

Supporting Information

Small Molecule Inhibition of the Archetypal UbiB protein COQ8

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Supplementary Tables

Supplementary Table 1. Inhibition data from Extended Data Figure 1a

Compound	IC ₅₀ (μM)	95% Confidence Interval (μM)
UNC-CA171	0.69	± 0.11
UNC-CA75	0.71	± 0.10
UNC-CA157	0.47	± 0.076
UNC-CA80	0.49	± 0.10
UNC-CA92	1.5	± 0.21
UNC-CA327	1.1	± 0.23
UNC-CA169	1.2	± 0.17
UNC-CA62	1.7	± 0.38

Supplementary Table 2. DSF binding data from Extended Data Figure 1b

Compound	K _{d,app} (μM)	95% Confidence Interval (μM)
UNC-CA171	26	± 9.1
UNC-CA75	15	± 2.7
UNC-CA157	23	± 9.2
UNC-CA80	7.6	± 4.3
UNC-CA92	21	± 6.5
UNC-CA327	27	± 6.6
UNC-CA169	20	± 7.5
UNC-CA62	26	± 9.9

Supplementary Table 3. NanoBRET apparent live cell affinity data from Figure 1e

Compound	COQ8A IC ₅₀ (nM)	COQ8B IC ₅₀ (nM)
PD173955	1080 ± 70	270 ± 30
UNC-CA157	580 ± 40	> 10,000
UNC-CA171	730 ± 40	> 10,000
UNC-CA75	1410 ± 90	> 10,000
UNC-CA81	7000 ± 3000	> 10,000
UNC-CA162	3600 ± 500	> 10,000

Supplementary Table 4. NanoBRET apparent live cell affinity data from Figure 4b

Target	UNC-CA157 IC ₅₀ (nM)	TPP-UNC-CA157 IC ₅₀ (nM)
COQ8A	640 ± 80	>2,000
COQ8B	>10,000	> 10,000
ACVR1	>2,000	> 10,000
GAK	150 ± 20	> 2,000
NLK	42 ± 2	> 2,000
PKN3	>2,000	> 10,000
RIPK2	58 ± 5	> 10,000

Supplementary Table 5. Oligonucleotides used in this study for site directed mutagenesis

Primer Name	Primer Sequence
COQ8A_F336A_F	GAGGAGCGGCCCGCCGCCGCCGCATCCATTG
COQ8A_F336A_R	GATGCGGCGGCCGCGGGCCGCTCCTCGAAGTA
COQ8A_A356G_F	GGCCGCGAGGTGGGCATGAAGATCCAGTAC
COQ8A_A356G_R	CTGGATCTTCATGCCACCTCGCGGCC
COQ8A_K358A_F	GAGGTGGCCATGGCGATCCAGTACCCTGGCGTG
COQ8A_K358A_R	AGGGTACTGGATCGCCATGGCCACCTC
COQ8A_K358E_F	GAGGTGGCCATGGAGATCCAGTACCCTGGC
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COQ8A_L443A_F	AGCCACATGTGGCGACCACAGAGCTG
COQ8A_L443A_R	CAGCTCTGTGGTCGCCACATGTGGGCT
COQ8A_T445A_F	CATGTGCTGACCGCAGAGCTGGTGTCTGGCTTC
COQ8A_T445A_R	AGACACCAGCTCTGCGGTCAGCACATGTGG
COQ8A_L447A_F	CTGACCACAGAGGCGGTGTCTGGCTTCCCC
COQ8A_L447A_R	GAAGCCAGACACCGCCTCTGTGGTCAGCACATG
COQ8A_V448P_F	ACCACAGAGCTGCCGTCTGGCTTCCCC
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COQ8A_F495A_R	CTGGGGGTCATAGGCCGAAGTTGGACCAGTTGGG

COQ8A_F495L_F	TGGTCCAACCTTCctgTATGACCCCCAGCAGCAC
COQ8A_F495L_R	CCCAACTGGTCCAACCTTCctgTATGACCCCCAG
Coq8p_L353F_F	TGGGCAAATTTTctcTATAACGGTAGGACAAAG
Coq8p_L353F_R	CCTACCGTTATAgaaGAAATTTGCCCAATTAGG

Supplementary Table 6. Crystallography data collection and refinement statistics

	7UDP	7UDQ
Data collection		
Space group	C2	P1
Cell dimensions		
a, b, c (Å)	151.21, 56.38, 48.68	48.27, 54.86, 80.01
α, β, γ (°)	90, 98.39, 90	109.79, 94.62, 92.11
Resolution (Å)	37.4 – 2.01 (2.082 – 2.01)*	38.72 – 1.90 (1.97 – 1.90)*
R_{merge}	0.0337 (1.45)	0.0632 (1.10)
$I / \sigma I$	21.35 (1.01)	9.38 (1.01)
Completeness (%)	99.86 (99.93)	96.99 (96.29)
Redundancy	6.7 (6.8)	3.6 (3.7)
Refinement		
Resolution (Å)	37.40 – 2.01	38.72 – 1.90
No. reflections	27131	58805
$R_{\text{work}} / R_{\text{free}}$	0.239 / 0.279	0.199 / 0.238
No. atoms	3096	6354
Protein	3053	6036
Ligand/ion	42	86
Water	18	266
B -factors	88.0	55.6
Protein	88.3	56.0
Ligand/ion	65.7	41.0
Water	65.2	50.9
R.m.s. deviations		
Bond lengths (Å)	0.002	0.006
Bond angles (°)	0.46	0.64

*Values in parentheses are for highest-resolution shell.

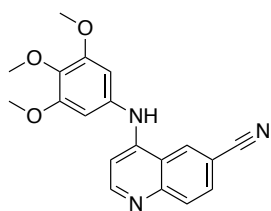
Ramachandran statistics for

7UDP: 94.3% favored, 5.7% allowed, 0% outliers

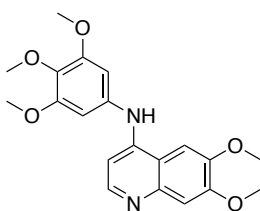
7UDQ: 98.0% favored, 1.9% allowed, 0.14% outliers

7UDP & 7UDQ data collection wavelength 1.033Å, data collection temperature 100K

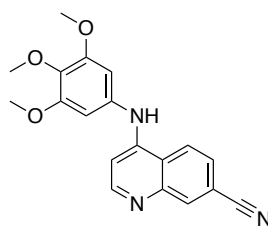
Supplementary Figures



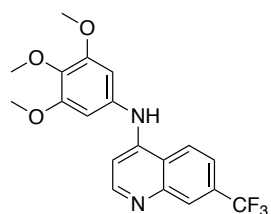
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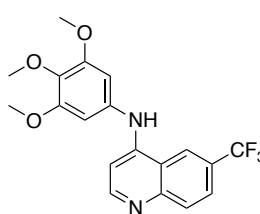
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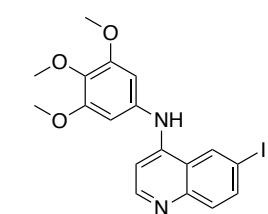
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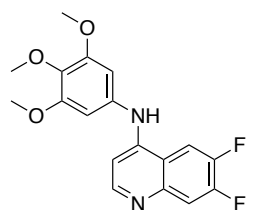
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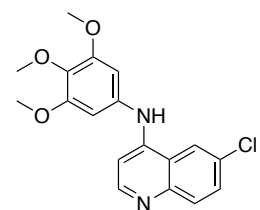
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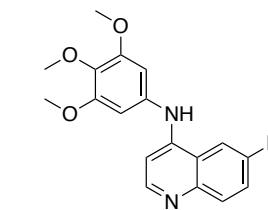
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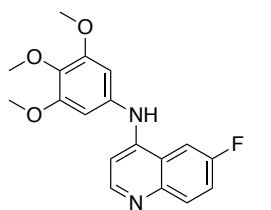
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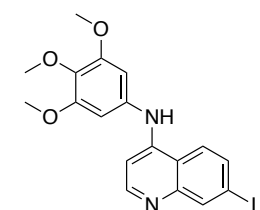
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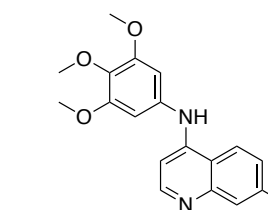
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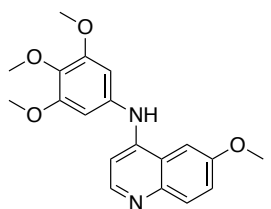
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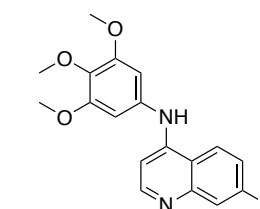
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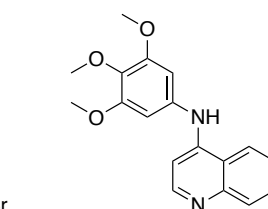
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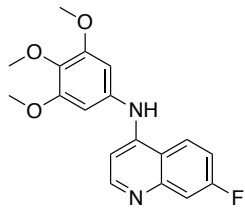
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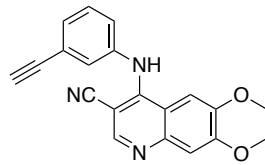
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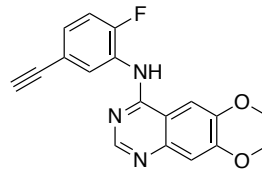
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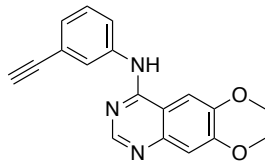
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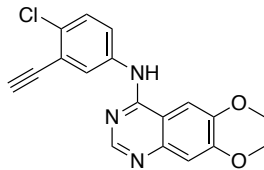
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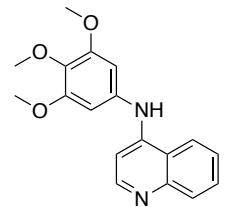
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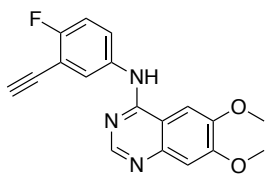
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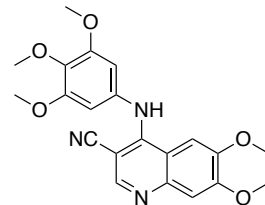
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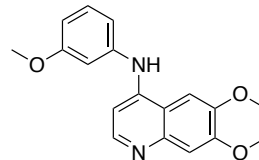
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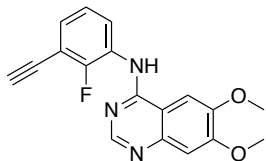
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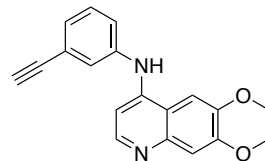
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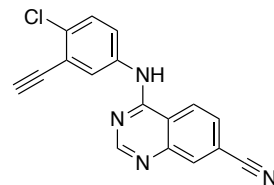
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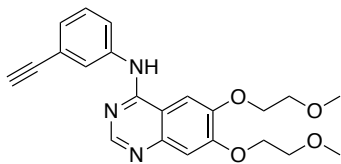
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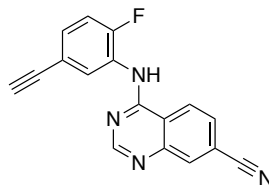
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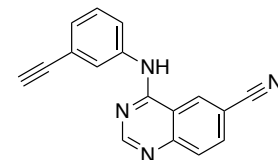
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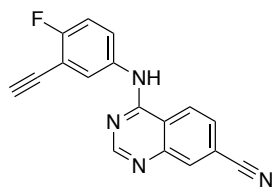
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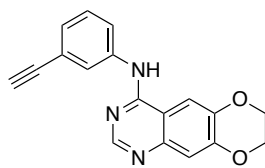
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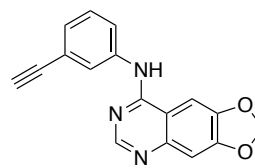
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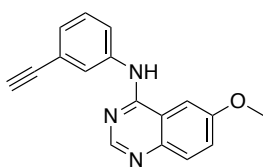
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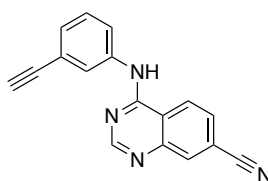
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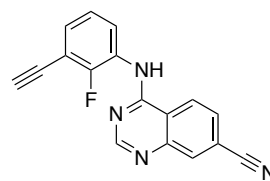
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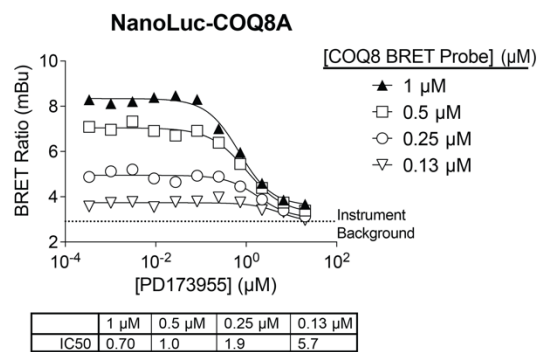
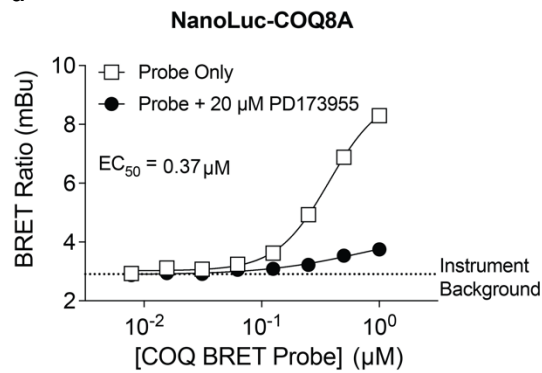
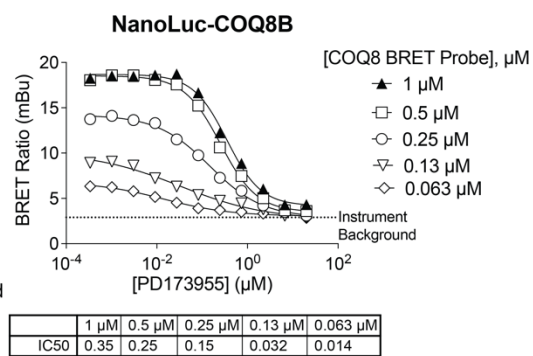
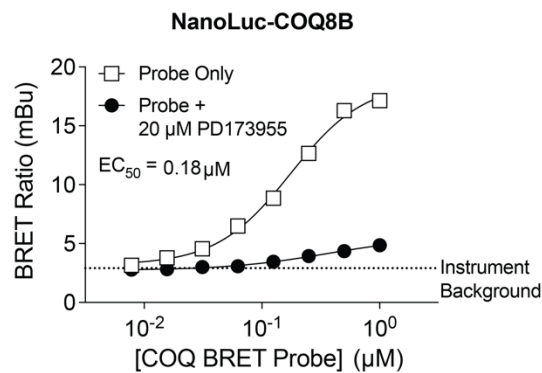


