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Photophysics and Light-Activated Biocidal Activity of Visible-Light-Absorbing Conjugated Oligomers

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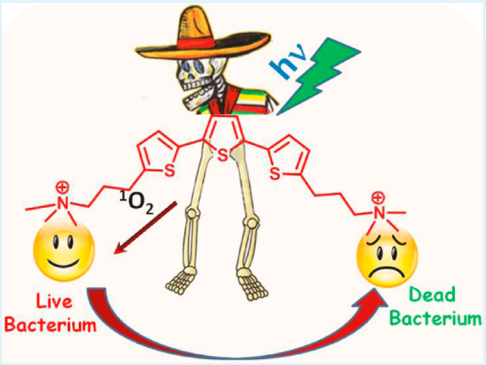
Supporting Information

ABSTRACT: The photophysical properties of three cationic π-conjugated oligomers were correlated with their visible light activated biocidal activity vs *S. aureus*. The oligomers contain three arylene units (terthiophene, **4a**; thiophene-benzotriazole-thiophene, **4b**; thiophene-benzothiadiazole-thiophene, **4c**) capped on each end by cationic $-(CH_2)_3NMe_3^+$ groups. The oligomers absorb in the visible region due to their donor–acceptor–donor electronic structure. Oligomers **4a** and **4b** have high intersystem crossing and singlet oxygen sensitization efficiency, but **4c** has a very low intersystem crossing efficiency and it does not sensitize singlet oxygen. The biocidal activity of the oligomers under visible light varies in the order **4a** > **4b** ≈ **4c**.



KEYWORDS: *conjugated oligomer, singlet oxygen, light activated biocide, donor–acceptor*

INTRODUCTION

Controlling bacterial infections is of immense importance with applications ranging from health care to improving the quality of day to day life.[1−6] Increased bacterial resistance to available antibiotics emphasizes the need for robust and versatile antimicrobials in controlling pathogenic bacteria. Different strategies have been taken to develop new antimicrobial agents, and polymers have emerged as an interesting class as they have the advantage of being fabricated in a variety of formats.[1−6] We and others have explored and confirmed the biocidal properties of cationic conjugated polyelectrolytes (CPEs).[7−11] Recently, we synthesized a series of cationic phenylene ethynylene oligomers (OPEs) with different lengths and examined structure–reactivity relationships between their photophysical properties and antibacterial activity.[12−14] This work shows that OPEs possess pronounced light activated biocidal activity, and photophysical studies reveal they are efficient sensitizers for singlet oxygen, which is believed to play an important role in their biocidal activity. One limitation of the OPEs studied previously is that their absorption is primarily in the near-UV, although it extends into the blue-violet region of the visible spectrum as the number of repeat unit increases. This limits their usefulness as light-activated biocides, as a UV source is required for their activation.

π-Conjugated, donor–acceptor electronic systems have received much attention owing to their utility in opto-electronic devices such as polymer and organic solar cells where visible light absorption and emission is important.[15−17] Although low band gap oligomers and polymers can be synthesized by this strategy, it also allows one to fine-tune the band gap by varying the strength of the acceptor.[15−17] In essence, the donor–acceptor motif provides the means to construct conjugated polymers and oligomers with controlled absorption throughout the visible region.[18]

Thiophene-based donor–acceptor systems have gained popularity mainly due to their useful optical and electronic properties.[17−22] In an effort to develop light activated biocidal oligomers that have strong absorption in the visible region, we turned our attention to thiophene based compounds featuring a donor–acceptor–donor (D–A–D) motif. Here we report the synthesis, photophysical properties and light-activated biocidal activity of a set of three cationic, water-soluble, thiophene containing oligomers that feature varying donor–acceptor interactions. The absorption of these oligomers in the visible region increases with the strength of the acceptor unit. The results show that the biocidal activity of the oligomers is correlated to their photophysical properties, especially their ability to sensitize singlet oxygen.[21,22] In addition, the results suggest the importance of other structure–property relation-

