Supporting information

Selenoglutathione Diselenide. Unique Redox Reactions in the GPx-Like Catalytic Cycle and Repairing of Disulfide Bonds in Scrambled Protein

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Supplementary Material

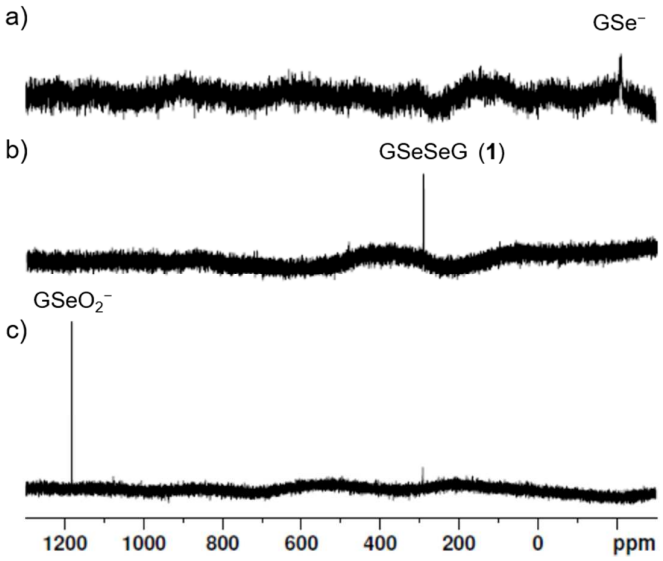


Figure S1. The reaction of GSe^- with GSSG or H_2O_2 in D_2O monitored by ^{77}Se NMR. a) A 1:3 mixture of GSeSeG and DTT^{red} in D_2O containing NaOH (pH 10). b) After addition of GSSG (3 eq) to a). c) After addition of H_2O_2 (6 eq) to a).

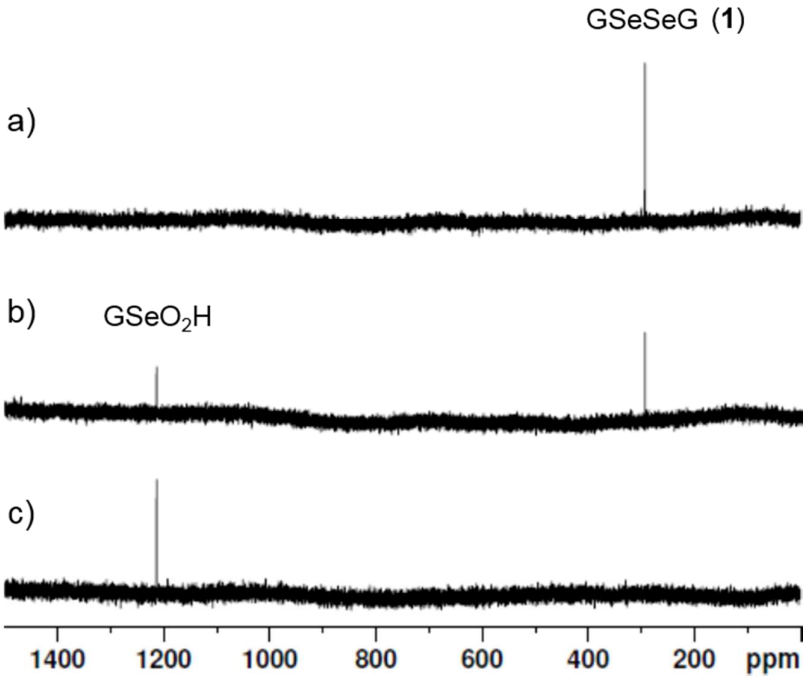


Figure S2. The reaction of GSeSeG with H_2O_2 in D_2O monitored by ^{77}Se NMR. a) GSeSeG (**1**). b) A 1:1 mixture of **1** and H_2O_2. c) A 1:3 mixture of **1** and H_2O_2.

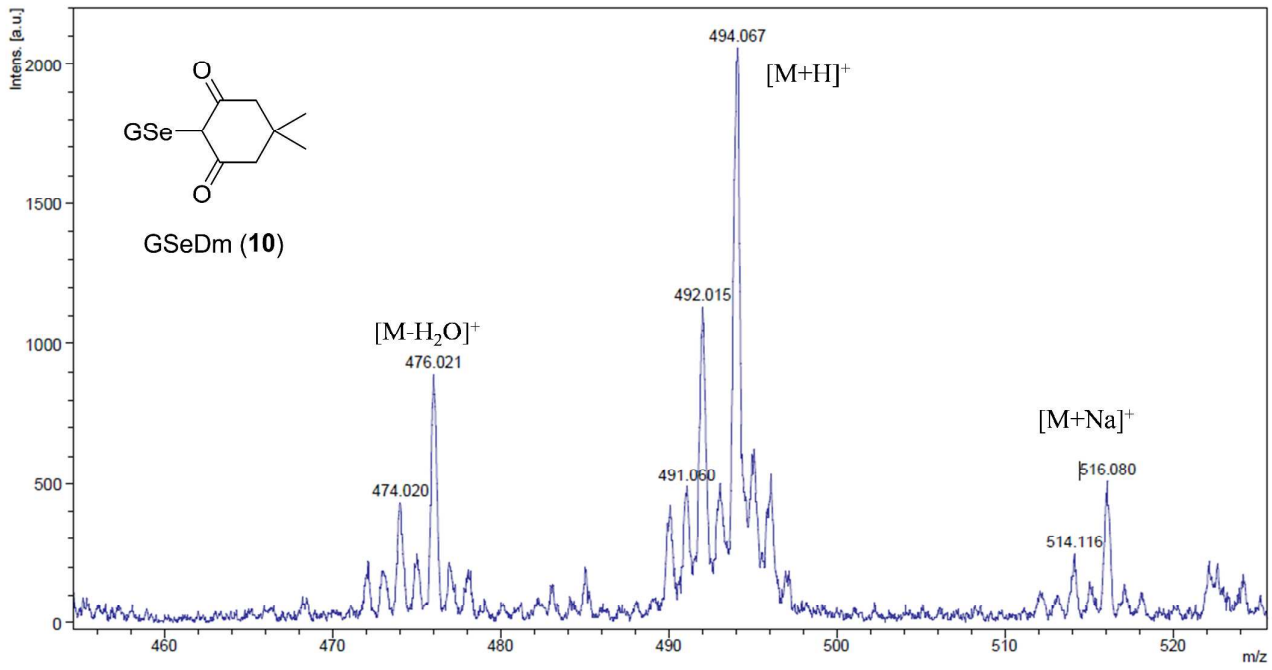


Figure S3. MALDI-TOF mass spectrum of a 1:1 mixture of GSeSeG and H_2O_2 in the presence of dimedone (6 eq) in H_2O.

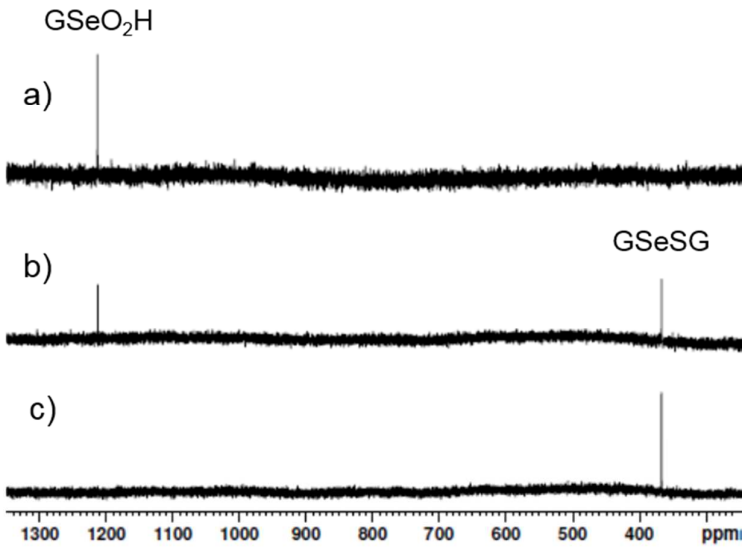


Figure S4. The reaction of $GSeO_2H$ with 1 or 2 eq GSH in D_2O monitored by ^{77}Se NMR. a) A 1:3 mixture of GSeSeG and H_2O_2. b) After addition of GSH (1 eq) to a). c) After addition of GSH (2 eq) to a).

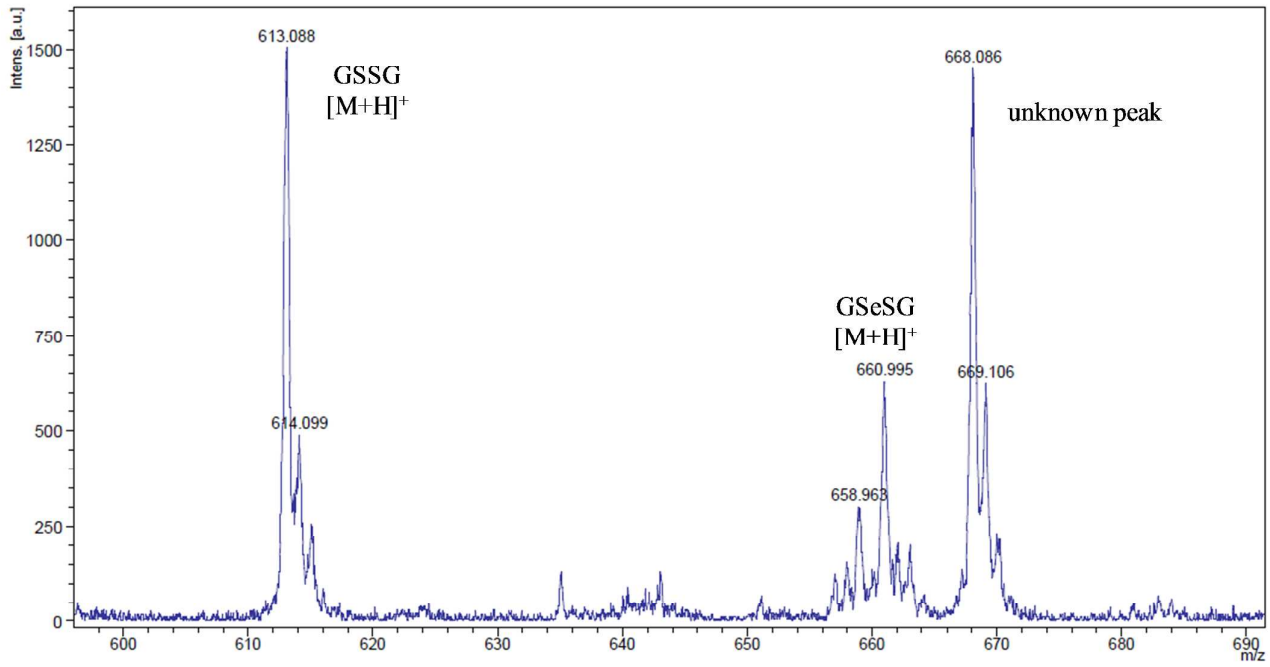


Figure S5. MALDI-TOF mass spectrum of a 1:3 mixture of $GSeO_2H$ and GSH in H_2O.

