Electronic Supplementary Information

Self-assembling Oligothiophene-Bolaamphiphiles for Loading and Controlled Release of Doxorubicin into Living Cells

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Physical measurements and instrumentation:

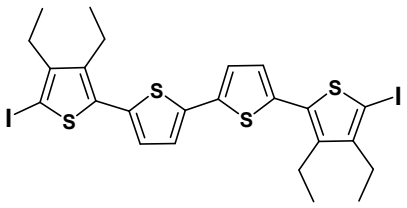
Nuclear magnetic resonance spectra were recorded on a *Bruker* AMX 500 spectrometer (^{1}H NMR: 500 MHz, ^{13}C-NMR 125 MHz), a *Bruker* Avance 400 (^{1}H NMR: 400 MHz, ^{13}C NMR: 100 MHz) at room temperature unless otherwise noted. Chemical shift values (δ) are given in parts per million using residual solvent protons (^{1}H NMR: δ_H = 7.26 for $CDCl_3$, δ_H = 2.49 for DMSO-d_6; δ_H = 3.33 for MeOD-d_4, ^{13}C NMR: δ_C = 77.0 for $CDCl_3$, δ_C = 49.05 for MeOD-d_4 and 39.43 for DMSO-d_6) as internal standard. The splitting patterns are described as follows: (s) singlet, (d) doublet, (t) triplet, qr (quartet), q (quintet), m (multiplet). Matrix-assisted laser desorption ionization time-of-flight mass spectrometry (MALDI-TOF MS) measurements were carried out on a *Bruker* Daltonik Reflex III mass spectrometer with the following matrices: 1,2,3-trihydroxyanthracene (dithranol), 2,5-dihydroxybenzoic acid (DHB) and T-2-(3-(4-t- Butyl-phenyl)- 2-methyl- 2-propenylidene) malononitrile (DCTB). Elemental analyses were performed on an *Elementar Vario* EL (Ulm University). Melting points are uncorrected and were determined using a *Büchi* B-545 apparatus or a Mettler Toledo Differential Scanning Calorimetry (DSC) 823^e measuring cell. Absorption spectra were recorded on a *Perkin Elmer* Lambda 19 spectrometer and fluorescence emission spectra on a *Perkin Elmer* LS 55 spectrometer using 1 cm cuvettes. All spectra are corrected. CD spectra were recorded on a JASCO J 600 spectropolarimeter. All reactions were monitored by TLC (aluminium plates, pre-coated with silica gel, *Merck* Si60 F254). Confocal laser scanning microscopy was performed using Zeiss LSM 710, Observer Z.1 using a 403 nm excitation laser and broad-band emission filter 450 nm – 650 nm with a 63x/1.40 oil immersion objective. Luminescent readouts for the quantification of cell viability were analyzed using GloMax® 96-well luminometer (Promega). The AFM images were obtained with a Nanoscope IIIa (Veeco

Instruments Inc.) using standard silicon-cantilevers (spring constant: 50 N/m, frequency: 300 kHz) in tapping mode. Height, phase and amplitude images were recorded simultaneously. The samples were spin-coated (2000 rpm) from solution (0.05-0.5 mg/mL) on freshly cleaved mica substrates. Theoretical calculations: Quantum chemical calculations were performed on a semiempirical basis with the INDO based method Austin Model 1 (AM1) from the Hyperchem (Hypercube, Inc., FL) software package.

Chemicals: Dichloromethane, toluene, diethylether (Merck) were dried over CaH_2 and distilled; tris(dibenzylideneacetone)dipalladium(0)-chloroform adduct, tris-tert-butylphosphonium tetraflouroborate, *N*,*N*-diisopropylamine and bis(triphenylphosphine) palladium(II)chloride were purchased from Merck. Ion exchange resin *Dowex marathon C* were purchased from *Sigma Aldrich*. For purification by column chromatography silica gel 60 (0.040-0.063 mm) from *Machery & Nagel* was used. Solvents were distilled prior to use. 2-propynyl-2,3,4,6-tetra-*O*-acetyl-α-D-(+)-mannopyranoside **2**, the enantiomeric 2-propynyl-2,3,4,6-tetra-*O*-acetyl-α-L-(-)-mannopyranoside **3** and 3,3’’’,4,4’’’tetraethyl-[2,2’;5’,2’’;5’’,2’’’] quaterthiophene were prepared as previously described.[1] Biodegradation studies were performed in vitro in the presence of simulated bodyfluid (SBF) prepared according ref. 2.

Abbreviations: Calcd: Calculated; DCM: Dichloromethane; DIPA: Diisopropylamine; DMSO: Dimethylsulfoxide; $CDCl_3$: Chloroform; PBS phosphate buffer saline; MeOH: Methanol; rt: room temperature; $Pd(PPh_3)_2Cl_2$: Bis(triphenylphosphine)palladium(II) dichloride; THF: Tetrahydrofuran.

