Electronic Supplementary Material (ESI) for Chemical Science.

Supporting Information

Diselenolane-Mediated Cellular Uptake

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1. Materials and methods

As in ref. S1, Supporting Information. Briefly, reagents for synthesis were purchased from Brunschwig, Acros, Alfa Aesar, Sigma-Aldrich, Fluka, Bachem and Apollo, buffers and salts of the best grade available from Fluka or Sigma-Aldrich and used as received.

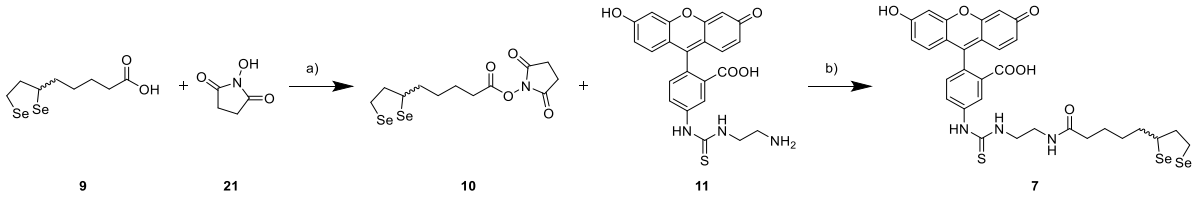
Column chromatography was carried out on silica gel (SiliaFlash® P60, SILICYCLE, 230–400 mesh). Analytical thin layer chromatography (TLC) were performed on silica gel 60 F254 (Merck). Semi-preparative HPLC was performed using JASCO LC-2000 Plus system equipped with quaternary pump (JASCO PU-2089) and UV/Vis detector (JASCO UV-2077 Plus). The chromatographic column used was a Phenomenex Jupiter Proteo (250 x 10 mm, 4.0 µm particles size, flow 3.0 mL/min with a linear elution gradient from 90% H_2O / 10% CH_3CN + 0.1% TFA to 10% H_2O / 90% CH_3CN + 0.1% TFA in 30 min). Reverse phase flash chromatography was performed on Biotage Isolera^{TM} Four (column: SNAP Cartridge, KP-C18-HS-60g, eluents: CH_3CN and H_2O with 0.1% TFA). Melting points (Mp) were measured on a Melting Point M-565 (BUCHI). IR spectra were recorded on a Perkin Elmer Spectrum One FT-IR spectrometer (ATR, Golden Gate) and are reported as wavenumbers ν in cm^{-1} with band intensities indicated as s (strong), m (medium) or w (weak). ^{1}H and ^{13}C NMR spectra were recorded (as indicated) either on a Bruker 300 MHz, 400 MHz or 500 MHz spectrometer and are reported as chemical shifts (δ) in ppm relative to TMS (δ = 0). Proton spin multiplicities are reported as a singlet (s), doublet (d), triplet (t), quartet (q) and quintet (quint) with coupling constants (J) given in Hz, or multiplet (m). ^{1}H and ^{13}C resonances were assigned with the aid of additional information from 1D & 2D NMR spectra (H,H-COSY, DEPT-135, HSQC and HMBC). UHPLC-MS (ESI) were recorded using a Thermo Scientific Accela HPLC with a LCQ Fleet three-dimensional ion trap mass spectrometer (ESI, Thermo scientific) and a diode array detector. The characterization of new

compounds are reported as mass-per-charge ratio *m/z* (intensity in %, [assignment]). Only the most intense ones among isotopic peaks are reported. The chromatographic column used was a Thermo C18 Hypersil gold column 1.9 μm, 5 cm x 2.1 mm, using an eluent gradient from 90% H_2O / 10% CH_3CN + 0.1% TFA to 10% H_2O / 90% CH_3CN + 0.1% TFA in 4 min at a flow rate 0.75 mL/min. UV-Vis spectra were recorded on a JASCO V-650 spectrophotometer equipped with a temperature controller. Deuterated sodium phosphate buffer was prepared by dissolving phosphoric acid (1 mmol, 119 mg, 85% D_3PO_4 in D_2O) in 10 mL D_2O; pD adjustments were made with DCl (20 wt.% solution in D_2O) or NaOD (30 wt.% solution in D_2O) and the pH meter reading in D_2O was corrected (pD = pH meter reading + 0.4).[S2] Fluorescence measurements were performed with a FluoroMax-4 spectrofluorometer (Horiba Scientific) equipped with a stirrer and a temperature controller (25 ± 1 ºC). Fluorescence spectra were corrected using instrument-supplied correction factors. Fluorescence cellular imaging was performed using Leica SP5 or SP8 confocal, 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.

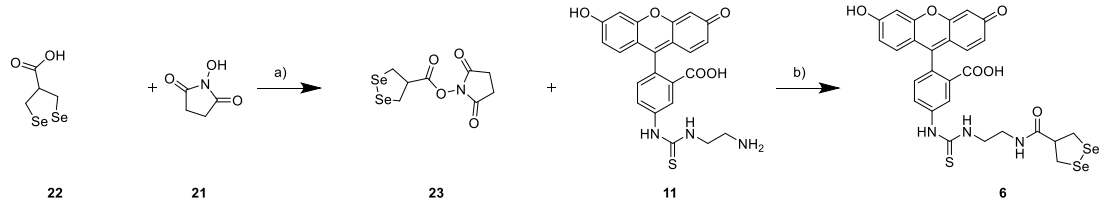
Abbreviations. CLSM: Confocal laser scanning microscopy; DCC: *N,N'*-Dicyclohexylcarbodiimide; DMF: *N*,*N*-Dimethylformamide; DMSO: Dimethyl sulfoxide; DTNB: 5,5-Dithio-bis-(2-nitrobenzoic acid) (Ellman's reagent); DTT: Dithiothreitol; EDTA: Ethylenediaminetetraacetic acid; FBS: Fetal bovine serum; MTT: 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide; NHS: *N*-Hydroxysuccinimide; ODT: Octadecanthiol; PBS: Phosphate-buffered saline; PCC: Pearson correlation coefficient; PE: Petroleum ether; PS: Penicillin/streptomycin; PTSA: *p*-Toluenesulfonic acid; quant: Quantitative; rt: Room temperature; SDS: Sodium dodecyl sulfate; SR: Sytox red; TCEP: Tris(2-

carboxyethyl)phosphine; TEA: Triethylamine; THF: Tetrahydrofuran; Tris: Tris(hydroxymethyl)aminomethane.

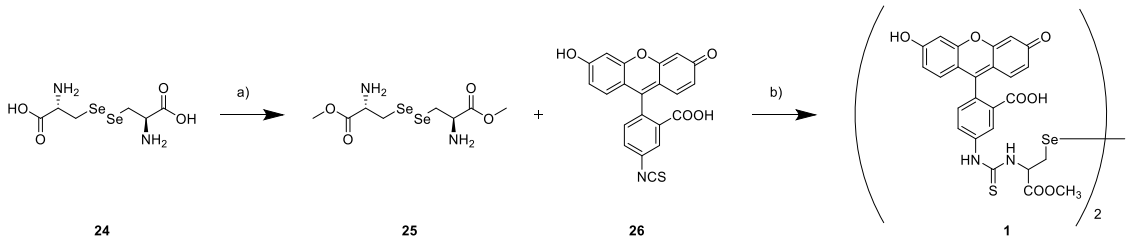
2. Synthesis



Scheme S1. Reagents and conditions: (a) DCC, THF, 18 h, rt, 53%; (b) DMF, 3 h, rt, 14%.



Scheme S2. Reagents and conditions: (a) DCC, THF, 22 h, rt, 49%; (b) DMF, 3 h, rt, 12%.



Scheme S3. Reagents and conditions: (a) PTSA, MeOH, 45 h, reflux, quant; (b) TEA, DMF, 3 h, rt, 44%.

Compound 9 was synthesized according to the procedure reported in the literature.[S3]

Compound 10. To a solution of **9** (44 mg, 150 μmol) in THF (5 mL), DCC (35 mg, 170 μmol) and **21** (23 mg, 200 μmol) were successively added and the solution was stirred at rt under Ar atmosphere. After 18 h and the formation of a precipitate, the mixture was filtered and dried under reduced pressure. The mixture was purified by two flash column

chromatography (SiO_2, CH_2Cl_2/acetone 97:3, R_f 0.6 in CH_2Cl_2/acetone 97:3 and PE/EtOAc 1:1, R_f 0.6 in PE/EtOAc 4:6) to give **10** as a dark brown oil (39 mg, 53%). IR (neat): 2933 (w), 1812 (w), 1781 (w), 1730 (s), 1456 (w), 1427 (w), 1364 (w), 1296 (w), 1248 (w), 1201 (s), 1152 (w), 1064 (s), 994 (w), 877 (w), 813 (w), 766 (w), 734 (w), 696 (w), 647 (m), 578 (w), 558 (w), 542 (w), 518 (w), 513 (w); ^{1}H NMR (400 MHz, $CDCl_3$): 3.91 – 3.78 (m, 1H), 3.31 – 3.20 (m, 2H), 2.95 – 2.84 (m, 1H), 2.77 (s, 4H), 2.61 – 2.42 (m, 3H), 1.82 – 1.65 (m, 4H), 1.57 – 1.43 (m, 2H); ^{13}C NMR (101 MHz, $CDCl_3$): 169.1 (C), 168.4 (C), 52.2 (CH), 45.6 (CH_2), 35.1 (CH_2), 30.8 (CH_2), 29.3 (CH_2), 25.6 (CH_2), 24.3 (CH_2); UHPLC-MS: 399 (100, $[M+H]^+$).

Compound 11 was synthesized according to the procedure reported in the literature.[S1]

Compound 7. To a solution of **11** (38 mg, 84 μmol) in DMF (1 mL), **10** (31 mg, 78 μmol) in DMF (1 mL) was added and the solution was stirred for 3 h at rt. The mixture was purified by trituration first in Et_2O (3 x 15 mL) and then in EtOAc (2 x 10 mL), and semi-preparative HPLC (90% H_2O / 10% CH_3CN + 0.1% TFA to 10% H_2O / 90% CH_3CN + 0.1% TFA in 30 min, R_t = 22.6 min) to yield **7** as a pale brown solid (8 mg, 14%). Mp: 131 – 132 °C; IR (neat): 3266 (w), 3059 (w), 2927 (w), 2585 (w), 1671 (w), 1590 (s), 1538 (s), 1455 (m), 1424 (m), 1384 (m), 1308 (s), 1272 (s), 1192 (s), 1175 (s), 1119 (s), 994 (w), 972 (w), 916 (m), 848 (m), 795 (m), 765 (m), 719 (m), 692 (m), 669 (m), 638 (m), 598 (s), 581 (s), 564 (s), 546 (s), 529 (s), 502 (s); ^{1}H NMR (500 MHz, DMSO-d_6): 10.13 (br s, 1H), 10.04 (s, 1H), 8.18 (s, 1H), 8.05 (br s, 1H), 8.00 (br s, 1H), 7.73 (br s, 1H), 7.19 (d, ^3J (H,H) = 8.2 Hz, 1H), 6.68 (d, ^4J (H,H) = 2.4 Hz, 2H), 6.62 (d, ^3J (H,H) = 8.7 Hz, 2H), 6.57 (dd, ^3J (H,H) = 8.7 Hz, ^4J (H,H) = 2.4 Hz, 2H), 3.94 – 3.88 (m, 1H), 3.63 – 3.53 (m, 2H), 3.32 – 3.25 (m, 4H), 2.87 – 2.80 (m, 1H), 2.44 – 2.36 (m, 1H), 2.09 (t, ^3J (H,H) = 7.4 Hz, 2H), 1.79 – 1.71 (m, 1H), 1.63 – 1.47 (m, 3H), 1.32 (quint, ^3J (H,H) = 7.7 Hz, 2H); ^{13}C NMR (126 MHz, DMSO-

d_6): 181.1 (C), 173.1 (C), 168.9 (C), 160.0 (C), 152.4 (C), 147.8 (C), 141.4 (C), 130.3 (CH), 129.5 (CH), 127.2 (C), 124.7 (CH), 117.5 (CH), 113.0 (CH), 110.2 (CH), 102.7 (CH), 53.3 (^1J (Se,C) = 62 Hz, CH), 45.6 (CH_2), 44.5 (CH_2), 38.1 (CH_2), 35.6 (CH_2), 35.3 (CH_2), 30.1 (^1J (Se,C) = 62 Hz, CH_2), 29.7 (CH_2), 25.4 (CH_2); UHPLC-MS: 734 (100, $[M+H]^+$).

Compound 22 was synthesized according to the procedure reported in the literature.[S4]

Compound 23. To a solution of **22** (111 mg, 456 μmol) in THF (5 mL), DCC (110 mg, 531 μmol) and **21** (60.5 mg, 526 μmol) were successively added and the solution was stirred at rt under Ar atmosphere. After 22 h and the formation of a precipitate, the mixture was filtered and dried under reduced pressure. The mixture was purified by flash column chromatography (SiO_2, CH_2Cl_2/acetone 49:1, R_f 0.6 in CH_2Cl_2/acetone 24:1) to yield **23** as a dark brown oil (75.7 mg, 49%). IR (neat): 2931 (w), 2854 (w), 1808 (w), 1779 (m), 1727 (s), 1652 (w), 1423 (w), 1408 (w), 1359 (m), 1293 (w), 1247 (w), 1197 (s), 1063 (s), 992 (m), 934 (w), 909 (m), 809 (m), 725 (s), 643 (s), 577 (m), 554 (m), 513 (s), 509 (s), 501 (m); ^{1}H NMR (400 MHz, $CDCl_3$): 3.83 (quint, ^3J (H,H) = 6.9 Hz, 1H), 3.63 – 3.57 (m, 4H), 2.78 (s, 4H); ^{13}C NMR (101 MHz, $CDCl_3$): 168.7 (C), 168.3 (C), 52.7 (CH), 31.6 (CH_2), 25.6 (CH_2); UHPLC-MS: 343 (100, $[M+H]^+$).

