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Photochemotherapeutic Properties of a Linear Tetrapyrrole Palladium(II) Complex displaying an Exceptionally High **Phototoxicity Index**

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Supporting Information

ABSTRACT: Photodynamic therapy (PDT) represents a minimally invasive and highly localized treatment strategy to ablate tumors with few side effects. In PDT, photosensitizers embedded within tumors are activated by light and undergo intersystem crossing, followed by energy transfer to molecular oxygen, resulting in the production of toxic singlet oxygen $({}^{1}O_{2})$. Previously, we reported a robust, linear tetrapyrrole palladium(II) complex, Pd[DMBil1], characterized by its facile and modular synthesis, broad absorption profile, and efficient ¹O₂ quantum yield of Φ_{Δ} = 0.8 in organic media. However, the insolubility of this porphyrinoid derivative in aqueous solution prevents its use under



biologically relevant conditions. In this work, we report the synthesis of Pd[DMBil1]-PEG750, a water-soluble dimethylbiladiene derivative that is appended with a poly(ethylene) glycol (PEG) functionality. Characterization of this complex shows that this PEGylated biladiene architecture maintains the attractive photophysical properties of the parent complex under biologically relevant conditions. More specifically, the absorption profile of Pd[DMBil1]-PEG₇₅₀ closely matches that of Pd[DMBil1] and obeys the Beer-Lambert Law, suggesting that the complex does not aggregate under biologically relevant conditions. Additionally, the emission spectrum of Pd[DMBil1]-PEG₇₅₀ retains the fluorescence and phosphorescence features characteristic of Pd[DMBil1]. Importantly, the PEGylated photosensitizer generates ${}^{1}O_{2}$ with $\Phi_{A} = 0.57$, which is well within the range to warrant interrogation as a potential PDT agent for treatment of cancer cells. The Pd[DMBil1]-PEG₇₅₀ is biologically compatible, as it is taken up by MDA-MB-231 triple negative breast cancer (TNBC) cells and has an effective dose (ED_{50}) of only 0.354 μ M when exposed to $\lambda_{ex} > 500$ nm light for 30 min. Impressively, the lethal dose (LD_{50}) of Pd[DMBil1]-PEG750 without light exposure was measured to be 1.87 mM, leading to a remarkably high phototoxicity index of ~5300, which is vastly superior to existing photosensitizers that form the basis for clinical PDT treatments. Finally, through flow cytometry experiments, we show that PDT with Pd[DMBil1]-PEG750 induces primarily apoptotic cell death in MDA-MB-231 cells. Overall these results demonstrate that Pd[DMBil1]-PEG750 is an easily prepared, biologically compatible, and well-tolerated photochemotherapeutic agent that can efficiently drive the photoinduced apoptotic death of TNBC cells.

INTRODUCTION

Photodynamic therapy (PDT) is used clinically as a noninvasive treatment for solid tumors. In PDT, photosensitizing compounds are either intratumorally or intraveneously injected and circulate throughout the body prior to irradiation of the tumor site.¹ Photosensitizers present within the illuminated tissue absorb light and transfer energy to ground-state molecular oxygen to produce toxic ${}^{1}O_{2}$ that ultimately induces localized cell death (Scheme 1). PDT is regarded as a promising treatment strategy for certain types of cancers² and skin conditions,³ because it is less invasive than surgical removal,⁴ has fewer side effects than radiation or chemotherapy,⁵⁻⁹ and can, in some cases, stimulate an antitumor immune response.^{10,11}

Several photosensitizers, most of which belong to the porphyrinoid family of macrocyclic tetrapyrroles, have been approved for use in PDT.^{12–14} However, widespread clinical use of PDT has been hindered, at least in part, because existing ¹O₂ photosensitizers lack the photophysical and pharmacological attributes required for an optimal phototherapeutic agent. An ideal photosensitizer would be simple to synthesize and purify, demonstrate strong absorption of 600-850 nm light, which can penetrate deeper into tissues, and generate ${}^{1}O_{2}$ with a high quantum yield during irradiation. It is also critical that a photosensitizer for PDT be relatively nontoxic in the absence of light to minimize off-target side effects. Lastly, water

