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Molecular Electronic Tuning of Photosensitizers to Enhance Photodynamic Therapy: Synthetic Dicyanobacteriochlorins as a Case Study

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Abstract

Photophysical, photostability, electrochemical, and molecular-orbital characteristics are analyzed for a set of stable dicyanobacteriochlorins that are promising photosensitizers for photodynamic therapy (PDT). The bacteriochlorins are the parent compound (**BC**), dicyano derivative (**(NC)₂BC**) and corresponding zinc (**(NC)₂BC-Zn**) and palladium chelate (**(NC)₂BC-Pd**). The order of PDT activity against HeLa human cancer cells *in vitro* is **(NC)₂BC-Pd** > **(NC)₂BC** > **(NC)₂BC-Zn** ≈ **BC**. The near-infrared absorption feature of each dicyanobacteriochlorin is bathochromically shifted 35–50 nm (748–763 nm) from that for **BC** (713 nm). Intersystem crossing to the PDT-active triplet excited state is essentially quantitative for **(NC)₂BC-Pd**. Phosphorescence from **(NC)₂BC-Pd** occurs at 1122 nm (1.1 eV). This value and the measured ground-state redox potentials fix the triplet excited-state redox properties, which underpin PDT activity via Type-1 (electron-transfer) pathways. A perhaps counterintuitive (but readily explicable) result is that of the three dicyanobacteriochlorins, the photosensitizer with the shortest triplet lifetime (7 μs), **(NC)₂BC-Pd**, has the highest activity. Photostabilities of the dicyanobacteriochlorins and other bacteriochlorins studied recently are investigated and discussed in terms of four phenomena: aggregation, reduction, oxidation, and chemical reaction. Collectively, the results and analysis provide fundamental insights concerning the molecular design of PDT agents.

INTRODUCTION

Photodynamic therapy (PDT) is an emerging treatment approach that uses a non-toxic photosensitizer and harmless visible or near-infrared (NIR) light to kill diseased cells by generating reactive oxygen species, such as singlet oxygen, superoxide and hydroxyl radicals (1). To enhance the efficacy and selectivity of response, molecular tuning of the

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Conflict of Interest Statement

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chemical and electronic-structure characteristics of photosensitizers is employed to produce (i) favorable photophysical properties, (ii) chemical and photochemical stability, (iii) preferential delivery to the destination tissue or cell type, including subcellular compartments, and (iv) production of reactive oxygen species that are especially lethal to the target cell.

An important consideration is to choose or tune the photosensitizer to have strong absorption in the NIR region where light penetration through tissue is maximal because both absorption and scattering of light at these wavelengths by endogenous chromophores are minimal. In this regard, bacteriochlorins and related macrocycles are ideal because of the intense NIR (720–850 nm) absorption resulting from the reduction of two pyrrolic rings in the tetrapyrrole macrocycle compared to chlorins (one reduced ring, modestly intense red absorption) and porphyrins (no reduced rings and weak or no red or NIR absorption). Naturally occurring bacteriochlorins or derivatives thereof, such as **WST9** (Tookad) and **WST11** (Chart 1) can have high PDT efficacy but have drawbacks due to instability (in the dark and in the light) and limitations on molecular tailoring because nearly all sites about the perimeter of the macrocycle already bear substituents.

A *de novo* synthetic route has been developed to access bacteriochlorins that are stabilized against adventitious oxidation by the presence of a geminal dimethyl group in each reduced ring (2–4). This route has been used to prepare bacteriochlorins containing a variety of positively charged substituents, groups to impart water solubility, and/or groups that can vary the balance between hydrophobicity and hydrophilicity. The PDT activity of these bacteriochlorins has been tested against B16 mouse melanoma cells (5), HeLa human cervical cancer cells (6) and microbial cells (Gram-positive, Gram-negative bacteria and yeast) (7) *in vitro*. A number of these synthetic bacteriochlorins are highly efficacious (i.e., LD₅₀ 100 nM) for cell killing against human cancer cell lines. Representative structures are shown in Chart 2. The bacteriochlorins employed are all the metal-free (free base) forms. The PDT efficacy of tetrapyrrole chromophores (e.g., porphyrins, chlorins, bacteriochlorins, phthalocyanines) often depends on the metalation state (8), as was found for a set of imidazole-substituted metalloporphyrins (Chart 3) targeted against HeLa and CT26 cancer cells (9,10).

Some of the synthetic bacteriochlorins are more readily photobleached than others. Photobleaching is defined as loss of absorption of the bacteriochlorin, and can stem from a variety of causes (oxidation, reduction, aggregation, chemical reaction). Understanding the interplay of molecular structure and origin of photobleaching in principle could lead to improvements in photostability and thereby afford increased PDT activity.

Recently we synthesized and examined the PDT efficacy against HeLa cancer cells of a set of free base and metal-substituted bacteriochlorins bearing electron-withdrawing, cyano-groups (Chart 4) (11). In addition to photoactivity, analyses were performed of cellular uptake, subcellular distribution, and the propensity of the compounds to form singlet oxygen (Type-2 photochemistry) or hydroxyl radicals (Type-1 photochemistry). The dicyanobacteriochlorins show (i) the same trend in efficacy with metal ion (e.g., Pd²⁺ > Zn²⁺) as determined for the imidazole-substituted (and other) porphyrins, (ii) increased photostability, and (iii) efficacies comparable to or surpassing those of the best of the synthetic bacteriochlorins studied previously and other common PDT agents.

In the present work, the photophysical, redox, and molecular-orbital characteristics of the unsubstituted and dicyanobacteriochlorins are presented along with more extensive photostability data. This information allows analysis of the recent results (11) concerning photoactivity and reactive oxygen species production in terms of fundamental electronic

properties, including excited-state redox potentials. To increase the generality of the findings, the analysis is extended to include results on the imidazole-substituted porphyrins and representative synthetic bacteriochlorins studied previously, for which photostability characteristics are presented here for the first time. The collective findings provide fundamental insights into the design and electronic tuning of bacteriochlorin photosensitizers for enhanced PDT efficacy.

MATERIALS AND METHODS

Photosensitizers

The syntheses of **BC** (4), **(NC)₂BC** (3), and **(NC)₂BC-Zn** (3) have been described. The synthesis of **(NC)₂BC-Pd** will be reported elsewhere. Absorption and emission studies indicate that samples of **(NC)₂BC-Pd** studied herein contain <1% of the free base **(NC)₂BC** starting material. Photofrin and Verteporfin (liposomal benzoporphyrin derivative, BPD) were gifts from QLT, Inc. (Vancouver, Canada) and Lutex was a gift from Pharmacyclics, Inc. (Sunnyvale, CA).

Photophysical Characterization

Static absorption (Varian Cary 100 or Shimadzu UV-1800) and fluorescence (Spex Fluorolog Tau 2 or PTI Quantamaster 40) measurements were performed at room temperature, as were all other studies. Determination of the fluorescence quantum yield (Φ_f), singlet excited-state lifetimes (τ_S) and triplet yields (Φ_T) utilized dilute (μM) Ar-purged toluene and methanol solutions. Measurements of the triplet lifetimes (τ_T) utilized Ar-purged 2-methyl tetrahydrofuran solutions. Samples for Φ_f measurements had an absorbance 0.1 at the excitation wavelength to minimize front-face effects and similarly low absorbance in the $Q_y(0,0)$ band to minimize inner-filter effects.

Static emission measurements employed 2–4 nm excitation- and detection-monochromator bandwidths and 0.2-nm data intervals. Emission spectra were corrected for detection-system spectral response. Some measurements employed an extended NIR sensitive detector that drives a lock-in amplifier frequency-referenced to the rate of chopping of the excitation light prior to the excitation monochromator. Fluorescence quantum yields were determined relative to several different standards. These standards are (i) chlorophyll *a* in deoxygenated toluene ($\Phi_f = 0.325$) (12), which is the value measured in benzene (13), (ii) free base *meso*-tetraphenylporphyrin (**FbTPP**) in nondegassed toluene, for which $\Phi_f = 0.070$ was established with respect to the zinc chelate **ZnTPP** in nondegassed toluene ($\Phi_f = 0.030$) (14), a value consistent with prior results on **FbTPP** (15), and (iii) 8,8,18,18-tetramethylbacteriochlorin (4) in Ar-purged toluene, for which $\Phi_f = 0.14$ was established with respect to chlorophyll *a* in benzene and **FbTPP** in toluene.

Singlet-excited state lifetimes (τ_S) for all compounds except **(NC)₂BC-Pd** were obtained using time-correlated-single-photon-counting detection on an apparatus with an approximately Gaussian instrument response function with a full-width-at-half-maximum of ~1 ns (Photon Technology International LaserStrobe TM-3). Samples were excited in the Soret or Q_x regions using excitation pulses at 337 nm from a nitrogen laser or in the blue to green spectral regions from a dye laser pumped by the nitrogen laser. The τ_S value for **(NC)₂BC-Pd** was obtained by ultrafast transient absorption spectroscopy, probing disappearance of the bleaching in the Q_y ground-state absorption band and excited-state absorption features following excitation in the Q_x band with an ~130 fs excitation flash (16). The τ_T values were similarly determined using transient absorption spectroscopy, probing the decay of bleaching of the Soret band and excited-state absorption features following

excitation in the Q_x band with ~5 ns pulses from a Q-switched Nd:YAG laser (532 nm) or a dye laser pumped by the Nd:YAG laser (9,16).

The Φ_{isc} values (triplet yields) were obtained using transient absorption spectroscopy. The extent of bleaching of the ground-state Q_x bands due to the formation of the lowest singlet excited state was measured immediately following a 130 fs flash in the $Q_y(0,0)$ band and compared with that due to the formation of the lowest triplet excited state at the asymptote of the singlet excited-state decay (9,16).