Compound 6. To a solution of **23** (61 mg, 180 μmol) in DMF (0.5 mL), **11** (82 mg, 180 μmol) in DMF (2.5 mL) was added and the solution was stirred for 4 h at rt. The mixture was purified by trituration in Et_2O (3 x 15 mL), reverse phase flash chromatography (90% H_2O / 10% CH_3CN + 0.1% TFA to 10% H_2O / 90% CH_3CN + 0.1% TFA) and semi-preparative HPLC (40% H_2O / 60% CH_3CN + 0.1% TFA to 30% H_2O / 70% CH_3CN + 0.1% TFA in 10 min, R_t = 4.8 min) to yield **6** as an orange solid (14 mg, 12%). Mp: 165 – 166 °C; IR (neat): 3244 (w), 3064 (w), 2929 (w), 2585 (w), 1635 (m), 1589 (m), 1534 (s), 1454 (m), 1383 (m),

1306 (m), 1268 (s), 1175 (s), 1117 (s), 992 (m), 966 (m), 915 (m), 843 (m), 795 (m), 764 (m), 718 (m), 694 (m), 668 (m), 639 (m), 596 (m), 574 (s), 546 (m), 522 (s), 514 (s), 509 (s); ^{1}H NMR (400 MHz, DMSO-d_6): 10.12 (br s, 1H), 10.01 (s, 1H), 8.29 (t, ^{3}J (H,H) = 5.7 Hz, 1H), 8.17 (d, ^{4}J (H,H) = 1.9 Hz, 1H), 8.05 (br s, 1H), 7.73 (br d, ^{3}J (H,H) = 8.3 Hz, 1H), 7.19 (d, ^{3}J (H,H) = 8.3 Hz, 1H), 6.68 (d, ^{4}J (H,H) = 2.3 Hz, 2H), 6.62 (d, ^{3}J (H,H) = 8.7 Hz, 2H), 6.57 (dd, ^{3}J (H,H) = 8.7, ^{4}J (H,H) = 2.3 Hz, 2H), 3.56 – 3.52 (m, 2H), 3.37 – 3.29 (m, 4H), 3.24 – 3.16 (m, 1H); ^{13}C NMR (126 MHz, DMSO-d_6): 181.2 (C), 172.2 (C), 168.9 (C), 159.9 (C), 152.3 (C), 147.9 (C), 141.4 (C), 130.5 (CH), 129.5 (CH), 127.1 (C), 124.7 (CH), 117.6 (CH), 113.0 (CH), 110.1 (CH), 102.7 (CH), 57.3 (CH), 43.9 (CH_2), 38.6 (CH_2), 33.9 (^{1}J (Se,C) = 68 Hz, CH_2); UHPLC-MS: 678 (100, $[M+H]^+$).

Compound 25 was synthesized according to the procedure reported in the literature.[S5]

Compound 1. To a solution of **25** (108 mg, 153 μmol) in dry DMF (4.5 mL), TEA (50 μL, 360 μmol) and **26** (119 mg, 305 μmol) in DMF (1.5 mL) were successively added and the solution was stirred for 3 h at rt under Ar atmosphere. The mixture was purified by trituration in Et_2O (3 x 15 mL) and reverse phase flash chromatography (90% H_2O / 10% CH_3CN + 0.1% TFA to 10% H_2O / 90% CH_3CN + 0.1% TFA) to afford **1** as an orange solid (77 mg, 44%). Mp: 143 – 144 °C; IR (neat): 3064 (br w), 2589 (w), 1719 (w), 1671 (w), 1637 (w), 1588 (m), 1537 (m), 1455 (m), 1385 (w), 1305 (m), 1269 (m), 1193 (m), 1174 (m), 1117 (m), 993 (w), 916 (w), 846 (m), 799 (w), 764 (w), 720 (m), 669 (m), 643 (w), 598 (m), 575 (m), 548 (m), 531 (m), 511 (s); ^{1}H NMR (500 MHz, DMSO-d_6): 10.36 (s, 2H), 10.14 (br s, 2H), 8.58 – 8.51 (m, 2H), 8.32 – 8.29 (m, 2H), 7.78 – 7.75 (m, 2H), 7.21 (d, ^{3}J (H,H) = 8.3 Hz, 2H), 6.68 (d, ^{4}J (H,H) = 1.8 Hz, 4H), 6.63 – 6.55 (m, 8H), 5.44 – 5.32 (m, 2H), 3.72 (s, 6H), 3.57 (dd, ^{2}J (H,H) = 12.8 Hz, ^{3}J (H,H) = 4.8 Hz, 2H), 3.46 (dd, ^{2}J (H,H) = 12.8 Hz, ^{3}J (H,H) = 8.2 Hz, 2H); ^{13}C NMR (126 MHz, DMSO-d_6): 181.2 (C), 171.3 (C), 168.9 (C), 160.0 (C),

152.3 (C), 148.0 (C), 141.4 (C), 130.0 (CH), 129.5 (CH), 127.0 (C), 124.7 (CH), 117.0 (CH), 113.1 (CH), 110.1 (C), 102.7 (CH), 57.6 (CH), 52.9 (CH_3), 31.3 (CH_2); UHPLC-MS: 1143 (10, $[M+H]^+$), 571 (100, $[M+2H]^{2+}$).

3. Computational studies

3.1. Computational details

All the structures were optimized in gas phase at B3LYP level using 6-311++G(d,p) Pople basis set for all atoms. Frequency calculations were performed at the same level for all intermediates and transition states to confirm minima (no negative frequencies) and first order saddle points (one negative frequency), respectively. In addition, all the transition states were connected to the corresponding intermediates by means of intrinsic reaction coordinate (IRC) calculations. All the energies presented in this study are single point zero-point corrected energies. The calculations were performed with the Gaussian09 package.

3.2. Computed energies and cartesian coordinates

1,2-dioxolane (COOC = 49.7°)

Zero-point correction= 0.091474 (Hartree/Particle)

Thermal correction to Energy= 0.096072

Thermal correction to Enthalpy= 0.097016

Thermal correction to Gibbs Free Energy= 0.063918

Sum of electronic and zero-point Energies= -268.260722

Sum of electronic and thermal Energies= -268.256124

Sum of electronic and thermal Enthalpies= -268.255180

Sum of electronic and thermal Free Energies= -268.288278

O	2.972000	-0.521000	-0.168000
O	3.084000	0.124000	1.137000

C	1.770000	0.049000	-0.691000
C	1.782000	-0.071000	1.692000
C	0.808000	0.129000	0.513000
H	1.978000	1.039000	-1.109000
H	1.438000	-0.628000	-1.480000
H	0.305000	1.097000	0.550000
H	0.050000	-0.655000	0.490000
H	1.703000	-1.079000	2.114000
H	1.680000	0.673000	2.483000

1,2-dithiolane (CSSC = 35.5°)

Zero-point correction= 0.086577 (Hartree/Particle)

Thermal correction to Energy= 0.092181

Thermal correction to Enthalpy= 093125

Thermal correction to Gibbs Free Energy= 0.055715

Sum of electronic and zero-point Energies= -914.309439

Sum of electronic and thermal Energies= -914.303835

Sum of electronic and thermal Enthalpies= -914.302891

Sum of electronic and thermal Free Energies= -914.340301

S	3.460000	-0.330000	-0.416000
S	3.381000	0.487000	1.544000
C	1.664000	-0.019000	-0.781000
C	1.685000	-0.171000	1.763000
C	0.882000	0.249000	0.527000
H	1.577000	0.824000	-1.466000

H	1.316000	-0.920000	-1.287000
H	0.664000	1.316000	0.599000
H	-0.075000	-0.283000	0.504000
H	1.737000	-1.257000	1.866000
H	1.277000	0.260000	2.679000

1,2-diselenolane (CSeSeC = 0°)

Zero-point correction= 0.084827 (Hartree/Particle)

Thermal correction to Energy= 0.090919

Thermal correction to Enthalpy= 0.091863

Thermal correction to Gibbs Free Energy= 0.052123

Sum of electronic and zero-point Energies= -4920.995845

Sum of electronic and thermal Energies= -4920.989753

Sum of electronic and thermal Enthalpies= -4920.988809

Sum of electronic and thermal Free Energies= -4921.028549

Se	3.569000	0.341000	-0.668000
Se	3.549000	0.160000	1.743000
C	1.622000	-0.074000	-0.766000
C	1.600000	-0.262000	1.744000
C	0.935000	0.364000	0.523000
H	1.244000	0.469000	-1.633000
H	1.515000	-1.145000	-0.935000
H	0.955000	1.454000	0.606000
H	-0.117000	0.053000	0.491000
H	1.491000	-1.347000	1.750000

H	1.206000	0.145000	2.677000

TS 1,2-dimethyldioxalane (COOC = 180°)

Zero-point correction= 0.081982 (Hartree/Particle)

Thermal correction to Energy= 0.086623

Thermal correction to Enthalpy= 0.087568

Thermal correction to Gibbs Free Energy= 0.055135

Sum of electronic and zero-point Energies= -230.135419

Sum of electronic and thermal Energies= -230.130778

Sum of electronic and thermal Enthalpies= -230.129834

Sum of electronic and thermal Free Energies= -230.162266

O	0.297003	0.251118	-0.066384
O	0.296854	0.408938	1.414728
C	1.566539	0.184900	-0.683889
C	1.566264	0.474890	2.032518
H	1.306857	0.074229	-1.739795
H	2.153468	1.102002	-0.566077
H	2.151852	-0.688803	-0.377824
H	1.306370	0.585566	3.088372
H	2.153045	-0.442319	1.914804
H	2.151813	1.348493	1.726616

1,2-dimethyldioxalane (COOC = 0°)

Zero-point correction= 0.082508 (Hartree/Particle)

Thermal correction to Energy= 0.088091

Thermal correction to Enthalpy= 0.089035

Thermal correction to Gibbs Free Energy= 0.054087

Sum of electronic and zero-point Energies= -230.153929

Sum of electronic and thermal Energies= -230.148345

Sum of electronic and thermal Enthalpies= -230.147401

Sum of electronic and thermal Free Energies= -230.182350

O	0.552842	0.385855	-0.624058
O	0.552628	-0.385973	0.624182
C	0.553748	-0.580544	-1.656147
C	0.553497	0.580426	1.656272
H	0.553633	0.003441	-2.578901
H	1.451302	-1.205949	-1.612060
H	-0.342997	-1.207114	-1.612662
H	0.553066	-0.003559	2.579026
H	1.451182	1.205656	1.612381
H	-0.343116	1.207171	1.612590

TS 1,2-dimethyldisulfane (CSSC = 180°)

Zero-point correction= 0.076991 (Hartree/Particle)

Thermal correction to Energy= 0.082755

Thermal correction to Enthalpy= 0.083699

Thermal correction to Gibbs Free Energy= 0.047492

Sum of electronic and zero-point Energies= -876.201643

Sum of electronic and thermal Energies= -876.195879

Sum of electronic and thermal Enthalpies= -876.194935

Sum of electronic and thermal Free Energies= -876.231142

S	1.494706	0.747282	0.243269
S	1.494701	-0.747281	1.786737
C	1.495152	-0.385075	-1.186690
C	1.495143	0.385075	3.216696
H	1.495113	0.254960	-2.070282
H	2.391324	-1.006235	-1.194496
H	0.599261	-1.006640	-1.194750
H	1.495128	-0.254960	4.100288
H	2.391305	1.006250	3.224490
H	0.599242	1.006624	3.224767

TS 1,2-dimethyldisulfane (CSSC = 0°)

Zero-point correction= 0.076850 (Hartree/Particle)

Thermal correction to Energy= 0.082557

Thermal correction to Enthalpy= 0.083501

Thermal correction to Gibbs Free Energy= 0.047435

Sum of electronic and zero-point Energies= -876.193655

Sum of electronic and thermal Energies= -876.187948

Sum of electronic and thermal Enthalpies= -876.187003

Sum of electronic and thermal Free Energies= -876.223069

S	3.103392	0.004656	-0.074086
S	3.103392	-0.004671	2.104090
C	1.346129	0.006205	-0.561407
C	1.346130	-0.006203	2.591413
H	1.378324	0.009330	-1.653262

H	0.823764	-0.892045	-0.233936
H	0.823927	0.902664	-0.228701
H	1.378326	-0.009342	3.683268
H	0.823776	0.892057	2.263953
H	0.823915	-0.902650	2.258697

1,2-dimethyldisulfane (CSSC = 90°)

Zero-point correction= 0.076964 (Hartree/Particle)

Thermal correction to Energy= 0.083514

Thermal correction to Enthalpy= 0.084459

Thermal correction to Gibbs Free Energy= 0.046383

Sum of electronic and zero-point Energies= -876.210234

Sum of electronic and thermal Energies= -876.203683

Sum of electronic and thermal Enthalpies= -876.202739

Sum of electronic and thermal Free Energies= -876.240814

S	-1.060160	0.628604	-0.819897
S	-1.025593	-0.493161	0.945994
C	0.111324	-0.273982	-1.903412
C	0.372452	0.244259	1.875013
H	0.077596	0.234861	-2.869472
H	1.127827	-0.231134	-1.512788
H	-0.203423	-1.309248	-2.028120
H	0.394828	-0.269268	2.838898
H	1.320185	0.081616	1.362275
H	0.206387	1.308735	2.035293

TS 1,2-dimethyldiselane (CSeSeC = 180°)

Zero-point correction= 0.074956 (Hartree/Particle)

Thermal correction to Energy= 0.081379

Thermal correction to Enthalpy= 0.082323

Thermal correction to Gibbs Free Energy= 0.042775

Sum of electronic and zero-point Energies= -4882.888115

Sum of electronic and thermal Energies= -4882.881692

Sum of electronic and thermal Enthalpies= -4882.880747

Sum of electronic and thermal Free Energies= -4882.920296

Se	1.495419	0.844940	0.156717
Se	1.495367	-0.844940	1.873289
C	1.495086	-0.415691	-1.358716
C	1.495070	0.415691	3.388722
H	1.495039	0.204854	-2.255209
H	2.393061	-1.030496	-1.337454
H	0.596983	-1.030297	-1.337203
H	1.494981	-0.204854	4.285215
H	2.393074	1.030454	3.367472
H	0.596996	1.030339	3.367195

TS 1,2-dimethyldiselane (CSeSeC = 0°)

Zero-point correction= 0.074984 (Hartree/Particle)

Thermal correction to Energy= 0.081294

Thermal correction to Enthalpy= 0.082238

Thermal correction to Gibbs Free Energy= 0.043074

Sum of electronic and zero-point Energies= -4882.882649

Sum of electronic and thermal Energies= -4882.876339

Sum of electronic and thermal Enthalpies= -4882.875395

Sum of electronic and thermal Free Energies= -4882.914559

Se	3.234233	0.003797	-0.202322
Se	3.234208	-0.003897	2.232363
C	1.310747	0.004610	-0.630086
C	1.310714	-0.004600	2.660088
H	1.281821	0.007166	-1.720993
H	0.824475	-0.895143	-0.260120
H	0.824344	0.902539	-0.255848
H	1.281764	-0.007140	3.750995
H	0.824498	0.895174	2.290100
H	0.824270	-0.902508	2.285852

1,2-dimethyldiselane (CSeSeC = 90°)

Zero-point correction= 0.074838 (Hartree/Particle)

Thermal correction to Energy= 0.082128

Thermal correction to Enthalpy= 0.083072

Thermal correction to Gibbs Free Energy= 0.041401

Sum of electronic and zero-point Energies= -4882.896702

Sum of electronic and thermal Energies= -4882.889412

Sum of electronic and thermal Enthalpies= -4882.888468

Sum of electronic and thermal Free Energies= -4882.930139

Se	1.135660	0.710390	-0.918195

Se	1.093289	-0.572262	1.055562
C	-0.133652	-0.292852	-2.061570
C	-0.419302	0.258815	2.027866
H	-0.102728	0.194529	-3.037920
H	0.193175	-1.326192	-2.151321
H	-1.139870	-0.235776	-1.652226
H	-0.445318	-0.225420	3.005900
H	-0.237325	1.324522	2.145363
H	-1.354117	0.078200	1.501784

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 $NaHCO_3$ (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.

