Synthesis and Evaluation of A Novel Rhodamine B Pyrene [2]Rotaxane as an Intracellular Delivery Agent for doxorubicin

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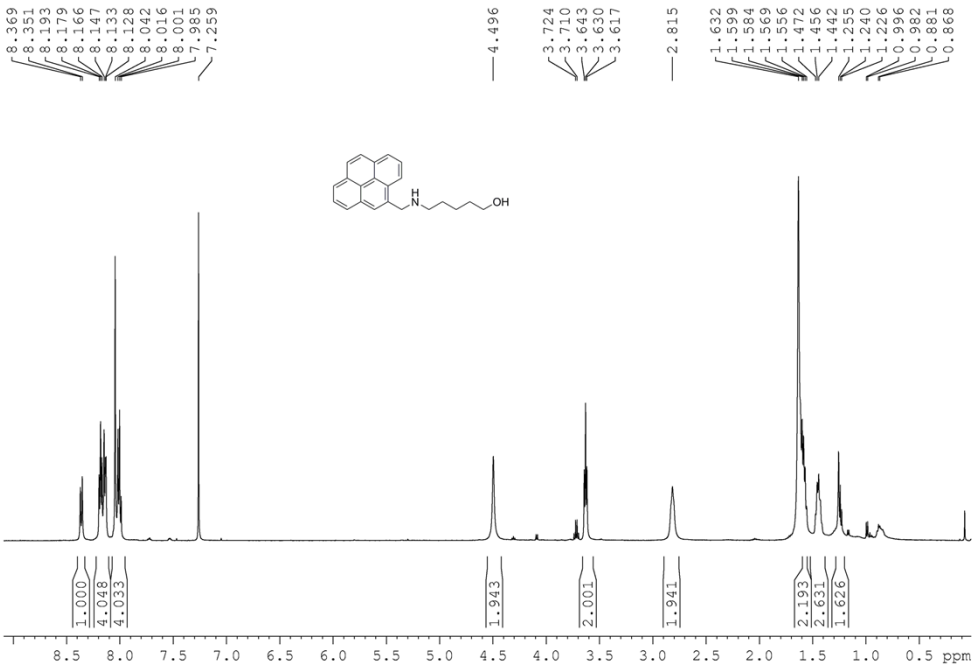

Figure S1. ^{1}H NMR spectrum of compound **1** in $CDCl_3$.

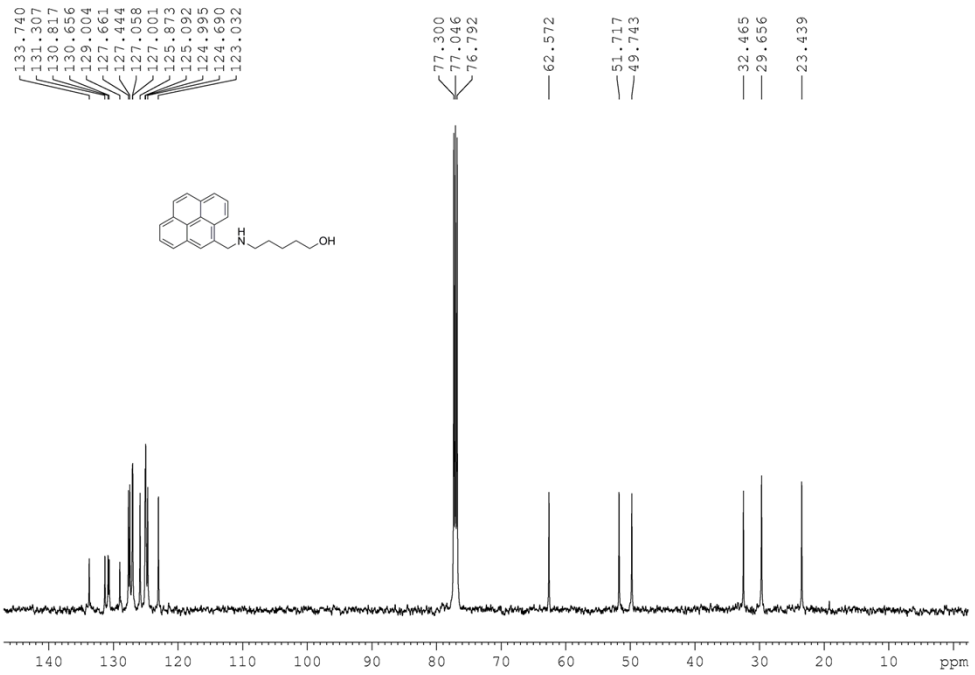

Figure S2. ^{13}C NMR spectrum of **1** in $CDCl_3$.