Photostability Studies

Photostability measurements utilized excitation light obtained from a 300W Xenon lamp (Model R300-3 lamp and PS300-1 power supply; ILC Technologies, Sunnyvale, CA) that passed through a 70 cm path cell containing deionized water followed by a monochromator with a 10 nm bandpass. The light intensity at the sample position was measured using a calibrated diode and optometer (Models 221 and S471, United Detector Technologies, San Diego, CA) and was typically ~5 mW/cm² at the wavelength (720–750 nm) of the NIR absorption maximum of the bacteriochlorin sample ($A \sim 0.5$ in 1 cm). The solutions contained ambient (atmospheric) O₂ or had the O₂ removed by purging with Ar for ~1 h or by repeated freeze-pump-thaw cycles on a high vacuum line that achieved a vacuum of <10⁻⁶ Torr. The O₂ was removed from all aqueous micellar solutions via the latter method. Samples were stirred using a micro magnetic bar at the bottom of the cuvette during illumination.

Electrochemistry

Electrochemical studies were performed using previously described instrumentation (17). The solvent was butyronitrile (Burdick and Jackson) containing 0.1 M tetrabutylammonium hexafluorophosphate (Aldrich; recrystallized three times from methanol and dried at 110 °C *in vacuo*) as the supporting electrolyte. The electrochemical cell was housed in a glovebox. The $E_{1/2}$ values were obtained with square wave voltammetry (frequency 10 Hz) under conditions where the ferrocene couple has a potential of +0.19 V.

Density Functional Theory Calculations

DFT calculations were performed with Spartan '10 for Windows version 1.2.0 in parallel mode on a PC equipped with an Intel i7-975 cpu, 24 GB ram, and three 300 GB, 10k rpm hard drives (18). The calculations employed the hybrid B3LYP functional and basis sets 6-31G* and LACVP (the former for atoms H to Kr and lan12dz for atoms Kr and above). The equilibrium geometries were fully optimized using the default parameters of the Spartan program. Molecular orbital images were plotted using an isovalue of 0.016.

RESULTS AND DISCUSSION

Sensitizer Activity, Uptake, Localization, and Reactive Oxygen Species Production

In our recent study (11), the PDT activity against HeLa cancer cells *in vitro* was studied using the parent bacteriochlorin (**BC**), dicyano derivative (**(NC)₂BC**) and the corresponding zinc chelate (**(NC)₂BC-Zn**) and palladium chelate (**(NC)₂BC-Pd**) (Chart 4). The photosensitizers were delivered either by (i) direct dilution (denoted “dd”) of a solution of bacteriochlorin in an organic solvent [5 mM in *N,N*-dimethylacetamide (DMA) or tetrahydrofuran (THF)] into serum-containing complete culture medium or (ii) encapsulation into aqueous Cremophore EL micelles (CrEL) followed by dilution into the same complete medium. For both delivery methods, the order of PDT activity against HeLa cells after incubation for 24 h and illumination with 10 J/cm² of NIR light is (**(NC)₂BC-Pd**) > (**(NC)₂BC**) > (**(NC)₂BC-Zn**) > **BC**. As can be seen from Table 1, the LD50 values improve using CrEL

versus direct dilution only modestly for **(NC)₂BC-Pd** (18 versus 25 nM) and **(NC)₂BC** (25 versus 60 nM) but significantly for **(NC)₂BC-Zn** (60 versus 1000 nM) and **BC** (350 versus 1800 nM).

Values for the uptake of 5 μ M bacteriochlorin at different time points in the incubation medium are also given in Table 1. The highest uptake using direct dilution is found for **BC** and **(NC)₂BC-Pd**, followed by **(NC)₂BC** and **(NC)₂BC-Zn**. The use of CrEL substantially decreases the uptake of **(NC)₂BC-Pd**, **(NC)₂BC** and **BC** but has little effect for **(NC)₂BC-Zn**. Table 1 also gives the PDT efficacy per unit uptake, obtained by dividing the reciprocal of the LD50 value by the uptake value and then normalizing to the value of **BC** using direct dilution. The tabulated values of efficacy corrected for uptake for both delivery motifs show the same order as the tabulated values for activity alone: **(NC)₂BC-Pd** > **(NC)₂BC** > **(NC)₂BC-Zn** > **BC**. Thus, although cell uptake certainly influences the overall PDT activity, other factors appear to dominate. In this regard, the greater efficacy of **(NC)₂BC-Pd** versus **(NC)₂BC-Zn** parallels our prior results on imidazole-substituted metalloporphyrins (Chart 3) wherein **2-Pd** is more active than **2-Zn**, exhibiting LD50 values of 55 nM and 833 nM under the excitation conditions employed (9,10). The studies on the dicyanobacteriochlorins complement and extend this prior work by inclusion of a free base analogue, providing additional information for analysis of activity.

Fluorescence microscopy studies reveal that subcellular localization of the unsubstituted and dicyanobacteriochlorins is in the endoplasmic reticulum, mitochondria and lysosomes depending on the compound. The least active compound, **BC**, is found mainly in the endoplasmic reticulum and lysosomes, and the next compound, **(NC)₂BC-Zn**, is also found mainly in lysosomes with little evidence for association with mitochondria. The second most active bacteriochlorin, **(NC)₂BC**, localizes mainly in the endoplasmic reticulum and mitochondria. The most active compound, **(NC)₂BC-Pd**, also targets mitochondria (and lysosomes) as deduced by the damage done by PDT activity monitored after illumination. The results suggest that the propensity for mitochondrial targeting contributes to the relative PDT efficacy of the four bacteriochlorins. Mitochondria are considered to be an important PDT target because of the vital cellular functions performed, including regulation of metabolism, cell-cycle control development, and induction of apoptotic cell death after mitochondrial damage.

The propensities of the bacteriochlorins to produce singlet oxygen by energy transfer (Type-2 mechanism) or hydroxyl radical by electron transfer (Type-1 mechanism) were probed using fluorescent dyes sensitive to the particular reactive oxygen species. Of the four bacteriochlorins, **(NC)₂BC-Pd** produces the most singlet oxygen and also the most hydroxyl radicals, with the balance favoring the latter (Type-1 photochemistry). The next active compound, **(NC)₂BC**, also produces a relatively high ratio of hydroxyl radicals to singlet oxygen. The two least active photosensitizers, **(NC)₂BC-Zn** and **BC**, give low ratios hydroxyl radicals versus singlet oxygen and therefore carry out less Type-1 than Type-2 photochemistry. In summary, the most active bacteriochlorins (against HeLa cells) produce the greatest amounts of hydroxyl radicals. Similar results were found previously for the imidazole-substituted porphyrins (Chart 3), for which **2-Pd** produces more hydroxyl radicals and, as noted above, has greater PDT activity than **2-Zn** (10).

Initial results of photobleaching studies obtained previously (11) on the four bacteriochlorins are also given in Table 1. Listed is the fraction of the NIR $Q_y(0,0)$ absorbance remaining after 100 J/cm² of illumination in the same optical band. Values are given for the compounds in DMA and CrEL solutions in the presence of ambient O₂ and for the latter in the absence of O₂. The most active compounds, **(NC)₂BC-Pd** and **(NC)₂BC**, are the most photostable, with typically >90% of the Q_y absorbance remaining even in the presence of

ambient O₂. The two lesser active compounds, (NC)₂BC-Zn and BC, show lower photostability in the presence of O₂ and marked improvement if O₂ is removed from the solution. Below, more extensive measurements of the photostability of the dicyanobacteriochlorins are presented and compared with those for representative synthetic bacteriochlorins whose PDT activity (but not photostability) have been studied previously.

Absorption and Fluorescence Spectra

Figure 1A shows the absorption spectra for BC, (NC)₂BC, (NC)₂BC-Zn, and (NC)₂BC-Pd in toluene. The wavelengths of all the major absorption features for the bacteriochlorins in toluene and methanol are given in Table 2. For each bacteriochlorin the spectral positions are similar in the two solvents, and the same is found for the compounds in DMA and in CrEL solutions. All four compounds show good solubility in the four media.

Figure 1B focuses on the NIR absorption feature, the Q_y(0,0) band. This band for the metal-free dicyanobacteriochlorin (NC)₂BC is substantially (35 nm) hypsochromically shifted versus the parent bacteriochlorin BC (748 versus 713 nm in toluene). The incorporation of the central Pd²⁺ ion of (NC)₂BC-Pd slightly bathochromically shifts the band to 751 nm while the Zn²⁺ of (NC)₂BC-Zn causes a larger shift to 761 nm. Figure 1B shows that the corresponding Q_y(0,0) fluorescence band for each bacteriochlorin lies only 2–5 nm to longer wavelength than the Q_y(0,0) absorption maximum (Table 2).

Fluorescence Yields, Singlet Excited-State Lifetimes, and Triplet Yields

Compounds BC, (NC)₂BC and (NC)₂BC-Zn in toluene and methanol have similar singlet excited-state lifetimes ($\tau_S = 3.9$ –4.1 ns) and fluorescence quantum yields ($\Phi_f = 0.14$ –0.15). The results are summarized in Tables 1 and 2. The lifetime of (NC)₂BC-Pd is dramatically reduced ($\tau_S = 23$ ps) as is the fluorescence yield ($\Phi_f = 0.007$). The latter value is an upper limit because the possibility cannot be excluded that some of the weak emission derives from trace demetalated analogue (NC)₂BC, which has spectral overlap with (NC)₂BC-Pd. Indeed, based on the relative τ_S values, one would have expected a Φ_f value of ~ 0.0008 for (NC)₂BC-Pd. The Φ_f and τ_S values of BC, (NC)₂BC and (NC)₂BC-Zn are comparable to those that we have found recently for synthetic free base bacteriochlorins (see e.g., Table 1) (5–7,16,19) and zinc bacteriochlorins (19) bearing a number of substituent patterns. The results for (NC)₂BC-Pd are in keeping with values for two other synthetic palladium bacteriochlorins (19), and palladium-substituted bacteriochlorophyll and the corresponding sulfonato derivative (20,21).

