Supplemental Information

Host defense peptide mimicking poly-β-peptides with fast, potent and broad spectrum antibacterial activities

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Synthesis of β-lactam monomers.

DMβ(±)-3-tert-Butyloxycarbonylaminomethyl-4,4-dimethyl azetidin-2-one **(1).** Compound 1 was prepared by following the procedure in literature.[1] The pure product of compound 1 was obtained as a white solid in 34% yield over 4 steps. 1H NMR (400 MHz, CDCl3): δ 6.32 (s, 1H), 5.00 (s, 1H), 3.58 (dt, J = 13.7, 7.0 Hz, 1H), 3.26 (t, J = 9.6 Hz, 1H), 2.94 (t, J = 7.7 Hz, 1H), 1.42 (d, J = 4.9 Hz, 12H), 1.34 (s, 3H).

Buβ(±)-4-Butyl-azetidine-2-one **(2).** Compound 2 was prepared by following the procedure in literature.[2] The pure product of compound 2 was obtained as a clear oil in 46% yield. 1H NMR (400 MHz, CDCl3): δ 6.95 (s, 1H), 3.58-3.40 (m, 1H), 2.93 (ddd, J = 14.7, 4.9, 2.0 Hz, 1H), 2.43 (ddd, J = 14.8, 2.3, 1.2 Hz, 1H), 1.62-1.39 (m, 2H), 1.31-1.09 (m, 4H), 0.81 (t, J = 7.0 Hz, 3H).

Synthesis poly-β-peptides.

The synthesis of a representative poly-β-peptide (20:80 Bu:DM, target length at 20-mer) is described below, and all other poly-β-peptides were synthesized similarly. Polymerization was performed in a nitrogen purged glove box at room temperature. Two β-lactams Bu (10.2 mg, 0.08 mmol) and DM (73.1 mg, 0.32 mmol) were mixed and dissolved in anhydrous THF (1 mL). After adding co-initiator *t*BuBzCl in THF (0.5 mL, 0.02 mmol) and the base catalyst LiHMDS in THF (0.5 mL, 0.06 mmol) into the reaction sequentially, the mixture was stirred overnight. Then the reaction mixture was removed from the glove box and quenched with MeOH. The crude product was purified by repeated dissolution-precipitation-centrifugation using THF and cold hexane. Finally, the side-chain NHBoc protected poly-β-peptide was obtained from vacuum drying. The side-chain NHBoc protected poly-β-peptide was used for GPC characterization using N,N-dimethylformamide (DMF) as the mobile phase. The side-chain NHBoc protection groups were removed by dissolving the sample in pure TFA (2mL) and shaking for 2 hours. After solvent was removed under air flow, the mixture was dissolved in MeOH (0.5 mL). Cold Et_2O (45 mL) was poured into the solution to precipitate out a white fluffy solid. This precipitate was collected by centrifugation, removal of the supernatant and drying under air flow. After three dissolution-precipitation-centrifugation cycles and drying

under vacuum, the final deprotected poly-β-peptide was collected as a white powder in a form of TFA salt.

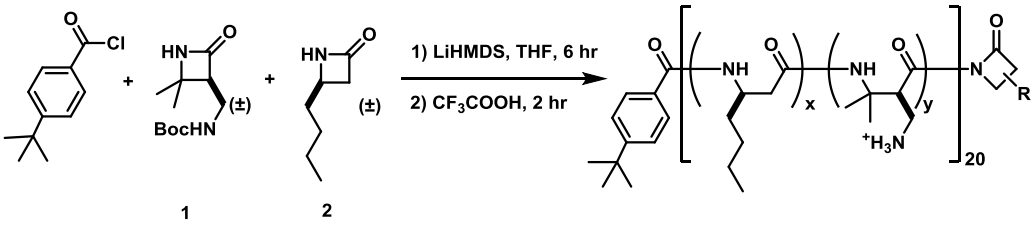


Figure S1. Synthesis of 20-mer Bu:DM series poly-β-peptides. x+ y = 1, x = 0.1, 0.2, 0.3 or 0.4. R represents the side chain of either Bu or DM subunit. All β-lactams are racemic mixtures and the resulting poly-β-peptides are heterochiral.

Cytoplasmic membrane depolarization assay.[3]

Staphylococcus aureus (*S. a*) USA300 and *Pseudomonas aeruginosa* (*P. a*) O1 was inoculated and cultured at 37 °C for 10 hr in LB medium, and then bacterial cells were diluted in HEPES buffer (5 mM HEPES, 20 mM glucose, pH=7.4) to generate the working suspension at 1×10^6 CFU/mL. Then the suspension was incubated with 0.4 μM diSC3(5) for 1 hour. KCl was added into the suspension at a final concentration of 100mM to balance the cytoplasmic and external K^+. An aliquot of 90 μL the suspension was added to each well of a 384-well plate. Changes in fluorescence were recorded (excitation λ= 622 nm, emission λ= 673 nm) on a SpectraMax® M2 plate reader (Molecular Devics, USA). When fluorescence intensity almost keep steady, 10 μL antimicrobial agent was added into the system (Time=0 s) and fluorescence signals were recorded continuously.

