Supporting Information

Synthesis of Guanidinium Functionalized Polycarbodiimides and Their Antibacterial Activities

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Experimental Section

Chemistry. S-Ethylisothiourea hydrobromide and dibromotriphenylphosphorane were purchased from Tokyo Chemical Industry, Co. Ltd., Tokyo, Japan and Strem Chemicals, MA, USA respectively. All other chemicals were purchased from commercial venders such as Sigma-Aldrich, Acros Organics and Fisher Scientific, and used as received unless stated otherwise. The solvents, tetrahydrofuran (THF) dichloromethane and chloroform were distilled prior to use. ^{1}H and ^{13}C NMR data were recorded on Mercury 300 MHz or 400 MHz spectrometers at room temperature. The chemical shift values were reported relative to TMS (δ = 0.00 ppm) or corresponding solvents as an internal standards. IR spectra were obtained from JASCO FT/IR-410. Wave numbers in cm^{-1} are reported for characteristic peaks. Mass spectra were obtained at the NCSU Department of Chemistry Mass Spectrometry Facility using electrospray ionization (ESI) on an Agilent Technologies 6210 LC-TOF mass spectrometer. Specific

optical rotation was recorded on a JASCO P-1010 polarimeter. All the manipulations for polymerizations were done inside an MBraun UNIlab drybox under nitrogen atmosphere.

Synthesis of Ureas and Monomers. Monomer was synthesized as shown in the scheme 1. Prop-2-yn-1-amine and n-alkyl isocyanate (1:1.2 mol equivalents) were mixed in dichloromethane and stirred under nitrogen. Solvent was removed and the resulting urea derivative was purified by recrystallization from ethanol at 0 °C and dried under vacuum. To dibromotriphenyl phosphorane and triethyl amine (1.2: 2.5 mol equivalents relative to the urea compound) suspended in dichloromethane at 0 °C under nitrogen atmosphere, the urea derivative was added in portions over 15 minutes. The reaction mixture was allowed to warm to room temperature and stirred under inert atmosphere. The dehydration of the urea compound into the carbodiimide monomer can be monitored by Infrared spectroscopy by the formation of strong absorption band corresponding to N=C=N at around 2130 cm^{-1}. After completion, the reaction was quenched by pentane. The resulting monomer was extracted from the solid by pentane/ether. The crude carbodiimide monomer sample was purified by column chromatography on silica gel using ethyl acetate and hexane (1:4) mixture or dichloromethane and dried under vacuum to provide colorless oil. The corresponding monomers of the polymers, **Poly-1**(**c-e**) are volatile and should be handled with care.

Synthesis of (R) and (S) – BINOL-titanium (IV) – diisopropoxide Catalyst. The catalysts were synthesized according to reported procedure in the literature.[1]

Synthesis of Precursor Polymers. Alkyne functionalized polymers, **Poly-1**(**a-e**) were synthesized as shown in the scheme 1. The monomer sample was mixed with the catalyst dissolved in a small amount of distilled chloroform (ca.0.4 – 0.6 mL for 0.09 g – 1.0 g scale monomers) with a monomer to catalyst ratio 100:1, 50:1, 40:1 or 25:1 inside a dry box filled with nitrogen. The polymerizations were performed at room temperature. Polymerization reaction to form **Poly-1e** is exothermic, needs halogenated solvent to dissolve the catalyst and should be handled carefully. The reaction mixture transformed into a dark red viscous liquid and solidified into light orange solid. The reaction mixture was allowed to remain at room temperature inside the dry box for 24 hrs. The resulting light orange

solid was taken out from dry box, dissolved in chloroform (or THF) and precipitated in methanol to purify the polymers. The polymer was collected by filtration and dried under vacuum overnight prior to click reaction.

Poly-1a: Monomer to catalyst ratio: 25:1; yield: 85%

Poly-1b: Monomer to catalyst ratio: 40:1; yield: 58%

Poly-1c: Monomer to catalyst ratio: 50:1; yield: 70%

Poly-1d: Monomer to catalyst ratio: 100:1; yield: 90%

Poly-1e: Monomer to catalyst ratio: 50:1; yield: 87%

Experimental details and characterizations of these ureas, monomers and precursor polymers are as described in our previous publication.[2]

