Autoinhibitory Feedback Control over Photodynamic Action

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Supporting Information

ABSTRACT: In biology, the activity of enzymes is usually regulated by feedback loops, which enables direct communication between enzymes and the state of the cell. In a similar manner, with the intention to have automated activity regulation, the therapeutic effect of a photosensitizer (BOD1) is shown to be reduced through a negative feedback loop initiated by the photosensitizer. Photodynamic action produces cytotoxic \(^1\)O\(_2\) and this reactive oxygen species reacts with ascorbate, generating H\(_2\)O\(_2\). Peroxide-mediated oxidation of the photosensitizer auxiliary group leads to the formation of inactive BOD2 from the parent photosensitizer. BOD1 is shown to accumulate in mitochondria, and cell viability is shown to decrease significantly with BOD1 compared to the loop end product, BOD2. Photoinduced enhancement of fluorescence indicates the formation of inactive BOD2 under cellular conditions, and enhanced fluorescence acts as a reporter for the activity of the photosensitizer. We present the first example of PDT autoinactivation, and such a feedback control mechanism would enable a decrease in post-therapy side effects.

INTRODUCTION

Autoacting or self-regulatory molecular networks provide higher level control over many complex systems of diverse disciplines, including biology, economics, and engineering. Various complex biological processes, including pattern formation, limb development, and circadian clocks, are controlled by the interaction between pathway elements via feedback loops, maintaining homeostasis at every level from molecular biology of the cell to ecology. Amplification of the signal or self-inhibition is maintained by self-acting elements mainly either by autoactivation through a positive loop or by product inhibition via a negative loop, respectively. Recently, this higher order control behavior has also been explored/mimicked in nonbiological systems. In the late 20th century, pioneering works on template-assisted self-replication with artificial replicants were reported. The first asymmetric autocatalysis was achieved when a chiral product acted as a chiral catalyst to make more of itself, and still many more autocatalytic molecular systems are being reported. Raymo reported autocatalytic fluorescence photoactivation based on product amplification through energy-transfer-dependent photochemical generation of anthracene derivatives. The Aprahamian group reported a communicating molecular system in which a Zn\(^{2+}\)-dependent hydrazone switch is regulated by a negative feedback loop; the loop is initiated when Zn\(^{2+}\)-mediated release of a proton from the switch results in the formation of an imine, which sequesters Zn\(^{2+}\), thereby inhibiting the switch. Feedback-dependent control over catalytic activity or amplification of the signal is also reported by several groups. In biology, the activity of enzymes is usually finely tuned by means of feedback regulation, which enables a communication among the state of the cell, concentration of the product, etc. Likely, control of activity by means of self-acting systems would enable selective and efficient therapies, and this potential is yet to be explored. Once optimized, such a therapeutic system would enable communication with the disease state and allow autocontrol over therapeutic activity.

Here, we report a photodynamic therapy (PDT) agent whose activity is regulated through a negative feedback loop (Scheme 1). PDT is an oxygen- and light-dependent noninnvasive method mainly used to treat surface tumors and certain skin diseases. To improve the selectivity of the therapy various activatable photosensitizers (PSs) are reported. Activity control in these literature examples is maintained by inducing disease-specific chemical and/or physical transformation on the PS. pH-, glutathione-, and enzyme-mediated conversions are the most widely used methods to change the PS from an inactive to an active state. To the best of our knowledge, so far, a PS with an autocontrol behavior has not been investigated. In this study, singlet oxygen (\(^1\)O\(_2\)) produced by a photosensitizer acts back
on the PS through a H$_2$O$_2$ intermediate, to reduce the efficiency of PDT action (Scheme 1).

