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Light-Enhanced Bacterial Killing and Wash-Free Imaging Based on AIE Fluorogen

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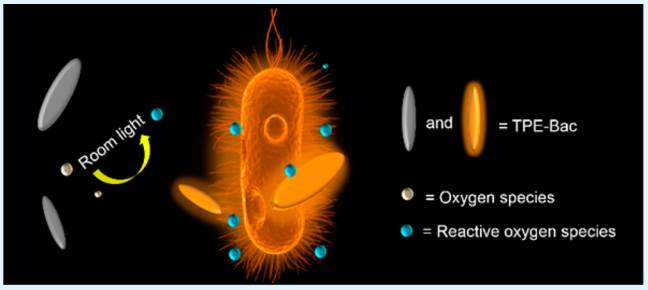
Supporting Information

ABSTRACT: The rapid acquisition of antibiotic resistance poses difficulties in the development of effective methods to eliminate pathogenic bacteria. New bactericides, especially those do not induce the emergence of resistance, are thus in great demand. In this work, we report an aggregation-induced emission fluorogen, TPE-Bac, for bacterial imaging and elimination. TPE-Bac can be readily dissolved in aqueous solution with weak emission. The presence of bacteria can turn on its emission, and thus no washing step is required in the imaging process. Meanwhile, TPE-Bac can be applied as a bactericide for elimination of bacteria. The amphiphilic TPE-Bac bearing two long alkyl chains and two positively charged amines can intercalate into the membrane of bacteria, increase membrane permeability and lead to dark toxicity. The efficiency of bacteria killing is greatly enhanced under light irradiation. TPE-Bac can serve as a photosensitizer to induce reactive oxygen species (ROS) generation, which ensures the efficient killing of bacteria. The TPE-Bac-containing agar plates can be continuously used for bacteria killing by applying light to induce ROS generation.



KEYWORDS: *aggregation-induced emission, photodynamic therapy, bactericide, bacterial imaging, reactive oxygen species*

INTRODUCTION

With the advancement of human living standards, hygiene and food safety issues are gaining increasing attention from both research scientists and ordinary people, owing to their close relationship with human health. Antibiotics are the most widely used materials for pathogen prevention and infection treatment, due to their high effectiveness in inhibiting bacteria growth and low interference to mammlian cells. The rapid emergence of antibiotic resistance,[1] however, pushes scientists to develop new antibiotics, which is usually a long-term and costly process. Recently, increasing research efforts have been spent to develop new disinfection methods which bacteria can hardly generate resistance. Photodynamic therapy (PDT), which utilizes a photosensitizer to produce toxic reactive oxygen species (ROS) for tumor treatment, was thus proposed and applied to localized pathogen elimination.[2−4] Currently, most PDT photosensitizers are based on porphyrin,[5−7] and phenothiazium.[8−10] Conjugated polymers[11−14] are also gaining attention to be used as photosensitizers. However, most of these materials are coplanar or extremely hydrophobic in nature, in which strong $\pi-\pi$ stacking interactions may take place. Such $\pi-\pi$ stacking interactions will lead to chromophore aggregation, decreased disinfection efficiency and fluorescence quenching.

We observed a phenomenon of aggregation-induced emission (AIE) in a group of propeller-shaped molecules. These molecules show faint or no emission in solution, but demonstrate enhanced emission upon aggregation.[15,16] Through systematic studies, we identified restriction of intramolecular motion as the main cause for the AIE effect.[17,18] The AIE phenomenon is of both scientific value and practical implications, and has thus attracted increasing research efforts on these materials. Owing to their good biocompatibility, excellent photostability and superb selectivity, the AIE luminogens (AIEgens)[19−21] have been applied to cell[22−25]

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and bacterial imaging,[26] cell apoptosis detection,[27] chemotherapy[28] and drug delivery.[29,30]

We recently noticed the light-induced ROS generation effect of some AIEgens and applied them to kill cancer cells through necrosis[31] and apoptosis[32] pathways, respectively. By selectively targeting the cytoplasmic membrane and mitochondria, the photosensitizers take effect at the key organelles regulating cell necrosis and apoptosis, respectively, and eliminate cancer cells efficiently. Besides, the AIEgens have also been fabricated into nanoparticles[33] and functionalized with short peptides[34] to increase their efficiency and specificity in tumor elimination.

Inspired by these successes of utilizing AIEgens in PDT, in this work, a new AIEgen, TPE-Bac, is applied to bacterial imaging and light-enhanced bacterial killing. Thanks to the good water dispersity and AIE feature of TPE-Bac, no washing process is required during the imaging of bacteria, which simplifies the imaging process. Besides imaging, TPE-Bac is also designed to be an effective antibacterial material. The amphiphilic molecule with two hydrophobic alkyl chains and two hydrophilic amines will intercalate into the membrane of the bacteria and increase the membrane permeability by distorting the phosphatidyl lipid arrangement of the membrane. TPE-Bac can serve as a photosensitizer for ROS generation, which exerts additional toxicity to the bacteria. After 1 h of irradiation with room light, more than 99% of both Gram-positive and -negative bacteria are eliminated. In addition, the agar plate containing TPE-Bac can be repeatedly applied to bacteria spray, followed by light-enhanced killing and recycling processes (bacteria spray-killing cycle) for at least five times and remains effective (see Scheme S1 of the Supporting Information). The good photostability of TPE-Bac guarantees its durability in continuous killing of bacteria and makes it an excellent candidate for a PDT photosensitizer.

EXPERIMENTAL SECTION

Materials. LB agar, LB broth, potassium phosphate dibasic anhydrous and sodium phosphate were purchased from USB Co. Singlet oxygen senor green (SOSG) was purchased from Invitrogen. Zinc dust, titanium tetrachloride, 4,4′-dihydroxybenzophenone, 4-bromobenzophenone, 1-bromoundecane, potassium carbonate, 4-formylphenylboronic acid, tetrakis(triphenylphosphine)palladium, piperidine and propidium iodide (PI) were purchased from Sigma-Aldrich and used as received. Tetrahydrofuran (THF) was purified by distillation from sodium benzophenone ketyl immediately prior to use. 1-(3-Trimethylammoniopropyl)-4-methylpyridinium dibromide was synthesized according to a literature method.[35] Other reagents used in this work, such as dimethyl sulfoxide, potassium chloride and sodium chloride, were purchased from Sigma-Aldrich.

