Structure–function mapping of host defense peptide mimics reveals strategies for antibacterial activity and eradication of mature bacterial biofilms

Xueyang Yin,[†,a] *Yufang Bi*,[†,a] *Xiehe Wang*,[†,a] *Jing Ren*,[b] *Jinrong Yao*,[*,a] *Xin Chen*,[a,c,d] *Shengjie Ling*,[*,a,c] *Zhengzhong Shao*[*,a,d]

[a]State Key Laboratory of Molecular Engineering of Polymers, Research Center of AI for Polymer Science, Department of Macromolecular Science, Fudan University, Shanghai 200433, China

[b]School of Physical Science and Technology, ShanghaiTech University, Shanghai 201210, China

[c]Stomatological Hospital & School of Stomatology, Fudan University, Shanghai 200433, China

[d]Laboratory of Advanced Materials, Fudan University, Shanghai 200433, China

E-mail: yaoyaojr@fudan.edu.cn (J. Yao); lingshengjie@fudan.edu.cn (S. Ling); zzshao@fudan.edu.cn (Z. Shao)

[†] These authors contributed equally.

S1 Definitions and Boundary

$$cPMI_{\text{gross}} = \frac{\sum m_{all\ inputs}}{m_{product}}$$

$$cPMI_{\text{net}}(r) = \frac{\sum m_{non-recoverables} + \sum(1-r_i)\, m_{\text{recoverables}}}{m_{product}}$$

Here 'all inputs' include reagents/acid scavenger/activator and the solvents/water explicitly reported in the manuscript. For net cPMI we account for solvent recovery with r_i=90% for THF and ethyl acetate; water is treated as non-recoverable.

Table S1. cPMI comparison between conventional α-pinene/HCl and epoxide-neutralized NCA synthesis routes.

Sample	cPMIgross	cPMInet(r=90%)
Moisture-Tolerant Epoxide-Neutralized Route (this work)	82.3	23.5
Conventional α-Pinene/HCl Route	210.1	133.8

(1) 'Materials PMI' reported in the main text excludes solvents/water; cPMI here complements it for full-process transparency.

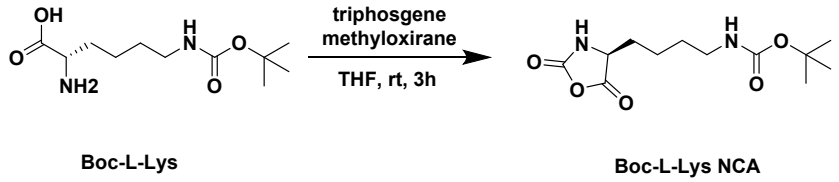
(2) If brine washes or recrystallization solvent volumes are later specified, cPMI scales linearly with the added mass: $\Delta cPMI_{gross}=V\rho/m_{product}$ and $\Delta cPMI_{net}=(1-r)V\rho/m_{product}$ for recoverable solvents (with recovery r).

S2 Methods of Cytotoxicity

NIH/3T3 cells (from the Chinese Academy of Sciences Cell Bank) were thawed, cultured, and passaged until normal cell viability was achieved. They were then dispersed in DMEM medium containing 10% fetal bovine serum and seeded at a density of 8,000 cells per well in a 96-well plate. After incubating at 37 °C in a CO_2 incubator for 1 day, the supernatant was carefully removed. Polymer stock solution (200 μg mL^{-1}) was diluted with DMEM and carefully transferred to the cell-seeded plates. After 24 hours of incubation, prepare a 10% CCK-8 solution in the dark and

replace the polymer-medium mixture in the wells. Incubate at 37 °C for 2 hours, then read the absorbance at 450 nm using a multifunctional microplate reader. Use wells containing only medium without cells as the blank group, and wells incubated with medium alone as the positive control group. Cell viability was calculated using formula.

$$\text{Bacterial viability (\%)} = \frac{A_{450,\text{sample}} - A_{450,\text{blank}}}{A_{450,\text{control}} - A_{450,\text{blank}}} \times 100\%$$



Boc-L-Lys　　Boc-L-Lys NCA

Fig. S1. Synthesis of Nε-tert-butyloxycarbonyl-L-lysine NCA (Boc-L-Lys NCA).