The reduced τ_S and Φ_f values for (NC)₂BC-Pd (like other palladium tetrapyrroles) derives from heavy-metal (and perhaps d-orbital) enhancement of singlet-to-triplet intersystem-crossing, which in turn makes the latter process virtually quantitative ($\Phi_T = 0.99$). The yield of intersystem crossing (i.e., the triplet yield) for (NC)₂BC-Pd can be compared with 0.63 for (NC)₂BC-Zn and 0.43 for (NC)₂BC (Table 1). The Φ_T value for BC (0.62) is greater than that for (NC)₂BC, reflecting the effect of the cyano-groups to draw electron density from the macrocycle and thereby diminish spin-orbit coupling, which underlies the intersystem-crossing process. The Φ_T values obtained here for the dicyanobacteriochlorins are consistent with the results of prior studies on synthetic bacteriochlorins, which give average yields of ~ 0.5 for free base bacteriochlorins, ~ 0.7 for zinc chelates, ~ 0.8 for indium chelates, and ~ 1 for palladium chelates (11–13,16,19). Essentially quantitative intersystem crossing is also found for palladium-substituted bacteriochlorophyll and derivatives thereof (20,21). These values for bacteriochlorins can be compared with the typical triplet yields of 0.7–0.8, 0.8–0.9 and 0.9–1 for free base, zinc, and palladium porphyrins such as tetraphenylporphyrins and imidazolium porphyrins (9,22).

These comparisons show that one can expect a maximal enhancement factor in triplet yield of ~1.5 for porphyrins and ~2 for bacteriochlorins upon changing the metalation state (i.e., palladium versus free base). The data in Table 1 indicate that PDT activity, as judged by LD50 or related measures that account for uptake, show variations in activity of one or two orders of magnitude. Although heavy metal (e.g., palladium) substitution may not give a significant gain in PDT efficacy due to an increased triplet yield, activity could be enhanced via accompanying changes in more critical characteristics, such as ground- and excited-state redox properties, shorter T_1 lifetimes, and altered axial ligation, as discussed previously (9) and below.

Phosphorescence Spectra and Triplet Excited-State Energies

Figure 2 shows an emission scan for $(\text{NC})_2\text{BC-Pd}$ in deoxygenated THF at room temperature; the scan was collected using a detection system with extended NIR sensitivity. The spectrum shows fluorescence (747 nm) close in position to that found in toluene (753 nm; Figure 1B) as well as weak phosphorescence at 1122 nm. The phosphorescence is verified as originating from $(\text{NC})_2\text{BC-Pd}$ using excitation spectra and is completely quenched (as expected) if the solution contains ambient O_2 . Phosphorescence was not observed for $(\text{NC})_2\text{BC-Zn}$ under the same conditions, and is expected to be much weaker.

Because of the rather small number of reports of bacteriochlorin phosphorescence, this emission was also measured for two other recently synthesized palladium chelates (**B9** and **B87**) for which the other photophysical properties have been obtained recently (19). The structures of these palladium bacteriochlorins and their zinc and free base analogues are shown in Chart 5. The phosphorescence, verified by excitation spectra, occurs at 1114 nm for **B9** and 1118 nm for **B87**, similar to 1122 nm for $(\text{NC})_2\text{BC-Pd}$. Each wavelength corresponds to a triplet excited-state (T_1) energy of 1.11 eV. The latter value is lower than that of the singlet excited state (S_1) for the three bacteriochlorins by 0.56, 0.52 and 0.54 eV, respectively.

The above-noted phosphorescence wavelengths, T_1 energies and S_1 – T_1 energy gaps for the three synthetic palladium bacteriochlorins are within the span of most reported values. The compounds include bacteriochlorophyll (BChl) and its zinc- and palladium-substituted analogues (1170–1221 nm, 1.01–1.06 eV; 0.56–0.58 eV), the free base analogue bacteriopheophytin (1097 nm; 1.13 eV; 0.50 eV), zinc tetraphenylbacteriochlorin (1157 nm; 1.07 eV; 0.57 eV), and free base tetraphenylbacteriochlorin (1053 nm; 1.17 eV; 0.50 eV) (23,24). Similar T_1 energies (1.15 and 1.22 eV) have been determined for two chlorinated tetrasulfonatophenylbacteriochlorins using photoacoustic spectroscopy (25). The T_1 energies for the latter two compounds could be slightly lower than reported because the analysis assumed that internal conversion is effectively zero (i.e., $\Phi_f + \Phi_T = 1$) whereas recent results on a large group of free base bacteriochlorins indicate that this nonradiative pathway typically has at least twice the quantum yield as fluorescence (16). The triplet energy of 1.30 eV for free base tetraphenylbacteriochlorin (22) is modestly higher than the above-noted phosphorescence energy of 1.17 eV (24). Elucidation of triplet excited-state energies is important for deducing excited-state redox potentials and assessing PDT mechanisms as discussed recently (22,25) and below.

Triplet Excited-State Lifetimes

The lifetime of the lowest triplet excited state (τ_T) in deoxygenated solution is in the range 80–170 μs for **BC**, $(\text{NC})_2\text{BC}$, and $(\text{NC})_2\text{BC-Zn}$ but is dramatically reduced to 7 μs for $(\text{NC})_2\text{BC-Pd}$ (Table 1). Similar triplet lifetimes (3–10 μs) have been reported for other synthetic palladium bacteriochlorins (19) and for palladium-substituted bacteriochlorophylls (20,21). The shorter lifetimes of the palladium versus zinc or free base bacteriochlorins can

be attributed to heavy-atom enhancement of both triplet excited-state decay pathways (phosphorescence emission and non-radiative intersystem crossing). The triplet lifetime is reduced to $<1\ \mu\text{s}$ for each of the bacteriochlorins in the presence of ambient O_2 , consistent with the added contribution of the energy/electron-transfer processes that underlie the formation of the reactive oxygen species for PDT.

The results of the present study of metal-containing dicyanobacteriochlorins along with the findings in our prior studies of free base bacteriochlorins (5–7) and imidazole-substituted porphyrins (9,10) show no clear correlation between triplet lifetime and PDT activity (Table 1). In fact, a seemingly counterintuitive result is that of the three dicyanobacteriochlorins, the photosensitizer with the shortest triplet lifetime ($7\ \mu\text{s}$), **(NC)₂BC-Pd**, has the highest activity. The same is true concerning imidazole-substituted porphyrins **2-Pd** ($\sim 10\ \mu\text{s}$) versus **2-Zn** ($\sim 6\ \text{ms}$) studied previously (9,10). The synthetic palladium tetrapyrroles, like native bacteriochlorophyll-derived **WST9** (Tookad) and **WST11**, apparently have such favorable general characteristics (e.g., uptake/distribution, reactive oxygen species production) that a triplet lifetime of $\sim 10\ \mu\text{s}$ allows sufficient encounters with O_2 to attain high PDT activity (22). A possible advantage to such short triplet lifetimes in reducing photobleaching is described below.

Photostability

The photostability properties of the four bacteriochlorins under study [**BC**, **(NC)₂BC**, **(NC)₂BC-Zn**, **(NC)₂BC-Pd**] (Chart 4) were measured, as were those of representative synthetic bacteriochlorins whose PDT activity was investigated previously (**B16**, **B17**, **B19**, **B22**, **B29**, **B31**; Chart 2) (5–7). The photostability properties of several standard PDT agents (Lutex, Photofrin, benzoporphyrin derivative BPD) (26) were also examined. Each compound was studied in multiple media [e.g., toluene, DMA, dimethylsulfoxide (DMSO), methanol (MeOH), ethanol (EtOH), acetonitrile (MeCN), CrEL] both in the presence and absence of ambient O_2 . The absorbance spectrum (350–900 nm) was monitored as a function of incident light fluence (up to $100\ \text{J}/\text{cm}^2$) in the $\text{Q}_y(0,0)$ band, and in most cases the fluorescence spectrum was followed as well. In each case, the fraction of the initial peak $\text{Q}_y(0,0)$ absorbance as a function of light fluence was plotted. Table 1 gives the value at $100\ \text{J}/\text{cm}^2$ for a number of bacteriochlorins in DMA and CrEL in the presence of ambient O_2 and CrEL in the absence of O_2 . Representative spectra and plots are shown in Figures 3–6.

Photobleaching is defined operationally as loss of absorption of the bacteriochlorin in the entire near-UV-to-NIR spectral region. Photobleaching can stem from a variety of sources, including reduction, oxidation, aggregation, or chemical reaction, which are referred to as photoreduction, photooxidation, photoaggregation, and photodegradation, respectively. Photoreduction and photooxidation may in part be reversed (by electron transfer with a species in the environment), as can photoaggregation (by dissociation), whereas photodegradation is typically irreversible. One photodegradation pathway entails $[2 + 2]$ cycloaddition of the ground-state bacteriochlorin with singlet oxygen (formed by energy transfer from the triplet excited-state bacteriochlorin) followed by ring opening to give a bilin species.

One of the goals with the dicyanobacteriochlorins (Chart 4) (11) was to improve photostability compared to the previously studied synthetic bacteriochlorins. The results bear out the success of that endeavor. The spectra in Figures 3A and 3C, the plots in Figures 4A, and the values in Table 1 show that **(NC)₂BC-Pd** and **(NC)₂BC** in the presence of ambient O_2 are extremely stable. The solutions retain 90% of the NIR absorbance in CrEL and 83% in DMA after exposure to $100\ \text{J}/\text{cm}^2$ of NIR light. In contrast, the previously studied bacteriochlorins **B16**, **B17**, **B22**, **B29**, and **B31**, as well as the parent unsubstituted

compound **BC** in the same media, have 20% absorbance remaining after illumination with 100 J/cm² (in some cases at 50 J/cm²) in the presence of ambient O₂ and 50–80% after removal of O₂ (Figures 3 and 4 and Table 1). Thus, for CrEL and DMA solutions, (NC)₂BC-Pd and (NC)₂BC in the presence of ambient O₂ are more photostable than the other bacteriochlorins even after stability has been markedly improved by the removal of O₂ (Figure 4). Bacteriochlorins (NC)₂BC-Pd and (NC)₂BC are also modestly more stable than common PDT agents (26) Photofrin and Lutex and considerably more stable than benzoporphyrin derivative (BPD) (Figures 4A and 4F).

