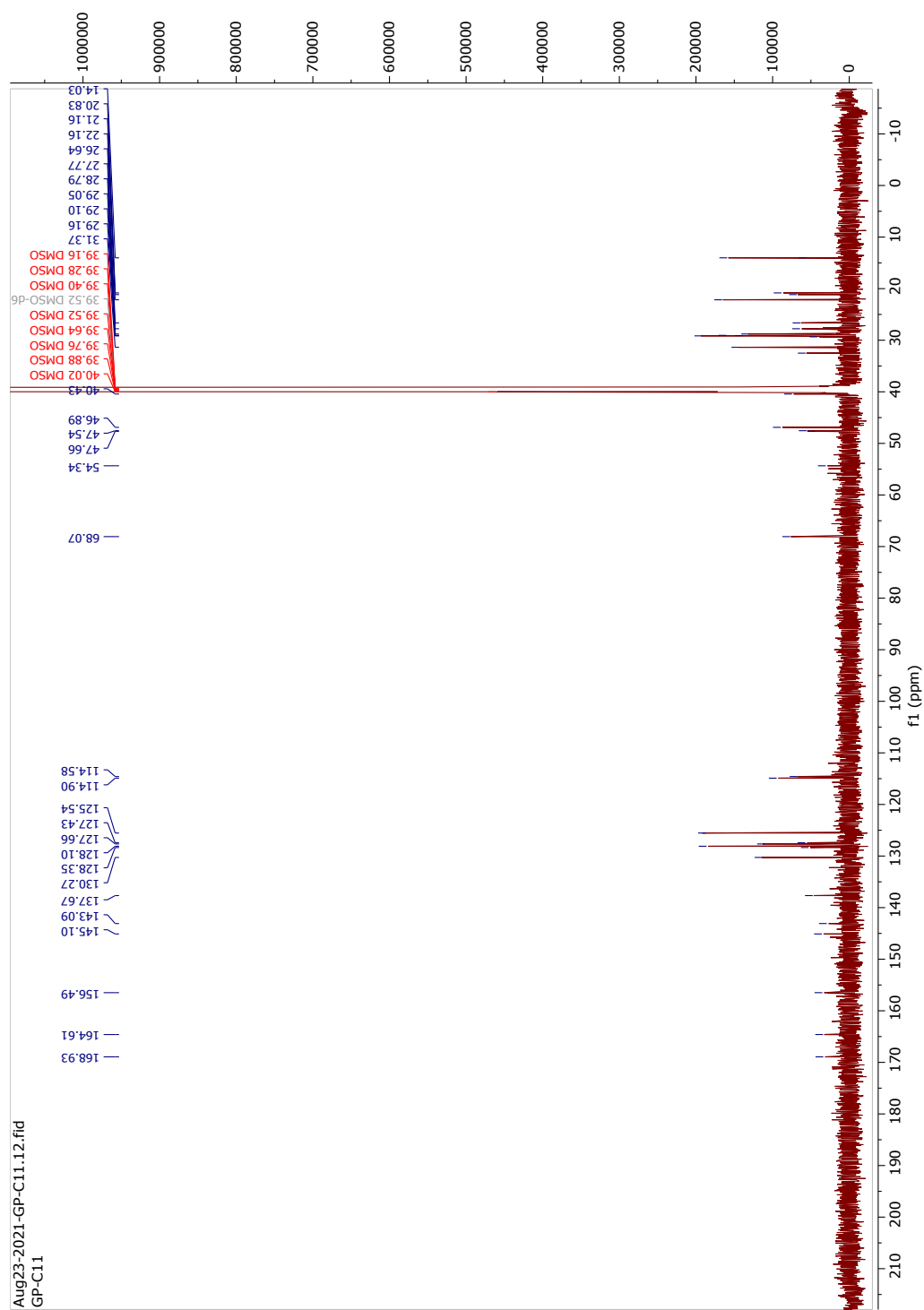
Compound 15



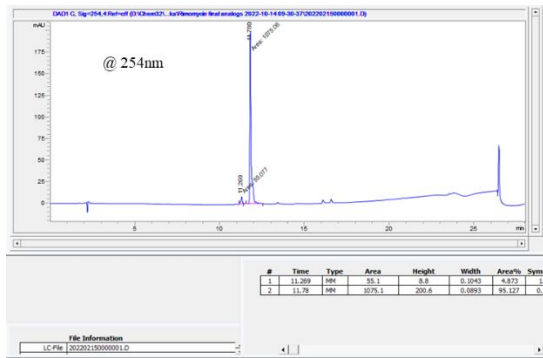
¹H-NMR of compound 15



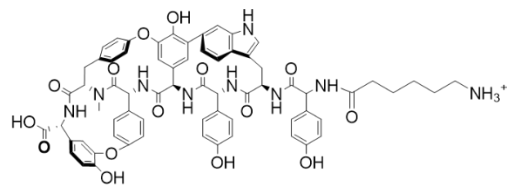
¹³C-NMR of compound 15



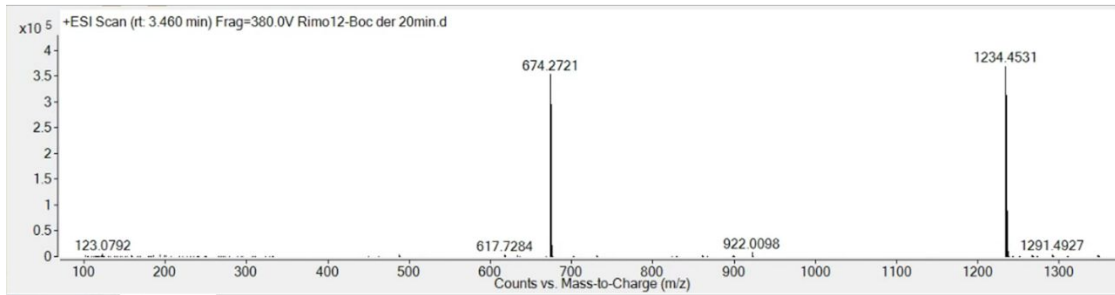
Compound 16



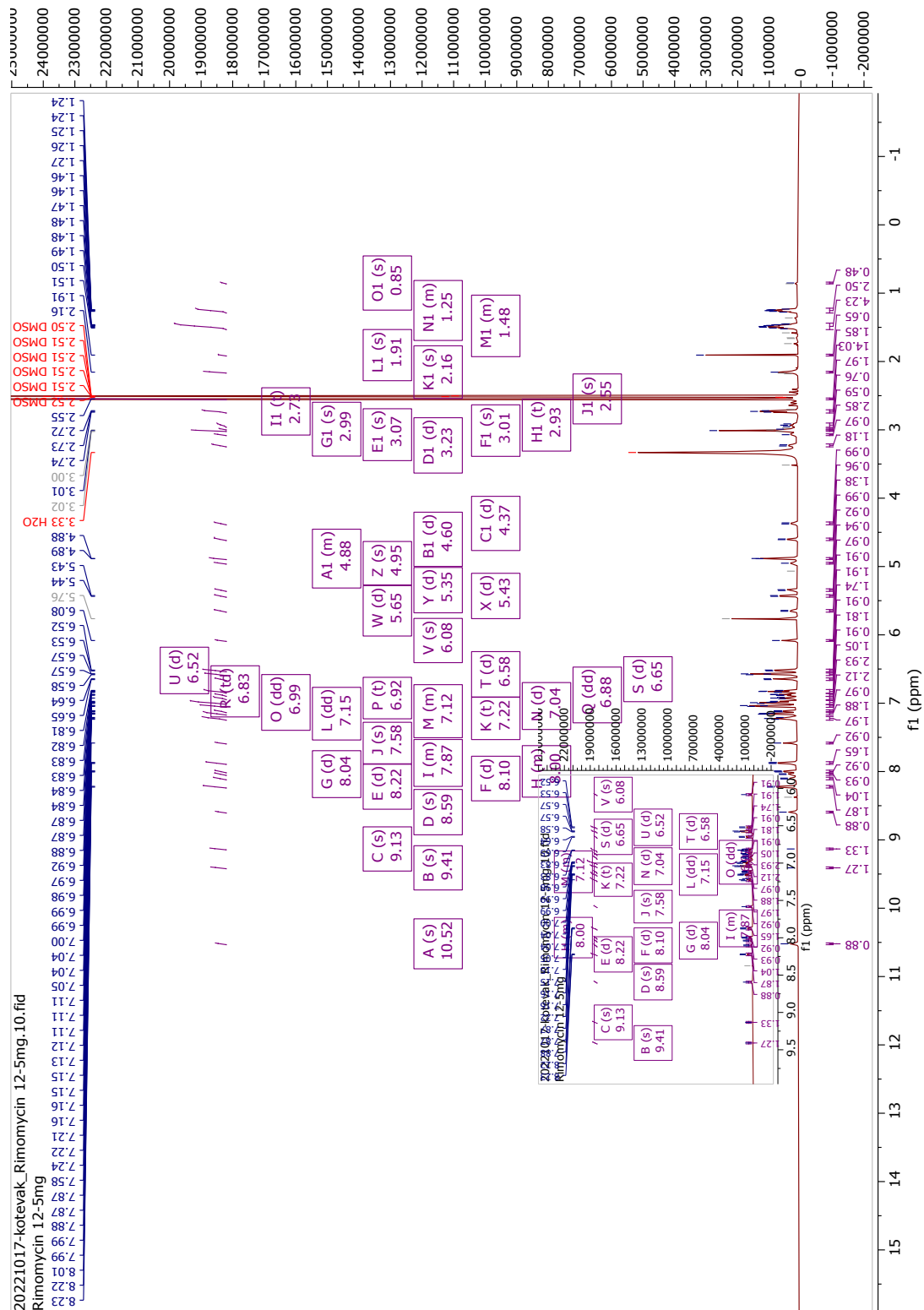
Compound 16



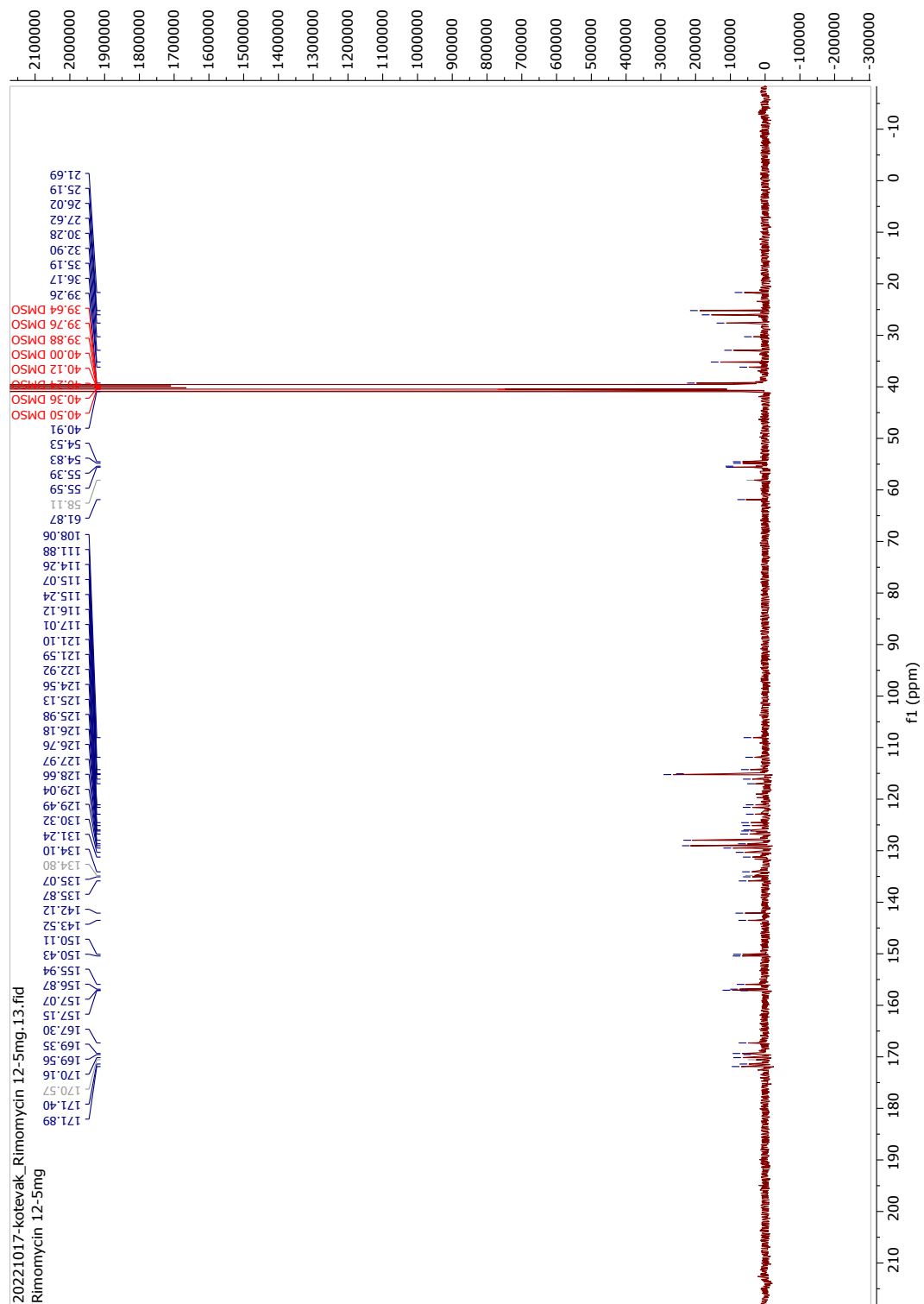
Chemical Formula: $C_{67}H_{64}N_9O_{15}^+$
Exact Mass: 1234.4516



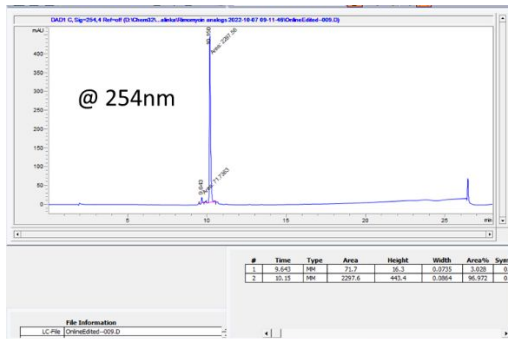
¹H-NMR of compound 16



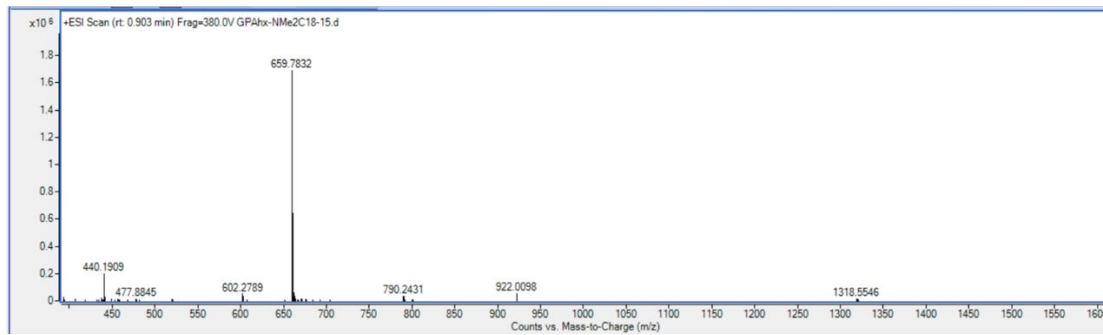
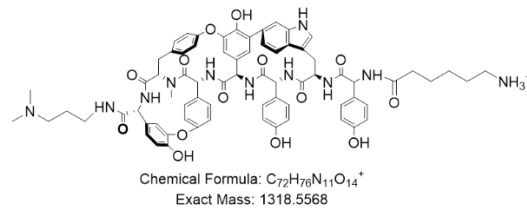
¹³C-NMR of compound 16