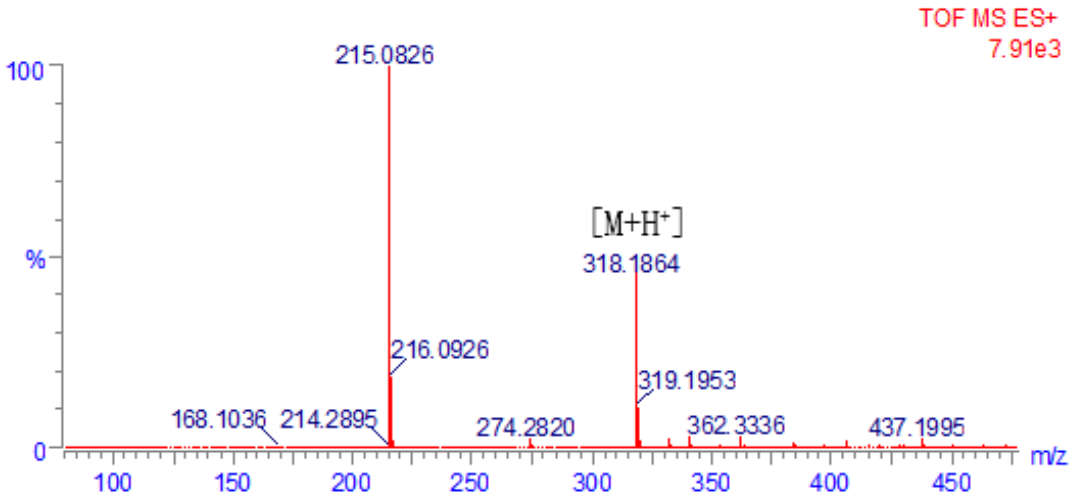

Figure S3. HRMS spectrum of compound 1

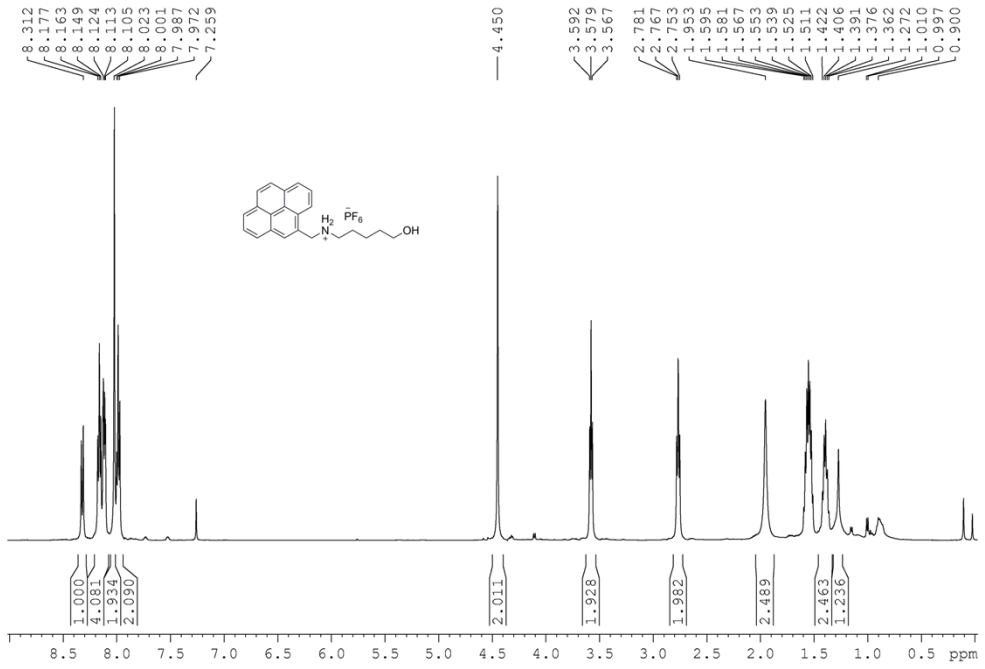

Figure S4. ^{1}H NMR spectrum of compound 2 in $CDCl_3$.

