Identification of Histone Deacetylase 10 (HDAC10) Inhibitors that Modulate Autophagy in Transformed Cells

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[#] contributed equally to the work

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Content

S1. Molecular modelling results **3-5**

S2. LysoTracker results **6**

S3. NMR, HPLC and HRMS characterization data **7-29**

S1: Molecular modelling results

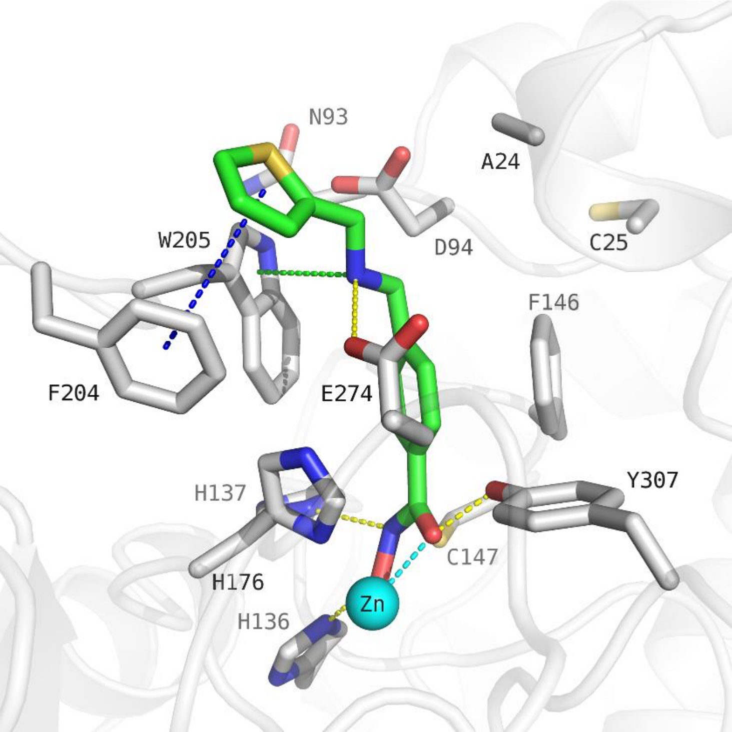


Figure S1: Obtained docking pose of benzhydroxamic acid derivatives carrying a methylene spacer. Predicted binding mode of **4b** in the catalytic pocket of drHDAC10 (PDB ID: 5TD7. The Zn ion is shown as cyan sphere and water molecules as red spheres. Yellow dashed lines depict hydrogen bond and salt-bridge interactions, cyan dashed lines metal chelation, green dashed lines cation-π interactions and blue-dashed lines π-π interactions.

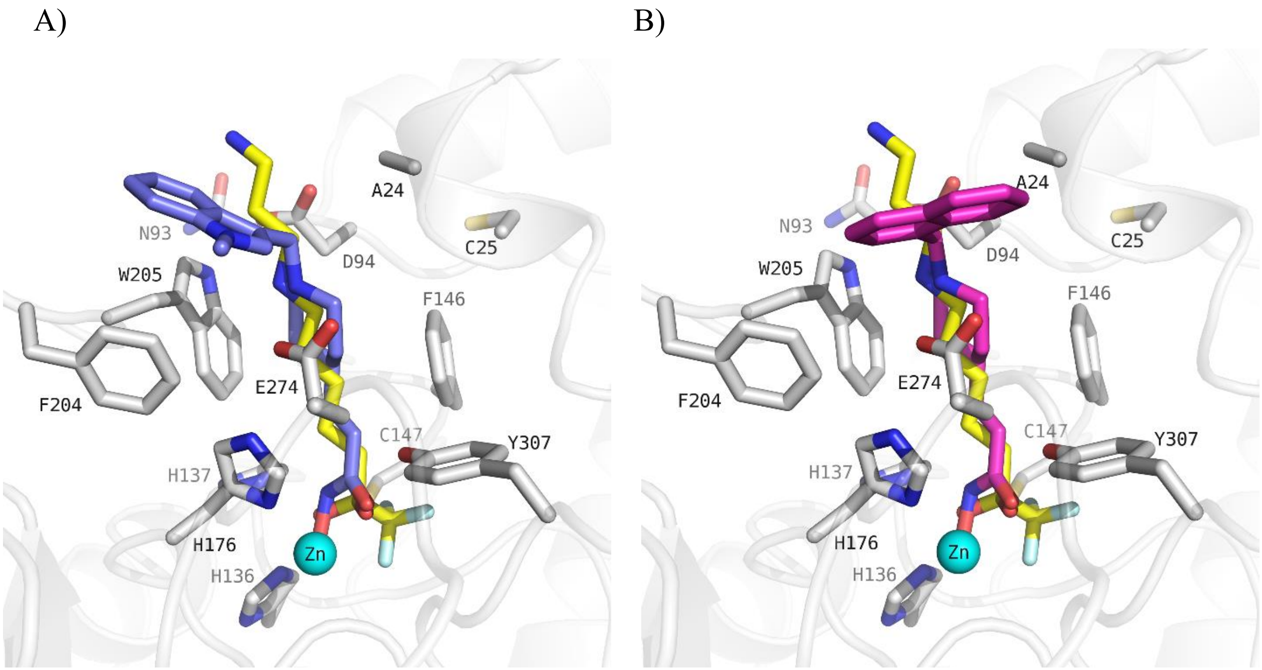


Figure S2: **Superimposition of docking solutions of 10c and 13b with the cocrystal structure of drHDAC10 in complex with *N*8-acetylspermidine analogue (PDB ID 5TD7).** a) Predicted binding mode of **13b** (state blue sticks) overlapped with the experimentally determined binding mode of *N*8-acetylspermidine analogue (yellow sticks) taken from the respective crystal structure (PDB ID 5TD7), c) Predicted binding mode of **10c** (pink sticks) overlapped with the experimentally determined binding mode of *N*8-acetylspermidine analogue (yellow sticks) taken from the respective crystal structure (PDB ID 5TD7). Binding site residues are shown as white sticks and the catalytic zinc ion as cyan sphere.

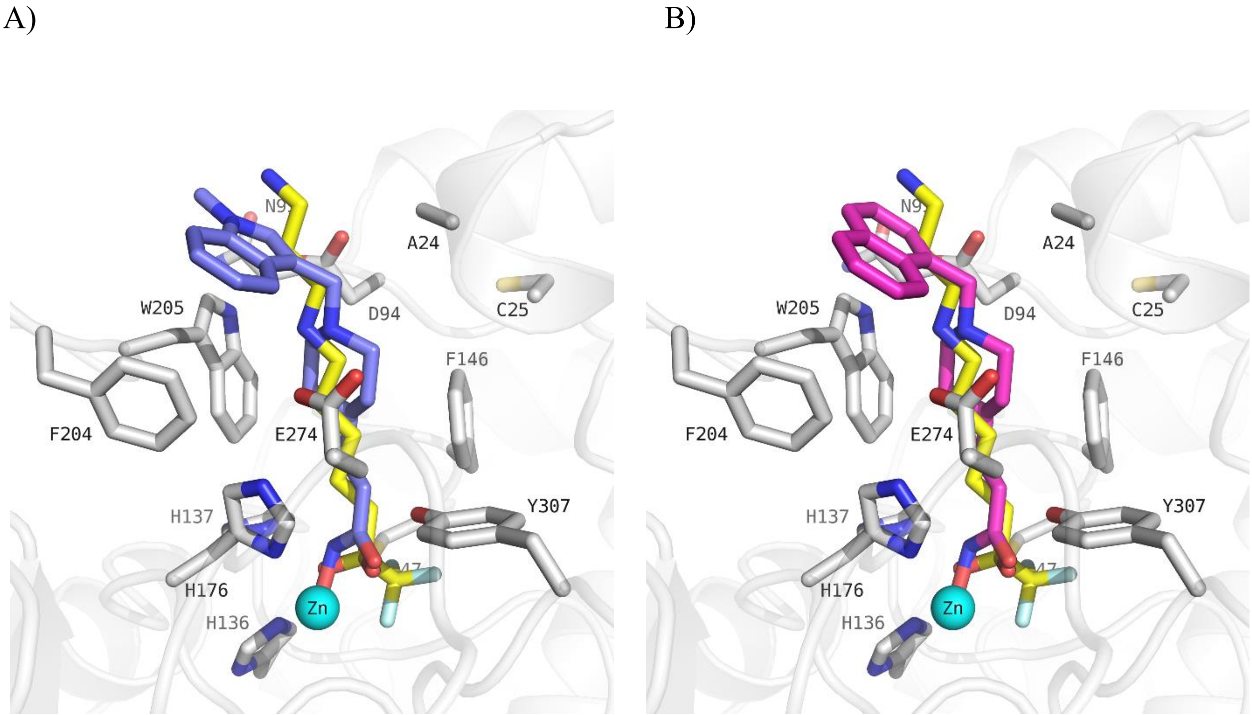


Figure S3: a) Predicted binding mode of **13b** (state blue sticks) and **10c** (pink sticks) in humanized drHDAC10 (PDB ID 6VNQ). b) Predicted binding mode of **13b** (state blue sticks) overlapped with the experimentally determined binding mode of *N*8-acetylspermidine analogue (green sticks) taken from the respective crystal structure (PDB ID 5TD7), b) Predicted binding mode of **10c** (pink sticks) overlapped with the experimentally determined binding mode of *N*8-acetylspermidine analogue inhibitor (green sticks) taken from the respective crystal structure (PDB ID 5TD7). Binding site residues are shown as white sticks and the catalytic zinc ion as cyan sphere.

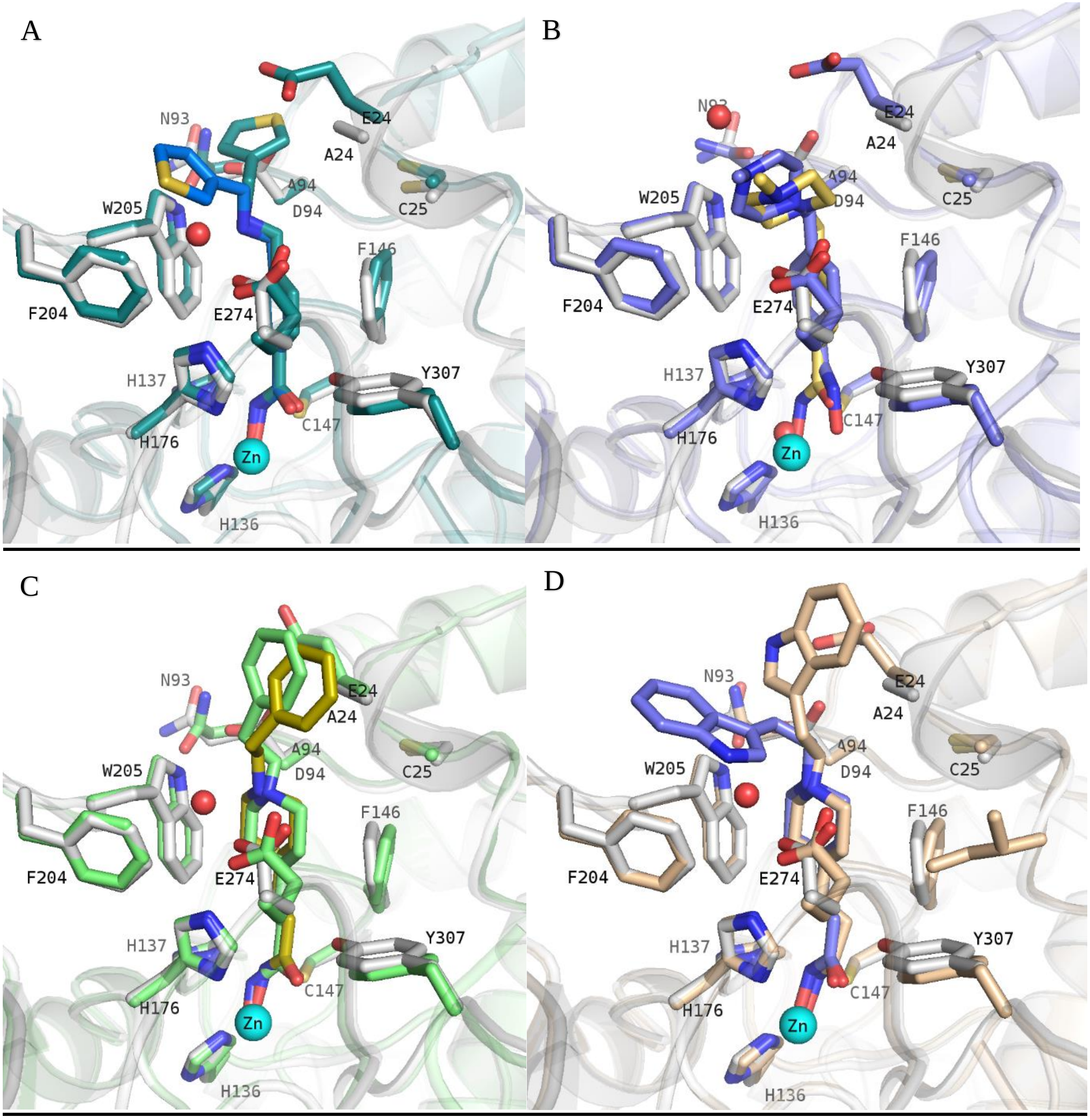


Figure S4: a) Predicted binding mode of **4c** in drHDAC10 (PDB ID 5TD7) (state blue sticks) overlapped with the experimentally determined binding mode of **4c** (oil green sticks) in humanized drHDAC10. b) Predicted binding mode of **6b** in drHDAC10 (PDB ID 5TD7) (yellow sticks) overlapped with the experimentally determined binding mode of **6b** (magenta sticks) in humanized drHDAC10. c) Predicted binding mode of **10a** in drHDAC10 (PDB ID 5TD7) (golden sticks) overlapped with the experimentally determined binding mode of **10a** (pale green sticks) in humanized drHDAC10. d) Predicted binding mode of **10b** in drHDAC10 (PDB ID 5TD7) (magenta sticks) overlapped with the experimentally determined binding mode of

10b (wheat sticks) in humanized drHDAC10. Binding site residues of in drHDAC10 (PDB ID 5TD7) are shown as white sticks, binding site residues of new crystals with the same colour of their own ligands respectively, water residues in the new crystals as red sphere and the catalytic zinc ion as cyan sphere.

