**Supplementary Material**

# Fluorescent Discrimination of Cysteine, Homocysteine, and Glutathione in Urine Samples Using a novel Seleno-BODIPY Probe

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## Summary

## 1. Photophysical Studies

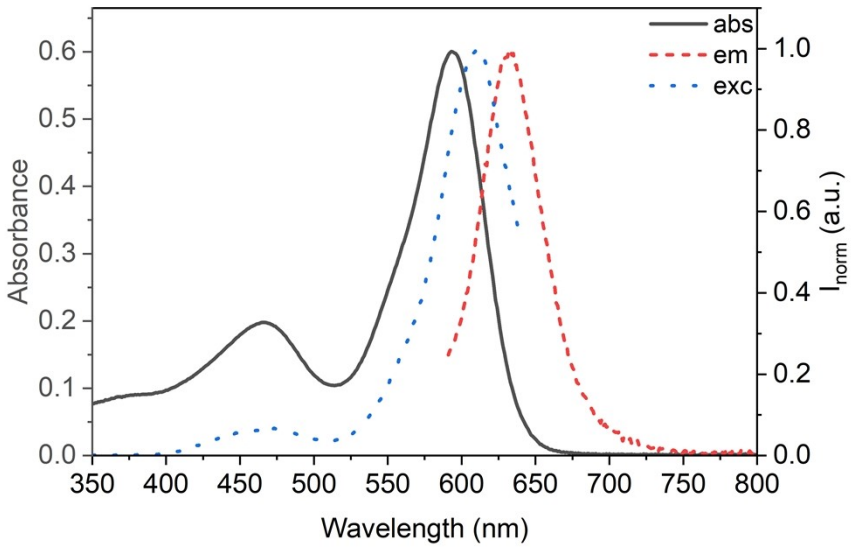


**Figure S1.** Absorption, emission, and excitation spectra of **Se-BODIPY** (12 μmol/L) in 40% DMSO/$H_2O$. $\lambda_{ex}$ = 570 nm, $\lambda_{em}$ = 650 nm, slit 5.0 / 5.0 nm.

**Table S1**. Photophysical data of **Se-BODIPY** in 40% DMSO in $H_2O$ (v/v) at 298 K

| Absorption data | | | Emission data | | | |
|---|---|---|---|---|---|---|
| $\lambda_{abs}$ (nm) | ε (L.mol$^{-1}$.cm$^{-1}$) | $Fwhm_{abs}$ (nm) | $\lambda_{em}$ (nm) | Stokes shift (nm) | $Fwhm_{em}$ (nm) | Φ |
| 593 | 50200.6988 | 60.366 | 633 | 40 | 50.019 | < 0.01 |
| 465 | 16575.4703 | 118.071 | | | | |

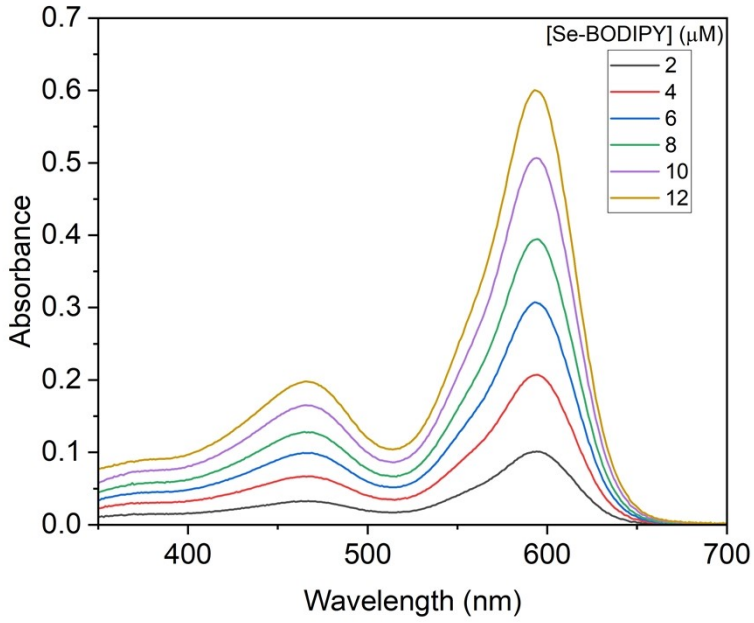


**Figure S2.** Absorption spectra of **Se-BODIPY** at different concentrations in 40% DMSO in water.

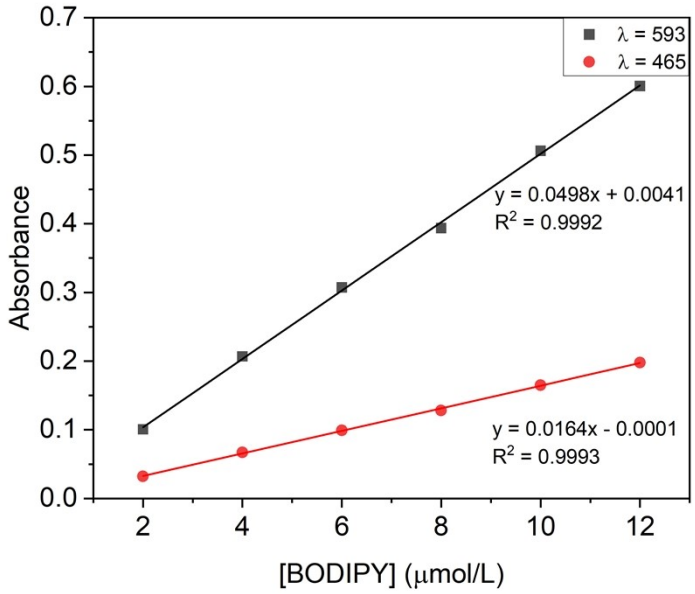


**Figure S3.** Absorbance at λ = 593 nm and λ = 465 nm as a function of **Se-BODIPY** concentration.

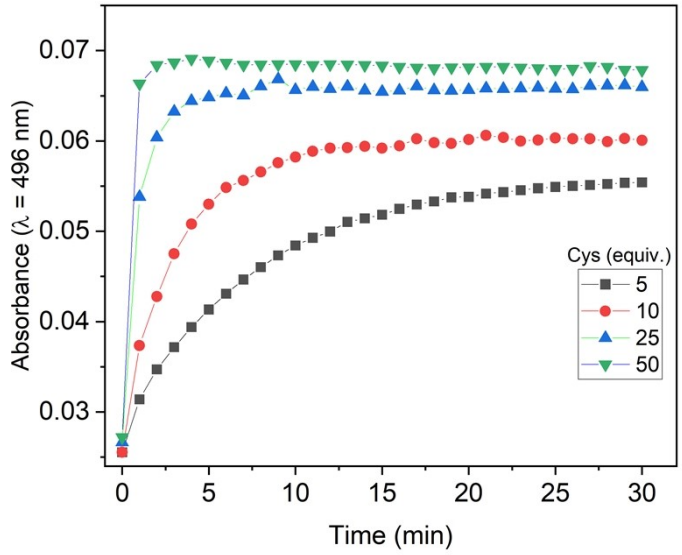


**Figure. S4.** Absorption kinetics of BODIPY **Se-BODIPY** (2 μmol/L) when in the presence of different concentrations (5, 10, 25 and 50 equiv.) of Cys (Abs = 496 nm) in 40% DMSO in water.

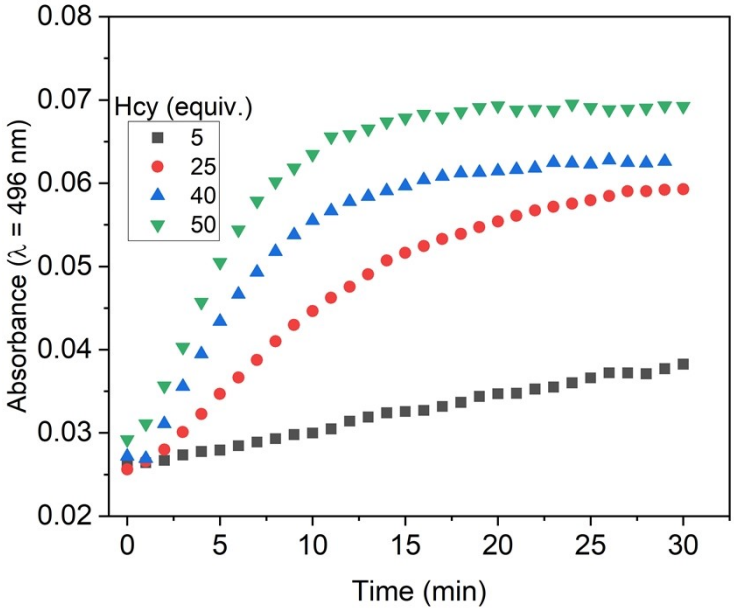


**Figure. S5.** Absorption kinetics of BODIPY **Se-BODIPY** (2 μmol/L) when in the presence of different concentrations (5, 25, 40, and 50 equiv.) of Hcy (Abs = 496 nm) in 40% DMSO in water.

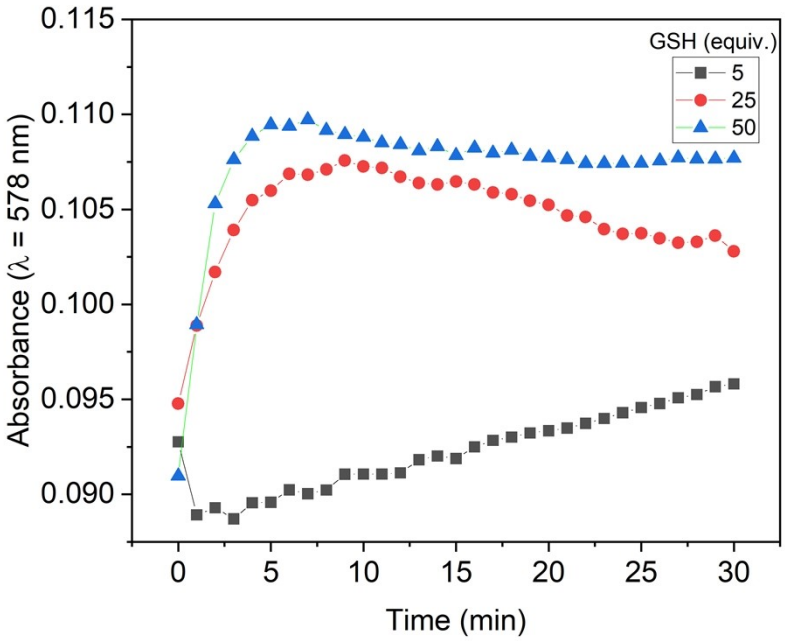


**Figure. S6.** Absorption kinetics of BODIPY **Se-BODIPY** (2 μmol/L) when in the presence of different concentrations (5, 25, and 50 equiv.) of GSH (Abs = 578 nm) in 40% DMSO in water.

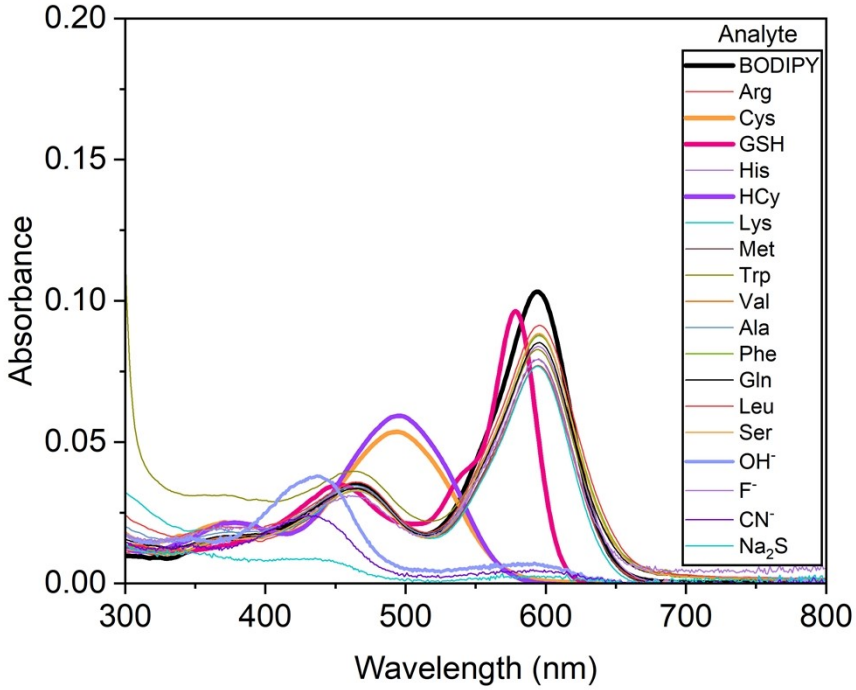


**Figure. S7.** Absorption spectra of **Se-BODIPY** (2 μmol/L) when in the presence of 50 equiv. (100 μmol/L) of analytes in 40% DMSO in water. Analytes: Cys, Hcy, GSH, Arg, His, Lys, Met, Trp, Val, Ala, Phe, Gln, Leu, Ser, $OH^-$, $F^-$, $CN^-$, and $Na_2S$.

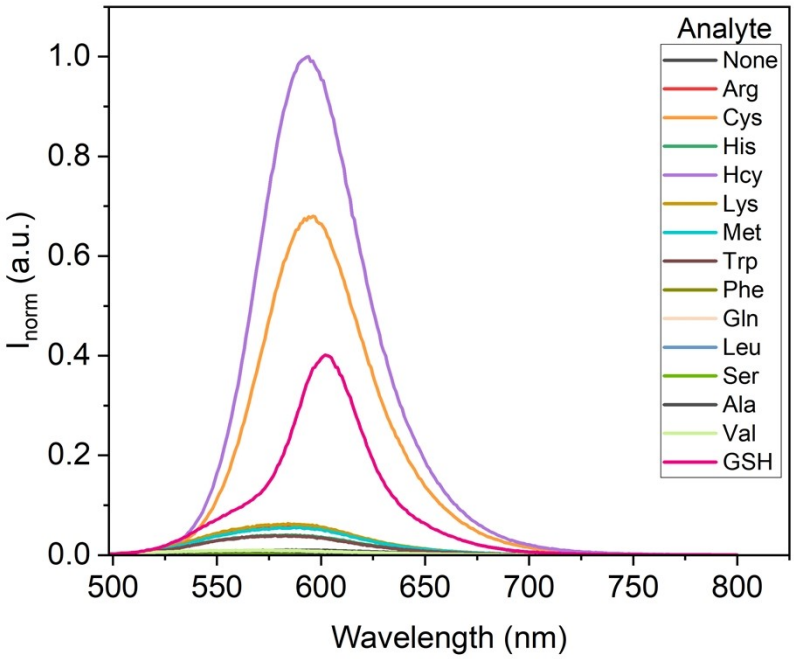


**Figure. S8.** Emission spectra of **Se-BODIPY** (2 μmol/L) when in the presence of 50 equiv. (100 μmol/L) of analytes in 40% DMSO in water. Analytes: Cys, Hcy, GSH, Arg, His, Lys, Met, Trp, Val, Ala, Phe, Gln, Leu, Ser. λex = 488 nm, slit 2.5.

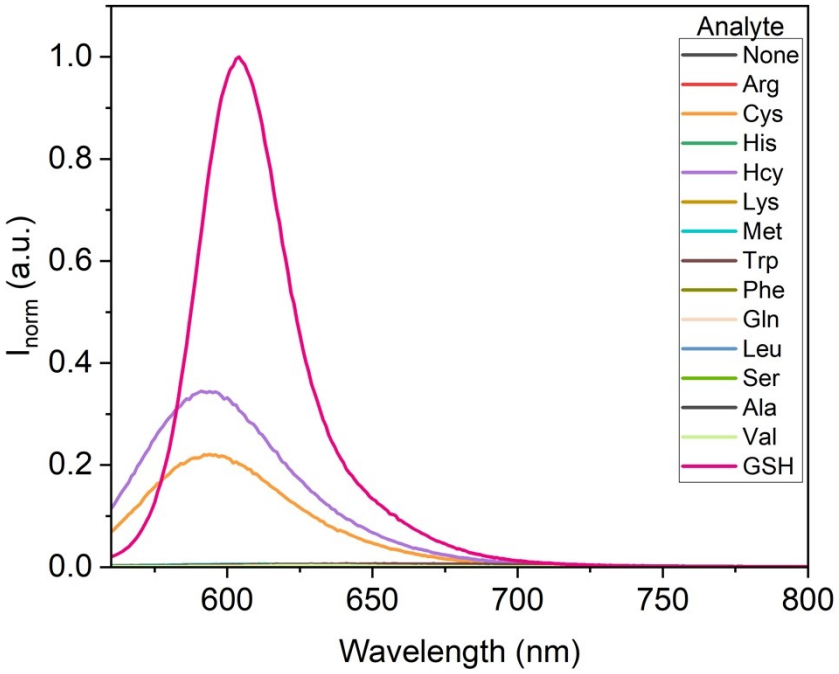


**Figure. S9.** Emission spectra of **Se-BODIPY** (2 μmol/L) when in the presence of 50 equiv. (100 μmol/L) of analytes in 40% DMSO in water. Analytes: Cys, Hcy, GSH, Arg, His, Lys, Met, Trp, Val, Ala, Phe, Gln, Leu, Ser. λex = 550 nm, slit 2.5

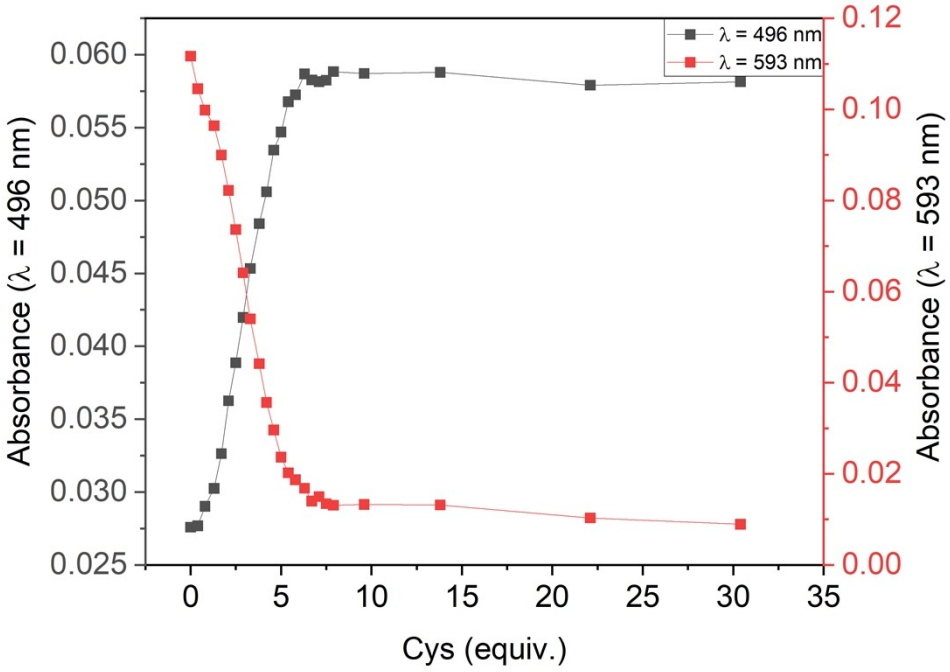


**Figure. S10.** Correlation between absorbance of **Se-BODIPY** (2 μmol/L) (λ = 496 nm and λ = 593 nm) and [Cys] in 40% DMSO in water.

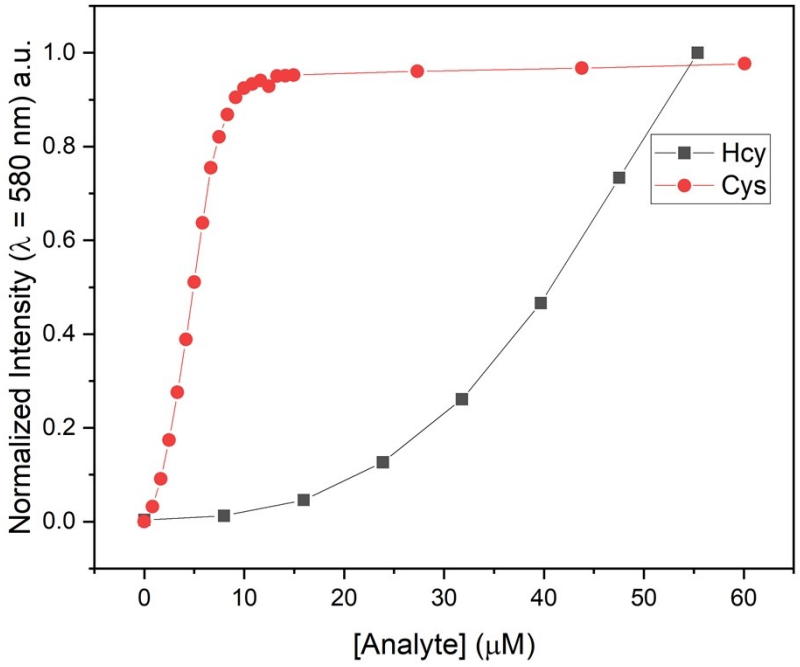


**Figure. S11.** Correlation between emission (λ = 580 nm) of **Se-BODIPY** (2 μmol/L) and [Cys] or [Hcy] in 40% DMSO in water.

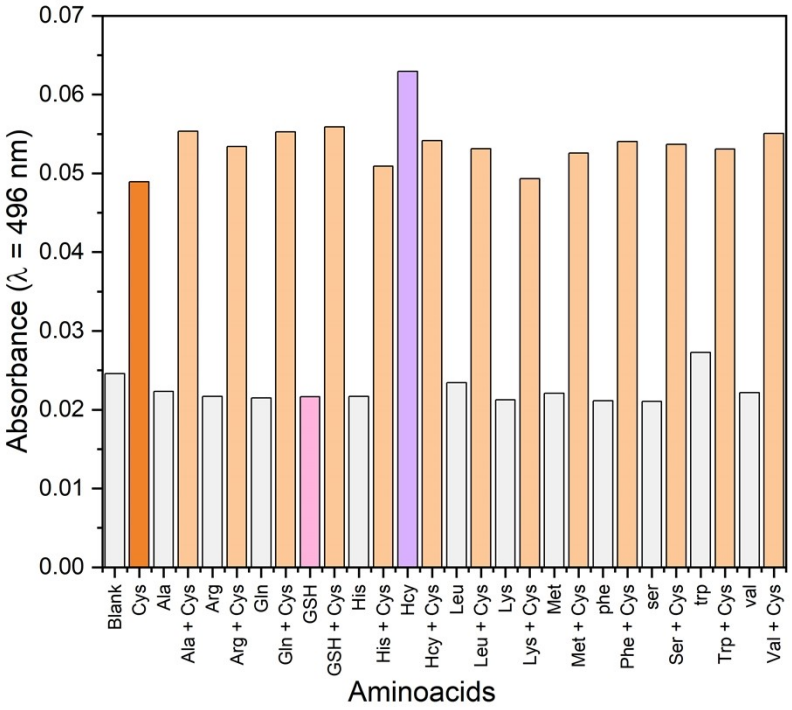


**Figure. S12**. Competition study of the reaction of **Se-BODIPY** (2 μmol/L) with Cys (5 equiv.) in the presence of other analytes (5 equiv.) by absorption (λ = 496 nm) in 40% DMSO/$H_2O$.

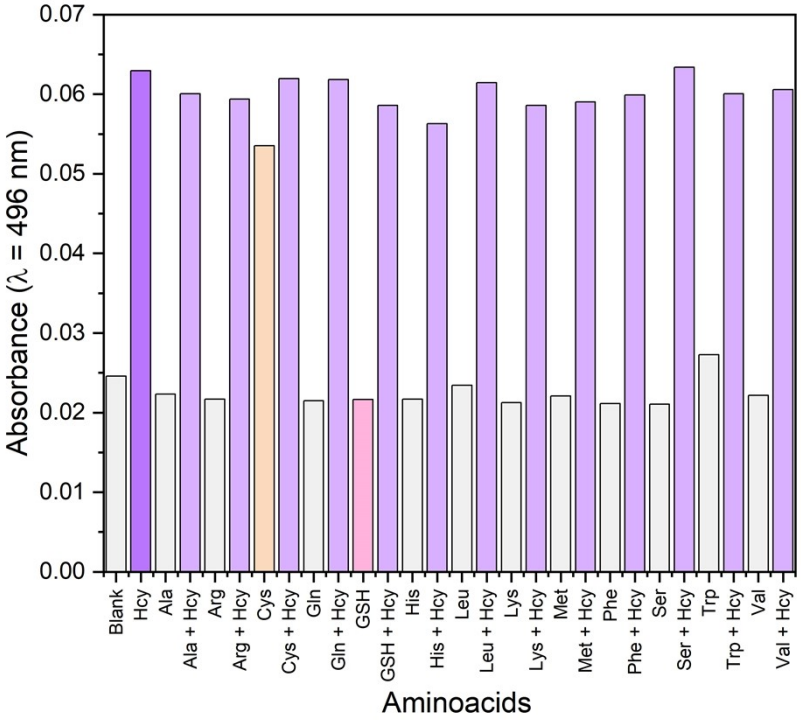


**Figure. S13**. Competition study of the reaction of **Se-BODIPY** (2 μmol/L) with Hcy (50 equiv.) in the presence of other analytes (50 equiv.) by absorption (λ = 496 nm) in 40% DMSO/$H_2O$.

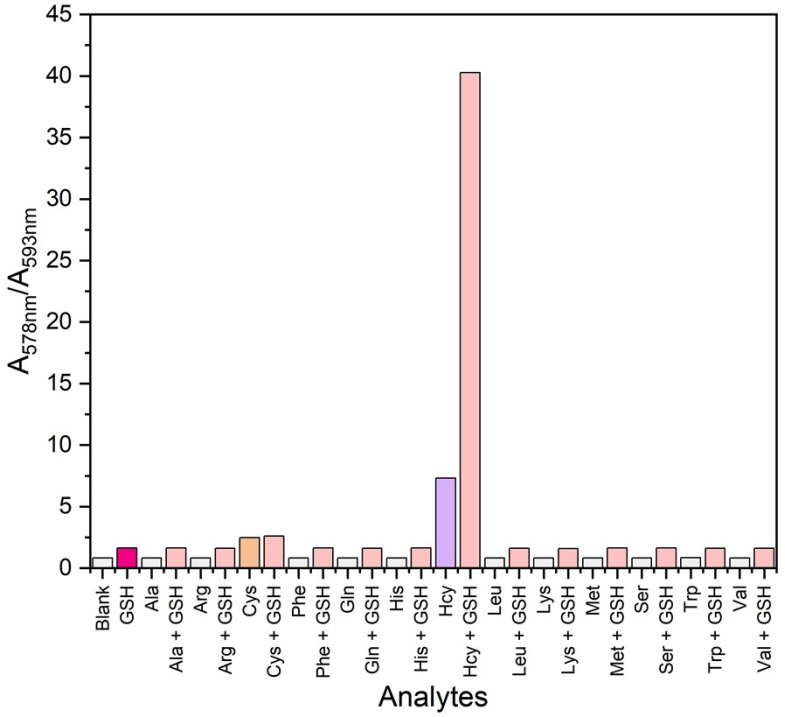


**Figure. S14.** Competition study of the reaction of **Se-BODIPY** (2 μmol/L) with GSH (50 equiv.) in the presence of other analytes (50 equiv.) by absorption ($A_{578nm}/A_{593nm}$) in 40 % DMSO/$H_2O$.

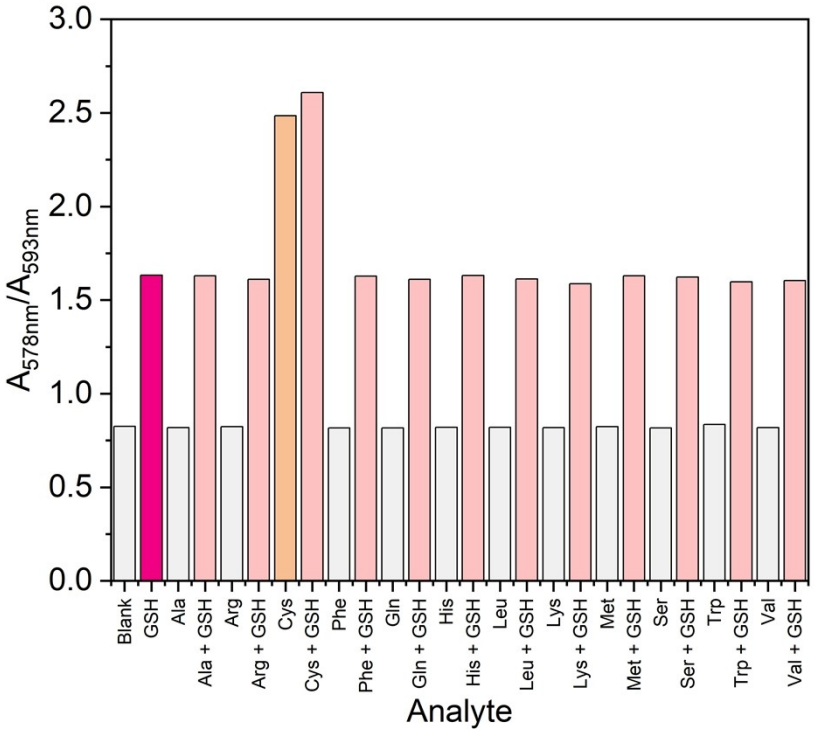


**Figure. S15.** Competition study of the reaction of **Se-BODIPY** (2 μmol/L) with GSH (50 equiv.) in the presence of other analytes (50 equiv.) by emission (λ = 615 nm; $\lambda_{ex}$ = 550 nm and slit 2.5 nm) in 40% DMSO/$H_2O$.

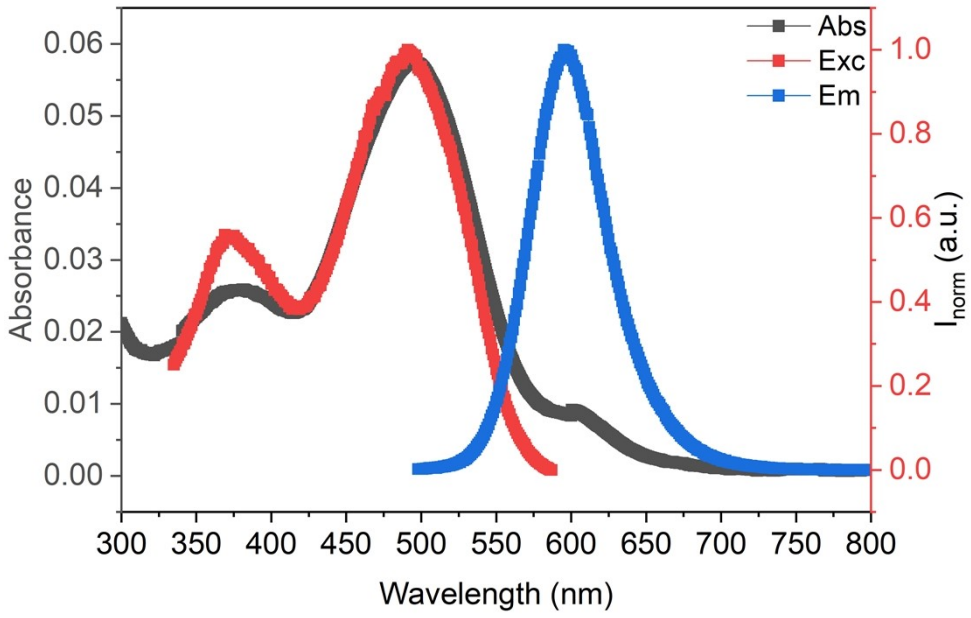


**Figure. S16.** Absorption, emission and excitation wavelength of the product **7** obtained after reaction between **Se-BODIPY** and Cys, in 40% DMSO in $H_2O$. $\lambda_{ex}$ = 488 nm, slit 2.5.

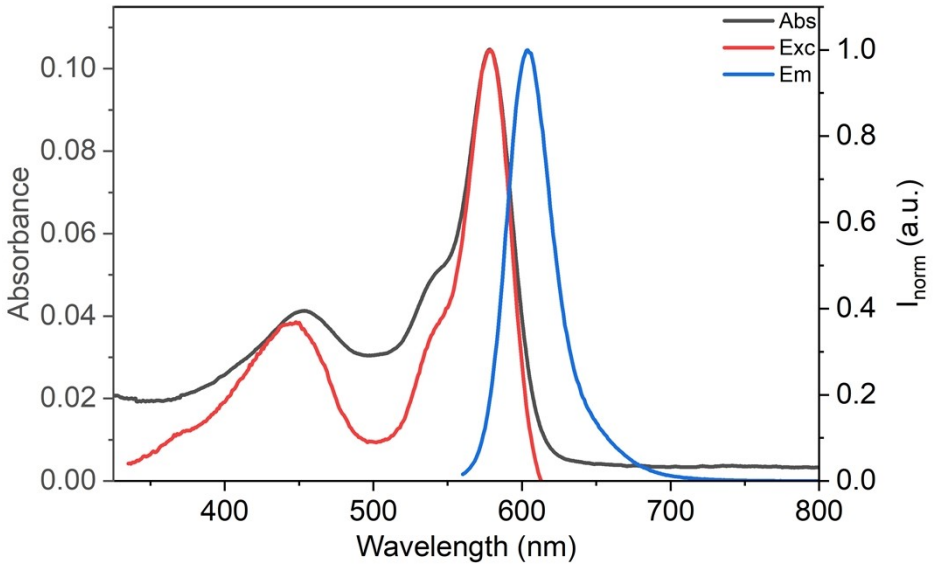


**Figure. S17.** Absorption, emission and excitation wavelength of the product **9** obtained after reaction between **Se-BODIPY** and GSH, in 40% DMSO in $H_2O$. λex = 550 nm, slit 2.5

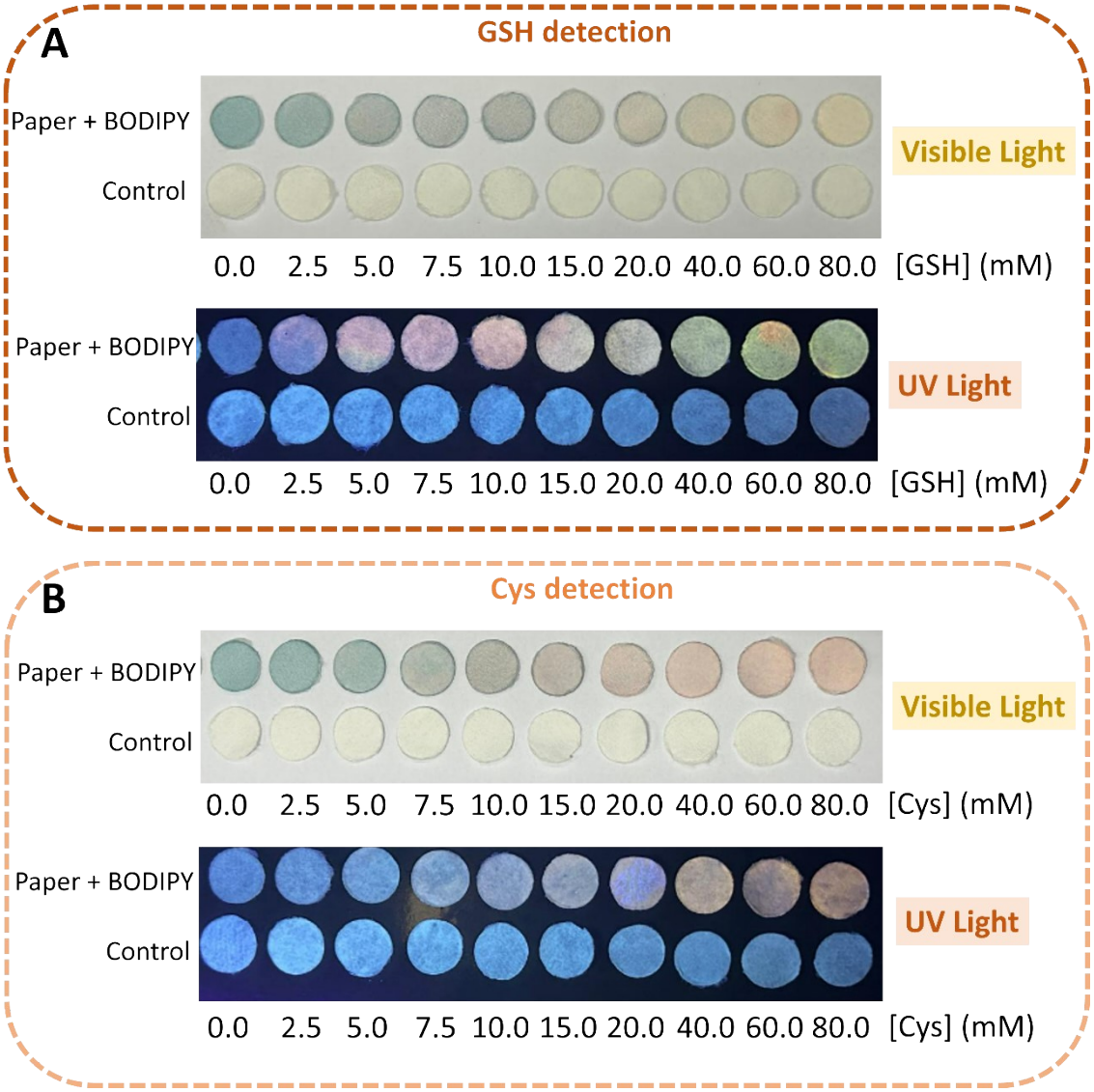


**Figure S18.** Paper discs containing **Se-BODIPY** ($5.10^{-4}$ mol/L) after reaction with different concentrations of **A)** GSH (after 2h reaction) and **B)** Cys (after 1h reaction) under visible light (top) and UV light (bottom). Obs: Control experiments were conducted by immersing paper discs without BODIPY in Cys or GSH solutions with different concentrations.

**Table S2.** Comparison of **Se-BODIPY** with other reported probes based on BODIPYs for detection of Cys, Hcy or GSH.

| Solvent | LOD Cys (μM) | LOD Hcy (μM) | LOD GSH (μM) | Application | Ref. |
|---|---|---|---|---|---|
| PBS (10 mM, pH 7.4, 10 % DMSO) | 0.64 | --- | --- | Images in HepG2 cells | [1] |
| PBS/MeCN (9/1, v/v) | --- | --- | 0.0017 | Images in HepG2 cells | [2] |
| PBS (0.1% F127 and 0.1% DMSO) | --- | --- | 3.4 | Images in A549 cells | [3] |
| MeCN/PBS buffer (4:6, v/v, pH 7.4, 10 mM) | 0.072 | --- | --- | Images in HeLa cells and *in vivo* | [4] |
| PBS/MeCN (v/v = 1:1, pH 7.4, 10 mM) | 0.0112 | --- | --- | Images in HepG2 cells, *in vivo* and quantification in food samples | [5] |
| MeCN/HEPES buffer (1:2 v/v, 20 mM, pH 7.4) | 0.67 | --- | --- | Images in HeLa cells and quantification in urine samples | [6] |
| DMSO/PBS (v/v = 3/7, pH 7.4) | 0.020 | --- | 0.034 | Images in HeLa cells and quantification in food samples | [7] |
| PBS/DMSO (v/v = 1:1, pH 7.4, 10 mM) | 0.049 | --- | --- | Quantification in food samples and images *in vivo* | [8] |
| DMF/$H_2O$ (1:9 v/v, 10 mM HEPES buffer, pH 8.0). | 0.029 | --- | --- | Removal of $Hg^{2+}$ from water | [9] |
| PBS buffer (pH 7.4, 1% DMSO) | --- | --- | 0.0023 | Images in HepG2 cells | [10] |
| MeCN (1 : 1 v/v, PBS) | 0.0627 | --- | --- | --- | [11] |
| 10 mM PBS (pH = 7.4) | 2.29 | --- | 1.37 | Images in HepG2 cells | [12] |
| 10 mM PBS (pH 7.4, containing 50 % MeCN) | 0.118 | --- | --- | Images in HeLa cells and *in vivo* | [13] |
| PBS (pH 7.4, 10 mM, containing 40% MeCN) | 0.052 | 0.071 | --- | Images in HeLa cells | [14] |
| MeCN /PBS buffer (1: 1, v/v, 10 mM, pH 7.4) | 0.044 | 0.063 | --- | Images in HeLa cells | [15] |
| MeCN and 20 mmol.$L^{-1}$ HEPES buffer (1:1, v/v, pH = 7.4) | 0.147 | 0.087 | 0.129 | Images in HeLa cells | [16] |
| DMSO (40% v/v in $H_2O$) | 0.8 | 20.4 | 35.9 | Quantification in human urine samples | This work |

## 2. Recovery values of biothiols in urine samples

**Table S3.** The recovery values of spiked Cys, Hcy, and GSH in real human urine samples by absorbance

| Urine Sample | Cys | | | Hcy | | |
|---|---|---|---|---|---|---|
| | Spiked (μM) | Recovery (μM) | Recovery (%) | Spiked (μM) | Recovery (μM) | Recovery (%) |
| 1 | 15.0 | 17.8 ± 0.2 | 118 | 20.0 | 13.4 ± 0.8 | 67 |
| | 17.5 | 19.6 ± 0.1 | 112 | 21.2 | 16 ± 1 | 76 |
| | 20.0 | 21.22 ± 0.01 | 106 | 25.2 | 20.4 ± 0.7 | 81 |
| 2 | 15.0 | 16.1 ± 0.5 | 107 | 20.0 | 15.8 ± 0.2 | 79 |
| | 17.5 | 19.1 ± 0.6 | 109 | 21.2 | 17.50 ± 0.01 | 83 |
| | 20.0 | 18.1 ± 0.5 | 91 | 25.2 | 21.6 ± 0.6 | 86 |
| 3 | 15.0 | 13.4 ± 0.2 | 89 | 20.0 | 17.1 ± 0.2 | 85 |
| | 17.5 | 18.0 ± 0.3 | 103 | 21.2 | 16.3 ± 0.3 | 77 |
| | 20.0 | 18.0 ± 0.3 | 90 | 25.2 | 20.5 ± 0.4 | 81 |
| 4 | 15.0 | 16.7 ± 0.1 | 111 | 20.0 | 11.9 ± 0.5 | 59 |
| | 17.5 | 17.56 ± 0.01 | 100 | 21.2 | 12.3 ± 0.5 | 58 |
| | 20.0 | 19.27 ± 0.01 | 96 | 25.2 | 18.0 ± 0.4 | 72 |

*Found: [mean ± SD, mM ($n$ = 3). [**Se-BODIPY**] = 20 μmol/ L, with 40% DMSO in Tris-HCl buffer pH 7.51. $\lambda_{abs}$ = 496 nm.

**Table S4.** The recovery values of spiked Cys and GSH in real human urine samples through R values obtained from pictures of paper discs impregnated with **Se-BODIPY**.

| Urine Sample | Cys | | | GSH | | |
|---|---|---|---|---|---|---|
| | Spiked (mM) | Recovery (mM) | Recovery (%) | Spiked (mM) | Recovery (mM) | Recovery (%) |
| Visible | 2.5 | 2.5 ± 0.5 | 101 | 5.0 | 3.7 ± 0.2 | 74 |
| | 5.0 | 5.4 ± 0.8 | 108 | 7.5 | 7.4 ± 0.3 | 99 |
| | 7.5 | 6.3 ± 0.8 | 84 | 10.0 | 10.6 ± 0.3 | 106 |
| UV | 2.5 | 2.5 ± 0.1 | 106 | 5.0 | 3.5 ± 0.2 | 70 |
| | 5.0 | 5.3 ± 0.2 | 106 | 7.5 | 6.1 ± 0.3 | 81 |
| | 7.5 | 8.0 ± 0.2 | 106 | 10.0 | 8.7 ± 0.3 | 87 |

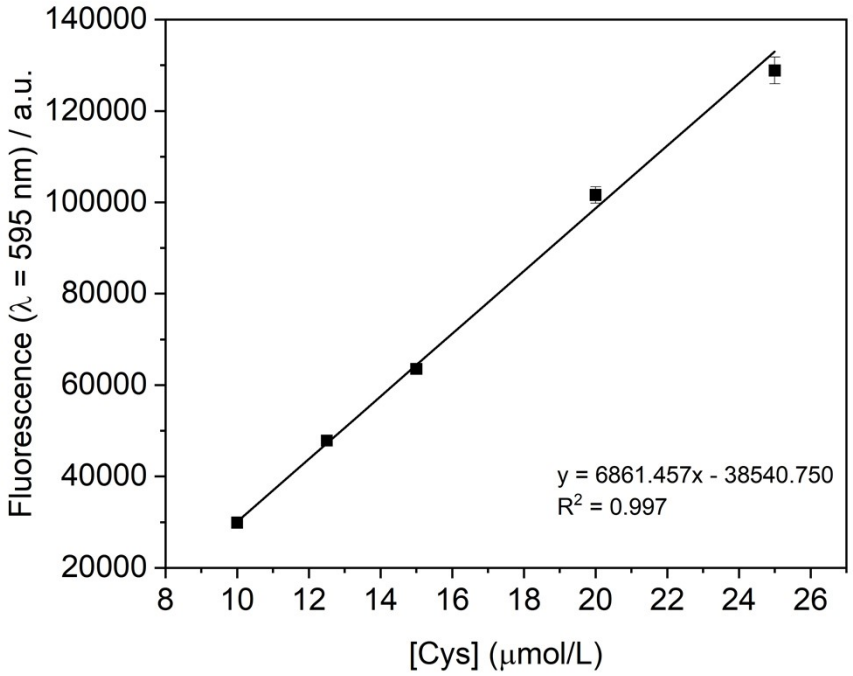


**Figure S19.** Calibration curve for Cys with Se-BODIPY as fluorescent probe used for obtaining the recovery values presented in the Table 1. Conditions: [Se-BODIPY] = 20 μmol/ L, with 40% DMSO in Tris-HCl buffer pH 7.5. $\lambda_{ex}$ = 486 nm, $\lambda_{em}$ = 595 nm.

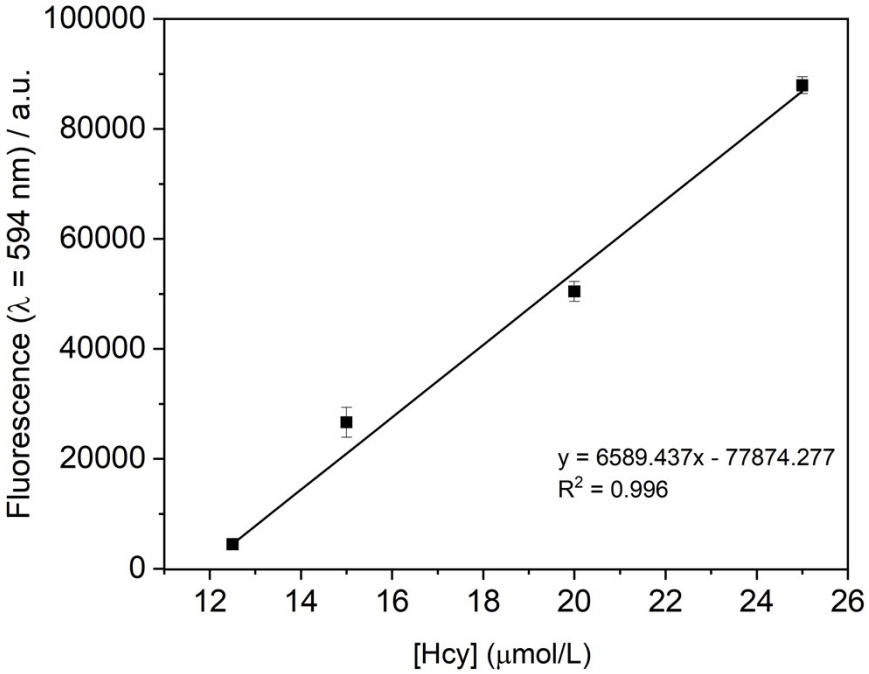


**Figure S20.** Calibration curve for Hcy with Se-BODIPY as fluorescent probe used for obtaining the recovery values presented in the Table 1. Conditions: [Se-BODIPY] = 20 μmol/L, with 40% DMSO in Tris-HCl buffer pH 7.5. $\lambda_{ex}$ = 486 nm, $\lambda_{em}$ = 594 nm.

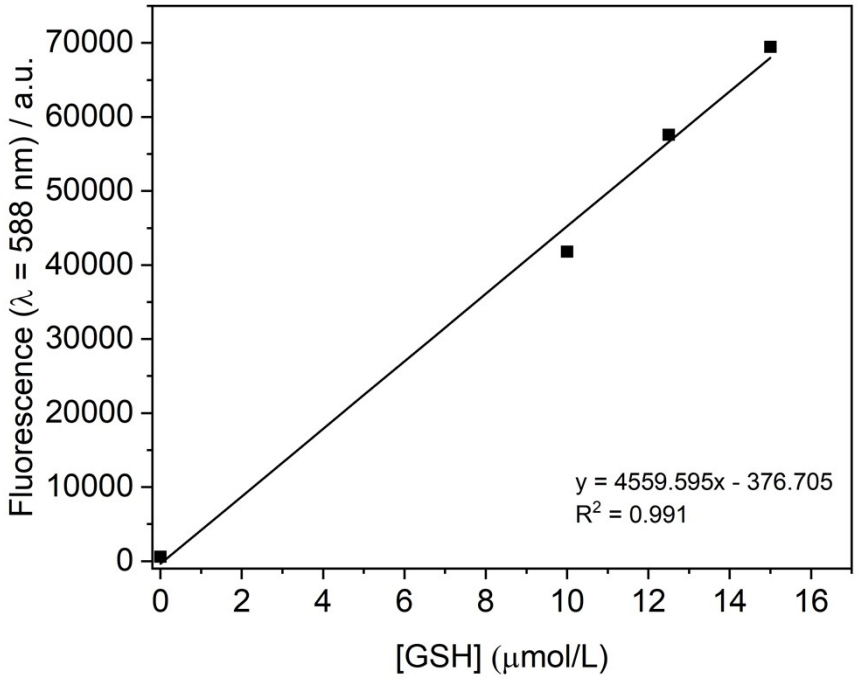


**Figure S21.** Calibration curve for GSH with Se-BODIPY as fluorescent probe used for obtaining the recovery values presented in the Table 1. Conditions: [Se-BODIPY] = 20 μmol/L, with 40% DMSO in Tris-HCl buffer pH 7.5. $\lambda_{ex}$ = 540, $\lambda_{em}$ = 588 nm.

## 3. Structural characterization of BODIPYs

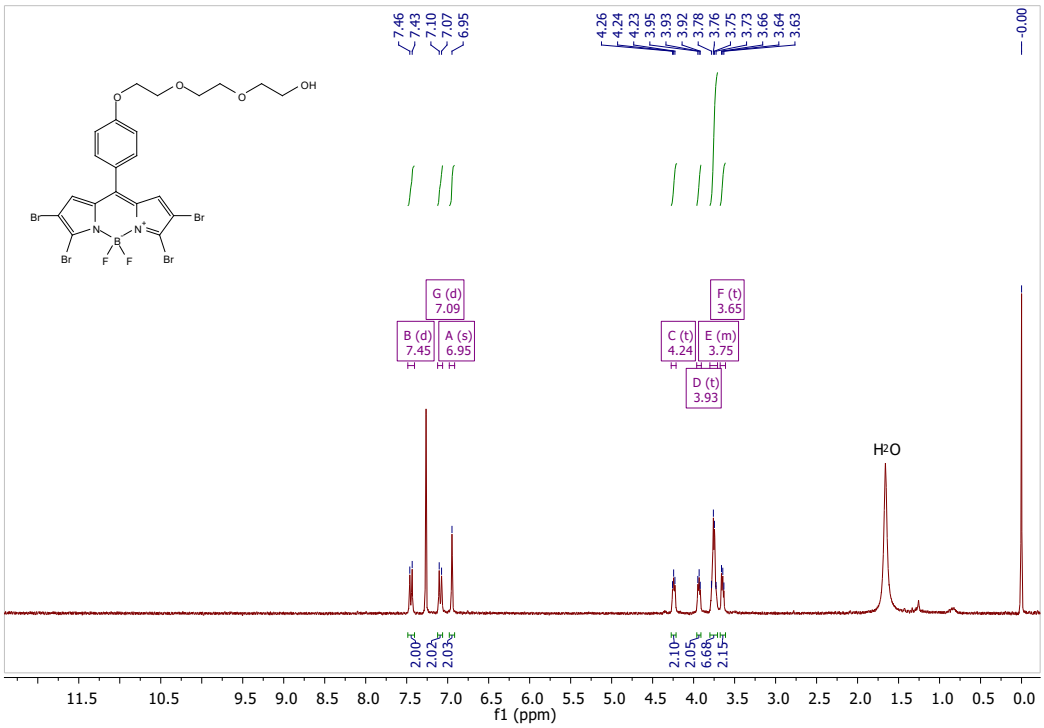


**Figure. S22.** $^{1}$H NMR spectra (300 MHz) of BODIPY **2** in $CDCl_3$.

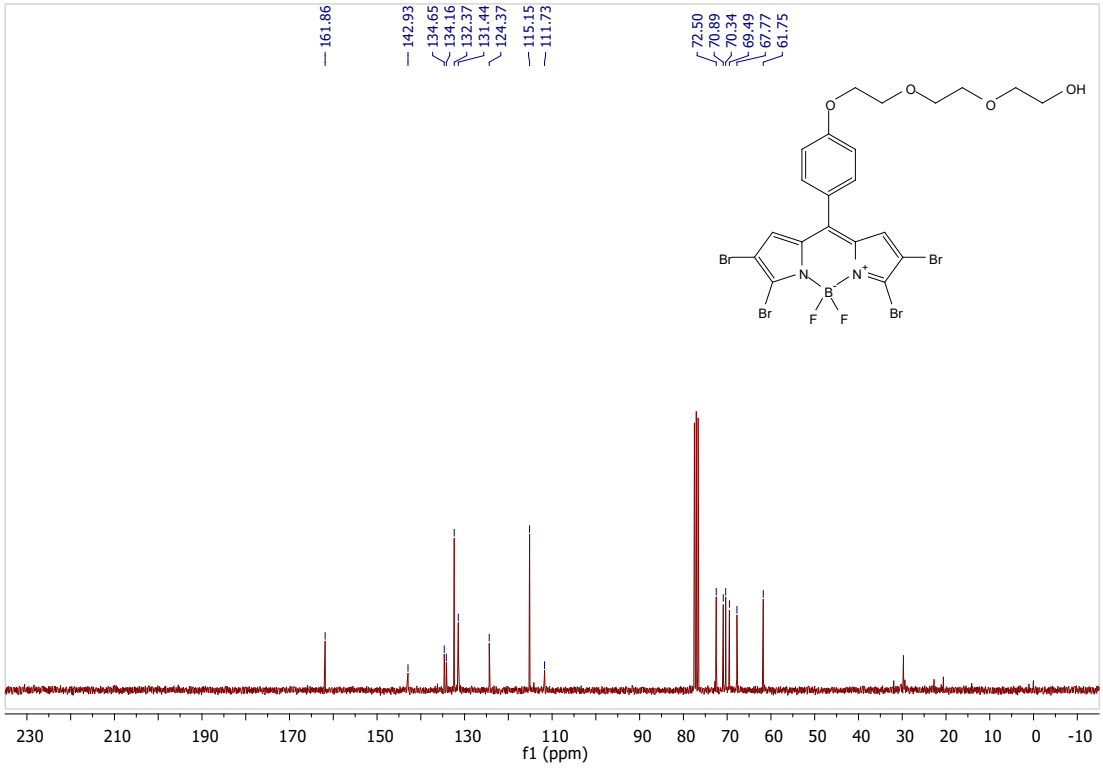


**Figure. S23.** $^{13}$C NMR spectra (75 MHz) of BODIPY **2** in $CDCl_3$.

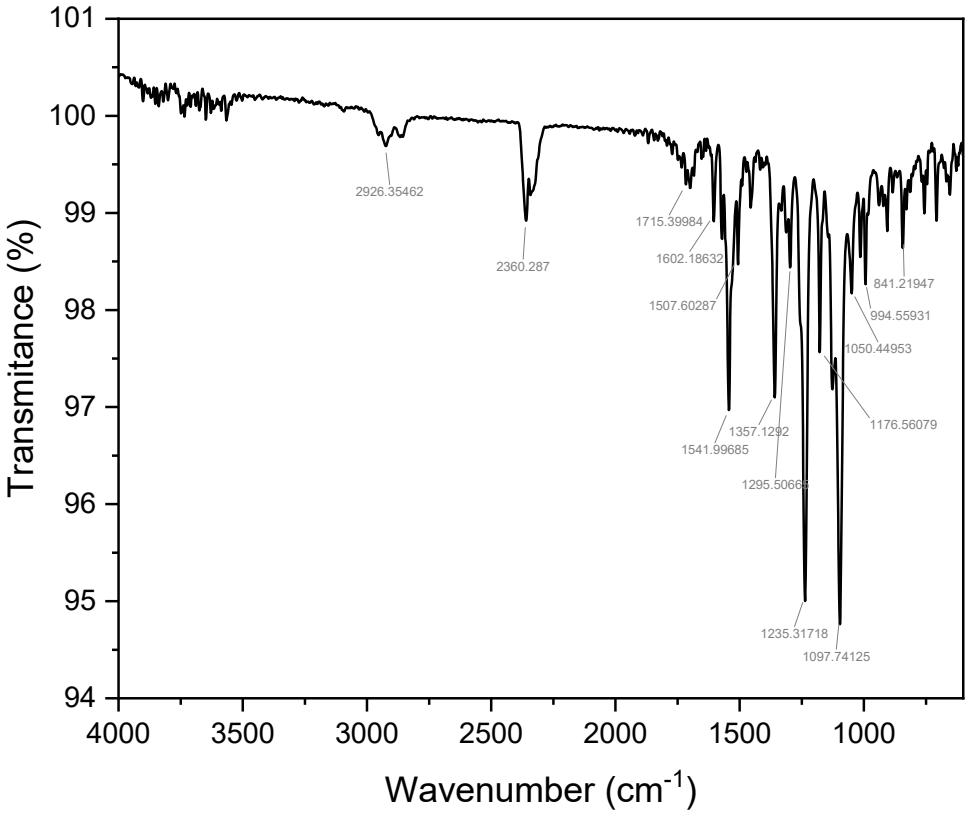


**Figure. S24.** IR of BODIPY **2**.

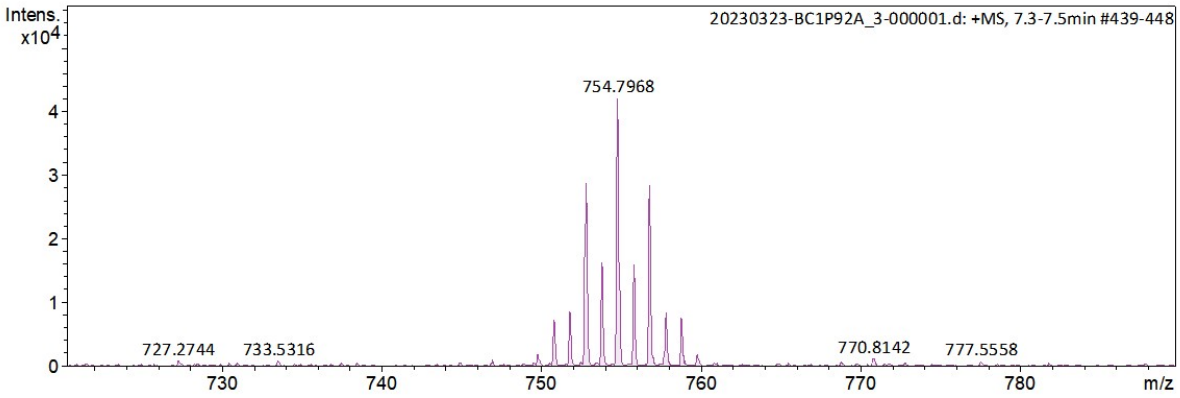


**Figure. S25.** HRMS of BODIPY **2**.

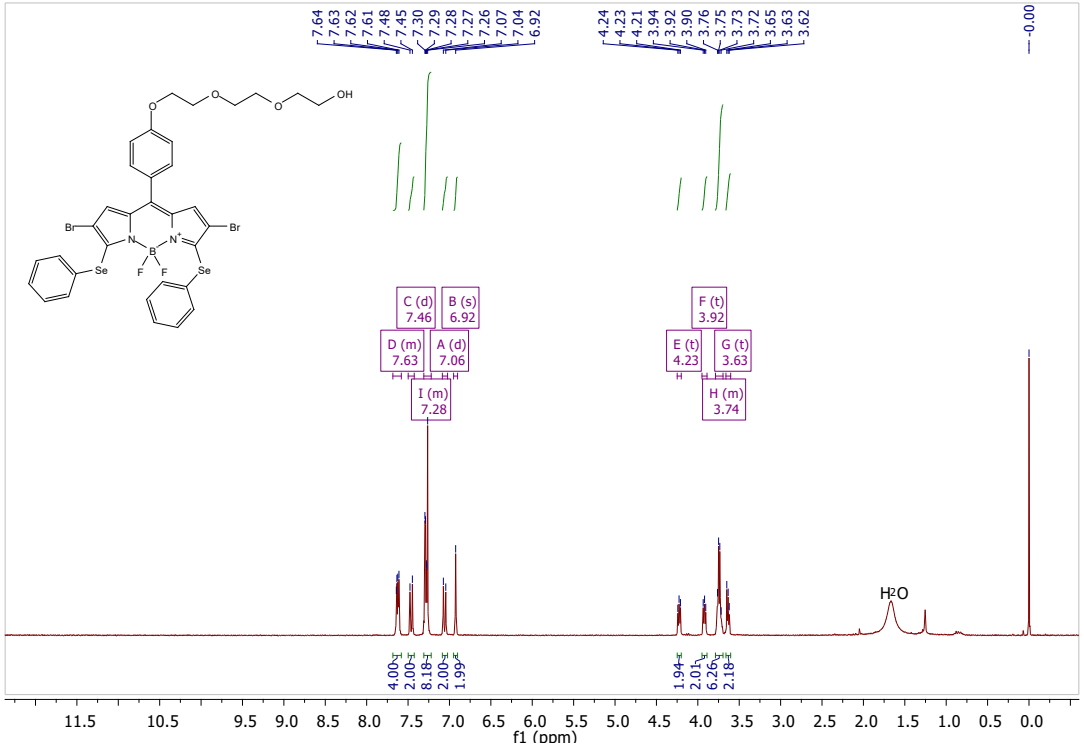


**Figure. S26.** $^{1}$H NMR spectra (300 MHz) of **Se-BODIPY** in $CDCl_3$.

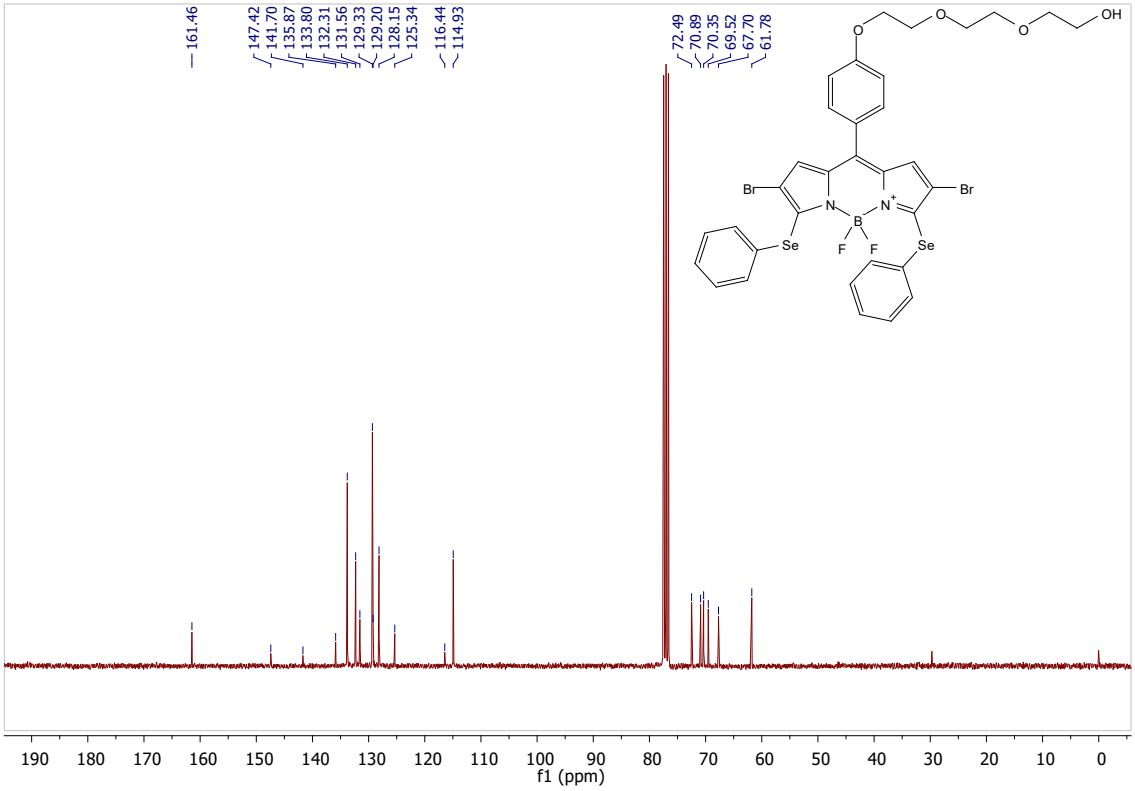


**Figure. S27**. $^{13}$C NMR spectra (75 MHz) of **Se-BODIPY** in $CDCl_3$.

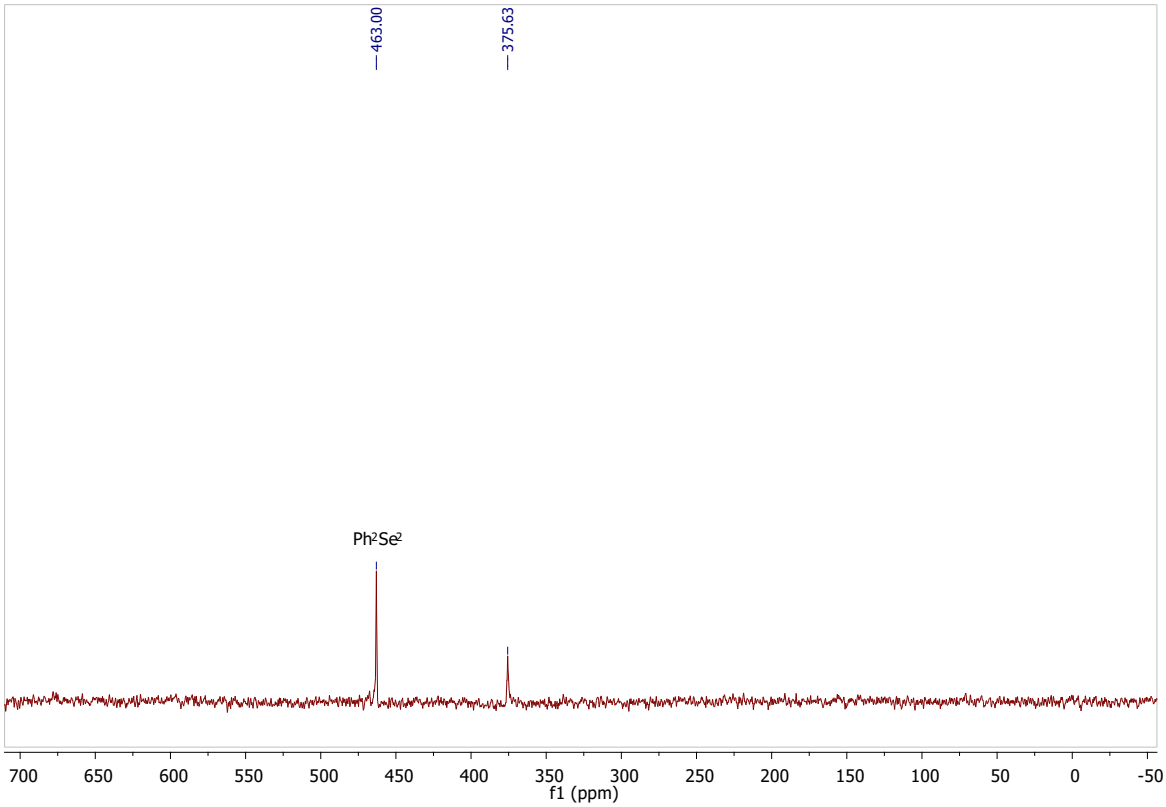


**Figure. S28.** HRMS of **Se-BODIPY** using $Ph_2Se_2$ as an external standard.

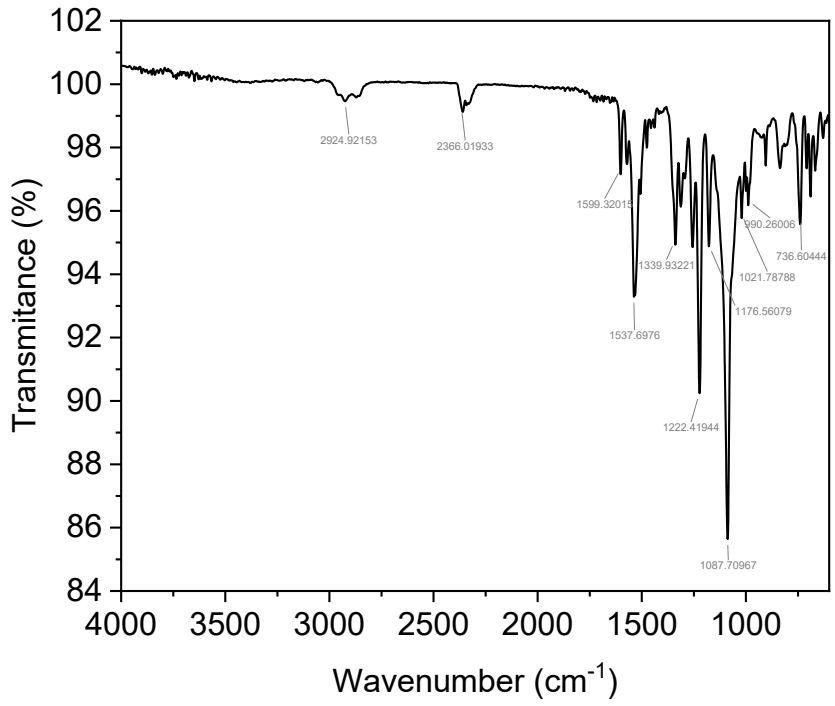


**Figure. S29.** IR of **Se-BODIPY**.

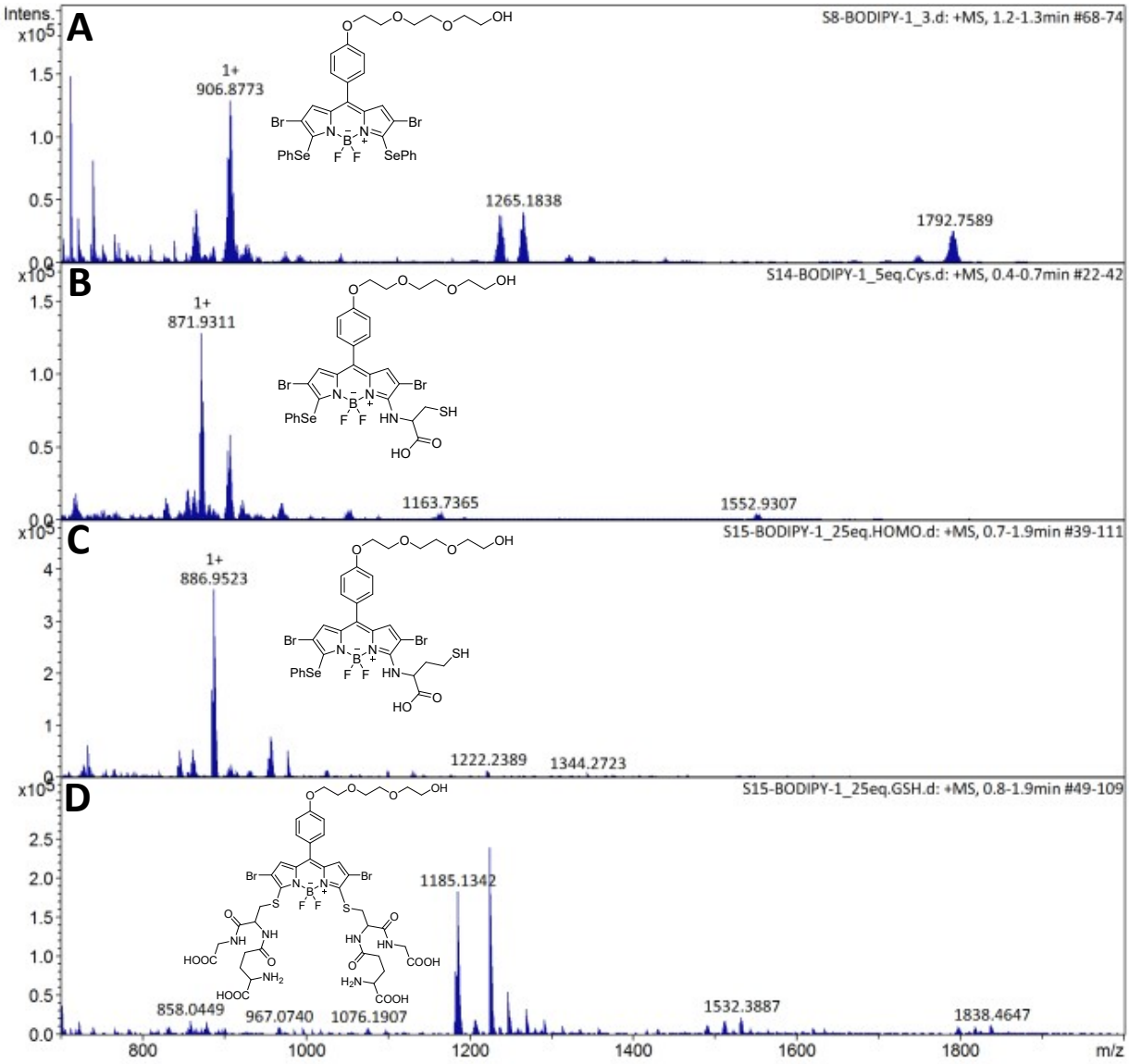


**Figure. S30.** High-resolution Q-TOF MS Spectra. **A. Se-BODIPY** $[M + Na]^+$ for $C_{33}H_{29}BBr_2F_2N_2O_4Se_2$ = 906.8773 m/z (-1.1 ppm). Calculated $[M+Na]^+$ for $C_{33}H_{29}BBr_2F_2N_2O_4Se_2$ = 906.878258 m/z. **B. Se-BODIPY-Cys** (Compound **7**) $[M + Na]^+$ for $C_{30}H_{30}BBr_2F_2N_3O_6SSe$ = 871.9311 m/z (-2.0 ppm). Calculated $[M+Na]^+$ for $C_{30}H_{30}BBr_2F_2N_3O_6SSe$ = 871.932839 m/z. **C. Se-BODIPY-Hcy** (Compound **8**) $[M + Na]^+$ for $C_{31}H_{33}BBr_2F_2N_3O_6SSe$ = 886.9523 m/z (-4.6 ppm). Calculated $[M+Na]^+$ for $C_{31}H_{33}BBr_2F_2N_3O_6SSe$ = 886.956353 m/z. **D. Se-BODIPY-GSH** (Compound **9**) $[M+H]^+$ for $C_{41}H_{51}BBr_2F_2N_8O_{16}S_2$ = 1185.1342 m/z (-1.2 ppm). Calculated $[M+H]^+$ for $C_{41}H_{51}BBr_2F_2N_8O_{16}S_2$ = 1185.135574 m/z.

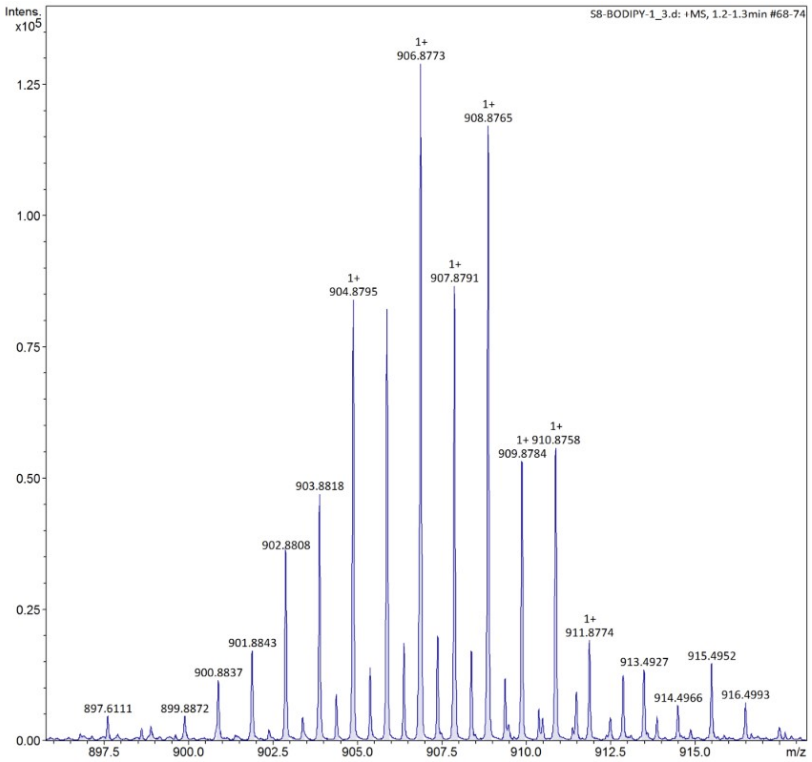


**Figure. S31.** HRMS of **Se-BODIPY**. HRMS m/z $[M + Na]^+$ for $C_{33}H_{29}BBr_2F_2N_2O_4Se_2$ = 906.8773 (-1.1 ppm). Calculated $[M + Na]^+$ for $C_{33}H_{29}BBr_2F_2N_2O_4Se_2$ = 906.878258.

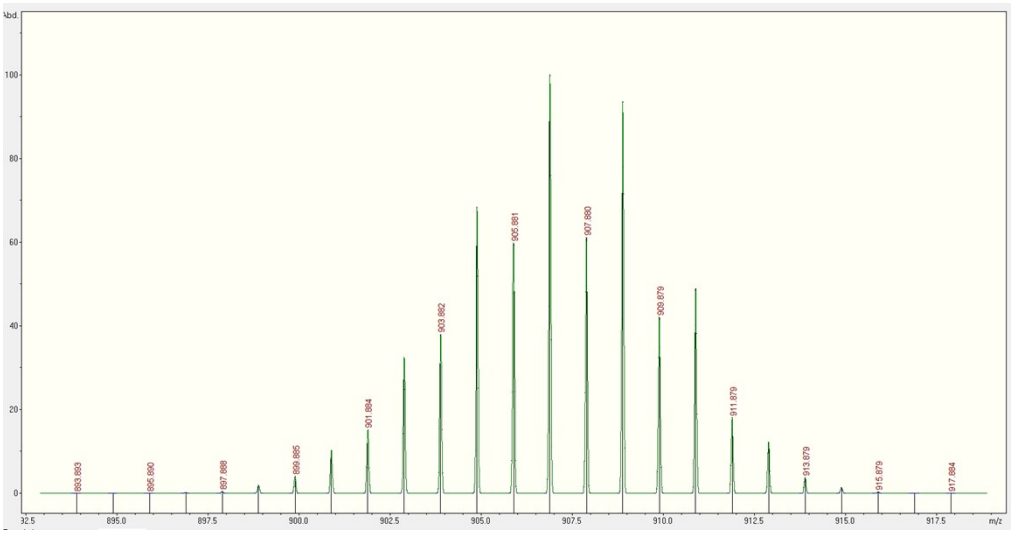

**Figure. S32.** Theoretical isotopic pattern for **Se-BODIPY**. obtained by software Compass IsotopePattern. HRMS (*m/z*) for $C_{33}H_{29}BBr_2F_2N_2O_4Se_2$: $[M+Na]^+$ =906.878258.

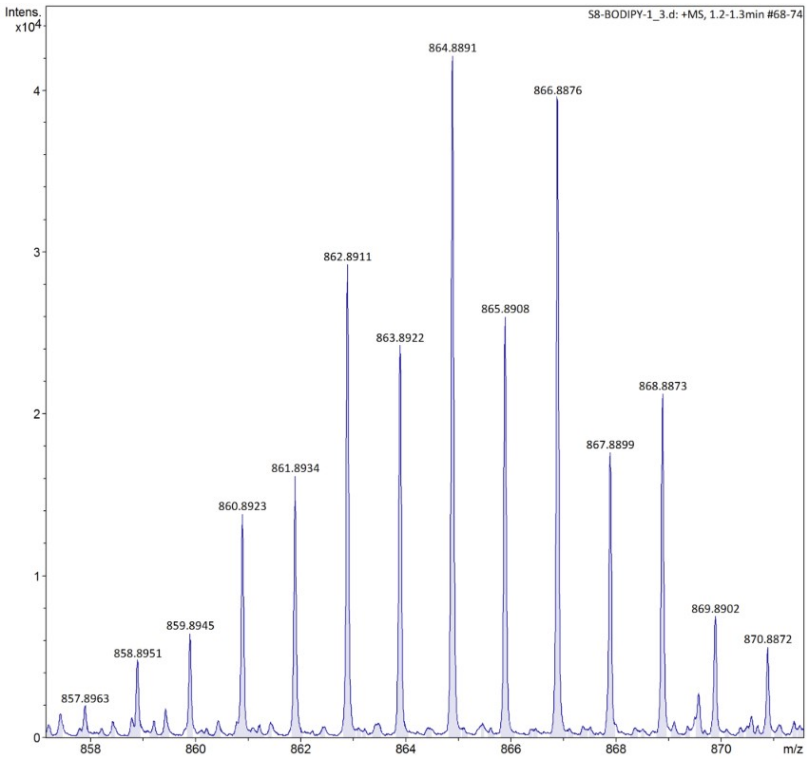


**Figure. S33.** HRMS of a fragment of **Se-BODIPY**. HRMS m/z $[M]^+$ for $C_{33}H_{29}BBr_2FN_2O_4Se_2$ = 864.8891 (-1.1 ppm). Calculated $[M]^+$ for $C_{33}H_{29}BBr_2FN_2O_4Se_2$ = 864.890085.

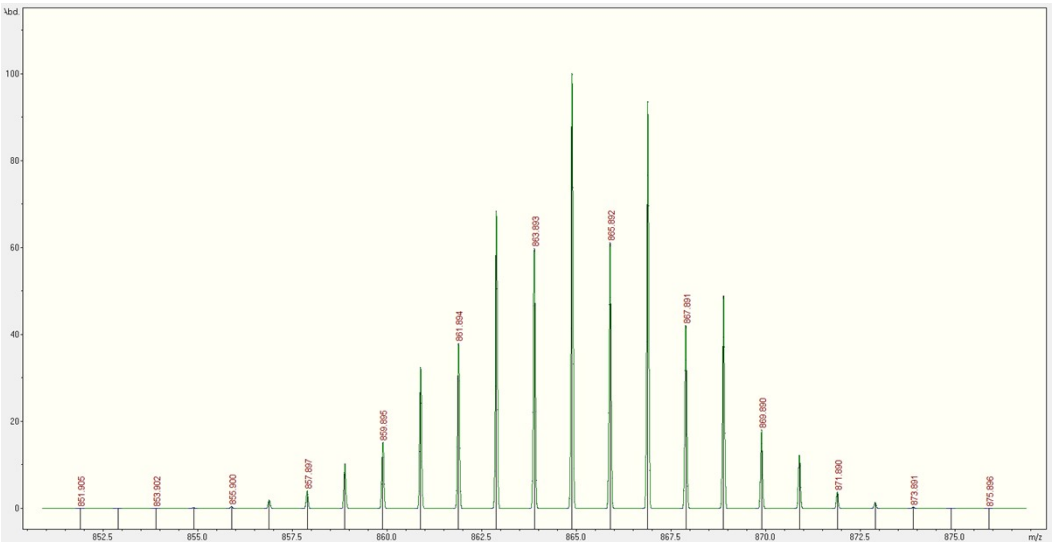


**Figure. S34.** Theoretical isotopic pattern for **Se-BODIPY**. obtained by software Compass IsotopePattern. HRMS (*m/z*) for $C_{33}H_{29}BBr_2FN_2O_4Se_2$: $[M]^+$ =864.890085.

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