Electronic Supplementary Material (ESI) for ChemComm.

Supplementary Information

An Eco-friendly In-situ Activable Antibiotic via Cucurbit[8]uril-Mediated Supramolecular Crosslinking of Branched Polyethyleimine

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Experimental Section

Materials. CB[8] was prepared according to a literature report.[1] Boc-$_L$-phenylalanine N-hydroxysuccinimide ester (Boc-Phe-OSu), and low M.W. PEI (800 Da) were purchased from Aladdin (Shanghai). All reagents and solvents were used as received without further purification.

General procedure for the synthesis of PhePEI (800 Da). To a solution of PEI (800 Da) (1.00 mmol, 800.00 mg) in DMF (10 mL), trimethylamine (n mmol) was added under ice-water bath conditions, subsequently Boc-$_L$-phenylalanine N-hydroxysuccinimide ester (Boc-Phe-OSu, n mmol) was added. The reaction mixture was stirred at room temperature for 18 hours, followed by the removal of the solvents with a rotary evaporator. Subsequently, a solution of 4 N HCl in Dioxane (8 mL, v/v = 1) was added to the residue and stirred at room temperature for another 2 hours for the deprotection of N-Boc in phenylalanine. The solvent was removed and the crude product was dialyzed (500 Da) against water for 3 days before being lyophilized. The approximate grafting percentage of PEI was calculated by the integration of aromatic proton against that of the ethylene protons in the ^{1}H NMR spectra of the product (Figure S1).

PhePEI-1. n = 10%, yellow viscous oily liquid, grafting percentage 2.2% (relative to primary amino group), 9.9% (molar ratio to PEI);

PhePEI-2. n = 20%, light yellow viscous oily liquid, grafting percentage 3.9% (relative to primary amino group), 17.7% (molar ratio to PEI);

PhePEI-3. n = 40% light yellow viscous oily solid, grafting percentage 7.5% (relative to primary amino group), 33.9% (molar ratio to PEI);

Strains and growth conditions

Staphylococcus aureus (ATCC 25904), *Pseudomonas aeruginosae* (PAO1), *Acinetobacter baumannii* (ATCC 17978) and *Escherichia coli* (DH5α) were used in this study. *P. seudomonas, A.baumannii* and *E.coli* were grown at 37°C in Luria broth (LB) medium (10 g tryptone, 5 g yeast extract and 5 g NaCl per liter). *S. aureus* was grown at 37 °C in Tryptic soy broth (TSB) medium (17g tryptone, 3g phytone, 5g NaCl, 2.5g glucose and 2.5g K_2HPO4 per liter). For the bacterial viability test, bacteria were cultured in 96-well microtiter plate containing 100 µl fresh medium and different concentrations of complexes, each well as inoculum to a starting optical density at 600 nm of 0.05. Cell numbers were determined by plate counting after 18 h incubation.

