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Synthetic bacteriochlorins bearing polar motifs (carboxylate, phosphonate, ammonium and a short PEG). Water-solubilization, bioconjugation, and photophysical properties†

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Bacteriochlorins are potentially excellent chromophores for near-infrared (NIR) photochemical and spectroscopic studies yet the intrinsically hydrophobic macrocycle core has stymied work in aqueous media. Herein, a set of bacteriochlorins bearing distinct polar motifs is reported. The motifs include phosphonate (pH-dependent anionic, **BC1**), carboxylate (pH-dependent anionic, **BC2**), ammonium (permanently cationic) without (**BC3**) or with (**BC4**) a linker ester moiety, and tetraethyleneoxy (a short PEG, polar non-ionic, **BC5**). The groups are located at the 3,5-positions of each of two aryl groups at the bacteriochlorin 3,13-sites. Synthesis of the bacteriochlorins entails the Suzuki coupling of a common 3,13-dibromobacteriochlorin building block with a set of aryl boronates. Five factors were selected for comparisons among the polar motifs upon attachment to the bacteriochlorins: (1) synthesis yield and ease of purification, (2) amenability toward subsequent derivatization, (3) water-solubility, (4) full-width-at-half-maximum (fwhm) of the long-wavelength (Q_y) absorption and fluorescence bands, singlet excited-state lifetime (τ_S) and fluorescence quantum yield (Φ_f), and (5) stability in the dark or under illumination. Water-solubility was assessed by examination of the absorption spectra across a 1000-fold concentration range (~0.2–0.6 μM to ~200–600 μM). With the exception of **BC4**, all displayed good aqueous solubility, photostability, and photophysical properties in aqueous solution (fwhm = 23–31 nm, Φ_f = 0.10–0.16, τ_S = 1.9–2.7 ns). The modestly lower Φ_f and τ_S values for the bacteriochlorins in aqueous *versus* organic (*N*,*N*-dimethylformamide) media are traced to an increased rate constant for excited-state internal conversion. Upon consideration of all factors, the ammonium (short linker) and short PEG groups were most attractive for solubilization of the bacteriochlorins in aqueous media. The studies prompted the synthesis of two water-soluble (ammonium-substituted) bacteriochlorins bearing *N*-hydroxysuccinimide esters.

Introduction

Methods for the synthesis of hydrophilic tetrapyrroles provide an entrée into diverse areas of scientific investigation ranging from energy sciences to photomedicine. A generic design of hydrophilic tetrapyrroles is to introduce polar groups at synthetically accessible positions about the perimeter of the macrocycle.[1–5] Representative polar groups include those that are ionic, such as ammonium,[3,6–11] sulfonate,[2,12–14] carboxylate[15,16] and phosphonate,[17,18] as well as those that are nonionic, such as glycoside[19,20] or polyethylene glycol.[21–24] While there are numerous reports concerning the synthesis of hydrophilic tetrapyrroles and their use in aqueous media,[1–5] as in photodynamic therapy or biomedical imaging, most such reports describe one or a few target compounds of similar structure. Independent studies of water solubility are rarely performed, and the absence of comparative studies of distinct motifs across a common molecular architecture precludes meaningful conclusions about relative merits.

Our interest in preparing hydrophilic tetrapyrroles stems first of all from our objectives in energy sciences, where such

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† Electronic supplementary information (ESI) available: Attempted synthesis of a phosphatidylcholine bacteriochlorin (**BC6**); mass spectrometry analysis for **BC1–BC5**, **BC7**, and **BC8**; and additional data concerning the photophysical properties of bacteriochlorins. See DOI: 10.1039/c5nj00759c

