Electronic Supplementary Material (ESI) for Chemical Communications.

Self-assembled benzoselenadiazole-capped tripeptide hydrogels with inherent *in vitro* anti-cancer and anti-inflammatory activity

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Experimental Sections

Preparation of Gels: In a 5 mL glass vial, BSeLYF (13.01 mg) and BSeLYW (13.79 mg) were dispersed in 1 mL of sodium phosphate buffer solution (0.1 M) of pH 7.4 and 6.7 respectively via alternate shaking, and ultrasonication treatments until it mix well. After sonication, the resulting solutions were then heated at 80 °C until all the gelators completely dissolved. Then the clear peptide solutions were kept at room temperature to initiate the hydrogel formation. All the hydrogels have final concentrations of 20 mmol/L. The formation of hydrogels was confirmed by vial inversion method.

Rheological Properties: The rheological experiments were performed at 25 °C on an Anton Paar Physica MCR 301 rheometer. The viscoelastic properties of hydrogels were measured by measuring the storage modulus (G′) and loss modulus (G″). Gels (0.5 mL) were transferred on a rheometer plate by using a microspatula and kept hydrated by using a solvent trap. A stainless steel parallel plate (diameter: 25 mm) was used to sandwich the hydrogels with TruGap (0.5 mm). The dynamic strain sweep experiment was performed to determine the region of deformation of hydrogels in which linear viscoelasticity is valid. The exact strains and stress resistivity for the gels were determined by linear viscoelastic regime at a constant frequency of 10 rad s^{-1}. The mechanical strengths of the gels were determined by frequency sweep experiment. In the frequency sweep measurement, the graph was plotted as a function of frequency in the range of 0.1-500 rad s^{-1}. The thixotropic properties of BSeLYW were investigated by step-strain experiments at the constant frequency of 10 rad s^{-1}, and applied strains were varied from 1 to 100%.

Fourier-transform Infrared (FTIR) Spectroscopy: Fourier-transform infrared (FTIR) spectroscopy was recorded on a Bruker Tensor 27 FTIR spectrophotometer. The lyophilized gels were used to perform the solid state experiments. The solid-state experiments were performed using the KBr pellet making technique with a scan range between 600 and 4000 cm^{-1} over 64 scans at a resolution of 4 cm^{-1} and an interval of 1 cm^{-1}.

Circular Dichroism Analysis of Hydrogel: The secondary structure of the self-assembled tripeptide hydrogels was analyzed by circular dichroism spectroscopy. Circular dichroism (CD) spectra were recorded at 25 °C using a JASCO J-815 spectropolarimeter at concentrations ranging from 0.015 to 1 mM. The spectra were recorded in a quartz cell (path length: 1 mm) within the range 400-190 nm with a data pitch of 0.1 nm. The bandwidth was set at 1 nm, the scanning speed was 20 nm min^{-1}, and the response time was 1 s. Before running the sample, respective solvent systems were run to correct the baseline.

Morphological Study of the Hydrogel: Field-emission Gun-scanning electron microscopic studies of gels were performed by using Carl Zeiss scanning electron microscope (FE-SEM Supra 55 Zeiss). The diluted aqueous gel samples were drop casted on a glass cover slip and dried followed by coated with gold for SEM analysis with an operating voltage of 5 kV.
Transmission electron microscopy (TEM) images were captured using a JEOL electron microscope (model: JEM-2100F), operated at an accelerating voltage of 200 kV. Gels (50 μL) were dissolved in 450 μL of water, and the dilute solution of the gels was dried on carboncoated copper grids (300 mesh) by slow evaporation in air and then allowed to dry separately under a reduced pressure at room temperature. The nanostructural morphology of gels was analyzed by TEM experiments using 0.2% phosphotungstic acid as a negative stain.

X-ray Diffraction (XRD) Measurement: The powder X-ray diffraction (XRD) patterns of the gels were measured using Rigaku Smart lab automated multipurpose X-ray diffractometer with a wavelength of 0.154 nm (Cu Ka) at 25 °C. PXRD analyses of the lyophilized gels were performed by placing the sample on a glass plate. The X-rays were produced using a sealed tube and detected using a fast-counting detector based on silicon strip technology (Scintillator NaI photomultiplier detector).

Cell cytotoxicity assay: The cell cytotoxicity was determined by MTT (3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide) assay. Concisely, MCF-7 and HEK 293T (2000 cells/well) were seeded in 96 well plates (Tarson, India) and incubated in specific (5% CO_2, 37 °C) cell culture condition overnight. BSeLYF, BSeLYW, LYF and LYW were diluted in the cell culture medium with a range of concentrations such as 16, 8, 4, 2, 1 mmol/L. The overnight grown cells were treated with the abovementioned concentrations for 24 h. After completion of the treatment period, we removed the media and added the 50 µl MTT reagent to each tube, and incubated at 37 °C for 4 h. Later, after 4 h we removed the MTT reagent and added 100 µL of the DMSO, incubated on the shaker for another 1 h, and then recorded the OD at 570 and 590 nm on a microplate reader.

Cell proliferation assay: We performed a cell proliferation assay through the crystal violet density and trypan blue exclusion methods as described earlier studies.[1] Briefly, an equal number of (0.25 million) cells were seeded and observed the cells till confluency reached ~50%. After reaching the confluency, we exposed the cells to I10 (MOI-100) and EBV (MOI-2.5) as mentioned in infection models, incubated at 37 °C for 24 h. Furthermore, trypsinized cells were mixed with trypan blue, and counted only the viable cells through a hemocytometer.

ROS formation assay: We performed a 2′-7′-dichlorodihydrofluorescein diacetate (DCFH-DA) assay to quantify the total cellular ROS. Briefly, we seeded the 0.25 million cells in 6-well plates overnight and then exposed the seeded cells with BSeLYF and BSeLYW for 24 h while taking the untreated cells as a negative control. Further, removed the media and washed twice with PBS, and incubated with 10 µg/ml DCFH-DA dye for 30 min. After 30 min of incubation, we removed the dye and washed it thrice to remove the unspecific bound dye and then captured the image by using Olympus IX83. Furthermore, we performed this experiment on two biological and three technical replicates and quantified the 9 images from each sample through ImageJ software, and plotted the ROI value in fold change through Graphpad PRISM software.

RNA isolation and qRT-PCR: We performed qRT-PCR for inflammatory genes such as (Tnf-α and Il-6); proapoptotic genes involved in the intrinsic and extrinsic pathway of programmed cell death (CytC, Caspase-9, Apaf-1, and Bax,) and (Caspase-8 and Fadd) respectively. Concisely,

we counted the MCF-7 and HEK 293T cells through the Neubauer chamber method, and further seeded the 0.25 million cells in 6 well plates, after reaching the confluency of 60 to 70% we treated the cells with 2mM of BSe-LYF and BSe-LYW for 24 h. Furthermore, we collected the above-mentioned samples and total RNA was isolated by the triazole method. We prepared the cDNA by converting mRNA into the cDNA as described earlier.[1a] Later, we performed qRT-PCR as described in earlier report.[2]

Table S1 Optimization of hydrogelation of BSe-tripeptides at different peptide concentrations

Peptides	Peptide Concentrations (mmol/L)	Remarks	Photographs
BSeLYF	10	S	
	15	S	
	20	H	
BSeLYW	10	S	
	15	S	
	20	H	
S = solution, H = hydrogel			

Fig. S1 Digital photographs of (a) BSeLYF hydrogel, (b) BSeLYW hydrogel.

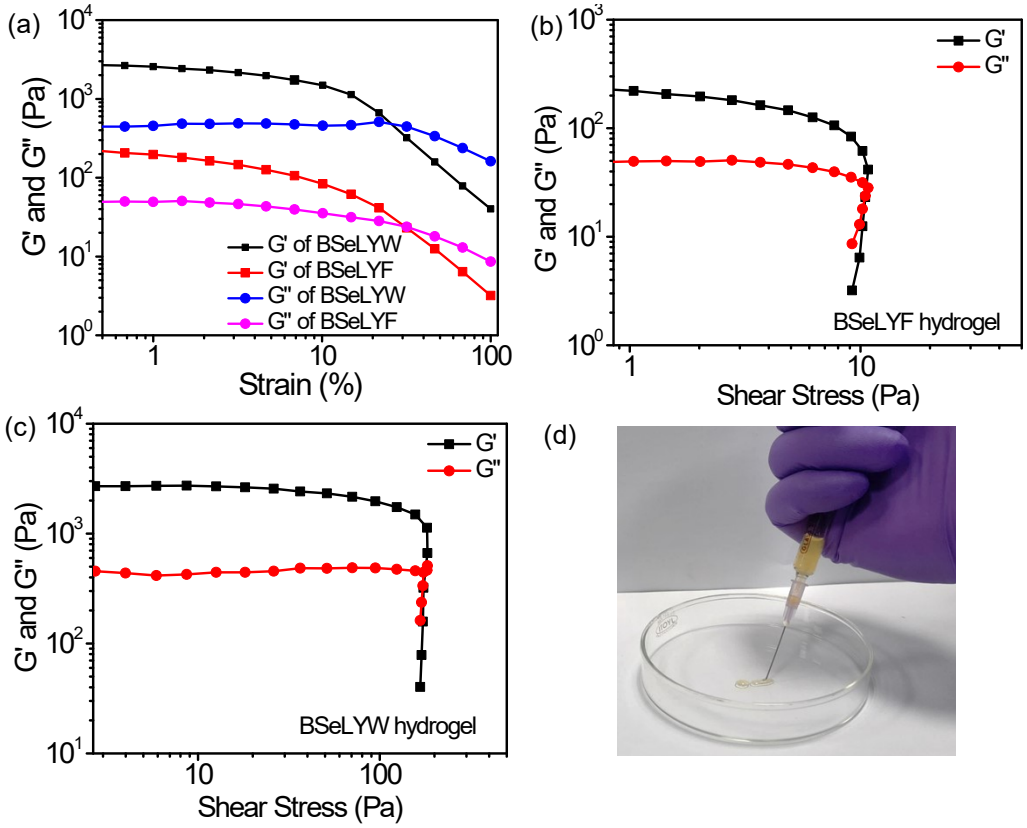


Fig. S2 (a) Amplitude strain sweep experiment of BSe-tripeptide hydrogels. Amplitude stress-sweep experiment of (b) BSeLYF and (c) BSeLYW hydrogels. (d) Injectability test of BSeLYW hydrogel through 22G needle.

Table S2 Relationship between the hydrophobicity[3] and storage modulus of two BSe capped tripeptide hydrogels.

Peptide Sequence	Hydrogel	Hydrophobicity (log *P*)	Storage Modulus (*G*′) (Pa)
BSeLYF	BSeLYF hydrogel	1.70	2000
BSeLYW	BSeLYW hydrogel	1.85	216

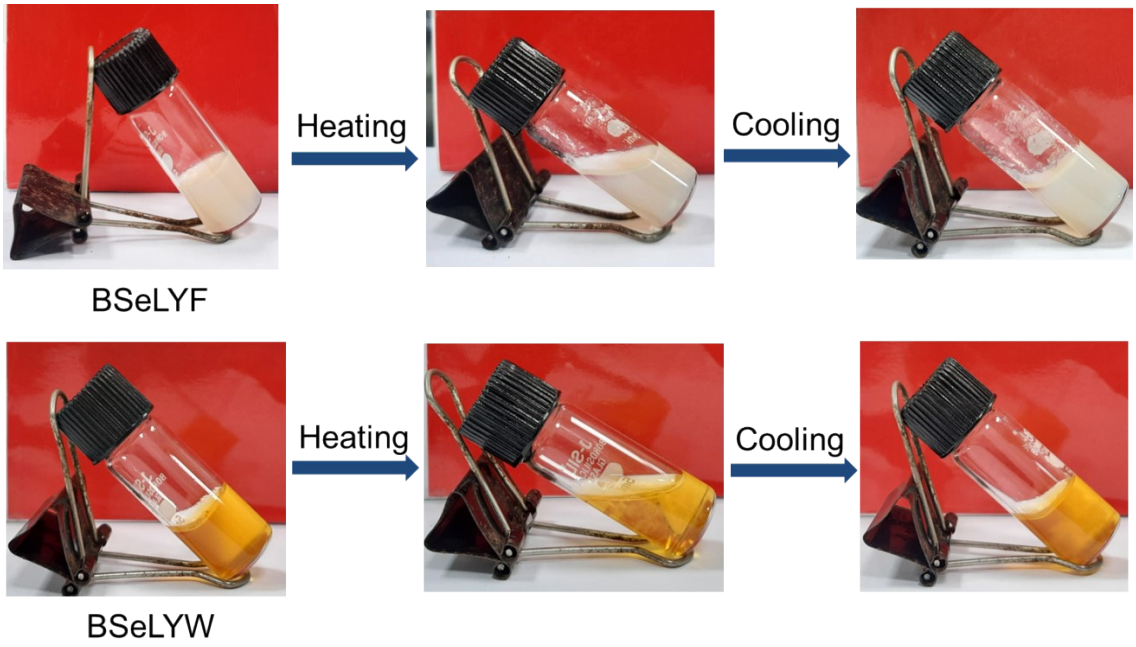


Fig. S3 Digital photographs of gel-sol transitions upon heating and cooling of BSeLYF hydrogel (upper) and BSeLYW hydrogel (lower).

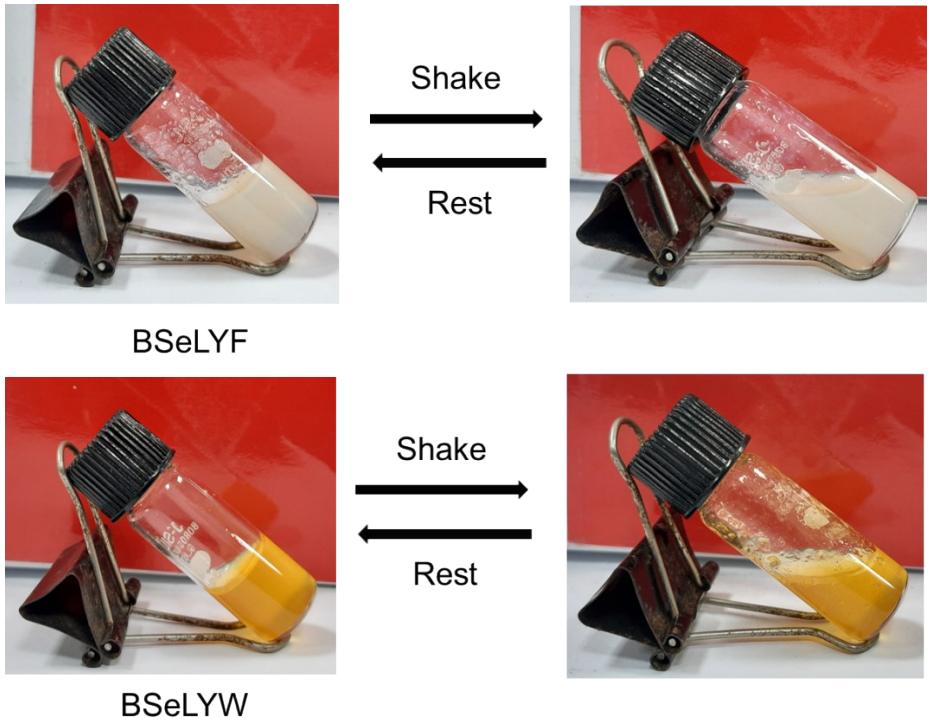


Fig. S4 Digital photographs of gel-sol-gel transitions upon vigorous shaking by hand followed by resting for 5 min. BSeLYF hydrogel (upper) and BSeLYW hydrogel (lower).

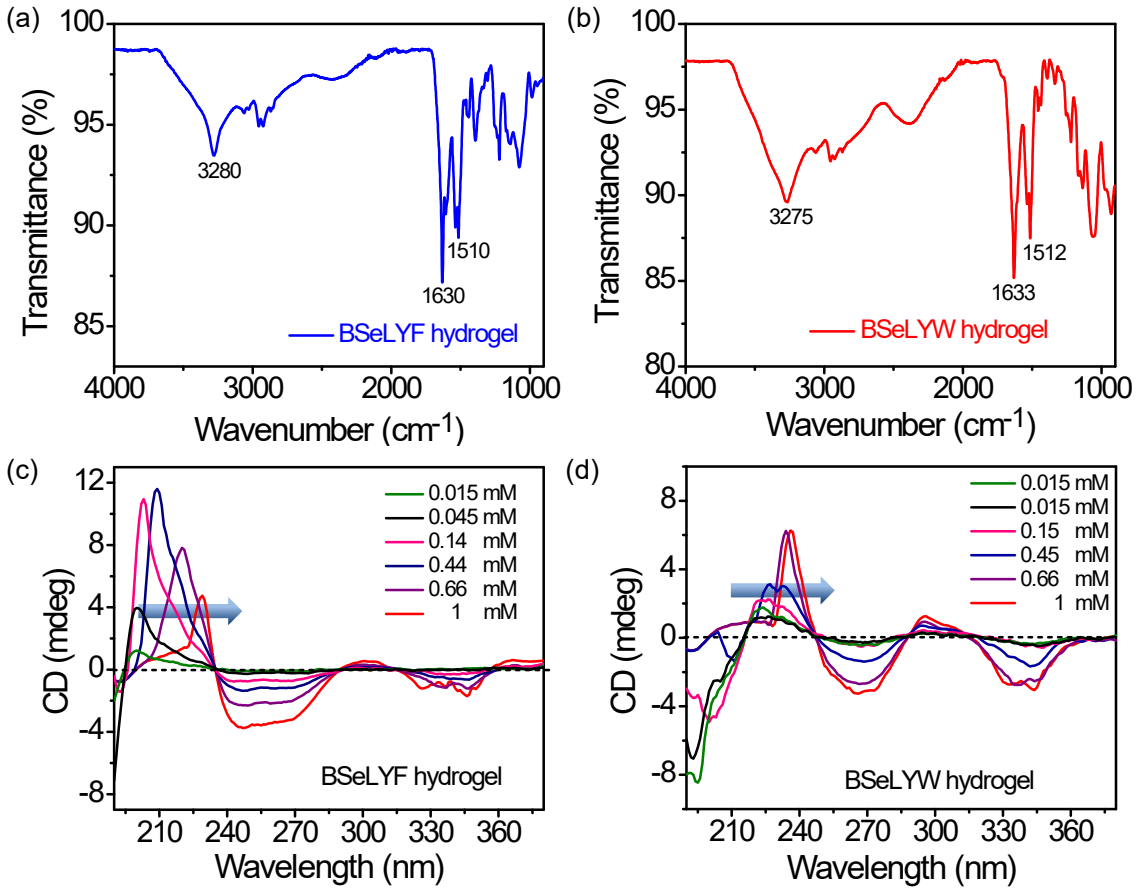


Fig S5 FT-IR spectra of dried (a) BSeLYF and (b) BSeLYW hydrogels. Concentration dependent CD spectra of (c) BSeLYF and (d) BSeLYW hydrogels.

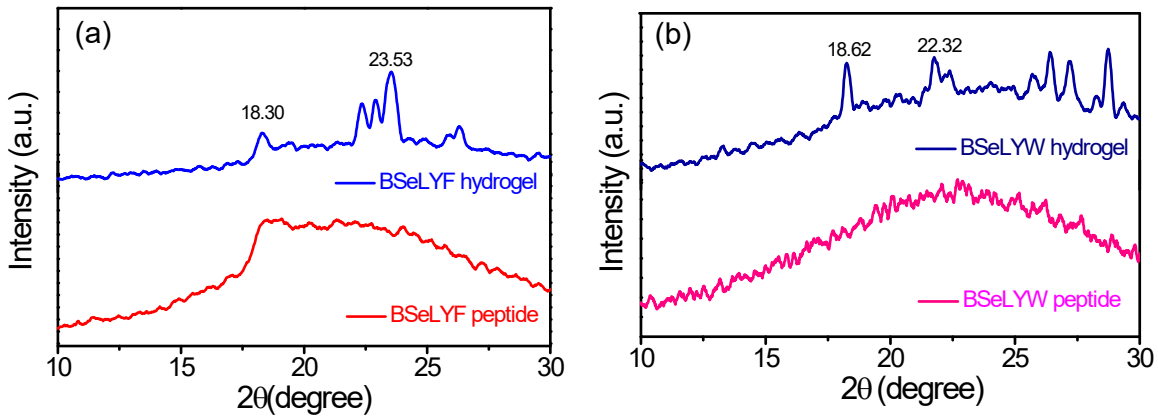


Fig. S6 PXRD profiles of dried (a) BSeLYF peptide and BSeLYF hydrogel and (b) BSeLYW peptide and BSeLYW hydrogel.

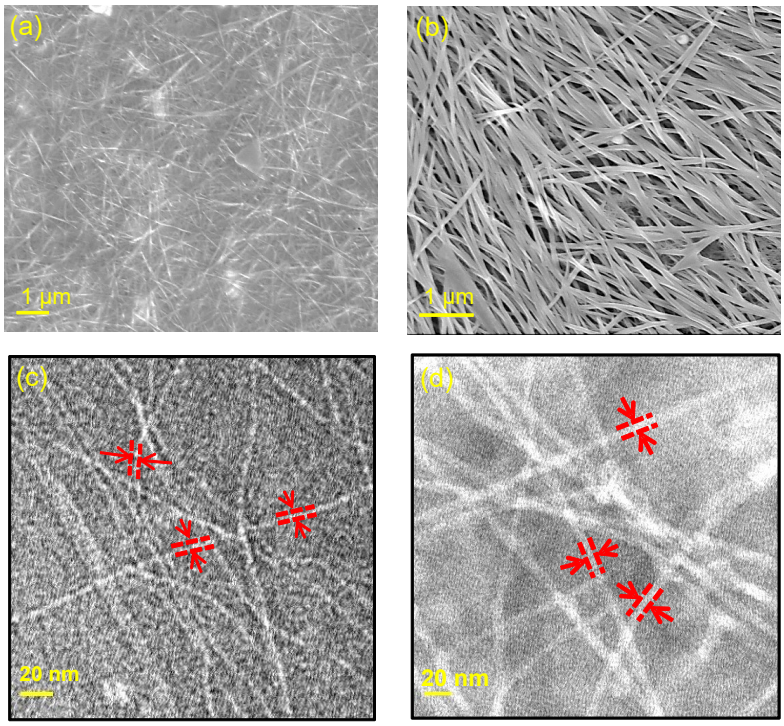


Fig. S7 FESEM images of (a) BSeLYF and (b) BSeLYW hydrogels. Enlarged HR-TEM images of (c) BSeLYF and (d) BSeLYW hydrogels.

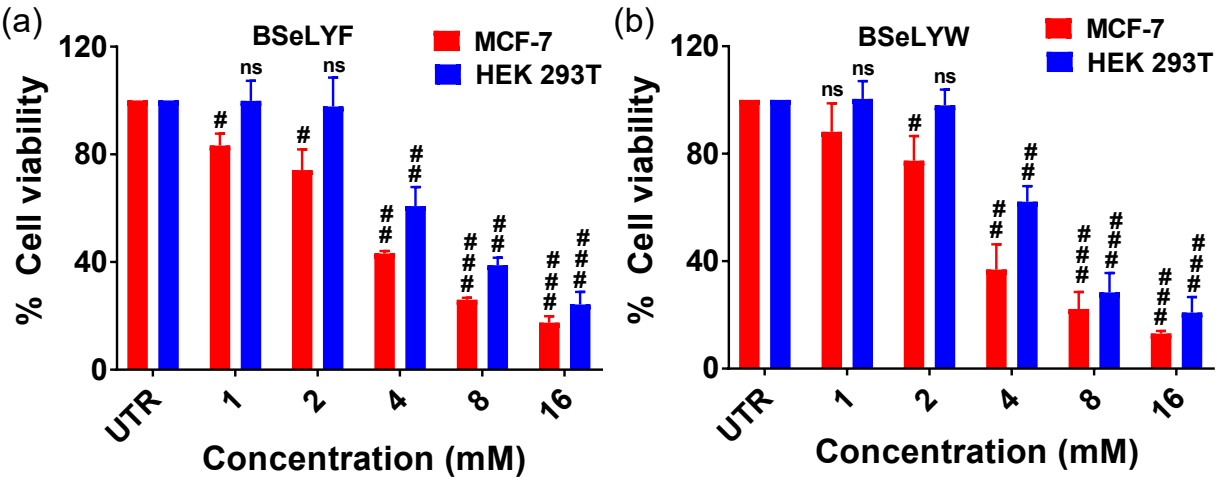


Fig. 8 Cell viability of MCF-7 and HEK 293T cells after treatment of different concentrations of (a) BSeLYF hydrogel and (b) BSeLYW hydrogel for 24 h. Data were statistically analyzed using a two-tailed Student t test. All the results were derived from a set of biological duplicates and two technical experiments. *P* values were estimated using GraphPad Prism version 6, and *P* values of 0.05, 0.01, and 0.001 were considered statistically significant and represented by #, ## and ### decreased respectively. The non-significant values are represented by ns.

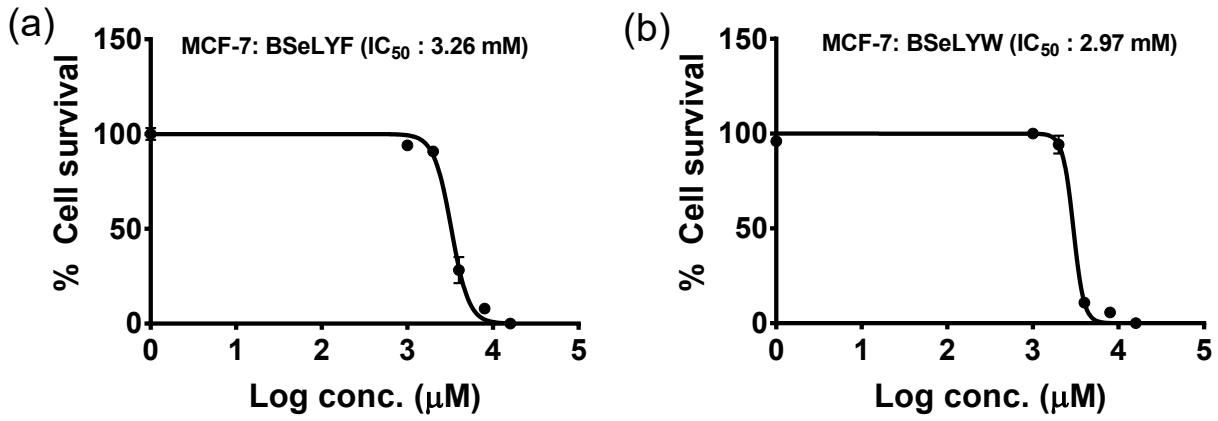


Fig. S9 IC_{50} values of the (a) BSeLYF and (b) BSeLYW hydrogels against MCF-7 cells.

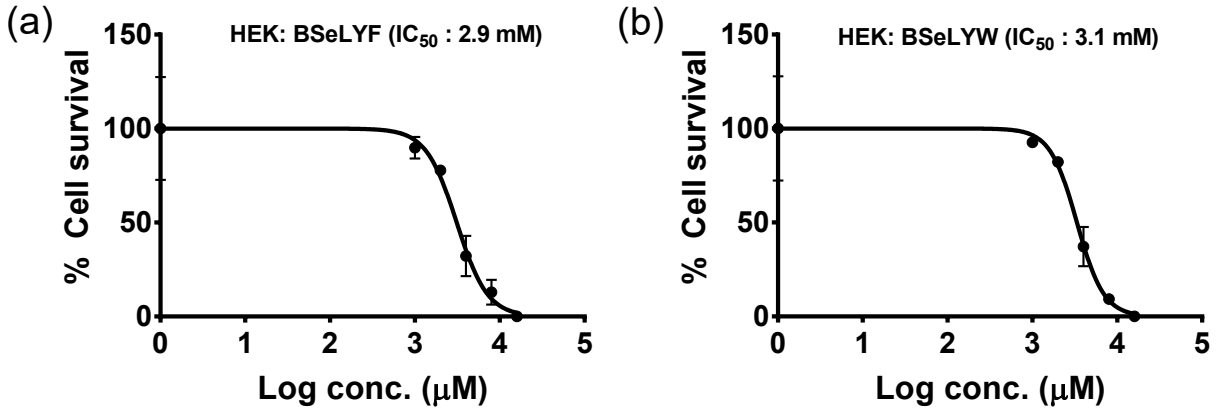


Fig. S10 IC_{50} values of the (a) BSeLYF and (b) BSeLYW hydrogels against HEK 293T cells.

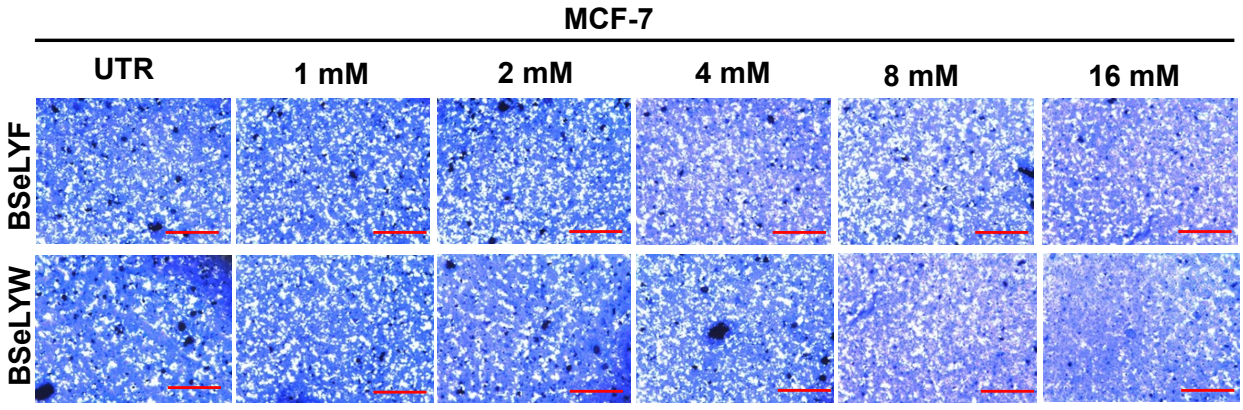


Fig. S11 Cellular morphology of MCF-7 cancer cell lines after treating different concentrations of BSeLYF and BSeLYW hydrogels (scale bar 500 nm).

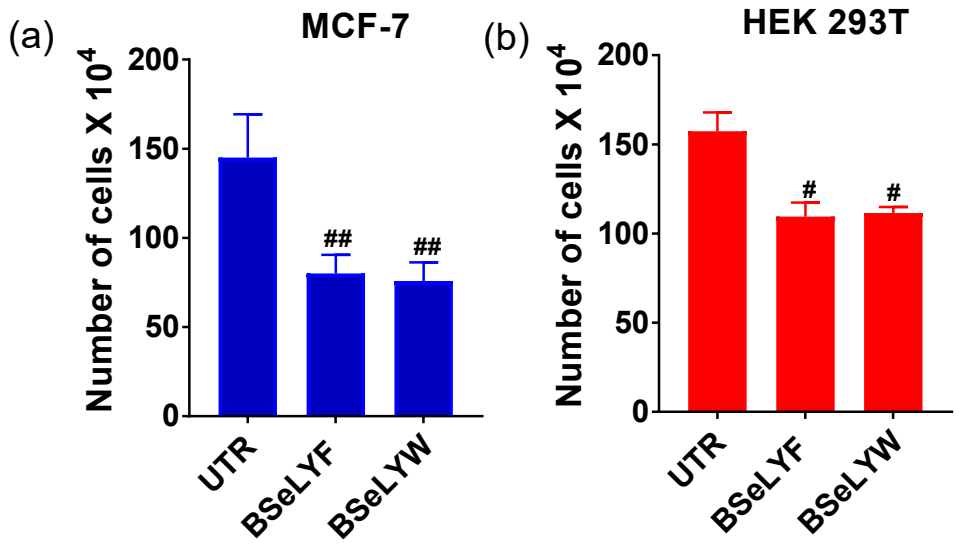


Fig. S12 Cell proliferation assay of (a) MCF-7 and (b) HEK 293T cells after treatment of BSeLYF and BSeLYW hydrogels. Data were statistically analyzed using a two-tailed Student t test. All the results were derived from a set of biological duplicates and two technical experiments. *P* values were estimated using GraphPad Prism version 6, and *P* values of 0.05, 0.01 and 0.001 were considered statistically significant and represented by #, ## and ### decreased respectively.

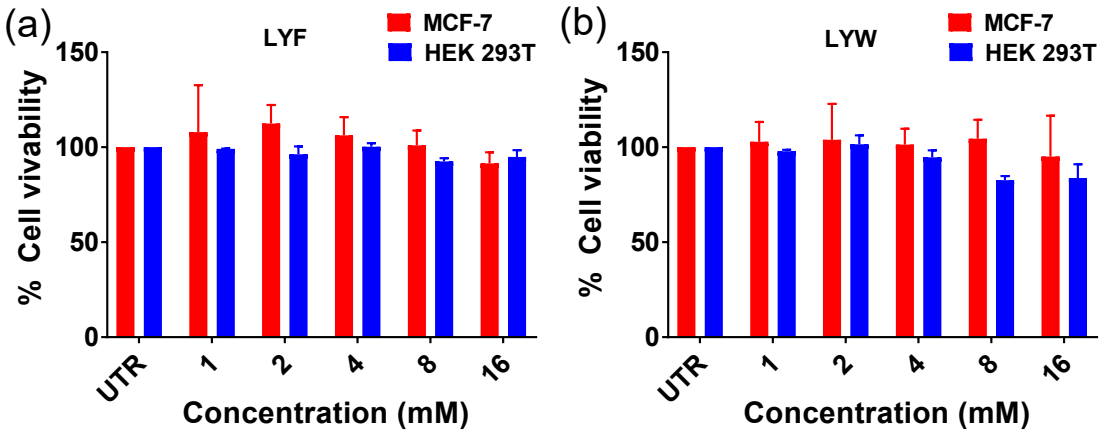


Fig. S13 Cell viability of MCF-7 and HEK 293T cells after treatment of different concentrations of (a) LYF and (b) LYW peptides for 24 h.

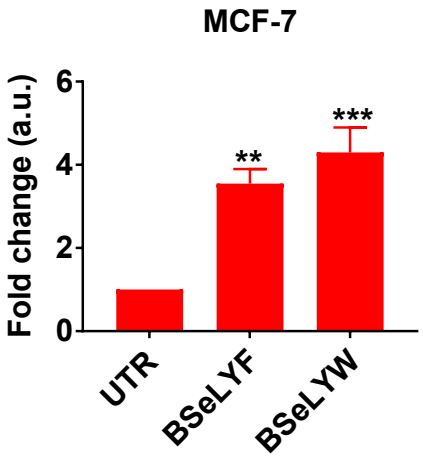


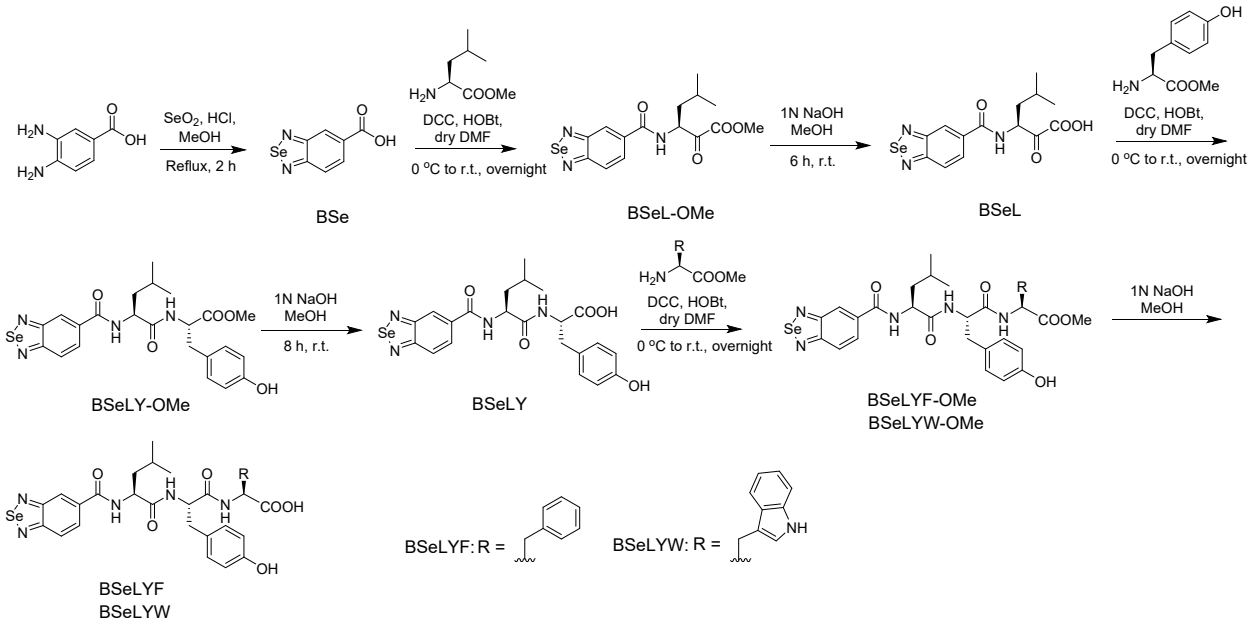
Fig. S14 Data are presented as fold change of DCFH-DA fluorescence levels of MCF-7 cells after treated with BSeLYF and BSeLYW hydrogels. Data were statistically analyzed using a two-tailed Student t test. All the results were derived from a set of biological duplicates and two technical experiments. P values were estimated using GraphPad Prism version 6, and P values of 0.05, 0.01, and 0.001 were considered statistically significant and represented by *, ** and ***, increased respectively.

Materials and Methods

In this work, all the reagents were used without further purifications. L-Leucine (L), L-tyrosine (Y), L-phenylalanine (F), L-tryptophan (W), 1-hydroxybenzotriazole (HOBt), *N,N*'-dicyclohexylcarbodiimide (DCC), sodium hydroxide (NaOH) and hydrochloric acid (HCl) were procured from SRL, India. 3,4-Diaminobenzoic acid and SeO_2 were purchased from Sigma-Aldrich, USA. All solvents were used as analytical grade, purchased from Merck chemicals and distilled prior to use. Thin-layer chromatography was performed on precoated silica gel plates (Kieselgel 60 F254, Merck). Column chromatography was performed on silica gel (100-200 mesh size). HPLC analysis was carried out using a Dionex HPLC-Ultimate 3000 (High Performance Liquid Chromatography) pump. A Dionex Acclaim TM 120 C18 column of 250 mm length with an internal diameter 4.6 mm and 5 µm fused silica particles at a flow rate of 1 mL min^{-1} coupled with UV-Vis detector. Bruker AV 500 MHz NMR spectrometer was used to characterize peptide molecules in $CDCl_3$ and DMSO-d_6. Chemical shifts (d) are reported in ppm, downfield of tetramethylsilane; peak multiplicities are reported as follows: singlet (s), doublet (d), doublet of doublets (dd), triplet (t), and multiplet (m). Electrospray ionization mass

spectrometry (ESI-MS) was performed using mass spectrometer (model: Bruker micrOTOF-Q II) by positive and negative modes.

Synthetic pathway of tripeptides BSeLYF and BSeLYW



Scheme S1 Synthetic pathway for liquid phase synthesis of BSe-capped tripeptides.

The intermediates benzo[2,1,3]selenadiazole-5-carboxylic acid (BSe), BSeL-OMe and BSeL were synthesized. The final tripeptides BSeLYF and BSeLYW were purified by column chromatography and fully characterized by ^{1}H, ^{13}C NMR and mass spectral studies.

Synthesis of BSe: In a 250 mL round bottom flask, 2 g of 3,4-diaminobenzoic acid (13.14 mmol) and 1.60 g of selenium dioxide (14.45 mmol) were dissolved in methanol (40 mL) and 1N HCl (20 mL). The whole mixture was then refluxed at 80 °C for 2 h. After completion of reaction, the reaction mixture was then cooled at room temperature followed by evaporation of methanol under reduced pressure. The brownish residue then filtered with D.I water and final BSe was obtained as precipitation. Yield: 89% (2.65 g). ^{1}H NMR (DMSO-d6, 500 MHz): δ 13.35 (bs, 1H), 8.40 (s, 1H), 7.97-7.90 (dd, 2H, J = 11.05, 11.55); ^{13}C NMR (DMSO-*d*6, 125

MHz): δ 167.3, 167.2, 159.6, 131.7, 128.3, 125.8, 123.7. ESI-MS m/z: $[M-H]^-$ calcd for $C_7H_3N_2O_2Se$ 226.9354; Found 226.9443.

General Procedure for Peptide Coupling: BSe-capped compound (1.0 equiv) was dissolved in dry-DMF (5 mL/g) and stirred on an ice-water bath. To this pre-cooled reaction mixture (1.0 equiv) HOBt, (1.1 equiv) dicyclohexylcarbodiimide (DCC) were added. Methyl ester protected amino acid was isolated from its corresponding methyl ester hydrochloride (2.0 equiv) by neutralization and subsequently extracted thrice with ethyl acetate (3 × 30 mL). The collected ethyl acetate extracts was dried over anhydrous Na_2SO_4 and concentrated to 5-7 mL. It was then added to the precooled reaction mixture. The reaction mixture was allowed to come to room temperature and stirred for overnight. After completion of the reaction, acetonitrile (40 mL) was added to the reaction mixture and dicyclohexylurea was filtered off. The filtrate was then concentrated under vaccum and the residue was diluted with 40 mL of ethyl acetate. The organic layer was washed with 1M HCl (3 × 30 mL), brine (2 × 30 mL), 1M sodium carbonate (3 × 30 mL) and brine (2 × 30 mL) and dried over anhydrous Na_2SO_4 and evaporated in a vacuum. The purification was done by using silica gel column (100-200 mesh).

General Procedure for Methyl Ester Hydrolysis: C-terminally protected peptide in methanol (20 mL/g) was taken in a round bottom flask (R.B) and 1N NaOH was added drop wise. The progress of hydrolysis was monitored by thin layer chromatography (TLC). After completion of the reaction, 15 mL of distilled water was added to the reaction mixture and methanol was removed under vacuum. The aqueous part was washed with diethyl ether (2 × 30 mL). Then aqueous part was cooled under ice-water bath for 15 minutes and then pH was adjusted to 2-3 by drop wise addition of 1M HCl. It was extracted with ethyl acetate (3 × 30 mL). The extracted ethyl acetate was dried over anhydrous Na_2SO_4 and evaporated under vacuum to yield corresponding carboxylic acid which was used for the next step without purification.

General Procedure for Boc Deprotection: N-terminally (Boc) protected peptide was first taken in a r.b. flask and dissolved in TFA/DCM (1:1). The reaction mixture was allowed to stir for 6 h. After completion of the reaction, the reaction mixture was concentrated under vacuum. The residue was then diluted with water and washed with diethyl ether. The pH of the water parts was

then adjusted to 9 by adding NH_4OH solution. Finally, the N-terminally free (Boc deprotected) peptide was obtained after lyophilization.

Synthesis of BSeL-OMe: BSeL-OMe was synthesized according to the above-mentioned general procedure for peptide coupling. BSeL-OMe was obtained as white solid and the purification was performed by silica gel column (100-200 mesh) using 50% ethyl acetate/hexane as eluent. Yield: 91% (0.786 g). ^{1}H NMR (DMSO-d_6, 500 MHz): δ 8.24 (s, 1H), 7.89-7.86 (m, 2H), 6.79-6.77 (d, 1H, J = 8.95 Hz) 4.89-4.88 (m, 1H), 3.80 (s, 3H), 1.81-1.76 (m, 2H), 1.69 (s, 1H), 1.02-0.99 (m, 6H); ^{13}C NMR (DMSO-d6, 125 MHz): δ 173.5, 166.2, 135.0, 127.9, 123.7, 122.1, 52.5, 51.4, 41.8, 25.1, 22.8, 22.1. ESI-MS m/z: $[M+Na]^+$ calcd for $C_{14}H_{17}N_3O_3NaSe$ 378.0327; Found 378.0637.

Synthesis of BSeL: BSeL was synthesized according to the above-mentioned general procedure for methyl ester hydrolysis. BSeL was obtained as off-white solid. Yield: 85% (0.556 g). ^{1}H NMR (DMSO-d_6, 500 MHz): δ 12.64 (bs, 1H), 8.93-8.91 (d, 1H), 8.44 (s, 1H), 7.97-7.90 (dd, 2H), 4.51-4.46 (t, 1H), 1.81-1.74 (m, 2H), 1.81-1.76 (m, 2H), 1.66-1.63 (m, 1H), 0.96-0.90 (dd, 6H, J = 7.67, 7.77 Hz); ^{13}C NMR (DMSO-d_6, 125 MHz): δ 174.8, 166.2, 160.8, 159.7, 134.5, 128.3, 123.4, 122.9, 51.6, 25.0, 23.4, 21.6. ESI-MS m/z: $[M-H]^-$ calcd for $C_{13}H_{14}N_3O_3Se$ 340.0195; Found 340.0312.

Synthesis of BSeLY-OMe: BSeLY-OMe was synthesized according to the above-mentioned general procedure for peptide coupling. BSeLY-OMe was obtained as off-white solid and the purification was performed by silica gel column (100-200 mesh) using 60% ethyl acetate/hexane as eluent. Yield: 94% (0.746 g). ^{1}H NMR (DMSO-d_6, 500 MHz): δ 9.20 (s, 1H), 8.77-8.76 (d, 1H, J = 7.9 Hz), 8.43 (s, 1H), 8.35-8.33 (d, 1H, J = 7.4 Hz), 7.96-7.90 (dd, 2H, J = 9.35, 9.3 Hz,), 7.02-7.00 (d, 2H, J = 8.4 Hz), 6.64-6.62 (d, 2H, J = 8.4 Hz), 4.61-4.58 (m, 1H), 4.43-4.39 (m, 1H), 3.58 (s, 3H), 2.94-2.87 (m, 2H), 1.70-1.66 (m, 2H), 1.55-1.53 (m, 1H), 0.94-0.89 (dd, 6H, J = 6.25, 6.3 Hz); ^{13}C NMR (DMSO-d_6, 125 MHz): δ 172.6, 172.4, 166.01, 160.8, 159.7, 156.5, 134.7, 130.5, 128.5, 127.53, 123.3, 123.0, 115.5, 54.4, 52.2, 36.3, 24.8, 23.5, 22.0. ESI-MS m/z: $[M+Na]^+$ calcd for $C_{23}H_{26}N_4O_5NaSe$ 541.0962; Found 541.1067.

Synthesis of BSeLY: BSeLY was synthesized according to the above-mentioned general procedure for methyl ester hydrolysis. BSeLY was obtained as faint white solid. Yield: 88% (0.600 g). ^{1}H NMR (DMSO-d_6, 500 MHz): δ 12.59 (bs, 1H), 9.16 (s, 1H), 8.78-8.76 (d, 1H, J = 8.25 Hz), 8.43 (s, 1H), 8.12-8.10 (d, 1H, J = 7.5 Hz), 7.96-7.90 (dd, 2H, J = 9.3, 9.3 Hz), 7.03-

7.01 (d, 2H, *J* = 8.3 Hz), 6.62-6.60 (d, 2H, *J* = 8.3 Hz), 4.61-4.56 (m, 1H), 4.39-4.35 (m, 1H), 2.97-2.84 (m, 2H), 1.70-1.65 (m, 2H), 1.56-1.53 (m, 1H), 0.93-0.88 (dd, 6H, *J* = 6.15, 6.25 Hz); ^{13}C NMR (DMSO-d_6, 125 MHz): δ 172.3, 166.0, 160.8, 159.7, 156.9, 156.4, 134.7, 130.5, 128.5, 123.3, 122.9, 155.4, 54.2, 52.3, 36.3, 24.8, 23.5, 21.9. ESI-MS m/z: $[M+Na]^+$ calcd for $C_{22}H_{24}N_4O_5NaSe$ 527.0805; Found 527.0899.

Synthesis of BSeLYF-OMe: BSeLYF-OMe was synthesized according to the above-mentioned general procedure for peptide coupling. BSeLYF-OMe was obtained as white solid and the purification was performed by silica gel column (100-200 mesh) using 60% ethyl acetate/hexane as eluent. Yield: 82% (0.358 g). ^{1}H NMR (DMSO-d_6, 500 MHz): δ 9.09 (s, 1H), 8.76-8.75 (d, 1H, *J* = 7.95 Hz), 8.41 (s, 1H), 8.40-8.38 (d, 1H, *J* = 7.45 Hz), 7.94-7.89 (dd, 2H, *J* = 9.35, 9.75 Hz), 7.26-7.17 (m, 5H), 6.99-6.97 (d, 2H, *J* = 8.35 Hz), 6.58-6.56 (d, 2H, *J* = 8.35 Hz), 4.51-4.44 (m, 3H), 3.55 (s, 3H), 3.02-2.86 (m, 4H), 1.66-1.60 (m, 2H), 1.47-1.44 (m, 1H), 0.90-0.85 (dd, 6H, *J* = 6.1, 6.1 Hz); ^{13}C NMR (DMSO-d_6, 125 MHz): 172.1, 172.0, 171.6, 166.1, 160.8, 159.7, 156.2, 137.4, 134.6, 130.6, 129.5, 128.7, 128.4, 128.0, 127.0, 123.4, 122.9, 115.2, 54.2, 54.0, 52.5, 52.3, 37.1, 24.8, 23.5, 21.9. ESI-MS m/z: $[M+Na]^+$ calcd for $C_{32}H_{35}N_5O_6NaSe$ 688.1647; Found 688.1676.

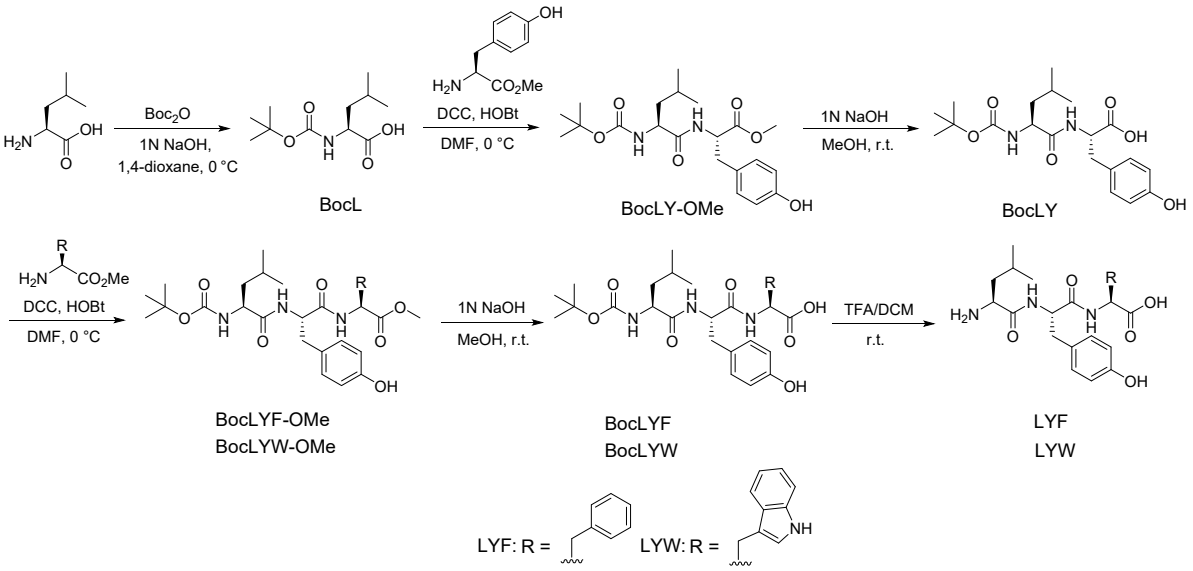
Synthesis of BSeLYW-OMe: BSeLYW-OMe was synthesized according to the above-mentioned general procedure for peptide coupling. BSeLYW-OMe was obtained as yellowish solid and the purification was performed by silica gel column (100-200 mesh) using 50% ethyl acetate/hexane as eluent. Yield: 85% (0.356 g). ^{1}H NMR (500 MHz, DMSO-d_6): δ 10.85 (s, 1H), 9.10 (s, 1H), 8.79-8.77 (d, 1H, *J* = 7.9 Hz), 8.43 (s, 1H), 8.37-8.36 (d, 1H, *J* = 7.25 Hz), 7.95-7.88 (dd, 2H, *J* = 9.25, 9.25 Hz), 7.46-7.45 (d, 1H, *J* = 7.9 Hz), 7.34-7.32 (d, 1H, *J* = 8.05 Hz), 7.16 (s, 1H), 7.07-7.00 (m, 4H), 6.58-6.57 (d, 2H, *J* = 8.4 Hz), 4.52-4.49 (m, 3H), 3.54 (s, 3H), 3.18-3.08 (m, 4H), 1.67-1.62 (m, 2H), 1.48 (m, 1H), 0.91-0.86 (dd, 6H, *J* = 6.2, 6.15 Hz); ^{13}C NMR (125 MHz; DMSO-*d*6): δ 172.5, 172.1, 171.7, 166.2, 160.8, 159.7, 156.2, 136.5, 134.7, 130.6, 128.4, 128.1, 127.5, 124.2, 123.3, 123.0, 121.4, 118.9, 118.4, 115.2, 111.9, 109.6, 60.2, 53.6, 52.6, 52.2, 37.1, 27.6, 24.9, 23.5, 21.9; ESI-MS m/z: $[M+Na]^+$ calcd for $C_{34}H_{36}N_6O_6NaSe$ 727.1757; Found 727.1797.

Synthesis of BSeLYF: BSeLYF was synthesized according to the above-mentioned general procedure for methyl ester hydrolysis. BSeLYF was obtained as off-white solid. Yield: 89% (0.136 g). ^{1}H NMR (DMSO-d_6, 500 MHz): δ 12.67 (s, 1H), 9.10 (s, 1H), 8.78-8.76 (d, 1H, *J* =

7.6 Hz), 8.41 (s, 1H), 8.22-8.20 (d, 1H, *J* = 7.25 Hz), 7.93-7.88 (dd, 2H, *J* = 6.6, 7.85 Hz), 7.25-7.20 (m, 5H), 7.01-6.99 (d, 2H, *J* = 7.55 Hz), 6.57-6.55 (d, 2H, *J* = 7.6 Hz), 4.50-4.33 (m, 3H), 3.06-2.67 (m, 4H), 1.63-1.61 (m, 2H), 1.48-1.46 (m, 1H), 0.91-0.85 (dd, 6H, *J* = 5.3, 5.25 Hz); ^{13}C NMR (DMSO-d_6, 125 MHz): δ 173.1, 172.0, 171.5, 166.1, 160.8, 159.7, 156.2, 137.8, 134.7, 130.6, 129.5, 128.6, 128.4, 126.9, 123.3, 122.9, 121.3, 115.2, 54.2, 53.9, 52.5, 37.2, 24.8, 23.4, 21.9. ESI-MS m/z: $[M+Na]^+$ calcd for $C_{31}H_{33}N_5O_6NaSe$ 674.1462; Found 674.1491.

Synthesis of BSeLYW: BSeLYW was synthesized according to the above-mentioned general procedure for methyl ester hydrolysis. BSeLYW was obtained as yellowish powder. Yield: 92% (0.140 g). ^{1}H NMR (DMSO-d_6, 500 MHz): δ 12.64 (bs, 1H), 10.83 (s, 1H), 9.09 (s, 1H), 8.78-8.77 (d, 1H, *J* = 8 Hz), 8.42 (s, 1H), 8.20-8.19 (d, 1H, *J* = 7.5 Hz), 7.94-7.88 (dd, 2H, *J* = 10.3, 9.35 Hz), 7.52-7.50 (d, 1H, *J* = 7.8 Hz), 7.33-7.32 (d, 1H, *J* = 8 Hz), 7.16 (s, 1H), 7.05-6.96 (m, 4H), 6.57-6.55 (d, 2H, *J* = 8.25 Hz), 4.98-4.78 (m, 3H), 3.09-2.92 (m, 2H), 2.74-2.64 (m, 2H), 1.63-161 (m, 2H), 1.26-1.24 (m, 1H), 0.90-0.85 (dd, 6H, *J* = 6.1, 6.05 Hz) ppm. ^{13}C NMR (125 MHz; DMSO-d_6): δ 172.1, 171.4, 166.2, 160.8, 159.7, 156.2, 136.5, 134.7, 130.6, 128.4, 128.1, 127.7, 124.0, 123.3, 122.9, 121.3, 118.8, 118.6, 115.2, 111.8, 54.4, 53.6, 52.6, 49.1, 46.0, 37.0, 24.8, 23.4, 21.9. ESI-MS m/z: $[M+Na]^+$ calcd for $C_{33}H_{34}N_6O_6NaSe$ 713.1597; Found 713.1570.

Synthetic pathway of tripeptide LYF and LYW



Scheme S2 Synthetic pathway for solution phase synthesis of LYF and LYW tripeptides. The tripeptides were synthesized by previously described methods.[4]

Synthesis of LYF: LYF was synthesized according to the above-mentioned general procedure for Boc-deprotection. LYF was obtained as off-white powder. Yield: 85% (0.160 g). ^{1}H NMR (DMSO-d_6, 500 MHz): δ 8.39 (d, 1H, J = 8 Hz), 8.04 (d, 1H, J = 7.25 Hz), 7.23-7.19 (m, 5H,), 7.01 (d, 2H, J = 8.45 Hz), 6.63 (d, 2H, J = 8.45 Hz), 4.44-4.28 (m, 3H), 3.46-3.43 (m, 2H), 3.10-2.94 (m, 2H), 1.62 (m, 1H), 1.42-1.33 (m, 2H), 0.85-0.82 (dd, 6H, J = 6.6, 6.5 Hz) ppm. ^{13}C NMR (125 MHz; DMSO-d_6): δ 173.4, 171.8, 170.9, 158.6, 156.3, 138.7, 130.6, 129.8, 128.4, 126.5, 116.5, 115.3, 54.8, 52.1, 42.0, 37.4, 24.1, 23.5, 22.1. ESI-MS m/z: $[M+H]^+$ calcd for $C_{24}H_{32}N_3O_5$ 442.2336; Found 442.2338.

Synthesis of LYW: LYW was synthesized according to the above-mentioned general procedure for Boc-deprotection. LYW was obtained as off-white powder. Yield: 81% (0.130 g). ^{1}H NMR (DMSO-d_6, 500 MHz): δ 10.62 (bs, 1H), 8.29 (d, 1H, J = 8 Hz), 8.12 (d, 1H, J = 7.2 Hz), 7.55 (d, 1H, J = 7.9 Hz), 7.33 (d, 1H, J = 8.1 Hz), 7.13 (d, 1H, J = 1.95 Hz), 7.05-6.96 (m, 4H), 6.62(d, 2H, J = 8.4 Hz), 4.48-4.38 (m, 3H), 3.22-3.18 (m, 2H), 3.07-2.94 (m, 2H), 1.62(m, 1H), 1.39-1.30 (m, 2H), 0.84-.80 (dd, 6H, J = 6.6, 6.5 Hz) ppm. ^{13}C NMR (125 MHz; DMSO-d_6): δ 172.3, 171.0, 158.7, 158.4, 156.3, 136.5, 130.6, 128.1, 124.0, 121.2, 118.9, 118.7, 116.5, 115.3, 111.7, 110.8, 54.5, 52.3, 42.3, 37.2, 24.1, 23.5, 22.0. ESI-MS m/z: $[M+H]^+$ calcd for $C_{26}H_{33}N_4O_5$ 481.2445; Found 481.2455.

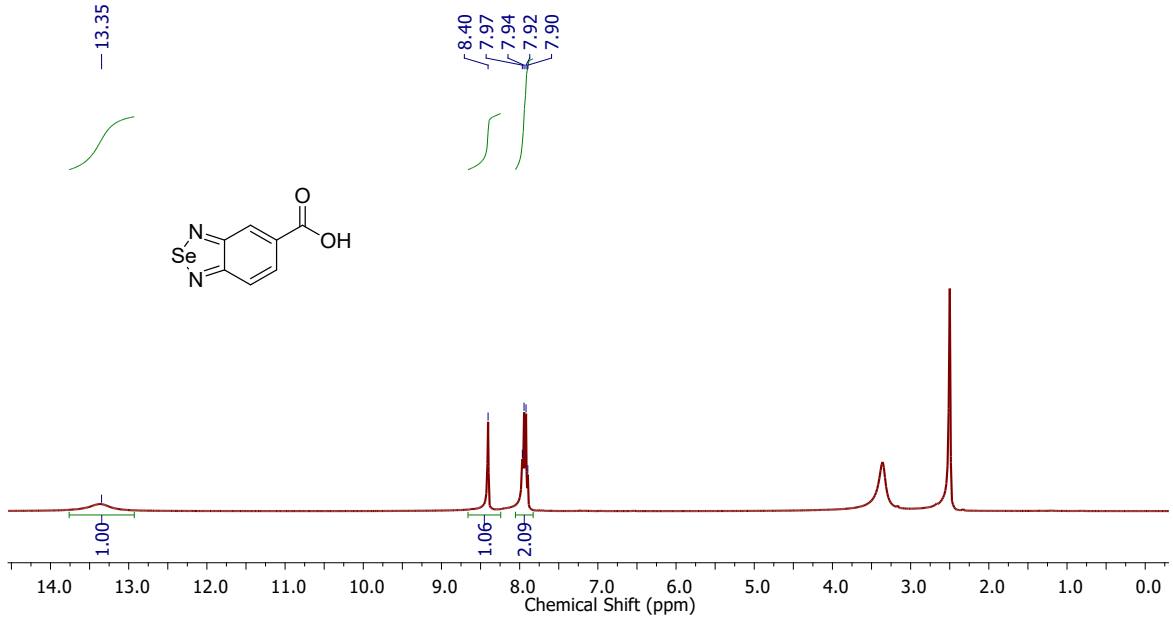


Fig. S15 ^{1}H NMR (DMSO-d_6, 500 MHz) spectrum of BSe.

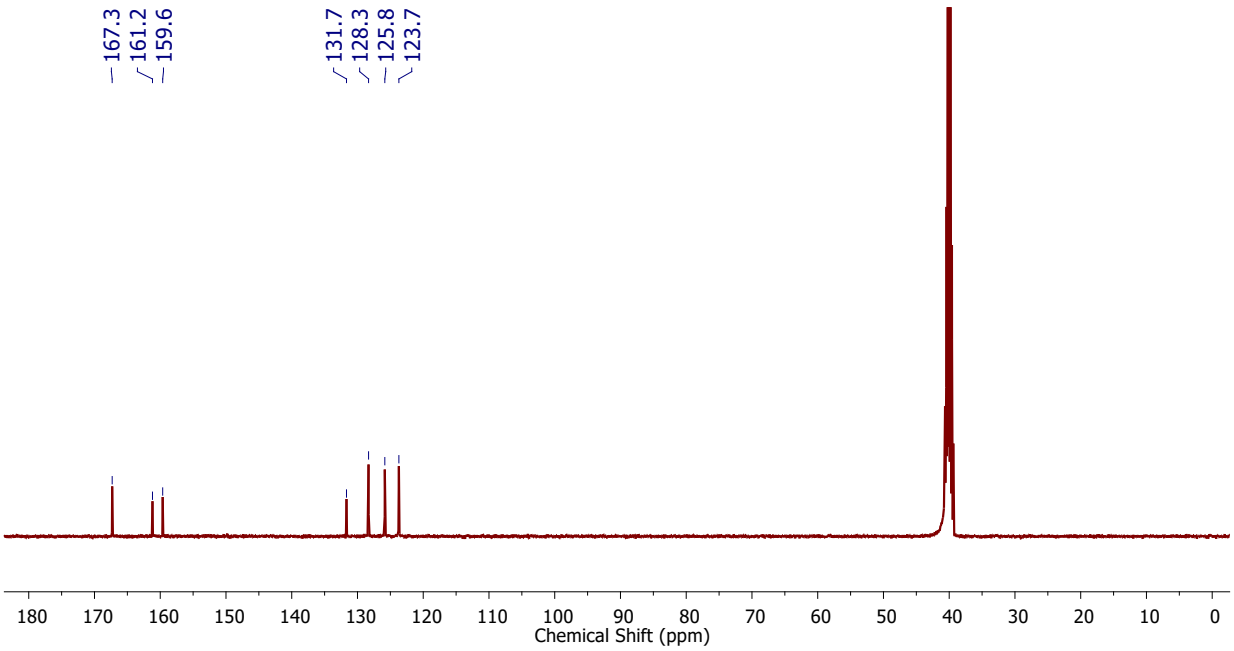


Fig. S16 ^{13}C NMR (DMSO-d_6, 125 MHz) spectrum of BSe.

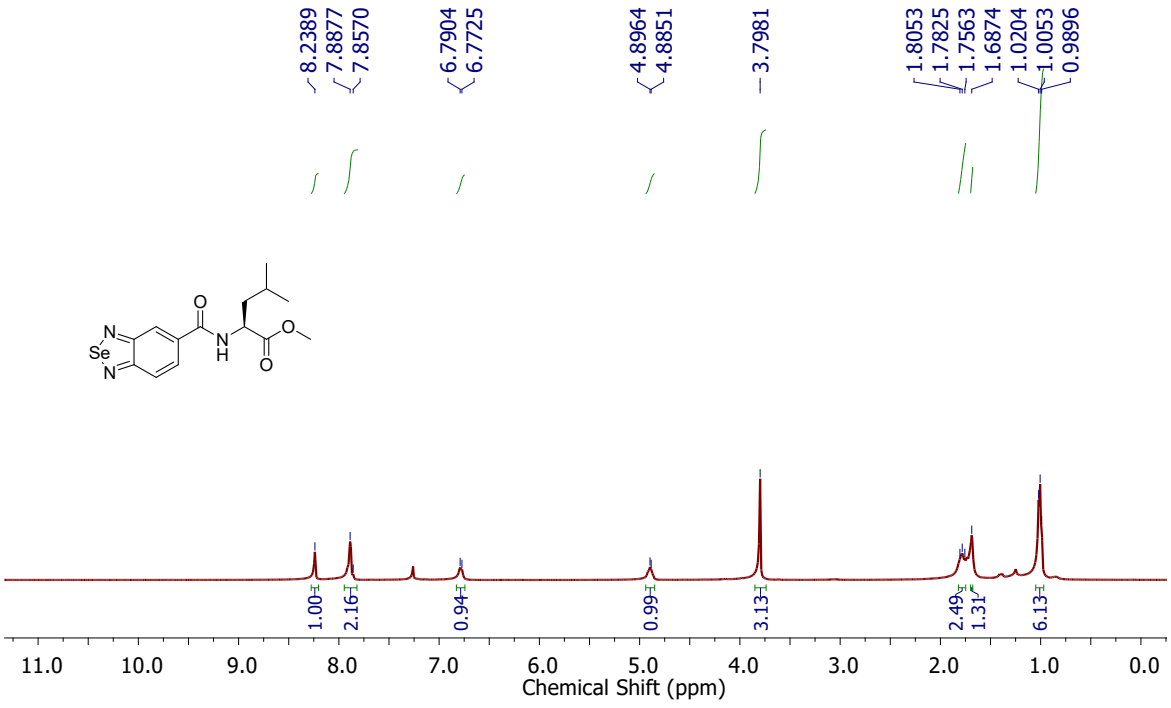


Fig. S17 ^{1}H NMR (DMSO-d_6, 500 MHz) spectrum of BSeL-OMe.

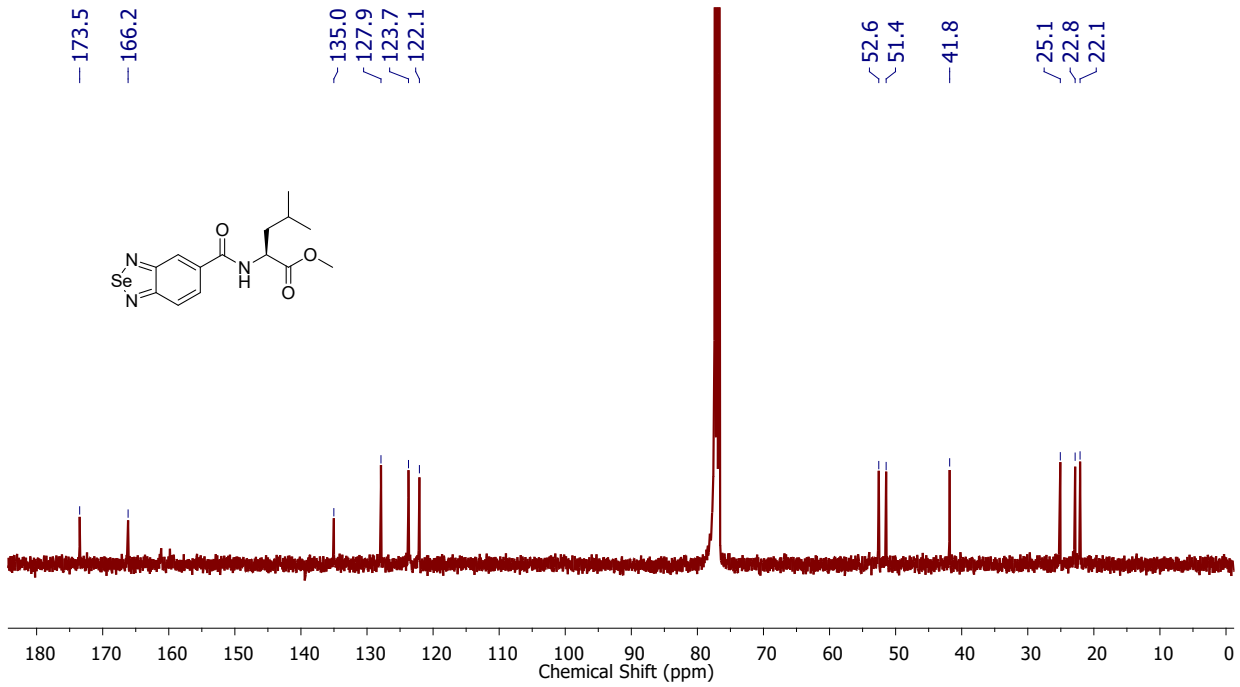


Fig. S18 ^{13}C NMR (DMSO-d_6, 125 MHz) spectrum of BSeL-OMe.

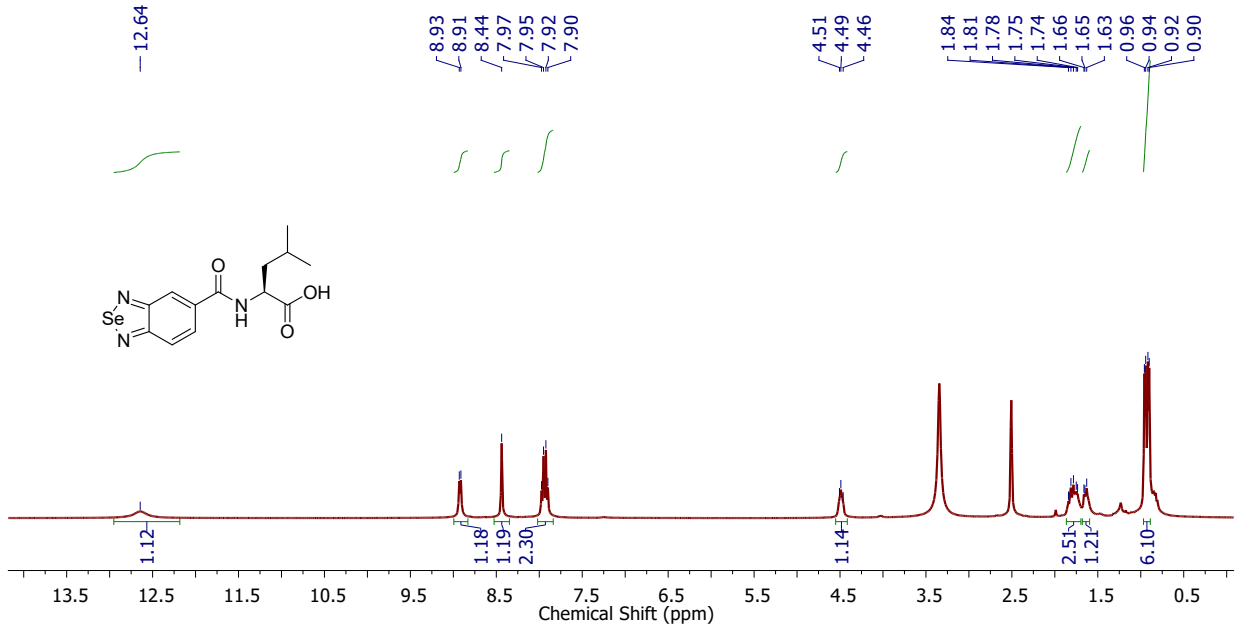


Fig. S19 ^{1}H NMR (DMSO-d_6, 500 MHz) spectrum of BSeL.

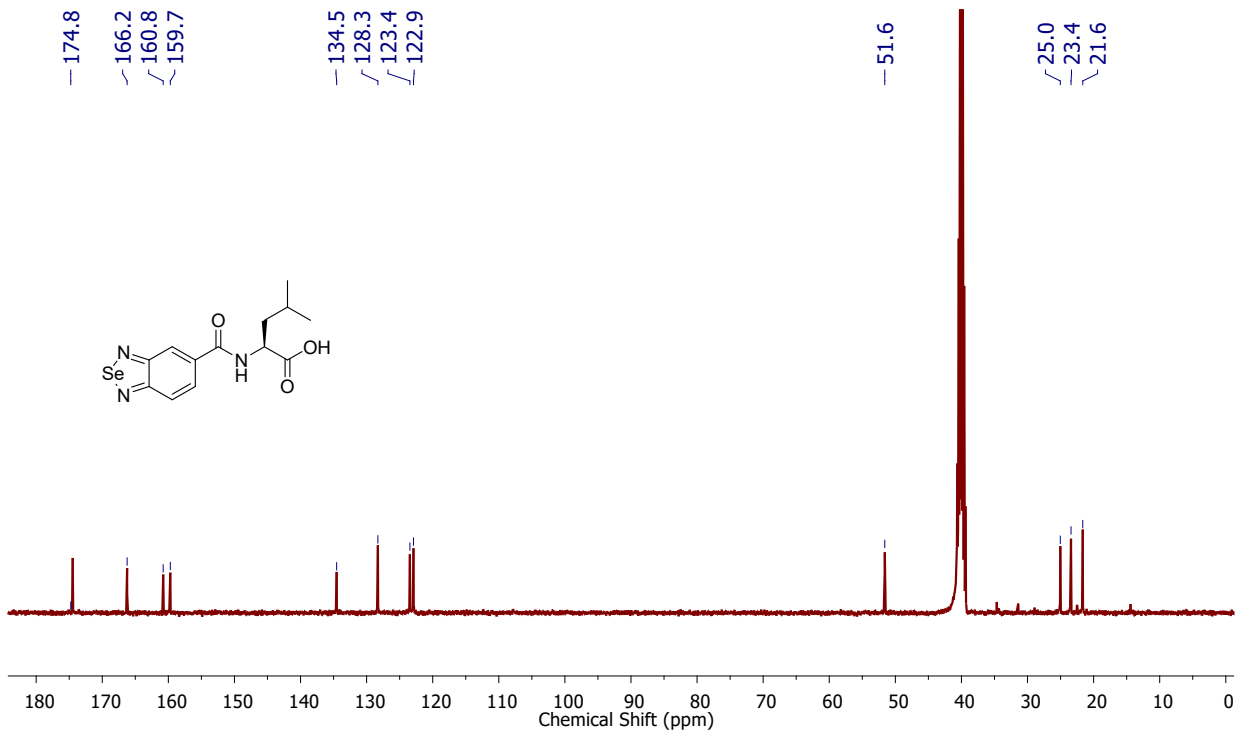


Fig. S20 ^{13}C NMR (DMSO-d_6, 125 MHz) spectrum of BSeL.

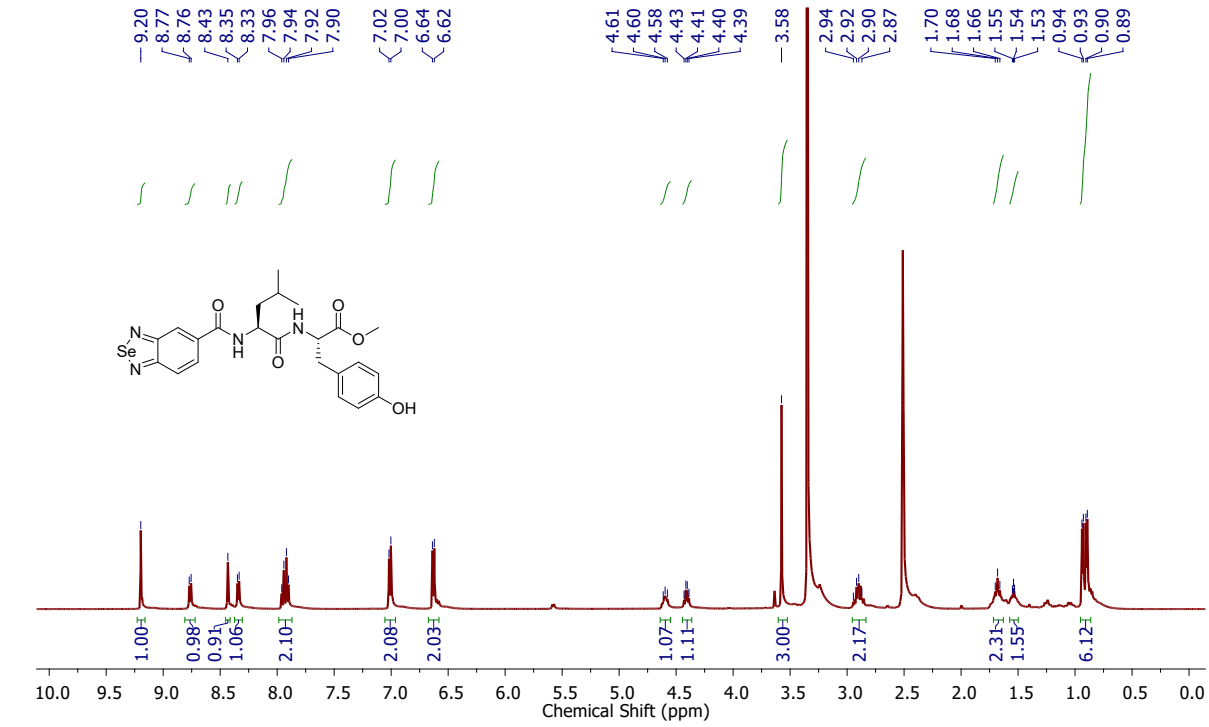


Fig. S21 ^{1}H NMR (DMSO-d_6, 500 MHz) spectrum of BSeLY-OMe.

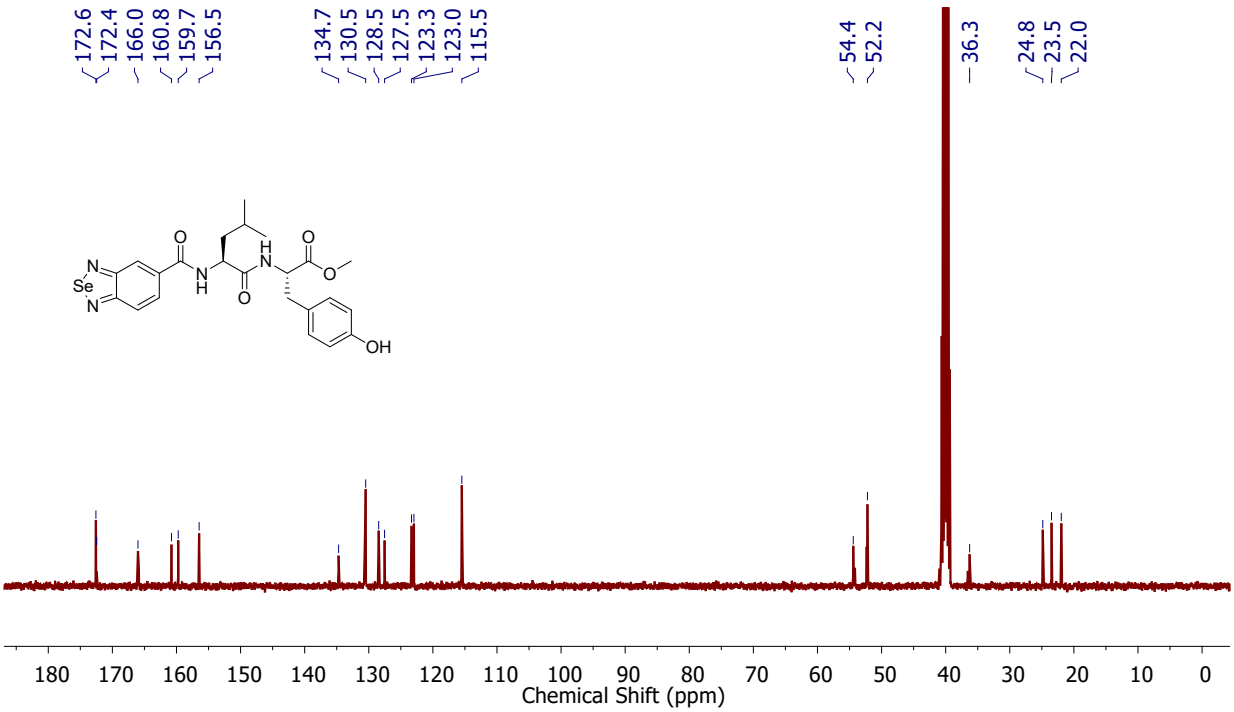


Fig. S22 ^{13}C NMR (DMSO-d_6, 125 MHz) spectrum of BSeLY-OMe.

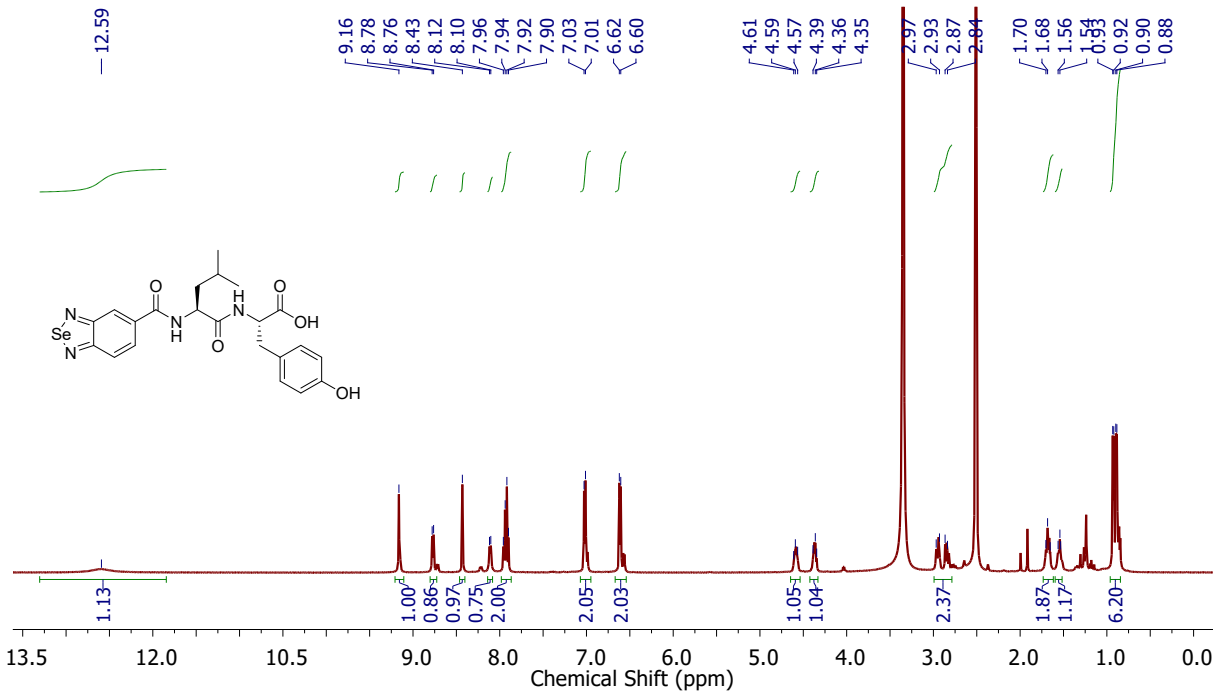


Fig. S23 ^{1}H NMR (DMSO-d_6, 500 MHz) spectrum of BSeLY.

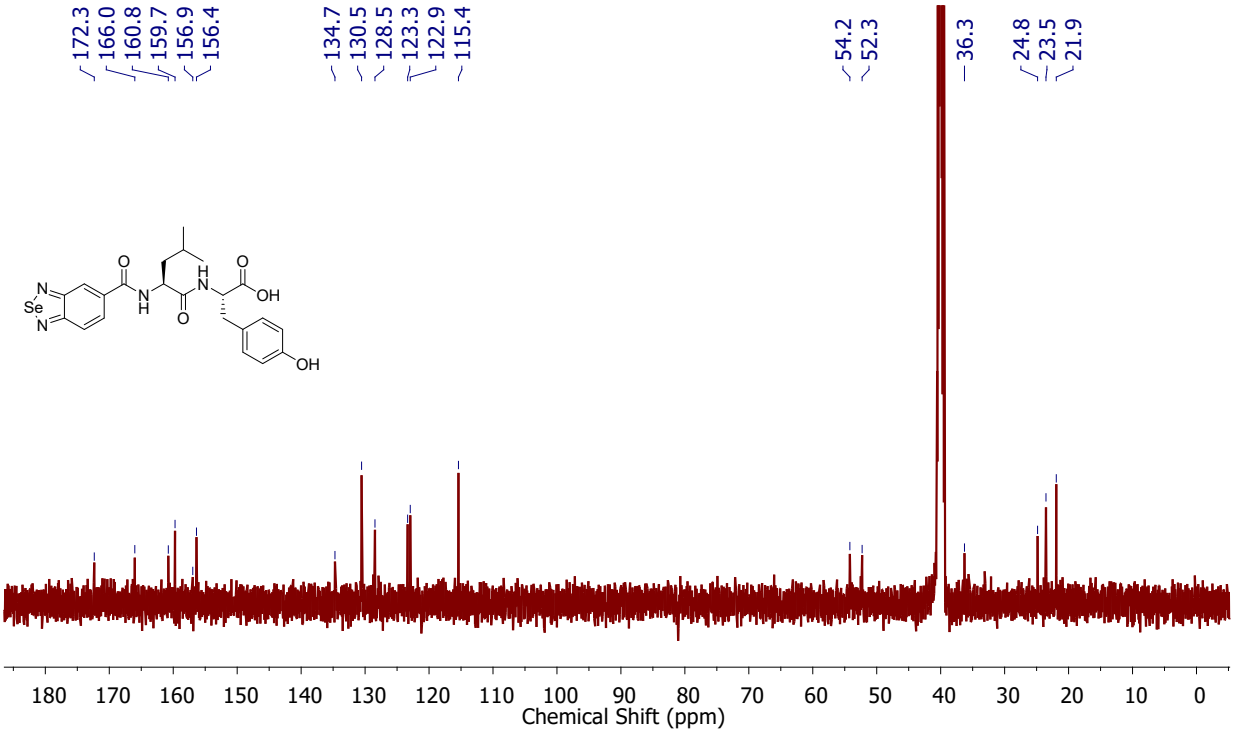


Fig. S24 ^{13}C NMR (DMSO-d_6, 125 MHz) spectrum of BSeLY.

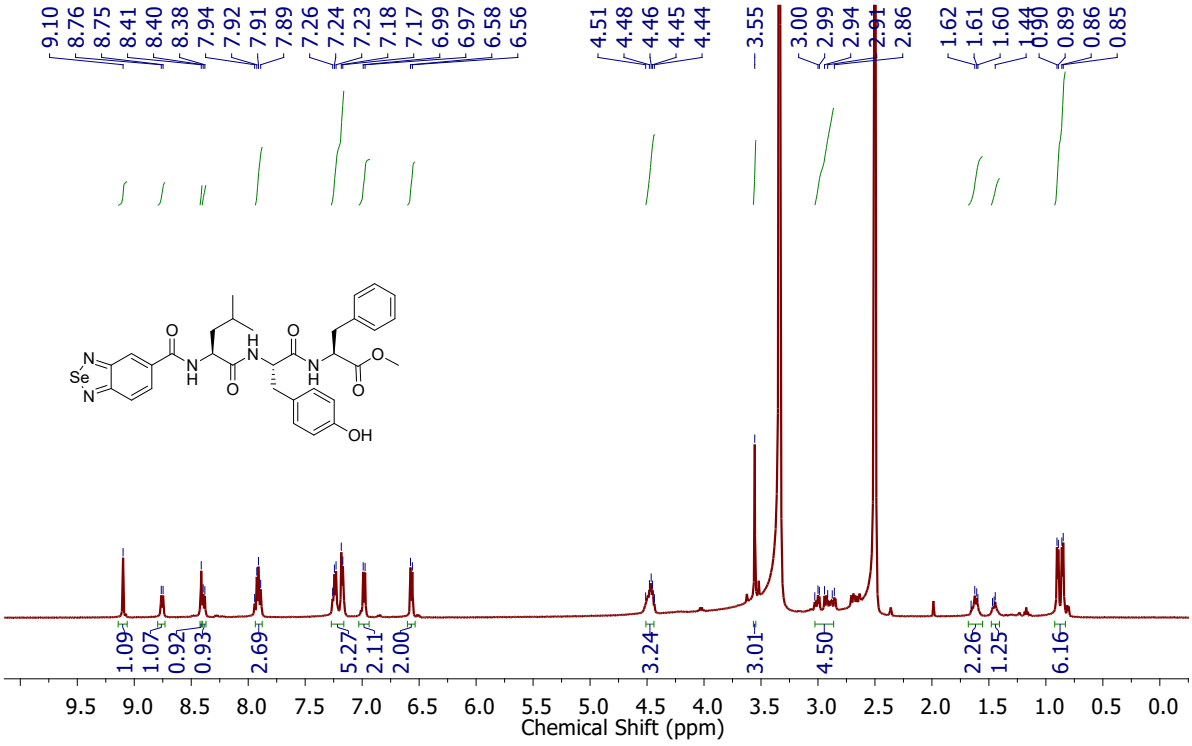


Fig. S25 ^{1}H NMR (DMSO-d_6, 500 MHz) spectrum of BSeLYF-OMe.

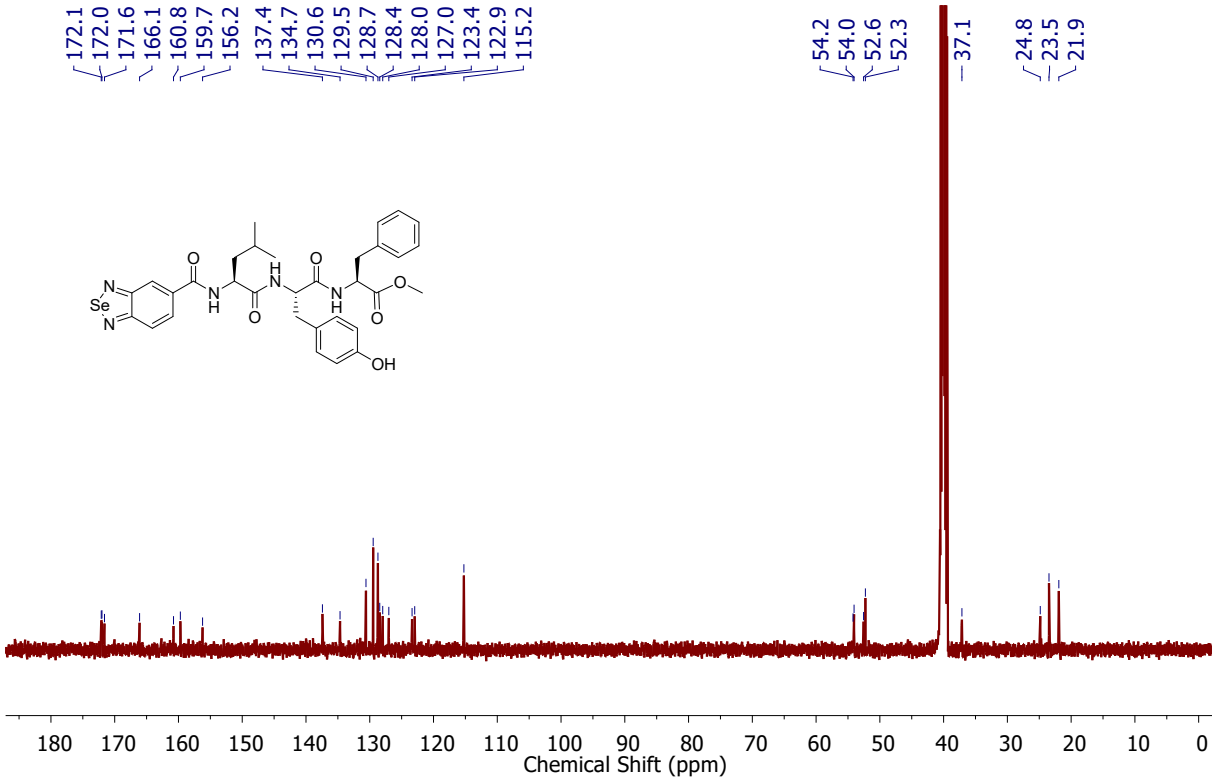


Fig. S26 ^{13}C NMR (DMSO-d_6, 125 MHz) spectrum of BSeLYF-OMe.

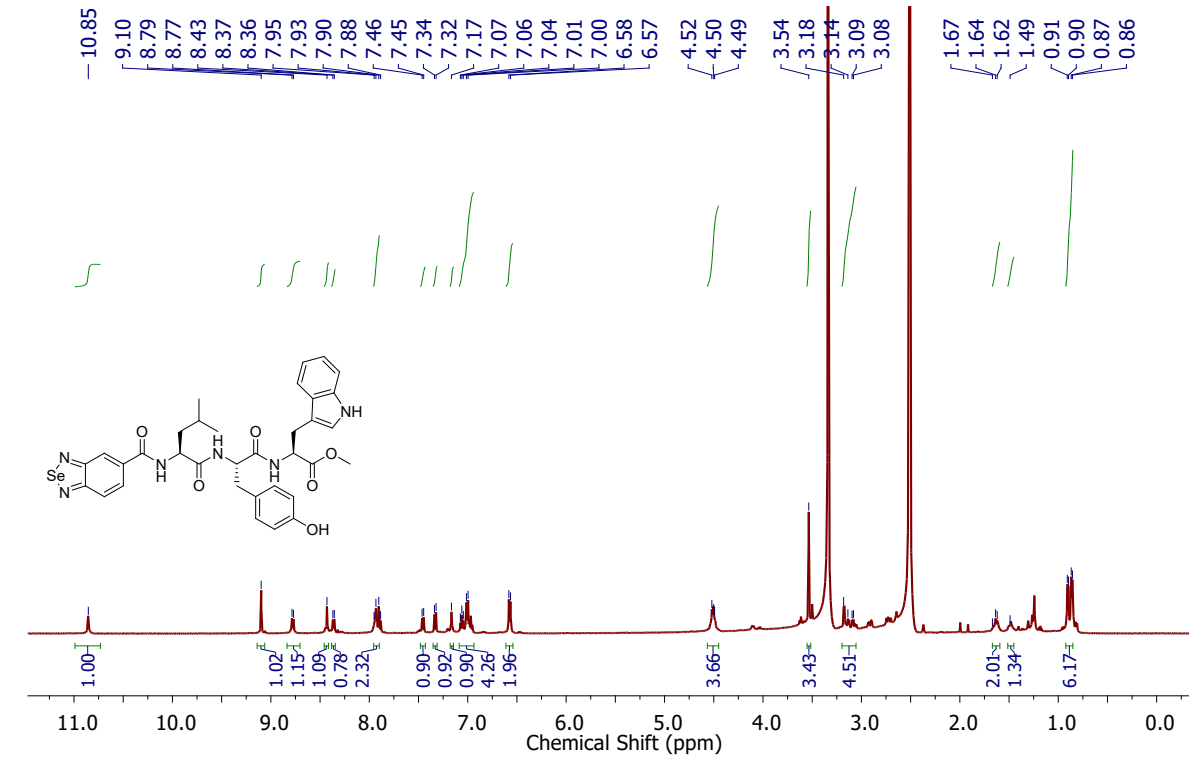


Fig. S27 ^{1}H NMR (DMSO-d_6, 500 MHz) spectrum of BSeLYW-OMe.

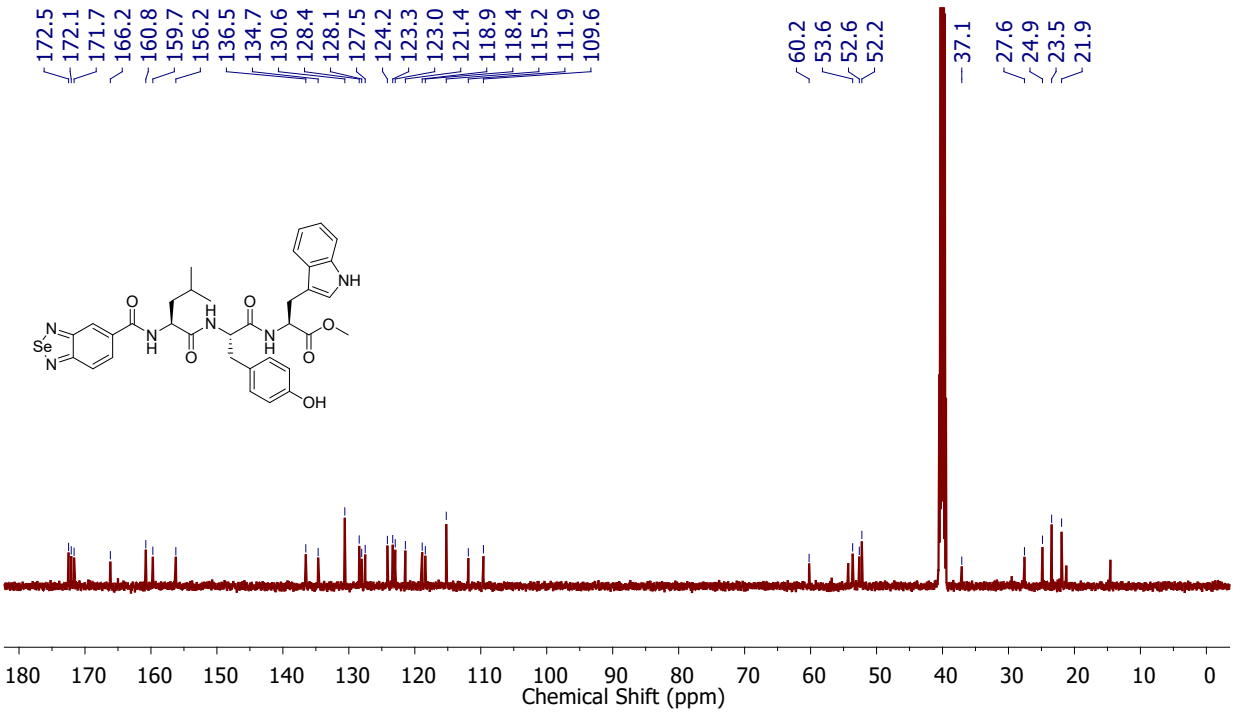


Fig. S28 ^{13}C NMR (DMSO-d_6, 500 MHz) spectrum of BSeLYW-OMe.

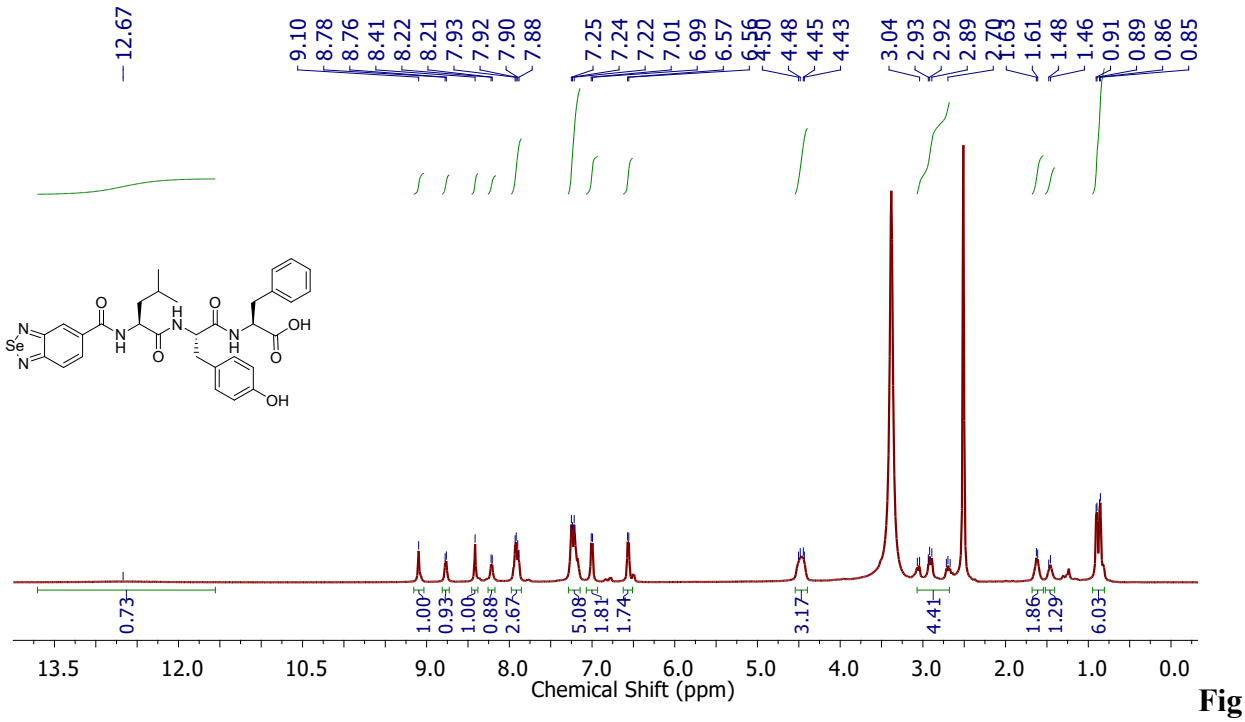


Fig. S29 ^{1}H NMR (DMSO-d_6, 500 MHz) spectrum of BSeLYF.

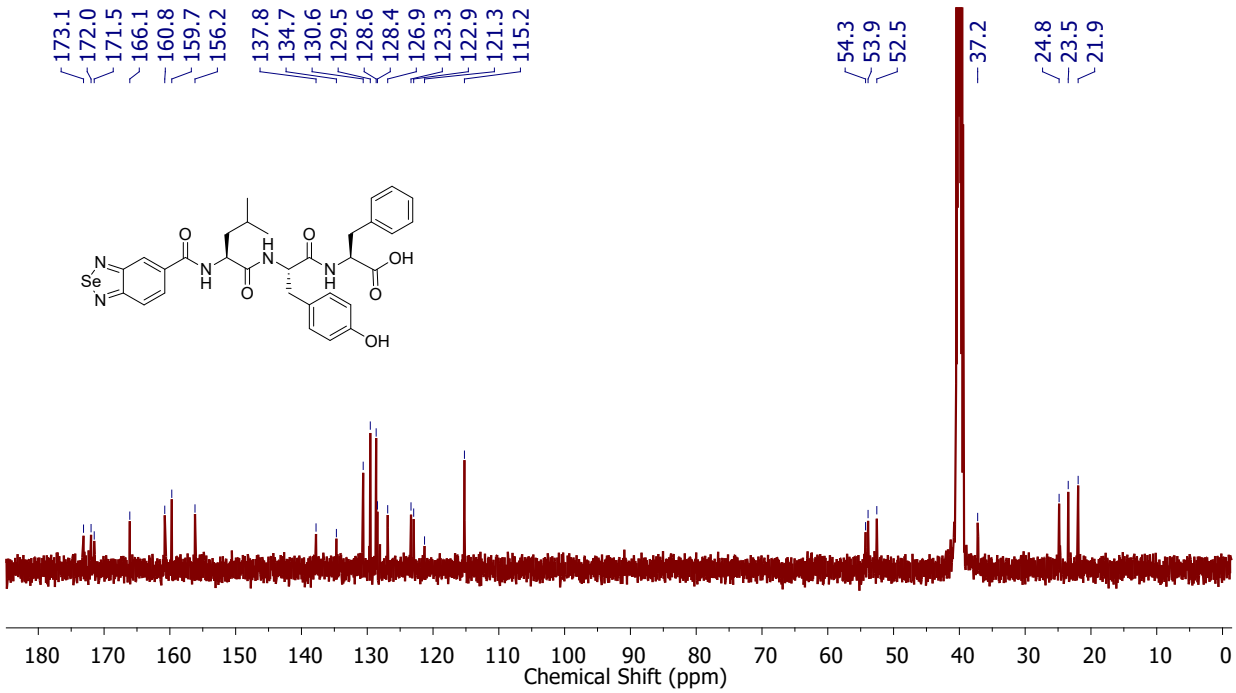


Fig. S30 ^{13}C NMR (DMSO-d_6, 125 MHz) spectrum of BSeLYF.

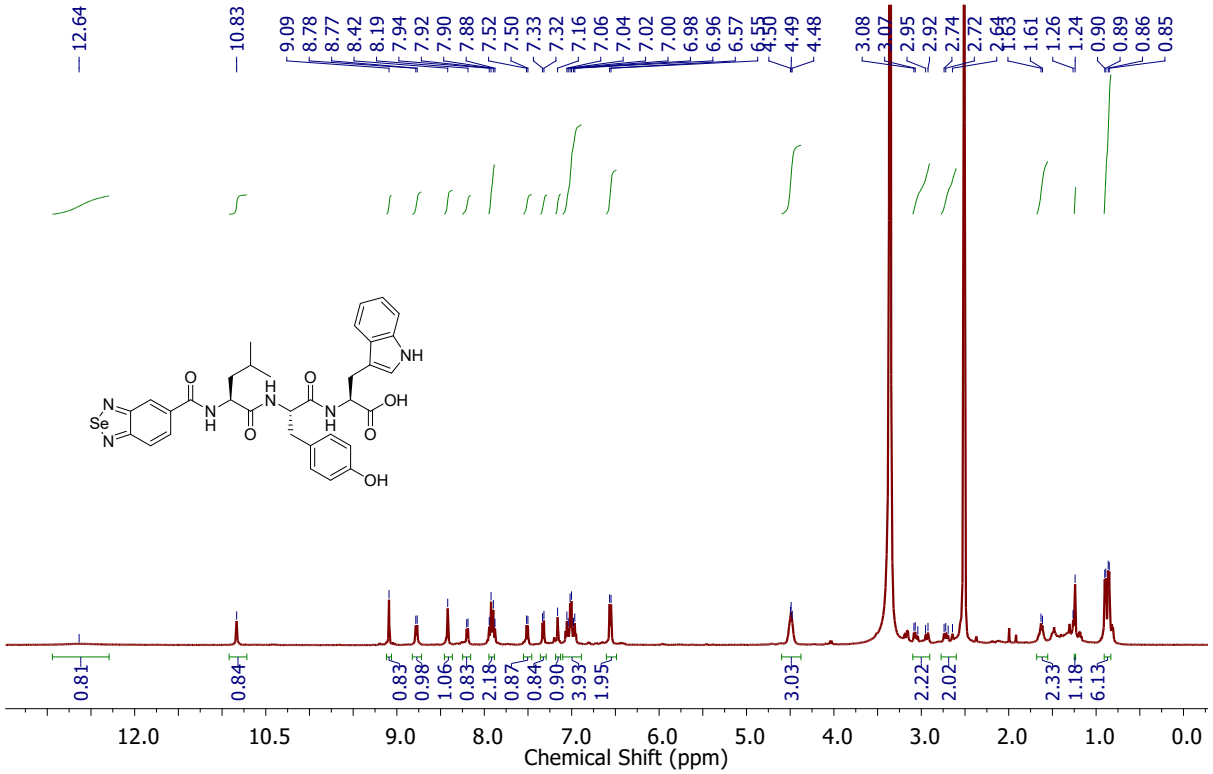


Fig. S31 ^{1}H NMR (DMSO-d_6, 500 MHz) spectrum of BSeLYW.

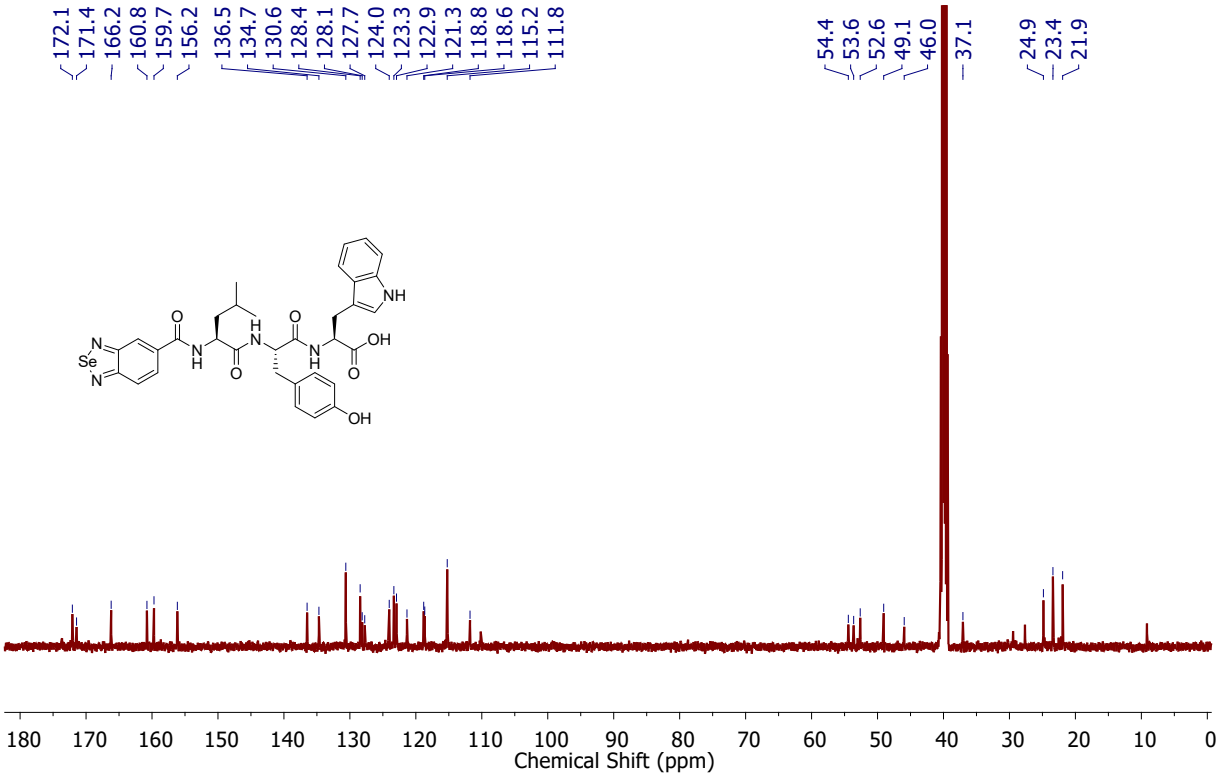


Fig. S32 ^{13}C NMR (DMSO-d_6, 125 MHz) spectrum of BSeLYW.

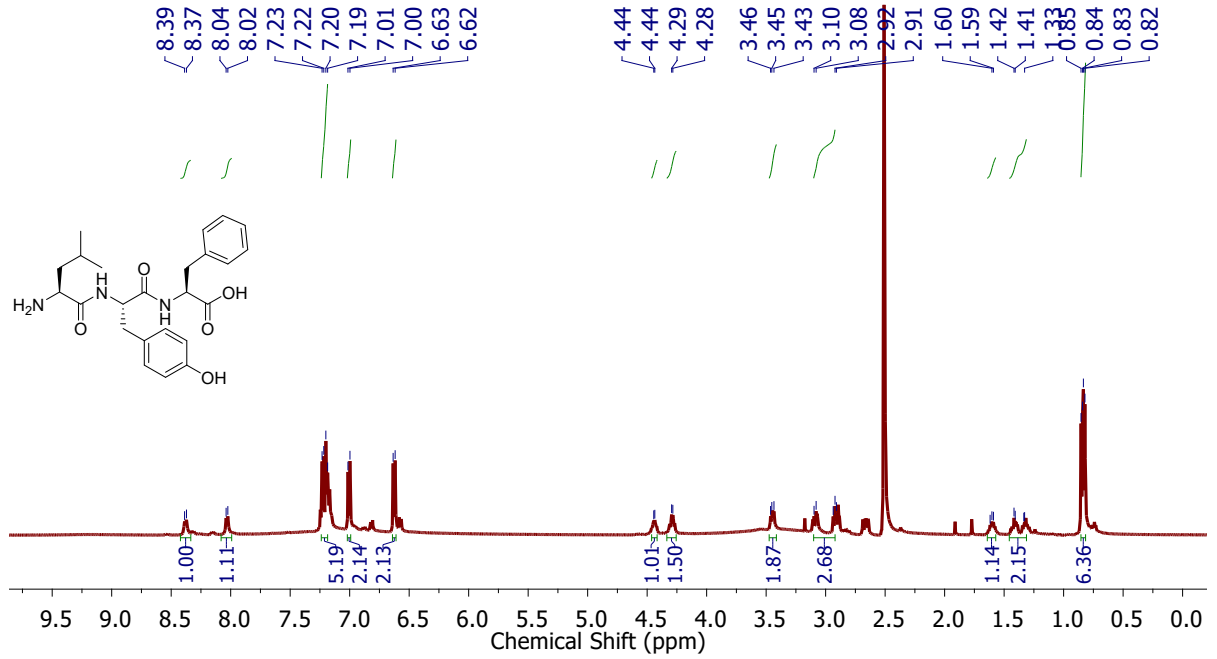


Fig. S33 ^{1}H NMR (DMSO-d_6, 500 MHz) spectrum of LYF.

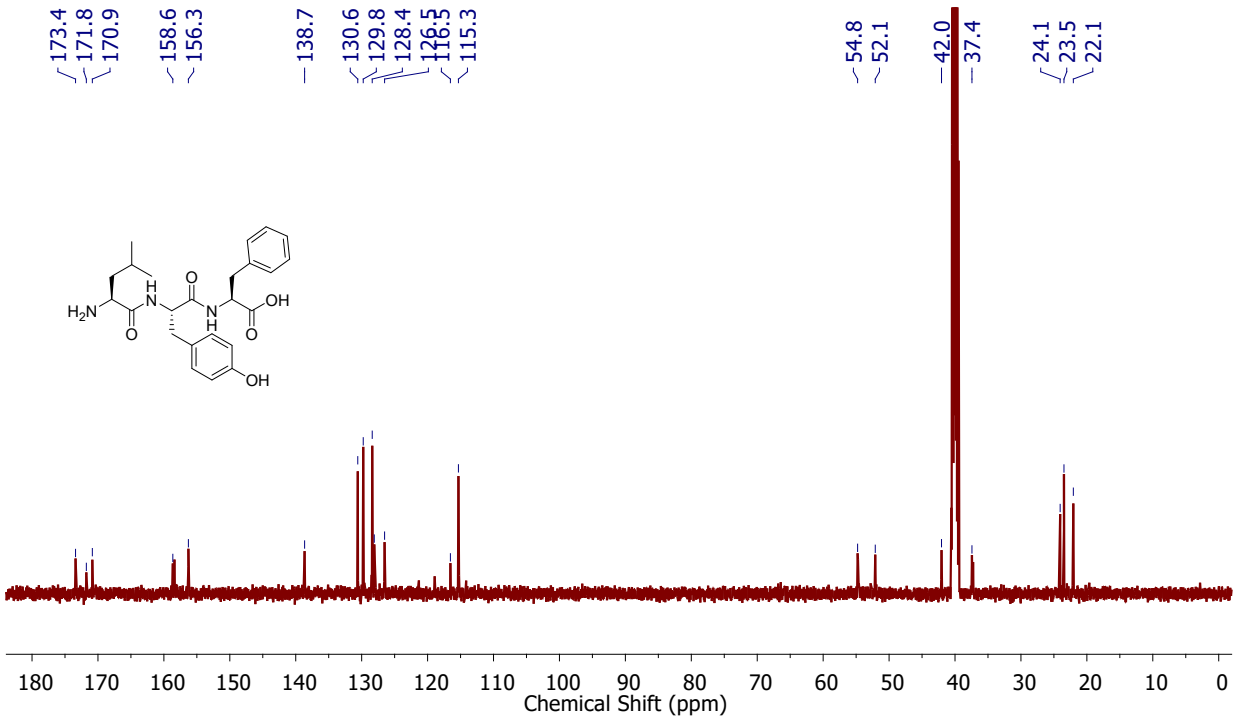


Fig. S34 ^{13}C NMR (DMSO-d_6, 125 MHz) spectrum of LYF.

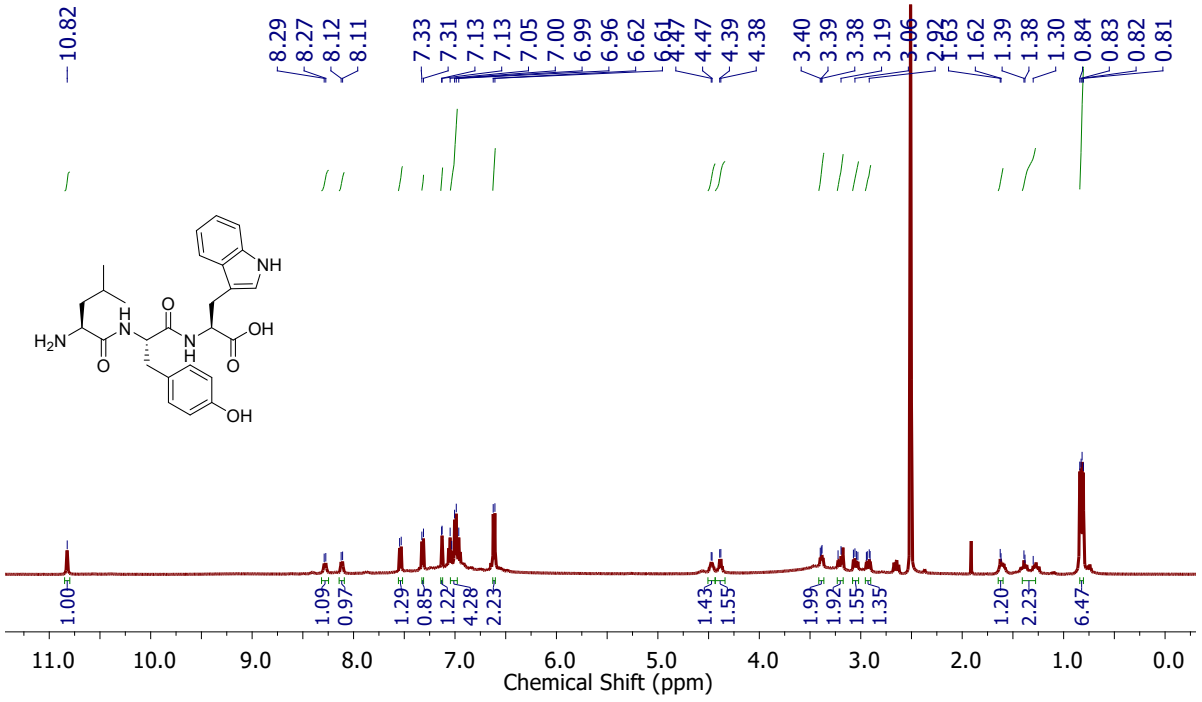


Fig. S35 ^{1}H NMR (DMSO-d_6, 500 MHz) spectrum of LYW.

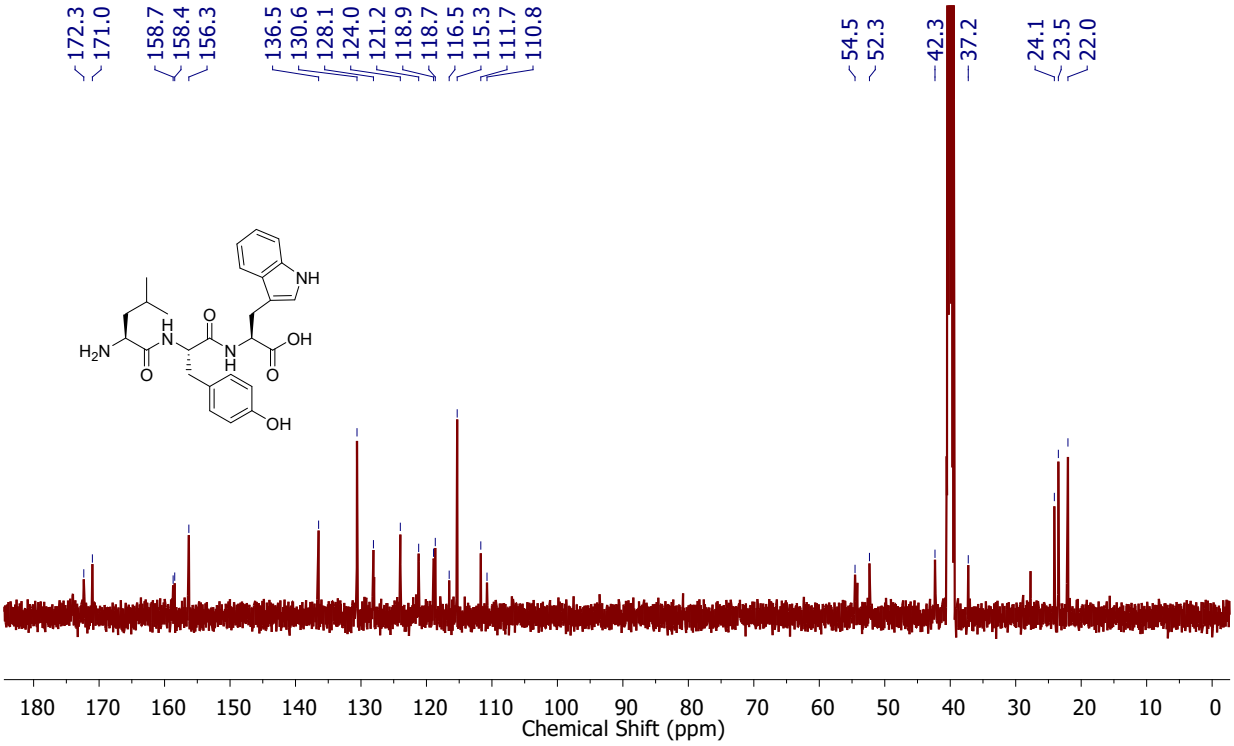


Fig. S36 ^{13}C NMR (DMSO-d_6, 125 MHz) spectrum of LYW.

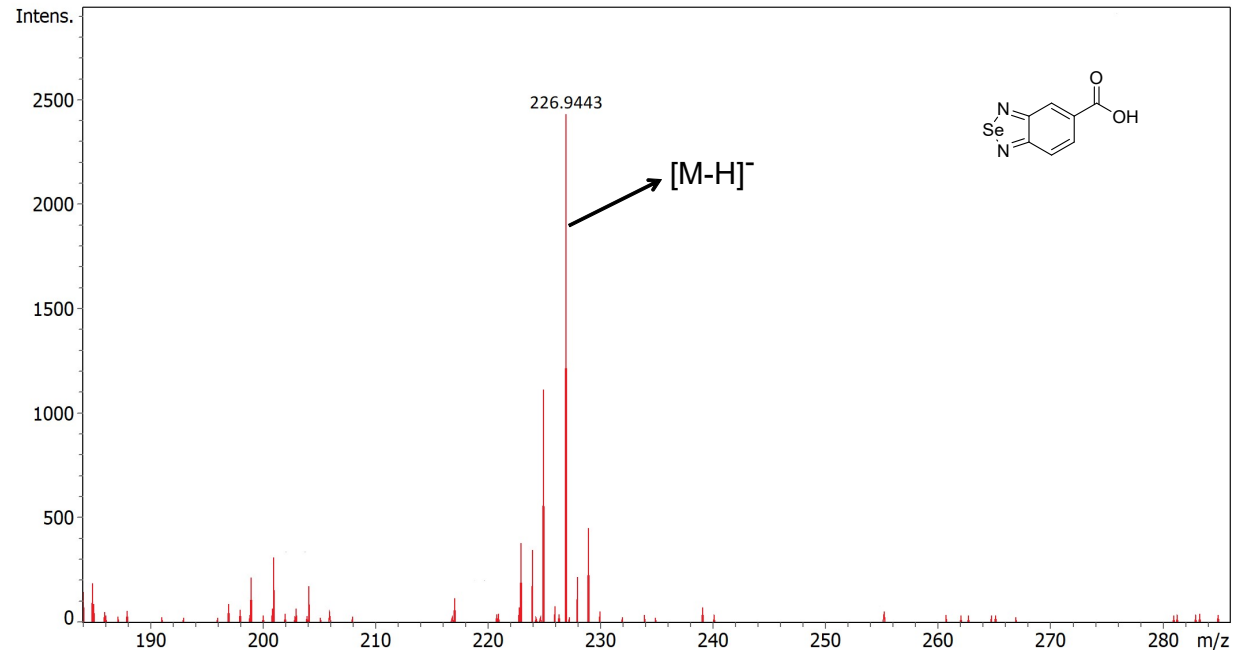


Fig. S37 ESI-MS Mass spectrum of BSe.

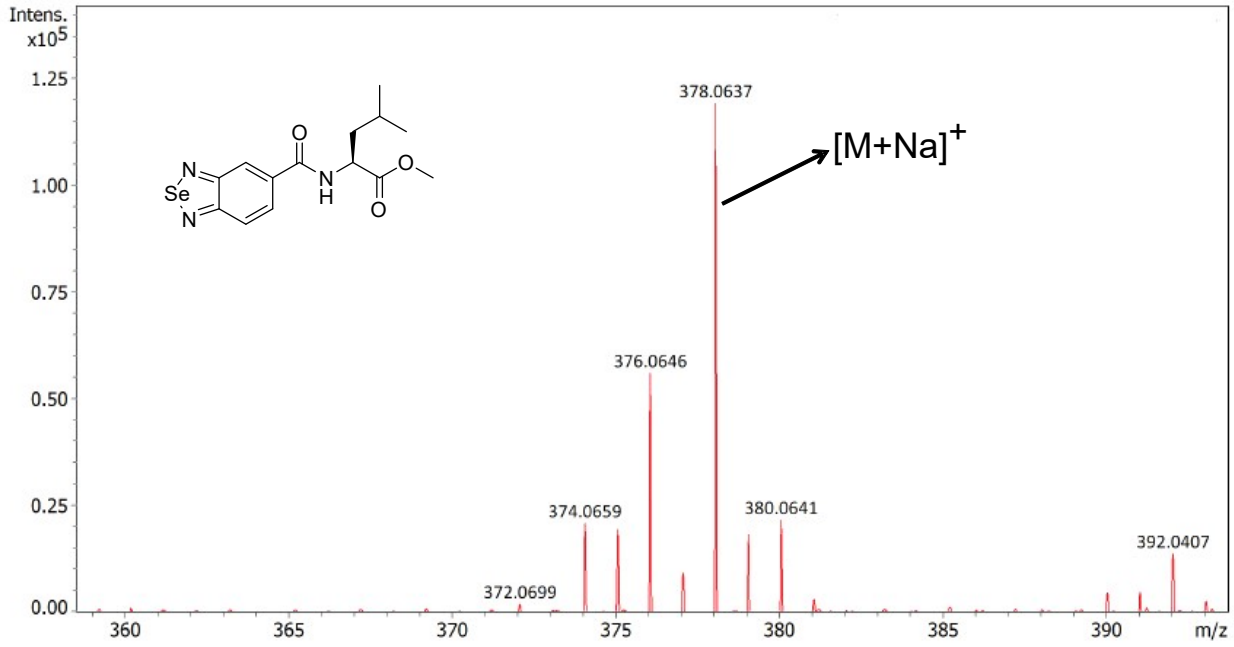


Fig. S38 ESI-MS Mass spectrum of BSeL-OMe.

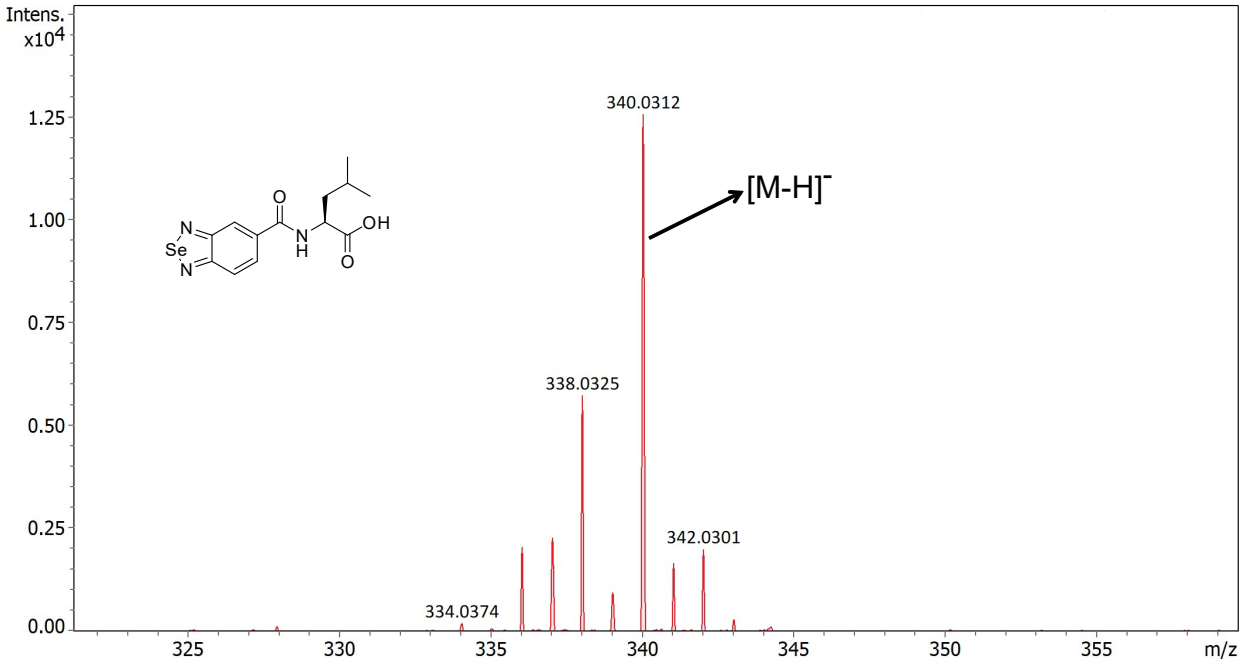


Fig. S39 ESI-MS Mass spectrum of BSeL.

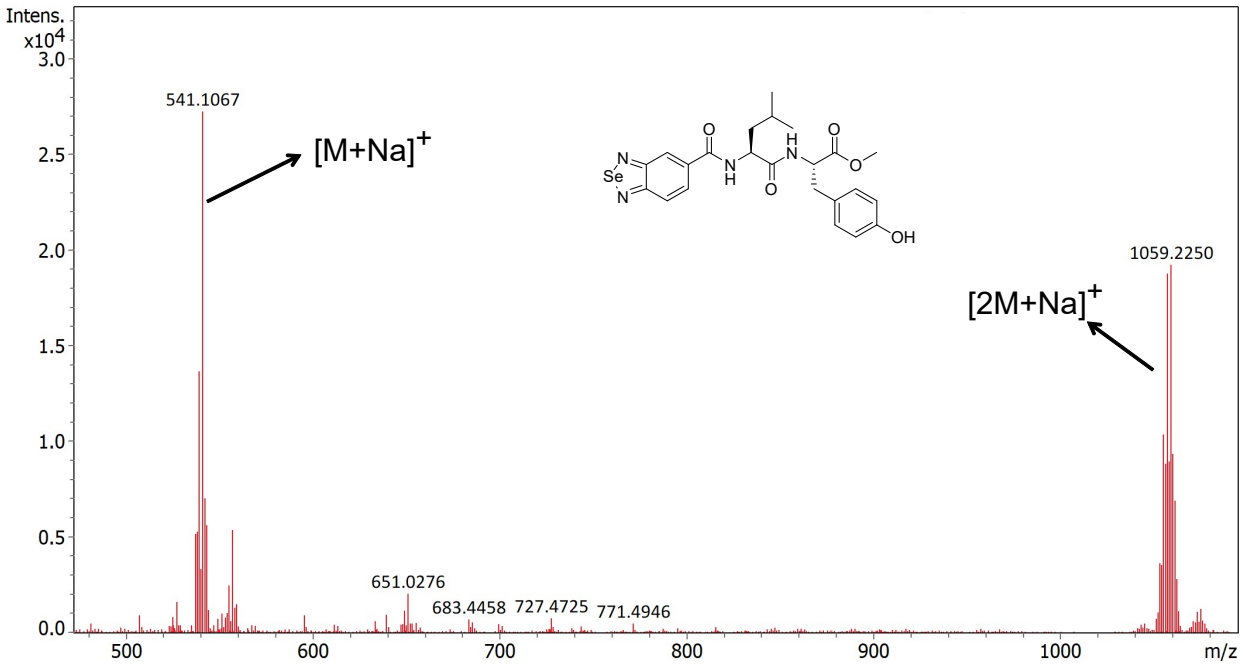


Fig. S40 ESI-MS Mass spectrum of BSeLY-OMe.

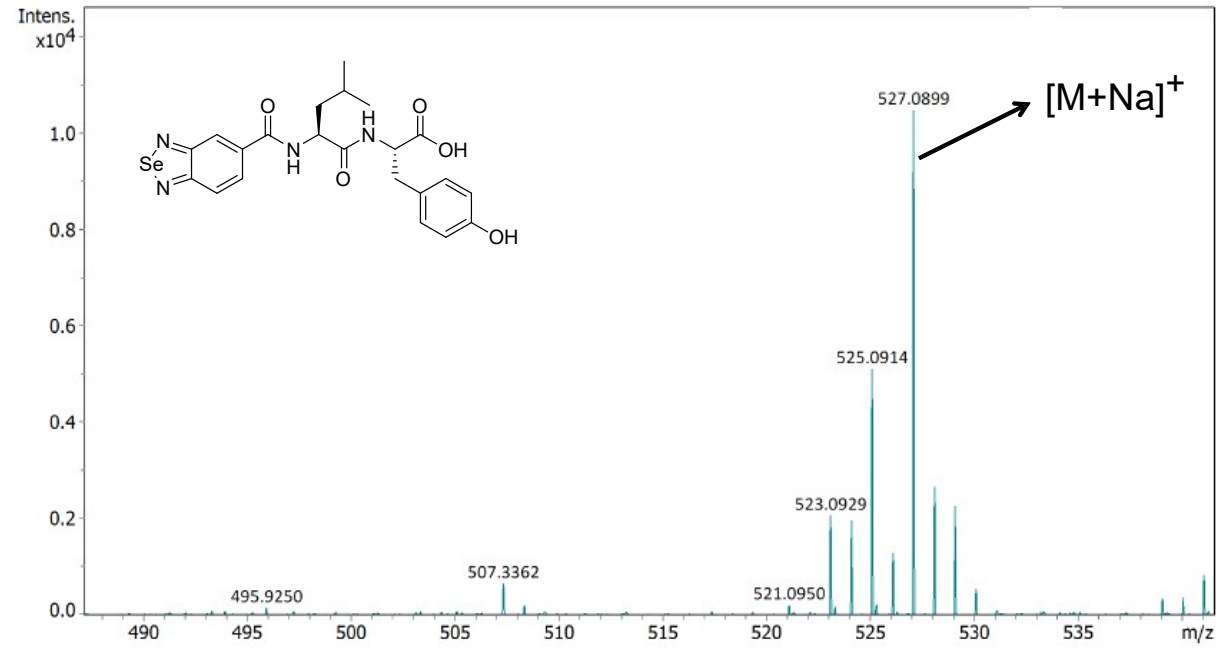


Fig. S41 ESI-MS Mass spectrum of BSeLY.

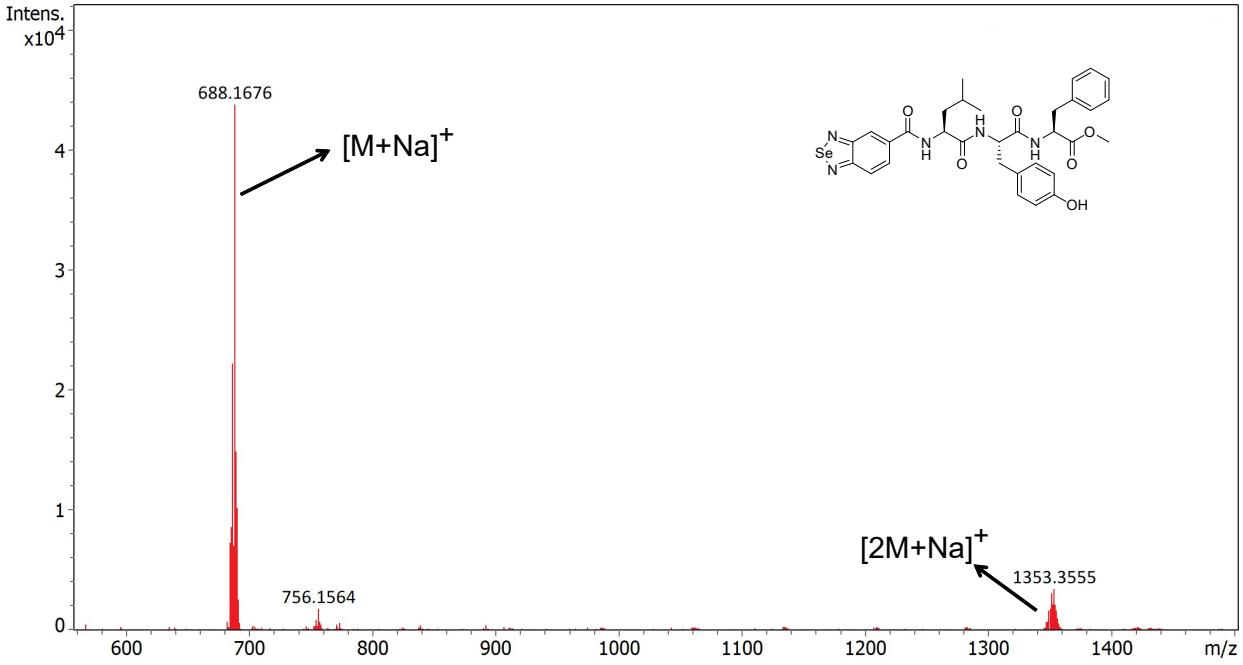


Fig. S42 ESI-MS Mass spectrum of BSeLYF-OMe.

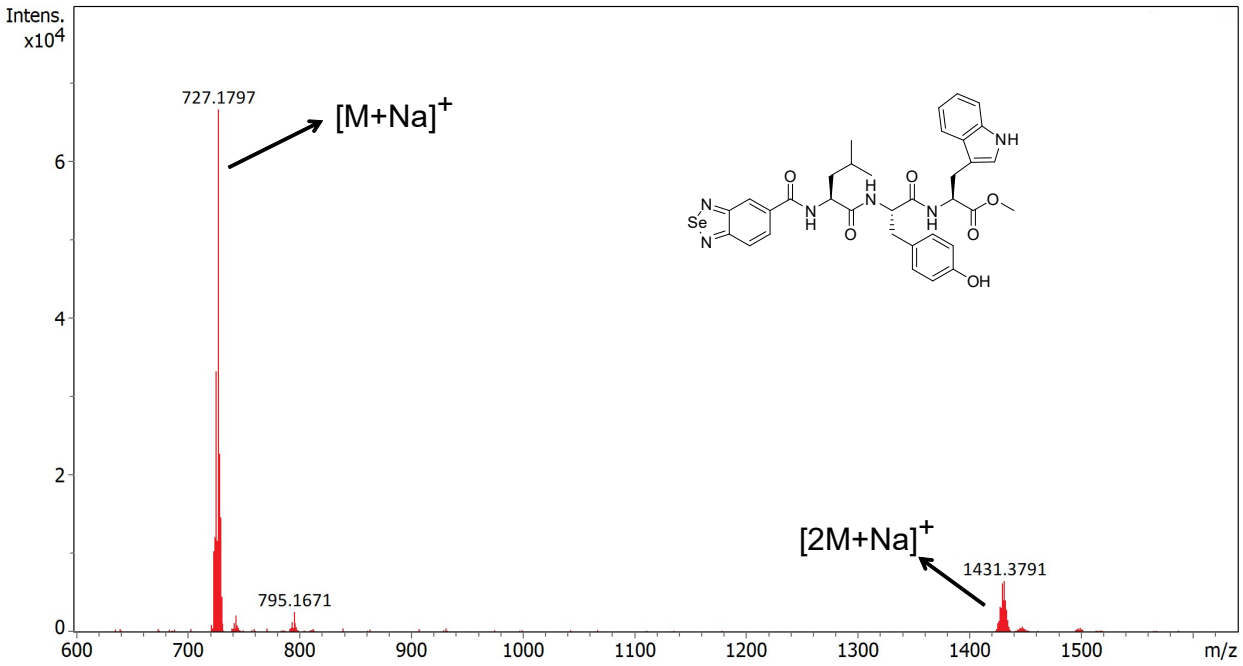


Fig. S43 ESI-MS Mass spectrum of BSeLYW-OMe.

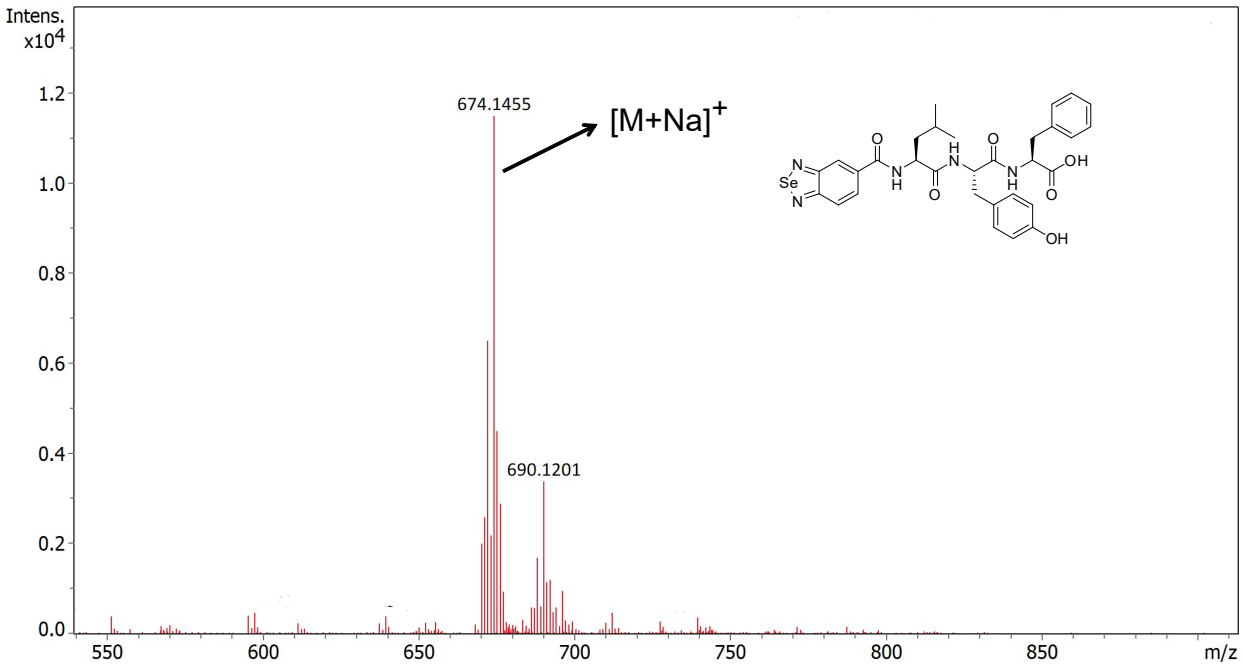


Fig. S44 ESI-MS Mass spectrum of BSeLYF.

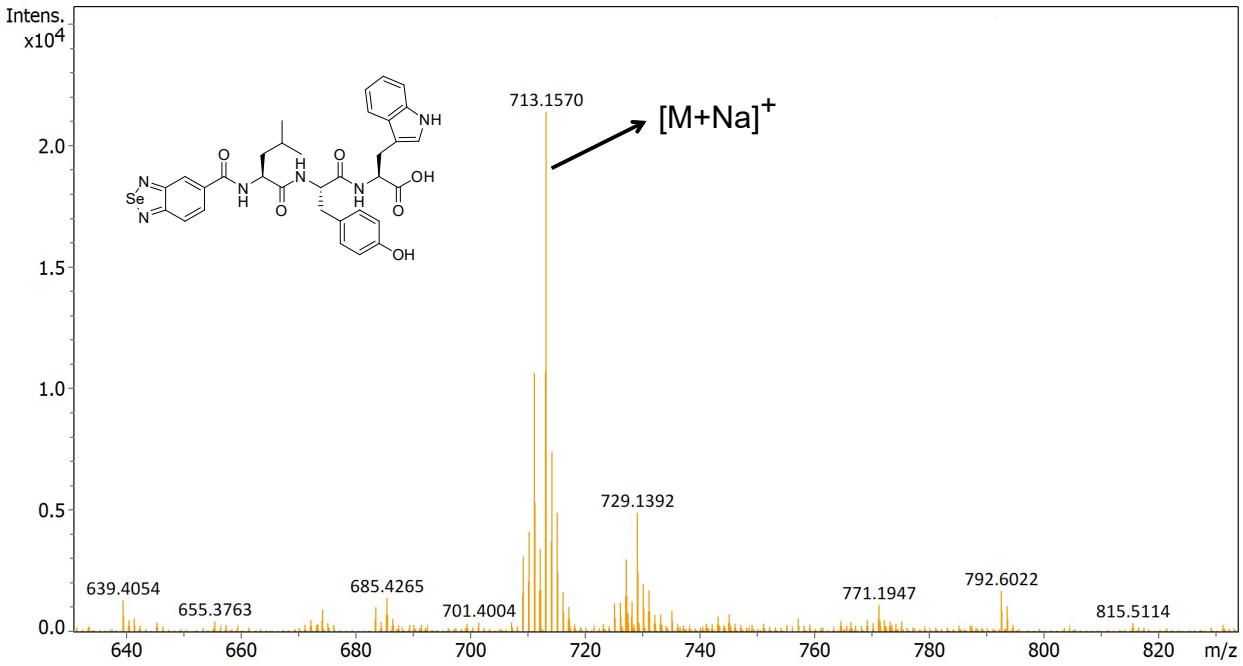


Fig. S45 ESI-MS Mass spectrum of BSeLYW.

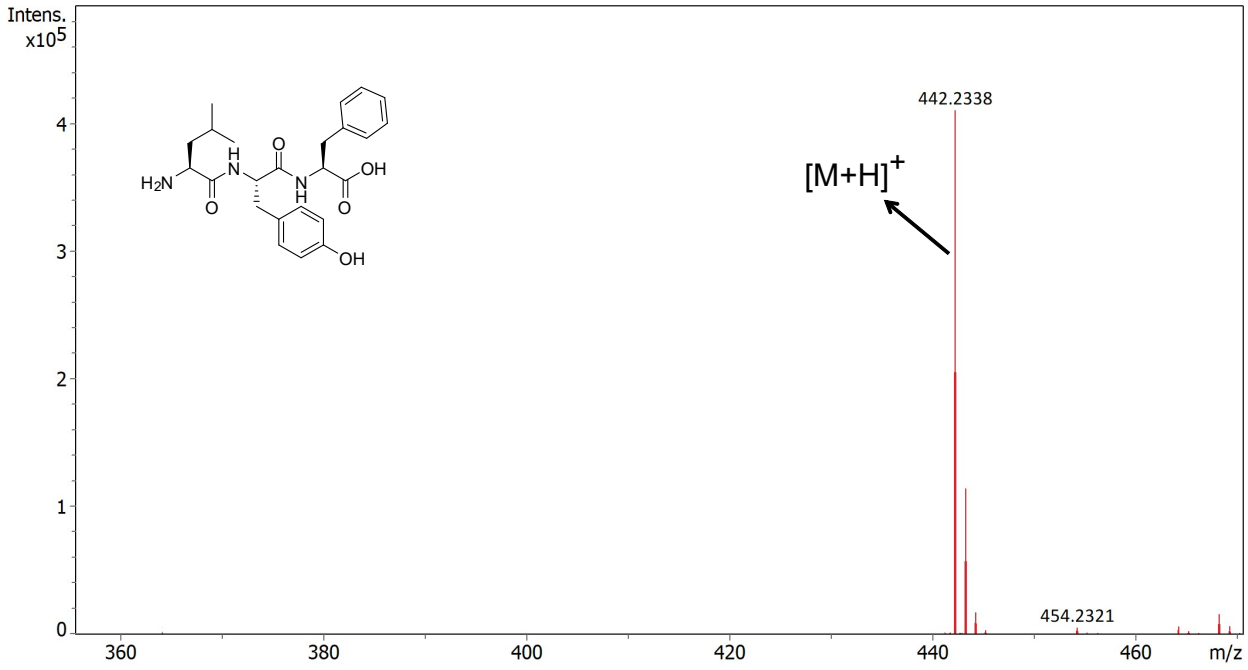


Fig. S46 ESI-MS Mass spectrum of LYF.

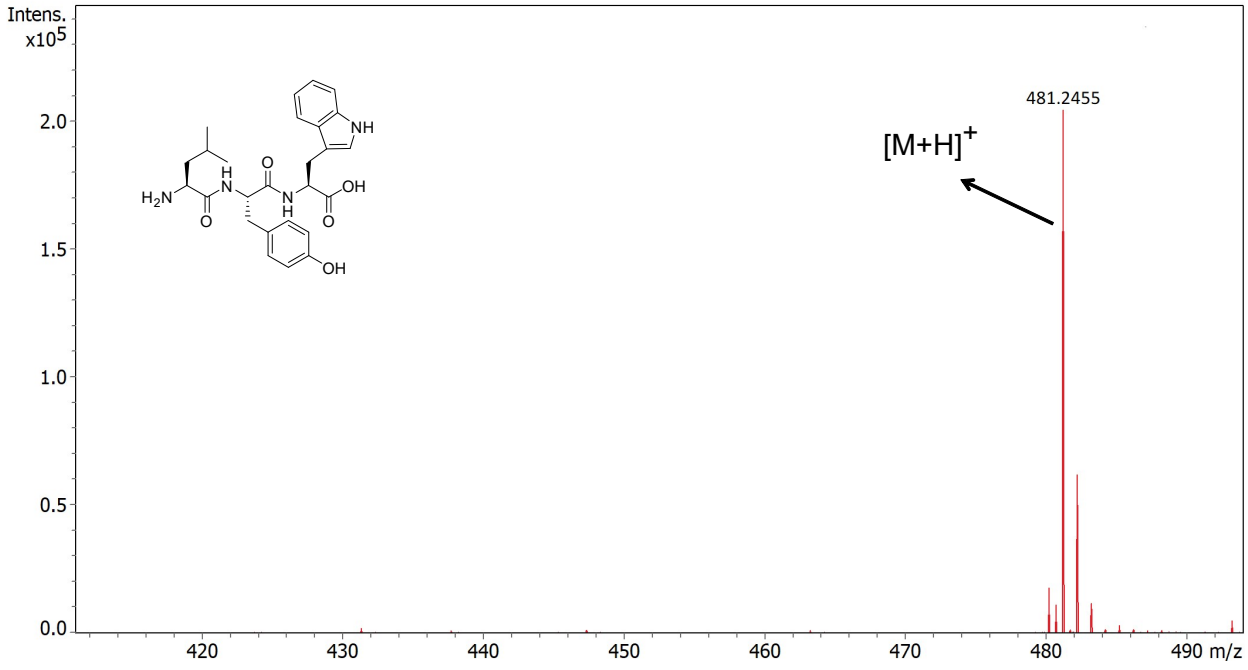


Fig. S47 ESI-MS Mass spectrum of LYW

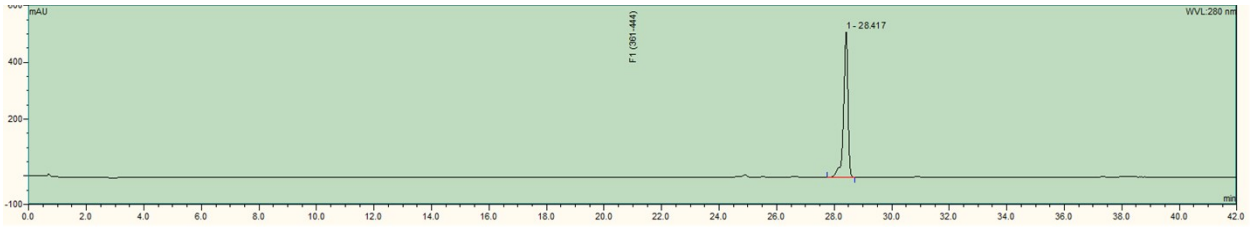


Fig. S48 HPLC chromatogram of synthesized BSeLYF tripeptide.

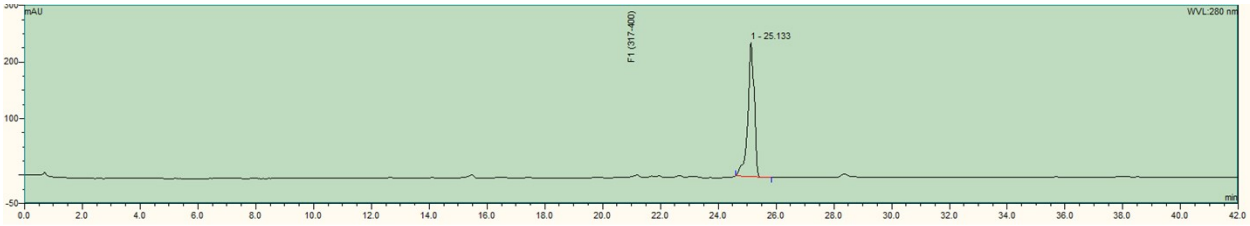


Fig. S49 HPLC chromatogram of synthesized BSeLYW tripeptide.

References

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3. The hydrophobicity (log P) values of tripeptides were calculated by using online prediction program. http://www.molinspiration.com.

4. B. Adhikari, G. Palui, and A. Banerjee, *Soft Matter,* 2009*,* **5***,* 3452-3460.