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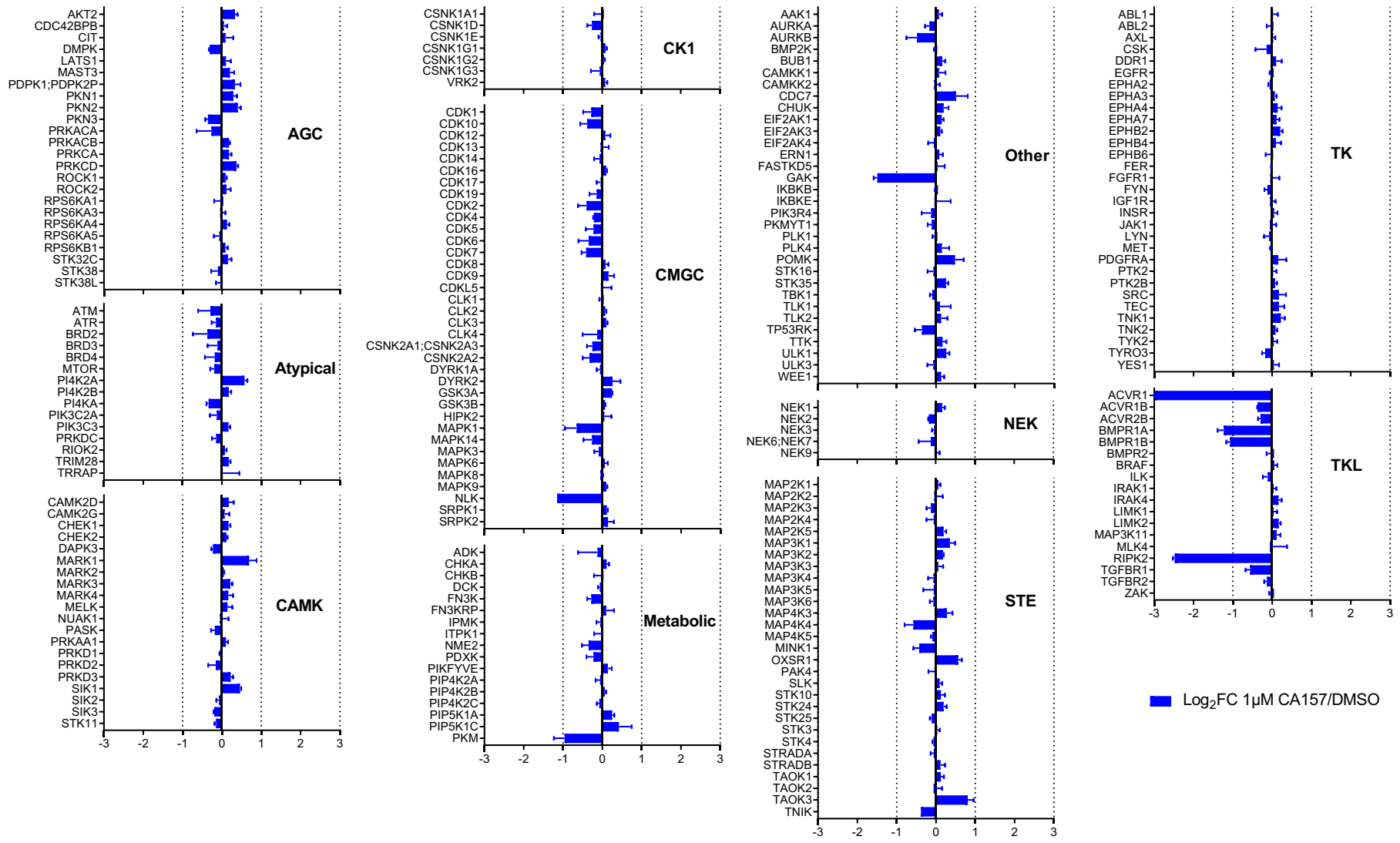


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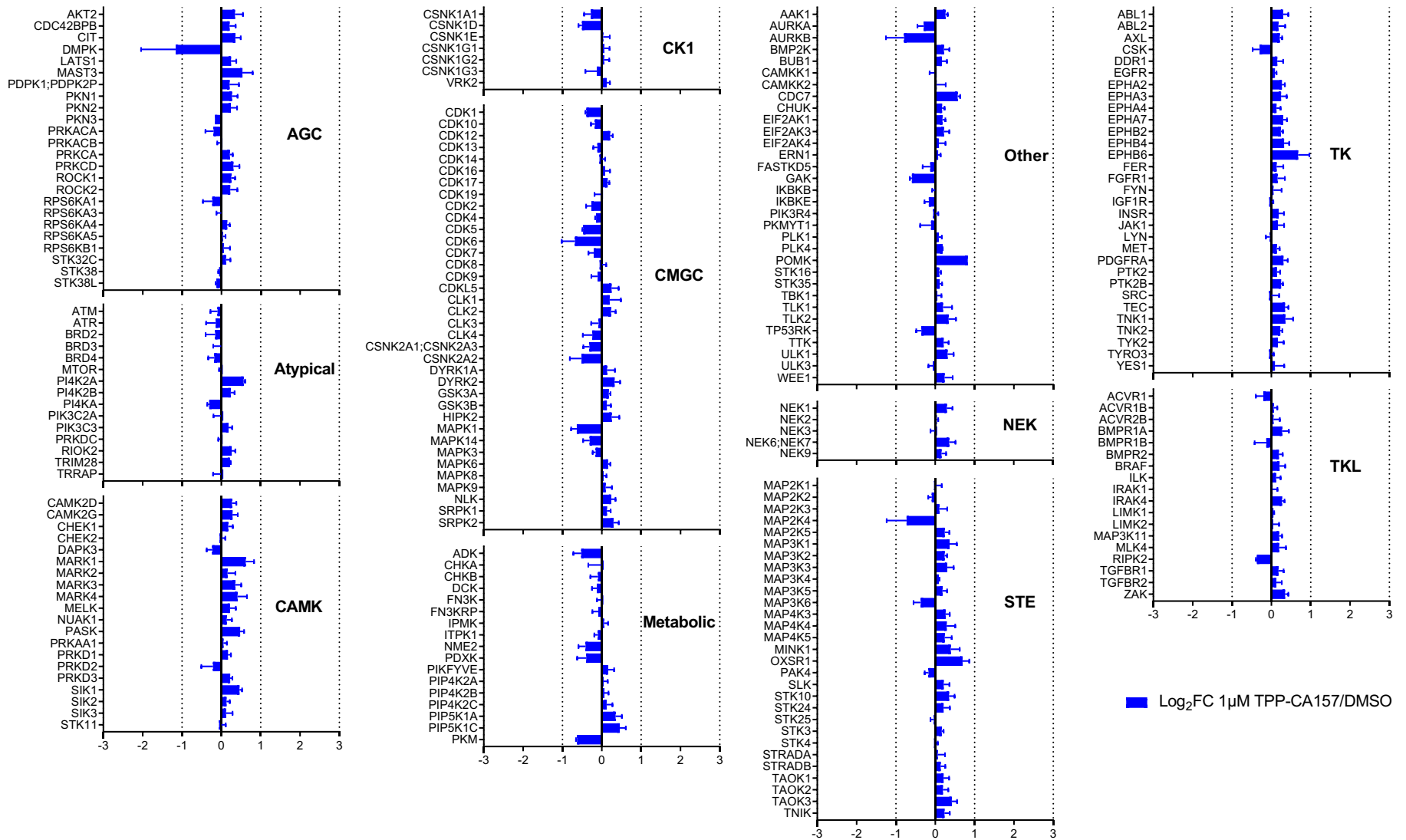
Supplementary Fig. 1 | Chemical structures of the compounds used in Fig. 1a

a**b**

Supplementary Fig. 2 | Validation of the BRET probe for COQ8A and COQ8B in HEK293 cells. Characterization of BRET between (A) NanoLuc-COQ8A and (B) NanoLuc-COQ8B and the COQ8 BRET probe. Probe titrations (left) produced specific BRET as demonstrated via competition with 20 μM PD173955. Individual data points are the mean of technical duplicates (n=1). PD173955 competed the BRET signal in a dose-responsive manner (right). Individual data points are technical singlicates (n=1). The probe concentrations used for subsequent affinity measurements for test compounds are highlighted with black triangles (right).

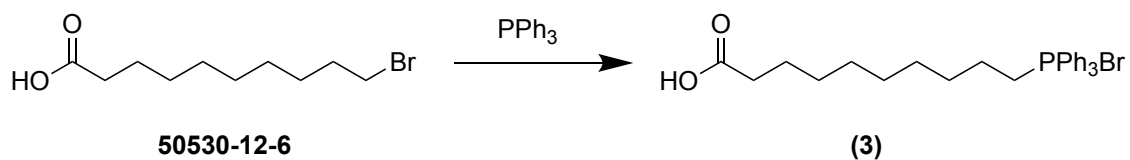


Supplementary Fig. 3 | Full MIBS-MS Profile for UNC-CA157



Supplementary Fig. 4 | Full MIBS-MS Profile for TPP-UNC-CA157

Supplementary Note: TPP-UNC-CA157 Synthesis

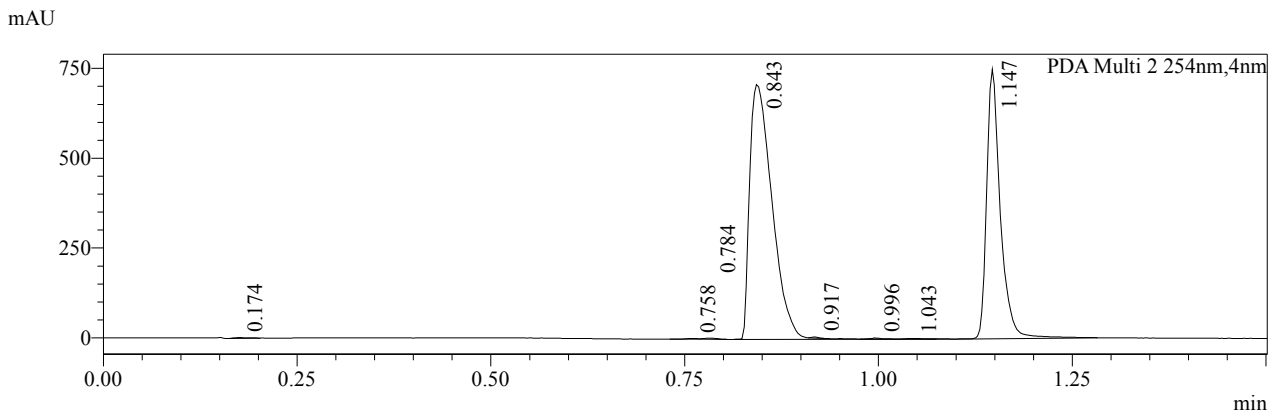
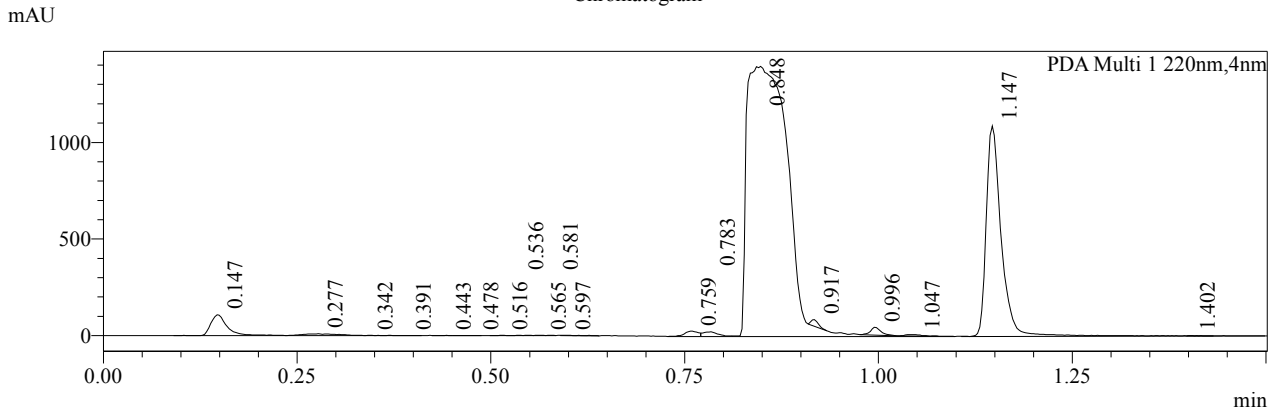


A mixture of 10-bromodecanoic acid (500 mg, 1.99 mmol, 1.00 eq) and PPh₃ (522 mg, 1.99 mmol, 1.00 eq) in MeCN (5.00 mL) was stirred at 90 °C for 70 h. LC-MS (EB3453-2-P1L5) showed the desired MS peak was detected. The mixture was concentrated to give crude compound (3) (1.10 g, 73.2% yield) as a light pink oil and used directly for the next step without further purification (LC-MS EB3453-2-P1A1).

LCMS REPORT

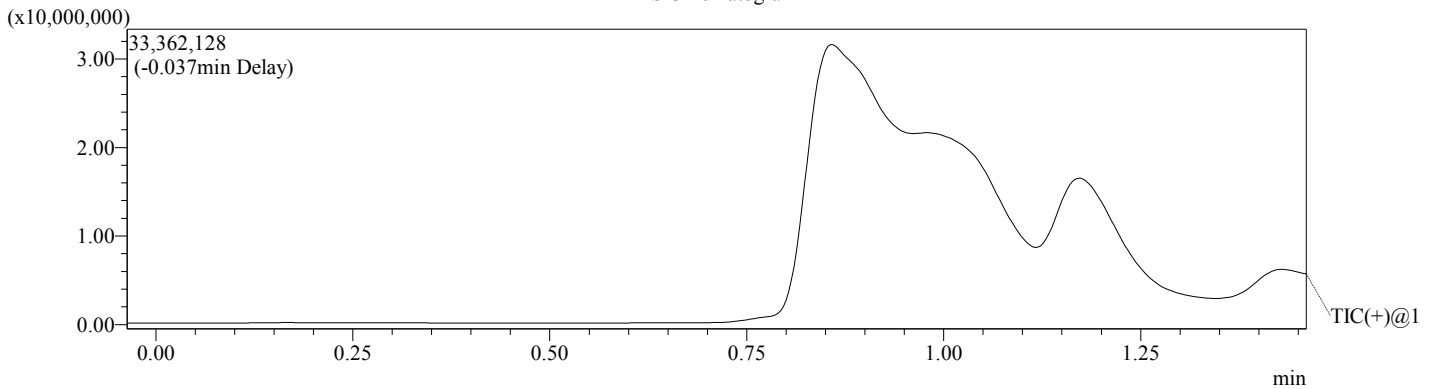
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Chromolith Flash RP-18, 5um,3.0*25mm

Chromatogram



- 1 PDA Multi 1 / 220nm,4nm
- 2 PDA Multi 2 / 254nm,4nm

MS Chromatogram



Integration Result

PDA Ch1 220nm

Peak#	Ret. Time	Height	Height%	USP Width	Area	Area%
1	0.147	108235	3.930	0.041	161442	2.336
2	0.277	9497	0.345	0.096	32294	0.467
3	0.342	2081	0.076	0.028	2786	0.040
4	0.391	1079	0.039	0.018	1445	0.021
5	0.443	1562	0.057	0.014	1111	0.016
6	0.478	1243	0.045	0.051	1837	0.027
7	0.516	1188	0.043	0.029	1051	0.015
8	0.536	1965	0.071	0.017	1240	0.018
9	0.565	2420	0.088	0.045	3040	0.044
10	0.581	1973	0.072	0.045	1023	0.015
11	0.597	2386	0.087	0.057	4086	0.059
12	0.759	27068	0.983	0.050	34544	0.500
13	0.783	23292	0.846	0.069	30304	0.439
14	0.848	1397055	50.728	0.081	5079105	73.507
15	0.917	34694	1.260	0.019	19673	0.285
16	0.996	39633	1.439	0.028	37682	0.545
17	1.047	7550	0.274	0.038	11078	0.160
18	1.147	1089692	39.568	0.038	1484392	21.483
19	1.402	1377	0.050	0.051	1555	0.023

PDA Ch2 254nm

Peak#	Ret. Time	Height	Height%	USP Width	Area	Area%
1	0.174	2401	0.163	0.036	2829	0.120
2	0.758	2472	0.168	0.491	2958	0.125
3	0.784	3245	0.220	0.044	3940	0.167
4	0.843	708910	48.085	0.057	1420317	60.142
5	0.917	2604	0.177	0.021	1406	0.060
6	0.996	3171	0.215	0.026	2983	0.126
7	1.043	1913	0.130	0.042	3037	0.129
8	1.147	749566	50.843	0.035	924120	39.131

