**Electronic Supplementary Information (ESI) for Polymer Chemistry**



# Doxorubicin Conjugated, Crosslinked, PEGylated Particles Prepared via One-Pot Thiol-Ene Modification of a Homopolymer Scaffold: Synthesis and In vitro Evaluation

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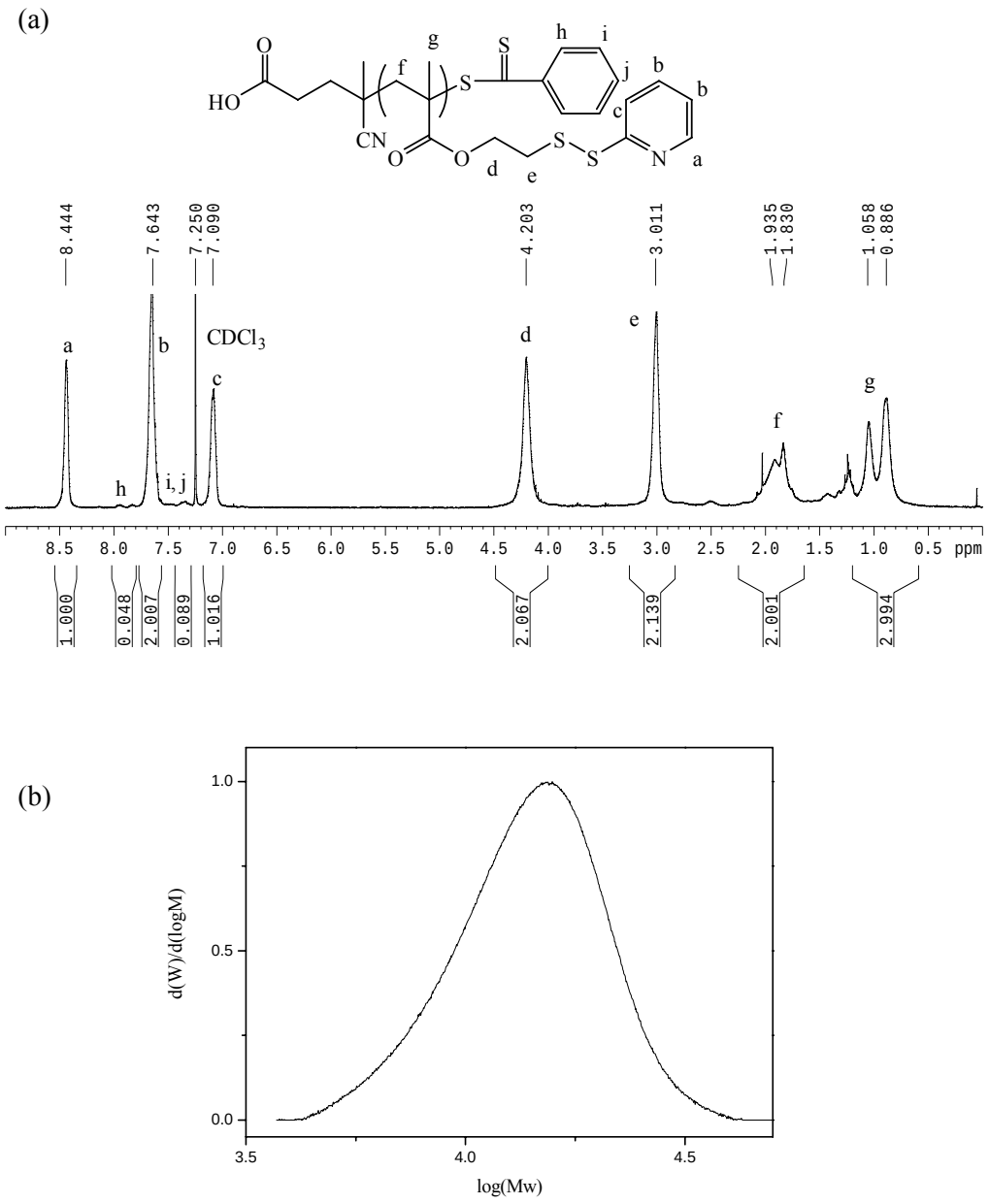
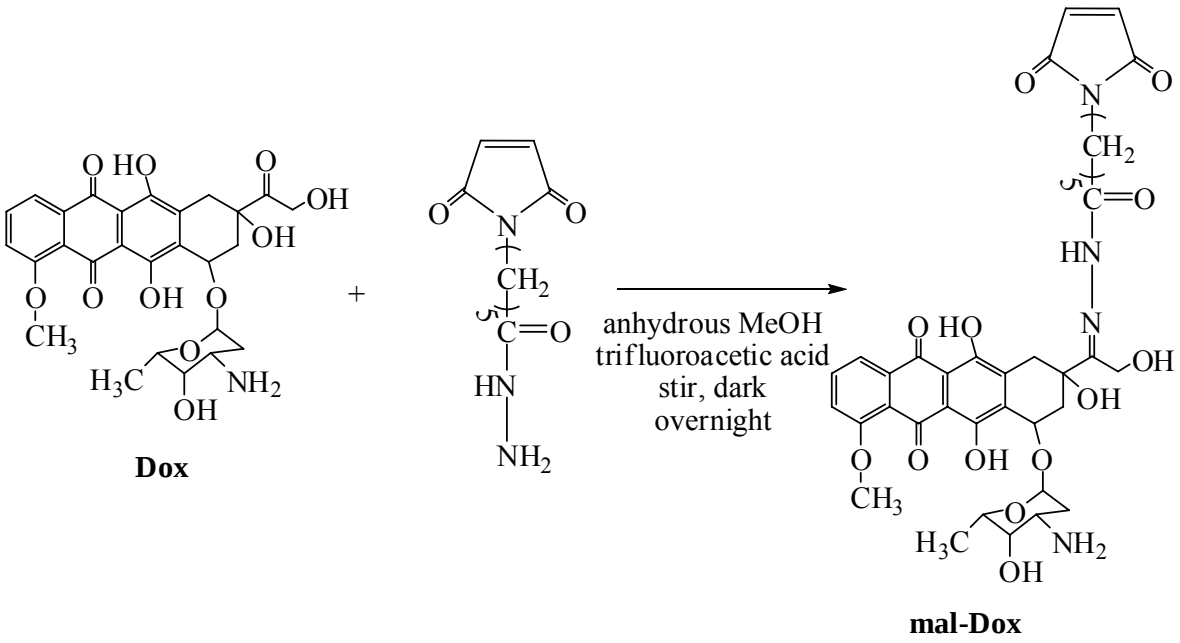



