Supporting information

Bioactivity Focus on α-Cyano-4-hydroxycinnamic acid (CHCA) Leads to Effective Multifunctional Aldose Reductase Inhibitors

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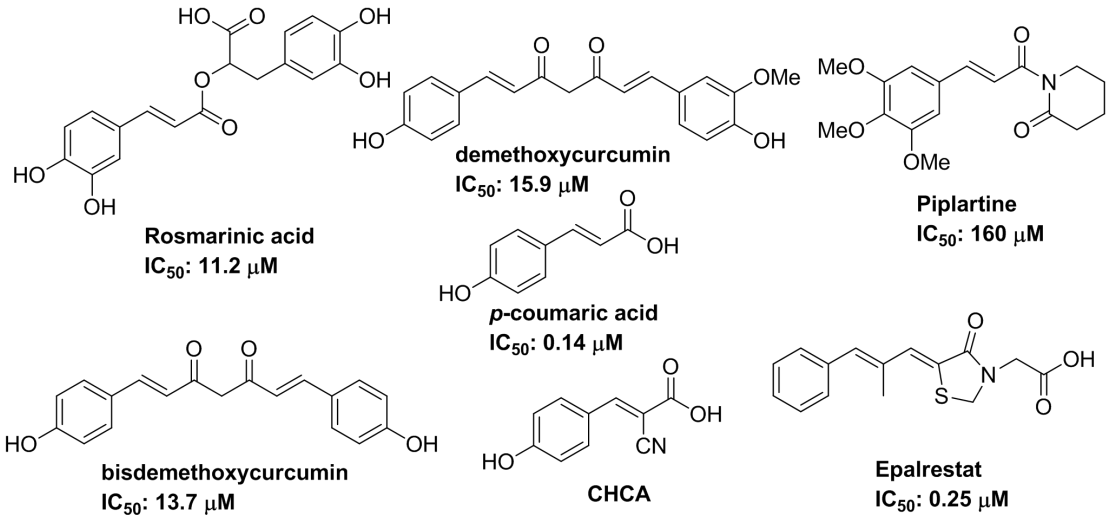


Fig. S1. Chemical structures of some representative ARIs.

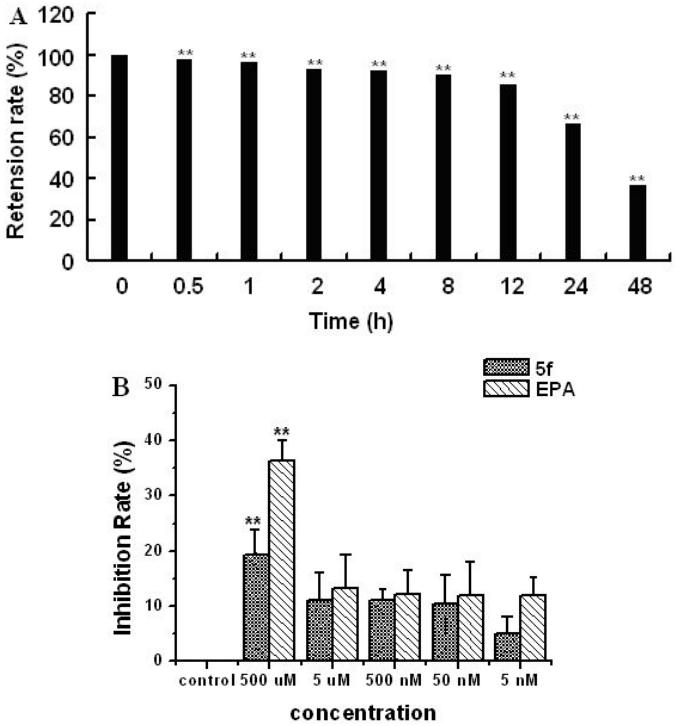


Fig. S2. Stability and toxicity of **5f**. (**A**) Stability studies with **5f** incubated in 5% FBS DMEM showing % retention over time. RP-HPLC was used as the assay. Analyses were scheduled at 0.5, 1.0, 4.0, 8.0, 12.0, 24.0, and 48.0 h. $^{**}P \leq 0.01$ *vs* control group. (**B**) Growth inhibition rate of **5f** and EPS against HEK293 cells. MTT assay was used to determine the cell viability. Testing samples and epalrestat at final concentrations of 5.0, 50, 500 nM, and 5.0, 50, and 500 μM, respectively, were assessed $^{**}P \leq 0.01$ *vs* control group.

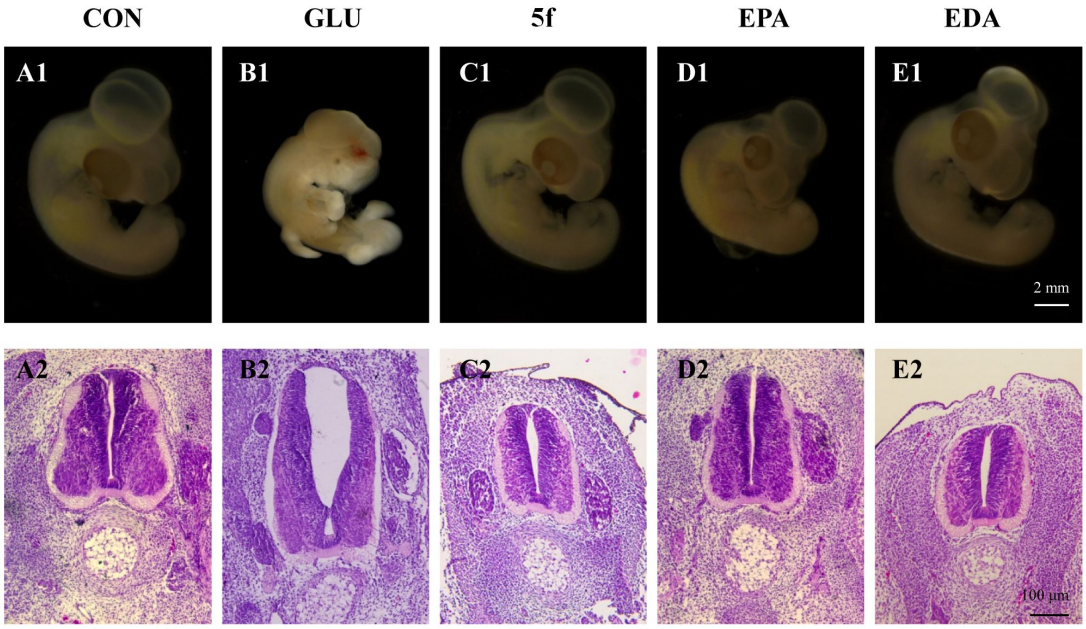
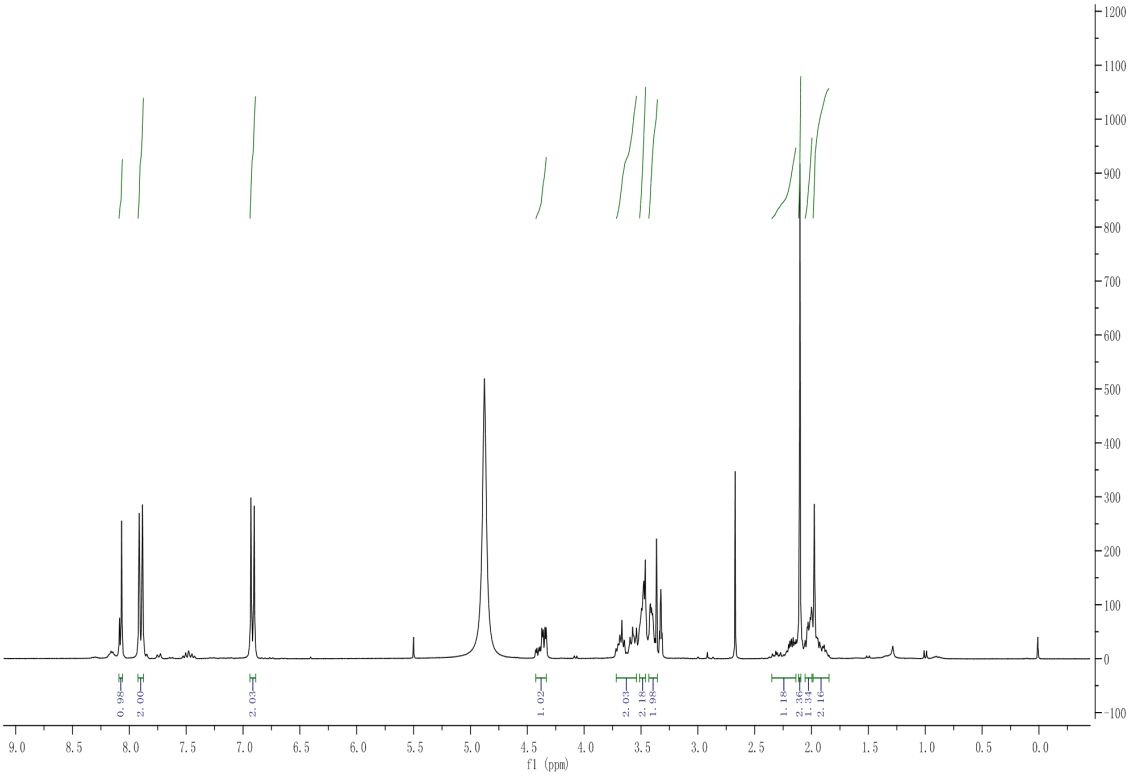
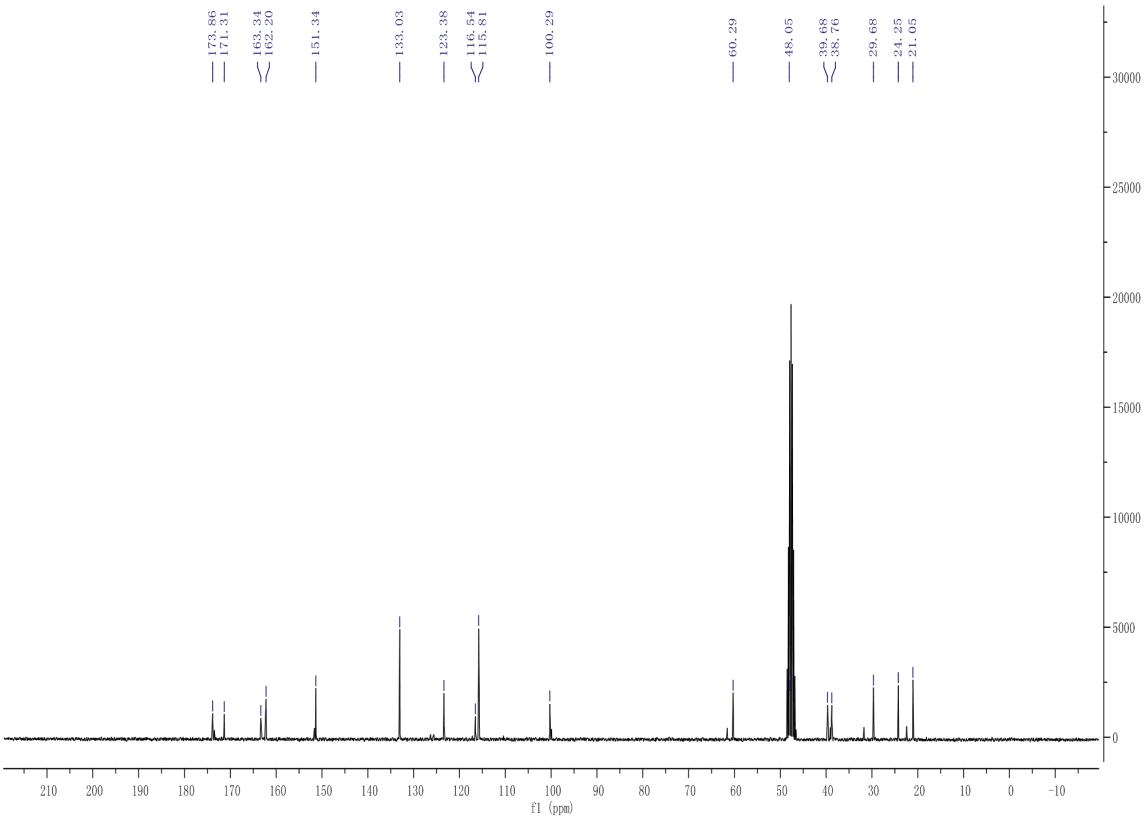


Fig. S3. 5f ameliorated high-glucose-induced NTD in chick embryos on EDD 5. Stereoscopic microscope measurement of whole body and Hematoxylin and eosin (H&E) staining of embryo sections. Scale bars of **A1-E1**: 2 mm. Scale bars of **A2-E2**: 100 μm.

II. The NMR and HRMS data of compounds 5a-j are listed in the following

5a (^{1}H NMR, ^{13}C NMR, HRMS)





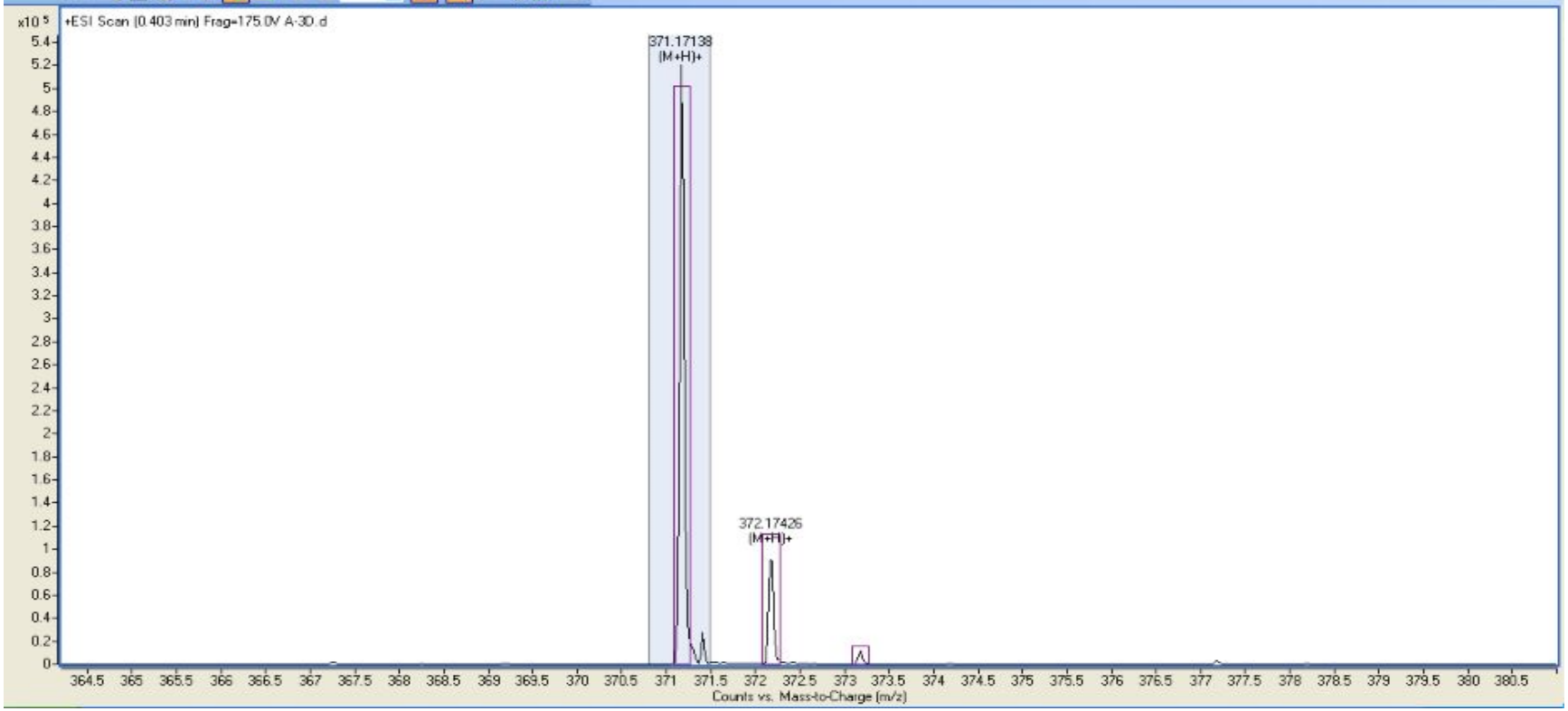
m/z	Ion	Formula	Abundance
371.17138	(M+H)+	C19 H23 N4 O4	5221121