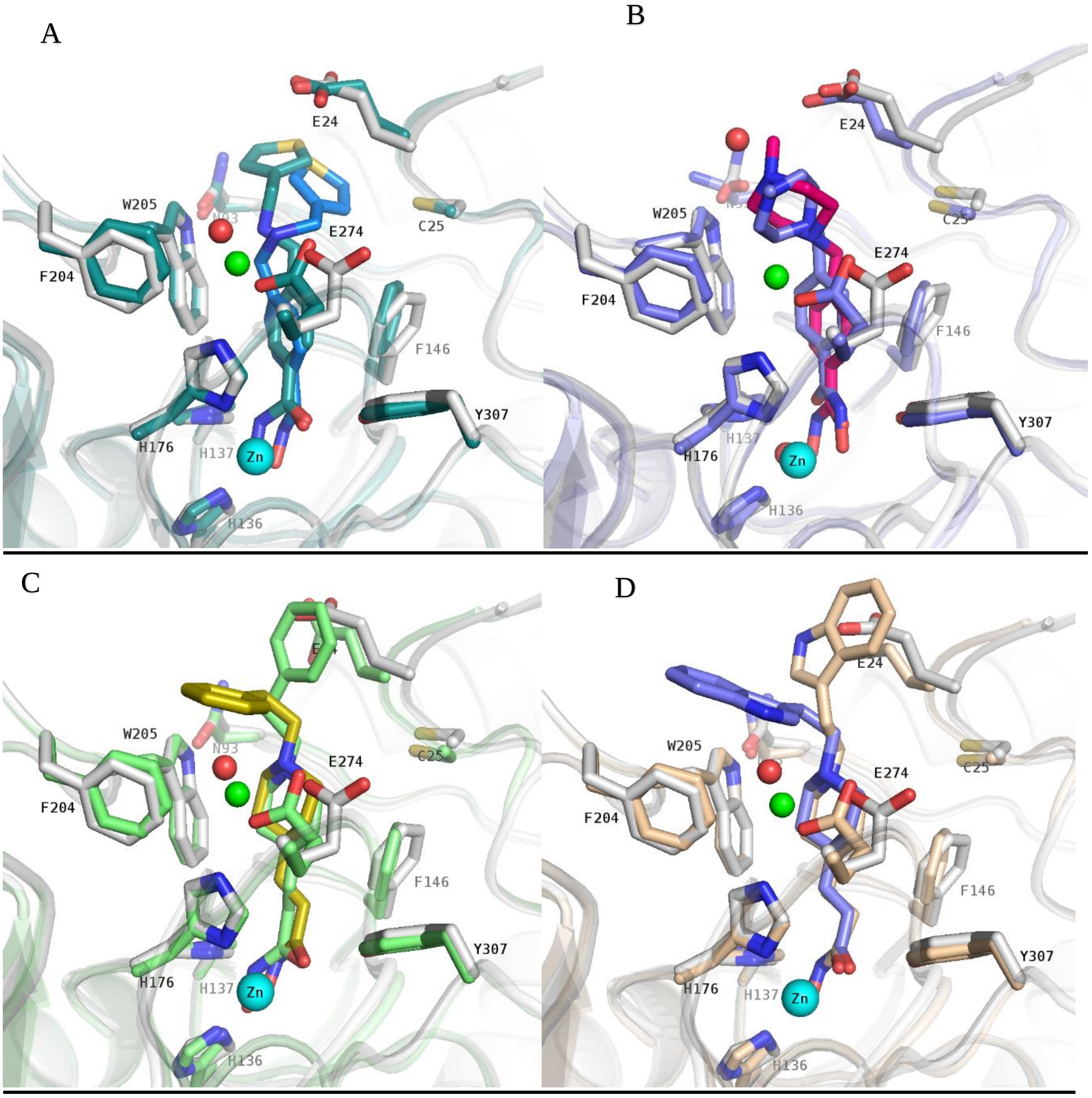


Figure S5 a) Predicted binding mode of **4c** in humanized drHDAC10 (PDB ID 6VNQ) (state blue sticks) overlapped with the experimentally determined binding mode of **4c** (oil green sticks) in humanized drHDAC10. b) Predicted binding mode of **6b** in humanized drHDAC10 (PDB ID 6VNQ) (pink sticks) overlapped with the experimentally determined binding mode of **6b** (magenta sticks) in humanized drHDAC10. c) Predicted binding mode of **10a** in humanized drHDAC10 (PDB ID 6VNQ) (golden sticks) overlapped with the experimentally determined binding mode of **10a** (pale green sticks) in humanized drHDAC10. d) Predicted binding mode of **10b** in humanized drHDAC10 (PDB ID 6VNQ) (magenta sticks) overlapped with the experimentally determined binding mode of **10b** (wheat sticks) in humanized drHDAC10. Binding site residues of in drHDAC10 (PDB ID 5TD7) are shown as white sticks, binding site residues of new crystals with the same colour of their own ligands respectively, water residues in the new crystals as red sphere, water residues in the humanized drHDAC10 (PDB ID 6VNQ) as pale green sphere and the catalytic zinc ion as cyan sphere.

S2. LysoTracker results

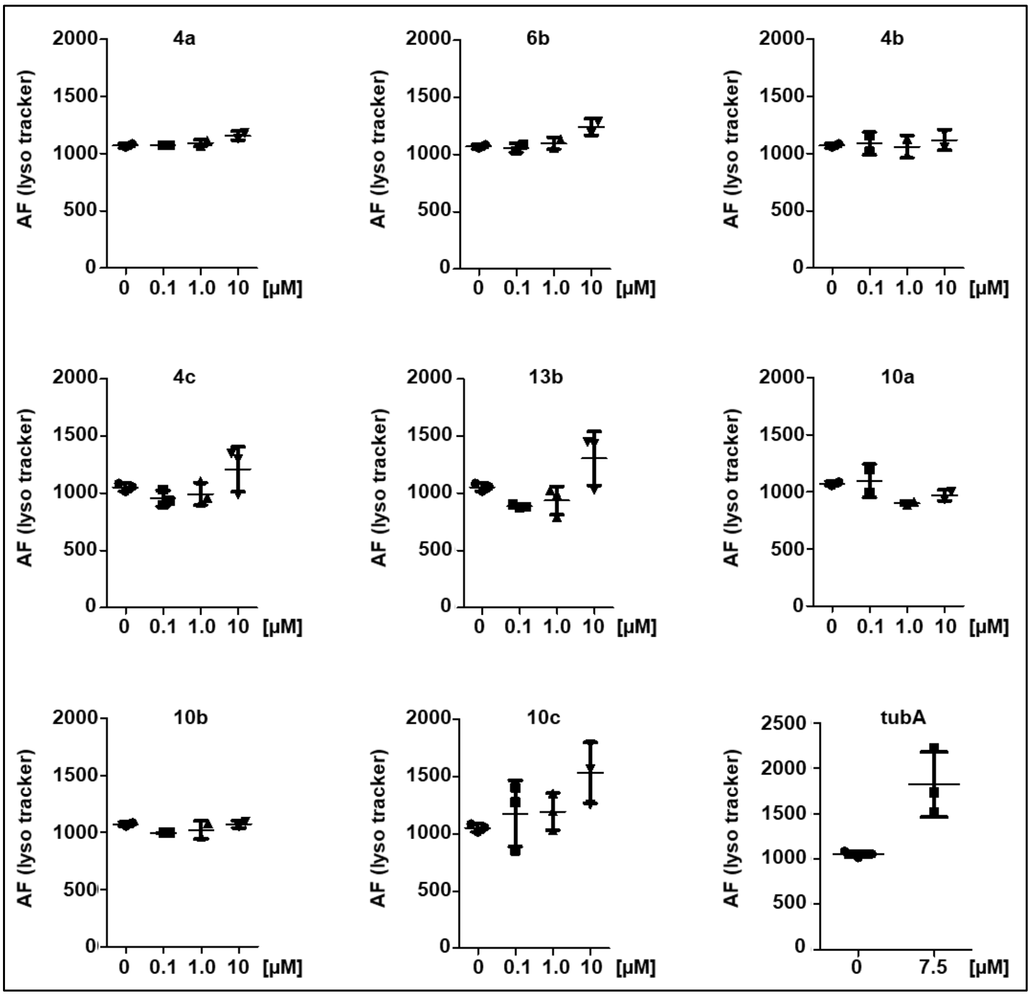
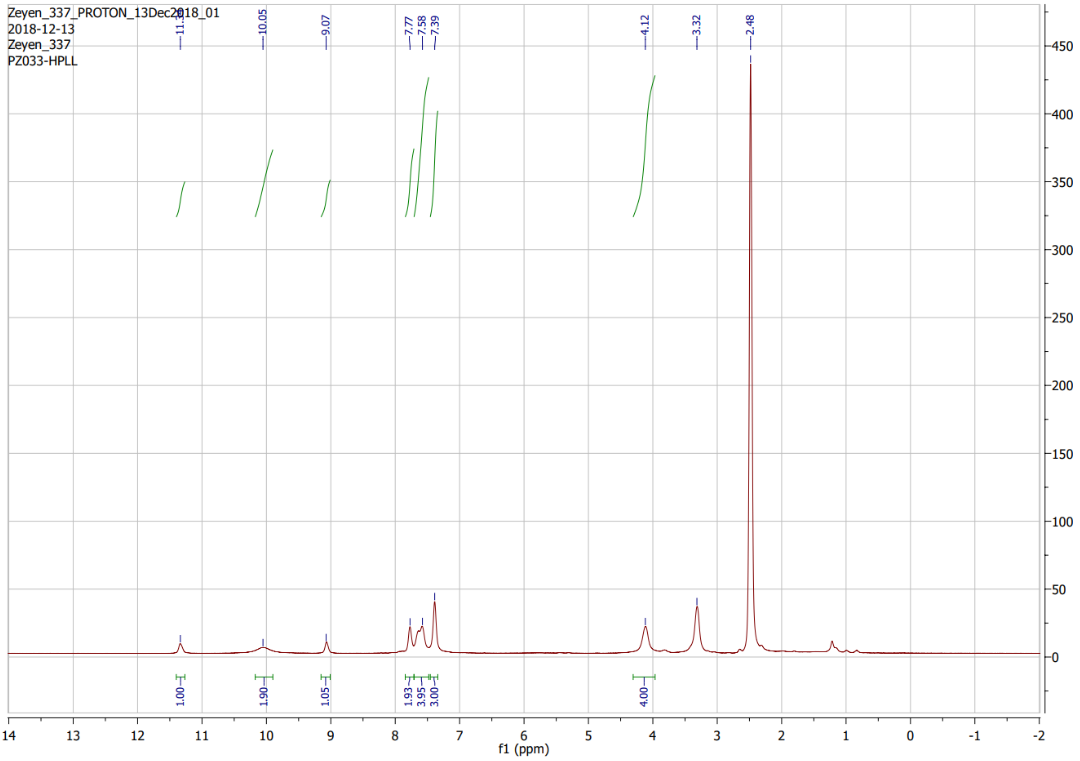
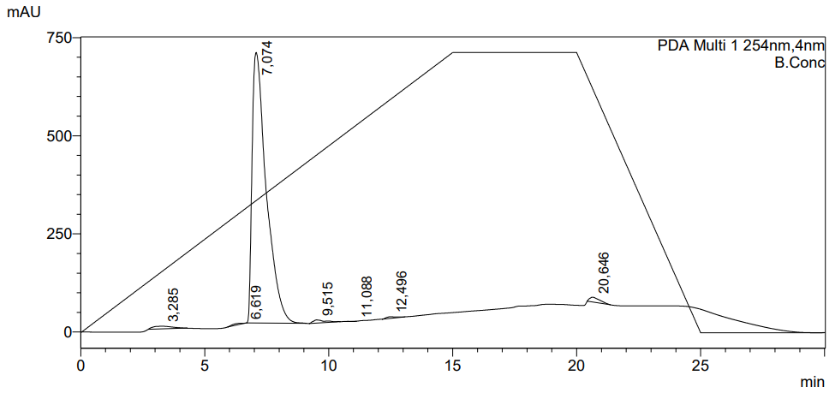


Figure S6: Expansion of the lysosomal compartment as a measure of HDAC10 inhibition, shown by increased fluorescence from the LysoTracker DND-99 probe. tubA: tubastatin A.

S3. NMR, HPLC and HRMS characterization data

4a





<Peak Table>

PDA Ch1 254nm

Peak#	Ret. Time	Area%	Area	Height
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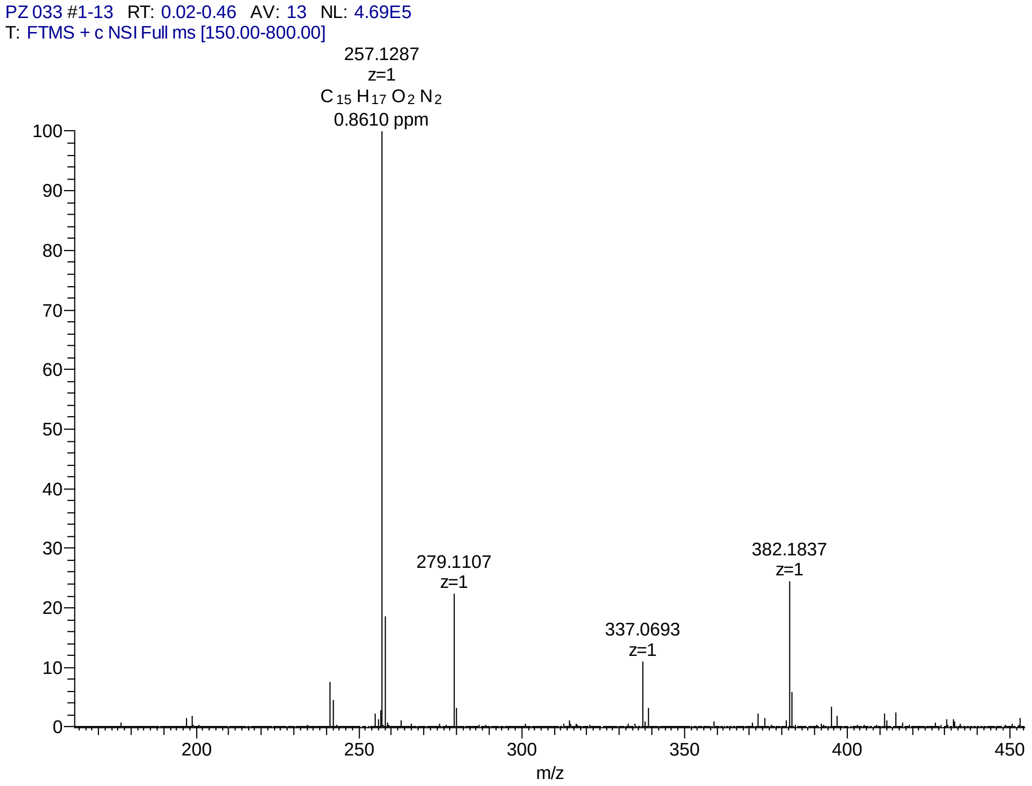
2	6,619	0,336	96489	16
3	7,074	95,877	27539326	688947
4	9,515	0,880	252858	7954
5	11,088	-0,014	-4003	-75
6	12,496	0,375	107625	4014
7	20,646	1,235	354868	12270
Total		100,000	28723494	719850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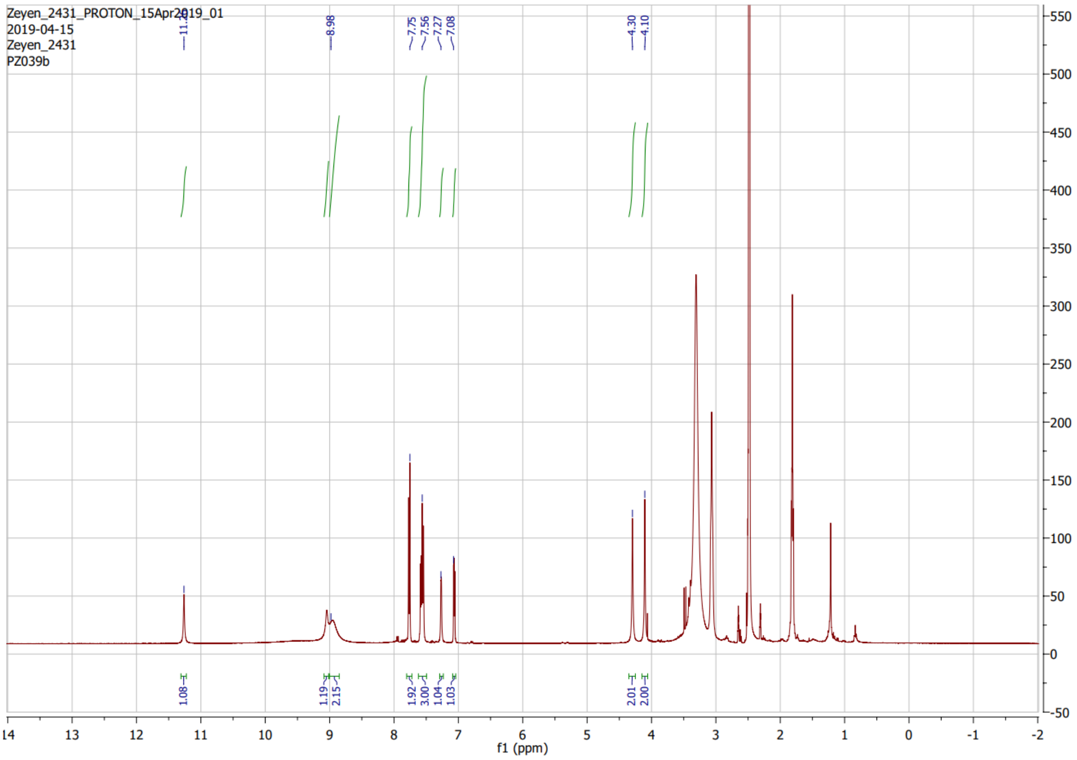
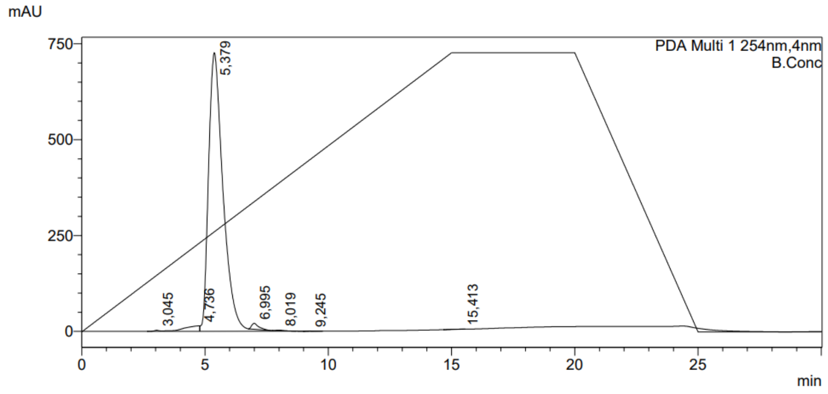
T: FTMS + c NSI Full ms [150.00-800.00]
257.1287
z=1
C 15 H 17 O 2 N 2
0.8610 ppm
279.1107
z=1
337.0693
z=1
382.1837
z=1
100
90
80
70
60
50
40
30
20
10
0
200
250
300
350
400
450
m/z