5,5’’’Diiodo-3,3’’’,4,4’’’-tetraethyl-[2,2’;5’,2’’;5’’,2’’’]quaterthiophene **1**[1]



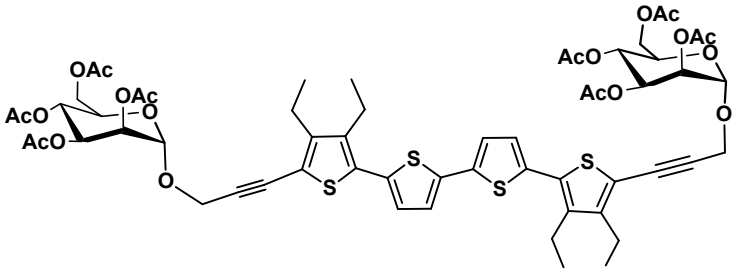
1

To an ice-cooled solution of 0.9 g (2 mM) of 3,3’’’,4,4’’’-tetraethyl-[2,2’;5’,2’’;5’’,2’’’] quaterthiophene in 80 mL dry chloroform 1.59 g (5 mM) of mercury(II) acetate was added (argon atmosphere). The reaction mixture was allowed to warm to room temperature and to stir overnight. The thick solution was cooled to 0° C again and 1.27 g (5 mM) of iodine was added under argon atmosphere. After 6h, the reaction was quenched by addition of saturated $NaHCO_3$-solution. The layers were separated and after extraction of the aqueous layer with dichloromethane the combined organic layers were washed with a saturated sodium bisulfate solution and water, and dried over Na_2SO_4. The solvent was removed in *vacuo*. Further purification of the crude product via column chromatography (silicagel, eluent: *n*-hexane) yielded 1.0 g (1.44 mM, 72 %) of the di-iodinated quaterthiophene **1** as an orange solid, mp 150° -151° C.

^{1}H-NMR (400 MHz, $CDCl_3$) δ [ppm]: 1.14, t, 6H, 7.55 Hz, 1.19, t, 6H, 7.56 Hz, 2.58, qr, 4H, 7.57 Hz, 2.79, qr, 4H, 7.55 Hz., 6.98, d, 2H, 3.78 Hz, 7.11, 2H, 3.78 Hz. ^{13}C-NMR (100 MHz, $CDCl_3$) δ [ppm]: 148.54, 139.95, 137.01, 135.75, 134.73, 126.77, 123.96, 24.52, 21.62, 15.41, 14.34 ppm.

MS (MALDI-TOF) calc. monoisotopic mass for $C_{24}H_{24}I_2S_4$ 694.52, found 694.5 [M^+]. Elemental analyses requires (%) C: 41.50, H: 3.48, S: 18.47 found C: 41.31, H: 3.40, S: 18.29.

3-(3,3'',4,4''-Tetraethyl-5''-{[(2,3,4,6-tetra-*O*-acetyl-α-D-(+)-mannopyranosyl)oxy]ethynyl}-2,2':5',2'':5'',2''-quaterthien-5-yl)prop-2-yn-1-yl -α-D-(+)-mannopyranoside **4a**



4a

To a solution of 3,4,3“,4“-tetraethyl-5,5’’-diiodo- [2,2’,5’,2“] quaterthiophene **1** (326 mg, 0.47 mmol) and 2-propynyl-2,3,4,6-tetra-*O*-acetyl-α-D-(+)-mannopyranoside (399 mg, 1.1 mmol) **2** in carfully degassed (3:1, v/v) DIPA /THF was added $PdCl_2(PPh_3)_2$ (4 mol%) and CuI (1.5 mol %). The solution was kept stirring at r.t. for 5h. After adding 80 ml water and 100 ml DCM, the phases were separated and the organic layer was dried over Na_2SO_4. The solvent was removed and the crude product was purified by chromatography using silicagel (DCM → DCM: EE 3:1). Traces of residual solvent (ethylacetate) were still present and observable in the NMR and could not be removed under vacuum. Applying high temperatures resulted in decomposition. **4a** could be isolated in a 72% yield (407 mg).

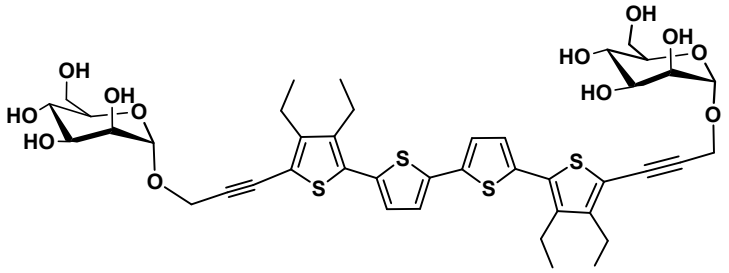
^{1}H-NMR (400 MHz $CDCl_3$) δ [ppm]: 1.20, m, 12H, 2.00, s, 6H, 2.05, s, 6H, 2.12, s, 6H, 2.17, s, 6H, 2.68, q, 4H, 7.58 Hz, 2.75, q, 4H, 7.58 Hz, 4.08, m, 2H, 4.15, dd, 2H, 12.38 Hz, 2.27 Hz, 4.35, dd, 2H, 12.37 Hz, 5.05 Hz, 4.56, m, 4H, 5.12, d, 2H, 1.51 Hz, 5.30-5.41, m, 6H, 7.05, d, 2H, 7.13, d, 2H .

^{13}C-NMR (101 MHz $CDCl_3$): δ 170.66, 169.87, 169.69, 150.70, 139.71, 137.02, 134.67, 132.15, 126.94, 124.03, 115.82, 96.14, 90.15, 80.24, 69.34, 68.99, 65.91, 62.27, 55.83, 21.86, 21.01, 20.88, 20.75, 20.70, 20.67, 15.17, 14.87, 14.20 [ppm].

MS (MALDI-TOF) m/z calc. monoisotopic mass for $C_{58}H_{66}O_20S_4$: 1210.30, found: 1210.8[M^+], 1234.4 [M+ Na] $^+$.

The opposite α-L-(-) enantiomer **5a** could be synthesized applying the same protocol, but using **3** as alkyne component. **5a** displayed identical NMR data.

