

Supplementary Information

Native chemical ligation at methionine bioisostere norleucine allows for N-terminal chemical protein ligation

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Supplementary Information

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1. Characterization of synthetic and expressed linear diubiquitin

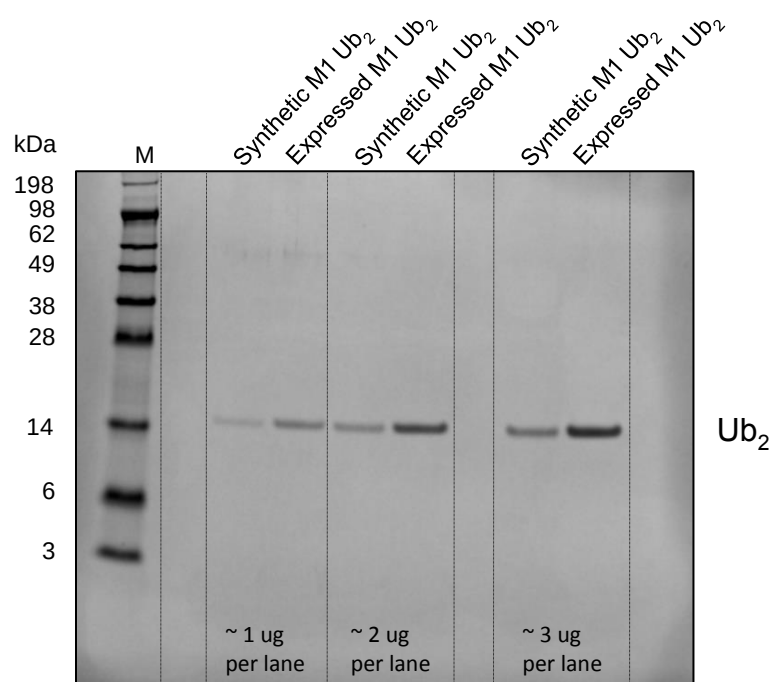


Figure S1. Characterization of synthetic linear diUb (**14**) and expressed linear diUb by SDS-PAGE analysis stained with InstantBlue™. Loading: ~1, ~2 and ~3 µg linear diUb/ lane (~ 5.85 µM, ~11.7 µM and ~17.55 µM). Concentration of expressed linear diUb was normalized to synthetic linear diUb according to InstantBlue™ band intensities from the 3 µg lanes.

2. DUB cleavage assays

USP16

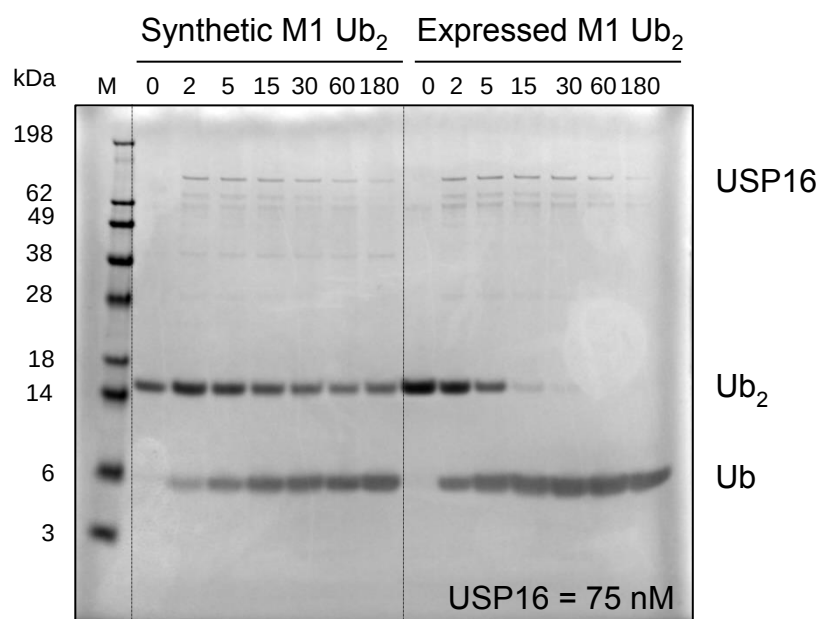


Figure S2. USP16 (75 nM) mediated hydrolysis of synthetic (**14**) and recombinant linear diubiquitin(30.8 μ M and 27.3 μ M respectively). Samples from each time point (2, 5, 15, 30, 60 and 180 minutes) were analysed by InstantBlue™ stained SDS-PAGE gel.

USP21

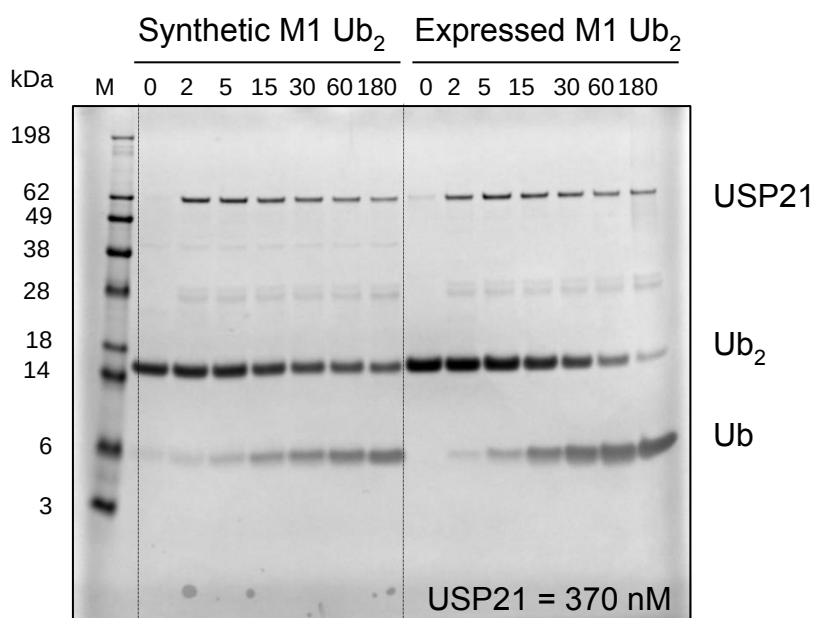


Figure S3. USP21 (370 nM) mediated hydrolysis of synthetic (**14**) and recombinant linear diubiquitin(30.8 μ M and 27.3 μ M respectively). Samples from each time point (2, 5, 15, 30, 60 and 180 minutes) were analysed by InstantBlue™ stained SDS-PAGE gel.

OTULIN

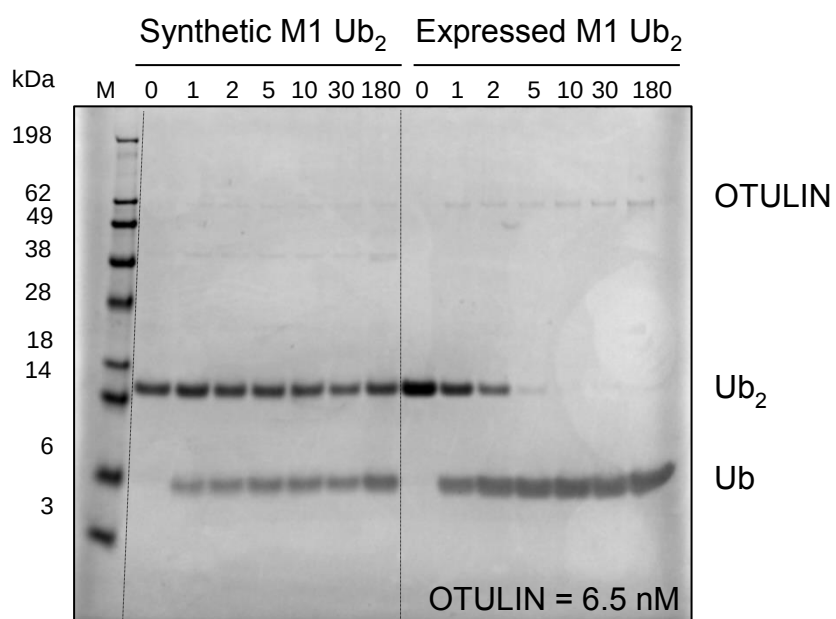
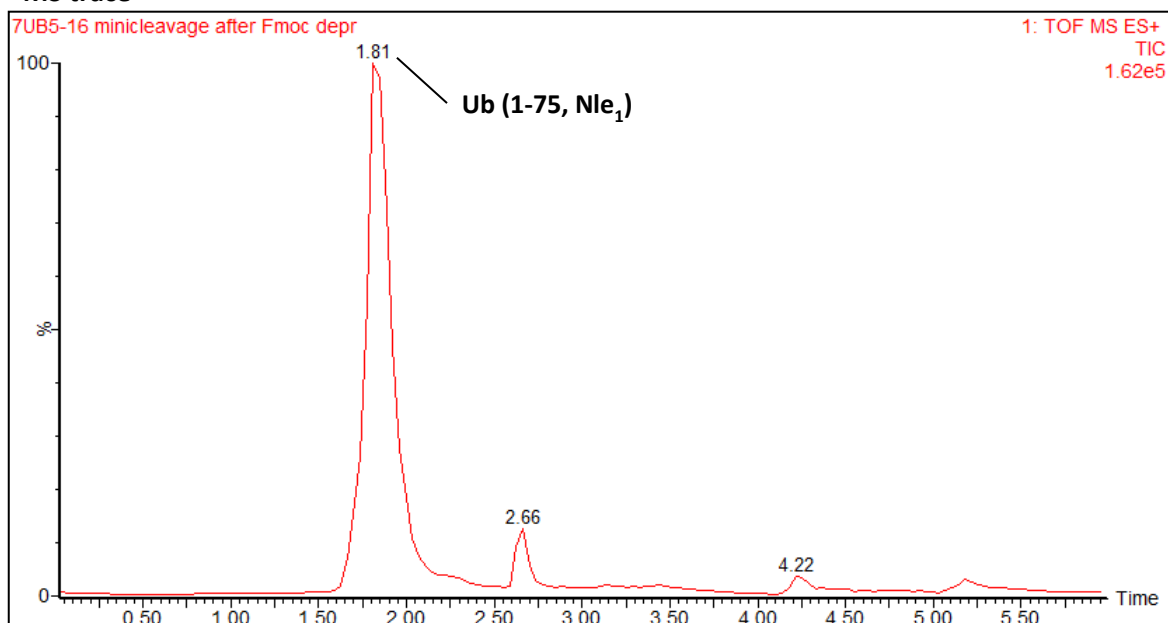


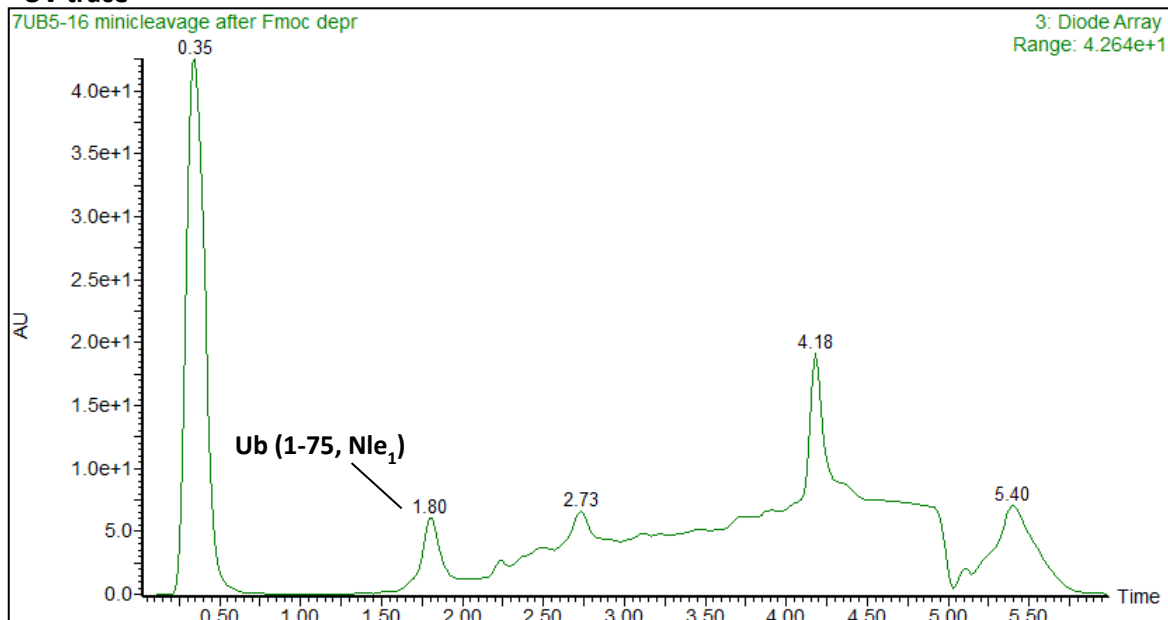
Figure S4. OTULIN (6.5 nM) mediated hydrolysis of synthetic (**14**) and recombinant linear diubiquitin (30.8 μ M and 27.3 μ M respectively). Samples from each time point (1, 2, 5, 10, 30 and 180 minutes) were analysed by InstantBlue™ stained SDS-PAGE gel. Related to Figure 2.

3. LC-MS data of the linear diubiquitin synthesis

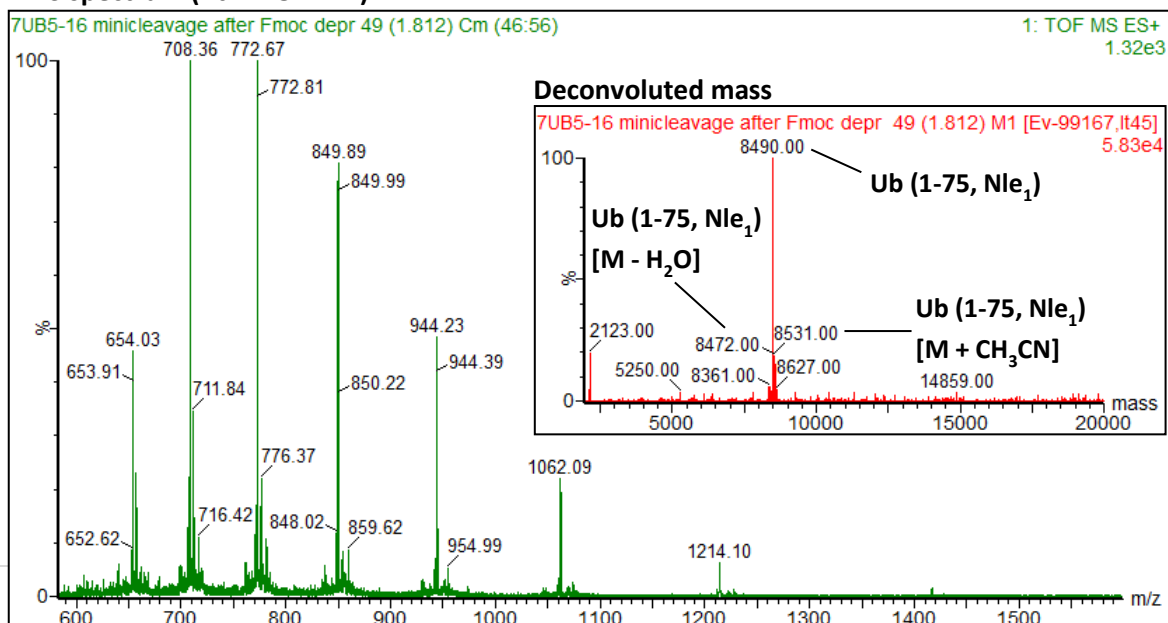
MS trace



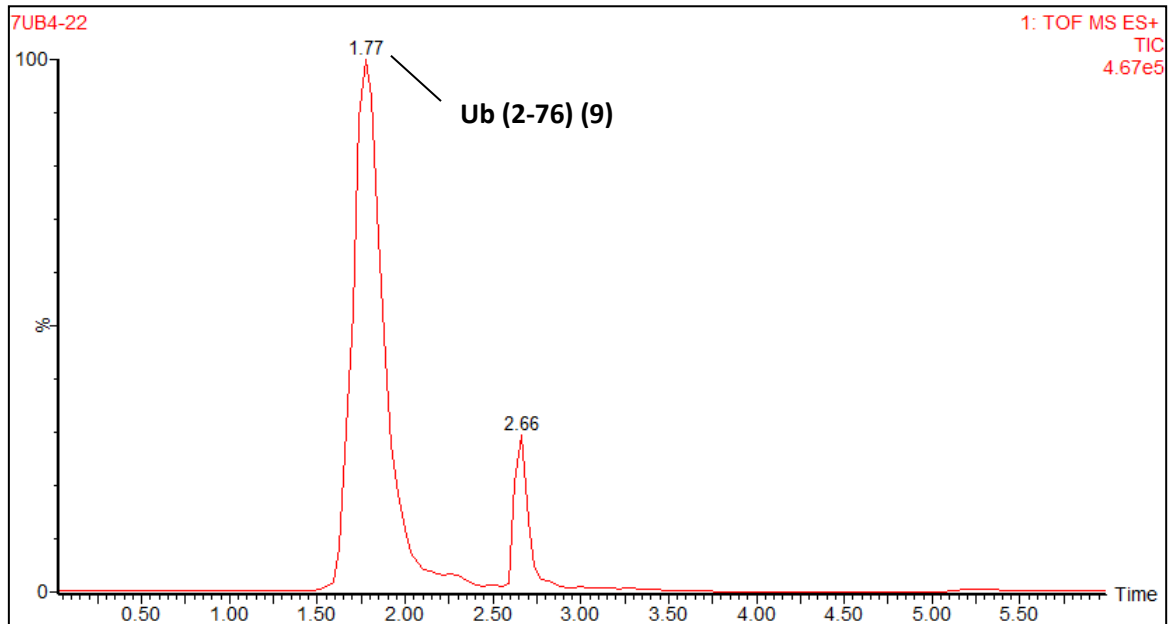
UV trace



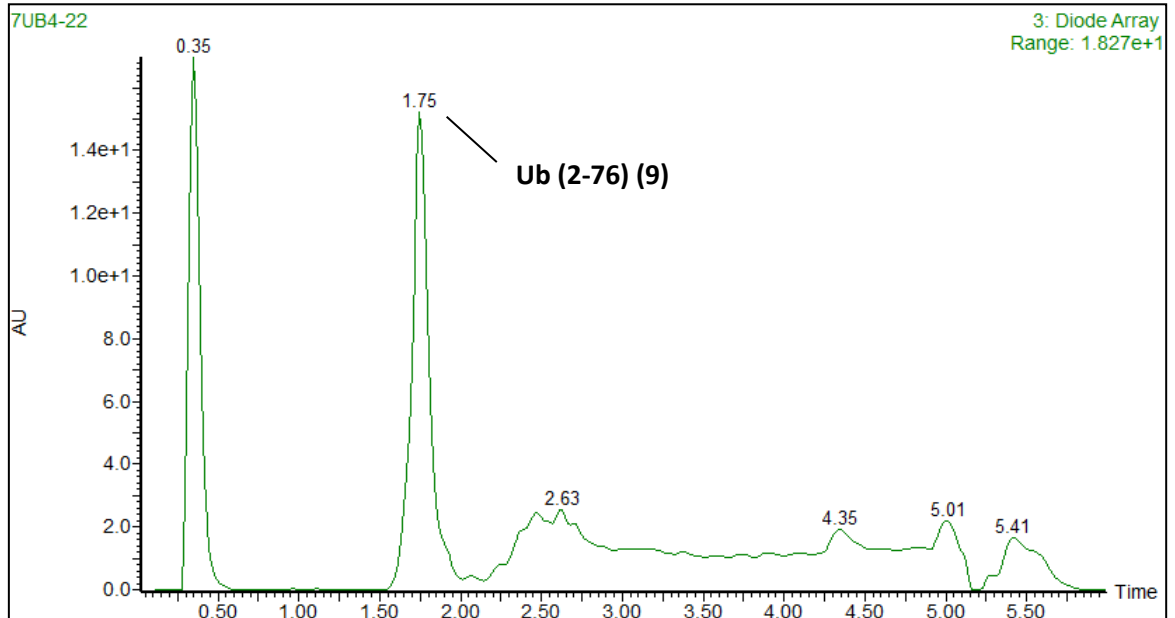
MS spectrum (Rt = 1.81 min)