Outer membrane permeabilization assay.[4]

1-N-phenyl-naphthylamine (NPN) was used in the experiment as a fluorescent probe. Fresh cultured *P. a O1* cells were used to prepare the working suspension at 1×10^9 CFU/mL followed by addition of NPN at a final concentration of 10 μM. An aliquot of 90 μL of the suspension was added to each well of a 384-well plate. Changes in fluorescence were recorded (excitation λ= 350 nm, emission λ= 420 nm) on a SpectraMax® M2 plate reader (Molecular Devics, USA). When fluorescence intensity almost keep steady, 10 μL antimicrobial agent was added into the system (Time=0 s) and fluorescence signals were recorded continuously.

Cytoplasmic membrane permeabilization assay.[5]

Propidium Iodide (PI) was used in experiment as a fluorescent probe (excitation λ= 535 nm, emission λ= 617 nm). *S. a* and *P. a* were used to prepare suspensions, and the procedure was the same as the outer membrane permeabilization assay.

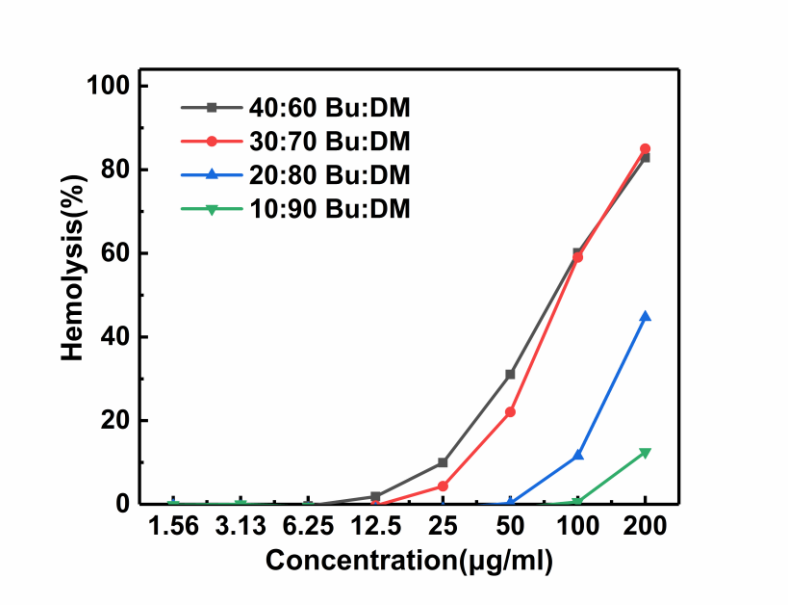


Figure S2. RBC hemolysis for Bu:DM series poly-β-peptides.

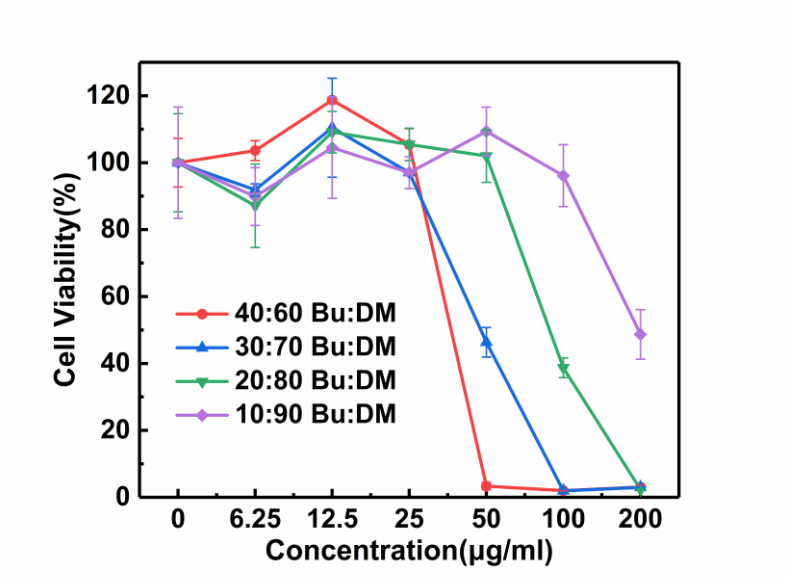


Figure S3. Cytotoxicity of Bu:DM series poly-β-peptides toward NIH 3T3 fibroblast cells.