3-{3,3''',4,4'''-Tetraethyl-5'''-[(α-D-(+) mannopyranosyloxy)ethynyl]-2,2':5',2'':5'',2'''-quaterthien-5-yl}prop-2-yn-1-yl α-D-(+) mannopyranoside **4b**



4b

121 mg (0.138 mmol) of **4a** were dissolved in 16 mL *abs*. THF/MeOH (1/1) and a catalytic amount of sodium methanolate (0.3 M) was added. After stirring for 1 hour the mixture was brought to pH 7 using ion exchanger *Dowex Marathon C*. After removal of the ion exchanger by filtration and evaporation of the solvent 84.0 mg of **5b** were afforded.

The opposite enantiomer **5b** could be synthesized according the same protocol. **5b** displayed identical NMR data.

^{1}H-NMR of **4b** (400 MHz, DMSO- d_6/D_2O 1 drop) δ [ppm]: 1.15, t, 6H 7.46 Hz, 1.19, t, 6H, 7.55 Hz, 2.76, q, 4H, 7.49 Hz, 2.74, q, 4H, 7.49 Hz, 3.38, m, 2H, 3.46-3.58, m, 6H, 3.66-3.76, m, 4H, 4.52, m, 4H, 4.88, d, 2H, 7.18, d, 3.78 Hz, 7.33, d, 3.78 Hz.
^{13}C-NMR (100 MHz, DMSO-d^6): 14.8, 14.9 18.8, 20.5, 21.3, 25.1, 30.7, 46.3, 53.7, 61.1, 66.8, 67.0, 70.1, 74.5, 78.0, 93.2, 98.3, 115.6, 125.3, 127.7, 130.8, 133.7, 136.0, 139.8, 150.2 [ppm]
HRMS ($C_{42}H_{49}NaO_{12}S_4$): 897.2083

Confocal Laser Scanning Microscopy

A549 cells were seeded at a density of 15,000 cells/well in an 8 well confocal microscopy chamber (Ibidi, Germany) using 300 µL of fortified DMEM medium and left to adhere overnight at 37 °C, (5% CO_2. The cells were treated with **DOX-4b, DOX-5b and DOX** (1 µM) in DMEM medium for 24 h at 37 °, 5% CO_2. The treated cells were washed three times with DMEM medium to remove non-specific adsorption and imaged using Zeiss LSM 710, Observer Z.1 confocal microscope and processed using the Zen software and ImageJ. The

respective filters were used to visualize independently for the oligothiophene (λ_{ex} = 405 nm, λ_{em} = 435 nm–540 nm), doxorubicin (λ_{ex} = 488 nm, λ_{em} = 540 nm–700 nm) and FRET (λ_{ex} = 405 nm, λ_{em} = 650 nm–750 nm). Laser gain was optimized to reduce residual signals from spectral overlap between the emission of oligothiophenes and doxorubicin.

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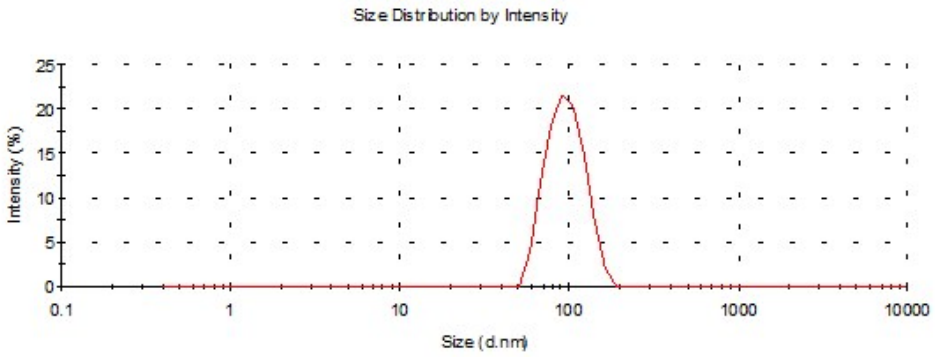
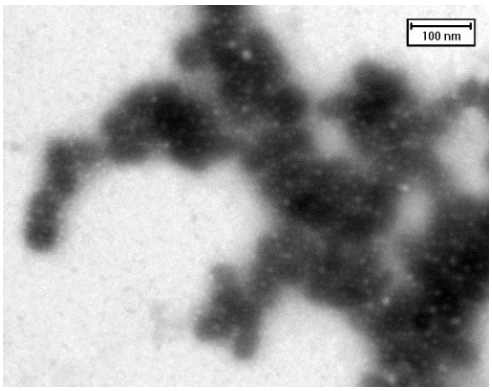
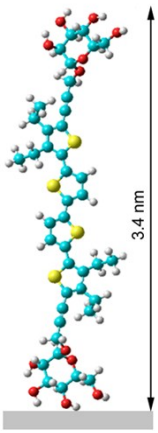


S1: Size distribution of **4b** in aqueous DMSO solutions [10^{-7} M] measured by DLS.



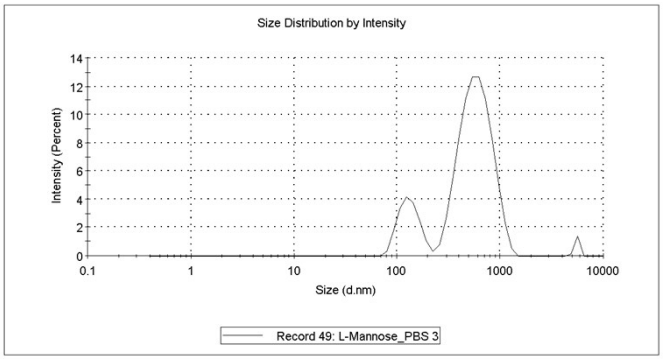
S2: TEM images of aggregates of **4b** deposited on carbon coated copper grids from an aqueous 5 % MeOH solution stained with uranylacetate. Scale bar represents 100 nm.