Best	Formula (M)	Ion Formula	Calc m/z	Score	Cross Score	Mass	Calc Mass	Diff (ppm)	Abs Diff (ppm	Abund Matc	Spacing Match	Mass Match	m/z	DBE
☑	C19 H22 N4 O4	C19 H23 N4 O4	371.17138	96.93		370.16...	370.16411	0.01	0.01	89.43	99.79	100	371.17138	11

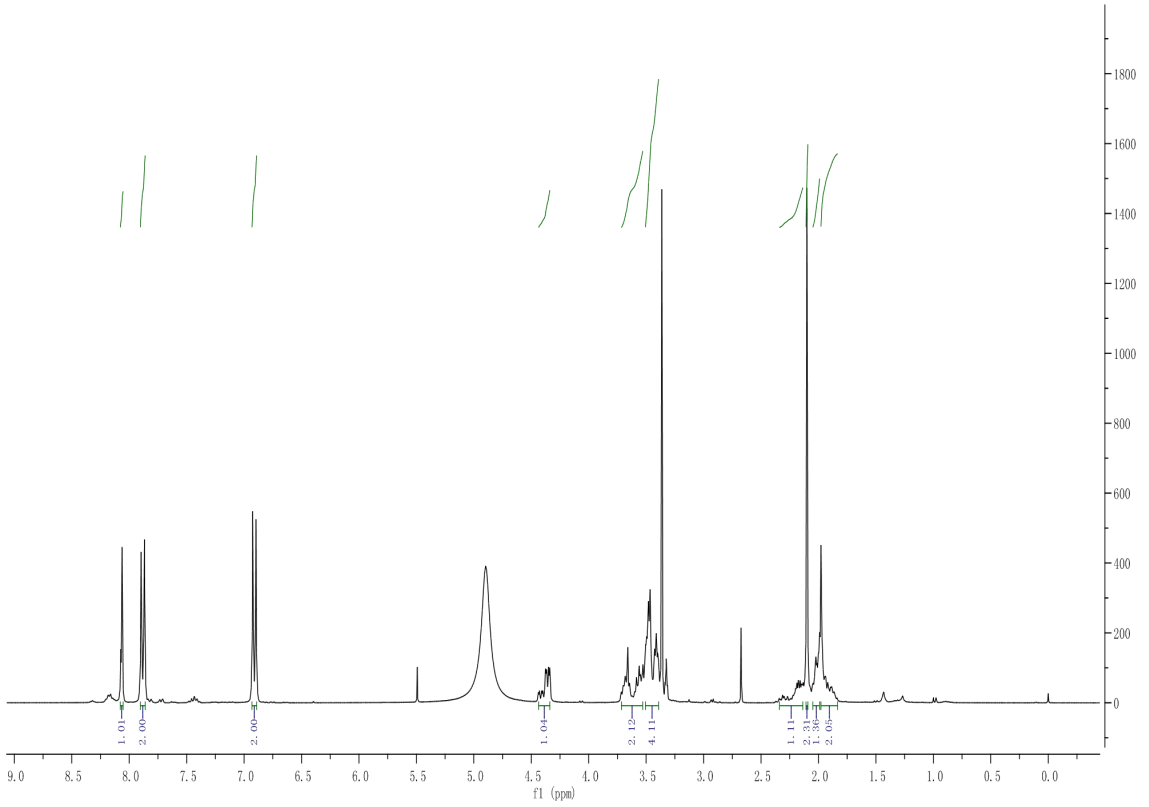
Isotope	Abund%	Calc Abund%	Calc Abund Sum%	m/z	Calc m/z	Diff (pp	Abund Sum%
1	100	100	79.59	371.17138	371.17138	0.01	82.77
2	18.64	22.43	17.85	372.17426	372.17437	0.29	15.42
3	2.19	3.22	2.57	373.17811	373.17689	-3.26	1.81

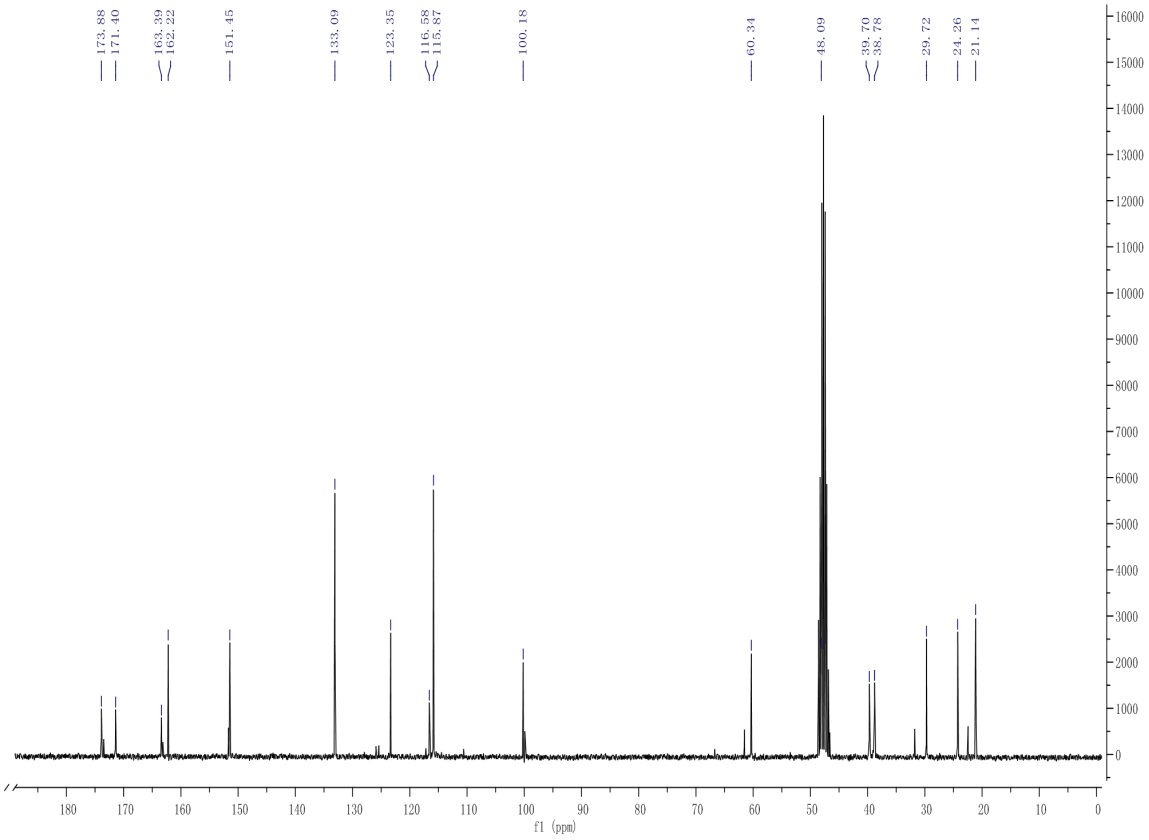
Chromatogram Results | MS Formula Results: + Scan (0.403 min)

MS Spectrum Results



5b (^{1}H NMR, ^{13}C NMR, HRMS)





m/z	Ion	Formula	Abundance
371.17138	(M+H)+	C19 H23 N4 O4	5221121

Best	Formula (M)	Ion Formula	Calc m/z	Score	Cross Score	Mass	Calc Mass	Diff (ppm)	Abs Diff (ppm	Abund Matc	Spacing Match	Mass Match	m/z	DBE
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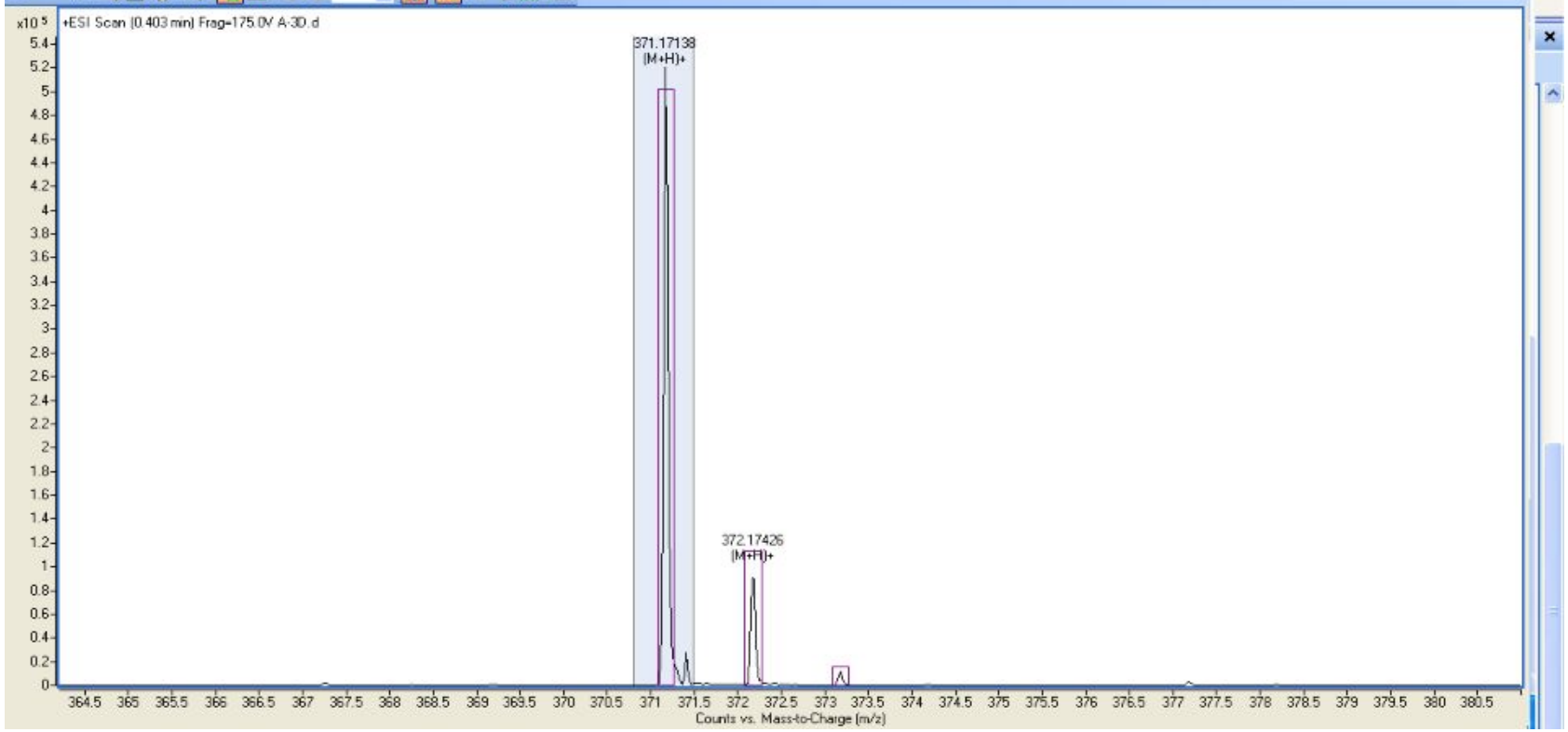
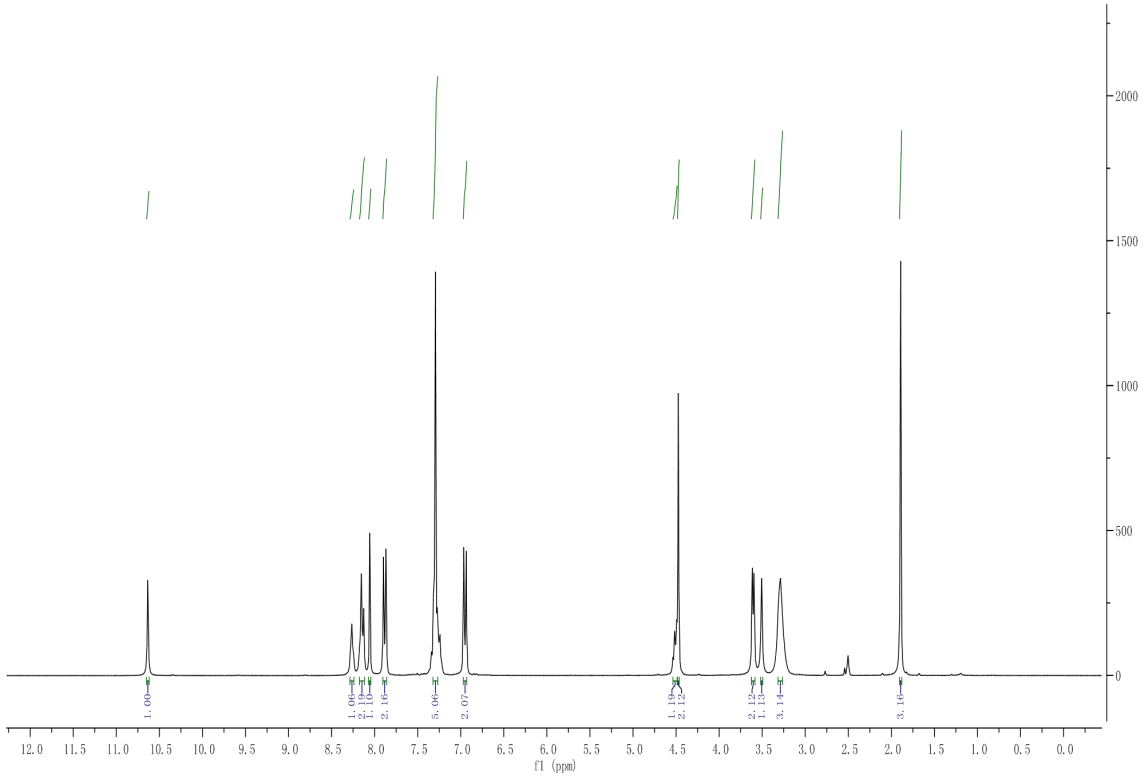
Isotope	Abund%	Calc Abund%	Calc Abund Sum%	m/z	Calc m/z	Diff (pp	Abund Sum%
1	100	100	79.59	371.17138	371.17138	0.01	82.77
2	18.64	22.43	17.85	372.17426	372.17437	0.29	15.42
3	2.19	3.22	2.57	373.17811	373.17689	-3.26	1.81

