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Supporting Information

Poly (2-Oxazoline)-Based Core Cross-Linked Star Polymers: Synthesis and Drug Delivery Applications

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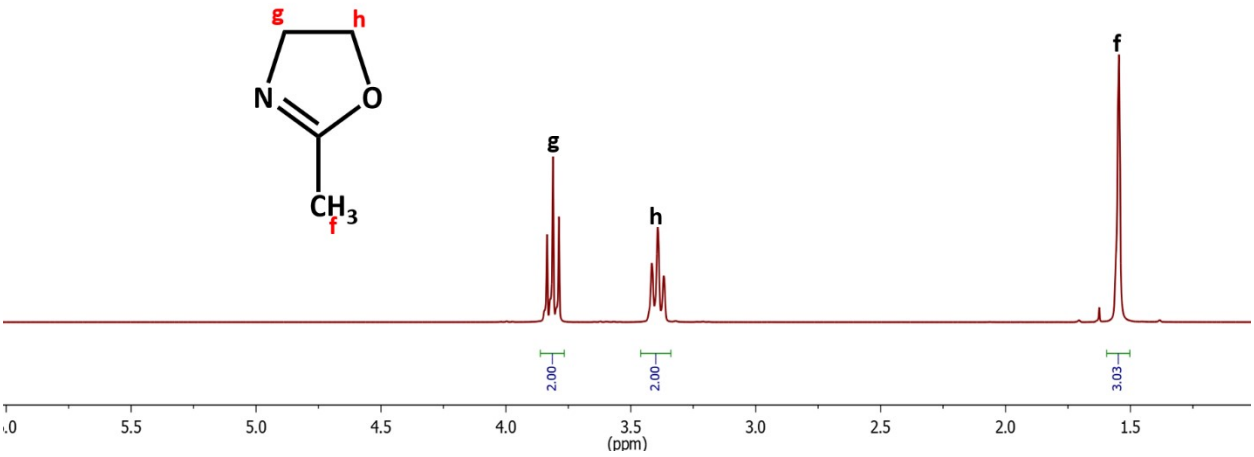


Figure S1. ^{1}H NMR of the MeOx monomer (500 MHz, $CDCl_3$, 25 °C).

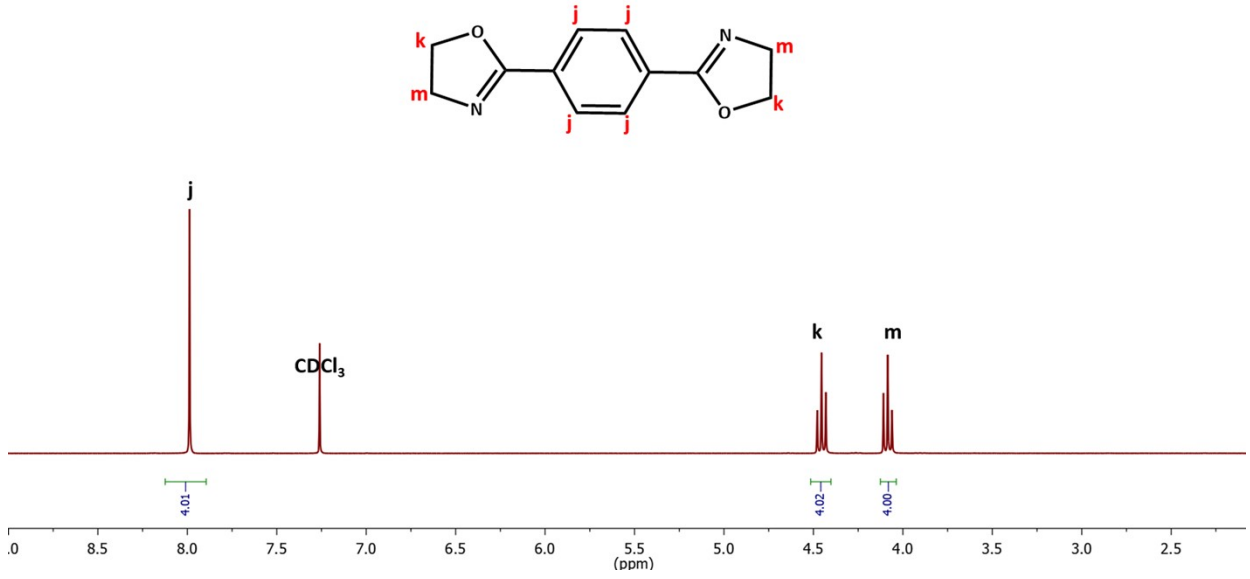


Figure S2. ^{1}H NMR of the PhBisOx monomer (500 MHz, $CDCl_3$, 25 °C).