The zinc chelate (NC)₂BC-Zn in DMA and CrEL both in the presence and absence of O₂ is less photostable than (NC)₂BC-Pd and (NC)₂BC in these media containing O₂ (Figure 3B and 4A). (NC)₂BC-Zn has comparable stability to the previously studied bacteriochlorins and **BC** in CrEL (with or without O₂) but is typically more stable in DMA (Table 1). In effect, the incorporation of a zinc ion counteracts the photostability enhancement obtained upon incorporation of the dicyano-groups; in contrast, a palladium ion can have a synergistic effect. These findings can be understood in terms of the redox properties of the chromophores, as described below. Collectively, the results show that the incorporation of (electron-withdrawing) cyano groups at the 3,13-positions, along with the appropriate choice of metalation state, provide an excellent design for improving photostability of bacteriochlorin photosensitizers. Chlorine atoms, which also are electron-withdrawing, have been incorporated at the *ortho*-positions of the aryl rings in synthetic *meso*-tetraaryl bacteriochlorins to improve photostability (Chart 1) (27,28).

Prior studies of the photodegradation of bacteriochlorophyll *a* and derivatives show that the process is highly solvent dependent and requires O₂. Typical photoproducts include chlorins such as 2-acetylchlorophyll *a* and other derivatives formed by two-electron oxidation of the macrocycle, bilin-like open-chain tetrapyrroles likely formed via addition of singlet oxygen and ring opening, and other species that have no featured absorption in the visible region (21,29). The geminal dimethyl groups in the synthetic bacteriochlorins are incorporated to avoid dehydrogenation to form chlorins and porphyrins (2,3), which when observed are produced in such low yield that the expected features are cleanly resolved only via fluorescence. Bacteriochlorin **B16** in DMA with ambient O₂ after prolonged illumination gives such an example, showing a weak fluorescence feature at ~640 nm (Figure 6C, inset) with no corresponding sharp feature in the absorption spectrum (Figure 6A). This example also reveals broad weak absorption and fluorescence-centered features around 600 nm that could represent open-chain tetrapyrrolic photoproducts.

Generally the photobleaching of the synthetic bacteriochlorins (bearing geminal dimethyl groups) is evidenced by decrease in the entire near-UV-to-NIR absorption spectrum along with a similar disappearance of fluorescence. The diminution of the absorption profile is often accompanied by an apparent increase in the baseline, particularly at wavelengths shorter than 500 nm and increasing toward the near-UV region. This behavior is illustrated by the absorption spectra for (NC)₂BC-Zn, **BC**, and **B16** in CrEL with ambient O₂ obtained after 100 J/cm² illumination (Figures 3B, 3D, and 5A; dark yellow). The featureless spectral profiles could reflect formation of nondescript photoproducts analogous to those for bacteriochlorophyll *a* (29) or light scattering due to photoaggregation. The analogous profiles for **B16** and **BC** in CrEL in the absence of O₂ (Figures 5B and 5C) show Soret, Q_x and Q_y features that are decreased in intensity, broadened, and bathochromically shifted after prolonged illumination. This behavior could reflect the formation of small aggregates (possibly as small as dimers) that are partially soluble and remain suspended in the micellar environment. The ability of CrEL to reduce aggregation or aid disaggregation underlies the motivation for its use as a delivery vehicle for the bacteriochlorins (6,11). Aggregates may

play a positive role in PDT by serving as reservoirs to provide fresh photosensitizer in place of those that have undergone irreversible photodegradation (29).

A relationship between photobleaching and the solubilization characteristics of the bacteriochlorin in a given medium is illustrated by the behavior of unsubstituted bacteriochlorin **BC** in several media in the presence of ambient O₂ (Figures 3D, 3E, 3F, and 4C). The photostability is poor in the CrEL (as noted above), MeCN and DMA but excellent in toluene, where 93% of the absorbance remains after 100 J/cm² illumination. Thus, in the (nonpolar) medium in which it is expected to be the most soluble, the highly nonpolar bacteriochlorin **BC** shows photostability that rivals that of dicyanobacteriochlorins **(NC)₂BC-Pd** and **(NC)₂BC** (Figures 3A, 3C, 3E and 4). Solubility characteristics may also contribute to the marked medium dependence of photobleaching of the charged bacteriochlorin **B31** (Chart 2 and Figure 4E). Solvent polarity can also impact photooxidation contributions to photobleaching via the concentration of ambient O₂ in the medium and stabilization of electron-transfer products. Collectively, these results and considerations suggest that the photostability of a photosensitizer may be much different in organic solvents, delivery media, and the cellular milieu.

A contribution of a photoaggregation mechanism to the observed photobleaching could occur by several pathways. One pathway is an extension of excimer formation (30) whereby a bacteriochlorin in the T₁ state associates with another bacteriochlorin in the ground state and so on to associate more chromophores. Such a pathway raises the possibility that photobleaching could be reduced for photosensitizers that have shorter T₁ lifetimes (such as palladium tetrapyrroles) and thus provide less time for such diffusional encounters. A second pathway would involve electron transfer from a bacteriochlorin in the T₁ excited state to O₂ (or another electron acceptor) to form the bacteriochlorin π -cation radical. Interaction of the latter species with a ground-state bacteriochlorin would form a dimeric complex, analogous to formation of the monocation dimer of magnesium octaethylporphyrin (31), and so on to associate more chromophores. Dimer (and aggregate) formation would be enhanced because the monomer cation radical has a half-filled HOMO and thus the bonding orbital of the interacting chromophore pair would be filled and the higher energy antibonding orbital would be half-filled (or empty), thereby giving a net bonding interaction that would not occur for two neutral species (where both orbitals are filled). The bacteriochlorin π -cation radical in the latter mechanism is presumably an intermediate in more common photooxidation pathways for degradation (e.g., to form chlorins). Thus, it is possible that multiple photobleaching pathways may be intertwined.

Electrochemical and Molecular-Orbital Characteristics of the Dicyanobacteriochlorins

The first oxidation potential (E_{ox}) and first reduction potential (E_{red}) of the four bacteriochlorins [**BC**, **(NC)₂BC**, **(NC)₂BC-Zn**, **(NC)₂BC-Pd**] were measured and are listed in Table 3. The table also gives the energy of the highest occupied molecular orbital (E_{HOMO}) and the lowest unoccupied molecular orbital (E_{LUMO}) obtained from DFT calculations for each photosensitizer. Electron densities (and energies) of the four frontier molecular orbitals for each of these four bacteriochlorins are shown in Figure 7. Studies of a large number of chlorins have shown essentially linear relationships between E_{ox} and E_{HOMO} and between E_{red} and E_{LUMO} (32). As E_{ox} becomes more positive, E_{HOMO} becomes more negative, and the molecule is harder to oxidize. As E_{red} becomes less negative, E_{LUMO} becomes more negative, and the molecule is easier to reduce. For comparison Table 3 also lists redox and MO data for two other sets of free base, zinc and palladium bacteriochlorins investigated recently (19). The Table also gives data acquired here or previously (9) for imidazole-substituted porphyrins and tetraphenylporphyrins.

Incorporation of the two cyano groups of **(NC)₂BC** make the molecule harder to oxidize than **BC** ($E_{\text{ox}} = 0.60$ versus 0.09 V; $E_{\text{HOMO}} = -5.22$ versus -4.45 eV). Similarly, the two cyano groups of **(NC)₂BC** make the molecule easier to reduce than **BC** ($E_{\text{red}} = -1.10$ versus -1.67 V; $E_{\text{LUMO}} = -3.10$ versus -2.20 eV). Compared to **(NC)₂BC**, zinc chelate **(NC)₂BC-Zn** is significantly easier to oxidize (-290 mV) and only slightly harder to reduce (-40 mV); these redox-potential shifts are in concert with the relative magnitudes and signs (to less negative values) of the energy shifts in the HOMO ($+140$ mV) and LUMO ($+30$ mV). Compared to **(NC)₂BC**, palladium chelate **(NC)₂BC-Pd** is slightly easier to oxidize (-80 mV) and also slightly easier to reduce ($+70$ mV); the former tracks the small shift to less negative HOMO energy ($+60$ mV) whereas the latter is not paralleled by the virtually unchanged LUMO energy (-10 mV). In summary, the data in Table 3 show that of the three dicyanobacteriochlorins, **(NC)₂BC-Zn** is the easiest to oxidize (although it is harder to oxidize than **BC**) and **(NC)₂BC-Pd** is the easiest to reduce (and is also easier to reduce than **BC**).

Figure 8A plots the orbital energies (Table 3) versus the cell-killing LD50 values (Table 1) for the bacteriochlorins obtained using direct dilution into the complete culture medium. A qualitatively similar plot is obtained using the LD50 values when the bacteriochlorins were first encapsulated in micelles upon treatment with CrEL before dilution into the complete culture medium. Figure 8B plots the redox potentials of the T_1 excited state versus LD50 values. The excited-state redox potentials are obtained from the ground-state redox potentials and the T_1 energies (Table 3). The correlations with LD50 are discussed below.