4b

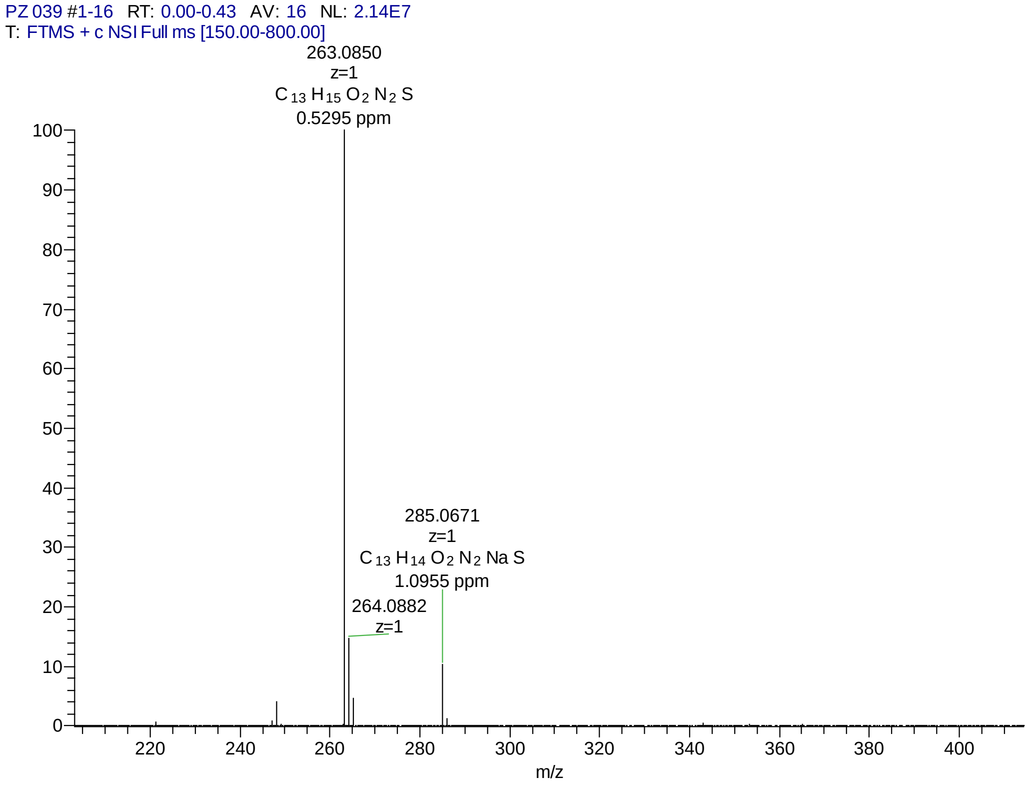

Zeyen_2431_PROTON_15Apr2019_01
2019-04-15
Zeyen_2431
PZ039b
11.2
8.98
7.75
7.56
7.27
7.08
4.30
4.10
1.08
1.19
2.15
1.92
3.00
1.04
1.03
2.01
2.00
550
500
450
400
350
300
250
200
150
100
50
0
-50
14
13
12
11
10
9
8
7
6
5
4
3
2
1
0
-1
-2
f1 (ppm)



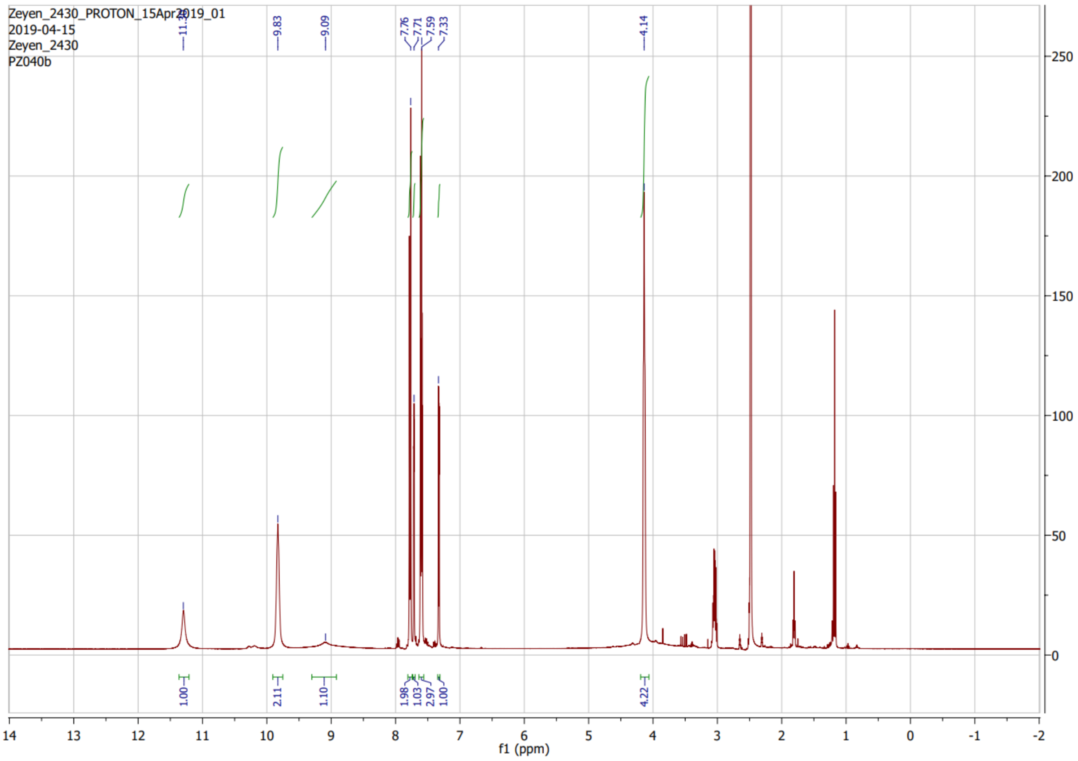
<Peak Table>

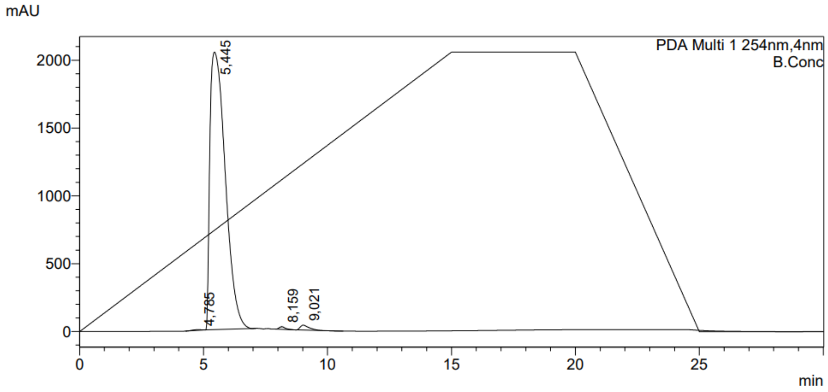
PDA Ch1 254nm

Peak#	Ret. Time	Area%	Area	Height
1	3,045	0,153	47139	3157
2	4,736	2,046	630557	14245
3	5,379	96,571	29760281	726285
4	6,995	1,028	316648	15671
5	8,019	0,121	37339	1925
6	9,245	0,044	13420	661
7	15,413	0,038	11699	141
Total		100,000	30817083	762085



4c





<Peak Table>

PDA Ch1 254nm

Peak#	Ret. Time	Area%	Area	Height
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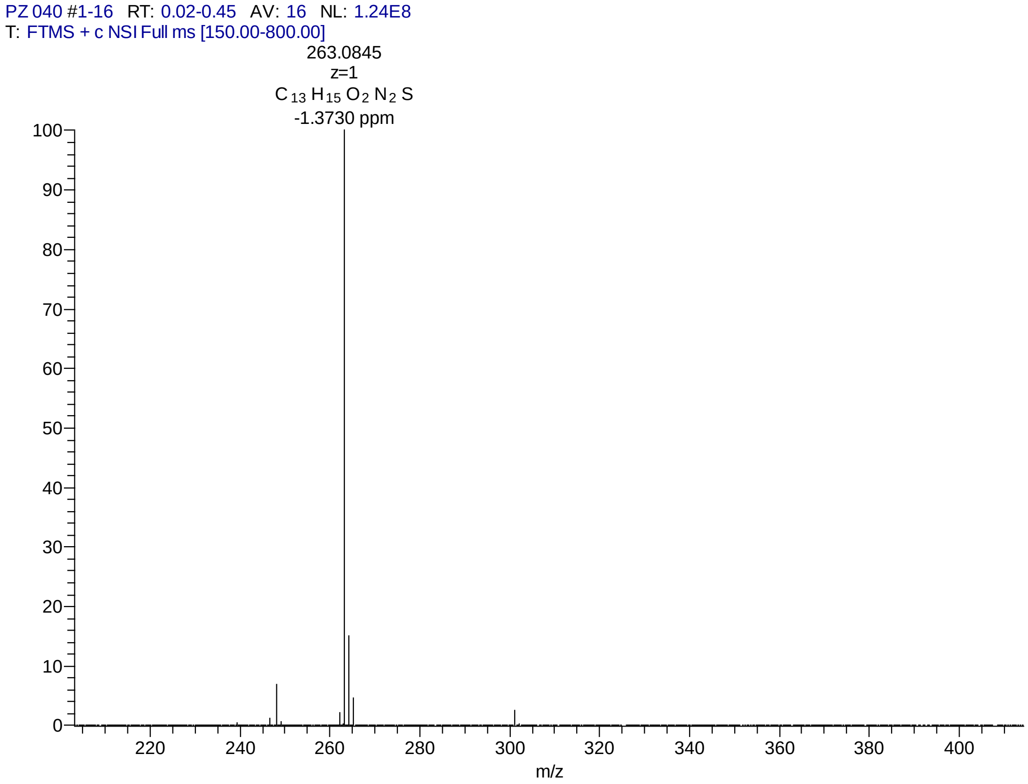
2	5,445	98,416	88367647	2045773
3	8,159	0,383	343688	19792
4	9,021	1,051	943727	36948
Total		100,000	89790206	2107251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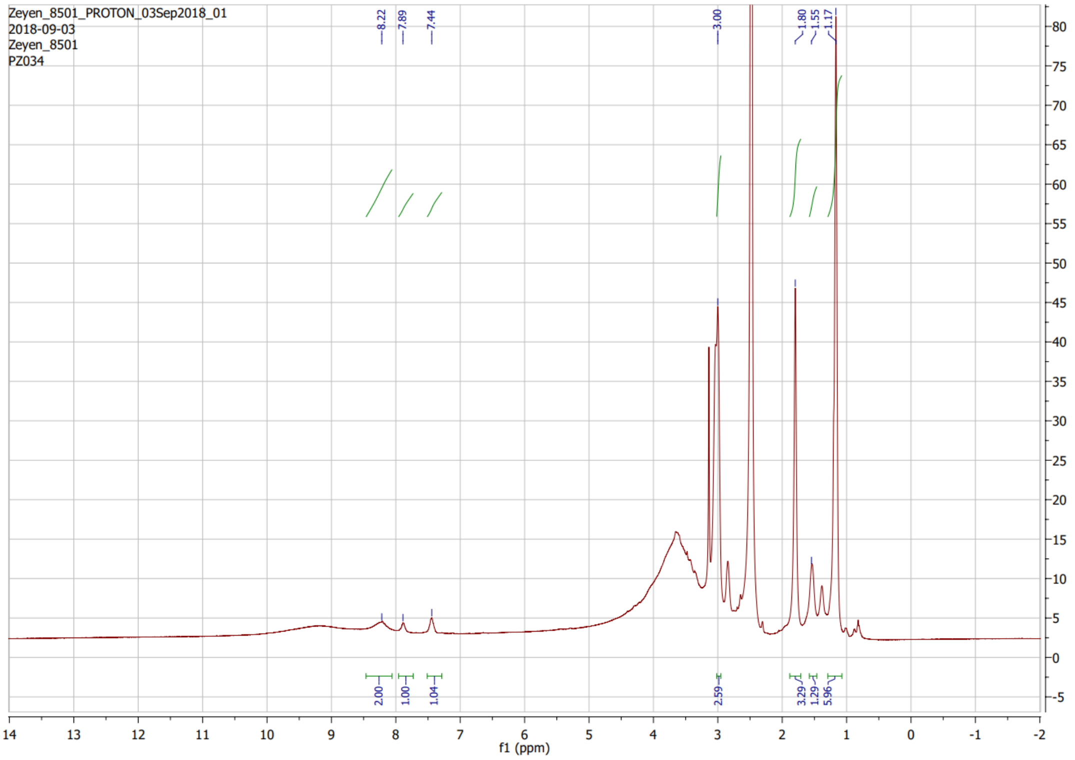
T: FTMS + c NSI Full ms [150.00-800.00]
263.0845
z=1
C 13 H 15 O 2 N 2 S
-1.3730 ppm
100
90
80
70
60
50
40
30
20
10
0
220
240
260
280
300
320
340
360
380
400
m/z

6a


Zeyen_8501_PROTON_03Sep2018_01
2018-09-03
Zeyen_8501
PZ034
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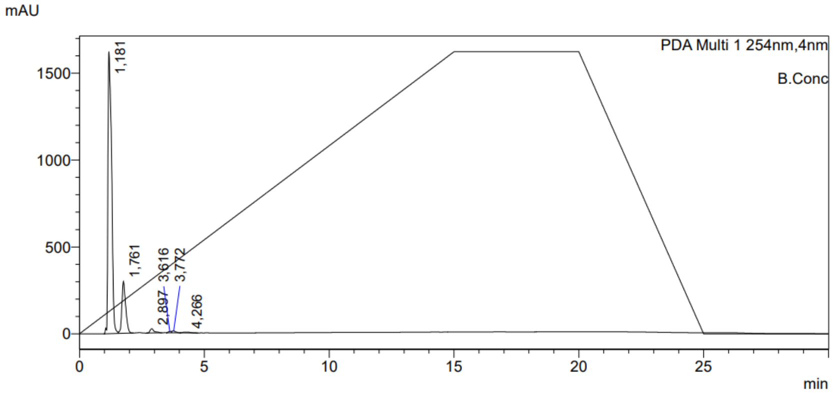
7.89
7.44
3.00
1.80
1.55
1.17
2.00
1.00
1.04
2.59
3.29
1.29
5.96
80
75
70
65
60
55
50
45
40
35
30
25
20
15
10
5
0
-5
14
13
12
11
10
9
8
7
6
5
4
3
2
1
0
-1
-2
f1 (ppm)