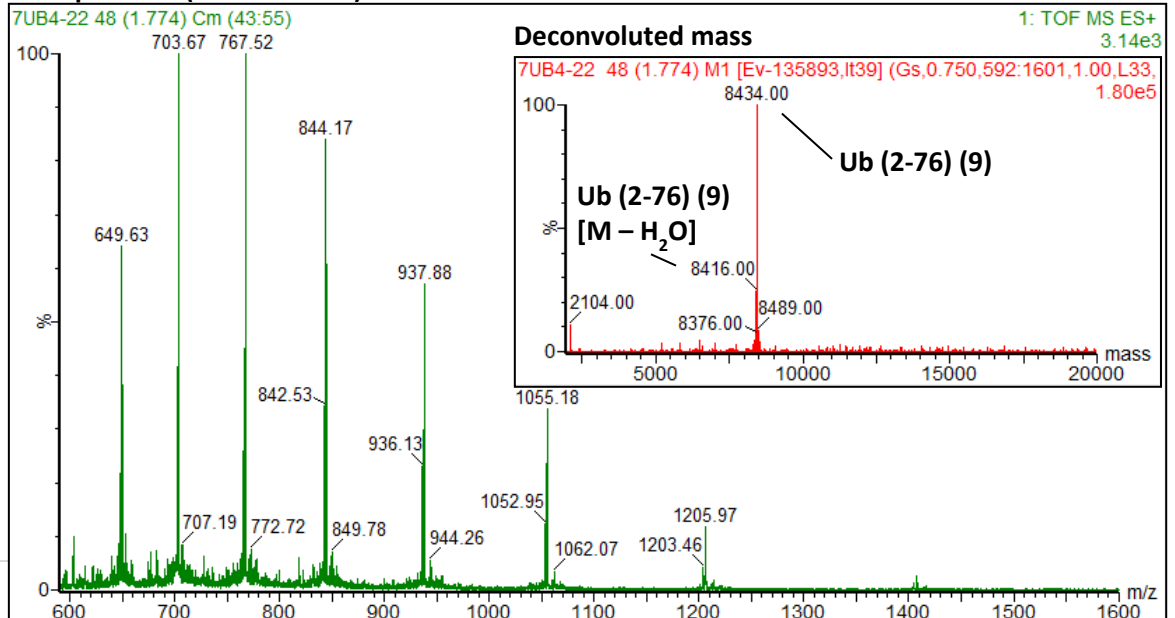
MS trace



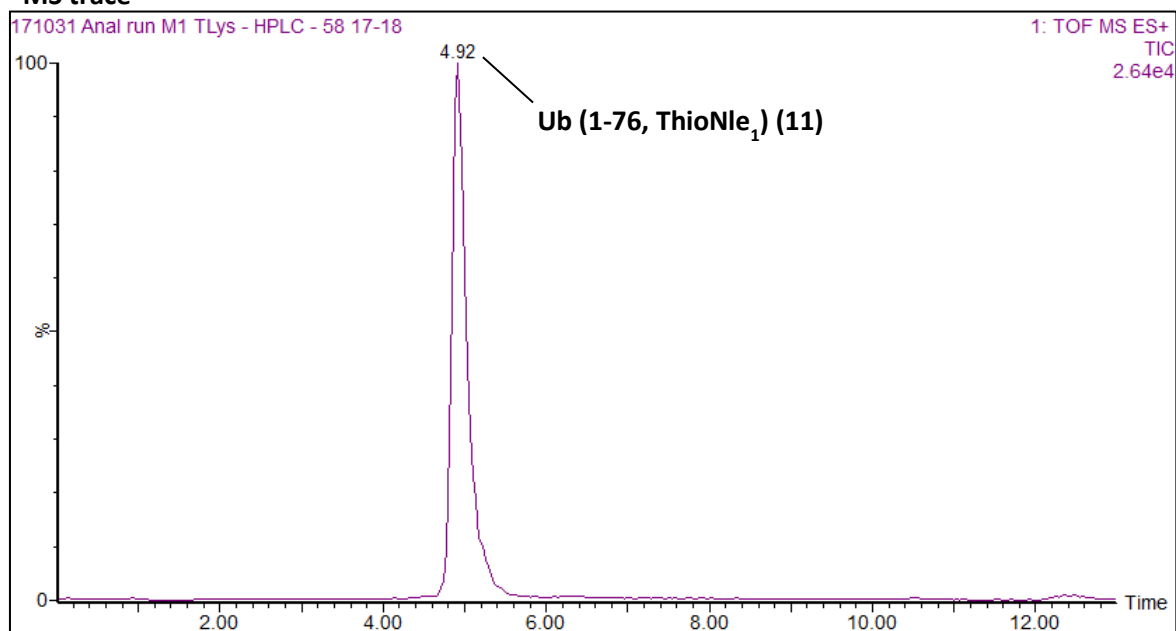
UV trace



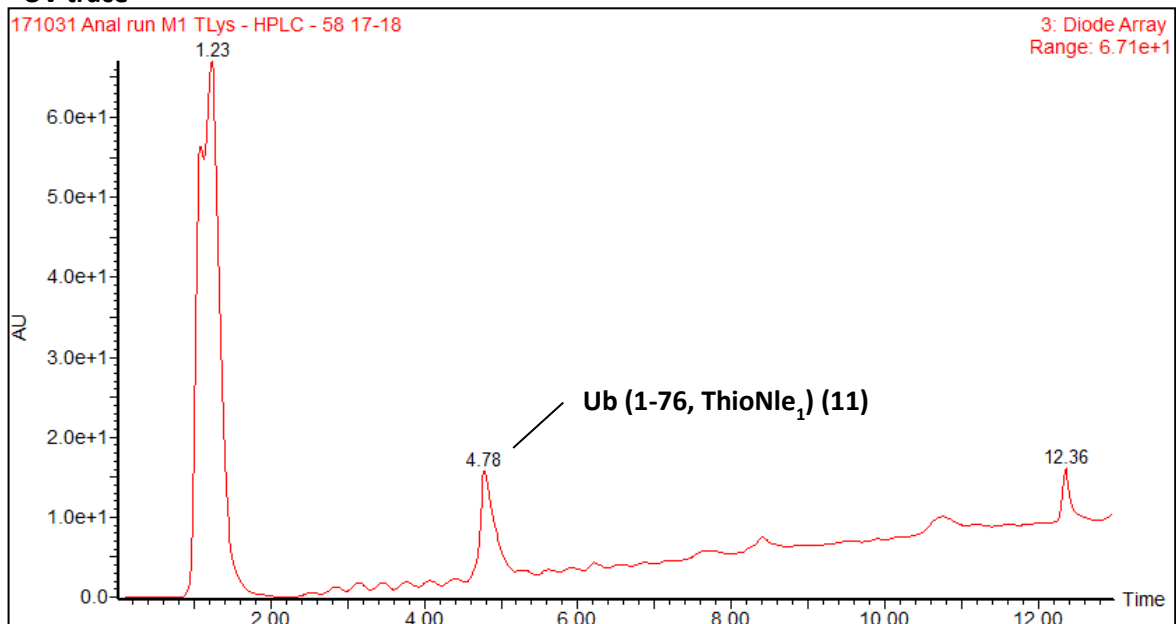
MS spectrum (Rt = 1.77 min)



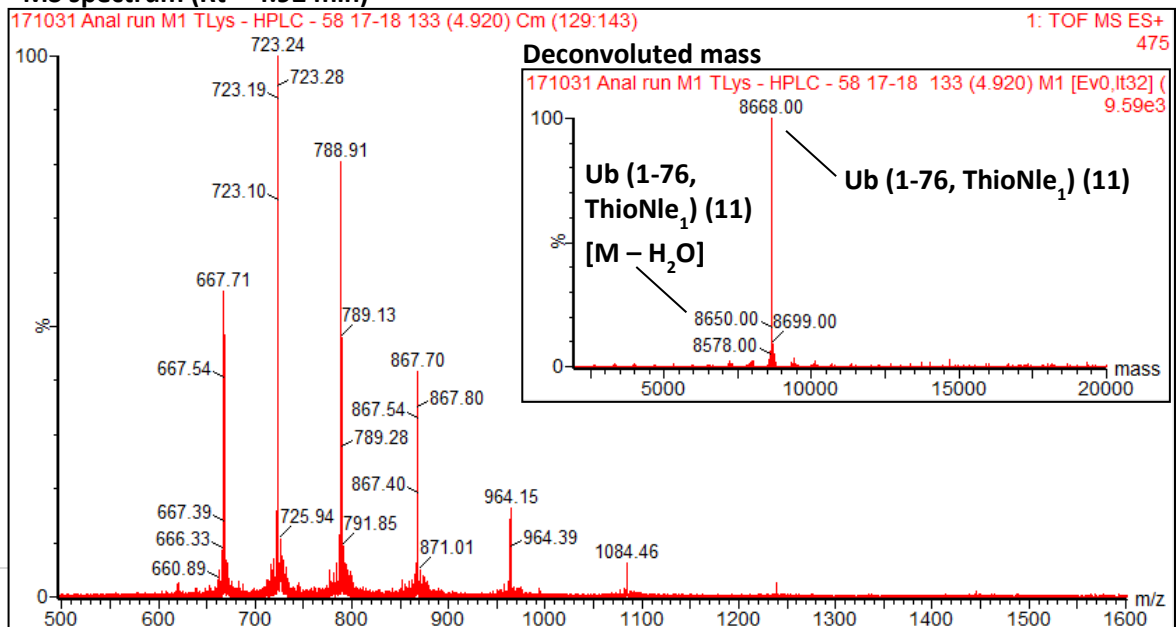
MS trace



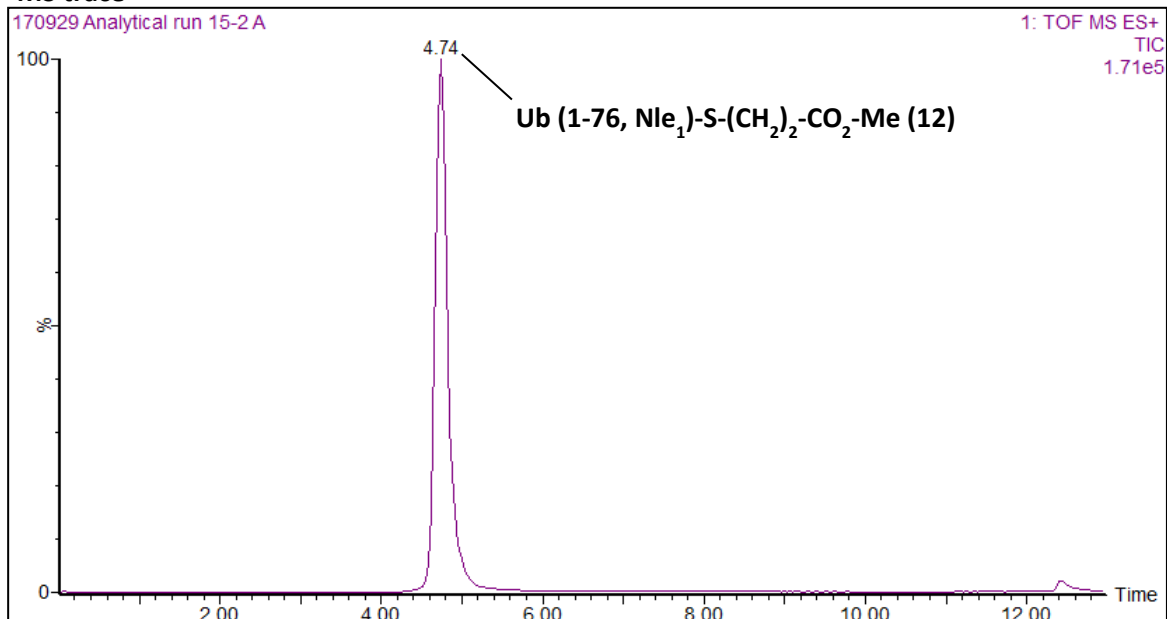
UV trace



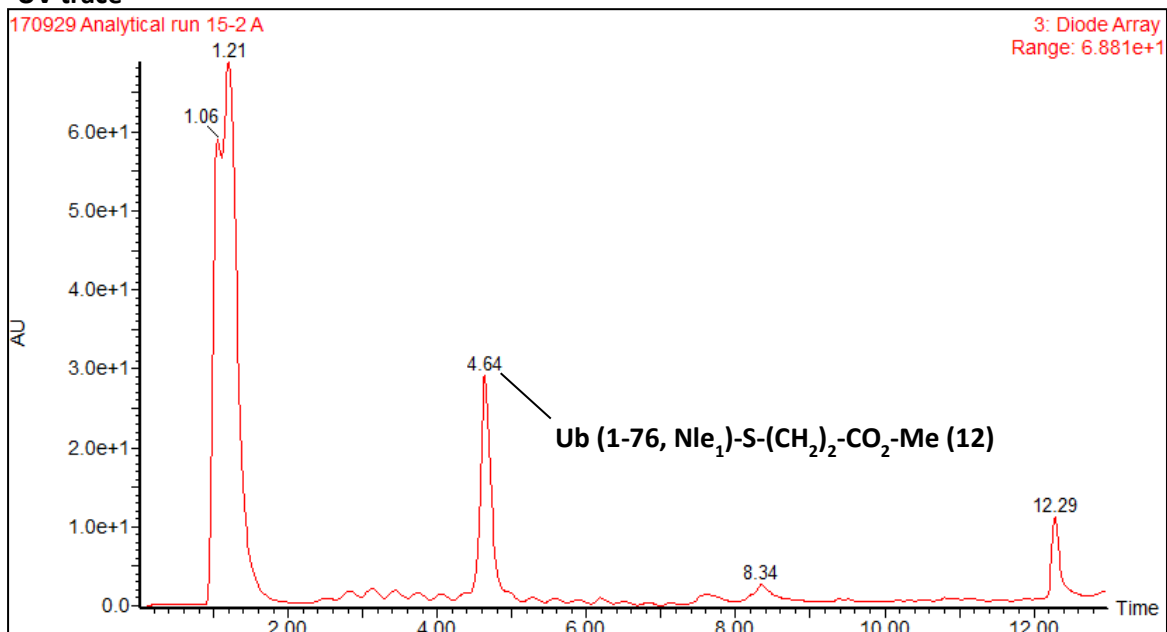
MS spectrum (Rt = 4.92 min)



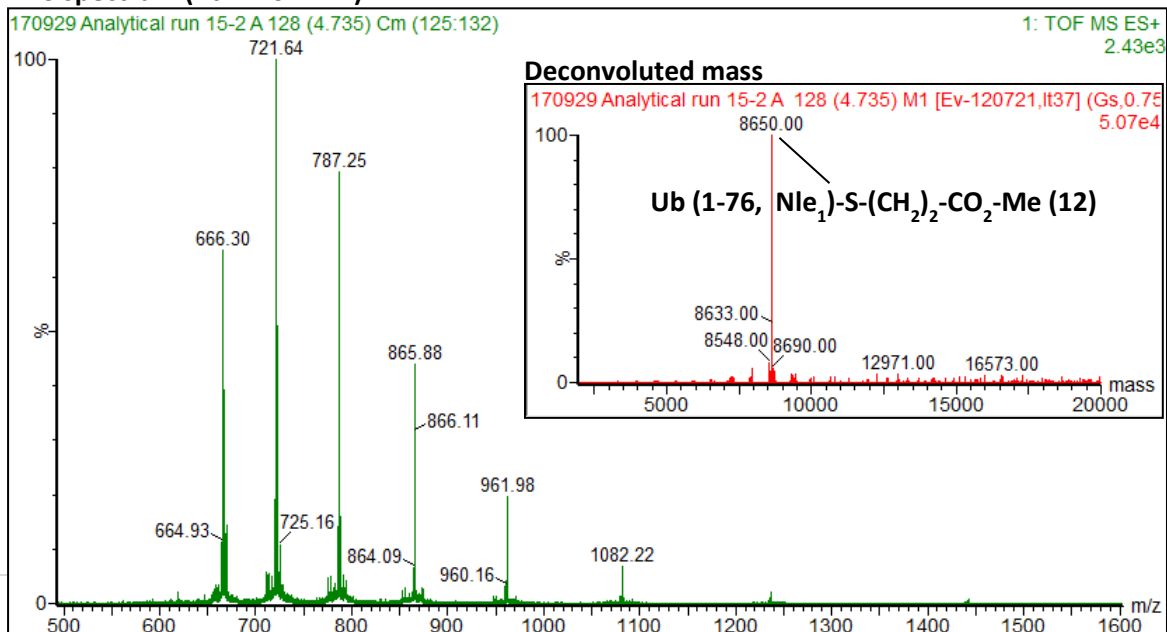
MS trace



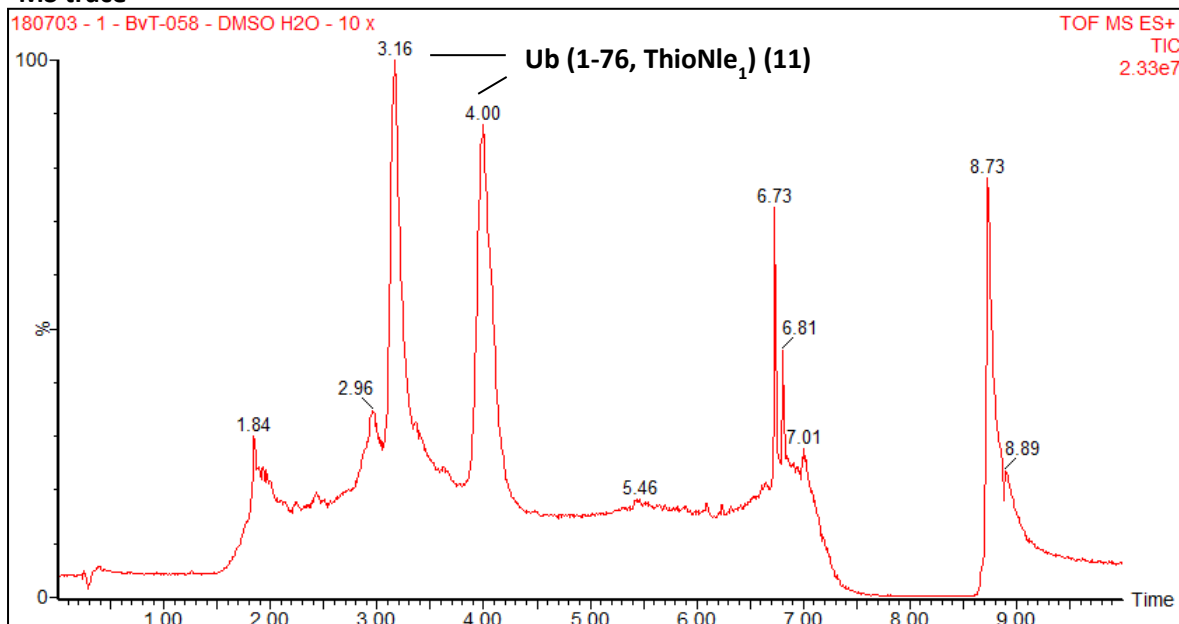
UV trace



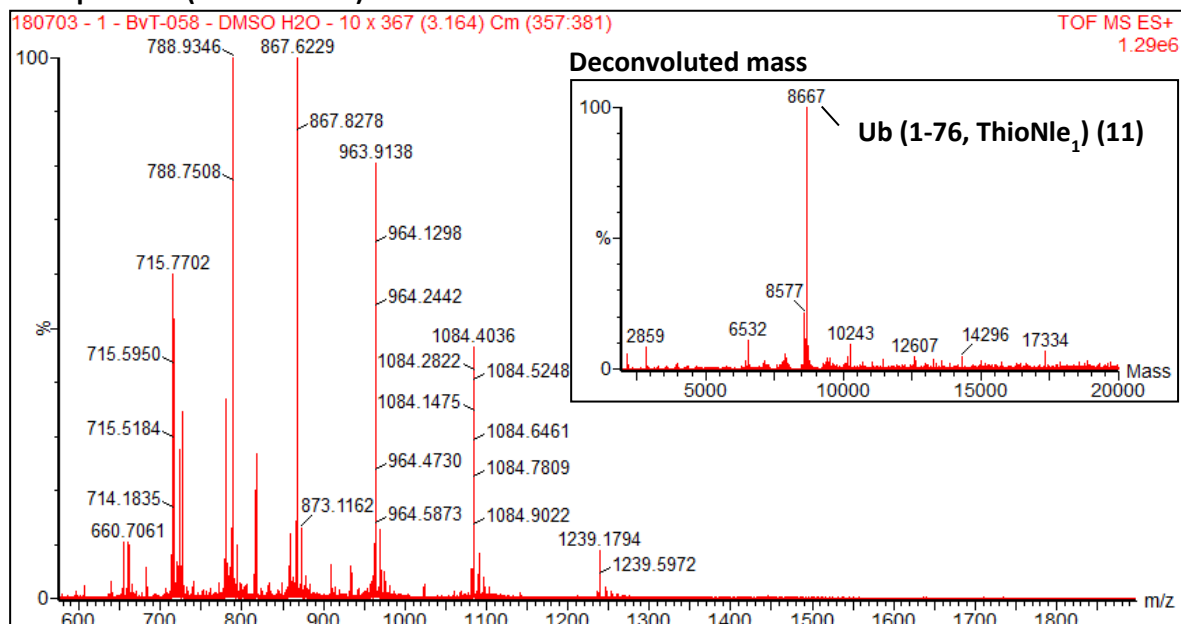
MS spectrum (Rt = 4.64 min)