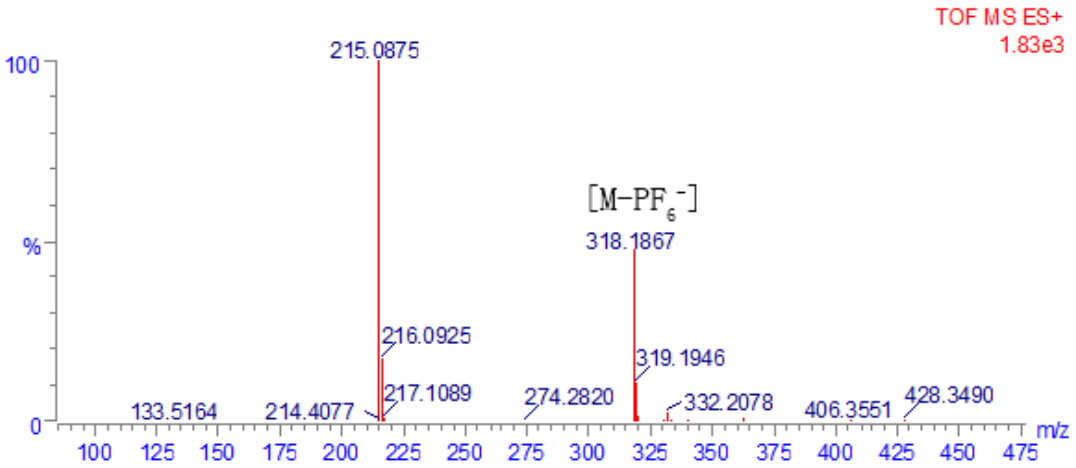

Figure S5. HRMS spectrum of the positive ion of compound 2

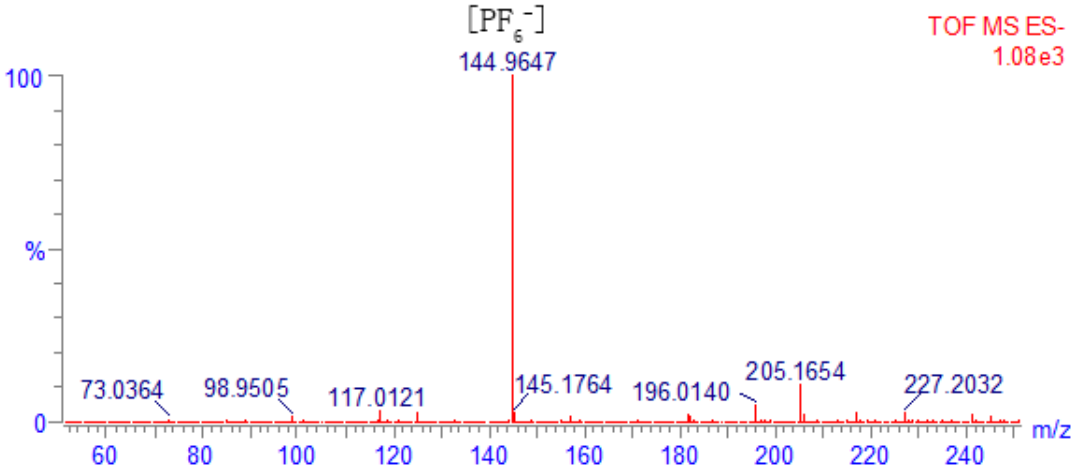

Figure S6. HRMS spectrum of the negative ion of compound 2 (PF_6^-)