To incorporate autoinhibition, H$_2$O$_2$-responsive BODIPY, BOD1, is used (Scheme 1). BOD1 bears a boronic ester in the structure, which is known to be oxidized by H$_2$O$_2$. Elimination on alkoxy methyl phenol follows spontaneously to generate pyridine BODIPY from a pyridinium precursor. This behavior is used to develop H$_2$O$_2$ sensors by others, since fluorescence is enhanced as pyridinium with charge transfer properties is transformed into pyridine. In this work, BOD1 is used as a PS. PDT action is mediated by $\cdot$O$_2$, which can only be generated when energy transfer to O$_2$ takes place following an intersystem crossing (ISC). Spin-forbidden ISC is either facilitated by a heavy atom or special orientation of the PSs. Ziessel et al. reported that halogen-free pyridinium BODIPYs also display high ISC through the charge transfer state. Despite this finding, heavy-atom-free pyridinium BODIPYs have not been used as PSs to date. Here, BOD1 with similar features is used for this purpose. Pyridine BODIPY lacking charge transfer character is not expected to display efficient $\cdot$O$_2$ generation. Based on this knowledge, we create a loop that generates BOD2 (inefficient PS) from BOD1 (active PS) through PDT activity of BOD1. Reactions involved in the loop are as follows: $\cdot$O$_2$ generated by BOD1 reacts with ascorbate to generate H$_2$O$_2$. H$_2$O$_2$ then reacts with boronate and ineffective BOD2 is produced as an end product (Scheme 1). Ascorbate, which is found in every cell, is chosen to be an intermediate loop element to enable communication between PS activity and the cell.

## RESULTS AND DISCUSSION

BOD1 and BOD2 are synthesized as described in the literature. Loop end product BOD2 is proposed to be less active, and it has been claimed that it can be generated by the PDT action of BOD1. To compare the PDT activities of these two compounds, relative $\cdot$O$_2$ generation efficiencies are analyzed using the $\cdot$O$_2$ trap molecule, 1,3-diphenylisobenzofuran (DPBF). The absorbance of DPBF is known to decrease as a result of the reaction of furan with $\cdot$O$_2$. A comparison of the change in DPBF absorption in the presence of BOD1 and BOD2 indicates that the latter has significantly lower activity, consistent with the design (Figure 1). None of the compounds produces $\cdot$O$_2$ in the dark.

In the design, product inhibition is expected to be mediated by the reaction of the PDT product ($\cdot$O$_2$) with ascorbate and this reaction generates H$_2$O$_2$. Selective reaction of BOD1 with peroxide produces fluorescent BOD2. Thus, feedback loop analysis is performed by following changes in the fluorescence of BOD1 in the presence or absence of ascorbate (Figure 2).

Fluorescence is expected to increase as BOD2 is produced. For this to take place, $\cdot$O$_2$ must react with ascorbate to generate H$_2$O$_2$ (Scheme 1). Fluorescence increase in the presence of ascorbate (blue) is significantly higher than that in the control lacking ascorbate (red). This result indicates that ascorbate is needed to generate BOD2.
Fluorescence quantum yields are calculated to be 0.04 and 0.31 for BOD1 and BOD2, respectively (Table S1). Consistently, upon irradiation in the presence of ascorbate, the quantum yield of BOD1 is increased to 0.16, approaching the value for BOD2.

We hypothesize that due to reactions given in Scheme 1, \( ^1O_2 \) generation efficiency is expected to decrease in the presence of ascorbate. Consistently, the decrease in the absorption of DPBF becomes less pronounced in the presence of increasing amounts of ascorbate (0, 0.2, and 1 mM, Figure 3).

This can be a result of a combinatorial effect: one reason being direct scavenging of \( ^1O_2 \) by ascorbate and the second one is conversion of BOD1 to a less efficient PS. Figure 3 supports this hypothesis, showing that the change in the absorption of DPBF upon irradiation decreases in the presence of ascorbate. As shown in Figure S1, during the PDT activity, the absorbance of BOD1 at 510 nm is shown to decrease and the absorbance at 505 nm is elevated. The new peak at 505 nm overlaps with BOD2 absorbance, supporting the formation of this molecule by PDT.

As mentioned above, one reason for the ascorbate-dependent decrease during PDT shown in Figure 3 can be scavenging, namely, ascorbate may react and remove \( ^1O_2 \). To show that the decrease in \( ^1O_2 \) generation by BOD1 in the presence of various amounts of ascorbate results mainly from conversion to BOD2 rather than removal of \( ^1O_2 \), a control experiment was done. BOD2 which lacks a peroxide-sensitive group is used as the PS and \(^1\)O2 generation efficiency is expected to decrease during PDT shown in Figure 3 can be calculated to be 32% in the presence of ascorbate (Figure S2).

