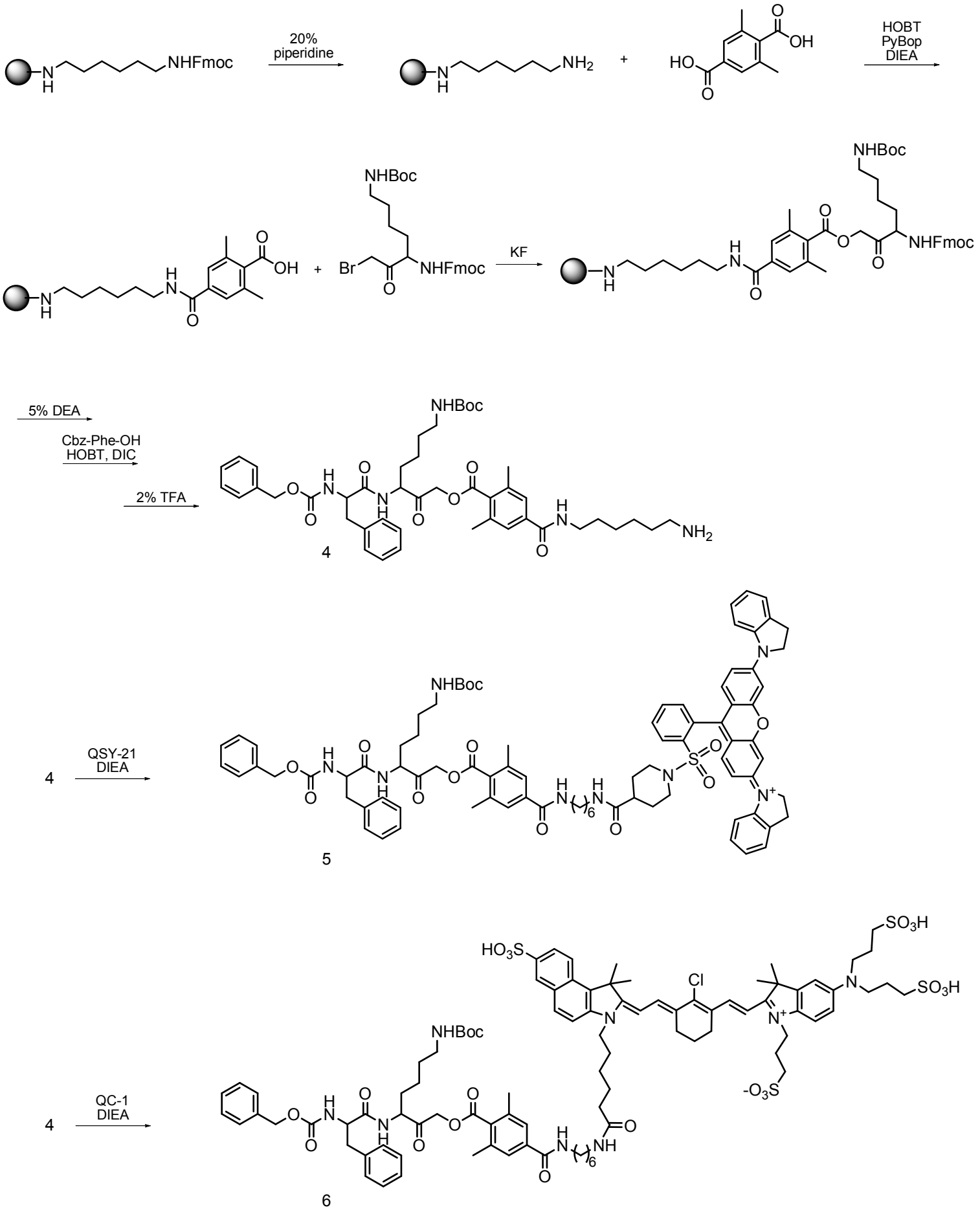
Supplementary Scheme 1



Synthesis of the PS quenched ABPs precursors 5 and 6. Fmoc, 9-fluorenylmethylcarbonyl; HOBT, 1-hydroxybenzotriazole; PyBOP, (benzotriazole-1-yl- oxy) tris (pyrrolidino) phosphonium hexafluorophosphate; DIEA, diisopropylethylamine;Boc, t-butyloxycarbonyl; DEA, Diethylamine; Cbz, carboxybenzyl; DIC, diisopropyl-carbodiimide; TFA, trifluoroacetic acid.

Supplementary Tables

Table 1. Synthesis conditions for PS coupling by activation to succinimide ester, method 1.

Probe	PS	PS [eq.]	DCC [eq.]	TSTU [eq.]	Activation time	Precursor, 1eq.	Coupling time	%ACN elution	Color	Yield [%]	M/z
YBN2	Pheophorbide-a	1.2	1.4	1.4	40 minutes	5	30 minutes	71-75	turquoise	66	1954
YBN6	Visudyne	1.2	1.4	1.4	1 hour	5	1.5 hours	57-63	turquoise	81	(M/z+1)/2 1040
YBN13	Bacteriochlorin	1.1	1.1	1.4	2.5 hours	3, GB111-NH_2	30 minutes	49-52	magenta	43	M/z+1 1291
YBN14	Bacteriochlorin	0.9	1.1	1.4	2 hours	6	over night	47-50	dark blue-purple	46	(M/z+2)/2 1249

Table 2. Synthesis conditions for PS coupling by coupling reagents, method 2

Probe	PS	PS [eq.]	DCC [eq.]	HOBT [eq.]	Precursor, 1eq.	Coupling time	Color	Yield [%]	Mw
YBN1	Pheophorbide-a	1.1	2	2	3, GB111-NH_2	2.5 hours	dark green	77	M/z+1 1148
YBN3	Pyropheophorbide-a	1.1	2	2	3, GB111-NH_2	2 hours	dark green	60	M/z+1 1090
YBN4	Pyropheophorbide-a	3	3	3	5	1 hour	turquoise	39	M/z+1 1897
YBN5	Visudyne	1.1	2	2	3, GB111-NH_2	1.5 hours	light green	81	M/z+1 1274
YBN7	Chlorin-*e*6	1.1	2	2	3, GB111-NH_2	1 hour	dark green	94	M/z+1 1152
YBN8	Chlorin-*e*6	3	3	3	5	1.5 hours	turquoise	49	M/z+1 1959

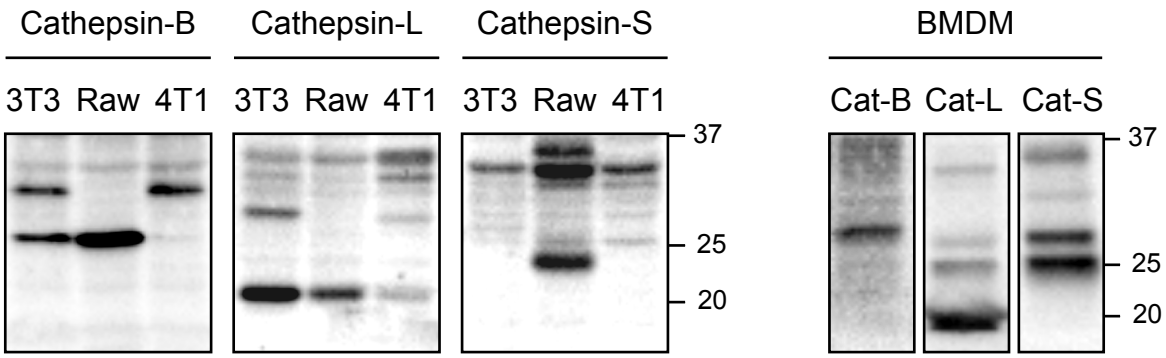
Table 3. R groups of YBN 1-8

R_1	Non-Quenched R_2 = H	Quenched R_2 = Linker-QSY-21
Pheophorbide-a	YBN1	YBN2
Pyropheophorbide-a	YBN3	YBN4
Visudyne	YBN5	YBN6
Chlorin-*e*6	YBN7	YBN8

Table 4. Quenching efficiency of YBN1-8:

Quenching Efficiency	**Pheophorbide-a**	**Pyropheophorbide-a**	**Visudyne**	**Chlorin-*e*6**
	YBN2	**YBN4**	**YBN6**	**YBN8**
pH = 7.5	54	88	363	40
pH = 5.5	363	26	164	14

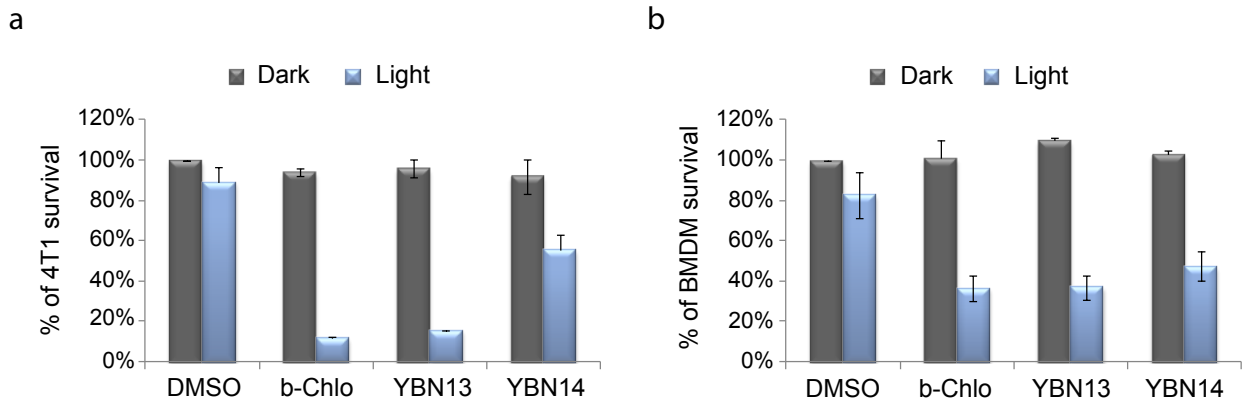
Supplementary Figure S1



Cathepsins expression in cell lines: NIH 3T3, Raw 264.7, 4T1 and BMDM were harvested in RIPA lysis buffer (1% Tergitol-type NP-40, 0.1% SDS, 0.5% sodium deoxycholate). Equal protein amounts were separated on a 12.5% SDS-PAGE and immunoblotted on PVDF membrane. The following antibodies were used: Cathepsin B (1:1000)[1], Cathepsin L (Santa Cruz Biotechnology, Inc, Santa Cruz, CA, USA, 1:500) Cathepsin S (R&D Systems Minneapolis, MN, USA 1:1000). Chemiluminescence was measured with a Chemi-Doc XRS imaging system and an Image Lab Software Bio-Rad, Hercules, CA, USA.

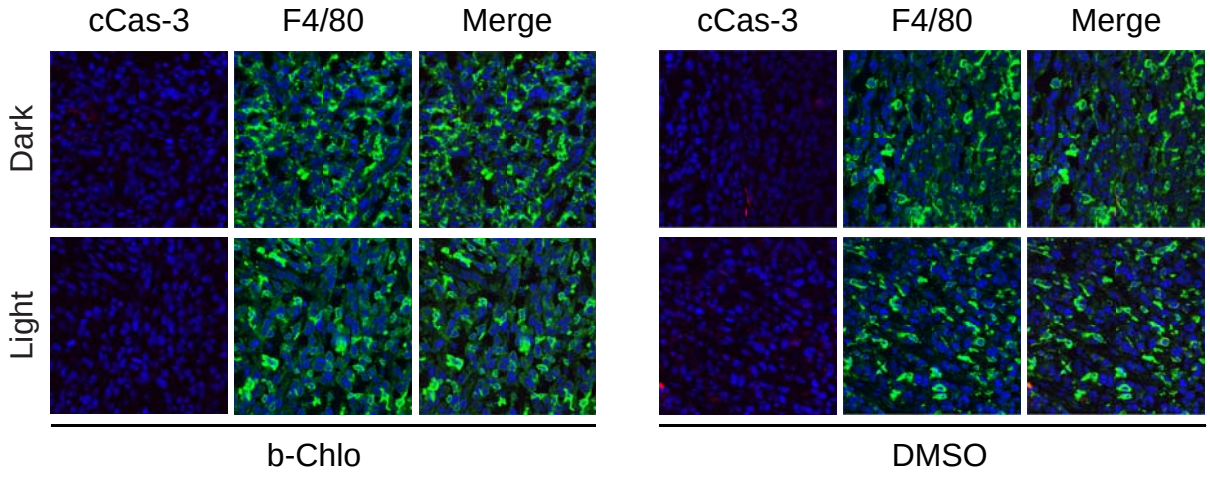
Refernce
1. Kos J, Smid A, Krasovec M, et al. Lysosomal proteases cathepsins D, B, H, L and their inhibitors stefins A and B in head and neck cancer. Biol Chem Hoppe Seyler. 1995; 376: 401-5.

