Electronic Supporting Information

Facile syntheses of stable selenocystine peptides and their solution state nmr studies

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Materials and Method:

Rink Amide Resin (loading 0.62 mmol/g) was purchased from Novabiochem. Hydroxybenzotriazole (HOBt), N,N′-Diisopropylcarbodiimide (DIC) were purchased from Sigma-Aldrich. Fmoc-Sec(Xan)-OH was prepared as previously described procedure.[1] The solvents used (Dimethylformamide (DMF), Methanol, Dichloromethane (DCM), Diethyl ether) were HPLC grade.

Solid phase peptide synthesis of selenocystine peptide:

Solid phase peptide synthesis (SPSS) of the selenocystine peptides was performed manually using Fmoc protocol.[2] In brief, 80 mg of Rink Amide resin was swelled for 1 hour in DMF followed by deprotection of amine group. Fmoc-AA-OH (AA- amino acid) (0.2976 mmol) dissolved in DMF and to this solution, HOBt (45.57 mg, 0.2976 mmol) and DIC (92.16 µL, 0.5952 mmol) were added. This mixture was added to the resin and stirred for 6 hours. After completion of reaction, the resin was filtered and washed with DMF, methanol, DCM, and diethyl ether consecutively using vacuum. Then, 30% v/v piperidine solution in DMF was used to remove the Fmoc protecting group by shaking for 25 minutes followed by the washing with DMF, methanol, DMF and the deprotection procedure was repeated two times. The same procedure was applied to introduce other two amino acid residues in the peptide chain with Fmoc-AA-OH (0.1488 mmol) HOBt (22.78 mg, 0.1488 mmol) and DIC (46.08 µL, 0.2976 mmol).

Resin cleavage and formation of Se-Se bond in peptides:

The peptide loaded on resin, was stirred in a solution of (1650 µL) TFA and (100 µL) water in the presence of 1,2 ethanedithiol, thioanisole and phenol scavengers for 3 hours and the cleaved peptide was precipitated in dry chilled diethyl ether. The mixture was centrifuged and diethyl

ether was then decanted. This step was repeated twice, resulting in an off-white precipitate. The purity of peptide was affirmed by reverse phase high performance liquid chromatography (HPLC) on a Phenomenex C4 column (250 mm × 10 mm 300 Å, 10 µm), eluting with a linear gradient of 0–1% solvent A (acetonitrile containing 0.1% trifluoroacetic acid) in solvent B (water containing 0.1% trifluoroacetic acid) over 30 min (flow rate of 1 ml/min). All the peptides show a single peak at retention time ~18 mins. Yield: 30-35% (with respect to resin): White colour solid. NMR spectra of all the reported peptides were recorded in DMSO-d_6 at room temperature with 50 mM concentration using diphenyl diselenide as external reference. Racemization of amino acids is well known during the coupling reaction under the SPPS conditions. It is very common for the Cys/Sec and His type of amino acids. We have used HOBt to avoid racemization, but still traces of racemized products have been seen in some cases.

Peptide 1

^{1}H NMR (500 MHz, DMSO) δ 8.81 (d, *J* = 8.0 Hz, 1H), 8.13 (s, 2H), 8.10 (d, *J* = 7.6 Hz, 1H), 7.46 (s, 1H), 7.13 (s, 1H), 4.61 (dd, *J* = 13.5, 8.5 Hz, 1H), 4.27 – 4.19 (m, 1H), 3.91 (q, *J* = 6.8 Hz, 1H), 3.39 (dd, *J* = 6.0, 3.8 Hz, 1H), 3.16 (dd, *J* = 12.1, 9.5 Hz, 1H), 1.38 (d, *J* = 6.9 Hz, 3H), 1.23 (d, *J* = 7.0 Hz, 3H). ^{13}C NMR (101 MHz, DMSO) δ 174.12, 170.18, 169.33, 53.78, 48.43, 32.32, 31.77, 18.97, 17.60. ^{77}Se NMR (76 MHz, DMSO): 304.171 ppm. HRMS (ESI^{+}): calcd. for $C_{18}H_{34}N_8NaO_6Se_2$ $(M+Na)^{+}$ 641.0824, found 641.0828.

Peptide 2

^{1}H NMR (400 MHz, DMSO) δ 8.82 (d, *J* = 8.0 Hz, 1H), 8.14 (d, *J* = 9.1 Hz, 1H), 7.97 (s, 2H), 7.59 (s, 1H), 7.18 (s, 1H), 4.79 (dd, *J* = 13.8, 8.0 Hz, 1H), 4.17 (dd, *J* = 8.9, 6.8 Hz, 1H), 3.66 (d, *J* = 5.0 Hz, 1H), 3.30 – 3.27 (m, 1H), 3.22 – 3.11 (m, *J* = 12.3, 9.1 Hz, 1H), 2.16 – 2.02 (m, *J* =

13.4, 6.6 Hz, 1H), 1.99 – 1.87 (m, *J* = 13.4, 6.7 Hz, 1H), 0.94 – 0.86 (m, *J* = 7.3 Hz, 6H), 0.85 – 0.78 (m, *J* = 9.7, 6.8 Hz, 6H). ^{13}C NMR (126 MHz, DMSO) δ 173.33, 169.76, 168.57, 57.91, 57.47, 54.14, 32.87, 31.78, 31.24, 30.46, 19.66, 18.56, 18.28, 17.86. ^{77}Se NMR (76 MHz, DMSO): 309.290 ppm. HRMS (ESI$^+$): calcd. for $C_{26}H_{51}N_8O_6Se_2$ $(M+H)^+$ 731.2262, found 731.2261.

Peptide 3

^{1}H NMR (400 MHz, DMSO) δ 8.81 (d, *J* = 8.0 Hz, 1H), 8.26 (d, *J* = 8.5 Hz, 1H), 8.04 (s, 2H), 7.46 (s, 1H), 7.09 (s, 1H), 4.67 (dd, *J* = 14.1, 8.0 Hz, 1H), 4.32 – 4.21 (m, *J* = 9.8, 4.8 Hz, 1H), 3.64 (d, *J* = 5.2 Hz, 1H), 3.33 – 3.28 (m, 1H), 3.19 – 3.03 (m, *J* = 28.9, 14.5 Hz, 1H), 2.13 – 1.97 (m, 1H), 1.62 – 1.49 (m, *J* = 13.7, 10.0 Hz, 1H), 1.47 – 1.35 (m, 2H), 0.93 – 0.88 (m, *J* = 6.6, 5.2 Hz, 6H), 0.84 (d, *J* = 6.6 Hz, 3H), 0.79 (d, *J* = 6.5 Hz, 3H).^{13}C NMR (101 MHz, DMSO) δ 200.28, 199.89, 174.50, 169.60, 168.35, 57.60, 53.92, 51.36, 32.47, 31.77, 30.44, 24.59, 23.58, 21.86, 18.47, 17.90. ^{77}Se NMR (76 MHz, DMSO): 306.768 ppm. HRMS (ESI$^+$): calcd. for $C_{28}H_{55}N_8O_6Se_2$ $(M+H)^+$ 759.2576, found 759.2579.

Peptide 4

^{1}H NMR (400 MHz, DMSO) δ 8.91 (d, *J* = 7.2 Hz, 1H), 8.14 (d, *J* = 8.4 Hz, 1H), 7.96 (s, 1H), 7.40 (s, 1H), 7.05 (s, 1H), 4.65 (dd, *J* = 6.5 Hz, 1H), 4.34 – 4.21 (m, *J* = 9.0, 5.3 Hz, 1H), 3.78 (t, *J* = 7.0 Hz, 1H), 3.33 (dd, 1H), 3.13 (dd, *J* = 12.2, 8.5 Hz, 1H), 1.68 – 1.61 (m, *J* = 12.4, 6.2 Hz, 1H), 1.60 – 1.55 (m, 1H), 1.54 – 1.49 (m, 2H), 1.47 – 1.41 (m, 2H), 0.89 – 0.84 (m, *J* = 6.1, 4.5 Hz, 6H), 0.84 – 0.79 (m, 6H). ^{13}C NMR (101 MHz, DMSO) δ 174.41, 169.67, 53.91, 51.29, 32.27, 31.77, 24.62, 23.96, 23.57, 23.07, 22.39, 21.91. ^{77}Se NMR (76 MHz, DMSO): 306.615 ppm. HRMS (ESI$^+$): calcd. for $C_{30}H_{58}N_8NaO_6Se_2$ $(M+Na)^+$ 809.2709, found 809.2700.

Peptide 5

^{1}H NMR (400 MHz, DMSO) δ 8.69 (d, 1H), 8.29 (d, *J* = 8.3 Hz, 1H), 7.93 (s, 2H), 7.60 (s, 1H), 7.21 (s, 1H), 7.19 – 7.16 (m, *J* = 6.4, 2.1 Hz, 5H), 4.70 (d, *J* = 5.1 Hz, 1H), 4.51 (dd, *J* = 13.4, 8.5 Hz, 1H), 3.64 (d, *J* = 5.2 Hz, 1H), 3.28-3.20 (m, 1H), 3.18-3.08 (m, 1H), 2.98 (dd, *J* = 13.7, 4.7 Hz, 1H), 2.81 (dd, *J* = 13.8, 8.8 Hz, 1H), 1.79 – 1.61 (m, *J* = 43.4 Hz, 1H), 1.48 – 1.38 (m, 1H), 1.07 – 0.96 (m, 1H), 0.82 – 0.80 (m, 6H). ^{13}C NMR (101 MHz, DMSO) δ 173.00, 169.47, 168.67, 137.92, 129.56, 128.48, 126.75, 57.04, 54.20, 53.82, 38.31, 36.86, 33.00, 31.77, 24.28, 14.93, 11.68. ^{77}Se NMR (76 MHz, DMSO): 308.634 ppm. HRMS (ESI^{+}): calcd. for $C_{36}H_{55}N_8O_6Se_2$ $(M+H)^{+}$ 855.2578, found 855.2566.

Peptide 6

^{1}H NMR (500 MHz, DMSO) δ 8.87 (d, *J* = 8.4 Hz, 1H), 8.23 (d, *J* = 8.3 Hz, 1H), 7.65 (s, 1H), 7.19 (s, 1H), 7.18 – 7.13 (m, 5H), 4.73 – 4.59 (m, *J* = 8.9, 5.1 Hz, 1H), 4.54 – 4.49 (m, *J* = 8.7, 4.8 Hz, 1H), 4.21 – 4.11 (s, 1H), 3.28 – 3.22 (m, 1H), 3.21 – 3.13 (m, 2H), 3.11 – 3.06 (m, 1H), 3.06 – 2.98 (m, 2H), 2.83 (dd, *J* = 13.7, 9.1 Hz, 1H), 2.27 – 2.16 (m, *J* = 14.7, 7.8 Hz, 1H), 1.94 – 1.77 (m, 2H), 1.77 – 1.54 (m, 2H). ^{13}C NMR (126 MHz, DMSO) δ 173.22, 169.31, 137.83, 129.71, 128.49, 126.77, 59.39, 54.06, 46.46, 44.04, 32.83, 31.55, 30.34, 24.11, 22.50. ^{77}Se NMR (76 MHz, DMSO): 308.240 ppm. HRMS (ESI^{+}): calcd. for $C_{34}H_{47}N_8O_6Se_2$ $(M+H)^{+}$ 823.1951, found 823.1916.

Peptide 7

^{1}H NMR (400 MHz, DMSO) δ 8.87 (d, *J* = 8.1 Hz, 1H), 8.30 (d, *J* = 7.9 Hz, 1H), 8.11 (s, 2H), 7.95 (s, 1H), 7.43 (s, 1H), 7.32 (s, 2H), 7.14 (s, 1H), 4.69 (dd, *J* = 13.5, 8.6 Hz, 1H), 4.18 (dd, *J* = 13.5, 8.0 Hz, 1H), 3.68 – 3.57 (m, 2H), 3.35 – 3.29 (m, *J* = 4.4 Hz, 1H), 3.15 – 3.11 (m, *J* = 9.8, 6.5 Hz, 1H), 3.10 – 3.08 (m, *J* = 6.3 Hz, 1H), 3.07 – 3.03 (m, *J* = 7.2 Hz, 3H), 1.74 – 1.56 (m, 2H), 1.55 – 1.39 (m, 2H). ^{13}C NMR (101 MHz, DMSO) δ 173.71, 169.90, 166.79, 157.34, 53.94, 52.87, 32.99, 31.75, 29.55, 25.45. ^{77}Se NMR (76 MHz, DMSO): 305.444 ppm. HRMS (ESI^{+}): calcd. for $C_{22}H_{45}N_{14}O_6Se_2$ $(M+H)^{+}$ 761.1976, found 761.1963.

Peptide 8

^{1}H NMR (400 MHz, DMSO) δ 8.79 (d, *J* = 7.4 Hz, 1H), 8.23 (d, *J* = 7.9 Hz, 1H), 7.41 (s, 1H), 7.14 (s, 1H), 4.61 (dd, *J* = 5.9 Hz, 1H), 4.21 (dd, *J* = 13.2, 8.3 Hz, 1H), 3.63 (d, *J* = 5.3 Hz, 1H), 3.36 – 3.30 (m, 1H), 3.21 – 3.11 (m, *J* = 12.1, 8.7 Hz, 1H), 2.22 – 2.15 (m, 2H), 2.12 – 2.04 (m, *J* = 12.6, 6.7 Hz, 1H), 1.95 – 1.85 (m, 1H), 1.79 – 1.67 (m, *J* = 15.2, 8.7 Hz, 1H), 0.91 (dd, *J* = 11.2, 6.9 Hz, 6H). ^{13}C NMR (101 MHz, DMSO) δ 174.41, 173.30, 169.75, 168.66, 57.79, 54.10, 52.43, 35.33, 31.77, 30.60, 30.43, 27.97, 18.77, 17.87. ^{77}Se NMR (76 MHz, DMSO): 305.112 ppm. HRMS (ESI^{+}): calcd. for $C_{26}H_{47}N_8O_{10}Se_2$ $(M+H)^{+}$ 791.1746, found 791.1748.

Peptide 9

^{1}H NMR (400 MHz, DMSO) δ 8.89 (d, 1H), 8.30 (t, *J* = 5.7 Hz, 1H), 7.28 (s, 1H), 7.11 (s, 1H), 4.55 (s, 1H), 3.82 (t, *J* = 6.3 Hz, 1H), 3.66 (d, *J* = 5.7 Hz, 2H), 3.40 – 3.34 (m, 1H), 3.22 – 3.13 (m, *J* = 12.3, 9.0 Hz, 1H), 2.40 – 2.30 (m, 2H), 2.00 – 1.87 (m, 2H). ^{13}C NMR (101 MHz, DMSO) δ 174.07, 171.04, 170.13, 169.41, 54.22, 52.24, 42.49, 32.00, 31.76, 29.71, 27.15. ^{77}Se NMR (76 MHz, DMSO): 303.618 ppm. HRMS (ESI^{+}): calcd. for $C_{20}H_{35}N_8O_{10}Se_2$ $(M+H)^{+}$ 707.0806, found 707.0812.

Peptide 10

^{1}H NMR (400 MHz, DMSO) δ 8.86 (s, 1H), 8.73 (d, *J* = 7.4 Hz, 1H), 8.39 (d, *J* = 8.0 Hz, 1H), 7.37 (s, 1H), 7.28 (s, 1H), 7.22 (s, 1H), 4.48 (dd, *J* = 14.0, 8.1 Hz, 1H), 4.31 (dd, *J* = 14.7, 7.6 Hz, 1H), 4.09 (dd, *J* = 8.2, 5.1 Hz, 1H), 3.41 – 3.33 (m, 1H), 3.19 – 3.09 (m, 1H), 3.09 – 3.02 (m, 1H), 2.97 – 2.87 (m, 1H), 1.59 (dt, *J* = 19.4, 6.4 Hz, 1H), 1.44 (d, *J* = 7.0 Hz, 1H), 1.43 (s, 1H), 0.84 (dt, *J* = 15.3, 7.7 Hz, 7H).^{13}C NMR (101 MHz, DMSO) δ 172.14, 171.94, 167.76, 134.24, 130.06, 117.32, 67.39, 52.83, 52.12, 31.77, 29.64, 27.56, 24.44, 23.45, 21.89. ^{77}Se NMR (76 MHz, DMSO): 290.596 ppm. HRMS (ESI^{+}): calcd. for $C_{30}H_{51}N_{12}O_6Se_2$ $(M+H)^{+}$ 835.2386, found 835.2404.