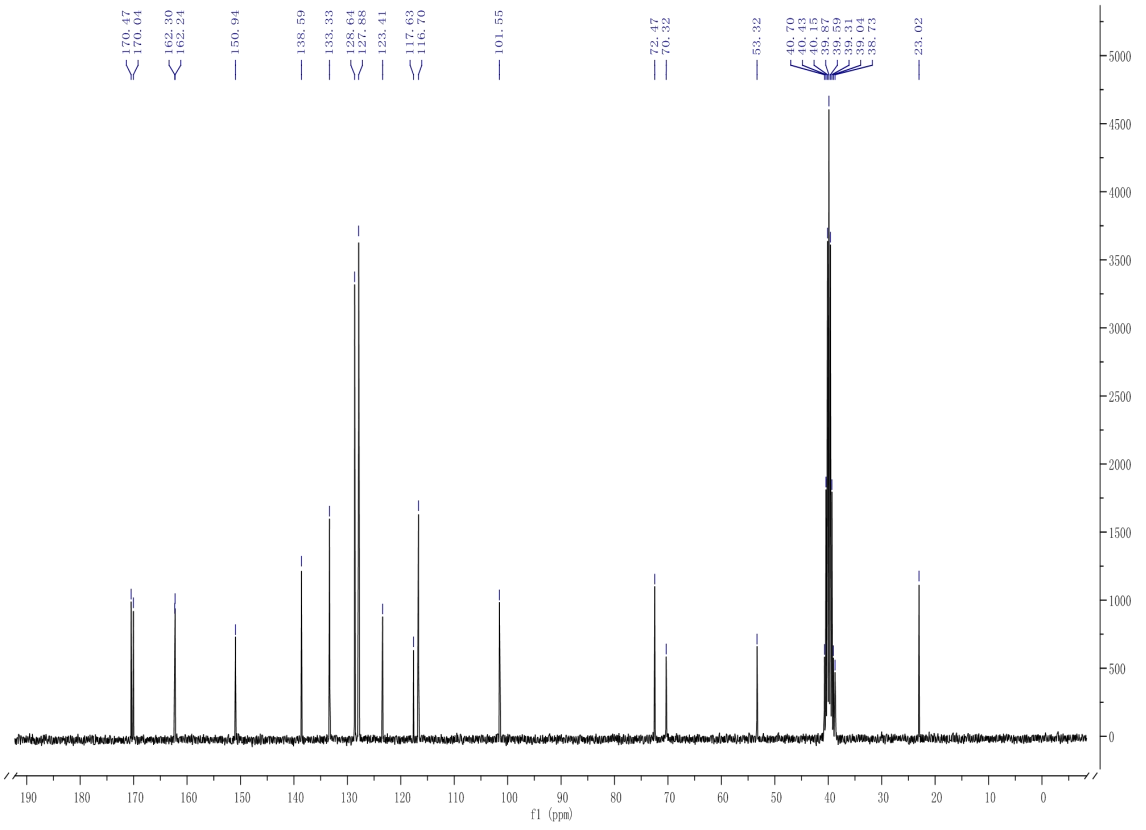
Chromatogram Results | MS Formula Results: + Scan (0.403 min)

MS Spectrum Results



5c (^{1}H NMR, ^{13}C NMR, HRMS)



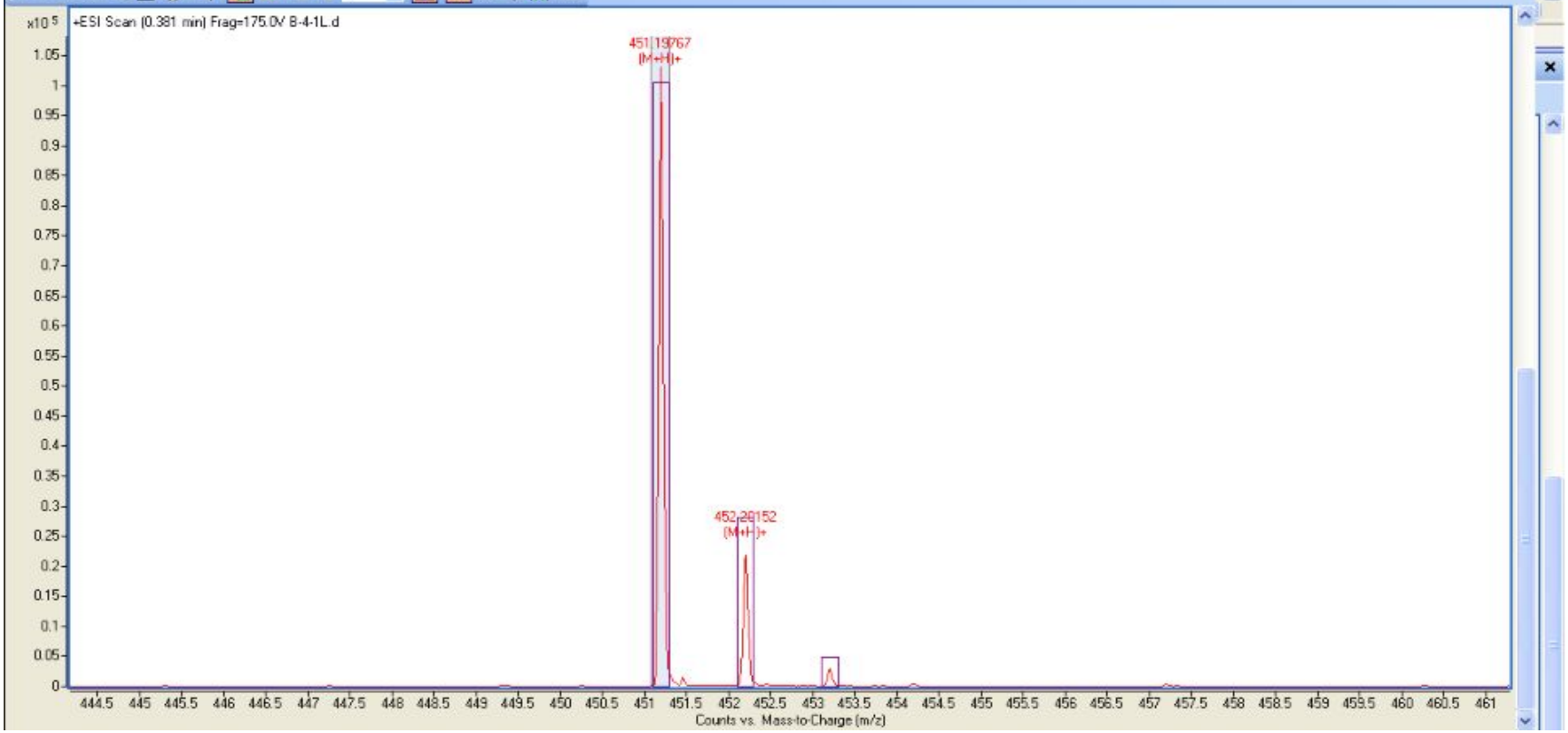


Best	Formula (M)	Ion Formula	Calc m/z	Score	Cross Score	Mass	Calc Mass	Diff (ppm	Abs Diff (ppm	Abund Match	Spacing Mat	Mass Match	m/z	DBE
☑	C24 H26 N4 O5	C24 H27 N4 O5	451.1976	92.64		450.1904	450.19032	-0.18	0.18	76.31	97.57	99.97	451.19767	14

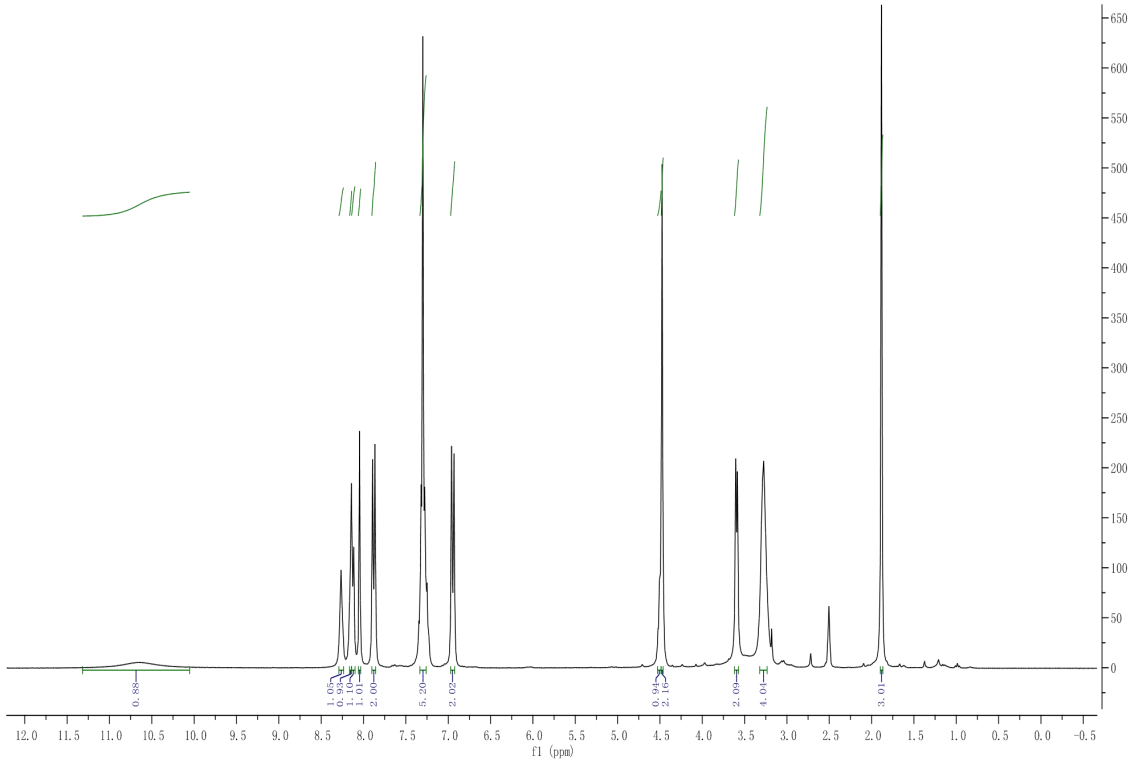
Isotope	Abund%	Calc Abund%	Calc Abund Sum%	m/z	Calc m/z	Diff (ppm)	Abund Sum%
1	100	100	75.36	451.19767	451.1976	-0.16	80.38
2	21.49	27.92	21.04	452.20152	452.20066	-1.91	17.28
3	2.91	4.78	3.6	453.20324	453.20331	0.14	2.34

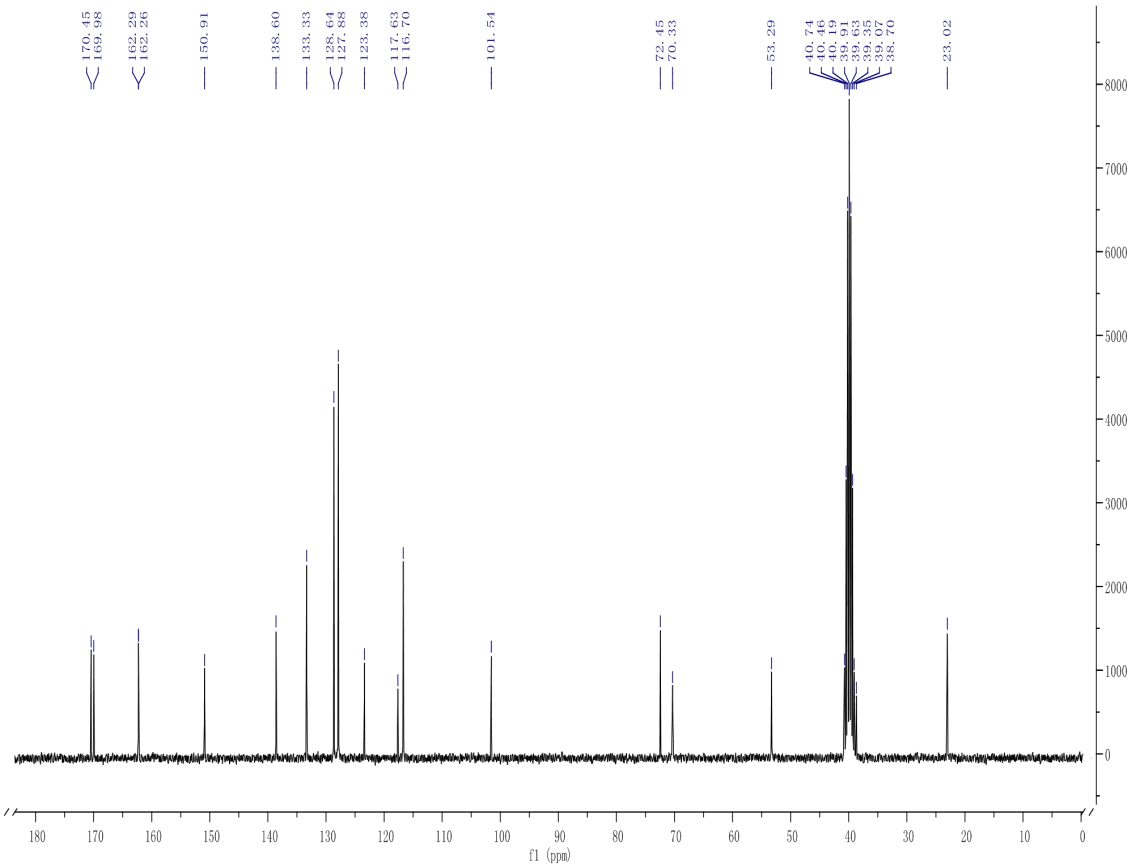
Chromatogram Results | MS Formula Results: + Scan (0.381 min)

