SUPPORTING INFORMATION

Carborane-Containing Hydroxamate MMP Ligands for the Treatment of Tumors using Boron Neutron Capture Therapy (BNCT): Efficacy Without Tumor Cell Entry

Sebastian Flieger[1], Mao Takagaki[2], Natsuko Kondo[3], Marlon R. Lutz, Jr.[1], Yash Gupta[4], Hiroki Ueda[3], Yoshinori Sakurai[3], Graham Moran[1], Prakasha Kempaiah[4], Narayan Hosmane[5], Minoru Suzuki[3], and Daniel P. Becker[1*]

[1]Department of Chemistry and Biochemistry, Loyola University Chicago, Chicago, IL, 60660, USA

[2]Research Center for Nuclear Physics, Osaka University, 10-1 Mihoga-oka, Ibaraki-city, Osaka 567-0047, JAPAN

[3]Particle Radiation Oncology Research Center, Institute for Integrated Radiation and Nuclear Science, Kyoto University, 2-1010 Asashiro-Nishi, Kumatori, Sennan-gun, Osaka 590-0494 JAPAN

[4]Department of Medicine, Infectious Diseases, Mayo Clinic, Jacksonville, Florida, USA

[5]Department of Chemistry and Biochemistry, Northern Illinois University, DeKalb IL 60115, USA

*Correspondence: dbecke3@luc.edu

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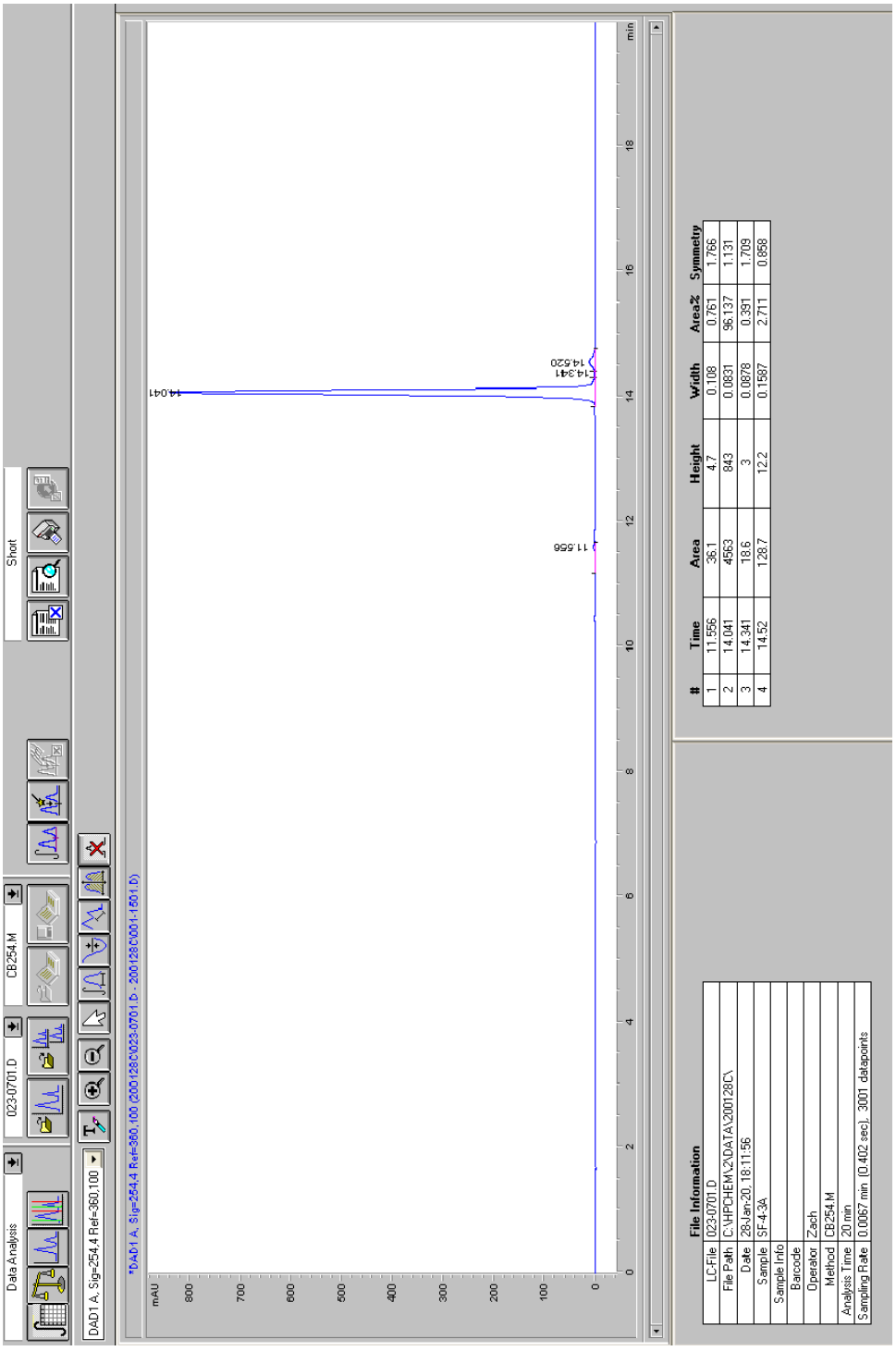
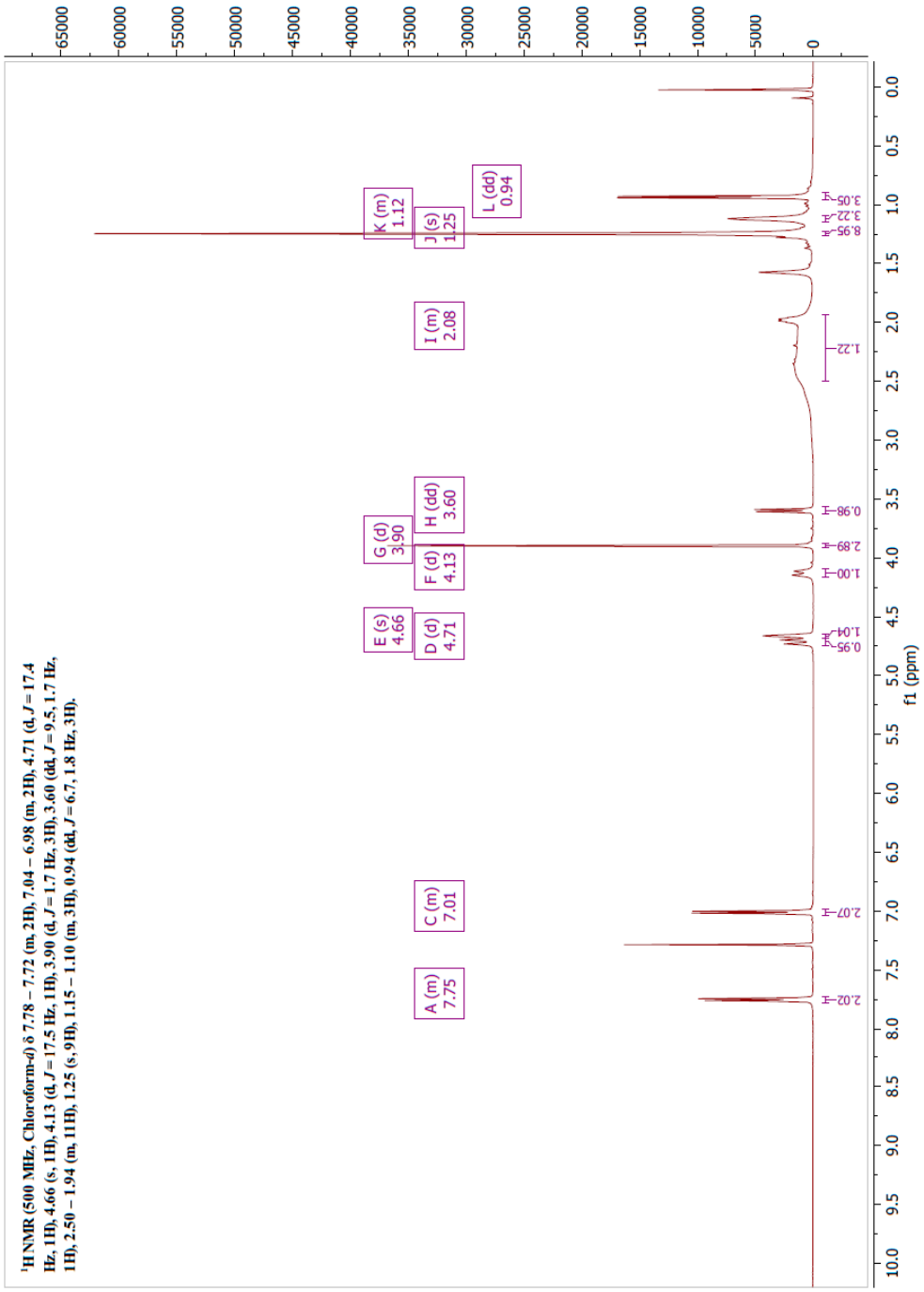
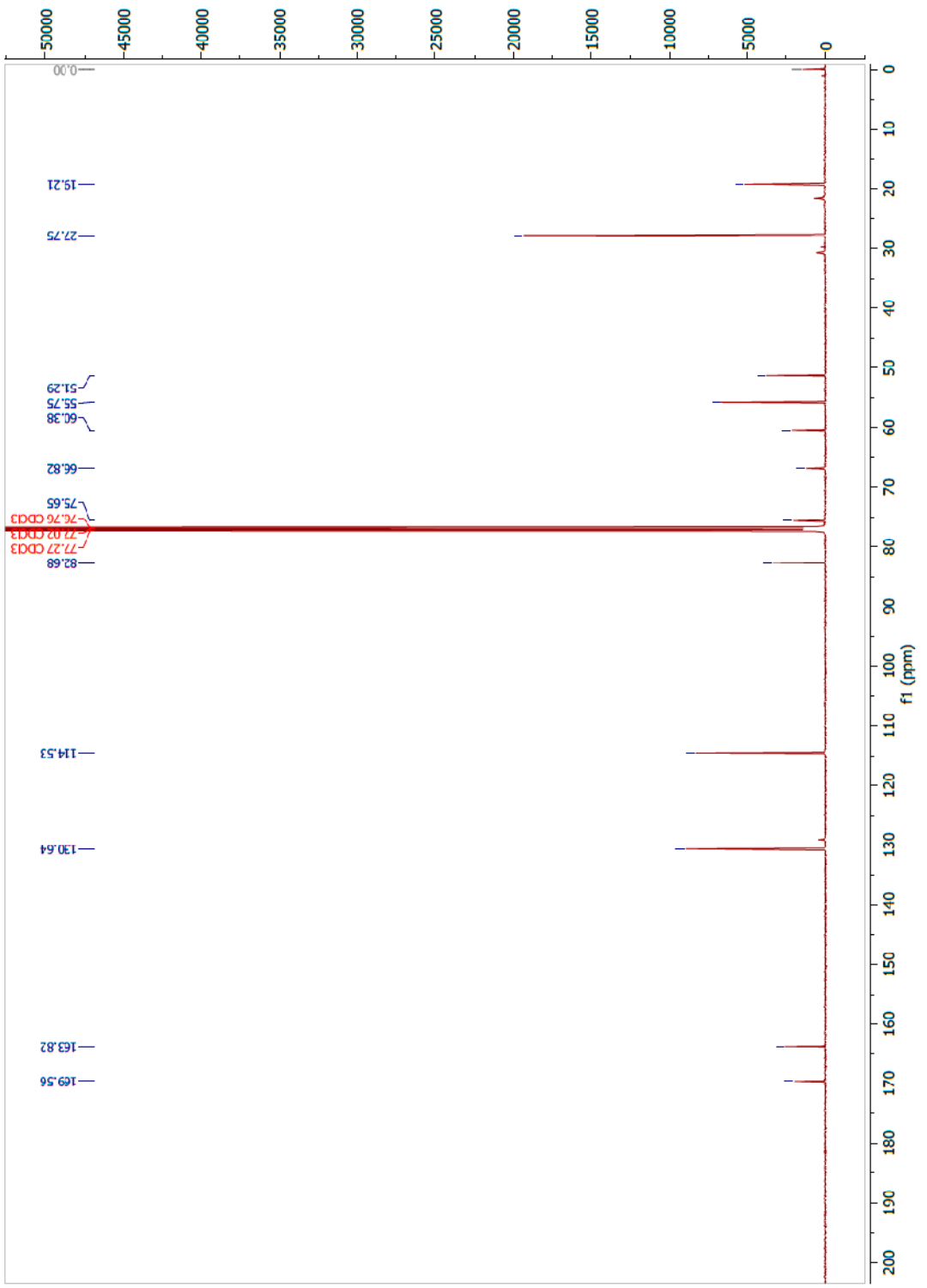
HPLC of Closo-carborane complex from (R)-tert-Butyl 2-(4-Methoxyphenylsulfonamido-N-(prop-2-yn-1-yl))-3-methylbutanoate **8.**



[1]H NMR of Closo-carborane complex from (R)-tert-Butyl 2-(4-Methoxyphenylsulfonamido-N-(prop-2-yn-1-yl))-3-methylbutanoate **8.**



^{13}C NMR of Closo-carborane complex from (R)-tert-Butyl 2-(4-Methoxyphenylsulfonamido-N-(prop-2-yn-1-yl))-3-methylbutanoate **8.**



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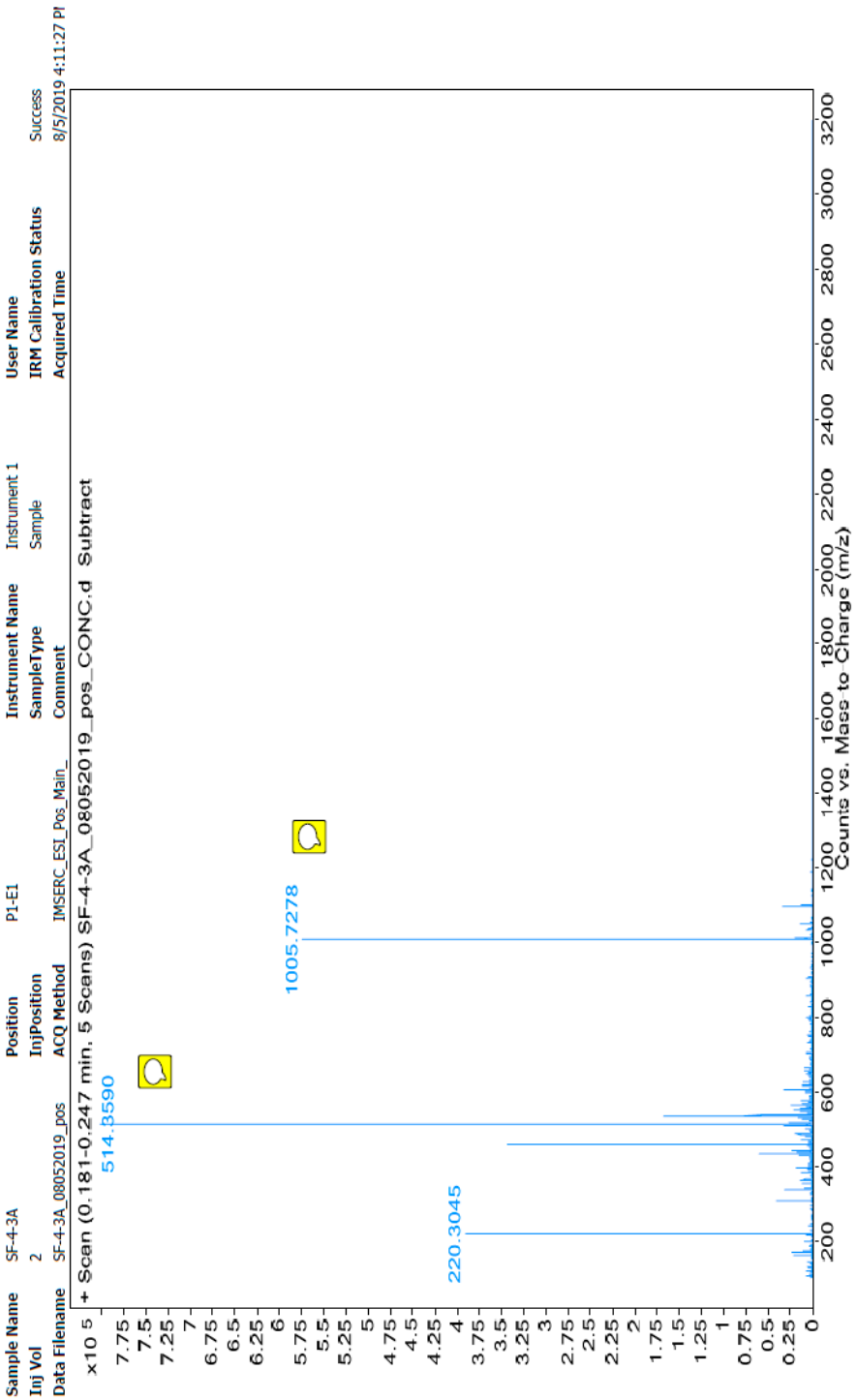
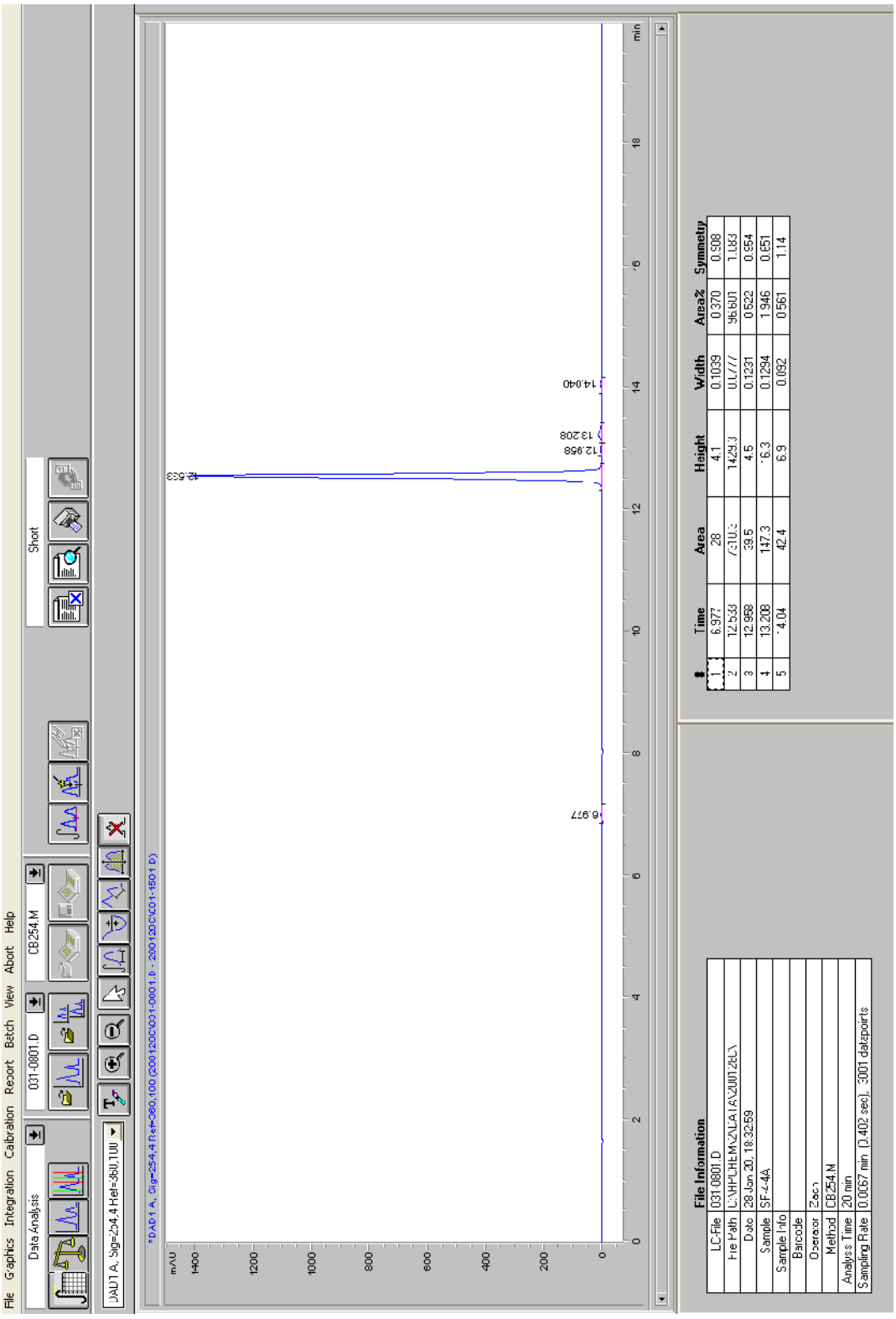


HPLC of Carboxylic Acid Closo-Carborane **9.**



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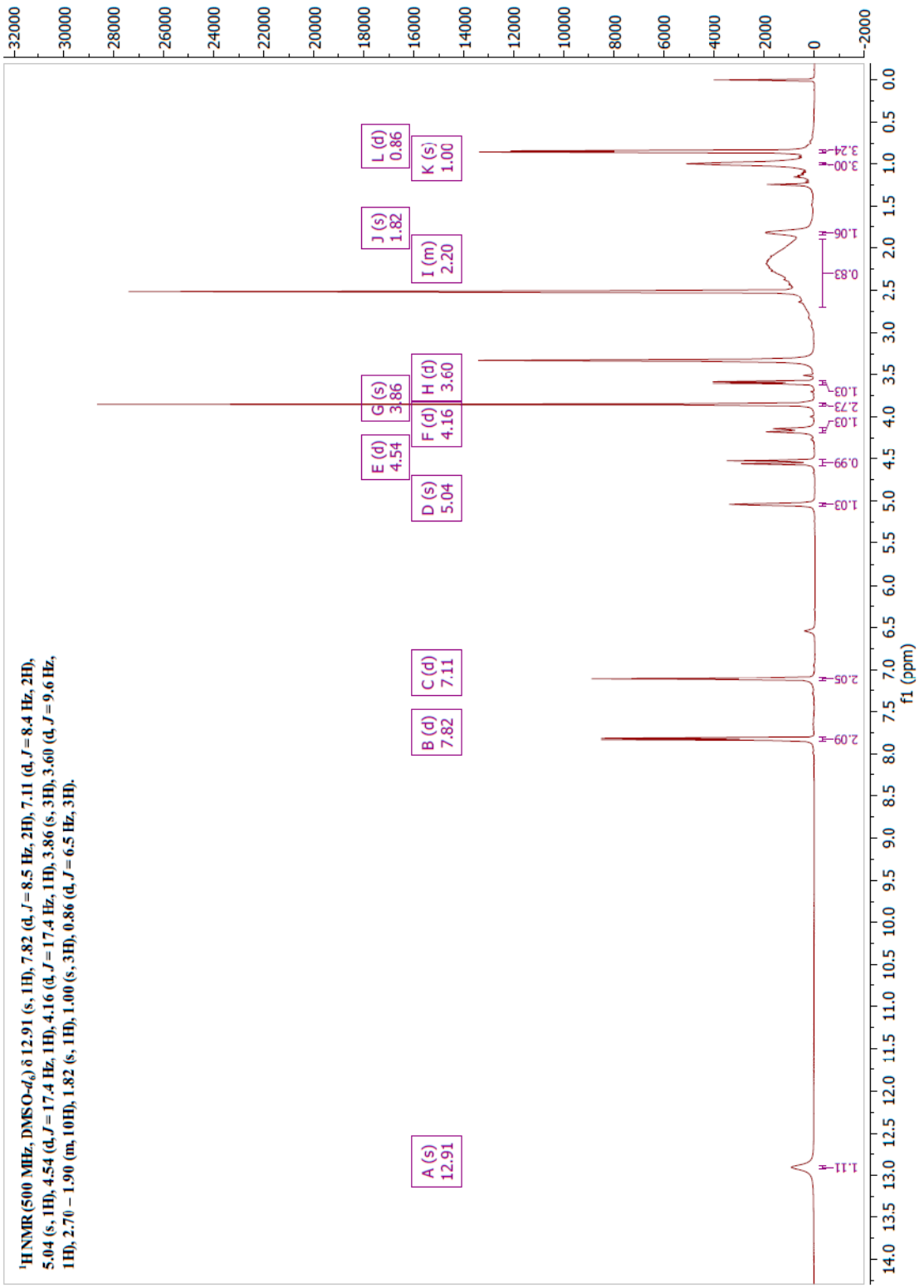
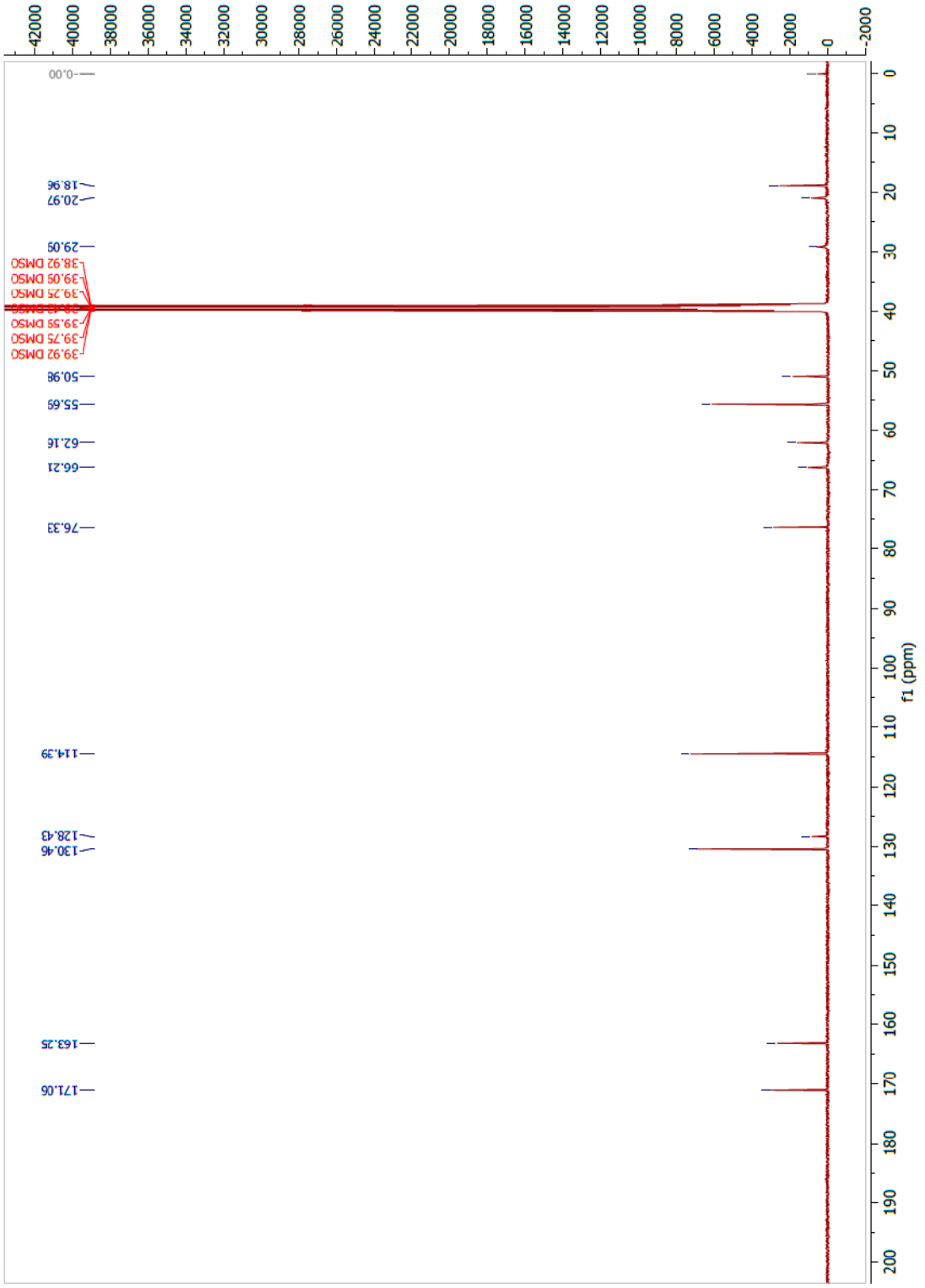
2	12.538	7310.3	1429.3	0.1777	96.601	1.083
3	12.958	39.5	4.5	0.1231	0.522	0.554
4	13.208	147.3	16.3	0.1294	1.946	0.651
5	14.04	42.4	6.9	0.092	0.561	1.14

