Electronic Supplementary Materials

Selenazolidine: a selenium containing proline surrogate in peptide science.

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Material and methods 3

Chemicals **3**

Mass spectrometry analyses **3**

Syntheses of compounds 1 to 10 4

Synthesis of Fmoc-selenazolidine 1a **4**

Synthesis of Boc-selenazolidine 1b **4**

Synthesis of dipeptide Boc-Sez-Phe-NH_2 3 in solution **4**

Synthesis of H-Sez-Phe-NH_2 4 ..... **4**

Synthesis of Boc-Ala-Sez-Phe-NH_2 5 ..... **5**

Synthesis of H-Ala-Sez-Phe-NH_2 6 ..... **5**

Synthesis of Fmoc-Sez-Phe-NH_2 7 ..... **5**

Synthesis of dipeptide Fmoc-Ala-Sez-OH 9 and dipeptide Fmoc-Arg(Pbf)-Sez-OH 10 ..... **5**

Supported synthesis of tripeptide H-Ala-Sez-Phe-NH_2 6 in Fmoc/tBu strategy **6**

Stability experiments protocols 6

Stability studies of N-Fmoc and N-Boc protected selenazolidine **6**

N-Boc removal of Boc-Sez-OH 1b in a solution of DMF/piperidine 80/20 v/v **6**

N-Fmoc removal of Fmoc-Sez-OH 1a in a solution of DMF/piperidine 80/20 v/v **6**

Stability of TFA,H-Sez-OH in a solution of DMF/piperidine 80/20 v/v **6**

Stability of Fmoc-Sez-Phe-NH_2 7 in a solution of DMF/piperidine 80/20 v/v **6**

Stability of Boc-Sez-Phe-NH_2 3 in a solution of DCM/TFA 50/50 v/v **7**

Stability of Boc-Ala-Sez-Phe-NH_2 5 in a solution of DCM/TFA 50/50 v/v **7**

Stability of H-Ala-Sez-Phe-NH_2 6 in TFMSA/TFA/TIS solution **7**

Study of several N-Fmoc removal conditions on Fmoc-Sez-Phe-NH-RA (RA: Rink amide resin) **7**

Stability of Boc-Ala-Sez-Phe-NH_2 5 in a solution of DMF/piperidine 80/20 v/v **8**

Figures S1 to S6 illustrating Sez stabilities experiments 9

Characterization of [Sez]^{6}-HO-Phpa-LVA 12
Isotopic cluster of [Sez]^{6}-HO-Phpa-LVA 12
^{13}C NMR of [Sez]^{6}-HO-Phpa-LVA : 13
MALDI-Tof/Tof MS/MS of [Sez]^{6}-HO-Phpa-LVA 13
Post proline enzymatic digestion 14
NMR spectra 15
Fmoc-selenazolidine 1a 15
Boc-selenazolidine 1b 16
Fmoc-Ala-Sez-OH 9 17
Fmoc-Arg(Pbf)-Sez-OH 10 18
[Sez]^{6}-HO-Phpa-LVA 19

Material and methods

Chemicals

N,N-dimethylformamide (DMF), anhydrous DMF, amine-free DMF were obtained from Sigma–Aldrich with the following specifications: DMF: Assay spec ≥ 99 %; impurity ≤ 0.1 % (H_2O). Anhydrous DMF: Assay spec ≥ 99.8 %; impurity ≤ 0.005 % (H_2O). Amine-free DMF: Assay spec ≥ 99.9 %; free amine as dimethyl amine < 8 ppm; acid ≤ 0.005 meq/g ; base ≤ 0.0003 meq/g; evaporation residue < 0.0005 %. All other solvents were purchased from Carlo Erba and were used without purification. Solvents used for HPLC and LC/MS analyses were of HPLC grade. Protected amino acids, L-selenocystine, resins and coupling reagents were purchased from Iris Biotech GmbH. Other reagents were purchased from Aldrich: Di-tert-butyl dicarbonate (Boc_2O), trifluoroacetic acid (TFA), N-9-Fluorenylmethoxycarbonyloxy-N-Hydroxysuccinimide (Fmoc,-OSu), Benzotriazol-1-yloxy)tris(dimethylamino)phosphonium hexafluorophosphate (BOP), 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU), diisopropylethylamine (DIEA), 2-(7-Aza-1H-benzotriazole-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate (HATU), hydroxybenzotriazole (HOBt), O-(1H-benzotriazol-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate (HBTU), 2,2,4,6,7-pentamethyldihydrobenzofuran-5-sulfonyl (Pbf), triisopropylsilane (TIS), tetramethylfluoroformamidinium hexafluorophosphate (TFFH).

Mass spectrometry analyses

Low resolution LC-UV-MS analyses were performed on a Waters Alliance 2690 HPLC coupled to a ZQ spectrometer (electrospray ionization mode, ESI+). Analyses were carried out with the column oven at 25°C, a Chromolith C18 Flash 25 x 4.6 mm column from Merk Millipore, a flow rate of 3 ml.min-1 and an injection volume of 1 µL. Elution solvent used were water and acetonitrile each supplemented with 0.1 % formic acid. Gradient elution was performed from 0 % to 100 % of acetonitrile 0.1 % formic acid in 2.5 min. Positive-ion electrospray mass spectra were acquired at a solvent flow rate of 100-200 µL/min. Nitrogen was used for both the nebulizing and drying gas. The data were obtained in a scan mode ranging from 200 to 1700 m/z in 0.1 s intervals; 10 scans were summed up to get the final spectrum. Retention times (RT) are given in minutes.

High resolution LC-UV-MS analyses were performed on UPLC Acquity H-Class from Waters with Kinetex C18 100 Å 2.1 x 2.6 µm column from Phenomenex hyphenated to a Synapt G2-S mass spectrometer with a dual ESI source from Waters. UV chromatograms were recorded with PDA detector from 200 to 400 nm. Analyses were carried out with the column oven at 25°C, with a flow rate of 500 $\mu l.min^{-1}$ and an injection volume of 1 µL. Elution solvent used were water and acetonitrile each supplemented with 0.1% formic acid. Gradient elution was performed from 0 % to 100 % of acetonitrile 0.1 % formic acid in 12 min. Mass spectrum was recorded in positive mode from 100 to 1500 Da with a capillary voltage of 3000 V and cone voltage of 30 V. Source and desolvation temperatures were respectively 140°C and 450°C.

MALDI mass spectra were recorded on an Ultraflex III TOF/TOF instrument (Bruker Daltonics, Wissembourg, France) equipped with LIFT capability. A pulsed Nd:YAG laser at a wavelength of 355 nm was operated at a frequency of 100 Hz (MS data) or 200 Hz (MS/MS data) with a delayed extraction time of 30 ns. The source was operated in the positive mode. Data were acquired with the Flex Control software and processed with the Flex Analysis software. A solution of the α-cyano-4-hydroxycinnamic acid (HCCA) matrix in water/acetonitrile (70/30, v/v) at a concentration of 10 mg ml^{-1} was mixed with the peptide sample in equal amount and 1.2 µl of this solution was deposited onto the MALDI target according to the dried droplet procedure. After evaporation of the solvent, the MALDI target was introduced into the mass spectrometer ion source. External calibration was performed with the commercial peptide mixture (Calibration peptide standard 2, Bruker Daltonics, Wissembourg, France). MS data were acquired under the following MS conditions. An acceleration voltage of 25.0 kV (IS1) was applied for a final acceleration of 21.95 kV (IS2). The reflectron mode was used for the Tof analyzer (voltages of 26.3 kV and 13.8 kV). Mass spectra were acquired from 700 laser shots, the laser fluence at 30 %. Ions were detected over a mass range from m/z 200 to 2000. MS/MS data were acquired under the following conditions. An acceleration voltage of 8.0 kV (IS1) was applied for a final acceleration of 7.25 kV (IS2). The reflectron mode was used for the Tof analyzer (voltages of 29.5 kV and 13.9 kV). Mass spectra were acquired from 700 laser shots, the laser fluence at 35 %. MS/MS experiments were performed under laser induced dissociation (LID) conditions with the LIFT cell voltage parameters set at 19.0 kV (LIFT 1) and 3.2 kV (LIFT 2) for a final acceleration of 29.5 kV (reflector voltage) and a pressure in the LIFT cell around 4×10^{-7} mbar. The precursor ion selector was set manually to the selenium isotope 80 signal of the protonated molecular ion pattern for all analyses. For LID experiments, no collision gas was added (gas off spectra).

Syntheses of compounds 1 to 10

Synthesis of Fmoc-selenazolidine 1a

0.60 mmol of L-selenocystine was suspended in 7.7 mL of NaOH 0.05 M and 3.3 mL of ethanol previously degassed under vacuum. Under stirring and inert atmosphere, 1.97 mmol of sodium borohydride were added portion wise. Stirring was continued until the solution became clear and decolorized. Flask was then placed in an ice-bath and pH was adjusted to 4-5 with HCl 6 M. Formaldehyde solution (37 %, 3.90 mmol) was added dropwise over 1 h and the reaction mixture was stirred for 3 h under argon atmosphere. pH was adjusted to 10 with K_2CO_3, then 1.80 mmol of Fmoc-OSu dissolved in 5 mL of dioxane was added to the reaction. The reaction was stirred for 4h30, another 1.80 mmol of Fmoc-OSu were added and the reaction was stirred overnight. 50 mL of water and 50 mL of diethyl ether were added to the solution. The aqueous layer was washed a second time with ether and the pH was adjusted to 3 with solid citric acid. The aqueous layer was extracted three times with ethyl acetate and the organic layer was washed with 10% citric acid, brine, dried over $MgSO_4$, filtered and evaporated under vacuum. The crude product was purified on reversed-phase preparative HPLC. After freeze-drying, 0.50 mmol of Fmoc-Sez-OH were obtained as a white powder (202.0 mg, 42 %). LC-UV-MS (ESI, negative mode): found m/z 402.0 $[M-H]^-$ (^{80}Se). ^{1}H NMR (DMSO, 600MHz, 100°C): δ (ppm): 7.87 (2H, d, $J_{8,7}$ = 7.5 Hz, 8-H) ; 7.65 (2H, d, $J_{5,6}$ = 7.5 Hz, 5-H); 7.42 (2H, t, $J_{7,8/6}$ = 7.5 Hz, 7-H) ; 7.32 (2H, t, $J_{6,7/5}$ = 7.5 Hz, 6-H); 5.12 (1H, dd, $J_{\alpha,\beta}$ = 6.6 Hz, $J_{\alpha,\beta'}$ = 3.3 Hz; α-H) ; 4.80 (1H, d, $J_{\delta,\delta'}$ = 7.5 Hz, δ-H) ; 4.4 (3H, m, δ'-H, 2-H) ; 4.3 (1H, t, $J_{3,2}$ = 6.7 Hz, 3-H); 3.3 (2H, m, β-H, β'-H). ^{13}C NMR (DMSO, 150MHz, 100°C): δ (ppm): 172.0 (CO); 155.0 (1-C); 145.3 (4-C); 142.3 (9-C); 129.3 (7-C); 128.8 (6-C); 126.7 (5-C); 121.7 (8-C); 69.2 (2-C); 64.4 (α-C); 48.5 (3-C); 40.4 (δ-C); 26.6 (β-C).