**Figure S-1:** (a) $^{1}$H NMR spectrum and (b) GPC chromatogram of the RAFT synthesized poly(pyridyldisulfide ethylmethacrylate) (PPDSM) used in this study. The mobile phase for GPC: dimethylacetamide (DMAc). The RAFT polymerization was performed at 70$^{o}$C, at a [PDSM]: [RAFT]: [Initiator] feed ratio of 0.4 M: 8.0 mM: 1.6 mM at a monomer conversion of 60mol% (by NMR). PPDSM has a number average molecular weight ($M_n$) of 8,900 g/mol and PDI of 1.18 (by GPC). Chemical shifts ($\delta$ = 8.4 (1H), 7.6 (2H) and 7.1 (1H) ppm) and the methyl/methylene protons of the polymer backbone ($\delta$ = 4.2 (2H), 3.0 (2H), 2.0-1.7 (2H), 1.1-0.7 (3H) ppm).




**Dox**

**mal-Dox**

**Scheme S-1:** Synthesis of maleimide-Doxorubicin (mal-Dox) using EMCH crosslinker as reported in previous publications. (*1-2*)

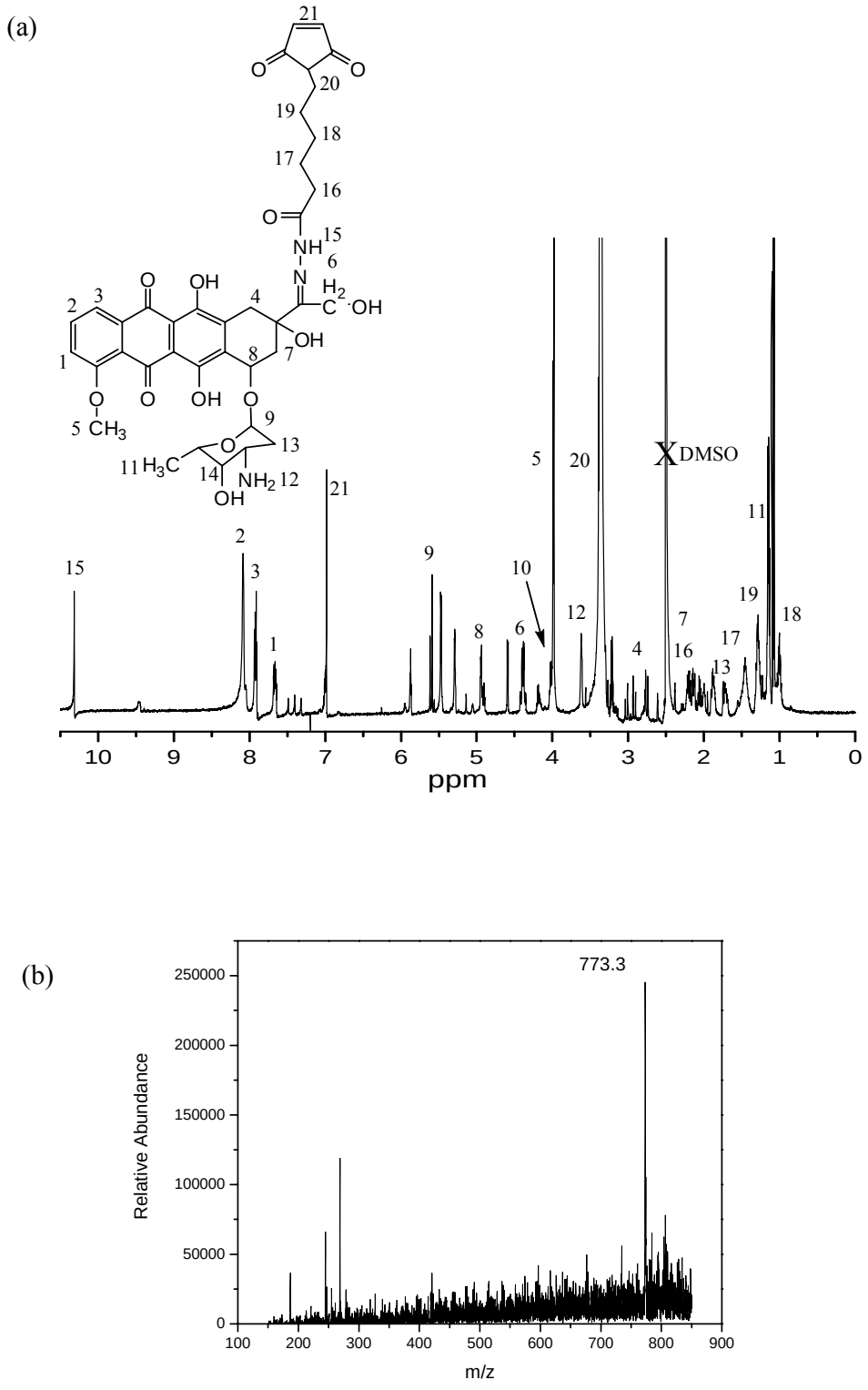



**Figure S-2:** (a) $^{1}$H-NMR spectrum of maleimide-modified Dox (mal-Dox) in DMSO-$d_6$. (b) ESI-MS spectrum of mal-Dox with the exact molecular mass of 773.3 Da (the sample was prepared in HPLC-grade methanol at a concentration of 1 mg/ml).

