Electronic Supplementary Material (ESI) for Biomaterials Science.

Supplementary Information

Biodegradable Peptide Polymers as Alternatives to Antibiotics Used in Aquaculture

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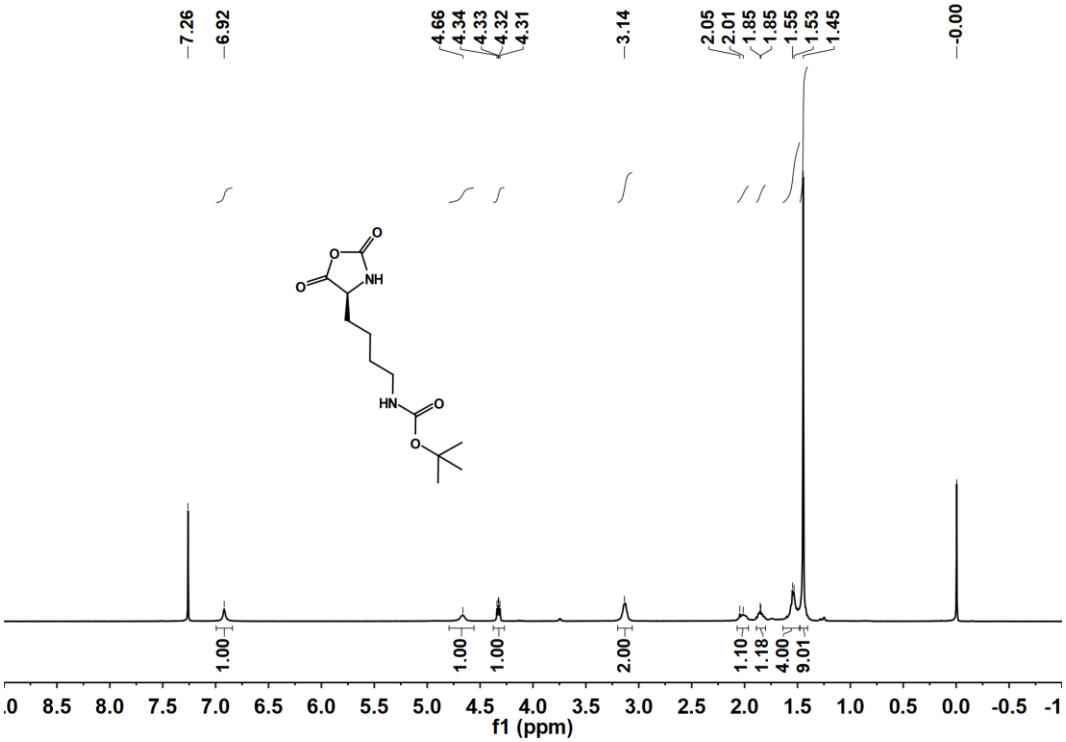
Materials

Anhydrous tetrahydrofuran (THF) and lithium hexamethyldisilazide (LiHMDS) were purchased from Sigma Aldrich and used without further purification; triphosgene and protease XXIII from *Aspergillus oryzae* were purchased from Shanghai Titan Technology Co., Ltd.; chymotrypsin was purchased from Shanghai Aladdin Biochemical Technology Co., Ltd.; trypsin, Nε-*tert*-butyloxycarbonyl-L-lysine and O-*tert*-butyl-L-serine were purchased from Bide Pharmatech Ltd; α-pinene was purchased from J&K Scientific Ltd; other solvents and reagents were purchased from Shanghai Adamas Reagent; all solvent used for N-carboxyanhydride (NCA) purification were dried over $MgSO_4$.