Note: Peaks at ~ 1.09 for t-BuOH, ~ 2.49 for DMSO, ~ 3.33 ppm for water

Peptide **1**

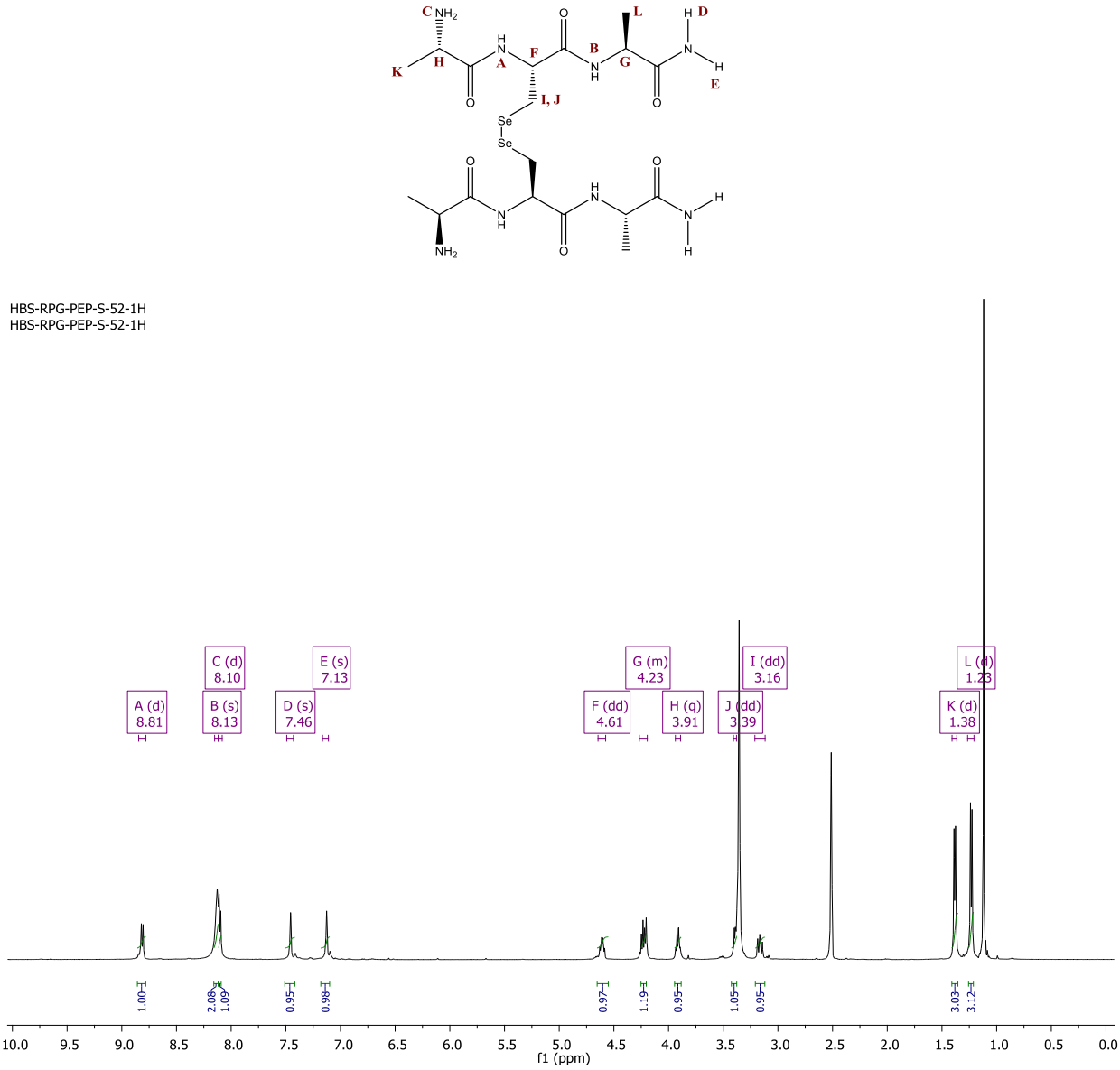


Fig. S1: ^{1}H NMR of Peptide **1**

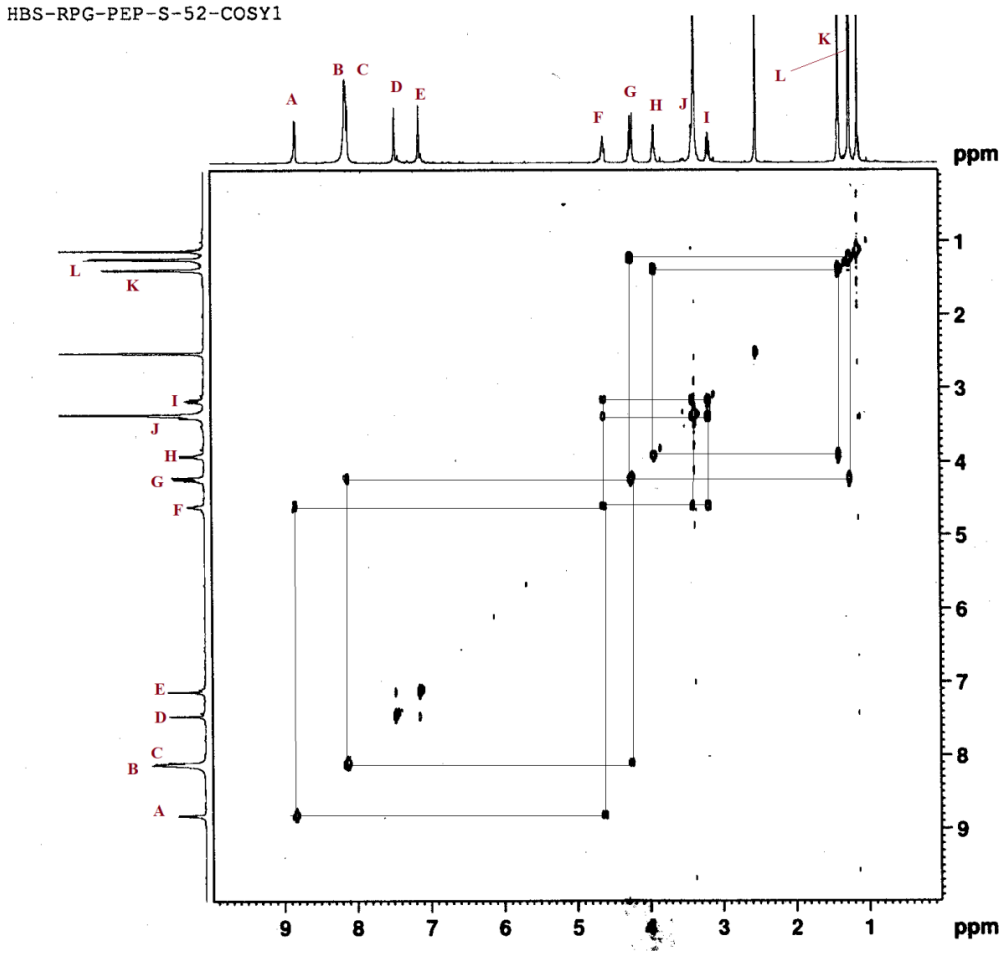
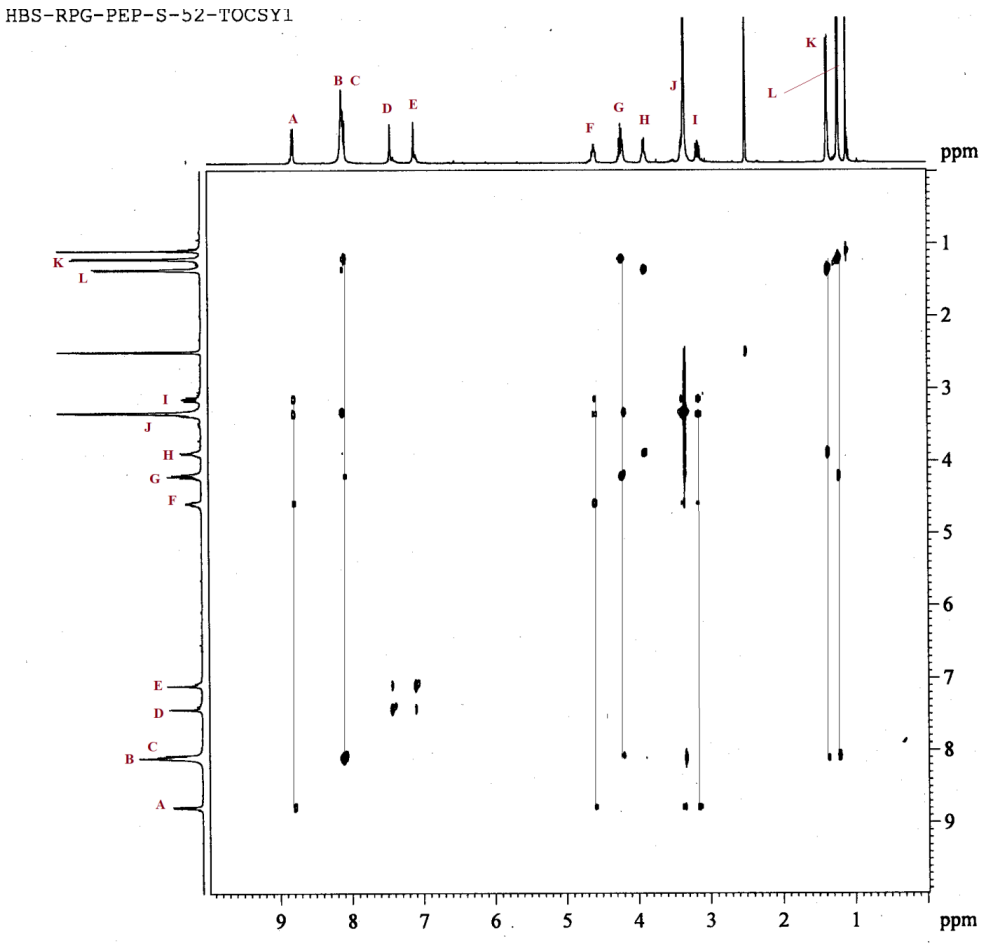


Fig. S2: ^{1}H-^{1}H COSY NMR of Peptide **1**



residue	NH	$C_{\alpha}H$	$C_{\beta}H$	others
Ala	8.13	4.23	1.23	-
Sec	8.81	4.61	3.39, 3.16	-
Ala	-	3.91	1.38	-

Fig. S3: ^{1}H-^{1}H TOCSY NMR of Peptide **1**

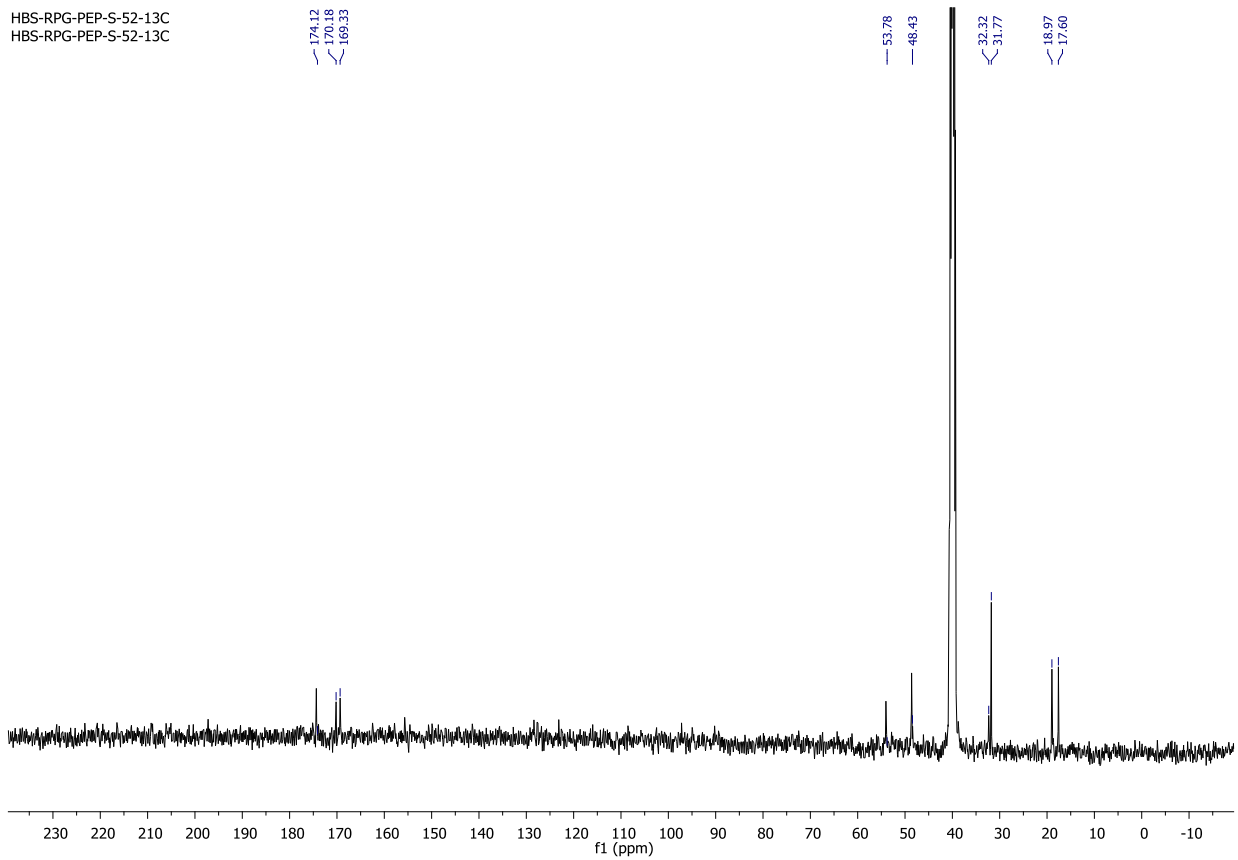


Fig. S4: ^{13}C NMR of Peptide **1**

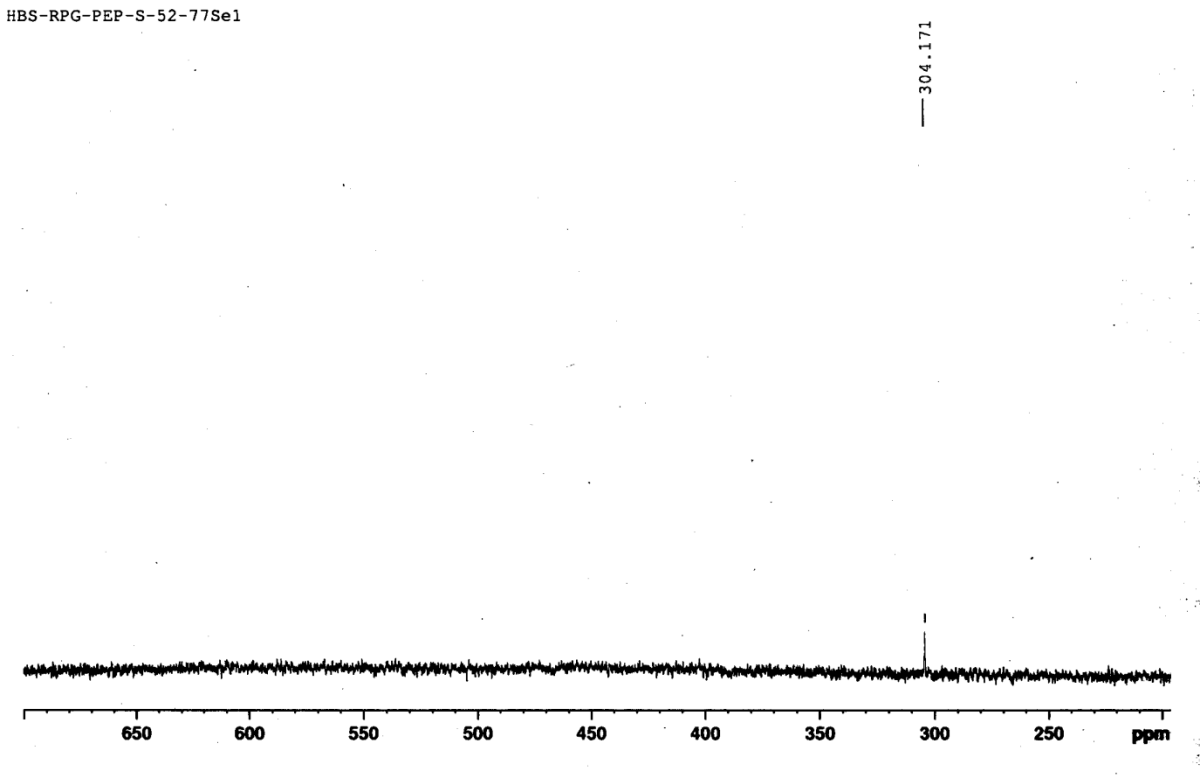
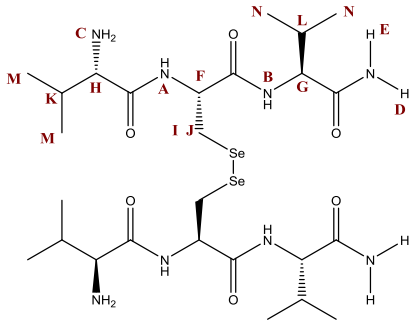


Fig. S5: ^{77}Se NMR of Peptide **1**

Peptide **2**



HBS-RPG-PEP-S-42-1H
HBS-RPG-PEP-S-42-1H

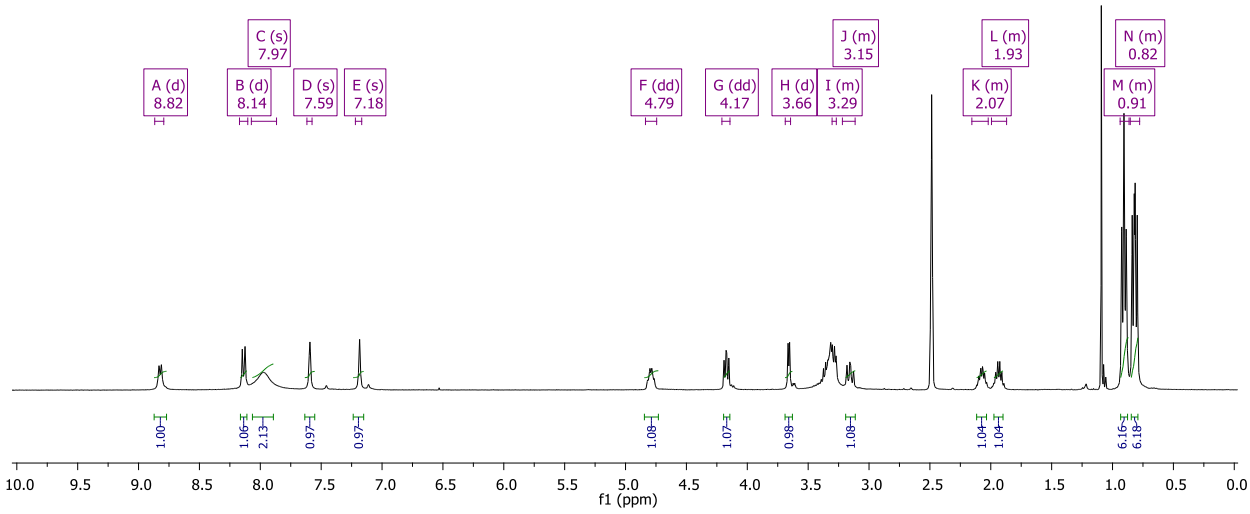


Fig. S6: ^{1}H NMR of Peptide **2**

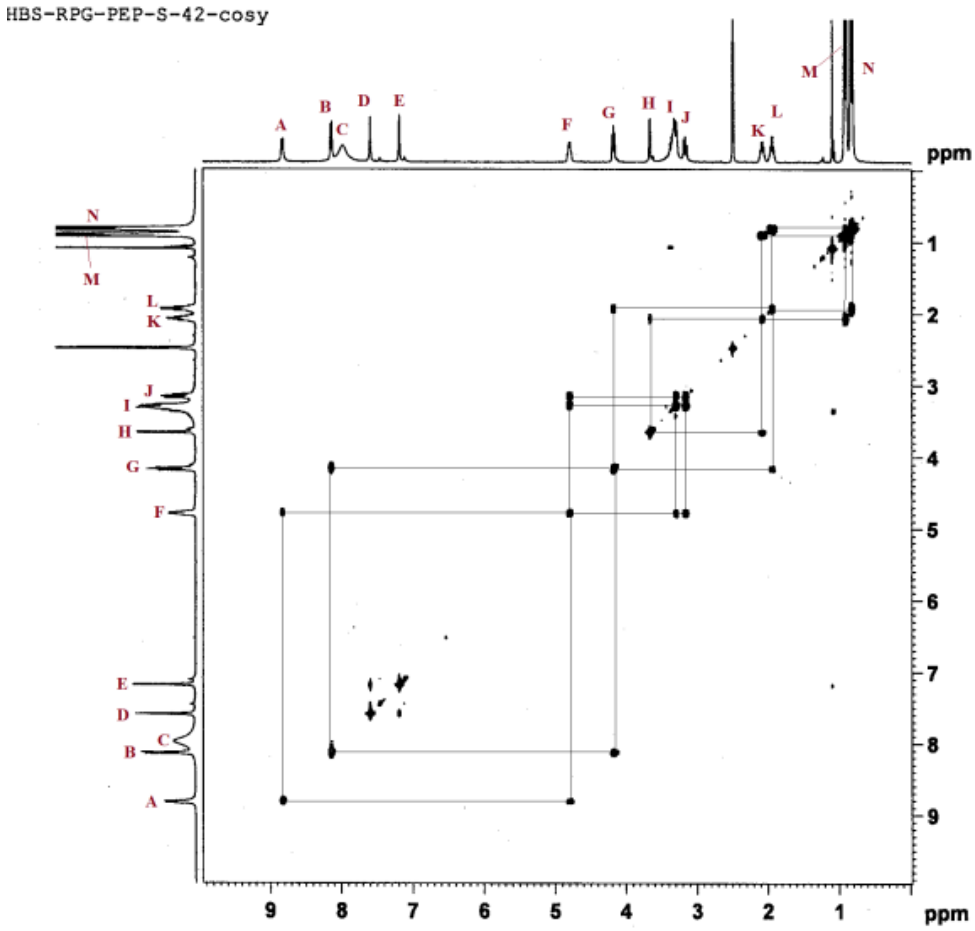
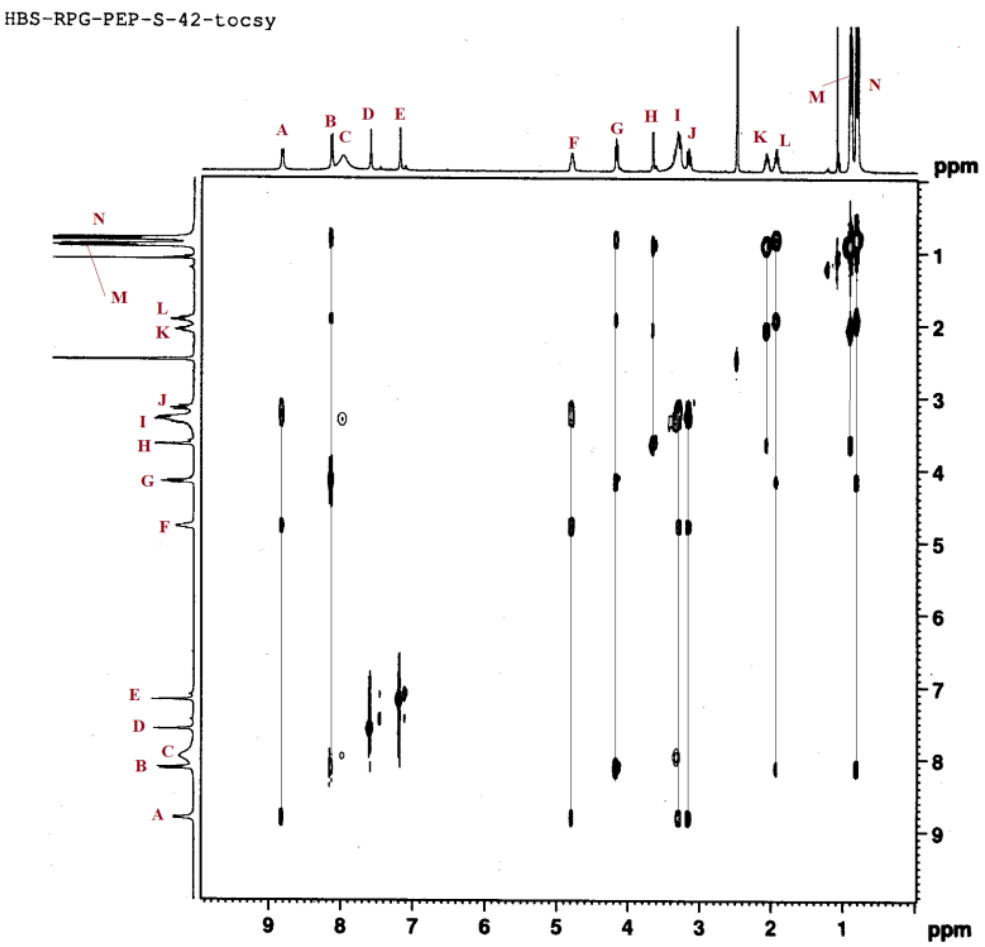


Fig. S7: ^{1}H-^{1}H COSY NMR of Peptide **2**



residue	NH	$C_{\alpha}H$	$C_{\beta}H$	$C_{\gamma}H$	others
Val	8.14	4.17	1.93	0.82	-
Sec	8.82	4.79	3.29, 3.15	-	-
Val	-	3.66	2.07	0.91	-

