

# **Glutathione-sensitive nanoplatform for monitored intracellular delivery and controlled release of Camptothecin†**

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## **Supporting Information**

## General methods

Reverse-phase high performance liquid chromatography (RP-HPLC) analysis was performed on an Agilent 1220 Infinity LC coupled to a fluorescence detector 1260 Infinity with an analytical column (Mediterranean Sea C18, 3 $\mu$ m, 100 x 21 mm). The products were eluted utilizing a constant solvent mixture (CH<sub>3</sub>CN/H<sub>2</sub>O-TFA pH 4.5 50:50 v/v) at 0.8 mL/min. NMR spectra were recorded on a Bruker AV300 Ultrashield<sup>TM</sup> spectrometer. <sup>1</sup>H NMR spectra were acquired at 300 MHz employing pulses of 15  $\mu$ sec and a recycle time of 1 sec. Data for <sup>1</sup>H spectra are reported as follows: chemical shift, multiplicity (s = singlet, d = doublet, dd = doublet of doublet, t = triplet, dt = doublet of triplet, m = multiplet) and integration. To obtain <sup>13</sup>C spectra 9  $\mu$ sec pulses at 75 MHz were applied with a recycle time of 2 seconds. Both <sup>1</sup>H and <sup>13</sup>C experiments were carried out using tetramethylsilane (TMS) as chemical shift reference. Electrospray ionization mass spectra (ESI-MS) were recorded on an Agilent 1100 Series LC/MSD/VL with a Mediterranean Sea C18 (3 $\mu$ m, 100 x 21 mm) column and a quadrupole ion trap mass spectrometer. Quadrupole time-of-flight mass spectra (Q-TOF) were recorded on an Aquity UPLC Waters coupled with Xevo Qtof MS with an Aquity UPLC BEH C18 (1.7 $\mu$ m, 50 x 21 mm) column and using positive electrospray ionization. The products were eluted utilizing a constant solvent mixture 50:50 v/v (solvent A = 0.1 % CH<sub>3</sub>COOH/CH<sub>3</sub>CN; solvent B = H<sub>2</sub>O) at 0.5 mL min<sup>-1</sup>.

## Synthesis of camptothecin prodrugs

### *Synthesis of compounds 2a-c*

Compound **2a**: <sup>1</sup>H NMR (300 MHz, CD<sub>3</sub>OD,  $\delta$ ): 8.76 (d, 1 H, Ar H), 8.46 (t, 1 H, Ar H), 8.37 (d, 1 H, Ar H), 7.84 (t, 1 H, Ar H), 3.85 (t, 2H, CH<sub>2</sub>), 3.16 (t, 2 H, CH<sub>2</sub>) ppm. <sup>13</sup>C NMR (75MHz, CDCl<sub>3</sub>,  $\delta$ ): 159.12 (C7), 149.88 (C6), 136.84 (C4), 121.97 (C5), 121.53 (C3), 58.22 (C1), 42.75 (C2) ppm. ESI-MS ( $m/z$ ): [ $M+H$ ]<sup>+</sup> calcd for C<sub>7</sub>H<sub>9</sub>NOS<sub>2</sub>, 187.0; found 187.0.

Compound **2b**: <sup>1</sup>H NMR (300MHz, CDCl<sub>3</sub>,  $\delta$ ): 8.39-8.37 (m, 1 H, Ar H), 7.59-7.56 (m, 2 H, Ar H), 7.04-7.01 (m, 1 H, Ar H), 3.70 (t, 2 H, CH<sub>2</sub>), 2.88 (t, 2 H, CH<sub>2</sub>), 1.87 (m, 2 H, CH<sub>2</sub>). <sup>13</sup>C NMR (75MHz, CDCl<sub>3</sub>,  $\delta$ ): 159.38 (C8), 147.67 (C7) 137.89 (C5), 122.08 (C6), 120.19 (C4), 60.54 (C1), 36.34 (C3), 31.40 (C2) ppm. ESI-MS ( $m/z$ ): [ $M+H$ ]<sup>+</sup> calcd for C<sub>8</sub>H<sub>11</sub>NOS<sub>2</sub>, 201.0; found 201.0.

Compound **2c**: <sup>1</sup>H NMR (300 MHz, MeOD,  $\delta$ ): 8.37-8.34 (m, 1 H, Ar H), 7.67-7.63 (m, 2H, Ar H), 7.01-7.68 (m, 1 H, Ar H), 3.54 (t, 2 H, CH<sub>2</sub>), 2.72 (t, 2H, CH<sub>2</sub>), 1.69-1.58 (m, 4H, CH<sub>2</sub>CH<sub>2</sub>). <sup>13</sup>C NMR (75 MHz, MeOD,  $\delta$ ): 161.41 (C9), 150.07 (C8), 140.84 (C6), 123.21 (C7), 122.38 (C5), 62.69 (C1), 40.50 (C4), 32.60 (C3), 26.81 (C2) ppm. ESI-MS ( $m/z$ ): [ $M+H$ ]<sup>+</sup> calcd for C<sub>9</sub>H<sub>13</sub>NOS<sub>2</sub>, 215.0; found 215.0.

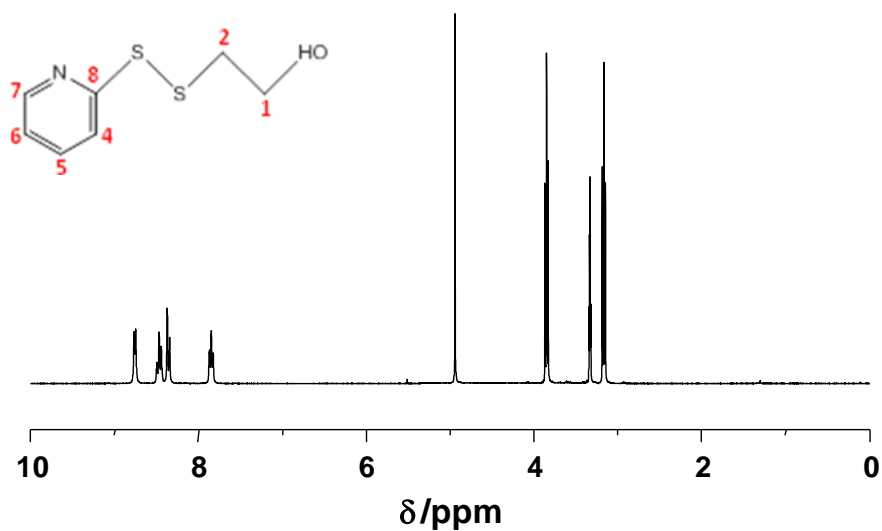
### Synthesis of compounds 4a-c

Compound **4a**: HPLC retention time: 3.0 min, Q-TOF MS (ESI,  $m/z$ ) [ $M-H$ ]<sup>-</sup> calcd for C<sub>28</sub>H<sub>22</sub>N<sub>3</sub>O<sub>6</sub>S<sub>2</sub>, 560.1; found 560.1. <sup>1</sup>H NMR (75 MHz, DMSO-*d*<sub>6</sub>, δ): 0.92 (t, 3H, CH<sub>3</sub>), 2.18 (m, 2H, CH<sub>2</sub>), 3.14 (t, 2H, S-CH<sub>2</sub>), 4.33 (t, 2H, O-CH<sub>2</sub>), 5.23 (dd, 2H, CH<sub>2</sub>), 5.52 (d, 2H, CH<sub>2</sub>), 7.09 (s, 1H, CH), 7.15 (m, 1H, Ar H), 7.70 (m, 2H, Ar H), 7.77 (m, 1H, Ar H), 7.85 (m, 1H, Ar H), 8.10 (m, 2H, 2 Ar H), 8.39 (dt, 1H, Ar H), 8.64 (s, 1H, Ar H) ppm. <sup>13</sup>C NMR (75 MHz, DMSO-*d*<sub>6</sub>, δ): 172.41 (C<sub>21</sub>), 166.99 (C<sub>A</sub>), 158.57 (C<sub>D</sub>), 156.40 (C<sub>16a</sub>), 152.70 (C<sub>2</sub>), 149.49 (C<sub>H</sub>), 147.80 (C<sub>15</sub>), 146.18 (C<sub>13</sub>), 144.64 (C<sub>3</sub>), 137.63 (C<sub>F</sub>), 131.48 (C<sub>7</sub>), 130.32 (C<sub>11</sub>), 129.62 (C<sub>6</sub>), 128.90 (C<sub>12</sub>), 128.42 (C<sub>9</sub>), 127.90 (C<sub>8</sub>), 127.66 (C<sub>10</sub>), 121.19 (C<sub>16</sub>), 119.32 (C<sub>G</sub>), 119.14 (C<sub>E</sub>), 94.35 (C<sub>14</sub>), 77.90 (C<sub>B</sub>), 72.34 (C<sub>20</sub>), 66.14 (C<sub>17</sub>), 50.22 (C<sub>5</sub>), 36.72 (C<sub>C</sub>), 30.29 (C<sub>19</sub>), 7.51 (C<sub>18</sub>) ppm.