Supplementary Figure S2



Evaluation of second generation probes for cell killing: 4T1 cells (7,000 cells/well, **a**) or bone marrow derived macrophages (BMDM, 22,300 cells/well, **b**) were cultured in 96 well plates one day prior to treatment. Triplicate samples of cells were incubated with 20 μM YBN13 or YBN14 or free photosensitizer (final concentration of 0.1%DMSO was kept constant) in growth medium without phenol for 4 hours. Cells were illuminated from the bottom with a 45 mW light dose at 760 nm for 9 minutes, or kept in the dark. Cell survival was determined a day after illumination by a WST-1 assay, relative to DMSO dark control. Probes demonstrated efficient cell killing after light treatment.

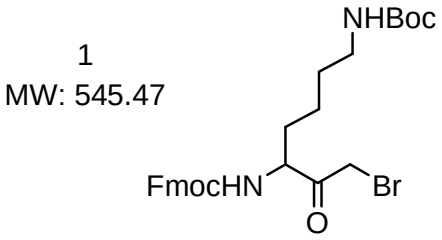
Supplementary Figure S3

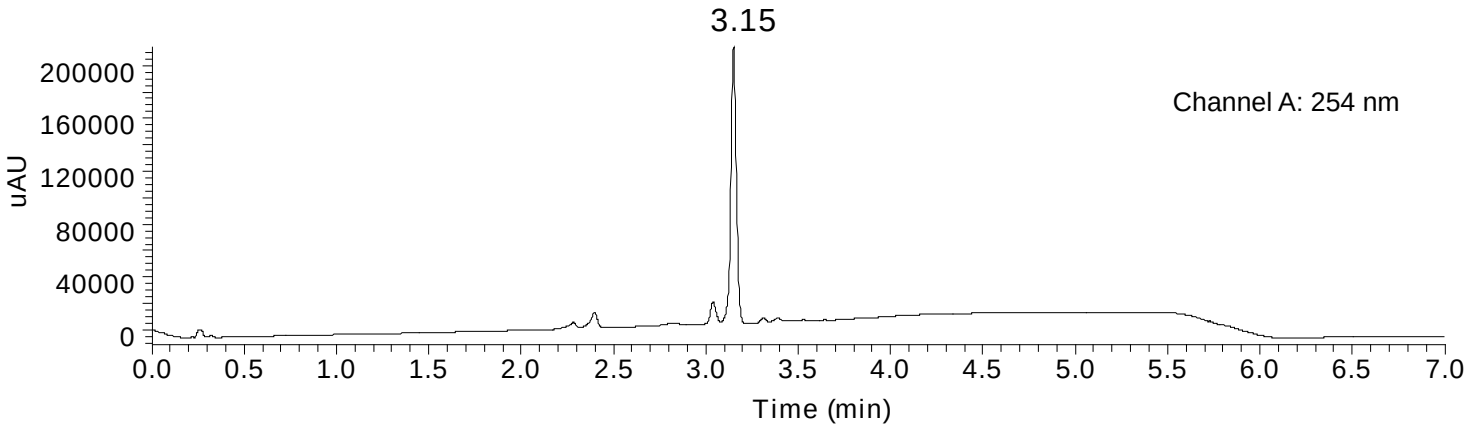


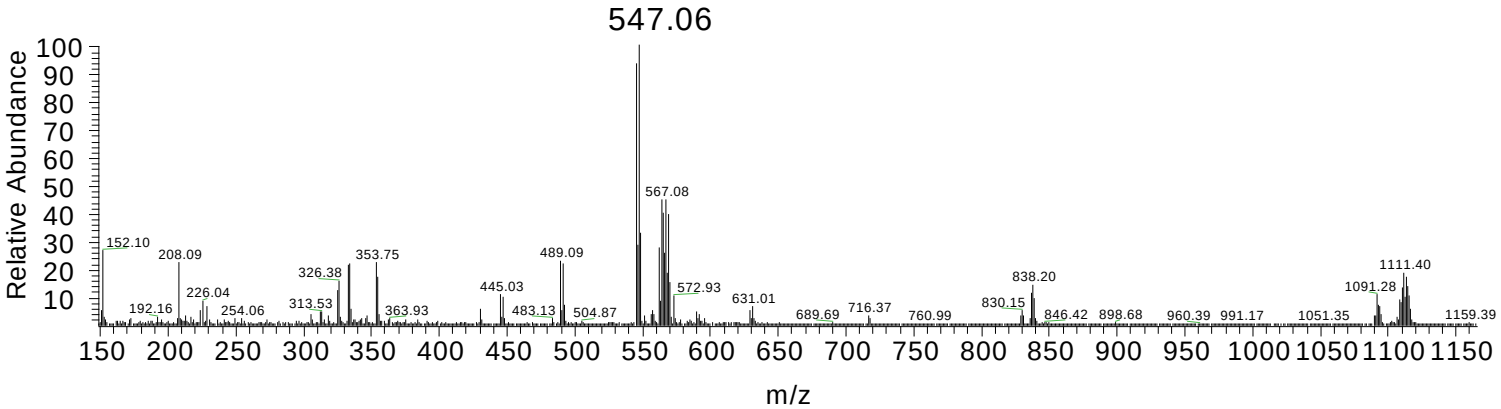
Photodynamic therapy effect in tumor bearing mice. Tumors from control mice injected with DMSO vehicle or bacteriochlorin were frozen in OCT, sectioned, and stained with cleaved Caspaese-3 and F4/80 antibodies labeled with Cy5 and Cy3 respectively. Representative fluorescent scan acquired with an Olympus FV10i confocal microscope are presented, green- F4/80, Red- Cleaved Caspase-3, Blue- DAPI. Apoptosis indicated by cleaved caspase 3 was not detected in F4/80 macrophages of light treated tumors of DMSO vehicle or bacteriochlorin.

Supplementary S4

Compound structure, UV trace, mass spetrometry analysis of the major peak and maximal fluorescent absorption (when applicable).







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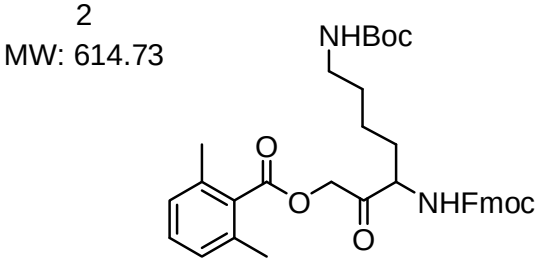
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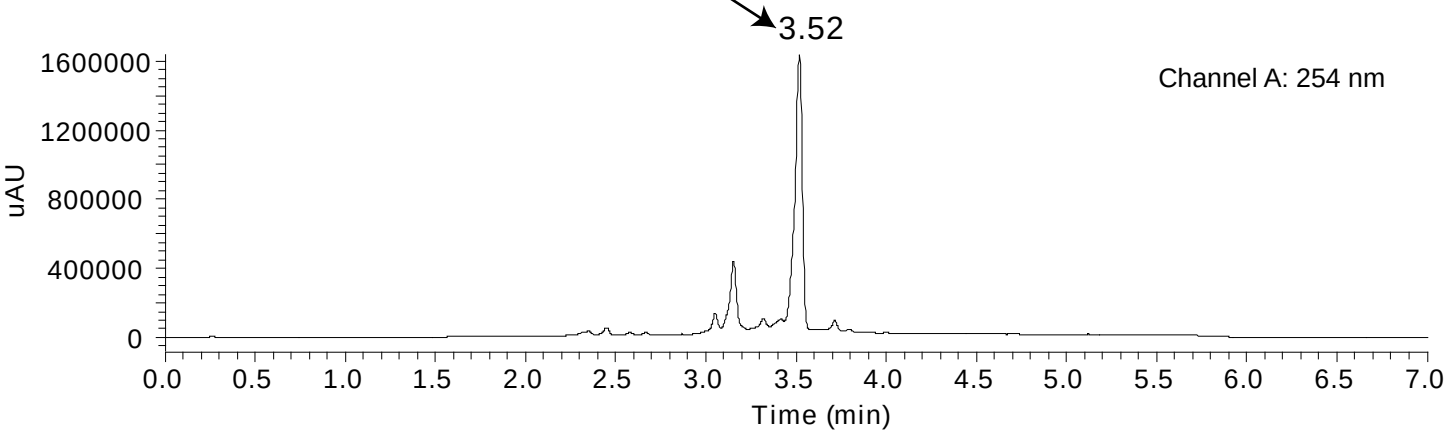
O
O
NHFmoc
O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1.5
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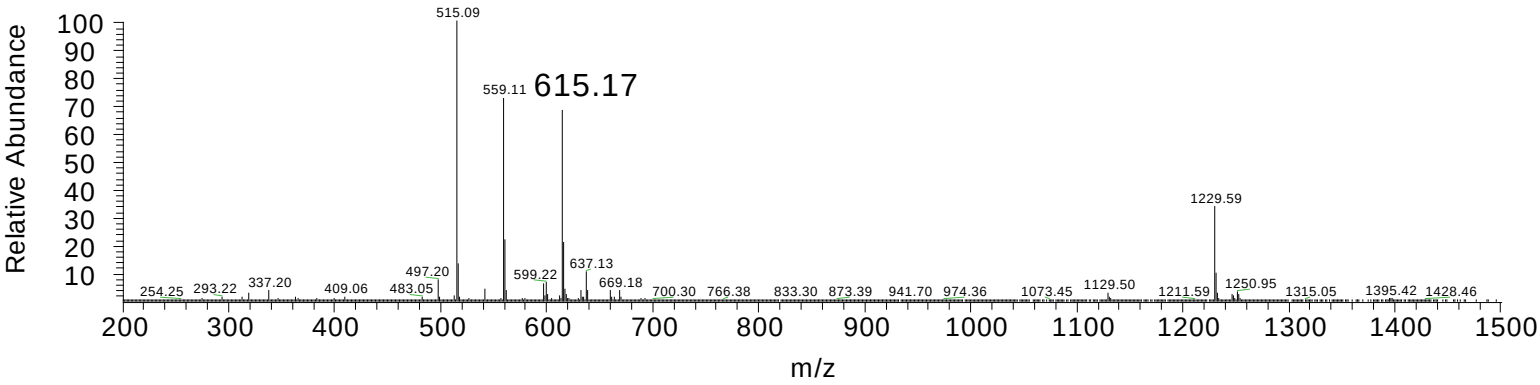
5.5
6.0
6.5
7.0
Time (min)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615.17
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599.22
637.13
669.18
254.25
293.22
337.20
409.06
483.05
700.30
766.38
833.30
873.39
941.70
974.36
1073.45
1129.50
1211.59
1250.95
1315.05
1395.42
1428.46
200
300
400
500
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900
1000
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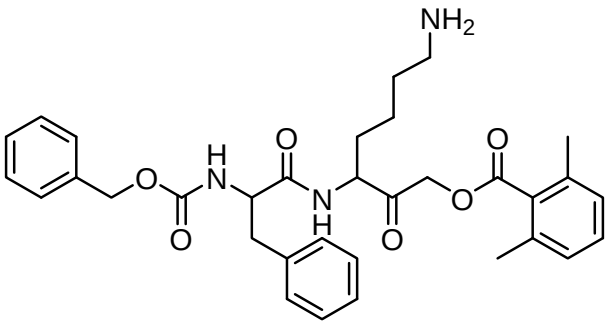
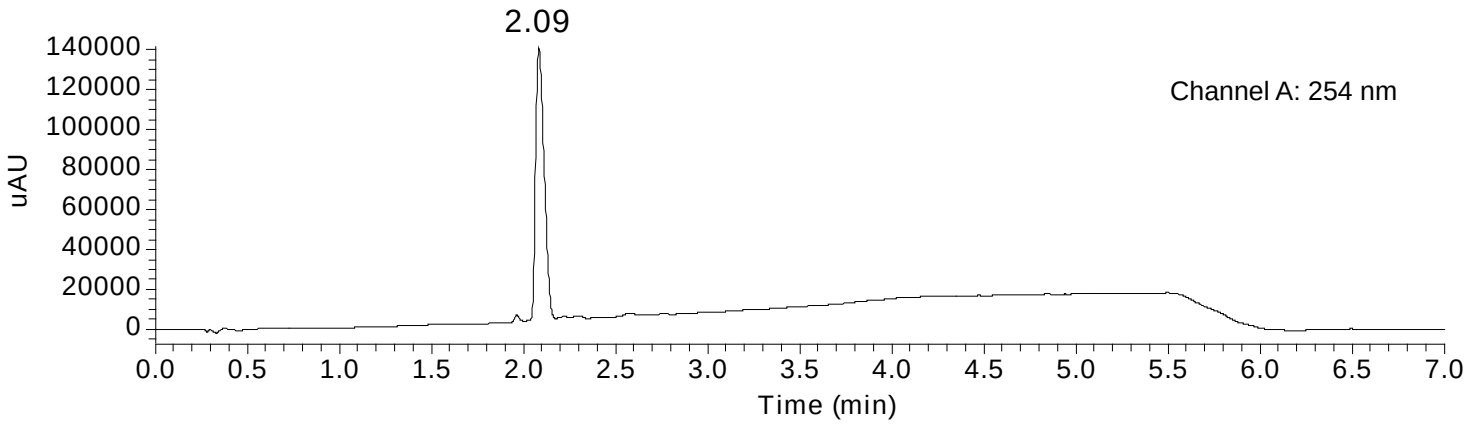
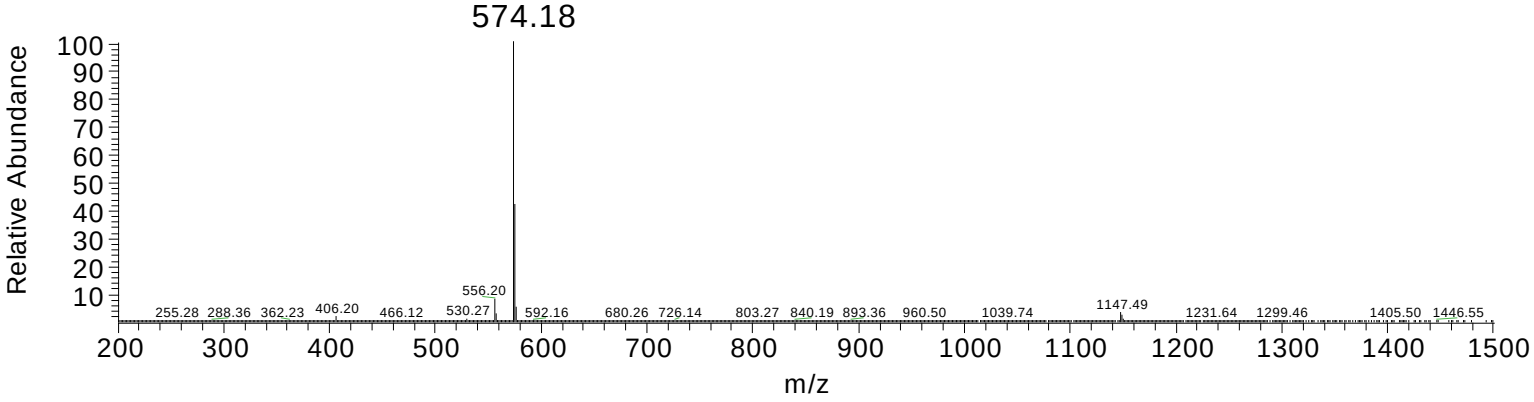
1400
1500
m/z