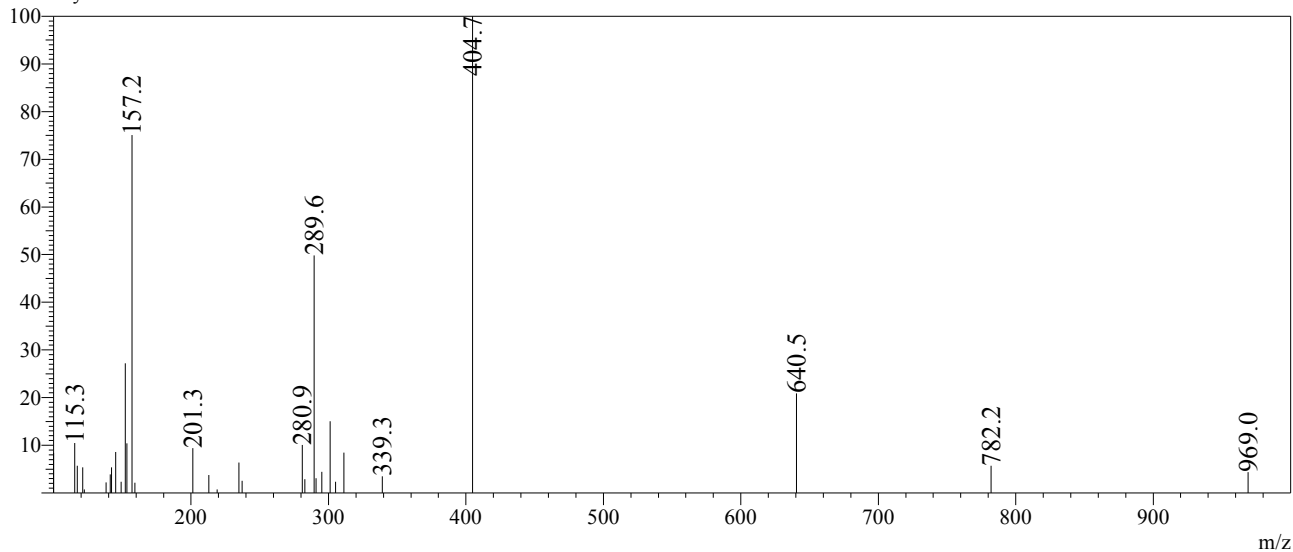
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Mass Spectrum

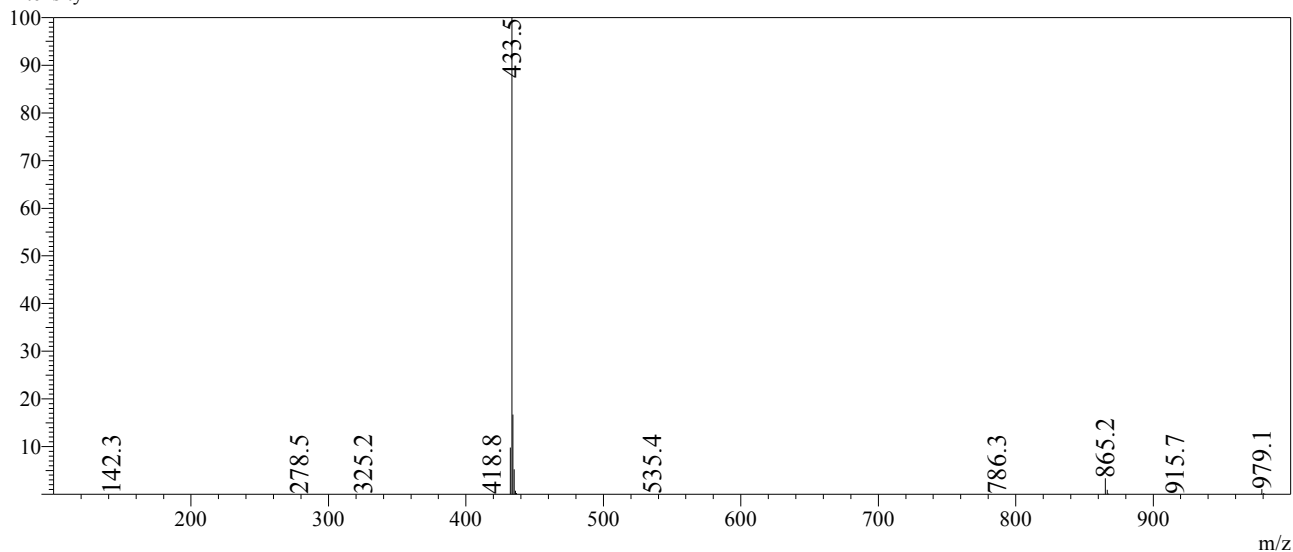
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Intensity



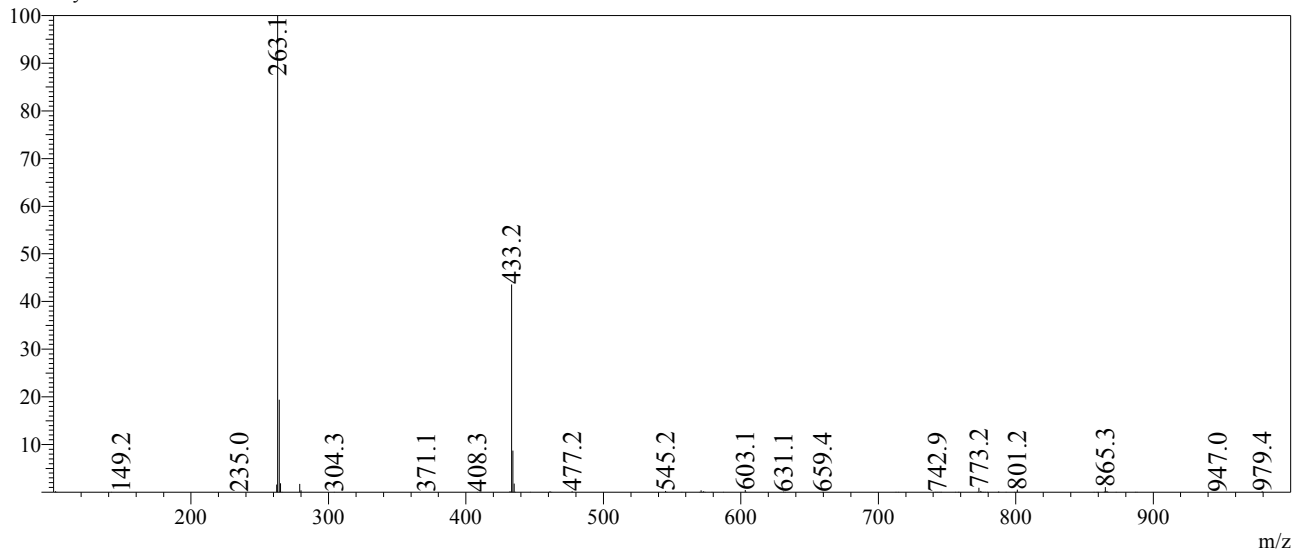
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Intensity



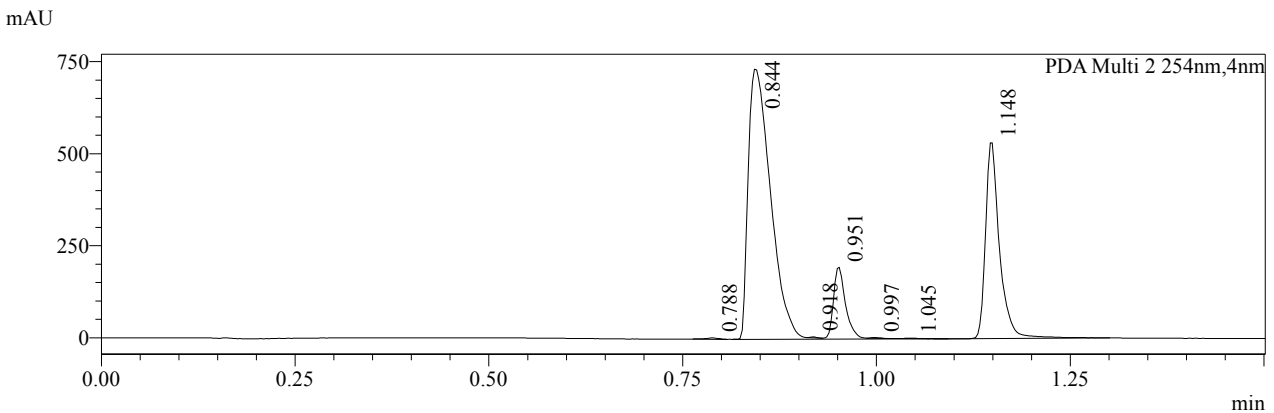
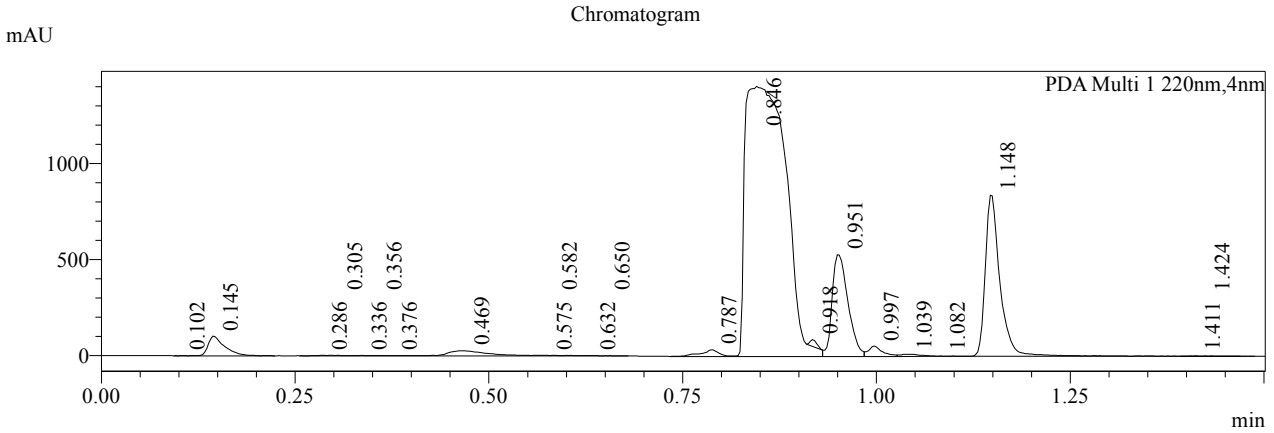
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Intensity

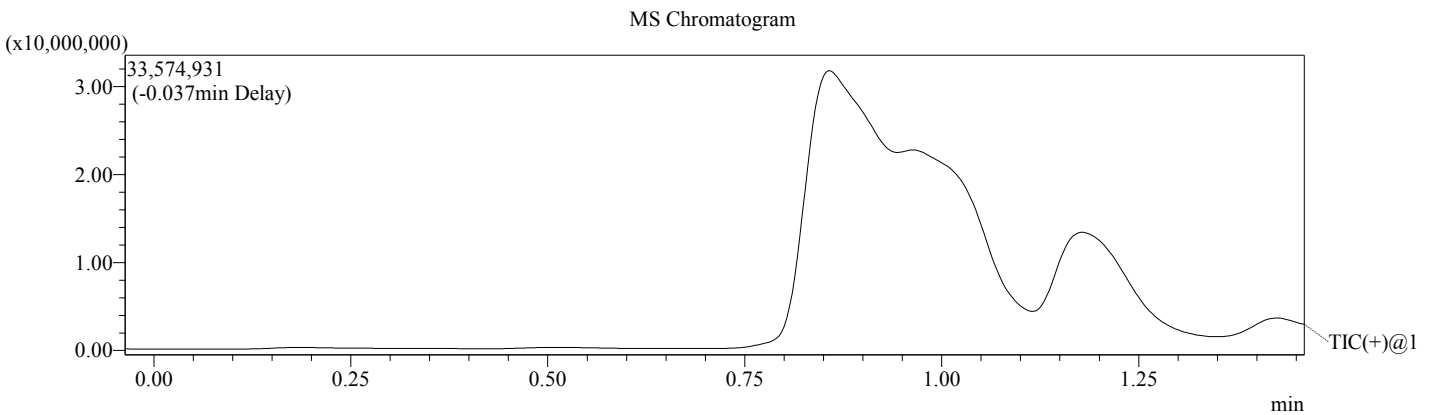


LCMS REPORT

Print time : 12/16/2020 15:02:03
Compound ID : 1
Sample ID : EB3453-2-P1A1
Injection Date : 12/16/2020 3:00:20 PM
Injection Vol : 2ul
Location : tray1 vail52
Acq Method : 5-95AB 1.5min 220&254 Shimadzu All.lcm
Org DataFile : D:\DATA\2020\2012\201216\EB3453-2-P1A1.lcd
Instrument & column: LCMS-SAW 1-2402
Chromolith Flash RP-18, 5um,3.0*25mm



- 1 PDA Multi 1 / 220nm,4nm
- 2 PDA Multi 2 / 254nm,4nm



Integration Result

PDA Ch1 220nm

Peak#	Ret. Time	Height	Height%	USP Width	Area	Area%
1	0.102	1705	0.055	0.009	1064	0.014
2	0.145	102222	3.327	0.042	150981	2.024
3	0.286	2815	0.092	0.057	3503	0.047
4	0.305	3463	0.113	0.065	5676	0.076
5	0.336	2992	0.097	0.061	2324	0.031
6	0.356	2153	0.070	0.018	1199	0.016
7	0.376	2235	0.073	0.050	3169	0.042
8	0.469	27121	0.883	0.094	101118	1.355
9	0.575	4194	0.137	0.066	5021	0.067
10	0.582	3680	0.120	0.106	8221	0.110
11	0.632	2437	0.079	0.051	2282	0.031
12	0.650	1733	0.056	0.133	1766	0.024
13	0.787	35117	1.143	0.038	56653	0.759
14	0.846	1404560	45.718	0.081	5128500	68.740
15	0.918	36998	1.204	0.019	19504	0.261
16	0.951	529232	17.226	0.041	755442	10.126
17	0.997	54070	1.760	0.037	66817	0.896
18	1.039	11525	0.375	0.059	21083	0.283
19	1.082	1746	0.057	0.040	2082	0.028
20	1.148	839200	27.316	0.037	1120789	15.022
21	1.411	1491	0.049	0.038	1471	0.020
22	1.424	1545	0.050	0.046	2088	0.028

PDA Ch2 254nm

Peak#	Ret. Time	Height	Height%	USP Width	Area	Area%
1	0.788	3905	0.266	0.037	5029	0.212
2	0.844	733198	49.868	0.057	1469731	61.975
3	0.918	2693	0.183	0.017	1369	0.058
4	0.951	194557	13.233	0.032	217540	9.173
5	0.997	2455	0.167	0.023	1908	0.080
6	1.045	1401	0.095	0.035	1916	0.081
7	1.148	532055	36.188	0.035	673986	28.420