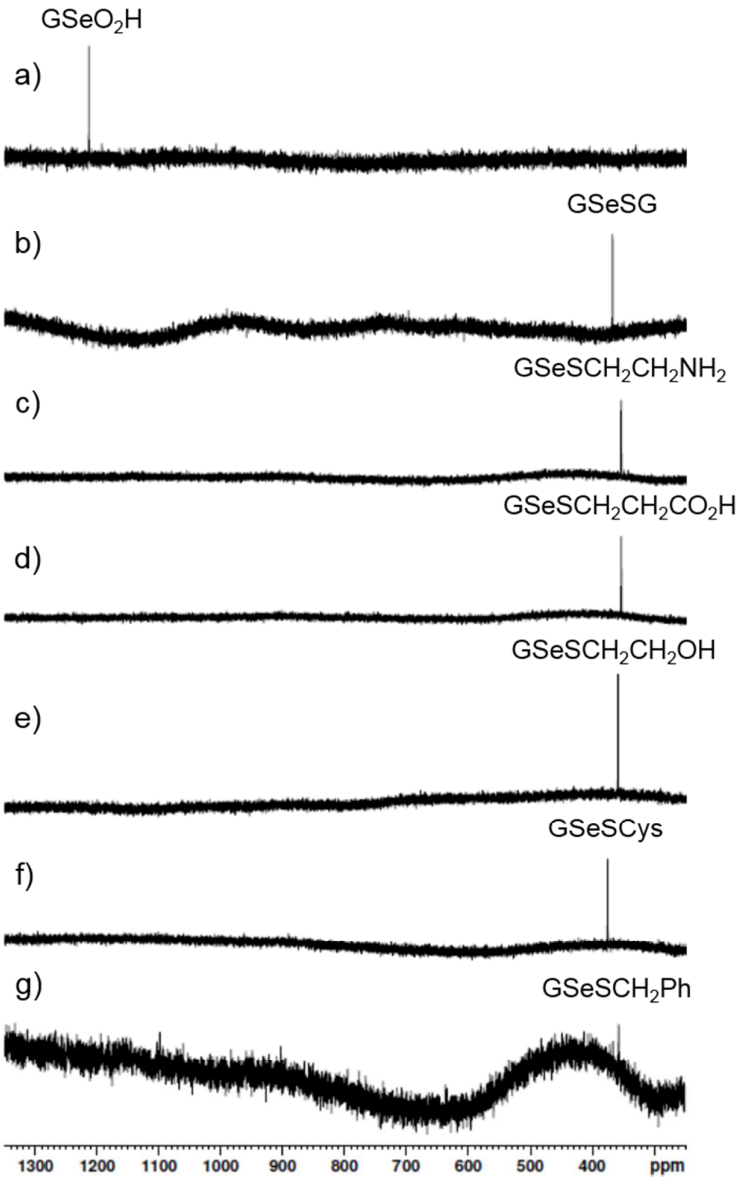


Figure S6. The reaction of $GSeO_2H$ with various thiols in D_2O monitored by ^{77}Se NMR. a) A 1:3 mixture of GSeSeG and H_2O_2 in D_2O. b) After addition of GSH (3 eq) to a). c) After addition of $HSCH_2CH_2NH_2$ (3 eq) to a). d) After addition of $HSCH_2CH_2CO_2H$ (3 eq) to a). e) After addition of $HSCH_2CH_2OH$ (3 eq) to a). f) After addition of CysSH (3 eq) to a). g) After addition of $HSCH_2Ph$ (3 eq) to a). Dibenzyl disulfide was precipitated.

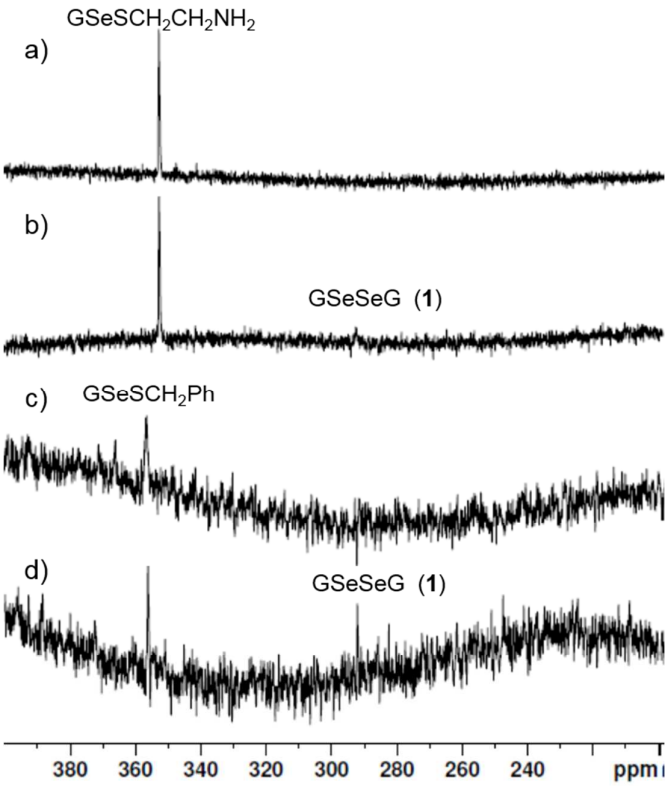


Figure S7. Disproportionation of $GSeSCH_2CH_2NH_2$ and $GSeSCH_2Ph$ in D_2O monitored by ^{77}Se NMR. a) A mixture of $GSeO_2H$ and $HSCH_2CH_2NH_2$ (1:3) after 10 min. b) A mixture of $GSeO_2H$ and $HSCH_2CH_2NH_2$ (1:3) after 24 h. c) A mixture of $GSeO_2H$ and $PhCH_2SH$ (1:3) after 10 min. d) A mixture of $GSeO_2H$ and $PhCH_2SH$ (1:3) after 24 h.

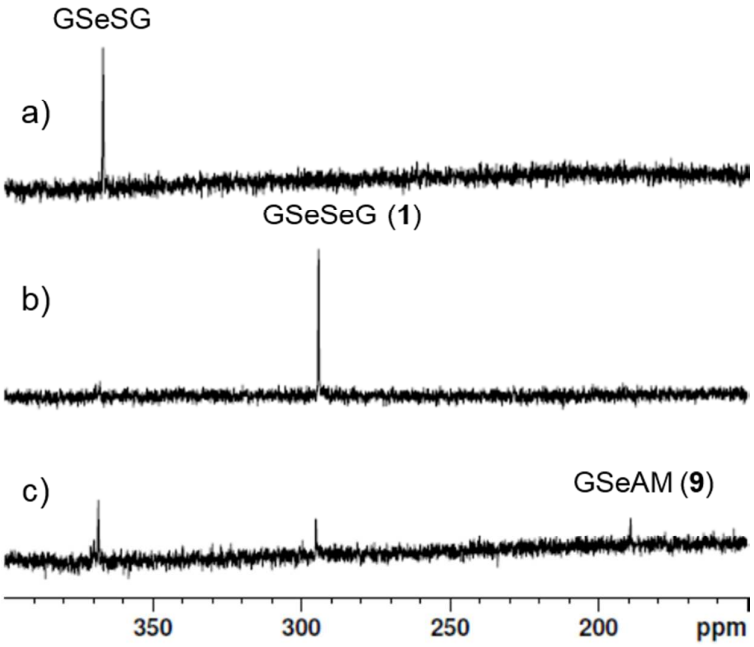


Figure S8. Reduction of GSeSG with DTT^{red} in the absence or presence of IAM in D_2O monitored by ^{77}Se NMR. a) A mixture of $GSeO_2H$ and GSH (1:3) after 10 min. b) After addition of DTT^{red} (2 eq) to a). c) After addition of DTT^{red} (2 eq) to a) in the presence IAM (5 eq).

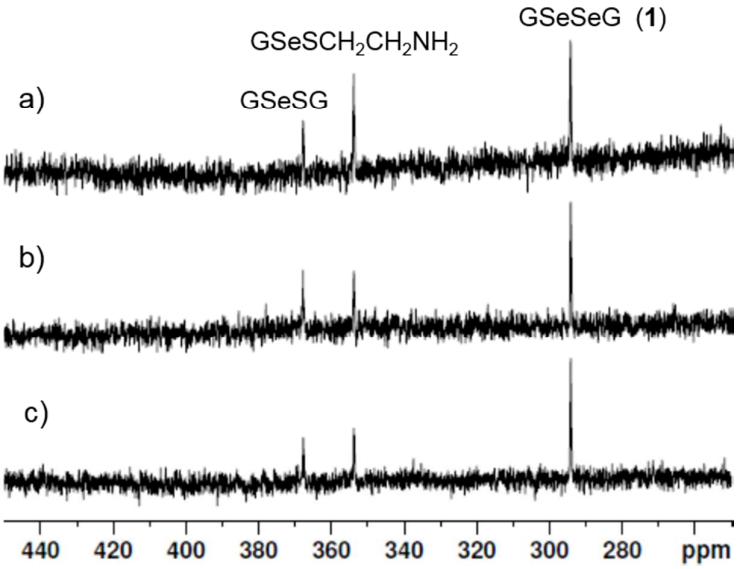


Figure S9. The reaction of GSeSG with $HSCH_2CH_2NH_2$ in D_2O monitored by ^{77}Se NMR. a) A mixture of GSeSG and $HSCH_2CH_2NH_2$ (1:3) after 10 min. b) After 6 h. c) After 24 h.

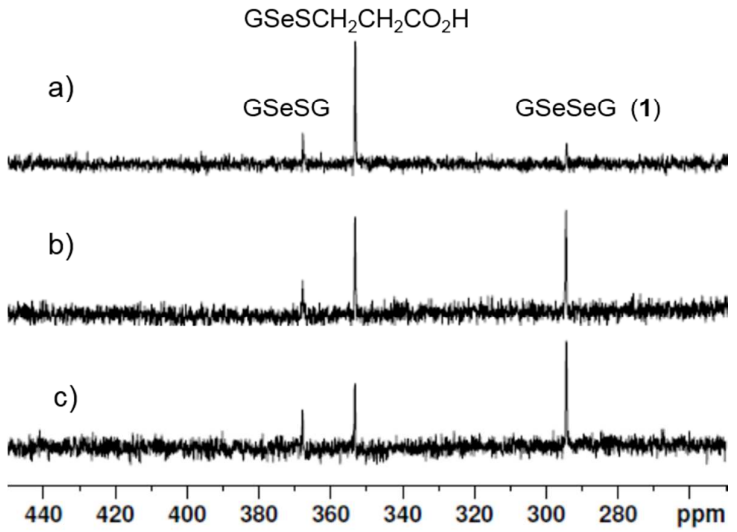


Figure S10. The reaction of GSeSG with $HSCH_2CH_2CO_2H$ in D_2O monitored by ^{77}Se NMR. a) A mixture of GSeSG and $HSCH_2CH_2CO_2H$ (1:3) after 10 min. b) After 6 h. c) After 24 h.

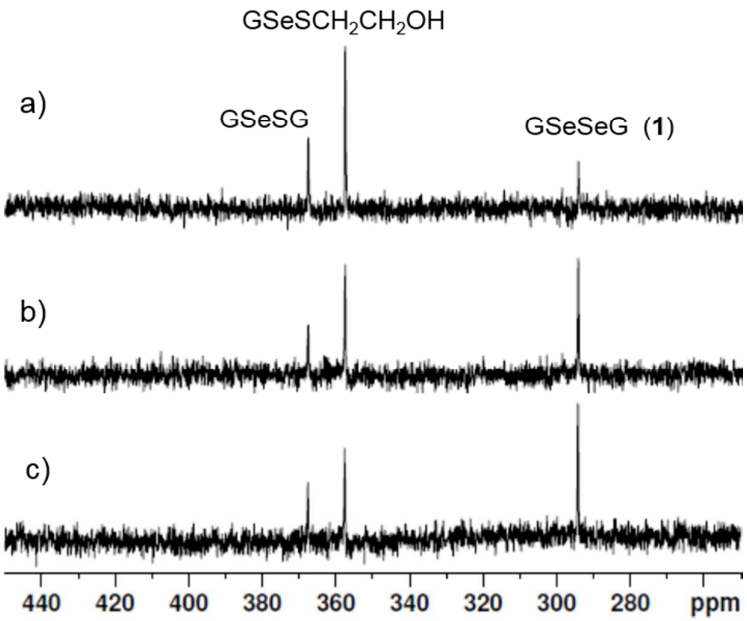


Figure S11. The reaction of GSeSG with $HSCH_2CH_2OH$ in D_2O monitored by ^{77}Se NMR. a) A mixture of GSeSG and $HSCH_2CH_2OH$ (1:3) after 10 min. b) After 6 h. c) After 24 h.