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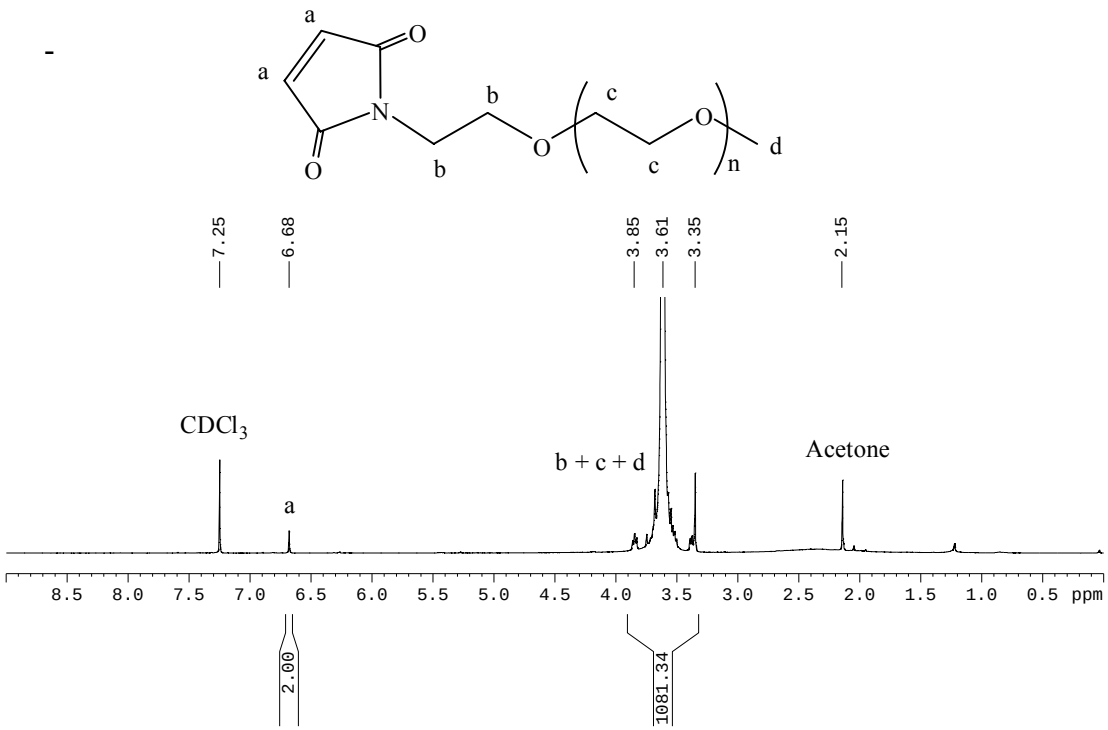
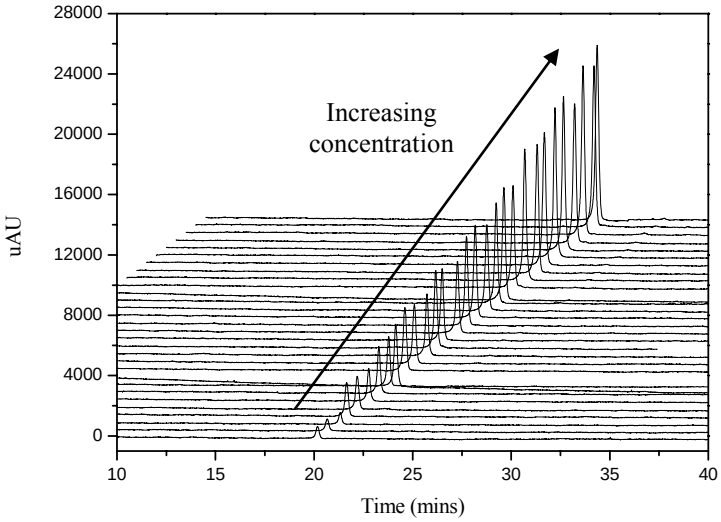



**Figure S-3:** $^{1}$H-NMR spectrum (in $CDCl_3$) of mal-PEG ($M_n$ = 5000 g/mol) (repeating units of 110) used in the study. The purity of maleimide modified PEG is 41 mol%.