Synthesis of Boc-selenazolidine 1b

1.98 mmol of L-selenocystine was suspended in 31 mL of NaOH 0.05 M and 9 mL of ethanol previously degassed under vacuum. Under stirring and inert atmosphere, 6.53 mmol of sodium borohydride were added portion wise. Stirring was continued until the solution became clear and decolorized. Flask was then placed in an ice-bath and pH was adjusted to 4-5 with HCl 6 M. Formaldehyde solution (37 %, 13.00 mmol) was added dropwise over 1 h and the reaction mixture was stirred for 3 h under argon atmosphere. pH was adjusted to 10 with K_2CO_3, then 4.00 mmol of Boc_2O dissolved in 5 mL of dioxane was added to the reaction. The reaction was stirred for 4 h 30, another 4.00 mmol of Boc_2O were added and the reaction was stirred overnight. 50 mL of water and 50 mL of diethyl ether were added to the solution. The aqueous layer was washed a second time with ether and the pH was adjusted to 3 with solid citric acid. The aqueous layer was extracted three times with ethyl acetate and the organic layer was washed with 10 % citric acid, brine, and then dried over $MgSO_4$. After filtration, solvent was evaporated under vacuum. The crude product was purified on reversed-phase preparative HPLC. After freeze-drying, 2.10 mmol of Boc-Sez-OH were obtained as a light yellow oil (591.5 mg, 53 %). LC-UV-MS (ESI, negative mode): found m/z 280.0 $[M-H]^-$ (^{80}Se). ^{1}H NMR (DMSO, 600MHz, 100°C) : δ (ppm) : 5.05 (1H, m, α-H) ; 4.81 (1H, d, $J_{\delta,\delta'}$ = 7.7 Hz, δ-H) ; 4.37 (1H, d, $J_{\delta',\delta}$ = 7.7 Hz, δ'-H) ; 3.31 (1H, dd, $J_{\beta,\beta'}$ = 10.5 Hz, $J_{\beta,\alpha}$ = 7.3 Hz, β-H) ; 3,25 (dd, $J_{\beta',\beta}$ = 10.5 Hz, $J_{\beta',\alpha}$ = 2.9 Hz, β'-H) ; 1.42 (9H, s, 3-H). ^{13}C NMR (DMSO, 600MHz, 100°C) : δ (ppm) : 170.9 (1-C); 152.6 (CO); 79.9 (2-C); 62.0 (α-C); 38.7 (δ-C); 27.5 (9-C); 24.4 (β-C).

Synthesis of dipeptide Boc-Sez-Phe-NH_2 3 in solution

Boc-Sez-OH **1b** (0.29 mmol), DIEA (0.29 mmol) and BOP (0.29 mmol) were successively added to H-Phe-NH_2 (0.29 mmol) in DMF. After 2 h stirring at room temperature, solvent was evaporated and residue was solubilized in ethyl acetate. Organic phase was successively washed with $KHSO_3$, $NaHCO_3$ and NaCl and then dried over $MgSO_4$. After filtration, solvent was evaporated under vacuum. 0.20 mmol of Boc-Sez-Phe-NH_2 were obtained as light yellow oil (85 mg, 69%). LC-UV-MS (ESI, positive mode): found m/z 427.9 $[M+H]^+$ (^{80}Se). Compound was used for the next step without further purification.

Synthesis of H-Sez-Phe-NH_2 4

A solution of DCM/TFA/TIS 50/50/2.5 v/v/v was applied to the previous crude compound Boc-Sez-Phe-NH2 3 for 30 min at room temperature. After solvent evaporation, the dipeptide 4 was obtained as an oil, LC-UV-MS (ESI, positive mode): found m/z 328.0 $[M+H]^+$ (^{80}Se), UV purity of 91% at 214 nm.

Synthesis of Boc-Ala-Sez-Phe-NH_2 5

Boc-Ala-OH (0.60 mmol), DIEA (1.50 mmol) and HATU (0.60 mmol) were successively added to dipeptide H-Sez-Phe-NH_2 **4** (0.20 mmol) in DMF. Solution was stirred for 2 h at room temperature and LC-UV-MS showed that coupling was performed with 88 % of conversion. Solvent was then evaporated under vacuum and residue was solubilized in ethyl acetate. Organic phase was successively washed with $KHSO_3$, $NaHCO_3$ and NaCl, and then dried over $MgSO_4$. After filtration, solvent was evaporated under vacuum. The crude product was purified on reversed-phase preparative HPLC. After freeze-drying, 0.14 mmol of Boc-Ala-Sez-Phe-NH_2 **5** were obtained as white powder (72 mg, 72 %). LC-UV-MS (ESI, positive mode): found m/z 520.9 $[M+Na]^+$ (^{80}Se).

Synthesis of H-Ala-Sez-Phe-NH_2 6

A solution of DCM/TFA/TIS 50/50/2.5 v/v/v was applied to the previous crude compound Boc-Ala-Sez-Phe-NH_2 (**5**) for 30 min. After solvent evaporation, the tripeptide 6 was obtained as colourless oil, LC-UV-MS (ESI, positive mode): found m/z 399.0 $[M+H]^+$ (^{80}Se).

Synthesis of Fmoc-Sez-Phe-NH_2 7

Fmoc-Sez-OH **1a** (0.13 mmol), DIEA (0.13 mmol) and BOP (0.13 mmol) were successively added to H-Phe-NH2 (0.08 mmol) in DMF. After 2 h stirring at room temperature, solvent was evaporated and residue was solubilized in ethyl acetate. Organic phase was successively washed with $KHSO_3$, $NaHCO_3$ and NaCl and then dried on $MgSO_4$. After filtration, solvent was evaporated under vacuum. The crude product was purified on reversed-phase preparative HPLC. After freeze-drying, 0.07 mmol of Fmoc-Sez-Phe-NH_2 were obtained as yellow/orange powder (41.2 mg, 87%). LC-UV-MS (ESI, positive mode): found m/z 550.1 $[M+H]^+$ (^{80}Se), calculated monoisotopic mass m/z 549.1 Da (^{80}Se).

Synthesis of dipeptide Fmoc-Ala-Sez-OH 9 and dipeptide Fmoc-Arg(Pbf)-Sez-OH 10

HATU/DIEA activation: 3 equivalents of Fmoc-L-AA-OH (N-α-Fmoc-L-alanine or N-α-Fmoc-N-g-(2,2,4,6,7-pentamethyldihydrobenzofuran-5-sulfonyl)-L-arginine for the syntheses of dipeptides **9** and **10** respectively) were solubilized in acetonitrile with 3 equivalents of DIPEA. Then 2.9 equivalents of HATU were added. Solution was stirred for 2 min at room temperature to form the activated OAt derivative. *Boc removal on Boc-Sez-OH **1b** to yield TFA,H-Sez-OH **2*** : 1 equivalent of Boc-Sez-OH was deprotected in a mixture of DCM/TFA 50/50 (v/v). After 15 min of stirring at room temperature, solvent was evaporated under vacuum. Residue was then suspended in ACN with 1 equivalent of DIPEA. *Coupling step*: Solution of H-Sez-OH **2** was added dropwise to the previous Fmoc-AA-OAt solution. The mixture was stirred for 1h30 at room temperature and finally evaporated to dryness. Residue was dissolved in DCM (30 mL/mmol) and the solution was washed three times with a 5% citric acid (60 mL/mmol) solution, three times with a NaCl saturated solution (60 mL/mmol) and dried over $MgSO_4$. After filtration and evaporation under vacuum, the crude product was purified on reversed-phase chromatography and freeze-dried.

Fmoc-Ala-Sez-OH **9** was obtained as a white powder (258.3 mg, 65 %). LC-UV-MS (ESI, positive mode) : found m/z 475.1 $[M+H]^+$ (^{80}Se). ^{1}H RMN (600 MHz, DMSO, 110°C): δ (ppm): 7.86 (2H, d, $J_{8,7}$ = 7.5 Hz, 8-H); 7.69 (2H, dd, $J_{5,6}$ = 7.5 Hz; $J_{5,7}$ = 3.9 Hz, 5-H); 7.41 (2H, t, J $J_{7,6/8}$ = 7.5 Hz, 7-H); 7.33 (2H, t, $J_{6,5/7}$ = 7.5 Hz, 6-H); 7.00 (1H, br s, NH); 5.40 (1H, dd, $J_{\alpha,\beta}$ = 7.0 Hz; $J_{\alpha,\beta'}$ = 3.1 Hz, α-H-Sez^2); 5.04 (1H, d, $J_{\delta,\delta'}$ = 7.7 Hz, δ-H-Sez^2); 4.49 (2H, m, δ'-H-Sez^2, α-H-Ala^1); 4.32 (2H, dd, $J_{2,2'}$ = 2.3 Hz; $J_{2,3}$ = 6.8 Hz, 2-H); 4.23 (1H, t, $J_{3,2/2'}$ = 6.8 Hz, 3-H); 3.29 (2H, m, β-H-Sez^2, β'-H-Sez^2); 1.28 (3H, d, $J_{\beta,\alpha}$ = 6.9 Hz, β-H-Ala^1). ^{13}C NMR (DMSO, 600MHz, 110°C) : δ (ppm): 170.3 (CO-Sez^2); 170.1 (CO-Ala^1); 154.8 (1-C); 143.4 (9-C); 140.24 (4-C); 126.9 (7-C); 126.3 (6-C); 124.5 (5-C); 119.3 (8-C); 65.4 (2-C); 61.5 (α-C- Sez^2); 47.57 (α-C- Ala^1); 45.5 (3-C); 39.5 (δ-C-Sez^2); 23.4 (β-C-Sez^2); 16.7 (β-C-Ala^1).