Fig. S8: ^{1}H-^{1}H TOCSY NMR of Peptide **2**

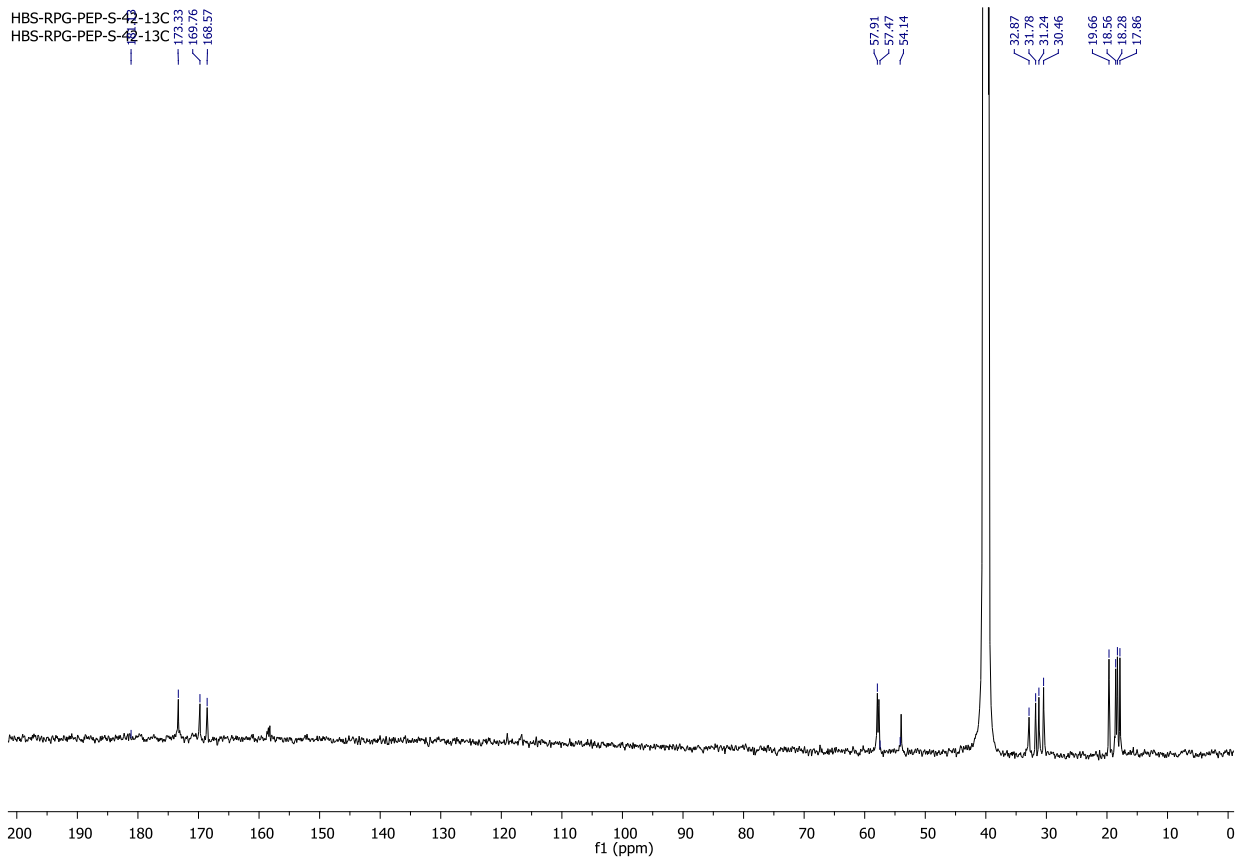


Fig. S9: ^{13}C NMR of Peptide **2**

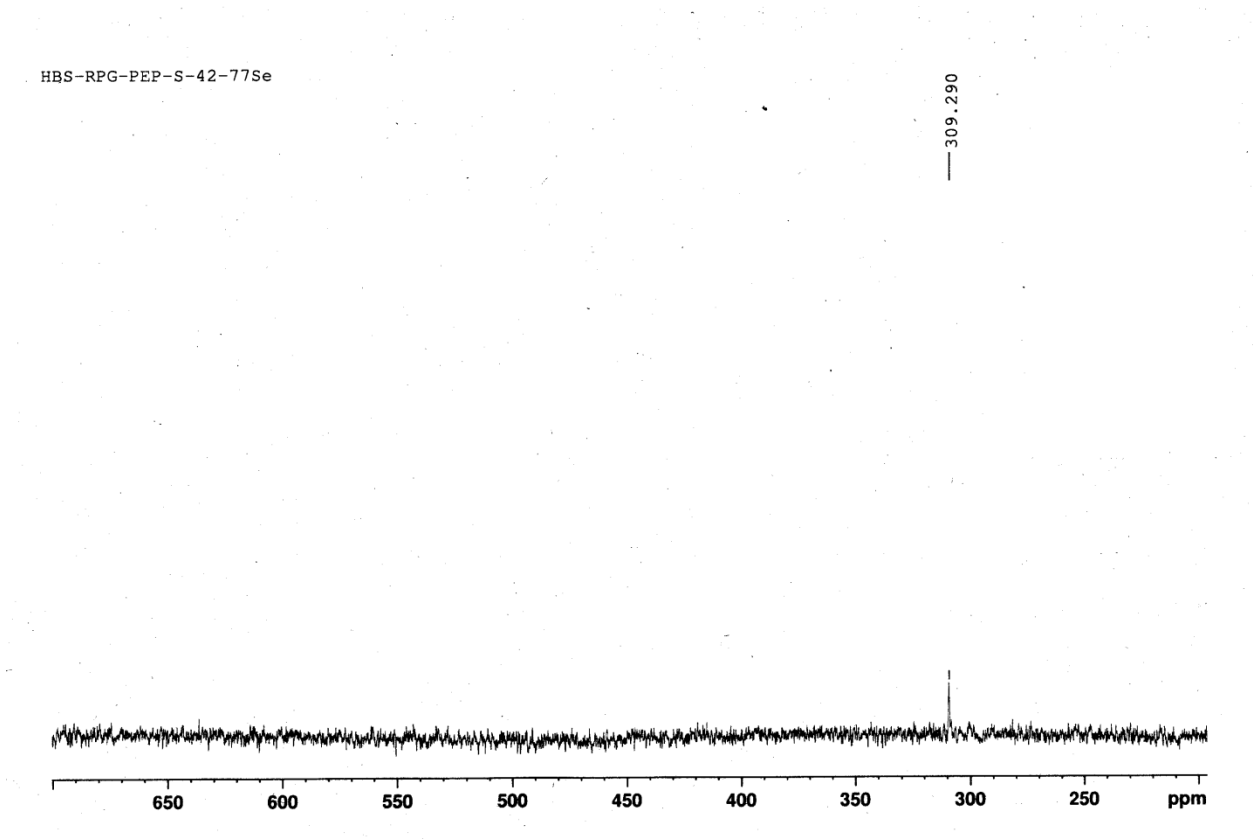
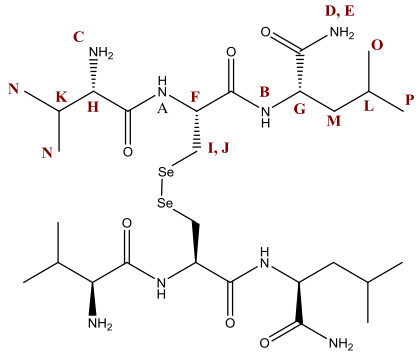


Fig. S10: ^{77}Se NMR of Peptide **2**

Peptide **3**



HBS-RPG-PEP-S-59-1H
HBS-RPG-PEP-S-59-1H

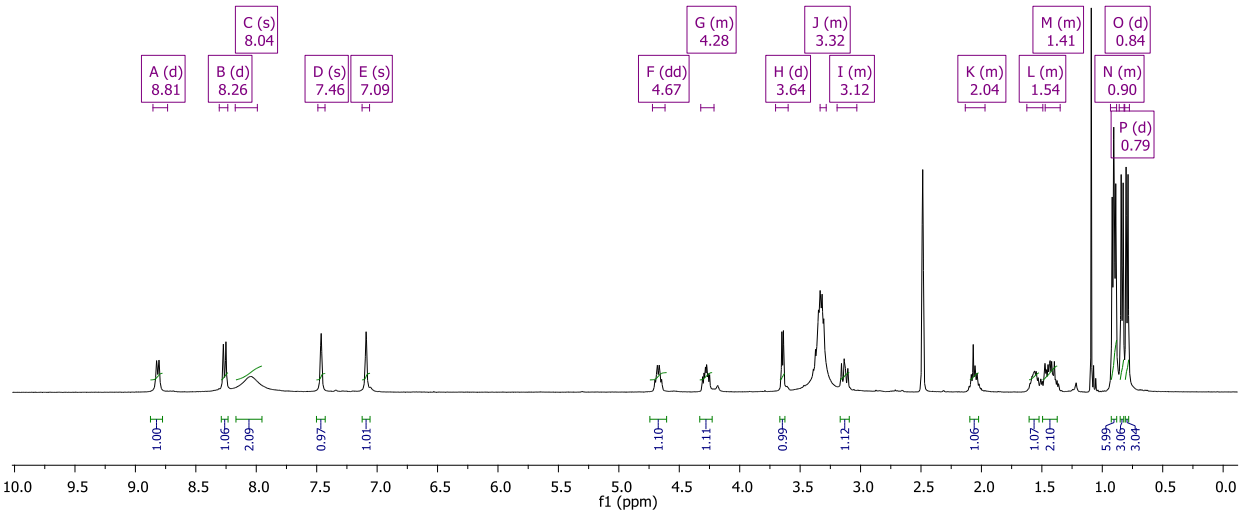


Fig. S11: ^{1}H NMR of Peptide **3**

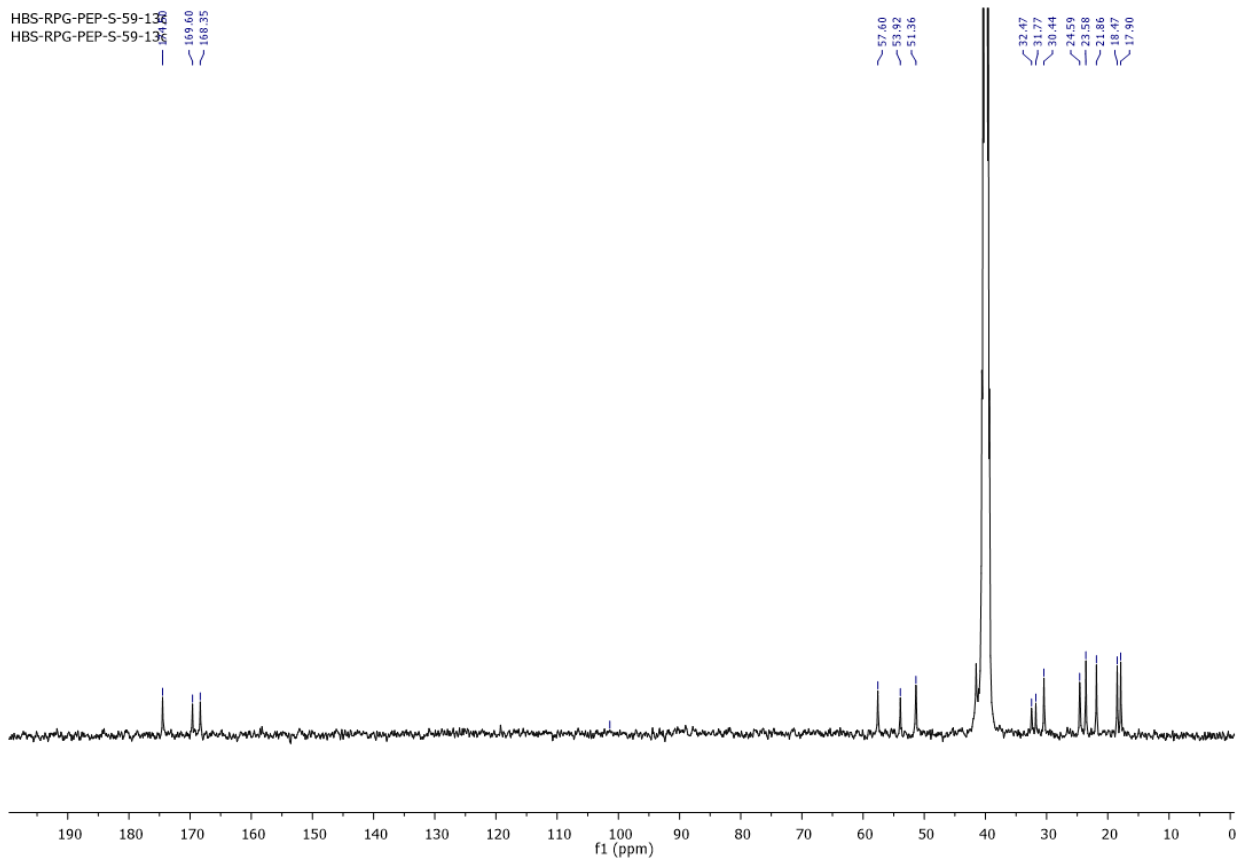
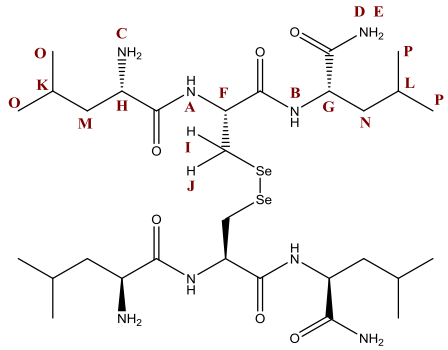


Fig. S12: ^{13}C NMR of Peptide **3**

Peptide **4**



HBS-RPG-PEP-S-60-1H
HBS-RPG-PEP-S-60-1H

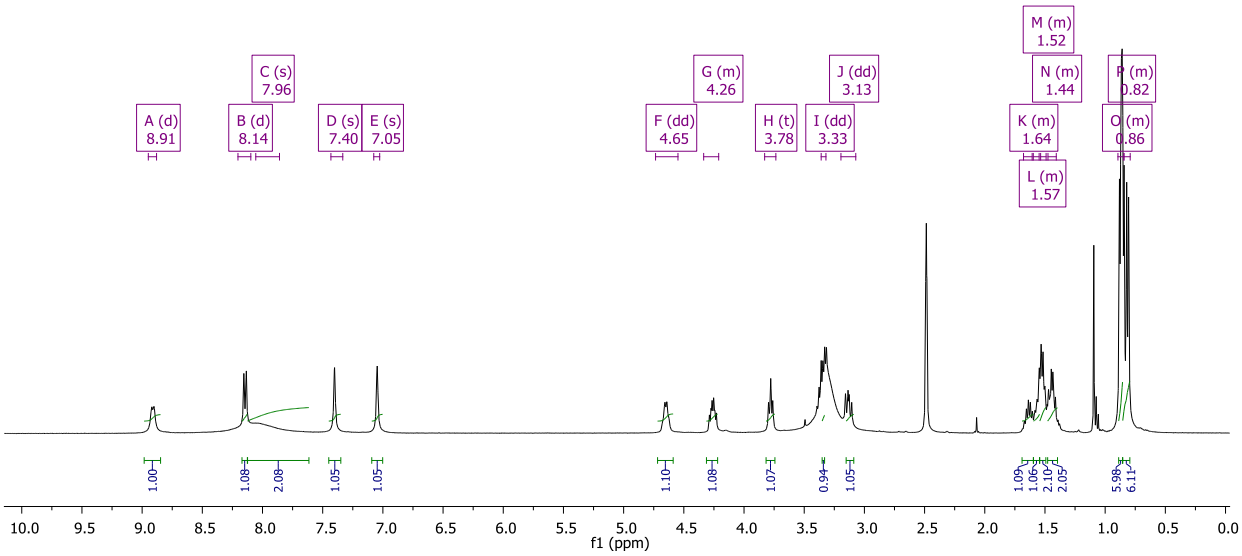


Fig. S13: ^{1}H NMR of Peptide **4**

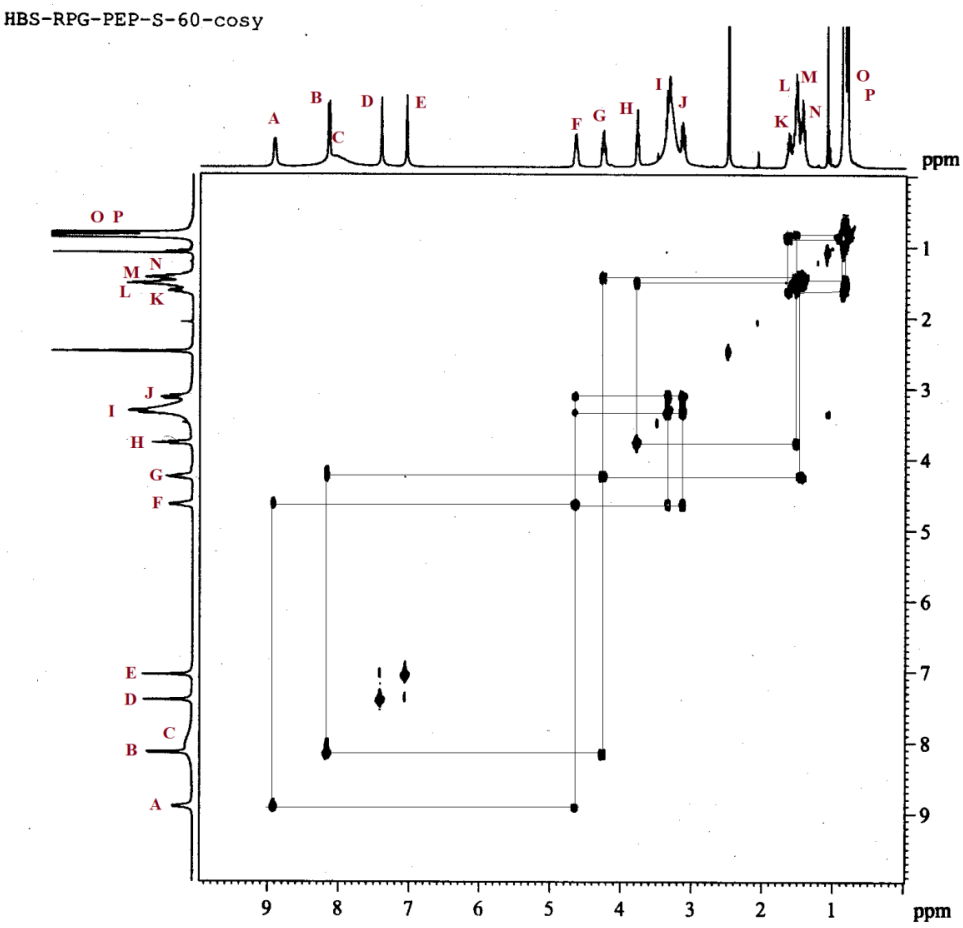
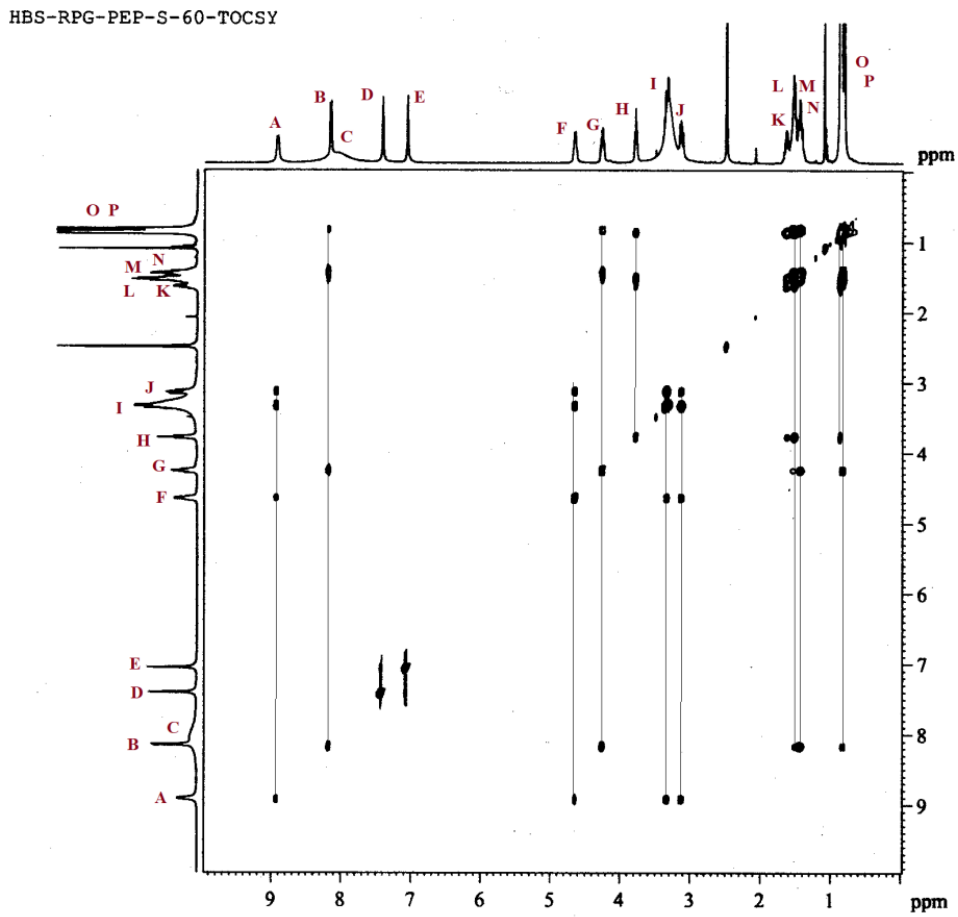