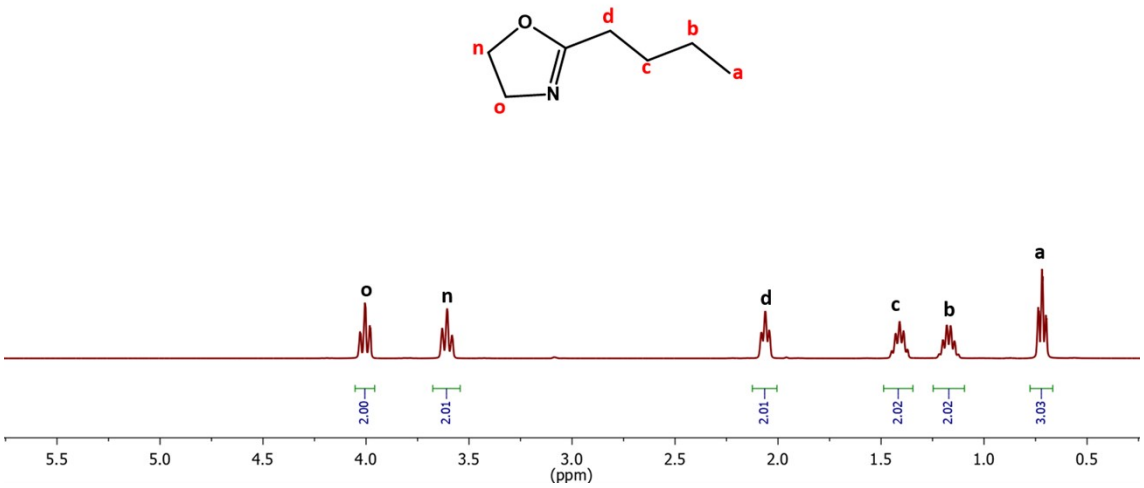


Figure S3. ^{1}H NMR of the ButOx monomer (500 MHz, $CDCl_3$, 25 °C).

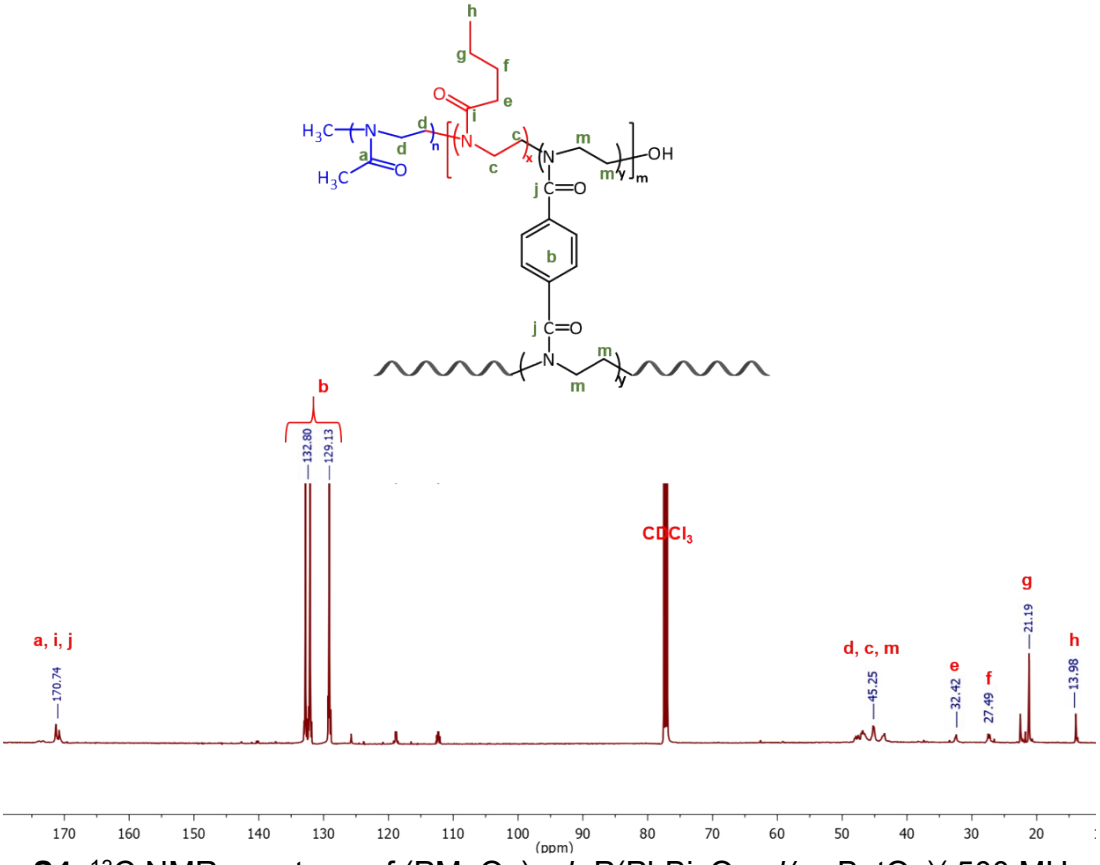


Figure S4. ^{13}C NMR spectrum of $(PMeOx)_{59}$-*b*-P(PhBisOx-*cl/co*-ButOx)(500 MHz, $CDCl_3$, 25 °C).