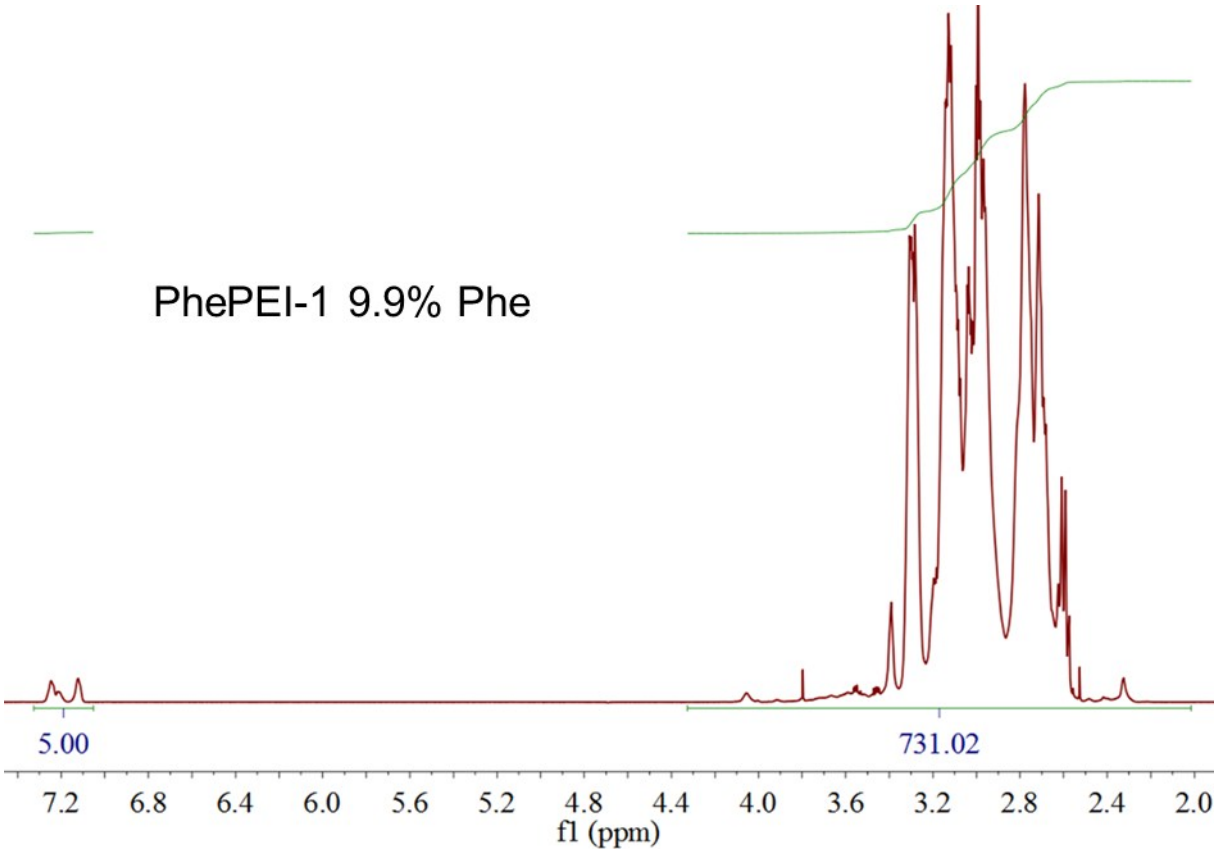
Cell culture and MTT assay

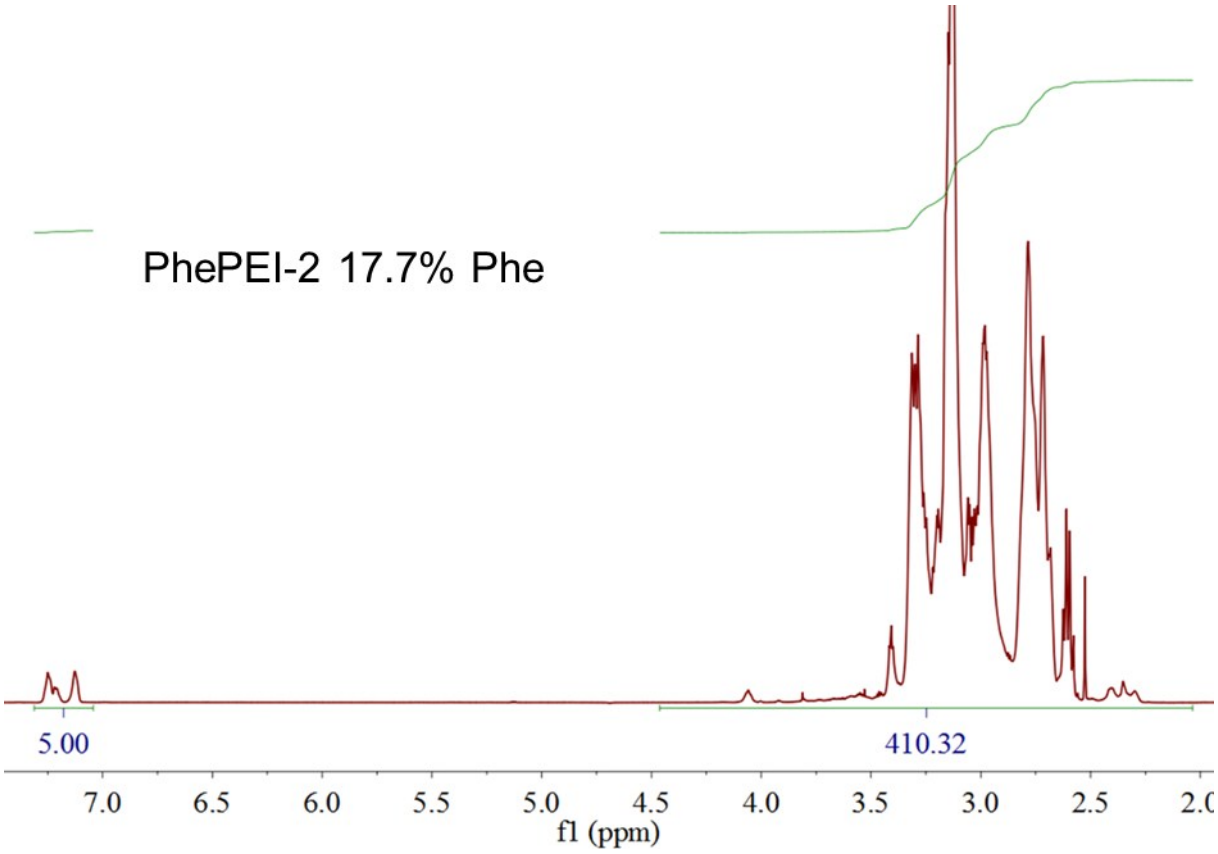
RAW 264.7 cell line was cultured in DMEM medium (Gibco) supplemented with 10% FBS (Gibco) and 1% penicillin–streptomycin (Gibco) in a humidified environment at 37 °C with 5% CO_2.
Cytotoxicity of PhePEI-3 in the absence and presence of CB[8] were tested by colorimetric 3-(4,5-dimethylthiazol-2-yl) 2,5-diphenyltetrazolium bromide (MTT) assay in vitro. Cells were plated in 96-well plates (1×10^4 per well) and were allowed to attach overnight. The test compounds were added (0.1 mg/ml PhePEI-3, in the absence and in the presence of 20 µM CB[8]), and the cells were incubated for 24 h at 37 °C. Following incubation, 5 mg/ml MTT solution was added to each well and incubated for 3 h. DMSO was then added to each well to dissolve the formazan crystals. The DMSO-dissolved formazan crystals were read immediately at 540 nm with a multiplate reader (SpectraMax M5 Microplate Reader, Molecular Devices, USA).