5. DTT oxidation kinetics

5.1. Oxidation assay

Solution of DTT (**19**, 250 µL, 1 mM) in deuterated sodium phosphate buffer (100 mM, pD 8.0) was mixed with solution of **9** or **22** (250 µL, 1 – 10 mM) in deuterated sodium phosphate buffer (100 mM, pD 8.0). The reaction mixture was transferred in a NMR tube and sealed with parafilm. Control experiment was performed by adding deuterated sodium phosphate buffer (250 µL, 100 mM, pD 8.0) to **19** (250 µL, 1 mM). ^{1}H NMR was measured on a Bruker 300 MHz spectrometer at different time until full conversion of **19** to **20**. The oxidation yield of DTT was calculated by comparing the integrals associated with protons of **19** (3.13 – 3.01 ppm, 2H) and of **20** (2.70 – 2.53 ppm, 4H).

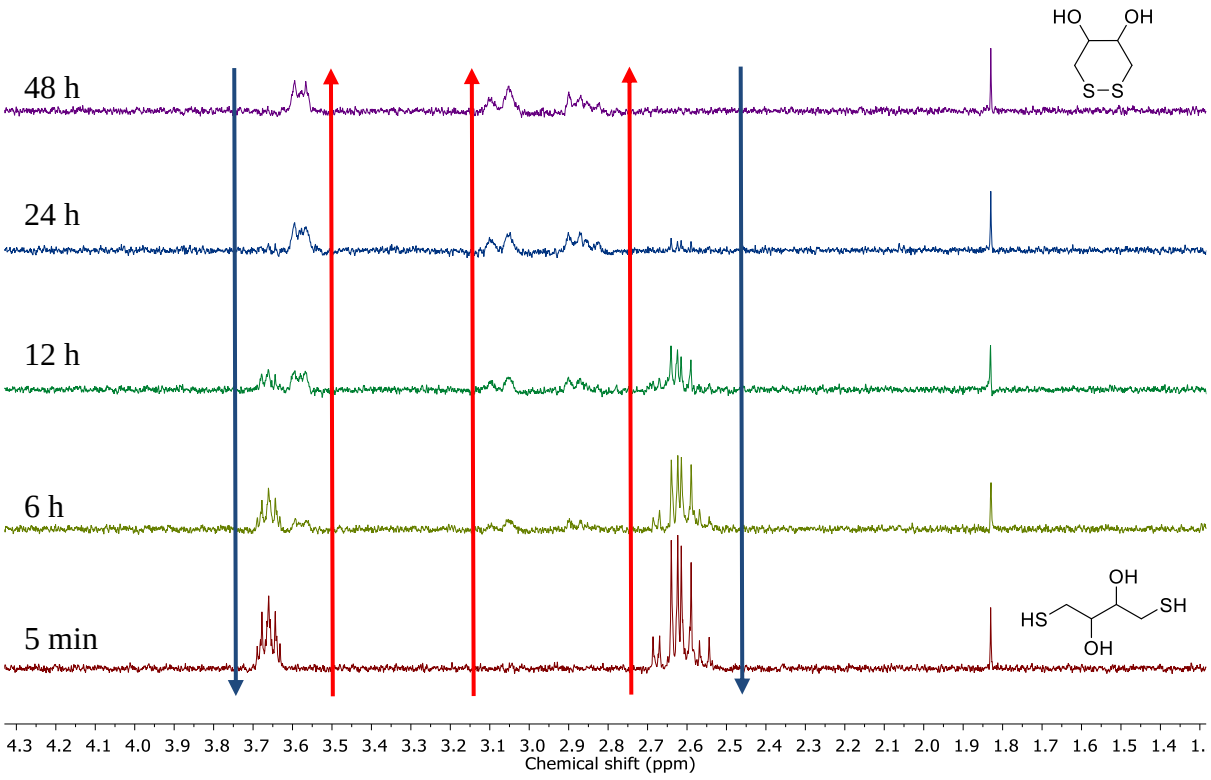


Fig. S1 Representative ^{1}H NMR showing the reaction evolution of the oxidation of **19** to **20** at different time in deuterated phosphate buffer (100 mM, pD 8.0). Signals from **19** and **20** are shown with blue and red arrow respectively.

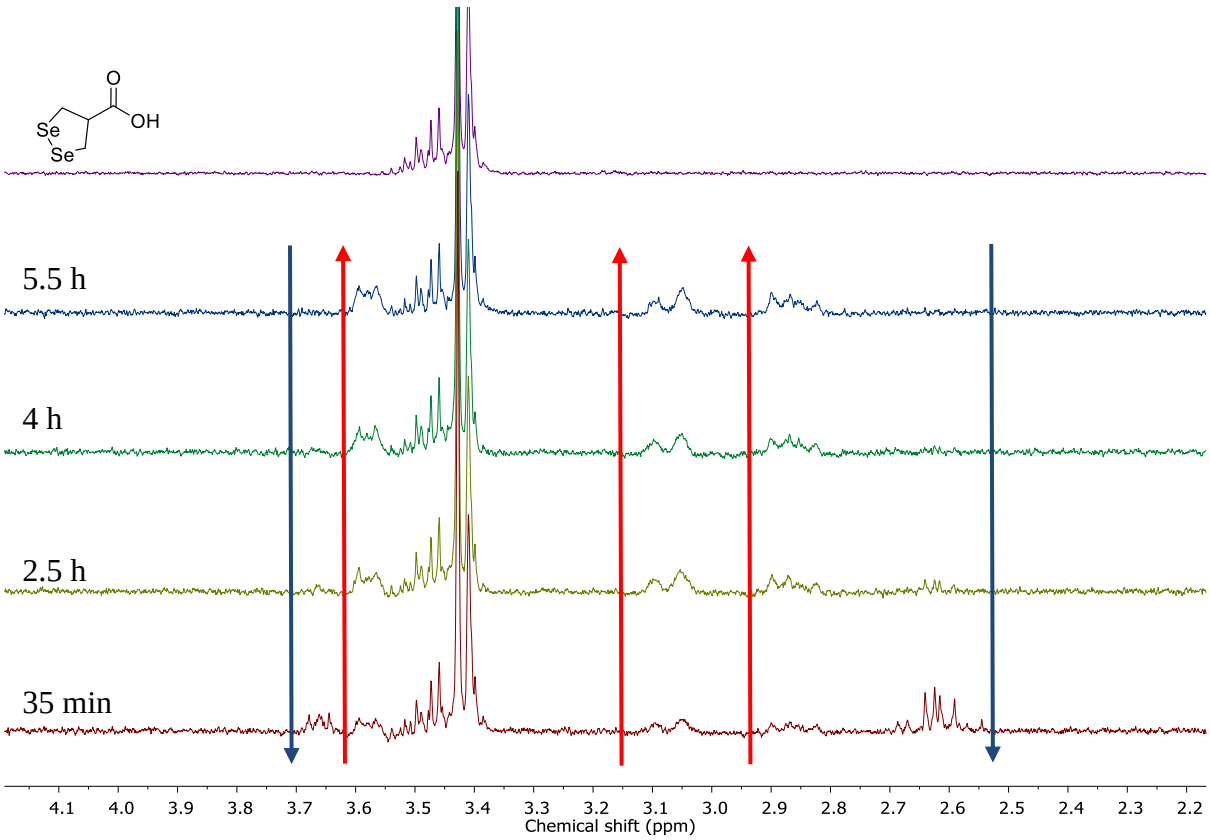


Fig. S2 Representative ^{1}H NMR showing SeA **22** and the reaction evolution of the oxidation of **19** to **20** in presence of **22** (1 mM) at different time in deuterated phosphate buffer (100 mM, pD 8.0). Signals from **19** and **20** are shown with blue and red arrow respectively.

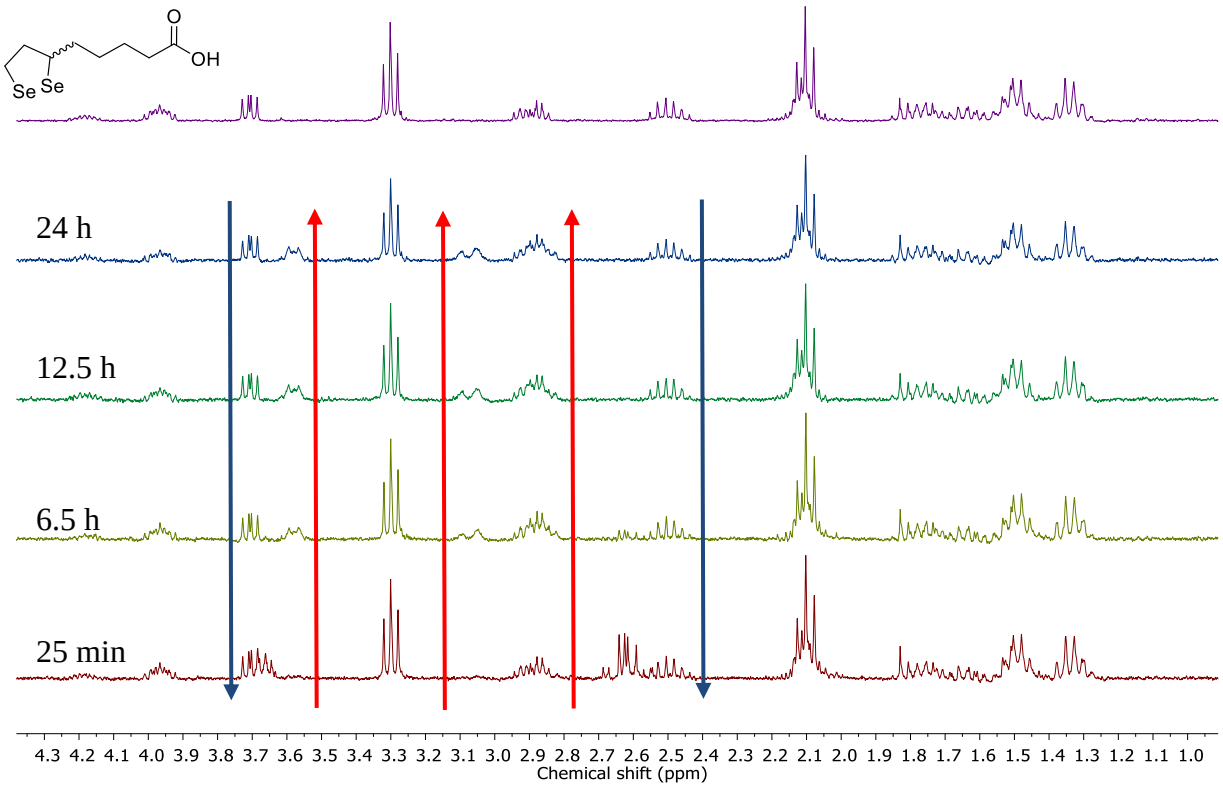


Fig. S3 Representative ^{1}H NMR showing SeL **9** and the reaction evolution of the oxidation of **19** to **20** in presence of **9** (2.5 mM) at different time in deuterated phosphate buffer (100 mM, pD 8.0). Signals from **19** and **20** are shown with blue and red arrow respectively.

The oxidation half-time t_{50} was determined by plotting the yield of **20** as a function of reaction time and solving the linear fit for 50% conversion (Figure S4). The t_{50} values are reported in Table S1.

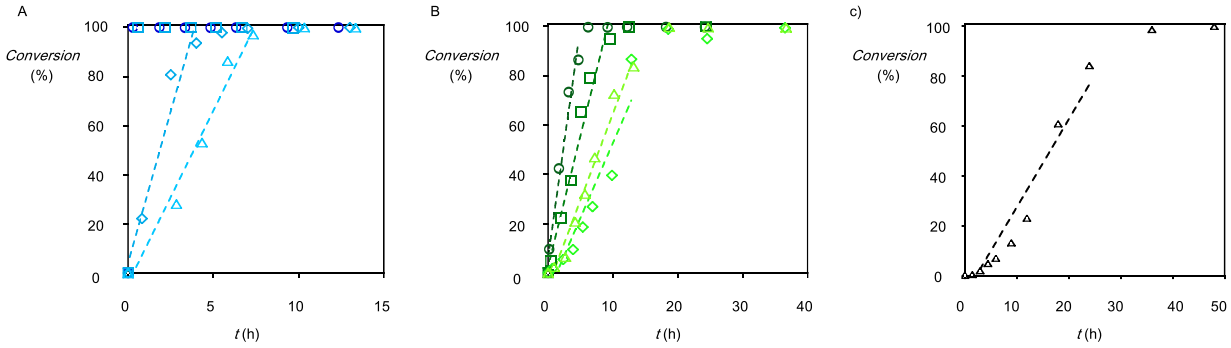


Fig. S4 DTT (0.5 mM) oxidation kinetics, catalyzed by **22** (A), **9** (B) and without catalyst (C) with linear fits. Concentration of catalysts: 5.0 (○), 2.5 (□), 1.0 (◊) and 0.5 (△) mM.

Table S1. Half-time t_{50} (h) of DTT oxidation as a function of the concentration of diselenide.