Characterization. ^{1}H and ^{13}C NMR spectra were measured with a Bruker ARX 400 NMR spectrometer using $CDCl_3$ and DMSO-d_6 as the deuterated solvent. High-resolution mass spectra (HRMS) were recorded on a Finnigan MAT TSQ 7000 mass spectrometer system operating in a matrix-assisted laser desorption/ionization time-of-flight (MALDI-TOF) mode. UV−visible absorption spectra were taken on a Milton Ray Spectronic 3000 array spectrophotometer. Steady-state fluorescence spectra were recorded on a PerkinElmer LS 55 spectrometer. Fluorescence images were collected with an Olympus BX 41 fluorescence microscope. Particle sizes were measured on a ζ-potential analyzer (Brookhaven, ZETAPLUS). The aggregation morphology of TPE-Bac was investigated using transmission electron microscopy (TEM, JEOL JEM 100CXII, Japan) at an accelerating voltage of 100 kV.

Synthesis. *4,4′-(2-(4-Bromophenyl)-2-phenylethene-1,1-diyl)-diphenol (**3**).* **3** was synthesized following the procedures reported in the literature[36] with some modifications. Into a 500 mL two-necked round-bottom flask was added 4,4′-dihydroxybenzophenone (3.21 g, 15 mmol), 4-bromobenzophenone (7.80 g, 30 mmol) and zinc dust (8.82 g, 135 mmol). The flask was vacuumed and purged with nitrogen for three times. Afterward, 200 mL of THF was injected into the flask, followed by cooling down to −78 °C with an acetone/dry ice bath. $TiCl_4$ (6.74 mL, 67.5 mmol) was added into the mixture in a dropwise way. The reaction was then refluxed overnight under nitrogen conditions. After the solution cooled to room temperature, hydrochloric acid (1 M) was added to the reaction mixture to adjust the pH to 2. The mixture was then extracted with DCM and dried with anhydrous sodium sulfate. The crude product was purified by silica column chromatography, using hexane and ethyl acetate (5:2 v/v) as the elusion solution to give **3** as a white solid (4.32 g, 65%). ^{1}H NMR (400 MHz, DMSO- d_6), δ (ppm): 9.43 (s, 2H), 7.30−7.24 (d, 2H), 7.14−7.02 (m, 4H), 6.92−6.88 (d, 2H), 6.85−6.81 (d, 1H), 6.75−6.68 (m, 4H). ^{13}C NMR (100 MHz, DMSO- d_6), δ (ppm): 155.869, 155.777, 143.953, 143.467, 143.300, 141.143, 136.233, 133.660, 133.601, 132.701, 131.887, 131.816, 130.564, 130.504, 129.223, 127.694, 127.549, 126.005, 118.871, 114.878, 114.539, 114.376. HRMS (MALDI-TOF) m/z: calcd, 442.0568 [M]; found, 442.0099 $[M]^+$.

*4,4′-(2-(4-Bromophenyl)-2-phenylethene-1,1-diyl)bis-((undecyloxy)benzene) (**4**).* Into a 250 mL two-necked round-bottom flask was added potassium carbonate (4.00 g, 28.25 mmol) and **3** (2.5 g, 5.65 mmol). The flask was vacuumed and purged with dry nitrogen three times. After 1-bromoundecane (5.31 mL, 22.6 mmol) and DMF (80 mL) were added, the reaction was stirred overnight under nitrogen conditions at 70 °C. After the mixture cooled to room temperature, it was extracted with dichloromethane (DCM), washed with distilled water several times and dried with anhydrous magnesium sulfate. The crude product was purified by silica column chromatography using hexane and DCM (10:1 v/v) as the elusion solvent to give **4** as a pale yellow viscous oil (3.30 g, 78%). ^{1}H NMR (400 MHz, $CDCl_3$), δ (ppm): 7.24−7.20 (m, 2H), 7.14−6.99 (m, 6H), 6.94−6.87 (m, 5H), 6.68−6.60 (m, 4H), 3.92−3.84 (m, 4H), 1.80−1.69 (m, 4H), 1.47−1.24 (m, 32H), 0.92−0.87 (t, 6H). ^{13}C NMR (100 MHz, $CDCl_3$), δ (ppm): 158.068, 157.981, 144.092, 143.608, 141.153, 137.843, 136.011, 135.926, 133.270, 132.755, 131.566, 131.033, 127.994, 127.847, 126.433, 120.140, 113.899, 113.717, 68.057, 68.007, 32.127, 29.829, 29.791, 29.639, 29.560, 29.511, 26.273, 22.910, 14.351. HRMS (MALDI-TOF) m/z: calcd, 750.4011 [M]; found, 750.4021 $[M]^+$.

*4′-(1-Phenyl-2,2-bis(4-(undecyloxy)phenyl)vinyl)-[1,1′-biphenyl]-4-carbaldehyde (**6**).* Into a 250 mL two-necked round-bottom flask was added **4** (2.00 g, 2.60 mmol), 4-formylphenylboronic acid (**5**) (0.59 g, 3.96 mmol), K_2CO_3 (2.28 g, 16.50 mmol) and $Pd(PPh_3)_4$ (0.23 g, 0.20 mmol). The flask was vacuumed and purged with nitrogen three times. Afterward, THF (80 mL) and water (20 mL) were injected into the flask, followed by refluxing overnight under nitrogen conditions. After the mixture cooled down to room temperature, it was extracted with DCM, washed with distilled water several times and dried with anhydrous magnesium sulfate. The crude product was purified by silica column chromatography using hexane and DCM (5:1 v/v) as the elusion solvent to give **6** as a yellow viscous oil (1.72 g, 85%). ^{1}H NMR (400 MHz, $CDCl_3$), δ (ppm): 10.03 (s, 1H), 7.93−7.89 (d, 2H), 7.74−7.70 (d, 2H), 7.42−7.39 (d, 2H), 7.16−7.05 (m, 7H), 7.00−6.92 (m, 4H), 6.68−6.62 (t, 4H), 3.90−3.83 (t, 4H), 1.78−1.70 (m, 4H), 1.47−1.24 (m, 32H), 0.92−0.87 (t, 6H). ^{13}C NMR (100 MHz, $CDCl_3$), δ (ppm): 191.240, 157.241, 157.155, 146.129, 144.338, 143.546, 140.432, 137.535, 136.140, 135.351, 134.342, 131.999, 131.967, 131.454, 130.824, 129.582, 127.160, 126.655, 125.880, 125.571, 113.032, 112.903, 67.222, 67.193, 31.280, 28.982, 28.943, 28.803, 28.715, 28.687, 25.439, 22.064, 13.501. HRMS (MALDI-TOF) m/z: calcd, 776.5168 [M]; found, 776.5176 $[M]^+$.