Zeta potential measurement

E. coli cells (500µL, OD600 =1) were centrifuged and resuspended in 500µL PBS containing PhePEI or PhePEI/CB[8] complex at room temperature for 30 min. For the disassembly group, AD was added into the buffer treated with PhePEI/CB[8] and incubated for 30 min. Bacterial cells were collected by centrifugation and then suspended in 1mL Milli-Q water. Membrane

potential was determined by the Malvern Zetasizer Nano ZS apparatus. All samples were performed with three biological replicates.




PhePEI-2 17.7% Phe
5.00
410.32
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
f1 (ppm)

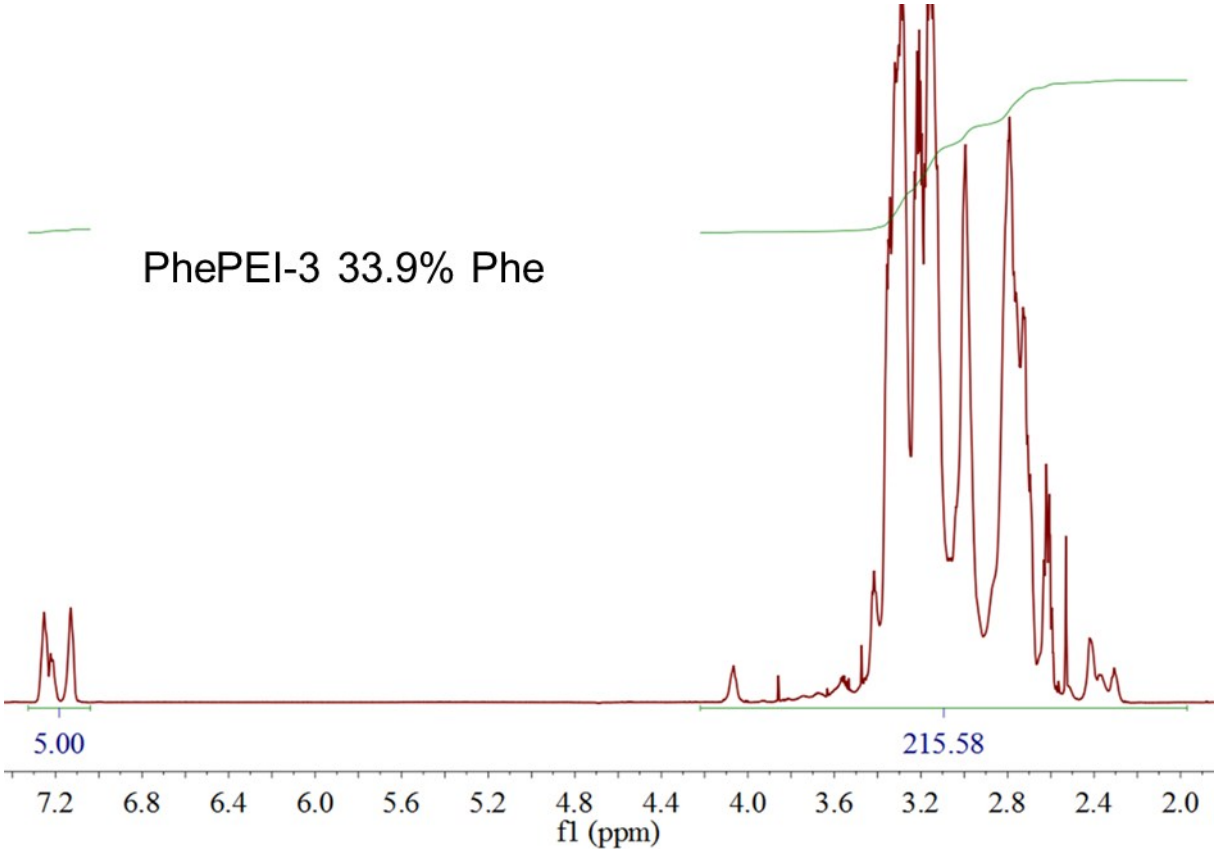

PhePEI-3 33.9% Phe
5.00
215.58
7.2
6.8
6.4
6.0
5.6
5.2
4.8
4.4
4.0
3.6
3.2
2.8
2.4
2.0
f1 (ppm)

Fig. S1 ^{1}H NMR spectra of the prepared PhePEI in D_2O with various molar ratio to PEI as indicated.

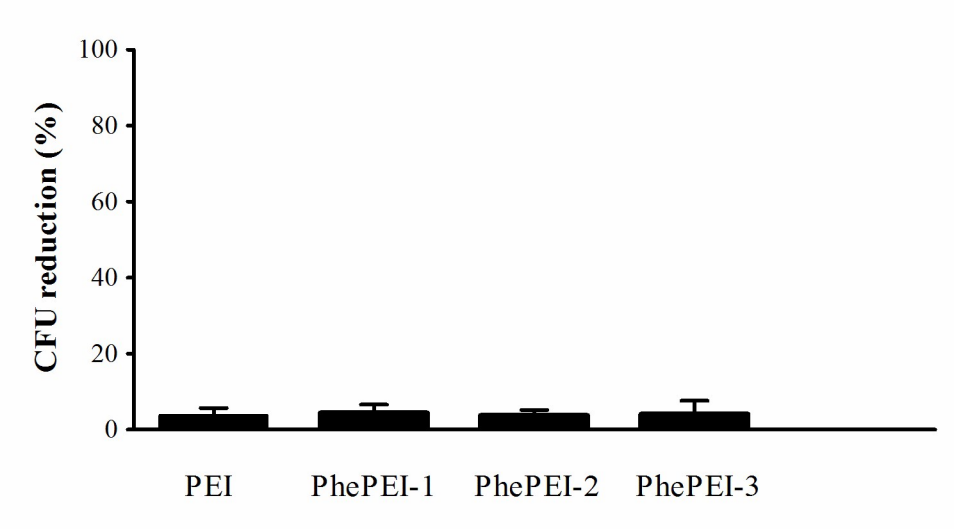


Fig. S2 Antibacterial activity of PEI and PhePEI with different grafting percentage.