Relative molecular weights of the polymers were determined using size exclusion chromatography(SEC) on Viscotek VE 3580 system equipped with ViscoGEL columns (GMHHR-M) connected to a refractive index (RI) detector at 30 °C using 0.12M diethanolamine in THF as an eluent. Polystyrene standards were used for the calibration of the instrument. Polymer samples were dissolved in (0.12M diethanolamine in THF) and the solutions were filtered through 0.45 µm PTFE filters prior to injection. The flow rate was 1.0 mL/min and injector volume was 100µL. Addition of diethanolamine in the eluent(THF) minimizes the adhesion of these polymers in the SEC column matrix.[4]

The specific optical rotation measured in these polymers synthesized using chiral catalysts clearly demonstrates excess helical sense. Typically, polymer sample was prepared by dissolving solid polymer sample in the mentioned solvent (generally 20 mg/10.0 mL; may vary sometimes) and stirred overnight for homogenous mixing. The solution was then filtered through 0.45µ syringe filter (PTFE) prior to data collection. For each polymer, data were collected twice. All the polymers were synthesized using *R*-form of the catalyst except **Poly-1c** which was polymerized using both *R*- and *S*- forms of catalysts separately.

Poly-1a, $[\alpha]_{589}^{25}$ = 10 (c = 0.22 in $CHCl_3$, l = 0.5 dm), **Poly-1b**, $[\alpha]_{589}^{25}$ = 16 (c = 0.22 in $CHCl_3$, l = 0.5 dm), **Poly-1c** (*S*- form of catalyst was used), $[\alpha]_{589}^{25}$ = -30 (c = 0.21 in DMSO, l = 0.5 dm), **Poly-1c**

(*R*- form of catalyst was used), $[\alpha]_{589}^{25}$ = 20 (c = 0.21 in DMSO, l = 0.5 dm), **Poly-1d**, $[\alpha]_{589}^{25}$ = -30 (c = 0.10 in DMSO, l = 0.5 dm), **Poly-1e**, $[\alpha]_{589}^{25}$ = 25 (c = 0.10 in DMSO, l = 0.5 dm)

Click chemistry in polymers. In a typical procedure, an alkyne functionalized polymer (1.0 equiv, generally 100mg), was dissolved in THF (about 6.0 mL) at room temperature in a clean and dry glass vial purged with nitrogen gas. Under nitrogen atmosphere was added guanidinium azide (prepared following literature procedure[3]), (1.3 equiv for **Poly-1**(**a-d**), and 2.6 equiv for **Poly-1e**. CuI and DBU (10 mol %, 4.0 equiv per repeat unit respectively) were added to the reaction mixture and stirred at room temperature under nitrogen overnight. A change in the solubility of the polymers during the course of the reaction also indicates the progress of the click reaction. The precursor polymers are soluble in THF whereas the guanidinylated polymers **Poly-2(a-e)** precipitate out of THF. The resulting solid polymer was extensively washed with THF to remove unreacted materials. **Poly-2**(**a-b**) were further washed with dilute acid HCl and water whereas **Poly-2**(**c-e**) are water soluble polymers and any small molecules contaminants remained were removed by dialysis of polymer aqueous solution against water using Thermo Scientific Slide – A – Lyzer Dialysis Cassettes with molecular weight cut off (MWCO) 3.5 K. Water was removed by using rotavap and polymer sample was dried overnight under reduced pressure prior to characterization and bioloactivity tests. Due to strong affinity of these polymers towards column in GPC, we were unable to provide the data for molecular weight and PDI of these polymers. Dialysis of the polymer samples in dialysis membrane with molecular weight cut off 35K implies sufficiently high molecular weight for the study.

The ^{1}H NMR of the polymer after click chemistry clearly shows the guanidinium functionalization(Fig. S1)