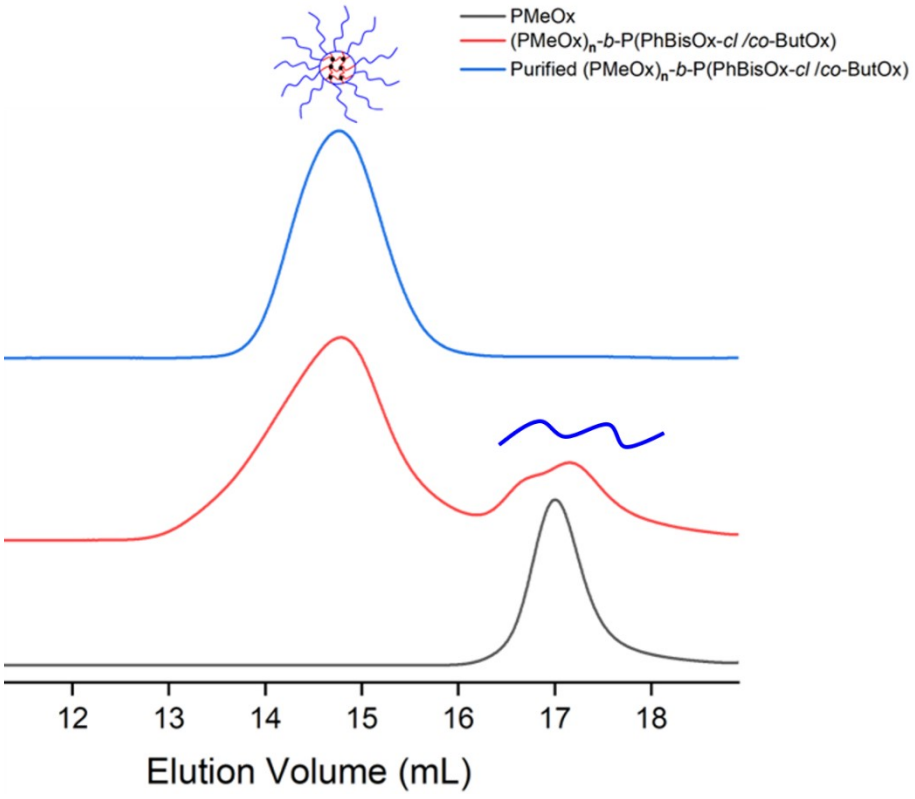


Figure S5. SEC traces for (a) PMeOx (black), unpurified $(PMeOx)_{106}$-*b*-P(PhBisOx-*cl/co*-ButOx)(red), and purified $(PMeOx)_{106}$-*b*-P(PhBisOx-*cl/co*-ButOx)(blue), (DMF, 45 °C, PS standard).

Equation S1. The precursor of the arm percentage.

The relative precursor percentage of the PMeOx arm was calculated from the SEC chromatogram integration method by using the following equation:

$$precursor\ arm\ \% = \frac{peak\ area\ of\ unreacted\ arm}{peak\ area\ of\ unreacted\ arm\ + peak\ area\ of\ star\ polymer} \times 100$$

Equation S2. Calculation of Number of Arms in CCS POxs

The average number of arms in the CCS POxs was calculated based on SEC, MDS-SEC, and ^{1}H NMR analysis of precursor polymers and star polymers. The number of PMeOx arms in CCS $(PMeOx)_n$-*b*-P(PhBisOx-*cl/co*-ButOx) was estimated by molecular weights of CCS polymer and PMeOx. The purified star polymer was analyzed

using (DA-SEC) in DMF to determine the absolute molecular weight ($M_{w,\,star}$). The average number of arms was calculated using the following equation:

$$N_{arm} = \frac{(M_{w,\,star} \times arm_{wf})}{M_{n,\,arm,NMR}}$$

Where, N_{arm}, $M_{w,\,star}$, arm $_{wf\,\%}$, and $M_{n,\,arm,\,NMR}$ represent the number of arms in CCS polymer, weight averaged molecular weight of CCS polymer measured by MDS-SEC, the weight fraction of PMeOx arms in CCS polymer, and weight averaged molecular weight of PMeOx arms $M_{n,\,arm,\,NMR}$ calculated from NMR, respectively. The weight fraction of PMeOx was calculated as follows:

$$arm_{wf} = \frac{W(PMeOx) \times yield\ of\ CCS\ polymer}{[W(PMeOx) \times yield\ of\ CCS\ polymer + W(PhBisOx) + W\ (ButOx)]}$$

Where, W(PMeOx), W(PhBisOx), and W(ButOx) are the weights of PMeOx, PhBisOx, and ButOx in the reaction solution.

