

Supporting Information

Short peptides with uncleavable peptide bond mimetics as photoactivatable caspase-3 inhibitors

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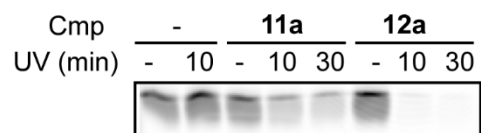
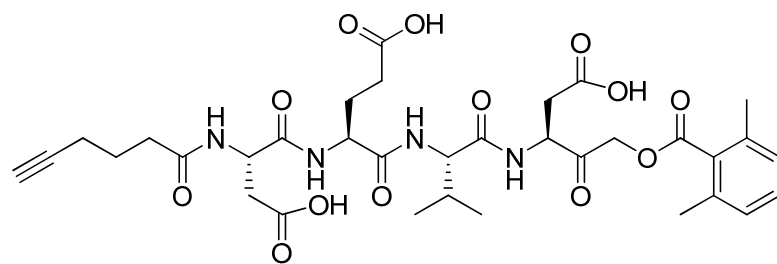


Figure S1. Competitive ABPP on caspase-3 using triazolo peptides **11a** and **12a** at 100 μ M. Although compound **12a** displays inhibition that is nearly quantitative, compound **11a** does not show efficient inhibition.



13

Figure S2. Caspase-directed ABP **13** with a clickable alkyne handle at the N-terminus.

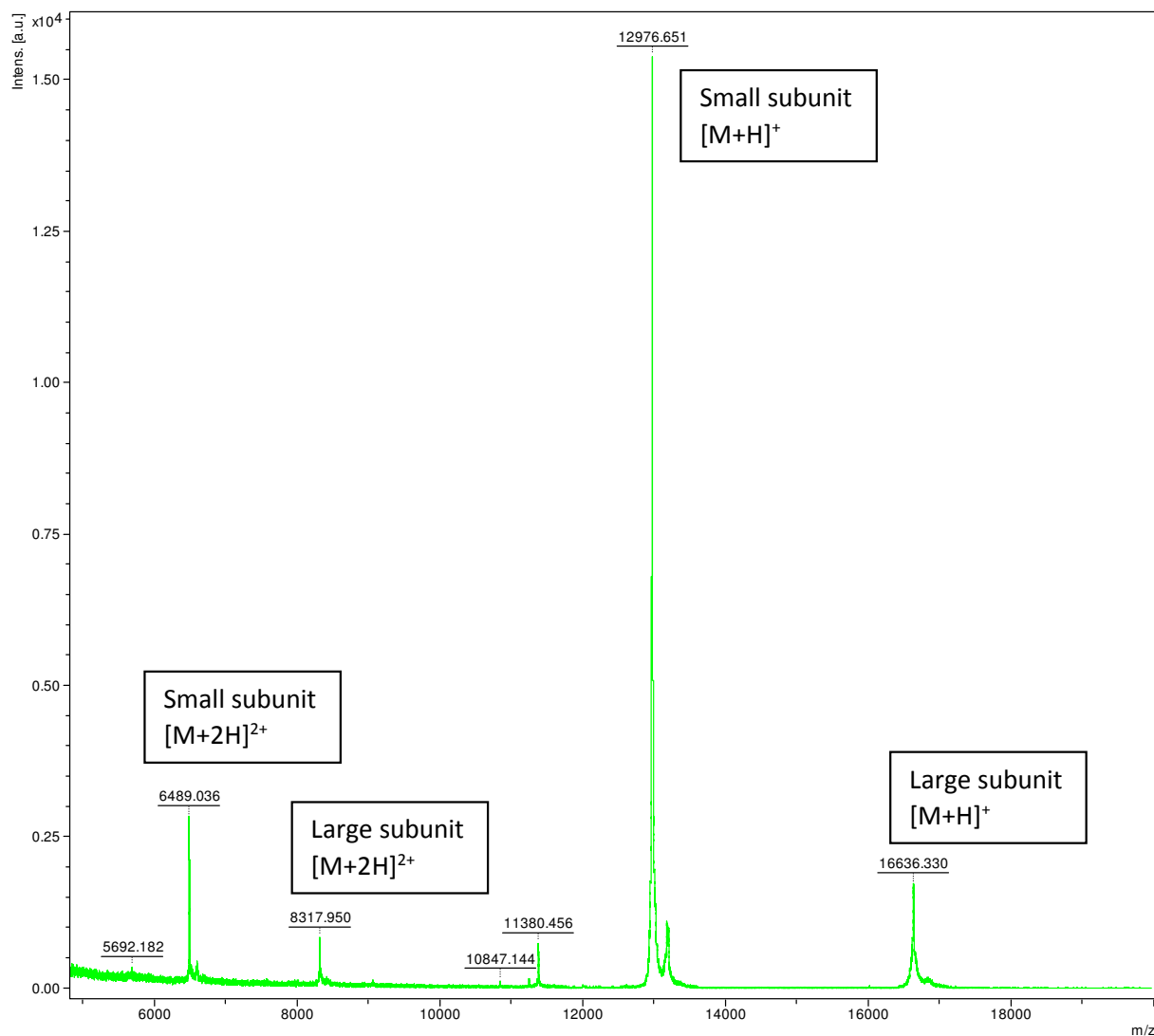


Figure S3. MALDI-TOF spectrum of purified, recombinant caspase-3 after 30 min of UV irradiation. Both the small and large subunit were detected.

Small subunit:

SGVDDDMACH KIPVEADFLY AYSTAPGYYS WRNSKDGSWF IQSLCAMLKQ YADKLEFMHI
LTRVNRKVAT EFESFSFDAT FHAKKQIPCI VSMLTKELYF YHLEHHHHHH

Monoisotopic mass: 12952.23; Average mass: 12960.73. Indicated mass likely corresponds to one methionine oxidation.

Large subunit (propeptide in grey):

MENTENSVDS KSIKNLEPKI IHGSESMDSG ISLDNSYKMD YPEMGLCIII NNKNFHKSTG
MTRSRTDGD AANLRETFRN LKYEVRNKND LTREEIVELM RDVSKEDHSK RSSFVCVLLS
HGEEGIIFGT NGPVDLKKIT NFFRGDRCS LTGKPKLFII QACRGTELDG GIETD

Monoisotopic mass: 16604.27; Average mass: 16614.85. Indicated mass likely corresponds to one methionine oxidation.