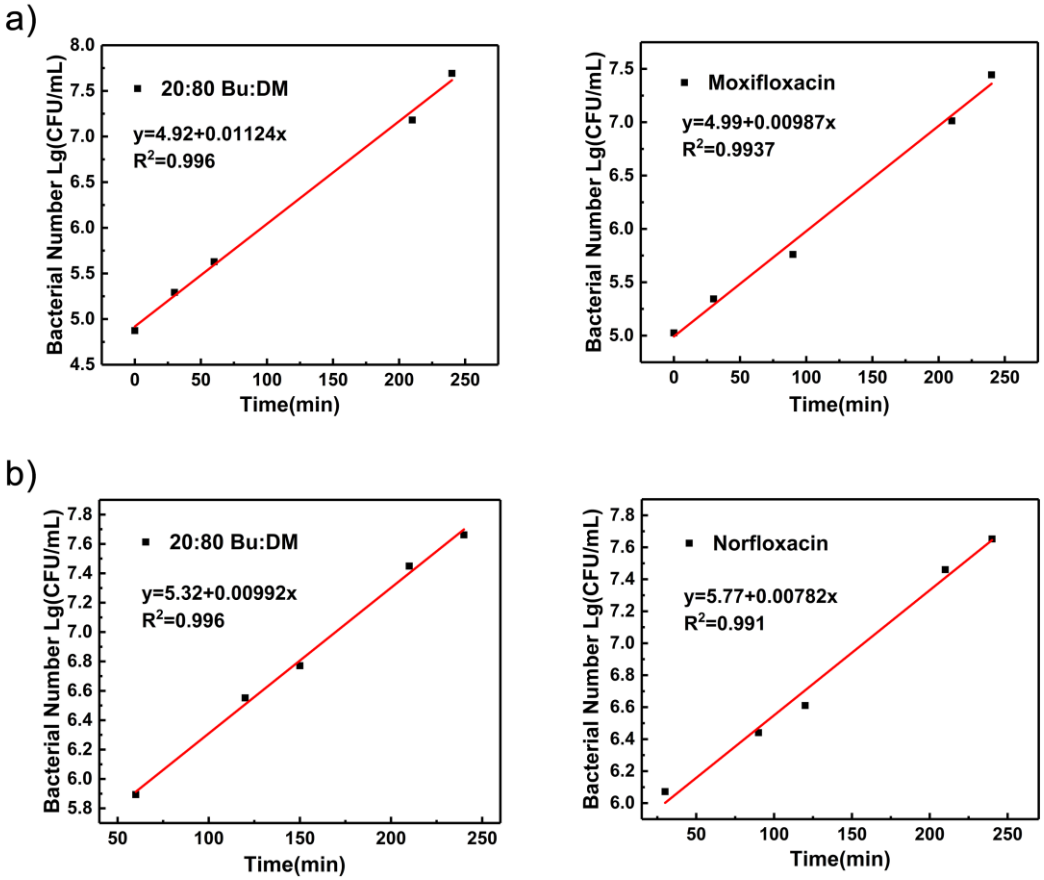


Figure S4. Rate of bacteria growth in the presence of antimicrobial agents. a) *P. a* ATCC9027 growth in the presence of poly-β-peptide 20:80 Bu:DM or moxifloxacin hydrochloride. b) *S. a* ATCC6538 growth in the presence of poly-β-peptide 20:80 Bu:DM or norfloxacin hydrochloride.

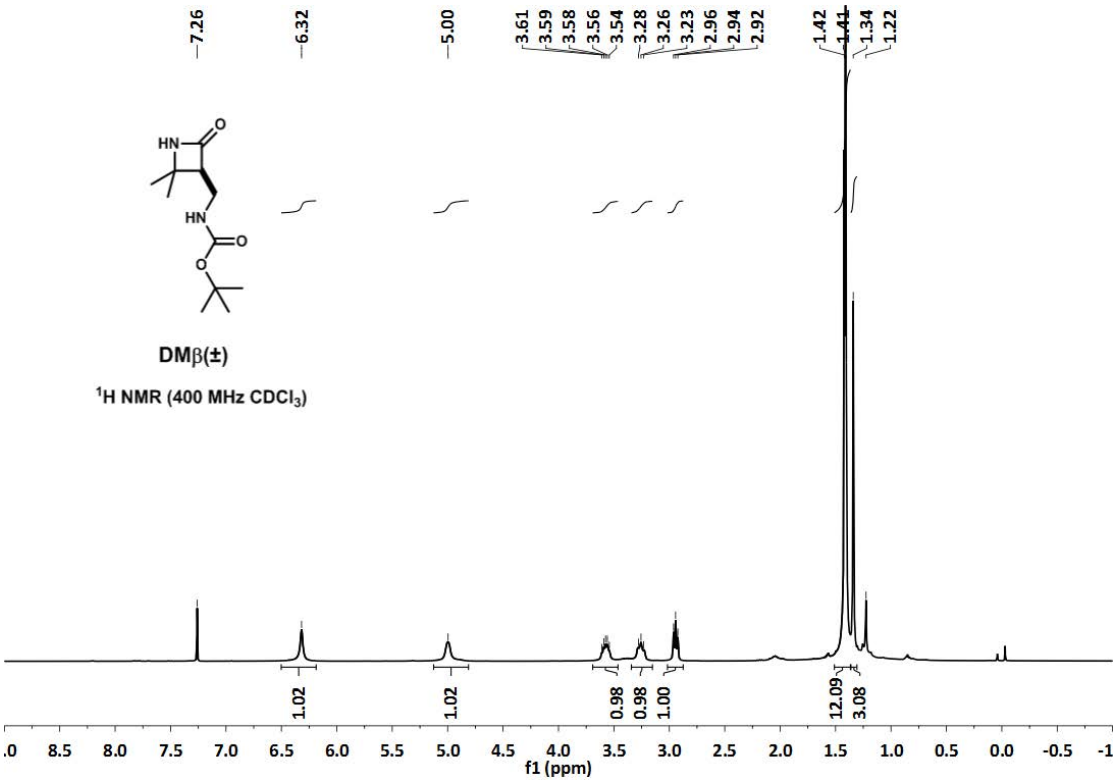


Figure S5. ^{1}H NMR spectrum of β-lactam monomer DMβ(±).

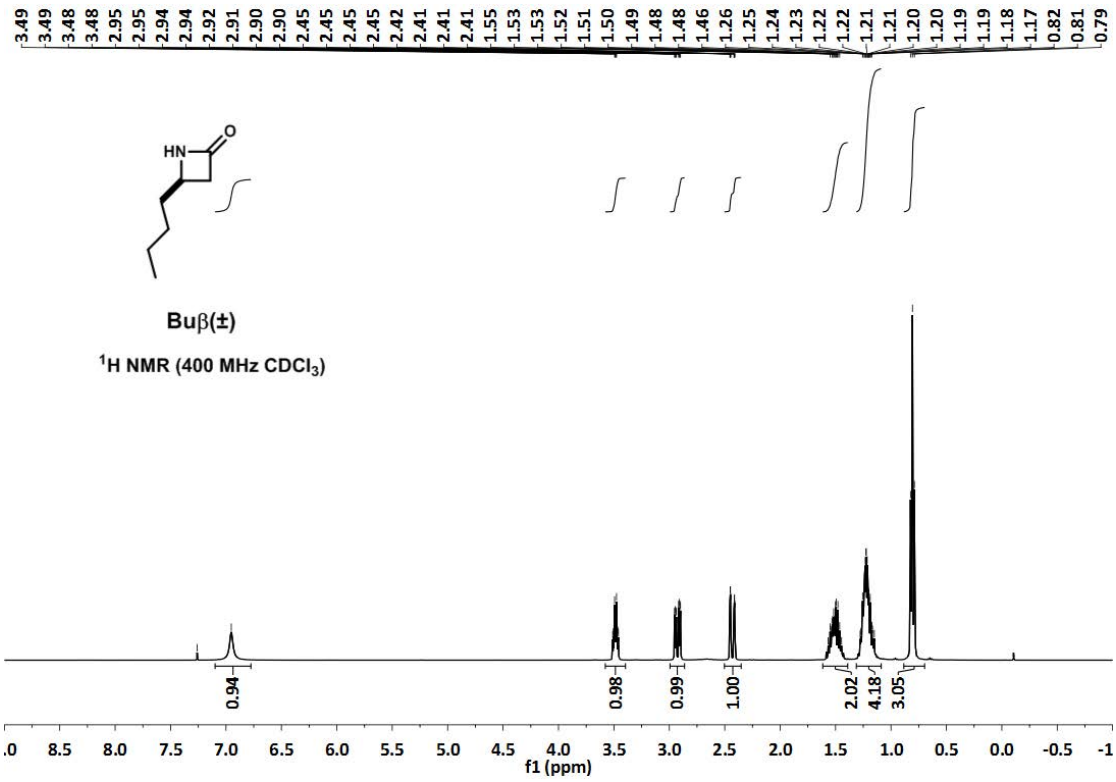


Figure S6. ^{1}H NMR spectrum of β-lactam monomer Buβ(±).

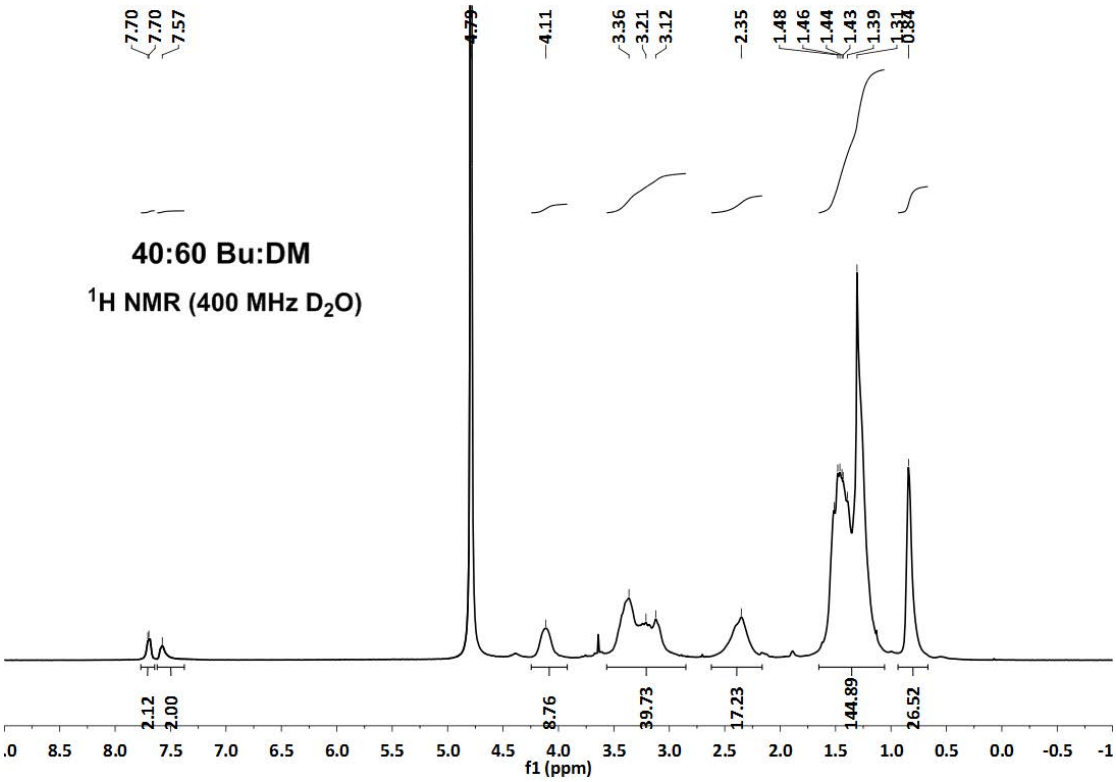


Figure S7. ^{1}H NMR spectrum of poly-β-peptide 40:60 Bu:DM.

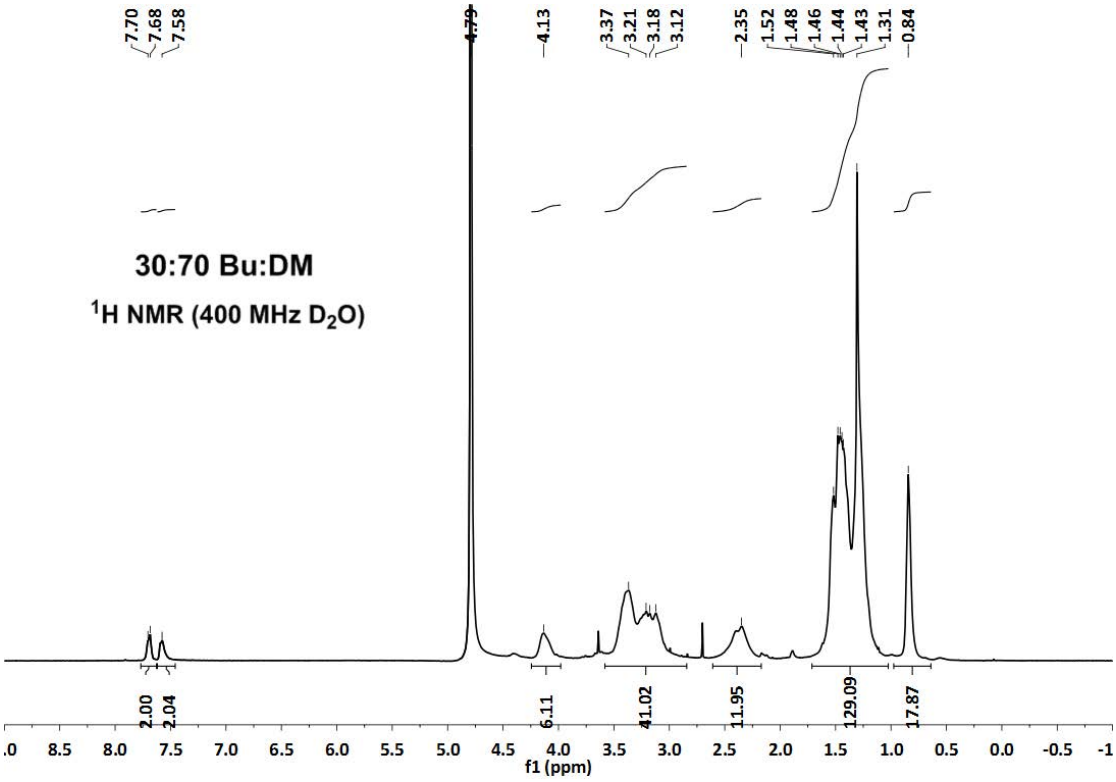


Figure S8. ^{1}H NMR spectrum of poly-β-peptide 30:70 Bu:DM.

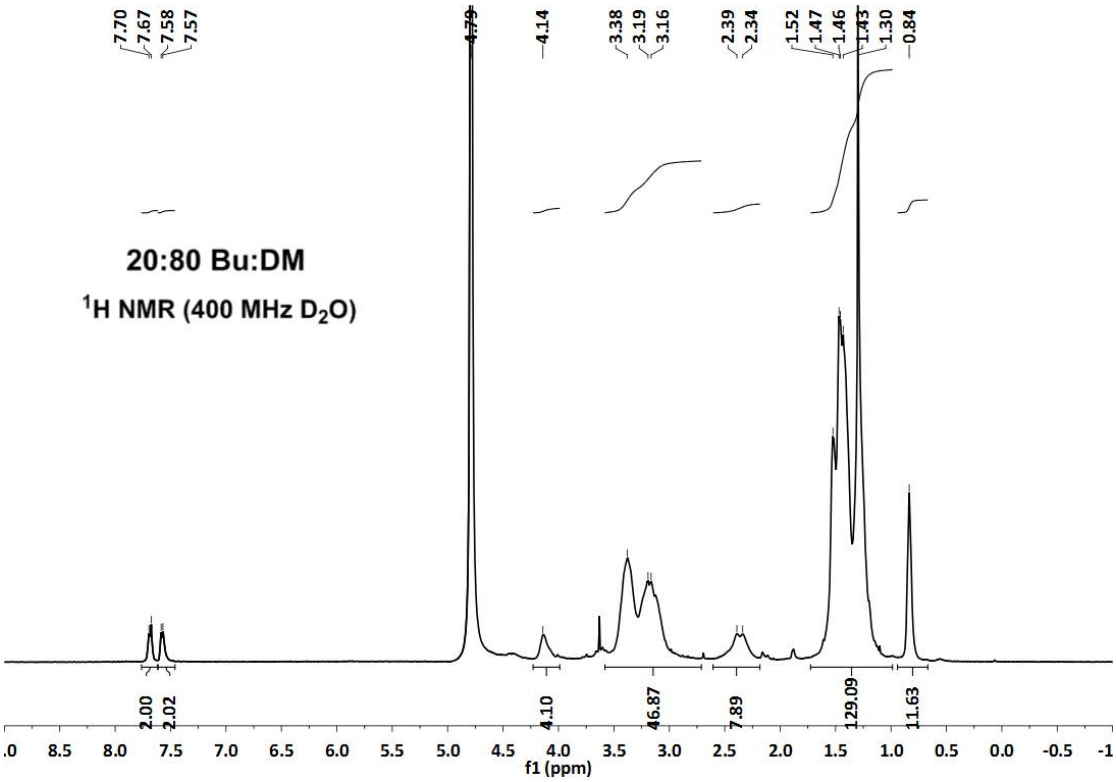


Figure S9. ^{1}H NMR spectrum of poly-β-peptide 20:80 Bu:DM.

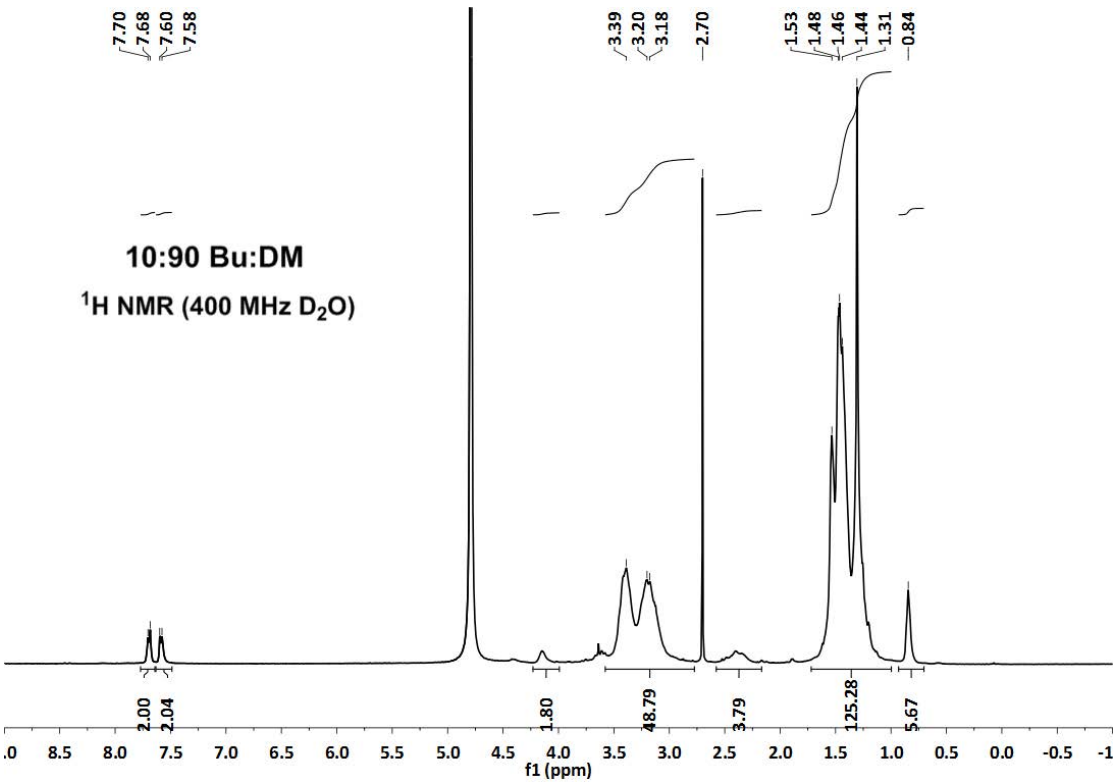
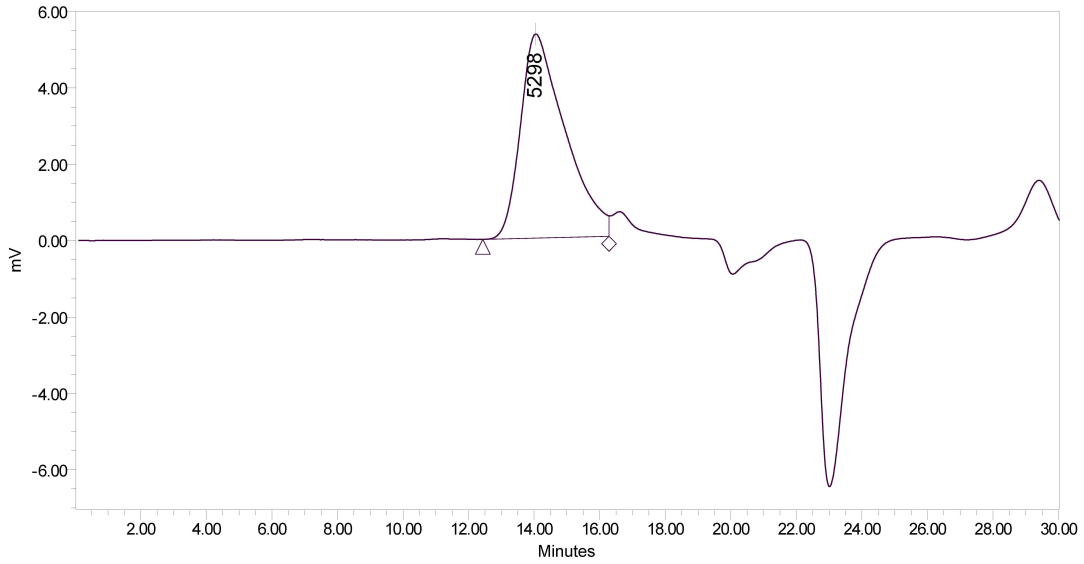


