Supporting Information

Pro-Antimicrobial Network via Degradable Acetals (PANDAs) using Thiol-Ene Photopolymerization.

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Experimental Section

Materials. Allyl alcohol, triethylamine (TEA), 4-chlorobenzaldehyde (pCB), 1,3,5-triallyl-1,3,5-triazine-2,4,6 (1H, 3H, 5H) trione (TTT), 2-Hydroxy-2-methyl-1-phenyl-propan-1-one (Darocure 1173), p-toluenesulfonic acid (pTSOH), molecular sieves (3Å), dry tetrahydrofuran (THF), hexane and ethyl acetate were acquired from Fisher Scientific. Pentaerythritol tetra(3-mercaptopropionate) (PETMP) were provided by Bruno Bock. Difco Agar, Mueller Hinton II agar (MHA), Mueller Hinton II broth (MHB), and Bacto Tryptone were from Becton, Dickinson and Company. All the materials were obtained at the highest purity available and used without further purification unless otherwise specified.

Characterization. ^{1}H NMR was recorded on a Varian Mercury Plus 300 MHz NMR in chloroform-d. Dynamic mechanical analysis (DMA) was performed using a TA Instruments Q800 dynamic mechanical analyzer in tension mode equipped with a gas cooling accessory. Samples were clamped and at a strain of 1 %. Samples were heated from -80 °C to 90 °C at a ramp rate of 3 °C min$^{-1.}$ Kinetic data was obtained using real-time FTIR (RT-FTIR) spectroscopy by determining the conversions of the thiol and ene functional groups. The RT-FTIR studies were conducted using a Nicolet 8700 FTIR spectrometer with a KBr beam splitter and a MCT/A detector with a 320–500 nm filtered ultraviolet light source. Each sample was exposed to a UV light with an intensity of 200 mW cm^{-2}. Series scans were recorded, where spectra were taken approximately 2 scan/s with a resolution of 4 cm^{-1}. Thiol conversions was monitored with integrating the SH conversion peak between 2500-2620 cm^{-1} while the conversion of the allyl group was monitored with conversion peak between 3050-3125 cm^{-1}. Optical density (OD) and fluorescence readings were performed in a BioTek Synergy 2 programmable microplate reader.

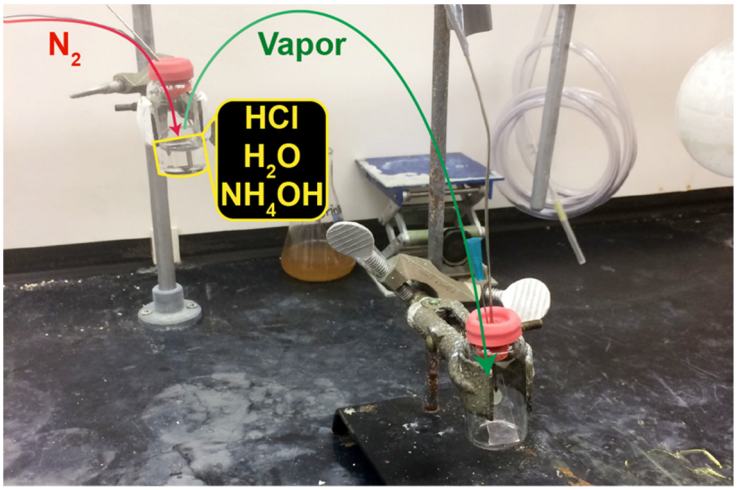
Synthesis of p-chlorobenzaldehyde diallylacetal (pCBA). pCB (10 g, 71.14 mmol, 1 equiv), pTSOH (4.3 g, 24.97 mmol, 0.35 equiv), 3Å molecular sieves (15 g), and allyl alcohol (16.52 g, 284.6 mmol, 4 equiv) were dissolved in dry THF (50 mL). The yellow reaction mixture was left to stir overnight then quenched with addition of TEA (19.8 mL, 142.3 mmol, 2 equiv), filtered and rotovapped before adding to silica gel column. The column was eluted with a 10:90 ethyl acetate/hexane to afford 8.3 g (48.9%) of the acetal. ^{1}H NMR (300 MHz, Chloroform-*d*) δ 7.46 (d, *J* = 8.4 Hz, 1H), 7.36 (d, *J* = 8.4 Hz, 1H), 5.95 (ddt, *J* = 16.2, 10.8, 5.6 Hz, 1H), 5.63 (d, *J* = 2.7 Hz, 1H), 5.33 (dt, *J* = 17.2, 1.8 Hz, 1H), 5.20 (dd, *J* = 10.5, 2.1 Hz, 1H), 4.06 (d, *J* = 5.1 Hz,

2H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 136.97, 134.27, 128.41, 128.21, 116.96, 99.63, 66.06. GC-MS observed: 238; calculated: 238.