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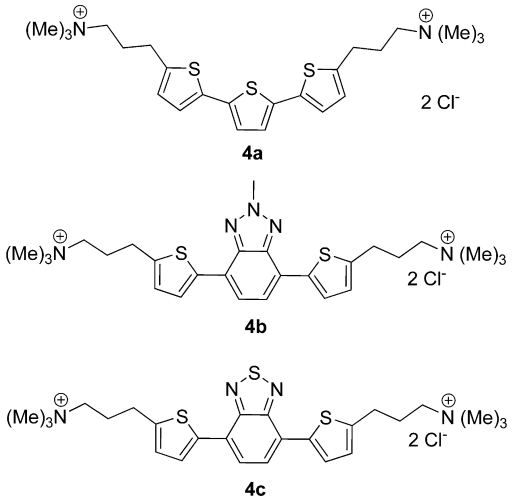
ships that influence the biocidal activity, including hydrophobic/hydrophilic balance and molecular structure/shape.

Chart 1



RESULTS AND DISCUSSION

The chemical structures of the thiophene based oligomers developed in the work are shown in Chart 1. Oligomers **4a–c** were synthesized following the method outlined in Supporting Information (Scheme S1). The synthesis involves 4 steps, with the key step involving a Stille coupling reaction. The final oligomers were fully characterized by chemical spectroscopy and these data are included as Supporting Information.

Oligomers **4a–4c** were designed with several features in mind. First, each structure is "end-capped" with cationic $-(CH_2)_3NMe_3^+$ groups which render the oligomers soluble in water and polar organic solvents. This structural motif is similar to that used in our earlier work on "end-only" OPEs.[12] The OPEs exhibit strong biocidal activity that is believed to be due in part to their linear structure capped by cationic quaternary ammonium groups which results in a strong propensity to bind and disrupt the bacterial membrane.[23,24] Second, the π-conjugated structures of **4a–4c** feature a varying degree of D–A–D character which influences their HOMO–LUMO gap and visible light absorption properties. In particular, **4a** features no D–A–D interaction, whereas **4b** with central benzotriazole (BTz) and **4c** with a benzothiadiazole (BTD) exhibit increased D–A–D character.[17]

The absorption and fluorescence spectra of **4a–4c** in methanol solution are shown in Figure 1. Oligomer **4a** exhibits strong absorption in the near UV region characteristic of terthiophene; the absorption of **4b** is red-shifted in comparison to **4a**, with the absorption band extending well into the visible region (Figure 1 and Table 1). The red shift induced by BTz is consistent with the study by Patel et al., where it was shown that BTz functions as a weak electron acceptor in π-conjugated systems.[17] The absorption of **4c** is further red-shifted to ca. 457 nm due to intramolecular charge transfer consistent with the strong electron accepting nature of the BTD unit.[17] The absorption of the oligomers in water is very similar to that seen in methanol; the absorption is slightly red-shifted for **4a** (ca. 4 nm) in water compared to methanol whereas a blue shift was observed for oligomers **4b** and **4c** (ca. 3–5 nm) in water.

Oligomers **4a** and **4b** show blue fluorescence (λ_{max} = 437 and 460 nm, respectively) in methanol whereas **4c** exhibits a strong, red fluorescence (λ_{max} = 615 nm, Figure 1 and Table 1). The fluorescence quantum yield of **4a** is low and comparable both in methanol and water; however, the fluorescence quantum yields of **4b** and **4c** are higher in methanol than in water (see Table S1 in the Supporting Information). Close inspection of the fluorescence quantum yield data for **4a–c** reveals an interesting correlation (Table 1). Specifically, the fluorescence quantum yield of oligomer **4a** is lower than **4b** and **4c** in methanol. A similar trend is observed for the fluorescence lifetimes, i.e., the lifetime of the oligomer **4a** is shorter than **4b** and **4c**.

