Supporting Information

Ugi Reaction-Assisted Rapid Assembly of Affinity-Based Probes (A*f*BPs) against Potential Protein Tyrosine Phosphatases (PTPs)

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Contents

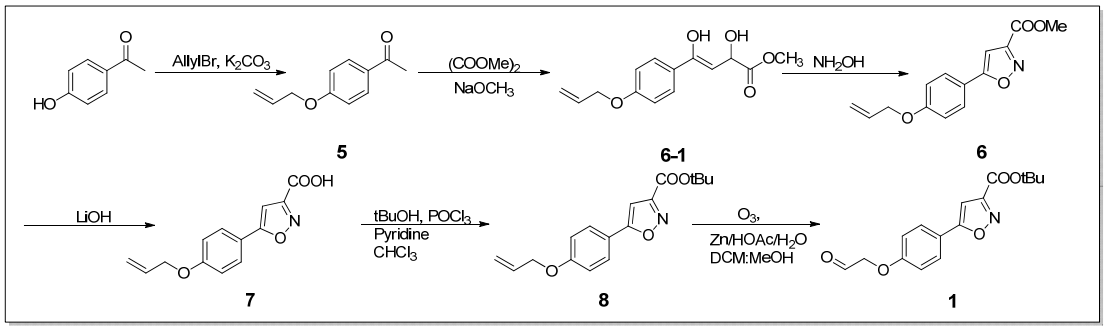
1. General Information

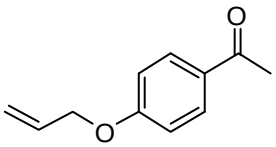
All chemicals were purchased from commercial vendors and used without further purification, unless otherwise noted. ^{1}H and ^{13}C NMR spectra were recorded on a Bruker 300 MHz, 500 MHz or DPX-300 NMR spectrometer. Chemical shifts are reported in parts per million referenced with respect to residual solvent ($CHCl_3$ = 7.26 ppm and DMSO-d_6 = 2.5 ppm) or from internal standard tetramethylsilane ($Si(CH_3)_4$ = 0.00 ppm). The following abbreviations were used in reporting spectra: s = singlet, d = doublet, t = triplet, q = quarter, qn = quintet, m = multiplet, dd = doublet of doublets, br = broad. All solvents used were of HPLC grade, all reactions

requiring anhydrous conditions were conducted under a nitrogen or argon atmosphere in flame dried glassware. All LC-IT-TOF profiles and mass spectra were recorded on a Shimadzu LC-IT-TOF system equipped with an autosampler, using reverse-phase Phenomenex Luna 5 C_{18} (2) 100 Å 50 × 3.0 mm column. Eluent A (0.1% trifluoroacetic acid/acetonitrile) and B (0.1% trifluoroacetic acid/water) were used as the mobile phase. The flow rate is 0.6 mL/min. All the enzymes were expressed in *E. coli* strain BL21-DE3 and purified by Ni-NTA technology. For enzyme activity measurements, Biotek microplate reader was used. IC_{50} curves were generated using the Graphpad Prism software v5 (GraphPad, San Diego, USA). Fluorescence scanning of the SDS-PAGE gels was carried out with Typhoon 9200 fluorescence gel scanner (GE Heathcare) and the bands were quantified with ImageQuant software installed on the scanner.

2. Synthetic procedures for aldehyde and isonitrile

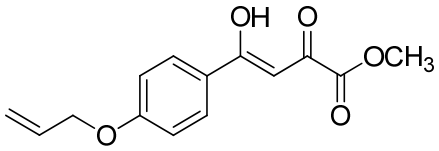
2.1 Synthesis of aldehyde warhead





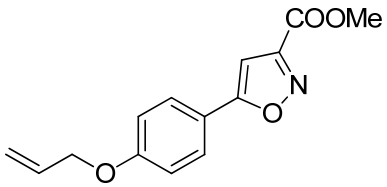
1-(4-(allyloxy)phenyl)ethanone (5)

p-Hydroxyacetophenone (2.0 g, 14.7 mmol) was dissolved in acetonitrile (30 mL). K_2CO_3 (4.06 g, 29.7 mmol) and allyl bromide (2.13 g, 17.6 mmol) were added into the solution. The reaction was heated the reaction for 1 hour at 50 °C, after which the organic solvent was removed under reduced pressure. The residue was dissolved in EtOAc and washed with water, brine. The organic layer was dried over Na_2SO_4 and the solvent was removed *in vacuo* to afford compound **5** (89%). ^{1}H-NMR (300 MHz, $CDCl_3$) δ 7.89 (d, J = 8.88 Hz, 2H), 6.90 (d, J = 8.88 Hz, 2H), 5.95 - 6.08 (m, 1H), 5.39 (dd, J_1 = 16.42, J_2 = 1.47 Hz, 1H), 5.29 (dd, J_1 = 10.51 Hz, J_2 = 1.32 Hz, 1H), 5.56 (td, J_1 = 5.28 Hz, J_2 = 1.47 Hz, 2H), 2.51 (s, 3H). ^{13}C-NMR (75 MHz, $CDCl_3$) δ 196.5, 162.3, 132.4, 130.4, 118.0, 114.2, 68.7, 26.2. IT-TOF: m/z $[M+1]^+$ calcd: 177.08, found: 177.09



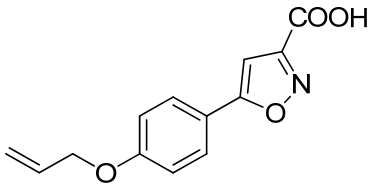
Methyl 4-(4-(allyloxy)phenyl)-4-hydroxy-2-oxobut-3-enoate (6-1)[1]

To a solution of compound **5** (2.0 g, 11.4 mmol) and dimethyl oxalate (1.47 g, 12.4 mmol) in MeOH (in an ice bath) under a nitrogen atmosphere was added a freshly prepared NaOMe (12.5 mmol, 0.5 M in MeOH) in small portions. The reaction was subsequently refluxed for 24 hours before being cooled down to room temperature. Upon filtration of the while precipitate formed, the solution was collected, concentrated *in vacuo* to provide the desired product **6-1** (48%). ^{1}H-NMR (300 MHz, $CDCl_3$) δ 7.97 (d, J = 8.85 Hz, 2H), 6.96 - 7.02 (m, 3H), 5.98 - 6.11 (m, 1H), 5.42 (dd, J_1 = 17.26 Hz, J_2 = 1.29 Hz, 1H), 5.32 (dd, J_1 = 10.44 Hz, J_2 = 1.23 Hz, 1H), 4.61 (d, J = 5.28 Hz, 2H), 3.92 (s, 3H). ^{13}C-NMR (75 MHz, $CDCl_3$) δ 190.3, 167.8, 163.3, 162.9, 132.2, 130.3, 127.7, 118.3, 114.9, 97.8, 69.0, 53.1



Methyl 5-(4-(allyloxy)phenyl)isoxazole-3-carboxylate (6)

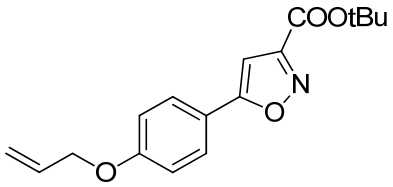
Compound **6-1** (0.64 g, 2.4 mmol) and $NH_2OH{\cdot}H_2O$ (0.17 g, 2.9 mmol) dissolved in MeOH was added a catalytic amount of $TsOH{\cdot}H_2O$ (0.01 g, 0.05 mmol). The reaction mixture was refluxed for 24 hours. After being cooled down, the resulting white precipitate was collected by suction filtration and washed with a mixture of ice-cold MeOH and deionized water to provide the desired product **6** as a solid (80%). ^{1}H-NMR (300 MHz, $CDCl_3$) δ 7.70 (d, J = 9.06 Hz, 2H), 6.97 (d, J = 9.06 Hz, 2H), 6.77 (s, 1H), 6.00-6.09 (m, 1H), 5.41 (dd, J_1 = 17.28 Hz, J_2 = 1.47 Hz, 1H), 5.30 (dd, J_1 = 10.53 Hz, J_2 = 1.17 Hz, 1H), 4.56 (d, J = 5.25 Hz, 2H), 3.97 (s, 3H) ^{13}C-NMR (75 MHz, $CDCl_3$) δ 171.6, 160.5, 160.4, 156.5, 132.5, 127.4, 119.3, 118.0, 115.1, 98.4, 68.7, 52.7. IT-TOF: m/z $[M+1]^+$ calcd: 260.08, found: 260.06



5-(4-(Allyloxy) phenyl) isoxazole-3-carboxylic acid (7)

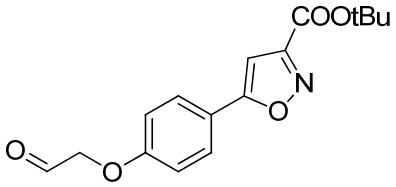
Compound **6** (1 g, 3.9 mmol) was dissolved in MeOH and water (1:1; 10 ml) in an ice bath. LiOH (0.47 g, 19.5 mmol) in 0.5 ml water was added slowly into the solution. After 2 hours when TLC indicated the starting material was completely consumed, the solution was neutralized with 1 M HCl. The resulting white precipitate was collected, washed with water and chilled methanol to provide the

desired compound **7** (89%). ^{1}H-NMR (300 MHz, DMSO-d_6) δ 7.87 (d, J = 8.70 Hz, 2H), 7.24 (s, 1H), 7.10 (d, J = 8.55 Hz, 2H), 5.99 - 6.11 (m, 1H), 5.41 (dd, J_1 = 17.26 Hz, J_2 = 1.15Hz, 1H), 5.28 (dd, J_1 = 10.53 Hz, J_2 = 0.99 Hz, 1H), 4.65 (d, J = 5.10 Hz, 2H). ^{13}C-NMR (75 MHz, DMSO-d_6) δ 170.8, 160.9, 160.1, 157.8, 133.2, 127.5, 119.0, 117.8, 115.4, 99.3, 68.4. IT-TOF: m/z [M+1] $^{+}$ calcd: 246.07, found: 246.06



***Tert*-butyl 5-(4-(allyloxy)phenyl)isoxazole-3-carboxylate(8)**

To a solution of compound **7** (500 mg, 2.0 mmol) in $CHCl_3$ was added *t*-BuOH (2 mL, 20 mmol) and pyridine (806 mg, 10.0 mmol) in an ice bath. After 5 min, $POCl_3$ (406 mg, 2.6 mmol) was slowly added into the solution. The reaction was stirred for 1 hour. Dichloromethane and 10 mL dilute HCl were poured into the solution. The organic layer was separated, washed with dilute HCl (10 mL × 2), water (10 mL × 2), and brine (10 mL × 2), dried over Na_2SO_4. The solvent was removed and the residue was purified by silica gel chromatography (10% - 35% EtOAc in hexane) to afford compound **8** as a white solid (85%). ^{1}H-NMR (300 MHz, $CDCl_3$) δ 7.49 (d, J = 8.85 Hz, 2H), 6.99 (d, J = 9.02 Hz, 2H), 6.72(s, 2H), 6.00 - 6.12 (m, 1H), 5.43 (dd, J_1 = 17.26, J_2 = 1.5 Hz, 1H), 5.32 (dd, J_1 = 10.53, J_2 = 1.32 Hz, 1H), 4.59 (d, J = 5.25 Hz, 2H), 1.63 (s, 9H). ^{13}C-NMR (75 MHz, $CDCl_3$) δ 171.4, 160.5, 159.2, 158.1, 132.6, 127.5, 119.8, 118.1, 115.3, 98.6, 83.5, 68.9, 28.1. IT-TOF: m/z [M+1] $^{+}$ calcd: 302.13, found: 302.10

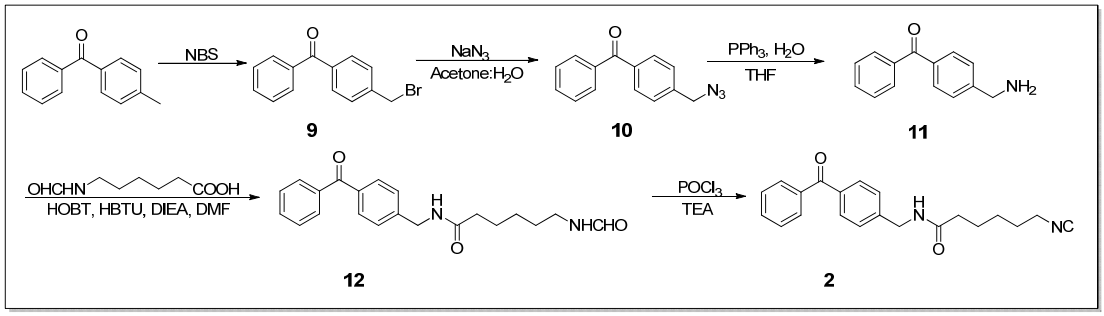


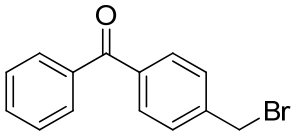
***Tert*-butyl 5-(4-(2-oxoethoxy)phenyl)isoxazole-3-carboxylate(1)**

O_3 gas was bubbled into a solution of compound **8** (450 mg, 1.5 mmol) in DCM (10 mL) at -78 °C until the solution became blue. Excess O_3 was purged out using argon. Then, zinc dust (340 mg, 5.2 mmol) and a mixture of glacial AcOH and water (17:3; 360 μL) were added to the solution in small portions with vigorous stirring. Subsequently, the reaction mixture was allowed to warm to room temperature, followed by stirring for a further 1 hour. The reaction mixture was then neutralized and filter through Celite to remove any residual solids. The filtrate was extracted with DCM and the organic layer was washed with saturated $NaHCO_3$ and brine, dried over Na_2SO_4 and concentrated *in vacuo*. The resulting crud product was purification by flash column chromatography (10% - 40% EtOAc in hexane) to afford the pure product **1** as an white solid (81%) ^{1}H-NMR (300 MHz, $CDCl_3$) δ 9.81 (s, 1H) , 7.68 (d, J = 8.88 Hz, 2H), 6.94 (d, J = 8.88 Hz, 2H), 6.70 (s, 1H), 4.63

(d, J = 0.81 Hz, 2H), 1.99 (s, 9H) δ ^{13}C-NMR (75 MHz, $CDCl_3$) δ 197.8, 170.8, 159.2, 158.9, 157.9, 127.5, 120.5, 115.0, 98.8, 83.5, 72.4, 27.9. IT-TOF: m/z [M+1] $^+$ calcd: 304.11, found: 304.09

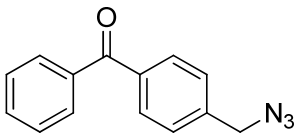
2.2 Synthesis of isonitrile





(4-(bromomethyl)phenyl)(phenyl)methanone (9)

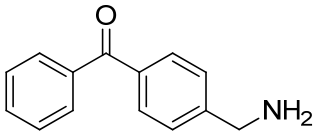
4-Methylbenzophenone (3 g, 15.3 mmol) and *N*-bromosuccinimide (2.72 g, 15.3 mmol) were dissolved in CCl_4 (30 mL) solution. The reaction was placed under an IR lamp and the solution was refluxed for ~3 hours when monitoring of the reaction by TLC indicated the complete consumption of the starting material. The resulting solution was cooled down and the solid filtered off. Upon concentration *in vacuo* to remove half of the solvent, the resulting solution was added hexane, and left standing for recrystalization to occur. The resulting solid was collected to give pure compound **9** (78%). ^{1}H-NMR (300 MHz, $CDCl_3$) δ 7.77 - 7.80 (m, 4H), 7.60 (t, J = 7.30 Hz, 1H), 7.46 - 7.51 (m, 4H), 4.53 (s, 2H) ^{13}C-NMR (75 MHz, $CDCl_3$) δ 195.9, 142.0, 137.4, 137.3, 132.5, 130.5, 130.0, 128.9, 128.3, 32.2. IT-TOF: m/z [M+1] $^+$ calcd: 275.00, 277.00 found: 274.99, 276.99.



(4-(Azidomethyl)phenyl)(phenyl)methanone (10)

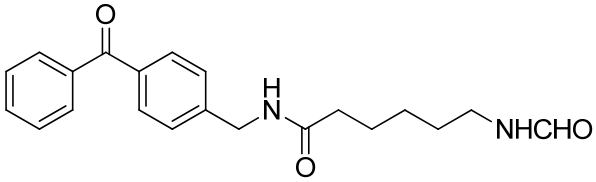
To a stirred solution of **9** (1.5 g, 5.5 mmol) in 16 mL mixture of acetone:H_2O (3:1) in an ice bath was added NaN_3 (0.7 g, 11 mmol; dissolved in water). After 1 hour, some precipitates formed. Water (50 mL) was then added, and the resulting solution was filtered. The solid collected was washed with water and then dried *in vacuo* to afford compound **10** (96%). ^{1}H-NMR (300 MHz, $CDCl_3$) δ 7.80 (t, J = 8.49 Hz, 4H), 7.59 (t, J = 7.30 Hz, 1H), 7.41 - 7.50 (m, 4H), 4.43 (s, 2H). ^{13}C-NMR (75

MHz, $CDCl_3$) δ 196.0, 139.8, 137.3, 132.5, 130.5, 130.0, 128.2, 127.7, 54.2. IT-TOF: m/z $[M+1]^+$ calcd: 238.09 found: 238.07.



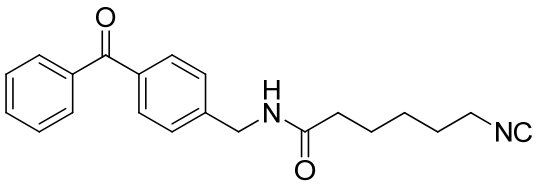
(4-(aminomethyl)phenyl)(phenyl)methanone (11)

10 (0.5 g, 2.1 mmol) dissolved in THF (10 mL) was added H_2O (0.16 g) at room temperature. PPh_3 was then added and the resulting solution was heated to 60 °C for 1 hour before being cooled down to 25 °C. Upon solvent removal under reduced pressure, the crude residue collected was subsequently purified by silica gel chromatography (30% EtOAc in hexane to 10% MeOH in DCM with 0.1% triethylamine) to give compound **11** (82%). ^{1}H-NMR (300 MHz, $CDCl_3$) δ 7.72 - 7.74 (d, *J* = 8.20 Hz, 4H), 7.53 (t, *J* = 7.25 Hz, 1H) , 7.38 - 7.42 (m, 4H), 3.91 (s, 2H), 1.20(s, 2H) ^{13}C-NMR (75 MHz, $CDCl_3$) δ 196.2, 147.5, 137.5, 135.9, 132.1, 130.2, 129.7, 128.1, 126.7, 45.8.



***N*-(4-benzoylbenzyl)-6-formamidohexanamide (12)**

6-formamidohexanoic acid (0.40 g, 2.5 mmol) was pre-activated with HOBT (0.37 g, 2.75 mmol), HBTU (1.0 g, 2.75 mmol) and DIEA (0.38 g, 3 mmol) in 15 mL DMF. After 10 min, compound **11** (0.53g, 2.5 mmol) was added into the solution. The reaction was stirred further for 2 hours. Upon DMF removal *in vacuo*, the resulting residue was purified by silica gel chromatography (10% to 40% EtOAc in hexane) to yield compound **12** (84%). ^{1}H-NMR (300 MHz, $CDCl_3$) δ 8.06 (s, 1H), 7.15 (t, *J* = 8.05 Hz, 4H), 7.57 (t, *J* = 7.40 Hz, 1H), 7.45 (t, *J* = 7.24 Hz, 2H), 7.34 (d, *J* = 8.22 Hz, 2H), 6.77 (br, 1H), 6.34 (br, 1H), 4.47 (d, *J* = 5.91 Hz, 2H), 3.25 (q, *J* = 6.66 Hz, 2H), 2.24 (t, *J* = 7.39 Hz, 2H), 1.64 (qn, *J* = 7.45 Hz, 2H), 1.49 (qn, *J* = 7.11 Hz, 2H), 1.31 (qn, *J* = 7.27 Hz, 2H) ^{13}C-NMR (75 MHz, $CDCl_3$) δ 196.5, 173.3, 161.6, 143.3, 137.3, 136.4, 132.5, 130.3, 129.3, 128.3, 127.3, 43.1, 37.7, 36.1, 28.9, 26.2, 25.0. IT-TOF: m/z $[M+1]^+$ calcd: 354.18, found: 353.17

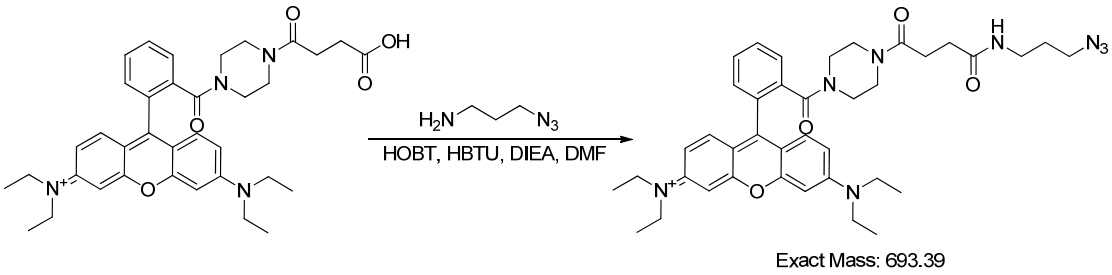


N-(4-benzoylbenzyl)-6-isocyanohexanamide (2)

Compound **12** (1.0 g, 2.9 mmol) dissolved in anhydrous DCM in an ice bath was

added triethylamine (1.86 g, 18.4 mmol) and $POCl_3$ (0.91 g, 5.9 mmol). The solution was stirred for 30 min. Subsequently, 5% $NaHCO_3$ was added and the reaction was stirred further for another 10 min. The resulting solution was extracted by DCM. The combine organic layer was washed with brine and dried over Na_2SO_4. The crude residue was purified by silica gel chromatography (5% to 30% EtOAc in hexane) to provide compound **2** (81%). ^{1}H-NMR (300 MHz, $CDCl_3$) δ 7.71 - 7.76 (m, 4H), 7.58 (t, *J* = 7.38 Hz, 1H), 7.46 (t, *J* = 7.20 Hz, 2H), 7.35 (d, *J* = 8.04 Hz, 2H), 6.23 (br, 1H), 4.49 (d, *J* = 5.91 Hz, 2H), 3.34 - 3.40 (m, 2H), 2.25 (t, *J* = 7.38Hz, 2H), 1.65 - 1.75 (m, 2H), 1.48 - 1.50 (qn, *J* = 7.11 Hz, 2H). ^{13}C-NMR (75 MHz, $CDCl_3$) δ 196.3, 172.6, 155.9, 143.2, 137.4, 136.6, 132.5, 130.4, 129.9, 128.3, 127.4, 43.1, 41.3, 36.0, 28.7, 25.9, 24.6. IT-TOF: m/z [M+1] $^+$ calcd: 335.17, found: 335.15

2.3 Synthesis of dye-azide



The acid (50 mg, 0.09 mmol; prepared based on reference 2) was pre-activated with HOBT (13.2 mg, 0.1 mmol), HBTU (37.2 mg, 0.1 mmol) and DIEA (18.55 mg, 0.1 mmol) in DMF for 10 min. Subsequently, 3-azidopropan-1-amine (9 mg, 0.09 mmol) was added and the resulting mixture was stirred for 2 hours. Upon DMF removal *in vacuo*, the residue was purified by flash chromatography (0.1% to 5% Methanol in DCM) to afford the desired rhodamine-N_3 (85%). ESI: m/z [M+1] $^+$ calcd: 694.4, found: 694.3.

3. Procedure and LC-MS profiles of probes' synthesis

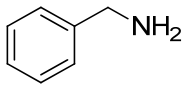
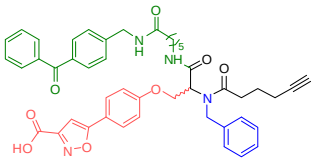
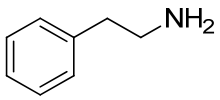
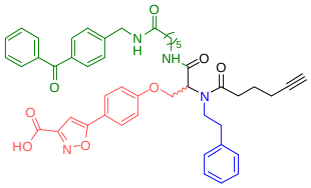
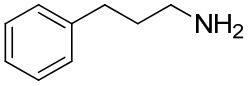
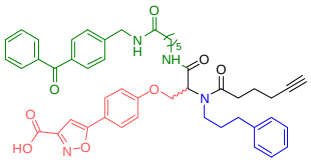
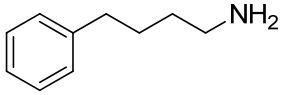
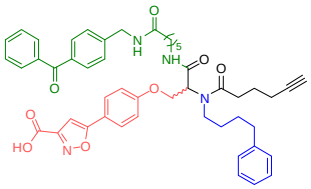
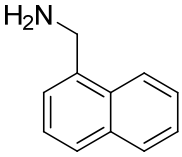
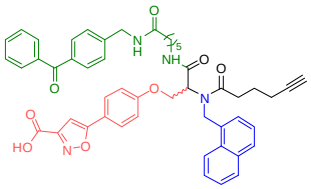
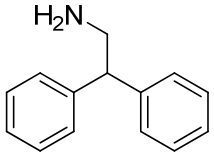
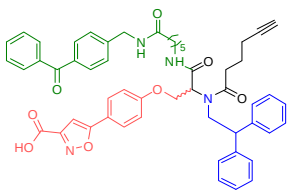
3.1 General Procedure for the synthesis of probes

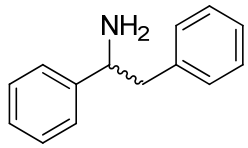
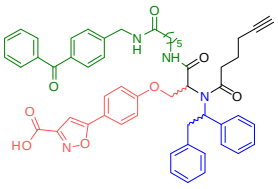
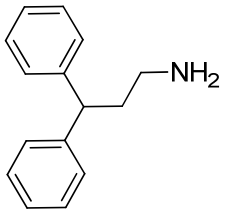
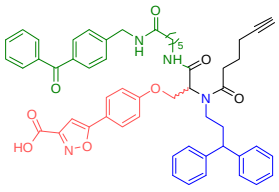
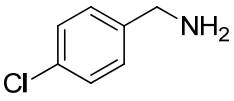
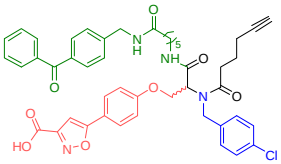
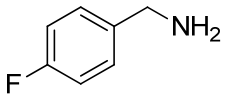
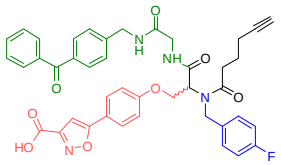
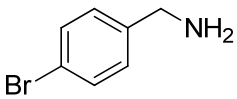
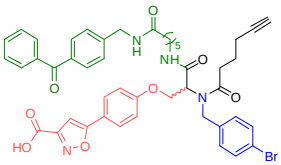
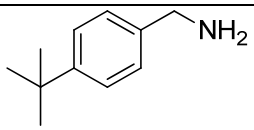
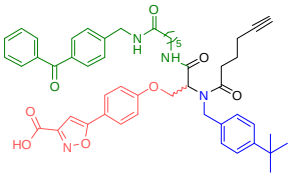
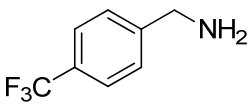
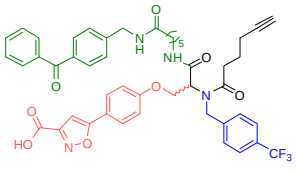
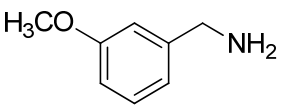
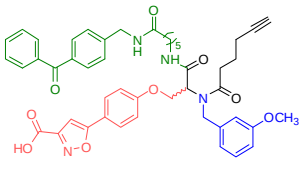
In 1.5 mL eppendorf tubes, a solution of amine (0.1 mmol, Table S1) was added into 400 µL MeOH. Then aldehyde **1** (0.1 mmol in 100 µL DMF) was added. After 1 hour, isonitrile **2** (0.1 mmol in 100 µL MeOH) and 5-Hexynoic acid (0.1 mmol in 100 µL MeOH) were added into the mixture. The reaction was stirred for ~9 hours. The crude residue was purified by semi-preparative reverse-phase HPLC. The product was next treated with 1 mL of a mixture of TFA/DCM (50/50) for 2 hours. TFA/DCM was next removed under reduced pressure with a GeneVac HT-4X Series II parallel evaporation system, affording the final product which was sufficiently pure and could be directly used in subsequent screening experiments. Yield (in two steps): 20 - 30%. Both LCMS and ^{1}H NMR were carried out to further characterize

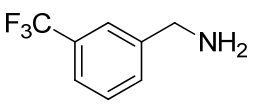
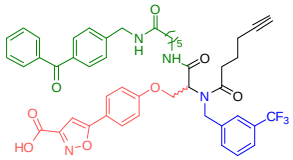
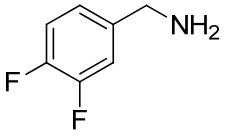
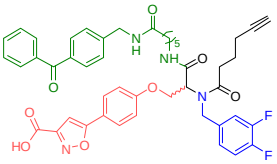
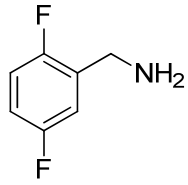
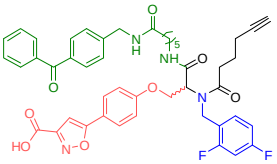
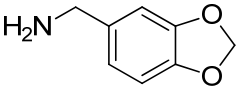
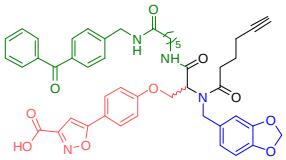
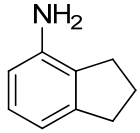
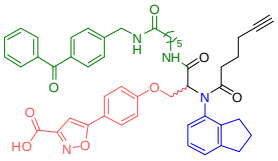
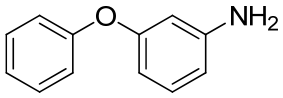
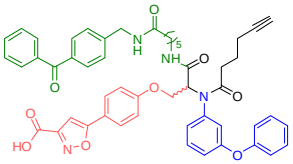
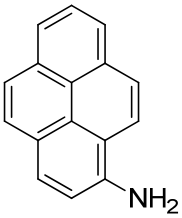
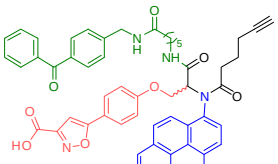
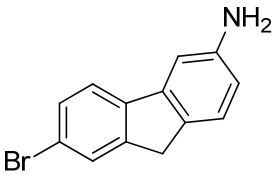
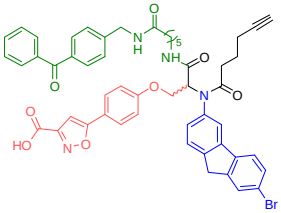
the final products and ensure the correct ID and purity (Table S1).