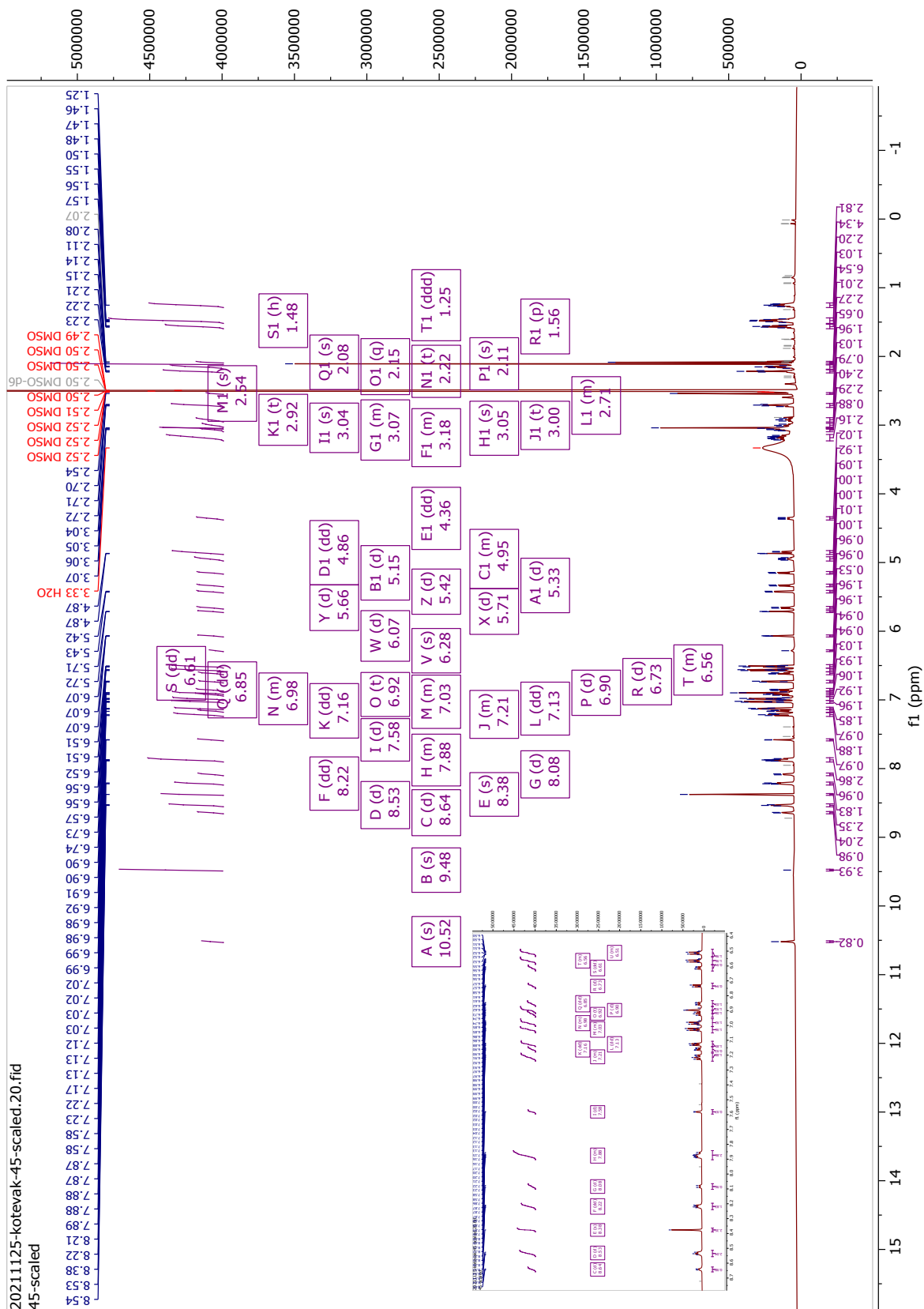
Compound 17



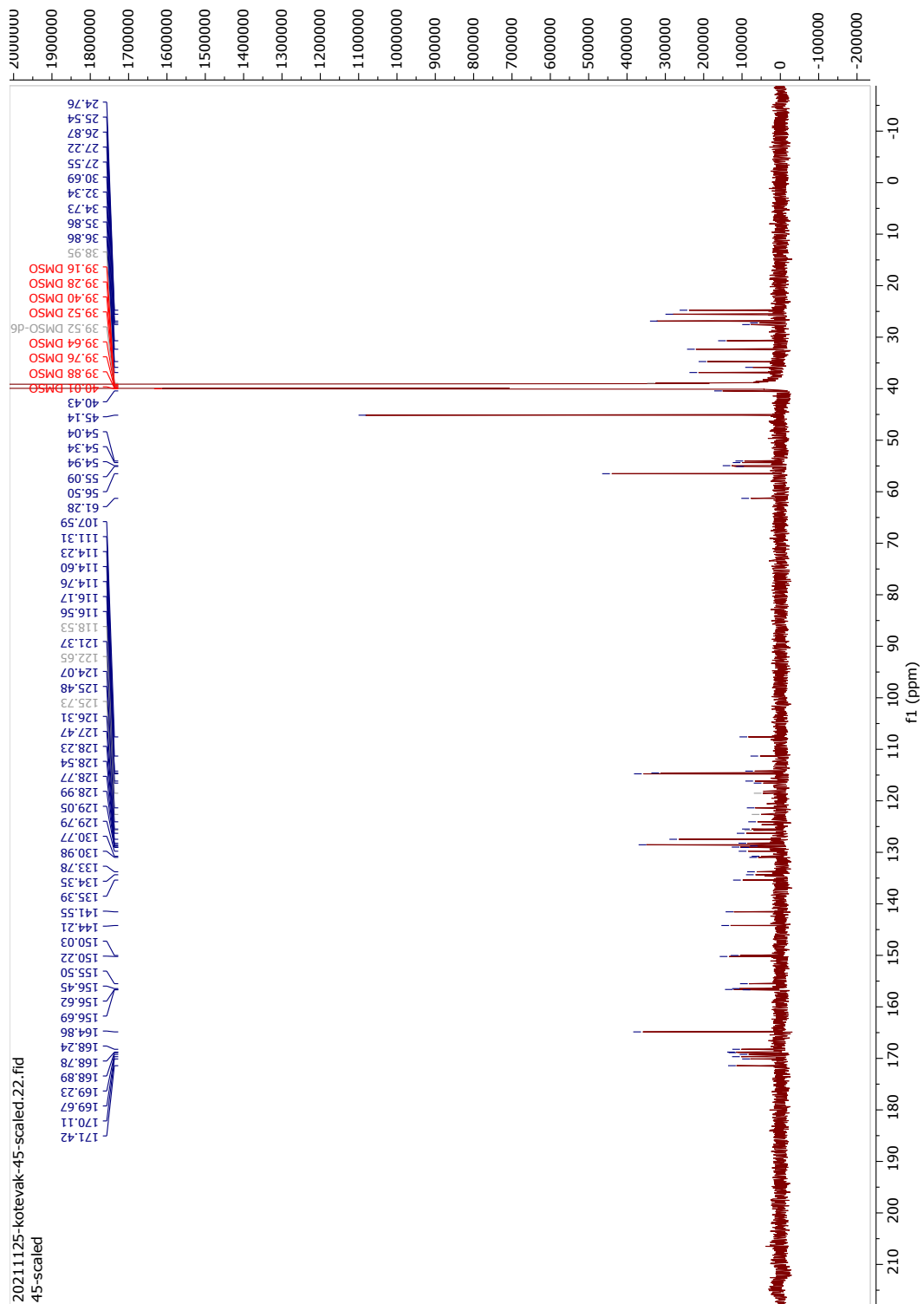
Compound 17



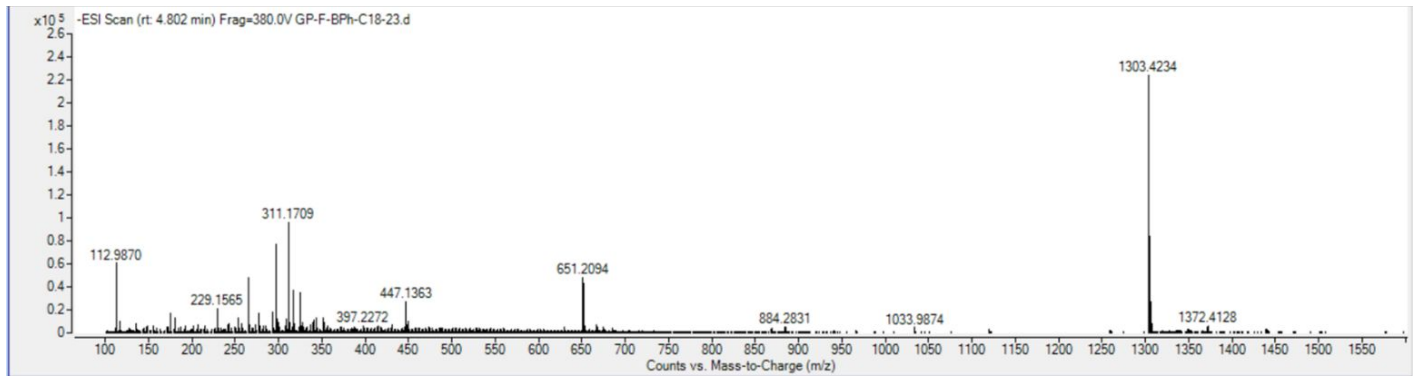
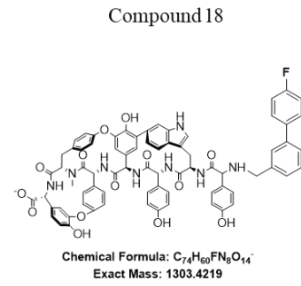
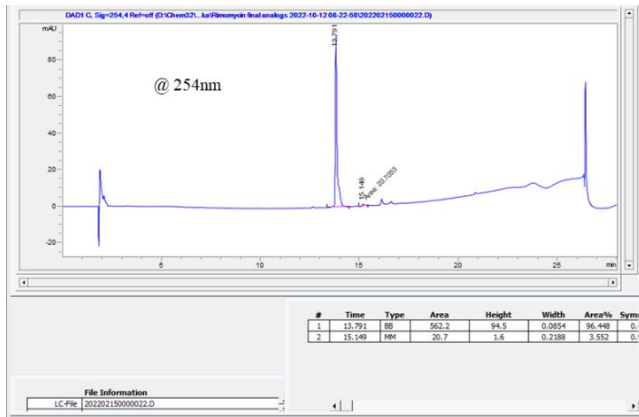
¹H-NMR of compound 17



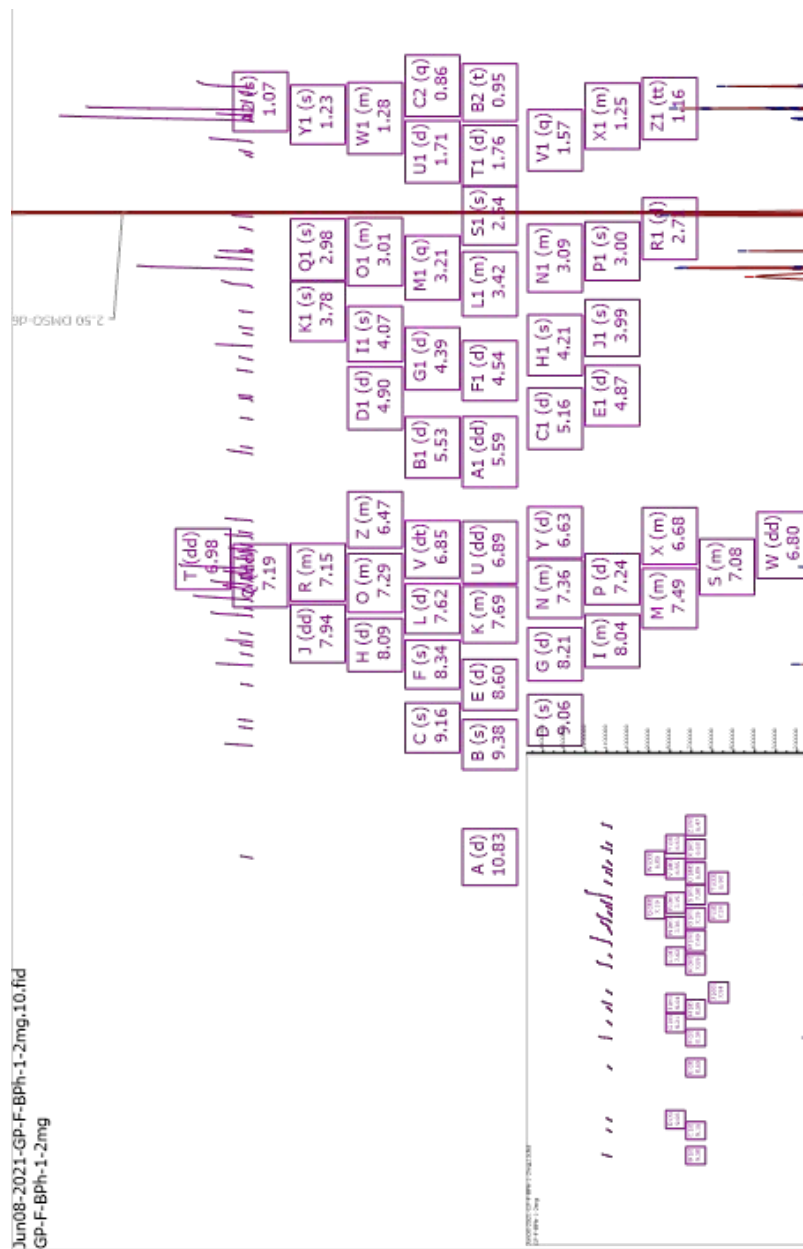
^{13}C -NMR of compound 17



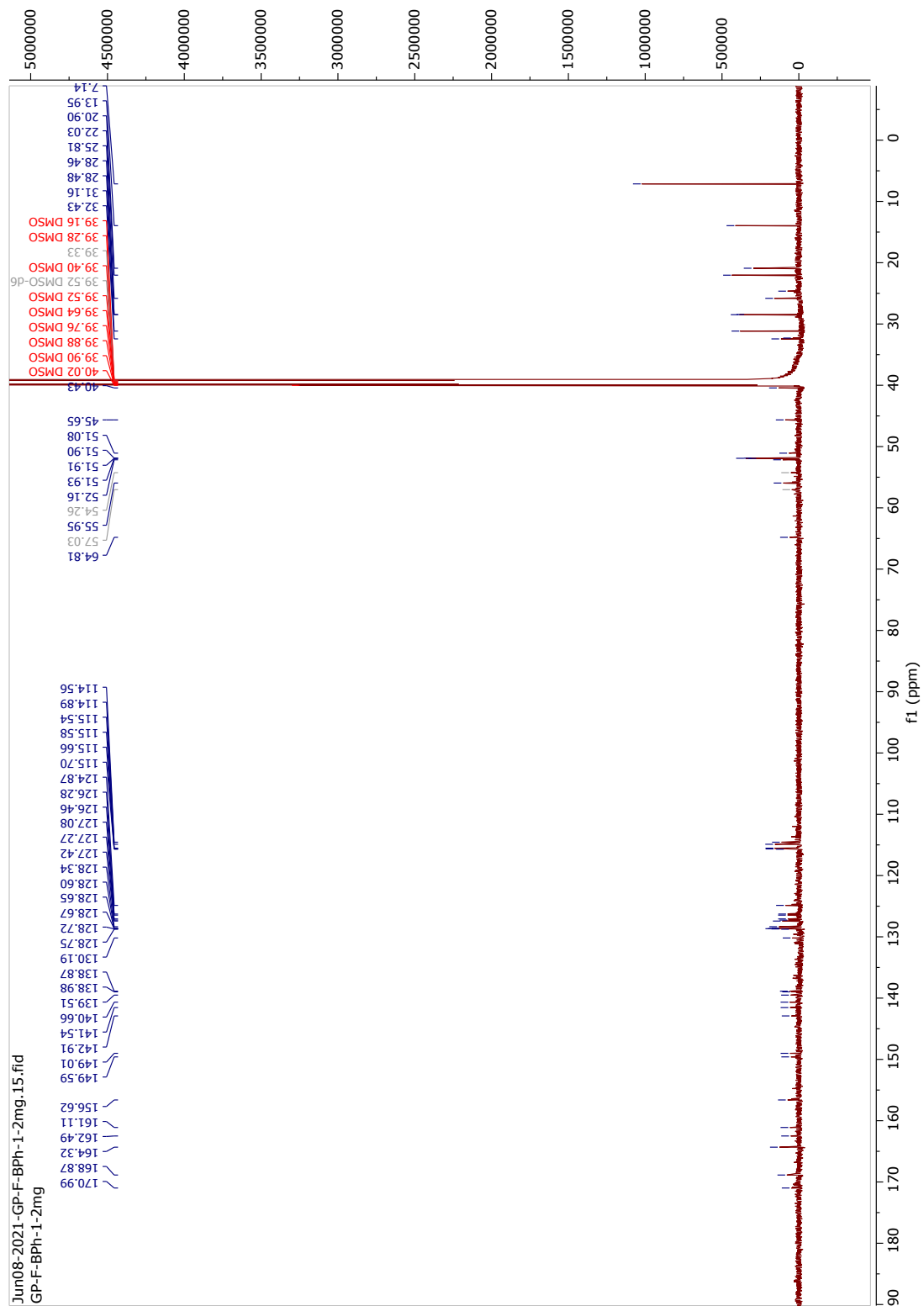
Compound 18



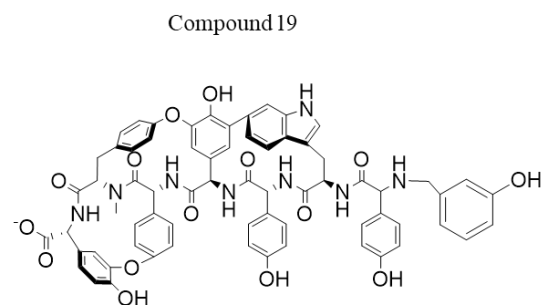
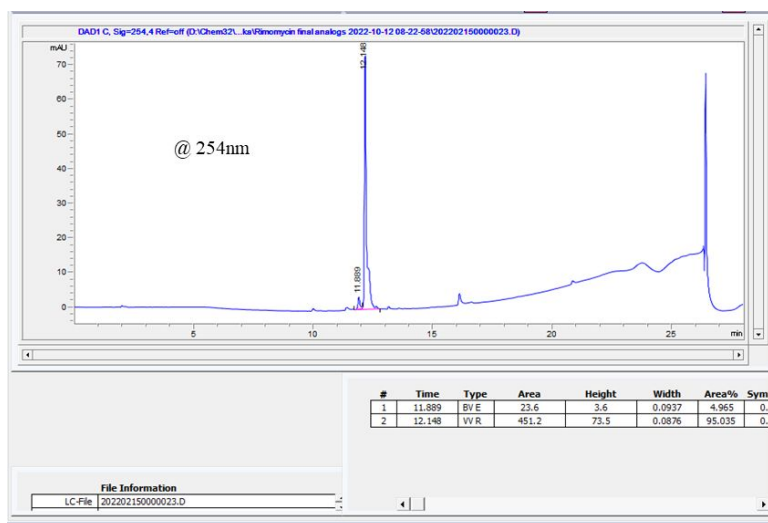
¹H-NMR of compound 18