Excited-State Redox Properties and Reactive Oxygen Species Production

Until recently it has been generally accepted that singlet oxygen (formed by energy transfer; i.e., Type-2 photochemistry) is the major mediator of toxicity in PDT. Studies have now shown that hydroxyl radicals, superoxide and other species formed by electron transfer (Type-1 photochemistry) also play an important role in PDT activity depending on the photosensitizer and specific disease or cell type. The palladium analogues of bacteriochlorophyll *a* (**WST9** and **WST11**; Chart 1) produce superoxide and hydroxyl radicals (20,21,33). Synthetic free base *meso*-tetraaryl bacteriochlorins (particularly halogenated sulfonamide analogues; Chart 1) also produce superoxide and hydroxyl radicals as well as the Type-2 product singlet oxygen (25,27). It has been suggested that the latter bacteriochlorins may be expected to produce both Type-1 and Type-2 photoproducts whereas porphyrins give significantly less electron-transfer (Type-1) products because porphyrins are harder to oxidize than bacteriochlorins (22,25).

The diverse free base synthetic bacteriochlorins shown in Chart 2 and free base and metal-containing dicyanobacteriochlorins (Chart 4) that are the subject of the present study also produce hydroxyl radicals as well as singlet oxygen (5,6,7,11). As noted above, the most active dicyanobacteriochlorin **(NC)₂BC-Pd** and the second most active analogue **(NC)₂BC** give a higher ratio of hydroxyl radicals versus singlet oxygen upon illumination, whereas the reverse is true for **(NC)₂BC-Zn** and **BC**. Indeed, **(NC)₂BC-Pd** has greater PDT efficacy and a much greater propensity to produce hydroxyl radicals than **(NC)₂BC-Zn**. In a prior study of imidazole-substituted metalloporphyrins (Chart 3), the palladium chelate (**2-Pd**) was similarly found to have greater PDT activity and produce much more hydroxyl radicals than the zinc analogue (**2-Zn**) (10).

The parallelism in relative photoactivity and hydroxyl-radical formation for the palladium versus zinc chelates of the dicyanobacteriochlorins and imidazole-substituted porphyrins gives insights into fundamental mechanistic issues. In particular, the palladium chelates of both the dicyanobacteriochlorins and porphyrins are found (or expected) to be harder to

oxidize and easier to reduce than the zinc chelates (both in the ground state and the T₁ states). These findings together with general comparisons of the excited-state redox properties of bacteriochlorins versus porphyrins, and the effects of the dicyano substituents of the bacteriochlorins on photoactivity (and photostability), must be considered collectively in assessing the mechanisms of production of reactive oxygen species via Type-1 photochemistry (e.g., hydroxyl radical formation) for these tetrapyrroles.

The Type-1 mechanism is virtually always thought to involve photooxidation of the photosensitizer (P) in the T₁ excited state (P^T) by electron transfer to O₂ via Eqn. (1A).

A number of reaction sequences have been considered for the subsequent formation of hydrogen peroxide and hydroxyl radicals from the superoxide ion (O₂^{-•}) (10,20–22,25,33). The one-electron oxidized photosensitizer is then returned to the starting condition by electron transfer from endogenous electron donor (D) [Eqn. (1B)]. The endogenous electron donor has been proposed to be human serum albumin for the palladium bacteriochlorophyll *a* derivatives, where complexes between the two species underlie a proposed photocatalytic role of such photosensitizers via Eqns. (1A) and (1B) (21). Photosensitizer oxidation mechanisms involving Eqn. (1A) would generally imply that photoactivity should track the T₁ excited-state oxidation potential of the photosensitizer. Therefore, more potent photosensitizers should be easier to oxidize in the T₁ excited state than less efficacious compounds, all other factors being equal.

An alternative mechanism is photoreduction of the photosensitizer in the T₁ excited state by an endogenous electron donor (D) followed by electron transfer from the photosensitizer anion to O₂, to again produce superoxide ion, via Eqns. (2A) and (2B).

Subsequent dark reactions of superoxide ion leading to hydrogen peroxide and hydroxyl radicals and other reactive oxygen species would proceed just as in the photooxidation mechanism described above [Eqn. (1A)]. Additionally, hydroxyl radicals potentially can be formed more directly from hydrogen peroxide and the reduced photosensitizer produced in Eqn. 2A (10). The photoreduction mechanism [(Eqns. (2A) plus (2B))] would also give a photocatalytic role for the photosensitizer in an analogous manner (to that noted above via the photooxidation pathway) for human serum albumin (as the electron donor) and palladium bacteriochlorophyll *a* derivatives (as photosensitizer) (21). Furthermore, ascorbate has been proposed to perform a similar role as a sacrificial electron donor to tetrapyrrole photosensitizers in the P^T state [Eqn. (2A)] or P⁺• forms [Eqn. (1A)] (22). Photosensitizer reduction mechanisms involving Eqn. (2A) would generally imply that photoactivity should track the T₁ excited-state reduction potential of the photosensitizer. Therefore, more potent photosensitizers should be easier to reduce in the T₁ excited state than less efficacious compounds, all other factors being equal.

We have considered these two alternative mechanisms (photosensitizer oxidation and reduction) previously in the study of imidazole-substituted palladium versus zinc porphyrins (9,10). The photoreduction pathway rather than the photooxidation pathway seems to best explain the findings that porphyrin **2-Pd** produces more hydroxyl radicals and is more efficacious than **2-Zn** (Chart 3). This follows because based on the T_1 energies and ground-state redox potentials for these compounds and **PdTPP** and **ZnTPP** (Table 3 and ref (35)), the **2-Pd** is expected to be ~150 mV easier to reduce and ~50 mV harder to oxidize than **2-Zn** in the T_1 state. Similarly, for the dicyanobacteriochlorins, **(NC)₂BC-Pd** is 130 mV easier to reduce and 190 mV harder to oxidize than **(NC)₂BC-Zn** in the T_1 state.

The latter trend can be extended to the entire set of unsubstituted and dicyanobacteriochlorins (Chart 4 and Table 3). The potential for first T_1 reduction becomes less negative or more positive (the excited bacteriochlorin becomes easier to reduce) in the order **BC** (−0.47 V) < **(NC)₂BC-Zn** (−0.05 V) < **(NC)₂BC** (0.01 V) < **(NC)₂BC-Pd** (0.08 V). The potential for first T_1 oxidation becomes more negative (the excited bacteriochlorin becomes easier to oxidize) in the order **(NC)₂BC** (−0.51 V) > **(NC)₂BC-Pd** (−0.59 V) > **(NC)₂BC-Zn** (−0.78 V) > **BC** (−1.11 V). Figure 8B shows the excited-state redox potentials versus LD50 (using the direct-dilution medium). A similar plot is obtained using the LD50 values obtained using CrEL delivery. Good correlations are seen for both the T_1 oxidation and reduction potentials, although the implications for activity are, at first glance, reversed. Increasing PDT activity (decreasing LD50) correlates with greater ease of reduction and greater difficulty of oxidation.

The simplest interpretation of the parallel findings for the dicyanobacteriochlorins and imidazole-substituted porphyrins is that the photoreduction of the T_1 excited state contributes significantly to inherent PDT efficacy (i.e., reactive oxygen species production once the photosensitizer is excited). However, at least two additional factors must be considered as follows.

- i. The absolute (and relative) rates of electron transfer will ultimately depend on the relationship between net free energy change for the excited-state electron-transfer reaction (which depends on the T_1 redox potentials) and the total reorganization energy involving the photosensitizer, O_2 , and the medium. In other words, depending on where electron transfer for a particular photosensitizer is on this curve (i.e., the Marcus plot), an increase in the free-energy driving force for the process (via a change in redox potentials or T_1 energy) could either increase or decrease the rate of the reaction. The standard considerations given above consider that a greater magnitude of the free energy change implies an increased rate and yield of electron transfer.
- ii. The overall PDT activity via the Type-1 mechanism may not be dictated by the rates and yields of electron transfer (or the Type-2 contribution by the rates and yields of energy transfer) if the limiting factor is photostability. In this regard, it is normally thought that photoactivity proceeds via photooxidation [Eqn. (1A)] and most electron-transfer routes to photobleaching proceed via a similar mechanism. In particular, for a bacteriochlorin the excited-state oxidation process in Eqn. (1A) involves one-electron oxidation of the macrocycle to form the π -cation radical, and ultimately formation of a typical chlorin photoproduct by two-electron oxidation (21,29). Even if formation of chlorin does not proceed via the bacteriochlorin π -cation radical, the general reasoning seems to be that, within the context of a Type-1 mechanism, both PDT activity and photobleaching proceed via a photooxidation (rather than photoreduction) pathway.

Electronic Tuning of Tetrapyrrole Photosensitizers for Photostability and Photoactivity

Ideally, the photoactivity and photostability of a photosensitizer could be independently controlled, at least to some degree. The data in Figure 8 and the reasoning given above suggest that such a situation could in fact contribute to the PDT efficacy of tetrapyrroles. In particular, photoactivity via the Type-1 mechanism could proceed at least in part via transient reduction of the photosensitizer [Eqn. (2A)] and thus would be enhanced if the photosensitizer is made easier to reduce in the excited state. On the other hand, photobleaching likely proceeds to a significant degree by photooxidation (rather than photoreduction) and photoaggregation (perhaps via the π -cation radical), in addition to photodegradation; thus photostability would be enhanced by making the photosensitizer harder to oxidize. Rendering a tetrapyrrole chromophore harder to oxidize and easier to reduce is, in fact, what generally occurs upon the incorporation of electron-withdrawing substituents or the incorporation of a central metal ion with a high electronegativity (e.g., palladium). Such trends can be seen in the redox properties and the energies of the frontier molecular orbitals (Table 3) (22,34,35). Furthermore, the strategic choice and placement of substituents allow some control over the relative oxidation and reduction potentials (via HOMO and LUMO energies), as has been shown for synthetic chlorins, bacteriochlorins and related oxophorbins and bacteriooxophorbins that incorporate the keto-bearing five-membered ring of the native photosynthetic chromophores (16,19,32,36–38).