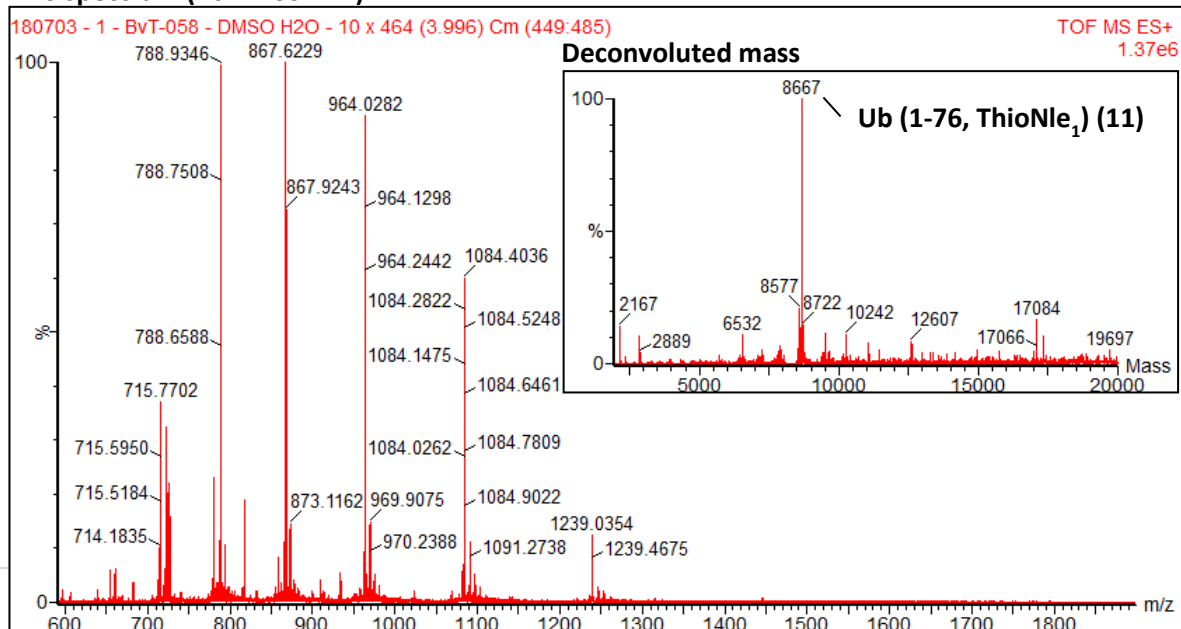
MS trace



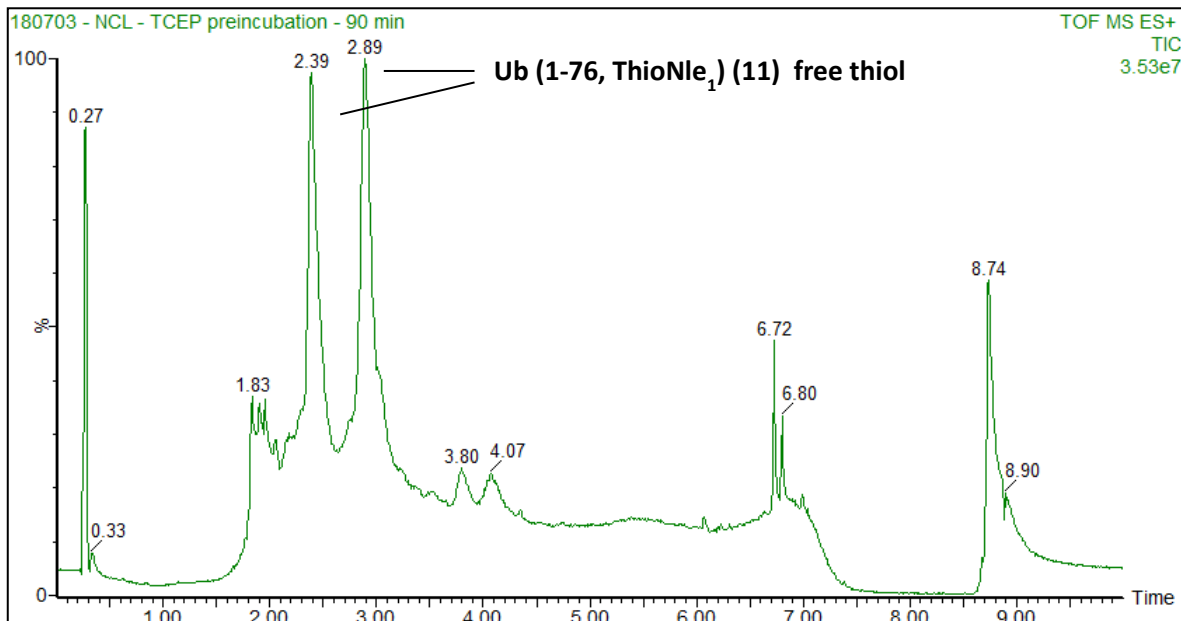
MS spectrum (Rt = 3.16 min)



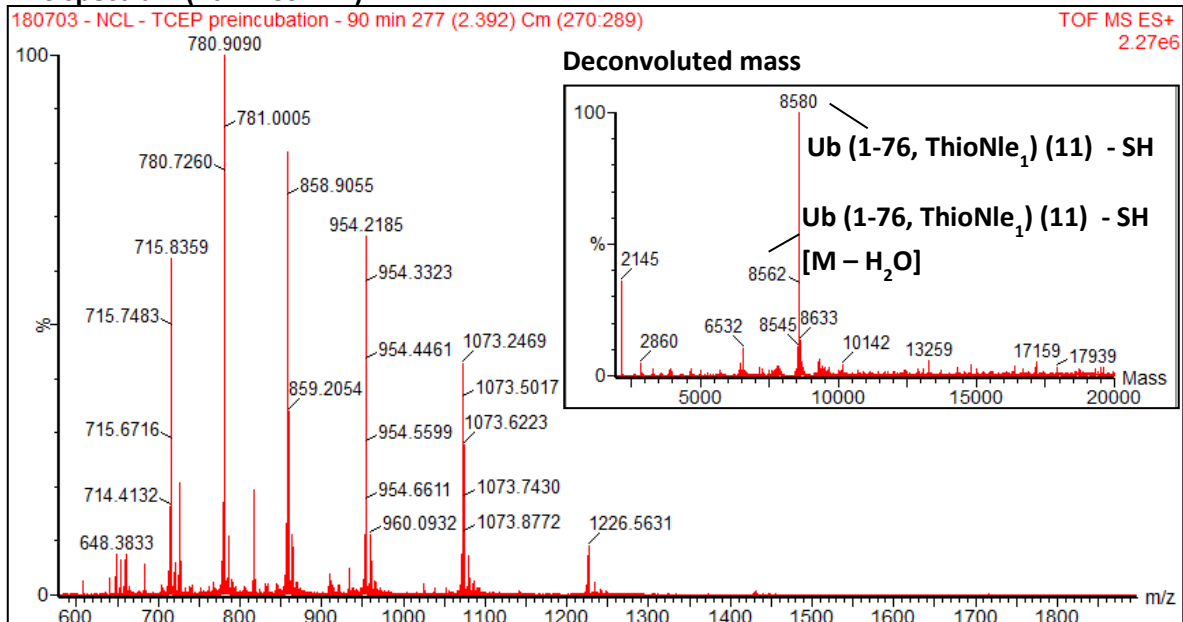
MS spectrum (Rt = 4.00 min)



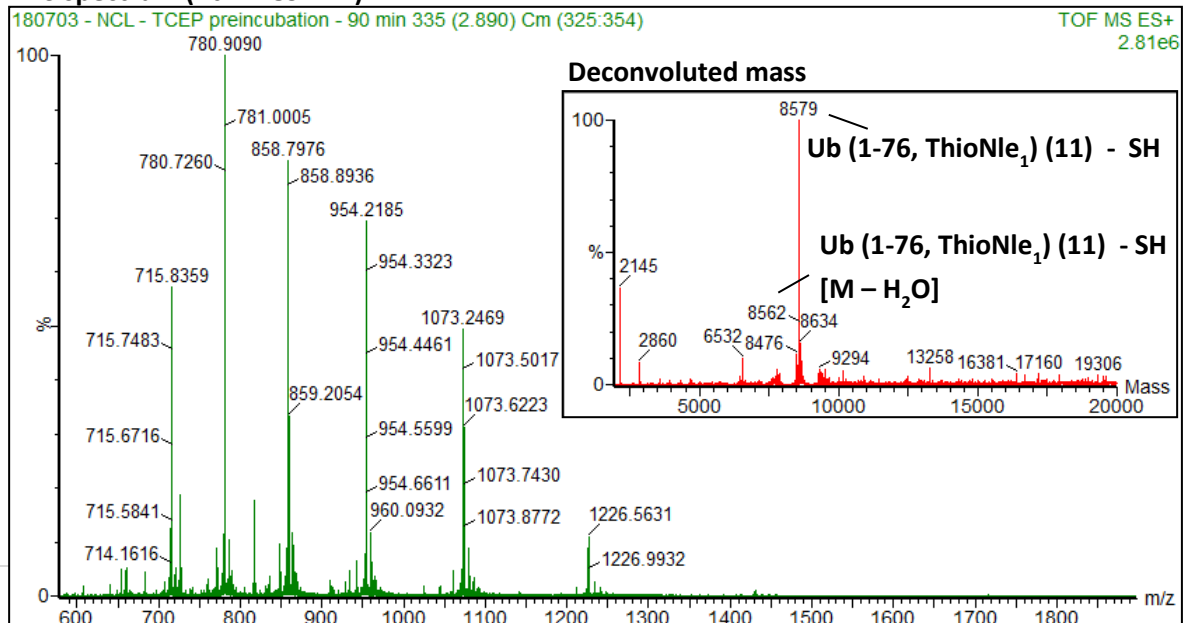
MS trace



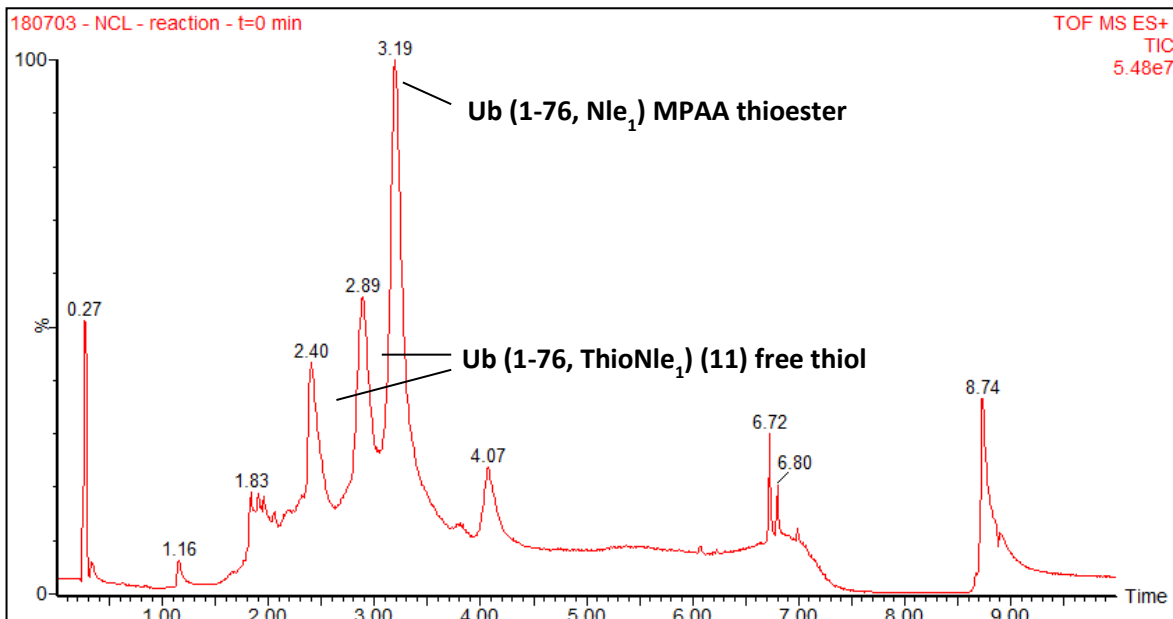
MS spectrum (Rt = 2.39 min)



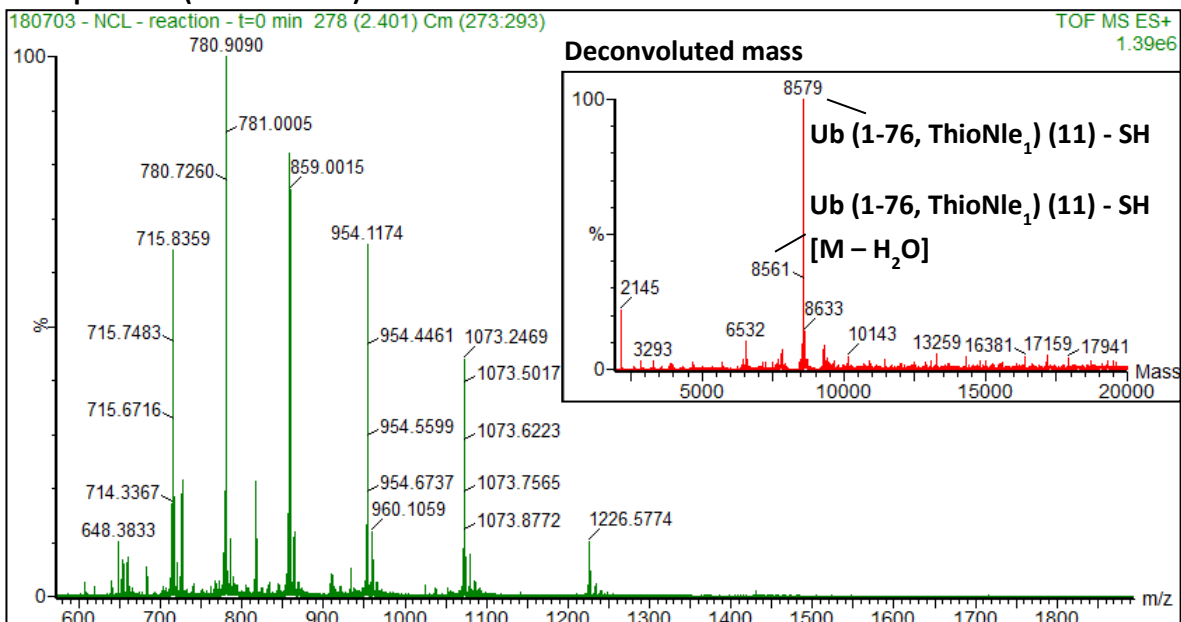
MS spectrum (Rt = 2.89 min)