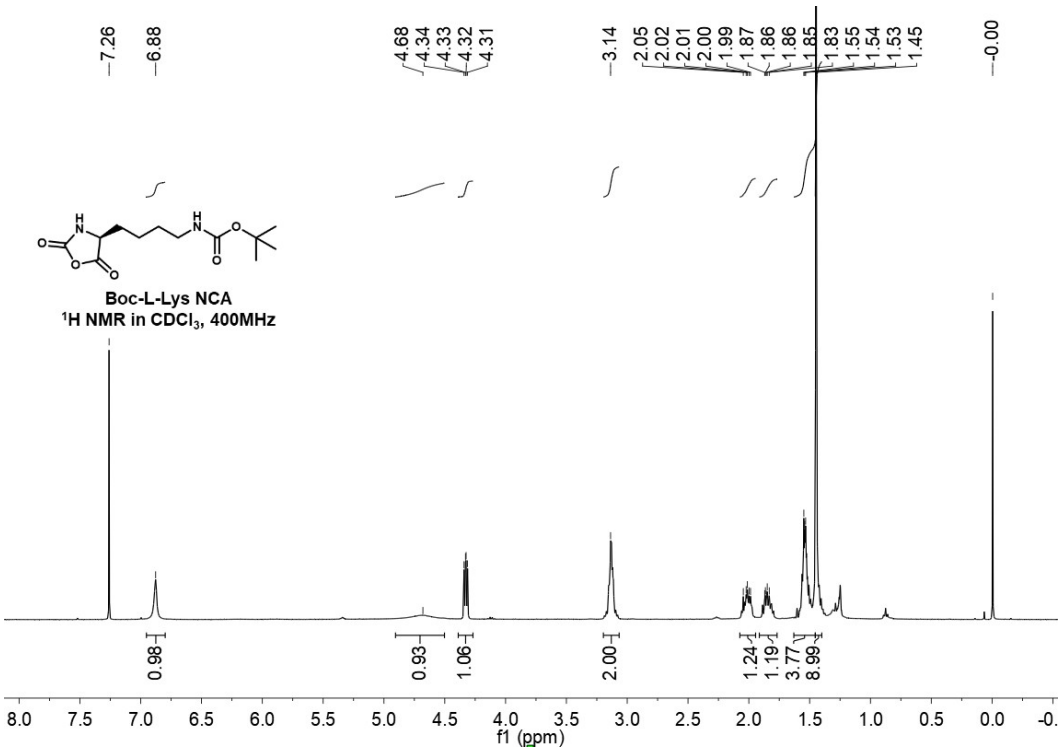


Fig. S2. ^{1}H NMR spectrum of Boc-L-Lys NCA in $CDCl_3$ (400MHz).

