Electronic Supplementary Material (ESI) for Medicinal Chemistry Communications

Supplementary information

Discovery of selective 2,4-diaminopyrimidine-based photoaffinity probes for glyoxalase I

Yiqing Zhou,[a] Tianlin Guo,[a] Xitao Li,[a] Yi Dong,[a] Paul Galatsis,[b] Douglas S. Johnson[b] and Zhengying Pan*[a]

[a] Key Laboratory of Chemical Genomics, School of Chemical Biology and Biotechnology, Peking University, Xili University Town, PKU Campus, Shenzhen, China, 518055. 86-755-26033072; panzy@pkusz.edu.cn.
[b] Neuroscience Medicinal Chemistry and Chemical Biology, Pfizer Worldwide Research and Development, Cambridge, MA 02139, USA.

Contents **Page No.**

Chemistry

General Information

All the reagents were purchased commercially and used without further purification. Anhydrous DMF was distilled from calcium hydride. Brine refers to a saturated solution of sodium chloride in distilled water.

Reactions were monitored by thin-layer chromatography (TLC) carried out on 0.2 mm Jiangyou silica gel plates ($HSGF_{254}$) using UV light as visualizing agent . Flash column chromatography was carried out using Puke silica (ZCX-II).

NMR spectra were recorded on a Bruker Advance 400 (^1H: 400 MHz, ^{13}C: 100 MHz) with chemical shift values in ppm relative to TMS (δ_H 0.00 and δ_C 0.0), residual chloroform (δ_H 7.26 and δ_C 77.16), dimethylsulfoxide (δ_H 2.50 and δ_C 39.52), or methanol (δ_H 3.31 and δ_C 49.00) as standard. HR-MS were obtained using Bruker Apex IV RTMS.

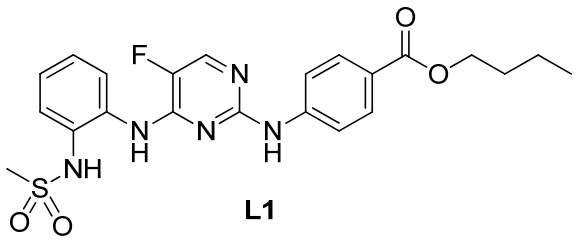
Purity of compounds was determined by HPLC chromatograms acquired on an Agilent 1200 LC. Analysis were conducted by an Agilent PN959990-902 Eclipse Plus C18 250 mm × 4.6 mm column, using a water–MeCN gradient containing 0.1% formic acid (FA) with MeCN from 30% to 98% in 10min. Detection was at 254 nm, and the average peak area was used to determine purity. All the compounds were determined to be >95% pure.

All the compounds can be synthesized from commercially available compounds para-diaminobenzene, 2,6-chlorol,3-fluorolpyrimidine and diaminobenzophenone.

Compound 1 was synthesized by two steps from ortho-diaminobenzene and 2,6-chlorol,3-fluorolpyrimidine. L1 was synthesized by refluxing para-aminobenzoic acid and Compound 1 under acidic condition in n-BuOH. L1-Bpyne was obtained from Buchwald-Hartwig amination under catalysis of $Pd_3(dba)_2$ and ligand DPBP. L1-$BpNH_2$ was obtained by S_NAr of Compound 1 with diaminobenzophenone. L1-biotin was obtained by amide bond formation between L1-$BpNH_2$ and biotin.

Experimental Procedures and Spectroscopic Data

L1

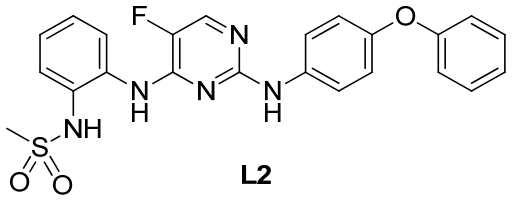


L1

Compound 1 (1.58 g, 5 mmol, 1 eq) and p-aminobenzoic acid (1.37 g, 10 mmol, 2 eq) were suspended in ^{n}BuOH (47 ml) and 2N HCl (6.25 ml). The mixture was heated at 120 ºC for 12 hours, and then cooled to rt. The product was collected by filtration and washed with ^{n}BuOH(10 ml×2) and Et_2O (10 ml×2), as a light brown solid (1.59 g, 76%). ^{1}H-NMR (400 MHz, DMSO-d_6) δ H 0.91 (3H, t), 1.40 (2H, tq), 1.65 (2H, tt),

2.92 (3H, s), 4.23 (2H, t), 7.22-7.27 (3H, m), 7.45-7.50 (2H, m), 7.73-7.76 (1H, m), 7.84 (1H, d), 8.04 (1H, s), 8.23(1H, d), 9.24(1H, s), 9.27(1H, s), 9.89(1H, s). ^{13}C-NMR (100 MHz, DMSO-d_6) δ C 166.17, 154.11, 152.15, 152.04, 142.48, 140.59, 140.03, 131.87, 131.68, 130.66, 129.14, 127.21, 126.74, 126.54, 125.60, 123.91, 122.77, 120.34, 64.77, 30.67, 19.16, 14.01. HRMS-ESI calcd. for $C_{22}H_{24}N_5O_4FNaSCl$ [M+H$^+$]: 496.1431; Found: 496.1423.

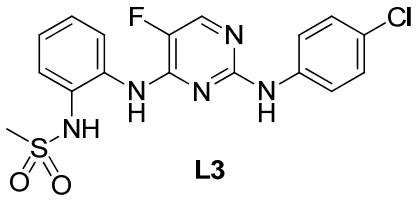
L2:



L2

Compound 1 (100 mg, 0.16 mmol, 1 eq) and p-Chlorobenzenamine (119 mg, 0.32 mmol, 2 eq) were suspended in ^{n}BuOH (2 ml) and 2N HCl (0.2 ml). The mixture was heated at 120 ºC for 12 hours, then cooled to rt. The product was collected by filtration and washed with ^{n}BuOH (2 ml×2) and Et_2O (2 ml×2), as a light brown solid (130 mg, 87%). ^{1}H-NMR (400 MHz, DMSO-d_6) δ H 2.91 (3H, s), 6.81 (2H, d), 6.91 (2H, d), 7.10 (1H, t), 7.23-7.38 (6H, m), 7.50(2H,t), 8.36 (1H, d), 9.38(1H, s), 10.46(1H, s), 10.64(1H, s). ^{13}C-NMR (100 MHz, DMSO-d_6) δ C 157.56, 154.84, 154.71, 152.77, 150.74, 141.19, 138.73, 133.75, 133.48, 130.43, 129.30, 128.91, 128.44, 125.82, 124.08, 123.59, 122.81, 119.67, 118.31. HRMS-ESI calcd. for $C_{23}H_{21}N_5O_3FSCl$ [M+H$^+$]: 466.1349; Found: 466.1341.

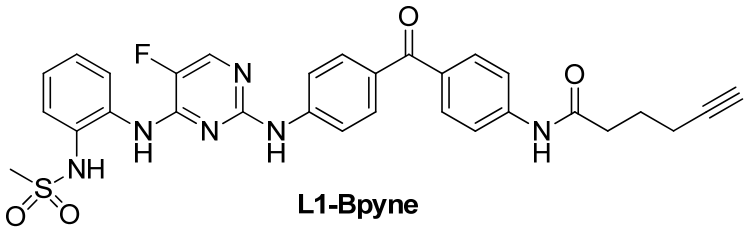
L3



L3

Compound 1 (50 mg, 0.16 mmol, 1 eq) and *p*-Chlorobenzenamine(41 mg, 0.32 mmol, 2 eq) were suspended in ^{n}BuOH (2 ml) and 2 N HCl (0.2 ml). The mixture was heated at 120 ºC for 12 h, then cooled to room temperature. The product was collected by filtration and washed with ^{n}BuOH (2 ml×2) and Et_2O (2 ml×2), as a light brown solid (47 mg, 72%). ^{1}H-NMR (400MHz, DMSO-d_6) δ H 2.92 (3H, s), 7.14 (2H, d), 7.31 (3H, d), 7.42 (1H, t), 7.50 (1H, d), 7.60 (1H, d), 8.40 (1H, d), 9.41 (1H, s). ^{13}C-NMR (100 MHz, DMSO-d_6) δ C 154.88, 154.75, 150.43, 141.27, 138.81, 136.96, 133.82, 129.31, 128.97, 128.82, 128.64, 127.72, 125.92, 124.25, 121.79. HRMS-ESI calcd. for $C_{17}H_{16}N_5O_2FSCl$ [M+H$^+$]: 408.0697; Found: 408.0691.