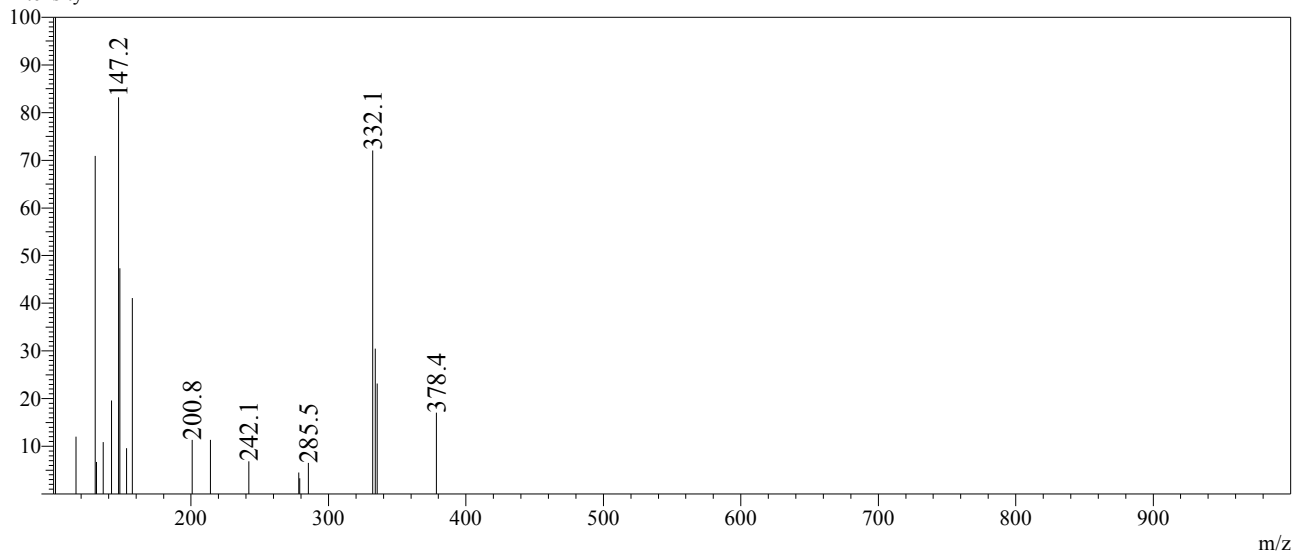
Operator: _____

Date: _____

Mass Spectrum

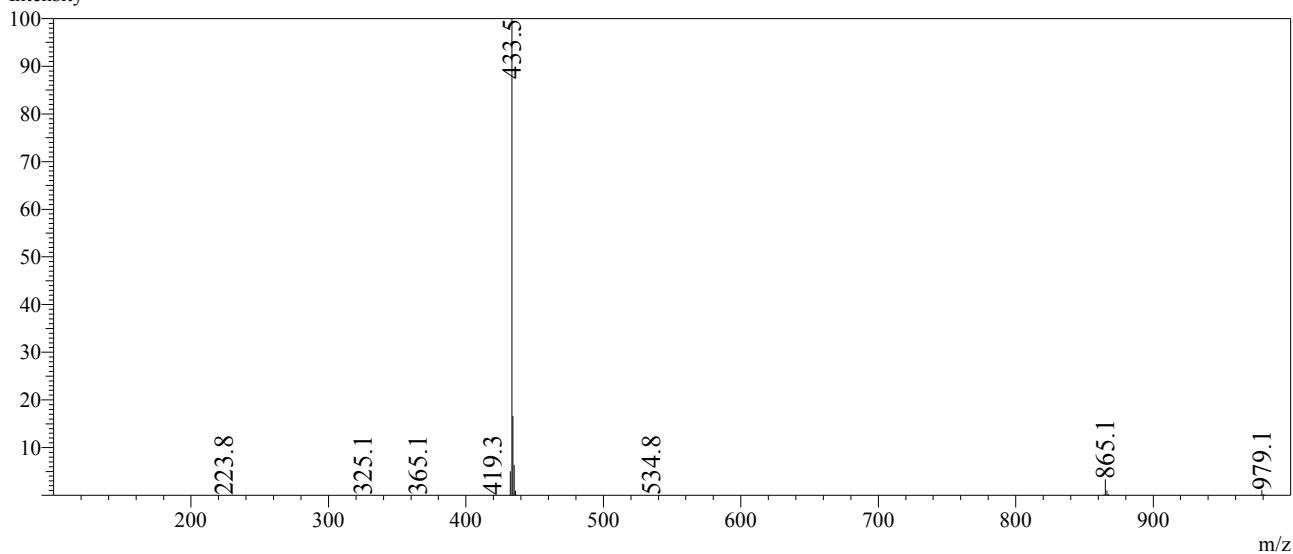
RefTime: 0.143 Datafile: D:\DATA\2020\2012\201216\EB3453-2-P1A1.lcd

Intensity



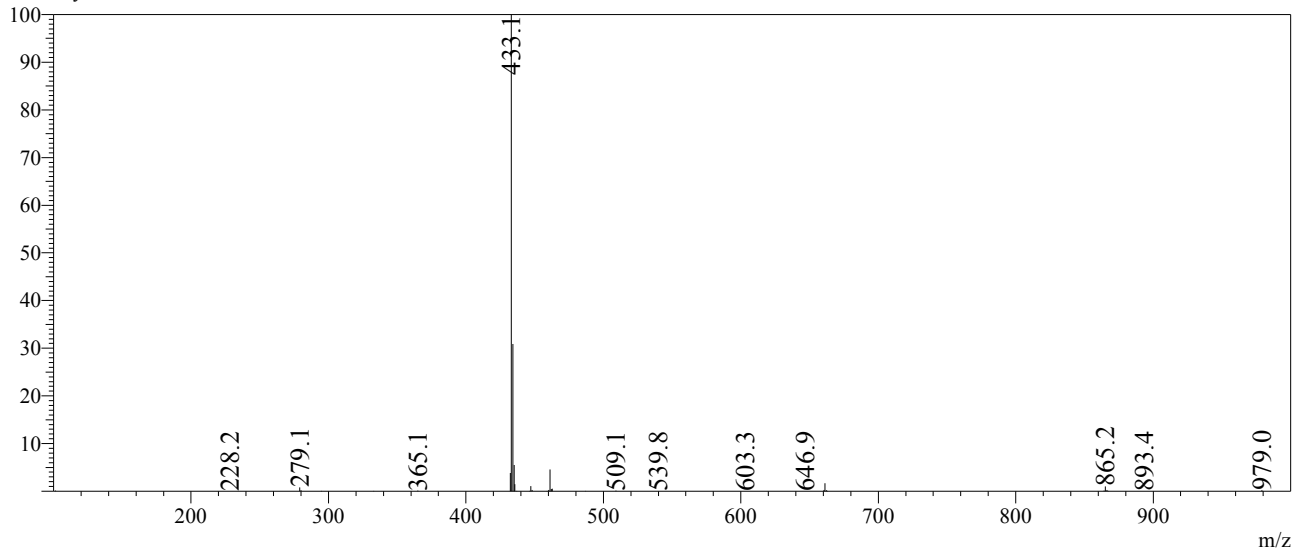
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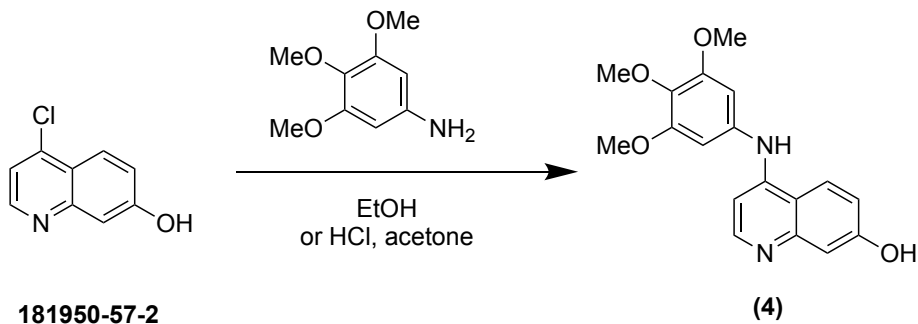
Intensity



RefTime: 0.950 Datafile: D:\DATA\2020\2012\201216\EB3453-2-P1A1.lcd

Intensity





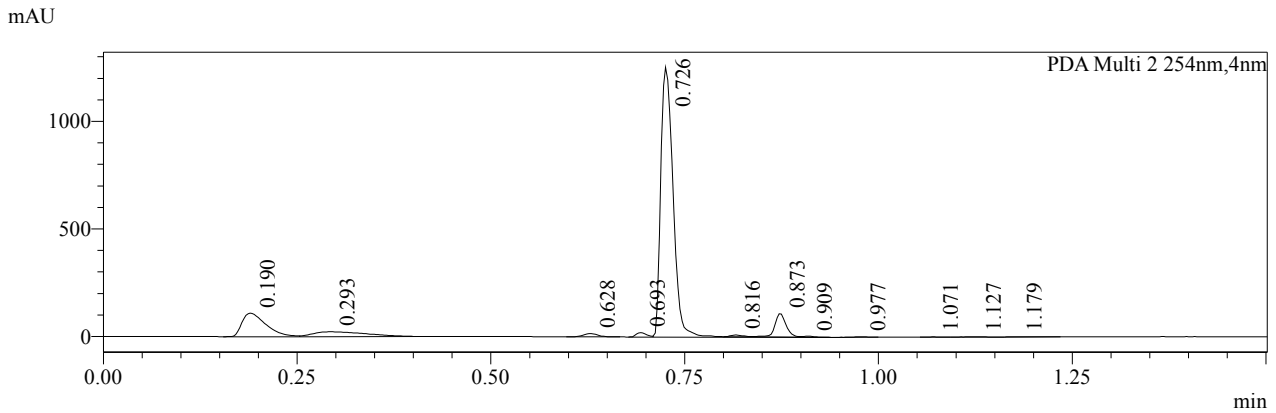
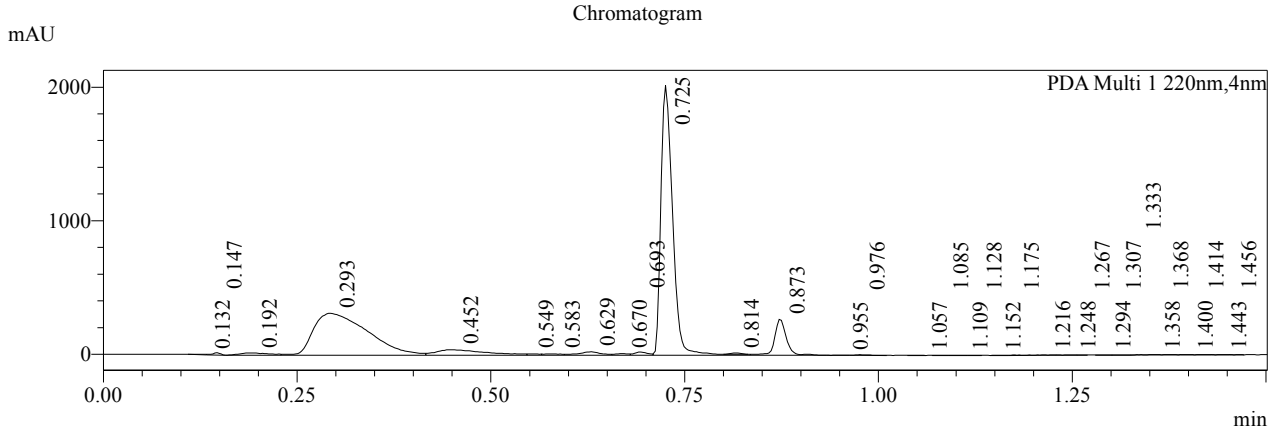
HCl (12.0 M, 1.00 mL, 4.31 eq) was added to a solution of 4-chloroquinolin-7-ol (500 mg, 2.78 mmol, 1.00 eq) and 3,4,5-trimethoxyaniline (2.04 g, 11.1 mmol, 4.00 eq) in acetone (30.0 mL). The mixture was stirred at 75 °C for 16 h. LC-MS (EB3453-3-P1L2) clearly showed the desired MS peak. The reaction was cooled to room temperature, poured into ice water, and sodium hydroxide solution was added to adjust pH to 9 (2 M NaOH). The sample was then extracted with ethyl acetate (100 mL*3). The organic phase was dried over Na₂SO₄, filtered and concentrated to dryness under reduced pressure. The residue was purified by flash silica gel chromatography (ISCO®; 12.0 g Sepa Flash® Silica Flash Column, Eluent of 0~75% Ethyl acetate/Petroleum ether gradient @ 45 mL/min) to afford Compound **4** (700 mg, 2.12 mmol, 76.3% yield) as a light yellow solid. LCMS (EB3453-3-P1C1) and NMR (EB3453-3-P1N2)

¹H NMR: (400 MHz, MeOD)

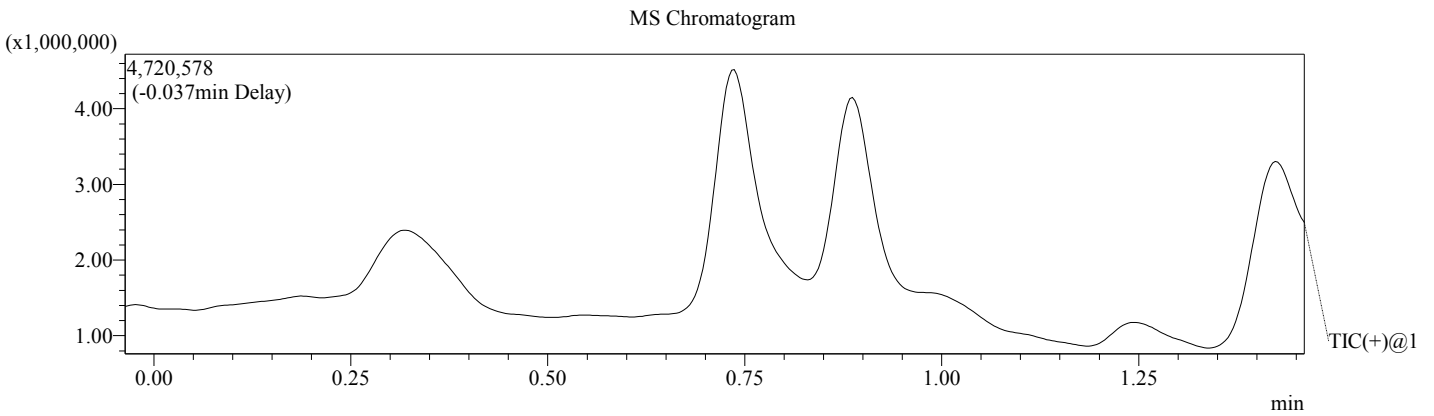
δ: 8.00-8.07 (m, 2H), 6.95-7.02 (m, 1H), 6.80-6.85 (m, 1H), 6.63-6.69 (m, 2H), 6.56-6.62 (m, 1H), 3.81 (s, 6H), 3.76 (s, 3H).

LCMS REPORT

Print time : 12/16/2020 11:08:38
Compound ID : 1
Sample ID : EB3453-3-P1L2
Injection Date : 12/16/2020 11:06:54 AM
Injection Vol : 3ul
Location : tray1 vail66
Acq Method : 5-95AB 1.5min 220&254 Shimadzu All.lcm
Org DataFile : D:\DATA\2020\2012\201216\EB3453-3-P1L2.lcd
Instrument & column: LCMS-SAW 1-2402
Chromolith Flash RP-18, 5um,3.0*25mm



- 1 PDA Multi 1 / 220nm,4nm
- 2 PDA Multi 2 / 254nm,4nm



Integration Result

PDA Ch1 220nm

Peak#	Ret. Time	Height	Height%	USP Width	Area	Area%
1	0.132	4390	0.155	0.000	3537	0.081
2	0.147	18092	0.639	0.022	12277	0.279
3	0.192	18235	0.644	0.099	49592	1.129
4	0.293	314814	11.113	0.132	1534325	34.920
5	0.452	42097	1.486	0.123	190695	4.340
6	0.549	10705	0.378	0.089	11229	0.256
7	0.583	10190	0.360	0.144	16943	0.386
8	0.629	26464	0.934	0.060	55177	1.256
9	0.670	12587	0.444	0.085	15670	0.357
10	0.693	25814	0.911	0.041	30335	0.690
11	0.725	2020450	71.321	0.032	2127222	48.414
12	0.814	10439	0.369	0.031	10374	0.236
13	0.873	268760	9.487	0.030	285959	6.508
14	0.955	1786	0.063	0.028	1488	0.034
15	0.976	5162	0.182	0.044	6805	0.155
16	1.057	1415	0.050	0.056	1052	0.024
17	1.085	2379	0.084	0.027	2045	0.047
18	1.109	1829	0.065	0.041	2032	0.046
19	1.128	1971	0.070	0.027	1754	0.040
20	1.152	2282	0.081	0.008	1992	0.045
21	1.175	2522	0.089	0.069	4524	0.103
22	1.216	2756	0.097	0.024	2703	0.062
23	1.248	3066	0.108	0.046	3046	0.069
24	1.267	2830	0.100	0.026	1833	0.042
25	1.294	2883	0.102	0.041	2881	0.066
26	1.307	2590	0.091	0.027	2980	0.068
27	1.333	2528	0.089	0.022	1438	0.033
28	1.358	2485	0.088	0.083	3731	0.085
29	1.368	2719	0.096	0.058	2884	0.066
30	1.400	2554	0.090	0.032	2243	0.051
31	1.414	2456	0.087	0.028	2027	0.046
32	1.443	2053	0.072	0.039	1976	0.045
33	1.456	1612	0.057	0.008	1084	0.025