It has been shown that when BOD2 is used as the photosensitizer, the change in the absorption of DPBF does not display a significant variation upon use of ascorbate. This clearly indicates that removal of \( ^1O_2 \) from the solution by reaction with ascorbate has a minimal effect on the observed data shown in Figure 3. Since \( ^1O_2 \) is catalytically produced by the PS, this effect is negligible. Instead, quenching of the \( ^1O_2 \) producer (PS) through loop control seems to be the major regulator of PDT activity.

Figure 3. \(^1O_2\) generation by BOD1 (10 \( \mu \)M, in isopropanol) in the presence or absence of sodium ascorbate (0.2 mM shown in blue or 1 mM shown in purple) as followed by the change in the absorption of DPBF (50 \( \mu \)M) at 411 nm. The sample lacking sodium ascorbate (red) produces more \(^1O_2\) and DPBF alone does not decompose under experimental conditions (green). Samples are kept in the dark for the first 30 min and then irradiated with 506 nm LED light for 110 min.

Fluorescence enhancement and spectral shift in absorption during PDT action of BOD1 give clues about BOD1-to-BOD2 conversion (Figures 2 and S1). To further demonstrate that BOD2 is generated via PDT activity, the \(^1\)H NMR spectrum of BOD1 is analyzed before and after irradiation in the presence of ascorbate (Figure 4). When the sample is irradiated for 80 min, BOD2 peaks resonating at 8.73, 7.44, and 6.22 ppm appear in the spectrum. Disappearance of BOD1 signals indicates that it is converted completely to either an intermediate oxidation product or BOD2. Calculations of the ratio of BOD2 peak integrals to other peaks indicate 32% conversion to BOD2. Considering the duration of PDT application (usually 1 or more hours), the product ratio within a given time is acceptable. Other oxidation products and 4-hydroxybenzyl alcohol are also produced consistent with the literature.

Figure 4. \(^1\)H NMR spectra (400 MHz, DMSO-\(d_6\)) of BOD1 (25 mM) (a) alone and (b) in the presence of saturated sodium ascorbate and light. (a) Upon irradiation with 506 nm light for 80 min, peaks of BOD2 appear. (c) \(^1\)H NMR spectrum of BOD2. Structures of products are given on the top of the spectra.

Although BOD1 is soluble in aqueous media in micromolar concentrations, in situ PDT experiments are performed in isopropanol due to the lack of solubility of BOD2 and DPBF in water. To check degradation-dependent fluorescence enhancement, a stability analysis is performed in neutral and slightly acidic buffer. Over 24 h, no significant fluorescence enhancement of BOD1 is observed, indicating BOD1 is stable over the experimental time scale (Figure S5). Selective degradation of BOD1 in the presence of ascorbate (Figure S6) suggests that the increase in fluorescence and spectral shift in absorption due to PDT action is not due to enzyme degradation but rather due to the increase in the quantum yield of BOD2.
oxidation of boronic ester bearing compounds (including BOD1) by H₂O₂ is reported by independent groups. To demonstrate that the loop requires H₂O₂, 1O₂-mediated conversion to BOD2 is analyzed by ¹H NMR in the presence of a H₂O₂ scavenger, sodium pyruvate. Even though the reaction mixture contains ascorbate, photoconversion to BOD2 failed, indicating the necessity of H₂O₂ for PS inactivation (Figure S6).

Cationic BOD1 (with IC₅₀ of 69 μM) localizes in the mitochondria of DLD-1 (Figure 5a). Mitochondrial targeting is an advantage for PDT, since in cancer tissues (to which this therapy is applied most), the oxygen level is much less than that in healthy tissues. On the other hand, PDT requires O₂. Indeed, targeting oxygen-rich organelle of hypoxic cancer tissue is reported to enhance PDT action.

In this work, BOD2 is produced by loop reactions and proposed to be less effective. To demonstrate the relative PDT actions of photosensitizers in cell culture, they are introduced into DLD-1 cells (Figure 5b). Cell viability assay indicates that neither of the compounds has dark toxicity. However, upon irradiation, viability drops down to 20% with BOD1. BOD2 has no significant cytotoxicity, consistent with the proposed design. That is, BOD1 is inactivated when it is converted into BOD2. Results of cell culture experiments indicate that within 2 h significant cell death is induced (Figure 5) and, at the same time, within this time scale, the photosensitizer is converted to inactive fluorescent BOD2 (Figure S6).