Collectively, these results suggest that a rapid and efficient nonradiative decay pathway is active in **4a** (possibly intersystem crossing to the triplet state). To further understand this phenomenan, we carried out transient absorption spectroscopy to proble the triplet excited state.

Transient abosrption experiments were carried out on **4a–4c** in methanol and in water solutions.[25] Figure 2 compares transient absorption difference spectra obtained immediately following the laser pulse in methanol (see the Supporting Information for data in aqueous solution). Oligomers **4a** and **4b** exhibit strong transient absorption throughout the visible region; the transients decay with τ = 1–3 μs in methanol (Table 1) and they are quenched in air saturated solution;

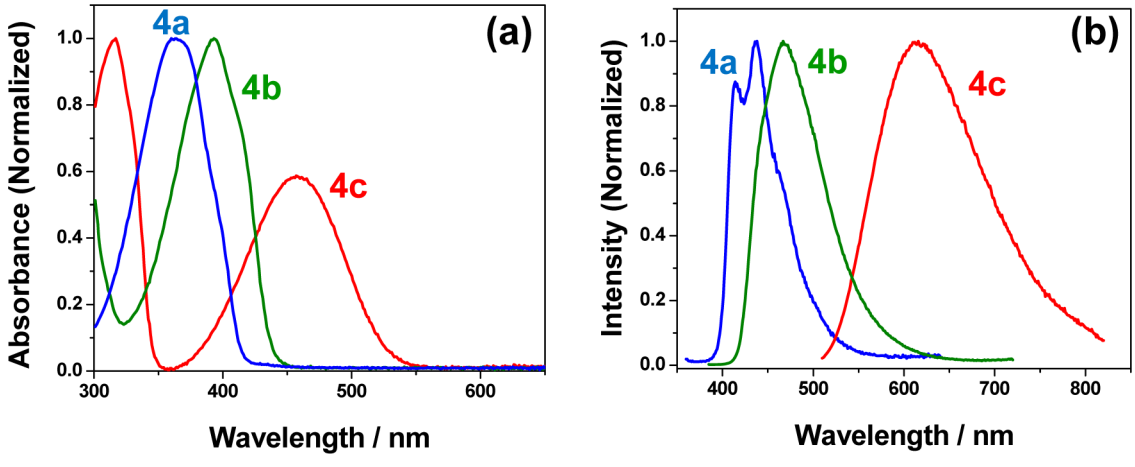


Figure 1. (a) Absorption, and (b) fluorescence spectra of the oligomers **4a–4c** in methanol.

Table 1. Photophysical Data of Substrates 4a−4c in Methanol Solution

		4a	4b	4c
λ_{max} (absorption/nm)	MeOH	364	390	457
λ_{max} (fluorescence/nm)	MeOH	437	460	615
Φ_F[a]	MeOH	0.06 ± 0.02	0.51 ± 0.02	0.27 ± 0.02[b]
Φ_Δ[c]	CD_3OD	0.74 ± 0.02	0.49 ± 0.03	[d]
τ_F/ns (MeOH)		0.18 (450 nm)	2.2 (94%), 4.6 (6%) (500 nm)	4.6 (85%) 8.2 (15%) (600 nm)
TT $_{Abs}$ (λ/nm) in MeOH		467	492	529
TT $_{Abs}$ (ΔA, t = 0) in MeOH		0.55	0.39	0.01
$\tau_{triplet}$ (μs)in MeOH		2.7	2.1	-

[a]Measured using quinine sulfate in 0.1 M sulfuric acid (Φ_F = 0.54) as actinometer. [b]ZnTPP in toluene (Φ_F = 0.033) was used as actinometer. [c]Measured in CD_3OD using 2′-acetonaphthone (Φ_Δ = 0.79) as actinometer. [d]No detectable singlet oxygen emission observed.