PDA Ch2 254nm

Peak#	Ret. Time	Height	Height%	USP Width	Area	Area%
1	0.190	110033	7.101	0.060	238411	12.499
2	0.293	22440	1.448	0.131	105033	5.506
3	0.628	15795	1.019	0.039	20575	1.079
4	0.693	20720	1.337	0.029	18938	0.993
5	0.726	1253347	80.886	0.033	1394772	73.122
6	0.816	8005	0.517	0.034	8763	0.459
7	0.873	108827	7.023	0.029	108944	5.712
8	0.909	4836	0.312	0.035	4843	0.254
9	0.977	2442	0.158	0.032	2612	0.137
10	1.071	1100	0.071	0.035	1364	0.071
11	1.127	1348	0.087	0.049	2160	0.113
12	1.179	637	0.041	0.031	1040	0.055

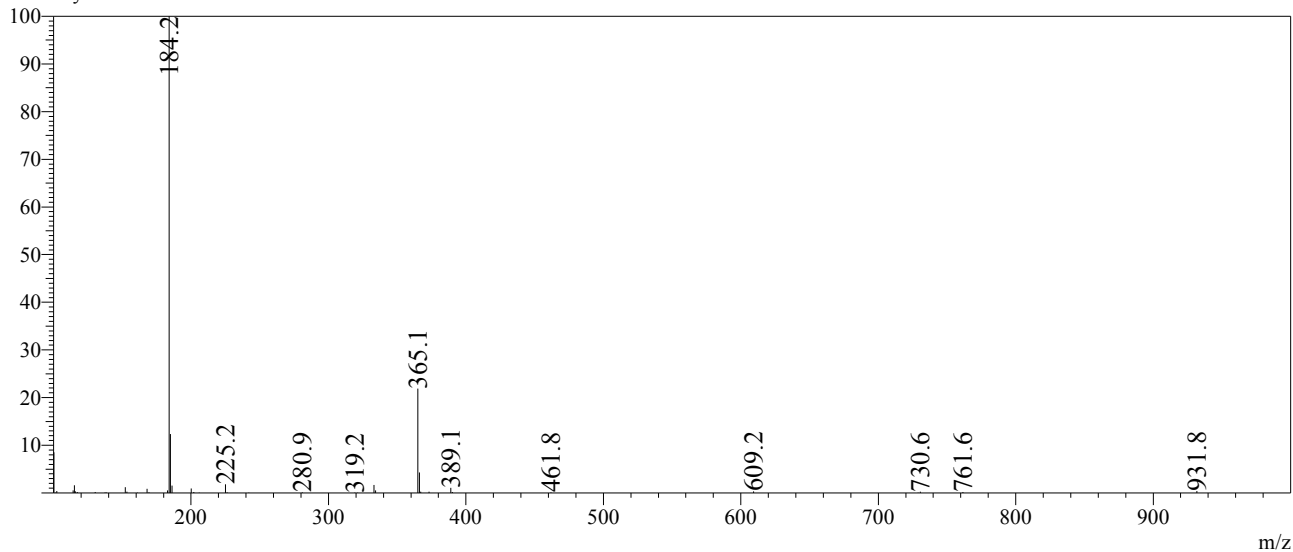
Operator: _____

Date: _____

Mass Spectrum

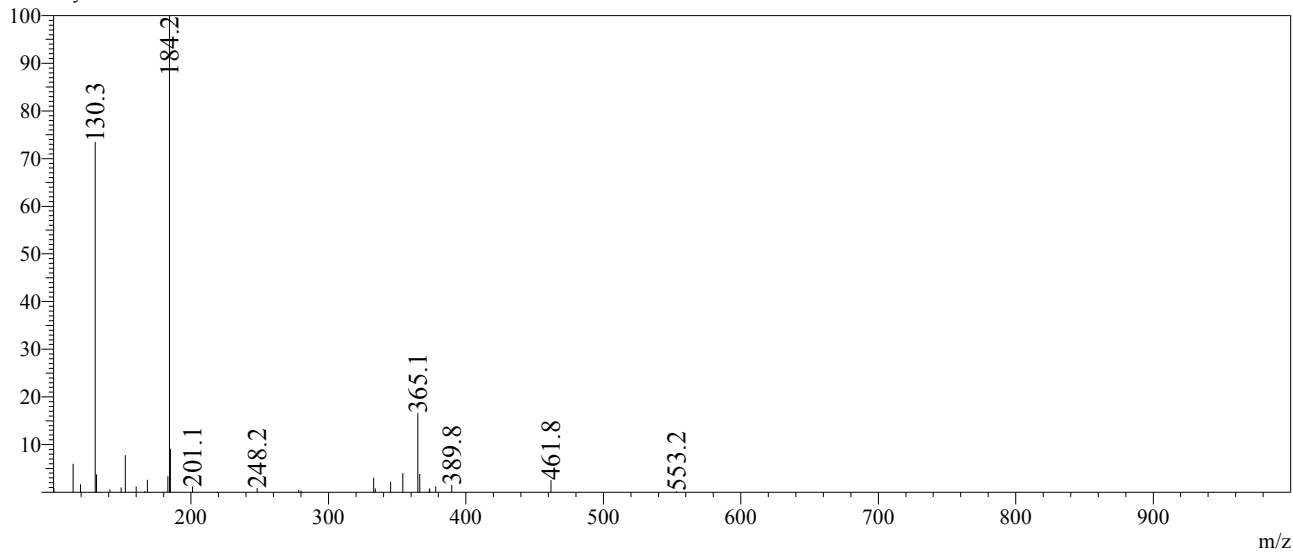
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Intensity



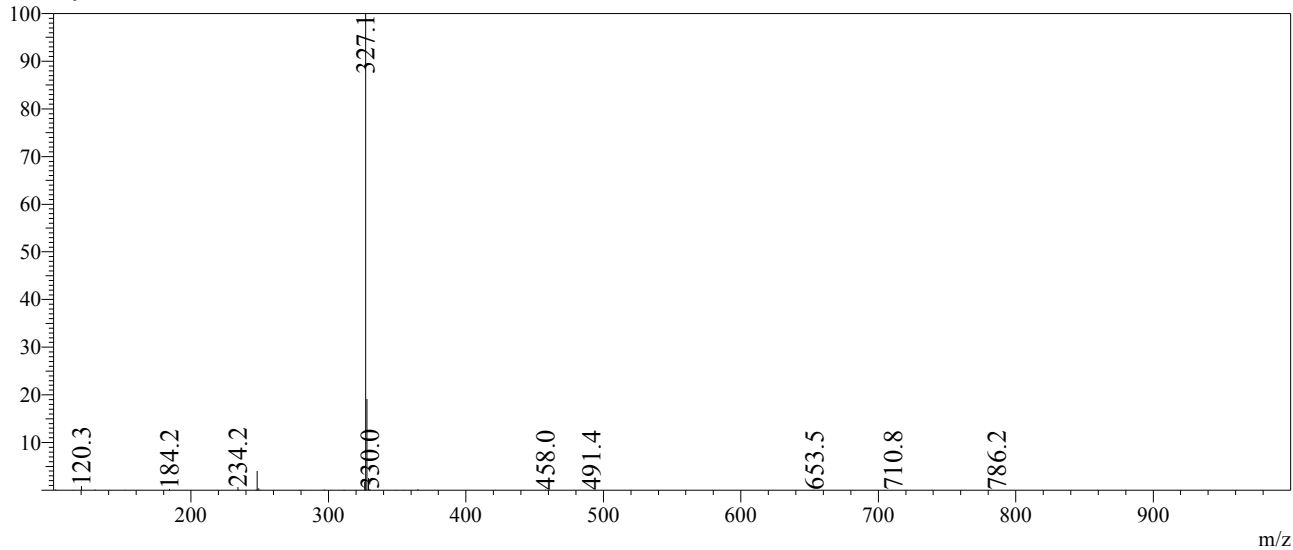
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Intensity



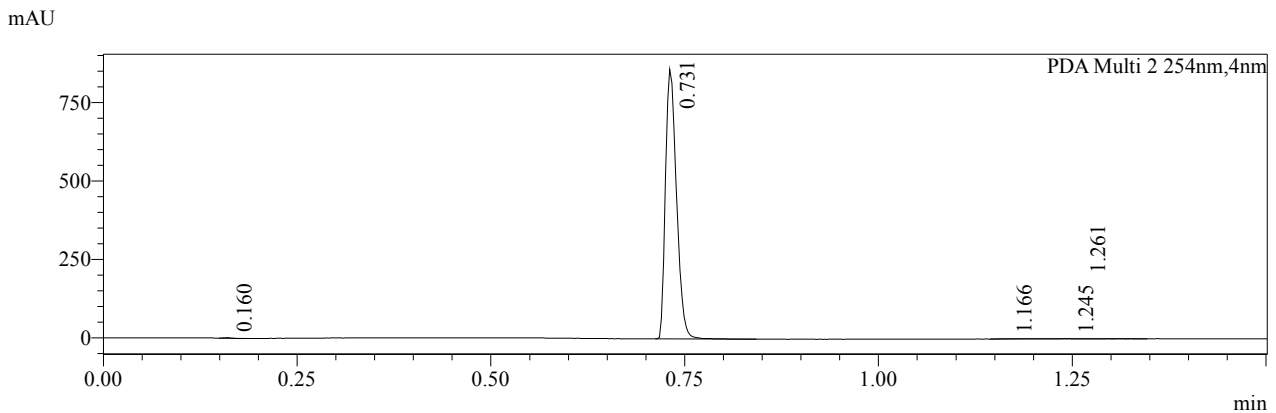
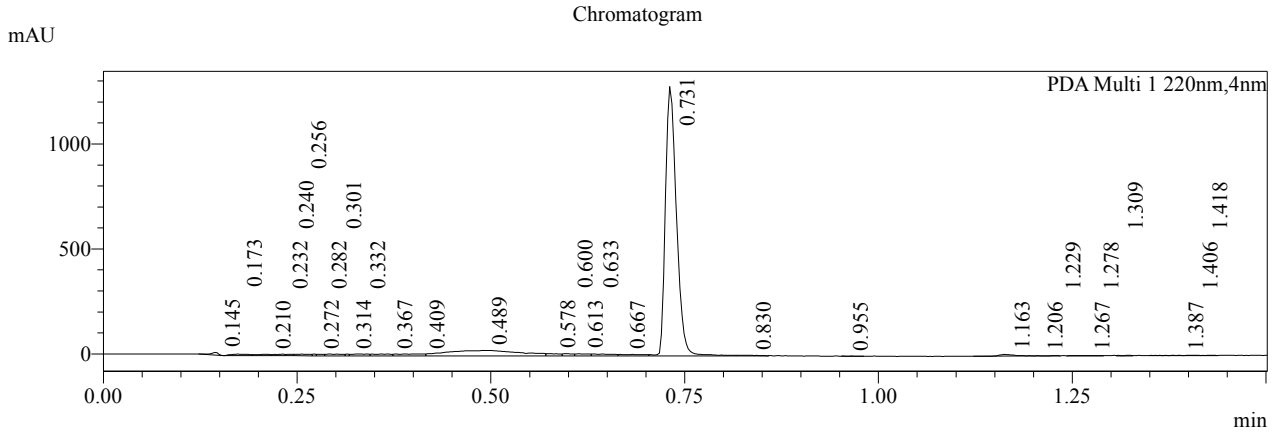
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Intensity

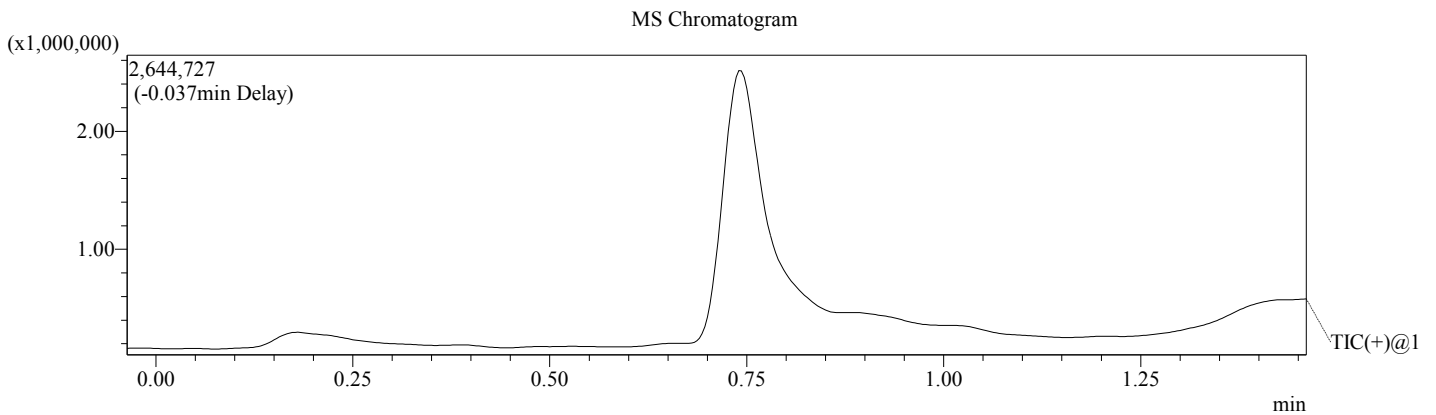


LCMS REPORT

Print time : 12/17/2020 15:44:29
Compound ID : 1
Sample ID : EB3453-3-P1C1
Injection Date : 12/17/2020 3:42:45 PM
Injection Vol : 2ul
Location : tray1 vail71
Acq Method : 5-95AB 1.5min 220&254 Shimadzu All.lcm
Org DataFile : D:\DATA\2020\2012\201217\EB3453-3-P1C1.lcd
Instrument & column: LCMS-SAW 1-2402
Chromolith Flash RP-18, 5um,3.0*25mm



- 1 PDA Multi 1 / 220nm,4nm
- 2 PDA Multi 2 / 254nm,4nm



Integration Result

PDA Ch1 220nm

Peak#	Ret. Time	Height	Height%	USP Width	Area	Area%
1	0.145	13135	0.883	0.019	9047	0.545
2	0.173	7732	0.520	0.075	12706	0.766
3	0.210	6549	0.440	0.151	8533	0.514
4	0.232	7782	0.523	0.243	7183	0.433
5	0.240	7102	0.478	0.225	4255	0.257
6	0.256	7817	0.526	0.119	10034	0.605
7	0.272	7844	0.527	0.033	2332	0.141
8	0.282	7580	0.510	0.053	4584	0.276
9	0.301	7879	0.530	0.000	11781	0.710
10	0.314	7763	0.522	0.086	2363	0.142
11	0.332	8121	0.546	0.085	12208	0.736
12	0.367	7902	0.531	2.083	13305	0.802
13	0.409	8749	0.588	0.000	20807	1.254
14	0.489	25819	1.736	0.206	168987	10.187
15	0.578	10221	0.687	0.334	12394	0.747
16	0.600	10407	0.700	0.108	9123	0.550
17	0.613	9532	0.641	0.187	11293	0.681
18	0.633	8814	0.593	0.123	15659	0.944
19	0.667	6903	0.464	0.114	14097	0.850
20	0.731	1283040	86.274	0.031	1277414	77.009
21	0.830	2858	0.192	0.070	4998	0.301
22	0.955	2110	0.142	0.034	1449	0.087
23	1.163	7875	0.529	0.038	10604	0.639
24	1.206	1525	0.103	0.016	1158	0.070
25	1.229	1657	0.111	0.029	1473	0.089
26	1.267	1911	0.129	0.017	2164	0.130
27	1.278	1798	0.121	0.052	1834	0.111
28	1.309	1972	0.133	0.016	1928	0.116
29	1.387	1924	0.129	0.012	2642	0.159
30	1.406	1728	0.116	0.088	1387	0.084
31	1.418	1124	0.076	0.016	1050	0.063