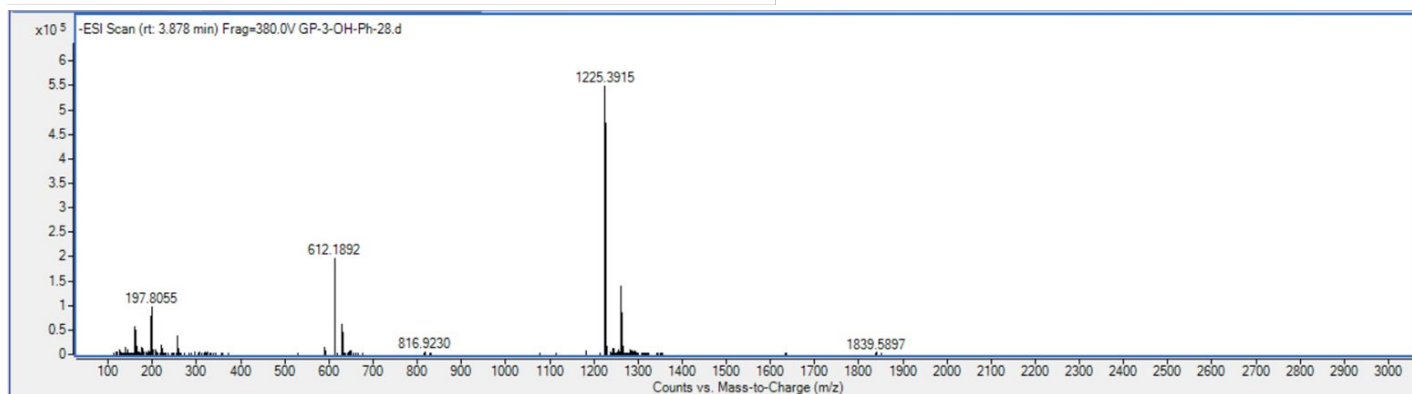
¹³C-NMR of compound 18



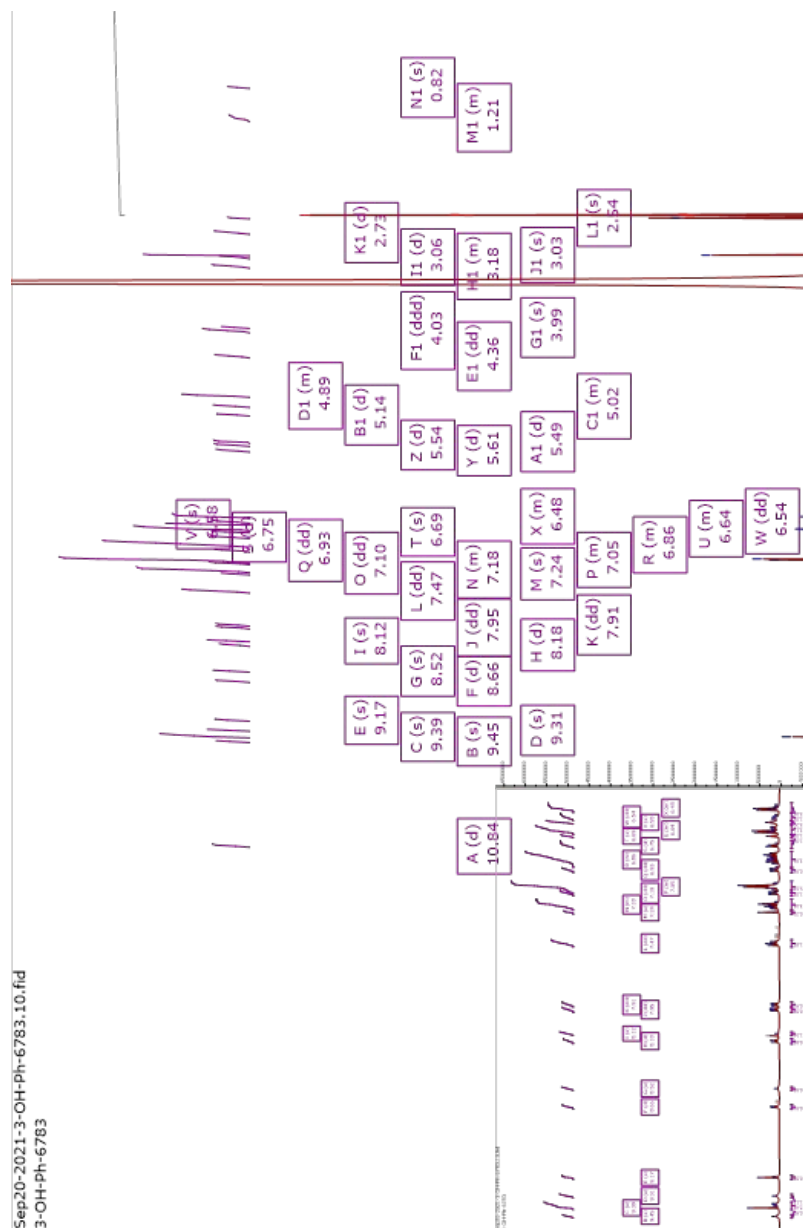
Compound 19



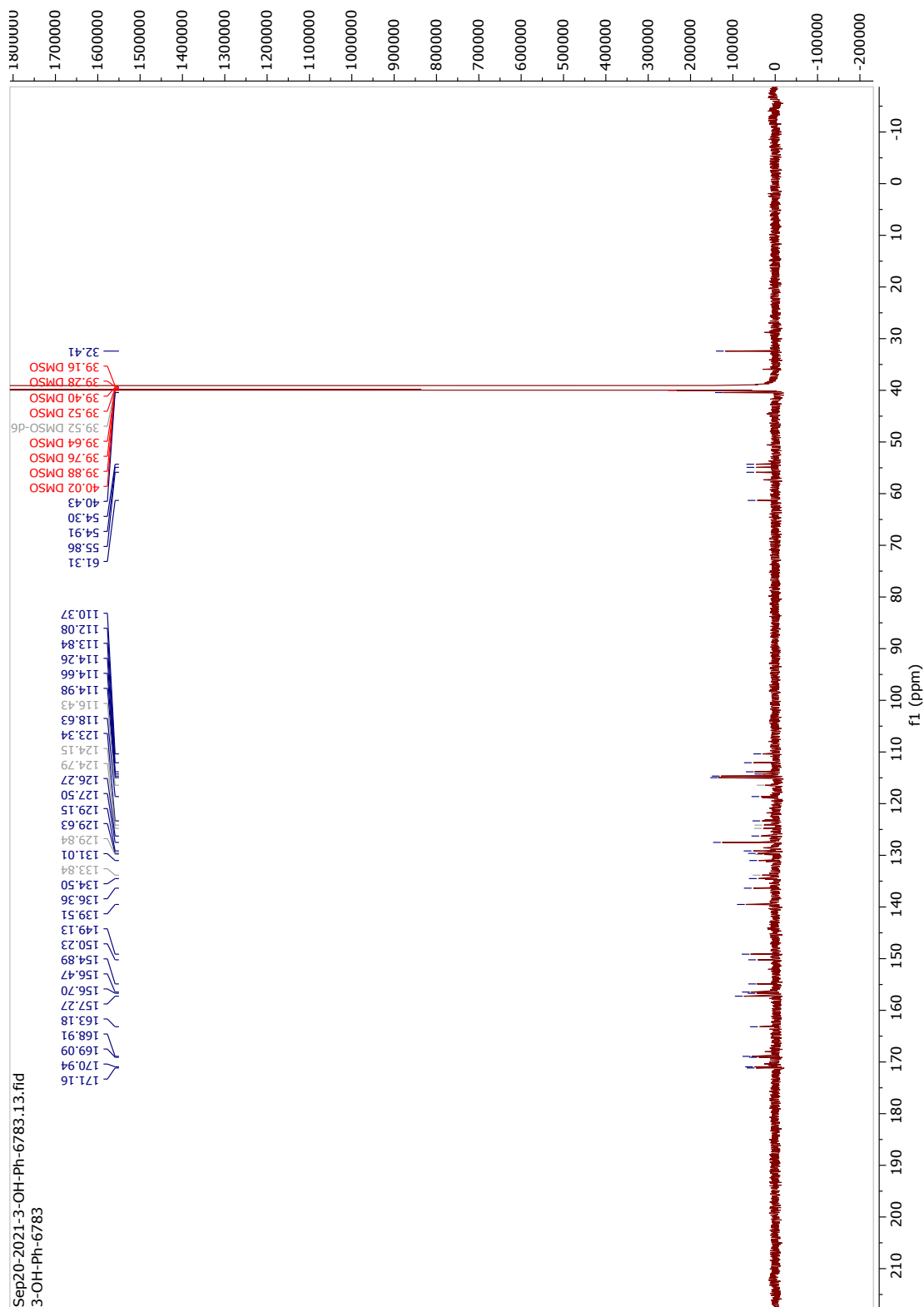
Chemical Formula: $C_{68}H_{57}N_8O_{15}$
Exact Mass: 1225.3949



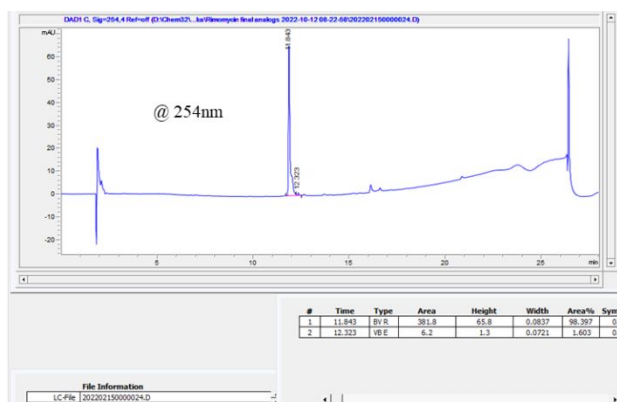
¹H-NMR of compound 19



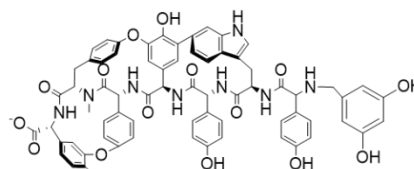
^{13}C -NMR of compound 19



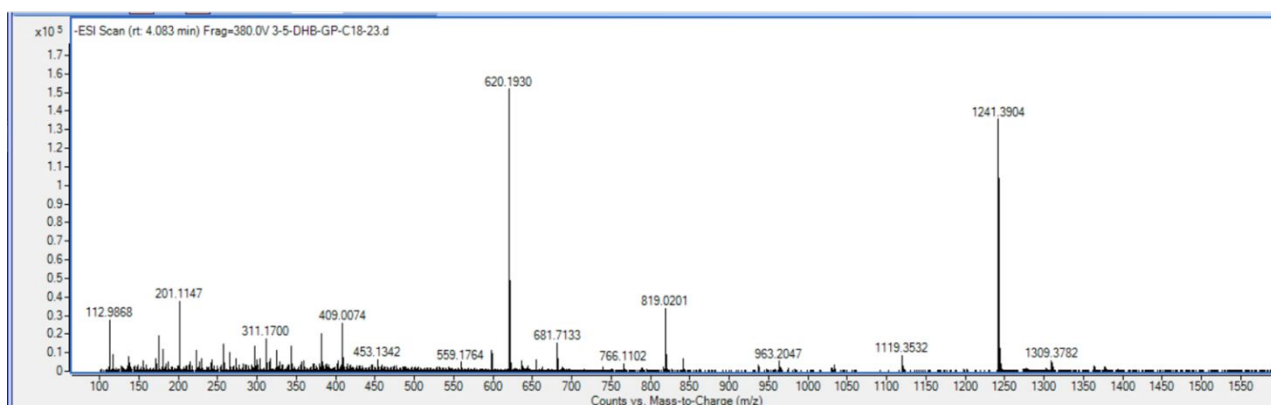
Compound 20



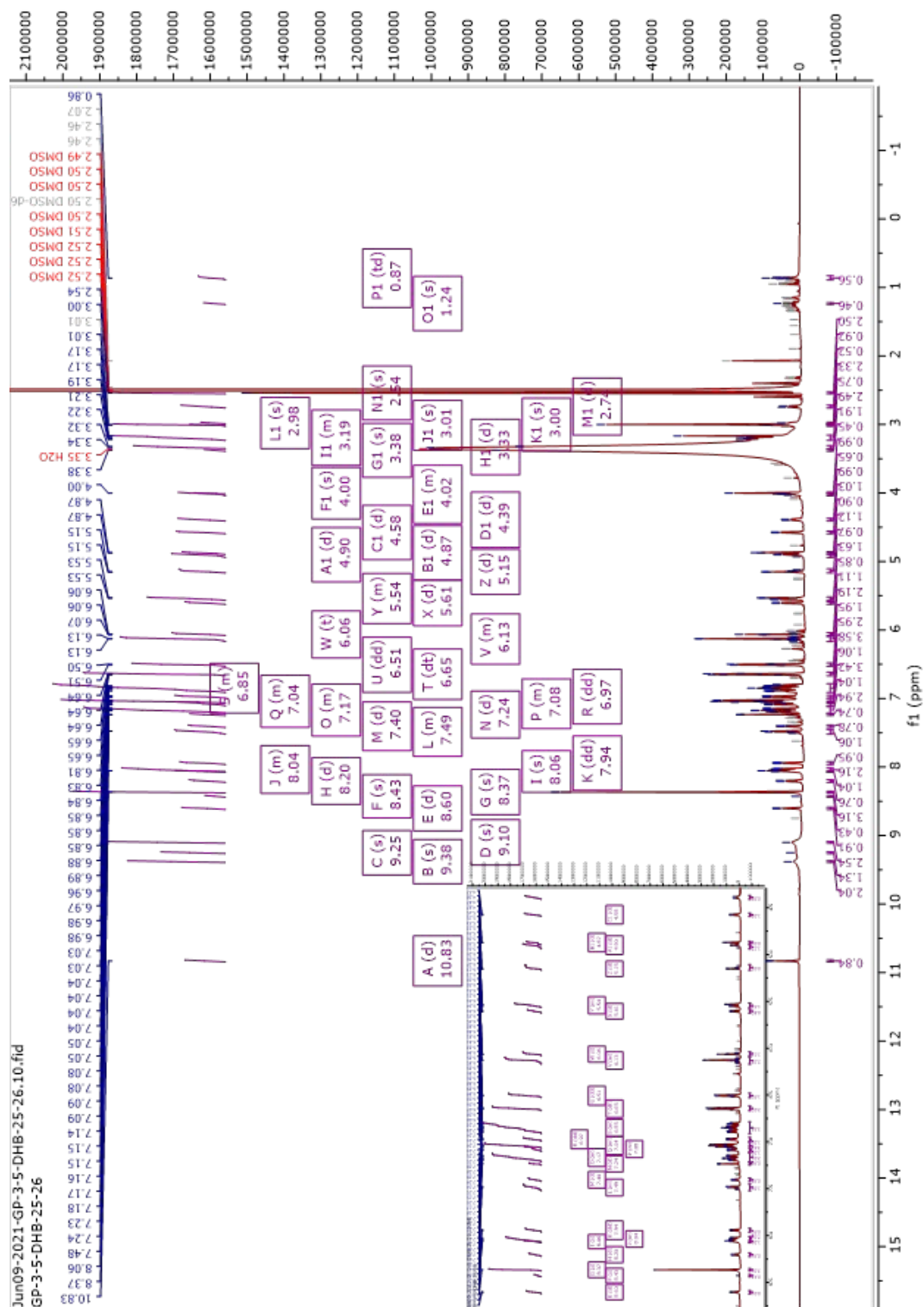
Compound 20



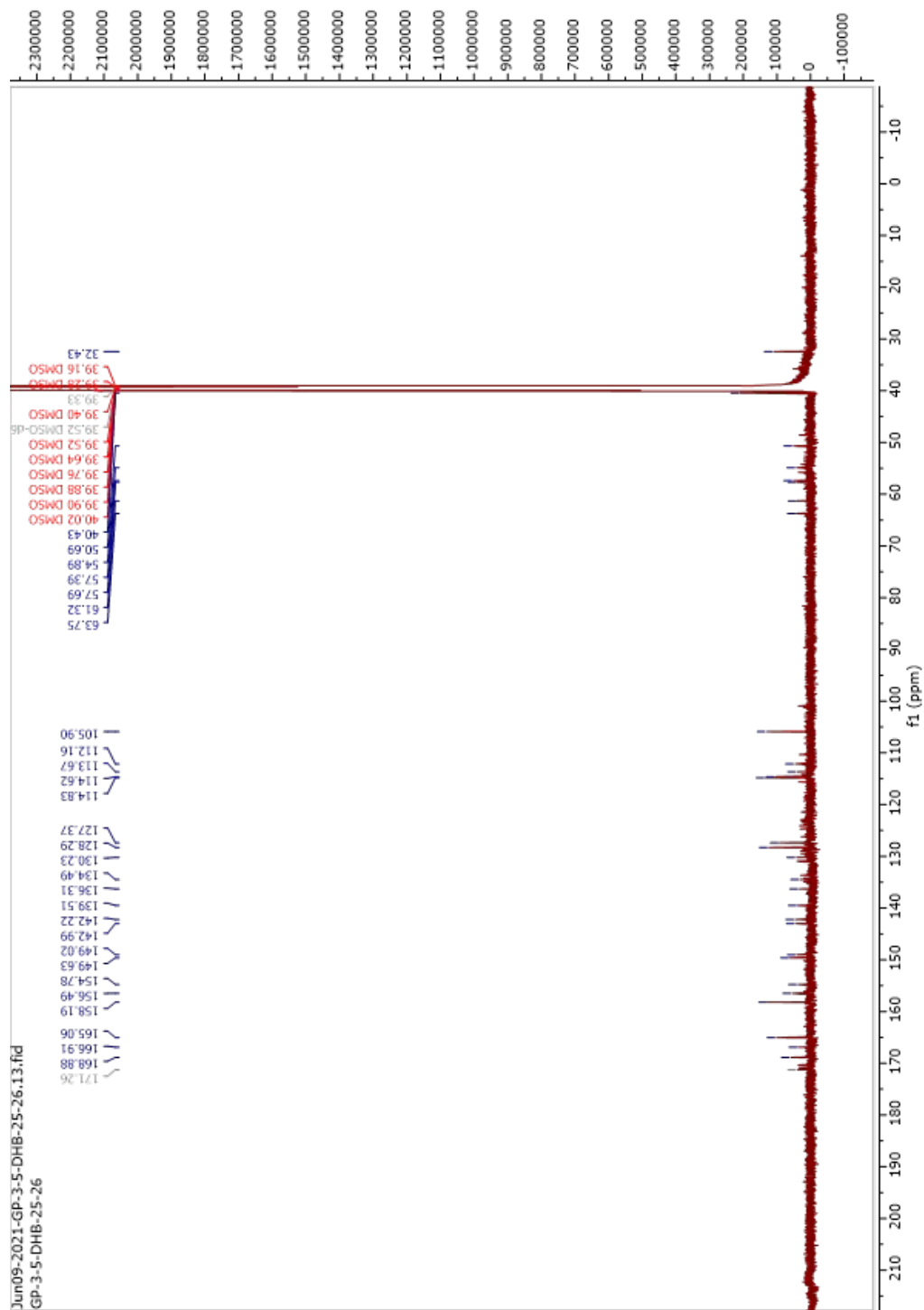
Chemical Formula: $C_{68}H_{57}N_8O_{16}^-$
Exact Mass: 1241.3898