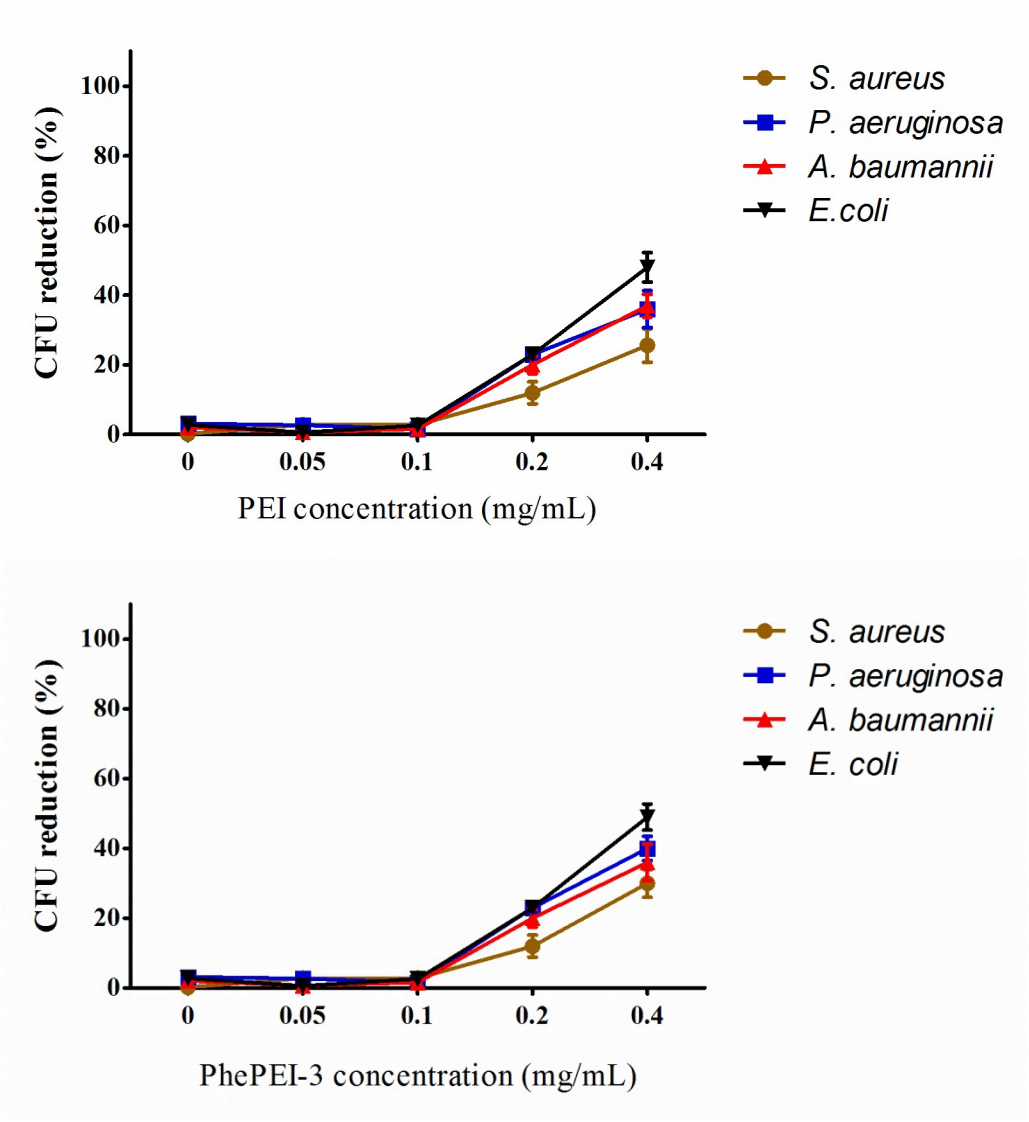


Fig. S3 Dose-dependent result of the antibacterial activity of PEI, and PhePEI-3.