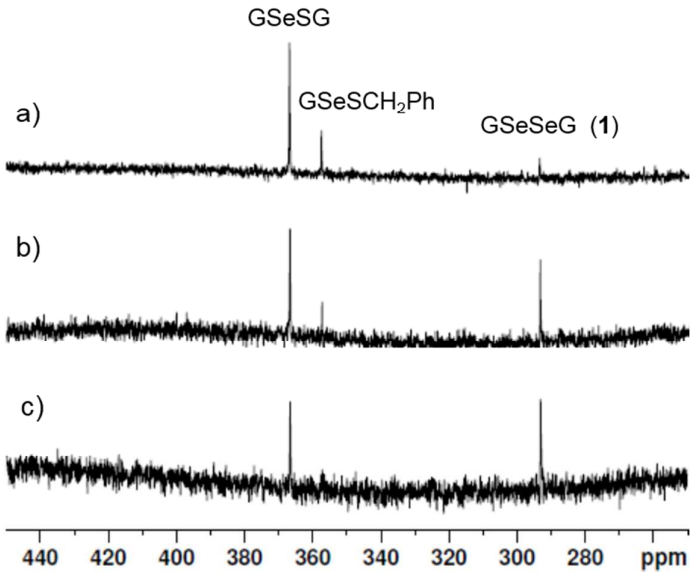


Figure S12. The reaction of GSeSG with $HSCH_2Ph$ in D_2O monitored by ^{77}Se NMR. a) A mixture of GSeSG and $HSCH_2Ph$ (1:3) after 10 min. b) After 6 h. c) After 24 h.

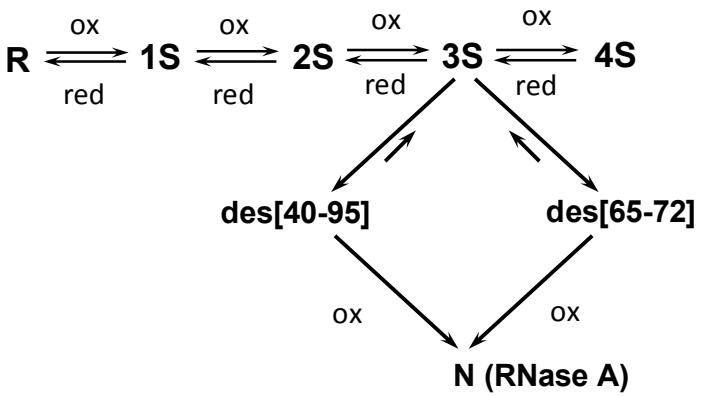


Figure S13. Oxidative folding pathways of RNase A. R, 1S, 2S, 3S, 4S, des[40-95], des[65-72], and N represent reduced RNase A, ensembles of one-disulfide, two-disulfide, three-disulfide, and four-disulfide intermediates, a three-disulfide intermediate having three native disulfide bonds but lacking one native disulfide bond of Cys40—Cys95, a three-disulfide intermediate having three native disulfide bonds but lacking one native disulfide bond of Cys65—Cys72, and native RNase A, respectively. (Rothwarf, D. M., Li, Y.-J., Scheraga, H. A. *Biochemistry* **1998**, *37*, 3760–3766; Rothwarf, D. M., Li, Y.-J., Scheraga, H. A. *Biochemistry* **1998**, *37*, 3767–3776.)

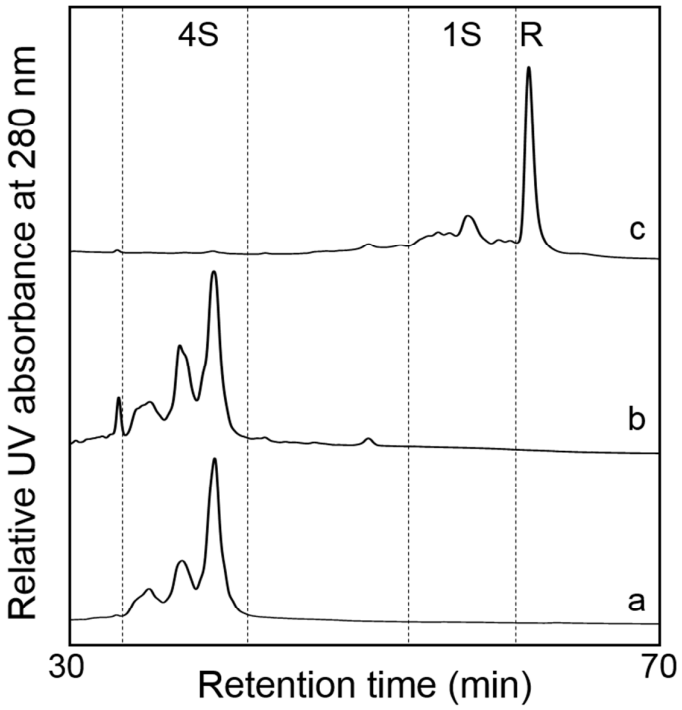
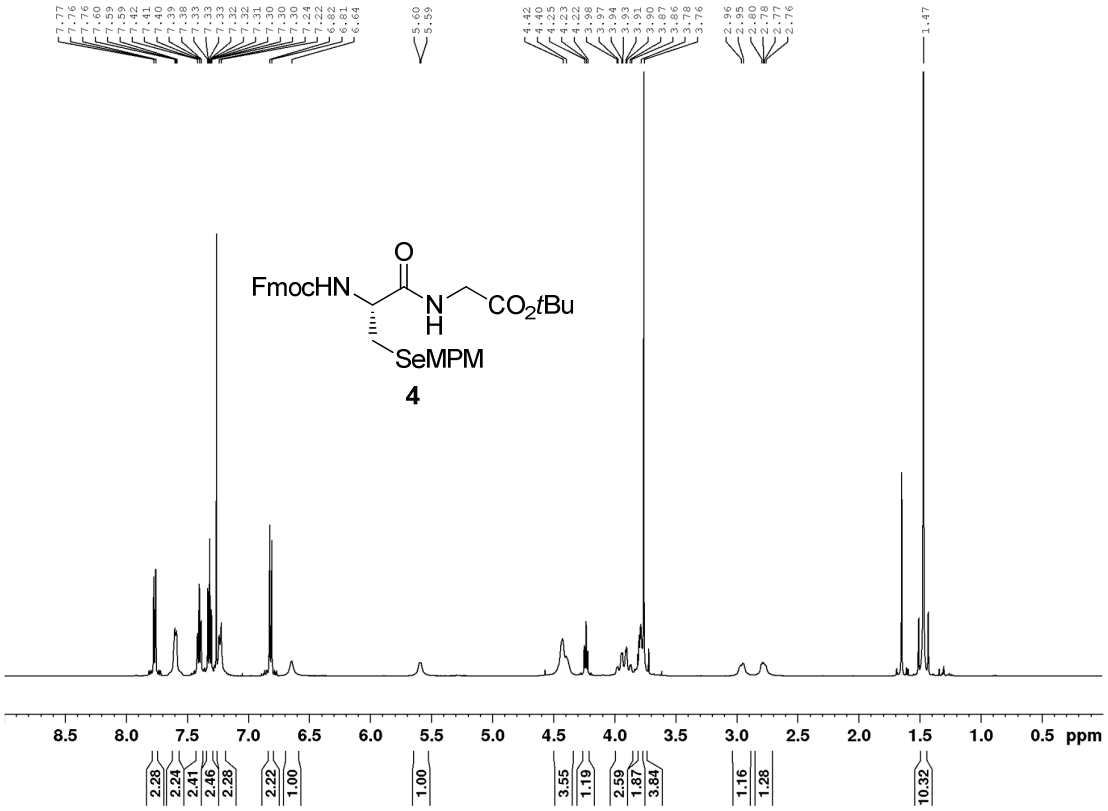


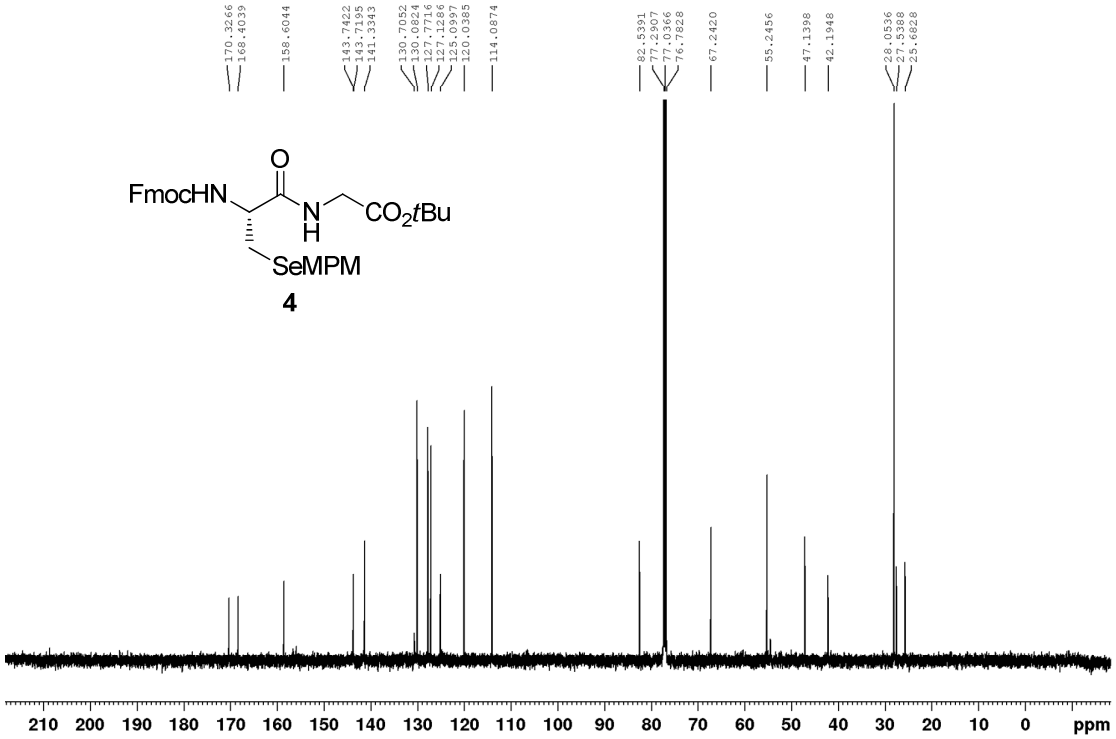
Figure S14. HPLC chromatograms obtained from the refolding experiment of 4S of RNase A using GSeSeG (**1**) or GSSG in presence of NADPH and GR at pH 7.5 and 25°C. The reaction conditions were $[4S]_0$ = 15 μM, $[NADPH]_0$ = 0.3 mM, and [GR] = 4U/mL. a) Before addition of the catalyst, b) 3 h after addition of GSSG (15 μM), and c) 15 min after addition of GSeSeG (15 μM).