Entry	Concentration (mM)[a]	t_{50} with **9**[b] (h)	t_{50} with **22**[c] (h)
1	5.0	2.3 ± 0.2	<0.28
2	2.5	4.5 ± 0.3	<0.55
3	1.0	9.0 ± 1.0	1.9 ± 0.3
4	0.5	6.9 ± 0.9	3.9 ± 0.4
5	0.0	16.0 ± 2.0	16.0 ± 2.0

[a]Concentration of diselenides (**9** or **22**) in NMR tube. DTT concentration was 0.5 mM. [b]See Scheme S1. [c]See Scheme S2.

5.2. Rate constants

The pseudo-first order rate constant k was estimated from t_{50} by using the equation S1:

$$k = 0.693 / t_{50} \qquad \text{(S1)}$$

The obtained k's were plotted as a function of the concentration (c) of diselenide, and fitted with the linear regression to equation S2 to give the oxidation rate constant k_{ox}:

$$k = k_0 + k_{ox}\, c \qquad \text{(S2)}$$

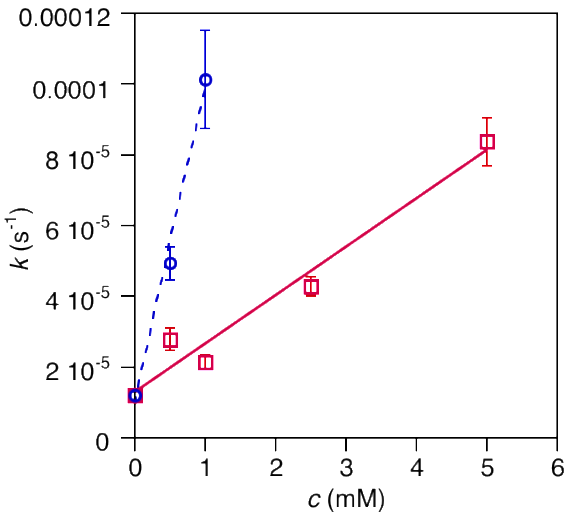


Fig. S5 Linear correlations between the rate constant k and the concentration c of **9** (□) and **22** (○).

6. Fluorescence calibration

6.1. Closed cycle correction factor

Stock solutions of **4**, **6** and **7** (10 mM in DMSO) were diluted to 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 was then recorded from 500 nm to 700 nm upon excitation at 488 nm (slits 2 nm : 2 nm). Closed cycle correction factors were calculated by dividing the integrated emission intensity through the recorded spectral region of compound **4** by that of **6** or **7** (Table S2).

Table S2. Fluorescence closed cycle correction factors for compounds **4**, **6** and **7**.

Entry	Compound[a]	Emission Intensity[b]	Correction factors[c]
1	**4**	$3.09\ 10^6$	1.00
2	**6**	$9.40\ 10^5$	3.29
3	**7**	$6.73\ 10^5$	4.59

[a]See Figure 1 and Scheme 1. [b]Emission Intensity recorded from 500 nm to 700 nm upon excitation at 488 nm (slits 2 nm : 2 nm). [c]Correction factors calculated by dividing the integrated emission intensity through the recorded spectral region of compound **4** by that of **6** or **7**.

6.2. Open cycle correction factor

Reduction of 9 and 22: TCEP (250 μL, 10 mM) in deoxygenated deuterated sodium phosphate buffer (100 mM, pD 8.0) was added to **9** or **22** (250 μL, 10 mM) in deoxygenated deuterated sodium phosphate buffer (100 mM, pD 8.0). The solution was transferred in a NMR tube and the spectrum was recorded on a Bruker 300 MHz spectrometer (Figure S6) to prove the reduction of diselenide using TCEP.

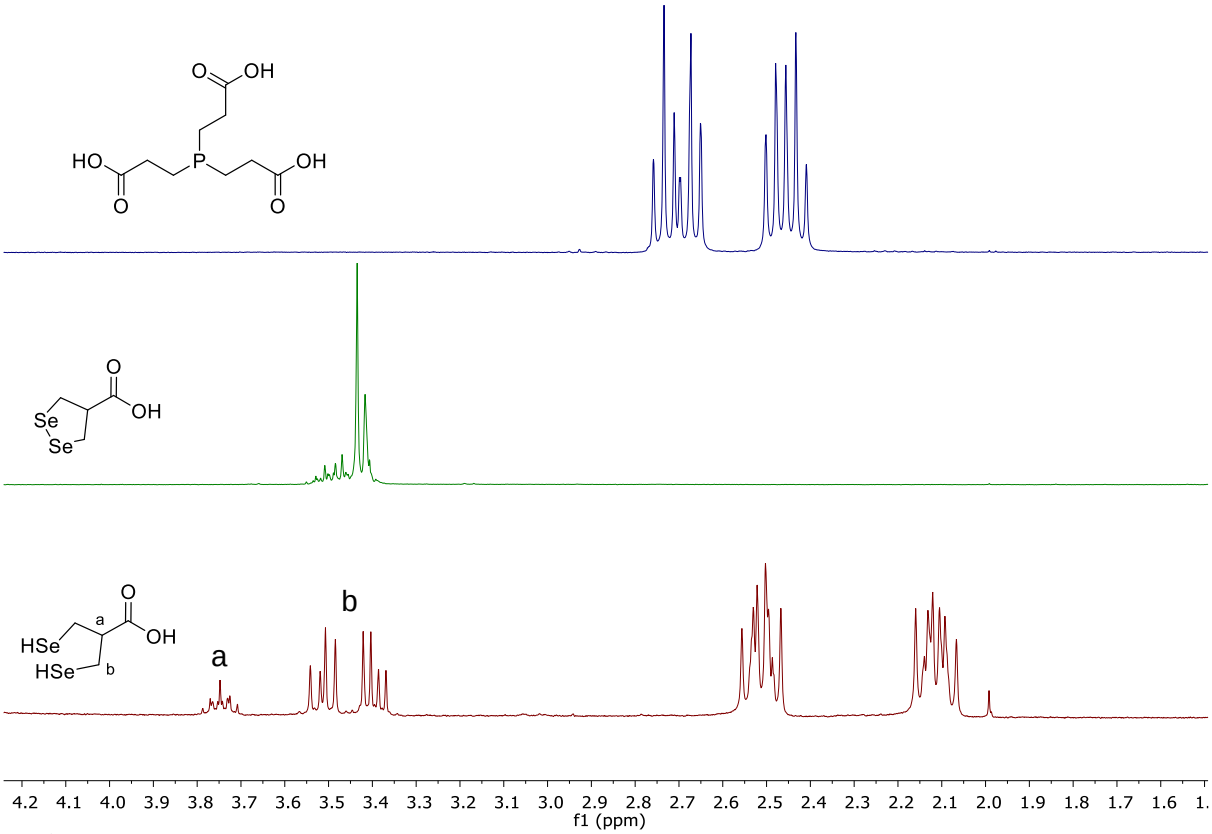


Fig. S6 ^{1}H NMR spectra of TCEP, **22**, and **22** reduced by TCEP in D_2O at pD 8.0.

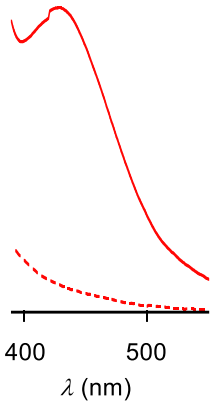


Fig. S7 Absorption spectrum of SeL (**9**) before (solid) and after treatment with TCEP (dashed).

To obtain the open cycle correction factor, the fluorescent compounds (2 µL, 100 µM) were treated for 30 min with TCEP (2 µL, 100 mM) before to be diluted to 1 µM solution of

fluorescent compounds in PBS buffer. The fluorescence emission was then recorded from 500 nm to 700 nm upon excitation at 488 nm (slits 1.2 nm : 1.2 nm). Open cycle correction factors were calculated by dividing the integrated emission intensity through the recorded spectral region of compound **4** by that of **6** or **10** (Table S3).

Table S3. Fluorescence open correction factors for compounds **4**, **6** and **7**.

Entry	Compound[a]	Emission Intensity[b]	Correction factors[c]
1	**4**	$1.80\ 10^6$	1.00
2	**6**	$1.21\ 10^6$	1.51
3	**7**	$9.54\ 10^5$	1.89

[a]See Figure 1 and Scheme 1 and S2. [b]Emission intensity recorded from 500 nm to 700 nm upon excitation at 488 nm (slits 1.2 nm : 1.2 nm), see Figure 3b. [c]Correction factors calculated by dividing the integrated emission intensity through the recorded spectral region of compound **4** by that of **6** or **7**.

7. Cellular uptake experiments

7.1. Cell culture

Human cervical cancer-derived HeLa Kyoto cells were cultured in minimum essential medium (MEM) containing 10% fetal bovine serum (FBS), 1% Penicillin / Streptomycin (PS) and 1% L-Glutamine. The cells were grown on a 25 cm^3 tissue culture flask (TPD corporation) at 37 °C under 5% CO_2.

7.2. Confocal microscopy

HeLa-Kyoto cells were seeded at 5×10^4 cells/well on 35 mm glass-bottomed dishes (MatTek Corporation) and cultured overnight. After removing the medium, the cells were washed with PBS (2 x 1 mL) and with Leibovitz's medium (1 mL) before being treated with compound **1**, **4**, **6** or **7** (1 mL, 1 – 100 μM in Leibovitz's medium). The cells were incubated for 1 – 4 h at 37 °C, then the media was removed by aspiration. For experiment with the

nucleus stained, Hoechst 33342 (1 μL for a 10 μg/mL in H_2O stock solution) was added to the incubation mixture for the last 10 min. Cells were washed with PBS containing 0.1 mg/mL heparin (3 x 1 mL) and with Leibovitz's medium (3 x 1 mL). The cells were kept in Leibovitz's medium during the microscope experiment. Fluorescence images were obtained without fixing using a confocal laser scanning microscope (Leica SP5 or SP8) equipped with 63× oil immersion objective lens. On Leica SP5 microscope, Ar laser was used as light source (15% laser power) with excitation wavelength 488 nm and emission 498 – 535 nm (Leica HyD™ detector) for CF distribution. On Leica SP8 microscope, OPSL488 laser was used as light source (0.84% laser power) with excitation wavelength 488 nm and emission 501 – 550 nm (PMT detector) for CF distribution and Diode 405 laser was used as light source (6.45% laser power) with excitation wavelength 405 nm and emission 437 – 470 nm (PMT detector) for Hoechst 33342 distribution. During CLSM analysis the sample was kept at 37 °C.

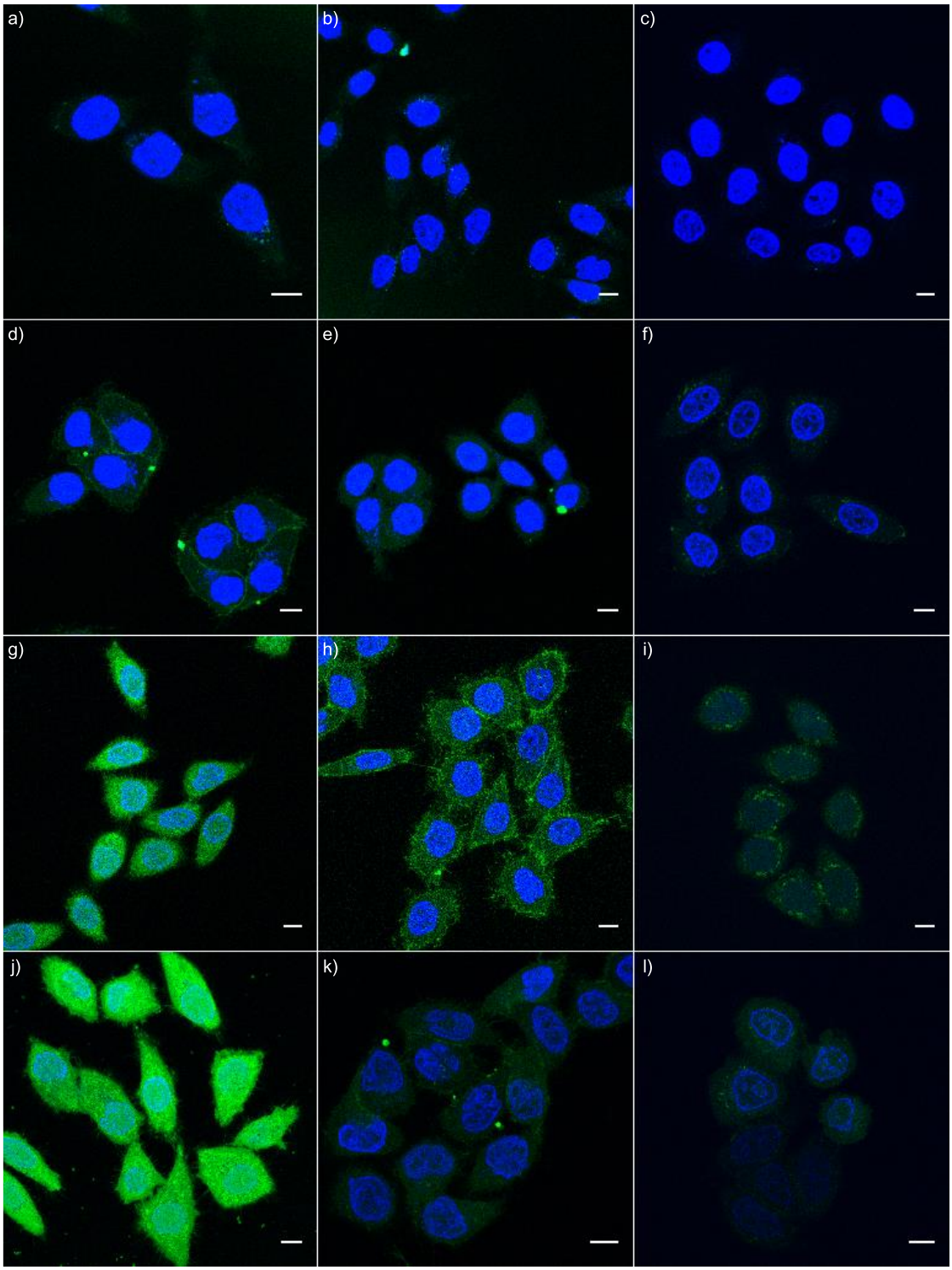


Fig. S8 Merged and pseudo-colored (green for $\lambda_{ex}/\lambda_{em}$ 488/517 ± 18 nm, blue for $\lambda_{ex}/\lambda_{em}$ 405/454 ± 16 nm) CLSM images of HeLa Kyoto cells incubated for 1 h with **7** (a, d, g and j), **6** (b, e, h and k) and **1** (c, f, i and l) at 1 (a – c), 10 (d – f), 50 (g – i) and 100 (j – l) µM. Scale bar: 10 µm.