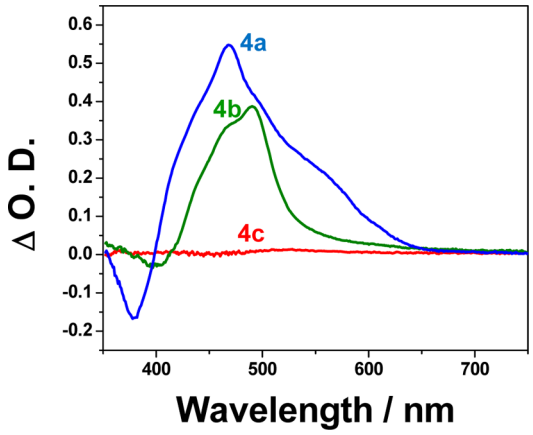


Figure 2. Transient absorption difference spectra of the oligomers (Abs = 0.7 at λ_{ex}) immediately following a 10 ns laser pulse (TT_{Abs} (ΔA, t = 0)) in methanol (λ_{ex} = 355 nm for **4a** and **4b**; 425 nm for **4c**). Pulse energy and ground-state absorption matched.

those features are consistent with the assignment of the absorption to the triplet state.

Interestingly, when the transient absorption is carried out using the same excitation energy, **4c** exhibits little or no transient absorption. Inspection of the initial amplitude of the TT absorption immediately following the laser pulse (TT_{Abs} (ΔA, t = 0), Table1) gives useful information regarding the relative triplet yield. The data reveals that the TT absorption of **4a** is substantially larger than for the other oligomers, suggesting a higher triplet yield. This result compares favorably with the fluorescence data, which also point to the notion that intersystem crossing is more efficient in this oligomer. The lack of a detectable transient absorption for **4c** strongly suggests that intersystem crossing in this oligomer is very inefficient.

As noted above, we have postulated that the light-activated biocidal activity of OPEs is correlated with their ability to sensitize singlet oxygen.[12] Thus, having established that direct excitation of **4a** and **4b** (but not **4c**) affords the triplet state, we assessed their ability to sensitize singlet oxygen in deuterated methanol (Φ_Δ, Table 1). These data show that **4a** and **4b** sensitize singlet oxygen efficiently, with the former giving the highest yield; not surprisingly, **4c** is unable to produce singlet oxygen within the limits of the measurements. From the above results it is evident that terthiophene oligomer **4a** is the most efficient singlet oxygen sensitizer and oligomer **4c**, with the strongest donor−acceptor interaction, is unable to act as a sensitizer.

After establishing the photophysical properties of the new oligomers, we assessed their antibacterial activity against *Staphylococcus aureus* (*S. aureus*) with visible light as the illumination source. Suspensions of *S. aureus* were incubated in the presence of **4a−c** (c = 1 μg mL^{-1}) in the dark and under visible light illumination (Sylvania T5 bulbs, λ = 350−800 nm). The effect of the oligomers on the bacterial viability was checked after 15, 30, 45, 60, and 90 min exposure by using flow cytometry (the bacterial suspensions were stained with live/dead fluorescent dyes prior to flow cytometry).[26] Figure 3 shows plots of the percentage of dead *S.aureus* cells versus light exposure time for the set of three oligomers, along with a control experiment in which the *S.aureus* suspension was

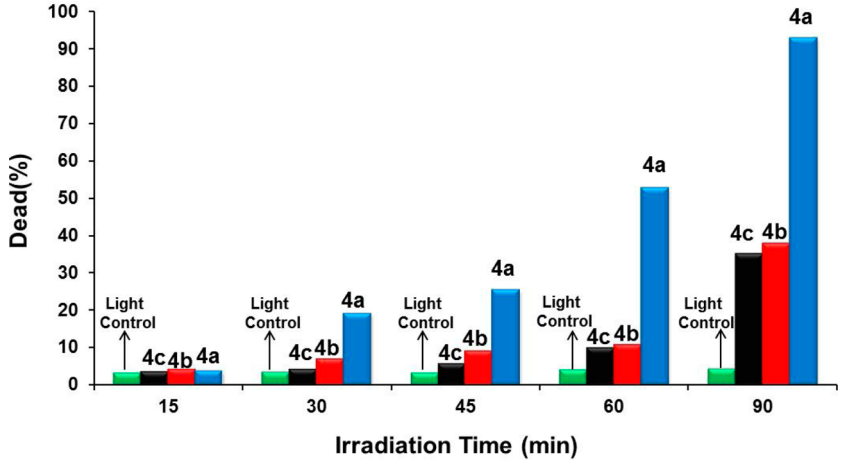


Figure 3. *S.aureus* viability against oligomers **4a−4c** upon exposure to visible light for various time intervals ([**4a−4c**] = 1 μg/mL).

irradiated in the absence of oligomer. These data reveal that each of the oligomers displays moderate biocidal activity at the (rather low) concentration used, in the sequence of efficacy **4a** > **4b** ≈ **4c**. (The dark control experiment is provided in Figure S6 in the Supporting Information, and the results show that only **4c** exhibits a biocidal effect in the dark, and it is relatively a modest effect with <30% killing after 90 min exposure.) An additional experiment was carried out with **4a** using UV light illumination (see Figure S7 in the Supporting Information) and the results show that this oligomer's efficacy is enhanced, killing ∼99.2% in 60 min exposure (>2log kill efficacy). Overall, the biocidal efficacy of **4a** observed here vs *S.aureus* under visible and UV light illumination is comparable in magnitude to that observed for the cationic "end-only" OPE oligomers displayed vs *E. coli* in an earlier investigation.[12]

Although the biocidal study presented here is limited in scope, we believe that several conclusions can be drawn based on the results. First, the enhanced light activated biocidal efficacy of **4a** compared to **4b** and **4c** is consistent with the photophysical data showing that the former is the most effective singlet oxygen sensitizer. We conclude here, and as previously suggested,[10,12,13] that singlet oxygen is at least partially responsible for the light activated biocidal activity for these cationic conjugated oligomers. Second, it is noteworthy that oligomer **4b** exhibits an attenuated light activated biocidal effect compared to **4a**, despite the fact that it is a very efficient singlet oxygen sensitizer ($\Phi_\Delta \approx 0.5$). The attenuated efficacy of **4b** may arise because this oligomer has a lower propensity to bind to the bacterial membrane due to its nonlinear shape and increased water solubility due to the heteroatoms in the benzotriazole unit. Third, although **4c** exhibits a biocidal effect under illumination, the killing efficacy is within experimental error the same as that observed for oligomer in the dark control experiment (see Figure S6 in the Supporting Information). Thus, we conclude that this compound exhibits little, if any light-activated killing, consistent with the fact that the compound does not sensitize singlet oxygen.

Finally, an important result from the photophysical experiments is that the triplet yield (via intersystem crossing) in D-A-D oligomer **4c** is very low. This result is in direct contrast to the result for the structurally related oligomers **4a** and **4b** which have significantly larger triplet yields (>50% in each case). The origin of the low triplet yield in **4c** is unclear; however, it is likely that this effect arises because the donor−acceptor electronic structure gives rise to a comparatively low HOMO−LUMO overlap, which may decrease spin−orbit coupling.[27] This interesting relationship between donor−acceptor structure and intersystem crossing efficiency certainly bears further investigation.

