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Supplementary Information For
Aminoluciferin 4-Hydroxyphenyl Amide Enables Bioluminescence Detection of Endogenous Tyrosinase

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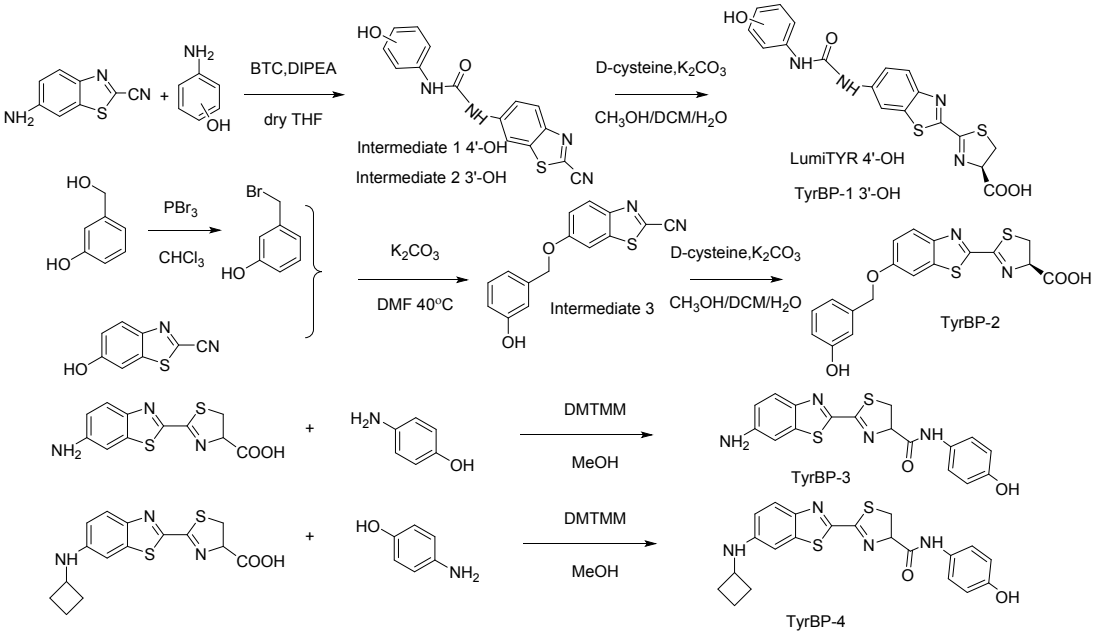
1. Materials and Instruments

Unless otherwise noted, all solvents and reagents available were purchased from commercial sources. 0.9% N.S represent 0.9% sodium chloride solution. Tyrosinase was obtained from Shyuanye company. B16F10-Fluc cellsexpressing firefly luciferase were employed from Beijing Biocytogen Co., Ltd. ES-2-Fluc cells (human ovarian cancer cell lines) expressing firefly luciferase, which were supplied with Cellcyto. NMR data was obtained for ^{1}H NMR (400 MHz) and ^{13}C NMR (100 MHz) on a Bruker AV-400 spectrometer. Mass spectral analyses were obtained on an API 4000 spectrometer (ESI-HRMS). Tris-HCl buffer (50 mM, pH 7.4, 10 mM $MgCl_2$) was used in the bioluminescent measurement. HPLC analysis was undertaken with a 1260 Infinity HPLC system (Agilent Technologies, Santa Clara, CA). The IVIS Lumina (Xenogen) imaging system (Caliper Life Sciences, USA) with a cooled CCD camera was used to capture bioluminescence signals.

2. Synthetic Procedures



Scheme S1 Synthesis of LumiTYR, TyrBP-1, TyrBP-2, TyrBP-3 andTyrBP-4.

The 3-(bromomethyl)phenol , aminoluciferin and cybLuc were synthesised according to the reported literature[1, 2].

Synthesis of Intermediate 1

2-Cyano-6-aminobenzothiazole（50mg，0.285mmol）and 140 uL DIPEA were dissolved in 3 mL THF, and to this solution was added the BTC（85 mg，0.285mmol）in THF, stirred at 0 °C for 30 min.，then stirred at r.t for 2 h. Finally, the p-aminophenol（93 mg，0.855mmol）was added and strred at r.t overnight. The organic solvent was removed in vacuo then diluted with ethylacetate (50 mL) and washed with H_2O (50 mL x 3).The organic layer was then dried over MgSO4, filtered, and concentrated in vacuo. The product was purified via column chromatography with hexane-ethyl acetate (8 : 1) to provide 2a as a pale yellow solid (15 mg, 17%),m.p.: 244-248 °C
^{1}H NMR (400 MHz, DMSO) δ 9.26 – 9.07 (m, 2H), 8.51 (dd, *J* = 24.8, 23.4 Hz, 2H), 8.16 (dd, *J* = 19.6, 9.0 Hz, 1H), 7.65 (dd, *J* = 9.0, 1.7 Hz, 1H), 7.25 (d, *J* = 8.7 Hz, 2H), 6.70 (t, *J* = 9.0 Hz, 2H).
^{13}C NMR (100 MHz, DMSO) δ 153.35, 152.98, 147.05, 141.48, 137.54, 133.96, 131.13, 125.25, 121.13, 120.44, 115.72, 114.21, 109.65.
For $C_{15}H_{10}N_4O_2S$ $(M+H)^+$, 310.0524 , found 311.0597

Synthesis of Intermediate 2

The preparation method was described as 1, povide a pale yellow solid (19 mg, 14%), m.p.: 184-189 °C。

^{1}H NMR (400 MHz, DMSO) δ 9.39 (d, *J* = 18.8 Hz, 1H), 9.18 (s, 1H), 8.78 (d, *J* = 21.8 Hz, 1H), 8.60 – 8.46 (m, 1H), 8.17 (t, *J* = 12.2 Hz, 1H), 7.66 (dd, *J* = 9.0, 1.8 Hz, 1H), 7.17 – 6.98 (m, 2H), 6.83 (d, *J* = 8.2 Hz, 1H), 6.40 (t, *J* = 9.5 Hz, 1H).
^{13}C NMR (100 MHz, DMSO) δ 158.26, 152.61, 147.22, 141.13, 140.80, 137.53, 134.25, 130.00, 125.30, 120.51, 114.20, 109.91, 109.55, 105.89.
For $C_{15}H_{10}N_4O_2S$ $(M+H)^+$, 310.0524 , found 311.0597

Synthesis of LumiTYR

To the solution of compound 1 (14 mg, 0.045 mmol) in 4 mL DCM/MeOH (1:1) was added the mixture of D-cysteine hydrochloride (13 mg, 0.09 mmol) and potassium carbonate (16 mg, 0.09mmol) dissolved in 2 mL H_2O/MeOH (1:1). The reaction was stirred for 30 min at r.t. DCM and MeOH were then removed from the flask under low pressure prior to the addition of 0.1 M aqueous HCl solution until pH 5-6 was reached. Precipitate formed immediately. The precipitate was filtered off and washed with ice water. The precipitate was dried under reduced pressure to provide a yellow soild (17 mg, 90%), m.p.: 186-190 °C Analytical RP HPLC (phenomenex, C8, 250 4.6 mm column) 60% Methanol with 0.1% trifluoracetic acid 0.6 mL/min at 330 nm, Rt: 14.40 min。
^{1}H NMR (400 MHz, DMSO) δ 13.20 (s, 1H), 9.11 (d, *J* = 12.8 Hz, 2H), 8.58 (s, 1H), 8.42 (d, *J* = 1.7 Hz, 1H), 8.04 (d, *J* = 8.9 Hz, 1H), 7.50 (dd, *J* = 8.9, 1.9 Hz, 1H), 7.25 (d, *J* = 8.8 Hz, 2H), 6.70 (d, *J* = 8.7 Hz, 2H), 5.43 (t, *J* = 9.0 Hz, 1H), 3.92 – 3.56 (m, 2H).
^{13}C NMR (100 MHz, DMSO) δ 171.66, 164.85, 158.29, 153.23, 153.11, 148.00, 140.22, 137.14, 131.28, 124.70, 121.00, 119.27, 115.70, 109.96, 78.59, 35.17.
For $C_{18}H_{14}N_4O_4S_2$ $(M+H)^+$, 415.0456 , found 415.0529

Synthesis of TyrBP-1

The preparation method was described as LumiTYR, povide a red solid (13 mg, 81%). m.p.: 188-192 °C。 Analytical RP HPLC (phenomenex, C8, 250 4.6 mm column) 70% Methanol with 0.1% trifluoracetic acid 0.8 mL/min at 350 nm, Rt: 3.61 min
^{1}H NMR (400 MHz, DMSO) δ 9.39 (s, 2H), 9.01 (s, 1H), 8.43 (s, 1H), 8.06 (d, *J* = 8.9 Hz, 1H), 7.53 (d, *J* = 8.8 Hz, 1H), 7.06 (t, *J* = 7.9 Hz, 2H), 6.85 (d, *J* = 8.0 Hz, 1H), 6.39 (d, *J* = 7.9 Hz, 1H), 5.43 (t, *J* = 9.0 Hz, 1H), 3.74 (dt, *J* = 19.4, 10.9 Hz, 2H).
^{13}C NMR (100 MHz, DMSO) δ 171.70, 164.78, 158.46, 158.24, 152.83, 148.12, 141.03, 140.00, 137.13, 129.94, 124.72, 119.29, 110.08, 109.67, 109.44, 105.77, 78.69, 35.21.
For $C_{18}H_{14}N_4O_4S_2$ $(M+H)^+$, 415.0456 , found 415.0531

Synthesis of Intermediate 3

3-(bromomethyl)phenol (106 mg，0.56 mmol），2-Cyano-6-hydroxybenzothiazole (100 mg, 0.56 mmol) and K_2CO_3 (74 mg，0.56 mmol) were dissolved in 2 mL DMF, then stir at 50 °C overnight, the reaction was diluted with 10 mL ethylacetate and H_2O (50 mL X 3), then the organic layer was dried over Na_2SO_4, filtered, and recrystallized from ethanol for purifying. White soild (45 mg, 28%), m.p.: 160-164 °C。
^{1}H NMR (400 MHz, DMSO) δ 9.48 (s, 1H), 8.17 (d, *J* = 9.1 Hz, 1H), 7.96 (d, *J* = 2.5 Hz, 1H), 7.40 (dd, *J* = 9.1, 2.6 Hz, 1H), 7.20 (t, *J* = 7.8 Hz, 1H), 6.88 (dd, *J* = 9.0, 4.8 Hz, 2H), 6.74 (dd, *J* = 8.1, 1.6 Hz, 1H).
^{13}C NMR (100 MHz, DMSO) δ 159.40, 157.97, 146.76, 138.07, 137.99, 134.24, 130.03, 125.81, 119.38, 118.73, 115.49, 114.96, 114.07, 106.11, 70.45.
For $C_{15}H_{10}N_2O_2S$ $(M+H)^+$, 282.0463 , found 283.0536

Synthesis of TyrBP-2

The preparation method was described as LumiTYR, povide a pale yellow soild (25 mg, 92%), m.p.: 80-86 °C. Analytical RP HPLC (phenomenex, C8, 250 4.6 mm column) 70% Methanol with 0.1% trifluoracetic acid 0.8 mL/min at 330 nm, Rt: 5.50 min
^{1}H NMR (400 MHz, DMSO) δ 8.05 (d, *J* = 9.0 Hz, 1H), 7.83 (d, *J* = 2.5 Hz, 1H), 7.26 (dd, *J* = 9.0, 2.6 Hz, 1H), 7.19 (t, *J* = 7.8 Hz, 1H), 6.88 (t, *J* = 5.3 Hz, 2H), 6.73 (dd, *J* = 8.1, 1.5 Hz, 1H), 5.45 – 5.33 (m, 1H), 5.14 (s, 2H), 3.82 – 3.63 (m, 2H).
^{13}C NMR (100 MHz, DMSO) δ 171.56, 164.18, 158.62, 158.40, 157.97, 147.66, 138.38, 137.52, 129.98, 125.23, 118.68, 117.90, 115.40, 114.93, 106.33, 79.33, 70.34, 35.41.
For $C_{18}H_{14}N_2O_4S_2$ $(M-H)^-$, 385.0395 , found 385.0322.

Synthesis of TyrBP-3

Aminoluciferin（62 mg，0.222 mmol）was dissolved in 6 mL MeOH，then added DMTMM（67.5 mg，0.244 mmol）and p-aminophenol（26.5 mg，0.244 mmol）in 2 mL MeOH，followed by stirring at room temperature overnight, the solvent was removed by evaporation, then was diluted with EA, washed with water (25 mL X 3), dried over anhydrous Na_2SO_4, The product was purified via column chromatography further recrystallized from EA for purifying, povided yellow soild (16 mg, 19.5%), m.p.: 240-244 °C。
Analytical RP HPLC (phenomenex, C8, 250 4.6 mm column) 70% Methanol with 0.1% trifluoracetic acid 0.8 mL/min at 350 nm, Rt: 3.38 min.
^{1}H NMR (400 MHz, DMSO) δ 10.09 (s, 1H), 9.28 (s, 1H), 7.78 (d, *J* = 8.8 Hz, 1H), 7.45 (d, *J* = 8.8 Hz, 2H), 7.07 (d, *J* = 1.7 Hz, 1H), 6.86 (dd, *J* = 8.8, 1.9 Hz, 1H), 6.73 (d, *J* = 8.8 Hz, 2H), 5.84 (s, 2H), 5.38 (t, *J* = 9.3 Hz, 1H), 3.73 (t, *J* = 11.0 Hz, 2H).
^{13}C NMR (100 MHz, DMSO) δ 167.46, 164.77, 154.17, 153.64, 149.74, 144.64, 138.34, 130.75, 125.05, 121.77, 116.44, 115.60, 103.48, 80.28, 34.47.
For $C_{17}H_{14}N_4O_2S_2$$(M+H)^+$, 371.0558 , found 371.0631.

