# Synthesis and Biological Activity of Highly Cationic Dendrimer Antibiotics

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## Supporting Information

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**General procedure for determining charge of $C_{16}$-DABCO dendrimers.**
A 0.01 mM solution of $C_{16}$-DABCO dendrimers was dissolved in filtered (0.022 μm filter) Millipore water. 100 μL of the solution was placed into Wyatt Technologies' Mobius Dip Cell to perform the electrophoretic mobility experiment. The triangle extending above the y-axis indicates $C_{16}$-DABCO dendrimers are positively charged. It is noteworthy, that the positive charge appears after the addition of $C_{16}$-DABCO. Generally speaking, unfunctionalized glycodendrimers are neutral in charge.

**General Procedure for Determining Critical Micelle Concentration**
Experiments were performed to ensure that $C_{16}$-DABCO was below the critical micelle concentration (CMC). Several concentrations of $C_{16}$-DABCO were dissolved in water (Millipore) and filtered through a 0.22 μm syringe filter to ensure no dust or other contaminants were present in the sample. Samples were analyzed on a 90 plus Particle Size Analyzer made by Brookhaven Instruments Corporation. The graph in Figure S4 represents several overlapping data acquisitions. The two regression lines represent the intensity changes as a function of concentration of $C_{16}$-DABCO. One line shows data prior to micelle formation, and the other line is after formation of micellular aggregates. The intersection point shows the concentration at which micelles begin to form. From this experimental data, the critical micelle concentration was determined to be 0.685 mM. This concentration is well above the concentrations reported throughout this publication for $C_{16}$-DABCO use in assays.

