Electronic Supplementary Material (ESI) for Chemical Communications

Supporting information

Glutathione triggered intracellular cleavage of bis-sulfide bioconjugates for tumor responsive drug release

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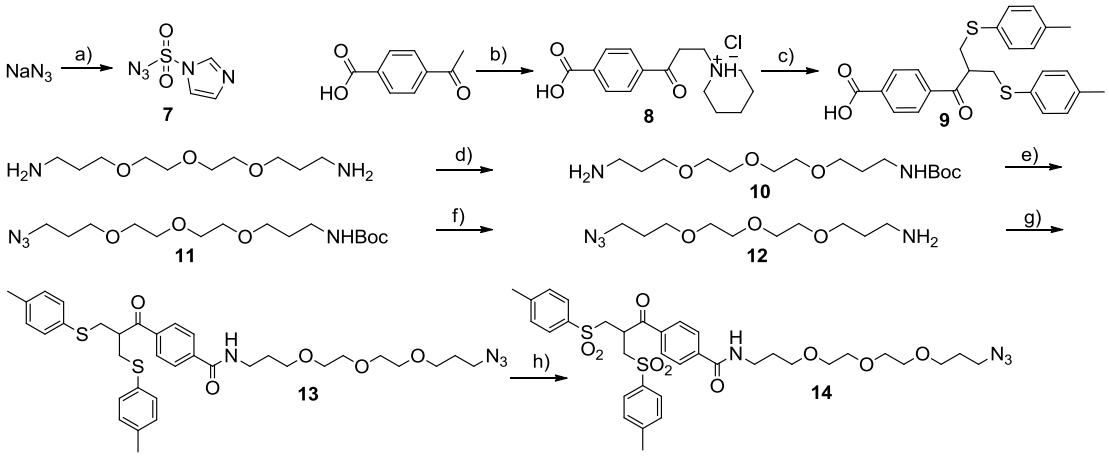
1. General experimental details

Unless otherwise noted, all operations were performed without taking precautions to exclude air and moisture. All solvents and reagents were purchased from commercial sources and were used without further purification. Reaction progress was monitored by thin layer chromatography (TLC) using Merck 60 F254 pre-coated silica gel plates illuminating under UV 254 nm or using appropriate stains. Flash column chromatography was carried out using Merck silica gel 70-230 mesh. NMR spectra were measured on Bruker 400 MHz or 500 MHz NMR spectrometer and the chemical shifts were referenced to residual solvent shifts in the respective deutero solvents. Chemical shifts are reported as parts per million referenced with respect the residual solvent peak. Mass spectra were acquired on a Finnigan Mat LCQ (ESI) spectrometer or a Bruker Reflex III (MALDI-TOF). LC-MS analysis was performed on a Shimadzu LC-MS 2020 equipped with an electrospray ionisation source and a SPD-20A UV-Vis detector (Shimadzu, Duisburg, Germany). The absorbance and emission were measured on Microplate Readers (Tecan Infinite M1000 PRO).

2. Synthesis of azido-SST (1b)



Scheme 1. Synthesis of azido-bissulfone (14). a) SO_2Cl_2, MeCN, imidazole, 72%; b) piperidine hydrochloride, paraformaldehyde, EtOH, 48%; c) 4-methylbenzenethiol, piperidine, 37 wt.% formaldehyde in water, EtOH-MeOH, 75%; d) Boc, DCM, 43%; e) **7**, K_2CO_3, $CuSO_4$, 75%; f) TFA, DCM, 95%; g) **3**, HBTU, DIEA, DMF, 70%; h) Oxone, MeOH-H_2O (1:1), 90%;

8 and **9** were synthesized according to the published procedure[1].

Synthesis of Imidazole-1-sulfony azide hydrochloride (7)[2]

Sulfuryl chloride (0.4ml, 5mol) was added drop-wise to an ice-cooled suspension of NaN_3 (0.32g, 5mmol) in ACN (5ml) and the mixture stirred overnight at RT. Imidazole (0.68g, 10mmol) was added portion-wise to the ice-cooled mixture and the resulting slurry stirred for 3 h at RT. The mixture was diluted with EtOAc, washed with H_2O then saturated aqueous $NaHCO_3$, dried over $MgSO_4$ and filtered. A solution of HCl in EtOH [obtained by the drop-wise addition of AcCl (0.533ml, 7.5mmol) to ice-cooled dry ethanol (2ml)] was added drop-wise to the filtrate with stirring, the mixture chilled in an ice-bath, filtered and the filter cake washed with EtOAc to give Imidazole-1-sulfonyl azide hydrochlorideas colourless needles.

^{1}H NMR (500MHz, $CDCl_3$):δ 9.0613(s, 1H), 7.9226 (t, 1H), 7.4972 (t, 1H); **^{13}C NMR** (100MHz, $CDCl_3$):δ 127.6,

135.3, 145.6; **ESI-MS**: (+) m/z= 173.9 [M+H]

Synthesis of tert-butyl (3-(2-(2-(3-aminopropoxy)ethoxy)ethoxy)propyl) carbamate (10)[3]

A solution of 4,7,10-trioxoxa-1,13 -tridecanediamine (1g, 1ml) in DCM (15 ml) was treated with Boc-anhydride (0.521ml) dropwise for 15min. The mixture was stirred at RT for 12h. The solvent was removed, and the resulting yellow oil was purified by silica gel flash chromatography (10% MeOH in DCM containing 1% NH_4OH) to produce 313 mg of **10** as oil product in 43% yield.

^{1}H NMR (500MHz, $CDCl_3$): δ 3.59(m, 12H), 3.21 (m, 2H), 2.79(t, 2H), 1.73 (m, 2H), 1.42 (s, 9H); **^{13}C NMR** (100MHz, $CDCl_3$):δ 28.5, 29.6, 32.7, 38.4, 39.6, 69.6, 70.2, 70.6, 78.9, 156.1

Synthesis of tert-butyl (3-(2-(2-(3-azidopropoxy)ethoxy)ethoxy)propyl)carbamate (11)

Imidazole-1-sulfonyl azide (**7**, 320 mg, 1.873mmol, 1.2eq.) was added to the Boc-ethylene glycol (**8**, 500 mg, 1.56mmol, 1eq.), K_2CO_3 (110 mg, 0.78mmol, 0.5eq.) and $CuSO_4.5H_2O$ (4mg, 0.0156mmol, 0.01eq.) in MeOH (10 ml) and the mixture stirred at RT. The mixture was concentrated, diluted with H_2O (15 ml) and extracted with EtOAc (3 × 10 ml). The combined organic layers were dried over $MgSO_4$, filtered and concentrated. The crude product was purified by flash chromatography using Hexane / EA (2:1) to give 389 mg of **11**in 72% yield.

^{1}H NMR (500MHz, $CDCl_3$):δ 1.41 (s, 9H), 1.73 (m, 2H), 1.83 (m,2H), 3.19 (m,2H), 3.37 (t, 2H), 3.50-3.64 (m, 12H); **^{13}C NMR** (100MHz, $CDCl_3$):δ 28.5, 29.1, 29.6, 38.6, 48.4, 67.9, 70.2, 70.4, 70.6, 78.9, 156.1;

Synthesis of 3-(2-(2-(3-azidopropoxy)ethoxy)ethoxy)propan-1-amine (12)

Boc-ethylene glycol (**11**, 350 mg, 1.01 mmol, 1 eq.) was dissolved in 5 ml of DCM and trifluoroacetic acid (TFA, 0.774 ml, 10.1 mmol, 10 eq.) was added. The resulting mixture was stirred at RT overnight. The solvent and TFA were removed under vacuumto afford 236 mg of in 95% yield.

^{1}H NMR (400MHz, $CDCl_3$):δ1.81 (m, 2H), 1.92 (m, 2H), 3.22 (m, 2H), 3.35 (t, 2H), 3.52-3.65 (m, 12H) ; **^{13}C NMR**(100MHz, $CDCl_3$):δ26.0, 28.5, 40.9, 48.4, 68.3, 69.7, 70.8;**FT-IR** 2095 cm^{-1} .

Synthesis of N-(3-(2-(2-(3-azidopropoxy)ethoxy)ethoxy)propyl)-4-(3-(p-tolylthio)- 2-((p-tolylthio) methyl)propanoyl)benzamide (13)

Bis-sulfide (**9**, 380 mg, 0.866mmol, 1 eq.) dissolved in 1ml of anhydrous DMF was added HBTU (530 mg, 1.386mmol, 1.6eq.) and DIEA (290 µl, 1.732mmol, 2eq.) at 0℃ under argon. The reaction mixture was stirred for 10mins before 3-(2-(2-(3-azidopropoxy)ethoxy) ethoxy) propan-1- amine (**12**, 210 mg, 0.866mmol, 1.2 eq.) was added. The resulting mixture was stirred overnight at RT. The solvent was reduced under high vacuum and the crude product was dissolved in DCM. The organic phase was washed with saturated $NaHCO_3$ twice, brine and dried over anhydrous Na_2SO_4. The solvent was evaporated under vacuum and crude product was purified by flash chromatography to afford 403 mg of **13** in 70% yield.