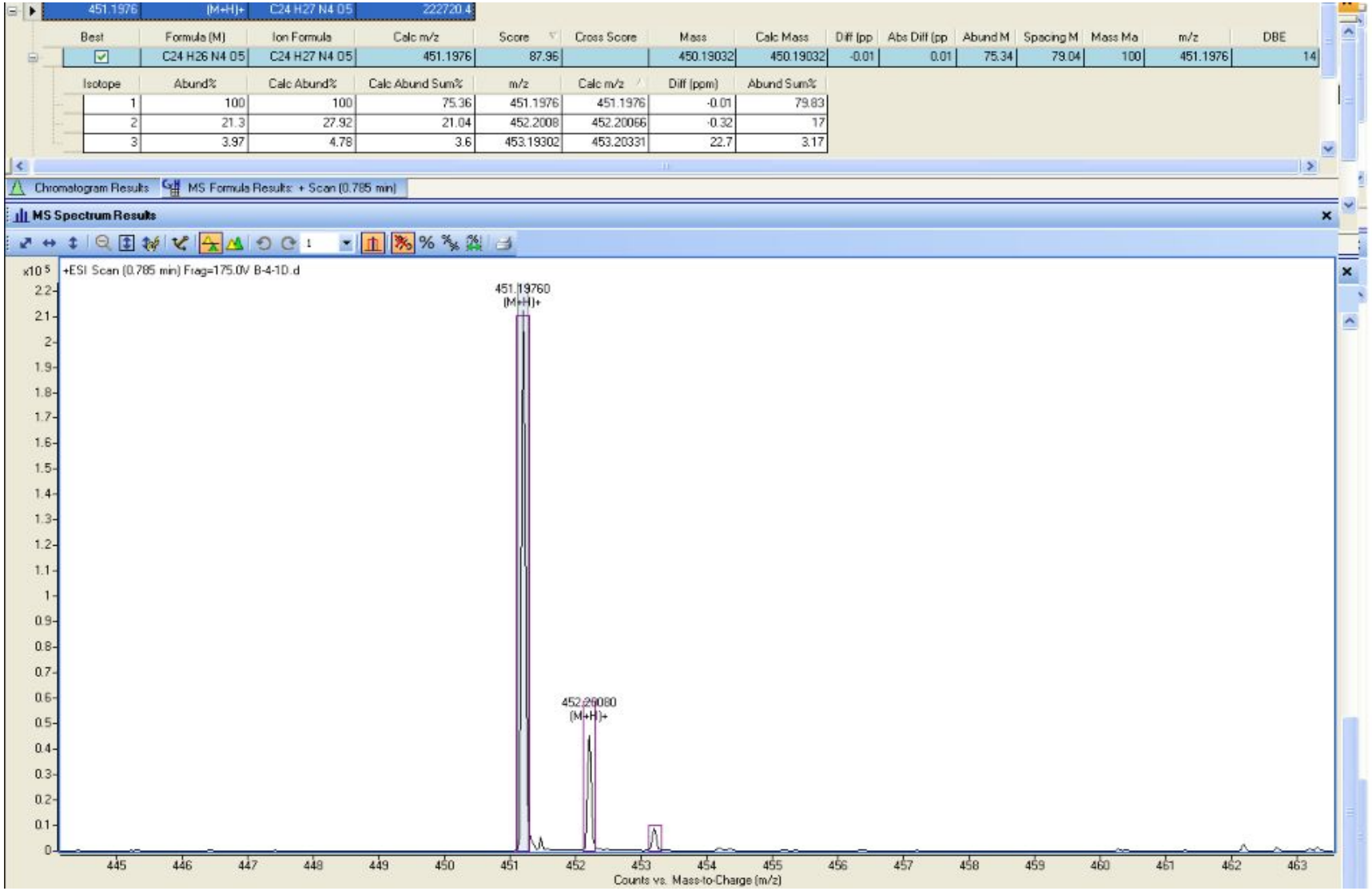
MS Spectrum Results



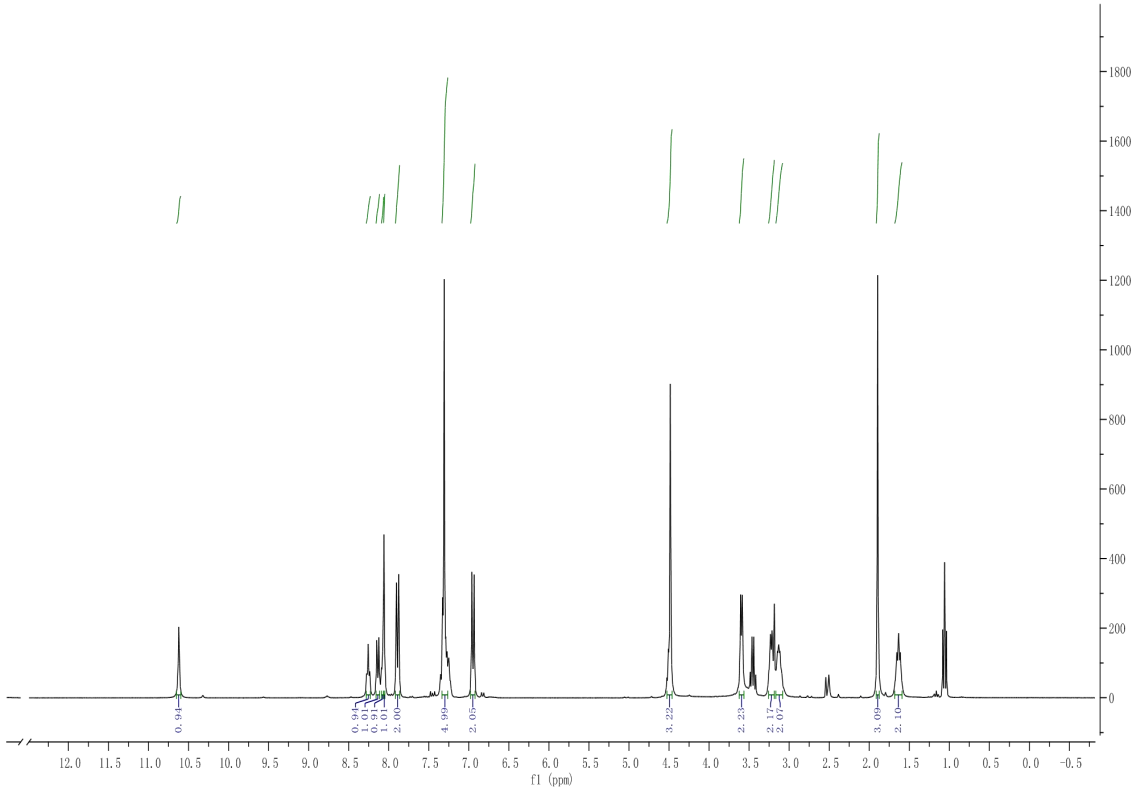
5d (^{1}H NMR, ^{13}C NMR, HRMS)

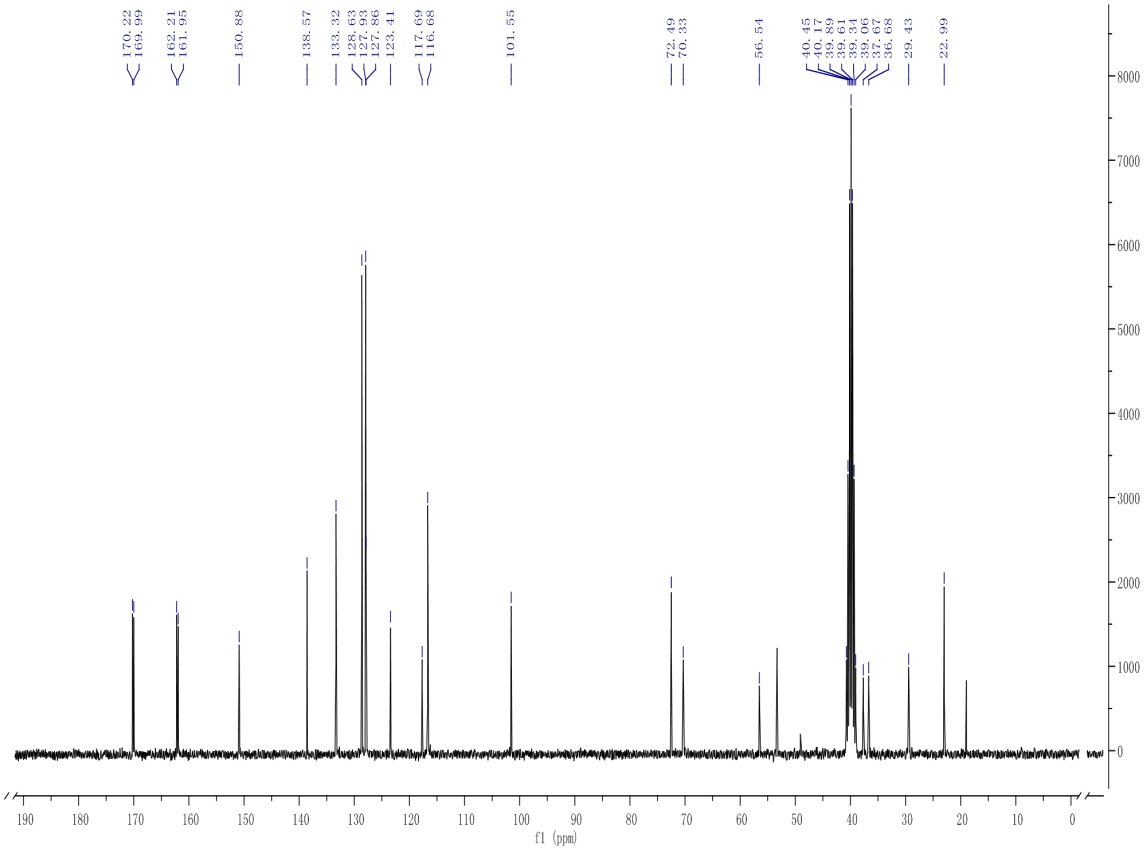


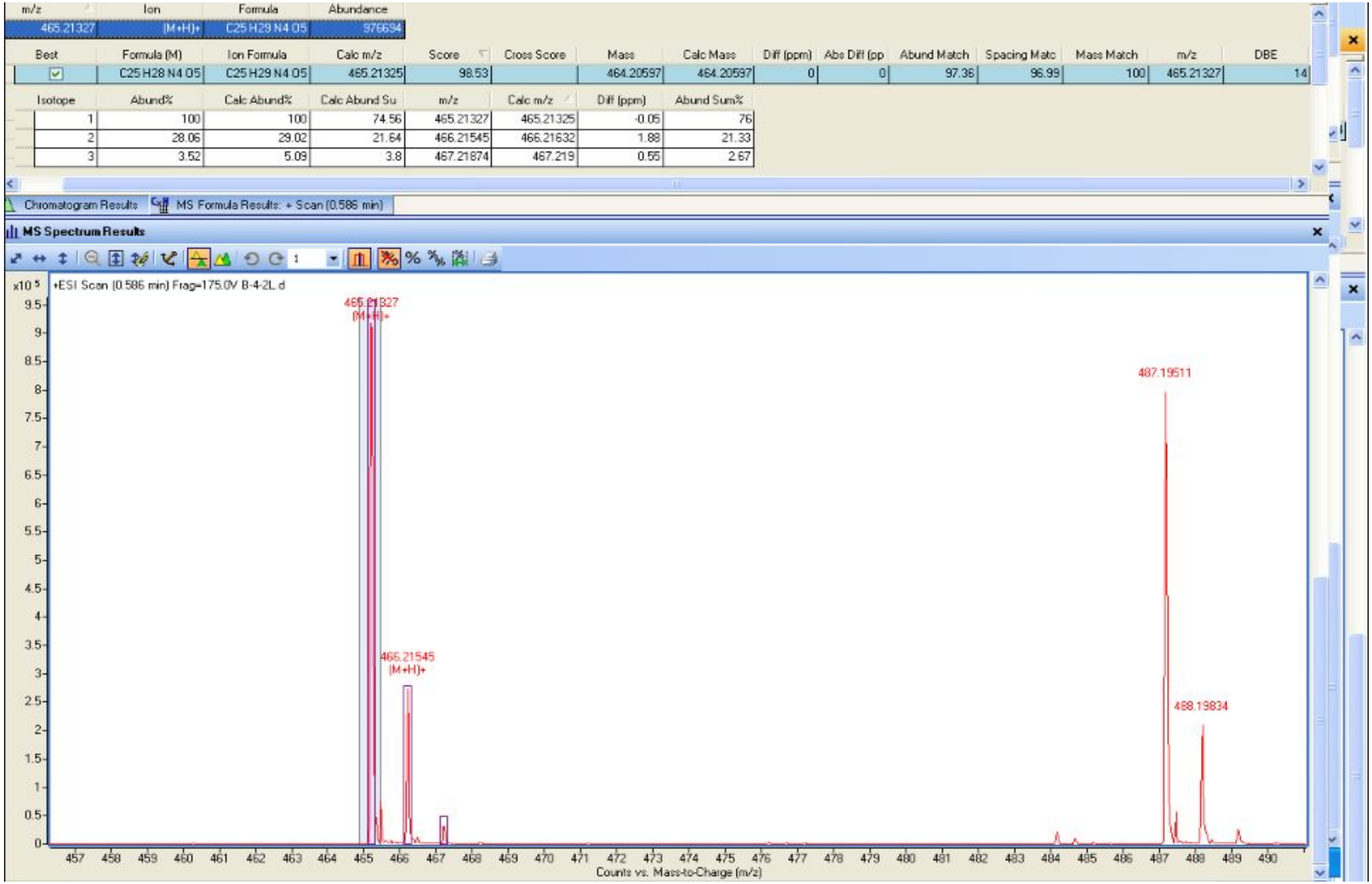



451.1976 (M+H)+ C24 H27 N4 05 222720.4
Best Formula (M) Ion Formula Calc m/z Score Cross Score Mass Calc Mass Diff (pp Abs Diff (pp Abund M Spacing M Mass Ma m/z DBE
C24 H26 N4 05 C24 H27 N4 05 451.1976 87.96 450.19032 450.19032 -0.01 0.01 75.34 79.04 100 451.1976 14
Isotope Abund% Calc Abund% Calc Abund Sum% m/z Calc m/z Diff (ppm) Abund Sum%
1 100 100 75.36 451.1976 451.1976 -0.01 79.83
2 21.3 27.92 21.04 452.2008 452.20066 -0.32 17
3 3.97 4.78 3.6 453.19302 453.20331 22.7 3.17
Chromatogram Results
MS Formula Results: + Scan (0.785 min)
MS Spectrum Results
x10 5
+ESI Scan (0.785 min) Frag=175.0V B-4-1D.d
451.19760
(M+H)+
452.20080
(M+H)+
Counts vs. Mass-to-Charge (m/z)

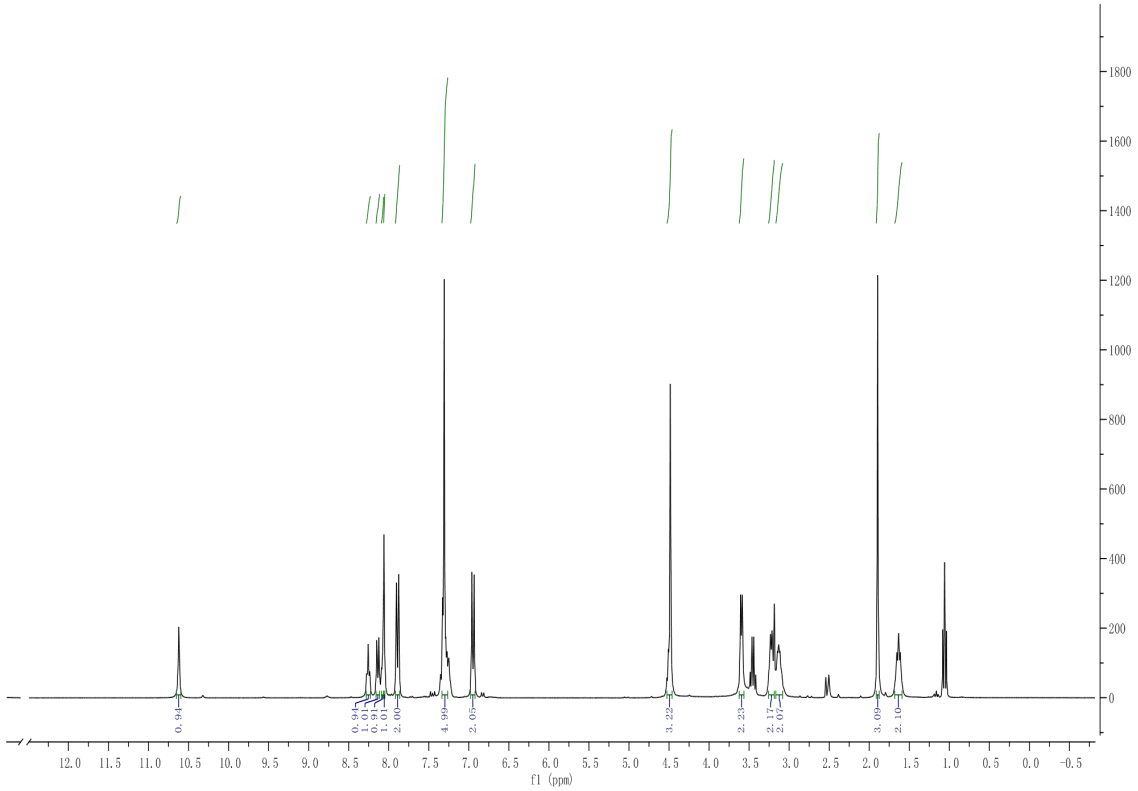
5e (^{1}H NMR, ^{13}C NMR, HRMS)