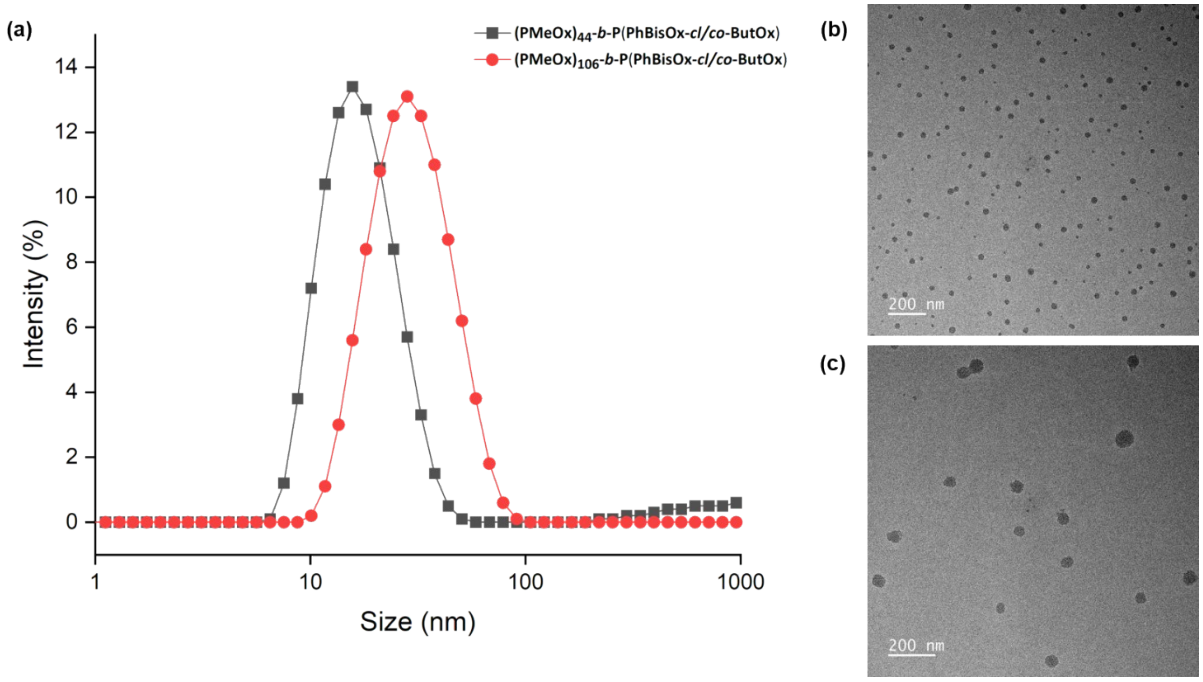


Figure S6. (a) DLS traces of $(PMeOx)_{44}$-*b*-P(PhBisOx-*cl/co*-ButOx) (black) and $(PMeOx)_{106}$-*b*-P(PhBisOx-*cl/co*-ButOx)(red) in water (concentration: 2 mg/mL) and TEM images (b) $PMeOx_{44}$-*b*-P(PhBisOx-*cl/co*-ButOx) and (c) $(PMeOx)_{106}$-*b*-P(PhBisOx-*cl/co*-ButOx).

Equation S3. Preparation of calibration Curve.

A series of free DOX solutions in water with known concentrations were used to prepare the calibration curve (Figure S7). The DOX concentration was calculated by the following equation:

$$Y = 0.0028\ X + 0.0172$$

Where X is the DOX concentration, and Y is the absorbance from UV-Vis.

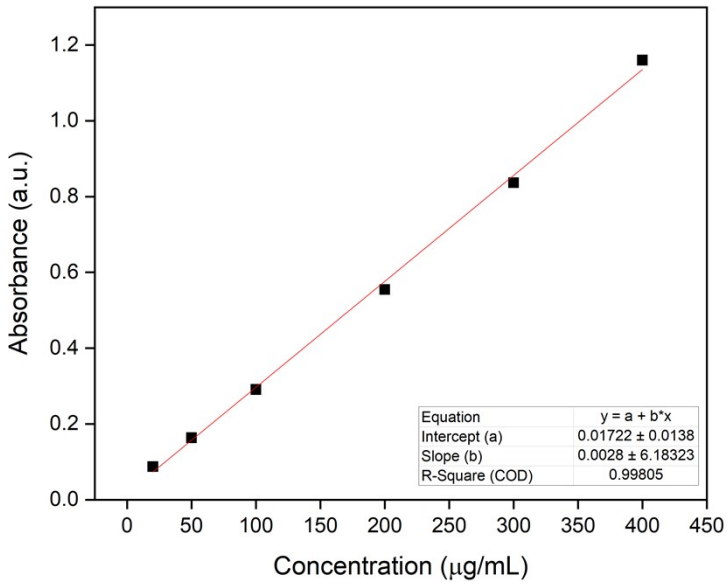
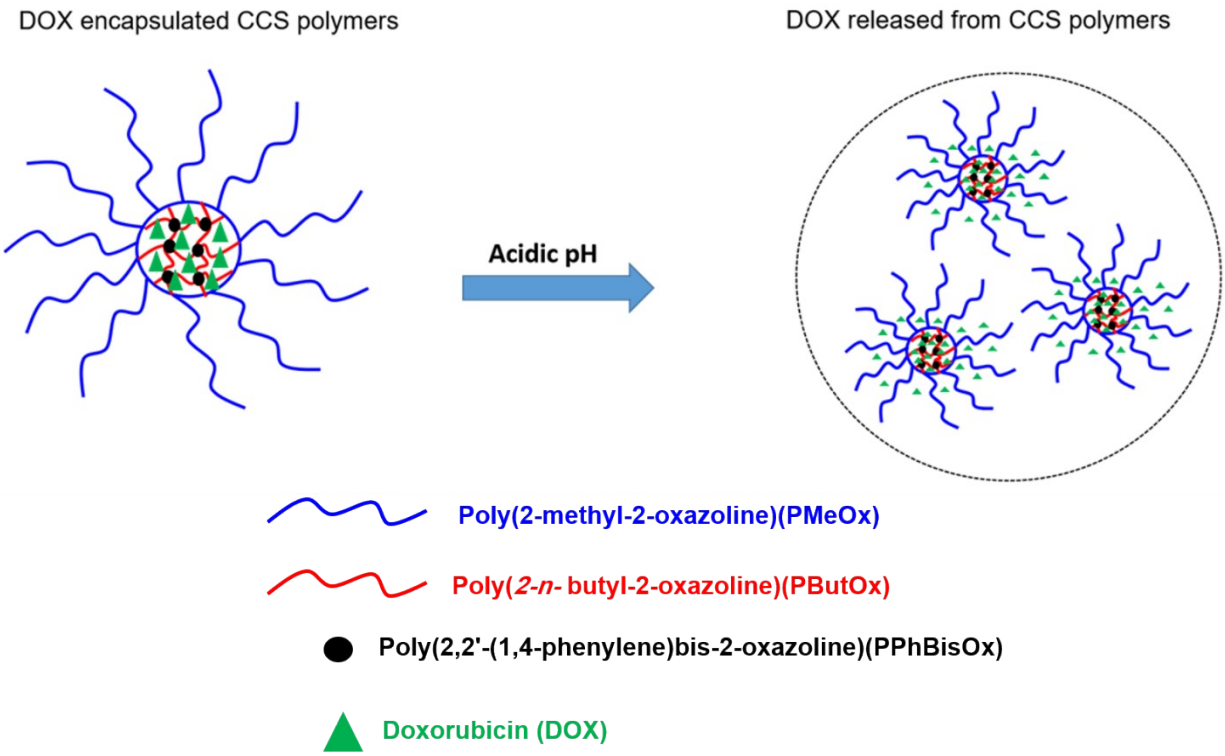


Figure S7. The standard calibration curve for free DOX with different concentrations in water.

