Synthesis, Structural Insights and Bioevaluation of Phenoxy Pendant Isatin Hydrazones as Potent α-Glucosidase Inhibitors

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(M. M. N.)

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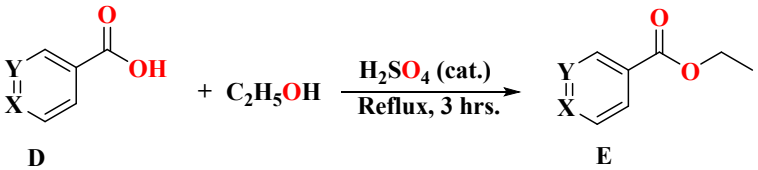
1) Procedure for the synthesis of substituted ethyl benzoates E

For the synthesis of ethyl benzoates **E**, the esterification of respective benzoic acids **D** (1 mM) was done by using ethanol (10 mL) with a catalytic amount of conc. H_2SO_4 (2 drops). The reaction mixture was refluxed for 3 hours to afford ethyl esters **E** of respective benzoic acids **D**[1]. The reaction mixture was cooled, evaporated at reduced pressure, and then extracted with ethyl acetate from the aqueous phase (2 × 30 mL). It was washed with sodium bicarbonate and then dried over anhydrous $MgSO_4$ and filtered. To afford respective ethyl benzoates **E**[2-4] ethyl acetate was evaporated at reduced pressure and used in the next step without any further purification. **Scheme S1**.



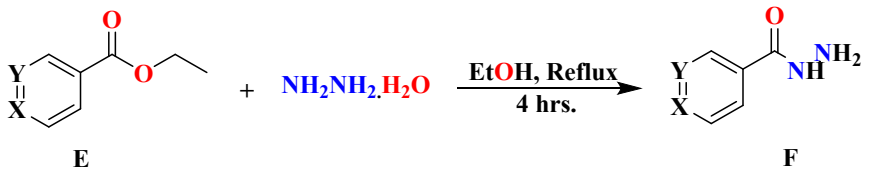
D **E**

E1 X = CH, Y = N
E2 X = N, Y = CH
E3 X = CBr, Y = CH

Scheme S1: Preparation of substituted ethyl benzoates **E**

2) Procedure for the synthesis of substituted benzohydrazides F

To a stirred solution of ethyl benzoates **E** (1mM) in ethanol (10 mL) was added hydrazine hydrate (3 mM). The reaction mixture was refluxed with continuous stirring for 4 hours. TLC was used to monitor the progress of the reaction. Upon completion, the reaction mixture was cooled, benzo hydrazides **F** were crystallized out, filtered the crystals, and washed with cold ethanol. The purity of the benzo hydrazides **F** was checked with TLC and confirmed with melting point compared with the literature[5-7]. **Scheme S2**.



E **F**

F1 X = CH, Y = N
F2 X = N, Y = CH
F3 X = CBr, Y = CH

Scheme S2: Preparation of substituted benzohydrazides **F**

3) **Table S1:** Pharmacokinetic Studies of Novel Hydrazide Conjugates **1 (a-l)**

ADMET Properties		1a	1b	1c	1d	1g	1h	1i	1j	1k	1l
Absorption	WS (log mol/L)	-4.234	-4.247	-5.839	-4.787	-4.875	-4.871	-6.35	-4.652	-4.593	-6.196
	IS (%abs)	94.73	94.738	90.949	93.815	93.748	93.748	89.96	94.911	94.911	91.123
	SP (log K_p)	-2.758	-2.74	-2.691	-2.786	-2.786	-2.77	-2.695	-2.807	-2.789	-2.744
Distribution	BBBP (Log BB)	-0.695	-0.695	-0.545	-0.882	-0.891	-0.891	-0.74	-0.915	-0.915	-0.765
	CNSP (Log PS)	-2.484	-2.496	-2.055	-2.375	-2.352	-2.364	-1.923	-2.547	-2.559	-2.118
	VDss (log L/kg)	-0.519	-0.464	-0.282	-0.476	-0.457	-0.405	-0.221	-0.553	-0.494	-0.387
Metabolism	CYP3A4 inhibitor	No	No	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
	CYP1A2 inhibitor	No	No	No	No	No	No	No	No	No	No
	CYP2C19 Inhibitor	No	No	No	No	No	No	No	No	No	No
	CYP2C9 inhibitor	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
Excretion	TC (log mL/min/kg)	0.358	0.414	-0.106	-0.028	-0.049	-0.124	-0.258	0.093	0.149	-0.251
Toxicity	AMES toxicity	No	No	No	No	No	No	No	No	No	No
	Max. TD. (log mg/kg/day)	0.076	0.103	0.27	0.03	0.031	0.059	0.238	0.174	0.203	0.331
	ORAT (LD_{50}) (mol/kg)	2.174	2.167	2.185	2.294	2.306	2.298	2.282	2.26	2.268	2.196
	HT	No	No	No	No	No	No	No	No	No	No
	SS	No	No	No	No	No	No	No	No	No	No
	T.Pyriformis Toxicity (log ug/L)	0.567	0.55	0.52	0.557	0.554	0.537	0.513	0.466	0.446	0.436

Abbreviations: **WS** = water solubility, **IS** = intestinal solubility, **SP** = skin permeability, **BBBP** = blood–brain barrier permeability, **CNSP** = central nervous system permeability, **TC** = total clearance, **ORAT** = oral rat acute toxicity, **HT** = hepatotoxicity, **SS** = skin sensitization

4) References:

[1]. Arjunan, V., Rani, T., Mythili, C., & Mohan, S. *Spectrochim. Acta A Mol. Biomol. Spectrosc.,* **2011**, *79*, 3, 486-496. 10.1016/j.saa.2011.03.018.

[2]. Yuan, C., Lu, Z., & Jin, Z. *Food Chem.,* **2014**, *152*, 140-145. 10.1016/j.foodchem.2013.11.139.

[3]. Lei, Y., Chen, G., Yang, Q., Fu, C., Pan, J., & Li, T. *J. Struc. Chem.,* **2013**, *54*, 829-833. 10.1134/S0022476613040288.

[4]. Yu, J. *J. Struct. Chem.,* **2013**, *54*, 581-585. 10.1134/S0022476613030177.

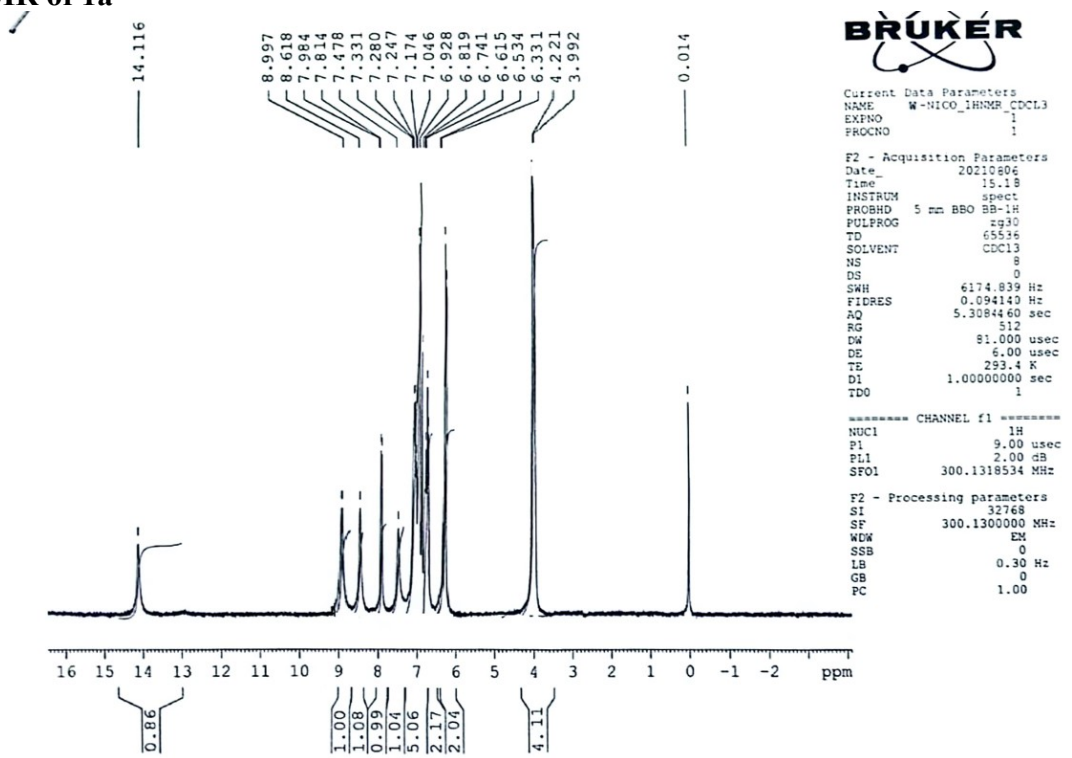
[5]. Kerzare, D., Chikhale, R., Bansode, R., Amnerkar, N., Karodia, N., Paradkar, A., & Khedekar, P. *J. Braz. Chem. Soc.,* **2016**, *27*, 1998-2010. 10.5935/0103-5053.20160090.

[6]. Umberger, E. *Anal. Chem.,* **1955**, *27*, 5, 768-773. 10.1021/ac60101a021.