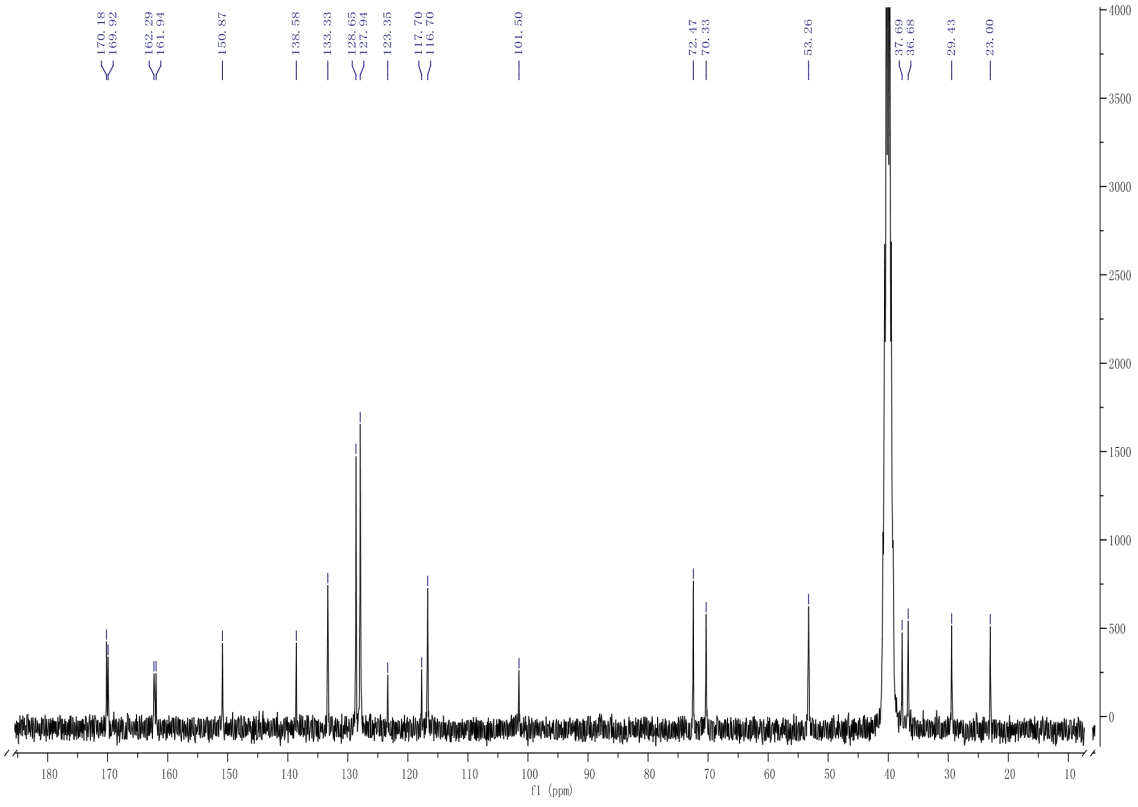


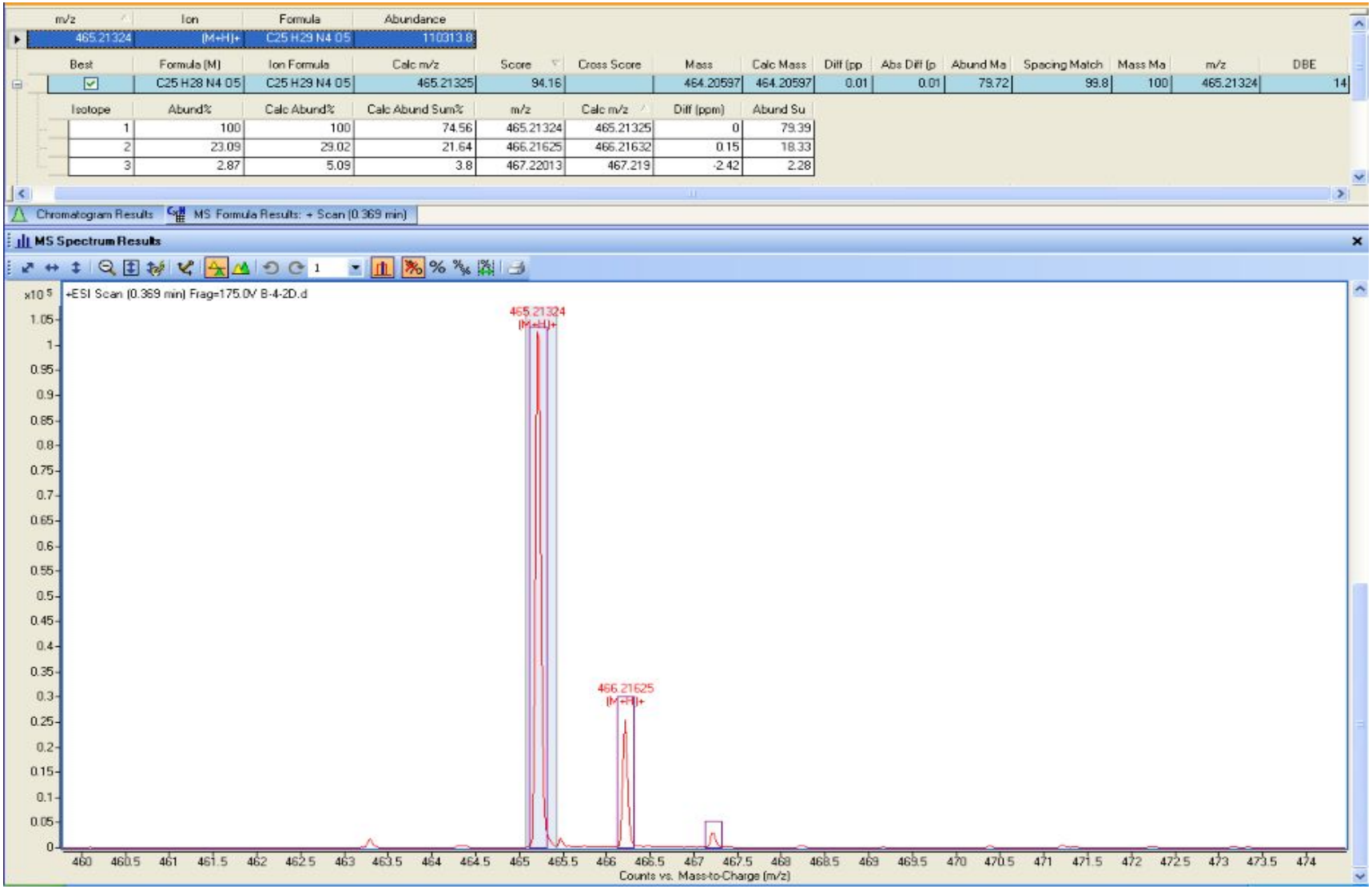



m/z
Ion
Formula
Abundance
465.21327
(M+H)+
C25 H29 N4 O5
97694
Best
Formula (M)
Ion Formula
Calc m/z
Score
Cross Score
Mass
Calc Mass
Diff (ppm)
Abs Diff (pp
Abund Match
Spacing Matc
Mass Match
m/z
DBE
C25 H28 N4 O5
C25 H29 N4 O5
465.21325
98.53
464.20597
464.20597
0
0
97.36
96.99
100
465.21327
14
Isotope
Abund%
Calc Abund%
Calc Abund Su
m/z
Calc m/z
Diff (ppm)
Abund Sum%
1 100 100 74.56 465.21327 465.21325 -0.05 76
2 28.06 29.02 21.64 466.21545 466.21632 1.88 21.33
3 3.52 5.09 3.8 467.21874 467.219 0.55 2.67
Chromatogram Results
MS Formula Results: + Scan (0.586 min)
MS Spectrum Results
x10 5
+ESI Scan (0.586 min) Frag=175.0V B-4-2L.d
465.21327
(M+H)+
466.21545
(M+H)+
487.19511
488.19834
Counts vs. Mass-to-Charge (m/z)

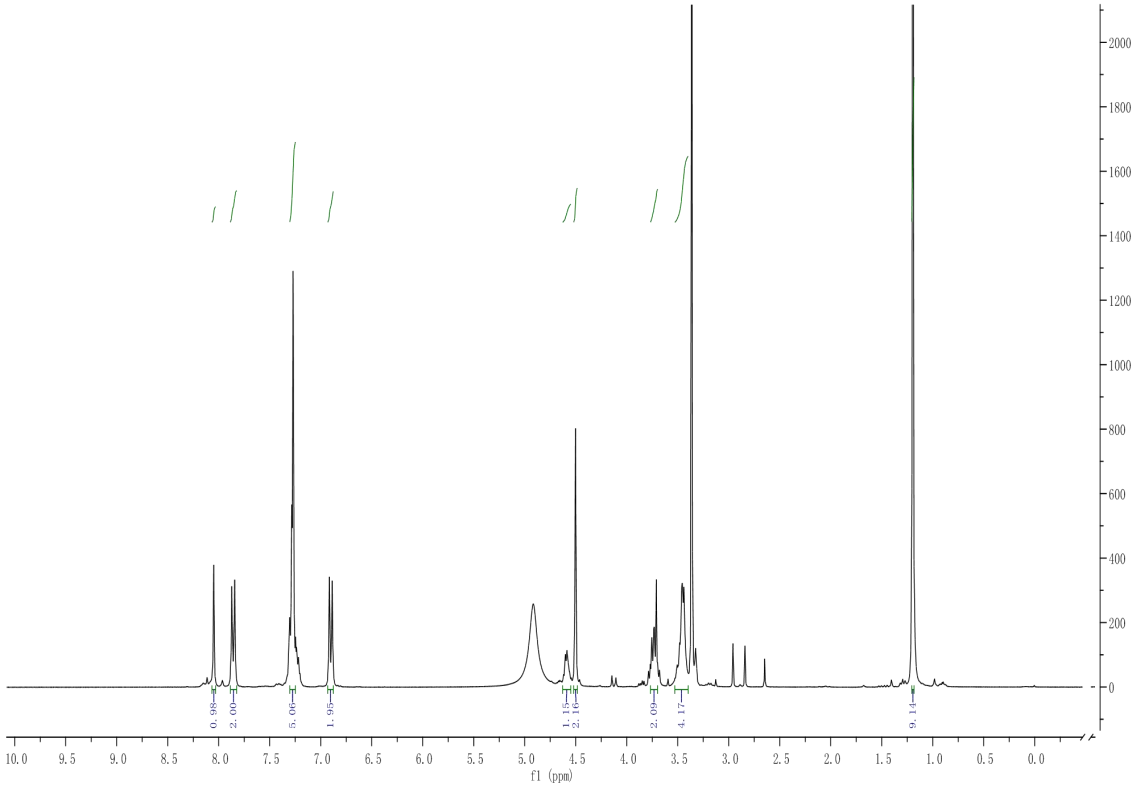
5f (^{1}H NMR, ^{13}C NMR, HRMS)