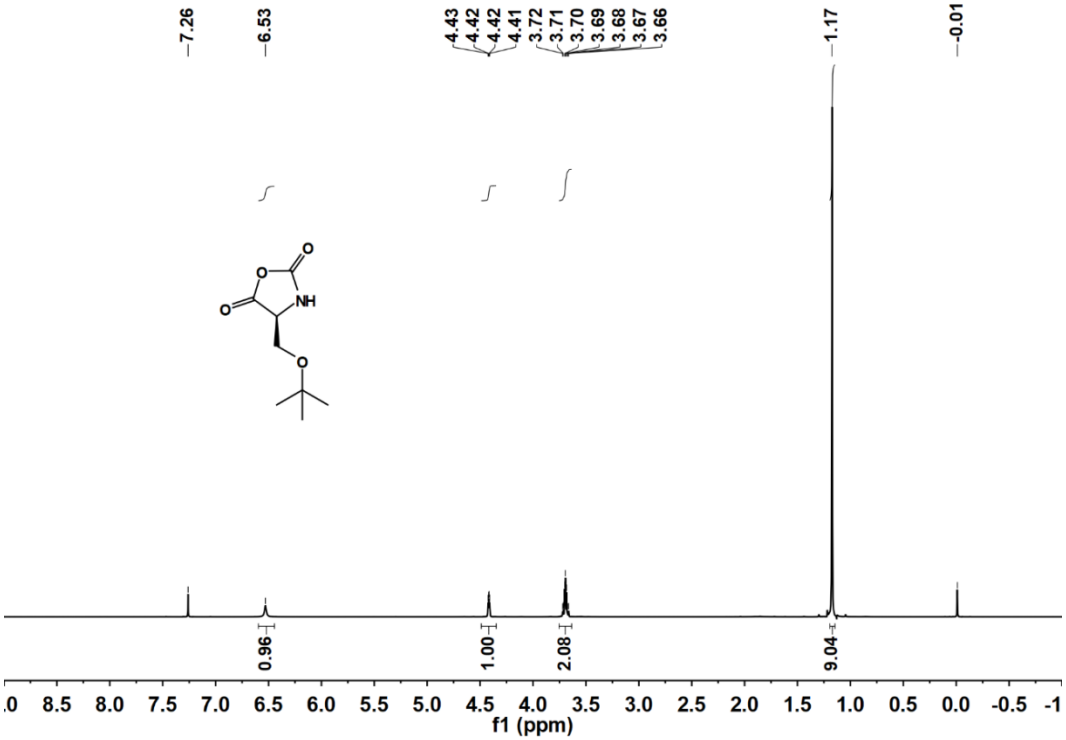
Instruments

^1H NMR spectra were recorded on a Bruker spectrometer at 400 MHz, using D_2O or $CDCl_3$ as the solvent. The chemical shifts are referred to the residual protons in the D_2O or $CDCl_3$. Gel permeation chromatography (GPC) was performed on a Waters GPC instrument equipped with a Waters 1515 isocratic HPLC pump and a Waters 2414 refractive index detector using DMF supplemented with 0.01 M LiBr as the mobile phase at a flow rate of 1 mL/min at 50 ℃. The GPC was equipped by a Tosoh TSKgel Alpha-2500 column (particle size 7 μm), a Tosoh TSKgel Alpha-3000 column (particle size 7 μm) and a Tosoh TSKgel Alpha-4000 column (particle size 10 μm) linked in series. Relative number-average molecular weight (M_n) and dispersion index ($Đ$) were calculated from the calibration curve with polymethylmethacrylate (PMMA) as the standard. The synthesized morpholino naphthalimide fluorophore and reaction intermediates were purified using a SepaBean machine equipped with a Sepaflash columns manufactured by Santai Technology Inc. in China. High resolution electrospray ionization time-of-flight mass spectrometry (HRESI-MS) was obtained on a Waters XEVO G2 TOF mass spectrometer. Time-laps fluorescent confocal imaging was performed by using LSM 880 with Airyscan system in ZEISS Microscopy Shanghai Customer Center.