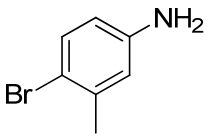
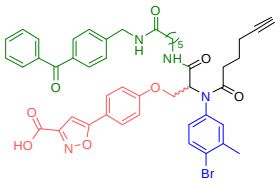
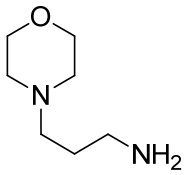
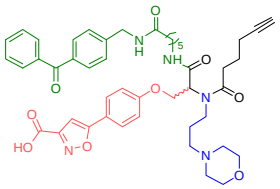
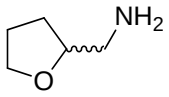
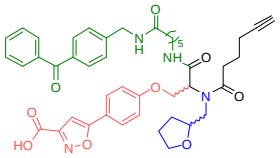
3.2 Characterization of the A*f*BPs.

Table S1. Summary of A*f*BPs and their characterizations

Amine structure	Probe structure	Cald(M+1) Found Mass	Purity (LCMS)	^{1}H NMR (Y/N)
 **4a**	 **P1**	Calcd: 783.33 Found: 783.30	> 90%	N
 **4b**	 **P2**	Calcd: 797.35 Found: 797.31	> 85%	Y
 **4c**	 **P3**	Calcd: 811.36 Found: 811.32	> 85%	N
 **4d**	 **P4**	Calcd: 825.38 Found: 825.34	> 85%	Y
 **4e**	 **P5**	Calcd: 833.35 Found: 833.32	> 90%	N
 **4f**	 **P6**	Calcd: 873.25 Found: 873.34	> 90%	N

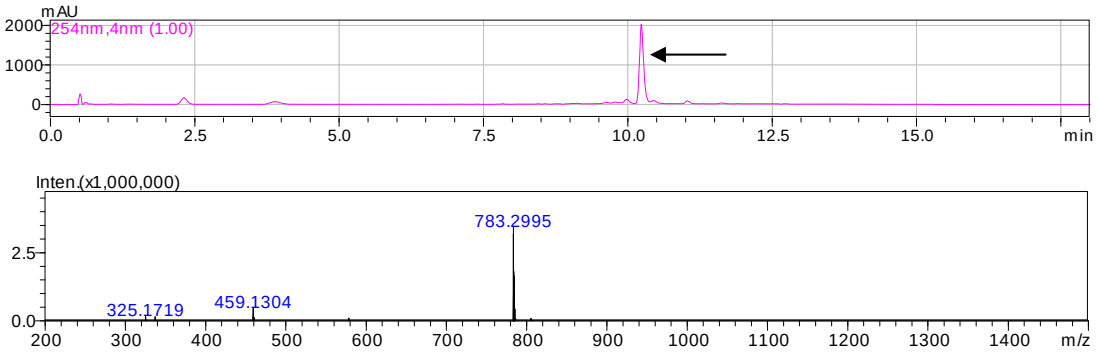
 **4g**	 **P7**	Calcd: 873.38 Found: 873.35	> 95%	N
 **4h**	 **P8**	Calcd: 887.39 Found: 887.37	> 90%	N
 **4i**	 **P9**	Calcd: 817.29 Found: 817.26	> 90%	Y
 **4j**	 **P10**	Calcd: 801.32 Found: 801.30	> 85%	Y
 **4k**	 **P11**	Calcd: 861.24 Found: 863.21 (Br)	> 85%	Y
 **4l**	 **P12**	Calcd: 839.39 Found: 839.37	> 90%	Y
 **4m**	 **P13**	Calcd: 851.32 Found: 851.29	> 95%	Y
 **4n**	 **P14**	Calcd: 813.34 Found: 813.33	> 90%	Y

 **4o**	 **P15**	Calcd: 851.32 Found: 851.28	> 90%	Y
 **4p**	 **P16**	Calcd: 819.31 Found: 819.29	> 90%	Y
 **4q**	 **P17**	Calcd: 819.31 Found: 819.29	> 90%	N
 **4r**	 **P18**	Calcd: 827.32 Found: 827.30	> 85%	Y
 **4s**	 **P19**	Calcd: 809.35 Found: 809.32	> 95%	Y
 **4t**	 **P20**	Calcd: 861.34 Found: 861.30	> 95%	Y
 **4u**	 **P21**	Calcd: 893.35 Found: 893.32	> 95%	N
 **4v**		Calcd: 935.26 Found: 937.21 (Br)	> 85%	N

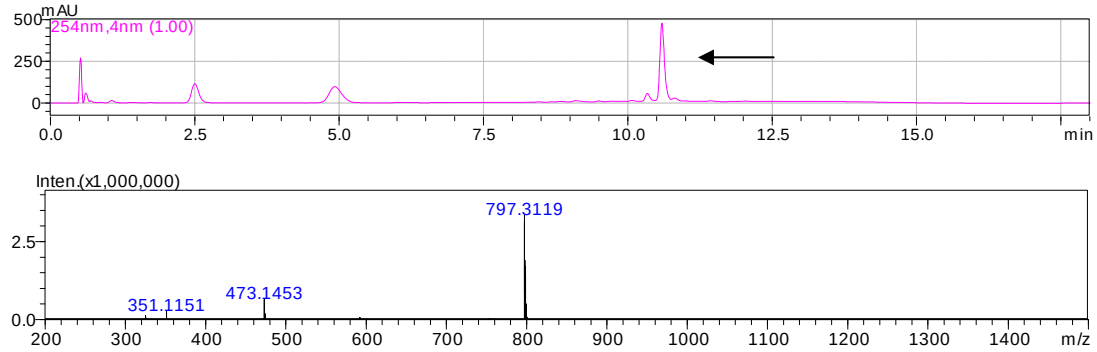
	P22			
 **4w**	 **P23**	Calcd: 861.24 Found: 863.22 (Br)	> 90%	N
 **4x**	 **P24**	Calcd: 820.38 Found: 820.36	> 50%	Y
 **4y**	 **P25**	Calcd: 777.34 Found: 777.32	> 60%	N

3.2.1 LCMS Data

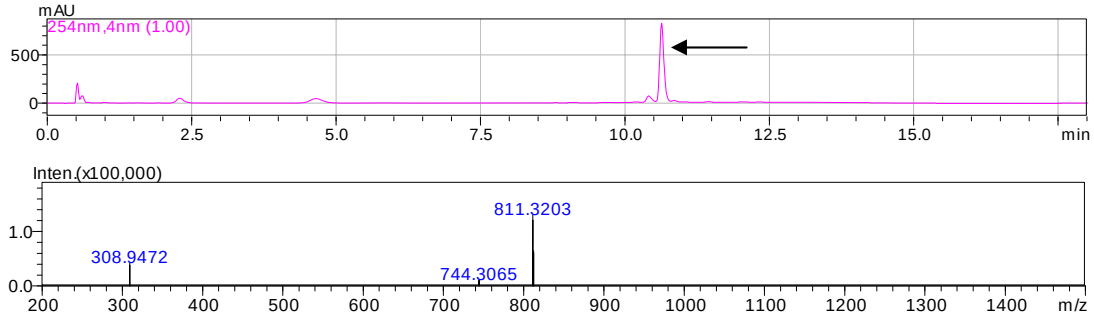
P1



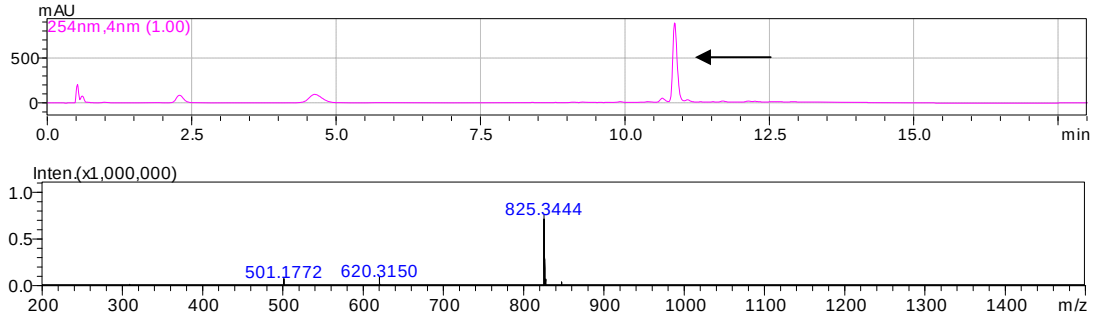
P2



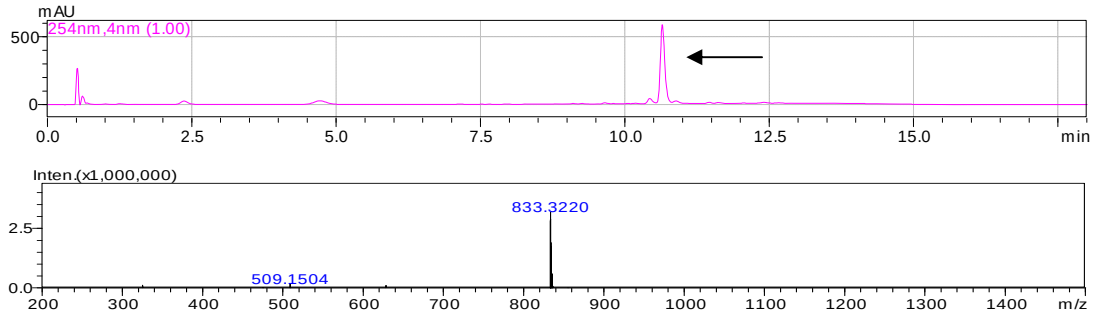
P3


mAU
254nm,4nm (1.00)
500
0
0.0
2.5
5.0
7.5
10.0
12.5
15.0
min
Inten.(x100,000)
811.3203
308.9472
744.3065
1.0
0.0
200
300
400
500
600
700
800
900
1000
1100
1200
1300
1400
m/z

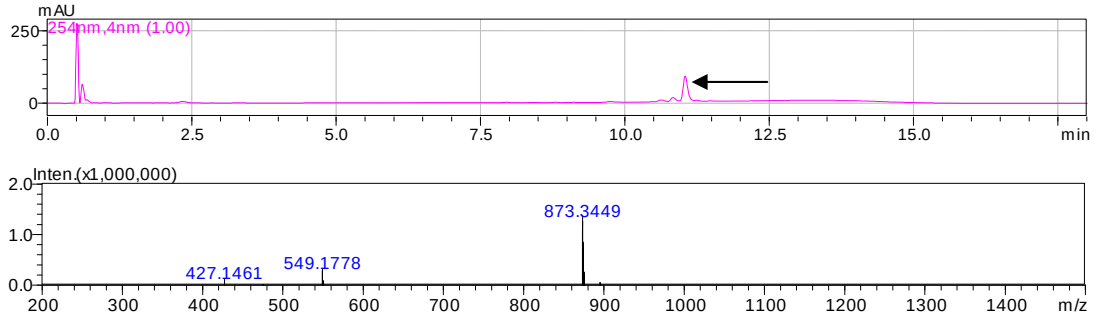
P4


mAU
254nm,4nm (1.00)
500
0
0.0
2.5
5.0
7.5
10.0
12.5
15.0
min
Inten.(x1,000,000)
825.3444
501.1772
620.3150
1.0
0.5
0.0
200
300
400
500
600
700
800
900
1000
1100
1200
1300
1400
m/z

P5


mAU
254nm,4nm (1.00)
500
0
0.0
2.5
5.0
7.5
10.0
12.5
15.0
min
Inten.(x1,000,000)
833.3220
509.1504
2.5
0.0
200
300
400
500
600
700
800
900
1000
1100
1200
1300
1400
m/z

P6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7.5
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12.5
15.0
min
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427.1461
549.1778
2.0
1.0
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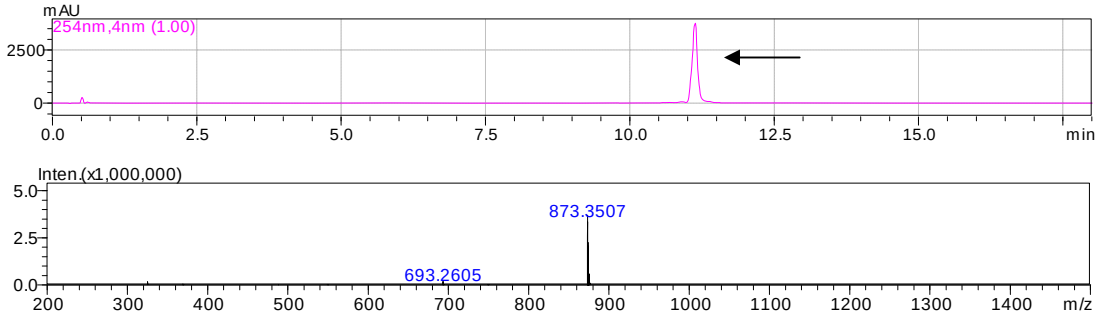
200
300
400
500
600
700
800
900
1000
1100
1200
1300
1400
m/z

P7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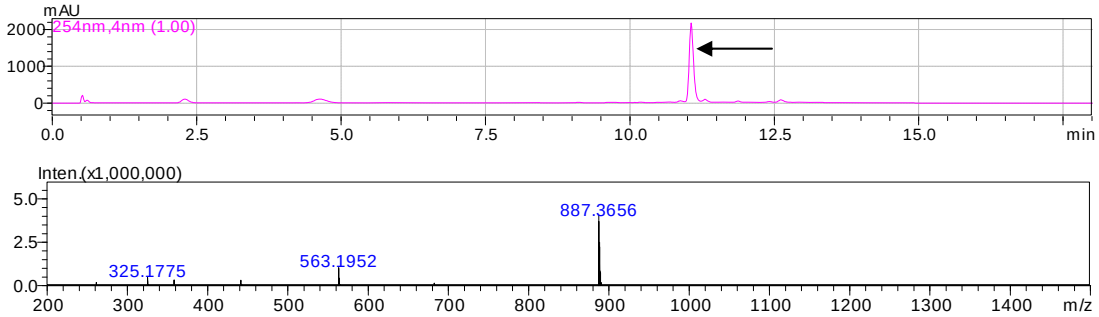
700
800
900
1000
1100
1200
1300
1400
m/z

P8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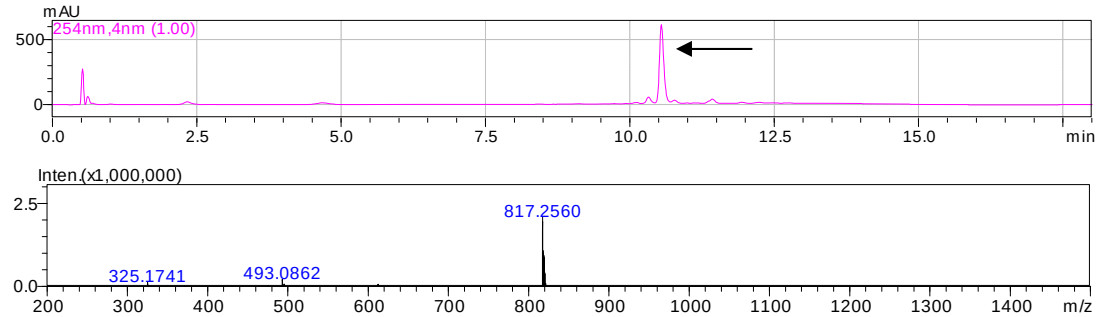
1100
1200
1300
1400
m/z

P9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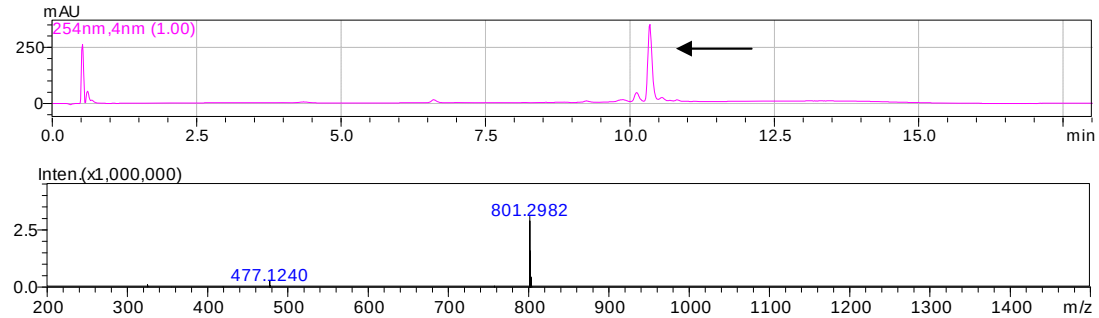
1000
1100
1200
1300
1400
m/z

P10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12.5
15.0
min
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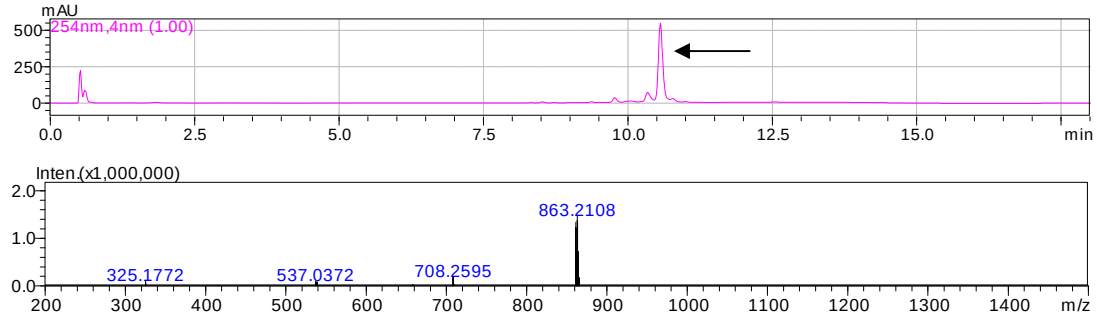
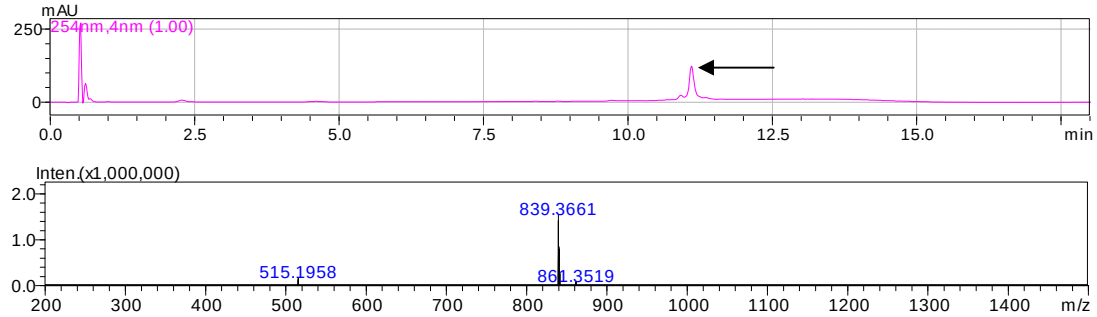
400
500
600
700
800
900
1000
1100
1200
1300
1400
m/z

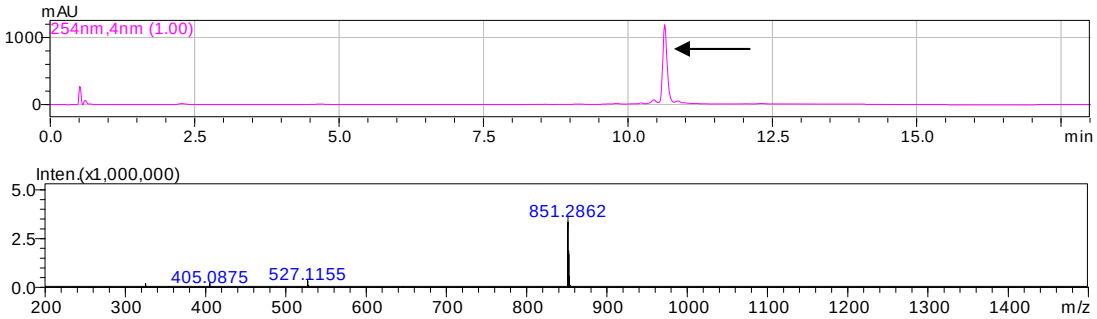
P11



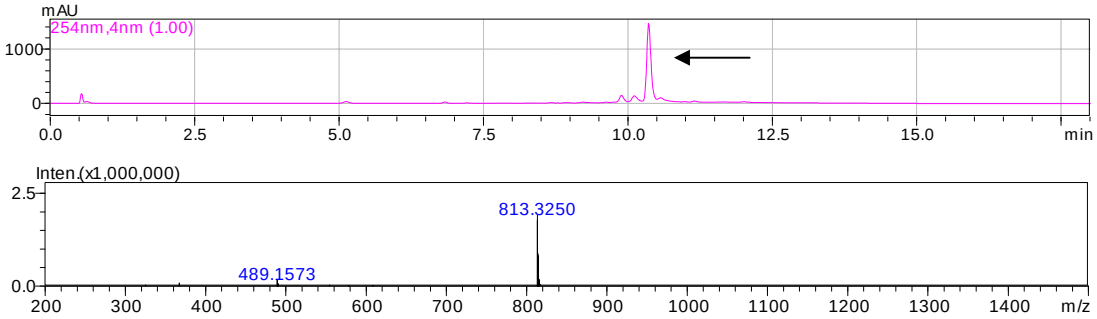
P12



P13



P14



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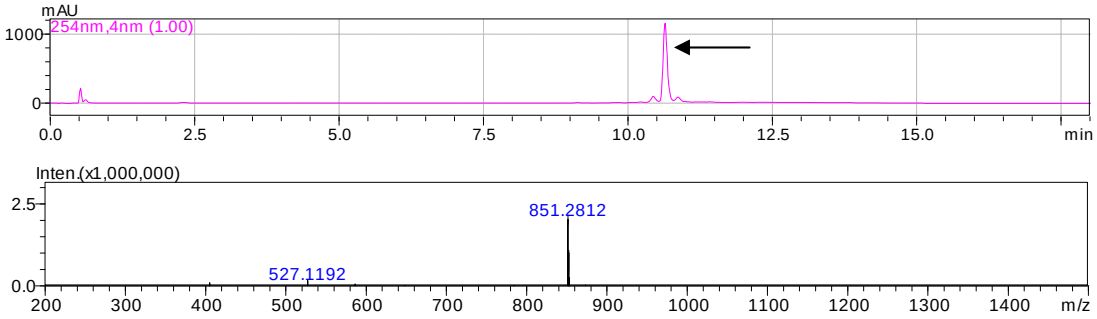

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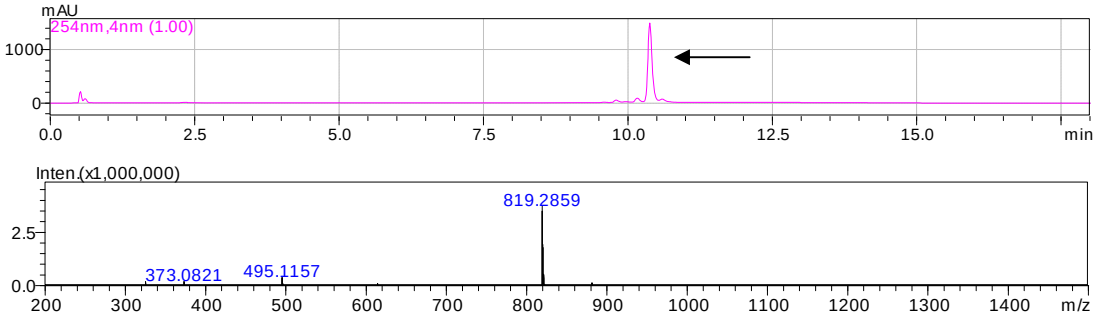

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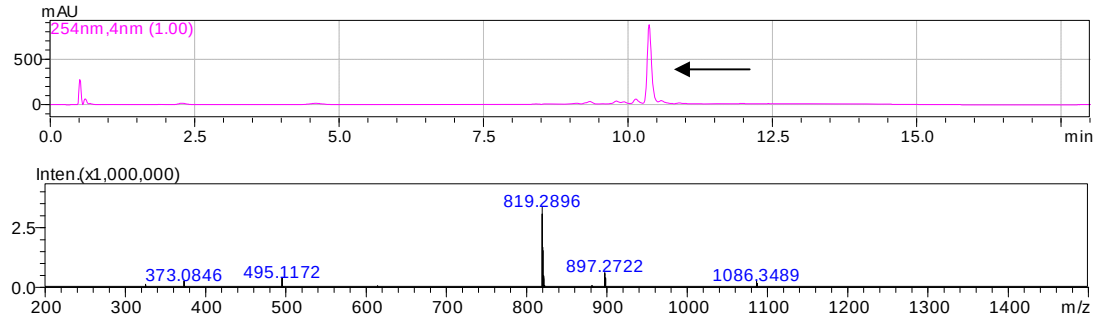
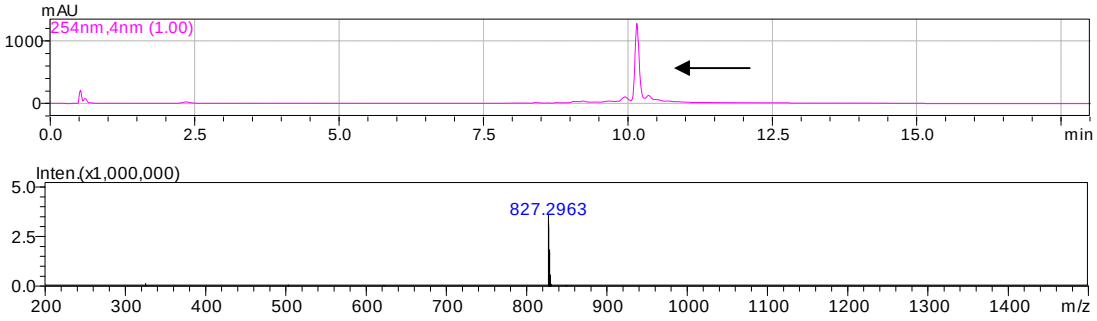


P18



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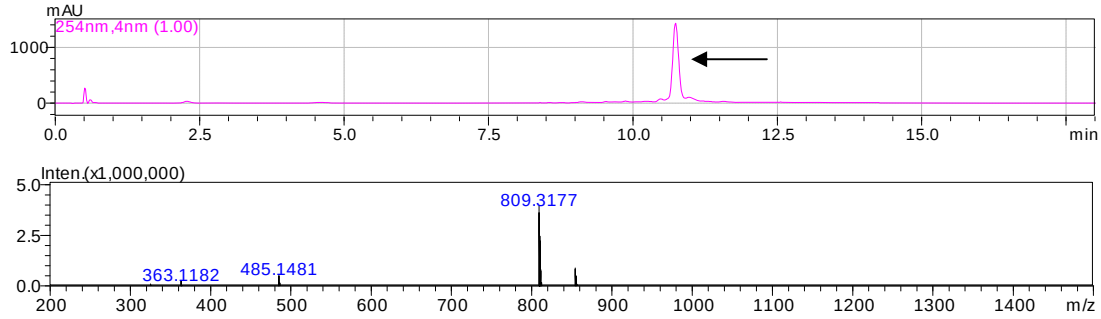