***In vitro* DOX release from CCS polymer.**



Scheme S1. Schematic of *in vitro* DOX release from CCS POxs at acidic pH.

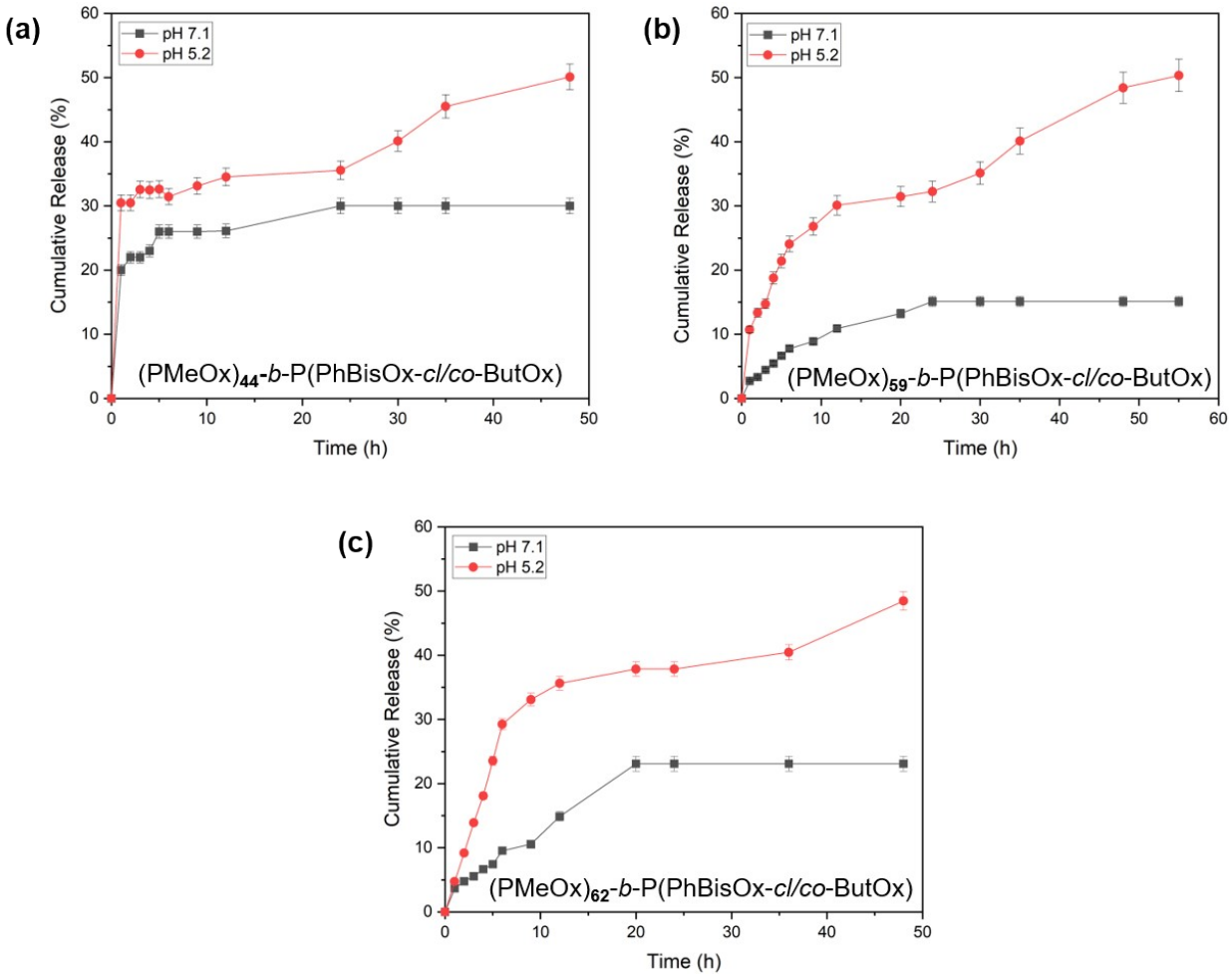


Figure S8. *In vitro* DOX release profile from CCS POxs (a) $(PMeOx)_{44}$-*b*-P(PhBisOx-*cl/co*-ButOx), (b) $(PMeOx)_{59}$-*b*-P(PhBisOx-*cl/co*-ButOx), and (c) $(PMeOx)_{62}$-*b*-P(PhBisOx-*cl/co*-ButOx) in PBS at different pH values at 37°C. The results are presented as average data with SD (n= 2).

