Electronic Supplementary Material (ESI) for RSC Advances.

Application of dehydroalanine as a building block for the synthesis of selenocysteine-containing peptides

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Electronic Supplementary

Information

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General procedure:

All the amino acids were purchased from GL Biochem (Shanghai) Ltd. Methanol was obtained from Merck. All other chemicals were of the highest purity available. Most reactions were carried out in a well-ventilated fume hood to avoid the unpleasant odour and toxic nature of the reaction mixtures involved. Thin-layer chromatography analyses were carried out on pre-coated silica gel plates (Merck), and spots were visualized under UV radiation. Column chromatography was performed on glass columns loaded with silica gel. ^{1}H (400 MHz), ^{13}C (100.56 MHz), and ^{77}Se (76.29 MHz) NMR spectra were obtained on a Bruker 400 MHz NMR spectrometer. Chemical shift values are cited with respect to $SiMe_4$ as internal (^{1}H and ^{13}C) and Me_2Se as external (^{77}Se) standard. A Perkin–Elmer Lambda 5 UV/Vis spectrophotometer and high-performance liquid chromatography (HPLC) having a 2695 separation module and UV detector were used. The HPLC system was controlled by EMPOWER software (Waters corporation, Milford, MA). Mass spectral studies were carried out on a Bruker Daltonics Esquire 6000 plus mass spectrometer with ESI-MS mode analysis.

General procedure for the peptide synthesis.

Boc-protection: The free amino acid (1 equiv.) was dissolved in the aq. $NaHCO_3$ solution and cooled the reaction mixture to 0 °C and added Boc anhydride (1.2 equiv.) in dioxane to the reaction mixture at 0 °C. After 5 min, removed the ice bath and reaction mixture was stirred at 27 °C for 6 h. The reaction mixture was acidified with dil. HCl and extracted with ethylacetate. The organic layer was washed with water and brine. The organic layer was dried over Na_2SO_4, filtered and concentrated in vacuo to afford the boc-protected amino acid in quantitative yield. This boc protected amino acid used directly for the next step.[1-2]

Esterification: The free aminoacid (1 equiv.) was added to methanol and to this was added thionyl chloride (1.5 equiv.) slowly at 0 °C. Removed the ice bath and refluxed the reaction mixture for 4 h. Methanol was removed, and the white ppt was washed with diethylether to remove the excess thionyl chloride to afford the methyl ester amino acid as white solid. This compound also used directly for the next step.[1-2]

Coupling of two amino acids: The Boc protected aminoacid (1 equiv.) was dissolved in dry dichloromethane and small amount of DMF. 1.1 equiv. of HOBt and 1.1 equiv. of EDC were added and stirred the reaction mixture at 0 °C for 2 h.

In another round bottom flask, methyl ester of amino acid (1.1 equiv.) was taken and dissolved in DCM. To this solution was added 5 equiv. of triethylamine and stirred for 30 min. This solution was added to the activated ester at 0 °C slowly and the continued the stirring at 27 °C for 16 h. The completion of reaction mixture was followed by TLC. The reaction mixture was added to $NaHCO_3$ solution and extracted with DCM 2 times. The combined organic layer was washed with brine and dried over Na_2SO_4. filtered and concentrated in vacuo. The crude product was subjected to column chromatography to afford the desired compound.[3]

Saponification of Esters: The methyl ester of peptide was saponified by dissolving the ester in MeOH and added to this 1N NaOH and MeOH (1:1) slowly at 0 °C. MeOH and NaOH should be in the ratio of 2:1. The reaction was stirred at 0 °C for 20 mins, the completion of reaction mixture was followed by TLC. The reaction mixture was washed with diethylether 2 times. And then the aqueous layer was acidified with $KHSO_4$ solution and extracted the compound with ethyl acetate. Washed the organic layer with brine and dried over Na_2SO_4, filtered and concentrated in vacuo to afford the saponified product and used the compound directly for the coupling with free amine containing amino acid ester.[4]

Boc-Deprotection: The Boc group of the peptide was removed by treating it with 1:1 ratio of DCM and TFA for 2 h. TFA was removed by high vacuum to afford the free amine containing peptide.[5-6]

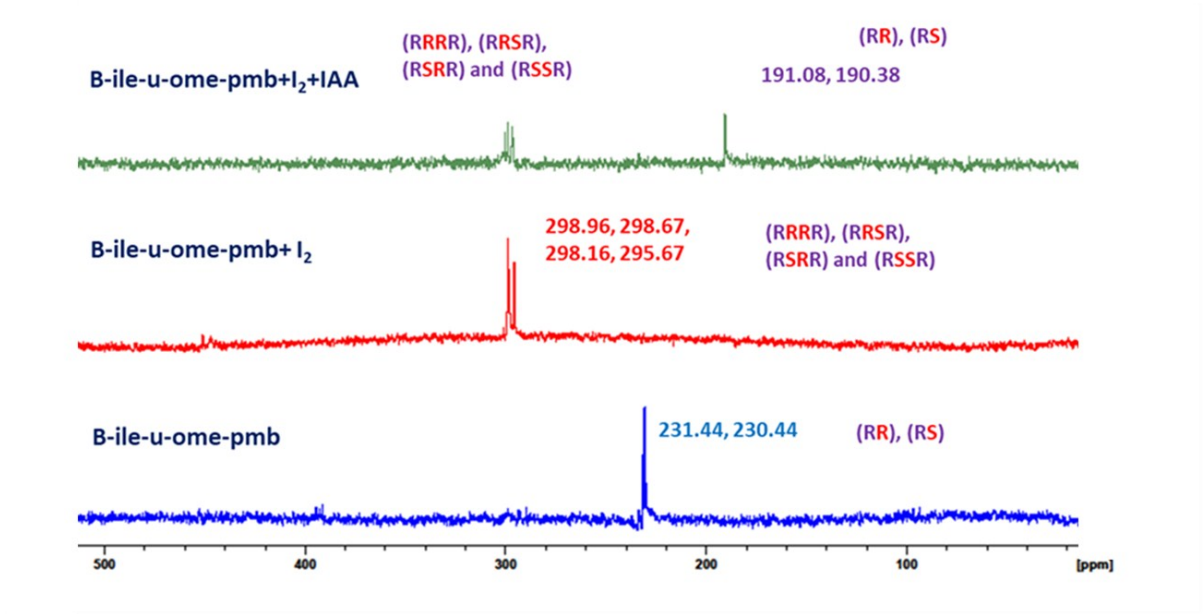

Figure **S1.** Reaction of the selenocysteine dipeptide **9d** with iodine in methanol and a subsequent reaction with sodium borohydride and iodoacetic acid to produce the alkylated derivative **21**.

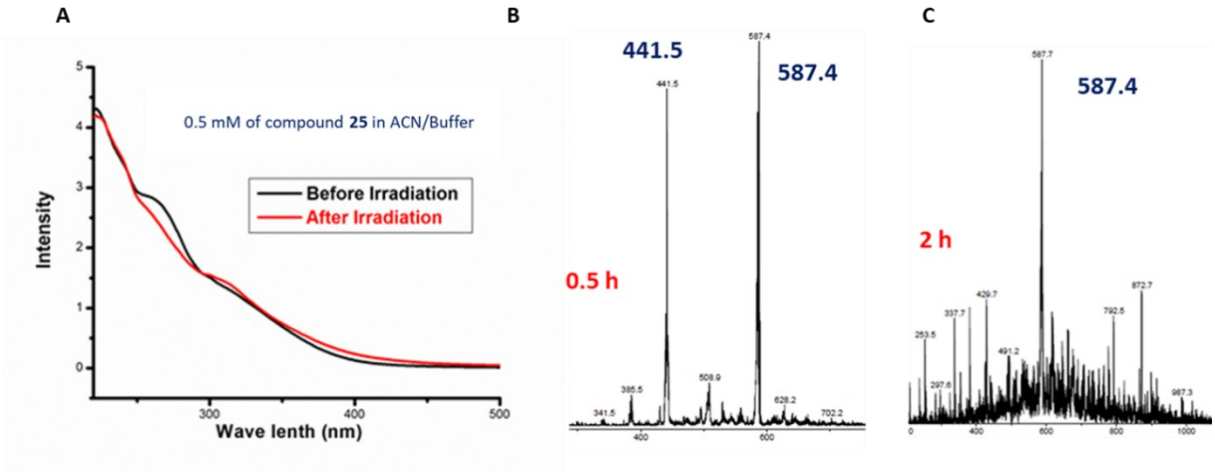

Figure **S2.** (A) UV spectrum of sec derivative **22** at 366 nm UV light in ACN and phosphate buffer pH 7 (1:1), before irradiation and after irradiation for 2 h. (B) ESI-MS of compound **22** after irradiation at 366 nm for 30 min. (C) ESI-MS of compound **22** after irradiation at 366 nm for 2 h.

HPLC Analysis:

All the compounds are subjected to HPLC analysis to see their purity. The selenium coupled compounds of dipeptides and tripeptides were seen as a single peak even though they are diasteriomers. A C18 column was used and the mobile phase was 60% Acetonitrile and 40% water having 0.1% TFA. Flow rate was 1 mL/min.