^{1}H NMR (500MHz, $CDCl_3$):δ 7.76 (d, 2H), 7.60 (d, 2H), 7.14(d, 4H), 7.06 (d, 4H), 3.79 (m, 1H), 3.64 (m, 10H), 3.47 (m, 4H), 3.32(t, 2H), 3.24(m, 2H), 3.15 (m, 2H), 2.35 (s, 6H), 1.91(m 2H), 1.78 (m, 2H) ; **^{13}C NMR**(125MHz, $CDCl_3$):δ 200.33, 166.03, 138.85, 138.45, 137.20, 131.46, 131.15, 129.84, 128.47, 127.22, 70.44, 70.41, 70.33, 70.22, 67.86, 48.38, 45.58, 39.35, 36.41, 29.03, 28.64, 21.08; **ESI-MS**: (+) m/z= 665.2 [M+H], 687.3[M+Na]

N-(3-(2-(2-(3-azidopropoxy)ethoxy)ethoxy)propyl)-4-(3-tosyl-2- (tosylmethyl)propanoyl) benzamide (14)

Azido-sulfide (**13**, 250 mg, 0.376mmol, 1 eq.) and OXONE (1.39g, 2.256mmol, 6 eq.) were added to a 1:1 methanol-deionized water solution (1ml). The reaction mixture was allowed to stir at RT for 48h. The mixture was diluted by deionized water (60 ml) and extracted with chloroform (4*50 ml). More water was added to dissolve all the inorganic solids and the aqueous layer was extracted once with chloroform (50 ml). The chloroform extracts were combined and wash once with Brine. Then the organic chloroform layer was dried over anhydrous sodium sulfate and filtered. The solvent was removed under vacuum to afford 247 mg of **14** in 90% yield.

^{1}H NMR (400MHz, $CDCl_3$):δ 1.72-1.79 (m, 2H), 1.87-1.92 (m,2H), 2.47 (s, 6H), 3.28-3.32 (t, 2H), 3.42-3.49 (m, 6H), 3.57-3.69 (m, 12H), 4.30-4.35 (m, 1H), 7.34 (d, 4H), 7.62-7.67 (m, 6H), 7.78 (d, 2H); **^{13}C NMR**(125MHz,

$CDCl_3$):δ 21.8, 28.5, 29.0, 35.5, 48.4, 55.5, 67.9, 70.2, 70.3, 70.5, 71.0, 127.6, 128.3, 128.7, 130.2, 132.9, 135.3, 136.0, 145.6, 165.8, 202.5; **MALDI-TOF:** m/z= 729.65 [M+H].

Synthesis of azido-SST (1b)

Azido ethylene glycol bissulfone (**14**，8.9 mg, 12.2 μmol, 2 eq.) was dissolved in 10 ml of 40% ACN in PB buffer (50 mM PB, 10 mM EDTA, pH 7.8) and shaken at RT overnight to generate azido monosulfone. Somatostatin (10mg, 6.1μmol, 1eq.) was dissolved in 5ml of 40% ACN in PB buffer. TCEP (3.5mg, 12.2μmol, 2eq.) and azido monosulfone solution above were added sequentially. The mixture was incubated at RT overnight. The mixture was purified by Prep HPLC using an Atlantis Prep OBD T3 Column (19 x 100 mm, 5 μm) with the mobile phase starting from 100% solvent A (0.1% TFA in MiliQ water) and 0% solvent B (0.1% TFA in ACN) (0-20 min), raising to 30% B in 1min, 30% B for 9mins, raising to 40% B in 1min, 40% solvent B for 11 mins and finally reaching 100% B in 5 mins with a flow rate of 10 ml/min. The absorbance was monitored at 280 nm and 254 nm. The retention time for **1** was 37.3 min. 5mg of**1** was obtained from lyophilisation in 40% yield. **MALDI-TOF-MS**: (+) m/z=2056.4 (M+H) (calc. exact mass = 2054.94) (Fig. S2)

3. HPLC study of reaction kinetics and MALDI-MS characterization.

The HPLC analysis was conducted using MerckChroCART 125-4 Column with mobile phase consisted of 0.1% TFA in water (A) and 0.1% TFA in ACN (B). The gradient was linearly increased from 5% to 95% B over 30 minand then immediately stepped back down to 5% for re-equilibration. The mobile phase flow rate was 1 ml /min and UV-VIS detection was monitored simultaneously at 280 nm.

Synthesized azido-SST (**1b**) was dissolved at concentration of 0.1 mM in 50 mM PB pH 7.8 containing 20μM L-GSH. The reaction was monitored by analytical HPLC (Fig. S1) after 72h and the peak was characterized by MALDI-MS using α-cyano-4-hydroxycinnamic acid (CHCA) (Fig. S2). As shown in Fig. S1 and 2, **1b** remained intact in 20μM GSH solution, which represents the highest GSH concentration in plasma.

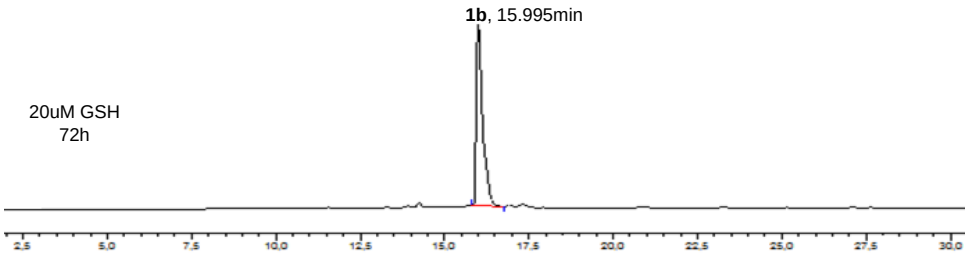


Fig. S1. HPLC profile of **1b** in 50mM PB with 20μM GSH pH 7.8 at 37℃ for 72h.

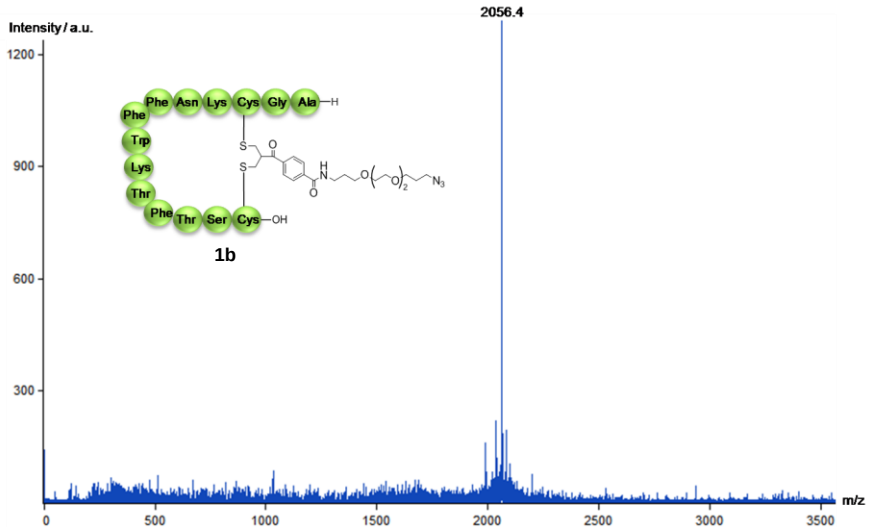


Fig. S2. MALDI-MS spectrum of **1b** using CHCA as matrix(calc. exact mass = 2054.94).

Synthesized azido-SST (**1b**) was dissolved at concentration of 0.1mM in 50 mM PB pH 7.8 containing 10 mM L-GSH. The reaction kinetics was measured three times by monitoring reactions incubated at 37 ℃. 200 µl samples were collected at different time points and 1 µl of 50% formic acid was added to quench the reaction. Samples were stored at -20 ℃ until analyzed. RP-HPLC injections were carried out and the areas of peaks were integrated to calculate conversion curves (Fig. S3). The identities of the compounds present in each peak were determined using MALDI-MS (Fig. S2, 4, 5).The reaction rate equation (1) has been converted to the integrated rate laws given in equations (2-3).