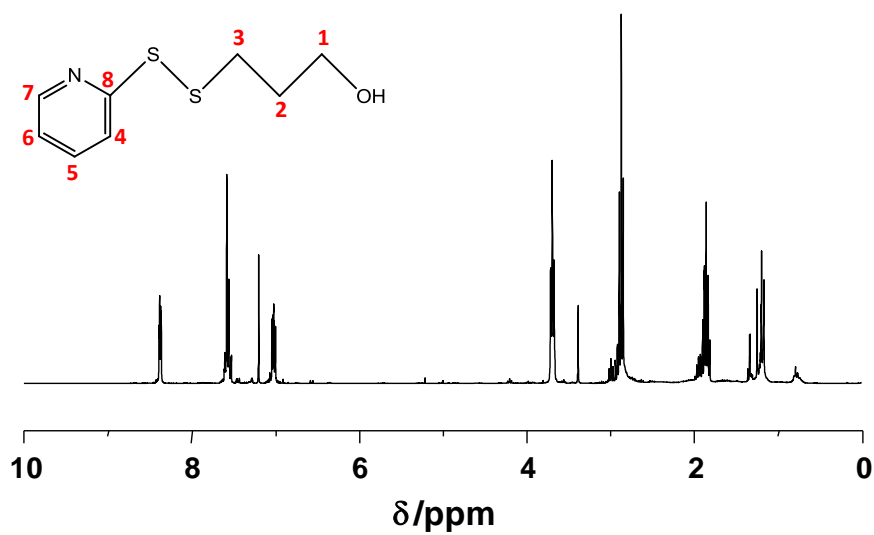
Compound **4b**: HPLC retention time: 7.3 min, Q-TOF MS (ESI,  $m/z$ ) [ $M-H$ ]<sup>-</sup> calcd for C<sub>29</sub>H<sub>24</sub>N<sub>3</sub>O<sub>6</sub>S<sub>2</sub>, 574.1; found 574.1. <sup>1</sup>H NMR (75 MHz, DMSO-*d*<sub>6</sub>, δ): 0.90 (t, 3H, CH<sub>3</sub>), 1.96 (m, 2H, CH<sub>2</sub>), 2.17 (m, 2H, CH<sub>2</sub>), 2.84 (t, 2H, S-CH<sub>2</sub>), 4.20 (t, 2H, O-CH<sub>2</sub>), 5.30 (dd, 2H, CH<sub>2</sub>), 5.51 (d, 2H, CH<sub>2</sub>), 7.07 (s, 1H, CH), 7.20 (m, 1H, Ar H), 7.72 (m, 3H, 3 Ar H), 7.86 (m, 1H, Ar H), 8.14 (m, 2H, 2 Ar H), 8.40 (dt, 1H, Ar H), 8.70 (s, 1H, Ar H) ppm. <sup>13</sup>C NMR (75 MHz, DMSO-*d*<sub>6</sub>, δ): 172.41 (C<sub>21</sub>), 166.99 (C<sub>A</sub>), 158.57 (C<sub>E</sub>), 156.40 (C<sub>16a</sub>), 152.70 (C<sub>2</sub>), 149.49 (C<sub>I</sub>), 147.80 (C<sub>15</sub>), 146.18 (C<sub>13</sub>), 144.64 (C<sub>3</sub>), 137.63 (C<sub>G</sub>), 131.48 (C<sub>7</sub>), 130.32 (C<sub>11</sub>), 129.62 (C<sub>6</sub>), 128.90 (C<sub>12</sub>), 128.42 (C<sub>9</sub>), 127.90 (C<sub>8</sub>), 127.66 (C<sub>10</sub>), 121.19 (C<sub>16</sub>), 119.32 (C<sub>H</sub>), 119.14 (C<sub>F</sub>), 94.35 (C<sub>14</sub>), 77.90 (C<sub>B</sub>), 72.34 (C<sub>20</sub>), 66.14 (C<sub>17</sub>), 50.22 (C<sub>5</sub>), 36.72 (C<sub>D</sub>), 31.74 (C<sub>C</sub>), 30.29 (C<sub>19</sub>), 7.51 (C<sub>18</sub>) ppm.

Compound **4c**: HPLC retention time: 10.2 min, Q-TOF MS (ESI,  $m/z$ ) [ $M-H$ ]<sup>-</sup> calcd for C<sub>30</sub>H<sub>26</sub>N<sub>3</sub>O<sub>6</sub>S<sub>2</sub>, 588.1; found 588.1. <sup>1</sup>H NMR (300 MHz, DMSO-*d*<sub>6</sub>, δ): 0.91 (t, 3H, CH<sub>3</sub>), 1.76 (m, 2H, CH<sub>2</sub>), 1.97 (m, 2H, CH<sub>2</sub>), 2.16 (m, 2H, CH<sub>2</sub>), 2.88 (t, 2H, SCH<sub>2</sub>), 4.20 (t, 2H, OCH<sub>2</sub>), 5.29 (s, 2H, CH<sub>2</sub>), 5.52 (d, 2H, CH<sub>2</sub>), 7.06 (s, 1H, CH), 7.19 (m, 1H, Ar H), 7.36 (m, 3H, 3 Ar H), 7.78 (m, 1H, Ar H), 8.11 (m, 2H, 2 Ar H), 8.44 (dt, 1H, Ar H), 8.69 (s, 1H, Ar H) ppm. <sup>13</sup>C NMR (75 MHz, DMSO-*d*<sub>6</sub>, δ): 172.39 (C<sub>21</sub>), 167.08 (C<sub>A</sub>), 158.86 (C<sub>E</sub>), 156.44 (C<sub>16a</sub>), 152.13 (C<sub>2</sub>), 149.49 (C<sub>I</sub>), 147.85 (C<sub>15</sub>), 146.22 (C<sub>13</sub>), 144.80 (C<sub>3</sub>), 137.59 (C<sub>G</sub>), 131.48 (C<sub>7</sub>), 130.36 (C<sub>11</sub>), 129.62 (C<sub>6</sub>), 128.95 (C<sub>12</sub>), 128.48 (C<sub>9</sub>), 127.99 (C<sub>8</sub>), 127.70 (C<sub>10</sub>), 121.11 (C<sub>16</sub>), 119.23 (C<sub>H</sub>), 119.11 (C<sub>F</sub>), 96.66 (C<sub>14</sub>), 77.76 (C<sub>B</sub>), 72.34 (C<sub>20</sub>), 66.96 (C<sub>17</sub>), 50.28 (C<sub>5</sub>), 34.15 (C<sub>E</sub>), 31.75 (C<sub>C</sub>), 30.22 (C<sub>19</sub>), 27.53 (C<sub>D</sub>), 7.50 (C<sub>18</sub>) ppm.