To understand the activity-reporting property of BOD1 and photogeneration of BOD2, cells are exposed to 506 nm light for 6 h. Although initially there is no fluorescence, in 1 h, fluorescence is enhanced significantly (Figure S7). Within 6 h, fluorescence starts to decrease slightly which would be due to leakage of uncharged BOD2 from the cell. Fluorescence increase within the first hours of irradiation is attributed to the formation of BOD2. Control samples kept in the dark for 6 h do not show any fluorescence, which supports the hypothesis that photogenerated H₂O₂ (not cellular peroxide) leads to the formation of fluorescent BOD2. Together with the relative PDT activities of BOD1 and BOD2, photo-autoinactivation of BOD1 under cellular conditions is evident (Figure 5 and S7). In addition, since fluorescence enhancement is a result of PDT action, it allows monitoring PS activity.

CONCLUSIONS

In conclusion, a halogen-free PS is shown to be self-inhibited. ¹O₂ generated by the PS is converted into H₂O₂, which in turn reacts with the PS and decreases the PDT activity. Fluorescence, UV−vis absorption spectra, ¹H NMR analysis, and HRMS results prove the formation of BOD2 in the presence of ascorbate and light. BOD2 is shown to be less effective at inducing apoptosis in cells. Fluorescence enhancement, as BOD1 is converted into BOD2, can be observed in the cell (Figure S7). It is demonstrated that H₂O₂ generated through PDT (not cellular peroxide) produces BOD2. The proposed feedback loop control is shown in Figure 6. Since the boronate group of BOD1 selectively reacts with hydrogen...
peroxo and cellular peroxide is not sufficient to react with this group, the results suggest that the activity can be autocontrolled via the proposed steps in vivo.

Taking the behavior of BOD1 into account, this PS can also be considered as a self-activity reporter, namely, it reports its own PDT activity by producing fluorescent output upon singlet oxygen generation.\(^{36–38}\)

Ascorbate, being an important element of cellular redox metabolism, enables communication of the PS with the cell and enables autoinhibition of the PS by reacting with the cytotoxic \(^1\text{O}_2\). Since inactivation is dependent on the PDT activity of the PS, the inhibitory effect of ascorbate is also highly dependent on the therapeutic activity. In other words, when \(^1\text{O}_2\) is produced to activate cell death, an autoinactivation pathway is initiated in parallel (Figure 6).

Even though PDT activity is controlled in many different ways, to the best of our knowledge, no one has yet reported PS activity regulation by such a feedback loop.\(^{16–22,39}\) This behavior can be used in actual applications such that, once a certain amount of \(^1\text{O}_2\) is generated sufficient to kill cancer cells, PS activity can be self-inhibited to diminish any further effect on neighboring healthy cells. This would improve the noninvasiveness of PDT. Activity-dependent inactivation, like we present here, would be a strategy to enable communication with the stage of therapy. With similar self-inhibitory PSs having reversible conversion between active/inactive states, an oscillatory activity can, in principle, be obtained. This behavior may find application in certain therapies, i.e., those requiring periodic drug release. This work may encourage the research in such directions.

EXPERIMENTAL SECTION

General Methods. All reagents and solvents were purchased from commercial sources and used without further purification. Compounds BOD1 and BOD2 were prepared using the literature procedures.\(^{24,27}\) Column chromatography was carried out using a silica stationary phase (230–400 mesh, SiliCycle Inc., Canada). Analytical thin layer chromatography was performed on 0.25 mm thick precoated silica gel plates (60F254, Merck, Germany). Compounds were visualized under UV light. All \(^1\text{H}\) NMR spectra were recorded on a Varian Inova instrument (400 MHz) at Selçuk University, Konya. Chemical shifts (\(\delta\)) are reported in parts per million (ppm) and referenced to the residual solvent peak. Coupling constants (\(J\)) are reported in hertz (Hz). Standard abbreviations indicating multiplicities are given: \(br = \) broad, \(d = \) doublet, \(m = \) multiplet, \(s = \) singlet, and \(t = \) triplet. High-resolution mass spectrometry was carried out using an Agilent 6530 Accurate-Mass Q-TOF LC/MS of the Eastern Anatolia Advanced Technology Research and Application Centre (DAYTAM, Erzurum, Turkey). For PDT, an LED from Bright LED Electronics Corp., model BL-BG43V4V, was used. Absorbance values at 506 nm were used as a light source. For cell culture experiments DLD-1 human colorectal adenocarcinoma cells (ATCC) were used.