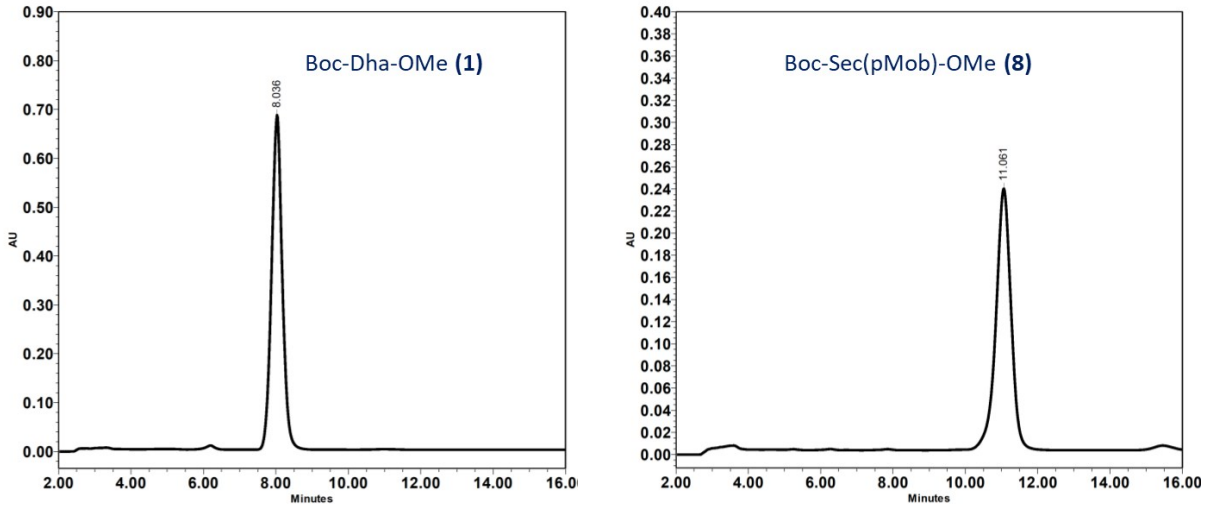

Figure **S3.** HPLC chromatograms of compound **1.0** and **8.0.**

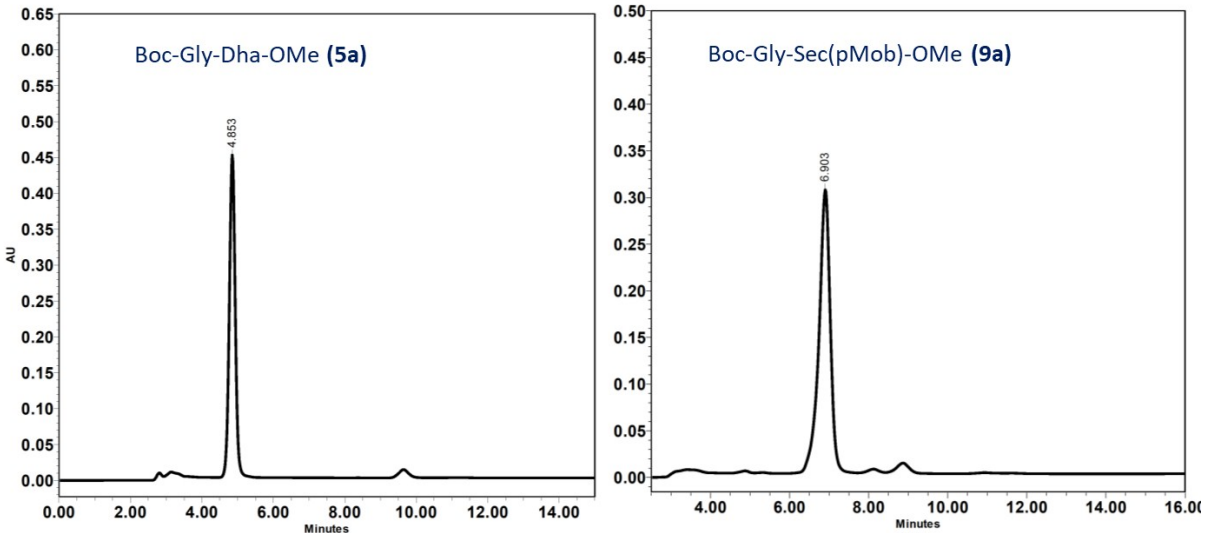

Figure **S4.** HPLC chromatograms of compounds **5a** and **9a.**

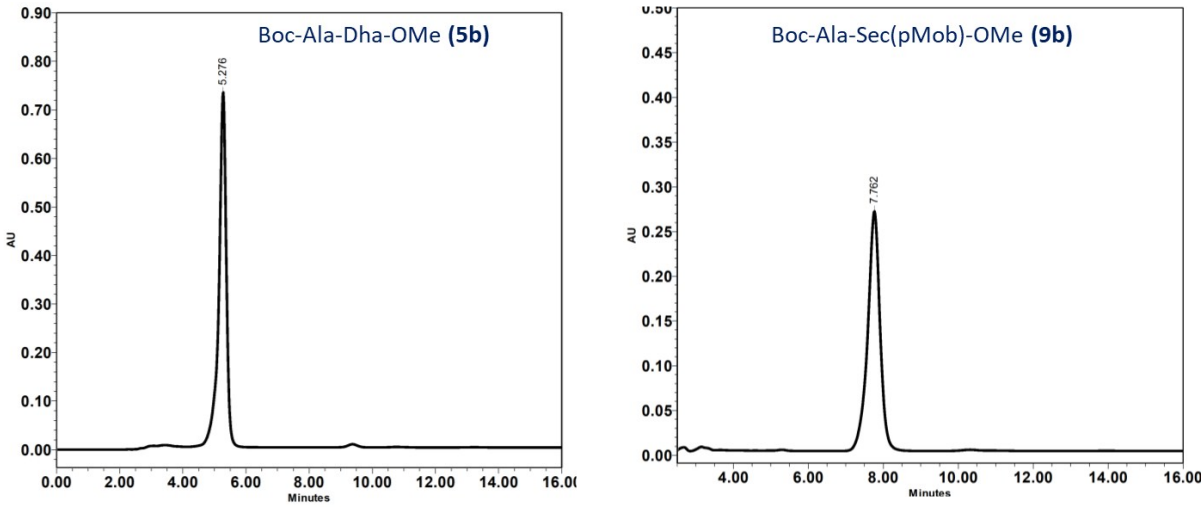

Figure **S5.** HPLC chromatograms of compounds **5b** and **9b.**

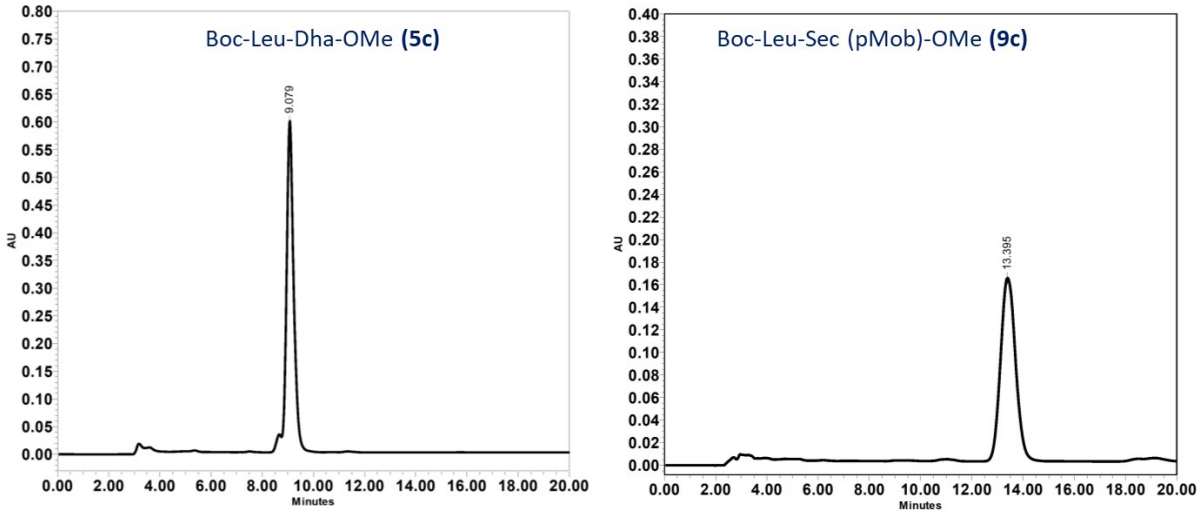

Figure **S6.** HPLC chromatograms of compounds **5c** and **9c.**

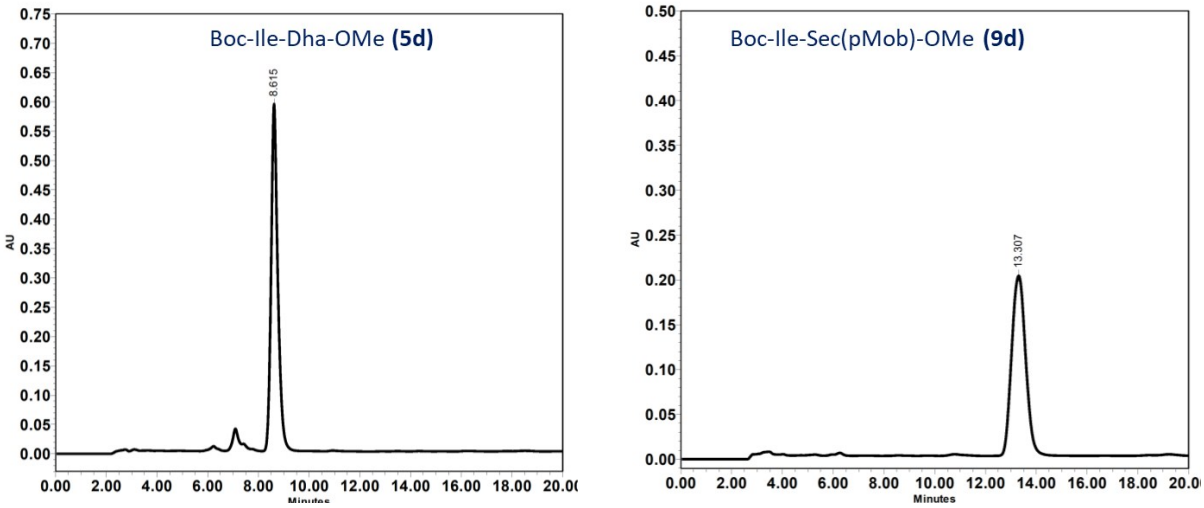

Figure **S7.** HPLC chromatograms of compounds **5d** and **9d.**

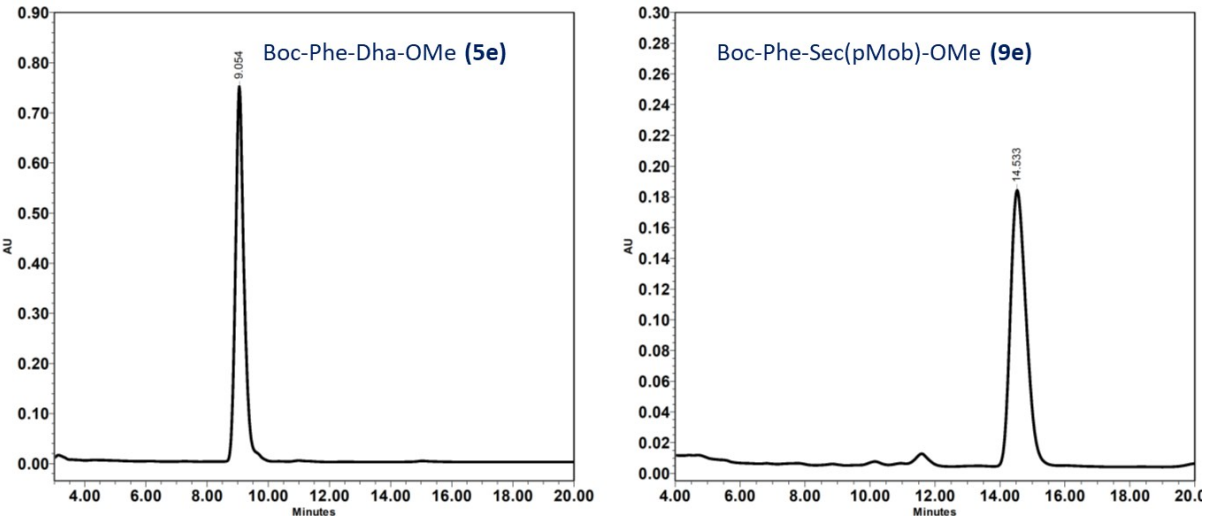

Figure **S8.** HPLC chromatogram of compounds **5e** and **9e.**

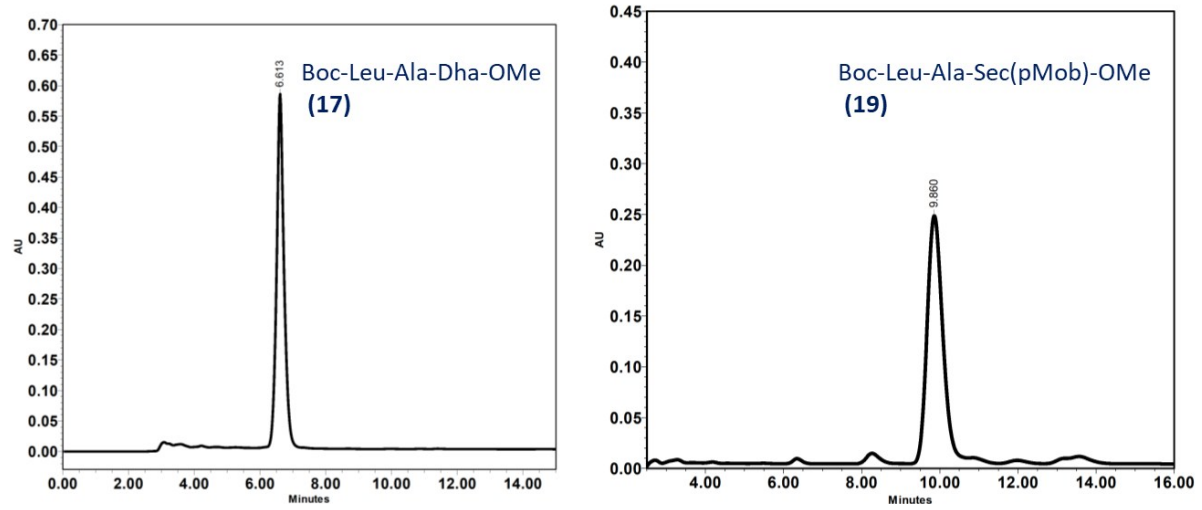

Figure **S9.** HPLC chromatogram of compounds **17** and **19.**

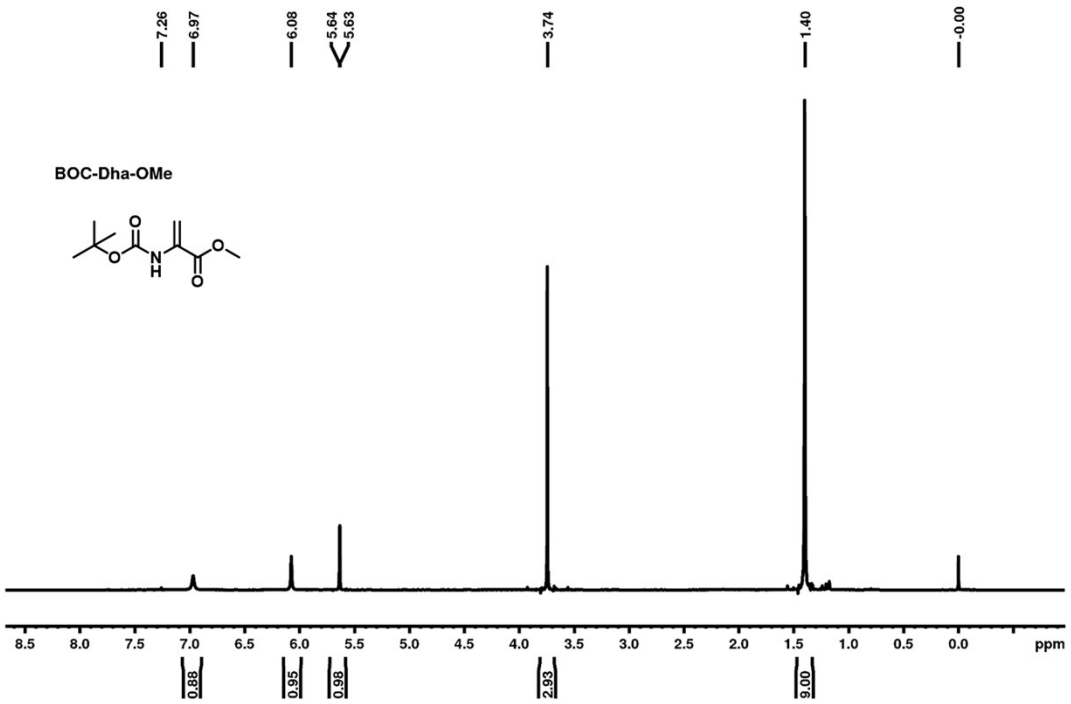

Figure **S10.** ^{1}H NMR spectrum (400 MHz, $CDCl_3$) of compound **1** [Boc-Dha-OMe]

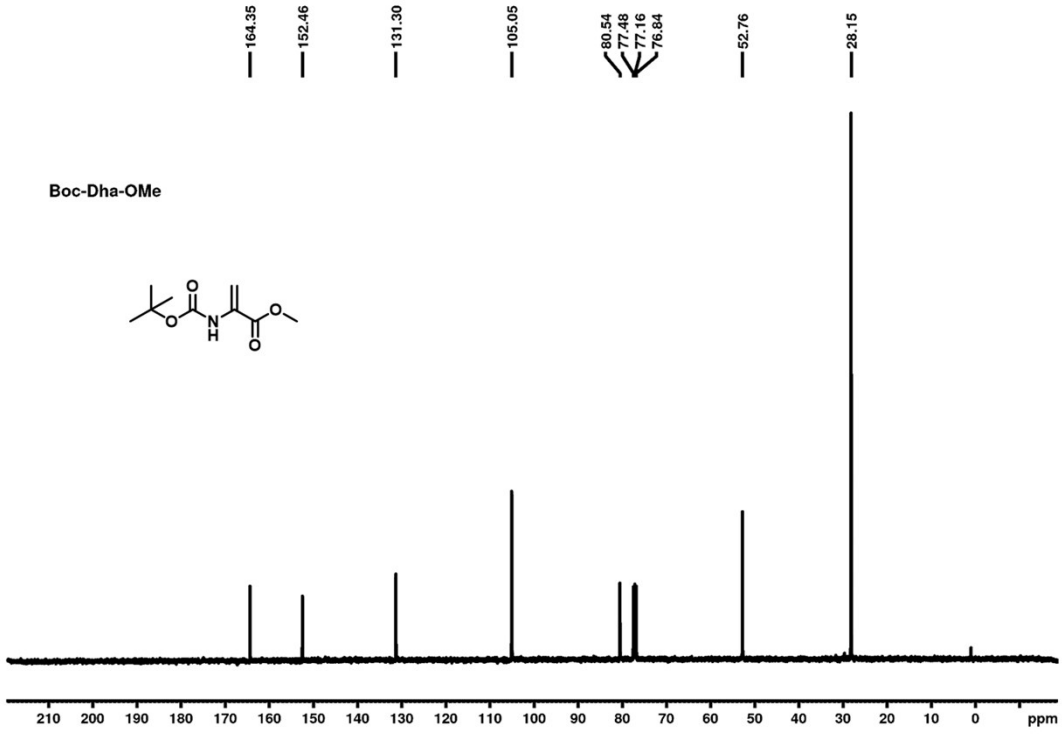

Figure **S11.** ^{13}C NMR spectrum (100.56 MHz, $CDCl_3$) of compound **1** [Boc-Dha-OMe]

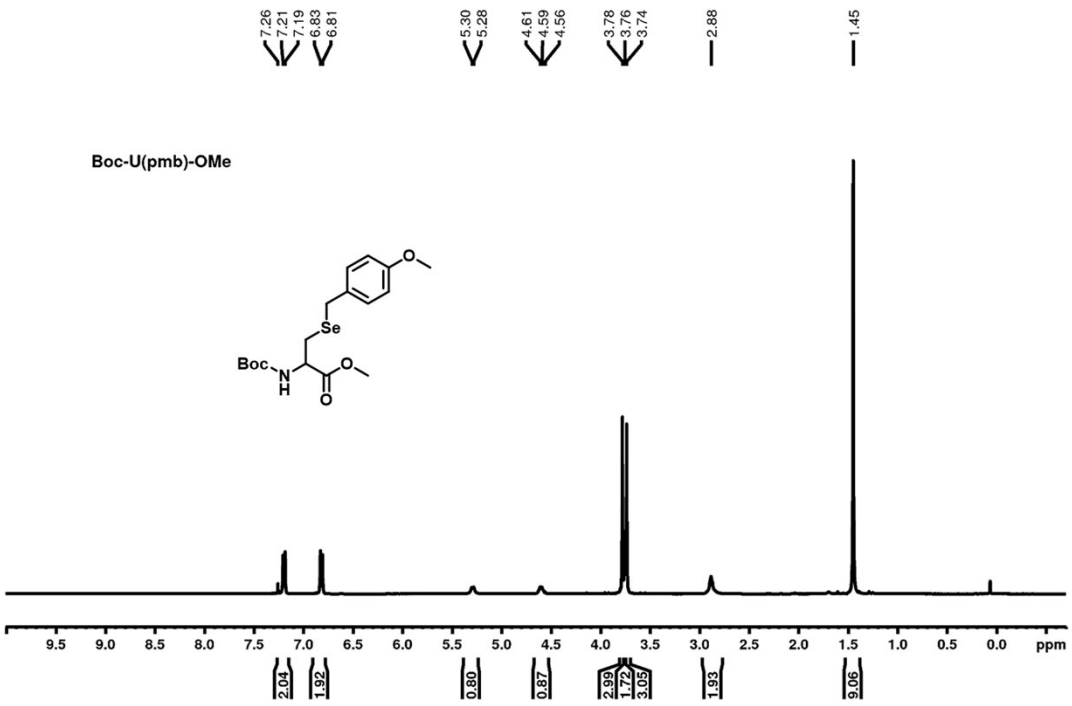

Figure **S12.** ^{1}H NMR spectrum (400 MHz, $CDCl_3$) of compound **8** [Boc-Sec(pMob)-OMe]

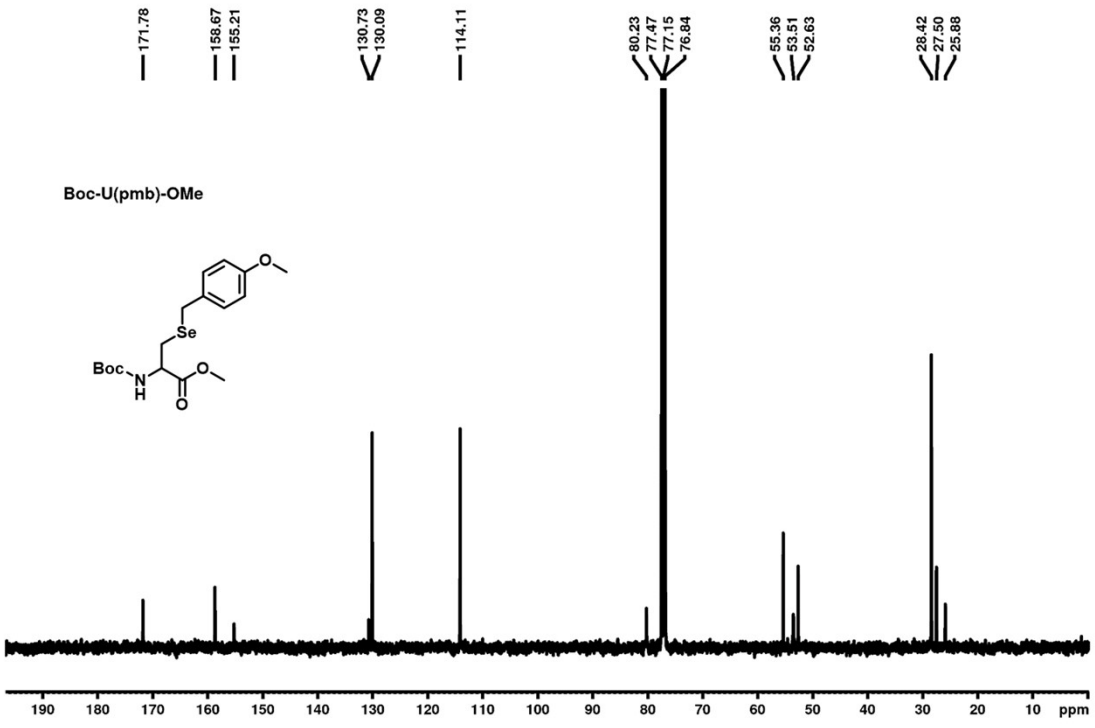

Figure **S13.** ^{13}C NMR spectrum (100.56 MHz, $CDCl_3$) of compound **8** [Boc-Sec(pMob)-OMe]

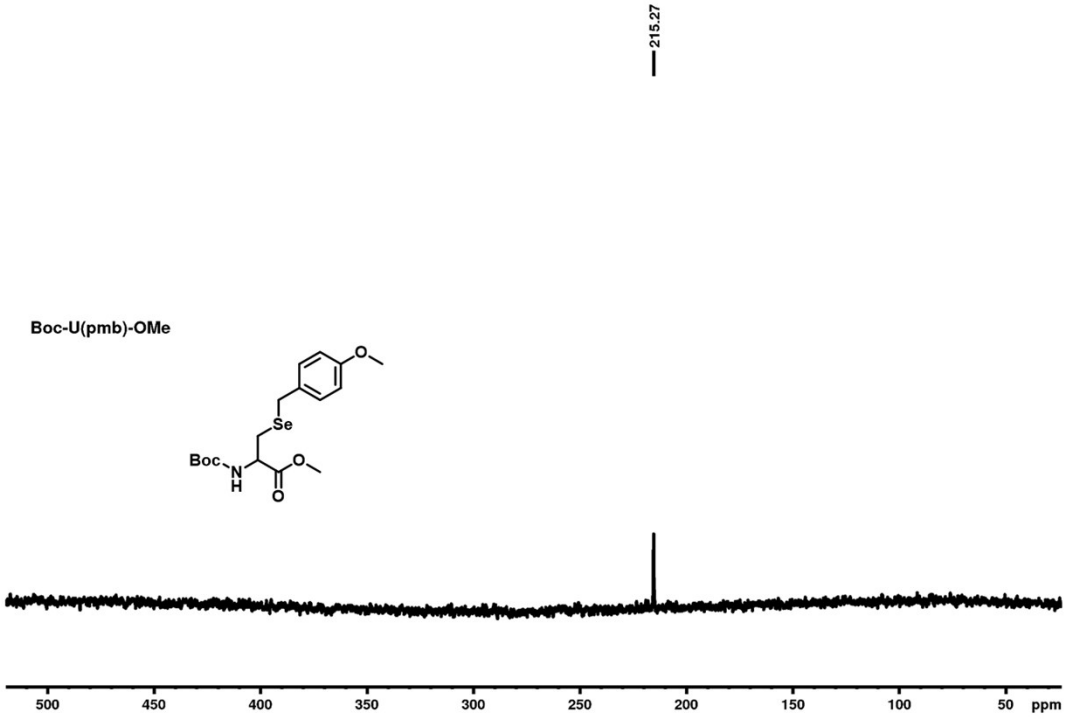

Figure **S14.** ^{77}Se NMR spectrum (76.29 MHz, $CDCl_3$) of compound **8** [Boc-Sec(pMob)-OMe]

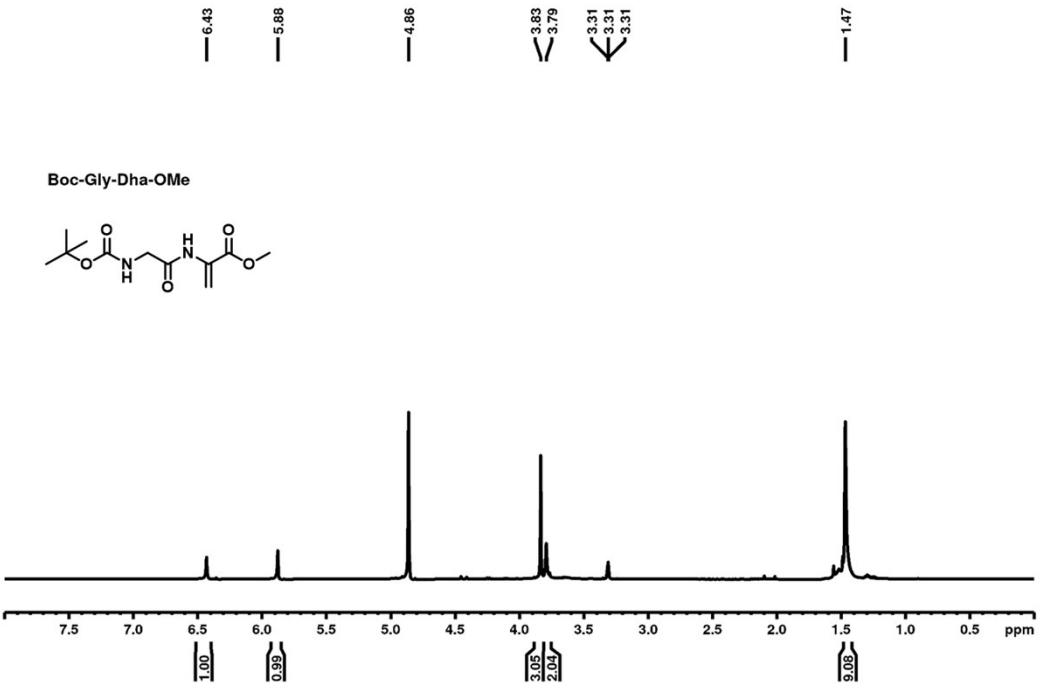

Figure **S15.** ^{1}H NMR spectrum (400 MHz, CD_3OD) of compound **5a** [Boc-Gly-Dha-OMe]