$$\frac{d[\mathbf{1b}]}{dt} = -k\,[\mathbf{1b}] \qquad (1)$$

$$[\mathbf{1b}] = [\mathbf{1b}]_0\, e^{-kt} \qquad (2)$$

$$ln\,[\mathbf{1b}] = -kt + ln\,[\mathbf{1b}]_0 \qquad (3)$$

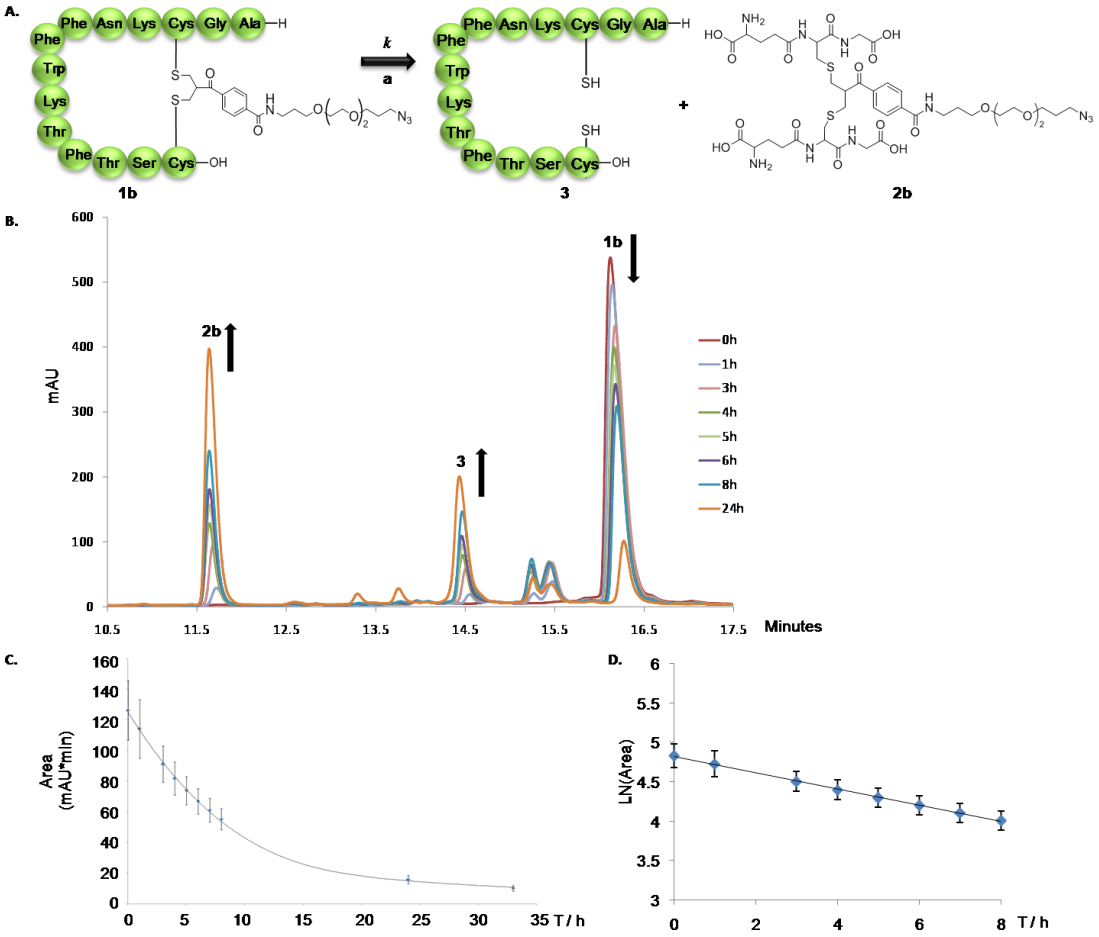


Fig. S3 A. The scheme of GSH mediated cleavage of azido-SST (**1b**). a. 10mM GSH, 50mM PB, pH 7.8, 37℃. B. HPLC profile of cleavage of **1b** in 50mM PB with 10mM GSH pH 7.8 at 37℃. The peak area for **1b** decreases with time while peak areas for **2b** and **3** increase. Arrows indicate the direction of peak area increase or decrease with time. C. The integrated peak area of **1b** was plot by time. D. The natural logarithm of peak area for **1b** was plot by time.

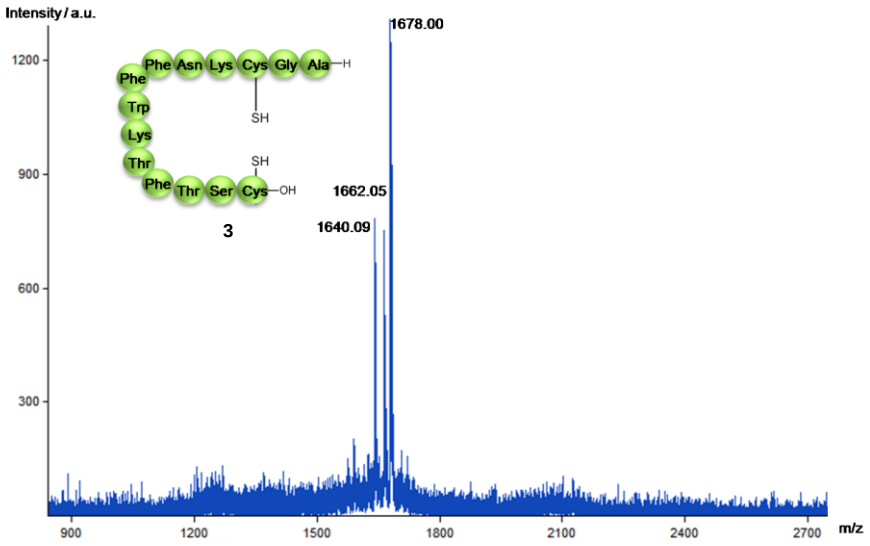


Fig. S4. MALDI-MS of **3** by using CHCA as matrix (calc. exact mass = 1638.73).

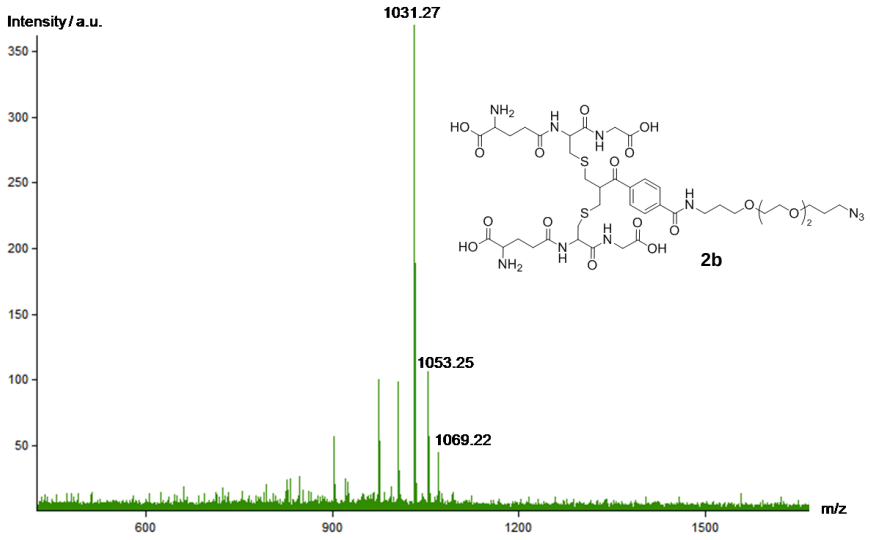
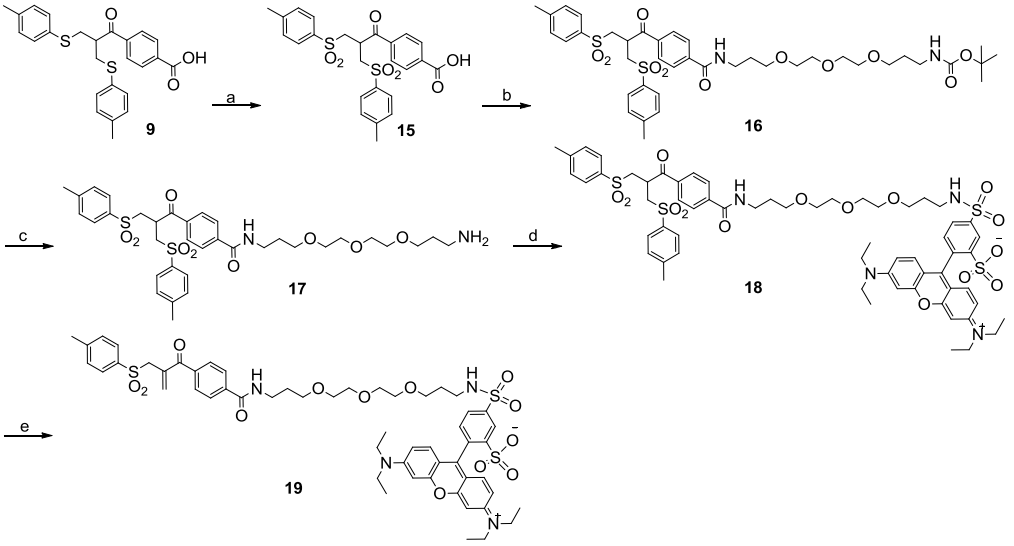


Fig. S5. MALDI-MS spectrum of **2b** by using 2, 5-dihydroxybenzoic acid (DHB) as matrix (calc. exact mass = 1030.37).

4. Synthesis of FITC-SR-SST (4)



Scheme 2. Synthesis of SR bissulfone (**18**). a. OXONE, MeOH-H_2O (1:1), 90%; b. **10**, HBTU, DIEA, anhydrous DMF, 82%; c. TFA, DCM, 95%; d. Lissamine, DIEA, DMF, 45%; e. 40% ACN in 50mM PB, 10mM EDTA, pH 7.8

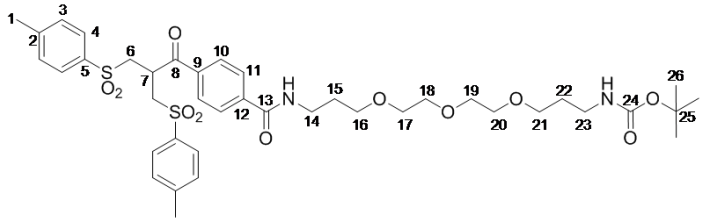
Synthesis of bissulfone (15)[4]

A 1:1 methanol-deionized water solution (50 ml) was prepared in a 10 ml round bottom flask. To this solution, bis-sulfide (**9**, 500 mg, 1.145 mmol), and OXONE (4.22 g, 6.87 mmol) were added. The reaction mixture was stirred at RT for 24 h and monitored by TLC plate using EA/Hexane (v/v=1:1). The mixture was poured into a separatory funnel and extracted with chloroform twice. Then, sufficient DI-water was added in order to dissolve the inorganic salts and extract twice. The organic extract was combined and extracted with Brine once. The solvent was removed under vacuum to afford 516 mg of **15** in 90 % yield.