Singlet Oxygen Generation Experiments. Singlet Oxygen generations were analyzed using the singlet oxygen trap molecule, 1,3-diphenylisobenzofuran. Absorption of this molecule decreased upon reaction with \(^1\text{O}_2\). Typically, 50 \(\mu\)M of this compound in isopropanol was used in the presence of the photosensitizer (1, 5, or 10 \(\mu\)M of BOD1 or BOD2). The absorption spectrum was recorded in the dark (for either 15 or 30 min). Then, samples were irradiated with a 506 nm LED lamp (Bright LED Electronics Corp., model BL-BG43V4V) from a distance of 20 cm. For samples that involve sodium ascorbate, a stock solution of this compound was prepared and added to the reaction cuvette so that the final ascorbate concentration was 0.2 or 1 mM.

\(^1\text{H}\) NMR spectra during PDT action were recorded to determine the generation of BOD2 in the presence of ascorbate. This experiment was performed in deuterated dimethyl sulfoxide (DMSO-\(d_6\)) instead of isopropanol because of the poor solubilities of ascorbate and photosensitizers in isopropanol at high concentrations. A saturated solution of sodium ascorbate (having concentration less than 100 mM) in DMSO-\(d_6\) was prepared. BOD1 (25 mM) in the presence of saturated sodium ascorbate (less than 100 mM) was irradiated for 80 min with the 506 nm LED lamp. After the experiment, the sample was extracted with water and dichloromethane. The organic phase was collected and dried over sodium sulfate. The \(^1\text{H}\) NMR spectrum of the organic phase contained peaks corresponding to only BOD2 (Figure S4).

To show that hydrogen peroxide was involved in the reaction series leading to BOD2 formation, an experiment involving a hydrogen peroxide scavenger was designed. Sodium pyruvate (NaPyr) was used as the scavenger.\(^{32}\) BOD1 (25 mM) in the presence of saturated sodium ascorbate and sodium pyruvate (each less than 100 mM) was irradiated for 80 min with the 506 nm LED lamp and \(^1\text{H}\) NMR was recorded. The presence of NaPyr inhibited the formation of BOD2 (Figure S6).

UV–Vis Absorption, Fluorescence Experiments, and Quantum Yield Calculations. UV–vis absorption spectra of 10 \(\mu\)M BOD1 in the presence of ascorbate (0.2 mM) in isopropanol were recorded using a Cary-60 UV-Vis Spectrometer. Samples were irradiated with the 506 nm LED lamp from 20 cm for 140 min until no further shift in the spectrum was observed.

Fluorescence spectra were recorded using an Agilent, Cary Eclipse fluorescence spectrometer with a xenon flash lamp. Briefly, 10 \(\mu\)M BOD1 samples in the presence or absence of 0.2 mM sodium ascorbate were prepared in isopropanol. Samples were irradiated with the 506 nm LED lamp from 20 cm for 200 min. Fluorescence spectra were recorded in 10 min intervals with excitation at 475 nm.

Quantum yields were determined using Rhodamine 6G as a reference fluorophore. Samples were excited at 488 nm. Rhodamine 6G was dissolved in water, whereas all other samples were dissolved in isopropanol. Absorbance values of the samples were kept below 0.1, to avoid an inner filter effect. Calculations were done using the following formula

\[
Q = Q_{R}(I/I_{R})^*\frac{A_{R}/A}{(n^2/n_{R}^2)}
\]

where \(Q_{R}\) is the quantum yield of the reference compound, \(I\) and \(I_{R}\) are the integrated areas of the emission spectrum of the sample and reference, respectively, \(A\) and \(A_{R}\) refer to the absorbance values of the sample and reference at excitation wavelength (488 nm), respectively, \(n\) and \(n_{R}\) are the refractive indices of solvents (1.333 for water and 1.3776 for isopropanol).\(^{30}\)

Cell Culture Experiments. DLD-1 human colorectal adenocarcinoma cells (ATCC) were cultured in T-75 culture flasks in RPMI 1640 culture medium at 37 °C in a humidified incubator containing 5% CO₂ (Binder CB Series CO₂ incubators). The standard RPMI medium was supplemented...
with heat-inactivated 10% fetal bovine serum, 2 mM l-glutamine, 100 units mL⁻¹ Penicillin G, and 100 μg mL⁻¹ streptomycin at 37 °C in a humidified incubator containing 5% CO₂. After incubation of the cell line for 24 h, the medium was washed with PBS buffer, and cell viability was tested for the 0–200 μM concentration range of BOD1 in a 24-well plate containing 10⁴ cells/well.