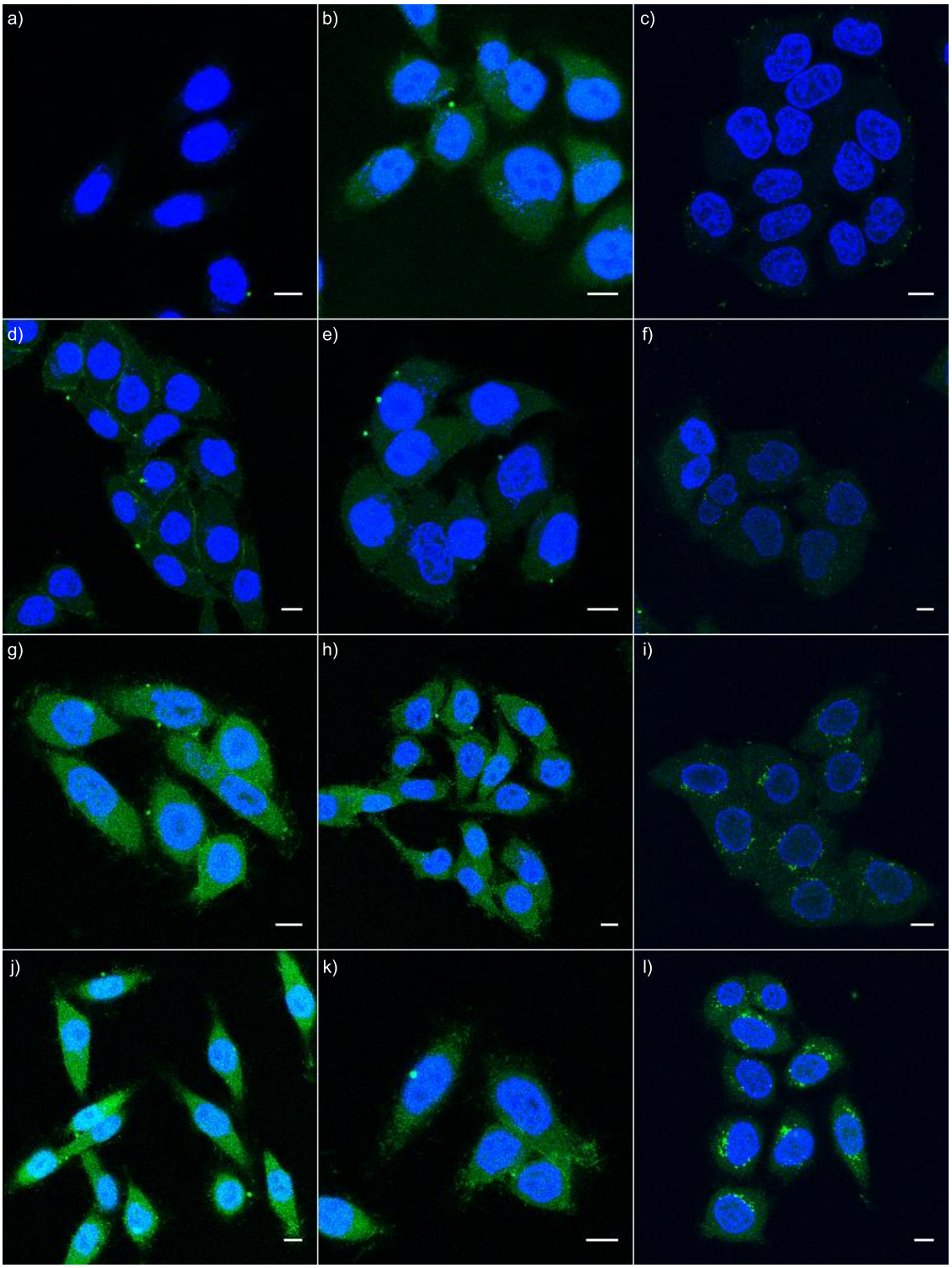


Fig. S9 Merged and pseudo-colored (green for $\lambda_{ex}/\lambda_{em}$ 488/517 ± 18 nm, blue for $\lambda_{ex}/\lambda_{em}$ 405/454 ± 16 nm) CLSM images of HeLa Kyoto cells incubated for 15 min (a – c), 1 (d – f), 2 (g – i) and 4 (j – l) h with **7** (a, d, g and j), **6** (b, e, h and k) and **1** (c, f, i and l) at 10 µM. Scale bar: 10 µm.

7.3. Colocalization assay

HeLa-Kyoto cells were seeded at 5×10^4 cells/well on 35 mm glass-bottomed dishes (MatTek Corporation) and cultured overnight. After removing the medium, the cells were washed with PBS (2 x 1 mL) and with Leibovitz's medium (1 mL) before being treated as following:

MitoTracker: cells were incubated with 1 mL of compounds **6** or **7** (10 μM in Leibovitz's medium) for 1 h at 37 °C. The last 20 min, MitoTracker Red CMXRos (Life Technologies) was added to the dish (final concentration: 500 nM).

LysoTracker: cells were incubated with 1 mL of compounds **6** or **7** (10 μM in Leibovitz's medium) for 1 h at 37 °C. The last 40 min, LysoTracker Red DND-99 (Life Technologies) was added to the dish (final concentration: 100 nM).

Dextran Red: cells were incubated with 1 mL of compounds **6** or **7** (10 μM in Leibovitz's medium) for 1 h at 37 °C. The last 20 min, Dextran Red (70 kDa, Life Technologies) was added to the dish (final concentration: 10 μM).

After the incubation, the media was removed by aspiration. Cells were washed with PBS containing 0.1 mg/mL heparin (3 x 1 mL) and with Leibovitz's medium (3 x 1 mL). The cells were kept in Leibovitz's medium during the microscope experiment. Fluorescence images were obtained without fixing using a confocal laser scanning microscope (Leica SP5) equipped with 63× oil immersion objective lens. Ar laser (10 – 15% laser power) was used as light source with excitation wavelength 488 nm and emission 492 – 534 nm (Leica HyD™ detector) for compounds **6** and **7**; DPSS laser (4 – 15%) with excitation wavelength 561 nm and emission 580 – 650 nm for colocalization dyes. During CLSM analysis the sample was kept at 37 °C.

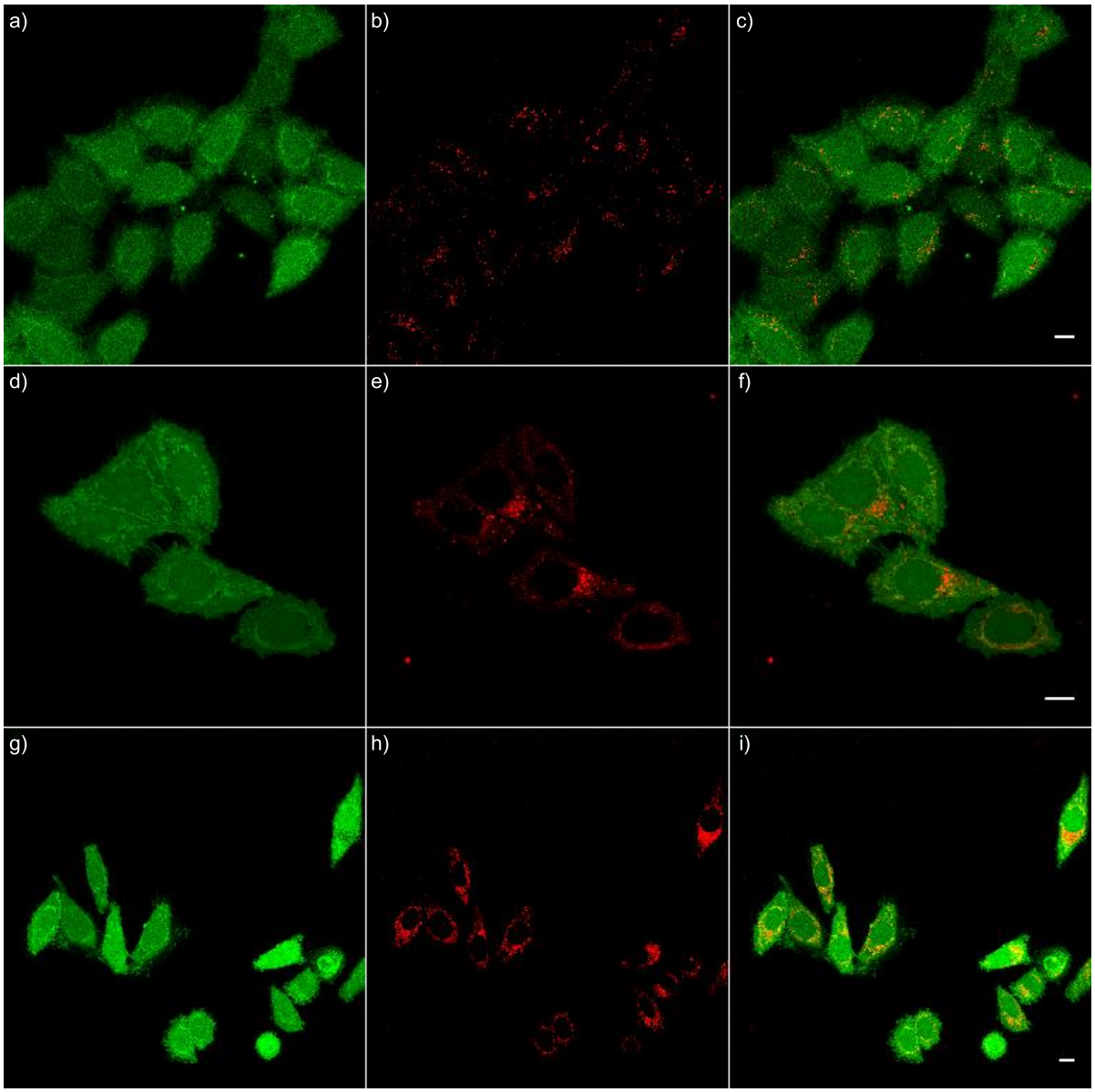


Fig. S10 CLSM images of HeLa Kyoto cells treated with **7** and lysosomes (a – c), endosomes (d – f) and mitochondria (g – i) probes. Images are pseudo-colored in green (a,d and g, $\lambda_{ex}/\lambda_{em}$ 488/517 ± 18 nm, for **7**) and red (b, e and f, $\lambda_{ex}/\lambda_{em}$ 516/615 ± 35 nm, for colocalization probes) and merged (c, f and i). Scale bar: 10 μm.

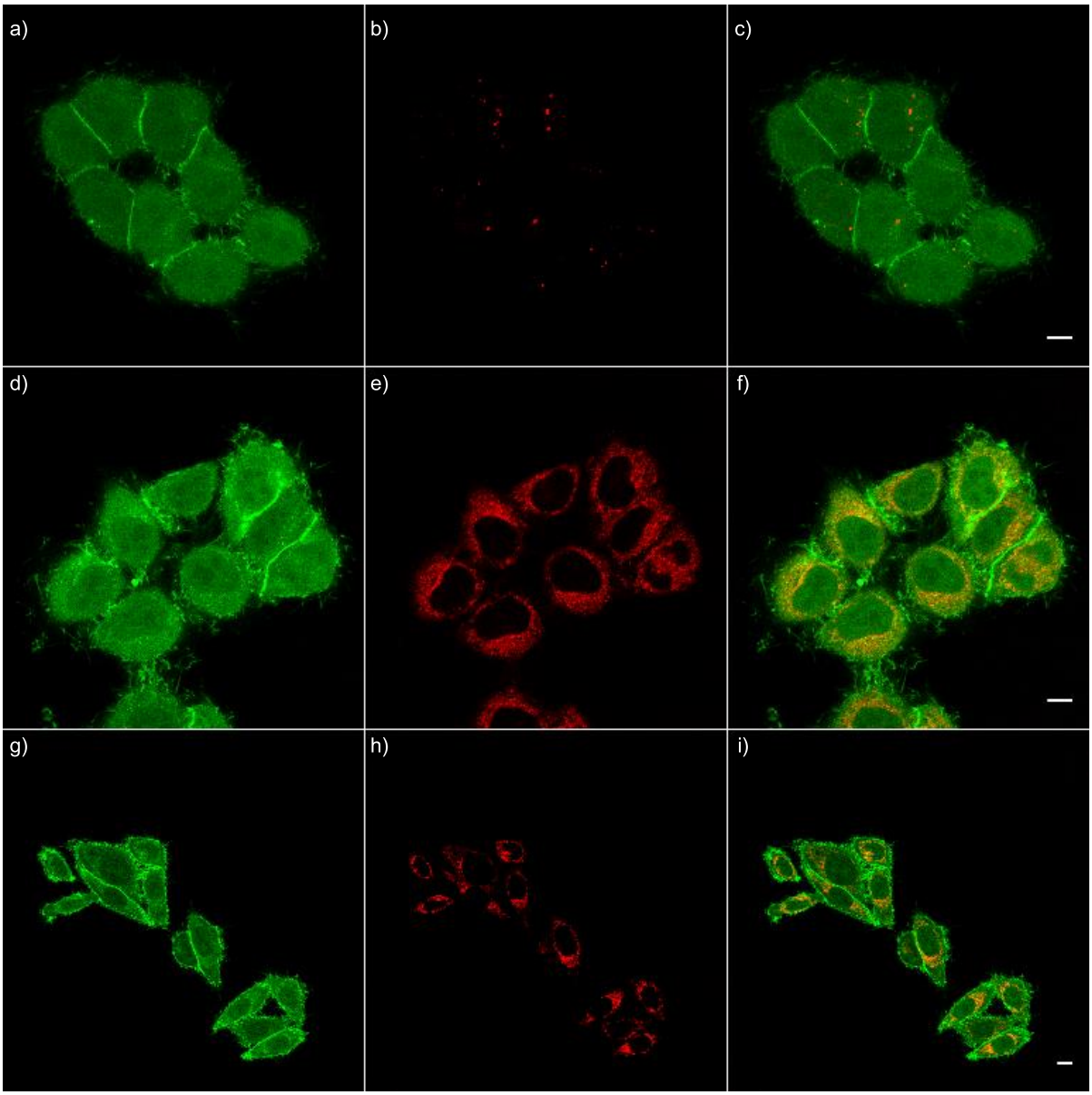


Fig. S11 CLSM images of HeLa Kyoto cells treated with **6** and lysosomes (a – c), endosomes (d – f) and mitochondria (g – i) probes. Images are pseudo-colored in green (a,d and g, $\lambda_{ex}/\lambda_{em}$ 488/517 ± 18 nm, for **6**) and red (b, e and f, $\lambda_{ex}/\lambda_{em}$ 516/615 ± 35 nm, for colocalization probes) and merged (c, f and i). Scale bar: 10 μm.

