Supporting Information

Cell-Penetrating Dynamic-Covalent Benzopolysulfane Networks
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Table of Content

1. Materials and Methods S4
2. Synthesis S7
   2.1. Synthesis of Benzopolysulfanes S7
   2.2. Synthesis of Epidithiodiketopiperazines S11
   2.3. Synthesis of Controls S18
   2.4. Synthesis of Biotinylated Fluorophores S19
3. Fluorescence Measurements S20
   3.1. Closed Cycle Quenching Factors S20
   3.2. Open Cycle Quenching Factors S20
4. Cellular Uptake Experiments S22
   4.1. Cell Culture S22
   4.2. Uptake into HeLa Kyoto Cells S22
      4.2.1. Kinetics S23
      4.2.2. Concentration Dependence S23
   4.3. Quantitative Analysis by Flow Cytometry S24
   4.4. Endocytosis Inhibition S25
   4.5. DTNB Assay S26
   4.6. MTT Assay S27
5. Thiol-Oligosulfide Exchange Reactions S28
   5.1. NMR Studies S28
      5.1.1. Reaction of BPS₅ with DTT S28
      5.1.2. Reaction of BPS₅ with GSH S30
      5.1.3. Reaction of BPS₅ with DTNB S32
      5.1.4. Reaction of BPS₅ with GSSG S32
   5.2. HPLC Studies S34
      5.2.1. Equilibration of BPS₅ in PBS Buffer S34
      5.2.2. Reaction of BPS₅ with DTT S35
      5.2.3. Reaction of BPS₅ with GSH S36
5.2.4. Reaction of BPS₅ with DTNB

5.2.5. Reaction of BPS₅ with GSSG

5.2.6. Reaction of BPS₅ with GSH and GSSG

5.2.7. Reaction of BPS₅ with Lipoic Acid

5.2.8. Reaction of BPS₅ with GSH and Lipoic Acid

5.2.9. Reaction of BPS₅ with Glycine

5.2.10. Reaction of BPS₅ with Glycine and GSSG

5.2.11. Reaction of BPS₅ with Histidine

5.2.12. Reaction of BPS₅ with Histidine and GSSG

5.2.13. Reaction of BPS₅ with n-BuNH₂

5.2.14. Reaction of BPS₅ with n-BuNH₂ and GSSG

5.2.15. Reaction of AspA with GSH

5.3. LC-MS Studies

5.3.1. LC-MS Analysis of BPS₅ in PBS Buffer

5.3.2. LC-MS Analysis of BPS₅ in the Presence of GSH

5.3.3. LC-MS Analysis of BPS₅ in the Presence of Catalytic Amounts of GSH and Lipoic Acid

5.4. Affinity Column Chromatography

6. Delivery Applications

6.1. Cellular Uptake of Fluorescent Proteins

6.1.1. Preparation of Streptavidin Complexes

6.1.2. Cellular Uptake of Streptavidin Complexes

6.2. Cellular Uptake of Artificial Metalloenzymes

6.2.1. Preparation of Biotinylated Catalysts

6.2.2. Preparation of Streptavidin Complexes

6.2.3. Catalytic Reactions in Cells

6.3. Cellular Uptake of Fluorescent CPS (Cell-Penetrating Streptavidin)

6.3.1. Preparation of CPS Complexes

6.3.2. Cellular Uptake of CPS Complexes
7. Supporting References S61
8. NMR Spectra S63
1. Materials and Methods

As in reference [S1], Supporting Information. Briefly, reagents for synthesis were purchased from Brunschwig, Alfa Aesar, Merck, TCI, Acros and Click Chemistry Tools. Buffers and salts of the best grade available from Fluka or Sigma-Aldrich were used as received. Phosphate buffered saline (PBS), MEM Alpha Medium, DMEM/high glucose Medium, Leibovitz’s L-15 Medium, Penicillin-Streptomycin, TrypLE Express Enzyme, and SYTOX Red Dead Cell Stain were obtained from Life Technologies. All reactions were performed under N₂ or Ar atmosphere. Deuterated sodium phosphate buffer was prepared by dissolving phosphoric acid (2 mmol, 238 mg, 85% D₃PO₄ in D₂O) in 20 mL of D₂O and adjusting pD with NaOD (30 wt% solution in D₂O). The pH meter reading in D₂O was corrected (pD = pH meter reading + 0.4).[S2,S3]

Unless stated otherwise, column chromatography was carried out on silica gel 60 (SiliaFlash® P60, SILICYCLE, 230–400 mesh). Analytical thin layer chromatography (TLC) were performed on silica gel 60 F254 (Merck). Fluorescence measurements were performed with a FluoroMax-3 spectrofluorometer (Horiba Jobin Yvon GmbH) or a FluoroMax-4 spectrofluorometer (Horiba Scientific) equipped with a stirrer and a temperature controller (25 ºC). Fluorescence spectra were corrected using instrument-supplied correction factors. UV-Vis spectra were recorded on a JASCO V-650 spectrophotometer equipped with a stirrer and a temperature controller (25 ºC) and are reported as maximal absorption wavelength λ in nm (extinction coefficient ε in M⁻¹cm⁻¹).

HPLC was performed using JASCO LC-2000 Plus system equipped with quaternary pump (JASCO PU-2089) and UV/Vis detector (JASCO UV-2077 Plus, λabs at 443 nm for detection). The chromatographic column used was a Phenomenex Jupiter Proteo (150 × 3 mm, 5 μm particles size), flow 0.7 mL/min with the following conditions: a linear elution gradient from 90% H₂O/10% CH₃CN + 0.1% TFA to 100% CH₃CN + 0.1% TFA in 6 min, 100% CH₃CN + 0.1% TFA for 1 min, then return to 90% H₂O/10% CH₃CN + 0.1% TFA. LCMS were recorded using a Thermo Scientific Accela HPLC equipped with a Thermo C18 Hypersil GOLD column (50 × 2.1 mm, 1.9 μm particles size) coupled with a LCQ Fleet three-dimensional ion trap mass spectrometer (ESI,
Thermo Scientific) with a linear elution gradient from 95% H$_2$O/5% CH$_3$CN + 0.1% TFA to 10% H$_2$O/90% CH$_3$CN + 0.1% TFA in 4.0 minutes at a flow rate of 0.75 mL/min. UHPLC-TOF MS analyses were performed using Waters Xevo G2-S ToF under the following conditions: Waters X-Select C18 column, 2.1 x 50 mm; 0.5 mL/min; 40 °C; CH$_3$CN/H$_2$O, with 5 mM NH$_4$OAc, 0:100 for 0.25 min, linear gradient to 95:5 in 2.5 min, 95:5 for 1.0 min, then return to 0:100; ESI-MS detector was calibrated using Leu-enkephalin as an internal standard. Reverse phase flash chromatography was performed on Biotage Isolera™ Four (column: SNAP Ultra C18 12 g, eluents: CH$_3$CN and H$_2$O with 0.1% TFA). pH values were measured with a Consort C832 multi-parameter analyzer equipped with a VWR glass membrane pH electrode calibrated with Titrisol solution from Merck at pH 4.00 and 7.00. Melting points (Mp) were measured on a Melting Point M-565 (BUCHI). IR spectra were recorded on a Perkin Elmer Spectrum 100 FT-IR spectrometer (ATR, Golden Gate, unless stated) and are reported as wavenumbers $\nu$ in cm$^{-1}$ with band intensities indicated as s (strong), m (medium), w (weak). $^1$H and $^{13}$C spectra were recorded (as indicated) either on a Bruker 300 MHz, 400 MHz or 500 MHz spectrometer and are reported as chemical shifts ($\delta$) in ppm relative to TMS ($\delta = 0$). Spin multiplicities are reported as a singlet (s), doublet (d), triplet (t) and quartet (q) with coupling constants ($J$) given in Hz, or multiplet (m). Broad peaks are marked as br. $^1$H and $^{13}$C resonances were assigned with the aid of additional information from 1D and 2D NMR spectra (H,H-NOESY, H,H-COSY, DEPT 135, HSQC and HMBC). ESI-MS for the characterization of new compounds was performed on an ESI API 150EX and are reported as mass-per-charge ratio $m/z$ (intensity in %, [assignment]). HR ESI-MS for the characterization of new compounds were performed on a QSTAR Pulsar (AB/MDS Sciex) or Xevo G2-S Tof (Waters) and are reported as mass-per-charge ratio $m/z$ calculated and observed. Fluorescence imaging was performed using Leica SP5 confocal microscope, equipped with 63X oil immersion objective lens. Flow cytometry measurements were performed using Beckman Coulter Gallios™ (6 colors 2 lasers) flow cytometer. Thiopropyl Sepharose 6B was from GE Healthcare.
**Abbreviations.** AIBN: Azobisisobutyronitrile; AspA: Asparagusic acid; BTTAA: 2-(4-((bis((1-(tert-butyl)-1H,1,2,3-triazol-4-yl)methyl)amino)methyl)-1H-1,2,3-triazol-1-yl)acetic acid; CLSM: Confocal laser scanning microscopy; DABCO: 1,4-Diazabicyclooctane; DCC: N,N’-Dicyclohexylcarbodiimide; DIAD: Diisopropyl azodicarboxylate; DIC: N,N’-Diisopropylcarbodiimide; DIPEA: N,N-Diisopropylethylamine; DMAc: N,N-Dimethylacetamide; DMAP: 4-Dimethylaminopyridine; DMF: N,N-Dimethylformamide; DMSO: Dimethyl sulfoxide; DTNB: 5,5’-Dithiobis(2-nitrobenzoic acid); DTT: 1,4-Dithio-DL-threitol; EDCI: 1-Ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride; EDTA: Ethylenediaminetetraacetic acid; EtOAc: Ethyl acetate; ETP: Epidithiodiketopiperazine; FBS: Fetal bovine serum; FL: Fluorescein; GSH: Glutathione; HOBT: Hydroxybenzotriazole; LA: Lipoic acid; mβCD: Methyl-β-cyclodextrin; MEM: Minimum essential media; MeOH: Methanol; MTT: 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide; NBS: N-Bromosuccinimide; NHS: N-Hydroxysuccinimide; PBS: Phosphate buffer saline; PE: Petroleum ether; PS: Penicillin/Streptomycin; rpm: Rotation per minute; rt: Room temperature; SDS: Sodium dodecyl sulfate; SR: Sytox red; TEA: Triethylamine; TFA: Trifluoroacetic acid; THF: Tetrahydrofuran; TNBSA: 2,4,6-Trinitrobenzene sulfonic acid.
2. Synthesis

2.1. Synthesis of Benzopolysulfanes

**Scheme S1.** (a) SOCl₂, MeOH, rt, 12 h, 95%; (b) Me₂NC(=S)Cl, DABCO, DMAc, 0 °C to rt, 12 h, 84%; (c) Ph₂O, 240 °C, 4 h, 63%; (d) NaOH, H₂O, 70 °C, 4 h, 78%; (e) Me₂SnCl₂, NaOH, EtOH/H₂O, rt, 2 h, 74%; (f) DCC, DMAP, 4-nitrophenol, CH₂Cl₂/DMF, rt, 12 h; (g) S₂Cl₂, 0 °C to rt, 24 h, 20 % (two steps from 39); (h) 40, DMF, rt, 12 h, 15%.