^{1}H NMR (500MHz, $CDCl_3$):δ 2.49 (s, 6H), 3.48–3.66 (m, 4H), 4.40 (q, 1H), 7.37 (d, 4H), 7.70–7.73 (m, 6H), 8.10 (d, 2H); **ESI-MS** (MeOH, 250^{o}C): m/z = 499$[M-H]^-$, 523$[M+Na]^+$

Synthesis of N-(3-(2-(2-(3-aminopropoxy)ethoxy)ethoxy)propyl)-4-(3-tosyl-2-(tosylmethyl) propanoyl)benzamide (16)

Bissulfone (**15**, 500mg, 1mmol, 1eq.) dissolved in 5ml of anhydrous DMF was added HBTU (606mg, 1.6mmol, 1.6eq.) and DIEA (330µl, 2mmol, 2eq.) at 0℃ The reaction mixture was stirred for 10mins before Boc-ethylene glycol (**8**, 384mg, 1.2mmol, 1.2eq.) was added. The resulting mixture was stirred overnight at RT. The solvent was removed under high vacuum, and the product was dissolved in DCM. The organic phase was washed with $NaHCO_3$, brine and dried over anhydrous Na_2SO_4. The solvent was evaporated under vacuum and the crude product was purified by column chromatography using 10% methanol in chloroform to afford 658 mg of **16** in 82% yield.**^{1}H NMR** (400MHz, $CDCl_3$):δ1.36 (s, 9H), 1.61-1.67 (m, 2H), 1.82-1.88 (m, 2H), 2.74 (s, 6H),3.11-3.13 (m, 2H), 3.40 (t, 2H), 3.44-3.45 (m, 2H), 3.54-3.63 (m, 11H), 4.29 (s, 4H), 7.28-7.31 (m, 2H), 7.57-7.66 (m, 4H), 7.71-7.78 (m, 4H), 7.82-7.84 (m, 2H);



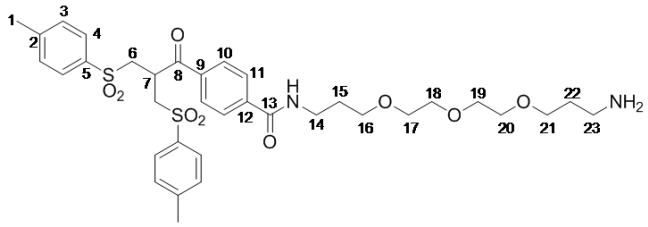
^{13}C NMR(100MHz, $CDCl_3$): δ21.62/21.70 (C-1), 28.40 (C-26), 28.71 (C-15), 29.58 (C-22), 38.34 (C-23), 39.03 (C-14), 57.65 (C-6), 69.39 (C-21), 70.03 (C-20), 70.22 (C-16), 70.37 (C-17), 70.39 (C-18), 70.56 (C-19), 78.81 (C-25), 127.06 (C-11), 128.26 (C-4), 129.58 (C-10), 129.88/130.17 (C-3), 135.20/135.93 (C-5), 135.72/135.76 (C-7), 138.31 (C-9), 138.37 (C-12), 145.10/145.54 (C-2), 156.04 (C-24), 166.16 (C-13), 194.23 (C-8);

LC-MS: m/z =669.3 [monosulfone+H], 825.3 [M + Na]

Synthesis ofN-(3-(2-(2-(3-aminopropoxy)ethoxy)ethoxy)propyl)-4-(3-tosyl-2-(tosylmethyl)propanoyl)benzamide(17)

Compound **16** (500 mg, 0.623 mmol, 1 eq.) was dissolved in 3 ml of DCM and TFA (476 µl, 10 eq.) was added. The DCM and TFA were removed under vacuum at 30℃ to afford 416 mg of amine bissulfone (**17**) as yellow oil in 95% yield.

^{1}H NMR (400MHz, $CDCl_3$):δ 1.84-1.90 (m, 2H), 1.94-1.99 (m, 2H), 2.41 (s, 6H), 3.20-3.25 (m,2H), 3.47-3.55 (m, 4H), 3.57-3.62 (m, 8H), 3.72 (t, 2H), 4.33 (s, 4H), 7.33-7.35 (m, 2H), 7.59-7.67 (m, 4H), 7.69-7.77 (m, 4H), 7.83-7.85 (m, 2H)



^{13}C NMR(100MHz, $CDCl_3$):δ 21.74 (C-1), 26.15 (C-22), 29.30 (C-15), 38.01 (C-14), 40.97 (C-23), 57.91 (C-6), 69.04 (C-16), 69.42 (C-17), 69.74 (C-18), 69.80 (C-19), 70.28 (C-20), 71.01 (C-21), 127.33 (C-11), 128.43 (C-4), 129.78 (C-10), 130.12 (C-3), 135.01 (C-5), 135.66/135.82 (C-7), 137.53 (C-9), 138.98 (C-12), 145.48 (C-2), 167.68 (C-13), 194.50 (C-8).

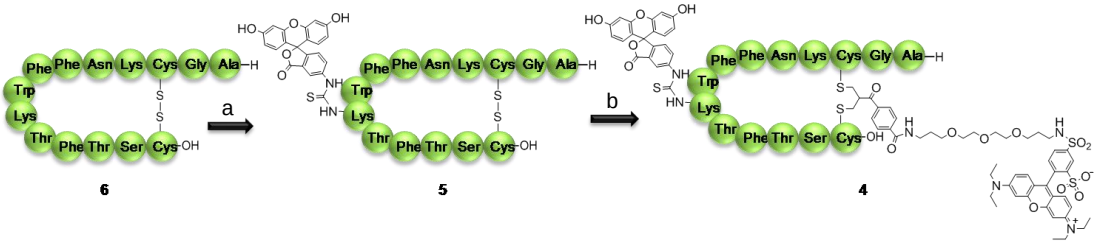
LC-MS: m/z = 547 [monosulfone + H], 703 [M+H]

Synthesis of SR bissulfone (18)

Lissamine rhodamine B chloride (60mg, 0.102mmol, 1eq.) was dissolved in 2ml of anhydrous DMF at 0℃. Then, amine bissulfone (**17**, 100mg, 0.142mmol, 1.4eq.) and DIEA (50µl, 0.306mmol, 3eq.) was added sequentially. The

reaction mixture was stirred at RT overnight. The solvent was removed under vacuum and the mixture was purified by column chromatography by using 10% methanol in chloroform to afford 57mg of the product **18** as pink solid in 45% yield.

LC-MS: m/z= 1087 [M+H$^+$] (monosulfone), 1243 [M+H$^+$]



Scheme 3. The synthesis of FITC-SR-SST (**4**). a. FITC, DMF, 65%; b. TCEP, **18**, 50 mM PB, 10 mM EDTA pH 7.8, 41%.

Synthesis of FITC-SST (5)[5]

Somatostatin (10 mg, 6.1 µmol) was dissolved in anhydrous DMF and fluorescein isothiocyanate (FITC, 2.4 mg, 6.1µmol) were added. The mixture was stirred under argon at RT overnight. The mixture was purified by Prep HPLC using an Atlantis Prep OBD T3 Column (19 x 100 mm, 5 µm) with the mobile phase0.1% TFA in water (A) and 0.1% TFA in ACN (B). 8mg of the product (**5**) was obtained in 65% yield from lyophilization. It was analyzed by analytical HPLC using a MerckChroCART 125-4 Column with the mobile phase starting from 95% solvent A (0.1% TFA in water) and 5% solvent B (0.1% TFA in ACN) (0-1 min) to 35% B at 2 min, increasing to 45% B at 10 min, reaching 95% at 16 min, returning back to 5% solvent B at 18 min and finally balancing the column for 2 mins with a flow rate of 1ml/min. The absorbance was monitored at 280 nm. The retention time were 7.96 and 8.206 min. Peak for FITC-SST (**5**) is split as a result of the formation of two diastereomers which was verified by the equivalence of masses determined from MALDI-MS using CHCA as matrix m/z= 2027.07 [M+H] (exact mass calc. = 2025.75). The attachment of FITC at K9 has been shown previously for reactions conducted under kinetically controlled conditions.[6]

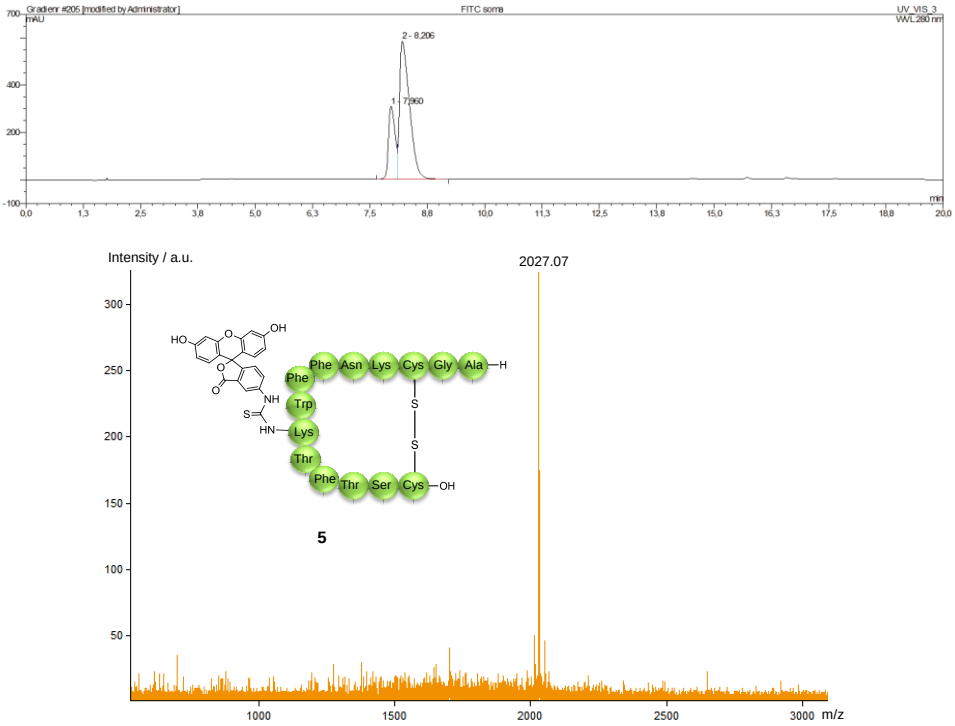


Fig. S6. HPLC profile and MALDI-MS of FITC-SST (**5**) by CHCA as matrix (calc. exact mass = 2025.75).

