Synthetic Mimic of Antimicrobial Peptide with Non-Membrane-Disrupting Antibacterial Properties

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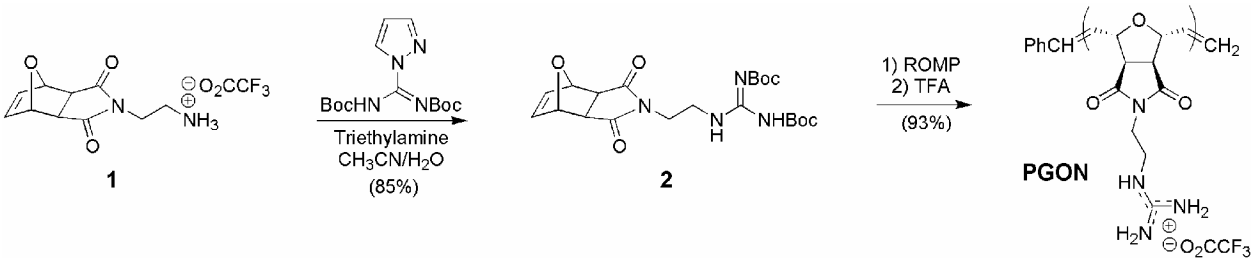
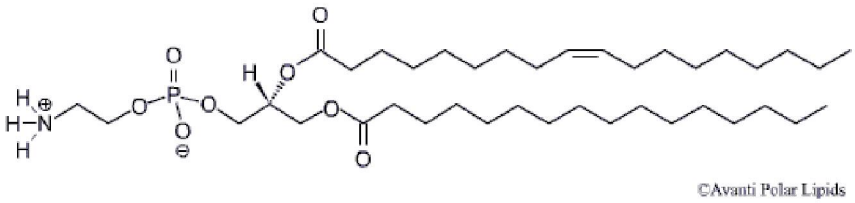
Supporting Information

I. Synthesis and characterization of PGON
II. Synthesis of NBD-labeled PGON and microscopy

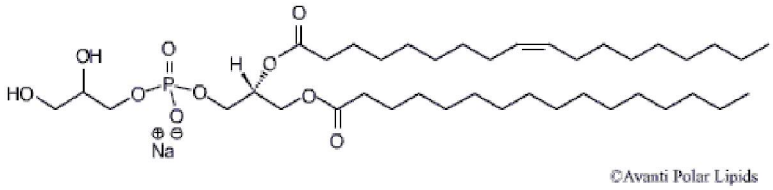
I. Synthesis and characterization of PGON

Instrumentation. Gel permeation chromatography (GPC) was performed on the Boc-protected polymers with a Polymer Lab LC1120 pump equipped with a Waters differential refractometer detector. The mobile phase was THF with a flow rate of 1.0 mL/min. Separations were performed with 105, 104, and 103 Å Polymer Lab columns and molecular weights were calibrated versus narrow molecular weight polystyrene standards. The Boc-protected **PGON** is THF soluble and GPC was used to approximate M_n and polydispersity index (PDI). ^{1}H-NMR and ^{13}C-NMR spectra were obtained on a Bruker DPX-300 NMR spectrometer. Spectra were calibrated to the DMSO-d_6 solvent signal. High-performance liquid chromatography (HPLC) using a Zorbax SB-C8 reverse-phase column (Agilent) was performed on the deprotected TFA-salt polymers. A Waters 2695 separations module was used along with a Waters 2996 Photodiode Array Detector. An aqueous/organic gradient was used (0.1% TFA in H_2O/0.1% TFA in CH_3CN, flow = 1 mL/min) in which the gradient was held for 1 min at 99% aqueous then ramped down to 20% aqueous over 45 min. Signal peaks were detected at 212 nm.





