

Supporting Information

6-Bromo-7-hydroxy-3-methylcoumarin (mBhc) is an efficient multi-photon labile protecting group for thiol caging and three-dimensional chemical patterning

M. Mohsen Mahmoodi¹, Stephanie A. Fisher², Roger Y. Tam², Philip C. Goff¹, Reid Anderson, Jane E. Wissinger¹, David A. Blank, Molly S. Shoichet², and Mark D. Distefano^{1*}

¹Department of Chemistry, University of Minnesota, Minneapolis, Minnesota, 55455, USA

²Department of Chemical Engineering & Applied Chemistry, Donnelly Centre for Cellular & Biomolecular Research, University of Toronto, Toronto, Ontario, M5S3E1 Canada

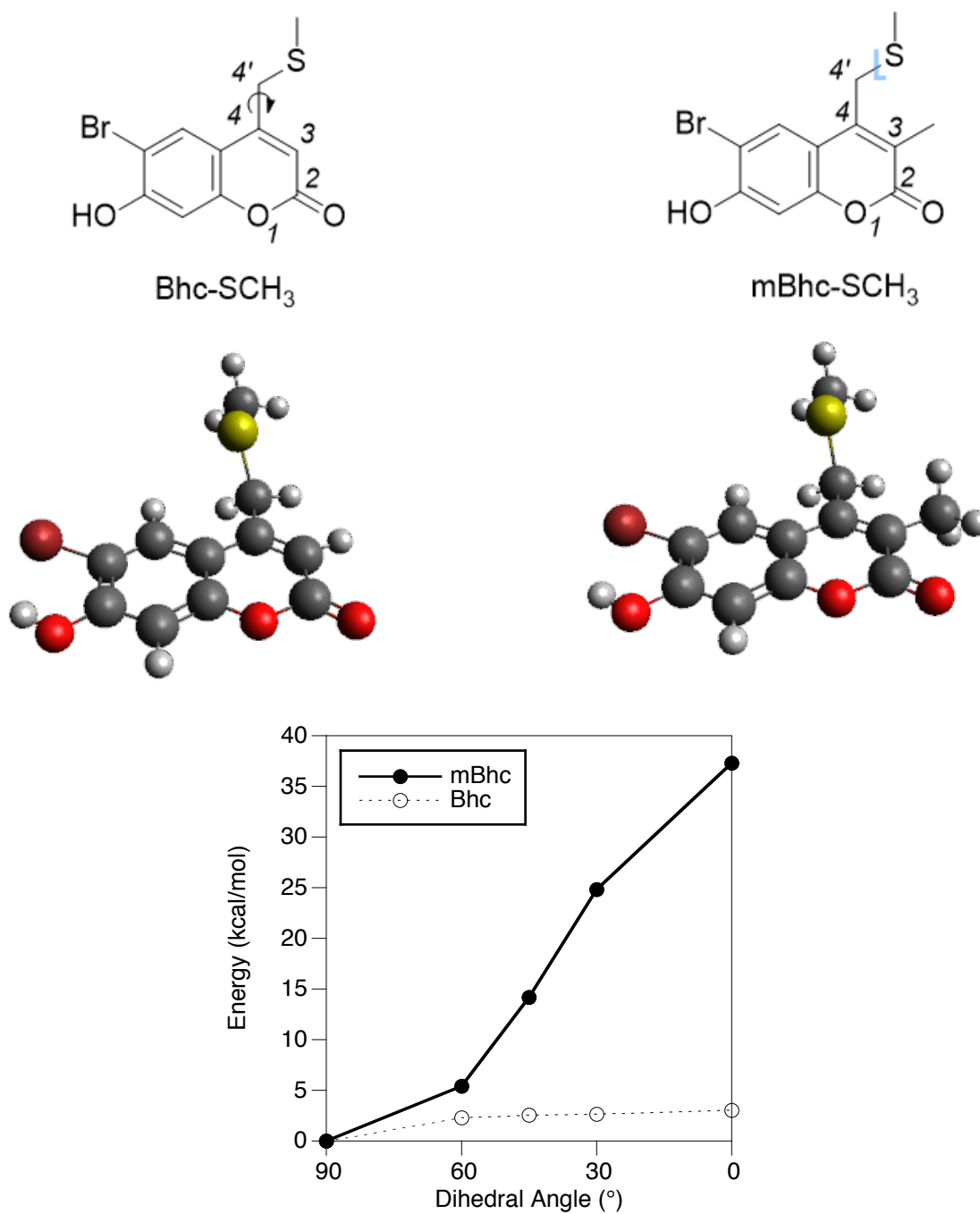


Figure S1. Computational analysis of the conformational energies of Bhc-SCH₃ and mBhc-SCH₃ showing the lowest energy conformer in each case, determined using Gaussian. A plot of energy versus dihedral angle (for the exocyclic thioether bond) is shown below. The conformations shown above in line angle form have dihedral angles for the C3-C4-C4'-S bond of 0° while the conformations shown in ball and stick form have dihedral angles for the C3-C4-C4'-S bond of 90° (lowest energy conformers).

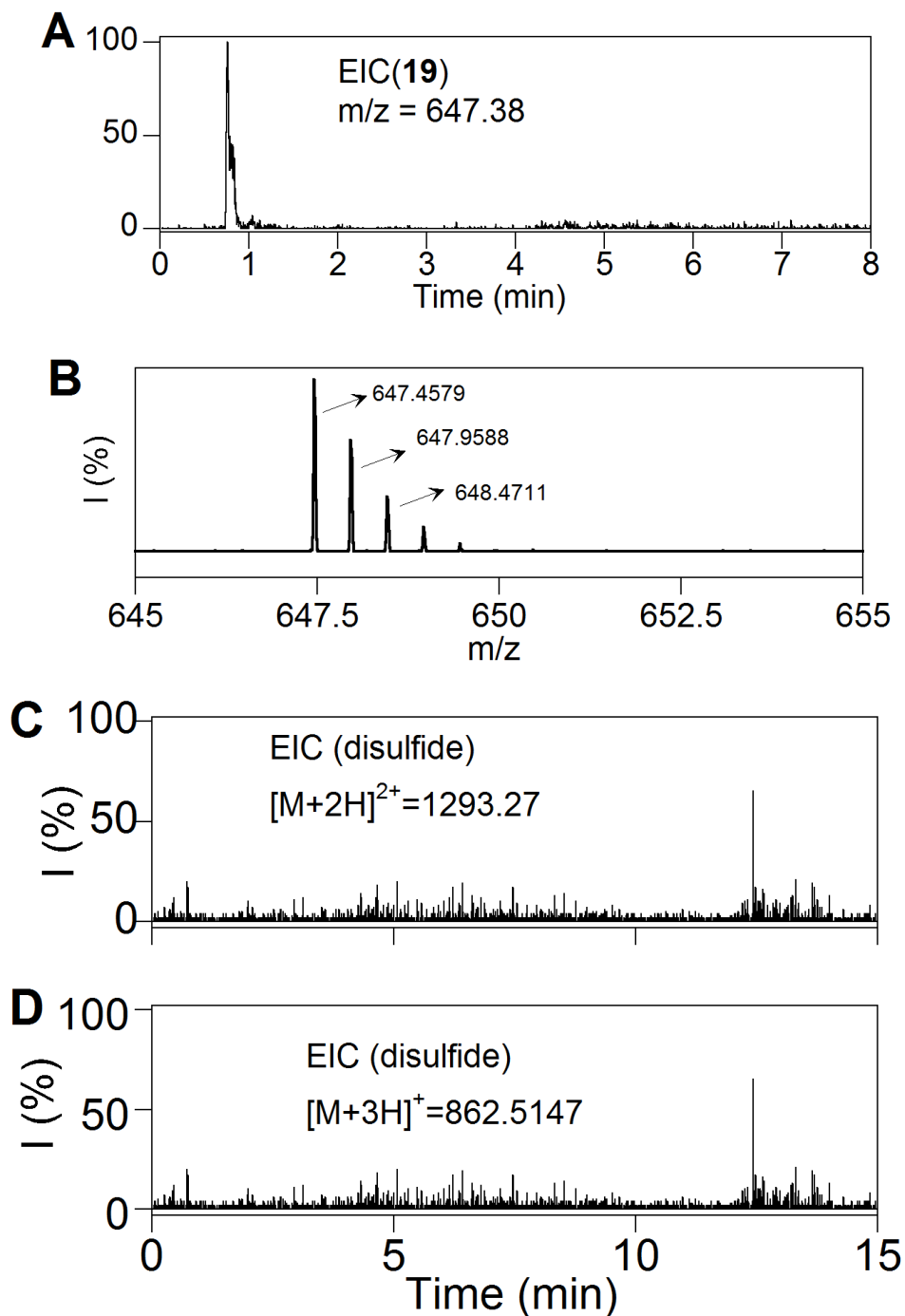


Figure S2. LC-MS analysis of a photolysis of **18** to probe for the formation of a disulfide-linked dimer. (A) EIC chromatogram ($m/z = 647.45$, calcd for $[M + 2H]^{2+} = 647.39$) of a $7.5 \mu\text{M}$ solution of **18** after 60 s irradiation at 365 nm, and (B) is the corresponding mass spectrum. 0.5 mass unit difference between the peaks clearly shows the observed ion has a +2 charge thus corresponds to the free thiol containing peptide with the expected mass and not the disulfide. (C) and (D) are EIC chromatograms corresponding to the $[M+2H]^{2+}$ and $[M+3H]^{3+}$ ions of the possible formed disulfide. This data clearly shows the absence of any significant disulfide bond formation in the photolysis reaction.