(a)




(b)

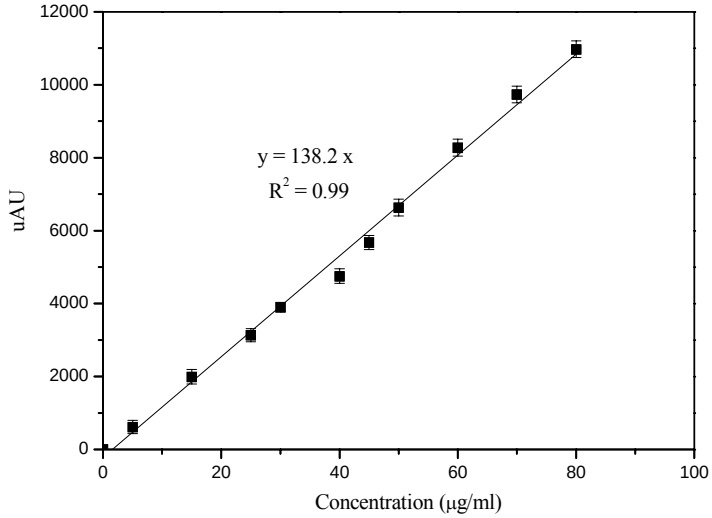



**Figure S-4:** (a) Representative HPLC traces of standard Dox solutions at increasing concentrations (5, 15, 30, 35 40, 60, 70 and 80 μg/mL) in HPLC-grade acetonitrile (in triplicate measurements). (b) The calibration curve built by HPLC using standard Dox solutions: UV-absorption at 490 nm versus concentration in μg/ml. The HPLC analysis was performed using 150 mm x 4.60 mm Phenomenex (R) column packed with 5 μm of IB-SIL C-8 beads. The column was eluted with the gradient mixture of HPLC-grade acetonitrile and milli-Q water at a flow rate of 1 mL/min.

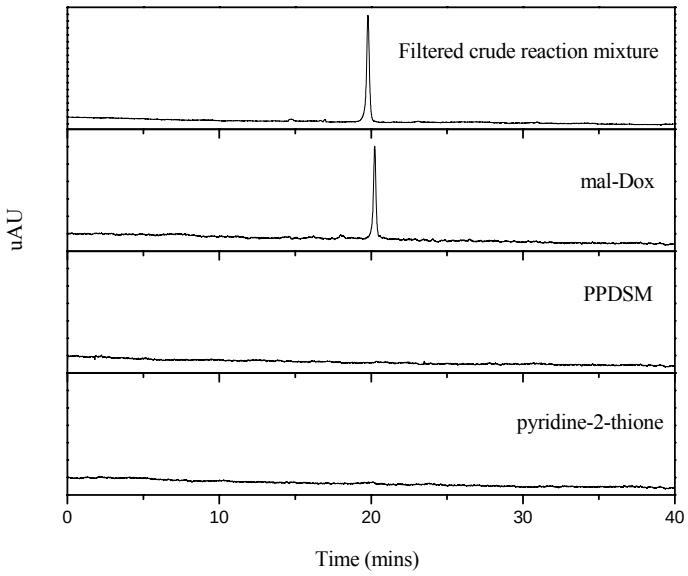



**Figure S-5:** HPLC traces of (a) filtered crude reaction mixture, (b) pure mal-Dox solution, (c) pure PPDSM solution, and (d) pure pyridine-2-thione solution. HPLC equipped with a UV-Visible photodiode array detector set at a wavelength of 490 nm was used for measurement. The HPLC analysis was performed using 150 mm x 4.60 mm Phenomenex (R) column packed with 5 μm of IB-SIL C-8 beads.

