Supporting Information

Cyclic Regulation of Sulfilimine Bond in Peptides and NC 1 Hexamers *via* HOBr/H_2Se Conjugated System

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1. Experimental Section

MTT cytotoxicity assay

HepG2 cells (20,000 cell/mL) were evaluated within replicate 96-well microtiter plates to a total volume of 200 μL/well. Plates were maintained at 37 °C in a 5% CO_2/95% air incubator. HepG2 cells were then incubated for 24 h upon different concentrations of the probes (0, 50, 100, 200, 300, 400 and 500 μM), respectively. Then the culture media were removed and MTT solution (5 mg/mL) was added to each well with 200 μL for 4 h at 37°C. After 4 h, the remaining MTT solution was removed, and 100 μL of DMSO was added to each well to dissolve the formazan crystals. After gently shaken for 10 min, absorbance was measured at 490 nm using a microliter plate reader.

The cell viability rate (VR) was calculated according to the following formula: VR = A490 (sample)/A490 (control) × 100%. The cell survival rate from the control group was considered to be 100%. Calculation of IC_{50} values is 582.04 μM according to Huber and Koella, which clearly demonstrated that the probe was of low toxicity to cultured cell lines under the experimental conditions.

2. Supplementary Figures

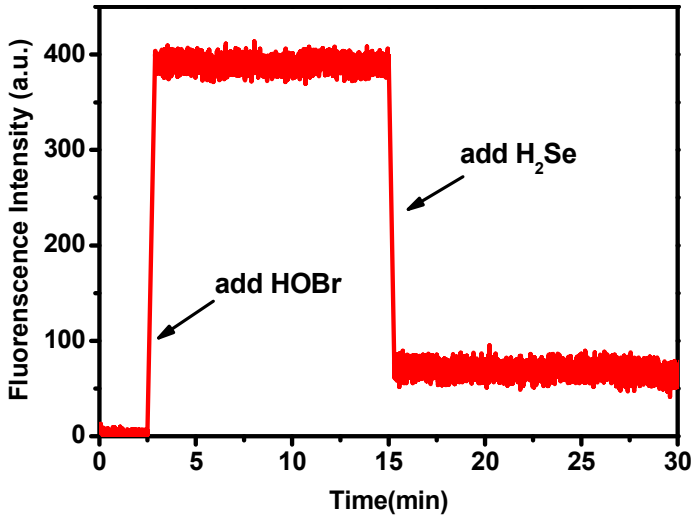

Figure S1. Time-dependent fluorescence intensities changes recorded at $\lambda_{ex}/\lambda_{em}$ = 480/525 nm for treatment of BPP (5 μM) with HOBr (20 μM) and cBPP (5 μM BPP reacted with 20 μM HOBr) with H_2Se (100 μM), respectively. Data was acquired in 10 mM PBS, pH 7.4, at room temperature. Slit widths: 10/10 nm.

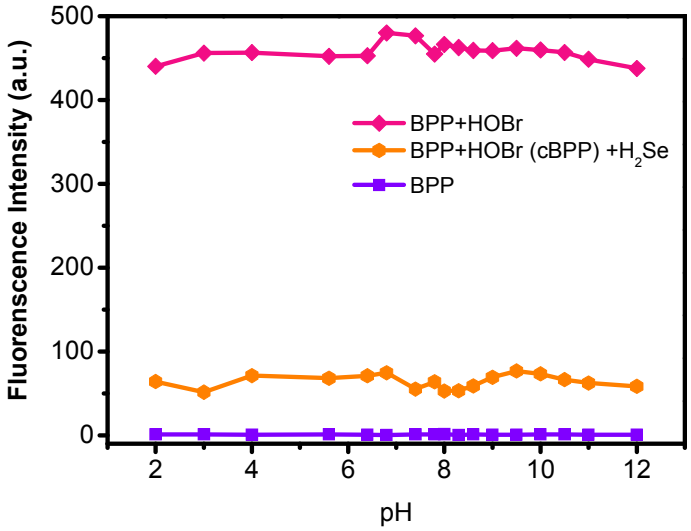

Figure S2. Fluorescence intensity changes of 5 μM BPP (purple line) at different pH values in the presence of 20 μM HOBr (pink line), 20 μM HOBr plus 100 μM H_2Se (orange line). Data was acquired in 10 mM PBS, pH 7.4, at room temperature. Slit widths: 10/10 nm. $\lambda_{ex}/\lambda_{em}$ = 480/525 nm.

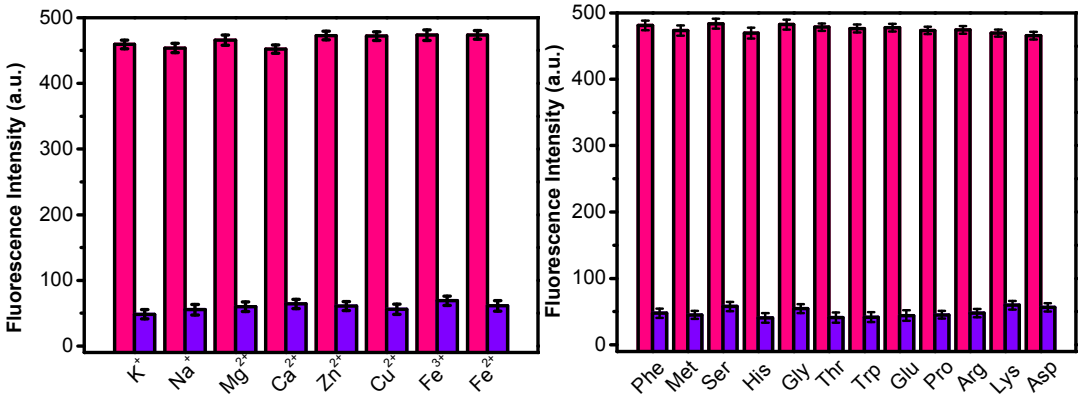

Figure S3. Fluorescent intensities of cBPP (5 μM BPP reacted with 20 μM HOBr) to metal ions and amino acids (pink bars). 100 μM H_2Se were added into the reaction mixture afterwards (violet bars): (left) Zn^{2+}, $Fe^{2+}$and Fe^{3+} (200 μM); K^+, Na^+, Mg^{2+}, Ca^{2+} and Cu^{2+} (1 mM); (right) phenylalanine (Phe), methionine (Met), serine (Ser), histidine (His), glycine (Gly), tyrosine (Thr), tryptophan (Trp), glutamic acid (Glu), proline (Pro), arginine (Arg) Lysine (Lys) and aspartic acid (Asp) (1 mM). Spectra were acquired in 10 mM phosphate buffer, pH 7.4 at room temperature. $\lambda_{ex}/\lambda_{em}$ = 480/525 nm.

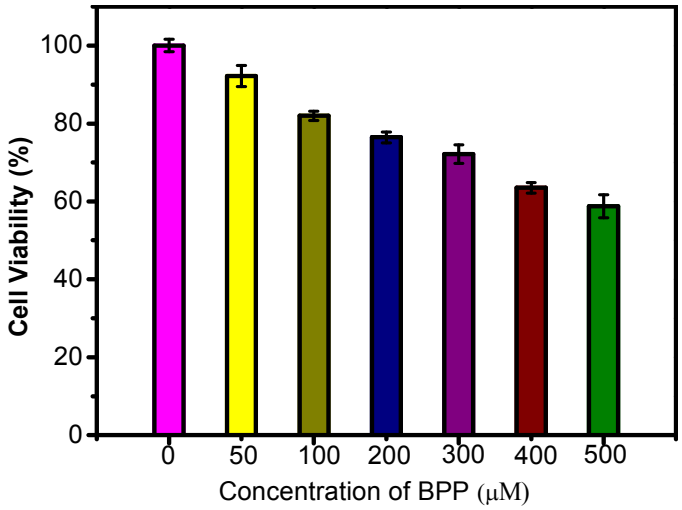

Figure S4. MTT assays of the HepG2 cells' viability upon incubation with different concentrations of BPP.

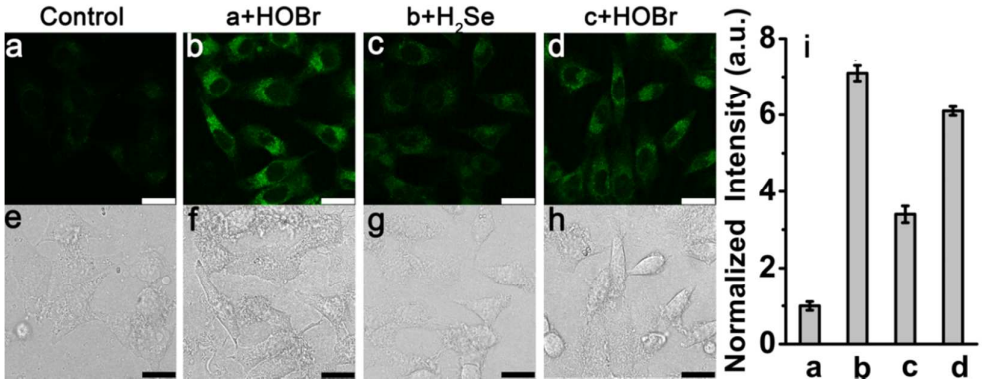

Figure S5. Fluorescence confocal microscopic images of HepG2 cells loaded with 25 μM BPP and exposed to cycles between HOBr and H_2Se in different visual fields. (a) HepG2 cells were incubated with 25 μM BPP under 37 °C for 30 min in DMEM; (b) Cells were treated with 50 μM HOBr for 30 min; (c) Cells incubated with 100 μM H_2Se for 30 min; (d) Cells were treated with another 50 μM HOBr for 30 min. (e-h) The bright-field images were corresponding to (a-d). (i) Graph showing quantification of mean fluorescence intensities in (a–d) correspondingly. Scale bars = 25 μm. Fluorescence images were acquired using a confocal microscope with 488 nm excitation and 500–600 nm collection.

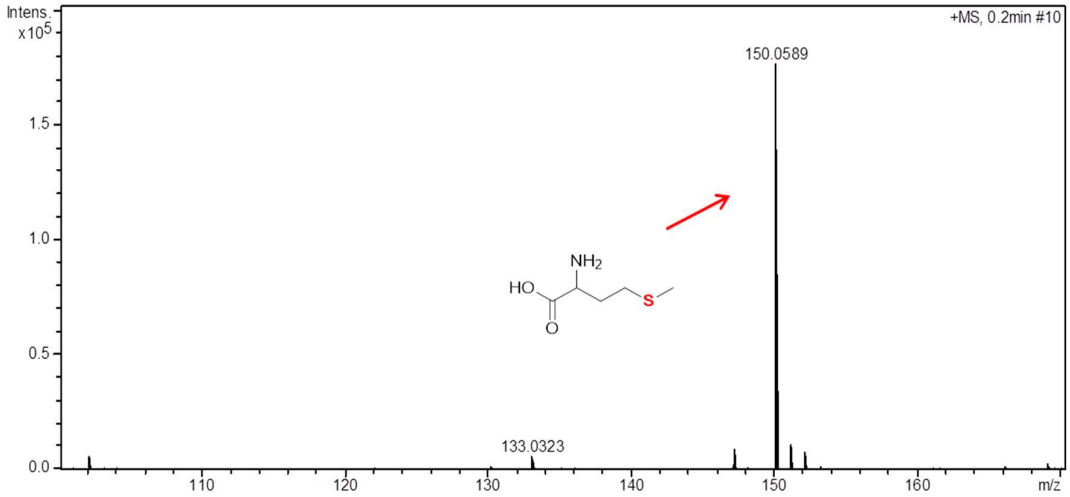

Figure S6. HRMS spectrum for DL-2-Amino-4-(methylthio) butyric acid (DL-Met)

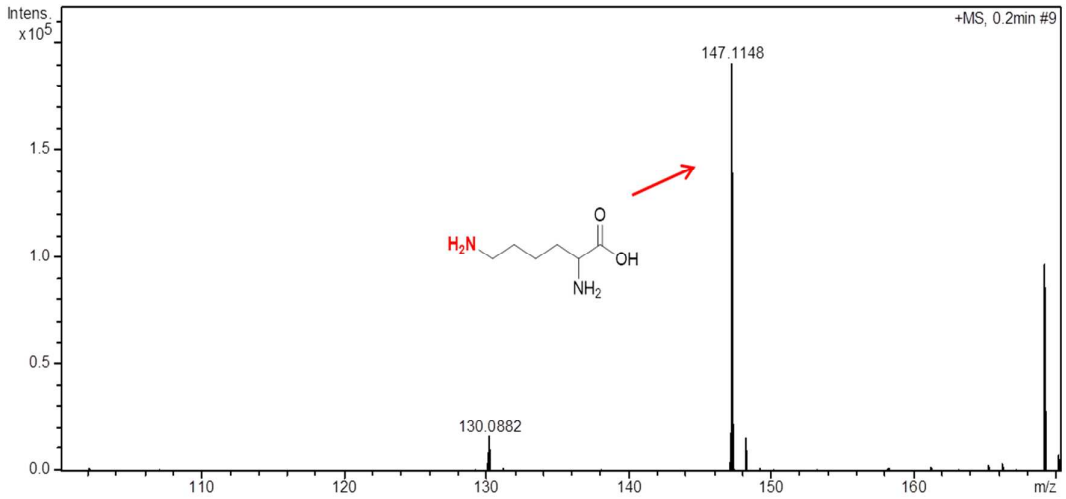

Figure S7. HRMS spectrum for L-2,6-Diaminohexanoic acid (L-Lys).

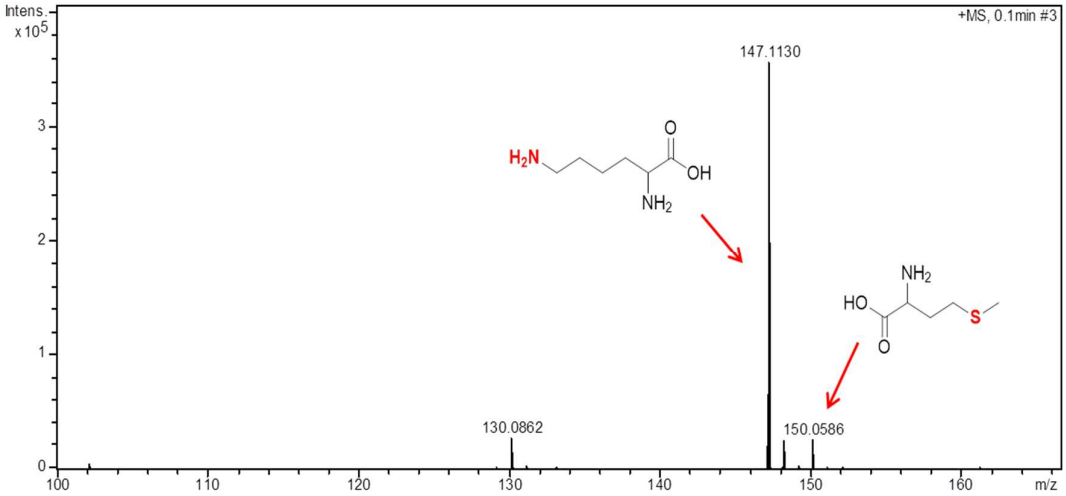

Figure S8. HRMS spectrum for the mixture of 1 mM DL-Met and 1 mM L-Lys.

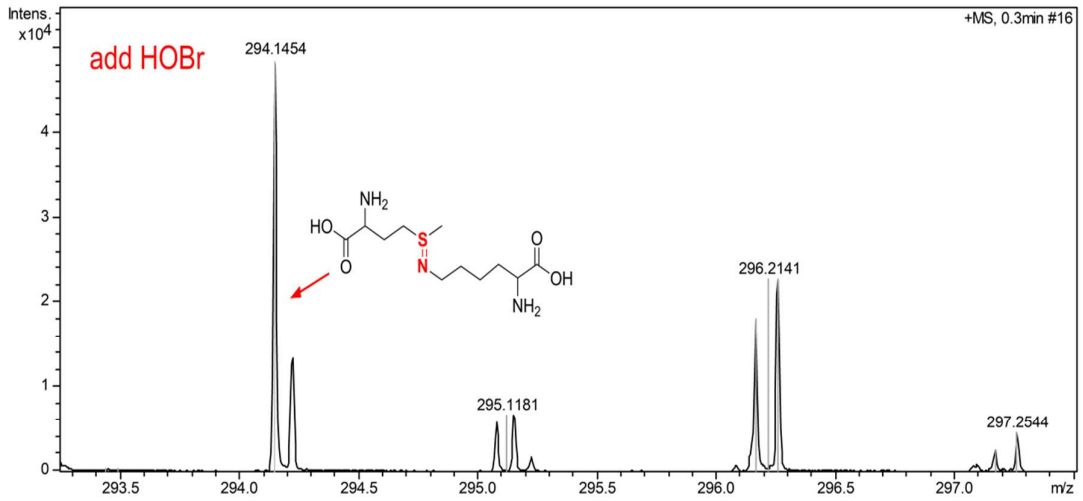

Figure S9. HRMS spectrum for the sulfilimine-cross-linked peptide by adding HOBr again.

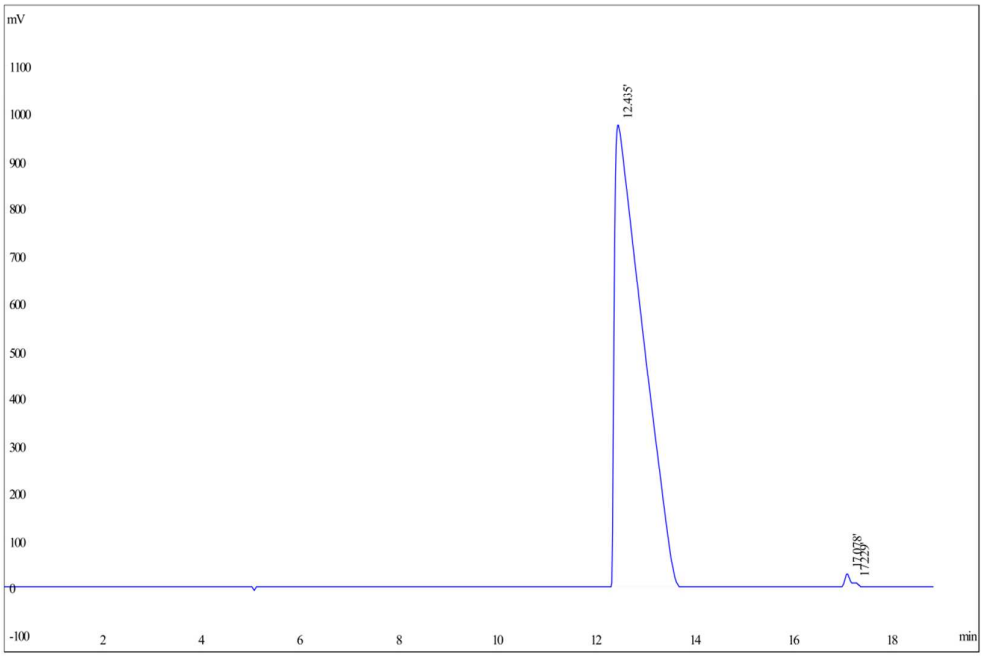

Figure S10. HPLC spectrum of the dipeptide Met-Lys.

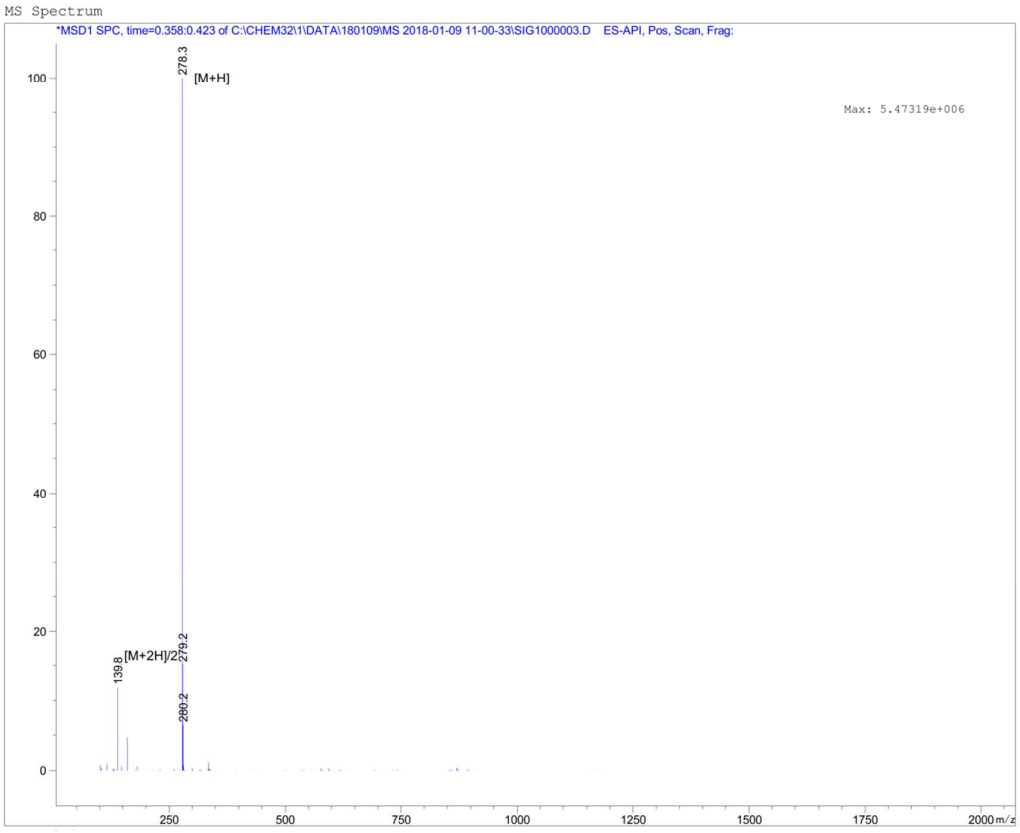

Figure S11. ESI-MS spectrum of the dipeptide Met-Lys.

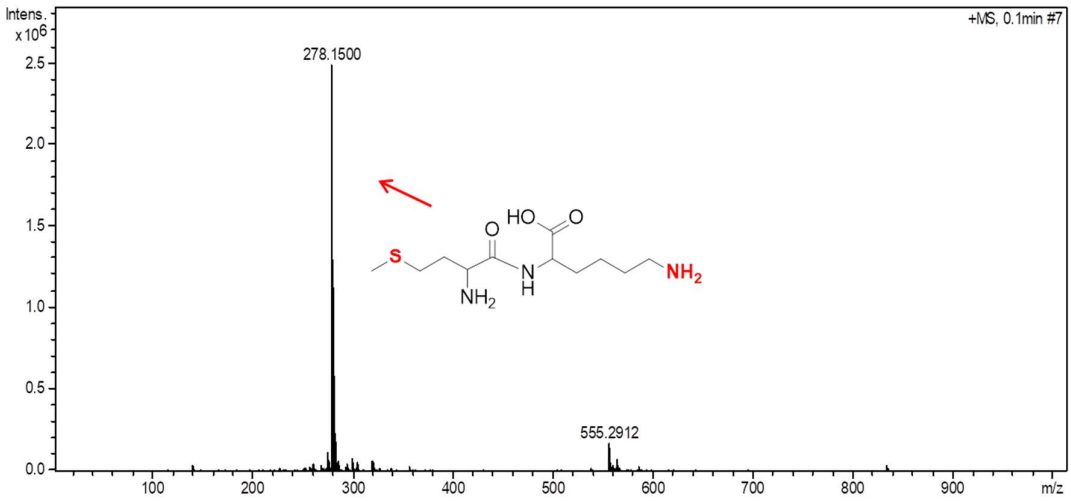

Figure S12. HRMS spectrum of the dipeptide Met-Lys.

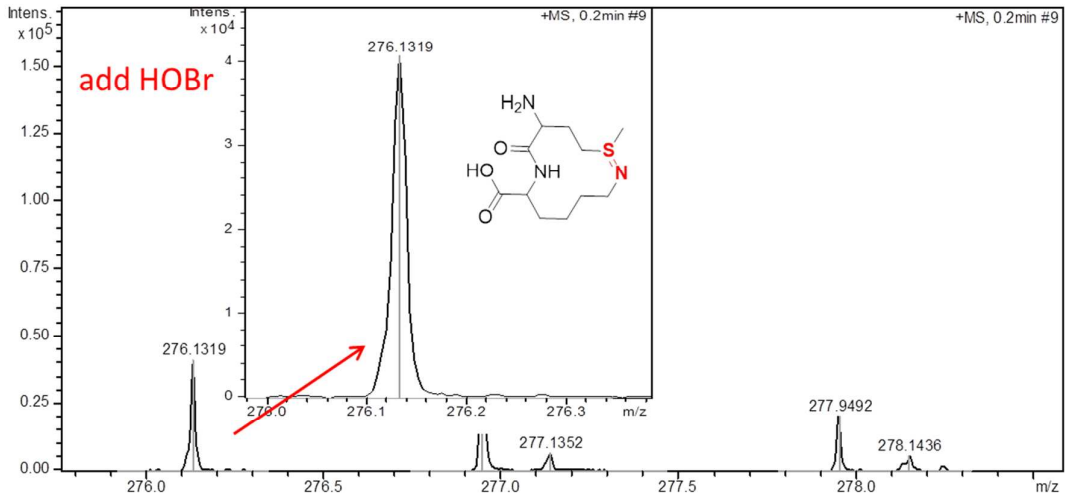

Figure S13. HRMS spectrum of the sulfilimine-cross-linked dipeptide Met-Lys by adding 2 mM HOBr again.

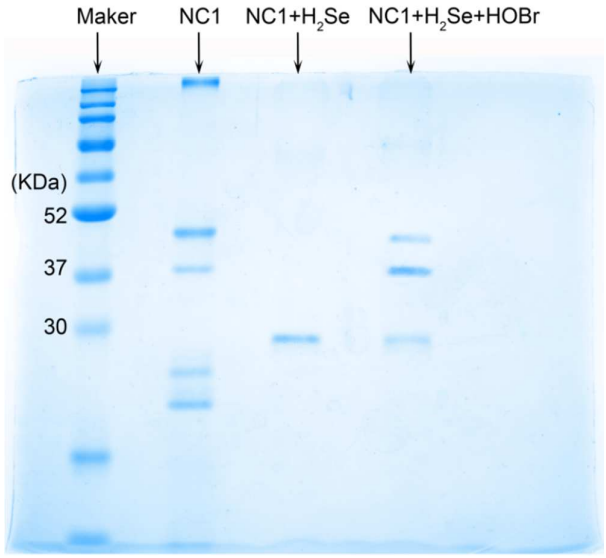

Figure S14. Uncropped SDS-PAGE gels scan for Figure 4a. SDS-PAGE comparison of NC1 banding patterns from bovine glomerular basement membrane (GBM), NC1 fraction (15 μg) treated with 100 μM H_2Se for 5 min, and NC1 fraction (15 μg) first treated with 100 μM H_2Se for 5 min and then reacted with freshly prepared HOBr (200 μM) for 5 min at room temperature. The reaction was quenched with 20 mM Met. NC1 fraction analyzed by 12% nonreducing SDS-PAGE and visualized by staining with Coomassie blue.

3. References:

(1) Xu, K.; Luan, D.; Wang, X.; Hu, B.; Liu, X.; Kong, F.; Tang, B. *Angew. Chem., Int. Ed.* **2016**, *55*, 12751-12754.