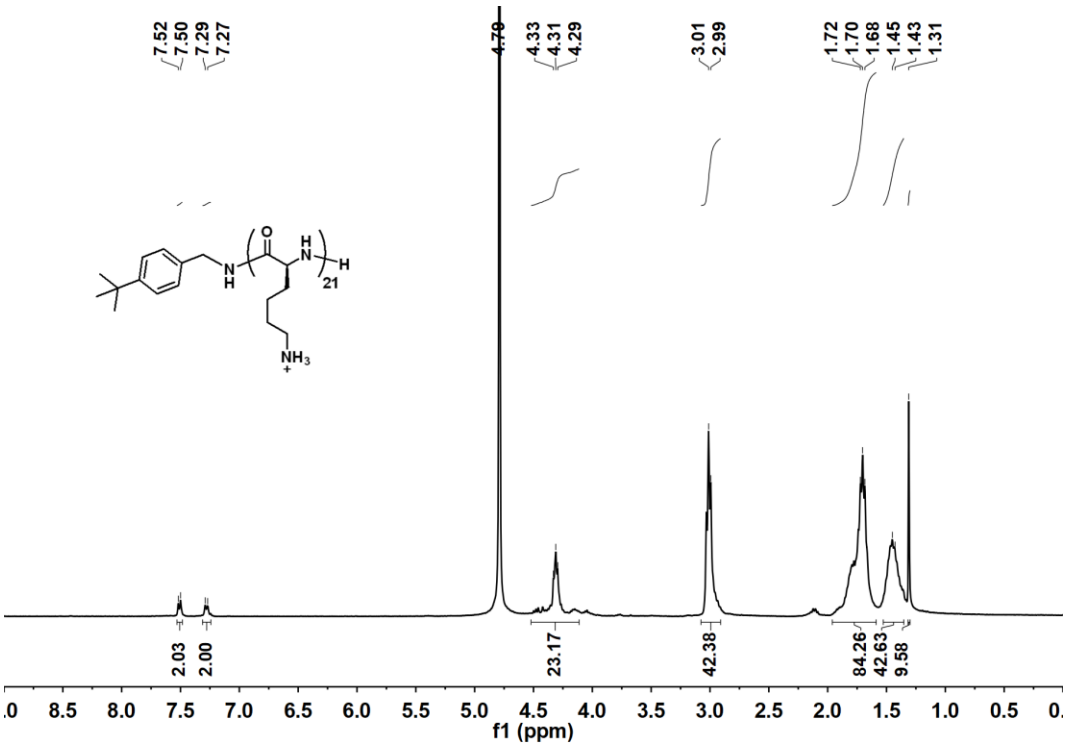
Figures



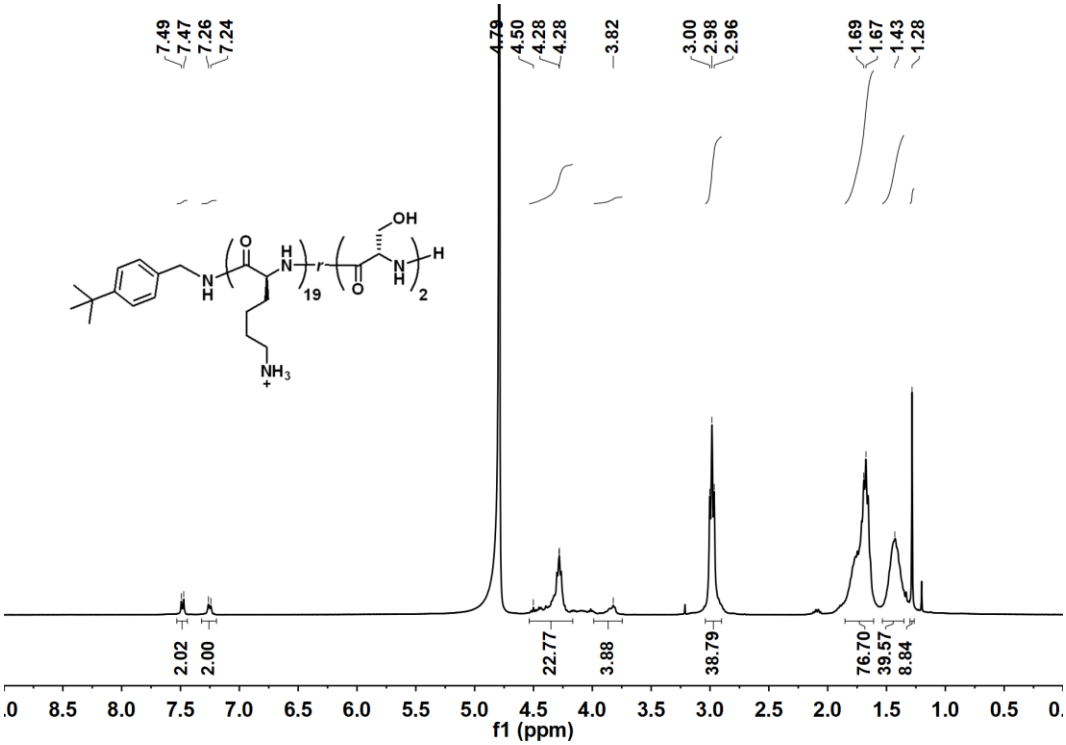
Supplementary Figure 1: ^{1}H NMR of Boc-L-Lys NCA in $CDCl_3$, 400 MHz.



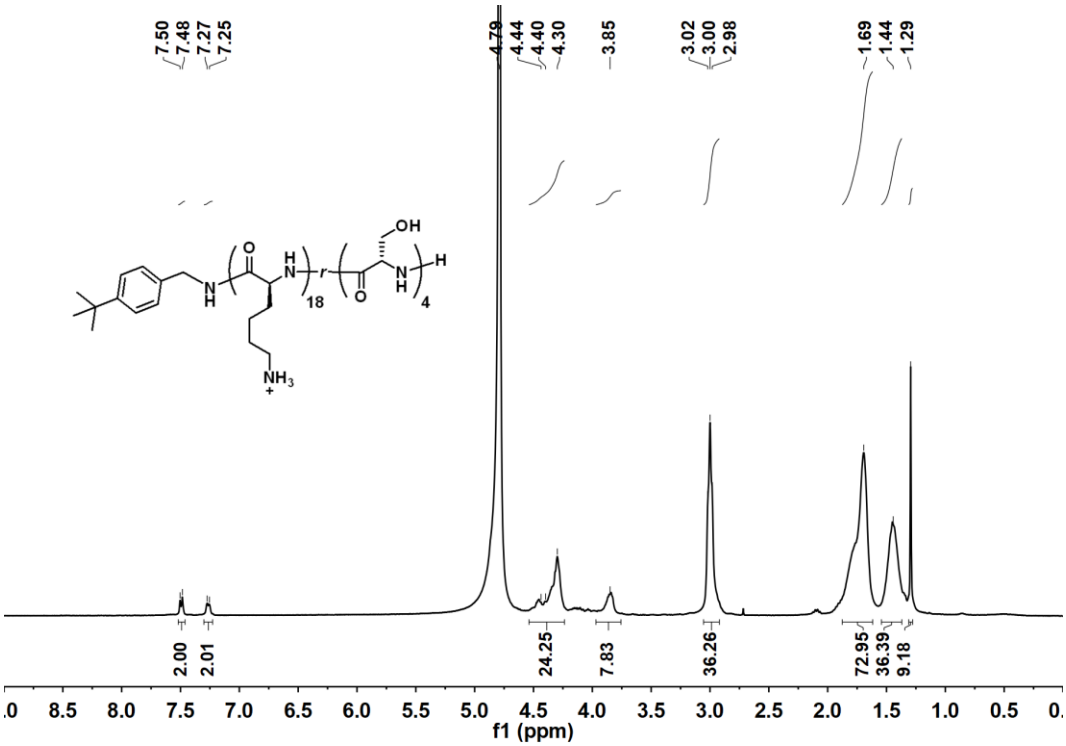
Supplementary Figure 2: ^{1}H NMR of *t*Bu-L-Ser NCA in $CDCl_3$, 400 MHz.



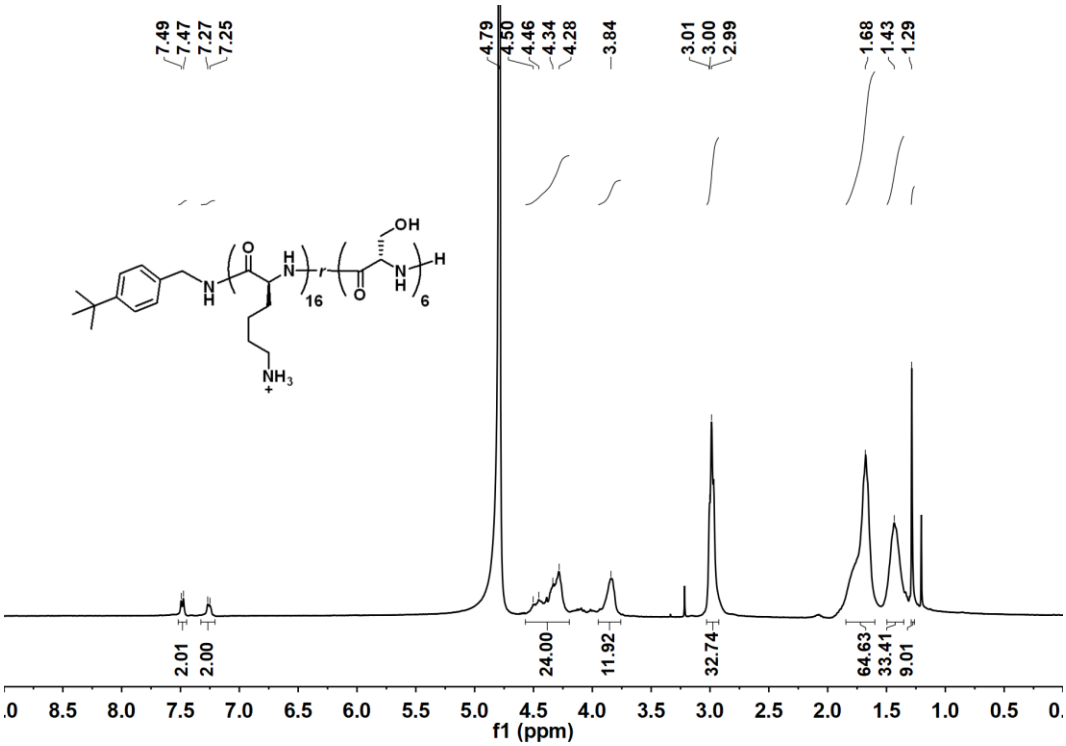
Supplementary Figure 3: ^{1}H NMR of C-terminus functionalized K_{21} in D_2O, 400 MHz.



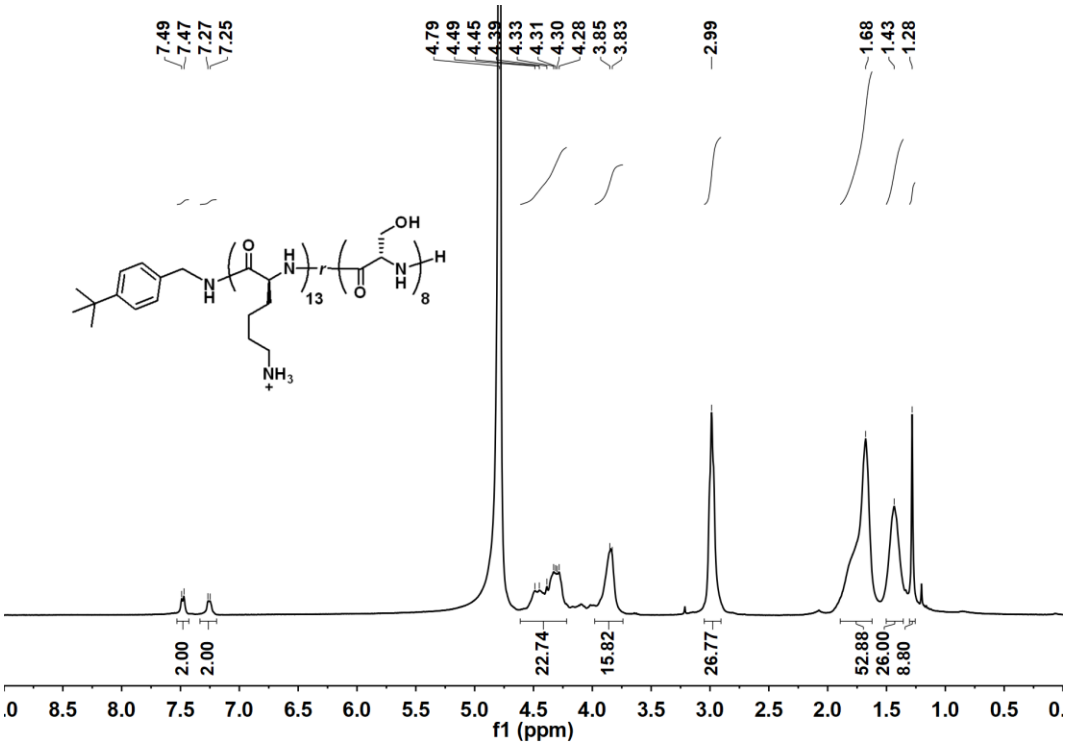
Supplementary Figure 4: ^{1}H NMR of C-terminus functionalized $K_{19}S_2$ in D_2O, 400 MHz.



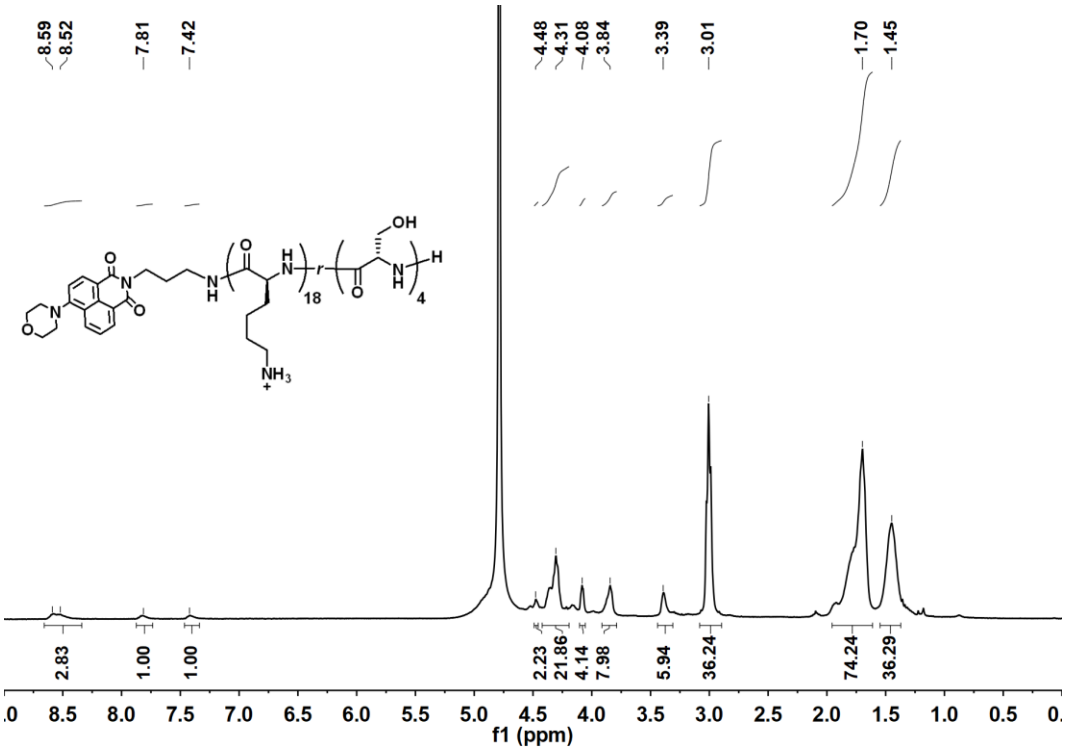
Supplementary Figure 5: ^{1}H NMR of C-terminus functionalized $K_{18}S_4$ in D_2O, 400 MHz.



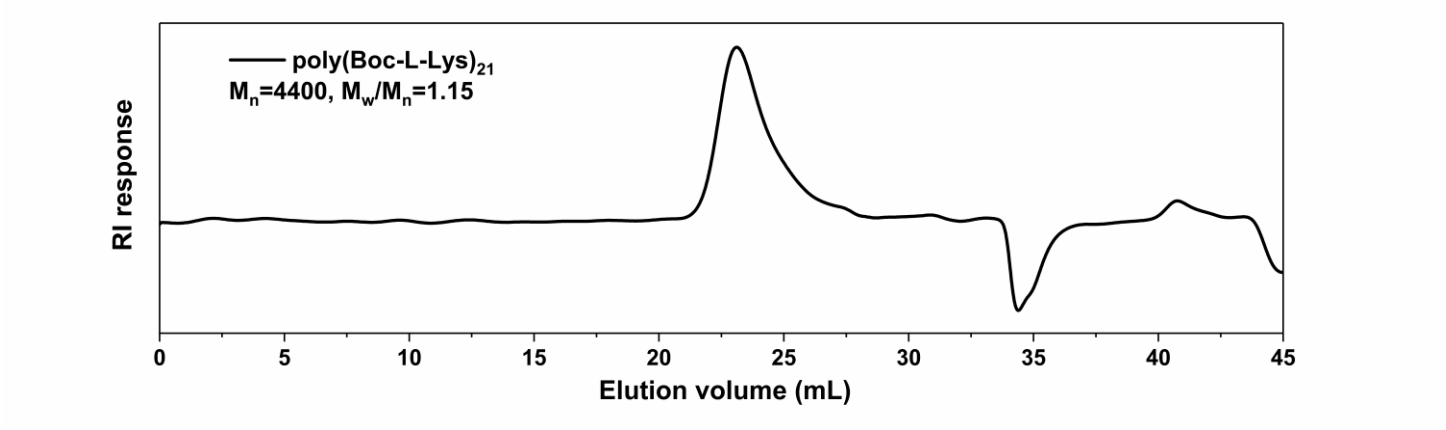
Supplementary Figure 6: ^{1}H NMR of C-terminus functionalized $K_{16}S_6$ in D_2O, 400 MHz.



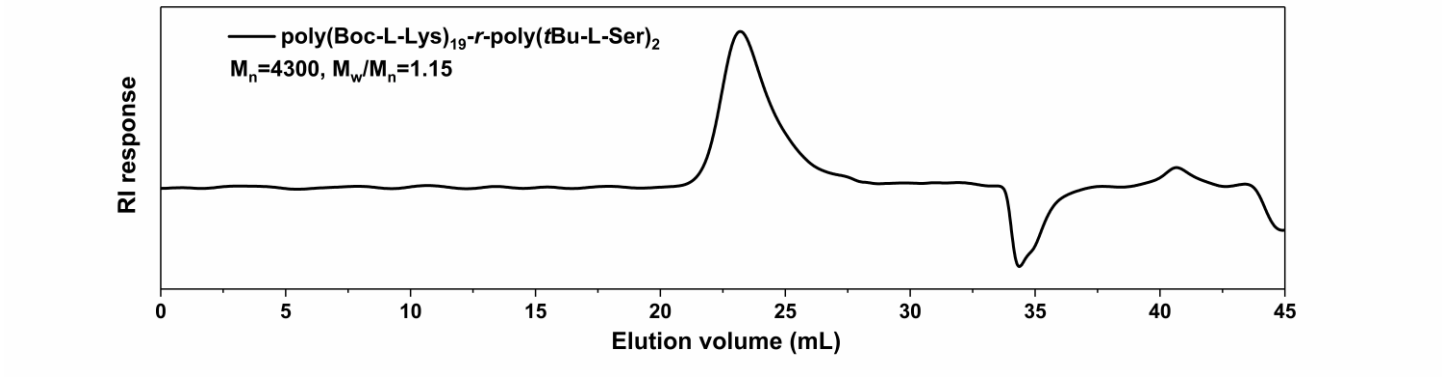
Supplementary Figure 7: ^{1}H NMR of C-terminus functionalized $K_{13}S_8$ in D_2O, 400 MHz.



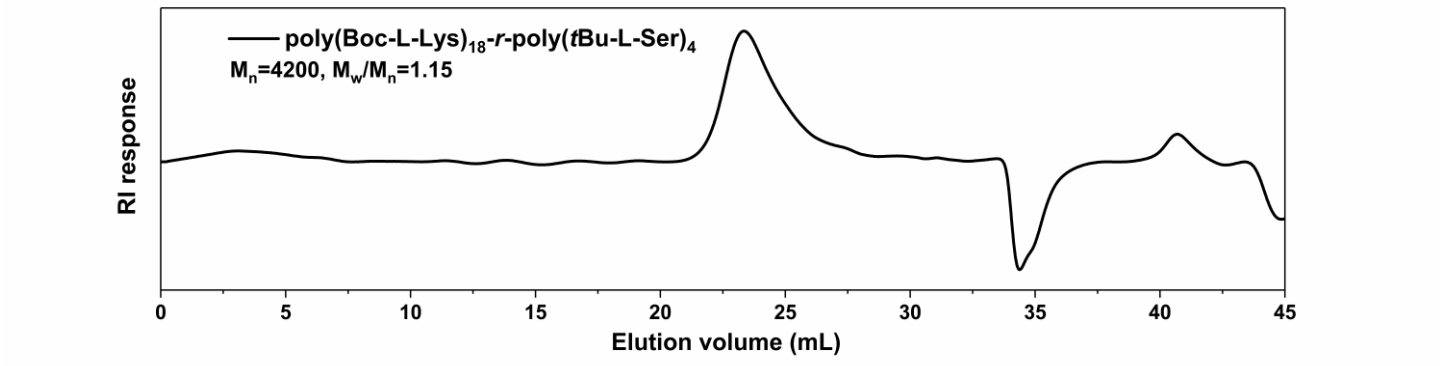
Supplementary Figure 8: ^{1}H NMR of Dye-$K_{18}S_4$ in D_2O, 400 MHz.



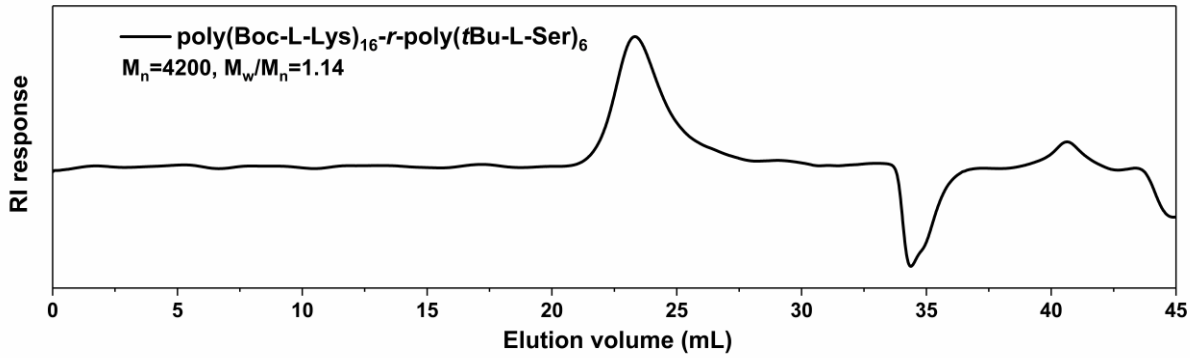
Supplementary Figure 9: GPC trace of poly(Boc-L-Lys)$_{21}$ using DMF as the mobile phase at a flow rate of 1 mL/min.



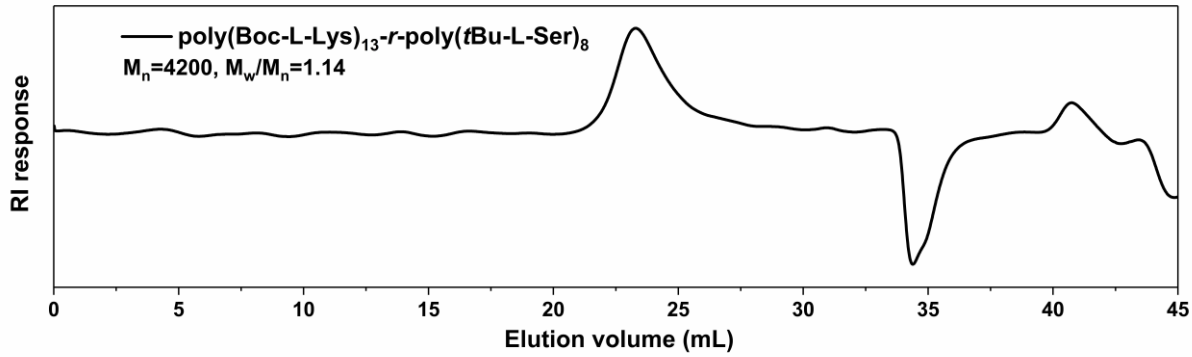
Supplementary Figure 10: GPC trace of poly(Boc-L-Lys)$_{19}$-*r*-poly(*t*Bu-L-Ser)$_2$ using DMF as the mobile phase at a flow rate of 1 mL/min.