2a



2b



2c

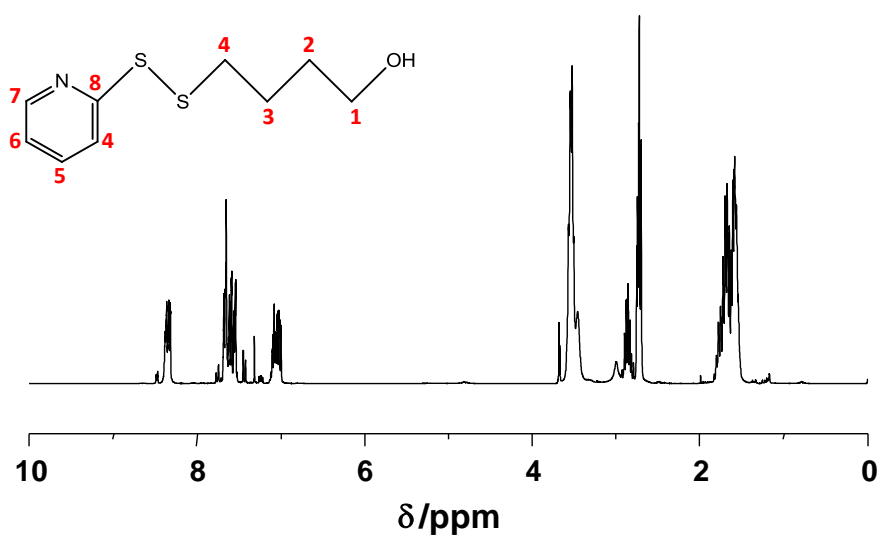
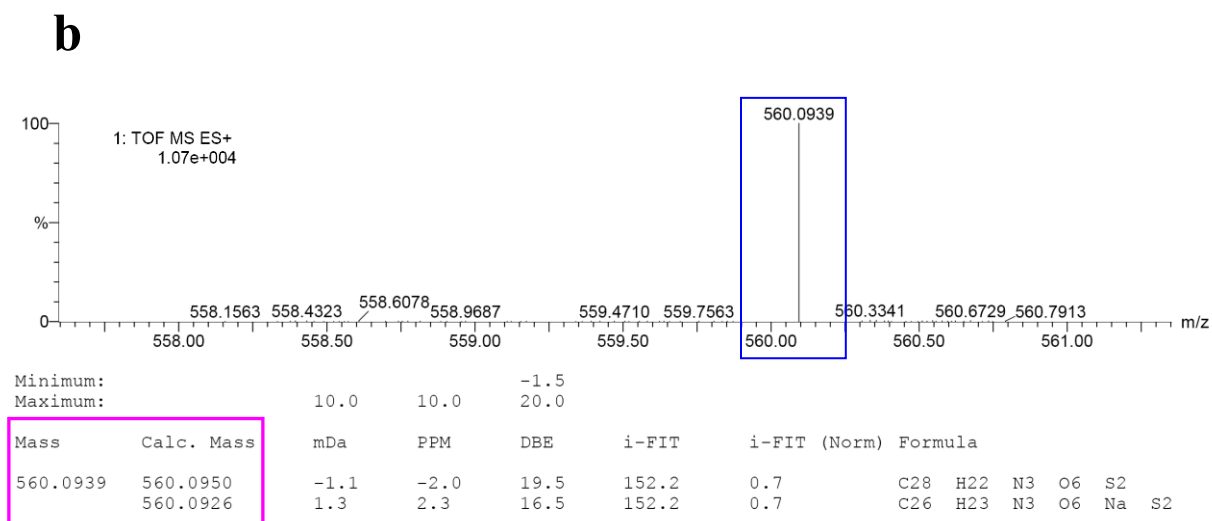
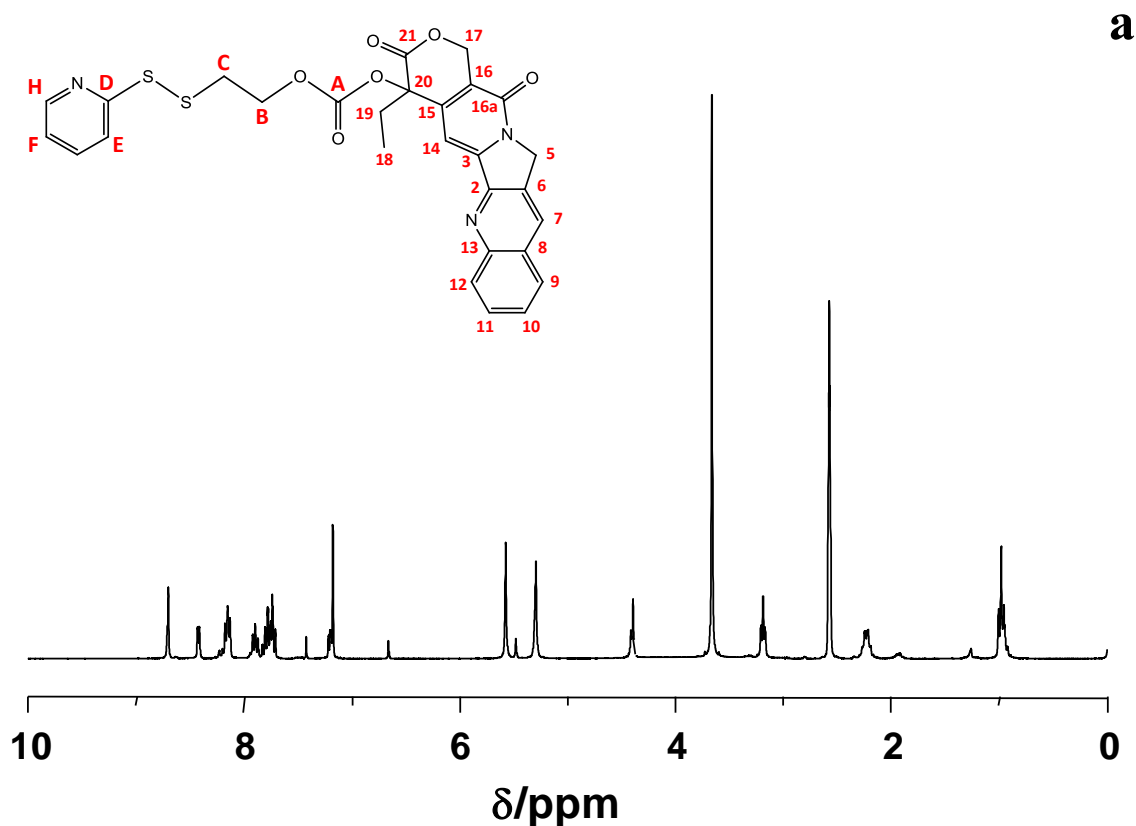
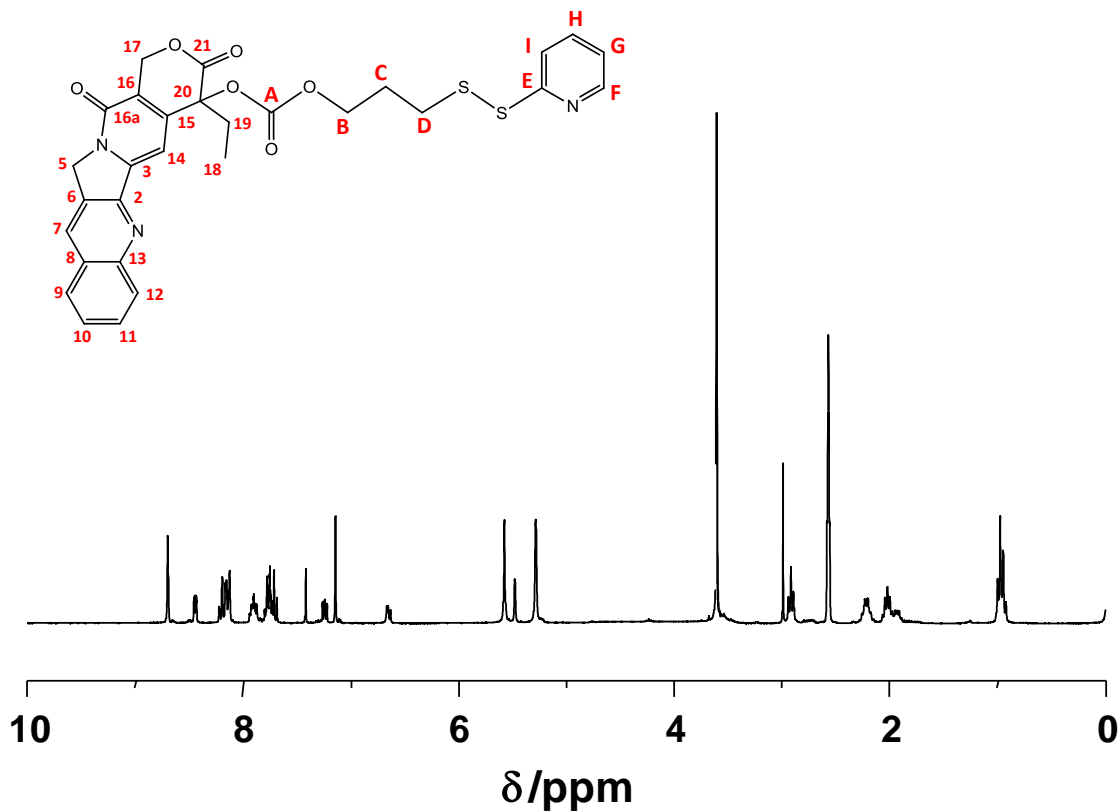