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Scheme 1. Photodynamic Therapy^a



^{*a*}Photosensitizers are intravenously or intratumorally administered. The tumor area is then irradiated, driving the formation of toxic ${}^{1}O_{2}$ to irreversibly damage/kill the surrounding cells.

solubility is critical to ensure stability during storage or intravenous injection and to prevent aggregation-induced attenuation of the ${}^{1}O_{2}$ quantum yield.

We recently demonstrated that the linear tetrapyrrole metal $c omplex palladium 10, 10 \cdot dimethyl \cdot 5, 15 \cdot bis$ (pentafluorophenyl)biladiene (**Pd[DMBil1**], structure shown in Scheme 2) is capable of absorbing light up to $\lambda \approx 600$ nm and sensitizing ${}^{1}O_{2}$ with a high quantum yield ($\Phi_{\Delta} \approx 0.8$) that is on par with those of photosensitizers currently used in PDT. 15 Importantly, **Pd[DMBil1**] is easily synthesized in four steps from commercially available starting materials, 15 and it can be purified and isolated using modular methods. While these traits suggest that the **Pd[DMBil1**] core is well-suited for interrogation as a PDT agent, this compound's complete lack of water solubility has precluded such studies.

The addition of poly(ethylene) glycol (PEG) substituents is commonly employed in the development of therapeutics, since PEGs generally show excellent biocompatibility and lend high water solubility.^{16–19} In the present study, we modified the **Pd[DMBil1]** photosensitizer with a PEG functionality to overcome the inherent hydrophobicity of the biladiene architecture. We demonstrate that the addition of this PEG functionality endows the dimethylbiladiene complex with water solubility while having little effect on its photophysical properties, thus generating a biocompatible compound, **Pd[DMBil1]**-**PEG**₇₅₀, that retains the ability to generate ¹O₂ with a high quantum yield under biologically relevant conditions. Additionally, we demonstrate that, while this biladiene complex is highly nontoxic in the dark, it can serve as an extremely potent chemotherapeutic agent for treatment of triple negative breast cancer (TNBC) cells and drives apoptotic cell death with a remarkable phototoxicity index of \sim 5300.

RESULTS AND DISCUSSION

Pd[DMBil1]-PEG₇₅₀ is readily accessible from unfunctionalized Pd[DMBil1], which is readily prepared (Scheme 2) in four steps using methodology previously developed for the synthesis of biladienes^{15,20} and related tetrapyrroles containing sp³ hybridized *meso*-carbons.^{21–23} PEGylation of the parent Pd[DMBil1] architecture was facilitated by the presence of the two pentafluorophenyl substituents at the 5- and 15-positions, which can be derivatized via nucleophilic aromatic substitution of the *para*-fluorine substituents.^{24–26} As shown in Scheme 2, treatment of Pd[DMBil1] with mercaptoacetic acid and NEt₃ at 90 °C in dimethylformamide (DMF) for 1 h afforded the monomercaptoacetic acid-substituted product (Pd[DMBil]- SCH_2CO_2H) in better than 75% yield following purification by flash chromatography. PEGylation of Pd[DMBil]-SCH₂CO₂H was achieved through conversion of the mercaptoacetic acid substituent into an N-(methoxyPEG)mercaptoacetamide using carbodiimide coupling chemistry.^{27,28} Pd[DMBil]-SCH₂CO₂H was initially reacted with N-hydroxysuccinimide (NHS) and 1-ethyl-3-(3'-dimethylaminopropyl)carbodiimide hydrochloride (EDC) to form an NHS mercaptoacetate intermediate. Further treatment with triethylamine and a methoxy-PEG-amine with an average molecular weight of 750 Da resulted in the formation of Pd[DMBil1]-PEG₇₅₀, as is shown in Scheme 2. Detailed synthetic procedures and characterization data for all new compounds shown in Figure 2 are detailed in the Supporting Information.