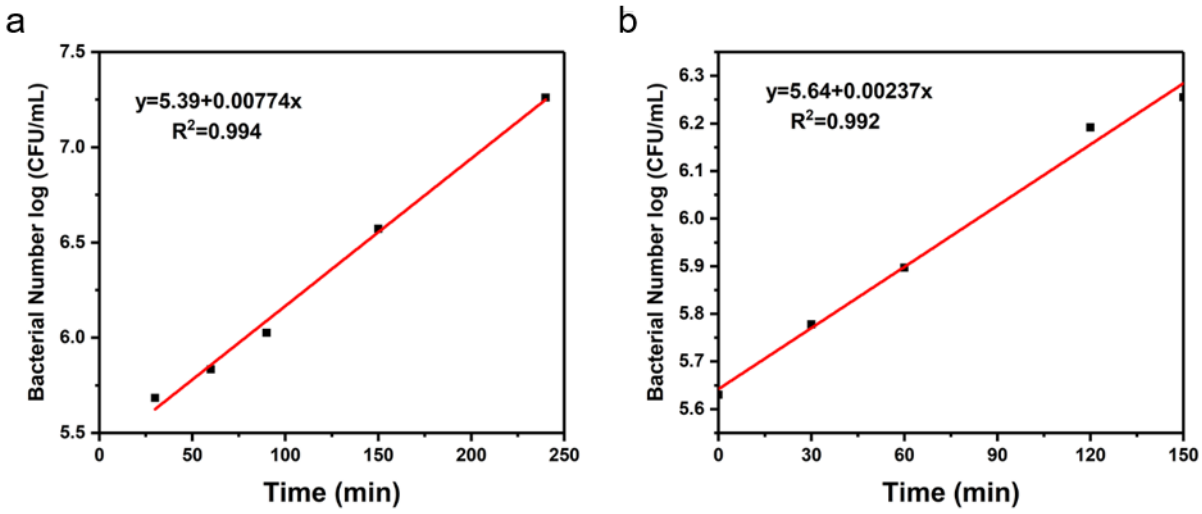
Supplementary Figure 11: GPC trace of poly(Boc-L-Lys)$_{18}$-*r*-poly(*t*Bu-L-Ser)$_4$ using DMF as the mobile phase at a flow rate of 1 mL/min.



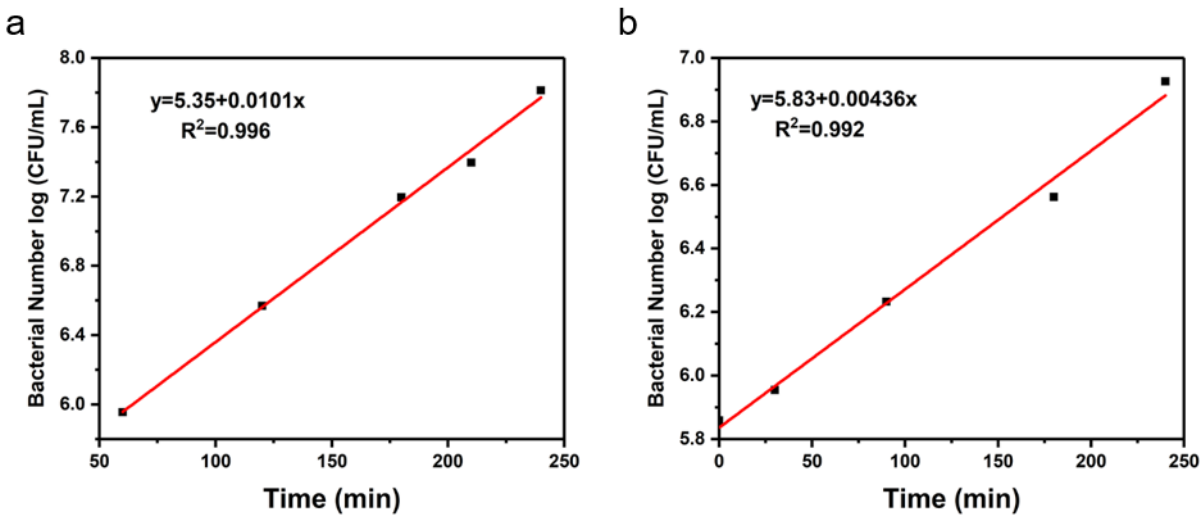
Supplementary Figure 12: GPC trace of poly(Boc-L-Lys)$_{16}$-*r*-poly(*t*Bu-L-Ser)$_6$ using DMF as the mobile phase at a flow rate of 1 mL/min.



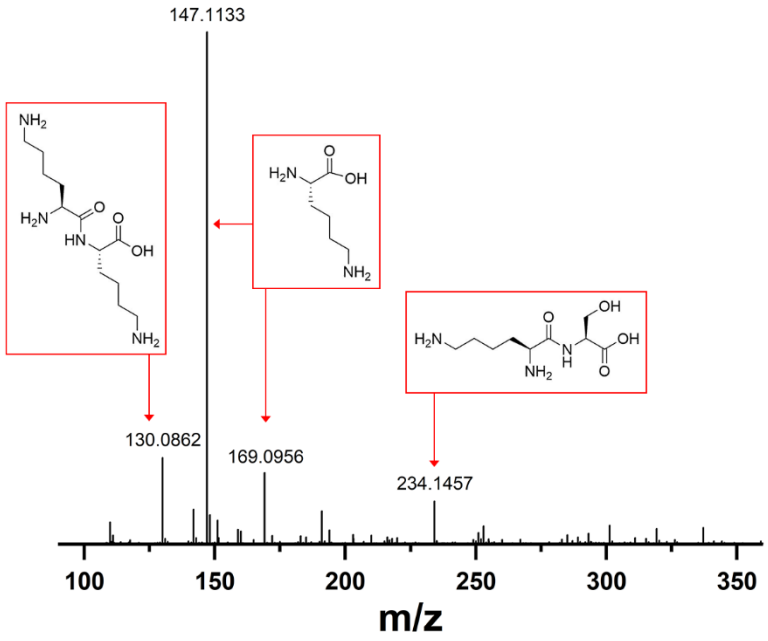
Supplementary Figure 13: GPC trace of poly(Boc-L-Lys)$_{13}$-*r*-poly(*t*Bu-L-Ser)$_8$ using DMF as the mobile phase at a flow rate of 1 mL/min.



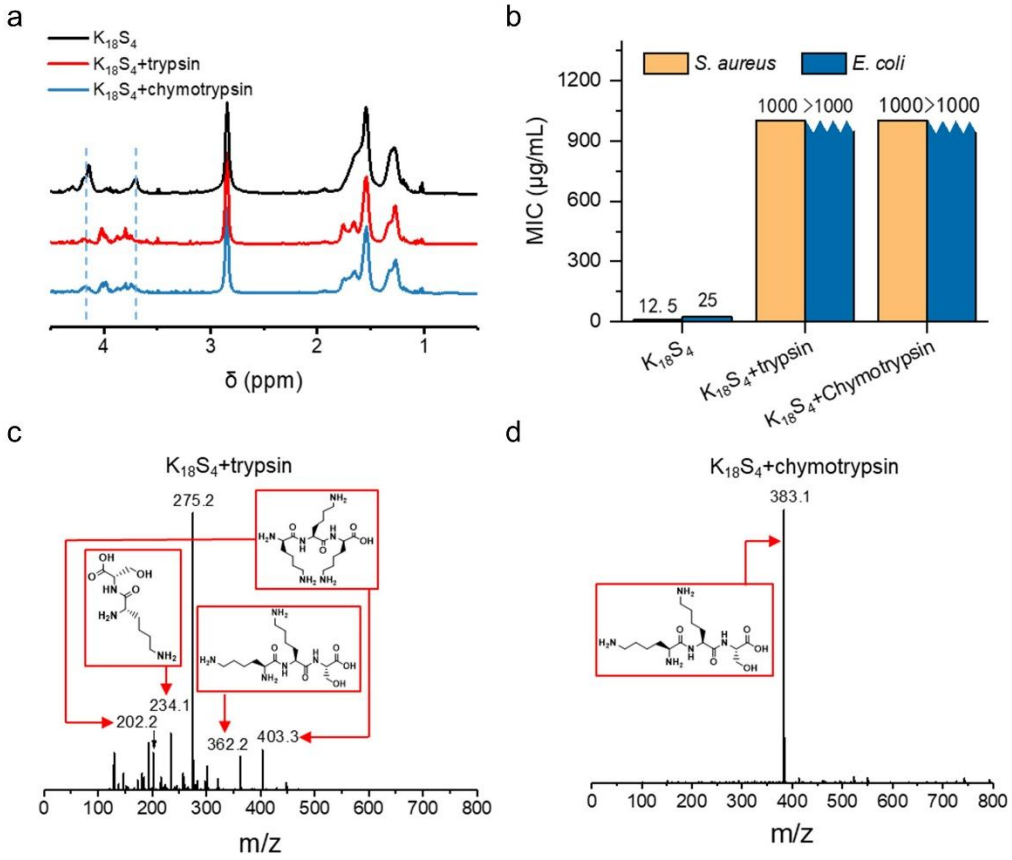
Supplementary Figure 14: The growth rate of *S. aureus* in M-H medium in the presence of $K_{18}S_4$ (a) or norfloxacin (b) at 1/2 MBC.



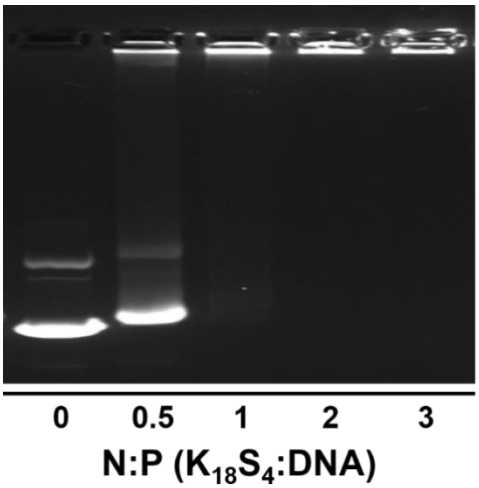
Supplementary Figure 15: The growth rate of *E. coli* in M-H medium in the presence of $K_{18}S_4$ (a) or ampicillin (b) at 1/2 MBC.



Supplementary Figure 16: The HRESI-MS spectrum of $K_{18}S_4$ after being degraded by protease XXIII for 6 hours.



Supplementary Figure 17: a) NMR spectra of $K_{18}S_4$ after 12 hours of degradation in the presence of trypsin or chymotrypsin, respectively. b) The MIC value change of $K_{18}S_4$ before and after enzymatic degradation in the presence of trypsin and chymotrypsin, respectively. c, d) The ESI-MS spectrum of $K_{18}S_4$ after being degraded by trypsin and chymotrypsin, respectively.



Supplementary Figure 18: The electrophoretic mobility shift assay of plasmid DNA and the mixture of $K_{18}S_4$ with plasmid DNA complexes at different ratios of N:P ($K_{18}S_4$:DNA).

Table

Supplementary Table 1: The minimum inhibitory concentration of the optimal peptide polymer $K_{18}S_4$ and amphotericin B against *Cryptococcus neoformans* and *Candida albicans*.

Strain	MIC (μg/mL)	
	$K_{18}S_4$	amphotericin B
C.neo MYA737	1.56	1.56
C.neo JEC21	1.56	1.56
C.neo H99	3.13	1.56
C.alb SC5314	>200	1.56
C.alb MYA737	>200	1.56
C.alb K1	>200	1.56

Supplementary Table 2: Acute toxicity of K_{21} and $K_{18}S_4$ to adult zebrafish in different time

Time (h)	K_{21}		$K_{18}S_4$	
	LC_{50} (μg/mL)	95 % confidence limit (μg/mL)	LC_{50} (μg/mL)	95 % confidence limit (μg/mL)
24	23.10	10.11-34.93	99.64	88.34-109.98
48	20.51	12.96-27.17	98.90	87.64-109.23
72	20.26	13.17-26.73	98.90	87.64-109.23
96	19.61	12.24-26.05	98.14	87.13-108.20