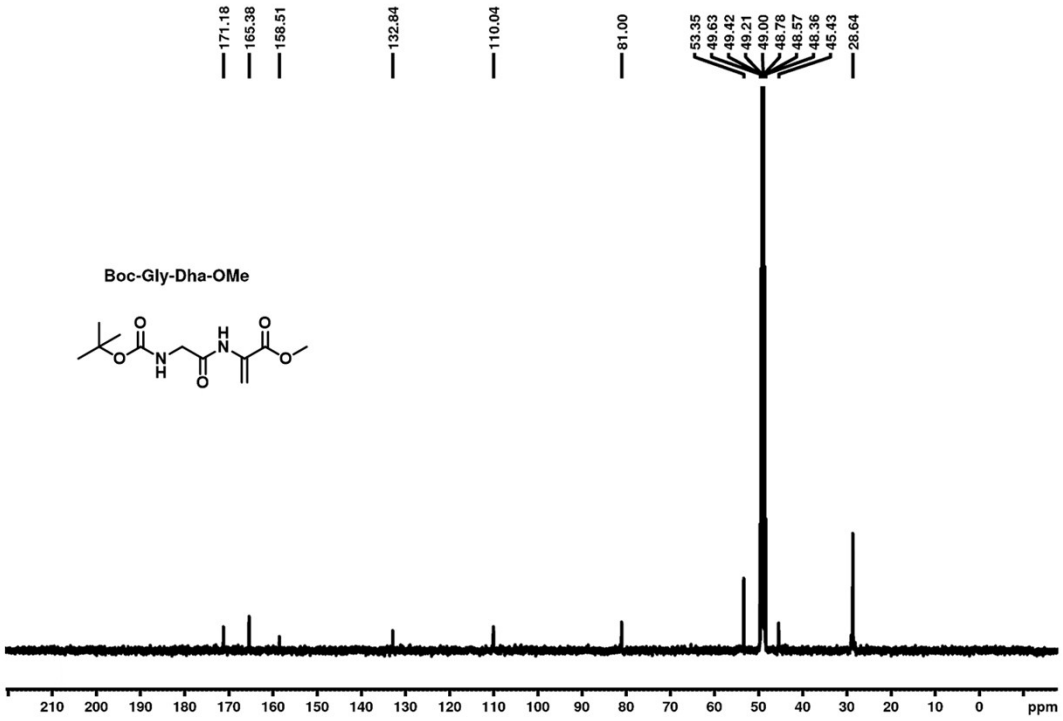

Figure **S16.** ^{13}C NMR spectrum (100.56 MHz, CD_3OD) of compound **5a** [Boc-Gly-Dha-OMe]

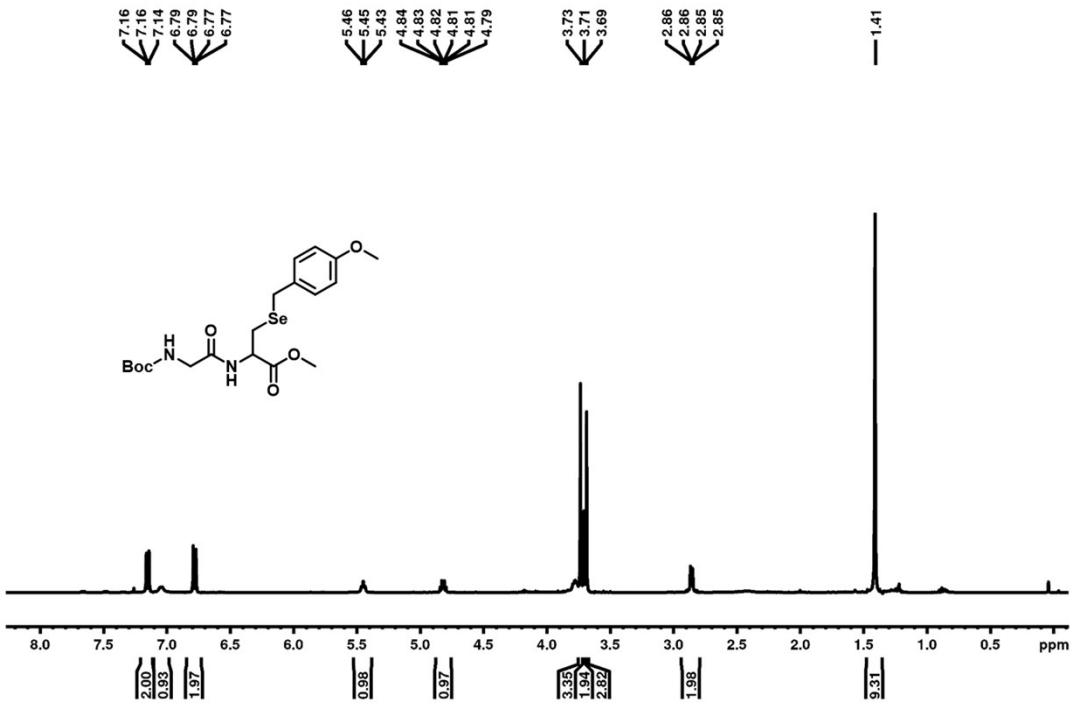

Figure **S17.** ^{1}H NMR spectrum (400 MHz, $CDCl_3$) of compound **9a** [Boc-Gly-Sec(pMob)-OMe]

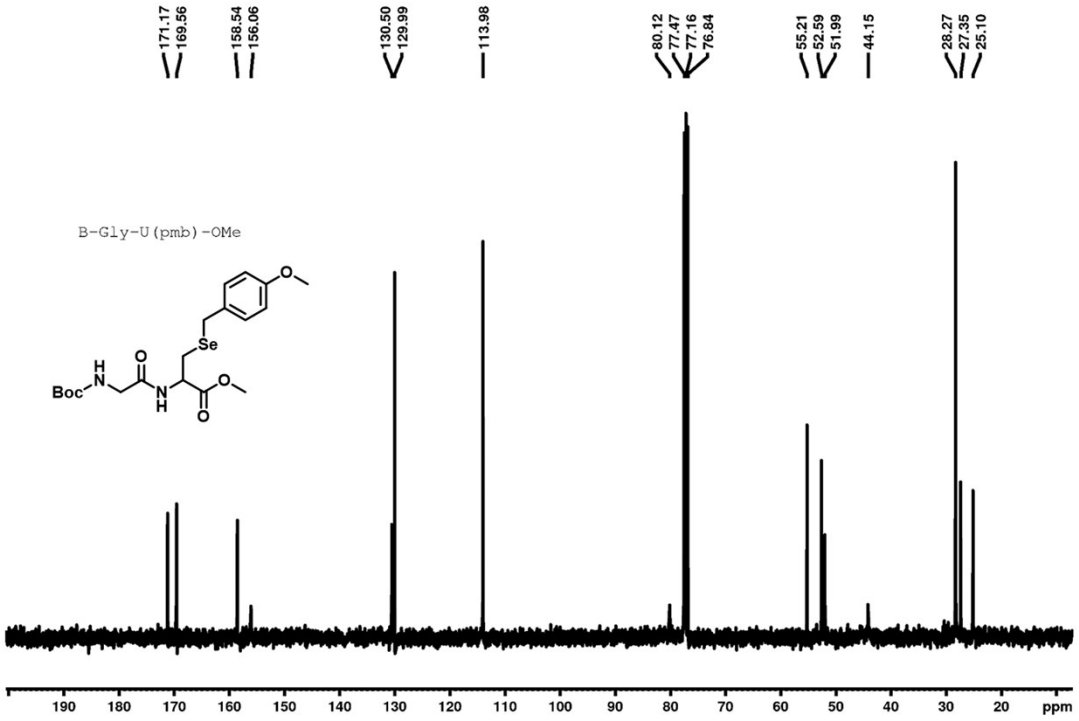

Figure **S18.** ^{13}C NMR spectrum (100.56 MHz, $CDCl_3$) of compound **9a** [Boc-Gly-Sec(pMob)-OMe]

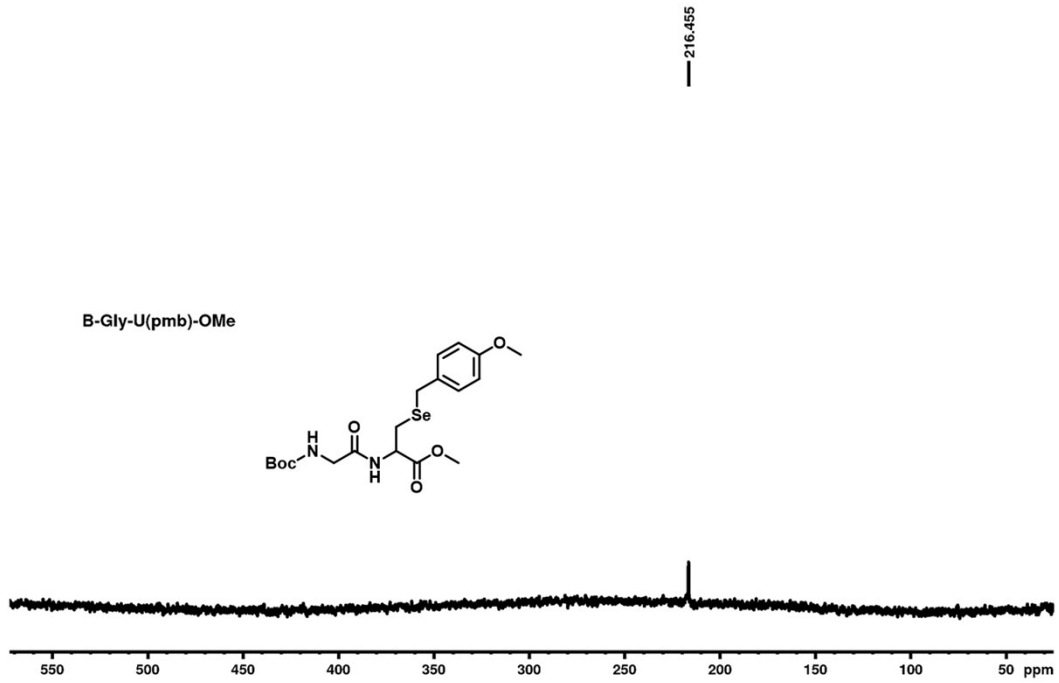

Figure **S19.** ^{77}Se NMR spectrum (76.29 MHz, $CDCl_3$) of compound **9a** [Boc-Gly-Sec(pMob)-OMe]

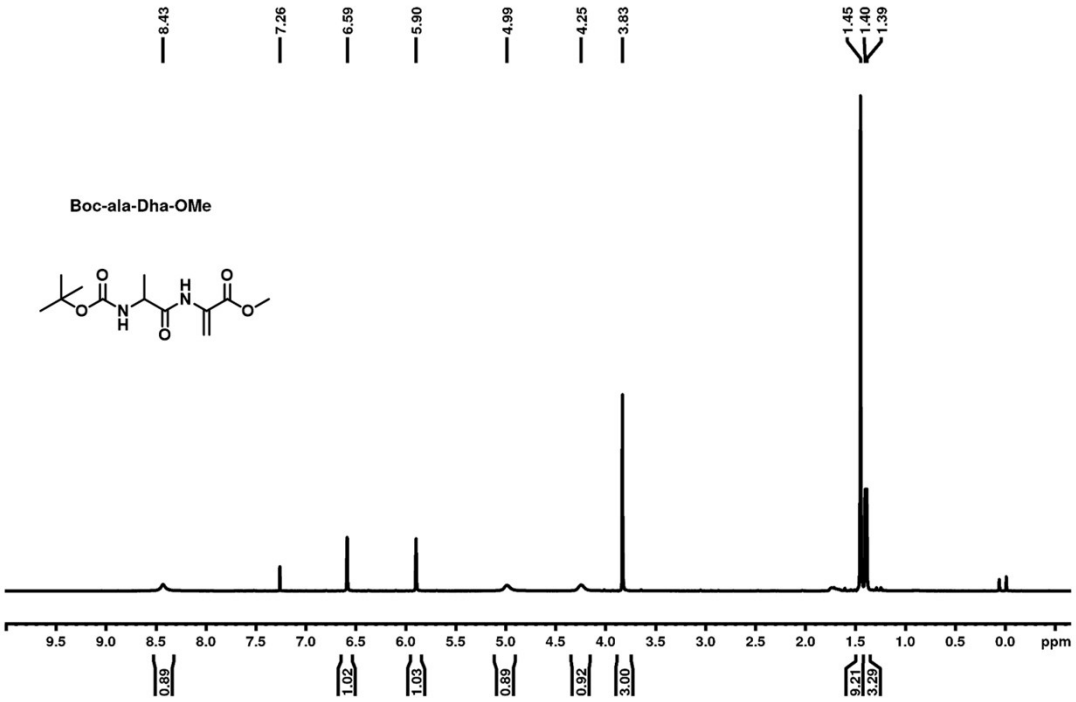

Figure **S20.** ^{1}H NMR spectrum (400 MHz, $CDCl_3$) of compound **5b** [Boc-Ala-Dha-OMe]

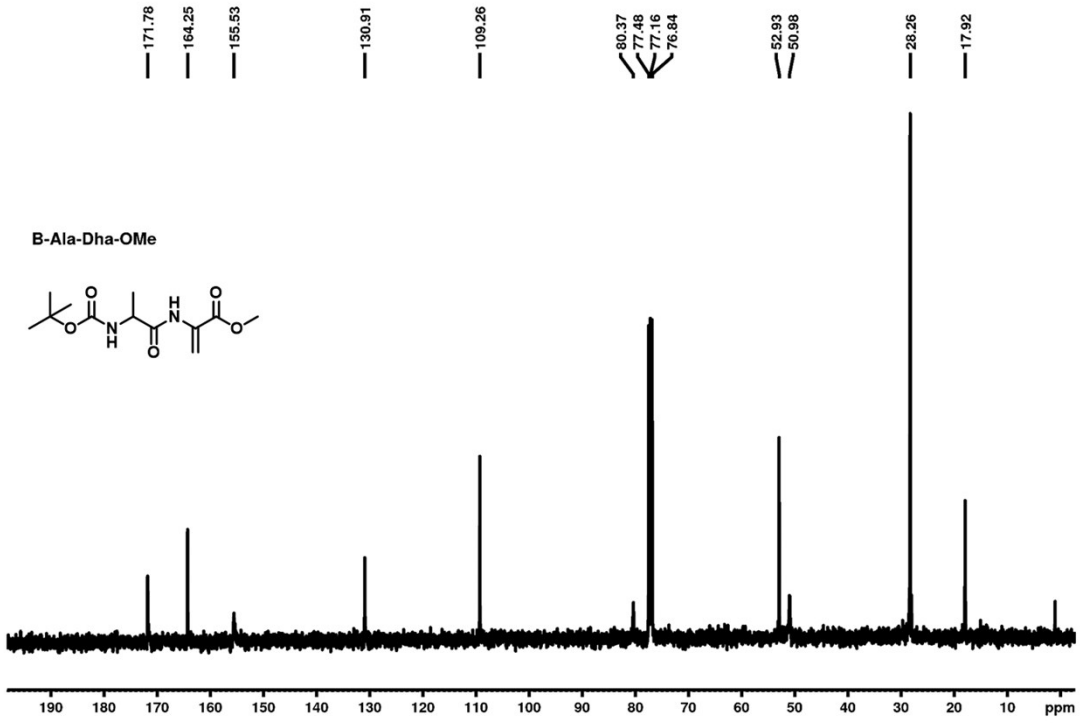

Figure **S21.** ^{13}C NMR spectrum (100.56 MHz, $CDCl_3$) of compound **5b** [Boc-Ala-Dha-OMe]

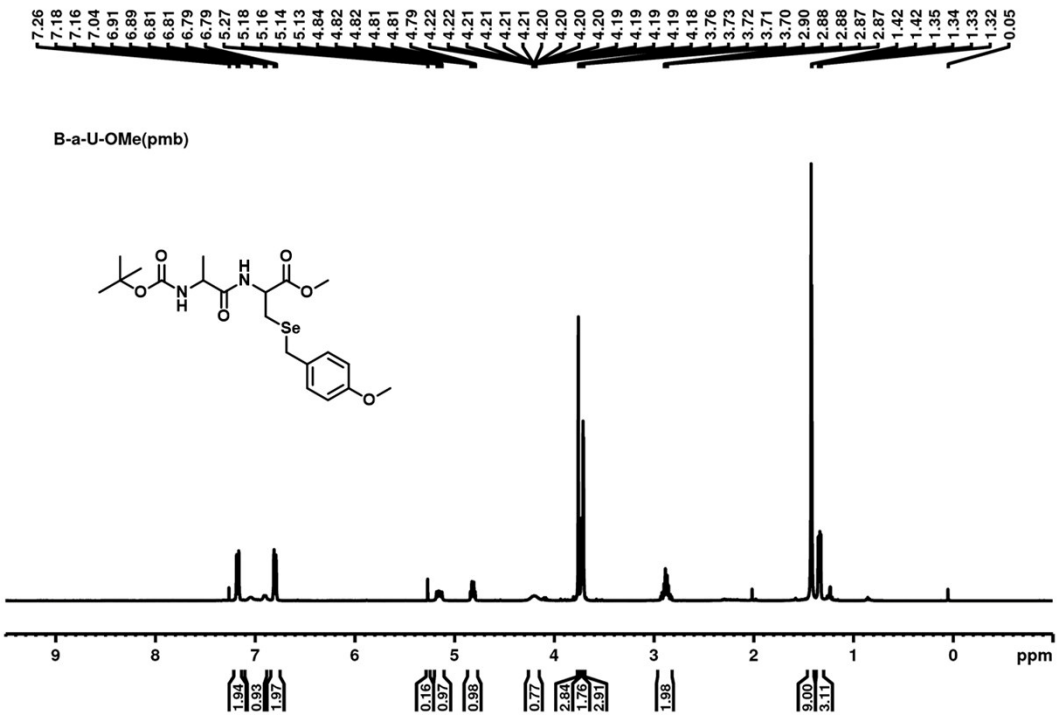

Figure **S22.** ^{1}H NMR spectrum (400 MHz, $CDCl_3$) of Compound **9b** [Boc-Ala-Sec(pMob)-OMe]

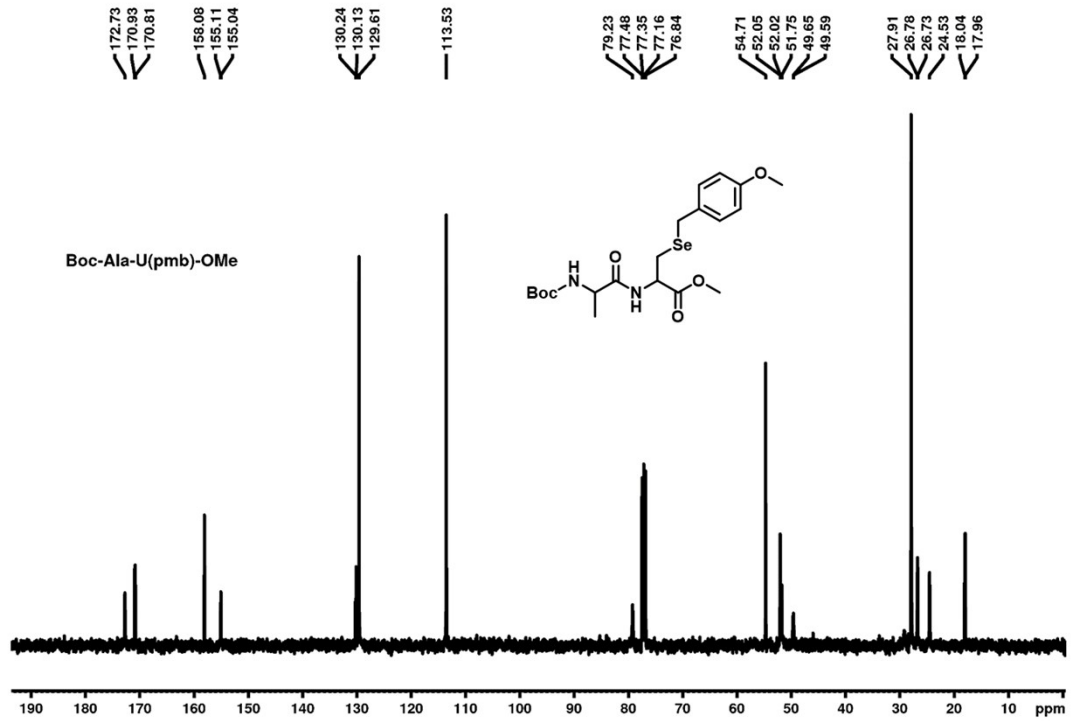

Figure **S23.** ^{13}C NMR spectrum (100.56 MHz, $CDCl_3$) of compound **9b** [Boc-Ala-Sec(pMob)-OMe]