**Compound 35.** SOCl₂ (9.40 mL, 130 mmol) was added dropwise to a solution of 3,4-dihydroxybenzoic acid 9 (5.0 g, 32 mmol) in MeOH (100 mL) at 0°C. The solution was stirred for 12 h at rt. Solvent was removed under reduced pressure. Water was added and the mixture was extracted with EtOAc (3 x 50 mL). The combined organic phase was washed with water, dried over Na₂SO₄, filtered and concentrated to afford compound 35 (5.2 g, 95%) as a colorless solid. Spectroscopic data are consistent with those reported in the literature.\[^{[S4]}\]

**Compound 36-39** were synthesized and purified according to procedures described in the literature.\[^{[S4]}\]

**Compound 10.** A catalytic amount of DMAP (30 mg) was added to a solution of compound 39 (440 mg, 1.32 mmol) in dry CH₂Cl₂ (10 mL)/DMF (4 mL). After 5 min, DCC (354 mg, 1.72 mmol) was added and the solution was stirred for another 10 min. 4-Nitrophenol (367 mg, 2.64 mmol) was added and the solution was stirred overnight.
The colorless precipitate of urea was filtered off. Solvent was removed under reduced pressure. EtOAc was added to the residue and the mixture was washed with saturated citric acid solution, saturated aqueous Na₂CO₃ solution, brine, dried over Na₂SO₄, filtered and concentrated to afford crude product 10, which was used in the next step without further purification. Spectroscopic data are consistent with those reported in the literature.[S4]

**Compound 11** was synthesized and purified according to procedures described in the literature.[S4]

**Compound 40** was synthesized and purified according to procedures described in the literature.[S1]

**Compound 1.** To a solution of 11 (5.0 mg, 0.012 mmol) in THF (0.4 mL)/DMF (0.4 mL) was added compound 40 (5.6 mg, 0.012 mmol). The mixture was stirred at rt overnight. After the removal of DMF *in vacuo*, the residue was purified by reverse phase flash chromatography (80% H₂O/20% CH₃CN + 0.1% TFA to 20% H₂O/80% CH₃CN + 0.1% TFA) to yield compound 1 (1.3 mg, 15%) as an orange solid. Mp: > 250 °C; IR (neat): 3263 (br), 3065 (br), 1635 (m), 1588 (s), 1535 (s), 1454 (m), 1386 (m), 1310 (s), 1263 (s), 1200 (s), 1178 (s), 1116 (s), 914 (m), 847 (m), 799 (m), 760 (m), 720 (m), 667 (m), 597 (m), 575 (m); ¹H NMR (400 MHz, CD₃CN/DMSO-*d₆*): 9.50 (s, 1H), 9.21 (br s, 1H), 8.32 (d, ⁴J (H,H) = 2.0 Hz, 1H), 8.16 (d, ⁴J (H,H) = 1.6 Hz, 1H), 8.04 (s, 1H), 7.91 (d, ³J (H,H) = 8.0 Hz, 1H), 7.83 (dd, ³J (H,H) = 8.0 Hz, ⁴J (H,H) = 2.0 Hz, 1H), 7.79 (s, 1H), 7.70 (d, ³J (H,H) = 8.0 Hz, 1H), 7.07 (d, ³J (H,H) = 8.0 Hz, ⁴J (H,H) = 1.2 Hz, 2H), 6.69 (d, ⁴J (H,H) = 2.0 Hz, 2H), 6.66 (dd, ³J (H,H) = 8.4 Hz, ⁴J (H,H) = 1.2 Hz, 2H), 6.55-6.51 (m, 2H), 3.84-3.80 (m, 2H), 3.62-3.56 (m, 2H); ¹³C NMR (100 MHz, CD₃CN/DMSO-*d₆*): 182.7 (C), 169.7 (C), 166.2 (C), 160.8 (C), 153.4 (C), 149.5 (C), 147.1 (C), 144.6 (C), 141.9 (C), 137.7 (C), 137.1 (CH), 135.8 (CH), 131.4 (CH), 130.4 (CH), 130.2 (CH), 128.3 (C), 125.1 (CH), 119.1 (C), 113.4 (CH), 111.1 (C), 103.4 (CH), 44.6 (CH₂), 40.2 (CH₂); HRMS (ESI, +ve) calcd for C₃₀H₂₅N₃O₆S₆ ([M+H]⁺): 711.9827, found: 711.9828.
Scheme S2. (a) TEA, THF/DMF, rt, 4 h, 15%.

**Compound 41** was synthesized and purified according to procedures described in the literature.[S5]

**Compound 2.** To a solution of compound 11 (30 mg, 0.075 mmol), compound 41 (23 mg, 0.050 mmol) in DMF (1.2 mL) was added a solution of TEA (3.5 μL, 0.025 mmol) in THF (0.1 mL). The reaction was stirred for 4 h at rt. After the removal of solvent in vacuo, the residue was purified by reverse phase flash chromatography (90% H2O/10% CH3CN + 0.1% TFA to 20% H2O/80% CH3CN + 0.1% TFA) to yield compound 2 (5.3 mg, 15%) as a pale-yellow solid. Mp: 130 – 132 °C; IR (neat): 3282 (br), 3104 (s), 2931 (m), 2860 (m), 1681 (s), 1634 (s), 1541 (s), 1438 (m), 1368 (m), 1312 (m), 1266 (m), 1202 (s), 1178 (s), 1132 (s), 840 (m), 801 (m), 757 (m), 722 (s), 684 (s), 592 (s), 569 (s), 540 (s), 523 (s); 1H NMR (400 MHz, DMSO-d6): 8.71 (t, J(H,H) = 6.4 Hz, 1H), 8.35 (d, J(H,H) = 2.0 Hz, 1H), 8.04 (d, J(H,H) = 8.0 Hz, 1H), 7.89 (dd, J(H,H) = 8.0 Hz, J(H,H) = 2.0 Hz, 1H), 7.72-7.69 (m, 2H), 6.40-6.36 (m, 2H), 4.32-4.28 (m, 1H), 4.13-4.10 (m, 1H), 3.24 (q, J(H,H) = 6.4 Hz, 2H), 3.11-3.06 (m, 1H), 3.02-2.97 (m, 4H), 2.82 (dd, J(H,H) = 12.4 Hz, J(H,H) = 5.2 Hz, 1H), 2.57 (d, J(H,H) = 12.4 Hz, 1H), 2.06-2.02 (m, 4H), 1.64-1.20 (m, 20H); 13C NMR (100 MHz, DMSO-d6): 171.8 (2xCH), 163.8 (C), 162.7 (C), 145.2 (C), 142.9 (C), 136.6 (C), 136.3 (CH), 134.5 (CH), 129.6 (CH), 61.0 (CH), 59.2 (CH), 55.4 (CH), 39.8 (CH2), 39.3 (CH2), 38.3 (CH2), 35.3 (CH2), 35.2 (CH2), 29.13 (CH2), 29.12 (CH2), 28.7 (CH2), 28.2 (CH2), 28.0 (CH2), 26.1 (2xCH2), 25.3 (CH2), 25.0 (CH2); HRMS (ESI, +ve) calcd for C29H44N2O4S6 ([M+H]+): 718.1712, found: 718.1716.
**Scheme S3.** (a) DIAD, PPh₃, THF, rt, 12 h; (b) N₂H₄, MeOH, reflux, overnight; (c) THF, rt, 12 h.

**Compound 44** was synthesized and purified according to procedures described in the literature.[S6]

**Compound 45** was synthesized and purified according to procedures described in the literature.[S7]

**Compound 3** was synthesized and purified according to procedures described in the literature.[S4]

**Scheme S4.** (a) DIPEA, HOBT, EDCI, 2-propynylamine, DMF, rt, 12 h; (b) S₂Cl₂, 0 °C to rt, 24 h, 12% (two steps from 39).

**Compound 47.** To a stirred solution of compound 39 (118 mg, 0.354 mmol) in dry DMF (3 mL) was added 2-propynylamine (32 μL, 0.46 mmol), HOBT (81.4 mg, 0.531 mmol), EDCI (101 mg, 0.531 mmol) and DIPEA (195 μL, 1.06 mmol). The reaction was stirred for 12 h at rt. EtOAc was added and the mixture was washed with citric acid solution (10%), saturated aqueous NaHCO₃ solution, brine, dried over Na₂SO₄, filtered and concentrated to afford crude product 46 (115 mg), which was used in the next step without further purification.

To a solution of crude 46 in dry CH₂Cl₂ (25 mL) at 0 °C was added dropwise a solution of S₂Cl₂ (62 μL, 0.78 mmol) in dry CH₂Cl₂ (4 mL) under N₂ atmosphere. The reaction mixture was allowed to reach rt and stirred overnight. CH₂Cl₂ was added and
the mixture was washed with water and brine. The organic layer was dried with over Na₂SO₄ and concentrated. The residue was purified by flash silica gel column chromatography (SiO₂, pentane/ethyl acetate 3:1, Rₜ 0.36), yielding compound 47 (13 mg, 12% from 39) as a pale-yellow solid. Mp: 170 °C (decop.); IR (neat): 3265 (m), 2923 (s), 2854 (s), 1714 (m), 1635 (s), 1584 (m), 1536 (s), 1477 (m), 1354 (m), 1296 (m), 1242 (m), 840 (m); ¹H NMR (400 MHz, CDCl₃): 8.23 (d, ²J (H,H) = 2.4 Hz, 1H), 7.92 (d, ³J (H,H) = 10.8 Hz, 1H), 7.73 (dd, ⁴J (H,H) = 10.8 Hz, ²J (H,H) = 2.8 Hz, 1H), 6.29 (br s, 1H), 4.26 (dd, ³J (H,H) = 6.8 Hz, ⁴J (H,H) = 3.6 Hz, 2H), 2.32 (t, ⁴J (H,H) = 3.6 Hz, 1H); ¹³C NMR (100 MHz, CDCl₃): 164.9 (C), 147.7 (C), 144.7 (C), 136.4 (CH), 135.5 (C), 134.5 (CH), 128.8 (CH), 79.0 (C), 72.6 (CH), 30.2 (CH₂); HRMS (ESI, +ve) calcd for C₁₀H₈NOS₅ ([M+H]⁺): 317.9209, found: 317.9213.

2.2. Synthesis of Epidithiodiketopiperazines

Scheme S5. (a) Methyl 2-chloroacetate, K₂CO₃, CH₃CN, rt, 12 h, 54%; (b) Boc-Gly-OH, DCC, DMAP, TEA, CH₂Cl₂, rt, 24 h, 74%; (c) 1. TFA, CH₂Cl₂, 0 °C to rt, 30 min; (d) toluene, reflux, 6 h, 79% (two steps from 49); (e) NaH, THF, tert-Butyl bromoacetate, 0 °C to rt, 12 h, 87%; (f) 1. NBS, AIBN, cyclohexane, reflux, 2 h, 2. Potassium thioacetate, CH₂Cl₂, rt, 12 h, 34%; (g) 1. NH₃, MeOH, rt, 30 min, 2. S₂Cl₂, CH₂Cl₂, 0 °C to rt, 2 h, 58%; (h) TFA, CH₂Cl₂, rt, 1 h, 100%; (i) NHS, DCC, DMF, rt, 24 h; (j) 40, THF/DMF, rt, 1.5 h, 48% (two steps from 53).

Compounds 48-52, and 13 were synthesized and purified according to procedures described in the literature.¹⁸⁸
**Compound 53.** Compound 13 (98 mg, 0.24 mmol) was stirred at rt for 30 min in 7 M methanolic ammonia (0.8 mL) for the complete deprotection of thioacetate group. After the removal of MeOH in vacuo, the residue was dissolved in CH$_2$Cl$_2$ (5 mL), cooled to 0 °C, and then treated with a solution of S$_2$Cl$_2$ (9.6 μL, 0.12 mmol) in CH$_2$Cl$_2$ (5 mL). After vigorous stirring for 2 h, water was added and the mixture was extracted with CH$_2$Cl$_2$ (3 × 10 mL). The combined organic extracts were washed with water and brine, dried over Na$_2$SO$_4$, filtered and concentrated. The residue was purified by flash silica gel column chromatography (SiO$_2$, pentane/ethyl acetate 67:33, R$_f$ 0.5), yielding compound 14 (44 mg, 58%) as a pale-yellow solid.

Compound 14 (23 mg, 0.060 mmol) was treated with a mixture of TFA/CH$_2$Cl$_2$ 1:2 (1.5 mL) and the mixture was stirred at rt for 1 h under N$_2$ atmosphere. Then the solvent was evaporated under reduced pressure. To the resulting oily residue was added water (0.5 mL) and the solvents was then removed by using lyophilization, yielding compound 53 (20 mg, quantitative yield) as a white solid. Mp: 66 – 68 °C; IR (neat): 3442 (w), 2932 (m), 2526 (w), 1731 (m), 1661 (s), 1449 (s), 1431 (s), 1402 (s), 1319 (m), 1273 (m), 1160 (s), 970 (m), 951 (m), 877 (m), 804 (s), 745 (m), 689 (m), 571 (s), 529 (s); $^1$H NMR (400 MHz, CD$_3$OD): 5.69 (s, 1H), 5.65 (s, 1H), 4.53 (d, $^2$J (H,H) = 17.4 Hz, 1H), 3.87 (d, $^2$J (H,H) = 17.4 Hz, 1H), 3.82-3.73 (m, 1H), 3.34-3.24 (m, 1H), 1.28 (t, $^3$J (H,H) = 7.2 Hz, 3H); $^{13}$C NMR (100 MHz, CD$_3$OD): 170.7 (C), 168.0 (C), 167.7 (C), 67.0 (CH), 66.1 (CH), 45.8 (CH$_2$), 41.7 (CH$_2$), 12.2 (CH$_3$); MS (ESI, CHCl$_3$/MeOH 1:1): 325 (50, [M–H$^-$]), 261 (100, [M–2S–H$^-$]).