Fig. S14: ^{1}H-^{1}H COSY NMR of Peptide **4**



residue	NH	$C_{\alpha}H$	$C_{\beta}H$	$C_{\gamma}H$	others
Leu	8.14	4.26	1.44	1.57	0.82
Sec	8.91	4.65	3.13, 3.33	-	-
Leu	-	3.78	1.64	1.49	0.86

Fig. S15: ^{1}H-^{1}H TOCSY NMR of Peptide **4**

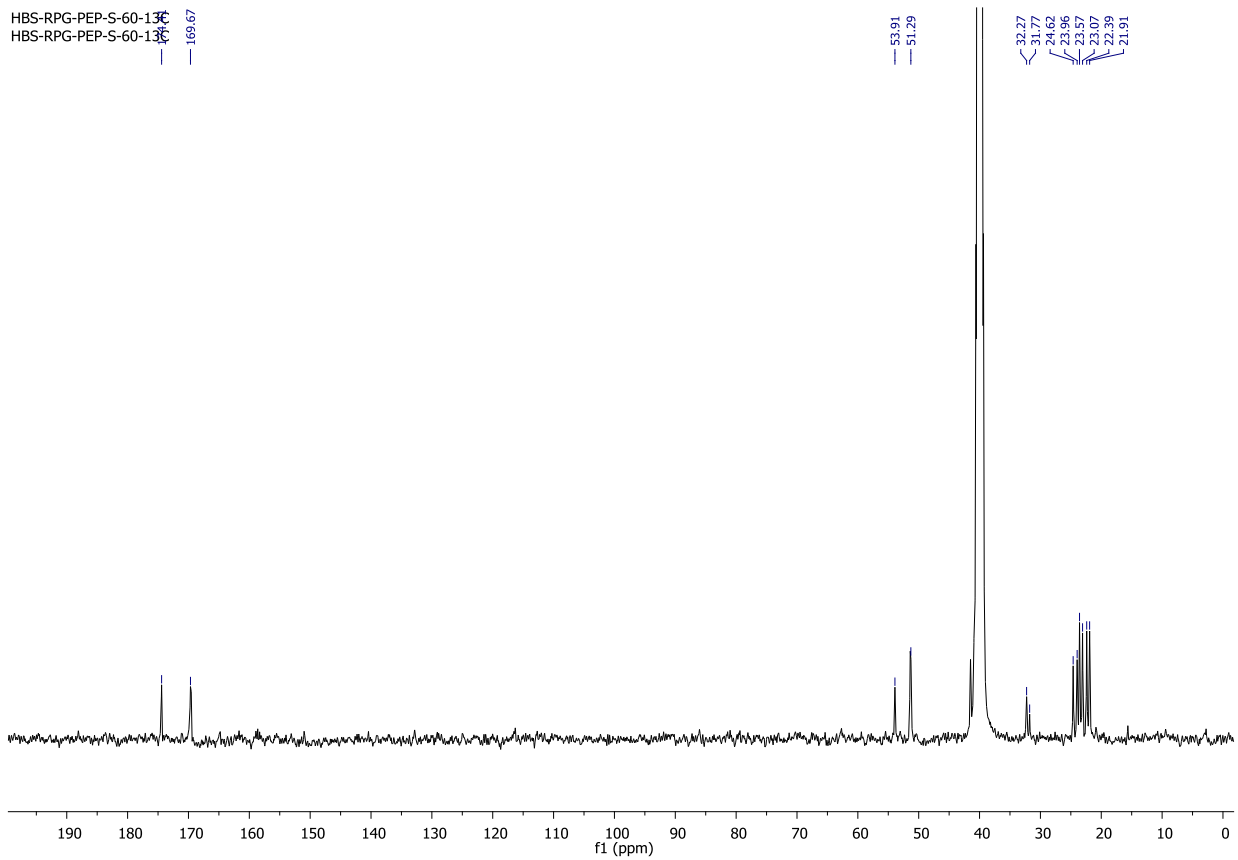


Fig. S15: ^{13}C NMR of Peptide **4**

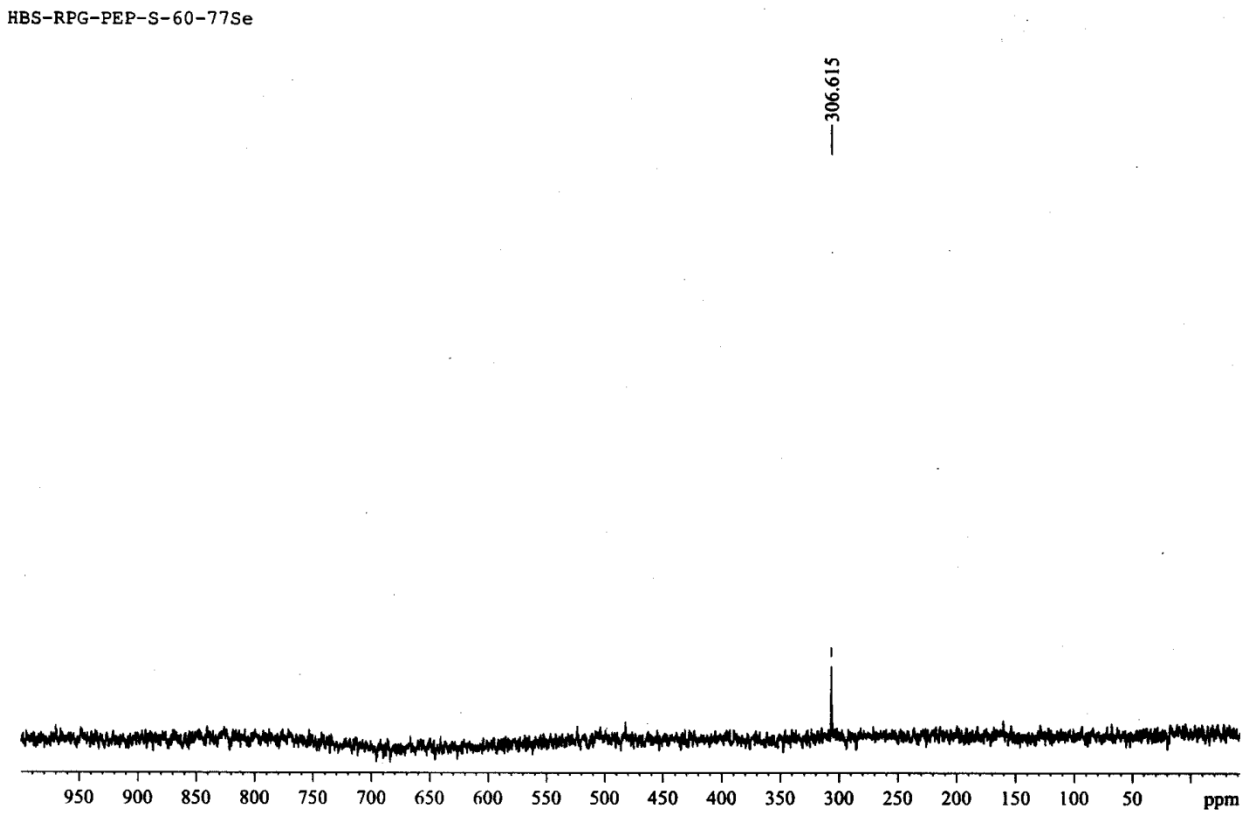
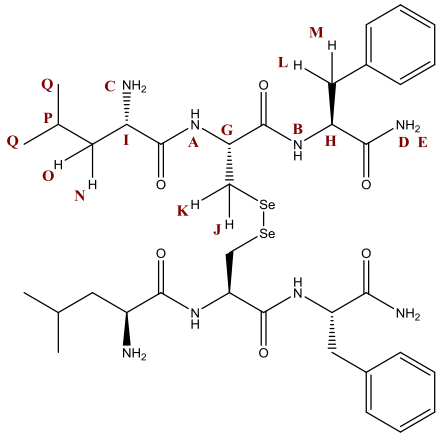


Fig. S16: ^{77}Se NMR of Peptide **4**

Peptide **5**



HBS-RPG-PEP-S-88-1H
HBS-RPG-PEP-S-88-1H

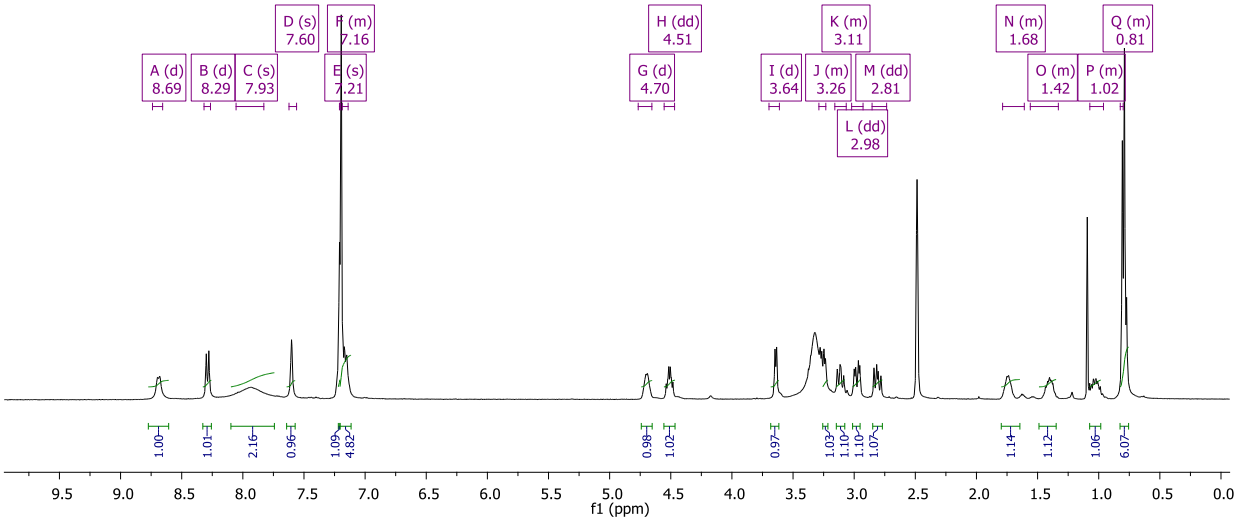


Fig. S17: ^{1}H NMR of Peptide **5**

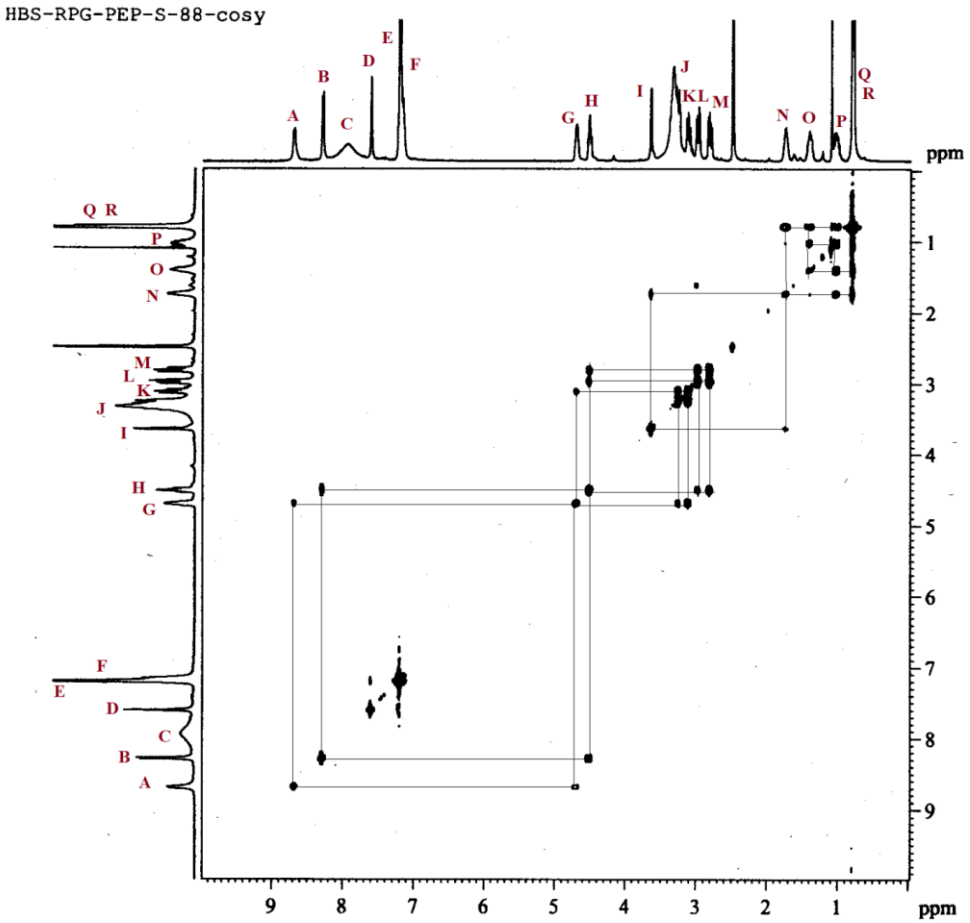
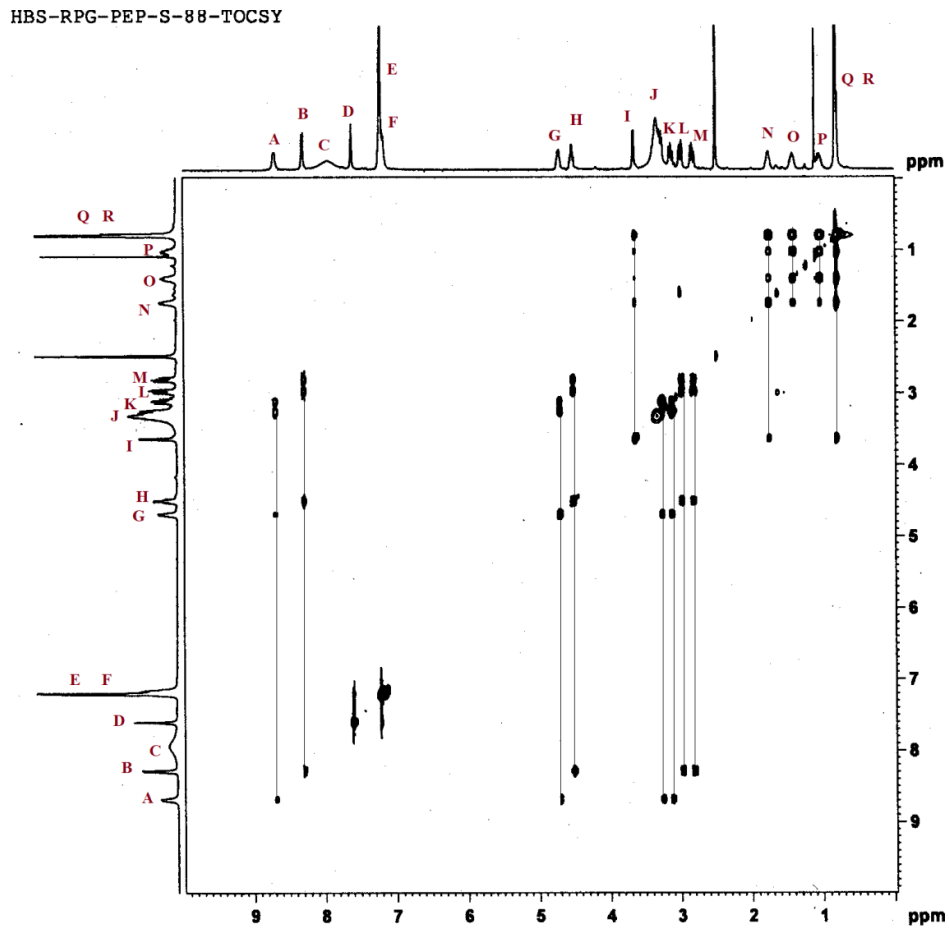


Fig. S18: ^{1}H-^{1}H COSY NMR of Peptide **5**



residue	NH	$C_{\alpha}H$	$C_{\beta}H$	$C_{\gamma}H$	others
Phe	8.29	4.51	2.81, 2.98	-	-
Sec	8.69	4.70	3.11, 3.26	-	-
Leu		3.64	1.42, 1.68	1.02	0.81

Fig. S19: ^{1}H-^{1}H TOCSY NMR of Peptide **5**

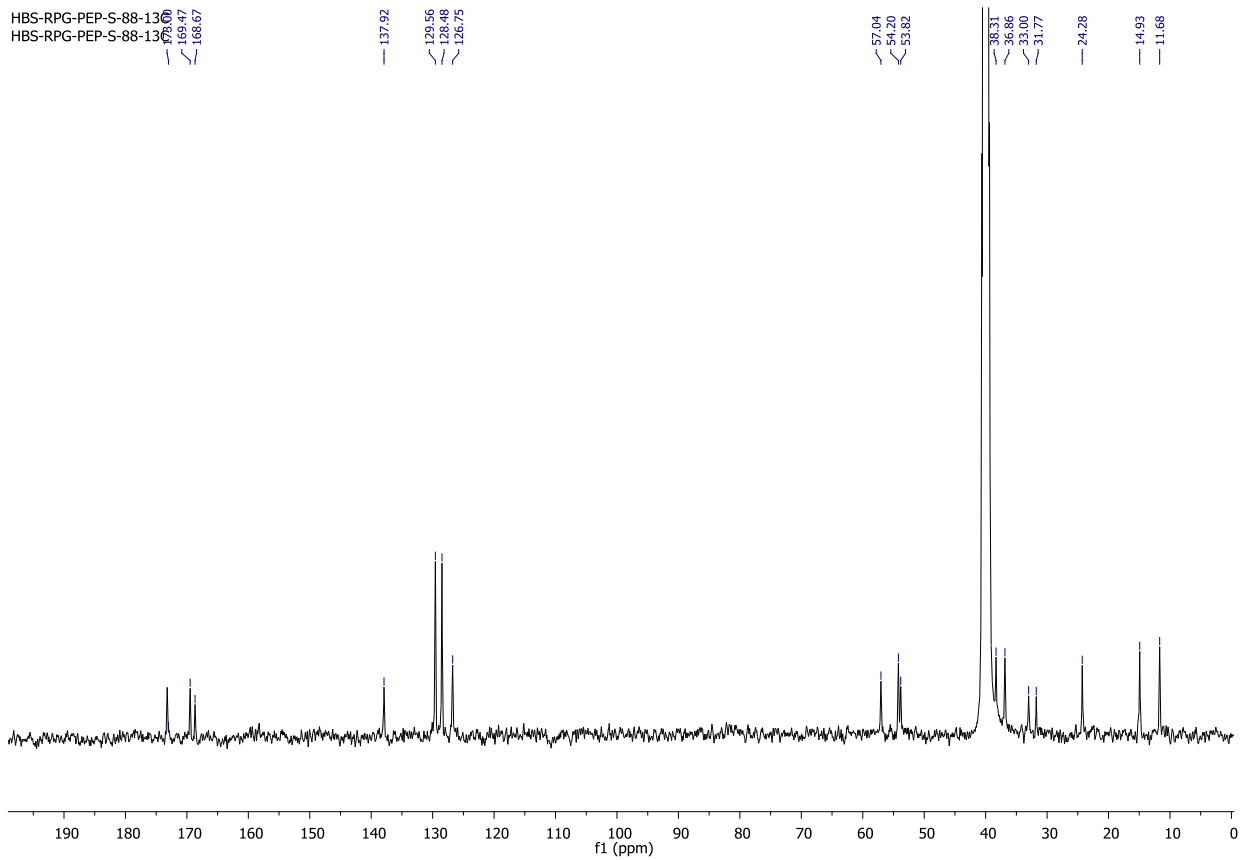


Fig. S20: ^{13}C NMR of Peptide **5**

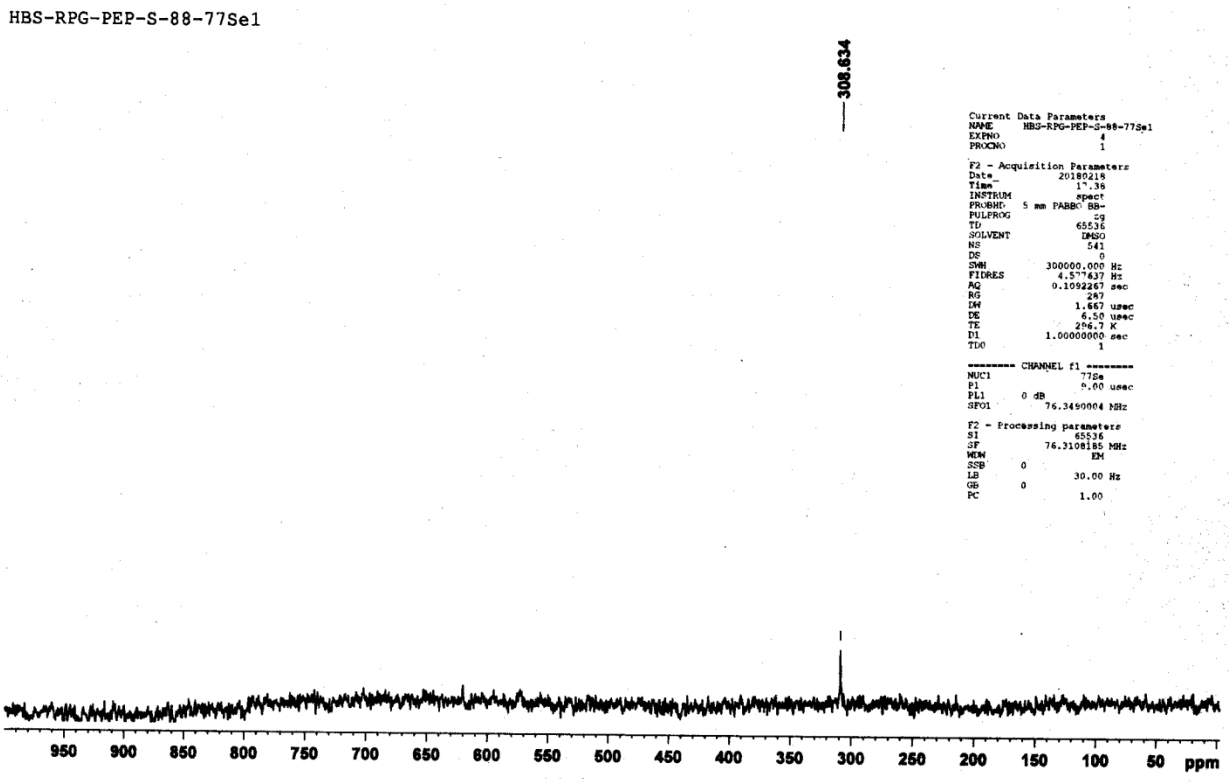
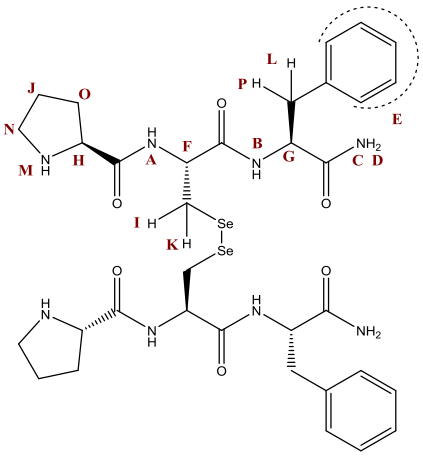