The increased photostability engendered by halogenation of the aryl rings of *meso*-tetraaryl bacteriochlorins (e.g., **TDCPBS** in Chart 1) has been noted previously (22,25). The more difficult oxidation (and increased photostability) of palladium bacteriochlorophyll *a* derivatives (e.g., **WST9** and **WST11** in Chart 1) than bacteriochlorophyll *a* also has been noted (20–22,33), and ascribed in part to the effect of the central palladium ion compared to the native magnesium ion. Additionally the native-like bacteriochlorins bear a 3-acetyl group and the related keto group in the fused five-membered ring spanning the 12- to 15-positions (Chart 1). These groups are basically electron withdrawing in character in that they lower the HOMO and LUMO energies and make the chromophore harder to oxidize and easier to reduce than bacteriochlorins that do not contain such substituents (16,19,38), and the same is true of chlorin analogues (9,37). Thus, the photostability of **WST9** and **WST11** is likely provided by effects of both substituents and the electronegative palladium ion to reduce photooxidation.

Again, the effectiveness of these previously studied photosensitizers to produce hydroxyl radicals (or other Type-1 photoproducts) would be diminished compared to compounds that lacked the electron-withdrawing substituents or highly electronegative central metal if the mechanism of that process involves photooxidation [Eqn. (1A)]; however, the activity would be enhanced if there is a contribution from the photoreduction pathway [Eqn. (2A)]. As described above, the same is true of the bacteriochlorins bearing 3,13 substituents that are the focus of the present work and our recent investigation of the PDT efficacy of these photosensitizers (11). On the basis of shifts in MO energy shifts and redox properties obtained upon incorporation of cyano versus acetyl groups at similar positions (Table 3),^{32,36–38} one would expect the combined effects on oxidation potentials [photostability and photoactivity via Eqn. (1A)] and reduction potentials [photoactivity via Eqn. (2A)] to be greater for **(NC)₂BC-Pd** versus the palladium bacteriochlorophyll *a* derivatives **WST9** and **WST11**, all other things being equal. The advantages of **(NC)₂BC-Pd** and related synthetic bacteriochlorins are (1) further enhanced photostability by the use of the geminal dimethyl groups that inhibit formation of chlorin photodegradation products and (2) the availability of macrocycle sites for additional electronic and chemical tuning.

In summary, a combination of favourable electronic effects make the palladium bacteriochlorin **(NC)₂BC-Pd** a photosensitizer with PDT activity (LD50 ~ 25 nM) and

photostability that rival or surpass those of synthetic bacteriochlorins that we have studied previously as well as many common PDT agents. The photostability of this compound is enhanced due to diminished photobleaching (via photooxidation, photoaggregation, and photodegradation), which stem from the effects of the peripheral cyano groups and central palladium ion. The photoactivity of (NC)₂BC-Pd, (NC)₂BC and other tetrapyrrole photosensitizers may derive in part from a contribution of a photoreduction pathway for production of lethal reactive oxygen species such as hydroxyl radicals. Collectively, the fundamental insights gained from the studies described herein may allow the design of bacteriochlorin photosensitizers that have even greater PDT efficacy.

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References

1. Agostinis P, Berg K, Cengel KA, Foster TH, Girotti AW, Gollnick SO, Hahn SM, Hamblin MR, Juzeniene A, Kessel D, Korbelik M, Moan J, Mroz P, Nowis D, Piette J, Wilson BC, Golab J. Photodynamic therapy of cancer: An update. *CA Cancer J Clin.* 2011; 61:250–281. [PubMed: 21617154]
2. Kim HJ, Lindsey JS. De novo synthesis of stable tetrahydroporphyrinic macrocycles: Bacteriochlorins and a tetradehydrocorrin. *J Org Chem.* 2005; 70:5475–5486. [PubMed: 15989329]
3. Ruzié C, Krayner M, Balasubramanian T, Lindsey JS. Tailoring a bacteriochlorin building block with cationic, amphipathic, or lipophilic substituents. *J Org Chem.* 2008; 73:5806–5820. [PubMed: 18576691]
4. Taniguchi M, Cramer DL, Bhise AD, Kee HL, Bocian DF, Holten D, Lindsey JS. Accessing the near-infrared spectral region with stable, synthetic, wavelength-tunable bacteriochlorins. *New J Chem.* 2008; 32:947–958.
5. Mroz P, Huang YY, Szokalska A, Zhiyentayev T, Janjua S, Nifli AP, Sherwood ME, Ruzié C, Borbas KE, Fan D, Krayner M, Balasubramanian T, Yang E, Kee HL, Kirmaier C, Diers JR, Bocian DF, Holten D, Lindsey JS, Hamblin MR. Stable synthetic bacteriochlorins overcome the resistance of melanoma to photodynamic therapy. *FASEB J.* 2010; 24:3160–3170. [PubMed: 20385618]
6. Huang YY, Mroz P, Zhiyentayev T, Sharma SK, Balasubramanian T, Ruzié C, Krayner M, Fan D, Borbas KE, Yang E, Kee HL, Kirmaier C, Diers JR, Bocian DF, Holten D, Lindsey JS, Hamblin MR. In vitro photodynamic therapy and quantitative structure-activity relationship studies with stable synthetic near-infrared-absorbing bacteriochlorin photosensitizers. *J Med Chem.* 2010; 53:4018–4027. [PubMed: 20441223]
7. Huang L, Huang YY, Mroz P, Tegos GP, Zhiyentayev T, Sharma SK, Lu Z, Balasubramanian T, Krayner M, Ruzié C, Yang E, Kee HL, Kirmaier C, Diers JR, Bocian DF, Holten D, Lindsey JS, Hamblin MR. Stable synthetic cationic bacteriochlorins as selective antimicrobial photosensitizers. *Antimicrob Agents Chemother.* 2010; 54:3834–3841. [PubMed: 20625146]
8. Nyman ES, Hynninen PH. Research advances in the use of tetrapyrrolic photosensitizers for photodynamic therapy. *J Photochem Photobiol B: Biol.* 2004; 73:1–28.
9. Kee HL, Bhaumik J, Diers JR, Mroz P, Hamblin MR, Bocian DF, Lindsey JS, Holten D. Photophysical characterization of imidazolium-substituted Pd(II), In(III), and Zn(II) porphyrins as photosensitizers for photodynamic therapy. *J Photochem Photobiol A: Chem.* 2008; 200:346–355. [PubMed: 20016663]
10. Mroz P, Bhaumik J, Dogutan DK, Aly Z, Kamal Z, Khalid L, Kee HL, Bocian DF, Holten D, Lindsey JS, Hamblin MR. Imidazole metalloporphyrins as photosensitizers for photodynamic

therapy: Role of molecular charge, central metal and hydroxyl radical production. *Cancer Lett.* 2009; 282:63–76. [PubMed: 19346065]

11. Huang Y-Y, Balasubramanian T, Yang E, Diers JR, Bocian DF, Lindsey JS, Holten D, Hamblin MR. Stable synthetic bacteriochlorins for photodynamic therapy: Role of dicyano peripheral groups, central metal substitution (2H, Zn, Pd), and Cremophor EL delivery. *ChemMedChem.* 2012; 7:2155–2157. [PubMed: 23065820]
12. Mass O, Taniguchi M, Ptaszek M, Springer JW, Faries KM, Diers JR, Bocian DF, Holten D, Lindsey JS. Structural characteristics that make chlorophylls green: Interplay of hydrocarbon skeleton and substituents. *New J Chem.* 2011; 35:76–88.
13. Weber G, Teale FWJ. Determination of the absolute quantum yield of fluorescent solutions. *Trans Faraday Soc.* 1957; 53:646–655.
14. Seybold PG, Gouterman M. Porphyrins XIII: Fluorescence spectra and quantum yields. *J Mol Spectrosc.* 1969; 31:1–13.
15. Gradyushko AT, Sevchenko AN, Solovyov KN, Tsvirko MP. Energetics of photophysical processes in chlorophyll-like molecules. *Photochem Photobiol.* 1970; 11:387–400. [PubMed: 5456267]
16. Yang E, Kirmaier C, Krayner M, Taniguchi M, Kim HJ, Diers JR, Bocian DF, Lindsey JS, Holten D. Photophysical properties and electronic structure of stable, tunable synthetic bacteriochlorins: Extending the features of native photosynthetic pigments. *J Phys Chem B.* 2011; 115:10801–10816. [PubMed: 21875047]
17. Strachan JP, Gentemann S, Seth J, Kalsbeck WA, Lindsey JS, Holten D, Bocian DF. Effects of orbital ordering on electronic communication in multiporphyrin arrays. *J Am Chem Soc.* 1997; 119:11191–11201.
18. Except for molecular mechanics and semi-empirical models the calculation methods used in Spartan '08 or '10 have been documented in the following: Shao Y, Molnar LF, Jung Y, Kussmann J, Ochsenfeld C, Brown ST, Gilbert ATB, Slipchenko LV, Levchenko SV, O'Neill DP, DiStasio RA Jr, Lochan RC, Wang T, Beran GJO, Besley NA, Herbert JM, Lin CY, Voorhis TV, Chien SH, Sodt A, Steele RP, Rassolov VA, Maslen PE, Korambath PP, Adamson RD, Austin B, Baker J, Byrd EFC, Dachsel H, Doerksen RJ, Dreuw A, Dunietz BD, Dutoi AD, Furlani TR, Gwaltney SR, Heyden A, Hirata S, Hsu CP, Kedziora G, Khalliulin RZ, Klunzinger PA, Lee M, Lee MS, Liang W, Lotan I, Nair N, Peters B, Proynov EI, Pieniazek PA, Rhee YM, Ritchie J, Rosta E, Sherrill CD, Simmonett AC, Subotnik JE, Woodcock HL III, Zhang W, Bell AT, Chakraborty AK, Chipman DM, Keil FJ, Warshel A, Hehre WJ, Schaefer HF III, Kong J, Krylov AI, Gill PMW, Head-Gordon M. Advances in methods and algorithms in a modern quantum chemistry program package. *Phys Chem Chem Phys.* 2006; 8:3172–3191. [PubMed: 16902710]
19. Chen CY, Sun E, Fan D, Taniguchi M, McDowell BE, Yang E, Diers JR, Bocian DF, Holten D, Lindsey JS. Synthesis and physicochemical properties of metallobacteriochlorins. *Inorg Chem.* 2012; 51:9443–9464. [PubMed: 22916839]
20. Vakrat-Haglili Y, Weiner L, Brumfeld V, Brandis A, Salomon Y, McIlroy B, Wilson BC, Pawlak A, Rozanowska M, Sarna T, Scherz A. The microenvironment effect on the generation of reactive oxygen species by Pd-bacteriopheophorbide. *J Am Chem Soc.* 2005; 127:6487–6497. [PubMed: 15853357]
21. Ashur I, Goldschmidt R, Pinkas I, Salomon Y, Szewczyk G, Sarna T, Scherz A. Photocatalytic generation of oxygen radicals by the water-soluble bacteriochlorophyll derivative WST11, noncovalently bound to serum albumin. *J Phys Chem A.* 2009; 113:8027–8037. [PubMed: 19545111]
22. Arnaut LG. Design of porphyrin-based photosensitizers for photodynamic therapy. *Inorg Photochem.* 2011; 63:187–233.
23. Hartwich G, Fiedor L, Simonin I, Cmiel E, Schäfer W, Noy D, Scherz A, Scheer H. Metal-substituted bacteriochlorophylls. 1 Preparation and influence of metal and coordination on spectra. *J Am Chem Soc.* 1988; 110:3675–3683.
24. Takiff L, Boxer SG. Phosphorescence from the primary donor in *Rhodobacter sphaeroides* and *Rhodospseudomonas viridis* reaction centers. *Biochim Biophys Acta.* 1988; 932:325–334.
25. Silva EFF, Serpa C, Dabrowski JM, Monteiro CJP, Formosinho SJ, Stochel G, Urbanska K, Simões S, Pereira MM, Arnaut LG. Mechanisms of singlet-oxygen and superoxide-ion generation