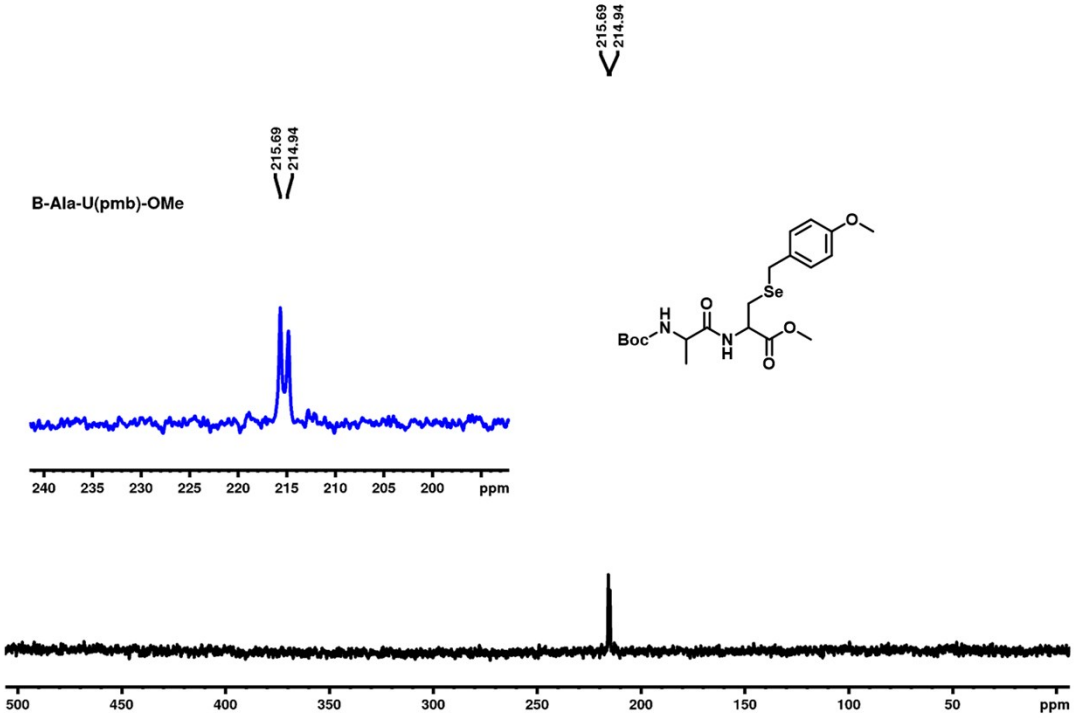

Figure **S24.** ^{77}Se NMR spectrum (76.29 MHz, $CDCl_3$) of compound **9b** [Boc-Ala-Sec(pMob)-OMe]

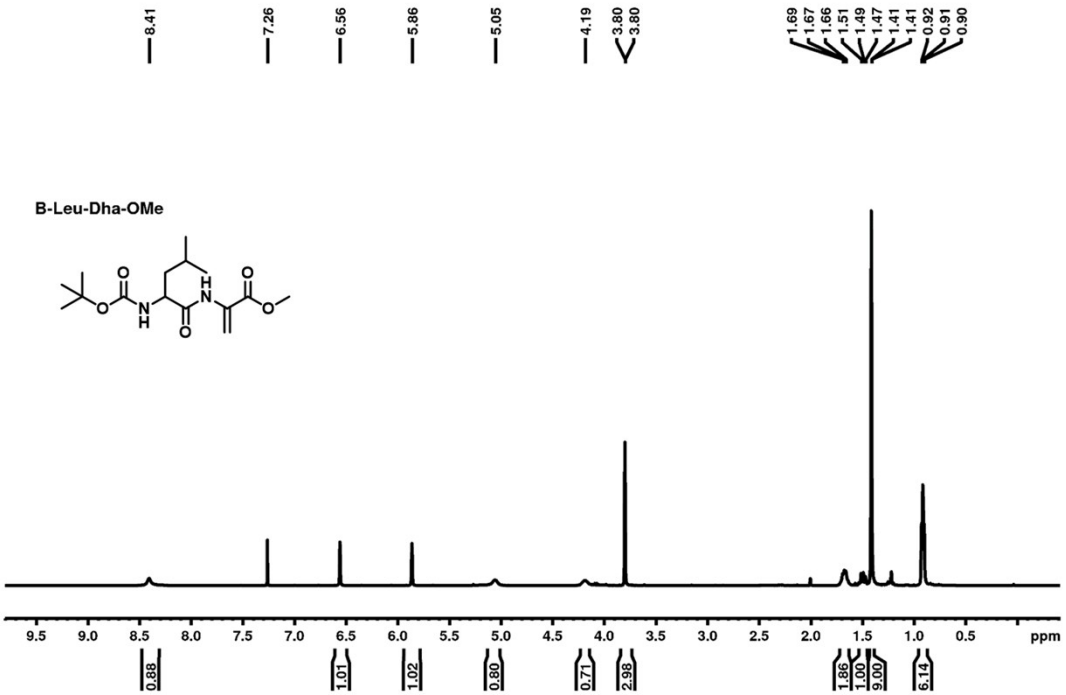

Figure **S25.** ^{1}H NMR spectrum (400 MHz, $CDCl_3$) of compound **5c** [Boc-Leu-Dha-OMe]

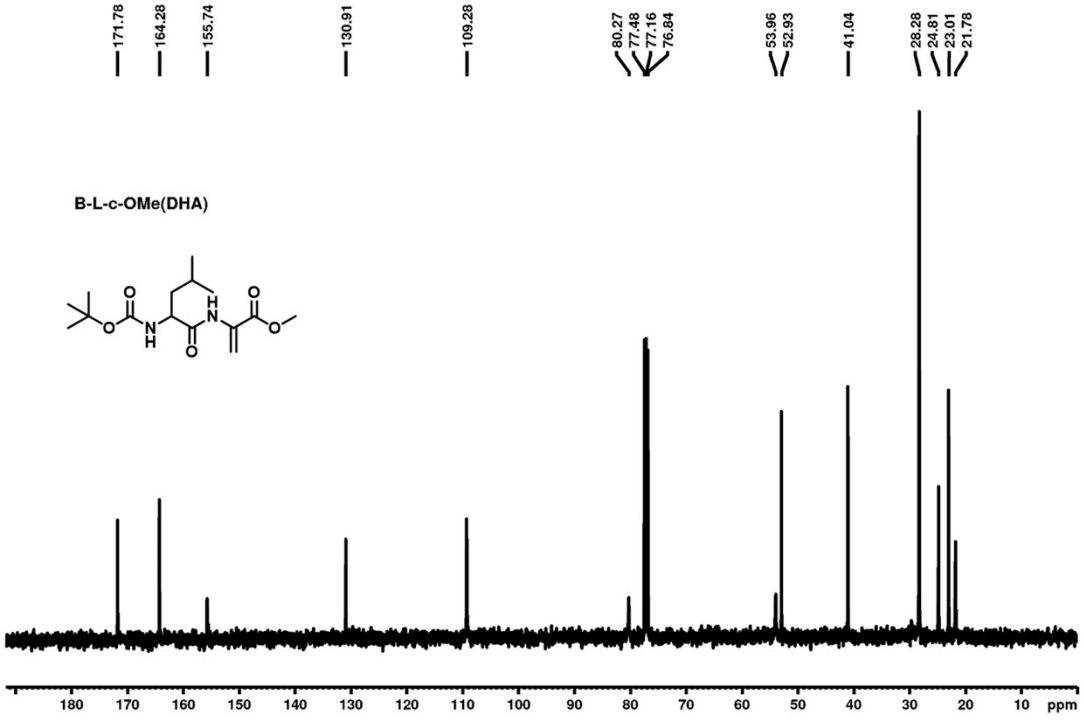

Figure **S26.** ^{13}C NMR spectrum (100.56 MHz, $CDCl_3$) of compound **5c** [Boc-Leu-Dha-OMe]

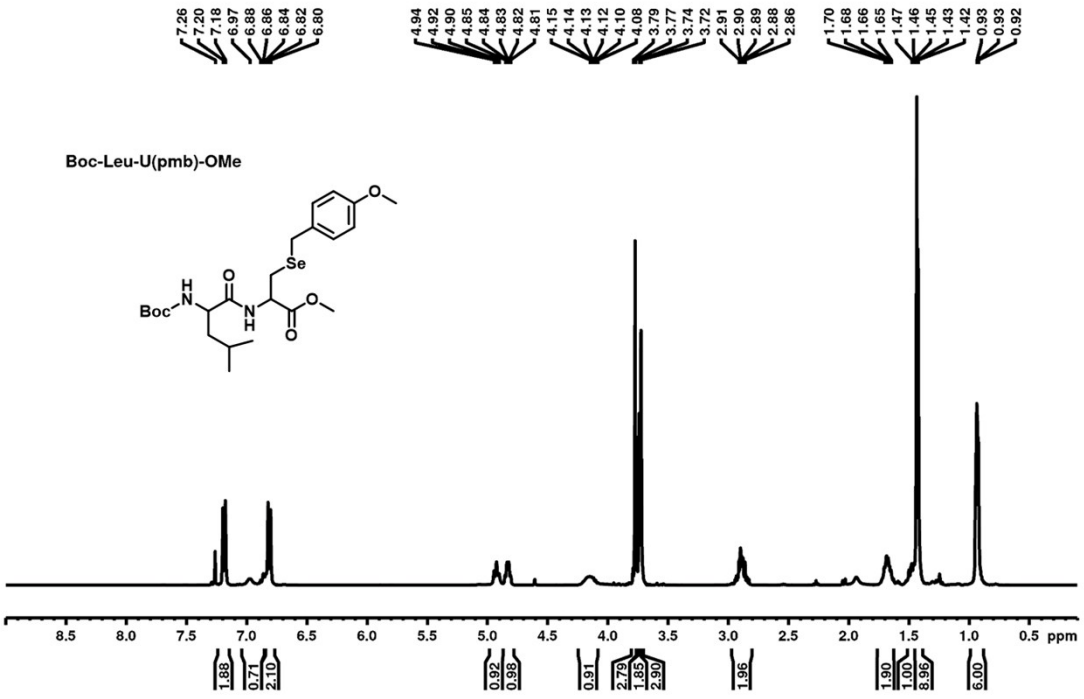

Figure **S27.** ^{1}H NMR spectrum (400 MHz, $CDCl_3$) of compound **9c** [Boc-Leu-Sec(pMob)-OMe]

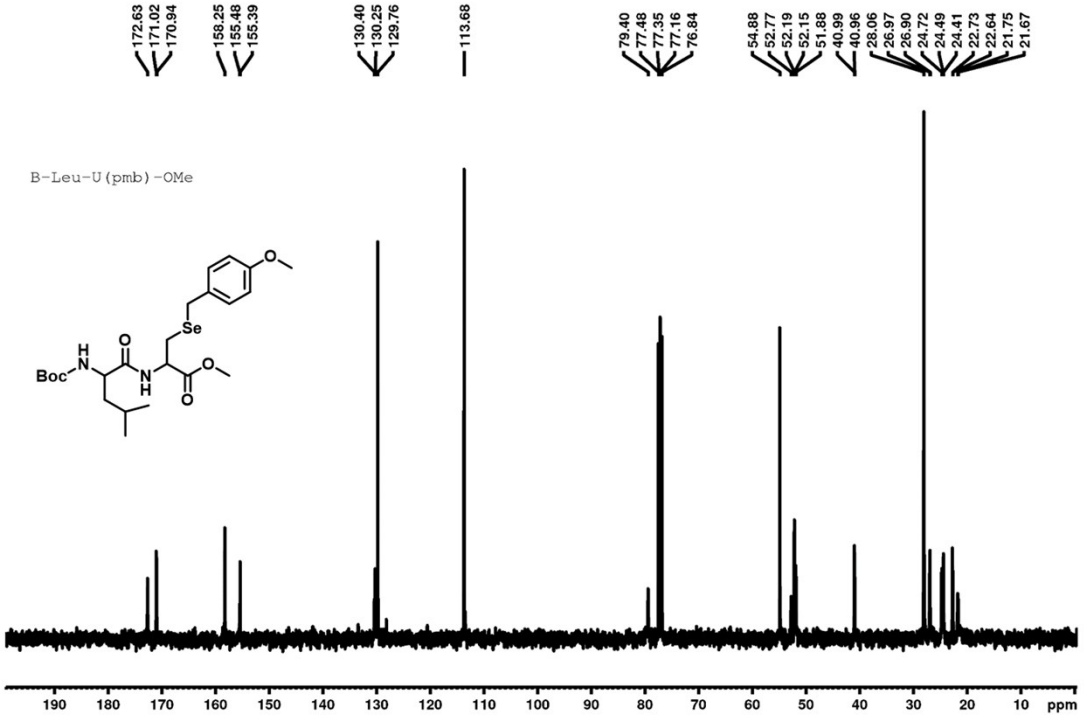

Figure **S28.** ^{13}C NMR spectrum (100.56 MHz, $CDCl_3$) of compound **9c** [Boc-Leu-Sec(pMob)-OMe]

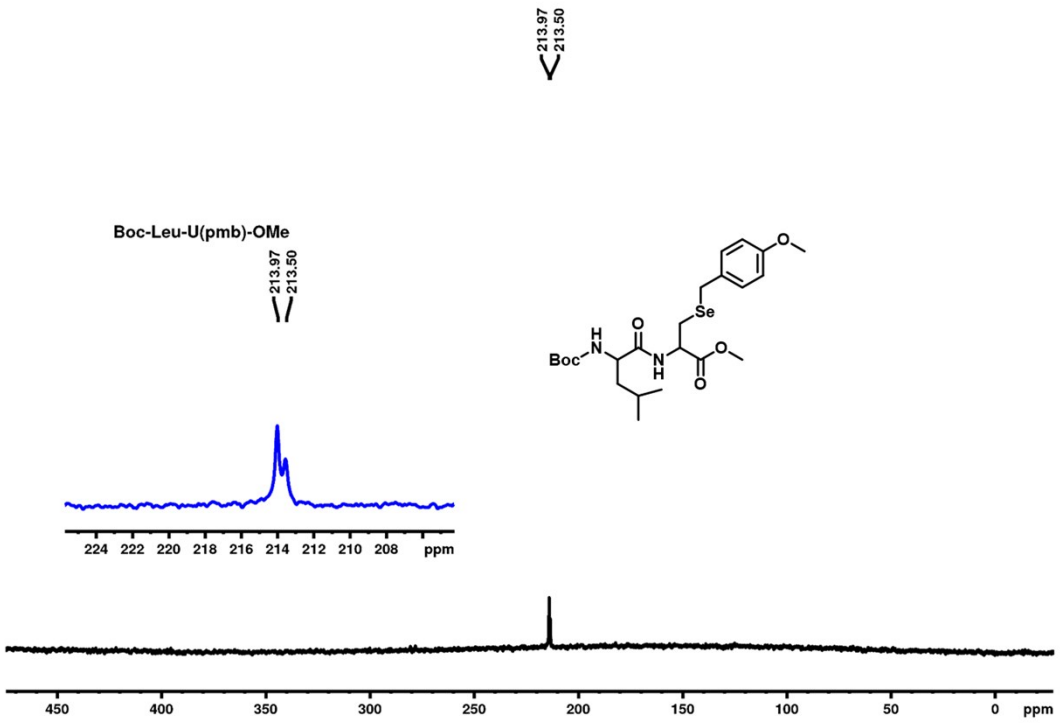

Figure **S29.** ^{77}Se NMR spectrum (76.29 MHz, $CDCl_3$) of compound **9c** [Boc-Leu-Sec(pMob)-OMe]

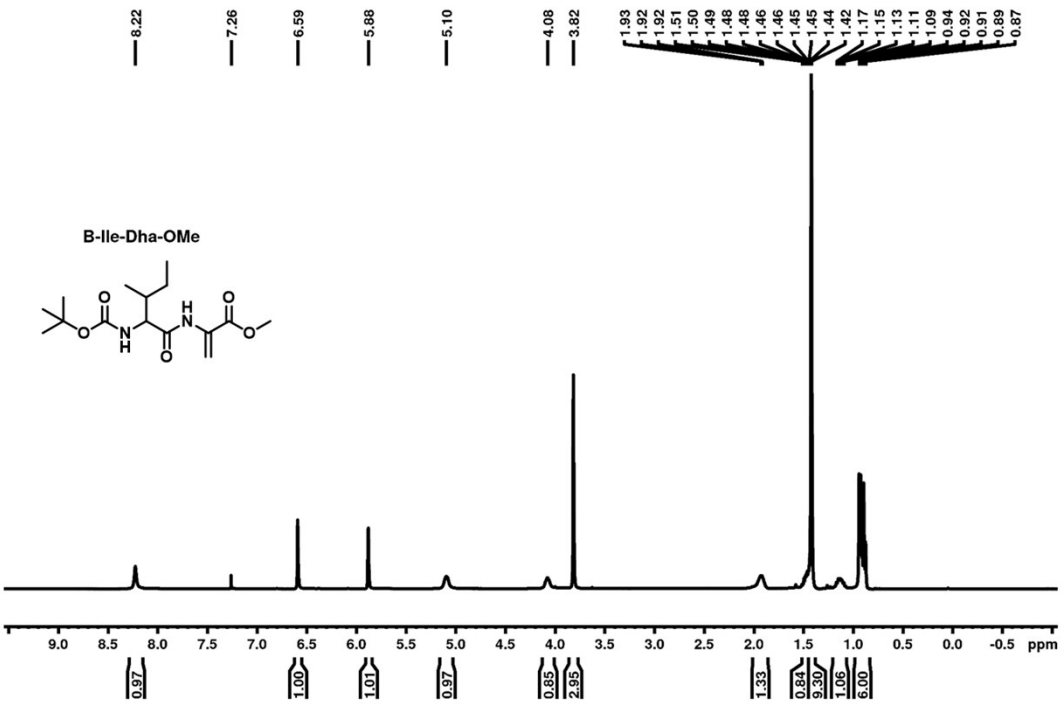

Figure **S30.** ^{1}H NMR spectrum (400 MHz, $CDCl_3$) of compound **5d** [Boc-Ile-Dha-OMe]

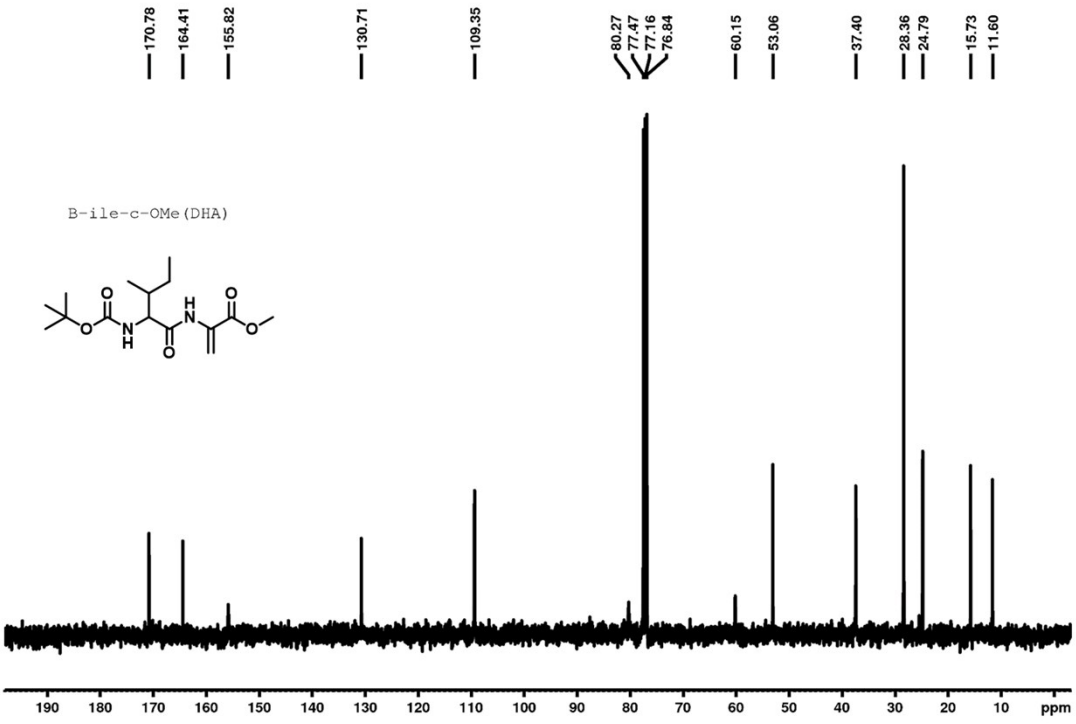

Figure **S31.** ^{13}C NMR spectrum (100.56 MHz, $CDCl_3$) of compound **5d** [Boc-Ile-Dha-OMe]