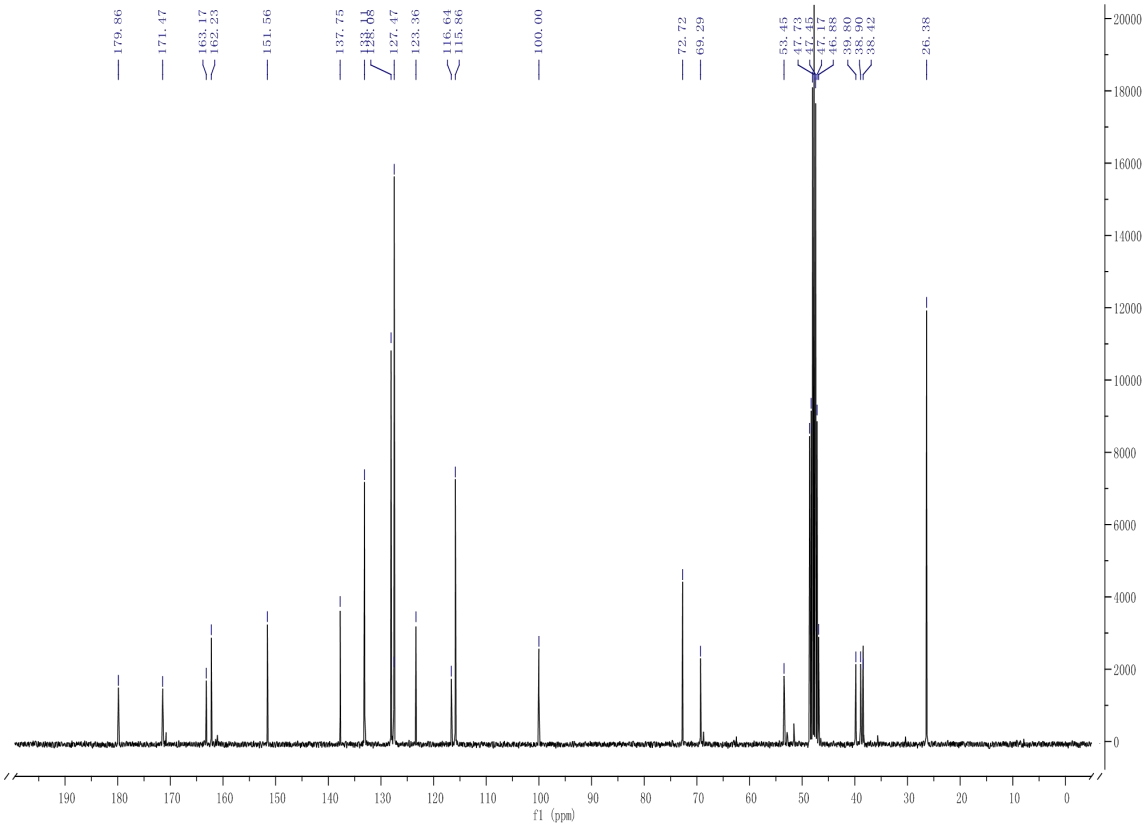


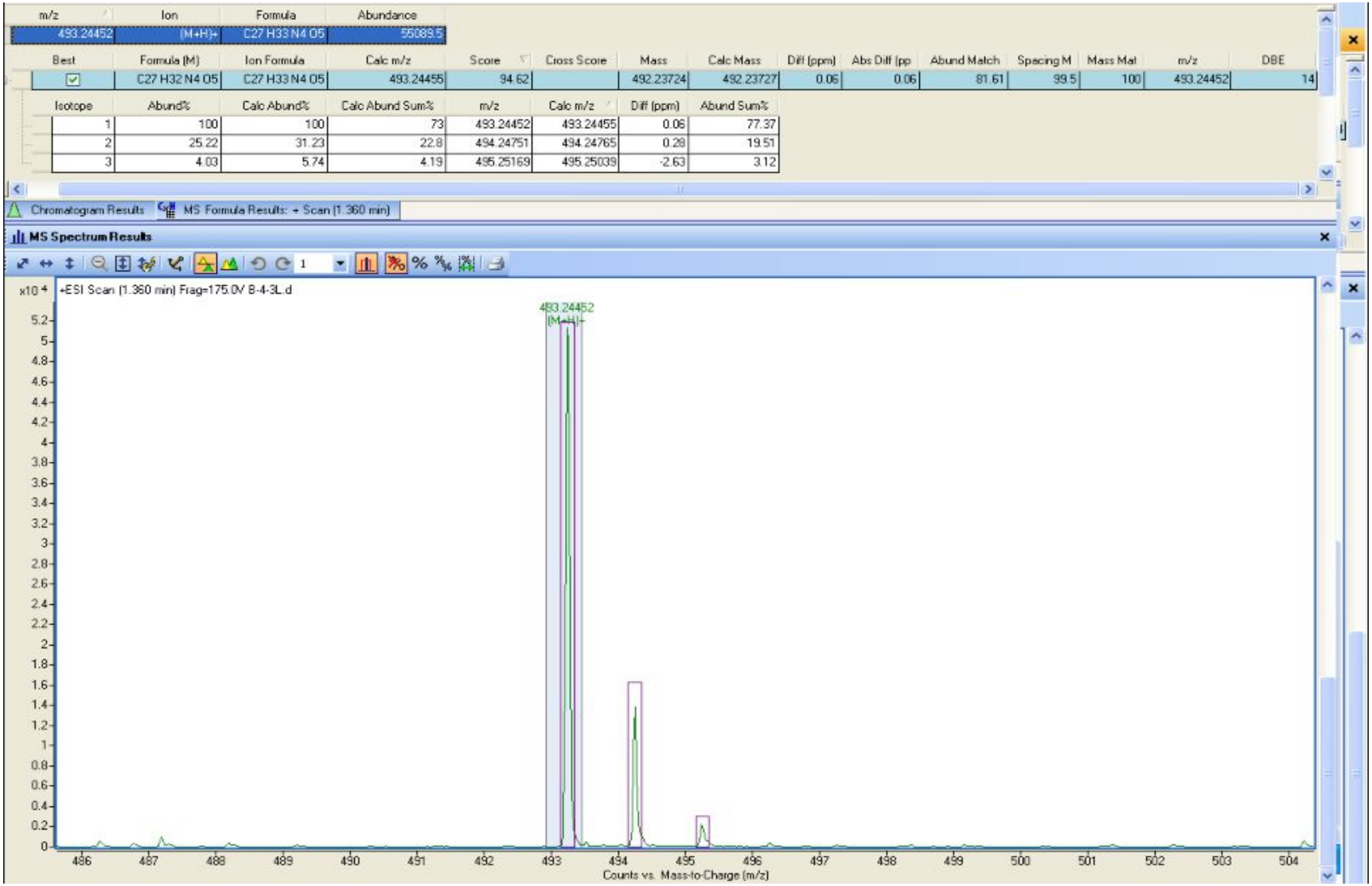



m/z
Ion
Formula
Abundance
465.21324
[M+H]+
C25 H29 N4 05
110313.8
Best
Formula (M)
Ion Formula
Calc m/z
Score
Cross Score
Mass
Calc Mass
Diff (pp
Abs Diff (p
Abund Ma
Spacing Match
Mass Ma
m/z
DBE
C25 H28 N4 05
C25 H29 N4 05
465.21325
94.16
464.20597
464.20597
0.01
0.01
79.72
99.8
100
465.21324
14
Isotope
Abund%
Calc Abund%
Calc Abund Sum%
m/z
Calc m/z
Diff (ppm)
Abund Su
1
100
100
74.56
465.21324
465.21325
0
79.39
2
23.09
29.02
21.64
466.21625
466.21632
0.15
18.33
3
2.87
5.09
3.8
467.22013
467.219
-2.42
2.28
Chromatogram Results
MS Formula Results: + Scan (0.369 min)
MS Spectrum Results
x10 5
+ESI Scan (0.369 min) Frag=175.0V B-4-2D.d
465.21324
[M+H]+
466.21625
[M+H]+
Counts vs. Mass-to-Charge (m/z)

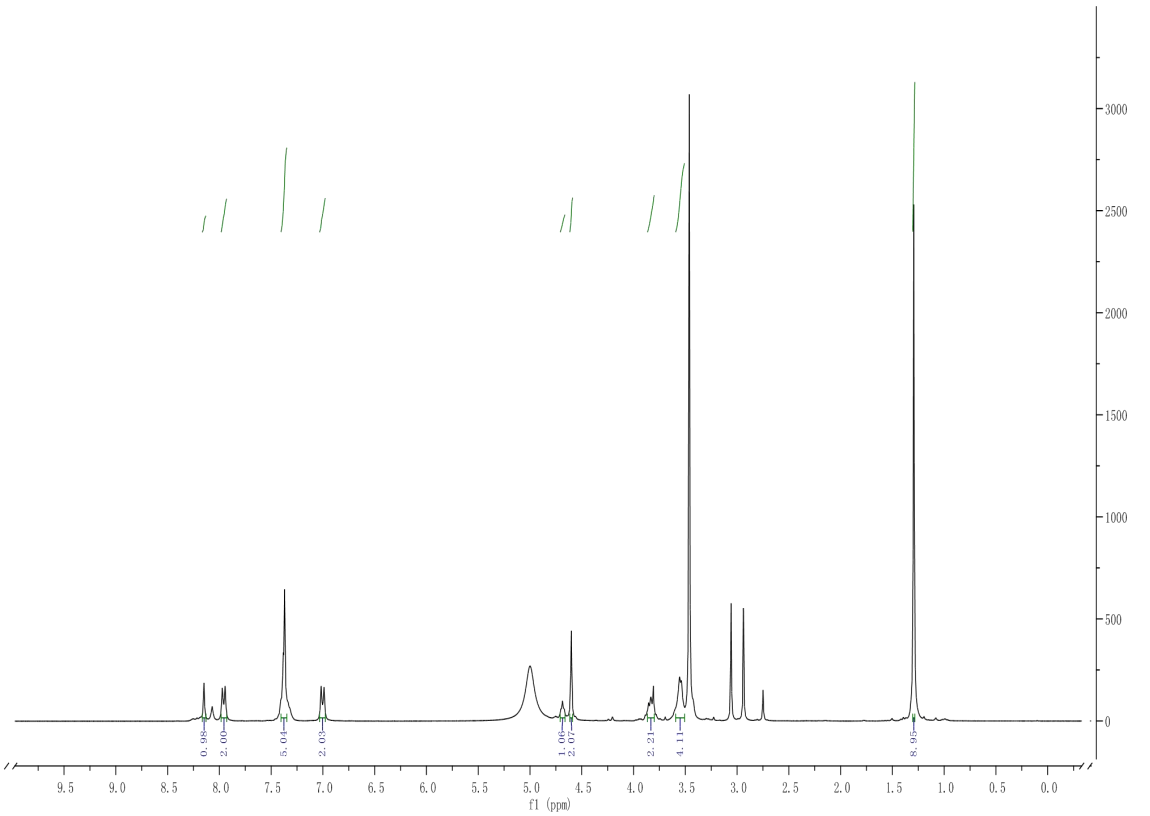
5g (^{1}H NMR, ^{13}C NMR, HRMS)

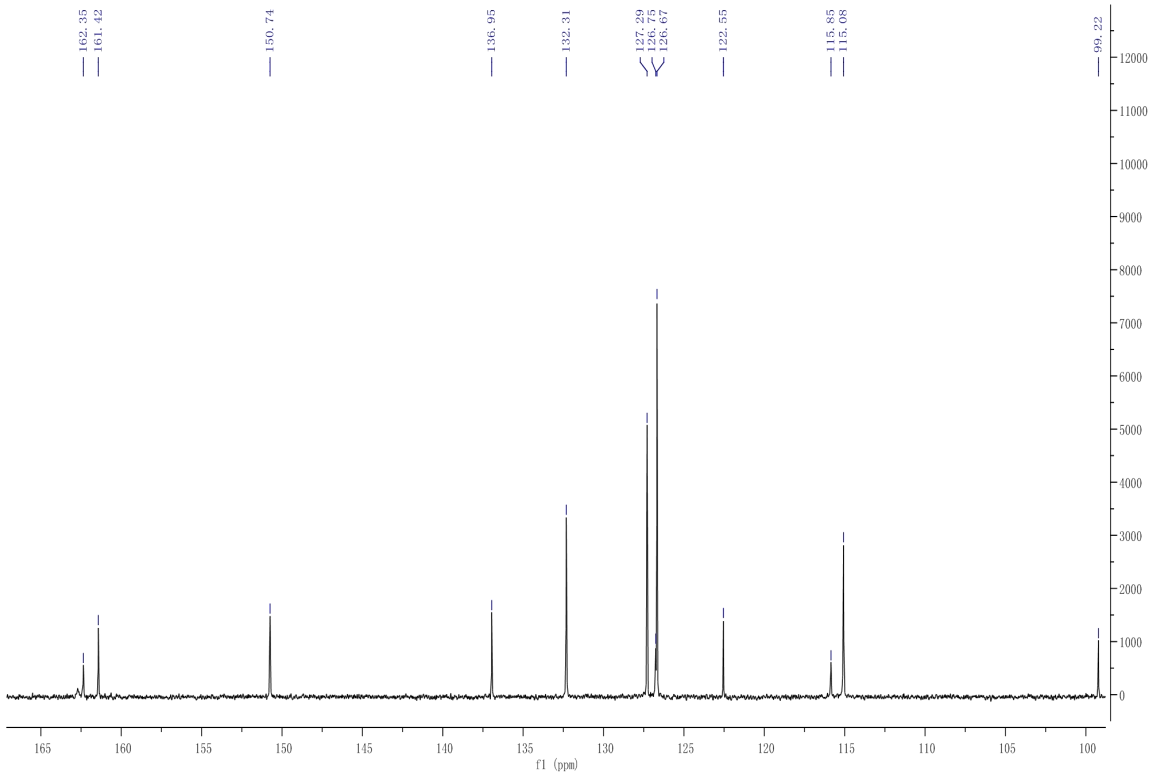





m/z
Ion
Formula
Abundance
493.24452
(M+H)+
C27 H33 N4 O5
55083.5
Best
Formula (M)
Ion Formula
Calc m/z
Score
Cross Score
Mass
Calc Mass
Diff (ppm)
Abs Diff (pp
Abund Match
Spacing M
Mass Mat
m/z
DBE
C27 H32 N4 O5
C27 H33 N4 O5
493.24455
94.62
492.23724
492.23727
0.06
0.06
81.61
99.5
100
493.24452
14
Isotope
Abund%
Calc Abund%
Calc Abund Sum%
m/z
Calc m/z
Diff (ppm)
Abund Sum%
1
100
100
73
493.24452
493.24455
0.06
77.37
2
25.22
31.23
22.8
494.24751
494.24765
0.28
19.51
3
4.03
5.74
4.19
495.25169
495.25039
-2.63
3.12
Chromatogram Results
MS Formula Results: + Scan (1.360 min)
MS Spectrum Results
x10 4
+ESI Scan (1.360 min) Frag=175.0V B-4-3L.d
493.24452
(M+H)+
Counts vs. Mass-to-Charge (m/z)

5h (^{1}H NMR, ^{13}C NMR, HRMS)





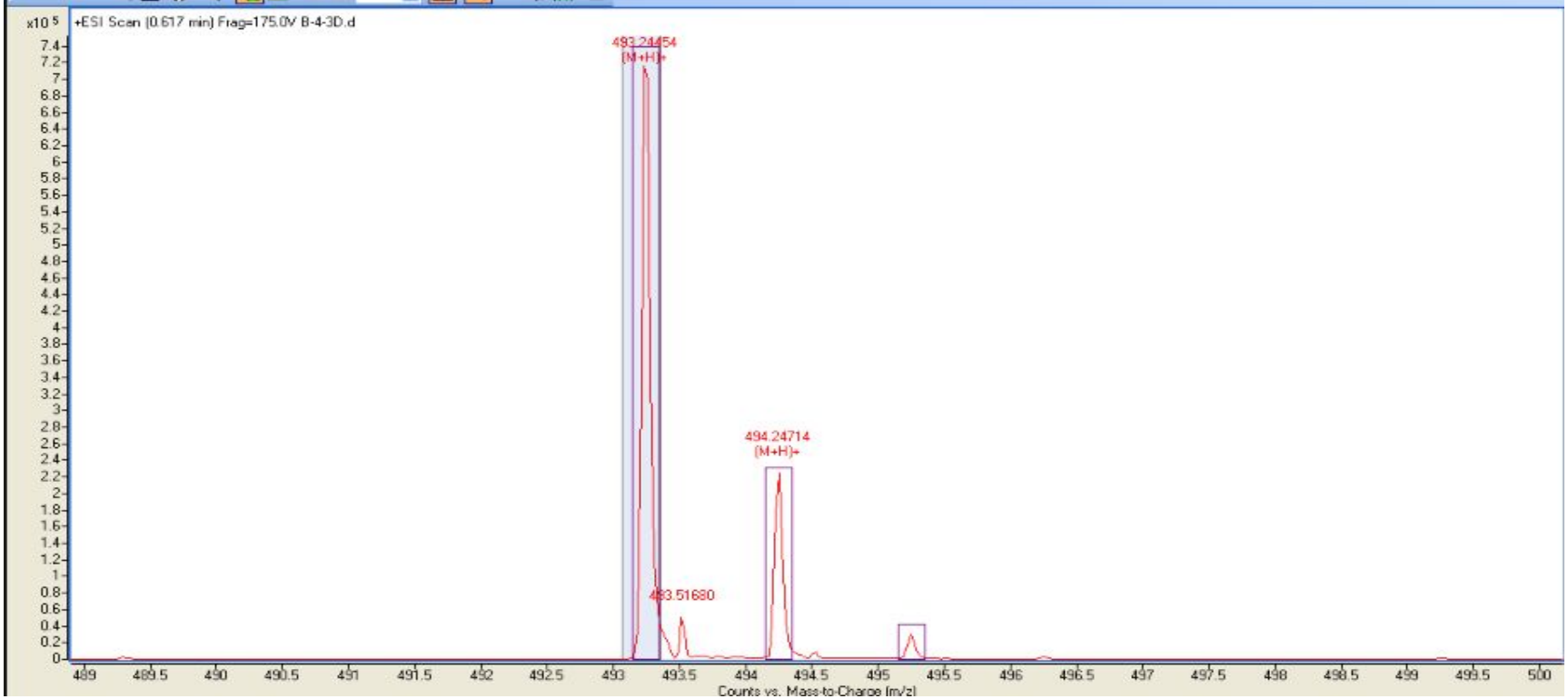
m/z	Ion	Formula	Abundance
493.24454	(M+H)+	C27 H33 N4 O5	758597.3