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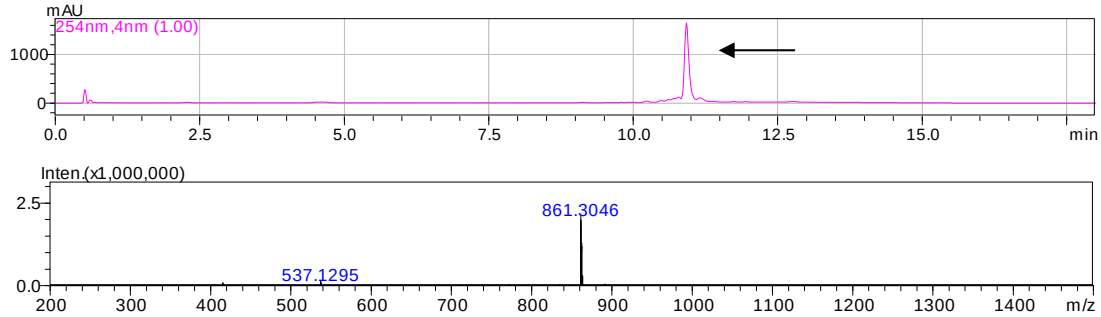
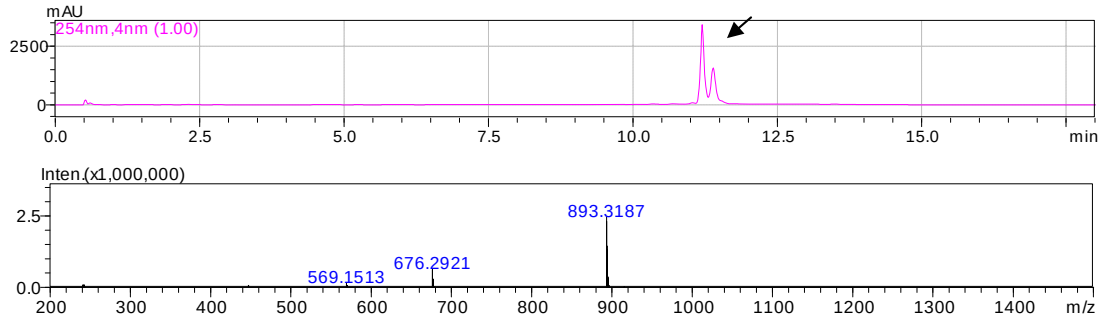
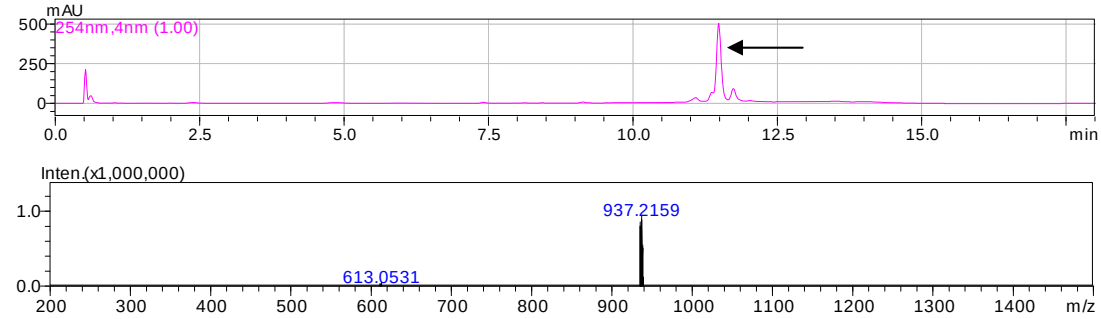


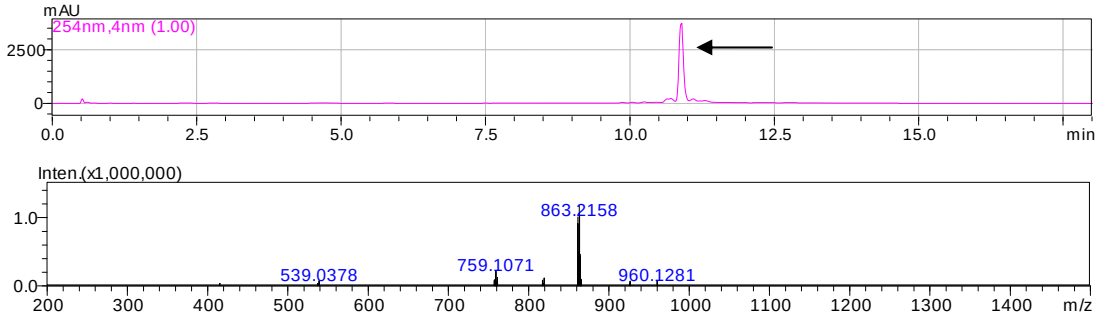
P21



P22



P23



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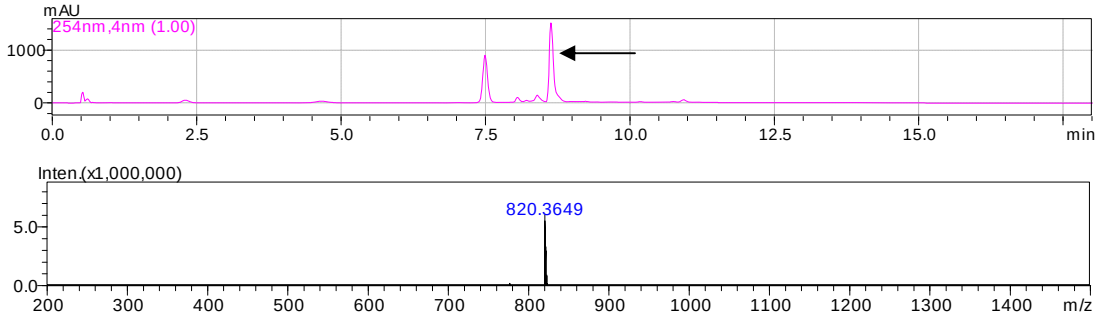
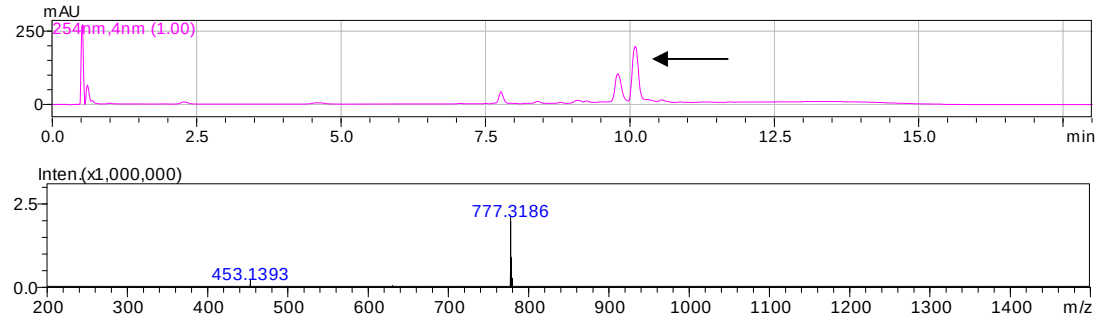


P25



3.2.2 ^{1}H NMR/MS Data

P2

^{1}H-NMR (500 MHz, DMSO-d_6) δ 8.41 - 8.42 (m, 1H), 7.91 - 8.28 (m, 1H), 7.65 - 7.72 (m, 7H), 7.55 (t, J = 7.55 Hz, 2H), 7.41 (d, J = 7.55 Hz, 2H), 7.11 - 7.29 (m, 6H), 6.88 (m, 2H), 4.80 - 5.04 (m, 1H), 4.23 - 4.46 (m, 4H), 3.53 (m, 2H), 3.06 - 3.11 (m, 2H), 2.78 - 2.85 (m, 3H), 2.11 - 2.21 (m, 6H), 1.69 (m, 2H), 1.50 - 1.51 (m, 2H), 1.39 - 1.42 (m, 2H), 1.33 (m, 2H). IT-TOF: m/z $[M+1]^{+}$ calcd: 797.35, found: 797.31.

P4

^{1}H-NMR (500 MHz, DMSO-d_6) δ 8.41 (m, 1H), 7.82 - 8.20 (m, 1H), 7.65 - 7.72 (m, 7H), 7.55 (t, J = 7.62 Hz, 2H), 7.41 (d, J = 8.20 Hz, 2H), 7.07 - 7.24 (m, 5H), 6.88 (m, 3H), 4.75 - 4.90 (m, 1H), 4.22 - 4.36 (m, 4H), 3.03 (m, 2H), 2.76 (m, 1H), 2.50 (m, 2H), 2.15 (m, 4H), 1.67 (m, 2H), 1.41 - 1.52 (m, 8H), 1.23 (m., 2H). IT-TOF: m/z [M+1] $^+$ calcd: 825.38, found: 825.34.

P9

^{1}H-NMR (500 MHz, DMSO-d_6) δ 8.41 (m, 1H), 8.08 - 8.30 (m, 1H), 7.70 - 8.07 (m, 7H), 7.66 (m, 2H), 7.42 (m, 2H), 7.28 (m, 2H), 7.14 (m, 2H), 6.83 - 6.88 (m, 3H), 4.94 - 5.25 (m, 1H), 4.58 - 4.80 (m, 2H), 4.15 - 4.39 (m, 4H), 2.99 (m, 2H), 2.70 (m, 1H), 2.34 - 2.40 (m, 1H), 2.25 (m, 1H), 2.14 - 2.18 (m, 4H), 1.67 - 1.80 (m, 2H), 1.52 (m, 2H), 1.33 (m, 2H), 1.24 (m, 2H). IT-TOF: m/z [M+1] $^+$ calcd: 817.29, found: 817.26.

P10

^{1}H-NMR (500 MHz, DMSO-d_6) δ 8.42 (m, 1H), 8.02 - 8.2 (m, 1H), 7.65 - 7.72 (m, 7H), 7.55 (t, J = 7.62 Hz, 2H), 7.41 (d, J = 8.20 Hz, 2H), 7.28 (m, 1H), 7.15 (m, 1H), 6.98 (dt, J_1 = 34.9, J_2 = 8.77 Hz, 2H), 6.81 (m, 3H), 4.89 - 5.21 (m, 1H), 4.63 - 4.78 (m, 2H), 4.09 - 4.37 (m, 4H), 2.98 (m, 2H), 2.68 (m, 1H), 2.23 - 2.32 (m, 2H), 2.08 - 2.15 (m, 4H), 1.65 - 1.80 (m, 4H), 1.51 (m, 2H), 1.33 (m, 2H), 1.22 (m, 2H). IT-TOF: m/z [M+1] $^+$ calcd: 801.32, found: 801.30.

P11

^{1}H-NMR (500 MHz, DMSO-d_6) δ 8.42 (m, 1H), 8.02-8.28 (m, 1H), 7.66 - 7.72 (m, 7H), 7.55 (t, J = 7.65 Hz, 2H), 7.41 (m, 3H), 7.20 - 7.32 (m, 2H), 7.07 (m, 1H), 6.82 (m, 3H), 4.91 - 5.23 (m, 1H), 4.63 - 4.77 (m, 2H), 4.15 - 4.37 (m, 4H), 3.00 (m, 2H), 2.70 (m, 1H), 2.23 - 2.34 (m, 2H), 2.14 (m, 4H), 1.66 - 1.68 (m, 2H), 1.52 (m, 2H), 1.32 (m, 2H), 1.21 (m. 2H). IT-TOF: m/z [M+1] $^+$ calcd: 861.24, found: 863.21. (Br isotope)

P12

^{1}H-NMR (500 MHz, DMSO-d_6) δ 8.42 (m, 1H), 7.96 - 8.22 (m, 1H), 7.63 - 7.96 (m, 7H), 7.55 (t, J = 7.55 Hz, 2H), 7.41 (d, J = 8.20 Hz, 2H), 7.22 (m,1H), 7.04 - 7.15 (m, 3H), 6.75 (m, 3H), 4.89 - 5.17 (m, 1H), 4.67 (m, 1H), 4.03 - 4.36 (m, 4H), 2.97 (m, 2H), 2.68 (s, 1H), 2.24 - 2.40 (m, 2H), 2.08 - 2.14 (m, 4H), 1.67 - 1.76 (m, 2H), 1.51 (m, 2H), 1.34 (m, 2H), 1,14 - 1,19 (m, 11H). IT-TOF: m/z [M+1] $^+$ calcd: 839.39, found: 839.37.

P13

^{1}H-NMR (500 MHz, DMSO-d_6) δ 8.41 (t, J = 5.80 Hz, 1H), 8.11 - 8.39 (m, 1H), 7.67 - 7.71 (m, 7H), 7.54 - 7.58 (m, 4H), 7.40 - 7.47 (m, 4H), 7.32 (d, J = 7.55 Hz, 1H), 6.84 (m, 2H), 4.96 - 5.30 (m, 1H), 4.77 - 4.91 (m, 2H), 4.16 - 3.37 (m, 4H), 2.93 - 2.99 (m, 2H), 2.66 - 2.69 (m, 1H), 2.23 - 2.26 (m, 1H), 2.15 (m, 1H), 2.13 -

2.14 (m, 4H), 1.68 - 1.79 (m, 2H), 1.50 (m, 2H), 1.32 (m, 2H), 1.22 (m, 2H). IT-TOF: m/z $[M+1]^+$ calcd: 851.32, found: 851.27.

P14

^{1}H-NMR (500 MHz, DMSO-d_6) δ 8.41 (m, 1H), 8.03 - 8.26 (m, 1H), 7.63 - 7.71 (m, 7H), 7.55 (m, 2H), 7.41 (d, J = 8.20 Hz, 2H), 6.61 - 7.15 (m, 7H), 4.91 - 5.22 (m, 1H), 4.61 - 4.78 (m, 2H), 4.13 - 4.37 (m, 4H), 3.57 - 3.65 (m, 3H), 2.99 (m, 2H), 2.67 (m, 1H), 2.24 - 2.35 (m, 2H), 2.11 - 2.15 (m, 4H), 1.65 - 1.69 (m, 2H), 1.51 (m, 2H), 1.35 (m, 2H), 1.23 (m, 2H). IT-TOF: m/z $[M+1]^+$ calcd: 813.34, found: 813.32.

P15

^{1}H-NMR (500 MHz, DMSO-d_6) δ 8.40 (m, 1H), 8.15 - 8.34 (m, 1H), 7.65 - 7.72 (m, 7H), 7.34 - 7.57 (m, 8H), 6.81 (m, 3H), 4.96 - 5.31 (m, 1H), 4.50 - 4.91 (m, 2H), 4.21 - 4.50 (m, 4H), 2.94 - 2.99 (m, 2H), 2.70 (m, 1H), 2.34 (m, 1H), 2.23 (m, 1H), 2.13 (m, 4H), 1.76 (m, 1H), 1.67 (m, 1H), 1.50 (m, 2H), 1.32 (m, 2H), 1.2 (m, 2H). IT-TOF: m/z $[M+1]^+$ calcd: 851.32, found: 851.28.

P16

^{1}H-NMR (500 MHz, DMSO-d_6) δ 8.42 (m, 1H), 8.08 - 8.31 (m, 1H), 7.65 - 7.72 (m, 7H), 7.56 (t, J = 7.60 Hz, 2H), 7.41 (d, J = 8.20 Hz, 2H), 7.29 (m, 1H), 7.11 (m, 2H), 6.98 (m, 1H), 6.83 (m, 2H), 4.92 - 5.24 (m, 1H), 4.65 - 4.74 (m, 2H), 4.17 - 4.37 (m, 4H), 2.99 (m, 2H), 2.67 (m, 1H), 2.36 (m, 1H), 2.24 (m, 1H), 2.14 (m, 4H), 1.65 - 1.78 (m, 2H), 1.51 (m. 2H), 1.34 (m, 2H), 1.24 (m, 2H). IT-TOF: m/z $[M+1]^+$ calcd: 819.31, found: 819.29.

P18

^{1}H-NMR (500 MHz, DMSO-d_6) δ 8.43 (m, 1H), 7.97 - 8.23 (m, 1H), 7.65 - 7.72 (m, 7H), 7.56 (t, J = 7.60Hz, 2H), 7.41 (d, J = 8.20 Hz, 2H), 6.61 - 6.82 (m, 6H), 5.77 - 5.92 (m, 2H), 4.87 - 5.14 (m, 1H), 5.53 - 4.67 (m, 2H), 4.06 - 4.37 (m, 4H), 2.30 (m, 2H), 2.64 (m, 1H), 2.23 - 2.37 (m, 2H), 2.14 (m, 4H), 1.66 - 1.68 (m, 2H), 1.51 (m, 2H), 1.34 (m, 2H), 1.22 (m, 2H). IT-TOF: m/z $[M+1]^+$ calcd: 827.32, found: 827.30.

P19

^{1}H-NMR (500 MHz, DMSO-d_6) δ 8.41 (m, 1H), 8.02 - 8.15 (m, 1H), 7.61 - 7.72 (m, 7H), 7.55 (t, J = 7.55 Hz, 2H), 7.41 (m, 3H), 7.12 - 7.22 (m, 2H), 6.93 - 7.03 (m, 1H), 6.73 (m, 1H), 5.21 - 5.26 (m, 1H), 3.84 - 4.36 (m, 4H), 2.81 - 2.88 (m, 4H), 2.68 - 2.71 (m, 2H), 1.98 - 2.18 (m, 8H), 1.52 - 1.63 (m, 4H), 1.40 (m, 2H), 1.25 (m, 2H). IT-TOF: m/z $[M+1]^+$ calcd: 809.35, found: 809.32.

P20

^{1}H-NMR (500 MHz, DMSO-d_6) δ 8.41 (m, 1H), 8.10 (m, 1H), 7.65 - 7.72 (m, 7H), 7.55 (t, J = 7.55 Hz, 2H), 7.32 - 7.42 (m, 6H), 6.89 - 7.12 (m, 8H), 5.31 (m, 2H), 4.00 - 4.36 (m, 4H), 3.02 (m, 2H), 2.68 (s, 1H), 2.07 - 2.17 (m, 6H), 1.61 (m, 2H),

1.52 (m, 2H), 1.38 (m, 2H), 1.23 (m, 2H). IT-TOF: m/z $[M+1]^+$ calcd: 861.34, found: 861.30.

P23

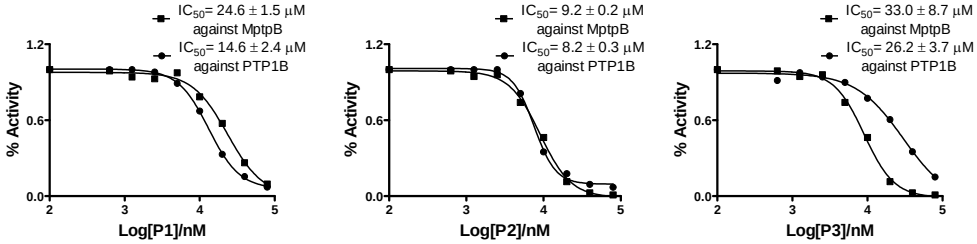
^{1}H-NMR (500 MHz, DMSO-d_6) δ 8.40 (m, 1H), 8.15 (m, 1H), 7.65 - 7.72 (m, 7H), 7.55 (m, 3H), 7.40 (m, 3H), 7.19 (s, 1H), 6.87 - 6.94 (m, 3H), 5.30 (m, 1H), 3.94 - 4.37 (m, 4H), 3.07(m, 2H), 2.68 (s, 1H), 2.05 - 2.23 (m, 9H), 1.53 - 1.63 (m, 4H), 1.41 (m, 2H), 1.27 (m, 2H). IT-TOF: m/z $[M+1]^+$ calcd: 861.24, found: 863.22. (Br isotope)

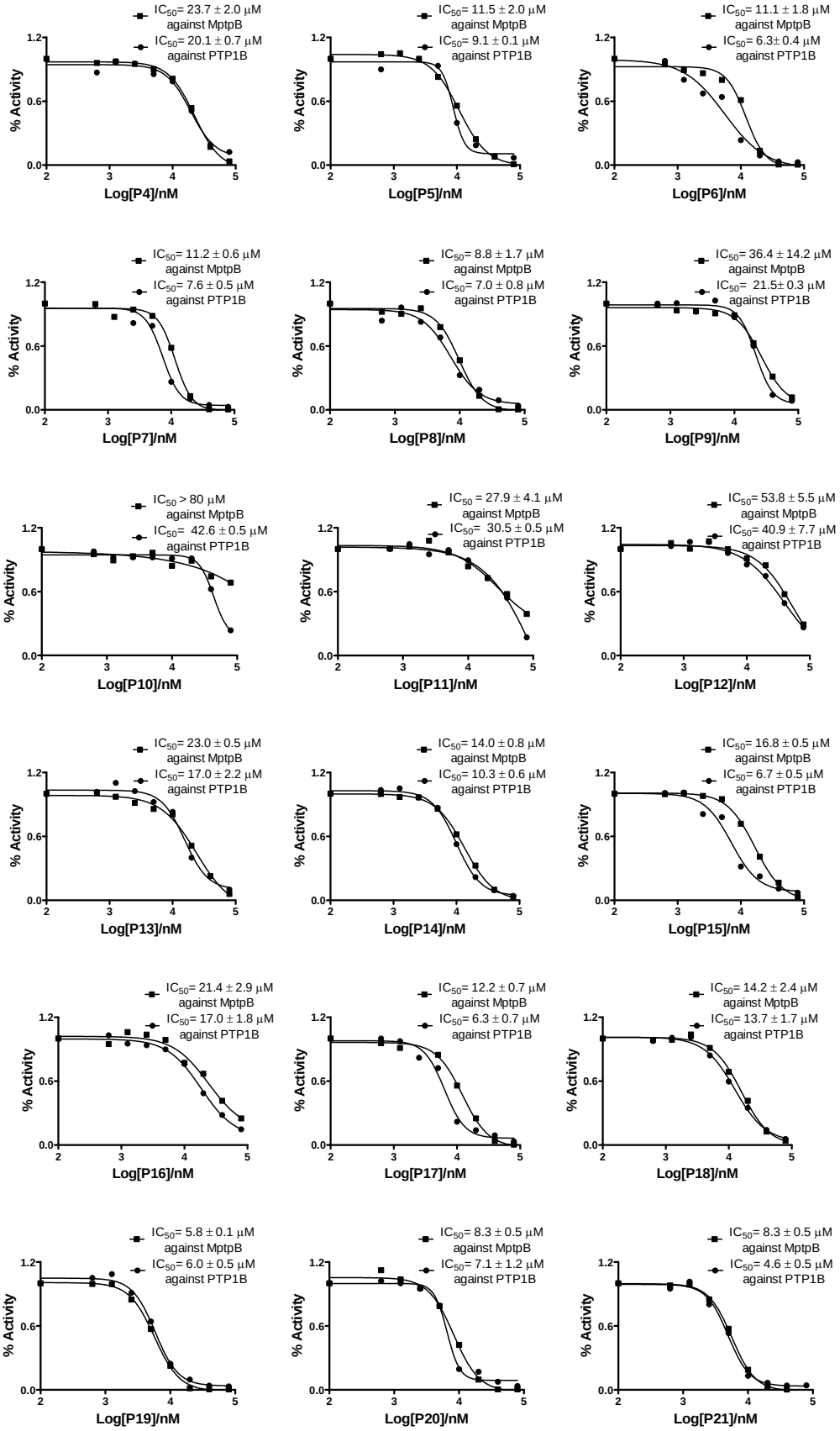
4. Procedures and results of inhibition assay[3]

The inhibition of MCR products to against protein tyrosine phosphatases (PTPs) was assessed by measuring the rate of hydrolysis of the fluorogenic substrate, 6,8-difluoromethylumbellifery phosphate (DIFMUP, Invitrogen, USA) in 25 μL reaction volumes in black polypropylene flat-bottom 384-well microtiter plates(Greiner, Germany), using PTP1B and MptpB as model proteins. For IC_{50} studies, dose-dependent reactions were set up by varying the concentration of each inhibitor while maintaining a fixed enzyme and substrate concentration. Briefly, a two-fold dilution series of an inhibitor, from approximately 400 μM to 3.125 μM (final concentrations) was prepared. The reaction conditions are shown below:

PTP1B (2 μg/mL) = 10 μL
DiFMUP (10 μM) = 10 μL
Inhibitor (Varied) = 5 μL (in 40% DMSO)
Assay buffer (PTP1B) = 25 mM HEPES, 150 mM NaCl, 0.1 mg/mL BSA *p*H=7.5
Assay buffer (MptpB) = 25 mM HEPES, 2.5 mM EDTA, 50 mM NaCl, 0.02% Triton-100, 2 mM DTT, *p*H = 7.4

Negative controls were performed in the absence of enzyme and positive controls were carried out in the presence of enzyme with DMSO (i.e. without inhibitor). The reactions were allowed to incubate at room temperature for 30 min before being initiated by addition of DIFMUP. The enzymatic reactions were immediately monitored with a Synergy™4 Multi-Mode Microplate Reader (BioTek, USA), at λ_{ex}= 355 nm and λ_{em}= 460 nm for a period of 15 min. Each IC_{50} plot was generated by averaging duplicates from two independent assays (Figure S1).




IC50= 23.7 ± 2.0 μM against MptpB
IC50= 20.1 ± 0.7 μM against PTP1B
% Activity
Log[P4]/nM
IC50= 11.5 ± 2.0 μM against MptpB
IC50= 9.1 ± 0.1 μM against PTP1B
Log[P5]/nM
IC50= 11.1 ± 1.8 μM against MptpB
IC50= 6.3± 0.4 μM against PTP1B
Log[P6]/nM
IC50= 11.2 ± 0.6 μM against MptpB
IC50= 7.6 ± 0.5 μM against PTP1B
Log[P7]/nM
IC50= 8.8 ± 1.7 μM against MptpB
IC50= 7.0 ± 0.8 μM against PTP1B
Log[P8]/nM
IC50= 36.4 ± 14.2 μM against MptpB
IC50= 21.5± 0.3 μM against PTP1B
Log[P9]/nM
IC50 > 80 μM against MptpB
IC50= 42.6 ± 0.5 μM against PTP1B
Log[P10]/nM
IC50 = 27.9 ± 4.1 μM against MptpB
IC50= 30.5 ± 0.5 μM against PTP1B
Log[P11]/nM
IC50= 53.8 ± 5.5 μM against MptpB
IC50= 40.9 ± 7.7 μM against PTP1B
Log[P12]/nM
IC50= 23.0 ± 0.5 μM against MptpB
IC50= 17.0 ± 2.2 μM against PTP1B
Log[P13]/nM
IC50= 14.0 ± 0.8 μM against MptpB
IC50= 10.3 ± 0.6 μM against PTP1B
Log[P14]/nM
IC50= 16.8 ± 0.5 μM against MptpB
IC50= 6.7 ± 0.5 μM against PTP1B
Log[P15]/nM
IC50= 21.4 ± 2.9 μM against MptpB
IC50= 17.0 ± 1.8 μM against PTP1B
Log[P16]/nM
IC50= 12.2 ± 0.7 μM against MptpB
IC50= 6.3 ± 0.7 μM against PTP1B
Log[P17]/nM
IC50= 14.2 ± 2.4 μM against MptpB
IC50= 13.7 ± 1.7 μM against PTP1B
Log[P18]/nM
IC50= 5.8 ± 0.1 μM against MptpB
IC50= 6.0 ± 0.5 μM against PTP1B
Log[P19]/nM
IC50= 8.3 ± 0.5 μM against MptpB
IC50= 7.1 ± 1.2 μM against PTP1B
Log[P20]/nM
IC50= 8.3 ± 0.5 μM against MptpB
IC50= 4.6 ± 0.5 μM against PTP1B
Log[P21]/nM

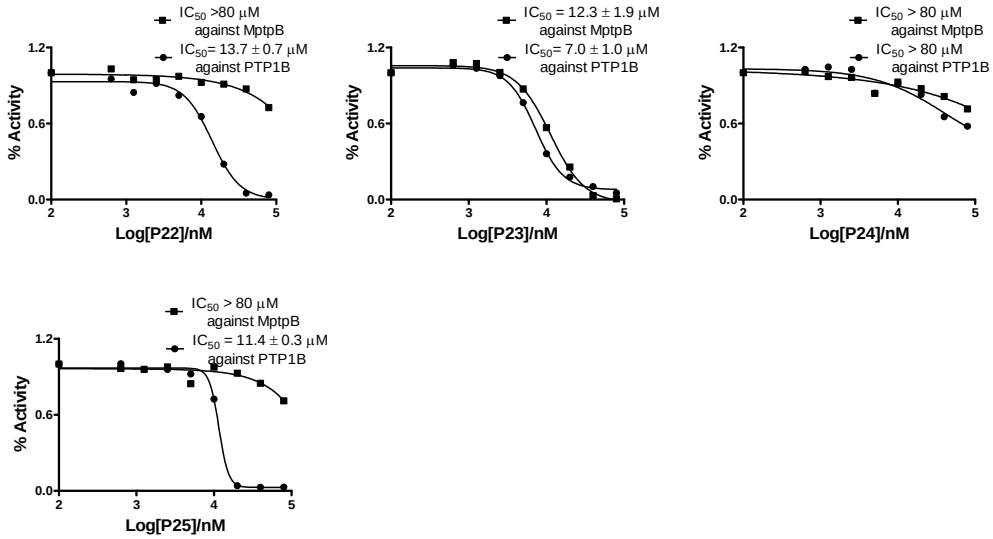


Figure S1. IC_{50} graphs of all 25 A*f*BPs against PTP1B and MptpB

Table S2 Summary of IC_{50} values of 25 A*f*BPs against PTP1B/MptpB

Probe ID	IC_{50} (μM)		Probe ID	IC_{50} (μM)	
	PTP1B	MptpB		PTP1B	MptpB
P1	14.6 ± 2.4	24.6 ± 1.5	**P14**	10.3 ± 0.6	14.0 ± 0.8
P2	8.2 ± 0.3	9.2 ± 0.2	**P15**	6.7 ± 0.5	16.8 ± 0.5
P3	26.2 ± 3.7	33.0 ± 8.7	**P16**	17.0 ± 1.8	21.4 ± 2.9
P4	20.1 ± 0.7	23.7 ± 2.0	**P17**	6.3 ± 0.7	12.2 ± 0.7
P5	9.1 ± 0.1	11.5 ± 2.0	**P18**	13.7 ± 1.7	14.2 ± 2.4
P6	6.3 ± 0.4	11.1 ± 1.8	**P19**	6.0 ± 0.5	5.8 ± 0.1
P7	7.6 ± 0.5	11.2 ± 0.6	**P20**	7.1 ±1.2	8.3 ± 0.5
P8	7.0 ± 0.8	8.8 ± 1.7	**P21**	4.6 ± 0.5	5.3 ± 0.5
P9	21.5 ± 0.3	36.4 ± 14.2	**P22**	13.7 ± 0.7	> 80
P10	42.6 ± 0.5	> 80	**P23**	7.0 ± 1.0	12.3 ± 1.9
P11	30.5 ± 0.5	27.9 ± 4.1	**P24**	> 80	> 80
P12	40.9 ± 7.7	53.8 ± 5.5	**P25**	11.4 ± 0.3	> 80
P13	17.0 ± 2.2	23.0 ± 0.5			

5. Procedures and results of labeling experiments

5.1 General Information

PTP1B and MptpB containing His_6-tag were expressed in *E.coli* strain BL21-DE3, as previously described,[1] and purified from the lysates on Nickel-nitrilotriacetic acid (Ni-NTA) metal-affinity chromatography matrices (Qiagen) according to the manufacturer's instructions. The purified proteins were then

dialyzed against dialysis buffer and stored in 30% glycerol at -20 °C before use. (Dialysis buffer: PTP1B: 10 mM Tris·HCl, 25 mM NaCl, *p*H = 7.5; MptpB: 50 mM Tris·HCl, 100 mM NaCl, *p*H = 7.4). Stock solutions of enzymes were prepared in final concentrations of 2-5 mg/mL (in dialysis buffer). Stock solutions of the probes were prepared in DMSO and stored at before use. UV photolysis experiments were carried out using a B100A UV lamp (UVP, USA). Fluorescence imaging was performed using a Typhoon 9410 fluorescence gel scanner at λ = 533 nm and analyzed with the ImageQuant Software.

