Supporting information for

PAC-FragmentDEL – Photoactivated covalent capture of DNA-encoded fragments for hit discovery

Huiyong Ma[b], James B. Murray[a], Huadong Luo[b], Xuemin Cheng[b], Qiuxia Chen[b], Chao Song[b], Cong Duan[b], Ping Tan[b], Lifang Zhang[b], Jian Liu[b], Barry A. Morgan[b], Jin Li[b], Jinqiao Wan[b],Lisa M. Baker[a], William Finnie[a], Lucie Guetzoyan[a], Richard Harris[a], Nicole Hendrickson[a], Natalia Matassova[a], Heather Simmonite[a], Julia Smith[a], Roderick E. Hubbard[a*], Guansai Liu[b]

[a]Vernalis (R&D) Ltd, Granta Park, Abington, Cambridge, CB21 6GB, UK

[b.]HitGen Inc., Building 6, No. 8 Huigu First East Road, Tianfu International Bio-Town, Shuangliu District, Chengdu 610000, Sichuan, P. R. China

*Corresponding author; r.hubbard@vernalis.com

Contents

Table S1: Summary of crystallographic data collection and refinement for the structures presented in the paper and supplementary information

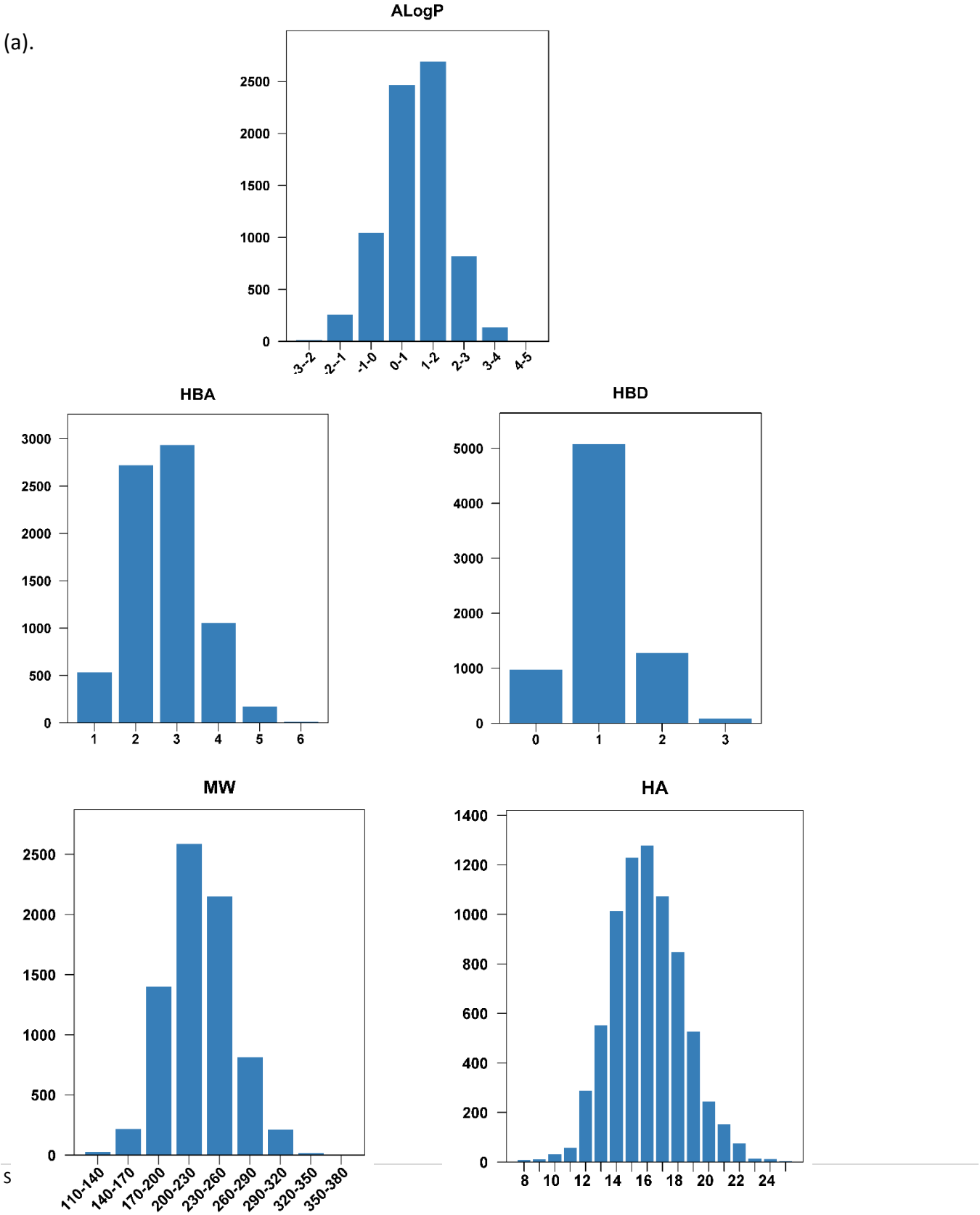
Data collection and refinement statistics for ligand-bound PAK4 and 2-Epimerase structures. Rfree is the R factor calculated using 5% of the reflection data chosen randomly and omitted from the refinement process. Rms bond lengths and angles are the deviations from ideal values. Values in parentheses are for the highest resolution bin.

Complex	**Pak4 + 6**	**Pak4 + 5**	**Pak4 + 7**	**Epi + 8**	**Epi + 9**
PDB code	8AHI	8AHH	8AHG	8AHE	8AHF
Data Collection					
Wavelength (Å)	1.54184	0.97629	0.97629	0.97627	0.97627
Space Group	P 41 21 2	P 41 21 2	P 41 21 2	P 41 21 2	P 41 21 2
Unit Cell dimensions (a,b,c)(Å)	61.913 91.913 181.903	61.897 61.897 180.162	61.822 61.822 180.790	109.119 109.119 136.207	109.315 109.315 135.866
Unit Cell dimensions (α,β,γ)(°)	90.0 90.0 90.0	90.0 90.0 90.0	90.0 90.0 90.0	90.0 90.0 90.0	90.0 90.0 90.0
Resolution (Å)	2.69	2.04	1.88	2.11	2.27
Rmerge	0.207 (0.555)	0.097 (2.747)	0.090 (3.058)	0.216 (2.657)	0.201 (3.536)
I/σ(I)	10.1 (3.5)	21.4 (1.4)	22.7 (1.2)	13.7 (1.5)	14.6 (1.2)
Completeness	99.2 (100.0)	94.6 (52.6)	95.3 (50.6)	95.6 (58.7)	95.7 (52.8)
CC ½	0.993 (0.889)	1.000 (0.699)	1.000 (0.638)	0.999 (0.582)	0.999 (0.590)
Redundancy	13.7 (14.3)	26.0 (26.3)	26.4 (27.2)	26.8 (25.5)	26.8 (27.7)
Refinement					
No. reflections all/free	10410 / 515	20091 / 1017	27811 / 1387	39374 / 1989	36587 / 1828
R-factor/R-free	0.155 / 0.249	0.184 / 0.250	0.179 / 0.226	0.184 / 0.235	0.177 / 0.238
Protein B-factor	32.73	53.19	43.16	47.71	51.57
Ligand B-factor	29.89	47.52	50.23	72.80	77.28
Water B-factor	27.07	56.48	48.12	43.67	47.01
RMS Deviations					
Bonds (Å)	0.0112	0.0089	0.0104	0.0119	0.0115
Angles (°)	2.027	1.531	1.6070	1.6320	1.639

Supplementary Figures

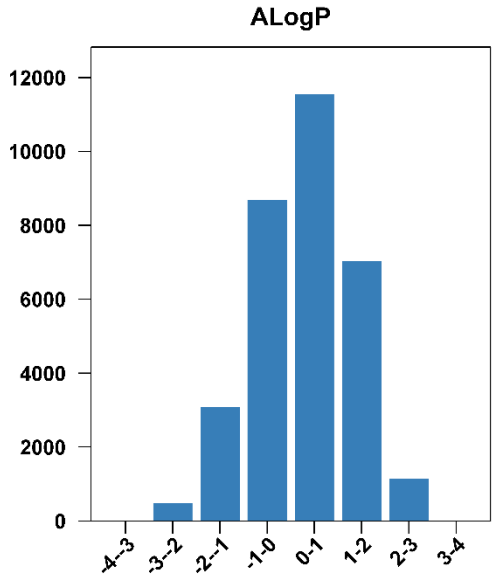
Figure S1 Properties of fragments in PAC-FragmentDELs. The properties of fragments in the collection of (a). Type 1 library, sub-libraries 1 and 2 and (b) Type 2 library, sub-libraries 3 and 4. See Figure S3 for the mapping between the fragments and corresponding PAC-FragmentDEL libraries

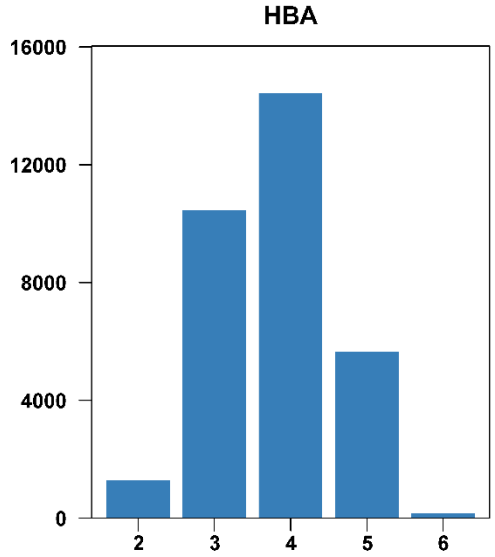
(a).

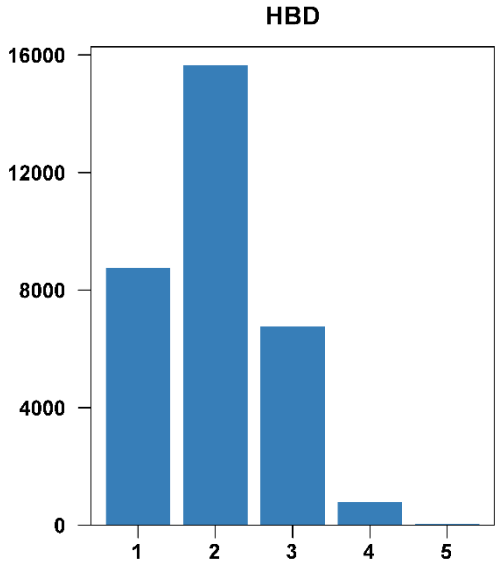


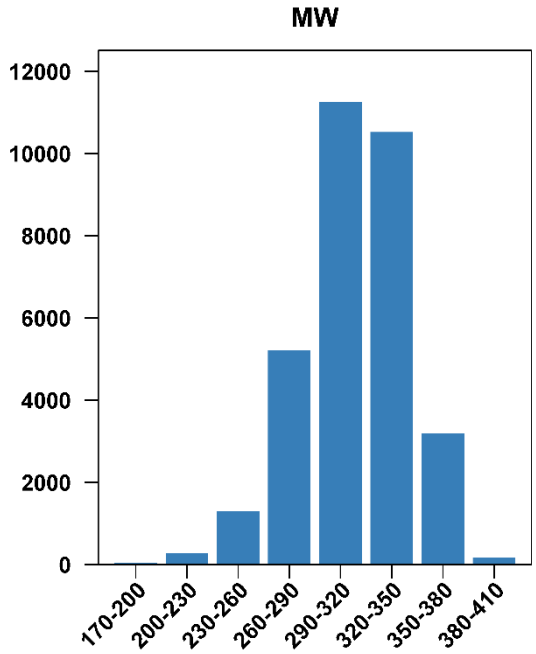
S

(b) .


ALogP
12000
10000
8000
6000
4000
2000
0
-4--3
-3--2
-2--1
-1-0
0-1
1-2
2-3
3-4


HBA
16000
12000
8000
4000
0
2
3
4
5
6


HBD
16000
12000
8000
4000
0
1
2
3
4
5


MW
12000
10000
8000
6000
4000
2000
0
170-200
200-230
230-260
260-290
290-320
320-350
350-380
380-410

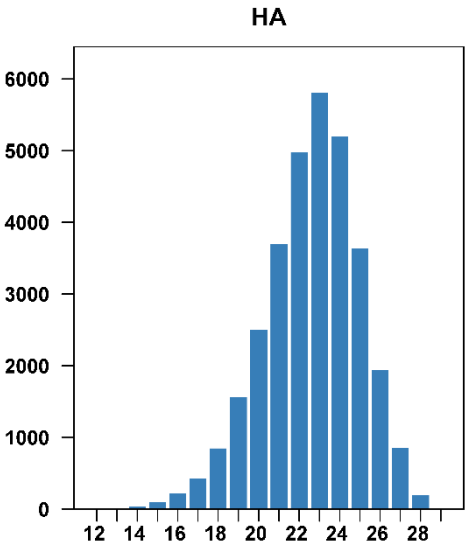
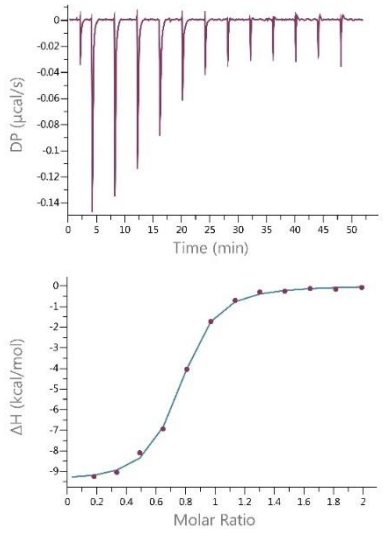

HA
6000
5000
4000
3000
2000
1000
0
12
14
16
18
20
22
24
26
28

Figure S2 characterization of the binding of 4 to 2-epimerase by (a) ITC and (b) LO-NMR. For ITC, the conditions were as described in the experimental section of the paper. For LO-NMR, the spectra were obtained as described in the experimental section of the paper for a sample containing 9µM *B. anthracis* 2-epimerase with 1mM UDP-GlcNAc in 20mM Tris pH7.5, 50mM NaCl and competition was determined by the addition of 20µM of **4**.

(a).



N (sites)	(+/-)	K_D (M)	(+/-)	ΔH (kcalmol^{-1})	(+/-)	ΔG (kcalmol^{-1})	(+/-)	-TΔS (kcal/mol^{-1})	(+/-)
0.77	0.06	9.1E-08	2.1E-08	-9.1E+00	5.0E-01	-9.6E+00	1.4E-01	-5.6E-01	6.3E-01

(b).

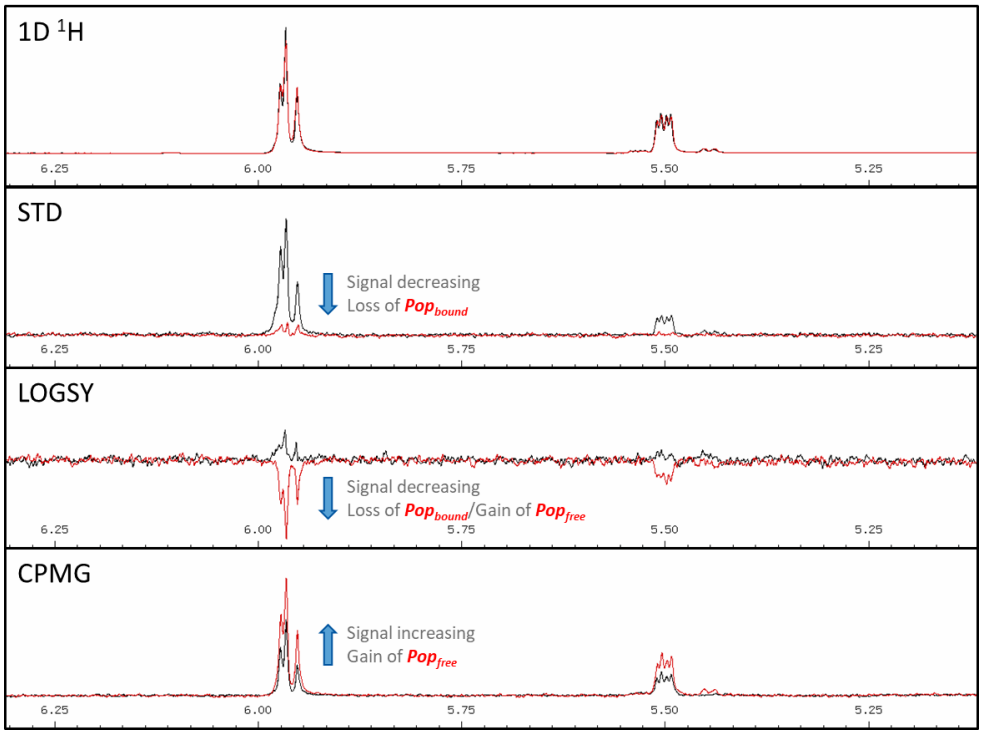
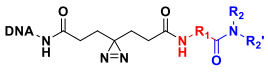
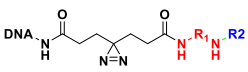
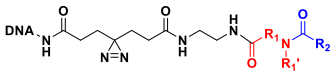
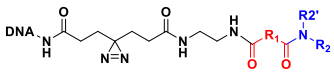
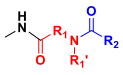


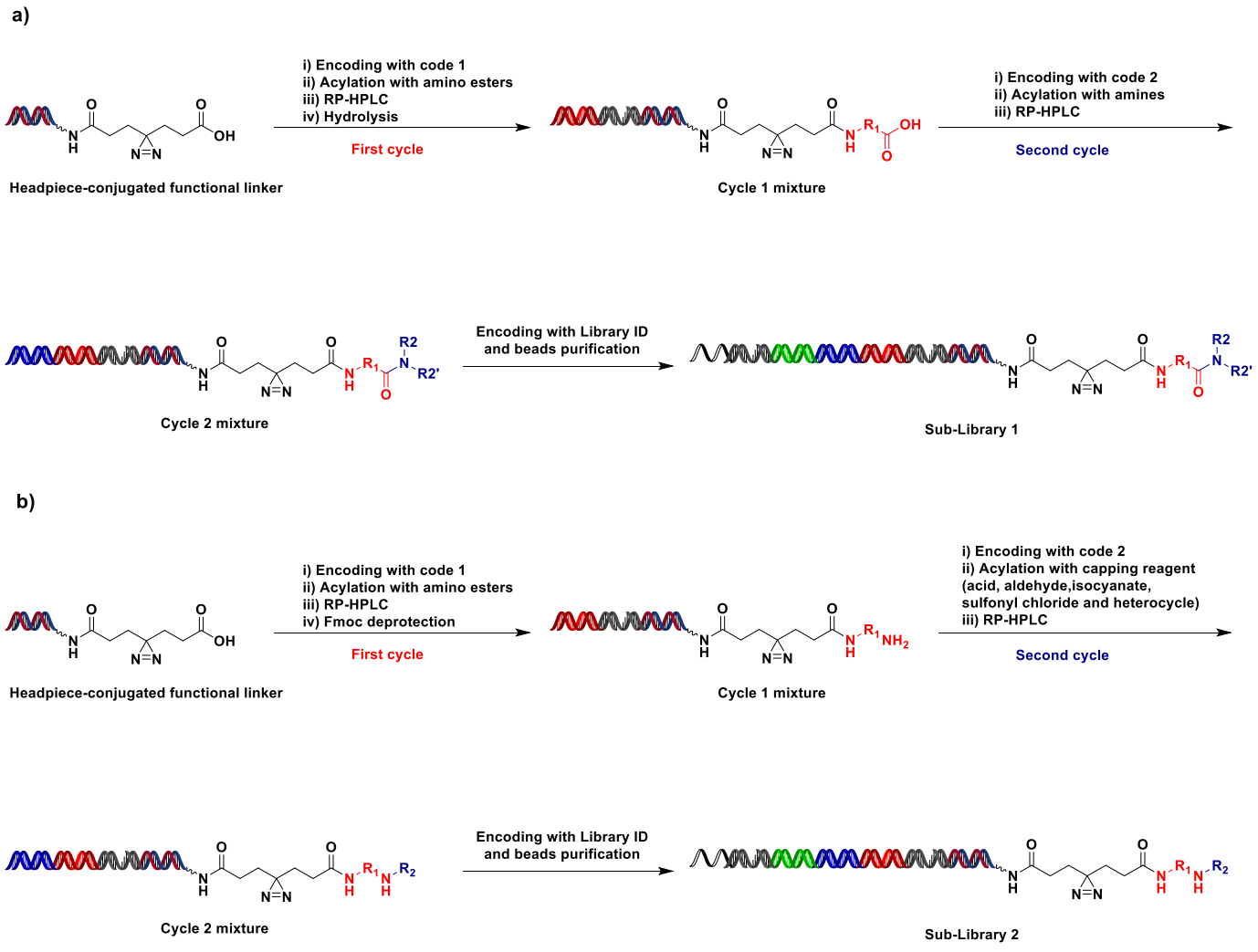
Figure S3 the off-DNA structure of fragments for PAC-FragmentDEL libraries

DEL structure	 sub-library 1	 sub-library 2	 sub-library 3	 sub-library 4
Off-DNA Structure				