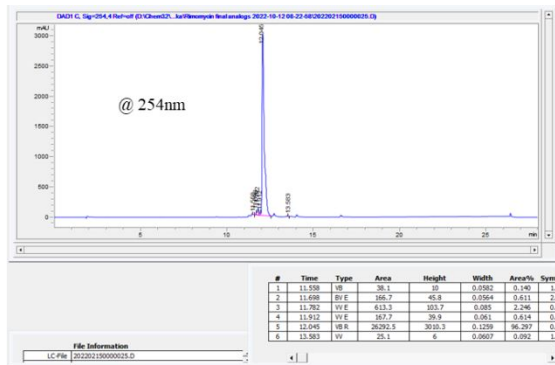
¹H-NMR of compound 20



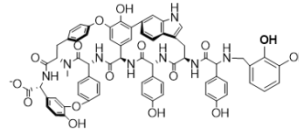
¹³C-NMR of compound 20



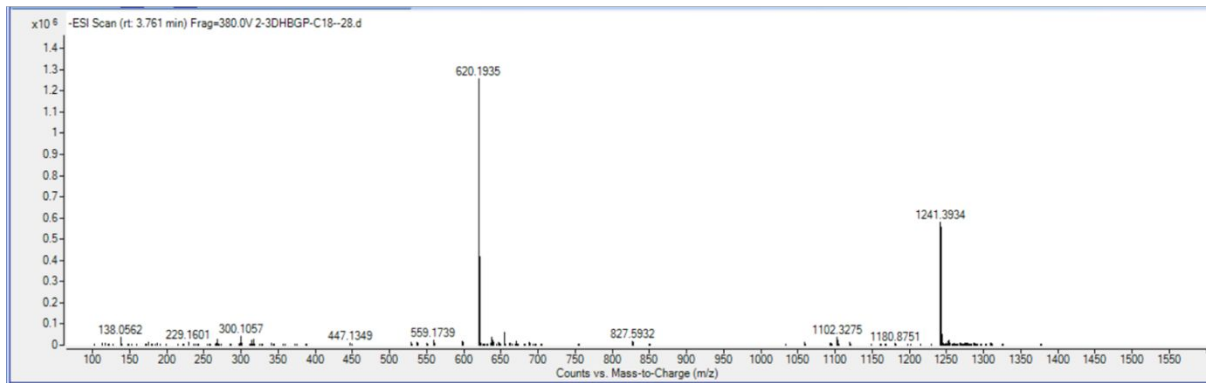
Compound 21



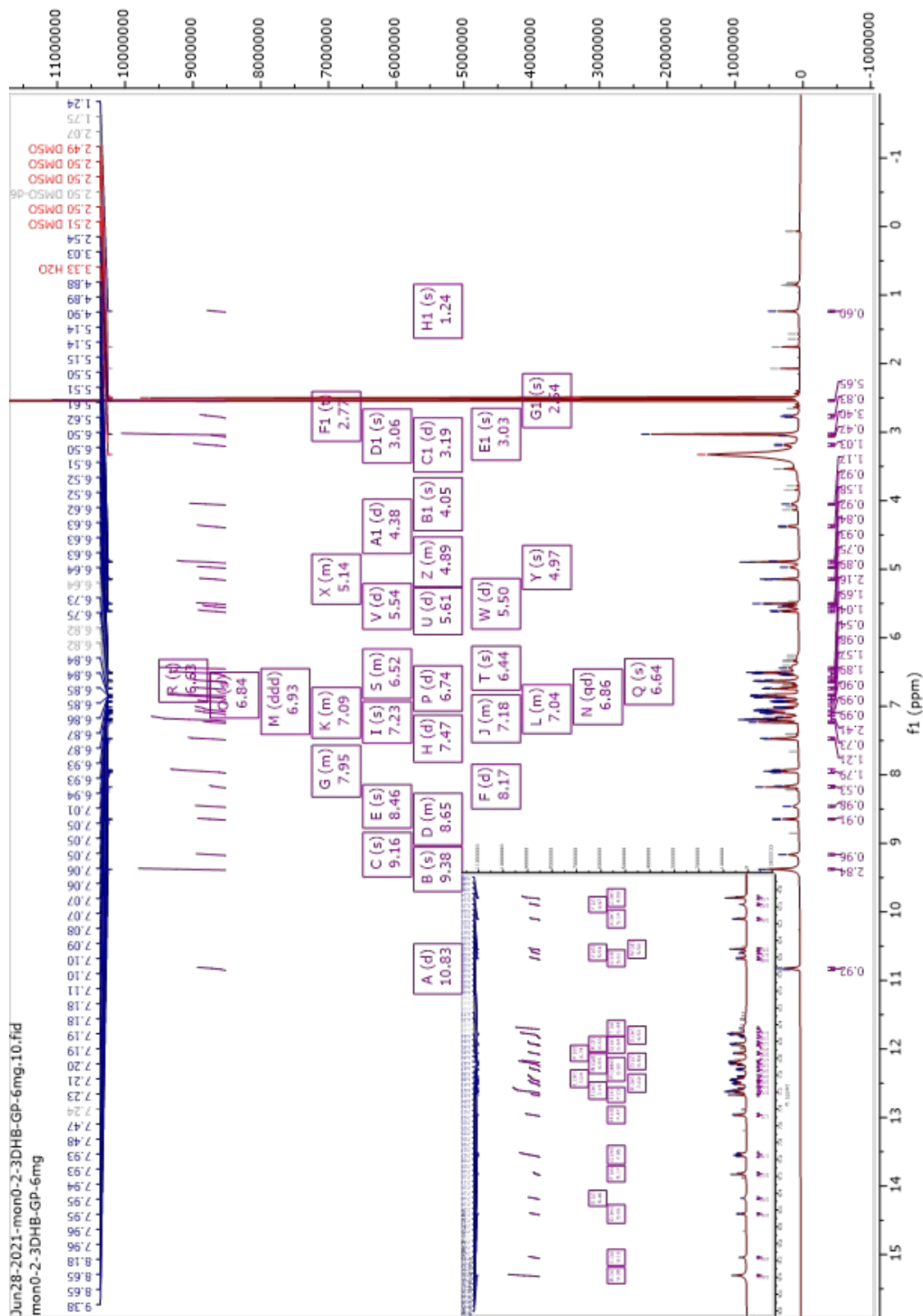
Compound 21



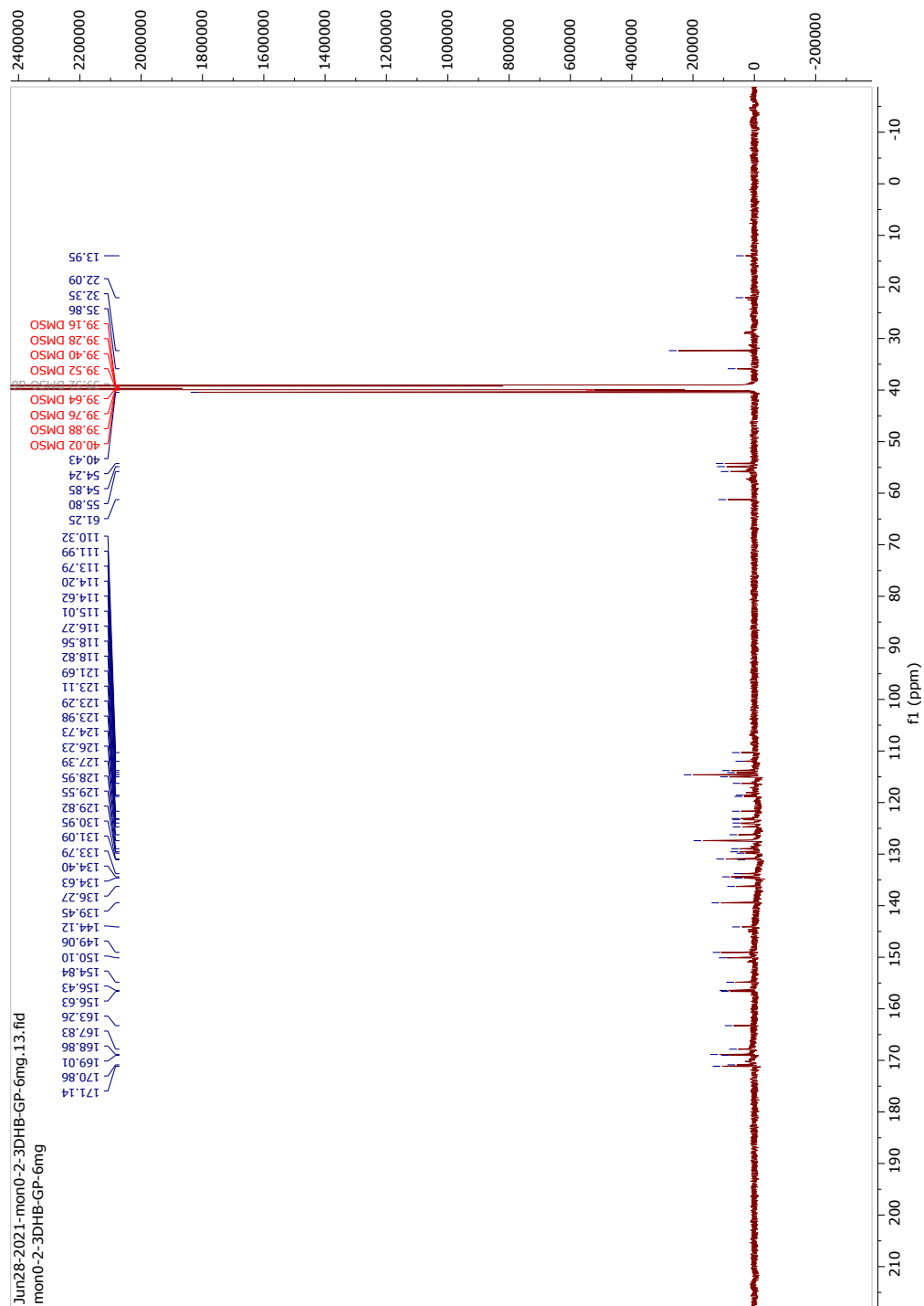
Chemical Formula: $C_{68}H_{57}N_8O_{16}$
Exact Mass: 1241.3898



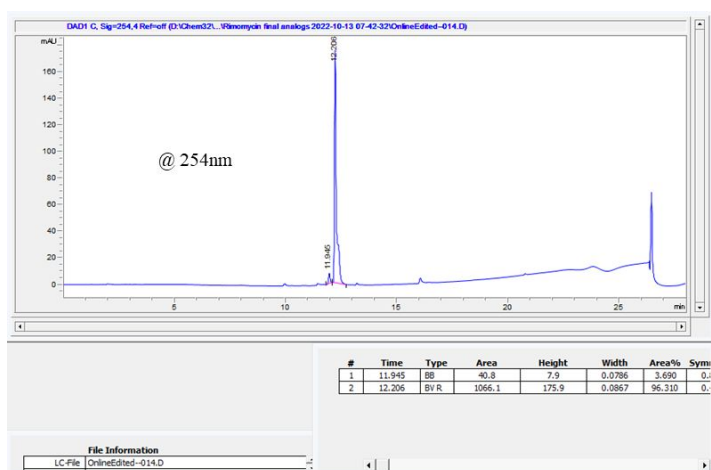
¹H-NMR of compound 21



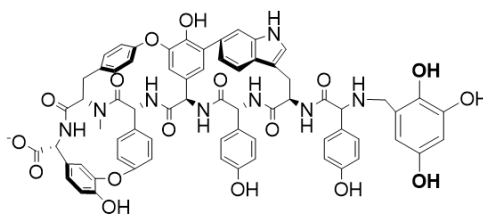
^{13}C -NMR of compound 21



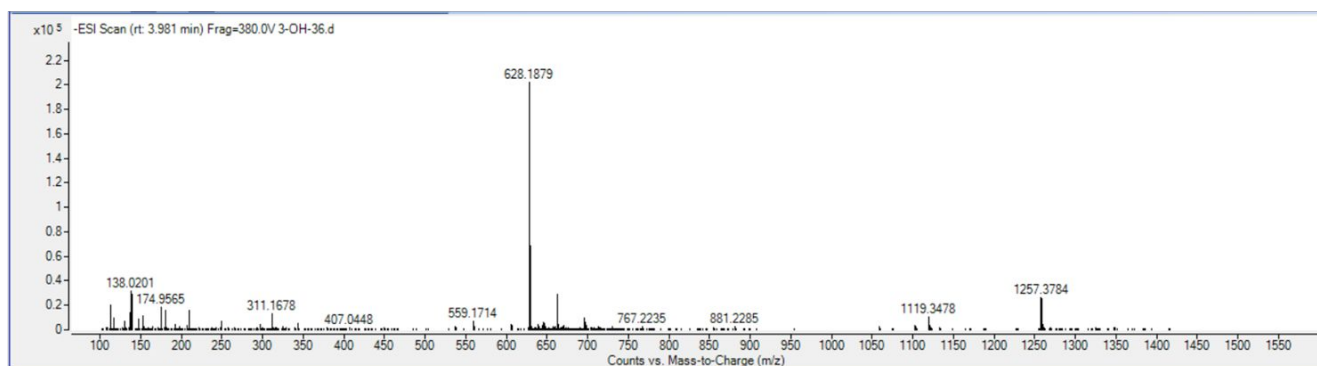
Compound 22



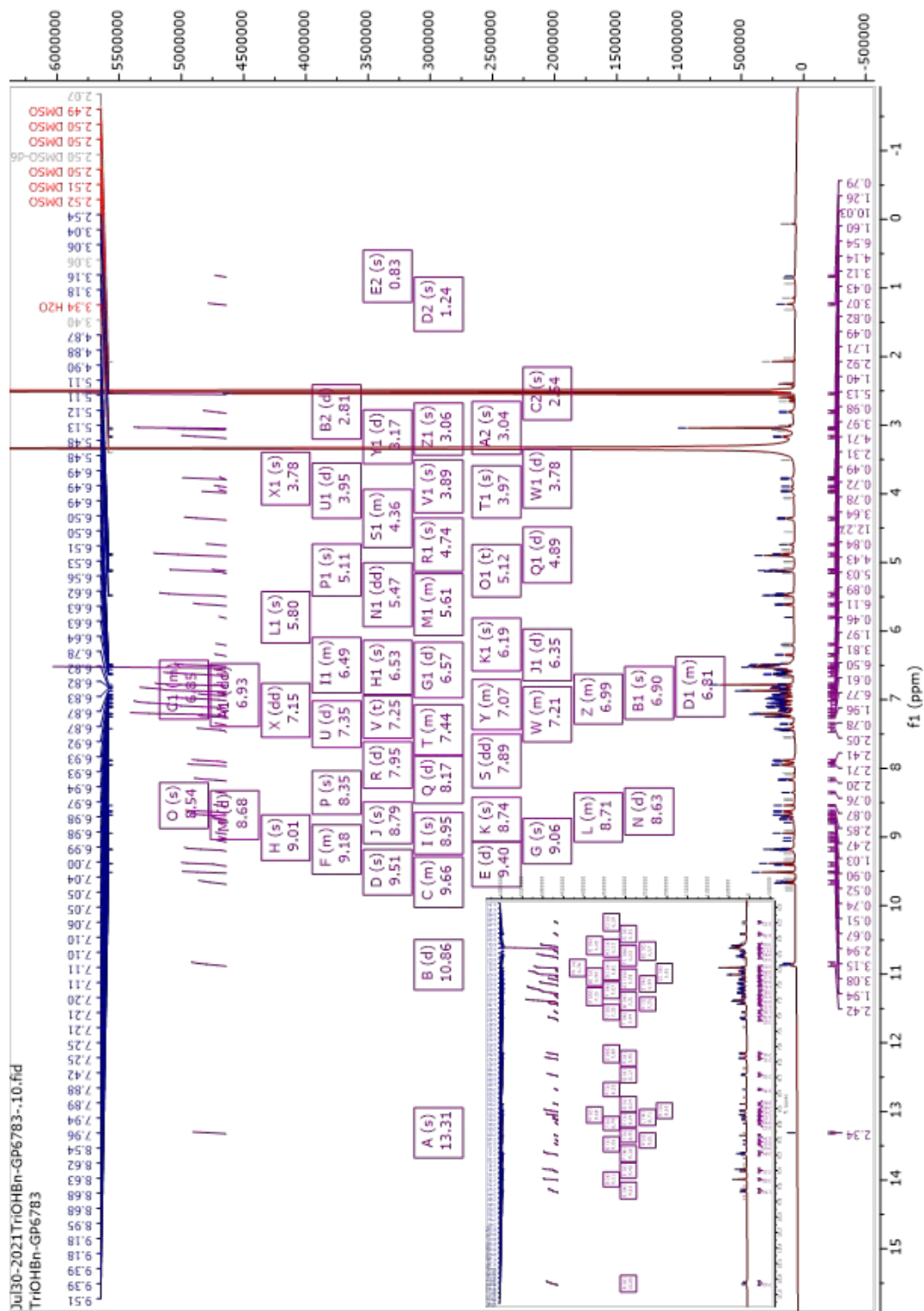
Compound 22



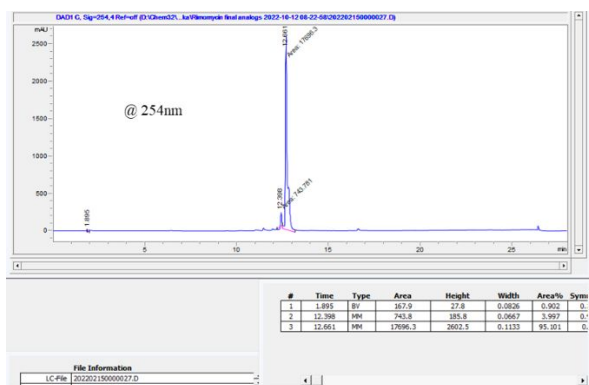
Chemical Formula: $C_{68}H_{57}N_8O_{17}$
Exact Mass: 1257.3847



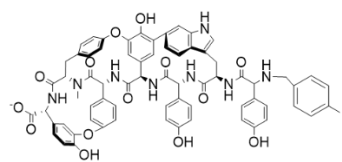
¹H-NMR of compound 22



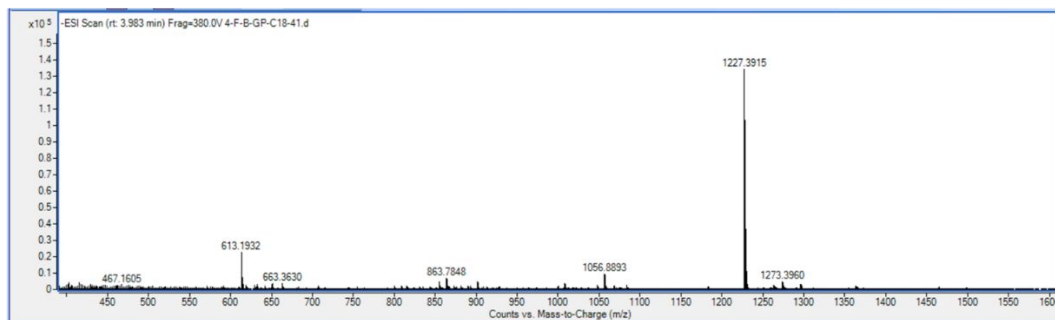
Compound 23



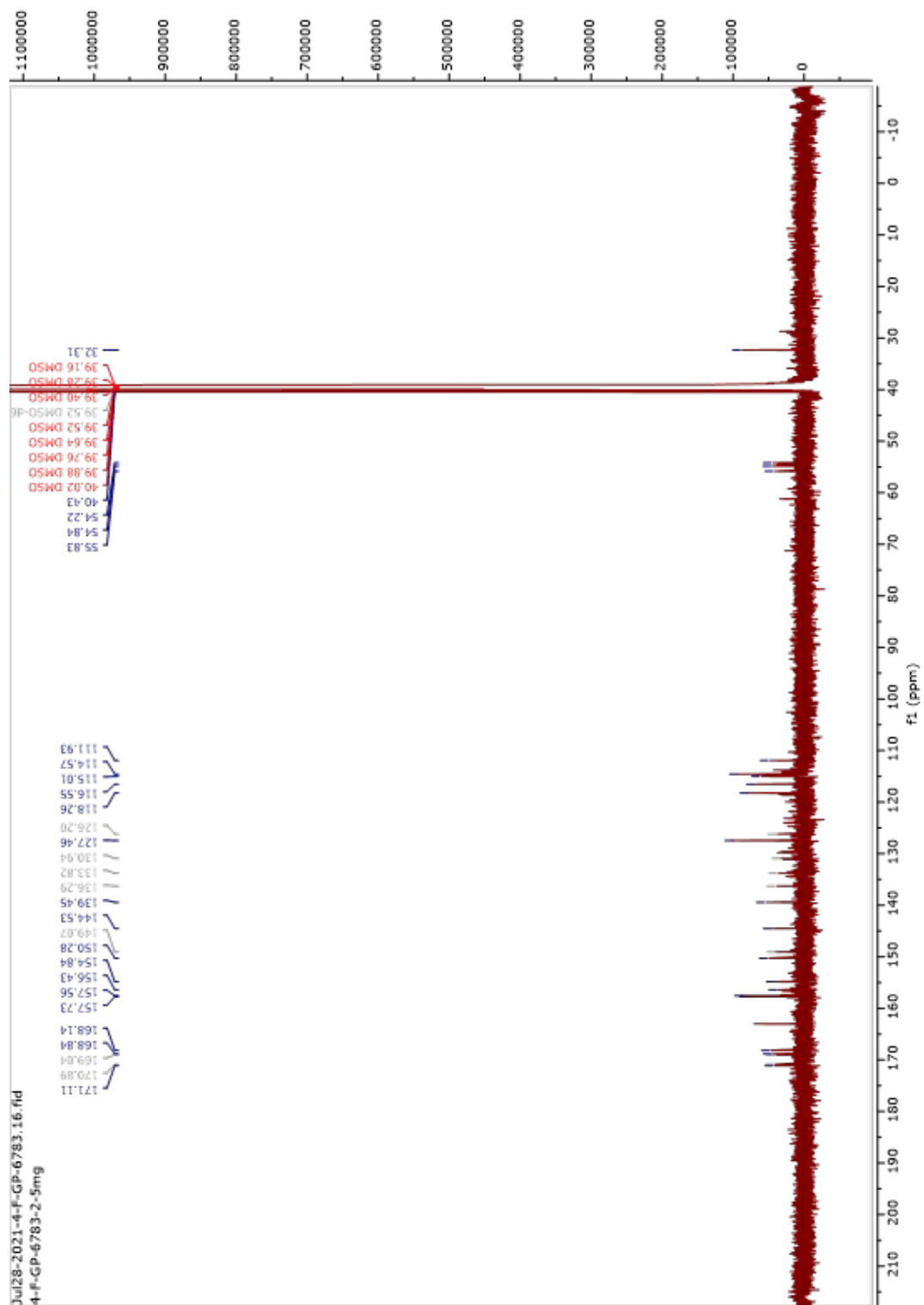
Compound 23



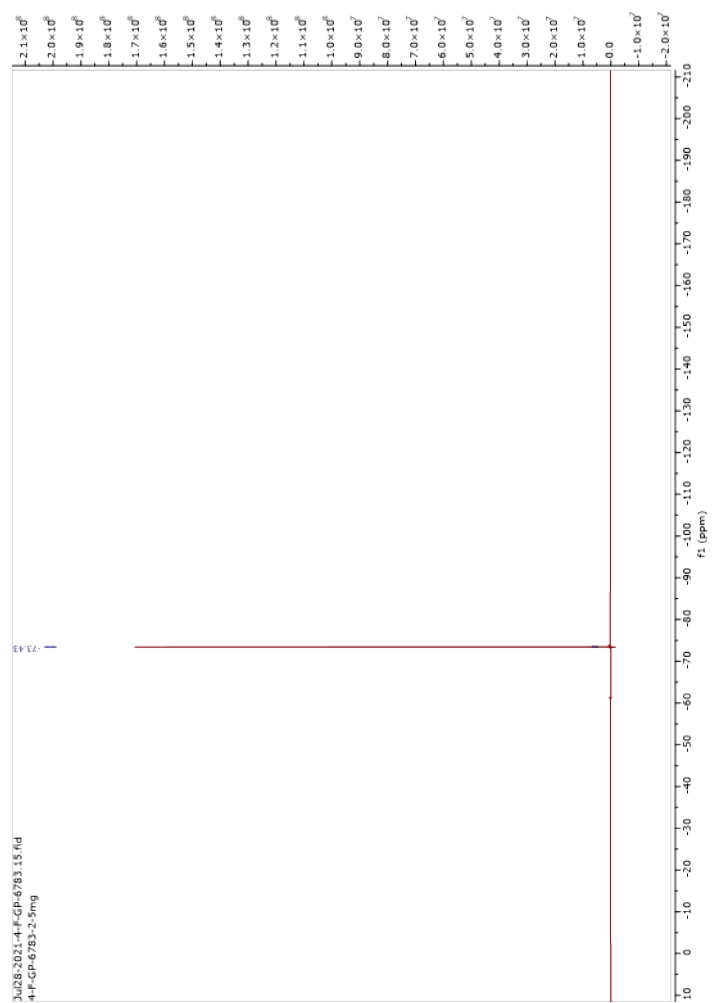
Chemical Formula: $C_{68}H_{56}FNa_{14}O_{14}^-$
Exact Mass: 1227.3906