4-(2-(4′-(1-Phenyl-2,2-bis(4-(undecyloxy)phenyl)vinyl)-[1,1′-biphenyl]-4-yl)vinyl)-1-(3-(trimethylammonio)propyl)pyridinium Bromide (TPE-Bac). A solution of 1-(3-trimethylammoniopropyl)-4-methylpyridinium dibromide (**7**) (0.31 g, 0.40 mmol) and **3** (0.28 g, 0.79 mmol) was refluxed under nitrogen in dry ethanol using piperdine as catalyst. After the mixture cooled to room temperature, it was evaporated under reduced pressure to remove the solvent. The residue was purified by a silica gel column chromatography using a

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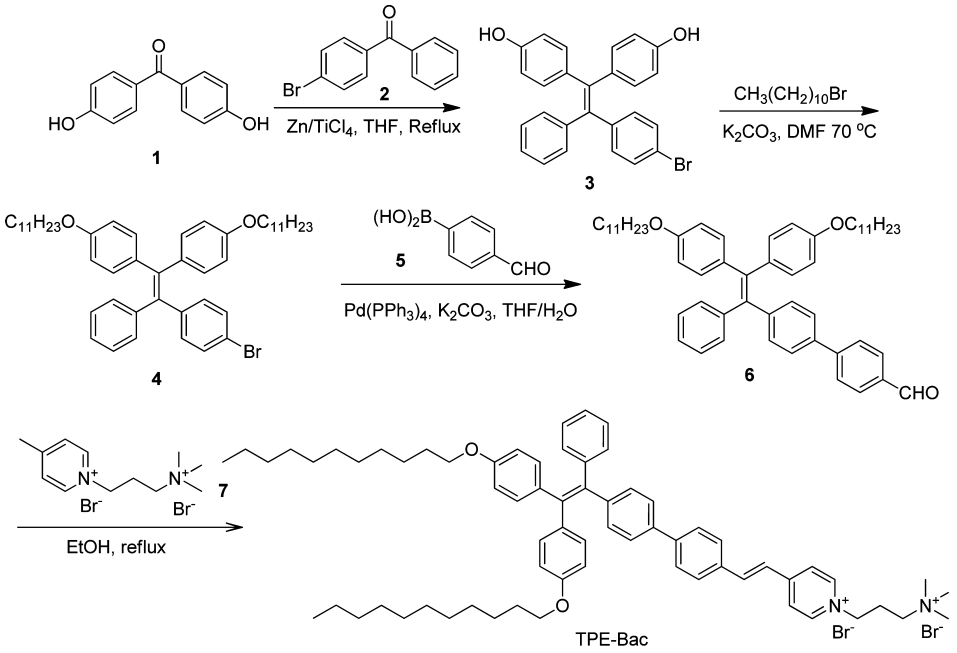
Scheme 1. Synthetic Route to TPE-Bac



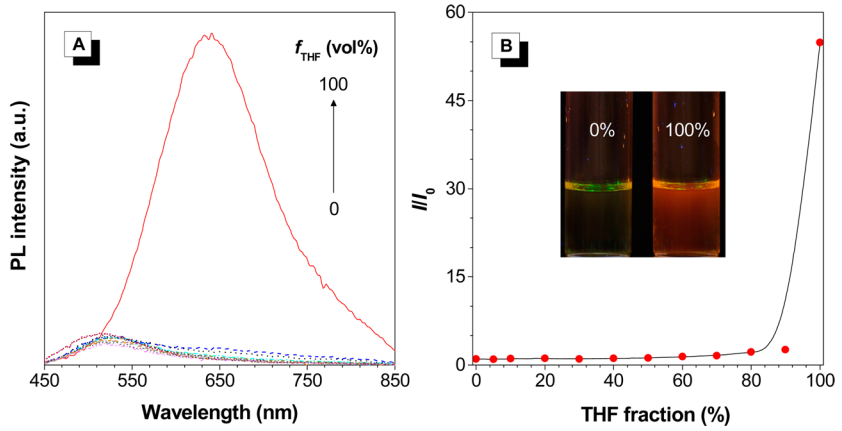


Figure 1. (A) PL spectra of TPE-Bac in THF and DMSO/THF mixtures with different THF fractions (f_{THF}). Concentration, 50 μM; excitation wavelength, 405 nm. (B) Plot of relative PL intensity (I/I_0) versus the composition of the DMSO/THF mixture of TPE-Bac. Inset: photographs of DMSO/THF mixtures of TPE-Bac with f_{THF} of 0% and 100% taken under 365 nm UV irradiation.

DCM and methanol mixture (2:1 v/v) as the elution solvent to give TPE-Bac as a red powder (0.24 g, 53%).^{1}H NMR (400 MHz, $CDCl_3$), δ (ppm): 9.18 (s, 2H), 8.01 (s, 2H), 7.69 (d, 1H), 7.47 (d, 2H), 7.35 (d, 2H), 7.17 (d, 2H), 7.10−6.83 (m, 12H), 6.55(dd, 4H), 4.90 (s, 2H), 3.94−3.77 (m, 4H), 3.77−3.68 (s, 2H), 3.42 (s, 9H), 2.77 (s, 2H), 1.75−1.56 (m, 4H), 1.45−1.14 (m, 32H), 0.90−0.77 (m, 6H). ^{13}C NMR (100 MHz, $CDCl_3$), δ (ppm): 157.160, 157.058, 152.857, 143.839, 143.592, 141.900, 140.753, 140.112, 137.596, 136.028, 135.497, 135.285, 132.764, 131.951, 131.356, 130.795, 128.403, 127.107, 126.416, 125.419, 123.627, 121.471, 112.998, 112.829, 67.096, 62.118, 53.823, 31.267, 31.214, 29.058, 28.980, 28.930, 28.890, 28.829, 28.727, 28.702, 28.648, 28.605, 25.448, 25.378, 22.040, 22.002, 13.487, 13.471. HRMS (MALDI-TOF) m/z: calcd, 1031.6024 $[M - Br]^+$; found, 1031.6024 $[M - Br]^+$.