_N_-(9-fluolenylmethoxycarbonyl)-_Se_-(_p_-methoxybenzyl)-L-selenocysteinyl-glycine-_tert_-butyl ester (4)

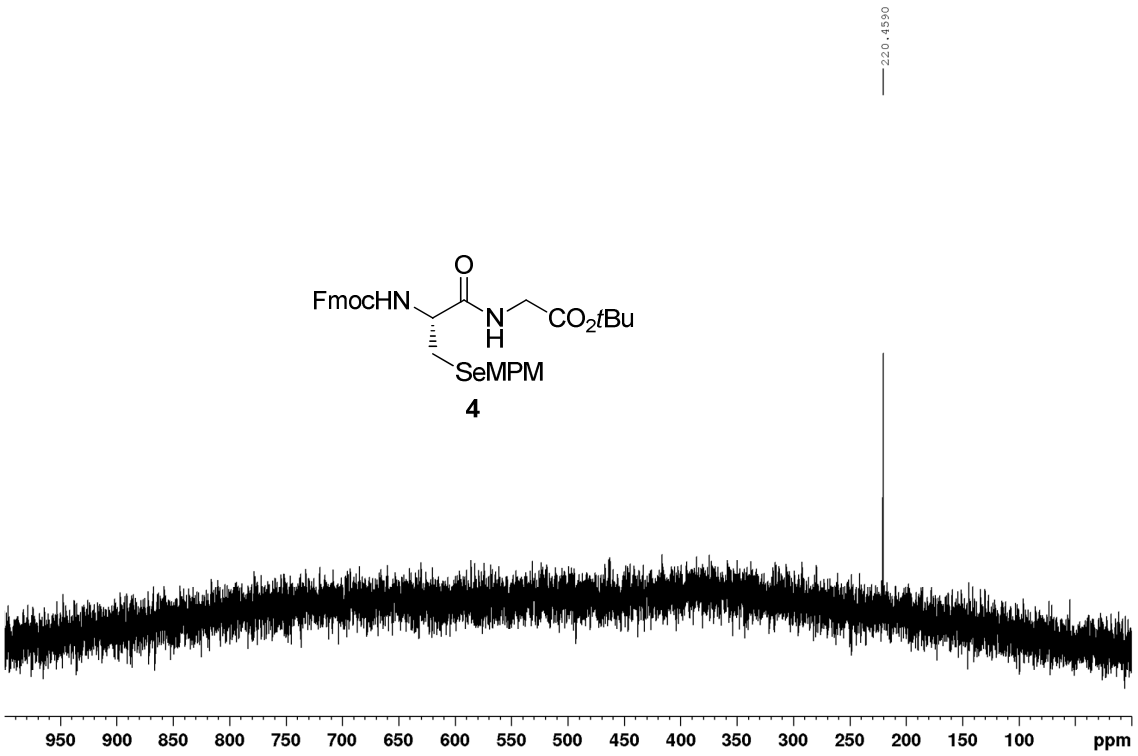
^{1}H in $CDCl_3$



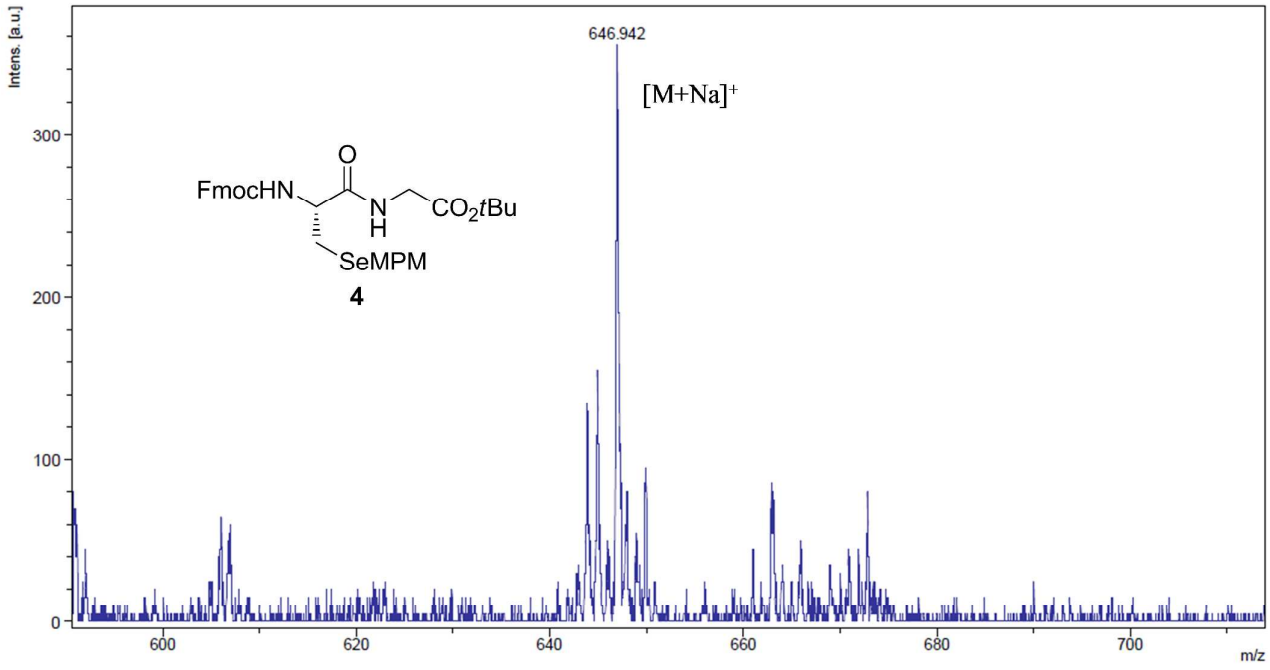
^{13}C in $CDCl_3$



^{77}Se in $CDCl_3$

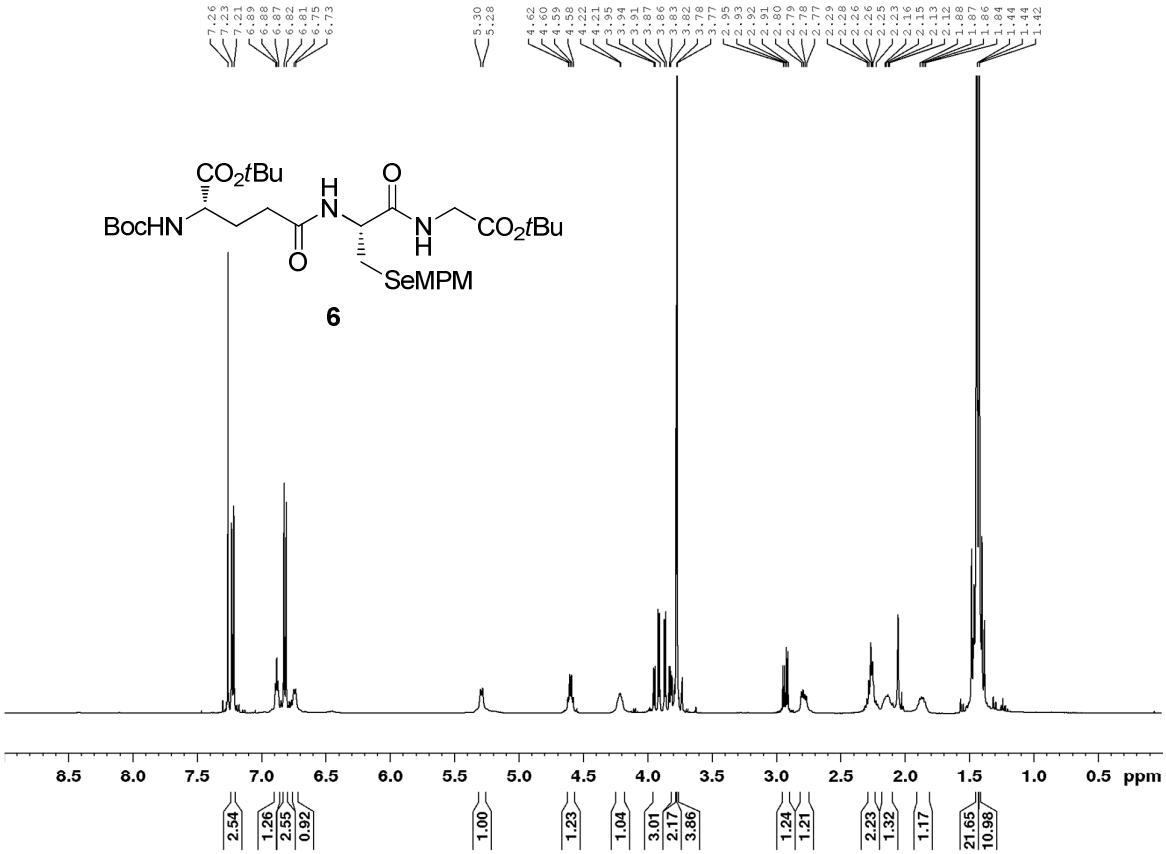


MALDI-TOF-MS

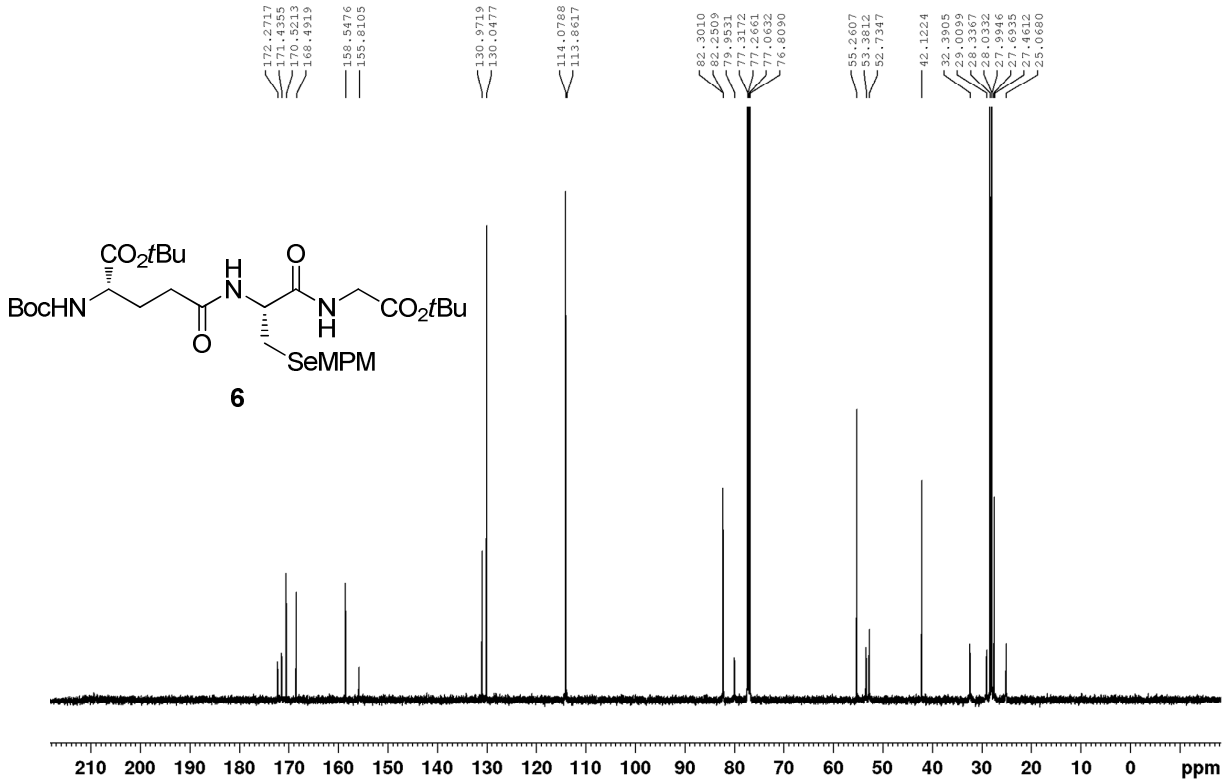


***N*-(*tert*-butoxycarbonyl)-*O*^{1}-*tert*-butyl-L-glutamyl-*Se*-(*p*-methoxybenzyl)-L-selenocysteinyl-glycine-*tert*-butyl ester (6)**