Fig. S21: ^{13}C NMR of Peptide **5**

Peptide **6**



HBS-RPG-PEP-S-95-1H1
HBS-RPG-PEP-S-95-1H1

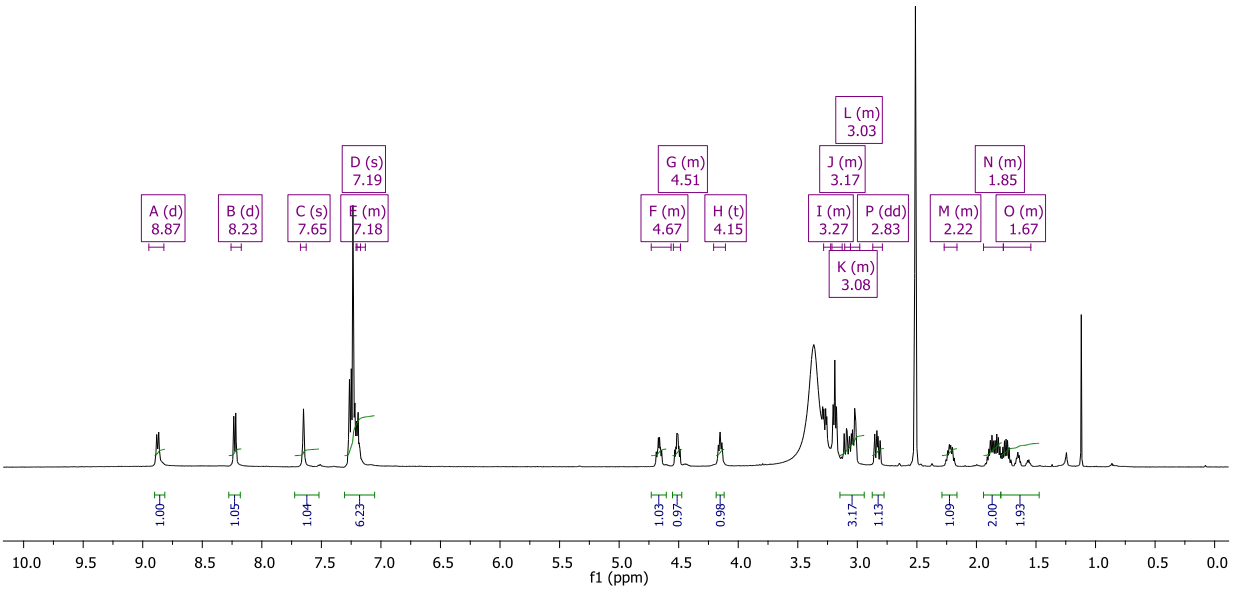


Fig. S22: ^{1}H NMR of Peptide **6**

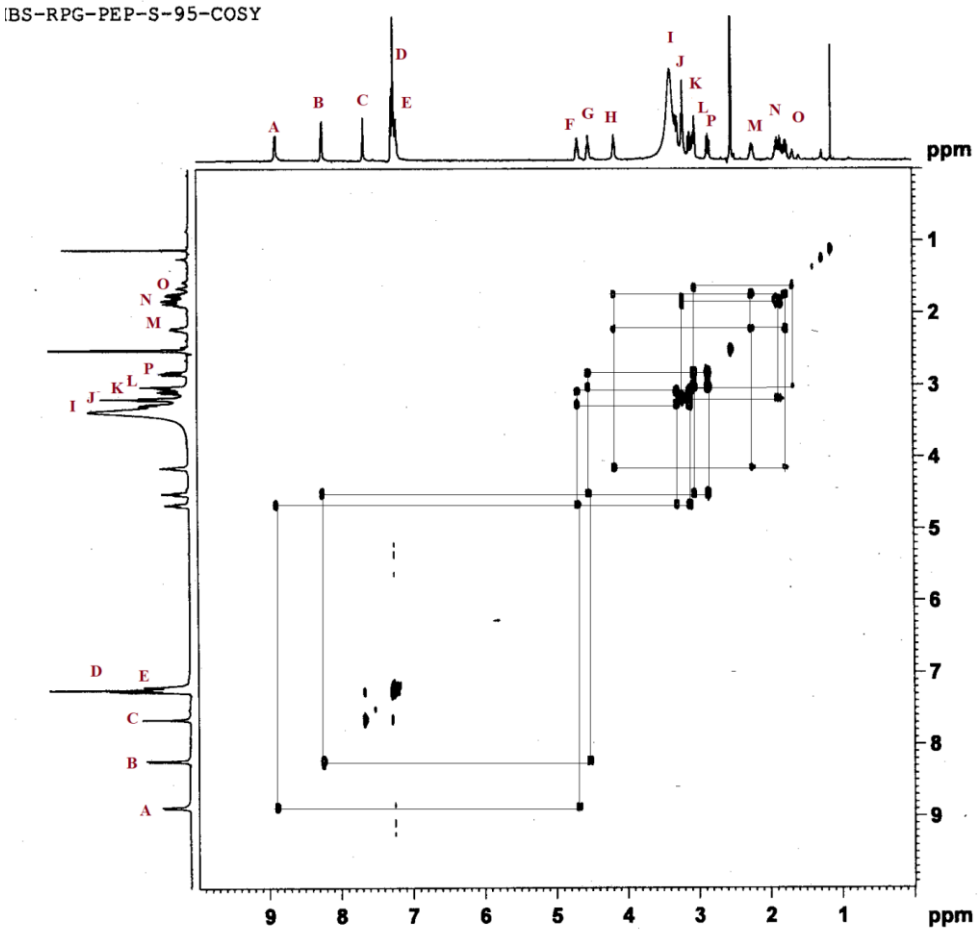
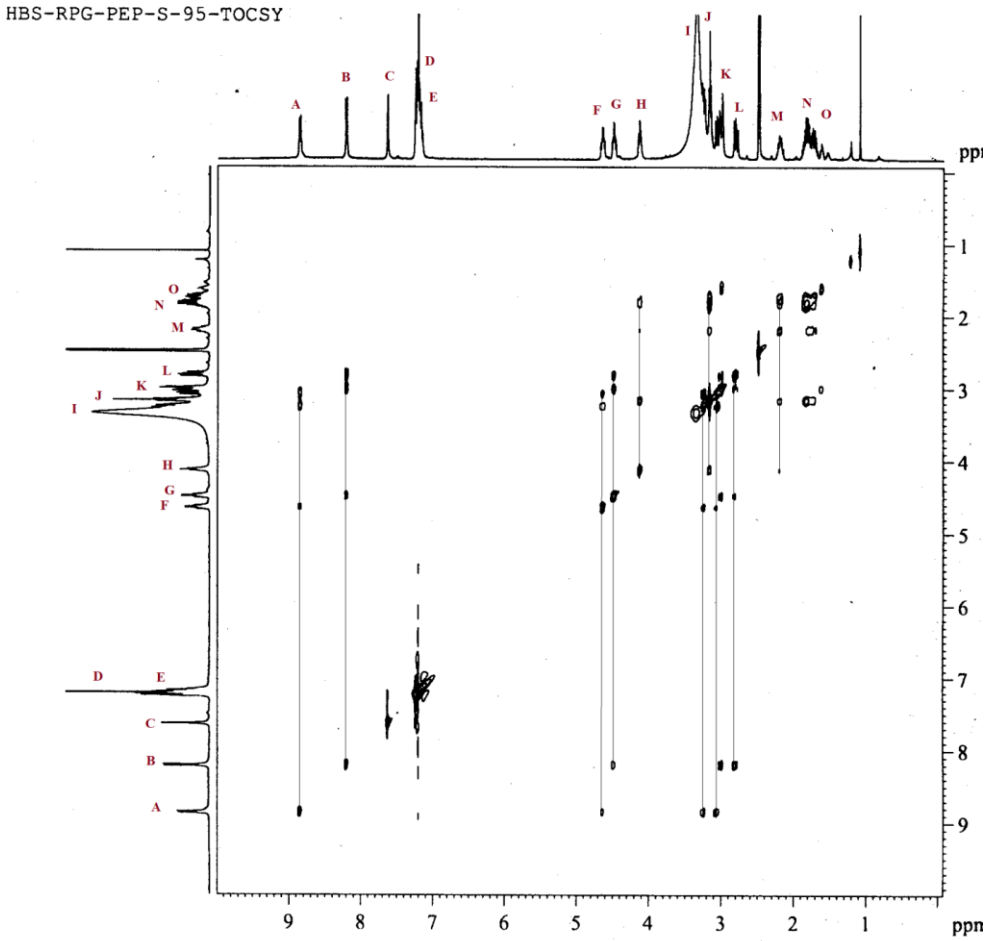


Fig. S23: ^{1}H-^{1}H COSY NMR of Peptide **6**



residue	NH	$C_{\alpha}H$	$C_{\beta}H$	$C_{\gamma}H$	others
Phe	8.23	4.51	2.83, 3.03	-	-
Sec	8.87	4.67	3.08, 3.17	-	-
Pro	2.22	4.15	1.67	3.17	-

Fig. S24: ^{1}H-^{1}H TOCSY NMR of Peptide **6**

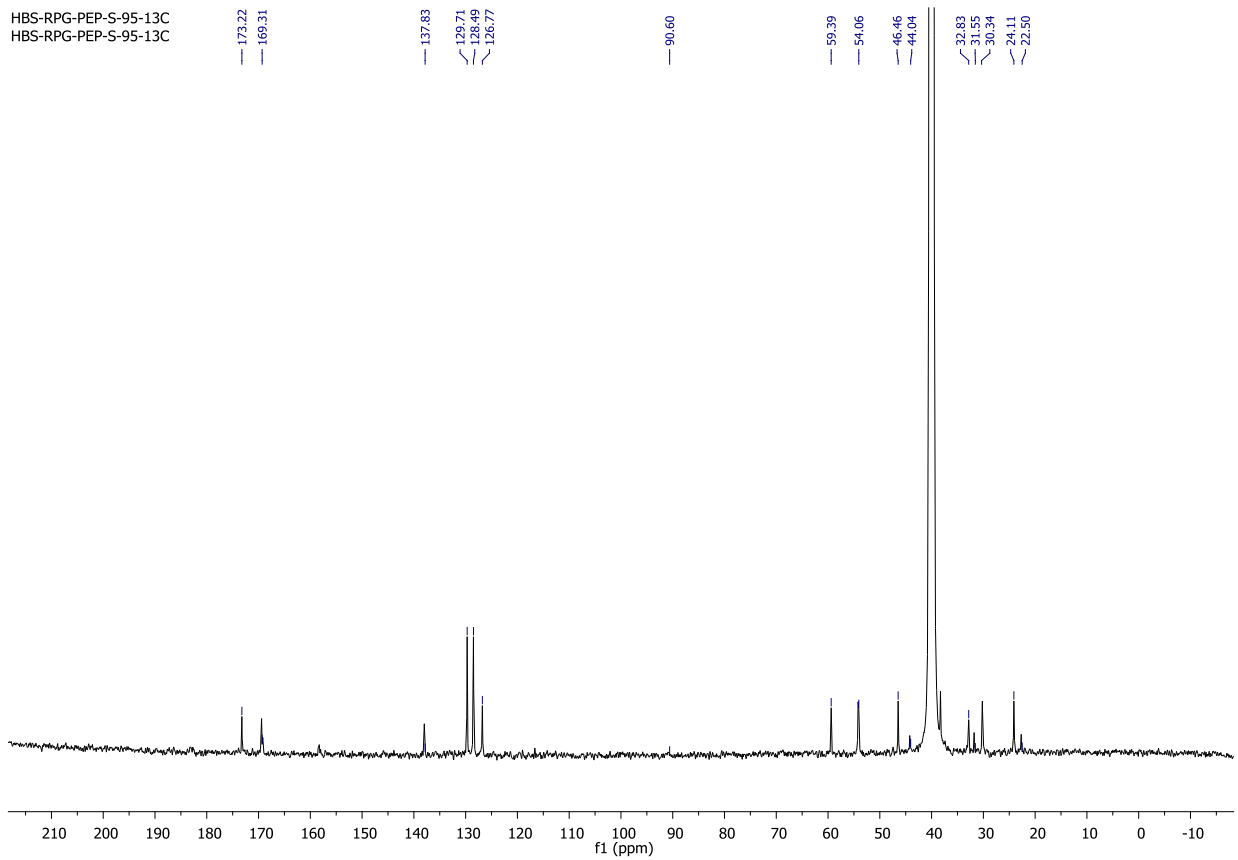


Fig. S25: ^{13}C NMR of Peptide **6**

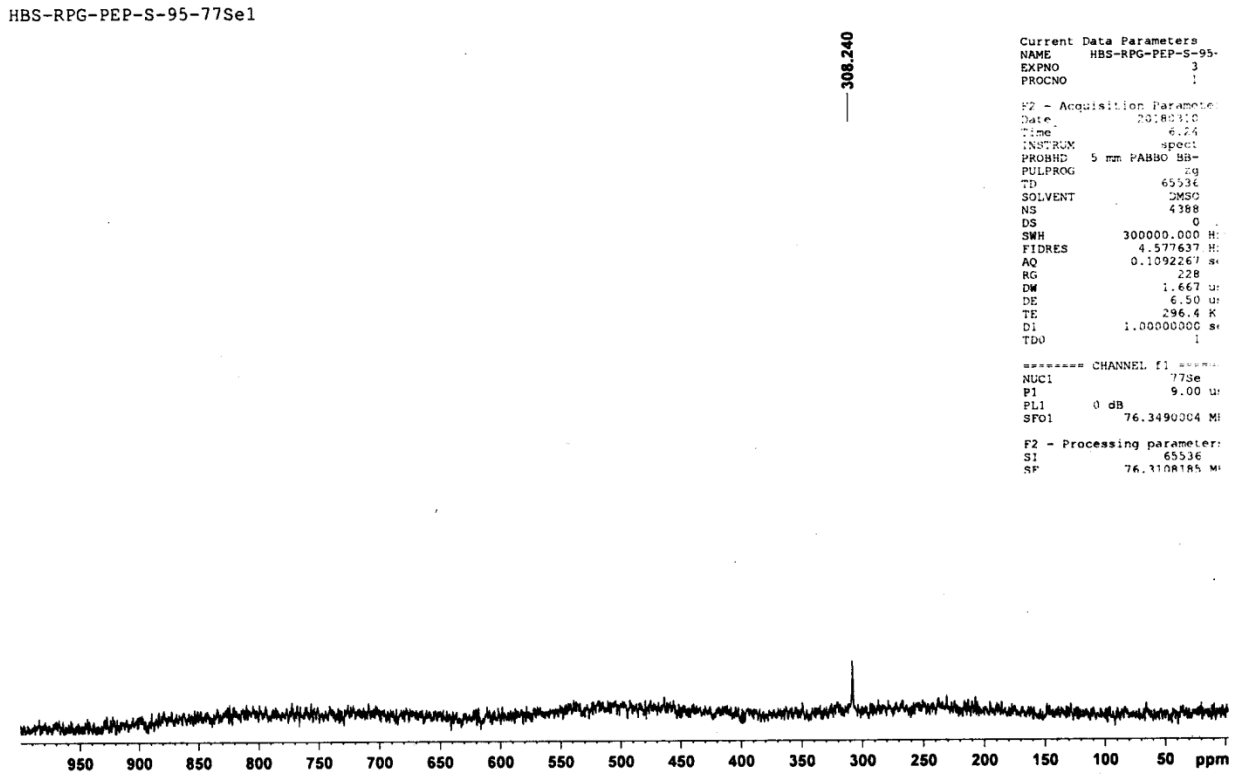
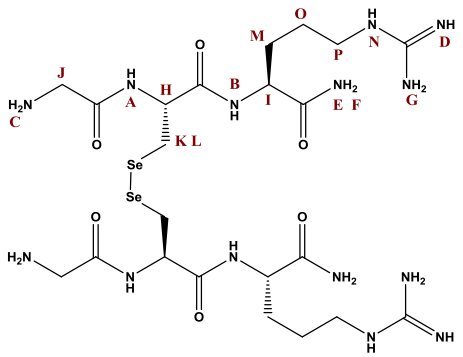