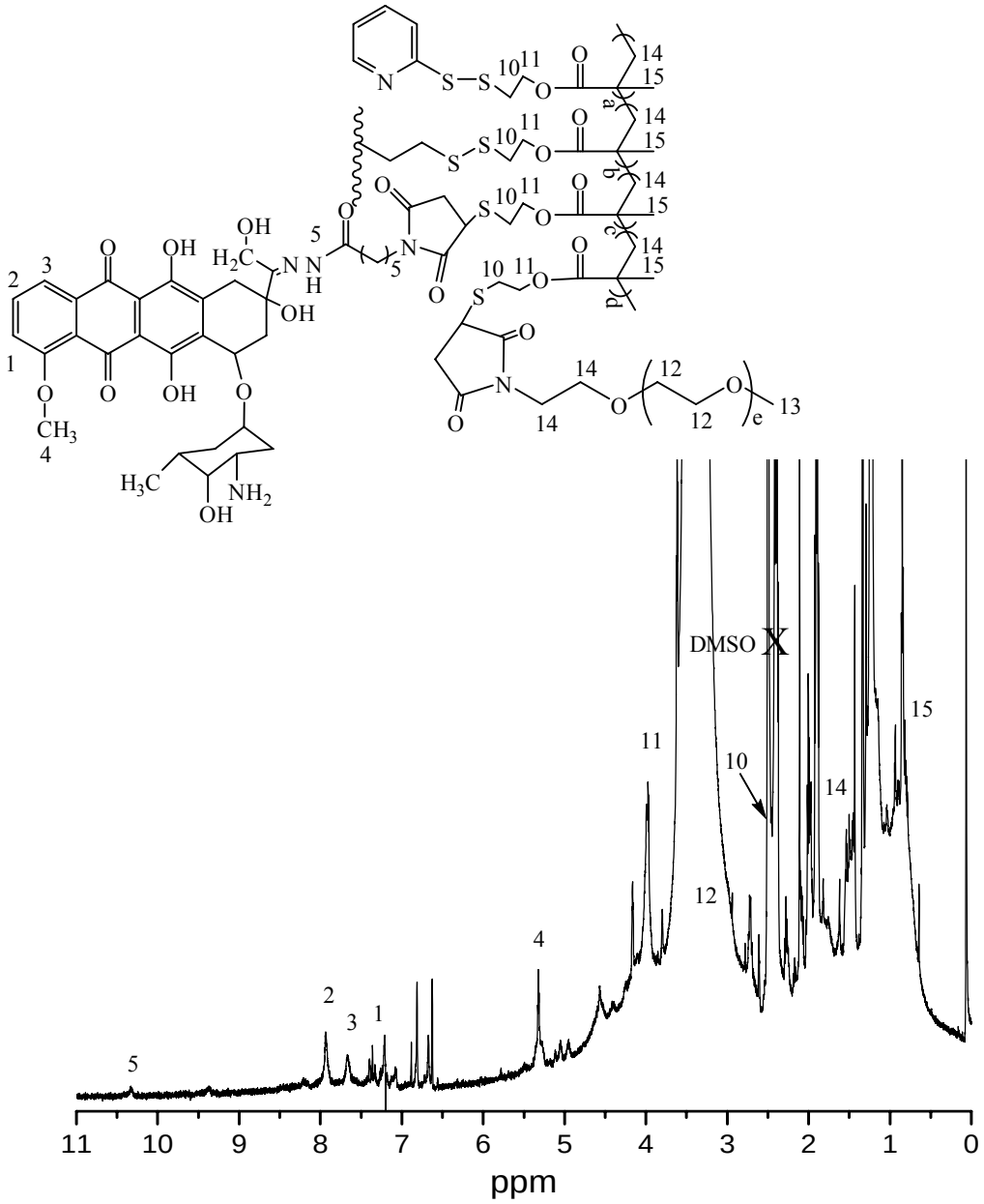
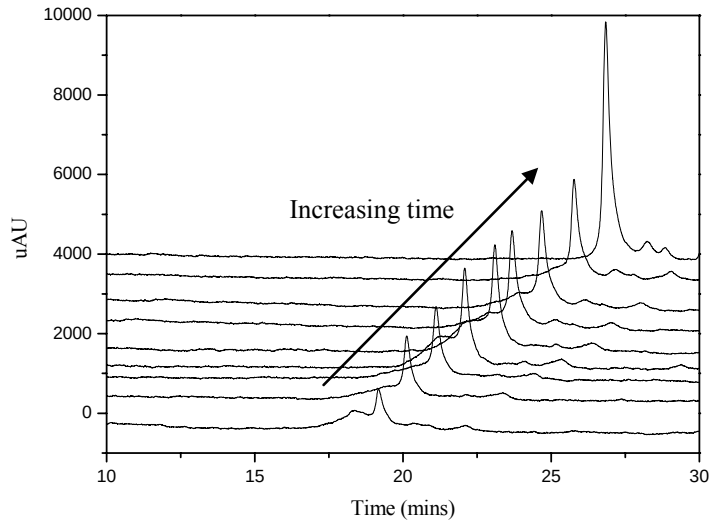