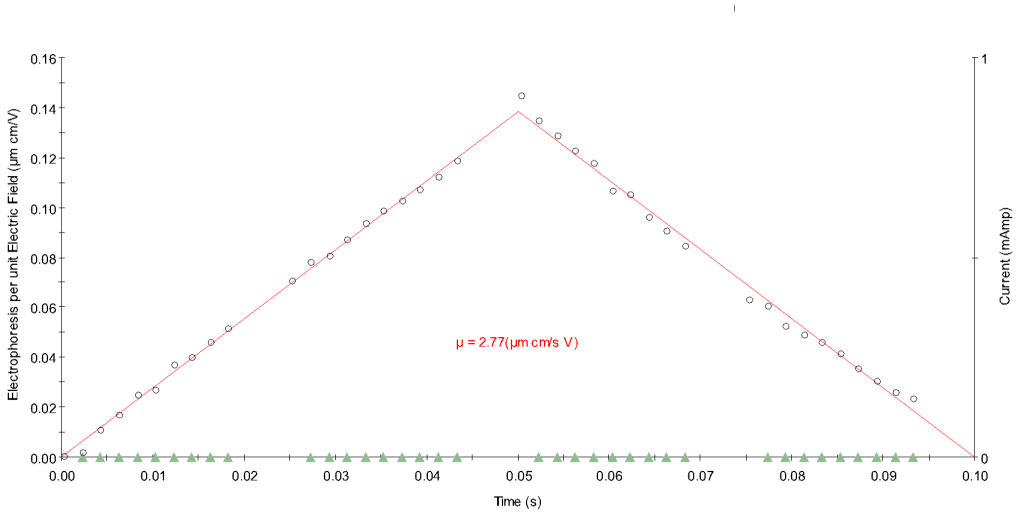



**Figure S1.** Electrophoretic mobility plot for $C_{16}$-DABCO dendrimers.

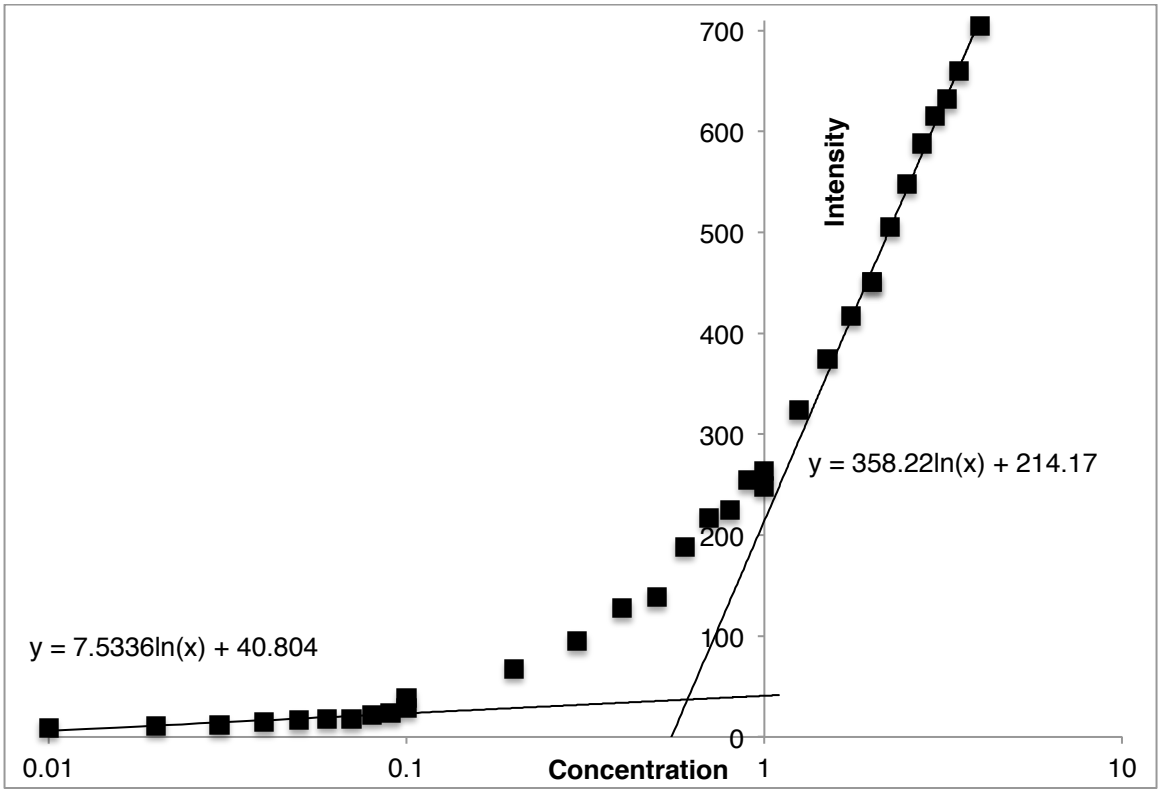



**Figure S2.** CMC determination for **1**.

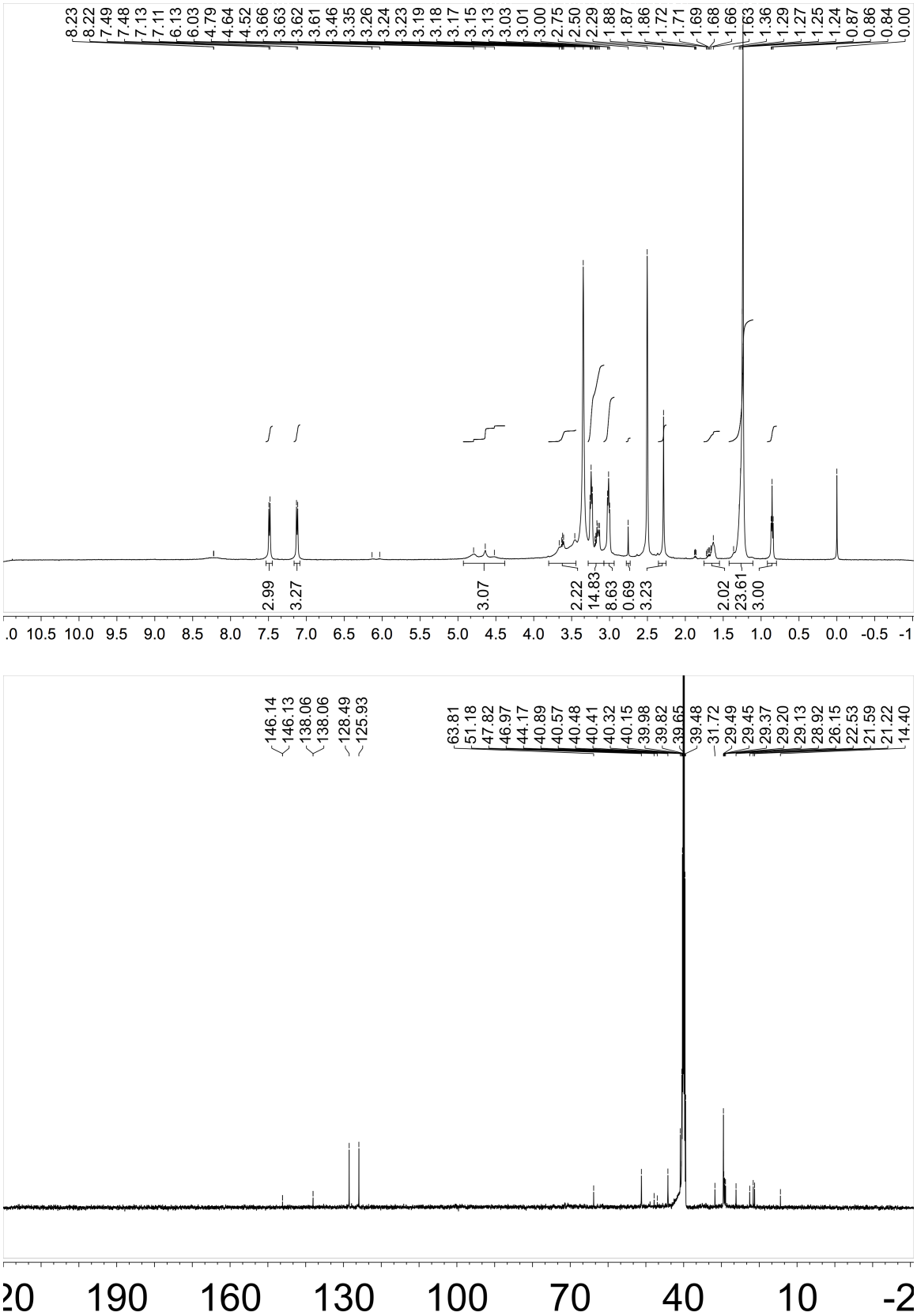


**Figure S3.** $^{1}$H NMR spectrum (top) and $^{13}$C NMR spectrum (bottom) of **1**.

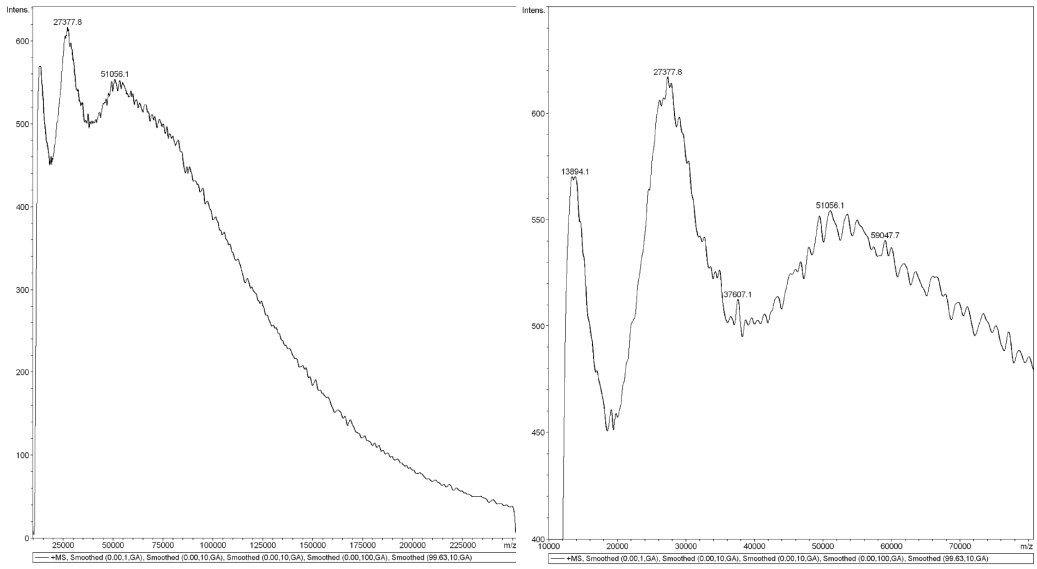



**Figure S4.** MALDI-TOF MS of **1**. Full spectrum on left and zoom view on right.

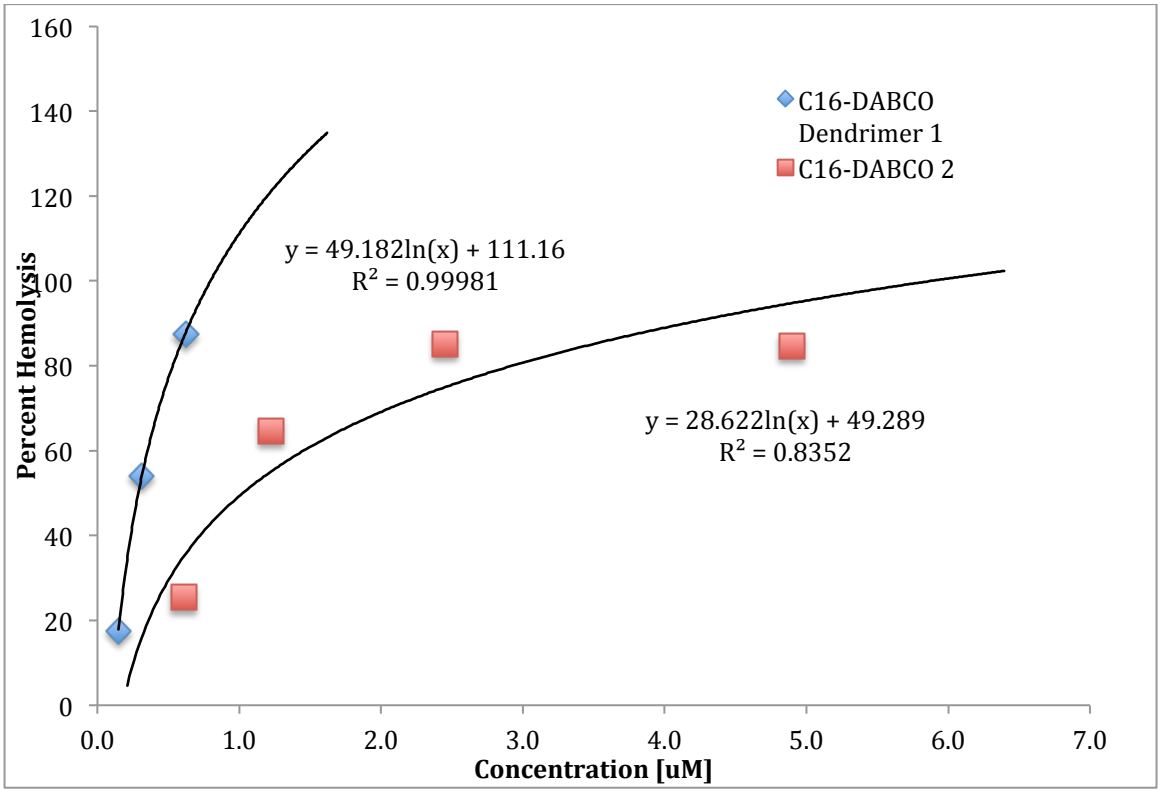