**Compound 4.** To a solution of compound 53 (20 mg, 0.060 mmol) and NHS (7.6 mg, 0.067 mmol) in THF (1.5 mL) was added dropwise a solution of DCC (14 mg, 0.066 mmol) in THF (1.5 mL). The mixture was stirred at rt for 24 h and the resulting solution containing compound 54 was added to another flask charged with compound 40 (16 mg, 0.036 mmol) through a syringe filter (13 mm, pore size: 0.22 μm). THF was removed in vacuo and DMF (1.0 mL) was added. The reaction mixture was stirred at rt for 1 h. After the removal of DMF in vacuo, the residue was treated with CH$_2$Cl$_2$ (25 mL) and the orange precipitate was collected by centrifugation. The crude product was
purified by reverse phase flash chromatography (80% H₂O/20% CH₃CN + 0.1% TFA to 20% H₂O/80% CH₃CN + 0.1% TFA) to yielding compound 4 (13 mg, 48%) as an orange solid. Mp: 188 – 190 °C; IR (neat): 3282 (br), 3083 (w), 2940 (w), 1667 (m), 1637 (m), 1591 (s), 1545 (m), 1384 (m), 1310 (s), 1273 (s), 1177 (s), 1130 (s), 917 (m), 850 (m), 798 (m), 765 (m), 720 (m), 671 (m), 595 (m), 576 (m); ¹H NMR (400 MHz, CD₃CN/D₂O): 8.12 (d, ⁴J (H,H) = 2.0 Hz, 1H), 7.71 (dd, ³J (H,H) = 8.0 Hz, ⁴J (H,H) = 2.0 Hz, 1H), 7.16 (dd, ³J (H,H) = 8.0 Hz, ⁵J (H,H) = 0.8 Hz, 1H), 6.75-6.71 (m, 4H), 6.59 (dd, ³J (H,H) = 8.8 Hz, ⁴J (H,H) = 2.8 Hz, 2H), 5.51 (s, 1H), 5.48 (s, 1H), 4.40 (d, ²J (H,H) = 16.4 Hz, 1H), 3.86-3.62 (m, 5H), 3.48-3.35 (m, 2H), 3.24-3.15 (m, 1H), 1.18 (t, ³J (H,H) = 7.2 Hz, 3H); ¹³C NMR (100 MHz, CD₃CN/D₂O): 182.4 (C), 169.7 (C), 169.6 (C), 168.0 (C), 167.5 (C), 166.2 (C), 160.5 (C), 153.4 (C), 149.4 (C), 140.9 (C), 132.0 (CH), 130.3 (CH), 128.3 (C), 125.4 (CH), 120.4 (CH), 113.4 (CH), 111.4 (C), 103.2 (CH), 66.6 (CH), 65.7 (CH), 47.2 (CH₂), 44.4 (CH₂), 41.2 (CH₂), 39.7 (CH₂), 12.0 (CH₃); HRMS (ESI, +ve) calcd for C₃₁H₂₈N₅O₈S₅ ([M+H]⁺): 758.0536, found: 758.0528.

**Scheme S6.** (a) Methyl 2-chloroacetate, K₂CO₃, CH₃CN, rt, 12 h, 89%; (b) Boc-Gly-OH, DCC, DMAP, Et₃N, CH₂Cl₂, rt, 24 h; (c) 1. TFA, CH₂Cl₂, 0 °C to rt, 2 h, 2. basified with K₂CO₃; (d) toluene, reflux, 6 h, 41% (three steps from 55); (e) NaH, THF, tert-Butyl bromoacetate, 0 °C to rt, 12 h, 62%; (f) 1. NBS, AIBN, CCl₄, reflux, 4 h, 2. Potassium thioacetate, CH₂Cl₂, rt, 12 h, 55%; (g) 1. NH₃, MeOH, rt, 45 min, 2. I₂, CHCl₃, rt, 2 h, 86%; (h) TFA, CH₂Cl₂, rt, 2 h, 91%; (i) NHS, DCC, DMF, rt, 24 h; (j) 40, DMF, rt, 3 h, 40% (two steps from 62).

**Compound 55.** To a mixture of 2-phenoxyethyamine 15 (617 mg, 4.50 mmol) and
K₂CO₃ (622 mg, 4.50 mmol) suspended in CH₃CN (9 mL) was added dropwise methyl 2-chloroacetate (0.40 mL, 4.5 mmol) at rt with stirring for 12 h. The inorganic salts were first filtered off, then washed with CH₃CN and the combined filtrate was concentrated under reduced pressure. The oily residue was purified by flash silica gel column chromatography (SiO₂, CH₂Cl₂/MeOH 40:1, Rf 0.28), yielding 55 (840 mg, 89%) as a colorless oil. IR (neat): 2950 (w), 1737 (m), 1599 (m), 1495 (m), 1459 (m), 1436 (w), 1371 (w), 1292 (w), 1242 (m), 1200 (m), 1173 (m), 1153 (m), 1080 (w), 1048 (m), 982 (w), 885 (w), 796 (w), 753 (m), 690 (m); ¹H NMR (400 MHz, CDCl₃): 7.30-7.26 (m, 2H), 6.97-6.90 (m, 3H), 4.08 (t, ³J (H,H) = 4.8 Hz, 2H), 3.73 (s, 3H), 3.52 (s, 2H), 3.04 (t, ³J (H,H) = 4.8 Hz, 2H), 1.94 (br s, 1H); ¹³C NMR (100 MHz, CDCl₃): 172.8 (C), 158.9 (C), 129.6 (CH), 121.0 (CH), 114.7 (CH), 67.6 (CH₂), 52.0 (CH₃), 50.9 (CH₂), 48.6 (CH₂); MS (ESI, CHCl₃/MeOH 1:1): 210 (90, [M+H]+), 116 (100, [M–OPh]+).

**Compound 58.** A solution of Boc-Gly-OH (671 mg, 3.83 mmol) and compound 55 (800 mg, 3.83 mmol) in CH₂Cl₂ (8 mL) was treated sequentially with TEA (531 μL, 3.87 mmol), DCC (790 mg, 3.83 mmol), and DMAP (140 mg, 1.15 mmol), and the reaction mixture was stirred at rt for 24 h. The white precipitate was removed by filtration. The filtrate was concentrated under reduced pressure. The solid residue was purified by flash silica gel column chromatography (SiO₂, CH₂Cl₂/MeOH 60:1-40:1, Rf 0.35), yielding 56 (913 mg, 65%) as a colorless oil. The obtained oil was then treated with a mixture of TFA/CH₂Cl₂ 1:1 (8 mL) and the resulting solution was stirred for 2 h. Then the solvent was evaporated under reduced pressure. After drying the residue in vacuo, compound 57 as a TFA salt was obtained as a colorless sticky foam.

To the solution of this sticky foam in MeOH (5 mL) was added anhydrous K₂CO₃ (1.38 g, 10.0 mmol) with vigorous stirring at rt. After 30 min, the excess salts were removed by filtration and the filtrate was concentrated under reduced pressure. The residue was suspended in toluene and the reaction mixture was allowed to reflux with vigorous stirring for 6 h. After cooling to rt, toluene was removed under reduced pressure. The residue was suspended in MeOH and filtered. The filtrate was
concentrated under reduced pressure. The solid residue was purified by flash silica gel column chromatography (SiO$_2$, CH$_2$Cl$_2$/MeOH 10:1, $R_t$ 0.41), yielding 58 (368 mg, 41% from 55) as a white solid. Mp: 142 – 143 °C; IR (neat): 3120 (w), 3064 (w), 2932 (w), 1686 (s), 1660 (s), 1601 (s), 1588 (m), 1499 (m), 1474 (s), 1430 (m), 1325 (m), 1303 (m), 1239 (s), 1199 (m), 1120 (m), 1080 (m), 1017 (s), 994 (m), 910 (m), 877 (m), 829 (s), 782 (m), 750 (s), 685 (s), 624 (m); $^1$H NMR (400 MHz, CDCl$_3$): 7.31 - 7.26 (m, 2H), 6.99 - 6.95 (m, 1H), 6.89 - 6.86 (m, 2H), 6.84 (br s, 1H), 4.25 (s, 2H), 4.19 (t, $^3$J (H,H) = 4.8 Hz, 2H), 4.03 - 4.02 (m, 2H), 3.79 (t, $^3$J (H,H) = 4.8 Hz, 2H); $^{13}$C NMR (100 MHz, CDCl$_3$): 166.4 (C), 163.8 (C), 158.2 (C), 129.8 (CH), 121.5 (CH), 114.4 (CH), 66.4 (CH$_2$), 52.1 (CH$_2$), 46.8 (CH$_2$), 45.3 (CH$_2$); MS (ESI, CHCl$_3$/MeOH 1:1): 235 (70, [M+H]$^+$), 141 (100, [M–OPh]$^+$).

**Compound 59.** A suspension of compound 58 (2.50 g, 10.7 mmol) in THF (100 mL) was cooled to 0 °C in an ice-water bath under N$_2$ atmosphere. Then NaH (860 mg, 60% dispersion in mineral oil, 21.4 mmol) was added in small portions, and the mixture was allowed to stir at 0 °C for 30 min. tert-Butyl bromoacetate (3.10 mL, 21.4 mmol) was added dropwise with vigorous stirring. Then the reaction was warmed to rt and stirred overnight. Ice was added slowly to quench the excess sodium hydride and the solvent was removed under reduced pressure. The mixture was extracted with CH$_2$Cl$_2$ (3 × 50 mL). The combined organic extracts were washed with water and brine, dried over Na$_2$SO$_4$, filtered and concentrated. The residue was purified by flash silica gel column chromatography (SiO$_2$, CH$_2$Cl$_2$/MeOH 20:1, $R_t$ 0.43), yielding compound 59 (2.30 g, 62%) as a colorless solid. Mp: 75 – 76 °C; IR (neat): 2976 (w), 1737 (m), 1726 (m), 1653 (s), 1599 (m), 1483 (s), 1367 (m), 137 (m), 1294 (m), 1232 (s), 1197 (m), 1153 (s), 1052 (m), 1036 (m), 962 (m), 862 (w), 839 (w), 794 (w), 750 (s), 690 (m), 603 (w), 574 (w), 535 (w); $^1$H NMR (400 MHz, CDCl$_3$): 7.30-7.26 (m, 2H), 6.99-6.94 (m, 1H), 6.88-6.86 (m, 2H), 4.30 (s, 2H), 4.19 (t, $^3$J (H,H) = 4.8 Hz, 2H), 4.07 (s, 2H), 4.03 (s, 2H), 3.80 (t, $^3$J (H,H) = 4.8 Hz, 2H), 1.46 (s, 9H); $^{13}$C NMR (100 MHz, CDCl$_3$): 167.0 (C), 164.5 (C), 163.8 (C), 158.2 (C), 129.7 (CH), 121.5 (CH), 114.5 (CH), 83.0 (C), 66.3 (CH$_2$), 52.2 (CH$_2$), 51.1 (CH$_2$), 47.9 (CH$_2$), 46.5 (CH$_2$), 28.1 (CH$_3$); MS (ESI, CHCl$_3$/MeOH 1:1): 235 (70, [M+H]$^+$), 141 (100, [M–OPh]$^+$).
CHCl₃/MeOH 1:1): 349 (50, [M+H]⁺), 199 (100, [M–OPh–t-Bu]⁺).