General preparation of PANDA disks. The 100% pCBA disks were prepared by adding PETMP and pCBA at 1:1 ratio of SH:alkene, along with 4 wt % of Darocure 1173. The 90% pCBA disks were prepared by adding pCBA and TTT as another source of alkene (TTT is 10% of total alkene while maintaining an overall 1:1 ratio of alkene:SH) with PETMP, and Darocure 1173. The mixtures were well mixed and 25 µL of monomer formulations were aliquoted onto a glass slide and covered with another glass slide spaced with Teflon spacers (0.76 ± 0.02 mm in thickness). The samples were then cured using an Omnicure S1000-1B with a 100W mercury lamp (λmax = 365 nm, 320–500 nm filter) at an intensity of 200 mW cm^{-2} for 20 minutes.

Degradation of PANDAs in Phosphate Buffer Saline (PBS). The hydrolysis of PANDA disks was done using slightly modified method by Carter and coworkers.[1] In general, the degradation study was performed in aqueous media (PBS) at pH 7.4 with a large excess of octanol to partition the aldehyde from the aqueous environment. Concentration was determined by measuring the absorbance of pCB released at 275 nm over 120 hours and extrapolated from a standard calibration curve. Experiments were done with n=5, with the mean and standard deviation reported. The 25 mm^3 disks were placed at the bottom of 20 mL scintillation vials filled with 3 mL of PBS and 15 mL of octanol. Immediately, 100 µL octanol aliquots were taken out form the vial and measured for t=0. After the measurement, aliquots were placed back to the scintillation vials to maintain the same concentration throughout the kinetic study. Aliquots were analyzed at t = 0, 20, 24, 30, 48, 54, 72, 78, 96, 102, 120 h.

Degradation of PANDAs in HCl, H_2O, and NH_4OH chambers. Humidity chambers were made via bubbling of nitrogen gas into either 12 M HCl, 30 wt% NH_4OH, or deionized H_2O with a cannula in the head space of the vial to transport into a vial containing a 90% pCBA PANDA disk on a glass slide.



Evaluation of antibacterial activity of PANDAs. The antimicrobial activity of PANDA disks were tested against several species of bacteria via the zone of inhibition method. The indicator microorganisms included E. coli ATCC 43895 (serotype O157:H7), Staphylococcus aureus RN6390, Burkholderia cenocepacia K56-2 (clinical isolate from Canada), and Pseudomonas aeruginosa PA-01. The testing was done on Mueller Hinton II agar (MHA) plates that have been overlaid with soft agar seeded with individual bacterial strains. The soft agar contained (per liter): 10 g of Bacto Tryptone, 6 g of Difco agar, and 8 g of sodium chloride. To create an overlay, the indicator organisms were grown overnight at 37°C in Mueller Hinton II broth (MHB). The overnight cultures were diluted 1:5 with fresh MHB, and mixed with molten soft agar to achieve the density of ~10^8 CFU mL^{-1}. From this mixture, 4-mL aliquots were overlaid onto MHA base plates and allowed to completely solidify. After solidifying of soft agar, 25 mm^3 disks were overlaid on the plates and incubated at 37°C. The zones of inhibition (ZOI) were measured after 30 h and reported as the radius from the edge of the disk to the edge of the zone. Five replicates were carried out for each disk treatment and bacterial strain with the mean and standard deviation reported.

Determination of Minimum Inhibitory Concentrations (MICs). MICs of the PANDA disks were determined using a modified broth macrodilution method. Briefly, overnight bacterial cultures in MHB were adjusted to ~10^5 CFU mL^{-1}. Various disks of different sizes (5-50 mm^3) containing pCB at concentrations ranging from 0.5 - 3 mg/mL of were added to 3 mL of bacterial solutions. The tubes then were incubated at 37 °C at 200 rpm for 30 hours. Bacteria suspended in MHB served as a positive control, while MHB without bacterial inoculum served as a negative control. The inoculated tubes were assessed by measuring optical density at 600 nm where OD < 0.05 considered negative for bacterial growth.