3, GB111-NH_2

MW: 573.68







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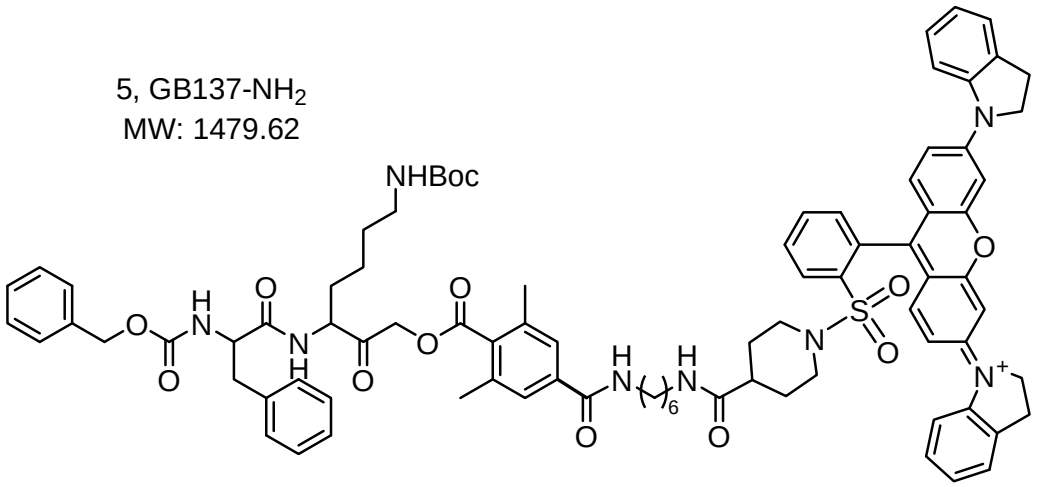