**Compound 60.** To a suspension of compounds 59 (2.1 g, 6.0 mmol) in carbon tetrachloride (120 mL) was added NBS (2.20 g, 12.3 mmol) and AIBN (50 mg, 0.31 mmol) at rt. The resulting mixture was stirred for 4 h under reflux. After the reaction mixture was cooled, the pale yellow precipitate was filtered off and the filtrate was concentrated under reduce pressure. The dibrominated intermediate was dissolved in anhydrous CH₂Cl₂ (80 mL) and potassium thioacetate (2.74 g, 24.0 mmol) was then added in one portion. After vigorous stirring overnight, the excess salts were removed by filtration and the filtrate was concentrated. The solid residue was purified by flash silica gel column chromatography (SiO₂, PE/EtOAc 50:1-2:1, Rf 0.46), yielding compound 60 (1.64 g, 55%) as a colorless sticky gum. IR (neat): 2978 (w), 2928 (w), 1740 (m), 1687 (s), 1599 (m), 1495 (m), 1452 (m), 1422 (m), 1367 (m), 1272 (m), 1231 (s), 1153 (s), 1118 (s), 1082 (m), 1037 (m), 946 (s), 847 (m), 753 (m), 692 (m), 608 (s), 516 (s); ¹H NMR (400 MHz, CDCl₃): 7.29–7.25 (m, 2H), 6.97–6.90 (m, 3H), 6.17 (s, 1H), 5.89 (s, 1H), 4.30 (d, J (H,H) = 17.6 Hz, 1H), 4.19–4.17 (m, 2H), 4.11–4.09 (m, 1H), 3.73 (d, J (H,H) = 17.6 Hz, 1H), 3.46–3.39 (m, 1H), 2.46 (s, 6H), 1.42 (s, 9H); ¹³C NMR (100 MHz, CDCl₃): 192.7 (C), 191.8 (C), 166.2 (C), 163.8 (C), 163.7 (C), 158.4 (C), 129.6 (CH), 121.4 (CH), 114.8 (CH), 82.9 (C), 65.2 (CH₂), 62.2 (CH), 62.1 (CH), 47.2 (CH₂), 45.1 (CH₂), 30.7 (CH₃), 30.6 (CH₃), 28.0 (CH₃); MS (ESI, CHCl₃/MeOH 1:1): 497 (70, [M+H]⁺), 261 (100, [M–(CH₂)₂OPh–CH₂COO–t-Bu]+H⁺).

**Compound 61.** Compound 60 (130 mg, 0.262 mmol) was stirred at rt for 30 min in 7 M methanolic ammonia (1.5 mL) for the complete deprotection of thioacetate group. After totally removing MeOH in vacuo, the residue was dissolved with CHCl₃ (18 mL) and a solution of I₂ (64 mg, 0.25 mmol) in CHCl₃ (12 mL) was added dropwise over 20 min. After vigorous stirring for 1 h, the pale-yellow precipitate was filtered off and the filtrate was concentrated. The oily residue was purified by flash silica gel column chromatography (SiO₂, PE/EtOAc 2:1, Rf 0.5), yielding compound 61 (92 mg, 86%) as a pale-yellow solid. Mp: 125 – 126 °C; IR (neat): 2976 (w), 1746 (m), 1685 (s), 1598 (m), 1496 (m), 1443 (m), 1410 (m), 1368 (m), 1294 (m), 1227 (s), 1153 (s), 1081 (m),
1032 (m), 957 (m), 843 (m), 787 (m), 691 (m), 673 (m), 614 (m), 581 (m), 540 (m); $^1$H NMR (400 MHz, CDCl$_3$): 7.32-7.27 (m, 2H), 7.00-6.96 (m, 1H), 6.93-6.91 (m, 2H), 5.71 (s, 1H), 5.38 (s, 1H), 4.49 (d, $^2$J (H,H) = 17.6 Hz, 1H), 4.26-4.22 (m, 1H), 4.16-4.11 (m, 1H), 4.02-3.97 (m, 1H), 3.94-3.87 (m, 1H), 3.86 (d, $^2$J (H,H) = 17.6 Hz, 1H), 1.44 (s, 9H); $^{13}$C NMR (100 MHz, CDCl$_3$): 166.5 (C), 163.6 (C), 163.5 (C), 158.1 (C), 129.8 (CH), 121.7 (CH), 114.7 (CH), 83.6 (C), 66.5 (CH), 66.4 (CH$_2$), 65.8 (CH), 45.5 (CH$_2$), 44.4 (CH$_2$), 28.1 (CH$_3$); MS (ESI, CHCl$_3$/MeOH 1:1): 411 (20, [M+H]+), 428 (80, [M+NH$_4$]+), 291 (100, [M–(CH$_2$)$_2$OPh]+H$^+$).

**Compound 62.** Compound 61 (103 mg, 0.250 mmol) was treated with a mixture of TFA/CH$_2$Cl$_2$ 1:1 (12 mL) and the reaction was stirred at rt for 2 h. Then the solvent was evaporated under reduced pressure. To the resulting oily residue was added water (1.0 mL) and the solvent was then removed by using lyophilization, yielding compound 62 (93.2 mg, 91%) as a gray solid. Mp: 155 – 157 °C; IR (neat): 2982 (w), 2932 (w), 2646 (w), 2549 (w), 1729 (m), 1684 (s), 1586 (m), 1488 (m), 1439 (m), 1414 (m), 1396 (m), 1364 (w), 1313 (w), 1296 (w), 1228 (s), 1186 (s), 1169 (s), 1077 (m), 1031 (m), 957 (m), 905 (m), 757 (s), 688 (m), 667 (s), 617 (m), 597 (m), 543 (m); $^1$H NMR (400 MHz, CD$_3$OD): 7.28-7.23 (m, 2H), 6.97-6.91 (m, 3H), 5.96 (s, 1H), 5.83 (s, 1H), 4.49 (d, $^2$J (H,H) = 18.0 Hz, 1H), 4.25-4.20 (m, 1H), 4.17-4.11 (m, 1H), 4.08 (d, $^2$J (H,H) = 18.0 Hz, 1H), 4.03-3.97 (m, 1H), 3.92-3.86 (m, 1H); $^{13}$C NMR (100 MHz, CD$_3$OD): 171.0 (C), 166.0 (C), 165.8 (C), 159.9 (C), 130.5 (CH), 122.3 (CH), 115.8 (CH), 67.1 (CH), 66.9 (CH$_2$), 66.8 (CH), 45.3 (CH$_2$), 45.2 (CH$_2$); MS (ESI, CHCl$_3$/MeOH 1:1): 353 (100, [M–H]).

**Compound 6.** To a solution of compound 62 (5.0 mg, 0.014 mmol) and NHS (2.4 mg, 0.021 mmol) in DMF (200 μL) was added DIC (3.3 μL, 0.022 mmol). The mixture was stirred at rt for 24 h. Compound 40 (3.2 mg, 0.0071 mmol) was added and the reaction mixture was stirred at rt for another 2 h. After the removal of DMF in vacuo, the residue was treated with CH$_2$Cl$_2$. The precipitate was collected by centrifugation and washed with CH$_2$Cl$_2$ two times. The crude product was purified by reverse phase flash chromatography (80% H$_2$O/20% CH$_3$CN + 0.1% TFA to 20% H$_2$O/80% CH$_3$CN
+ 0.1% TFA) to yield compound 6 (2.2 mg, 40%) as an orange solid. Mp: 156 – 158 °C; IR (neat): 3328 (w), 3270 (w), 3093 (w), 1680 (s), 1638 (m), 1591 (s), 1540 (s), 1494 (m), 1455 (m), 1388 (m), 1240 (s), 1178 (s), 1117 (s), 1080 (m), 1034 (m), 995 (m), 952 (m), 911 (m), 848 (m), 800 (m), 757 (m), 721 (m), 691 (m), 671 (m), 601 (m), 567 (m), 556 (m); ¹H NMR (400 MHz, CD₂CN/DMSO-d₆): 9.75 (s, 1H), 8.30 (d, ⁴J (H,H) = 1.6 Hz, 1H), 7.83 (s, 1H), 7.76-7.74 (m, 1H), 7.58 (s, 1H), 7.28-7.24 (m, 2H), 7.09 (d, ³J (H,H) = 8.4 Hz, 1H), 6.95-6.91 (m, 3H), 6.69 (d, ⁴J (H,H) = 2.4 Hz, 2H), 6.66 (d, ³J (H,H) = 8.8 Hz, 2H), 6.56 (dd, ³J (H,H) = 8.8 Hz, ⁴J (H,H) = 2.4 Hz, 2H), 5.84 (s, 1H), 5.76 (s, 1H), 4.36 (d, ²J (H,H) = 16.4 Hz, 1H), 4.20-4.07 (m, 2H), 3.91-3.77 (m, 3H), 3.70-3.68 (m, 2H), 3.48-3.31 (m, 2H); ¹³C NMR (100 MHz, CD₂CN/DMSO-d₆): 182.6 (C), 169.8 (C), 168.2 (C), 165.0 (C), 164.8 (C), 160.7 (C), 159.3 (C), 153.3 (C), 149.2 (C), 142.1 (C), 131.1 (CH), 130.4 (CH), 130.1 (CH), 128.0 (C), 124.9 (CH), 122.0 (CH), 115.5 (CH), 113.4 (CH), 111.1 (C), 103.3 (CH), 66.8 (CH), 66.7 (CH), 66.6 (CH₂), 46.4 (CH₂), 44.6 (CH₂), 44.4 (CH₂), 39.4 (CH₂); HRMS (ESI, +ve) calcd for C₃₇H₃₂N₅O₉S₃ ([M+H]⁺): 786.1357, found: 786.1361.

2.3. Synthesis of Controls

Scheme S7. (a) TEA, DMF, rt, 2 h, 71%.

Compound 64 was synthesized and purified according to procedures described in the literature.⁸⁹

Compound 8. To a solution of compound 40 (22.4 mg, 0.0499 mmol), compound 64 (16.4 mg, 0.0748 mmol) in DMF (0.9 mL) was added dropwise a solution of TEA (7 μL, 0.05 mmol) in DMF (0.1 mL). The reaction was stirred for 2 h at rt. After the removal of solvent in vacuo, the residue was purified by reverse phase flash chromatography (80% H₂O/20% CH₃CN + 0.1% TFA to 20% H₂O/80% CH₃CN + 0.1%
TFA) to yield compound 8 (20.7 mg, 71%) as a yellow solid. Mp: 158 – 160 °C; IR (neat): 3061 (w), 2595 (w), 1634 (m), 1597 (s), 1539 (s), 1490 (m), 1455 (m), 1385 (m), 1310 (s), 1272 (s), 1176 (s), 1119 (s), 969 (m), 917 (m), 849 (m), 797 (m), 764 (m), 717 (m), 668 (m), 598 (m), 566 (m), 547 (m); ¹H NMR (400 MHz, DMSO-d₆): 9.56-9.55 (m, 1H), 8.22 (s, 1H), 7.88-7.80 (m, 4H), 7.71 (d, ³J (H,H) = 8.4 Hz, 1H), 7.53-7.48 (m, 1H), 7.45-7.41 (m, 2H), 7.09 (d, ³J (H,H) = 8.4 Hz, 1H), 6.69 (d, ⁴J (H,H) = 2.4 Hz, 2H), 6.66 (d, ³J (H,H) = 8.4 Hz, 2H), 6.54 (dd, ³J (H,H) = 8.8 Hz, ⁴J (H,H) = 2.4 Hz, 2H), 3.81 (br s, 2H), 3.59 (q, ³J (H,H) = 6.4 Hz, 2H); ¹³C NMR (100 MHz, DMSO-d₆): 182.7 (C), 169.7 (C), 168.5 (C), 160.8 (C), 153.4 (C), 149.5 (C), 142.0 (C), 135.6 (C), 132.2 (CH), 131.4 (CH), 130.2 (CH), 129.3 (CH), 128.2 (CH), 125.1 (CH), 113.5 (CH), 111.2 (C), 103.4 (CH), 45.1 (CH₂), 40.1 (CH₂); HRMS (ESI, +ve) calcd for C₃₀H₂₄N₅O₆S ([M+H]⁺): 554.1380, found: 554.1387.