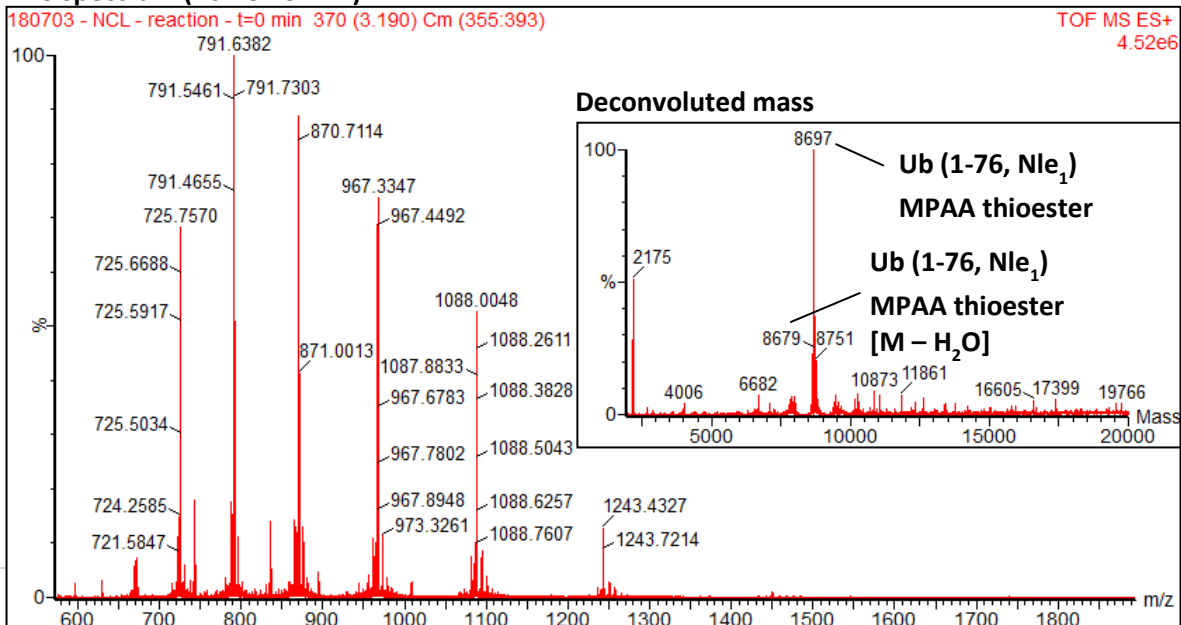
MS trace



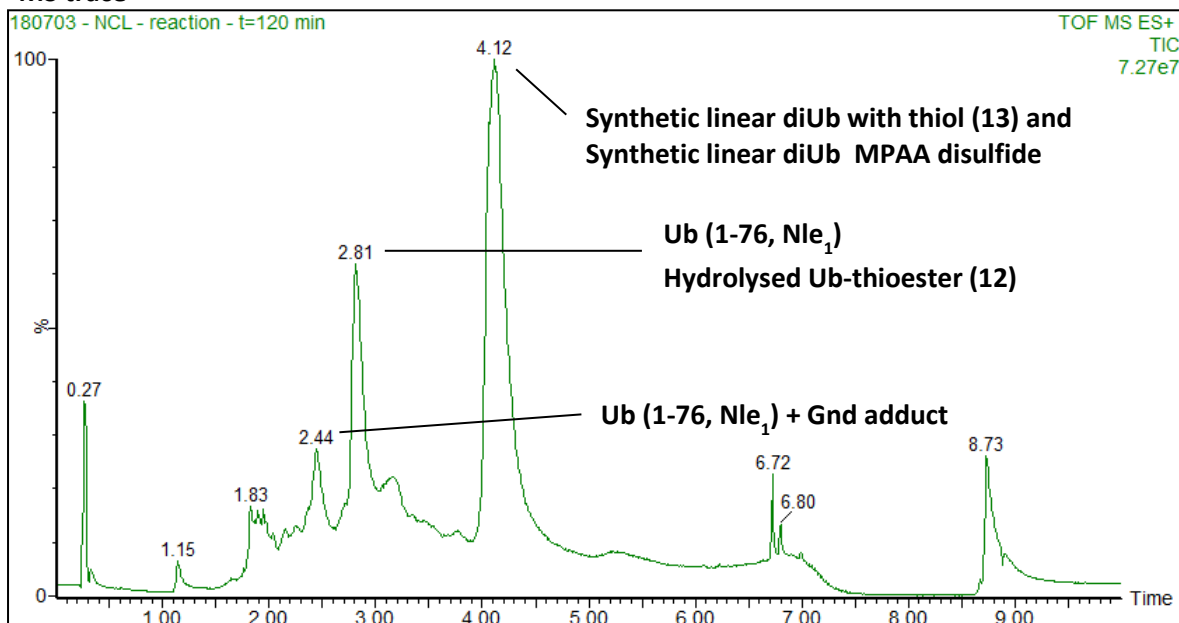
MS spectrum (Rt = 2.40 min)



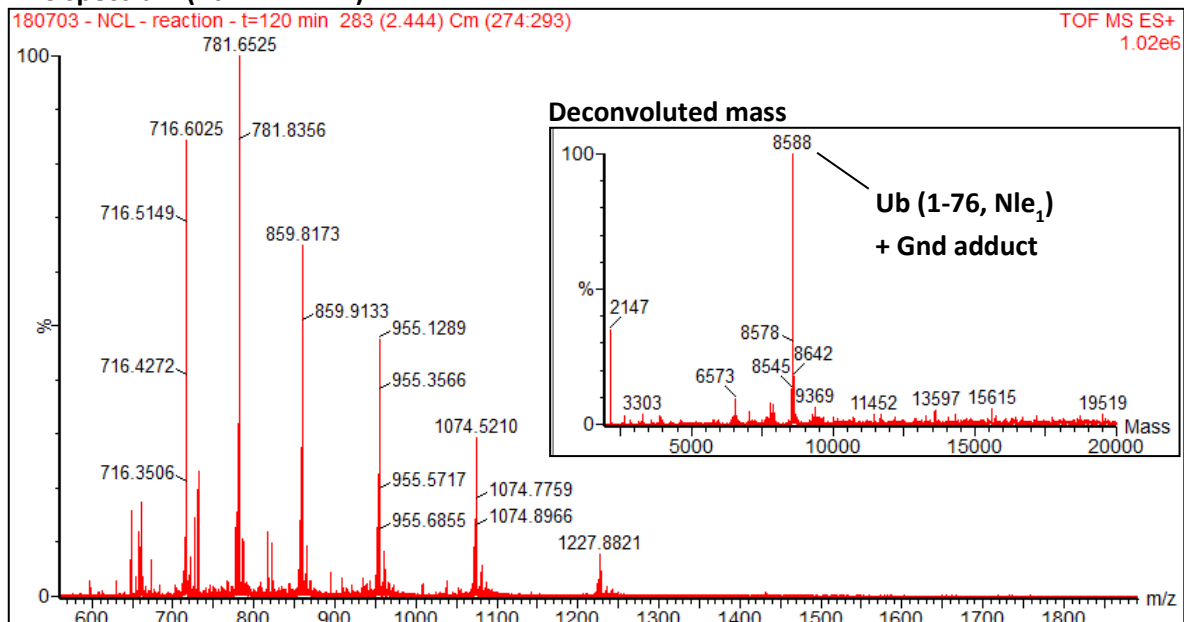
MS spectrum (Rt = 3.19 min)



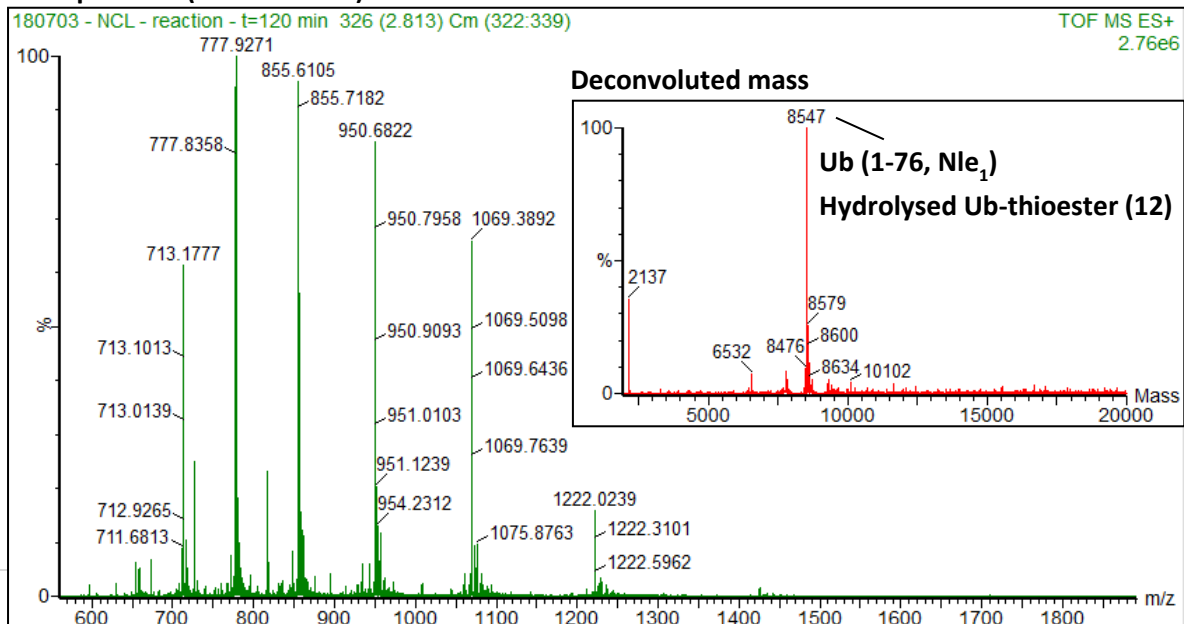
MS trace



MS spectrum (Rt = 2.44 min)



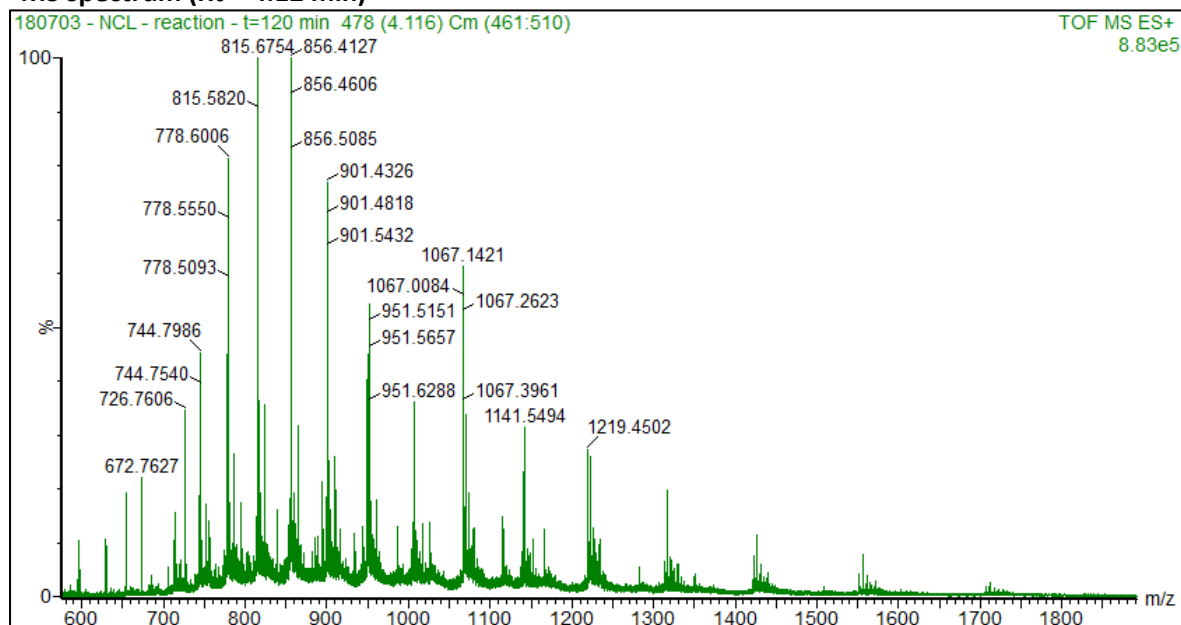
MS spectrum (Rt = 2.81 min)



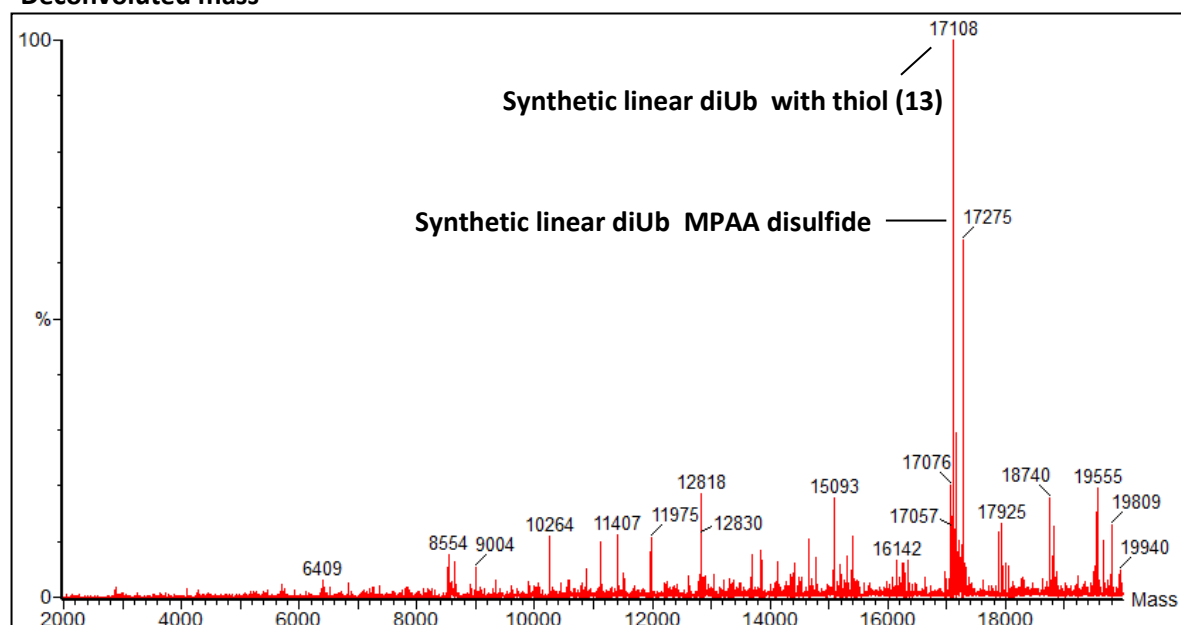
Native Chemical Ligation reaction – t = 120 minutes

XEVO

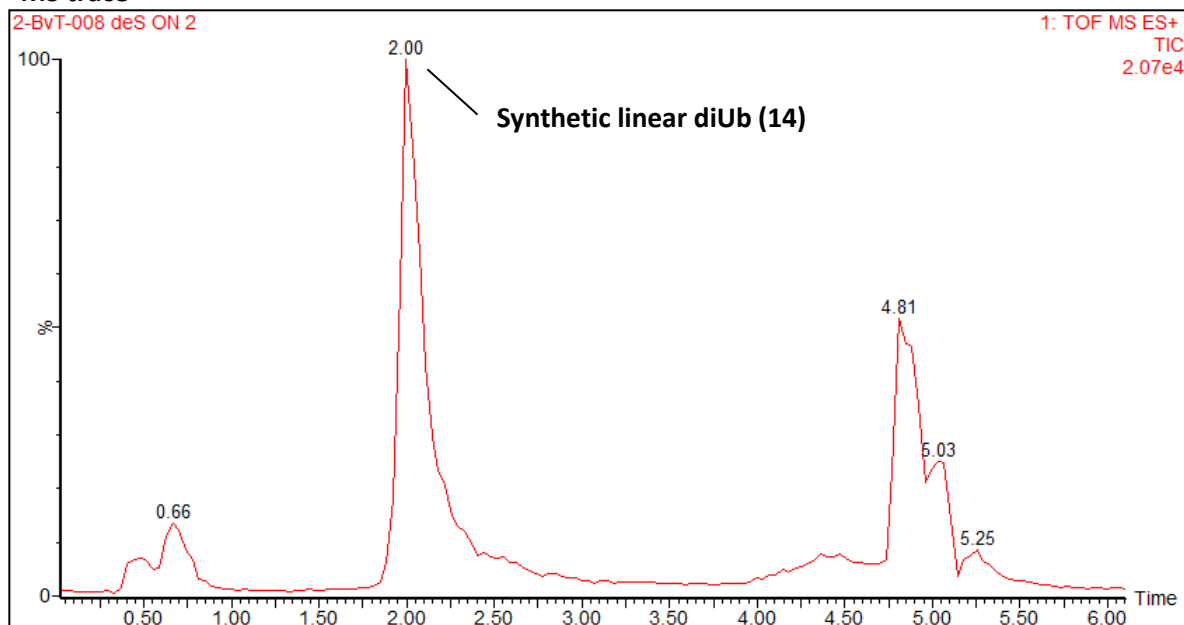
MS spectrum (Rt = 4.12 min)