**Figure S5.** Red blood cell hemolysis for $C_{16}$-DABCO dendrimer **1** and $C_{16}$-DABCO monomer **2**.

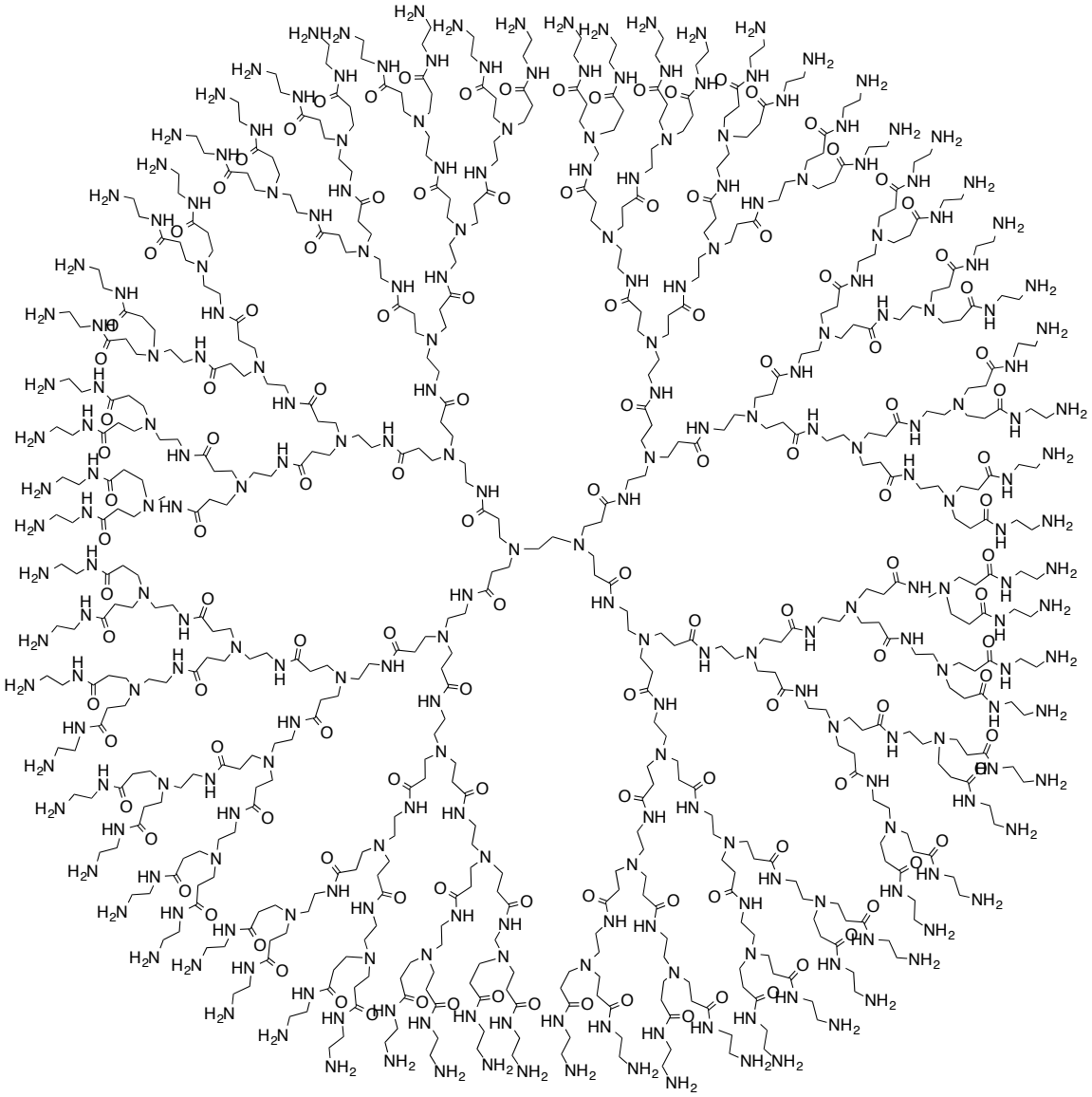


**Figure S6.** Fourth generation poly(amidoamine) dendrimer, i.e. G(4)-PAMAM.

**Table S1.** Minimum Inhibitory Concentrations (MICs) for Dendrimer **1** and Monomer **2**.

| Microorganism (Gram + or -) | $C_{16}$-DABCO Dendrimer **1** (per $C_{16}$-DABCO group) | $C_{16}$-DABCO Dendrimer **1** (per