Fmoc-Arg(Pbf)-Sez-OH **10** was obtained as a white powder(128.4 mg, 63 %). LC-UV-MS (ESI, positive mode): found m/z 812.2 $[M+H]^+$ (^{80}Se). ^{1}H NMR (600 MHz, DMSO, 110°C): δ (ppm): 7.89-7.88 (2H, d, J8,7 = 7.5 Hz, 8-H); 7.71 (2H, t, J5,6 = 7.5 Hz); 7.41 (2H, m, 7-H); 7.32 (2H, m, 6-H); 6.40 (1H, br s, NH); 5.37 (1H, m, α-H-Sez^2); 5.05 (1H, m, δ-H-Sez^2); 4.52 (1H, m, m, δ'-H-Sez^2); 4.42 (1H, m, α-H-Arg^1); 4.27 (1H, m, 2-H); 4.22-4.21 (3H, m, 2-H, 3-H); 3.23-3.24 (2H, m, β-H-Sez^2, β'-H-Sez^2); 3.05 (2H, m, δ-H-Sez^2); 2.94 (2H, s, 16-H), 2.50 (3H, s, 11-Me), 2.49 (3H, s, 15-Me); 2.00 (3H, s, 12-Me); 1.68 (1H, br s, β-H-Arg^1); 1.52-1.51 (2H, br s, β'-H-Arg^1, γ-H-Arg^1); 1.39 (6H, s, 17-Me). ^{13}C NMR (600 MHz, DMSO, 110°C): δ (ppm): 171.0 (CO-Arg^1); 170.3 (CO-Sez^2); 157.4 (13-C); 156.0 (1-C); 143.8 (4-C); 140.7 (9-C); 137.3 (11-C); 134.1 (10-C); 131.4 (15-C); 127.6 (7-C); 127.1 (6-C); 125.2 (5-C); 124.3 (14-C); 120.0 (8-C); 116.2

(12-C); 86.3 (17-C); 65.7 (2-C); 62.3 (α-C- Sez[2]); 51.8 (α-C-Arg[1]); 46.3 (3-C); 42.3 (16-C); 39.6 (δ-C-Arg[1]); 38.1 (δ-C-Sez[2]); 28.3 (17-Me); 27.9 (β-C-Arg[1]); 24.9 (γ-C-Arg[1]); 23.3 (β-C-Sez[2]); 18.9 (15-Me); 17.6 (11-Me); 12.2 (12-Me); ς-C ND.

Supported synthesis of tripeptide H-Ala-Sez-Phe-NH_2 6 in Fmoc/tBu strategy

Synthesis was carried out at a 0.25 mmol scale. The general procedure for SPPS was applied except for the Sez residue which was introduced as a dipeptide building block. Fmoc-Ala-Sez-OH 10, was activated with 2 equivalents of HATU/DIEA, during 1 h. After freeze drying, 0.19 mmol of crude product were obtained as a white powder (75.1 mg, 75 %). LC-UV-MS (ESI, positive mode): found m/z 399.1 $[M+H]^+$ (^{80}Se), calculated monoisotopic mass m/z 398.1 Da (^{80}Se).

Stability experiments protocols

Stability studies of N-Fmoc and N-Boc protected selenazolidine

With Fmoc-Sez-OH and Boc-Sez-OH in hands, we evaluated their stability during removal of the protecting group. These observations were confirmed by treatment of Boc-Sez-OH **1b** with a DCM/TFA (50/50 v/v) solution. No by-product was observed and the Boc N-protecting group was cleanly removed (Fig. S1 in ESI). On the contrary, Sez was not stable upon Fmoc removal. Indeed, the LC-MS analysis of the reaction mixture of Fmoc-Sez-OH **1a** in DMF/piperidine 80/20 v/v after 20 min showed the degradation of Sez, along with the formation of selenocystine (Fig. S2 in ESI). The dimer was probably formed after removal of the Fmoc group through a Schiff-base intermediate as it has already been described for the thiazolidine ring.[27] In such basic conditions, the unprotected Sez undergoes a ring opening, generating selenocysteine. Then, free selenol groups formed a diselenide bridge by prompt oxidation. This hypothesis was confirmed by the treatment of TFA.H-Sez-OH (obtained after Boc deprotection of **1b**) with a DMF/piperidine (80/20 v/v) solution, which afforded selonocystine within 20 min (Fig. S3). The same side-reaction was also observed during Fmoc deprotection when the Sez was the N-terminal residue of a dipeptide (Fmoc-Sez-Phe-NH_2 was prepared for that purpose, see Fig. S4).

N-Boc removal of Boc-Sez-OH 1b in a solution of DMF/piperidine 80/20 v/v

N-Boc removal of the Boc-Sez-OH **1b** was performed in a solution of DCM/TFA 50/50 v/v during 15 min. After solvent evaporation under vacuum, residue was dissolved in ACN/water 50/50 v/v solution, LC-UV-MS (ESI positive mode): found m/z 181.9 $[M+H]^+$ (^{80}Se). See Figure S1

N-Fmoc removal of Fmoc-Sez-OH 1a in a solution of DMF/piperidine 80/20 v/v

N-Fmoc removal of the Fmoc-Sez-OH **1a** was performed in a solution of DMF/piperidine 80/20 v/v during 20min. After solvent evaporation under vacuum, residue was dissolved in ACN/water 50/50 v/v solution, LC-UV-MS (ESI positive mode): found m/z 337.0 $[M+H]^+$ (^{80}Se). See Figure S2

Stability of TFA,H-Sez-OH in a solution of DMF/piperidine 80/20 v/v

N-Boc removal was performed on Boc-Sez-OH **1b** to obtain TFA,H-Sez-OH. Solvent was evaporated under vacuum and then the residue was dissolved in a DMF/piperidine 80/20 v/v solution. After 20 min, the solvent was evaporated under vacuum and compound was solubilized in in ACN/water 50/50 v/v solution, LC-UV-MS (ESI positive mode) found m/z 336.9 $[M+H]^+$ See Figure S3

Stability of Fmoc-Sez-Phe-NH_2 7 in a solution of DMF/piperidine 80/20 v/v

Fmoc-Sez-Phe-NH_2 **7** (0.07 mmol, 41 mg) was poured into a DMF/piperidine (80/20 v/v) solution (0.7 ml) and stirred for 15 min at room temperature. The solvent was evaporated under vacuum. The residue was dissolved in an ACN/water (50/50 v/v) solution for analysis. We have not succeeded in removing the Fmoc protecting group from Fmoc-Sez-Phe-NH_2 **7**, using a DMF/piperidine (80/20 v/v) solution. LC-UV-MS (ESI, positive mode): found m/z 629.2 $[M+H]^+$ (^{80}Se) corresponding to dimeric compound. See Figure S4

Stability of Boc-Sez-Phe-NH_2 3 in a solution of DCM/TFA 50/50 v/v

Boc-Sez-Phe-NH_2 ***3*** (0.1 mmol) was poured into a DCM/TFA 50/50 v/v solution (0.5 ml) and stirred for 15 min at room temperature. The solvent was evaporated under vacuum. The residue was dissolved in an ACN/water (50/50 v/v) solution for analysis. LC-UV-MS (ESI, positive mode): found m/z 328.0 $[M+H]^+$ (^{80}Se). See Figure S5

Stability of Boc-Ala-Sez-Phe-NH_2 5 in a solution of DCM/TFA 50/50 v/v

Boc-Ala-Sez-Phe-NH_2 **5** (0.1 mmol) was poured into a DCM/TFA 50/50 v/v solution (0.5 ml) and stirred for 15 min at room temperature. The solvent was evaporated under vacuum. The residue was dissolved in an ACN/water (50/50 v/v) solution for analysis. LC-UV-MS (ESI, positive mode): found m/z 399.0 $[M+H]^+$ (^{80}Se). See Figure S6

Stability of H-Ala-Sez-Phe-NH_2 6 in TFMSA/TFA/TIS solution

Tripeptide H-Ala-Sez-Phe-NH_2 **6** was poured into a TFA/TFMSA/TIS 73/10/17 v/v/v solution. The solution was stirred for 3h and then solvent was evaporated under nitrogen flow. Compound was then dissolved in a ACN/water 50/50 v/v solution for analysis. LC-UV-MS (ESI, positive mode): found m/z 399.1 $[M+H]^+$ (^{80}Se), calculated monoisotopic mass m/z 398.1 Da (^{80}Se). See Figure S7

Study of several N-Fmoc removal conditions on Fmoc-Sez-Phe-NH-RA (RA: Rink amide resin)

The dipeptide Fmoc-Sez-Phe-NH_2 **9** was synthesized on rink amide resine according to the general Fmoc/tBu SPPS protocol described above. Resin was then divided in three aliquots and different N-Fmoc removal conditions were evaluated.

DMF/piperidine 80/20 v/v. The dipeptide-anchored resin (40 mg) was stirred in a DMF/piperidine solution (80/20 v/v) (1 mL) for 5 min at room temperature. The resin was then filtered and washed three times with DMF and once with DCM.

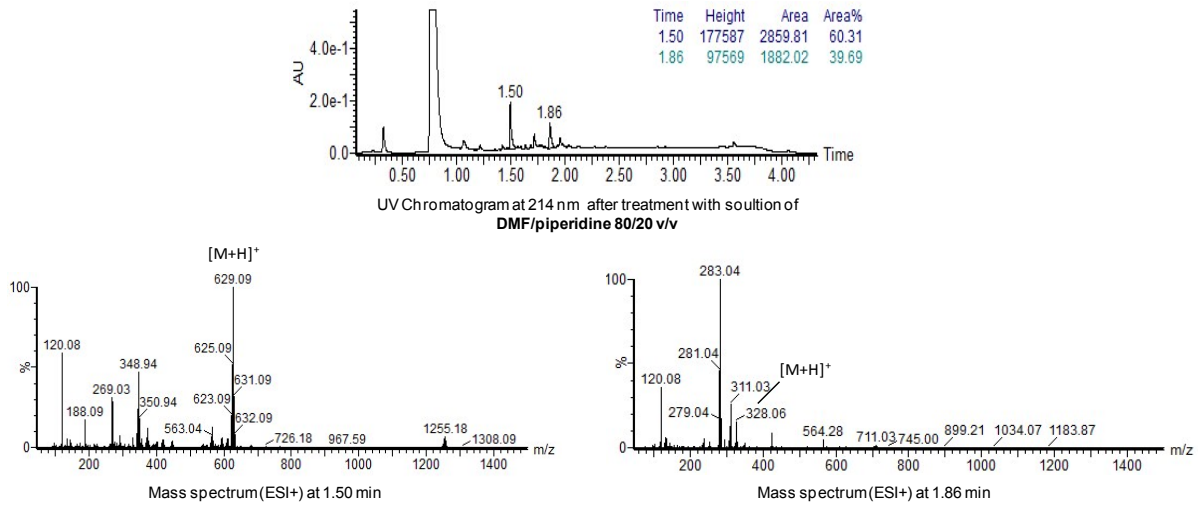
DMF/DBU 95/5 v/v. The dipeptide-anchored resin (40 mg) was stirred in a DMF/DBU (95/5 v/v) (1 mL) for 5 min at room temperature. The resin was then filtered and washed three times with DMF and once with DCM.