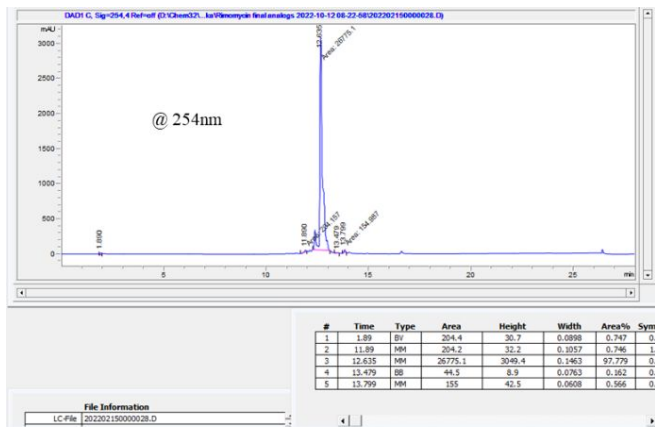
^{13}C -NMR of compound 23



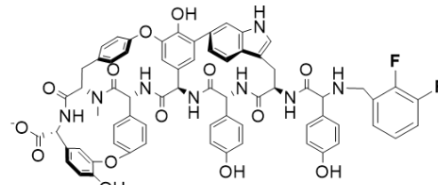
¹⁹F-NMR of compound **23**



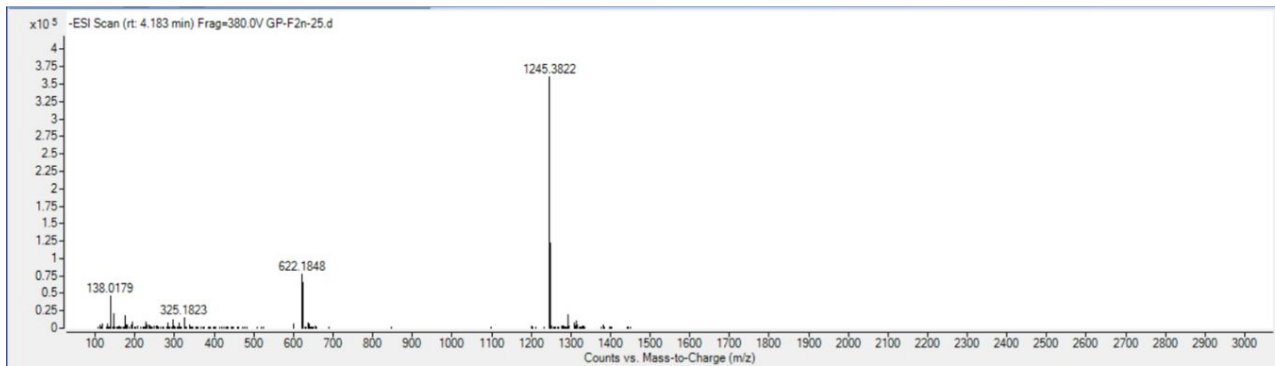
Compound 24



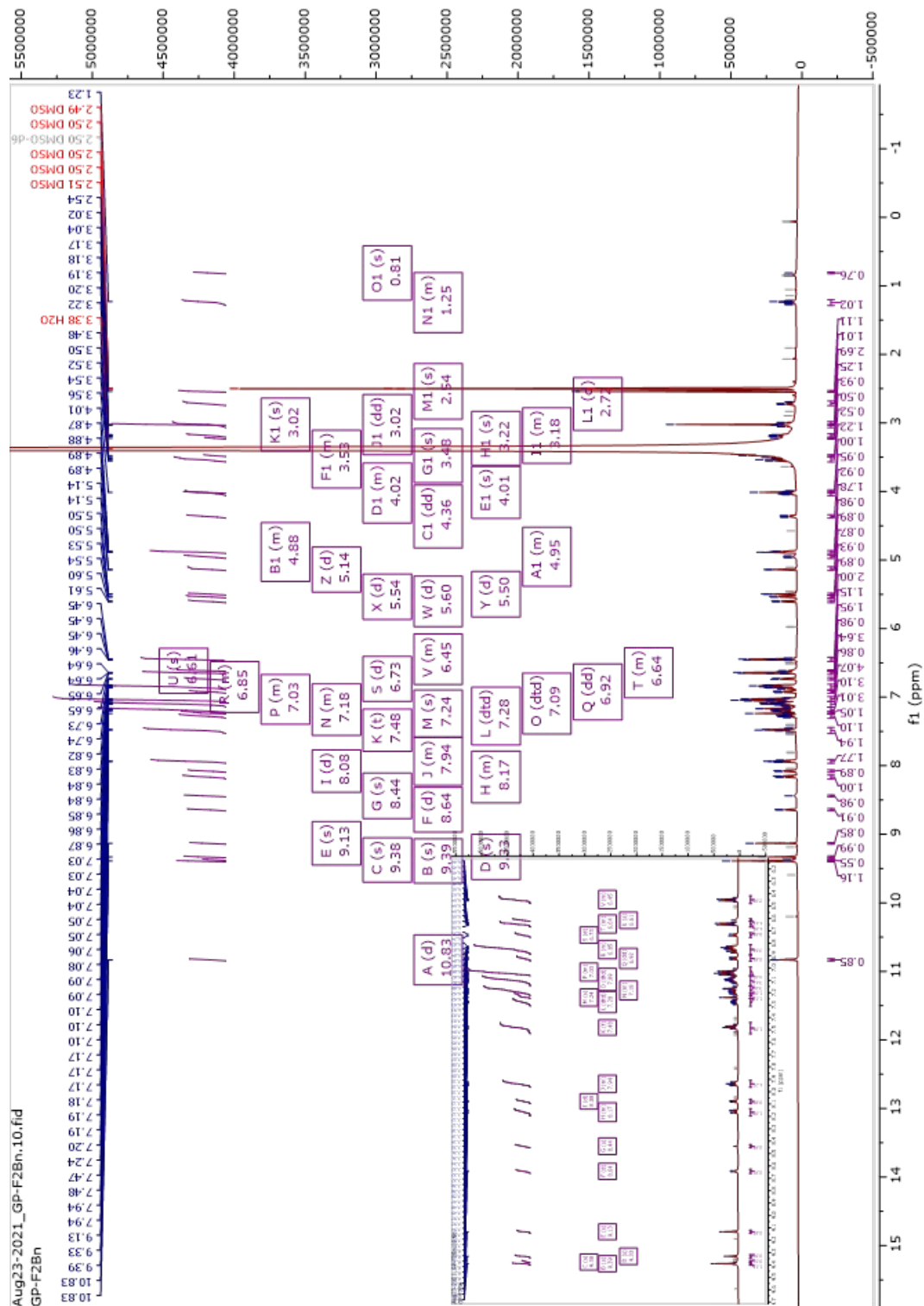
Compound24



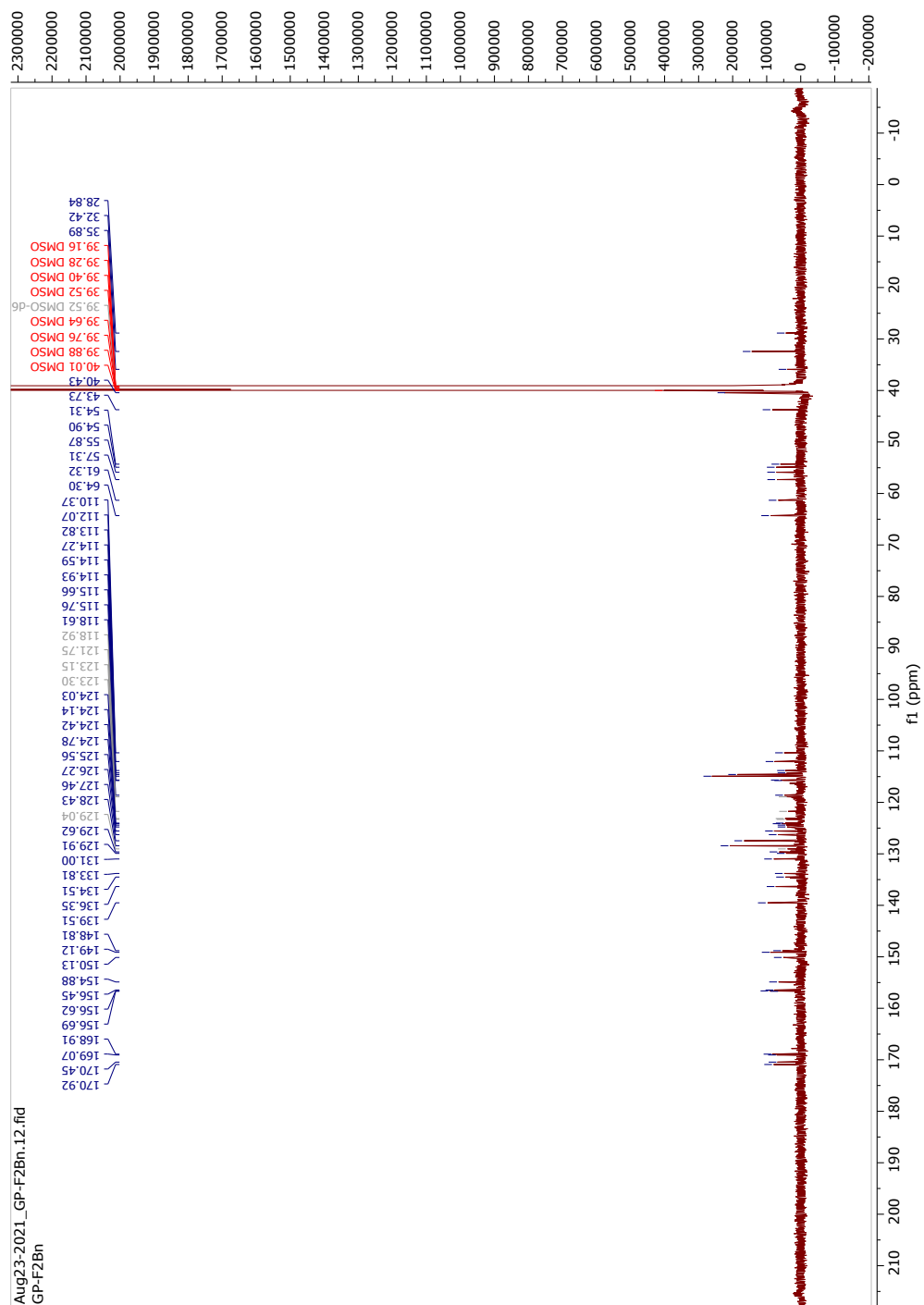
Chemical Formula: $C_{68}H_{55}F_2N_8O_{14}^-$
Exact Mass: 1245.3811



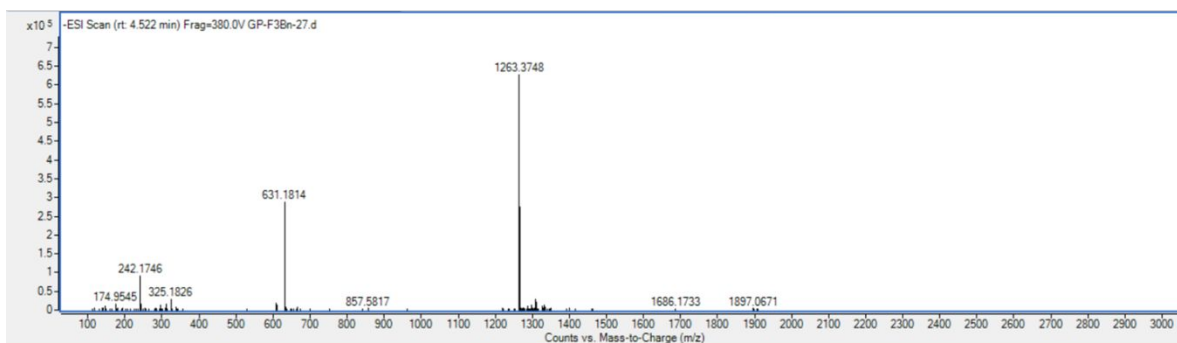
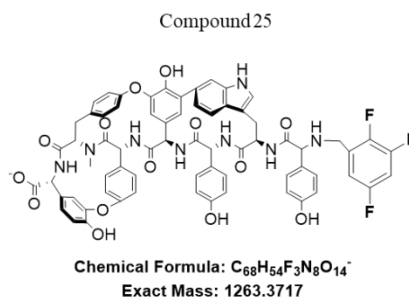
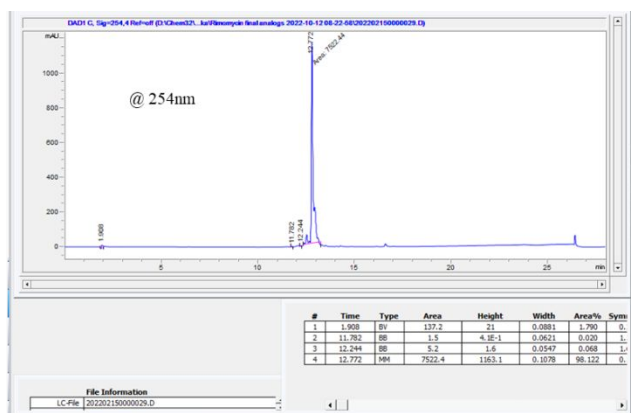
¹H-NMR of compound 24



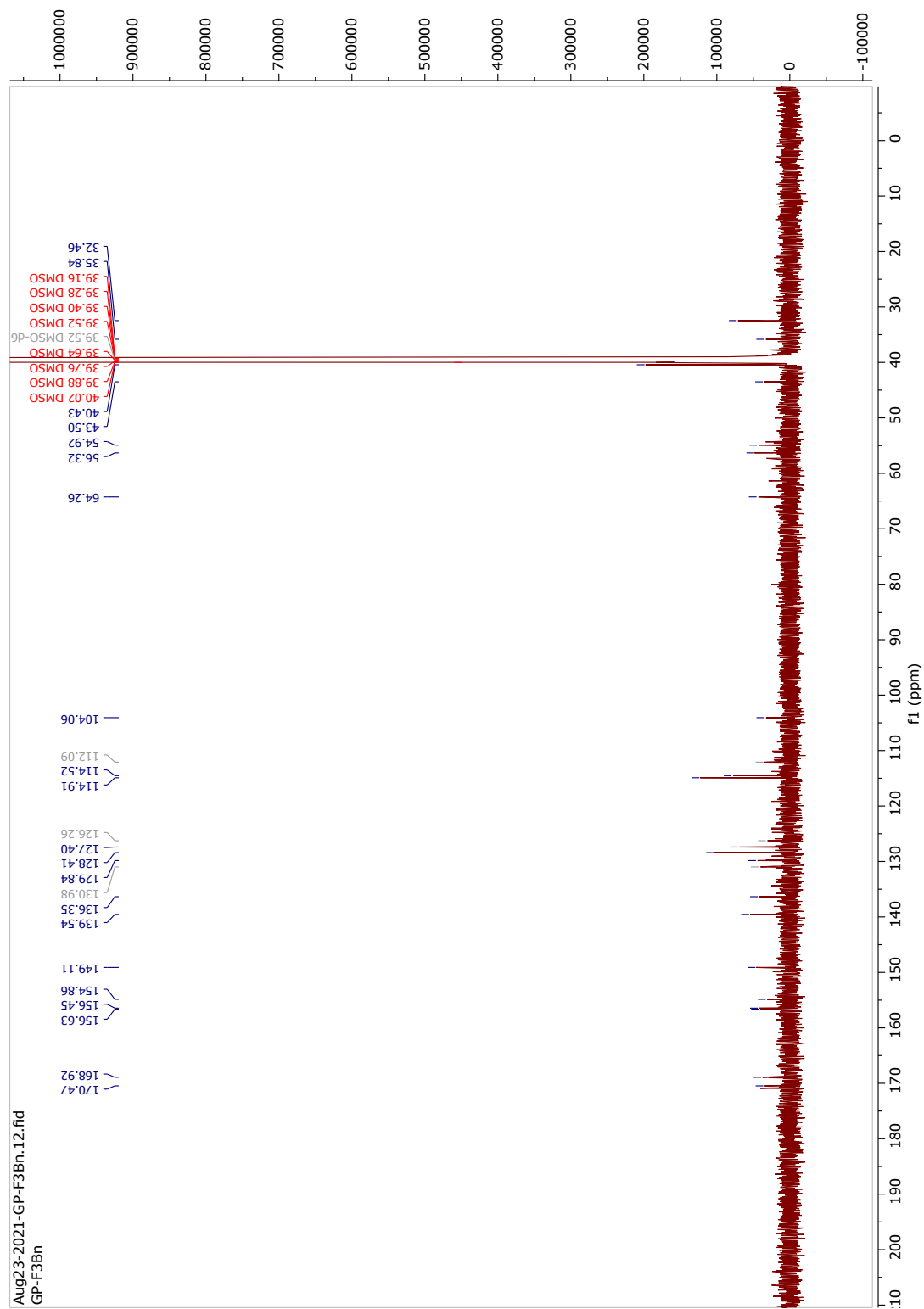
¹³C-NMR of compound 24



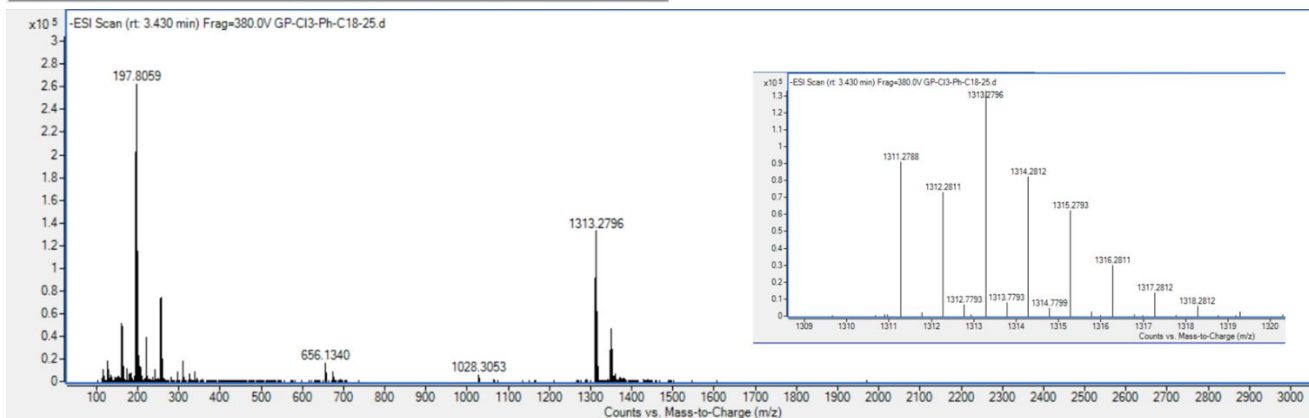
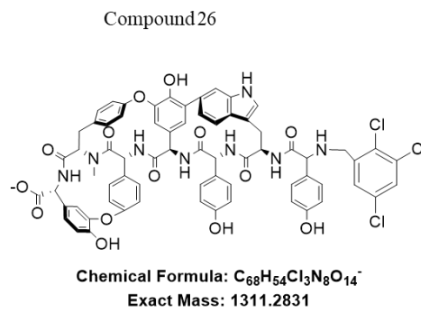
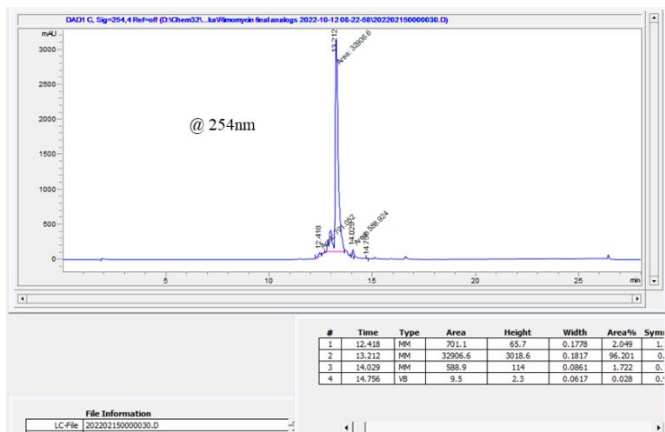
Compound 25