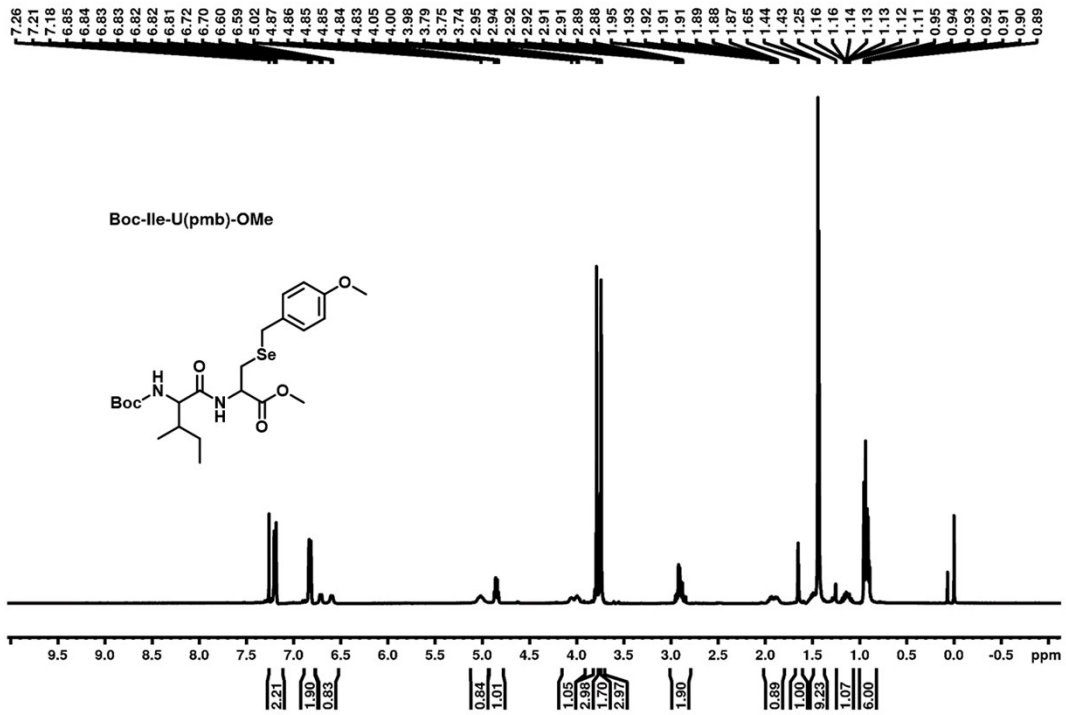

Figure **S32.** ^{1}H NMR spectrum (400 MHz, $CDCl_3$) of compound **9d** [Boc-Ile-Sec(pMob)-OMe]

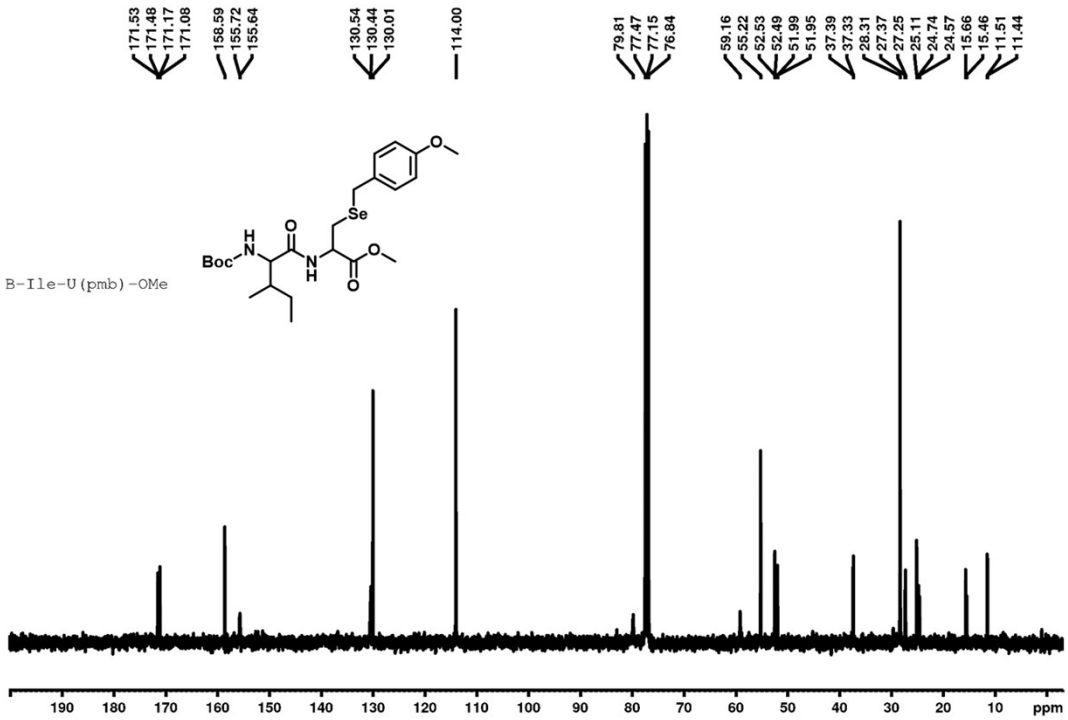

Figure **S33.** ^{13}C NMR spectrum (100.56 MHz, $CDCl_3$) of compound **9d** [Boc-Ile-Sec(pMob)-OMe]

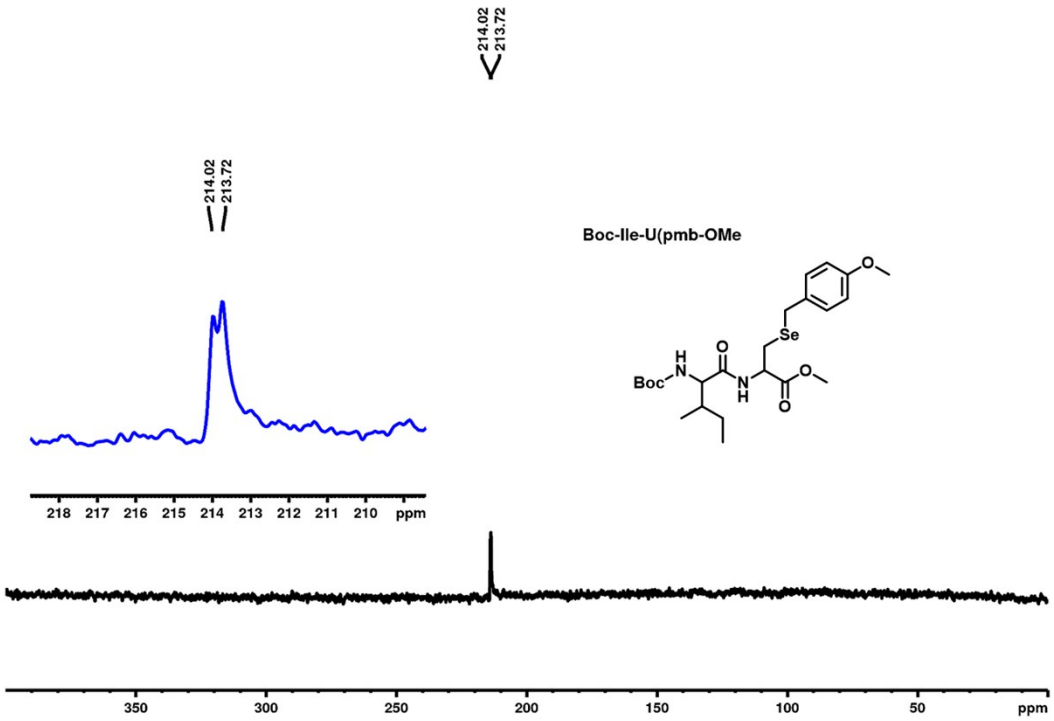

Figure **S34.** ^{77}Se NMR spectrum (76.29 MHz, $CDCl_3$) of compound **9d** [Boc-Ile-Sec(pMob)-OMe]

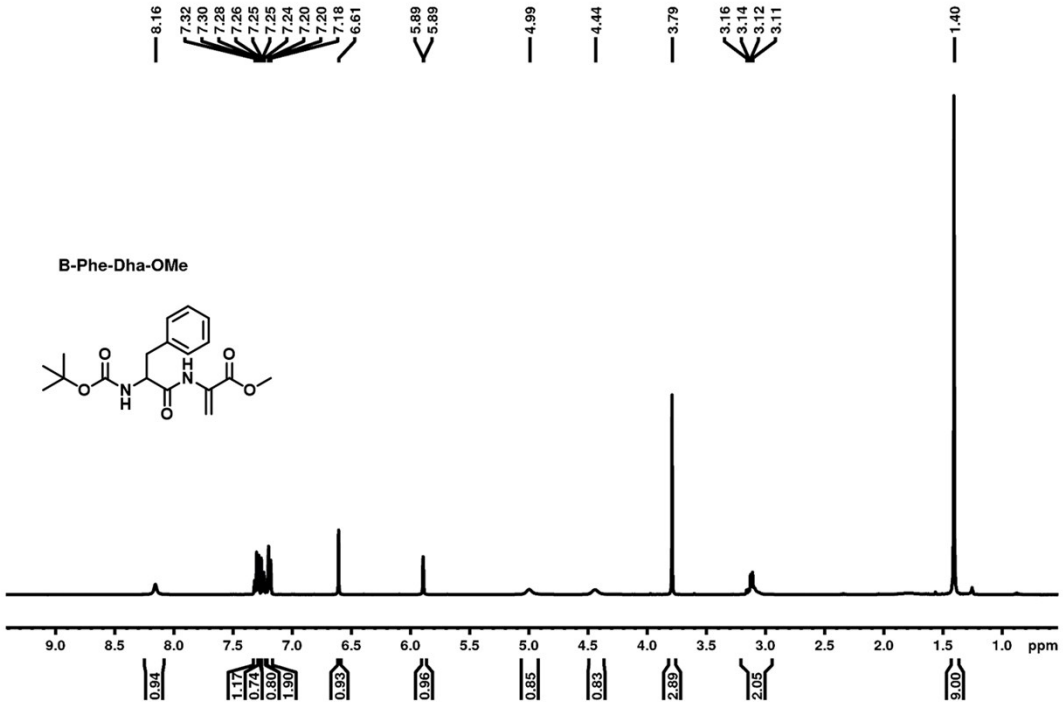

Figure **S35.** ^{1}H NMR spectrum (400 MHz, $CDCl_3$) of compound **5e** [Boc-Phe-Dha-OMe]

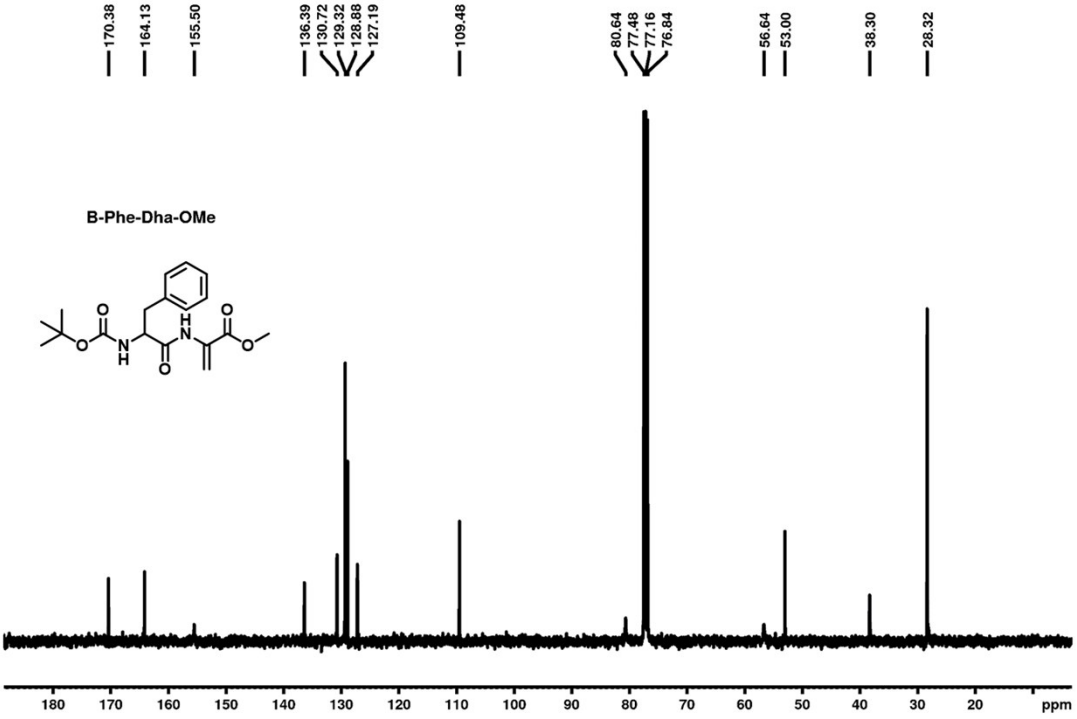

Figure **S36.** ^{13}C NMR spectrum (100.56 MHz, $CDCl_3$) of compound **5e** [Boc-Phe-Dha-OMe]

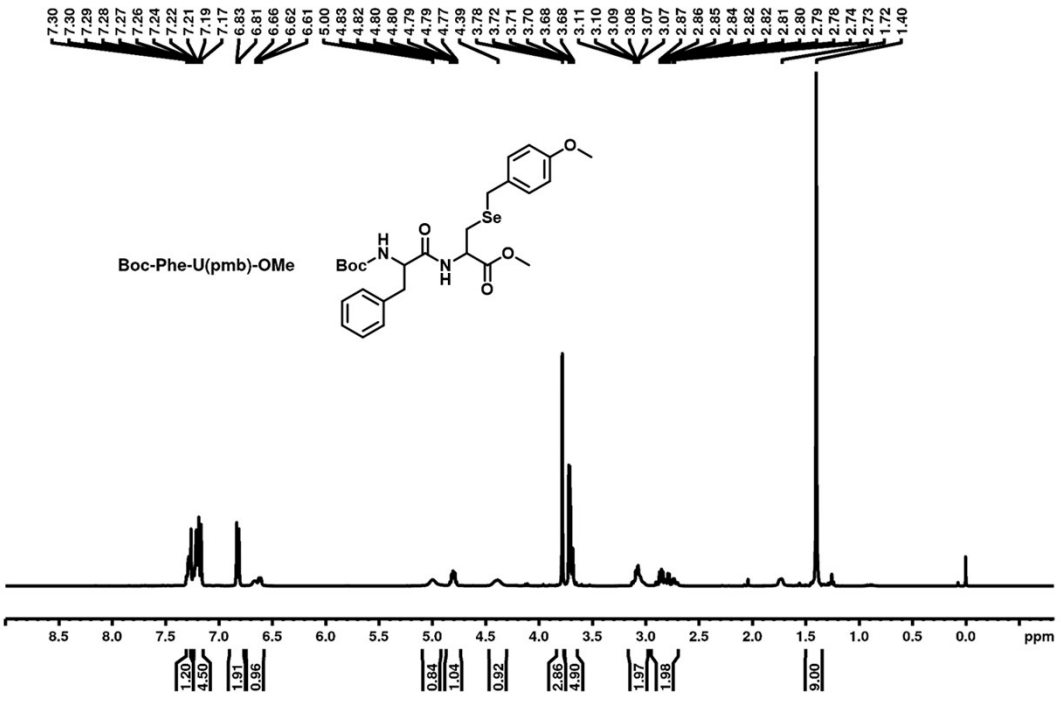

Figure **S37.** ^{1}H NMR spectrum (400 MHz, $CDCl_3$) of compound **9e** [Boc-Phe-Sec(pMob)-OMe]

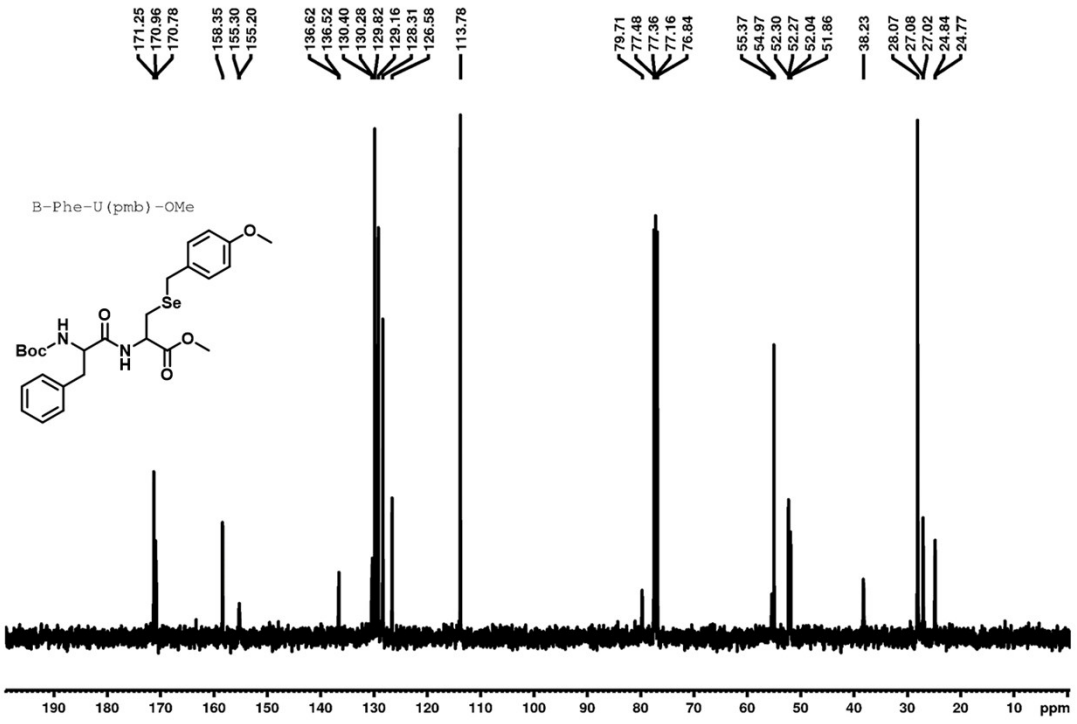

Figure **S38.** ^{13}C NMR spectrum (100.56 MHz, $CDCl_3$) of compound **9e** [Boc-Phe-Sec(pMob)-OMe]