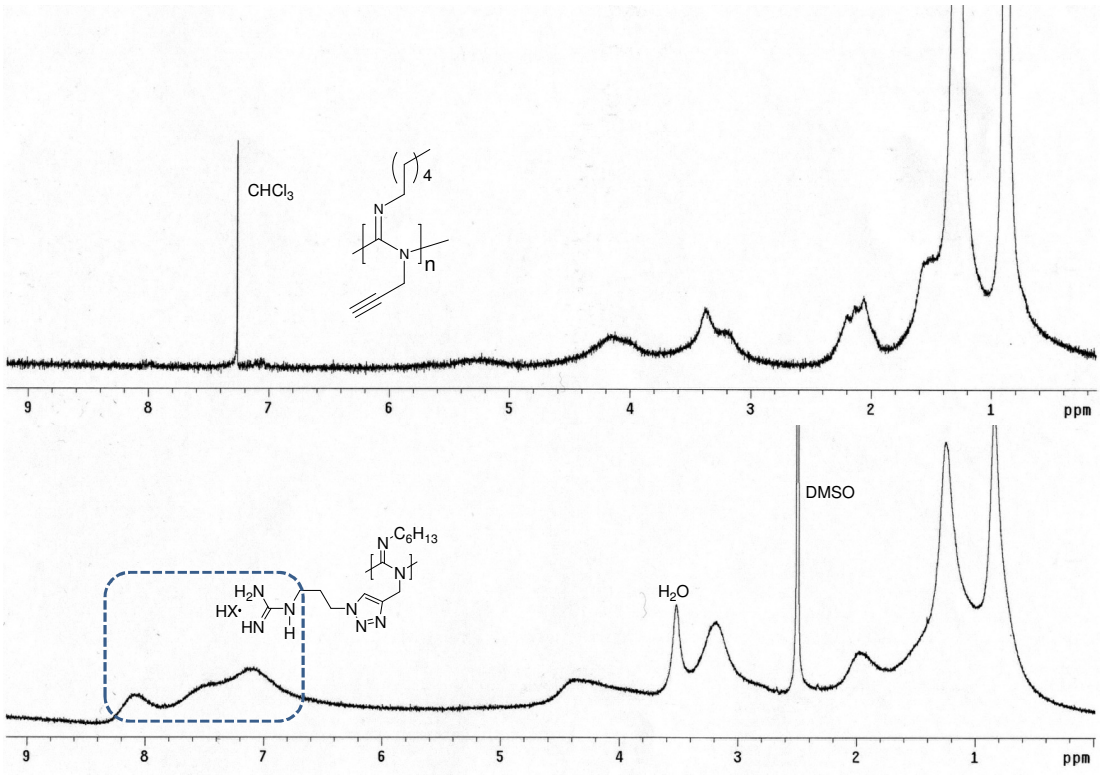

Figure S1. The ^{1}H NMR of precursor polymer **Poly-1b** in $CDCl_3$ (top) and guanidinium polymer **Poly-2b** in DMSO-d_6 (bottom). (X = Br^- or Cl^-)

The degree of functionalization as observed in FTIR appears quantitative. (Fig. S2) Absorption corresponding to carbon-carbon triple bond (2121 cm^{-1}) disappears as click reaction completes.

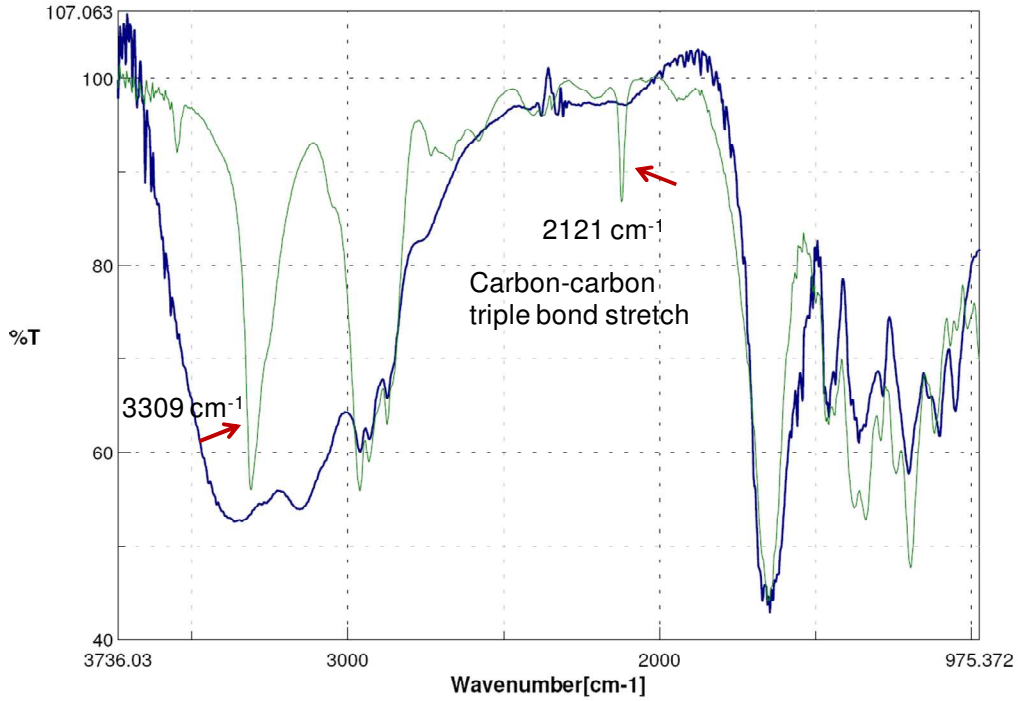

Figure S2. FTIR spectra of precursor polymer **Poly-1c** (shown in green) and guanidinium polymer **Poly-2c** (shown in blue).