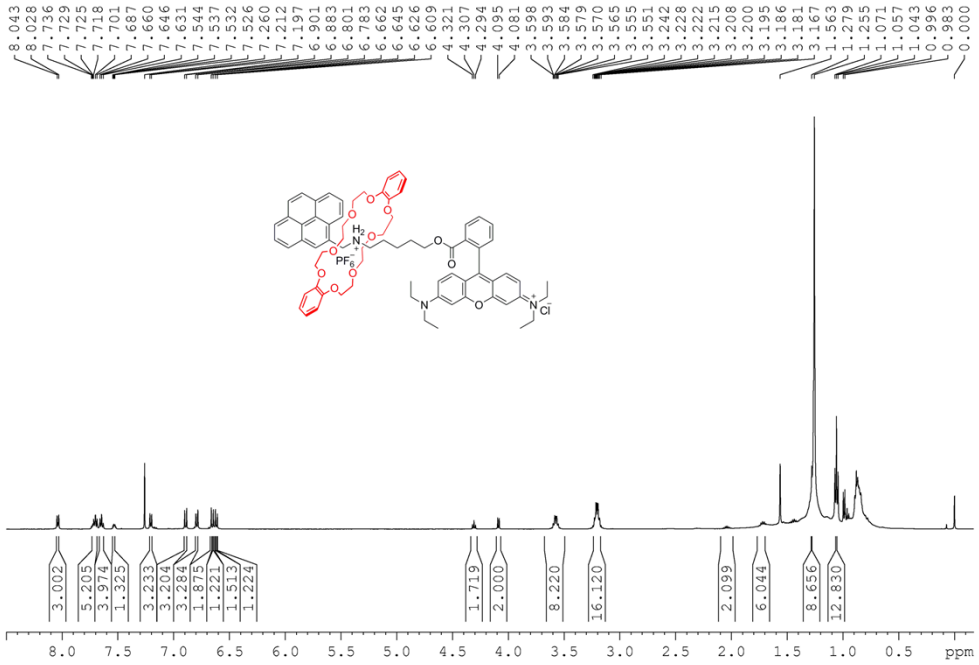

Figure S7. ^{1}H NMR spectrum of the RhBPy [2] rptaxane in $CDCl_3$.

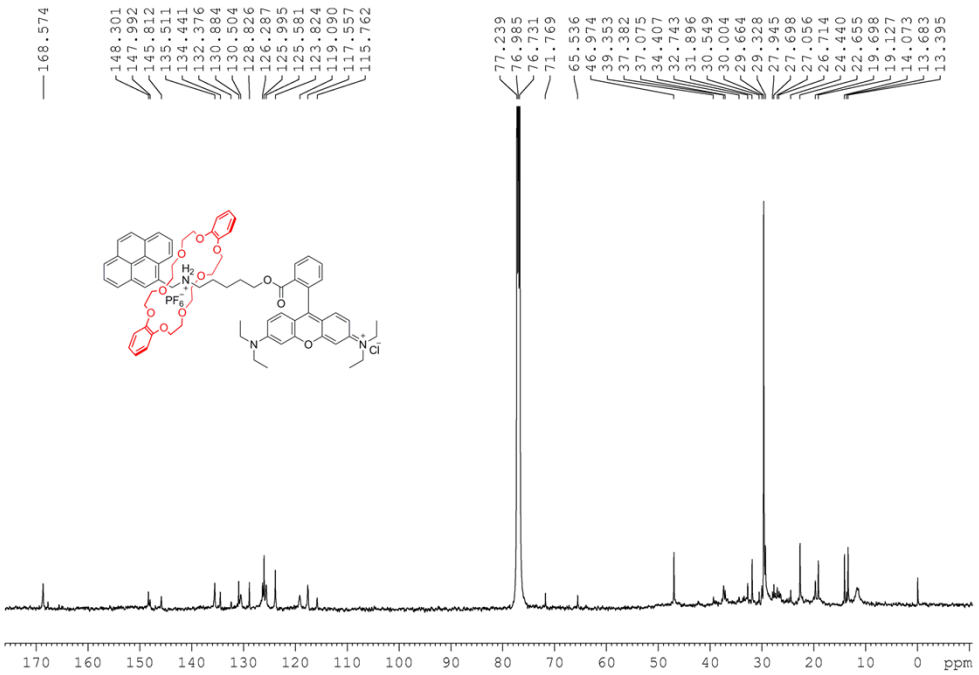

Figure S8. ^{13}C NMR spectrum of the RhBPy [2] rotaxane in $CDCl_3$.