- by porphyrins and bacteriochlorins and their implications in photodynamic therapy. *Chem Eur J*. 2010; 16:9273–9286. [PubMed: 20572171]
26. MacDonald IJ, Dougherty TJ. Basic principles of photodynamic therapy. *J Porphyrins Phthalocyanines*. 2001; 5:105–129.
27. Dabrowski JM, Urbanska K, Arnaut LG, Pereira MM, Abreu AR, Simoes S, Stochel G. Biodistribution and photodynamic efficacy of a water-soluble, stable, halogenated bacteriochlorin against melanoma. *ChemMedChem*. 2011; 6:465–475. [PubMed: 21265022]
28. Dabrowski JM, Arnaut LG, Pereira MM, Monteiro CJ, Urbanska K, Simoes S, Stochel G. New halogenated water-soluble chlorin and bacteriochlorin as photostable PDT sensitizers: synthesis, spectroscopy, photophysics, and in vitro photosensitizing efficacy. *ChemMedChem*. 2010; 5:1770–1780. [PubMed: 20715283]
29. Limantara L, Koehler P, Wilhelm B, Porra RJ, Scheer H. Photostability of bacteriochlorophyll a and derivatives: Potential sensitizers for photodynamic tumor therapy. *Photochem Photobiol*. 2006; 82:770–780. [PubMed: 16438618]
30. Birks, JB. *Photophysics of Aromatic Molecules*. Wiley-Interscience; London: 1970. p. 492-517.
31. Fajer, J.; Davis, MS. Electron spin resonance of porphyrin π cations and anions. In: Dolphin, D., editor. *The Porphyrins*. Vol. IV. Academic Press; New York: 1978. p. 197-256.
32. Kee HL, Kirmaier C, Tang Q, Diers JR, Muthiah C, Taniguchi M, Laha JK, Ptaszek M, Lindsey JS, Bocian DF, Holten D. Effects of substituents on synthetic analogs of chlorophylls. Part 2: Redox properties, optical spectra and electronic structure. *Photochem Photobiol*. 2007; 83:1125–1143. [PubMed: 17880507]
33. Posen Y, Kalchenko V, Seger R, Brandis A, Scherz A, Salomon Y. Manipulation of redox signaling in mammalian cells enabled by controlled photogeneration of reactive oxygen species. *J Cell Sci*. 2005; 118:1957–1969. [PubMed: 15840654]
34. Gouterman, M. Optical spectra and electronic structure of porphyrins and related rings. In: Dolphin, D., editor. *The Porphyrins*. Vol. III. Academic Press; New York: 1978. p. 1-165.
35. Felton, RH. Primary redox reactions of metalloporphyrins. In: Dolphin, D., editor. *The Porphyrins*. Vol. V. Academic Press; New York: 1978. p. 53-125.
36. Mass O, Taniguchi M, Ptaszek M, Springer JW, Faries KM, Diers JR, Bocian DF, Holten D, Lindsey JS. Structural characteristics that make chlorophylls green: Interplay of hydrocarbon skeleton and substituents. *New J Chem*. 2011; 35:76–88.
37. Kraye M, Yang E, Diers JR, Bocian DF, Holten D, Lindsey JS. *De novo* synthesis and photophysical characterization of annulated bacteriochlorins. Mimicking and extending the properties of bacteriochlorophylls. *New J Chem*. 2011; 35:587–601.
38. Springer JW, Faries KM, Diers JR, Muthia C, Mass O, Kee HL, Kirmaier C, Lindsey JS, Bocian DF, Holten D. The effects of substituents on synthetic analogs of chlorophylls. Part 3: The distinctive impact of auxochromes at the 7- versus 3-positions. *Photochem Photobiol*. 2012; 88:651–674. [PubMed: 22248176]

Figure 1. Absorption spectra (solid) and fluorescence spectra (dashed) of bacteriochlorins in toluene: **BC** (black), **(NC)₂BC** (green), **(NC)₂BC-Zn** (blue), **(NC)₂BC-Pd** (red). Panel A shows the full near-UV to NIR absorption spectrum. Panel B focuses on the NIR (Q_y) region of the absorption spectra and the companion fluorescence spectra.

Figure 2.

Emission spectrum of $(\text{NC})_2\text{BC-Pd}$ in 2-methyltetrahydrofuran. The band at 747 nm is fluorescence and the band at 1122 nm is phosphorescence. The spectra were acquired using excitation in the Soret band (374 nm). The phosphorescence was also observed using excitation in the Q_y band (740 nm).

Figure 3.

Photobleaching studies showing the absorption spectrum of bacteriochlorins in media containing ambient O_2 as a function of the fluence of the light delivered at the Q_y maximum (720–750 nm). The following fluences (J/cm^2) are shown in each panel: 0 (black), 5 (red), 25 (blue), 50 (green) and 100 (dark yellow). The insets to panels E and F show the corresponding fluorescence spectra elicited by excitation in the Q_x band (480–530 nm) for the same fluences used during acquisition of the absorption spectra.

Figure 4.

Photostability of bacteriochlorins and standard PDT agents in various media in the presence (closed symbols) or absence (open symbols) of atmospheric O_2 . The absorbance at the maximum of the NIR $Q_y(0,0)$ absorption band (720–750 nm) relative to that prior to illumination is plotted as a function of the fluence of incident light in that band. The panels are organized as follows: (A) unsubstituted and dicyanobacteriochlorins in CrEL and DMA, (B) **B16** and **B17** in CrEL, DMA and DMSO, (C) **BC** in various media, (D) **B31** in various media, (E) **B22**, **B29** and **B31** in CrEL, (F) standard PDT agents. The data for the bacteriochlorins are colorized as follows: $(NC)_2BC-Pd$ (red), $(NC)_2BC-Zn$ (purple), $(NC)_2BC$ (blue), **BC** (green), **B16** (magenta), **B17** (dark cyan), **B22** (light magenta), **B29** (wine), **B31** (navy blue). The data for the standard PDT agents are colorized as follows: Photofrin (orange), Lutex (violet), benzporphyrin derivative (BPD, sky blue). The symbols for the media are as follows: CrEL (circles), *N,N*-dimethylacetamide (DMA, squares), dimethylsulfoxide (DMSO, stars), acetonitrile (MeCN, down triangles), MeCN/H₂O = 50/50 (pentagons), ethanol (EtOH, diamonds), methanol (MeOH, diamonds), toluene (up triangles), acetone (right triangles), benzonitrile (PhCN, down triangles), H₂O (left triangles).

Figure 5.

Photobleaching studies in aqueous micellar solutions (CrEL) of **B16** with ambient O₂ (A), **B16** with no O₂ (B), and **BC** with no O₂ (C) showing the absorption spectrum as a function of light fluence (J/cm^2) in the Q_y band (~720 nm). The insets to each panel show expanded views of the 400–600 nm region. The absorbance scale is 0–0.8 in each panel.

Figure 6. Photobleaching studies of **B16** in *N,N*-dimethylacetamide (DMA) with ambient O_2 showing the absorption spectra (A) and fluorescence spectra using excitation at the 490-nm Q_x -band maximum (B) or at 376 nm on the long-wavelength side of the Soret band (C) as a function of light fluence (J/cm^2) in the Q_y band (720 nm): 0 (black), 5 (red), 25 (blue), 50 (green), 75 (purple) and 100 (dark yellow). The insets to each panel show expanded views of certain regions of each spectrum. The absorbance scale is 0–0.6 in panel (A).

Figure 7.

Electron densities and energies of frontier molecular orbitals. The DFT calculations employed basis sets 6-31G* for **BC**, **(NC)₂BC**, and **(NC)₂BC-Zn** and LACVP* for **(NC)₂BC-Pd**. Effectively the same electron densities and the same MO energies (-5.74, -5.09, -3.09, -1.45 eV) were obtained for **(NC)₂BC-Zn** using LACVP*. The LUMO+2 for **(NC)₂BC-Pd** has similar macrocycle electron density and energy (-1.41 eV) as the LUMO +1 for the other molecules.