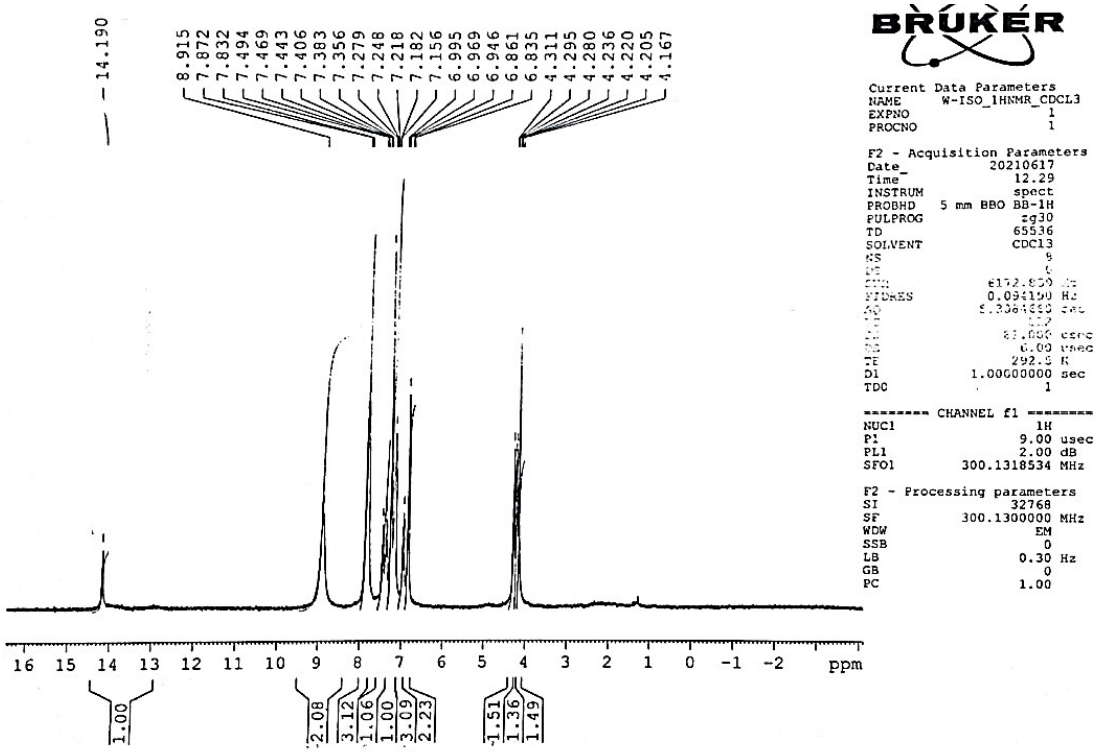
[7]. Kuznetsov, V., Skibina, L., & Khalikov, R. *Prot. Met. Phys. Chem. Surf.,* **2009**, *45*, 283-288. 10.1134/S2070205109030034.

5) Copies of ^{1}H-NMR,^{13}C-NMR spectra of compounds 1(a-l)

^{1}H-NMR of 1a



^{1}H-NMR of 1b



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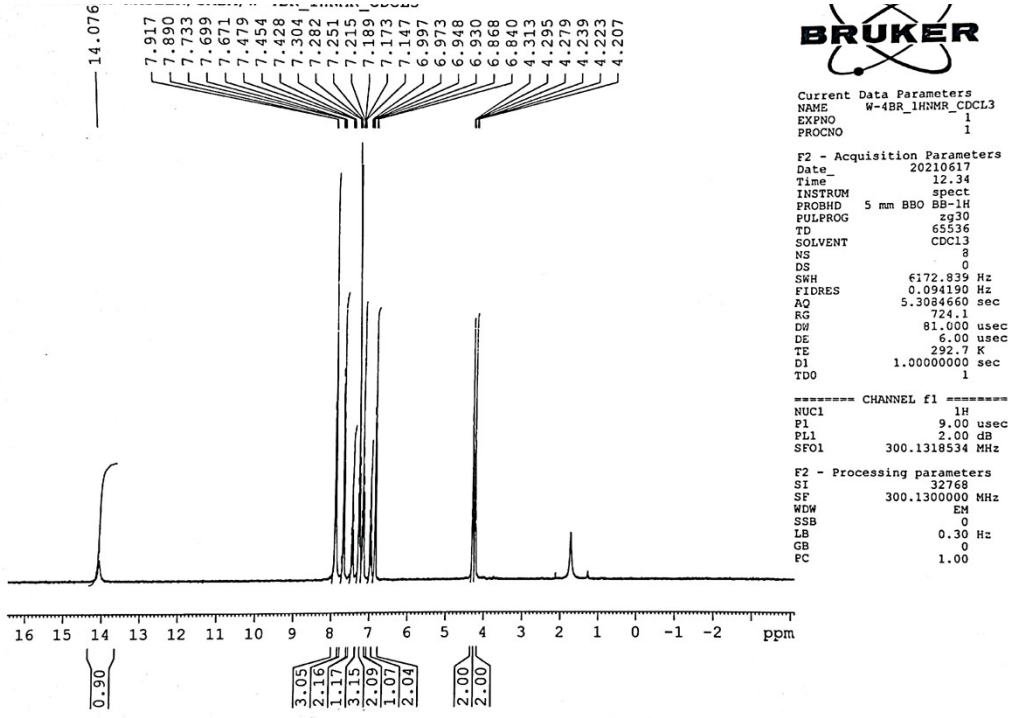

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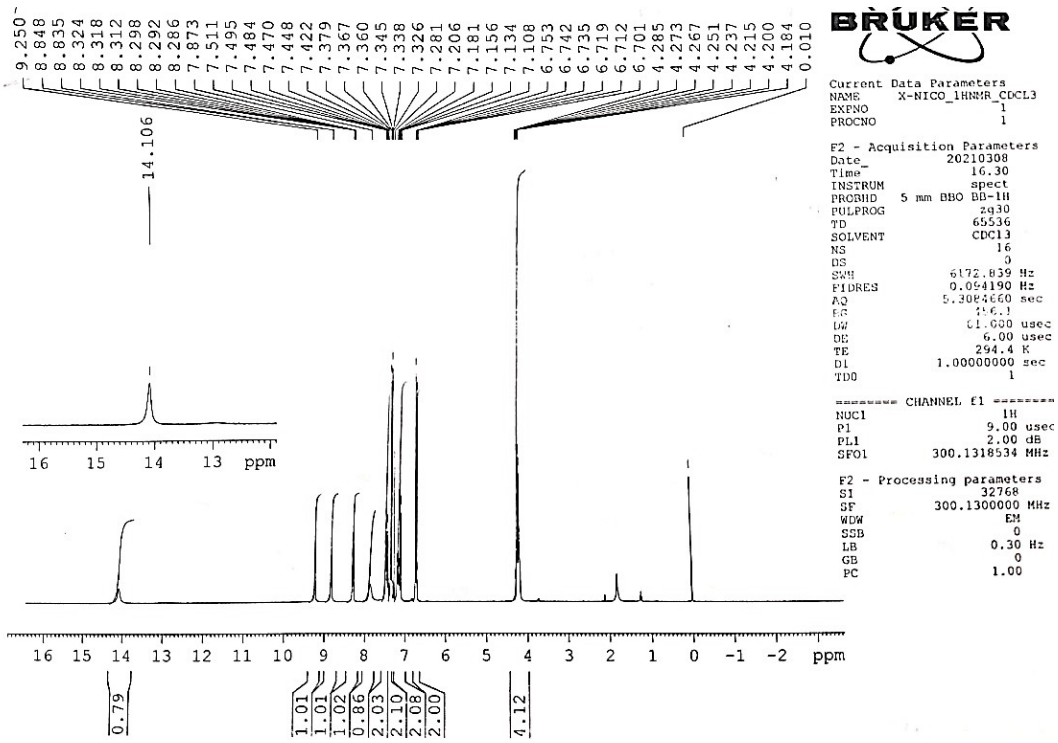
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8.318
8.312
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7.511
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7.470
7.448
7.422
7.379
7.367
7.360
7.345
7.338
7.326
7.281
7.206
7.181
7.156
7.134
7.108
6.753
6.742
6.735
6.719
6.712
6.701
4.285
4.273
4.267
4.251
4.237
4.215
4.200
4.184
0.010
14.106
16 15 14 13 ppm
16 15 14 13 12 11 10 9 8 7 6 5 4 3 2 1 0 -1 -2 ppm
0.79
1.01
1.01
1.02
0.86
2.03
2.10
2.08
2.00
4.12
BRUKER
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DE 6.00 usec
TE 294.4 K
D1 1.00000000 sec
TD0 1
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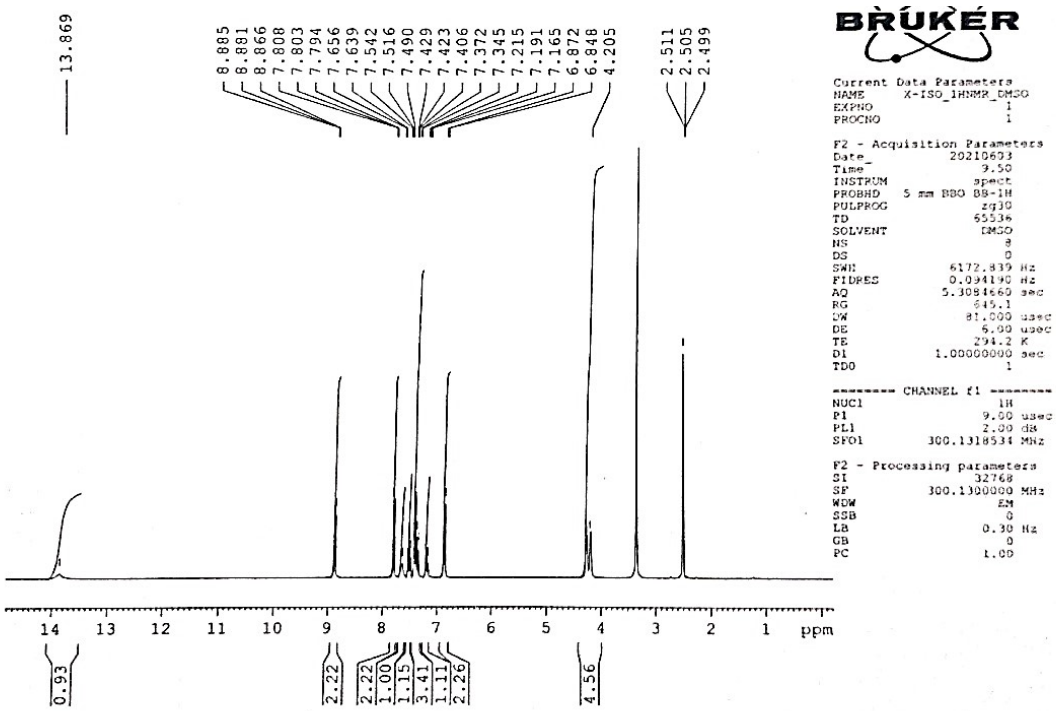
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7.429
7.423
7.406
7.372
7.345
7.215
7.191
7.165
6.872
6.848
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2.511
2.505
2.499
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RG 645.1
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DE 6.00 usec
TE 294.2 K
D1 1.00000000 sec
TD0 1
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P1 9.00 usec
PL1 2.00 dB
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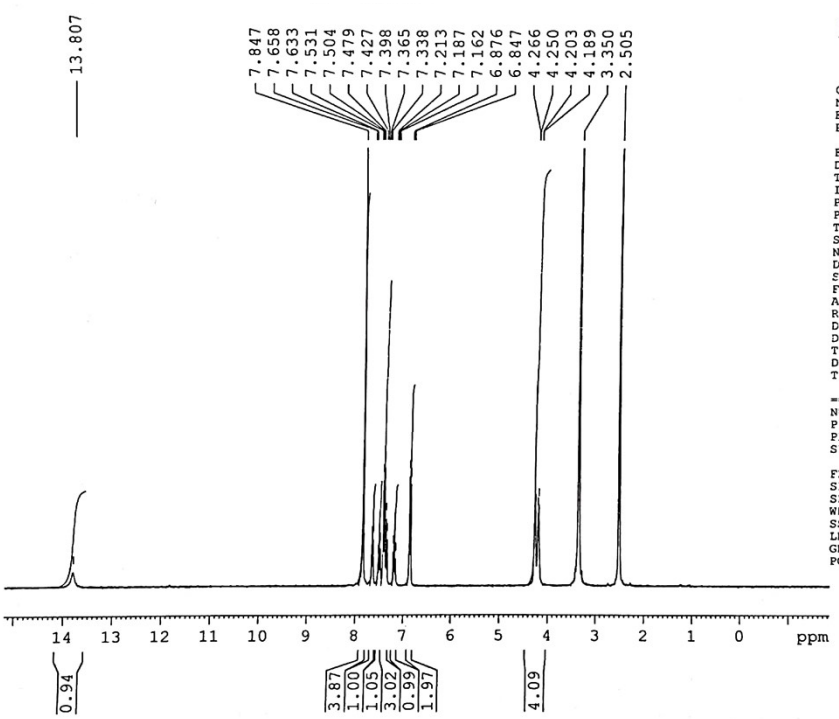
1.00
1.15
3.41
1.11
2.26
4.56