The visible absorption profile of Pd[DMBil1]-PEG₇₅₀ has three features centered at 402, 483, and 540 nm in methanol (Figure 1 and Table 1). Aside from showing a slight enhancement of the feature at 483 nm, this absorption profile is nearly identical to that of Pd[DMBil1] in methanol, suggesting that introduction of the thioether and PEG chain at the para position of one biladiene C_6F_5 substituent has little effect on the electronic structure of the palladium tetrapyrrole core. In a pH 7.4 phosphate-buffered saline (PBS) solution the

Scheme 2. Synthesis of Pd[DMBil1], Pd[DMBil1]-SCH₂CO₂H, and Pd[DMBil1]-PEG₇₅₀





Figure 1. Electronic absorption spectra of Pd[DMBil1] in methanol (black line), Pd[DMBil1]-PEG₇₅₀ in methanol (red line), and Pd[DMBil1]-PEG₇₅₀ in pH 7.4 PBS (dashed blue line).

Table 1. Photophysical Properties of Pd[DMBil] Complexes

compound (solvent)	λ_{abs} , nm ($\varepsilon \times 10^3$ M ⁻¹ cm ⁻¹)	$\lambda_{ m fl}$, nm $(\Phi_{ m fl})$	$\lambda_{\rm ph}$, nm $(\Phi_{\rm ph})$	$\Phi_{\Delta}{}^{a}$
Pd[DMBil1] (methanol)	401 (17.4), 483 (31.9), 540 (7.5)	(1.3×10^{-4})	(1.3×10^{-4})	0.80
Pd[DMBil1]- PEG ₇₅₀ (methanol)	402 (16.6), 483 (34.6), 540 (7.5)	568 (1.4 × 10 ⁻⁴)	(7.8×10^{-5})	0.57
Pd[DMBil1]- PEG ₇₅₀ (PBS)	405 (13.6), 496 (33.2), 551 (8.3)	$580 \\ (2.0 \times 10^{-4})$		0.23
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^{*a*}Measured upon irradiation with $\lambda_{exc} = 500$ nm.

absorption spectrum of Pd[DMBil1]-PEG₇₅₀ shows a bathochromic shift relative to its appearance in methanol. Although the full width at half-maximum (fwhm) of the most prominent absorption feature of the PEGylated derivative increases slightly from 63 nm in methanol to 67 nm in PBS, both of these values are smaller than the fwhm of the corresponding absorption feature of Pd[DMBil1] in methanol (73.5 nm). Furthermore, solutions of Pd[DMBil1]-PEG750 behave in accordance with the Beer-Lambert Law when dissolved in either methanol or PBS at concentrations ranging from 4 to 24 μM (Supporting Information Figure S2). Self-aggregation of other tetrapyrroles under aqueous conditions has been associated with broadened absorption features²⁹⁻³¹ and deviations from the Beer-Lambert Law,³² so the observation that Pd[DMBil1]-PEG750 does not exhibit such behavior indicates that aggregation for this system should be insignificant. The Pd[DMBil1]-PEG750 complex is also resistant to photodegradation, as the UV-vis absorption spectrum for this construct in PBS (24.0 μ M) showed no substantial changes over the course of 2 h of irradiation with 500 or 550 nm light (Figure S3).

The emission properties of the Pd[DMBil1] complex in nitrogen-saturated methanol are also largely unaffected by PEGylation. As has been previously observed for Pd-[DMBil1],¹⁵ excitation of Pd[DMBil1]-PEG₇₅₀ with 500 nm light elicits weak fluorescence from 500 to 700 nm as well as phosphorescence from 700 to 850 nm (Figure 2). Table 1 shows that, while the fluorescence emission maximum of Pd[DMBil1]-PEG₇₅₀ in methanol is red-shifted by 11 nm compared with that of Pd[DMBil1], the fluorescence quantum yield of the PEGylated derivative ($\Phi_{\rm fl} = 1.4 \times 10^{-4}$) is essentially identical to that of the parent compound ($\Phi_{\rm fl} = 1.3 \times 10^{-4}$). The phosphorescence emission maximum shows a