Sample Preparation. A stock solution of TPE-Bac in DMSO with a concentration of 10 mM was prepared and stored in the 4 °C fridge. Phosphate buffer saline (PBS) was prepared by dissolving NaCl (8 g), KCl (0.2 g), Na_2HPO_4 (1.44 g) and KH_2PO_4 (0.24 g) in 800 mL of distilled water, adjusting pH to 7.4 with HCl and calibrating to 1 L by adding H_2O. PBS was sterilized by autoclaving for 20 min at 15 psi (1.05 kg/cm^2) on liquid cycle and stored at room temperature.

Bacteria Culturing, Imaging and Killing. *Bacteria Staining.* A single colony of bacteria on solid culture medium [Luria broth (LB) for *Escherichia coli* and *Staphylococcus epidermidis*] was transferred to 5 mL of liquid culture medium and grown at 37 °C for 10 h. The concentrations of bacteria were determined by measuring optical density at 600 nm (OD_{600}) and then a 10^9 colony forming unit (CFU) of bacteria was transferred to a 1.5 mL microcentrifudge tube. Bacteria were harvested by centrifuging at 7000 rpm for 3 min. After removal of the supernatant, 1 mL of TPE-Bac solution in PBS at the concentration of 10 μM was added into the microcentrifudge tube. After dispersion with vortex, the bacteria were incubated at room temperature for 10 min.

To take fluorescence images, 2 μL of stained bacteria solution was transferred to glass slide and then covered by a coverslip. The image was collected using 100× objective. The bacteria were imaged under an FL microscope (BX41 Microscope) using different combinations of excitation and emission filters for each dye: for TPE-Bac, excitation filter = 460−490 nm, dichroic mirror = 505 nm, emission filter = 515 nm long pass; for PI, excitation filter = 510−550 nm, dichroic mirror = 570 nm, emission filter = 590 nm long pass.

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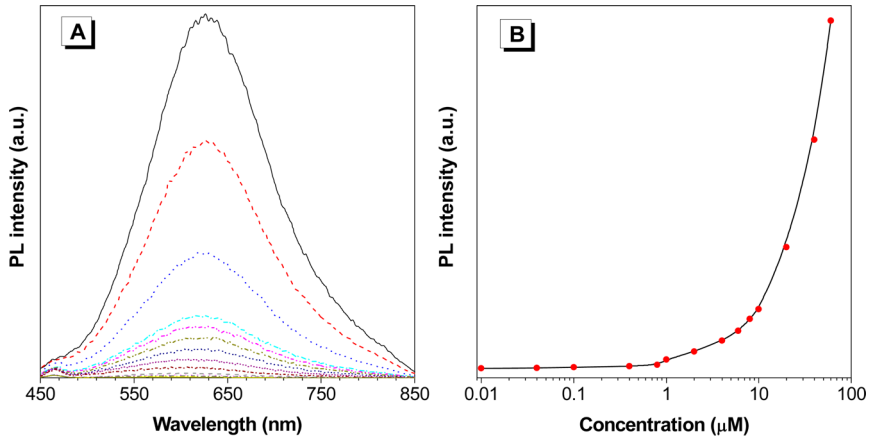

Figure 2. (A) PL spectra of aqueous solutions of TPE-Bac with different concentrations. Excitation wavelength: 405 nm. (B) Plot of PL intensity versus the solution concentration.

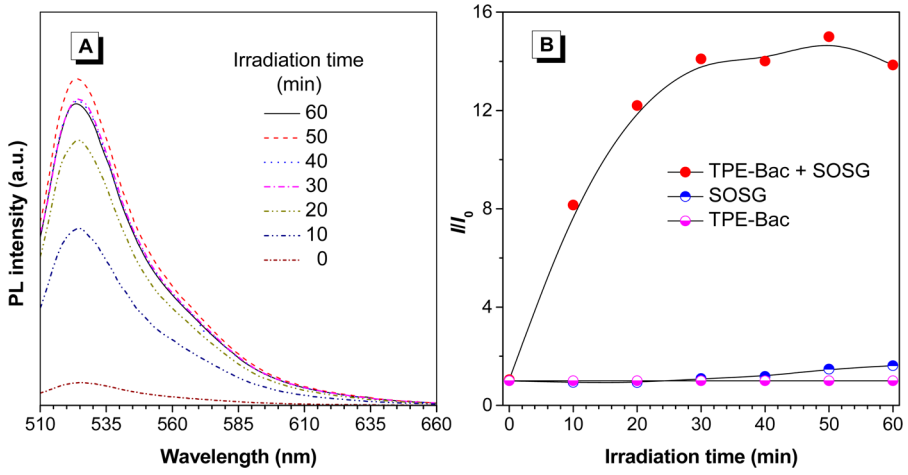

Figure 3. Light-induced singlet oxygen generation of TPE-Bac evaluated by SOSG. (A) PL spectra of mixtures of SOSG (5 μM) and TPE-Bac (5 μM) after room light irradiation for different times. Excitation wavelength: 505 nm. (B) Plot of relative PL intensity (I/I_0) at 530 nm versus the irradiation time.

For the PI staining experiment, after 10 min of incubation with 10 μM of TPE-Bac, the bacteria were exposed to room light for 10 min, while the control group was put in the dark. Afterward, PI was added to both the experiment and control group at a final concentration of 1.5 μM, followed by incubation in the dark for another 10 min. Then the bacteria were imaged under the fluorescent microscope with the following setting: excitation filter = 510–550 nm, dichroic mirror = 570 nm, emission filter = 590 nm long pass.

For the light-induced toxicity experiment, 10^8 CFU bacteria were dispersed in 1 mL of PBS. After incubation with 10 μM TPE-Bac for 10 min, the solution was centrifuged at 7000 rpm for 3 min, followed by removal of the supernatant and PBS washing. Then the bacteria were dispersed in PBS and exposed to room light for designed periods of time, while the control groups were put in the dark. Then the viability of bacteria was quantified by the plate-count method. The bacteria was diluted with a dilution factor of 10^5. Then 50 μL of the bacteria solution was sprayed onto a LB agar plate, which was then subjected to culturing at 37 °C for 24 before quantification or taking images.