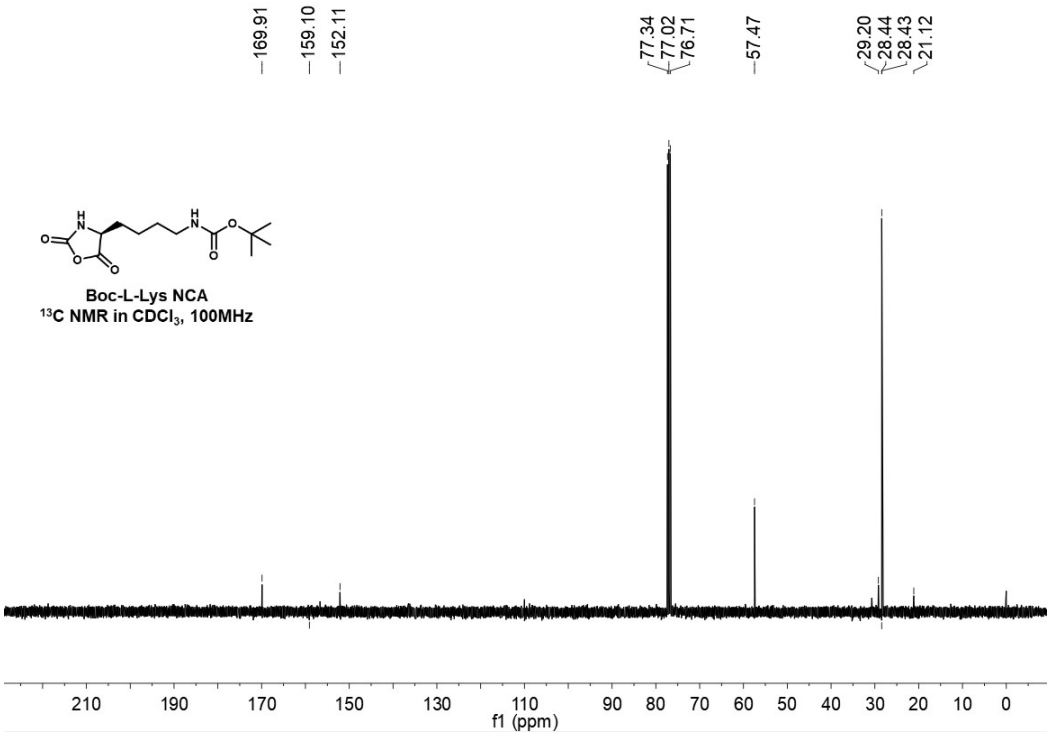


Fig. S3. ^{13}C NMR spectrum of Boc-L-Lys NCA in $CDCl_3$ (100MHz).

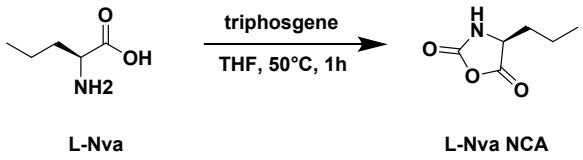


Fig. S4. Synthesis of L-Norvaline NCA (L-Nva NCA).