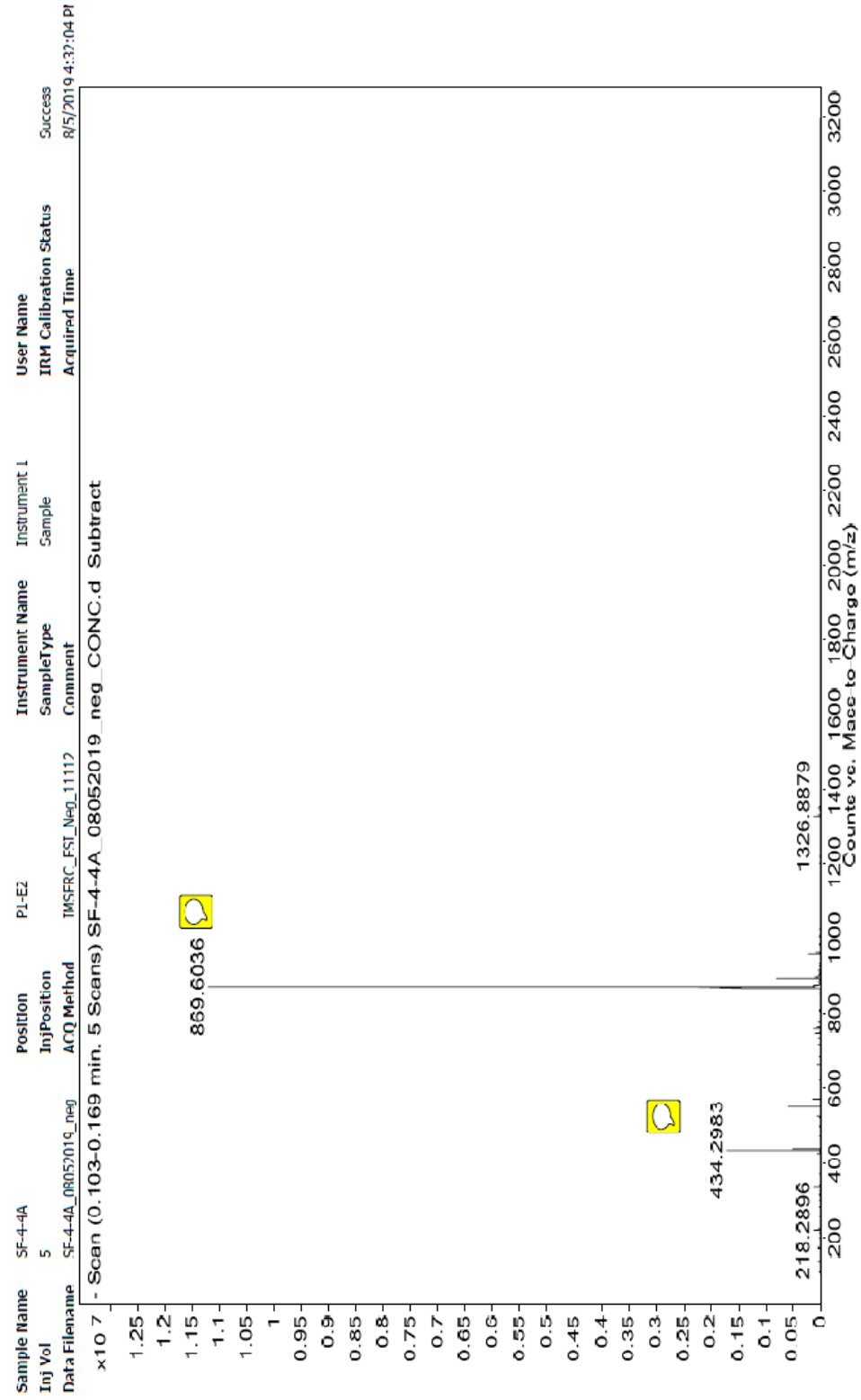
[1]H NMR of Carboxylic Acid Closo-Carborane **9.**



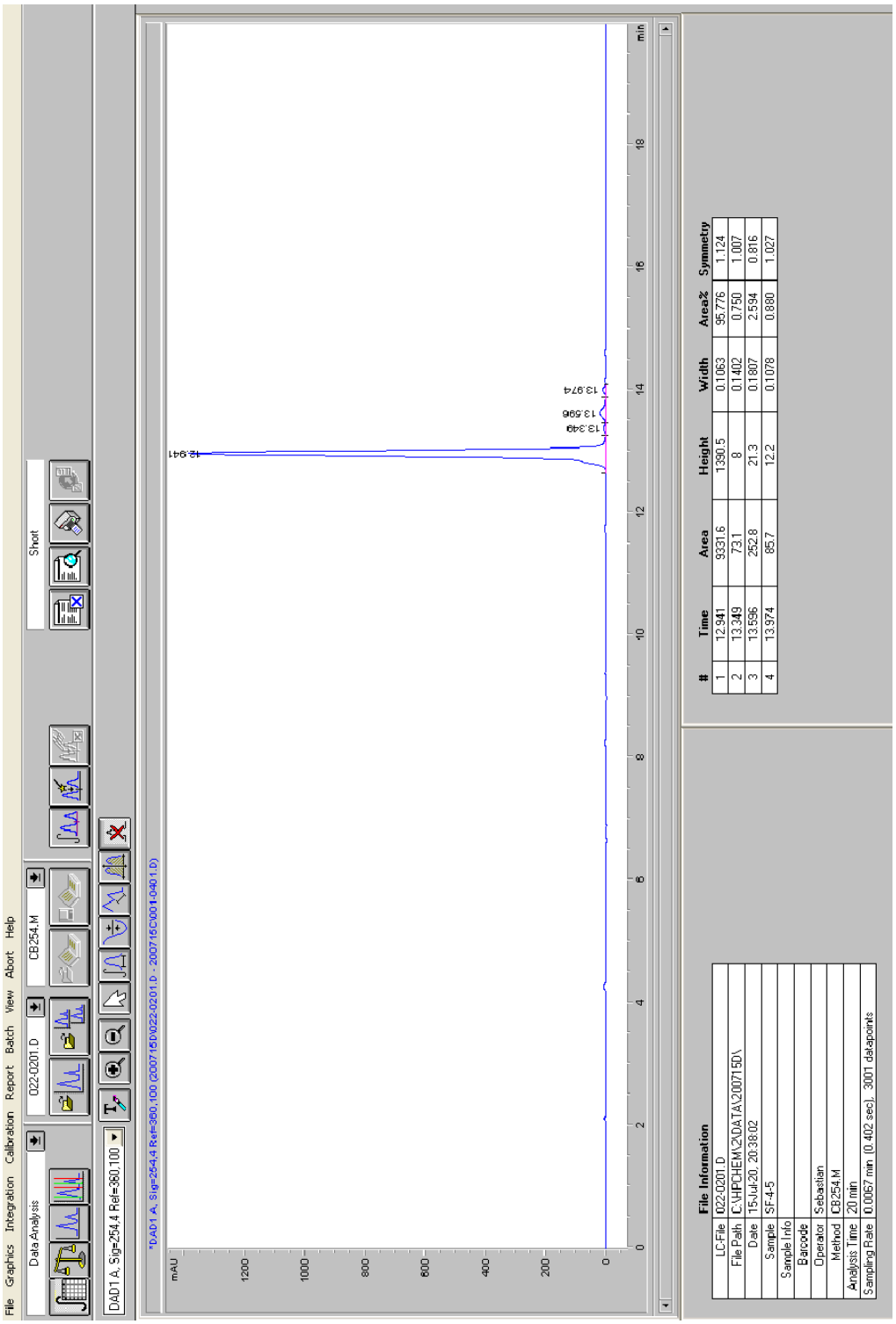
^{13}C NMR of Carboxylic Acid Closo-Carborane **9.**



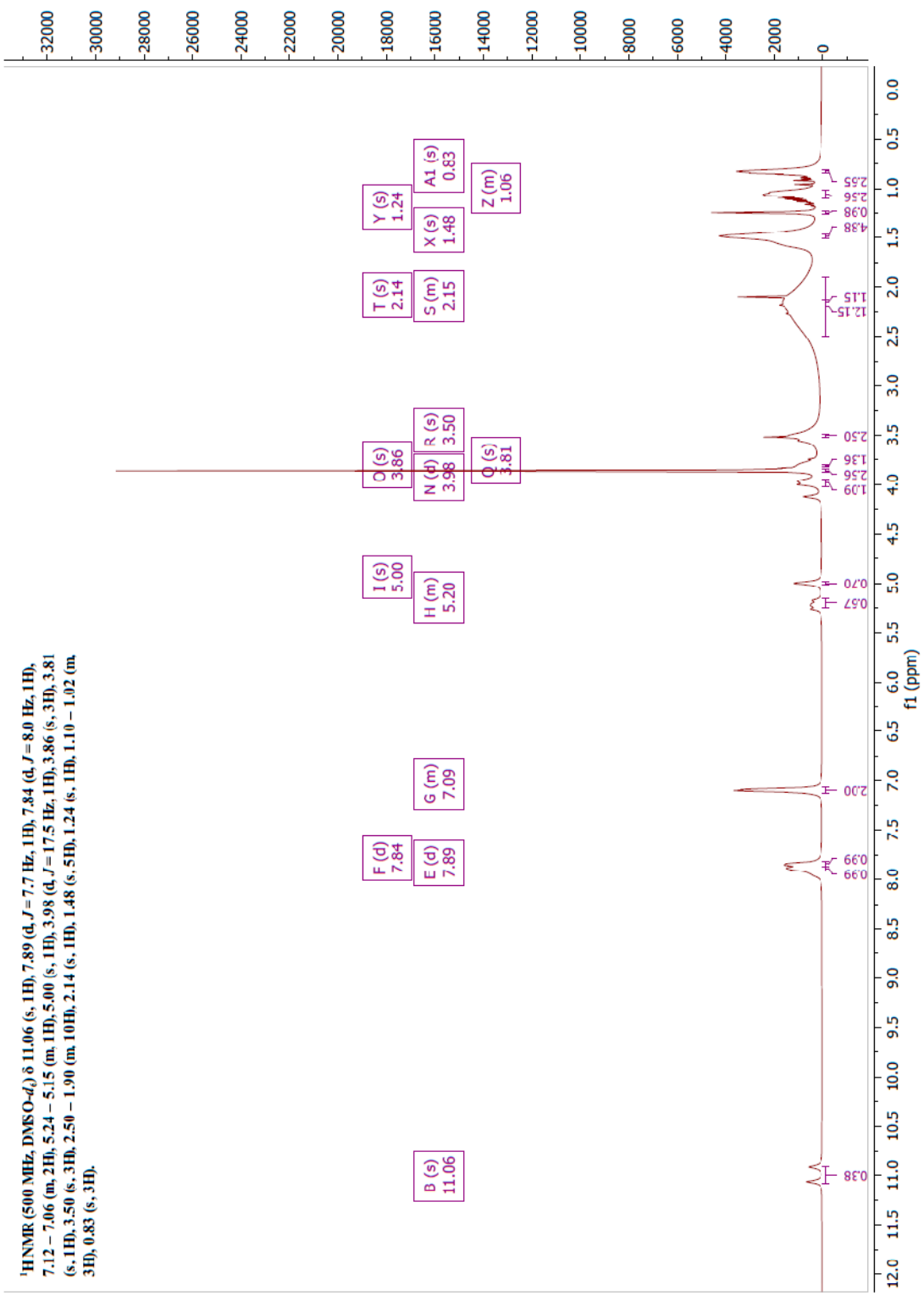
HRMS of Carboxylic Acid Closo-Carborane **9.**



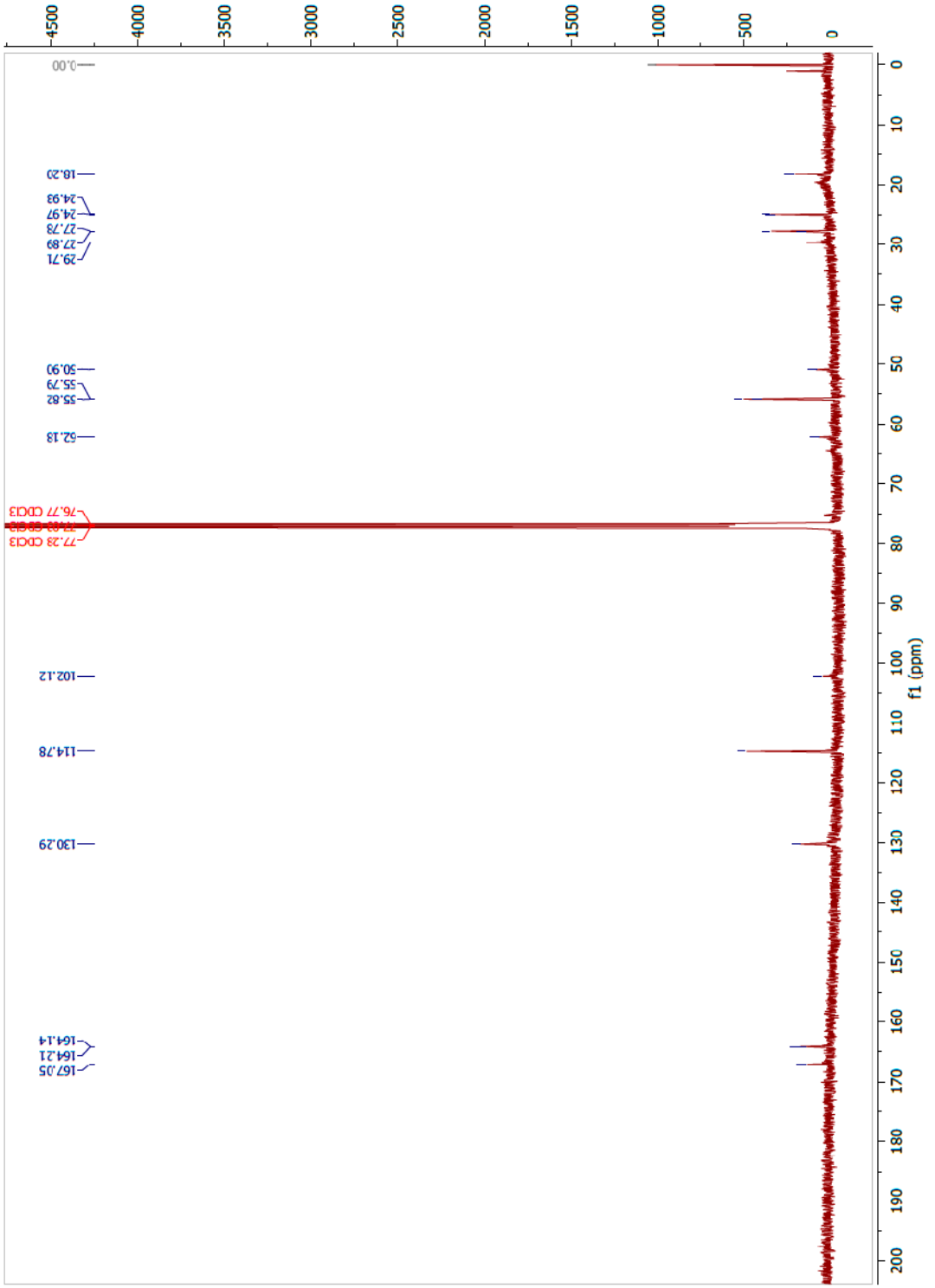
HPLC of THP-Protected Hydroxamate Closo-Carborane **10.**



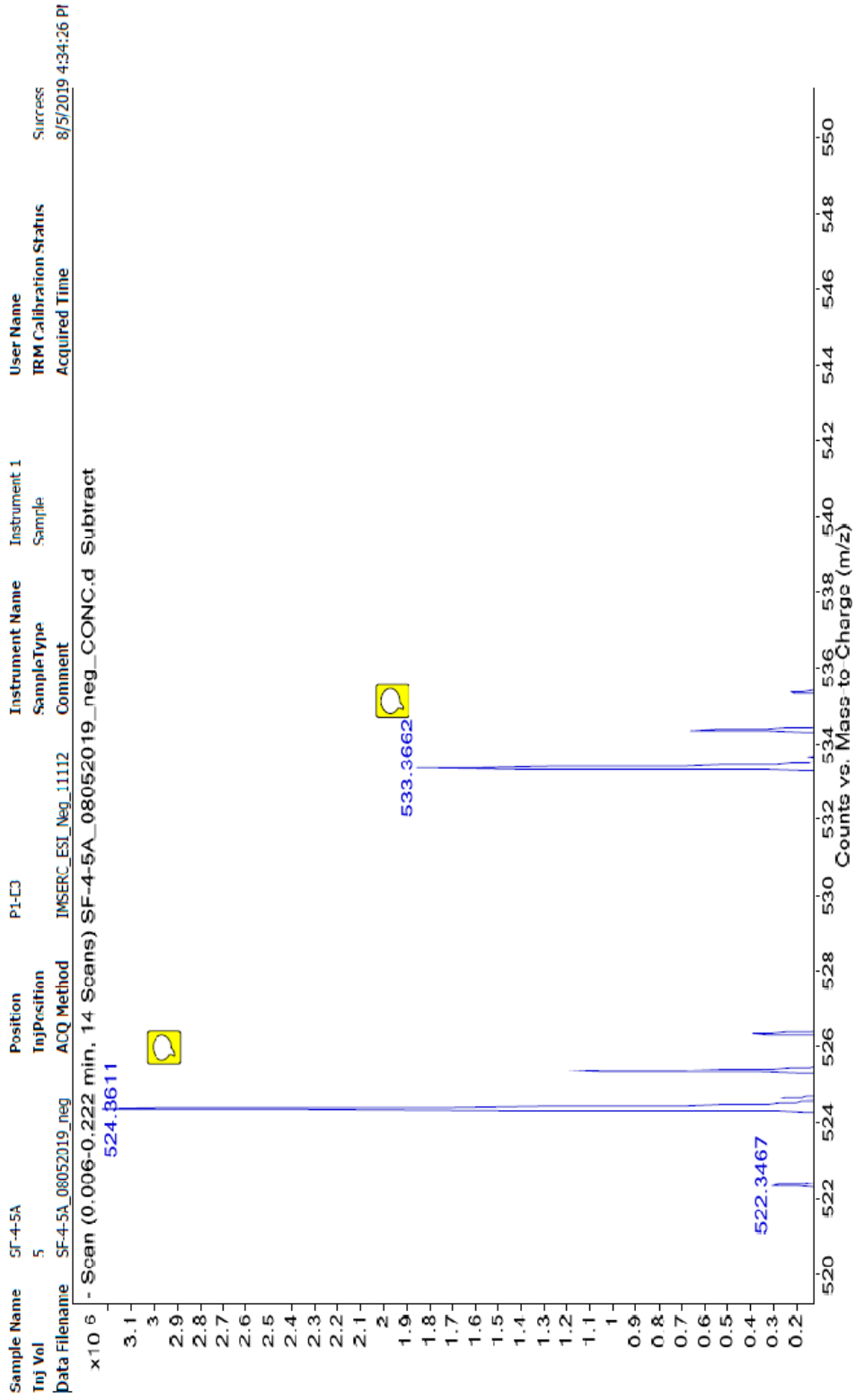
^{1}H NMR of THP-Protected Hydroxamate Closo-Carborane **10.**