5.2 General Procedure for enzyme labeling

The enzyme stock solutions were diluted to 1 mg/mL. Generally 1 μL enzyme solution was added into 14 μL dialysis buffer solution with 1 μL probe and shaken at room temperature in the dark for 30 min. The mixture was next irradiated under the long-rang wavelength UV channel for 20 min on the ice. Subsequently, 1 μL of rhodamine-N_3 and the click reagents (total 3 μL; see reference 4) were added into the solution (final solution contained $CuSO_4$ (500 μM), TBTA liagnd (100 μM) and the reducing reagent (PTP1B: sodium ascorbate 250 μM; MptpB: DTT 500 μM)). The whole solution (total 20 μL) was shaken at room temperature for 2 hours. The reaction was then quenched by addition of 4 μL of 6 × SDS loading dye followed by boiling at 95 °C for 10 min. The samples were analyzed on a 10% denaturing SDS-PAGE gel. The fluorescence was detected with fluorescence gel scanner.

To determine the concentration-dependent labeling with varied amounts of the enzyme, 1 μL of MptpB (2000 ng, 1000 ng, 500 ng, 250 ng) was added into different reaction vessels, each containing 5 μM of the probe **P15**. The samples were then treated as mentioned above. To determine the concentration-dependent labeling with varied amounts of the probe **P15**, 1 μg of MptpB was incubated with probe **P15** (20, 10, 5, 2.5, 1.25, 0.6, 0.3, 0 μM). The reaction was incubated for 30 min and treated as mentioned above.

For time-dependent experiments, identical reaction mixtures containing 1 μL of MptpB and 5 μM of the probe **P15** were similarly set up. The reactions were incubated at room temperature in the dark for 30 min. The mixtures were then irradiated for varied lengths of time (10, 15, 20, 30, 60 min) with long-wavelength UV light. Then the mixture was analyzed as mentioned above.

For labeling experiments with different click reagents and click reaction time, the reactions were prepared as mentioned above by using the probe **P15**. Varied amounts of $CuSO_4$ (100, 200, 500 μM) were used. Then the mixture was treated as described above. Probes **P12** and **P17** were used to test the optimized amount of the ligand (TBTA, tris[(1-benzyl-1H-1,2,3-triazol-4-yl)methyl]amine) in the labeling of MptpB. Probe **P15** was used to test the difference in the reducing agent (i.e. DTT vs sodium ascorbate) in the labeling reaction. Different amounts of sodium ascorbate (250, 500, 1000 μM) and DTT (125, 250, 500, 1000 μM) were used. All other conditions were similar as above mentioned. To optimize the click reaction time, after addition of the click reagent as mentioned above, the reaction was further incubated for 0.5, 1, 1.5, 2, and 3 hours.

For heat-denaturing experiments, MMptpB (in Tris buffer) was heated at 95 °C for 10 min and allowed to cool to room temperature. Each reaction was set up using 1000 ng denatured and normal MptpB. Different probes (**P13**, **P14**, **P19** and **P20**) were used. Other procedures were the same as above described.

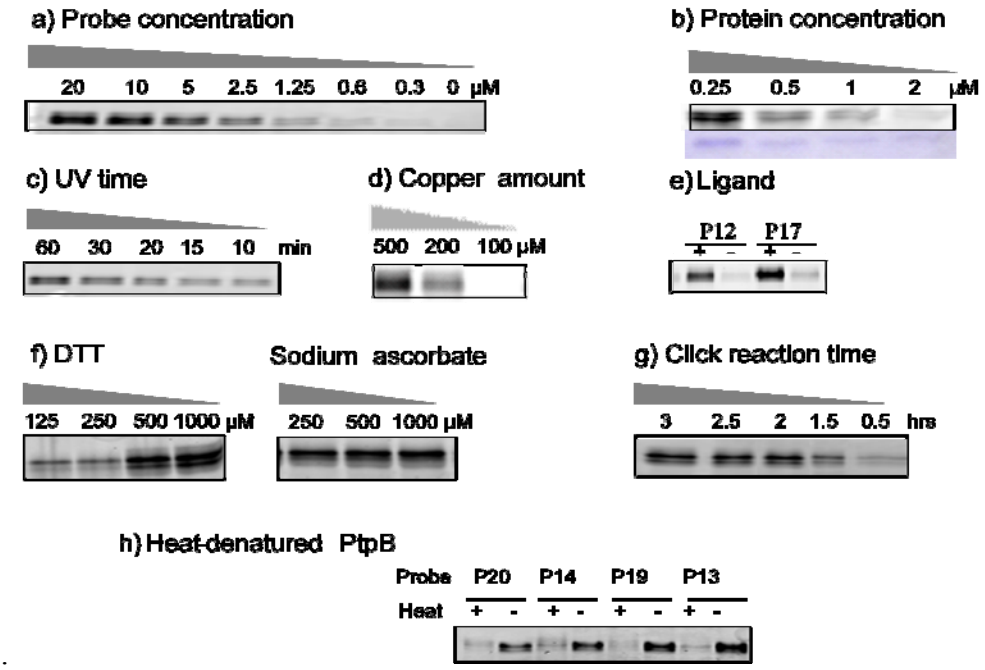


Figure S2 a) Effect of probe **P15** concentration on labeling intensity: MptpB was incubated with decreasing concentrations of **P15** (final concentration: 20, 10, 5, 2.5, 1.25, 0.6, 0.3, and 0 μM, respectively); b) Effect of protein concentrations on labeling intensity: different MptpB concentrations (final concentration: 2, 1, 0.5, and 0.25 μg, respectively); c) Effect of UV irradiation time on labeling intensity: MptpB was exposed to long-wavelength UV light for varying amounts of time (60, 30, 20, 15, 10 min, respectively); d) Effect of copper amount on labeling intensity: click reaction was carried out with increasing amounts of $CuSO_4$ (final concentration: 100, 200, and 500 μM); e) Effect of TBTA ligand on labeling intensity: two different probes were incubated with or without the TBTA ligand (final concentration: 125 μM); f) Effect of reducing reagents on labeling intensity: MptpB was incubated with different concentrations of DTT (final concentration: 125, 250, 500, and 1000 μM, respectively); PTP1B was incubated with different concentrations of sodium ascorbate (final concentration: 250, 500, and 1000 μM); g) Effect of click time on the labeling intensity: click reactions were carried with increasing time (3, 2.5, 2, 1.5 and 0.5 hours, respectively); h) Different probes (**P13**, **P14**, **P19**, and **P20**; final probe concentration: 5 μM) against heat-denatured and native MptpB.

5.3 Affinity-based profiling of MptpB/PTP1B by using 25 A*f*BPs.

The following optimized labeling conditions were used. The labeling procedure was the same as mentioned above. Results are shown in Figure 3 in the maintext.

PTP1B: protein 1 μg; probe 5 μM; dye 40 μM; Copper sulfate 500 μM; ligand 100 μM; sodium ascorbate 500 μM; UV time 20 min; click time 2 hours.

MptpB: protein 1 μg; probe 5 μM; dye 40 μM; Copper sulfate 500 μM; ligand 100 μM; DTT 500 μM; UV time 20 min; click time 2 hours.

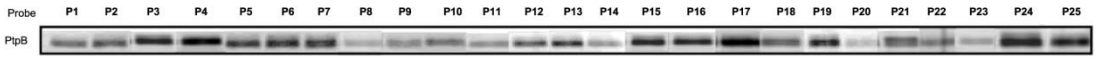


Figure S3 Gel profiling of MptpB generated by labeling against 25 probes

5.4 *In vitro* labeling mammalian proteomes and pull-down/LC-MS/MS analysis.

In vitro proteome labeling experiments were carried out with MCF-7 cell lysates, as previously described.[4] **P23** (20 μM) was incubated with MCF-7 cell lysates (10 mg/10 mL in a chilled buffer of 50 mM Tris·HCl, 150 mM NaCl, *p*H 8.0, 1.0% NP-40, 100 μM PMSF) for 20 min at room temperature. Then the samples were irradiated for 45 min under UV light. Subsequently, a small amount of the irradiated sample was treated with rhodamine-N_3 (100 μM) and click reagent (1 mM $CuSO_4$, 1 mM TCEP, 100 μM TBTA) for 3 hours. Following acetone precipitation, washing (2x with methanol), resolubilization (in 1 × SDS loading buffer) and heating (10 min at 95 °C), the sample was separated by SDS-PAGE (10% gel) and visualized by in-gel fluorescence scanning on a typhoon 9410 variable mode imager scanner. The fluorescent gel was shown in Figure 4a in maintext. The remaining labeled lysates (prior to click chemistry) were used for large-scale pull-down/LC-MS analysis. Briefly, the sample was treated with biotin-N_3 (100 μM) and click reagent (1 mM $CuSO_4$, 1 mM TCEP, 100 μM TBTA) for 3 hours.[4] Subsequently, it was acetone-precipitated, washed (2x methanol) and resolubilized in PBS (containing 0.1% SDS) with sonication. The sample was then incubated with avidin-agarose beads (Thermo Scientific) for 4 hours at room temperature. After centrifugation, the supernatant was removed and the beads were washed with washing buffers (3x with 1 M NaCl, 20 mM Tris·HCl, 5 mM EDTA, 0.1% NP-40, *p*H = 7.3; 6x with 2 mM Tris·HCl, 0.5 mM EDTA, 0.1% NP-40, *p*H = 7.3; 6x with 4 M urea, 10 mM Tris·HCl, 1 mM EDTA, 0.1% NP-40, *p*H = 7.3; 2x with 2 mM Tris·HCl, 0.5 mM EDTA, *p*H = 7.3). The beads were then boiled in 1 × SDS loading buffer for 10 min. Samples were separated by SDS-PAGE gels and stained by colloidal blue solution. Gel lanes corresponding to both DMSO- and probe-treated samples were then cut into small slices, respectively. Next, trypsin digestion (In-Gel Trypsin Digestion Kit, Pierce Co., USA) was performed for the whole pull-down proteins. Digested peptides were then extracted from the gel with 50% acetonitrile and 1% formic acid in H_2O. Two samples was then dried *in vacuo* and stored at -20 °C for further LCMS analysis. Briefly, samples were analyzed as previously described.[4] The samples were resuspended in 0.1% formic acid in H_2O. The peptides were separated and analyzed on a Shimadzu UFLC system (Shimadzu, Kyoto, Japan) coupled to an LTQ-FT Ultra (Thermo Electron, Germany). Peptides were then analyzed on LTQ-FT with an ADVANCE™ CaptiveSpray™Source (Michrom BioResources, USA) and the raw data were analyzed using an in-house Mascot Server (version 2.2.07, Matrix Science,

UK) with MS tolerance of 10 ppm and MS/MS tolerance of 0.8 Da. Two missed cleavage sites of trypsin were allowed. Carbamidomethylation (C) was set as a fixed modification. And oxidation (M) was set as variable modifications. After data analysis, a large number of proteins were identified from each LCMS experiments, many of which are non-specific binders and need to be removed. For those proteins with cores of < 50, they were automatically removed from the list. For those proteins that appeared in the negative run (pull-down/LCMS experiments with DMSO only), they also were excluded from the list as well. Highly abundant proteins that commonly appeared in irrelevant pull-down experiments were further removed.[4] The final list was shown in ESI_2, with selected hits shown in Figure 4 in the maintext.

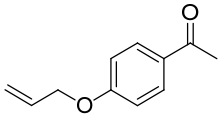
5..5 Target validation by pull-down/western blotting analysis

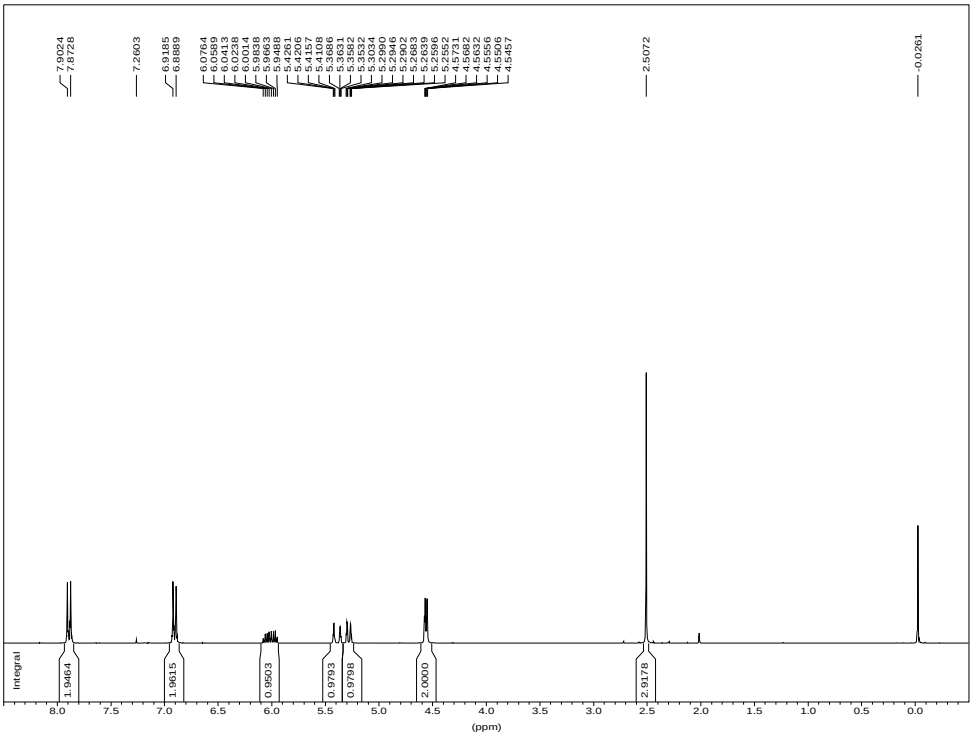
Pull-down samples were similarly prepared as described above. After SDS-PAGE gel separation, proteins were transferred to a PVDF membrane and subsequently blocked with 5% non-fat milk at room temperature for 1 hour. The membrane were further incubated for 1 hour at room temperature with anti-Biotin (Cell Signaling technology) and washed with TBST three times, then developed using Western blotting kit (Amersham ECL™ Advance).[4] For target validation, the membranes (prepared as before, prior to incubation with primary antibody) were incubated for 1 hour at room temperature with the respective antibodies (anti-PTP1B (Abcam, ab52650) [5], anti-prohibitin (Santa Cruz, sc-18198), and anti-Cathepsin D (Santa Cruz, sc-70513)). After washing, proper secondary antibody was applied for 1 hour at room temperature. The blot was developed as before. Results are shown in Figure 4 in the maintext.

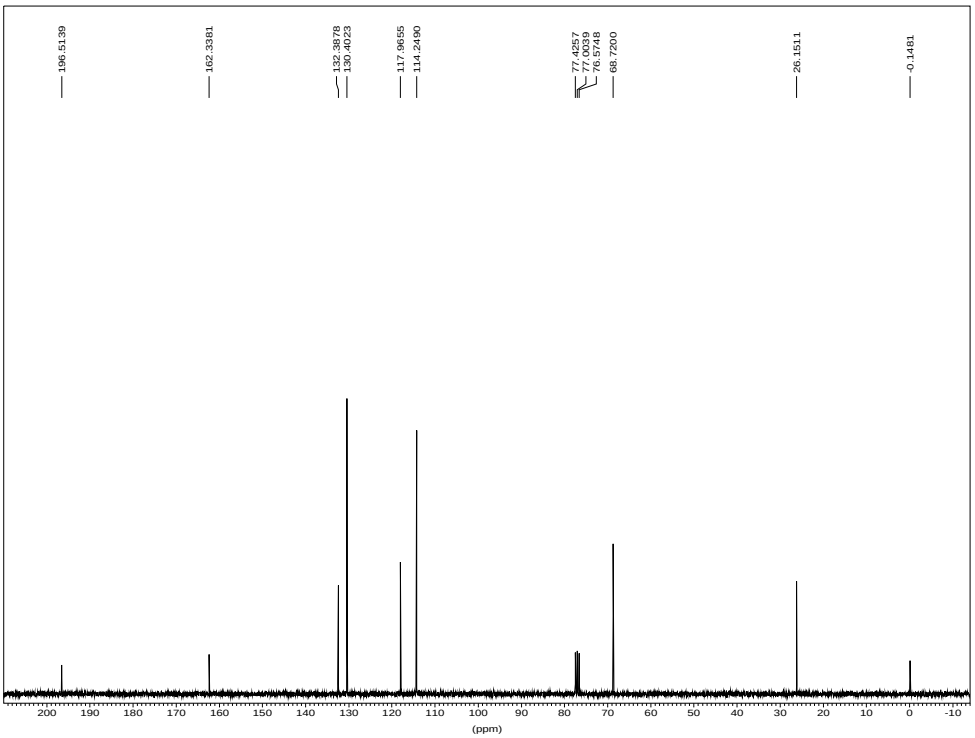
6. References

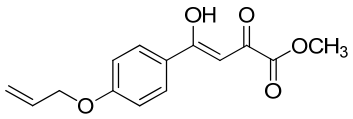
1. (1) R. Srinivasan, L. P. Tan, H, Wu, S. Q. Yao, *Org. Lett.*, 2008, **10**, 2295-2298; (b) L. P. Tan, H. Wu, P.-Y. Yang, K. A. Kalesh, X. Zhang, M. Hu, R. Srinivasan, S. Q. Yao, *Org. Lett.*, **2009**, *11*, 5102-5105.
2. M. B. Francis, T. Nguyen, *Org. Lett.*, 2003, **5**, 3245-3248.
3. R. Srinivasan, M. Uttamchandani, S. Q. Yao, *Org. Lett.*, 2006, **8**, 713-716.
4. P. -Y. Yang, K. Liu, M. H. Ngai, M. J. Lear, M. Wenk, S. Q. Yao, *J. Am. Chem. Soc.*, 2010, **132**, 656-666.
5. K. A. Kalesh, L. P. Tan, K. Liu, L. Gao, J. Wang, S. Q. Yao, *Chem. Comm.*, 2010, **46**, 1118-1120.