### 2.4. Synthesis of Biotinylated Fluorophores

![Scheme S8](image)

**Scheme S8.** (a) TEA, DMF, rt, 4 h, 62%.

**Compound 33.** To a solution of compound 65 (39 mg, 0.10 mmol) and compound 41 (45.5 mg, 0.100 mmol) in DMF (4 mL) was added a solution of TEA (42 μL, 0.30 mmol) in DMF (1 mL). The reaction was stirred for 4 h at rt. After the removal of solvent in vacuo, the residue was purified by reverse phase flash chromatography (80% H₂O/20% CH₃CN + 0.1% TFA to 20% H₂O/80% CH₃CN + 0.1% TFA) to yield compound 33 (52.3 mg, 62%) as a yellow solid. Mp: 128 – 130 °C; IR (neat): 3364 (br), 1650 (m), 1437 (w), 1408 (w), 1318 (w), 1011 (s), 950 (s), 614 (s); ¹H NMR (400 MHz, DMSO-d₆): 10.1 (br s, 1H), 9.86 (s, 1H), 8.23 (s, 1H), 8.06 (s, 1H), 7.73-7.69 (m, 3H), 7.17 (d, ³J (H,H) = 8.0 Hz, 1H), 6.67 (d, ⁴J (H,H) = 2.0 Hz, 2H), 6.61-6.55 (m, 4H),
6.41-6.36 (m, 2H), 4.31-4.28 (m, 1H), 4.13-4.10 (m, 1H), 3.51-3.48 (m, 2H), 3.11-3.06 (m, 1H), 3.03-2.98 (m, 4H), 2.81 (dd, \(^2J\) (H,H) = 12.4 Hz, \(^3J\) (H,H) = 4.8 Hz, 1H), 2.57 (d, \(^2J\) (H,H) = 12.4 Hz, 1H), 2.08-2.02 (m, 4H), 1.64-1.21 (m, 20H); \(^{13}\)C NMR (100 MHz, DMSO-\(d_6\)): 180.3 (C), 171.76 (C), 171.75 (C), 168.5 (C), 162.7 (C), 159.4 (C), 151.8 (C), 147.0 (C), 141.4 (C), 129.4 (CH), 129.0 (CH), 126.5 (C), 124.0 (CH), 116.3 (CH), 112.6 (CH), 109.7 (C), 102.2 (CH), 61.0 (CH), 59.2 (CH), 55.4 (CH), 43.8 (CH\(_2\)), 39.8 (CH\(_2\)), 38.31 (CH\(_2\)), 38.28 (CH\(_2\)), 35.4 (CH\(_2\)), 35.2 (CH\(_2\)), 29.1 (CH\(_2\)), 28.20 (CH\(_2\)), 28.15 (CH\(_2\)), 28.0 (CH\(_2\)), 26.2 (CH\(_2\)), 26.12 (CH\(_2\)), 26.10 (CH\(_2\)), 25.3 (CH\(_2\)), 25.1 (CH\(_2\)); HRMS (ESI, +ve) calcd for C\(_{43}\)H\(_{53}\)N\(_6\)O\(_8\)S\(_2\) ([M+H]\(^{+}\)): 845.3301, found: 845.3357.

3. Fluorescence Measurements

3.1. Closed Cycle Quenching Factors

Stock solutions of compound 1, 4, 5, 6, 7, and 8 (10 mM in DMSO) were diluted to give 1 μM solution of fluorescent compounds in PBS buffer (10 mM phosphate, 137 mM sodium chloride, 2.7 mM potassium chloride, pH 7.4). The fluorescence emission spectra were then recorded from 498 nm to 600 nm upon excitation at 488 nm (slits 1.5 nm, Figure S1). Closed cycle quenching factors were calculated by first dividing the value of emission intensity at 512 nm by the absorbance at 494 nm, which is then normalized against that of 8 (Table S1).

![Figure S1](image)

*Figure S1.* Absorption (dashed) and emission (solid) spectra of 1 (ocher), 4 (green), 5 (purple), 6 (red), 7 (blue), and 8 (black) in PBS buffer upon excitation at 488 nm.

3.2. Open Cycle Quenching Factors

To obtain the open cycle quenching factor, solutions of fluorescent compounds (10 μL, 100 μM in PBS buffer) were mixed with DTT (10 μL, 2 mM in PBS buffer) for 30
S
min before to be diluted to 1 μM in PBS buffer. The fluorescence emission was then recorded from 498 nm to 600 nm upon excitation at 488 nm (slits 1.5 nm, Figure S2). Open cycle quenching factors were calculated by first dividing the value of emission intensity at 512 nm by the absorbance at 494 nm, which is then normalized against that of 8 (Table S1).

![Emission spectra](image)

**Figure S2.** Emission spectra of 1 (ocher), 4 (green), 5 (purple), 6 (red), 7 (blue), and 8 (black) in PBS buffer after treatment with DTT upon excitation at 488 nm.

| Entry | Compound | $I_{em\ (closed)}$<sup>[a]</sup> | $I_{em\ (open)}$<sup>[b]</sup> | Abs<sup>[c]</sup> | QF<sub>(closed)</sub><sup>[d]</sup> | QF<sub>(open)</sub><sup>[e]</sup> |
|-------|----------|-------------------|-------------------|---------|-----------------|-----------------|
| 1     | 8        | 9.25 × 10<sup>5</sup> | 9.22 × 10<sup>5</sup> | 0.0193  | 1.00            | 1.00            |
| 2     | 7        | 2.81 × 10<sup>5</sup> | 1.03 × 10<sup>6</sup> | 0.0229  | 3.91            | 1.06            |
| 3     | 6        | 6.96 × 10<sup>5</sup> | 1.18 × 10<sup>5</sup> | 0.0194  | 1.34            | 7.90            |
| 4     | 5        | 7.80 × 10<sup>5</sup> | 2.17 × 10<sup>5</sup> | 0.0224  | 1.38            | 4.96            |
| 5     | 4        | 6.60 × 10<sup>5</sup> | 2.75 × 10<sup>5</sup> | 0.0230  | 1.68            | 4.01            |
| 6     | 1        | 4.68 × 10<sup>4</sup> | 5.05 × 10<sup>4</sup> | 0.0197  | 20.2            | 18.6            |

<sup>[a]</sup>Closed cycle emission intensity, $\lambda_{em} = 512$ nm. <sup>[b]</sup>Open cycle emission intensity, $\lambda_{em} = 512$ nm. <sup>[c]</sup>Absorbance, $\lambda_{abs} = 494$ nm. <sup>[d]</sup>Closed cycle quenching factors. <sup>[e]</sup>Open cycle quenching factors.
4. Cellular Uptake Experiments

4.1. Cell Culture

Human cervical cancer-derived HeLa Kyoto cells were cultured in MEM Alpha Medium containing 10% fetal bovine serum (FBS), 1% Penicillin/Streptomycin (PS) and 1% L-Glutamine. The cells were grown at 37 °C under 5% CO₂ on a 25 cm³ tissue culture flask (TPD Corporation). Cells were detached by treatment with TrypLE Express at 37 °C for 10 min, followed by the addition of 10 mL MEM Alpha. The supernatant was discarded after centrifugation at 1400 × g for 2 min at 4 °C. The cells were resuspended in MEM Alpha and plated according to the concentration needed.

4.2. Uptake into HeLa Kyoto Cells

HeLa Kyoto cells were seeded at 9 × 10⁴ cells/mL on 35 mm glass-bottomed dishes (MatTeK) and cultured overnight. After removing the medium, the cells were washed twice with PBS and once with Leibovitz’s medium and treated with a solution of compound 1, 4, 5, 6, 7 or 8 (1 mL of 10 μM in Leibovitz’s medium). The cells were incubated for 45 min at 37 °C under 5% CO₂, then the medium was removed by aspiration. Cells were washed twice with Leibovitz’s medium and finally kept in Leibovitz’s medium. Distribution of fluorescent compounds was analyzed without fixing using confocal laser scanning microscope (Leica SP5) equipped with 63X oil immersion objective lens. Argon laser was used as light source (15% laser power) with excitation wavelength 488 nm and emission 498 – 550 nm (Leica HyD™ detector). During the imaging, the samples were kept at 37 °C.

![Figure S3. Confocal laser scanning microscopy images of HeLa Kyoto cells treated with compound 8 (a), 7 (b), 5 (c), 6 (d), 4 (e), and 1 (f) (10 μM in Leibovitz’s medium) for 45 min at 15% laser power. Scale bars: 10 μm.](image-url)
4.2.1. Kinetics

HeLa Kyoto cells were seeded at $9 \times 10^4$ cells/mL on 35 mm glass-bottomed dishes (MatTeK) and cultured overnight. After removing the medium, the cells were washed twice with PBS and once with Leibovitz’s medium and treated with a solution of compound 1 (1 mL of 10 μM in Leibovitz’s medium). The cells were incubated for 15 min, 30 min, 1 h, 1.5 h, 2 h or 4 h at 37 °C under 5% CO$_2$, then the medium was removed by aspiration. Cells were washed twice with Leibovitz’s medium and finally kept in Leibovitz’s medium. Distribution of fluorescent compounds was analyzed without fixing using confocal laser scanning microscope (Leica SP5) equipped with 63X oil immersion objective lens. Argon laser was used as light source (15% laser power) with excitation wavelength 488 nm and emission 498 – 550 nm (Leica HyD™ detector). During the imaging, the samples were kept at 37 °C. Fluorescence intensities of cells were quantified with ImageJ.

![Figure S4.](image)

Figure S4. Confocal laser scanning microscopy images (left) of HeLa Kyoto cells treated with compound 1 (10 μM in Leibovitz’s medium) for 15 min (a), 30 min (b), 1 h (c), 1.5 h (d), 2 h (e), and 4 h (f) at 15% laser power. Scale bars: 10 μm. Fluorescent intensities in cells as a function of time (right).

4.2.2. Concentration Dependence

HeLa Kyoto cells were seeded at $9 \times 10^4$ cells/mL on 35 mm glass-bottomed dishes (MatTeK) and cultured overnight. After removing the medium, the cells were washed twice with PBS and once with Leibovitz’s medium and treated with a solution of compound 1 (1 mL of 1, 2.5, 5, 10, 20, or 50 μM in Leibovitz’s medium). The cells
were incubated for 45 min at 37 °C under 5% CO₂, then the medium was removed by aspiration. Cells were washed twice with Leibovitz’s medium and finally kept in Leibovitz’s medium. Distribution of fluorescent compounds was analyzed without fixing using confocal laser scanning microscope (Leica SP5) equipped with 63X oil immersion objective lens. Argon laser was used as light source (15% laser power) with excitation wavelength 488 nm and emission 498 – 550 nm (Leica HyD™ detector). During the imaging, the samples were kept at 37 °C. Fluorescence intensities in cells were quantified with ImageJ.

Figure S5. Confocal laser scanning microscopy images (left) of HeLa Kyoto cells treated with compound 1 (1 μM (a), 2.5 μM (b), 5 μM (c), 10 μM (d), 20 μM (e), 50 μM (f) in Leibovitz’s medium) for 45 min at 15% laser power. Scale bars: 10 μm. Fluorescent intensities in cells as a function of BPS 1 concentration (right).

4.3. Quantitative Analysis by Flow Cytometry

HeLa-Kyoto cells were seeded at 9 × 10⁴ cells/well in a 6-well plate (BD Falcon) and cultured overnight. After removing the medium, the cells were washed twice with PBS and once with Leibovitz’s medium before being treated with 1 mL of compound 1, 4, 5, 6, 7, or 8 (20 μM in Leibovitz’s medium). The cells were incubated for 45 min at 37 °C then the medium was removed by aspiration. Cells were washed three times with PBS (2 x 1 mL) before detachment by treatment with 0.05% trypsin-EDTA (1 mL) at 37 °C for 10 min. Cold MEM (1 mL) and cold PBS (2 mL) were added and the cells were collected and pelleted by centrifugation at 1400 × g for 2 min. The supernatant was removed and the cells were washed with cold PBS (1 mL). The cells were re-
suspended in PBS (600 μL) containing Sytox Red (SR, 1 μL/mL) and EDTA (0.02%). Fluorescent signals of cells were detected by laser excitation at 488 nm (at least 10000 events of live cells were collected) on a Beckman Coulter Gallios cytometer. Cells staining positive for SR were excluded from analysis.

For 4 °C experiments, cells were pre-incubated in growing medium (MEM Alpha Medium containing 10% fetal bovine serum (FBS), 1% Penicillin/Streptomycin (PS) and 1% L-Glutamine) at 4 °C for 1 h. After removing the medium, the cells were washed with cold PBS (2 x 1 mL, 4 °C) and with cold Leibovitz’s medium (1 mL, 4 °C) before being treated with 1 mL of compound 1 (20 μM in Leibovitz’s medium). The cells were incubated for 45 min at 4 °C then the medium was removed by aspiration. Cells were washed three times with cold PBS (2 x 1 mL, 4 °C) before detachment by treatment with 0.05% trypsin-EDTA (1 mL) at 4 °C for 10 min. The following procedure was performed as same as described previously.

**Figure S6.** Flow cytometry analysis of cellular uptake (left) of 1, 4, 5, 6, 7 and 8 (20 μM in Leibovitz’s medium) at 37 °C for 45 min and 1 (20 μM in Leibovitz’s medium) at 4 °C for 45 min. Flow cytometry analysis of compounds (right) with closed cycle quenching factors (see Table S1); blank: Cells alone.