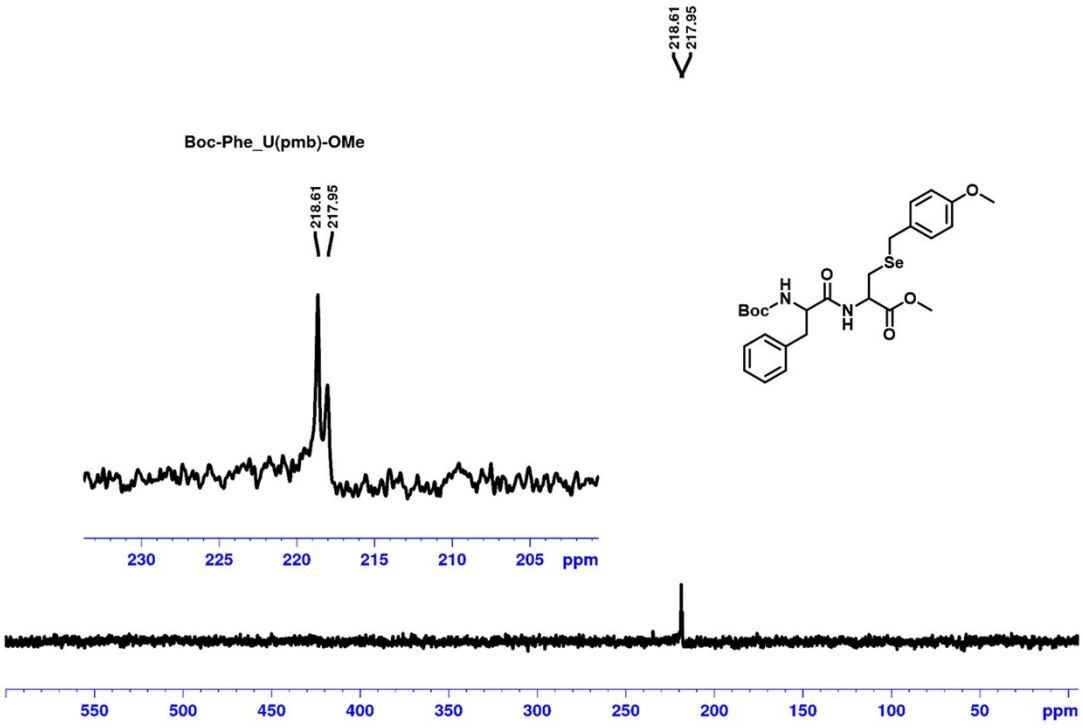

Figure **S39.** ^{77}Se NMR spectrum (76.29 MHz, $CDCl_3$) of compound **9e** [Boc-Phe-Sec(pMob)-OMe]

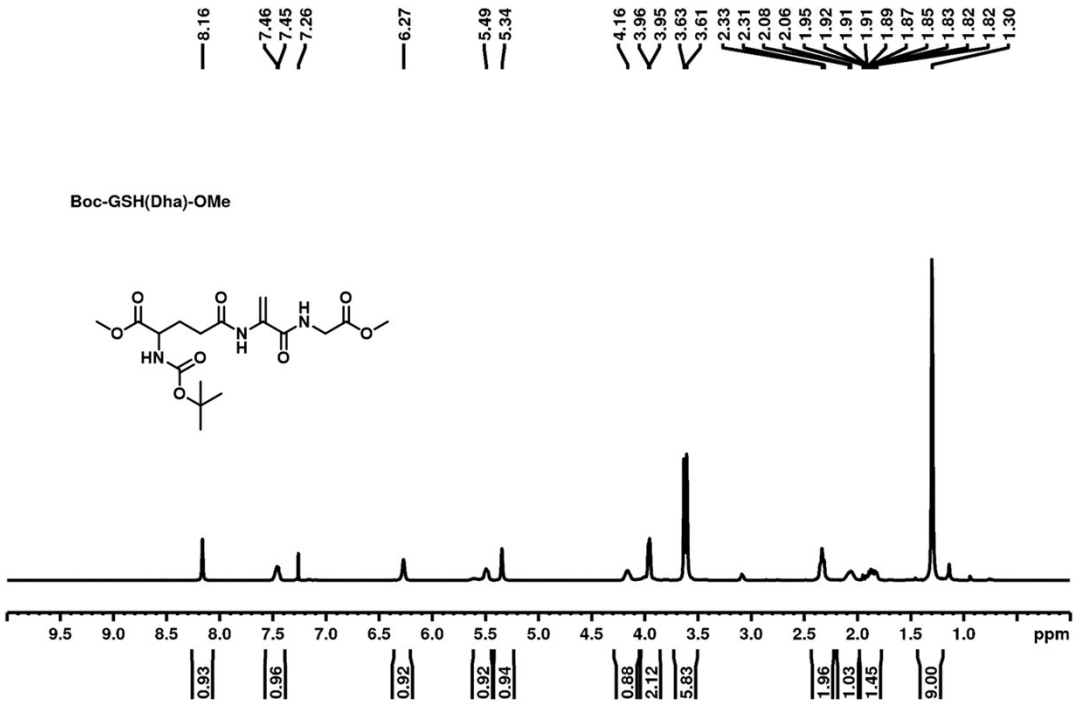

Figure **S40.** ^{1}H NMR spectrum (400 MHz, $CDCl_3$) of compound **13**

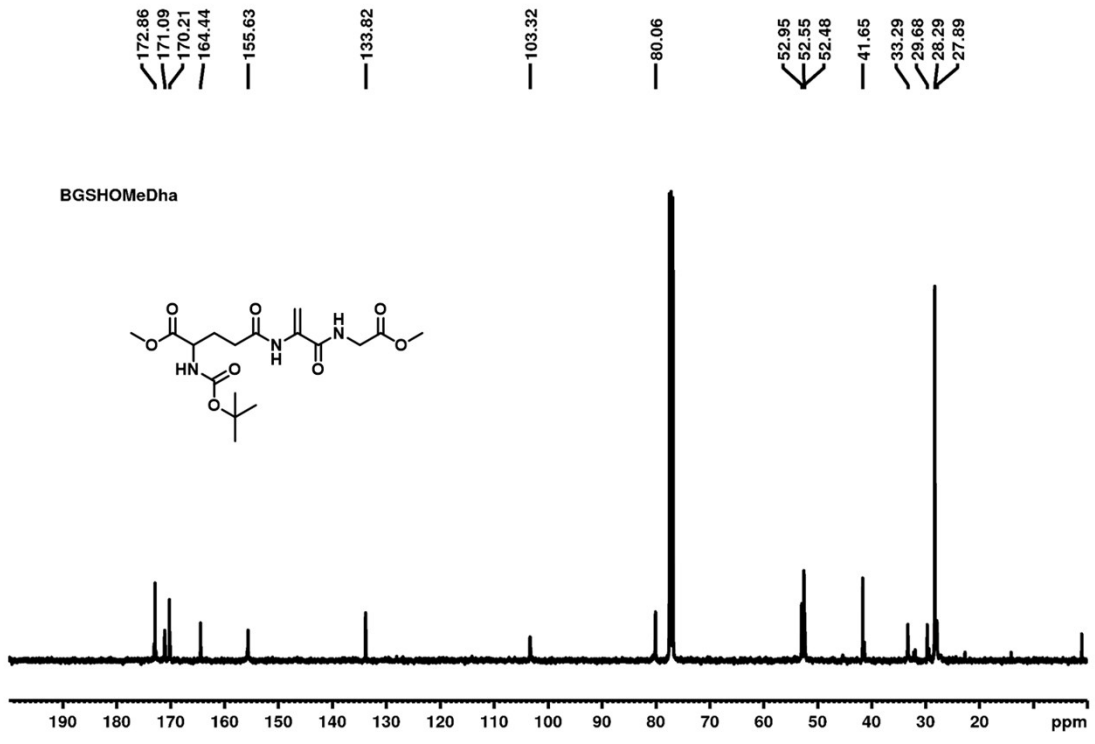

Figure **S41.** ^{13}C NMR spectrum (100.56 MHz, $CDCl_3$) of compound **13**

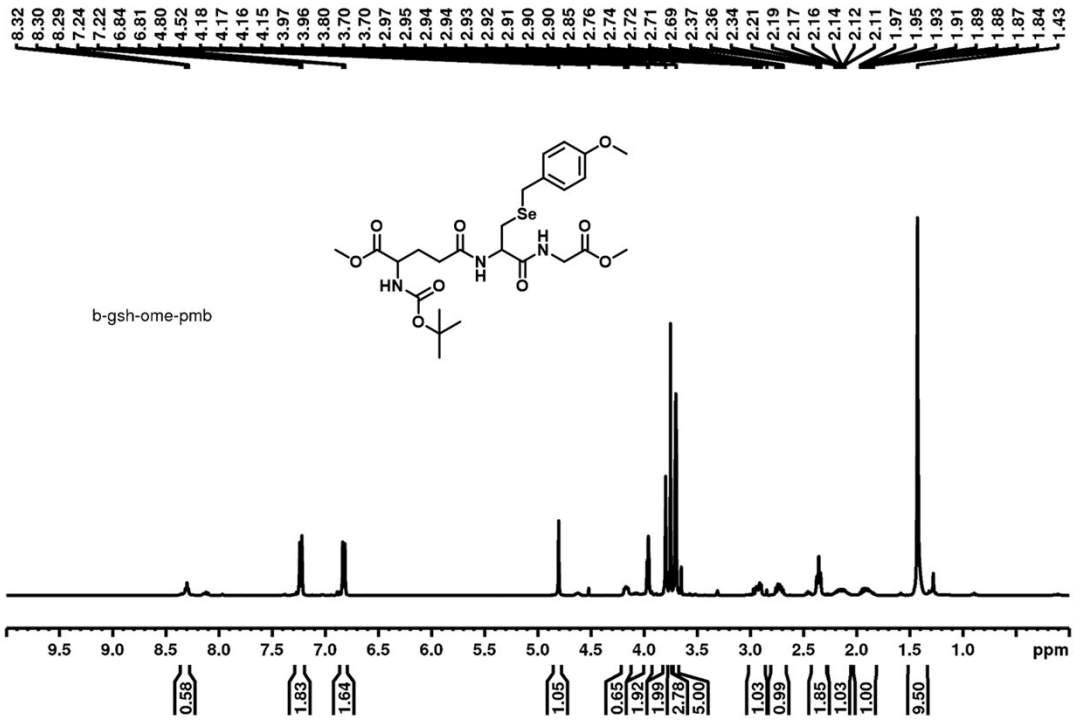

Figure **S42.** ^{1}H NMR spectrum (400 MHz, CD_3OD) of compound **18** [Boc-GSeH(pMob)-OMe]

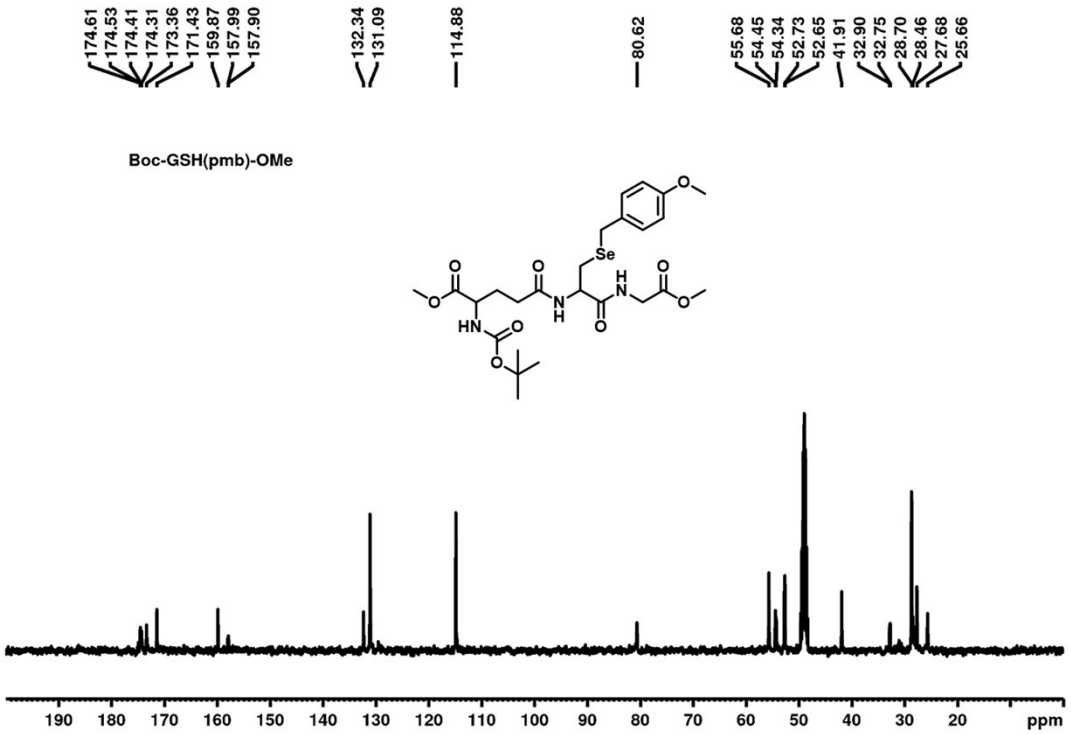

Figure **S43.** ^{13}C NMR spectrum (100.56 MHz, CD_3OD) of compound **18** [Boc-GSeH(pMob)-OMe]

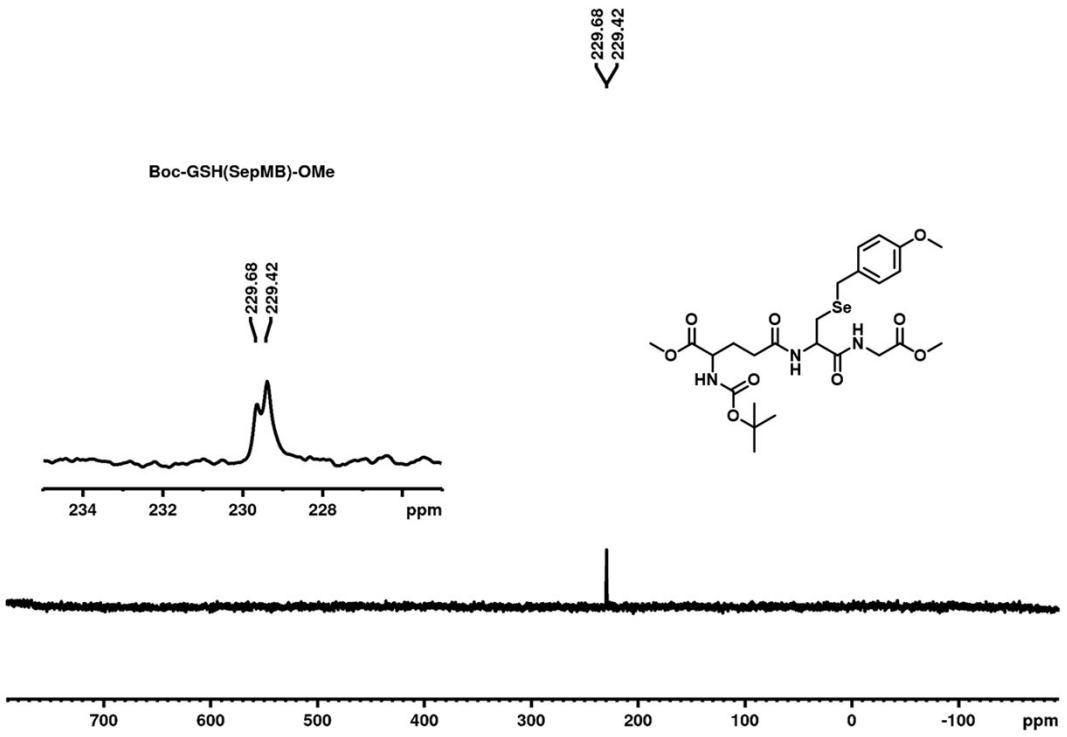

Figure **S44.** ^{77}Se NMR spectrum (76.29 MHz, CD_3OD) of compound **18** [Boc-GSeH(pMob)-OMe]

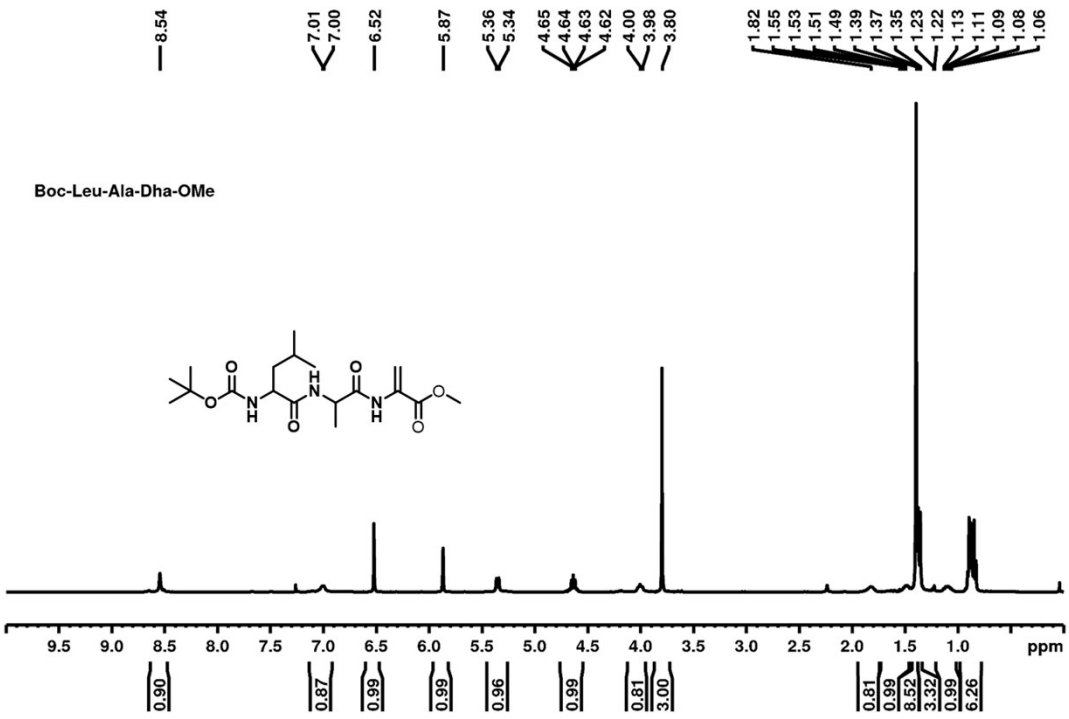

Figure **S45.** ^{1}H NMR spectrum (400 MHz, $CDCl_3$) of compound **17** [Boc-Leu-Ala-Dha-OMe]

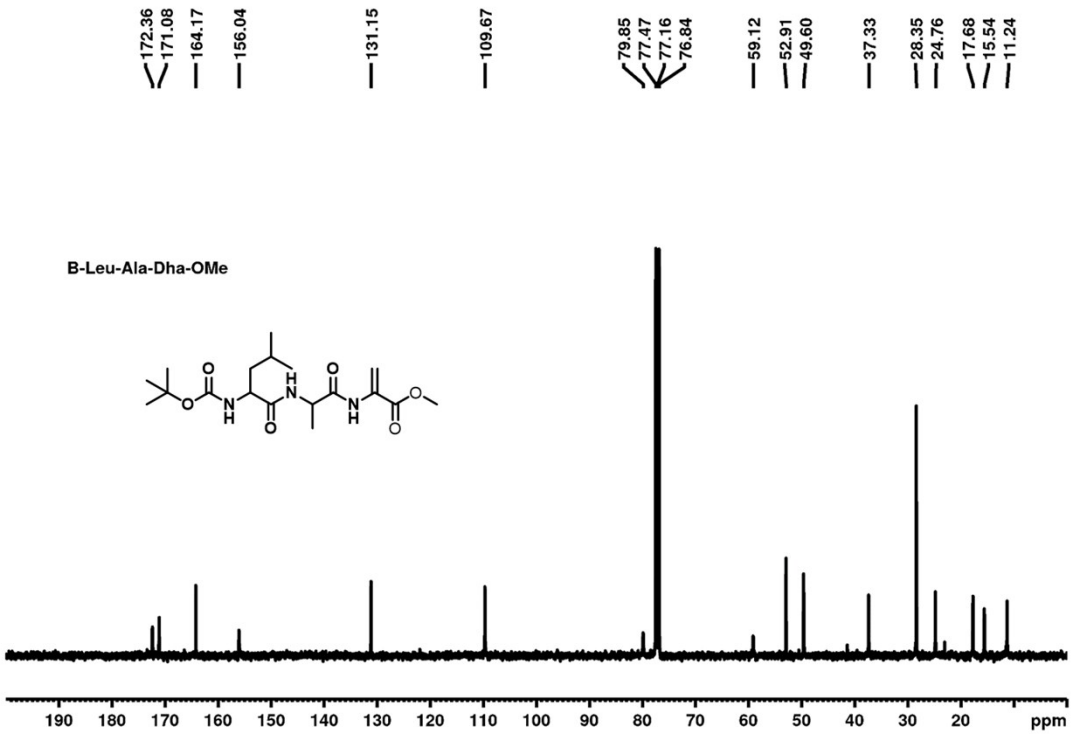

Figure **S46.** ^{13}C NMR spectrum (100.56 MHz, $CDCl_3$) of compound **17** [Boc-Leu-Ala-Dha-OMe]