**Figure S-6:** $^{1}$H NMR spectrum of purified, Dox-conjugated and PEGylated PPDSM in DMSO-$d_6$.

(a)




(b)

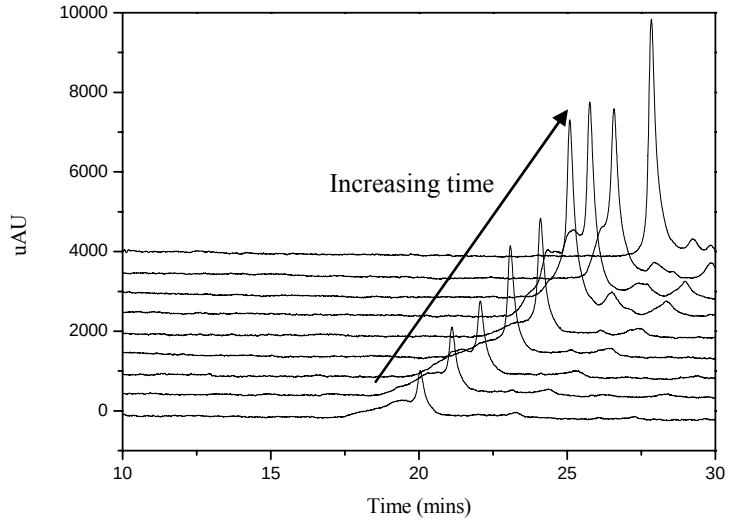



**Figure S-7:** Representative HPLC traces of Dox released from the PEGylated particles after incubation in (a) PBS pH = 7.4 and (b) Citrate-phosphate buffer saline pH = 5.0 for 2, 4, 6, 8, 12, 24, 48, 72 hours. The last chromatograms in both a and b were recorded after the complete release of Dox. The complete release was obtained by incubating the particles, that were already incubated for 72 hours in relevant buffer solution, in an acidic solution (pH = 3.0) for further 24 hours. All release experiments and following HPLC measurements were performed in duplicate.

**References:**

1. Willner, D., Trail, P. A., Hofstead, S. J., King, H. D., Lasch, S. J., Braslawsky, G. R., Greenfield, R. S., Kaneko, T., and Firestone, R. A. (1993) (6-Maleimidocaproyl)hydrazone of doxorubicin. A new derivative for the preparation of immunoconjugates of doxorubicin. *Bioconjugate Chem. 4*, 521-527.
2. Jia, Z., Wong, L., Davis Thomas, P., and Bulmus, V. (2008) One-pot conversion of RAFT-generated multifunctional block copolymers of HPMA to doxorubicin conjugated acid- and reductant-sensitive crosslinked micelles. *Biomacromolecules 9*, 3106-3113.