7. ^{1}H and ^{13}C NMR spectra





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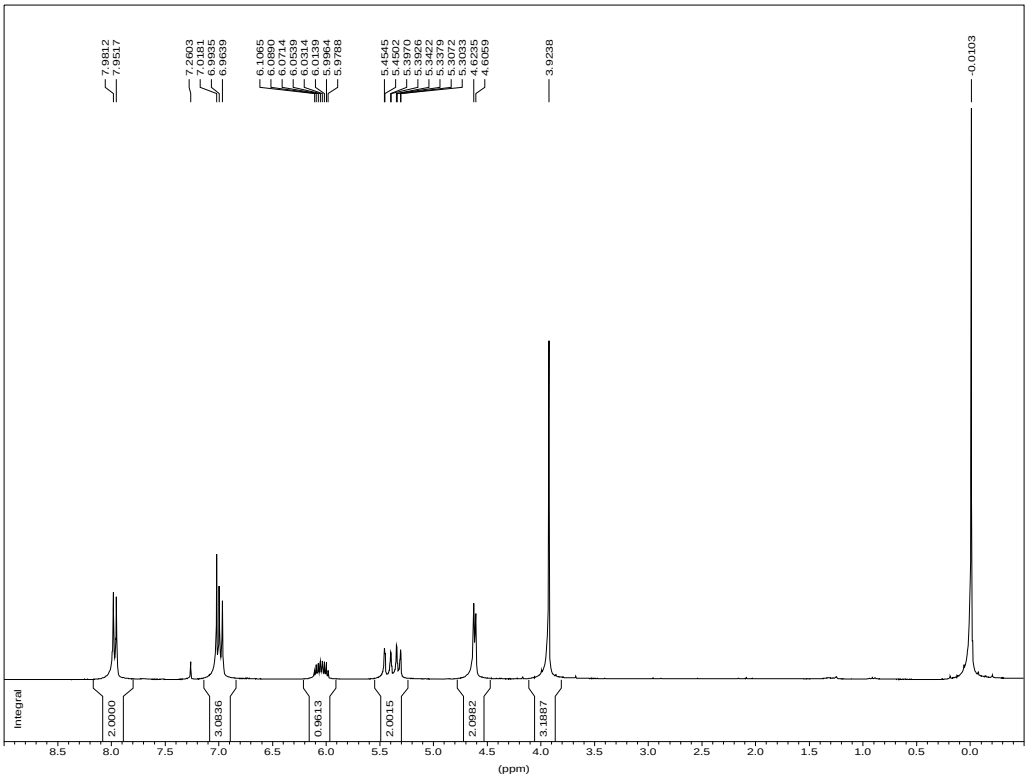
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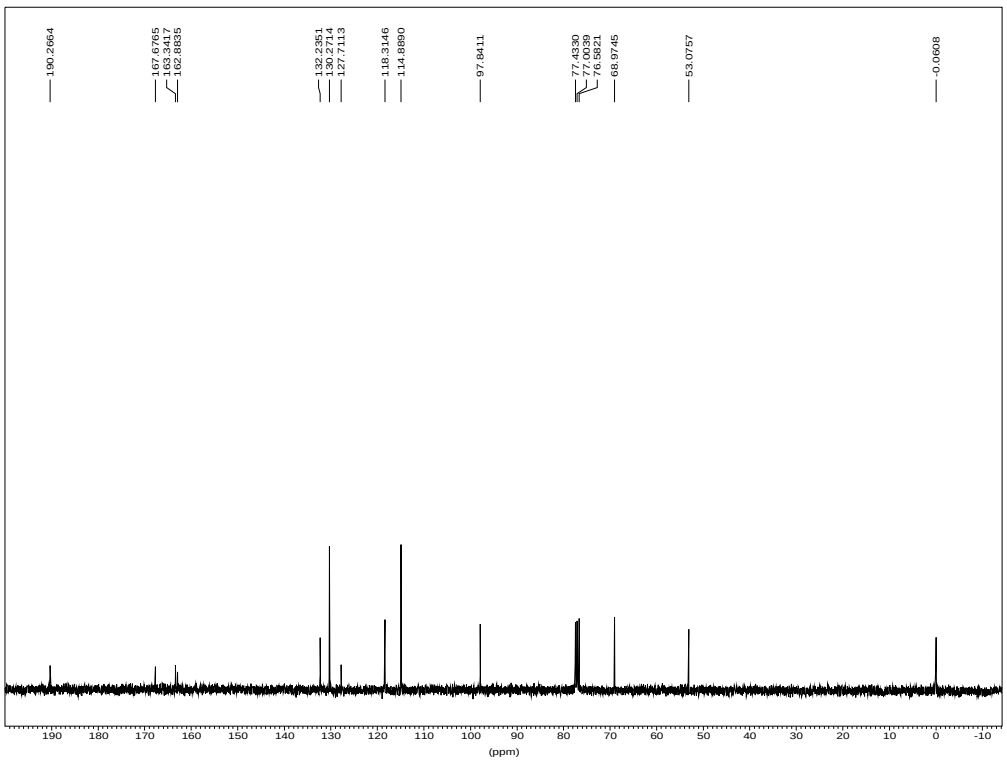
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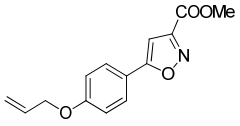
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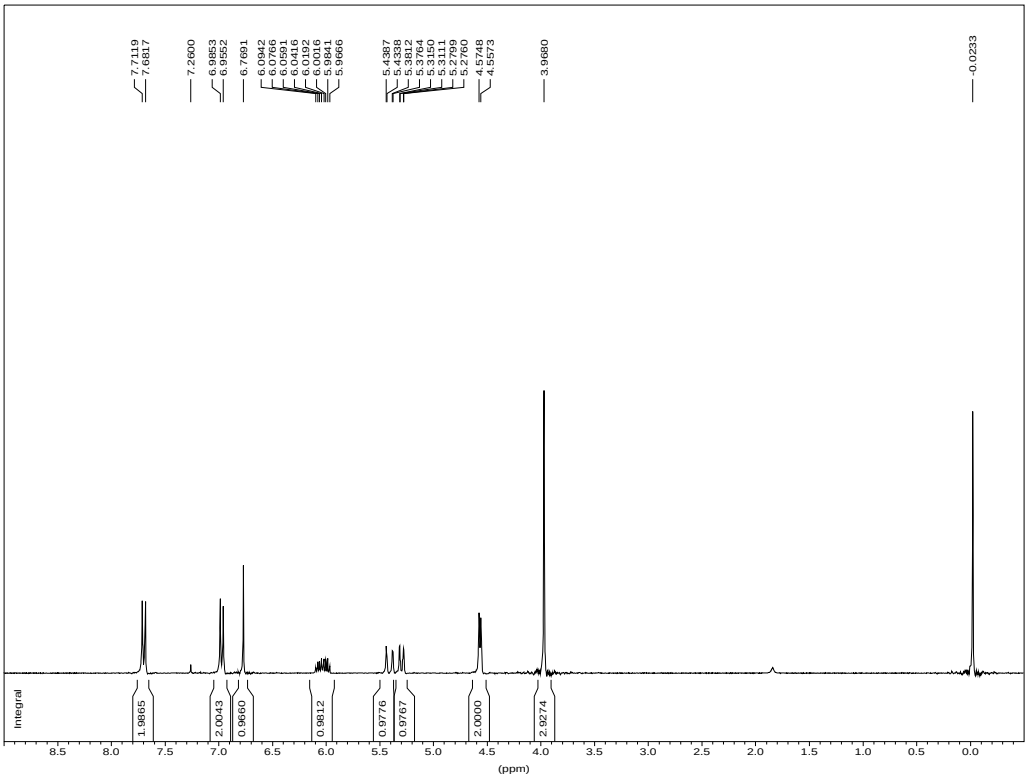
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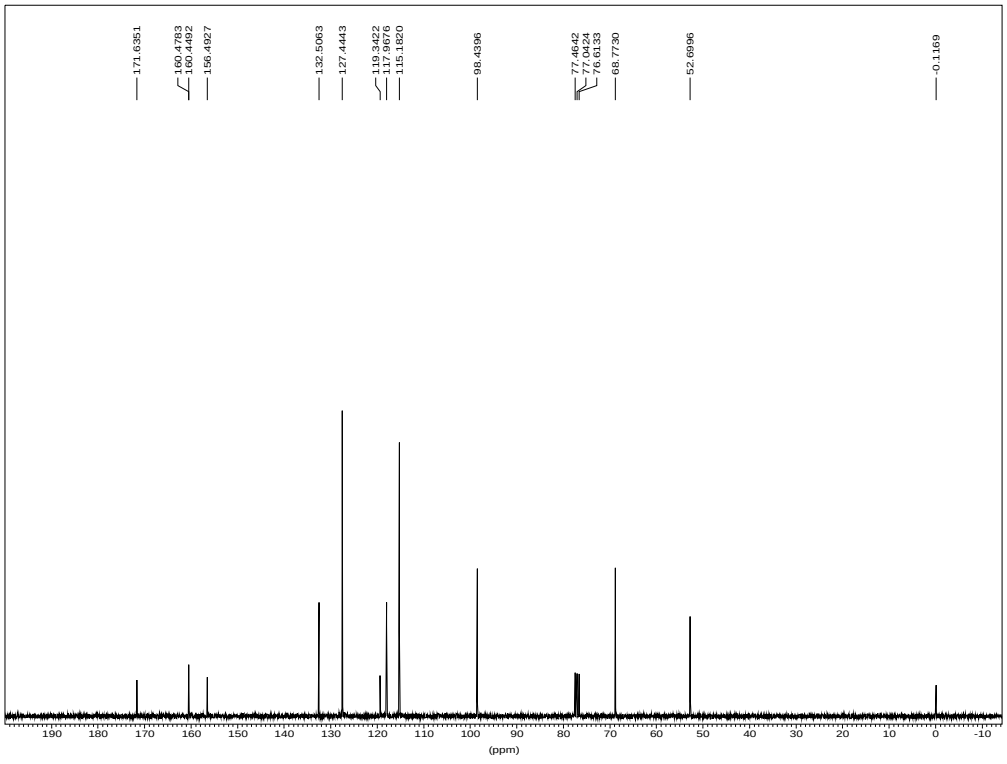
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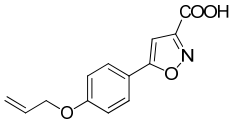
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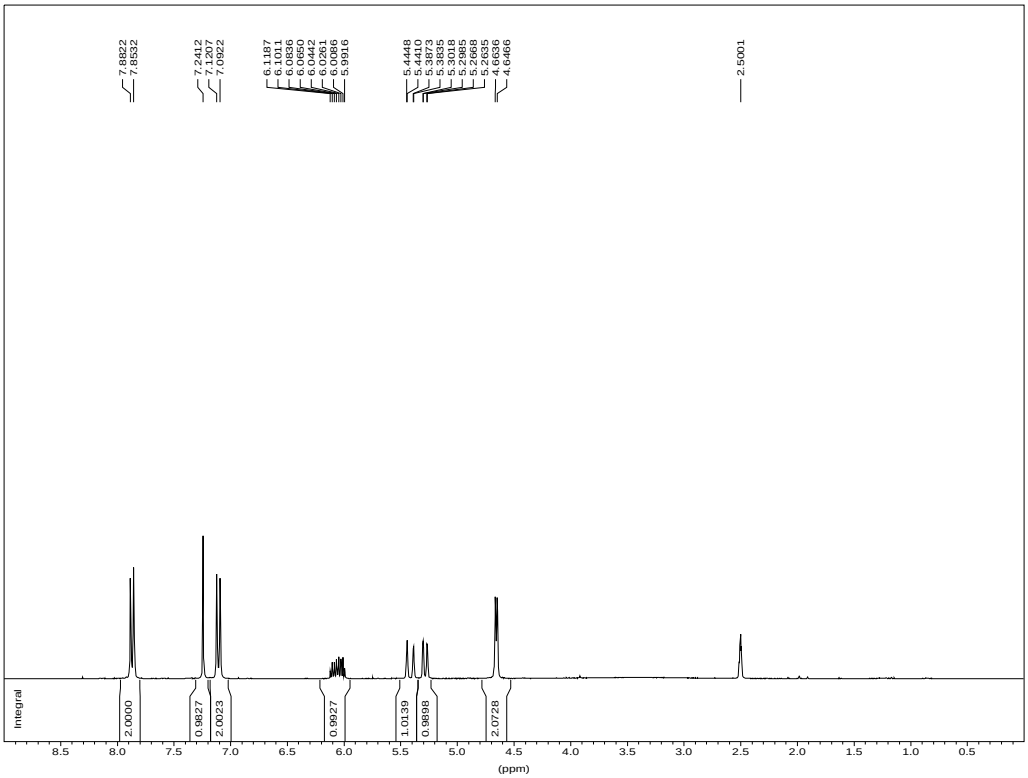
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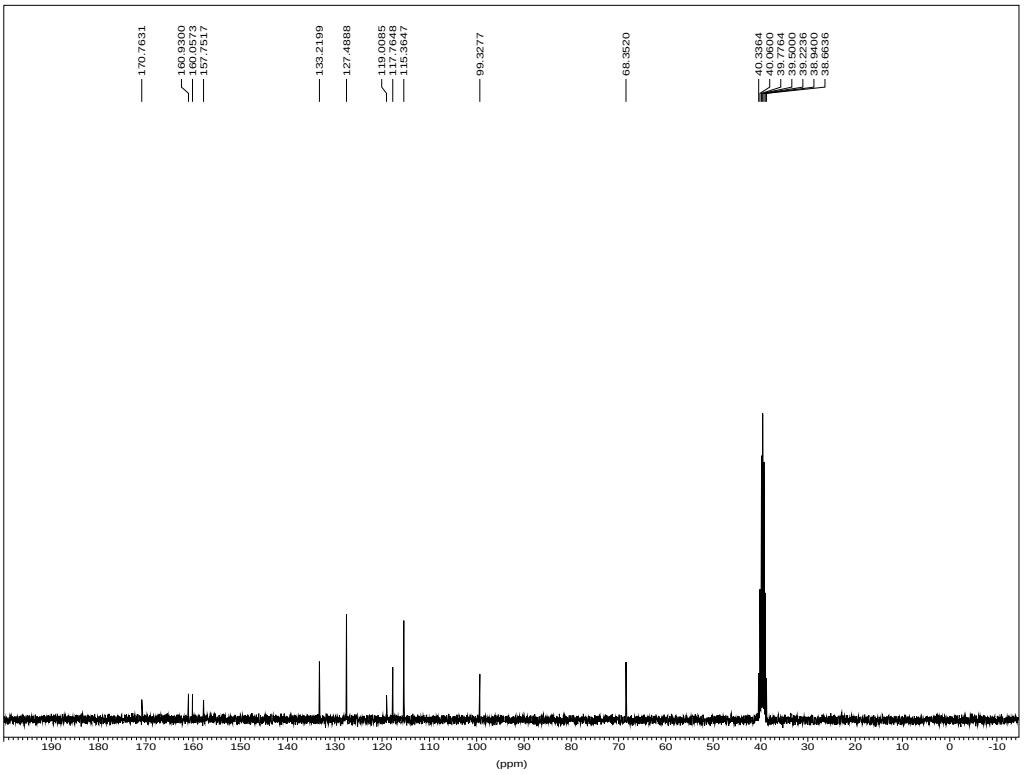
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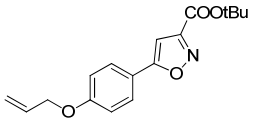
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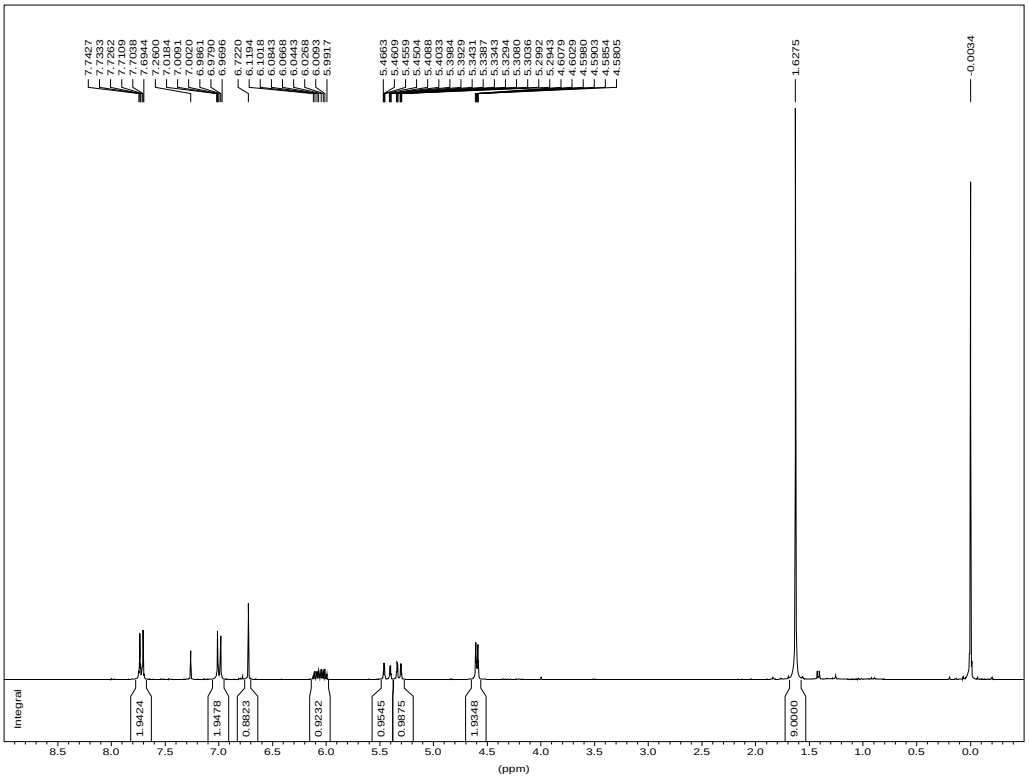
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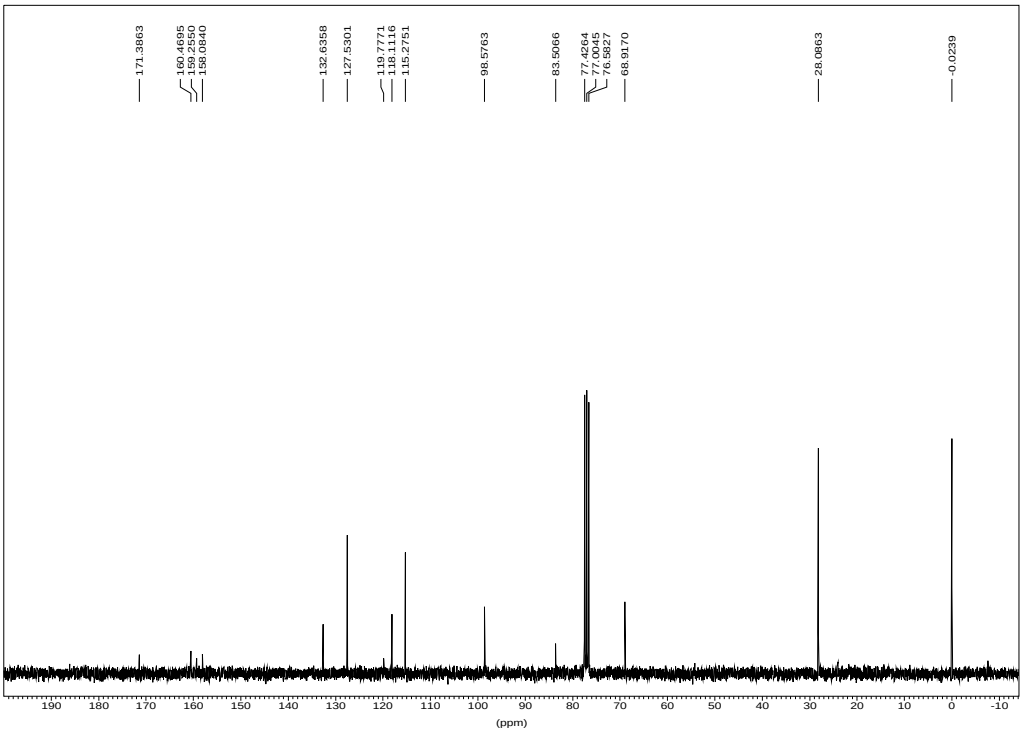
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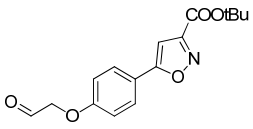
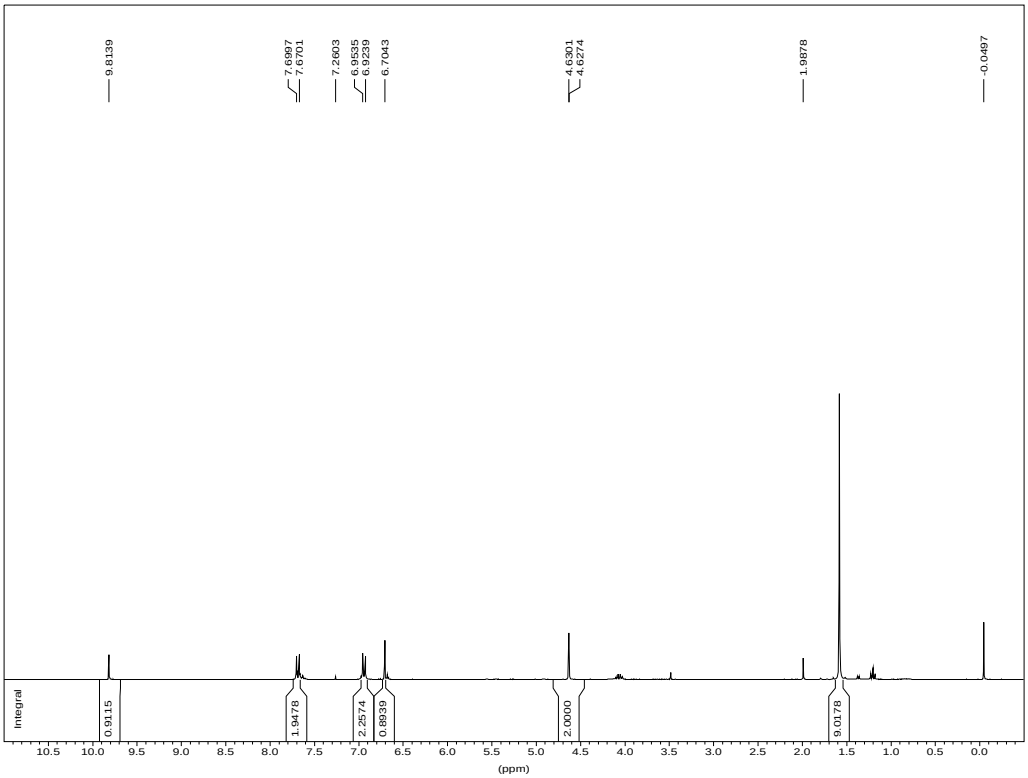
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
-10
(ppm)


COOtBu
N
O
O
O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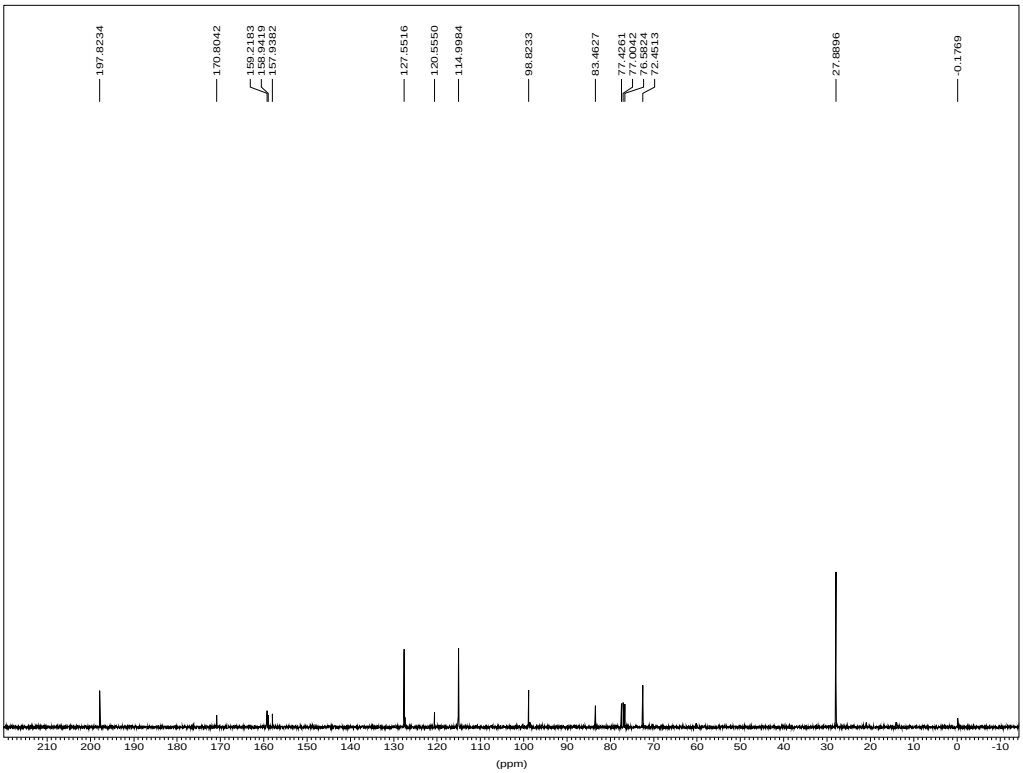
7.6997
7.6701
7.2603
6.9535
6.9239
6.7043
4.6301
4.6274
1.9878
-0.0497
Integral
0.9115
1.9478
2.2574
0.8939
2.0000
9.0178
(ppm)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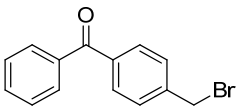
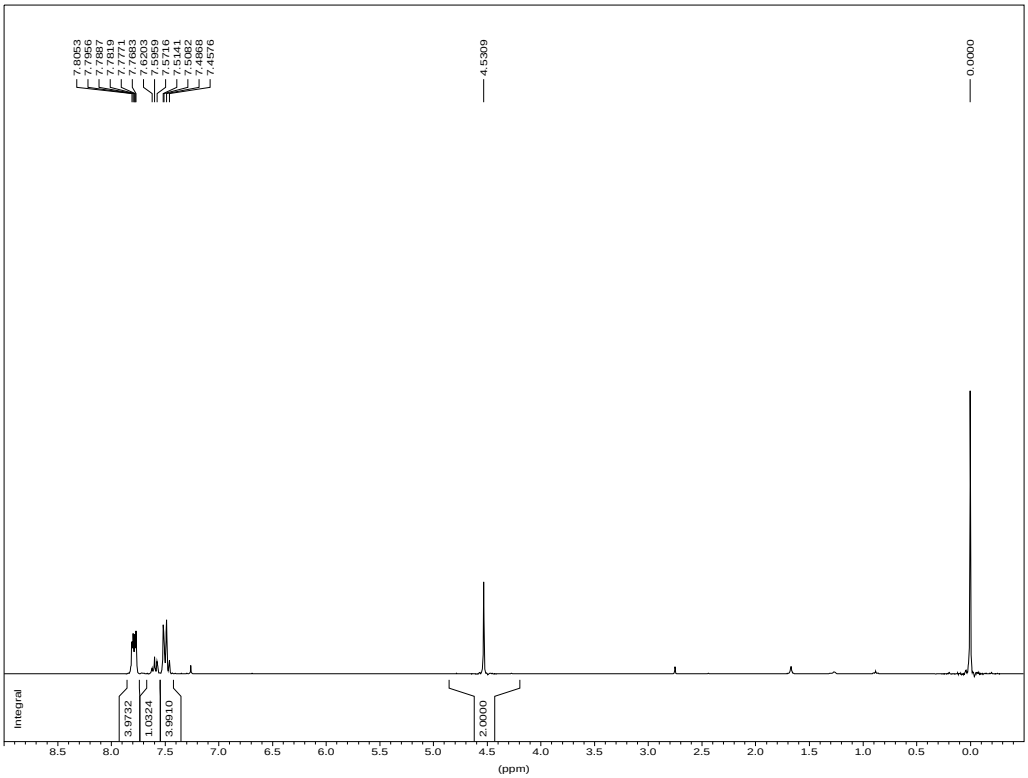
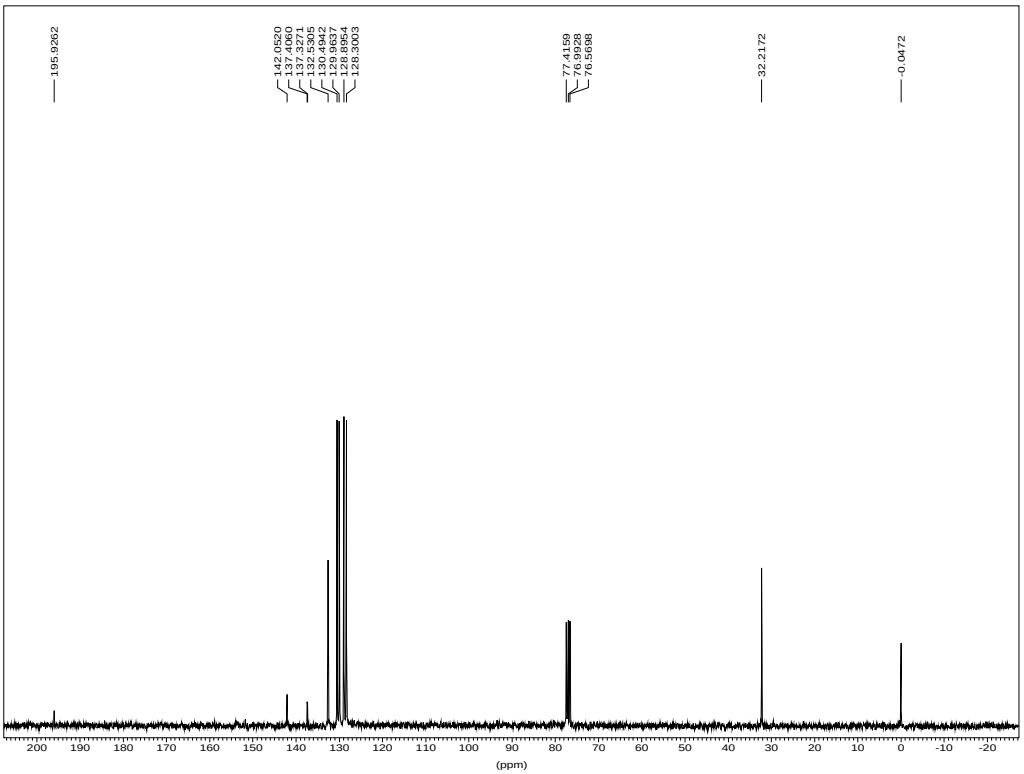
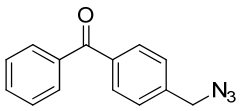
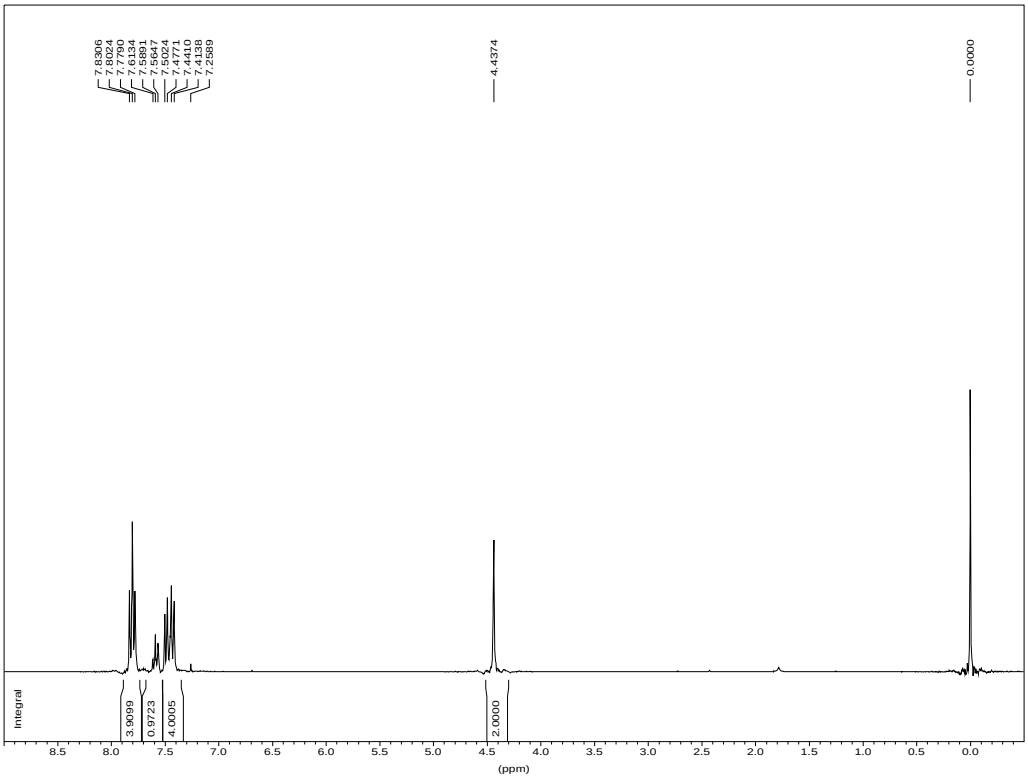
170.8042
159.2183
158.9419
157.9382
127.5516
120.5550
114.9984
98.8233
83.4627
77.4261
77.0042
76.5824
72.4513
27.8896
-0.1769
(ppm)

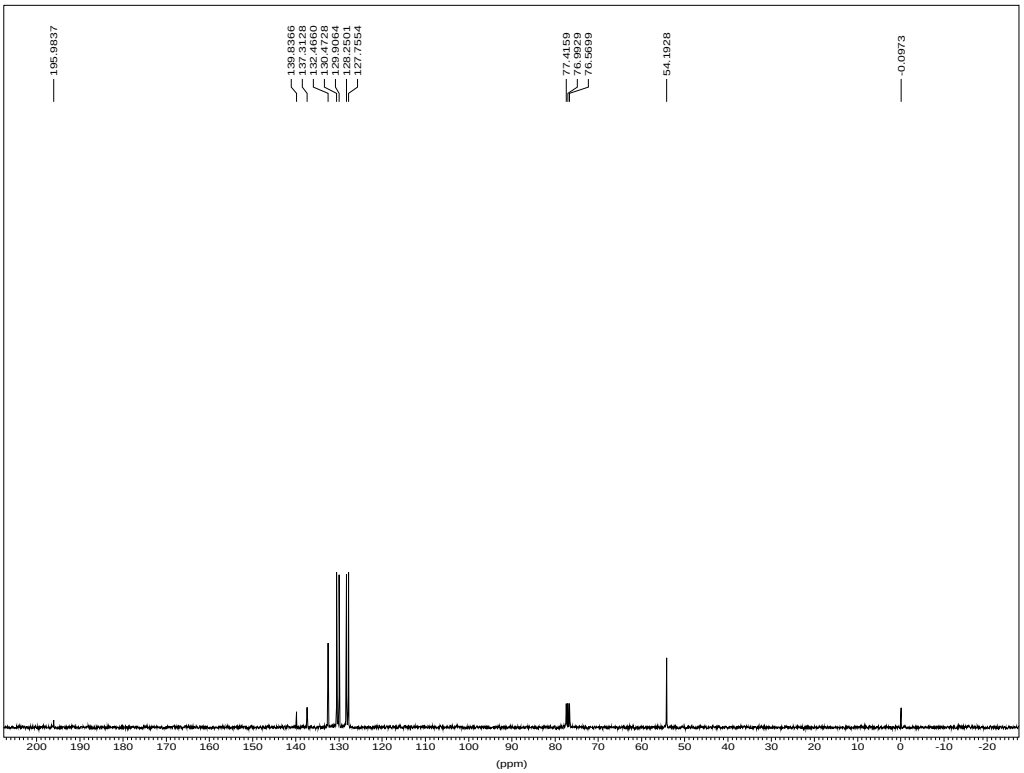

O
Br

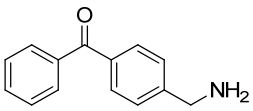

7.8053
7.7956
7.7887
7.7819
7.7771
7.7683
7.6203
7.5959
7.5716
7.5141
7.5082
7.4868
7.4576
4.5309
0.0000
Integral
3.9732
1.0324
3.9910
2.0000
(ppm)