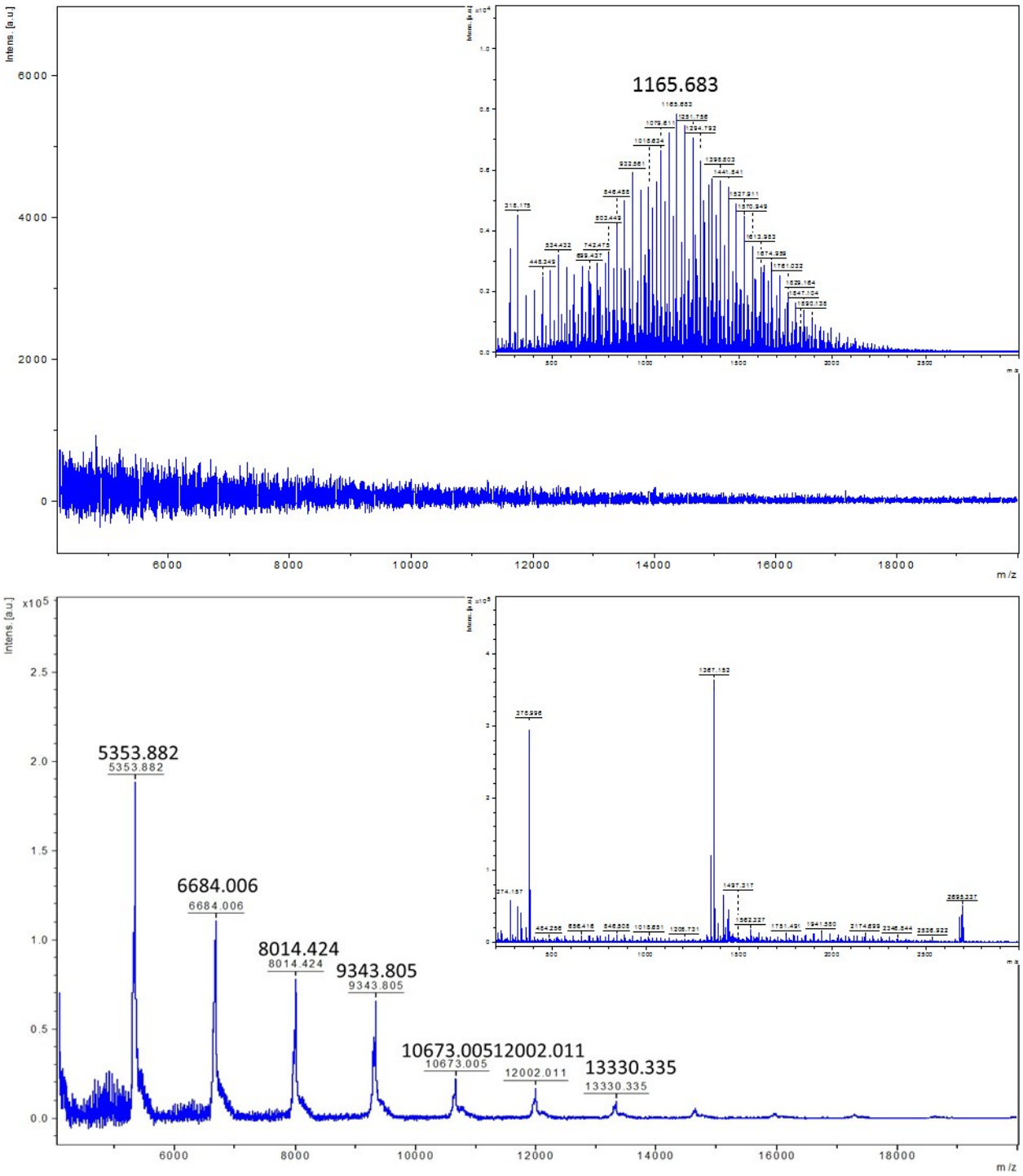


Fig. S4 MALDI-TOF spectrum (BRUKER, CHCA as matrix, range 4000~20000, inset range 200~3500)) of PhePEI-3 in the absence (top) and presence (bottom) of CB[8].