PDA Ch2 254nm

Peak#	Ret. Time	Height	Height%	USP Width	Area	Area%
1	0.160	2281	0.264	0.023	1485	0.172
2	0.731	859783	99.461	0.031	857604	99.169
3	1.166	877	0.101	0.034	1337	0.155
4	1.245	837	0.097	0.081	2358	0.273
5	1.261	665	0.077	0.045	2002	0.232

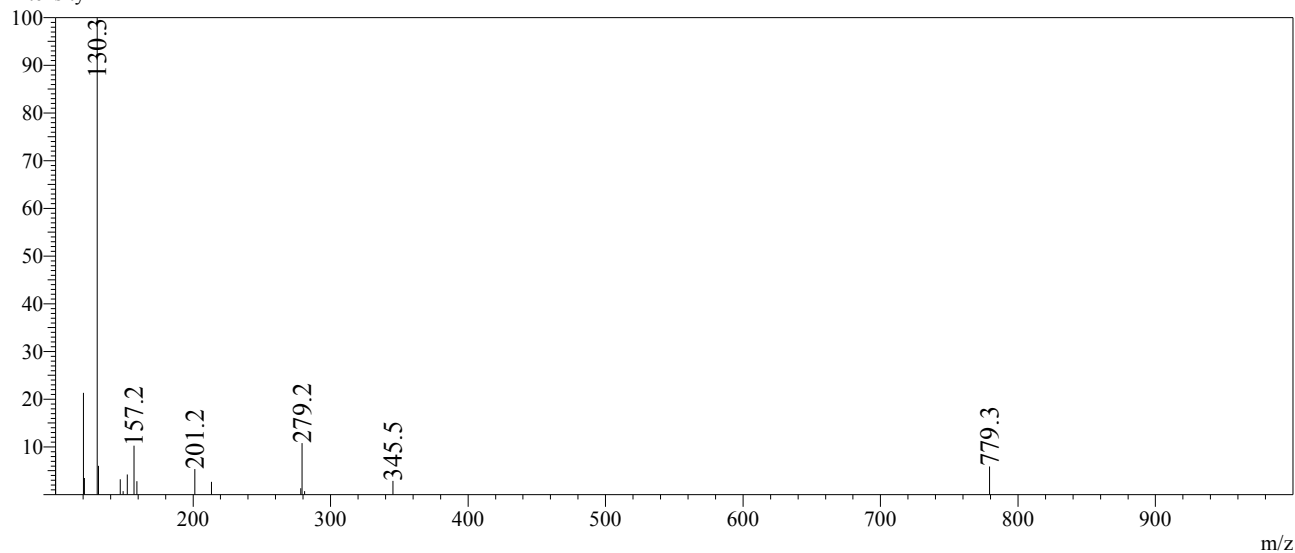
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Date: _____

Mass Spectrum

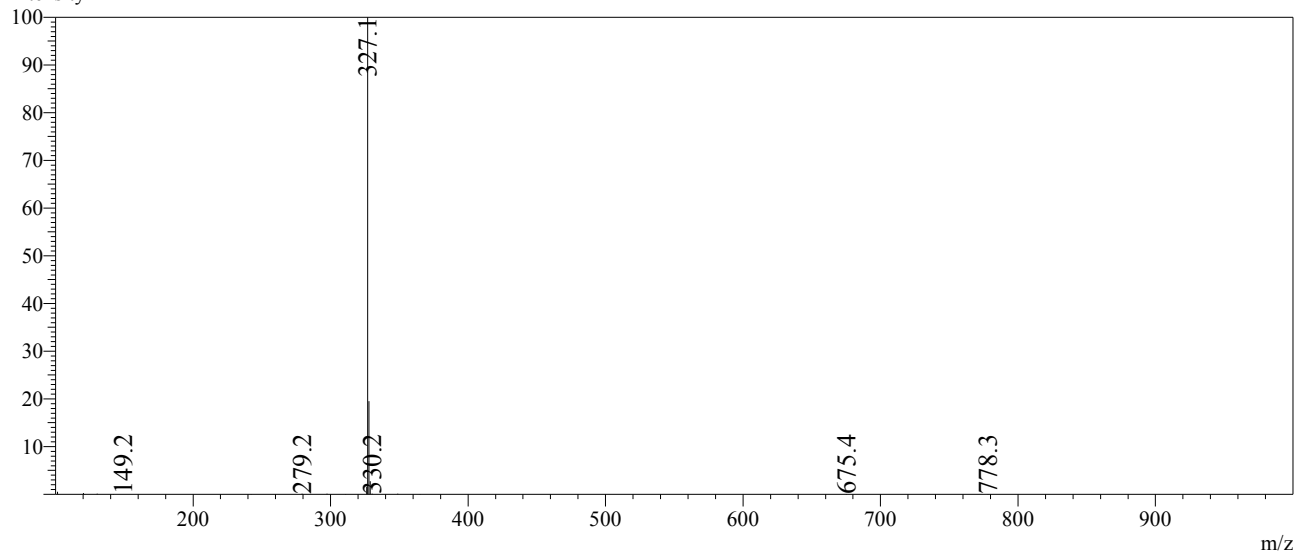
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Intensity



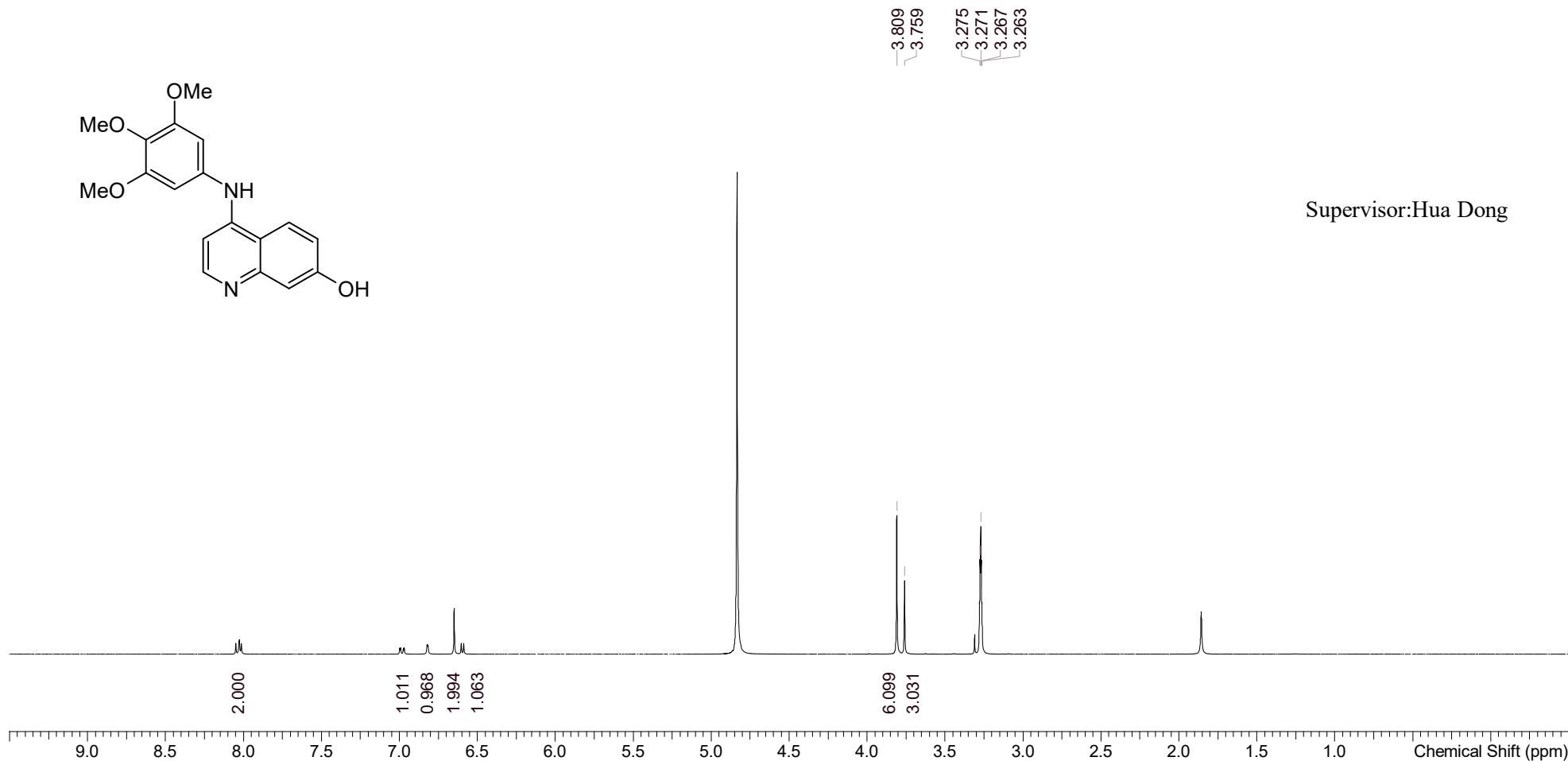
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Intensity



Compound ID:

EB3453-3-P1N2 MeOD Bruker_NT-B_400MHZ



Operator:

Date:

If you have any question related to this spectrum, please contact Dong_Hua@wuxiapptec.com for support.



WuXi V-Lab

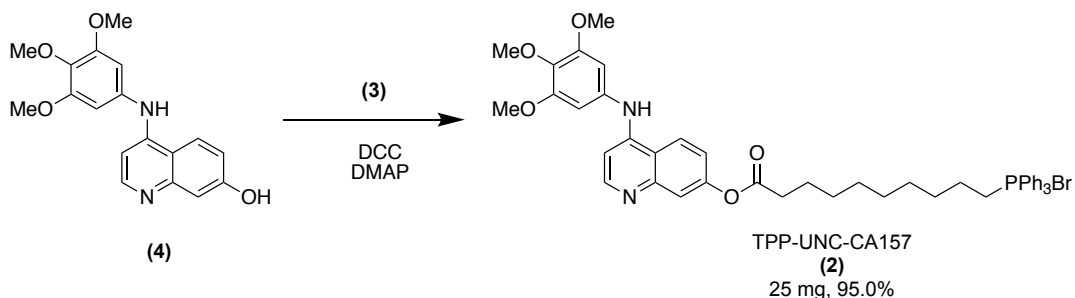
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A mixture of compound **(4)** (200 mg, 613 μmol , 1.00 eq), compound **(3)** (315 mg, 613 μmol , 1.00 eq), DMAP (3.74 mg, 30.6 μmol , 0.05 eq) and N,N'-dicyclohexylmethanediimine (126 mg, 613 μmol , 124 μL , 1.00 eq) in CH_2Cl_2 (10 mL) was stirred at 20 $^\circ\text{C}$ for 24 h under N_2 atmosphere. LC-MS showed that the desired compound was detected (EB3453-5-P1L1). The mixture was concentrated under reduced pressure to afford crude. The crude was purified by chromatography column (SiO_2 , EtOAc/Methanol = 20:1~5:1), and then prep-HPLC (column: Phenomenex Luna 30*30mm*10 μm +YMC AQ 100*30*10 μm ; mobile phase: [water (0.1%TFA)-ACN]; B%: 15%-65%, 26 min) to afford TPP-UNC-CA157 **(2)** (150 mg, 157 μmol , 25.6% yield) as a yellow oil.

^1H NMR: (400 MHz, CDCl_3) (EB3453-6-P1N1)

δ : 11.27 (s, 1H), 8.96 (d, $J = 9.26$ Hz, 1H), 8.19 (d, $J = 4.75$ Hz, 1 H), 7.77-7.86 (m, 4 H), 7.57-7.74 (m, 12H), 7.27-7.34 (m, 1H), 6.81 (d, $J = 6.50$ Hz, 1H), 6.68 (s, 2H), 3.87 (s, 3H), 3.82 (s, 5H), 3.13 - 3.30 (m, 2H), 2.54 (t, $J = 6.82$ Hz, 2H), 1.68 (m, 4H), 1.55 (d, $J = 6.00$ Hz, 2H), 1.19-1.45 (m, 10H).

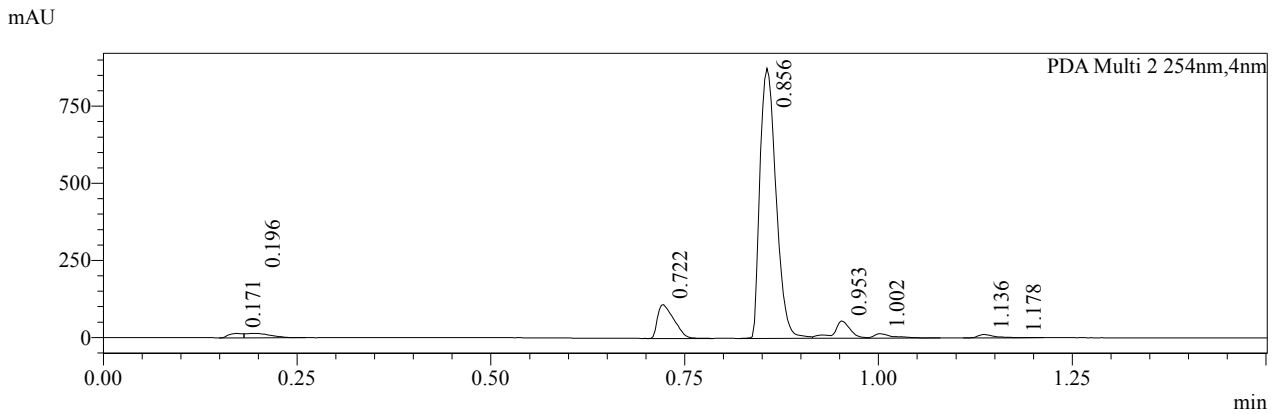
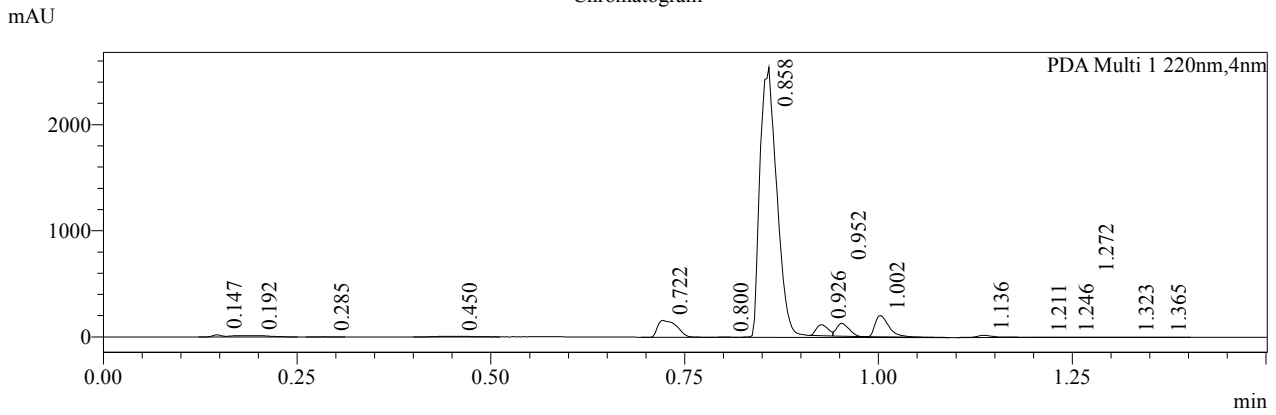
^{13}C NMR (CDCl_3) (ET58533-153-P1C2)

δ : 171.6, 155.6, 153.9, 142.9, 140.1, 136.9, 135.3, 133.3, 130.6, 126.5, 122.1, 118.3, 117.4, 115.8, 112.4, 102.5, 99.7, 61.0, 56.3, 34.0, 30.2, 30.1, 28.3, 28.0, 24.2, 22.6, 22.4, 22.2