dendrimer) | $C_{16}$-DABCO Monomer **2** | $C_{16}$-DABCO Dendrimer **1** (per dendrimer) | $C_{16}$-DABCO Monomer **2** | Ampicillin | Cephalexin | Streptomycin |
|---|---|---|---|---|---|---|---|---|
| *Streptococcus oralis* (+) | >165 μM | >20.0 uM | 3033 μM | >600 (μg/mL) | 1024 (μg/mL) | N/A | N/A | <34 μM |
| *Staphylococcus aureus* (+) | 1.1 | 0.133 | 11.8 | 4 | 4 | <67 μM | <68 μM | <17 |
| *Bacillus cereus* (+) | 1.1 | 0.133 | 17.7 | 4 | 6 | 27 | 41 | N/A |
| *Pseudomonas aeruginosa* (–) | 16 | 2.00 | 331.7 | 60 | 112 | N/A | N/A | <17 |
| *Escherichia coli* (–) | 11 | 1.09 | 148.1 | 40 | 50 | 11 | 22 | N/A |

**Sample Calculation.** Determining Concentration of Active Group.

From mass concentration of dendrimer to molar

$$x\frac{mg}{mL} * \frac{1\ mol\ dendrimer}{30{,}074\ g\ dendrimer} * \frac{1\ g\ dendrimer}{10^3\ mg\ dendrimer} * \frac{1\ mL}{10^{-3}L} * \frac{1\ umol}{10^{-6}\ mol} = y\ \frac{umol}{L} = y\ uM$$

Equation 1. Solving for concentration of active group (per dendrimer basis)

$$(mass\ of\ a\ tosyl\ group) * (number\ of\ tosyl\ groups) + (mass\ of\ a\ C16DABCO\ group) * (number\ of\ tosyl\ groups) = (mass\ after\ tosyl\ and\ C16DABCO\ addition - mass\ before\ tosyl\ and\ C16DABCO\ addition)$$

Equation 2. Determining ratio active group to non-active group vis NMR analysis

$$ratio\ of\ tosyl\ methyl\ to\ C16DABCO\ methyl\ (NMR) = \frac{number\ of\ tosyl\ groups}{number\ of\ C16DABCO\ groups}$$