Figure S1.  $^1\text{H}$  NMR spectra of compounds 2a-c.

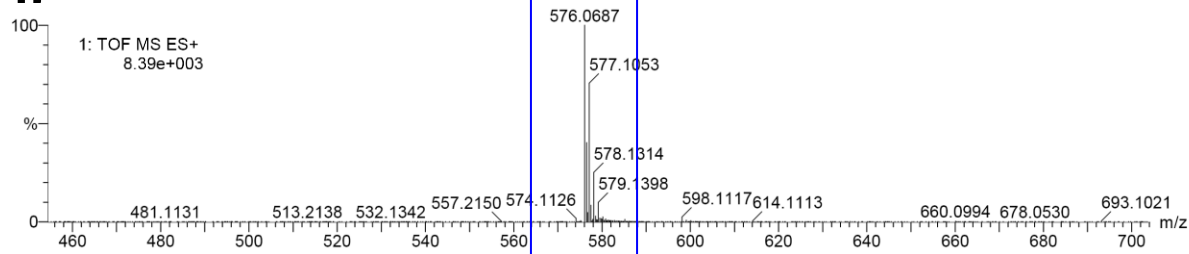


**Figure S2.** Compound **4a**: (a)  $^1\text{H}$  NMR spectrum. (b) Q-TOF report.

**a**



**b**



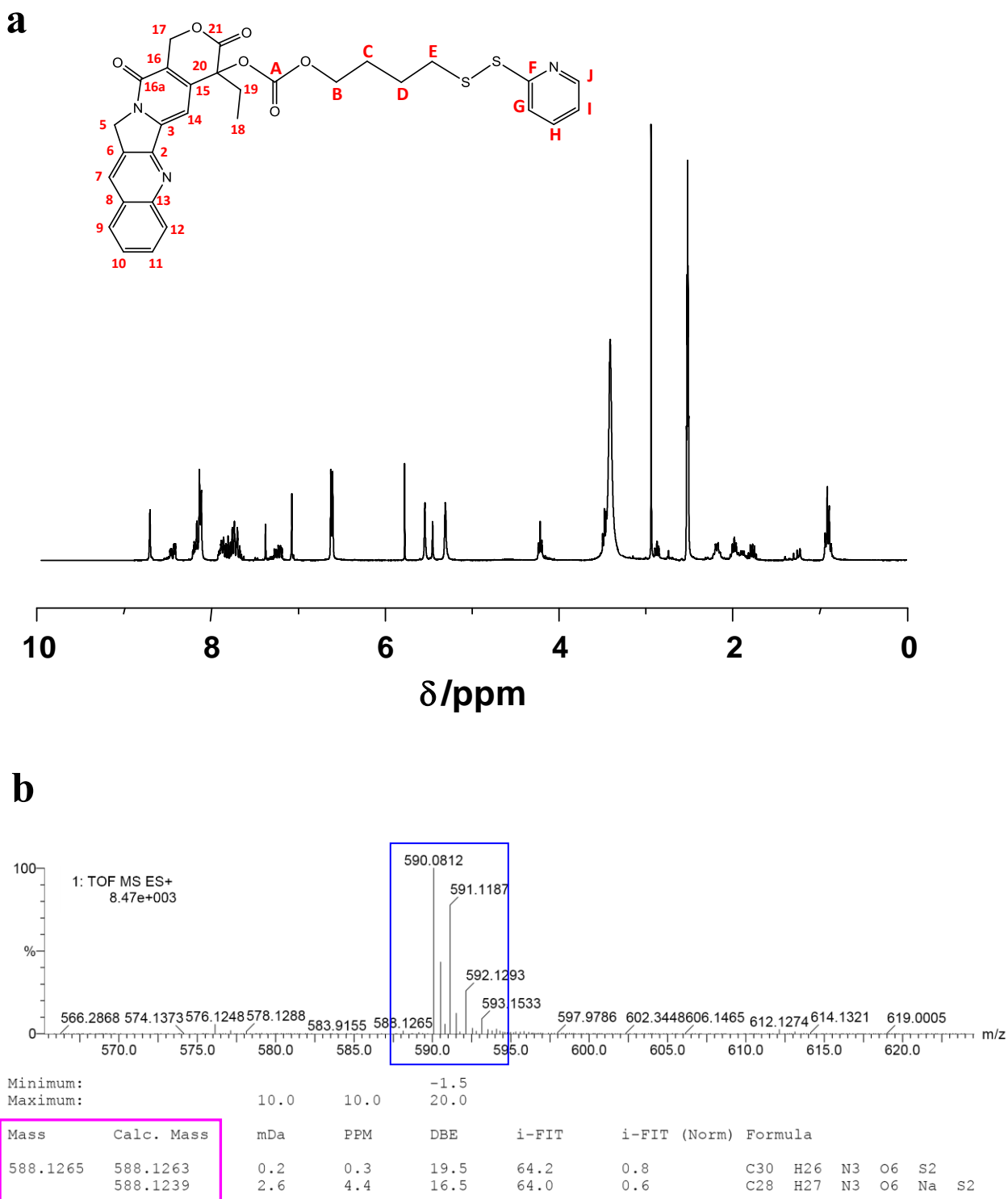
Minimum:  
Maximum:

10.0 10.0 -1.5  
20.0

Mass	Calc. Mass
574.1126	574.1107
	574.1082

mDa	PPM	DBE	i-FIT	i-FIT (Norm)	Formula
1.9	3.3	19.5	66.6	0.5	C <sub>29</sub> H <sub>24</sub> N <sub>3</sub> O <sub>6</sub> S <sub>2</sub>
4.4	7.7	16.5	67.0	0.9	C <sub>27</sub> H <sub>25</sub> N <sub>3</sub> O <sub>6</sub> Na S <sub>2</sub>