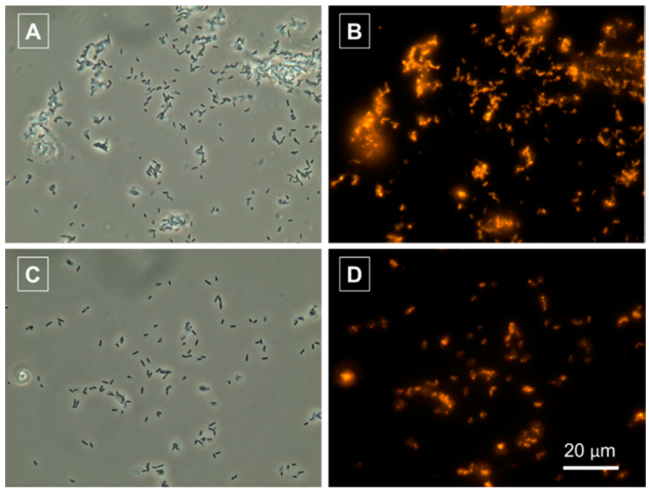

Figure 4. (A and C) Bright field and (B and D) fluorescence images of (A and B) *S. epidermidis* and (C and D) *E. coli* incubated with 10 μM TPE-Bac for 10 min. Excitation wavelength: 460–490 nm.

RESULTS AND DISCUSSION

Synthesis and Photophysical Properties. TPE-Bac was prepared following the synthetic route shown in Scheme 1. **3** was prepared by the cross McMurry coupling reaction of 4,4′-dihydroxybenzophone (**1**) and 4-bromobenzophenone (**2**), which then underwent Williamson ether synthesis reaction by treatment of 1-bromoundecane to form **4**. Compound **4** was transformed to **6** by Suzuki coupling reaction with 4-bromobenzaldehyde (**5**). The subsequent condensation

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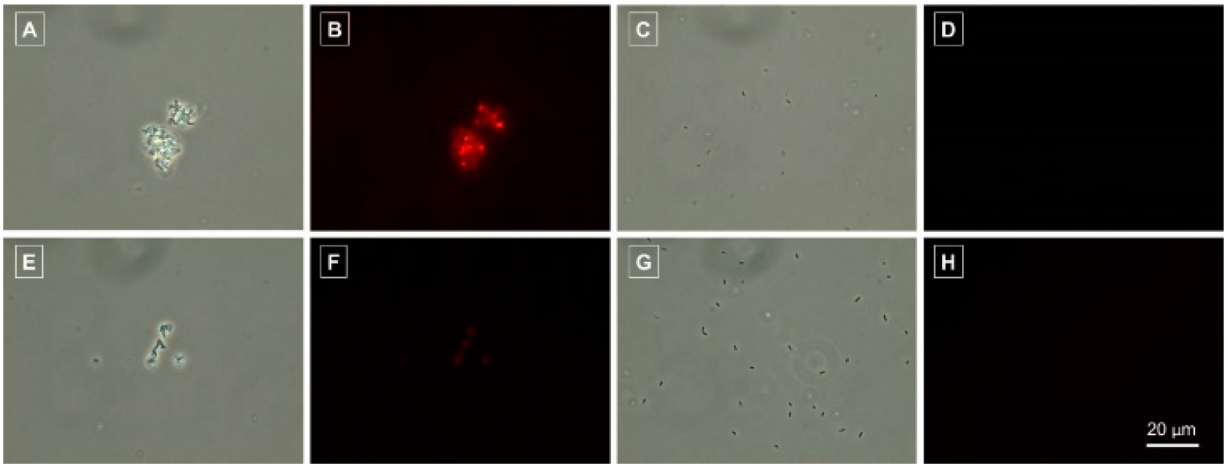

Figure 5. (A, C, E and G) Bright-field and (B, D, F and H) fluorescence images of (A–D) *S. epidermidis* and (E–H) *E. coli* incubated (A, B, E and F) with and (C, D, G and H) without 10 μM TPE-Bac for 10 min followed by room light exposure for 10 min, and staining with 1.5 μM PI for 10 min. Excitation wavelength: 510–550 nm.

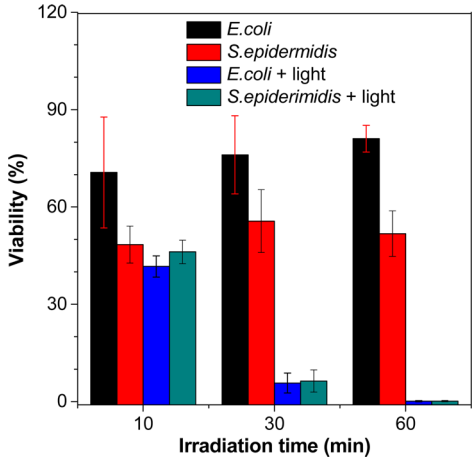

Figure 6. Killing efficiency of TPE-Bac on *E. coli* and *S. epiderimidis* in the absence and presence of room light irradiation for different times. The bacteria were incubated with 10 μM TPE-Bac for 10 min prior to light irradiation.

between **6** and pyridinium salt (**7**) readily gave TPE-Bac. All the intermediates were obtained in medium to high yield. ^{1}H NMR, ^{13}C NMR and high resolution mass (HRMS) were utilized to characterize the intermediates and TPE-Bac (Figure S1 of the Supporting Information), and satisfactory results corresponding to their structures were obtained.

Tetraphenylethene (TPE), which contains four phenyl rings, is hydrophobic in nature. The positively charged pyridinium salt and quaternary amine endow TPE with good hydrophilicity, making TPE-Bac readily soluble in polar solvents such as DMSO, methanol and ethanol. In nonpolar solvents such as THF, *n*-hexane and chloroform, TPE-Bac is insoluble.