Figure 2. Emission spectra following excitation with 500 nm light of (a) **Pd[DMBil1**] and (b) **Pd[DMBil1**]-**PEG**₇₅₀ in methanol at 298 K under an atmosphere of air (black) or nitrogen (red).

much smaller red shift from 753 nm for Pd[DMBil1] to 756 nm for Pd[DMBil1]-PEG₇₅₀, and the phosphorescence quantum yield decreases slightly from $\Phi_{\rm ph} = 1.3 \times 10^{-4}$ for Pd[DMBil1] to $\Phi_{\rm ph} = 7.8 \times 10^{-5}$ for Pd[DMBil1]-PEG₇₅₀.

The emission characteristics of Pd[DMBil1]-PEG₇₅₀ in nitrogen-saturated PBS (pH 7.4) were also investigated; however, irradiation with 460 nm light under these conditions only resulted in one emission feature stretching from 500 to 700 nm corresponding to fluorescence (Table 1 and Figure S4). As compared with its fluorescence in methanol, the fluorescence of Pd[DMBil1]-PEG₇₅₀ in PBS is further redshifted, with an emission maximum at 580 nm and a quantum yield of $\Phi_{\rm fl} = 2.0 \times 10^{-4}$ in (Table 1). Given that fluorescence quenching is a well-documented consequence of porphyrinoid self-aggregation,³³ the lack of attenuation of $\Phi_{\rm fl}$ in PBS provides further evidence that Pd[DMBil1]-PEG₇₅₀ does not tend to aggregate in aqueous environments. Failure to detect phosphorescence from the PEGylated derivative in PBS may be attributed to shortening of the triplet excited-state lifetime via energy transfer to a H₂O overtone.

In prior work, we showed that air efficiently quenches the excited triplet state of Pd[DMBil1] in methanol, resulting in quenching of the biladiene's phosphorescence due to efficient energy transfer to molecular oxygen.¹⁵ This phenomenon was also manifest in the ability of Pd[DMBil1] to photosensitize $^{1}O_{2}$ generation with an impressive quantum yield of $\Phi_{\Lambda} = 0.8$, as quantified using diphenylisobenzofuran $(DPBF)^{34}$ as an $^{1}O_{2}$ probe and $[\text{Ru}(\text{bpy})_3][\text{PF}_6]_2$ as an actinometer ($\Phi_{\Delta} = 0.81$).³⁵ Similarly, the phosphorescence observed for Pd[DMBil1]-PEG₇₅₀ in methanol under an inert atmosphere was quenched upon introduction of air (Figure 2b) to the sample. Quantification of the ability of the Pd[DMBil1]-PEG750 complex to sensitize the formation of ¹O₂ produced a quantum yield of Φ_{Δ} = 0.57 following irradiation with 550 nm light. The slight decrease in Φ_Δ value for the PEGylated biladiene complex may be attributed to a decrease in the O₂ diffusion rate within the immediate vicinity of the photosensitizer due to partial shielding by the PEG moiety.³⁶ Nonetheless, the ability of Pd[DMBil1]-PEG₇₅₀ to sensitize the formation of ${}^{1}O_{2}$ (Φ_{Δ} = 0.57) is competitive with commercial photosensitizers employed for PDT.13

Although Pd[DMBil1]-PEG₇₅₀ does not exhibit phosphorescence in PBS solutions (vide supra) this complex is capable of sensitizing the formation of ${}^{1}O_{2}$ under aqueous conditions. Through use of the probe singlet oxygen sensor green (SOSG)³⁷ and methylene blue as a reference photosensitizer ($\Phi_{\Delta} = 0.52$),^{38,39} the ability of Pd[DMBil1]-PEG₇₅₀ to sensitize singlet oxygen was determined to be $\Phi_{\Delta} \approx 0.23$ upon irradiation with 550 nm light (Table 1). The apparent attenuation of Φ_{Δ} in PBS relative to that in methanol is not surprising, given that detecting ${}^{1}O_{2}$ in aqueous environments is more challenging due to its shorter lifetime^{40,41} as well as the lower solubility of oxygen in water as compared to organic solvents.⁴² Importantly, however, the aqueous Φ_{Δ} measured for Pd[DMBil1]-PEG₇₅₀ is high enough to enable PDT in biological samples (vide infra).