Synthesis of TyrBP-4

The preparation method was described as TyrBP-3, povide pale yellow soild (11.2 mg, 22%), m.p.: 184-188 °C.Analytical RP HPLC (phenomenex, C8, 250 4.6 mm column) 70% Methanol with 0.1% trifluoracetic acid 0.8 mL/min at 380 nm, Rt: 8.95 min.
^{1}H NMR (400 MHz, DMSO) δ 10.09 (s, 1H), 9.27 (s, 1H), 7.81 (d, *J* = 8.9 Hz, 1H), 7.44 (t, *J* = 7.6 Hz, 2H), 6.99 (d, *J* = 1.9 Hz, 1H), 6.86 (dd, *J* =

9.0, 2.0 Hz, 1H), 6.79 – 6.66 (m, 3H), 5.35 (dt, *J* = 9.8, 7.0 Hz, 1H), 3.90 (dd, *J* = 16.3, 8.9 Hz, 2H), 3.72 (dd, *J* = 14.9, 7.3 Hz, 2H), 2.47 – 2.30 (m, 2H), 1.95 – 1.82 (m, 2H), 1.82 – 1.69 (m, 2H).
^{13}C NMR (100 MHz, $CDCl_3$) δ 168.84, 167.15, 154.35, 152.96, 147.02, 145.85, 138.86, 130.18, 125.35, 122.23, 115.76, 101.24, 79.20, 48.85, 35.29, 31.05, 29.71, 29.33, 15.37.
For $C_{21}H_{20}N_4O_2S_2(M-H)^-$, 423.1028 , found 423.0956.

3. Bioluminescent Assay

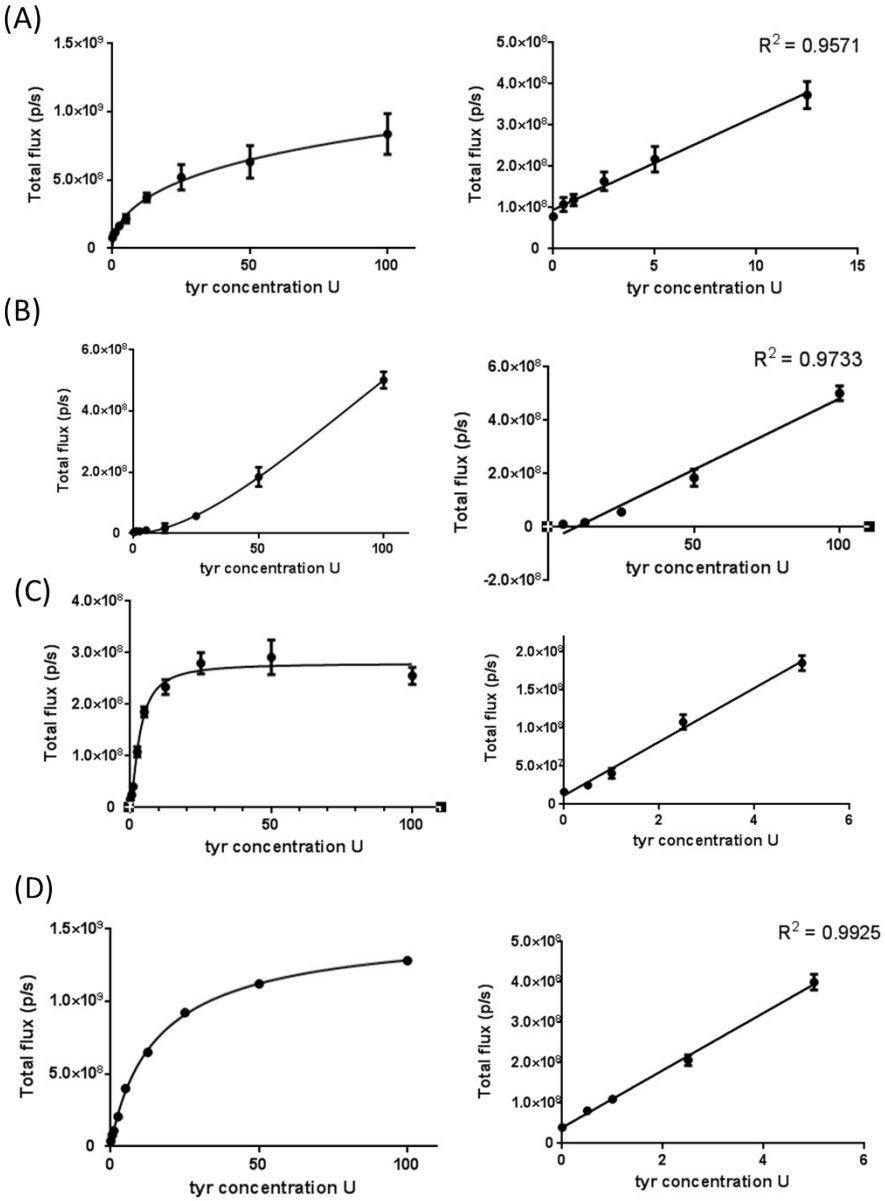


Figure S1 Tyrosinase concentration-dependent bioluminescence value changes from Figure 1. **(A)** The linear relationshion of LumiTYR is described as Y = 2.272e+007 × X + 9.504e+007 (R^2 = 0.9571), and the linearity range of tyrosinase concentration is from 0 to 0-12.5 U·mL^{-1}. **(B)** The linear relationshion of TyrBP-2 is described as Y = 5.322e+006 × X – 5.027e+007 (R^2 = 0.9733), and the linearity range of tyrosinase concentration is from 0 to 5-100 U·mL^{-1}. **(C)**The linear relationshion of TyrBP-3is described as Y = 3.528e+007 × X + 1.171e+007 (R^2 = 0.9841), and the linearity range of tyrosinase concentration is from 0 to 0-5 U·mL^{-1}. **(D)** The linear relationshion of TyrBP-4 is described as Y = 7.116e+007 × X + 3.934e+007 (R^2 = 0.9925), and the linearity range of tyrosinase concentration is from 0 to 0-5 U·mL^{-}.

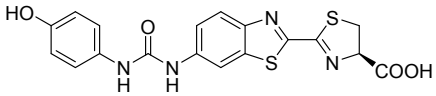
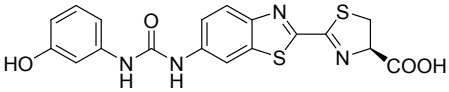
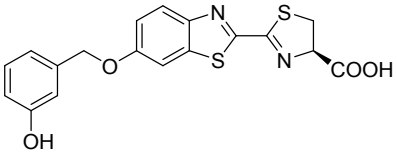
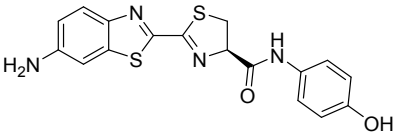
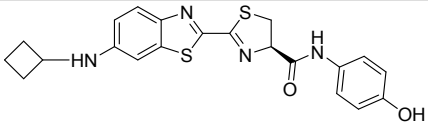
Number	TYR Probes	The Structures of Probes	LOD (U·mL-1)
1	LumiTYR		8.4
2	TyrBP-1		
3	TyrBP-2		12.5
4	TyrBP-3		0.65
5	TyrBP-4		1.1

Table S1 The limit detection (LOD)of LumiTYR, TyrBP-1, TyrBP-2,TyrBP-3 and TyrBP-4 about tyrosianse

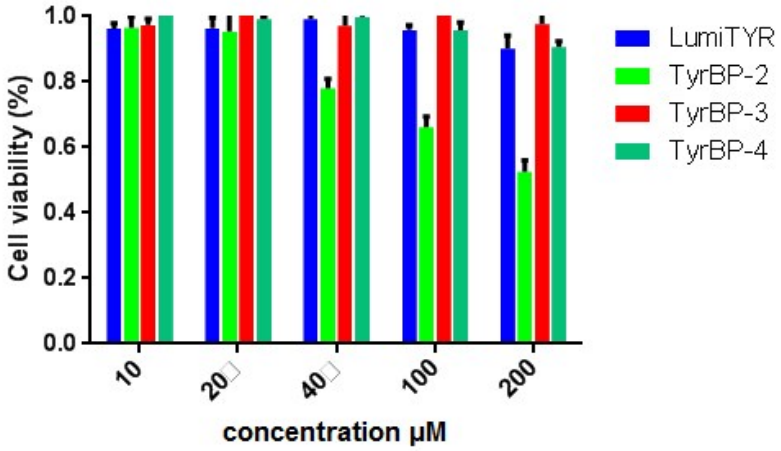


Figure S2 The cytotoxic effect of four BL probe**s** was determined by CCK8 assay. All assays were performed in triplicate and represented as the mean ± SEM.

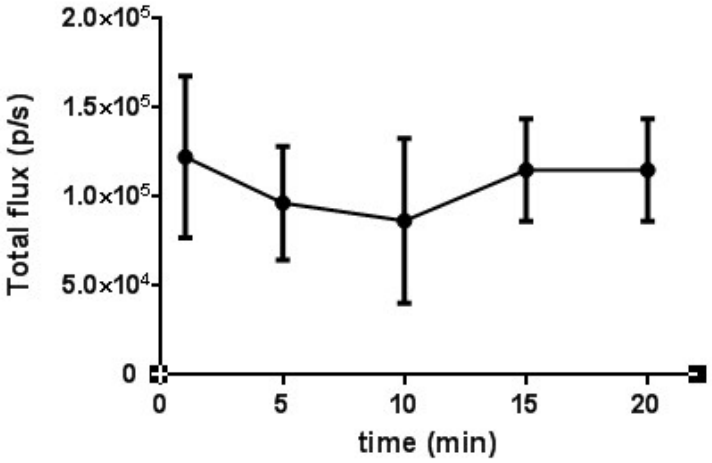


Figure S3 Total flux of bioluminescence imaging of TyrBP-3 in a real-time manner in ES-2-Fluc cells. All assays were performed in quintuplicate and represented as the mean ± SEM.

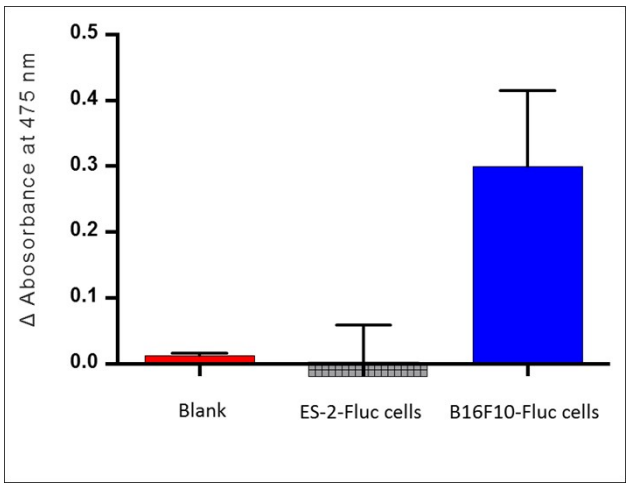
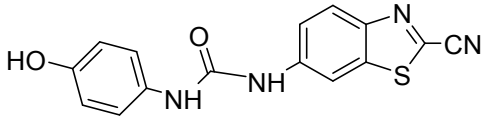
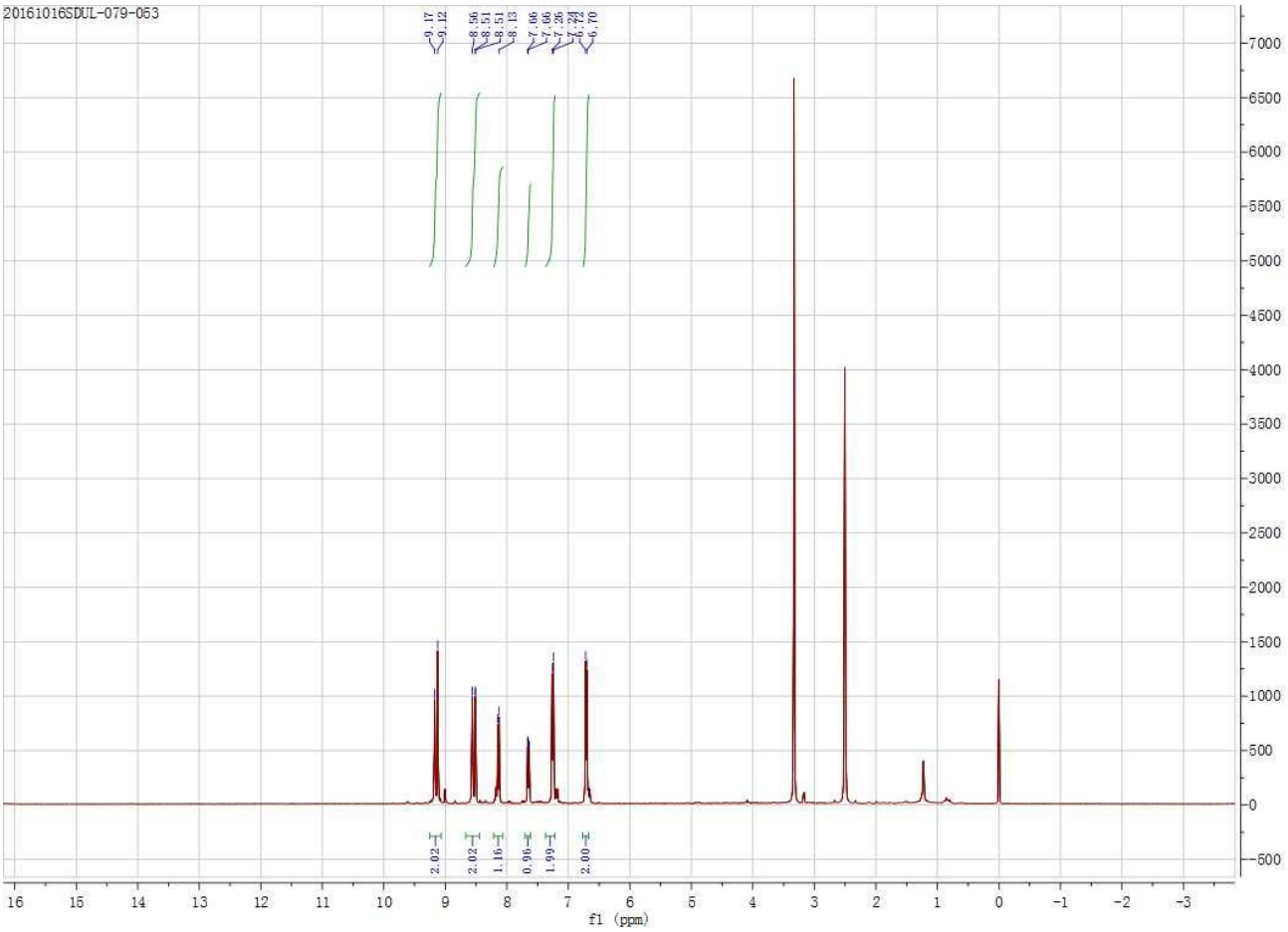