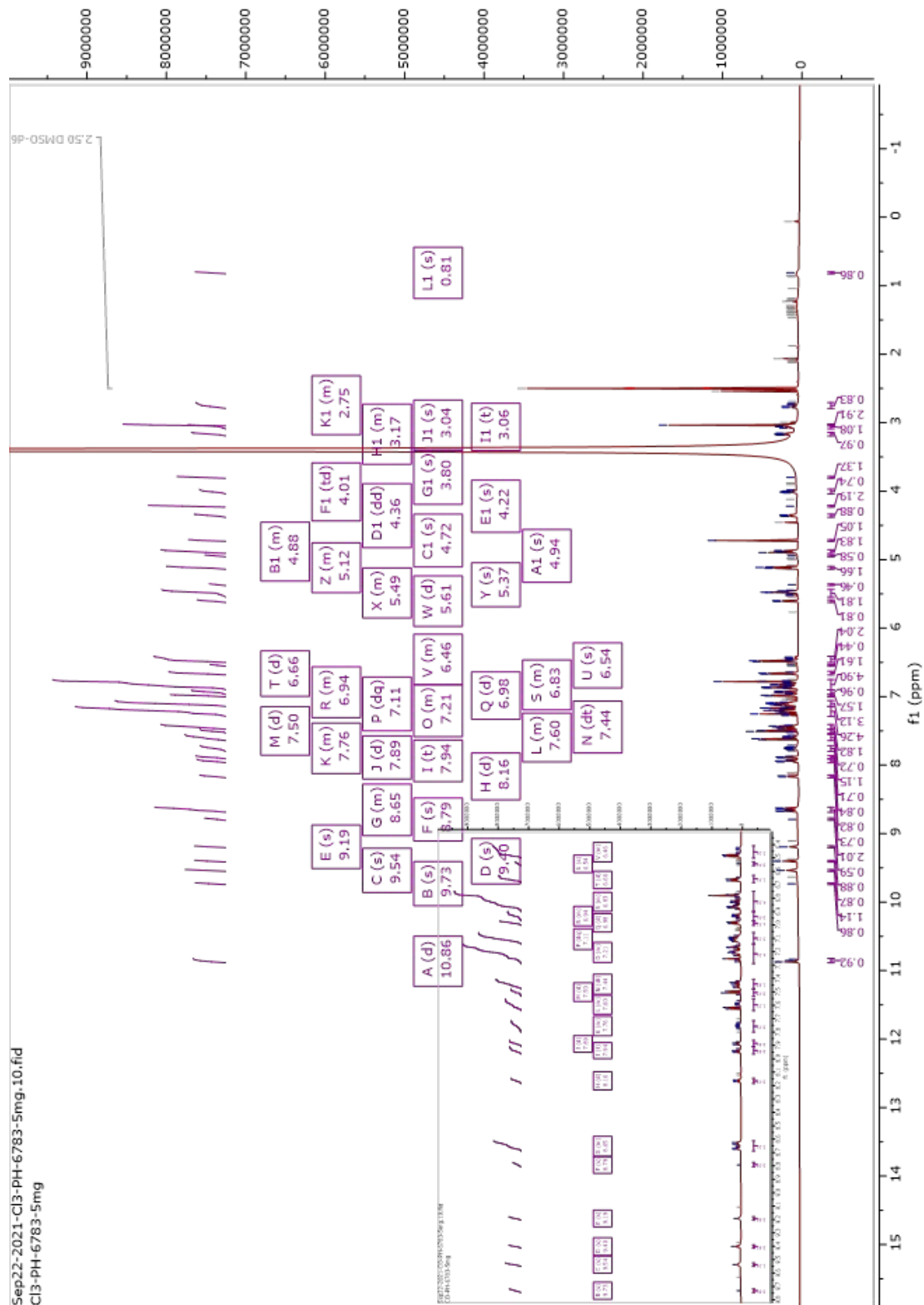
^{13}C -NMR of compound **25**



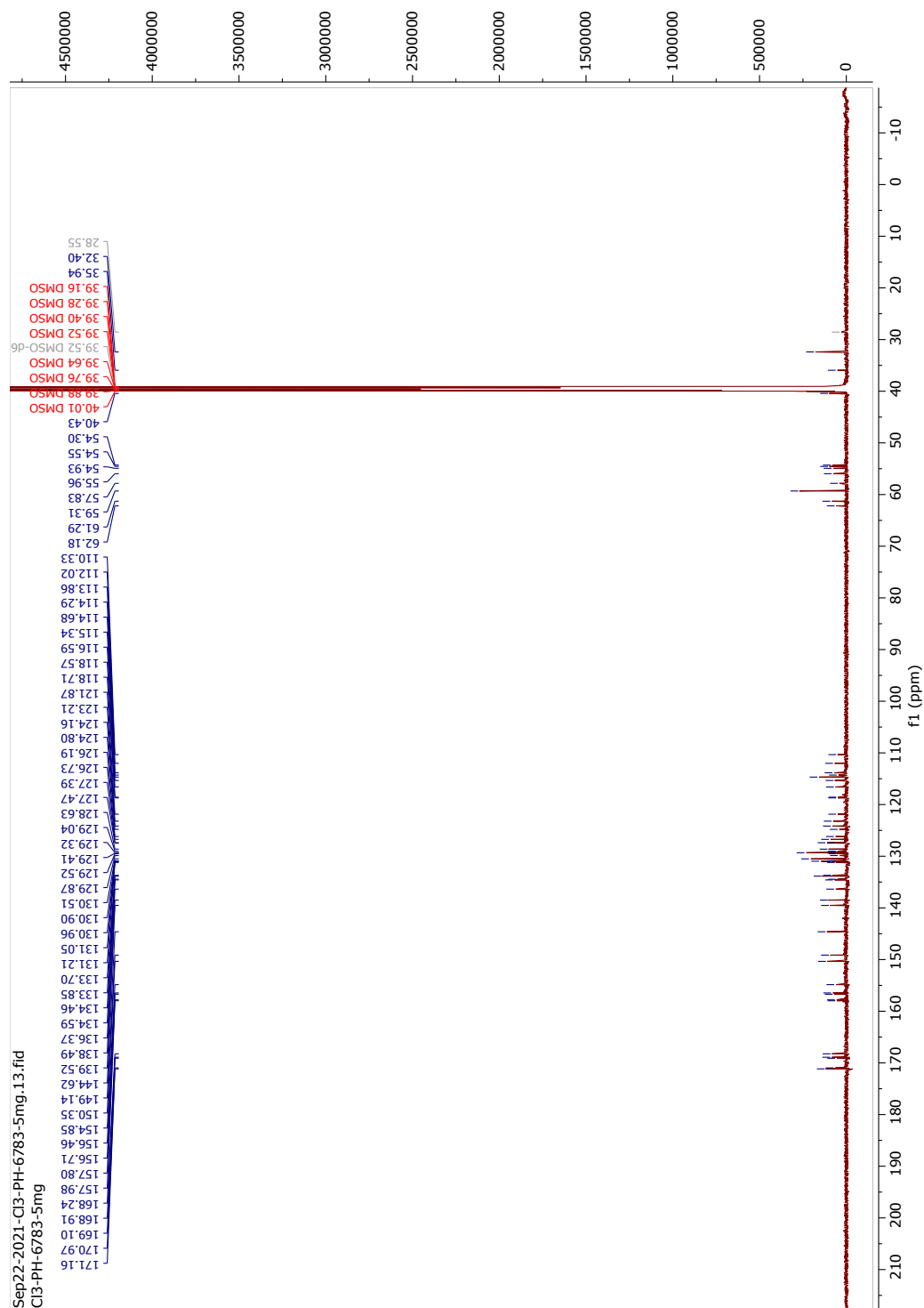
Compound 26



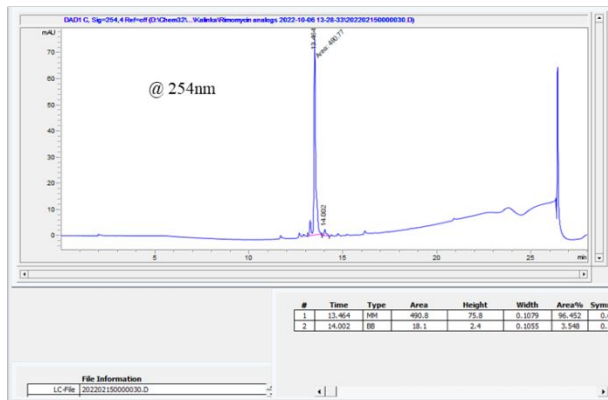
¹H-NMR of compound 26



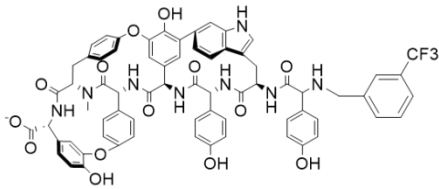
¹³C-NMR of compound 26



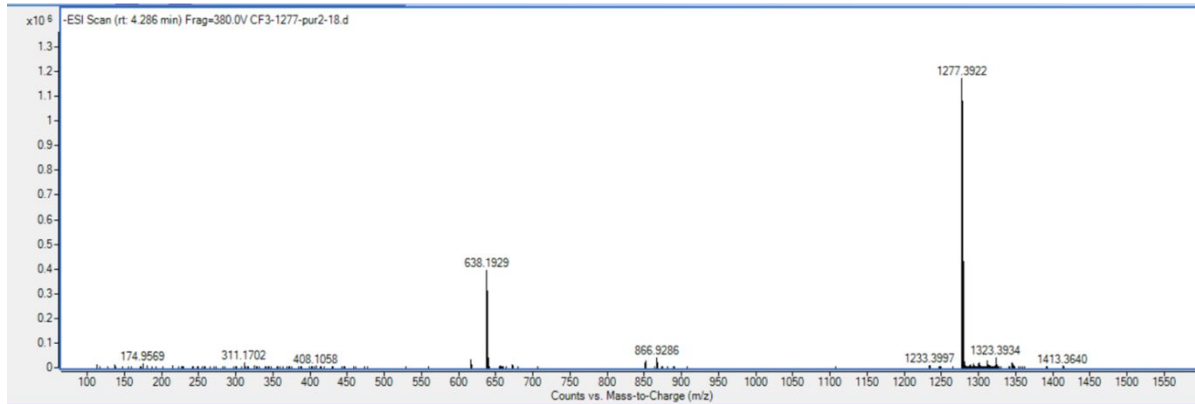
Compound 27



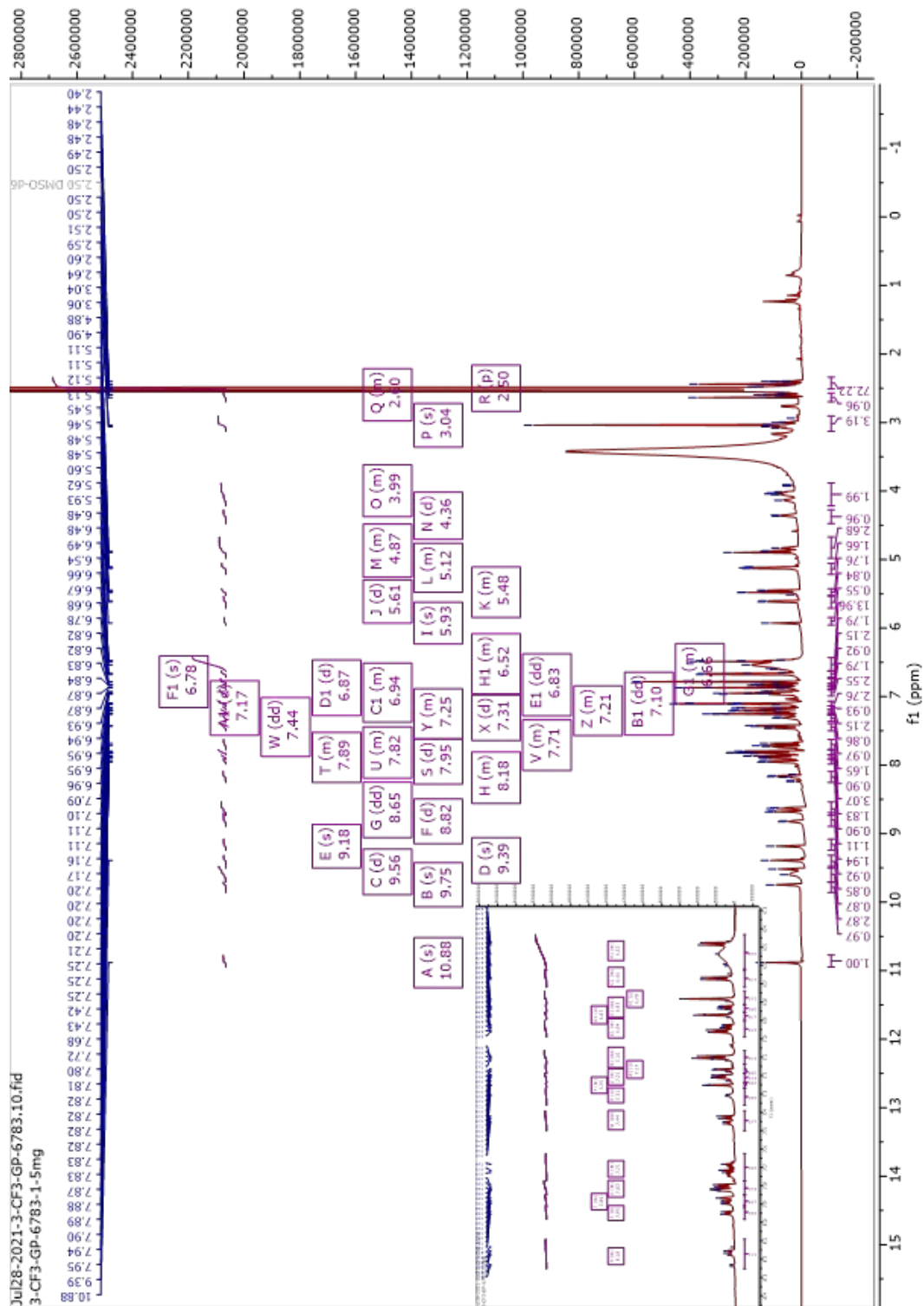
Compound 27



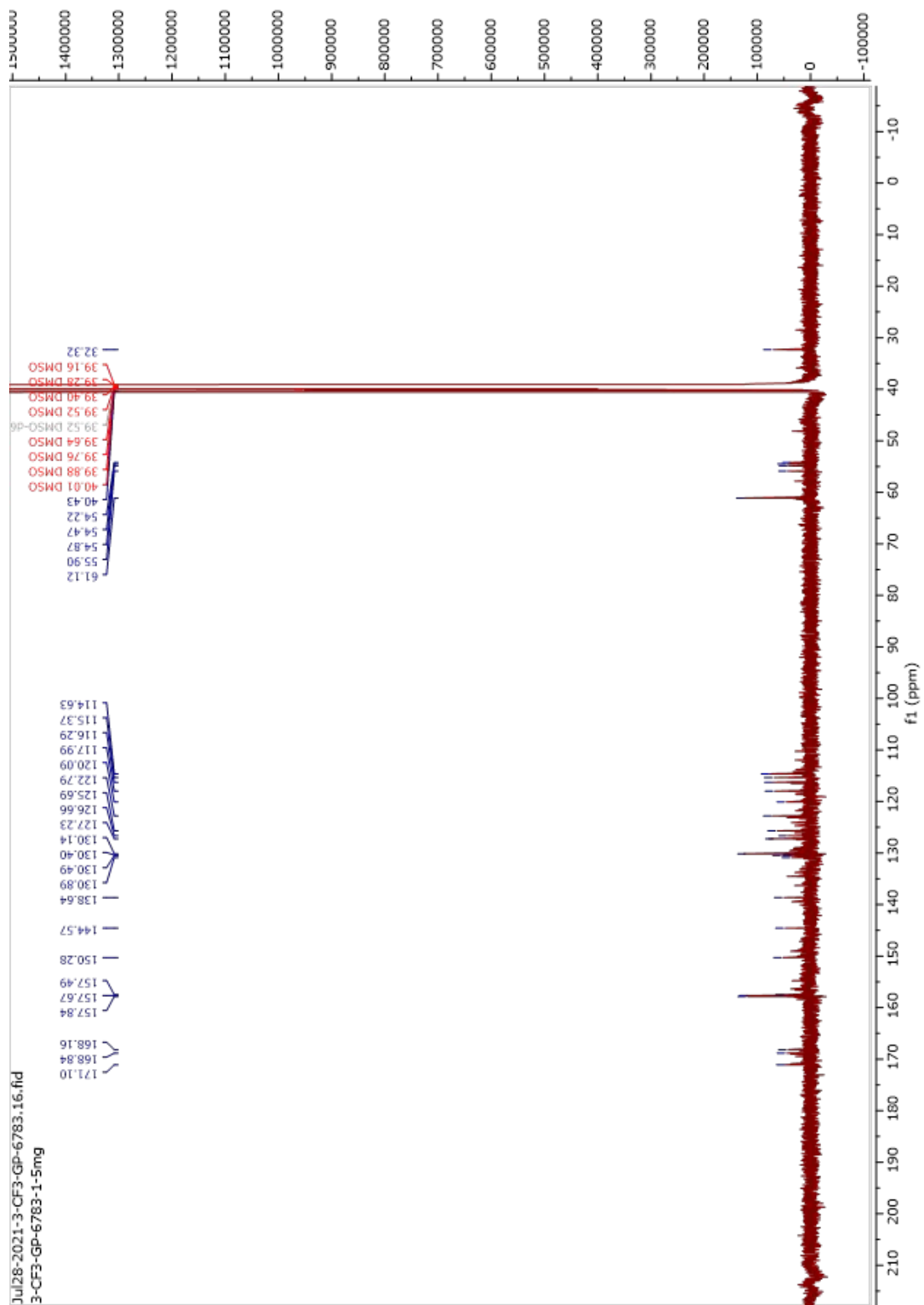
Chemical Formula: $C_{69}H_{56}F_3N_8O_{14}^-$
Exact Mass: 1277.3874



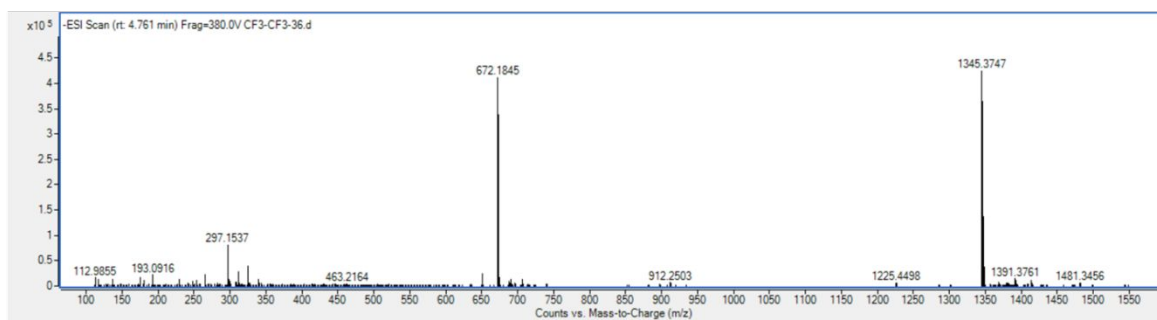
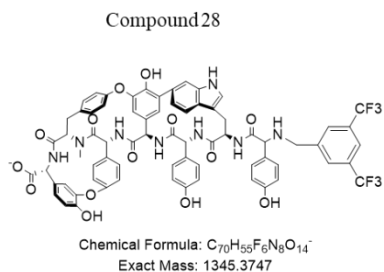
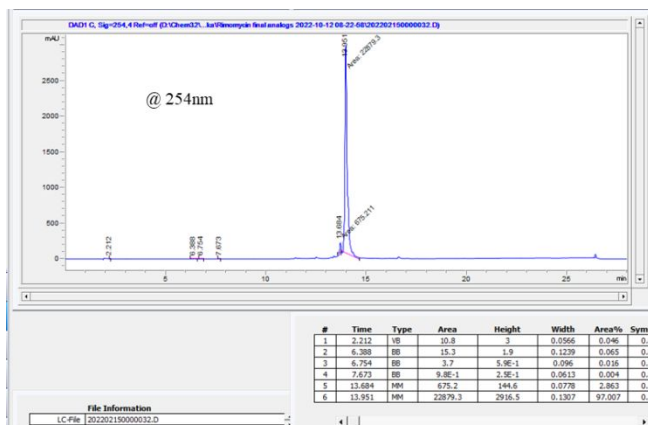
¹H-NMR of compound 27