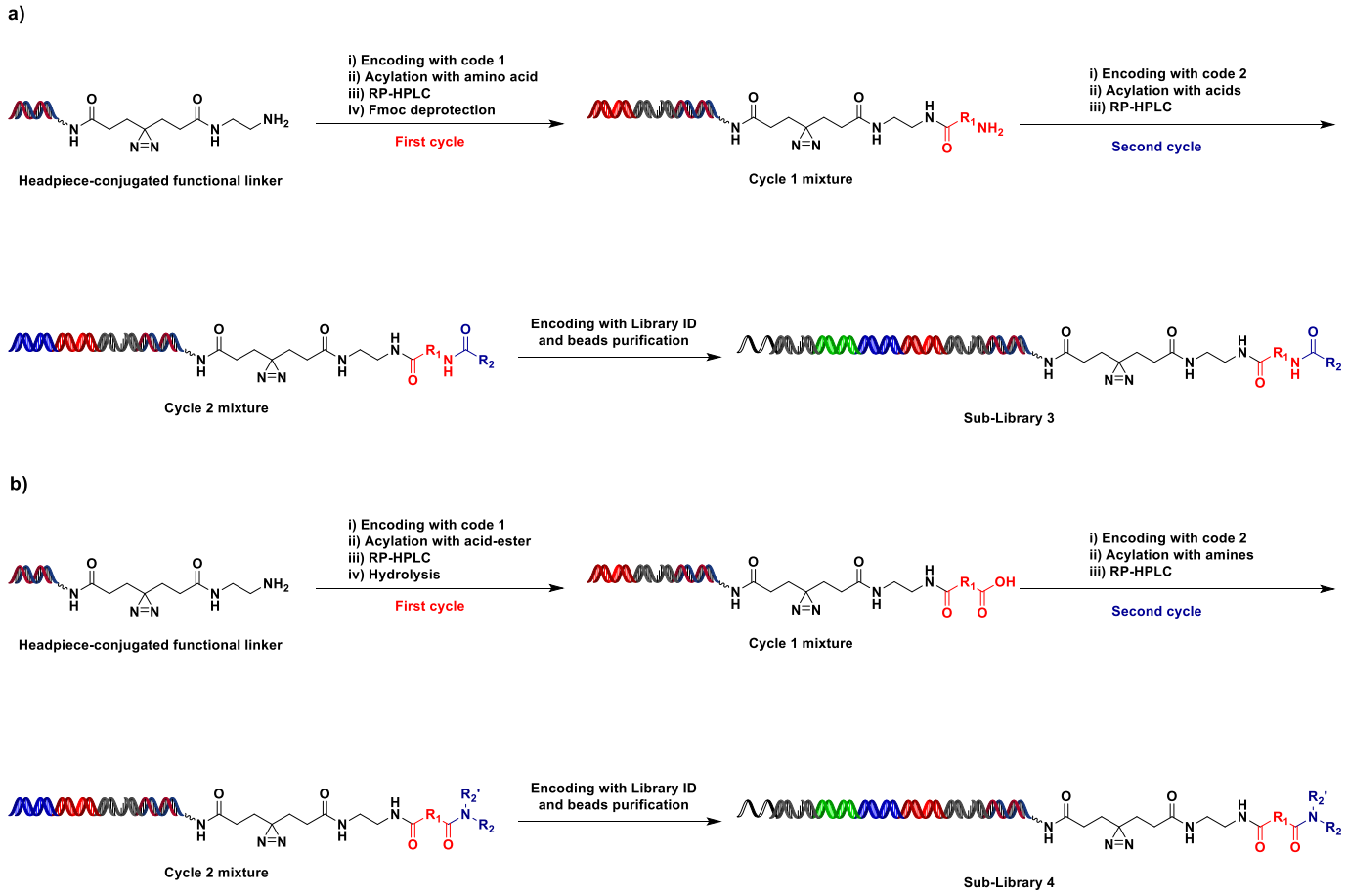
Chemical Synthesis

Synthetic schemes for library construction

Scheme S1. General Synthetic Scheme of Type 1 PAC-FragmentDEL (a) sub-library 1 and (b) sub-library 2



Scheme S2. General Synthetic Scheme of Type 2 PAC-FragmentDEL (a) sub-library 1 and (b) sub-library 2



General synthetic information

Materials

The chemically modified DNA oligonucleotide headpiece (HP, 5'-/5Phos/GAGTCA/iSp9/iUniAmM/iSp9/TGACTCCC-3', **Figure SA**) which used for DEL construction was synthesized at HitGen Inc. T4 ligase which used for DNA ligation was expressed at HitGen Inc. Fmoc-15-amino-4, 7, 10, 13-tetraoxapentadecanoic acid (Fmoc-AOP), 2-(7-Azabenzotriazol-1-yl)-N, N, N', N'-tetramethyluronium hexafluorophosphate (HATU), N, N-Diisopropylethylamine (DIPEA), Sodium hydroxide (NaOH), 4-(4,6-Dimethoxy-1,3,5-triazin-2-yl)-4-methylmorpholinium chloride (DMT-MM), piperidine, N,N-Dimethylaniline (DMA) were purchased from Bidepharm. All buffer and ionic solutions, including ligation buffer, aq. NaCl (5 M), aq. HCl (1 M), basic borate buffer (250 mM sodium borate/boric acid, pH = 9.4), dipotassium phosphate buffer (300 mM, pH = 9.4) were prepared in-house.

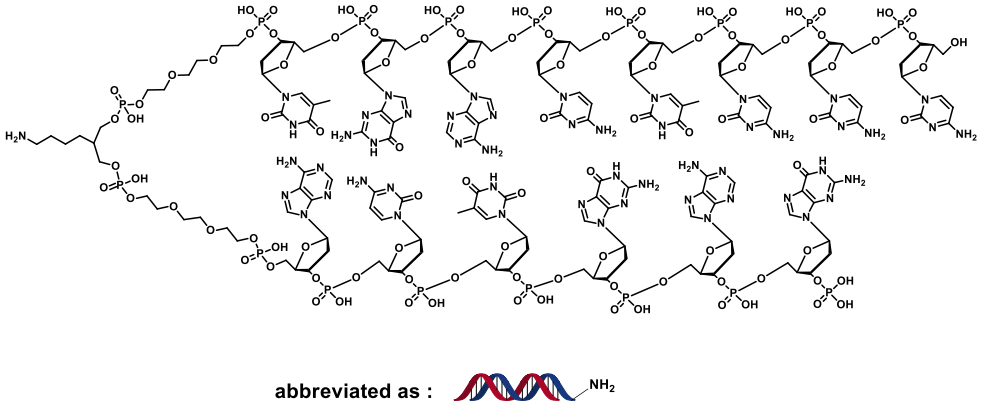


Figure SA. Headpiece structure and sequence

General procedure for library construction

DNA ligation

To the solution of DNA (10 nmol in water) were added T4 ligase (0.5 ug/10 nmol) and code (10 nmol). And the mixture was vibrated and proceeded at 20 ℃ for 4-16 hours until the gel image indicated the starting material was mostly consumed.

Acylation

To the solution of DNA-conjugated amine (1 eq, 1 mM in BBS buffer, 250 mM, pH=9.4) was added the mixed solution of acid (10-100 eq, 200 mM in DMA), HATU (10-100 eq, 400 mM in DMA), DIPEA (10-100 eq, 40 mM in DMA) which was pre-reacted at 5 ℃ for 10 min. After being vibrated for seconds, the mixture was allowed to proceed at room temperature for 2-24 h.

To the solution of DNA –conjugated acid (1 eq, 1 mM in BBS buffer, 250 mM, pH=9.4), amine (100 eq, 200 mM in DMSO) and DMT-MM (200 eq, 200 mM in H2O) were added. After being vibrated for 0.5 min, the reaction mixture was

allowed to react at 25 ℃ overnight.

Hydrolysis

To a solution of DNA-conjugated ester (1 eq, 1 mM in water) was added NaOH (100 eq, 1 M in water). After being vibrated for seconds, the reaction mixture was proceeded at room temperature overnight.

Fmoc deprotection

To a solution of DNA (1 eq, 1 mM in water) was added 10% v\v piperidine. The reaction was allowed to proceed at rt for 1-2 h.

Reductive amination

To a solution of DNA-conjugated amine (1 eq, 1 mM in BBS buffer, 250 mM, pH=9.4) was added aldehyde (100 eq, 200 mM in DMSO). After being vibrated for seconds, the mixture was proceed for 30 min at room temperature. And then NaBH4 (300 eq, 400 mM in DMSO) was added. The reaction was allowed to proceed for 5-12 hour at room temperature.

Urea Formation

To a solution of DNA-conjugate amine (1 eq, 1 mM in BBS buffer, 250 mM, pH=9.4) was added isocyanate (100 eq, 500 mM in ACN). And the reaction mixture was allowed to react at room temperature for 16 h.

Sulfonylation

To a solution of DNA-conjugate amine (1 eq, 1 mM in BBS buffer, 250 mM, pH=9.4) were sequentially added sulfonyl chloride (100 eq, 500 mM in ACN) and DIPEA (100 eq, 500 mM in ACN) which pre-mixed for 1 min. The mixture was vortexed and then reacted at 20-30 ℃ for 16 h.

SnAr Replacement

To a solution of DNA-conjugate amine (1 eq, 1 mM in BBS buffer, 250 mM, pH=9.4) were added heterocyclce (100 eq, 500 mM in ACN) and DIPEA (100 eq, 500 mM in ACN). After being vibrated for seconds, the mixture was heat up to react at 50-60 ℃ for 16 h.

Ethanol precipitation

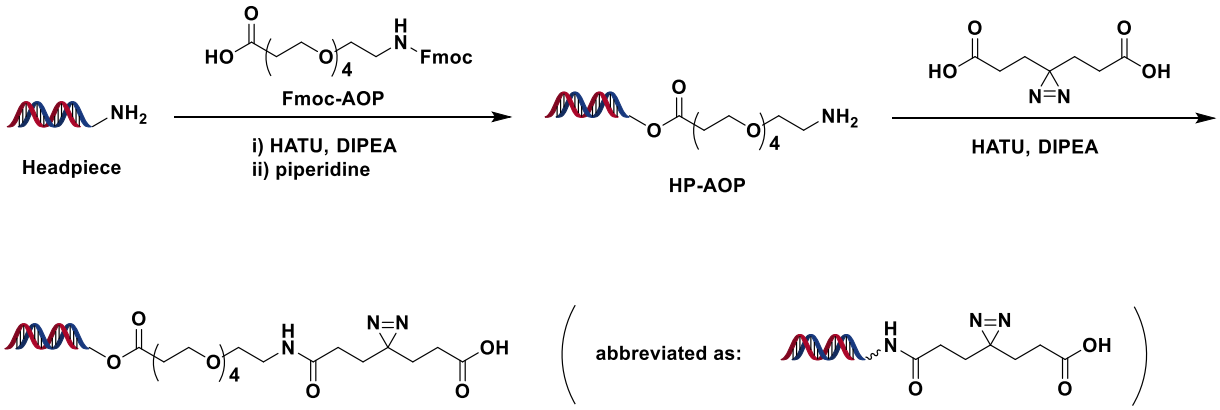
To a DNA reaction mixture was added 10% (v/v) 5 M NaCl solution and 3 times volume of absolute ethanol. The suspension was then incubated at -20℃ for 2 h. The mixture was then centrifuged at 4.0℃ for 30.0 min at 4000.0 g; and the supernatants were discarded. The residue was dried at room temperature for 1 h in vacuum to afford the crude product. Generally, ethanol precipitation was performed after each chemical reaction and the precipitate was re-dissolved with water or buffer to use for next step or pooling.

General method of analysis for on-DNA reaction

The analysis sample was prepared as follows: To the precipitated DNA material was added deionized water to afford a 1 mM solution. And then took 2-3 μL solution to dissolve into 100 μL of deionized water to get the analysis sample. The analysis samples were performed on a Waters Acquity UPLC H-Class Bio System coupled with a Waters Acquity UPLC Tunable Ultraviolet (TUV) detector and a Waters Xevo G2-XS QTof Mass Spectrometer. The UPLC system was set as following. Column: Waters Acquity UPLC Oligonucleotide BEH C18 Column (130 Å, 1.7 um, 2.1 × 50 mm; flow rate: 0.4 mL/min; column temperature: 40℃). Mobile phases A: 0.75 % HFIP/0.0375 % DIPEA/10 μM EDTA in HPLC grade water. Mobile phases B: 0.75 % HFIP/0.0375 % DIPEA/10 μM EDTA in 80/20 HPLC grade methanol/water. Eluting gradient: from 24 % to 44 % of B in 1.2 minutes, flow rate 0.4 ml/min. Absorption was detected at 260 nm. All the target

molecule yield was used to calculate the efficiency of one-step reaction according to the proportion of peak intensity of Mass Spectrum by UPLC-MS analysis at 260 nm. Unless otherwise specified, all the DNA-tagged substrates were purified by HPLC.

The preparation of headpiece-conjugated functional linker for sub-library 1 and 2



To a solution of headpiece (0.5 mmol) in sodium borate buffer (250 mM, pH = 9.4, 500 mL) were added the mixed solution of Fmoc-AOP (10 eq, 200 mM in DMA), HATU (10 eq, 400 mM in DMA), and DIPEA (10 eq, 400 mM in DMA) which was pre-reacted at 5℃ for 20 min. After being vibrated for seconds, the mixture was allowed to proceed at room temperature overnight. The reaction was monitored by UPLC/MS. Upon completion, the crude target product was obtained by ethanol precipitation as described before. The DNA precipitate was re-dissolved in 500 mL deionized water and piperidine (50 mL) was added. After being vibrating for seconds, the mixture was proceeded at room temperature for 2 h until UPLC-MS analysis showed the reaction was completed. The crude product HP-AOP was obtained by ethanol precipitation in yield = 95%.

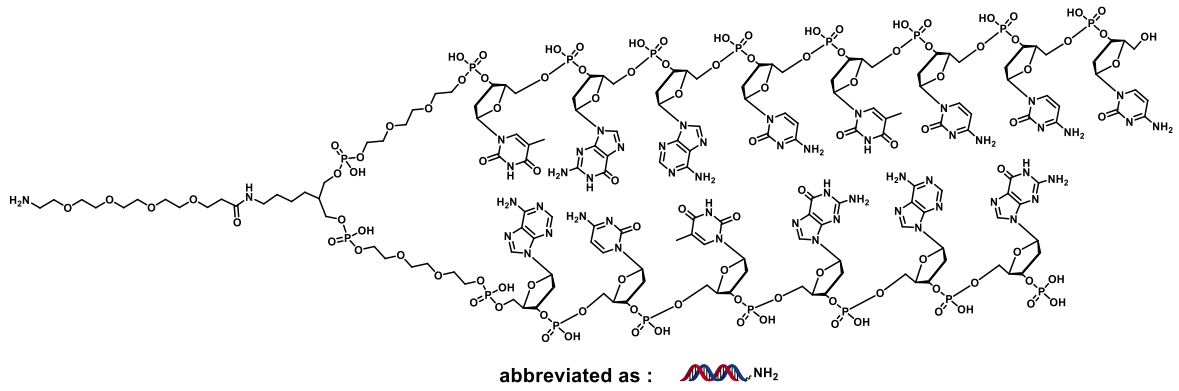


Figure A2. HP-AOP

To the solution of HP-AOP (0.2 mmol) in sodium borate buffer (250 mM, pH = 9.4, 200 mL) were added the mixed solution of 3,3'-(3H-diazirine-3,3-diyl)dipropionic acid (10 eq, 200 mM in DMA), HATU (10 eq, 400 mM in DMA), and DIPEA (10 eq, 400 mM in DMA) which was pre-reacted at 5℃ for 20 min. After being vibrated for 1 min, the mixture

was allowed to react at room temperature for 1 hour. UPLC-MS analysis indicated the reaction was completed. The product was obtained by ethanol precipitation in yield = 80%.

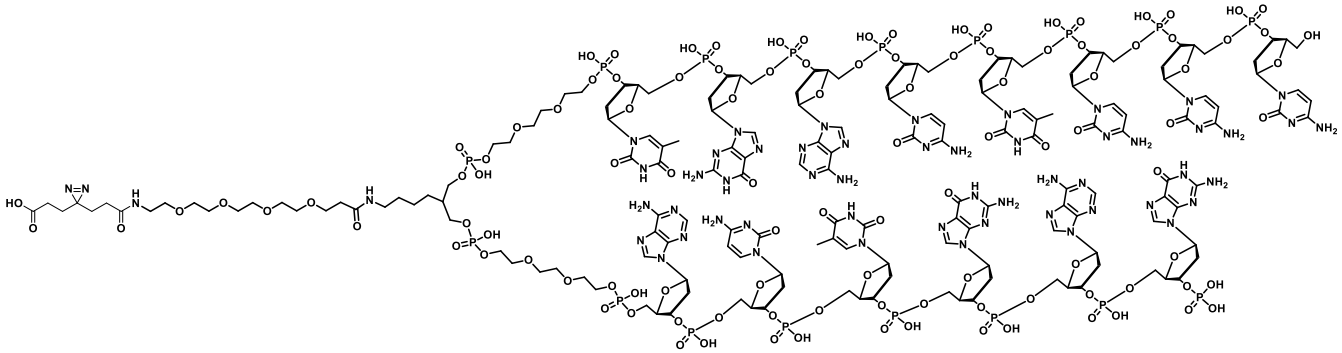
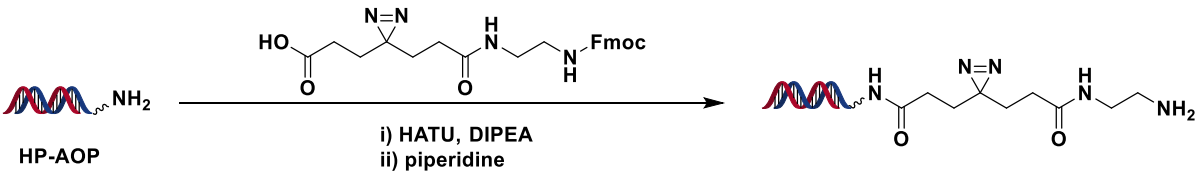


Figure A3. Headpiece-conjugated functional linker for sub-library 1 and 2

The preparation of headpiece-conjugated functional linker for sub-library 3 and 4



To a solution of HP-AOP (0.2 mmol) in sodium borate buffer (250 mM, pH = 9.4, 200 mL) were added the mixed solution of 3-(3-(3-((2-((((9H-fluoren-9-yl)methoxy)carbonyl)amino)ethyl)amino)-3-oxopropyl)-3H-diazirin-3-yl)propanoic acid (30 eq, 200 mM in DMA), HATU (30 eq, 400 mM in DMA), and DIPEA (30 eq, 400 mM in DMA) which was pre-reacted at 5℃ for 20 min. After being vibrated for seconds, the mixture was allowed to proceed at room temperature overnight. The reaction was monitored by UPLC/MS. Upon completion, the crude target product was obtained by ethanol precipitation as described before. The DNA precipitate was re-dissolved in 200 mL deionized water and piperidine (20 mL) was added. After being vibrating for seconds, the mixture was proceeded at room temperature for 2 h until UPLC-MS analysis showed the reaction was completed. The product was obtained by ethanol precipitation in yield = 70%.

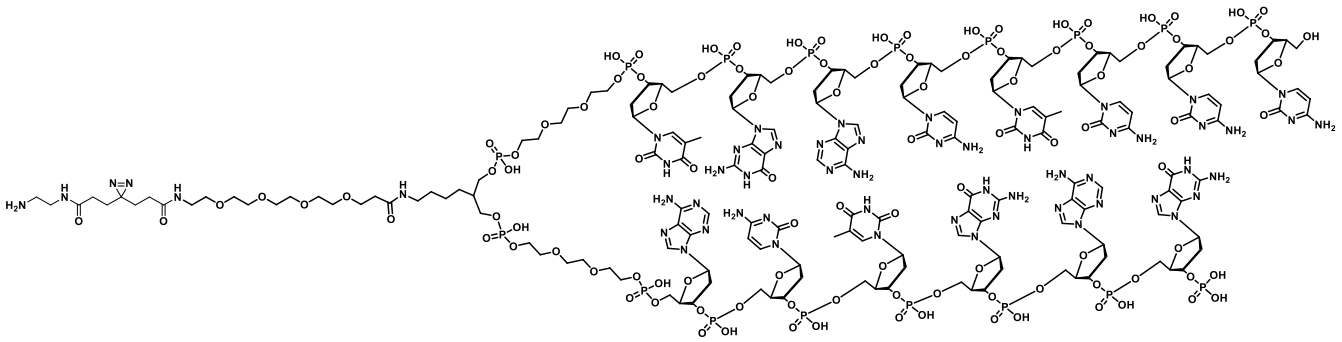
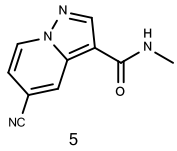


Figure A4. Headpiece-conjugated functional linker for sub-library 3 and 4

Synthesis of fragments not commercially available.