DMF/piperazine 94/6 v/v with 0.1 M HOBt. The dipeptide-anchored resin (40 mg) was stirred in a 6 % piperazine in DMF (v/v) solution containing 0.1 M HOBt (1 mL) for 5 min at room temperature. The resin was then filtered and washed three times with DMF and once with DCM.

Removal of the peptide from the resin concerning each aliquot of peptide-resin submitted to Fmoc deprotection conditions described above, was performed according to the general procedure already described. After cleavage, the TFA solution was evaporated under vacuum, the residue were dissolved in a ACN/water 50/50 v/v solution and directly analyzed by LC-UV-MS (ESI, positive mode).

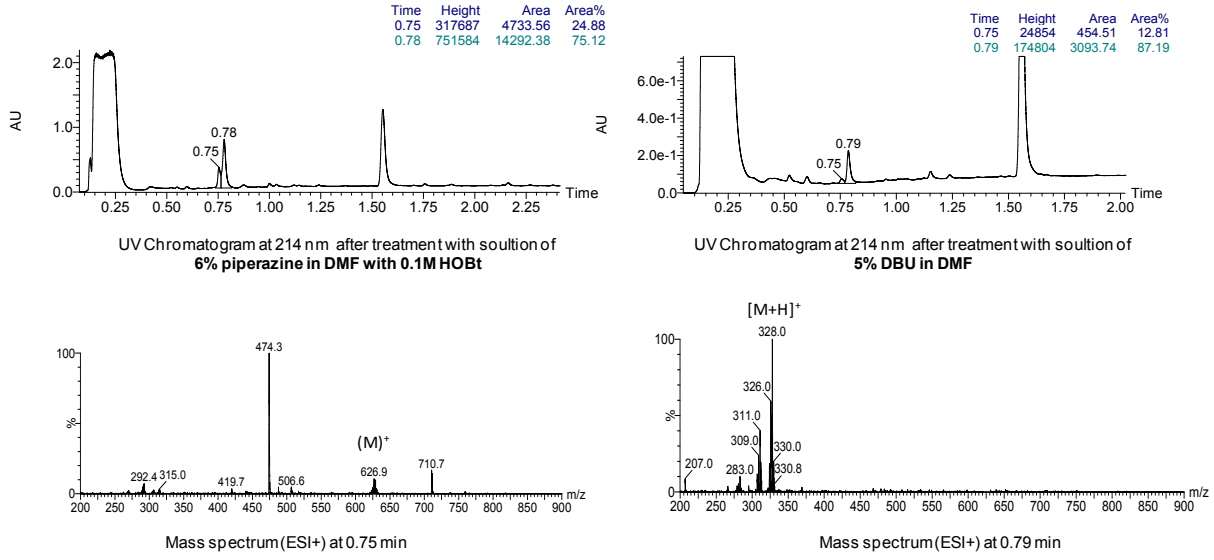
DMF/piperidine 80/20 v/v :

LC-UV-MS analyses were realized on UPLC Acquity H-Class from Waters with Acquity BEH C18 50 x 2.1, 1.7 µm column from Waters. UV chromatogram was recorded with photodiode detector TUV at 214nm. Analyses were carried out with the column oven at 25°C, with a flow rate of 500 $\mu l.min^{-1}$ and an injection volume of 1 µL. Elution solvent used were water and acetonitrile each supplemented with 0.1 % formic acid. Gradient elution was performed from 0 % to 100 % of acetonitrile 0.1 % formic acid in 3 min. Mass spectrometer hyphenated to the UPLC system was a Synapt G2-S from Waters with a dual ESI source. Mass spectrum was recorded in positive mode from 100 to 1500 Da with a capillary voltage of 3000 V and cone voltage of 30 V. Source and desolvation temperatures were respectively 140°C and 450°C.



6% piperazine in DMF with 0.1M HOBt and 5% DBU in DMF

LC-UV-MS analyses were performed on a Waters Alliance 2690 HPLC coupled to a Micromass ZQ spectrometer (electrospray ionization mode, ESI+). Analyses were carried out with the column oven at 25°C, a Chromolith C18 Flash 25 x 4.6 mm column from MerkMillipore, a flow rate of 3 ml.min^{-1} and an injection volume of 1 µL. Elution solvent used were water and acetonitrile each supplemented with 0.1 % formic acid. Gradient elution was performed from 0 % to 100 % of acetonitrile 0.1 % formic acid in 2.5 min. Positive-ion electrospray mass spectra were acquired at a solvent flow rate of 100-200 µL/min. Nitrogen was used for both the nebulizing and drying gas. The data were obtained in a scan mode ranging from 200 to 1700 m/z in 0.1 s intervals; 10 scans were summed up to get the final spectrum.



Stability of Boc-Ala-Sez-Phe-NH$_2$ 5 in a solution of DMF/piperidine 80/20 v/v

DMF/piperidine 80/20 v/v solution was applied to tripeptide Boc-Ala-Sez-Phe-NH$_2$ **5**. After 20 min, solvent was evaporated under vacuum and residue was dissolved in ACN/water 50/50 v/v solution, LC-UV-MS (ESI positive mode) found m/z 520.9 [M+Na]$^+$ (^{80}Se). See Figure S8

Figures S1 to S6 illustrating Sez stabilities experiments

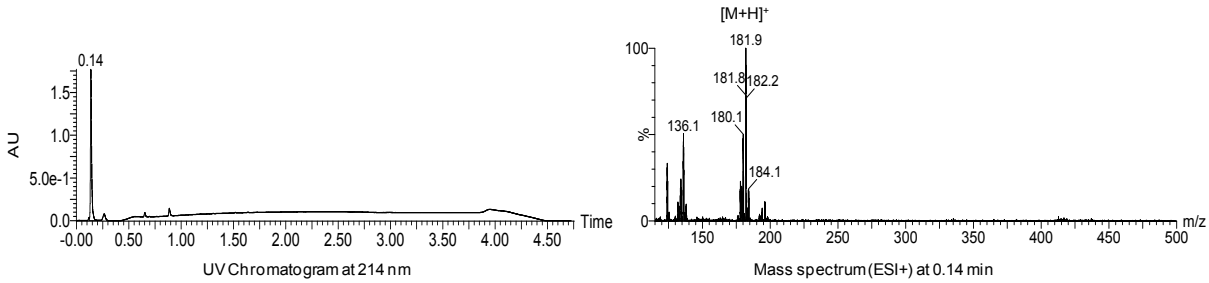


Figure S1: Stability of Boc-Sez-OH **1b** to N-Boc removal in a solution of DCM/TFA 50/50 v/v

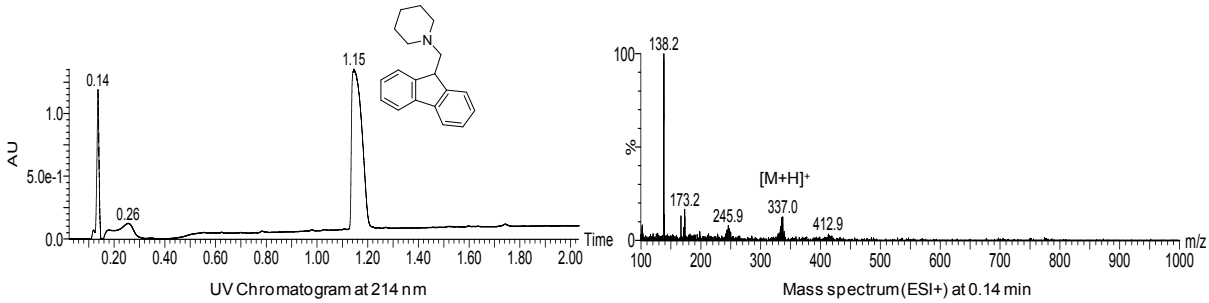


Figure S2: Stability of Fmoc-Sez-OH (**1a**) and H-Sez-OH to N-Fmoc removal in a solution of DMF/piperidine 80/20 v/v

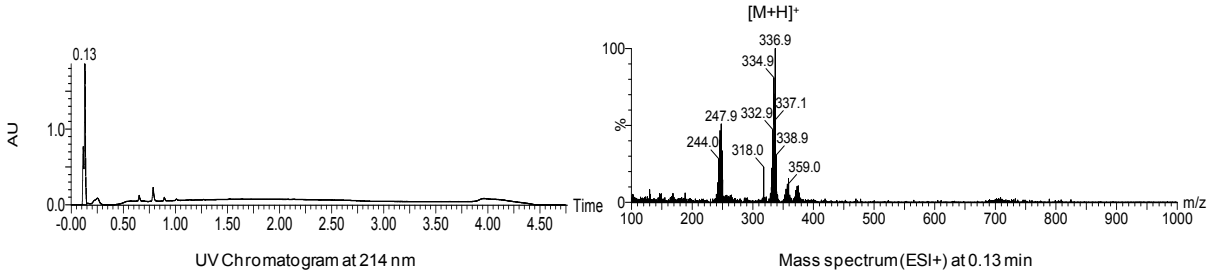


Figure S3: Stability of TFA,H-Sez-OH in a solution of DMF/piperidine 80/20 v/v

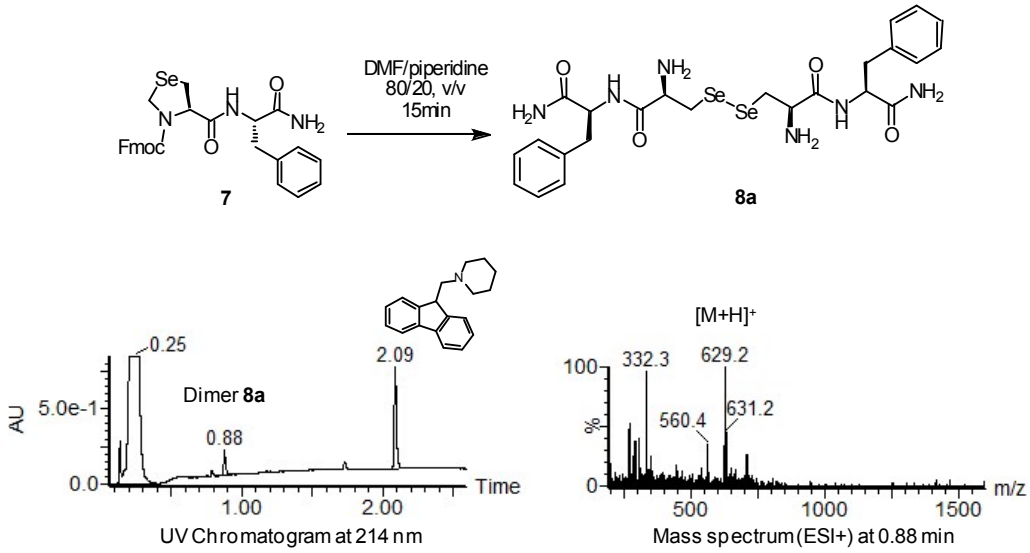


Figure S4: Stability of Fmoc-Sez-Phe-OH **7** in a solution of DMF/piperidine 80/20 v/v

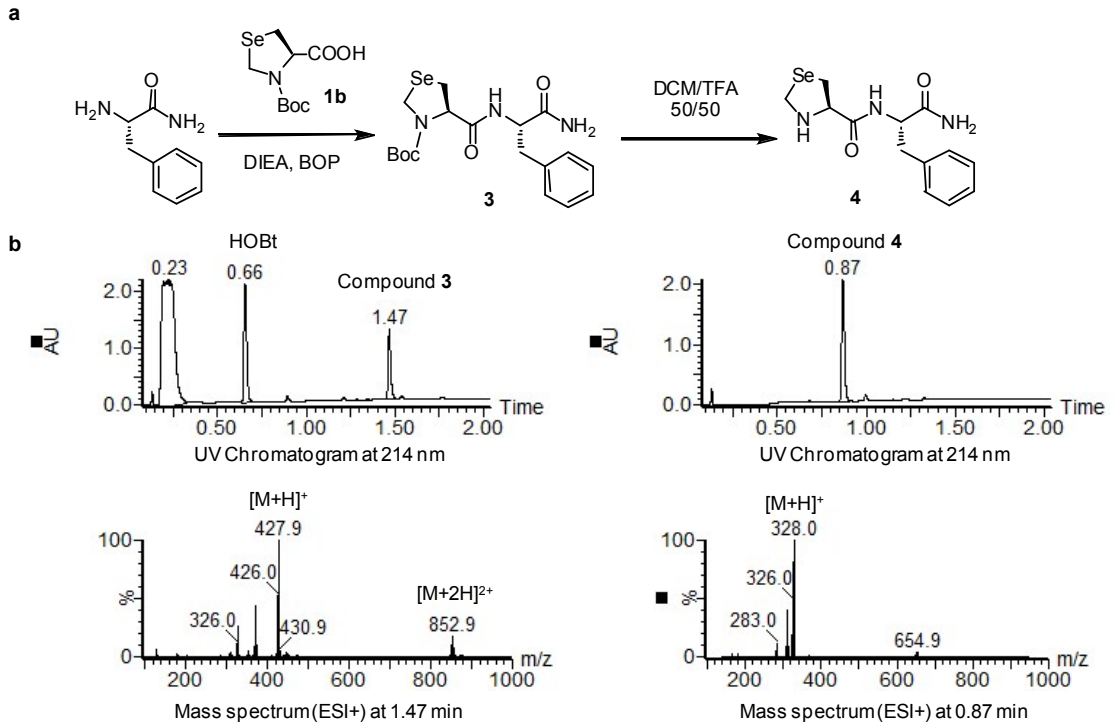


Figure S5: Synthesis of H-Sez-Phe-NH_2 **4** and stability to N-Boc deprotection condition in a solution of DCM/TFA 50/50

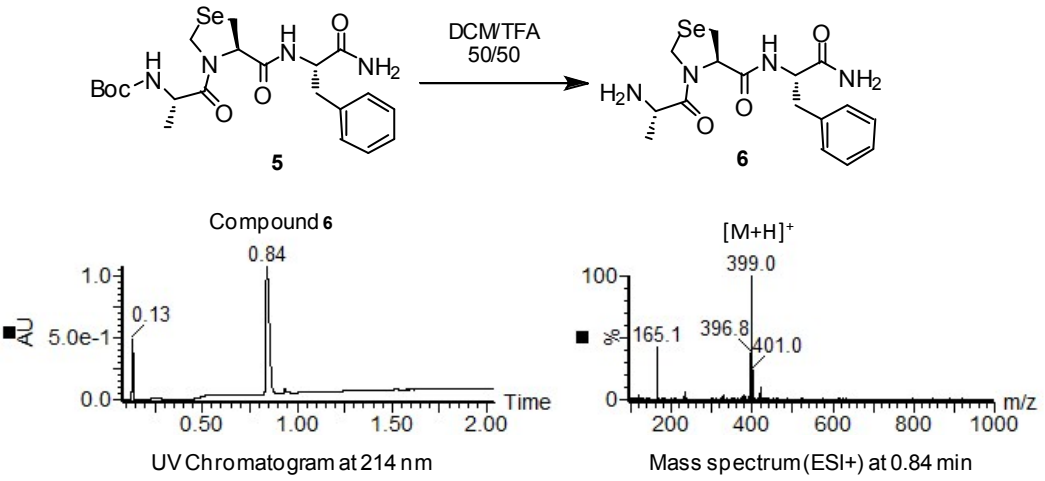
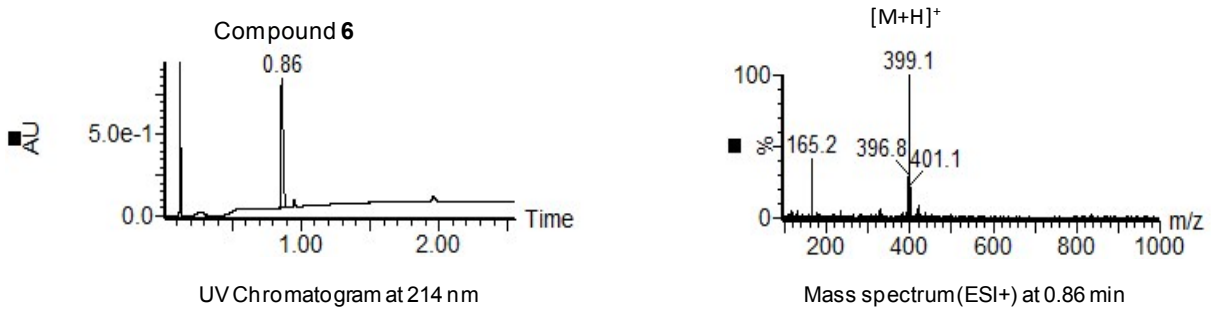
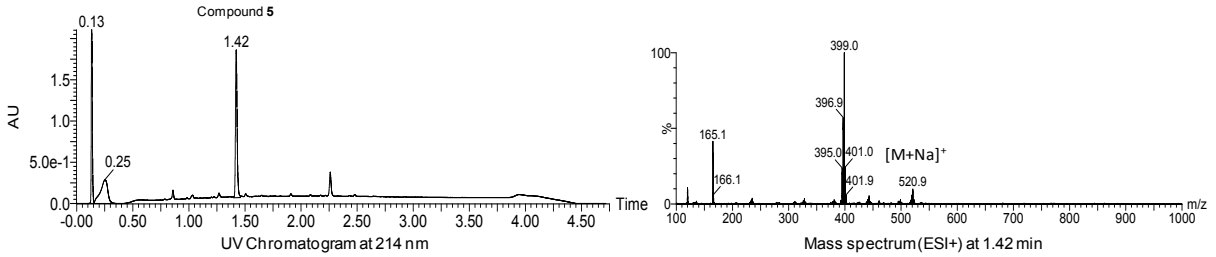


Figure S6: Stability of Boc-Ala-Sez-Phe-NH_2 **5** to N-Boc deprotection condition in a solution of DCM/TFA 50/50 v/v



UV Chromatogram at 214 nm Mass spectrum (ESI+) at 0.86 min

Figure S7: Stability of H-Ala-Sez-Phe-NH_2 **6** to in TFMSA/TFA/TIS solution



UV Chromatogram at 214 nm Mass spectrum (ESI+) at 1.42 min

Figure S8: Stability of Boc-Ala-Sez-Phe-NH_2 **5** in a solution of DMF/piperidine 80/20 v/v

Characterization of [Sez]^{6}-HO-Phpa-LVA

Isotopic cluster of [Sez]^{6}-HO-Phpa-LVA

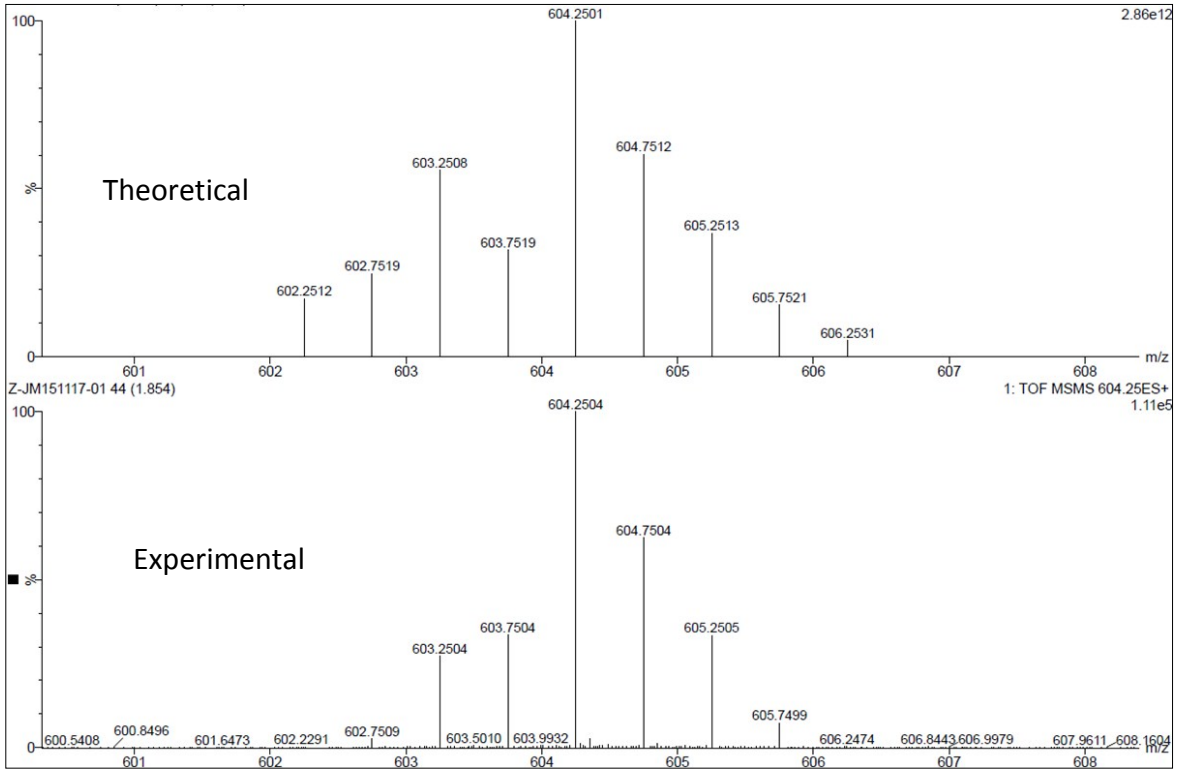


Figure S9: Isotopic cluster of the doubly charged ion of the selenium analog of a bioactive peptide: [Sez]^{6}-HO-Phpa-LVA

^{13}C NMR of [Sez]^{6}-HO-Phpa-LVA :

δ (ppm) ^{13}C (500 MHz, DMSO, 25°C) :						
Residue	**CO**	**αC**	**βC**	**γC**	**δC**	**other**
3-hydroxyphenylpropionyl	171.6					7-C, 155.4 4-C, 131.2 6, 8-CH, 128.9 5, 9-CH, 115.0 2-C, 37.2 3-C, 30.2
(D)-Tyr(Me)[1]	171.3	54.1	36.7			1-C, 129.6 2, 6-C, 130.1 3, 5-C, 113.3 4-C, 171.3 OMe, 54.9
Phe[2]	171.2	53.7	37.5			1-C, 137.8 2, 6-C, 129.3 3, 5-C, 128.0 4-C, 126.3
Asn[3]	170.9	49.5	36.7	171.3		
Gln[4]	171.3	52.2	27.9	31.4	174.0	
Arg[5]	169.6	50.0	28.1	25.1	40.5	ς-C, 156.6
Sez[6]	169.4	63.1	24.9		39.7	
Arg[7]	173.1	52.3	28.9	24.4	40.5	ς-C, 156.6

Fig S10: ^{13}C NMR of 3-hydroxyphenylpropionyl-D-Tyr(Me)-Phe-Gln-Asn-Arg-Sez-Arg-NH_2

MALDI-Tof/Tof MS/MS of [Sez]^{6}-HO-Phpa-LVA

#	a	b	b*	Residues*	y	y*	#
1	298.158	326.158		**3-hydroxyphenylpropionyl-(D)-Tyr(Me)**			**7**
2		473.257		**Phe**	337.092		**6**
3			573.237	**Gln**	493.229		**5**
4				**Asn**	607.297	590.265	**4**
5	843.557	871.564		**Arg**		718.350	**3**
6				**Sez**			**2**
7				**Arg**			**1**
Immonium ions and neutral loss are displayed in Fig. S11							

***Residues are listed downward from N-ter to C-terminal position**

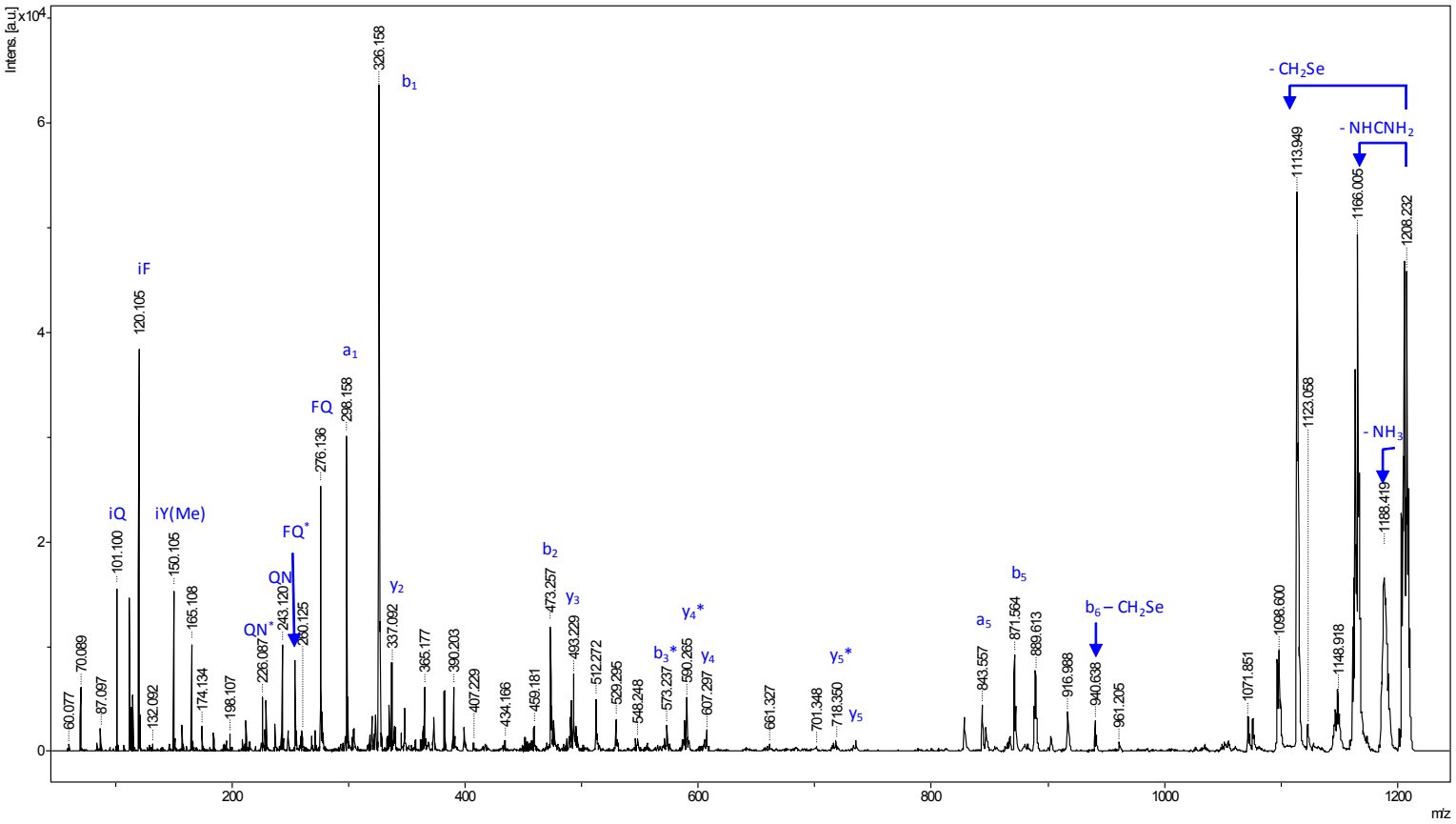


Fig S11: MALDI-Tof MS/MS of [Sez]^{6}-HO-Phpa-LVA

Post proline enzymatic digestion

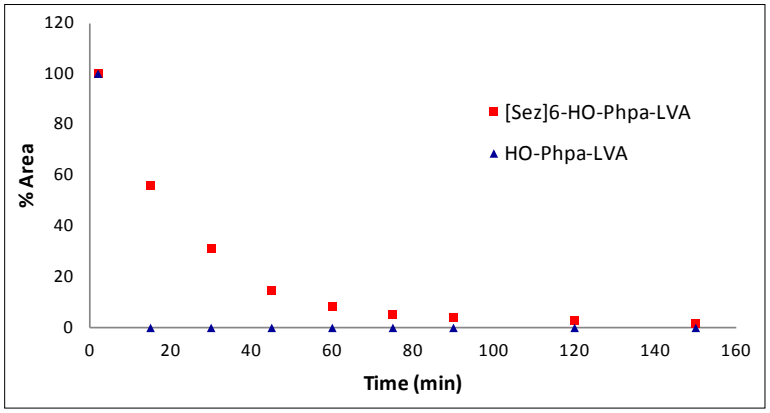
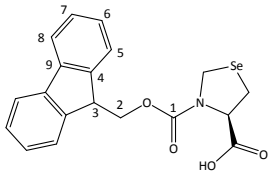
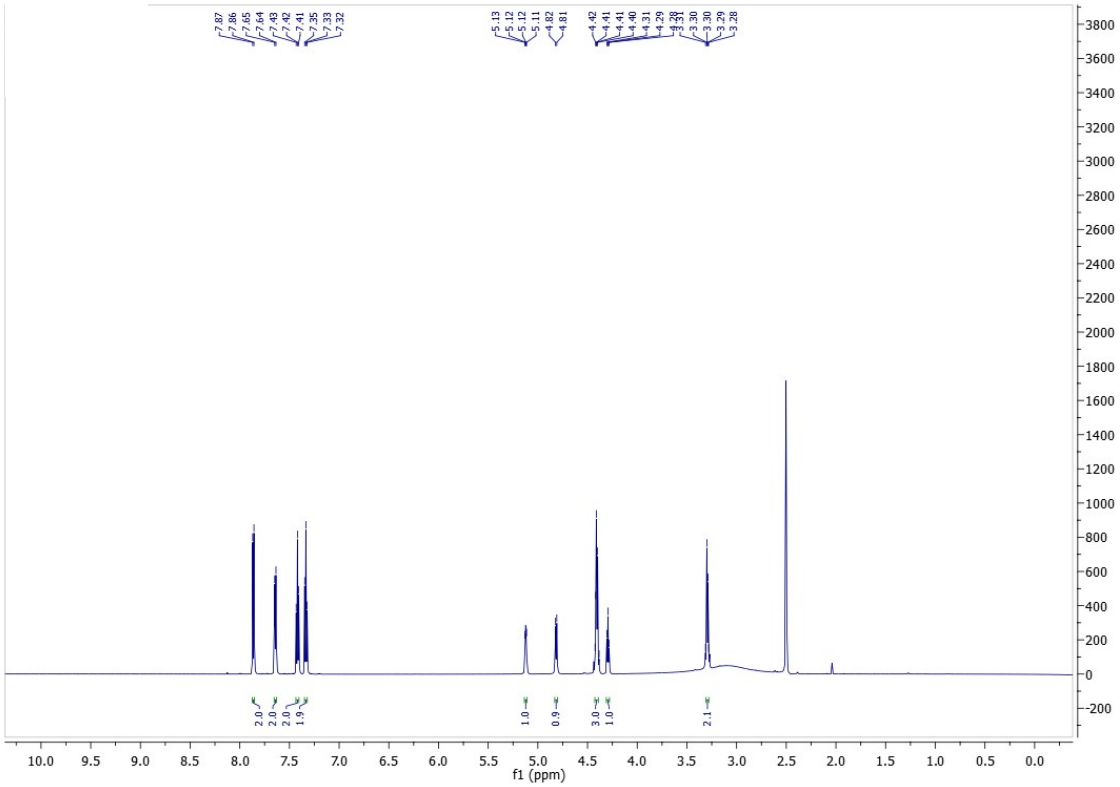


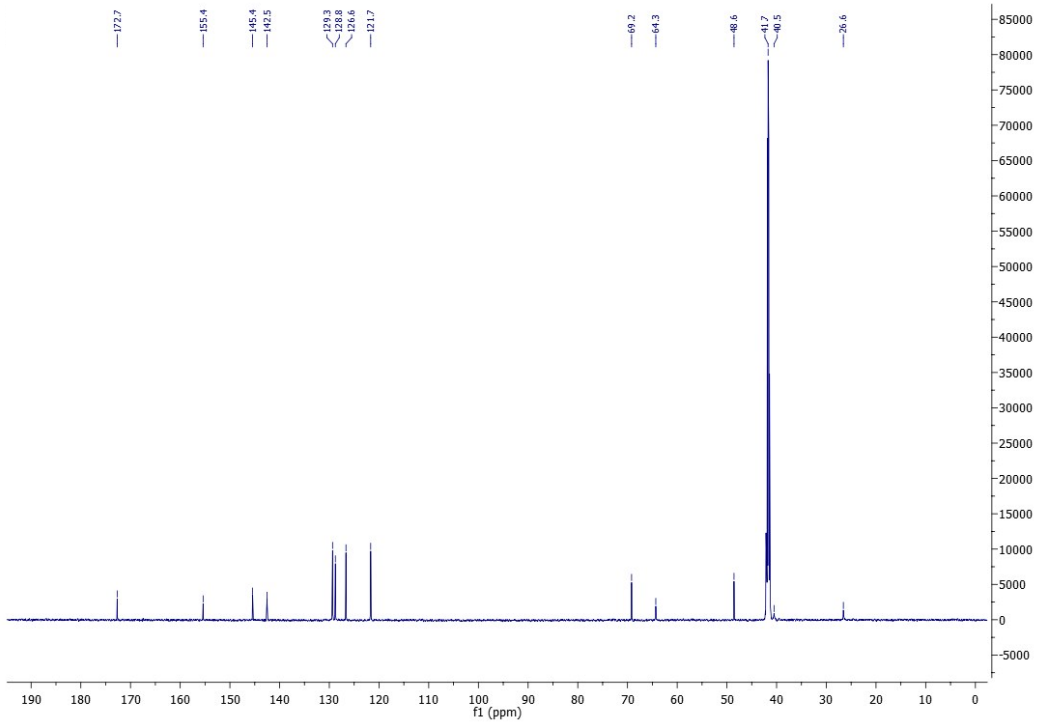
Fig S12: Digestion of [Sez]^{6}-HO-Phpa-LVA and HO-Phpa-LVA with Prolyl endopeptidase

NMR spectra

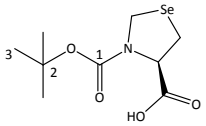
Fmoc-selenazolidine 1a

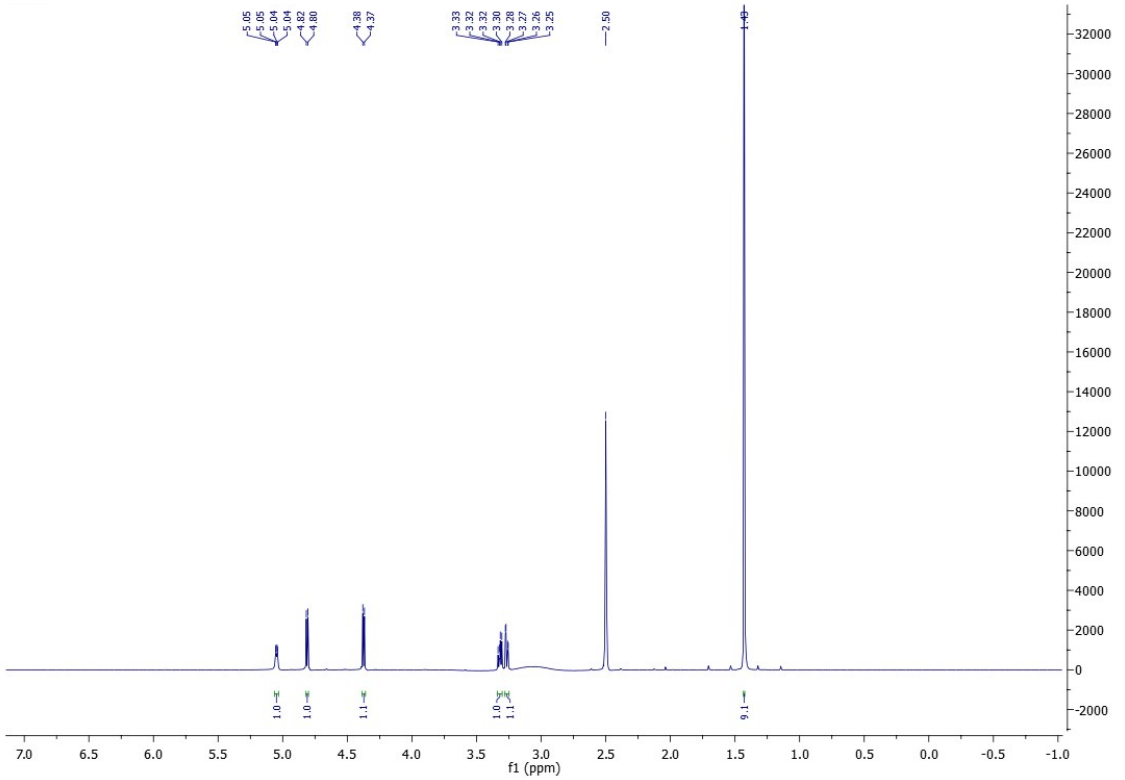


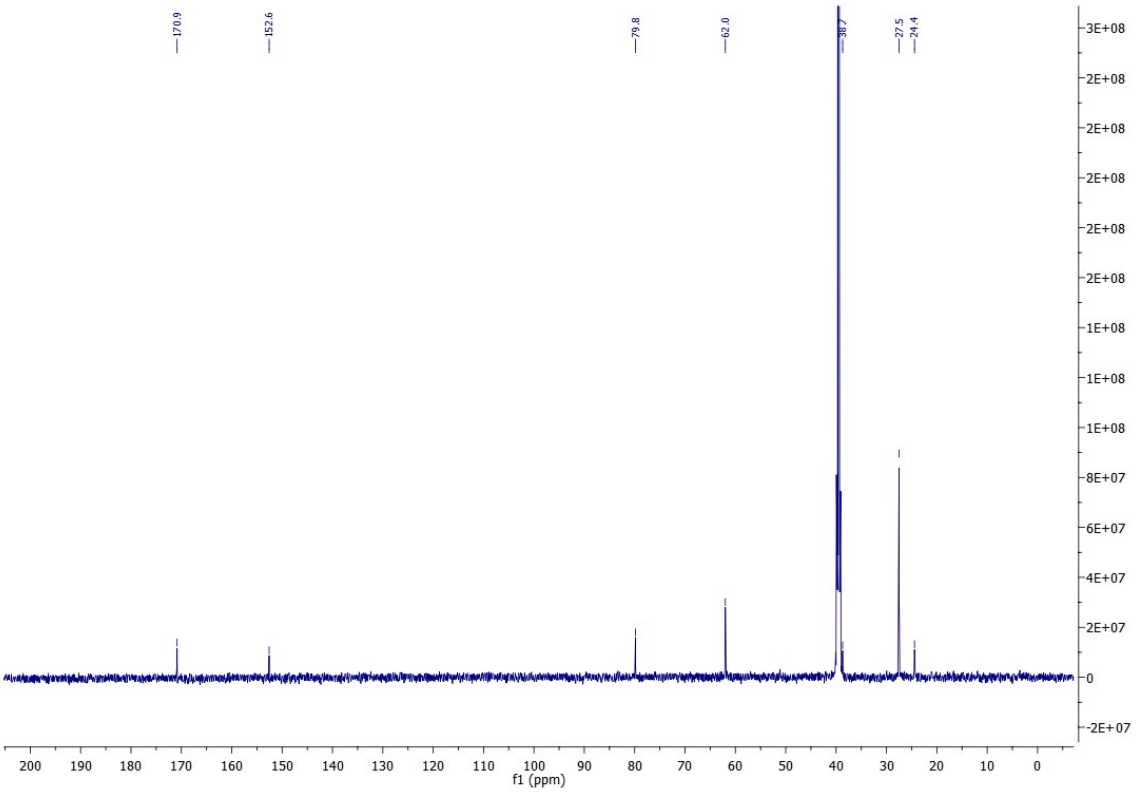




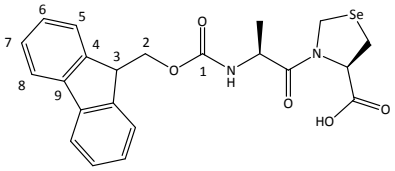
Boc-selenazolidine 1b

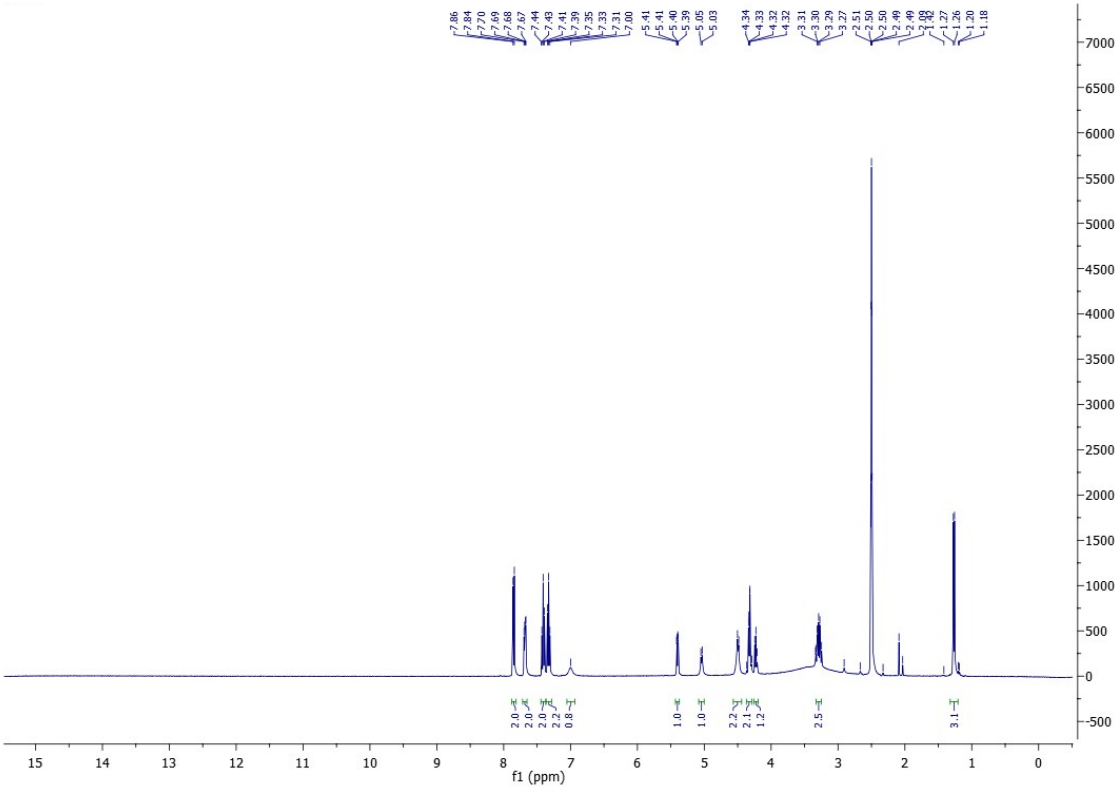


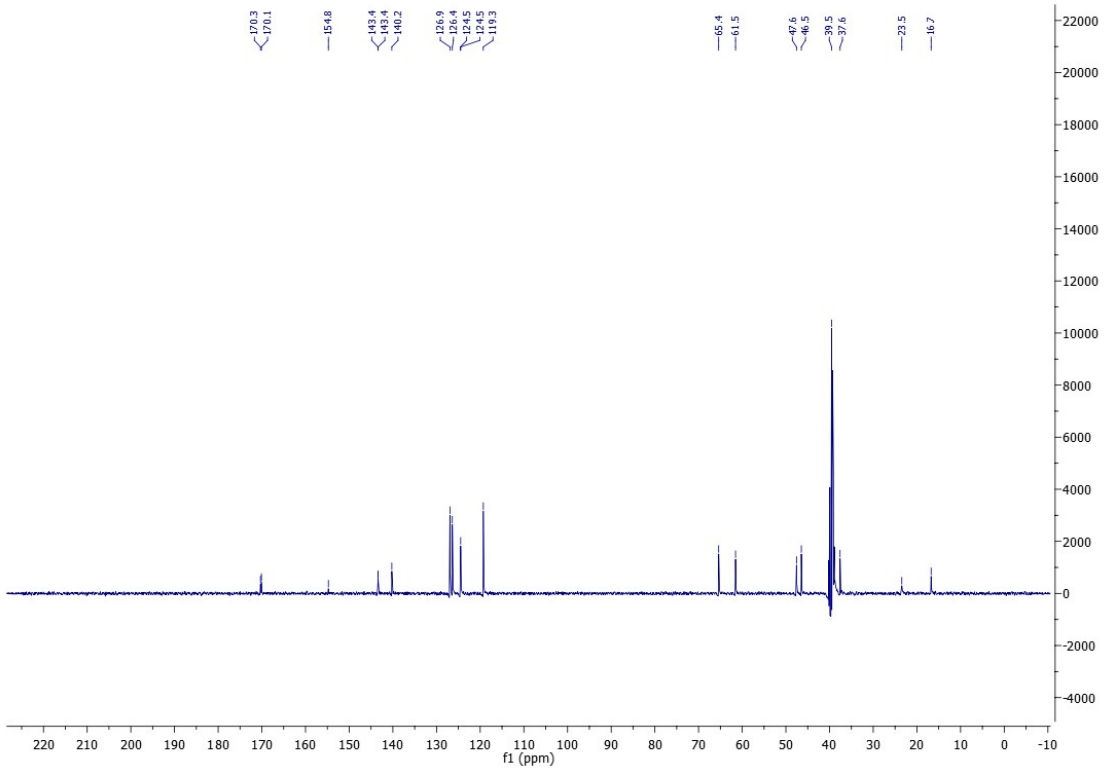




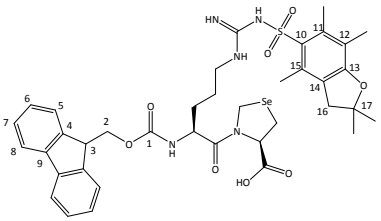
Fmoc-Ala-Sez-OH 9

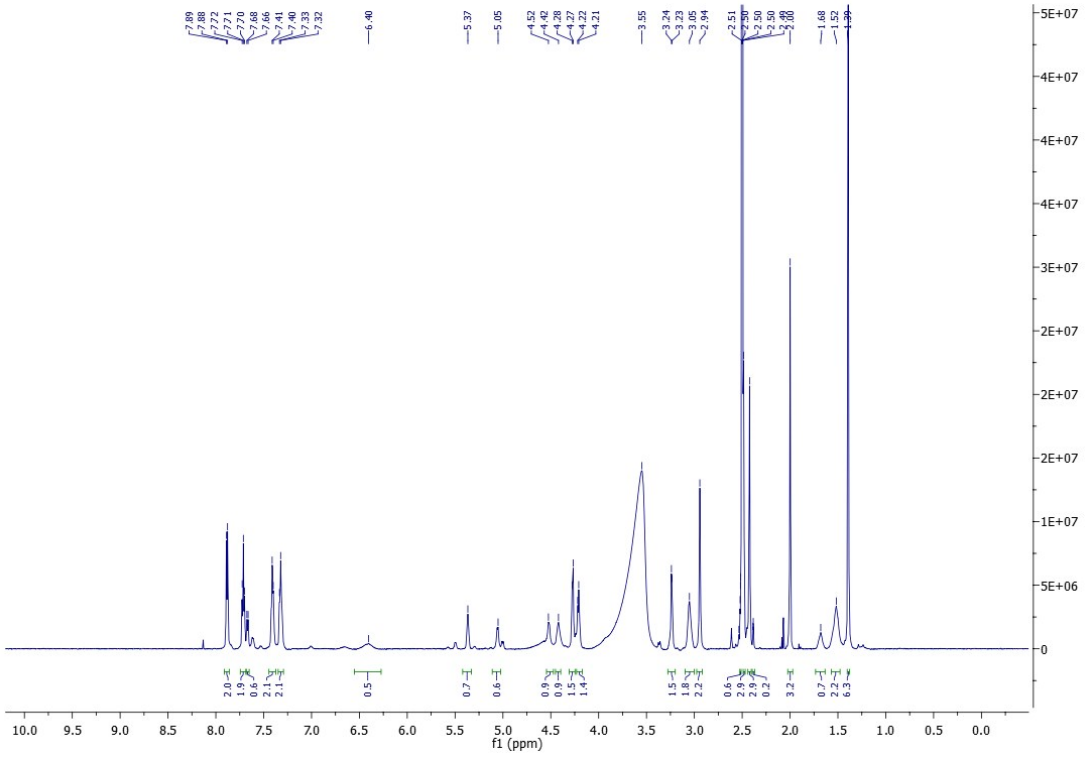


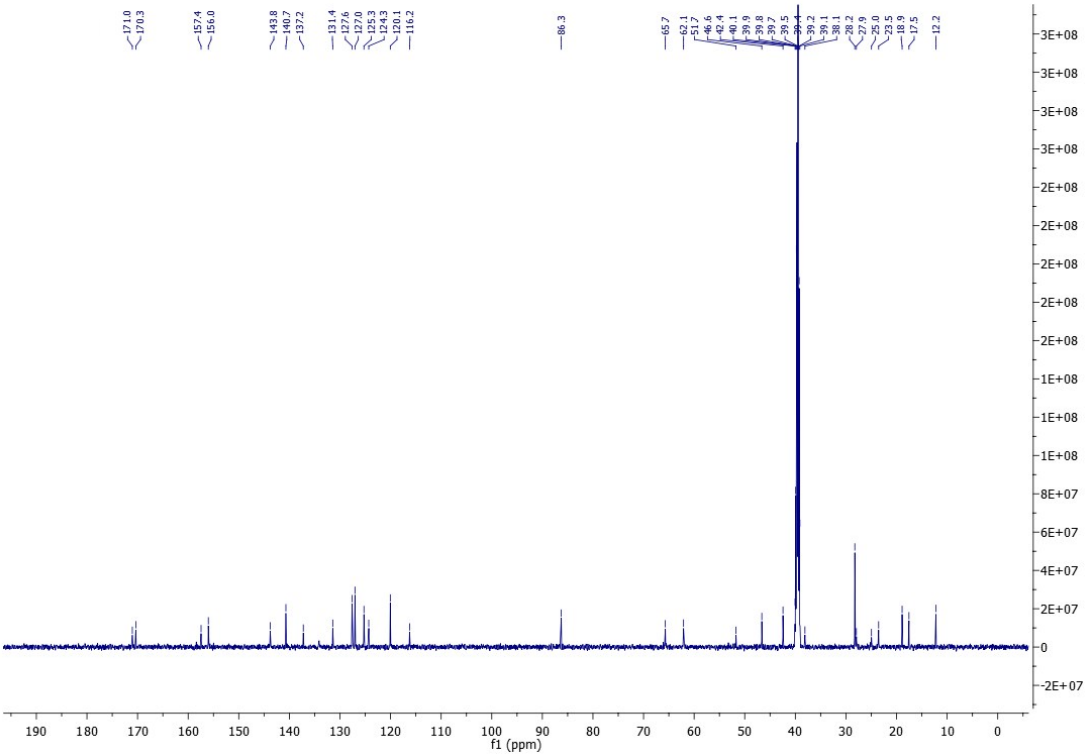




Fmoc-Arg(Pbf)-Sez-OH 10







[Sez]^{6}-HO-Phpa-LVA