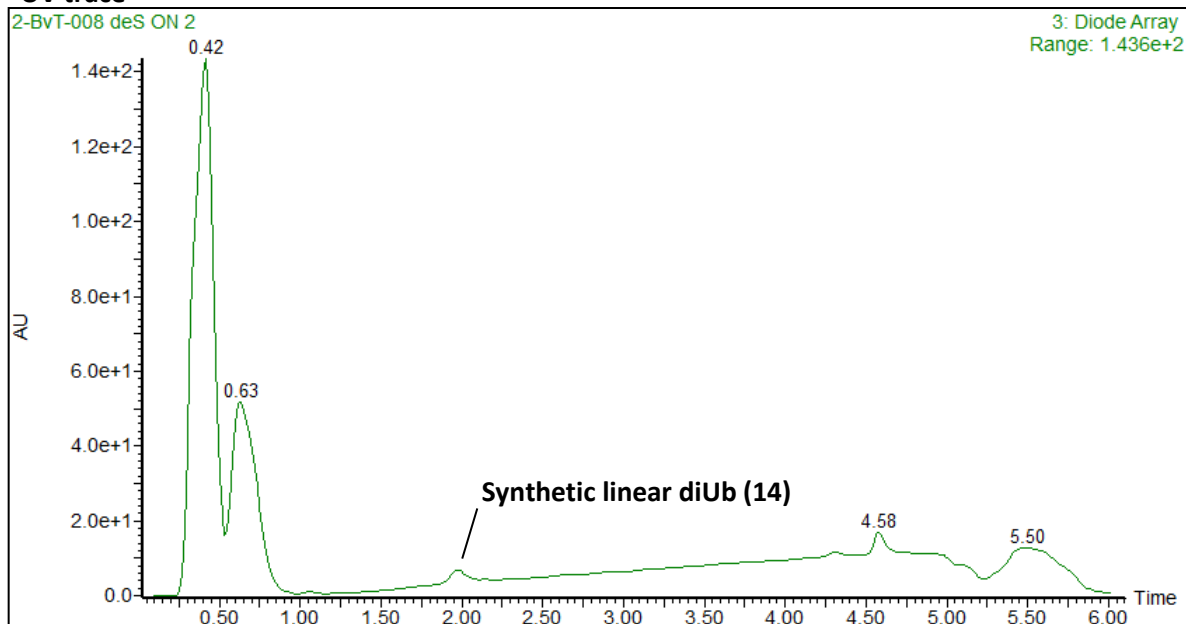
Deconvoluted mass



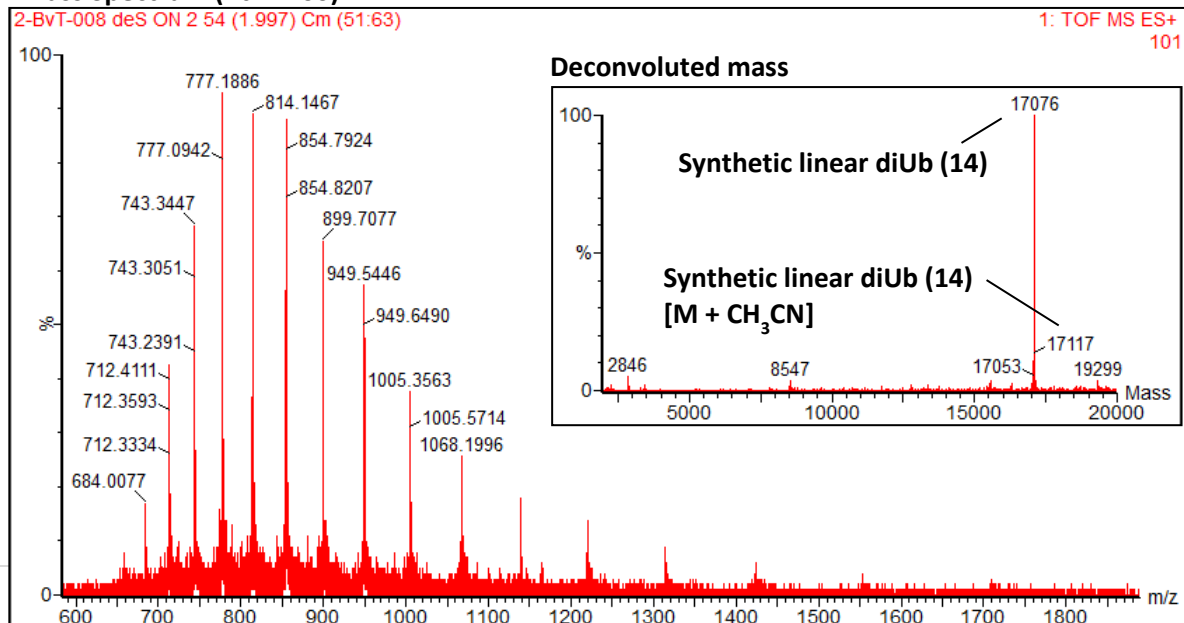
MS trace



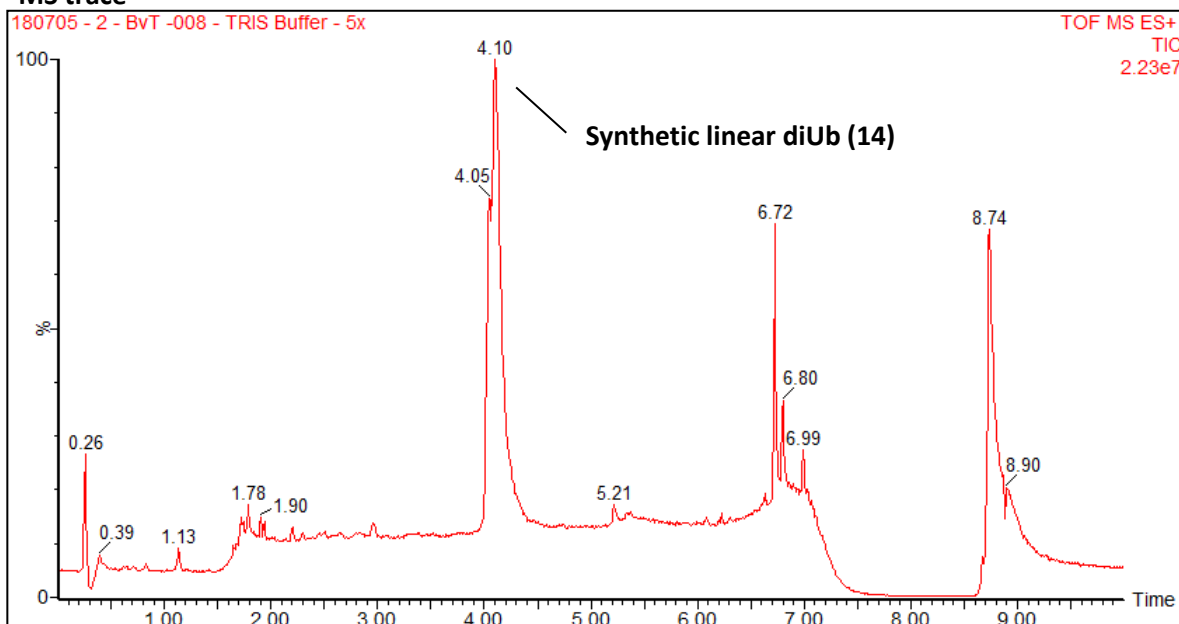
UV trace



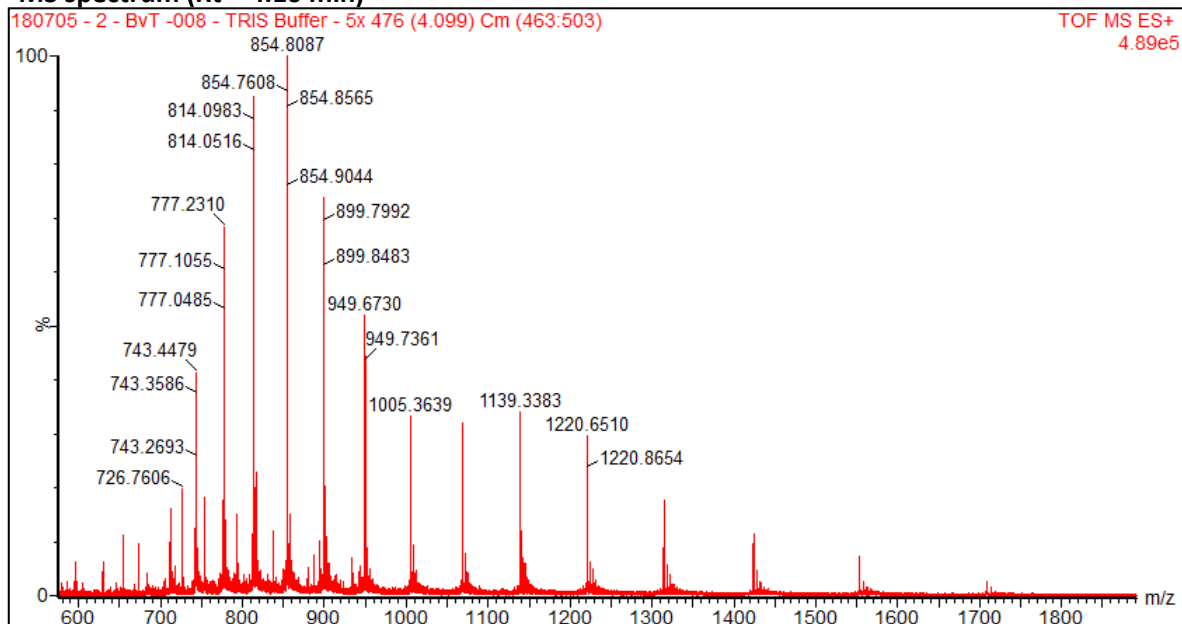
Mass Spectrum (Rt = 2.00)



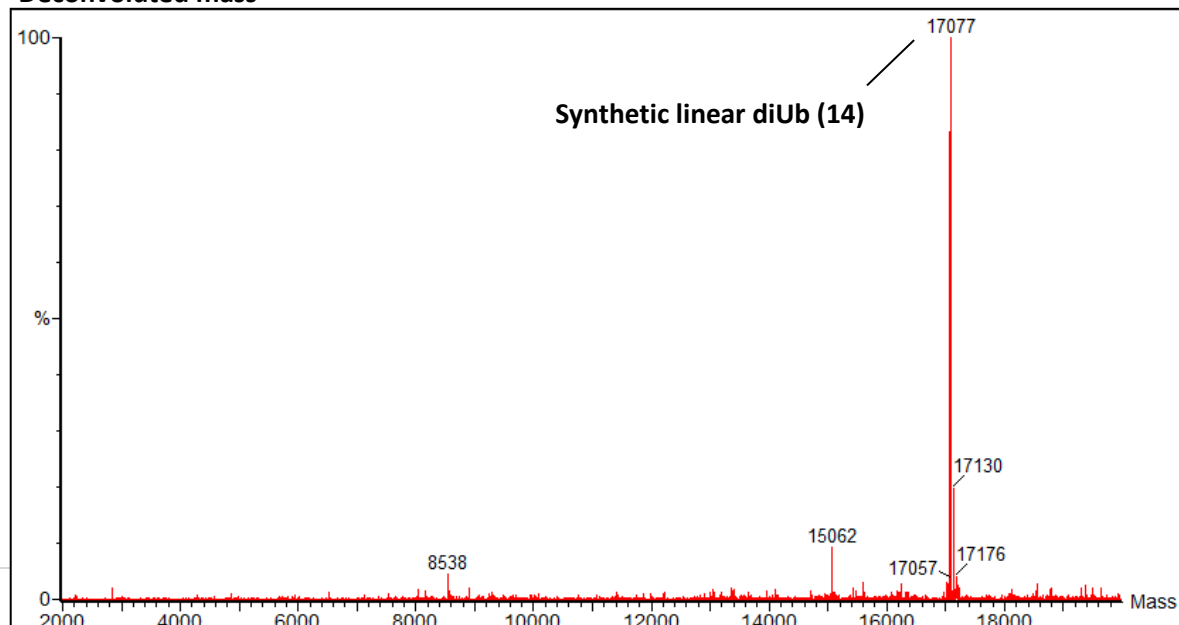
MS trace



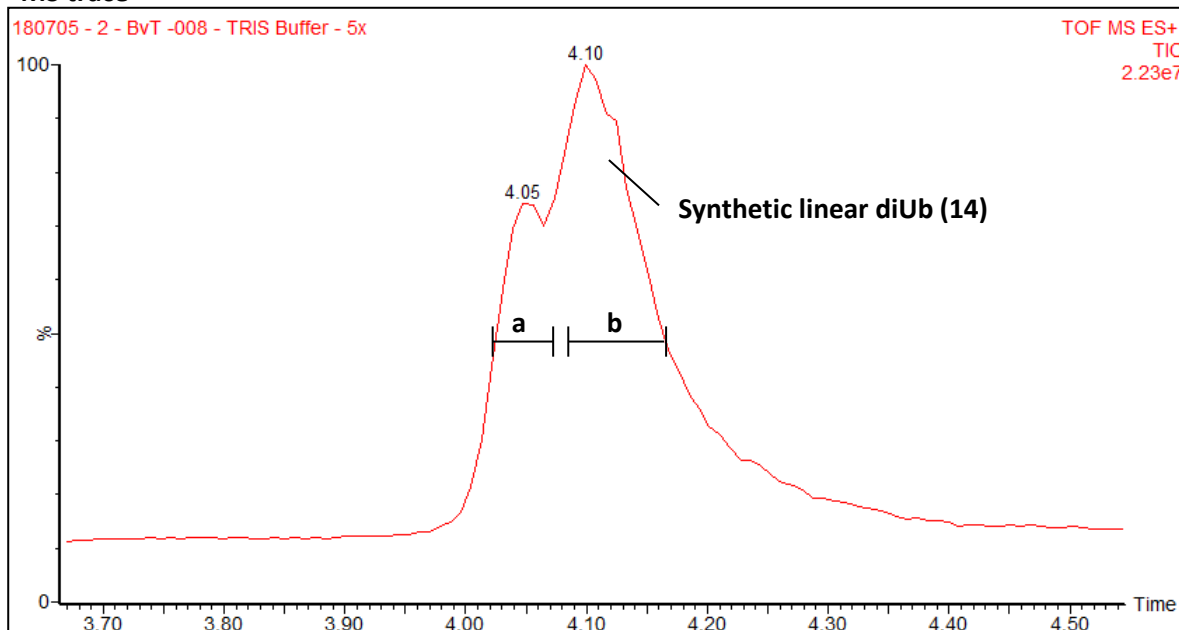
MS spectrum (Rt = 4.10 min)



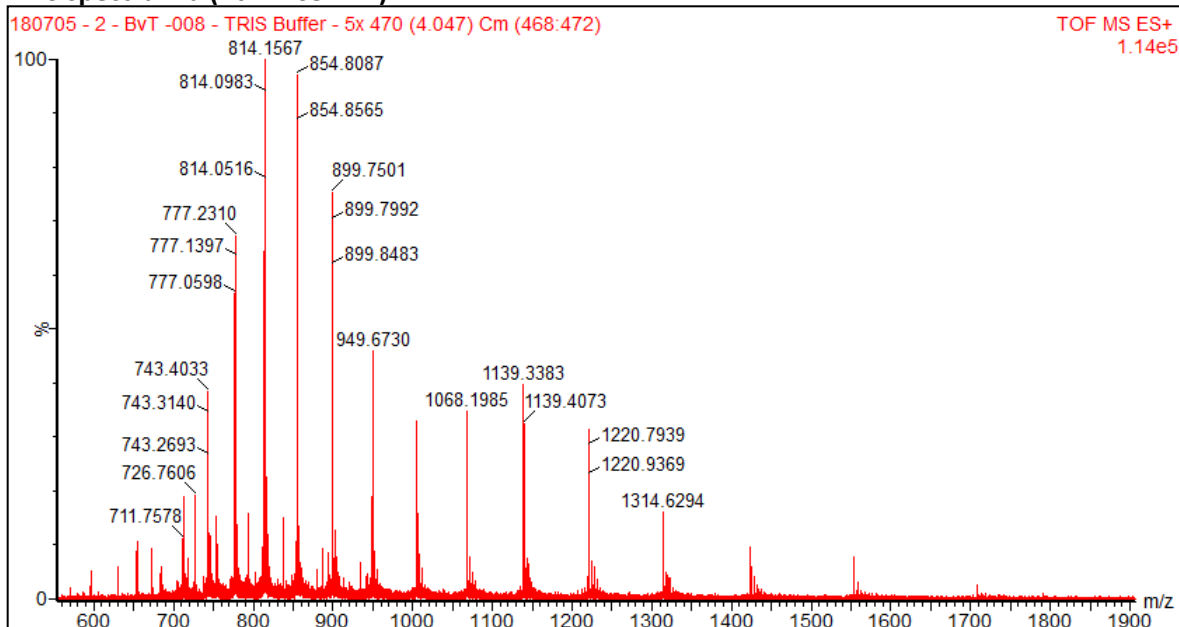
Deconvoluted mass



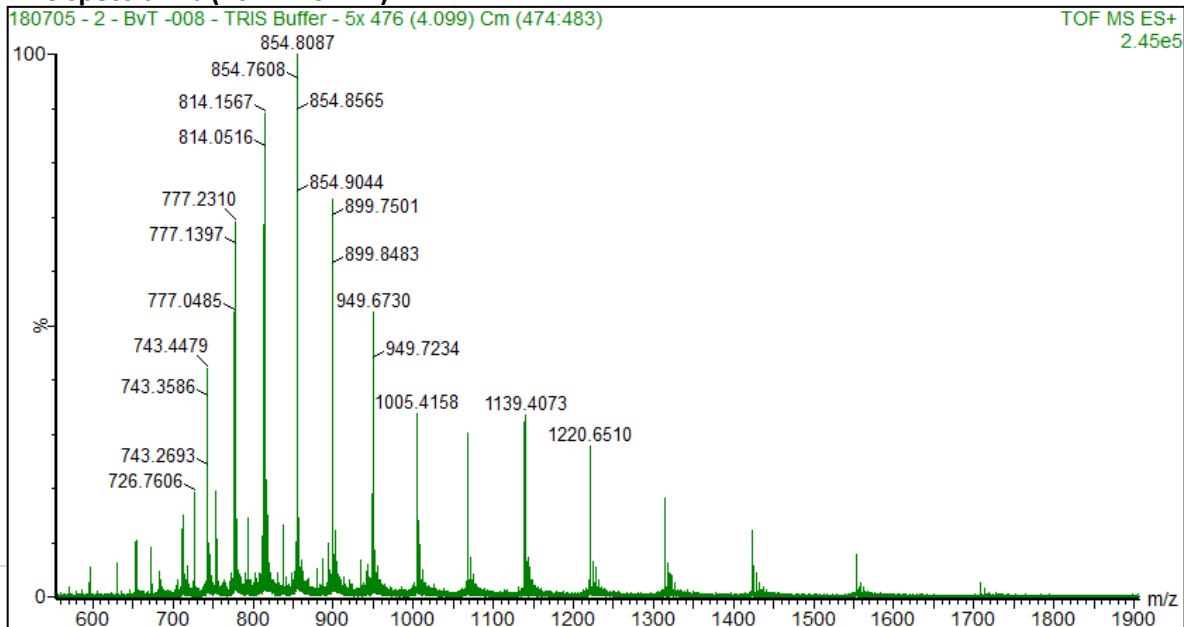
MS trace



MS spectrum a (Rt = 4.05 min)



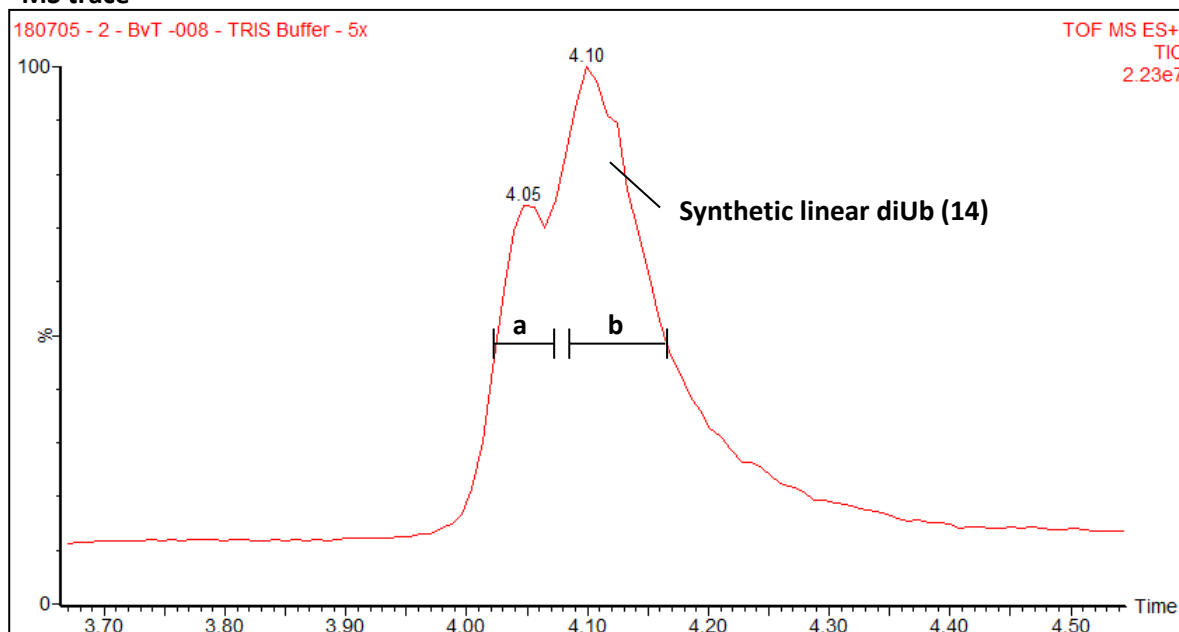
MS spectrum b (Rt = 4.10 min)



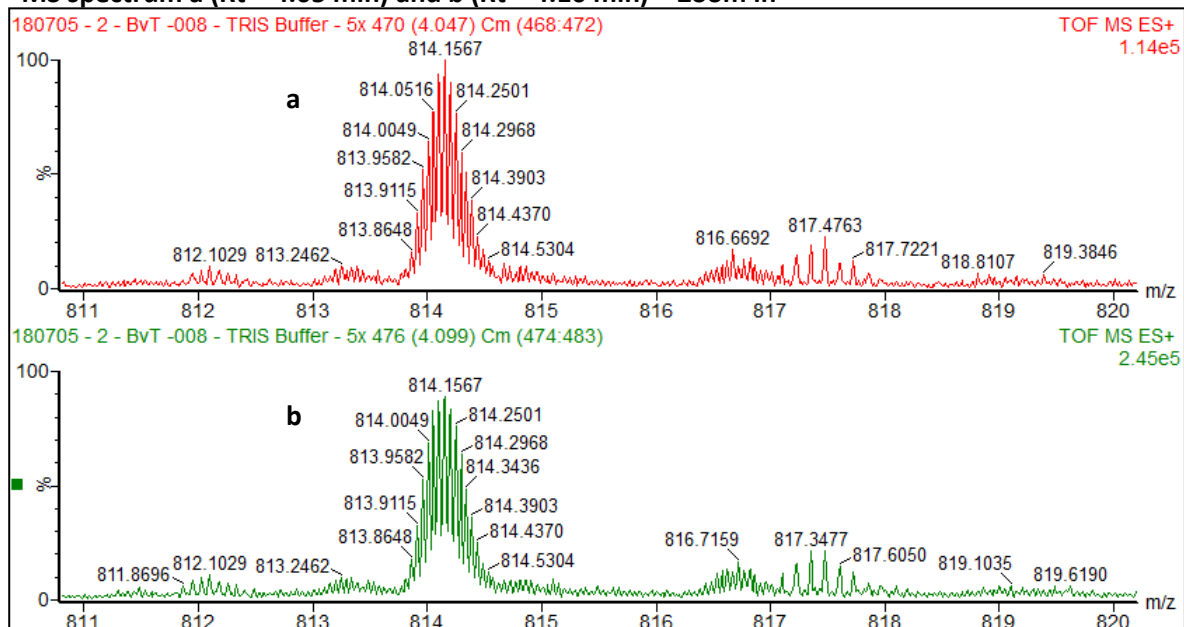
Synthetic linear diUb (14)

XEVO

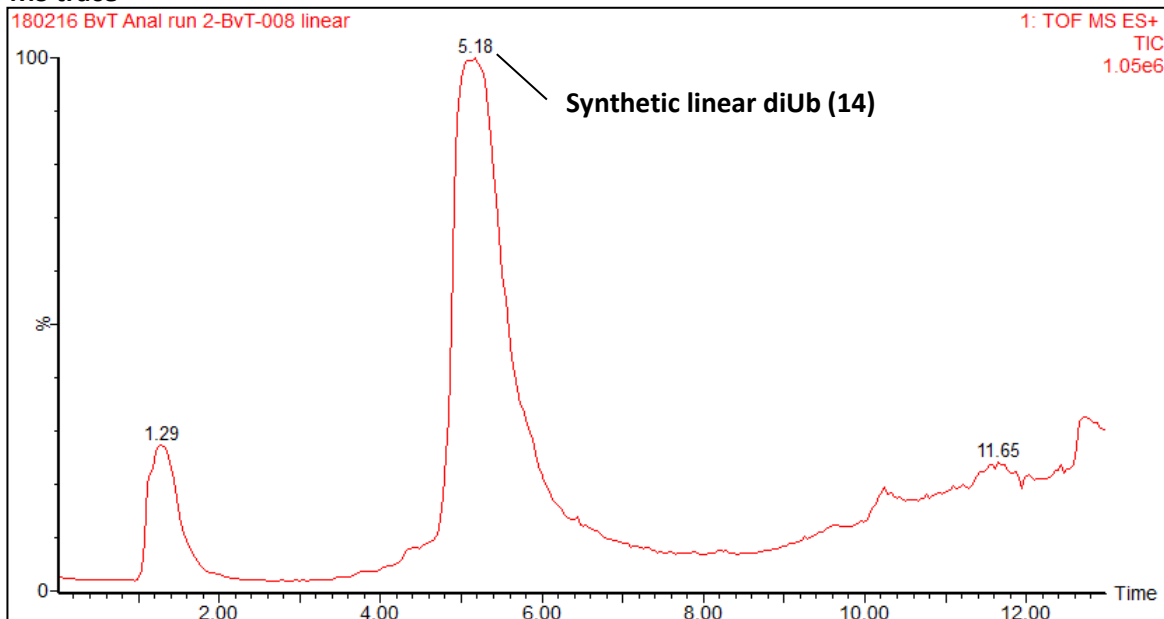
MS trace



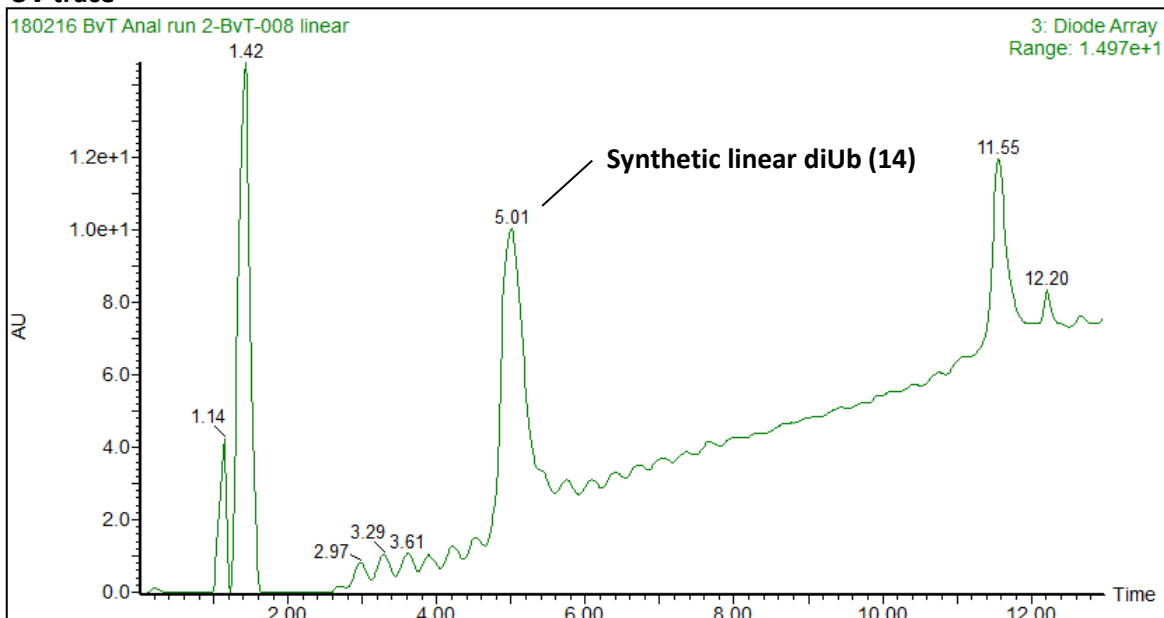
MS spectrum a (Rt = 4.05 min) and b (Rt = 4.10 min) – Zoom in



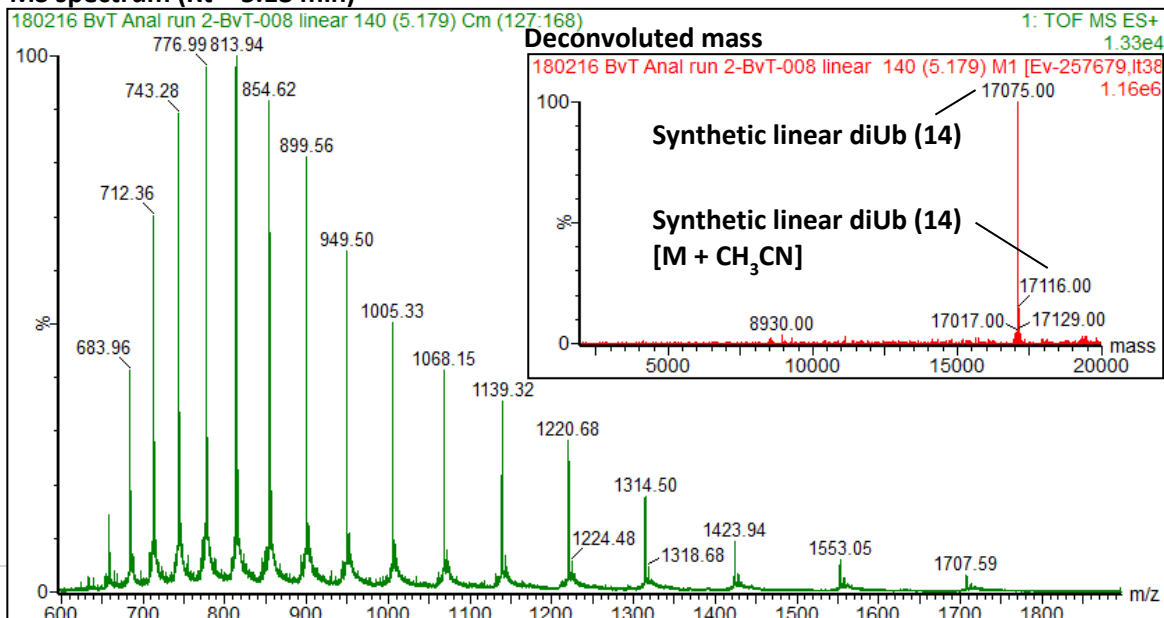
MS trace



UV trace

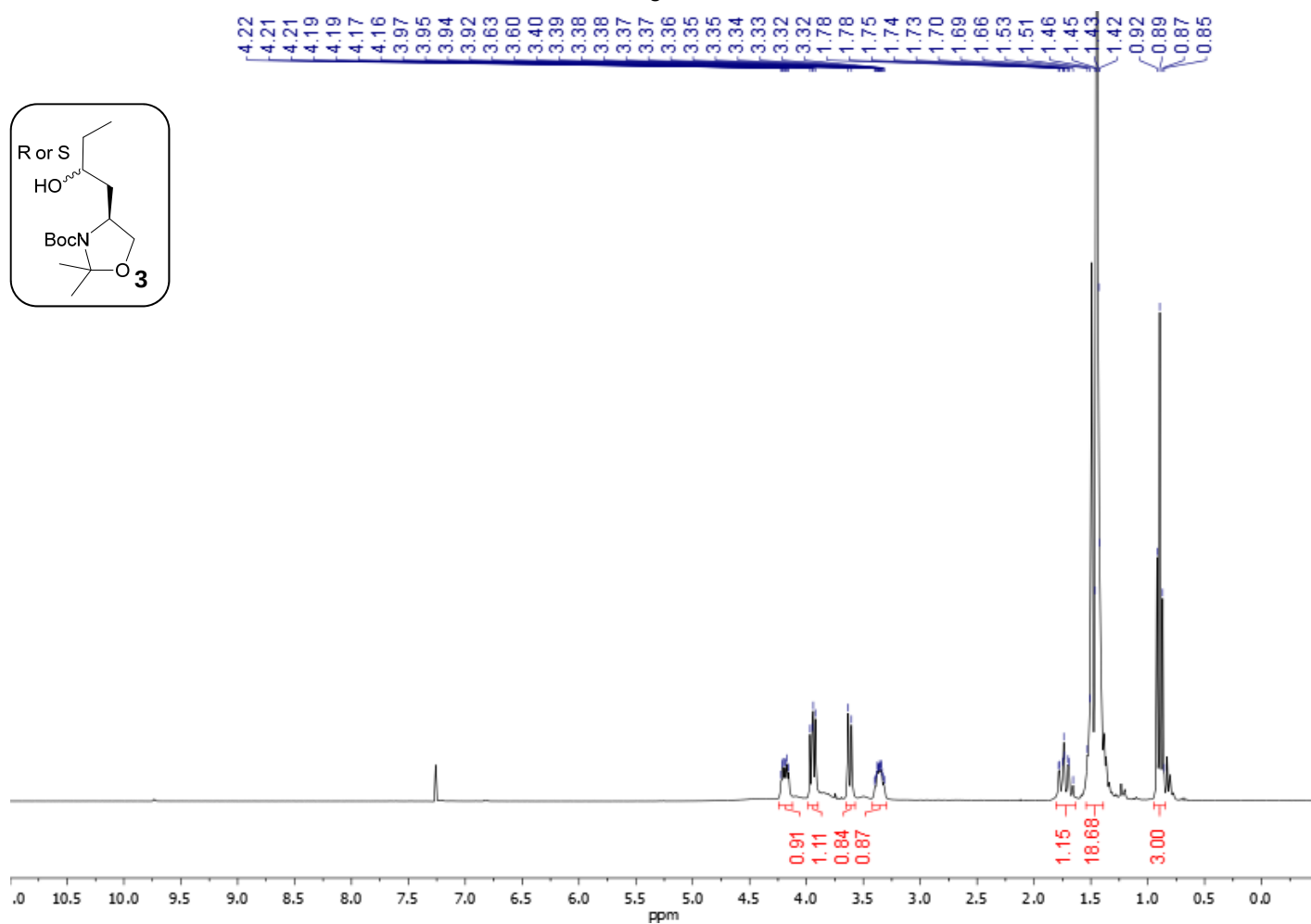
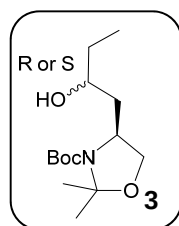


MS spectrum (Rt = 5.18 min)

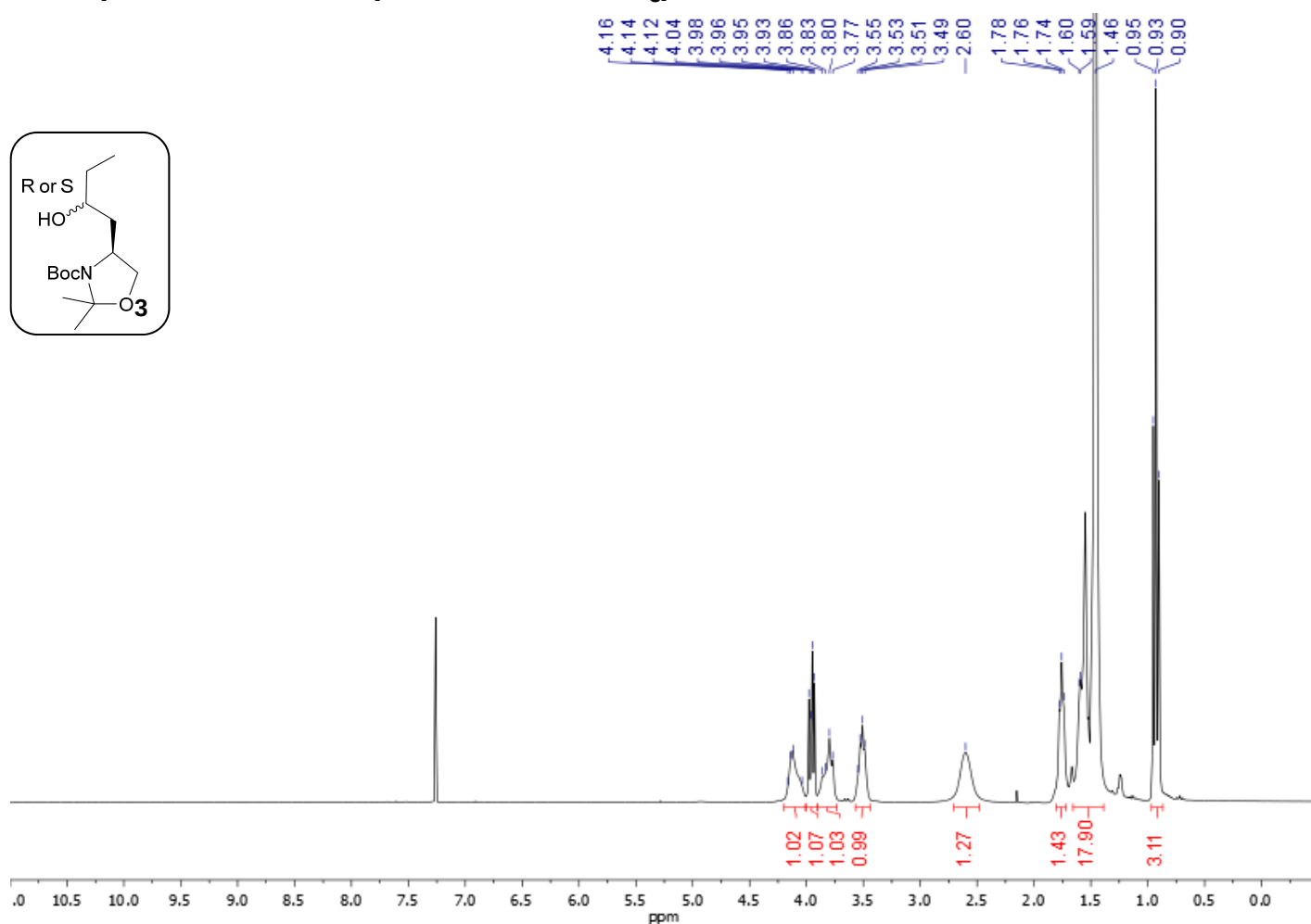
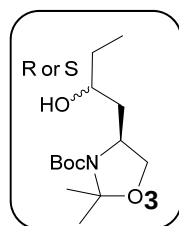


4. NMR spectra of the *N*-Boc, *S*-*tert*-butylsulfide γ -thionorleucine synthesis

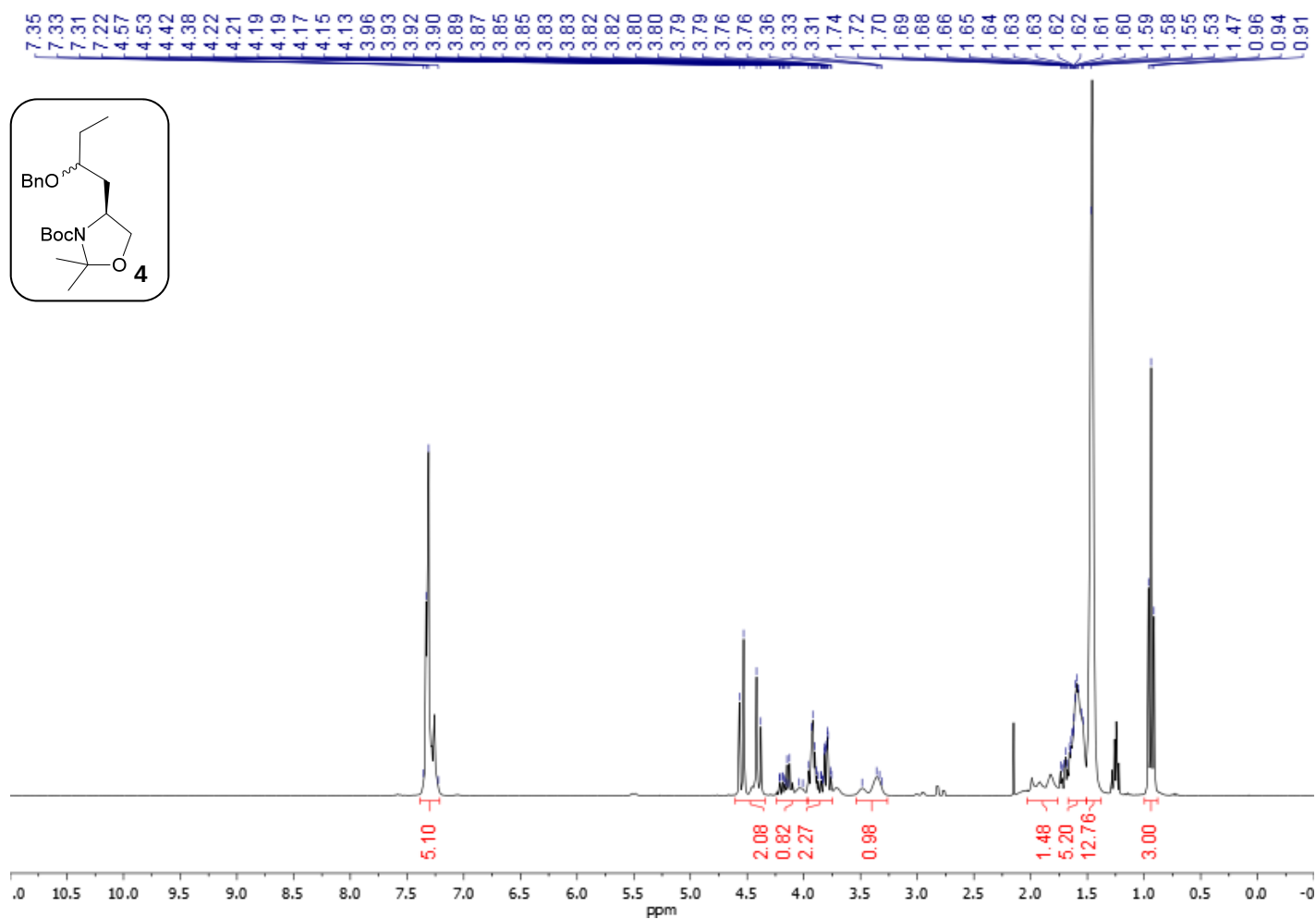
Compound 3: ^1H NMR (300.17 MHz, CDCl_3)



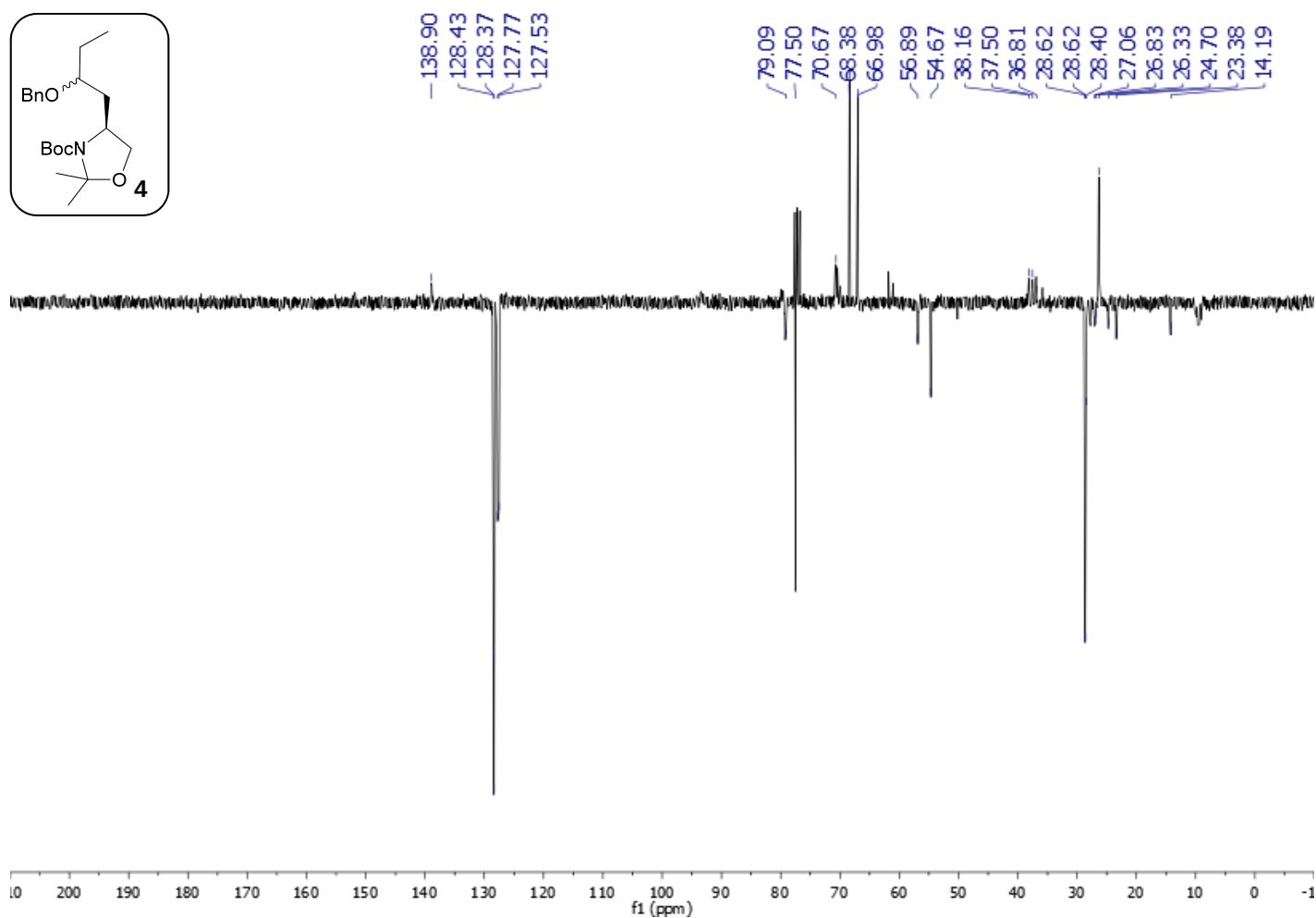
Compound 3: ^1H NMR (300.17 MHz, CDCl_3)



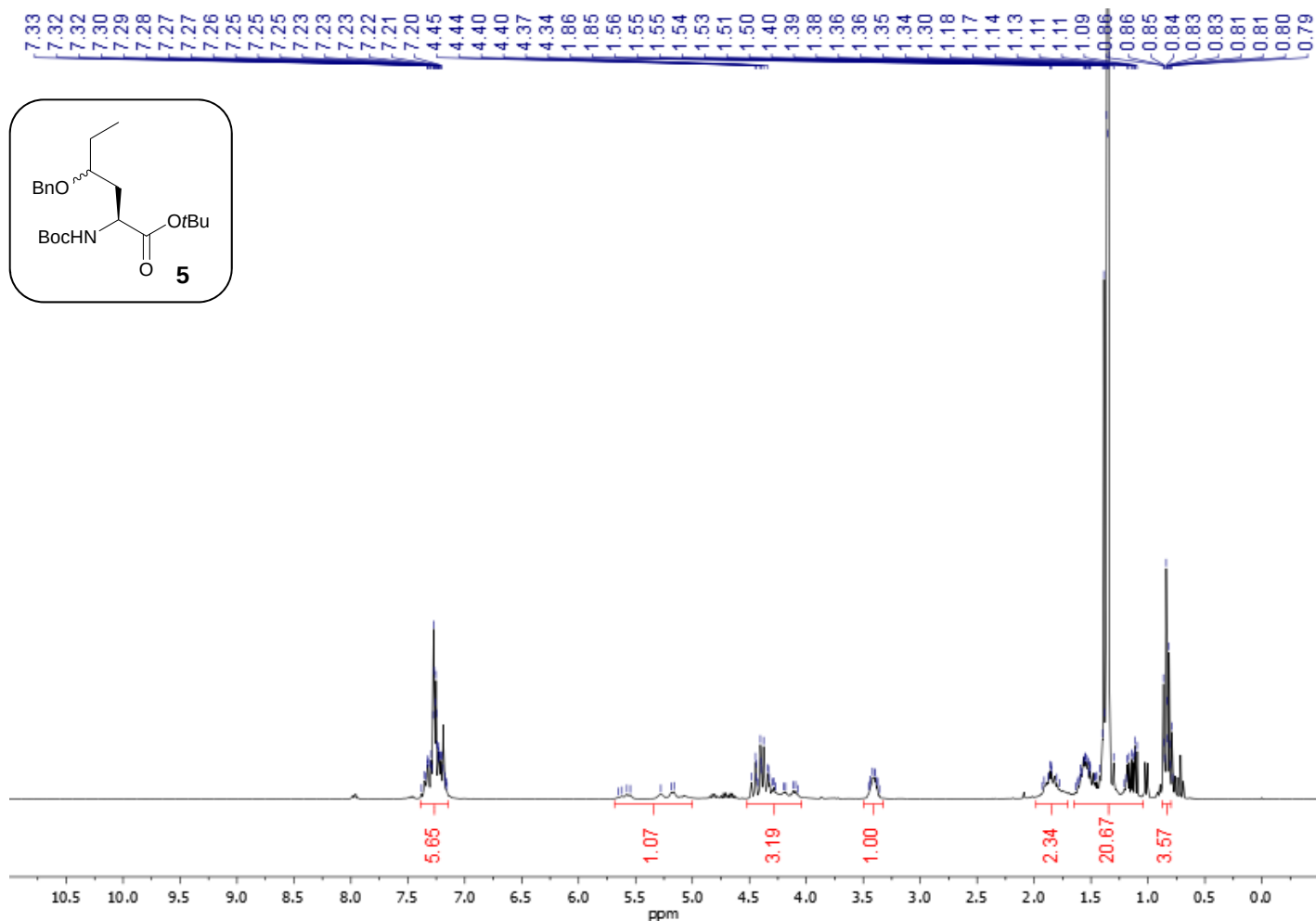
Compound 4: ^1H NMR (300.17 MHz, CDCl_3)



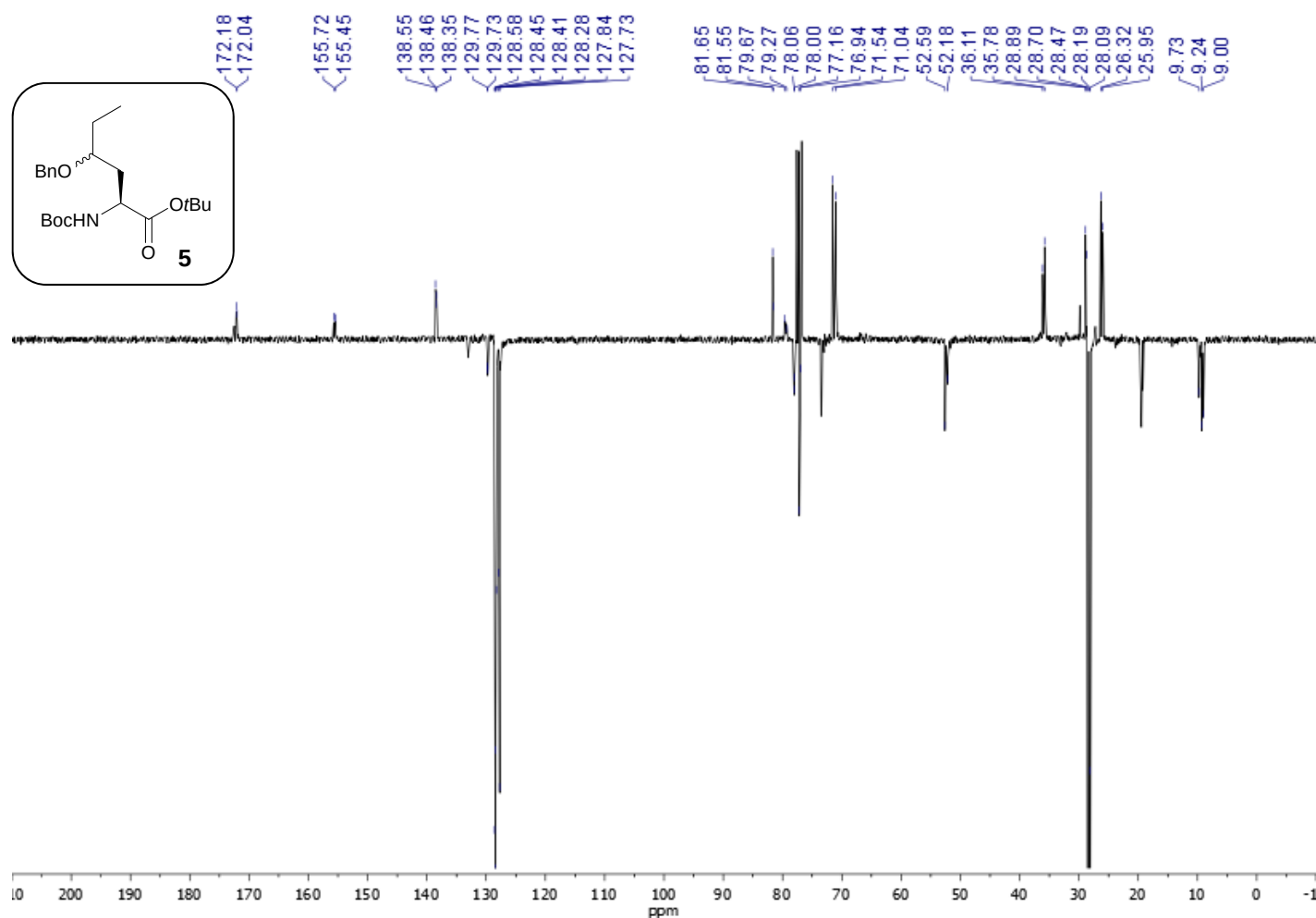
Compound 4: ^{13}C NMR (75.47 MHz, CDCl_3)



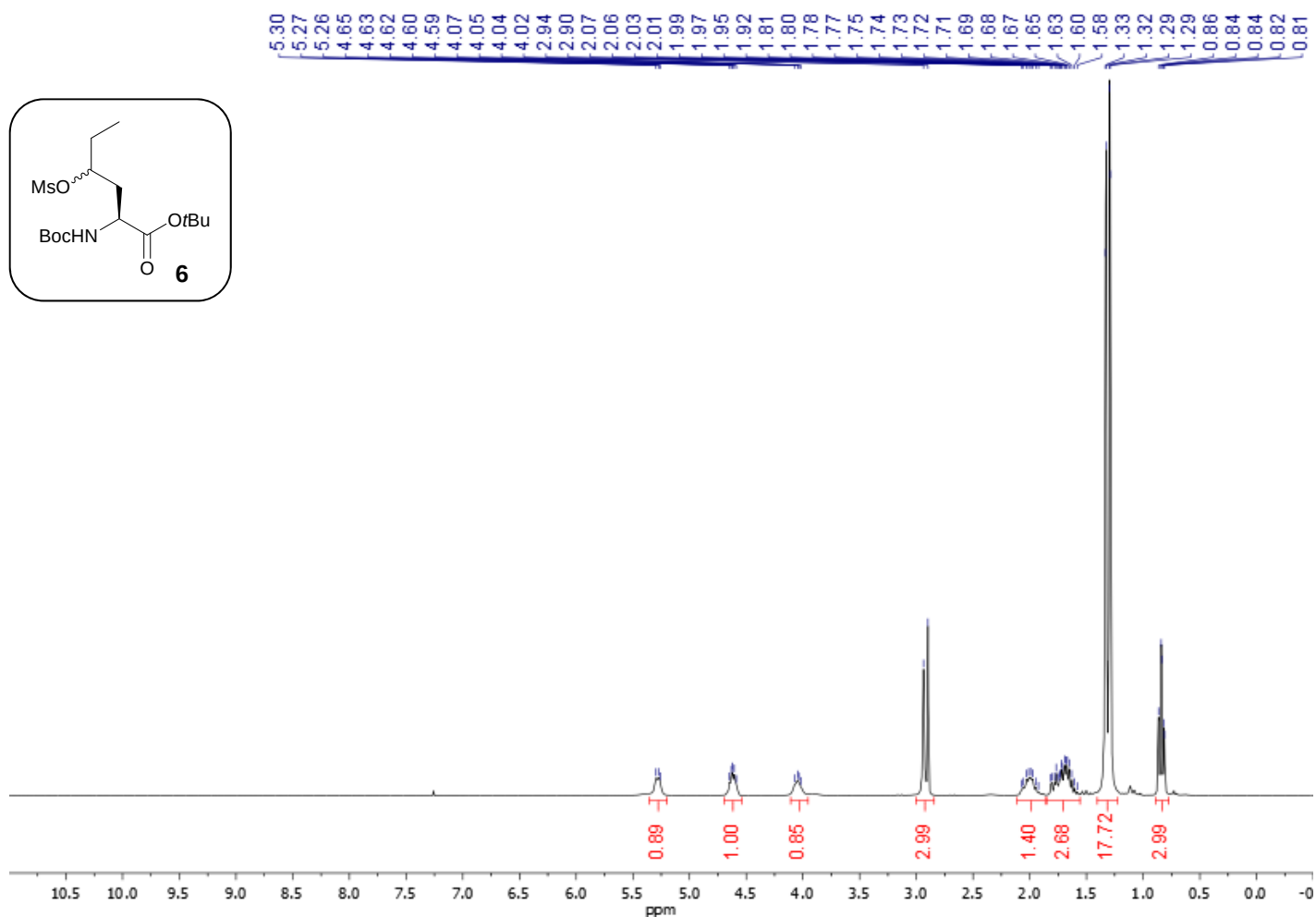
Compound 5: ^1H NMR (300.17 MHz, CDCl_3)



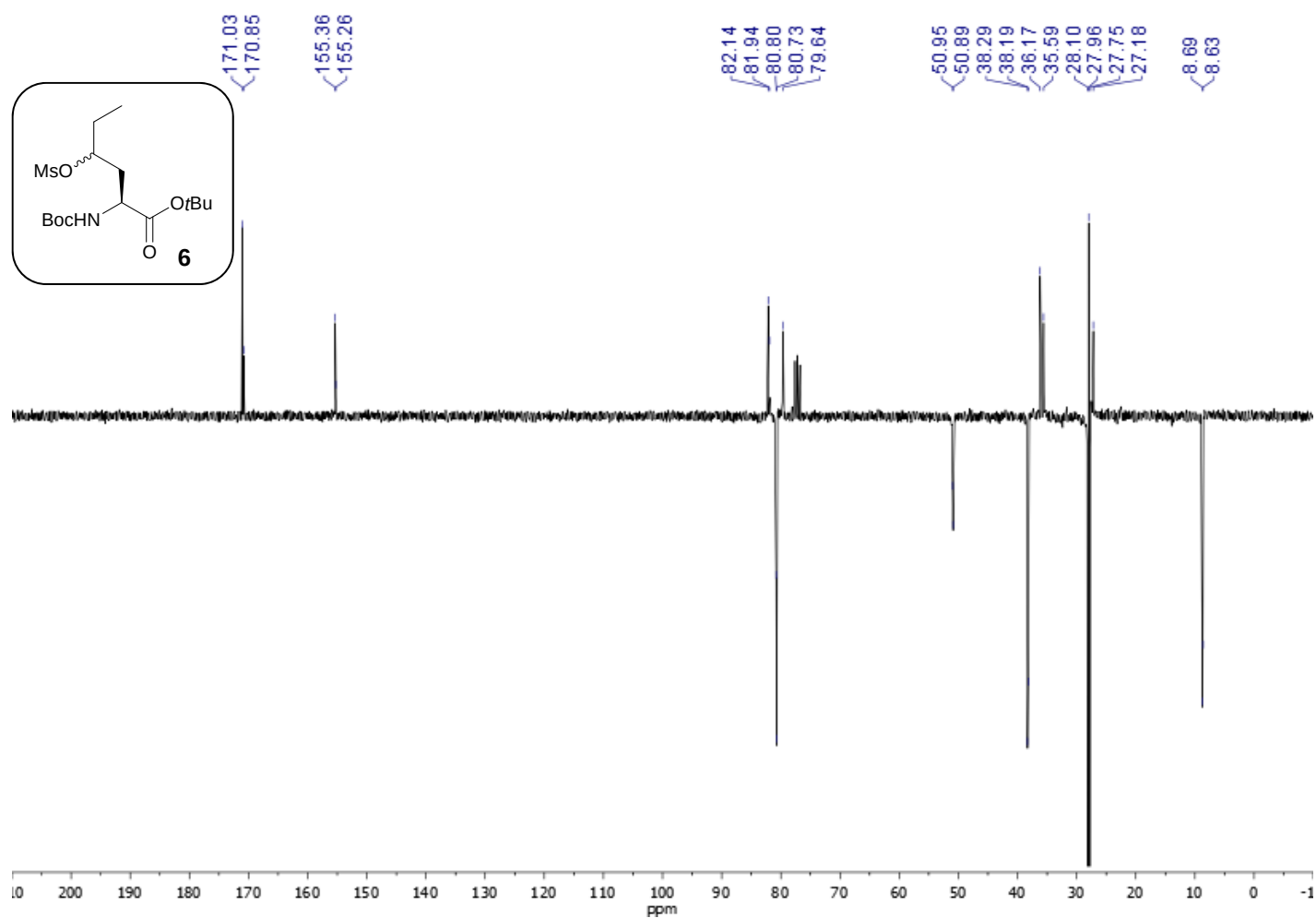
Compound 5: ^{13}C NMR (75.47 MHz, CDCl_3)



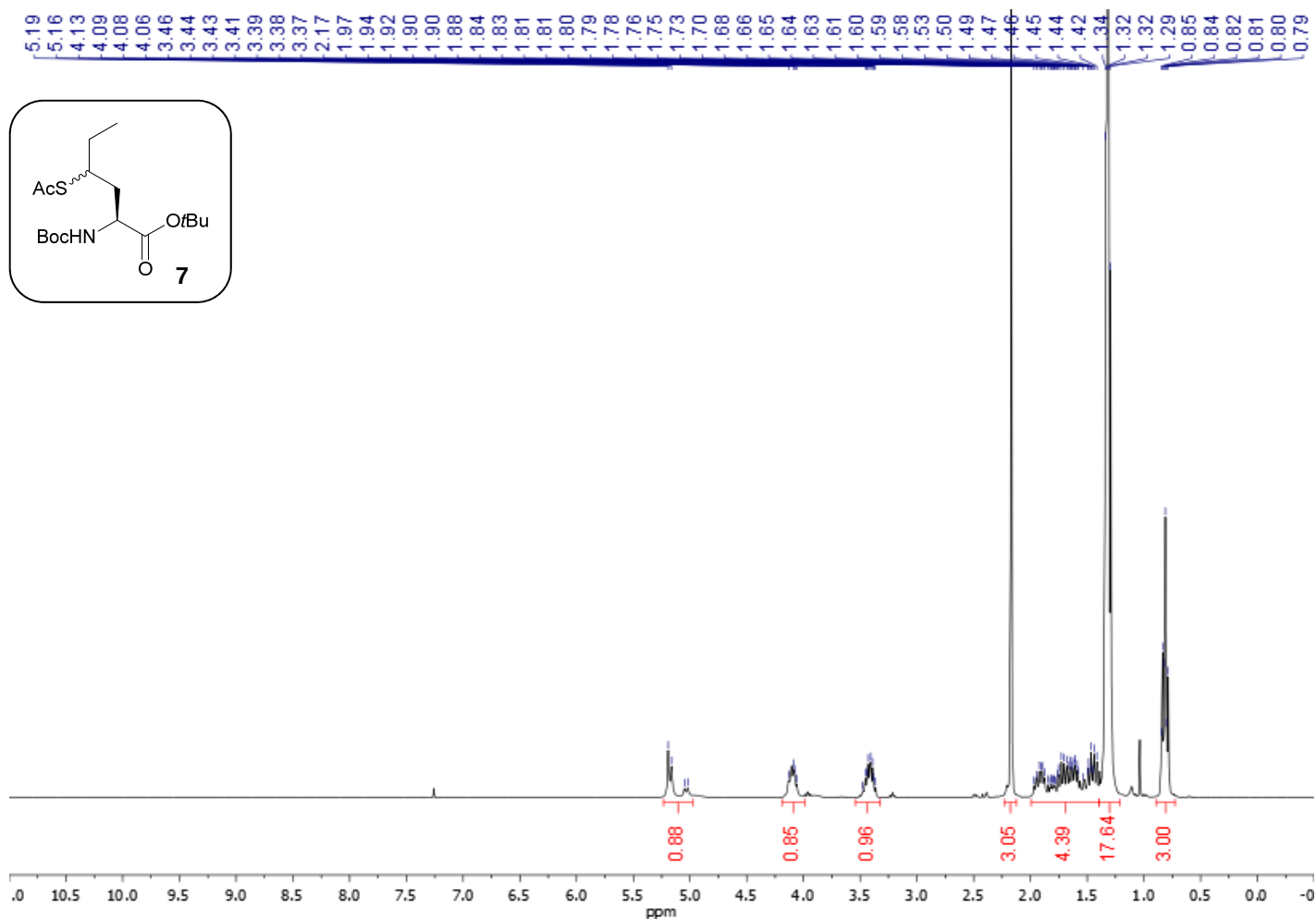
Compound 6: ^1H NMR (300.17 MHz, CDCl_3)



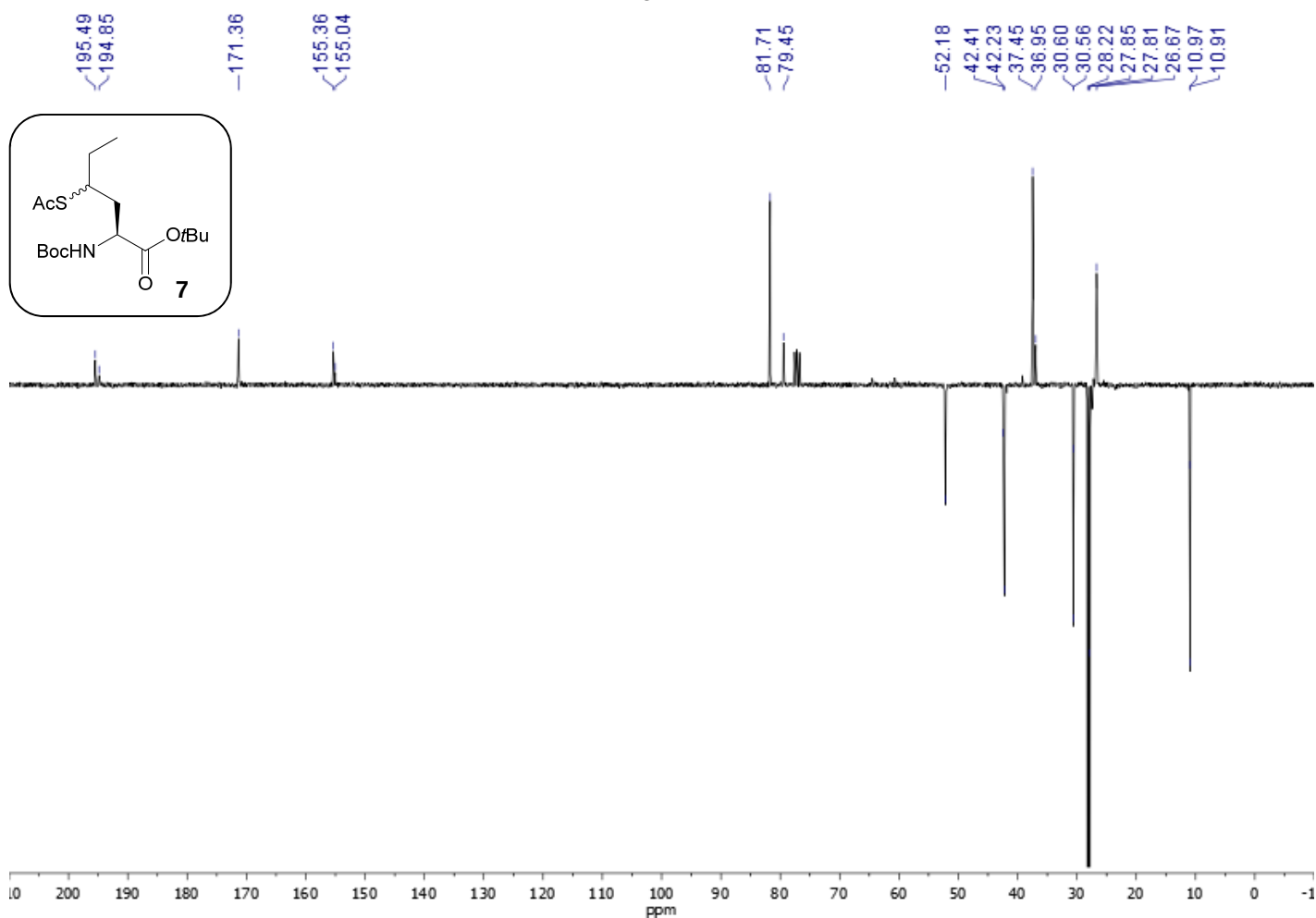
Compound 6: ^{13}C NMR (75.47 MHz, CDCl_3)



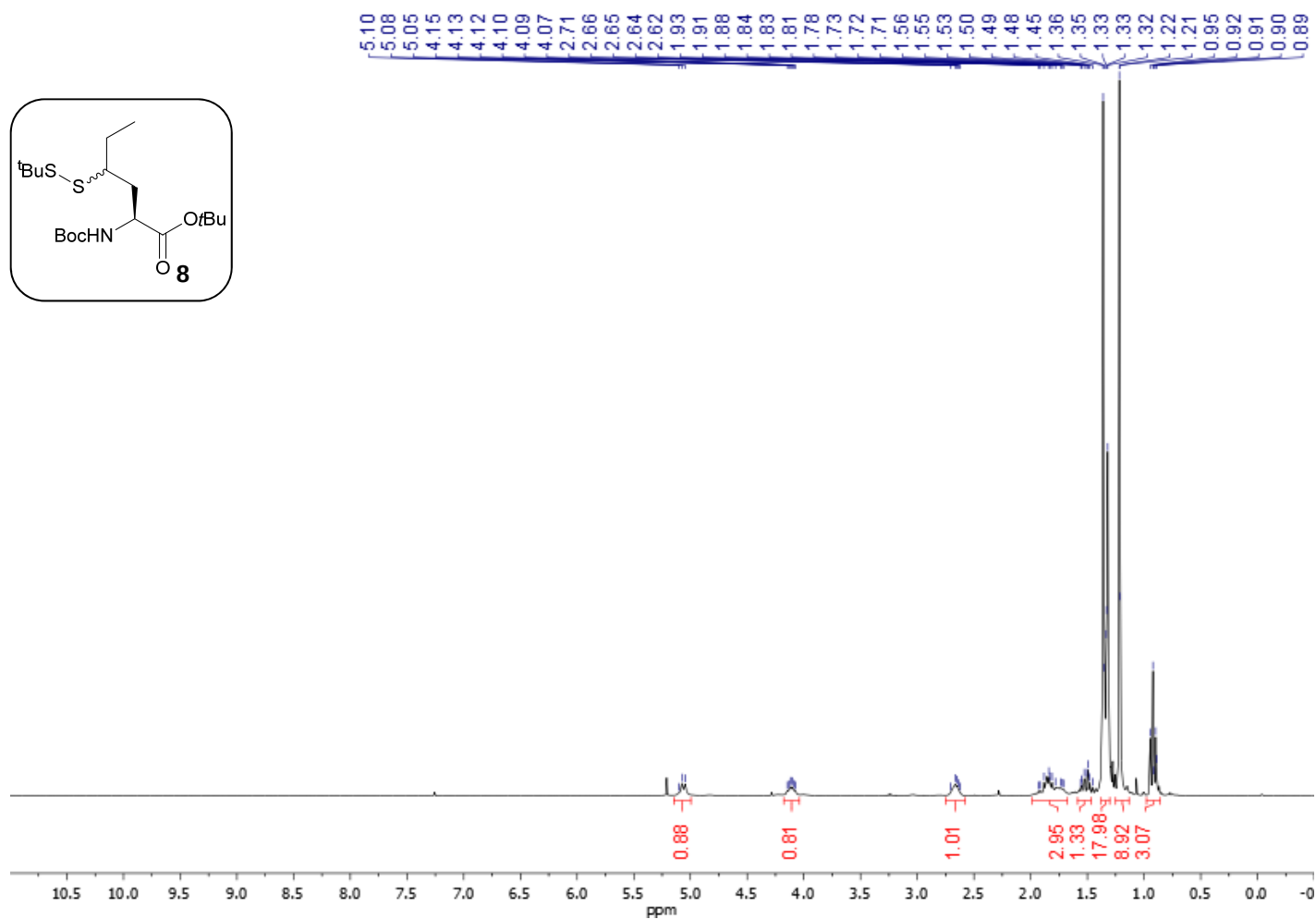
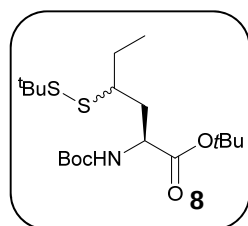
Compound 7: ^1H NMR (300.17 MHz, CDCl_3)



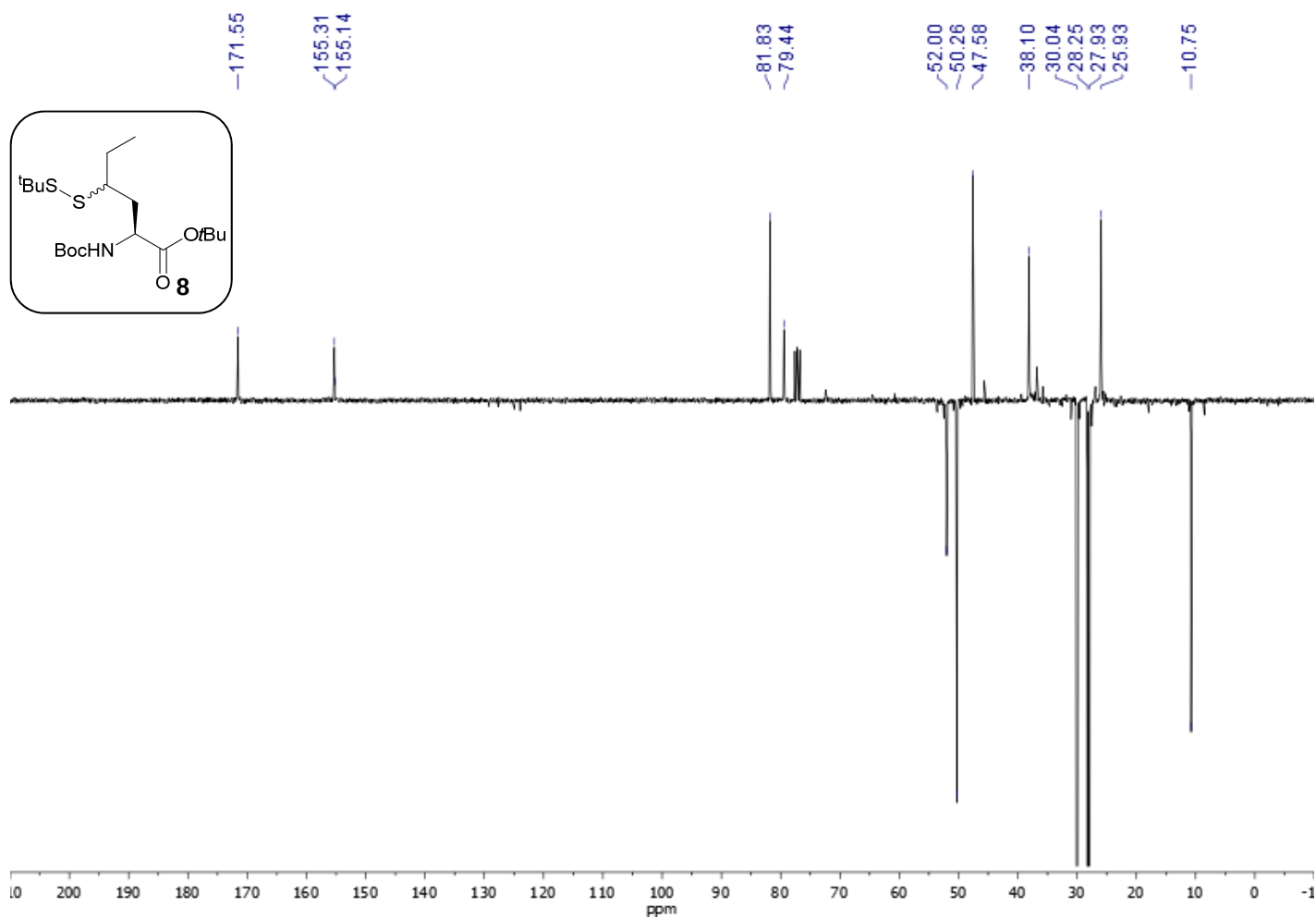
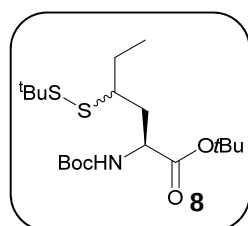
Compound 7: ^{13}C NMR (75.47 MHz, CDCl_3)



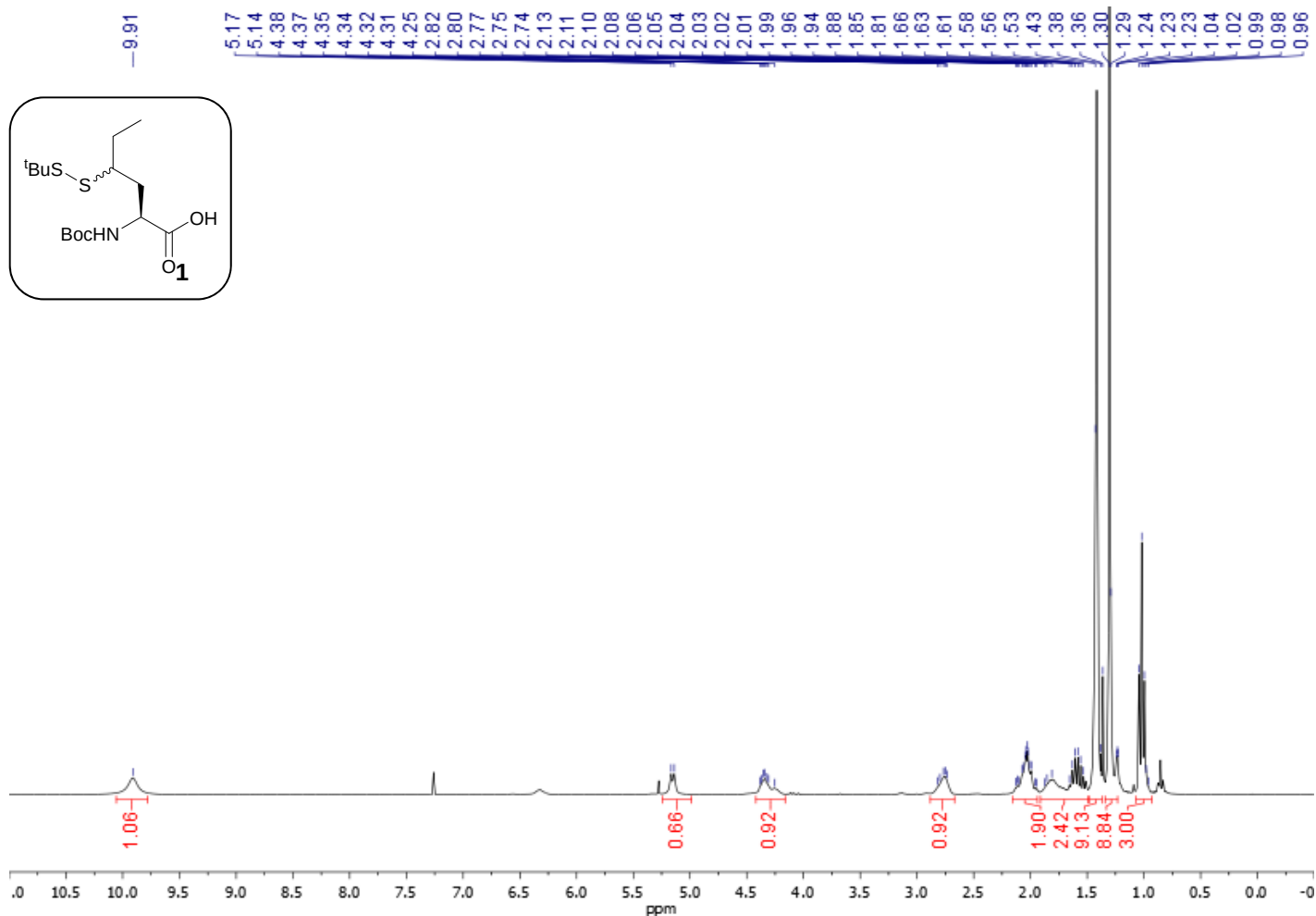
Compound 8: ^1H NMR (300.17 MHz, CDCl_3)



Compound 8: ^{13}C NMR (75.47 MHz, CDCl_3)



Compound 1: ^1H NMR (300.17 MHz, CDCl_3)



Compound 1: ^{13}C NMR (75.47 MHz, CDCl_3)