To determine cellular localization, cells were treated with 10 μM H₂O₂ (to obtain a fluorescence image), then the medium was washed with PBS buffer. Cells were stained with MitoTracker (ThermoFisher) and after washing with PBS, 5 μM BOD1 was introduced. After incubation with BOD1, cells were washed with PBS buffer and visualized with a fluorescence microscope at 20x and 80x (Thermo Multiskan Go).

To determine cell viability in the dark and after irradiation, standard Alamar Blue (ThermoFisher) assay was performed. After incubation of the cells, the medium was renewed with 10 μM of either BOD1 or BOD2 and irradiated with the 506 nm LED array for 2 h. Cell viability was determined using Alamar Blue assay.²

To determine fluorescence enhancement due to the formation of BOD2, cells were incubated with 10 μM of BOD1 and either irradiated with the 506 nm LED array for 6 h or kept in the dark (control). Fluorescence of the cells were visualized with a fluorescence microscope at 20x and 80x (Thermo Multiskan Go).

Synthesis. Synthesis was performed using modified literature procedures.²⁴,²⁷

Synthesis of BOD2. Dichloromethane (150 mL) was purged with N₂ for 30 min. 4-Pyridinecarboxyaldehyde (12.7 mmol, 1.36 g, 1.2 mL) was mixed with 2,4-dimethylpyrrole (26.2 mmol, 2.5 g, 2.6 mL) in degased dichloromethane. Three drops of trifluoroacetic acid (TFA) were added, and the color of the solution turned red. The solution was stirred at room temperature for 16 h under an inert atmosphere. p-Chloranil (6.9 mmol, 1.7 g) was added to the solution, and the solution was stirred for additional 2.5 h, followed by the addition of triethylamine (7.5 mL) and a boron trifluoride—diethyl ether complex (7.5 mL). The mixture was stirred for additional 2.5 h at room temperature. The reaction mixture was extracted with water and dichloromethane. The organic layer was collected, dried with sodium sulfate, and dichloromethane was evaporated in vacuo. The crude product was purified by silica gel column chromatography using 5% methanol in dichloromethane as the eluent. The fraction containing BOD2 was collected, and then the solvent was evaporated under reduced pressure. The product was obtained as glitter red powder (2.5 mmol, 823 mg, 20%).

¹H NMR (400 MHz, chloroform-d) δ 8.78 (d, J = 5.9 Hz, 2H), 7.33 (d, J = 6.0 Hz, 2H), 6.00 (s, 2H), 2.55 (s, 6H), 1.40 (s, 6H). High-resolution ESI-MS values are 326.1640 for theoretical m/z of (M + H)+ and 326.1679 for experimental (Δ: 12 ppm).

Synthesis of BOD1. 4-(4,4,5,5-Tetramethyl-1,3,2-dioxaborol-olan-2-yl)benzyl bromide (0.16 mmol, 47 mg) and BOD2 (0.10 mmol, 34 mg) were dissolved in 10 mL of toluene and then the solution was refluxed at 90 °C for 16 h. After cooling, the desired product was obtained by filtering the precipitate. The red−orange solid was further washed with toluene to afford pure BOD1 (0.05 mmol, 30.8 mg, 49.5%).

¹H NMR (400 MHz, DMSO-d₆) δ 9.39 (d, J = 5.9 Hz, 2H), 8.47 (d, J = 6.0 Hz, 2H), 7.72 (d, J = 7.5 Hz, 2H), 7.47 (d, J = 7.4 Hz, 2H), 6.26 (s, 2H), 6.00 (s, 2H), 1.35 (s, 6H), 1.33−1.19 (s, 12H). High-resolution ESI-MS values are 542.2962 for theoretical m/z of (M)+ and 542.2961 for experimental (Δ: 0.18 ppm).

ASSOCIATED CONTENT

Supporting Information
The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acsomega.9b01410.

Detailed experimental procedures, additional figures, UV−vis absorption, NMR, and HRMS spectra (PDF)

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Notes
The authors declare no competing financial interest.

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