Electronic supplementary information

Tailor-made Legumain/pH dual-responsive prodrug-embeded nanoparticles for efficient anticancer drug delivery and theranostic application

Yang Li[a,b,c#], Yimin Niu[c#], Jianhua Zhu[a], Cuicui Gao[d], Qunwei Xu[a], Zhiyu He[f], Dawei Chen[d], Ming Xu[b,e]*, Yang Liu[a]*

a. School of Pharmacy, Nanjing Medical University, Nanjing 211166, Jiangsu, China.

b. Department of Occupational Disease Prevention, Jiangsu Provincial Center for Disease Control and Prevention, Nanjing 210009, Jiangsu, China.

c. Department of Pharmacy, Zhongda hospital, School of Medicine, Southeast University, Nanjing 210009, Jiangsu, China.

d. School of Pharmacy, Shenyang Pharmaceutical University, Shenyang 110016, Liaoning, China.

e. School of Public Health, Nanjing Medical University, Nanjing 211166, Jiangsu, China.

f. Institute for NanoBioTechnology, Johns Hopkins University, Baltimore, MD 21218, USA.

Corresponding author:

* E-mail: yangliunano@njmu.edu.cn. (Yang Liu)

* E-mail: sosolou@126.com. (Ming Xu)

# These authors contributed equally to this work.

Contents:

METHODS

S1. Synthesis of CDs (CDs-NH_2)

Citric acid (2.1 g) and diethylenetriamine (3.5 g) was mixed and reacted at 170 °C under argon atmosphere for 3 h. After being cooled to room temperature, 10 mL deionized water was added into the reaction mixture. Then, the mixture was purified by dialyzed (MWCO: 3500 Da) against water for 48 h, followed by vacuum frozen drying to obtain the product[5-6].

S2. Synthesis of CDs-C9-DOX

The synthetic route of DOX conjugated CDs (CDs-C9-DOX) is demonstrated in Fig. S3. For synthesis of DOX-C6-MAL, 60 mg DOX and 40 mg 6-maleimidohexanoic acid N-hydroxysuccinimide ester (MAL-C6-NHS) was dissolved in DMF, and then 40 μL triethylamine was added into the mixture. The solution was stirred at room temperature for 12 h under argon atmosphere. Subsequently, DMF was concentrated by rotary evaporator, and the concentrate were precipitate by ether. The product was obtained by centrifugation and vacuum drying.

For synthesis of DOX-C9-COOH, 30 mg DOX-C6-MAL was dissolved in 10 mL DMF, and then 8 μL 3-Mercaptopropionic Acid was added. The mixture was stirred at room temperature for 24 h, followed with dialysis against water (MWCO: 500 Da) for 24 h. The solid was obtained by freeze drying.

For synthesis of CDs-C9-DOX, CDs functionalized with exterior amino groups were coupled with DOX-C9-COOH by coupling agent. Typically, 20 mg DOX-C9-COOH was dissolved in DMSO, followed with 9.22 mg EDC and 5.72 mg NHS was added. The mixture was stirred at room temperature for 2 h for activating carboxyl group. Then, triethylamine and 13 mg CDs was added into the solution. The mixture was allowed to stirred at room temperature for 48 h, followed with dialysis against water (MWCO: 3500 Da) for 24 h. The solid was obtained by freeze drying. The intermediate product and the final product were dissolved in DMSO-d6 for ^1H NMR analysis (Fig. S4).

S3. Synthesis of PEG-*b*-PBLA

PEG-*b*-PBLA, OPBLA and OAPI polymers was synthesis via ring-opening polymerization[1-4]. Briefly, PEG-NH_2 (50 mg, 0.01 mmol) was completely dissolved in 5 mL dry DMF. The newly prepared BLA-NCA (75 mg, 0.3 mmol) was also dissolved in 20 mL DMF. Then, the two solutions were mixed and reacted for 48 hours (h) at 40 °C under argon atmosphere, followed with the reaction mixture was slowly added dropwise into excess ice-cold diethyl ether and the white precipitation were collected by centrifugation. Subsequently, the crude product was re-dissolved in DMF and precipitated with ice-cold diethyl ether again. The solids were obtained by centrifugation and dried under vacuum at room temperature. The structure was confirmed by ^{1}H NMR analysis (Fig. 1). Additionally, Cy5.5 labeled conjugates were prepare by the reaction of Cy5.5-NHS ester with terminal amino group in PEG-*b*-PBLA copolymers.

S4. Synthesis of OPBLA and OAPI

Octadecylamine (0.1 g) was dissolved in 25 mL dry DCM. BLA-NCA (1g) was dissolved in 10 mL dry DMF. Then, the two solutions were mixed and stirred for 48 h at 40 °C under argon atmosphere, followed with DCM in the reaction mixture was removed by rotary evaporation and the remaining liquids were precipitated with ice-cold diethyl ether. After collected the precipitation by centrifugation, the crude product was re-dissolved in DMF and precipitated with ice-cold diethyl ether again. The solid product (Octadecylamine-PBLA, OPBLA) was obtained by vacuum drying. The structure was confirmed by ^{1}H NMR analysis (Fig. S2).

OPBLA (0.2 g) and 1-(3-aminopropyl) imidazole (1 g) was dissolved in 5 mL DMSO, followed by stirred at 25 °C for 12 h under argon atmosphere. The reaction mixture was purified by dialyzed (MWCO: 1000 Da) against water for 48 h and the solid (OAPI) was obtained by lyophilization. The structure was confirmed by ^{1}H NMR analysis (Fig. 1) .

S5. Preparation of SE-NA

2 mg CDs-C9-AANL-DOX and 10 mg PEG-*b*-PBLA was dissolved in 6 mL chloroform, and then stirred at room temperature for 30 min. Subsequently, 20 mg OPBLA was dissolved in 2 mL chloroform and added slowed into the above solution, followed with stirred at room temperature for 2 h. Then, the mixture was rotated to form a film at 70 °C. Finally, 10 mL PBS (0.01 M, pH 7.4) was added into the flask and the nanosuspension was obtained by ultrasonic dispersion for 5 minutes and then passed through syringe-driven Filters.

S6. Preparation of SA-NA

2 mg CDs-C9-DOX was dissolved in 3 mL methanol and 10 mg PEG-*b*-PBLA was dissolved in 3 mL chloroform, then the two solutions was mixed and stirred for 30 min. Subsequently, 20 mg OAPI dissolved in 300 μL methanol was added into the mixture, followed with stirred at room temperature for 2 h. Then, the mixture was rotated to form a film at 70 °C. Finally, 10 mL PBS (0.01 M, pH 7.4) was added into the flask and the nanosuspension was obtained by ultrasonic dispersion for 5 minutes and then passed through syringe-driven filters.

S7. Statistical analysis

Data were presented as mean ± standard deviation (SD), and statistically analyzed using two-tailed Student's t-test. The difference was defined as significant at $*p < 0.05$, $** p < 0.01$ and $*** p < 0.001$.

SUPPLEMENTAL FIGURES

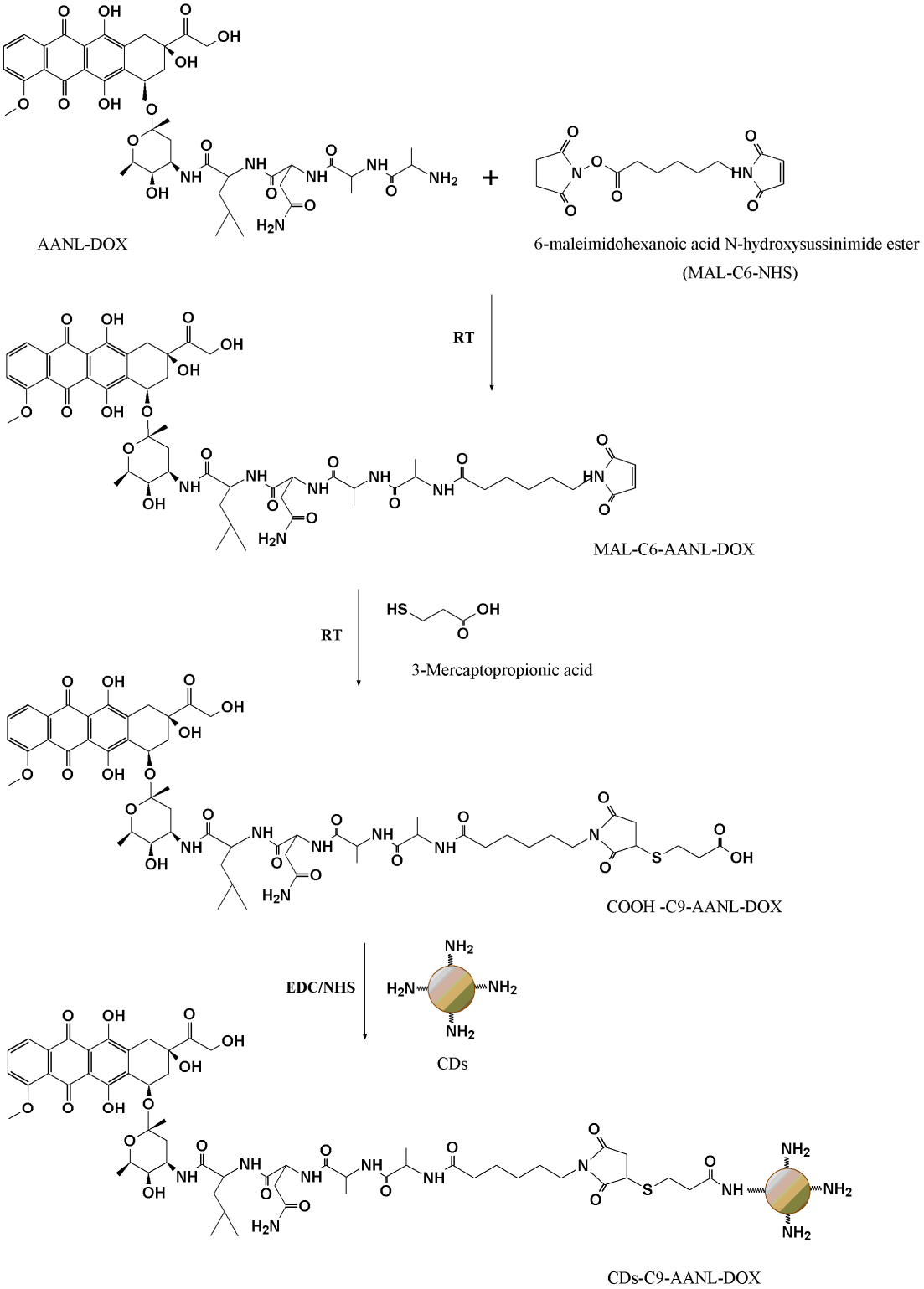

Fig. S1. The general synthetic route of CDs-C9-AANL-DOX and intermediate products

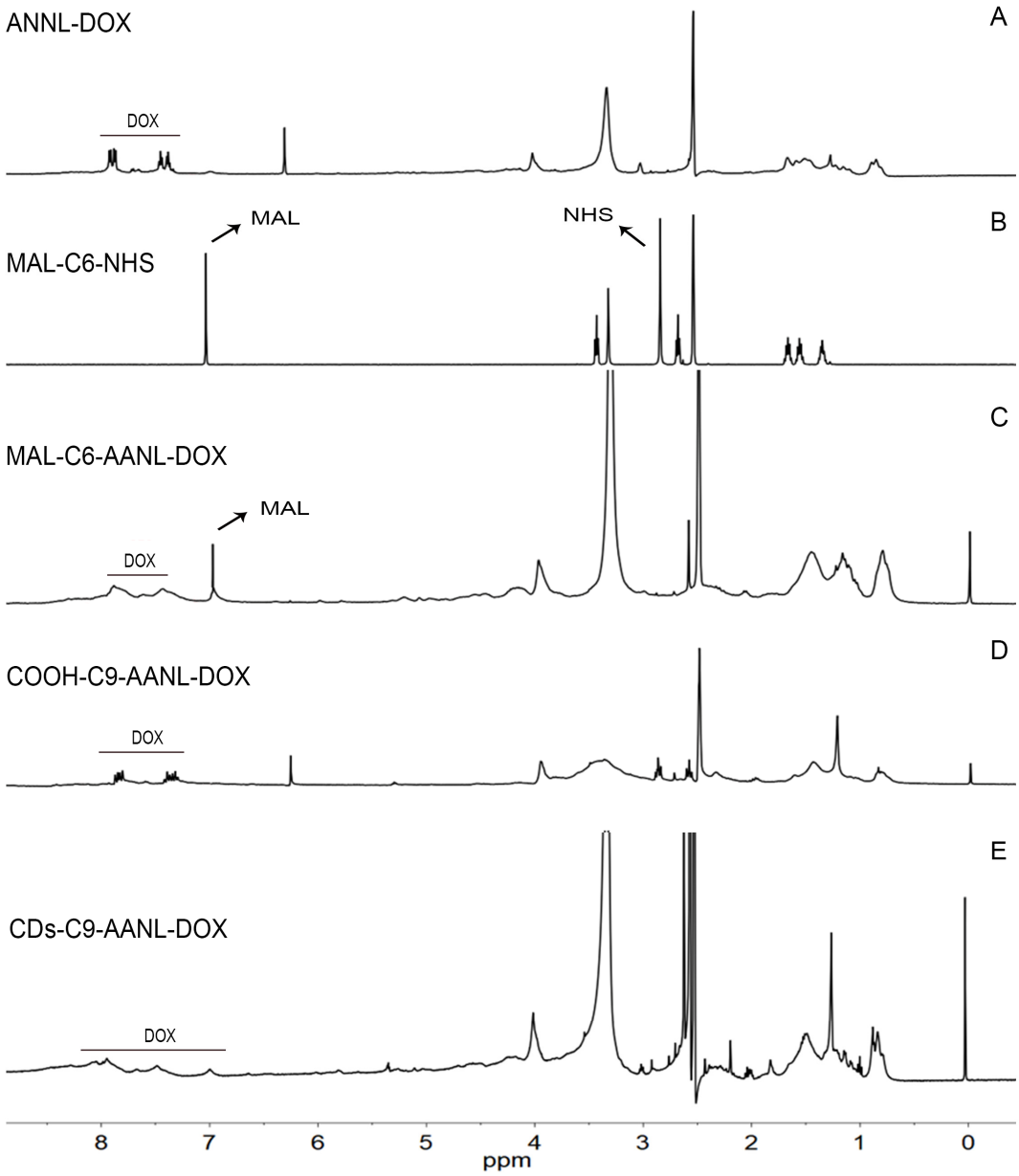

Fig. S2. ^{1}H NMR spectrum of CDs-C9-AANL-DOX and intermediate products.

The amino group of AANL-DOX was reacted with the NHS ester of 6-(maleimido)hexanoic acid succinimide ester (MAL-C6-NHS), and thus the NHS ester peak disappeared (C). Meanwhile, the imide group (MAL) peak also indicated the conjugation of AANL-DOX and 6-(maleimido)hexanoic acid succinimide ester(C). Next, the sulfhydryl group of the tridecylpropionic acid was reacted with MAL and the MAL peak was disappeared (D). Finally, COOH-link-AANL-DOX and NH_2-CDS reacted with an amide to form an enzyme-sensitive prodrug CDs-C9-AANL-DOX (E).

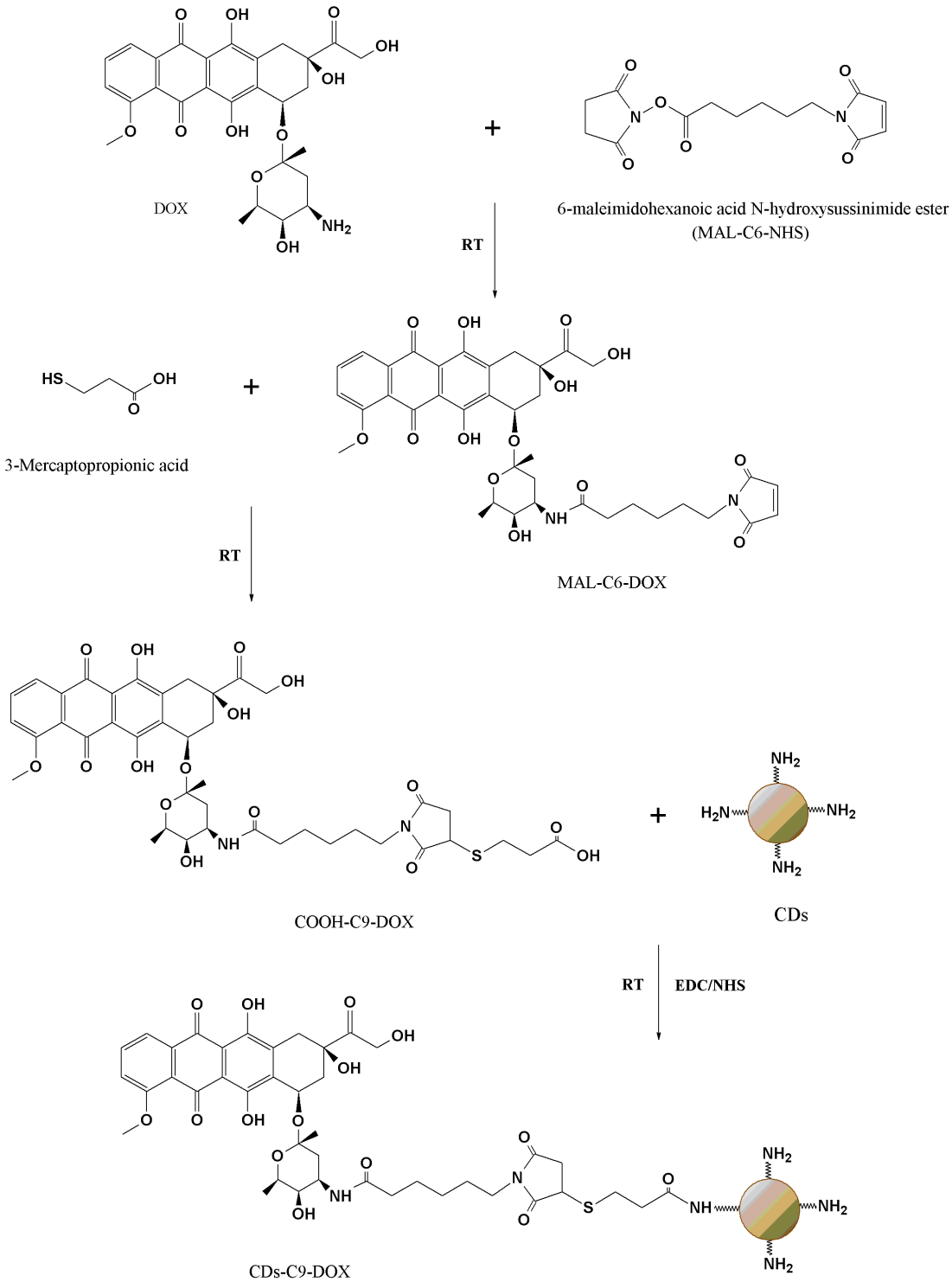

Fig. S3. The general synthetic route of CDs-C9-DOX and intermediate products

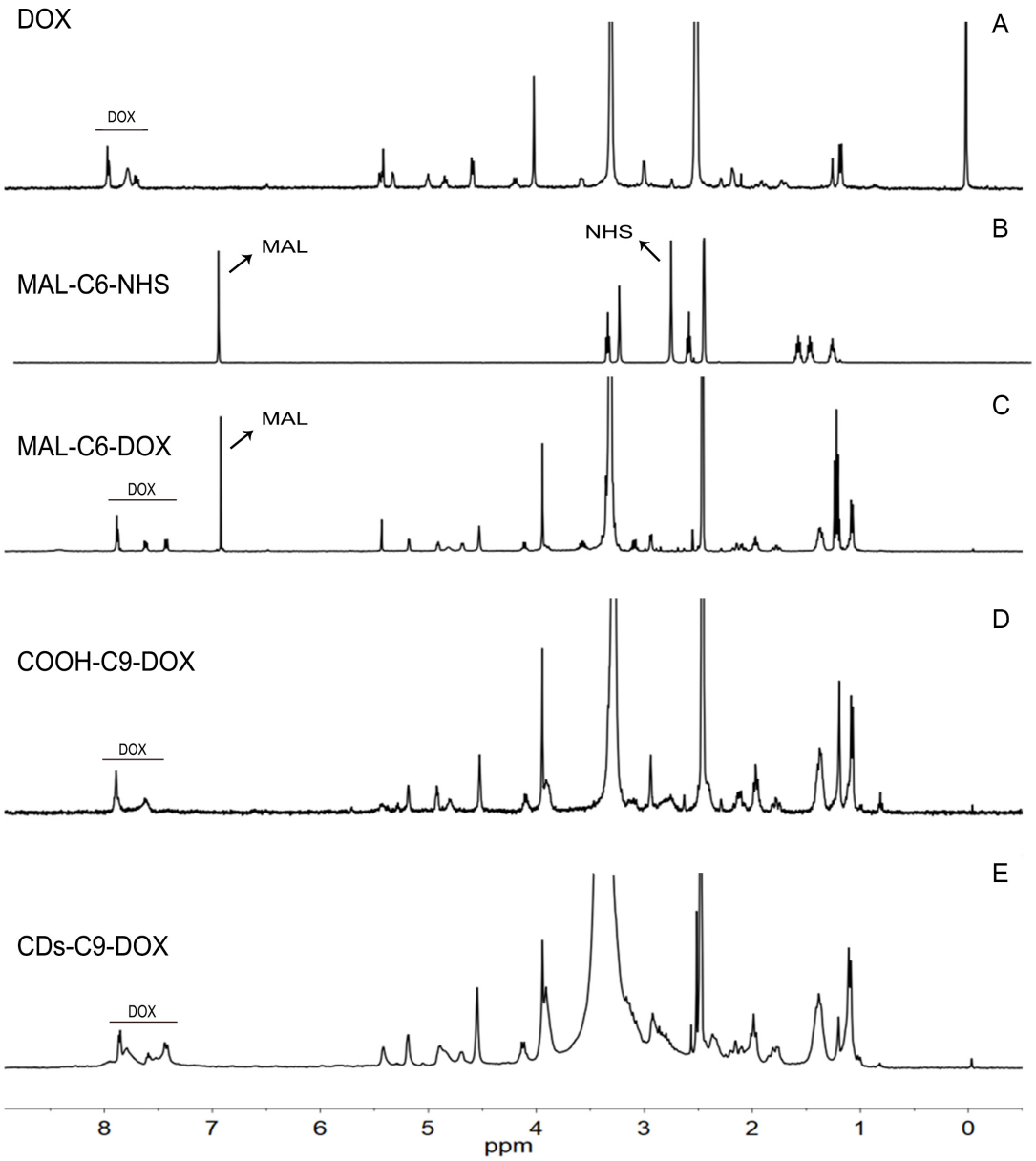

Fig. S4. ^{1}H NMR spectrum of CDs-C9-DOX and intermediate products.

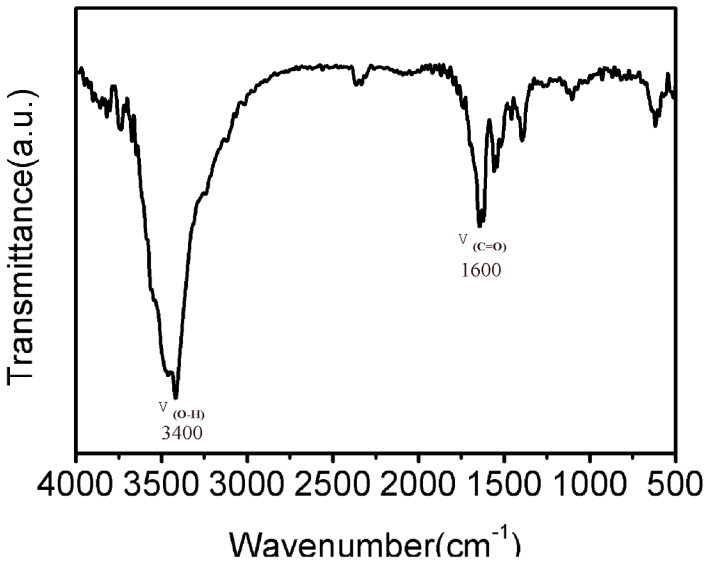

Fig. S5. FT-IR spectrum of CDs

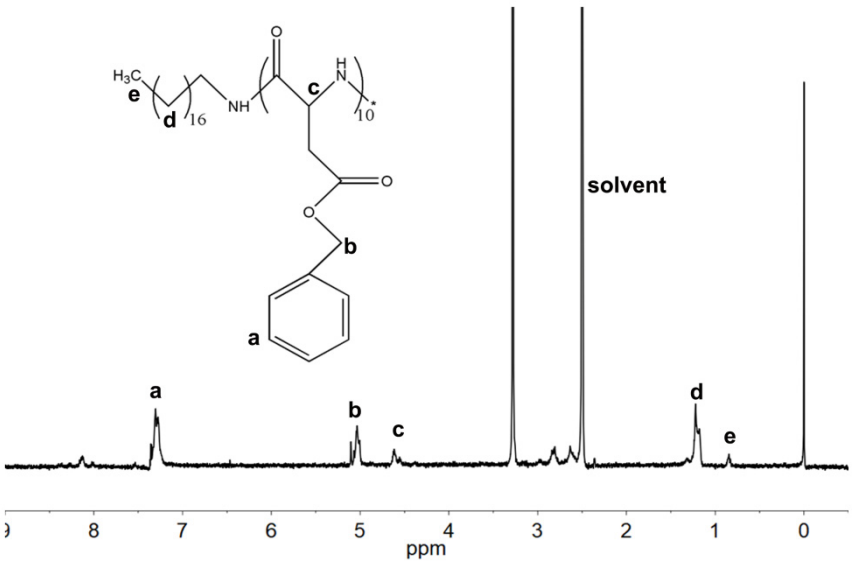

Fig. S6. ^{1}H NMR spectrum of OPBLA

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