PDA Ch1 254nm

Peak#	Ret. Time	Area	Height	Area%
1	1,181	16472524	1623019	81,625
2	1,761	3130190	298272	15,511
3	2,897	296083	24683	1,467
4	3,616	70273	8348	0,348
5	3,772	112655	10969	0,558
6	4,266	99058	4628	0,491
Total		20180783	1969919	100,000

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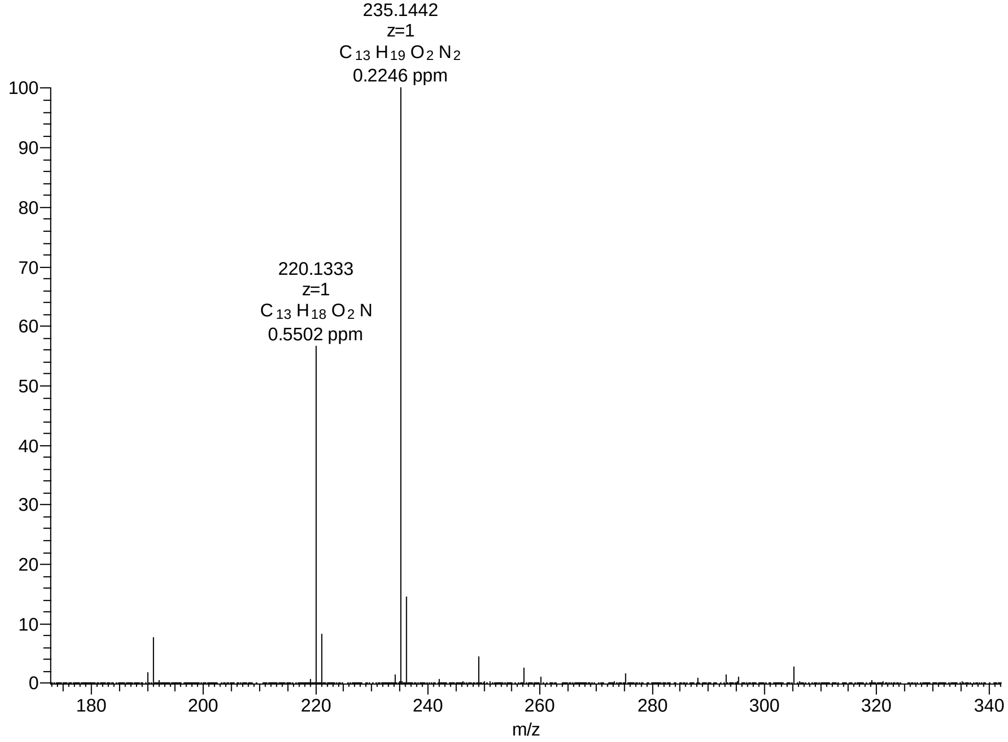
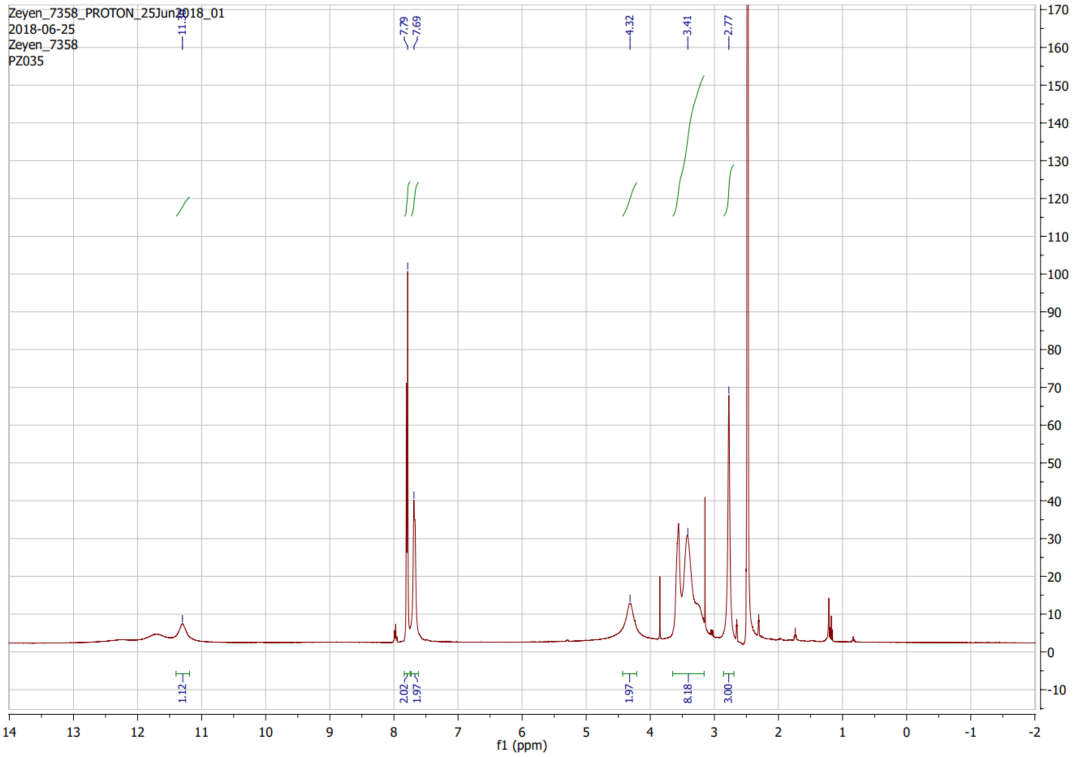
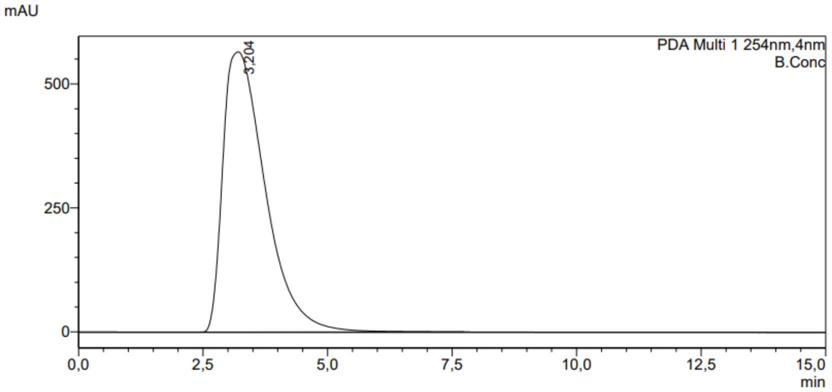
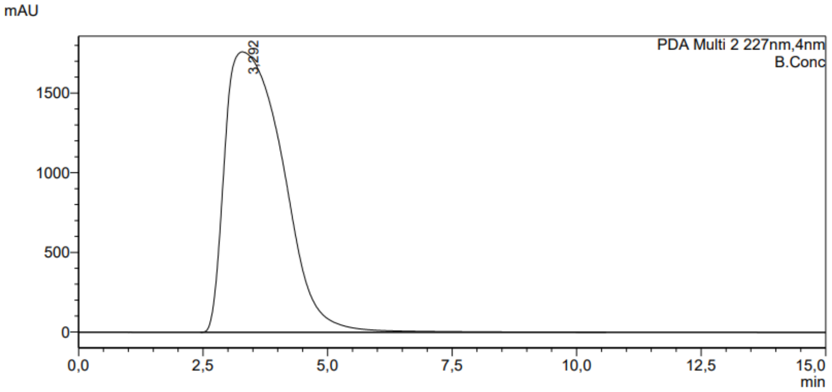
T: FTMS + c NSI Full ms [150.00-800.00]



6b







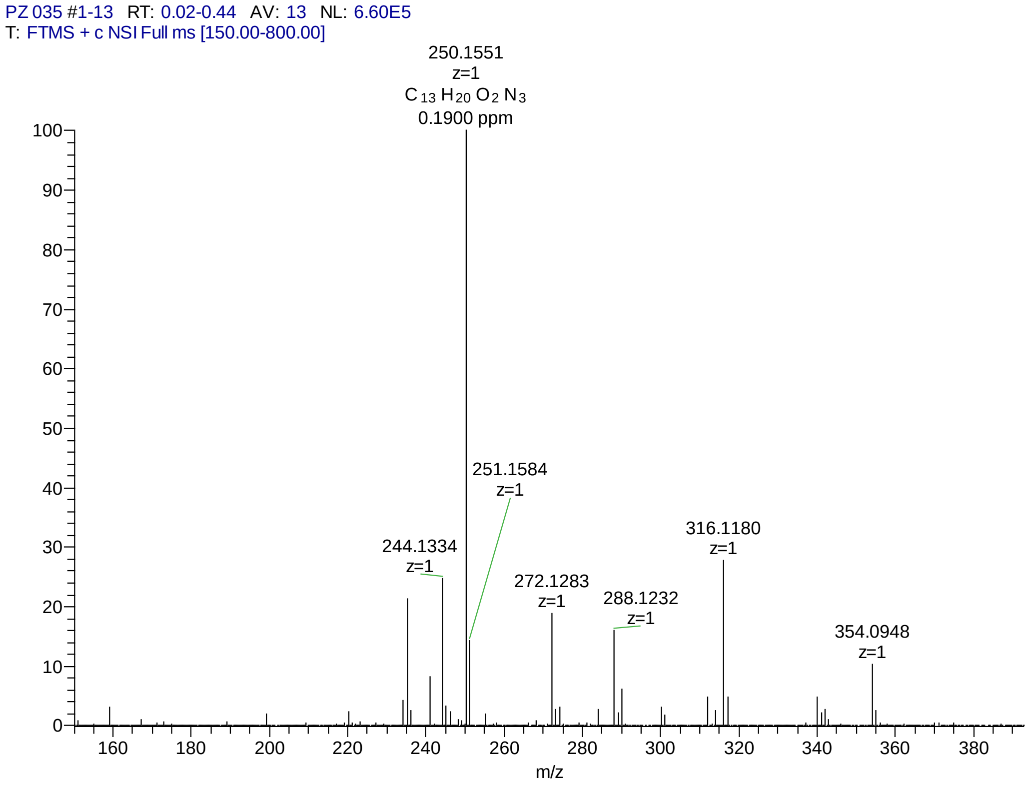
<Peak Table>

PDA Ch1 254nm

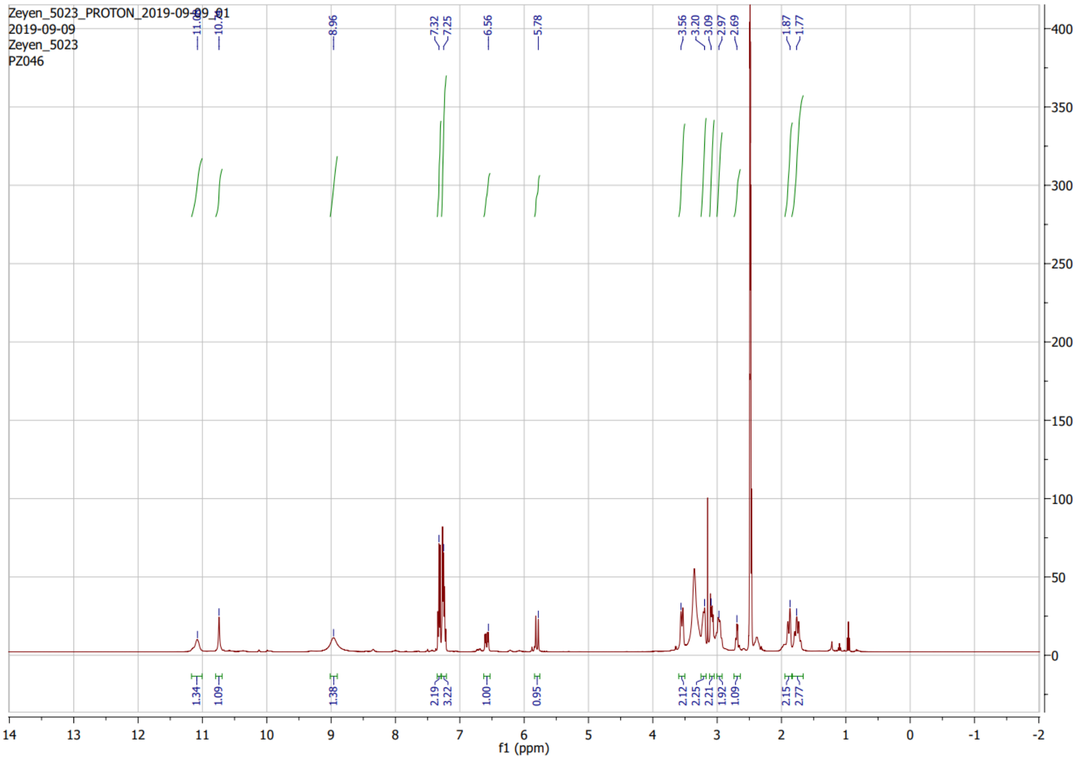
Peak#	Ret. Time	Area%	Area	Height
1	3,204	100,000	33400290	565109
Total		100,000	33400290	565109

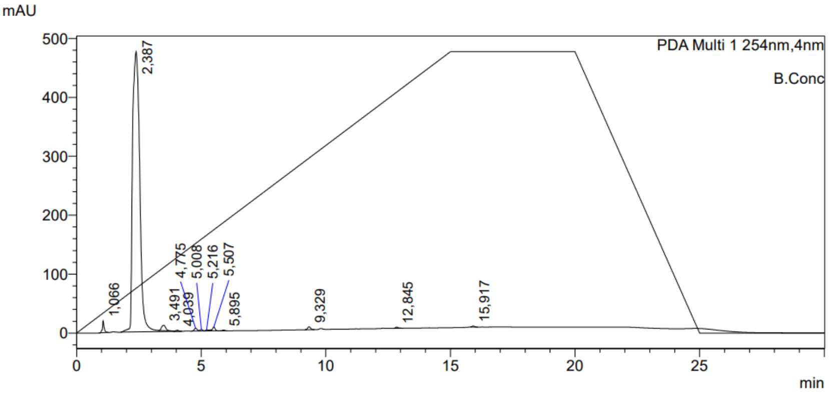
PDA Ch2 227nm

Peak#	Ret. Time	Area%	Area	Height
1	3,292	100,000	142730137	1760264
Total		100,000	142730137	1760264



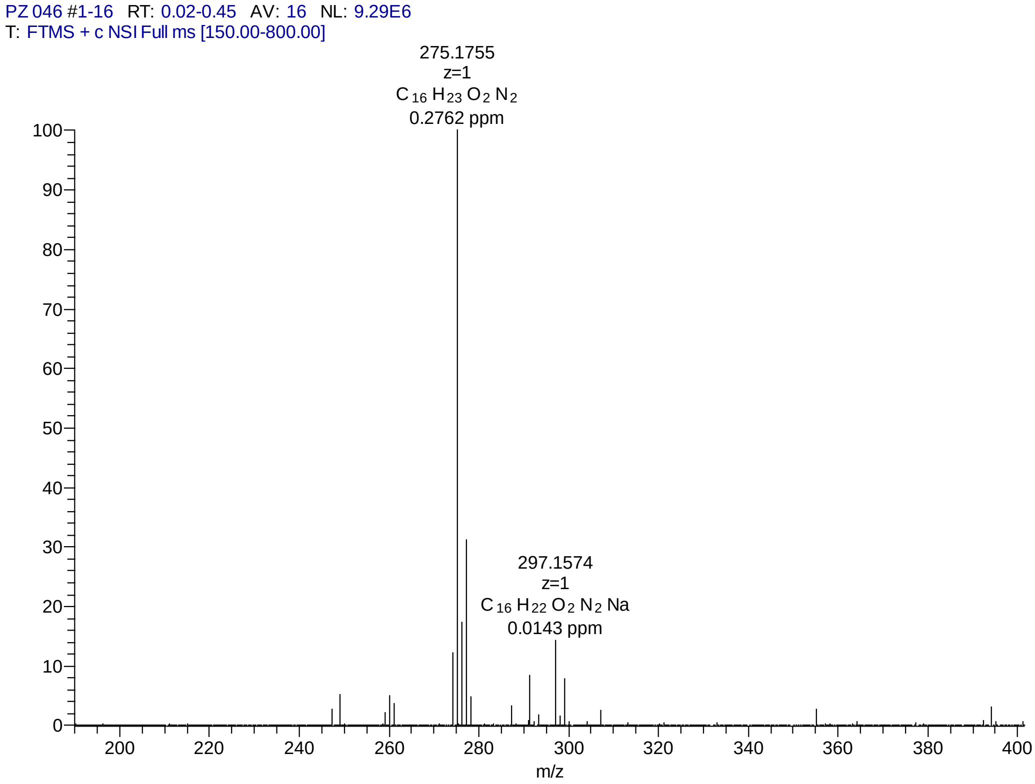
10a





PDA Ch1 254nm

Peak#	Ret. Time	Area	Height	Area%
1	1,066	92850	20050	0,931
2	2,387	9548788	475509	95,776
3	3,491	108298	9145	1,086
4	4,039	12772	1706	0,128
5	4,775	39381	4704	0,395
6	5,008	20544	2510	0,206
7	5,216	26441	1763	0,265
8	5,507	45325	6716	0,455
9	5,895	10286	1365	0,103
10	9,329	39721	4887	0,398
11	12,845	15067	1983	0,151
12	15,917	10494	1538	0,105
Total		9969967	531876	100,000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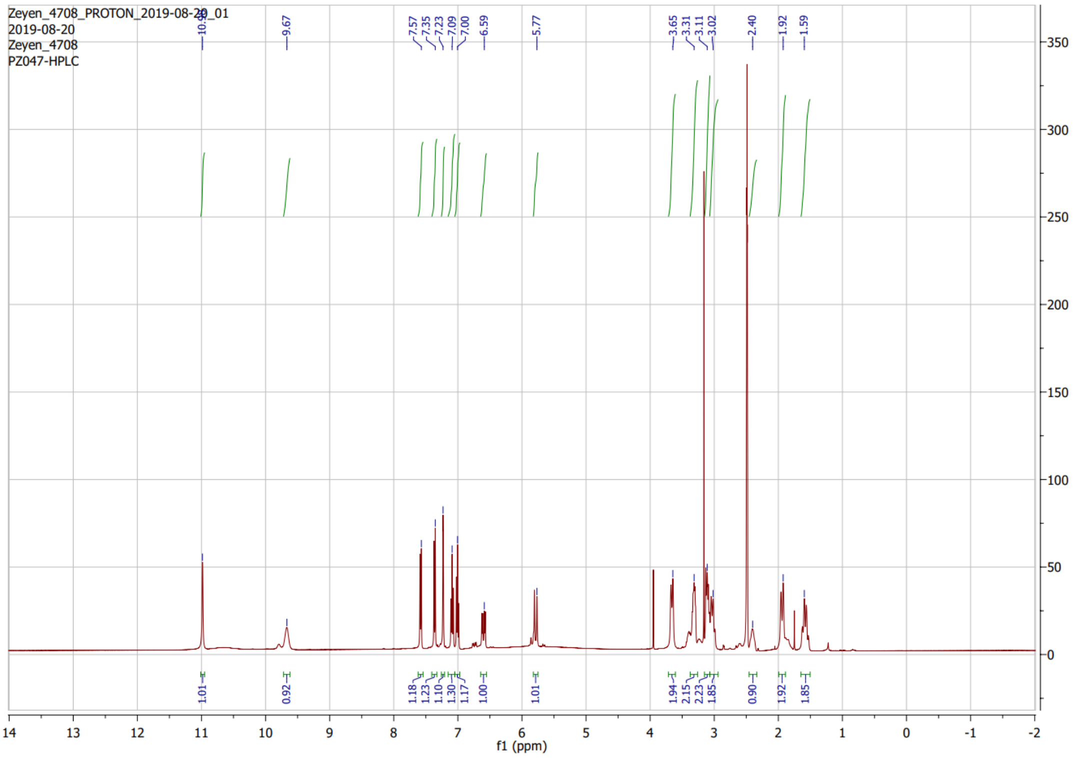
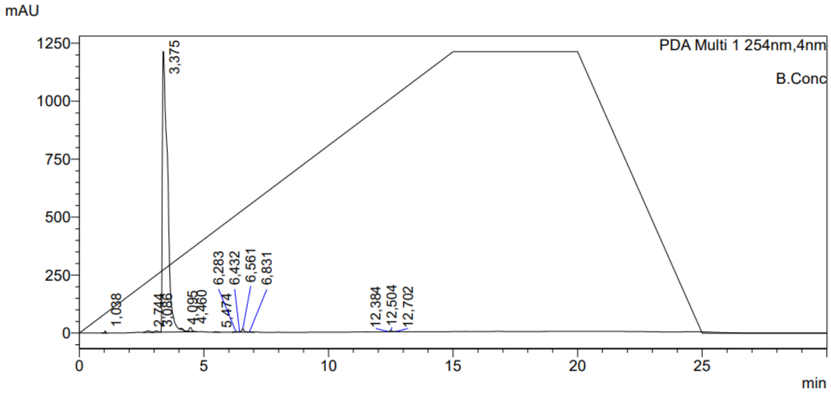
C 16 H23 O2 N2
0.2762 ppm
297.1574
z=1
C 16 H22 O2 N2 Na
0.0143 ppm
100
90
80
70
60
50
40
30
20
10
0
200
220
240
260
280
300
320
340
360
380
400
m/z

10b


Zeyen_4708_PROTON_2019-08-20_01
2019-08-20
Zeyen_4708
PZ047-HPLC
10.99
9.67
7.57
7.35
7.23
7.09
7.00
6.59
5.77
3.65
3.31
3.11
3.02
2.40
1.92
1.59
1.01
0.92
1.18
1.23
1.10
1.30
1.17
1.00
1.01
1.94
2.15
2.23
1.85
0.90
1.92
1.85
350
300
250
200
150
100
50
0
14
13
12
11
10
9
8
7
6
5
4
3
2
1
0
-1
-2
f1 (ppm)



PDA Ch1 254nm

Peak#	Ret. Time	Area	Height	Area%
1	1,038	26772	9753	0,149
2	2,744	69589	6203	0,386
3	3,086	59964	5420	0,333
4	3,375	17485190	1209874	97,038
5	4,095	37720	5097	0,209
6	4,460	126787	17183	0,704
7	5,474	12706	2437	0,071
8	6,283	23964	2308	0,133
9	6,432	14535	2785	0,081
10	6,561	88594	15847	0,492
11	6,831	24889	2370	0,138
12	12,384	6378	1198	0,035
13	12,504	34648	6878	0,192
14	12,702	7087	1406	0,039
Total		18018823	1288758	100,000

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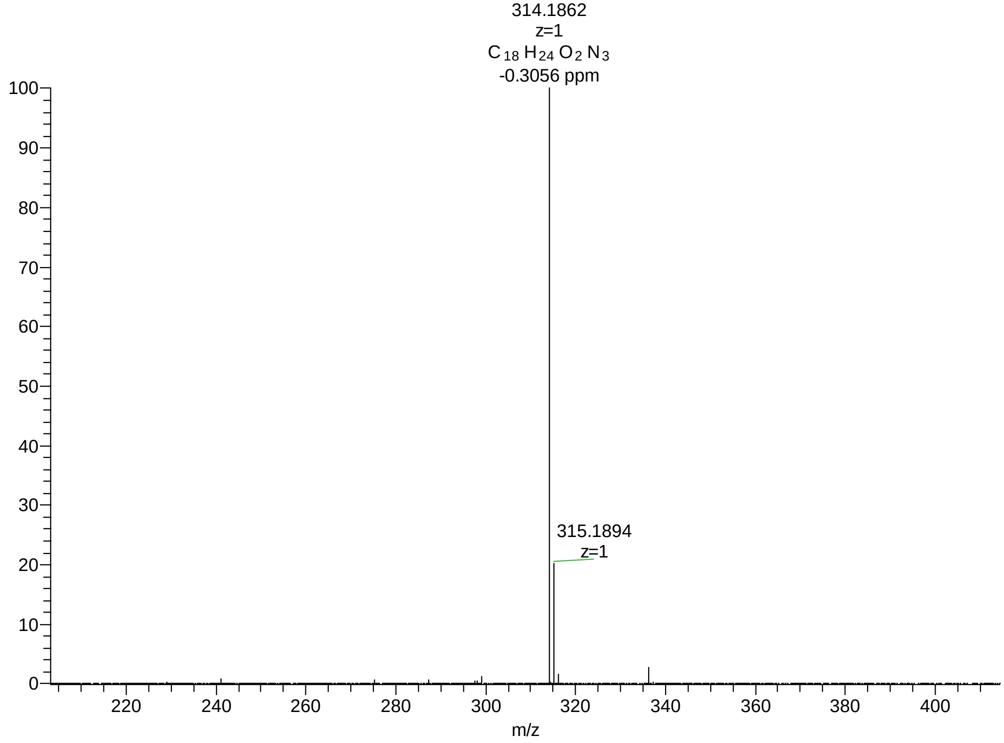
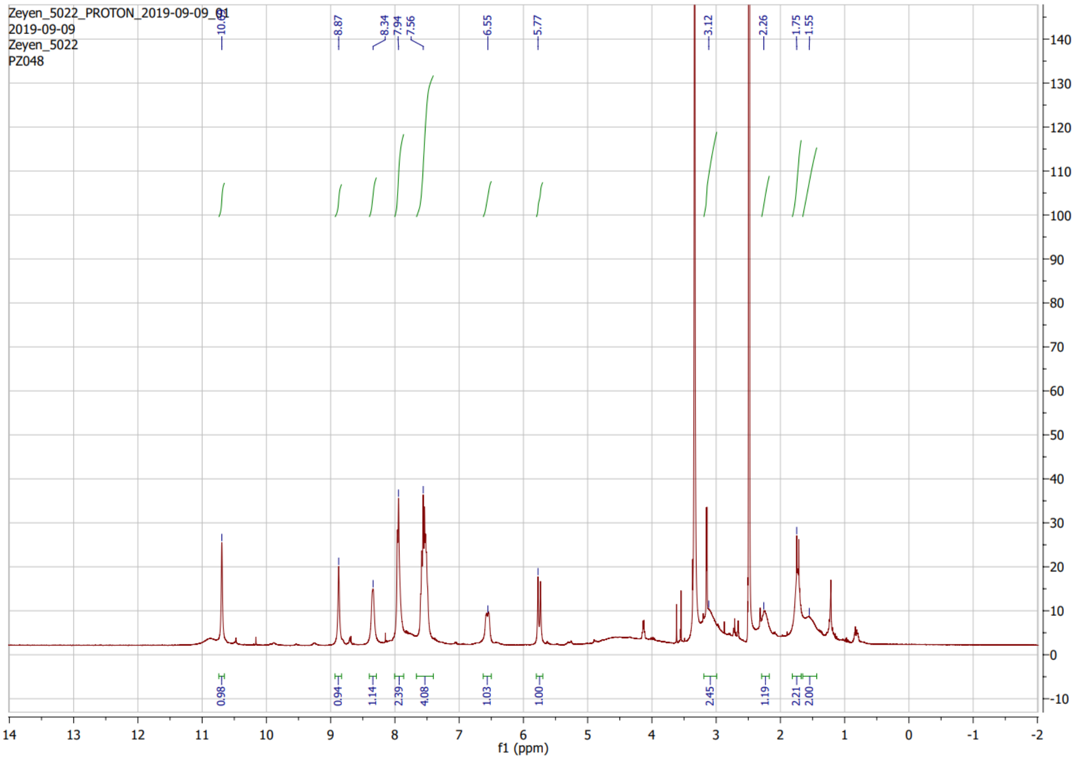
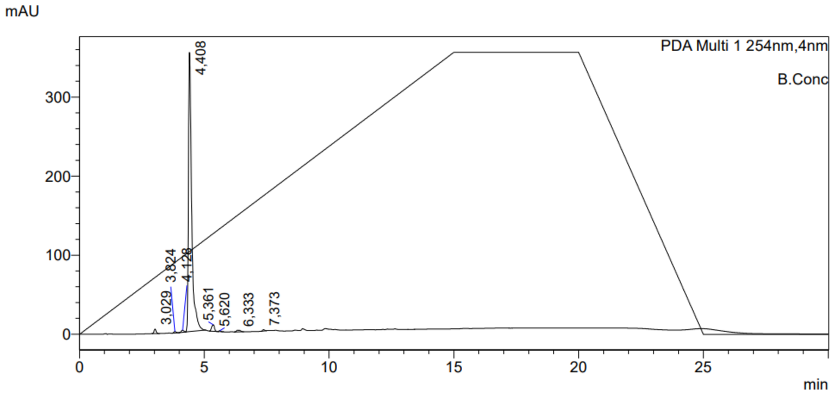
T: FTMS + c NSI Full ms [150.00-800.00]



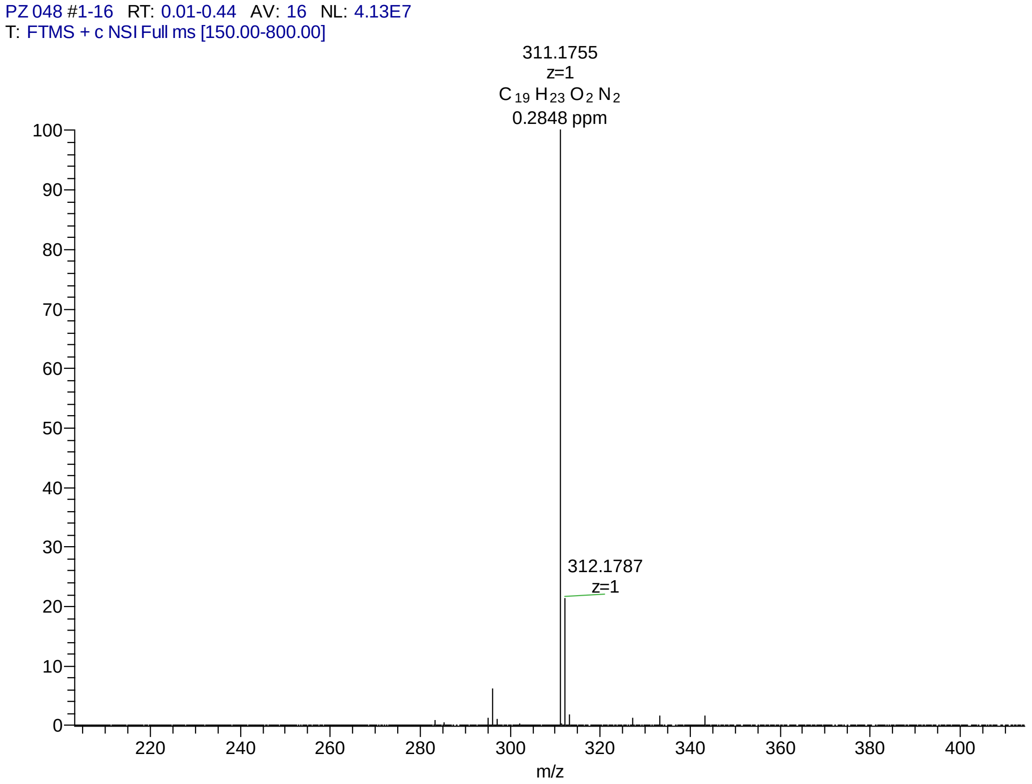
10c





PDA Ch1 254nm

Peak#	Ret. Time	Area	Height	Area%
1	3,029	31630	5674	0,936
2	3,824	12062	1848	0,357
3	4,128	19469	2645	0,576
4	4,408	3214969	353339	95,147
5	5,361	63437	8237	1,877
6	5,620	6890	1340	0,204
7	6,333	21209	1991	0,628
8	7,373	9291	1762	0,275
Total		3378956	376836	100,000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T: FTMS + c NSI Full ms [150.00-800.00]
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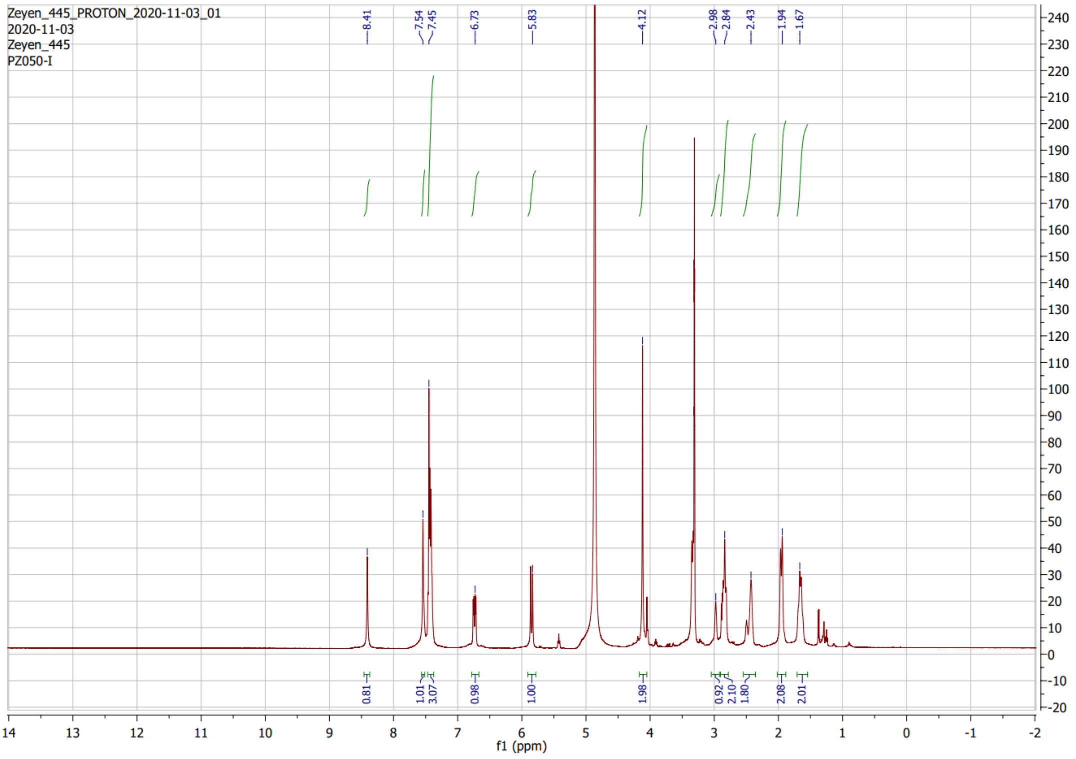
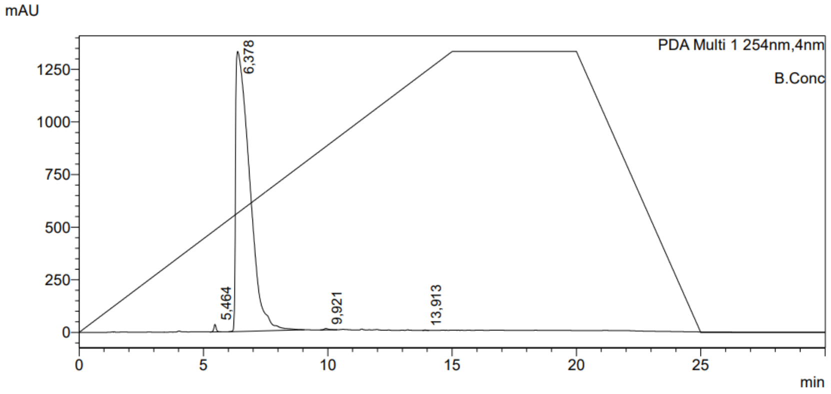
z=1
C 19 H 23 O 2 N 2
0.2848 ppm
312.1787
z=1
m/z

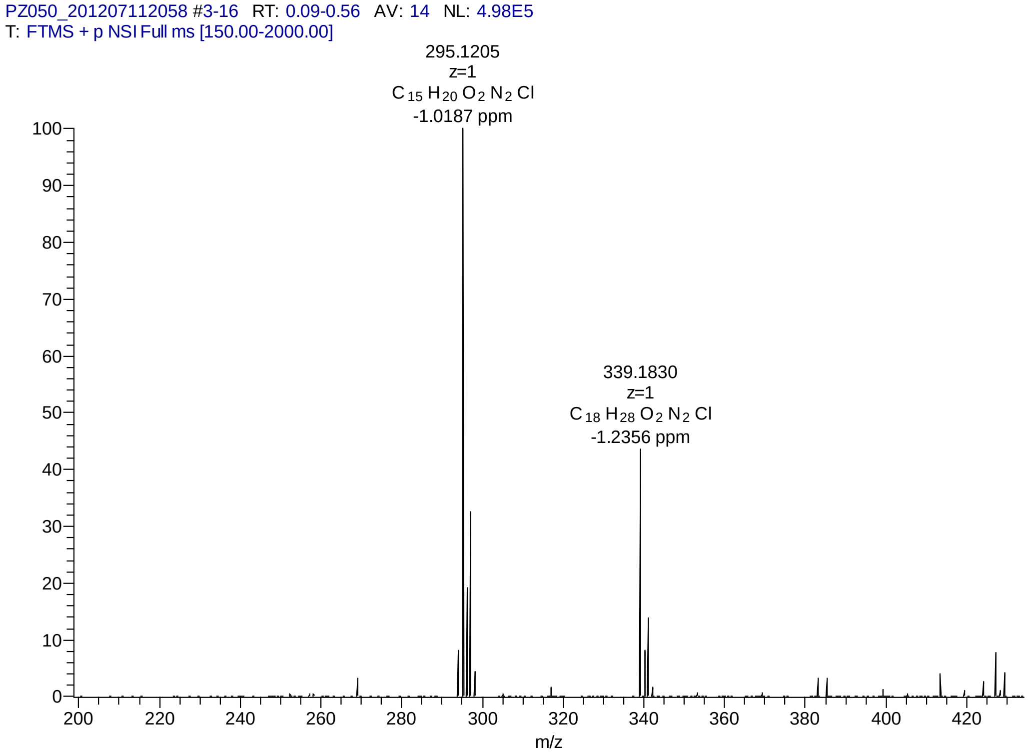
10d


Zeyen_445_PROTON_2020-11-03_01
2020-11-03
Zeyen_445
PZ050-I
8.41
7.54
7.45
6.73
5.83
4.12
2.98
2.84
2.43
1.94
1.67
0.81
1.01
3.07
0.98
1.00
1.98
0.92
2.10
1.80
2.08
2.01
f1 (ppm)

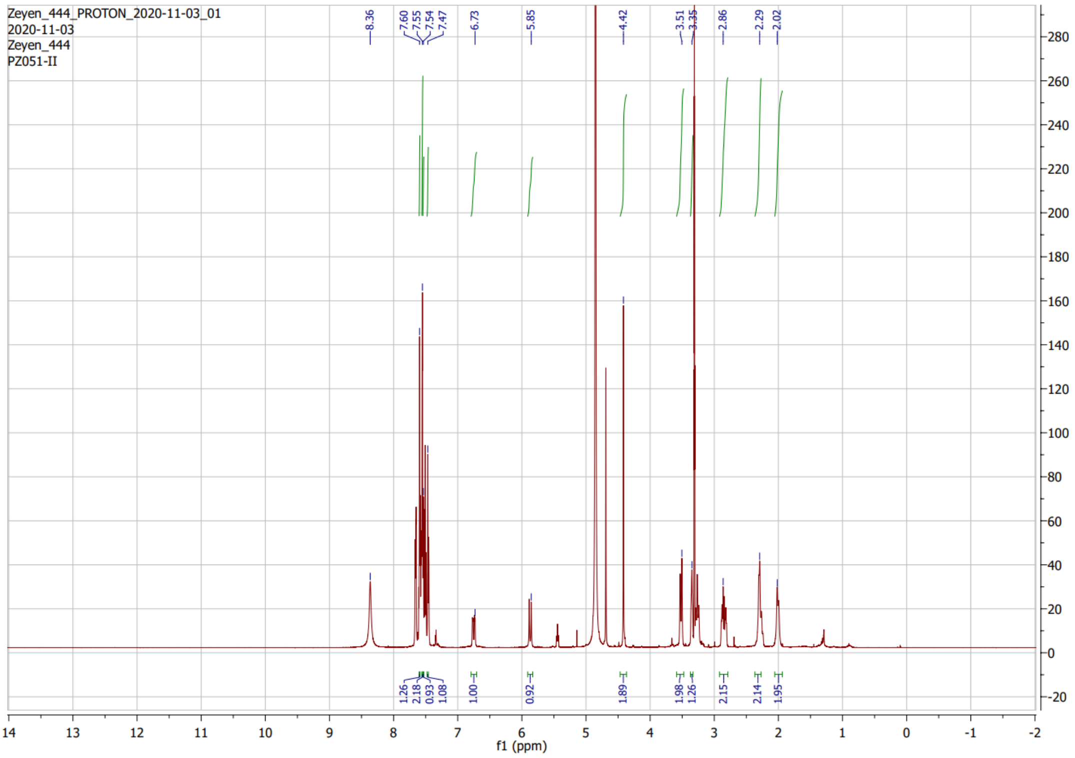


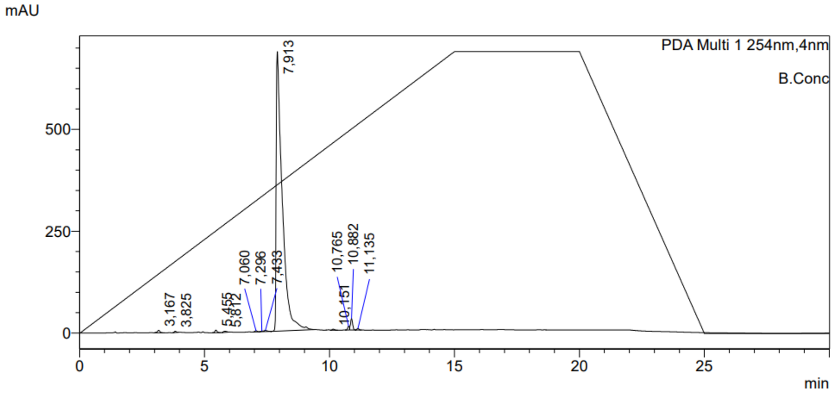
PDA Ch1 254nm

Peak#	Ret. Time	Area	Height	Area%
1	5,464	225687	35371	0,430
2	6,378	52138947	1330955	99,388
3	9,921	87689	6886	0,167
4	13,913	7480	1361	0,014
Total		52459803	1374573	100,000



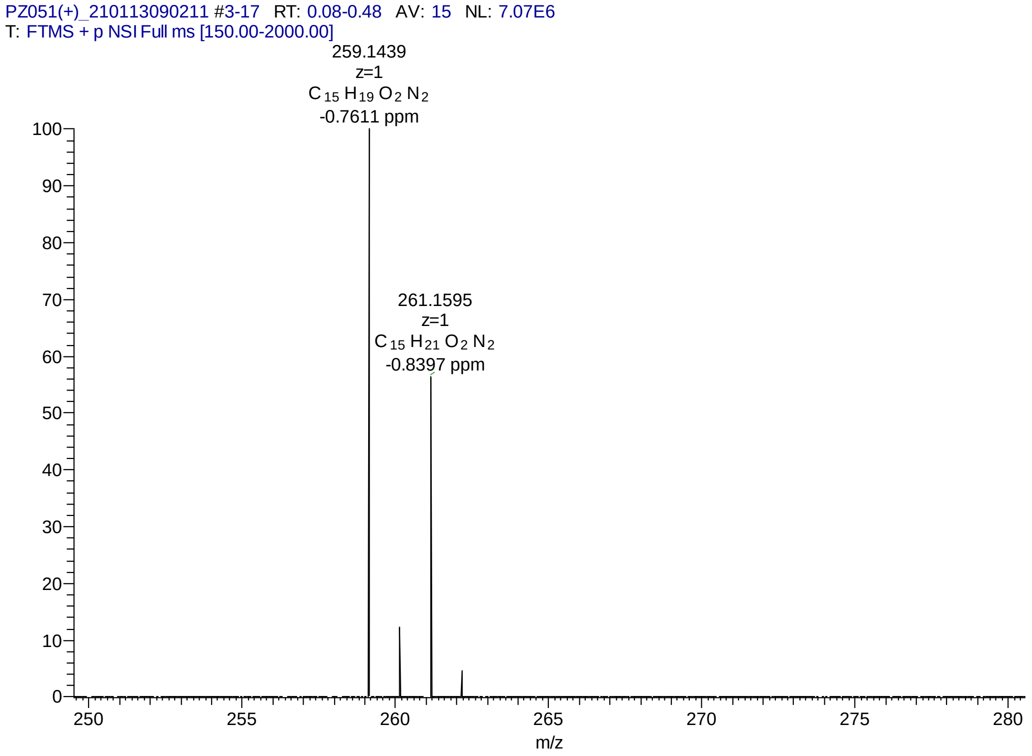
10e



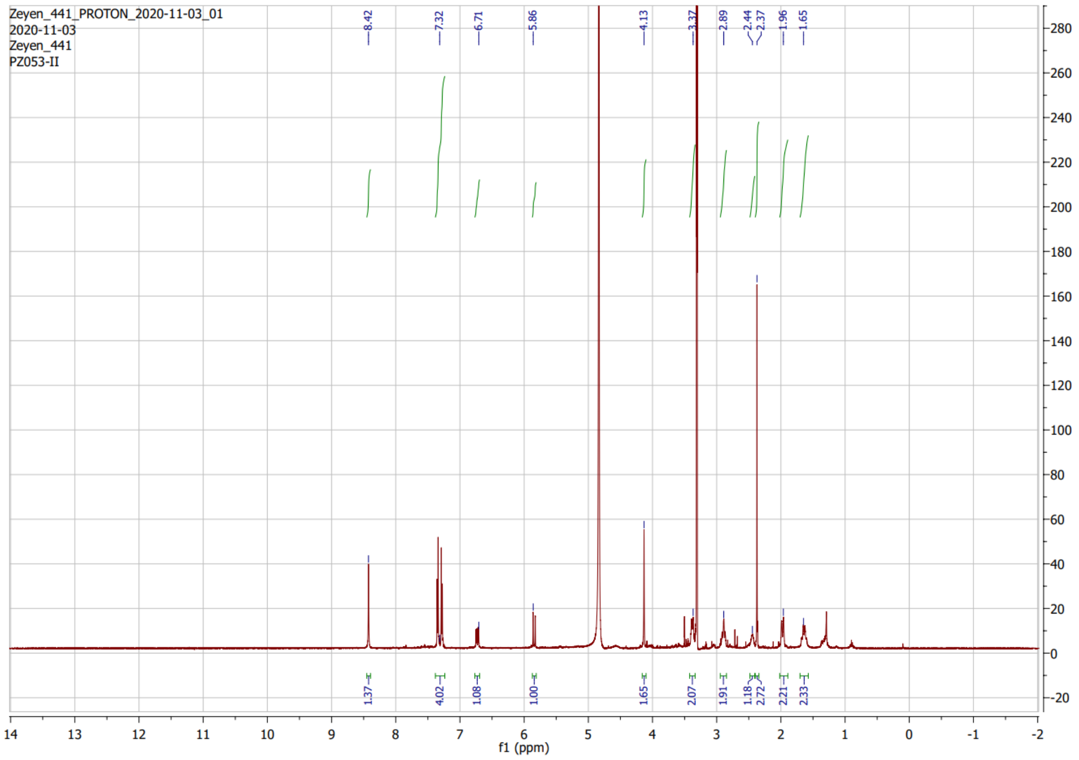


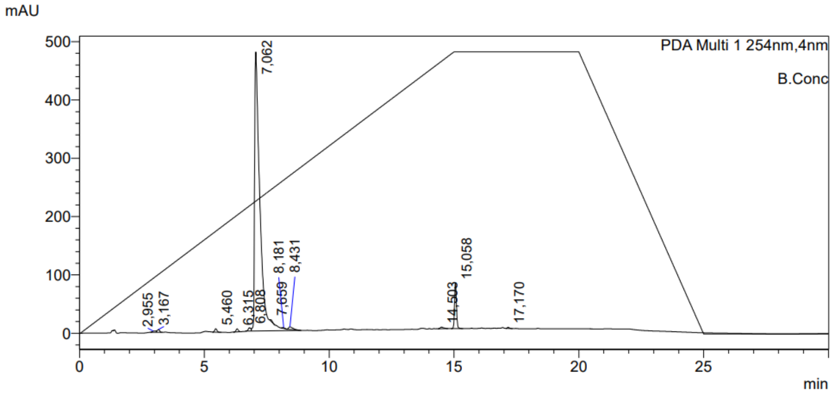
PDA Ch1 254nm

Peak#	Ret. Time	Area	Height	Area%
1	3,167	46538	6287	0,388
2	3,825	17129	3404	0,143
3	5,455	38762	6404	0,323
4	5,812	27129	3047	0,226
5	7,060	21124	3133	0,176
6	7,296	15977	1905	0,133
7	7,433	23503	2874	0,196
8	7,913	11565423	686388	96,382
9	10,151	14657	2326	0,122
10	10,765	46393	9092	0,387
11	10,882	163459	28591	1,362
12	11,135	19496	3839	0,162
Total		11999589	757289	100,000