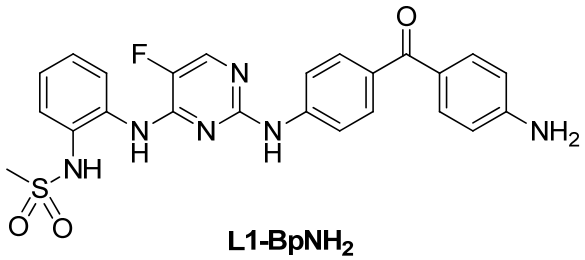
L1-Bpyne



L1-Bpyne

A mixture of Compound 1 (100 mg, 0.32 mmol, 1 eq), Compound 2 (108 mg, 0.35 mmol, 1.1 eq), $Pd_2(dba)_3$(20 mg, 9%), DPBP (32 mg, 9%), K_2CO_3 (200 mg, 1.28 mmol, 4 eq) were dissolved in 3 ml ^{t}BuOH and degassed. The mixture was refluxed under N_2 at 100 ºC for 4 h. Then the reaction was stopped and filtered on celite, washed with MeOH, concentrated and purified by flash column chromatography, providing L1-BpNH_2 as a white solid (56 mg, 29.83%). ^{1}H-NMR (400 MHz, DMSO-d_6) δ H 1.77 (3H, t), 2.24 (2H, dt), 2.46 (2H), 2.82 (1H, t), 2.92 (3H, s), 3.81 (1H, s), 7.25-7.34 (2H, m), 7.46-7.54 (3H, m), 7.64-7.77 (8H, m), 8.18(1H, d), 8.81 (1H, s), 9.22 (1H, s), 9.68 (1H, s), 10.28 (1H, s). ^{13}C-NMR (100 MHz, DMSO-d_6) δ C 193.51, 171,63, 155.37, 155.34, 151.24, 151.13, 145.45, 143.21, 143.12, 141.16, 140.96, 140.66, 132.58, 132.56, 131.83, 131.16, 129.63, 127.57, 126.59, 126.46, 125.76, 118.62, 118.47, 84.42, 72.12, 35.66, 24.24, 17.80. HRMS-ESI calcd. for $C_{30}H_{27}N_6O_4FNaSCl$ [M+H$^+$]: 609.1696; Found: 609.1690.

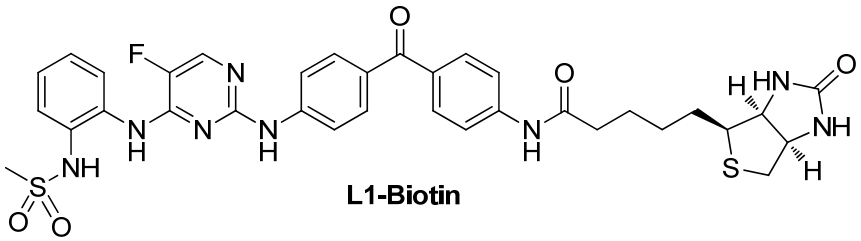
L1-BpNH_2



L1-BpNH_2

Compound 1 (1.00 g, 3.2 mmol, 1 eq) and 4,4'-diaminobenzopheonone (1.36 g, 6.4 mmol, 2 eq) were suspended in ^{n}BuOH (25 ml) and 2 N HCl (3 ml). The mixture was heated at 120 ºC for 12 hours, then cooled to rt. The product was collected by filtration and washed with ^{n}BuOH (10 ml×2) and Et_2O (10 ml×2), providing as a yellow solid (800 mg, 50.95%). ^{1}H-NMR (400 MHz, DMSO-d_6) δ H 2.92 (3H, s), 6.65 (2H, d), 7.31 (2H, dd), 7.42-7.51 (4H, m), 7.60-7.70 (3H, m), 8.24(1H, d), 9.26 (1H, s), 9.31(1H, s), 9.95(1H, s). ^{13}C-NMR (100 MHz, DMSO-d_6) δ C 192.76, 159.07,158.69, 153.89, 152.81, 143.38, 142.59, 132.67, 132.54, 131.90, 131.46, 130.48, 128.06, 127.11, 126.32, 125.48, 125.05, 118.12, 113.49. HRMS-ESI calcd. for $C_{24}H_{21}N_6O_3FNaSCl$ [M+H$^+$]: 515.1278; Found: 515.1275.

L1-Biotin



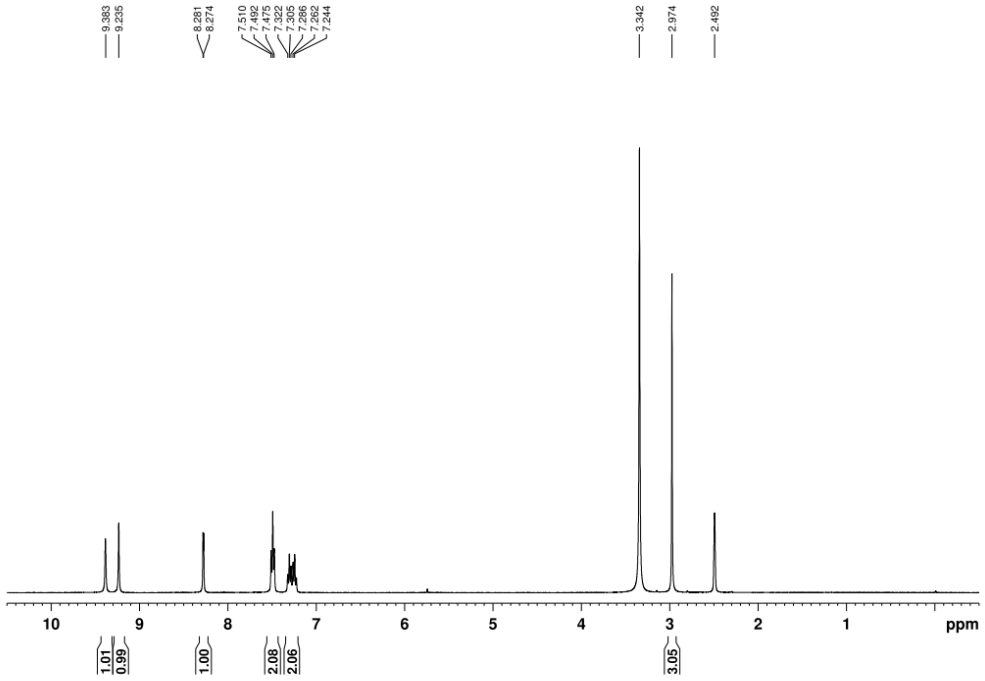
L1-Biotin

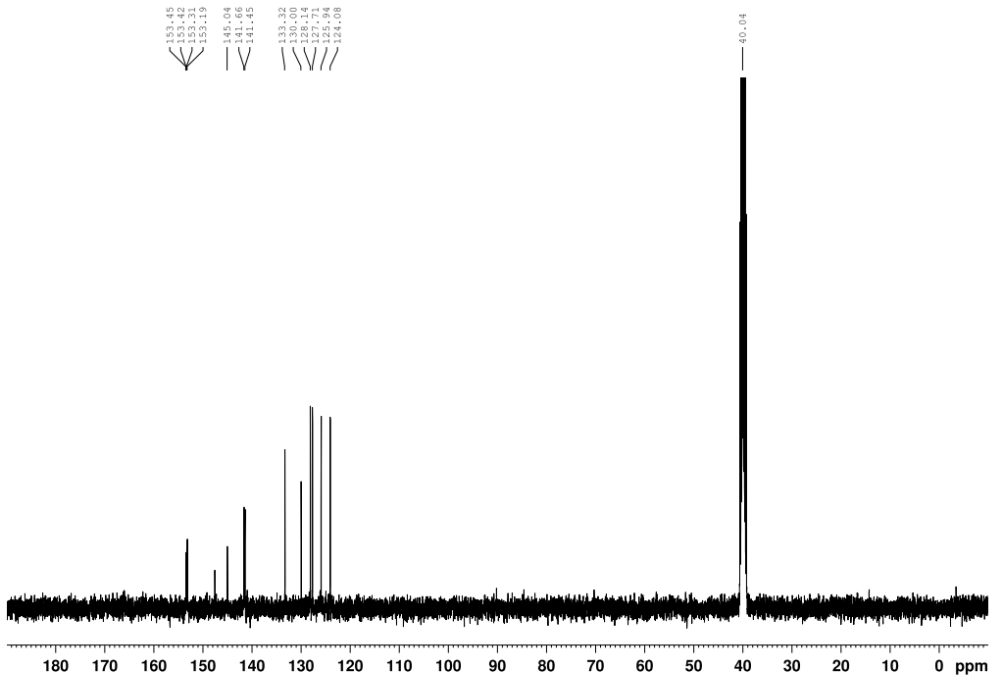
L1-BpNH$_2$ (98 mg, 0.2 mmol, 1 eq) and biotin (118 mg, 0.24 mmol, 1.2 eq) were dissolved in 2 ml DMF and DIPEA (139 µl, 0.8 mmol, 4 eq) was added. Then HATU was added to the mixture at 0 ºC and the mixture was warmed to room temperature and stirred overnight. The reaction was quenched by H_2O and extracted with ethyl acetate. The organic phase was washed by H_2O, brine, dried, concentrated, purified by flash column chromatography, providing L1-Biotin as a white solid (64 mg, 33.33%). ^{1}H-NMR (400 MHz, DMSO-d_6) δ H 1.37-1.68 (7H, m), 2.37 (2H, t), 2.58 (1H, d), 2.82 (1H, dd), 2.92 (3H, s), 3.10-3.17 (2H, m), 3.38-3.39 (1H, m), 4.12-4.15 (1H, m), 4.29-4.32 (1H, m), 6.37 (1H, s), 6.45 (1H, s), 7.29 (2H, dt), 7.46 (1H, dd), 7.53 (2H, d), 7.65-7.78 (7H, m), 8.18 (1H, d), 9.24 (1H, s), 9.69(1H, s), 10.24 (1H, s). ^{13}C-NMR (100 MHz, DMSO-d_6) δ C 193.51, 172.23, 163.19, 155.37, 155.34, 151.23, 151.11, 145.45, 143.29, 13.12, 141.14, 140,94, 140.66, 132.52, 131.92, 131.19, 129.62, 127.53, 126.50, 126.45, 125.69, 118.59, 117.46, 67.46, 63.24, 61.51, 59.66, 55.84, 49.05, 36.79, 28.67, 28.55, 25.57, 25.46. HRMS-ESI calcd. for $C_{34}H_{35}N_8O_5FNaS_2$ [M+Na$^+$]: 741.2054; Found: 741.2048.

^{1}H NMR, ^{13}C NMR and HR-MS Spectra of New Compounds

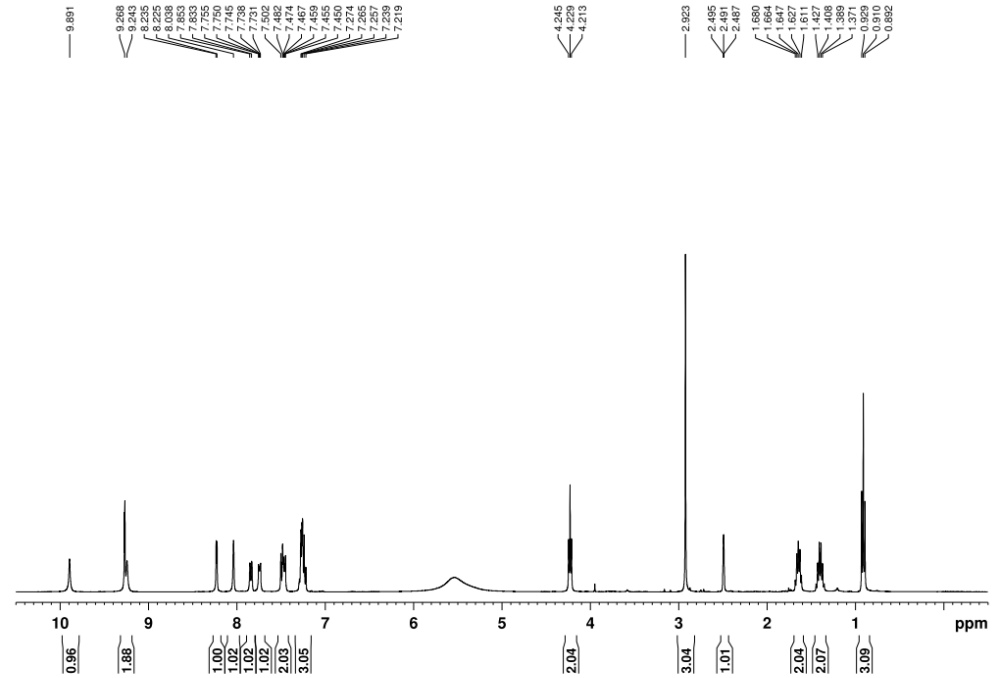
^{1}H NMR and ^{13}C NMR Spectra

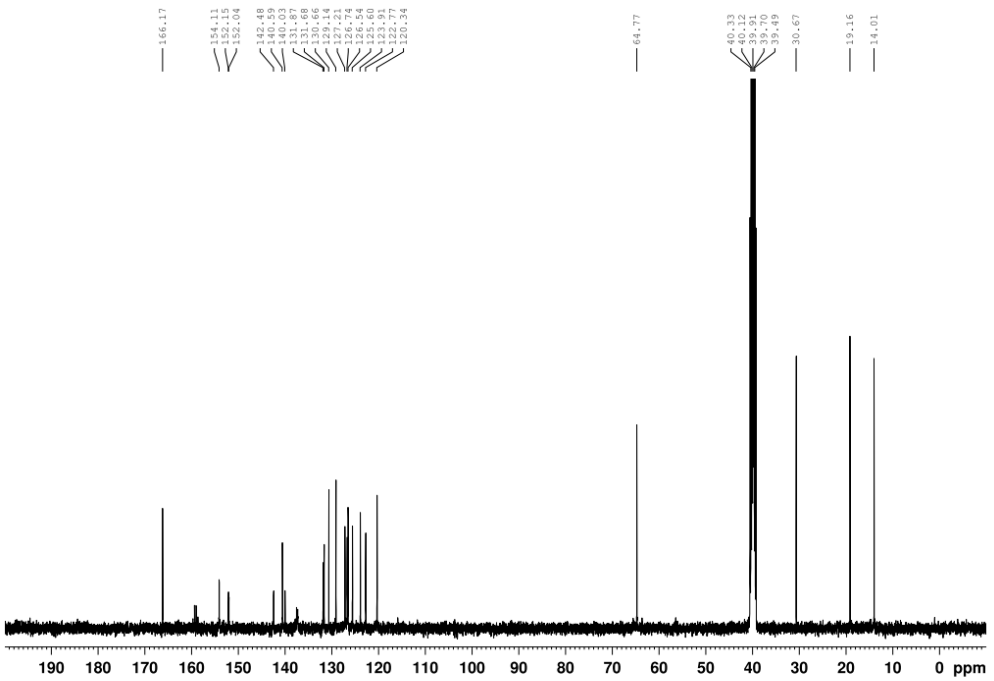
Compound 1



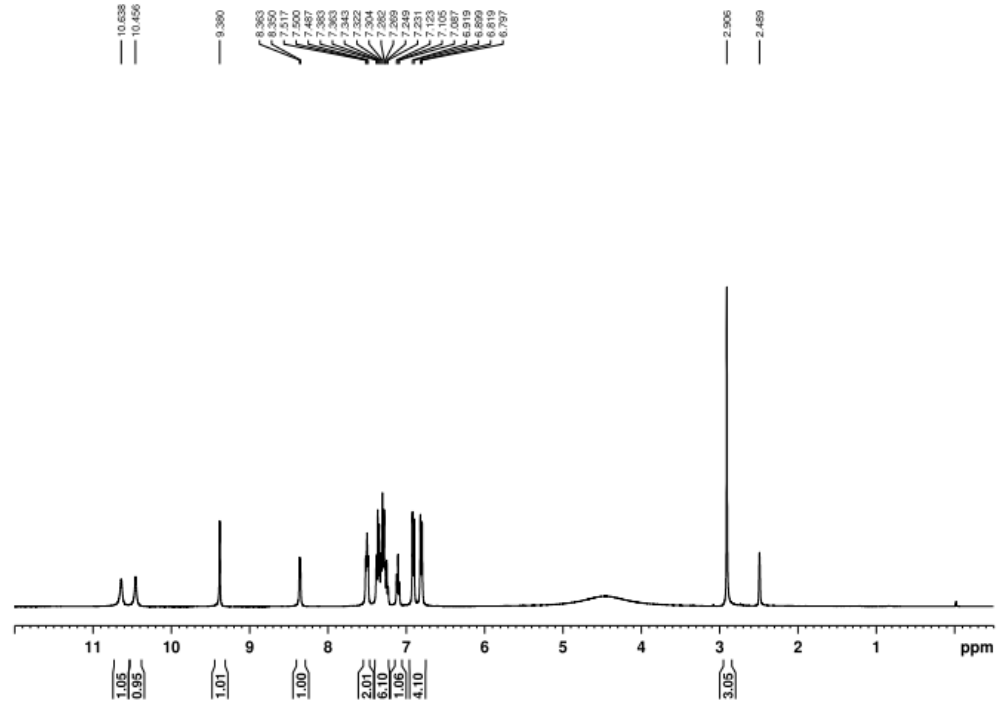


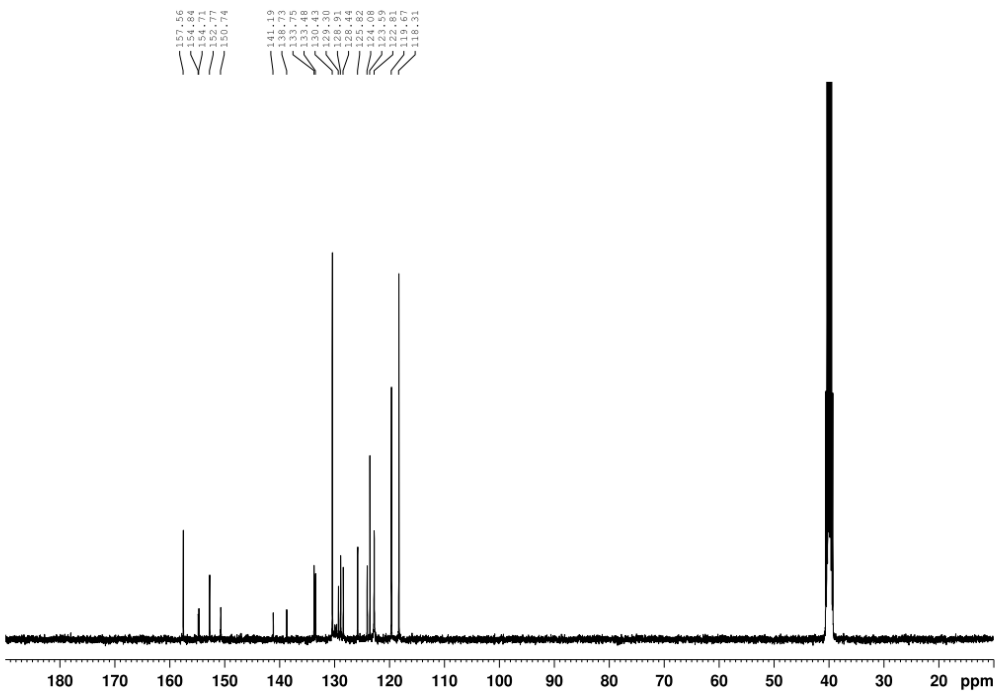
L1


9.891
9.268
9.243
8.235
8.225
8.038
7.853
7.833
7.755
7.750
7.745
7.738
7.731
7.502
7.482
7.474
7.467
7.459
7.455
7.450
7.274
7.265
7.257
7.239
7.219
4.245
4.229
4.213
2.923
2.495
2.491
2.487
1.680
1.664
1.647
1.627
1.611
1.427
1.408
1.389
1.371
0.929
0.910
0.892
10
9
8
7
6
5
4
3
2
1
ppm
0.96
1.88
1.00
1.02
1.02
1.02
2.03
3.05
2.04
3.04
1.01
2.04
2.07
3.09

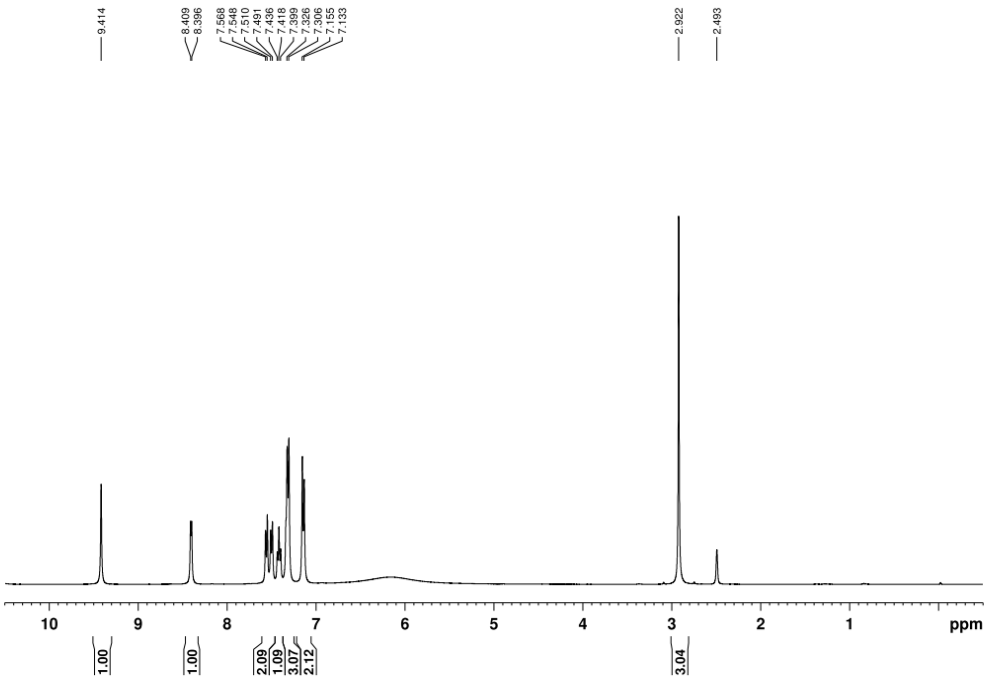

166.17
154.11
152.15
152.04
142.48
140.59
140.03
131.87
131.68
130.66
129.14
127.21
126.94
126.54
125.60
123.91
122.77
120.34
64.77
40.33
40.12
39.91
39.70
39.49
30.67
19.16
14.01
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0 ppm

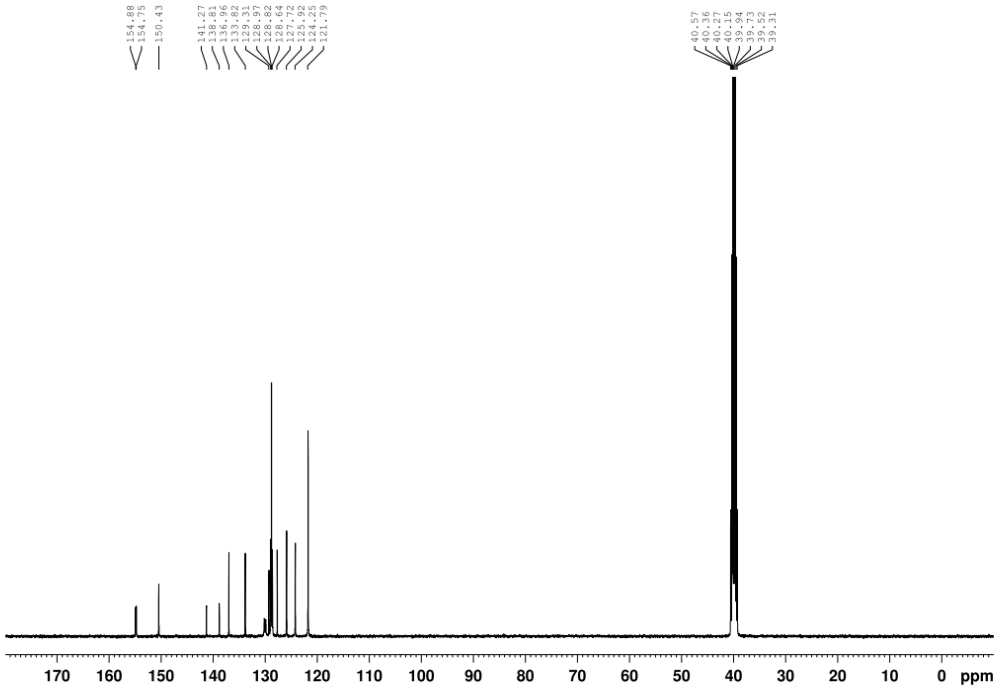
L2


10.638
10.456
9.360
8.363
8.350
7.517
7.500
7.487
7.363
7.343
7.322
7.304
7.282
7.269
7.249
7.231
7.123
7.105
7.087
6.919
6.899
6.819
6.797
2.906
2.489
11
10
9
8
7
6
5
4
3
2
1
ppm
1.05
0.95
1.01
1.00
2.01
6.10
1.06
4.10
3.05

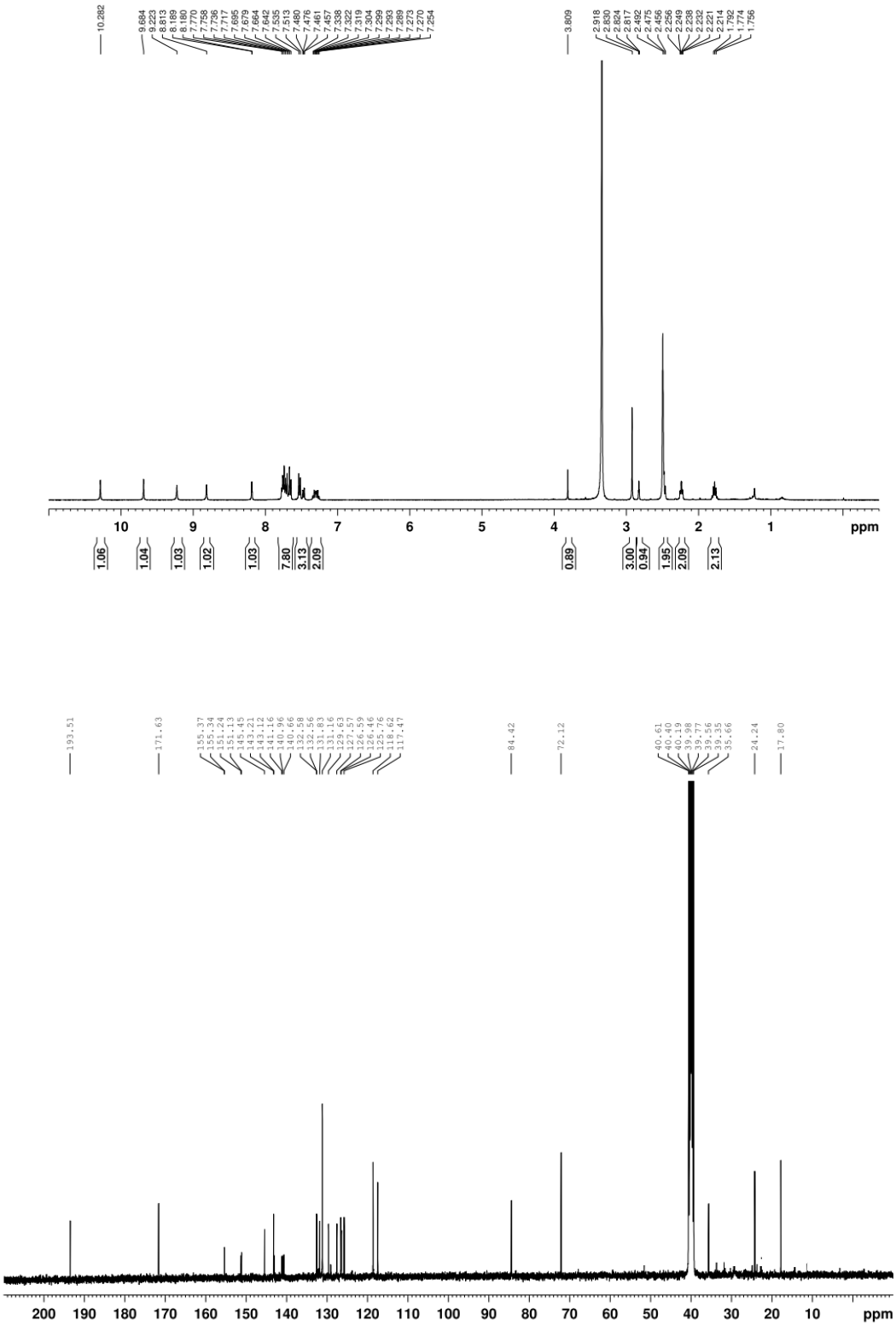

157.56
154.84
154.71
152.77
150.74
141.19
138.73
137.75
133.48
130.43
129.30
128.91
128.44
125.82
124.08
123.59
122.81
119.67
118.31
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
ppm

L3

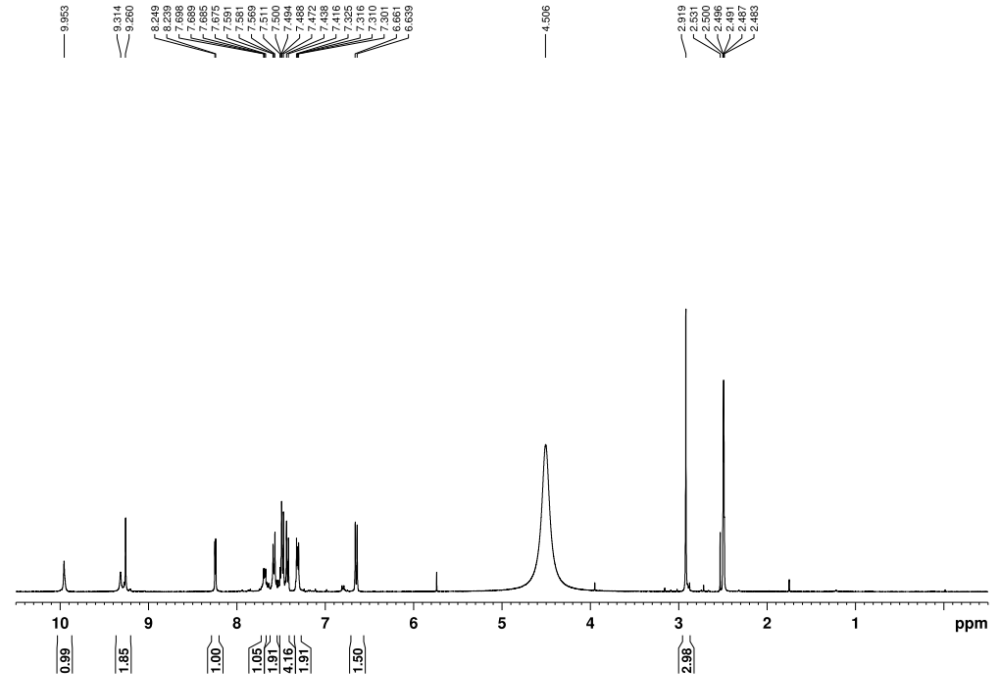

9.414
8.409
8.396
7.568
7.548
7.510
7.491
7.436
7.418
7.399
7.326
7.306
7.155
7.133
2.922
2.493
10
9
8
7
6
5
4
3
2
1
ppm
1.00
1.00
2.09
1.09
3.07
2.12
3.04

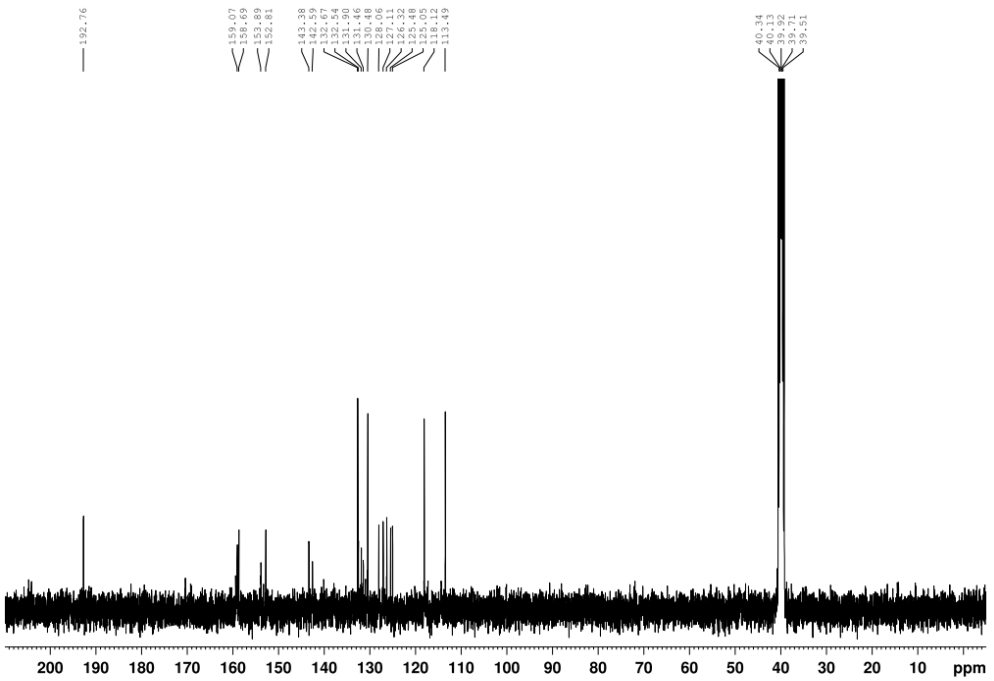

154.88
154.75
150.43
141.27
138.81
136.66
135.82
133.92
129.31
128.97
128.82
128.64
127.72
125.92
124.25
121.79
40.57
40.36
40.27
40.15
39.94
39.73
39.52
39.31
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
ppm

L1-Bpyne

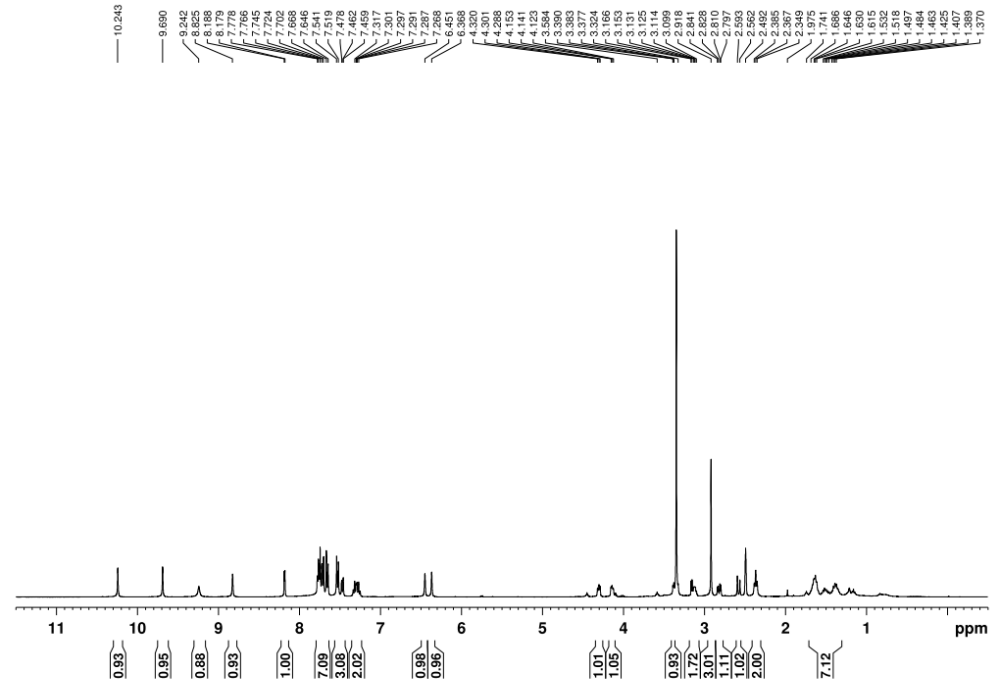

10.282
9.684
9.223
8.813
8.189
8.180
7.770
7.758
7.739
7.717
7.695
7.679
7.664
7.642
7.535
7.513
7.480
7.476
7.461
7.457
7.338
7.322
7.319
7.304
7.299
7.293
7.289
7.273
7.270
7.254
3.809
2.918
2.830
2.824
2.817
2.492
2.475
2.456
2.385
2.249
2.238
2.232
2.221
2.214
1.792
1.774
1.756
10
9
8
7
6
5
4
3
2
1
ppm
1.06
1.04
1.03
1.02
1.03
7.80
3.13
2.09
0.89
3.00
0.94
1.95
2.09
2.13
193.51
171.63
155.37
155.34
151.24
151.13
145.45
143.21
143.12
141.16
140.96
140.66
132.58
132.56
131.83
131.16
129.63
127.57
126.59
126.46
125.76
118.62
117.47
84.42
72.12
40.61
40.40
40.19
39.98
39.77
39.56
39.35
35.66
24.24
17.80
200
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
ppm

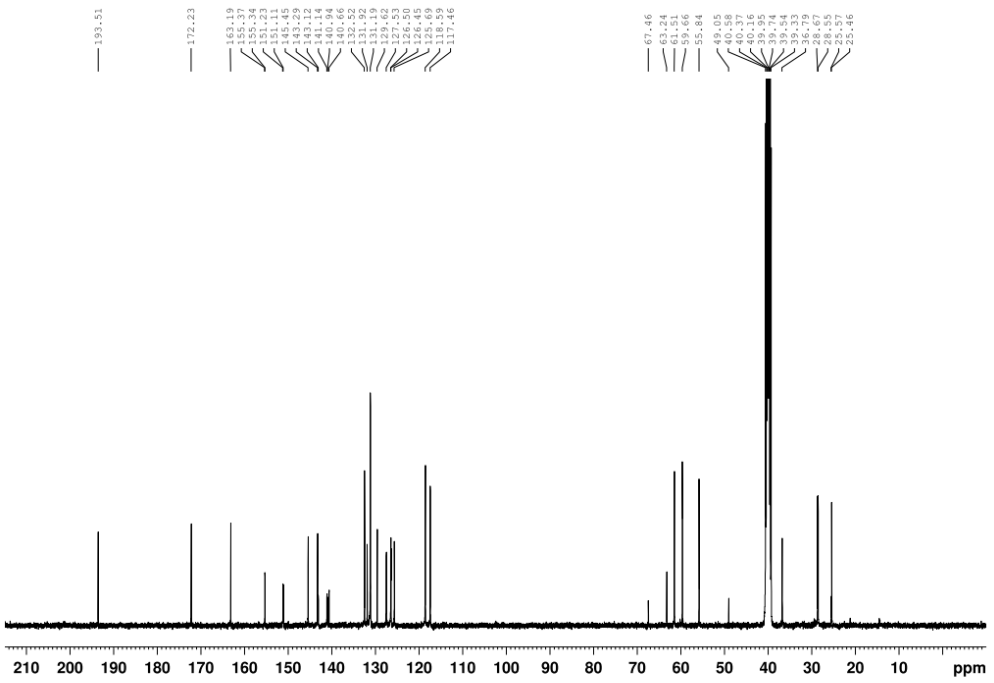
L1-BpNH$_2$


9.953
9.314
9.260
8.249
8.239
7.698
7.689
7.685
7.675
7.591
7.581
7.569
7.511
7.500
7.494
7.488
7.472
7.438
7.416
7.395
7.316
7.310
7.301
6.661
6.639
4.506
2.919
2.531
2.500
2.496
2.491
2.487
2.483
10
9
8
7
6
5
4
3
2
1
ppm
0.99
1.85
1.00
1.05
1.91
4.16
1.91
1.50
2.98


192.76
159.07
158.69
153.89
152.81
143.38
142.59
132.67
132.44
131.90
131.46
130.48
128.06
127.11
126.32
125.48
125.05
118.12
113.49
40.34
40.13
39.92
39.71
39.51
200
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
ppm

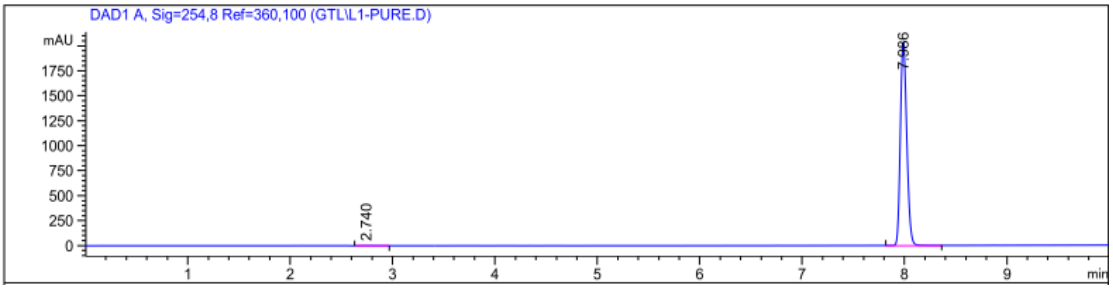
L1-Biotin


10.243
9.690
9.242
8.895
8.685
8.179
7.778
7.766
7.745
7.724
7.702
7.688
7.646
7.541
7.519
7.478
7.462
7.459
7.377
7.301
7.297
7.291
7.287
7.268
6.451
6.388
4.300
4.301
4.288
4.153
4.141
4.123
3.384
3.360
3.383
3.377
3.324
3.166
3.153
3.131
3.125
3.114
3.099
2.918
2.841
2.828
2.810
2.797
2.593
2.562
2.492
2.385
2.367
2.349
1.975
1.741
1.686
1.646
1.630
1.615
1.532
1.518
1.497
1.484
1.463
1.425
1.407
1.389
1.370
11
10
9
8
7
6
5
4
3
2
1
ppm
0.93
0.95
0.88
0.93
1.00
7.09
3.08
2.02
0.98
0.96
1.01
1.05
0.93
1.72
3.01
1.11
1.02
2.00
7.12


193.51
172.23
163.19
155.37
155.24
151.33
151.11
145.45
143.29
143.12
141.14
140.94
140.66
132.52
131.92
131.19
129.62
127.53
126.50
126.45
125.69
118.59
117.46
67.46
63.24
61.51
59.66
55.84
49.05
40.58
40.37
40.16
39.95
39.74
39.54
39.33
36.79
28.67
28.55
25.57
25.46
210
200
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
ppm

HPLC Spectra

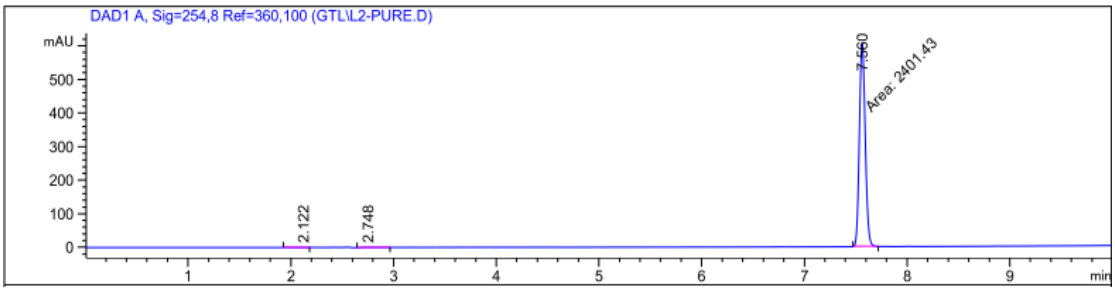
L1



Signal 1: DAD1 A, Sig=254,8 Ref=360,100

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	2.740	VB	0.1574	12.34653	1.16727	0.1386
2	7.986	VB	0.0667	8894.95410	2045.33093	99.8614
Totals :				8907.30063	2046.49821	

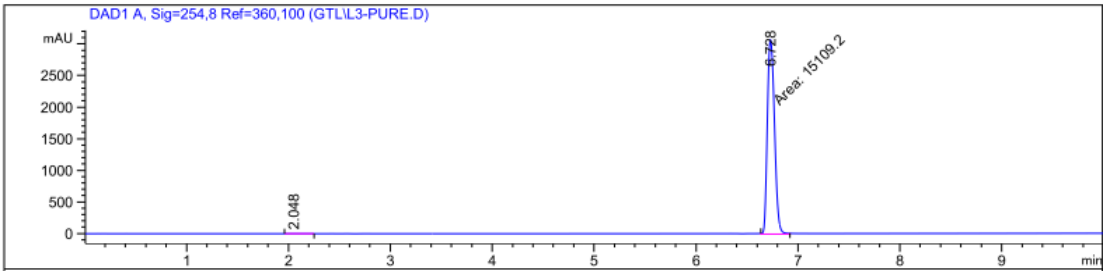
L2



Signal 1: DAD1 A, Sig=254,8 Ref=360,100

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	2.122	BV	0.1294	4.15850	4.13304e-1	0.1721
2	2.748	VB	0.1485	10.37378	1.03853	0.4294
3	7.560	MM	0.0659	2401.43286	607.38928	99.3985
Totals :				2415.96514	608.84111	

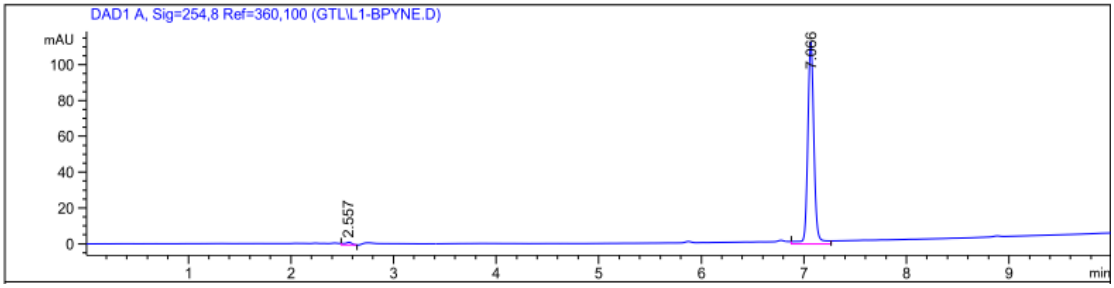
L3



Signal 1: DAD1 A, Sig=254,8 Ref=360,100

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	2.048	BB	0.2250	5.85457	3.26780e-1	0.0387
2	6.728	MM	0.0814	1.51092e4	3095.43481	99.9613
Totals :				1.51150e4	3095.76159	

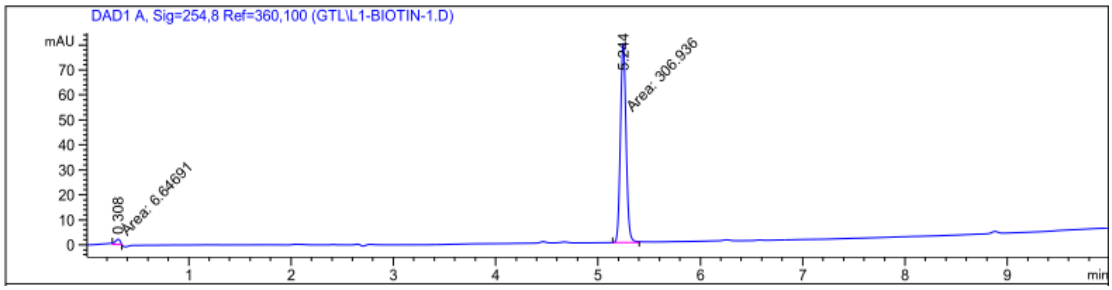
L1-Bpyne



Signal 1: DAD1 A, Sig=254,8 Ref=360,100

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	2.557	VV	0.0800	7.18641	1.39813	1.4812
2	7.066	VV	0.0699	477.99176	111.85812	98.5188
Totals :				485.17817	113.25624	

L1-Biotin



Signal 1: DAD1 A, Sig=254,8 Ref=360,100

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	0.308	MM	0.0532	6.64691	2.08225	2.1197
2	5.244	MM	0.0638	306.93619	80.13609	97.8803
Totals :				313.58310	82.21834	

Biology

Detection and purification of L1-biotin binding proteins

HEK293T cell was harvested and washed twice with ice cold PBS, then resuspended in binding buffer [50 mM HEPES pH 7.4, 150 mM NaCl, 5 mM $MgCl_2$, 1 mM EDTA, and complete protease inhibitor (Roche)]. After cell lysis the insoluble material was removed by centrifuge at 14000 g at 4 ºC for 5 min. The supernatant was collected as cell lysate. 0.5 ml of HEK293T lysate (5 mg/ml) was incubated with 0.1 µM or 1 µM L1-biotin dissolved in DMSO with or without competitor at 4 ºC overnight. The protein samples were transferred to a 24-well plate and irradiated by five 365 nm UV lights (UVP CL-1000L, 8W) for 1 hour on ice. The protein samples were centrifuged at 14000 g at 4 ºC for 10 min and passed through 0.45 µM filters (Millipore). The samples were collected and left for near western-blot analysis or affinity column purification.

For near western-blot detection, 7.5 µl of each protein sample was boiled with 2.5 µl 4×SDS-PAGE sample loading buffer (Invitrogen) for 5 min and applied to NUPAGE 4-12% Bis-tris denaturing gels (Invitrogen). After electrophoresis, the proteins were transferred to a nitrocellulose membrane by Trans-Blot Semi-Dry system (Bio-rad). Membranes were blocked using 5% BSA dissolved in tris-buffered saline containing 0.05 % Tween-20 (TBST) overnight in a cold room and incubated with streptavidin-HRP (Invitrogen) in block solution at room temperature for 1 hour. After washing 3 times by TBST the membrane was detected using an enhanced chemiluminescence detection system (GE Healthcare).

For pull-down experiment, streptavidin-sepharose beads (GE Healthcare) dissolved in 25% EtOH was equilibrated 3 times with 150 mM NaCl for 5 min at room temperature. After centrifuge, the beads were incubated with 0.2 M glycine, pH 10.6 at room temperature overnight. Before usage, the beads were equilibrated in ice-cold 50 mM HEPES pH 7.4, 1% Triton X-100. Biotinylated protein samples were incubated with streptavidin-sepharose beads at 4 ºC for 2 hours in a rotator with the speed of 60 rpm. The beads were collected by short centrifuge and the supernatant was removed by carefully aspiration without disturbing the beads. The beads were washed 3 times by 50 mM HEPES pH 7.4, 1.5% Triton X-100, followed by 50 mM HEPES pH 7.4, 1.5% Triton X-100 and 0.5 M NaCl for 3 times and 50 mM HEPES pH 7.4 once. Each washing step was performed at 4 ºC for 10 min in a rotator with the speed of 60 rpm. After the final wash, the beads were eluted by boiling in SDS-PAGE sample loading buffer for 15 min with frequently shaking. The eluted samples in SDS-PAGE sample loading buffer were applied to 4-12% Bis/Tris gradient denaturing gel and the gel was visualized by silver stain.

In vitro and *in situ* labelling by L1-Bpyne

For *in vitro* labelling, HEK293T cell lysates were prepared as previous described and incubated with 1 μM of L1-Bpyne with or without 20 μM of compound **L1** at 4 ºC overnight. The protein samples were irradiated by five 365 nm UV lights (UVP CL-1000L, 8W) in 24-well plate for 1 hour on ice. For *in situ* labelling, HEK293T cells were cultured in 10-cm dishes until ~90% confluence. The cells were washed with PBS and treated with 1 ml of Dulbecco's Modified Eagle Medium (DMEM, Invitrogen) containing 1 μM of L1-Bpyne with or without 20 μM of compound **L1**. After 6 hours incubation at 37 ºC, the cells were irradiated by five 365 nm UV lights (UVP CL-1000L, 8W) at room temperature for 1 hour. The medium containing probe was removed and the cells were washed with PBS gently. The attached cells were harvested by trypsinization and cell lysates were collected same as above. Both the protein samples from *in vitro* and *in situ* labelling were subjected to "click" reaction: For each reaction, 96 μl of protein samples were added 1 μl each of TAMRA-N_3 (10 mM stock in DMSO, Lumiprobe), $CuSO_4$ (100 mM stock in H_2O, Sigma), TBTA (10 mM stock in t-butanol:DMSO 4:1, Sigma) and ascorbic sodium (100 mM stock in H_2O, Sigma). The samples were transferred to 96-well plate and incubated for 2 hours in dark at room temperature with continuously shaking. The mixture was passed through 7-kDa Zeba desalting column (Pierce) to deplete excess TAMRA and salts and then the reaction was quenched by addition of 30 μL 4×SDS loading buffer (Invitrogen) and boiling at 95 ºC for 15 min. Samples were applied to NUPAGE 4-12% Bis-tris denaturing gels and in-gel fluorescence scanning was performed with Pharos FX imaging system (Bio-rad).