The colocalization quantification was performed using the ImageJ software (Ver. 1.51q), equipped with Coloc 2 plugin. The test was performed on at least 3 different pictures containing at least 5 cells each.

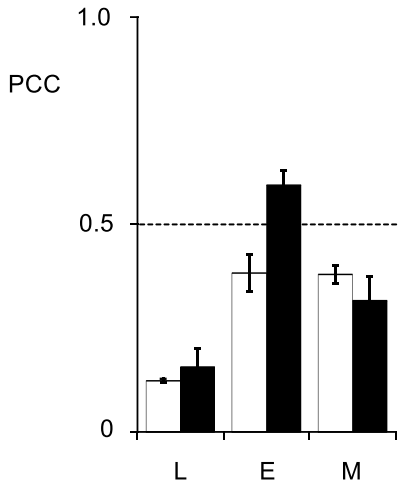


Fig. S12 Colocalization of compounds **7** (white) and **6** (black) with lysosomes (L, LysoTracker Red), endosomes (E, Dextran Red) and mitochondria (M, Mitotracker Red), quantified with PCC.

7.4. Flow cytometry

HeLa-Kyoto cells were seeded at 1×10^5 cells/well in a 6-well plate (BD Falcon) and cultured overnight. After removing the medium, the cells were washed with PBS (2 x 1 mL) and with Leibovitz's medium (1 mL) before being treated with 1 mL of compound **4**, **6** or **7** (10 μM in Leibovitz's medium). The cells were incubated for 1 – 4 h at 37 °C then the media was removed by aspiration. Cells were washed with PBS containing 0.1 mg/mL heparin (1 mL) and with PBS (2 x 1 mL) before detachment by treatment with 0.05% trypsin-EDTA (1 mL) at 37 °C for 5 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 in cells were detected by laser excitation at 488 nm and emission at 525 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. The experiments were done in triplicate.

For experiment in presence of serum, the procedure described before was applied, adding 10% FBS in the incubation medium. For 4 °C experiments, cells were preincubated in growing medium at 4 °C for 1 h. The same procedure described before was applied, using PBS solution at 4 °C for washing and performing the incubation with compound **7** in a 4 °C refrigerator. For inactivation of cell surface thiols, cells were preincubated with DTNB solution (1.2 mM in MEM) before incubation with **7**. After incubation, the cells were treated in the same way as described before.

7.5. Endocytosis inhibition

HeLa Kyoto cells were seeded at 1×10^5 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% CO_2 with 1 mL of one of the specific endocytosis inhibitors in MEM: chlorpromazine (CPZ, 30 μM), methyl-*β*-cyclodextrin (m*β*CD, 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 **7** (10 μM in MEM, 1 mL), containing the same amount of inhibitor. The cells were incubated for 1 h at 37 °C. The media was then removed by aspiration and the cells were washed three times with PBS before detachment with trypsin for 5 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 supernatant was removed and the cells re-suspended in 600 μL PBS containing 1 μL/mL SR and 0.02% EDTA. Fluorescent signals in cells were detected by laser excitation at 488 nm and emission at 525 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. The experiments were done in triplicate.

7.6. MTT assay

HeLa Kyoto cells were seeded with 100 μL of cells suspension at 5×10^4 cells/mL in a 96-well plate (BD Falcon) and cultured 2 days. After removing the medium, the cells were incubated with 100 μL of the desired CF-compound solution (10 or 100 μM in MEM) for 24 h at 37 °C under 5% CO_2. Then, MTT solution (10 μL of 12 mM in sterile PBS) was added to each well. The cells were incubated for 4 h at 37 °C under 5% CO_2. Finally, SDS solution (100 μL of 100 g/L SDS in 0.01 M HCl) was added to each well and the cells were incubated for 2 h in the dark. The absorbance of the resulting solution was measured at 570 nm.

8. Supporting references

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(S2) (*a*) P. K. Glasoe and F. A. Long, *J. Phys Chem.*, 1960, **64**, 188−190. (*b*) W. J. Lees and G. M. Whitesides, *J. Org. Chem.*, 1993, **58**, 642−647.

(S3) F. Xu, Z.-Z. Yang and S.-J. Zhang, *Phosphorus Sulfur Silicon Relat. Elem.*, 2013, **188**, 1312−1319.

(S4) (*a*) G. Bergson, *Ark. Kemi*, 1962, **19**, 195−214. (*b*) A. F. Ferris, *J. Org. Chem.*, 1955, **20**, 780−787.

(S5) M. Haratake, S. Matsumoto, M. Ono and M. Nakayama, *Bioconjugate Chem.*, 2008, **19**, 1831−1839.

9. NMR spectra

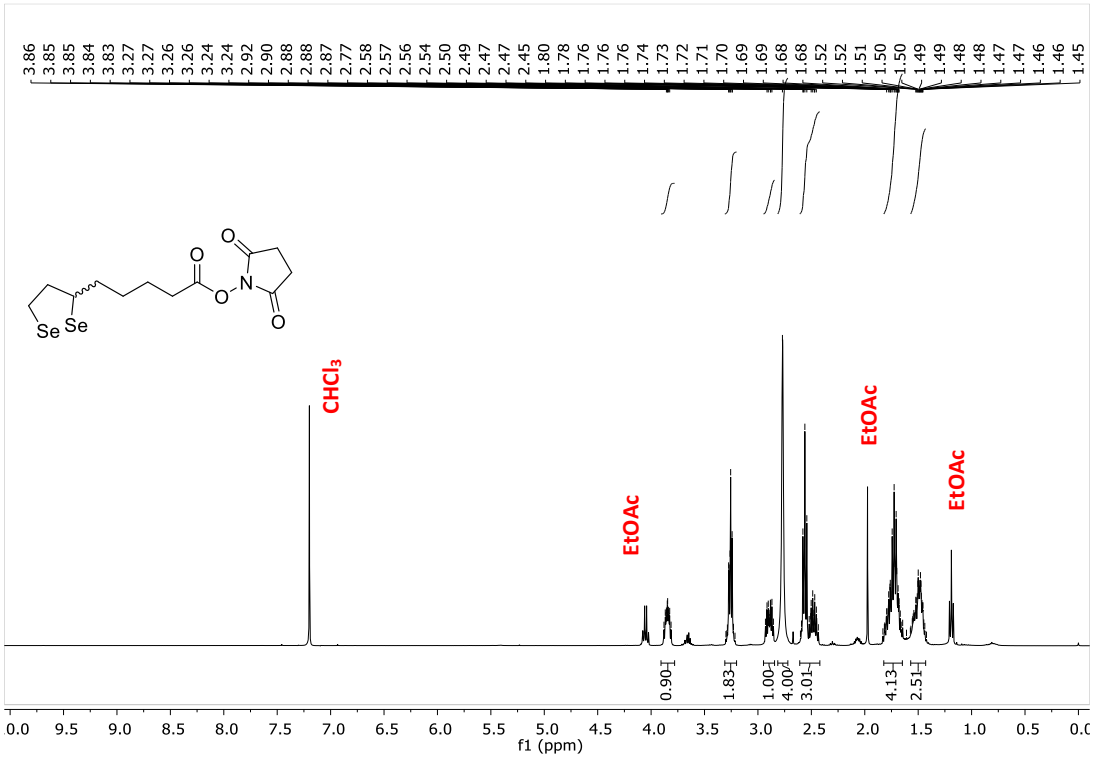

Fig. S13. ^{1}H NMR spectrum of **10** in $CDCl_3$.

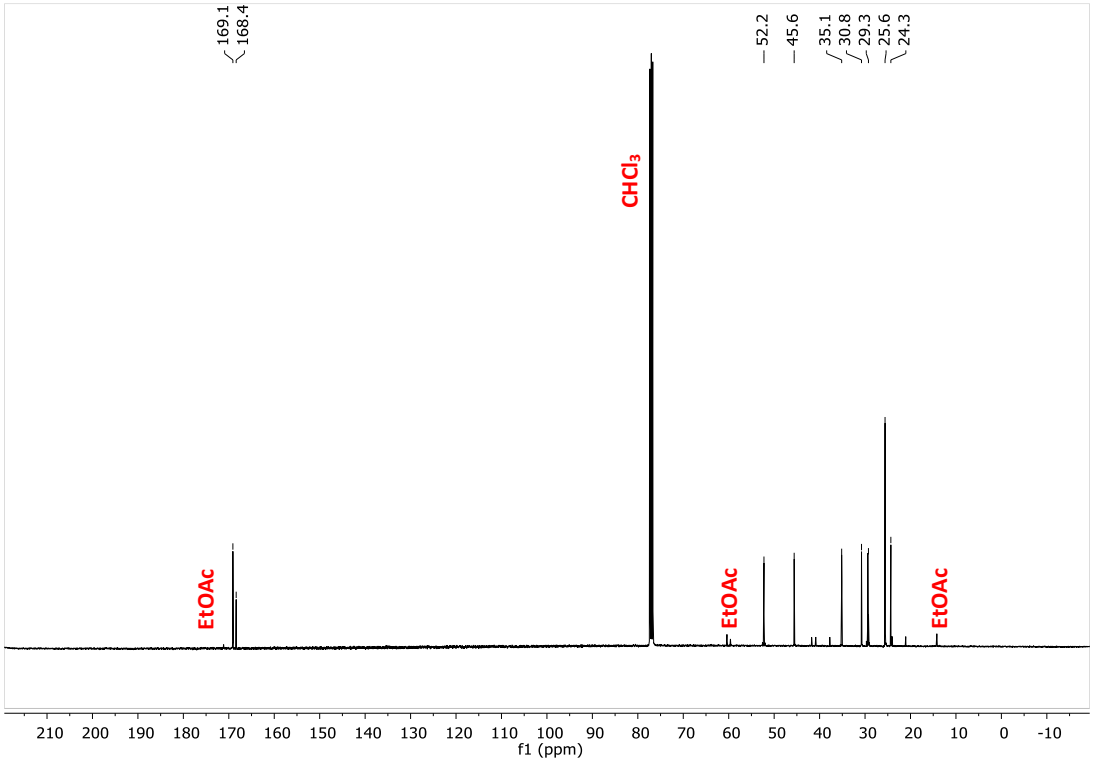

Fig. S14 ^{13}C NMR spectrum of **10** in $CDCl_3$.

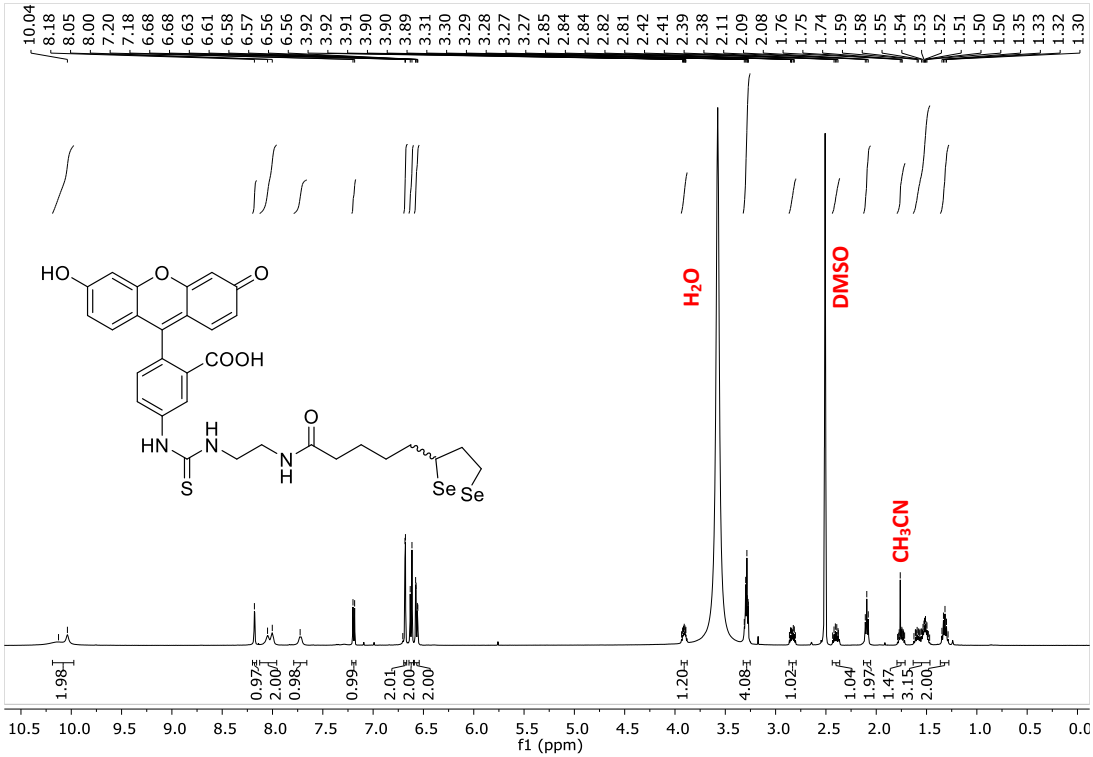

Fig. S15 ^{1}H NMR spectrum of **7** in DMSO-d_6.