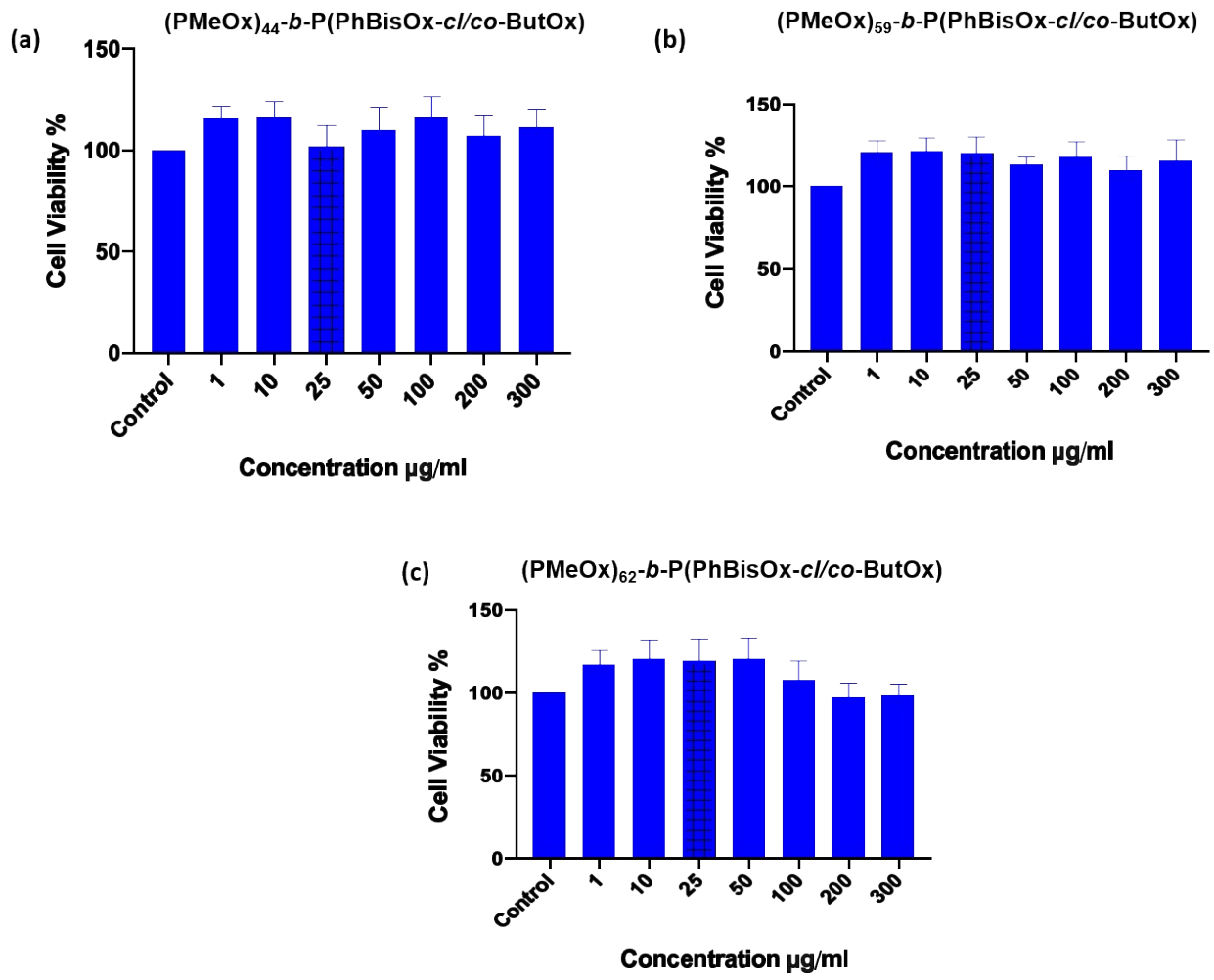

(a)
(PMeOx)44-b-P(PhBisOx-cl/co-ButOx)
Cell Viability %
150
100
50
0
Control
1
10
25
50
100
200
300
Concentration µg/ml
(b)
(PMeOx)59-b-P(PhBisOx-cl/co-ButOx)
Cell Viability %
150
100
50
0
Control
1
10
25
50
100
200
300
Concentration µg/ml
(c)
(PMeOx)62-b-P(PhBisOx-cl/co-ButOx)
Cell Viability %
150
100
50
0
Control
1
10
25
50
100
200
300
Concentration µg/ml

Figure S9. Cell viability of HeLa cells with different concentrations of a series of CCS POxs for 24 h. Error bars are based on SD (n=2).