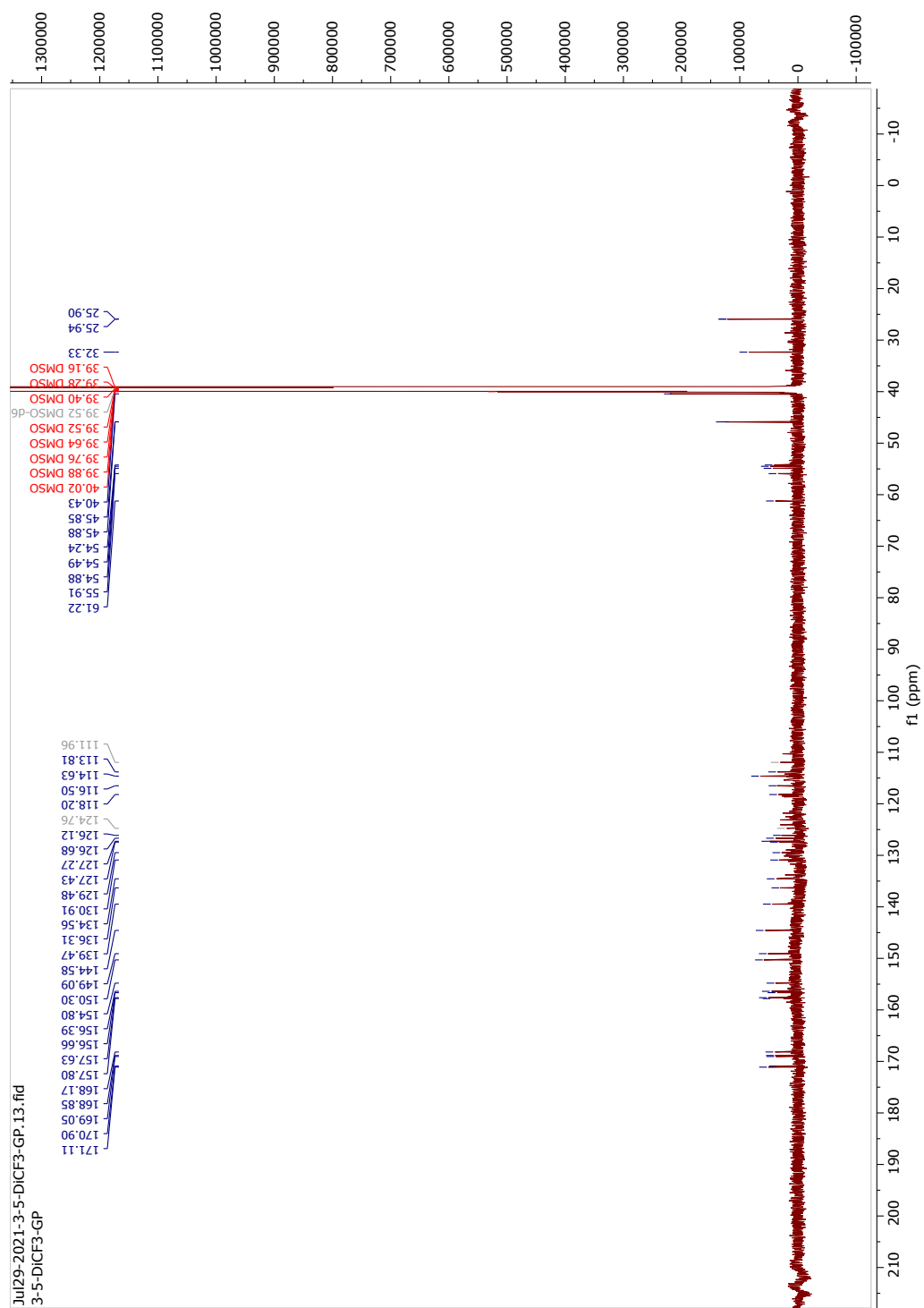
^{13}C -NMR of compound 27



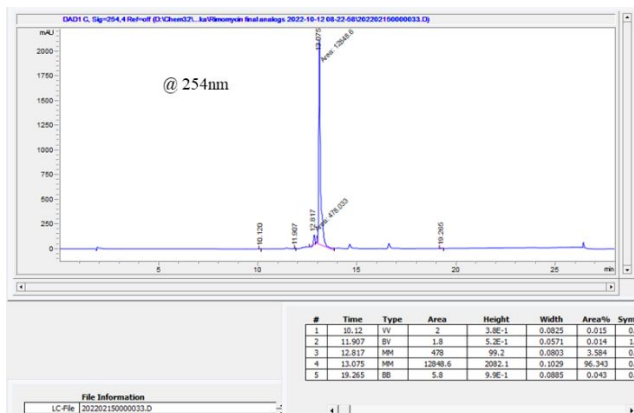
Compound 28



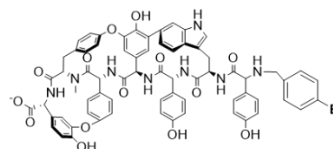
^{13}C -NMR of compound 28



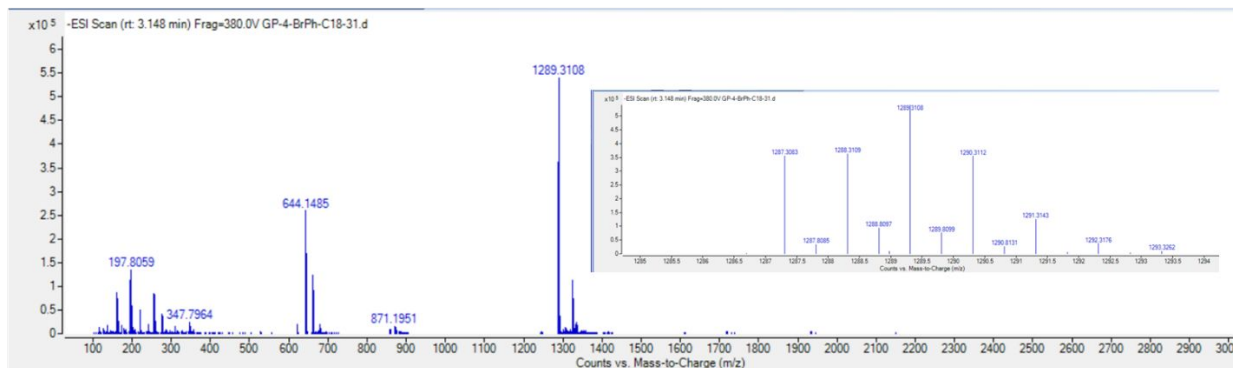
Compound 29



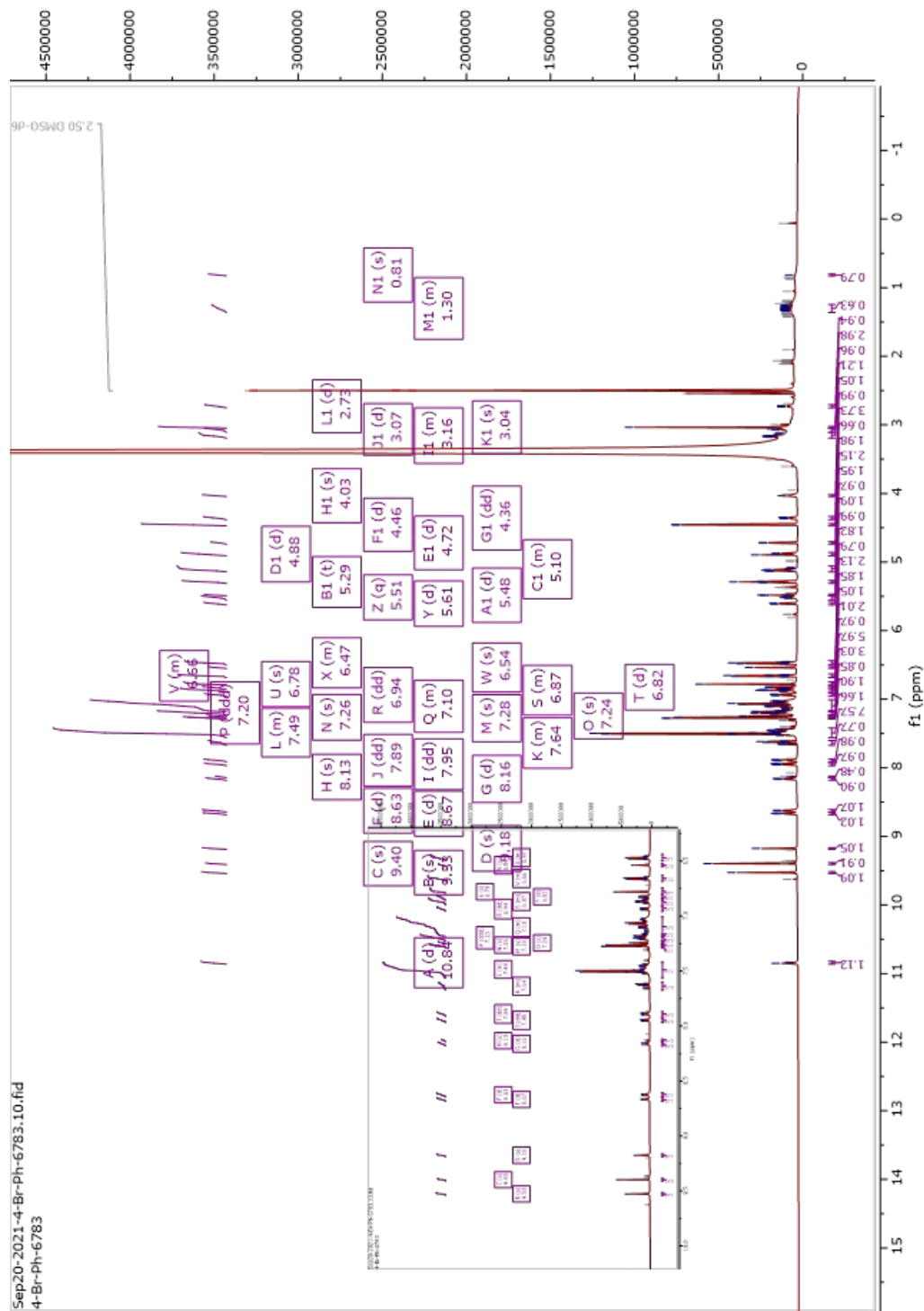
Compound 29



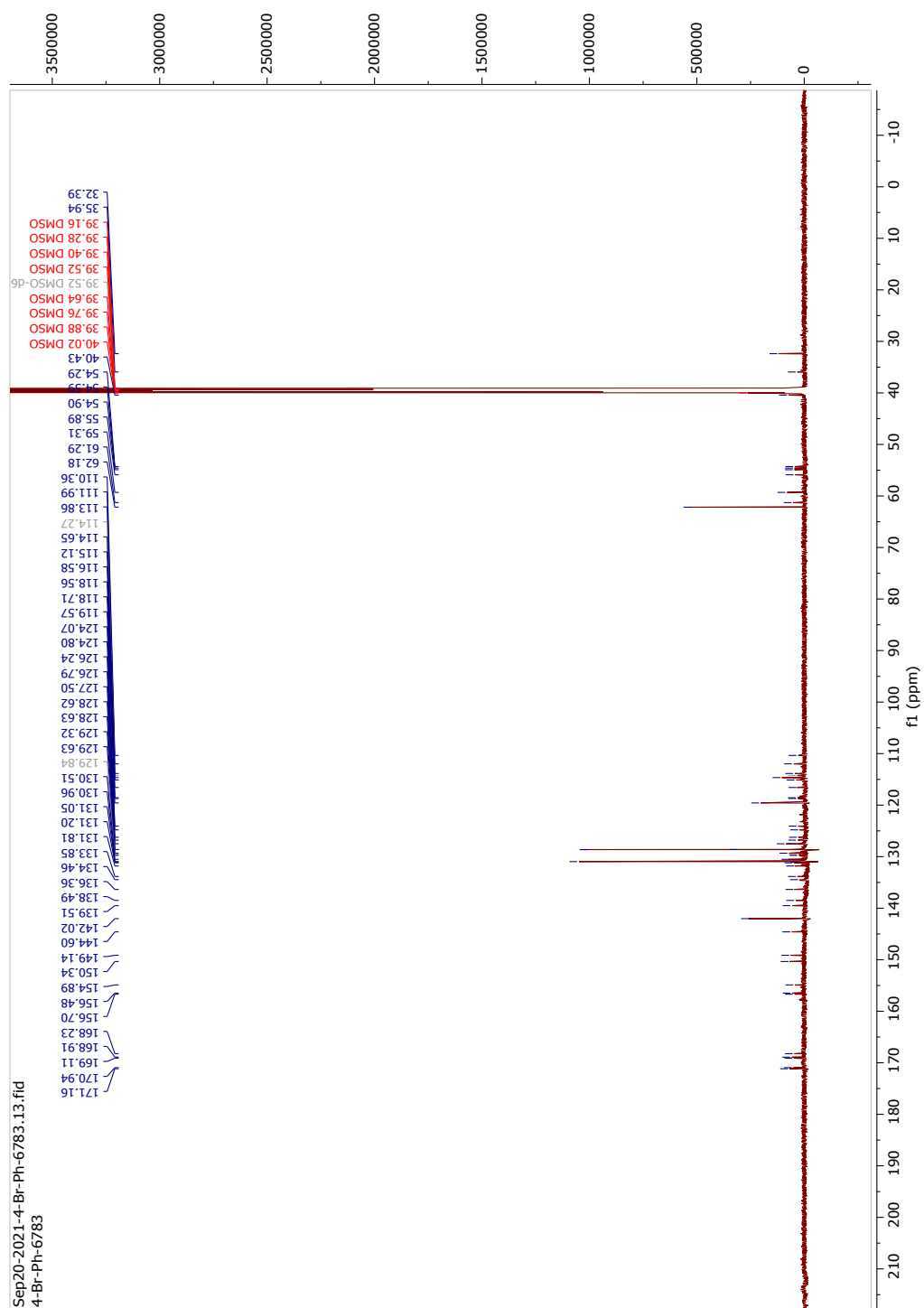
Chemical Formula: $C_{68}H_{56}BrN_6O_{14}$
Exact Mass: 1287.3105



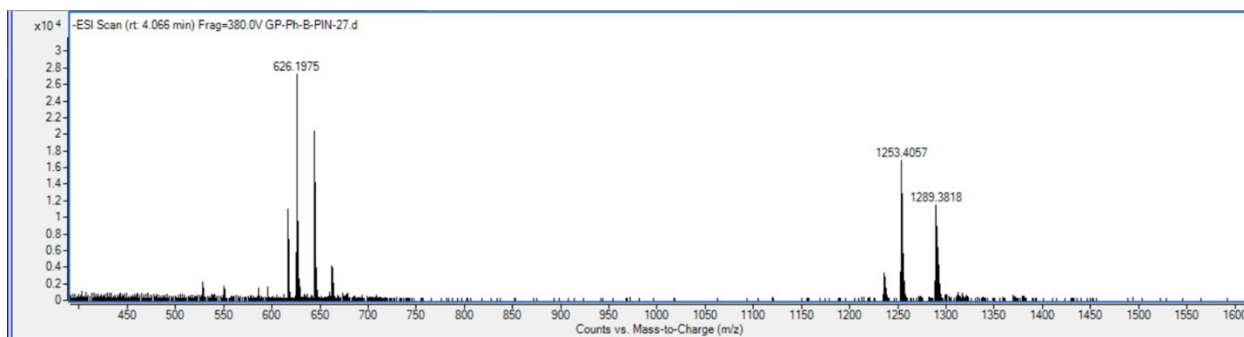
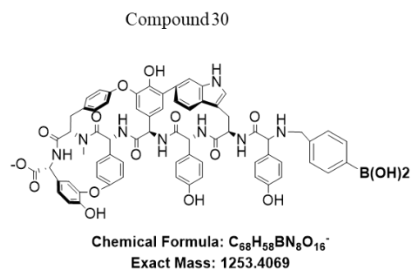
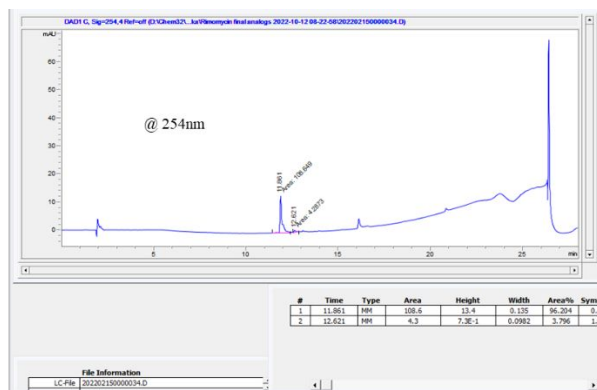
¹H-NMR of compound 29