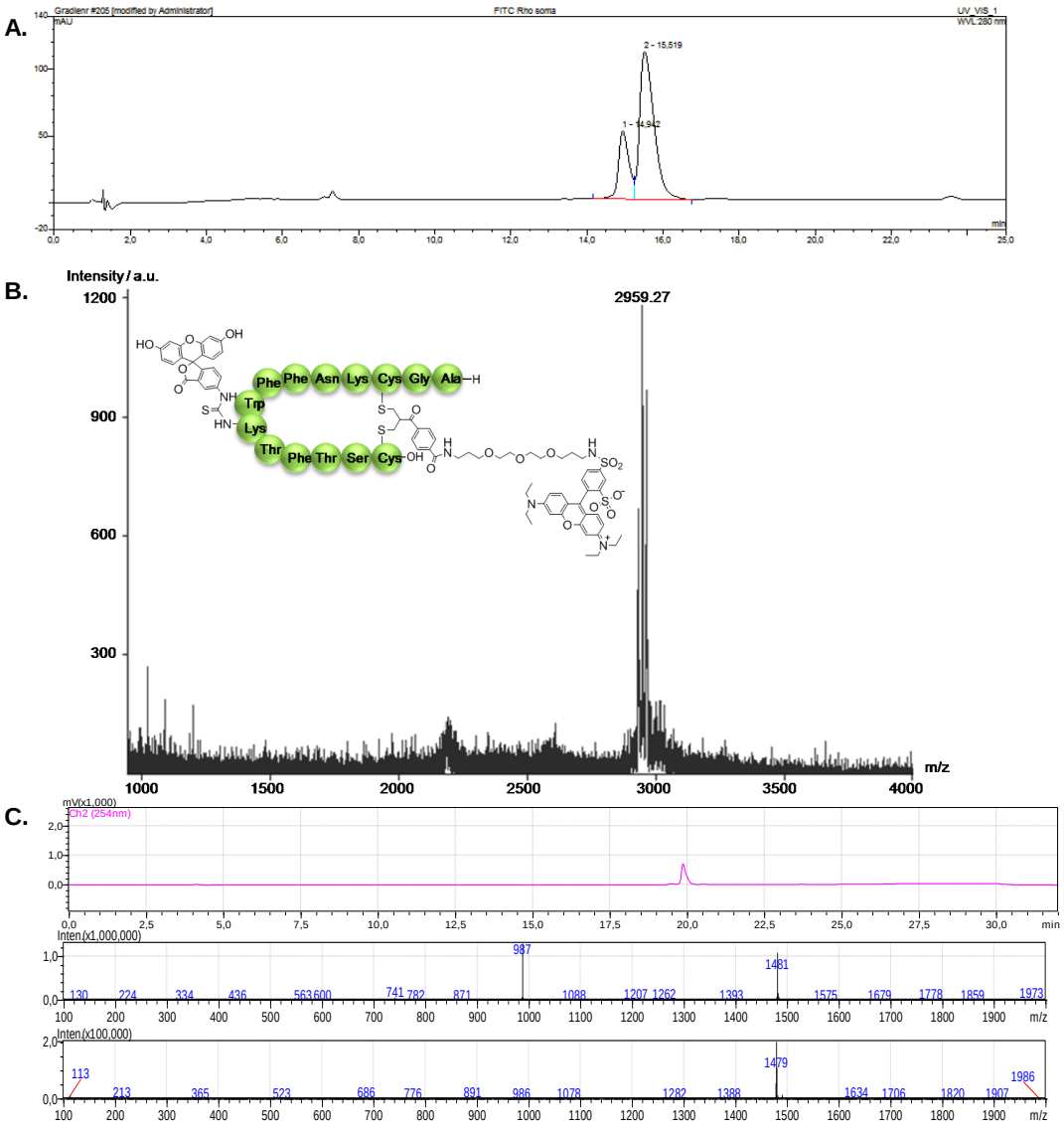


Fig. S7. A. HPLC profile of FITC-SR-SST (**4**). B. MALDI-MS spectrum of **4** by using DHB as matrix. C. LC-MS of **4**.

Synthesis of FITC-SR-SST (4)

Rhodamine bissulfone (**18**，6.1 mg, 4.94μmol, 2 eq.) was dissolved in 4ml of 1:1 ACN-PB (50mM PB, 10mM EDTA, pH 7.8) and shaken overnight to generate monosulfone **19** in situ. FITC-SST (5 mg, 2.47μmol, 1eq.) was dissolved in 3ml of 1:1 ACN-PB. TCEP (1.4 mg, 4.94μmol, 2eq.) and monosulfone **19** solution were added sequentially. The mixture was shaken gently for 24h at RT. The mixture was purified by Prep HPLC using an Atlantis Prep OBD T3 Column (19 x 100 mm, 5 μm) with the mobile phase 0.1% TFA in water (A) and 0.1% TFA in ACN (B).3 mg of product (**4**) was obtained in 41% yield from lyophilization. It was analyzed by analytical HPLC using a MerckChroCART 125-4 Column with the mobile phase starting from 95% A (0.1% TFA in water) and 0% B (0.1% TFA in ACN) (0-1 min) to 40% B at 3 min, increasing to 55% B at 26 min, returning back to 5% B at 28 min and finally balancing the column for 2 mins with a flow rate of 1ml/min. The absorbance was monitored at 280 nm. The retention time was 7.96 and 8.206 min (Fig. S7A). Peak for FITC-SR-SST (**4**) is split as a result of the formation of two diastereomers which was verified by the equivalence of masses determined from MALDI-MS using DHB as matrix m/z= 2959.27 [M+H] (exact mass calc. = 2958.12, Fig. S7B). LC-MS analysis was performed on a Shimadzu LC-MS 2020 equipped with an electrospray ionisation source and a SPD-20A UV-Vis detector (Shimadzu, Duisburg, Germany). The column temperature was set at 40°C. The mobile phase

consisted of 0.1% formic acid in water (A) and 0.1% formic acid in ACN (B). The gradient was starting from 0% B, raising to 10% B at 2 min and further to 15% B at 7 min, held at 85% for 7 min, then raising to 40% B at 15 min, 70% B at 21 min and finally to 100% B at 22 min. The column was washed for 4 mins with 100% B and then immediately stepped back down to 0% for re-equilibration. The mobile phase flow rate was 0.4 ml/min. Identification of products was performed simultaneously by UV-VIS detection at 254 nm. m/z = 1481 [M^{2+} + H], 987 [M^{3+} + H], 1479 [M^{2+} - H] (Fig. S7C).

5. Optical property of FITC-SR-SST (4) and its cleavage in 50 mM PB with 10 mM GSH at pH 7.8 and 37℃

The optical properties of FITC-SR-SST (**4,** 5.7μM) and 1:1 FITC-SST (**5**) and SR mixture (8.3μM)in 50 mM PB pH 7.8 were measured and the intensities were normalized to FITC-SR-SST (**4**) (Fig. S8).

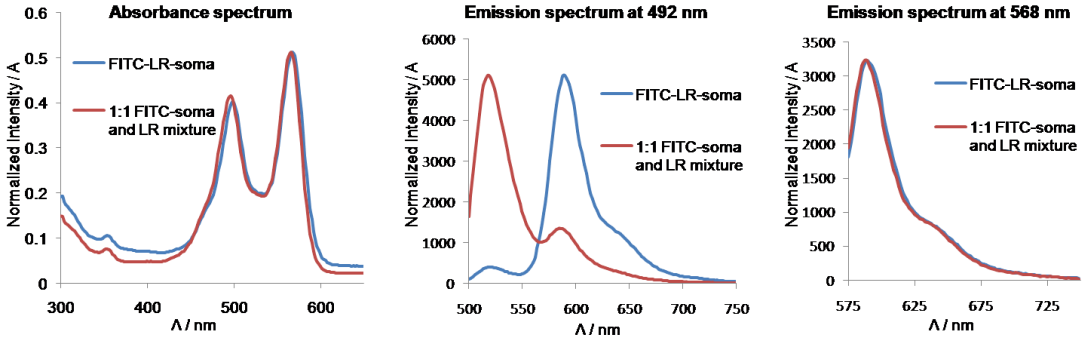


Fig. S8. The absorbance and emission spectra of **4** compared with 1:1 FITC SST (**5**) and SR mixture in 50 mM PB pH7.8.

FITC-SR-SST (**4,** 2.9μM) and 10 mM GSH at 37°C in 50 mM PB pH7.8 and the absorbance and emission were collected at 0h and 24h separately (Fig. S9). The intensities of absorbance and emission were normalized to FITC-SR-SST (**4**). An increase in fluorescein emission (excited at 492nm) after 24h, indicating FRET is disrupted and SR was released from SST.

