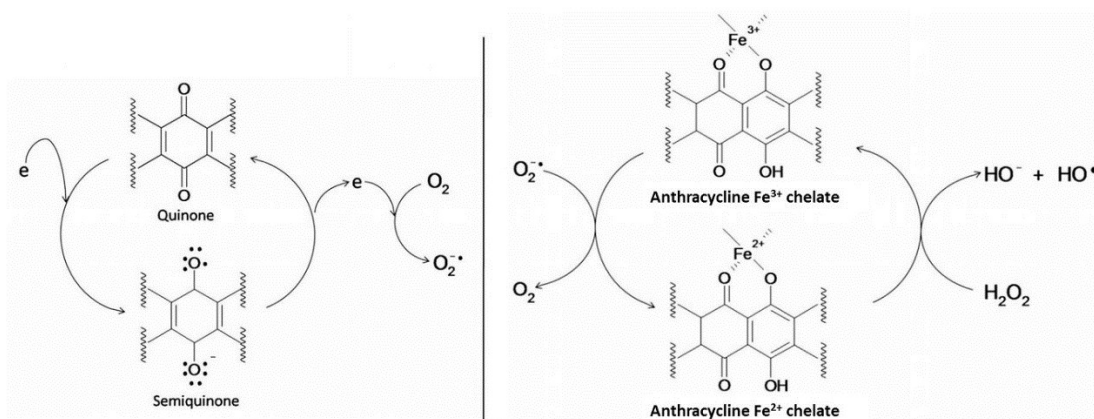


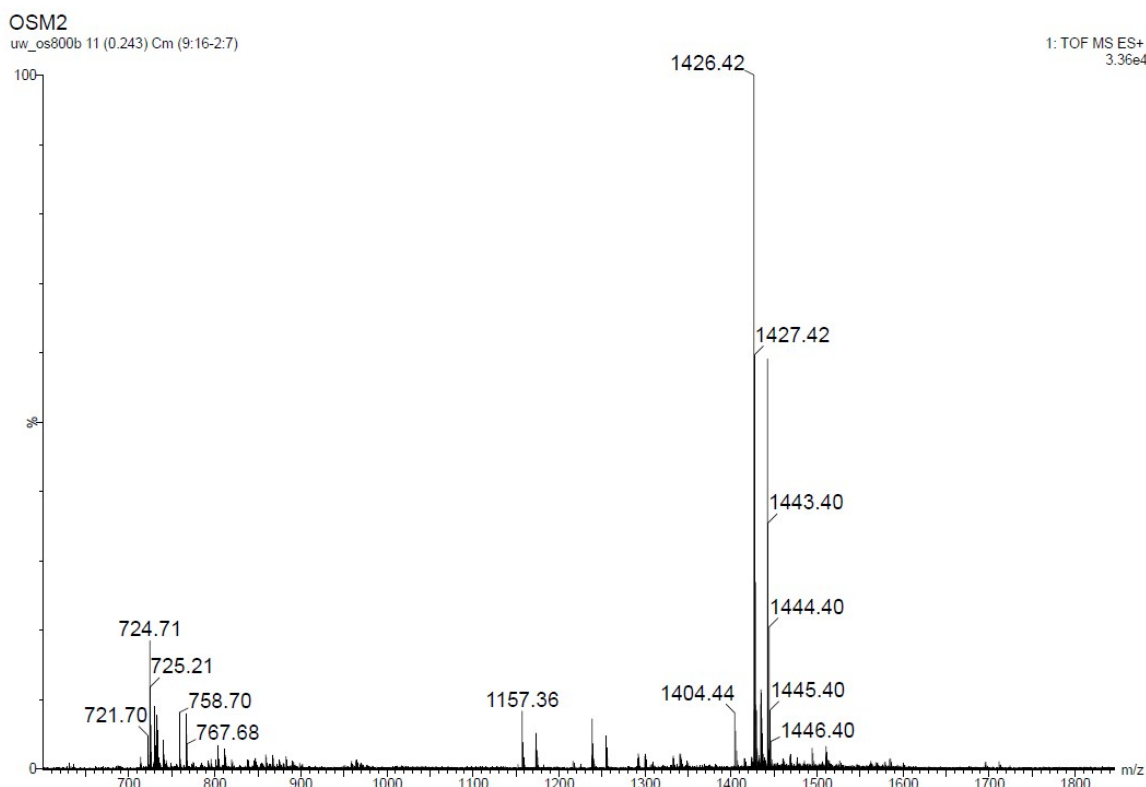
Swiech et al

**“Competition between self-inclusion and drug binding explains the pH dependence of cyclodextrin drug carrier - molecular modelling and electrochemistry studies”.**