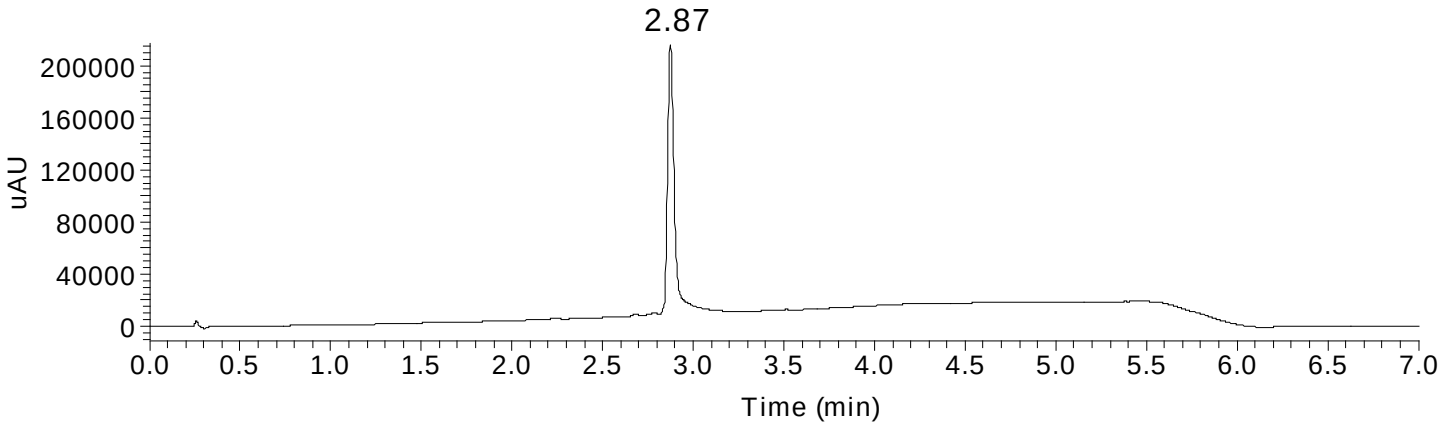

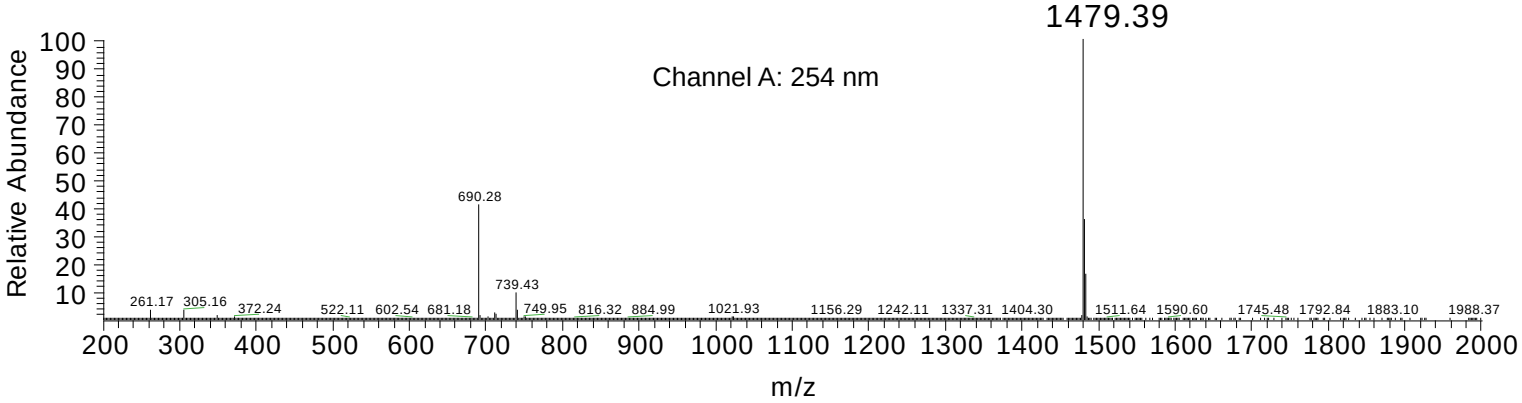

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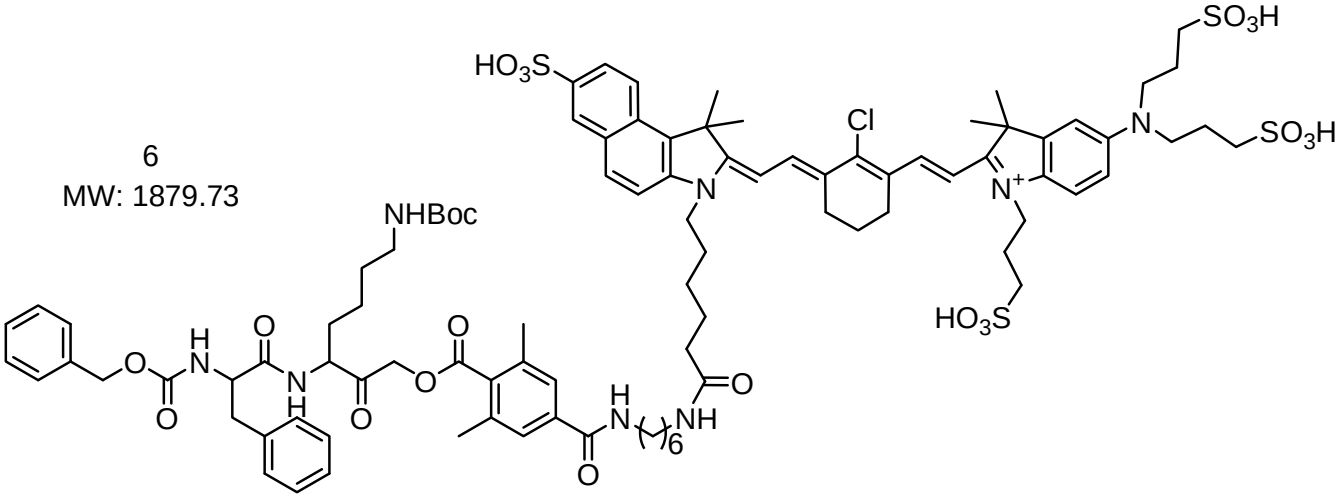
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HO3S
Cl
N
SO3H
N+
NHBoc
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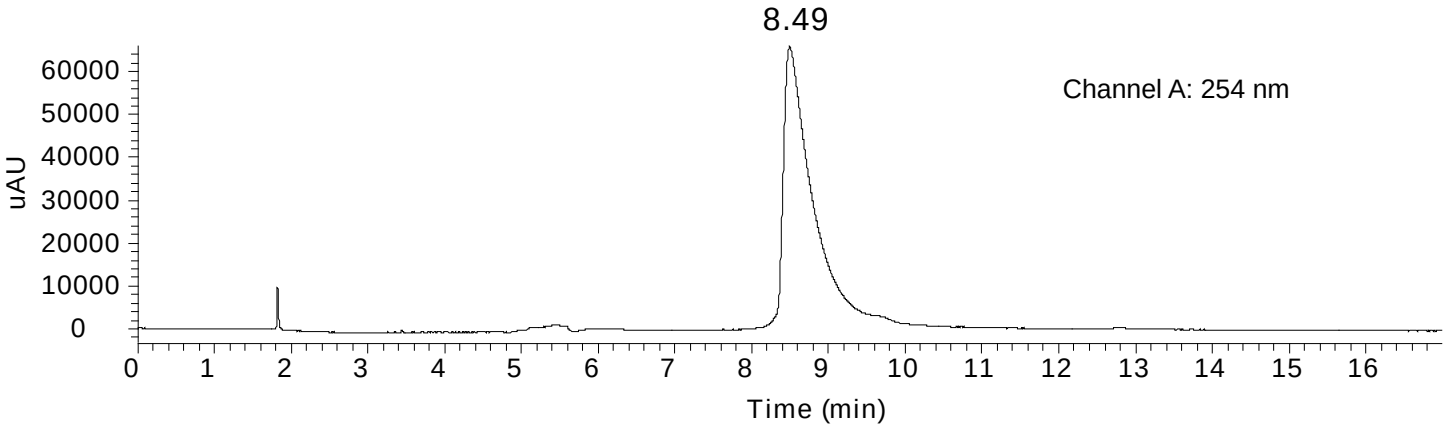
H
N
NH
6
O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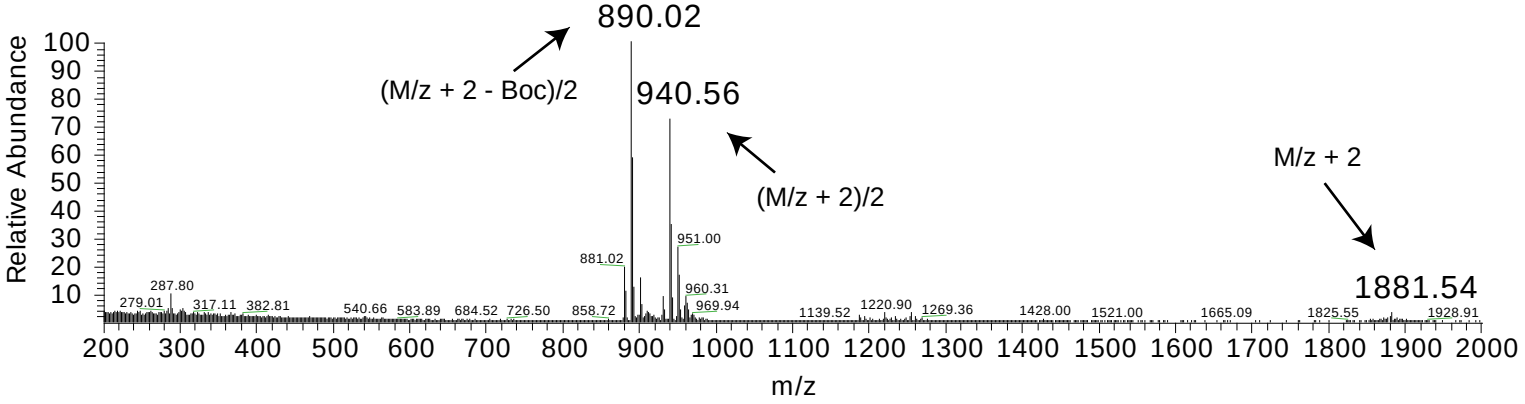
0 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16
Time (min)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(M/z + 2 - Boc)/2
940.56
(M/z + 2)/2
M/z + 2
1881.54
951.00
881.02
960.31
969.94
287.80
279.01
317.11
382.81
540.66
583.89
684.52
726.50
858.72
1139.52
1220.90
1269.36
1428.00
1521.00
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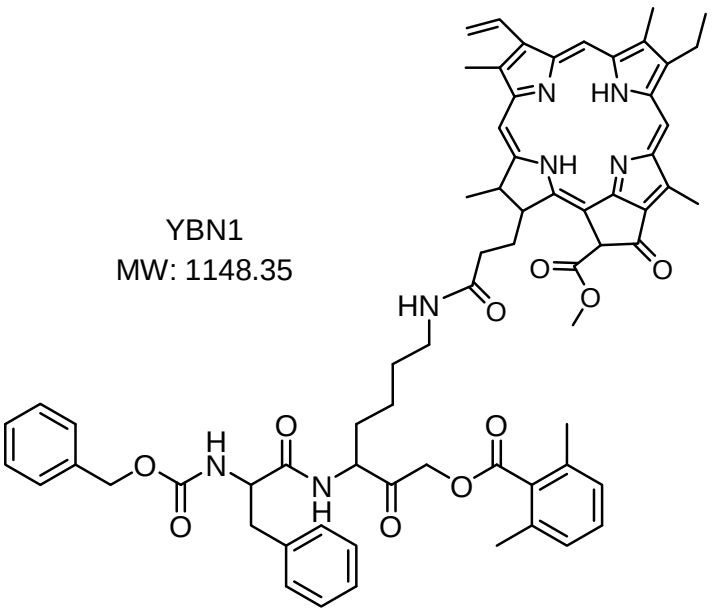
200 300 400 500 600 700 800 900 1000 1100 1200 1300 1400 1500 1600 1700 1800 1900 2000
m/z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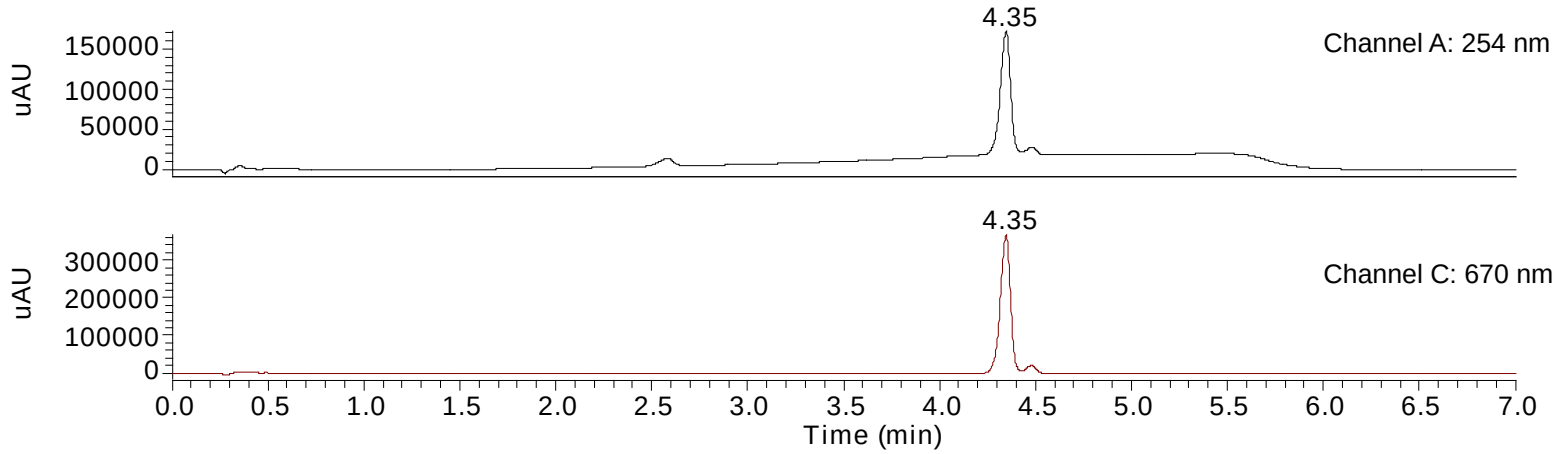
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4.35
Channel C: 670 nm
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300000
200000
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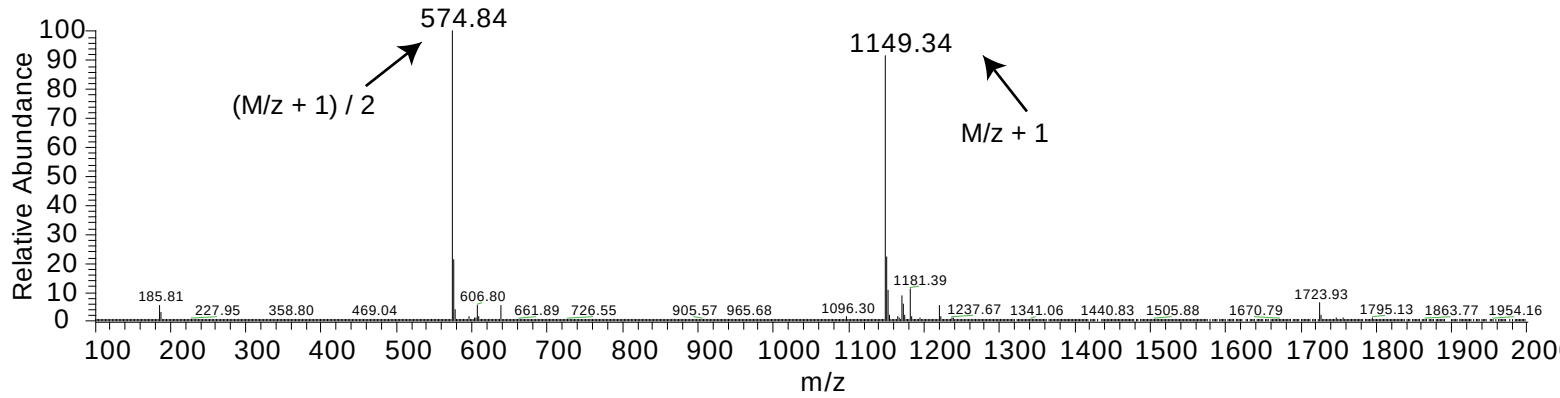
Time (min)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(M/z + 1) / 2
1149.34
M/z + 1
Relative Abundance
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m/z

YBN2
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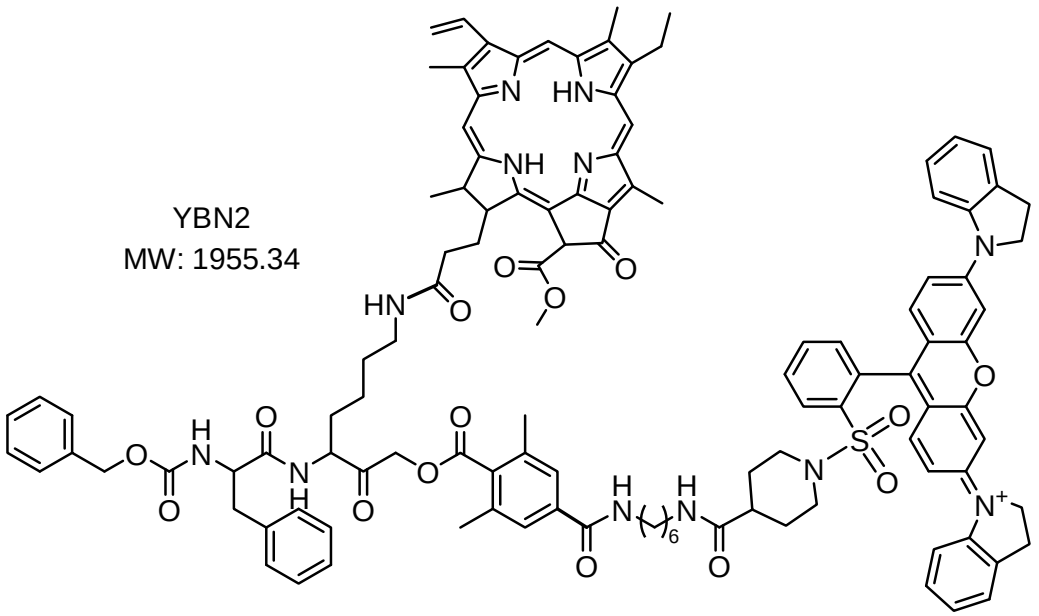