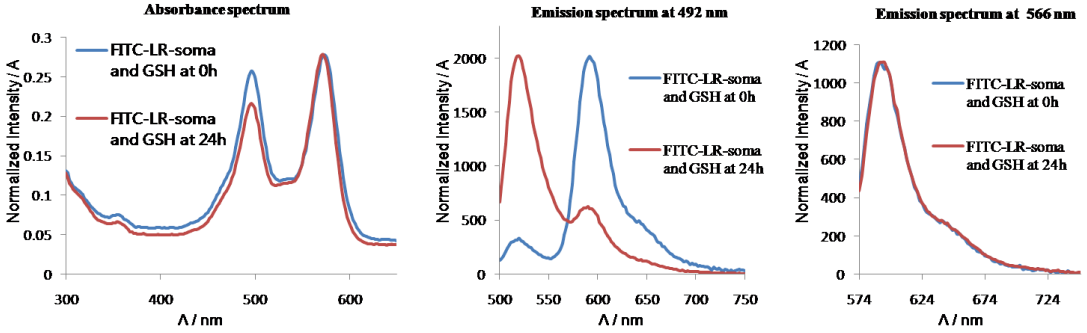


Fig. S9. The absorbance and emission spectra of **4** in 50mM PB pH 7.8 with 10mM GSH pH7.8 and 37°C for 24h compared with FITC-SR-SST (**4**) in PB without GSH.

6. Confocal laser scanning microscope (CLSM)

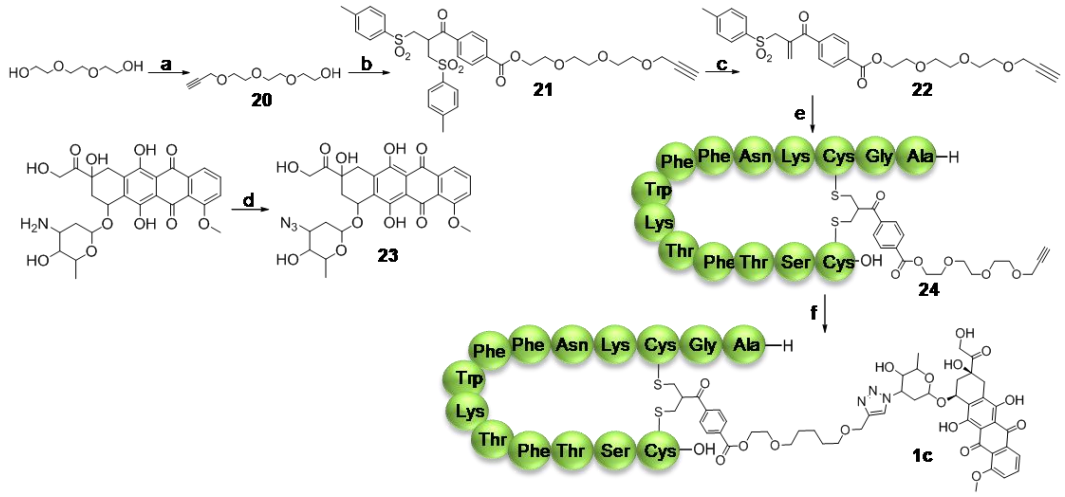
Cell culture

MCF-7 (human breast adenocarcinoma cell line) were obtained from DSMZ (German Collection of Microorganisms and Cell Cultures, Braunschweig) and cultured in DMEM medium with high glucose supplemented and with 10% fetal bovine serum (FBS), 100 U/mL Penicillin, 0.1 mg/mL Streptomycin, 0.1 mM non-essential amino acids at 37°C in a humidified 5% CO_2 incubator.

Confocal laser scanning microscope (CLSM)

Thirty thousands of MCF-7 cells were plated in a Cover glass Lab-Tek 8-well chamber (Nunc, Denmark) in 300 μL medium. The cells were cultured overnight to allow adhesion. The next day, cells was treated with 10mM of GSH-OEt in fresh and fully supplemented medium and incubated for 18h. 100 nM of FITC-SR-SST was then added to the cells and further incubated for 24 h in the incubator. Before imaging, cells were washed with PBS buffer for 3 times. The living cell imaging was then performed using a LSM 710 laser scanning confocal microscope system (Zeiss, Germany) coupled to an XL-LSM 710 S incubator and equipped with a 63× oil immersion objective. The FITC and SR were excited with 488nm and 514nm Argon laser in separated tracks, and the emission was collected using 500-582 nm and 525-759 nm filters, respectively. The acquired images were processed with Zen software developed by Carl Zeiss.

7. Synthesis of SST-DOX (1c)



Scheme 4. Synthesis of SST-DOX (**1c**). a. NaH, propargyl alcohol, 75%; b. **15**, EDC, DMAP, 68%; c. 40% ACN in 50mM PB, 10mM EDTA, pH 7.8; d. Imidazole-1-sulfonyl azide (**7**), K_2CO_3, $CuSO_4$, 70%; e. ACN in 50mM PB, 10mM EDTA, 41%; f. sodium ascorbate, $CuSO_4$, **23**, 1:1 THF-H_2O, 47%.

Synthesis of ethnyl triethylene glycol (20)[6]

To a solution of triethylene glycol (3 g, 20 mmol) in anhydrous THF (15 ml) at 0℃ under argon was slowly added sodium hydride (0.52 g, 13 mmol, 60%). The mixture was stirred at 0℃ for 30 min and propargyl bromide (1.08 ml, 1.49 g, 10 mmol) was injected slowly into the flask. The mixture was stirred at 0℃ for another 2 h and at RT for 20 h. The mixture was poured into water, extracted with DCM and dried over Na_2SO_4. The crude product was purified by flash chromatography eluting with EtOAc/hexane (3:2) to afford 7.102 g of **20** as yellow oil in 75% yield.

^{1}H NMR (400 MHz, $CDCl_3$): 4.13 (d, J = 2.5 Hz, 2H), 3.58-3.65 (m, 10H), 3.51-3.53 (m, 2H), 2.94 (s, 1H), 2.40 (t, J = 2.5 Hz, 1H); **^{13}C NMR** (100 MHz, $CDCl_3$): 79.5, 74.7, 72.5, 70.5, 70.3, 70.2, 69.0, 61.5, 58.3

Synthesis of 2-(2-(2-(prop-2-yn-1-yloxy)ethoxy)ethoxy)ethyl 4-(3-tosyl-2-(tosylmethyl) propanoyl)benzoate (21)

Bissulfone (**15,** 200mg, 0.4mmol, 1eq.), DMAP (6mg, 48μmol, 0.12eq.), 1-ethyl-3-(3-dimethylaminopropyl)carbodimide (EDC, 128mg, 0.48mmol, 1.2eq.) and ethnyl triethylene glycol (**20**, 90mg, 0.48mmol, 1.2eq.) were dissolve in 4ml of anhydrous DCM under argon at 0℃. The reaction mixture was stirred at RT overnight. The mixture was purified by column chromatography using Hexane/Ethylacetate (1:2) to afford 182 mg of product (**21**) as slightly yellow oil in 68% yield.

^{1}H NMR (400MHz, $CDCl_3$):δ 2.39 (s, 6H), 3.44 (m, 1H), 3.65 (m, 8H), 3.83 (m, 4H), 4.07 (m, 2H), 4.32 (m, 2H),

4.49 (m, 2H), 7.34 (d, 4H), 7.66 (m, 6H), 8.09 (d, 2H); **^{13}C NMR** (400MHz, $CDCl_3$):δ 21.4, 30.5, 60.1, 63.6, 64.4, 69.3, 69.4, 69.5, 77.0, 78.5, 128.5, 128.9, 129.9, 130.0, 134.6, 139.6, 141.1, 145.2, 165.7, 201.9; **MALDI-TOF:** m/z=671.39 [M+H];

Synthesis of ethynyl-SST (24)

Ethynyl ethylene glycol bissulfone (**21**，33 mg, 0.0488 mmol, 2eq.) was dissolved in 40 ml of 40% ACN in PB buffer (50 mM PB, 10 mM EDTA, pH 7.8) and shaken at RT overnight to generate monosulfone (**22**) in situ. Somatostatin (40 mg, 0.0244 mmol, 1eq.) was dissolved in 20 ml of 40% CAN in PB. TCEP (14 mg, 0.0488 mmol, 2eq.) and monosulfone **22** solution above were added sequentially. The mixture was gently shaken at RT overnight. The mixture was concentrated and purified by Prep HPLC using an Atlantis Prep OBD T3 Column (19 x 100 mm, 5 µm) with the mobile phase starting from 100% solvent A (0.1% TFA in water) and 0% solvent B (0.1% TFA in acetonitrile) (0-20 min), raising to 30% B in 1min, 30% B for 9mins, raising to 40% B in 1min, 40% B for 11 mins and finally reaching 100% B in 5 mins with a flow rate of 10 ml/min. The absorbance was monitored at 280 nm and 254 nm. The retention time for **25** was 37.3 min. 20 mg of the product **24** was obtained from lyophilisation in 41% yield. **MALDI-TOF-MS**:(+) m/z=1998.20 [M+H] (calc. exact mass = 1996.87, Fig. S11)