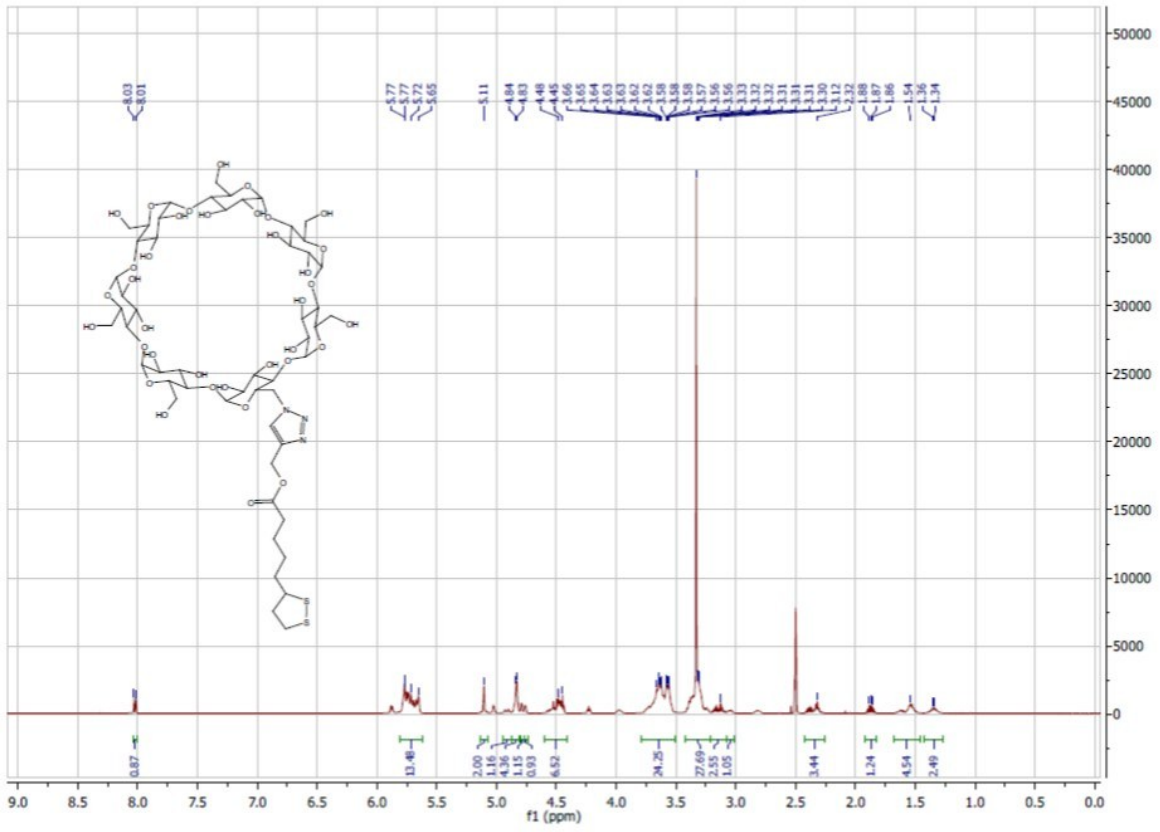
**Supplementary Material**



**S1. Reactions of ROS formation catalyzed by molecules which contain a quinone group.**



**S2. MS spectra of  $\beta$ CDLip**

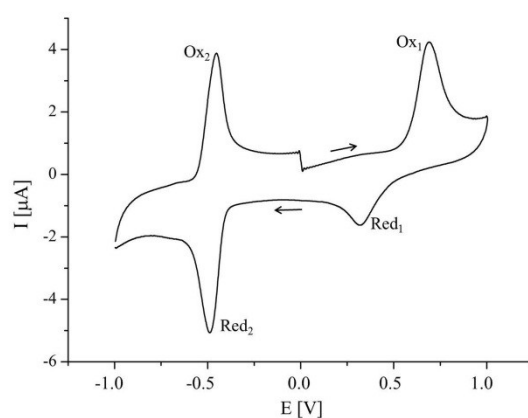


S3.  $^1\text{H}$ NMR spectra of  $\beta$ CDLip



**Solubility of the compounds (S5).** The solubility of Dox was determined by LC Laboratory (Woburn, USA) at 10 mg/mL and 100 mg/mL in water and DMSO, respectively. The solubility of native  $\beta$ CD in water is well known and is equal to 18.5 mg/mL. The solubility increases in an irregular manner after the addition of DMSO. At less than 30% DMSO, the solubility remains constant (20 mg/mL). Between 30 and 40% of DMSO, the solubility rapidly increases to 770 mg/mL and remains constant up to 86% [ ]. The solubility of  $\beta$ CDLip were determined using UV–vis spectroscopy. The value for the solubility in pure water are less than that of native  $\beta$ CD, equal to  $0.72 \pm 0.13$  mg/mL. Because of the limited solubility of  $\beta$ CDLip complex in water, all electrochemical experiments were conducted in a mixture of Britton–Robinson buffer and DMSO (2:1 ratio).