### 4.4. Endocytosis Inhibition

HeLa Kyoto cells were seeded at 9 × 10⁴ cells/mL in a 6-well plate (BD Falcon) and cultured overnight. After removing the medium, the cells were incubated for 30 min at 37 °C under 5% CO₂ with 1 mL of one of the specific endocytosis inhibitors in MEM: chlorpromazine (CPZ, 30 μM), methyl-β-cyclodextrin (mβCD, 50 μM), wortmannin
(wort, 50 nM) and cytochalasin B (cytoB, 10 μM). The solution was then removed by aspiration and the cells were washed twice with PBS. The cells were then treated with a solution of compound 1 (20 μM) and the same amount of inhibitor in MEM (1 mL). The cells were incubated for 45 min at 37 °C. The medium was then removed by aspiration and the cells were washed three times with PBS before treatment with 0.05% trypsin-EDTA (1 mL) at 37 °C for 10 min. Cold MEM (1 mL) and cold PBS (2 mL) were added to each well and the cells were collected and pelleted by centrifugation at 1400 × g for 2 min at 4 °C. The supernatant was removed and the cells were washed with cold PBS (1 mL). The cells were re-suspended in 600 μL PBS containing 1 μL/mL SR and 0.02% EDTA. Fluorescent signals of cells were detected by laser excitation at 488 nm (at least 10000 events of live cells were collected) on a Beckman Coulter Gallios cytometer. Cells staining positive for SR were excluded from analysis.

**Figure S7.** Flow cytometry analysis of cellular uptake of 1 upon endocytosis inhibition; blank: Stained cells with 1 in the absence of endocytosis inhibitor.

### 4.5. DTNB Assay

DTNB aqueous solution was neutralized and lyophilized. HeLa Kyoto cells were seeded at 9 × 10⁴ cells/mL in a 6-well plate (BD Falcon) and cultured overnight. After removing the medium, the cells were incubated at 37 °C under 5% CO₂ with 1 mL of a solution of DTNB (1.2, 2, or 5 mM, pre-neutralized) in DMEM for 30 min. The solution was then removed by aspiration and the cells were washed three times with PBS. The cells were then treated with compound 1 (20 μM) in DMEM. The cells were incubated for 1 h at 37 °C under 5% CO₂. The media was then removed by aspiration and the cells
were washed three times with PBS before detachment with trypsin for 10 min. Cold MEM (1 mL) and cold PBS (2 mL) were added to each well and the cells were collected and pelleted by centrifugation at 1400 × g for 2 min at 4 °C. The supernatant was removed and the cells resuspended in 600 μL PBS containing 1 μL/mL Sytox Red and 0.02% EDTA. Fluorescent signals of cells were detected by laser excitation at 488 nm (at least 10000 events of live cells were collected) on a Beckman Coulter Gallios cytometer. Cells staining positive for SR were excluded from analysis.

**Figure S8.** Flow cytometry analysis of cellular uptake of 1 upon modification of cell surface with DTNB.

### 4.6. MTT Assay

HeLa Kyoto cells (100 μL at 9 × 10⁴ cells/mL) were seeded in a 96-well plate (BD Falcon) and cultured overnight. After removing the medium, the cells were incubated with 100 μL of the 1, 4, 6, or 7 solution (10 μM in Leibovitz’s medium) for 24 h at 37 °C under 5% CO₂. Then, MTT solution (10 μL of 12 mM in sterile PBS) were added to each well. The cells were incubated for 4 h at 37 °C under 5% CO₂. The cells were then treated with SDS solution (100 μL of 100 g/L SDS in 0.01 M HCl) and incubated for 2 h in the dark at 37 °C under 5% CO₂. The absorbance of the resulting solution was measured at 570 nm.
Figure S9. Cell viability of 1 (brown triangles), 4 (yellow triangles), 6 (red diamonds), or 7 (pink circles) from MTT assay in HeLa Kyoto cells; control (cells alone): Blue filled square; polyarginine (pR): Blue open square.

5. Thiol-Oligosulfide Exchange Reactions

5.1. NMR Studies

5.1.1. Reaction of BPS₅ with DTT

Compound 3 (4.25 mg, 0.0100 mmol) was added to a 5-mL flask, then the flask was sealed with a septum and flushed with argon for 3 min. THF-d₈ (0.5 mL) was added and the mixture was stirred for 5 min at rt. A solution of DTT (1.54 mg, 0.00998 mmol) in deuterated sodium phosphate buffer (0.5 mL, 20 mM, pD = 5.5 or 7.8) was then added to the flask under argon atmosphere. ¹H NMR spectra were measured after 5 or 30 min of stirring (Figures S10-S13).

Figure S10. ¹H NMR spectra of (a) compound 3, (b) with DTT after 5 min, (c) and after 30 min in THF-d₈/D₂O at pD 5.5, and (d) DTT in THF-d₈/D₂O at pD 5.5.
Figure S11. Zoom of spectra in Figure S10.

Figure S12. $^1$H NMR spectra of (a) compound 3, (b) with DTT after 5 min, (c) and after 30 min in THF-$d_8$/D$_2$O at pD 7.8, and (d) DTT in THF-$d_8$/D$_2$O at pD 7.8.
Figure S13. Zoom of spectra in Figure S12.

5.1.2. Reaction of BPS₅ with GSH

Compound 3 (4.25 mg, 0.0100 mmol) was added to a 5-mL flask, then the flask was sealed with a septum and flushed with argon for 3 min. THF-₅ (0.5 mL) was added and the mixture was stirred for 5 min at rt. A solution of GSH (12.4 mg, 0.0403 mmol) in deuterated sodium phosphate buffer (0.5 mL, 80 mM, pD = 5.5 or 8.0) was then added to the flask under argon atmosphere. ¹H NMR spectra were measured after 5 or 30 min of stirring (Figures S14-S19).

Figure S14. ¹H NMR spectra of (a) compound 3, (b) with GSH after 5 min, (c) and after 30 min in THF-₅/D₂O at pD 5.5, (d) GSH in THF-₅/D₂O at pD 5.5, and (e) GSSG in THF-₅/D₂O at pD 5.5.
**Figure S15.** Zoom of spectra in Figure S14.

**Figure S16.** $^1$H NMR spectra of (a) compound 3, (b) with GSH after 5 min, (c) and after 30 min in THF-$d_8$/D$_2$O at pD 8.0, (d) GSH in THF-$d_8$/D$_2$O at pD 8.0, and (e) GSSG in THF-$d_8$/D$_2$O at pD 8.0.
5.1.3. Reaction of BPS₅ with DTNB

Compound 3 (2.13 mg, 0.00501 mmol) was added to a 5-mL flask, then the flask was sealed with a septum and flushed with argon for 3 min. THF-d₈ (0.5 mL) was added and the mixture was stirred for 5 min at rt. A solution of neutral DTNB disodium salt (4.40 mg, 0.0100 mmol or 22.0 mg, 0.0500 mmol) in deuterated sodium phosphate buffer (0.5 mL, 20 mM or 100 mM, pD = 8.0) was then added to the flask under argon atmosphere. ¹H NMR spectra were measured after 5 or 30 min of stirring (Figures S18-S19).

5.1.4. Reaction of BPS₅ with GSSG

Compound 3 (2.13 mg, 0.00501 mmol) was added to a 5-mL flask, then the flask was sealed with a septum and flushed with argon for 3 min. THF-d₈ (0.5 mL) was added and the mixture was stirred for 5 min at rt. A solution of neutral GSSG (30.7 mg, 0.0501 mmol) in deuterated sodium phosphate buffer (0.5 mL, 100 mM, pD = 8.0) was then added to the flask under argon atmosphere. ¹H NMR spectra were measured after 5 or 30 min of stirring (Figures S20-S21).
Figure S18. $^1$H NMR spectra of (a) compound 3, (b) with DTNB disodium salt (0.01 mmol) after 5 min, (c) with DTNB disodium salt (0.01 mmol) after 30 min, (d) with DTNB disodium salt (0.05 mmol) after 5 min, (e) and with DTNB disodium salt (0.05 mmol) after 30 min in THF-$_d_8$/D$_2$O at pD 8.0, (f) DTNB disodium salt in THF-$_d_8$/D$_2$O at pD 8.0.

Figure S19. Zoom of spectra in Figure S18.
Figure S20. $^1$H NMR spectra of (A) compound 3, (B) with GSSG after 5 min, (C) and after 30 min in THF-$d_8$/D$_2$O at pD 8.0, (D) GSH in THF-$d_8$/D$_2$O at pD 8.0, (F) GSSG in THF-$d_8$/D$_2$O at pD 8.0.

Figure S21. Zoom of spectra in Figure S20.

5.2. HPLC Studies

5.2.1. Equilibration of BPS$_5$ in PBS Buffer

A solution of FL-BPS$_5$ 1 in DMSO (100 μM, 14.0 μL) was mixed with PBS buffer (128 μL, pH 7.4). HPLC were measured after 0 min, 30 min, 2 h, 4 h, 6 h, 12 h or 24 h.
Figure S22. HPLC analysis of FL-BPS₅ 1 in PBS buffer for 0 min, 30 min, 2 h, 4 h, 6 h, 12 h, and 24 h (left, from top to bottom) and plot of relative content of FL-BPS₅ 1 (blue circles), FL-BPS₃ 16 (red squares) and others (green triangles) at different time (right).

5.2.2. Reaction of BPSs with DTT

A solution of FL-BPS₅ 1 in DMSO (100 μM, 4.00 μL, 1.0 eq.) was mixed with a solution of DTT in PBS buffer (5.56 μM (0.5 eq.), 11.1 μM (1.0 eq.), 22.2 μM (2.0 eq.), 111 μM (10 eq.), or 1.11 mM (100 eq.), 36 μL, pH 7.4). HPLC were measured after 5 or 30 min.

Figure S23. HPLC analysis of FL-BPS₅ 1 in the presence of 0, 0.5, 1, 2, 10, and 100 equivalent of DTT (from top to bottom) for 5 min (left) or 30 min (right); dashed line: FL-BPS₅ 1 for 30 min.
5.2.3. Reaction of BPS₅ with GSH

A solution of FL-BPS₅ 1 in DMSO (100 μM, 4.00 μL, 1.0 eq.) was mixed with a solution of GSH in PBS buffer (5.56 μM (0.5 eq.), 11.1 μM (1.0 eq.), 22.2 μM (2.0 eq.), 111 μM (10 eq.), or 1.11 mM (100 eq.), 36 μL, pH 7.4). HPLC were measured after 5 or 30 min.

Figure S24. HPLC analysis of FL-BPS₅ 1 in the presence of 0, 0.5, 1, 2, 10, and 100 equivalent of GSH (from top to bottom) for 5 min (left) or 30 min (right); dashed line: FL-BPS₅ 1 for 30 min.

5.2.4. Reaction of BPS₅ with DTNB

A solution of FL-BPS₅ 1 in DMSO (100 μM, 4.00 μL, 1.0 eq.) was mixed with a solution of DTNB in PBS buffer (5.56 μM (0.5 eq.), 11.1 μM (1.0 eq.), 22.2 μM (2.0 eq.), 111 μM (10 eq.), or 1.11 mM (100 eq.), 36 μL, pH 7.4). HPLC were measured after 5 or 30 min.

Figure S25. HPLC analysis of FL-BPS₅ 1 in the presence of 0, 0.5, 1, 2, 10, and 100 equivalent of DTNB (from top to bottom) for 5 min (left) or 30 min (right). dashed line: FL-BPS₅ 1 for 30 min.
5.2.5. Reaction of BPS₅ with GSSG

A solution of FL-BPS₅ 1 in DMSO (100 μM, 4.00 μL, 1.0 eq.) was mixed with a solution of GSSG in PBS buffer (1.11 mM (100 eq.) or 5.55 mM (500 eq.), 36 μL, pH 7.4). HPLC were measured after 5 or 30 min.

Figure S26. HPLC analysis of FL-BPS₅ 1 in the presence of 0, 100, and 200 equivalent of GSSG (from top to bottom) for 5 min (solid) or 30 min (dashed).

5.2.6. Reaction of BPS₅ with GSH and GSSG

Firstly, a solution of FL-BPS₅ 1 in DMSO (100 μM, 4.00 μL, 1.0 eq.) was mixed with a solution of GSH in PBS buffer (22.7 μM (2.0 eq.), 35.2 μL, pH 7.4) for 30 min. Then, a solution of GSSG in PBS buffer (1.0 mM (2.0 eq.), 5 mM (10 eq.), or 50 mM (100 eq.), 0.8 μL, pH 7.4) was added. HPLC were measured after 30 min.

Figure S27. HPLC analysis of FL-BPS₅ 1 in the presence of GSH for 30 min followed by 0, 2, 10, and 100 equivalent of GSSG (from top to bottom) for 30 min; dashed line: FL-BPS₅ 1 in the presence of GSH for 60 min.
5.2.7. Reaction of BPS5 with Lipoic Acid

A solution of FL-BPS5 1 in DMSO (100 μM, 4.00 μL, 1.0 eq.) was mixed with a solution of lipoic acid (LA) in PBS buffer (22.2 μM (2.0 eq.), 111 μM (10 eq.), or 1.11 mM (100 eq.), 36 μL, pH 7.4). HPLC were measured after 30 min.