^{1}H-NMR of 1f



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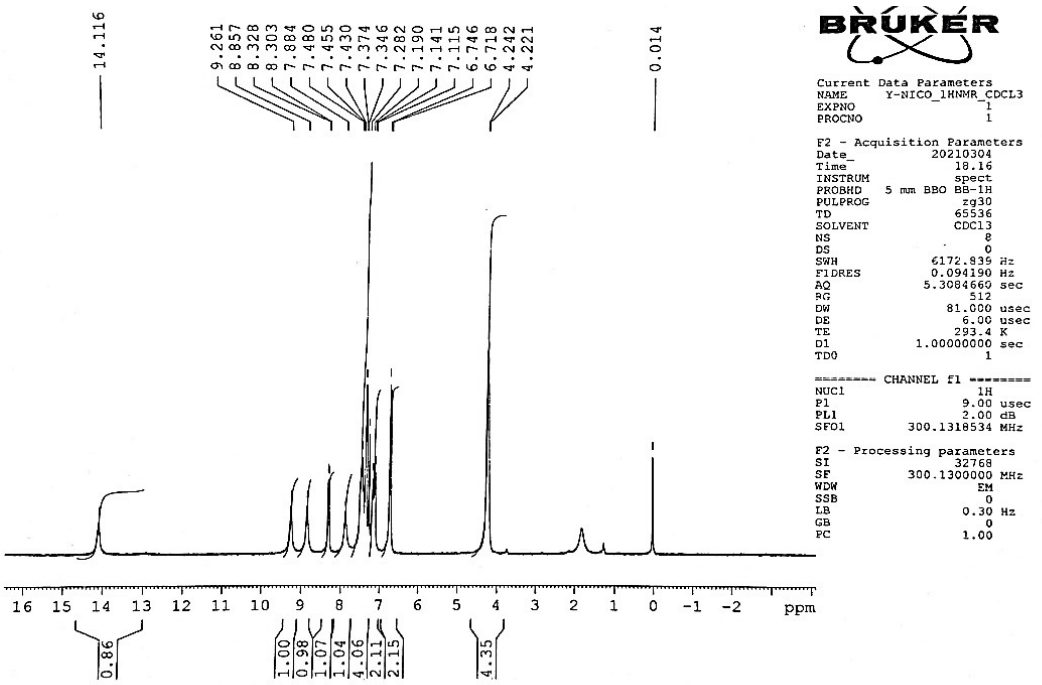

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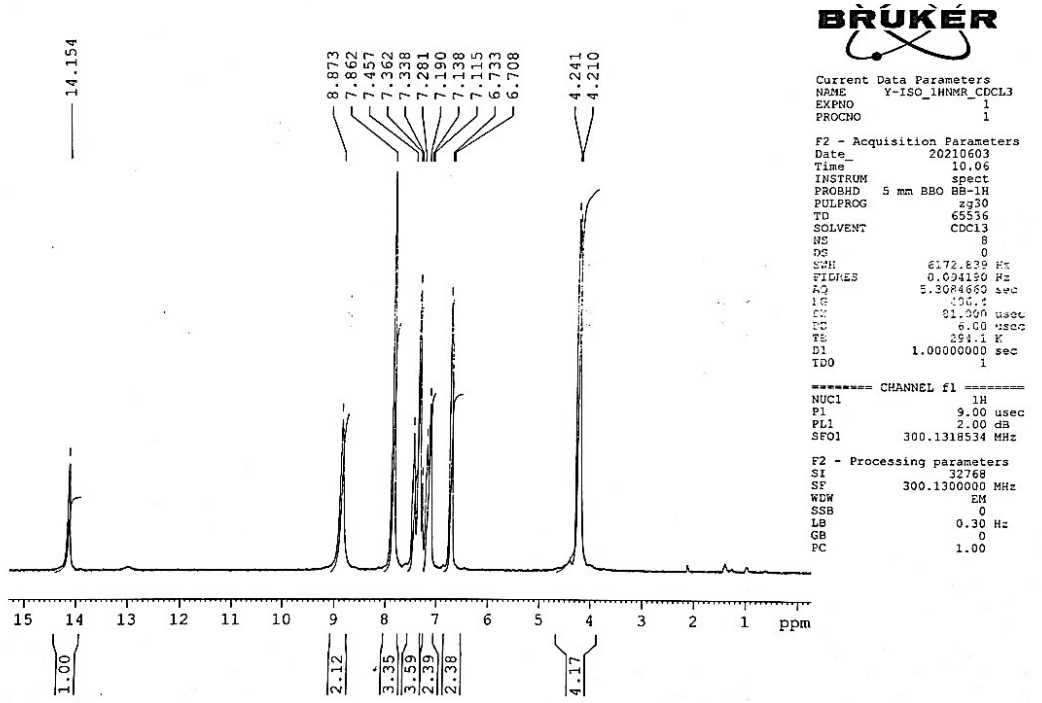
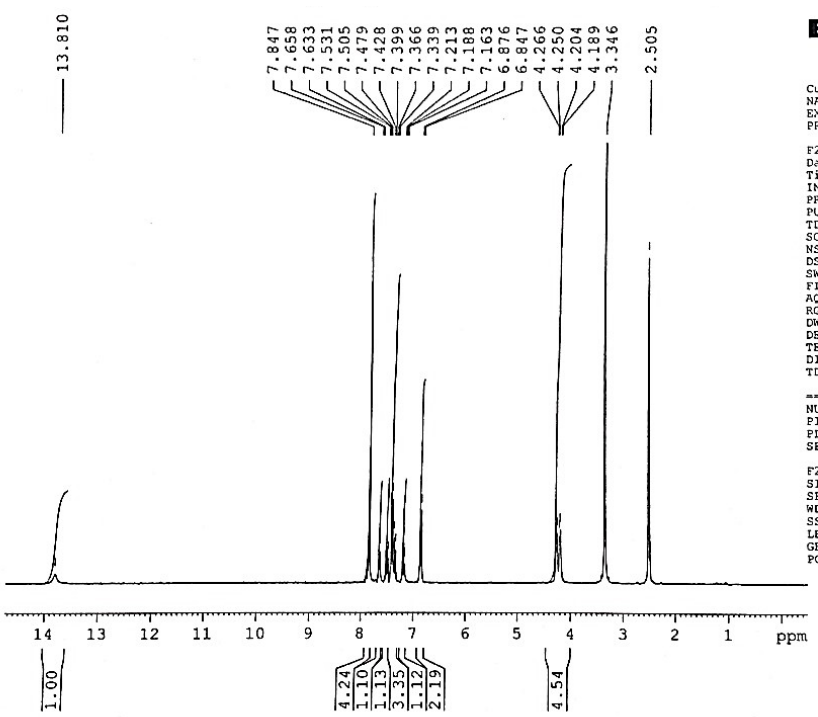