TNBC accounts for ~15–20% of diagnosed breast cancer cases and is associated with earlier relapse, higher mortality rates, and significantly decreased progression-free survival compared to non-TN breast cancers.⁴³ TNBC patients are unsusceptible to available targeted or hormonal therapies, because the cells in these tumors do not express the necessary surface receptors. Therefore, these patients are treated with aggressive chemotherapies and surgeries that have harmful side effects and are often unsuccessful, necessitating the development of new treatment strategies for this disease. Because of its high potency and specificity, PDT has been recognized as a promising therapeutic approach for TNBC.⁴⁴

To evaluate the inherent toxicity of Pd[DMBil1]- PEG_{750} TNBC MDA-MB-231 cells were treated with up to 5.0 mM Pd[DMBil1]- PEG_{750} for 48 h in the dark and then subjected to an Alamar blue cell viability assay. MDA-MB-231 cells were completely viable at Pd[DMBil1]- PEG_{750} concentrations up to 0.5 mM (Figure 4A). Further, the lethal (LD) dose required for 50% cell death was found to be $LD_{50} = 1.87$ mM, an exceptionally high value, which demonstrates that, in the dark, Pd[DMBil1]- PEG_{750} is biocompatible and well-tolerated by the TNBC cells.

To further demonstrate the safety profile of Pd[DMBil1]-PEG₇₅₀ and facilitate a comparison with a set of commercially available ${}^{1}O_{2}$ photosensitizers that form the basis for common PDT treatments, cell viability assays were also conducted for hematoporphyrin dihydrochloride (HPDC) and isohematoporphyrin (IHP). Cell viability following treatment with up to 3.0 mM of either HPDC or IHP revealed lethal dose values of LD₅₀ = 1.22 mM and LD₅₀ = 0.64 mM, respectively, which are both lower than the LD₅₀ obtained for Pd[DMBil1]-PEG₇₅₀ (Figure S5). These results demonstrate that Pd[DMBil1]-PEG₇₅₀ is less toxic than two commercially available photosensitizers, suggesting that it may be used as a photochemotherapeutic without causing off-target side effects that often limit the efficacy of PDT agents.

In an effort to assess whether Pd[DMBil1]-PEG₇₅₀ is taken up by TNBC cells, MDA-MB-231 cells were treated with up to 1.5 mM Pd[DMBil1]-PEG₇₅₀ for 48 h, and the cellular fluorescence was analyzed by fluorescence imaging and flow cytometry. As discussed above, Pd[DMBil1]-PEG750 retains the emission properties of Pd[DMBil1], enabling its detection by fluorescence microscopy and flow cytometry. Fluorescence imaging revealed that Pd[DMBil1]-PEG₇₅₀ is taken up by cells during the incubation period, as indicated by the red fluorescent signal (Figure 3). Further, flow cytometry analysis confirmed this result, as cells treated with Pd[DMBil1]-PEG₇₅₀ experienced a twofold increase in median fluorescence intensity (MFI) compared to untreated cells (Figure S6). These results were encouraging given that cellular uptake is important for any compound to be used for PDT,45 and they lead us to probe the efficacy of Pd[DMBil1]-PEG₇₅₀ as a photochemotherapeutic agent.