Kill kinetics via terminal dilution assays. To compare the rate of bacterial killing by 90% pCBA PANDA disks, the test organisms were exposed to disks and change of viable bacteria was determined by a modified terminal dilution method. The bacterial cultures were prepared and adjusted to ~10^5 CFU mL^{-1} as described above. The adjusted bacterial cultures (3 mL) were mixed with (2 x 25 mm^3) 90% pCBA PANDA disks. The inoculated tubes were incubated at 37°C with shaking (200 rpm) and bacterial populations were determined immediately after the addition of disks (0 h), and at 2, 4, 8, 12, 24, and 30 hours of exposure. At each time point, six 100 µL aliquots of the bacterial suspensions exposed to PANDAs were transferred into 96-well microplates prefilled with 200 µL of MHB and serially diluted. The inoculated microplates were incubated for 48 h at appropriate growth temperature, after which the turbidity was measured with a BioTek Synergy 2 microplate reader. An optical density at 600 nm of ≥ 0.05 was considered positive for bacterial growth. Populations of viable bacteria were calculated from the final dilution (terminal dilution, or TD), in which bacterial growth was observed using equation 1:

$$\frac{\text{CFU}}{\text{mL}} = \frac{10 \times 3^{\text{TD}}}{1\text{mL}} \quad (1)$$

Where TD is the terminal dilution factor obtained from microplate readings. The calculated CFU/mL was taken as the average of the 6 replicates and the experiment was repeated 3 times for each bacteria.

Evaluation of antifungal activity of PANDA disks. The antifungal activity of 90% pCBA PANDA disks was tested against several species of fungus using zone of inhibition method. The indicator microorganisms included Candida albicans and Trichoderma harzianum. Fungus cultures were adjusted to OD_{600} 0.1 for C. albicans and 10^5 ppg/mL for T. harzianum. Mueller Hinton II agar (MHA) plates have been spread with 100 µL of fungus culture and 25 mm^3 disks were placed on the plates. Zone of inhibition was measured after 72 hours of incubation at 30°C.

Resistance Development Study. The study was done using slightly modified method by Haldar and coworkers.[2] MIC values for 90% pCBA PANDA disks were determined for P. aeruginosa PAO-1 as described above. For the subsequent MIC determination, bacteria solutions were made by using bacteria from sub-MIC concentrations of the disks (at MIC/2). The bacteria solution was readjusted to ~10^5 via OD_{600} readings. Into 3 separate vials, 3 mL of adjusted bacteria solution was added along with disks of various sizes. After another 24 h incubation period, bacteria solutions again were made by using bacteria from sub-MIC concentrations of the disks (at MIC/2). The resistance development study was repeated twice, each with 20 total passages. The fold increase of MIC was reported.

3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) Cytotoxicity assay. The anti-proliferative activities of pCB and 90% pCBA PANDA degradation products in 3% DMSO solution were determined following a standard literature procedure.[3] Briefly, 48 hours prior to treatment, KB cells (100,000 cells/mL, 100 µL) were seeded in a 96 well plate (Corning Inc.). Cells were treated with 50 µL of pCB and 50 µL of PANDA byproducts (media from 7 days incubation at pH 7.4). KB cells treated with 3% DMSO solution served as control. Cell proliferation was determined via a standard MTT assay (Vybrant MTT Cell Proliferation Assay Kit; Invitrogen). Cells were incubated for 30 h before adding 10 µL of a 12 mM MTT reagent to each well. The cells were further incubated for an additional 4 h, followed by removal of the media and addition of 50 µL of DMSO. The absorbance was then determined utilizing a Biotek Synergy2 MultiMode Microplate Reader. Experiment was performed in quadruplicate.