^{1}H-NMR of 1i



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DE                   6.00 usec
TE                  294.1 K
D1                1.00000000 sec
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PL1                  2.00 dB
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F2 - Processing parameters
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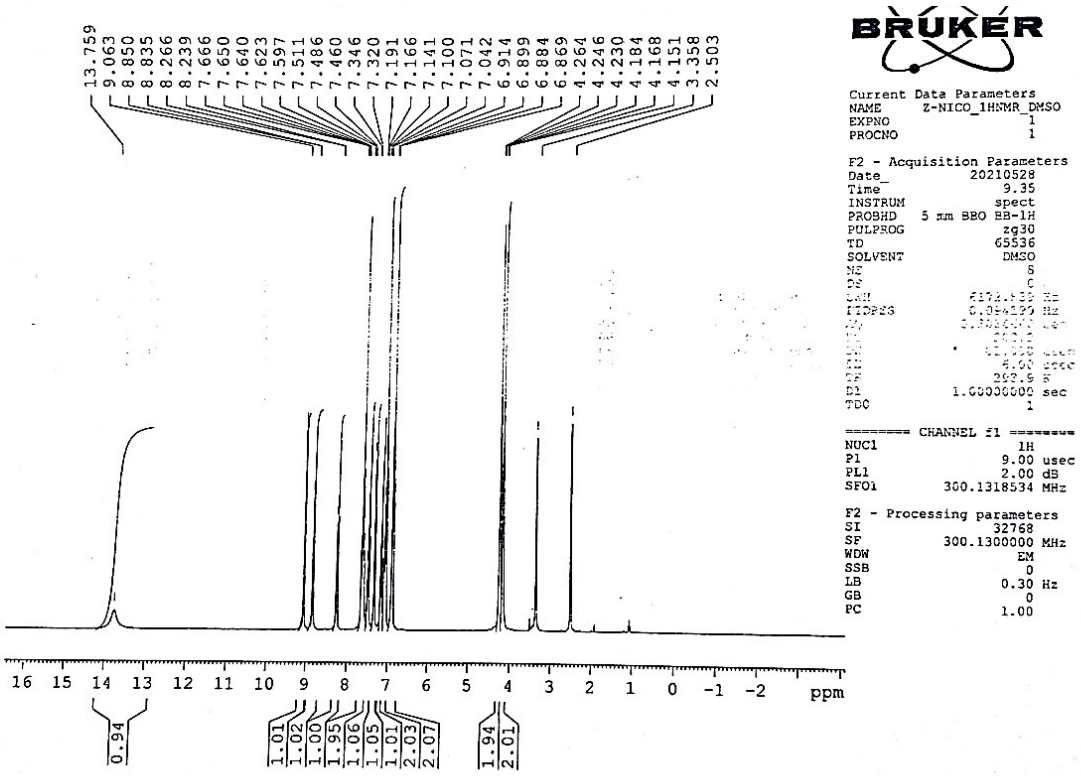
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7.460
7.346
7.320
7.191
7.166
7.141
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7.071
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6.899
6.884
6.869
4.264
4.246
4.230
4.184
4.168
4.151
3.358
2.503
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0.94
1.01
1.02
1.00
1.95
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1.05
1.01
2.03
2.07
1.94
2.01
BRUKER
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F2 - Processing parameters
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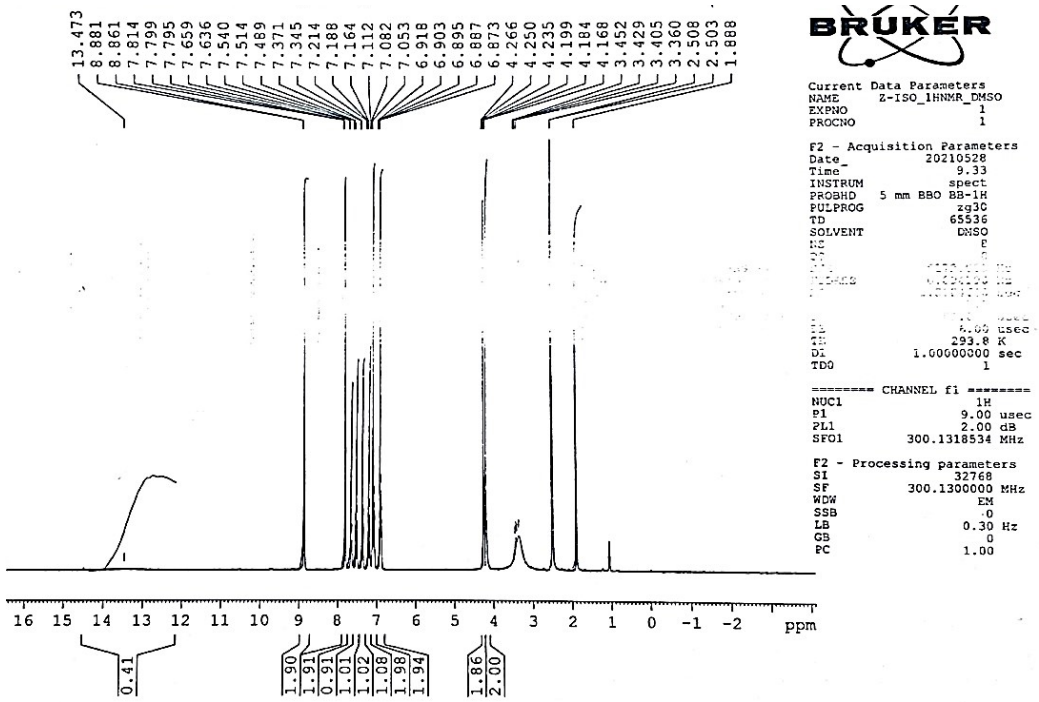
^{1}H-NMR of 1k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7.214
7.188
7.164
7.112
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6.895
6.887
6.873
4.266
4.250
4.235
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3.452
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2.508
2.503
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BRUKER
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PROCNO 1
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TD0 1
======== CHANNEL f1 ========
NUC1 1H
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PL1 2.00 dB
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F2 - Processing parameters
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SF 300.1300000 MHz
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0.41
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1.01
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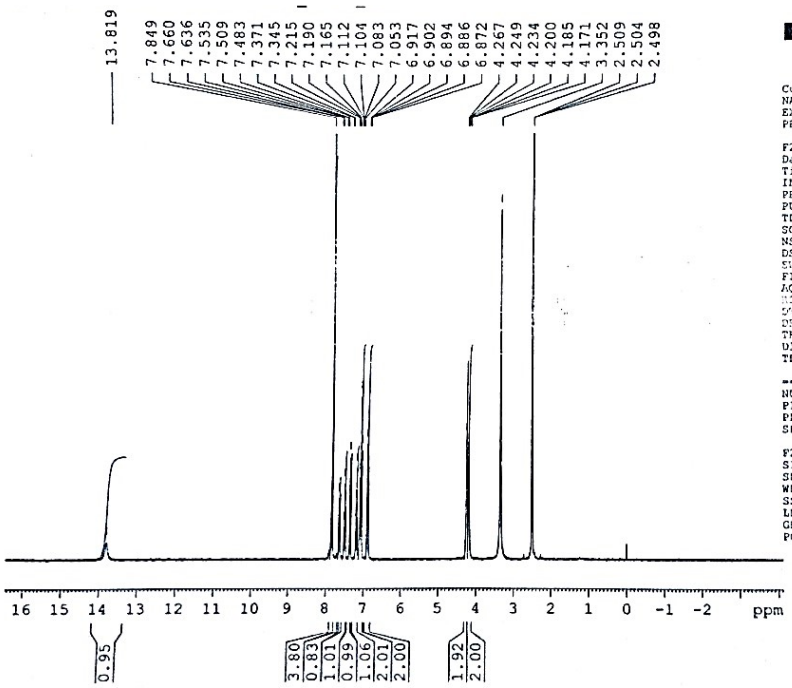
1.94
1.86
2.00

^{1}H-NMR of 1l



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PROCNO                   1

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AQ                5.3084860 sec
RG                    412.7
DW                   81.000 usec
DE                    6.00 usec
TE                   293.9 K
D1                1.00000000 sec
TD0                       1

======== CHANNEL f1 ========
NUC1                      1H
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PL1                   2.00 dB
SFO1          300.1318534 MHz