Best	Formula (M)	Ion Formula	Calc m/z	Score	Cross Score	Mass	Calc Mass	Diff (ppm)	Abs Diff (ppm)	Abund Match	Spacing Match	Mass Match
☑	C27 H32 N4 O5	C27 H33 N4 O5	493.24455	98.72		492.23725	492.23727	0.03	0.03	96.33	99.02	100

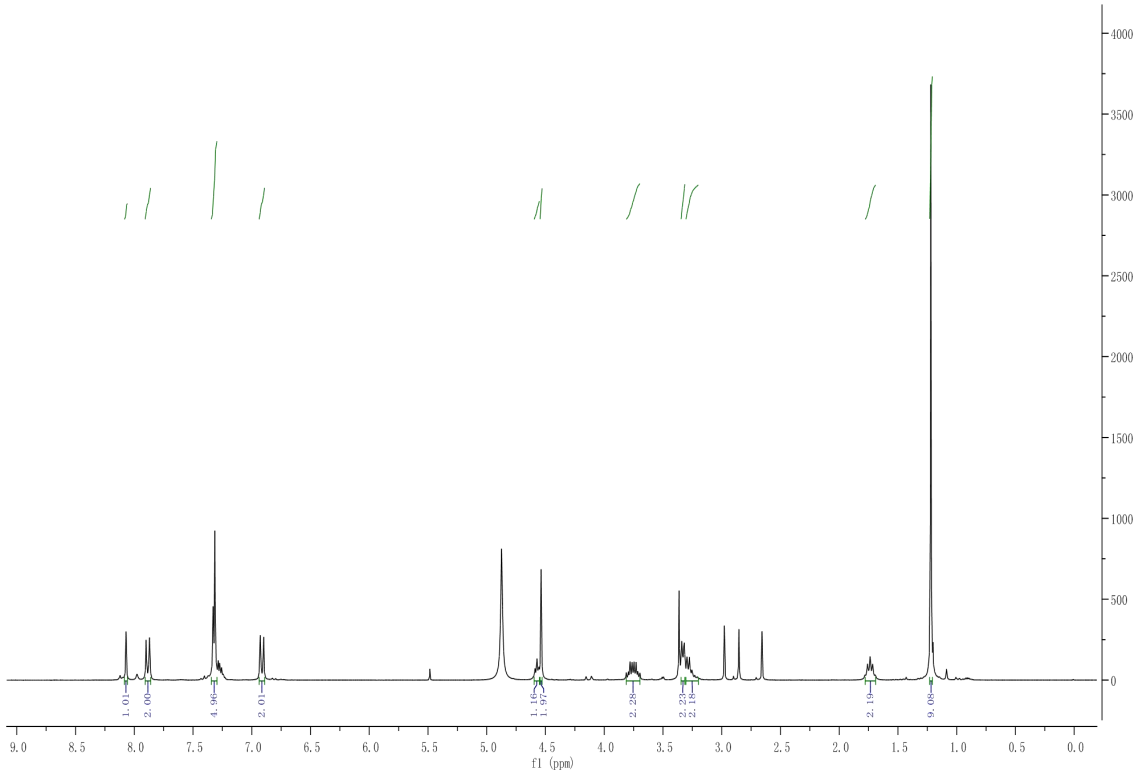
Isotope	Abund%	Calc Abund%	Calc Abund Sum%	m/z	Calc m/z	Diff (ppm)	Abund Sum%
1	100	100	73	493.24454	493.24455	0	74.86
2	29.69	31.23	22.8	494.24714	494.24765	1.03	22.23
3	3.89	5.74	4.19	495.24986	495.25039	1.06	2.91

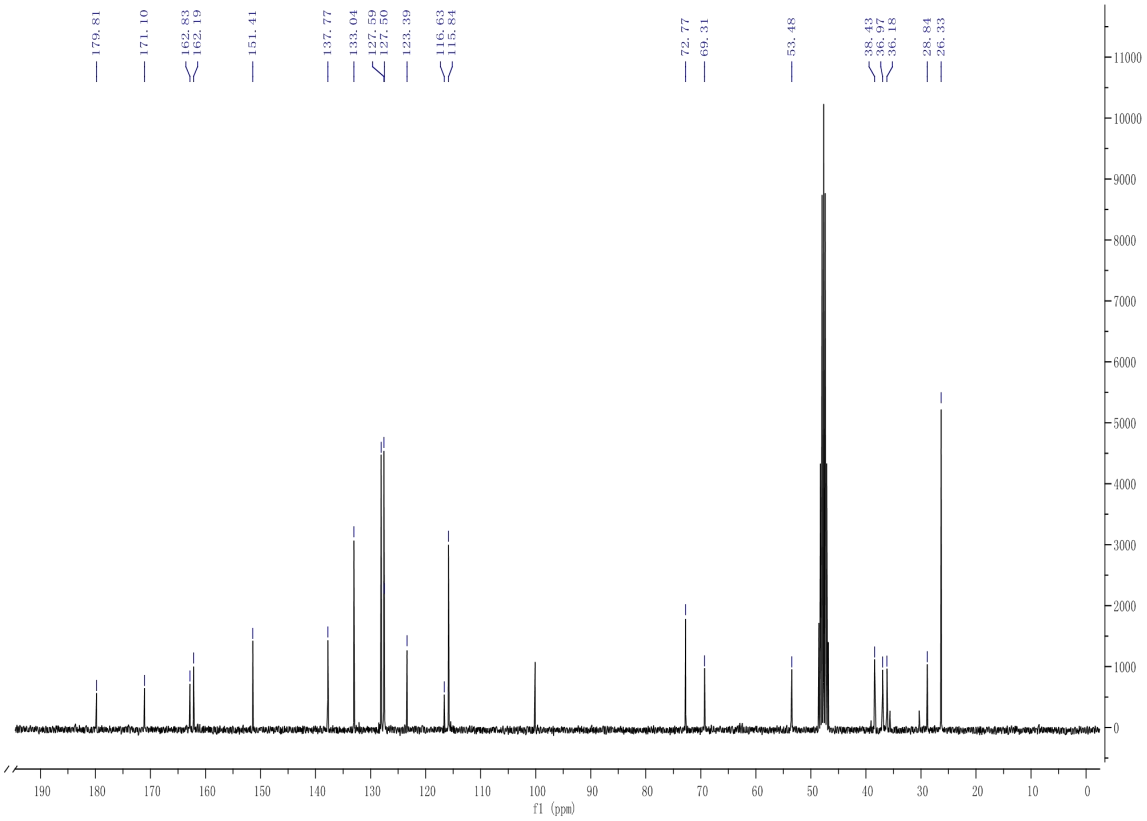
Chromatogram Results | MS Formula Results: + Scan (0.617 min)

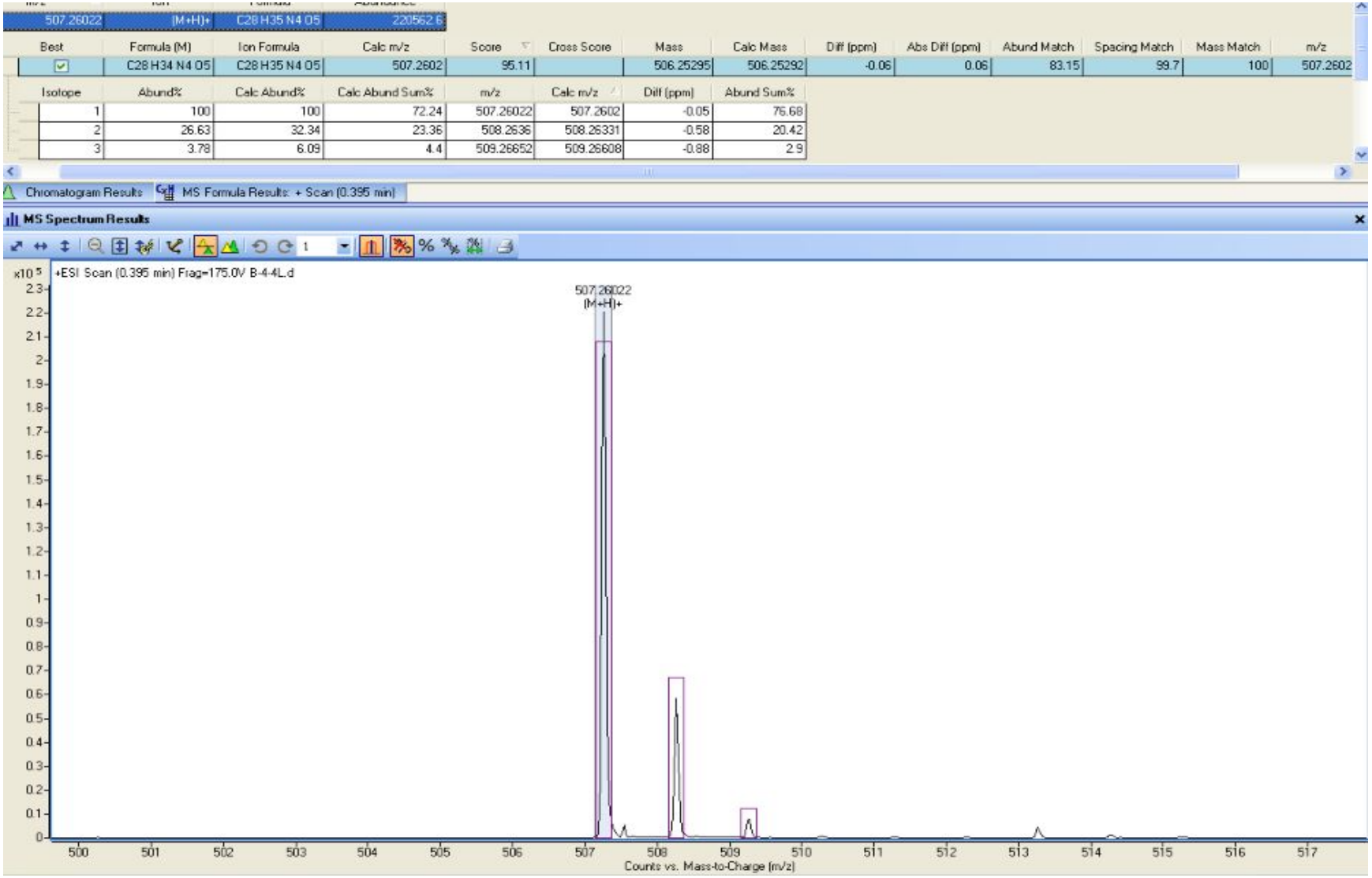
MS Spectrum Results



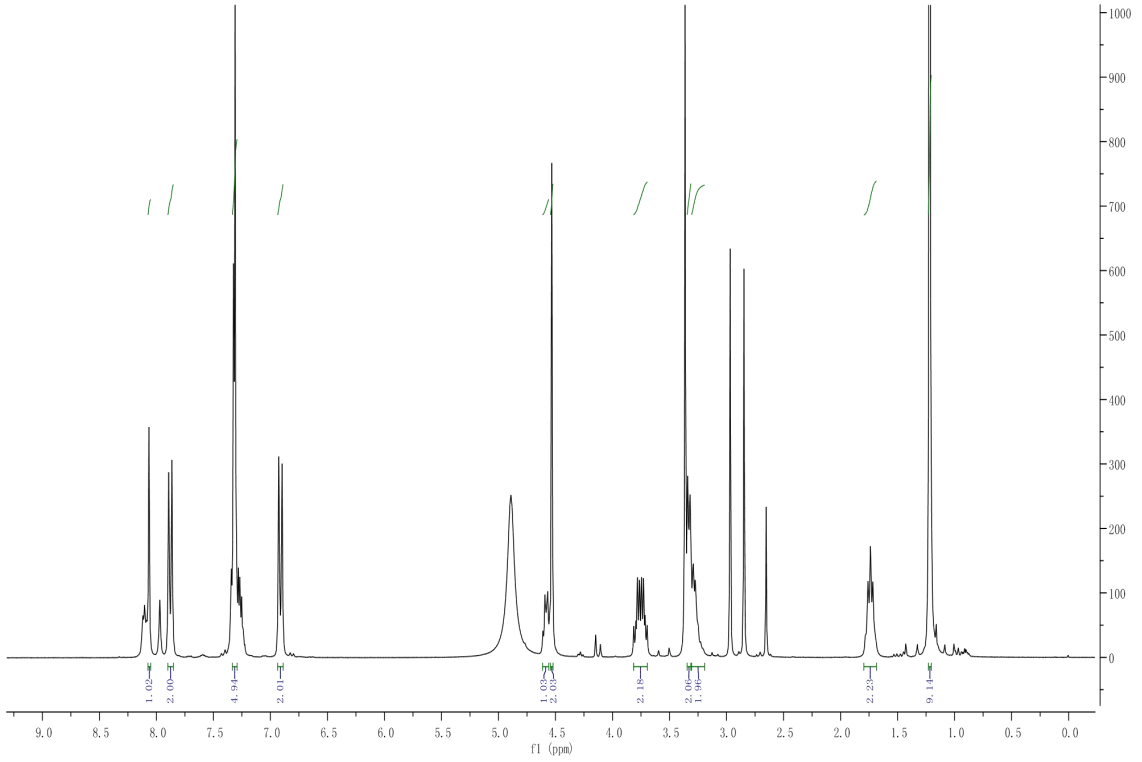
5i (^{1}H NMR, ^{13}C NMR, HRMS)