^{13}C NMR of THP-Protected Hydroxamate Closo-Carborane **10.**



HRMS of THP-Protected Hydroxamate Closo-Carborane **10.**



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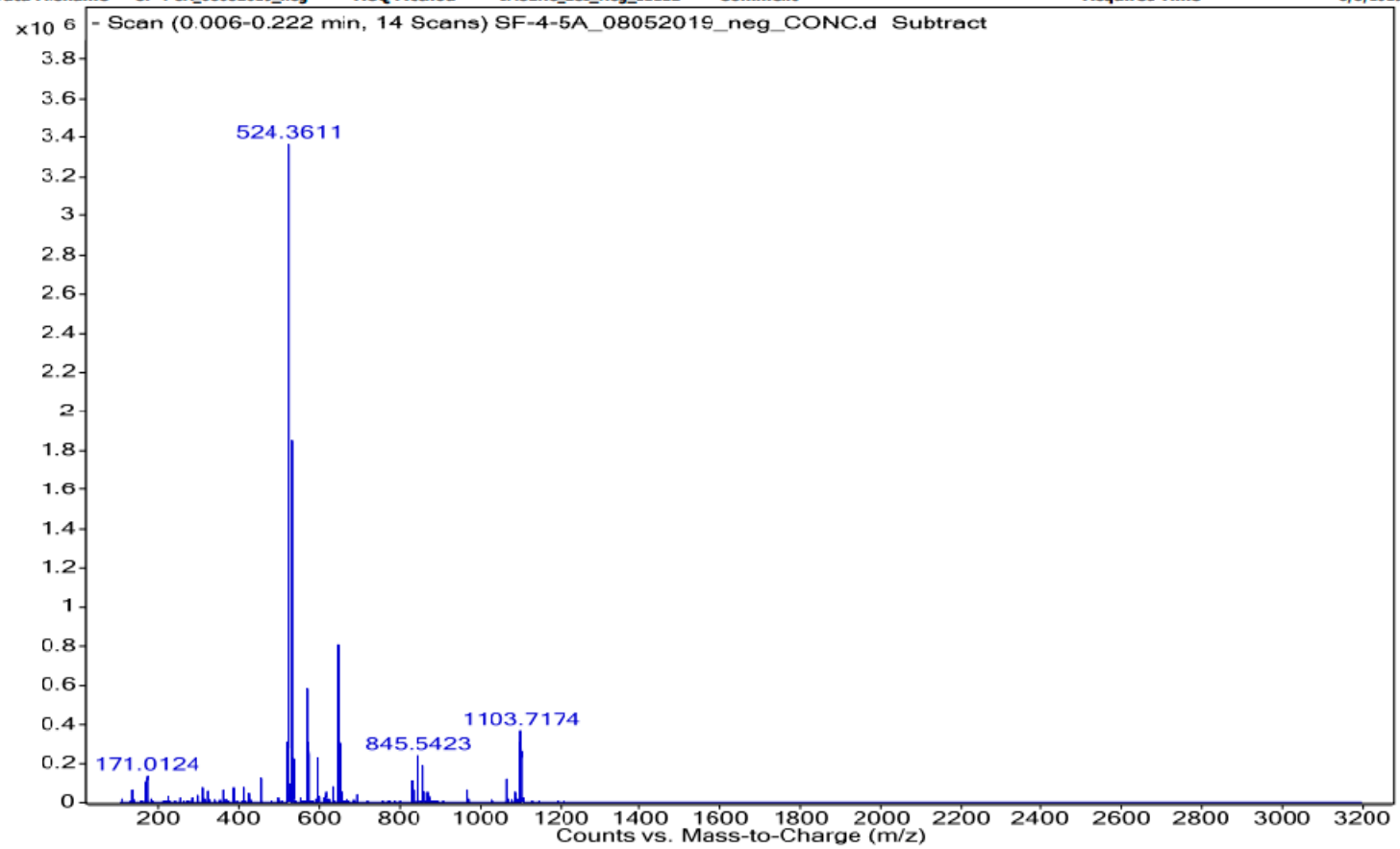
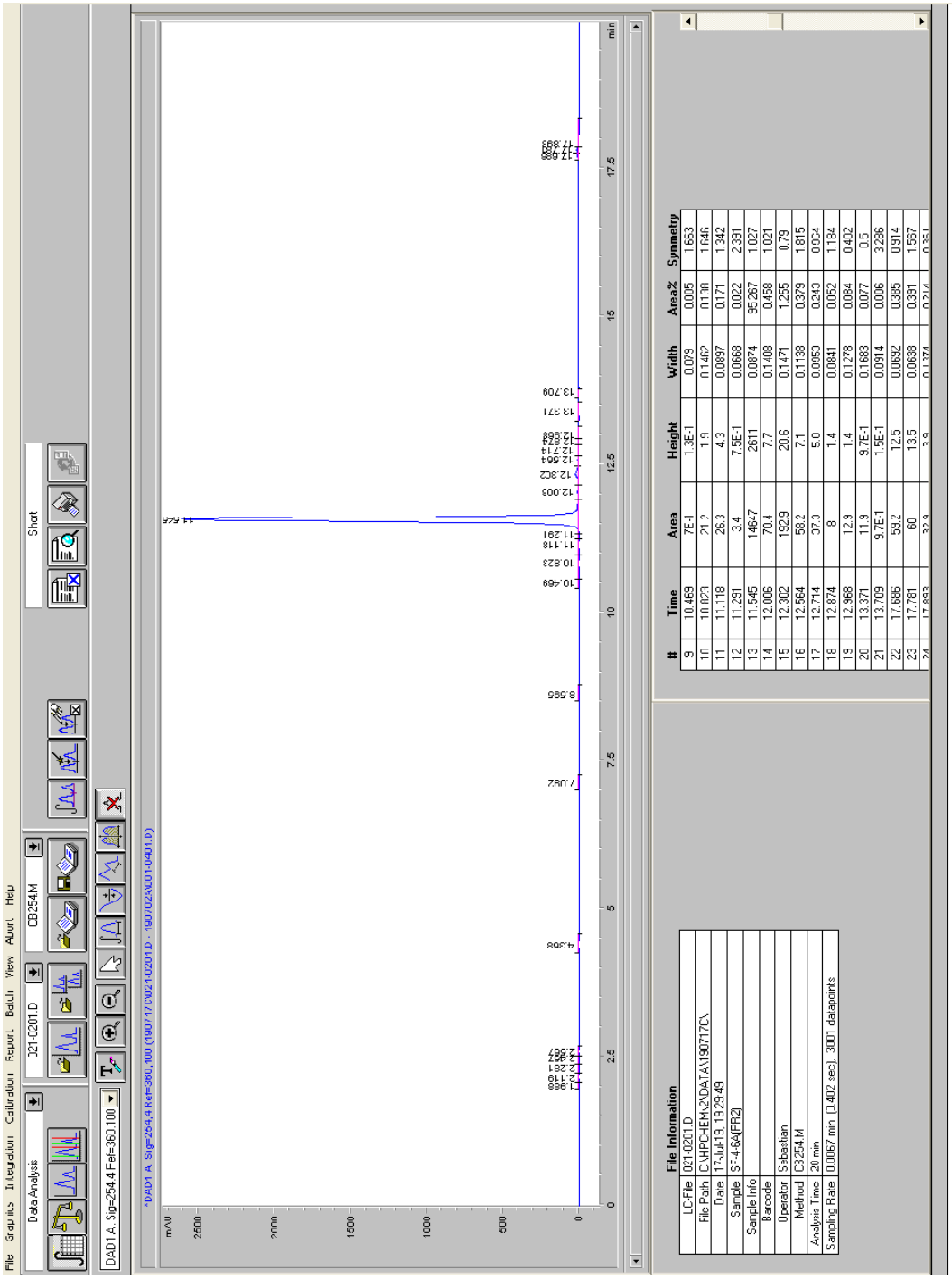
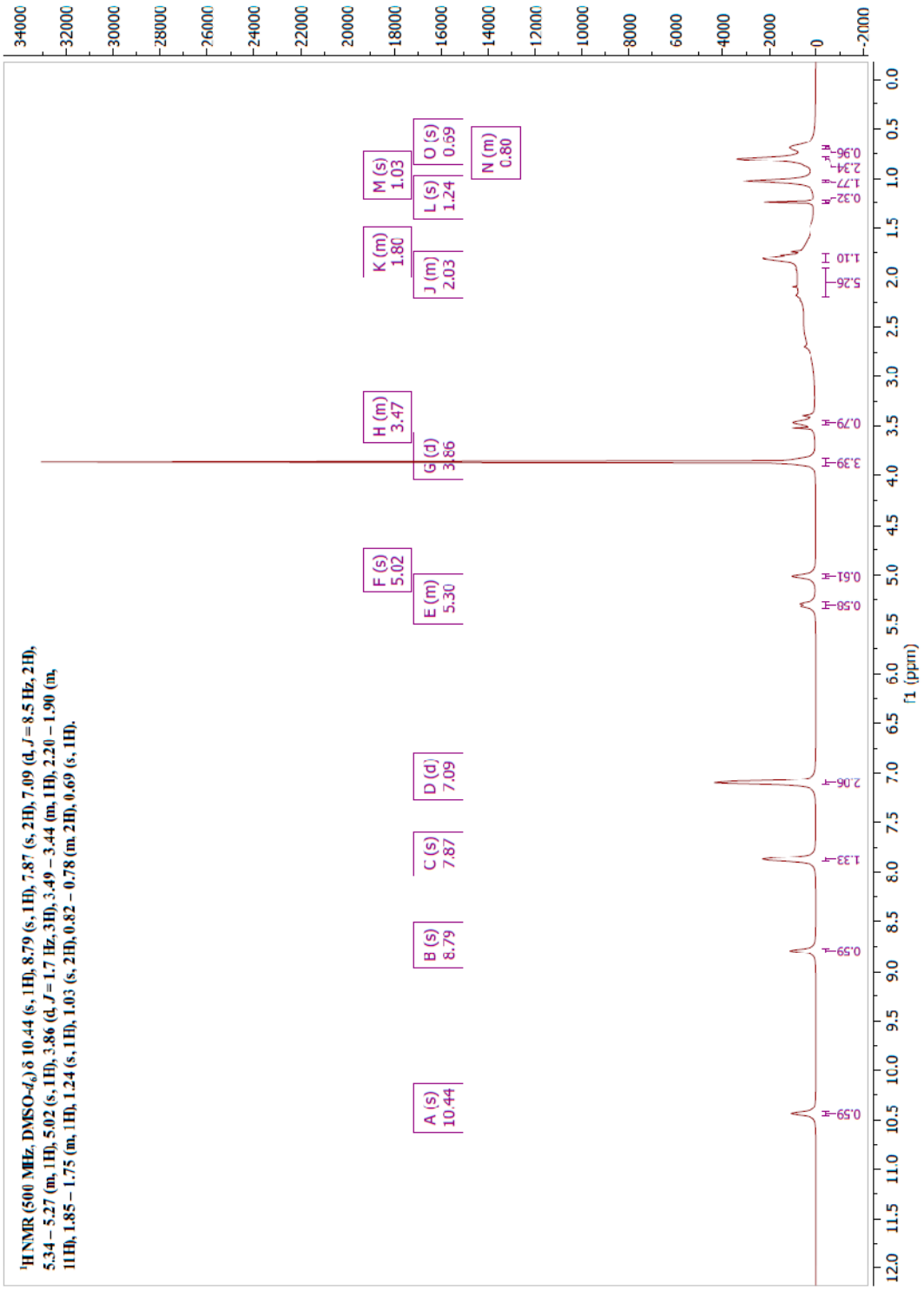


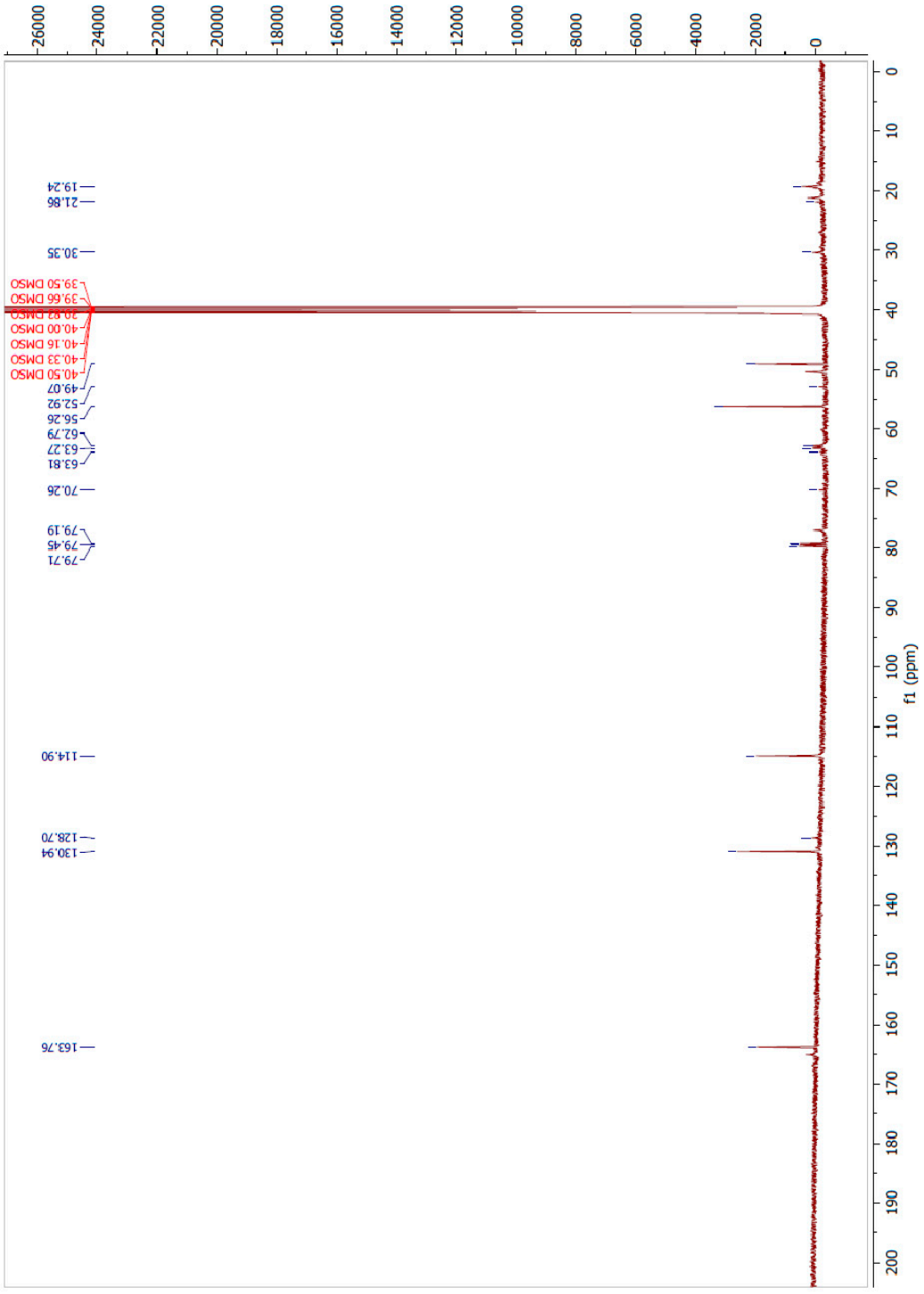
HPLC of Hydroxamic Acid Closo-Carborane **3.**



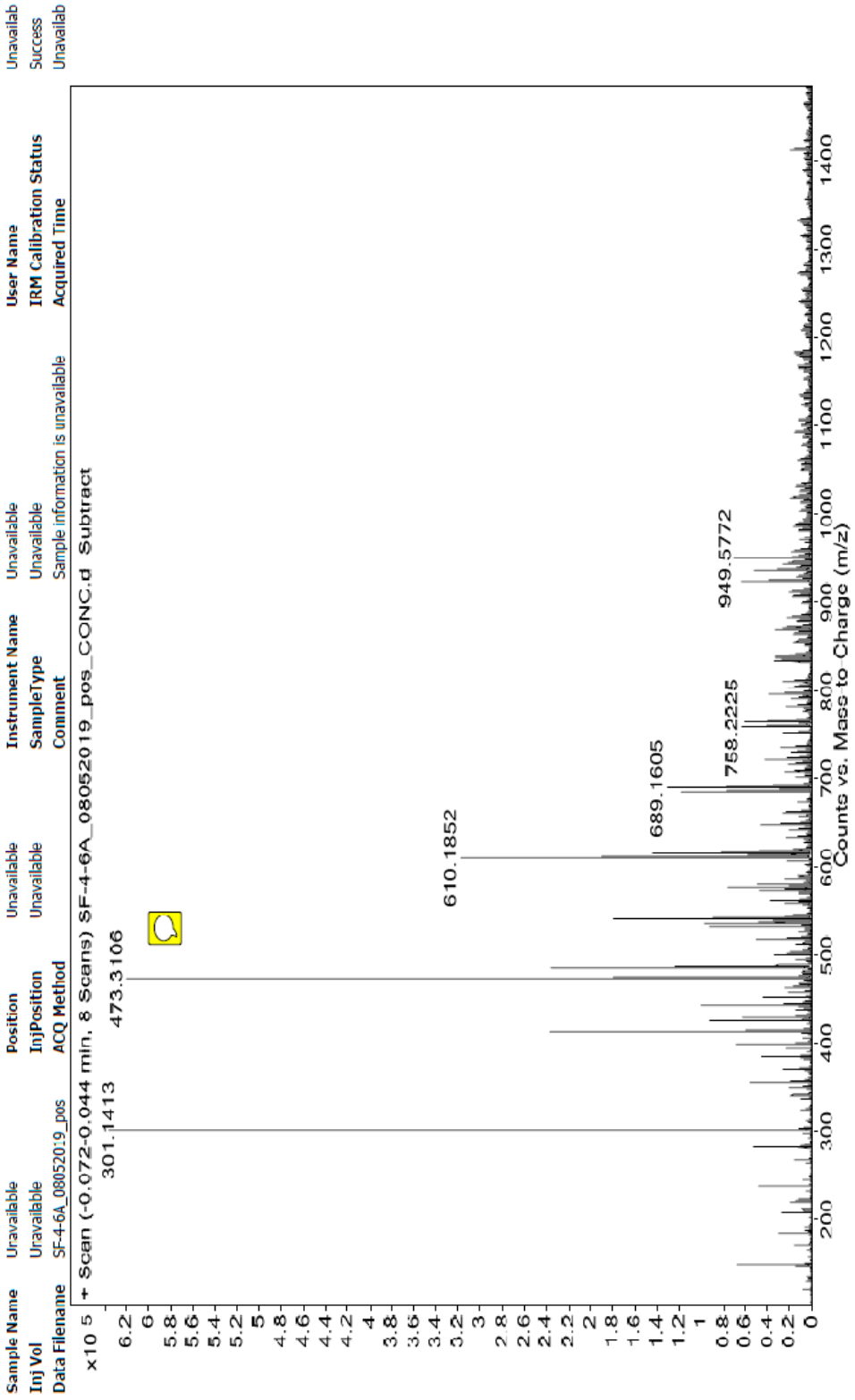
^{1}H NMR of Hydroxamic Acid Closo-Carborane **3.**



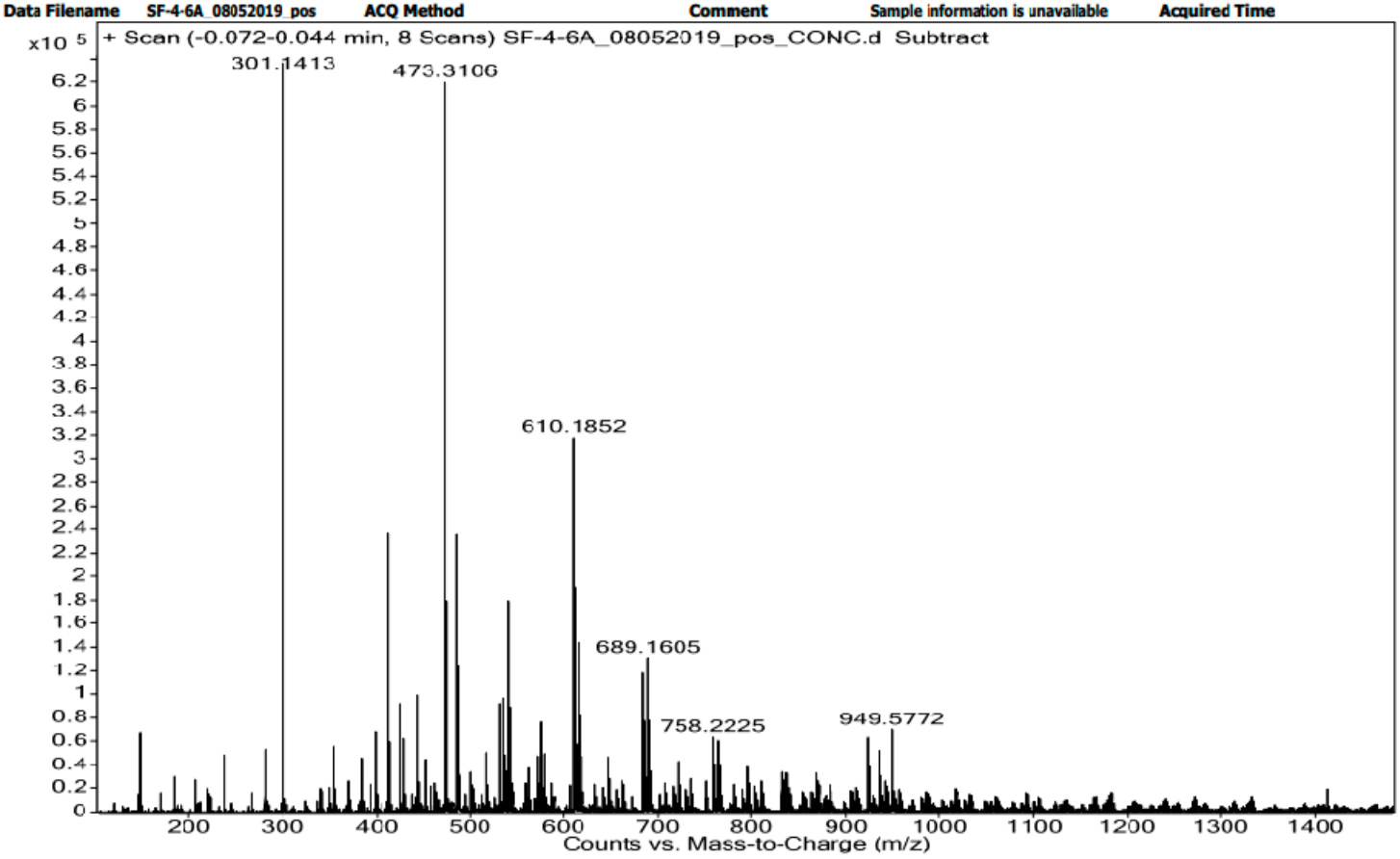
^{13}C NMR of Hydroxamic Acid Closo-Carborane **3.**



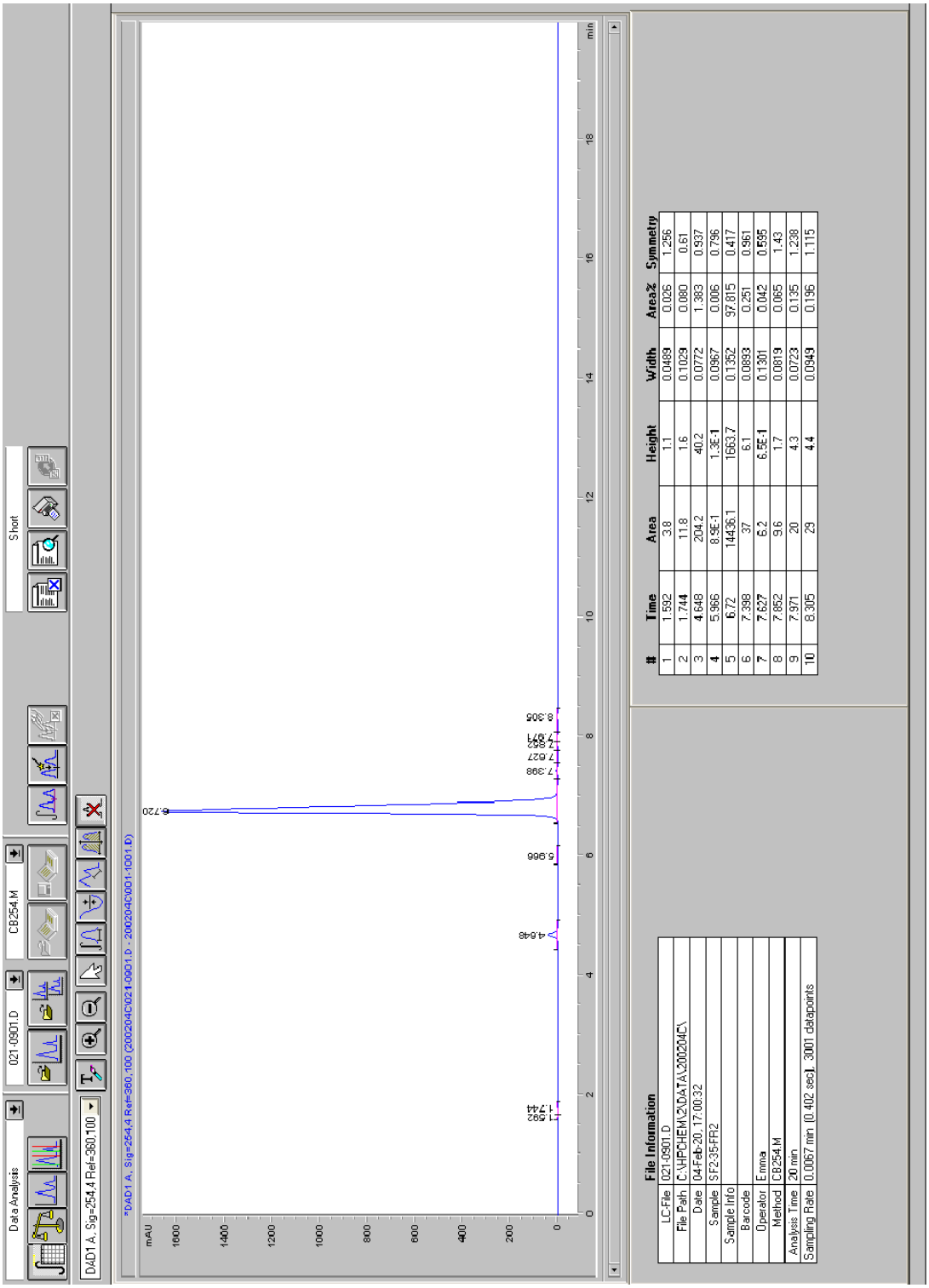
HRMS of Hydroxamic Acid Closo-Carborane **3.**



Sample Name	Unavailable	**Position**	Unavailable	**Instrument Name**	Unavailable	**User Name**	Unavailab
Inj Vol	Unavailable	**InjPosition**	Unavailable	**SampleType**	Unavailable	**IRM Calibration Status**	Success
Data Filename	SF-4-6A_08052019_pos	**ACQ Method**		**Comment**	Sample information is unavailable	**Acquired Time**	Unavailab

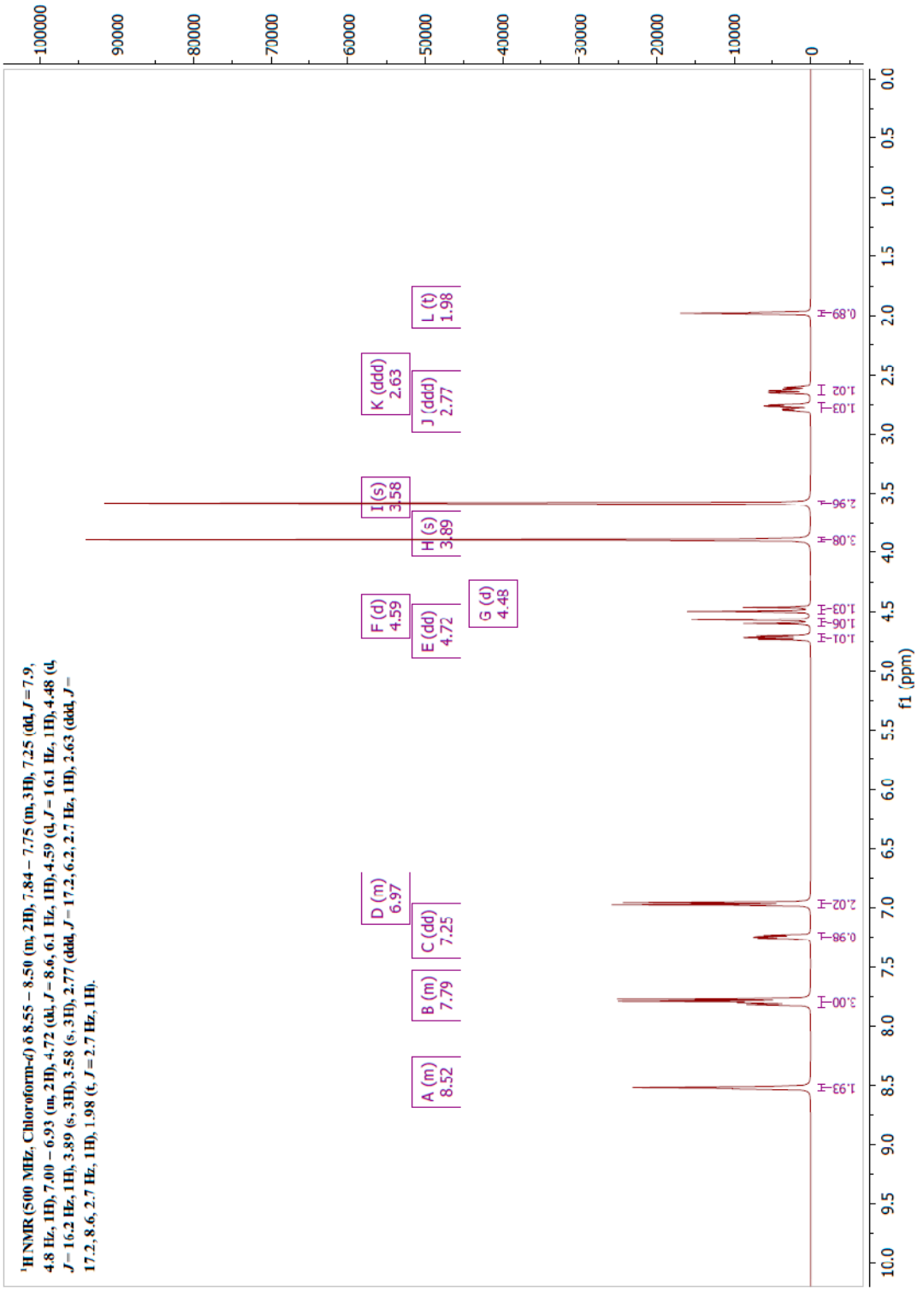


HPLC of Methyl (*R*)-2-((4-methoxy-*N*-(pyridine-3-ylmethyl) phenyl) sulfonamido) pent-4-ynoate **(14).**