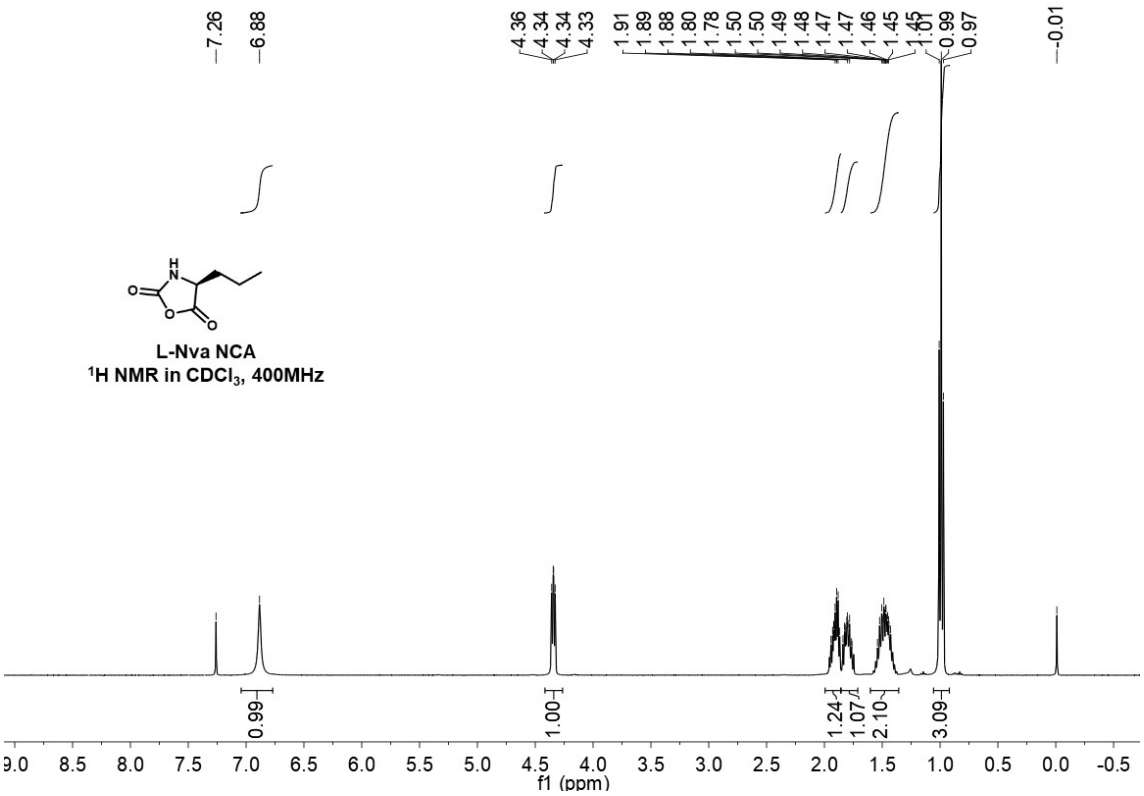


Fig. S5. ^{1}H NMR spectrum of L-Nva NCA in $CDCl_3$ (400MHz).

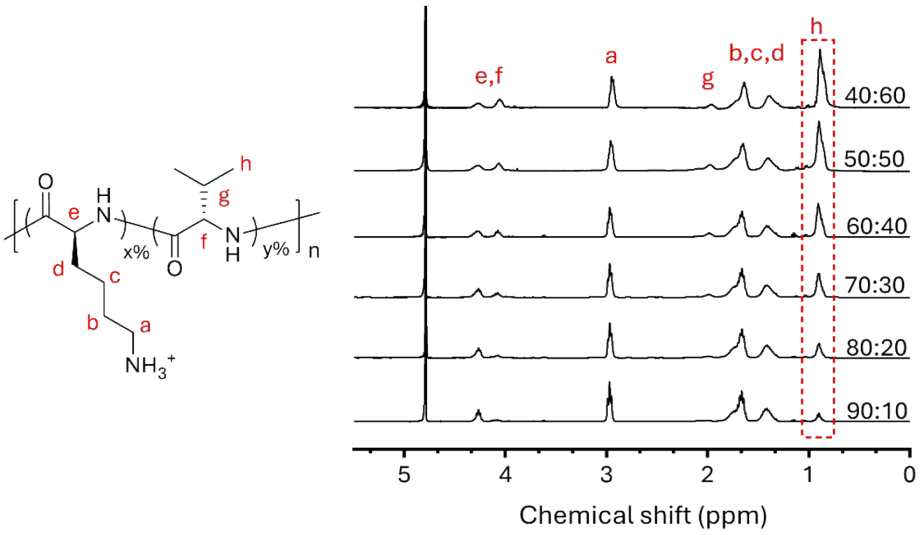


Fig. S6. ^{1}H NMR spectrums (D_2O, 400 MHz) of Lys_xVal_y (x + y = 100, x = 40, 50, 60, 70, 80, and 90). Reproduced from Ref. 52, with permission from the Royal Society of Chemistry.

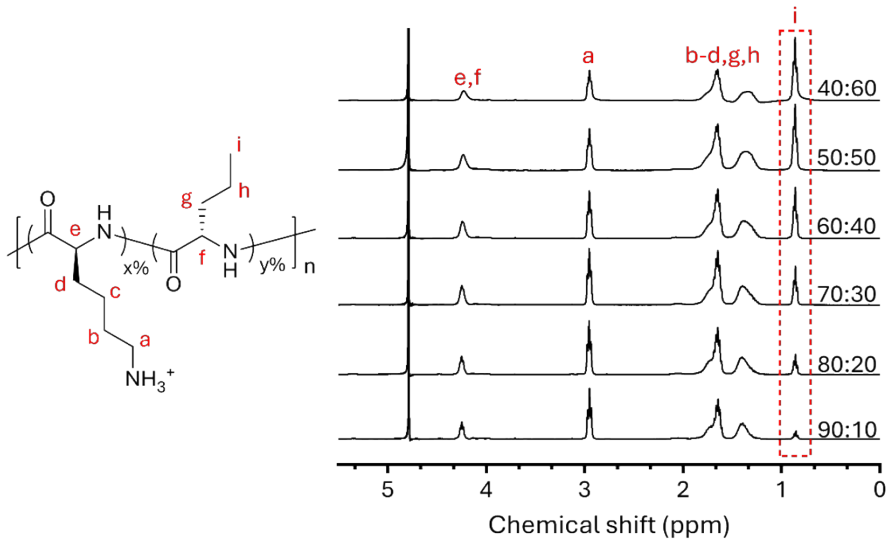


Fig. S7. ^{1}H NMR spectrums (D_2O, 400 MHz) of Lys_xNva_y (x + y = 100, x = 40, 50, 60, 70, 80, and 90).

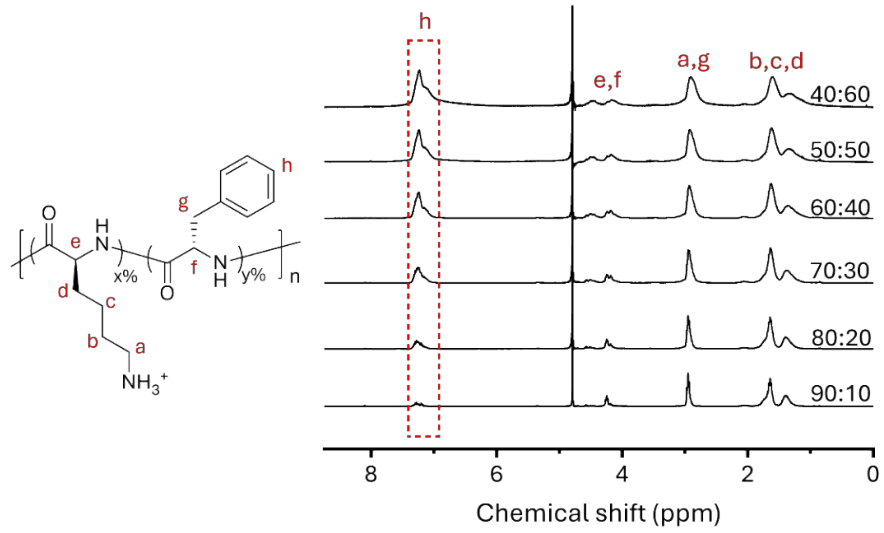


Fig. S8. ^{1}H NMR spectrums (D_2O, 400 MHz) of Lys_xPhe_y (x + y = 100, x = 40, 50, 60, 70, 80, and 90).

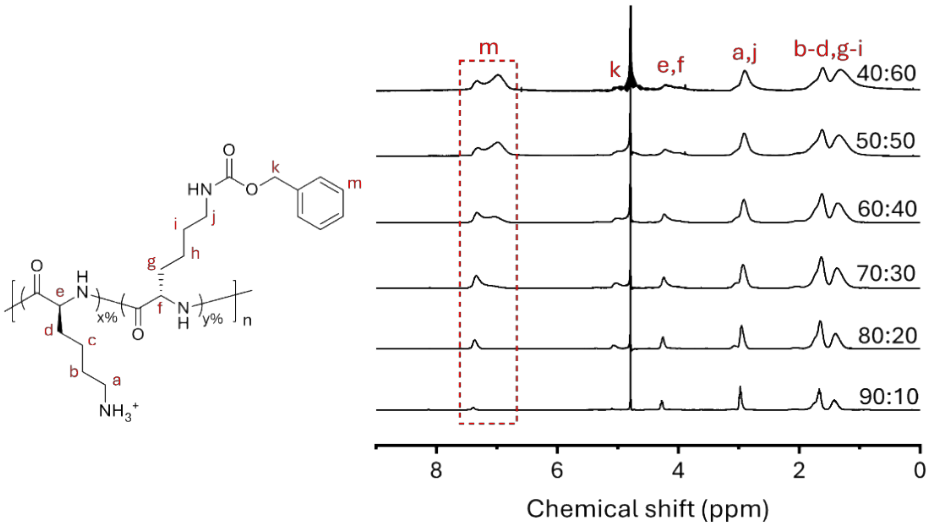


Fig. S9. ^{1}H NMR spectrums (D_2O， 400 MHz) of Lys_xCBL_y (x + y = 100, x = 40, 50, 60, 70, 80, and 90).