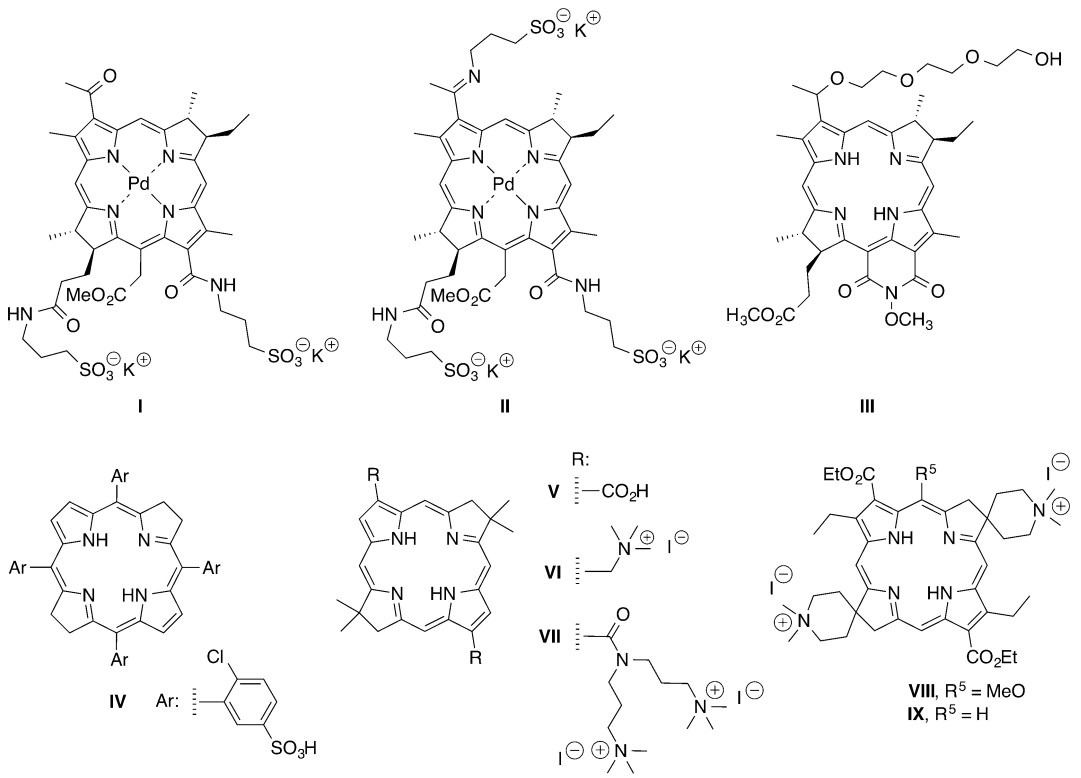


Chart 1 Representative hydrophilic bacteriochlorins.

macrocycles can be incorporated with peptides to give self-assembled light-harvesting architectures.[25,26] The tetrapyrroles of present interest are bacteriochlorins, which absorb strongly in the near-infrared (NIR) region. A second objective – a spinoff thereof – aims to use hydrophilic bacteriochlorins in biomedical applications. A variety of bacteriochlorins with appended polar motifs has been previously prepared; representative members are shown in Chart 1. Compounds **I**,[27] **II**[27] and **III**[28] were derived by semisynthesis[29,30] from bacteriochlorophyll *a*; **IV**[13] by hydrogenation of the corresponding porphyrin;[31] and **V–IX** by *de novo* synthesis.[32,33] Methods of bacteriochlorin synthesis have been reviewed.[34,35]

An attractive design element for solubilization of tetrapyrrole macrocycles entails installation of groups that project above and below the plane of the π-chromophore. The groups can be hydrophobic to enhance solubility in nonpolar organic solvents, or quite polar to enhance solubility in polar or even aqueous media. One motif for facial encumbrance employs a 2,6-disubsituted aryl unit; the steric bulk of the 2,6-substituents suppresses rotation[36] of the aryl group toward coplanarity with the tetrapyrrole macrocycle, thereby thrusting the 2,6-substituents above and below the tetrapyrrole plane. Such a "facial encumbrance" design feature has been employed extensively with porphyrins,[37,38] but to much lesser extent with chlorins, and hardly at all with bacteriochlorins. A 2,6-disubstituted arene for aqueous solubilization was employed with synthetic chlorins (Chart 2),[39] albeit with some difficulty,[40] and the synthetic approach is not yet applicable with bacteriochlorins.

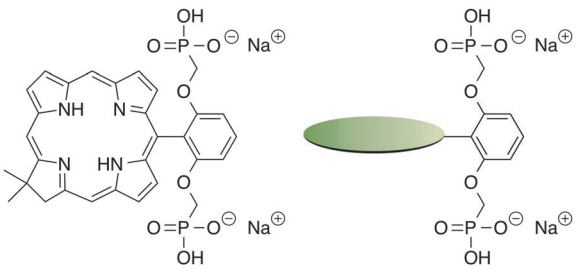


Chart 2 A facially encumbered chlorin bearing a 2,6-disubstituted aryl motif (line drawing at left; perspective illustration at right).[39]

Given the present challenge in constructing bacteriochlorins that bear synthetically malleable 2,6-disubstituted aryl units (mesityl groups have been installed in one instance[41]), we resorted to the use of 3,5-disubstituted aryl units. A 3,5-disubstituted arene is expected to have greater conformational motion about the carbon–carbon single bond that joins the bacteriochlorin, enabling the polar motifs to sweep out lateral conformations *versus* the more constrained motions of the 2,6-disubstituted arenes. Unlike 2,6-disubstituted arenes, 3,5-disubstituted aryl units are readily installed *via* Suzuki coupling reactions.[40] Nine such bacteriochlorins have been prepared; representative examples are shown in Chart 3. Eight bacteriochlorins include 3,5-dicarboxyphenyl groups at the 2,12-positions (*e.g.*, **X**, **XI**) or 3,13-positions (*e.g.*, **XII**) of the macrocycle,[42] whereas one contains 3,5-bis(PEG)aryl groups attached to the bacteriochlorin 15-position (**XIII**).[43] (We employ the term "PEG" here to describe a tetraethyleneoxy unit