**CDLip-Dox formation constant evaluation:** All calculations were based on the reduction peak current (Red<sub>2</sub>) of the quinone group of doxorubicin, (Figure S4).

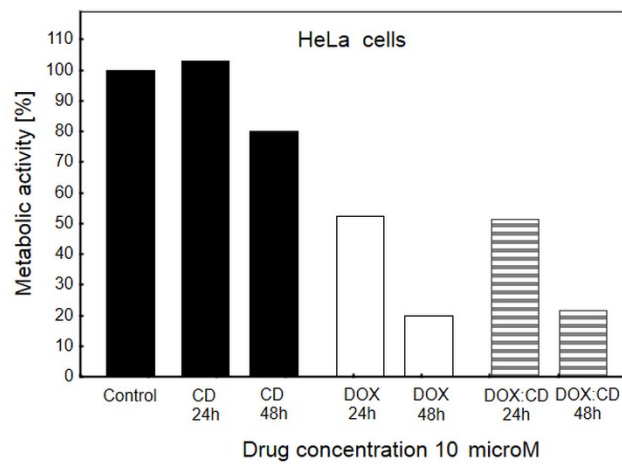
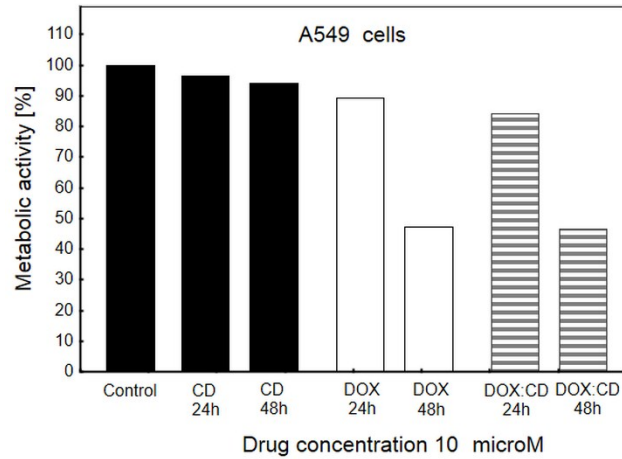


**Figure S6. Cyclic voltammograms for  $5 \cdot 10^{-5}$  M Dox recorded in BR buffer at pH 5.5.**

**Scan rate 0.2V/s.**

### **MTT measurements (S7).**

MTT [3-(4,5-dimethyldiazol-2-yl)-2,5-diphenyl tetrazolium bromide] test, which measures activity of mitochondrial dehydrogenases, was used to evaluate a short-term cellular toxicity of the synthesized NSs. The says was carried out as described elsewhere.<sup>24</sup> In brief, human epithelial lung carcinoma cell line A 549 and human cervix carcinoma cell line HeLa were purchased from American Type Tissue Culture Collection (ATCC, Rockville, MD, USA) and cultured in F12 or DMEM medium, respectively, according to the ATTC recommendations. The cells were plated at density of 50000 cells per well in 96-well plates and treated with the tested compounds in concentration  $1 \times 10^{-6}$  mol/dm<sup>3</sup>  $5 \times 10^{-6}$  mol/dm<sup>3</sup> and  $1 \times 10^{-5}$  mol/dm<sup>3</sup> or vehicle (control) for 24 or 48 h at 37°C in 5 % CO<sub>2</sub> humidified atmosphere. After incubation, 10 µL of MTT (5 mg/mL in PBS, pH 7) was added to each well and cells were incubated at 37°C for 4 h in a humidified atmosphere. Then, the growth medium was removed, 100 µl of DMSO was added to each well to dissolve the purple crystals of formazan. The absorbance was measured in a plate reader spectrophotometer (Infinite M200, Tecan, Morrisville, NC) at a wavelength of 570 nm. The cell metabolic activity, which roughly relates to the cell viability, was expressed as ratio of the absorbance of the treated cells to the absorbance of the cells treated with vehicle, both after subtraction of the reagent control and multiplied by 100%.



**S8. Toxicity of the synthesized complex Dox-CDLip (Dox:CD) and its components as measured by MTT assay on A549 and HeLa cell lines. Whiskers correspond to variation of raw, not standardized data, to illustrate the measurement variability.**

**Student's t-test evaluation was performed on raw data,  $p < 0.05$ ,  $n = 3$ .**