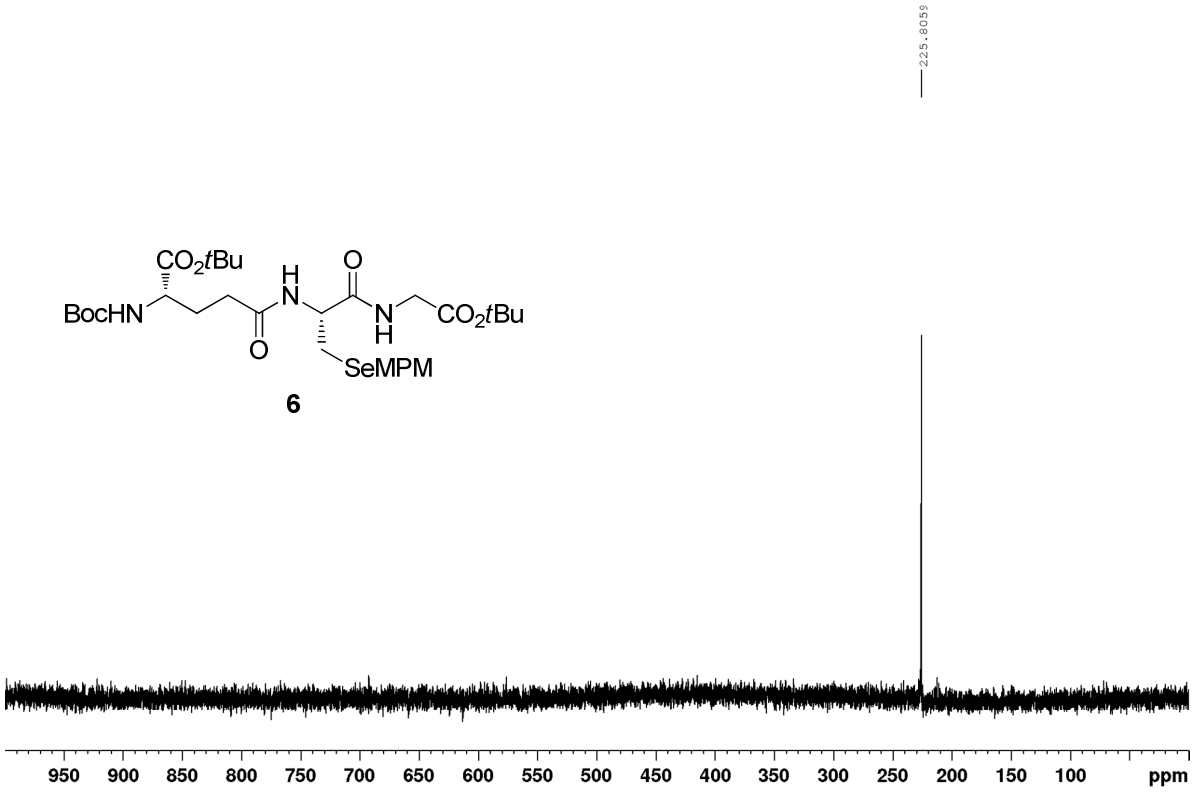
^{1}H in $CDCl_3$



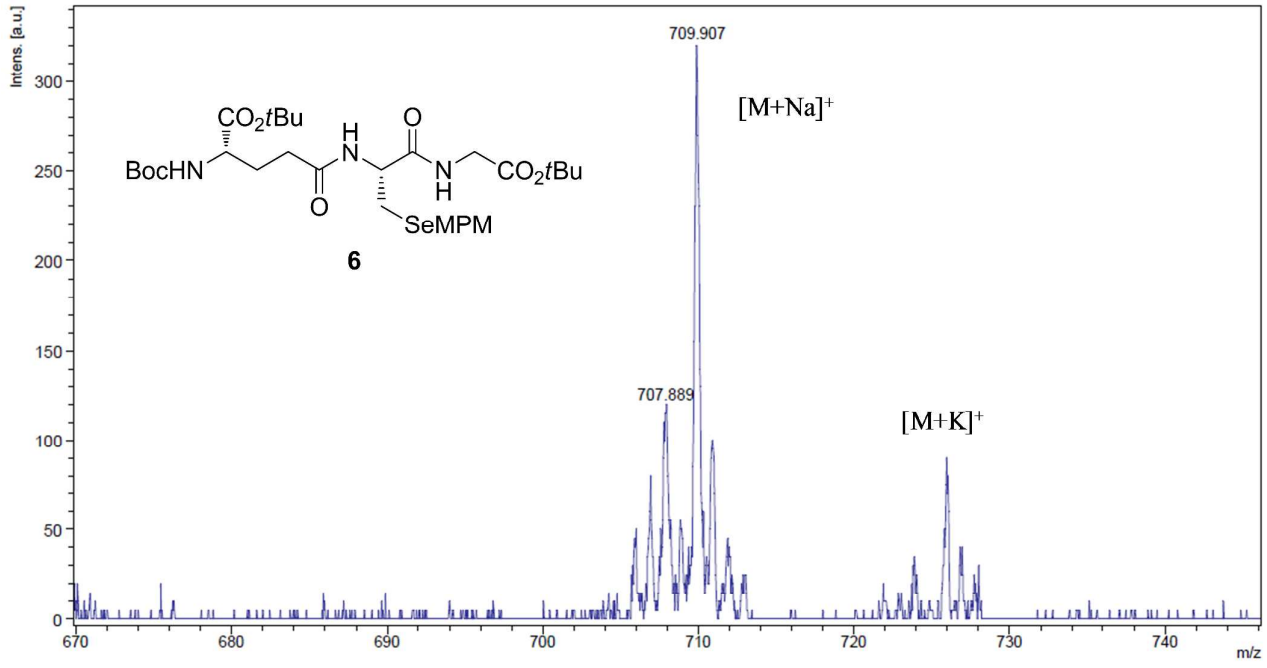
^{13}C in $CDCl_3$



^{77}Se in $CDCl_3$

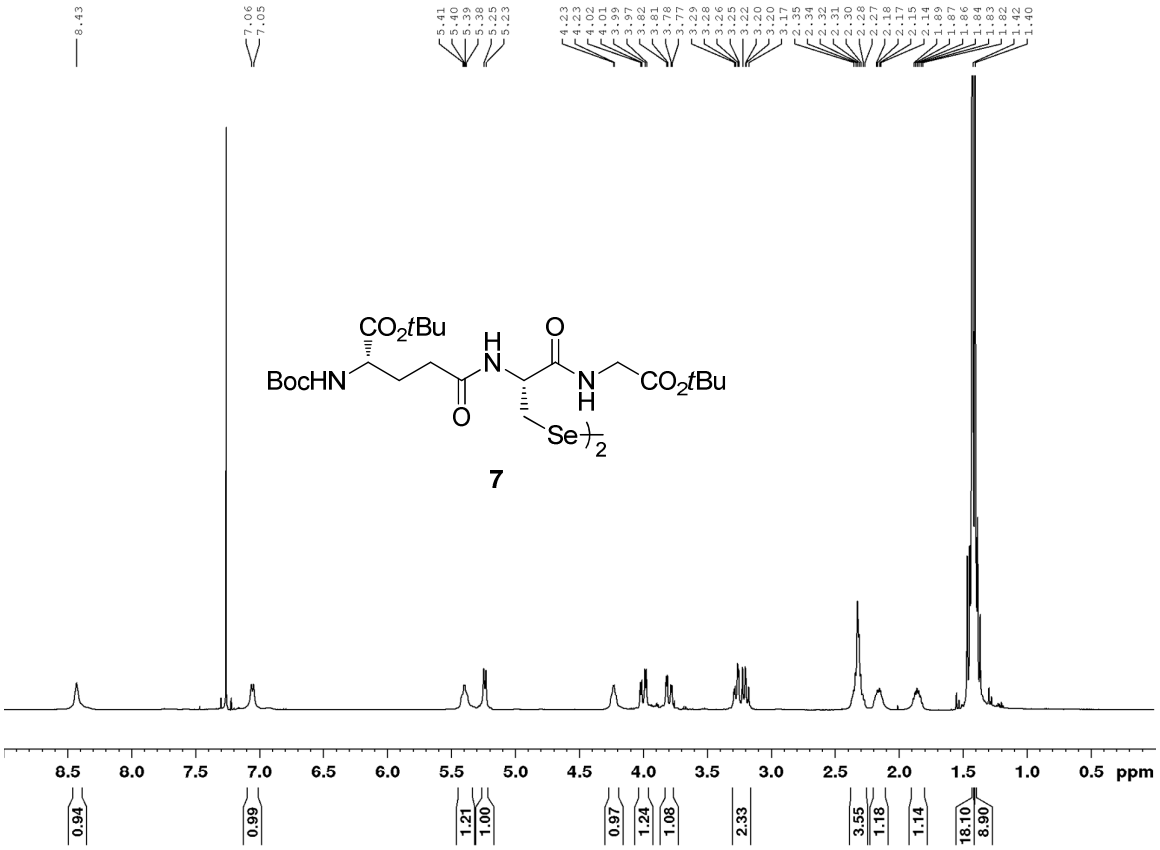


MALDI-TOF-MS

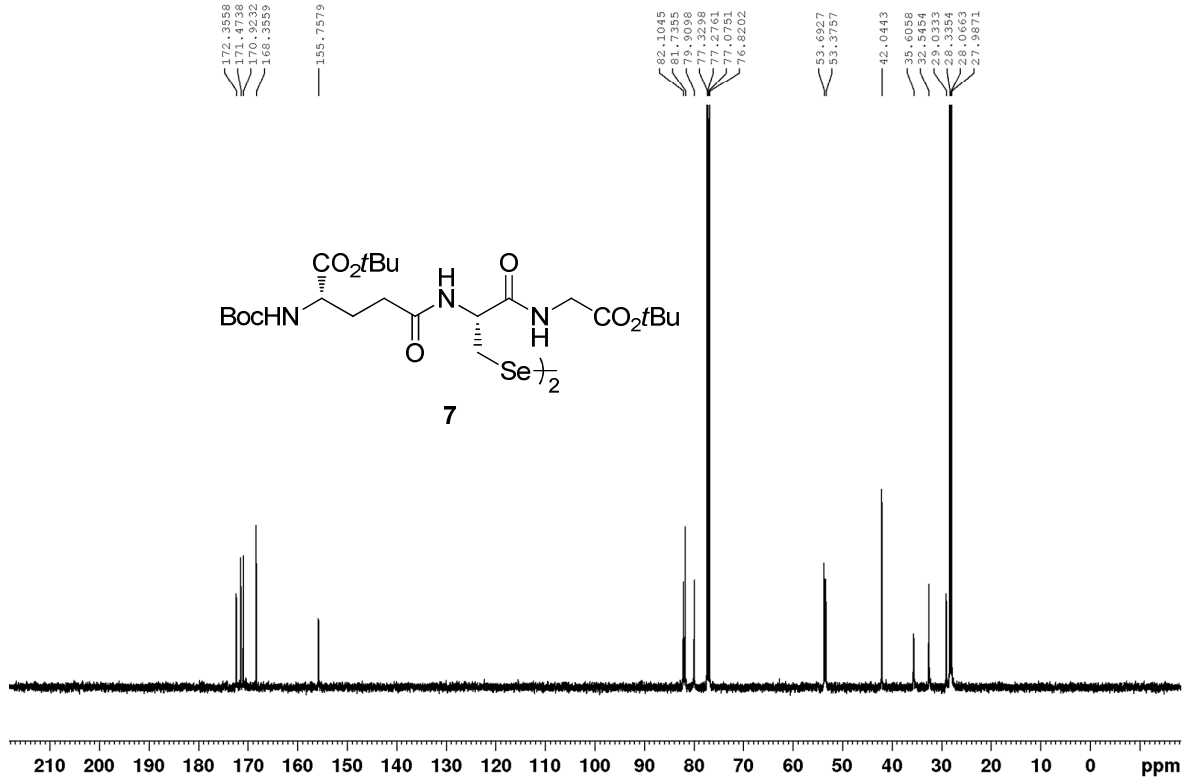


***N*-(*tert*-butoxycarbonyl)-*O*^{1}-*tert*-butyl-L-glutamyl-L-selenocysteinyl-glycine-*tert*-butyl ester diselenide (7)**