CONCLUSIONS

In summary, we report the results of a photophysical investigation and comparative biocidal study of a series of three cationic thiophene containing π-conjugated oligomers. By incorporating fused ring BTz and BTD heterocycles in the core of the oligomers, we introduce donor−acceptor electronic interactions, leading to a significant red-shift of the primary absorption band. The BTD based oligomer **4c**, which contains the strongest donor−acceptor interaction, features a strong absorption in the mid-visible, making the compound strongly (yellow-orange) colored in white light. Photophysical studies reveal that terthiophene oligomer **4a** and the BTz oligomer **4b** undergo efficient intersystem crossing and they are able to sensitize singlet oxygen efficiently. By contrast, the BTD oligomer **4c** does not undergo intersystem crossing and it is also unable to sensitize singlet oxygen to any measurable extent. Biocidal studies using *S.aureus* show that **4a** and **4b** exhibit light-activated biocidal activity, with terthiophene oligomer **4a** being the most efficient. The results provide unique insight into the relationship between molecular/electronic structure, photophysics and biocidal efficacy for this novel class of cationic antibacterial agents. The results of this study will serve as an important guide for future development of new molecular and polymeric materials for the destruction of pathogens.

EXPERIMENTAL METHODS

Instrumentation and Methods. NMR spectra were recorded using a Varian VXR-300 FT-NMR, operating at 300 MHz for ^{1}H NMR and at 75.4 MHz for ^{13}C NMR. UV−Visible spectra were recorded using a Varian Cary 100 dual beam spectrophotometer. Corrected steady-state fluorescence spectra were obtained with a PTI spectrometer. A 1 cm square quartz cuvette was used for solution spectra, and emission was collected at 90° relative to excitation beam. Fluorescence quantum yields are reported relative to known standards. The optical density of solutions at the excitation wavelength was ≤0.1, and corrections were applied for differences in the refractive index of standard and sample solutions. Transient absorption spectra were collected using a laser systems that is described elsewhere.[28,29] The optical density of the solutions was adjusted to ∼0.7 at the excitation wavelength (355 nm) with the laser energy set at 6−7 mJ. Solutions were purged with argon for 45 min before making transient absorption spectroscopy measurements. Singlet oxygen quantum yields were measured using a Photon Technology International Quantamaster near-IR spectrophotometer equipped with an InGaAs photodiode detector, optical chopper and a lock in amplifier.

Antibacterial Studies, Dead/Live Assays. *Staphylococcus aureus* (ATCC 10832, American Type Culture Collection) was grown in Brain Heart Infusion (BHI, Difco) and stored at −70 °C with 20% (v/v) glycerol (EMD) before use. Stock cultures maintained on agar plates (2%, Difco) were used to inoculate 50 mL cultures in liquid BHI. The cultures were incubated at 37 °C for 18 h. The bacteria were then centrifuged and washed twice with 0.85% NaCl followed by counting in a hemocytometer to normalize bacterial concentration in 20 mL of 0.85% NaCl (8×10^6 cells/mL) for antibacterial tests. Control samples containing only bacterial cells, and desired concentrations of oligomer/bacteria mixture samples were exposed to the visible light for 15, 30, 45, 60, and 90 min in a LuzChem ORG photoreactor using Sylvania T5 bulbs (λ = 350−799 nm). Another set of samples were incubated in the dark for 15, 30, 45, 60, and 90 min. Then dead/live assays were conducted using SYTO21 (green fluorescence = live) and propidium iodide (red fluorescence = dead) purchased from Invitrogen. After bacteria were incubated with the oligomers in the light and dark conditions, a 1:1 ratio of dyes (2.4 μL) were added to the samples and kept in the dark for 15 min. Finally, the bacteria were examined using an Accuri C6 flow cytometer where 2×10^4 cells were analyzed from 8×10^6 cell solution. The number of live cells and dead cells corresponding to green and red fluorescence respectively were counted and compared by flow cytometry.

ASSOCIATED CONTENT

Supporting Information

Detailed description of the synthesis and characterization of **4a**−**4c**, additional photophysical data, and UV light activated biocidal results with **4a**. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

K.S.S. and D.G.W. each hold >5% interest in a start-up company (Oligocide, Inc.) that is commericalizing biocidal technology based on polymers and compounds related to those described in this work.

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