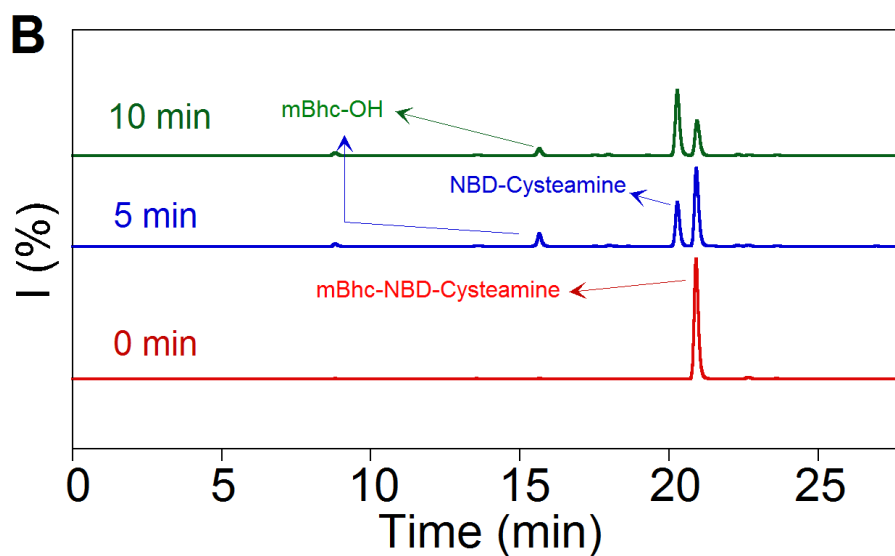
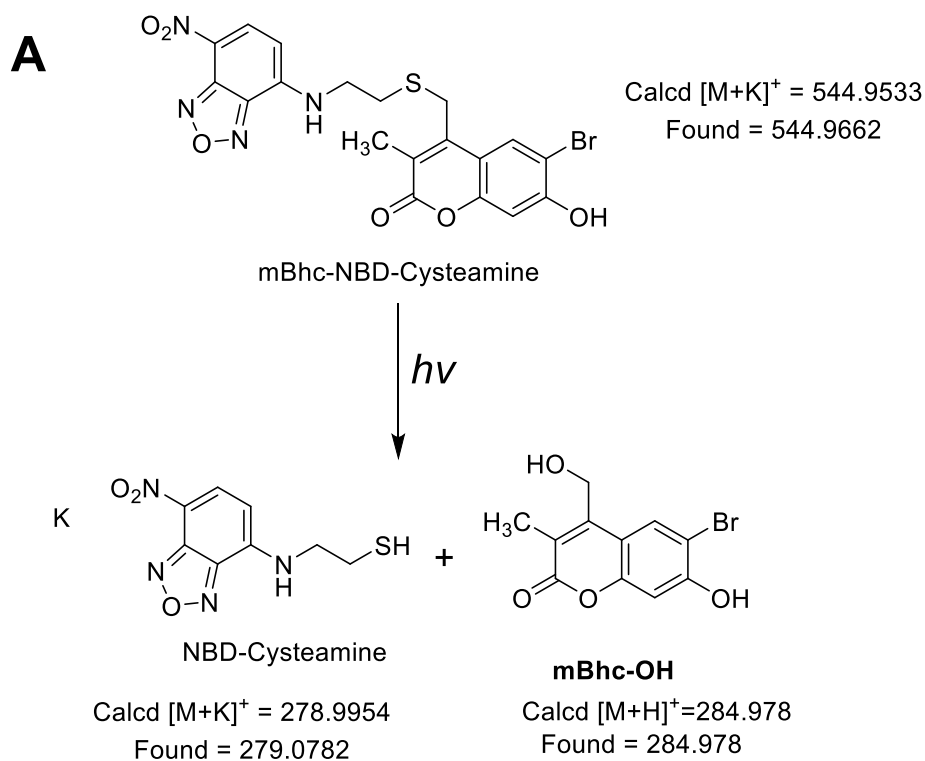


Figure S3. Analysis of the photolysis of mBhc-NBD-Cysteamine. (A) Scheme showing photo-triggered uncaging of mBhc-protected cysteamine. (B) fluorescence HPLC traces representing time-course photolysis of mBhc-protected cysteamine. These results show clean conversion of the caged peptide to the free thiol with no appearance of any unexpected byproducts. Each peak was isolated and analyzed by ESI-MS confirming that the observed masses were in agreement with the calculated values. mBhc-OH produced in the photolysis reaction was also purified by preparative RP-HPLC and characterized by LC-MS and NMR (See Figure S5).

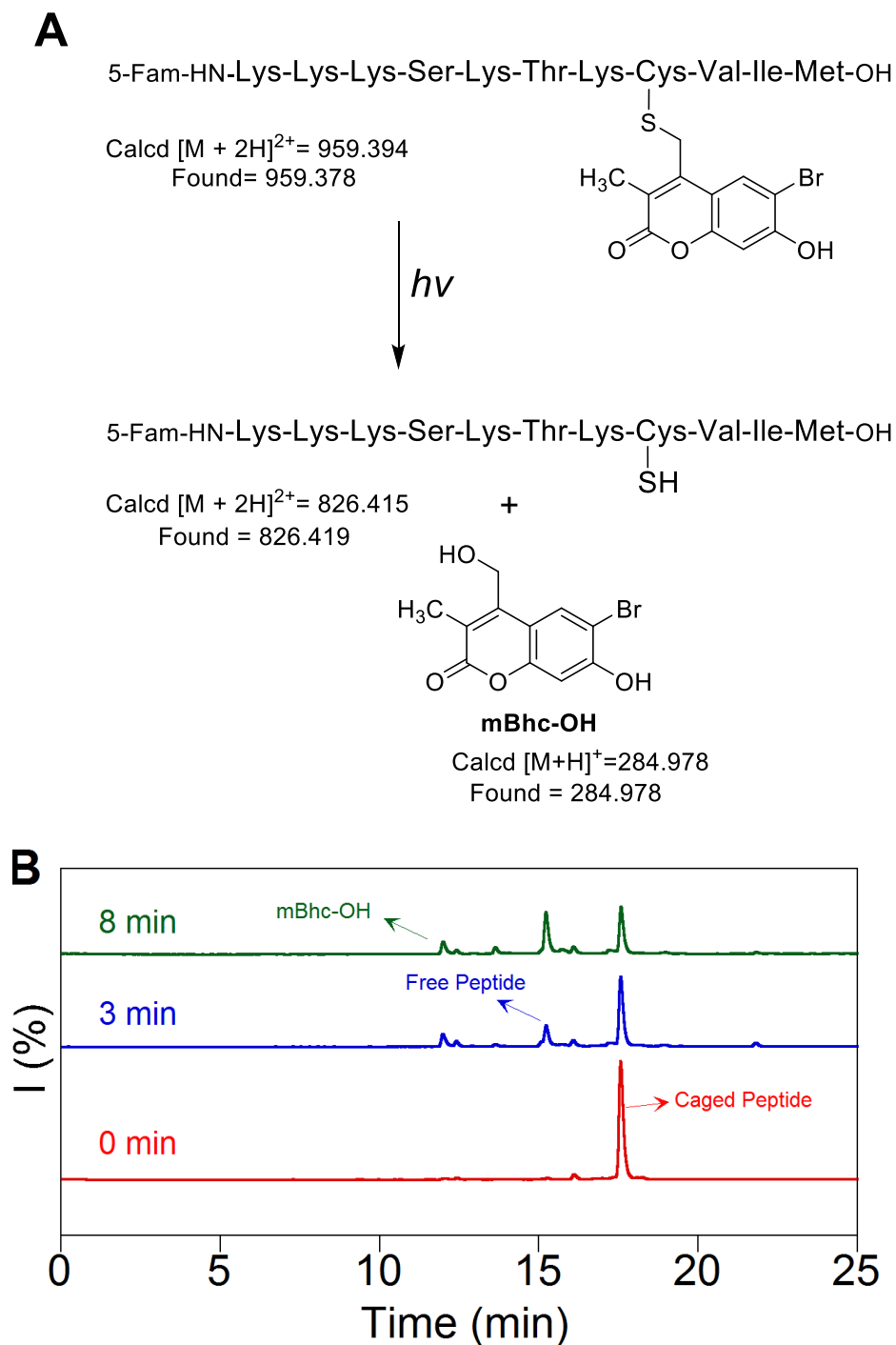


Figure S4. Analysis of the photolysis of a 5-Fam-labeled peptide containing a mBhc-protected cysteine. (A) Scheme showing photo-triggered uncaging of mBhc-protected 5-Fam labeled K-Ras peptide. (B) fluorescence HPLC traces representing the time-course of photolysis of 5-Fam-KKKSKTKC(mBhc)VIM. These results show clean conversion of caged peptide to the free compound with minimal byproduct formation. Each peak was isolated and analyzed by ESI-MS confirming that the observed masses were completely in agreement with the calculated values.