To investigate the ability of Pd[DMBil1]-PEG₇₅₀ to mediate PDT, MDA-MB-231 TNBC cells were treated with

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Figure 3. Fluorescence images showing Pd[DMBil1]-PEG₇₅₀ uptake by MDA-MB-231 cells following a 24 h incubation period. Nuclei are stained blue (DAPI), F-actin in the cytoskeleton is stained green (phalloidan), and Pd[DMBil1]-PEG₇₅₀ appears red. Scale = 25 μ m.

the biladiene complex at concentrations ranging from 0 to 10.0 μ M for 4 h. At each concentration surveyed, cells were irradiated (λ_{ex} > 500 nm) for either 0, 10, 20, or 30 min. Viability assays were conducted to assess cell death 16 h after irradiation. Cells incubated with Pd[DMBil1]-PEG₇₅₀ for 24 h prior to irradiation were highly susceptible to PDT-mediated cell death compared to cells irradiated immediately after adding Pd[DMBil1]-PEG750. More specifically, cells irradiated immediately following the addition of Pd[DMBil1]-PEG₇₅₀ required concentrations of at least 1 μ M and 30 min of light exposure before any loss of cell viability was observed (Figure S7). By contrast, cells incubated with Pd[DMBil1]-PEG₇₅₀ for 24 h prior to light application were susceptible to PDT with concentrations of photosensitizer as low as 0.25 μ M, and 10 μ M treatment resulted in complete loss of viability (Figure 4B). This enhancement in photoinduced toxicity in cells incubated with the photosensitizer before light treatment provides further evidence of cellular uptake of Pd[DMBil1]-PEG₇₅₀. From these results, we also determined that the effective dose (ED) of Pd[DMBil1]-PEG₇₅₀ required to reduce cell viability by 50% to be $ED_{50} = 0.354 \ \mu M$ for cells incubated with the photosensitizer for 24 h and then subjected to 30 min of light exposure. The ratio of LD₅₀/ED₅₀ delivers the phototoxicity index (PI) of a given photochemotherapeutic agent, which provides an indication of the potency of a photodrug in relation to its inherent dark toxicity. For Pd[DMBil1]-PEG₇₅₀ the phototoxicity index is determined to be PI \approx 5300, which is exceptionally high,⁴⁶ especially when compared to porphyrinoids typically employed for PDT (vide infra).

To compare the phototoxicity index of Pd[DMBil1]-PEG₇₅₀ with those for the commercially available photosensitizers, we conducted the same photodynamic activity experiments with HPDC and IHP. Treatment of MDA-MB-231 cells with each photosensitizer for 24 h followed by a 30 min period of irradiation ($\lambda_{ex} > 500$ nm) revealed effective doses of ED₅₀ = 48.65 μ M for HPDC and ED₅₀ = 327.56 μ M for IHP (Figure S8). The LD₅₀ and ED₅₀ values for HPDC and IHP correlate to phototoxicity indicies of PI \approx 25 for HPDC and PTI \approx 2 for IHP. By comparison, the phototoxicity index of Pd[DMBil1]-PEG₇₅₀ is quite impressive, as it is ~200 and 3000 times higher than those of HPDC and IHP, respectively.

With the realization that Pd[DMBil1]-PEG₇₅₀ is highly potent and effective for PDT via ¹O₂ sensitization, we sought to evaluate the mechanism by which the biladiene phototriggers cell death. Ideally PDT will induce cellular apoptosis as

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Figure 4. Normalized cell viability after incubation with Pd-[DMBil1]-PEG₇₅₀ for (a) 48 and (b) 24 h followed by light exposure for 0 (black), 10 (red), 20 (beige), or 30 (blue) min. Cells were incubated overnight post-irradiation and prior to the viability analyses. *p < 0.0001 by two-way ANOVA with post hoc Tukey-Kramer compared to 0 min light exposure for each concentration.