Fig. S26: ^{77}Se NMR of Peptide **6**

Peptide **7**



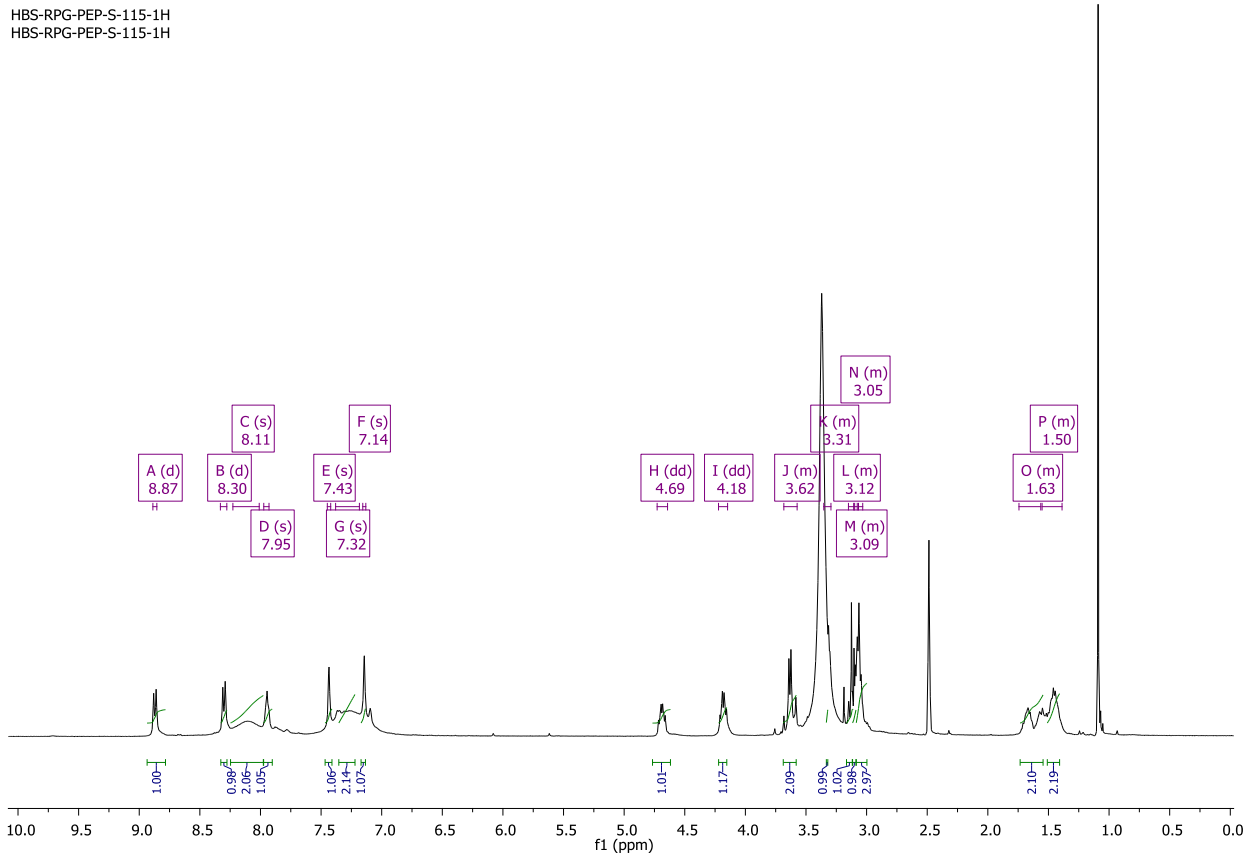


Fig. S27: ^{1}H NMR of Peptide **7**

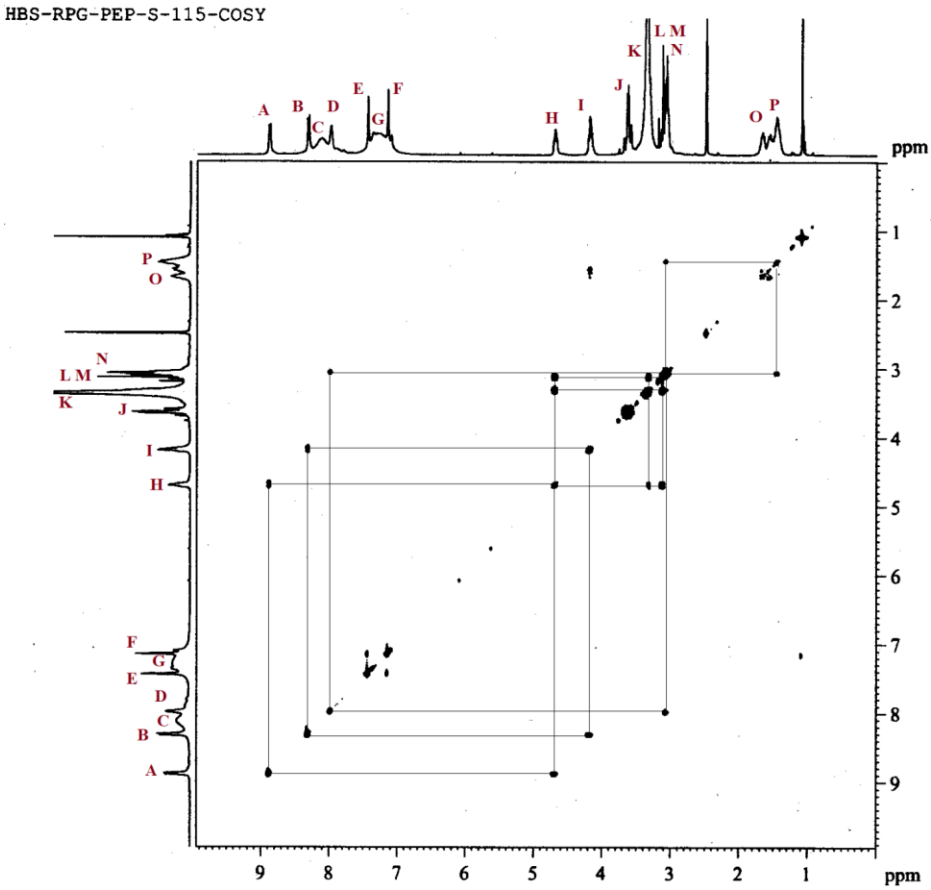
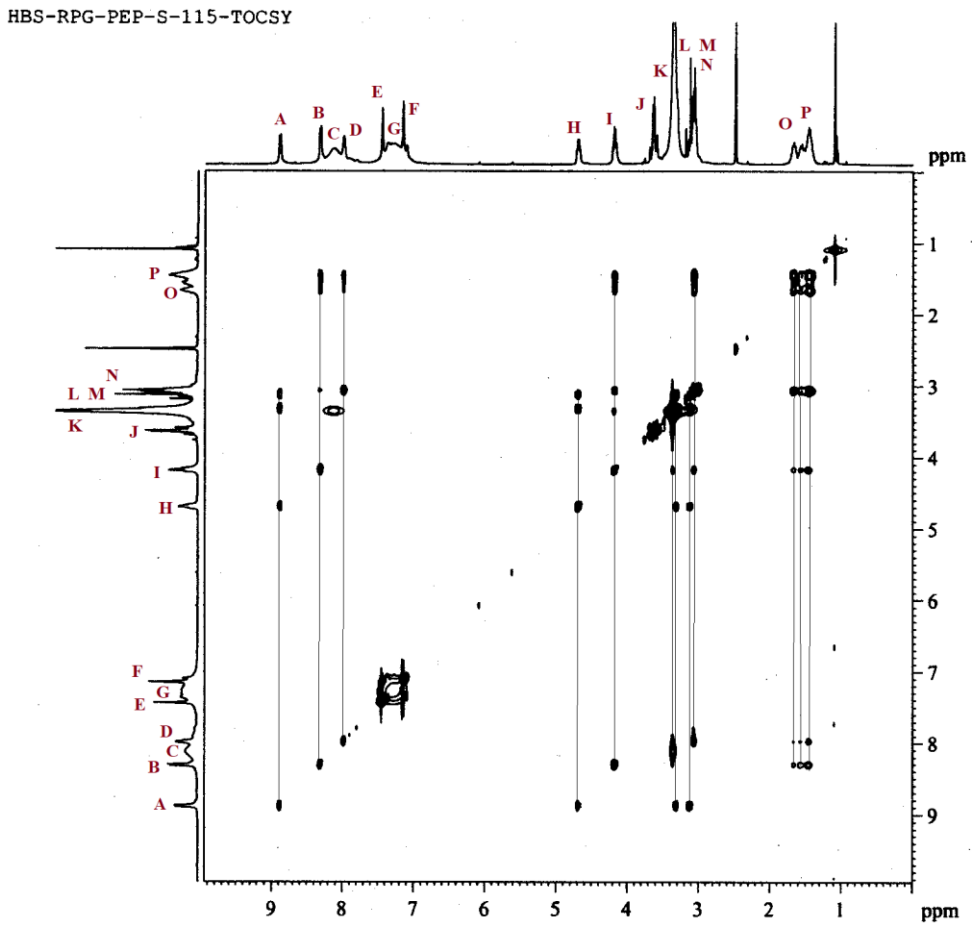


Fig.. S28: ^{1}H-^{1}H COSY NMR of Peptide **7**



residue	NH	$C_{\alpha}H$	$C_{\beta}H$	$C_{\gamma}H$	others
Arg	8.30	4.18	3.09	1.63	1.50, 3.05, 7.95
Sec	8.87	4.69	3.12, 3.31	-	-
Gly		3.62	-	-	-

Fig. S29: ^{1}H-^{1}H TOCSY NMR of Peptide **7**

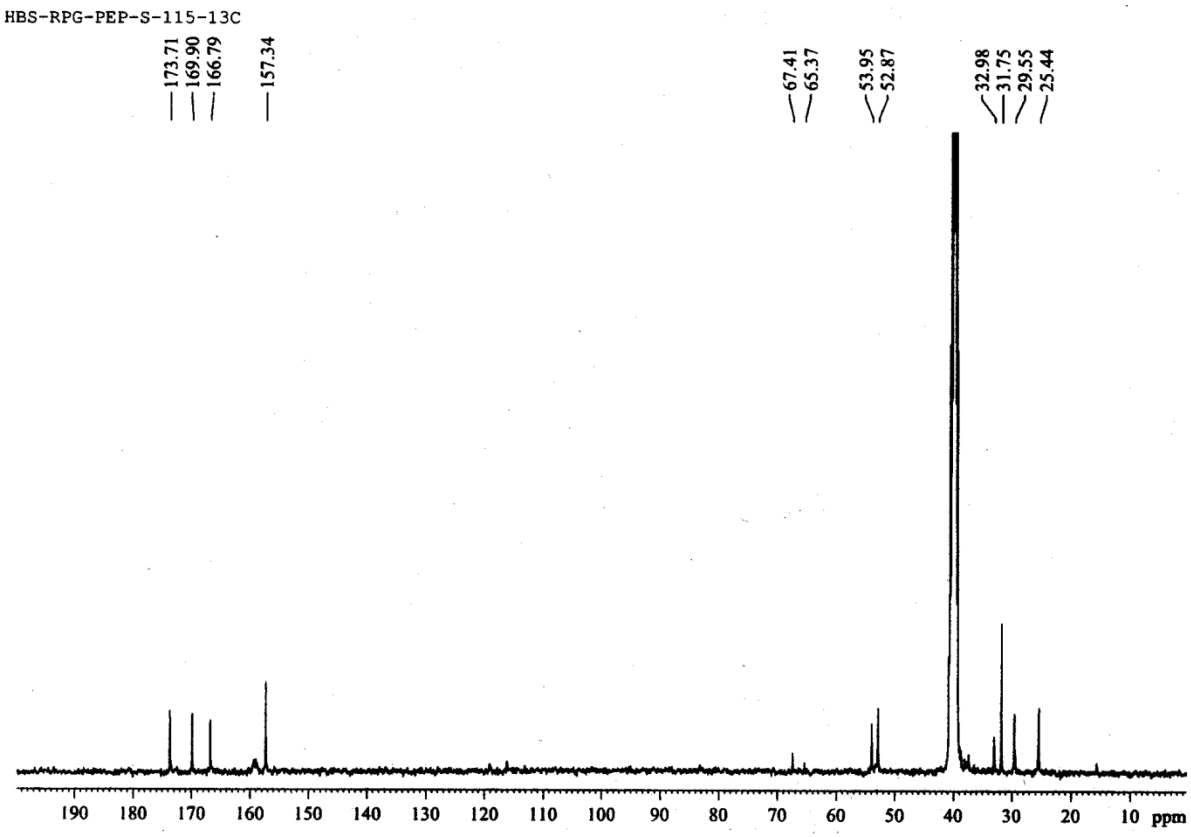


Fig. S30: ^{13}C NMR of Peptide **7**

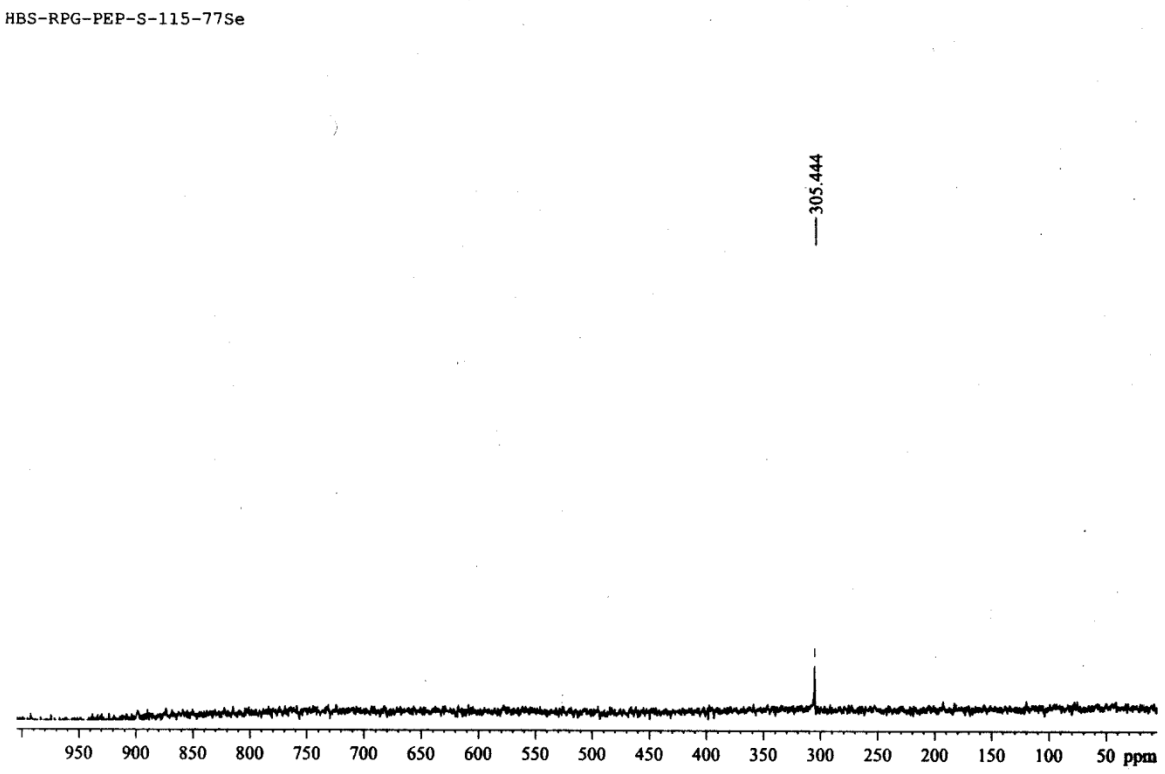


Fig. S31: ^{77}Se NMR of Peptide **7**

Peptide **8**

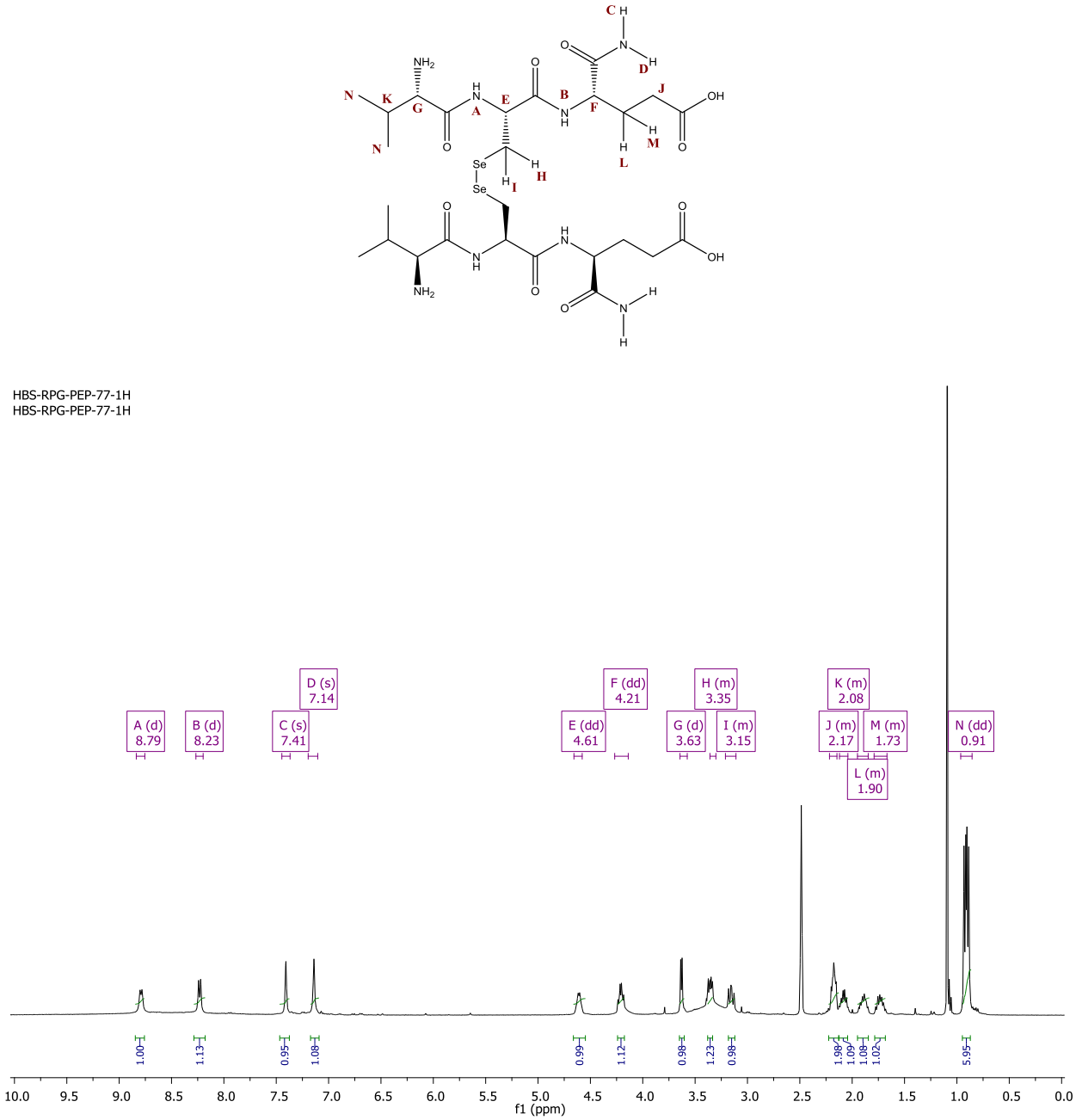


Fig. S32: ^{1}H NMR of Peptide **8**

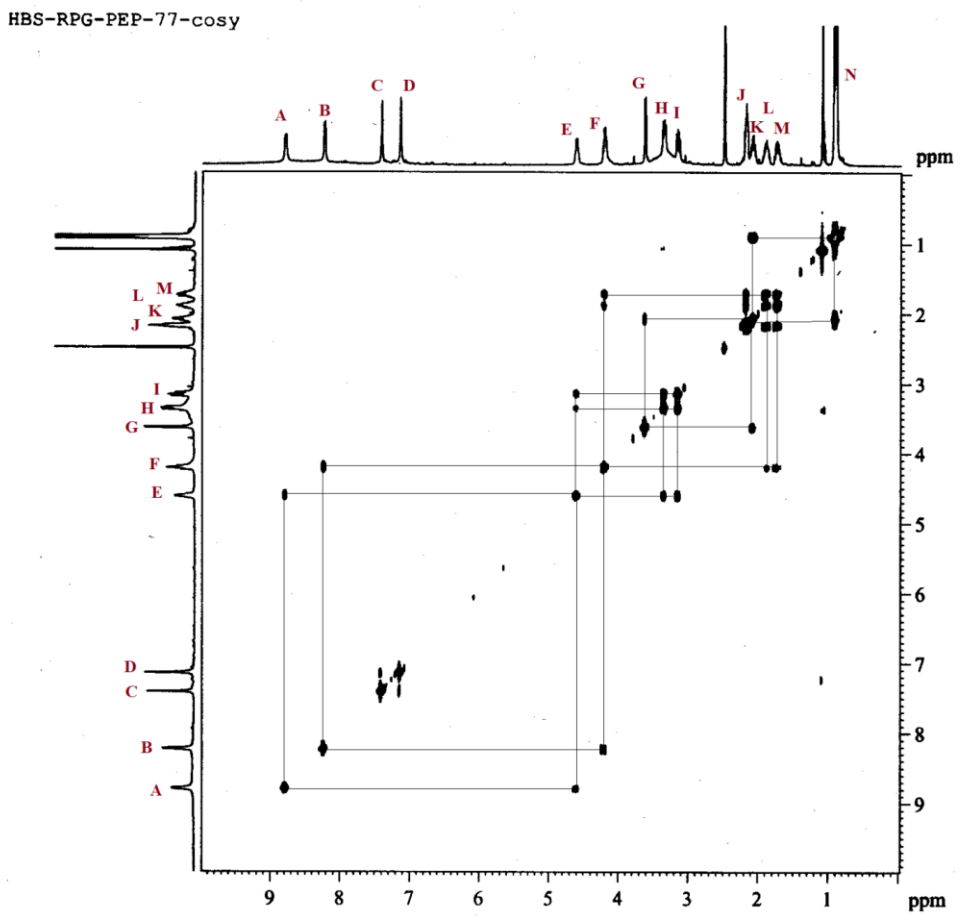
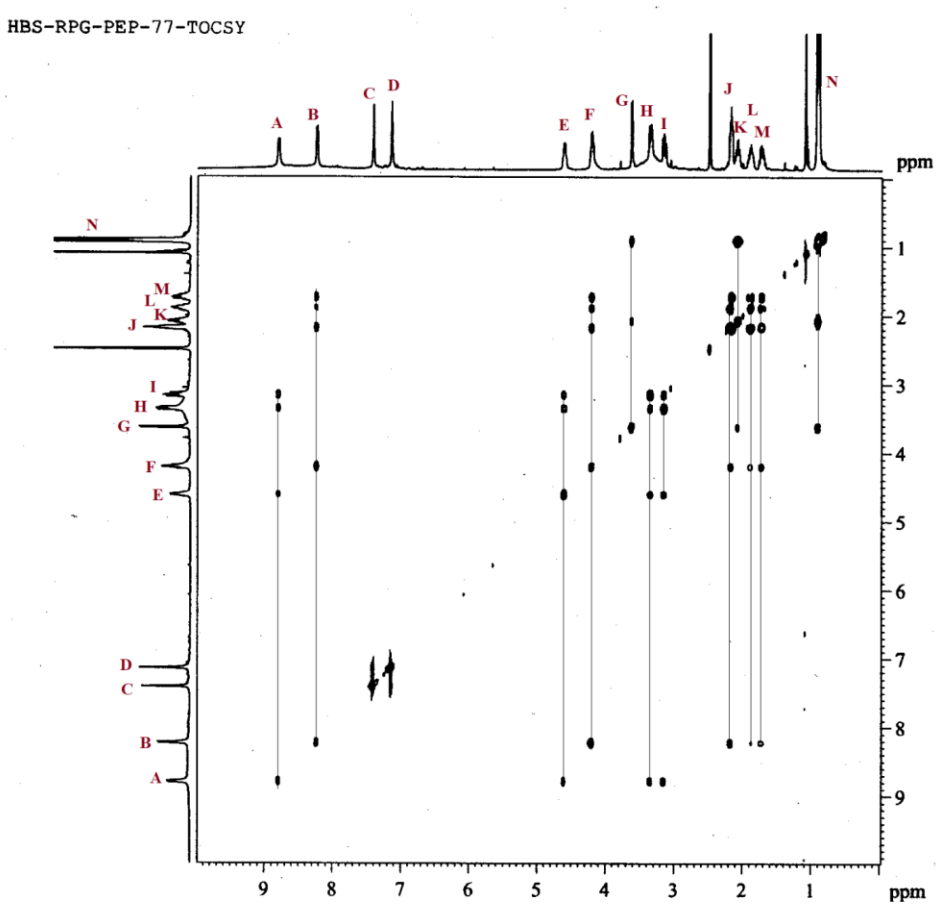


Fig. S33: ^{1}H-^{1}H COSY NMR of Peptide **8**



residue	NH	$C_{\alpha}H$	$C_{\beta}H$	$C_{\gamma}H$	others
Glu	8.23	4.21	1.73, 1.90	2.17	-
Sec	8.79	4.61	3.15, 3.35	-	-
Val	-	3.63	2.08	0.91	-

Fig. S34: ^{1}H-^{1}H TOCSY NMR of Peptide **8**

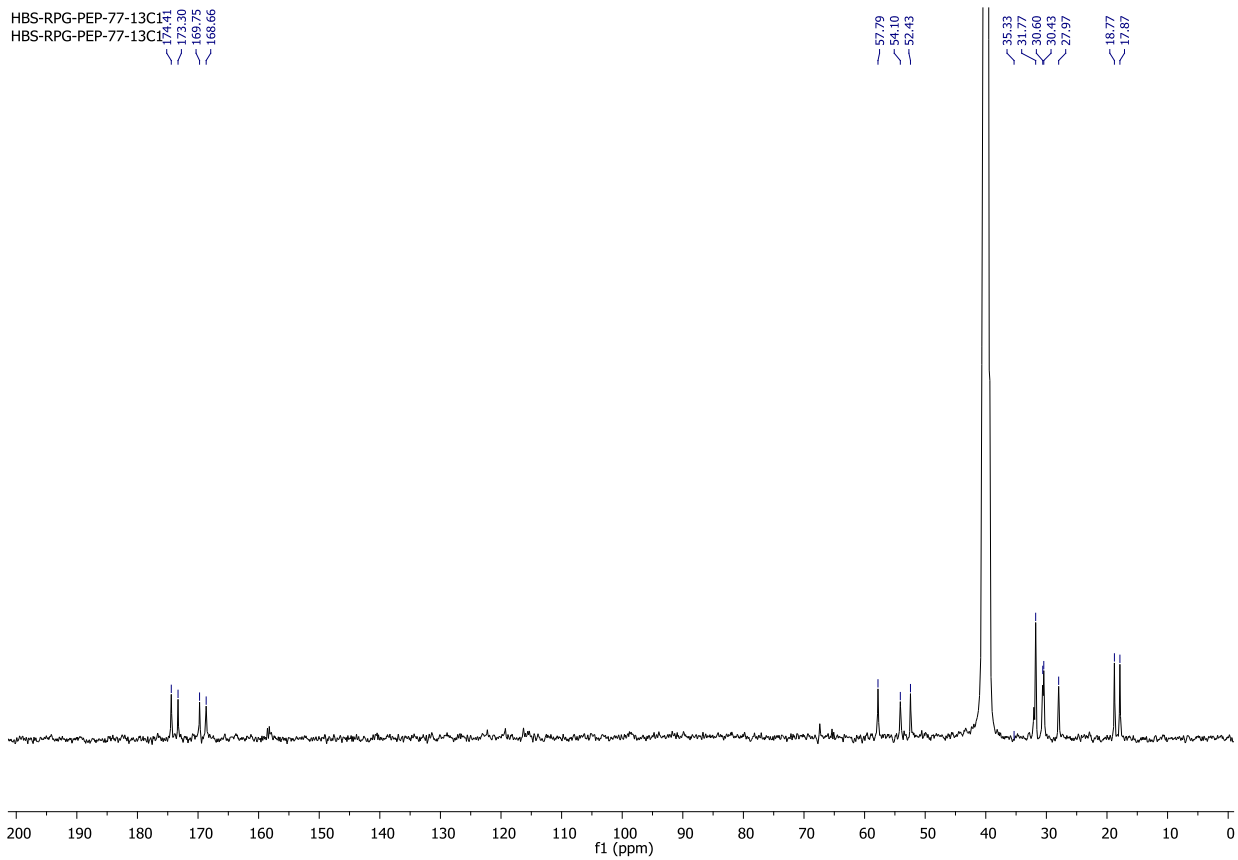


Fig. S35: ^{13}C NMR of Peptide **8**

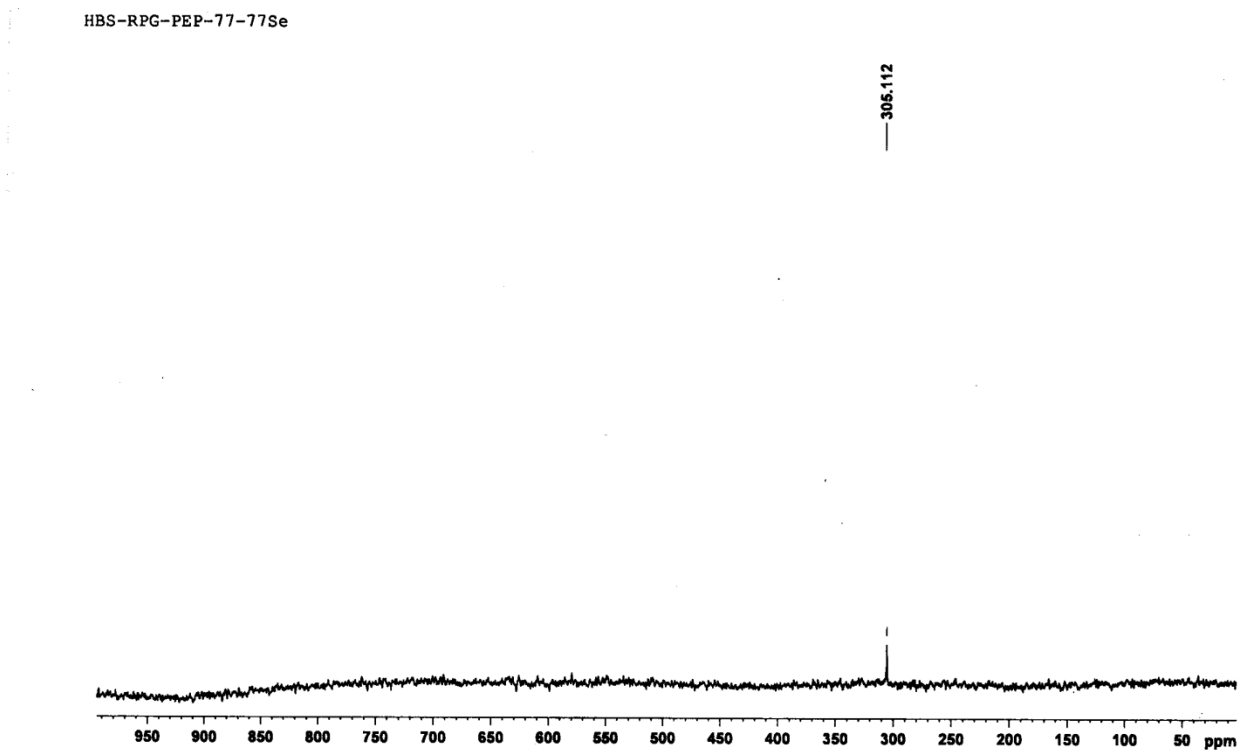


Fig. 36: ^{77}Se NMR of Peptide **8**

Peptide **9**

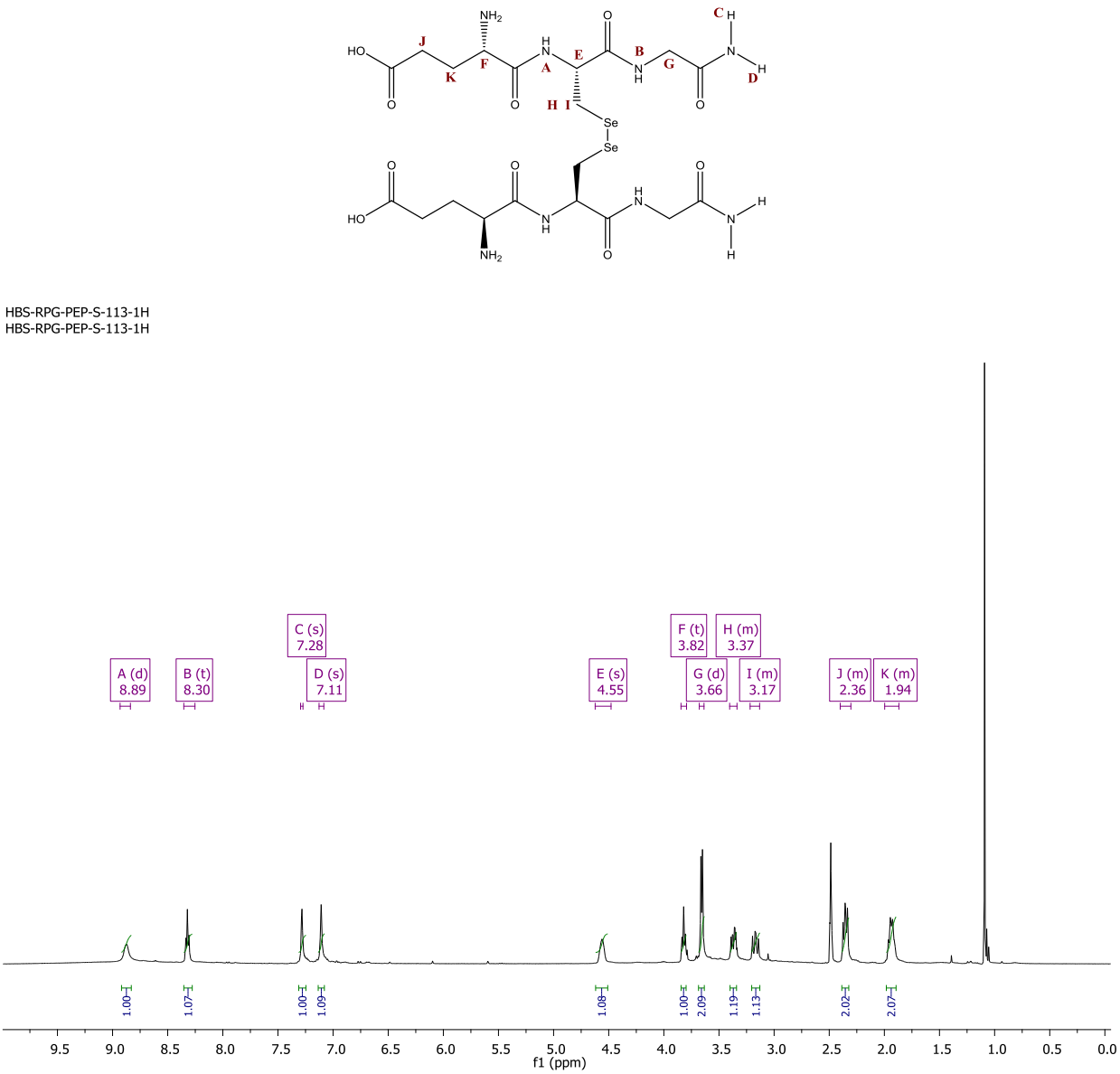


Fig. S37: ^{1}H NMR of Peptide **9**

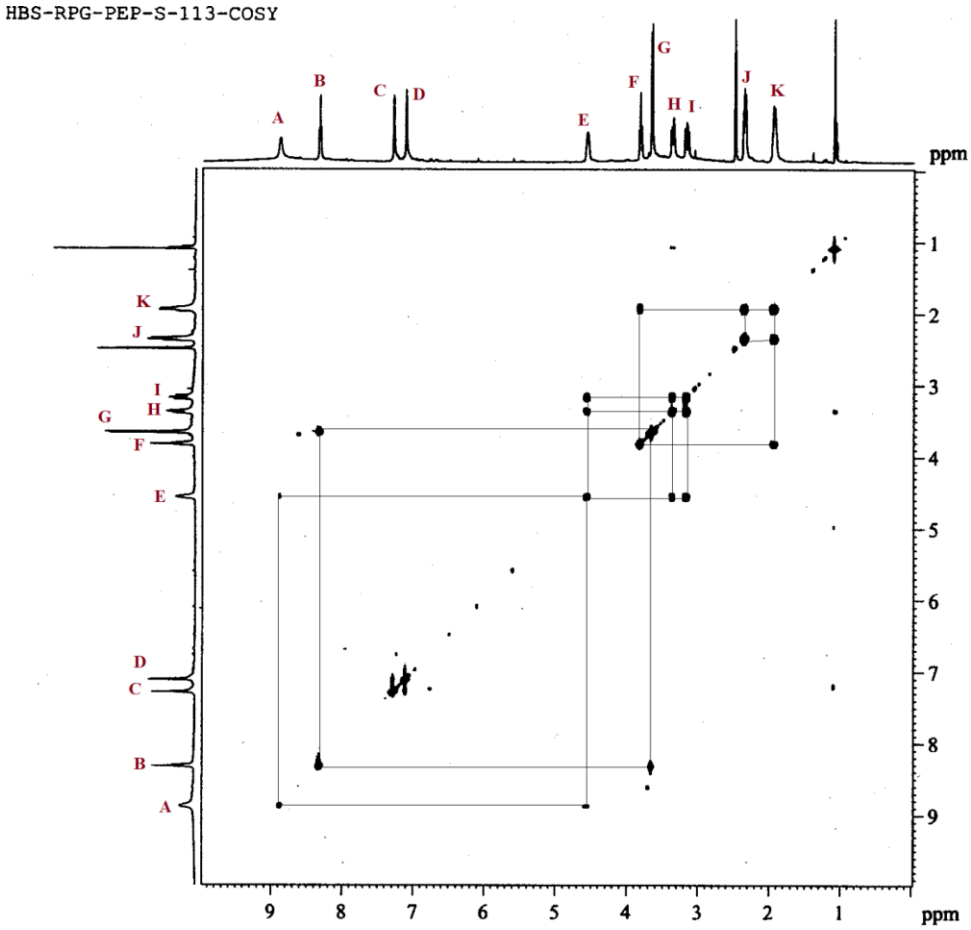
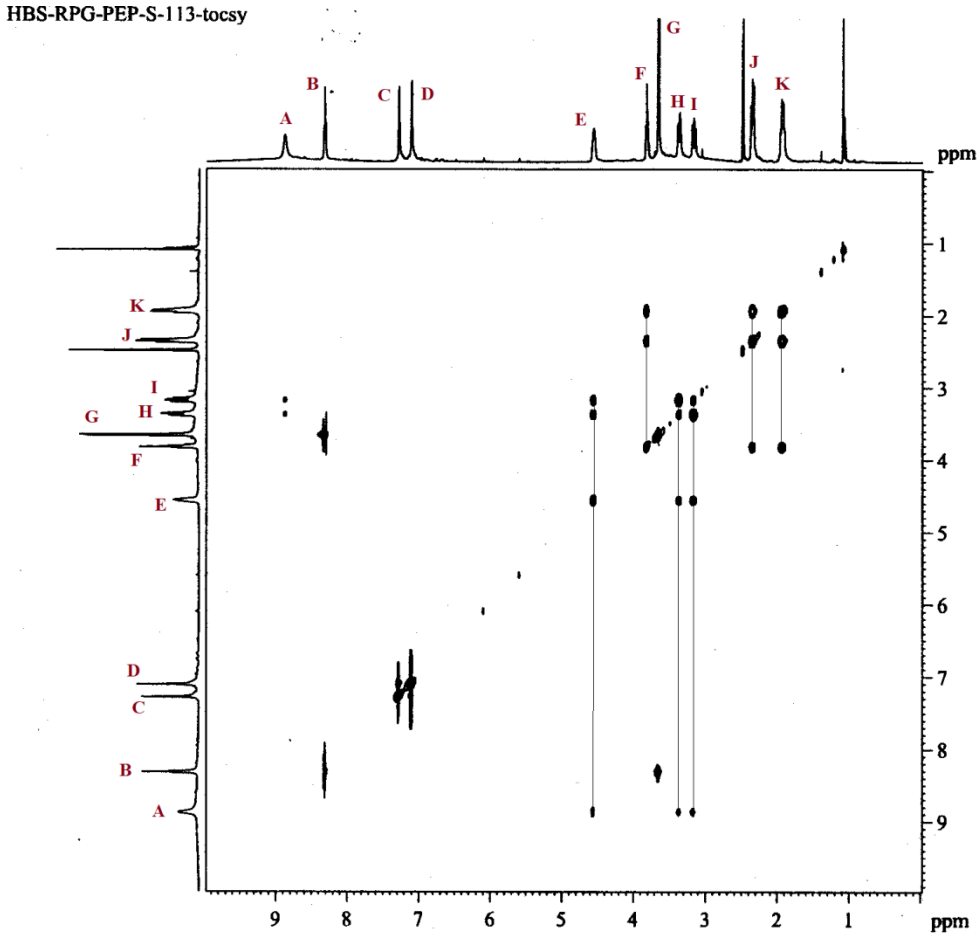


Fig. S38: ^{1}H-^{1}H COSY NMR of Peptide **9**



residue	NH	$C_{\alpha}H$	$C_{\beta}H$	$C_{\gamma}H$	others
Gly	8.30	3.66	-		-
Sec	8.89	4.55	3.17, 3.37	-	-
Glu		3.82	1.94	2.36	-