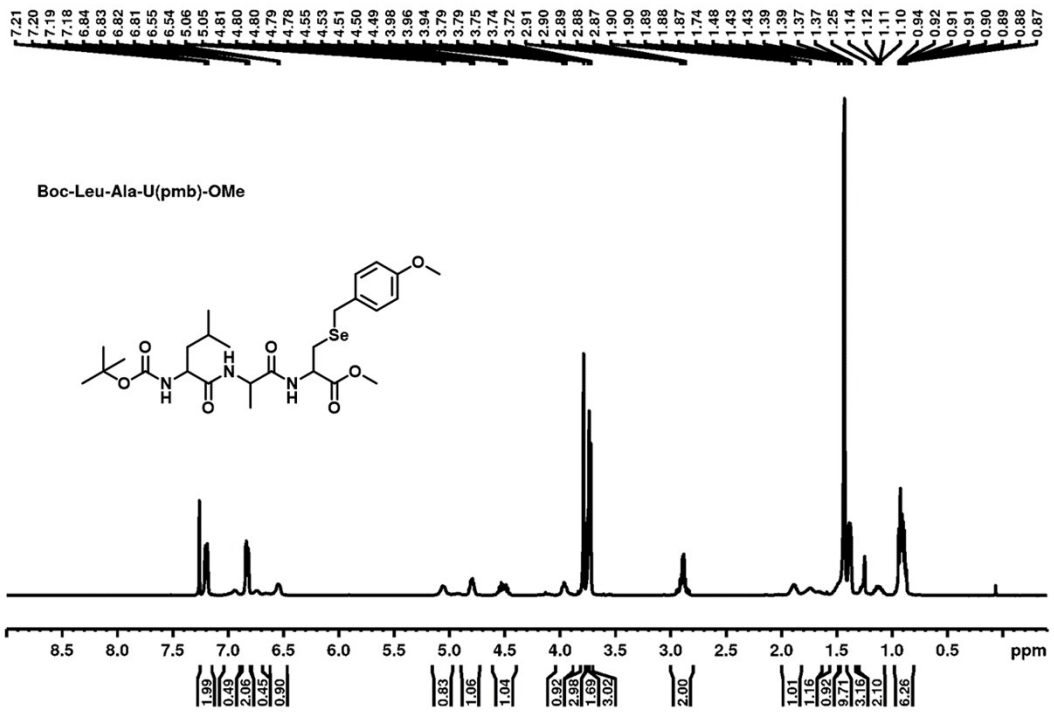

Figure **S47.** ^{1}H NMR spectrum (400 MHz, $CDCl_3$) of compound **19** [Boc-Leu-Ala-Sec(pMob)-OMe]

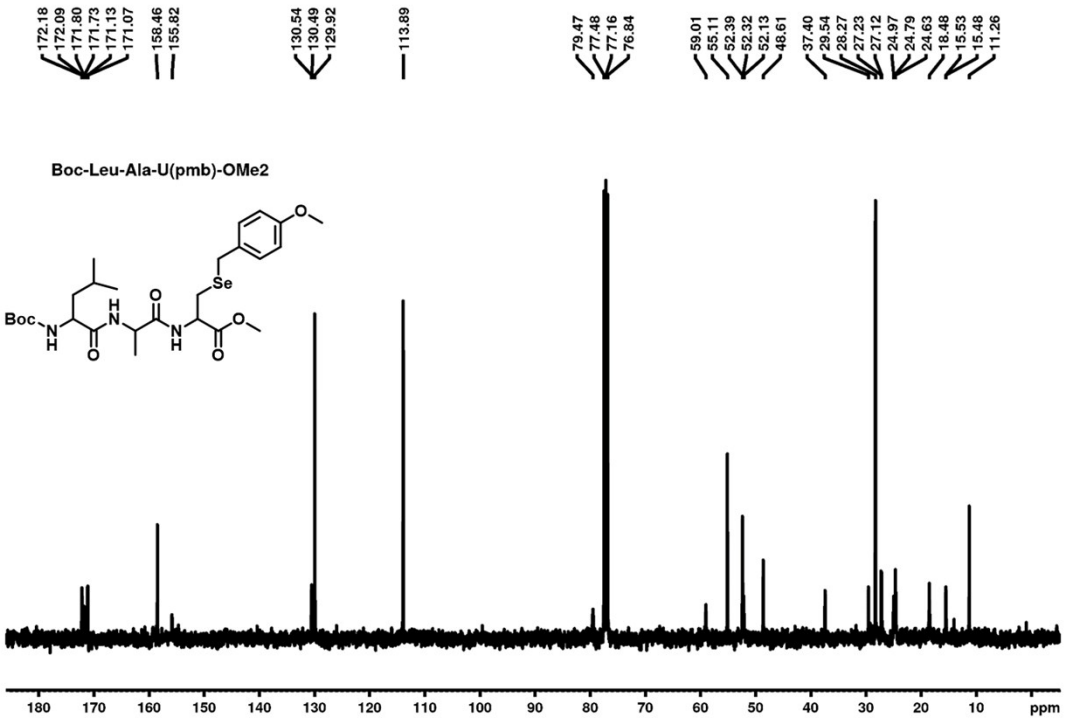

Figure **S48.** ^{13}C NMR spectrum (100.56 MHz, $CDCl_3$) of compound **19** [Boc-Leu-Ala-Sec(pMob)-OMe]

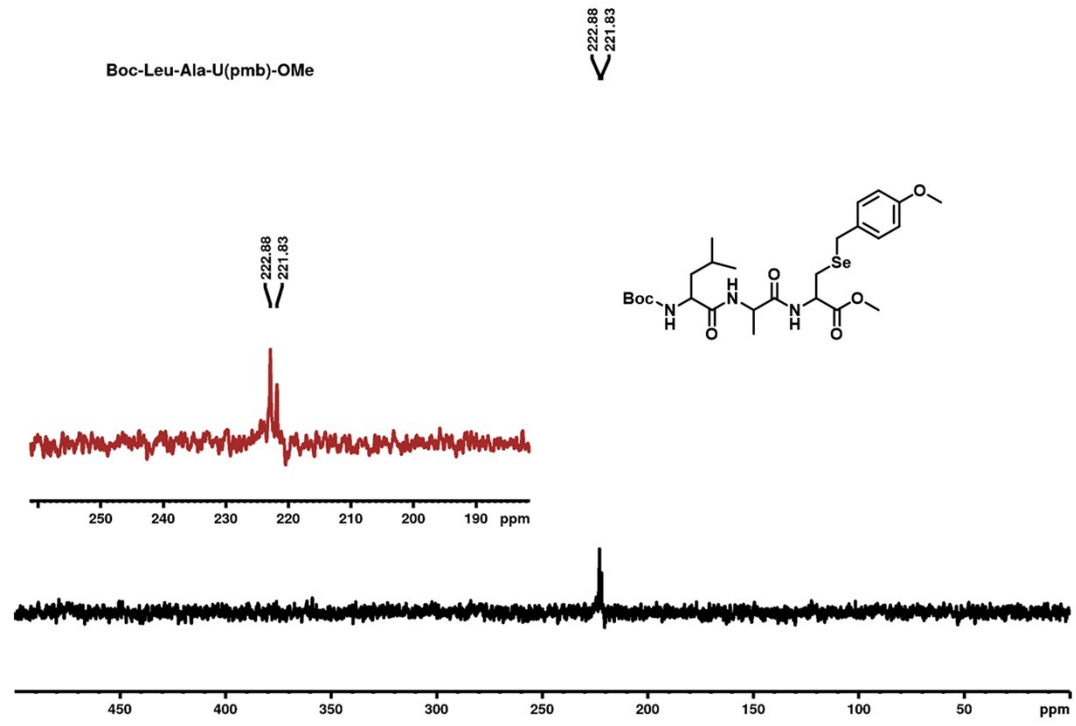

Figure **S49.** ^{77}Se NMR spectrum (76.29 MHz, $CDCl_3$) of compound **19** [Boc-Leu-Ala-Sec(pMob)-OMe]

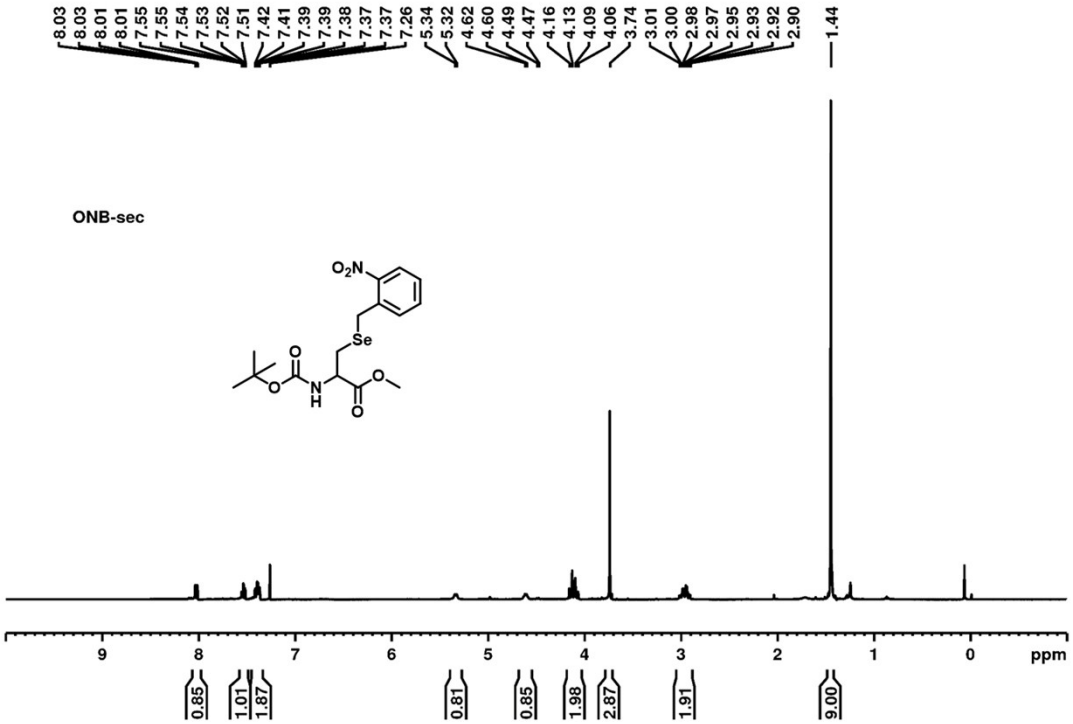

Figure **S50.** ^{1}H NMR spectrum (400 MHz, $CDCl_3$) of compound **22** [Boc-Sec(oNB)-OMe]

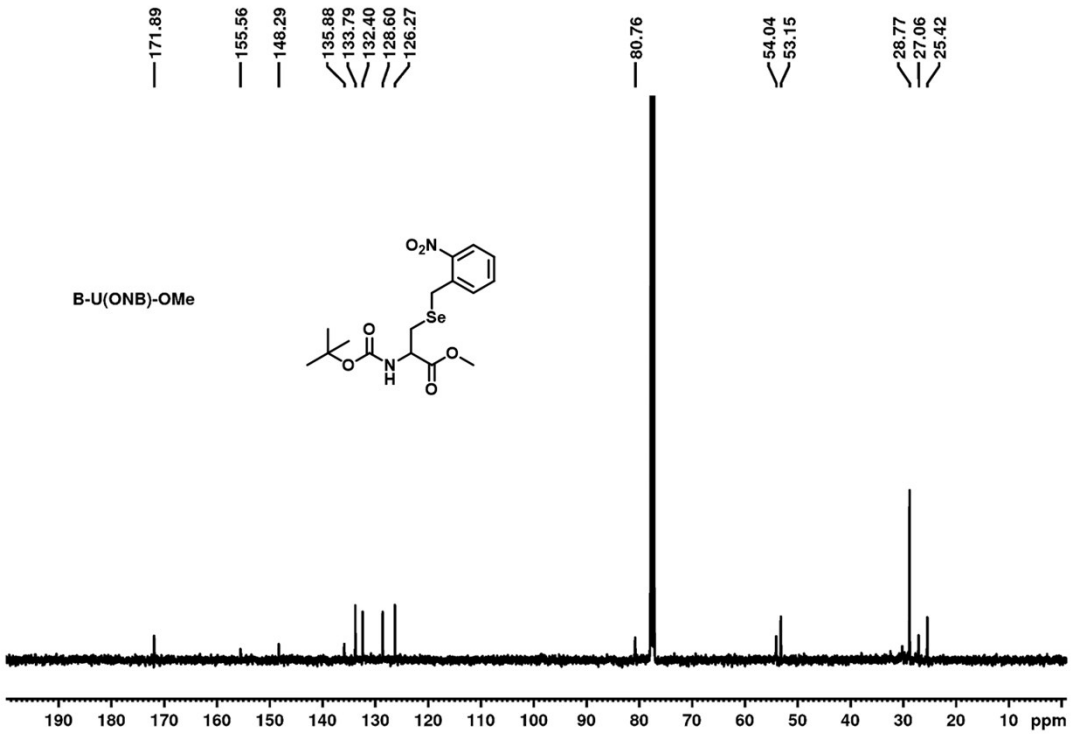

Figure **S51.** ^{13}C NMR spectrum (100.56 MHz, $CDCl_3$) of compound **22** [Boc-Sec(oNB)-OMe]

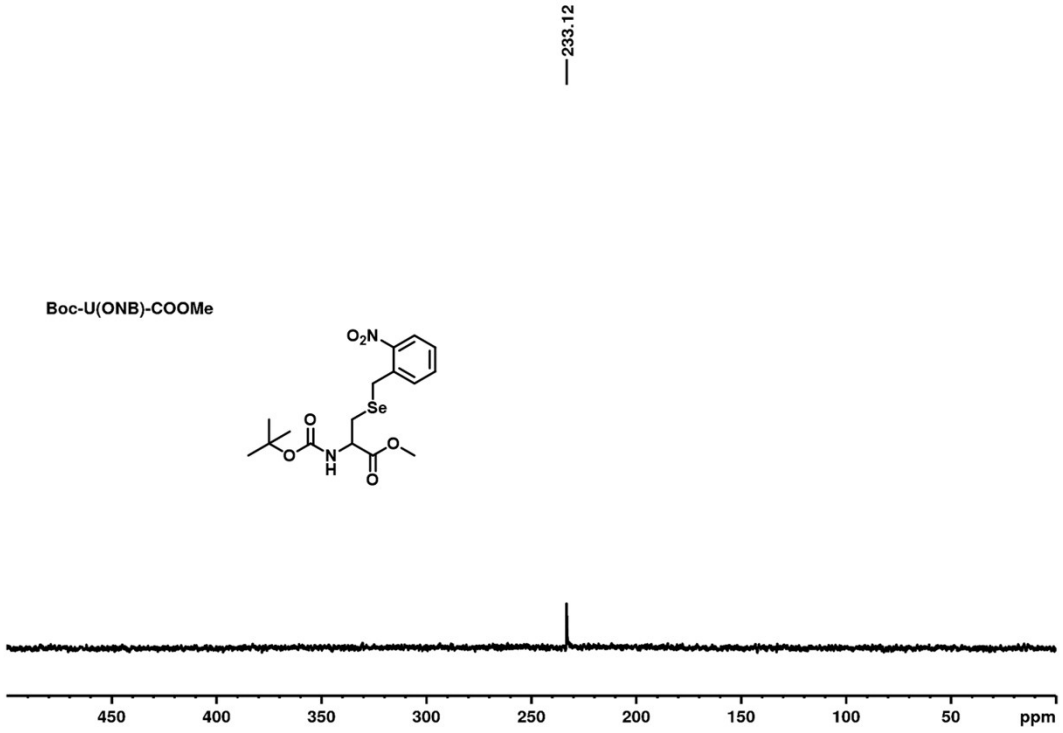

Figure **S52.** ^{77}Se NMR spectrum (76.29 MHz, $CDCl_3$) of compound **22** [Boc-Sec(oNB)-OMe]

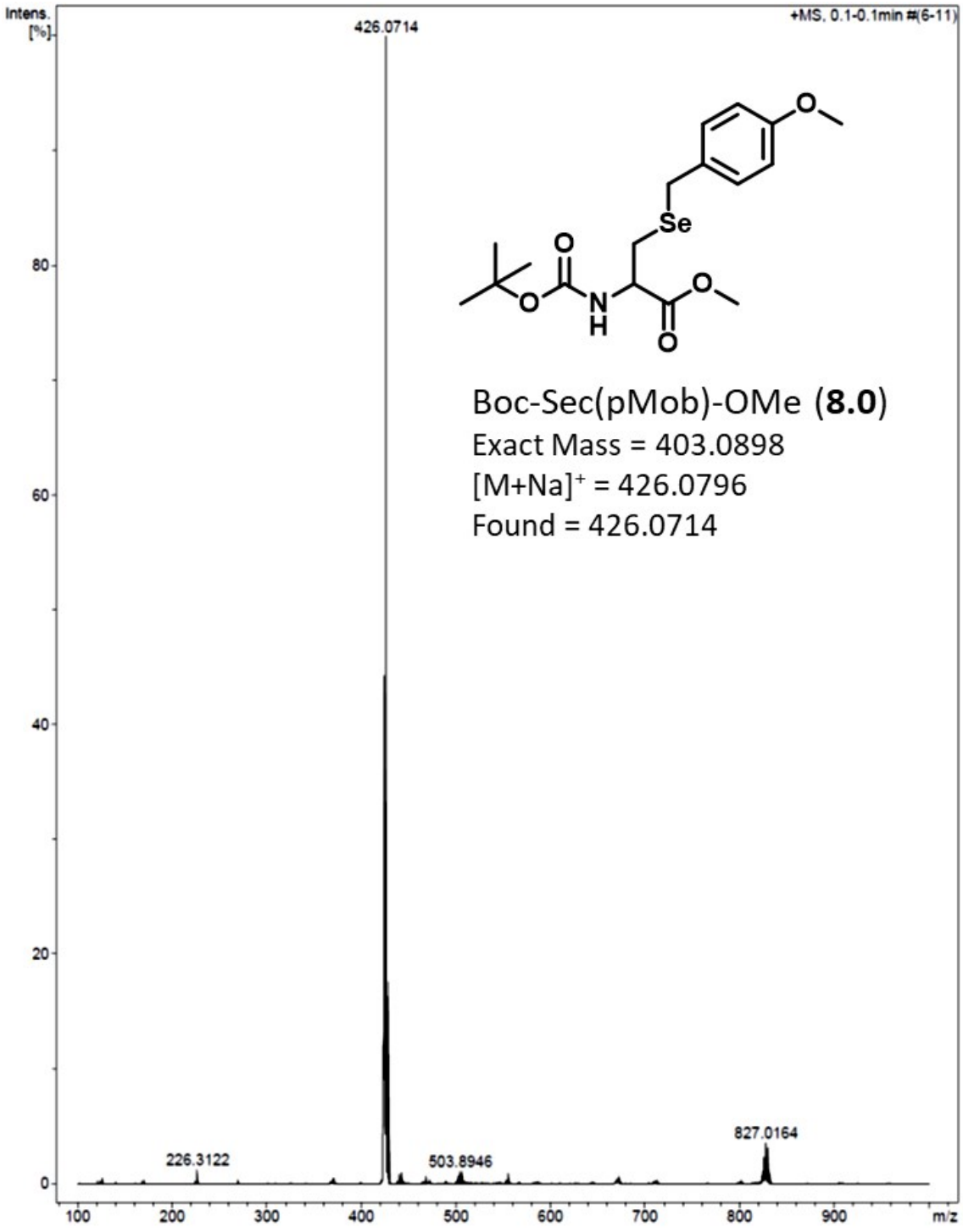

Figure **S53.** ESI- Mass Spectrum of compound **8** [Boc-Sec(pMob)-OMe]