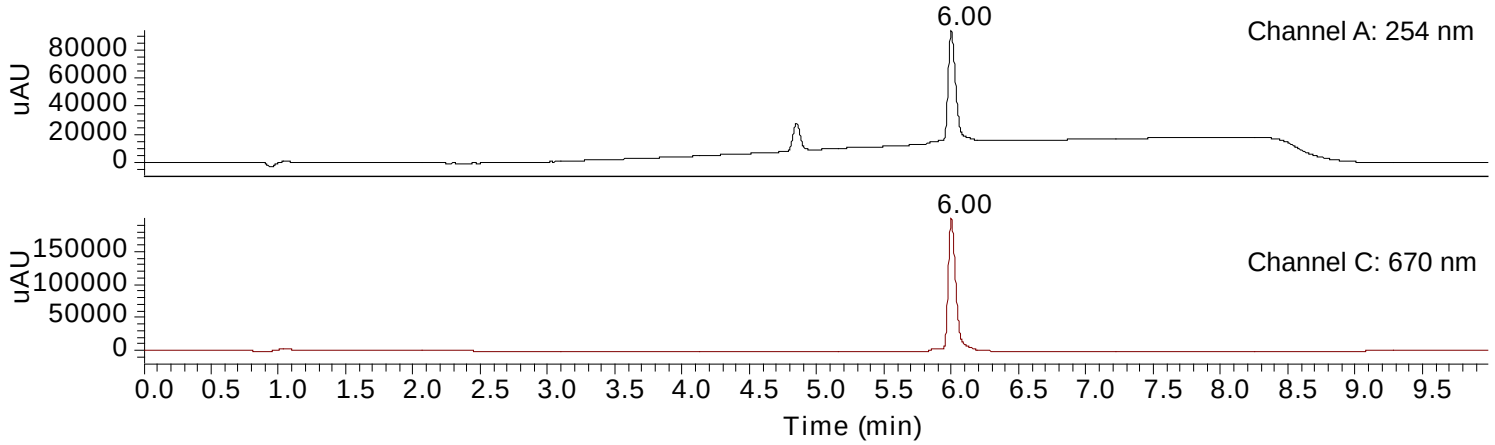

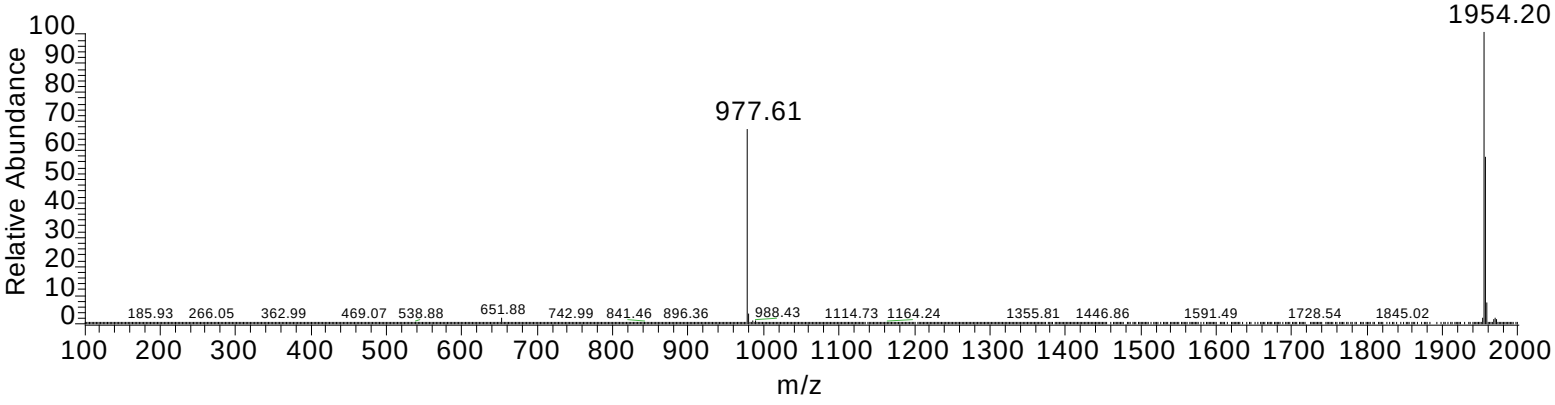

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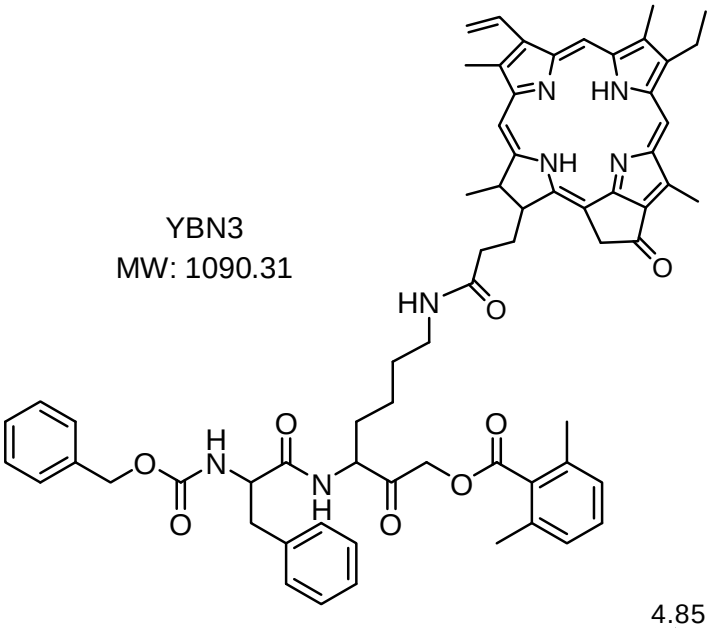

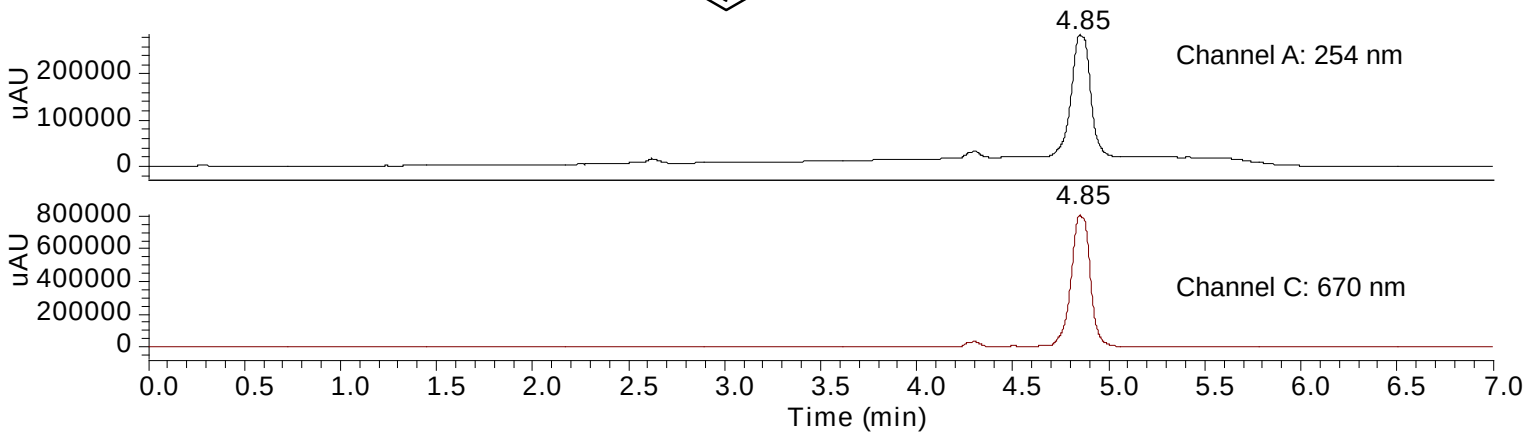

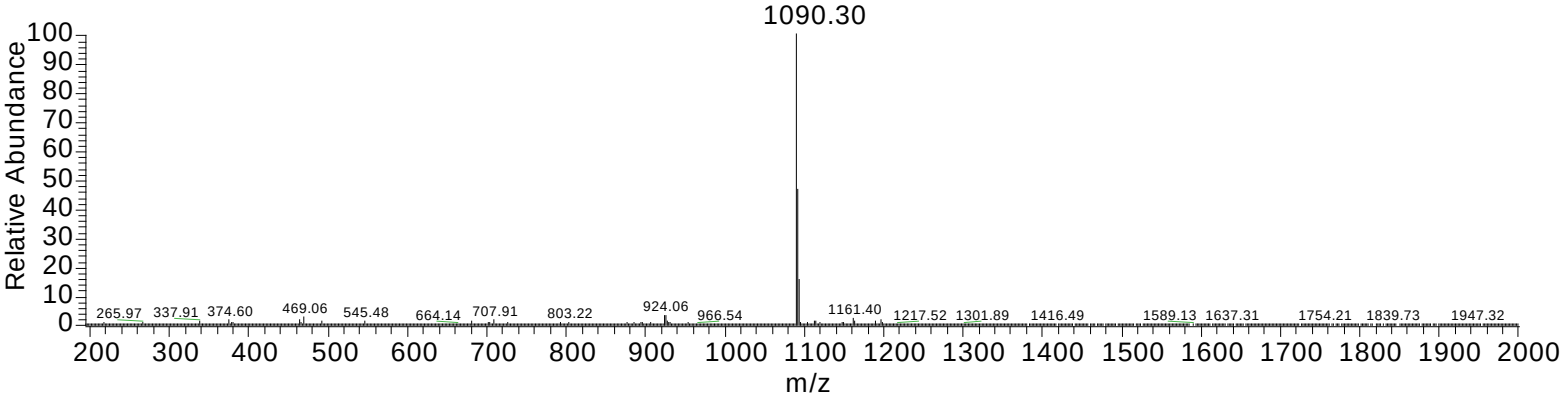

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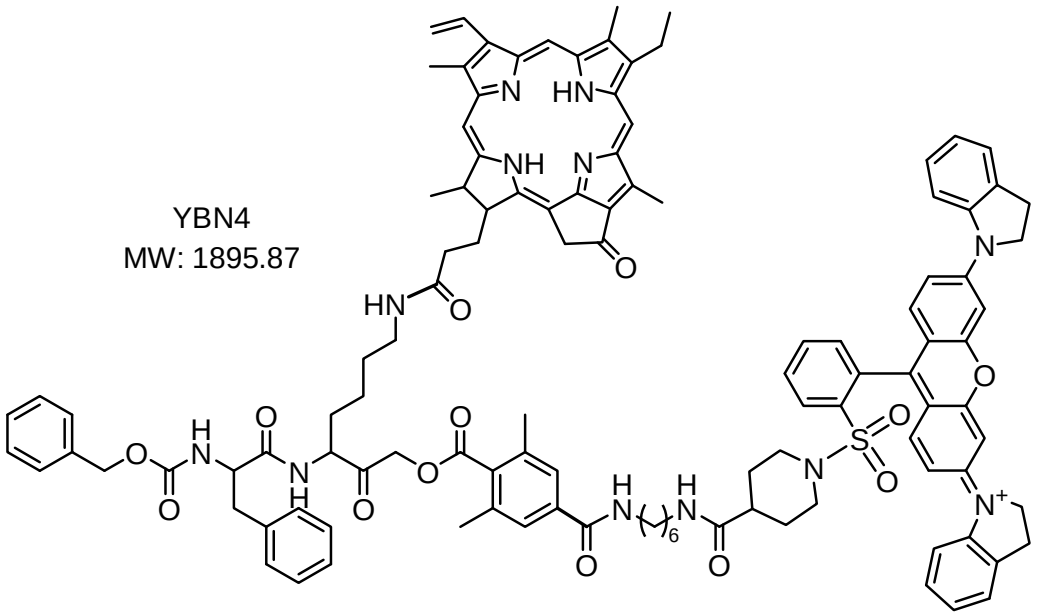

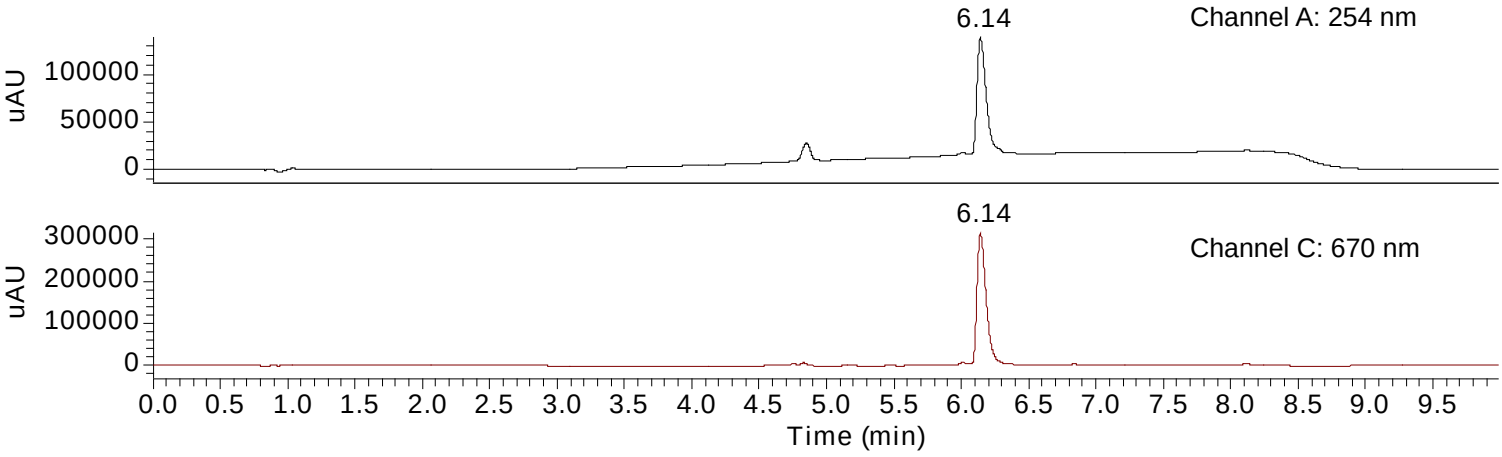

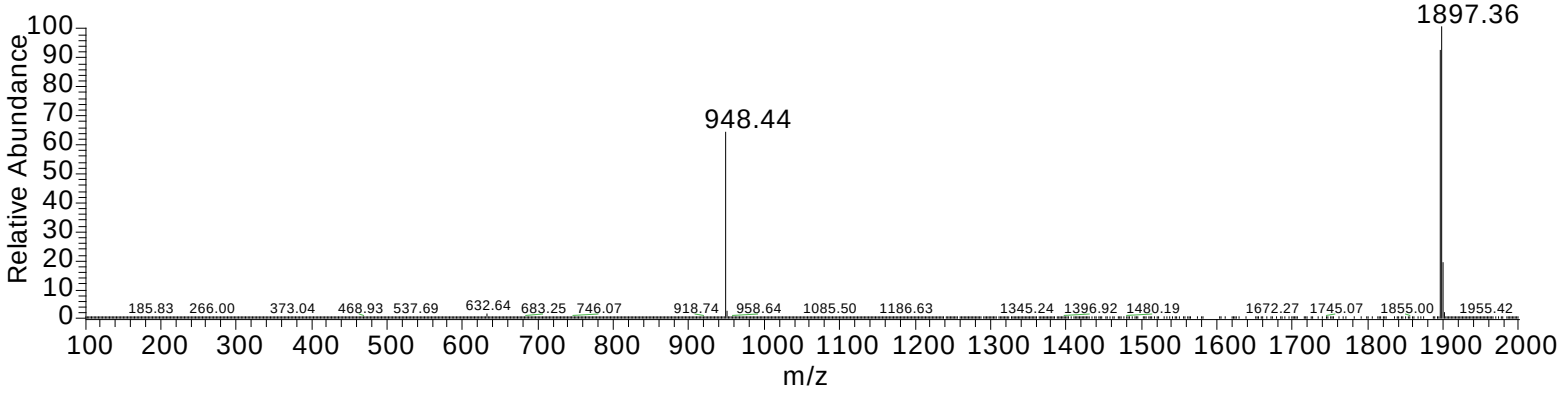

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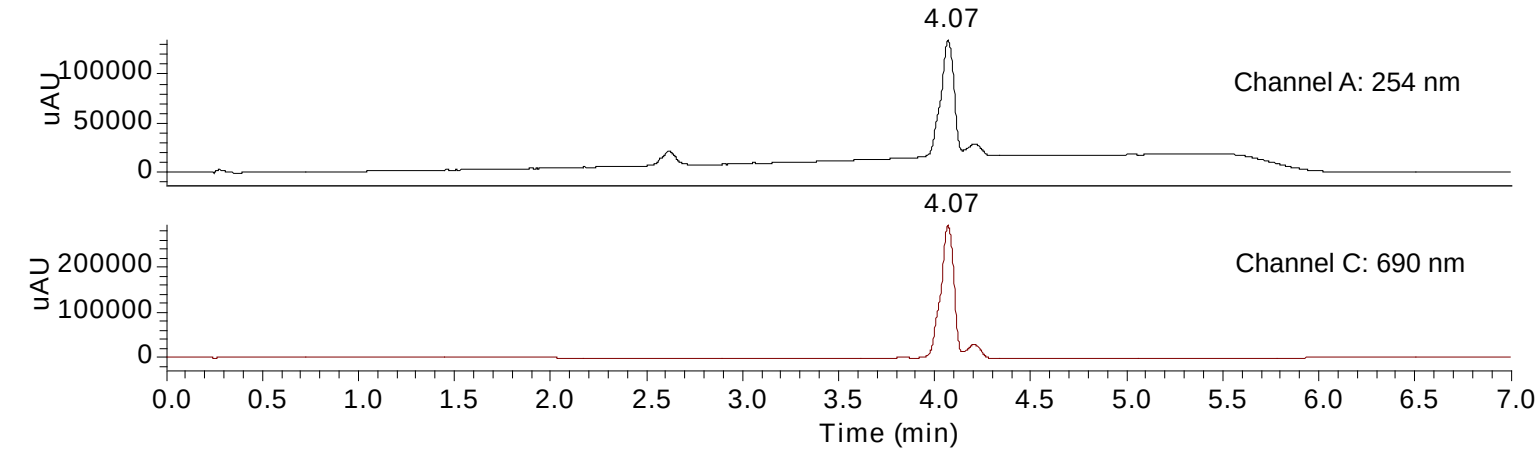

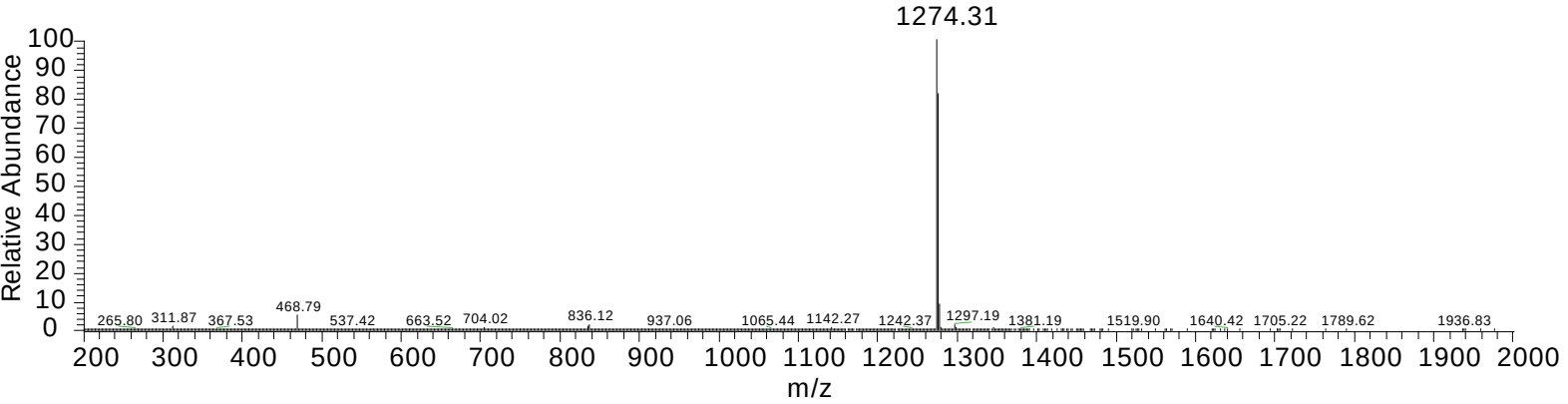

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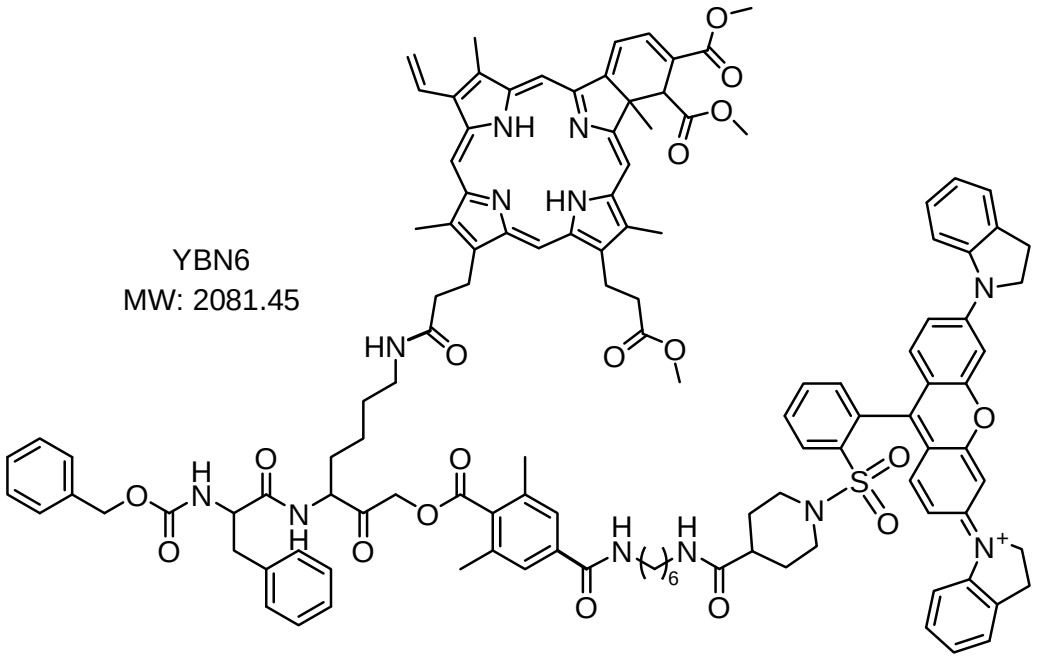

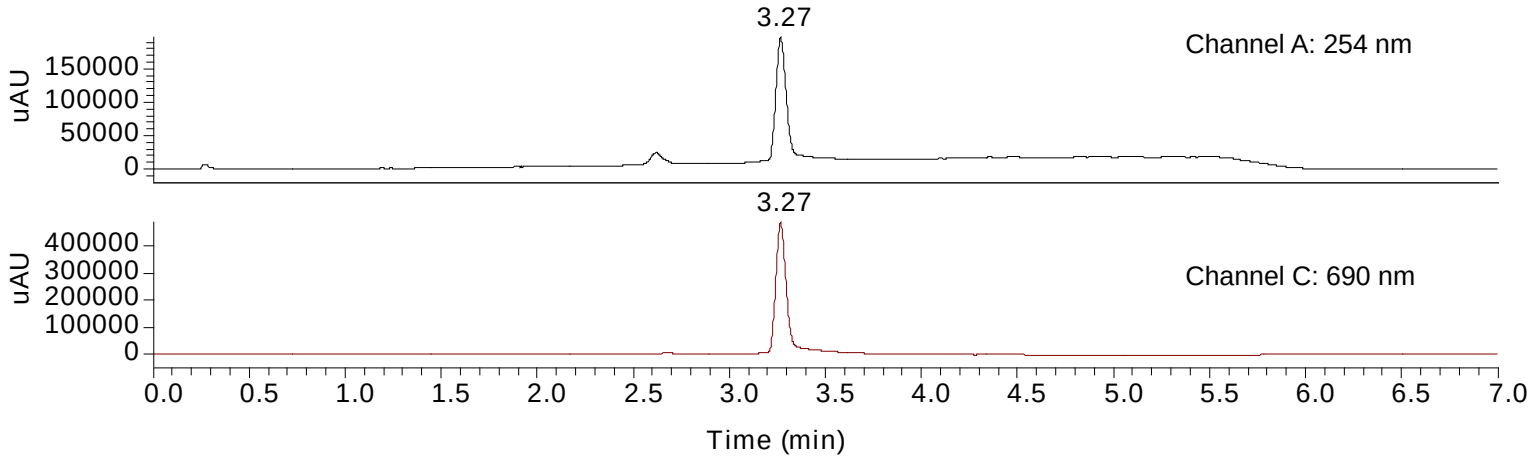

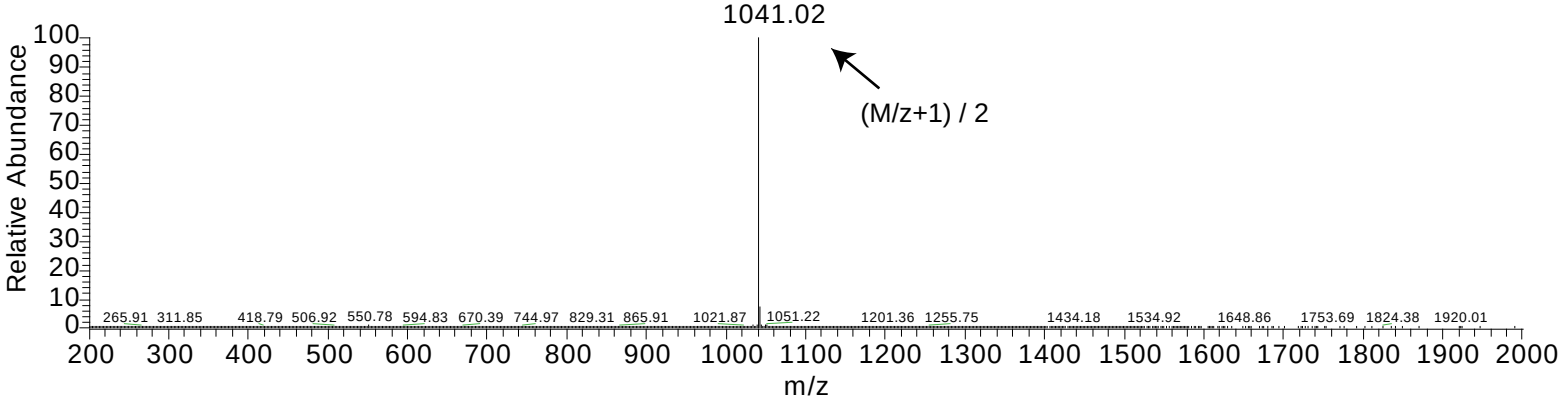

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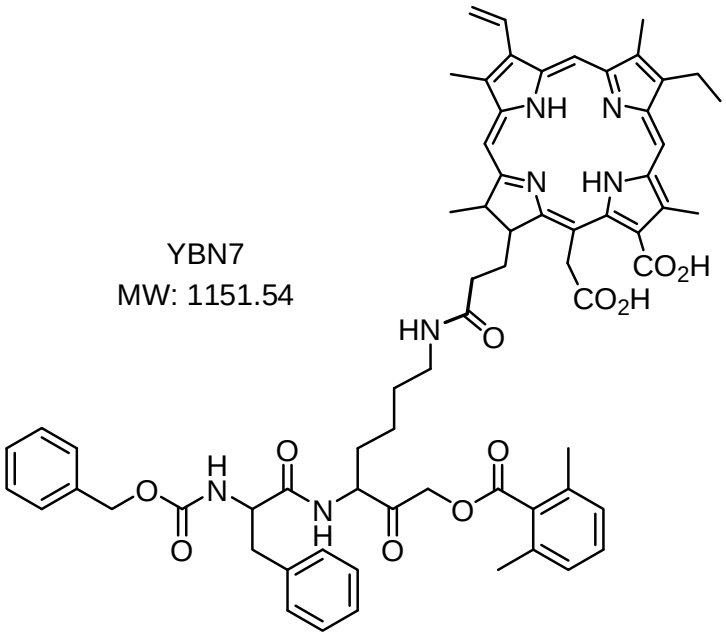

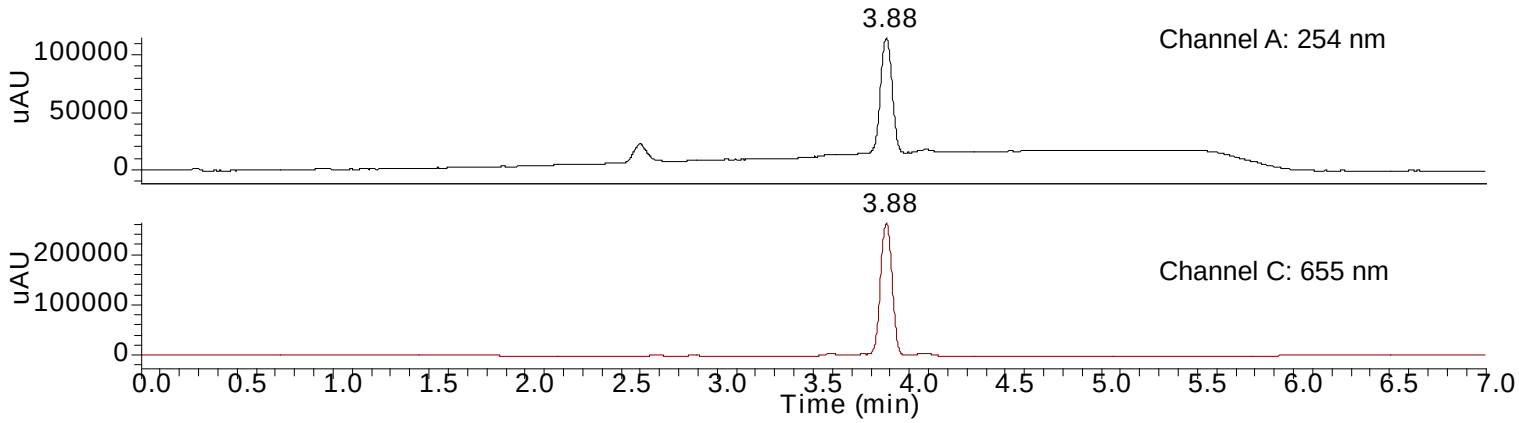

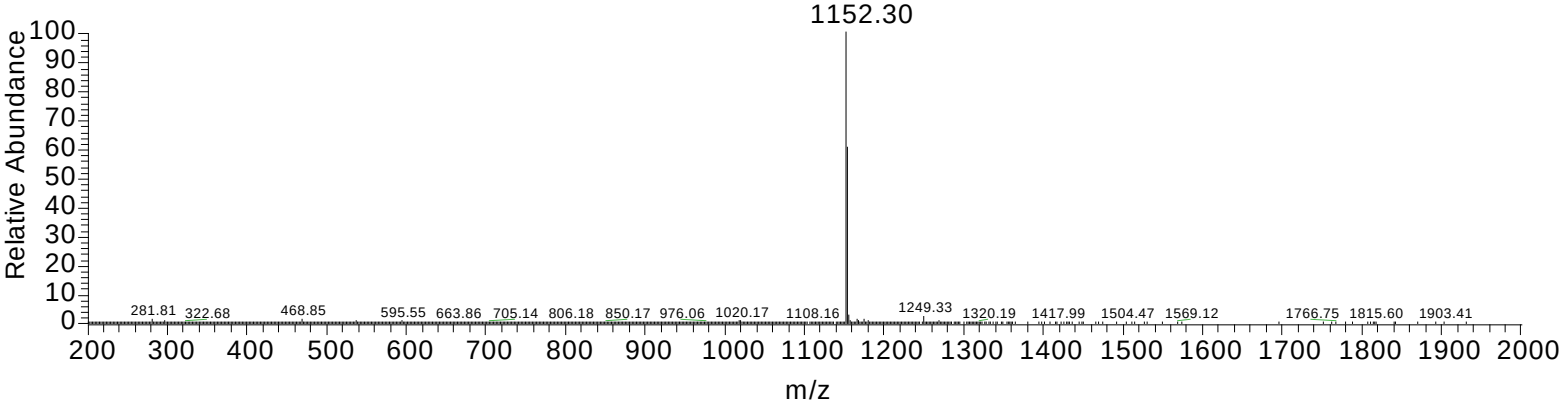

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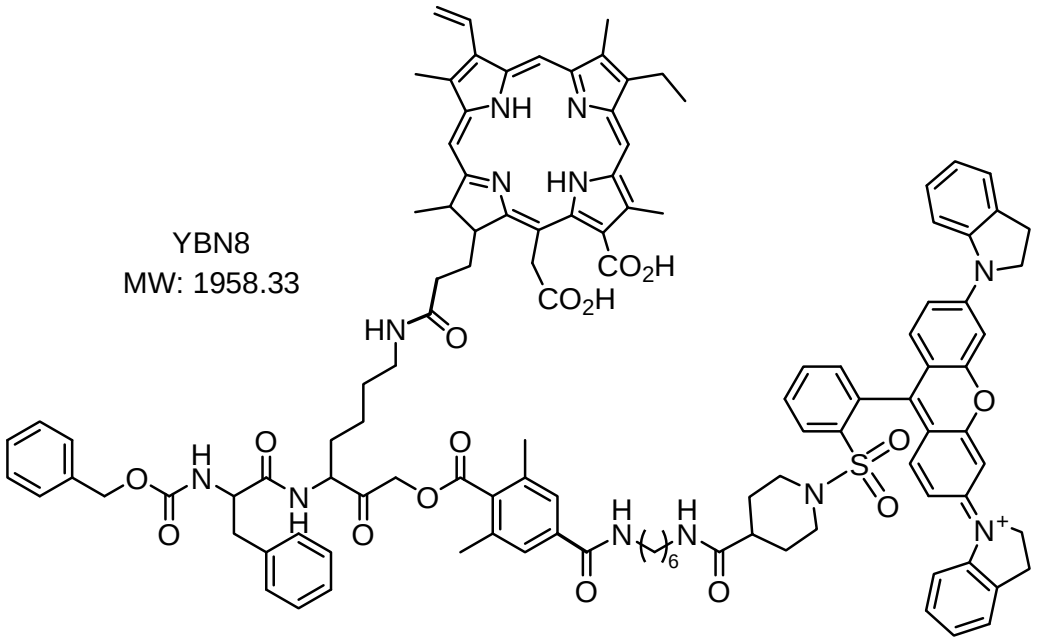
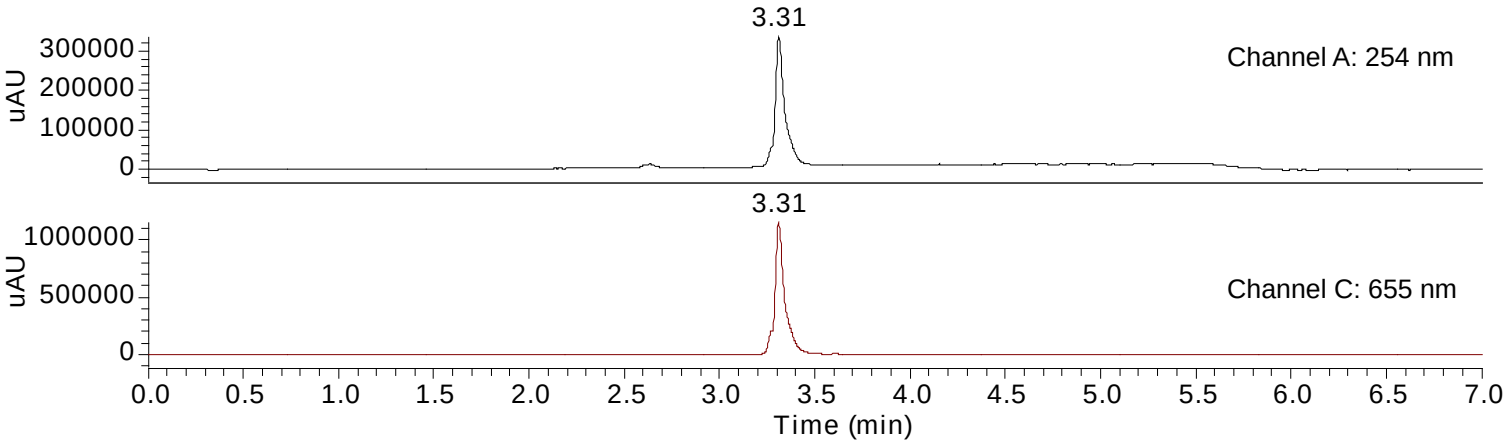
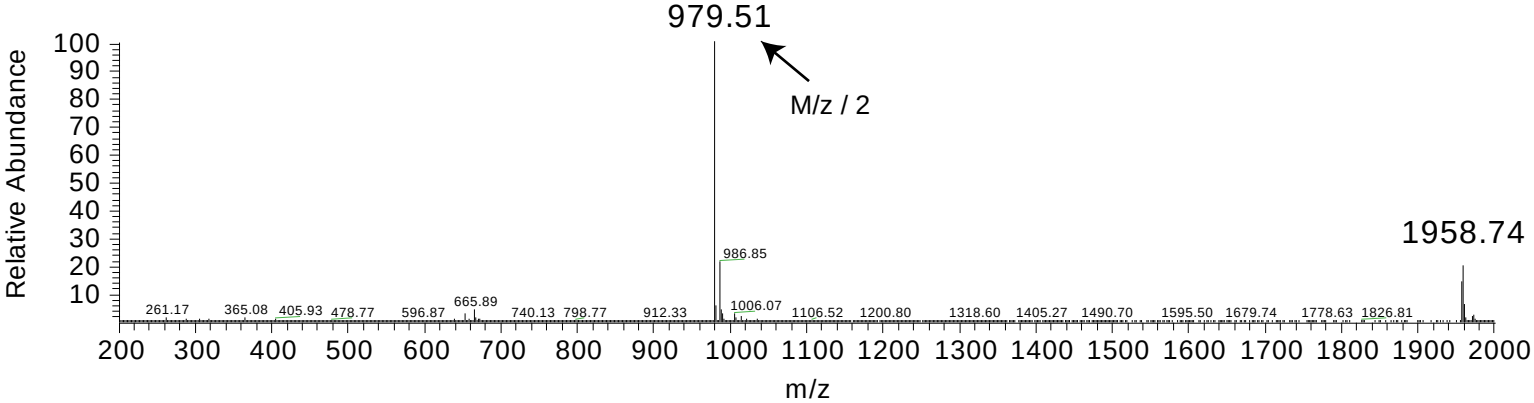
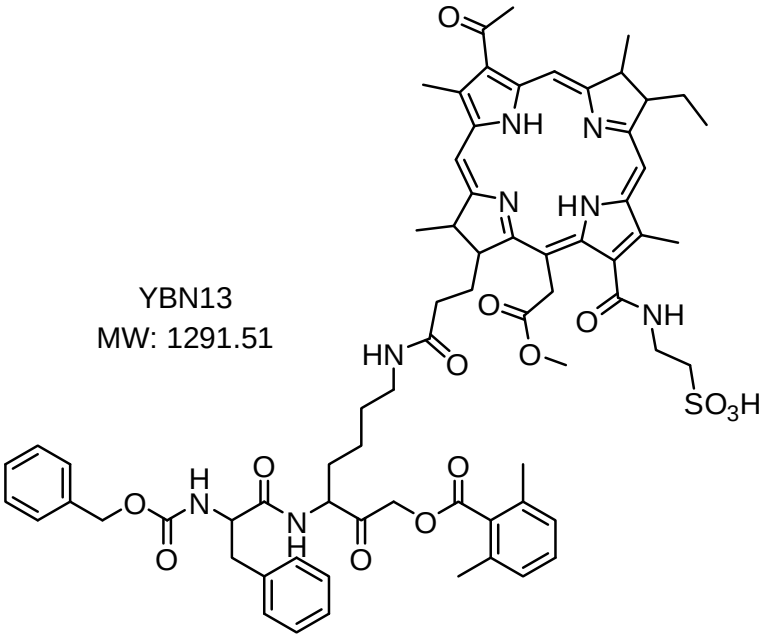
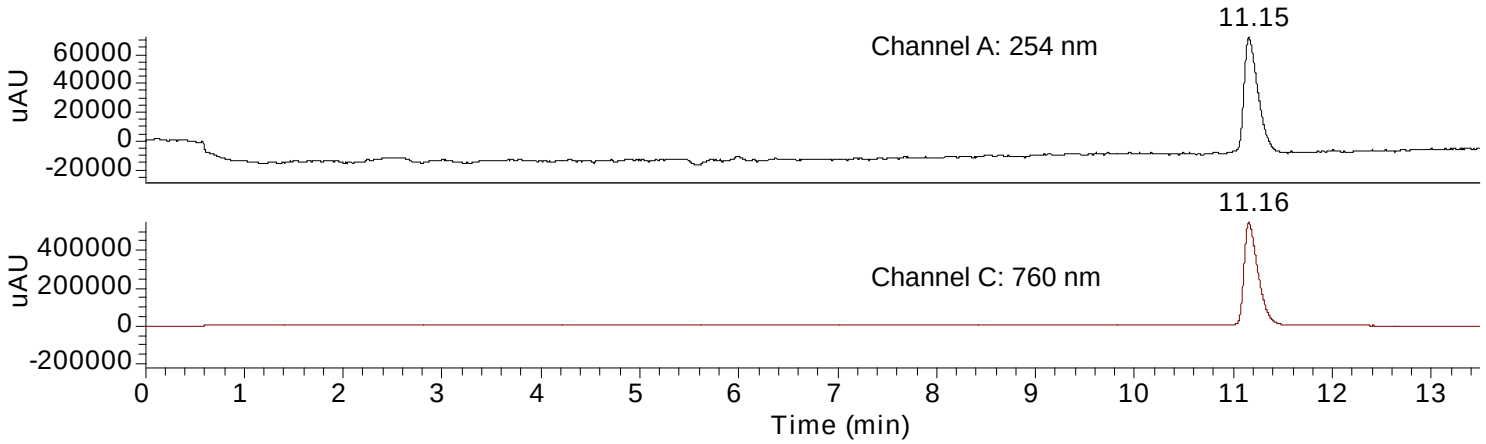


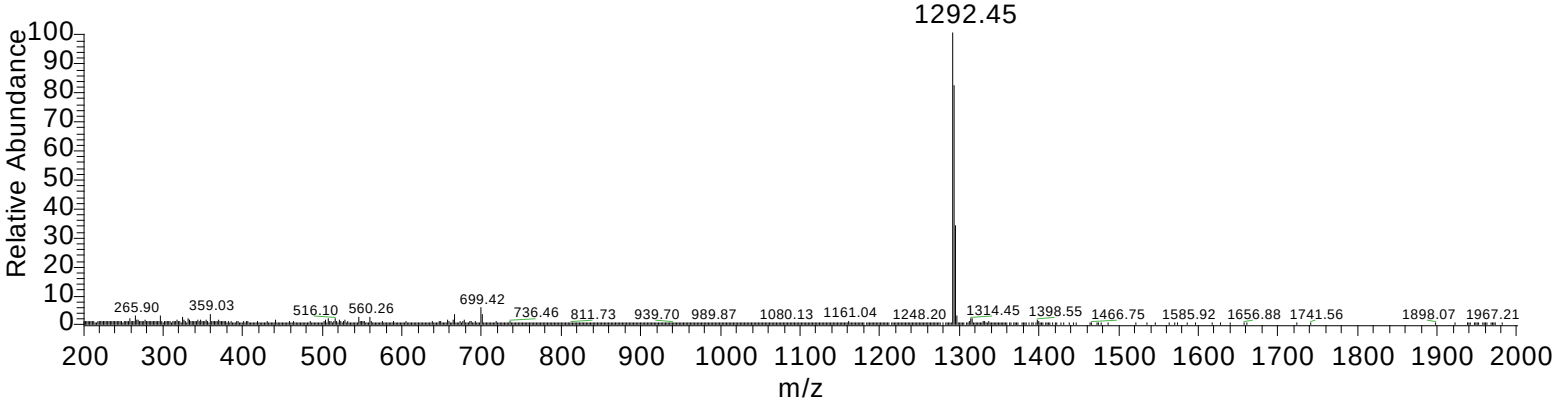


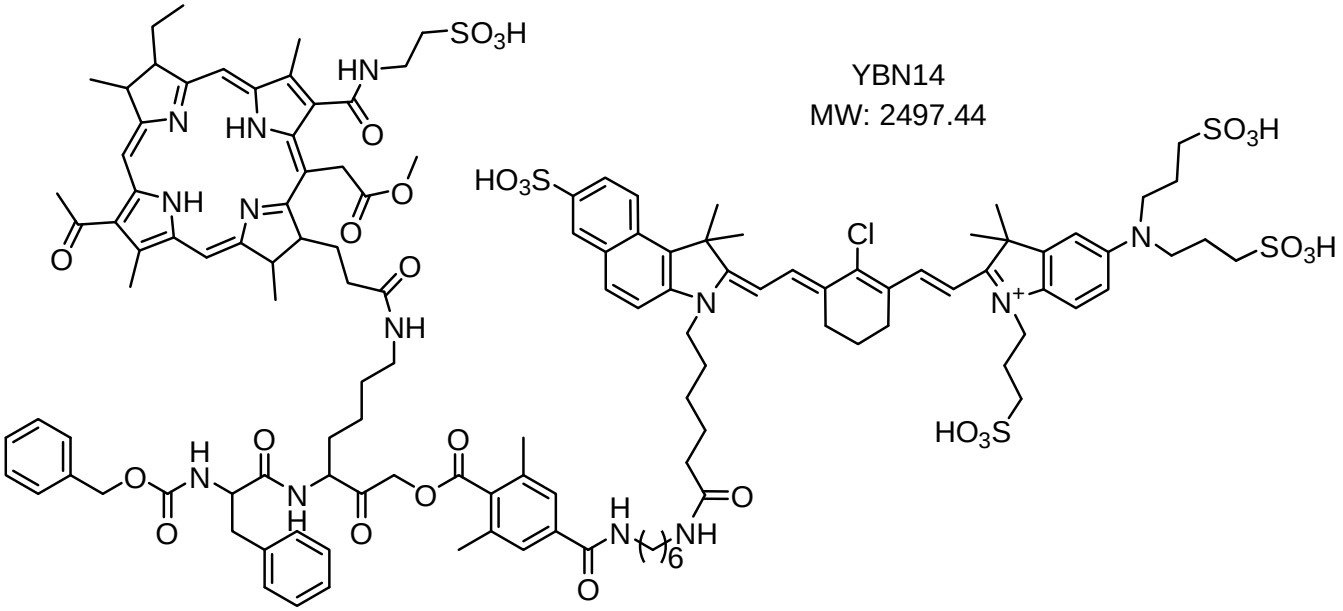


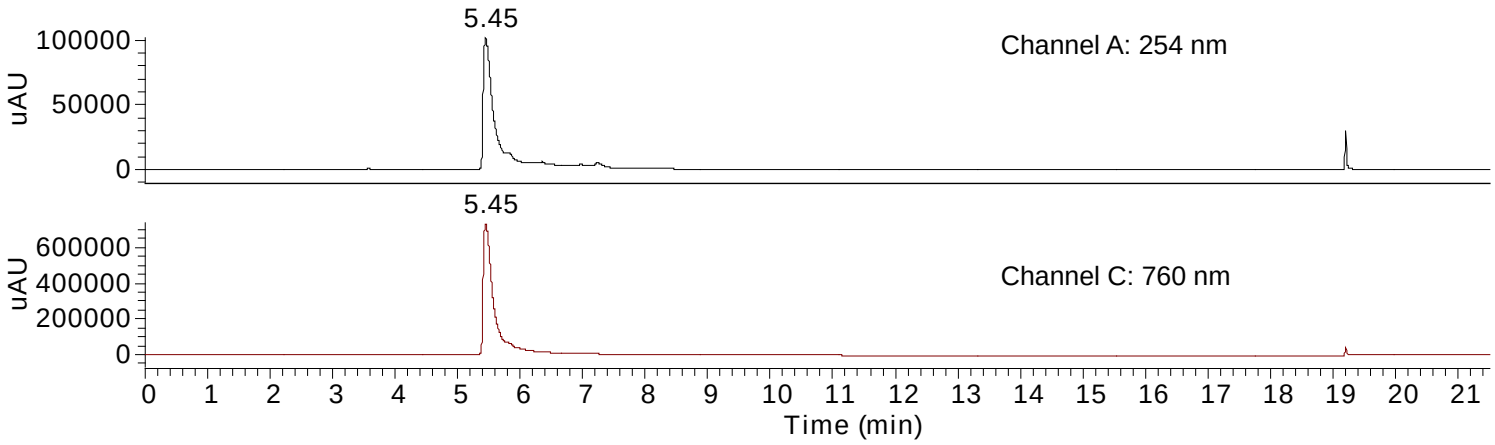
YBN13
MW: 1291.51

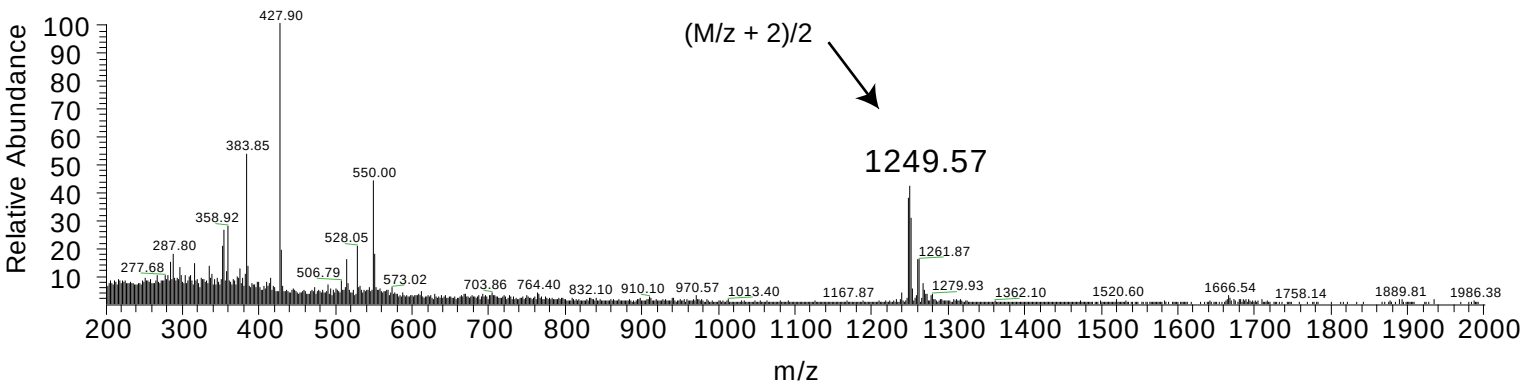







YBN14
MW: 2497.44
SO_3H
HO_3S
Cl
N
N^+
HN
NH
O
H


5.45
Channel A: 254 nm
uAU
100000
50000
0
5.45
Channel C: 760 nm
uAU
600000
400000
200000
0
0 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21
Time (min)


Relative Abundance
100 90 80 70 60 50 40 30 20 10
427.90
383.85
550.00
358.92
287.80
528.05
277.68
506.79
573.02
703.86
764.40
832.10
910.10
970.57
1013.40
1167.87
(M/z + 2)/2
1249.57
1261.87
1279.93
1362.10
1520.60
1666.54
1758.14
1889.81
1986.38
200 300 400 500 600 700 800 900 1000 1100 1200 1300 1400 1500 1600 1700 1800 1900 2000
m/z