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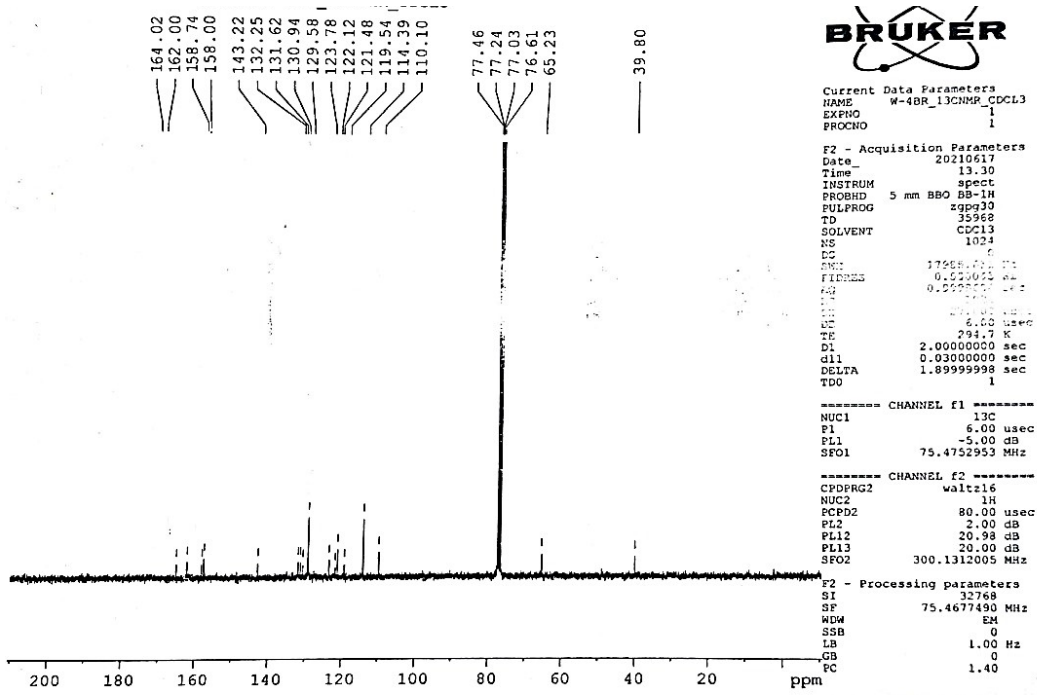

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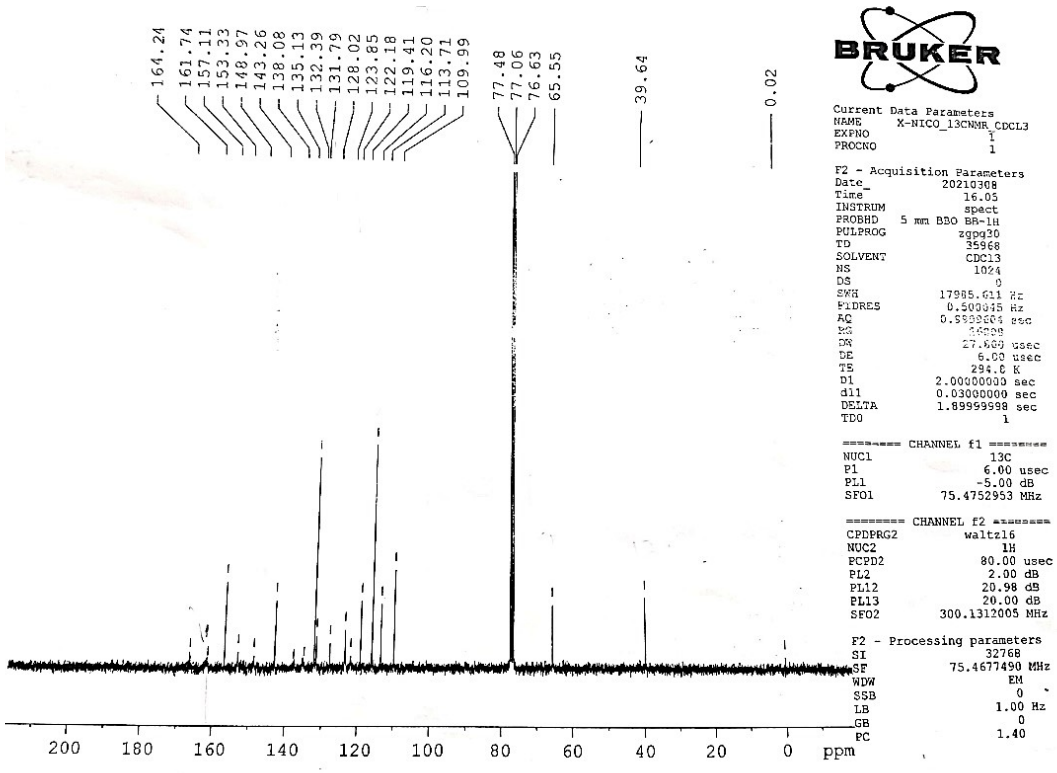

6) Copies of HRMS spectra

High-resolution electrospray ionization mass spectra recorded *via* Agilent 6530 LC Q-TOF.

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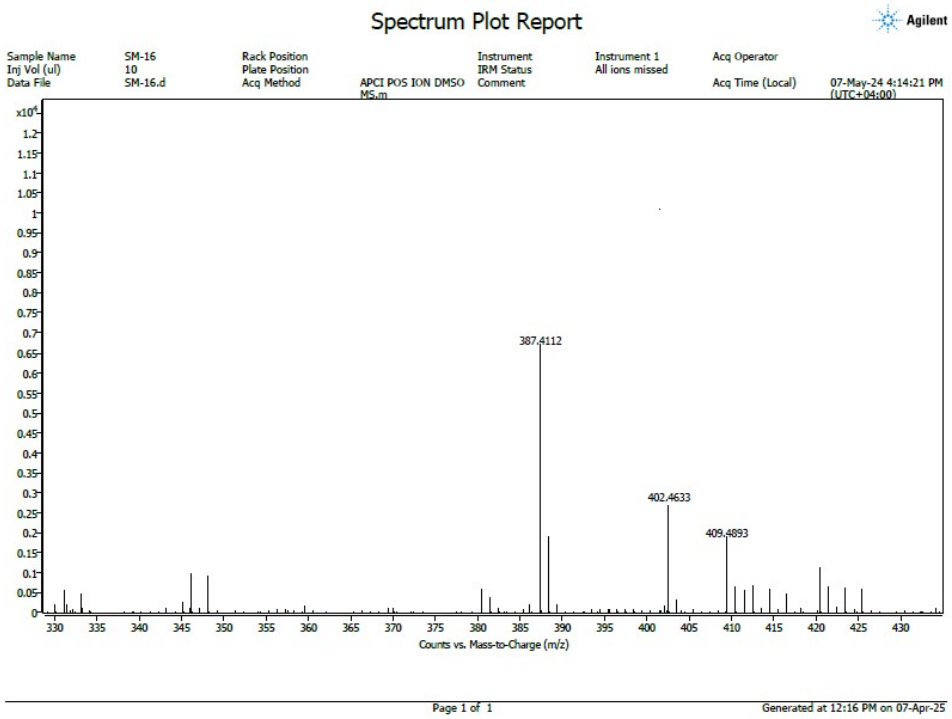
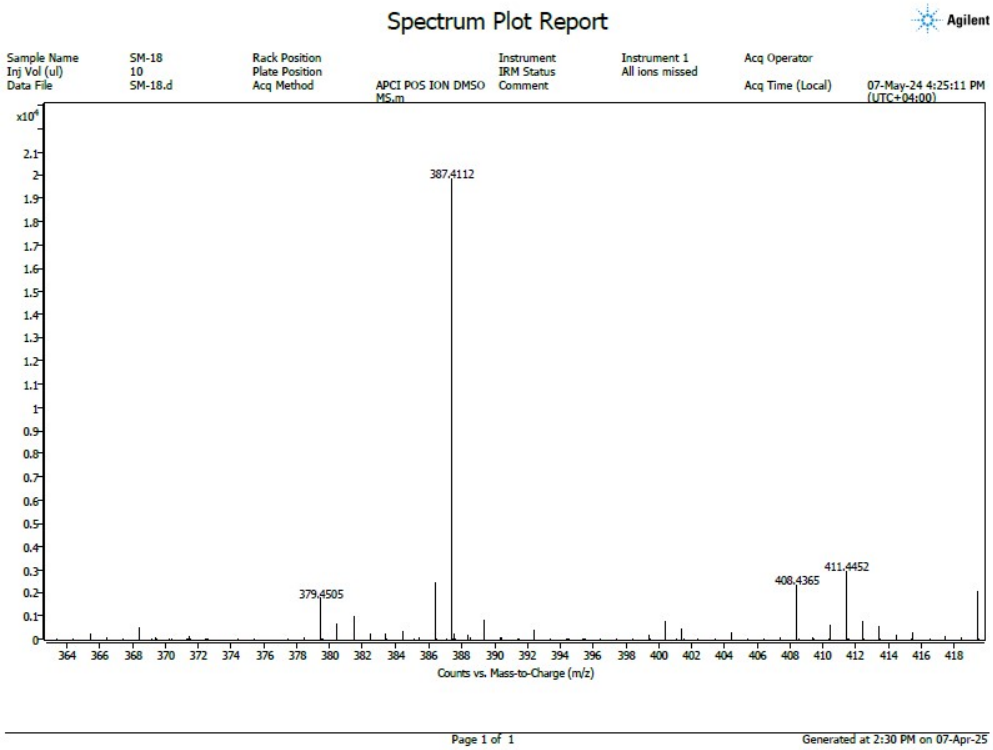