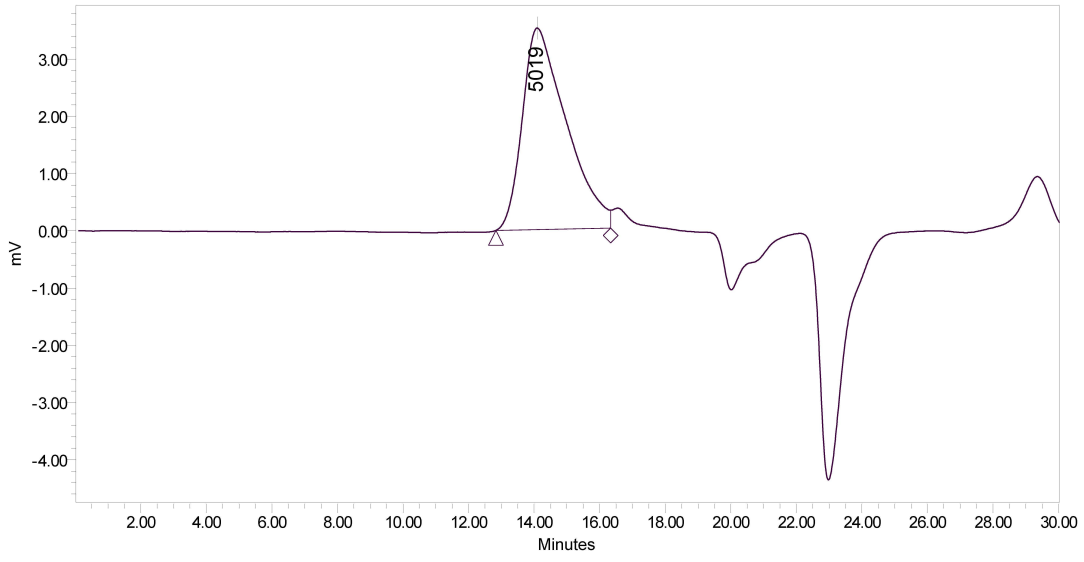
Figure S10. ^{1}H NMR spectrum of poly-β-peptide 10:90 Bu:DM.



GPC results

	Distribution Name	Mn (Daltons)	Mw (Daltons)	MP (Daltons)	Mz (Daltons)	Mz+1 (Daltons)	Polydispersity	Mz/Mw	Mz+1/Mw
1		3715	4495	5298	5396	6259	1.210022	1.200530	1.392437

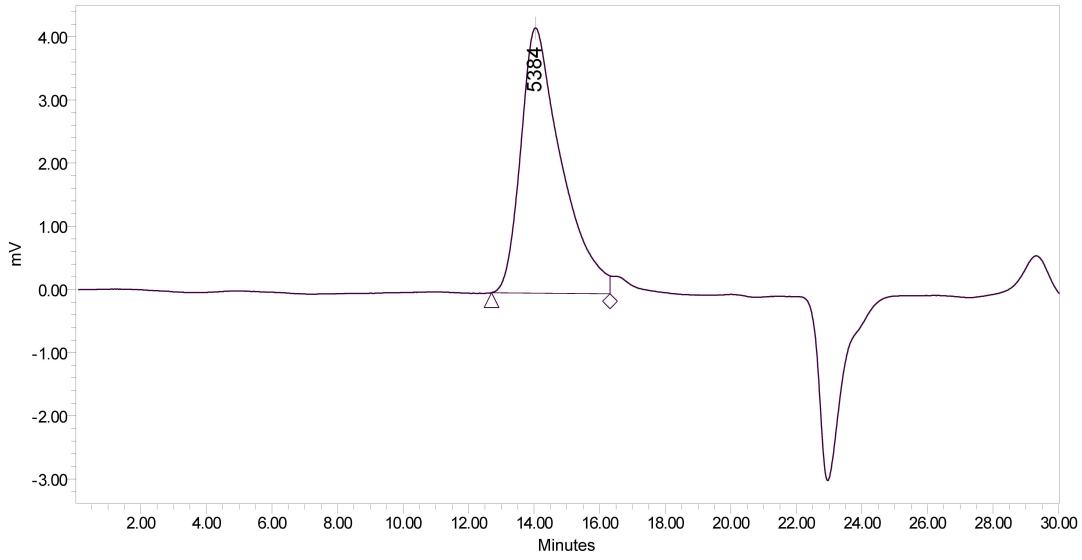
Figure S11. GPC trace of poly-β-peptide 40:60 Bu:DM at amine protected stage.



GPC results

	Distribution Name	Mn (Daltons)	Mw (Daltons)	MP (Daltons)	Mz (Daltons)	Mz+1 (Daltons)	Polydispersity	Mz/Mw	Mz+1/Mw
1		3657	4407	5019	5289	6152	1.205277	1.200059	1.395740

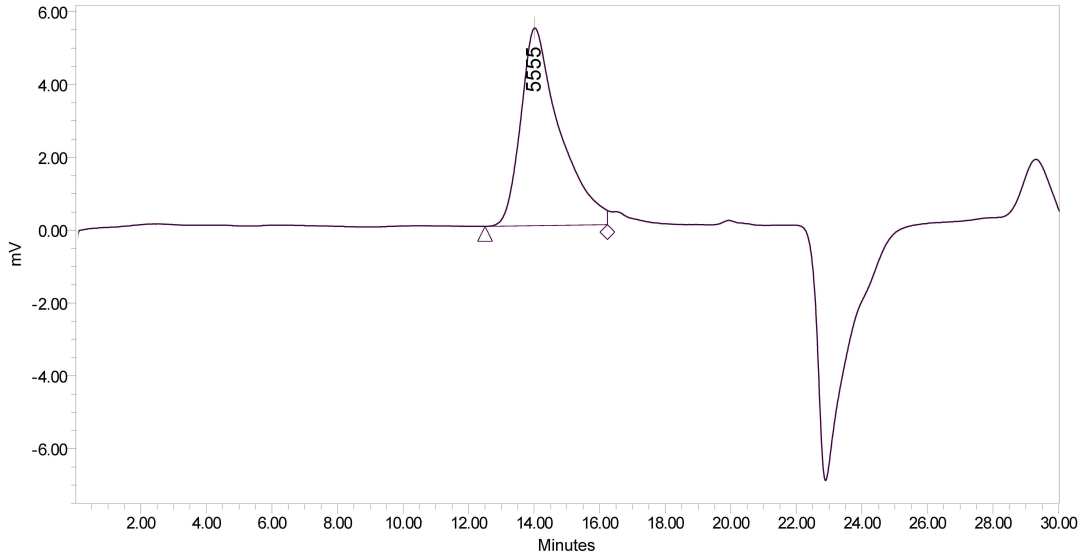
Figure S12. GPC trace of poly-β-peptide 30:70 Bu:DM at amine protected stage.



GPC results

	Distribution Name	Mn (Daltons)	Mw (Daltons)	MP (Daltons)	Mz (Daltons)	Mz+1 (Daltons)	Polydispersity	Mz/Mw	Mz+1/Mw
1		3820	4600	5384	5477	6304	1.204034	1.190570	1.370496

Figure S13. GPC trace of poly-β-peptide 20:80 Bu:DM at amine protected stage.



GPC results

	Distribution Name	Mn (Daltons)	Mw (Daltons)	MP (Daltons)	Mz (Daltons)	Mz+1 (Daltons)	Polydispersity	Mz/Mw	Mz+1/Mw
1		3875	4667	5555	5539	6350	1.204433	1.186971	1.360648

Figure S14. GPC trace of poly-β-peptide 10:90 Bu:DM at amine protected stage.

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2. D. F. Shellhamer, K. L. Alexander, S. A. Bunting, S. L. Elwin, C. J. Licata, J. C. Milligan, R. D. Robinson, D. E. Shipowick, L. B. Smith and M. C. Perry, *Synthesis*, 2015, **47**, 1944-1950.
3. M. H. Wu, E. Maier, R. Benz and R. E. W. Hancock, *Biochemistry*, 1999, **38**, 7235-7242.
4. D. L. Lee, J. P. S. Powers, K. Pflegerl, M. L. Vasil, R. E. W. Hancock and R. S. Hodges, *J. Peptide Res.*, 2004, **63**, 69-84.
5. V. Yarlagadda, G. B. Manjunath, P. Sarkar, P. Akkapeddi, K. Paramanandham, B. R. Shome, R. Ravikumar and J. Haldar, *ACS Infect. Dis.*, 2016, **2**, 132-139.