**Figure S3.** Compound **4b**: (a) <sup>1</sup>H NMR spectrum. (b) Q-TOF report.



**Figure S4.** Compound **4c**: (a)  $^1\text{H}$  NMR spectrum. (b) Q-TOF report.

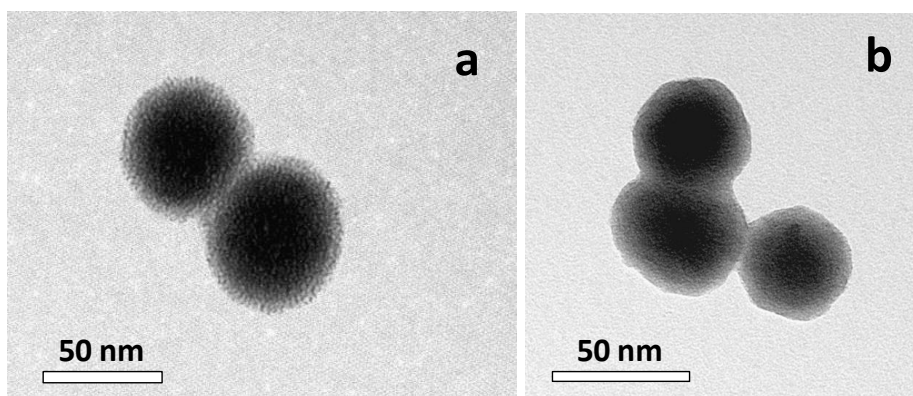
## Characterization of materials

**Table S1.** Textural characteristics of mesoporous silica shell in as-synthesized materials.

Material	$V_p^a$ ( $\text{cm}^3 \text{g}^{-1}$ ) [a]	$S_{\text{BET}}^a$ ( $\text{m}^2 \text{g}^{-1}$ ) [a]	$D^b$ ( $\text{\AA}$ ) [b]
RhB-SiO <sub>2</sub> @MSN	0.31	282.8	39.1
RhB-SiO <sub>2</sub> @MSN-SH	0.14	190.2	38.7
RhB-SiO <sub>2</sub> @MSN-SS-CPT	0.16	139.4	33.7
RhB-SiO <sub>2</sub> @MSN-COOH	0.21	237.3	31.2
RhB-SiO <sub>2</sub> @MSN-COO-CPT	0.11	149.6	29.9

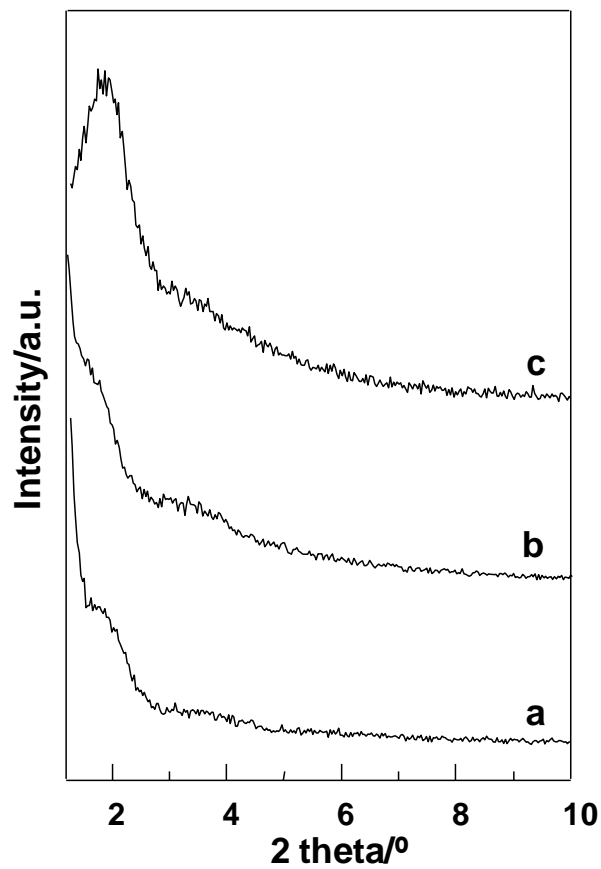
<sup>a</sup> As determined by nitrogen adsorption data.

<sup>b</sup> Pore diameter of the mesoporous silica wall. Calculated by the Kruk-Jaroniec-Sayari method [1].

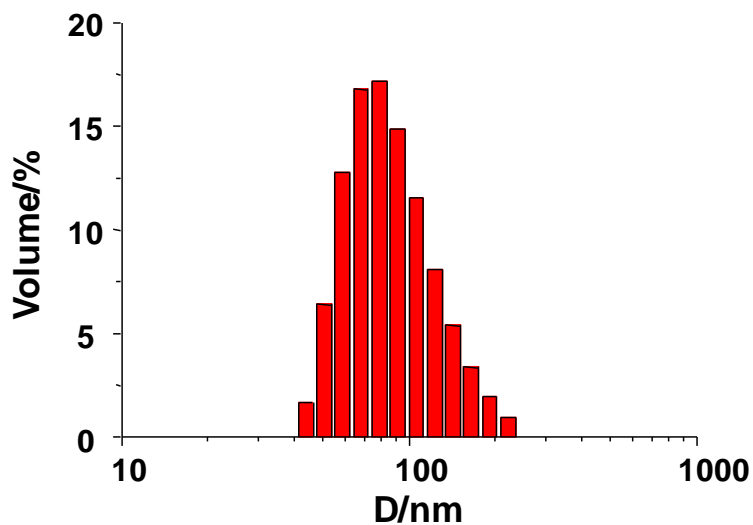


**Figure S5.** TEM images of small particle clusters found in the preparation of RhB-SiO<sub>2</sub>@MSN-SS-CPT nanoplateform: (a) dimer; (b) trimer.