The helical structure has been observed to be persistent before and after click reaction in circular dichroism spectra in polycarbodiimides.[2] In this study, we present optical rotation data of one of the representative polymer before and after click reaction to illustrate recurring tendency. e.g. **Poly-1d**, $[\alpha]_{589}^{25}$ = -30 (c = 0.10 in DMSO, l = 0.5 dm); **Poly-2d**, $[\alpha]_{589}^{25}$ = -23 (c = 0.21 in DMSO, l = 0.5 dm)

Biology. *Acinetobacter baumannii* (ATCC # 19606) , *Staphylococcus aureus* (ATCC # 29213),

MRSA (methicillin-resistant *Staphylococcus aureus*) (ATCC # BAA-44TM) were purchased from ATCC. *Escherichia coli* (*E. coli* K12, ER2738) was purchased from New England Biolabs. Mechanically difibrinated sheep blood (DSB100) was obtained from Hemostat Labs. All other supplies were purchased from commercially available sources. Stock solutions (32 mg/mL) of all compounds assayed for biological activity were prepared in DMSO and stored at 5 °C in frig. Mueller-Hinton medium was purchased from Fluka (# 70192).

Broth Microdilution Method for MIC Determination. Overnight cultures of four bacterial strains were subcultured to 5 x 10^5 CFU/mL in Mueller-Hinton medium (Fluka # 70192). The resulting bacterial suspension was aliquoted (0.5 mL) into culture tubes. Samples were prepared from these culture tubes containing 1024μg/mL of each specified polymer. Samples were then aliquoted (200 μL) into the first row of wells(two wells for each compound) of a 96-well microtiter plate in which subsequent wells were prefilled with 100 μL of Mueller-Hinton medium based 5 x 10^5 CFU/mL bacterial subculture. Mixed the medium in row one wells 6-8 times by a multichannel pipettor (set at 100 μL). Then 100 μL of medium were withdrawn after that and transferred to row two. Row two wells were mixed 6-8 times followed by a 100 μL transfer from row two to row three. This procedure was repeated to serial dilute the rest of the rows (except the last two rows, which were used as control) of the microtier plate. The microtiter plate sample was then covered with a microtiter plate lid and placed in a

covered plastic container. The chamber was incubated under stationary conditions at 37 °C for 16 hours. After that, the lid was removed and MIC values were recorded comparing turbidity vs. clarity of the solutions. Each test was performed in duplicate and repeated two times with different batches of polymer samples. MIC values were further confirmed by colony count method.

Red Blood Cell Hemolysis Assay. Hemolysis assays were performed on mechanically difibrinated sheep blood (Hemostat Labs: DSB100). 1.5 mL of blood was placed into a microcentrifuge tube and centrifuged at 10000 rpm for ten minutes. The supernatant was removed and then the cells were resuspended with 1 mL of phosphate-buffered saline (PBS) (Hyclone®, Cat NO.:SH30256.02). The suspension was centrifuged, the supernatant was removed and cells were resuspended two more times. The final cell suspension was then diluted tenfold. The test compounds solution were prepared in a serial of concentrations in PBS in small tubes (0.5 mL PBS for each tube, doubled the test concentration at first). 0.5 mL PBS alone was used as a negative control (zero hemolysis marker) whereas a 2%V Triton X in 0.5 mL PBS was used as a positive control (100 % lysis marker). Then 0.5 mL of the tenfold suspension dilution of blood cells was added to each of all these small tubes. Samples solutions of **Poly-2(b-e)** when mixed with blood caused precipitation of blood within minute of mixing. The analysis procedure for the sample solutions containing **Poly-2a** was continued as described. Samples were then placed in an incubator at 37 °C while being shaken at 200 rpm for one hour. After that, the samples were transferred to microcentrifuge tubes and then centrifuged at 10000 rpm for ten minutes. The resulting supernatant was diluted by a factor of 40 in distilled water. The absorbance of the supernatant was measured with a UV spectrometer at a 540 nm wavelength.

References

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