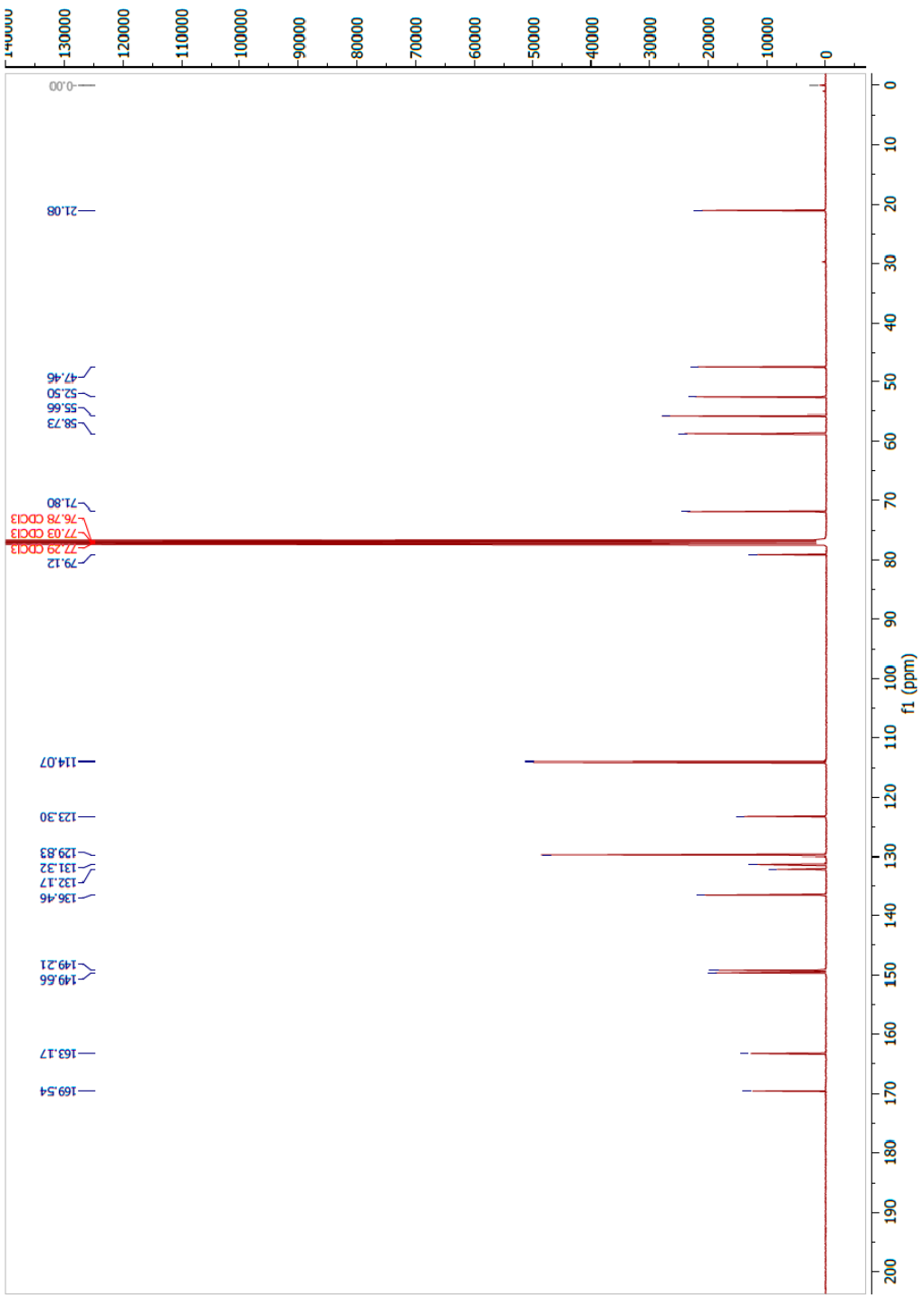


#	Time	Area	Height	Width	Area%	Symmetry
1	1.592	3.8	1.1	0.0489	0.026	1.256
2	1.744	11.8	1.6	0.1029	0.080	0.61
3	4.648	204.2	40.2	0.0772	1.383	0.937
4	5.966	8.9E-1	1.3E-1	0.0967	0.006	0.796
5	6.72	14436.1	1663.7	0.1352	97.815	0.417
6	7.398	37	6.1	0.0893	0.251	0.961
7	7.627	6.2	6.5E-1	0.1301	0.042	0.595
8	7.852	9.6	1.7	0.0819	0.065	1.43
9	7.971	20	4.3	0.0723	0.135	1.238
10	8.305	29	4.4	0.0949	0.196	1.115

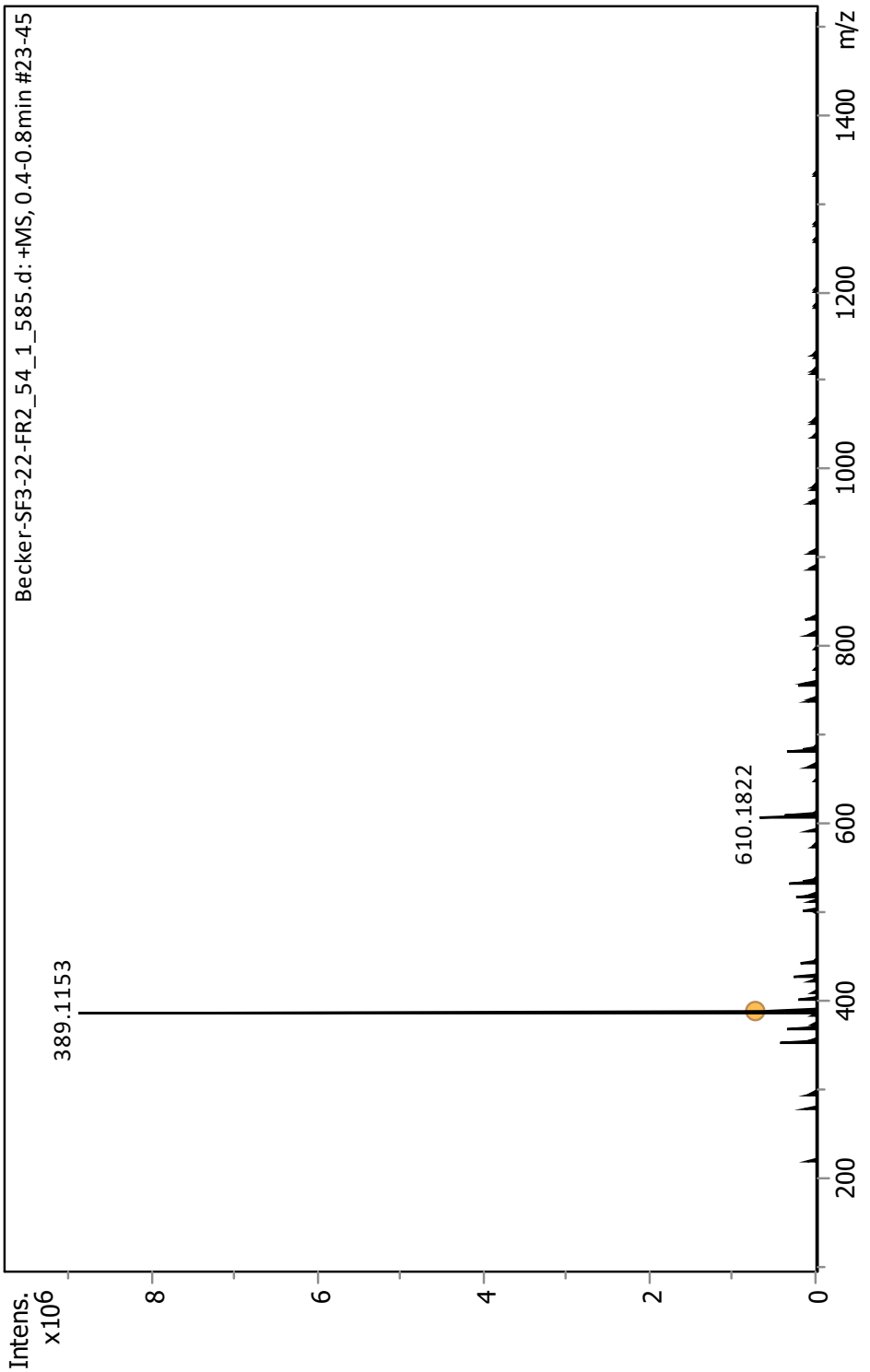
[1]H NMR of Methyl (*R*)-2-((4-methoxy-*N*-(pyridine-3-ylmethyl) phenyl) sulfonamido) pent-4-ynoate **(14).**

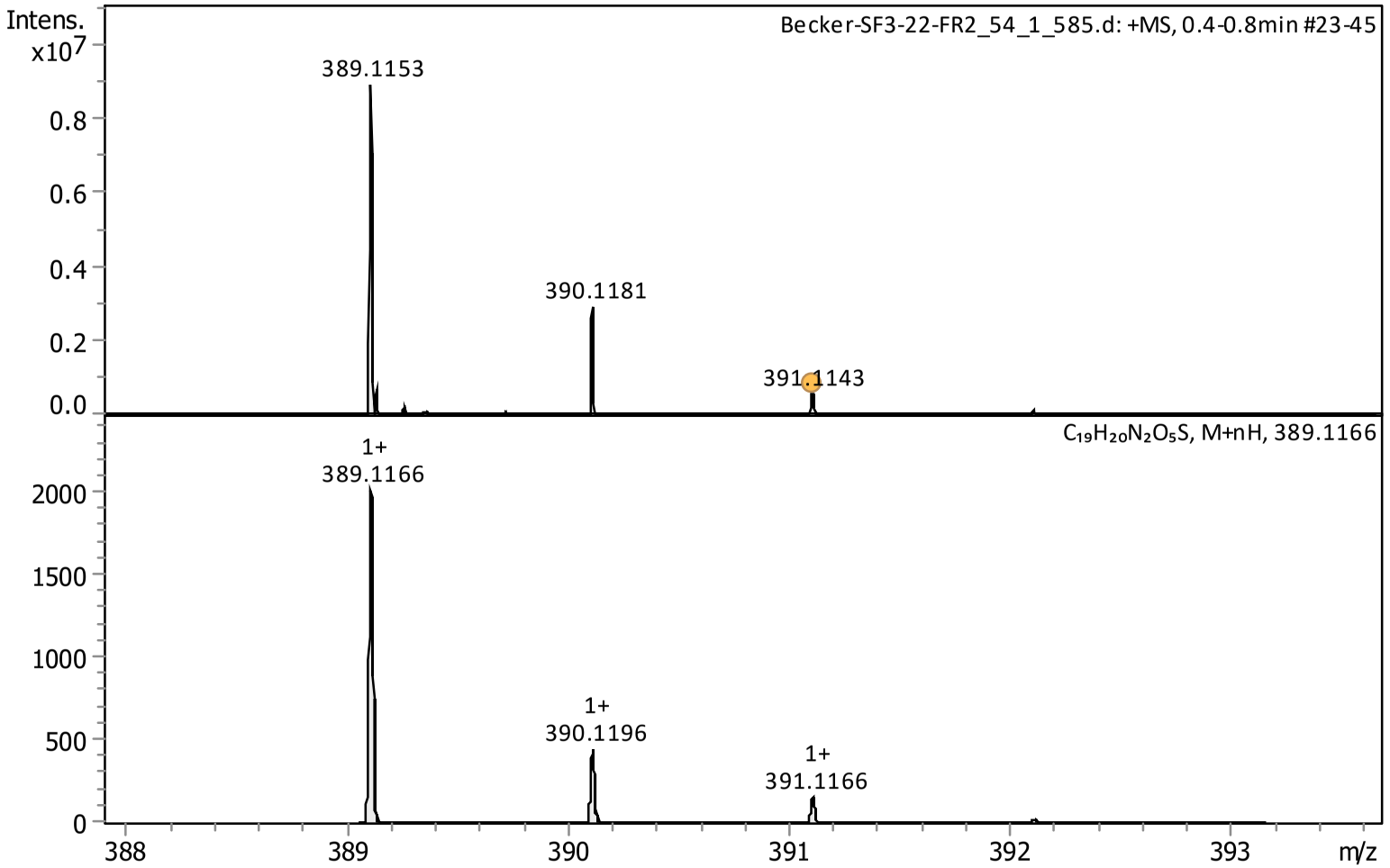


^{13}C NMR of Methyl (*R*)-2-((4-methoxy-*N*-(pyridine-3-ylmethyl) phenyl) sulfonamido) pent-4-ynoate **(14).**



HRMS of Methyl (*R*)-2-((4-methoxy-*N*-(pyridine-3-ylmethyl) phenyl) sulfonamido) pent-4-ynoate **(14).**

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0.6
0.4
0.2
0.0
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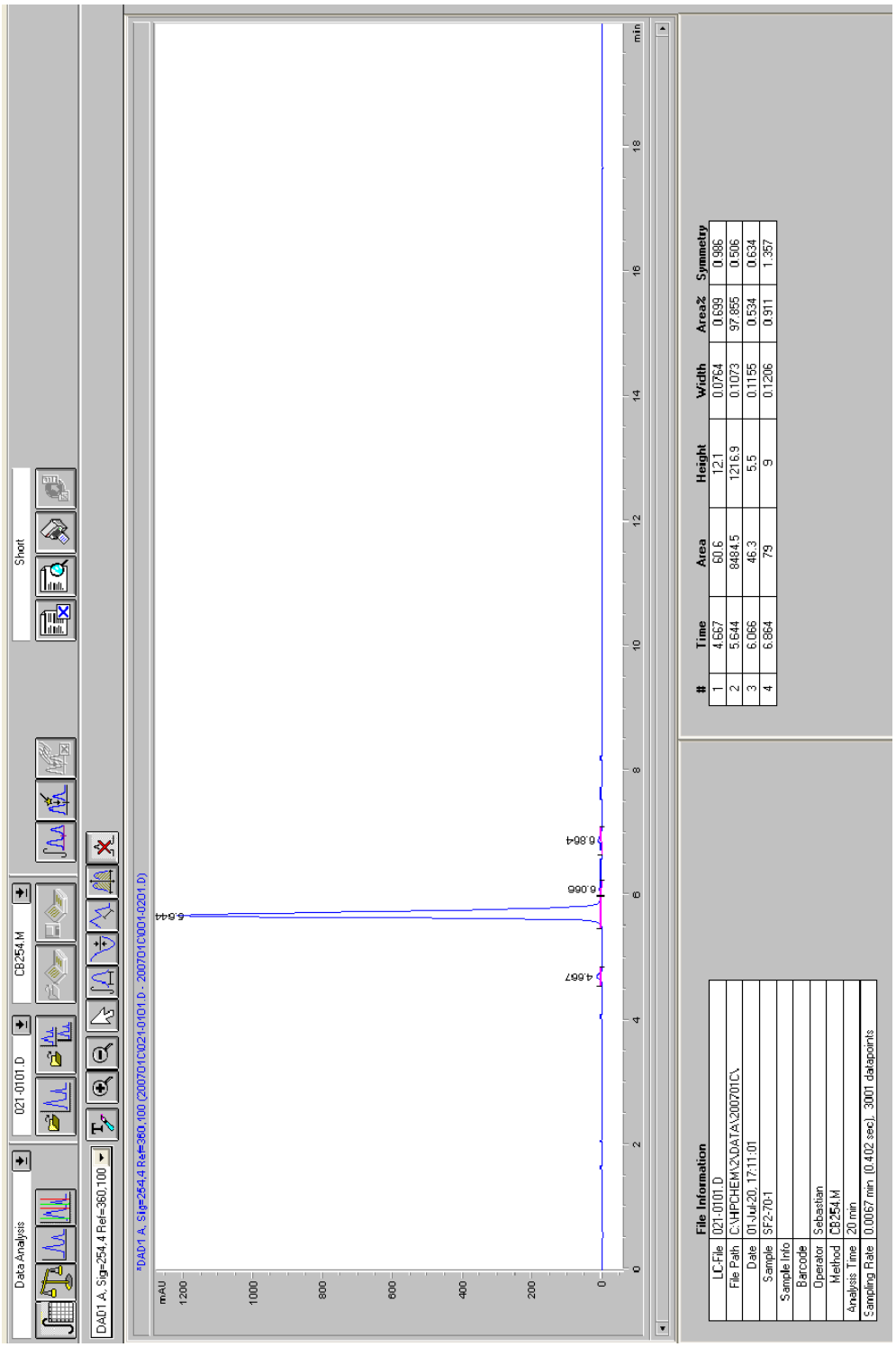
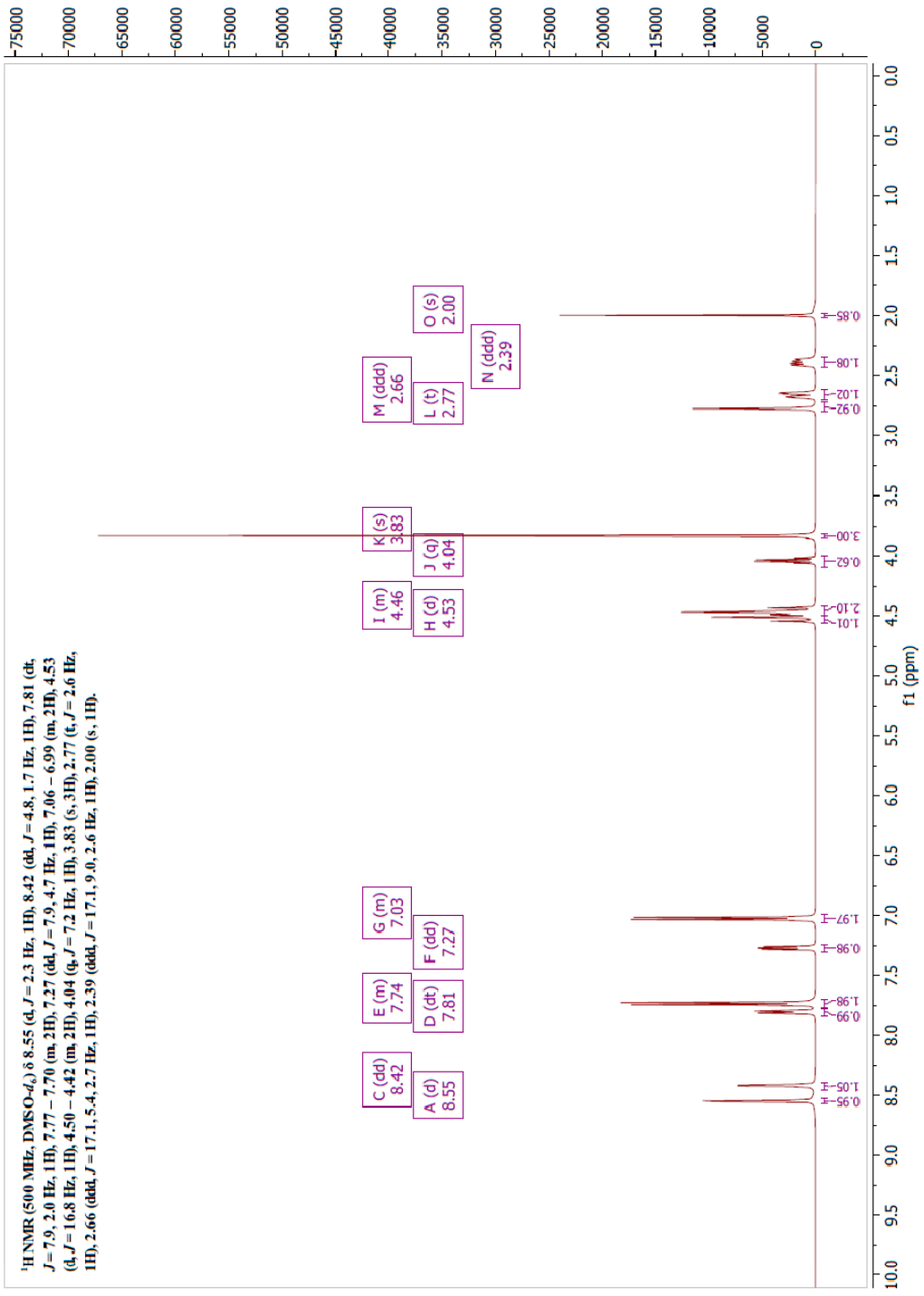
389.1153
390.1181
391.1143
C19H20N2O5S, M+nH, 389.1166
2000
1500
1000
500
0
1+
389.1166
1+
390.1196
1+
391.1166
388
389
390
391
392
393
m/z

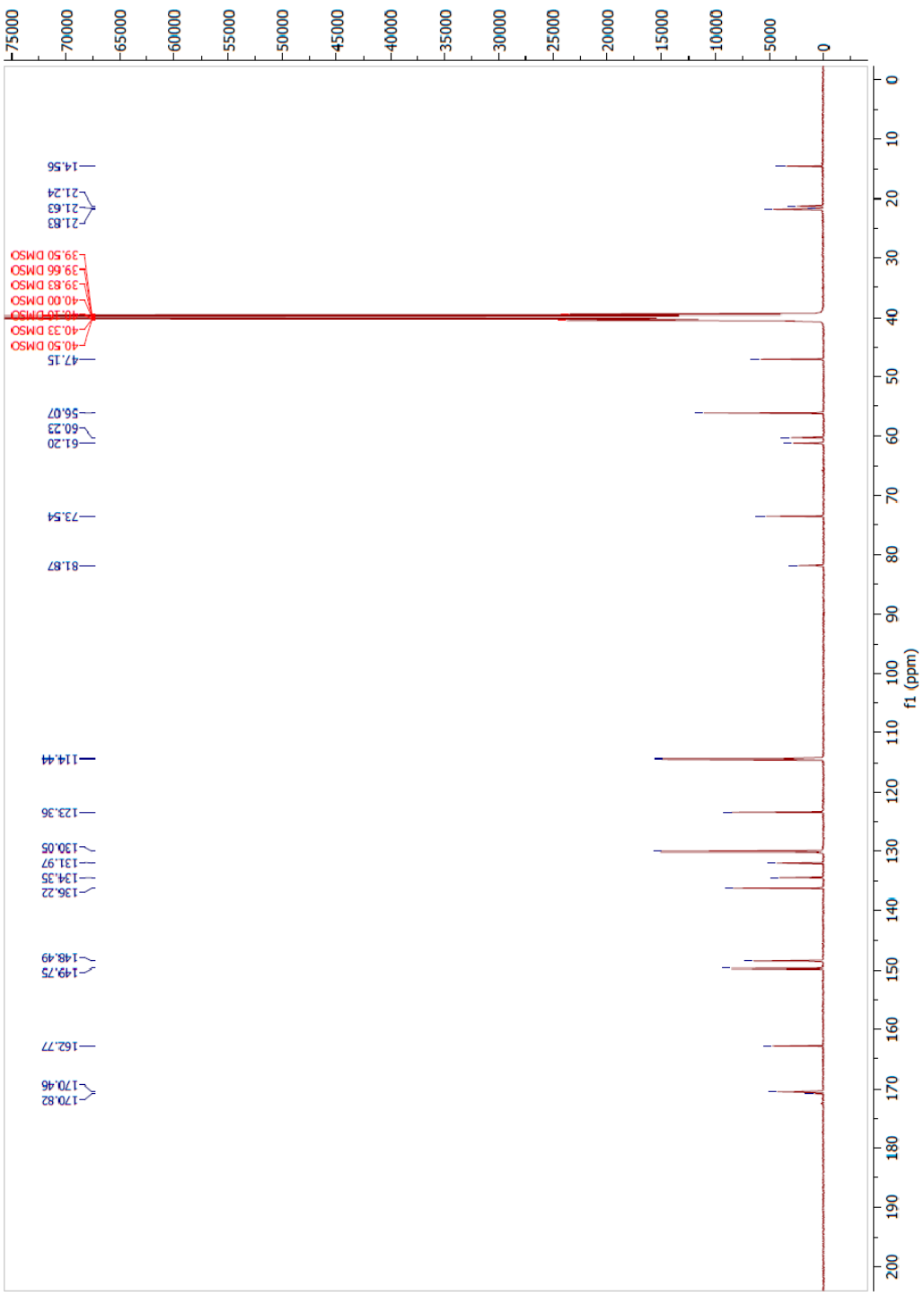
HPLC of (R)-2-((4-methoxy-N-(pyridin-3-ylmethyl)phenyl)sulfonamido)pent-4-ynoic acid **(15).**



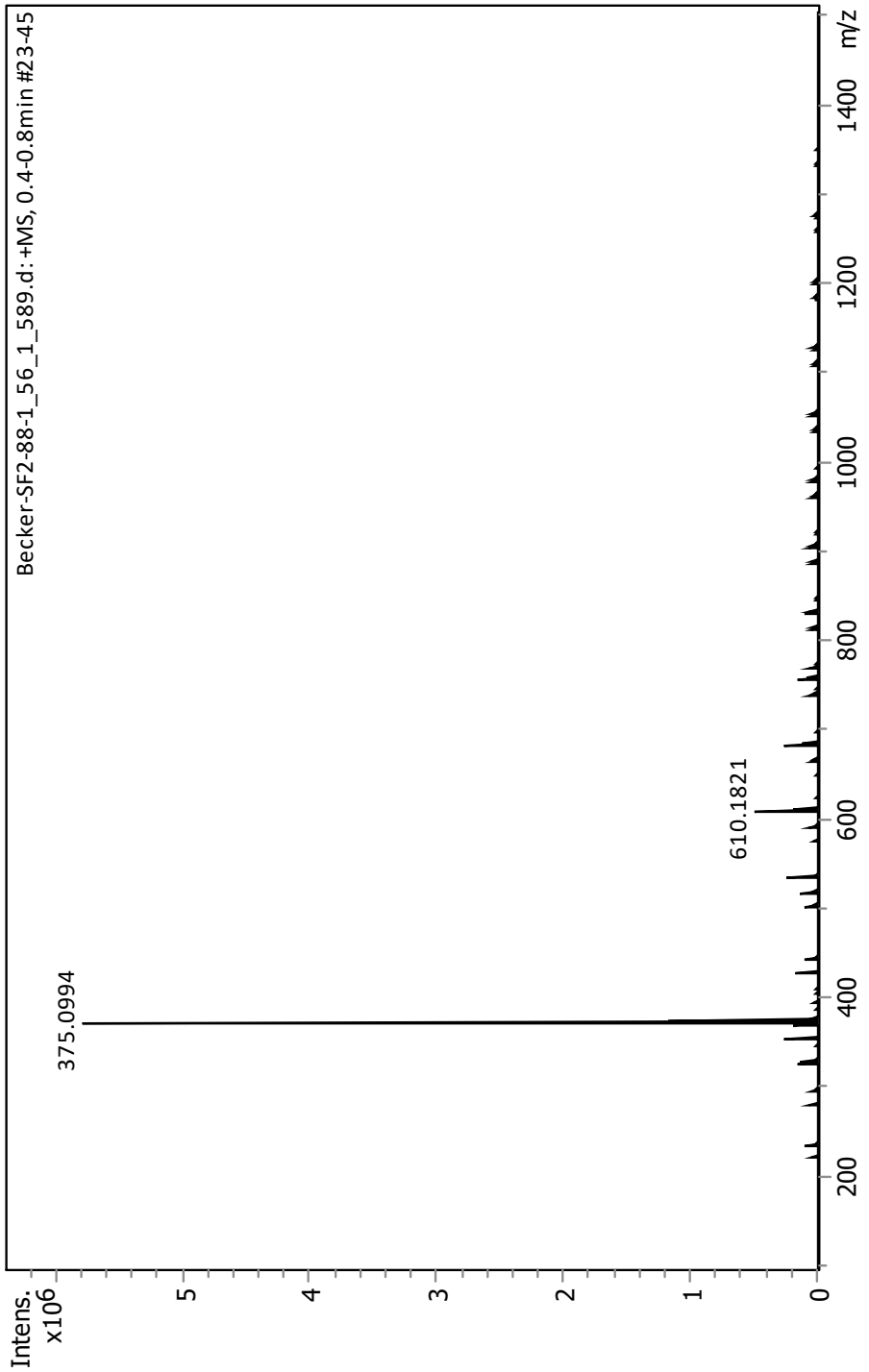
[1]H NMR of (R)-2-((4-methoxy-N-(pyridin-3-ylmethyl)phenyl)sulfonamido)pent-4-ynoic acid **(15).**