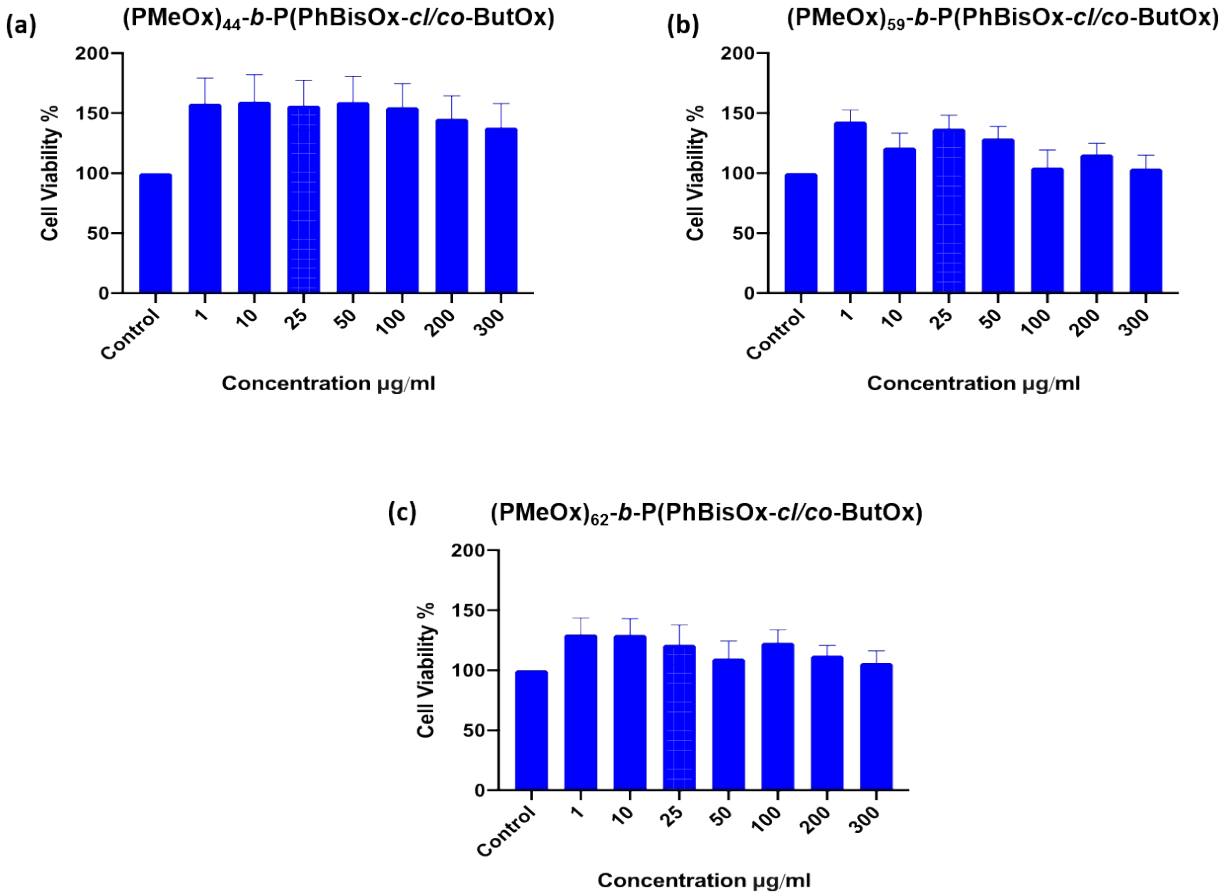

(a)
(PMeOx)44-b-P(PhBisOx-cl/co-ButOx)
Cell Viability %
200
150
100
50
0
Control
1
10
25
50
100
200
300
Concentration µg/ml
(b)
(PMeOx)59-b-P(PhBisOx-cl/co-ButOx)
Cell Viability %
200
150
100
50
0
Control
1
10
25
50
100
200
300
Concentration µg/ml
(c)
(PMeOx)62-b-P(PhBisOx-cl/co-ButOx)
Cell Viability %
200
150
100
50
0
Control
1
10
25
50
100
200
300
Concentration µg/ml

Figure S10. Cell viability of HeLa cells with different concentrations of a series of CCS POxs for 48 h. Error bars are based on SD (n=2).