The DMSO solution of TPE-Bac shows an absorption maximum at 428 nm (Figure S2 of the Supporting Information), which is red-shifted by around 100 nm compared with TPE. The pyridinium salt is a strongly electron-withdrawing group, while the ethers are moderately electron-donating groups. The strong donor–acceptor interaction across the TPE core facilitates the intramolecular charge transfer and lowers the energy gap, resulting in the bathochromic shift in absorption spectrum. The tail of the absorption peak extends to around 550 nm. Such a broad absorption peak almost covers

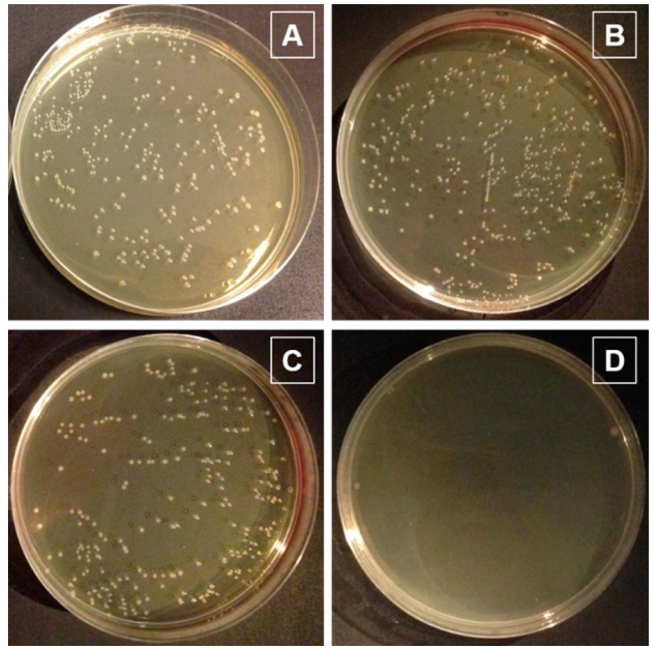

Figure 7. Plates of *E. coli* (A) without and (B) with light irradiation for 1 h in the absence of TPE-Bac. Those treated with 10 μM TPE-Bac for 10 min, followed by (C) storage in the dark or (D) irradiation with room light for 1 h were given in panels C and D.

the UV, blue and green light regions, which makes TPE-Bac a good candidate for photodynamic therapy. The absorption spectrum of TPE-Bac in its nonsolvent was also studied for comparison. As shown in Figure S2 of the Supporting Information, the absorption spectrum of TPE-Bac in THF is blue-shifted compared with that in DMSO, which may result from the relatively low polarity of THF.

The DMSO solution of TPE-Bac emits faintly with a small emission peak centered at around 520 nm. With the gradual increase in bad solvent (THF) ratio, the photoluminescence (PL) spectra remain unchanged. The THF solution of TPE-Bac, however, shows distinctively different emission profile. A strong emission peak at 641 nm can be easily identified from the PL spectrum (Figure 1A). The emission intensity of TPE-Bac in THF solution is more than 53 times higher than its

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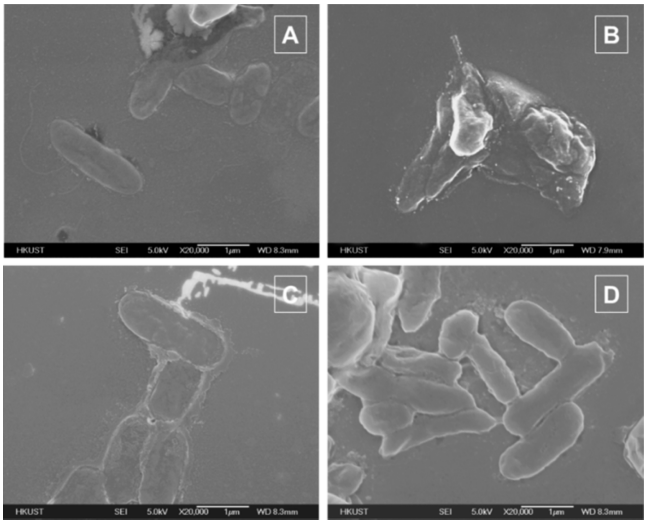

Figure 8. SEM images of (A and B) *S. epidermidis* and (C and D) *E. coli* incubated (A and C) without and (B and D) with 10 μM TPE-Bac for 10 min. The bacteria were then (A and C) stored in the dark or (B and D) exposed to room light for 1 h.

DMSO solution, and the difference can be easily distinguished by naked eyes (as shown in the inset photo of Figure 1B). The results clearly demonstrate that TPE-Bac is AIE-active and decorating TPE with the water-soluble functional group does not change the AIE characteristics.

The long alkyl chains and the positively charged amines add to the hydrophobicity and hydrophilicity of TPE, respectively, making TPE-Bac an amphiphilic molecule. TPE-Bac readily dissolves in water at low concentrations. At high concentrations, however, micelle formation takes place and the emission of TPE-Bac is turned on. Following the fluorescence changes of TPE-Bac upon aggregation, the critical micelle concentration (CMC) can be easily determined. When the concentration is below the CMC, TPE-Bac molecularly

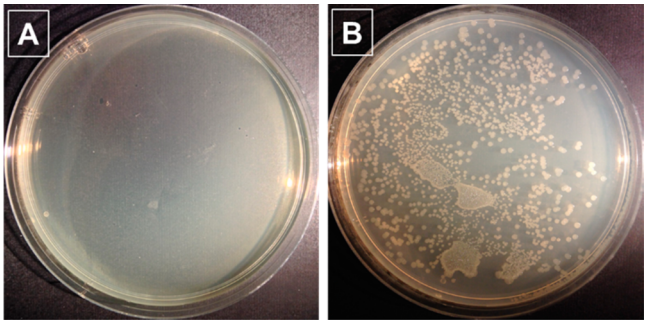

Figure 10. Agar plates containing 10 μM TPE-Bac sprayed with *S. epidermidis*. The plates were first irradiated (A) with or (B) without room light for 1 h, sprayed with bacteria and then incubated for 24 h.