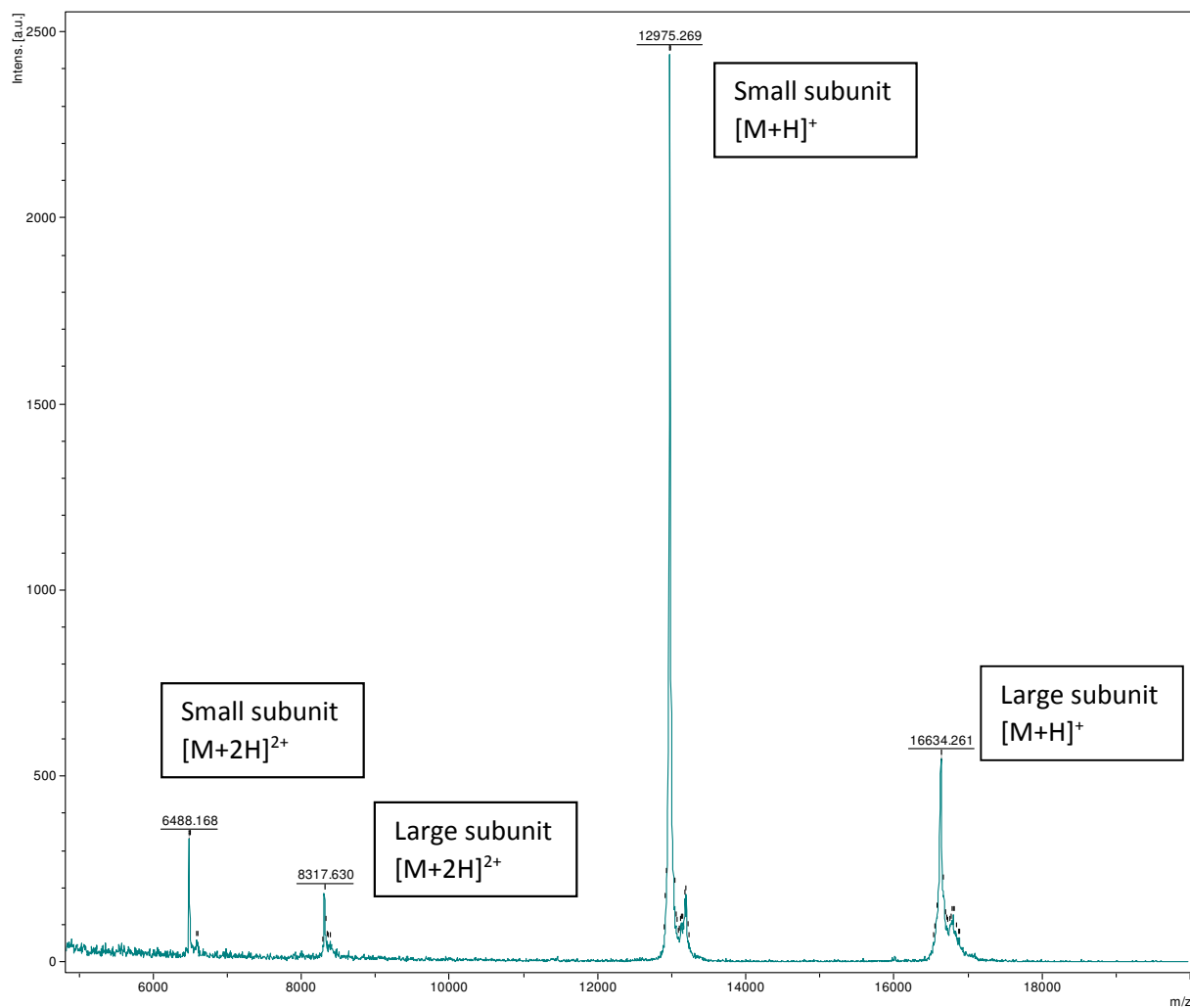
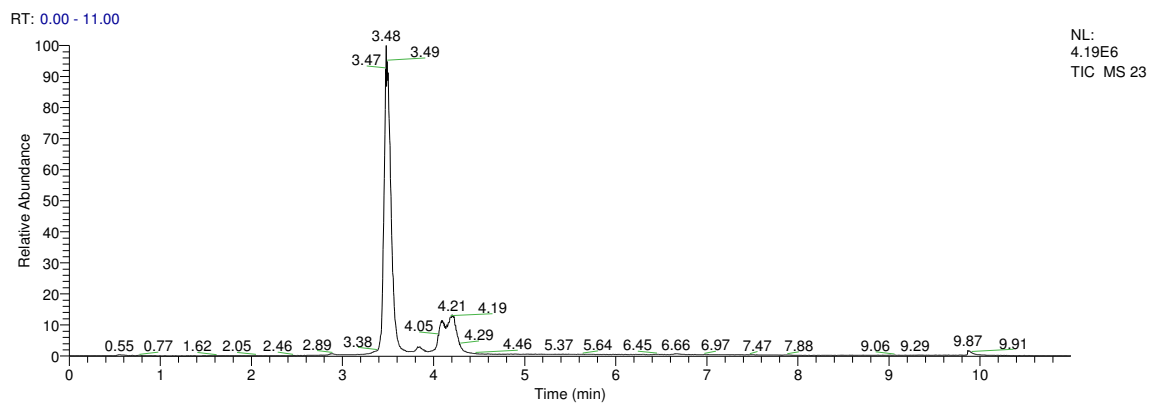
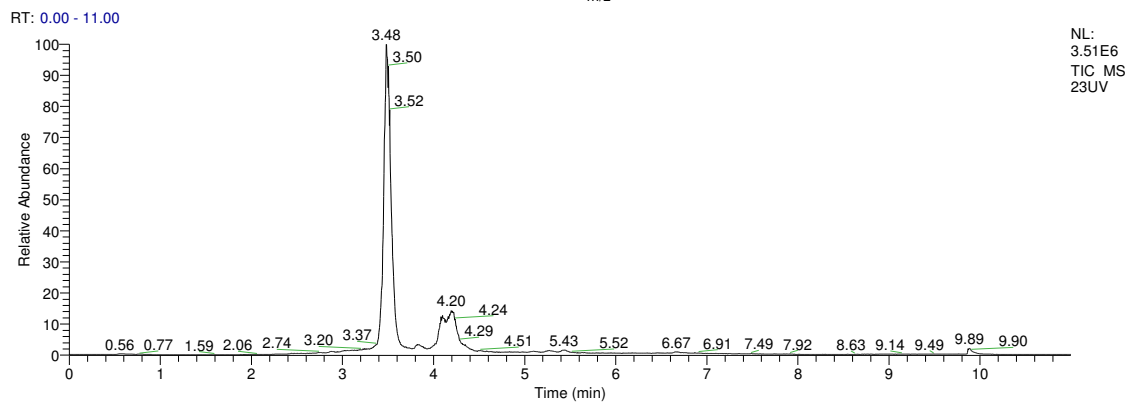
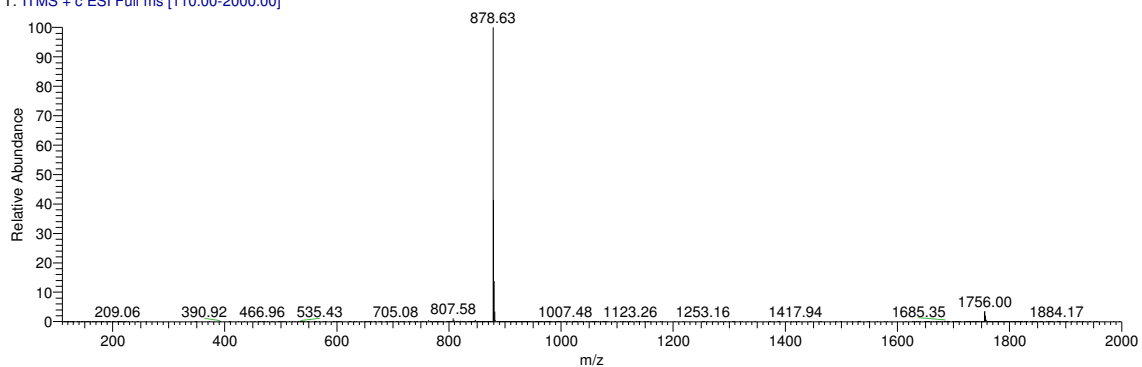


Figure S4. MALDI-TOF spectrum of purified, recombinant caspase-3 after 30 min of UV irradiation in the presence of diazirine probe **9b**. No shifts in the mass of the subunits is detected compared with the caspase-3 alone (compare with Figure S3).



23 #623-646 RT: 3.43-3.52 AV: 24 NL: 1.55E6
T: ITMS + c ESI Full ms [110.00-2000.00]



23UV #645-672 RT: 3.43-3.54 AV: 28 NL: 1.32E6
T: ITMS + c ESI Full ms [110.00-2000.00]

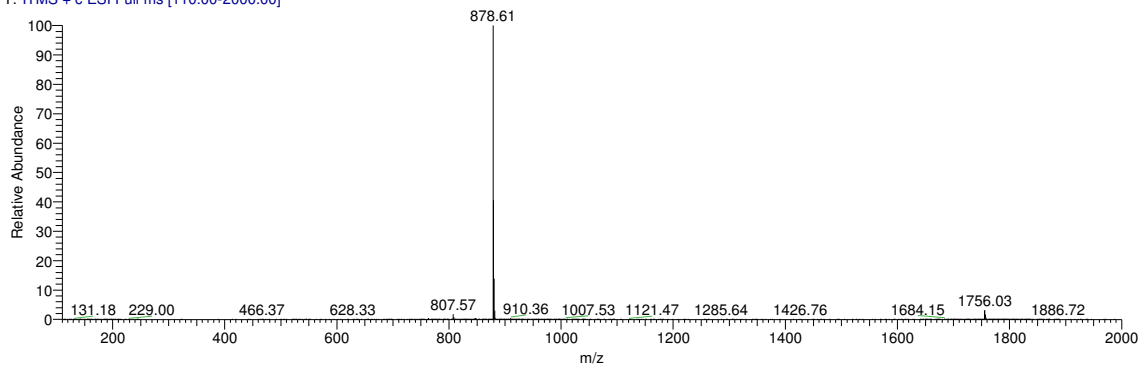
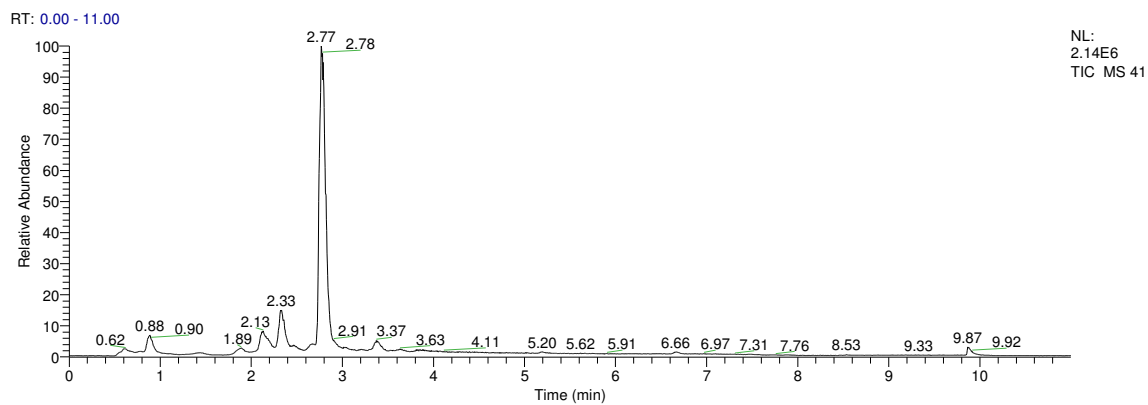
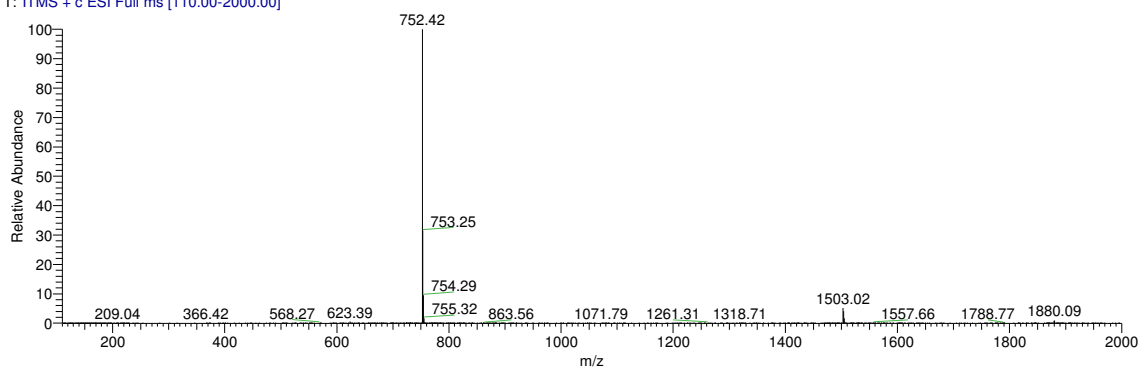


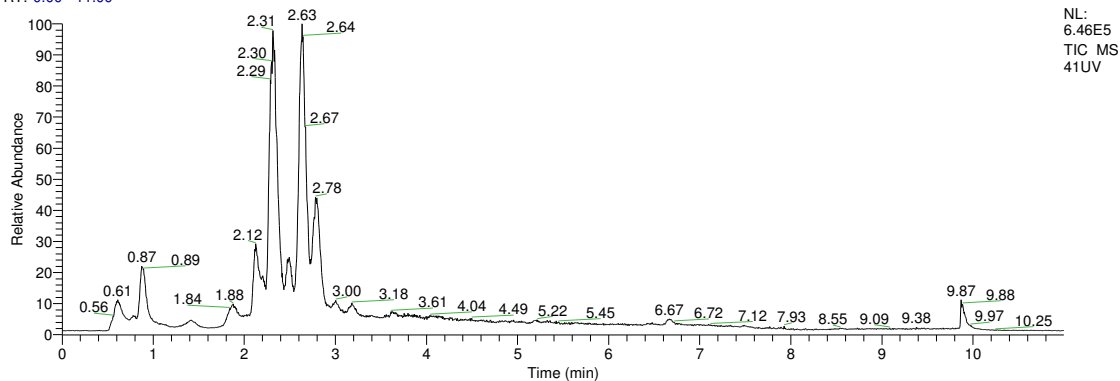
Figure S5. LC-MS data of compound **9a**, before and after UV irradiation.