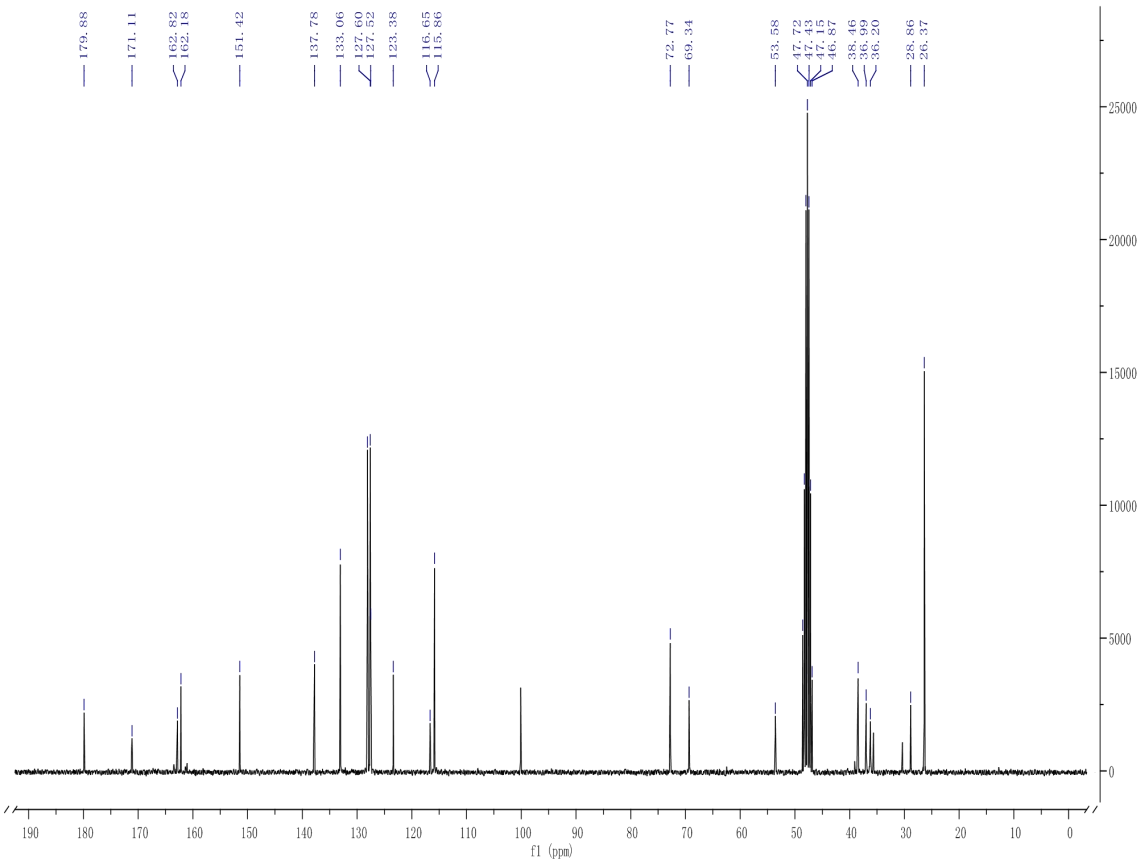





507.26022 (M+H)+ C28 H35 N4 O5 220562.6
Best Formula (M) Ion Formula Calc m/z Score Cross Score Mass Calc Mass Diff (ppm) Abs Diff (ppm) Abund Match Spacing Match Mass Match m/z
C28 H34 N4 O5 C28 H35 N4 O5 507.2602 95.11 506.25295 506.25292 -0.06 0.06 83.15 99.7 100 507.2602
Isotope Abund% Calc Abund% Calc Abund Sum% m/z Calc m/z Diff (ppm) Abund Sum%
1 100 100 72.24 507.26022 507.2602 -0.05 76.68
2 26.63 32.34 23.36 508.2636 508.26331 -0.58 20.42
3 3.78 6.09 4.4 509.26652 509.26608 -0.88 2.9
Chromatogram Results
MS Formula Results: + Scan (0.395 min)
MS Spectrum Results
x10 5
+ESI Scan (0.395 min) Frag=175.0V B-4-4L.d
507.26022 (M+H)+
Counts vs. Mass-to-Charge (m/z)

5j (^{1}H NMR, ^{13}C NMR, HRMS)





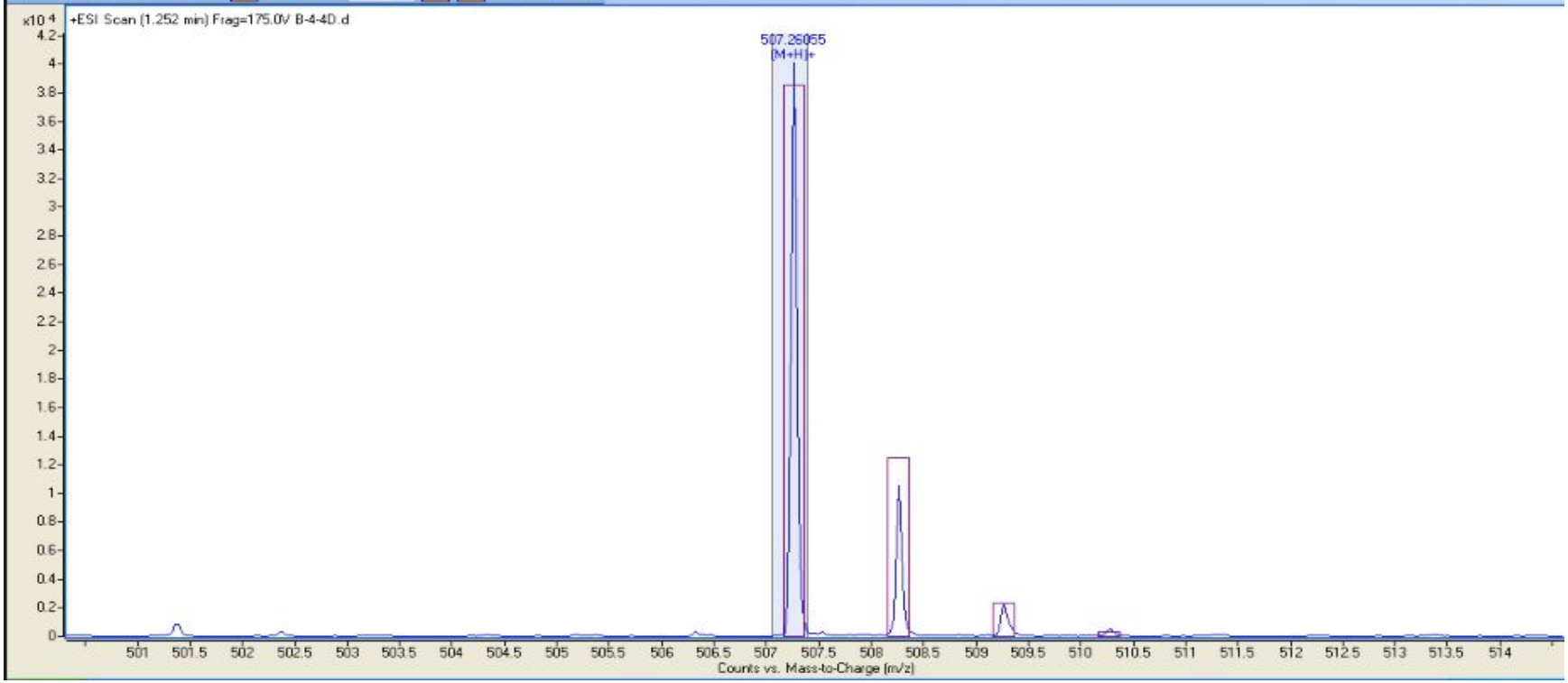
m/z	Ion	Formula	Abundance
507.26055	(M+H)+	C28 H35 N4 O5	40224.8

Best	Formula (M)	Ion Formula	Calc m/z	Score	Cross Score	Mass	Calc Mass	Diff (ppm)	Abs Diff (ppm)	Abund Match	Spacing Match	Mass Match	m/z
☑	C28 H34 N4 O5	C28 H35 N4 O5	507.2602	92.01		506.25329	506.25292	-0.72	0.72	79.83	91.77	99.43	507.26055

Isotope	Abund%	Calc Abund%	Calc Abund Sum%	m/z	Calc m/z	Diff (ppm)	Abund Sum%
1	100	100	71.8	507.26055	507.2602	-0.7	74.91
2	26.25	32.34	23.22	508.26431	508.26331	-1.98	19.67
3	5.93	6.09	4.37	509.27012	509.26608	-7.94	4.45
4	1.3	0.84	0.61	510.27483	510.26873	-11.94	0.98

Chromatogram Results | MS Formula Results + Scan (1.252 min)

MS Spectrum Results



II. Molecular docking

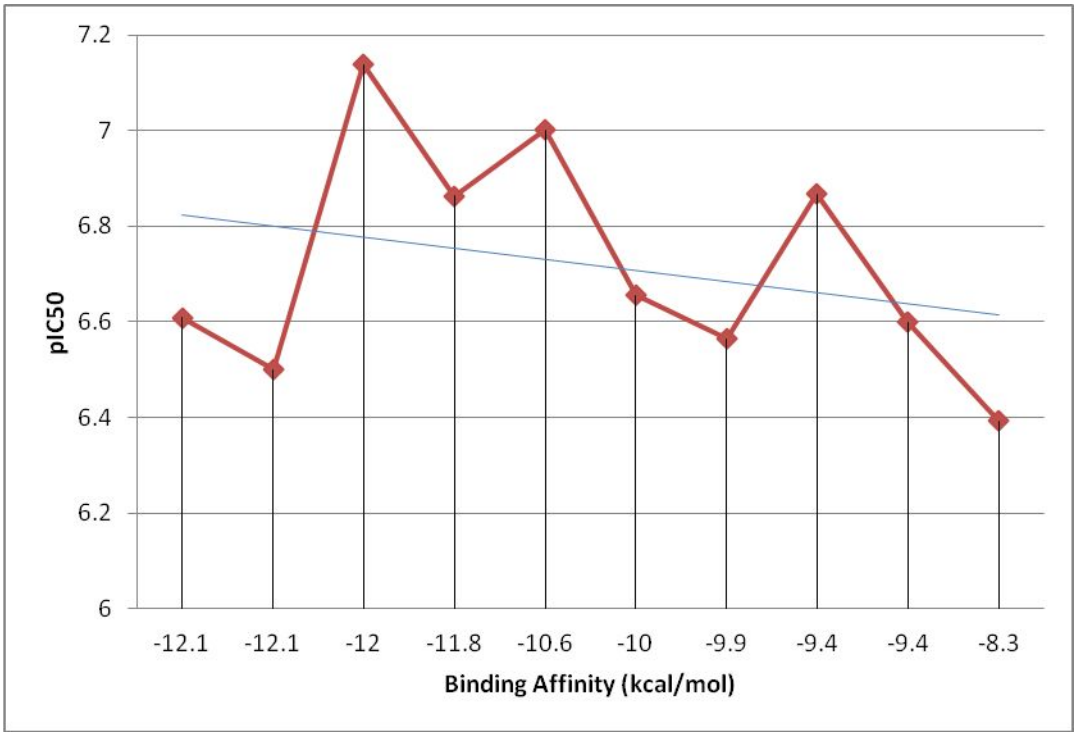


Fig. S4. The plot of binding affinity (kcal/mol) and pIC_{50} (the blue line shows the linear correlation between the binding affinity and pIC50 value).

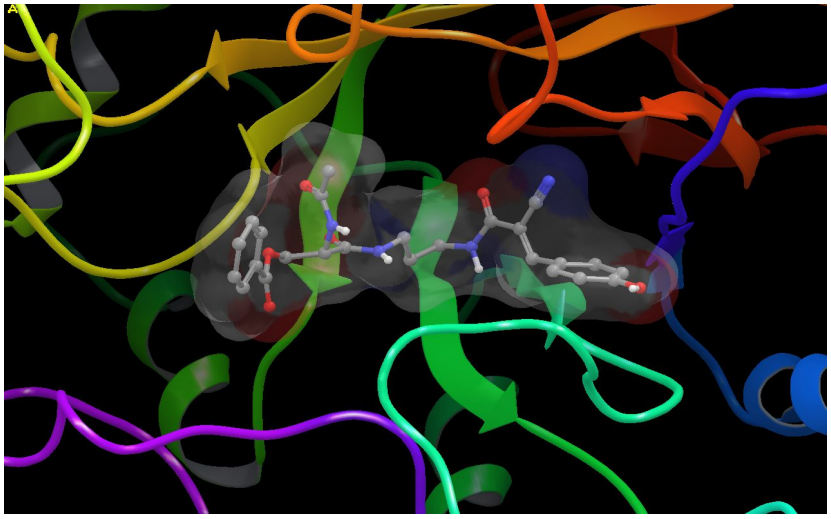


Fig. S5. **5e** poses at the binding sites.

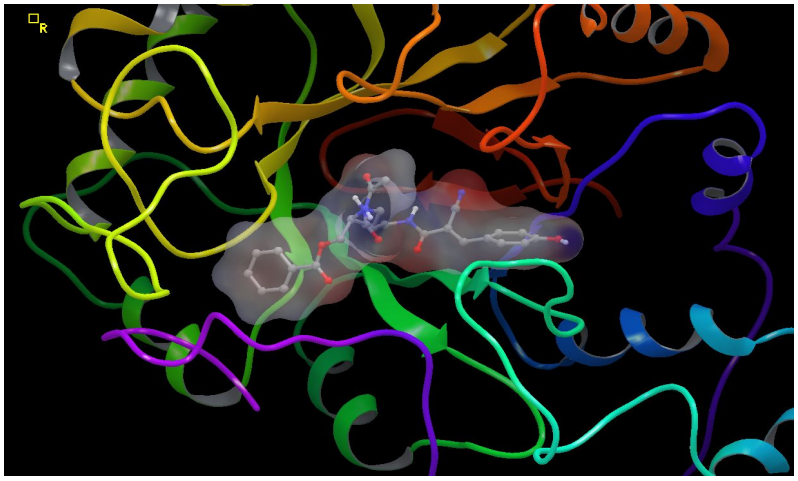


Fig. S6. **5f** poses at the binding sites.

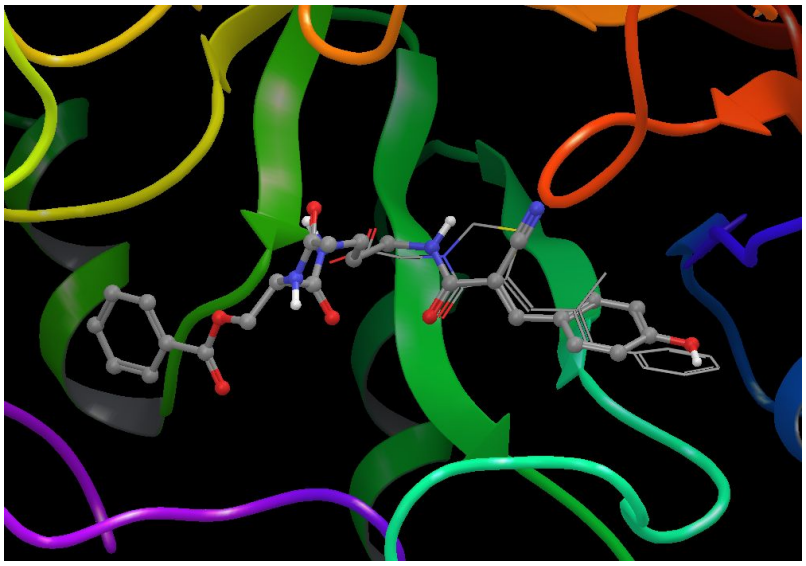


Fig. S7. **5f** superimpositions with Epalrest at the binding sites.

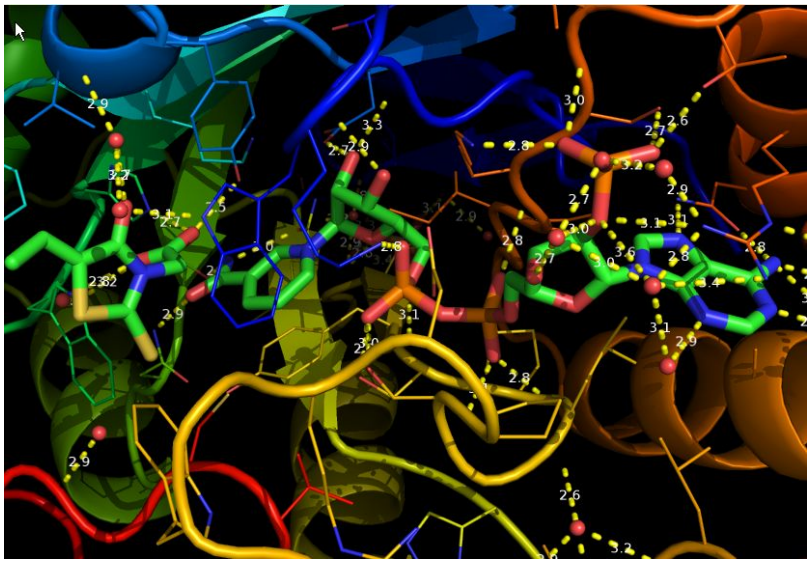


Fig. S8. Aldose reductase with Epalrestat which was catalyzed in the active site