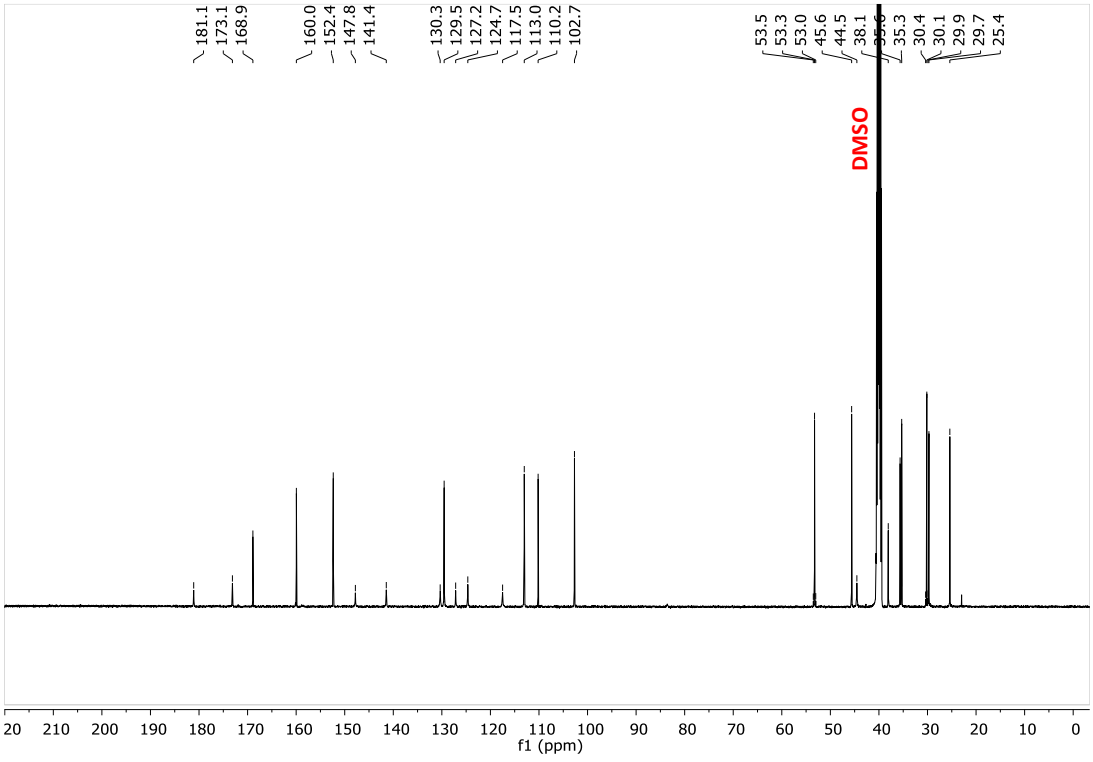

Fig. S16 ^{13}C NMR spectrum of **7** in DMSO-d_6.

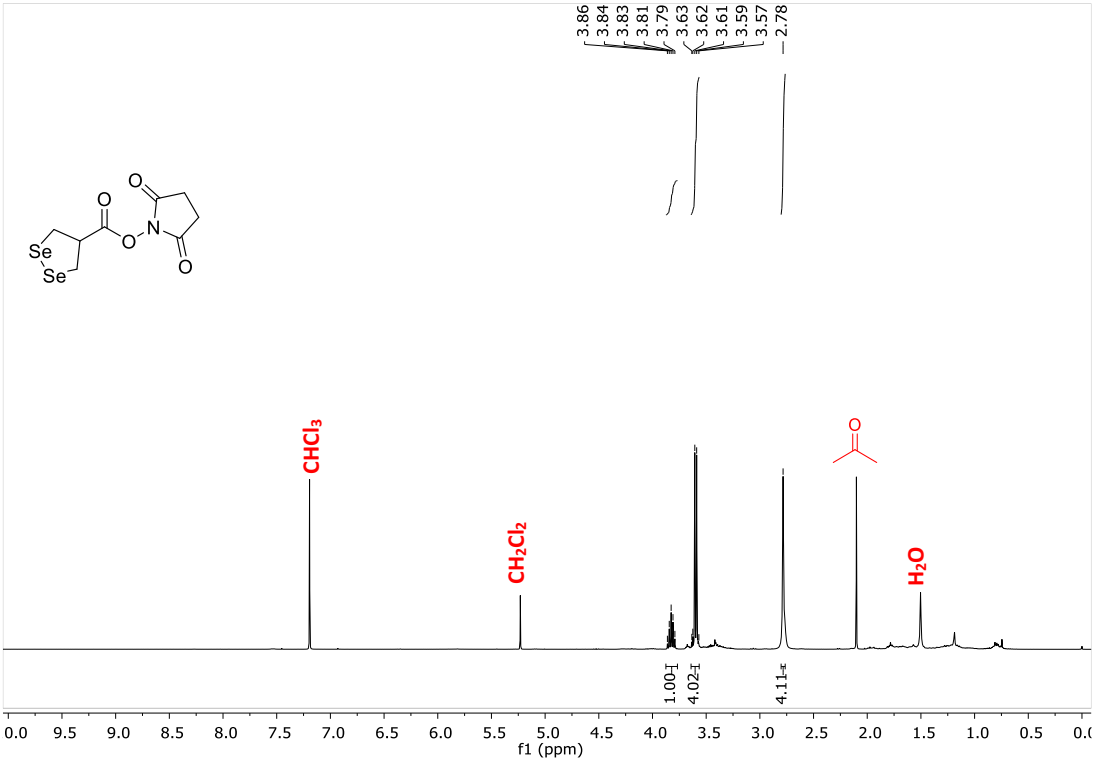

Fig. S17 ^{1}H NMR spectrum of **23** in $CDCl_3$.

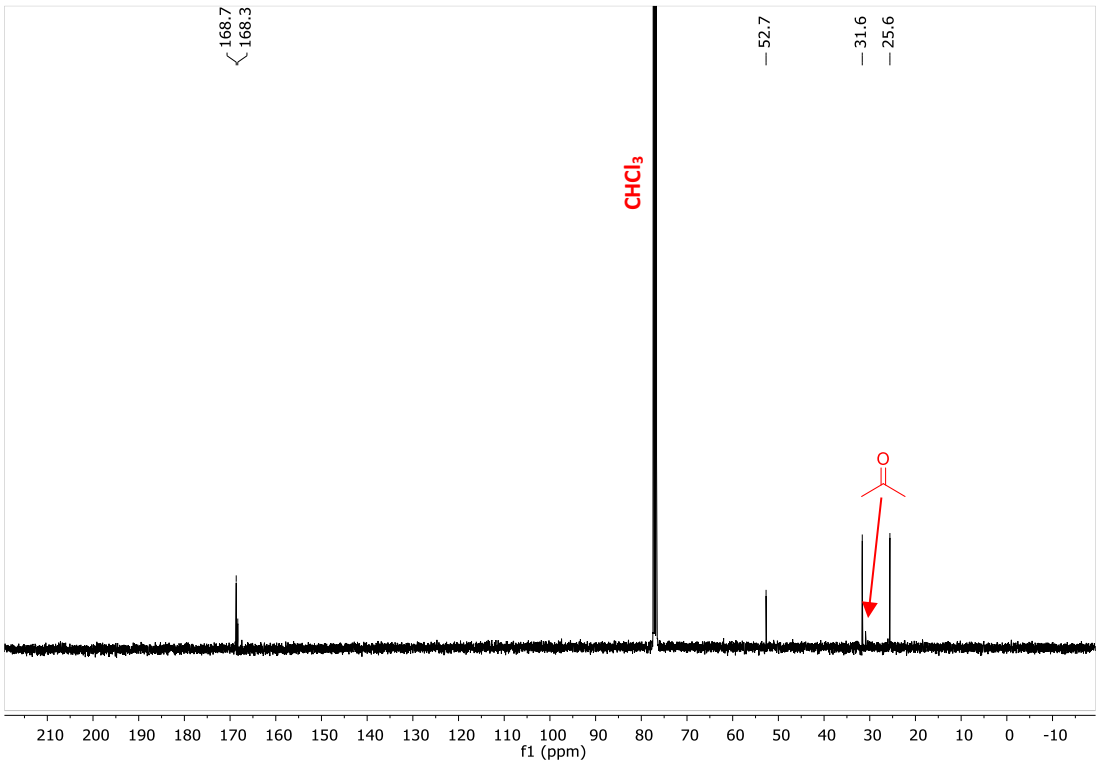

Fig. S18 ^{13}C NMR spectrum of **23** in $CDCl_3$.

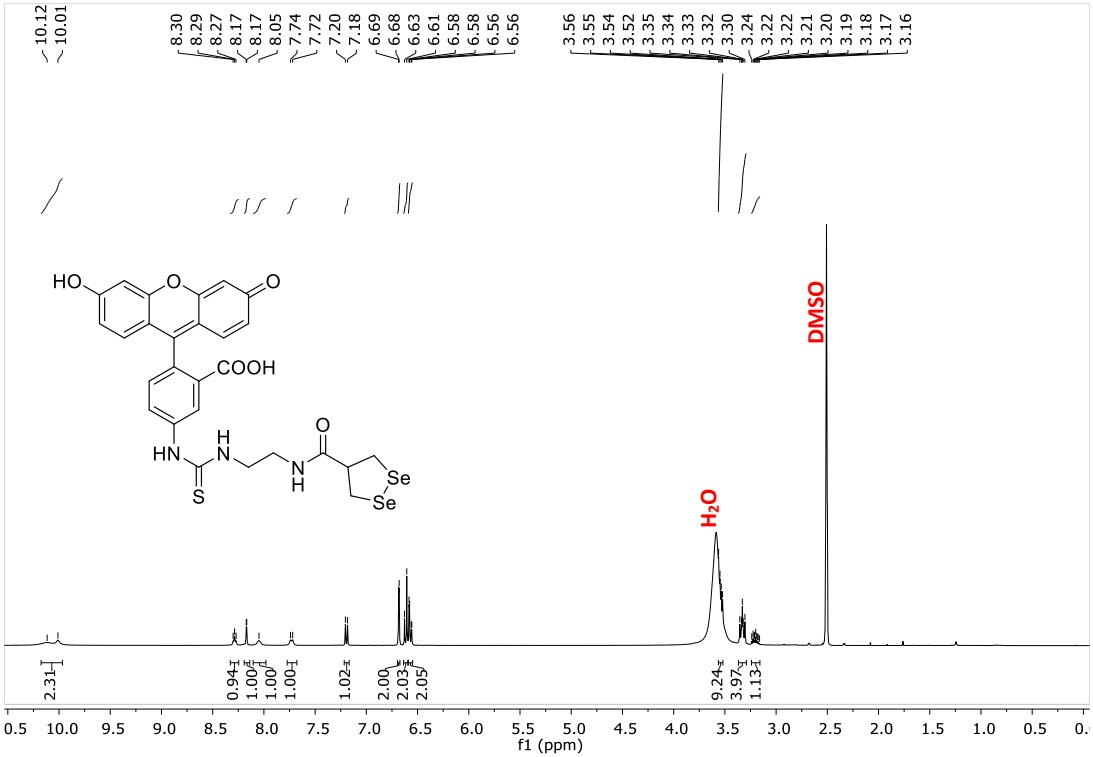

Fig. S19 ^{1}H NMR spectrum of **6** in DMSO-d_6.

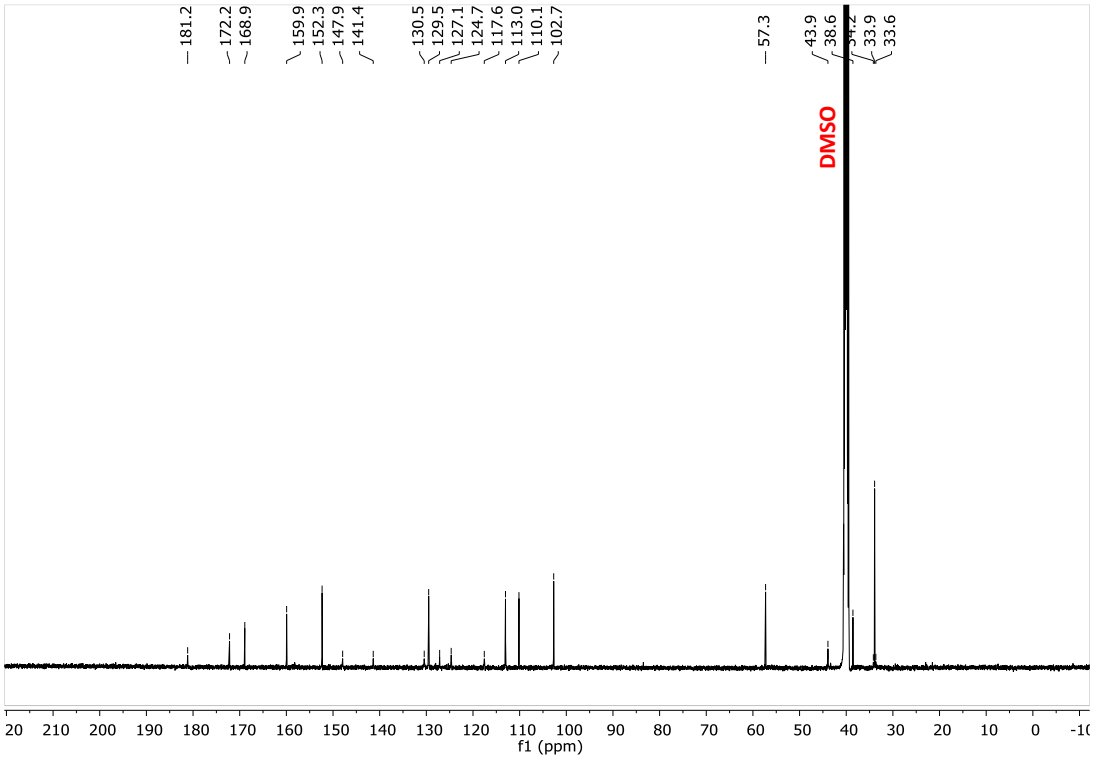

Fig. S20 ^{13}C NMR spectrum of **6** in DMSO-d_6.