LCMS REPORT

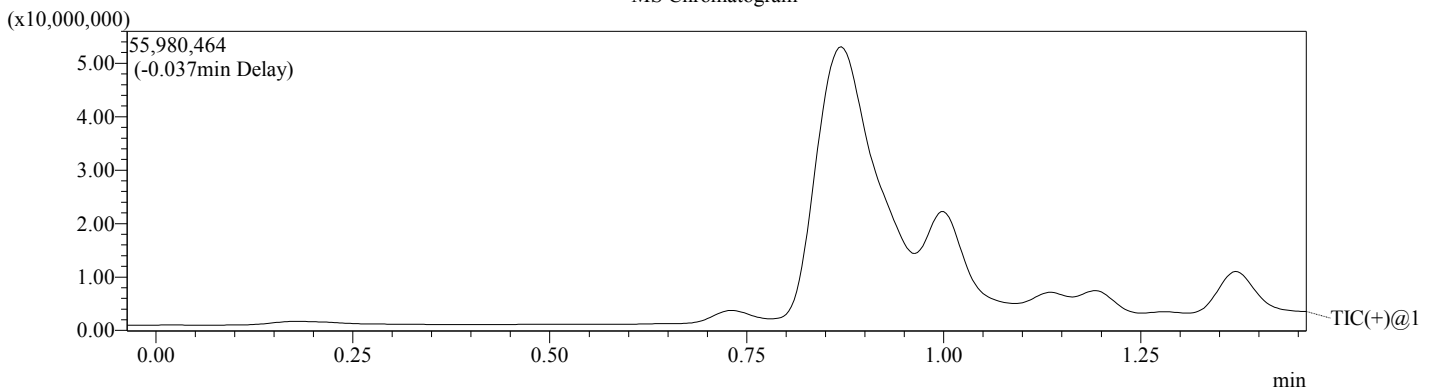
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Injection Date : 12/29/2020 10:10:13 AM
Injection Vol : 3ul
Location : tray1 vail48
Acq Method : 5-95AB 1.5min 220&254 Shimadzu All.lcm
Org DataFile : D:\DATA\2020\2012\201229\EB3453-5-P1L1.lcd
Instrument & column: LCMS-SAW 1-2402
Chromolith Flash RP-18, 5um,3.0*25mm

Chromatogram



- 1 PDA Multi 1 / 220nm,4nm
- 2 PDA Multi 2 / 254nm,4nm

MS Chromatogram



Integration Result

PDA Ch1 220nm

Peak#	Ret. Time	Height	Height%	USP Width	Area	Area%
1	0.147	20478	0.640	0.024	16165	0.335
2	0.192	12968	0.405	0.129	42983	0.890
3	0.285	1076	0.034	0.034	1290	0.027
4	0.450	6483	0.203	0.080	18895	0.391
5	0.722	159002	4.968	0.047	273181	5.658
6	0.800	3216	0.100	0.034	3123	0.065
7	0.858	2542386	79.431	0.128	3925121	81.301
8	0.926	104186	3.255	0.033	115002	2.382
9	0.952	121395	3.793	0.036	134747	2.791
10	1.002	201813	6.305	0.037	250754	5.194
11	1.136	21226	0.663	0.042	37486	0.776
12	1.211	1840	0.057	0.000	1540	0.032
13	1.246	1334	0.042	0.064	2045	0.042
14	1.272	1217	0.038	0.036	1063	0.022
15	1.323	909	0.028	0.148	2722	0.056
16	1.365	1219	0.038	0.051	1786	0.037

PDA Ch2 254nm

Peak#	Ret. Time	Height	Height%	USP Width	Area	Area%
1	0.171	14208	1.297	0.150	19134	1.199
2	0.196	14328	1.308	0.222	31705	1.986
3	0.722	109485	9.996	0.045	159994	10.022
4	0.856	875242	79.910	0.043	1265463	79.267
5	0.953	55954	5.109	0.037	80355	5.033
6	1.002	14151	1.292	0.042	22712	1.423
7	1.136	10941	0.999	0.041	15874	0.994
8	1.178	973	0.089	0.294	1212	0.076

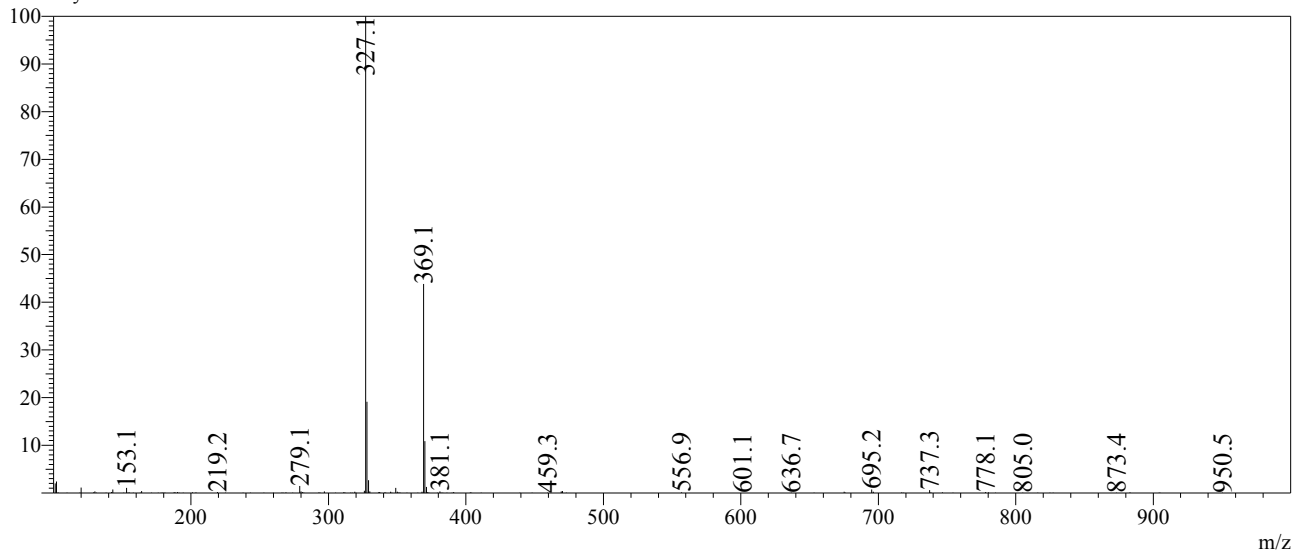
Operator: _____

Date: _____

Mass Spectrum

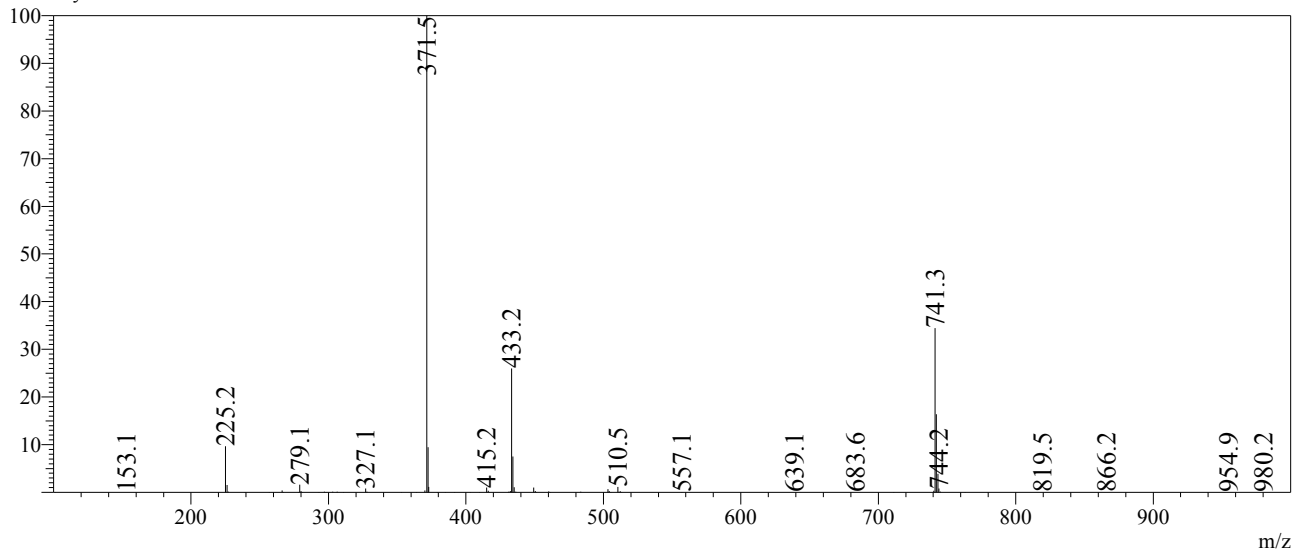
RefTime: 0.723 Datafile: D:\DATA\2020\2012\201229\EB3453-5-PIL1.lcd

Intensity



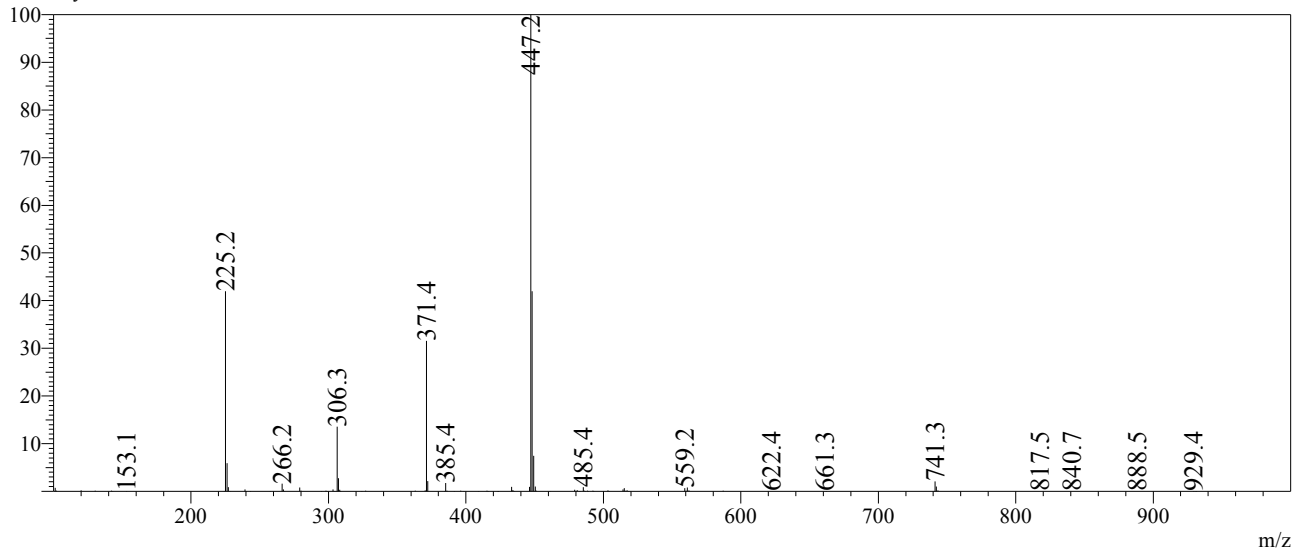
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Intensity



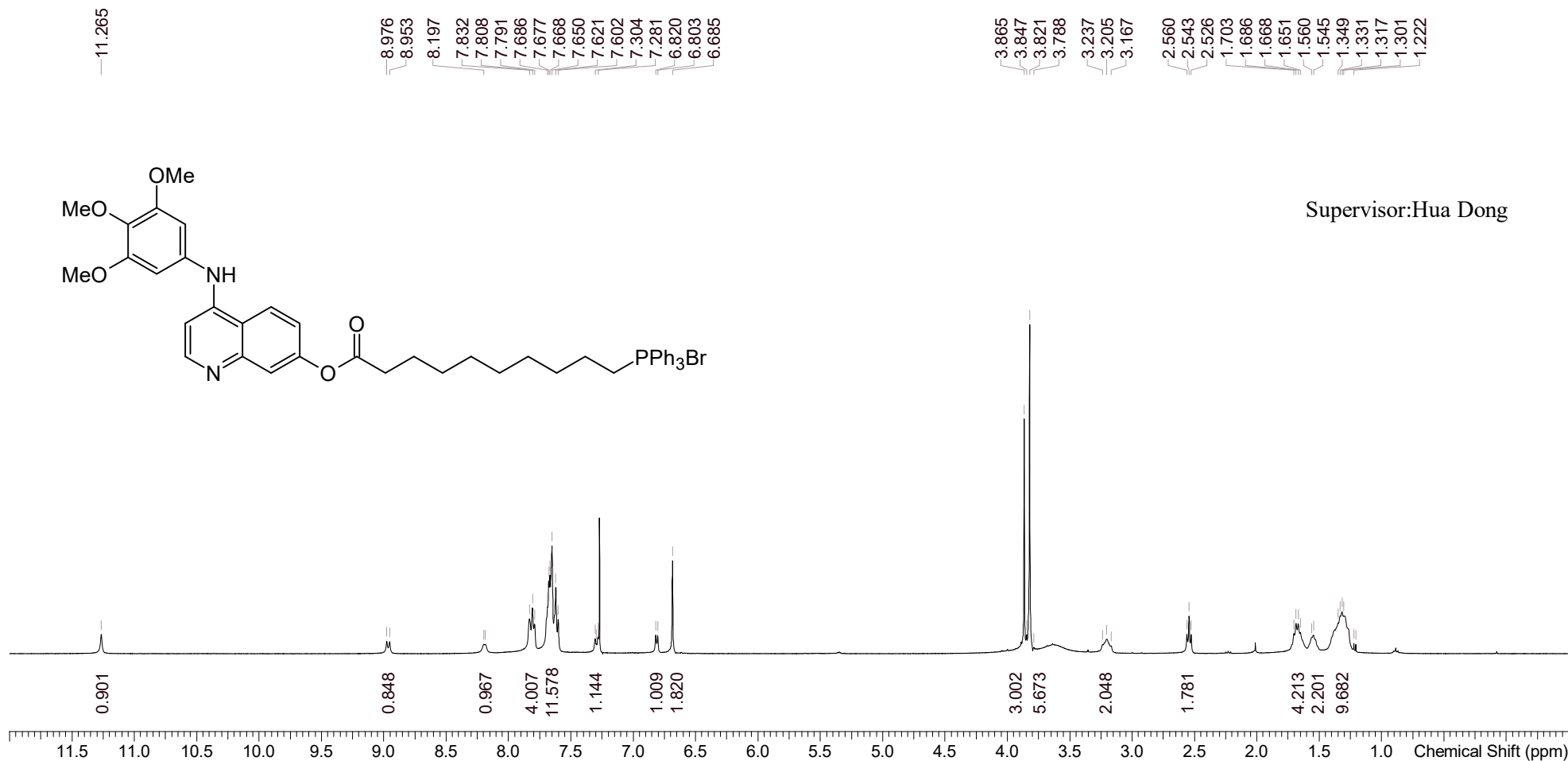
RefTime: 0.927 Datafile: D:\DATA\2020\2012\201229\EB3453-5-PIL1.lcd

Intensity



Compound ID: target (3)

EB3453-6-P1N1 CDCl3 Bruker_NT-B_400MHZ



Operator:

Date:

If you have any question related to this spectrum, please contact Dong_Hua@wuxiapptec.com for support.



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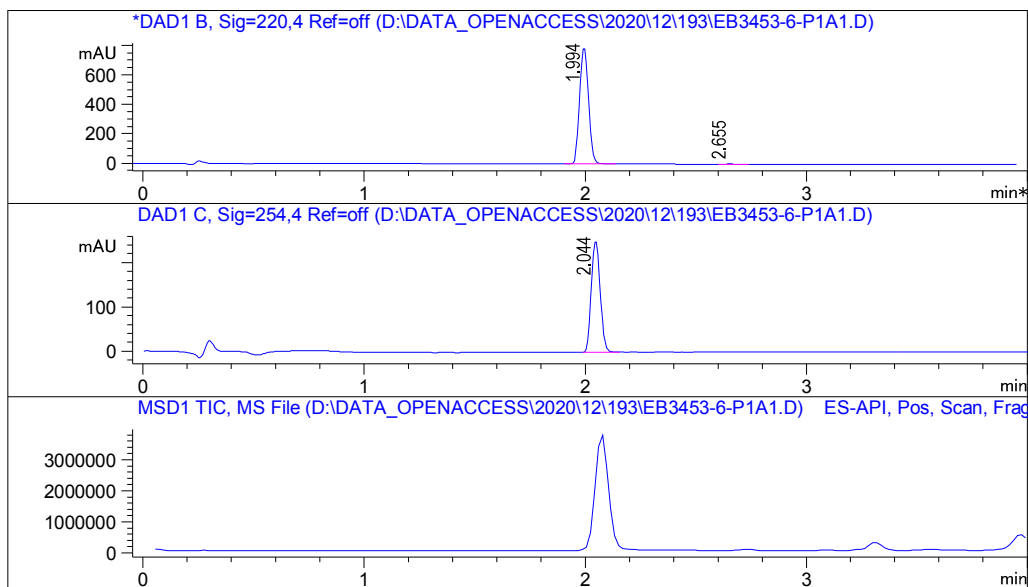
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LCMS REPORT

```

=====
Print Time       : 1/4/2021      11:25:23
Compound ID     : 1
Sample ID      : EB3453-6-P1A1
Injection Date  : Mon, 4- Jan- 2021    09:52:23
Acq Operator   : Admin
Location       : P2-D-09
Inj. Vol.      : 3.0 u1
Acq Method     : D:\DATA_OPENACCESS\2020\12\193\10-80AB_4min_Lilly_
                Agilent.M
Data Filename   : D:\DATA_OPENACCESS\2020\12\193\EB3453-6-P1A1.D

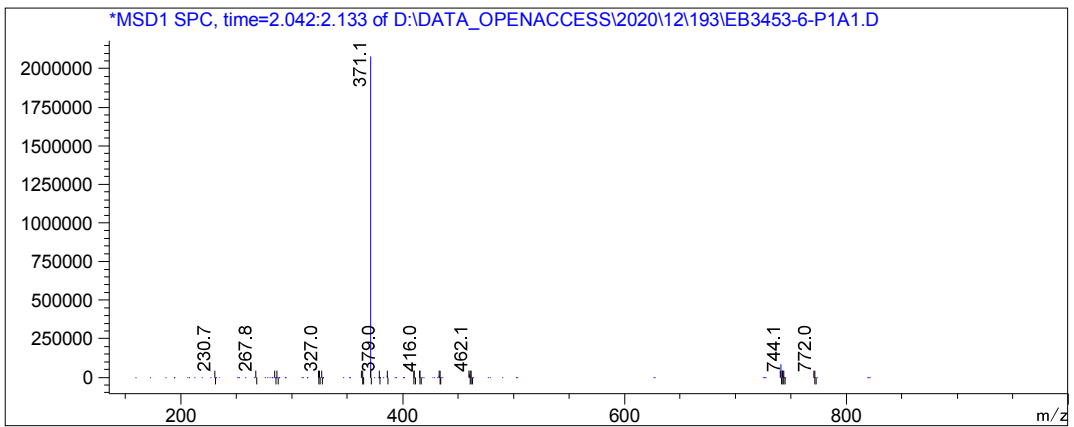
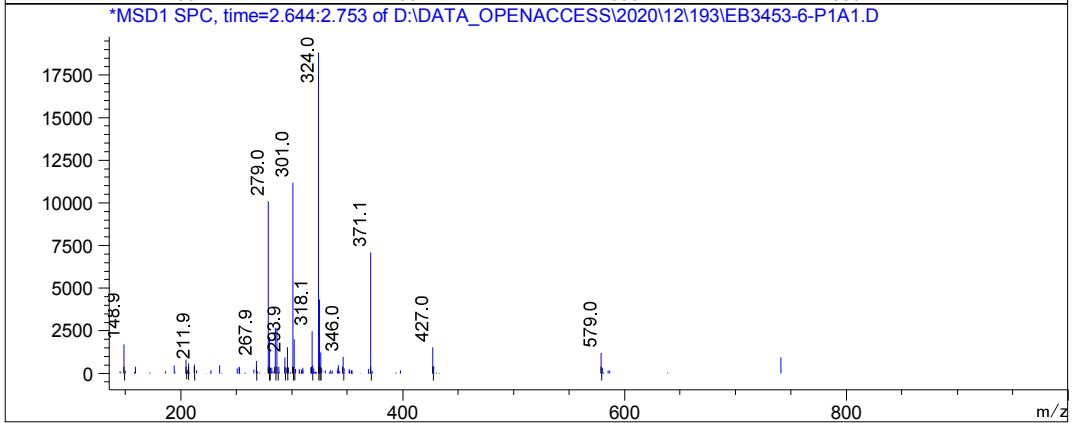
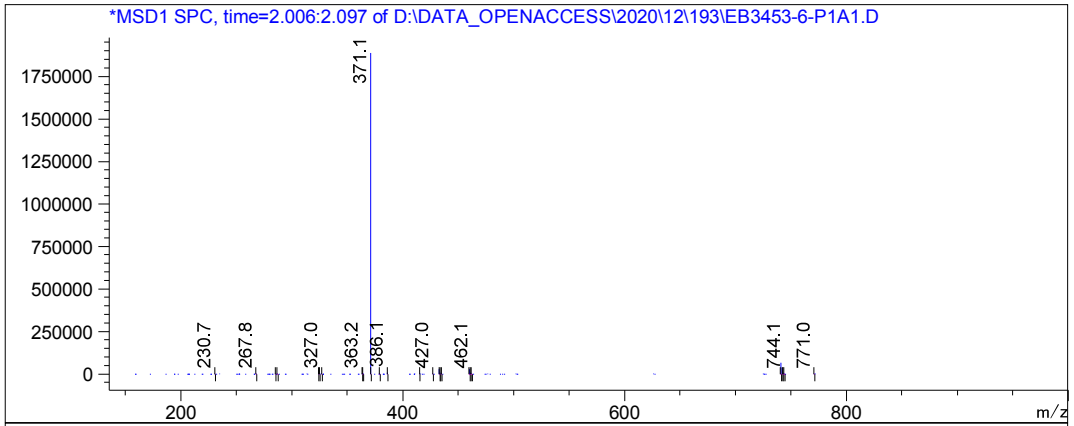
Instrument      : LCMS_NA Agilent 1290 Infinity II
    
```



Report

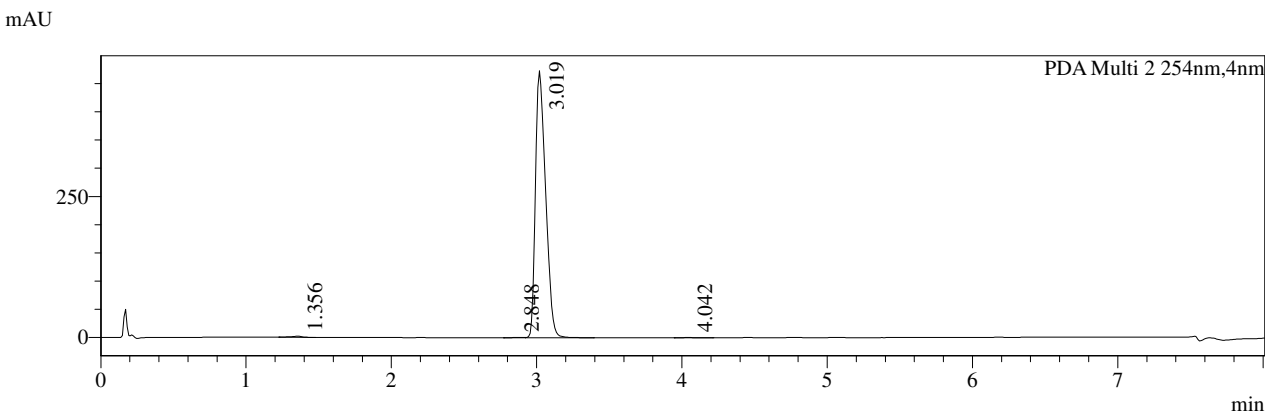
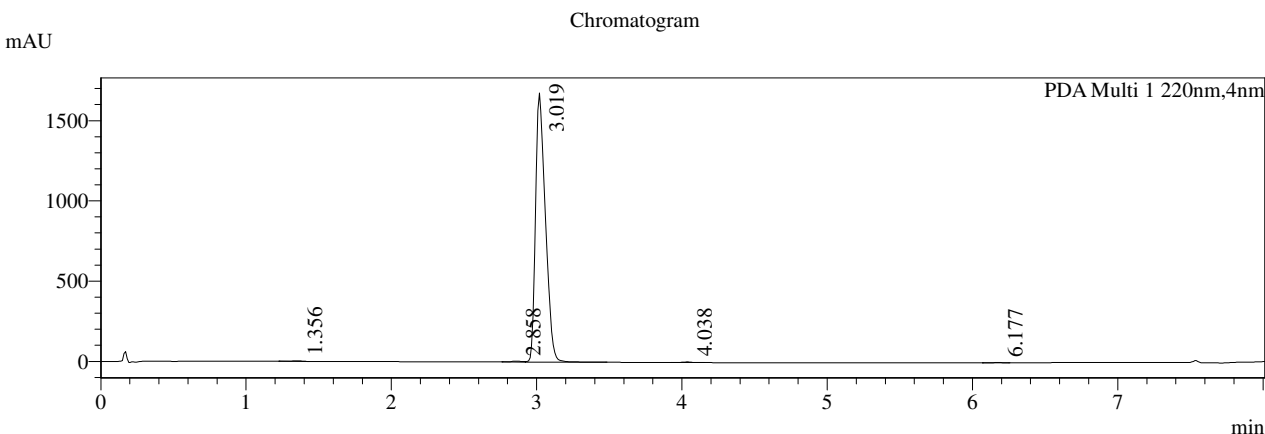
```

=====
Signal 1 : DAD1 B, Sig=220,4 Ref=off
-----
Peak #   RT [min]   Height   Height %   Width [min]   Area   Area %
-----
1       1.994   793.884   99.849     0.045   2245.362  99.810
2       2.655     1.203     0.151     0.053     4.272    0.190
-----
Signal 2 : DAD1 C, Sig=254,4 Ref=off
-----
Peak #   RT [min]   Height   Height %   Width [min]   Area   Area %
-----
1       2.044   253.261  100.000    0.045     712.811  100.000
-----
    
```

HPLC REPORT

Print time : 01/04/2021 14:13:00
 Compound ID : 1
 Sample ID : EB3453-6-P1F1
 Injection Date : 2021/1/4 14:04:50
 Injection Vol : 6ul
 Location : tray1 vail7
 Acq Method : 10-80AB_8min_220&254.lcm
 Org Data File : D:\DATA\2021\2101\210104\EB3453-6-P1F1.lcd
 Instrument & column:HPLC_01 1-2402
 XBridge Shield RP18 (2.1x50mm 5µm)



- 1 PDA Multi 1 / 220nm,4nm
- 2 PDA Multi 2 / 254nm,4nm

=====
 Integration Result
 =====

Peak Table

PDA Ch1 220nm

Peak#	Ret. Time	Height	Height%	USP Width	Area	Area%
1	1.356	2983	0.176	0.134	13114	0.164
2	2.858	5821	0.344	0.162	27875	0.349
3	3.019	1676212	99.158	0.145	7914695	99.203
4	4.038	3928	0.232	0.128	16380	0.205
5	6.177	1494	0.088	0.124	6234	0.078

PDA Ch2 254nm

Peak#	Ret. Time	Height	Height%	USP Width	Area	Area%
1	1.356	1995	0.419	0.134	8788	0.379
2	2.848	477	0.100	0.290	2726	0.118

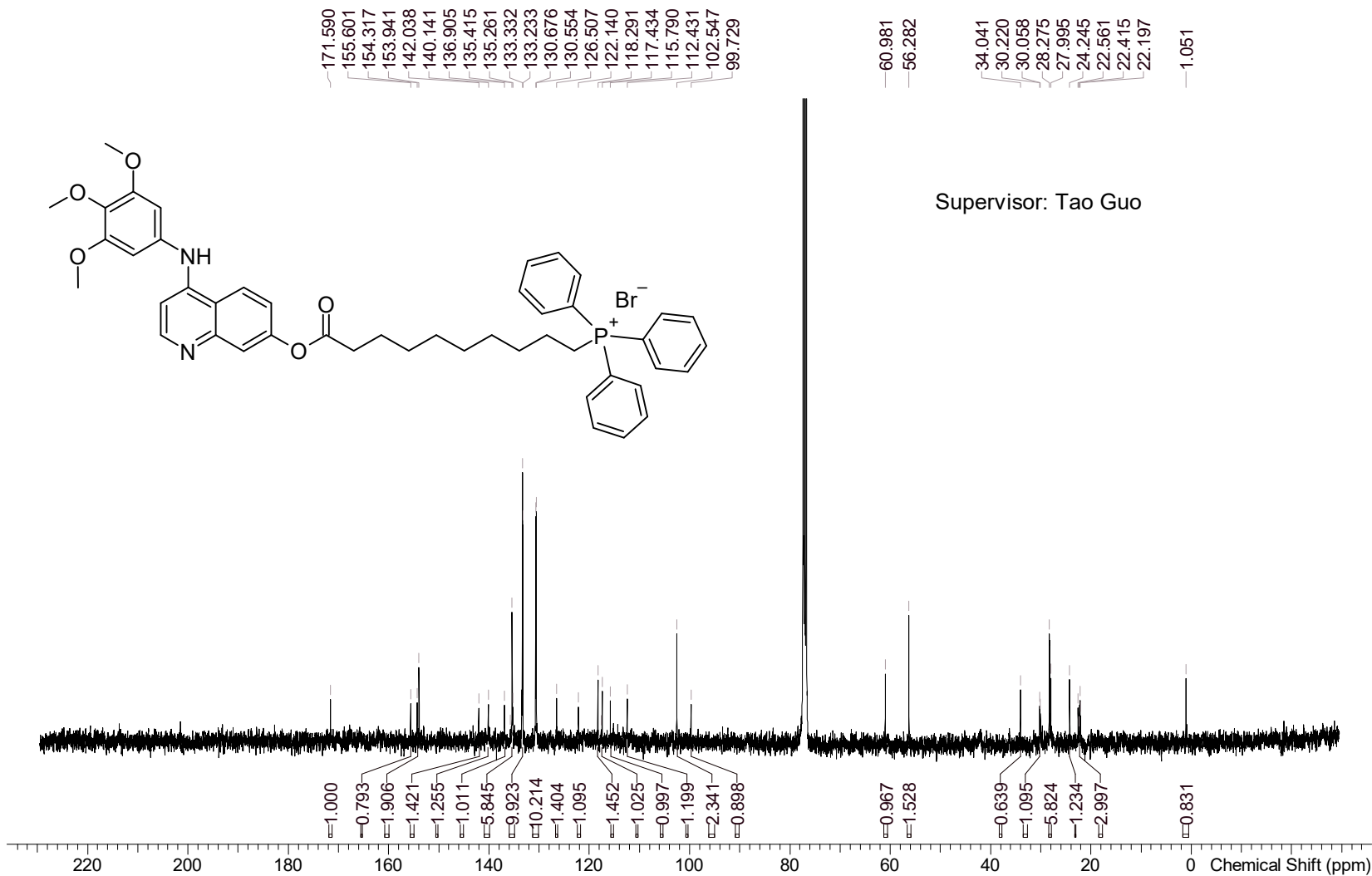
Peak#	Ret. Time	Height	Height%	USP Width	Area	Area%
3	3.019	472638	99.332	0.149	2300799	99.329
4	4.042	705	0.148	0.155	4036	0.174

Operator: _____

Date: _____

Compound ID: WUCDD-0001031

ET58533-153-P1C2 CDCI3 ZKNJ_N_400MHz



Supervisor: Tao Guo

Acquisition Time (sec)	1.0000
Comment	ET58533-1 53-P1C2 CDCI3 ZKNJ_N_ 400MHz
Date	16 Aug 2022 13:59:57 (GMT)
Frequency (MHz)	100.5482
Nucleus	13C
Number of Transients	3072
Origin	QUANTU M-I
Original Points Count	26042
Owner	admin
Points Count	65536
Pulse Sequence	s1pulg30
Receiver Gain	80.00
SW(cyclical) (Hz)	26041.00
Solvent	CHLORO FORM-d
Spectrum Offset (Hz)	10054.824 2
Spectrum Type	standard
Sweep Width (Hz)	26040.60
Temperature (degree C)	22.550

If you have any questions related to this NMR, please contact IDSU_CS@wuxiapptec.com for support.

Operator:

Date: