Supporting Information

Bacterial Membrane Selective Antimicrobial Peptide-Mimetic Polyurethanes: Structure-Property Correlations and Mechanisms of Action

Steven Mankoci,[1] Jason Ewing,[1] Punam Dalai,[1] Nita Sahai,[1] Hazel A. Barton,[2] and Abraham Joy[1*]*

[1]Department of Polymer Science, The University of Akron, Akron, OH, USA 44325

[2]Department of Biology, The University of Akron, Akron, OH, USA 44325

Corresponding authors: abraham@uakron.edu

bartonh@uakron.edu

Materials

All solvents and reagents used were purchased from Fisher Scientific (Waltham, MA, USA) unless otherwise specified. The 6-aminohexanoic acid, diethanolamine, and methyl isovalerate were obtained from Alfa Aesar (Ward Hill, MA, USA); hexamethylene diisocyanate (HDI) and the anhydrous 4N HCl in dioxane were obtained from Acros Organics (Fair Lawn, NJ, USA); tin 2-ethylhexanoate and dibutyltin dilaurate were obtained from Sigma-Aldrich (St. Louis, MO, USA); di-tert-butyl dicarbonate was obtained from Oakwood Chemical (Estill, SC, USA); and thionyl chloride was obtained from TCI (Tokyo, Japan). Methylene chloride was dried by distillation after preliminary drying with CaH_2 and stored over molecular sieves. Dry methanol was obtained from EMD Millipore (Billerica, MA, USA). The HDI was used as received. For the preparation of phosphate buffered saline (PBS), sodium phosphate dibasic, potassium phosphate monobasic, and potassium chloride were obtained from Sigma (St. Louis, MO, USA). The Mueller Hinton Broth (MHB) was purchased from Himedia (Mumbai, India). The trypticase soy broth

(TSB) was purchased from Becton-Dickinson (Franklin Lakes, NJ, USA). The nutrient broth was purchased from Hardy Diagnostics (Santa Maria, CA, USA). Agar was added separately to the medium and was purchased from Sigma. The bacteria used for MIC testing were *Staphylococcus epidermidis* (ATCC 12228), *Staphylococcus aureus* (ATCC 25923), *Enterococcus faecalis*, *Escherichia coli* K12 (ATCC 10798), *Pseudomonas aeruginosa* PAO1, *Stenotrophomonas maltophilia* (ATCC 13637), and *Serratia marcescens* (ATCC 13880). The bacteria used for the outer membrane permeability assay was *E. coli* K12 (ATCC 10798). The bacteria used in the cytoplasmic membrane depolarization assays were *E. coli* UB1005 and *S. aureus* (ATCC 25923). For the hemolysis assays, defibrinated sheep blood was purchased from Hardy Diagnostics. The phospholipids 1-palmitoyl-2-oleoyl-sn-glycero-3-phosphocholine (POPC), 1-palmitoyl-2-oleoyl-sn-glycero-3-phosphoethanolamine (POPE), and1-palmitoyl-2-oleoyl-sn-glycero-3-phospho-(1'-rac-glycerol) (POPG) were obtained dissolved at a known concentration in chloroform and purchased from Avanti Polar Lipids (Alabaster, AL, USA).

Instrumentation

^{1}H NMR spectra were acquired using a 300 MHz Varian Mercury spectrometer. The chemical shifts are reported in ppm relative to the signal of the residual protons of the deuterated solvent. Molar mass determinations were performed via size exclusion chromatography on a Tosoh EcoSec HLC-8320 instrument equipped with two PSS Gram Analytical SEC columns in series using 25 mM LiBr in N,N-dimethylformamide as the mobile phase at a flow rate of 0.8 mL/min. The column and detector were kept at 50 °C for the experiments. All molar mass values were obtained using a standard curve generated from polystyrene standards. All absorbance and fluorescence spectroscopy for the biological experiments were obtained using either a BioTek Synergy H1 or a Molecular Devices Spectramax M2 multimode plate reader.