In cell "click" reaction and imaging

HeLa cells were cultured in 6-well plates containing glass cover slips and grown until ~90% confluence. Cells were treated with DMSO (positive) or 100 μM of compound **L1** (competition) at 37 ºC for 6 hours. Subsequently, the medium was removed and fresh medium containing 20 μM of L1-Bpyne was added. After incubation at 37 ºC for 1 hour, cells were irradiated for 30 min with the same condition of *in situ* labelling. The cells were washed with ice-cold PBS buffer twice, and dead cells were aspired and discarded. After fixation with 4% paraformaldehyde in PBS for 15 min, cells were washed with PBS twice and permeabilized by 0.1% Triton X-100 in PBS for 15 min. Then cells were washed with PBS again and blocked with 3% BSA in PBS for 30 min, and washed with PBS twice to remove excess BSA. Subsequently, the cells were treated with freshly prepared cocktail containing 10 μM of TAMRA-N_3, 1 mM of $CuSO_4$, 100 μM of TBTA and 1 mM of ascorbic sodium dissolved in 200 μL PBS for 2 hours at room temperature with gentle shaking. Cells were washed with PBS five times and imaged with Zeiss Imager A1 fluorescence microscope.

GLO-1 enzyme activity assay

The GLO-1 enzyme activity assay was performed according to a spectrophotometric method monitoring the increase in absorbance at 240 nm due to the formation of S-D-lactoylglutathione at 25 ºC with slight changes. In brief, 7.9 mM MG, 1 mM glutathione, 14.6 mM magnesium sulfate, and 182 mM imidazole-HCl, were mixed at pH 7.0 for 10 min to ensure the equilibration of hemithioacetal formation. The reaction was initiated by adding 20 ng of recombinant GLO-1 protein (Sigma) pre-incubated with DMSO or inhibitor for 1 hour. After 9 min the reaction mixture were transferred to a cuvette and absorbance data at 240 nm were read by Smartspec Plus (Bio-rad).

Cell proliferation assay

HeLa cells were cultured in DMEM with glucose concentration of 5 mM (normal) or 25 mM (high glucose) for three passages and diluted in culture medium to 8000 cells/ml. 100 µl of cell suspension were seeded to each well of 96-well plate and incubated at 37 ºC overnight. Different concentrations of compounds were dissolved in culture medium containing 0.5% DMSO. Cells in 96-well plate were treated with 100 µL of different concentrations of compounds and DMSO (negative control) for 48 hours in a 37 ºC incubator. Cell viability was assessed by CellTiter-Glo® Luminescent Kit (Promega).

Cellular MG measurement

Total cellular MG was determined according to literature procedure with slight modification. HeLa cells were cultured in DMEM and treated with DMSO or 5 µM of L1-Bpyne for 24 hours. Cells were harvested by trypsinisation and washed twice with PBS and equalized by cell counting (~2×10^7 cells/sample), then resuspended in ddH_2O. Cells were boiled for 5 min and centrifuged at 14000 g for 3 min. The supernatant was collected as the source of MG. All the conditions were as same as GLO-1 enzyme activity assay mentioned above except that the GLO-1 amount for each sample was 50 ng in order to increase the sensitivity.

Apoptosis study

HeLa cells were treated with 5 µM of L1-Bpyne in DMEM (high glucose) for 24 hours in 6-well plates with glass cover slips. Cells were washed twice with PBS and treated with 10 µg/ml of 4',6-diamidino-2-phenylindole dihydrochloride (DAPI, Invitrogen) dissolved in PBS for 15 min at room temperature, followed by imaging with Zeiss Imager A1 fluorescence microscope.

For detection of caspase-3 degradation, HeLa cells were treated with different concentrations of L1-Bpyne in DMEM (high glucose) for 24 hours. Cell lysates were

collected, separated by SDS-PAGE and transferred to nitrocellulose membrane as mentioned above. Caspase-3 degradation was detected by anti-caspase-3 antibody (Abcam).

MALDI-TOF-MS Results of ~28 kD band

Protein View: gi|15030212
Glyoxalase I [Homo sapiens]
Database: NCBInr
Score: **99**
Expect: 3.10E-05
Nominal mass (Mr): 20824
Calculated pI: 5.12
Taxonomy: Homo sapiens

Protein sequence coverage: **56%**
Matched peptides shown in red.

1	MAEPQPPSGG	LTDEAALSYC	SDADPSTKDF	LLQQTMLRVK	DPKKSLDFYT
51	RVLGMTLIQK	CDFPIMKFSL	YFLAYEDKND	IPKEKDEKIA	WALSRKATLE
101	LTHNWGTEDD	ETQSYHNGNS	DPRGFGHIGIA	VPDVYSACK	RFEELGVKFV
151	KKPDDGKMKG	LAFIQDPDGY	WIEILNPNKM	ATLM	

Start	End	Observed	Mr(expt)	Mr(calc)	Delta	M	Peptide
29	38	1264.6986	1263.6913	1263.6645	0.0268	0	K.DFLLQQTMLR.V
29	38	1280.7212	1279.7139	1279.6595	0.0545	0	K.DFLLQQTMLR.V + Oxidation (M)
44	51	1029.5531	1028.5458	1028.5291	0.0167	1	K.KSLDFYTR.V
45	51	901.4729	900.4656	900.4341	0.0315	0	K.SLDFYTR.V
52	60	1002.6265	1001.6192	1001.5943	0.0249	0	R.VLGMTLIQK.C
52	60	1018.6089	1017.6016	1017.5892	0.0124	0	R.VLGMTLIQK.C + Oxidation (M)
68	78	1395.7605	1394.7532	1394.6758	0.0774	0	K.FSLYFLAYEDK.N
68	83	1963.0098	1962.0025	1961.9774	0.025	1	K.FSLYFLAYEDKNDIPK.E
89	95	816.5134	815.5061	815.4653	0.0407	0	K.IAWALSR.K
89	96	944.5788	943.5715	943.5603	0.0112	1	K.IAWALSRK.A
124	140	1733.9534	1732.9461	1732.8607	0.0854	0	R.GFGHIGIAVPDVYSACK.R
141	148	977.5585	976.5513	976.5342	0.0171	1	K.RFEELGVK.F
142	148	821.4567	820.4494	820.4331	0.0163	0	R.FEELGVK.F
152	159	918.5021	917.4948	917.464	0.0308	1	K.KPDDGKMK.G
160	179	2303.1333	2302.126	2302.1634	-0.0373	0	K.GLAFIQDPDGYWIEILNPNK.M

MALDI-TOF-MS Results of ~55 kD band

Protein View: gi|15030212

Glyoxalase I [Homo sapiens]

Database:	NCBInr
Score:	**47**
Expect:	5.3
Nominal mass (Mr):	20824
Calculated pI:	5.12
Taxonomy:	Homo sapiens

Protein sequence coverage: **53%**

Matched peptides shown in red.

1	MAEPQPPSGG	LTDEAALSYC	SDADPSTKDF	LLQQTMLRVK	DPKKSLDFYT
51	RVLGMTLIQK	CDFPIMKFSL	YFLAYEDKND	IPKEKDEKIA	WALSRKATLE
101	LTHNWGTEDD	ETQSYHNGNS	DPRGFGHIGIA	VPDVYSACK	RFEELGVKFV
151	KKPDDGKMKG	LAFIQDPDGY	WIEILNPNKM	ATLM	

Start	End	Observed	Mr(expt)	Mr(calc)	Delta	M	Peptide
29	38	1264.7076	1263.7004	1263.6645	0.0358	0	K.DFLLQQTMLR.V
29	38	1280.6959	1279.6886	1279.6595	0.0292	0	K.DFLLQQTMLR.V + Oxidation (M)
29	40	1491.8345	1490.8272	1490.8279	-0.0007	1	K.DFLLQQTMLRVK.D
44	51	1029.5557	1028.5484	1028.5291	0.0193	1	K.KSLDFYTR.V
45	51	901.4688	900.4615	900.4341	0.0274	0	K.SLDFYTR.V
52	60	1002.5782	1001.571	1001.5943	-0.0234	0	R.VLGMTLIQK.C
52	60	1018.6083	1017.6011	1017.5892	0.0118	0	R.VLGMTLIQK.C + Oxidation (M)
68	78	1395.7185	1394.7112	1394.6758	0.0354	0	K.FSLYFLAYEDK.N
68	83	1963.0178	1962.0105	1961.9774	0.0331	1	K.FSLYFLAYEDKNDIPK.E
89	95	816.4999	815.4926	815.4653	0.0273	0	K.IAWALSR.K
124	140	1733.8934	1732.8862	1732.8607	0.0255	0	R.GFGHIGIAVPDVYSACK.R
141	148	977.5669	976.5596	976.5342	0.0255	1	K.RFEELGVK.F
142	148	821.4649	820.4576	820.4331	0.0246	0	R.FEELGVK.F
158	179	2562.3843	2561.377	2561.2988	0.0782	1	K.MKGLAFIQDPDGYWIEILNPNK.M
160	179	2303.2117	2302.2044	2302.1634	0.041	0	K.GLAFIQDPDGYWIEILNPNK.M