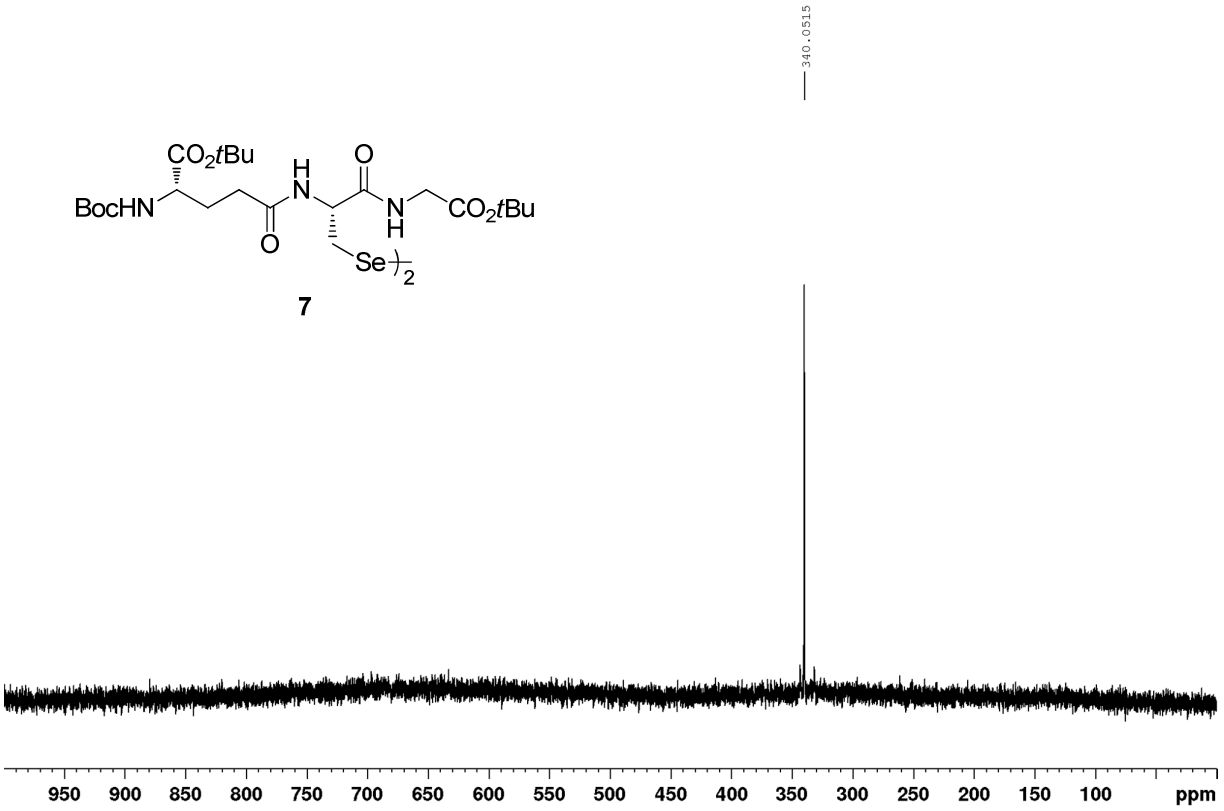
^{1}H in $CDCl_3$



^{13}C in $CDCl_3$



^{77}Se in $CDCl_3$



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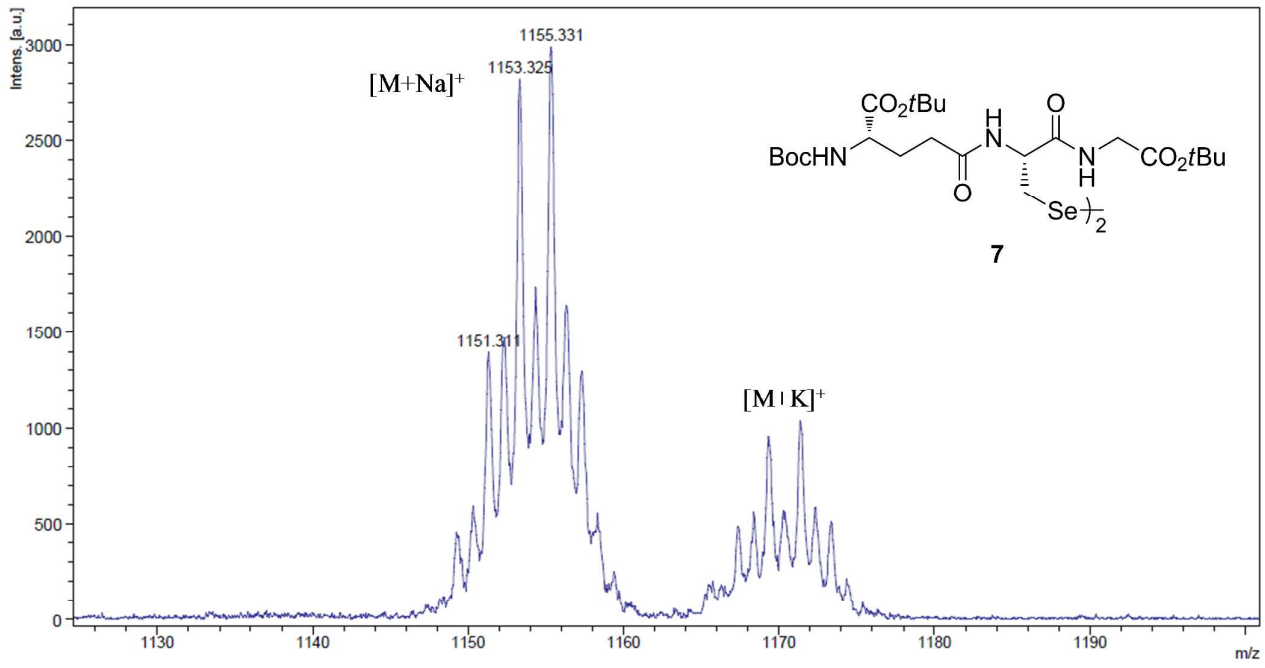
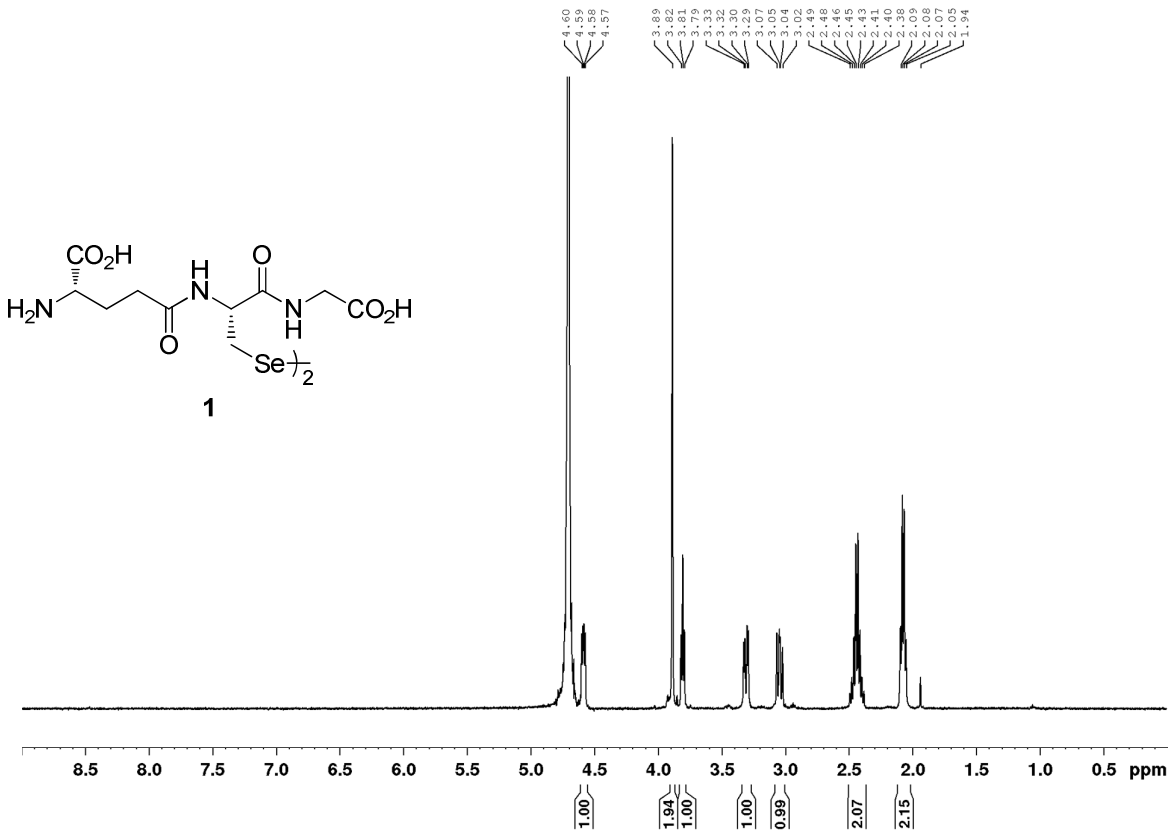
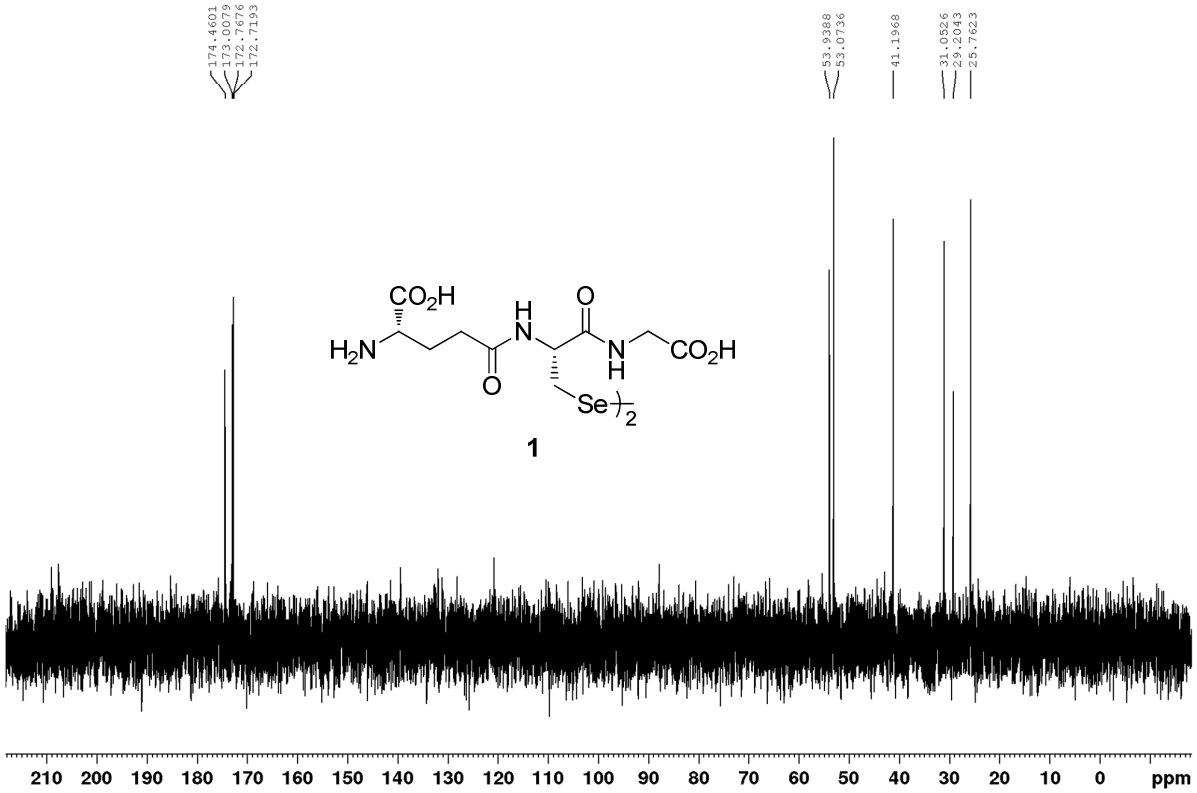


selenoglutathione diselenide (1)