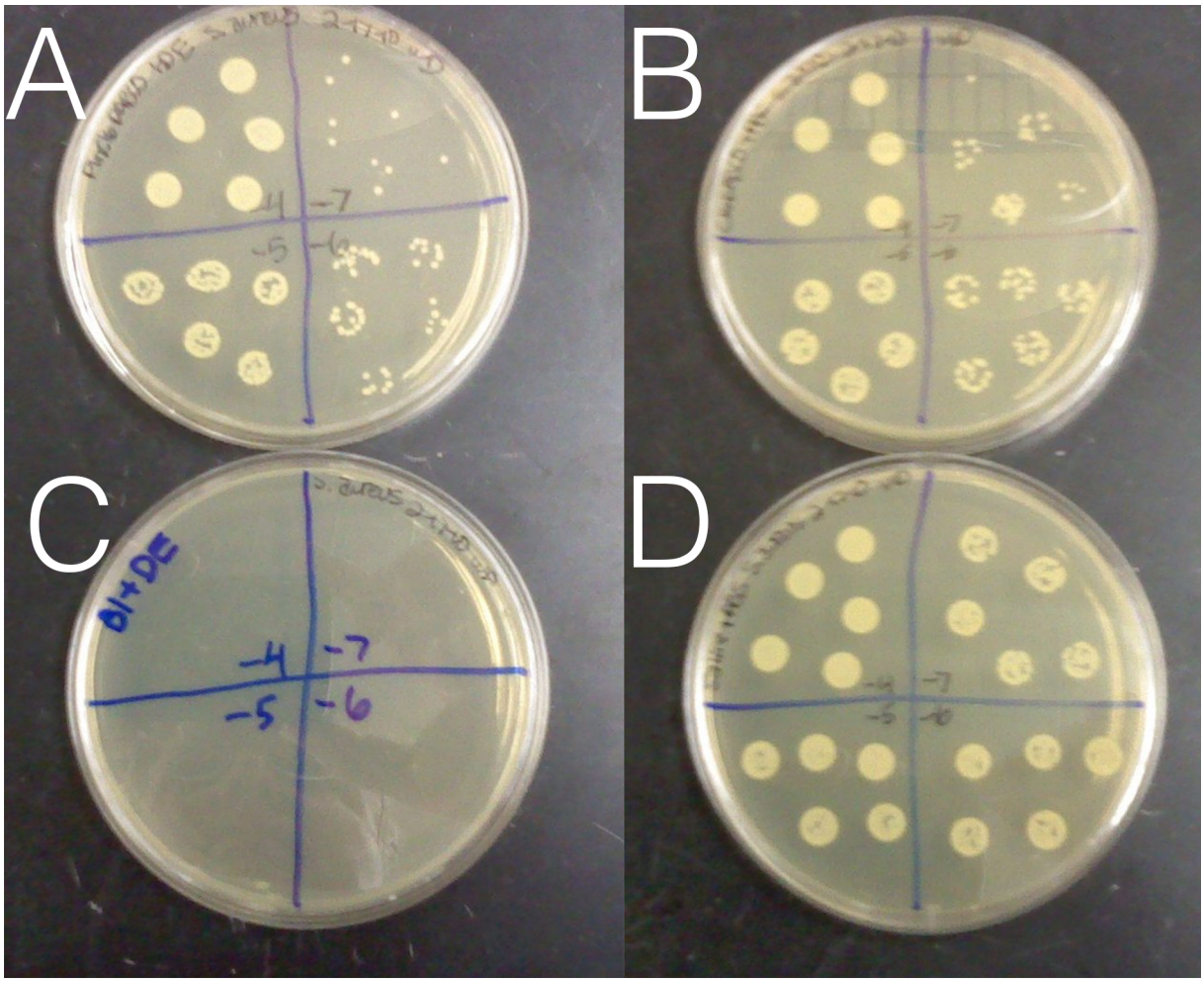
Two equations with two unknowns.

Solve for one variable in **equation 2** in terms of the other variable; put answer in **equation 1**; solve for the only variable and put answer back into **equation 1** to determine second variable. Variables are the number of tosyl and C16DABCO groups.

Finally,

$$number\ of\ C16DABCO\ per\ dendrimer * molarity\ of\ dendrimer\ (y) = molarity\ of\ C16DABCO \text{ per dendrimer}$$

**Figure S7**. Biofilm Disruption Studies (log-kill platings) for *S. aureus*.




A) $C_{16}$-DABCO dendrimer **1.** B) $C_{16}$-DABCO monomer **2**. C) Bleach. D) Saline.