HRMS of 1b



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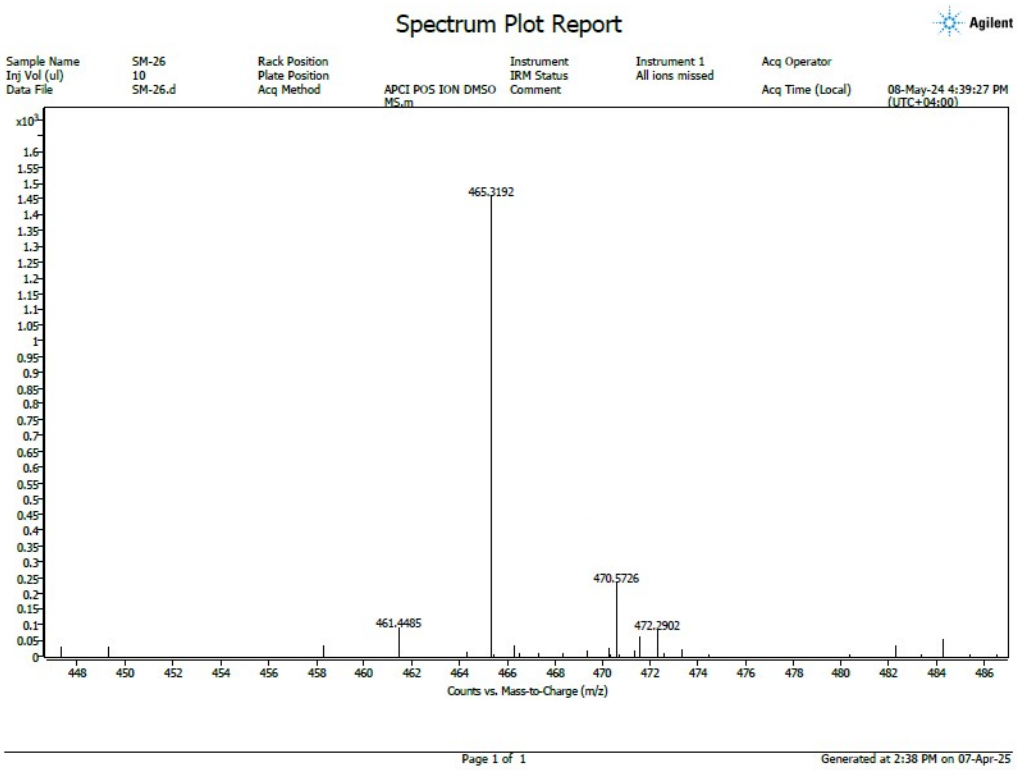

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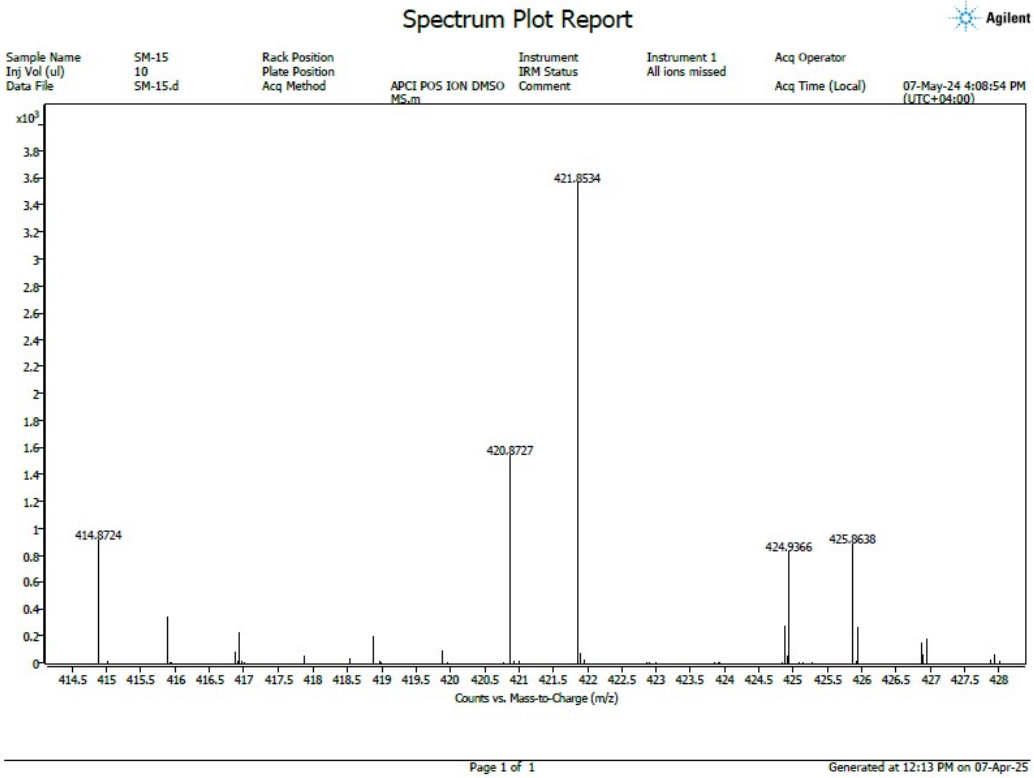

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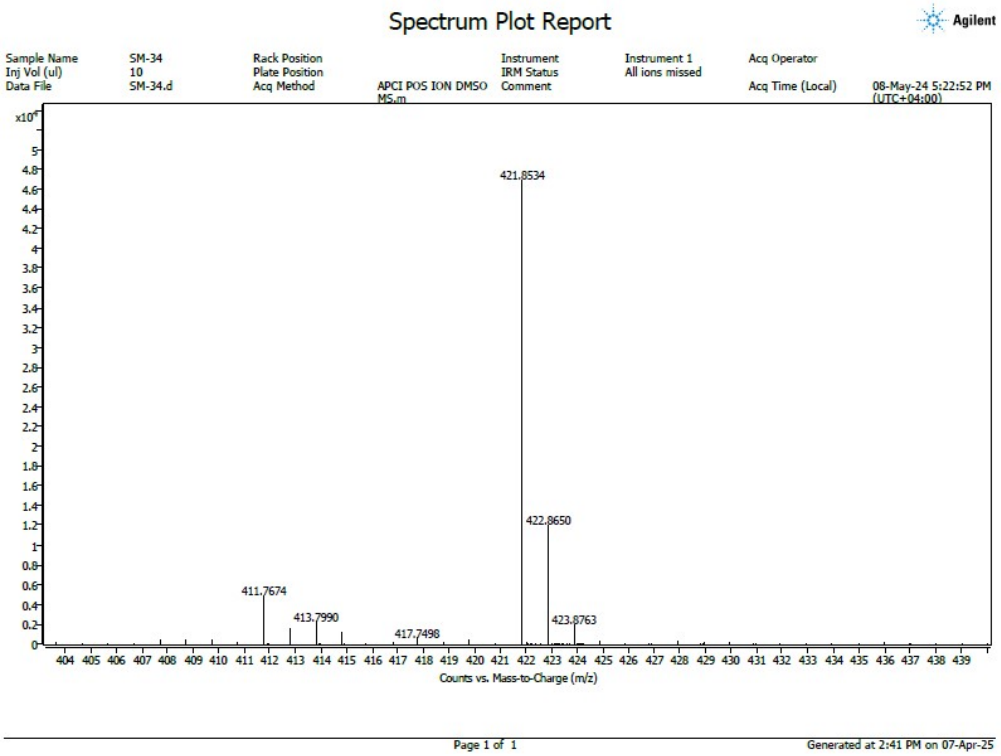
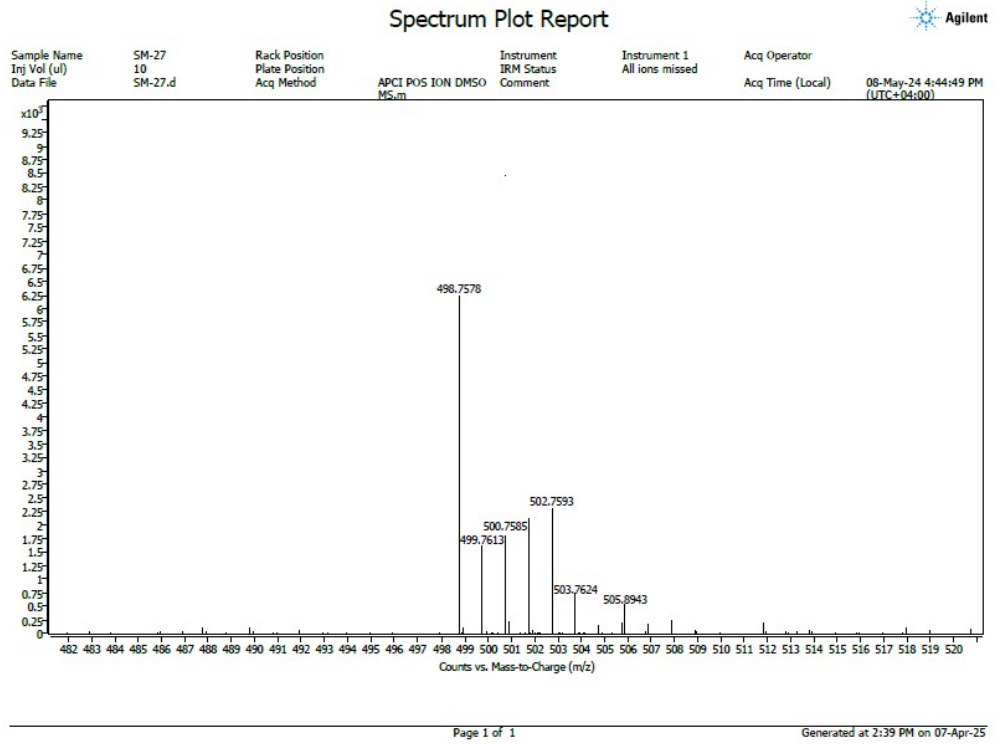
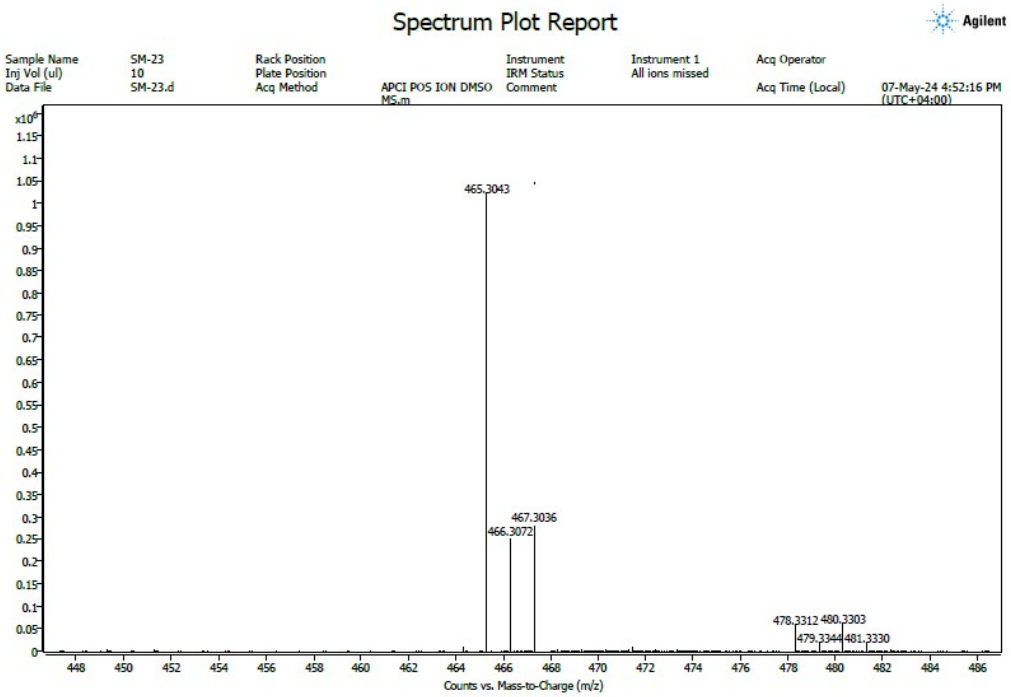