presents in solvent and remains nonfluorescent. When the concentration approches the CMC, the proximate packing of TPE-Bac restricts intramolecular motion, thus turning on the emission of TPE-Bac. This is really the case: when the concentration of TPE-Bac is below 1 μM, increasing the concentration of TPE-Bac does not influence the PL intensity of TPE-Bac very much (Figure 2). The emission enhances gradually when the concentration of TPE-Bac reaches 1 μM. Plotting the PL intensity at 632 nm versus the TPE-Bac concentration gives an estimation of CMC to be ca. 10 μM. Both the absorption and PL spectra exert little change when the TPE-Bac molecules form michelles at concentrations higher than the CMC, revealing that the same species dominate the absorption and light emission processes (Figure S3 of the Supporting Information).The nanoaggregates formed at concentrations higher than the CMC were characterized by the ζ-potential particle size analyzer (Figure S4 of the Supporting Information). Particles with effective diameter of 219 nm were detected with a polydispersity index (PDI) of 0.278. The presence of nanoaggregates was verified by the TEM measurement. As shown in the inset image of Figure S4

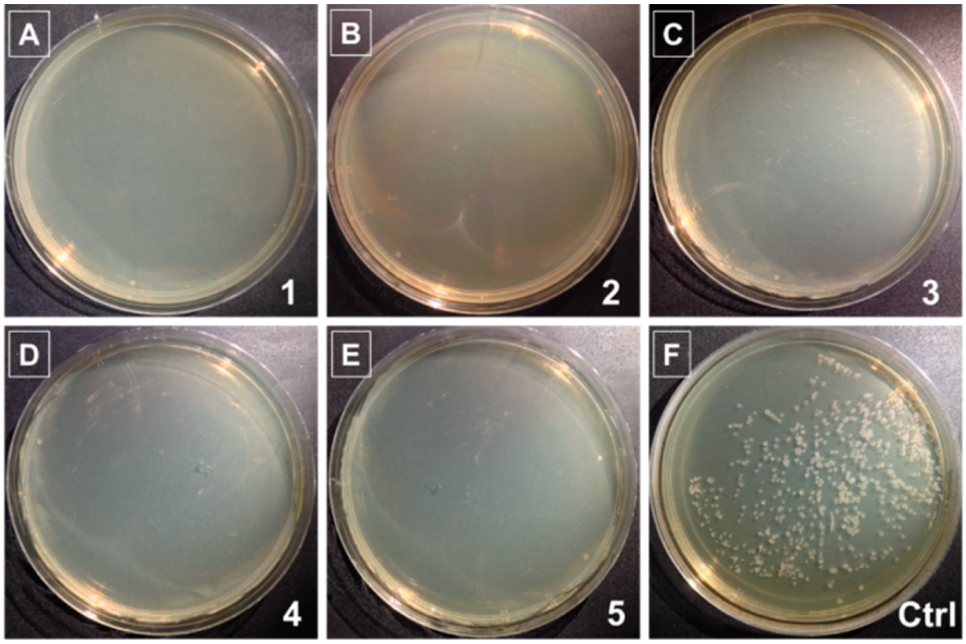

Figure 9. Agar plates containing 10 μM TPE-Bac plated with bacteria. The plates were first sprayed with *S. epidermidis* irradiated (A–E) with or (F) without room light for 1 h, and then incubated for 24 h.

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ACS Appl. Mater. Interfaces 2015, 7, 7180–7188

of the Supporting Information, black spots with sizes of 100–200 nm are clearly observed in the TEM image, corrobating the particle formation of TPE-Bac.

TPE-Bac Sensitized ROS Generation. ROS generation promoted by a photosensitizer is a simple and controllable method for ROS production. This method involves the photoexcitation of the sensitizer from the ground state to the singlet excited state, which can then undergo intersystem crossing to the sensitizer triplet state. The long lifetime of the triplet state allows it to react with various species to form ROS. We demonstrated that TPE with positively charged pyridinium could work as an efficient photosensitizer and it was utilized for selective cell membrane imaging and PDT.[31] TPE-Bac shares the same chromophore with that molecule, though it bears two longer alkyl chains. Similar light-induced ROS-generation property can thus be expected on TPE-Bac. To verify this hypothesis, singlet oxygen sensor green (SOSG) was used for the detection of singlet oxygen, which is one of the ROS. SOSG is nonemissive, but oxidation by singlet oxygen turns on its emission at around 530 nm. As shown in Figure 3, irradiating SOSG or TPE-Bac alone with room light does not affect the fluorescence intensity at 530 nm very much (Figures S5 and S6 of the Supporting Information). However, in the presence of both SOSG and TPE-Bac, the intensity at 530 nm increases accordingly with prolonged irradiation time, which proves that TPE-Bac promoted singlet oxygen generation.

Light-Enhanced Bacterial Imaging and Killing. TPE-Bac was then applied to bacterial imaging. After incubation with 10 μM TPE-Bac for 10 min, Gram-positive bacteria, *S. epidermidis*, are clearly visualized under fluorescent microscope (Figure 4B). Thanks to the AIE characteristics and water solubility of TPE-Bac, the unbounded TPE-Bac emits weakly in the buffer solution, which greatly lowers the background emission. Binding to the bacteria restricts the intramolecular motion and enhances the emission of TPE-Bac. In this way, the bacteria can be clearly distinguished without involving the washing process. This simplifies the imaging process and decreases the loss of bacteria during the washing process, which enables its potential in high-throughput applications. When apply TPE-Bac to Gram-negative bacteria, *E. coli*, we can obtain similar fluorescence images (Figure 4D), indicating TPE-Bac stains both Gram-positive and Gram-negative bacteria.

Afterward, the killing effect of TPE-Bac on both Gram-positive and -negative bacteria was evaluated with the aid of propidium iodide (PI). PI is a cell membrane impermeable fluorescent bioprobe. The cytoplasmic membrane of living bacteria is intacted and keeps PI from entering. The cytoplasmic membrane of dead bacteria, however, is compromised, which allows access of PI to approach its DNA, and thus selectively lights up the dead bacteria. To assess the killing effect of TPE-Bac, bacteria were first incubated with TPE-Bac for 10 min. The bacteria were washed three times with phosphate buffer solution (PBS) and redispersed in PBS to get rid of the ROS generated from unbounded TPE-Bac. Afterward, the bacteria were illuminated under room light for 10 min and then stained with PI. For comparison, a control group was also included, in which the bacteria were exposed to room light in the absence TPE-Bac. The Gram-positive bacteria, *S. epidermidis*, agglomerate after treatment with both TPE-Bac and the light (Figure 5A), presumely due to the exposure of the hydrophobic part of the cell membrane. After PI staining, a red emission is clearly observed, indicating the dentrimental effect of TPE-Bac on bacterial membrane integrity (Figure 5B). In the control group, however, no bacteria agglomeration or PI emission can be observed (Figure 5C,D). The results prove that TPE-Bac indeed serves as photosensitizer to kill *S. epidermidis* upon light irradiation. The killing effect of TPE-Bac on *E. coli* is less significant than on *S. epidermidis*. As shown in Figure 5, the morphological change of *E. coli* is quite obvious, but relative low PI emission from the bacteria is observed. That is because Gram-negative bacteria have an outer membrane, which serves as an additional protecting layer to the bacteria.[4]