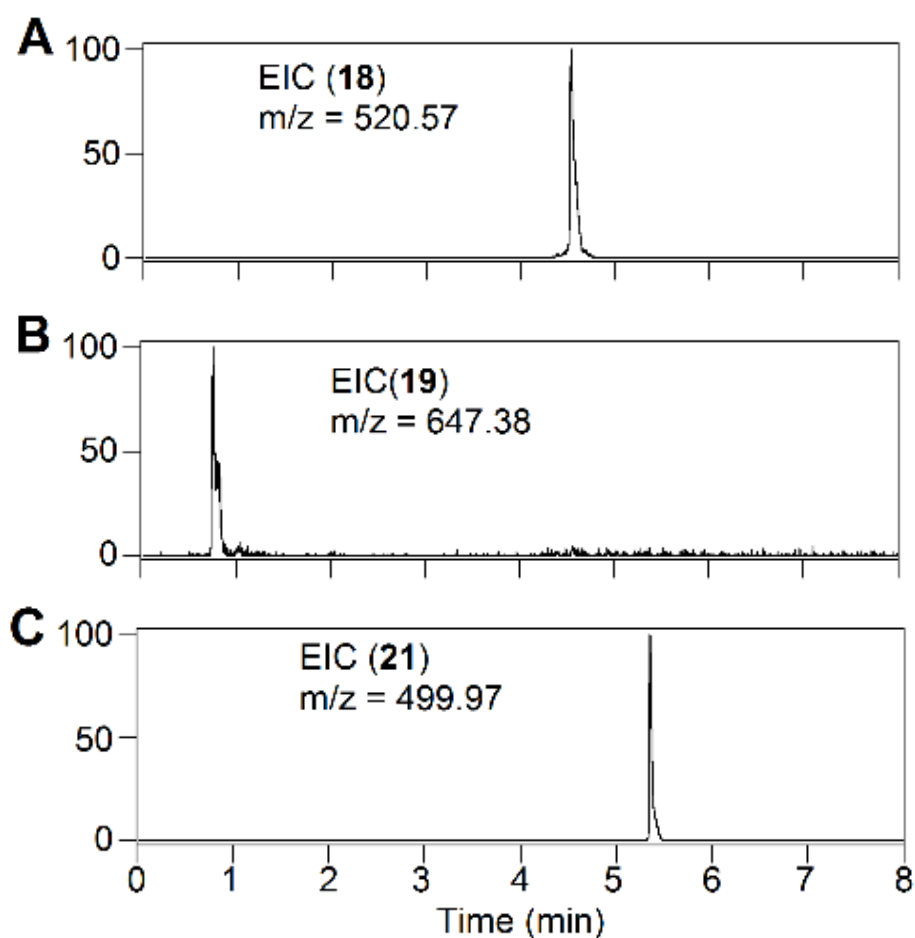


Figure S5. Analysis of a photolysis reaction of **18** showing the formation of the uncaged product and its subsequent enzymatic prenylation by PFTase. (A) EIC chromatogram (m/z = 520.57, calcd for $[M + 3H]^{3+} = 520.58$) of a 7.5 μM solution of **18** in a prenylation buffer containing PFTase with no irradiation (B) EIC chromatogram (m/z = 647.38, calcd for $[M + 2H]^{2+} = 647.39$) of a 7.5 μM solution of **18** after 5 min irradiation at 800 nm in prenylation buffer without any PFTase, indicates the generation of free peptide **19**, (C) EIC chromatogram (m/z = 499.97, calcd for $[M + 3H]^{3+} = 499.99$) of a 7.5 μM of **18** after 5 min irradiation at 800 nm in presence of PFTase indicates the formation of farnesylated peptide **19**.

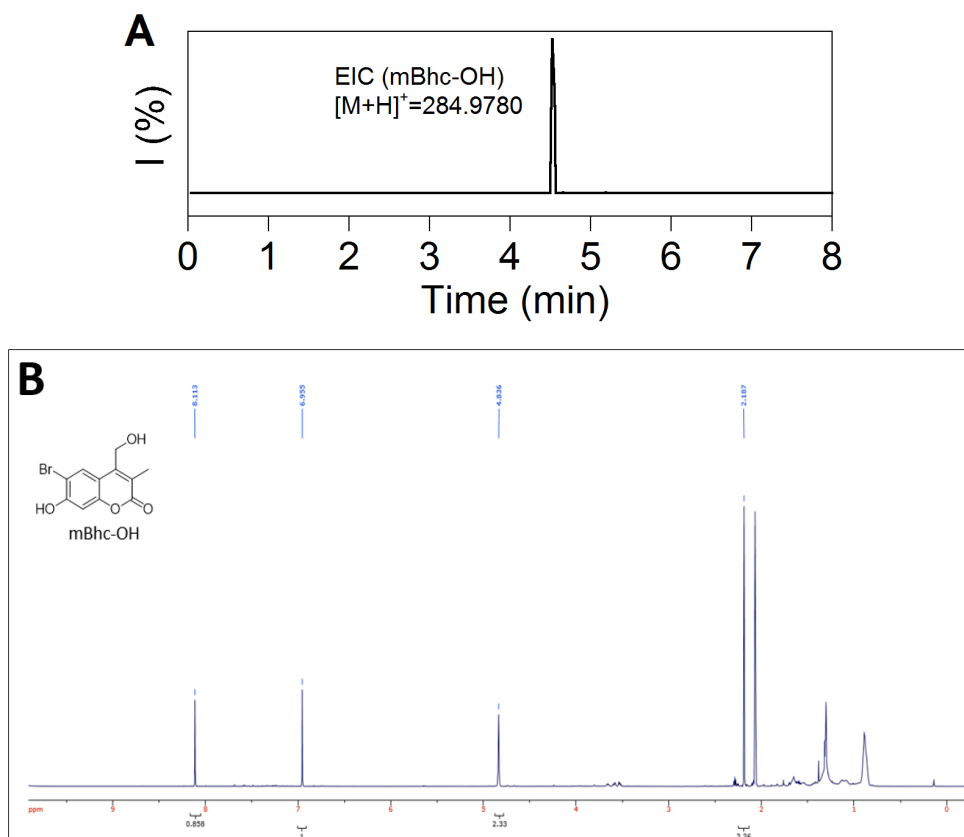
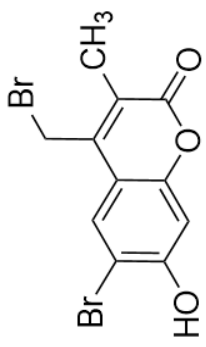
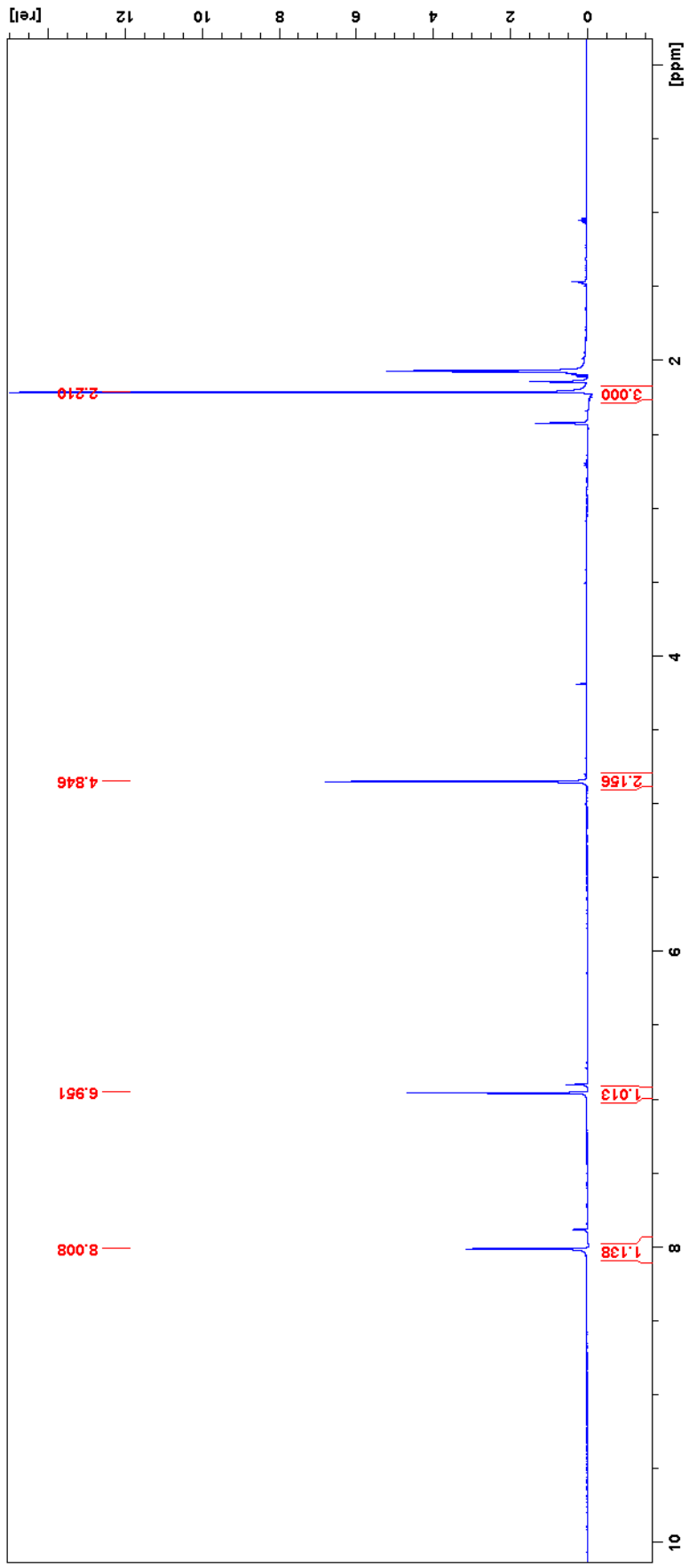
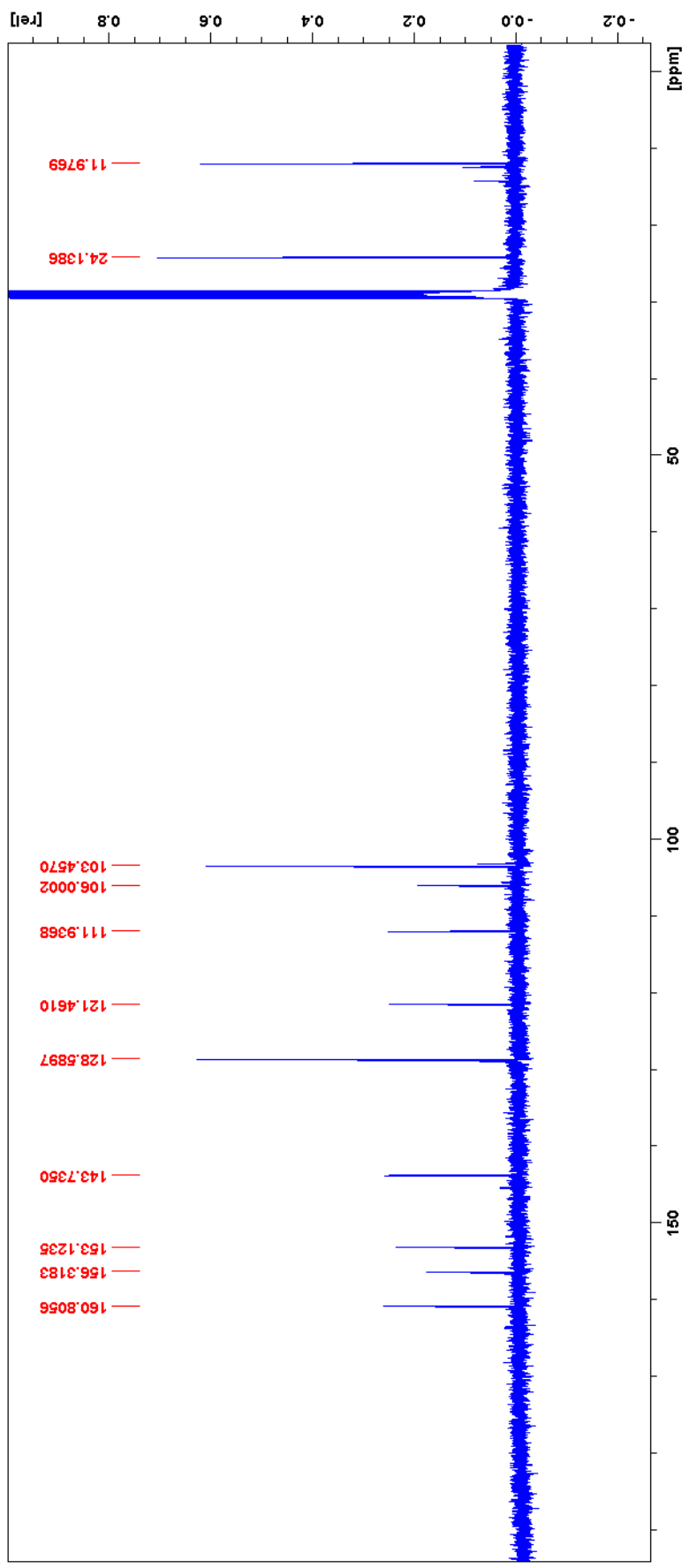
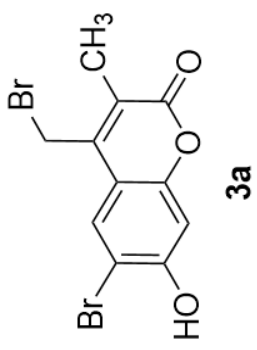