41 #553-576 RT: 2.73-2.82 AV: 24 NL: 9.32E5
T: ITMS + c ESI Full ms [110.00-2000.00]



RT: 0.00 - 11.00



41UV #522-541 RT: 2.57-2.65 AV: 20 NL: 2.36E5
T: ITMS + c ESI Full ms [110.00-2000.00]

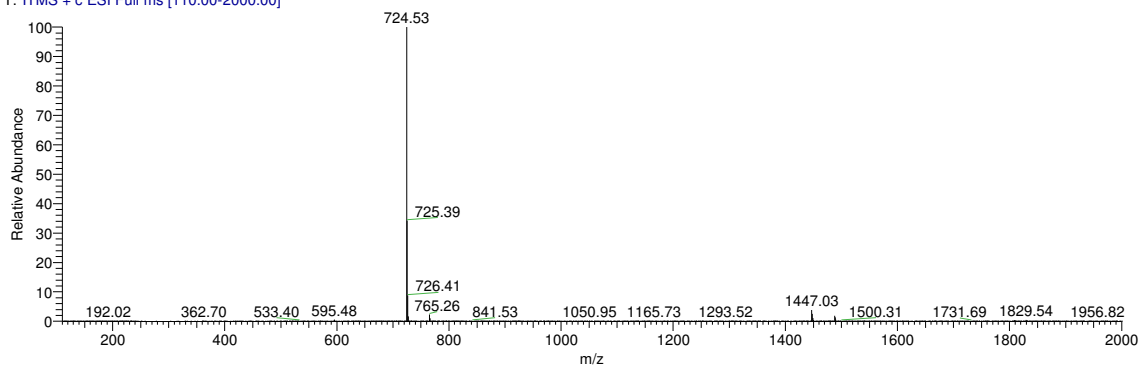
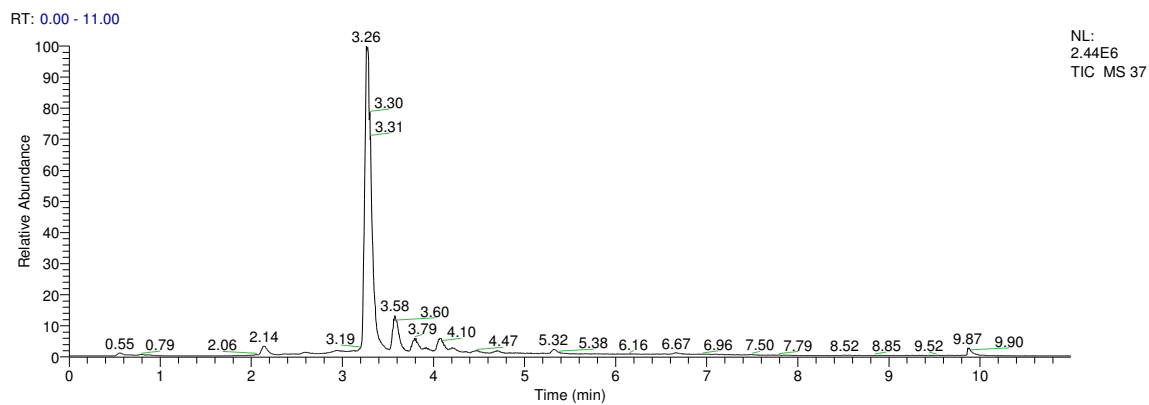
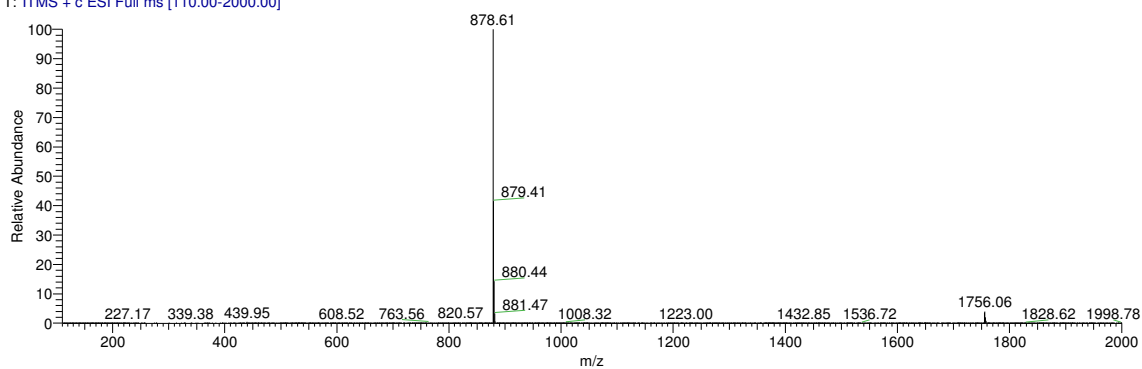


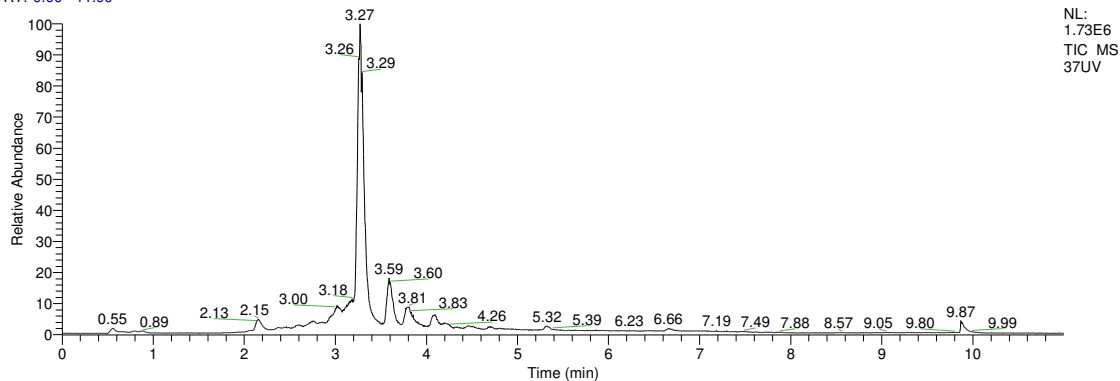
Figure S6. LC-MS data of compound **9b**, before and after UV irradiation



37 #599-632 RT: 3.20-3.33 AV: 34 NL: 7.66E5
T: ITMS + c ESI Full ms [110.00-2000.00]



RT: 0.00 - 11.00



37UV #631-651 RT: 3.23-3.31 AV: 21 NL: 7.41E5
T: ITMS + c ESI Full ms [110.00-2000.00]

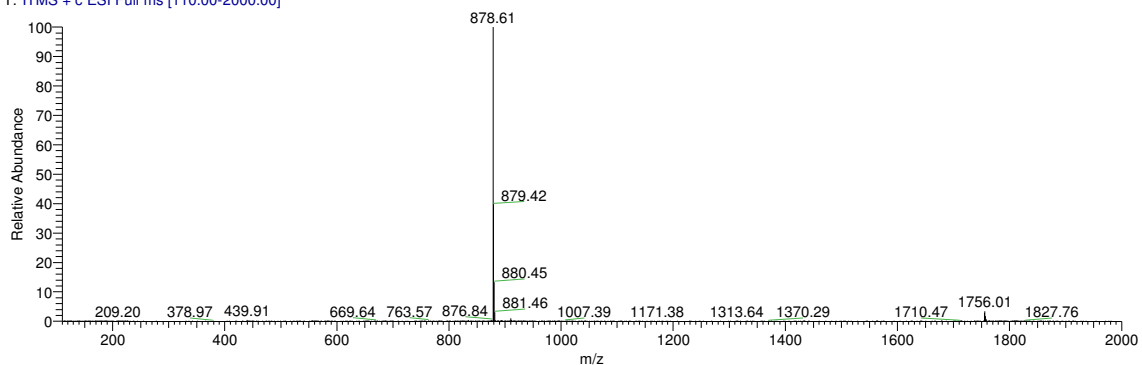
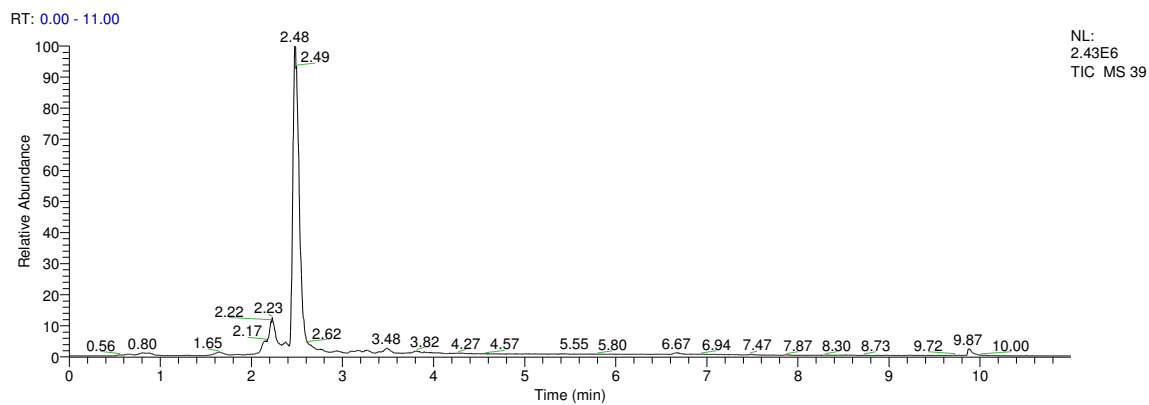
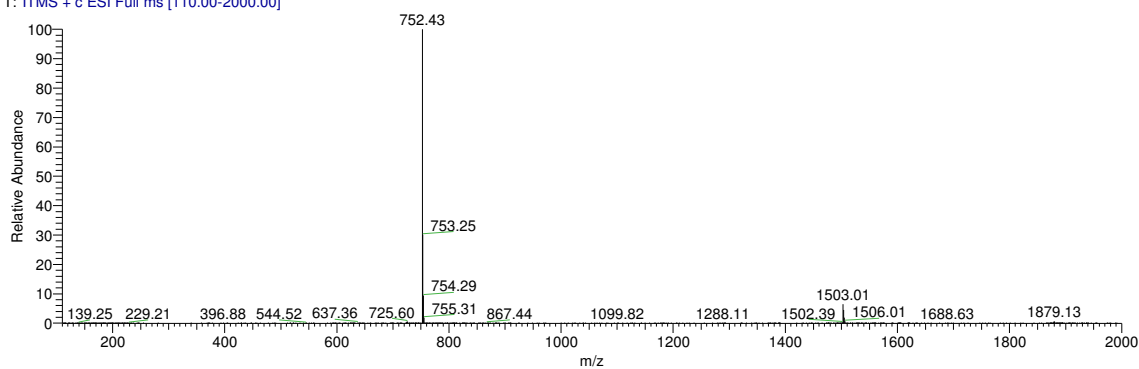