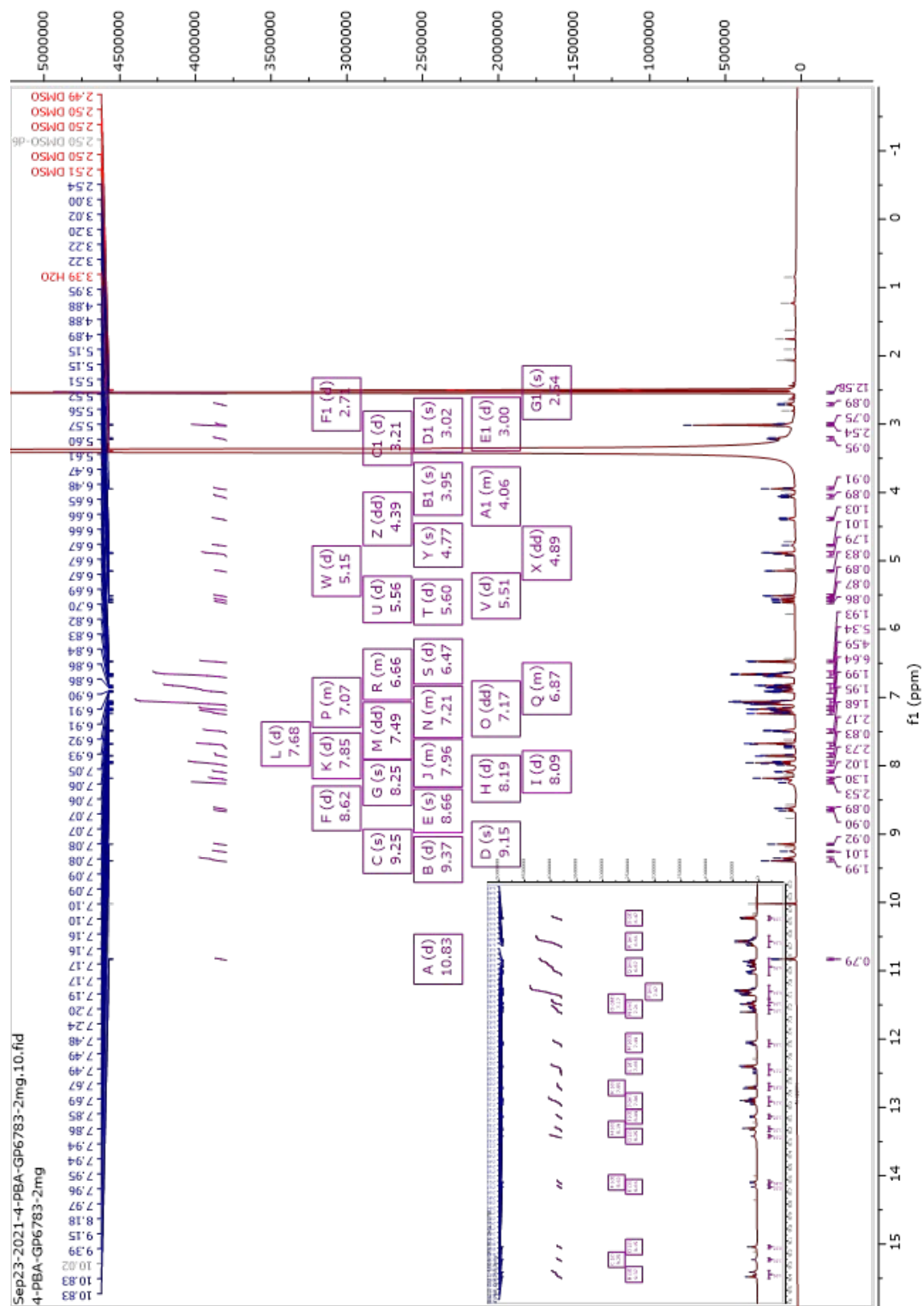
¹³C-NMR of compound 29



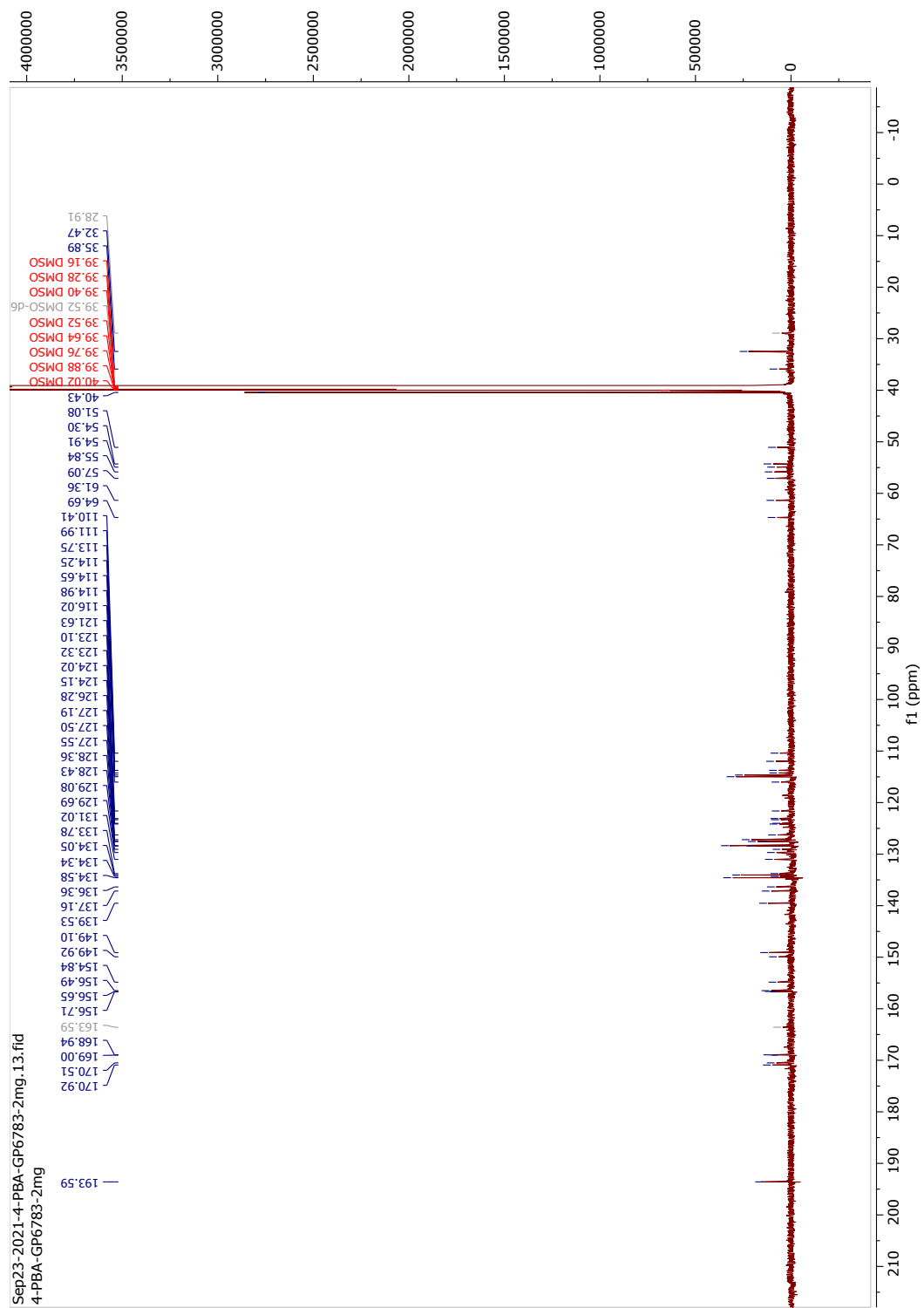
Compound 30



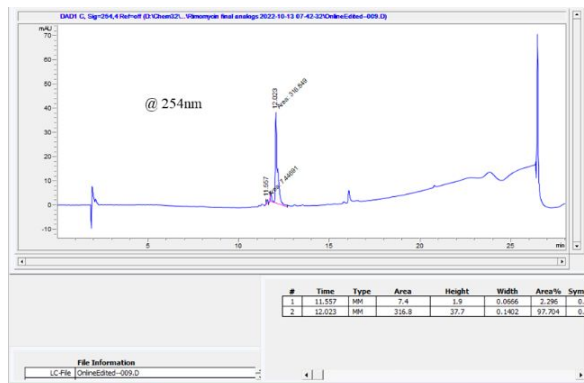
¹H-NMR of compound 30



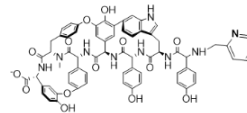
^{13}C -NMR of compound **30**



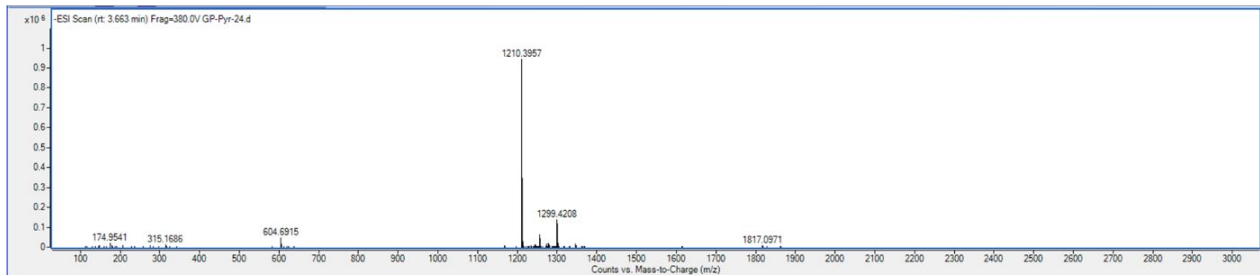
Compound 31



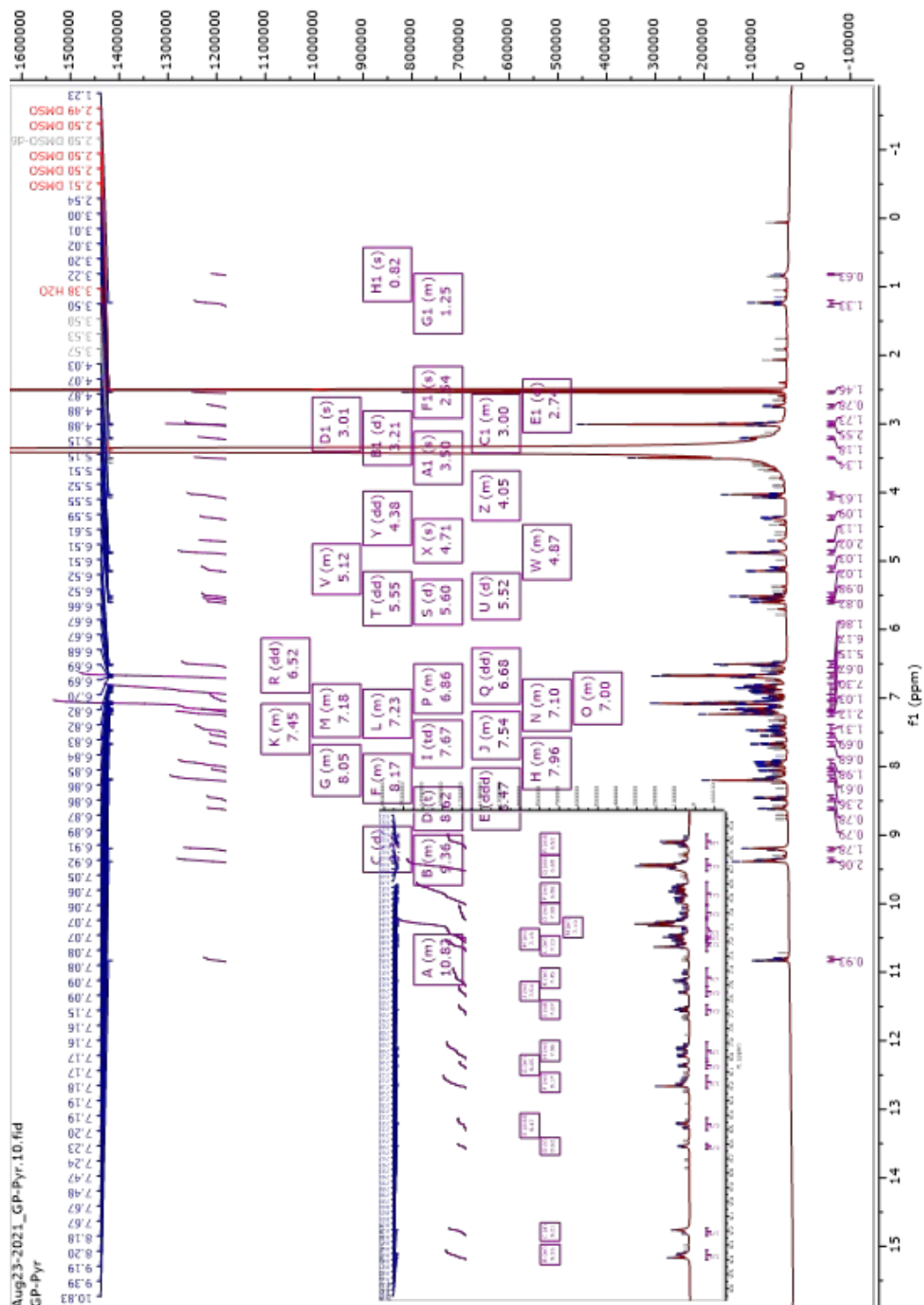
Compound 31



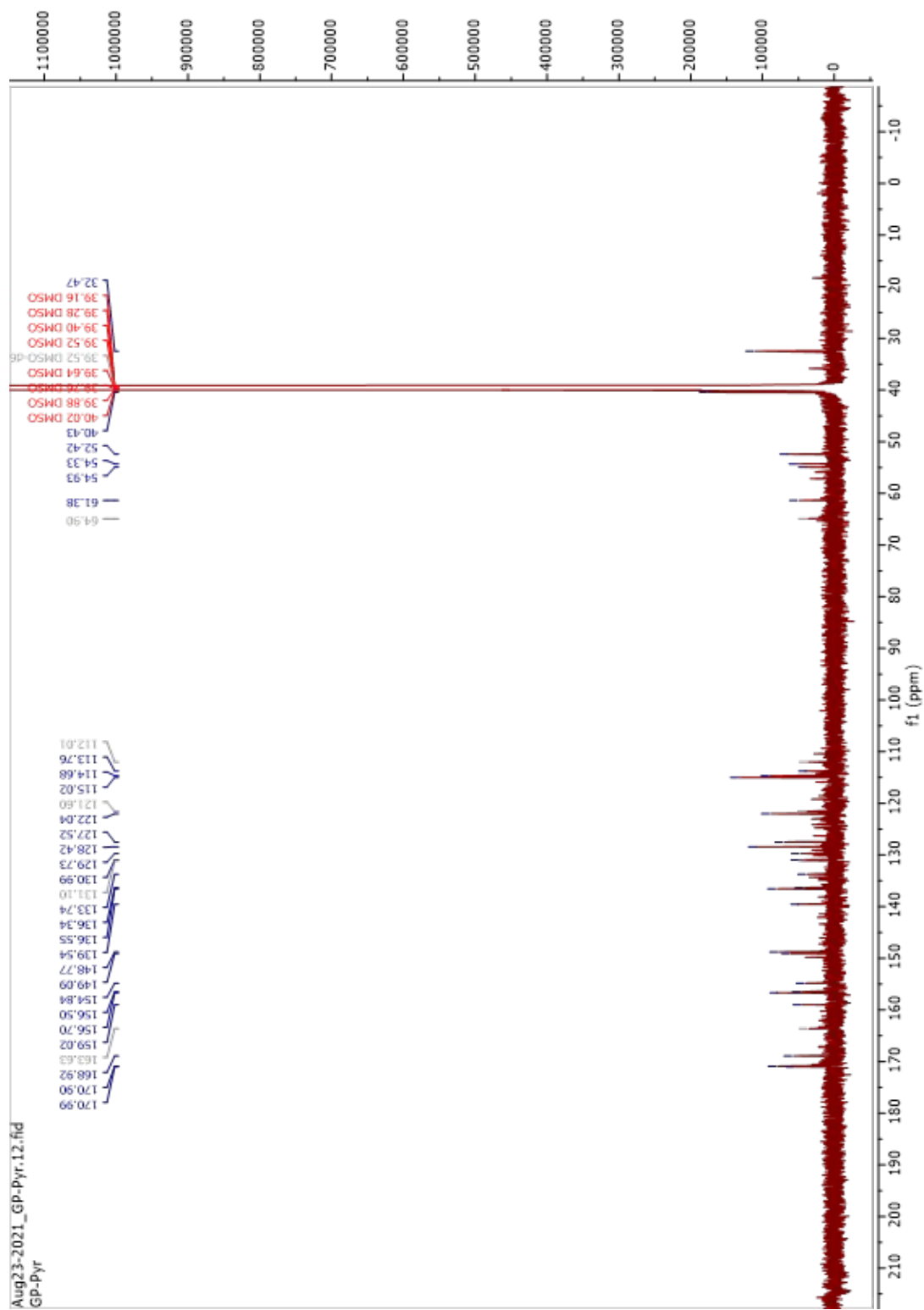
Chemical Formula: $C_{27}H_{26}N_6O_{14}$
Exact Mass: 1210.3952



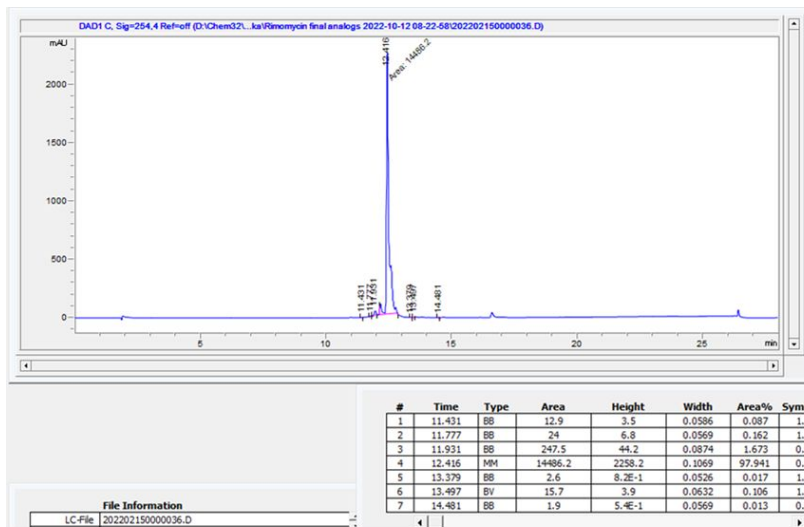
¹H-NMR of compound 31



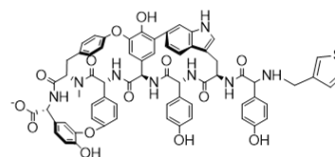
^{13}C -NMR of compound **31**



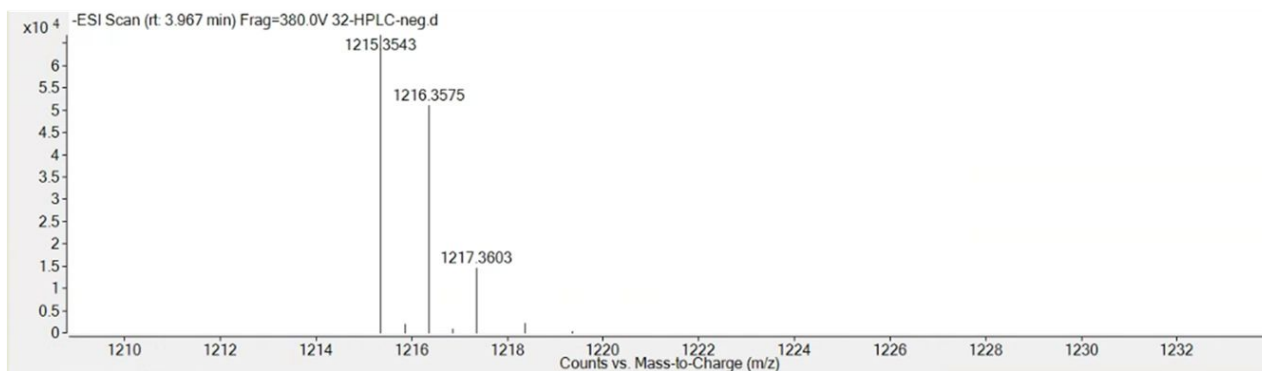
Compound 32



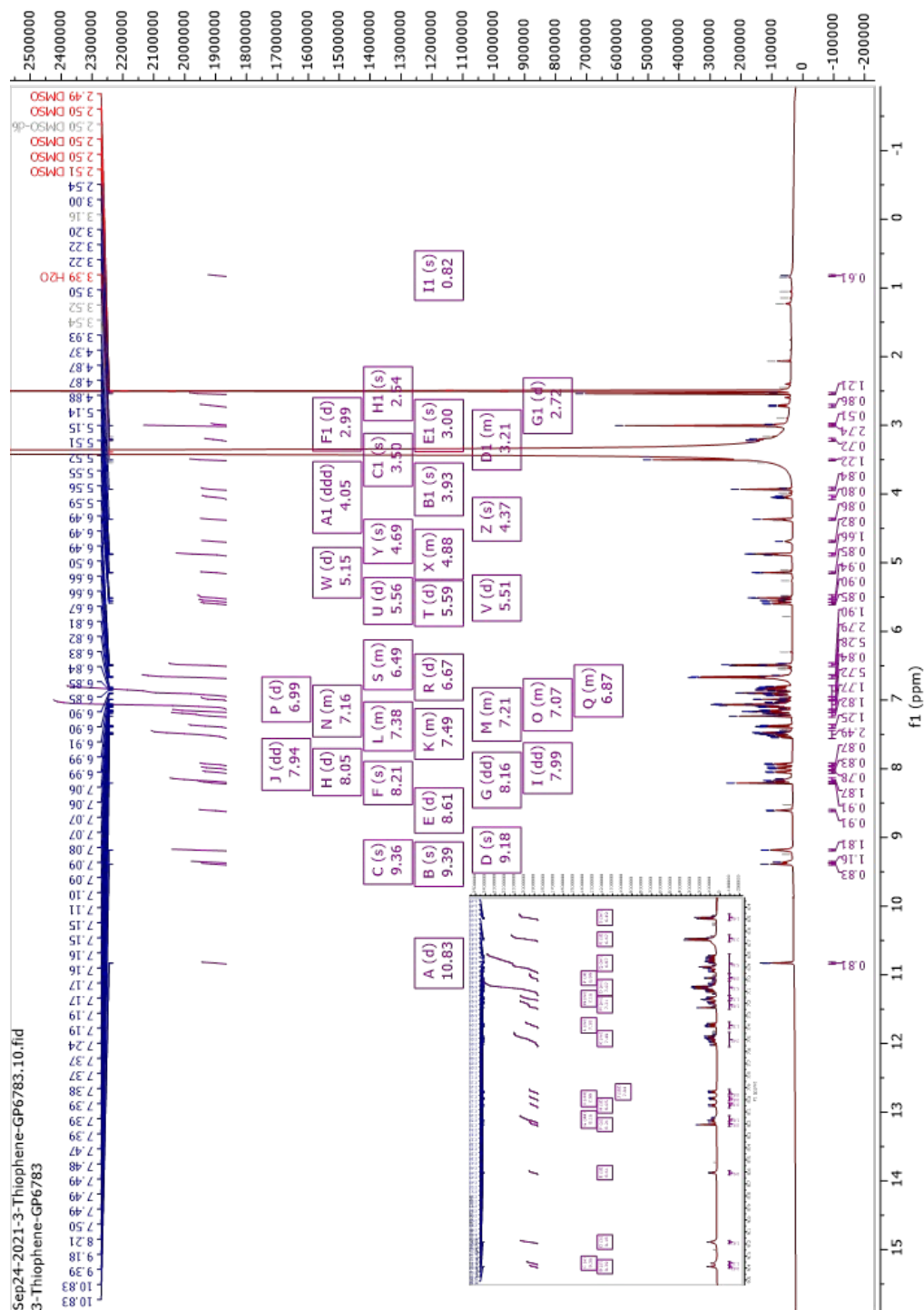
Compound 32



Chemical Formula: $C_{66}H_{56}N_8O_{14}S$
Exact Mass: 1215.3564



¹H-NMR of compound 32



¹³C-NMR of compound 32