PZ051(+)_210113090211 #3-17 RT: 0.08-0.48 AV: 15 NL: 7.07E6
T: FTMS + p NSI Full ms [150.00-2000.00]
259.1439
z=1
C 15 H19 O2 N2
-0.7611 ppm
261.1595
z=1
C 15 H21 O2 N2
-0.8397 ppm
100
90
80
70
60
50
40
30
20
10
0
250
255
260
265
270
275
280
m/z

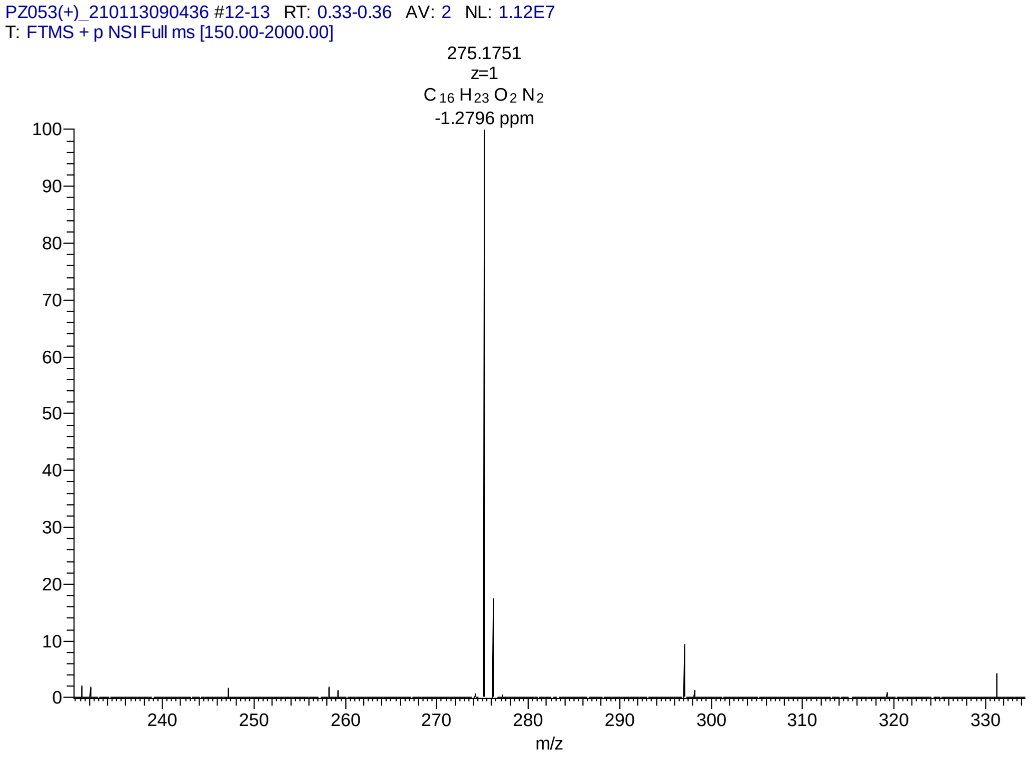
10f


Zeyen_441_PROTON_2020-11-03_01
2020-11-03
Zeyen_441
PZ053-II
8.42
7.32
6.71
5.86
4.13
3.37
2.89
2.44
2.37
1.96
1.65
1.37
4.02
1.08
1.00
1.65
2.07
1.91
1.18
2.72
2.21
2.33
280
260
240
220
200
180
160
140
120
100
80
60
40
20
0
-20
14
13
12
11
10
9
8
7
6
5
4
3
2
1
0
-1
-2
f1 (ppm)

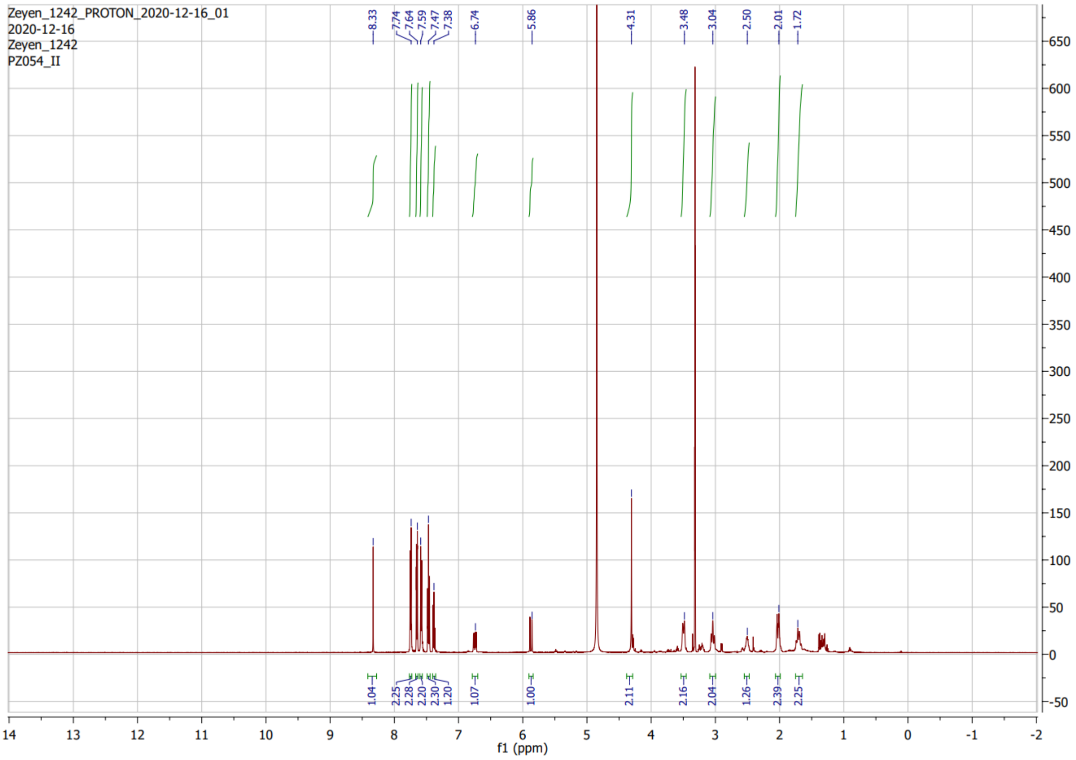


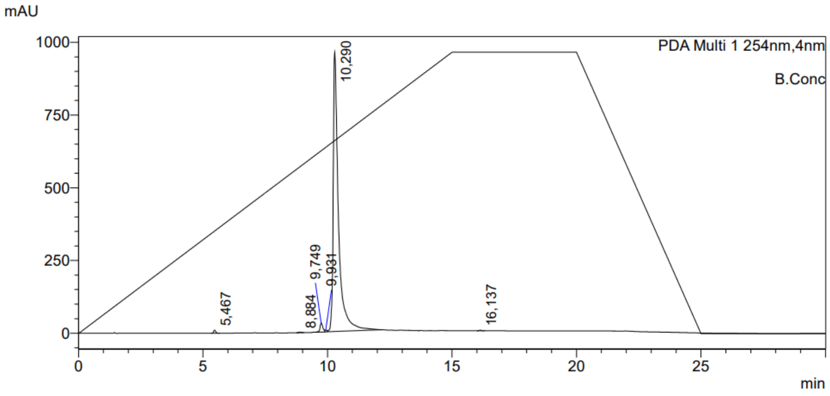
PDA Ch1 254nm

Peak#	Ret. Time	Area	Height	Area%
1	2,955	21576	1876	0,277
2	3,167	32110	4852	0,412
3	5,460	36463	5804	0,468
4	6,315	34223	5248	0,439
5	6,808	41505	5558	0,533
6	7,062	7091610	478695	91,033
7	7,659	14813	2409	0,190
8	8,181	8977	1621	0,115
9	8,431	53105	4388	0,682
10	14,503	23757	2598	0,305
11	15,058	420888	77318	5,403
12	17,170	11151	2074	0,143
Total		7790178	592441	100,000



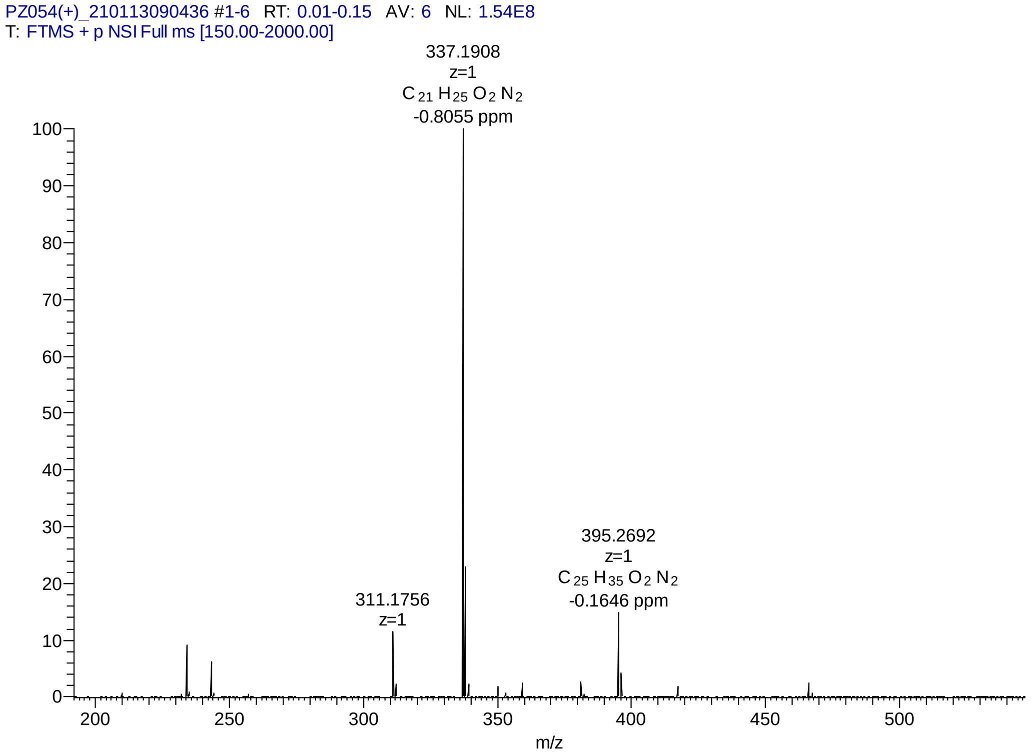
10g



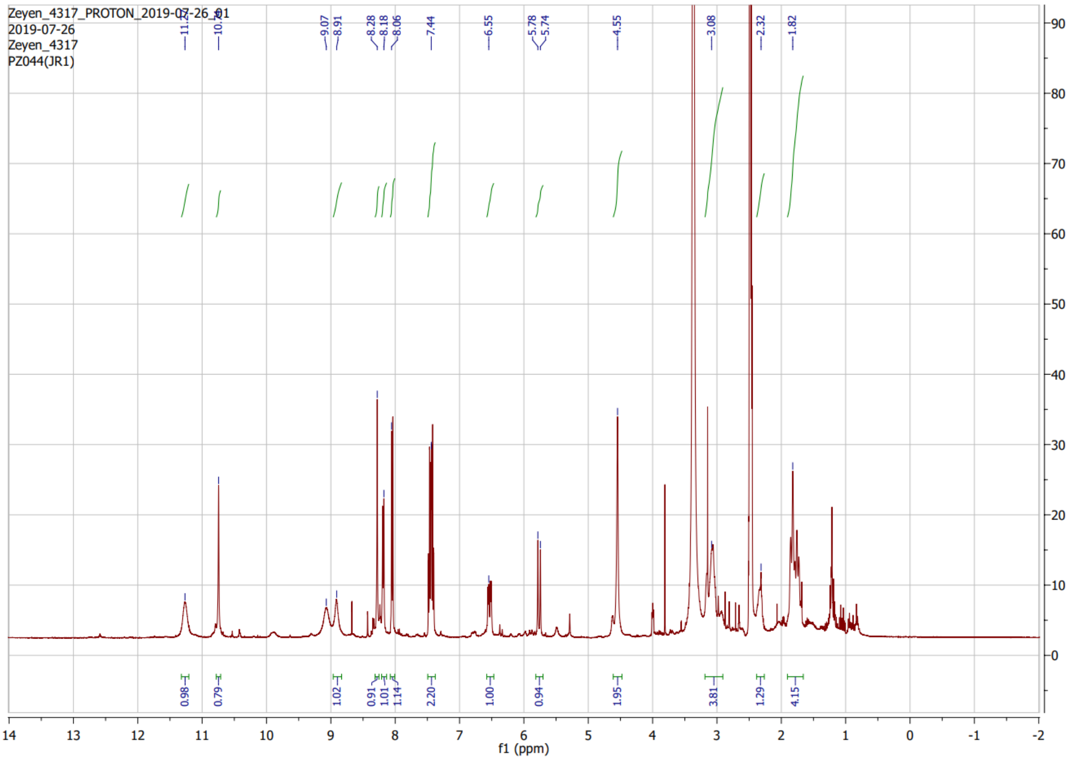


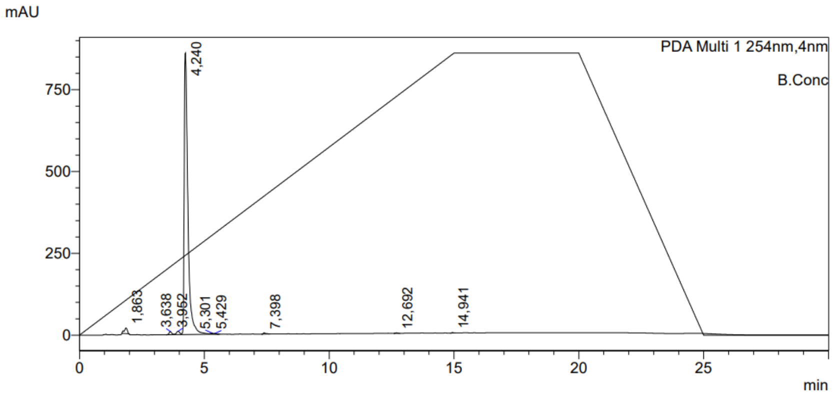
PDA Ch1 254nm

Peak#	Ret. Time	Area	Height	Area%
1	5,467	61540	10399	0,422
2	8,884	13735	1998	0,094
3	9,749	241532	30719	1,658
4	9,931	38304	6166	0,263
5	10,290	14199298	960025	97,473
6	16,137	13061	2030	0,090
Total		14567471	1011338	100,000