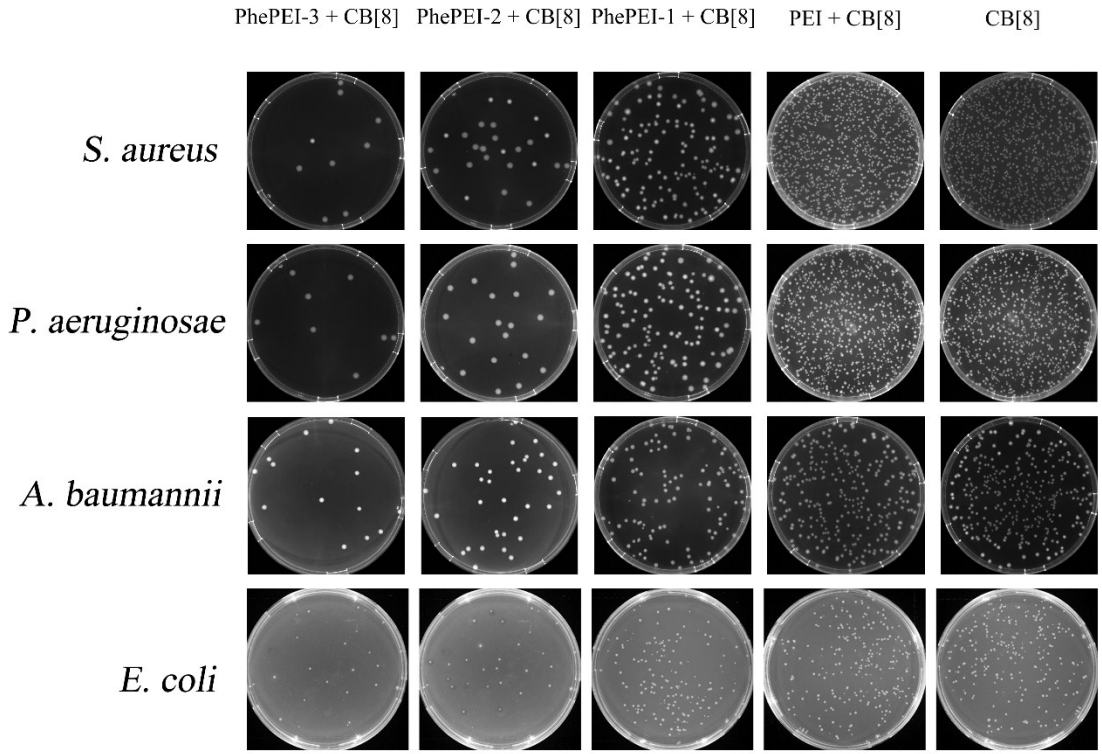


Fig. S5 The associated CFU on LB agar plate for antibacterial activity study of PEI and PhePEI with different grafting percentages in the presence of 20 μM CB[8].

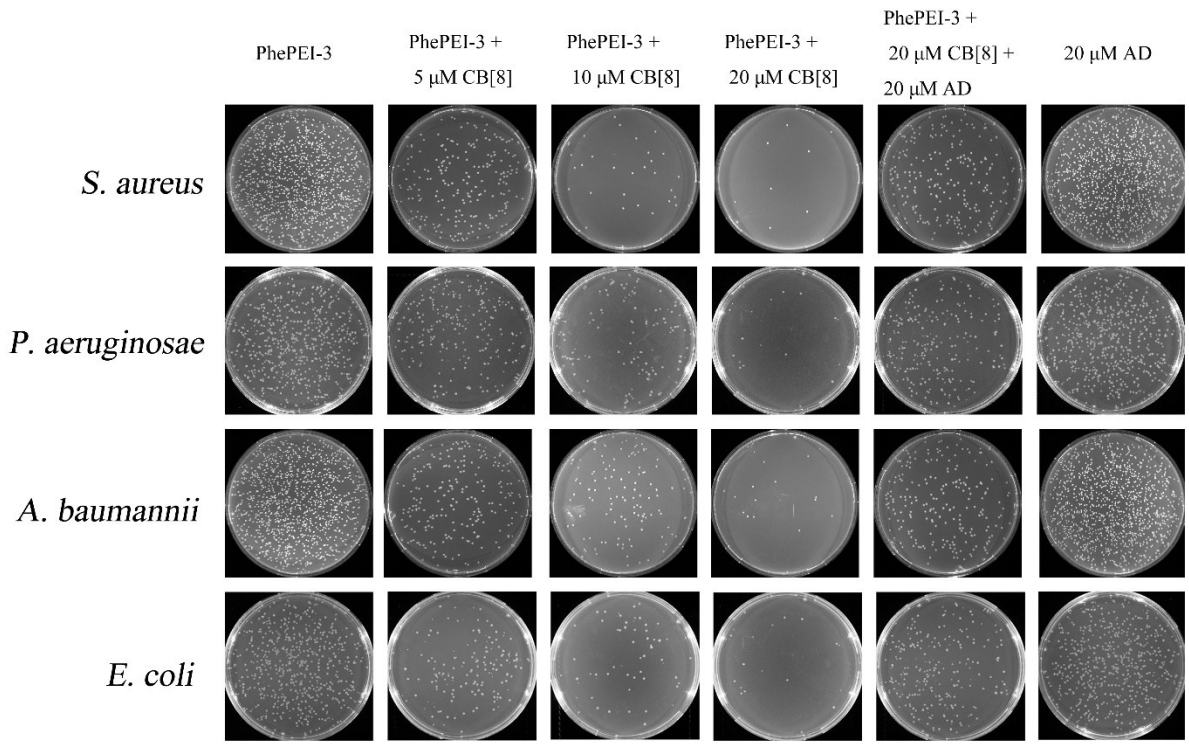


Fig. S6 The associated CFU on LB agar plate for antibacterial activity study of PhePEI-3 upon addition of different amount of CB[8].