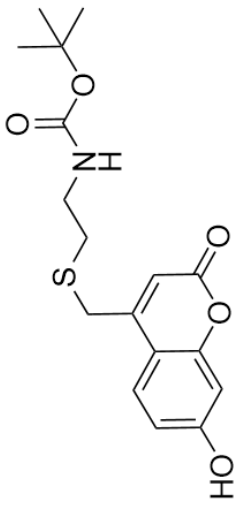
Figure S6. Characterization of mBhc-OH generated in the photolysis reaction and isolated via preparatory RP-HPLC. (A) EIC chromatogram showing high purity of the sample and confirming the observed mass exactly matches with the calculated one ($[M+H]^+$ *cacd,obs* = 284.978). (B) ^1H NMR of the isolated mBhc-OH obtained in deuterated acetone.



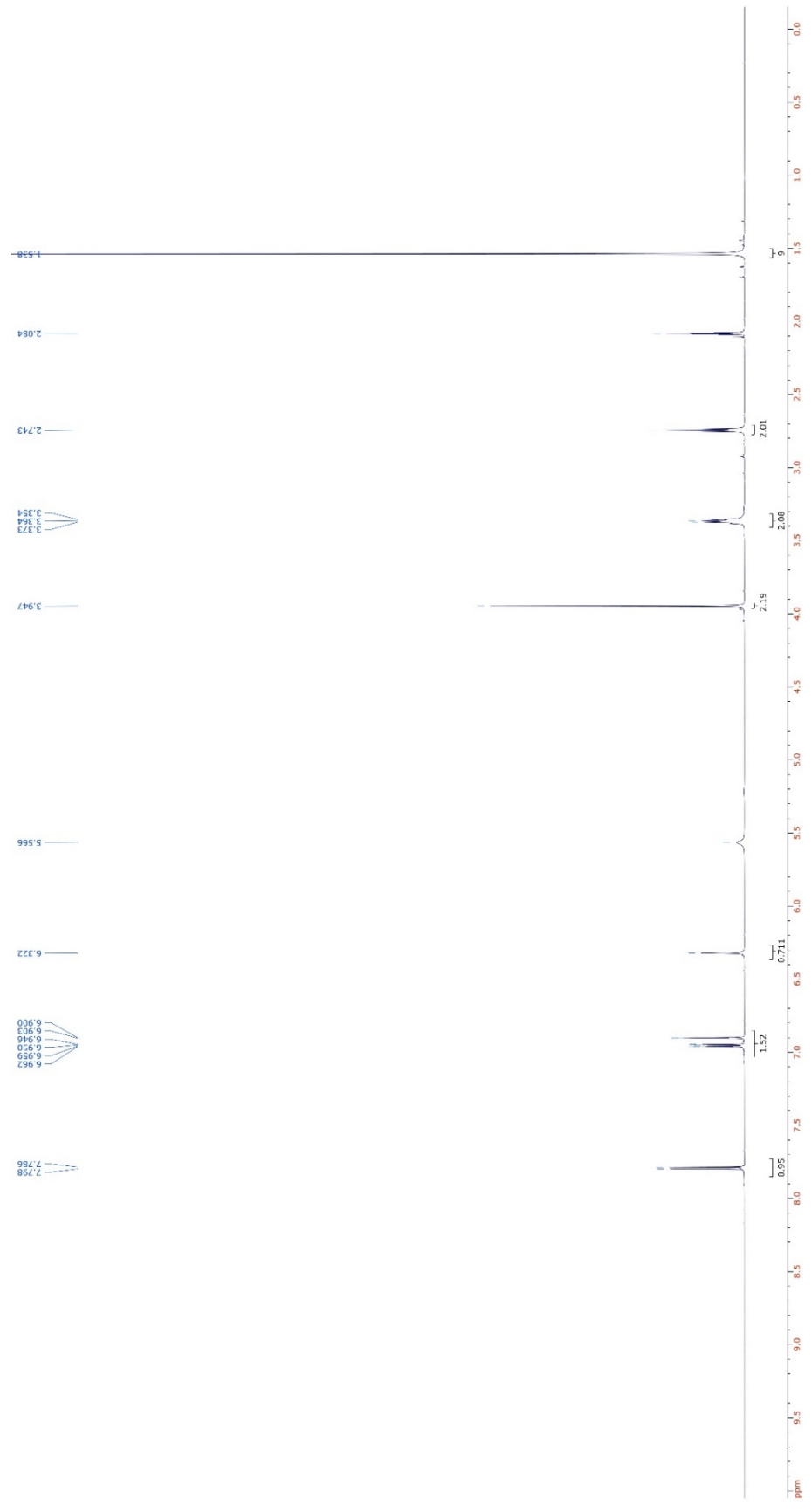
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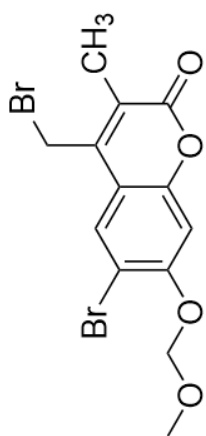




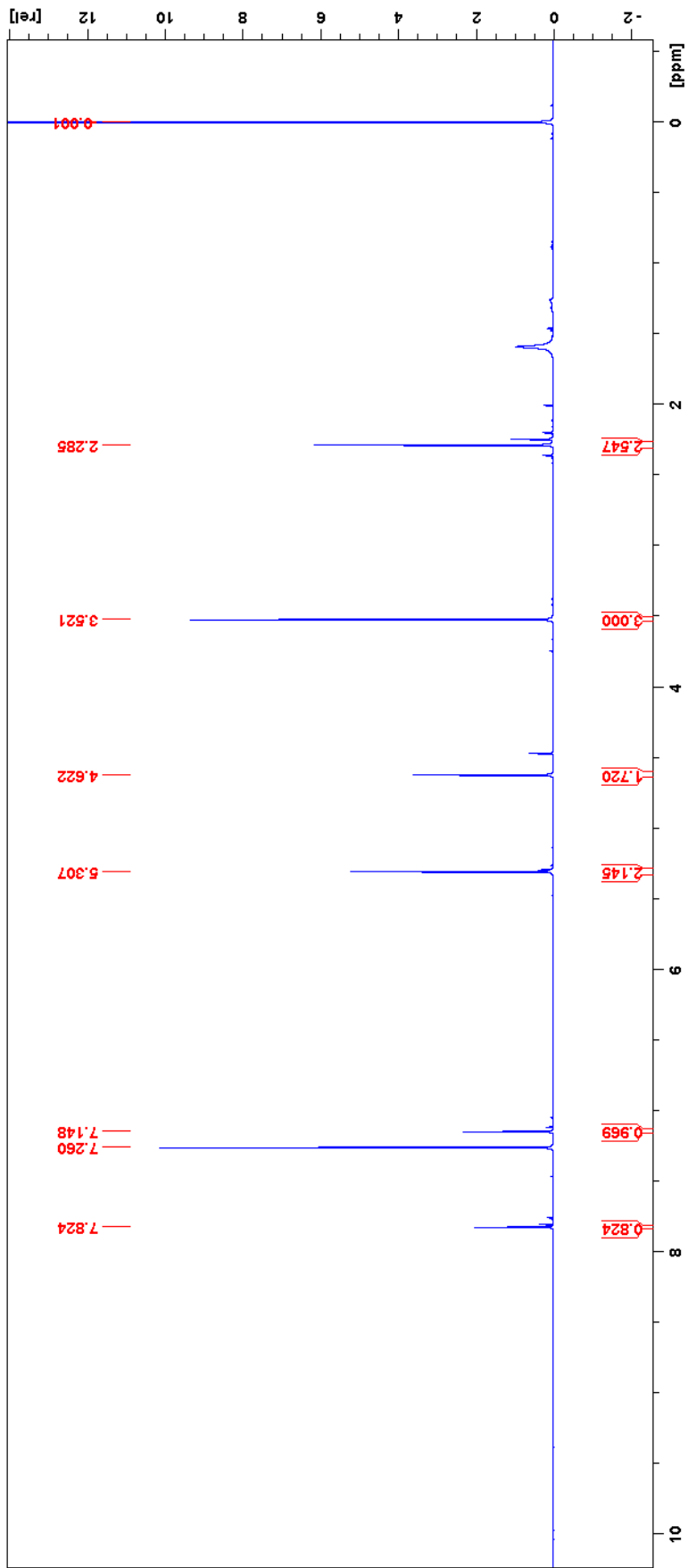


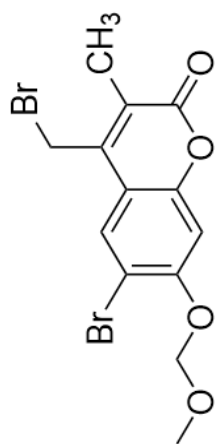
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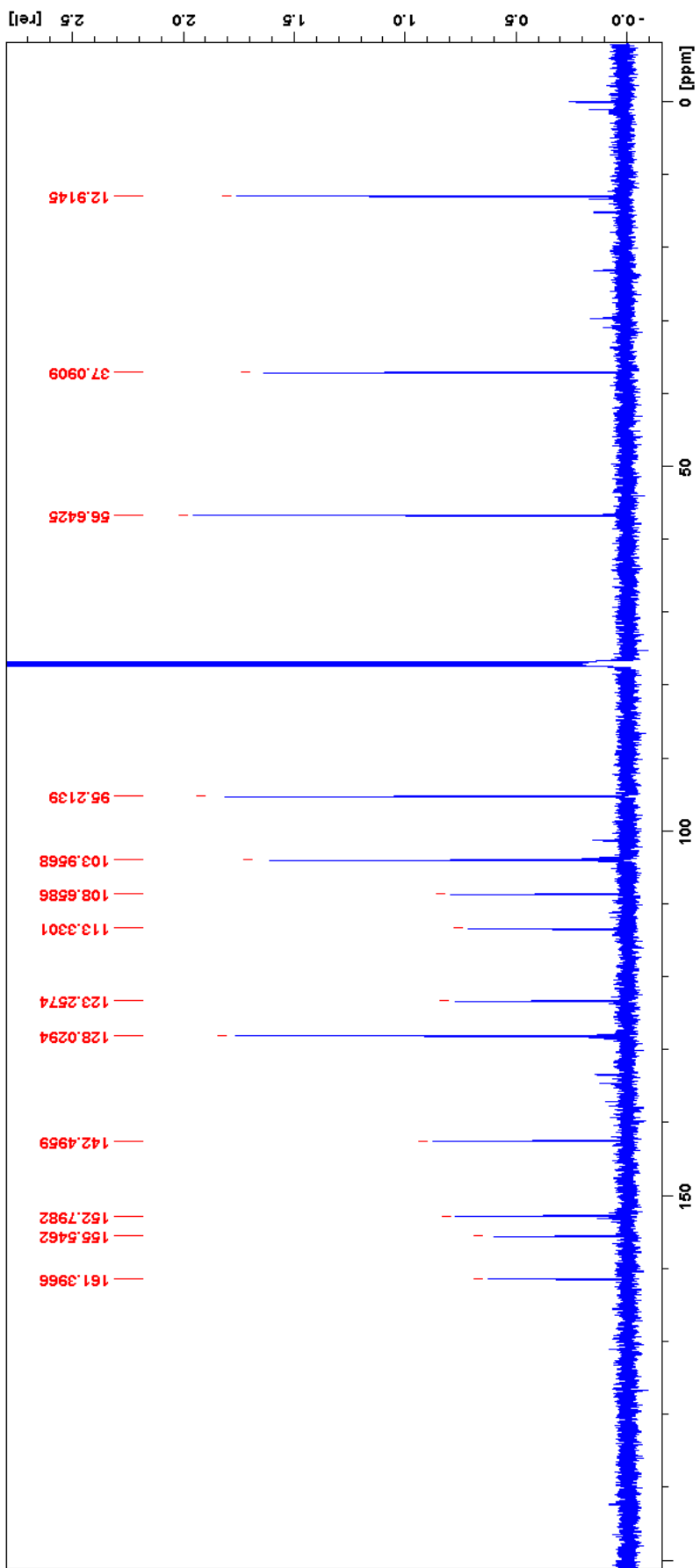


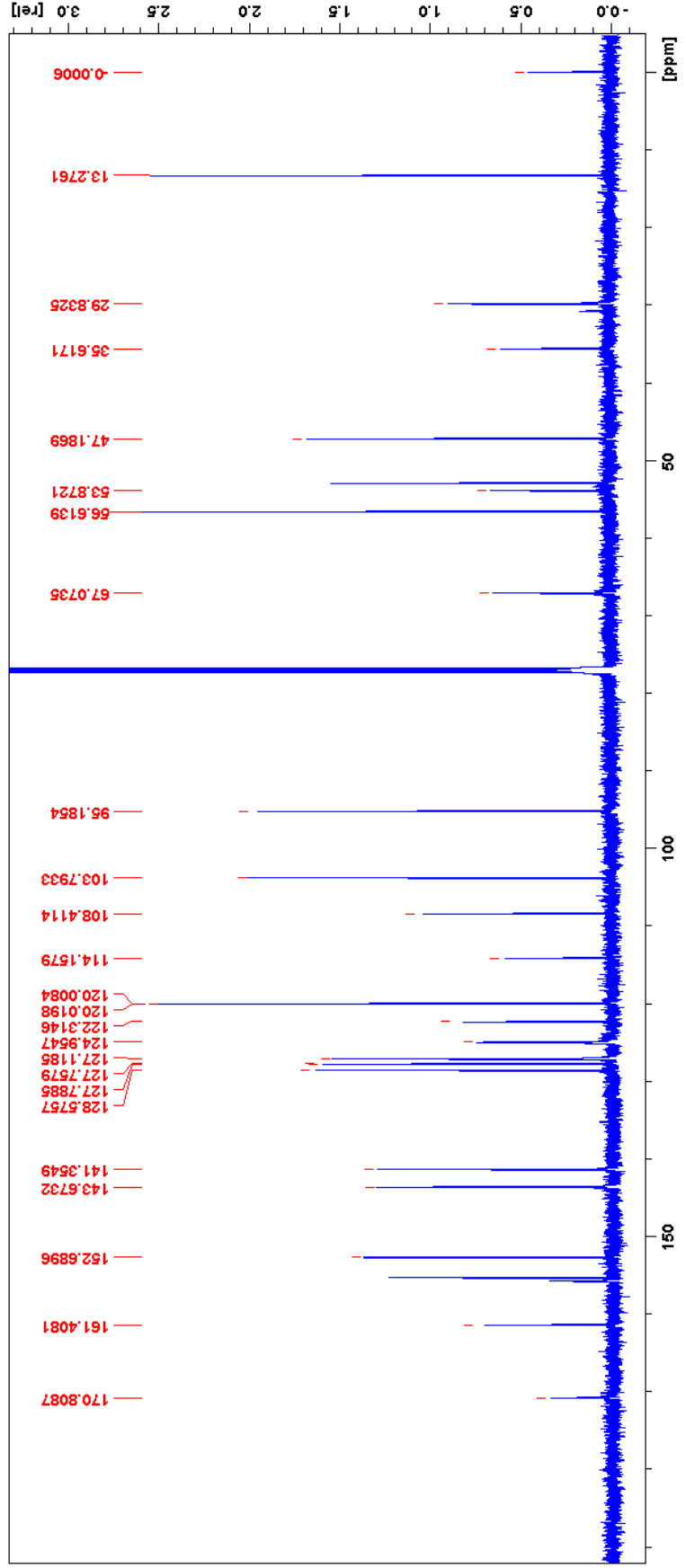
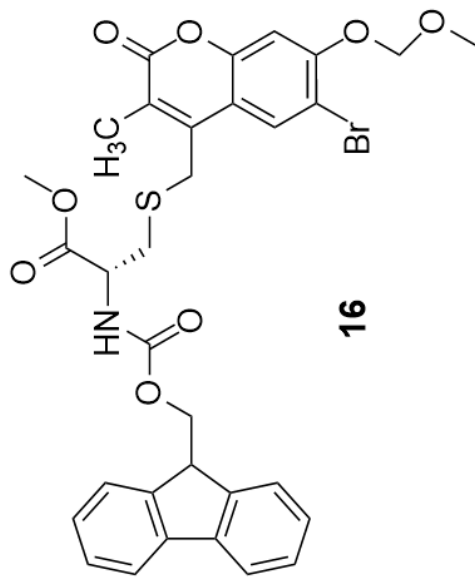
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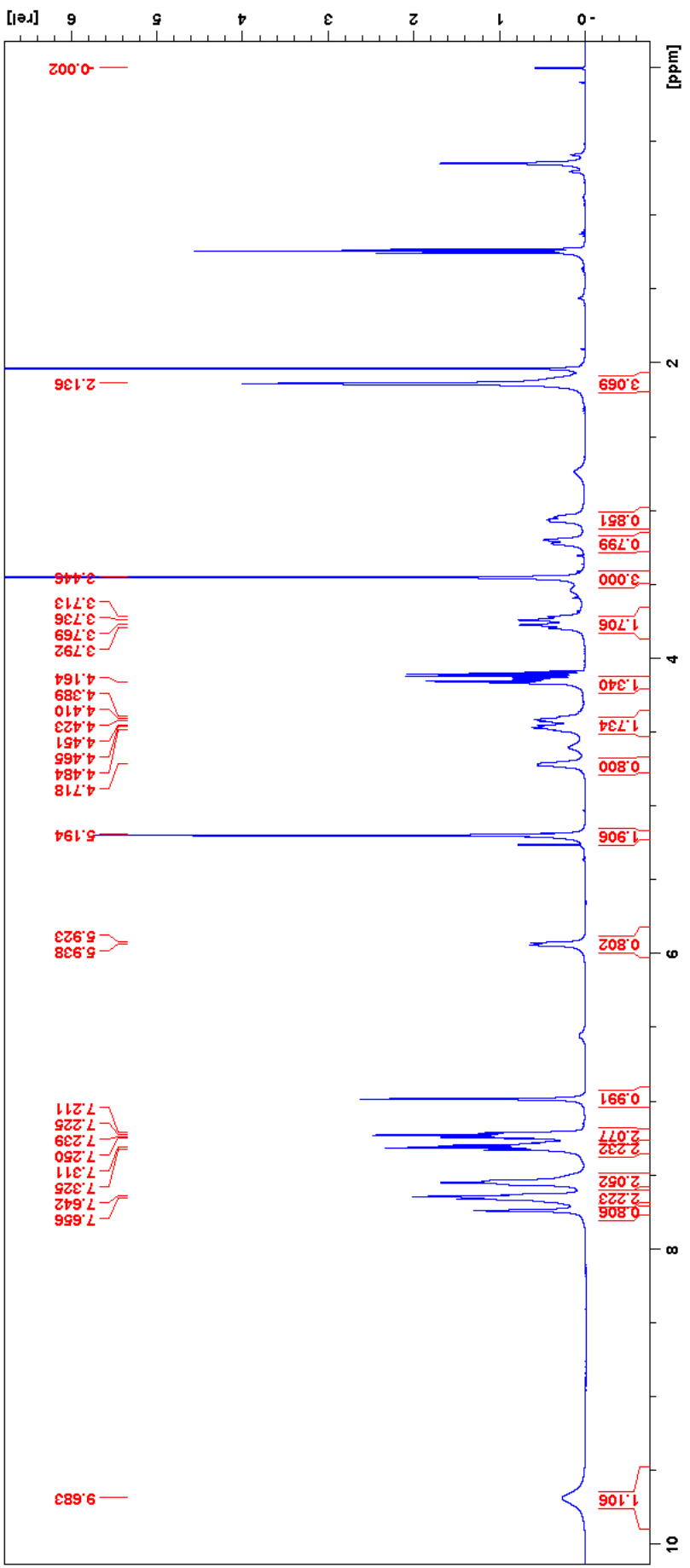
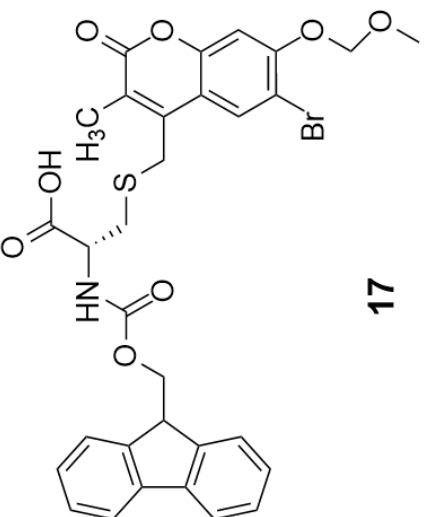


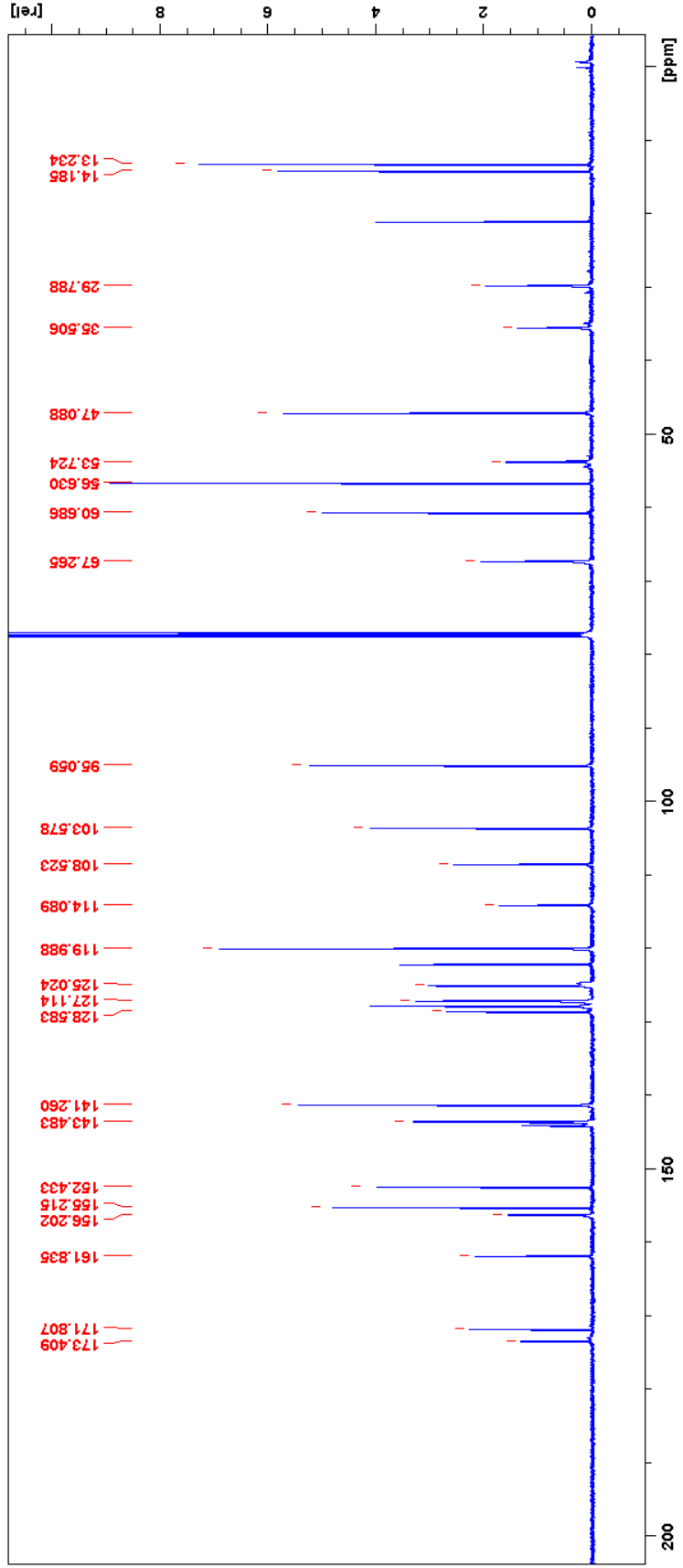
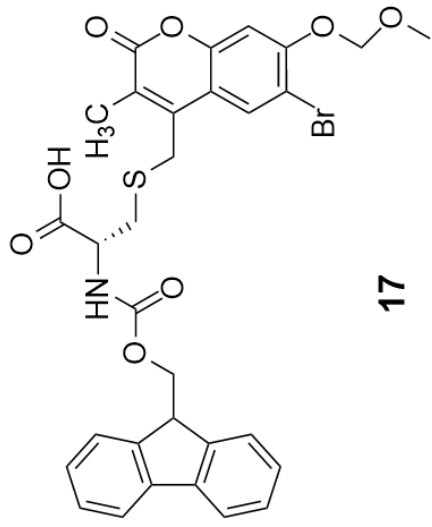


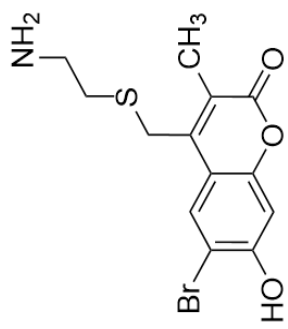
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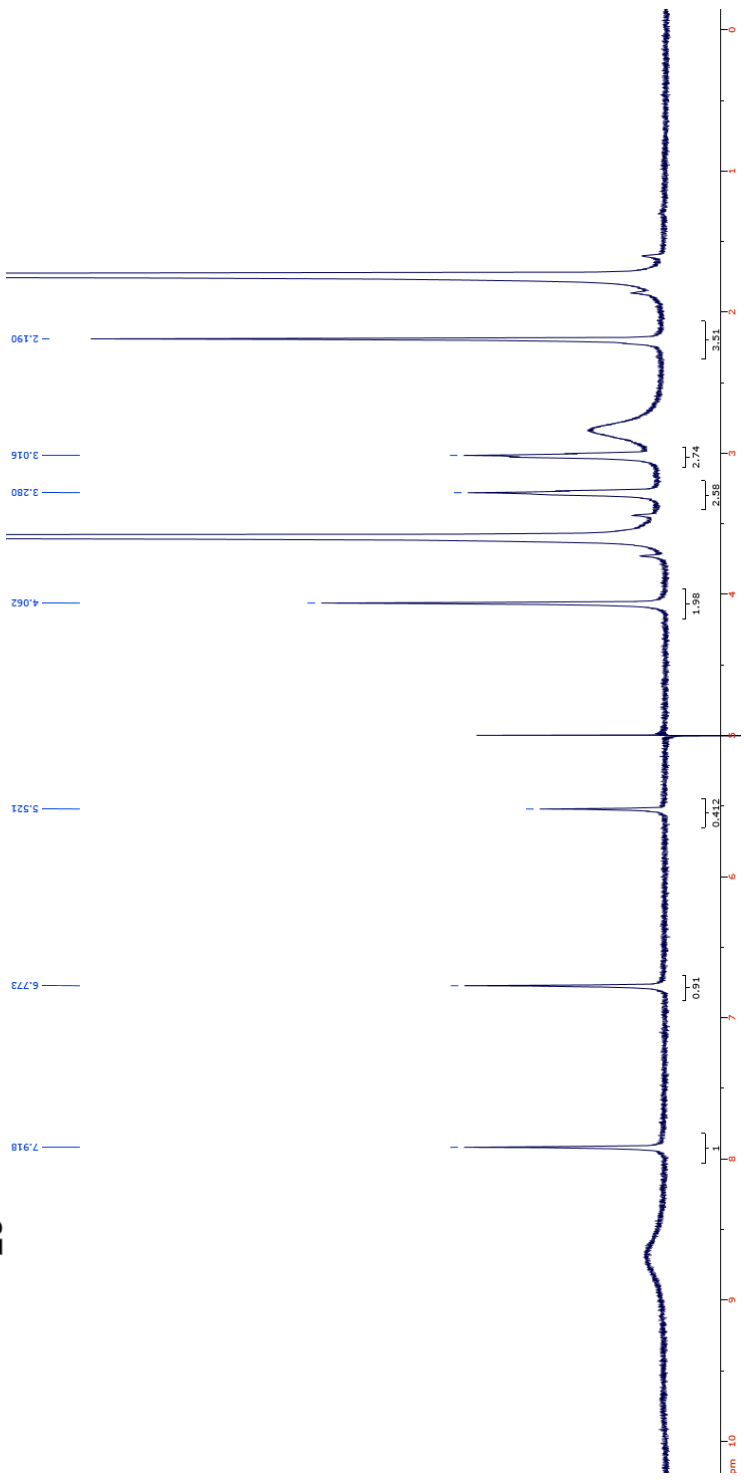


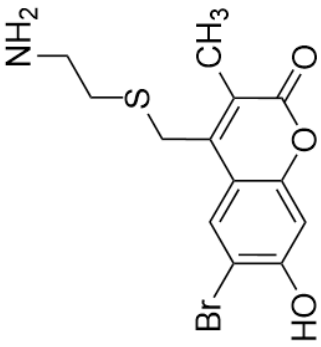




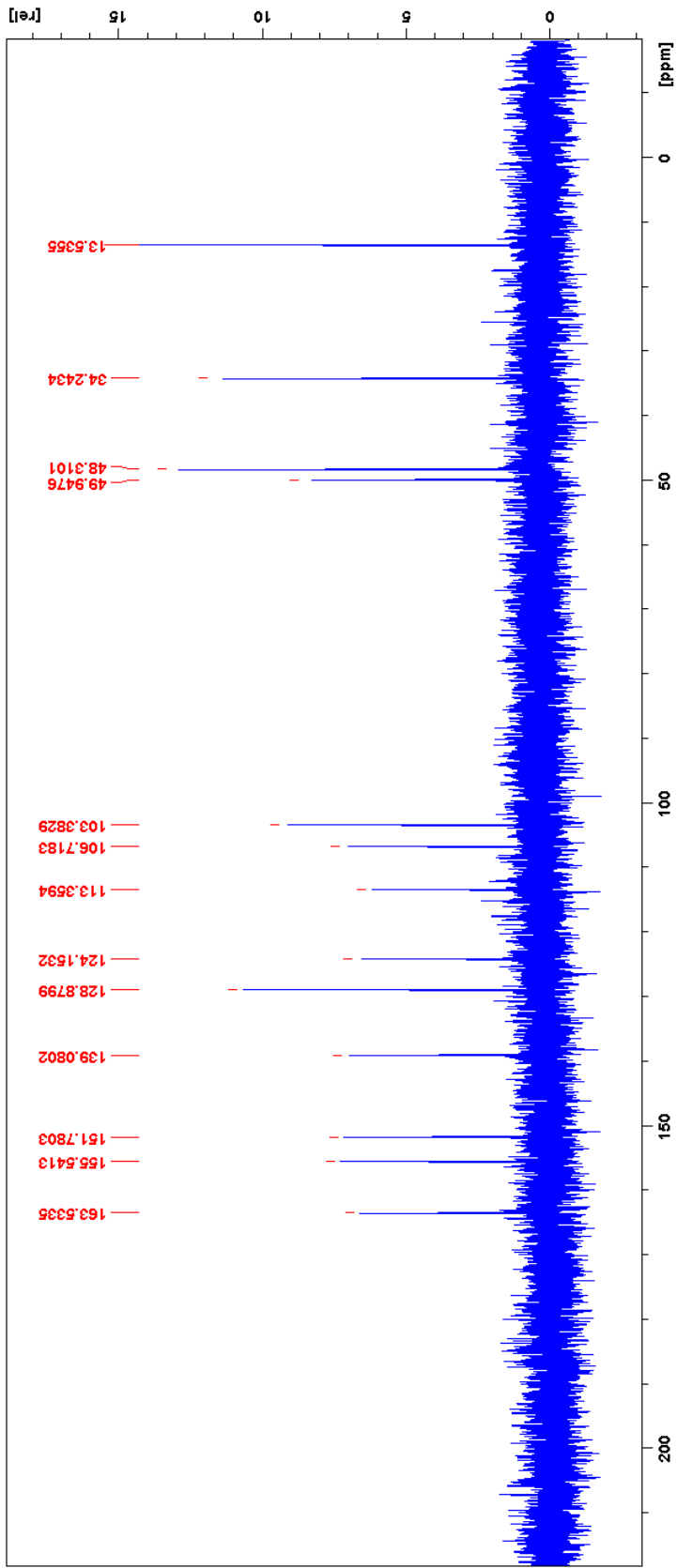


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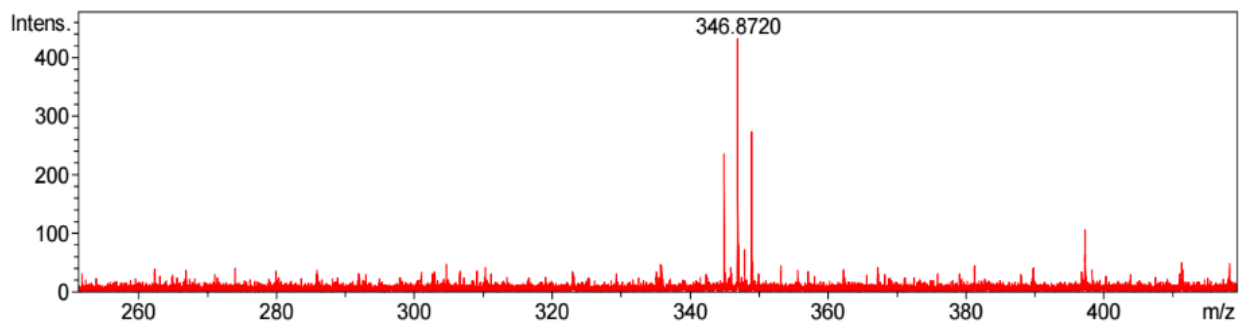




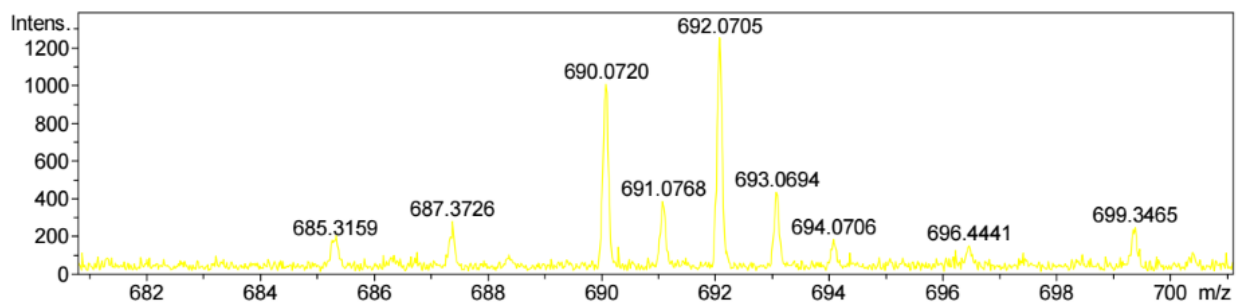
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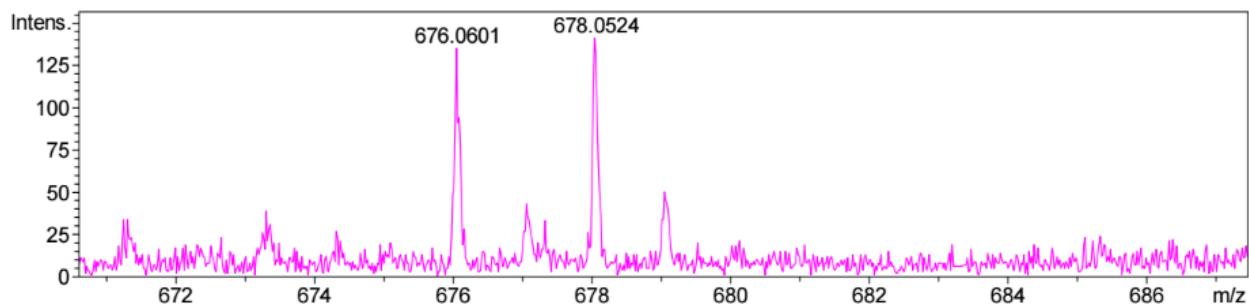
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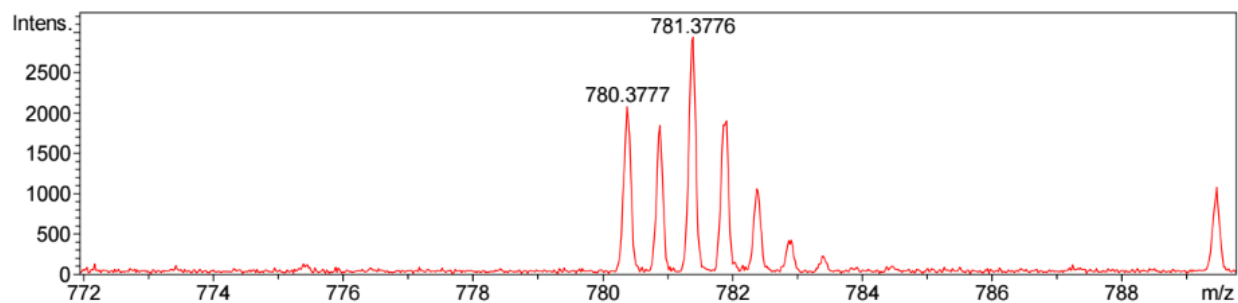
HR-MS of Compound **3a** $[M]^{1-}$



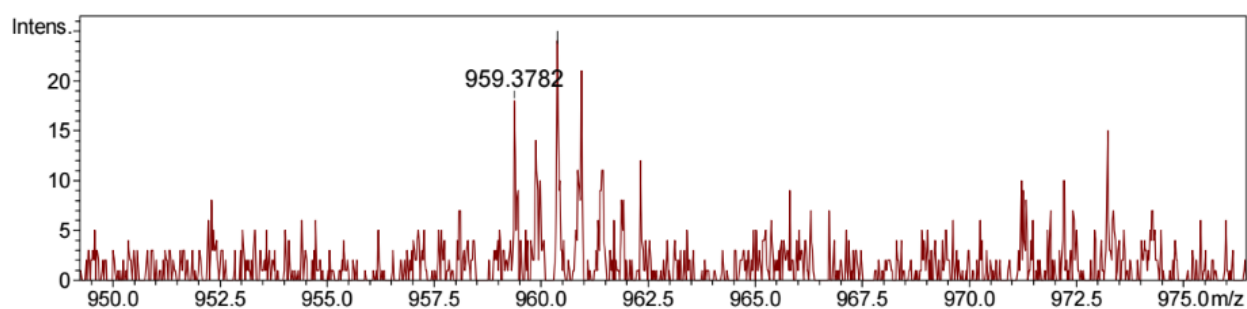
HR-MS of Compound **16** $[M+Na]^+$



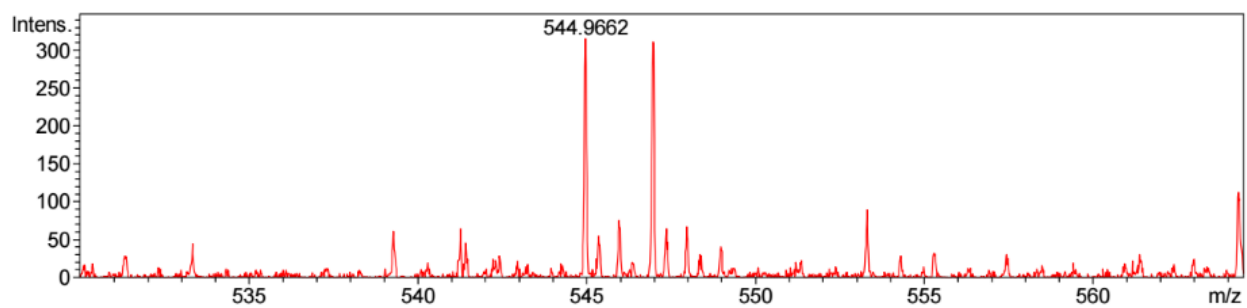
HR-MS of Compound **17** $[M+Na]^+$



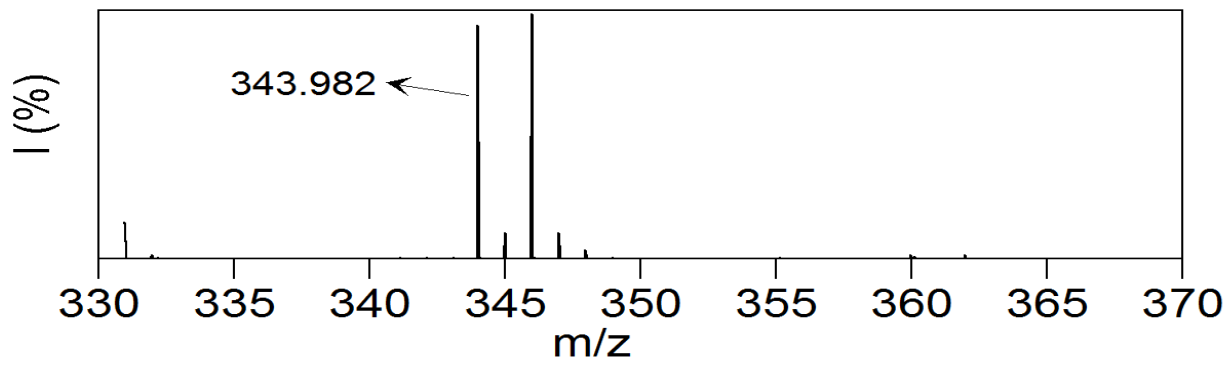
HR-MS of Compound **18** $[M+2H]^+$



HR-MS of Compound **5-Fam-KKKSATKC(mBhc)VIM** $[M+2H]^+$



HR-MS of Compound **mBhc-NBD-cysteamine** $[M+K]^+$



HR-MS of Compound **mBhc-cysteamine 23** $[M+Na]^+$