The killing efficiency of TPE-Bac was then assessed by the plate-count method. We first investigated the effect of light irradiation on bacteria viability. With room light illumination for 1 h, both *E. coli* and *S. epidermidis* remain healthy and no obvious decrease in bacteria viability is observed (see Figure S7 of the Supporting Information). When *E. coli* are treated with TPE-Bac and then stored in the dark for 10 min, their viability decreases to around 70% (Figure 6), demonstrating the dark toxicity of TPE-Bac toward *E. coli*. Further increasing the storage time in the dark does not add to its killing effect on the bacteria. The dark toxicity of TPE-Bac toward *S. epidermidis* is even higher. Only 50% of bacteria survives after incubation with TPE-Bac for 10 min. Light irradiation can significantly increase the killing efficiency on both Gram-positive and -negative bacteria. The viability of Gram-positive and -negative bacteria treated with TPE-Bac and 10 min of room light illumination are 40% and 45%, respectively, both of which drop to below 10% after 30 min of light irradiation, and further decrease to less than 1% when the irradiation time is increased to 1 h.

To gain a direct impression of the killing effect, the images of plates for the quantification of the killing effect on *E. coli* is shown in Figure 7. Without treatment, the bacteria grow healthily on the plate. Light irradiation alone does not exert obvious effect on bacteria viability (Figure 7B), and both the size and number of colony are similar to the blank control group (Figure 7A). Treatment with TPE-Bac alone decrease the amount of *E. coli* to some extent, but there are still some alive bacteria that can grow to form colonies on the plate (Figure 7C), suggesting the inefficient killing of TPE-Bac on *E. coli* in the dark. In the presence of both TPE-Bac and light irradiation, *E. coli* is killed effectively and almost no colony forms on the plate, which is a good sign of high bacterial killing efficiency under light irradiation. A similar effect is observed on the *S. epidermidis* (see Figure S8 of the Supporting Information). All these results prove that together with light irradiation, TPE-Bac could kill both Gram-positive and -negative bacteria efficiently.

The morphology of bacteria, which indicates bacteria viable state, was then investigated by scanning electron microscopy (SEM). Both *E. coli* and *S. epidermidis* were treated with TPE-Bac and illuminated with room light for 1 h, followed by drying, and collection of SEM images. Bacteria without treatment were also imaged under SEM for comparison. As shown in Figure 8, without treatment (Figure 8A,C), the morphology of both Gram-positive and -negative bacteria remains regular with clear borders and smooth bodies. When bacteria overlap with each other, a well-defined border can be clearly resolved, which indicates the healthy state of the bacteria. After treatment (Figure 8B,D), however, bacteria shrunk and fusion take place, resulting in enormous changes in morphology. The SEM results clearly demonstrate that the presence of TPE-Bac and light together can lead to bacteria death charcterized by the morphological changes.

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The superb performance of TPE-Bac for bacterial killing encouraged us to further investigate its photostability during photosensitizing process. For the purpose of demonstration, *S. epidermidis* was utilized in the following experiment. TPE-Bac was introduced to the agar plate at a final concentration of 10 μM and then *S. epidermidis* was sprayed onto the agar plate, followed by room light illumination for 1 h and culturing in the 37 °C incubator for 24 h. In the blank control group (see Figure S9A of the Supporting Information), after 24 h of incubation, bacteria grow into small colonies. In the experimental group (Figure 9A), however, room light illumination promotes ROS generation, which kills bacteria efficiently, and no colony is formed on the agar plate. Then the experimental agar plate was subjected to a new bacteria spray-killing cycle. After four cycles, there is still no colony formation. In the fifth cycle, the amount of *S. epiderimis* is doubled, but the agar plate remains clean. Note that five times is not the limit of repeatable cycles, and the TPE-Bac-containing plate can be subjected to more bacteria spray-killing cycles without a decrease in performance. Despite the excellent disinfection effect under light irradiation, TPE-Bac alone is less toxic. When kept in the dark, *S. epidermidis* can grow to form colonies (Figure 9G) in TPE-Bac-containing agar plate, even though the amount of conlony is less than the blank control. Interestingly, if we illuminate the plate containing TPE-Bac with room light first and then spray bacteria onto the plate, there is no colony formed on the plate after 24 h of incubation (Figure 10A). Though the underlying mechanism is still unclear, we postulate that some toxins to the bacteria have been generated by the room light induced ROS, which kills the bacteria.

CONCLUSION

In this work, we report the synthesis of an AIE-active fluorogen, TPE-Bac, and its application in bacterial imaging and killing studies. Thanks to the AIE characteristics of TPE-Bac, it can image bacteria without the involvement of washing procedure. The amphiphilic TPE-Bac with two long alkyl chains and positively charged amine groups may intercalate into bacteria membrane and destroy the membrane integrity, which is responsible for the dark toxicity of TPE-Bac. The ROS genernation sensitized by TPE-Bac upon light irradiation can efficiently kill both Gram-positive and -negative bacteria. The agar plate containing TPE-Bac can undergo more than five cycles of bacteria spray-killing and remain effective. These results demonstrate that TPE-Bac is an efficient and photostable photosensitizer for ROS generation, which may serve as a vibrant material for bacteria elimination.

ASSOCIATED CONTENT

Supporting Information

^{1}H, ^{13}C NMR, UV−visible absorption spectra, and particle size analysis of TPE-Bac, PL spectra of SOSG and TPE-Bac alone in buffer solution, effect of light on bacteria viability, agar plates image of *S. epidermidis* treated without/with light in the absence/presence of TPE-Bac, illustration of bacteria spray-killing cycle, and control group of Figure 10 in which agar plates are sprayed with bacteria and incubated in the dark. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

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