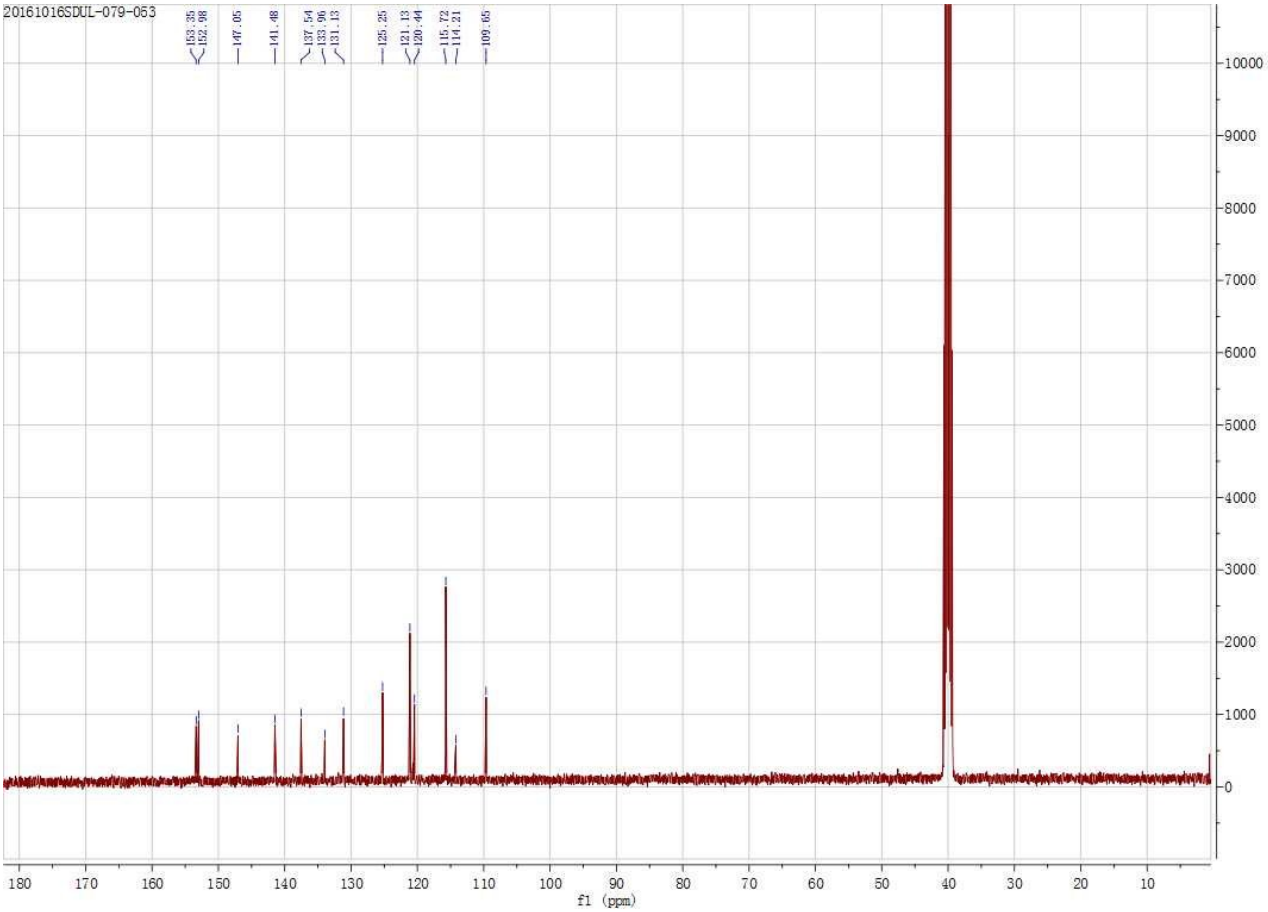
Figure S4 The assay of tyrosinase activity in ES-2-Fluc cells and B16F10-Fluc cells in the presence of L-DOPA. The absorbance was read with amicroplate reader at 475 nm.The results are the mean± SEM (n=3)

4. ^{1}H-NMR, ^{13}C-NMR, HPLC and ESI-MS spectra of compounds

Intermediate 1



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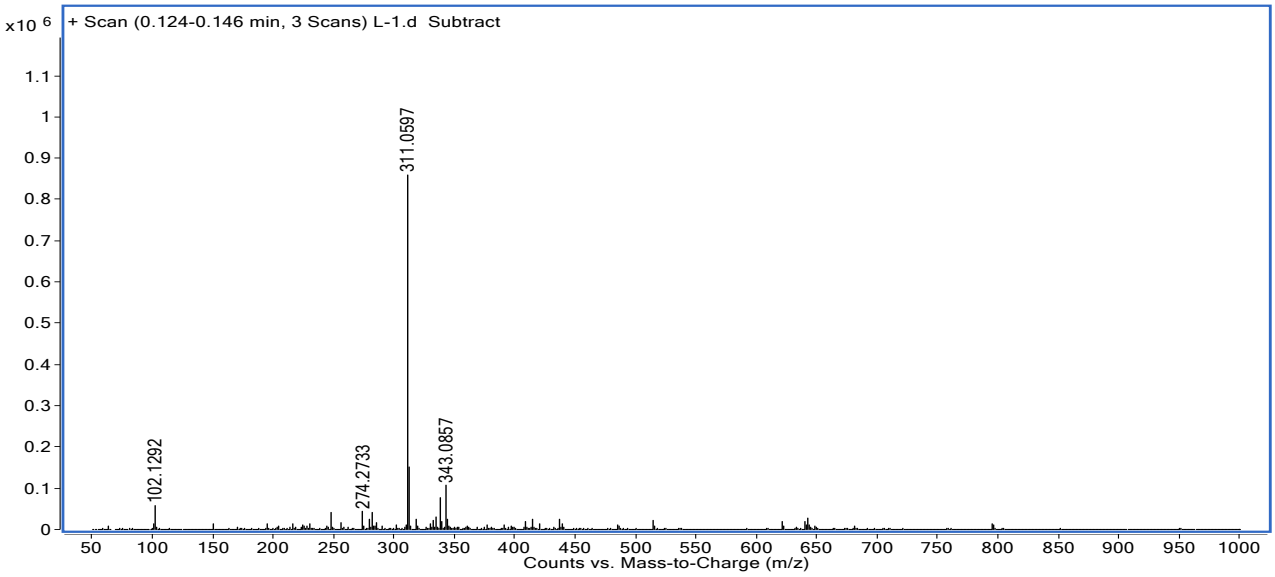
f1 (ppm)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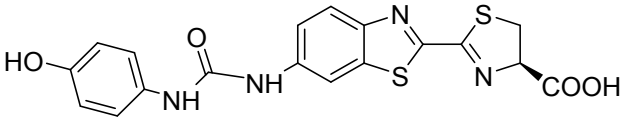
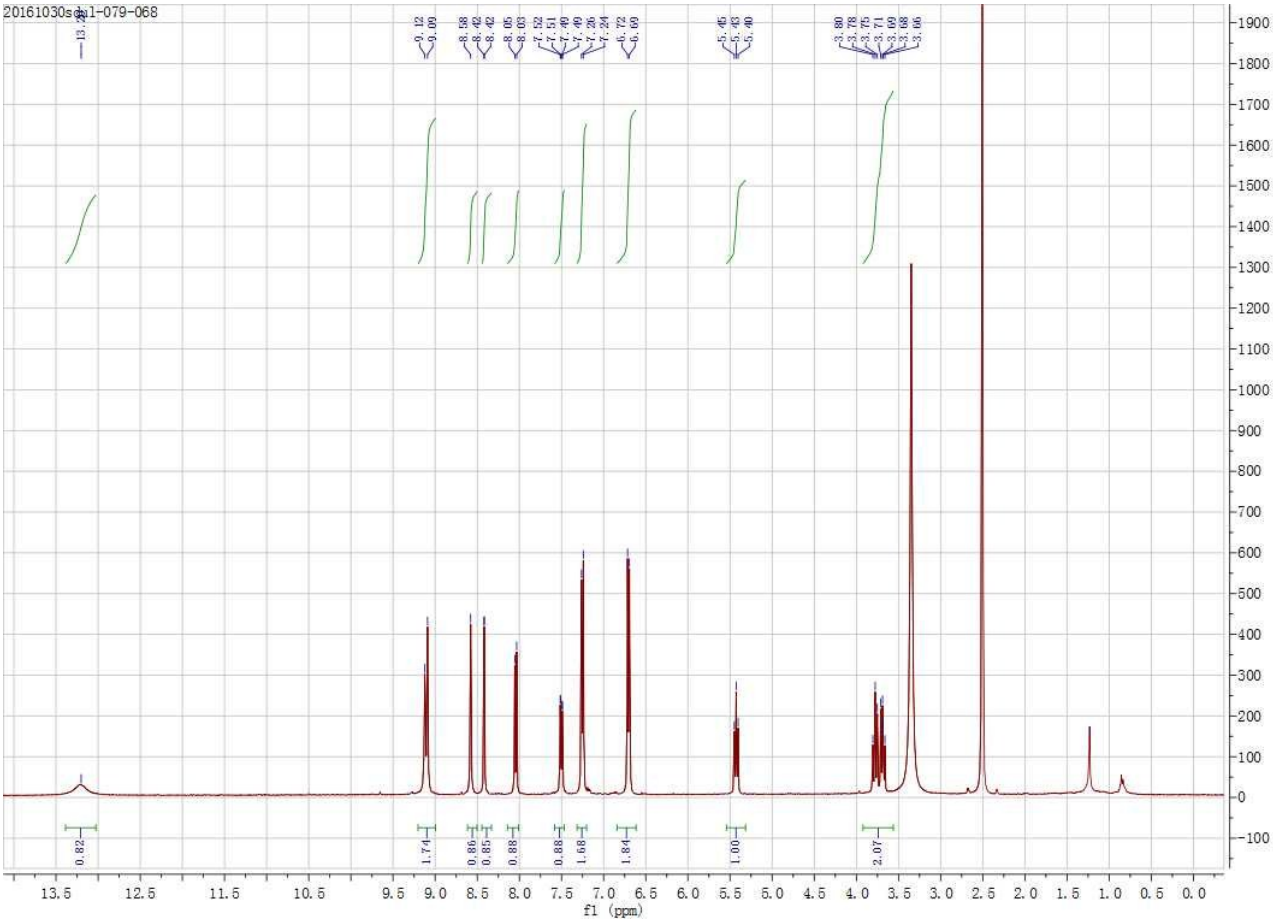
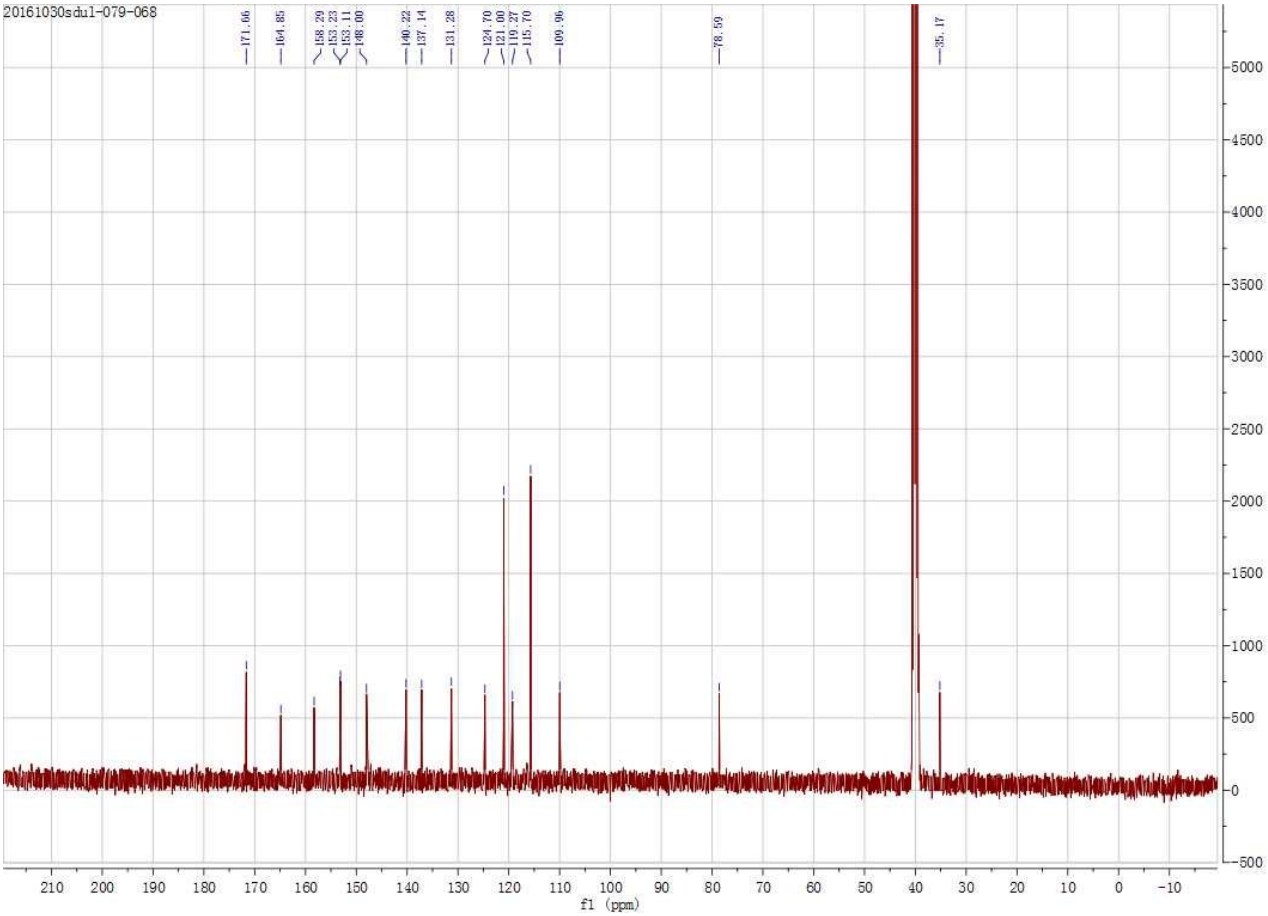
Counts vs. Mass-to-Charge (m/z)

LumiTYR



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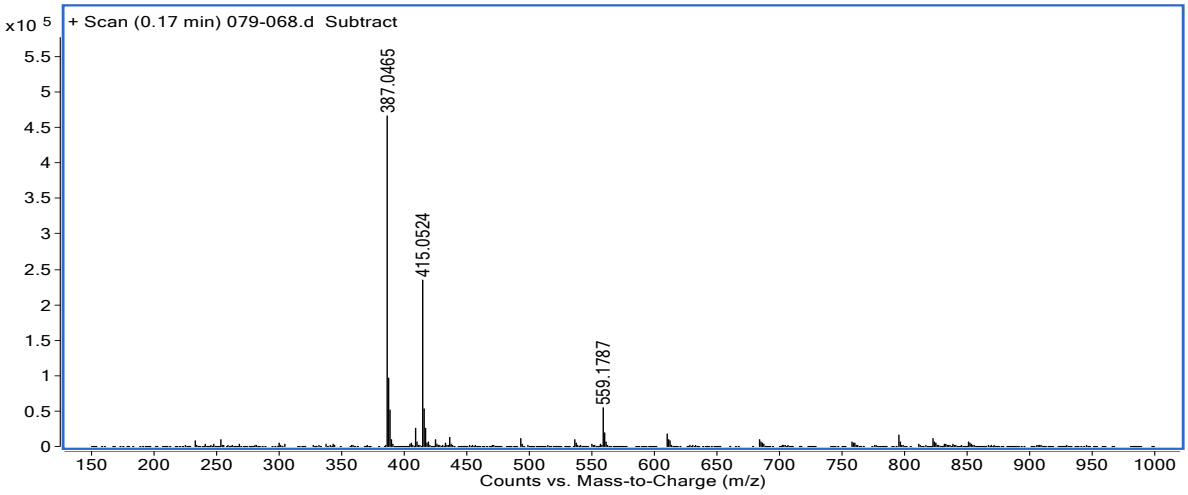
35.17
f1 (ppm)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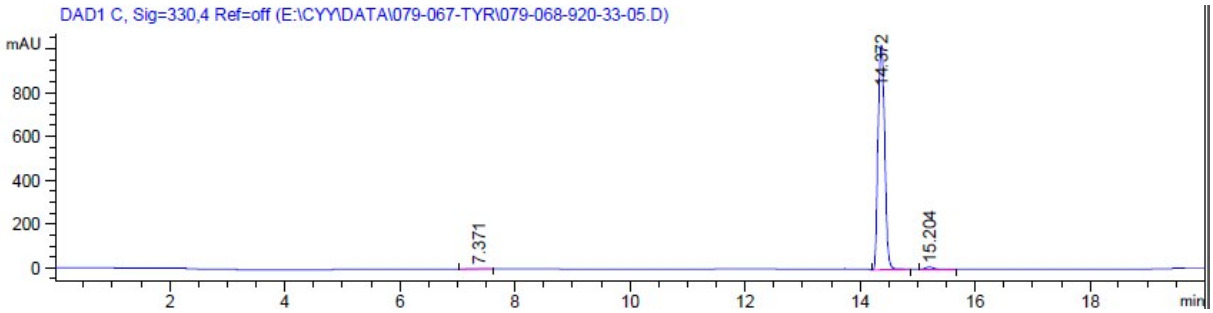
Counts vs. Mass-to-Charge (m/z)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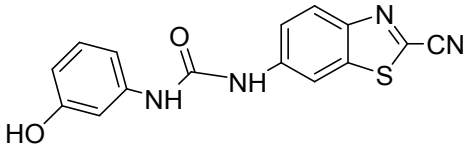
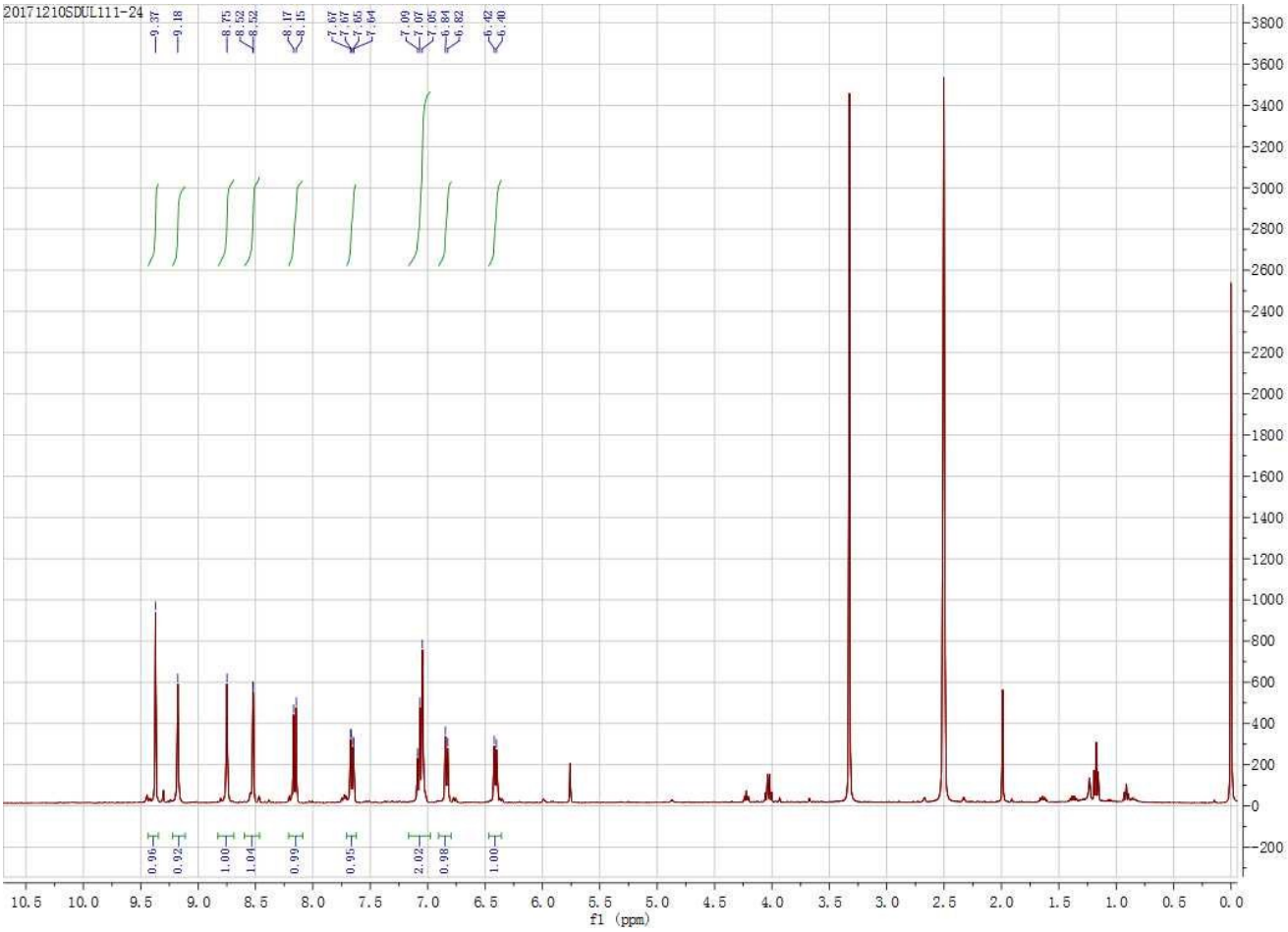
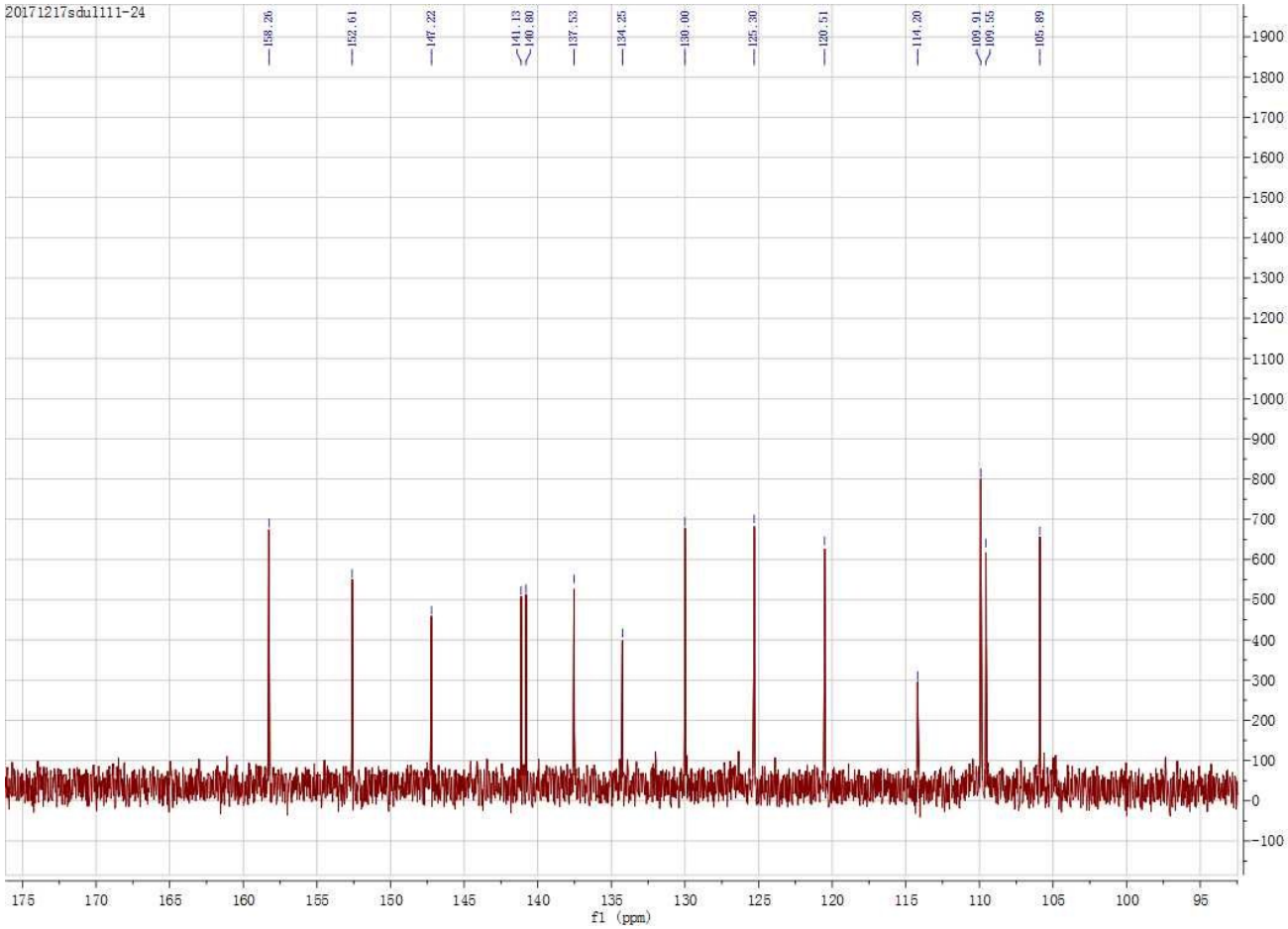
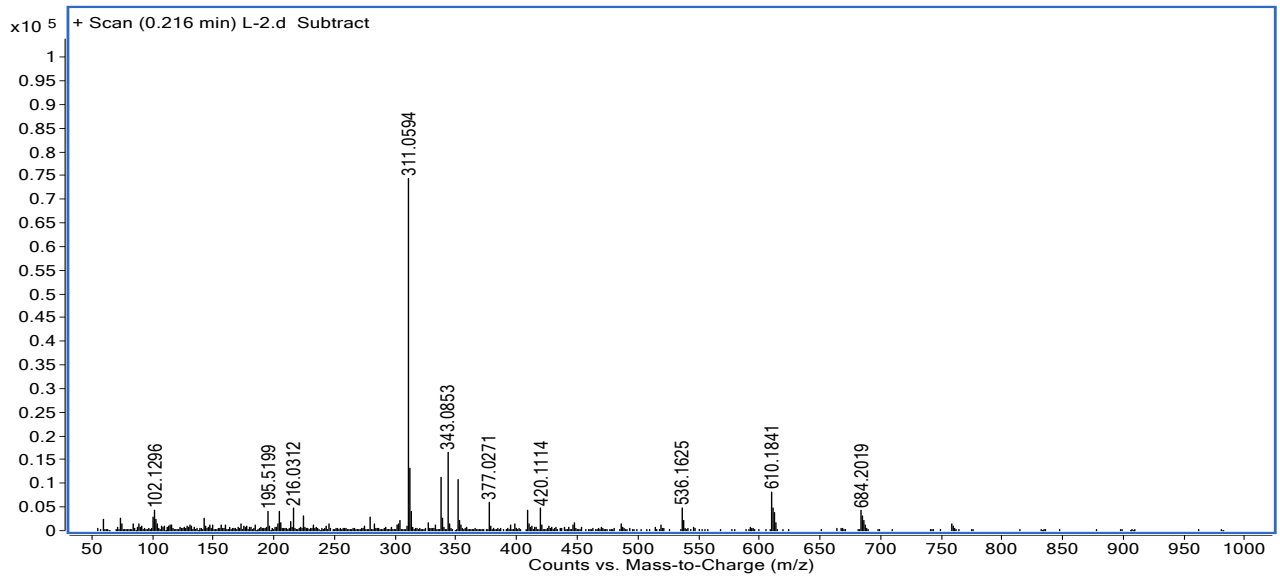
15.204
min

Intermediate 2

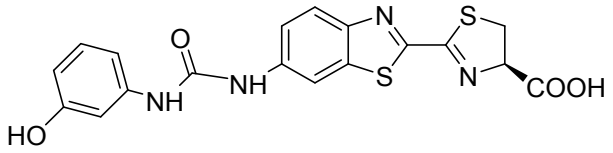


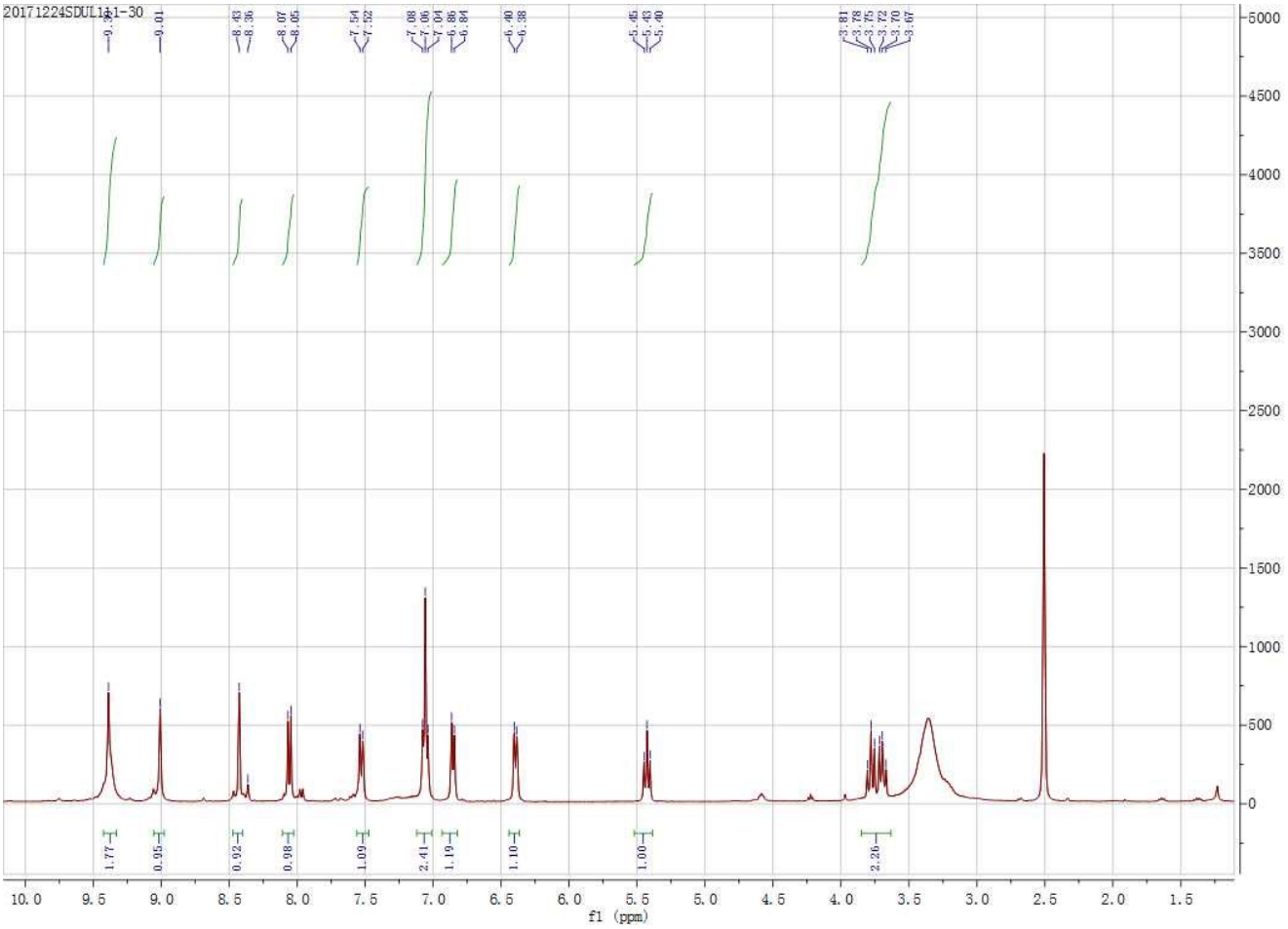


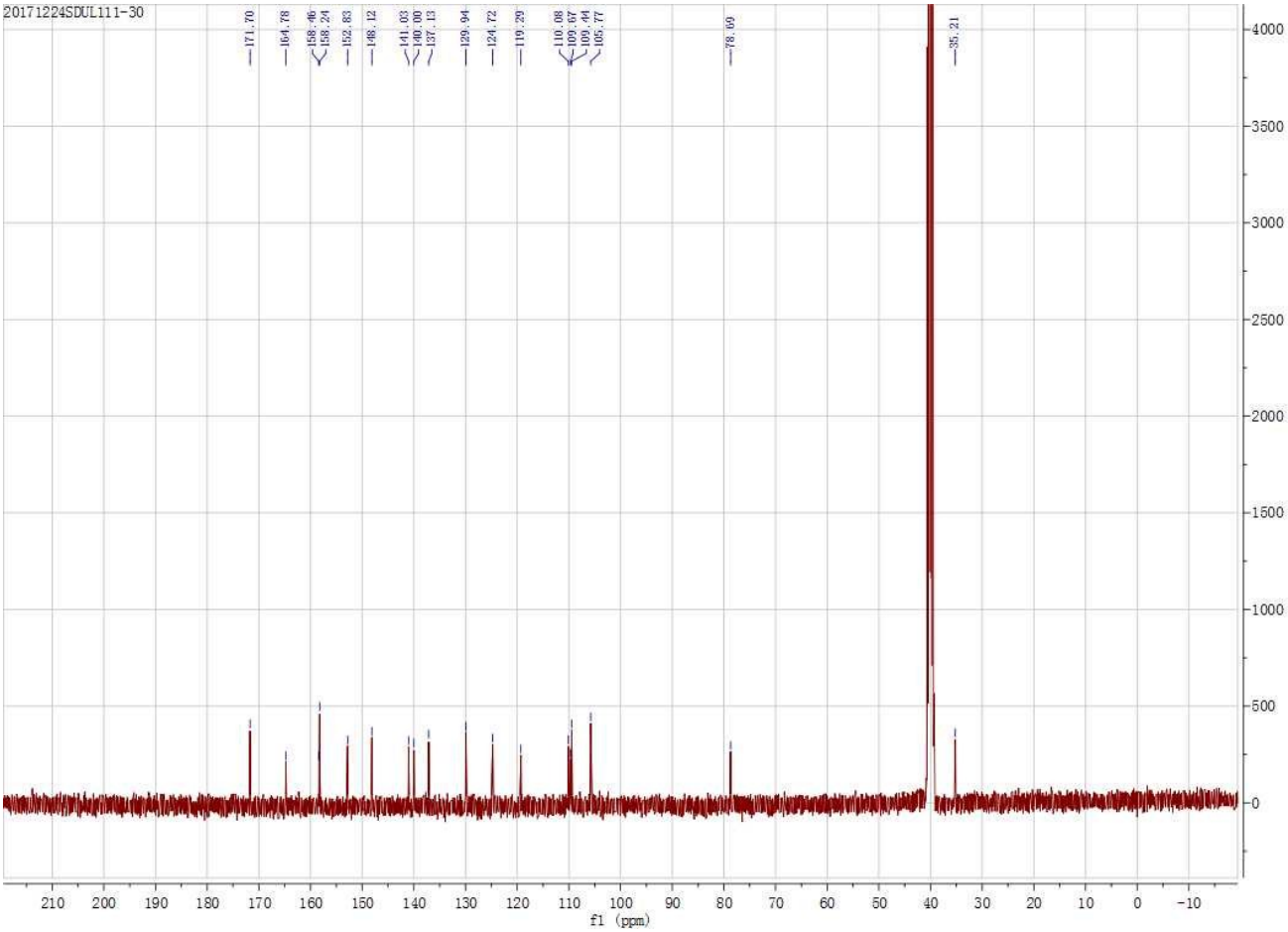

20171217sdu1111-24
158.26
152.61
147.22
141.13
140.80
137.53
134.25
130.06
125.30
120.51
114.20
109.91
109.55
105.89
f1 (ppm)


+ Scan (0.216 min) L-2.d Subtract
x10 5
311.0594
343.0853
102.1296
195.5199
216.0312
377.0271
420.1114
536.1625
610.1841
684.2019
Counts vs. Mass-to-Charge (m/z)

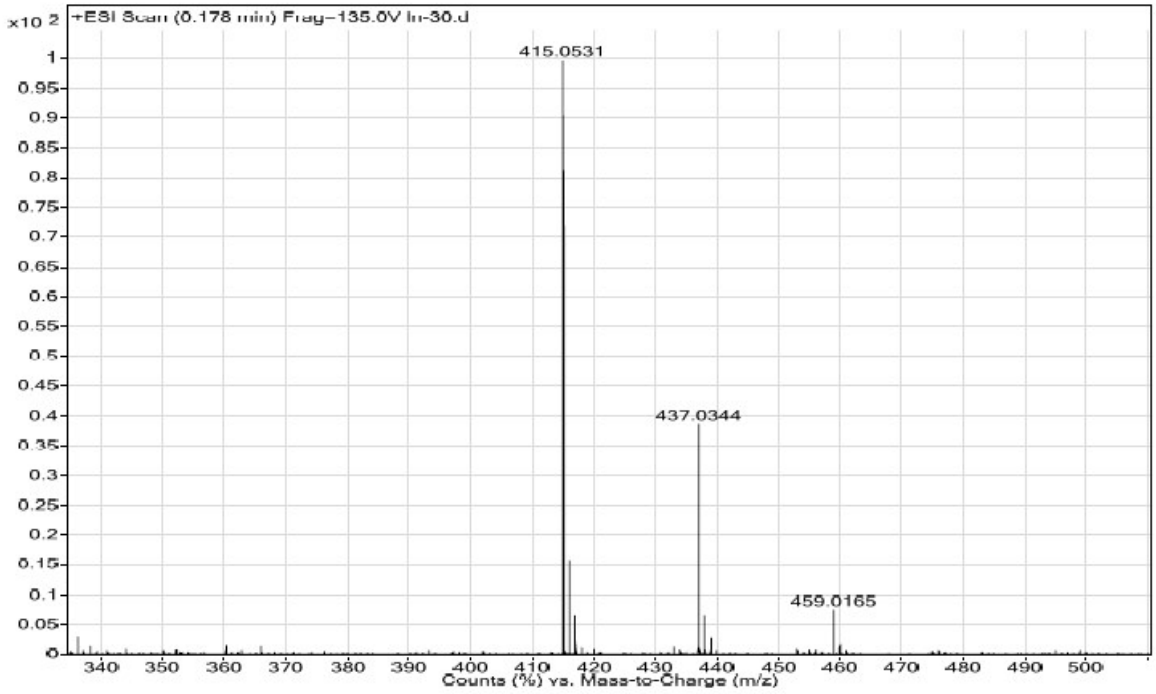
TyrBP-1

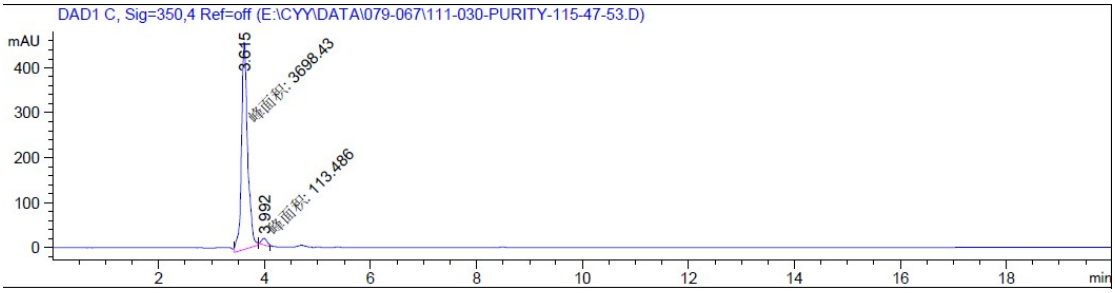

O
NH
NH
N
S
S
N
COOH
HO


20171224SDUL1a1-30
1.77
0.95
0.92
0.98
1.09
2.41
1.19
1.10
1.00
2.26
f1 (ppm)

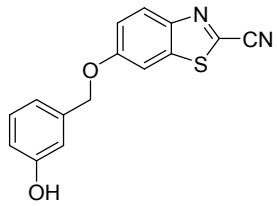


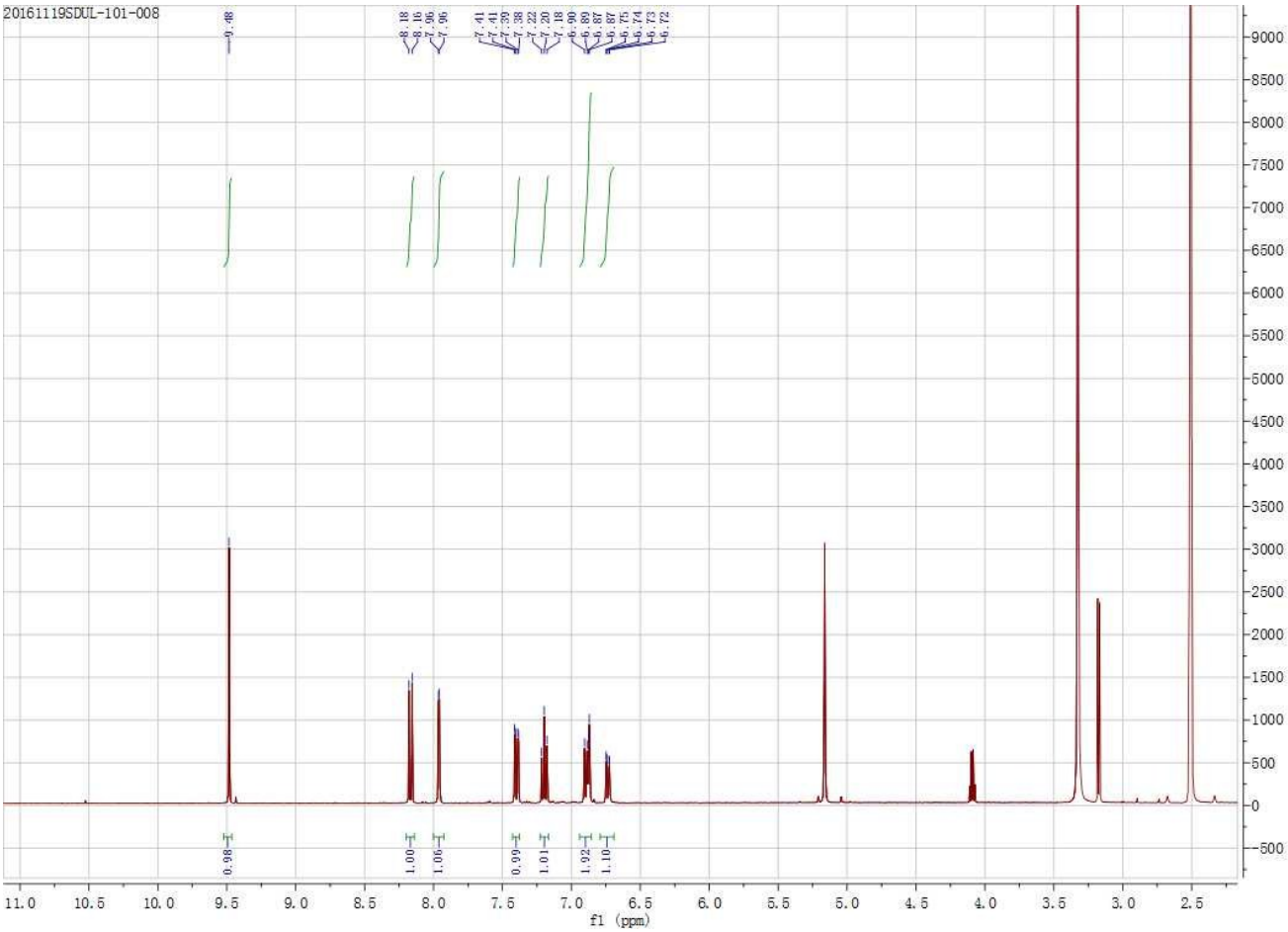
Sample Name	In-30	**Position**	P1-D4	**Instrument Name**	Instrument 1	**User Name**	
Inj Vol	-1	**InjPosition**		**SampleType**	Sample	**IRM Calibration Status**	Success
Data Filename	In-30.d	**ACQ Method**	0103.m	**Comment**		**Acquired Time**	3/29/2018 8:11:11 AM

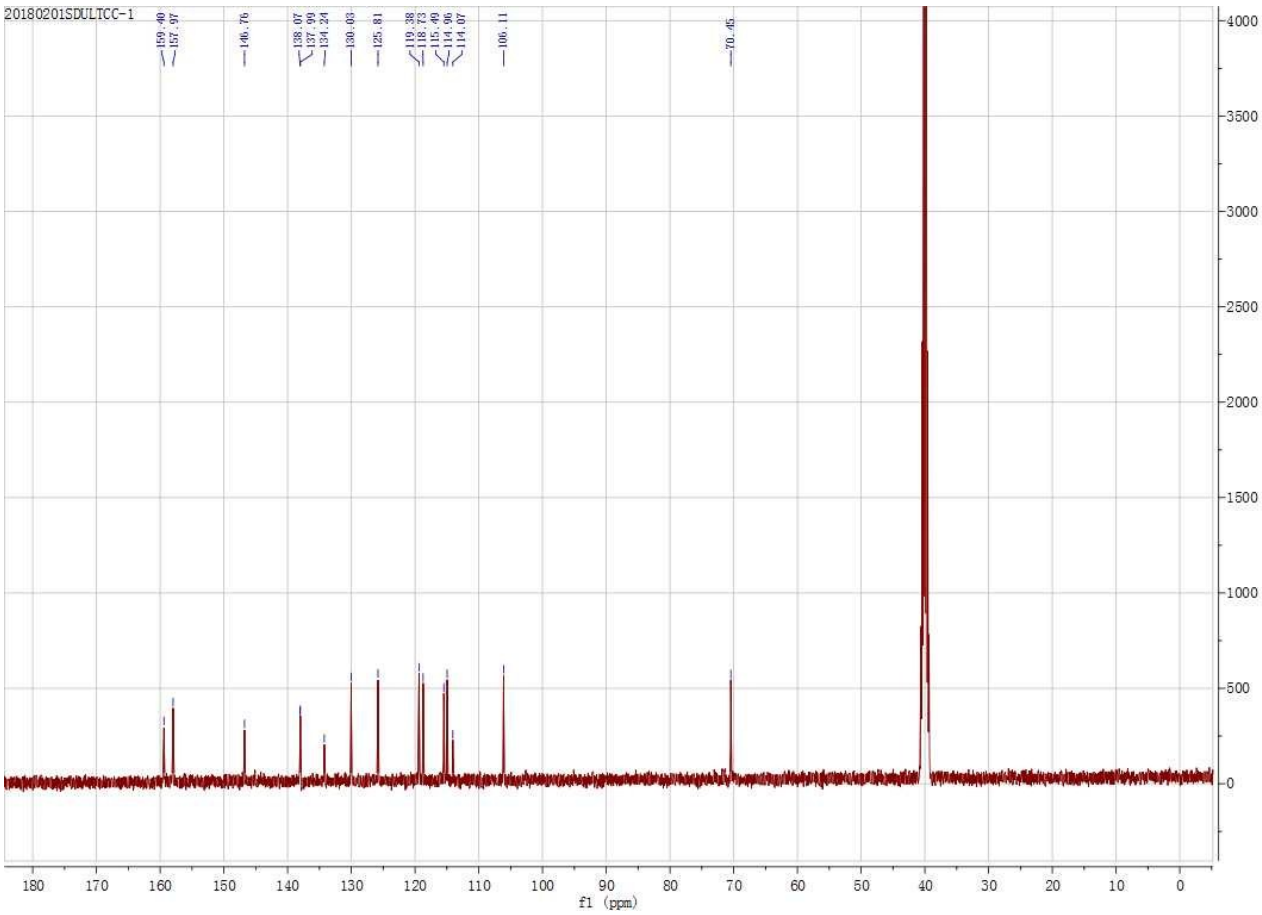


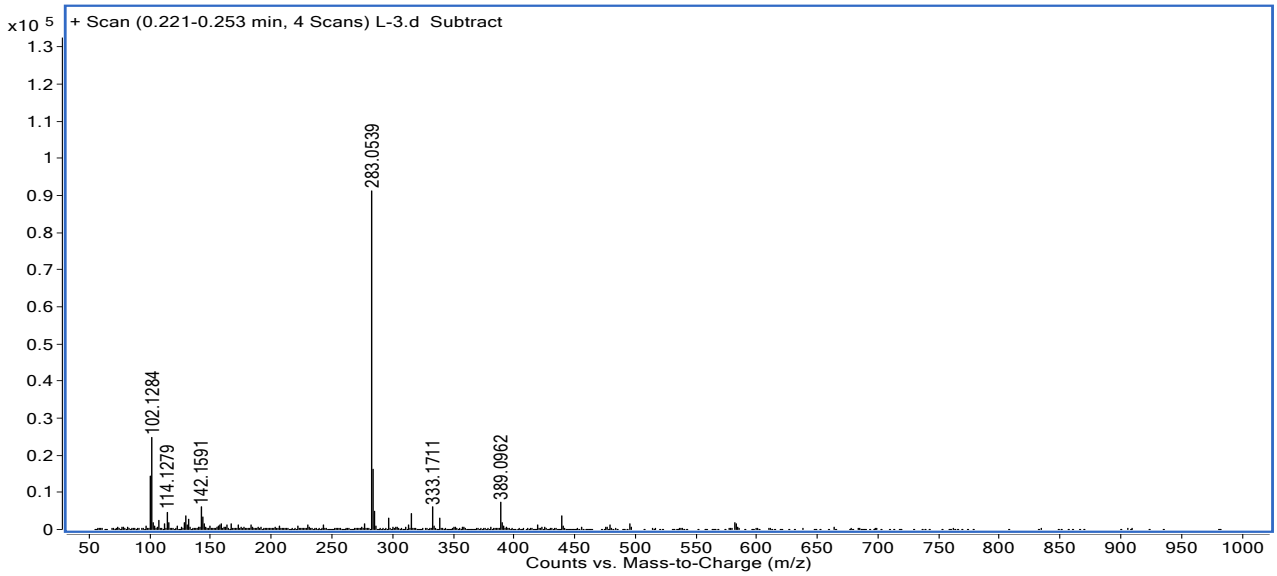


Intermediate 3

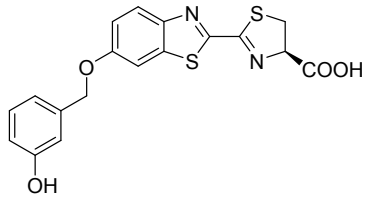


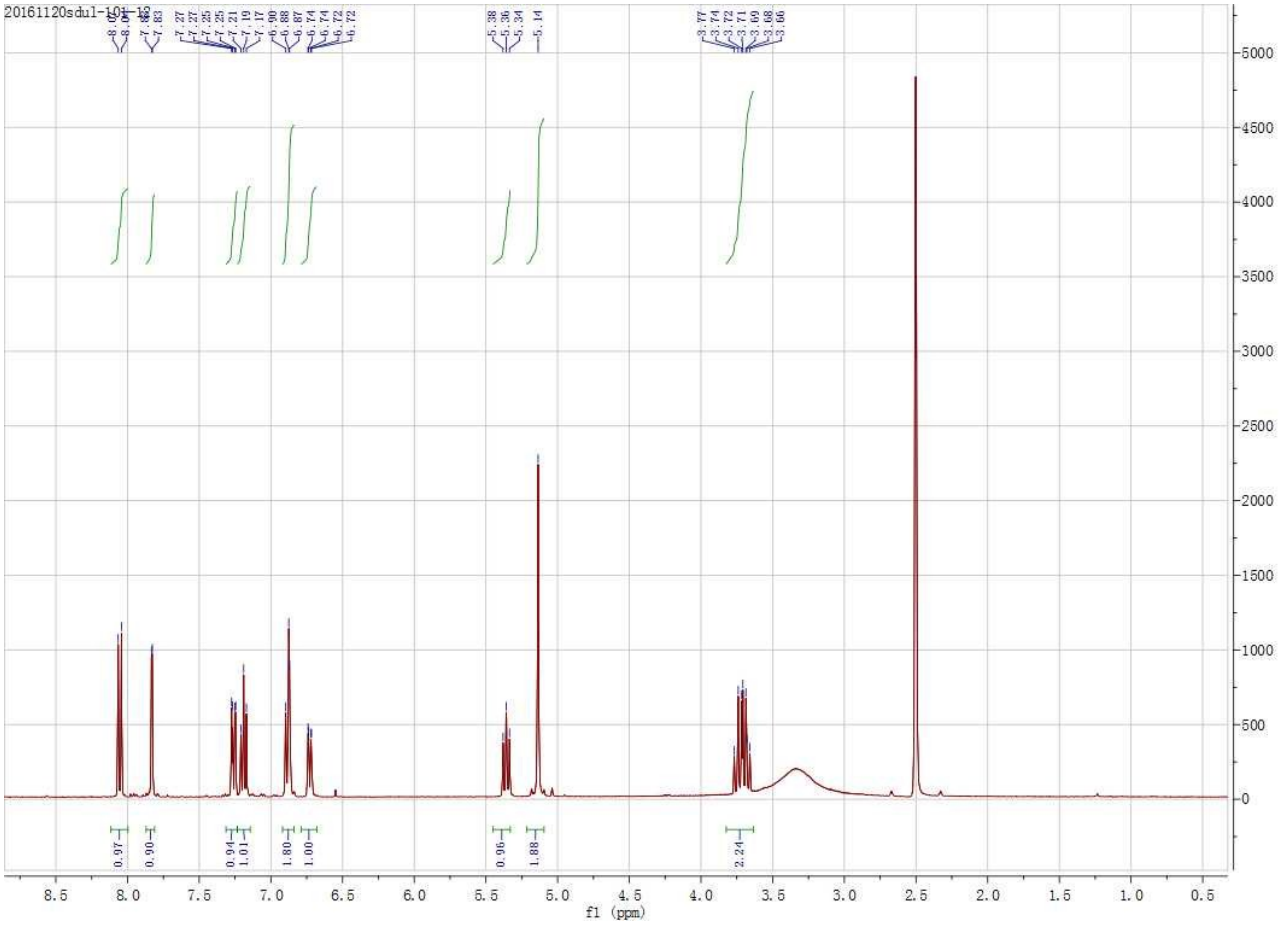


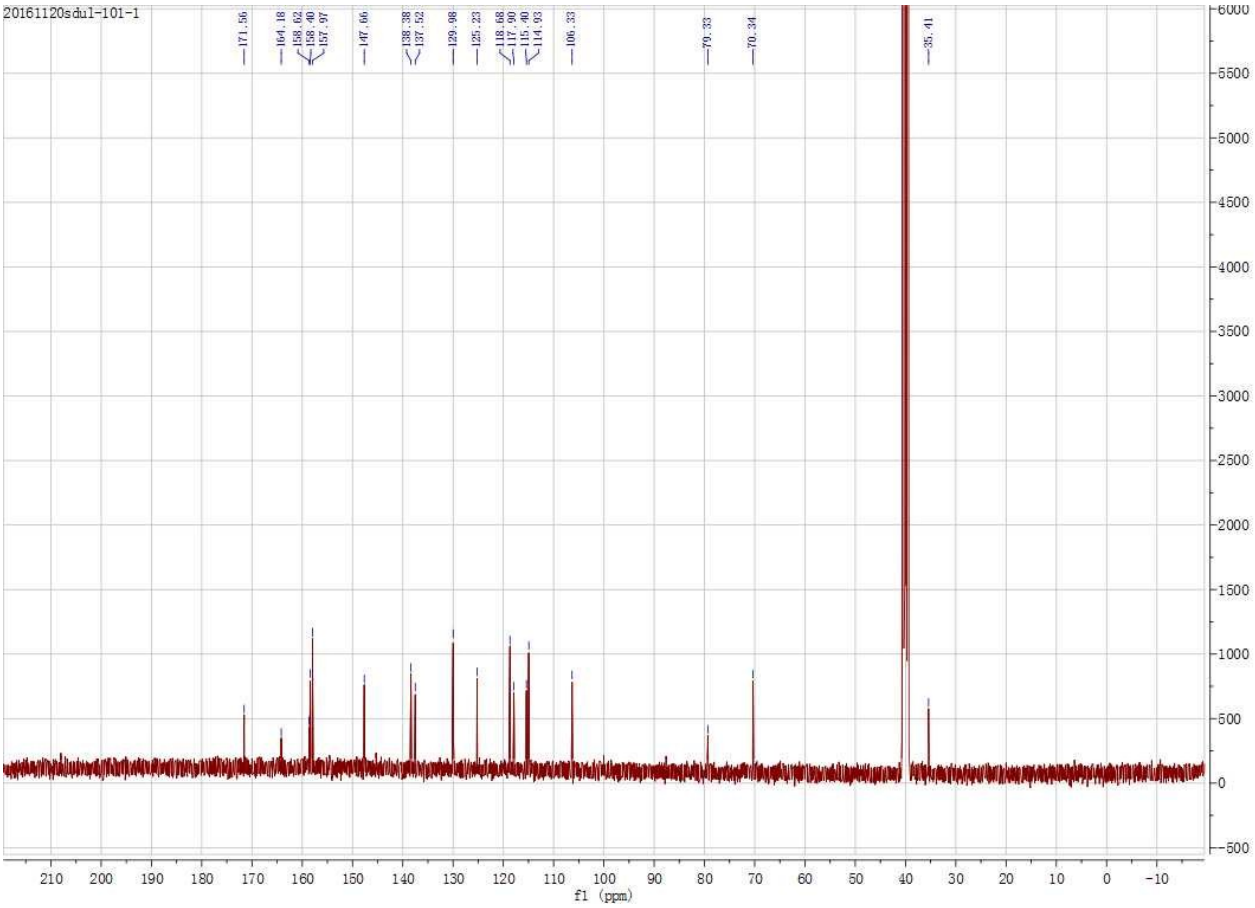

20180201SDULTCC-1
159.40
157.97
146.76
138.07
137.99
134.24
130.03
125.81
119.38
118.73
115.49
114.96
114.07
106.11
70.45
f1 (ppm)

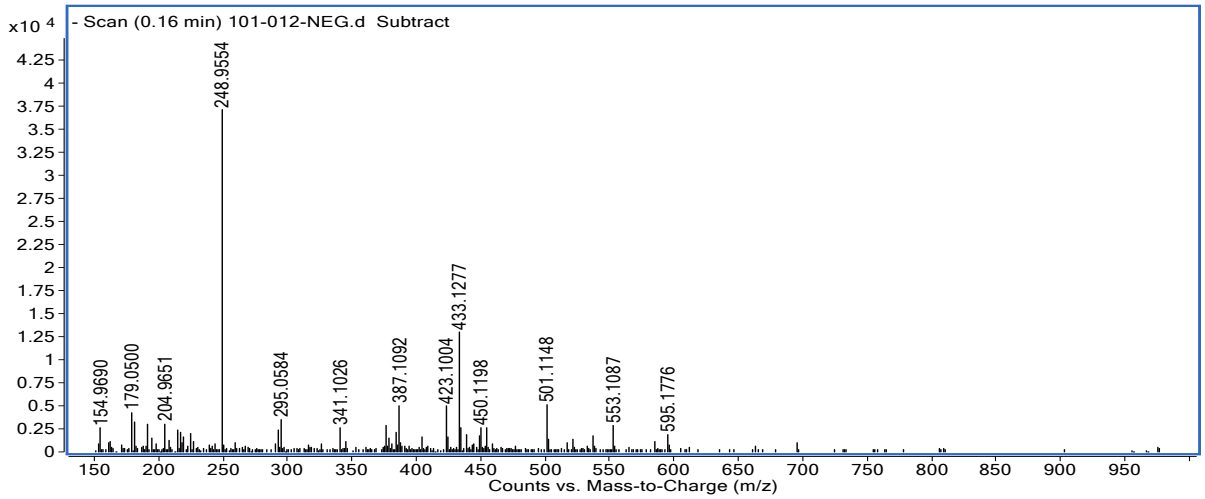

+ Scan (0.221-0.253 min, 4 Scans) L-3.d Subtract
x10 5
283.0539
102.1284
114.1279
142.1591
333.1711
389.0962
Counts vs. Mass-to-Charge (m/z)

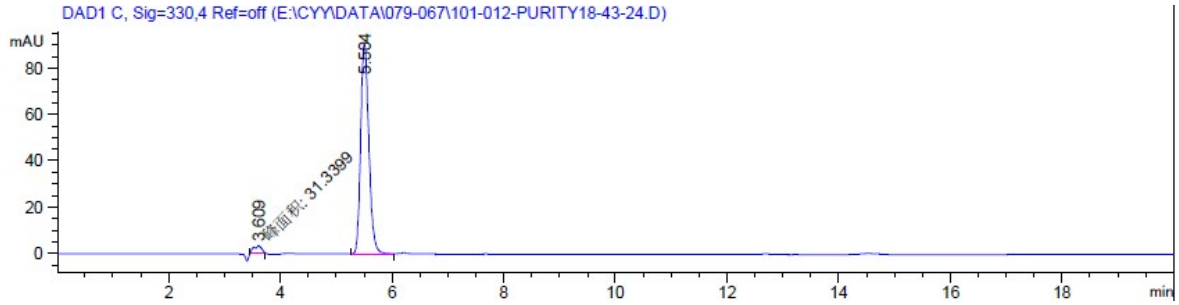
TyrBP-2

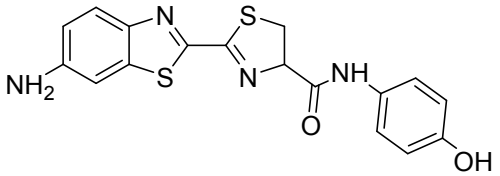


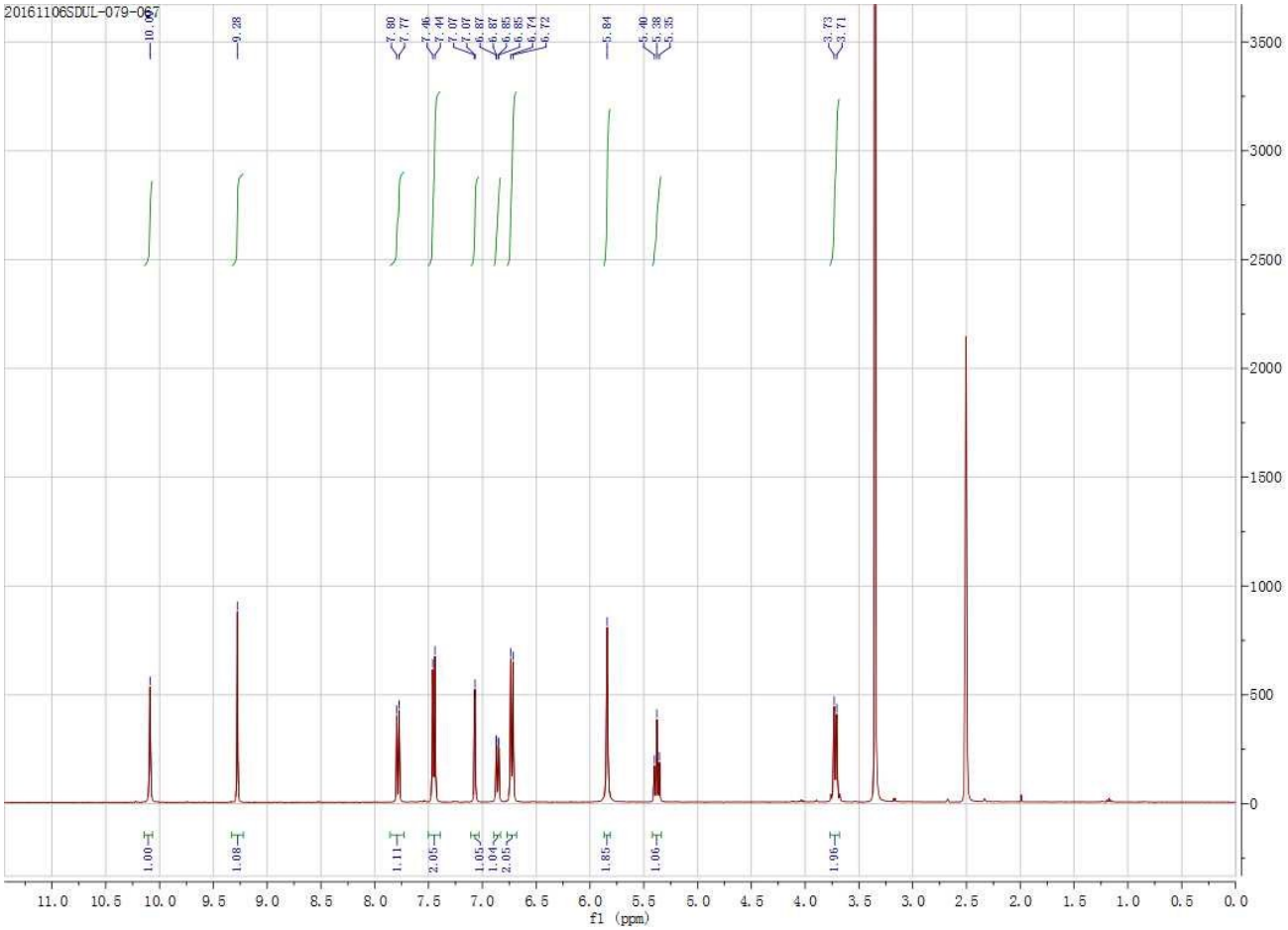


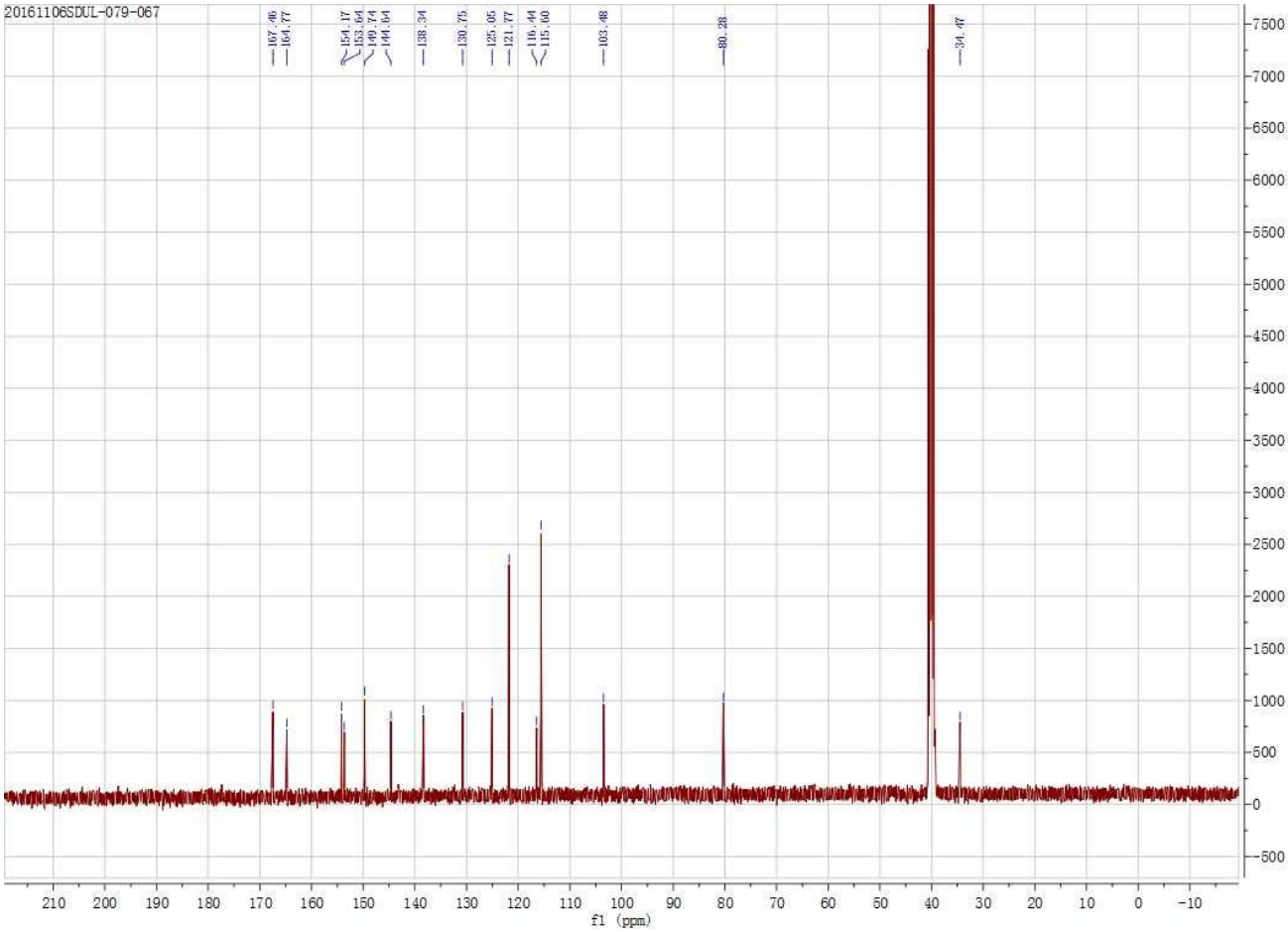

20161120sdu1-101-1
171.56
164.18
158.62
158.40
157.97
147.66
138.38
137.52
129.98
125.23
118.68
117.90
115.40
114.93
106.33
79.33
70.34
35.41
f1 (ppm)

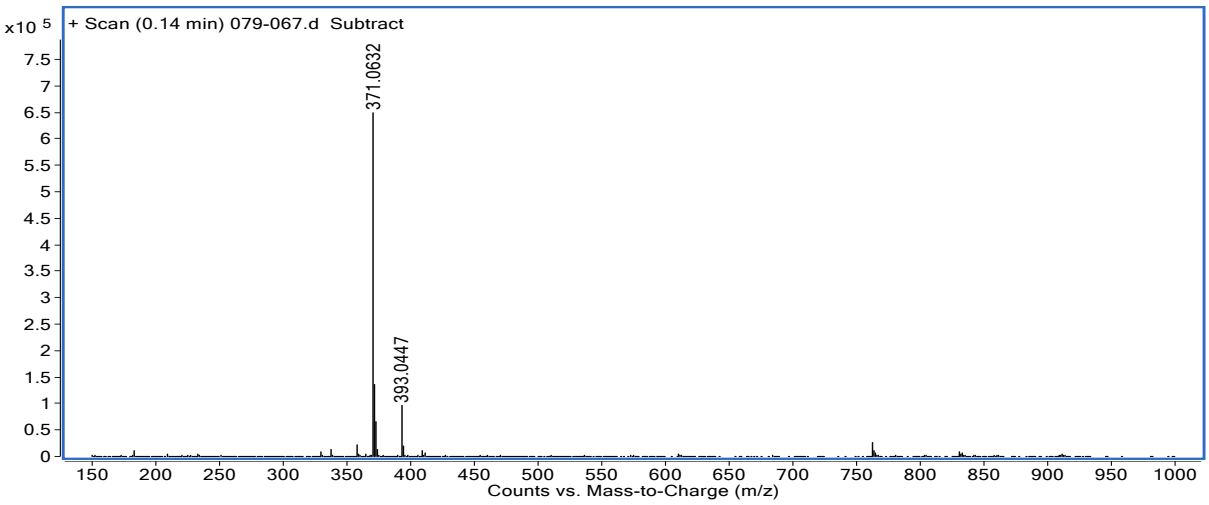

x10 4
- Scan (0.16 min) 101-012-NEG.d Subtract
248.9554
154.9690
179.0500
204.9651
295.0584
341.1026
387.1092
423.1004
433.1277
450.1198
501.1148
553.1087
595.1776
Counts vs. Mass-to-Charge (m/z)

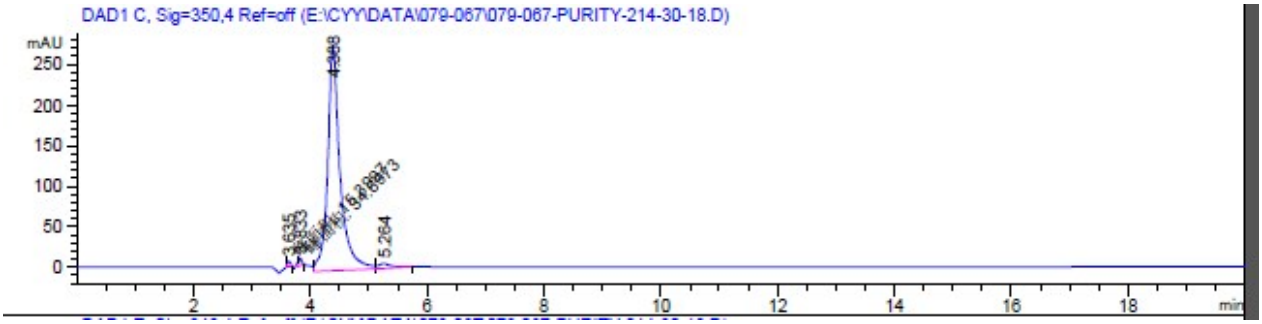

DAD1 C, Sig=330,4 Ref=off (E:\CYY\DATA\079-067\101-012-PURITY18-43-24.D)
mAU
5.504
3.609
峰面积: 31.3399
min

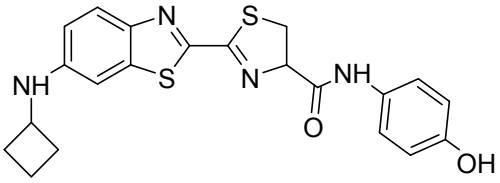

N
S
NH2
S
N
NH
O
OH

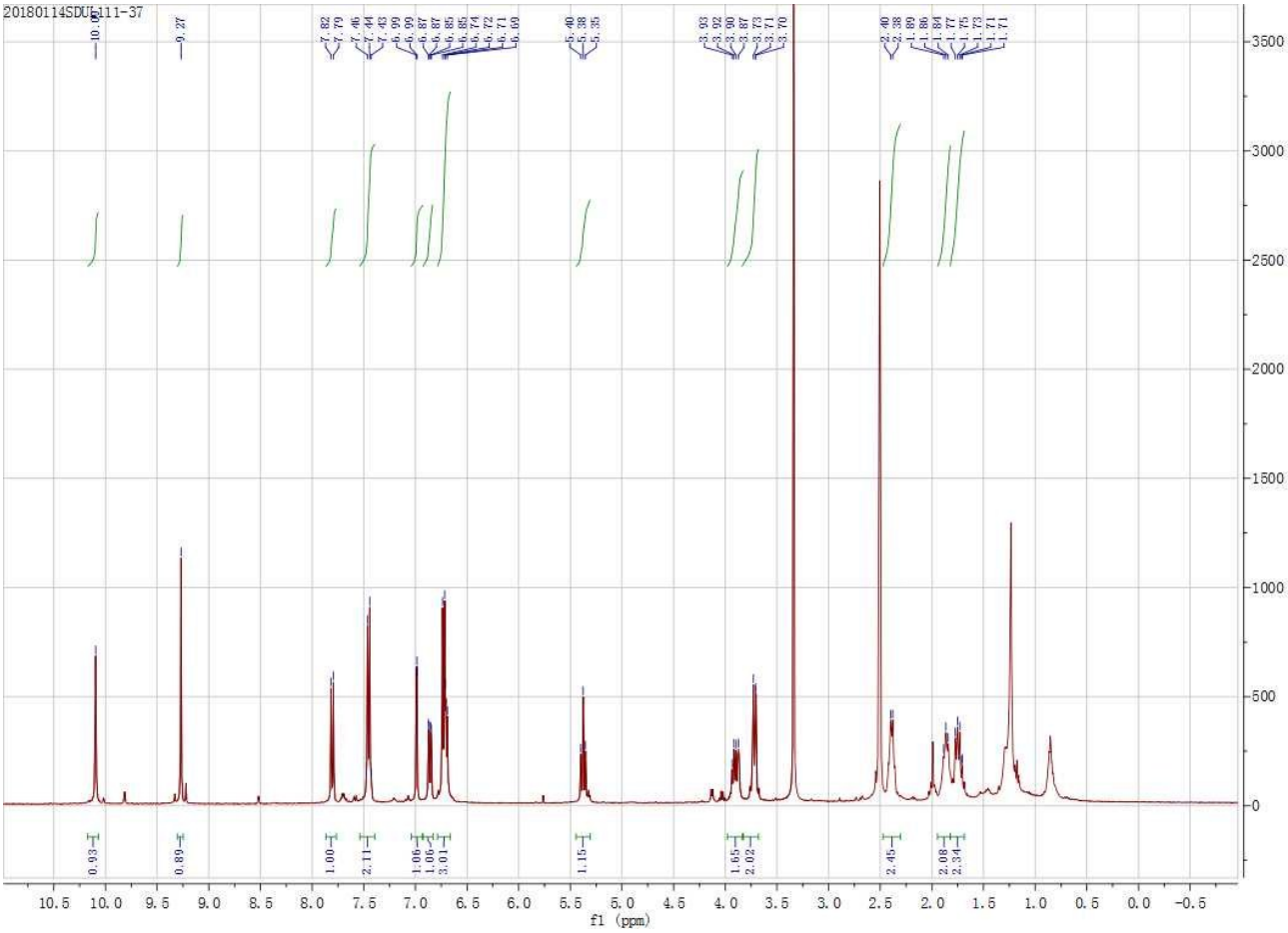

20161106SDUL-079-067
10.067
9.28
7.80
7.77
7.46
7.44
7.07
7.07
6.87
6.87
6.85
6.85
6.74
6.72
5.84
5.40
5.38
5.35
3.73
3.71
1.00
1.08
1.11
2.05
1.05
1.04
2.05
1.85
1.06
1.96
3500
3000
2500
2000
1500
1000
500
0
11.0 10.5 10.0 9.5 9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0.0
f1 (ppm)

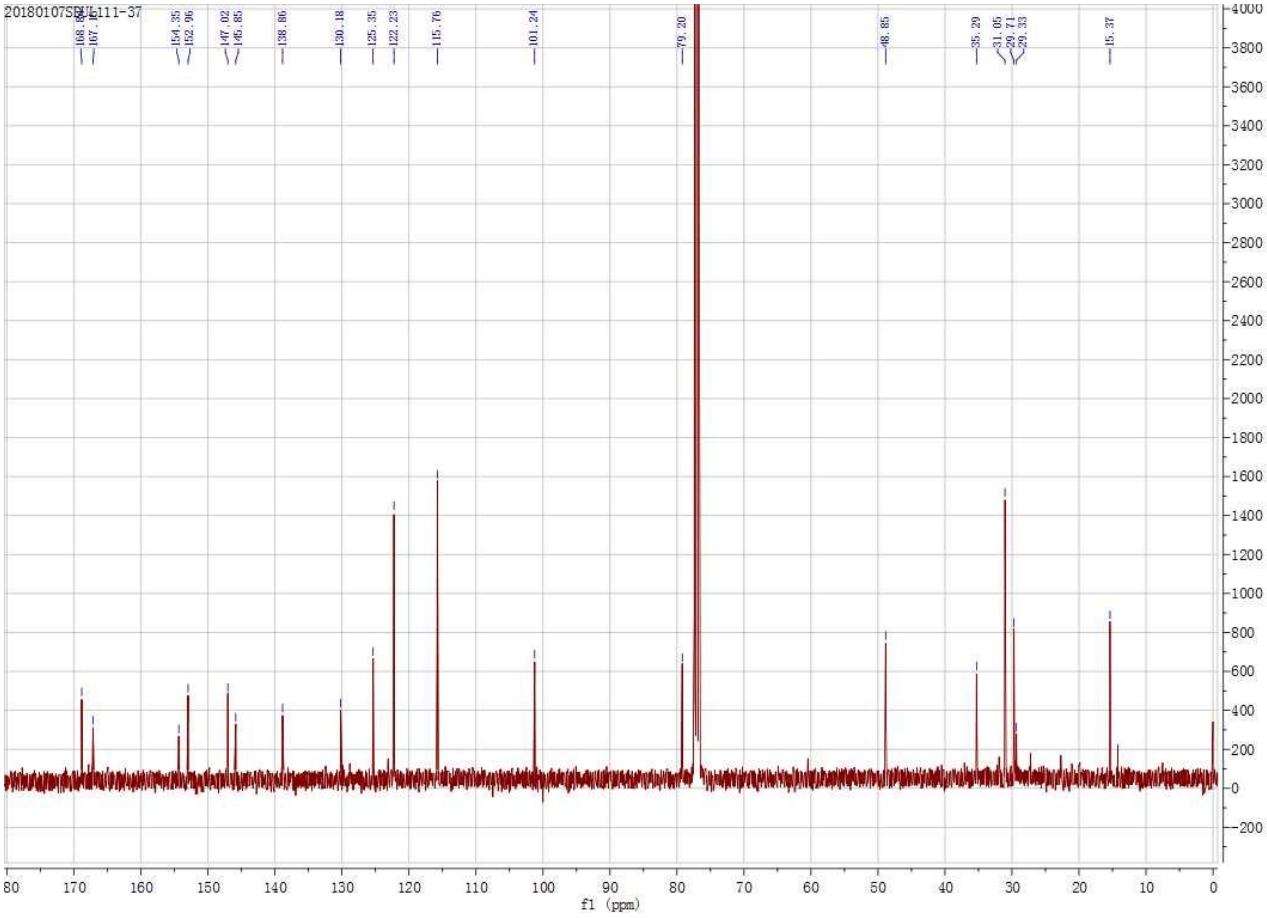

20161106SDUL-079-067
167.46
164.77
154.17
153.64
149.74
144.64
138.34
130.75
125.05
121.77
116.44
115.60
103.48
80.28
34.47
f1 (ppm)

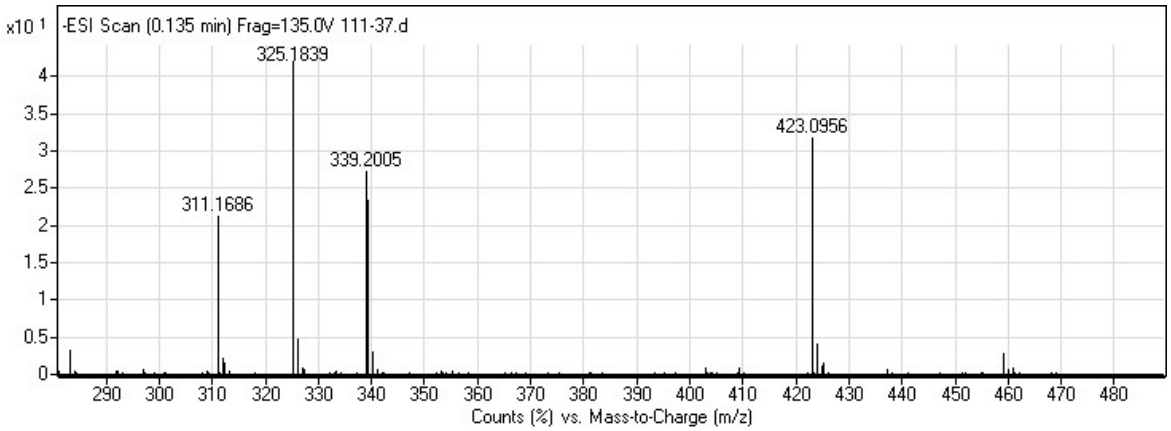

x10 5
+ Scan (0.14 min) 079-067.d Subtract
371.0632
393.0447
Counts vs. Mass-to-Charge (m/z)

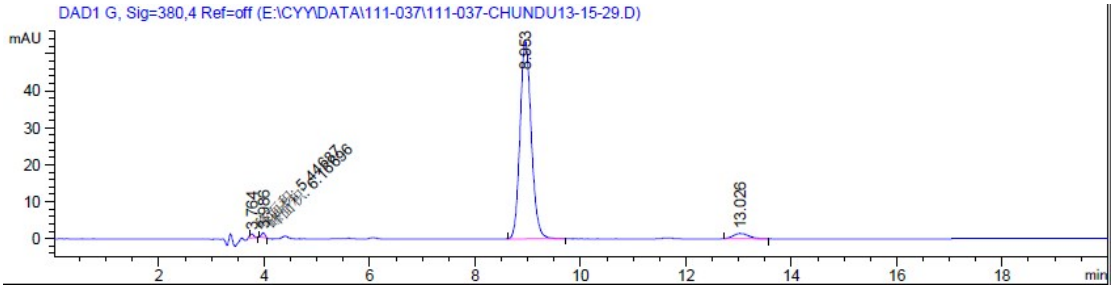

DAD1 C, Sig=350,4 Ref=off (E:\CYY\DATA\079-067\079-067-PURITY-214-30-18.D)
mAU
5.264
min


N
S
S
N
NH
NH
O
OH


20180114SDUL111-37
10.08
9.27
7.82
7.79
7.46
7.44
7.43
6.99
6.99
6.87
6.87
6.85
6.85
6.74
5.72
5.71
5.60
5.40
5.38
5.35
3.93
3.92
3.90
3.87
3.73
3.71
3.70
2.40
2.38
1.89
1.86
1.84
1.77
1.75
1.73
1.71
1.71
0.93
0.89
1.00
2.11
1.06
1.06
3.01
1.15
1.65
2.02
2.45
2.08
2.34
3500
3000
2500
2000
1500
1000
500
0
10.5
10.0
9.5
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
-0.5
f1 (ppm)







1. X. Wu, L. Li, W. Shi, Q. Gong and H. Ma, *Angew Chem Int Ed Engl*, 2016, 55, 14728-14732.
2. W. Wu, J. Su, C. Tang, H. Bai, Z. Ma, T. Zhang, Z. Yuan, Z. Li, W. Zhou, H. Zhang, Z. Liu, Y. Wang, Y. Zhou, L. Du, L. Gu and M. Li, *Anal Chem*, 2017, 89, 4808-4816.