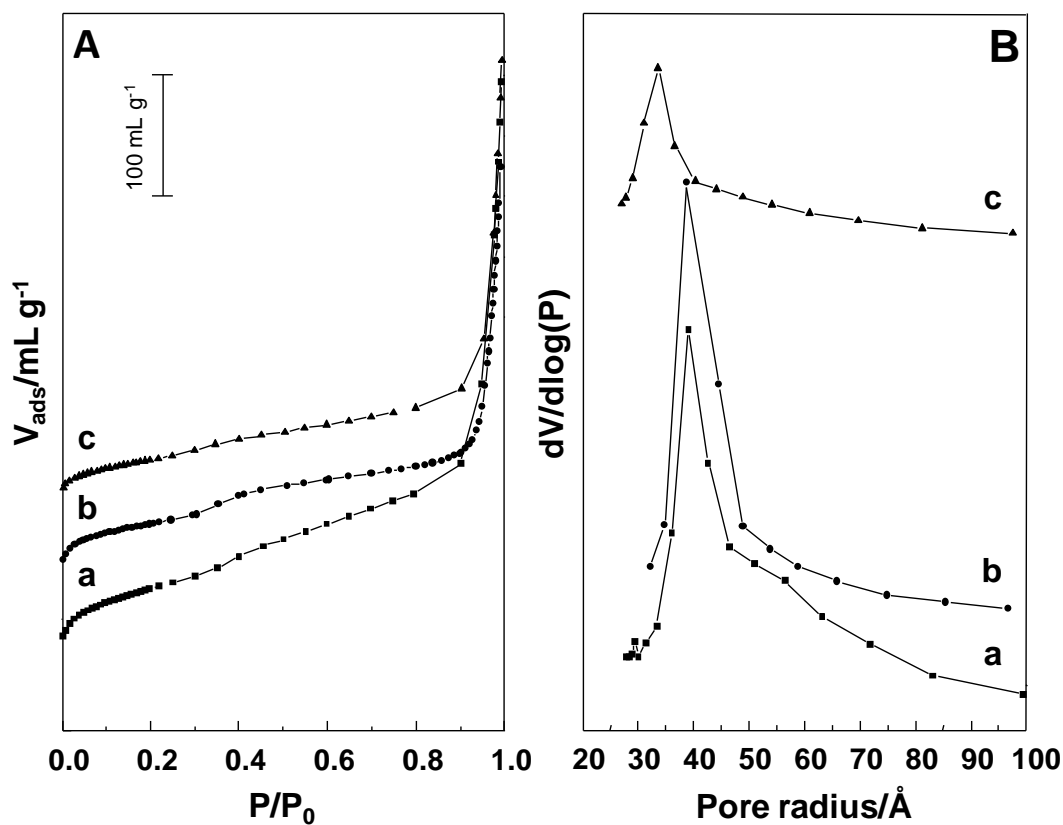




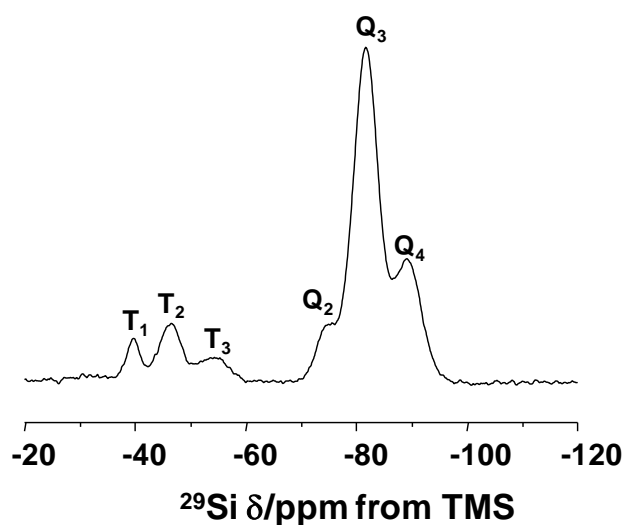
**Figure S6.** Powder XRD patterns of as-synthesized materials: (a) RhB-SiO<sub>2</sub>@MSN; (b) RhB-SiO<sub>2</sub>@MSN-SH; (c) RhB-SiO<sub>2</sub>@MSN-SS-CPT.



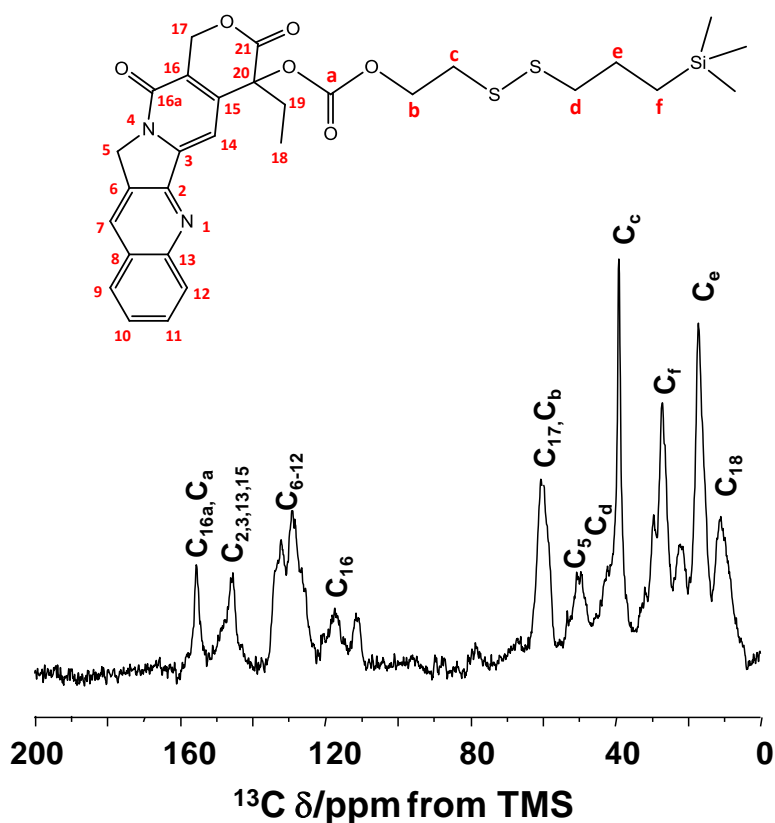
**Figure S7.** Particle hydrodynamic diameter of RhB-SiO<sub>2</sub>@MSN-SS-CPT as determined in water by DLS (volume output).



**Figure S8.** BET N<sub>2</sub> adsorption isotherms (A) and pore size distribution (B) of as-synthesized materials. (a) RhB-SiO<sub>2</sub>@MSN; (b) RhB-SiO<sub>2</sub>@MSN-SH; (c) RhB-SiO<sub>2</sub>@MSN-SS-CPT.



**Figure S9.**  $^{29}\text{Si}$  CP-MAS-NMR spectrum of RhB-SiO<sub>2</sub>@MSN-SS-CPT exhibits characteristic bands of silica species having a Si-C bond (T<sub>1</sub>, T<sub>2</sub> and T<sub>3</sub>) and siloxane species (Q<sub>2</sub>, Q<sub>3</sub> and Q<sub>4</sub>).



**Figure S10.**  $^{13}\text{C}$ -MAS-NMR spectrum of RhB-SiO<sub>2</sub>@MSN-SS-CPT material. The spectrum shows the covalent linking of CPT to a terminal carbonate group. Peaks at 34.5 and 42.1 ppm are assigned to carbons neighbour to the disulfide bridge. The peak at 156.5 ppm corresponds to the C=O group of the carbonate moiety.

## Synthesis of CPT nanoplatfoms

### *Synthesis of RhB-SiO<sub>2</sub>@MSN-COO-CPT*

To a mixture of 53 mL Triton-X100, 54 mL hexanol and 225 mL cyclohexane it was added 2 mL of a TMR-APTES solution (7 mg mL<sup>-1</sup>), 15 mL of water and 3 mL of NH<sub>4</sub>OH 30 % (v/v) [2]. The mixture was allowed to stir for 30 minutes. Then, 3 mL of TEOS was added and the reaction was allowed to stir for 24 h. The fluorescent cores were isolated by centrifugation (30000 g, 15 min) and washed twice with ethanol and deionised water. These cores were suspended in 3 mL of ultra-pure water and mixed with 100 mL 8 mM CTAB solution and 1 mL 100 mM NaOH. Then, 0.3 mL of a TEOS solution in ethanol (20% v/v) was added every 30 min for three times. The mixture was allowed to stir for 2 h at 60 °C and then, a solid was separated by centrifugation (12500 g, 15 min), washed five times with ethanol and freeze-dried. 170 mg of core-shell nanoparticles (RhB-SiO<sub>2</sub>@MSN) were dried under vacuum at 75 °C for 6 hours.

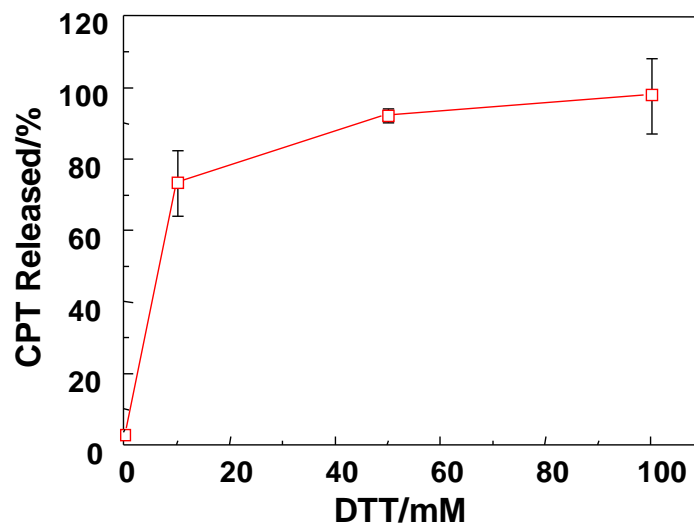
To obtain nanoparticles with a coating of carboxylic moieties attached to surface (RhB-SiO<sub>2</sub>@MSN-COOH), 1.00 g of RhB-SiO<sub>2</sub>@MSN was dried at 60 °C and vacuum for 3 h. Afterwards, 10 mL of anhydrous toluene was added and the mixture was heated to reflux. Then, 78 µL of (3-cyanopropyl)trichlorosilane (0.50 mmol) was added and the mixture was stirred for 3 h. The obtained product was filtered off, washed with toluene and methanol and dried at room temperature and vacuum for 16 h. This solid was dispersed in 150 mL of sulfuric acid (60%) and heated at 150 °C for 3 h in a reflux system. Then, the acid suspension was diluted with 200 mL of distilled water, filtered off, washed with toluene and methanol and finally freeze-dried.