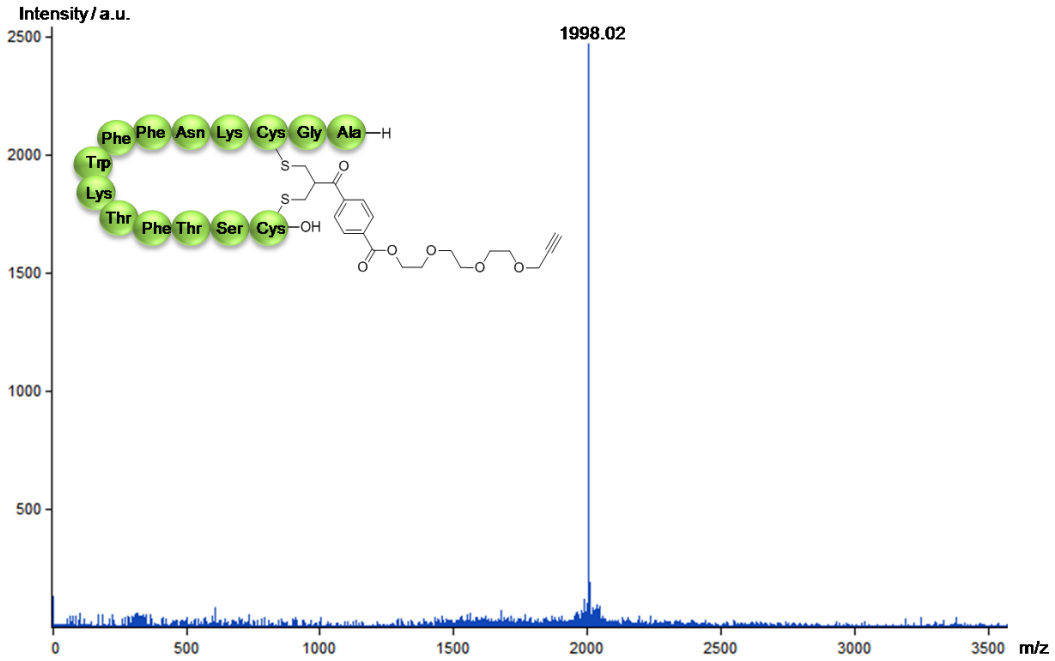


Fig. S11. MALDI-MS spectrum of ethynyl-SST (**24**) using CHCA as matrix

Synthesis of azido-DOX (23).

Imidazole-1-sulfonyl azide hydrochloride (22mg, 0.1035mmol) was added to the doxorubicin hydrochloride (50mg, 0.08621mmol), potassium carbonate (18mg, 0.1293mmol) and copper sulfate (0.14mg, 0.8621µmol) in 1ml of methanol. The reaction mixture was stirred at RT overnight. The mixture was concentrated, diluted with water (30ml) and extracted with DCM (3* 20ml). The combined organic layers were washed by saturated brine and dried over anhydrous sodium sulfate, filtered and concentrated. The crude product was purified by flash chromatography using 10% methanol in chloroform to give 34mg of the azido-DOX (**23**) in 70% yield.

^{1}H NMR (400MHz, $CDCl_3$): δ 1.34 (d, 3H), 1.933 (m, 2H), 2.14 (m, 2H), 2.34 (d, 1H), 3.02 (m, 2H), 3.28 (d, 1H), 3.59 (m, 1H), 3.74 (s, 1H), 3.92 (s, 1H), 4.09 (s, 3H), 4.51 (s, 1H), 4.75 (d, 2H), 5.31 (d, 1H), 5.59 (d, 1H), 7.41 (d, 1H), 7.80 (t, 1H), 8.04 (d, 1H), 13.24 (s, 1H), 14.01 (s, 1H). **^{1}C NMR** (100MHz, $CDCl_3$): δ 16.76, 28.18, 33.50,

35.25, 56.44, 65.30, 67.27, 69.34, 69.68, 76.49, 77.20, 100.59, 110.99, 111.11, 118.37, 119.57, 120.17, 133.25, 134.85, 135.65, 155.04, 155.88, 160.72, 186.04, 186.33, 213.35. **ESI-MS:** 568 [M-H].

Synthesis of SST-DOX (1c)

Azido-DOX (**23**, 6mg, 0.01mmol, 2eq.) and ethnyl-SST (**24**, 10mg, 5µmol, 1 eq.) were dissolved in 5ml of 1:1 THF-H_2O. Sodium ascorbate (1mg, 5µmol, 1eq.) and copper sulfate (0.4mg, 2.5µmol, 0.5eq.) were added to the mixture sequentially. The reaction mixture was stirred at RT for 24h. The mixture was purified by Prep HPLC using an Atlantis Prep OBD T3 Column (19 x 100 mm, 5 µm) with the mobile phase solvent A (0.1% TFA in water) and solvent B (0.1% TFA in ACN). 6 mg of the product **1c** was obtained in 47% yield from lyophilisation. LC-MS analysis was performed on a Shimadzu LC-MS 2020 equipped with an electrospray ionisation source and a SPD-20A UV-Vis detector (Shimadzu, Duisburg, Germany). The column temperature was set at 40°C. The mobile phase consisted of 0.1% formic acid in water (A) and 0.1% formic acid in ACN (B). The gradient was starting from 0% B, raising to 10% B at2 min and further to 15% B at 7 min, held at 85% for 7 min, then raising to 40% B at 15 min, 70% B at 21 min and finally to 100% B at 22 min. The column was washed for 4 mins with 100% B and then immediately stepped back down to 0% for re-equilibration. The mobile phase flow rate was 0.4 ml /min. Identification of products was performed simultaneously by UV-VIS detection at 254 nm. m/z = 1284 [M^{2+} + H], 857 [M^{3+} + H] (Fig. S12).

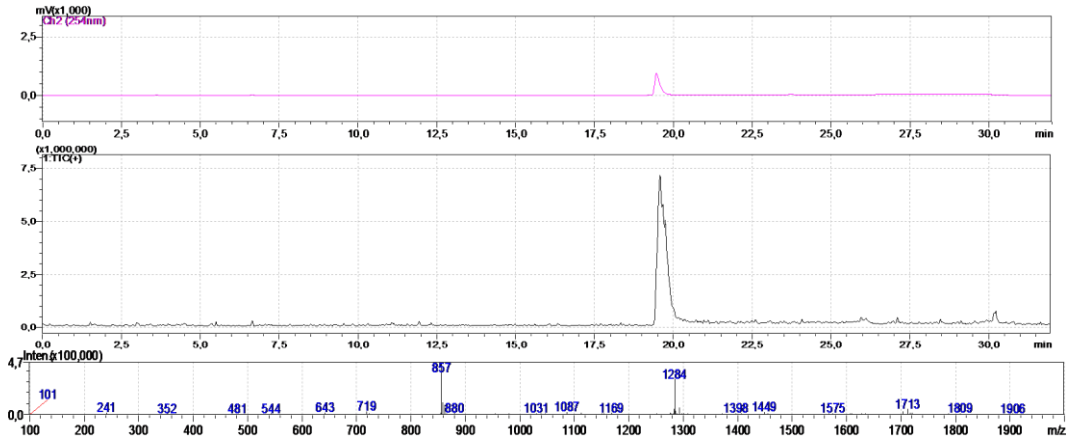


Fig. S12. LC-MS spectra of SST-DOX (**1c**)

8. HPLC study of SST-DOX (1c) and its application for targeted drug delivery

The HPLC analysis was conducted using MerckChroCART 125-4 Column with mobile phase consisted of 0.1% TFA in water (A) and 0.1% TFA in ACN (B). The gradient was linearly increased from 5% to 95% Bover 30 minand then immediately stepped back down to 5% for re-equilibration. The mobile phase flow rate was 1 ml /min and UV-VIS detection was monitored simultaneously at 488 nm and 554 nm.

Synthesized SST-DOX (1c) was dissolved at concentration of 50µM in 50 mM PB pH 7.8 containing 10mM L-GSH. The reaction kinetics was measured three times by monitoring reactions incubated at 37°C. 100 µl samples were collected at different time points and 5 µl of Rhodamine B (0.1 mM) was added as internal standards. RP-HPLC injections were carried out and the areas of peaks were integrated to calculate conversion curves. The identities of the compounds present in each peak were determined using MALDI-MS (Fig. S14).The cleavage of 1c was taken as pseudo first-order rate reaction in the case of large excess of L-GSH (10 mM). The dissociation constant k was calculated as -0.111 ±0.00114 (h^{-1}) and the reaction half-life was determined as 6.225 ± 0.001 h (Fig. S13, R^2 = 0.999).

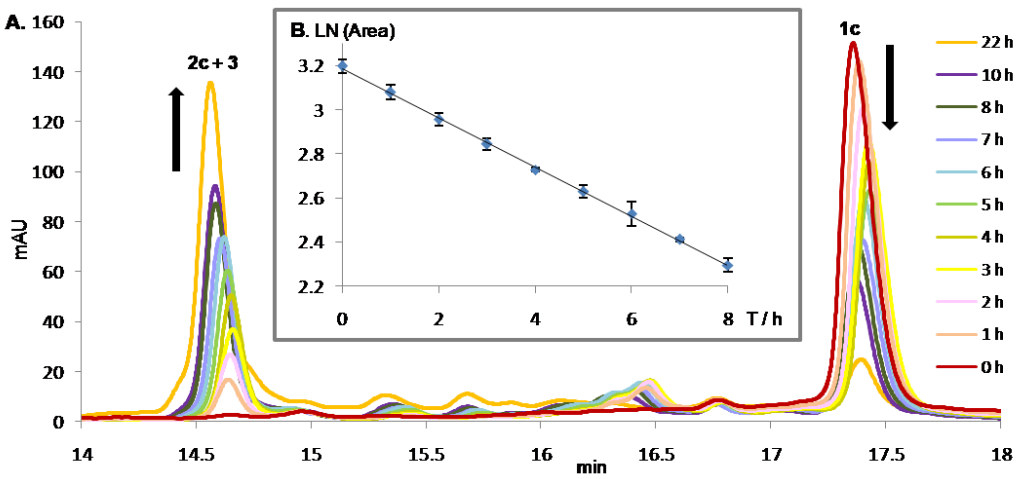


Fig. S13. A. HPLC profile of cleavage of SST-DOX (**1c**, 50 μM) in 50mM PB with 10mM GSH pH 7.8 at 37℃. The peak area for **1c** decreases with time while peak areas for **2c** and **3** increase. Arrows indicate the direction of peak area increase or decrease with time. B. The natural logarithm of peak area for **1c** was plot by time.