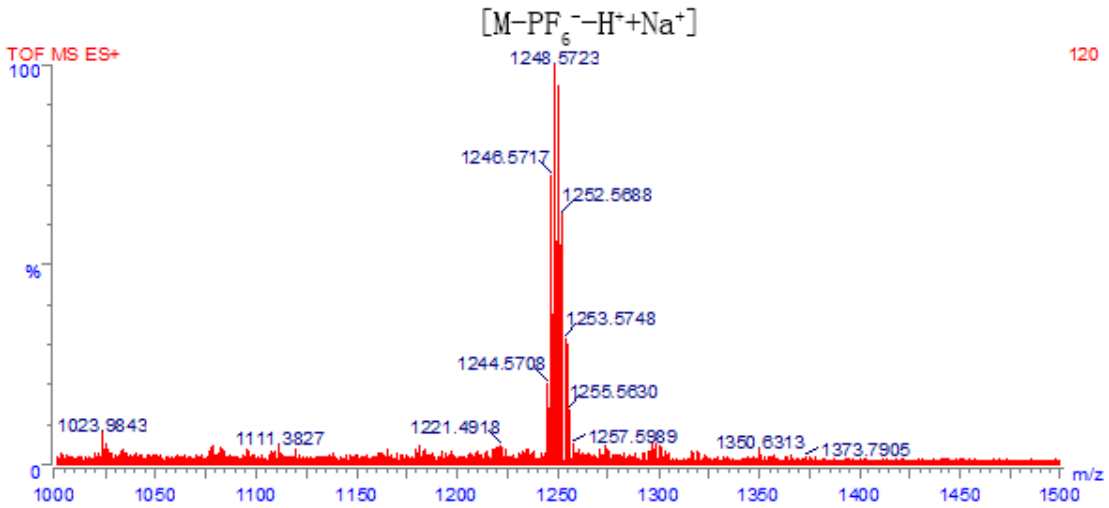

Figure S9. HRMS spectrum of the RhBPy [2] rotaxane.

The calculation of Kinetic and thermodynamic parameters

It is important to establish the rate of the movement of the macrocycle, and this can be obtained using the coalescence methods. This method was used, initially, to the equally populated two-site system formed by uncoupled nuclei and undergoing exchange with a rate constant ($k_1=k_{-1}$). The academics also proved that it was much greater than the line width in the absence of exchange by the chemical shift difference ($\Delta\nu$). So, there is a simple relationship between the observed separation of the peaks ($\Delta\nu_e$) and the rate constant for the system below and up to coalescence.

$$k = \frac{\pi}{\sqrt{2}} (\Delta\nu^2 - \Delta\nu_e^2)^{1/2} \qquad \text{eq 1}$$

We could apply the equation 1 to extract the rate constants, but it can not get accurate results due to the insensitive changes in k. Moreover, it is difficult to determine the accurate values of $\Delta\nu$ because the peaks of H_{10} and $H_{10'}$ overlap in the range 278K to 298K. As a consequence, the equation 1 can further simplify to a much simpler expression (equation 2) at coalescence ($\Delta\nu_e=0$).

$$k_1 = k_{-1} = k = \frac{\pi\Delta\nu}{\sqrt{2}} = \frac{\pi(\nu_A - \nu_B)}{\sqrt{2}} \qquad \text{eq 2}$$

The Δν of the equation 2 is the frequency difference between the split peaks obtained by the NMR spectroscopy measured at low temperature. The k is the rate constant of the move process of the macrocycle.

To further investigate the thermodynamic property of the move process, we also calculated the free energy of activation ΔG^{θ} by the equation 3

$$\Delta G^{\Theta} = -RT_c \ln\left(\frac{kh}{T_c k_B}\right) \qquad \text{eq 3}$$

Where h is the Planck constant, R is the gas constant, k_B is the Boltzmann constant, Tc is the coalescence temperature, and the k is the rate constant of the move of the cycle.

Based on the above speculative knowledge, the kinetic parameters which calculated by the coalescence temperature of the ^{1}H NMR spectra for the dynamic process can be obtained in **Table S1**. The rate constant (k) move behavior of the DB24C8 between two bulky stoppers been calculated on the basis of the equation 2 is 333.22 s^{-1}. Moreover, the free energy of activation (ΔG^{θ}= 58.60 kJ/mol) was also calculated at the coalescence temperature (Tc=298K) by the coupling constants of the H_{10}. Above all of these data, we draw a conclusion that the ring (DB24C8), driven by the entropy at more high temperature, was moving through the axis between the two bulky stoppers.