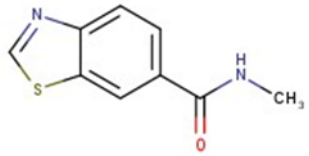
5-cyano-N-methylpyrazolo[1,5-a]pyridine-3-carboxamide (5)



5

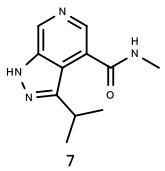
5-cyano-N-methylpyrazolo[1,5-a]pyridine-3-carboylic acid (100 mg, 0.5 mmol) was stirred in DCM (0.25 M) at 0°C. To the stirring solution were added DIPEA (258.5 mg, 2.0 mmol) and HATU (285.2 mg, 0.75 mmol). After 0.5 h, methylamine hydrochloride (67.5 mg, 1.0 mmol) was added. Then the reaction was stirred at 25°C for 16h. The reaction was monitored to be complete by TLC and LCMS. Water (5 mL) was added to the mixture and extracted with ethyl acetate (2 × 5 mL). Then the combined organic extracts were washed with brine (2 × 5 mL), dried over anhydrous Na_2SO_4, and concentrated in vacuo. Then the crude was purified by column chromatography to afford **5** (100 mg, white solid). White solid, yield: 35 %. ^{1}H NMR (600 MHz, DMSO-d_6) δ 8.99 (d, J = 7.2 Hz, 1H), 8.67 (d, J = 1.9 Hz, 1H), 8.66 (s, 1H), 8.40 (d, J = 5.0 Hz, 1H), 7.37 (dd, J = 7.2, 1.9 Hz, 1H), 2.81 (d, J = 4.5 Hz, 3H). LCMS (E+) m/z: 201.2 $[M+H]^+$. HPLC: RT = 7.25 min, purity: 99.2 %.

N-methyl-1,3-benzothiazole-6-carboxamide (6)



To a suspension of Benzothiazole-6-carboxylic acid, (70 mg, 0.39 mmol) in DCM (10 mL) under N_2 were added Thionyl Chloride (0.28 mL, 3.91 mmol) and DMF (0.5 mL). The reaction was stirred for 1 hour at rt, then the volatiles were evaporated. The crude was dissolved in Acetonitrile (5 mL) before adding Methylamine, (0.02 mL, 0.86 g/mL, 2M in THF, 0.59 mmol) and DIPEA (340.22 µL, 1.95 mmol). After 2 hours (reaction gone to completion by LCMS), water was added (5mL) to the mixture and extracted with DCM (2 x 5mL). The combined organics were dried through a phase separator and evaporated. The crude was dissolved in DMSO/MeOH (9:1) and purified by preparative HPLC automated chromatography (ISCO ACCQ HP125, Prep HPLC Column: Gemini-NX C18 Dimensions: 21 mm x 150 mm 5 µM; Sample: 2.00 ml from tube 2) eluting with 5 to 95% 0.1% Ammonia/ACN in 0.1% Ammonia/Water. Fractions containing product were combined and the volatiles were evaporated before freeze drying to afford **6** (23.8 mg, white solid). Yield 32%. 1H NMR (400MHz, DMSO-d6) δ 9.53 (s, 1H), 8.62 (dd, J = 18.9, 3.2 Hz, 2H), 8.15 (d, J = 8.5 Hz, 1H), 7.99 (dd, J = 8.6, 1.8 Hz, 1H), 2.83 (d, J = 4.5 Hz, 3H). LCMS (E+) m/z: 193.2 $[M+H]^+$.

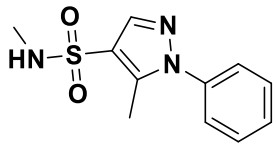
3-isopropyl-N-methyl-1H-pyrazolo[3,4-c]pyridine-4-carboxamide (7)



7

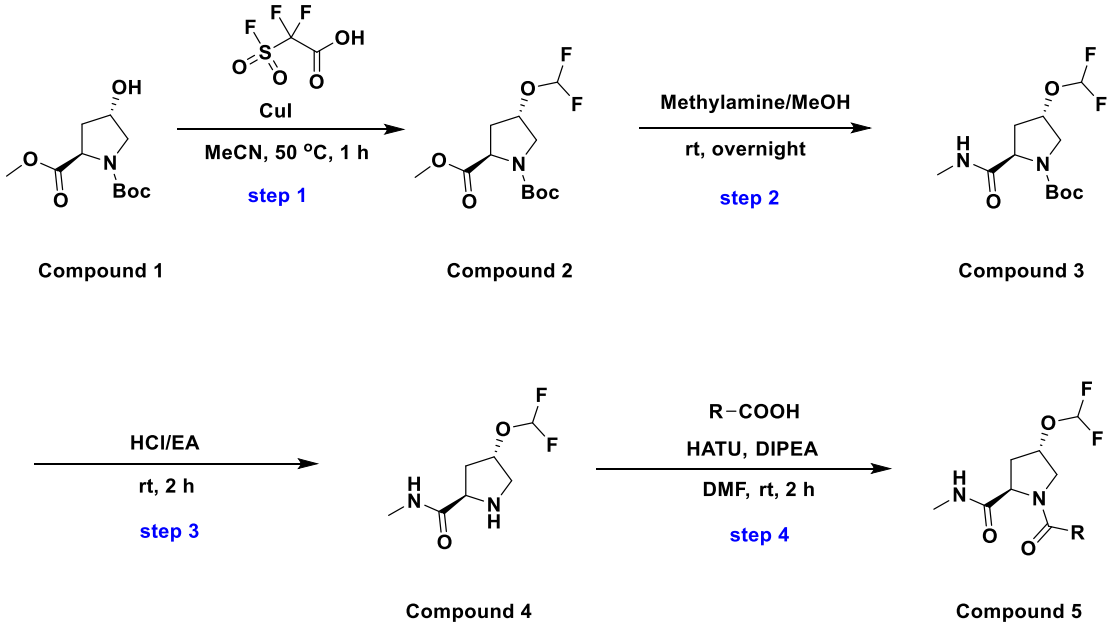
Was prepared from 3-isopropyl-N-methyl-pyrazolo[3,4-c]pyridine-4-carbolyic acid using the same procedure as 5. White solid, yield: 6 %. ^{1}H NMR (600 MHz, DMSO-d_6) δ 13.40 (s, 1H), 9.02 (s, 1H), 8.60 (d, J = 4.9 Hz, 1H), 8.20 (s, 1H), 3.54 (h, J = 6.7 Hz, 1H), 2.83 (d, J = 4.6 Hz, 3H), 1.26 (d, J = 6.8 Hz, 6H). LCMS (E+) m/z: 219.0 $[M+H]^+$. HPLC: RT = 3.90 min, purity: 99.7 %.

N,5-dimethyl-1-phenyl-1H-pyrazole-4-sulfonamide.(8)



5-methyl-1-phenyl-1H-pyrazole-4-sulfonyl chloride (100 mg, 0.39 mmol) was stirred in DCM (0.25 M) at 0 °C. To the stirring solution were added TEA (118.4 mg, 1.17 mmol) and methylamine hydrochloride (29.0 mg, 0.43 mmol). Then the reaction was stirred at 25 °C for 4h. The reaction completion was monitored by TLC and LCMS. Water (5 mL) was added to the mixture and extracted with ethyl acetate (3 ×5 mL). Then the combined organic extracts were washed with brine (2 × 5 mL), dried over anhydrous Na_2SO_4, and concentrated in vacuo. The residue was purified by column chromatography to afford N,5-dimethyl-1-phenyl-1H-pyrazole-4-sulfonamide (75 mg, white solid). Yield: 76%. ^{1}H NMR (600 MHz, Methanol-d4) δ 7.90 (s, 1H), 7.61 – 7.51 (m, 3H), 7.49 (dd, J = 7.5, 2.2 Hz, 2H), 2.61 (s, 3H), 2.47 (s, 3H). LCMS (E+) m/z: 252.2 $[M+H]^+$. HPLC: RT = 6.09 min, purity: 98.7%.

General procedure for synthesis of fragments from Type 2 libraries



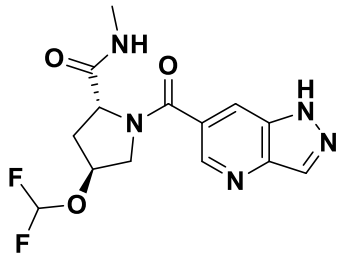
Step 1: To a solution of 1-(tert-butyl) 2-methyl (2R,4S)-4-hydroxypyrrolidine-1,2-dicarboxylate (4.8g, 19.57 mmol) in acetonitrile (66 mL) was added cuprous iodide (0.74g, 3.91 mmol) at 50 °C under nitrogen. Then a solution of 2,2-difluoro-2-(fluorosulfonyl)acetic acid (4,18g, 23.48 mmol) in acetonitrile (19.8 mL) was added and stirred at 50 °C for 1 hour. The reaction was concentrated, diluted with ethyl acetate (50 mL), washed with water (50 mL), brine (50 mL). Then organic layer was dried over anhydrous Na_2SO_4, concentrated under reduced pressure to afford the desired crude **Compound 2** as pale yellow solid (2.9 g, 9.8 mmol, 50% yield).

Step 2: To a solution of methylamine in methyl alcohol (30% mw%, 100 mL) was added **Compound 2** (2.9 g, 9.8 mmol). The mixture was then stirred at room temperature overnight until LCMS indicated the starting material was almostly consumed. The mixture was concentrated under reduced pressure to afford **compound 3** as grey solid and used for next step without further purification.

Step 3: To a flask charged with the crude **Compound 3** was added HCl ethyl acetate solution (4 M, 50 mL), and the mixture was then stirred at room temperature for 2 hours until TLC showed the starting material was compeletly consumed. The mixture was concentrated, and washed with diethyl ether to afford **Compound 4** as off while solid (1.7 g, 8.7 mmol, 89% yield over last two steps).

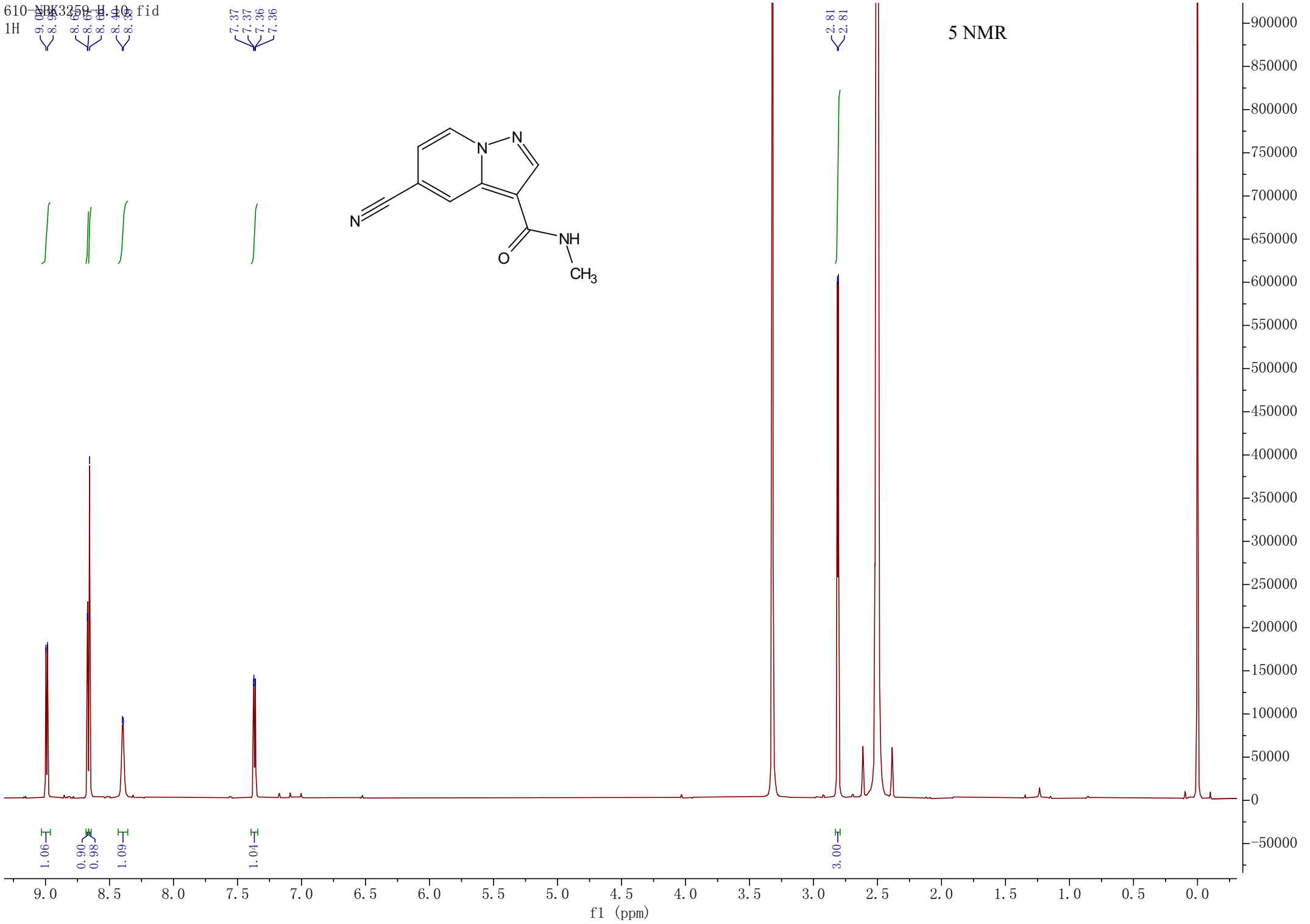
Step 4: To a stirred solution of acid (0.1 mmol) in DMF (2 mL) were added DIPEA (0.5 mmol) and HATU (0.15 mmol) at 0 °C. After being stirred for 0.5 hour, **Compound 4** (0.12 mmol) were added. The mixture was allowed to warm to room temperature and stirred for 2 hours until water (0.2 mL) was added. The mixture was then purified by column chromatography to afford the desired **Compound 5**.

(2R,4S)-4-(difluoromethoxy)-N-methyl-1-(1H-pyrazolo[4,3-b]pyridine-6-carbonyl)pyrrolidine-2-carboxamide (9)



White solid, yield: 26%.
^{1}H NMR (600 MHz, DMSO-d_6) δ 13.58 (s, 1H), 8.75-7.70 (m, 4H), 7.00-6.50 (m, 1H), 5.00-4.78 (m, 1H), 4.65-4.30 (m, 1H), 4.00-3.55 (m, 2H), 2.70-2.05 (m, 5H). LCMS (E+) m/z: 340.1 $[M+H]^+$. HPLC: RT = 4.08 min, purity: 98.2 %.

^{1}H NMR, and LCMS data for synthesized fragments


610-NBK3259-H.10.fid
1H
5 NMR
9.08
8.67
8.66
8.44
8.43
7.37
7.37
7.36
7.36
2.81
2.81
N
N
N
NH
O
CH3
1.06
0.90
0.98
1.09
1.04
3.00
900000
850000
800000
750000
700000
650000
600000
550000
500000
450000
400000
350000
300000
250000
200000
150000
100000
50000
0
-50000
9.0
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
f1 (ppm)

LCMS Report

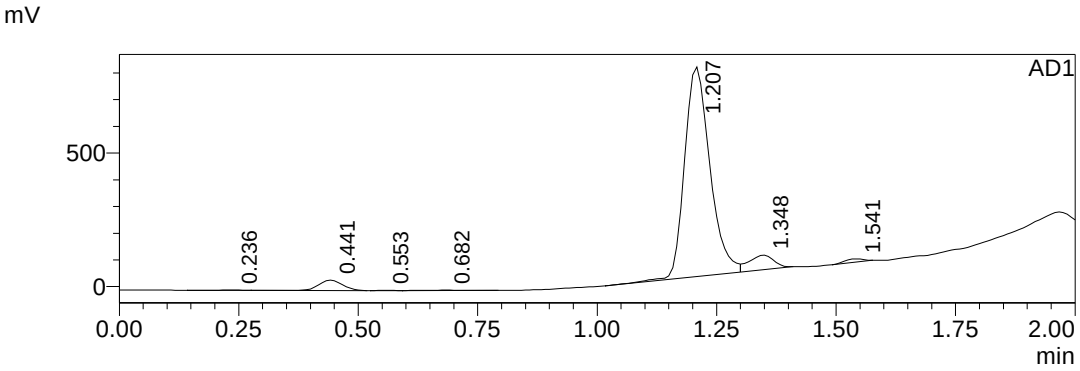
5 LCMS

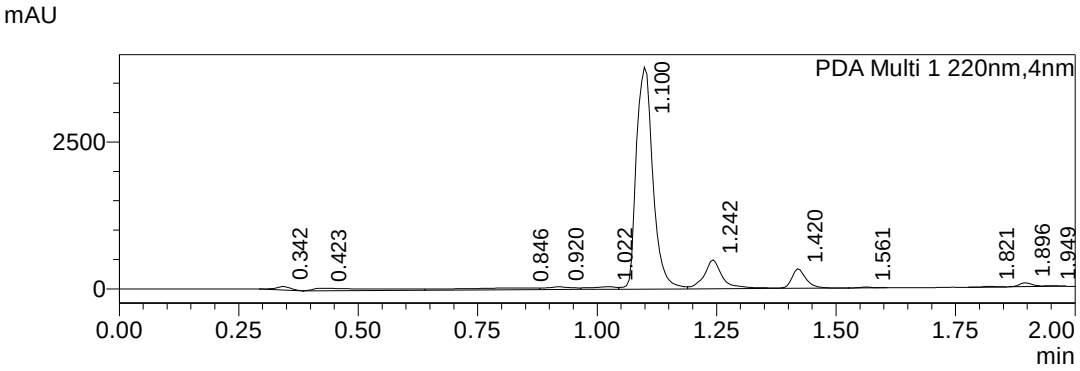
<Sample Information>

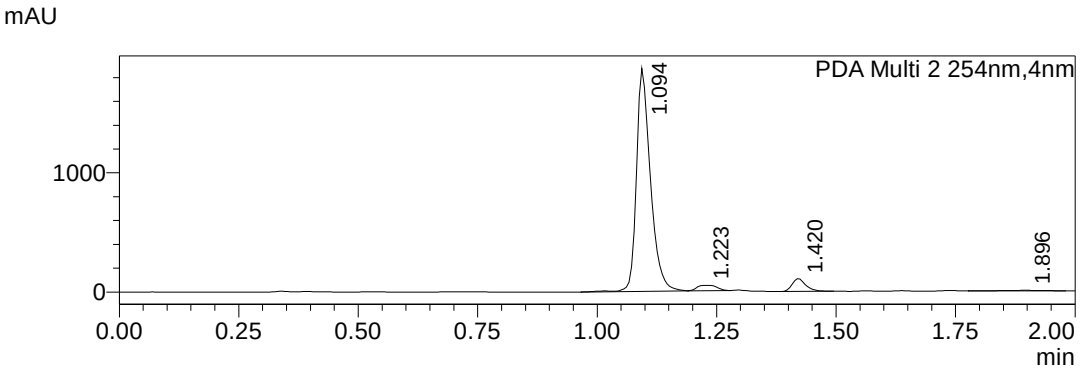
Sample ID	: 610-NBK3259-H-F		
Batch ID:			
Project ID	: 2058	Instrument: LCMS-3	
Method Filename	: 5-95%_2MIN.lcm		
Vial #	: 2-23	Injection Volume	: 10 uL
Date Acquired	: 2021/6/24 16:33:19	Acquired by	:Fang.Jiao
Date Processed	: 2021/6/24 16:35:22	Processed by	: Ling.Wang

Method : Column: XBridge BEH Shield RP18 Column,3.5μm, 50mm*4.6mm.
Mobile Phase: A:0.05%TFA Water B:0.05%TFA ACN
Gradient:5-95%B in 1.2min,and hold 95%B for 0.4min,
then re-equilibrate column at 5%B for 0.4min.
Flow Rate: 2.5 mL/min; Column Temperature:40 ℃.

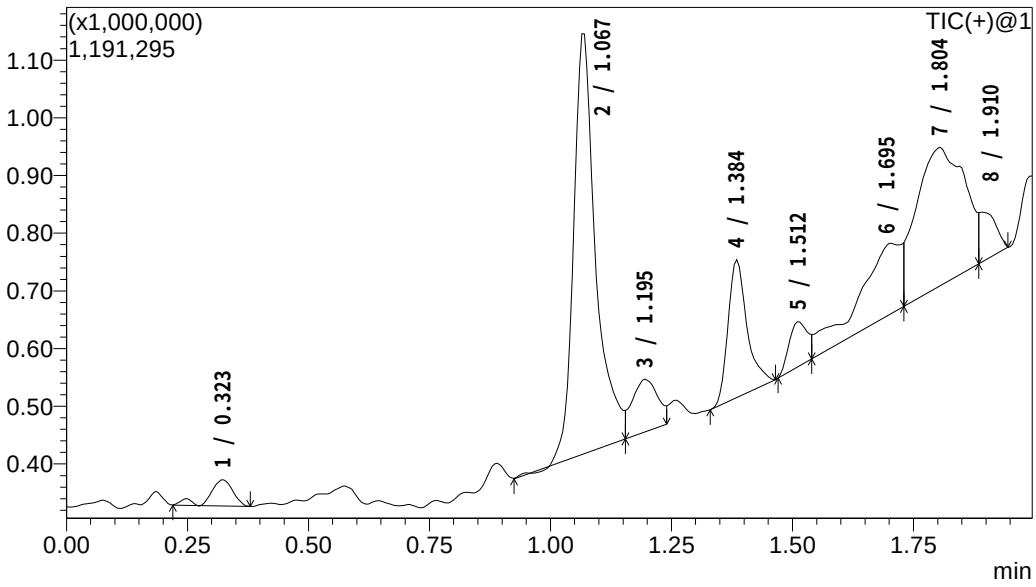
<Chromatogram>







<MS Chromatogram>


(x1,000,000)
1,191,295
TIC(+)@1
1.10
1.00
0.90
0.80
0.70
0.60
0.50
0.40
1 / 0.323
2 / 1.067
3 / 1.195
4 / 1.384
5 / 1.512
6 / 1.695
7 / 1.804
8 / 1.910
0.00
0.25
0.50
0.75
1.00
1.25
1.50
1.75
min

<Chromatogram Peak Table>

Peak Table

AD1

Peak#	Ret. Time	Area	Height	Area%
1	0.236	3269	857	0.099
2	0.441	135735	39256	4.096
3	0.553	1276	584	0.038
4	0.682	8923	1354	0.269
5	1.207	2924897	784984	88.256
6	1.348	201866	54997	6.091
7	1.541	38137	12559	1.151
总计		3314102	894592	100.000

PDA Ch1 220nm

Peak#	Ret. Time	Area	Height	Area%
1	0.342	116151	57375	0.925
2	0.423	426931	42650	3.402
3	0.846	369133	30651	2.941
4	0.920	173075	44567	1.379
5	1.022	163296	43346	1.301
6	1.100	9095304	3776306	72.470
7	1.242	1310533	492556	10.442
8	1.420	695593	330333	5.542
9	1.561	29715	14640	0.237
10	1.821	19588	9956	0.156
11	1.896	127379	64595	1.015
12	1.949	23822	13256	0.190
总计		12550520	4920231	100.000

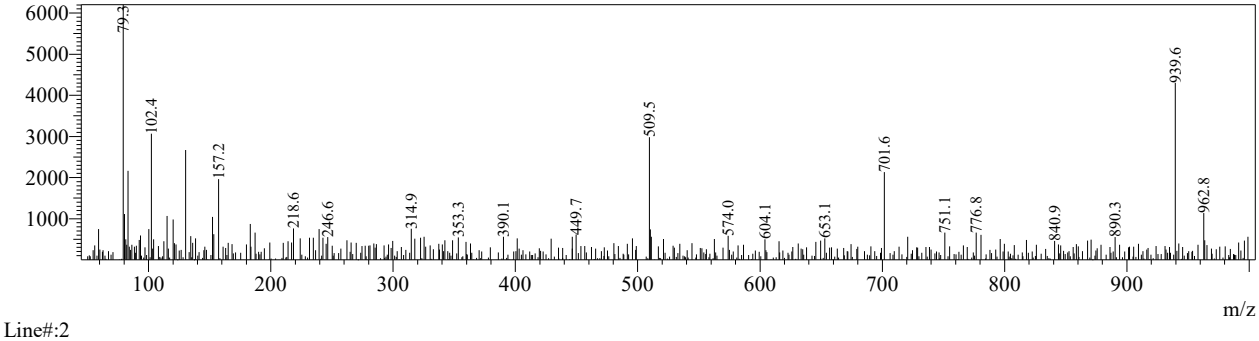
PDA Ch2 254nm

Peak#	Ret. Time	Area	Height	Area%
1	1.094	3788441	1870329	91.334
2	1.223	126678	44890	3.054
3	1.420	215651	106414	5.199
4	1.896	17140	4614	0.413
总计		4147909	2026247	100.000

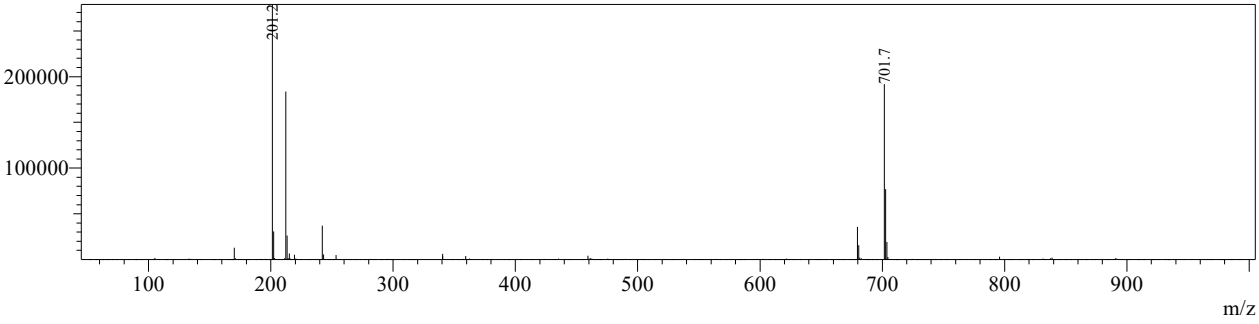
<MS Spectrum>

MS Spectrum

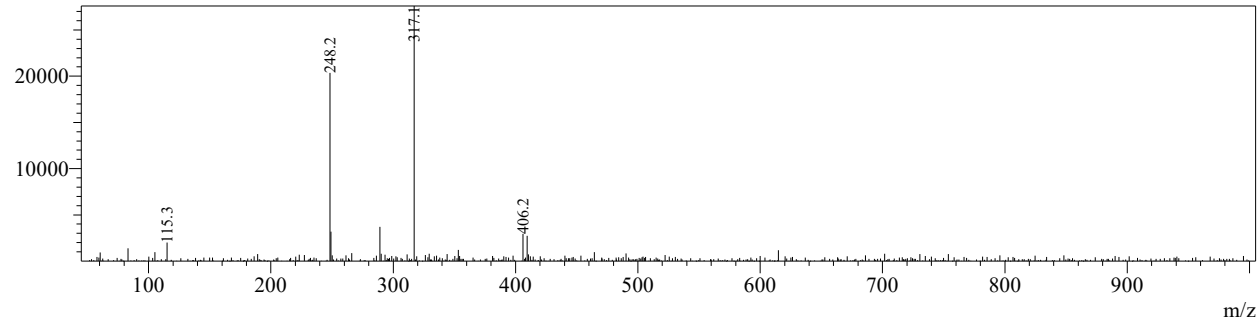
Line#:1
Rt:0.320-0.330(65-67)



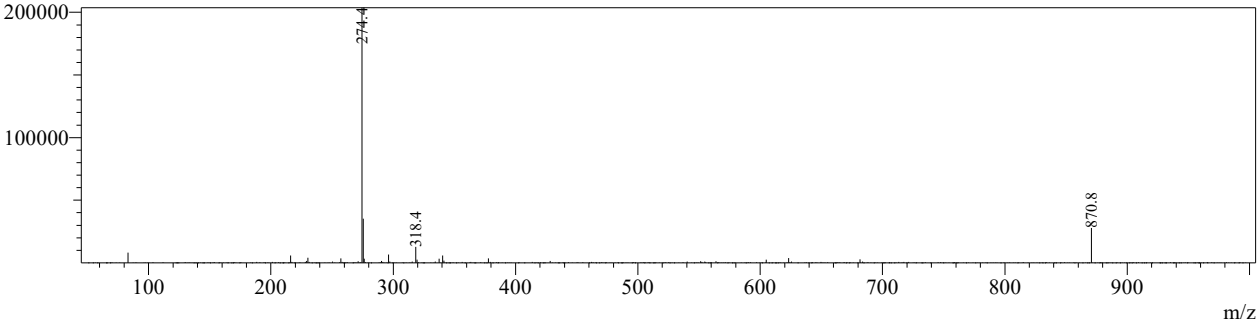
Line#:2
Rt:1.060-1.070(213-215)



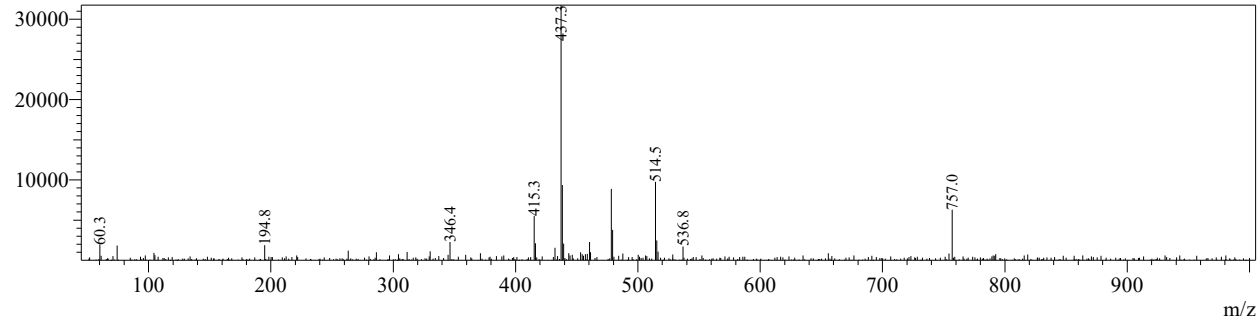
Line#:3
Rt:1.190-1.200(239-241)



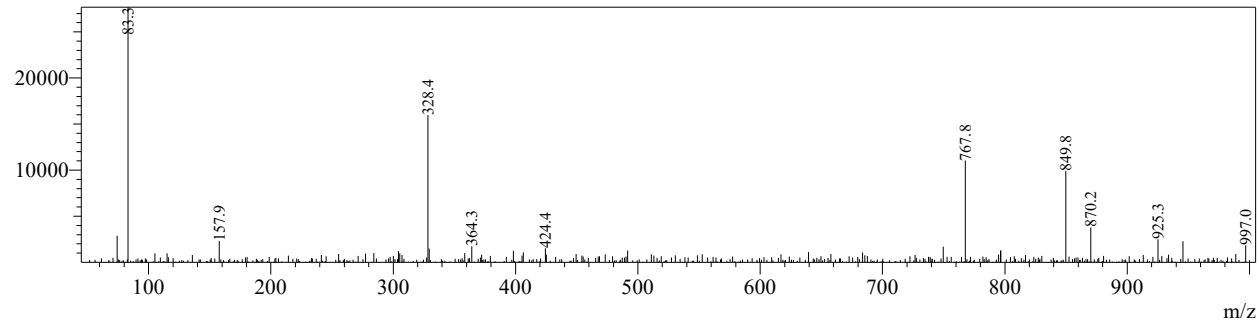
Line#:4
Rt:1.380-1.390(277-279)



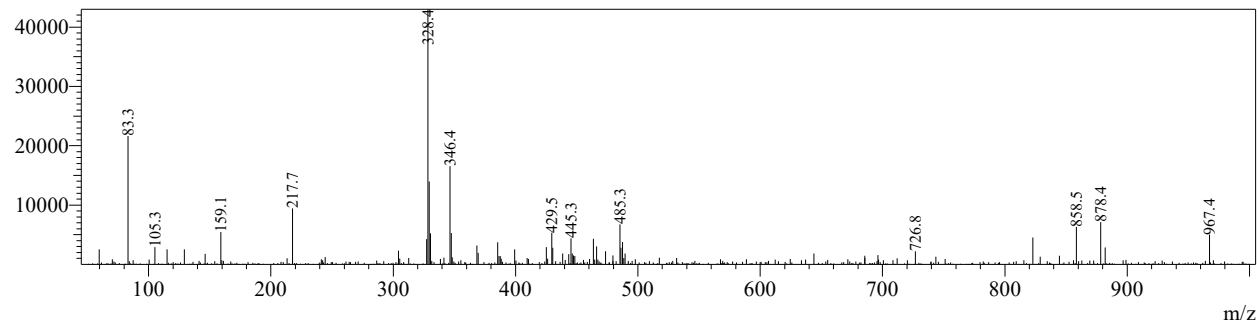
Line#:5
Rt:1.505-1.515(302-304)



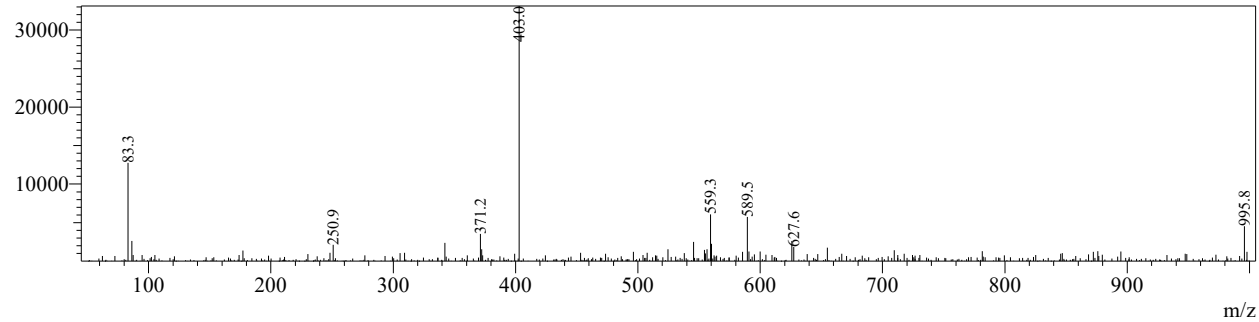
Line#:6
Rt:1.690-1.700(339-341)

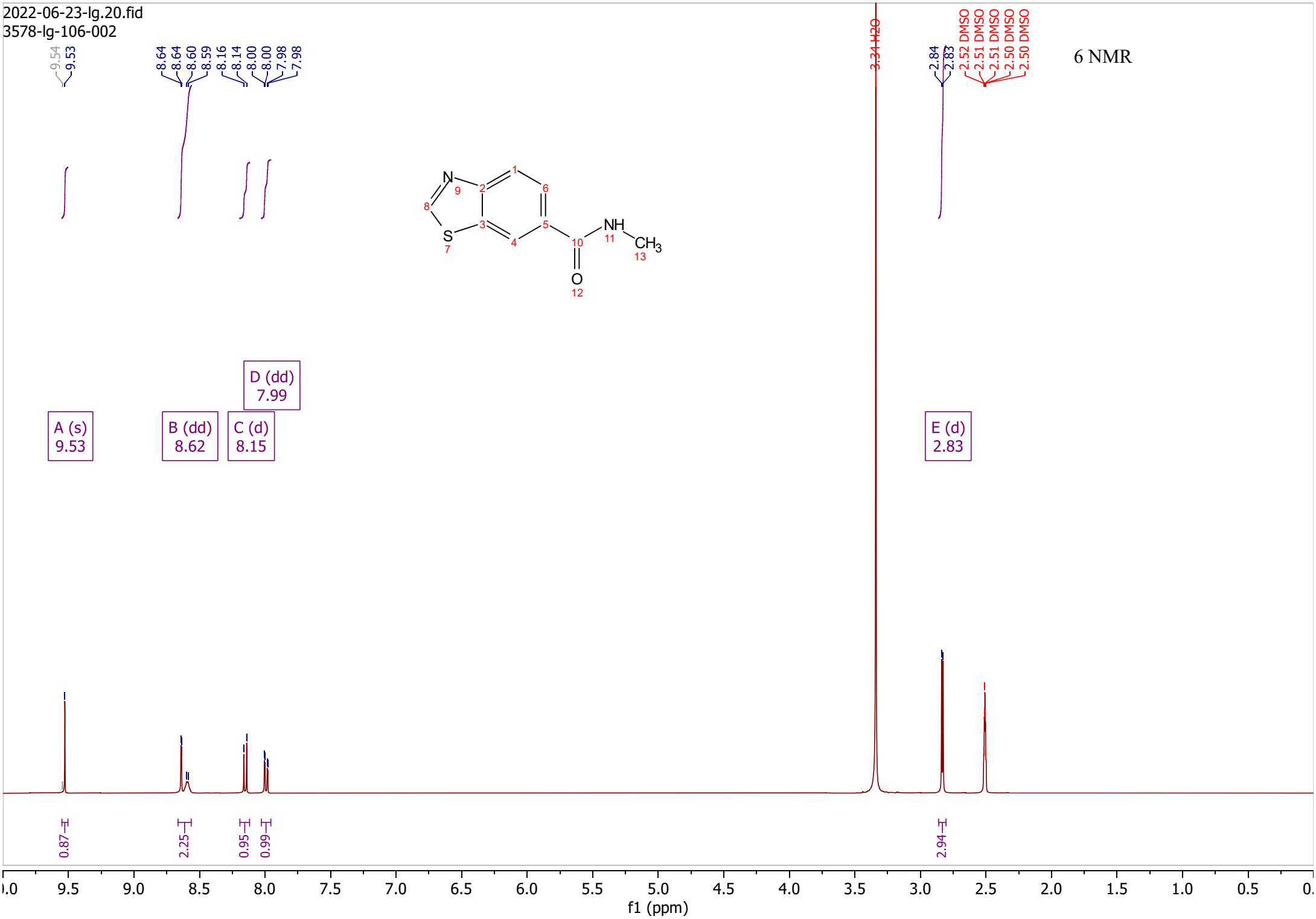


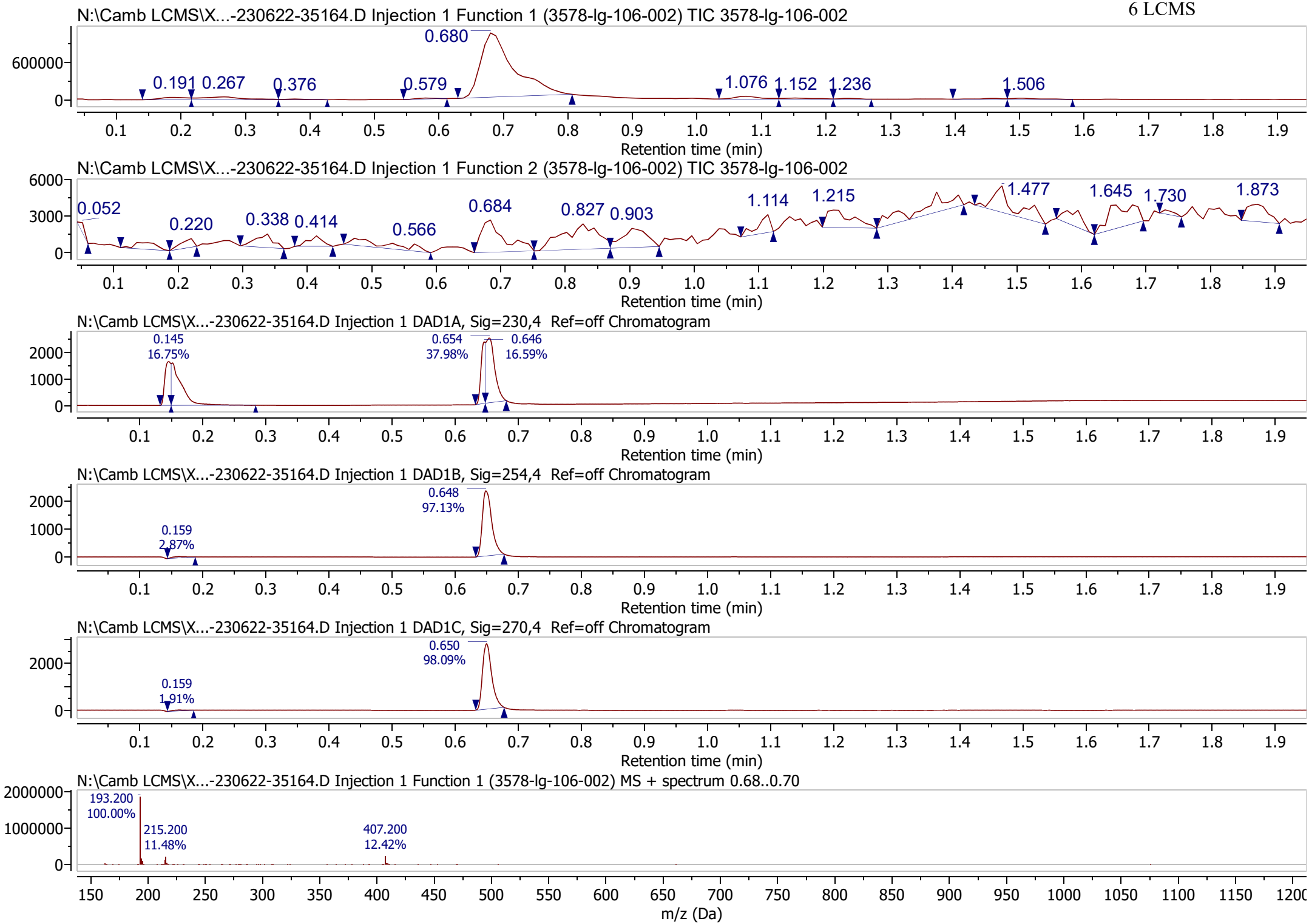
Line#:7
Rt:1.800-1.810(361-363)



Line#:8
Rt:1.905-1.915(382-384)

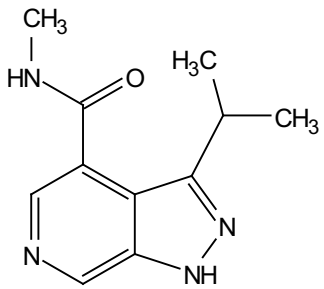



2022-06-23-lg.20.fid
3578-lg-106-002
6 NMR
9.54
9.53
8.64
8.64
8.60
8.59
8.16
8.14
8.00
8.00
7.98
7.98
3.34 H2O
2.84
2.83
2.52 DMSO
2.51 DMSO
2.51 DMSO
2.50 DMSO
2.50 DMSO
A (s)
9.53
B (dd)
8.62
C (d)
8.15
D (dd)
7.99
E (d)
2.83
0.87
2.25
0.95
0.99
2.94
f1 (ppm)


N:\Camb LCMS\X...-230622-35164.D Injection 1 Function 1 (3578-lg-106-002) TIC 3578-lg-106-002
0.680
0.191
0.267
0.376
0.579
1.076
1.152
1.236
1.506
Retention time (min)
N:\Camb LCMS\X...-230622-35164.D Injection 1 Function 2 (3578-lg-106-002) TIC 3578-lg-106-002
0.052
0.220
0.338
0.414
0.566
0.684
0.827
0.903
1.114
1.215
1.477
1.645
1.730
1.873
Retention time (min)
N:\Camb LCMS\X...-230622-35164.D Injection 1 DAD1A, Sig=230,4 Ref=off Chromatogram
0.145
16.75%
0.654
37.98%
0.646
16.59%
Retention time (min)
N:\Camb LCMS\X...-230622-35164.D Injection 1 DAD1B, Sig=254,4 Ref=off Chromatogram
0.648
97.13%
0.159
2.87%
Retention time (min)
N:\Camb LCMS\X...-230622-35164.D Injection 1 DAD1C, Sig=270,4 Ref=off Chromatogram
0.650
98.09%
0.159
1.91%
Retention time (min)
N:\Camb LCMS\X...-230622-35164.D Injection 1 Function 1 (3578-lg-106-002) MS + spectrum 0.68..0.70
193.200
100.00%
215.200
11.48%
407.200
12.42%
m/z (Da)

610-NBK3259-L-F.10.fid
1H

7 NMR



f1 (ppm)

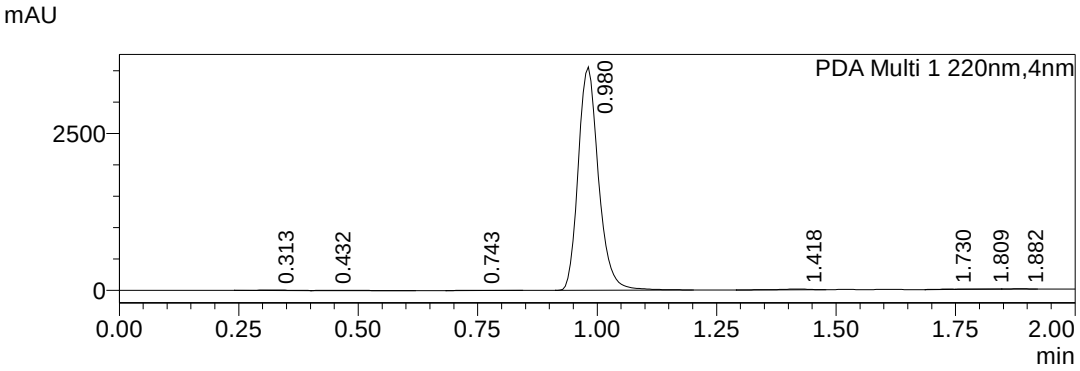
LCMS Report

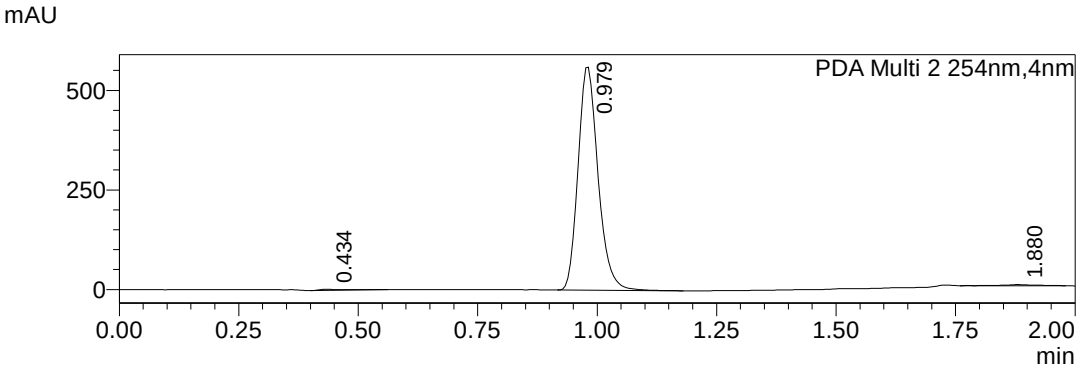
<Sample Information>

Sample ID	: 610-HG060267-L-F-F-2		
Batch ID:			
Project ID	: 2355	Instrument: LCMS-3	
Method Filename	: 5-95%_2MIN.lcm		
Vial #	: 1-19	Injection Volume	: 5 uL
Date Acquired	: 2022/5/26 10:42:50	Acquired by	:Yeping.Shen
Date Processed	: 2022/5/26 10:44:52	Processed by	:Fang.Jiao

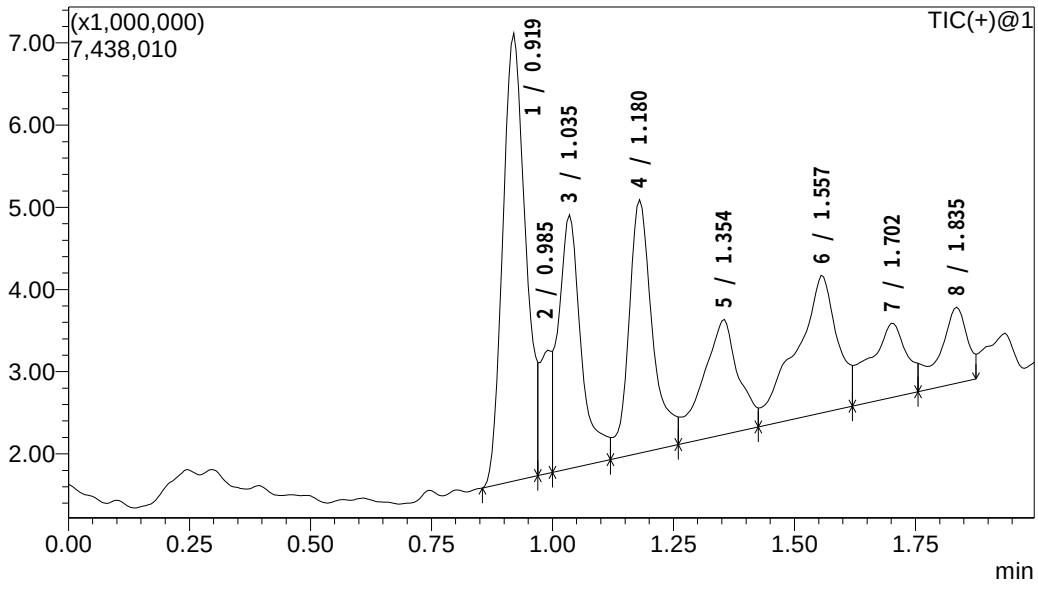
Method : Column: Waters XBridge Protein BEH C4 100mm*4.6mm 3.5um
Mobile Phase: A:0.05%TFA Water B:0.05%TFA ACN
Gradient:5-95%B in 1.2min,and hold 95%B for 0.4min,
then re-equilibrate column at 5%B for 0.4min.
Flow Rate: 2.5 mL/min; Column Temperature:40 ℃.

<Chromatogram>





<MS Chromatogram>



<Chromatogram Peak Table>

Peak Table

PDA Ch1 220nm

Peak#	Ret. Time	Area	Height	Area%
1	0.313	35526	9249	0.332
2	0.432	15218	4075	0.142
3	0.743	12834	2686	0.120
4	0.980	10531240	3559749	98.531
5	1.418	46548	13538	0.436
6	1.730	11283	5013	0.106
7	1.809	20830	5746	0.195
8	1.882	14734	6474	0.138
总计		10688213	3606529	100.000

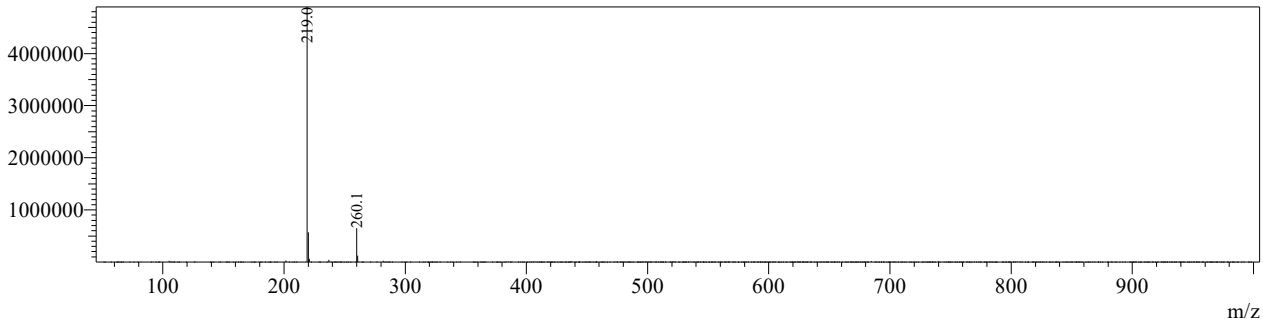
PDA Ch2 254nm

Peak#	Ret. Time	Area	Height	Area%
1	0.434	11235	2659	0.675
2	0.979	1639341	559838	98.534
3	1.880	13149	2613	0.790
总计		1663725	565110	100.000

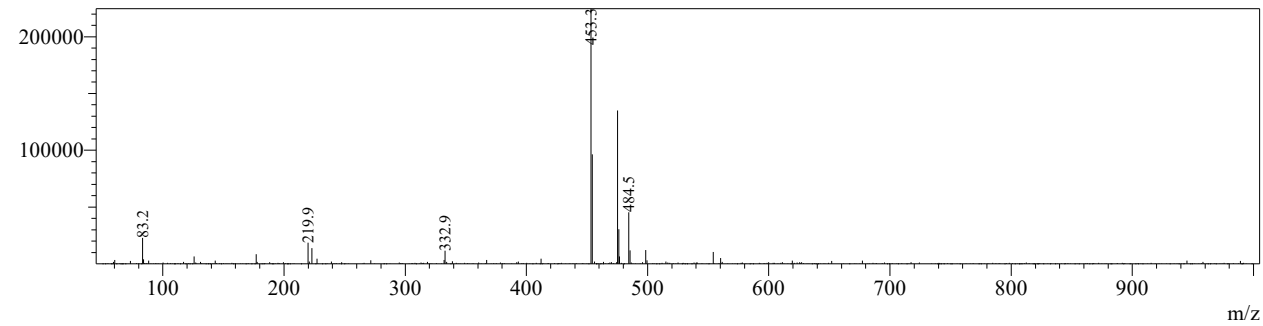
<MS Spectrum>

MS Spectrum

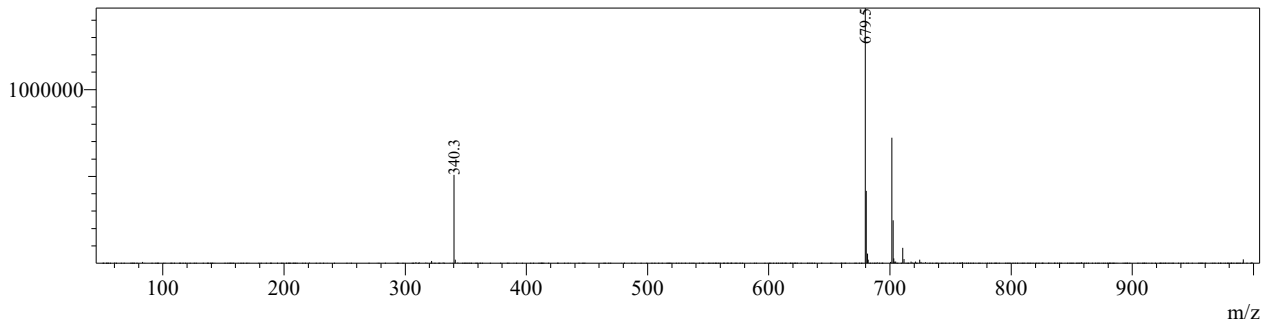
Line#:1
Rt:0.915-0.925(184-186)



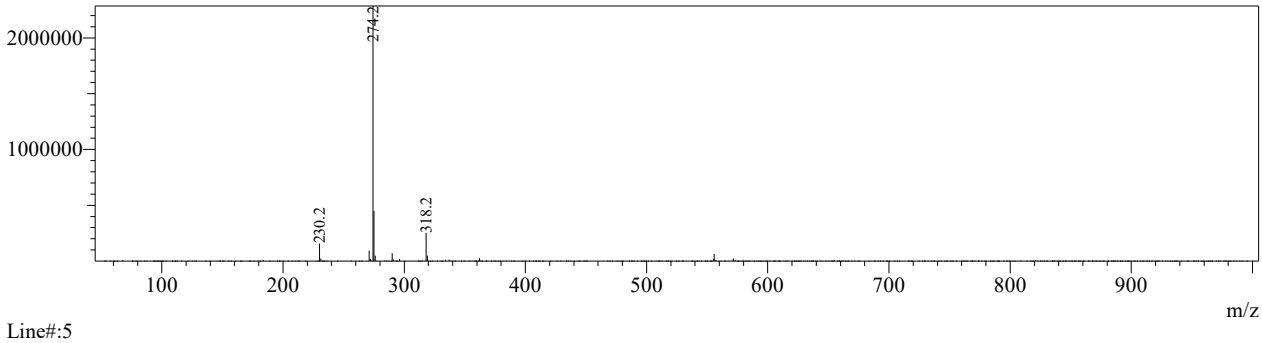
Line#:2
Rt:0.980-0.990(197-199)



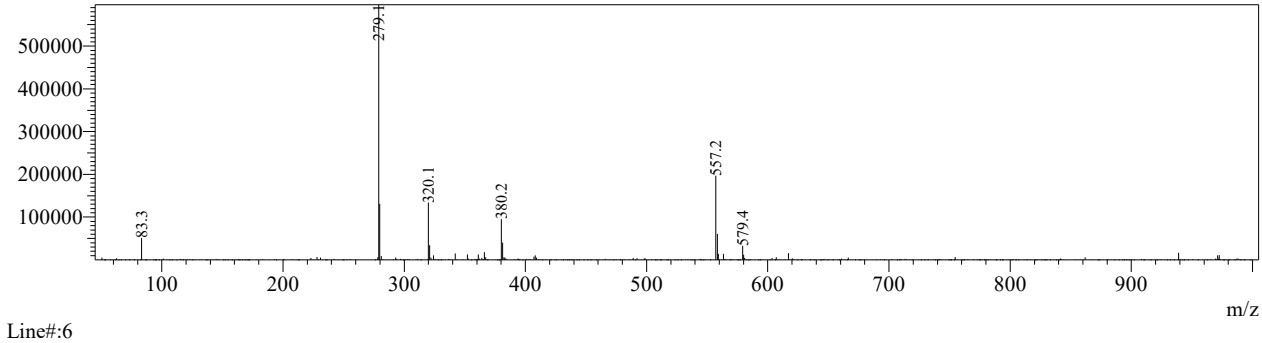
Line#:3
Rt:1.030-1.040(207-209)



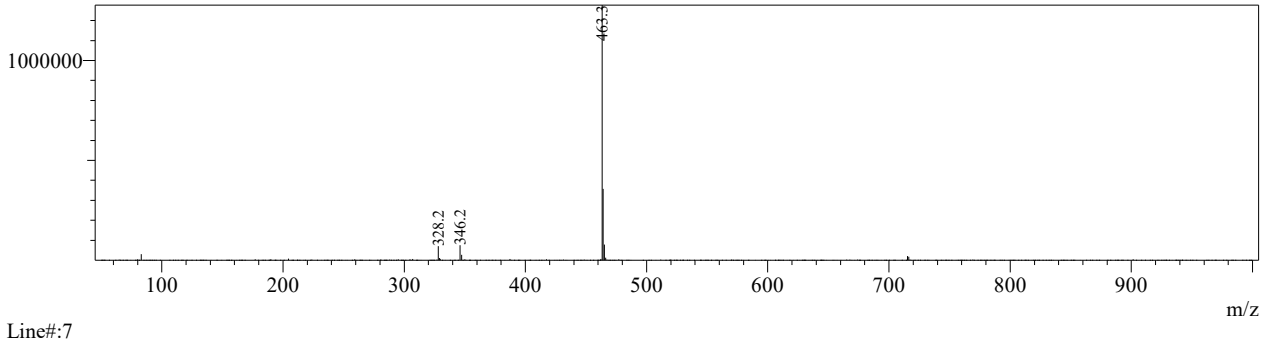
Line#:4
Rt:1.175-1.185(236-238)



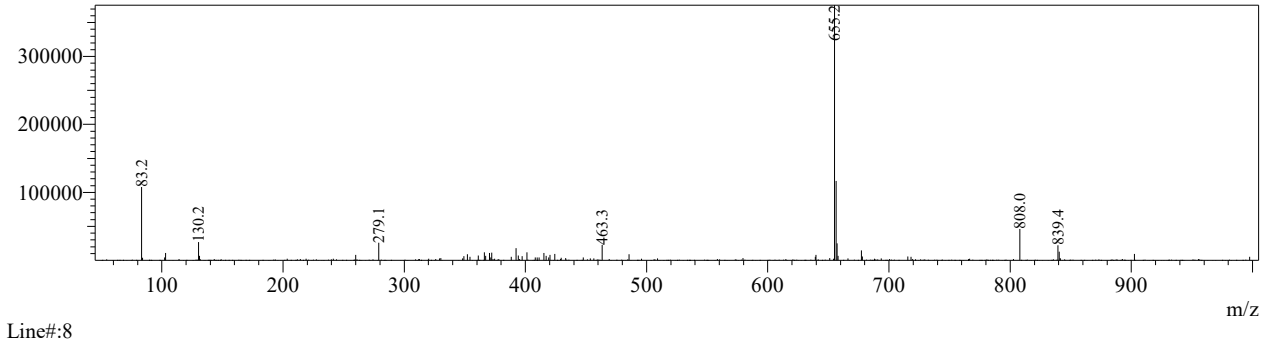
Line#:5
Rt:1.350-1.360(271-273)



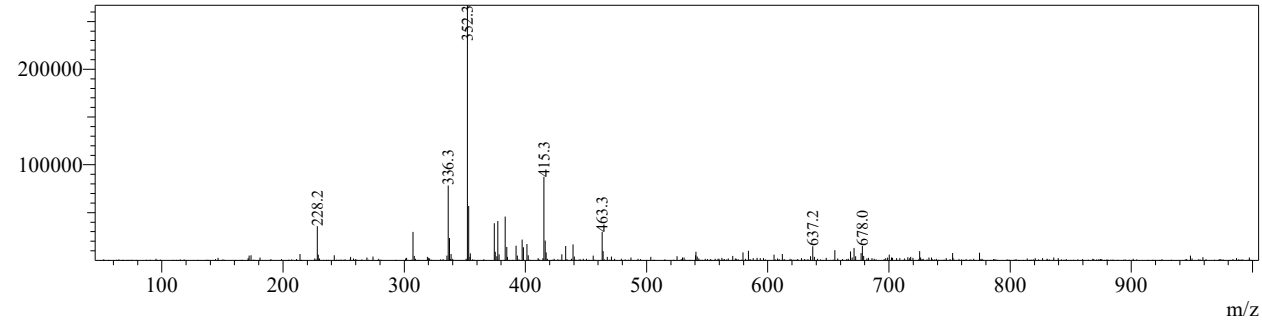
Line#:6
Rt:1.550-1.560(311-313)

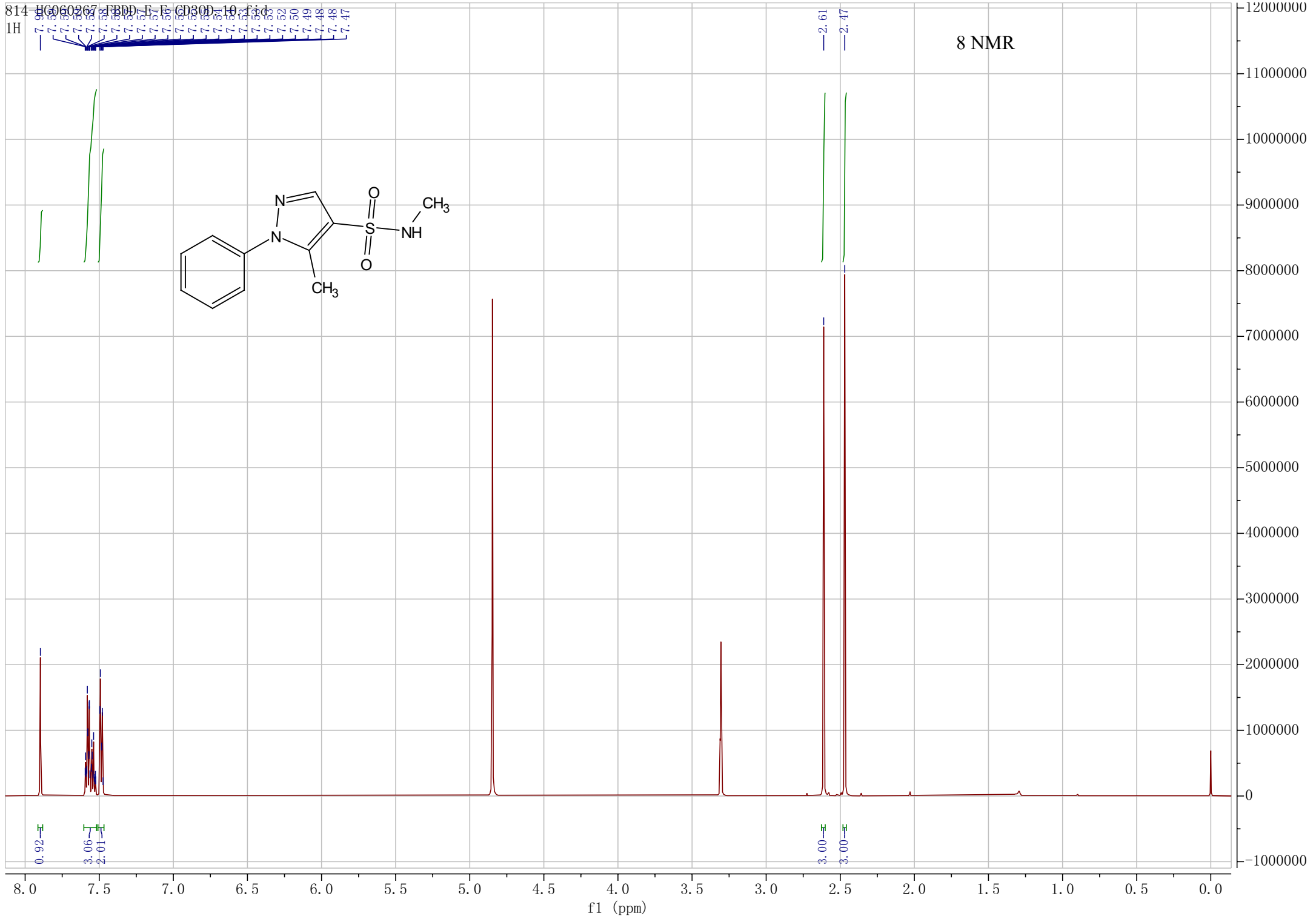


Line#:7
Rt:1.695-1.705(340-342)



Line#:8
Rt:1.830-1.840(367-369)




814-HG060267-FBDD-F-F-CD3OD-10.fid
1H
8 NMR
N
N
O
S
O
NH
CH3
CH3
f1 (ppm)

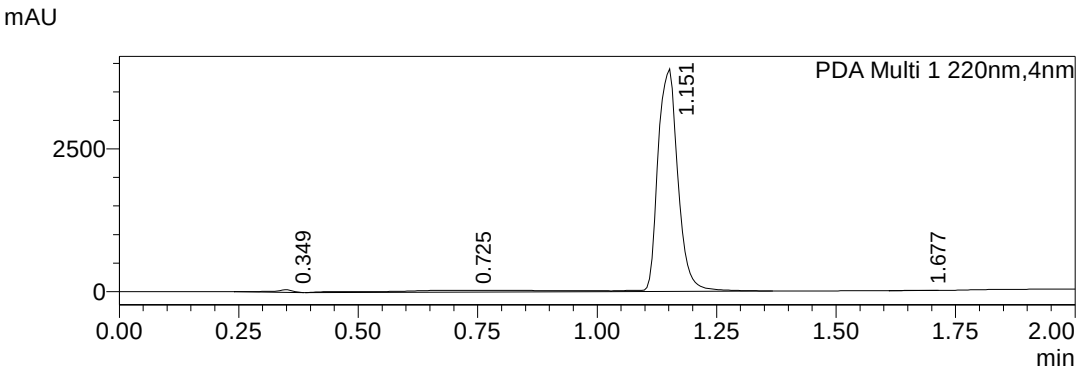
LCMS Report

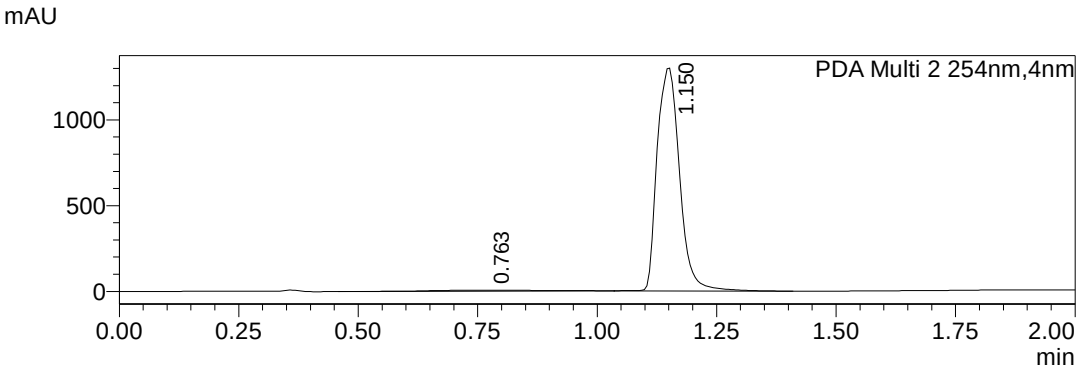
<Sample Information>

Sample ID	: 610-HG060267-FBDD-E-FINAL-1		
Batch ID:			
Project ID	: 2355	Instrument: LCMS-4	
Method Filename	: 5-95%_2MIN.lcm		
Vial #	: 3-27	Injection Volume	: 15 uL
Date Acquired	: 2022/6/17 16:35:47	Acquired by	:Yeping.Shen
Date Processed	: 2022/6/17 16:37:49	Processed by	: Fang.Jiao

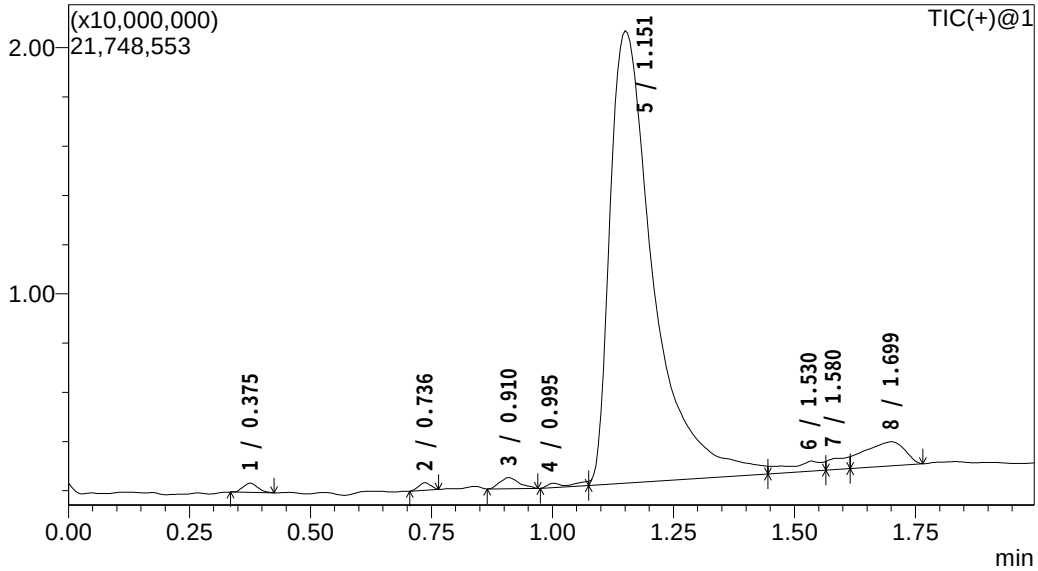
Method : Column: XBridge BEH Shield RP18 Column,3.5μm, 50mm*4.6mm.
Mobile Phase: A:0.05%TFA Water B:0.05%TFA ACN
Gradient:5-95%B in 4.0min,hold at 95%B for 1.0min.
Flow Rate: 2.5 mL/min; Column Temperature:40 °C.

<Chromatogram>





<MS Chromatogram>



<Chromatogram Peak Table>

Peak Table

PDA Ch1 220nm

Peak#	Ret. Time	Area	Height	Area%
1	0.349	105892	42585	0.861
2	0.725	749507	30964	6.095
3	1.151	11429211	3896734	92.936
4	1.677	13270	4208	0.108
总计		12297880	3974492	100.000

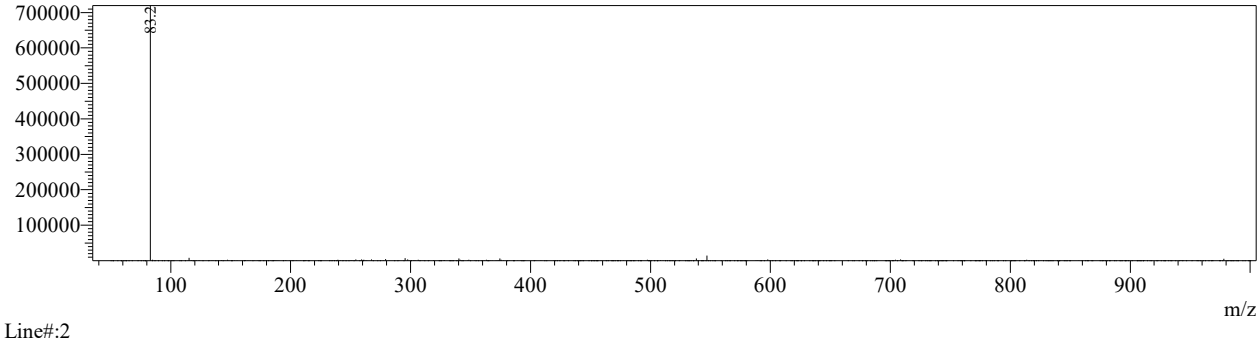
PDA Ch2 254nm

Peak#	Ret. Time	Area	Height	Area%
1	0.763	86407	5297	1.977
2	1.150	4284605	1298493	98.023
总计		4371012	1303790	100.000

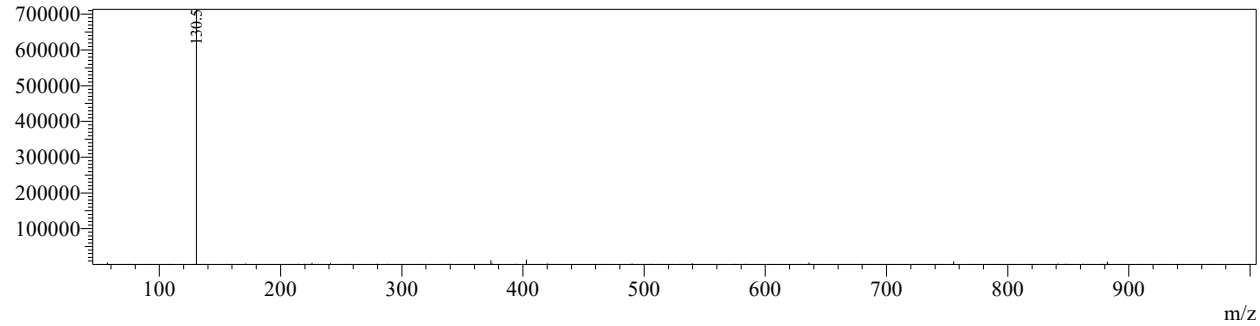
<MS Spectrum>

MS Spectrum

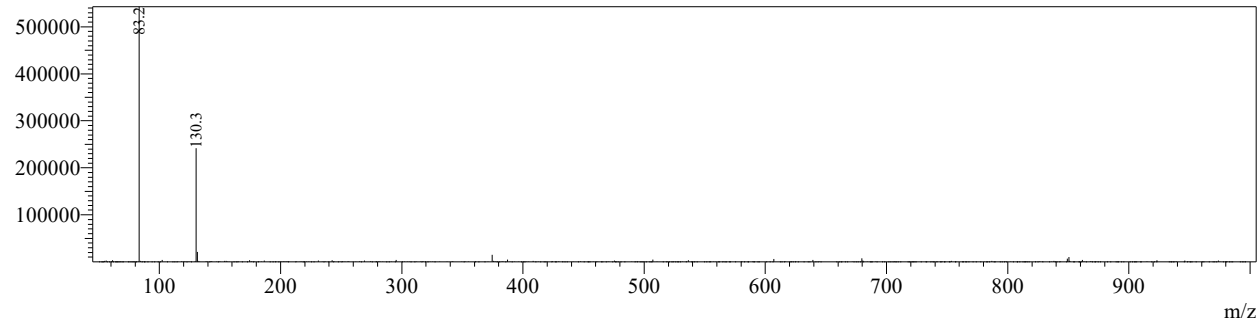
Line#:1
Rt:0.370-0.380



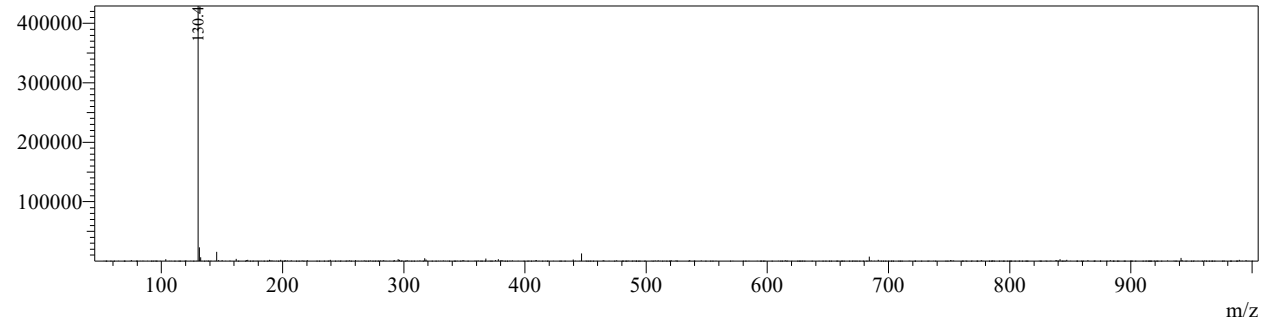
Line#:2
Rt:0.730-0.740



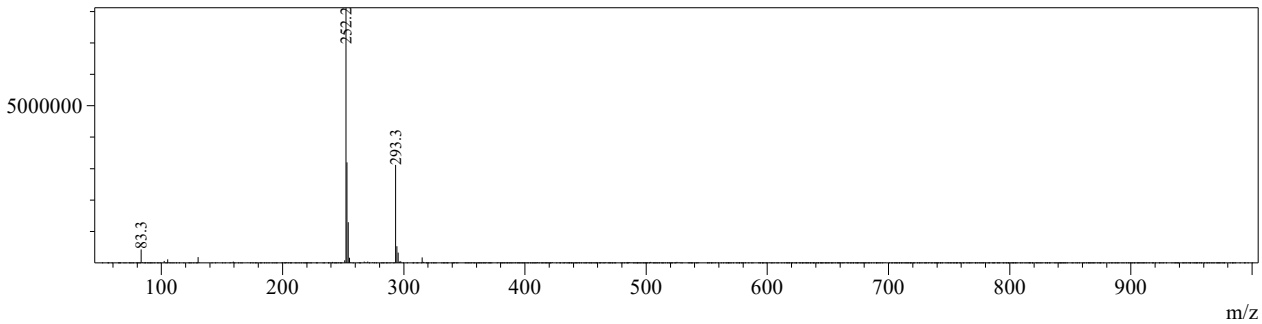
Line#:3
Rt:0.905-0.915



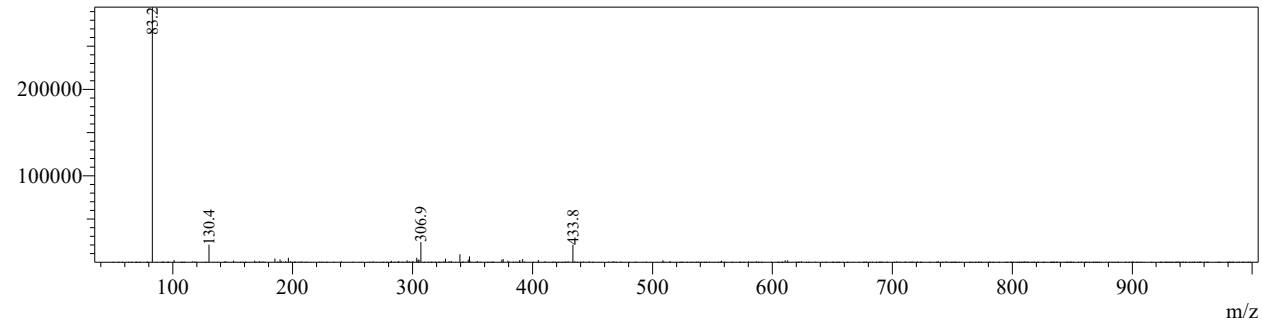
Line#:4
Rt:0.990-1.000



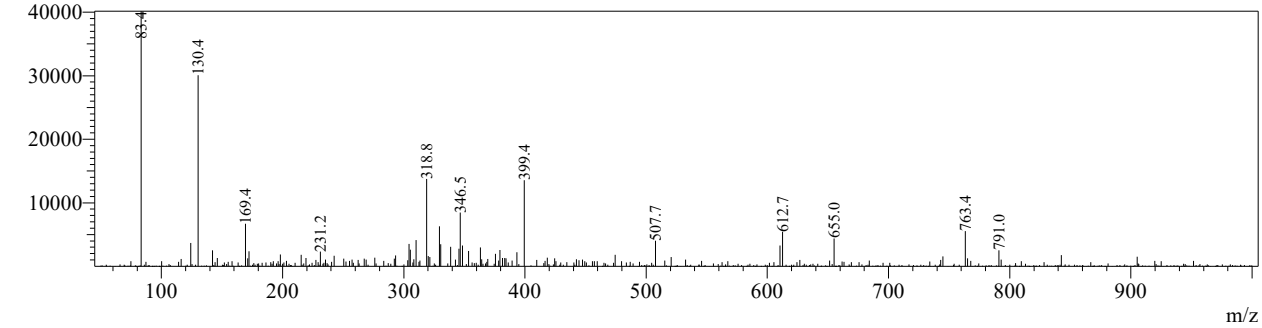
Line#:5
Rt:1.145-1.155



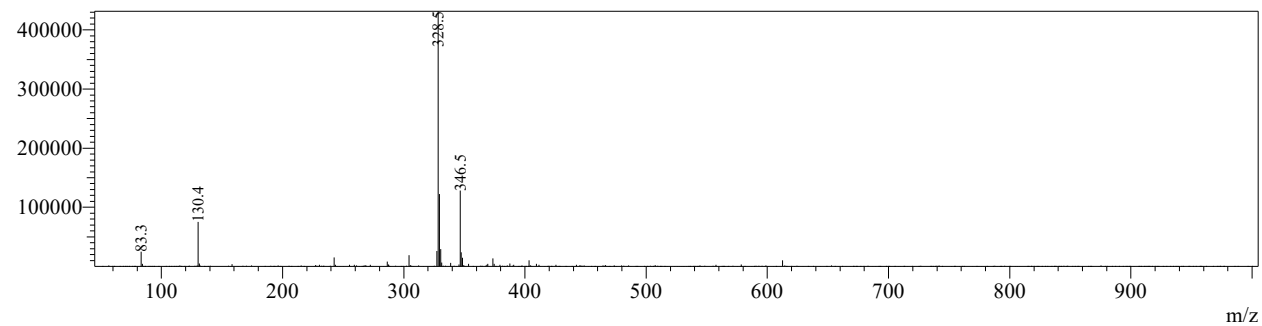
Line#:6
Rt:1.525-1.535

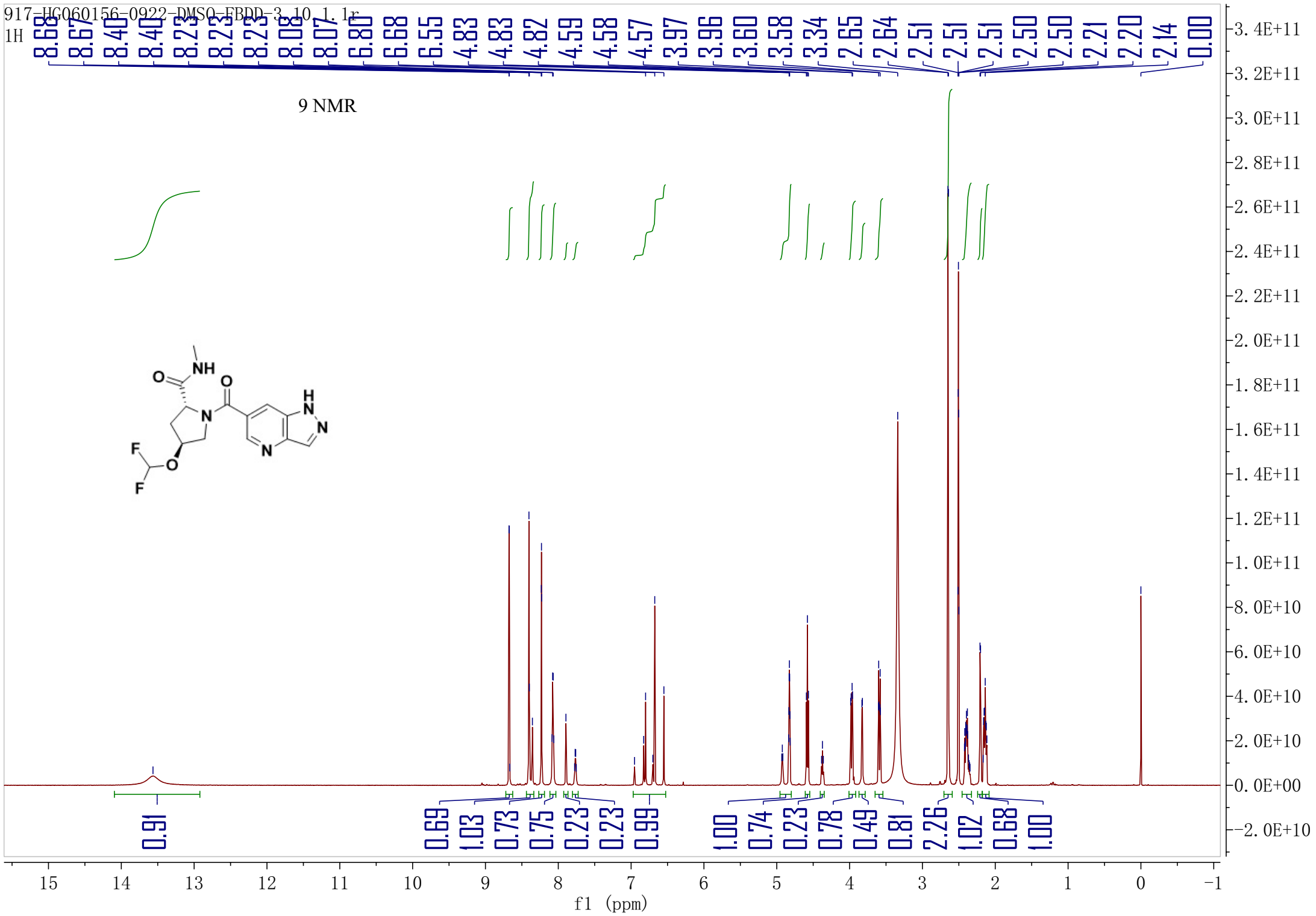


Line#:7
Rt:1.575-1.585



Line#:8
Rt:1.695-1.705




917-HG060156-0922-DMSO-FBDD-3_10_1.1r
1H
9 NMR
f1 (ppm)

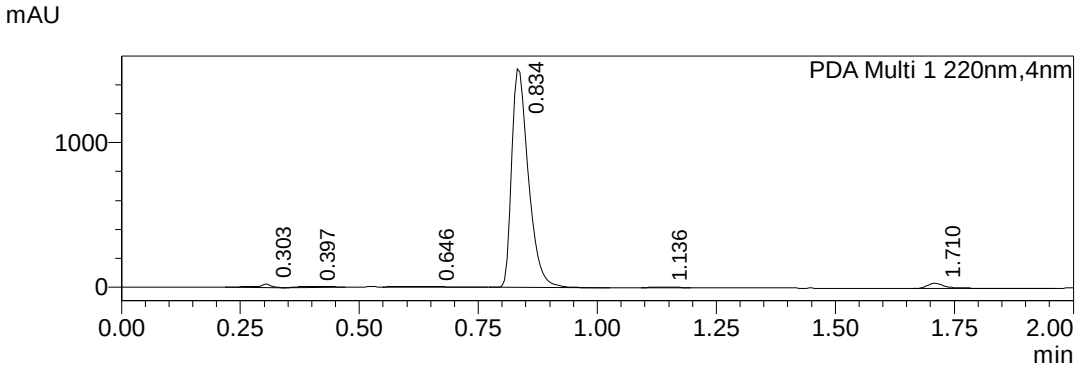
LCMS Report

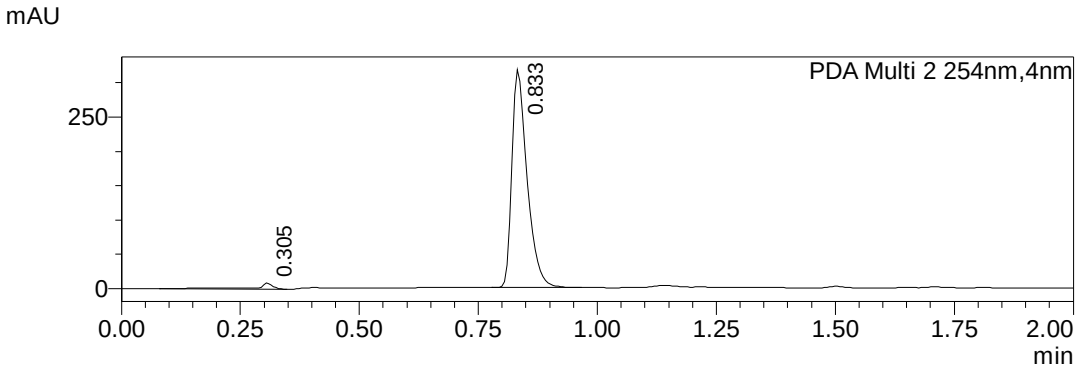
<Sample Information>

Sample ID	: 610-HG060156-0922-FBDD-3		
Batch ID:			
Project ID	: 1352	Instrument: LCMS-1	
Method Filename	: 5-95%_2MIN.lcm		
Vial #	: 1-18	Injection Volume	: 10 uL
Date Acquired	: 2021/9/22 10:35:30	Acquired by	:Fang.Jiao
Date Processed	: 2021/9/22 10:37:32	Processed by	: Ling.Wang

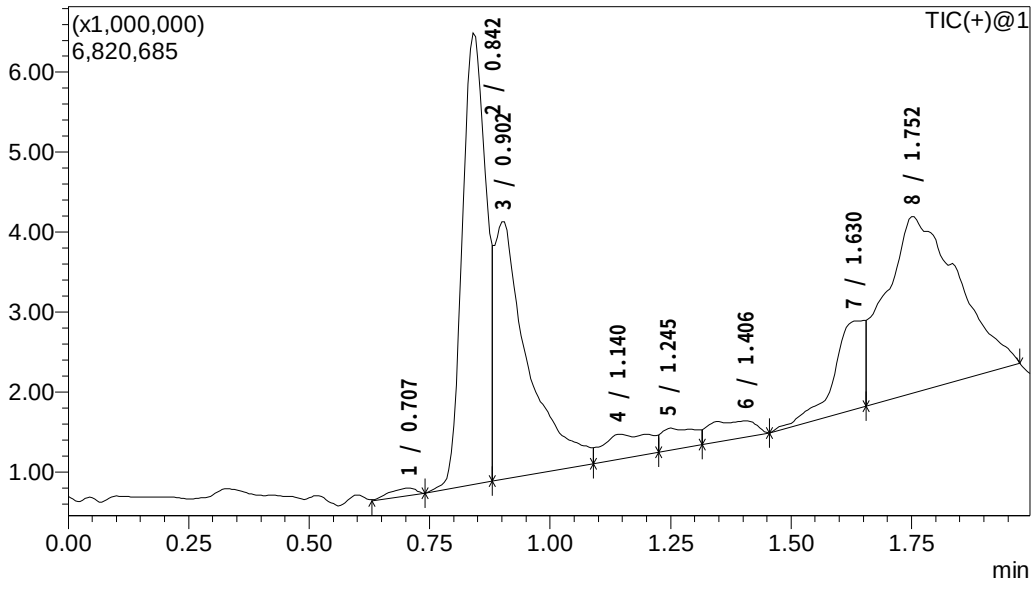
Method : Column: XBridge BEH Shield RP18 Column,3.5μm, 50mm*4.6mm.
Mobile Phase: A:10mM NH4HCO3 WATER B: ACN
Gradient:5-95%B in 1.2min,and hold 95%B for 0.4min,
then re-equilibrate column at 5%B for 0.4min.
Flow Rate: 2.5 mL/min; Column Temperature:40 ℃.

<Chromatogram>





<MS Chromatogram>



<Chromatogram Peak Table>

Peak Table

PDA Ch1 220nm

Peak#	Ret. Time	Area	Height	Area%
1	0.303	38483	23326	0.995
2	0.397	32907	7192	0.851
3	0.646	20139	4378	0.521
4	0.834	3673632	1516141	95.012
5	1.136	10541	3355	0.273
6	1.710	90800	34420	2.348
总计		3866502	1588812	100.000

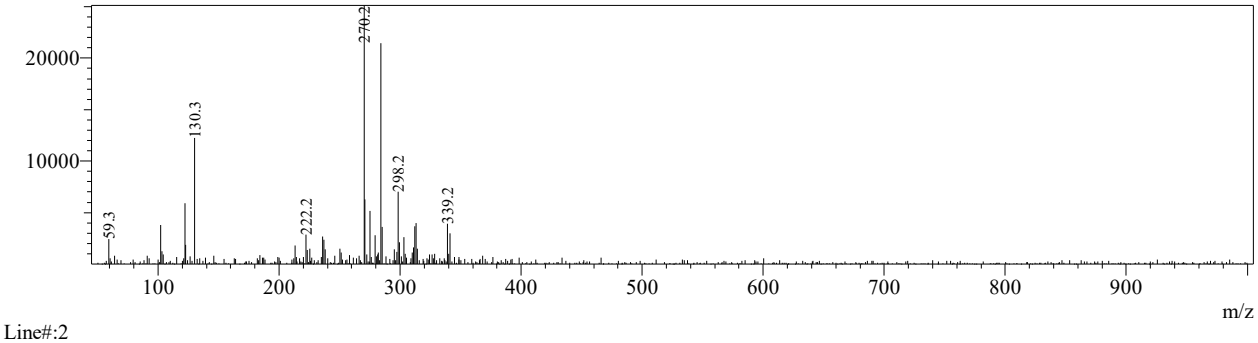
PDA Ch2 254nm

Peak#	Ret. Time	Area	Height	Area%
1	0.305	24545	9844	3.368
2	0.833	704299	318194	96.632
总计		728844	328038	100.000

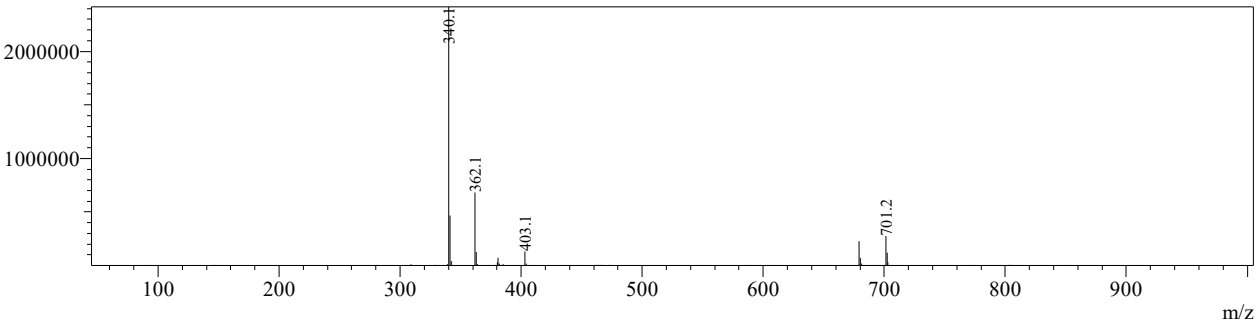
<MS Spectrum>

MS Spectrum

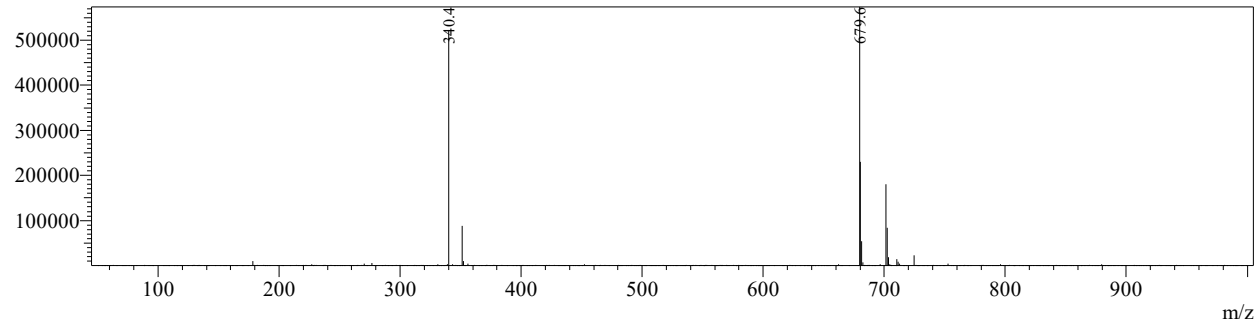
Line#:1
Rt:0.700-0.710



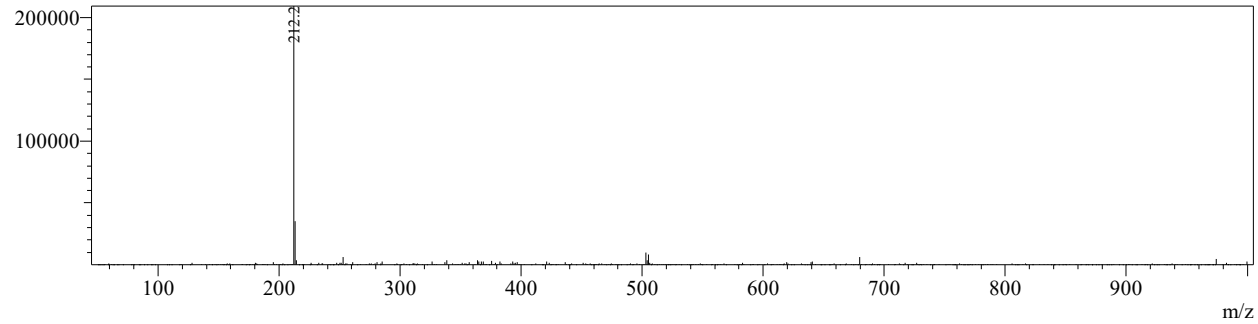
Line#:2
Rt:0.835-0.845



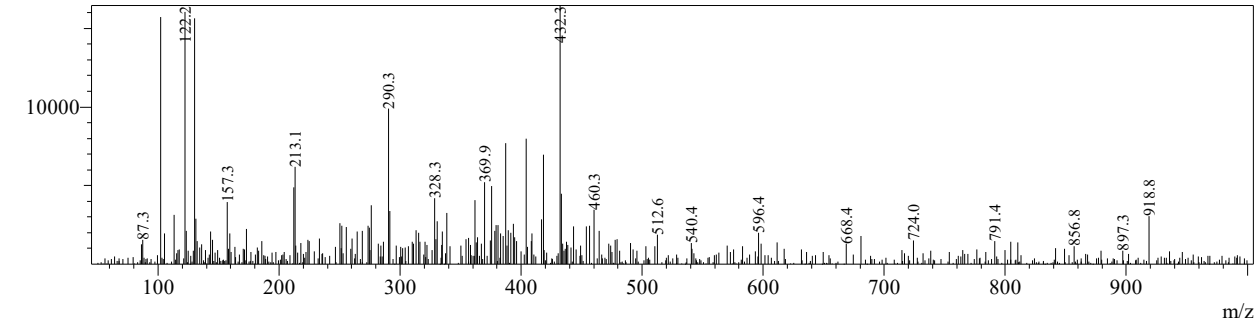
Line#:3
Rt:0.895-0.905



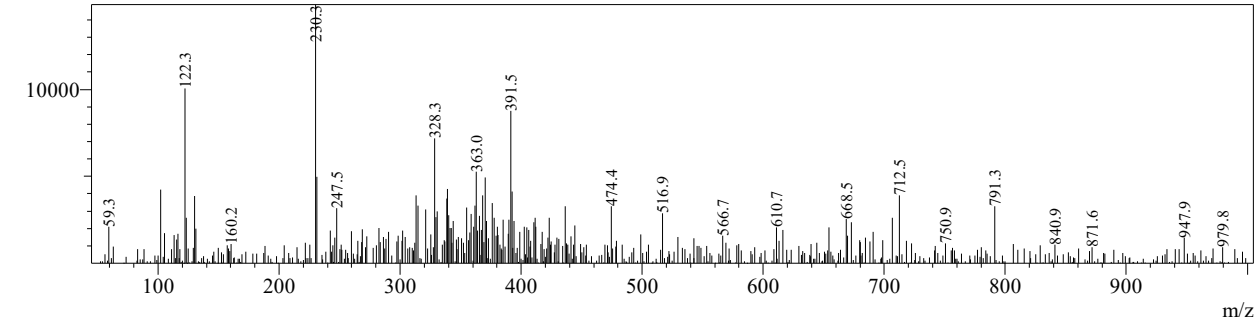
Line#:4
Rt:1.135-1.145



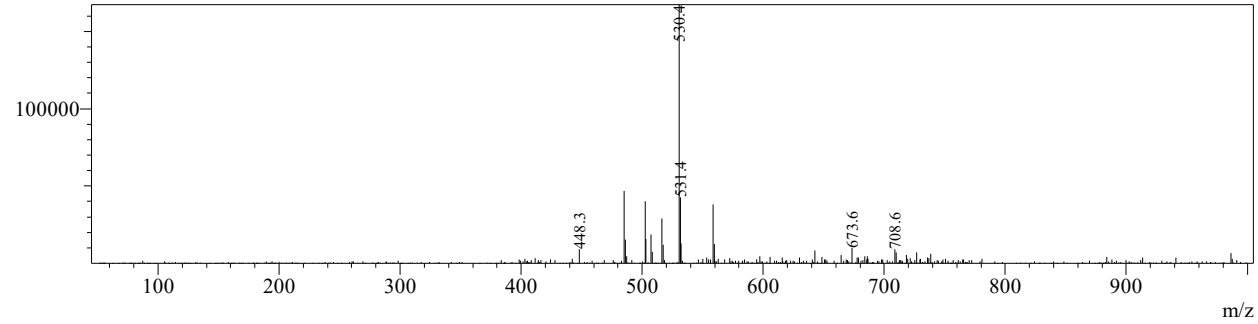
Line#:5
Rt:1.240-1.250



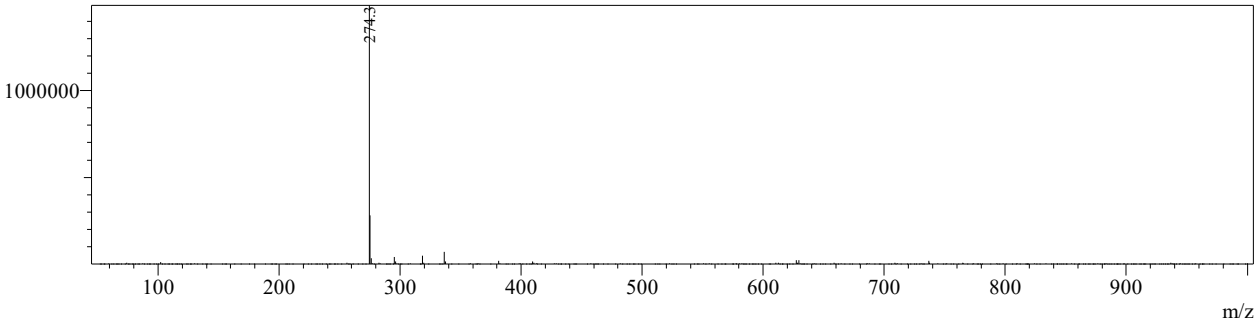
Line#:6
Rt:1.400-1.410

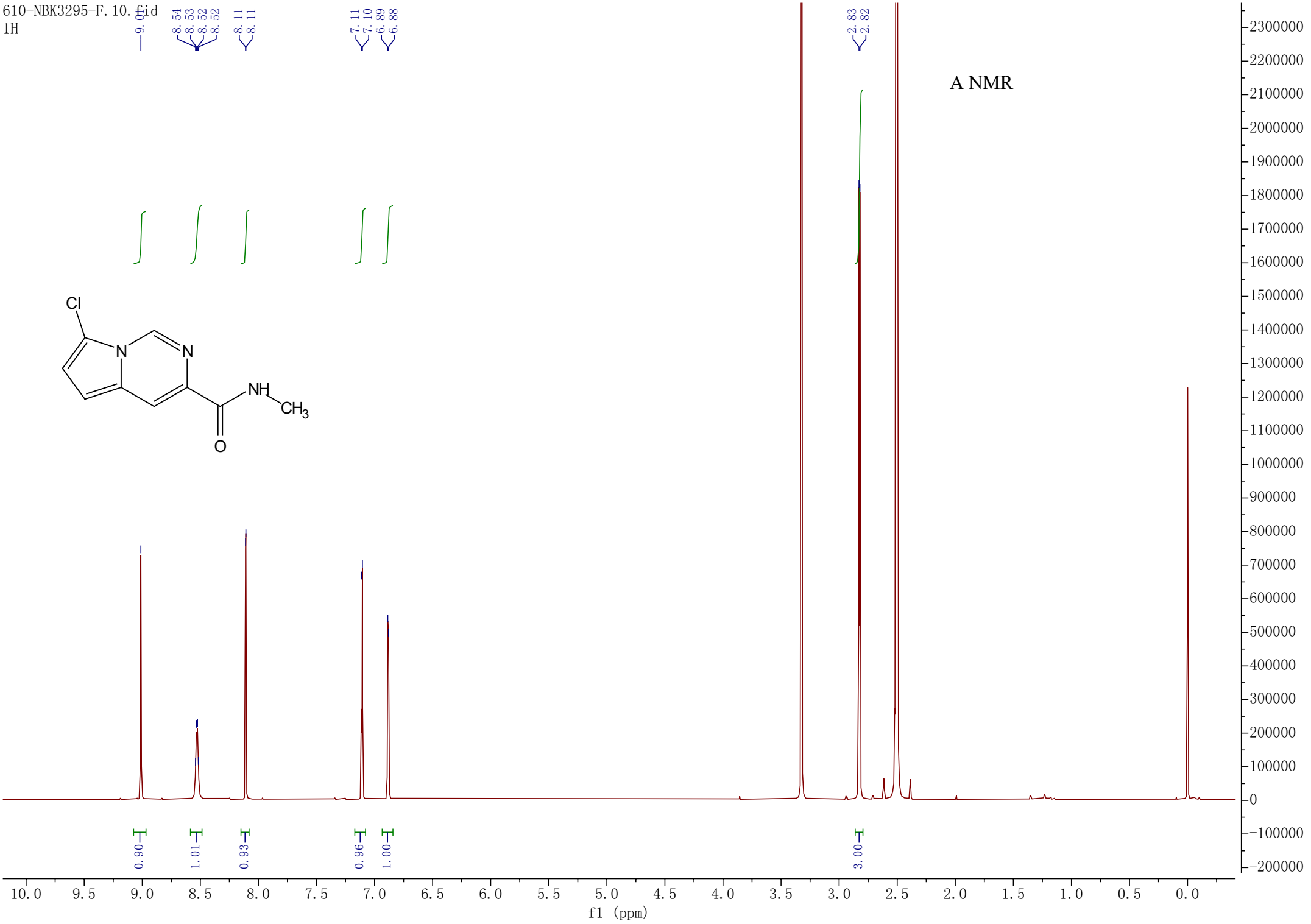


Line#:7
Rt:1.625-1.635



Line#:8
Rt:1.745-1.755




610-NBK3295-F.10.fid
1H
A NMR
9.00
8.54
8.53
8.52
8.52
8.11
8.11
7.11
7.10
6.89
6.88
2.83
2.82
Cl
N
N
NH
CH3
O
0.90
1.01
0.93
0.96
1.00
3.00
f1 (ppm)