Fig. S39: ^{1}H-^{1}H TOCSY NMR of Peptide **9**

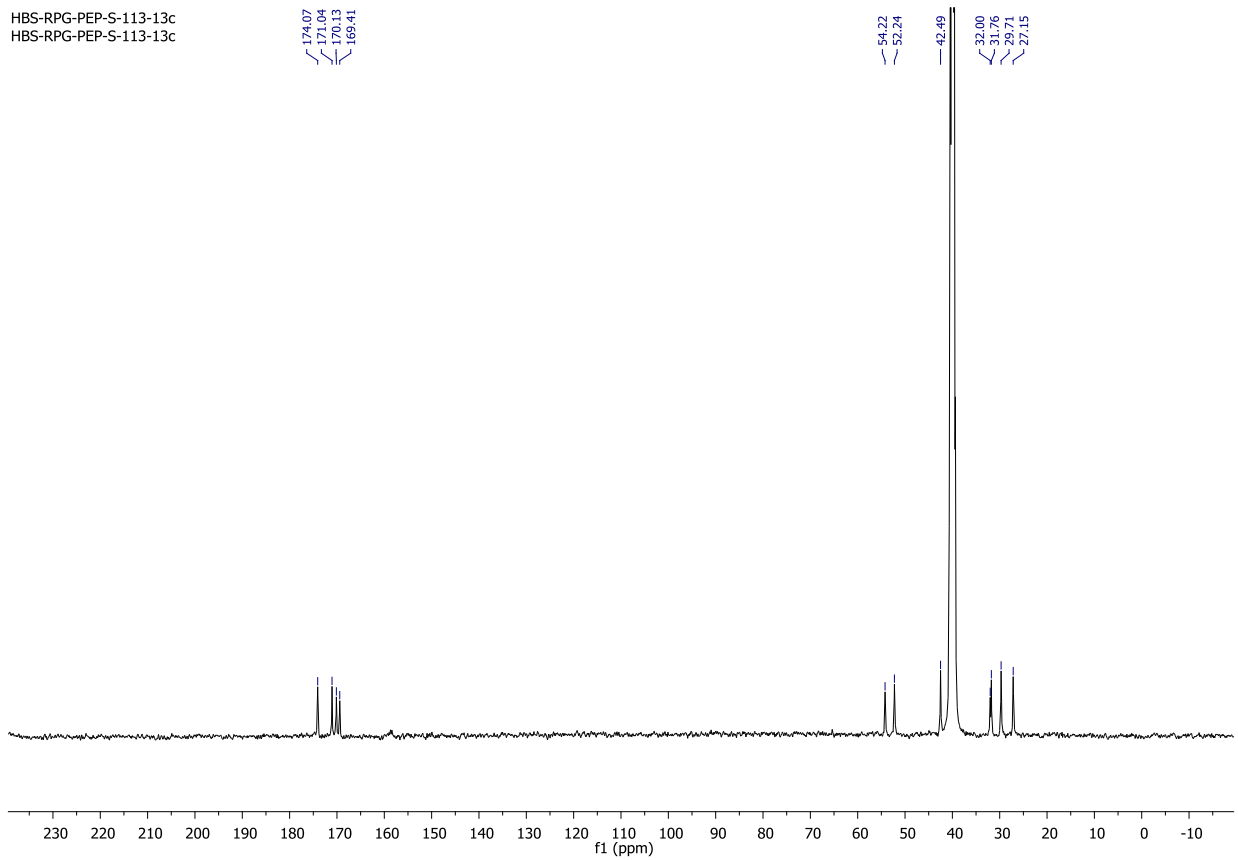


Fig. S40: ^{13}C NMR of Peptide **9**

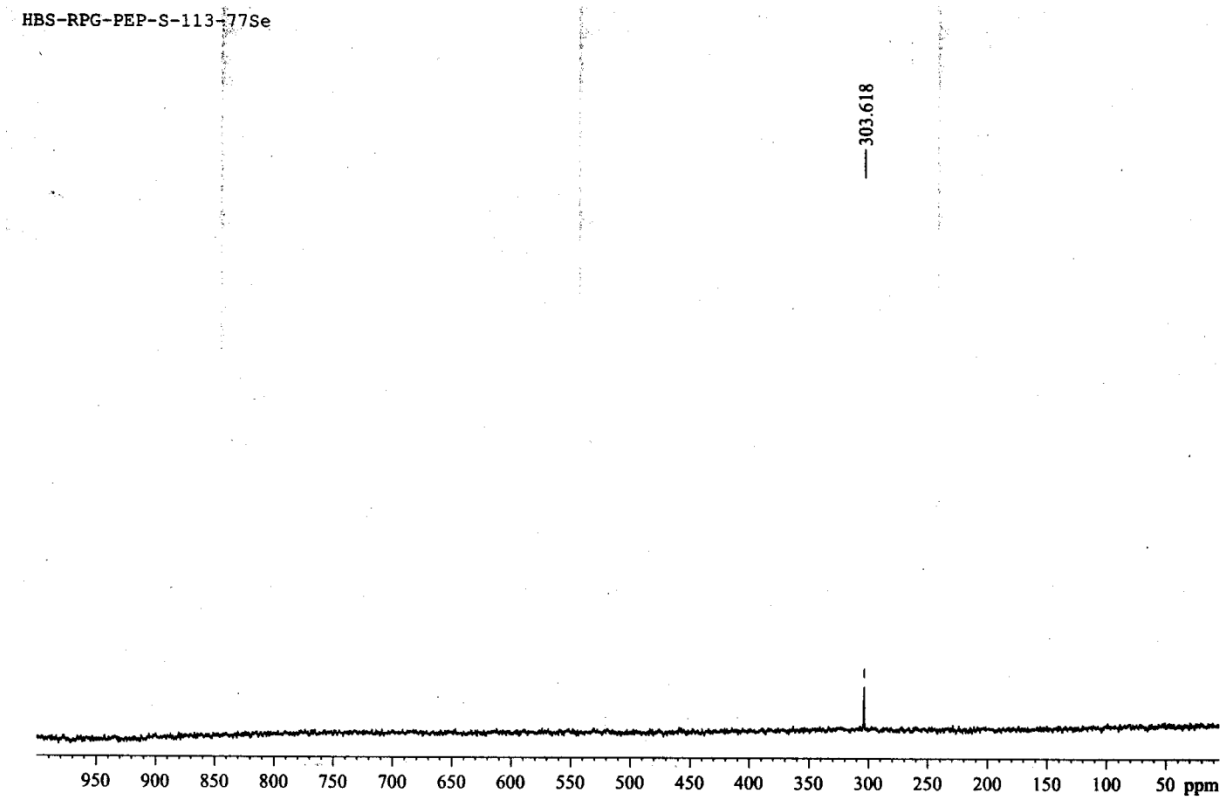
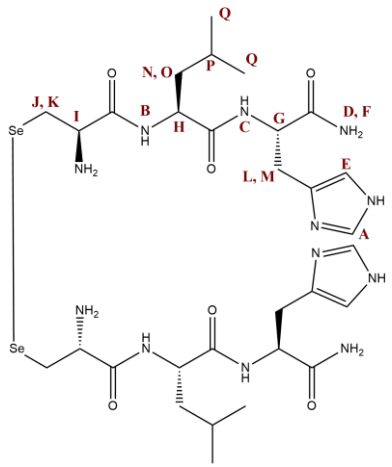


Fig. S41: ^{77}Se NMR of Peptide **9**

Peptide **10**



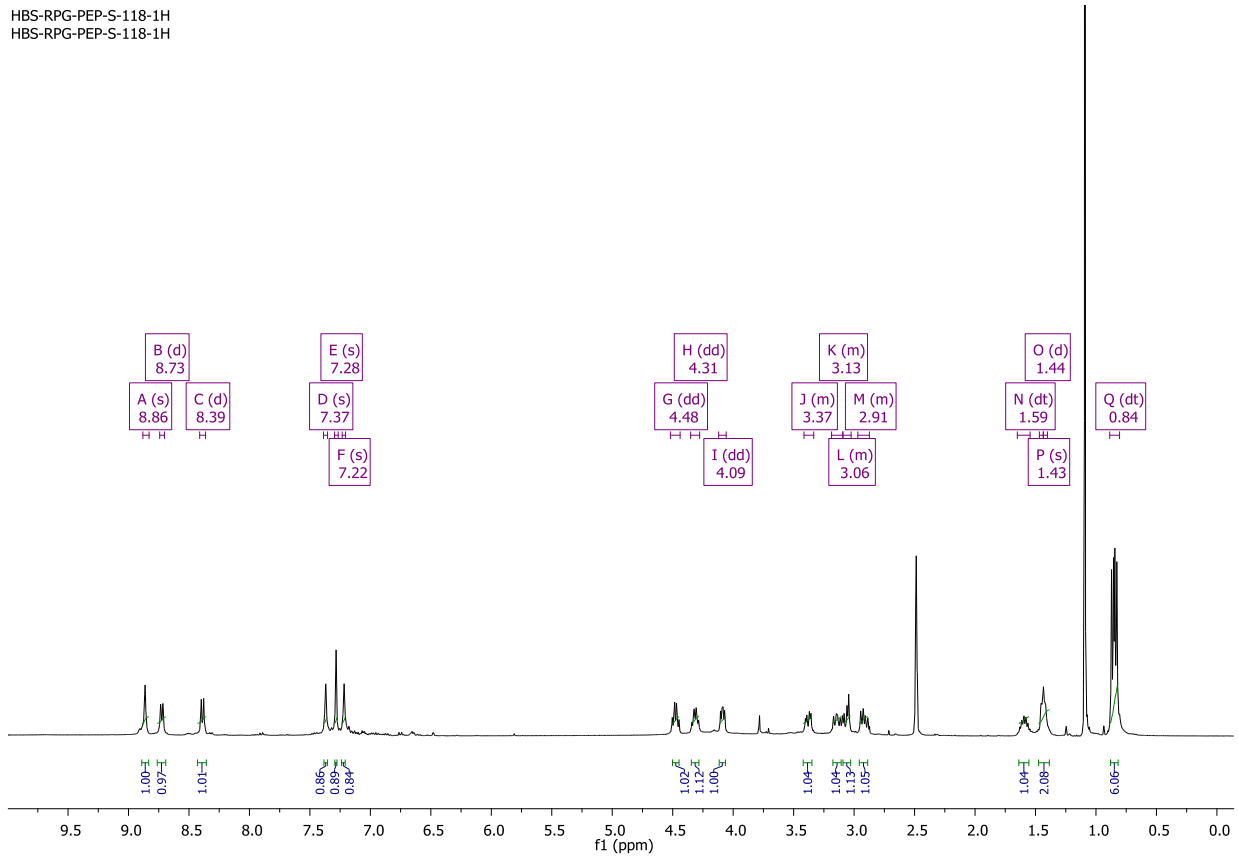


Fig. S42: ^{1}H NMR of Peptide **10**

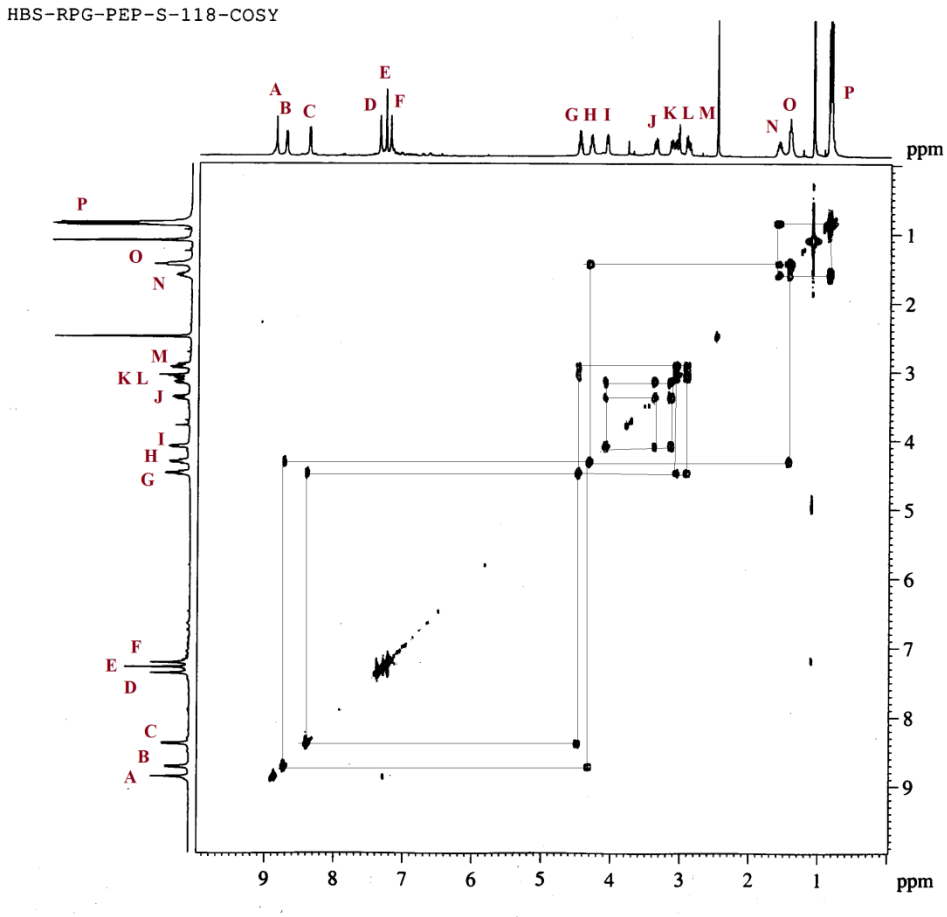
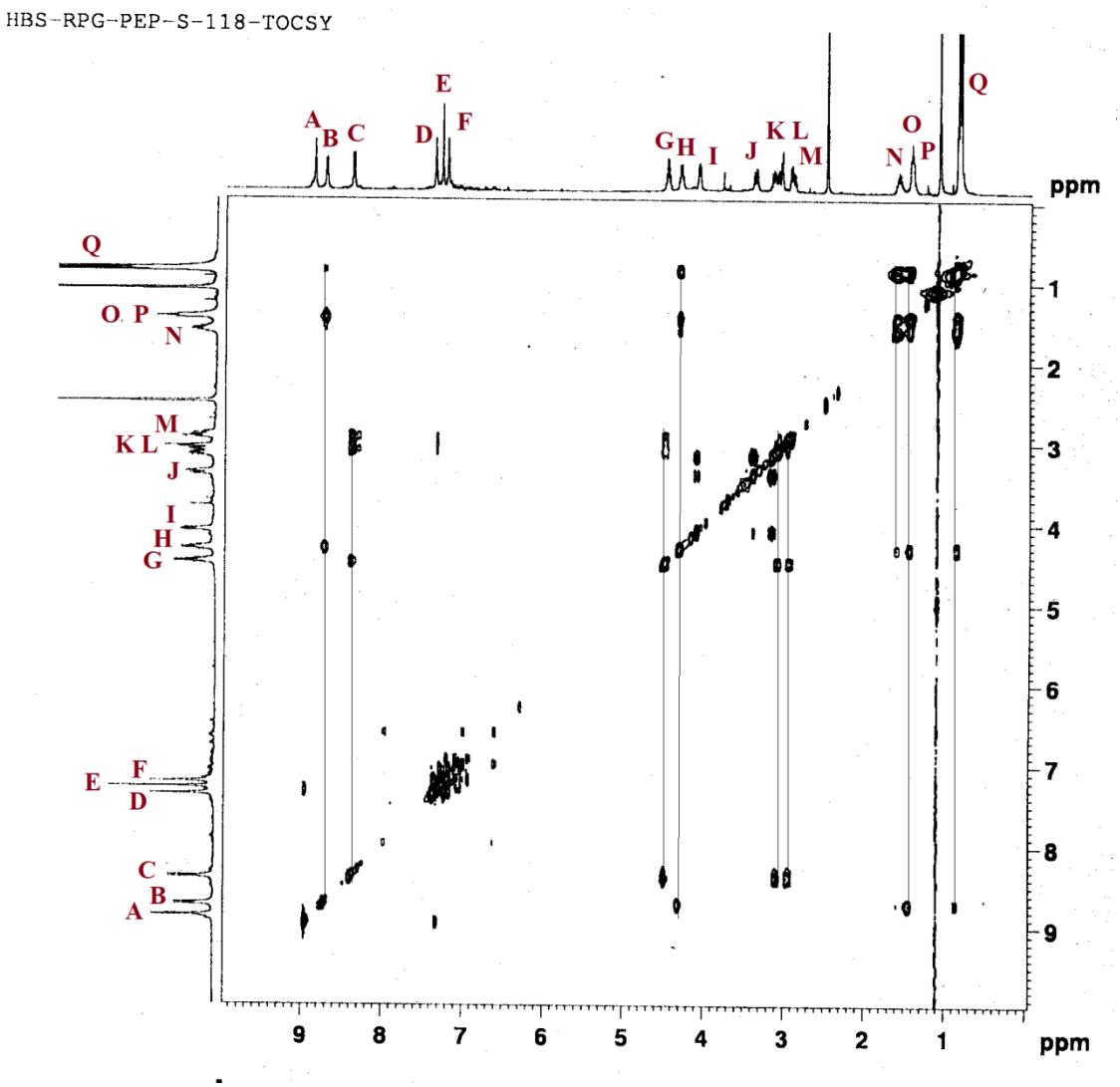


Fig. S43: ^{1}H-^{1}H COSY NMR of Peptide **10**



residue	NH	$C_{\alpha}H$	$C_{\beta}H$	$C_{\gamma}H$	others
His	8.39	4.31	2.91, 3.06		-
Leu	8.73	4.48	1.44, 1.59	1.43	0.84
Sec	-	4.09	3.13, 3.37	-	-

Fig. S44: ^{1}H-^{1}H TOCSY NMR of Peptide **10**

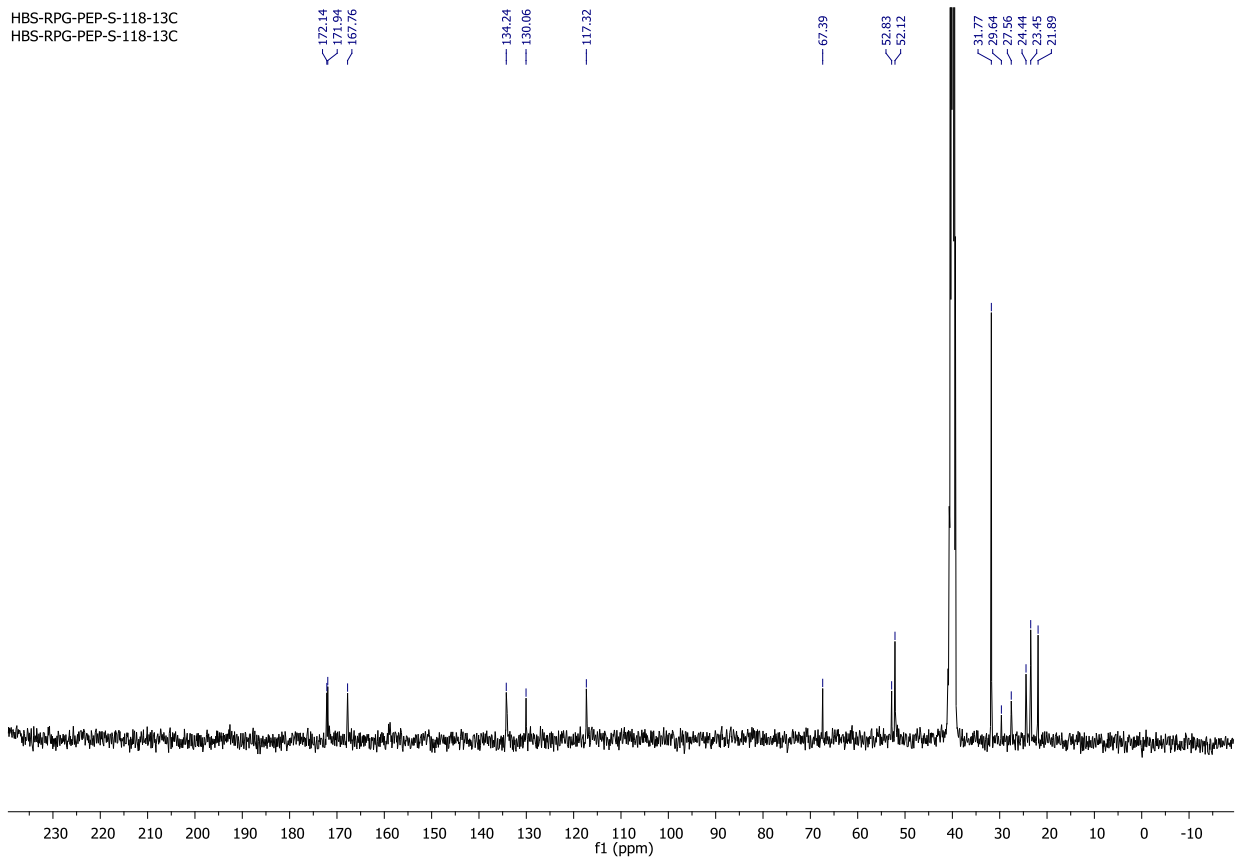


Fig. S45: ^{13}C NMR of Peptide **10**

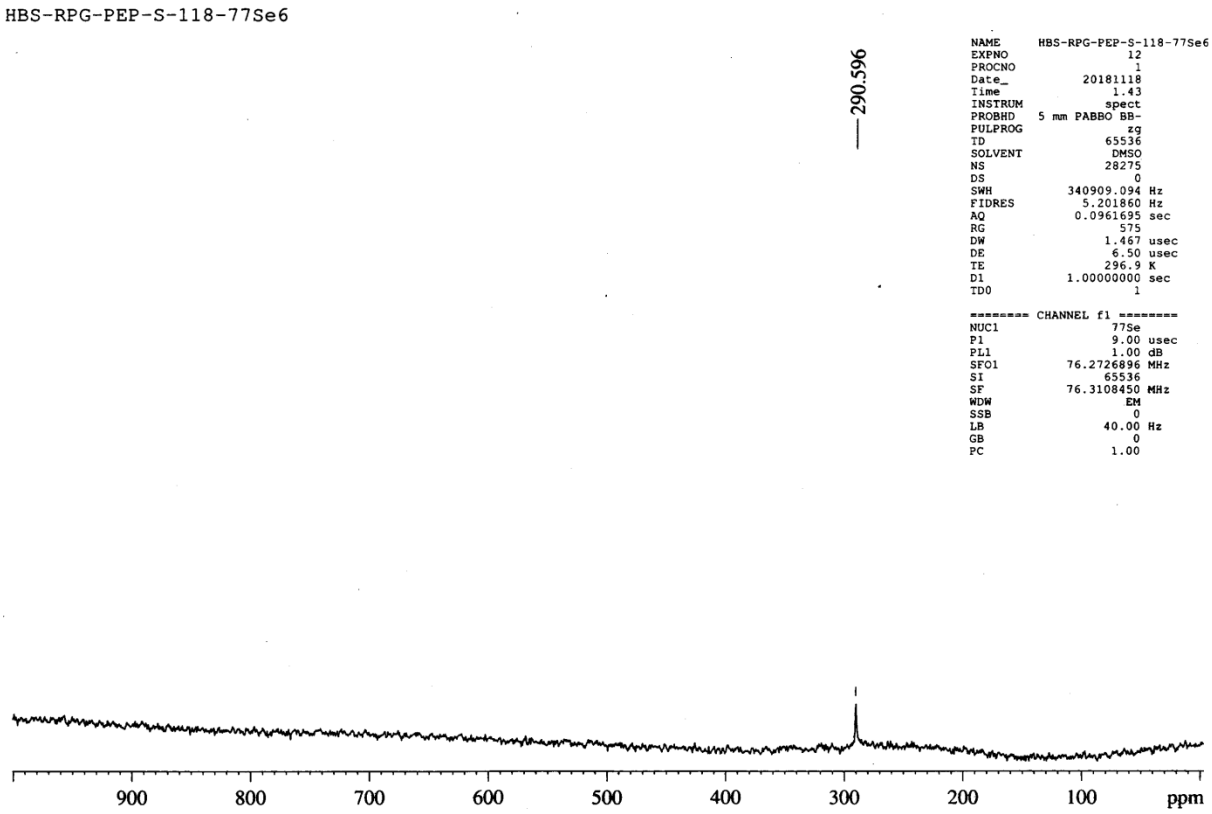


Fig. S46: ^{77}Se NMR of Peptide **10**

Supplementary References:

(1) S. Jr Flemer, *J. Pept. Sci.*, 2015, **21**, 53–59.

(2) M. Muttenthaler and P. F. Alewood, *J. Pept. Sci.*, 2008, **14**, 1223–1239.