Table S1 Kinetic and thermodynamic parameters for the move behavior of the macrocyclic ring between the two bulky stoppers in the [2] rotaxane by using the

coalescence method

Proton H	Tc (K)	Δν (Hz)	k (s^{-1})	ΔG^{θ}(kJ/mol)
H_{10}	298	150	333.22	58.60

Turning then to the understanding of the thermodynamic process, the entropy and enthalpy of the migration of the DB24C8 ring were also investigated. Therefore, the experiments of the ^{1}H NMR spectroscopy have to be measured above coalescence temperature (Tc). Since the speed of movement become faster and faster at T > Tc, it made the signal of proton of the relevant NMR lines wide. So the formula between the rate constant and difference of chemical shift is given by the equation 4. Therefore, information on rate constant can only be obtained from the half line width according to this formula.

$$\Delta v_{1/2} = \frac{\pi(v_A - v_B)}{2} k^{-1} = \frac{\pi \Delta v}{2} k^{-1} \qquad \text{eq 4}$$

Where $\Delta v_{1/2}$ is the half line width of the ^{1}H NMR of the signal for proton (H_{10}) at T > Tc, k is the rate constant, Δv values observed in Table 1.

The entropy and enthalpy of the migration of the DB24C8 ring can be investigated by means of the Eyring-Polanyi equation (eq 5) after calculating the rate constants at various temperatures.

$$\ln \frac{k}{T} = \frac{\Delta S}{R} + \ln \frac{k_B}{h} - \frac{\Delta H}{R} \bullet \frac{1}{T} \qquad \text{eq 5}$$

Where kB is the Boltzman constant; h is the Planck constant; R is the gas constant; ΔH andΔS is the enthalpy and entropy in the process, respectively.

Table S2 listed the estimated rates constant of the shutting ring using the

observed Δv and $\Delta v_{1/2}$ obtained at different temperature above 288K. The rate constants increase as the temperature is increased. Moreover, it was also noted that the values of ΔH and ΔS are unchangeable under a small range of the temperature. As a result, the values for ΔH=24.93 kJ/mol and ΔS=-112.64 J/(mol•K) were calculated on the basis of the Eyring-polanyi equation (eq 5).

Table S2. $\Delta v_{1/2}$ and k estimated according to the Eyring-polanyi equation for H_{10} protons at various temperatures

Temperature (K)	$\Delta v_{1/2}$	k (s^{-1})	ΔH (kJ/mol)	ΔS (J/(mol•K))
298	98	356.99[a]	24.93	-112.64
308	70	504.90		
318	51	693.00		

[a] Errors exist between the values of the rate constant (k) at 298K in **Table 1** and **Table 2**. So, average value was taken in the process of calculation of the ΔH and ΔS.