RhB-SiO<sub>2</sub>@MSN-COOH (500 mg) was dehydrated at 60 °C and vacuum for 6 h. Then, 10 mL of DCM, 122 mg of 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide (EDCI, 0.64 mmol) and 130 mg of hydroxybenzotriazole (HOBT, 0.96 mmol) were added and the mixture was stirred at 0 °C for 30 min. Next, 65 mg of 20-O-trifluoroglycincamptothecin (Gly-CPT, 0.125 mmol) and 87 µL of N,N'-diisopropylethylamine (DIPEA, 0.50mmol) were introduced, and the system was allowed to warm to room temperature and remain stirring for 16 h [3]. The resulting RhB-SiO<sub>2</sub>@MSN-COO-CPT material was centrifuged (12500 g, 15 min) and the obtained solid was washed with methanol repeatedly until no rest of Gly-CPT was detected by UV-Vis (A<sub>368</sub>), and freeze-dried.

### **DTT-sensitive release of CPT**

5 mg of RhB-SiO<sub>2</sub>@MSN-SS-CPT was placed in a vial with 1 mL of PBS solution. The tube was vigorously shaken using a VORTEX (mvor-01) for 30 seconds. Then the suspension was placed in a Thermomixer® at 37° C and 1350 rpm. After 2 hours, DTT was added to the solution to reach 10-100 mM concentration of reducing agent. At the corresponding time the sample was centrifuged (12500 g,

15 min) and the supernatant was freeze-dried and further dissolved with 950  $\mu\text{L}$  of methanol and 50  $\mu\text{L}$  of HCl 1 M solution. This sample was analyzed by RP-HPLC and ESI-MS. Triplicate samples were run for every experiment.



**Figure S11.** Effect of DTT concentration in the CPT release profile of RhB-SiO<sub>2</sub>@MSN-SS-CPT (37 °C, 3 h).

### Stability of the CPT nanoplatform in cell uptake conditions

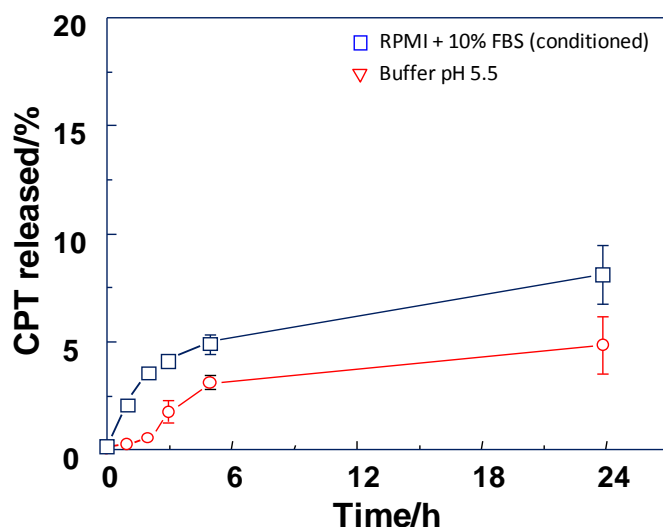
#### *Stability in conditioned cell culture medium*

HeLa cells (3000 cells per p10 plate) were seeded and stabilized for 24 h in RPMI supplemented with 10% FBS. Next, growth medium was exchanged and the used (conditioned) medium was aliquoted and frozen. 5 mg of RhB-SiO<sub>2</sub>@MSN-SS-CPT was placed in a vial with 1 mL of thawed conditioned cell culture medium. The tube was vigorously shaken using a vortex for 30 seconds. Then the suspension was placed in a Thermomixer® at 37° C and 1350 rpm. At the corresponding time the sample was centrifuged (12500 g, 15 min) and 500  $\mu\text{L}$  of 5% TCA aqueous solution at 4 °C was added to the supernatant. The protein precipitate was removed by centrifugation (9000 g, 10 min, 4 °C), and the supernatant was freeze-dried and further dissolved with 950  $\mu\text{L}$  of methanol and 50  $\mu\text{L}$  of HCl 1 M solution. Then, the released CPT was determined by RP-HPLC and ESI-MS. Triplicate samples were run for every experiment.

#### *Stability in acid conditions*

5 mg of RhB-SiO<sub>2</sub>@MSN-SS-CPT was placed in a vial with 1 mL of buffer solution (pH 5.5, obtained by mixing 1:1 v/v buffer solutions for HPCE of pH 5.0 and pH 6.0). The tube was vigorously shaken using a vortex for 30 seconds. Then the suspension was placed in a Thermomixer® at 37° C and 1350 rpm. At the corresponding time the sample was centrifuged (12500 g, 15 min), and the supernatant

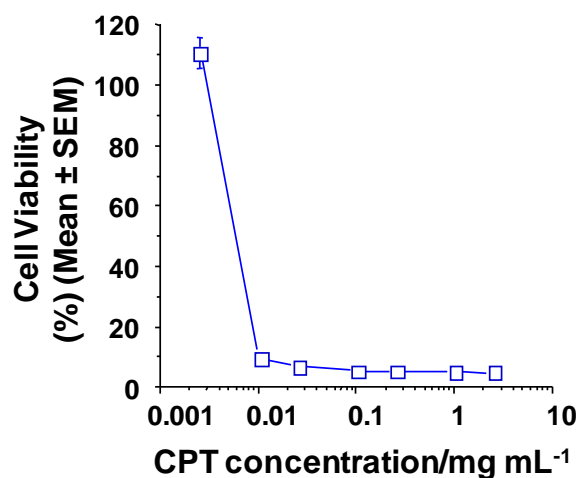
was freeze-dried and further dissolved with 950  $\mu\text{L}$  of methanol and 50  $\mu\text{L}$  of HCl 1 M solution. Then, the released CPT was determined by RP-HPLC and ESI-MS. Triplicate samples were run for every experiment.



**Figure S12.** Cumulative non-specific release profile of CPT at 37 °C from RhB-SiO<sub>2</sub>@MSN-SS-CPT in conditioned cell culture medium or in acid medium (pH 5.5).

### Cytotoxicity study of nanocarriers

HeLa cells (4000 cells/well, 96-well plates) were seeded and stabilized for 48 h in RPMI supplemented with 10% fetal bovine serum (FBS) at 37 °C in 95% air and 5% CO<sub>2</sub> environment. After incubation, growth medium was exchanged before further additions. Then, cells with fresh medium were treated with CPT (in DMSO, final doses ranging from 0.025 to 2.5  $\mu\text{g mL}^{-1}$ ) and RhB-SiO<sub>2</sub>@MSN-SH (0.1-70.0  $\mu\text{g mL}^{-1}$ , corresponding to the concentration range of CPT-loaded nanoparticles) during 72 hours. At the end of the incubation period, 5 mg mL<sup>-1</sup> of MTT solution in PBS was added to the wells and 4 h later formazan crystals were dissolved in DMSO and spectrophotometrically measured at 550 nm (Perkin-Elmer VictorX5 Microplate Reader). IC<sub>50</sub> calculation survival data were evaluated by variable slope curve-fitting using Prism 5.0 software (GraphPad, San Diego, CA). Three independent experiments were performed for every sample, and each experiment was carried out with five points per concentration.