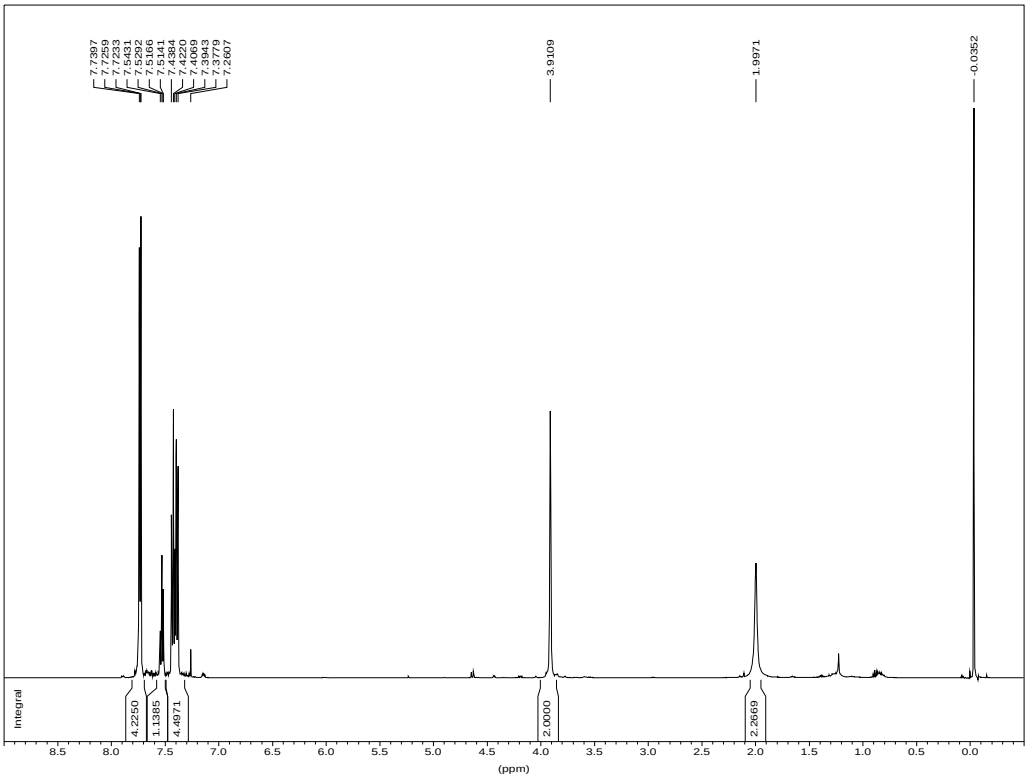

195.9262
142.0520
137.4060
137.3271
132.5305
130.4842
129.9657
128.8954
128.3003
77.4159
76.9928
76.5698
32.2172
-0.0472
(ppm)

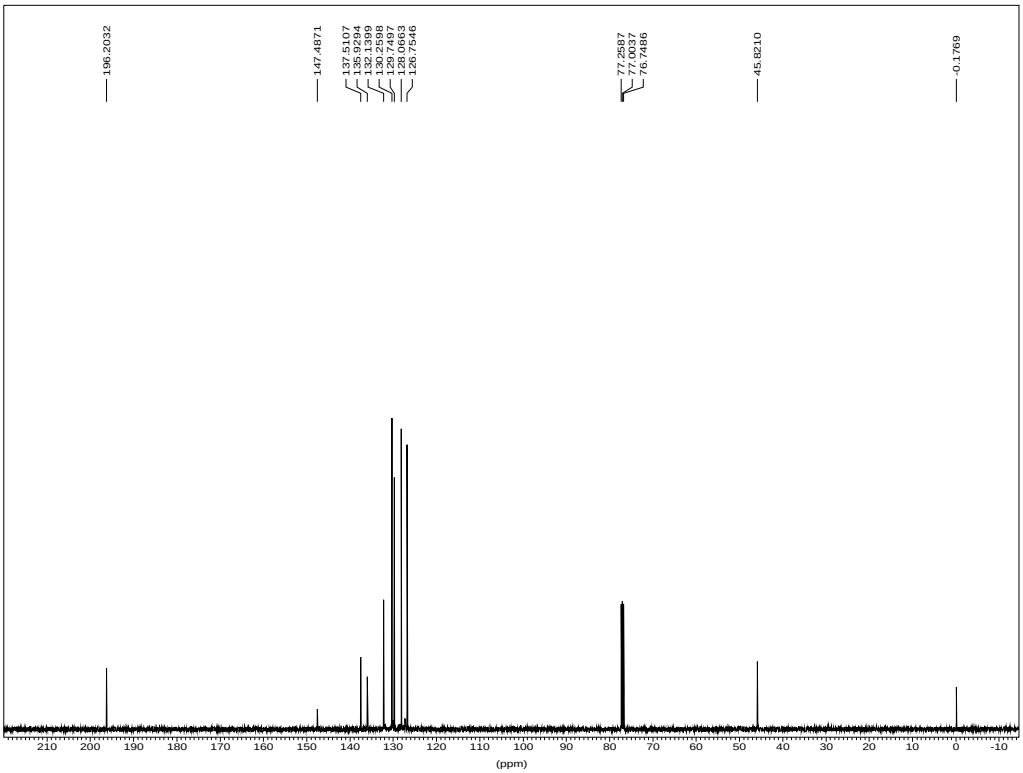

O
N3

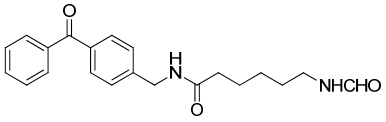

7.8306
7.8004
7.7790
7.6134
7.5891
7.5647
7.5004
7.4771
7.4410
7.4138
7.2589
4.4374
0.0000
Integral
3.9099
0.9723
4.0005
2.0000
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
(ppm)

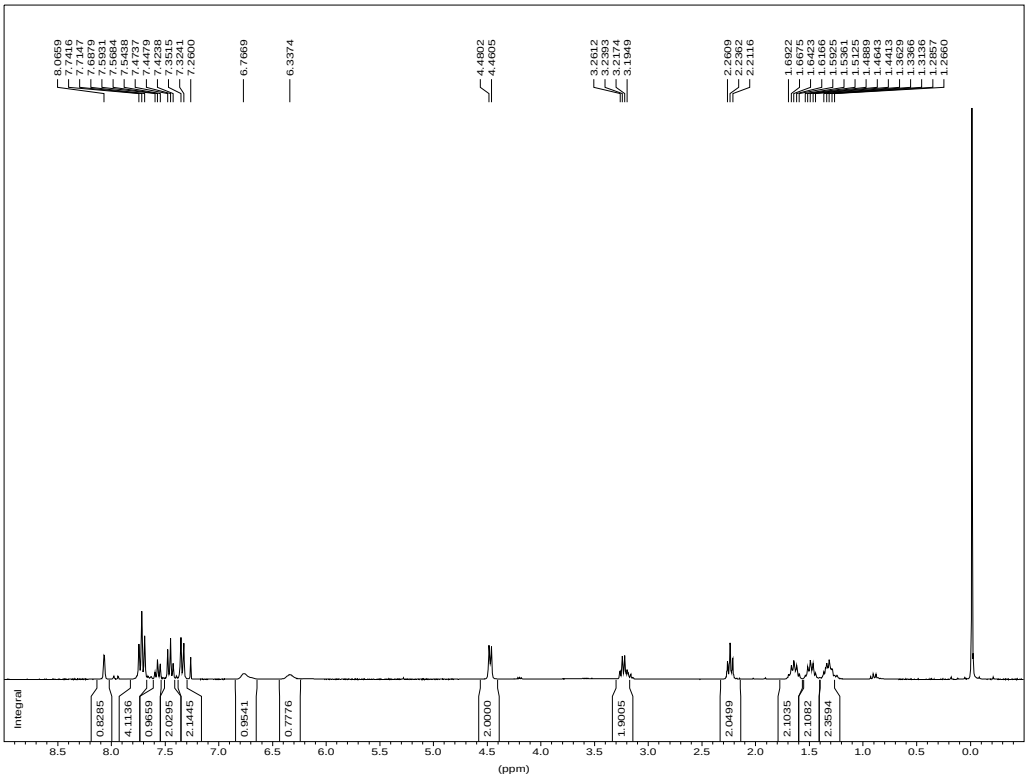

195.9837
139.8366
137.3128
132.4660
130.4728
129.0664
128.2501
127.7554
77.4159
76.9929
76.5699
54.1928
-0.0973
200
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
-10
-20
(ppm)


O
NH2


7.7397
7.7259
7.7233
7.5431
7.5322
7.5166
7.5141
7.4384
7.4220
7.4066
7.3943
7.3779
7.2607
3.9109
1.9971
-0.0352
Integral
4.2250
1.1385
4.4971
2.0000
2.2669
(ppm)


196.2032
147.4871
137.5107
135.9294
132.1399
130.2598
129.7497
128.0663
126.7546
77.2587
77.0037
76.7486
45.8210
-0.1769
(ppm)


O
H
N
O
NHCHO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7.7445
7.7147
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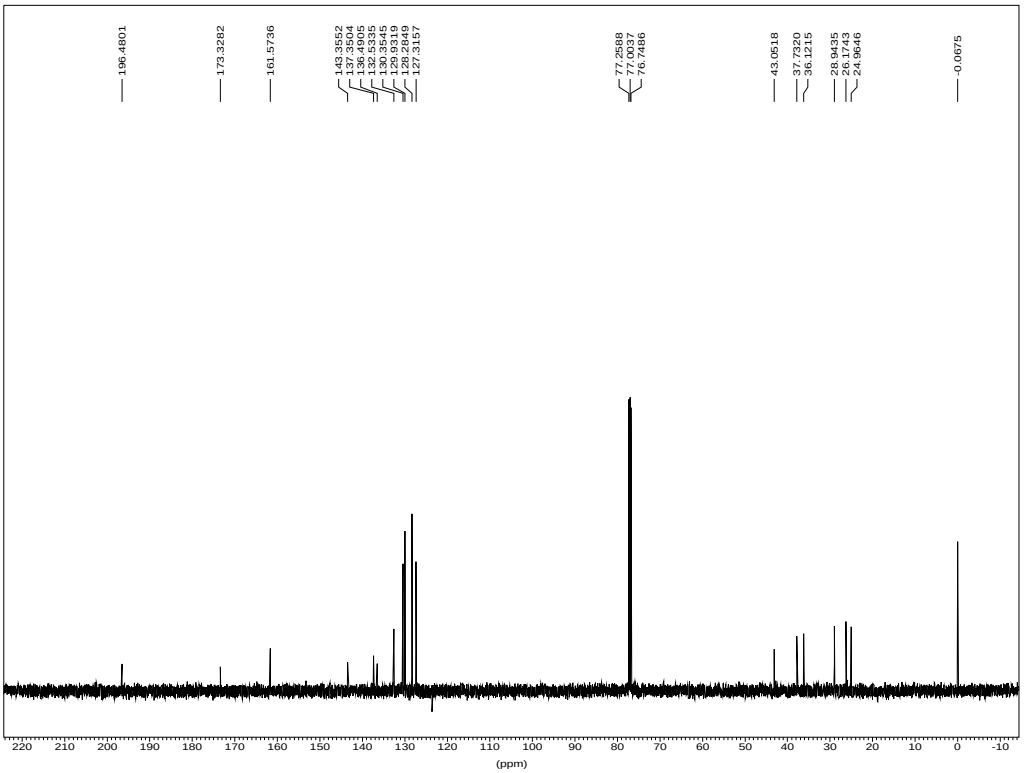
7.5931
7.5684
7.5438
7.4737
7.4479
7.4238
7.3515
7.3241
7.2600
6.7669
6.3374
4.4802
4.4605
3.2612
3.2393
3.2174
3.1949
2.2609
2.2382
2.2116
1.6922
1.6675
1.6423
1.6166
1.5925
1.5361
1.5125
1.4888
1.4643
1.4413
1.3629
1.3386
1.3136
1.2857
1.2660
Integral
0.8285
4.1136
0.9659
2.0295
2.1445
0.9541
0.7776
2.0000
1.9005
2.0499
2.1035
2.1082
2.3594
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
(ppm)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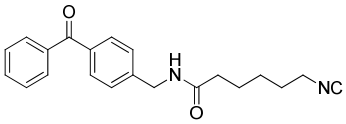
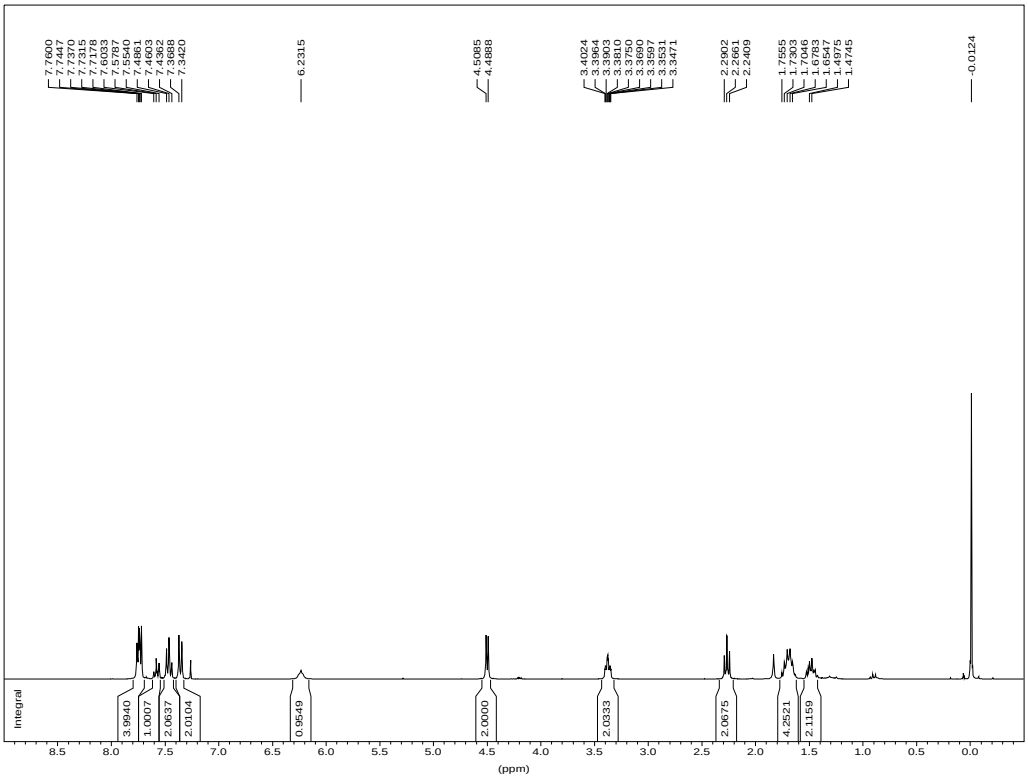
173.3282
161.5736
143.3552
137.3504
136.4505
132.5335
130.3545
129.9319
128.8449
127.3157
77.2588
77.0037
76.7486
43.0518
37.7320
36.1215
28.9435
26.1743
24.9646
-0.0675
220
210
200
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
-10
(ppm)


O
H
N
O
NC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7.7178
7.6033
7.5787
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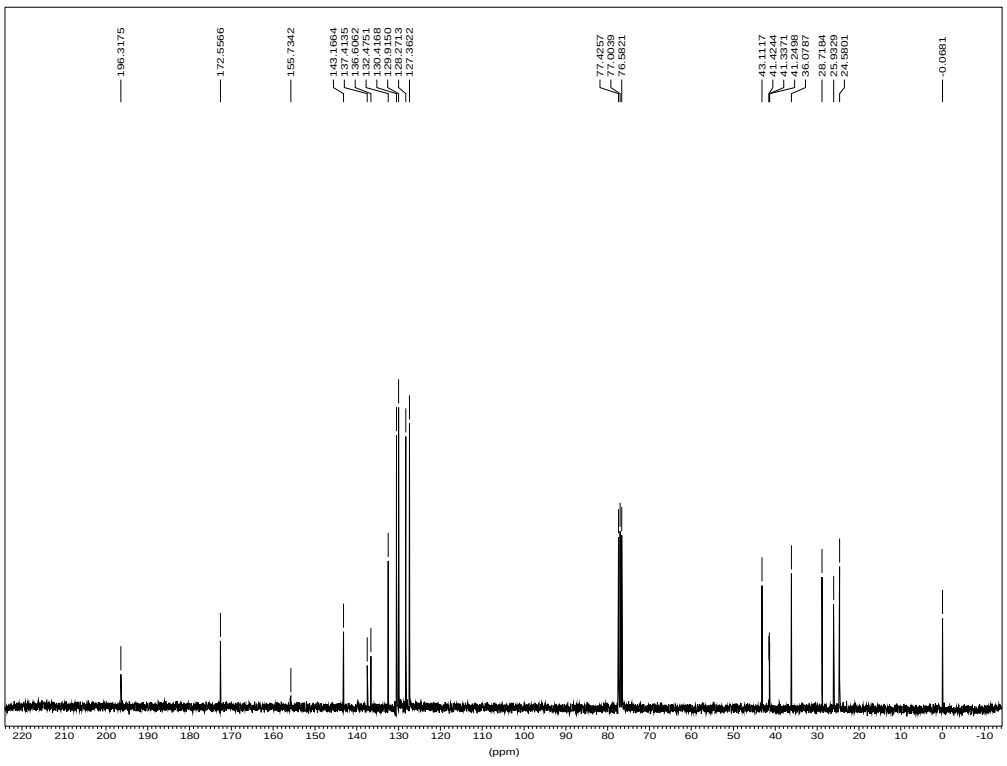
7.4481
7.4463
7.4362
7.3688
7.3420
6.2315
4.5085
4.4868
3.4024
3.3964
3.3903
3.3810
3.3755
3.3690
3.3597
3.3531
3.3471
2.2902
2.2661
2.2409
1.7555
1.7303
1.7046
1.6783
1.6547
1.6405
1.4745
-0.0124
Integral
3.9940
1.0007
2.0637
2.0104
0.9549
2.0000
2.0333
2.0675
4.2521
2.1159
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
(ppm)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155.7342
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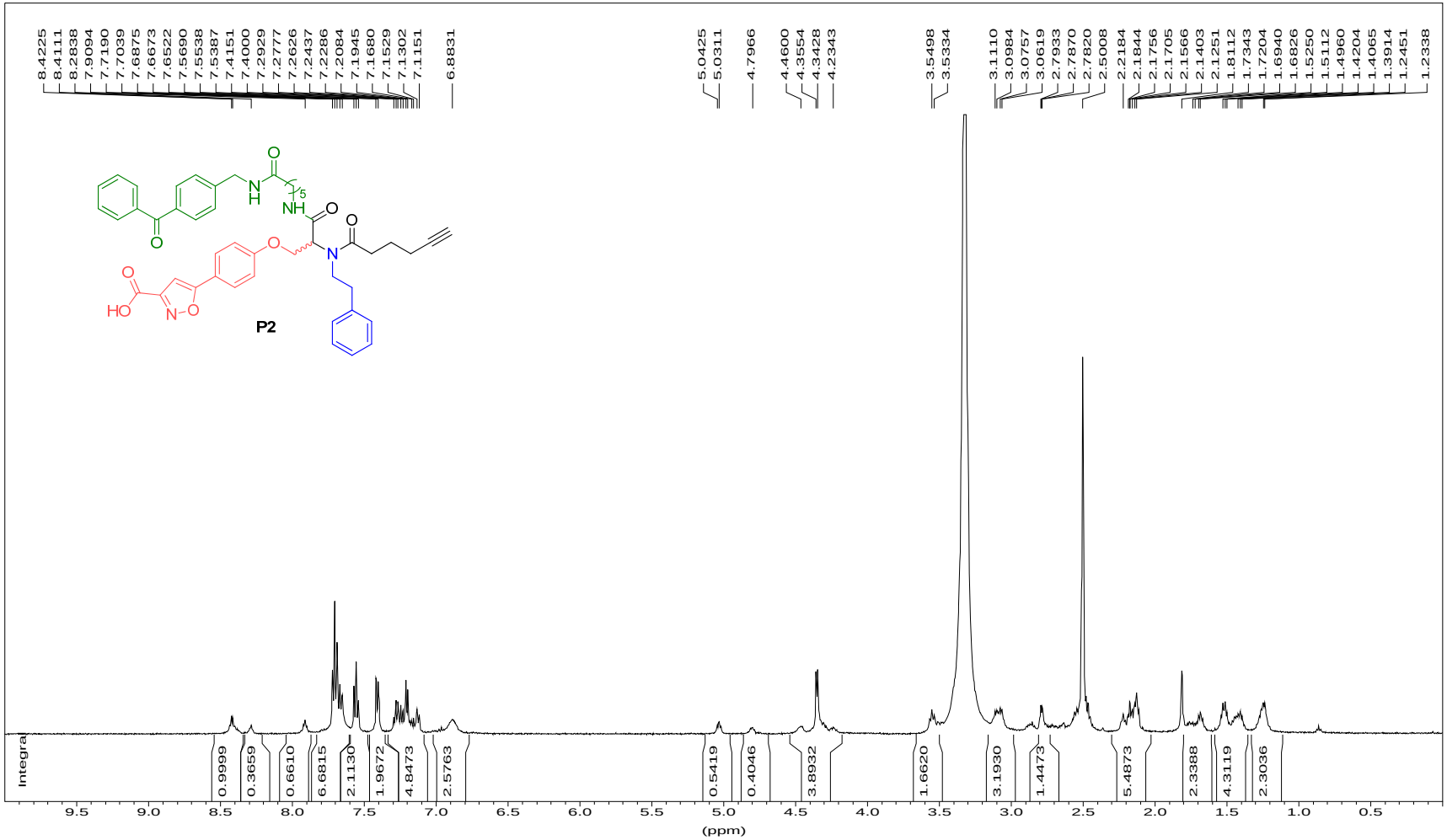
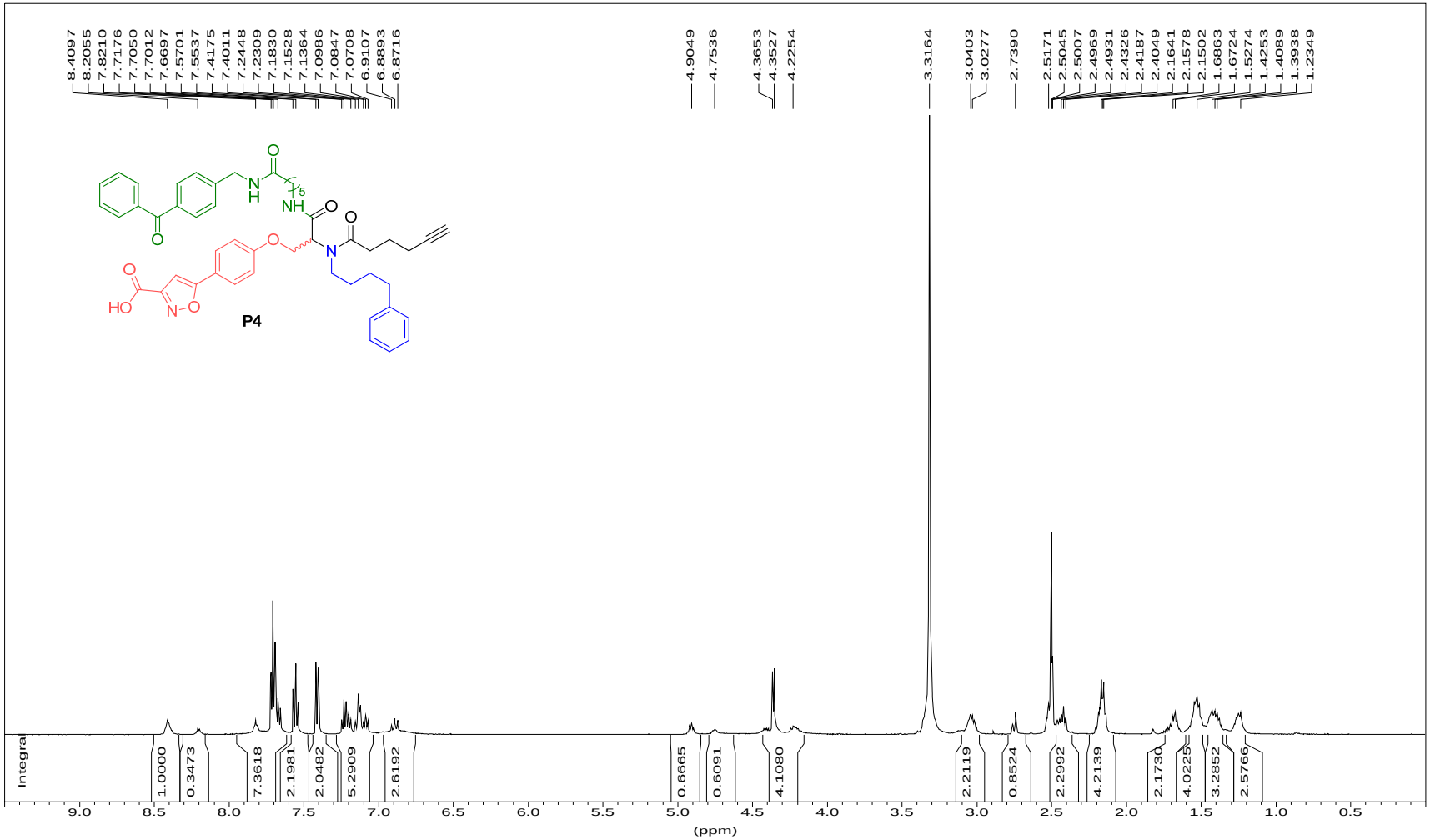
132.4751
130.4158
129.9150
128.2713
127.3622
77.4257
77.0039
76.5821
43.1117
41.4244
41.3271
41.2498
36.0787
28.7184
25.9329
24.5801
-0.0681
220
210
200
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
-10
(ppm)