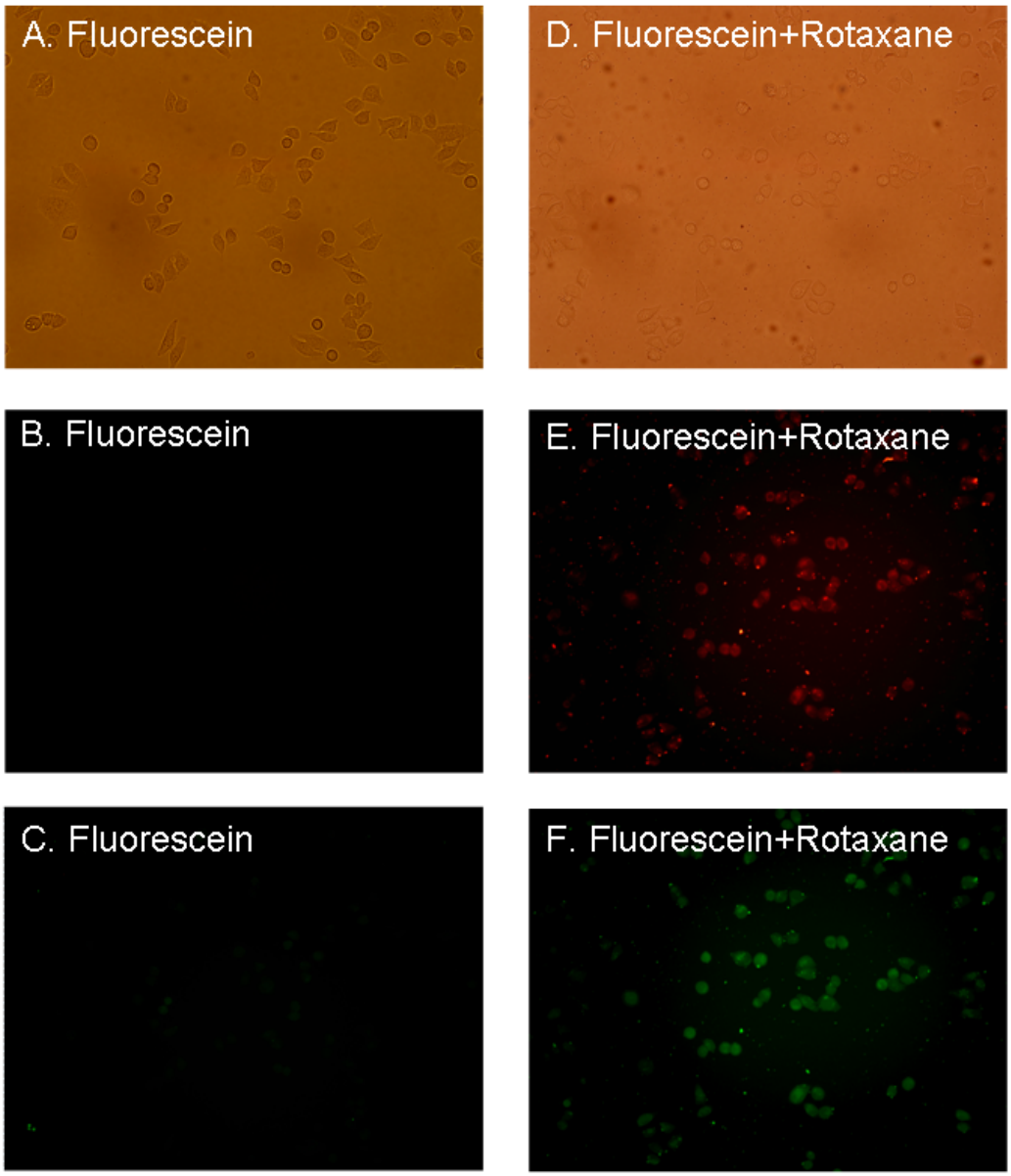

Figure S10. Fluorescence microscopy of HeLa cells exposed to fluorescein and the **RhBPy** [2]rotaxane(Rotaxane). The cells were incubated with fluorescein (20 μM) for 2 h or preincubated with the **RhBPy** [2]rotaxane (5 μM) for 1 h followed by exposure to fluorescein (20 μM) for an additional 2 h. Representative pictures are displayed (A, D: white light; B, E: fluorescence of **RhBPy** [2]rotaxane; C, F: fluorescein emission).

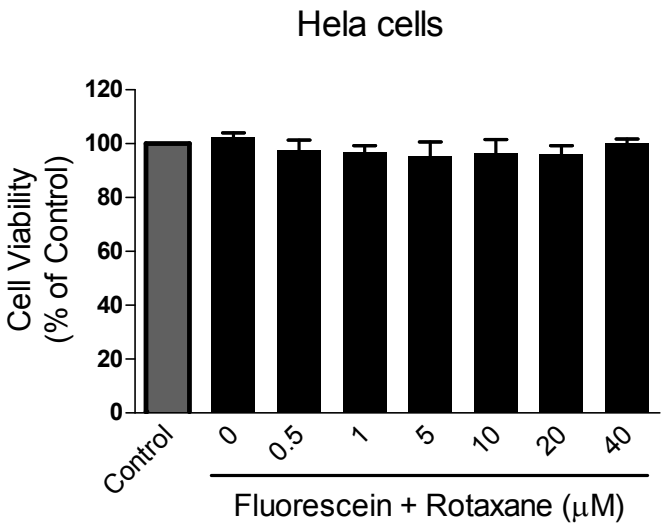

Figure S11. Cytotoxicity of the Fluorescein and **RhBPy** [2]rotaxane in HeLa cells**.** The cells were incubated with 20 μM Fluorescein with different concentrations of the **RhBPy** [2]rotaxane(Rotaxane) for 24 h, and cell viability was determined by an MTT assay. Data are expressed as means ± SD.