Figure 8.

Electronic properties versus cell-killing LD50 value for bacteriochlorins using direct dilution of stock solution into complete culture medium. The data points for increasing LD50 (left to right) reflecting a decrease in photosensitizer activity are $(NC)_2BC-Pd$ (a) > $(NC)_2BC$ (b) > $(NC)_2BC-Zn$ (c) > BC (d). (A) Calculated HOMO energy (open triangles) and LUMO energy (closed triangles). (B) Redox potential of the T_1 excited state (Table 3). The lines in both panels reflect the linear fits to the data.

WST9 **WST11** **TDCPBS****Chart 1.**

Representative native bacteriochlorin derivatives (**WST9** = Tookad, **WST11**) (20,21) and a synthetic bacteriochlorin (**TDCPBS**) (27,28) that have been examined in PDT studies.

Chart 2.
Representative synthetic bacteriochlorins examined previously for PDT activity (5–7).

Chart 3.
Imidazole-substituted porphyrins examined previously for PDT activity (9,10).

BC **(NC)₂BC** (M = H, H)**(NC)₂BC-Zn** (M = Zn)**(NC)₂BC-Pd** (M = Pd)**Chart 4.**

Synthetic bacteriochlorins examined recently for PDT activity (11).

B1 (M = H, H)**B4** (M = Zn)**B9** (M = Pd) **B88** (M = H, H)**B84** (M = Zn)**B87** (M = Pd) **Chart 5.**

Two sets of synthetic bacteriochlorins prepared and studied recently (19).

Table 1

PDT activity, photostability and photophysical properties of the bacteriochlorins.^a

Compound	LD50 dd (nM)	LD50 CrEL (nM)	Uptake dd (nmol/mg)	Uptake CrEL (nmol/mg)	Efficacy/uptake dd ^b	Efficacy/uptake CrEL ^b	Photostability DMA ^c	Photostability CrEL ^c	Photostability CrEL no O ₂ ^c	Φ _T	τ _S (ns)	Φ _T	τ _T (μs)
<i>Unsubstituted & dicyano BCs</i>													
BC	1800	350	5.3	3.7	1.0	7.3	0.13	0.11	0.54	0.14	4.0	0.62	169
(NC) ₂ BC	60	25	4.3	3.3	37.1	114.3	0.91	0.90		0.15	4.1	0.43	84
(NC) ₂ BC-Zn	1000	60	2.1	2.1	4.5	76.4	0.52	0.04	0.67	0.15	3.9	0.63	121
(NC) ₂ BC-Pd	25	18	4.8	2.3	79.6	227.2	0.83	0.94		0.007	0.023	0.99	7
<i>Representative prior studied BCs</i>													
B16	100		11.7		8.1		0.02	0.01	0.56	0.11	3.6	0.40	190
B17	80		13.5		8.8		0.09	0.21	0.71	0.08	3.0	0.56	198
B19	15		0.001		632400					0.15	3.0	0.55	159
B22	3000		7.7		0.4		0.60	0.11 ^d	0.76	0.11	4.0	0.48	77
B29	100		8.2		11.6		0.09	0.11 ^d	0.78	0.12	3.8	0.53	190
B31	800		1.8		6.6		0.02	0.05 ^d	0.73	0.09	3.5	0.53	54

^a All measured quantities were obtained at room temperature. Data for **B16-B31** are taken from refs (5), (6), and (16). LD50 and uptake data for the unsubstituted and dicyano bacteriochlorins are taken from ref (11). For the LD50, uptake, and efficacy/uptake values, “dd” reflects direct dilution of the DMA stock solution of the photosensitizer into complete medium and CrEL reflects dilution of the micellar solution into complete medium. Measurements of the fluorescence yields (Φ_F), singlet excited state lifetimes (τ_S) and triplet yields (Φ_T) employed Ar-purged toluene solutions and of the triplet lifetimes (τ_T) Ar-purged THF solutions.

^b Calculated by dividing the reciprocal of the LD50 by the uptake value and normalizing to the value for **BC** dd.

^c Photostability as measured by the percentage of the initial Q_y absorbance remaining after 100 J/cm² fluence of light given at that wavelength.

^d The values are reached at 50 J/cm² fluence.

Table 2

Absorption and fluorescence properties of dicyanobacteriochlorins.^a

Cmpd	Solvent	B _y abs (nm)	B _x abs (nm)	Q _x abs (nm)	Q _y abs (nm)	Q _y em (nm)	I ⁰ ₅ /I ₆	Φ _s	τ _s (ns)
BC	Tol	340	365	489	713	716	0.85	0.14	4.0
	MeOH	335	359	485	708	712	0.72	0.095	4.1
(NC) ₂ BC	Tol	347	372	515	748	752	1.3	0.15	4.1
	MeOH	342	367	510	743	747	1.3	0.095	4.1
(NC) ₂ BC-Zn	Tol	343	380	546	761	763	1.8	0.15	3.9
	MeOH	341	374	553	752	757	1.9	0.15	4.1
(NC) ₂ BC-Pd	Tol	326	374	518	751	753	3.4	0.007	0.023
	MeOH	323	371	514	744	749	2.8	0.007	

^a All measurements were made at room temperature.

Table 3

Spectral, state-energy, redox, and molecular-orbital properties of the bacteriochlorins.^a

Compound	Q _y ^b abs (nm)	Q _y ^c flu (nm)	T ₁ phos (nm)	S ₁ ^d energy (eV)	T ₁ ^e energy (eV)	S ₁ -T ₁ energy (eV)	S ₀ Redox ^f Potentials		T ₁ Redox ^g Potentials		Orbital ^h Energy		
							E _{ox} (V)	E _{red} (V)	T ₁ E _{ox} (V)	T ₁ E _{red} (V)	HOMO (eV)	LUMO (eV)	
DicyanoBC set													
BC	713	716		1.74		1.20	+0.09	-1.67	-1.11	-0.47	-4.45	-2.20	
(NC) ₂ BC	748	752		1.65		1.11	+0.60	-1.10	-0.51	+0.01	-5.22	-3.10	
(NC) ₂ BC-Zn	761	763		1.63		1.09	+0.31	-1.14	-0.78	-0.05	-5.08	-3.07	
(NC) ₂ BC-Pd	751	753	1122	1.65		1.11	+0.52	-1.03	-0.59	+0.08	-5.16	-3.11	
Other FbBC's													
B16	717	722		1.72							-4.36	-2.12	
B17	731	737		1.69							-4.46	-2.28	
RefBC-T set													
B1 (Fb)	736	742		1.68		1.12	+0.21	-1.49	-0.91	-0.37	-4.40	-2.22	
B4 (Zn)	749	756		1.65		1.09	-0.04	-1.60	-1.13	-0.51	-4.26	-2.20	
B9 (Pd)	739	745	1114	1.67		1.11	+0.43	-1.14	-0.68	-0.03	-4.36	-2.26	
RefBC-MME set													
B88 (Fb)	758	765		1.63		1.11	+0.38	-1.29	-0.73	-0.18	-4.65	-2.48	
B84 (Zn)	773	780		1.60		1.08	+0.08	-1.41	-1.00	-0.33	-4.55	-2.51	
B87 (Pd)	758	765	1118	1.63		1.11	+0.29	-1.29	-0.82	-0.18	-4.63	-2.54	
Imidazole porphyrins													
1-Zn	576	579	738	2.14		1.68					-4.97	-2.35	
2-Zn	576	579	738	2.14		1.68	+0.36	-1.39	-1.32	+0.29	-5.72 ⁱ	-2.81 ⁱ	
2-Pd	547	550	672	2.25		1.84					-5.81 ^j	-2.83 ^j	
Ref porphyrins													
FbTPP	647	650		1.91		1.47 ^k	+0.83	-1.45	-0.64	+0.02	-4.90	-2.20	
ZnTPP	588	599	784	2.08		1.58	+0.56	-1.58	-1.02	0	-4.96	-2.12	
PdTPP	553	560	696	2.21		1.78	+0.82	-1.61	-0.96	+0.17	-5.36	-2.42	

^aProperties of bacteriochlorins. All measured quantities were obtained at room temperature.

^bPeak wavelength of the Q_y(0,0) absorption band in toluene.

^cPeak wavelength of the Q_y(0,0) fluorescence band in toluene.

^dEnergy of the S₁ excited state calculated from the average energy positions of the Q_y(0,0) absorption and fluorescence bands in the prior two columns.

^eEnergy of the T₁ excited state determined by phosphorescence for values indicated in normal font. The values in italics for the free base and zinc bacteriochlorins were obtained from the S₁–T₁ energy gap as in the palladium analogue.

^fGround-state redox potentials measured in butyronitrile/0.1 M *n*-BuN₄PF₆ versus FeCp₂⁺/FeCp₂⁺ = +0.19 V.

^gRedox potentials for the T₁ excited state calculated from the ground state redox potentials and the T₁ energy.

^hFrontier molecular orbital energies from DFT calculations obtained using the 6-31G* basis set except for (NC)**2BC-Pd**, for which the LACVP basis set was used. For **PdTPP** and **ZnTPP** the values listed are for the C₂ symmetry structures, which are slightly lower in energy than the C₄ symmetry structures.

ⁱThe HOMO and LUMO energies listed for **2-Zn** were calculated using the actual iodide counterion; for comparison, the values calculated using a chloride counterion are –5.58 and –2.67 eV, respectively.

^jThe HOMO and LUMO energies listed for **2-Pd** were calculated using the actual iodide counterion; for comparison, the values calculated using a chloride counterion are –5.67 and –2.68 eV, respectively.

^kFrom refs (22) and (34).