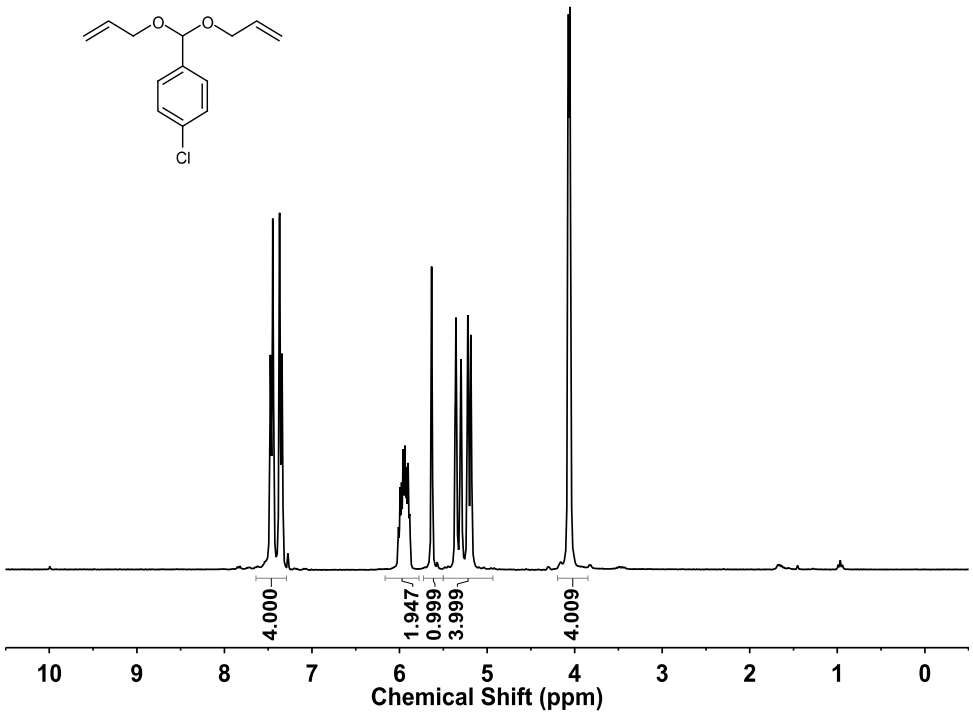


Figure S1. ^{1}H NMR of diallyl p-chlorobenzaldehyde acetal.

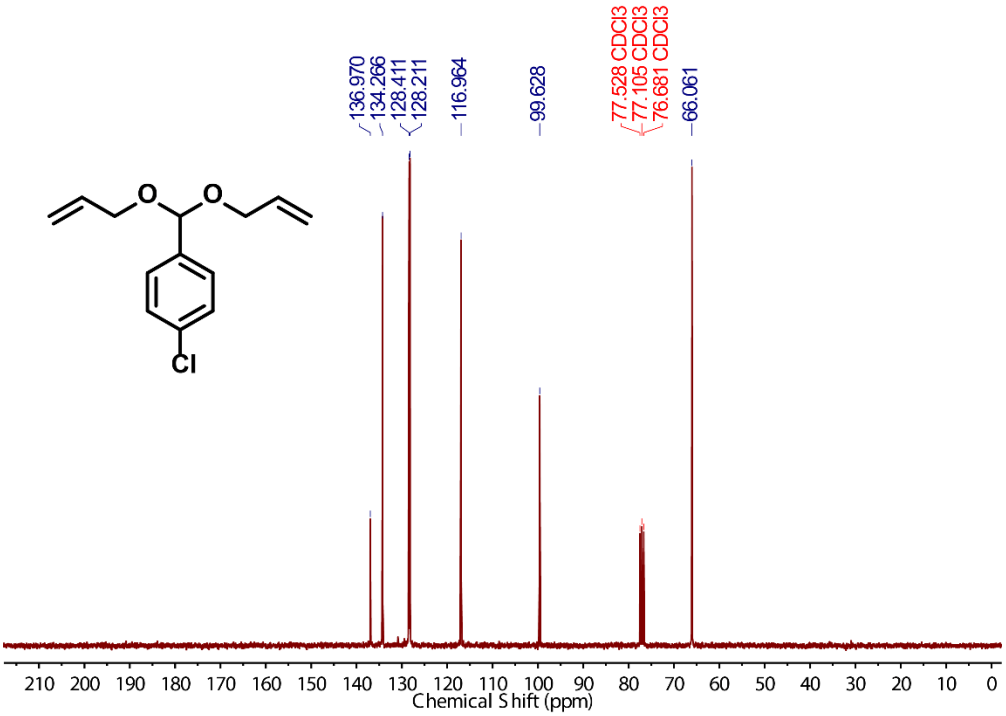


Figure S2. ^{13}C NMR of diallyl p-chlorobenzaldehyde acetal.