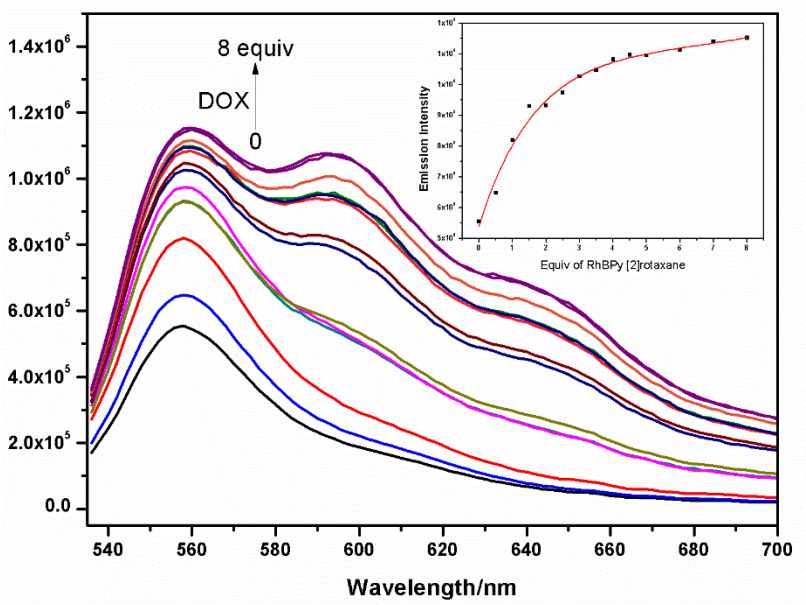

Figure S12. the fluorescence spectra of the RhBPy [2]rotaxane (10 μM) with different concentrations of DOX in buffered water solution (1 mM, pH 7.0). Inset: plot of the fluorescence intensity versus the equivalent of DOX.

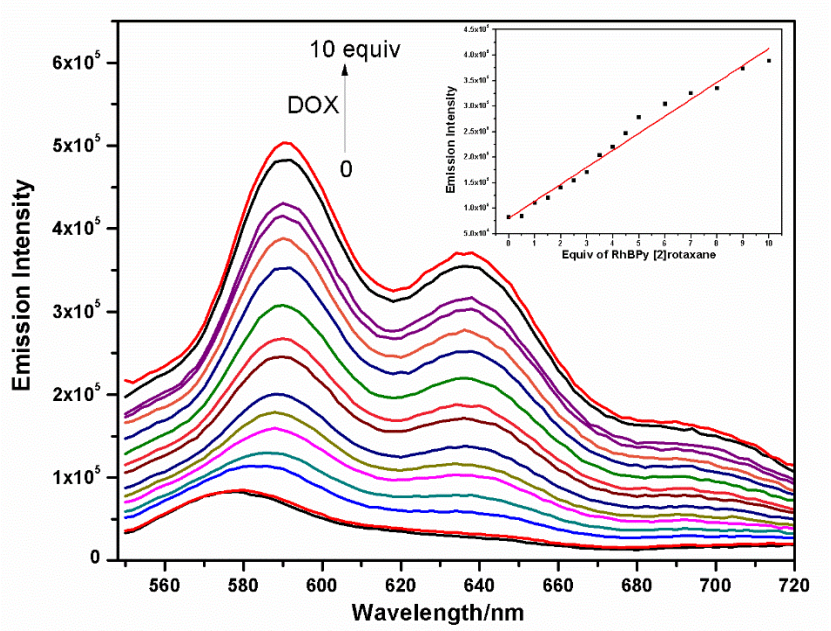

Figure S13 the fluorescence spectra of the RhBPy [2]rotaxane (10 μM) with different concentrations of DOX in CH_3CN. Inset: plot of the fluorescence intensity versus the equivalent of DOX.