POPE = 1-Palmitoyl-2-Oleoyl-sn-Glycero-3-Phosphoethanolamine

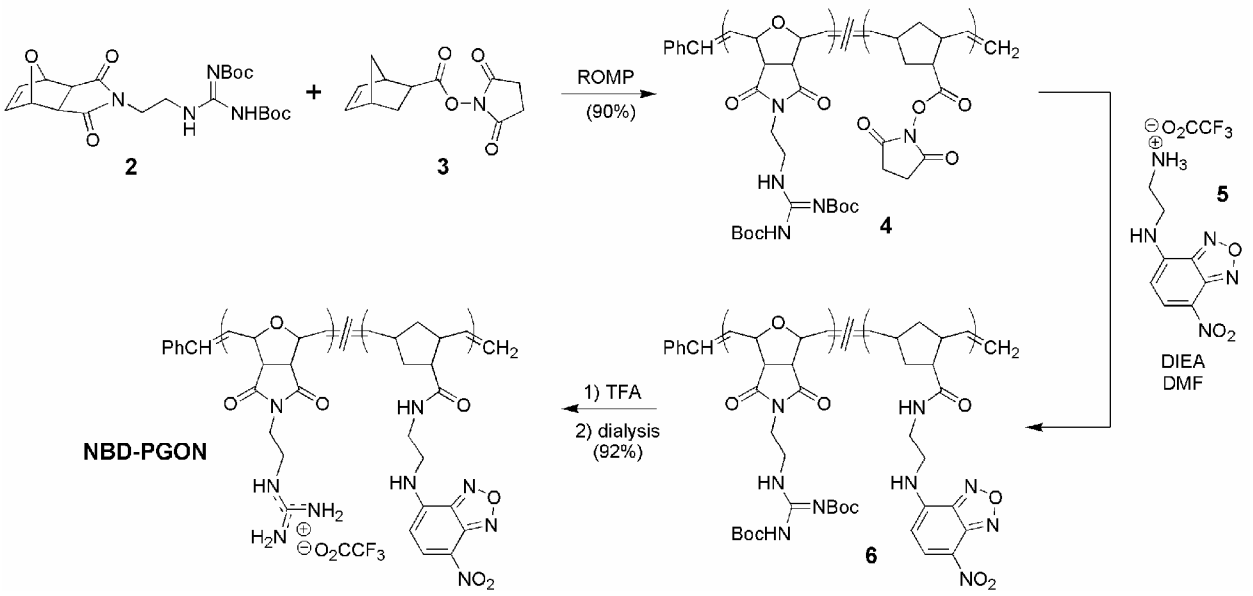


POPG = 1-Palmitoyl-2-Oleoyl-sn-Glycero-3-[Phospho-rac-(1-glycerol)] (Sodium Salt)
(Image from http://www.avantilipids.com)

Figure S1. Chemical structure of POPE and POPG.

II. Synthesis of NBD-labeled PGON and microscopy

An NBD-labeled **PGON** was synthesized to confirm interactions between the polymer and *Sa* cells at the identical experimental conditions as the fluorescence microscopy studies described above. The synthesis utilizes a previously reported norbornene monomer, **3**,[4] to incorporate *N*-hydroxysuccinimide esters onto **PGON** (Figure S1). Random polymer **4** was synthesized in a similar fashion as **PGON** with a **2**:**3**:catalyst molar ratio of 7:0.5:1 which theoretically corresponds to an average DP of 7.5 units with half the polymer chains containing one *N*-hydroxysuccinimide ester unit. ROMP gave **4**. This polymer was then reacted with NBD amine, **5**, according to literature.[5] The NBD-labeled random polymer, **6**, was collected by precipitation in pentane and treated with TFA to de-Boc the polymer to give **NBD-PGON**. Residual TFA and unreacted **5** was removed by dialysis against Milli-Q water over 48 h (Spectra/Por® cellulose ester dialysis tube, MW cutoff = 500 g/mol).



Random polymer, 4. ^{1}H NMR (300 MHz, $CDCl_3$): δ = 11.50 (1H, br), 8.58 (1H, br), 6.06 (trans) and 5.77 (cis) (2H total, br), 5.05 (1H, br), 4.26 (1H, br), 3.72 (4H, br), 3.61 (2H, br), 2.84 (0.4H, br), 1.49 (18H, br).
GPC: M_n = 3.1 kDa, PDI = 1.11.

Boc-protected NBD-labeled polymer, 6. ^{1}H NMR (400 MHz, $CDCl_3$): δ = 11.41 (1H, br), 10.02 (1H, br), 6.02 (trans) and 5.79 (cis) (2H total, br), 5.06 (1H, br), 4.78-4.12 (3H, br), 3.87 (3H, br), 3.46 (2H, br), 1.56 (9H, br), 1.54 (9H, br).
GPC: M_n = 2.6 kDa, PDI = 1.14.

NBD-PGON. ^{1}H NMR (400 MHz, DMSO-d_3): δ = 8.49 (0.1H, s), 7.71 (1H, br), 7.28 (5H, br), 6.65 (0.1H, d), 5.94 (1H, s), 5.72 (1H, s), 5.35 (0.1H, d), 5.24 (0.1H, d), 4.87 (1H, br), 4.4 (1H, br), 3.47 (2H, br).

Discussion of microscopy with NBD-PGON. As with the fluorescence microscopy studies with the SYTO9:propidium iodide stain described above, an initial *Sa* bacterial concentration of ~10^8 cells/mL was used (Figure S2). Once again the cells remain fairly isolated and non-aggregated. The fact that the cells possess green emission demonstrates that **NBD-PGON** binds to the cells. It cannot be determined though whether the polymer is internalized by bacterial cells or that the polymer just adheres to the membrane surface. The image does support that the membrane remains relatively intact compared to when *Sa* cells are mixed with **Poly-3** (Figure 2 in the text). In that case, the **Poly-3** treated cells appeared aggregated and their emission less distinct and spherical likely due to membrane disruption followed by the release of cellular material. Recent work by Kiessling and co-workers has shown that their dye-labeled poly-guanidino polynorbornene does indeed enter HeLa cells as evidenced by confocal microscopy studies.[6] Higher resolution experiments using **NBD-PGON** are being pursued.

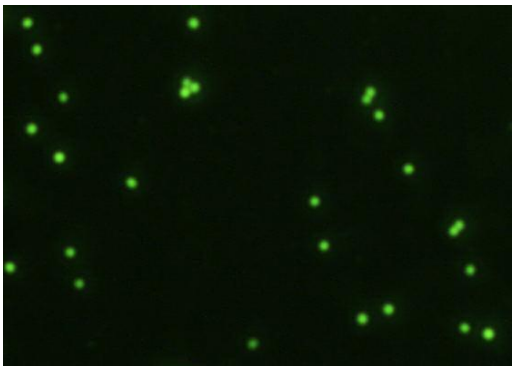


Figure S2. Fluorescence microscopy of *Sa* mixed with **NBD-PGON** (75 μg/mL) for 30 min. Cells visualized using a green filter setting.

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