opposed to necrosis, as the latter can cause the release of intracellular compartments and local inflammation that can stimulate tumor growth.47 By contrast, apoptosis is antiinflammatory and therefore discourages disease progression.⁴⁸ To assess the mechanism by which Pd[DMBil1]-PEG₇₅₀ photoinduces cell death, MDA-MB-231 cells were treated with 4.0, 6.0, or 8.0 µM Pd[DMBil1]-PEG₇₅₀ for 24 h, irradiated for 30 min (λ_{ex} > 500 nm), and then incubated for 1 h prior to AnnexinV (fluorescein isothiocyanate (FITC) channel) and PI (PerCP channel) staining. Experiments in which none of the biladiene photosensitizer was added to the cells were also performed as controls. For these experiments, the MDA-MB-231 cells were treated with higher Pd[DMBil1]-PEG₇₅₀ concentrations than in the viability experiments because we analyzed the cells only 1 h post light treatment (as opposed to overnight). Although these photosensitizer concentrations are higher than required for effective PDT, they are still more than 2 orders of magnitude lower than the LD₅₀ determined for Pd[DMBil1]-PEG750. In these experiments, each mechanism of cell death appears in a distinct quadrant of Figure 5A depending on their level of staining. For example, live cells are negative for both FITC and PI (lower left quadrant), apoptotic cells stain positive for only FITC (lower right quadrant), late apoptotic cells stain positive for both FITC and PI (upper right quadrant), and necrotic cells stain positive for only PI (upper left quadrant).



Figure 5. Apoptosis and necrosis assay after incubation with 0.0, 4.0, 6.0, or 8.0 μ M of Pd[DMBil1]-PEG₇₅₀ for 24 h, followed by light treatment for 30 min and incubation for 1 h. (A) Representative flow cytometry density plot of cells treated with 8.0 μ M Pd[DMBil1]-PEG₇₅₀. (B–D) Flow cytometry analysis of cell populations in early apoptosis, late apoptosis, and necrosis following treatment with the photosensitizer. *p < 0.05 by *t*-tests compared to no light treatment for each concentration.

The results of the apoptosis and necrosis assay show that PDT with Pd[DMBil1]-PEG₇₅₀ induces primarily apoptotic cell death and that the percentage of apoptotic cells increases with higher photosensitizer concentrations (Figures 5 and S9). The maximum amount of positively stained cells resulted from treatment with 8.0 µM Pd[DMBil1]-PEG₇₅₀. The average of three separate assays showed that 20.21% of cells were positive for early apoptosis, 17.08% of cells were positive for late apoptosis, and 8.17% of cells stained positive for necrosis only. Alternatively, cells that did not undergo light treatment experienced only minimal cell death by any mechanism. These results demonstrate that almost half of the total cell populations treated with Pd[DMBil1]-PEG₇₅₀ that are exposed to light for 30 min experience primarily apoptotic cell death, and \sim 82% of the cells killed by the phototreatment expire via apoptosis rather than necrosis. Given that apoptosis is much preferred over necrosis for cancer treatment, these results make Pd[DMBil1]-PEG₇₅₀ even more attractive as a potential photosensitizer for use in PDT.

CONCLUSIONS AND FUTURE DIRECTIONS

The development of new biologically compatible ${}^{1}O_{2}$ sensitizers for use as PDT agents remains a topic of significant interest,⁴⁹ given the benefits that phototherapeutics can hold over more conventional treatment options for certain cancers. Despite these advantages and the fact that PDT has been approved to treat cancers of the skin, head, neck, digestive system, ovaries, prostate, bladder, lungs, breast, and brain,² its adoption as a mainstream cancer treatment has been hampered by a general lack of ${}^{1}O_{2}$ photosensitizers that are efficient, biologically tolerated, and pose minimal side effects and toxicity in the dark. In this work we described our efforts to address these shortcomings through development of a water-

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soluble dimethylbiladiene derivative (Pd[DMBil1]-PEG₇₅₀) that can sensitize the formation of singlet O₂ with high quantum yield under biologically relevant conditions.

Preparation of the Pd[DMBil1]-PEG₇₅₀ complex was accomplished in two straightforward, high-yielding steps from the parent palladium dimethylbiladiene complex, and photophysical studies revealed that PEGylation of the tetrapyrrole core has little effect on the electronic properties of the parent Pd[DMBil1] complex. In the absence of oxygen, Pd-[DMBil1]-PEG₇₅₀ displays both fluorescence and phosphorescence when dissolved in methanol. Notably, the triplet emission signal is completely quenched upon introduction of air to the sample due to efficient sensitization of ${}^{1}O_{2}$ with a quantum yield of $\Phi_{\Delta} = 0.57$, which is competitive with traditional sensitizers employed for PDT, including Photofrin.

Pd[DMBil1]-PEG₇₅₀ is highly soluble in water as well as in biological media. Measurement of the photophysical properties of Pd[DMBil1]-PEG₇₅₀ in pH 7.4 PBS solutions showed that the complex does not aggregate and can photosensitize ¹O₂ under aqueous conditions. The efficacy of Pd[DMBil1]-PEG₇₅₀ as a photochemotherapeutic agent was assessed using TNBC MDA-MB-231 cells, which readily take up the biladiene construct as evidenced by fluorescence microscopy and flow cytometry. In the dark, the Pd[DMBil1]-PEG₇₅₀ complex is extremely well-tolerated by the TNBC cells and has an LD₅₀ of 1.87 mM. By contrast, the PEGylated palladium biladiene is an exceptionally potent photochemotherapeutic agent, as the effective dose of this complex required to reduce the TNBC viability by 50% was determined to be $ED_{50} = 0.354 \ \mu M$ after 30 min of irradiation. Consideration of both the ED_{50} and LD₅₀ metrics delivers the PI of Pd[DMBil1]-PEG₇₅₀, which is a benchmark of the efficacy of a photochemotherapeutic agent in relation to its inherent dark toxicity. Pd[DMBil1]-PEG750 has a remarkably high phototoxicity index of PI \approx 5300. This value dwarfs those determined for molecular surrogates of existing commercial PDT agents, as HPDC and IHP were found to have photoxicity indices of just PI \approx 25 and PI \approx 2, respectively.

Since comparison of the PI of Pd[DMBil1]-PEG₇₅₀ to those for common porphyrin-based PDT models suggests that the linear palladium(II) tetrapyrrole can be a vastly superior photochemotherapeutic agent, the mechanism by which Pd[DMBil1]-PEG₇₅₀ phototriggers TNBC cell death was probed. Cell death assays show that PDT with Pd[DMBil1]-PEG₇₅₀ induces primarily apoptotic cell death as opposed to necrosis, as ~82% of the TNBC cells that die as a result of PDT do so via apoptosis. This finding serves to further distinguish Pd[DMBil1]-PEG₇₅₀ as a photochemotherapeutic agent, since apoptosis is much preferred over necrosis for cancer treatments, because the latter can result in local inflammation that stimulates tumor growth.

When viewed as a whole, this study demonstrates that Pd[DMBil1]-PEG₇₅₀ holds promise as a photochemotherapeutic agent and should warrant further investigation as a PDT photosensitizer for treatment of cancers including TNBC. From a broader perspective, while macrocyclic tetrapyrroles such as porphyrins and phthalocyanines have been well-studied as potential PDT agents, this work demonstrates that properly engineered linear oligopyrrole complexes may represent a privileged class of ${}^{1}O_{2}$ sensitizers for the treatment of human disease. Building off our findings that Pd[DMBil1]-PEG₇₅₀ is well-tolerated by TNBC cells and is a potent ${}^{1}O_{2}$ sensitizer, future efforts will be focused on pushing the absorption envelope for this and related complexes further into the therapeutic window, either via modulation of the tetrapyrrole core or by using photon upconversion strategies, to better enable the use of such platforms in tissue and animal models.

ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications Web site. The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.inorgchem.8b01225.

Experimental procedures, additional Pd[DMBil1]-PEG₇₅₀ characterization data, and photodynamic therapy data (PDF)

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Notes

The authors declare no competing financial interest.

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