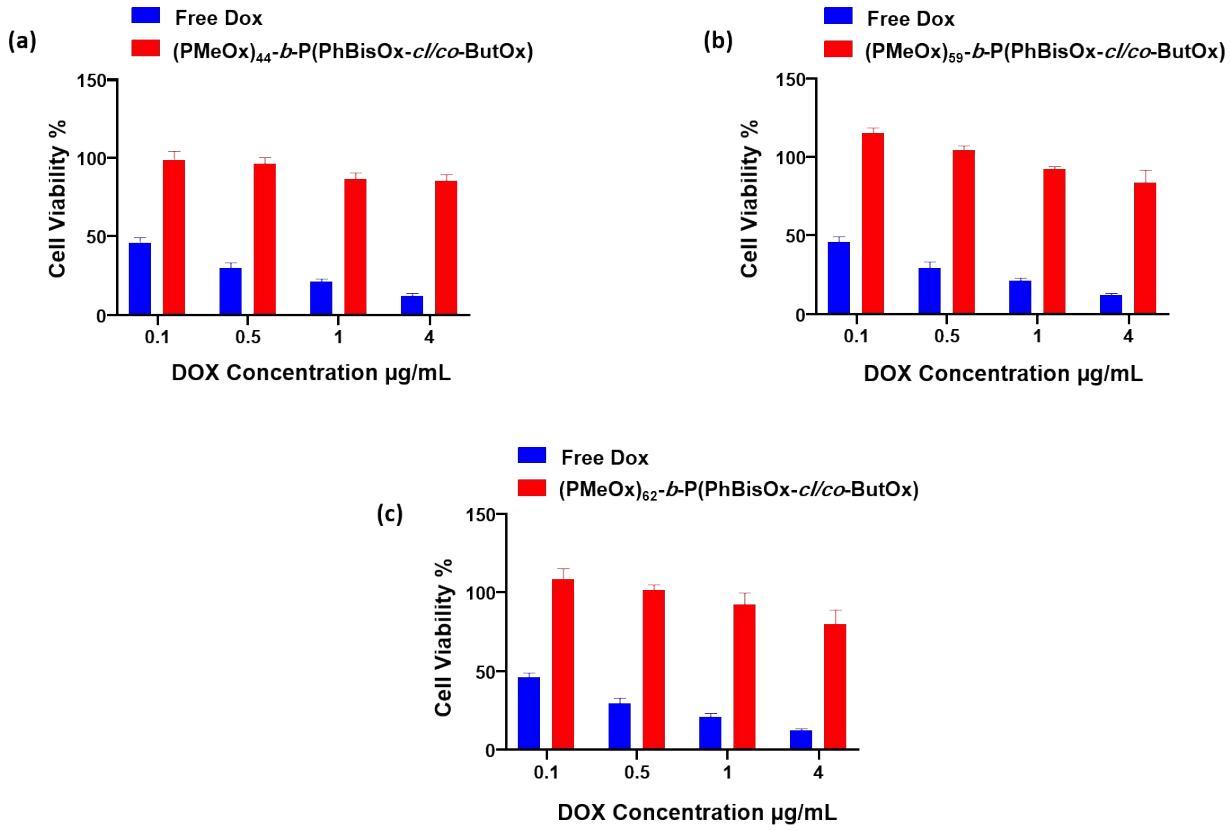


Figure S11. Cytotoxicity of DOX-loaded series of CCS POxs and free DOX in Hela cells for 24 h. (a) $(PMeOx)_{44}$-*b*-P(PhBisOx-*cl/co*-ButOx), (b) $(PMeOx)_{59}$-*b*-P(PhBisOx-*cl/co*-ButOx), and (c) $(PMeOx)_{62}$-*b*-P(PhBisOx-*cl/co*-ButOx). Error bars are based on SD (n=2).

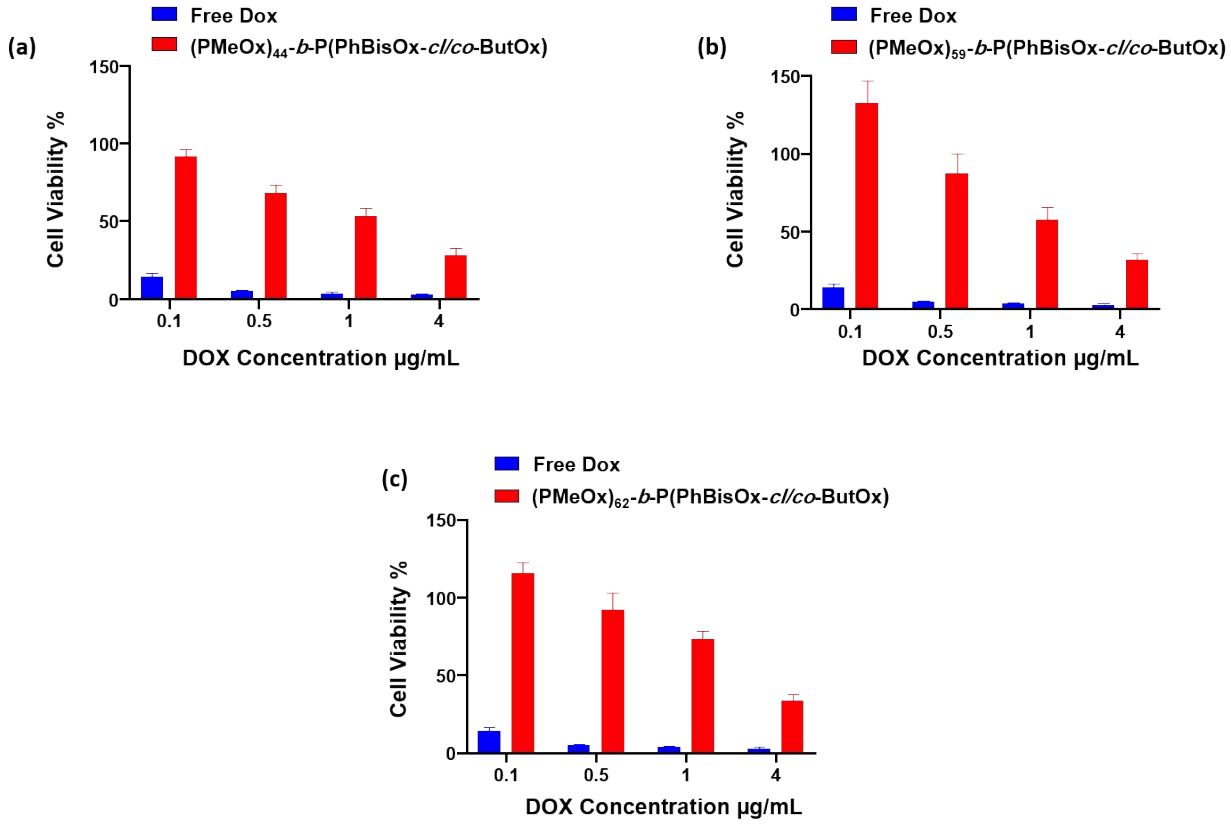

(a)
Free Dox
(PMeOx)44-b-P(PhBisOx-cl/co-ButOx)
Cell Viability %
150
100
50
0
0.1
0.5
1
4
DOX Concentration µg/mL
(b)
Free Dox
(PMeOx)59-b-P(PhBisOx-cl/co-ButOx)
Cell Viability %
150
100
50
0
0.1
0.5
1
4
DOX Concentration µg/mL
(c)
Free Dox
(PMeOx)62-b-P(PhBisOx-cl/co-ButOx)
Cell Viability %
150
100
50
0
0.1
0.5
1
4
DOX Concentration µg/mL

Figure S12. Cytotoxicity of DOX-loaded a series of CCS POxs and free DOX in Hela cells for 48 h. (a) $(PMeOx)_{44}$-*b*-P(PhBisOx-*cl/co*-ButOx), (b) $(PMeOx)_{59}$-*b*-P(PhBisOx-*cl/co*-ButOx), and (c) $(PMeOx)_{62}$-*b*-P(PhBisOx-*cl/co*-ButOx). Error bars are based on SD (n=2).