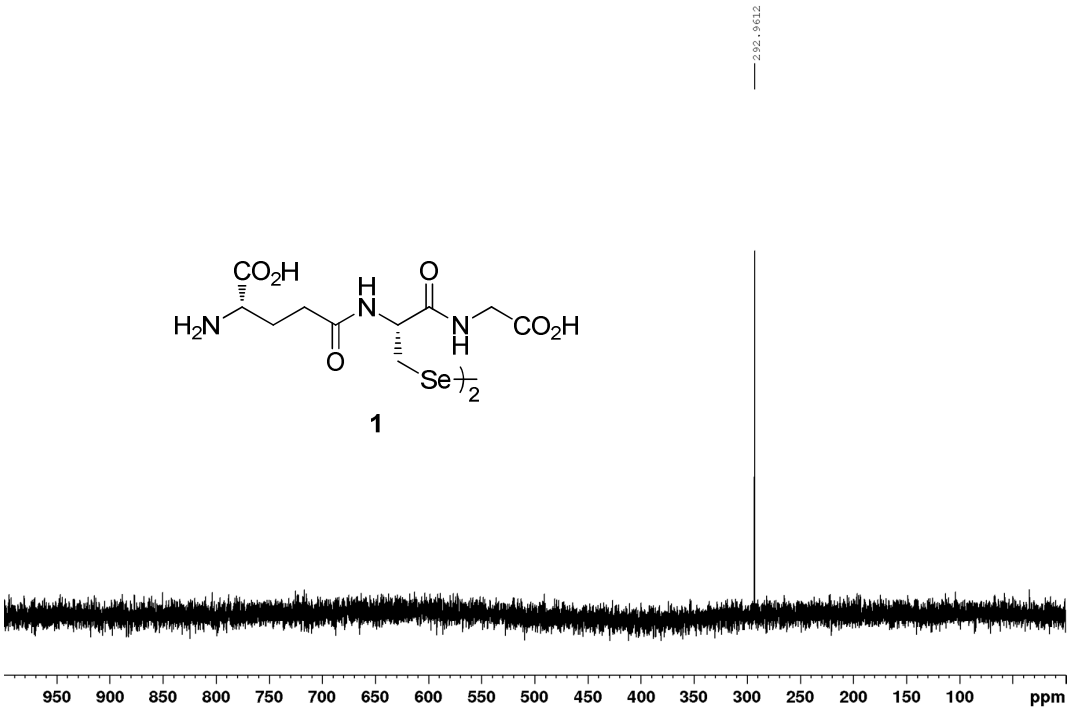
^{1}H in D_2O



^{13}C in D_2O



^{77}Se in D_2O



1

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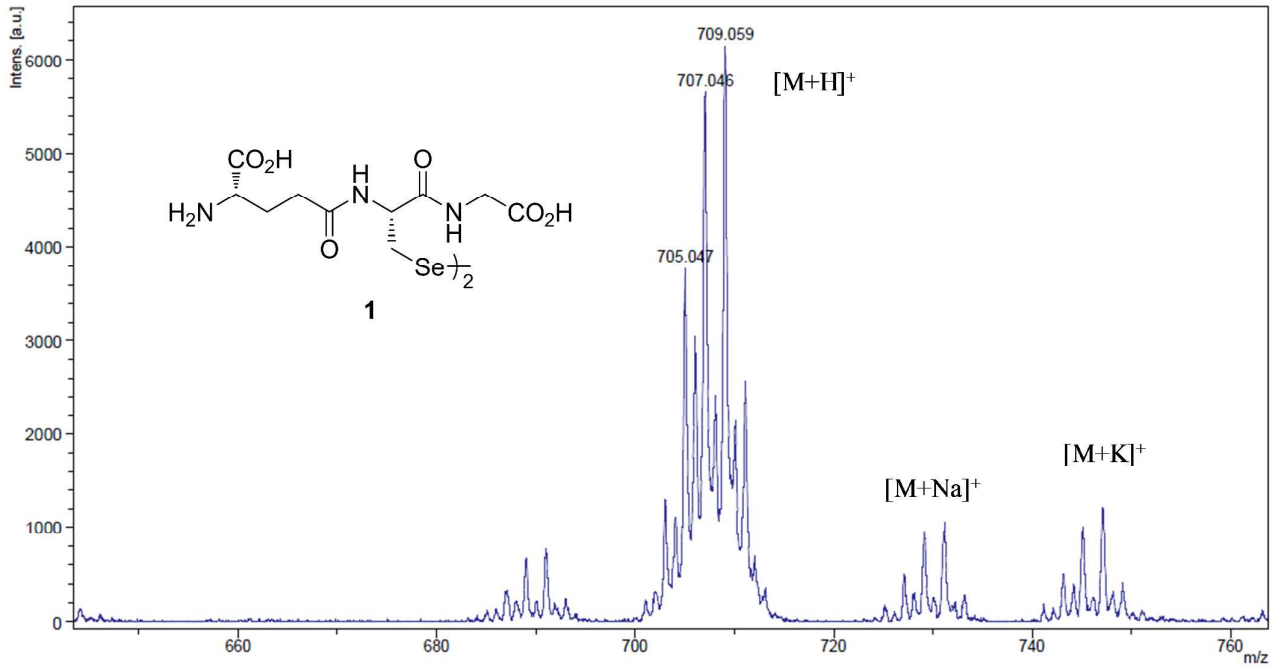
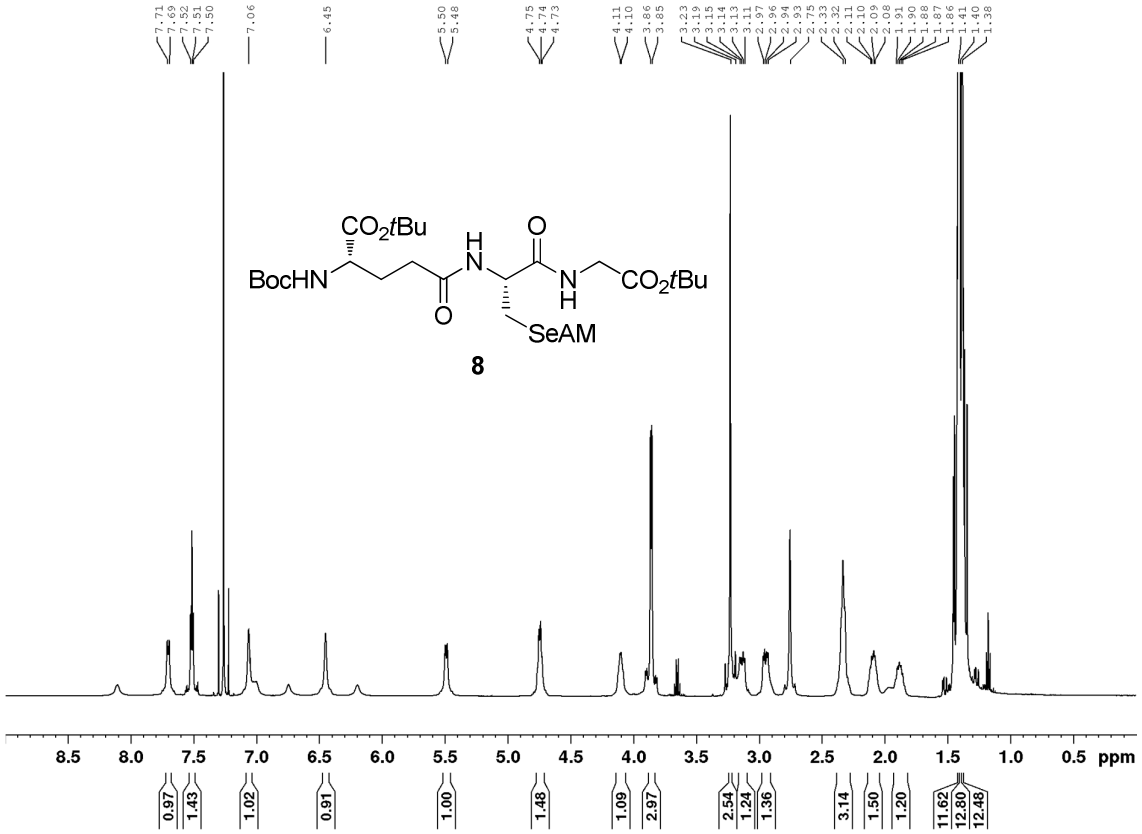


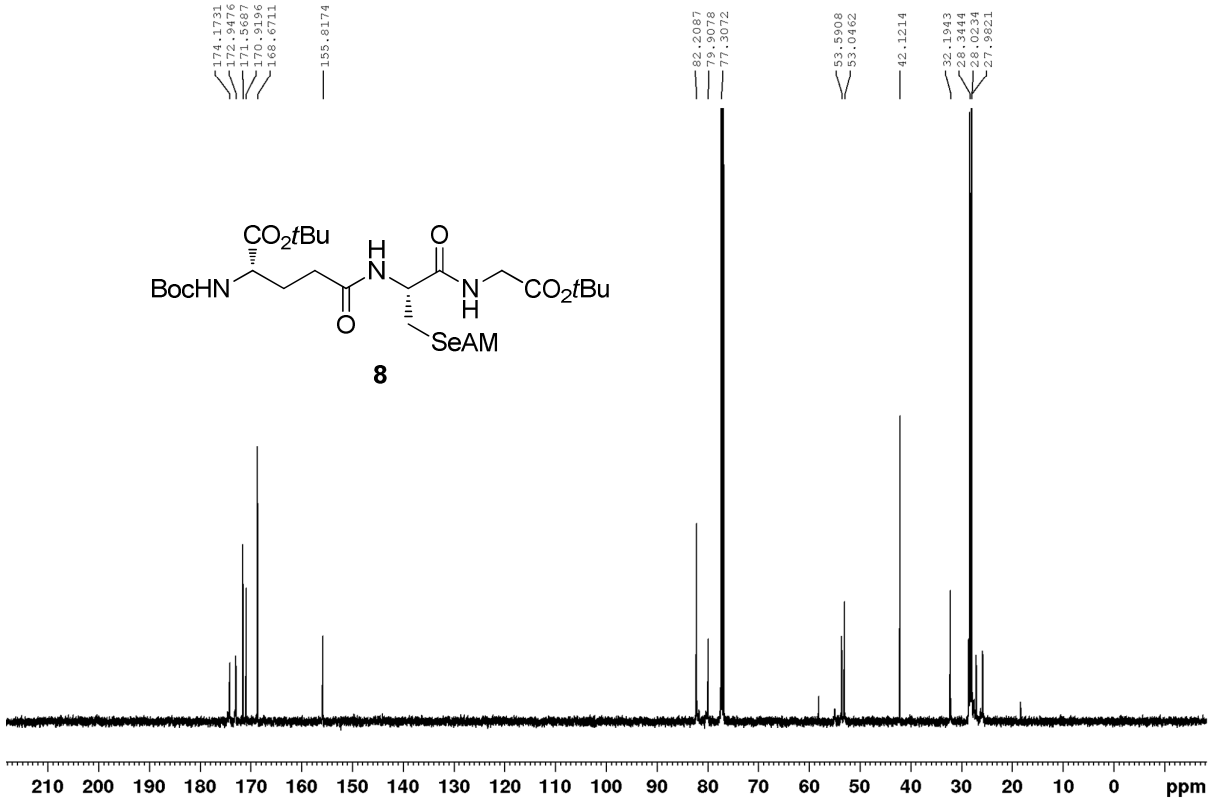
1

***N*-(*tert*-butoxycarbonyl)-*O*^{1}-*tert*-butyl-L-glutamyl-*Se*-(acetamide)-L-selenocysteinyl-glycine-*tert*-butyl ester (8)**