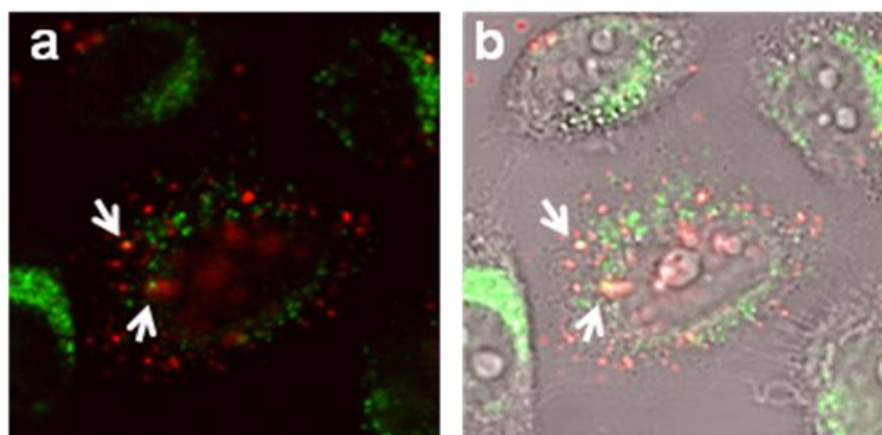
**Figure S13.** *In vitro* MTT cell viability assay after 72 h co-incubation of CPT and RhB-SiO<sub>2</sub>@MSN-SH with HeLa cells. The concentration of RhB-SiO<sub>2</sub>@MSN-SH is calculated on the base of CPT-loaded nanocarrier (0.1-70.0 µg mL<sup>-1</sup>).

**Table S2:** IC<sub>50</sub> values for tested samples in HeLa cells.

Entry	IC <sub>50</sub> <sup>a</sup>
CPT	0.0064±0.0007
CPT + RhB-SiO <sub>2</sub> @MSN-SH	0.0063±0.0009

<sup>a</sup> µg mL<sup>-1</sup>. Data indicate the mean ± SEM (µg mL<sup>-1</sup>) of three experiments.

### Cell internalization

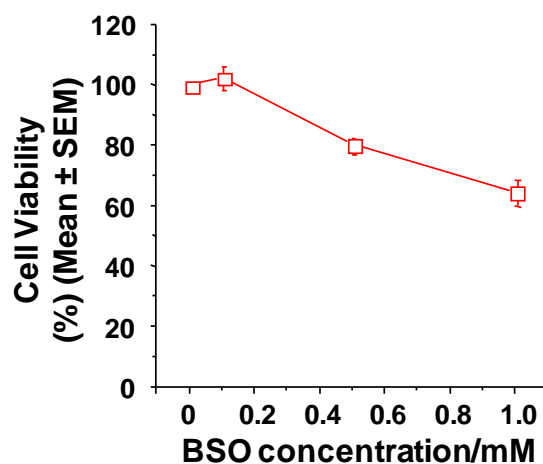


**Figure S14.** Co-localization studies of RhB-SiO<sub>2</sub>@MSN-SS-CPT in HeLa cells. Confocal images of the same Z plane were obtained for (a) Lysotracker and for Rhodamine B-labeled nanoparticles, and (b) merged with the transmitted light image to show that nanoparticles inside cells can be localized inside acidic organelles (see arrows) or outside them (red dots not co-localizing with the green signal).

## Inhibition of intracellular release

### *Effect of BSO concentration in cell viability*

HeLa cells (4000 cells/well, 96-well plates) were seeded and stabilized for 24 h in RPMI supplemented with 10% fetal bovine serum (FBS) at 37 °C in 95% air and 5% CO<sub>2</sub> environment. Next, L-buthionine sulfoximine (BSO) was added (0-1 mM) and cells were incubated further 24 h. After incubation, growth medium was exchanged and cells with fresh medium incubated 72 hours more. At the end of the incubation period IC<sub>50</sub> calculation survival data were evaluated as described above. Three independent experiments were performed for every sample, and each experiment was carried out with five points per concentration.

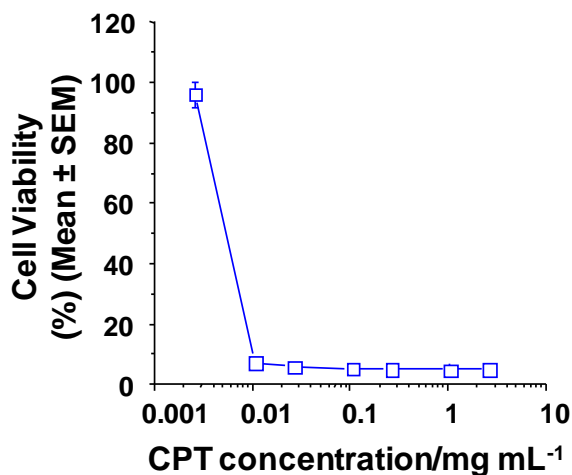


**Figure S15.** Effect of BSO concentration in cytotoxicity on HeLa cell line as stated by *in vitro* MTT cell viability assay.

### *Effect of BSO in CPT activity*

HeLa cells (4000 cells/well, 96-well plates) were seeded and stabilized for 24 h in RPMI supplemented with 10% fetal bovine serum (FBS) at 37 °C in 95% air and 5% CO<sub>2</sub> environment. Next, L-buthionine sulfoximine (BSO) was added (0.1 mM) and cells were incubated further 24 h. After incubation, growth medium was exchanged and cells with fresh medium were treated with CPT (in DMSO, final doses ranging from 0.025 to 2.5 µg mL<sup>-1</sup>) during 72 hours. At the end of the incubation period IC<sub>50</sub> calculation survival data were evaluated as described above. Three independent experiments were performed for every sample, and each experiment was carried out with five points per concentration.





**Figure S16.** Effect of BSO 0.1 mM in the cytotoxic activity of CPT on HeLa cell line as stated by *in vitro* MTT cell viability assay.

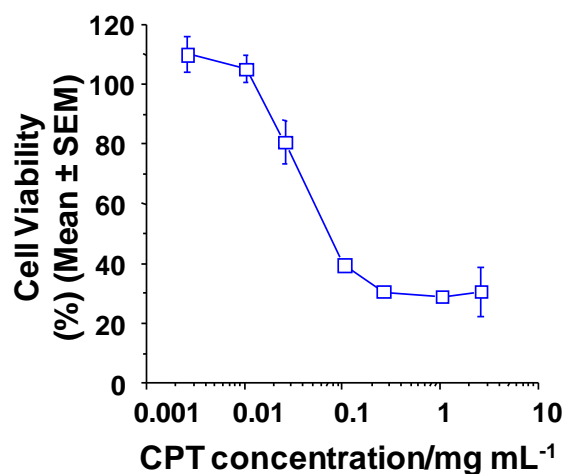
**Table S3:** IC<sub>50</sub> values for tested samples in HeLa cells.

Entry	IC <sub>50</sub> <sup>a</sup>
CPT	0.0064±0.0007
CPT + BSO 0.1 mM	0.0063±0.0012

<sup>a</sup> μg mL<sup>-1</sup>. Data indicate the mean ± SEM (μg mL<sup>-1</sup>) of three experiments.

#### *Effect of CLQ the cytotoxic activity*

HeLa cells (4000 cells/well, 96-well plates) were seeded and stabilized for 24 h in RPMI supplemented with 10% fetal bovine serum (FBS) at 37 °C in 95% air and 5% CO<sub>2</sub> environment. Next, CLQ was added (150 μM) and cells were incubated further 24 h. After incubation, growth medium was exchanged and cells with fresh medium were treated with RhB-SiO<sub>2</sub>@MSN-SS-CPT with final doses ranging from 0.025 to 2.5 μg mL<sup>-1</sup> (in CPT equivalents) during 72 hours. At the end of the incubation period IC<sub>50</sub> calculation survival data were evaluated as described above. Three independent experiments were performed for every sample, and each experiment was carried out with five points per concentration.



**Figure S17.** Effect of CLQ in the cytotoxic activity of RhB-SiO<sub>2</sub>@MSN-SS-CPT as stated by *in vitro* MTT cell viability.

**Table S4:** IC<sub>50</sub> values for tested samples in HeLa cells.

Entry	IC <sub>50</sub> <sup>a</sup>
RhB-SiO <sub>2</sub> @MSN-SS-CPT	0.0064±0.0007
RhB-SiO <sub>2</sub> @MSN-SS-CPT + CLQ	0.0265±0.0027

<sup>a</sup> μg mL<sup>-1</sup>. Data indicate the mean ± SEM (μg mL<sup>-1</sup>) of three experiments

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