PZ054(+)_210113090436 #1-6 RT: 0.01-0.15 AV: 6 NL: 1.54E8
T: FTMS + p NSI Full ms [150.00-2000.00]
337.1908
z=1
$C_{21}H_{25}O_2N_2$
-0.8055 ppm
395.2692
z=1
$C_{25}H_{35}O_2N_2$
-0.1646 ppm
311.1756
z=1
m/z

13a

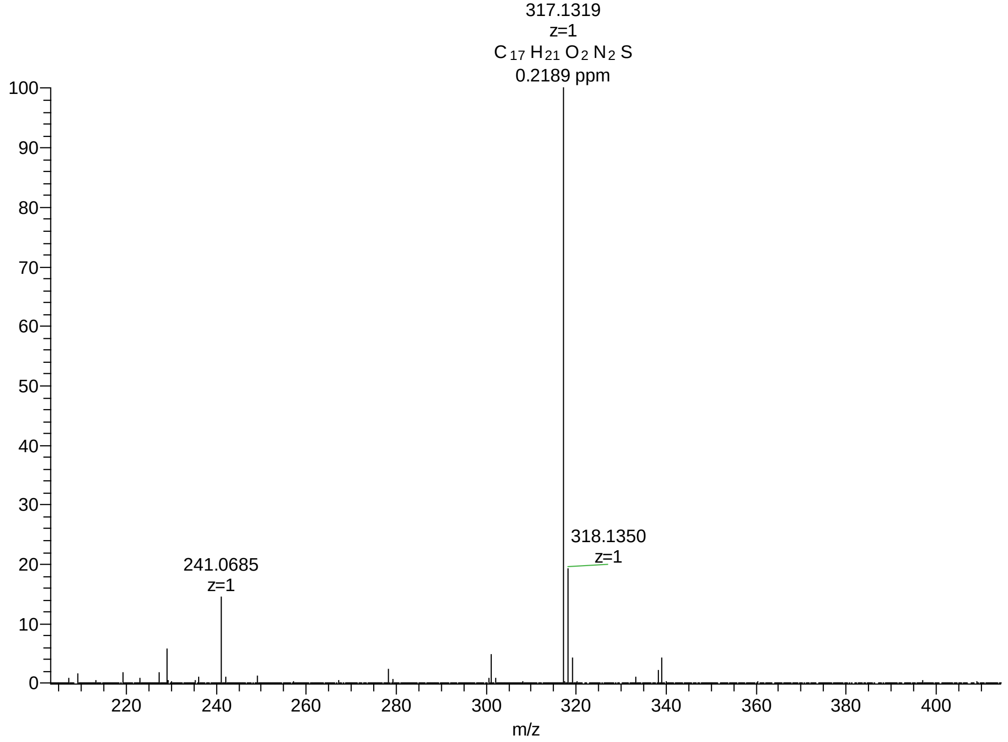

Zeyen_4317_PROTON_2019-07-26_01
2019-07-26
Zeyen_4317
PZ044(JR1)
9.07
8.91
8.28
8.18
8.06
7.44
6.55
5.78
5.74
4.55
3.08
2.32
1.82
0.98
0.79
1.02
0.91
1.01
1.14
2.20
1.00
0.94
1.95
3.81
1.29
4.15
f1 (ppm)



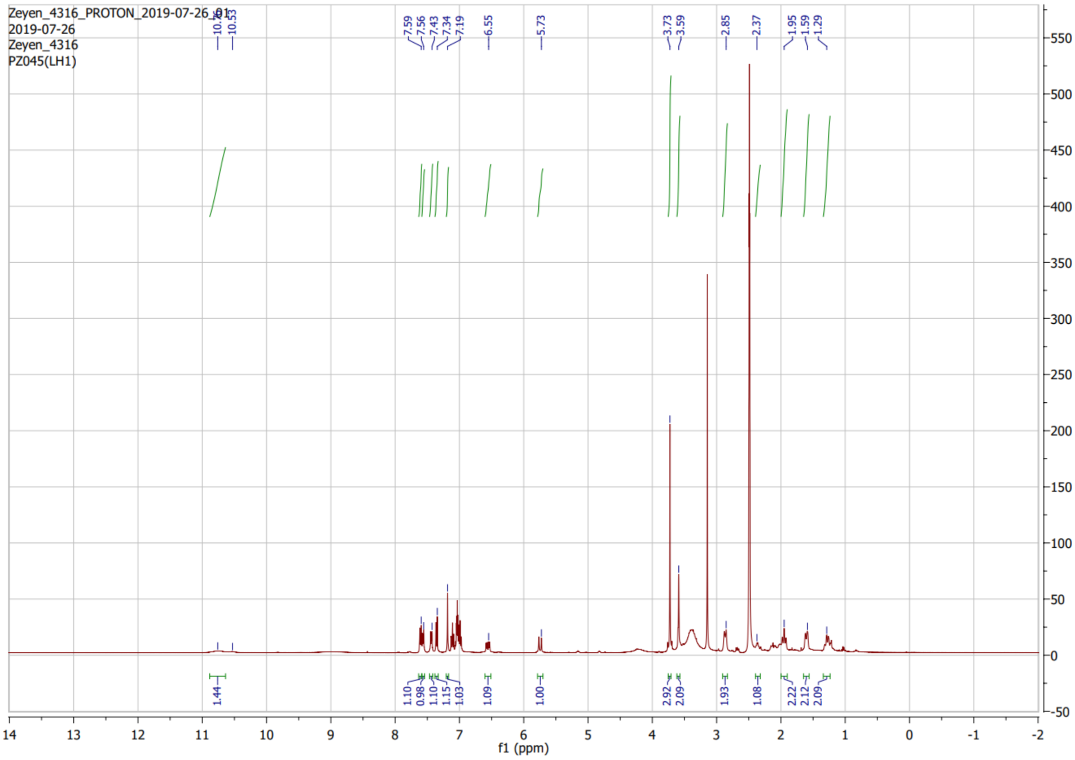
PDA Ch1 254nm

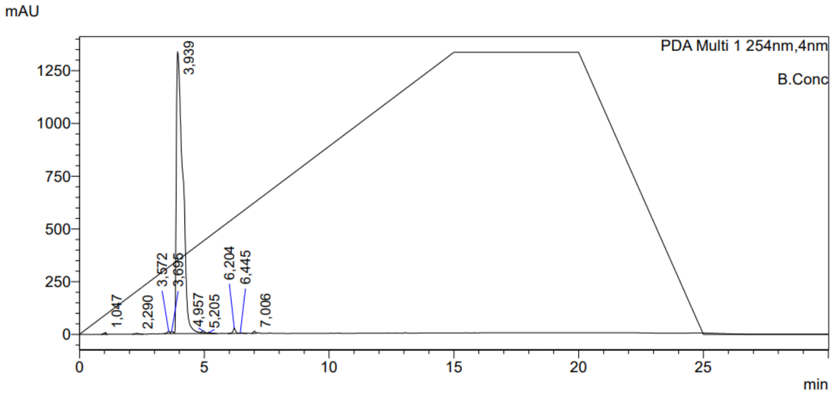
Peak#	Ret. Time	Area	Height	Area%
1	1,863	151215	17296	1,644
2	3,638	70842	10053	0,770
3	3,952	94539	10207	1,028
4	4,240	8813809	859997	95,824
5	5,301	13973	2841	0,152
6	5,429	11876	1968	0,129
7	7,398	27749	4004	0,302
8	12,692	7608	1484	0,083
9	14,941	6288	1577	0,068
Total		9197898	909425	100,000

PZ 044 #1-16 RT: 0.01-0.44 AV: 16 NL: 5.27E7
T: FTMS + c NSI Full ms [150.00-800.00]



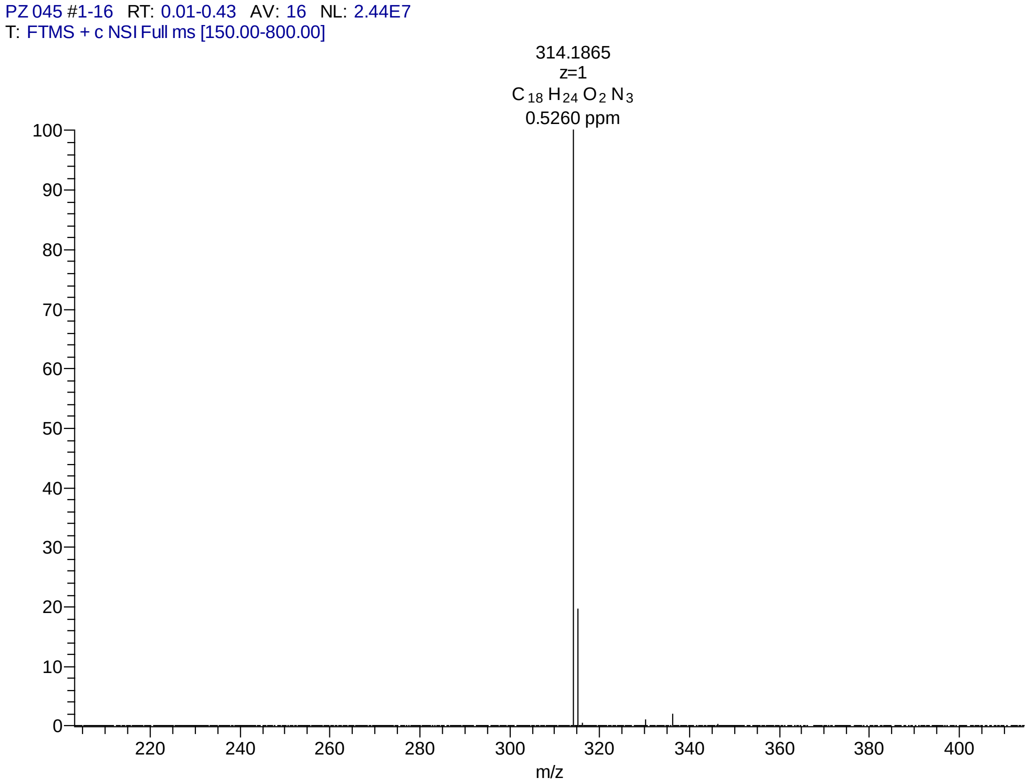
13b



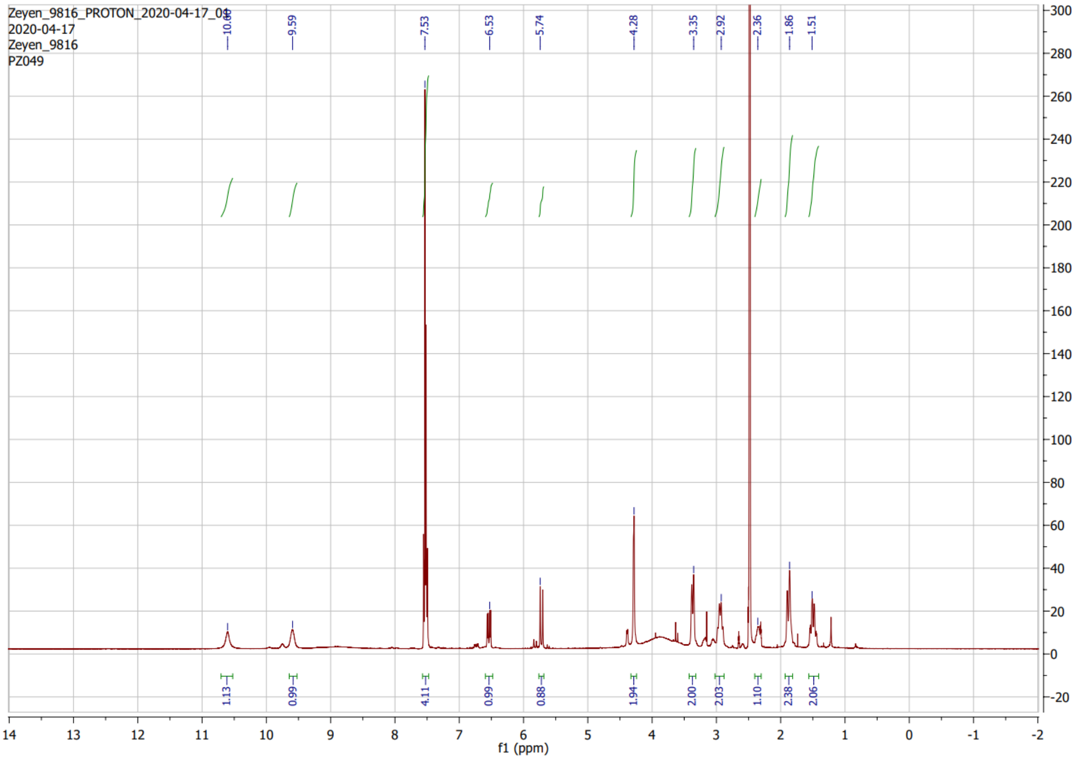


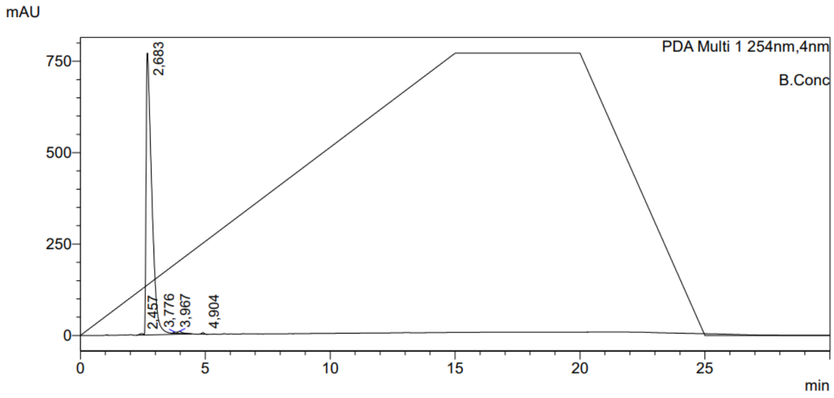
PDA Ch1 254nm

Peak#	Ret. Time	Area	Height	Area%
1	1,047	43796	9514	0,179
2	2,290	36301	3240	0,148
3	3,572	72412	9646	0,296
4	3,695	73037	8499	0,298
5	3,939	23903548	1333536	97,643
6	4,957	56242	8448	0,230
7	5,205	20033	3611	0,082
8	6,204	181677	24940	0,742
9	6,445	28417	2444	0,116
10	7,006	65067	10784	0,266
Total		24480530	1414661	100,000


PZ 045 #1-16 RT: 0.01-0.43 AV: 16 NL: 2.44E7
T: FTMS + c NSI Full ms [150.00-800.00]
314.1865
z=1
$C_{18}H_{24}O_2N_3$
0.5260 ppm
100
90
80
70
60
50
40
30
20
10
0
220
240
260
280
300
320
340
360
380
400
m/z

13c


Zeyen_9816_PROTON_2020-04-17_01
2020-04-17
Zeyen_9816
PZ049
10.60
9.59
7.53
6.53
5.74
4.28
3.35
2.92
2.36
1.86
1.51
1.13
0.99
4.11
0.99
0.88
1.94
2.00
2.03
1.10
2.38
2.06
300
280
260
240
220
200
180
160
140
120
100
80
60
40
20
0
-20
14
13
12
11
10
9
8
7
6
5
4
3
2
1
0
-1
-2
f1 (ppm)



PDA Ch1 254nm

Peak#	Ret. Time	Area	Height	Area%
1	2,457	42056	3905	0,338
2	2,683	12267434	770734	98,591
3	3,776	32634	3541	0,262
4	3,967	68413	5525	0,550
5	4,904	32271	4322	0,259
Total		12442809	788027	100,000

PZ 049 #1-16 RT: 0.00-0.43 AV: 16 NL: 2.93E7
T: FTMS + c NSI Full ms [150.00-800.00]