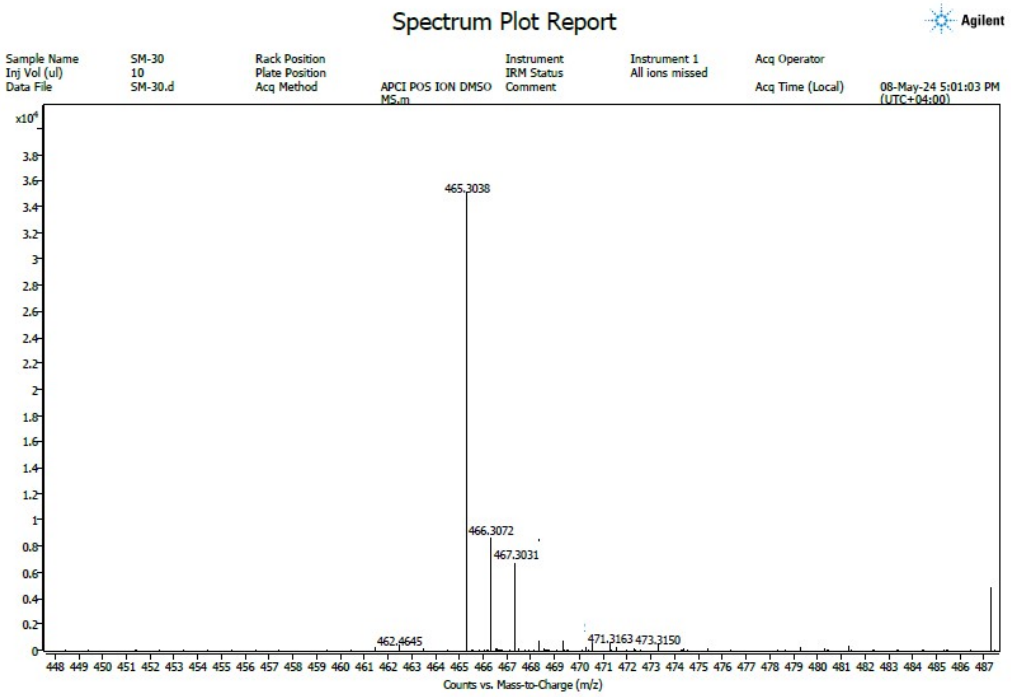
HRMS of 1f



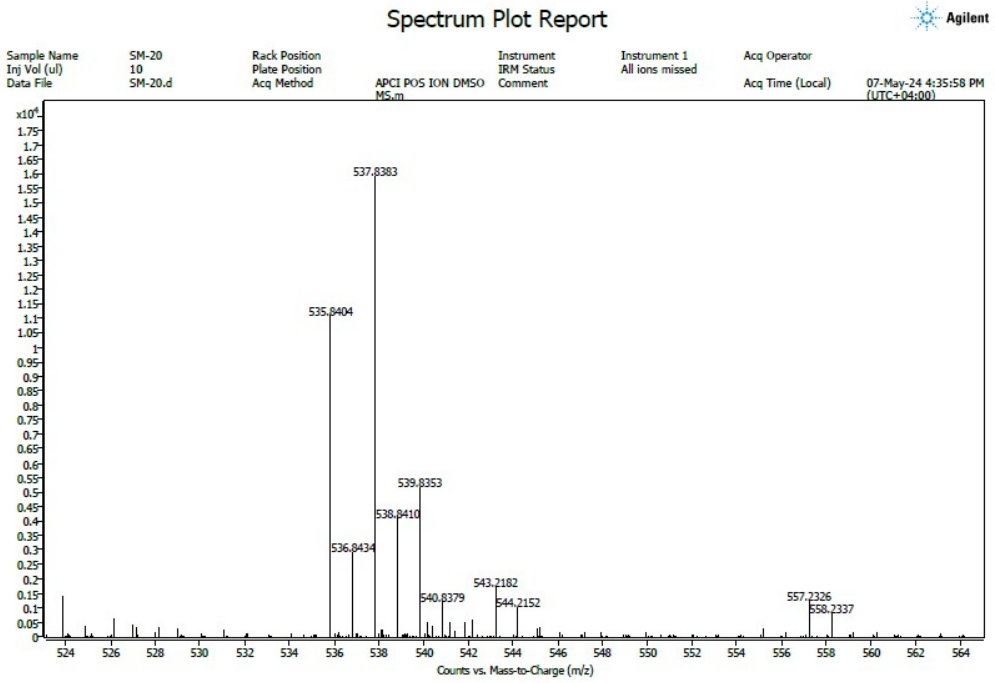
HRMS of 1g



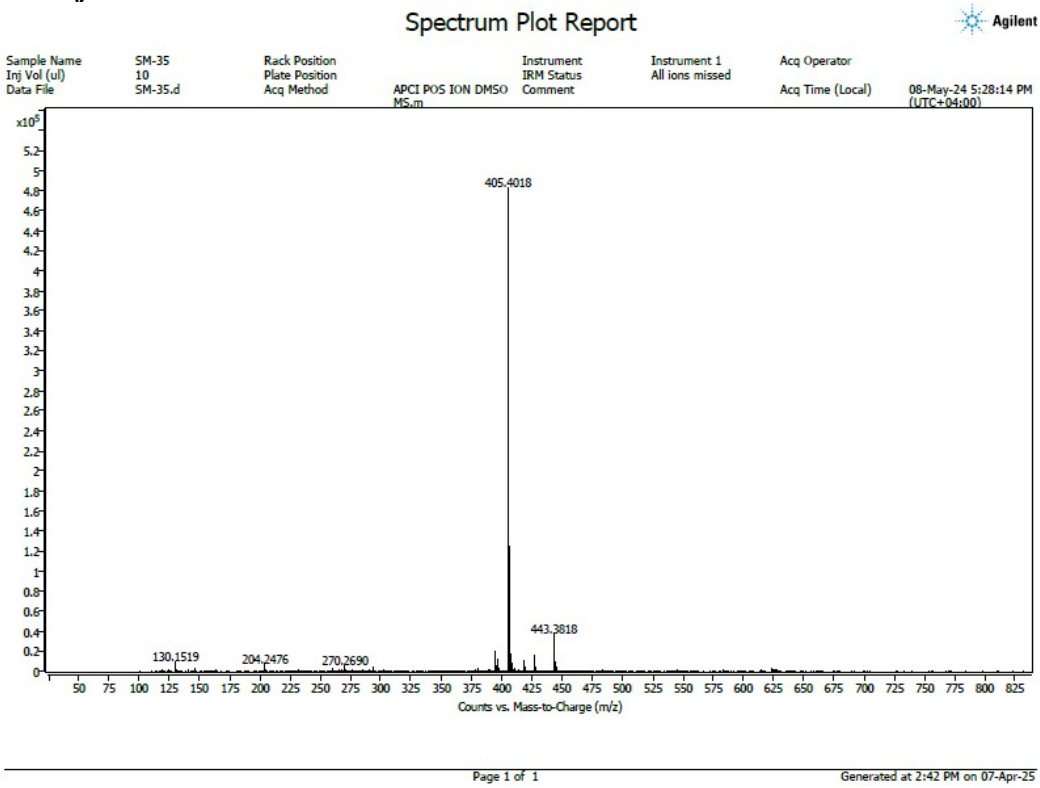
HRMS of 1h



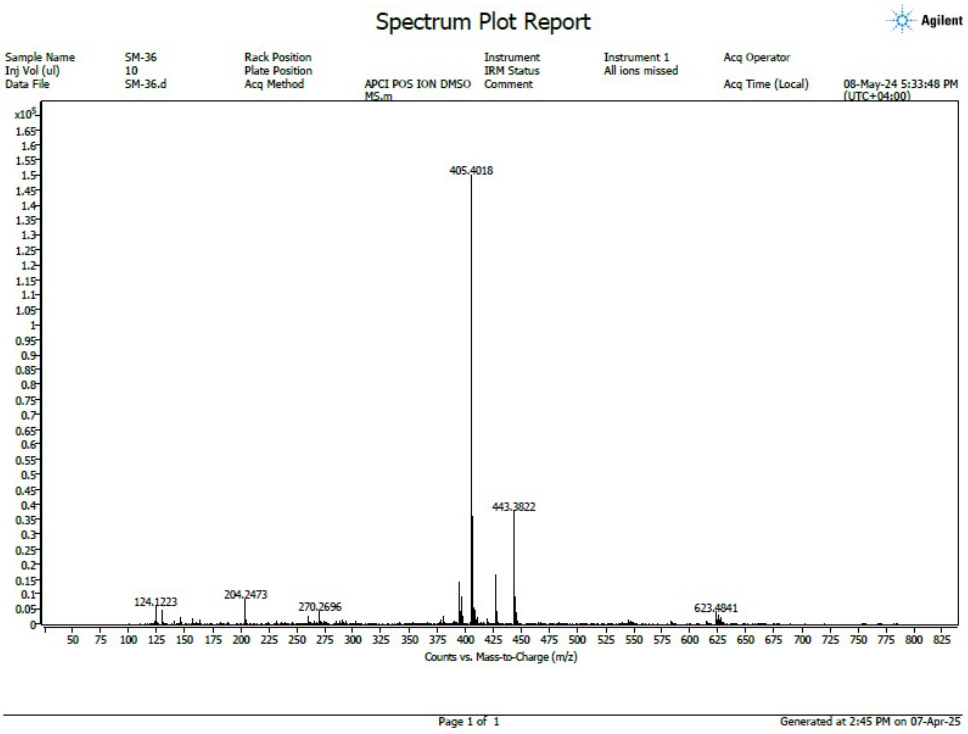
HRMS of 1i



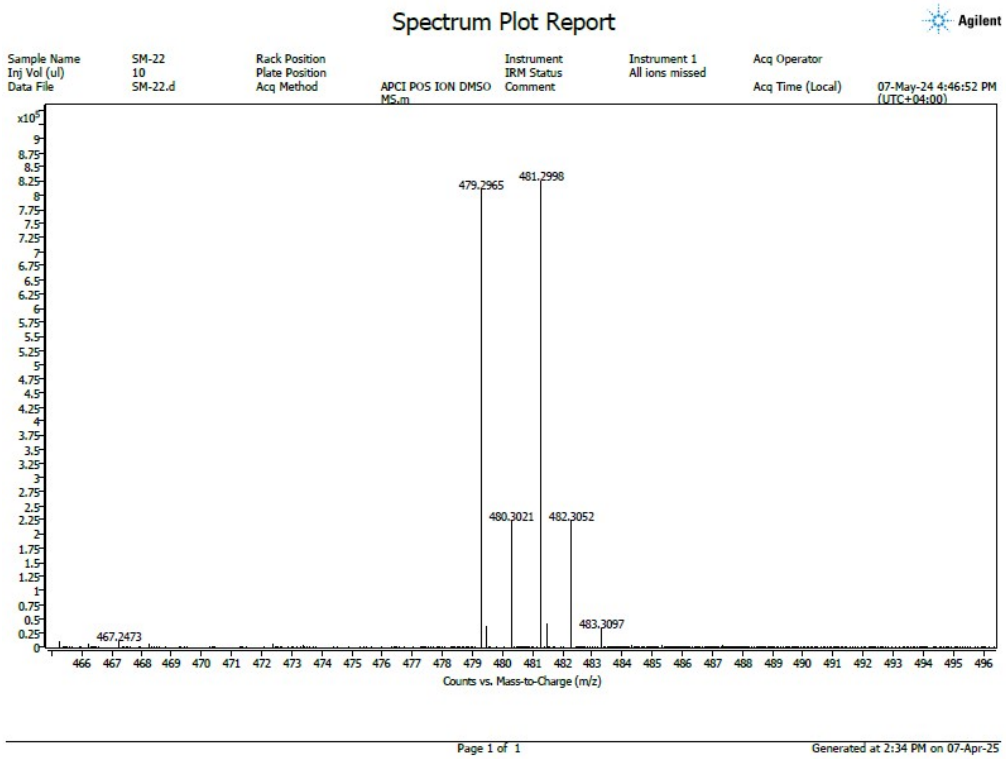
HRMS of 1j



HRMS of 1k

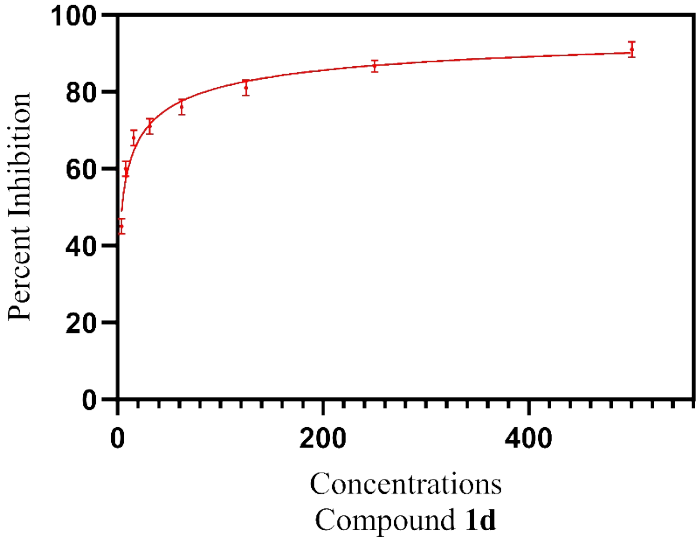


HRMS of 1l