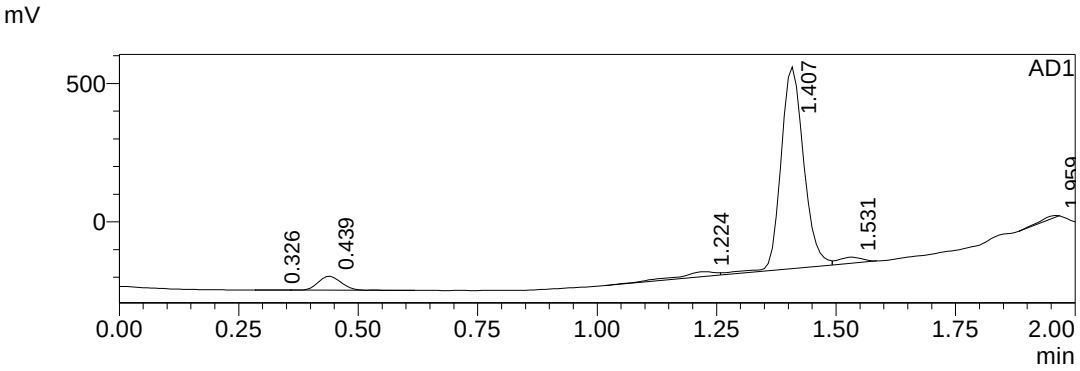
LCMS Report

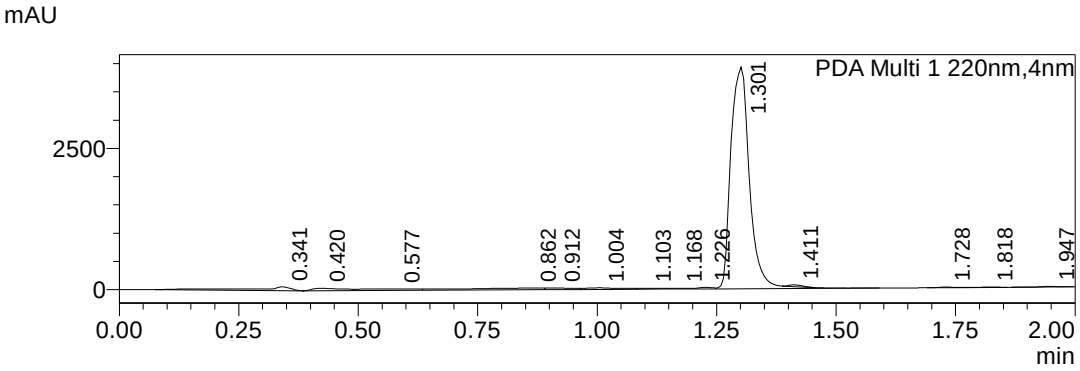
<Sample Information>

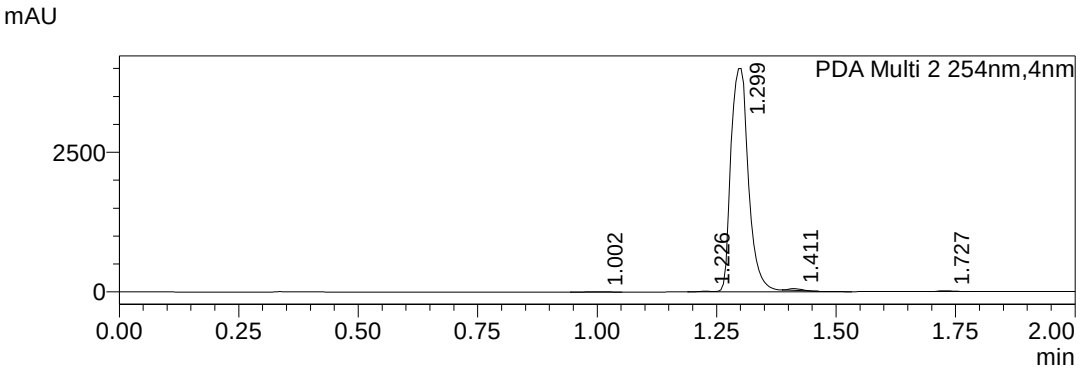
Sample ID	: 610-NBK3259-F-F		
Batch ID:			
Project ID	: 2057	Instrument: LCMS-3	
Method Filename	: 5-95%_2MIN.lcm		
Vial #	: 2-22	Injection Volume	: 10 uL
Date Acquired	: 2021/6/24 16:30:29	Acquired by	:Fang.Jiao
Date Processed	: 2021/6/24 16:32:32	Processed by	: Ling.Wang

Method : Column: XBridge BEH Shield RP18 Column,3.5μm, 50mm*4.6mm.
Mobile Phase: A:0.05%TFA Water B:0.05%TFA ACN
Gradient:5-95%B in 1.2min,and hold 95%B for 0.4min,
then re-equilibrate column at 5%B for 0.4min.
Flow Rate: 2.5 mL/min; Column Temperature:40 ℃.

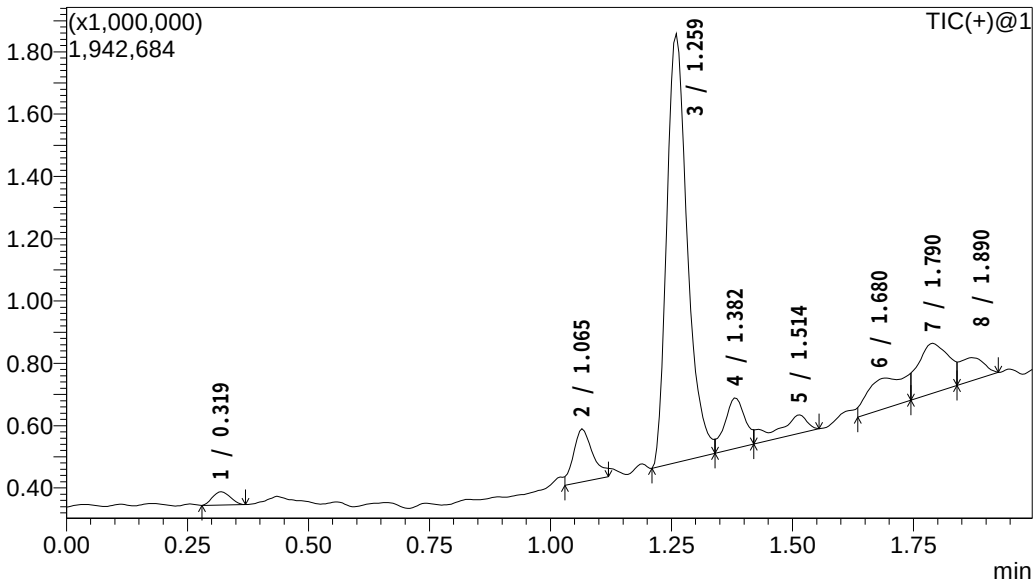
<Chromatogram>







<MS Chromatogram>


(x1,000,000)
1,942,684
TIC(+)@1
1.80
1.60
1.40
1.20
1.00
0.80
0.60
0.40
1 / 0.319
2 / 1.065
3 / 1.259
4 / 1.382
5 / 1.514
6 / 1.680
7 / 1.790
8 / 1.890
0.00
0.25
0.50
0.75
1.00
1.25
1.50
1.75
min

<Chromatogram Peak Table>

Peak Table

AD1

Peak#	Ret. Time	Area	Height	Area%
1	0.326	1321	497	0.047
2	0.439	169510	50265	5.984
3	1.224	115321	17749	4.071
4	1.407	2427729	728197	85.701
5	1.531	77945	22076	2.752
6	1.959	40973	6218	1.446
总计		2832798	825003	100.000

PDA Ch1 220nm

Peak#	Ret. Time	Area	Height	Area%
1	0.341	389164	69140	3.140
2	0.420	191620	43164	1.546
3	0.577	199271	22762	1.608
4	0.862	364182	27853	2.938
5	0.912	110660	26035	0.893
6	1.004	147516	28093	1.190
7	1.103	46650	14228	0.376
8	1.168	31276	11181	0.252
9	1.226	67484	28427	0.544
10	1.301	10719391	3918605	86.486
11	1.411	62996	33462	0.508
12	1.728	23475	11201	0.189
13	1.818	15378	6580	0.124
14	1.947	25246	9611	0.204
总计		12394308	4250341	100.000

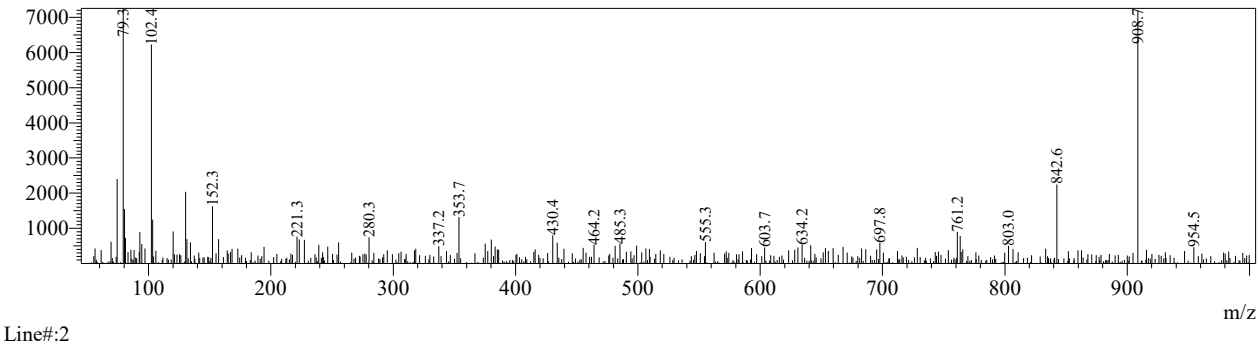
PDA Ch2 254nm

Peak#	Ret. Time	Area	Height	Area%
1	1.002	12230	5525	0.116
2	1.226	23427	12563	0.222
3	1.299	10440094	3998575	98.772
4	1.411	64573	35114	0.611
5	1.727	29595	14566	0.280
总计		10569919	4066343	100.000

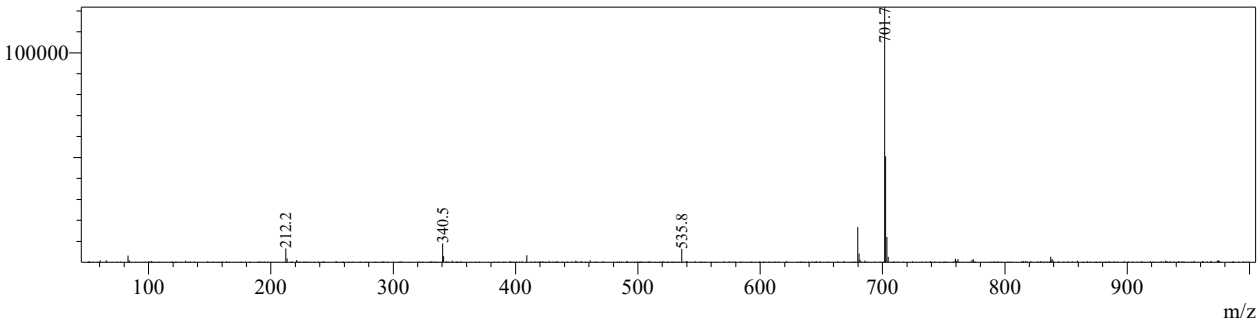
<MS Spectrum>

MS Spectrum

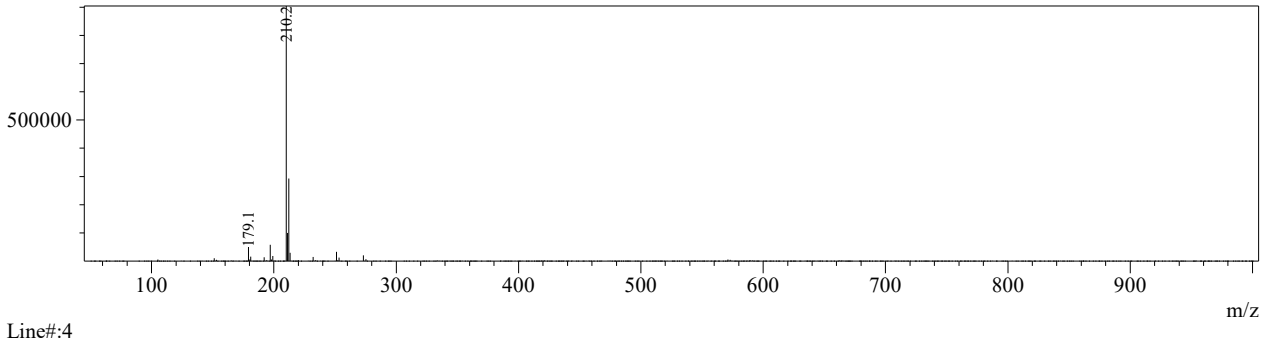
Line#:1
Rt:0.315-0.325(64-66)



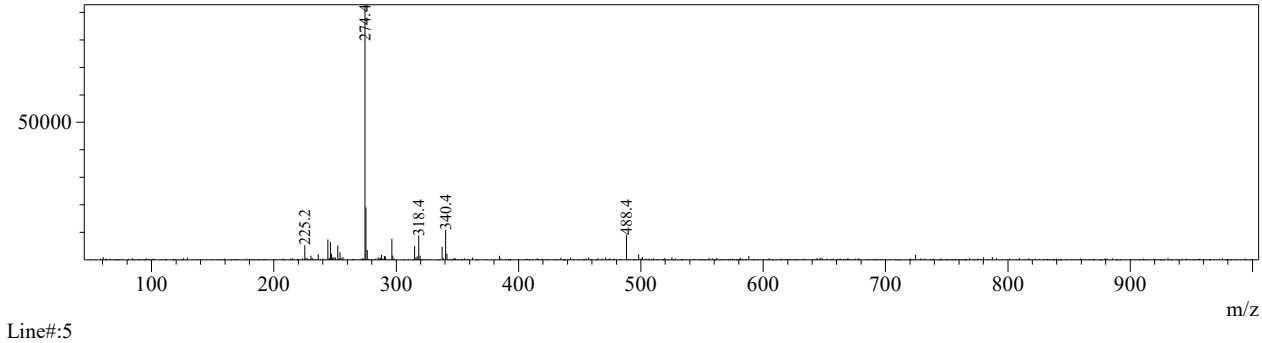
Line#:2
Rt:1.060-1.070(213-215)



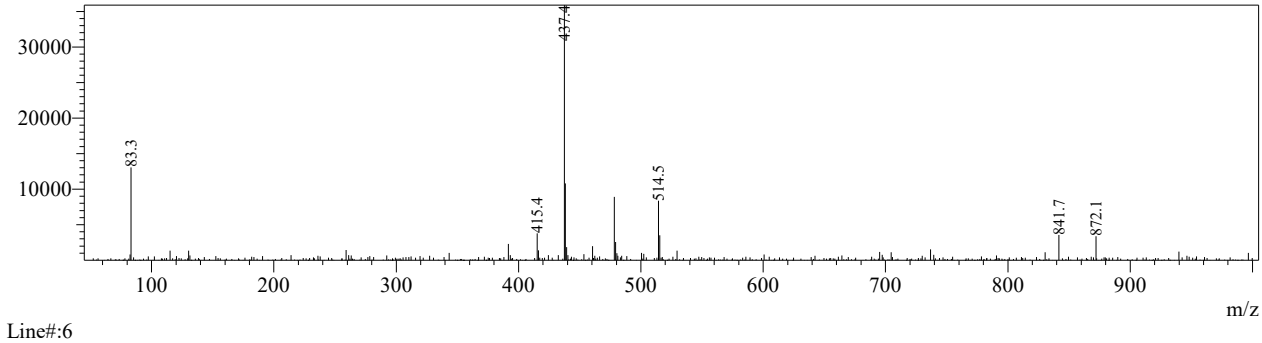
Line#:3
Rt:1.255-1.265(252-254)



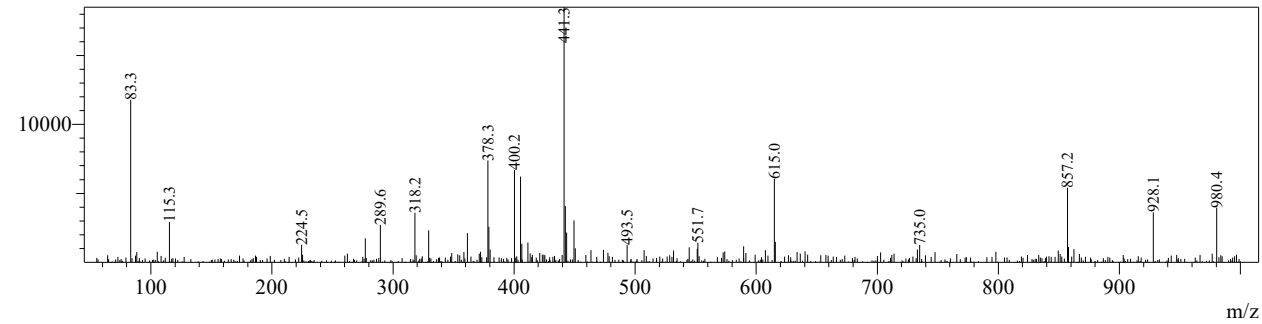
Line#:4
Rt:1.375-1.385(276-278)



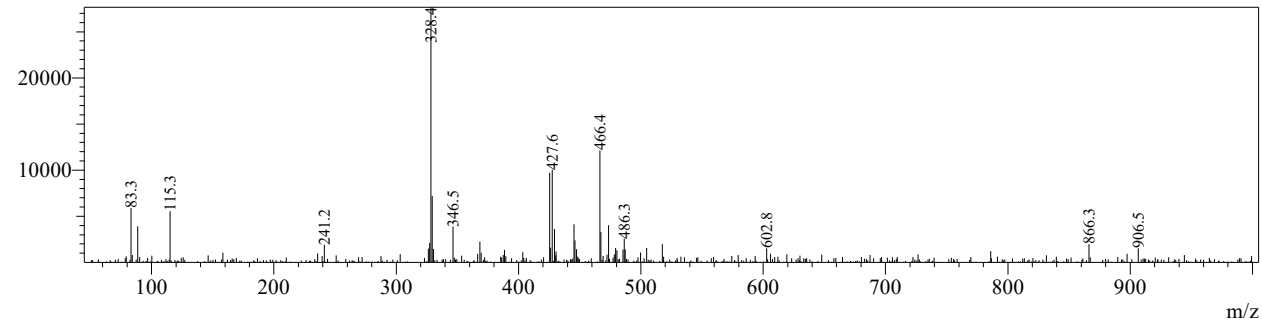
Line#:5
Rt:1.510-1.520(303-305)



Line#:6
Rt:1.675-1.685(336-338)



Line#:7
Rt:1.785-1.795(358-360)



Line#:8
Rt:1.885-1.895(378-380)