S3: Orientation of a semiempirical calculated model of **4b** onto the surface.

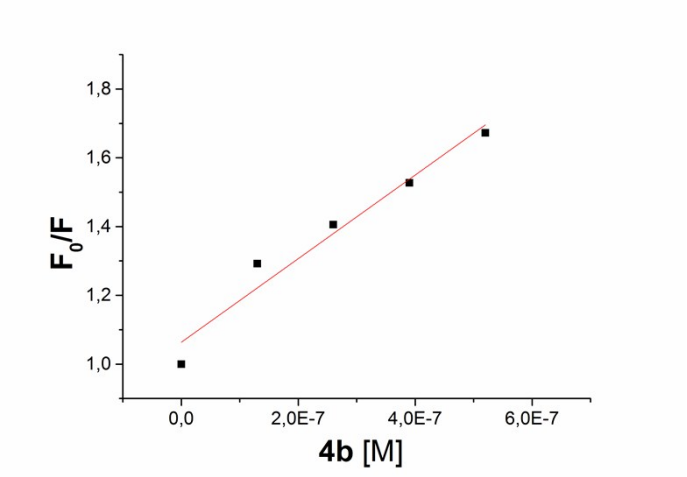
Results

			Size (d.nm...	% Intensity:	St Dev (d.n...
Z-Average (d.nm):	429,6	**Peak 1:**	598,6	81,1	205,1
PdI:	0,565	**Peak 2:**	131,4	17,3	30,14
Intercept:	0,957	**Peak 3:**	5488	1,6	222,6
Result quality	**Good**				

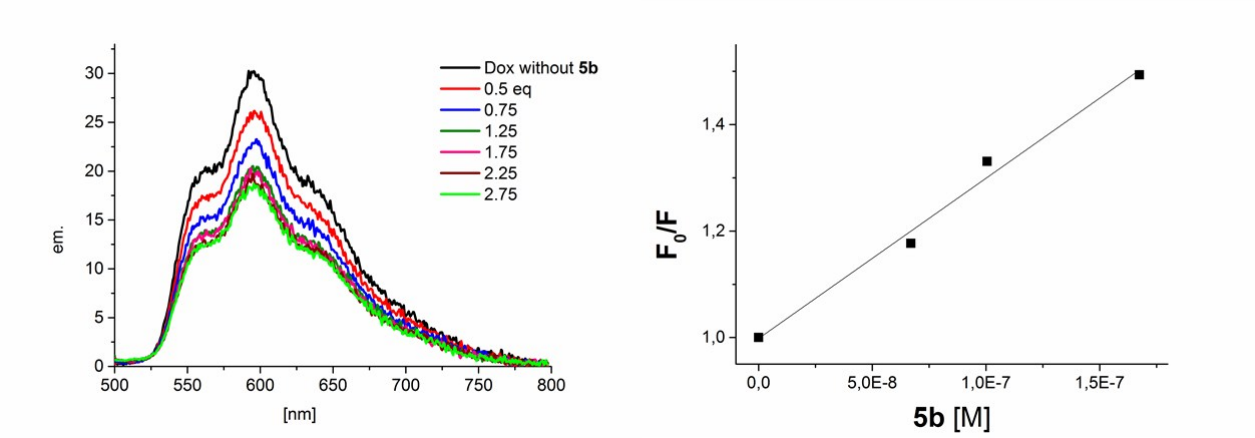


S4: Size distribution of the L-(-)-mannosidic 4T **5b** in PBS solutions [10^{-7} M] measured by DLS.

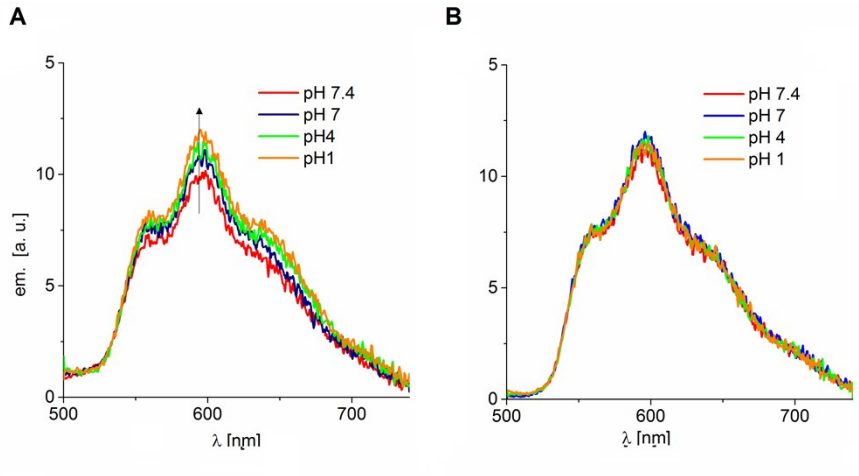
S5 Titration experiment**:** The **4b** or **5b** –DOX interactions were studied by fluorescence spectroscopy. Each titration experiment was made in triplicate using 10^{-6} to 10^{-8} M solutions of DOX. After addition of aliquots of **4b** or **5b**, respectively and ultrasonification (3 minutes) the emission spectra of DOX were recorded (500 nm to 800 nm, λ_{exc} 480 nm).



S5a Stern-Vollmer plot of DOX-**4b** complex formation resulting K_b= 1.2±0.1 x 10^6 mol^{-1}, [c] DOX = 6.5∗E 10^{-7} mol/L in PBS.

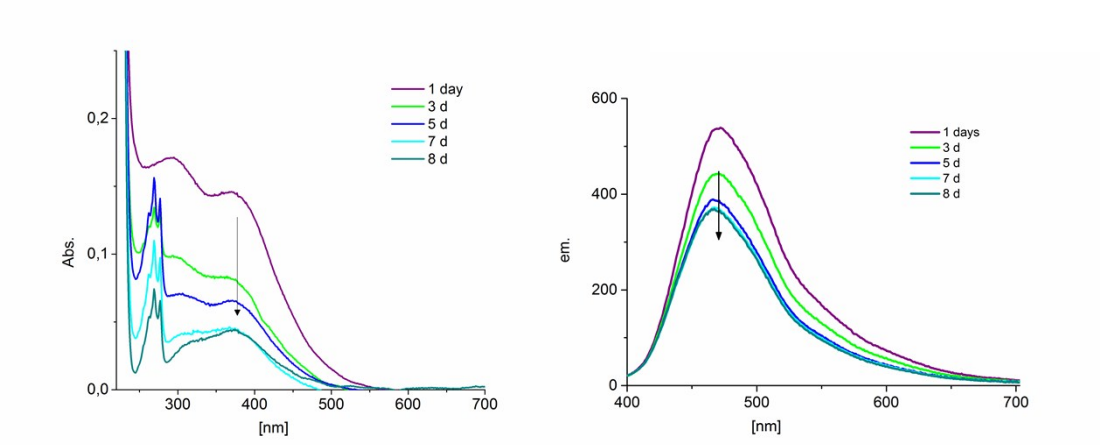


S5b Stern-Vollmer plot of DOX-**5b** complex formation resulting K_b=3.0±0.2x10^6 mol^{-1}, [c] DOX = 1.25∗E 10^{-8} mol/L in PBS.



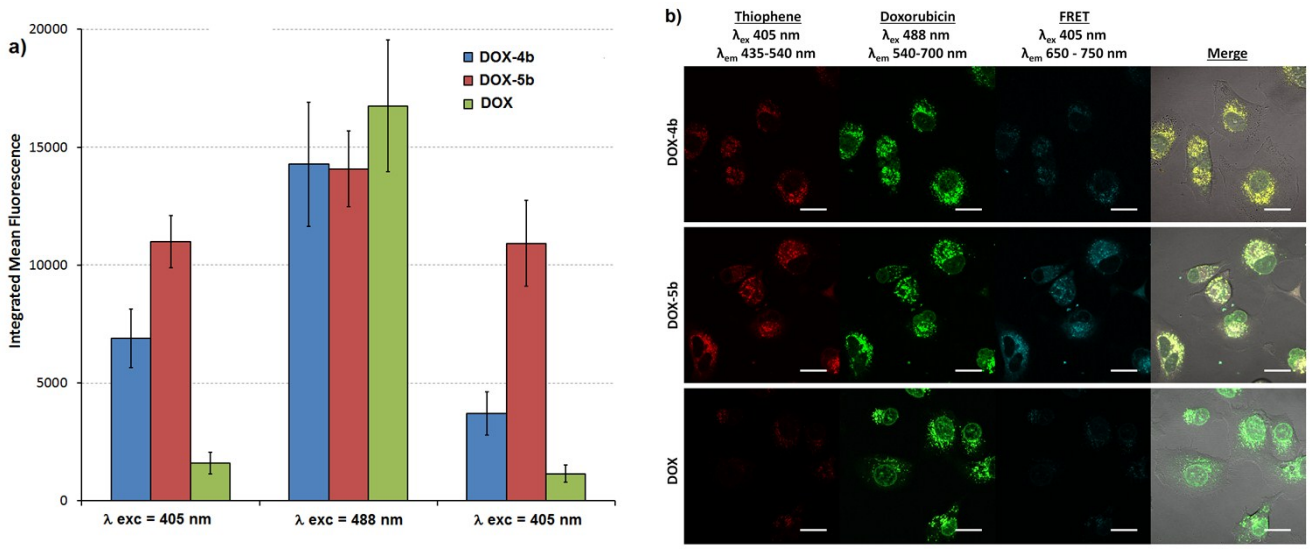
S6: A) Emission spectra of DOX with **4b** at pH 7.4 (red), pH 7 (blue), pH 4 (green), and pH 1 (orange). B) Emission spectra of DOX without **4b** at pH 7.4 (red), pH 7 (blue), pH 4 (green), and pH 1(orange).

Biodegradation experiment



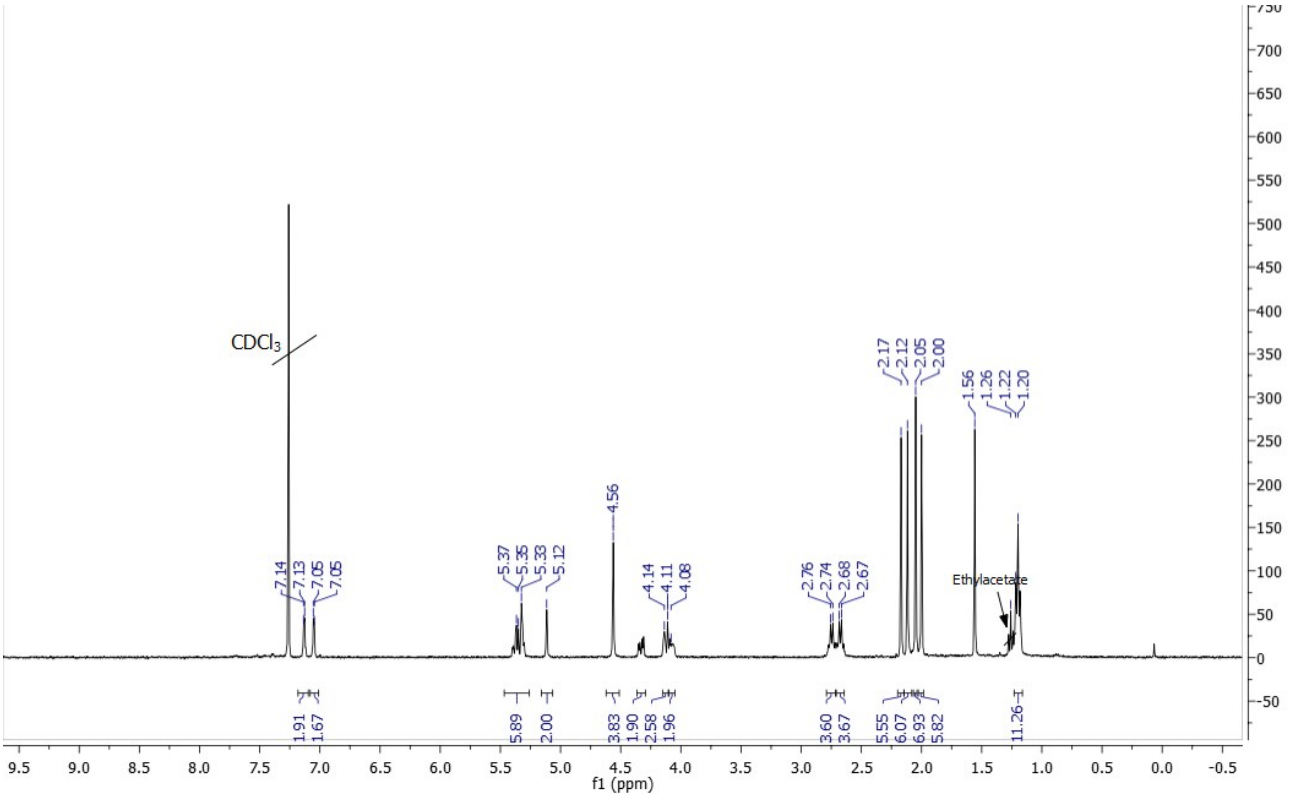
S7: Absorption (left) and fluorescence spectra (right) of **4b** [10^{-5} M] in simulated bodyfluid.

Cytotoxicity Assay: A549 cells were pre-cultured in high glucose DMEM medium fortified with 10% fetal boving serum, 1% penicillin/streptomycin and 1% MEM. The cells were seeded at a density of 6000 cells/well in a half-area 96-well plate using 50 µL of medium in each well and left to adhere overnight at 37 °C, 5% CO_2. Samples of **4b** and **5b** at various concentrations (1 – 40 µM) in DMEM medium were used to treat the cells for 36 h at 37 °C, 5% CO_2. Subsequently, the treated cells were subjected to CellTiter-Glo® (Promega, Germany) luminescent assay according to manufacturer's protocol to quantify the cell viability.

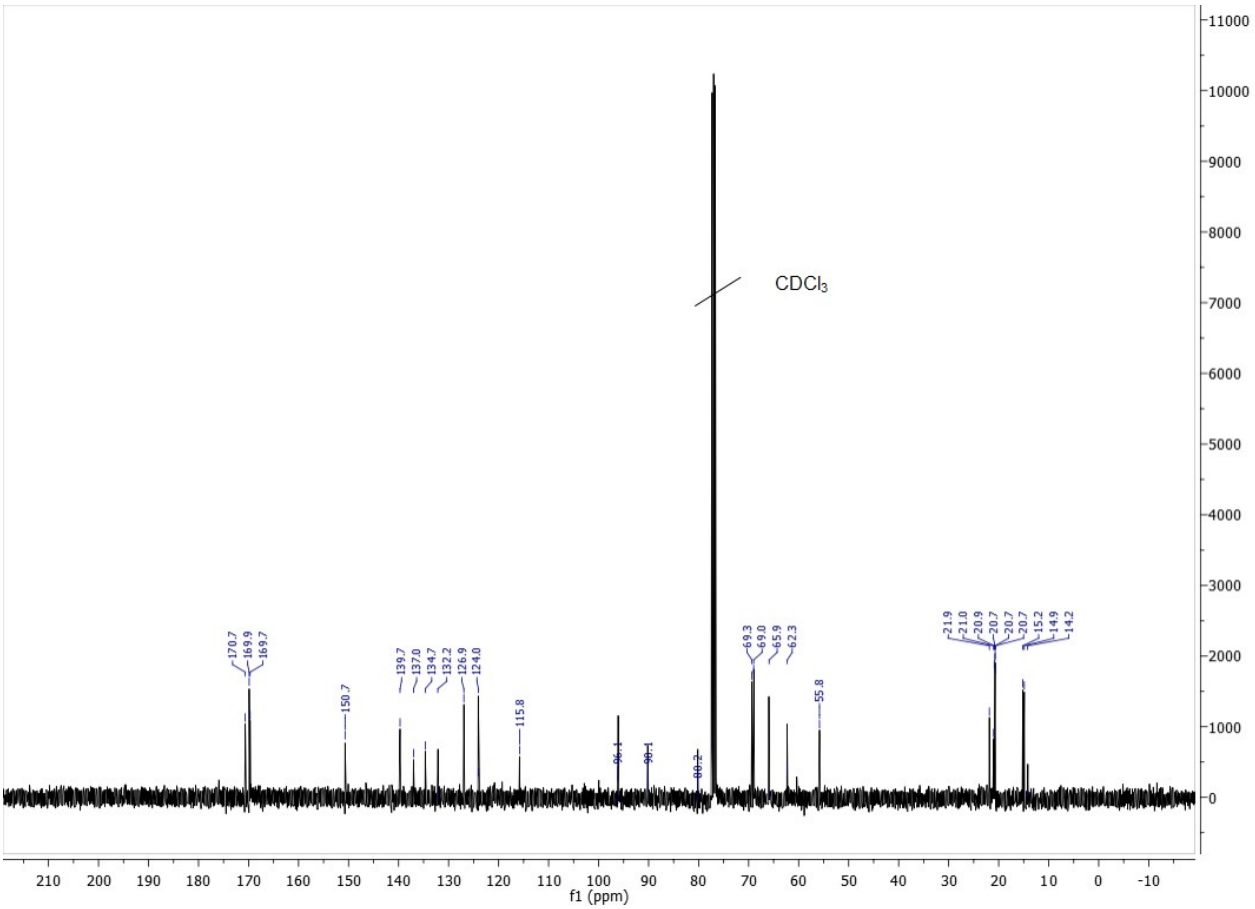


S8 a) ImageJ statistical analysis (left) and b) confocal laser scanning micrograph (right) of A549 cells treated with **DOX-4b**, **DOX-5b** and **DOX** over 36 h (37 °C, 5% CO_2) at 1 µM, scale bars represent 20 µm.

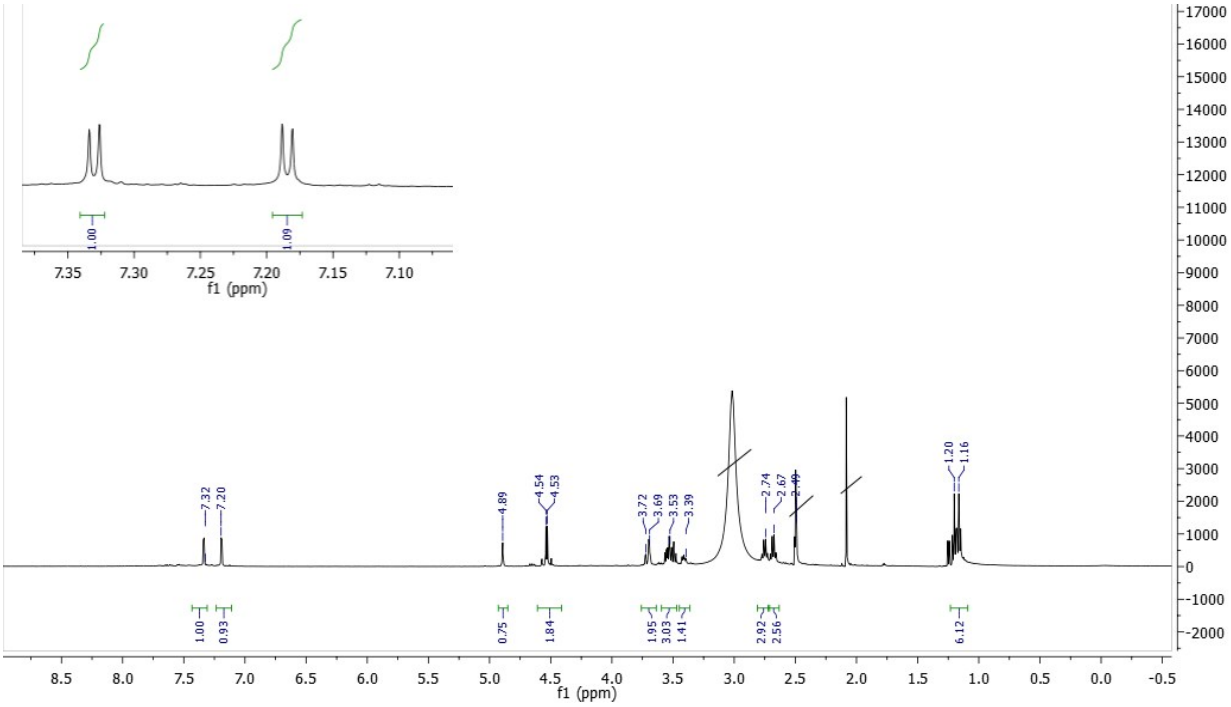
S9 ^{1}H-NMR of **4a** in $CDCl_3$



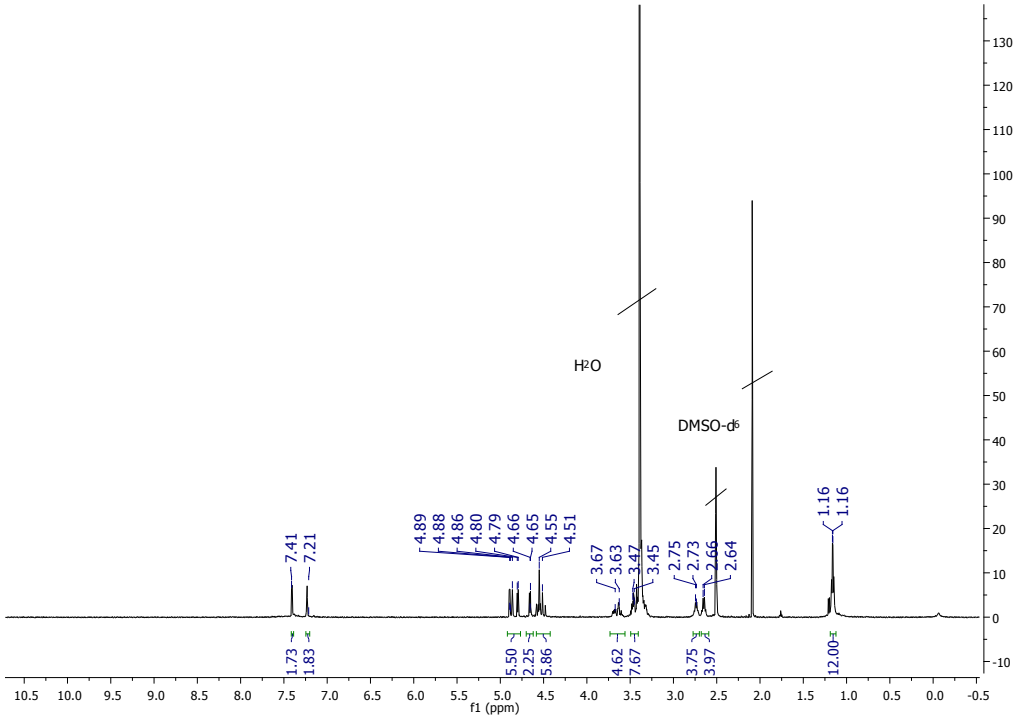
S10 ^{13}C-NMR of **4a** in $CDCl_3$



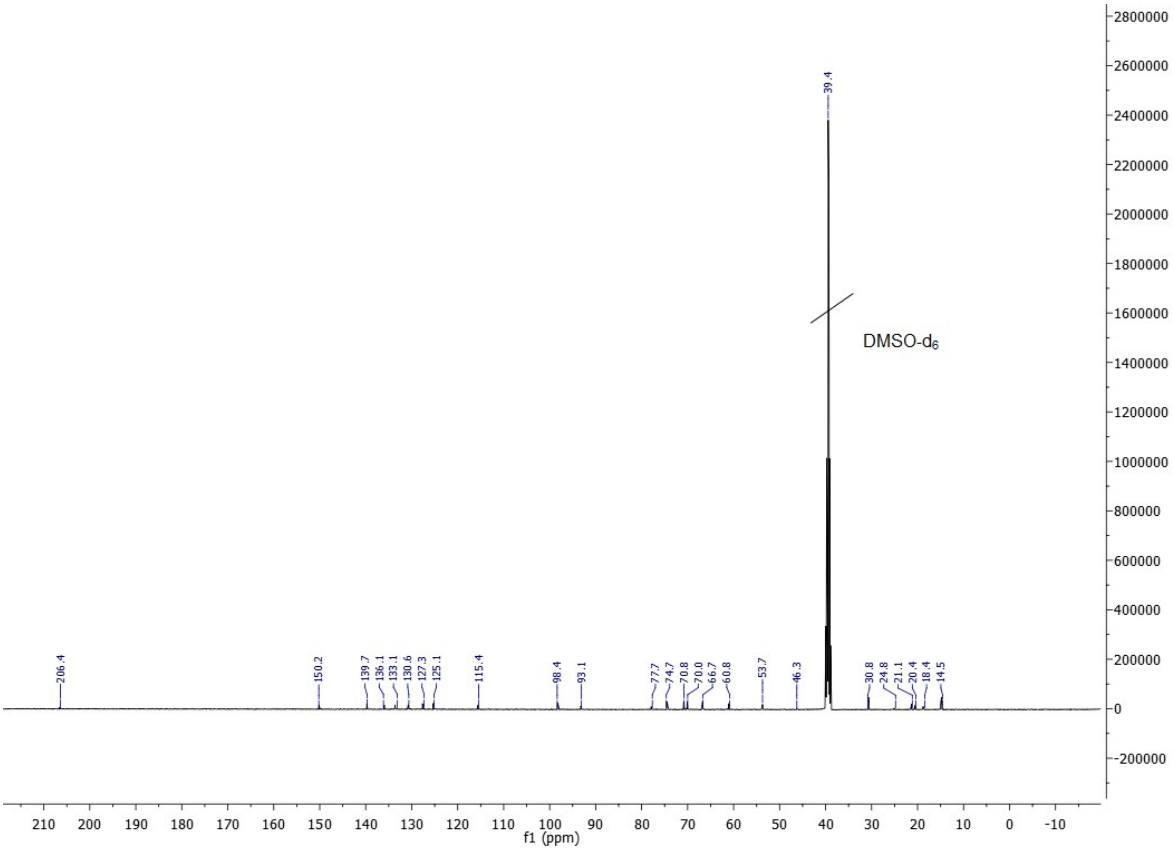
S11 ^{1}H-NMR of **4b** in DMSO



S12 ^{1}H-NMR of **4b** in DMSO/1 drop D_2O



S13 ^{13}C-NMR of **4b** in DMSO



References

[1] S. Schmid, A. Kopychev, E. Mena-Osteritz and P. Bäuerle, *Org. Lett*. 2009, **11**, 7146.
[2] T. Kokubo, H. Kushitani, S. Sakka, T. Kitsugi and T. Yamamuro, *J. Biomed. Mater. Res.* 1990*, 24*, 721-734.