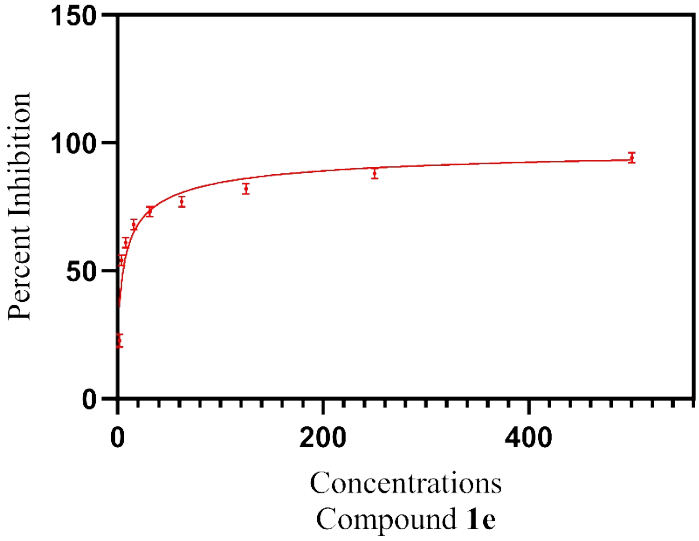


7) Dose Response Curves

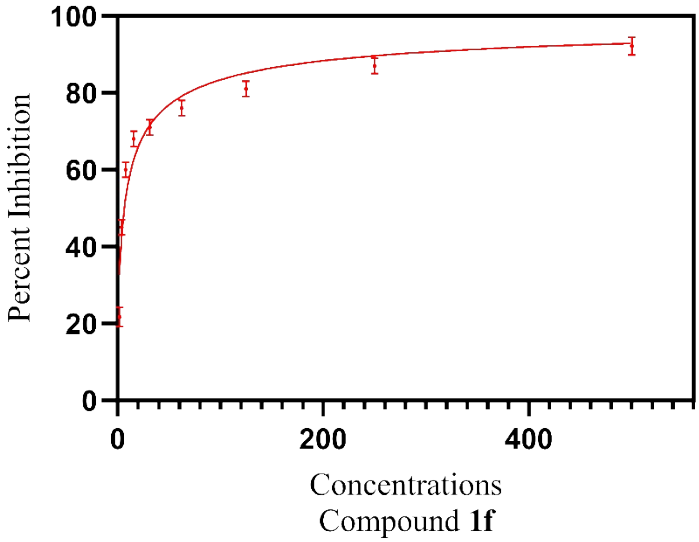
Dose curve response of comound **1d** against α-glucosidase:



Dose curve response of comound **1e** against α-glucosidase:



Dose curve response of comound **1f** against α-glucosidase:



Dose curve response of comound **1l** against α-glucosidase: