Supporting information

Biodegradable polymeric nanoparticle vaccine for combating *Acinetobacter baumannii* infection

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Materials

Unless otherwise noted, all chemicals and reagents were obtained commercially and used without further purification. 2,2'-Dithiodiethanol, 1,2,4,5-cyclohexanetetracarboxylic dianhydride, 4-aminomethylphenylboronic acid, 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (EDCl), N-hydroxysuccinimide (NHS), L-lysine diisocyanate and 3-dimethylamino-1,2-propanediol were purchased from Bidepharm.com. Methoxypolyethylene glycol

($mPEG_{5000}$-OH), anhydrous N, N-dimethylformamide (DMF), and deionized water were purchased from Aladdin. 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) was purchased from Aladdin. RPMI 1640 medium, 0.25% trypsinEDTA, and penicillin/streptomycin (P/S) were purchased from Procell Life Science & Technology Co., Ltd. QS21 was purchased from Med Chem Express. The *A. baumannii* fragments were obtained from the Academy of Military Medical Sciences. The antibodies were purchased from Cell Signaling Technology (CST). The detection kit and immunofluorescence reagents were purchased from Beyotime (Shanghai, China).

Instrumentation

Nuclear magnetic resonance spectroscopy (^{1}H-NMR) spectra were conducted by a Bruker Avance 400 NMR spectrometer (Bruker, USA) at room temperature. Transmission electron microscopy (TEM) images were captured using a JEM-2200FS transmission electron microscope (JEOL, Japan). The size, Zeta potential, and polydispersity index of the nanoparticles were measured by dynamic light scattering on an ALV/CGS-3 goniometry system (ALV, Germany). Cell imaging was conducted by Confocal Laser Scanning Microscopy (LSM-800, ZEISS, Germany).

Cells and mice

The 293T and L-02 cells were cultured in a complete RPMI-1640 culture medium containing 10% FBS and 1% penicillin-streptomycin in an artificial environment (5% CO_2 at 37 ℃). The 293T and L-02 cell lines were purchased from IMMOCELL (Xiamen, Fujian, China). BALB/c and BALB/c nu mice (5 weeks old, female) were purchased from SPF Biotechnology Co., Ltd. (Beijing, China). All animal experiment protocols were reviewed and approved by the Institutional Animal Care and Use Committee of the Academy of Military Medical Sciences (Permit number: IACUC-DWZX-2023-P025).

Synthesis of Polymer M1

2, 2'-Dithiodiethanol (0.208 g, 1.35 mmol) was dissolved in anhydrous N, N-dimethylformamide (DMF, 5 mL), followed by the rapid addition of 1,2,4,5-cyclohexanetetracarboxylic dianhydride (0.31 g, 1.41 mmol). The mixture was magnetically stirred at room temperature for 8 h. Subsequently, methoxypolyethylene glycol ($mPEG_{5000}$-OH, 0.68 g, 0.13 mmol) was added to the reaction mixture, and magnetic stirring was continued at 50 °C for an additional 12 h. After completion of the reaction, the mixture was transferred to a dialysis bag (molecular weight cutoff: 8000-14000 Da) for dialysis. After 72 h of dialysis, the resulting solution was freeze-dried under reduced pressure to obtain polymer M1 as a white solid .

Synthesis of Polymer POB

4-Aminomethylphenylboronic acid (8.8 mg), 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (EDCl, 15 mg), and N-hydroxysuccinimide (NHS, 9 mg) were dissolved in anhydrous DMF (5 mL). Then, polymer M1 (122 mg) was added to the reaction mixture. The mixture was stirred at room temperature for 12 h, after which the reaction was terminated. The mixture was poured into 10 mL of deionized water and then transferred to a dialysis bag (molecular weight cutoff: 8000-14000 Da) for dialysis. After 72 h of dialysis, the solution was freeze-dried under reduced pressure to yield polymer POB as a white solid.

Synthesis of Polymer POQ

L-Lysine diisocyanate (0.249 g, 1.1 mmol) was dissolved in anhydrous DMF (5 mL), and 3-dimethylamino-1,2-propanediol (0.119 g, 1.0 mmol) was added to the solution. The mixture was magnetically stirred at room temperature for 8 h. Then, $mPEG_{5000}$-OH (1.00 g, 0.2 mmol) was added to the reaction mixture, and magnetic stirring was maintained at 50 °C for another 12 h. After the reaction finished, the mixture was transferred to a dialysis bag (molecular weight cutoff: 8000-14000 Da) for dialysis. Following 72 h of dialysis, the solution was freeze-dried under reduced pressure to obtain polymer POQ as a white solid.

Preparation of QS21+AB

QS21 (1 mg) was dissolved in 1 mL of DMSO, and the solution was then slowly added dropwise to a rapidly stirred PBS suspension (10 mL) containing *A. baumannii* fragments (1×10^7 CFU mL^{-1}). The mixture was subsequently dialyzed using a dialysis bag (MWCO: 8000-14000 Da). After 72 h, the mixture was concentrated by ultrafiltration and then passed through a 0.22 μm syringe-driven filter to obtain QS21+AB.

Preparation of NP-VAC

POB (10 mg), POQ (10 mg), and QS21 (1 mg) were respectively weighed and dissolved in 1 mL of DMSO. This solution was then slowly added dropwise to 10 mL of a rapidly stirring PBS suspension containing Acinetobacter baumannii fragments (1×10^7 CFU·mL^{-1}). The mixture was dialyzed using a dialysis bag (MWCO: 8000–14000 Da) for 72 h. After dialysis, the solution was concentrated via ultrafiltration and subsequently filtered through a 0.22 μm syringe-driven filter to obtain NP-VAC.

Stability Test of NP-VAC

Take the NP-VAC dispersion, shake well, then dilute with deionized water to an appropriate concentration for DLS measurement. Take samples at a fixed time each day, equilibrate them in the test environment for 30 min, then gently shake. After confirming that there is no obvious agglomeration or precipitation, inject the sample into a cuvette. Place the cuvette into the DLS sample holder, start the test using preset parameters, test each sample three times consecutively, and record the Z-average particle size and PDI.

Degradation Test of NP-VAC

Add 30.7 mg of GSH to 10 mL of the NP-VAC dispersion, shake well, and incubate in a shaker at 37 °C for 24 h. Dilute the mixture to an appropriate concentration and transfer it into a cuvette. Place the cuvette into the DLS sample holder, start the measurement with preset parameters, test each sample three times consecutively, and record the Z-average particle size and PDI.

Cell viability

The cytotoxicity of NanoIr under US irradiation was evaluated on 293T cells as well as L-02 both cultivated in normoxic conditions and hypoxia conditions by MTT assay. The 293T cells or L-02 (5×10^3 per well) were seeded into 96-well plates. 293T cells or L-02 cells were incubated with various concentrations of QS21+AB and NP-VAC (0.1, 1, 5, 25, 50, and 100 μM). After 24 h, the cell viability was measured by MTT assay. After 4 h of incubation, 10% SDS (100 μL) was added to each well, and the cells were incubated for 12 h. The absorbance of wells was measured by a microplate reader (SpectraMax) at 570 nm (peak absorbance) and substracted at 650 nm (background absorbance). The cell viability was expressed as the ratio of the absorbance of the test wells and control wells.

Immunofluorescence staining

Coverslips were placed at the bottom of a 24-well plate. RAW264.7 cells (1×10^5 per well) in 1 mL of medium were added and incubated at 37 °C for 12 h. The cells were then treated with PBS, QS21+AB (with QS21at 25 μM), or NP-VAC (25 μM) for 24 h. After washing with PBS, fixation was performed using 4% paraformaldehyde. The fixed cells were blocked with 1% bovine serum albumin (Beyotime) and permeabilized with 0.1% Tween 80 (Beyotime), followed by incubation with primary antibodies against NLRP3 and Caspase-1 at 4 °C for 12 h. Nuclei were counterstained with DAPI, and images were captured with a confocal laser scanning microscope (CLSM).

Bacterial Cultivation

A. baumannii was cultured in liquid LB culture medium in a shaking incubator (200 rpm) at 37 °C and harvested at the logarithmic growth phase by centrifugation at 3000 g for 10 min. After washing with PBS three times, the bacteria were resuspended in PBS for further use. The bacteria were diluted with the liquid LB culture medium for further use until the OD at 600 nm reached 0.5. ($OD_{600} \approx 0.5$, 10^8 CFU mL^{-1}). Take 1

mL of Acinetobacter baumannii at a concentration of 1×10^7, disrupt it by ultrasonic treatment for 10 min, and prepare bacterial fragments for subsequent use.

Animal Lung Delivery Model

For immunological analysis in mice: Four doses were administered via pulmonary delivery over 9 days, with one-day intervals between doses. The experimental groups were as follows: (1) PBS control group; (2) QS21+AB group; (3) NP-VAC group. Each group consisted of 5 mice (n = 5), followed by immunological analysis. In the protective efficacy study, Mice first underwent a 7-day vaccine pretreatment period, during which they were dosed every other day. Seven days after the final dose, the mice were challenged via pulmonary delivery with an *Acinetobacter baumannii* suspension (1×10^6 ~ 1×10^7 CFU mL^{-1}), and their survival status was monitored and recorded. In these experiments, QS21 and NP-VAC were each administered at a concentration of 50 μM, with 50 μL delivered to the lungs.

Flow cytometry analysis of the animal tissue

Mice were randomly assigned to groups (n = 5) and received the same treatment as previously described. Mice were euthanized on day 9. Lung tissue, lymph nodes, and spleen were harvested and used to prepare single-cell suspensions. The single-cell suspensions were further incubated with multiple antibodies targeting immune cells. To analyze T cells in the lung and spleen, cells were stained with anti-CD8-FITC. For analysis of dendritic cells in the lungs and lymph nodes, cells were stained with anti-CD11c-PE, anti-CD80-FITC, and anti-CD86-APC. All antibodies were purchased from Elabscience. Flow cytometry data acquisition and analysis were performed using CytExpert software.

Histopathological analysis

9 days after pulmonary delivery (day 14 post-vaccination), major organs were harvested and analyzed *via* routine *H&E* and immunofluorescence staining.. For *H&E* staining, the excised tumor and organs were fixed in 4% paraformaldehyde solution, embedded in paraffin, sectioned, and stained with hematoxylin and eosin (*H&E*).

Statistical analysis

All the animal studies were performed after randomization. Quantitative data were collected at least three times. Data were analysed by two-way ANOVA or one-way ANOVA test with Prism 10.0 (GraphPad Software). Data were normally distributed, and the variance between groups was similar. All the values were reported as mean ± SD (*$p < 0.05$, **$p < 0.01$, ***$p < 0.001$, ****$p < 0.0001$, $n = 3$). No sample in any representative experiment was excluded from the analysis.

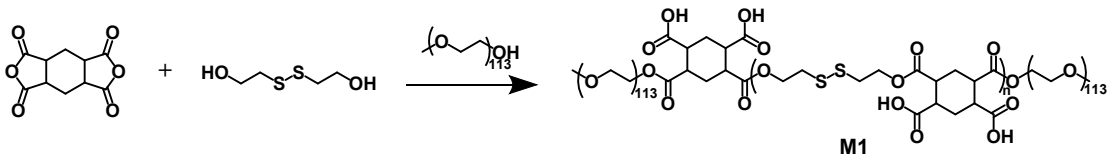

Fig. S1. Synthesis Route of M1

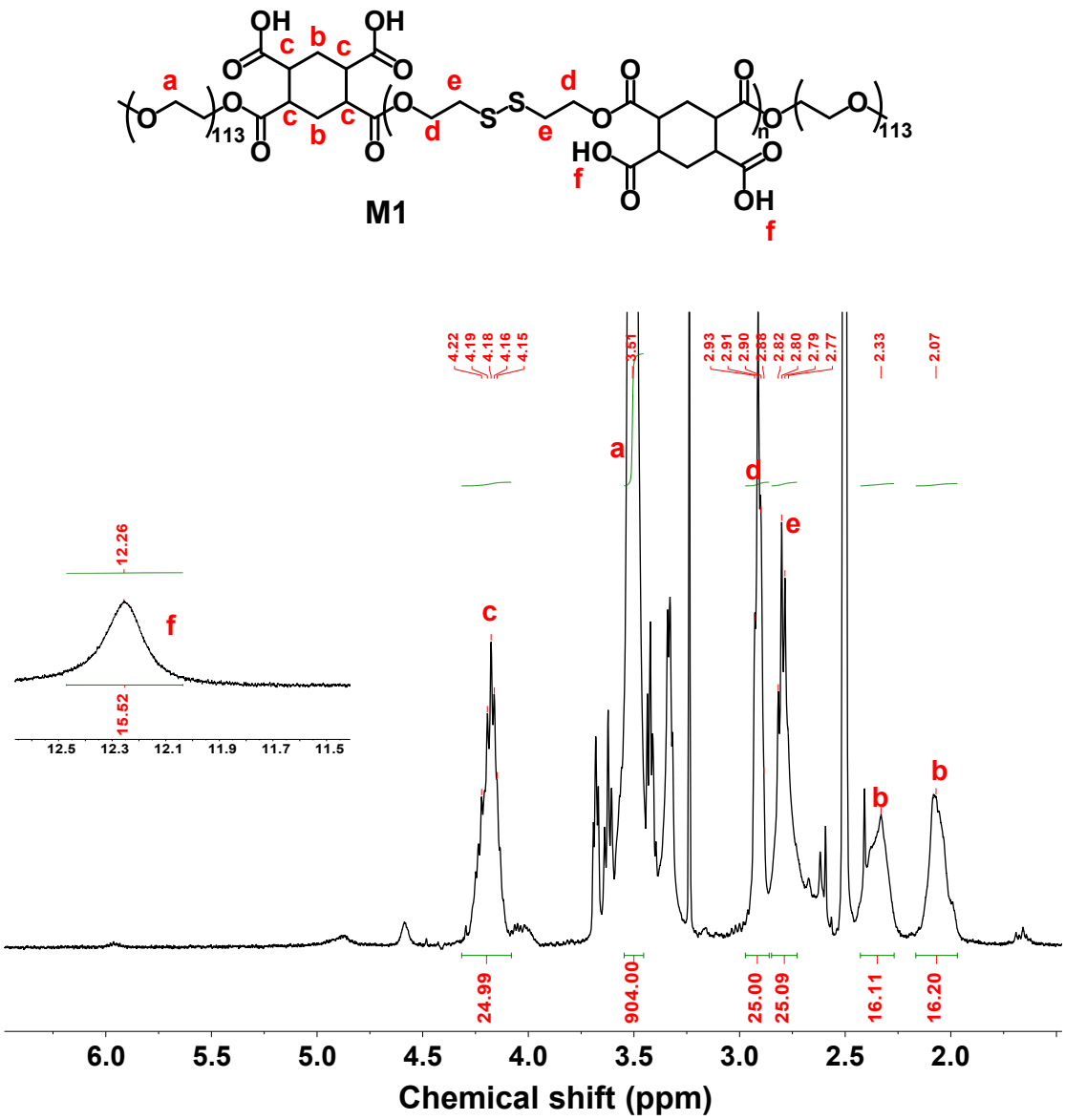

Fig. S2. ^{1}H NMR of M1 in DMSO-*d6*

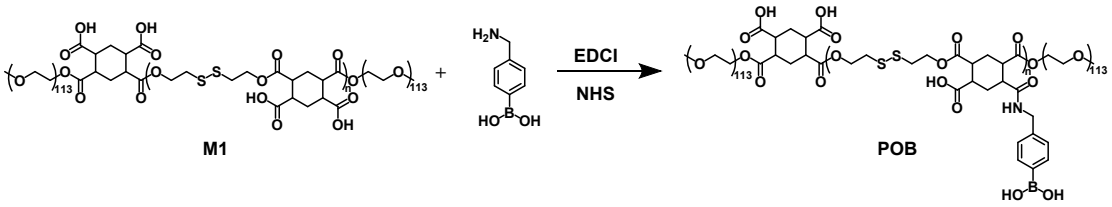

Fig. S3. Synthesis Route of POB

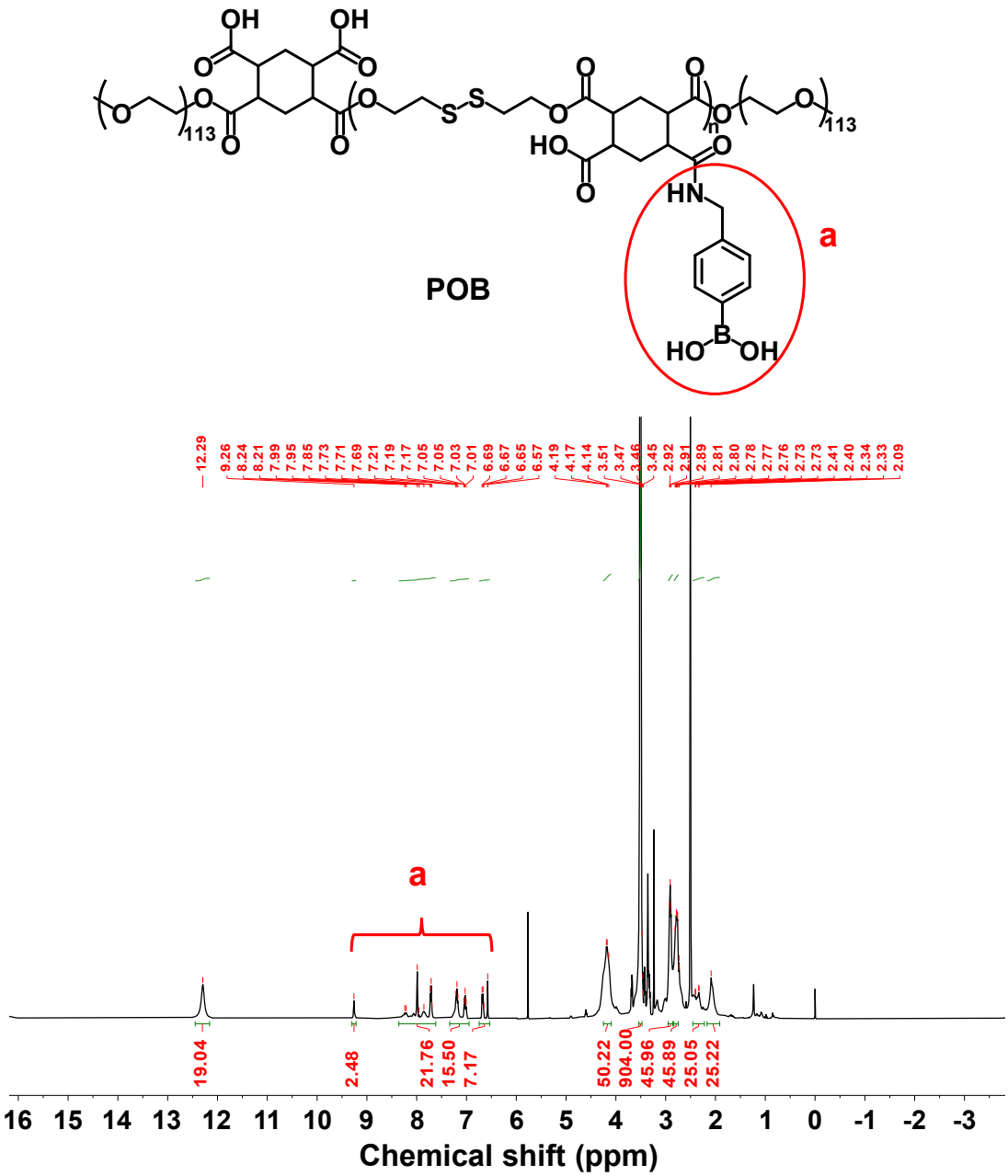

Fig. S4. ^{1}H NMR of POB in DMSO-*d6*

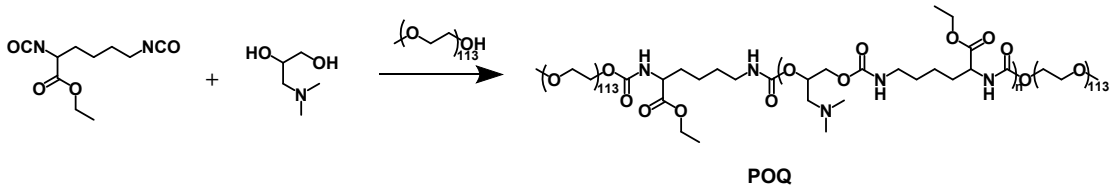

Fig. S5. Synthesis Route of POQ

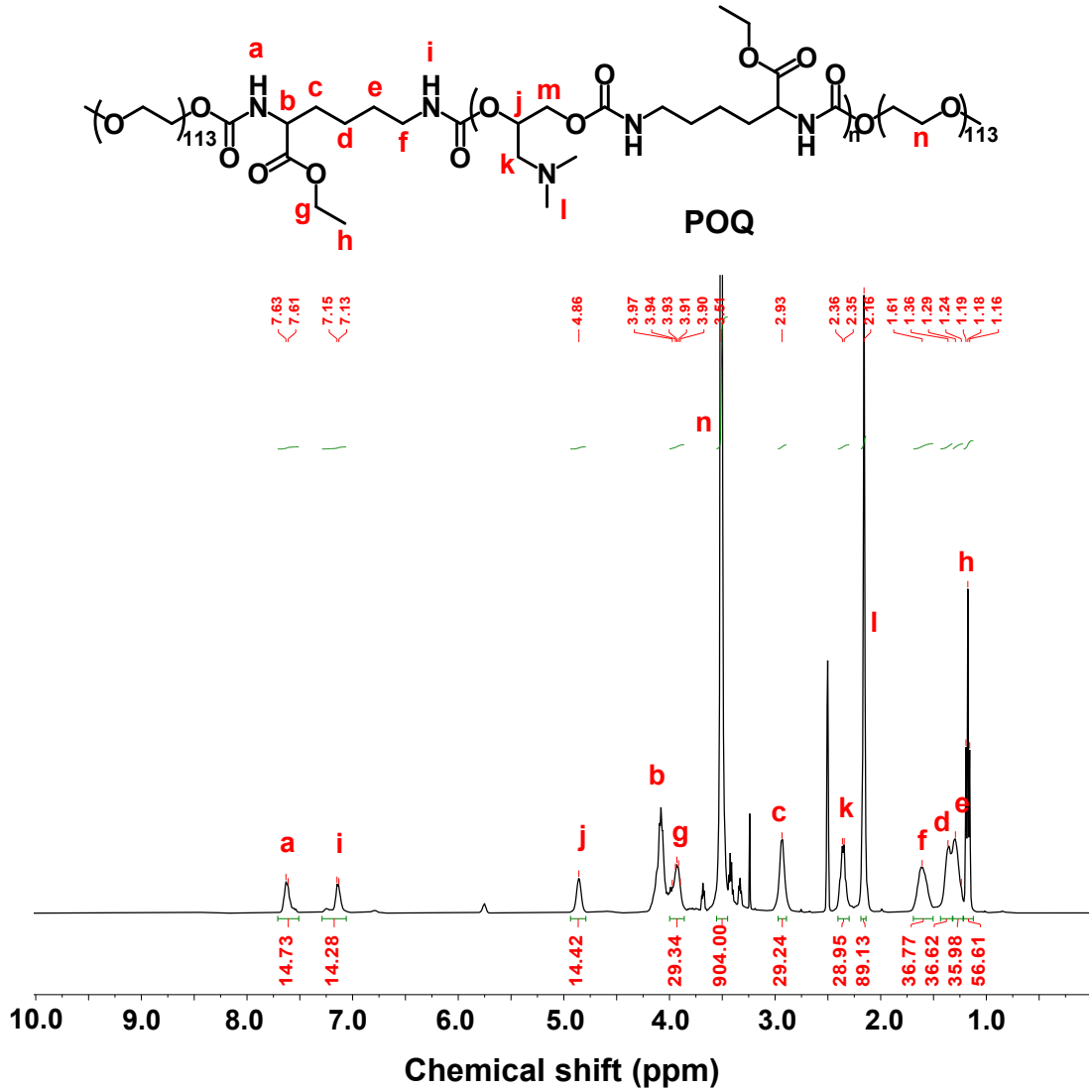

Fig. S6. ^{1}H NMR of POB in DMSO-*d6*

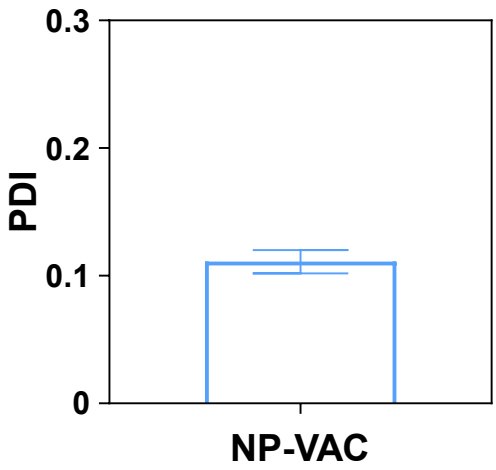

Fig. S7. NP-VAC of PDI