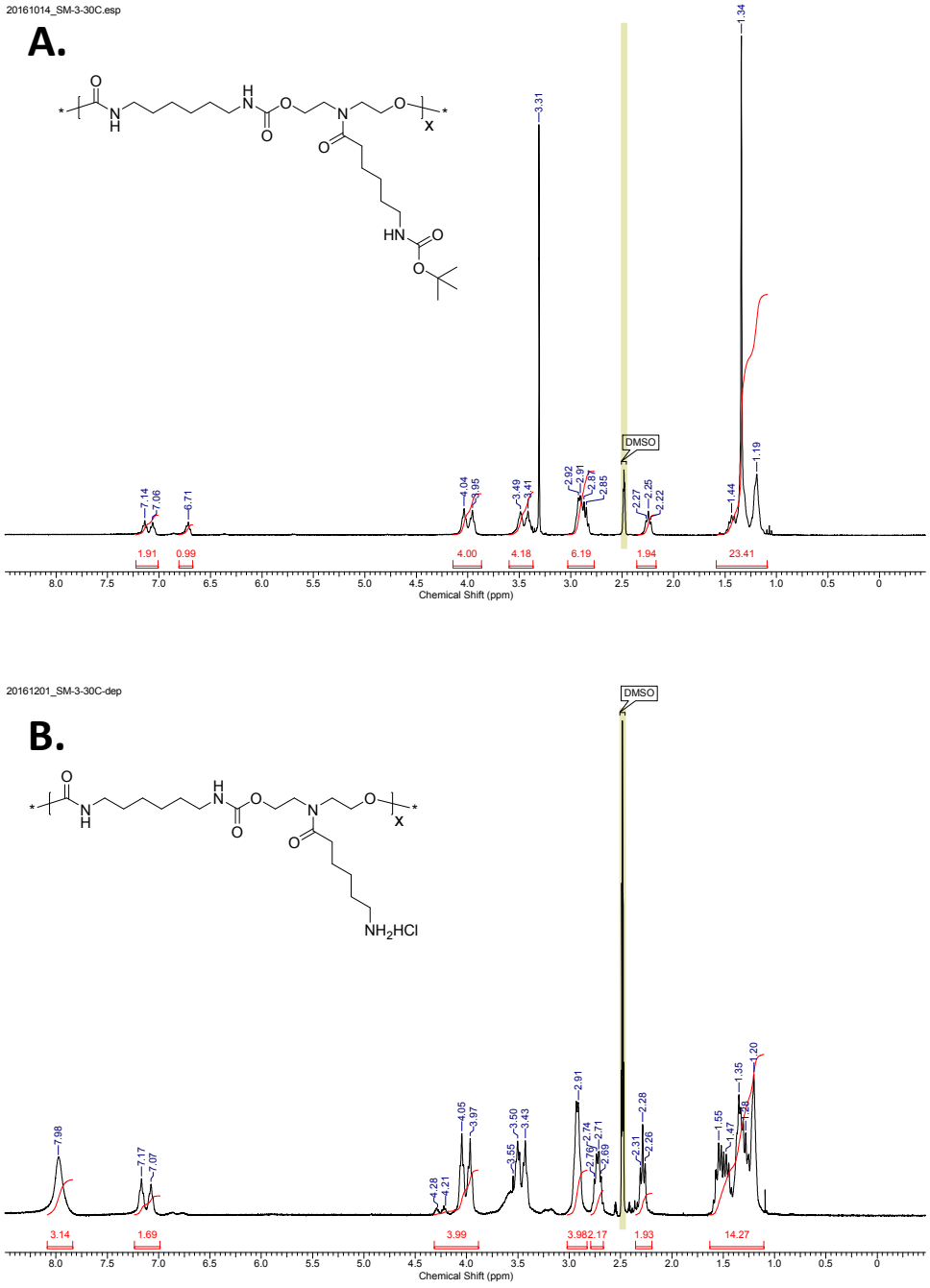
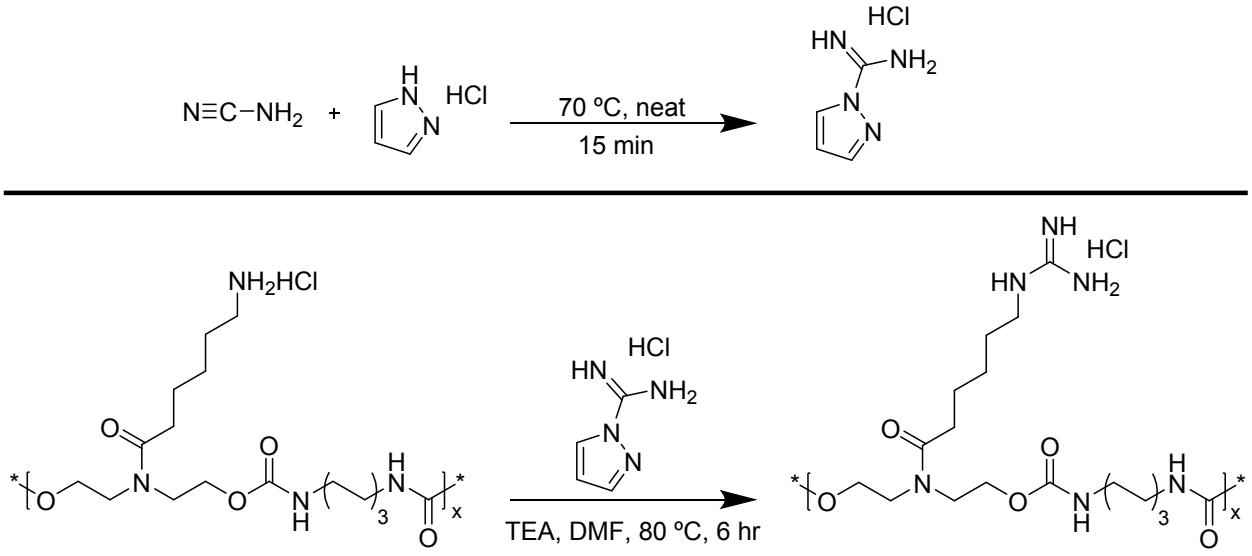


Figure S1. A typical ^{1}H NMR spectrum of an antimicrobial polyurethane A.) after polymerization and B.) after post-polymerization amine deprotection.



Scheme S1. The synthetic route for the post-polymerization guanylation of mLys HDIPU into mArg HDIPU.

Table S1. The theoretical vs. actual degrees of guanylation of mArg containing polyurethanes.

Polyurethane		*Theoretical mArg %*	*Actual mArg %**
80/20 HDIPU	mLys/mArg	20	15-20
50/50 HDIPU	mLys/mArg	50	50-60
100/0 mArg HDIPU		100	95-100

* These values were calculated from ^{1}H NMR spectroscopy from peaks that correspond to the mLys and mArg repeat units.

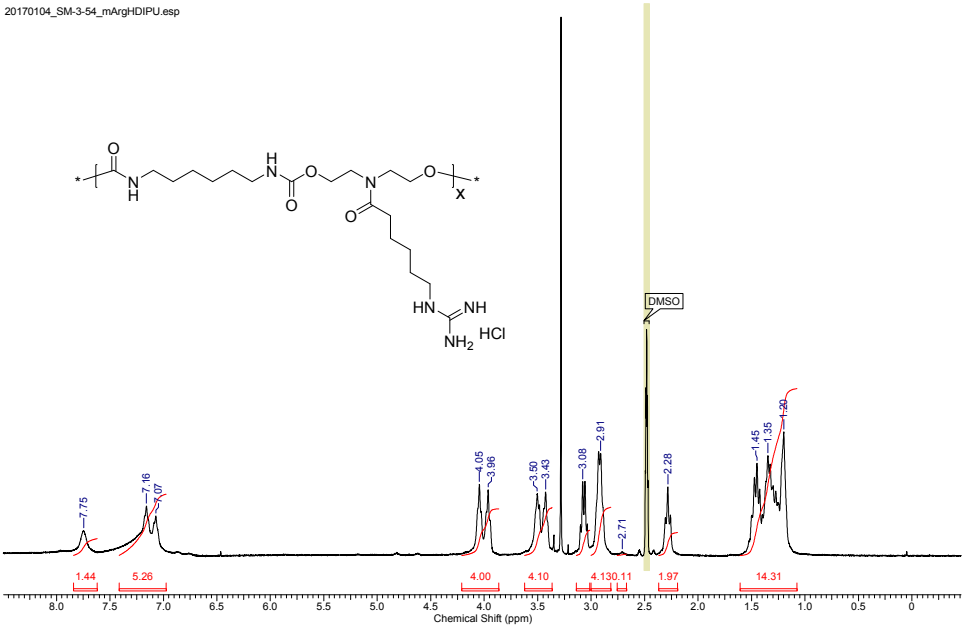


Figure S2. A typical ^{1}H NMR spectrum of an antimicrobial polyurethane after reaction with 1-amidinopyrazole to yield a polyurethane with an mArg repeat unit.

Table S2. The molecular mass and dispersity of the synthesized antimicrobial polyurethanes.

Polyurethane	M_n *(kDa)*	*Đ*
100/0 mLys HDIPU	35	1.4
80/20 mLys/mAla HDIPU	36	1.4
80/20 mLys/mVal HDIPU	26	1.4

80/20 mLys/mPhe HDIPU	33	1.4
80/20 mLys/mTrp HDIPU	28	1.4
80/20 mLys/mSer HDIPU	45	1.5
80/20 mLys/QL1 HDIPU	33	1.4
80/20 mLys/cPrDEA HDIPU	29	1.4
80/20 mLys/nPrDEA HDIPU	32	1.4
80/20 mLys/mAsp HDIPU	27	1.4
80/20 mLys/mArg HDIPU	*	*
50/50 mLys/mArg HDIPU	*	*
100/0 mArg HDIPU	*	*

*These polyurethanes were synthesized via a post-polymerization reaction on 100/0 mLys HDIPU.

Statistical Analysis

All experiments were performed at least twice independently and all were performed using at least three replicates. Values shown are expressed as the mean with their standard deviation of a single experiment. Comparisons were made among experimental data using one way ANOVA with MATLAB. Groups of data were considered significantly different for $p < 0.05$.