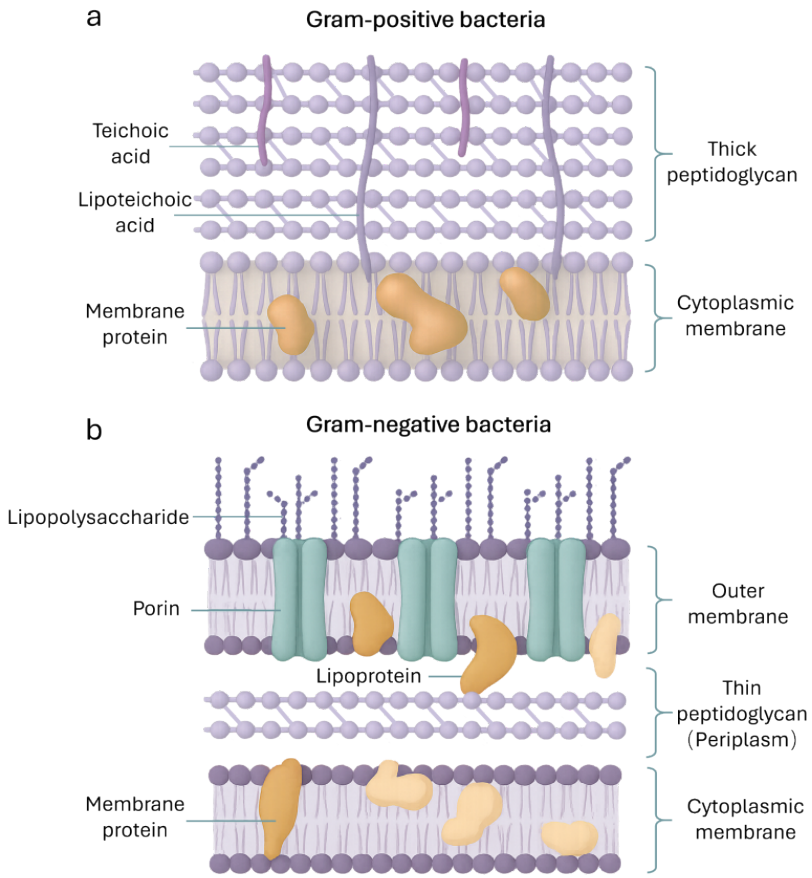


Fig. S10. Bacterial membrane structures of gram-positive (a) and gram-negative bacteria (b).

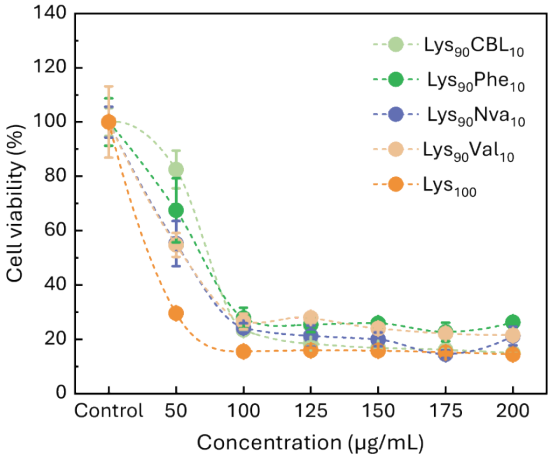


Fig. S11. Viability of NIH/3T3 cells after incubating with cationic peptide polymers. Cytotoxicity varies with hydrophobic pendant chemistry at equal charge density, demonstrating that pendant identity can be toggled to modulate mammalian toxicity. Dashed lines are guides to the eye.