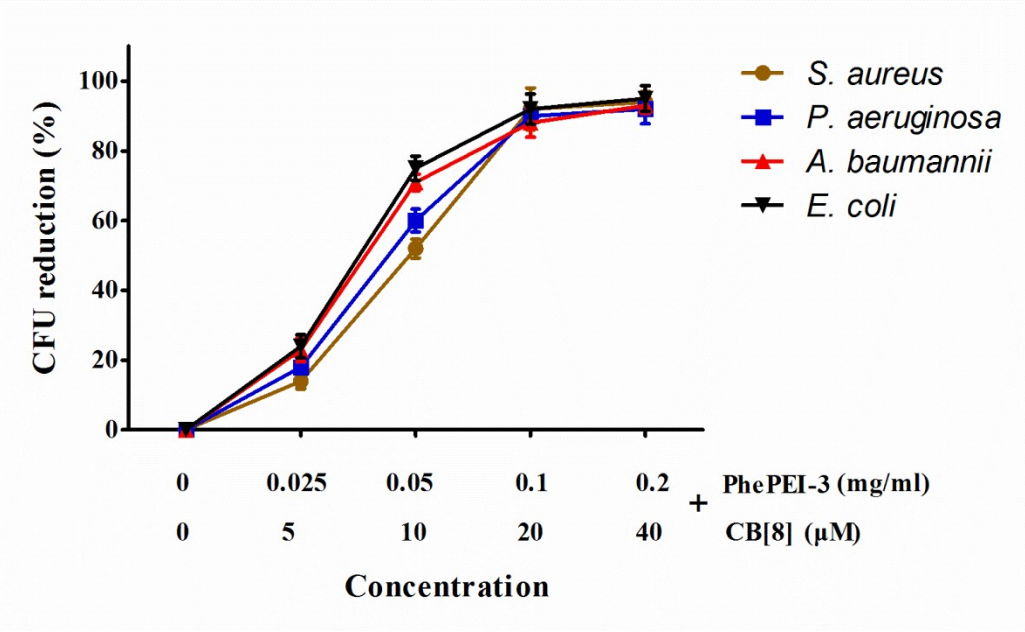


Fig. S7 Dose-dependent result of the antibacterial activity of PhePEI-3 with CB[8].

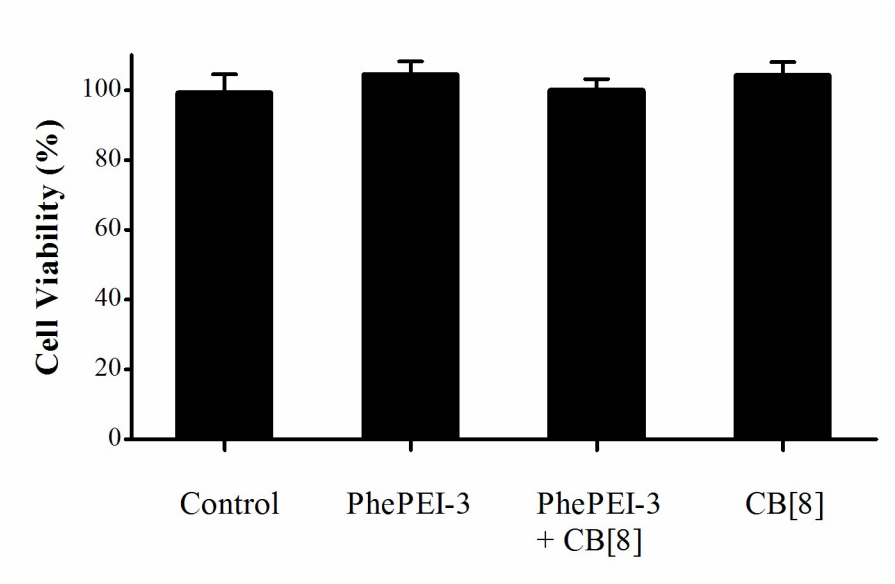


Fig. S8 MTT assay of PhePEI-3 (0.1 mg/ml) in the presence and absence of CB[8] (20 μM) by using RAW 264.7 cell line murine (macrophage from blood), 24h.

Reference

1 A. Day, A. P. Arnold, R. J. Blanch and B. Snushall, *J. Org. Chem.*, 2001, **66**, 8094-8100.