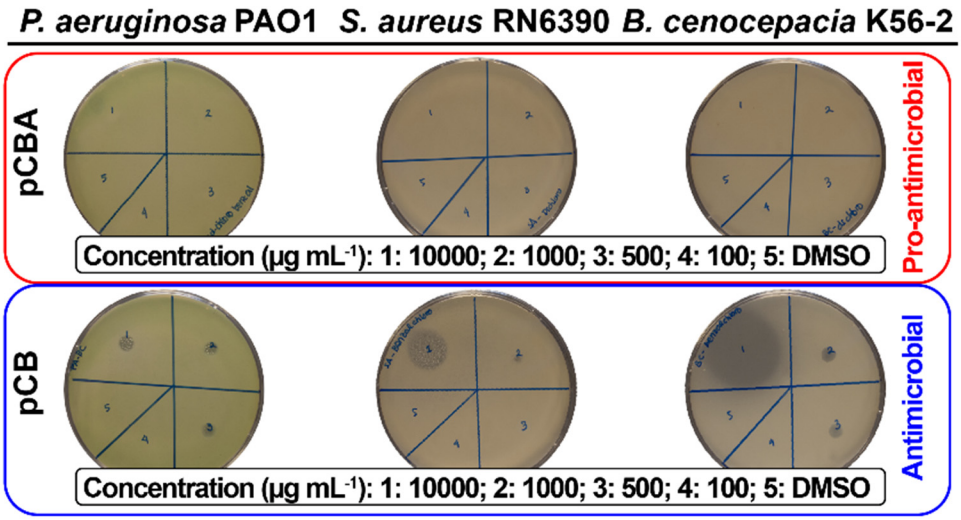


Figure S3. Zones of inhibition for small molecule precursors within PANDA formulation.

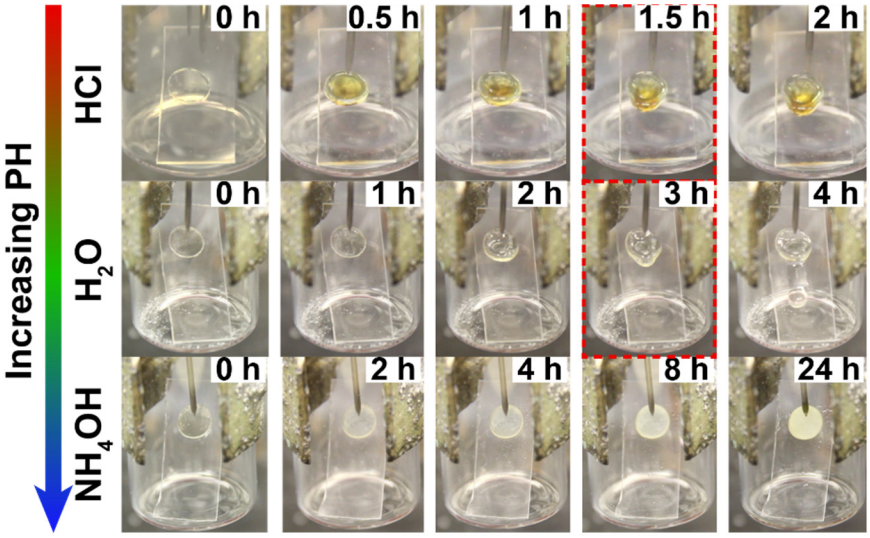


Figure S4. PANDAs exposed to HCl vapor led to complete bulk degradation in minutes while ammonium hydroxide vapor led to retarded degradation with no flow observed within 24 h.

References

(1) Kim, S.; Linker, O.; Garth, K.; Carter, K. R. *Polym. Degrad. Stab.* **2015**, *121*, 303.
(2) Yarlagadda, V.; Samaddar, S.; Paramanandham, K.; Shome, B. R.; Haldar, J. *Angew. Chem. Int. Ed.* **2015**, *54*, 13644.
(3) Riss, T. L.; Moravec, R. A.; Niles, A. L.; Benink, H. A.; Worzella, T. J.; Minor, L. Cell viability assays. In *Assay Guidance Manual*; Sittampalam, G. S., Coussens N.P., Nelson H., Eds.; Bethesda (MD), **2015**.