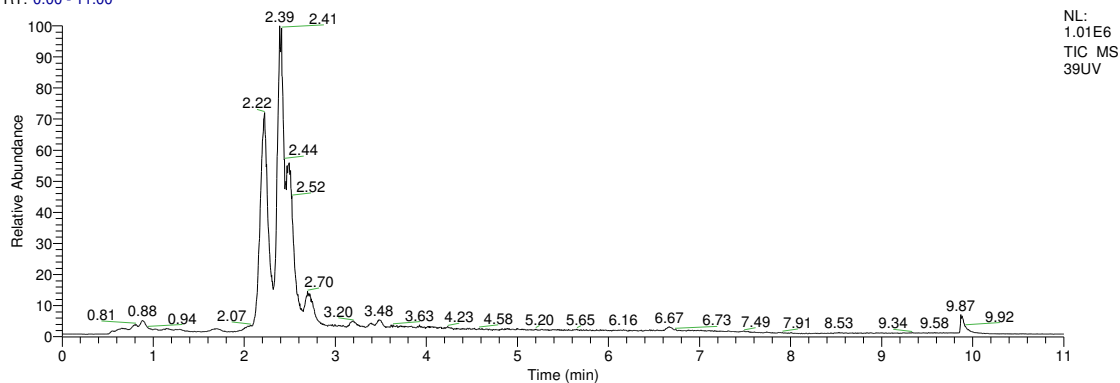
Figure S7. LC-MS data of compound 10a, before and after UV irradiation



39 #464-487 RT: 2.44-2.53 AV: 24 NL: 1.06E6
T: ITMS + c ESI Full ms [110.00-2000.00]



RT: 0.00 - 11.00



39UV #459-479 RT: 2.36-2.44 AV: 21 NL: 4.53E5
T: ITMS + c ESI Full ms [110.00-2000.00]

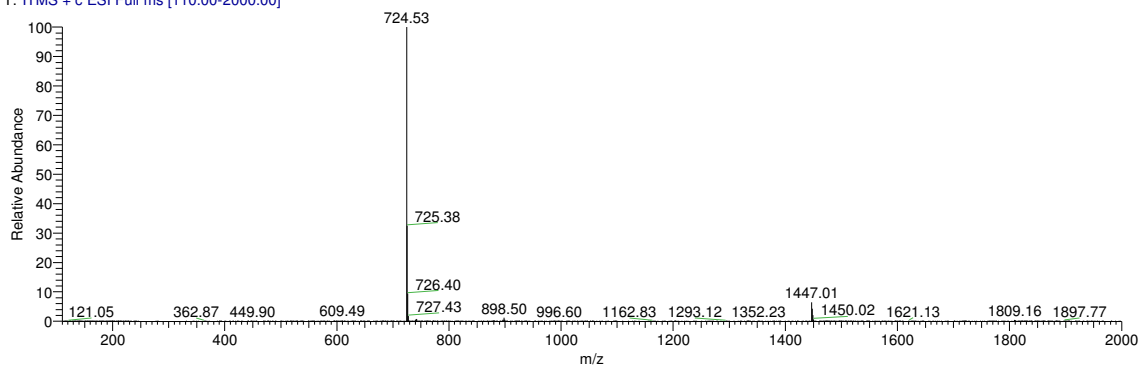
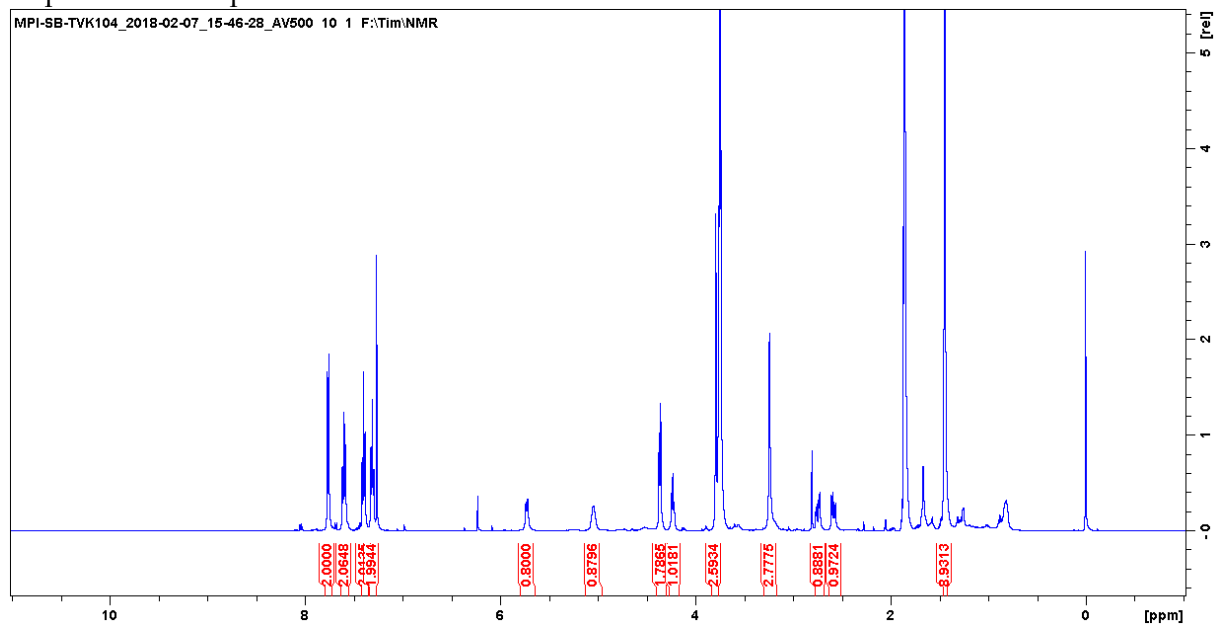
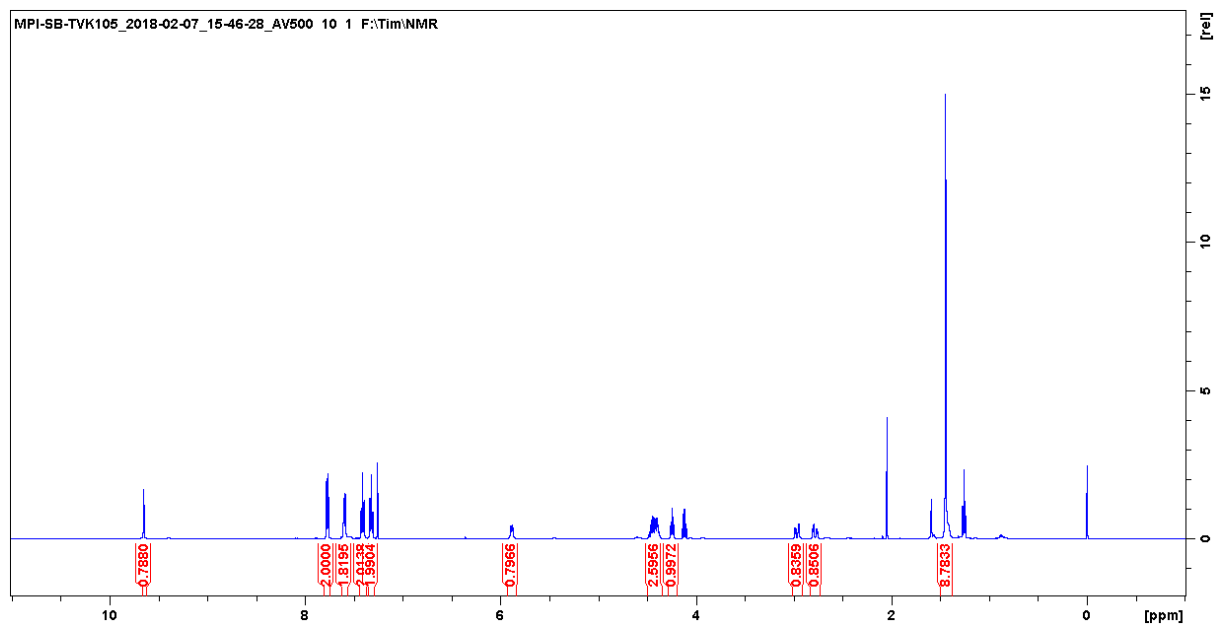


Figure S8. LC-MS data of compound **10b**, before and after UV irradiation

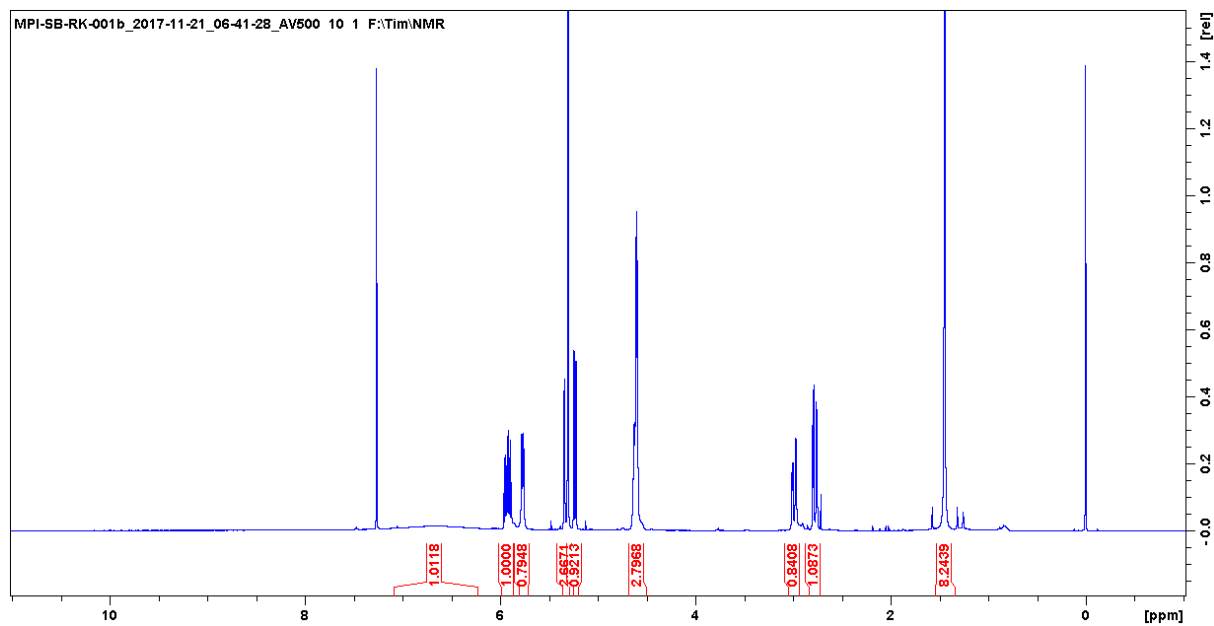
Copies of NMR spectra



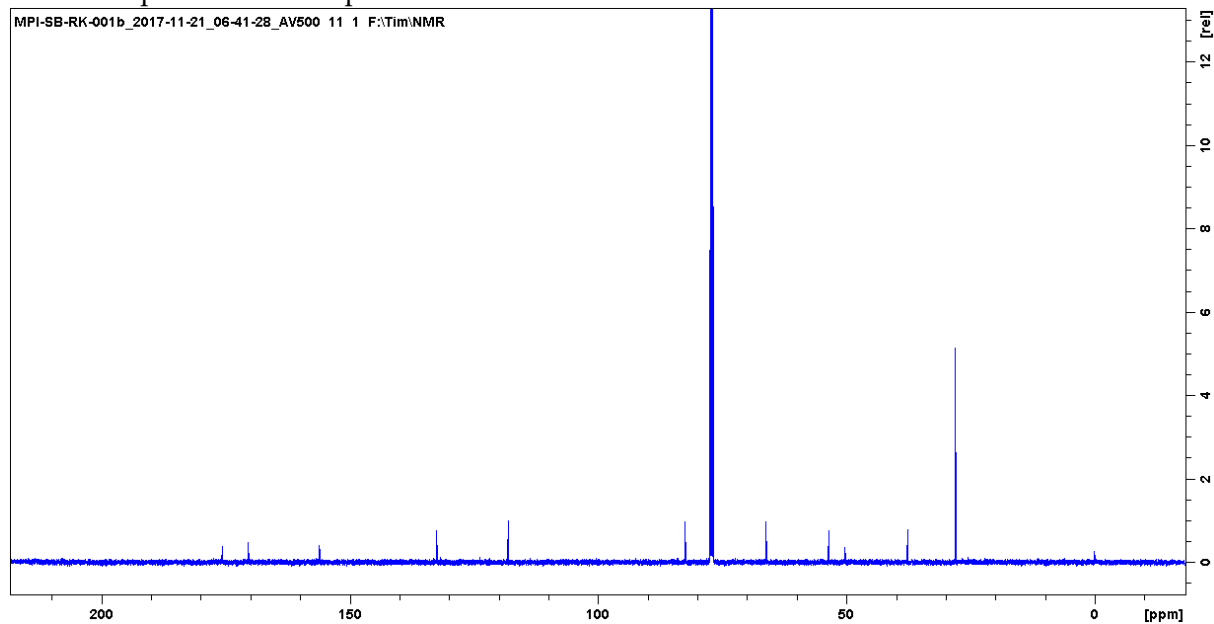
¹H NMR spectrum of compound 2



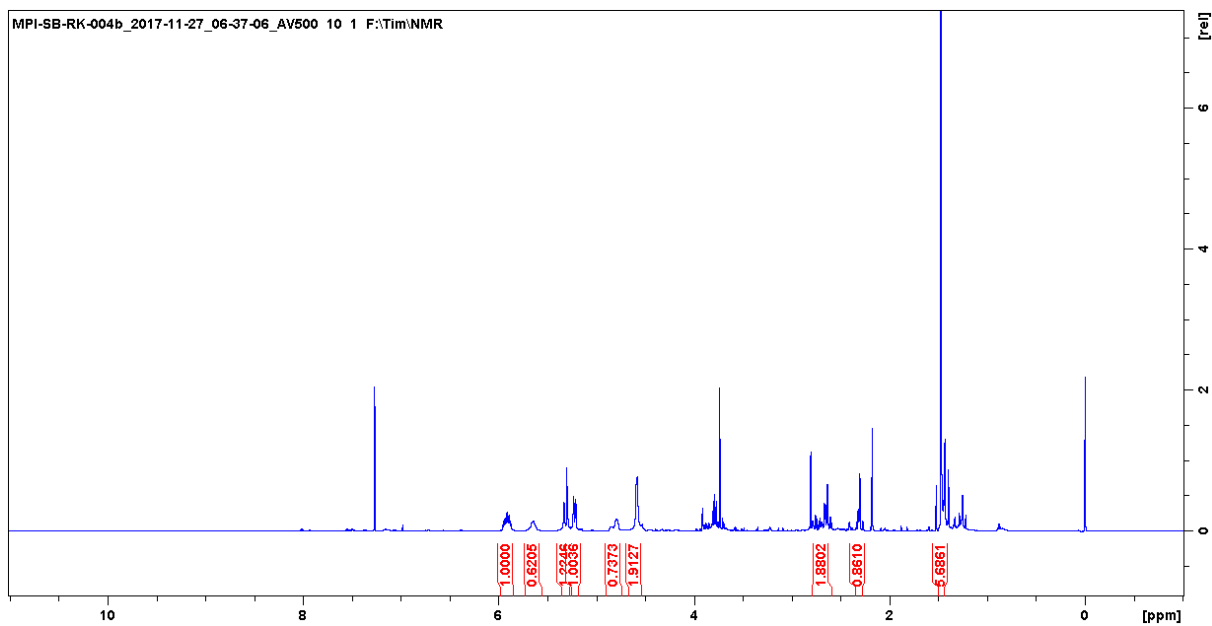
^1H NMR spectrum of compound **3**



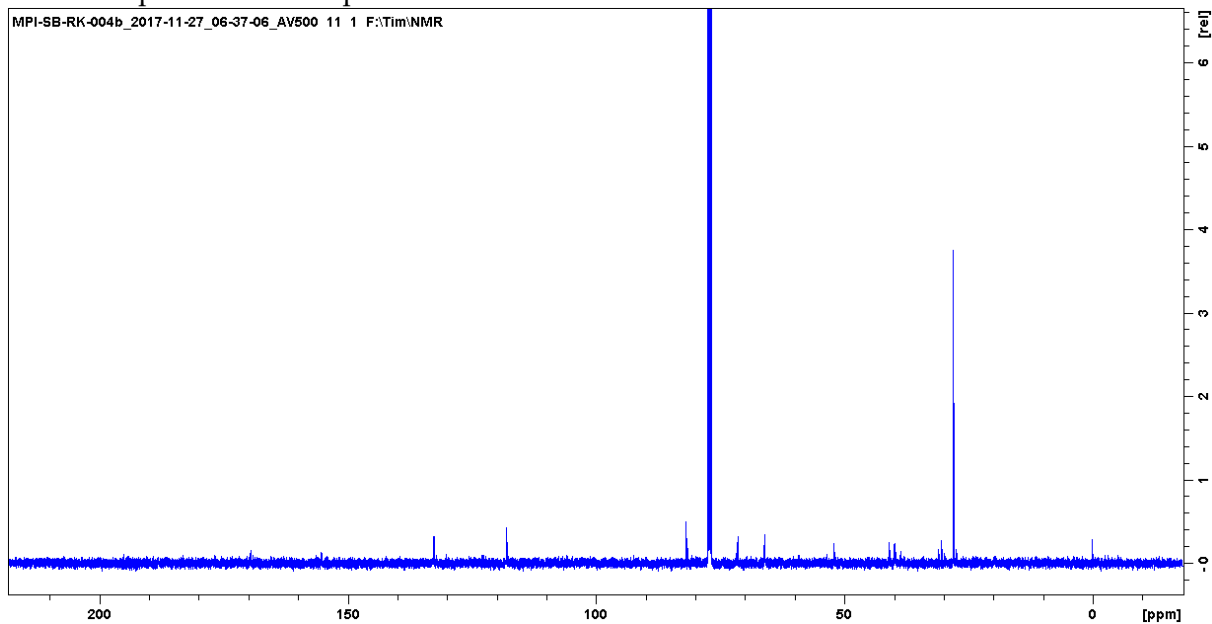
¹H NMR spectrum of compound 4



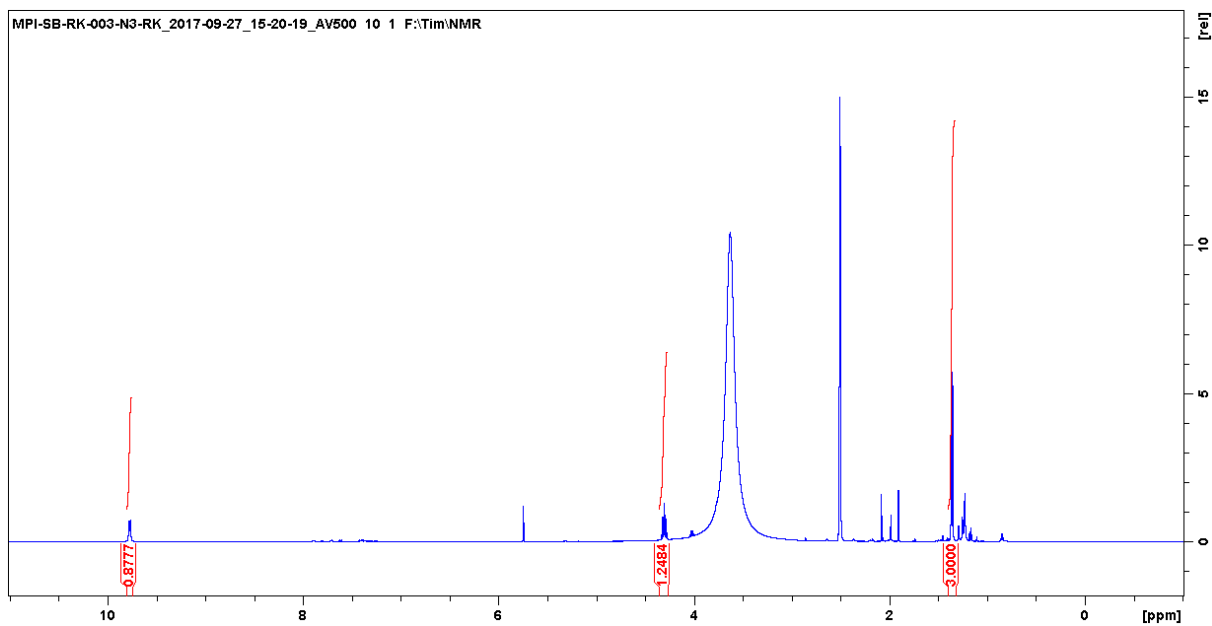
¹³C NMR spectrum of compound 4



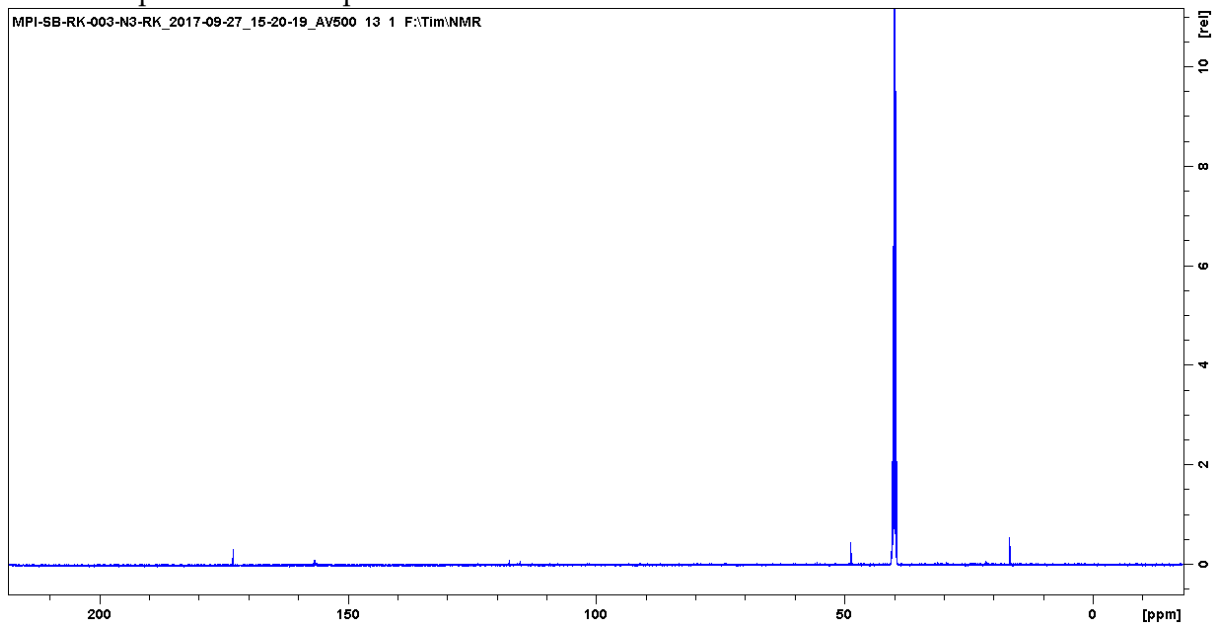
^1H NMR spectrum of compound 7



^{13}C NMR spectrum of compound 7



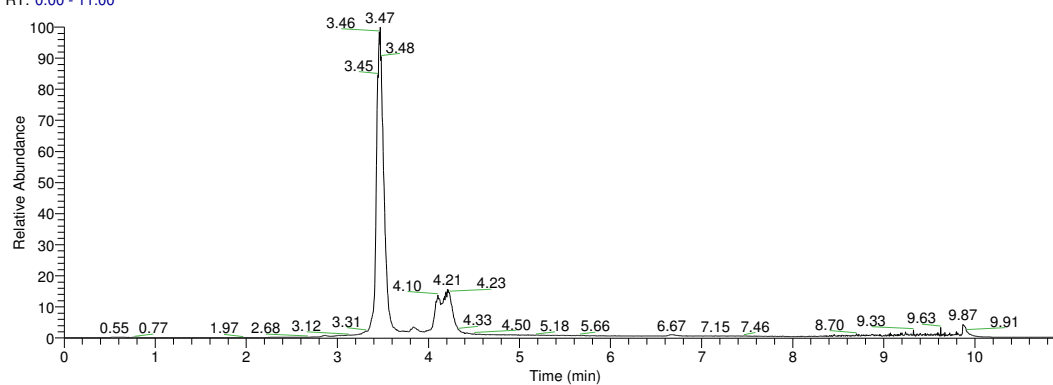
¹H NMR spectrum of compound 8



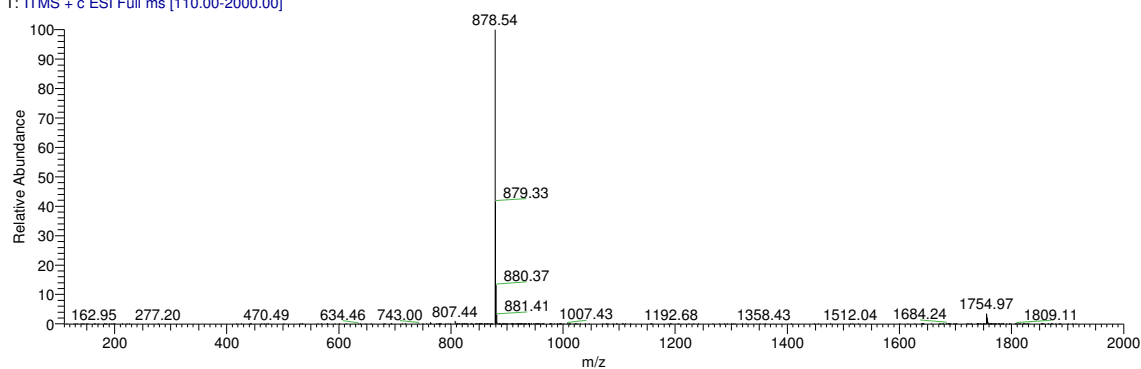
¹³C NMR spectrum of compound 8

Copies of LC-MS data

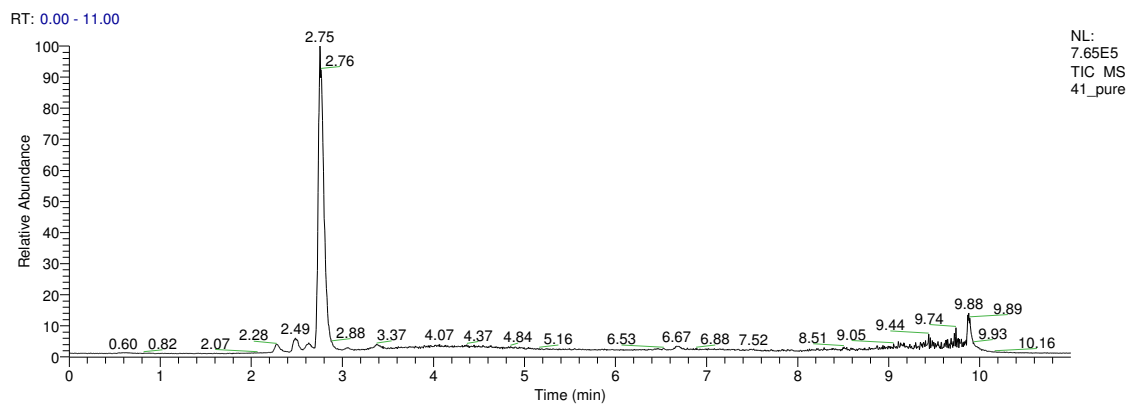
RT: 0.00 - 11.00



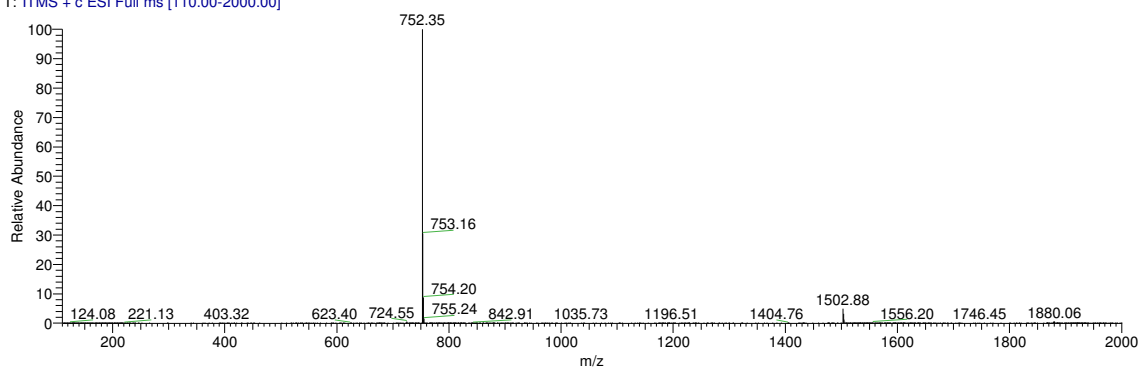
23-0 #637-650 RT: 3.45-3.50 AV: 14 NL: 2.23E6
T: ITMS + c ESI Full ms [110.00-2000.00]



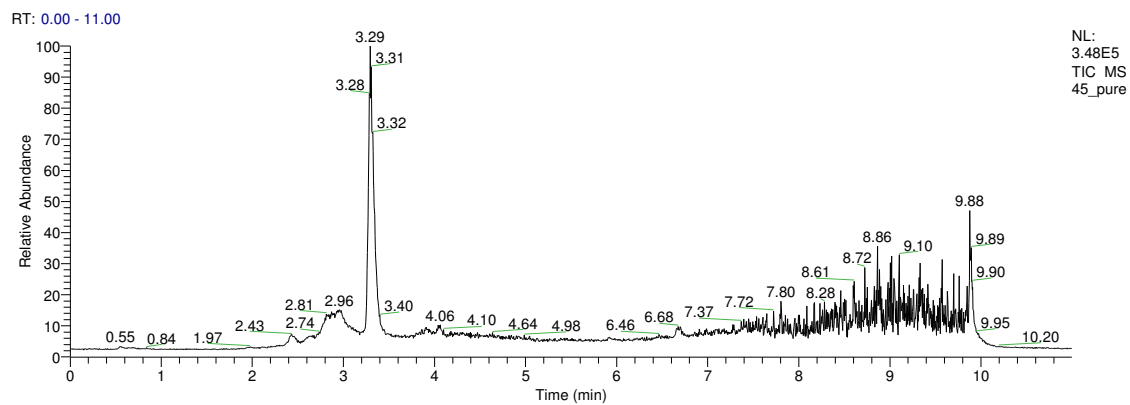
LC-MS data of probe 9a



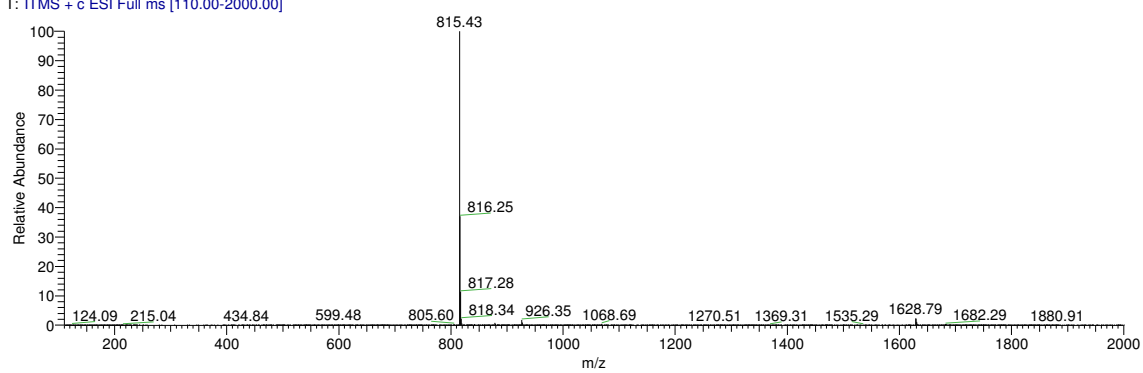
41_pure #493-515 RT: 2.73-2.82 AV: 23 NL: 3.17E5
T: ITMS + c ESI Full ms [110.00-2000.00]



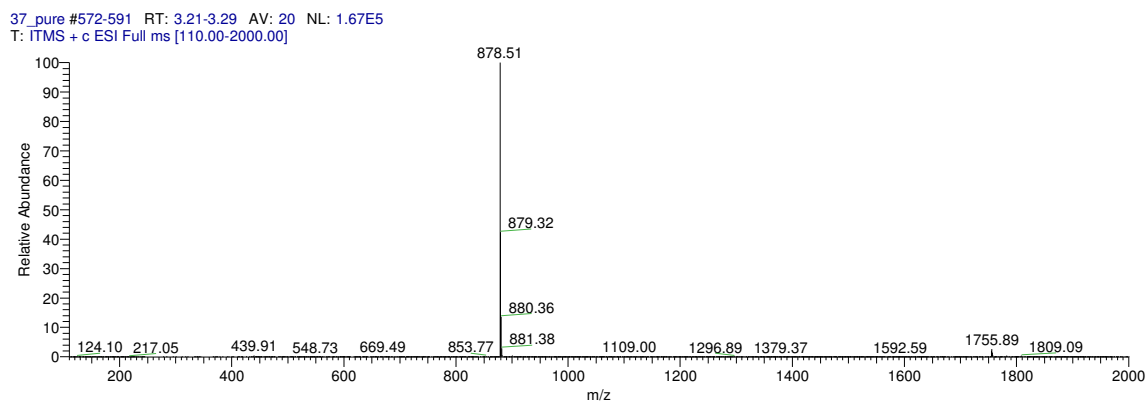
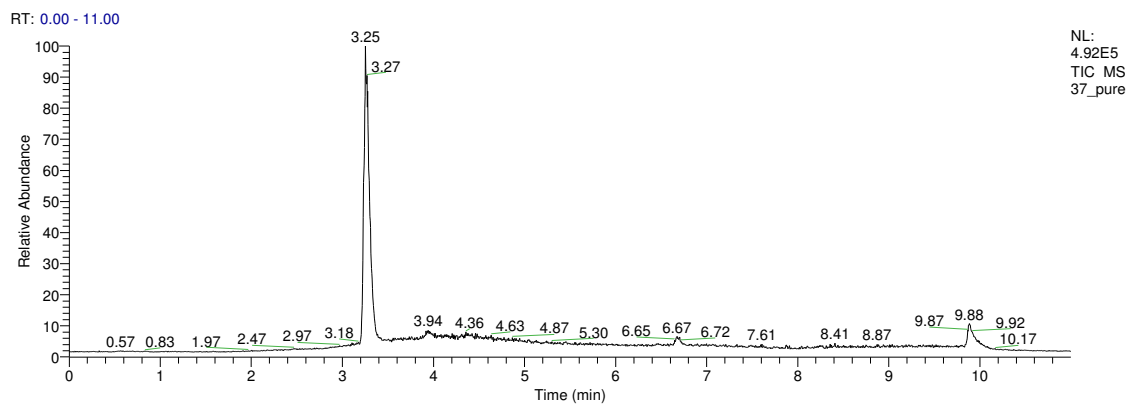
LC-MS data of probe **9b**



45_pure #599-615 RT: 3.26-3.33 AV: 17 NL: 1.25E5
T: ITMS + c ESI Full ms [110.00-2000.00]

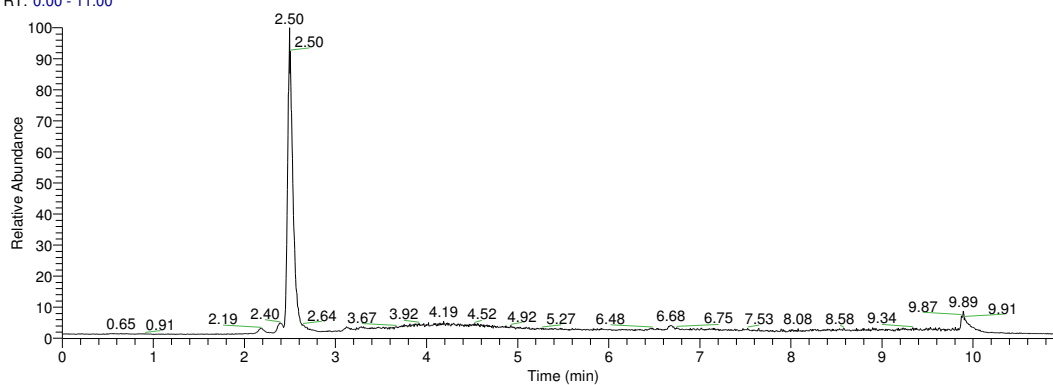


LC-MS data of probe **9c**



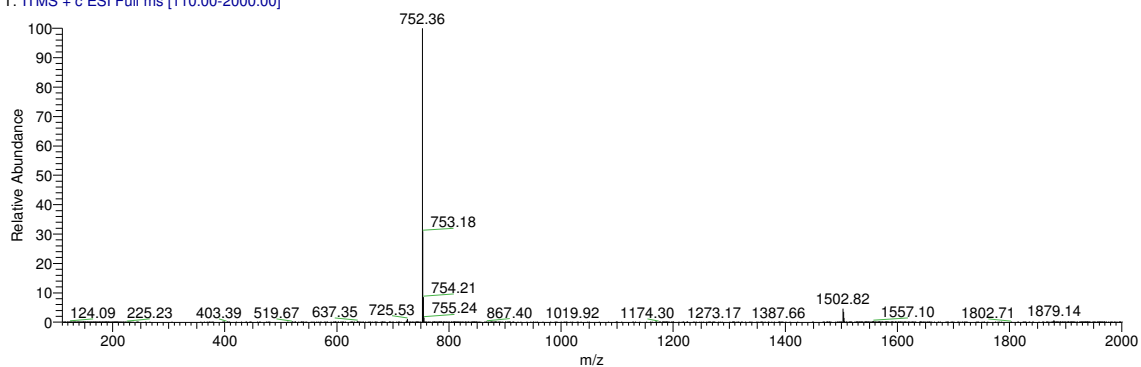
LC-MS data of probe **10a**

RT: 0.00 - 11.00



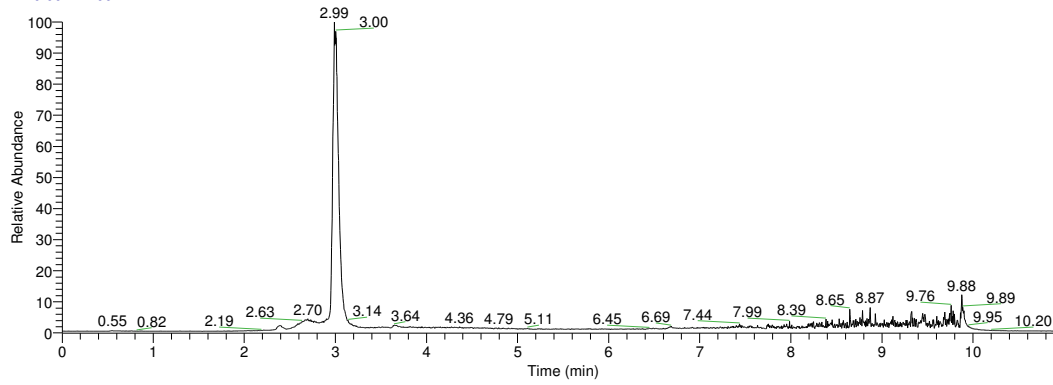
NL:
6.33E5
TIC MS
39_pure

39_pure #441-460 RT: 2.47-2.54 AV: 20 NL: 2.56E5
T: ITMS + c ESI Full ms [110.00-2000.00]



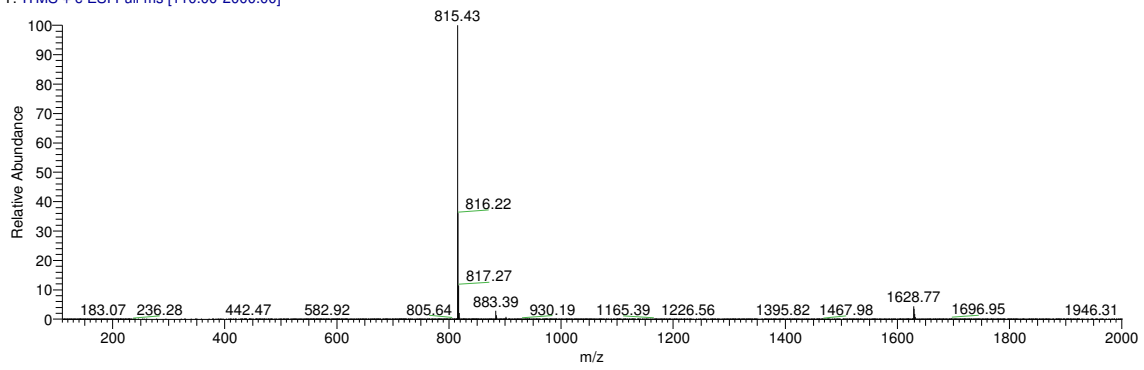
LC-MS data of probe 10b

RT: 0.00 - 11.00

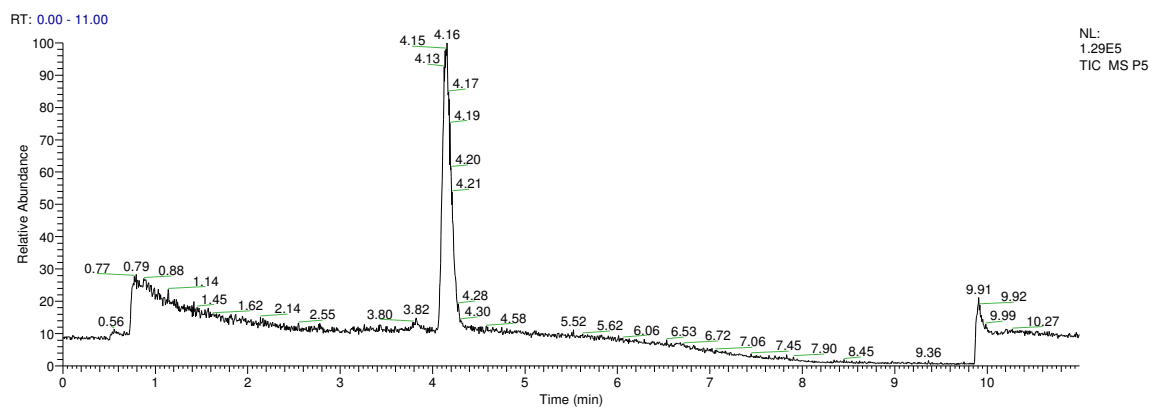


NL:
1.51E6
TIC MS
43-1_pure

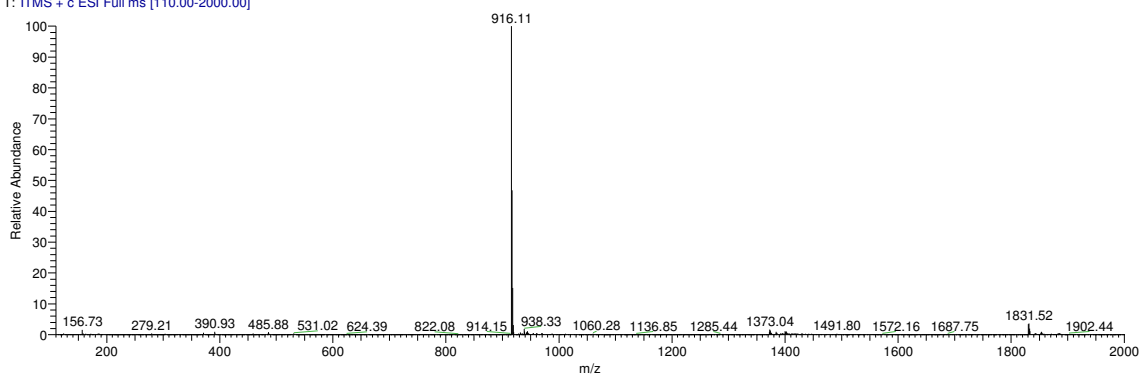
43-1_pure #540-563 RT: 2.93-3.03 AV: 24 NL: 5.04E5
T: ITMS + c ESI Full ms [110.00-2000.00]



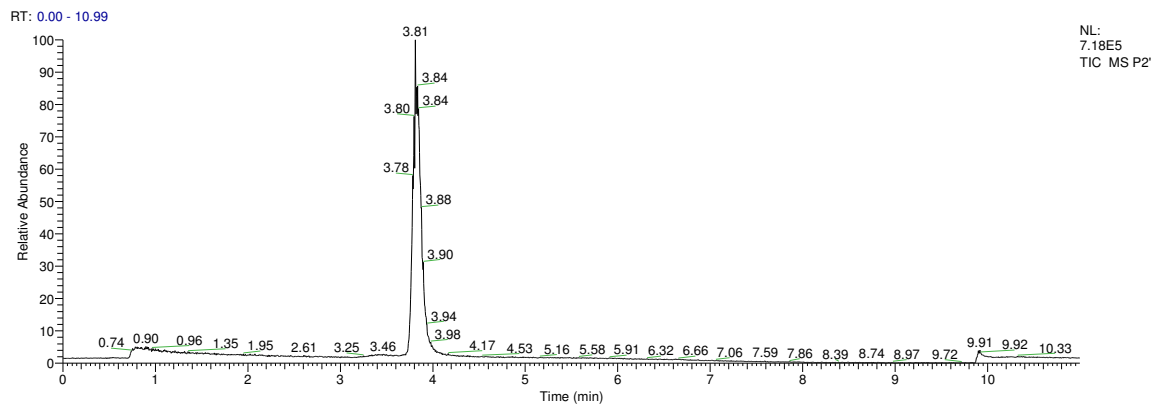
LC-MS data of probe 10c



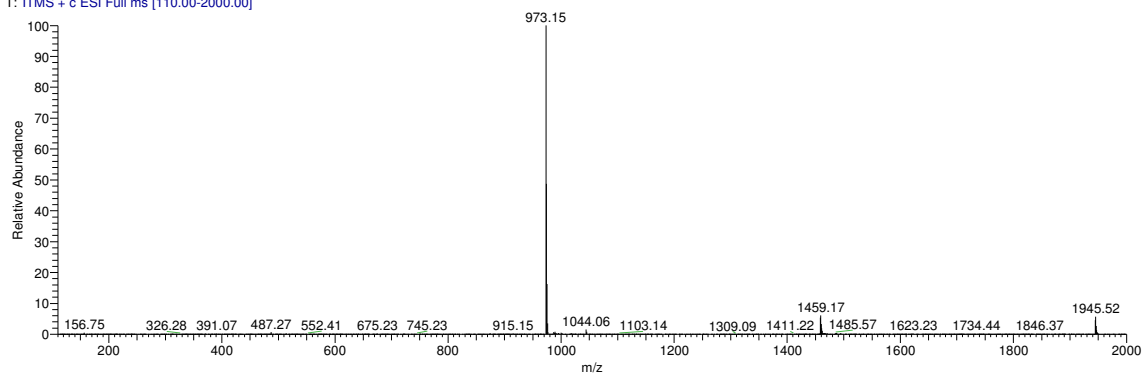
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T: ITMS + c ESI Full ms [110.00-2000.00]



LC-MS data of probe 11a



P2' #712-748 RT: 3.75-3.90 AV: 37 NL: 1.64E5
T: ITMS + c ESI Full ms [110.00-2000.00]



LC-MS data of probe 12a