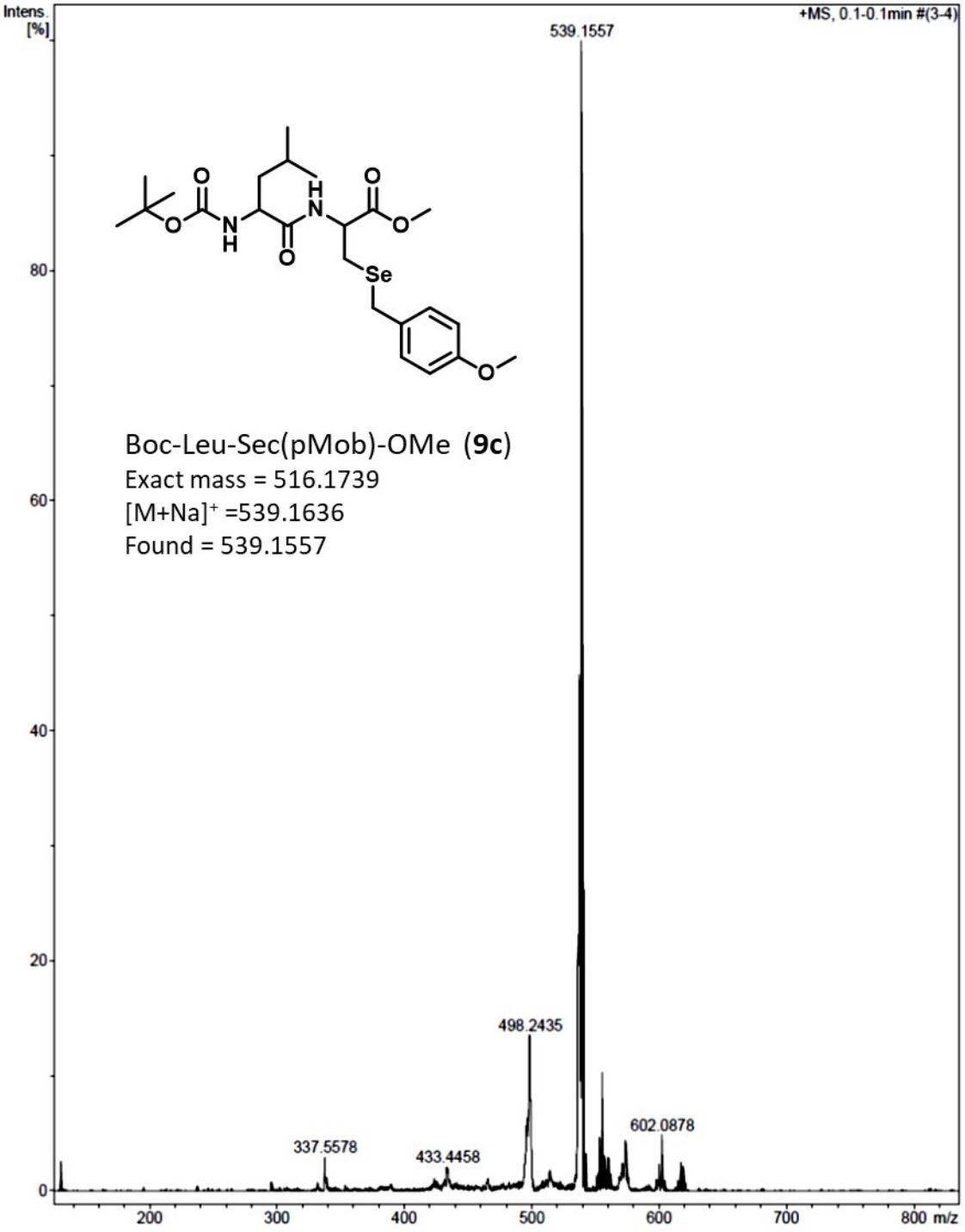

Figure **S54.** ESI- Mass spectrum of compound **9c** [Boc-Leu-Sec(pMob)-OMe]

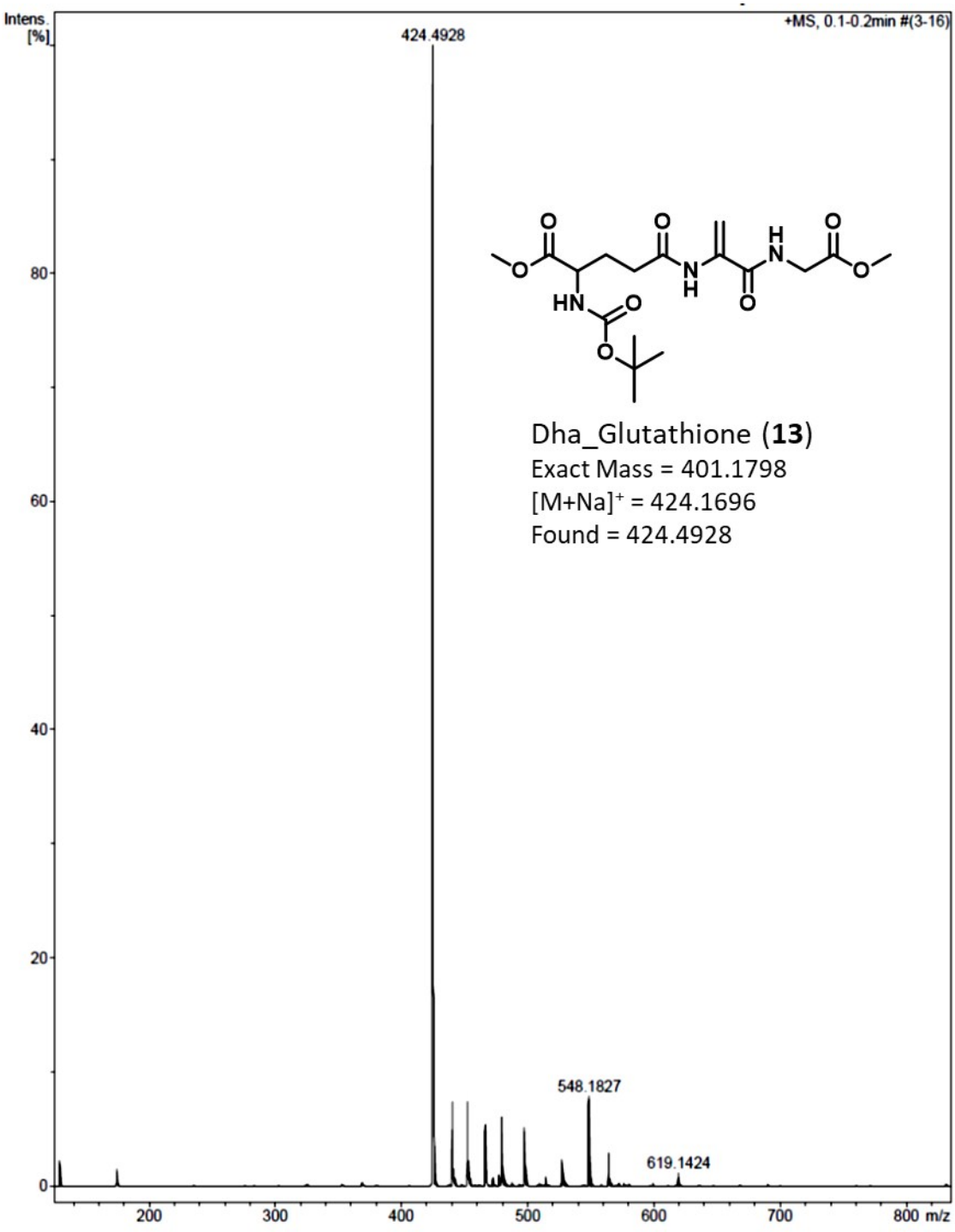

Figure **S55.** ESI- Mass spectrum of compound **13** [pMob-Selenoglutathione]

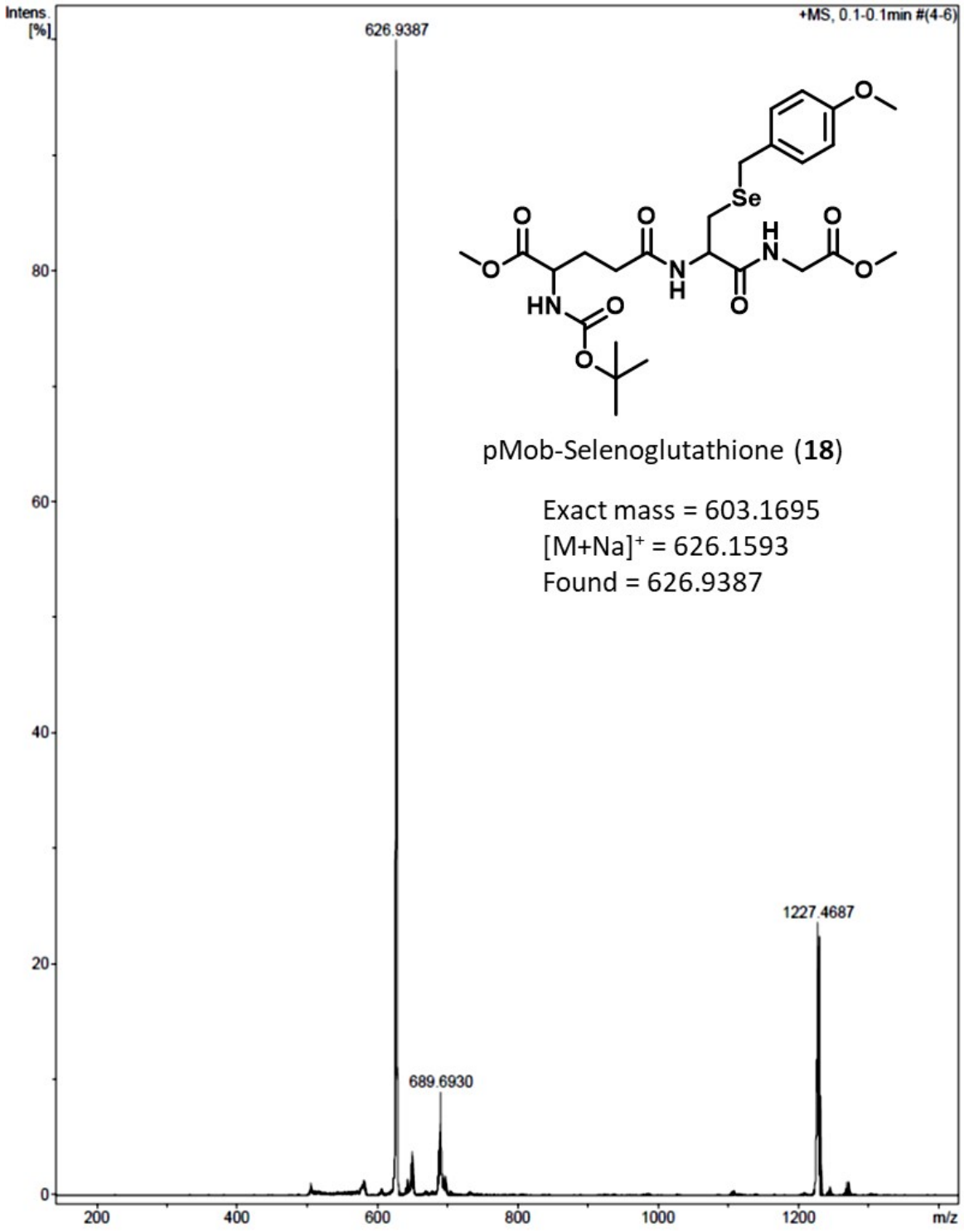

Figure **S56.** ESI- MS of compound **18** [pMob-Selenoglutathione]

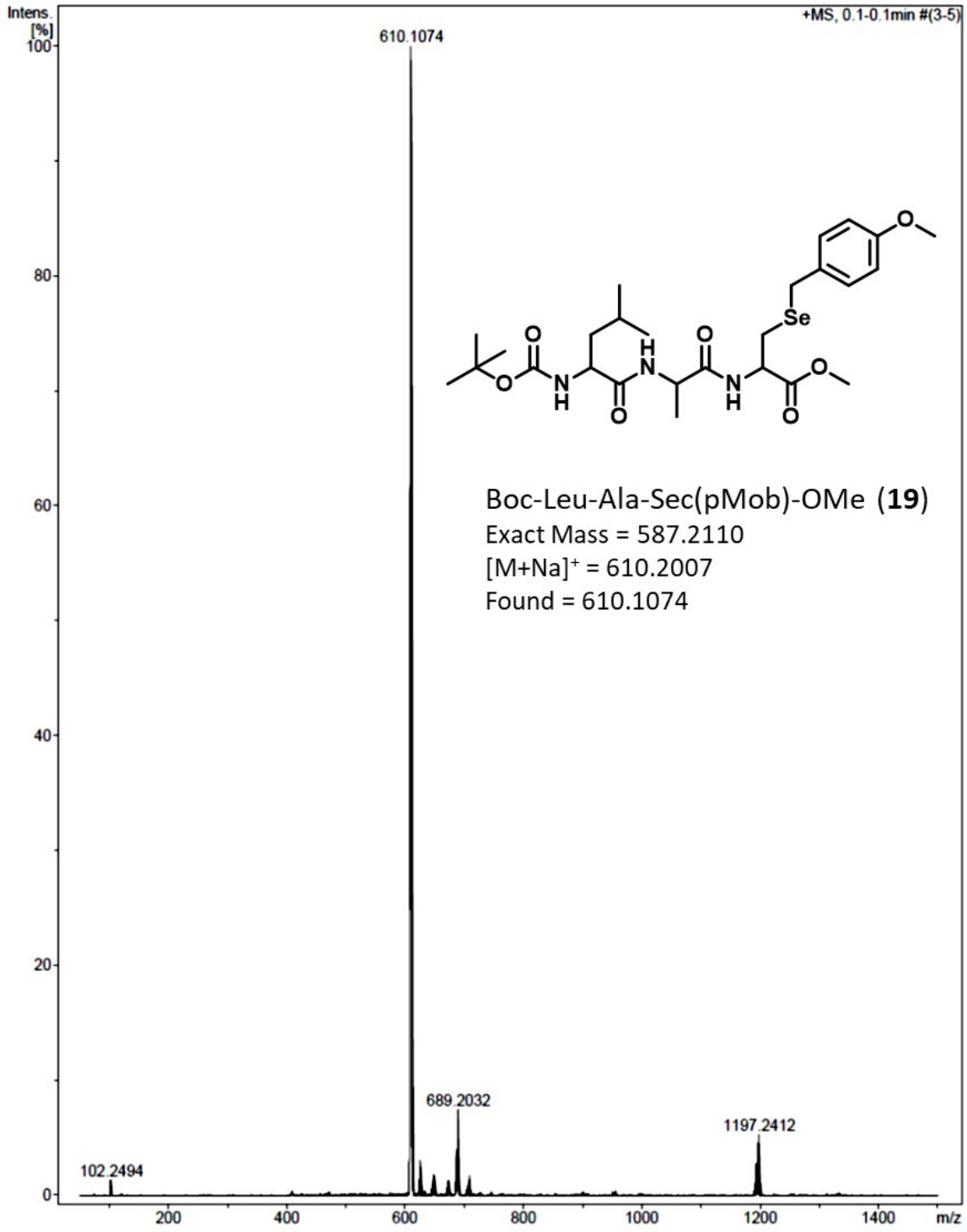

Figure **S57.** ESI- Mass Spectrum of compound **19** [Boc-Leu-Ala-Sec(pMob)-OMe]

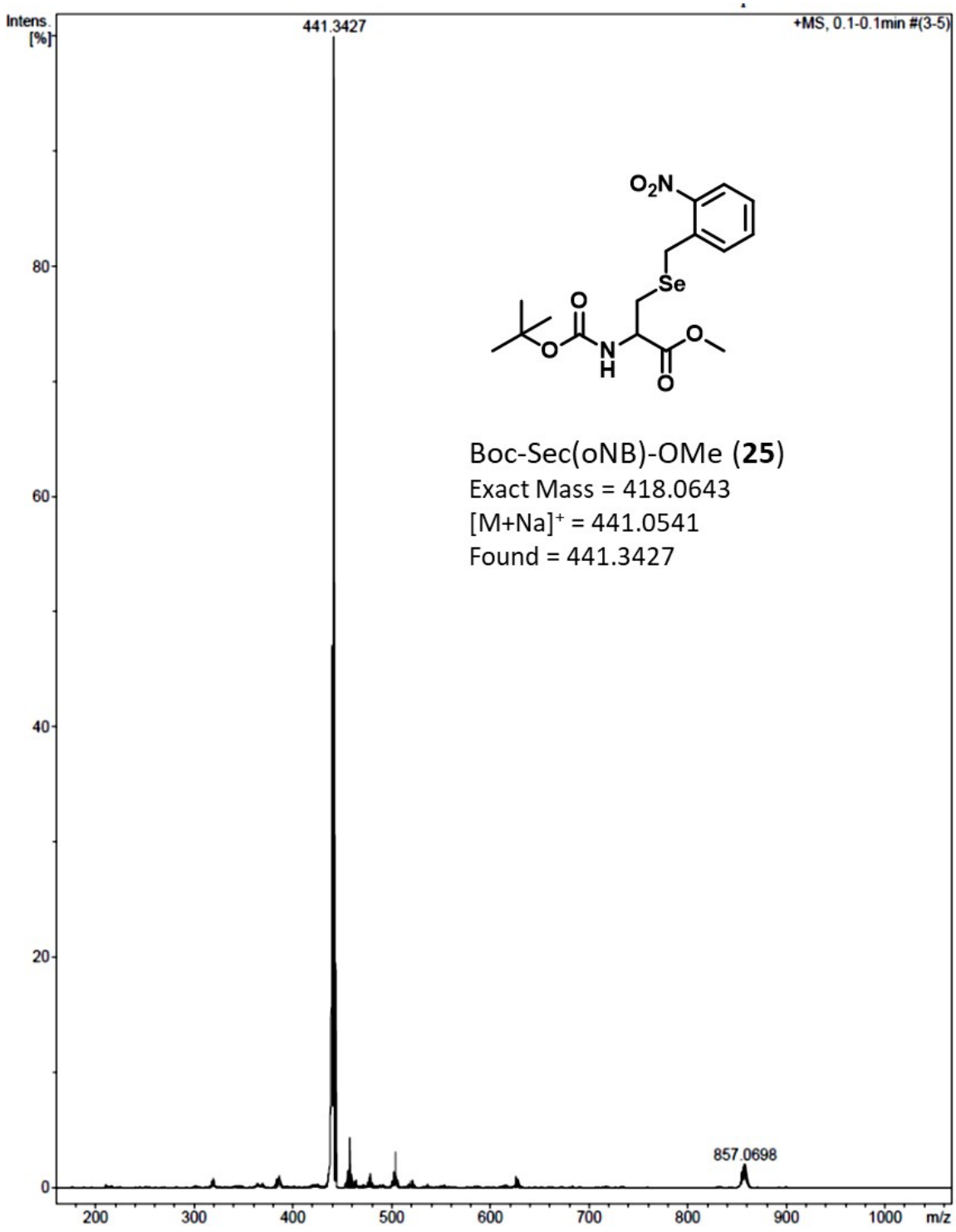

Figure **S58.** ESI- Mass Spectrum of compound **22** [Boc-Sec(oNB)-OMe]

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