Figure S28. HPLC analysis of FL-BPS5 1 in the presence of 0, 2, 10, and 100 equivalent of LA (from top to bottom).

5.2.8. Reaction of BPS5 with GSH and Lipoic Acid

To a solution of FL-BPS5 1 in DMSO (100 μM, 4.00 μL, 1.0 eq.) was added successively a solution of catalytic amount of GSH in PBS buffer (1.14 μM (0.1 eq.), 35.2 μL, pH 7.4) and a solution of LA in DMSO (1.0 mM (2.0 eq.), 5 mM (10 eq.), or 50 mM (100 eq.), 0.8 μL, pH 7.4). HPLC were measured after 5 or 30 min.

Figure S29. HPLC analysis of FL-BPS5 1 in the presence of 0.1 equivalent of GSH as well as 0, 2, 10, and 100 equivalent of LA (from top to bottom) for 5 min (solid) or 30 min (dashed).
5.2.9. Reaction of BPS₅ with Glycine

A solution of FL-BPS₅ 1 in DMSO (100 μM, 4.00 μL, 1.0 eq.) was mixed with a solution of Glycine (Gly) in PBS buffer (22.2 μM (2.0 eq.) or 111 μM (10 eq.), 36 μL, pH 7.4). HPLC were measured after 5 or 30 min.

Figure S30. HPLC analysis of FL-BPS₅ 1 in the presence of 0, 2, and 10 equivalent of Gly (from top to bottom) for 5 min (solid) or 30 min (dashed).

5.2.10. Reaction of BPS₅ with Glycine and GSSG

To a solution of FL-BPS₅ 1 in DMSO (100 μM, 4.00 μL, 1.0 eq.) was added successively a solution of Gly in PBS buffer (22.7 μM (2.0 eq.), 35.2 μL, pH 7.4) and a solution of GSSG in PBS buffer (5 mM (10 eq.), 0.8 μL, pH 7.4). HPLC were measured after 5 or 30 min.

Figure S31. HPLC analysis of FL-BPS₅ 1 in the absence (top) and presence of Gly and GSSG (bottom) for 5 min (solid) or 30 min (dashed).
5.2.11. Reaction of BPS₅ with Histidine

A solution of FL-BPS₅ 1 in DMSO (100 μM, 4.00 μL, 1.0 eq.) was mixed with a solution of Histidine (His) in PBS buffer (22.2 μM (2.0 eq.) or 111 μM (10 eq.), 36 μL, pH 7.4). HPLC were measured after 5 or 30 min.

**Figure S32.** HPLC analysis of FL-BPS₅ 1 in the presence of 0, 2, and 10 equivalent of Gly (from top to bottom) for 5 min (solid) or 30 min (dashed).

5.2.12. Reaction of BPS₅ with Histidine and GSSG

To a solution of FL-BPS₅ 1 in DMSO (100 μM, 4.00 μL, 1.0 eq.) was added successively a solution of His in PBS buffer (22.7 μM (2.0 eq.), 35.2 μL, pH 7.4) and a solution of GSSG in PBS buffer (5 mM (10 eq.), 0.8 μL, pH 7.4). HPLC were measured after 5 or 30 min.

**Figure S33.** HPLC analysis of FL-BPS₅ 1 in the absence (top) and presence of His and GSSG (bottom) for 5 min (solid) or 30 min (dashed).

5.2.13. Reaction of BPS₅ with n-BuNH₂

A solution of FL-BPS₅ 1 in DMSO (100 μM, 4.00 μL, 1.0 eq.) was mixed with a solution of n-BuNH₂ in PBS buffer (22.2 μM (2.0 eq.) or 111 μM (10 eq.), 36 μL, pH 7.4). HPLC were measured after 5 or 30 min.
5.2.14. Reaction of BPS₅ with n-BuNH₂ and GSSG

To a solution of FL-BPS₅ 1 in DMSO (100 μM, 4.00 μL, 1.0 eq.) was added successively a solution of n-BuNH₂ in PBS buffer (22.7 μM (2.0 eq.), 35.2 μL, pH 7.4) and a solution of GSSG in PBS buffer (5 mM (10 eq.), 0.8 μL, pH 7.4). HPLC were measured after 5 or 30 min.

Figure S35. HPLC analysis of FL-BPS₅ 1 in the absence (top) and presence of n-BuNH₂ and GSSG (bottom) for 5 min (solid) or 30 min (dashed).

5.2.15. Reaction of AspA with GSH

A solution of FL-AspA 7 in DMSO (100 μM, 4.00 μL, 1.0 eq.) was mixed with a solution of GSH in PBS buffer (22.2 μM (2.0 eq.), 111 μM (10 eq.), or 1.11 mM (100 eq.), 36 μL, pH 7.4). HPLC were measured after 30 min.
**Figure S36.** HPLC analysis of FL-AspA 7 in the presence of 0, 2, 10, and 100 equivalent of GSH (from top to bottom); dashed line: FL-AspA 7 for 30 min.

### 5.3. LC-MS Studies

**Figure S37.** Plausible structures of library components identified by LC-MS analyses.
5.3.1. LC-MS Analysis of BPSs in PBS Buffer

A solution of FL-BPSs 1 in DMSO (1 μL, 10 mM) was mixed with PBS buffer (99 μL, pH 7.4). LC-MS was measured after 4 h.
5.3.2. LC-MS Analysis of BPS₅ in the Presence of GSH

A stock solution of compound 1 in DMSO (1 μL, 10 mM, 1.0 eq.) was mixed with a solution of GSH in PBS buffer (99 μL, 200 μM (2.0 eq.), pH 7.4). LC-MS was measured after 30 min.
**Figure S39.** LC-MS analysis of 1 in the presence of GSH.

**Figure S40.** UHPLC-TOF HRMS analysis of BPS₃ 1 in the presence of GSH (2 eq.). Top: Total ion chromatogram. Bottom: ESI mass spectra obtained at Rₜ; a) ≈2.5 min, b) ≈2.4 min, c) ≈2.3 min, d) ≈2.2 min and e) 2.1 min. Note, the mass intensities were increased 4 times for m/z > 900 Da.
**Figure S41.** Observed (bottom) and simulated MS (top) of BPS$_5$ 1.

**Figure S42.** Observed (bottom) and simulated MS of BPS$_4$ 17 (middle) and BPS$_4$ dimer 23$_2$e (top).

**Figure S43.** Observed (bottom) and simulated MS of BPS$_3$ 16 and BPS dimers 23$_2$a,b,d (top).
Figure S44. Observed (bottom) and simulated MS of BPS$_3$ 16 and BPS dimers 23$_2$d-f (top).

Figure S45. Observed (bottom) and simulated MS of BPS trimers 23$_3$b-e (top).

Figure S46. Observed (bottom) and simulated MS of BPS tetramers 23$_4$c-f (top).
**Figure S47.** Plausible structures of BPS-LA adducts identified by LC-MS analyses. Only one regio-isomer of each structure is shown as an example.

### 5.3.3. LC-MS Analysis of BPSs in the Presence of Catalytic Amounts of GSH and Lipoic Acid

To a stock solution of compound 1 in DMSO (1 μL, 10 mM) was added successively both a solution of GSH in PBS buffer (49 μL, 20 μM (0.1 eq.), pH 7.4) and a solution of LA in DMSO (50 μL, 10 mM (50 eq.)). LC-MS was measured after 30 min.
Figure S48. LC-MS analysis of 1 in the presence of catalytic amount of GSH and LA.

5.4. Affinity Column Chromatography

The medium was prepared according to the supplier's procedures. Namely, thiopropyl Sepharose 6B (3 g) was suspended in bidistilled water and filtered. The swollen medium was then suspended in an aqueous solution (12 mL) of DTT (1% w/v), EDTA (1 mM) and NaHCO₃ (0.3 M) at pH 8.4. The mixture was shaken for 40 min at rt, and then filtered. The medium was thoroughly rinsed with an aqueous solution of AcOH (0.1 M), NaCl (0.5 M) and EDTA (1 mM). Thus obtained medium was
suspended in a buffer (10 mM Tris, 100 mM NaCl, 1 mM EDTA, pH 7.5), and packed in a column (Omnifit EZ, 10 x 110 mm).

Analyses were performed using JASCO LC-2000 systems at rt, under the following conditions: 0.4 mL/min, a buffer (10 mM Tris, 100 mM NaCl, 1 mM EDTA, pH 7.5) with DTT (gradient: 0 – 60 min: 0 mM, 60 – 70 min: 0 – 50 mM, 70–140 min: 50 mM; or isocratic: 50 mM) as an eluent, λ_{abs} at 490 nm for detection. Sample solutions (50 μM) were prepared in the eluent without DTT, and injected (50 μL) for analyses.

6. Delivery Applications
6.1. Cellular Uptake of Fluorescent Proteins
6.1.1. Preparation of Streptavidin Complexes

![Scheme S9](image)

**Scheme S9.** Complex 70 generated with both biotinylated BPS_{5} 2 and biotinylated FL 33 and for testing its cellular uptake efficiency. The indicated complex stoichiometries are simplifying the understood overall more complex distributions.[S10-S13]

**Complex 70.** A mixture of 2 (7.6 μL, 2 mM in DMSO, 15.1 nmol) and 33 (19 μL, 2 mM in DMSO, 38 nmol) was diluted with Leibovitz’s medium (26 μL). The mixture was added to an aqueous solution of Streptavidin 69 (500 μL, 30.2 μM in H_{2}O, 15.1 nmol). The resulting mixture was shaken at 4 °C with vigorous agitation (1000 rpm) for 2 h, filtered through an Amicon Ultracentrifugal filter® (cut off: 3 kDa, 10 min, 14.5 krpm) and washed 5 times with Leibovitz’s medium (40 μL each) to give a solution of complex 70 (248 μM in Leibovitz’s medium). The concentration was estimated from the volume of the solution assuming the complete recovery of the protein.
6.1.2. Cellular Uptake of Streptavidin Complexes

HeLa Kyoto cells were seeded at $8 \times 10^4$ cells/mL on 35 mm glass-bottomed dishes (MatTeK) and cultured overnight. After removing the medium, the cells were washed twice with PBS and once with Leibovitz’s medium and treated with a solution of complex 70 (2.5 μM in Leibovitz’s medium). The cells were incubated for 1 h, 2 h, 4 h, or 6 h at 37 °C under 5% CO₂, then the media was removed by aspiration. Cells were washed twice with Leibovitz’s medium and finally kept in Leibovitz’s medium. Distribution of fluorescent compounds was analyzed without fixing using confocal laser scanning microscope (Leica SP5) equipped with 63X oil immersion objective lens. Argon laser was used as light source with excitation wavelength 488 nm and emission 492 – 534 nm (Leica HyD™ detector). During the imaging, the samples were kept at 37 °C.

![Confocal laser scanning microscopy images of HeLa Kyoto cells treated with streptavidin complex 70 (2.5 μM in Leibovitz’s medium) for 1 h (a), 2 h (b), 4 h (c), or 6 h (d) at 30% laser power. Scale bars: 10 μm.](image)

**Figure S49.** Confocal laser scanning microscopy images of HeLa Kyoto cells treated with streptavidin complex 70 (2.5 μM in Leibovitz’s medium) for 1 h (a), 2 h (b), 4 h (c), or 6 h (d) at 30% laser power. Scale bars: 10 μm.

6.2. Cellular Uptake of Artificial Metalloenzymes

![Scheme S10](image)

**Scheme S10.** Complex and artificial metalloenzyme tested for the Alloc deprotection of 27 in cells. The indicated complex stoichiometries are simplifying the understood overall more complex distributions. [S10-S13]
6.2.1. Preparation of Biotinylated Catalysts

Scheme S11. (a) Ligand 71/catalyst precursor 72 1:1, rt, 10 min.

Biotinylated Catalyst 29. This compound was prepared following the procedure in ref. S10. Namely, freshly prepared solutions of 71 (10 mM) and 72 (10 mM) in DMF were mixed and stirred for 10 min at rt to afford 29 (5 mM).