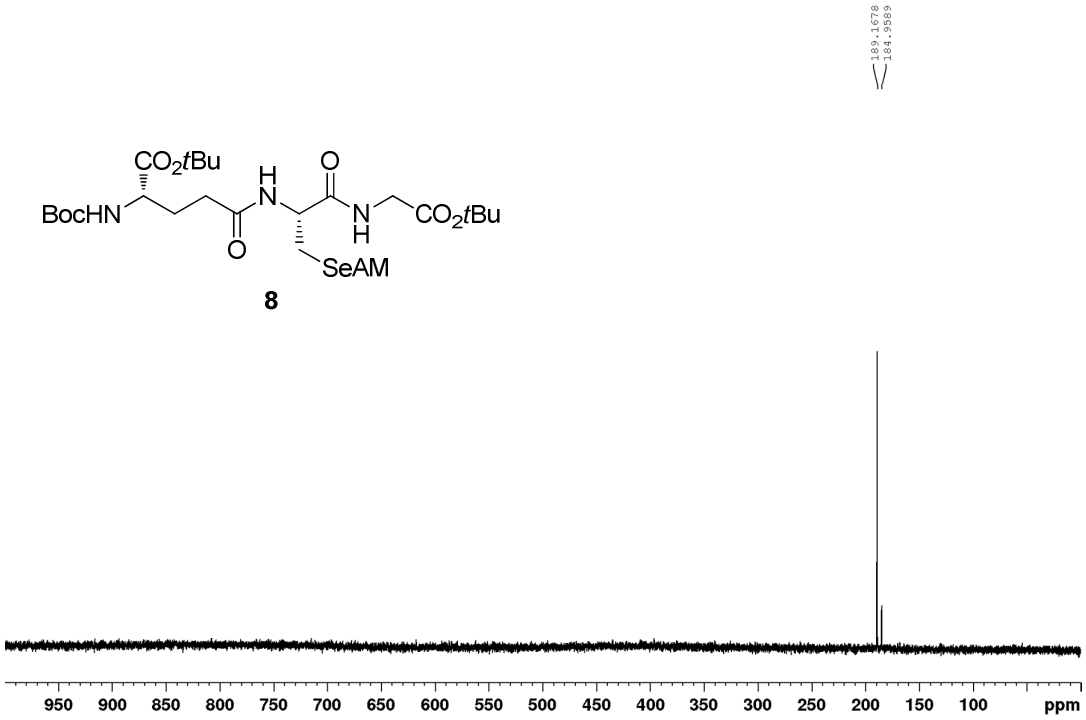
^{1}H in $CDCl_3$



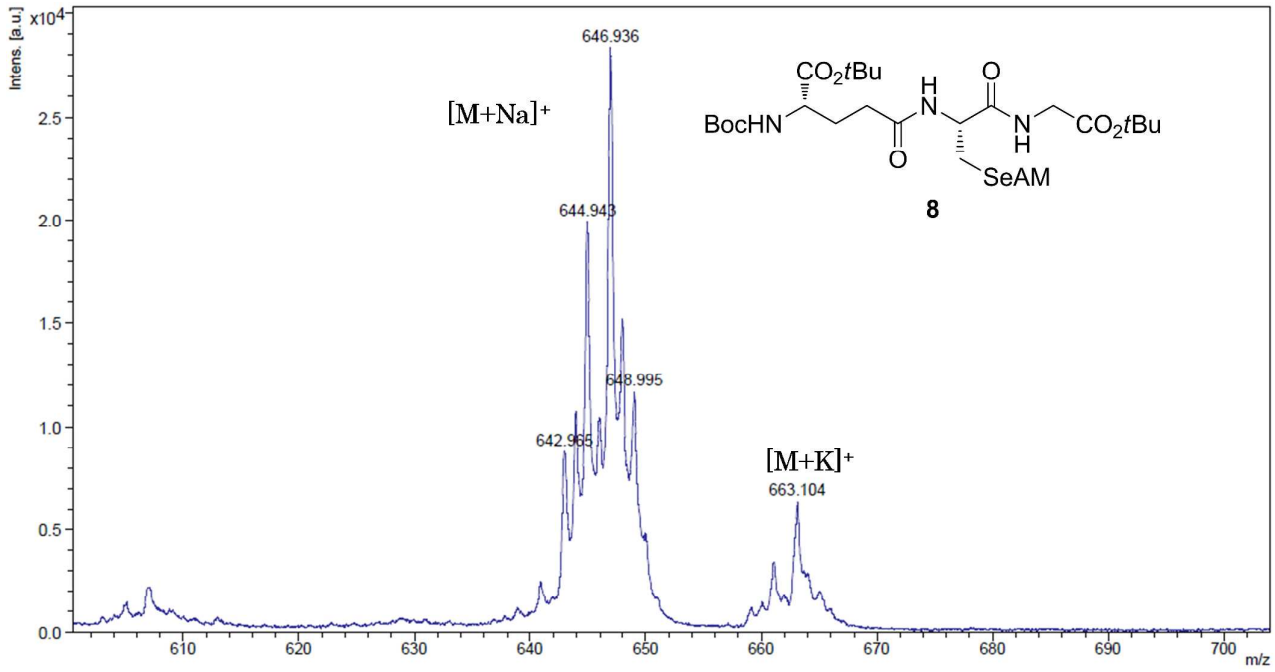
^{13}C in $CDCl_3$



^{77}Se in $CDCl_3$

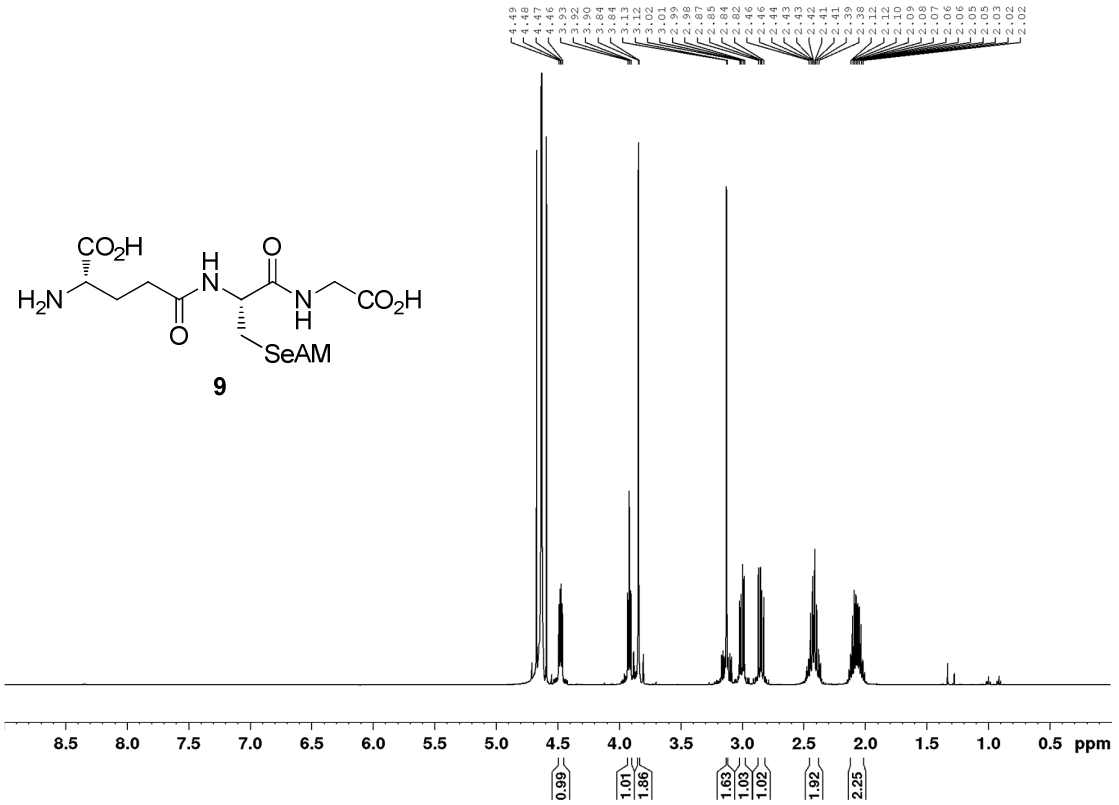


MALDI-TOF-MS

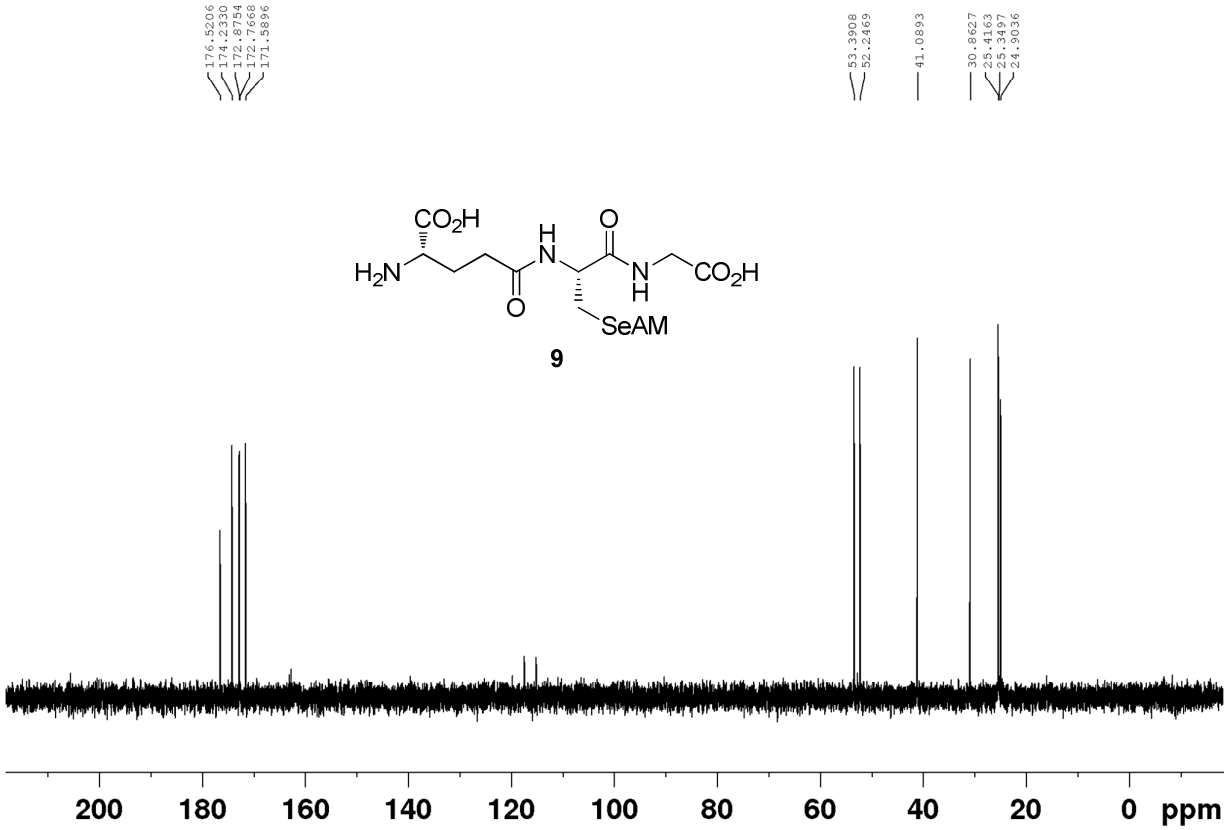


gamma-L-glutamyl-*Se*-(acetamide)-L-selenocysteinyl-glycine (9)

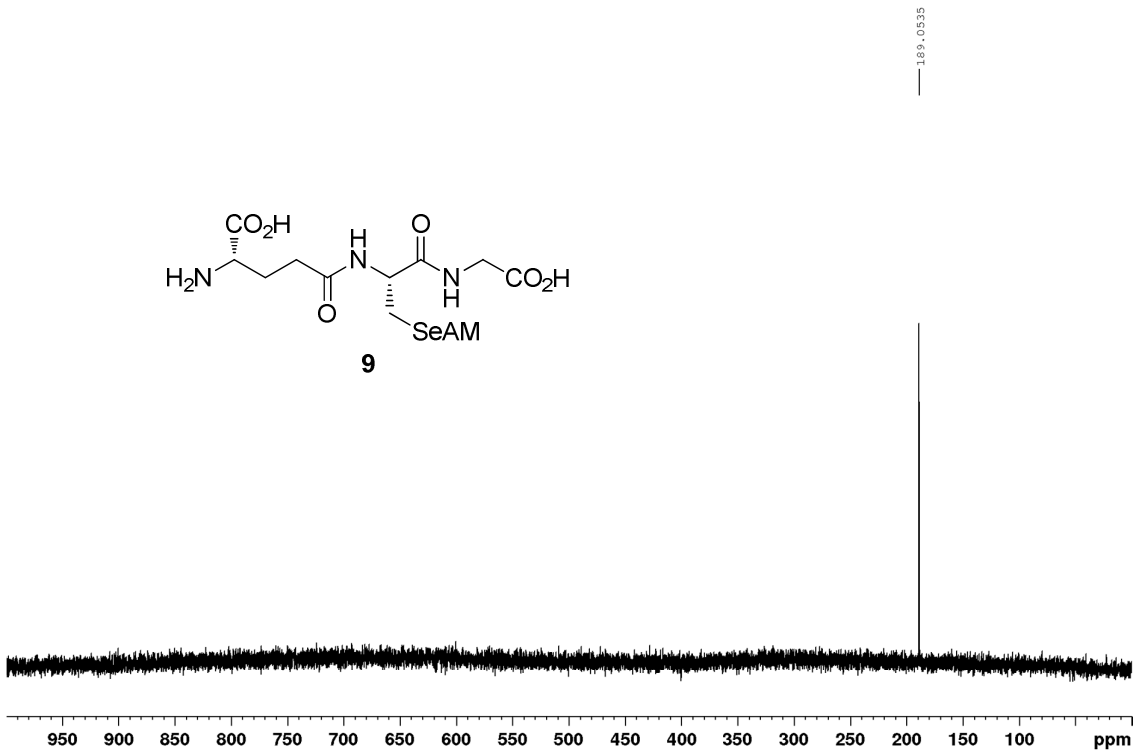
^{1}H in D_2O



^{13}C in D_2O



^{77}Se in D_2O



MALDI-TOF-MS