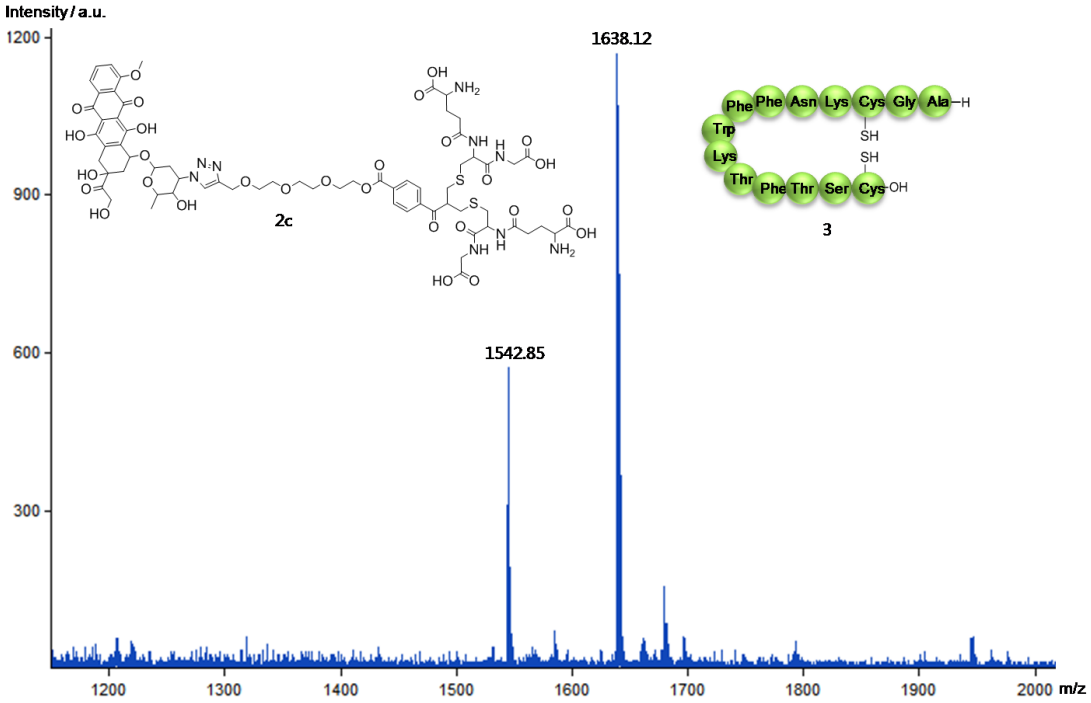


Fig. S14. The MALDI-MS spectrum of the peak at 14.6 min in the HPLC profile of SST-DOX (1c) using DHB as matrix. The calculated exact masses for 2c and 3 were 1541.47 and 1638.73 respectively.

Cytotoxicity Assay

MCF-7 cells were pre-cultured in DMEM medium (10% FBS, 1% Penicillin/Streptomycin, 1% MEM) and were seeded in a 96-well plate at a density of 6500 cells/well. The cells were allowed to adhere overnight and were pre-treated with 10 mM glutathione for 18 h. The medium was removed and added SST-DOX (1 – 100 μM). Additionally, MCF-7 cells were prepared separately without glutathione pre-treatment and added SST-DOX (1 – 100 μM) to elucidate the induced drug release and the difference in drug efficacy. MCF-7 cells were also separately treated with doxorubicin (1-100μM) (Fig. S13). The cells were subsequently incubated at 37°C, 5% CO_2 for 24 h before analyzing using Cell-Titer Glo® (Promega, Germany) following manufacturer's protocol.

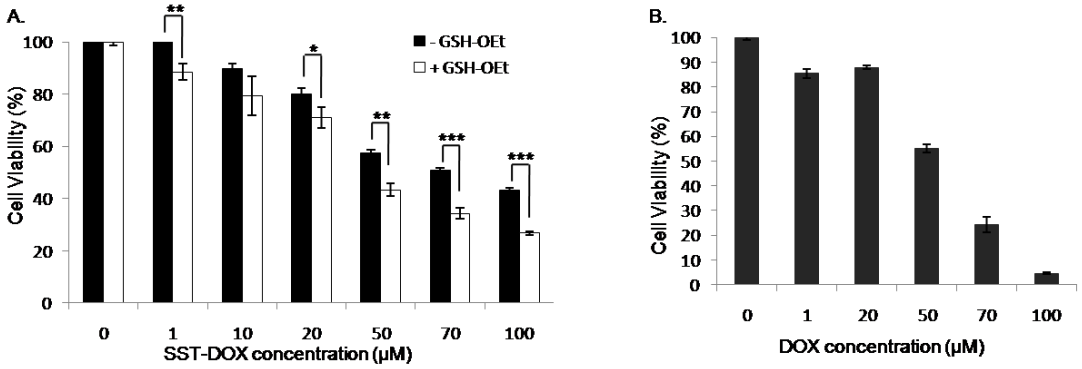


Fig. S15. A. Cytotoxicity of SST-DOX on MCF-7 cells tested without GSH-OEt and pretreatment of 10 mM GSH-OEt. The results indicate significant differences in the cytotoxicities of SST-DOX (1c) treated or without treated high level of GSH-OEt, (*) $p < 0.05$, (**) $p < 0.01$, (***) $P<0.001$ respectively. B. Cytotoxicity of DOX on MCF-7 cells

9. Confocal microscopy imaging for the uptake of SST-DOX in MCF-7 cells

Thirty thousands of MCF-7 cells were plated in a Cover glass Lab-Tek 8-well chamber (Nunc, Denmark) in 300 μL medium. The cells were cultured overnight to allow adhesion. The next day, cells was treated with 1μM of SST-DOX in fresh and fully supplemented medium and incubated for 24h in the incubator. Before imaging, cells were washed with PBS buffer for 3 times. The living cell imaging was then performed using a LSM 710 laser scanning confocal microscope system (Zeiss, Germany) coupled to an XL-LSM 710 S incubator and equipped with a 63× oil immersion objective. The DOX were excited with 488nm Argon laser in separated tracks, and the emission was collected using 500-582 nm filters. The acquired images were processed with Zen software developed by Carl Zeiss.

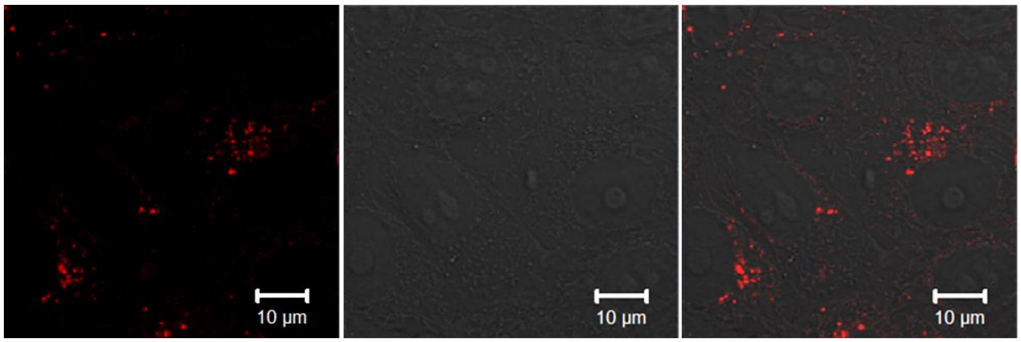


Fig S16. Confocal microscopy imaging of SST-DOX (1 μM) incubated with MCF-7 cells for 24h.

10. Selectivity of SST-DOX on sst2 expressing cells

Millipore's cloned human SSTR2-expressing cell line is made in the Chem-1 host, which supports high levels of recombinant SSTR2 expression on the cell surface and contains high levels of the promiscuous G protein Gα15 to couple the receptor to the calcium signaling pathway. It was used for screening for agonists of interactions between SSTR2 and its ligands.

SST-DOX is used for this SSTR2 functional assay on SST2 expressing Chem-1 cells and wild-type Chem-1 cells by Millipore to demonstrate the selectivity of SSTR2 expressing cells. As shown in Fig S17, SST-DOX displayed activation of the SSTR2 receptor with a predicted EC_{50} potency value of 13 nM, which is compatible to SST with EC50 of 1.3 nM.

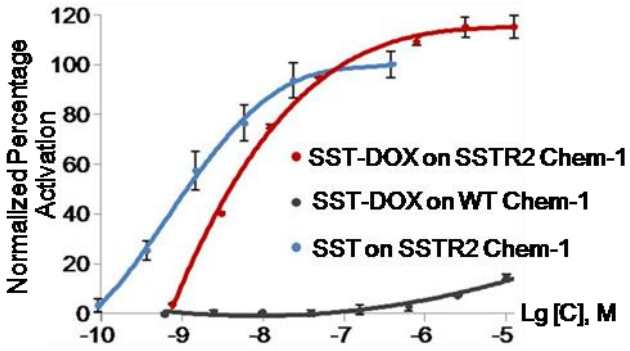


Fig S18. Calcium flux induced by SST-DOX in SSTR2 expressing Chem-1 cells (red) and wide type Chem-1 cells (black); calcium flux induced by SST in SSTR2 expressing Chem-1 cells (blue).

11. References

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