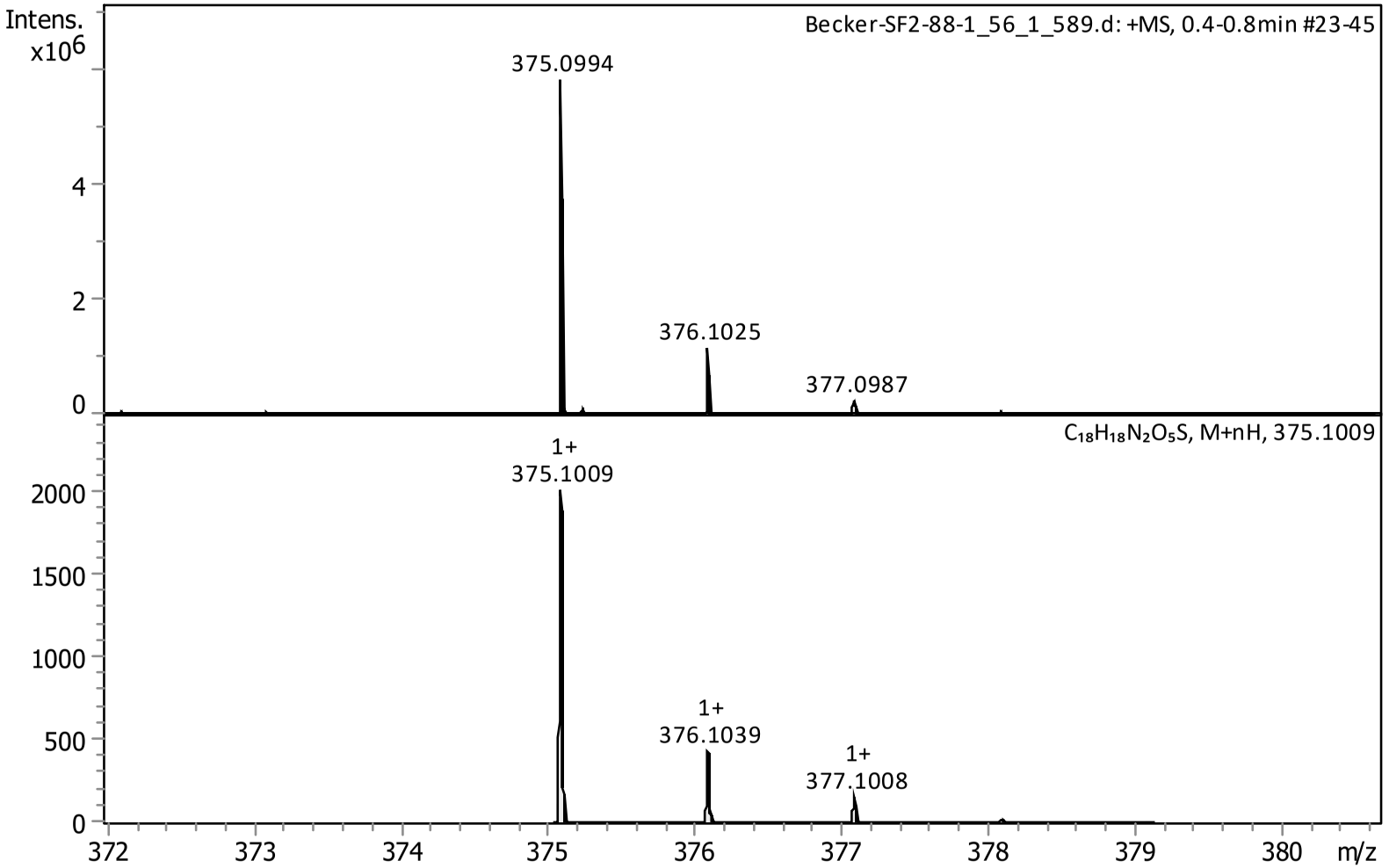


^{13}C NMR of (R)-2-((4-methoxy-N-(pyridin-3-ylmethyl)phenyl)sulfonamido)pent-4-ynoic acid **(15).**



HRMS of (R)-2-((4-methoxy-N-(pyridin-3-ylmethyl)phenyl)sulfonamido)pent-4-ynoic acid **(15).**

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377.0987
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0
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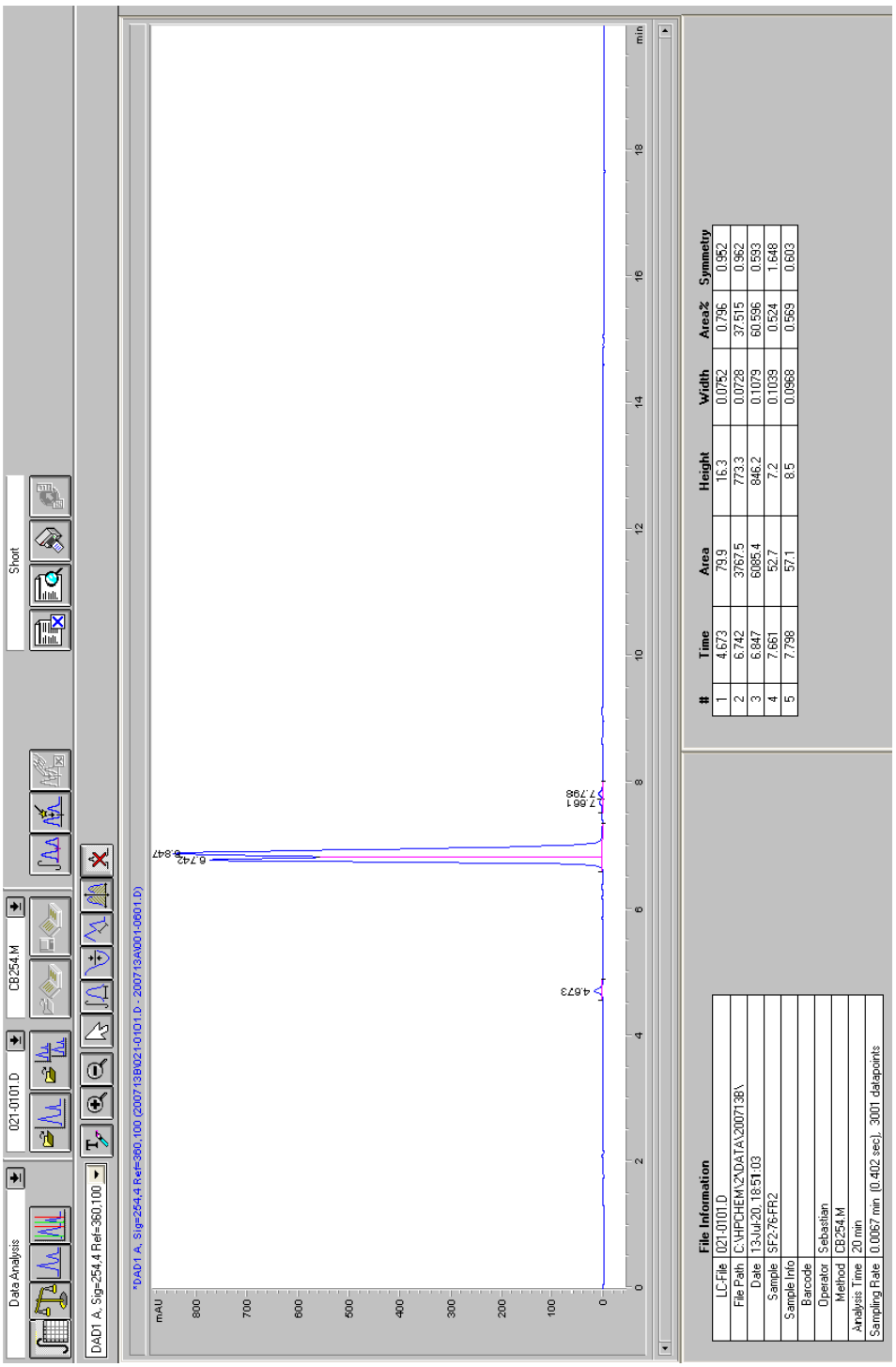
1+
375.1009
2000
1500
1000
1+
376.1039
500
1+
377.1008
0
372
373
374
375
376
377
378
379
380
m/z

HPLC of 4-methoxy-N-((2R)-1-oxo-1-(((tetrahydro-2H-pyran-2-yl)oxy)-l2-azaneyl)pent-4-yn-2-yl)-N-(pyridin-3-ylmethyl)benzenesulfonamide **(16).**



#	Time	Area	Height	Width	Area%	Symmetry
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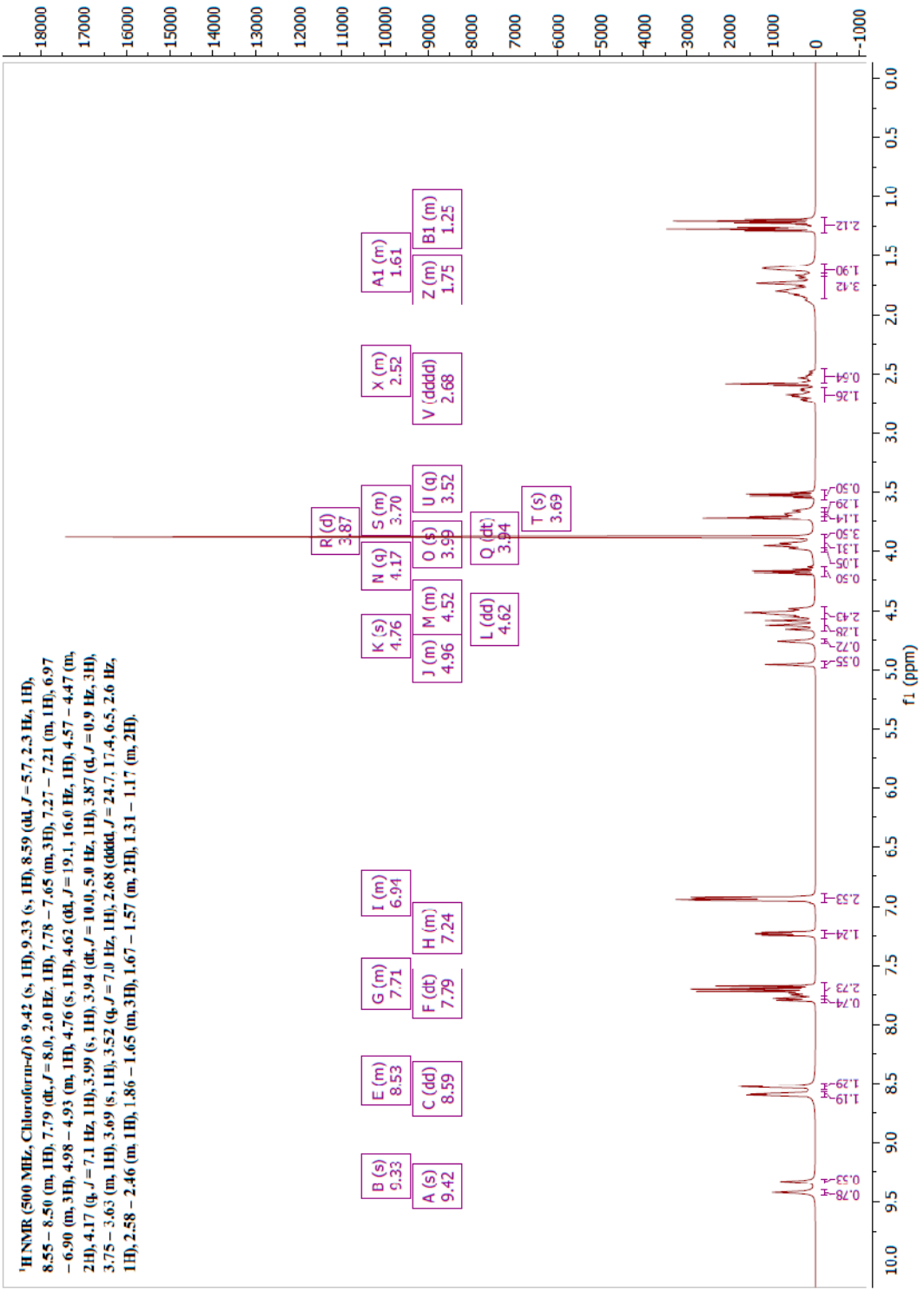
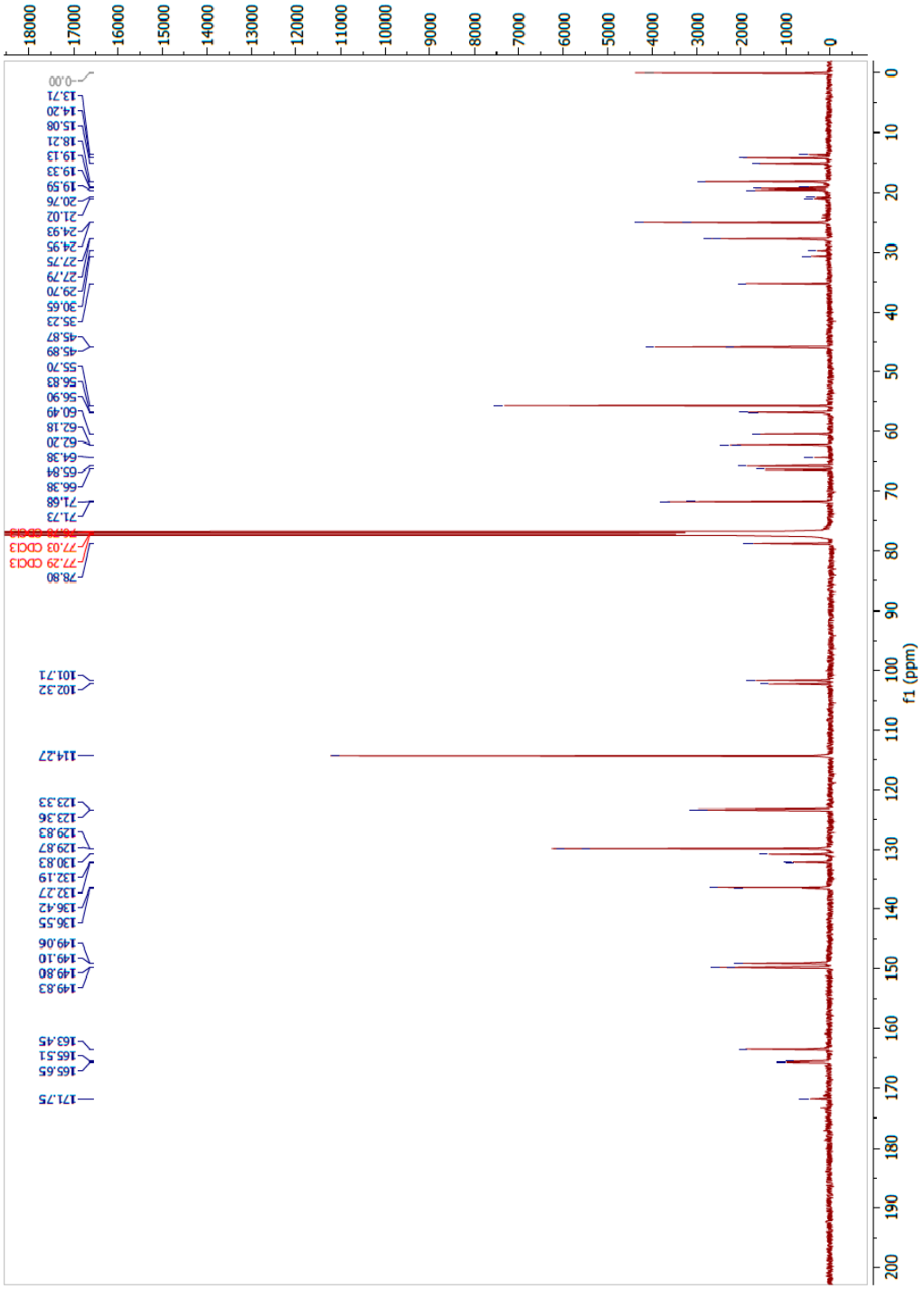
2	6.742	3767.5	773.3	0.0728	37.515	0.962
3	6.847	6095.4	846.2	0.1079	60.596	0.593
4	7.661	52.7	7.2	0.1029	0.524	1.648
5	7.798	57.1	8.5	0.0968	0.569	0.603

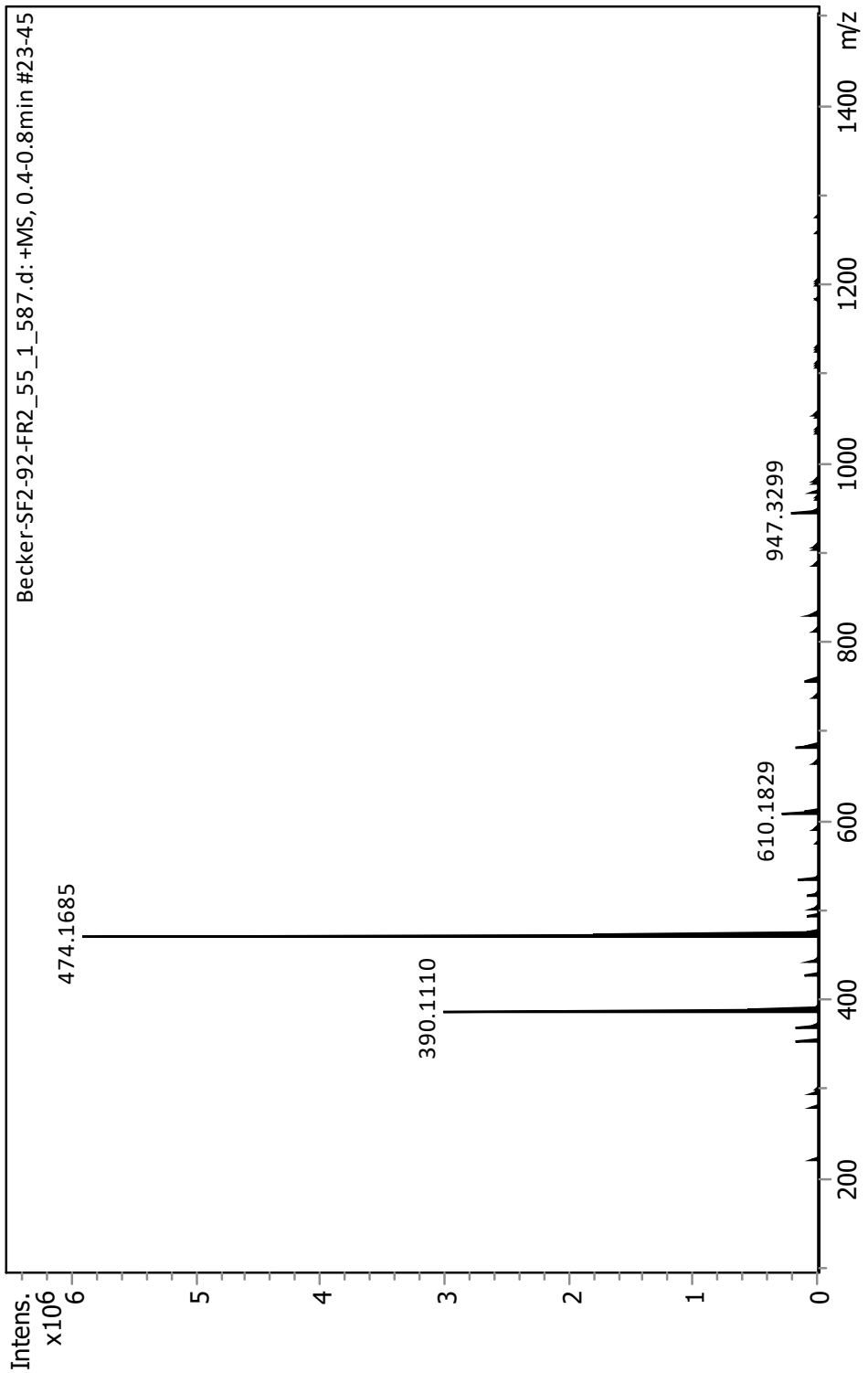
[1]H NMR of 4-methoxy-N-((2R)-1-oxo-1-(((tetrahydro-2H-pyran-2-yl)oxy)-l2-azaneyl)pent-4-yn-2-yl)-N-(pyridin-3-ylmethyl)benzenesulfonamide **(16).**

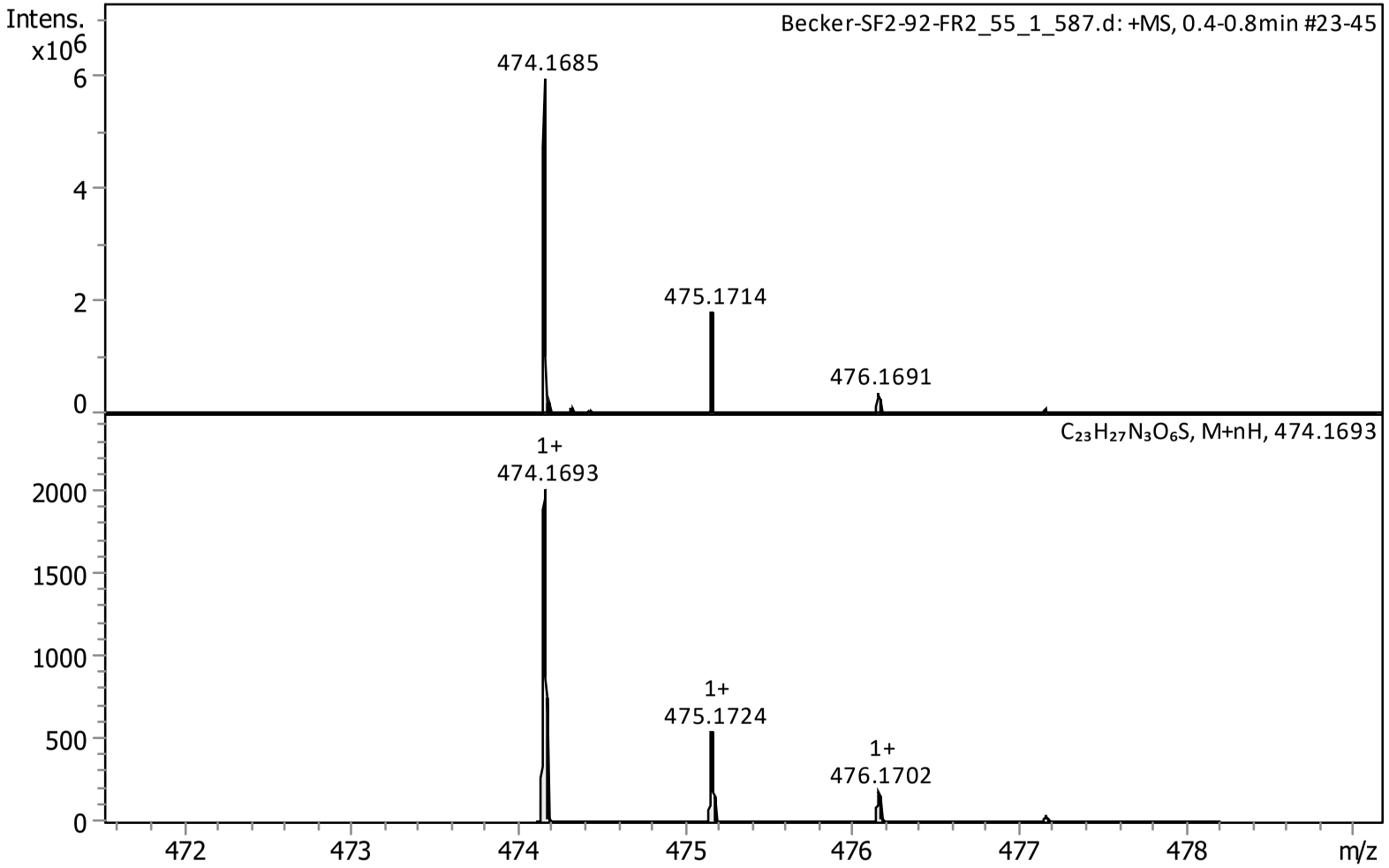


^{13}C NMR of 4-methoxy-N-((2R)-1-oxo-1-(((tetrahydro-2H-pyran-2-yl)oxy)-l2-azaneyl)pent-4-yn-2-yl)-N-(pyridin-3-ylmethyl)benzenesulfonamide **(16).**



HRMS of 4-methoxy-N-((2R)-1-oxo-1-(((tetrahydro-2H-pyran-2-yl)oxy)-l2-azaneyl)pent-4-yn-2-yl)-N-(pyridin-3-ylmethyl)benzenesulfonamide **(16).**




Intens.
x10^6
6
4
2
0
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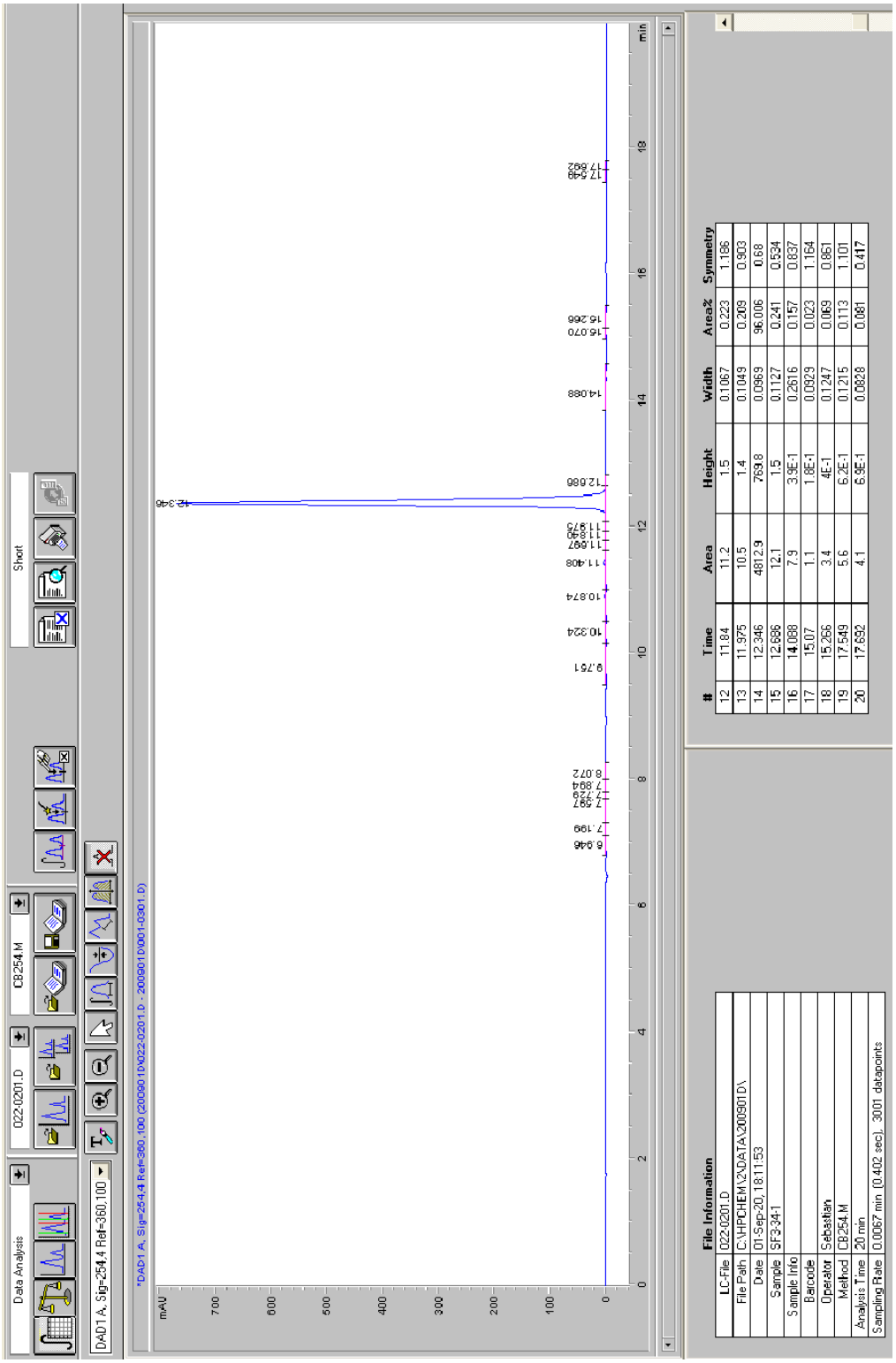
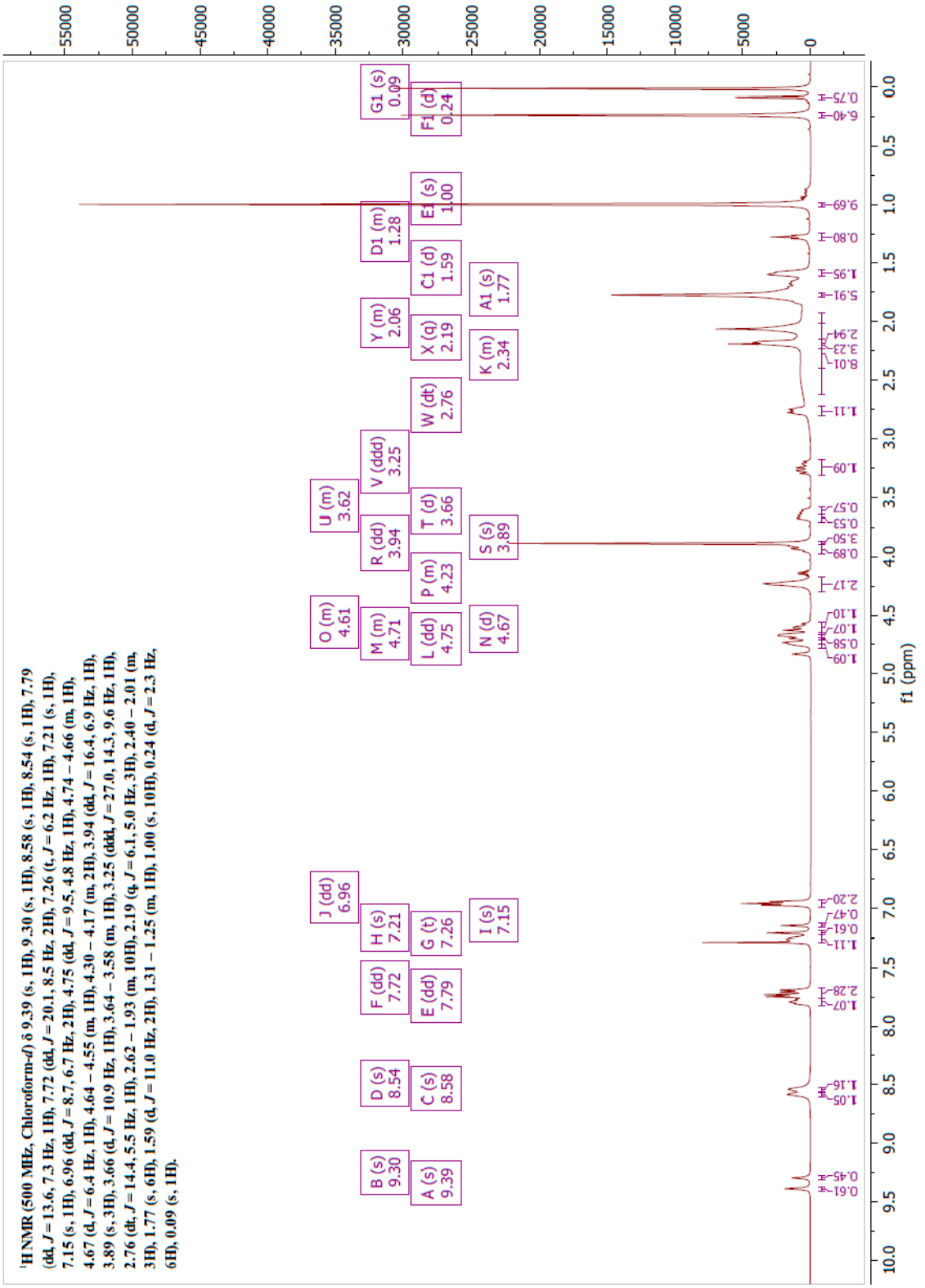
474.1685
475.1714
476.1691
C23H27N3O6S, M+nH, 474.1693
1+
474.1693
2000
1500
1000
500
0
1+
475.1724
1+
476.1702
472
473
474
475
476
477
478
m/z

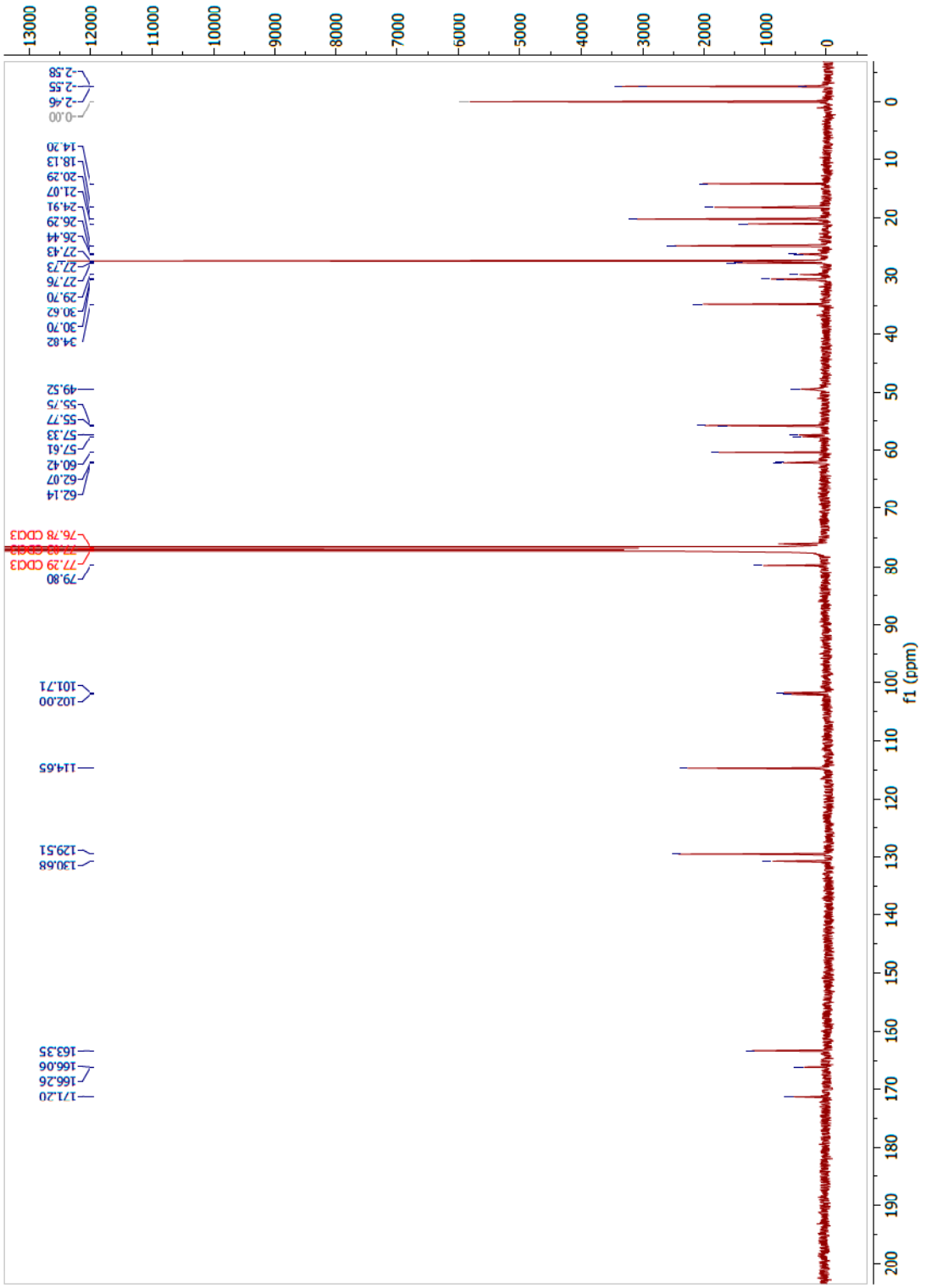
HPLC of THP-protected 1,4-Click Isomer **18.**



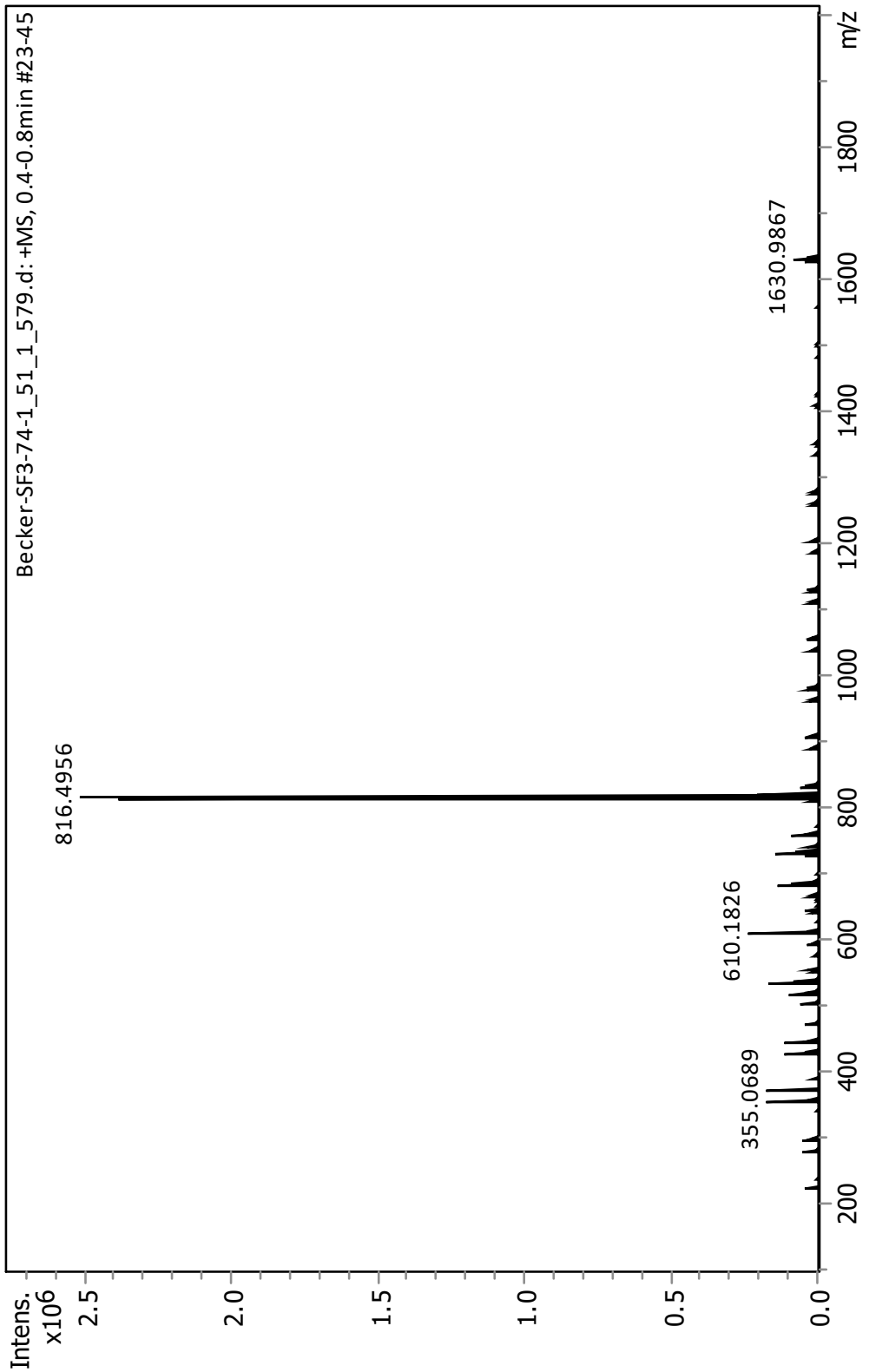
[1]H NMR of THP-protected 1,4-Click Isomer **18.**

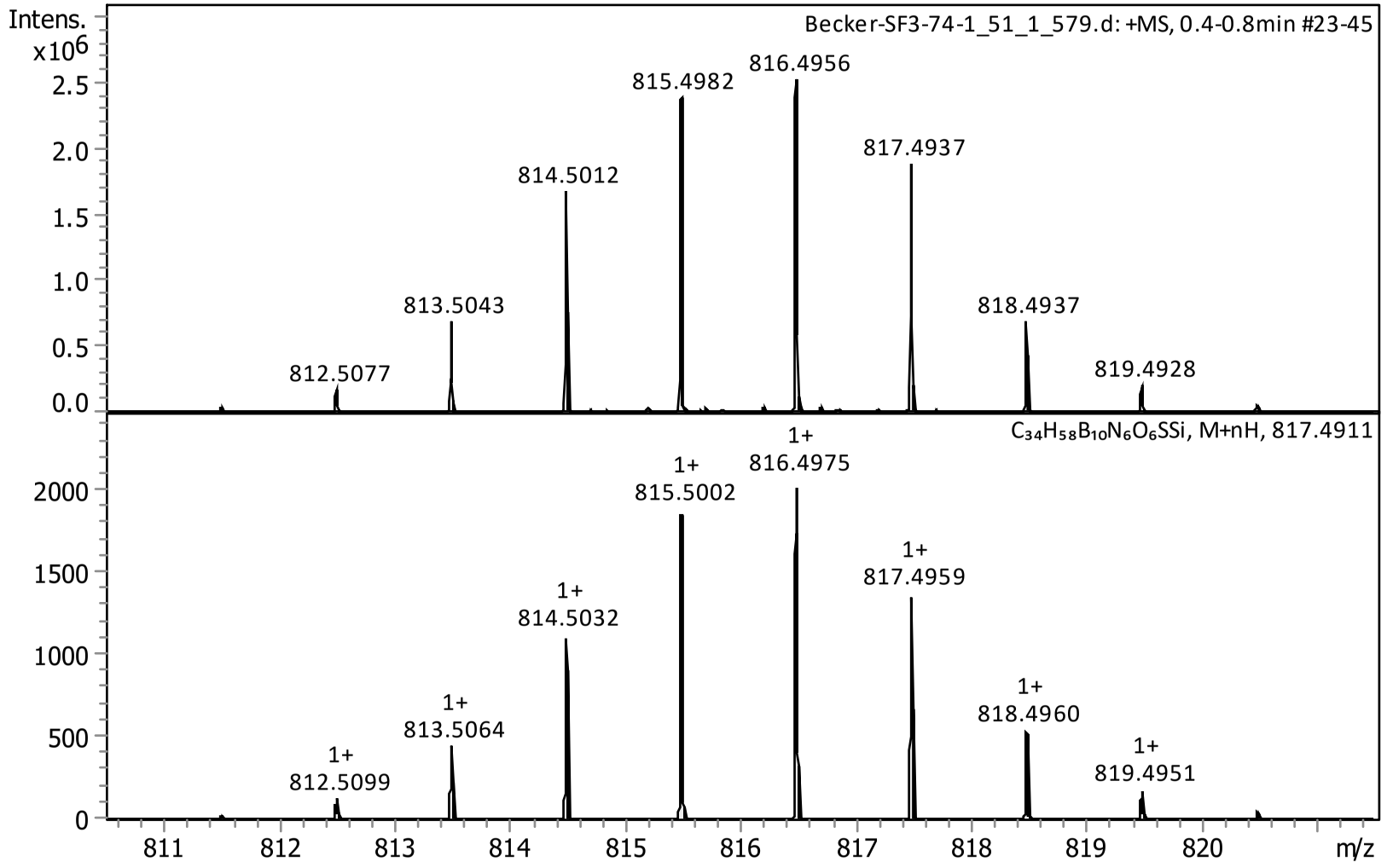


^{13}C NMR of THP-protected 1,4-Click Isomer **18.**

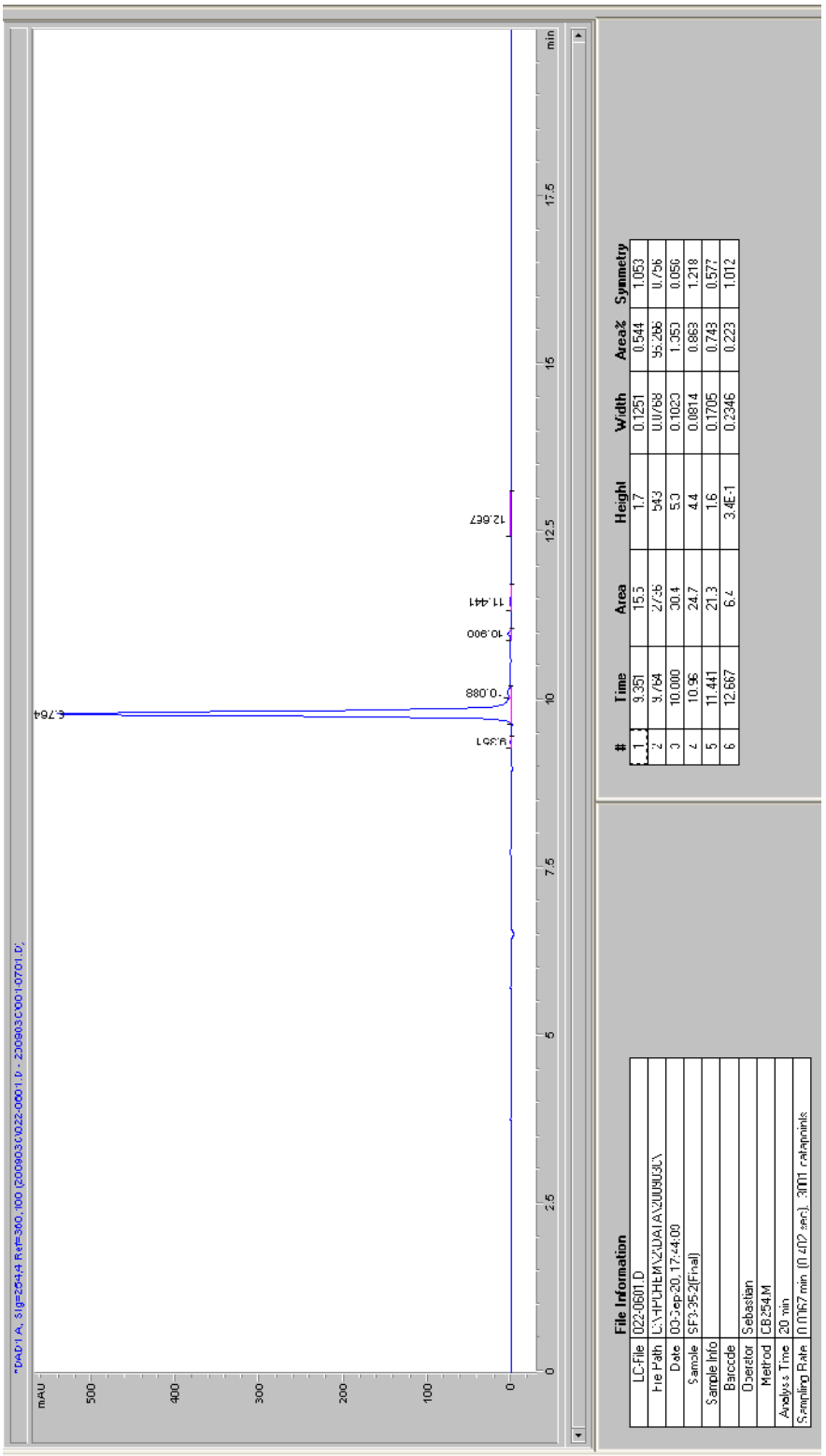


HRMS of THP-protected 1,4-Click Isomer **18.**



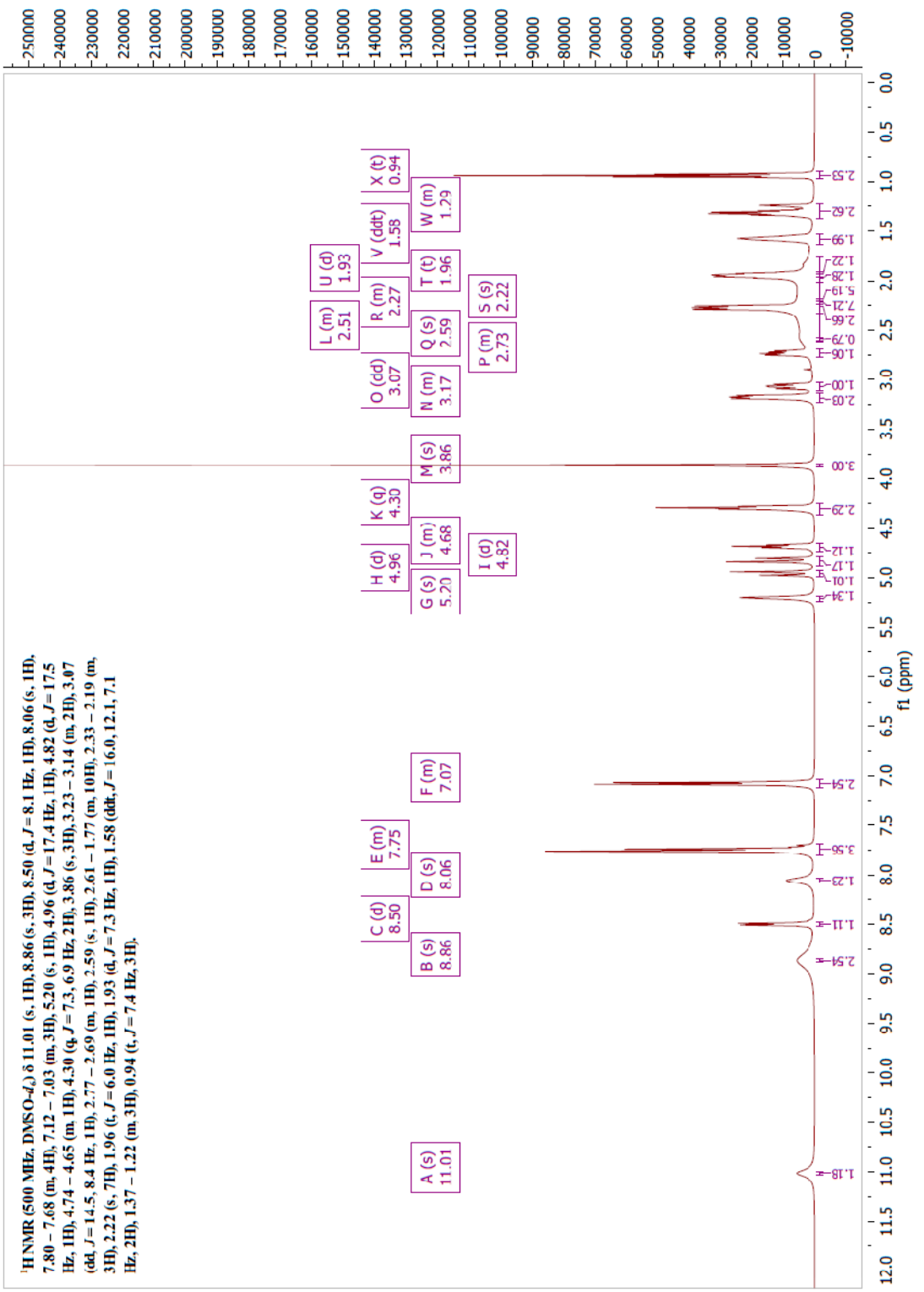

Intens.
x10^6
2.5
2.0
1.5
1.0
0.5
0.0
Becker-SF3-74-1_51_1_579.d: +MS, 0.4-0.8min #23-45
815.4982
816.4956
817.4937
814.5012
813.5043
818.4937
812.5077
819.4928
2000
1500
1000
500
0
C34H58B10N6O6SSi, M+nH, 817.4911
1+
816.4975
1+
815.5002
1+
817.4959
1+
814.5032
1+
818.4960
1+
813.5064
1+
819.4951
1+
812.5099
811
812
813
814
815
816
817
818
819
820
m/z

HPLC of Hydroxamic Acid 1,4-triazole **4.**

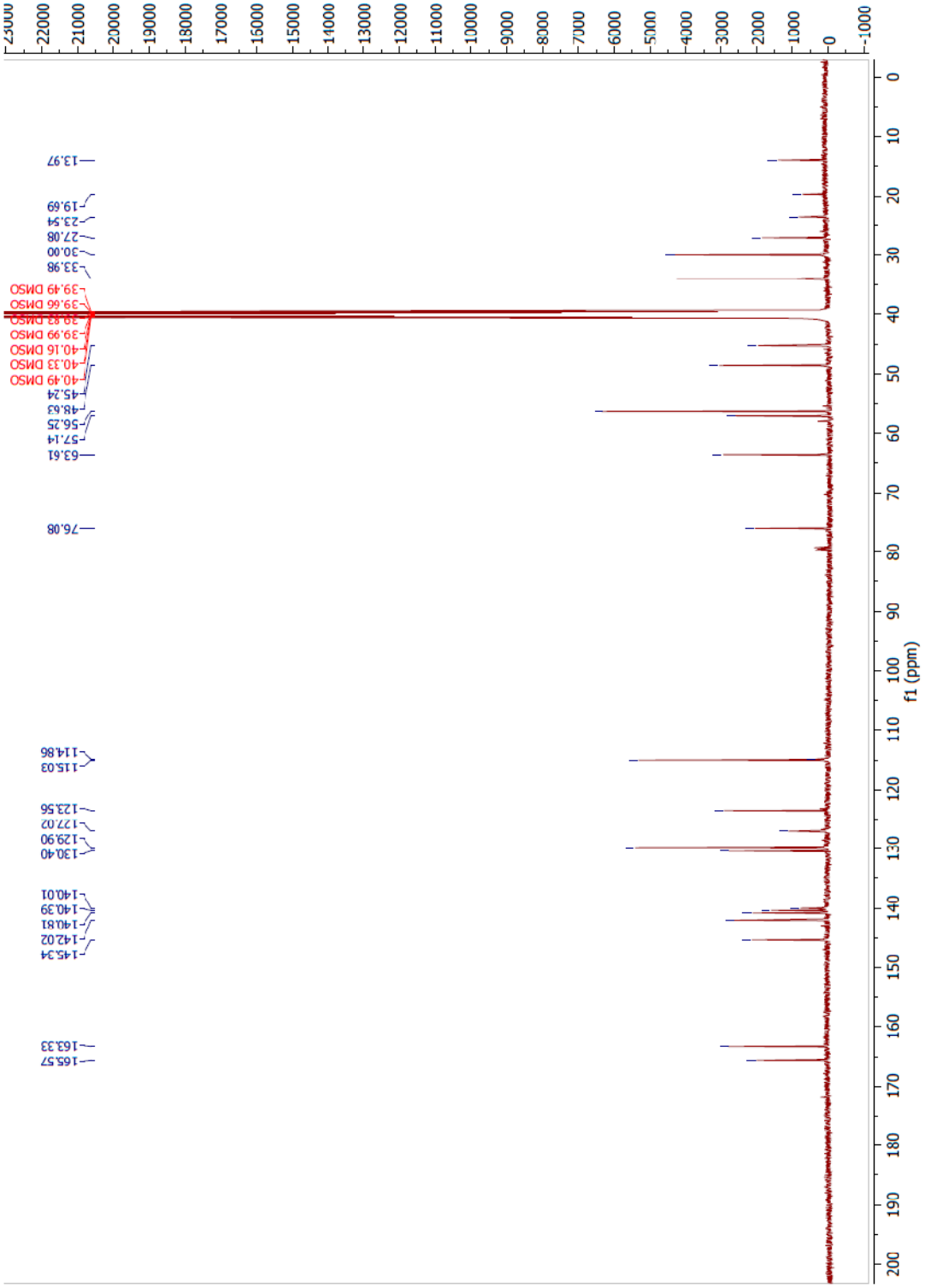


#	Time	Area	Height	Width	Area%	Symmetry
1	9.351	15.5	1.7	0.1251	0.544	1.053
2	9.764	2736	943	0.0768	95.266	0.756
3	10.088	30.4	5.3	0.1023	1.063	0.056
4	10.96	24.7	4.4	0.0814	0.863	1.218
5	11.441	21.3	1.6	0.1705	0.743	0.577
6	12.667	6.4	3.4E-1	0.2346	0.223	1.012

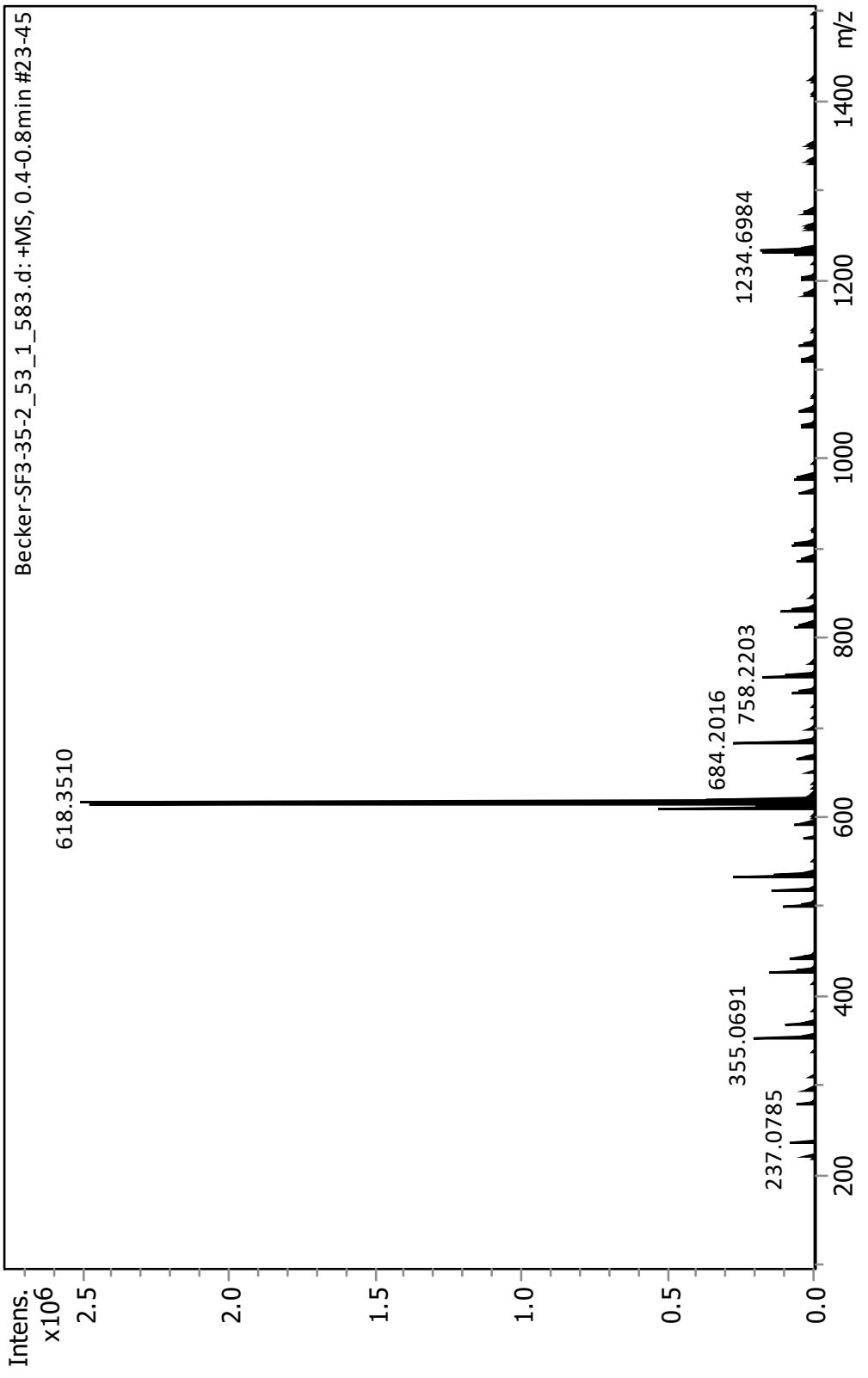
[1]H NMR of Hydroxamic Acid 1,4-triazole **4.**



^{13}C NMR of Hydroxamic Acid 1,4-triazole **4.**



HRMS of Hydroxamic Acid 1,4-triazole **4.**



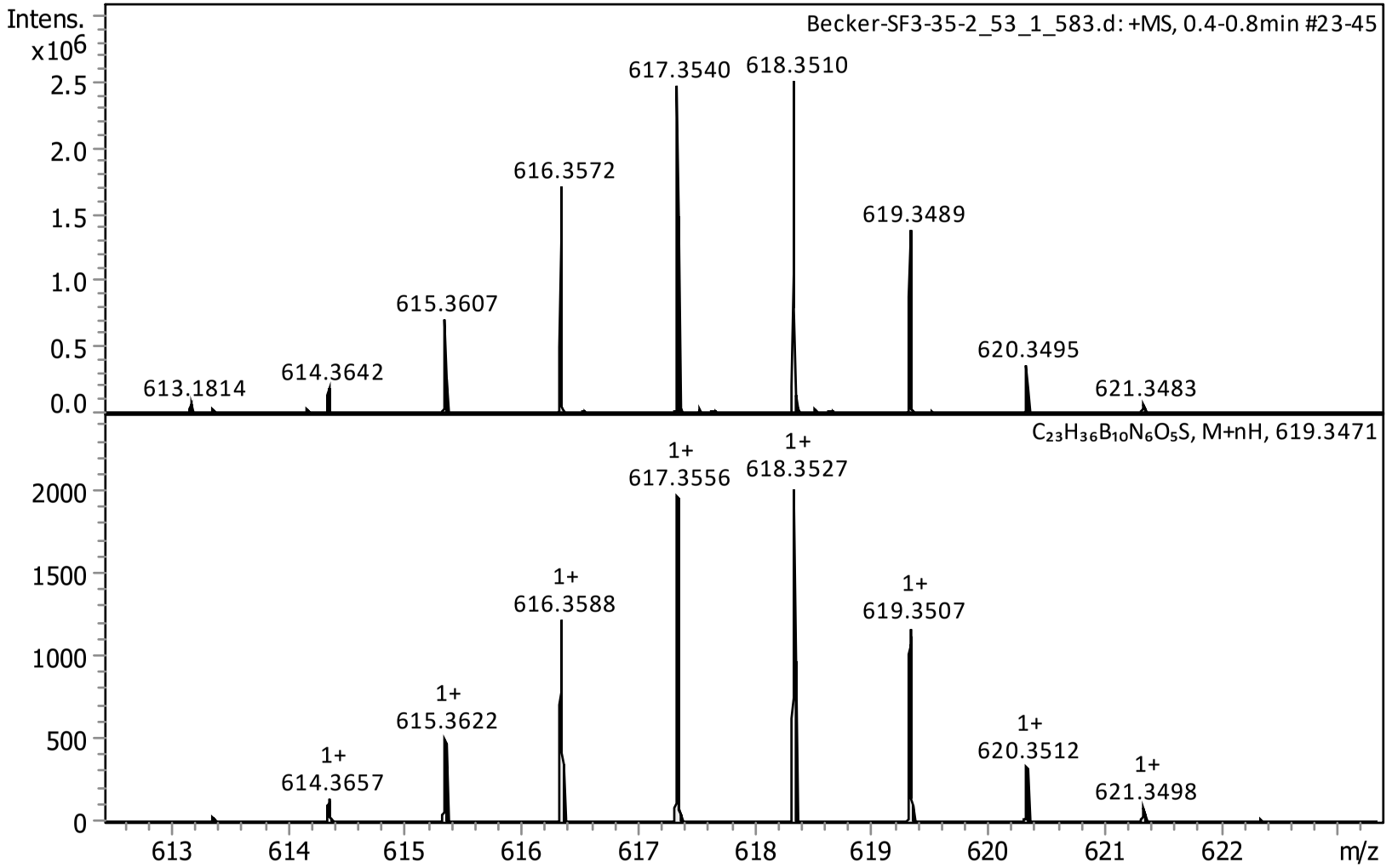

Intens.
x10^6
2.5
2.0
1.5
1.0
0.5
0.0
Becker-SF3-35-2_53_1_583.d: +MS, 0.4-0.8min #23-45
617.3540
618.3510
616.3572
619.3489
615.3607
620.3495
614.3642
621.3483
613.1814
C23H36B10N6O5S, M+nH, 619.3471
2000
1500
1000
500
0
1+
617.3556
1+
618.3527
1+
616.3588
1+
619.3507
1+
615.3622
1+
620.3512
1+
614.3657
1+
621.3498
613
614
615
616
617
618
619
620
621
622
m/z

Figure S1a. Superposition of BNCT Agent **20** and SC-74020 compound **19** in 1HOV (MMP-2).

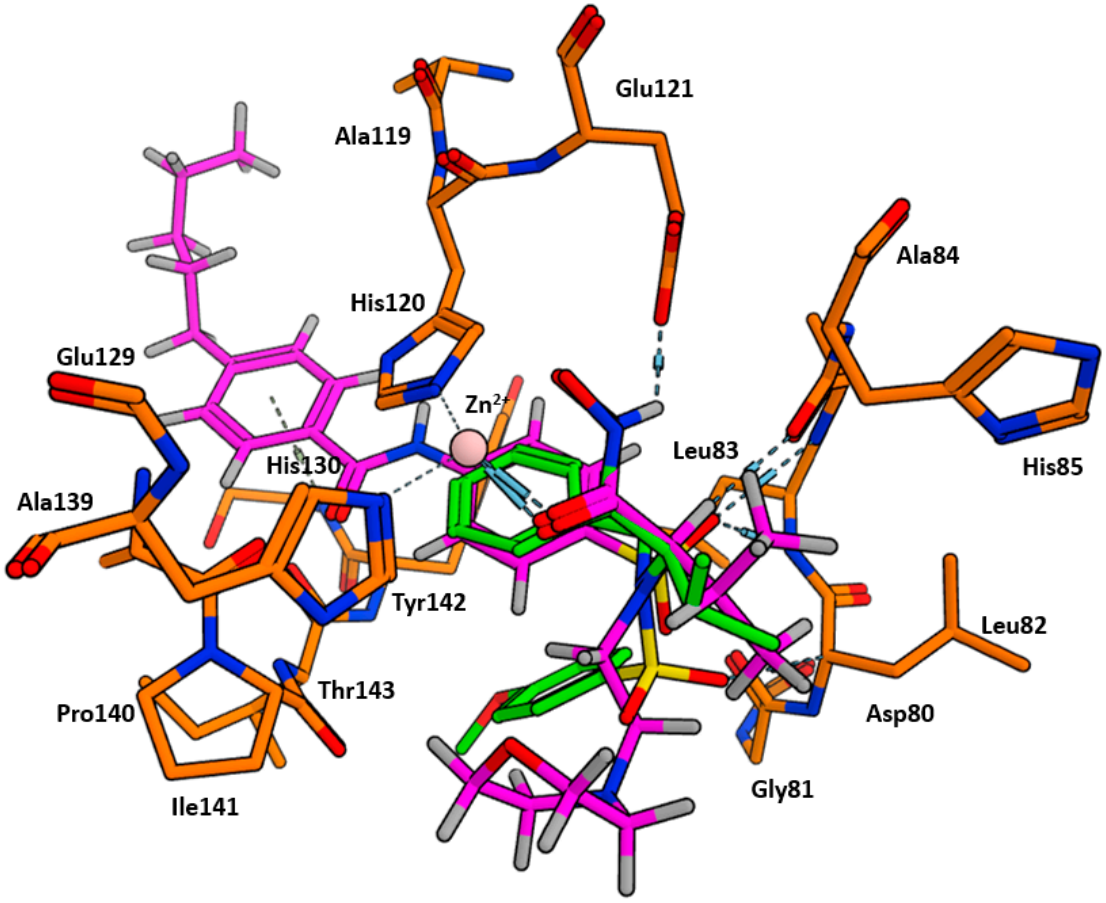


Figure S1b. Ligand interactions of 1HOV (MMP-2) with the bound ligand, BNCT Agent **20**, and Zn^{2+}.

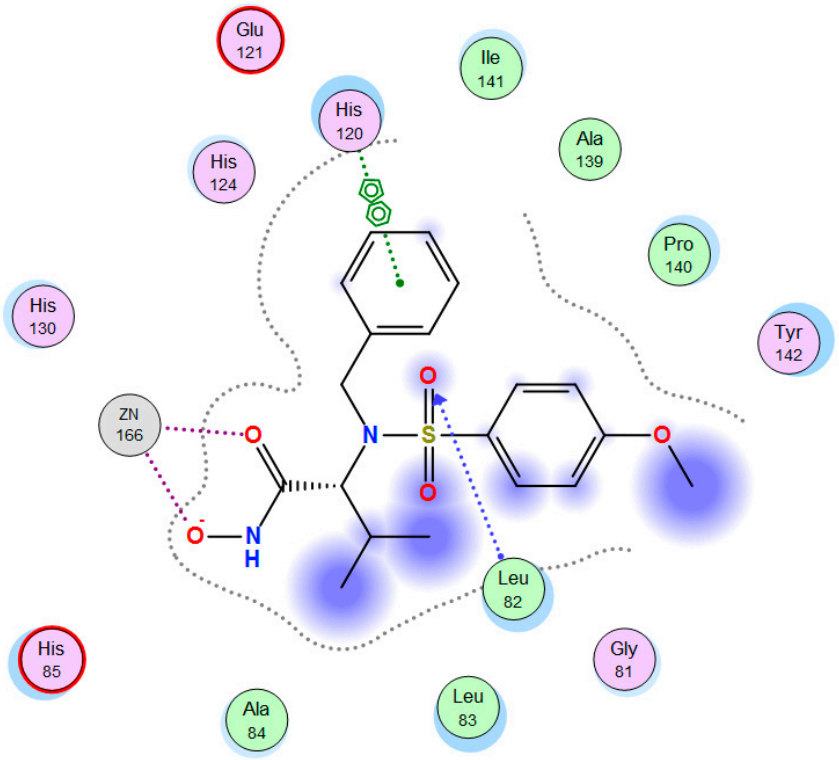


Figure S2a. Superposition of 1,4-click isomer **21** and SC-74020 compound **19** in 1HOV (MMP-2).

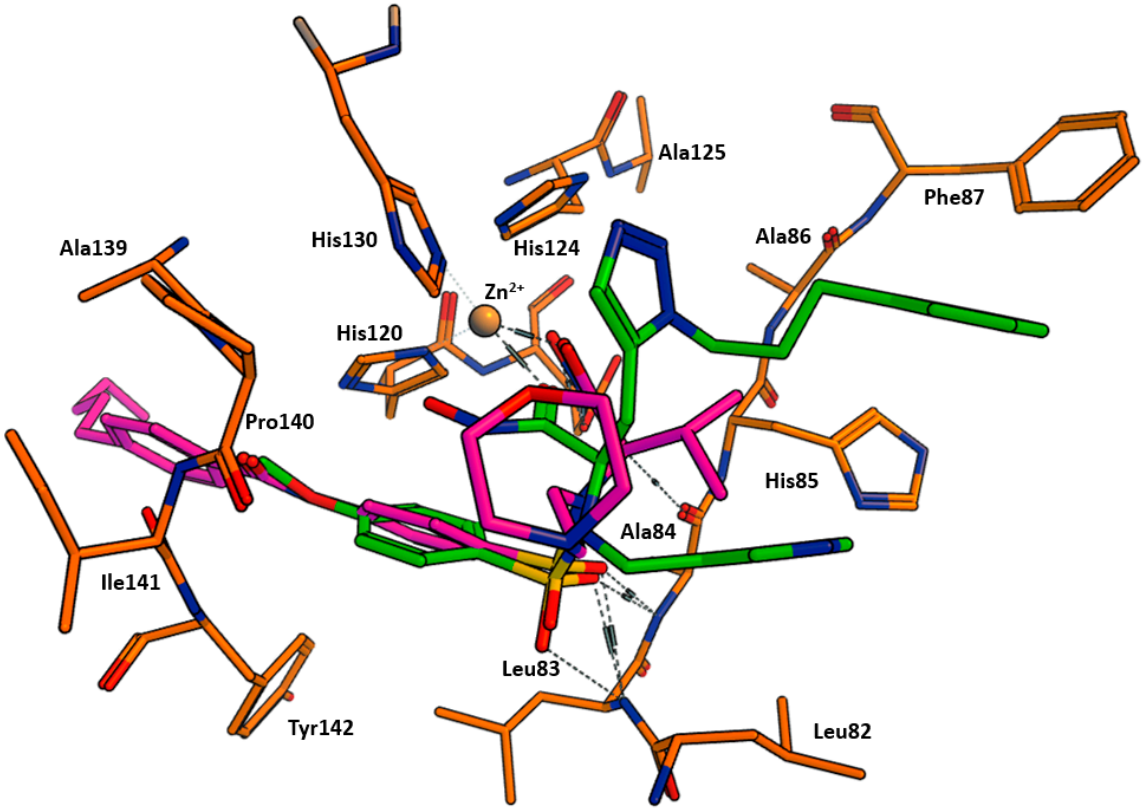
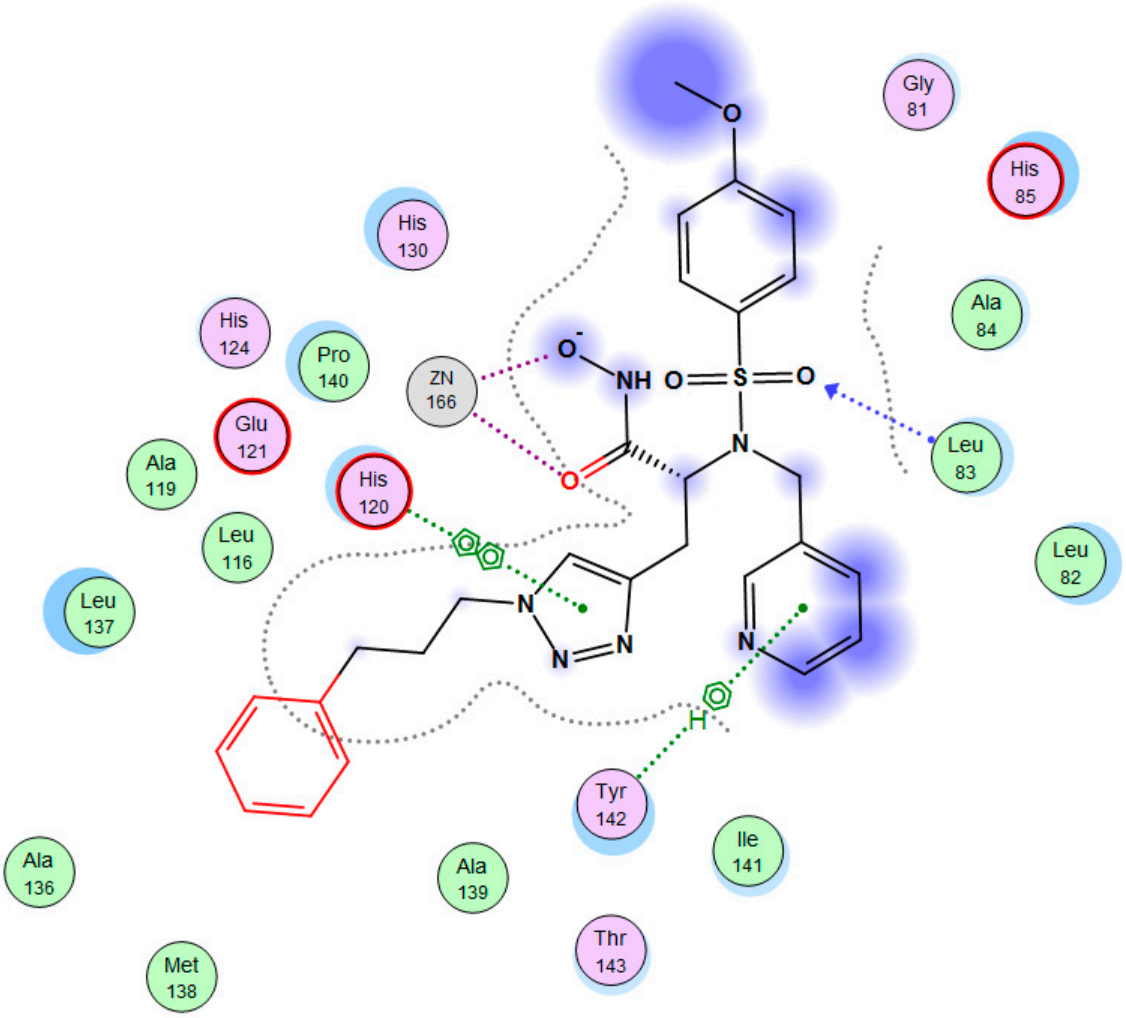


Figure S2b. Ligand interactions of 1HOV (MMP-2) with the bound ligand, 1,4-click isomer **21**, and Zn^{2+}.



PDB coordinates for docked compounds are available

- **PDB of BNCT Agent 20 Docked in 1HOV.pdb**
- **PDB of BNCT Agent 20 and SC-74020 compound 19 Docked in 1HOV.pdb**
- **PDB of 1,4-Click Isomer 21 Docked in 1HOV.pdb**
- **PDB of 1,4-Click Isomer 21 and SC-74020 compound 19 Docked in 1HOV.pdb**

Compound **3** (SF-4-6A), carborane conjugated MMP inhibitor

Our standardized experimental methods are online at the following URL,

https://1458ab30-7501-42df-8c2e-ff59d20cecb7.filesusr.com/ugd/ddd07a_cbe194d92fd14397a5db1690d68a185c.pdf , which appears on the subsequent pages of this Supplemental Information packet.

The minimum requirement for our screening protocol of Boron compound samples (B-coms) for BNCT is water (and/or DMSO)-soluble 30 mg for first look screening of experiments 1, 2 and 3. Whole serial steps are as follows. Compound **3** (SF-4-6A), was eliminated from further screening at step 2 due to its toxicity.