P2
(ppm)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7.7012
7.6697
7.5701
7.5537
7.4175
7.4011
7.2448
7.2309
7.1830
7.1528
7.1364
7.0986
7.0847
7.0708
6.9107
6.8893
6.8716
4.9049
4.7536
4.3653
4.3527
4.2254
3.3164
3.0403
3.0277
2.7390
2.5171
2.5045
2.5007
2.4969
2.4931
2.4326
2.4187
2.4049
2.1641
2.1578
2.1502
1.6863
1.6724
1.5274
1.4253
1.4089
1.3938
1.2349
P4
Integral
1.0000
0.3473
7.3618
2.1981
2.0482
5.2909
2.6192
0.6665
0.6091
4.1080
2.2119
0.8524
2.2992
4.2139
2.1730
4.0225
3.2852
2.5766
9.0
8.5
8.0
7.5
7.0
6.5
6.0
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5.0
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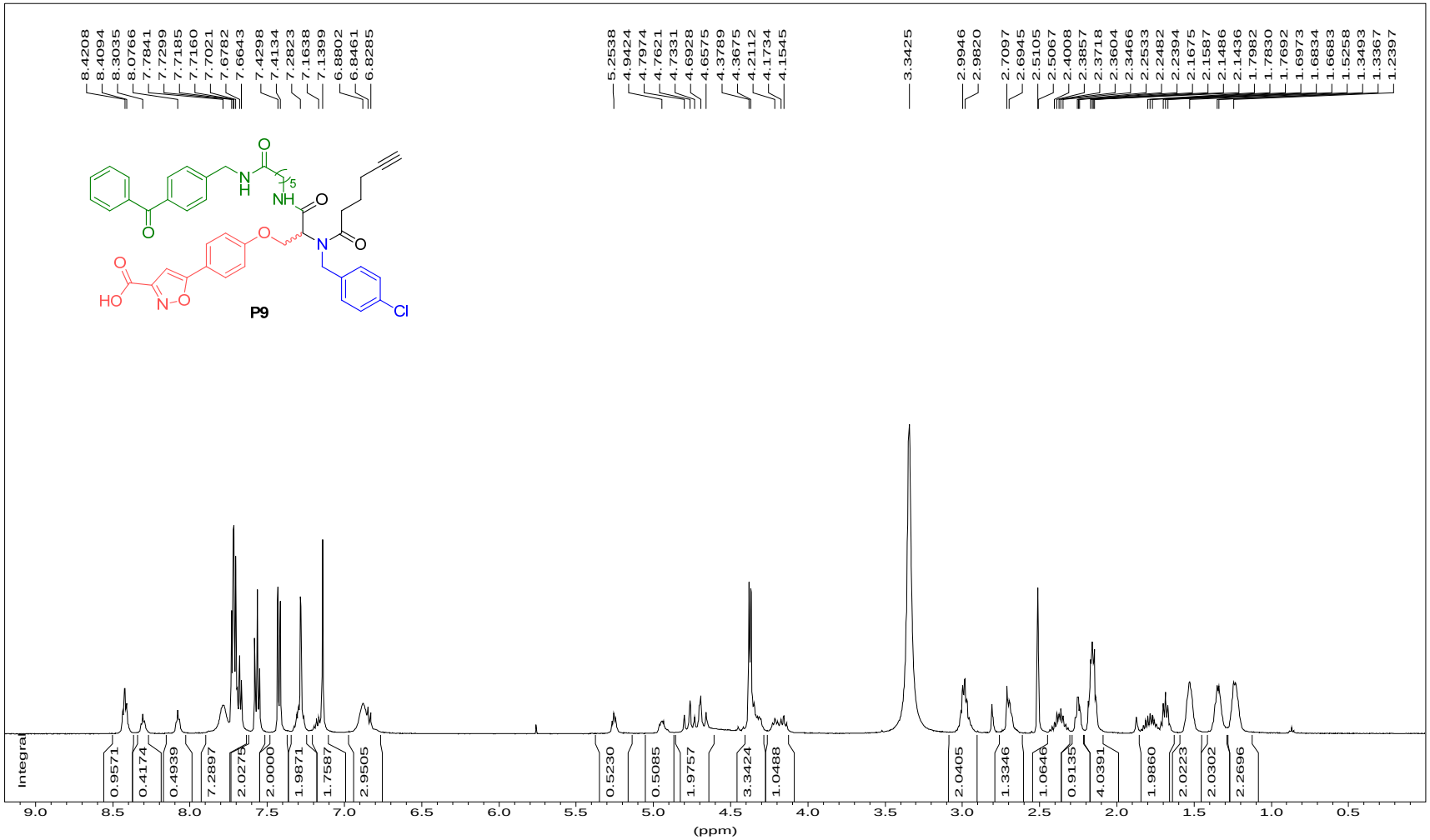
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
(ppm)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7.6643
7.4298
7.4134
7.2823
7.1638
7.1399
6.8802
6.8461
6.8285
5.2538
4.9424
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4.6575
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4.3675
4.2112
4.1734
4.1545
3.3425
2.9946
2.9820
2.7097
2.6945
2.5105
2.5067
2.4008
2.3857
2.3718
2.3604
2.3466
2.2533
2.2482
2.2394
2.1675
2.1587
2.1486
2.1436
1.7982
1.7830
1.7692
1.6973
1.6834
1.6683
1.5258
1.3493
1.3367
1.2397
P9
Integral
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0.4174
0.4939
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2.0279
2.0000
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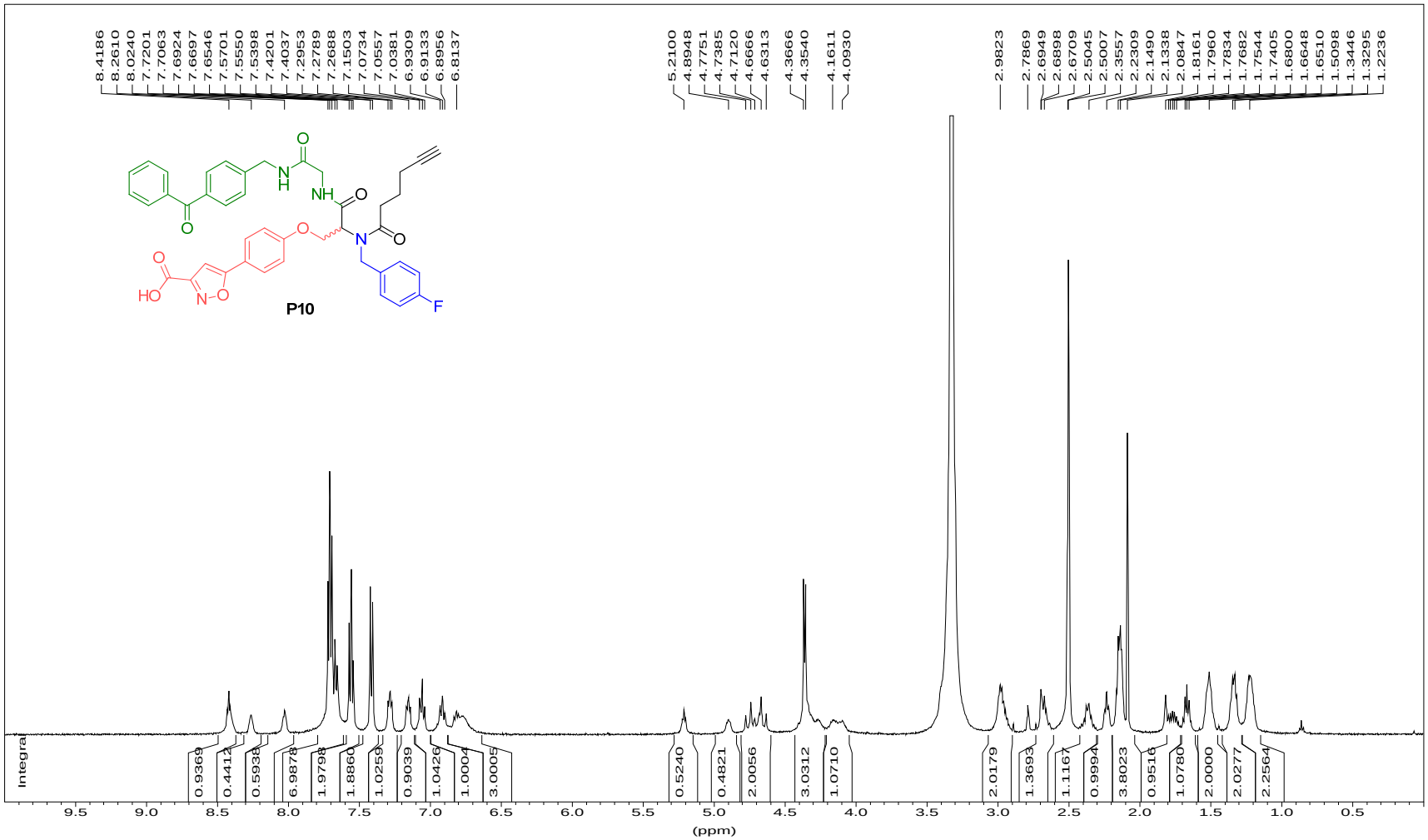
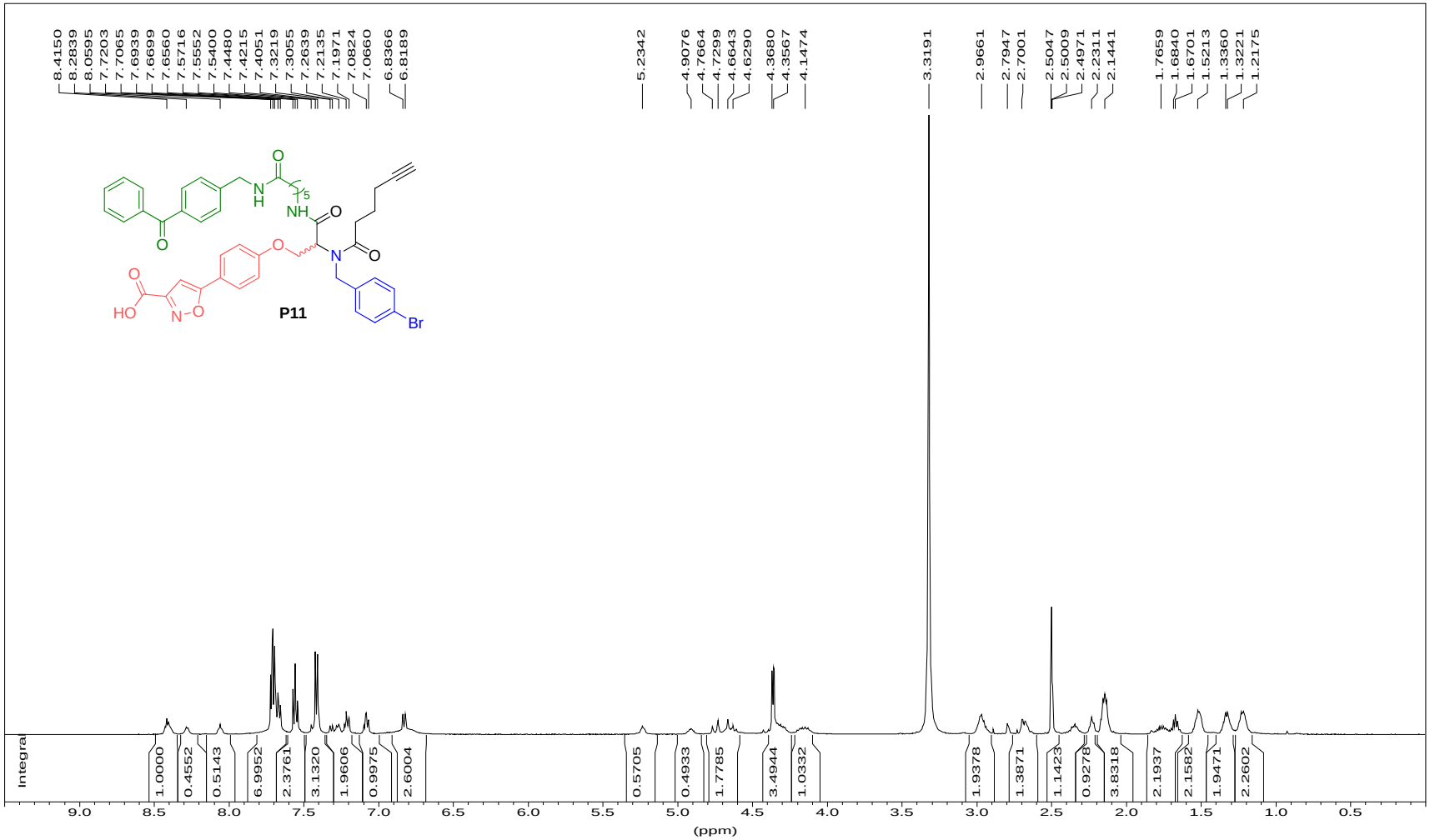
1.7587
2.9505
0.5230
0.5085
1.9757
3.3424
1.0488
2.0405
1.3346
1.0646
0.9135
4.0391
1.9860
2.0223
2.0302
2.2696
9.0
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
(ppm)

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7.4480
7.4215
7.4051
7.3219
7.3055
7.2639
7.2135
7.1971
7.0824
7.0660
6.8366
6.8189
5.2342
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4.7664
4.7299
4.6643
4.6290
4.3680
4.3567
4.1474
3.3191
2.9661
2.7947
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2.5009
2.4971
2.2311
2.1441
1.7659
1.6840
1.6701
1.5213
1.3360
1.3221
1.2175
P11
Integral
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0.5143
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2.3761
3.1320
1.9606
0.9975
2.6004
0.5705
0.4933
1.7785
3.4944
1.0332
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1.1423
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2.1582
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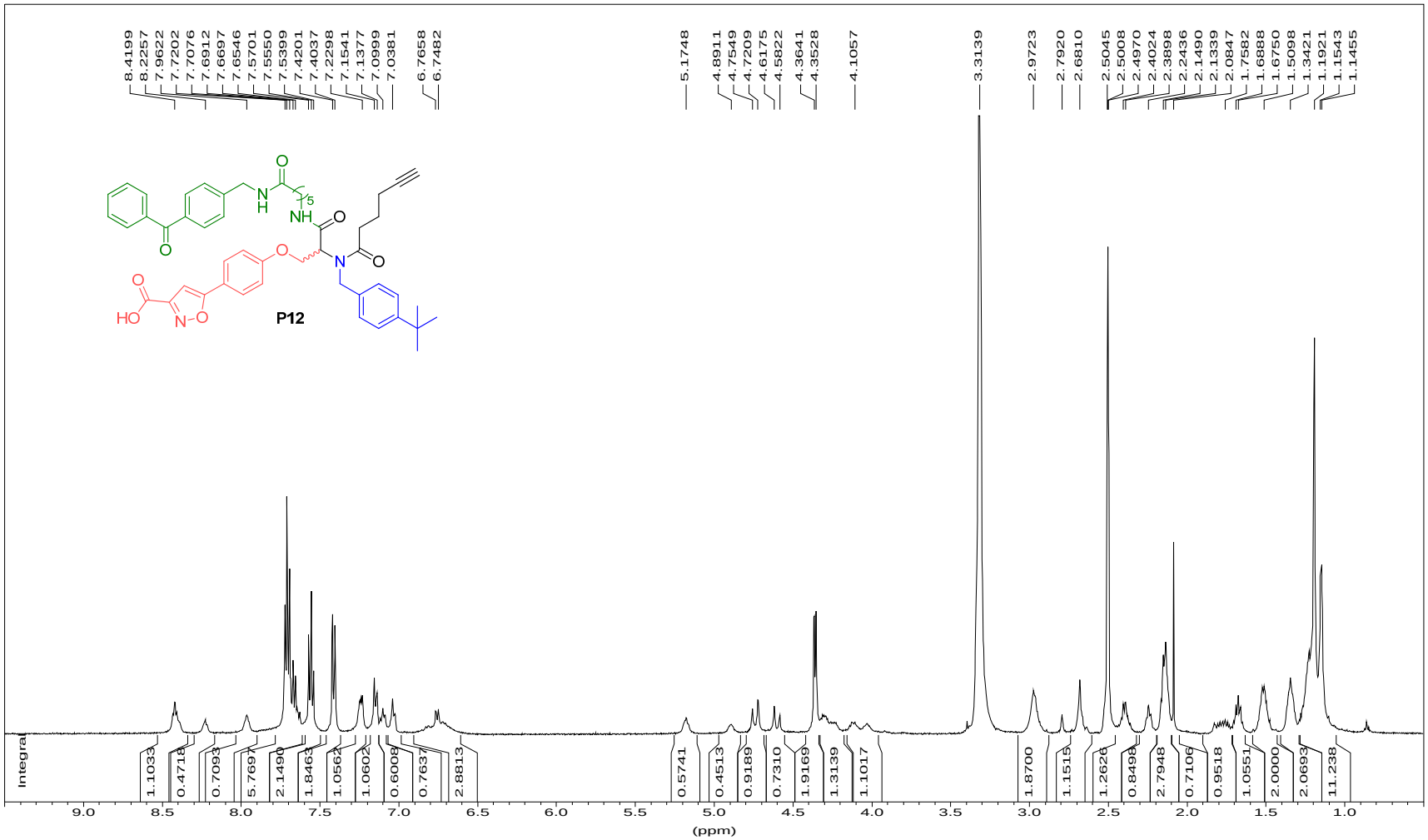
2.2602
(ppm)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7.6697
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7.5701
7.5550
7.5399
7.4201
7.4037
7.2298
7.1541
7.1377
7.0999
7.0381
6.7658
6.7482
5.1748
4.8911
4.7549
4.7209
4.6175
4.5822
4.3641
4.3528
4.1057
3.3139
2.9723
2.7920
2.6810
2.5045
2.5008
2.4970
2.4024
2.3898
2.2436
2.1490
2.1339
2.0847
1.7582
1.6888
1.6750
1.5098
1.3421
1.1921
1.1543
1.1455
P12
Integral
1.1033
0.4718
0.7093
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1.8463
1.0562
1.0602
0.6008
0.7637
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0.5741
0.4513
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0.7310
1.9169
1.3139
1.1017
1.8700
1.1515
1.2626
0.8498
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0.7106
0.9518
1.0551
2.0000
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11.238
9.0
8.5
8.0
7.5
7.0
6.5
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5.0
4.5
4.0
3.5
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2.5
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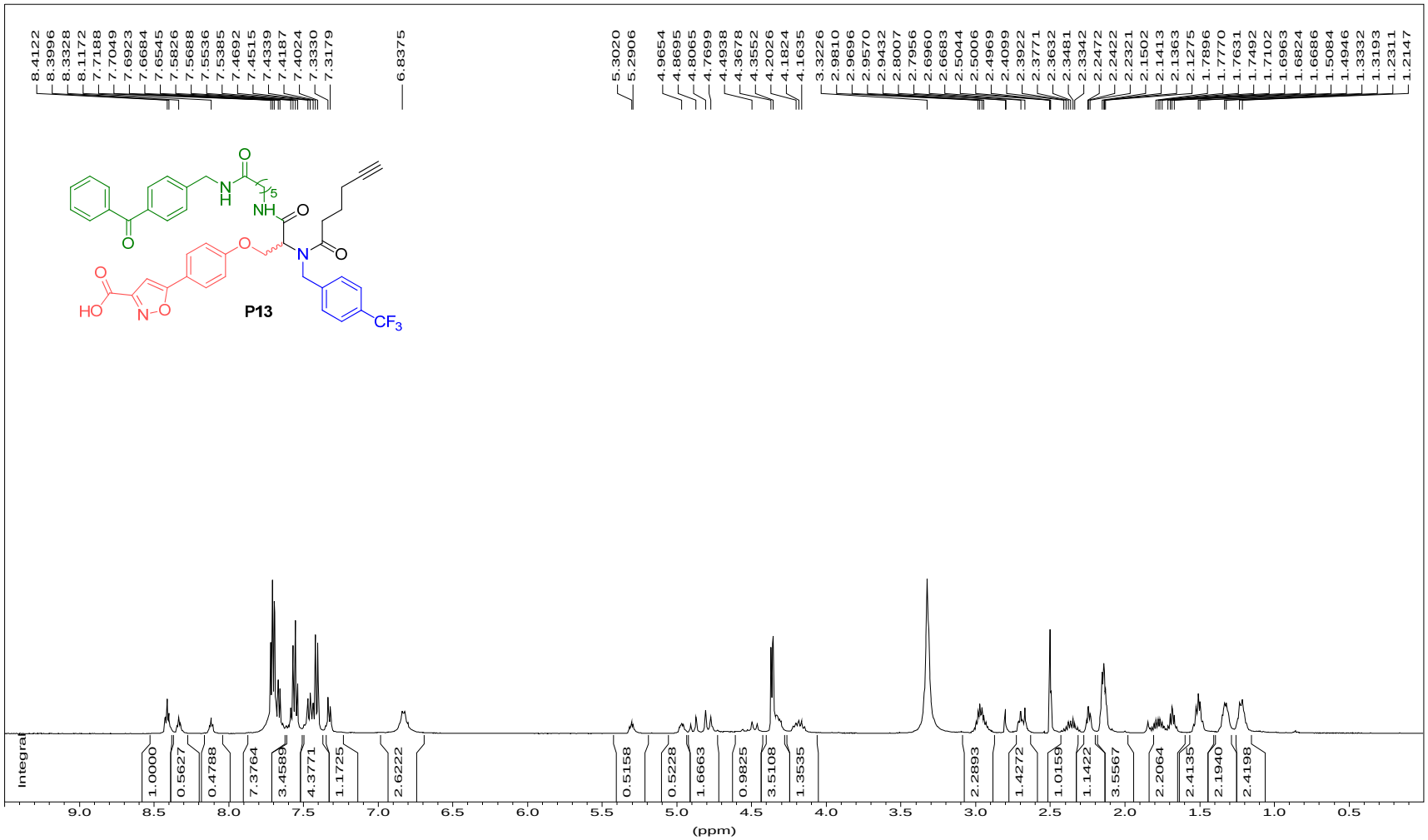
(ppm)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7.3179
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5.2906
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4.8695
4.8065
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4.2026
4.1824
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3.3226
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2.4969
2.4099
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2.3771
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2.3481
2.3342
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2.2422
2.2321
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2.1413
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1.6824
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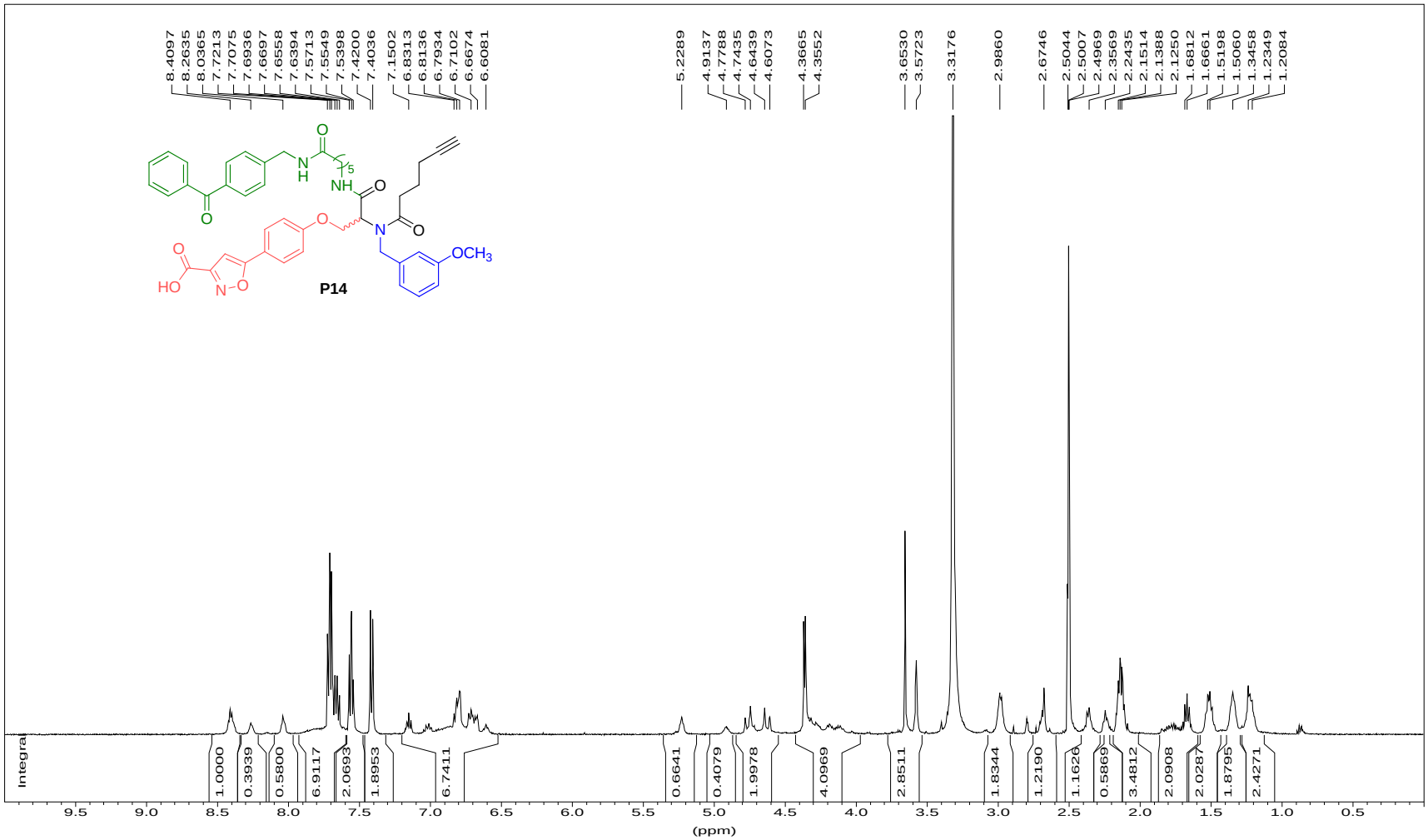
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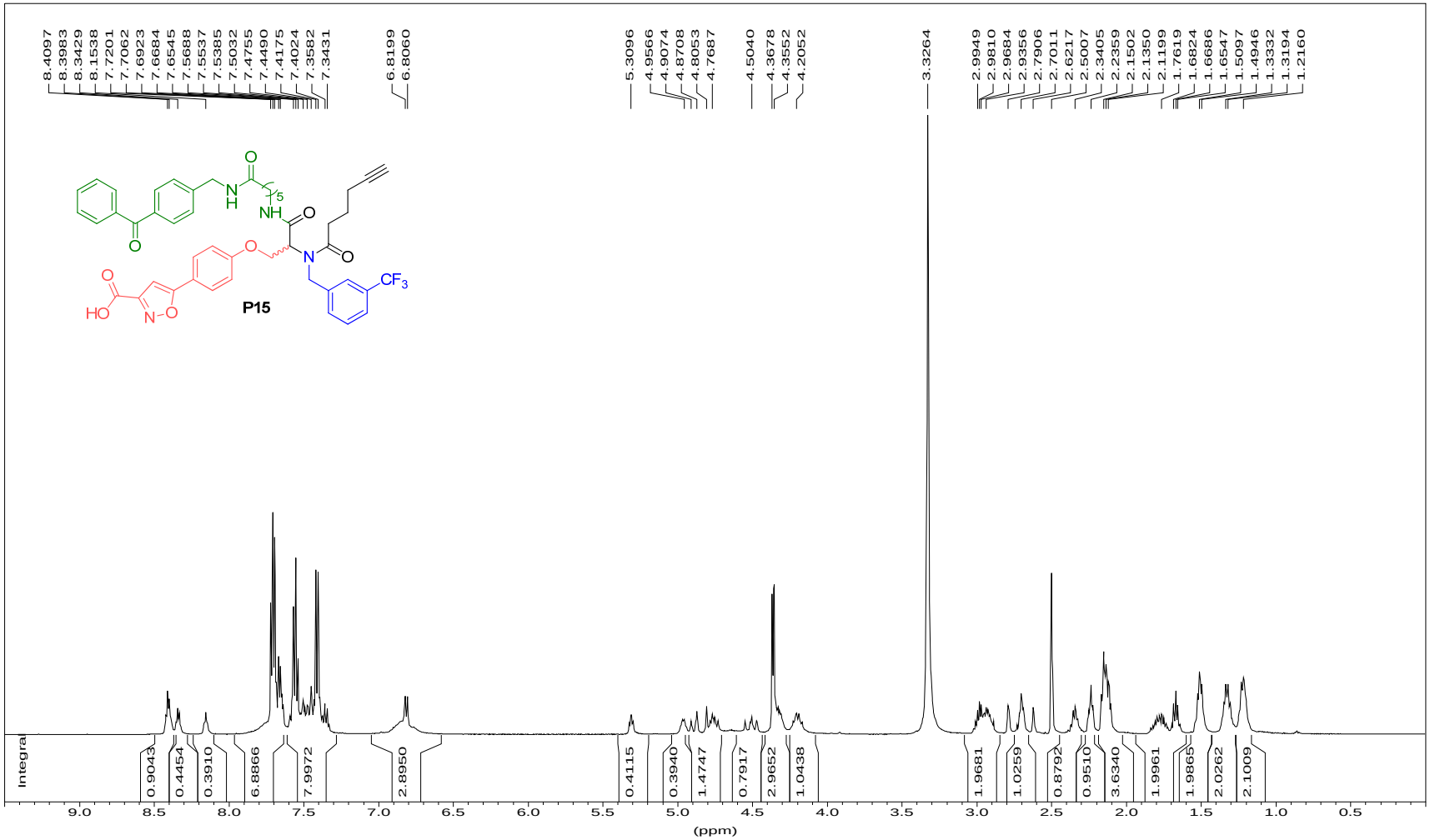
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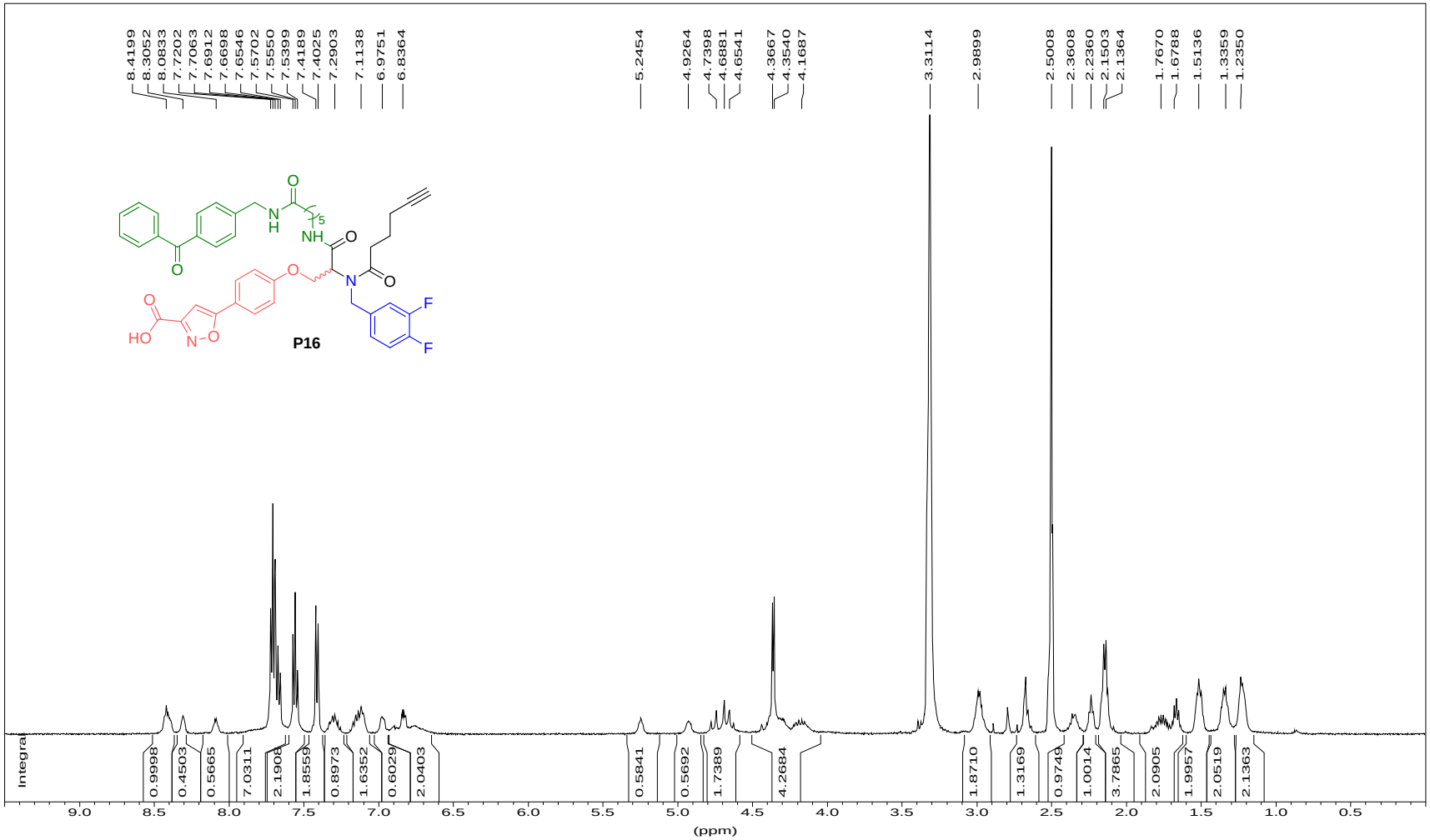
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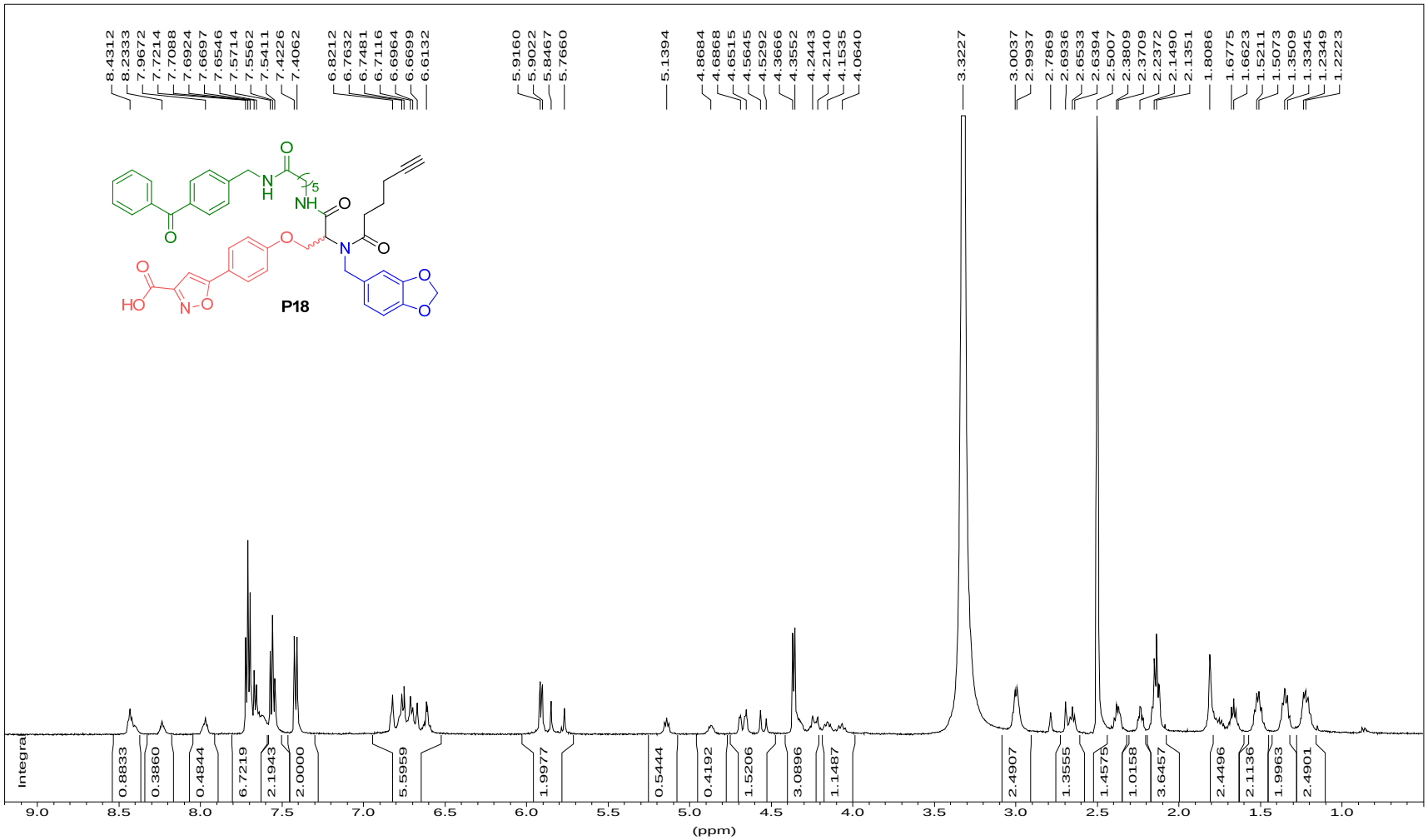
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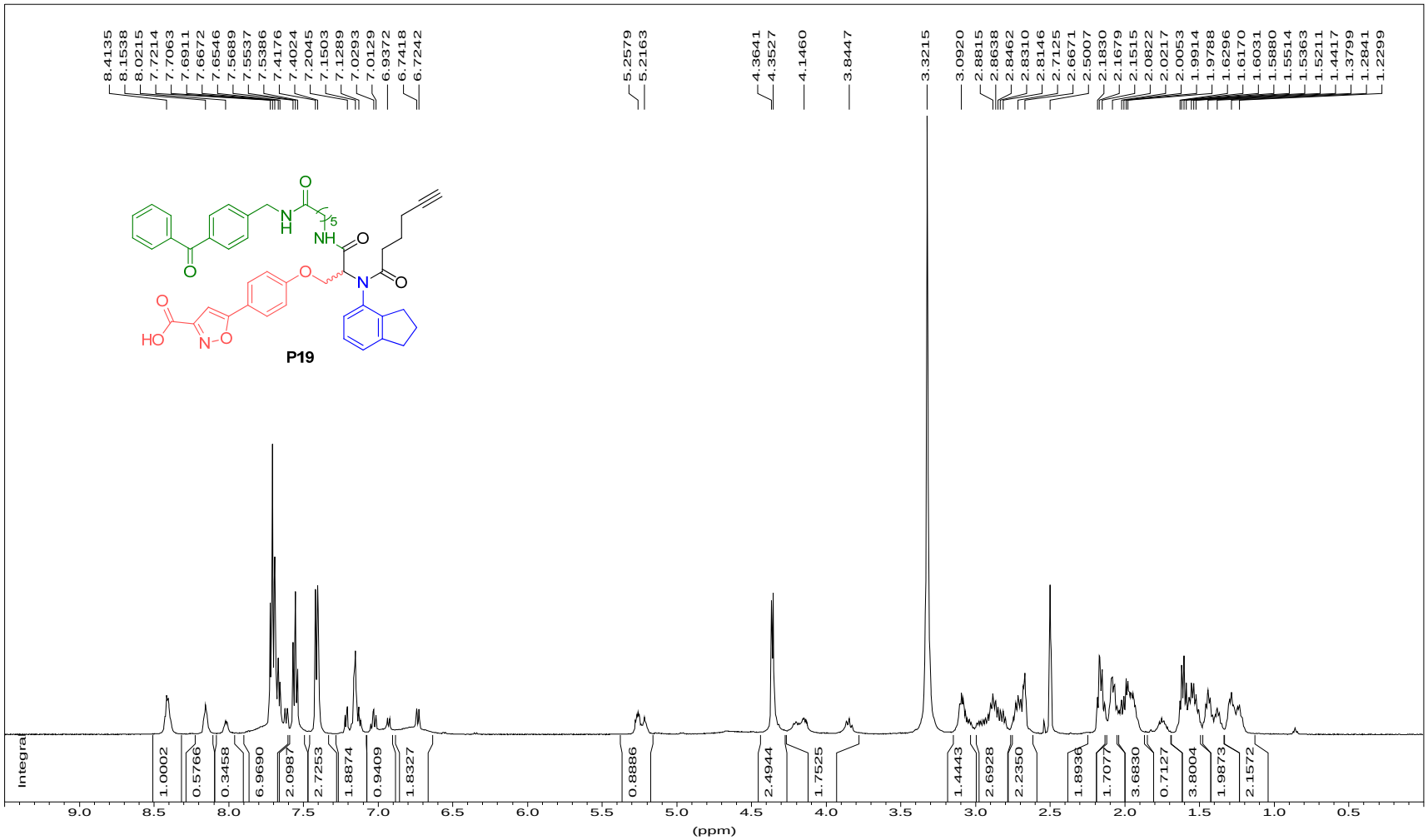