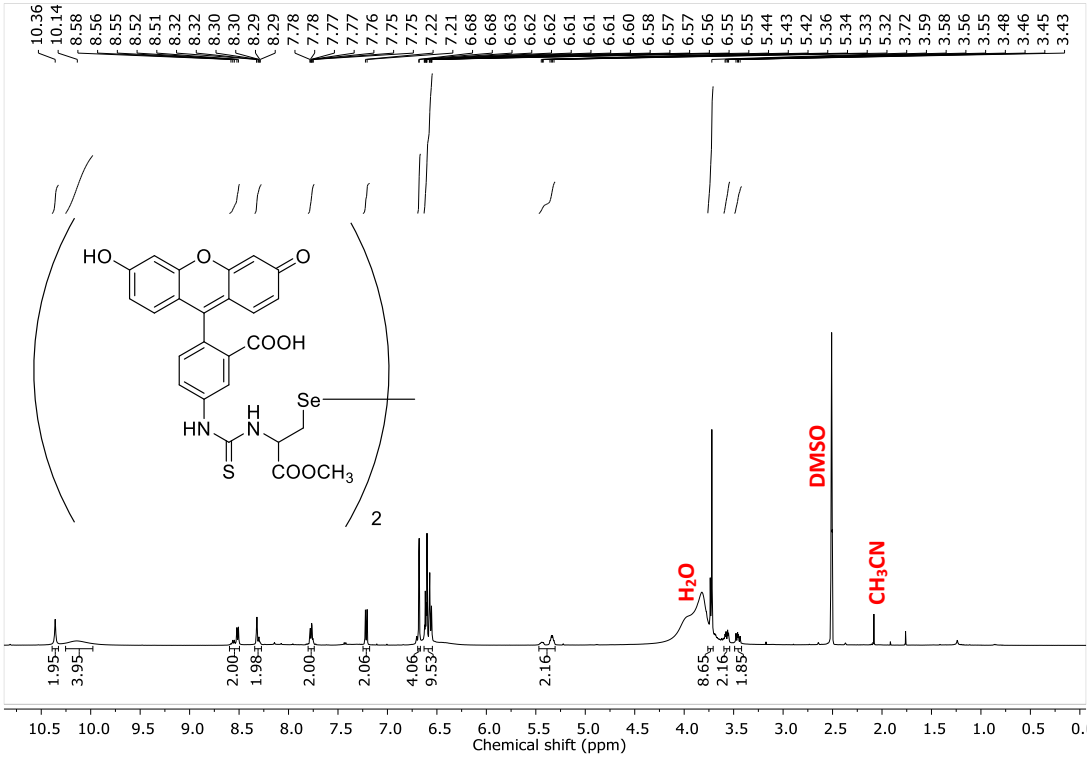

Fig. S21 ^{1}H NMR spectrum of **1** in DMSO-d_6.

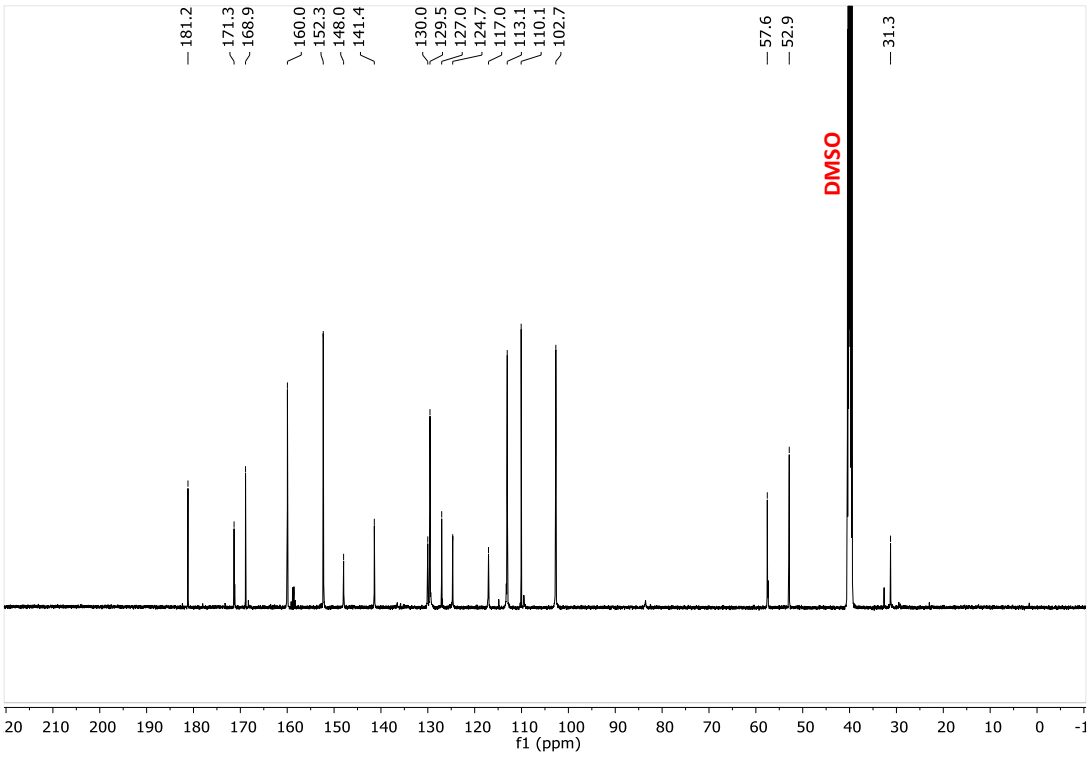

Fig. S22 ^{13}C NMR spectrum of **1** in DMSO-d_6.

10. UHPLC-MS spectra

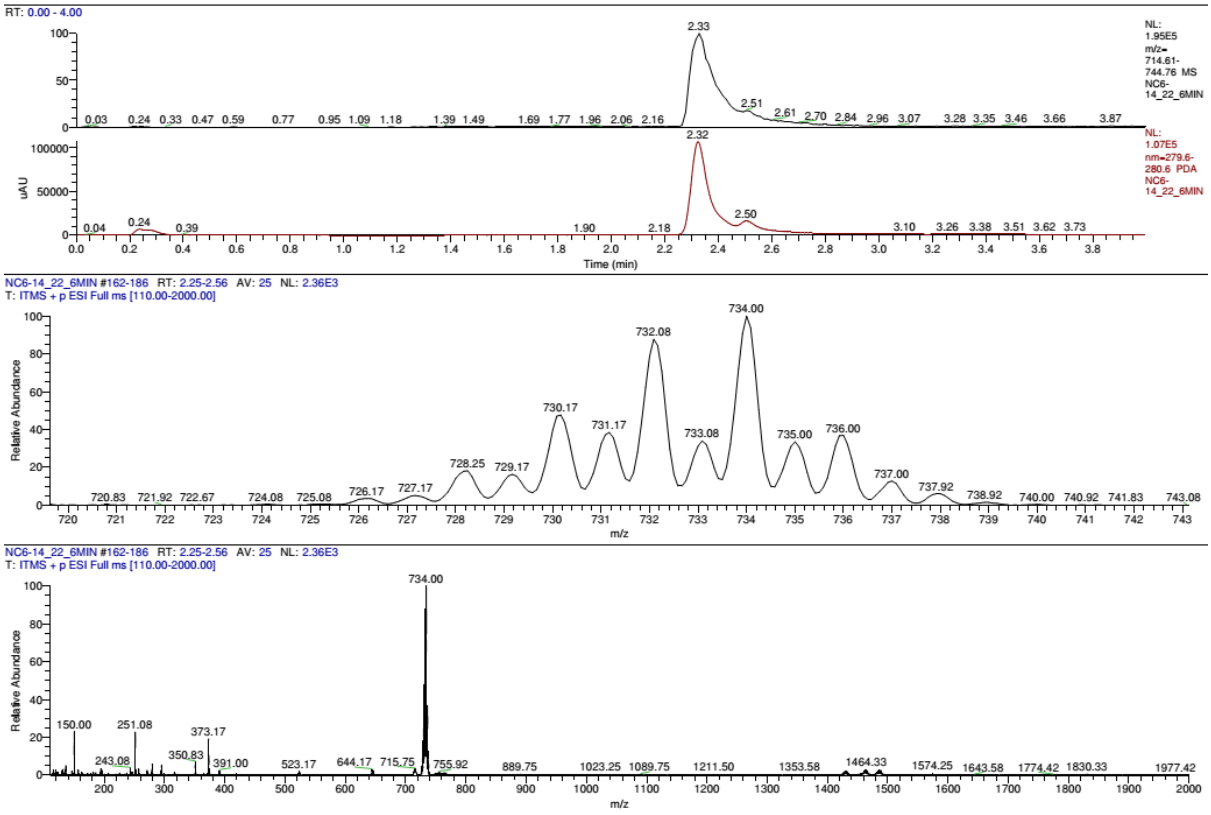

Fig. S23 UHPLC-MS spectrum of **7**.

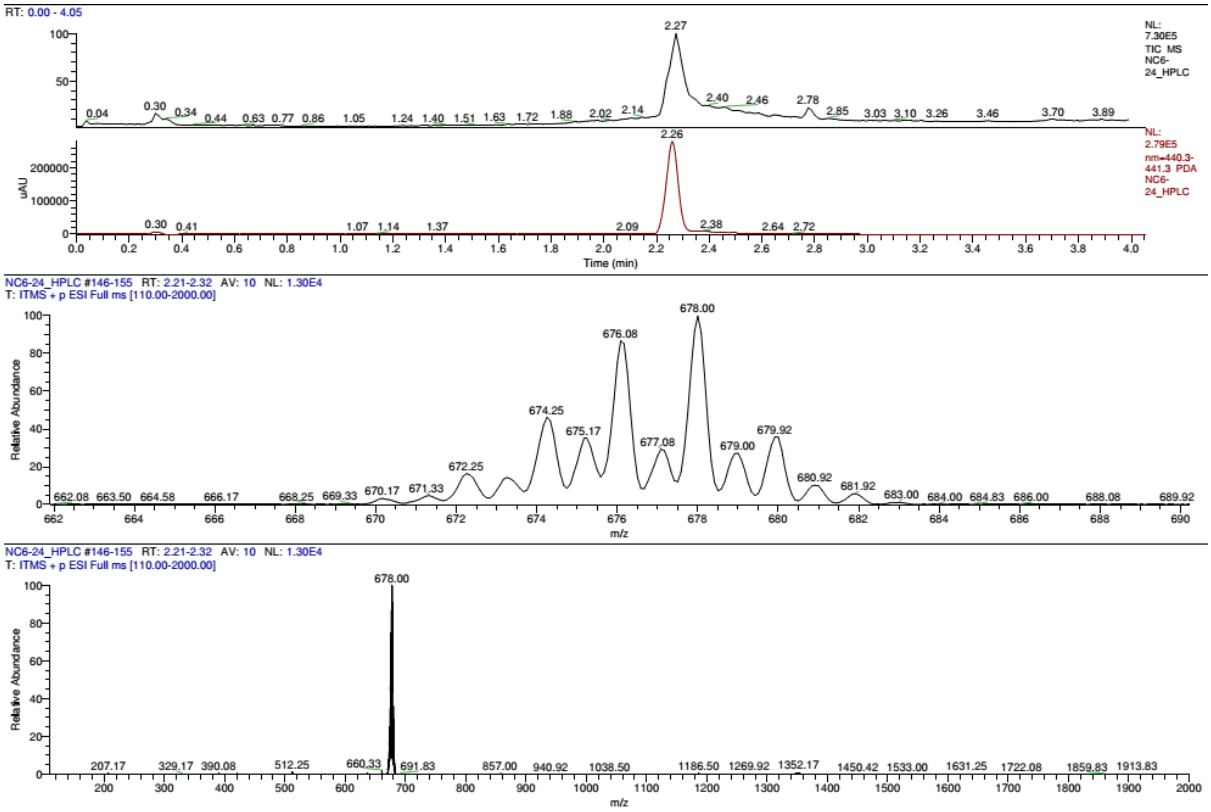

Fig. S24 UHPLC-MS spectrum of **6**.

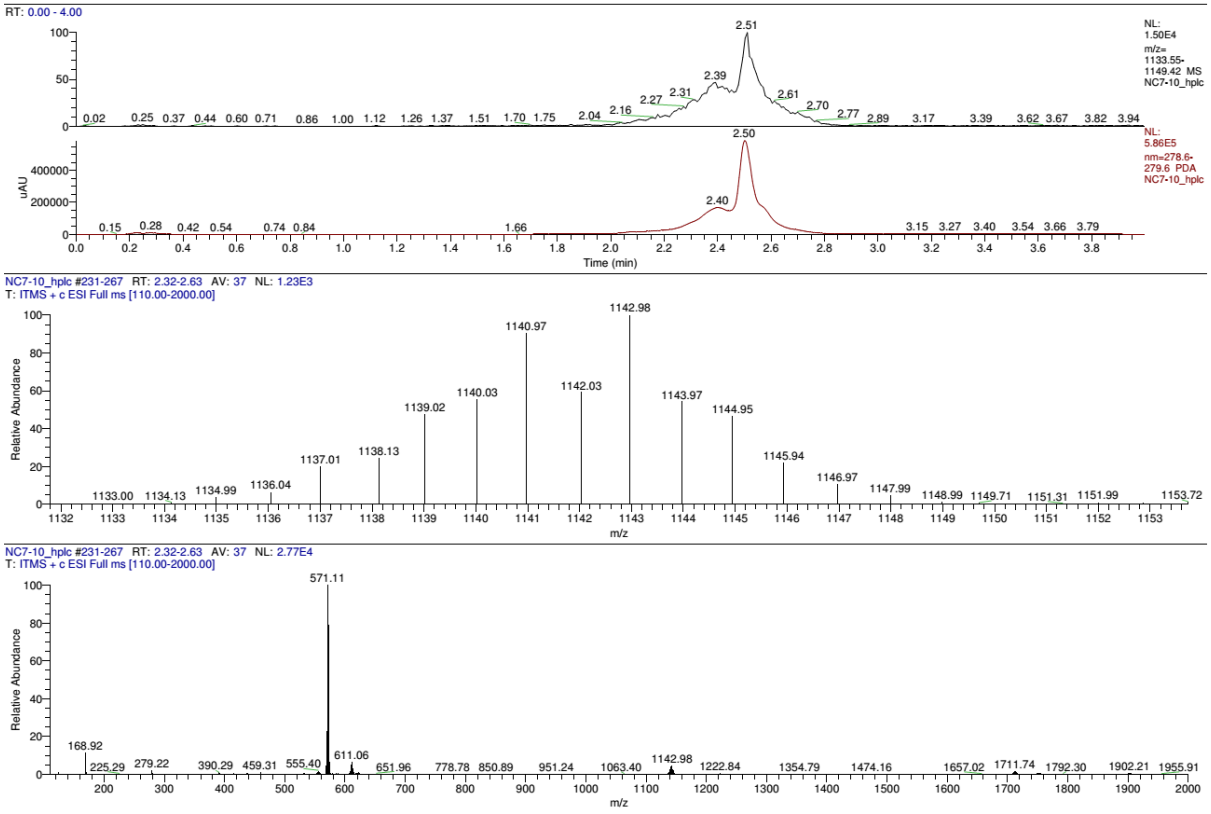

Fig. S25 UHPLC-MS spectrum of **1**.