1. Solubility in a physiological condition
2. Cell toxicity; IC_{50}
3. Cellular BNCT (in-vitro BNCT)
4. Bio-distribution study (neutron induced boron autoradiography)
5. Animal BNCT (in-vivo BNCT)
6. Pre-clinical study

Experimental summary for carborane-containing MMP ligand **3** (SF-4-6A)

Compound **3** (SF-4-6A, abbreviated SF46A) information:

1) The carborane cluster has been prepared with enriched ^{10}B decaborane (>99% ^{10}B)

2) See compound **3** (SF-4-6A) in manuscript and experimental.

3) Molecular Formula: $C_{15}H_{30}{}^{\mathbf{10}}B_{10}N_2O_5S$ boron content=10x10/450.32=22.2%

4) Molecular Weight: 450.32 g/mol (calculated based on enriched-^{10}B)

	Experiment 1	Experiment 2	Experiment 3
sample	**3** (SF46A)	**3** (SF46A)	**3** (SF46A)
Solvent (mg)	20mg	20mg	20mg
Dissolved in DMSO	1ml	1ml	1ml
Add（x μl）into culture dish	50μl	100	200 =4mg/ml SF46A =0.8ppm ^{10}B/ml
Solubility	Completely and easily dissolved	Completely and easily dissolved	Completely and easily dissolved

Filtering for sterilization through 0.022 m pore	Easily done	Easily done	Easily done
Pulse 23:20	50µ	100µ	200µ
Turbidity after adding	+ (clouded)	++ (strongly clouded)	+++ (strongly clouded)
Cell condition 3hrs after pulsation 01:50	Round shaped (toxic)	Detached from the bottom of the culture dish (toxic)	Detached from the bottom of the culture dish (toxic)
04:20 incubation stop and cell condition	Floating from the bottom of the culture dish (toxic)	Floating from the bottom of the culture dish (toxic)	Floating from the bottom of the culture dish (toxic)
ppm Boron in medium	22.2	44.4	66.6

*20mgx0.1x0.1x.22.2%=44.4µg/ml

IC50

Boron compound	n	$0.5x(1/2)^{n-1}$	A mg/ml	$IC_{50}=Ax0.5x(1/2)^{n-1}$ mg/ml
SF-4-6A	10.5	$6.91x10^{-4}$	20	$1.38x10^{-2}$ ($3.07x10^{-5}$ mM) ($3.07x10^{-3}$ ppm ^{10}B)
DSMO	5	$3.13x10^{-2}$		$3.13x10^{-1}$

Water Solubility Determination of 1,4-click triazole **1**

Solvent	1,4-Click Triazole **1**			
	Sample	Concentration (mg/mL)	Trial #1	Trial #2
			Peak Area	Peak Area
Acetonitrile	**A-0**	2.00	9964.6	9772.6
	A-1	1.00	5061.8	4915.7
	A-2	0.50	2534.7	2473.0
	A-3	0.25	1276.0	1250.7
	A-4	0.10	503.0	478.3
	A-5	0.05	247.9	233.2
	A-6	0.01	8.3	8.8
Nanopure H_2O	**A-Aq**	Unknown	44	23.3

Figure S3A. Raw data from HPLC analysis to determine drug water solubility of 1,4-click triazole **1**.

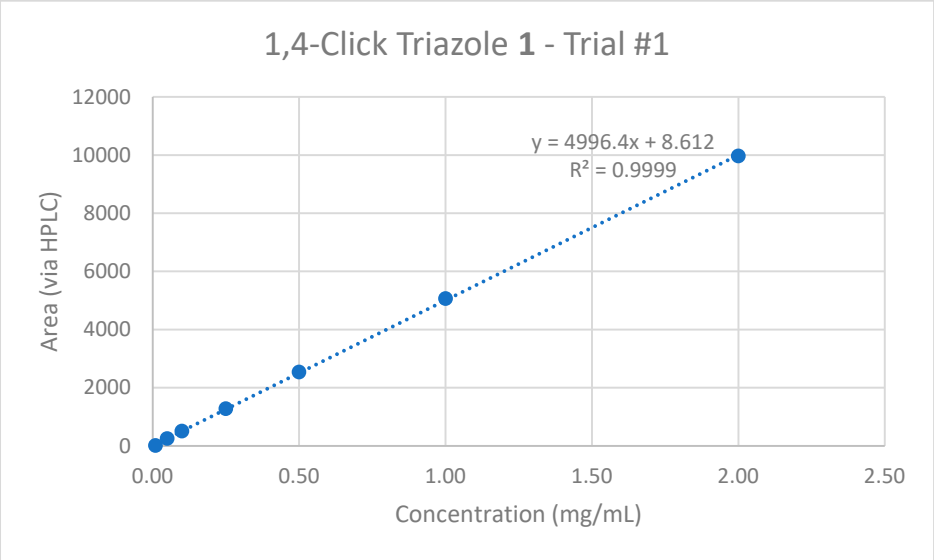


Figure S3B. Calibration graph (Trial 1) generated via HPLC analysis to determine drug water solubility of 1,4-click triazole **1**.

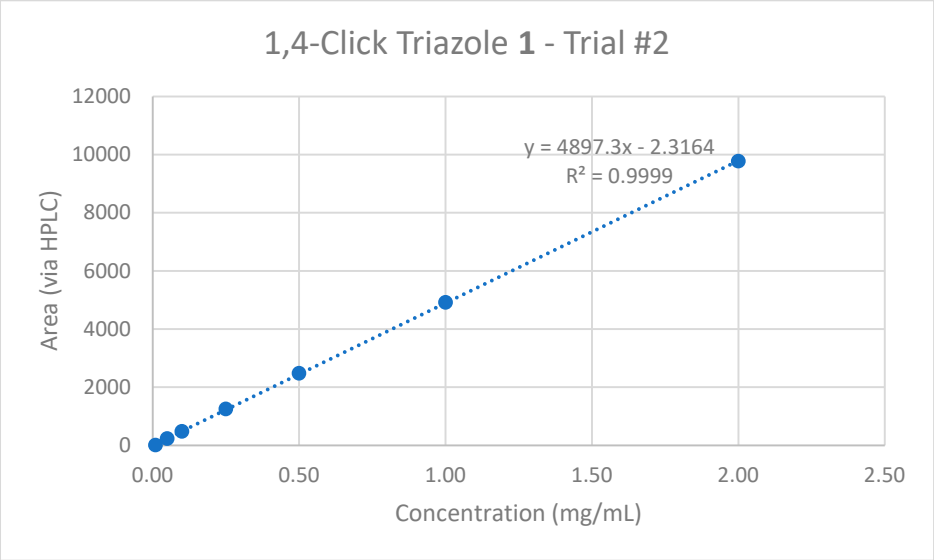


Figure S3C. Calibration graph (Trial 2) generated via HPLC analysis to determine drug water solubility of 1,4-click triazole **1**.

Water Solubility Determination of 1,5-click triazole **2**

Solvent	1,5-Click Triazole **2**			
	Sample	Concentration (mg/mL)	Trial #1	Trial #2
			Peak Area	Peak Area
Acetonitrile	**B-0**	2.00	11789.5	11884.4
	B-1	1.00	5930.6	6000.2
	B-2	0.50	2975.9	2993.6
	B-3	0.25	1473.9	1521.9
	B-4	0.10	583.7	625.6
	B-5	0.05	91.7	334.8
	B-6	0.01	10.6	22.4
Nanopure H_2O	**B-Aq**	Unknown	29.5	20.5

Figure S4A. Raw data generated via HPLC analysis to determine drug water solubility of 1,5-click triazole **2**.

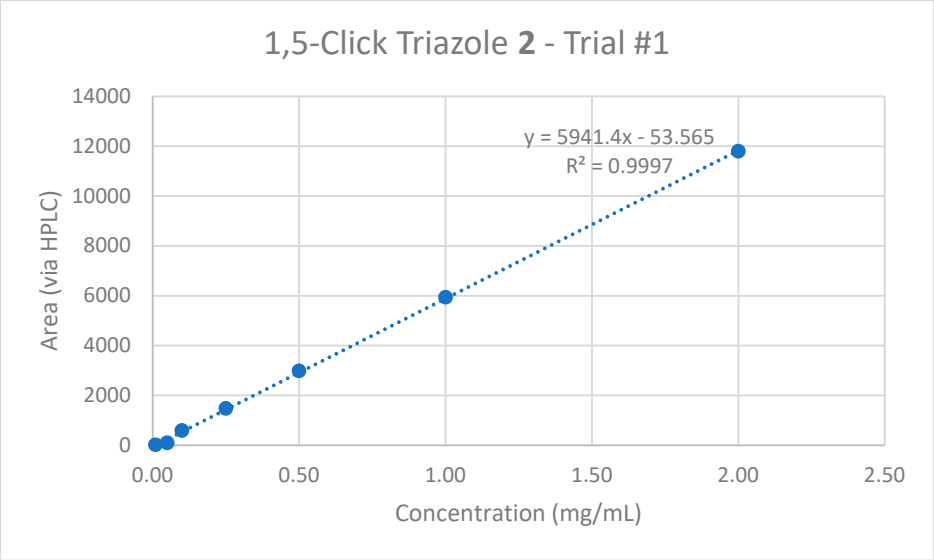


Figure S4B. Calibration graph (Trial 1) generated via HPLC analysis to determine drug water solubility of 1,5-click triazole **2**.

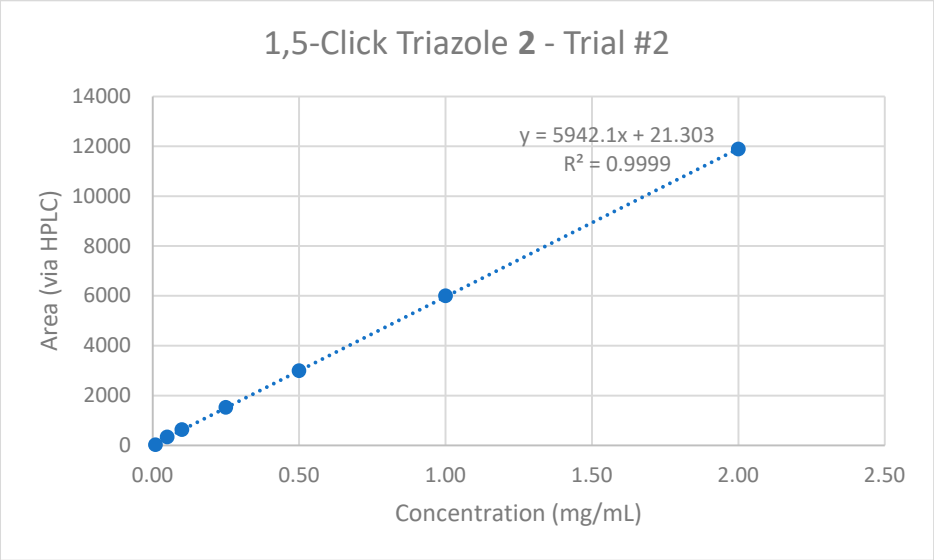


Figure S4C. Calibration graph (Trial 2) generated via HPLC analysis to determine drug water solubility of 1,5-click triazole **2**.

Water Solubility Determination of CGS-27023A analog **3**.

Solvent	CGS-27023A Enriched ^{10}B Analog **3**			
	Sample	Concentration (mg/mL)	Trial #1	Trial #2
			Peak Area	Peak Area
Acetonitrile	**C-0**	2.00	5863.2	5942.2
	C-1	1.00	2992.0	3044.7
	C-2	0.50	1526.5	1601.9
	C-3	0.25	762.2	845.1
	C-4	0.10	308.6	351.7
	C-5	0.05	154.1	174.0
	C-6	0.01	7.0	23.3
Nanopure H_2O	**C-Aq**	Unknown	17.8	91.8

Figure S5A. Raw data generated via HPLC analysis to determine drug water solubility of CGS-27023A analog **3**.

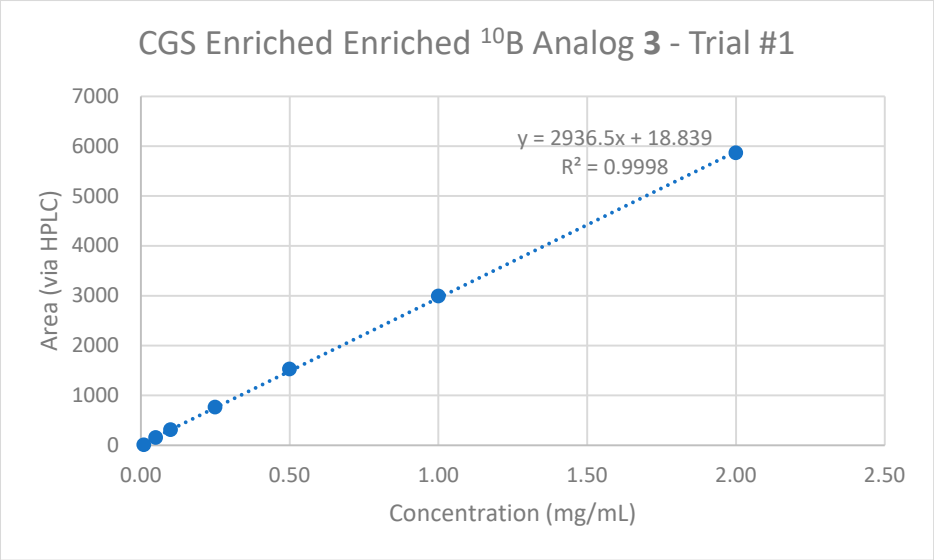


Figure S5B. Calibration graph (Trial 1) generated via HPLC analysis to determine drug water solubility of CGS-27023A analog **3**.

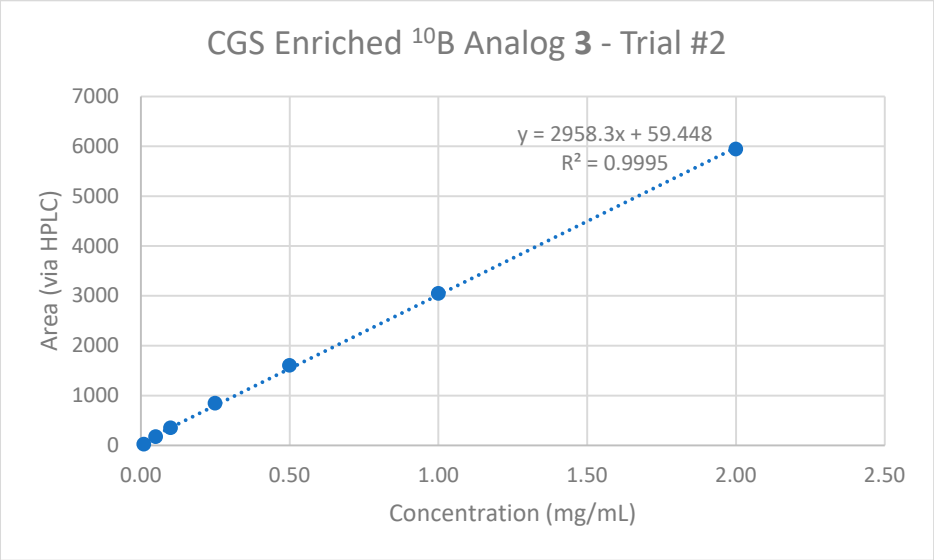


Figure S5C. Calibration graph (Trial 2) generated via HPLC analysis to determine drug water solubility of CGS-27023A analog **3**.

Water Solubility Determination of 1,4-Click triazole **4**.

Solvent	CGS-27023A 1,4-Click Triazole **4**			
	Sample	Concentration (mg/mL)	Trial #1	Trial #2
			Peak Area	Peak Area
Methanol	**D-0**	2.00	6666.0	6821.1
	D-1	1.00	3315.6	3381.9
	D-2	0.50	1617.4	1635.8
	D-3	0.25	799.8	810.5
	D-4	0.10	307.3	310.0
	D-5	0.05	150.2	156.2
	D-6	0.01	5.9	7.5
Nanopure H_2O	**D-Aq**	Unknown	321.1	257.9

Figure S6A. Raw data generated via HPLC analysis to determine drug water solubility of CGS-27023A 1,4-Click triazole **4**.

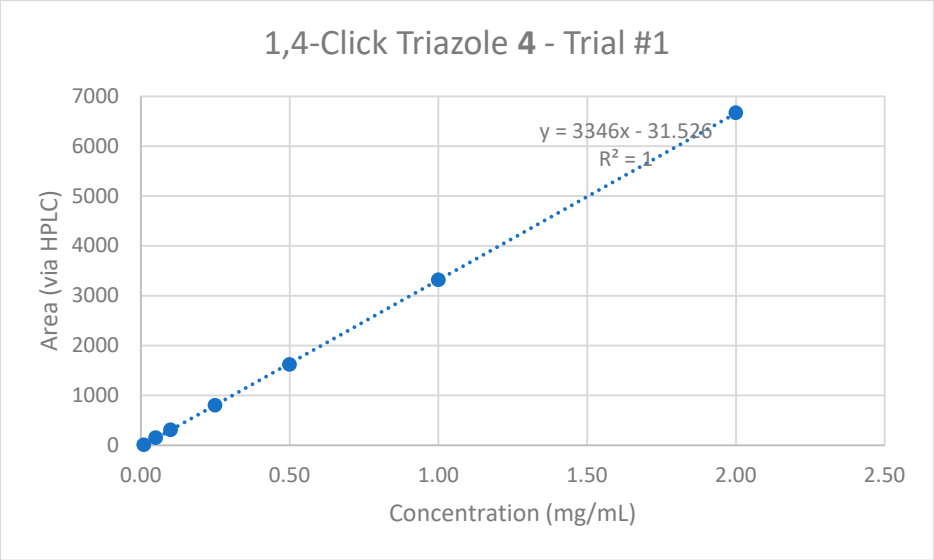


Figure S6B. Calibration graph (Trial 1) generated via HPLC analysis to determine drug water solubility of CGS-27023A 1,4-Click triazole **4**.

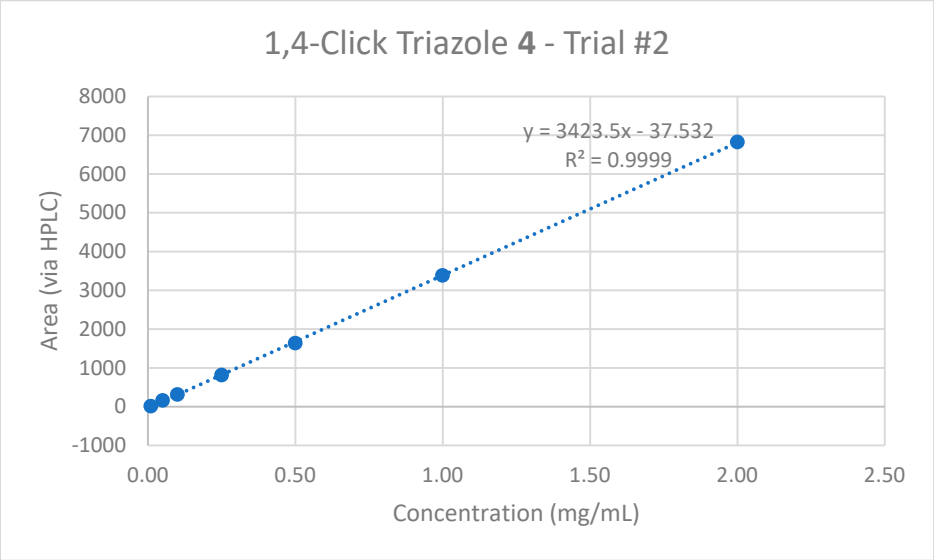
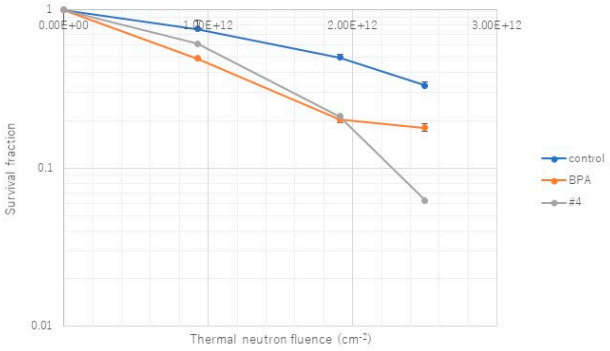


Figure S6C. Calibration graph (Trial 2) generated via HPLC analysis to determine drug water solubility of CGS-27023A 1,4-Click triazole **4**.

U87 MG *delta EGFR* (colony efficiency 4 %)

U87	X (Thermal Fluence)	Y (Survival fraction)	std in SF
control	0.00E+00	1	0.090138782
	9.24E+11	0.758333333	0.038188131
	1.91E+12	0.5	0.066143783
	2.50E+12	0.333333333	0.057735027
BPA(^{10}B 20ppm)	0.00E+00	1	0.070168517
	9.24E+11	0.494382022	0.07784498
	1.91E+12	0.202247191	0.033707865
	2.50E+12	0.179775281	0.03892249
#4 (^{10}B 0.48ppm)	0.00E+00	1	0.021650635
	9.24E+11	0.6125	0.229128785
	1.91E+12	0.2125	0.057282196
	2.50E+12	0.0625	0.021650635

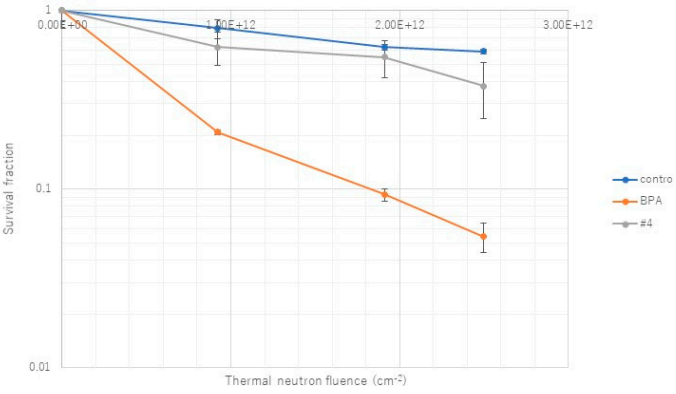


Survival fraction in U87 MG cells. BPA: 20 ppm ^{10}B; compound **4**: 0.48 ppm ^{10}B treated with U87 *delta EGFR* cells for 4 hours

Figure S7a. Survival fraction in U87 MG cells. BPA: 20 ppm ^{10}B; compound **4**: 0.48 ppm ^{10}B treated with U87 delta EGFR cells for 4 hours with raw data and standard deviation values.

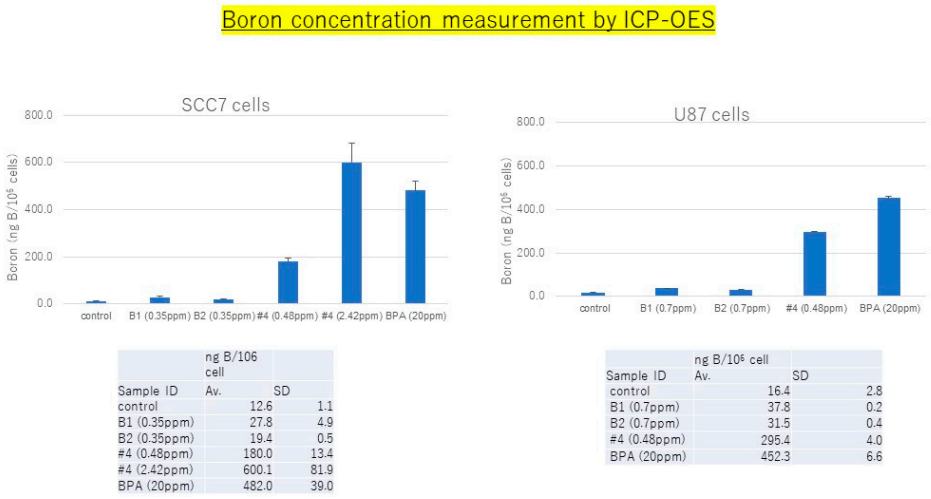
SCC 7 (colony efficiency 34 %)

SCC7	X (Thermal Fluence)	Y (Survival fraction)	std in SF
control	0.00E+00	1	0.120983443
	9.24E+11	0.79296875	0.097578094
	1.91E+12	0.62109375	0.025540423
	2.50E+12	0.58984375	0.018835255
BPA (^{10}B 20ppm)	0.00E+00	1	0.195263916
	9.24E+11	0.20824295	0.006507592
	1.91E+12	0.093275488	0.00751432
	2.50E+12	0.054229935	0.009940511
#4(^{10}B 0.48ppm)	0.00E+00	1	0.111704296
	9.24E+11	0.621880998	0.077468085
	1.91E+12	0.548944338	0.008795731
	2.50E+12	0.378119002	0.01198656



Survival fraction in SCC7 cells. BPA: 20 ppm ^{10}B; compound **4**: 0.48 ppm ^{10}B treated with SCC7 cells for 4 hours.

Figure S7b. Survival fraction in SCC7 cells. BPA: 20 ppm ^{10}B; compound **4**: 0.48 ppm ^{10}B treated with SCC7 cells for 4 hours with raw data and standard deviation values.



	ng B/106 cell	
Sample ID	Av.	SD
control	12.6	1.1
B1 (0.35ppm)	27.8	4.9
B2 (0.35ppm)	19.4	0.5
#4 (0.48ppm)	180.0	13.4
#4 (2.42ppm)	600.1	81.9
BPA (20ppm)	482.0	39.0

	ng B/10⁶ cell	
Sample ID	Av.	SD
control	16.4	2.8
B1 (0.7ppm)	37.8	0.2
B2 (0.7ppm)	31.5	0.4
#4 (0.48ppm)	295.4	4.0
BPA (20ppm)	452.3	6.6

Figure S8. Boron concentrations in SCCVII cells and in U87 cells determined by ICP-OES with standard deviation values.

Calculation of ^{10}B concentration in Medium

1. B1 and B2 : 6 mg/ml DMSO stock solution. If the medium final concentration is 2 micro L/ml, the medium include 0.012 mg/ml of B1 or B2. In 1 mol of B1 or B2 (MW 450.32), ^{10}B weighs 26.4 (22 % of natural boron) g in 450.32 g. Therefore, the medium includes 0.703 ppm (=0.012*26.4/450.32 mg/ml) of ^{10}B.

2. Compound #**4**: 12mg/ml DMSO stock solution. If the medium final concentration is 1 micro L/ml, the medium include 0.012mg/ml of #4. In 1 mol of #4 (MW 652.19), ^{10}B weighs 26.4 (22 % of natural boron) g in 652.19 g. Therefore, the medium includes 0.485ppm (=0.012*26.4/652.19 mg/ml)) of ^{10}B.