6.2.2. Preparation of Streptavidin Complexes

Scheme S12. Complex 28 generated with both biotinylated catalyst 29 and biotinylated BPS5 2. The indicated complex stoichiometries are simplifying the understood overall more complex distributions.\[^{[S10-S13]}\]

Complex 28. To a stock solution of 29 (2.4 μL of 5 mM in DMF, 12 nmol) diluted in Milli-Q H₂O (117 μL) was added a solution of streptavidin 69 (31 μL, 400 μM in H₂O). The mixture was agitated vigorously for 5 min at 25 °C. From this protein mixture, 100 μL (containing 8 nmol of 69) were pipetted and mixed with a solution of 2 (100 μL of 160 μM in H₂O, 16 nmol). The resulting mixture was shaken (1000 rpm) for 2 h at 25 °C to afford the complex 28 (40 μM).
Scheme S13. Complex 31 generated with biotinylated catalyst 29. The indicated complex stoichiometries are simplifying the understood overall more complex distributions.\[S10-S13\]

**Complex 31.** This complex was prepared following the literature procedure described in ref. S14. Namely, to a stock solution of 29 (2.4 μL of 5 mM in DMF, 12 nmol) diluted in Milli-Q H₂O (117 μL) was added a solution of 69 (31 μL, 400 μM in H₂O). The mixture was agitated vigorously for 5 min at 25 °C. From this protein mixture, 100 μL (containing 8 nmol of 69) were pipetted and mixed with Milli-Q H₂O (100 μL). The resulting mixture was shaken (1000 rpm) for 2 h at 25 °C to afford the complex 31 (40 μM).

**6.2.3. Catalytic Reactions in Cells**

HeLa Kyoto cells were seeded at 8 × 10⁴ cells/mL on 35 mm glass-bottomed dishes (MatTeK) and cultured overnight. After removing the medium, the cells were washed twice with PBS and once with Leibovitz’s medium and treated with a solution of complex 31 (2.5 μM in Leibovitz’s medium) or 28 (0.5 to 10 μM in Leibovitz’s medium). The cells were incubated for 8 h at 37 °C under 5% CO₂, then the media was removed by aspiration. The cells were washed three times with Leibovitz’s medium (1 mL). Then, a solution of 27 (1 μL of 10 mM in DMSO) in Leibovitz’s medium (999 μL) was added. The cells were incubated for 24 h at 37 °C, 5% CO₂. The medium was then removed by aspiration. Cells were washed twice with Leibovitz’s medium and finally kept in Leibovitz’s medium. Distribution of fluorescent compounds was analyzed without fixing using confocal laser scanning microscope (Leica SP5) equipped with 63X oil immersion objective lens. Argon laser was used as light source.
with excitation wavelength 488 nm and emission 499 – 565 nm (Leica HyD™ detector). During the imaging, the samples were kept at 37 °C. Fluorescence intensities in cells were quantified with ImageJ.

**Figure S50.** Confocal laser scanning microscopy images of HeLa Kyoto cells treated with complex 28 (0.5 μM (a), 1 μM (b), 2.5 μM (c), 5 μM (d), 10 μM (e) in Leibovitz’s medium) or complex 31 (2.5 μM (f) in Leibovitz’s medium) for 8 h followed by 24 h incubation of 27 (10 μM) in Leibovitz’s medium at 30% laser power. Scale bars: 10 μm.

6.3. Cellular Uptake of CPS (Fluorescent Cell-Penetrating Streptavidin)

6.3.1. Preparation of CPS Complexes

**Scheme S14.** Complex 74 generated with azide-substituted activated ester 73.

**Complex 74.** A solution of 69 (10 mL, 10 μM in PBS buffer) was added to a Falcon™ 15 mL conical centrifuge tube containing 73 (0.6 mg, 2 μmol)[S15], reaching a final concentration of 73 of 200 μM. The resulting mixture was shaken (1000 rpm) for 2 h at 25 °C, filtered through an Amicon® Ultra 15 mL centrifugal filter (cut off: 30 kDa, 10 min, 4.4 krpm) and washed 3 times with PBS buffer (10 mL each). Final concentration of adduct 74 was calculated by UV-vis absorption spectroscopy ($\varepsilon_{280} = 166636 \text{ M}^{-1} \text{ cm}^{-1}$)
Number of functionalized lysines was estimated using TNBSA colorimetric assay.

Scheme S15. Synthesis of Complex 75 using click reaction.

Complex 75. Stock solutions of the reagents for the click reaction were prepared:

Complex 74: 40 μM (in PBS buffer);
Compound 47: 20 mM (in DMSO);
CuSO₄·5H₂O: 10 mM (in bidistilled water);
Ligand BTTAA: 20 mM (in bidistilled water);
Sodium ascorbate: 50 mM (in bidistilled water);
Aminoguanidine hydrochloride: 50 mM (in bidistilled water)

In a 2 mL Eppendorf tube containing a solution of 74 and 47 in PBS buffer, the premixed click reagents (CuSO₄·5H₂O; BTTAA; sodium ascorbate; aminoguanidine hydrochloride) were added, reaching the following final concentration of all the reagents:

Complex 74: 10 μM;
Compound 47: 100 μM;
CuSO₄·5H₂O: 0.2 mM;
Ligand BTTAA: 1.5 mM;
Sodium ascorbate: 3 mM;
Aminoguanidine hydrochloride: 3 mM

The resulting mixture was shaken (1000 rpm) for 1 h at 25 °C. During this time, the premixed click reagents were added twice again (t = 20 min, t = 40 min), in the same amount that at the beginning of the reaction. After shaking, the mixture was filtered through an Amicon® Ultra 0.5 mL centrifugal filter (cut off: 30 kDa, 5 min, 14.5 krpm)
and washed 5 times with PBS buffer (0.4 mL each). Concentration of the CPS was calculated in the next step.

**Scheme S16.** Complex 76 generated with streptavidin 69 and biotinylated-FL 33.

**Complex 76.** To a solution of 69 (1000 μL, 10 μM in PBS), a solution of biotinylated-FL 33 (25 μL, 2 mM in DMSO) was added. The mixture was vortex for 5 seconds and then shaken (1000 rpm) for 15 min at 4 °C. Excess of 33 was removed using Amicon® Ultra 0.5 mL centrifugal filter (cut off: 30 kDa, 5 min, 14.0 krpm) and complex 76 was washed 2 times with PBS buffer (0.4 mL each). Assuming the quantitative formation and recovery of 76, the extinction coefficient of streptavidin conjugate was estimated ε_{498} = 163000 M^{-1} cm^{-1}.

**Scheme S17.** Complex 32 generated with complex 75 and biotinylated-FL 33.

**Complex 32.** To a solution of 75 (1000 μL, theoretical 10 μM in PBS), a solution of biotinylated-FL 33 (25 μL, 2 mM in DMSO) was added. The mixture was vortex for 5 seconds and then shaken (1000 rpm) for 15 min at 4 °C. Excess of 33 was removed using Amicon® Ultra 0.5 mL centrifugal filter (cut off: 30 kDa, 5 min, 14.0 krpm) and complex 32 was washed 2 times with PBS buffer (0.4 mL each). Final concentration of complex 32 was calculated by UV-vis absorption spectroscopy, compared with standard compound 76.
**Scheme S18.** Complex 34 generated with complex 74 and biotinylated-FL 33.

**Complex 34.** To a solution of 74 (1000 μL, theoretical 10 μM in PBS), a solution of biotinylated-FL 33 (25 μL, 2 mM in DMSO) was added. The mixture was vortex for 5 seconds and then shaken (1000 rpm) for 15 min at 4 °C. Excess of 33 was removed using Amicon Ultra 0.5 mL centrifugal filter (cut off: 30 kDa, 5 min, 14.0 krpm) and complex 34 was washed 2 times with PBS buffer (0.4 mL each). Final concentration of complex 34 was calculated by UV-vis absorption spectroscopy, compared with standard compound 76.

### 6.3.2. Cellular Uptake of CPS Complexes

HeLa Kyoto cells were seeded at $8 \times 10^4$ cells/mL on µ-Slide 8-Well Glass Bottom (ibidi) and cultured overnight. After removing the medium, the cells were washed twice with PBS and once with Leibovitz’s medium and treated with a solution of complex 32 or 34 (150 μL, 5 or 10 μM in Leibovitz’s medium). The cells were incubated for 8 h at 37 °C under 5% CO₂, then the medium was removed by aspiration. The cells were washed three times with Leibovitz’s medium (200 μL) and finally kept in Leibovitz’s medium. Distribution of fluorescent compounds was analyzed without fixing using confocal laser scanning microscope (Leica SP5) equipped with 63X oil immersion objective lens. Argon laser was used as light source with excitation wavelength 488 nm and emission 499 – 565 nm (Leica HyD™ detector). During the imaging, the samples were kept at 37 °C.
Confocal laser scanning microscopy images of HeLa Kyoto cells treated with complex 32 (5 μM (a), 10 μM (c) in Leibovitz’s medium) or complex 34 (5 μM (b), 10 μM (d) in Leibovitz’s medium) for 8 h at 30% laser power. Scale bars: 10 μm.

7. Supporting References

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8. NMR Spectra

Figure S52. $^1$H NMR spectrum of compound 1 in CD$_3$CN/DMSO-$d_6$ 95:5.

Figure S53. $^{13}$C NMR spectrum of compound 1 in CD$_3$CN/DMSO-$d_6$ 91:9.
**Figure S54.** DEPT spectrum of compound 1 in CD$_3$CN/DMSO-$d_6$ 91:9.

**Figure S55.** $^1$H NMR spectrum of compound 2 in DMSO-$d_6$. 
Figure S56. $^{13}$C NMR spectrum of compound 2 in DMSO-$d_6$.

Figure S57. DEPT spectrum of compound 2 in DMSO-$d_6$. 
Figure S58. $^1$H NMR spectrum of compound 47 in CDCl$_3$.

Figure S59. $^{13}$C NMR spectrum of compound 47 in CDCl$_3$. 
**Figure S60.** $^1$H NMR spectrum of compound 53 in CD$_3$OD.

**Figure S61.** $^{13}$C NMR spectrum of compound 53 in CD$_3$OD.
Figure S62. $^1$H NMR spectrum of compound 4 in CD$_3$CN/D$_2$O 96:4.

Figure S63. $^{13}$C NMR spectrum of compound 4 in CD$_3$CN/D$_2$O 96:4.
Figure S64. DEPT spectrum of compound 4 in CD$_3$CN/D$_2$O 96:4.

Figure S65. $^1$H NMR spectrum of compound 55 in CDCl$_3$. 

S69
Figure S66. $^{13}$C NMR spectrum of compound 55 in CDCl$_3$.

Figure S67. $^1$H NMR spectrum of compound 58 in CDCl$_3$. 
Figure S68. $^{13}$C NMR spectrum of compound 58 in CDCl$_3$.

Figure S69. $^1$H NMR spectrum of compound 59 in CDCl$_3$. 
**Figure S70.** $^{13}$C NMR spectrum of compound 59 in CDCl$_3$.

**Figure S71.** $^1$H NMR spectrum of compound 60 in CDCl$_3$. 

S72
Figure S72. $^{13}$C NMR spectrum of compound 60 in CDCl$_3$.

Figure S73. $^1$H NMR spectrum of compound 61 in CDCl$_3$. 

S73
Figure S74. $^{13}$C NMR spectrum of compound 61 in CDCl$_3$.

Figure S75. DEPT spectrum of compound 61 in CDCl$_3$. 
Figure S76. $^1$H NMR spectrum of compound 62 in CD$_3$OD.

Figure S77. $^{13}$C NMR spectrum of compound 62 in CD$_3$OD.
**Figure S78.** DEPT spectrum of compound 62 in CD$_3$OD.

**Figure S79.** $^1$H NMR spectrum of compound 6 in CD$_3$CN/DMSO-$d_6$ 91:9.
Figure S80. $^{13}$C NMR spectrum of compound 6 in CD$_3$CN/DMSO-$d_6$ 91:9.

Figure S81. DEPT spectrum of compound 6 in CD$_3$CN/DMSO-$d_6$ 91:9.
**Figure S82.** $^1$H NMR spectrum of compound 8 in CD$_3$CN/DMSO-$d_6$ 95:5.

**Figure S83.** $^{13}$C NMR spectrum of compound 8 in CD$_3$CN/DMSO-$d_6$ 95:5.
Figure S84. DEPT spectrum of compound 8 in CD$_3$CN/DMSO-$d_6$ 95:5.

Figure S85. $^1$H NMR spectrum of compound 33 in DMSO-$d_6$. 
Figure S86. $^{13}$C NMR spectrum of compound 33 in DMSO-$d_6$.

Figure S87. DEPT spectrum of compound 33 in DMSO-$d_6$. 

S80