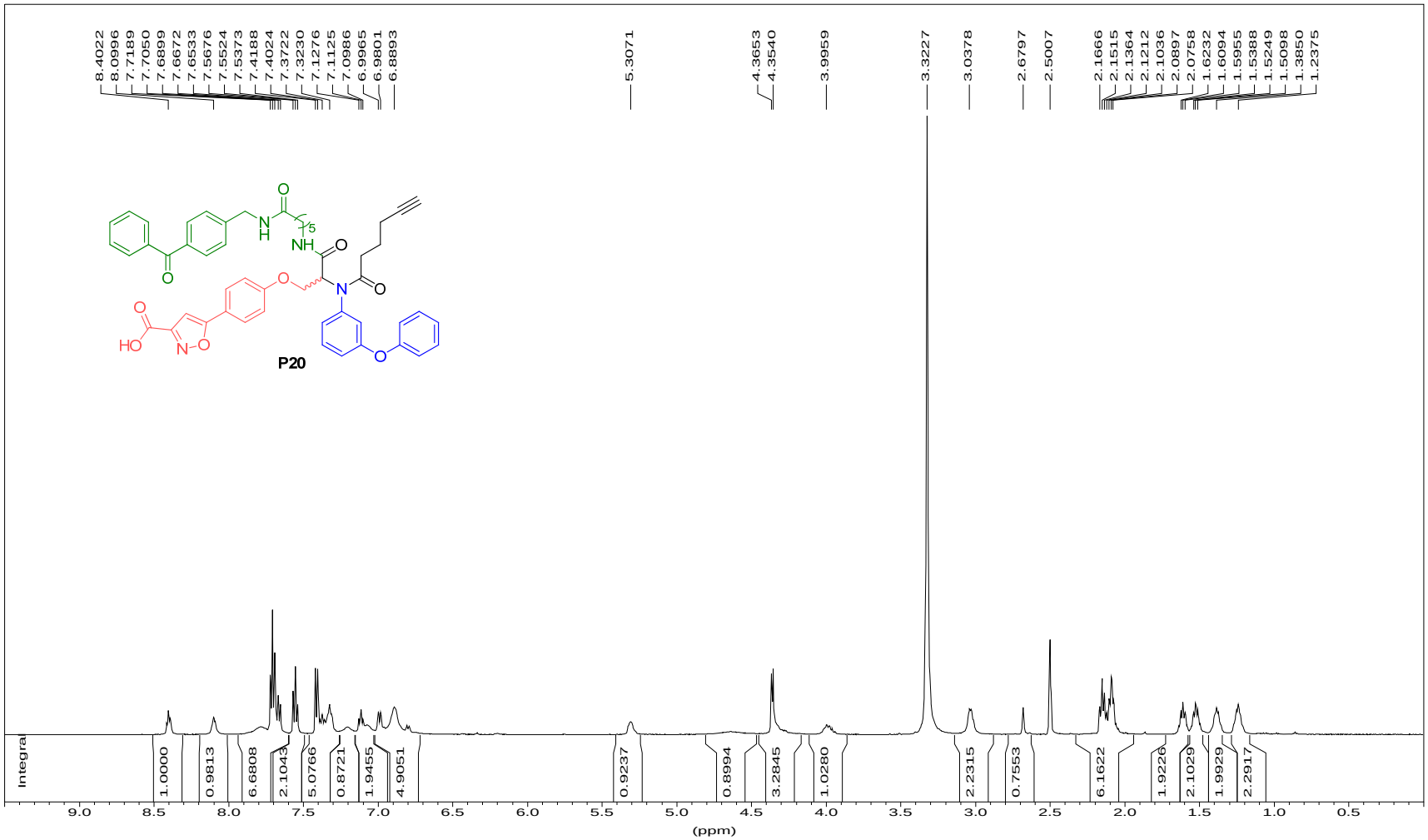
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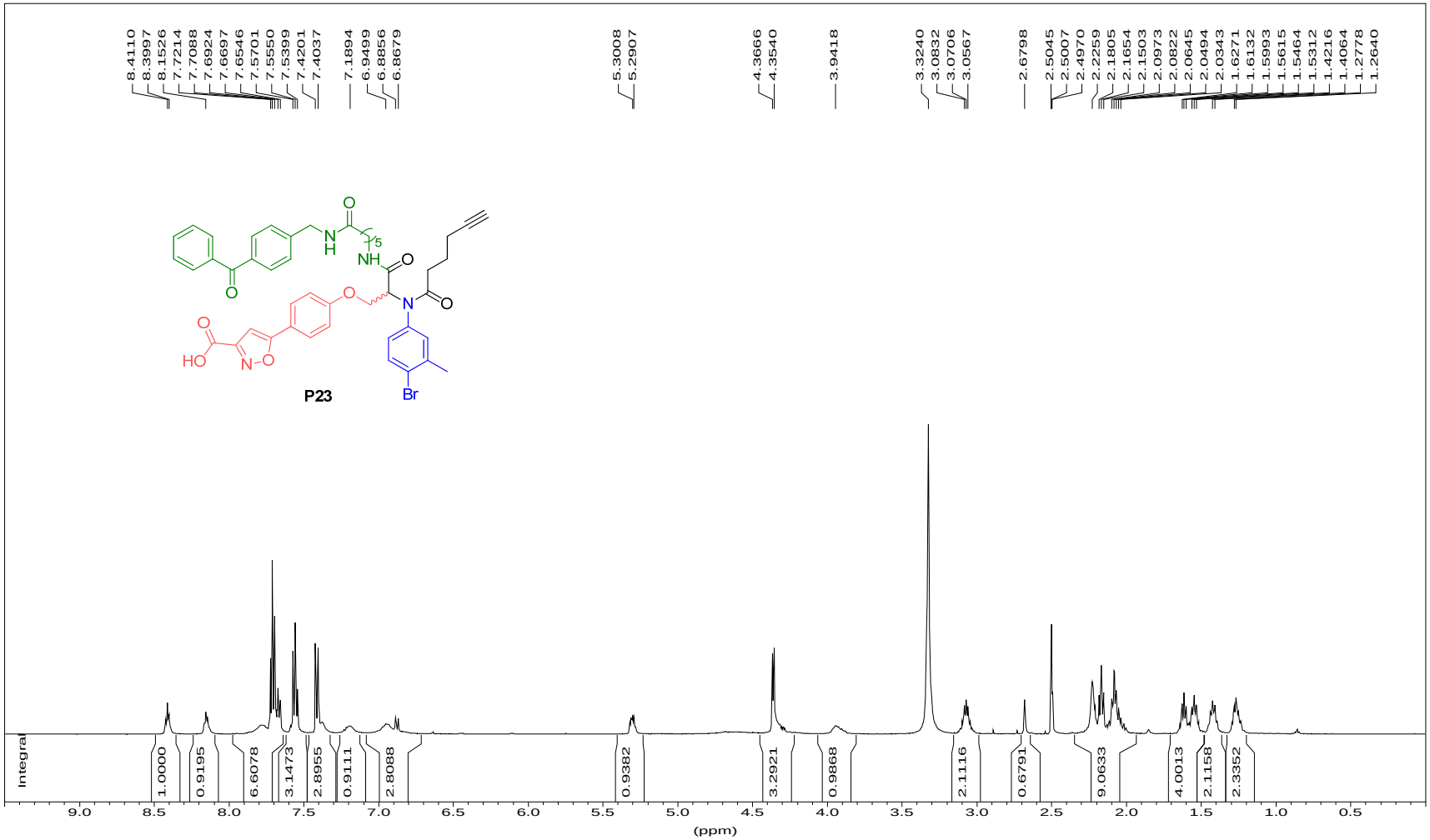
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IPI00219005.3	FKBP4 FK506-binding protein 4	52057	738	29	3.14	immunophilin protein family
IPI00156689.3	VAT1 Synaptic vesicle membrane protein VAT-1 homolog	42122	387	7	0.31	zinc-containing alcohol dehydrogenase family
IPI00295400.1	WARS Tryptophanyl-tRNA synthetase, cytoplasmic	53474	354	10	0.45	class-I aminoacyl-tRNA synthetase family
IPI00011229.1	CTSD Cathepsin D precursor	45037	338	13	0.46	peptidase family
IPI00014151.3	PSMD6 26S proteasome non-ATPase regulatory subunit 6	45787	313	16	1.1	proteasome subunit S10 family
IPI00216308.5	VDAC1 Voltage-dependent anion-selective channel protein 1	30868	289	9	0.85	eukaryotic mitochondrial porin family
IPI00220503.9	DCTN2 dynactin 2	44906	275	8	0.46	dynactin subunit 2 family
IPI00006865.3	SEC22B Vesicle-trafficking protein SEC22b	24896	271	7	1.13	synaptobrevin family
IPI00026154.2	PRKCSH Glucosidase 2 subunit beta precursor	60357	251	6	0.15	
IPI00009032.1	SSB Lupus La protein	46979	245	7	0.35	
IPI00294380.5	PCK2 Phosphoenolpyruvate carboxykinase [GTP], mitochondrial precursor	71447	242	7	0.25	phosphoenolpyruvate carboxykinase [GTP] family.
IPI00152441.3	HM13 Isoform 1 of Minor histocompatibility antigen H13	41747	229	10	0.58	peptidase family
IPI00008986.1	SLC7A5 Large neutral amino acids transporter small subunit 1	55659	223	6	0.11	amino acid-polyamine-organocation (APC) superfamily
IPI00218200.7	BCAP31 B-cell receptor-associated protein 31	34901	217	7	0.89	BCAP29/BCAP31 family
IPI00785096.2	BZW1 similar to basic leucine zipper and W2 domains 1	51420	208	15	0.55	BZW family
IPI00031812.3	YBX1 Nuclease sensitive element-binding protein 1	35903	208	5	0.17	may invovle in cancer
IPI00438229.2	TRIM28 Isoform 1 of Transcription intermediary factor 1-beta	90261	177	8	0.21	TRIM/RBCC family
IPI00029601.4	CTTN Src substrate cortactin	61896	175	7	0.32	
IPI00008274.7	CAP1 Adenylyl cyclase-associated protein 1	52222	173	5	0.18	CAP family
IPI00010896.3	Chloride intracellular channel protein 1	27248	169	4	0.41	chloride channel CLIC family.
IPI00030154.1	PSME1 Proteasome activator complex subunit 1	28876	150	11	1.4	PA28 family
IPI00011107.2	IDH2 Isocitrate dehydrogenase [NADP], mitochondrial precursor	51333	149	7	0.32	isocitrate and isopropylmalate dehydrogenases family
IPI00005719.1	RAB1A Isoform 1 of Ras-related protein Rab-1A	22891	149	6	0.51	Rab family
IPI00002520.1	SHMT2 Serine hydroxymethyltransferase, mitochondrial precursor	56414	147	5	0.22	SHMT family
IPI00027851.1	HEXA Beta-hexosaminidase alpha chain precursor	61106	145	9	0.32	glycosyl hydrolase 20 family
IPI00027442.4	AARS Alanyl-tRNA synthetase, cytoplasmic	107484	145	8	0.17	class-II aminoacyl-tRNA synthetase family
IPI00012303.2	SELENBP1 Selenium-binding protein 1	52928	144	8	0.38	selenium-binding protein family
IPI00025366.4	CS Citrate synthase, mitochondrial precursor	51908	141	4	0.18	citrate synthase family
IPI00215998.5	CD63 CD63 antigen	26474	140	10	0.81	tetraspanin (TM4SF) family
IPI00010471.5	LCP1 Plastin-2	70815	135	5	0.22	actin-binding proteins
IPI00028055.4	TMED10 Transmembrane emp24 domain-containing protein 10 precursor	25131	133	6	0.65	EMP24/GP25L family.
IPI00550069.3	Ribonuclease inhibitor	51766	131	3	0.12	
IPI00013122.1	CDC37 Hsp90 co-chaperone Cdc37	44953	131	7	0.46	CDC37 family
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IPI00004503.5	LAMP1 lysosomal-associated membrane protein 1	45367	126	6	0.32	LAMP family
IPI00003527.5	SLC9A3R1 Ezrin-radixin-moesin-binding phosphoprotein 50	39130	124	6	0.43	solute carrier family
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IPI00105598.3	PSMD11 Proteasome 26S non-ATPase subunit 11 variant (Fragment)	47790	120	3	0.19	proteasome family.
IPI00017334.1	PHB Prohibitin	29843	118	5	0.7	prohibitin family.
IPI00096066.2	SUCLG2 Succinyl-CoA ligase [GDP-forming] beta-chain, mitochondrial precursor	46824	116	4	0.27	succinate/malate CoA ligase beta subunit family
IPI00024664.1	USP5 Isoform Long of Ubiquitin carboxyl-terminal hydrolase 5	96638	116	5	0.09	peptidase family
IPI00100160.3	CAND1 Isoform 1 of Cullin-associated NEDD8-dissociated protein 1	137999	115	7	0.12	CAND family
IPI00299024.9	BASP1 Brain acid soluble protein 1	22680	111	5	0.74	BASP1 family

IPI00020719.2	VISA Isoform 1 of Mitochondrial antiviral-signaling protein	57063	110	4	0.16	
IPI00219913.10	USP14 Ubiquitin carboxyl-terminal hydrolase 14	56489	109	4	0.22	peptidase family
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IPI00031804.1	VDAC3 Isoform 1 of Voltage-dependent anion-selective channel protein 3	30981	108	3	0.23	eukaryotic mitochondrial porin family
IPI00006211.4	VAPB Isoform 1 of Vesicle-associated membrane protein-associated protein B/C	27439	107	6	0.77	VAMP-associated protein family
IPI00000690.1	AIFM1 Isoform 1 of Apoptosis-inducing factor 1, mitochondrial precursor	67144	107	5	0.15	FAD-dependent oxidoreductase family
IPI00216184.3	PICALM Isoform 2 of Phosphatidylinositol-binding clathrin assembly protein	68892	106	2	0.09	
IPI00021258.2	CNDP2 Cytosolic non-specific dipeptidase	41770	103	5	0.24	peptidase family
IPI00103599.1	BRI3BP BRI3-binding protein	27932	102	3	0.12	
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IPI00221234.6	ALDH7A1 Similar to Antiquitin	59020	99	2	0.1	aldehyde dehydrogenase family
IPI00009822.1	SRP54 Signal recognition particle 54 kDa protein	55953	99	4	0.23	GTP-binding SRP family
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IPI00000816.1	YWHAE 14-3-3 protein epsilon	29326	98	4	0.38	14-3-3 family
IPI00296215.1	TACSTD1 Tumor-associated calcium signal transducer 1 precursor	35582	97	3	0.17	EPCAM family
IPI00219301.7	MARCKS Myristoylated alanine-rich C-kinase substrate	31707	96	3	0.22	MARCKS family.
IPI00002460.2	ANXA7 Isoform 1 of Annexin A7	52991	95	6	0.24	annexin family
IPI00216520.1	ARFIP1 Isoform A of Arfaptin-1	38632	92	3	0.24	
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IPI00008223.3	RAD23B UV excision repair protein RAD23 homolog B	43202	90	8	0.39	RAD23 family
IPI00045511.1	CLCC1 Isoform 1 of Chloride channel CLIC-like protein 1 precursor	62667	87	4	0.11	chloride channel MCLC family
IPI00095891.2	GNAS Isoform XLas-1 of Guanine nucleotide-binding protein G(s) subunit alpha isc	111697	85	5	0.08	G-alpha family
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IPI00021926.2	PSMC6 26S protease regulatory subunit S10B	44430	85	3	0.21	AAA ATPase family
IPI00008475.1	HMGCS1 Hydroxymethylglutaryl-CoA synthase, cytoplasmic	57828	85	5	0.16	HMG-CoA synthase family
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IPI00014053.3	TOMM40 Isoform 1 of Probable mitochondrial import receptor subunit TOM40 hom	38211	83	4	0.34	Tom40 family
IPI00007682.2	ATP6V1A Vacuolar ATP synthase catalytic subunit A	68660	82	3	0.09	ATPase alpha/beta chains family
IPI00022334.1	OAT Ornithine aminotransferase, mitochondrial precursor	48846	81	3	0.19	class-III pyridoxal-phosphate-dependent aminotransferase family
IPI00375441.2	FUBP1 Isoform 1 of Far upstream element-binding protein 1	67690	80	5	0.18	
IPI00220365.5	EIF4G1 EIF4G1 variant protein (Fragment)	178843	77	4	0.06	
IPI00030131.3	TMPO Isoform Beta of Lamina-associated polypeptide 2, isoforms beta/gamma	50696	77	3	0.18	LEM family
IPI00027626.3	CCT6A T-complex protein 1 subunit zeta	58444	77	2	0.12	TCP-1 chaperonin family
IPI00013698.1	ASAH1 Acid ceramidase precursor	45077	77	2	0.15	acid ceramidase family
IPI00008167.1	ATP1B3 Sodium/potassium-transporting ATPase subunit beta-3	31834	77	2	0.22	potassium ATPases subunit beta family
IPI00073772.5	FBP1 Fructose-1,6-bisphosphatase 1	37190	76	5	0.29	FBPase class 1 family
IPI00028004.2	PSMB3 Proteasome subunit beta type-3	23219	76	2	0.31	peptidase family
IPI00027444.1	SERPINB1 Leukocyte elastase inhibitor	42829	76	2	0.14	serpin family
IPI00384280.5	PCYOX1 Prenylcysteine oxidase 1 precursor	57003	75	4	0.22	prenylcysteine oxidase family
IPI00107357.6	CLPTM1 Isoform 2 of Cleft lip and palate transmembrane protein 1	79791	74	2	0.07	CLPTM1 family
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IPI00012102.1	GNS N-acetylglucosamine-6-sulfatase precursor	62840	74	3	0.15	sulfatase family

IPI00296191.1	ATP6V1H Isoform 1 of Vacuolar ATP syntha	56417	73	2	0.05	V-ATPase H subunit family
IPI00784119.1	ATP6AP1 Vacuolar ATP synthase subunit S1 precursor	52164	72	4	0.24	vacuolar ATPase subunit S1 family
IPI00299095.2	SNX2 Sorting nexin-2	58549	72	3	0.18	sorting nexin family
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IPI00013895.1	S100A11 Protein S100-A11	11847	72	3	0.66	S-100 family
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IPI00141318.2	CKAP4 Isoform 1 of Cytoskeleton-associated protein 4	66097	70	6	0.03	
IPI00000494.6	RPL5 60S ribosomal protein L5	34569	70	2	0.2	ribosomal protein L18P family
IPI00021728.3	EIF2S2 Eukaryotic translation initiation factor 2 subunit 2	38706	69	2	0.16	eIF-2-beta/eIF-5 family.
IPI00021766.4	RTN4 Isoform 1 of Reticulon-4	130420	68	5	0.09	
IPI00010740.1	PPT1 Palmitoyl-protein thioesterase 1 precursor	76216	68	4	0.13	palmitoyl-protein thioesterase family
IPI00003818.1	KYNU Kynureninase	52831	68	2	0.13	kynureninase family.
IPI00024502.2	UBQLN4 Ubiquilin-4	63869	67	2	0.05	
IPI00217766.3	SCARB2 Lysosome membrane protein 2	54712	62	3	0.11	CD36 family
IPI00016255.4	FLJ22662 hypothetical protein LOC79887	63499	62	2	0.09	phospholipase B-like family
IPI00419237.3	LAP3 Isoform 1 of Cytosol aminopeptidase	56530	61	2	0.11	peptidase family
IPI00009960.6	IMMT Isoform 1 of Mitochondrial inner membrane protein	84026	61	4	0.11	
IPI00305383.1	UQCRC2 Ubiquinol-cytochrome-c reductase complex core protein 2, mitochondrial	48584	59	2	0.12	peptidase family
IPI00009030.1	LAMP2 Isoform LAMP-2A of Lysosome-associated membrane glycoprotein 2 precu	45503	59	2	0.13	LAMP family
IPI00009104.7	RUVBL2 RuvB-like 2	51296	58	2	0.12	peptidase family
IPI00217960.1	PRKACA Isoform 2 of cAMP-dependent protein kinase, alpha-catalytic subunit	39911	55	2	0.15	protein kinase superfamily