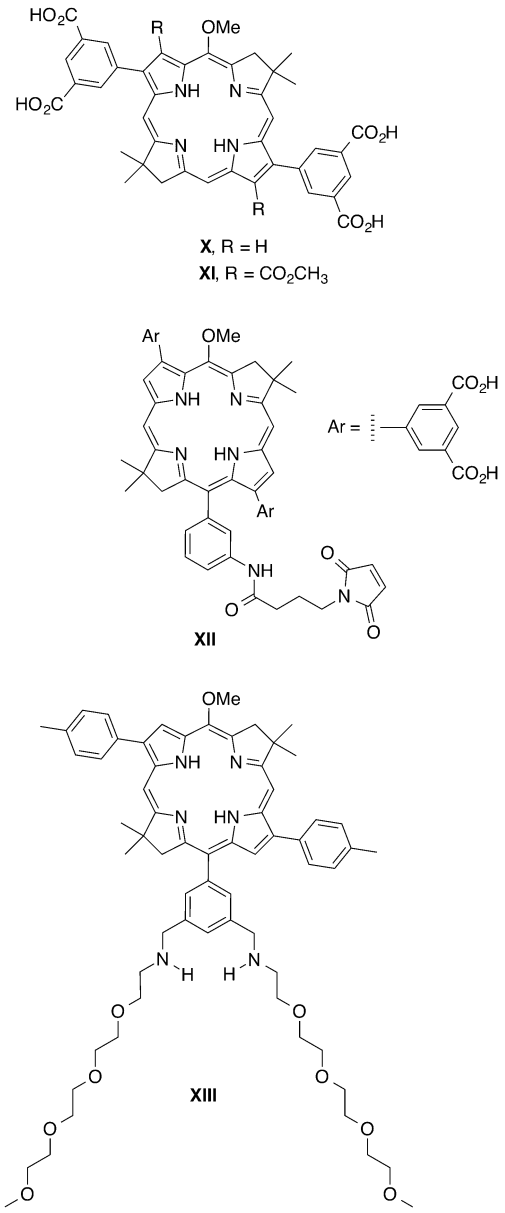


Chart 3 Representative hydrophilic bacteriochlorins with 3,5-disubstituted aryl motifs.

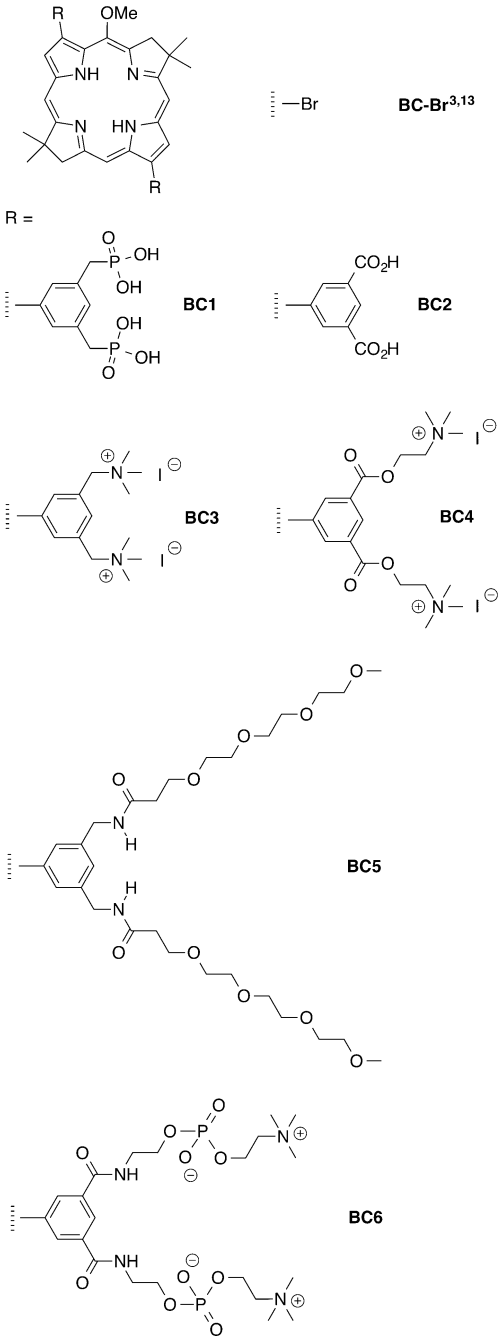


Chart 4 Bacteriochlorin scaffold and distinct polar groups.

given the widespread usage of this term.) Five of the bacteriochlorins contain a bioconjugatable tether, for which **XII** is representative.[42]

To gain a better understanding of the virtues and limitations of the various types of polar motifs for water-solubilization, we prepared a set of bacteriochlorins (**BC1**–**BC5**) encompassing phosphonate (**BC1**), carboxylate (**BC2**), ammonium (for **BC3** and **BC4**), or PEG (**BC5**) moieties (Chart 4). The reaction to incorporate phosphatidylcholine moieties (**BC6**) was incomplete. Bacteriochlorins **BC3** and **BC4** both bear four ammonium groups, but differ in that the former is more compact and has benzylammonium units, whereas the latter contains alkylammonium groups attached *via* ester moieties. Each bacteriochlorin in the set contains a common scaffold and was derived by Suzuki coupling with a known[44] 3,13-dibromobacteriochlorin (**BC-Br**^{3,13}).

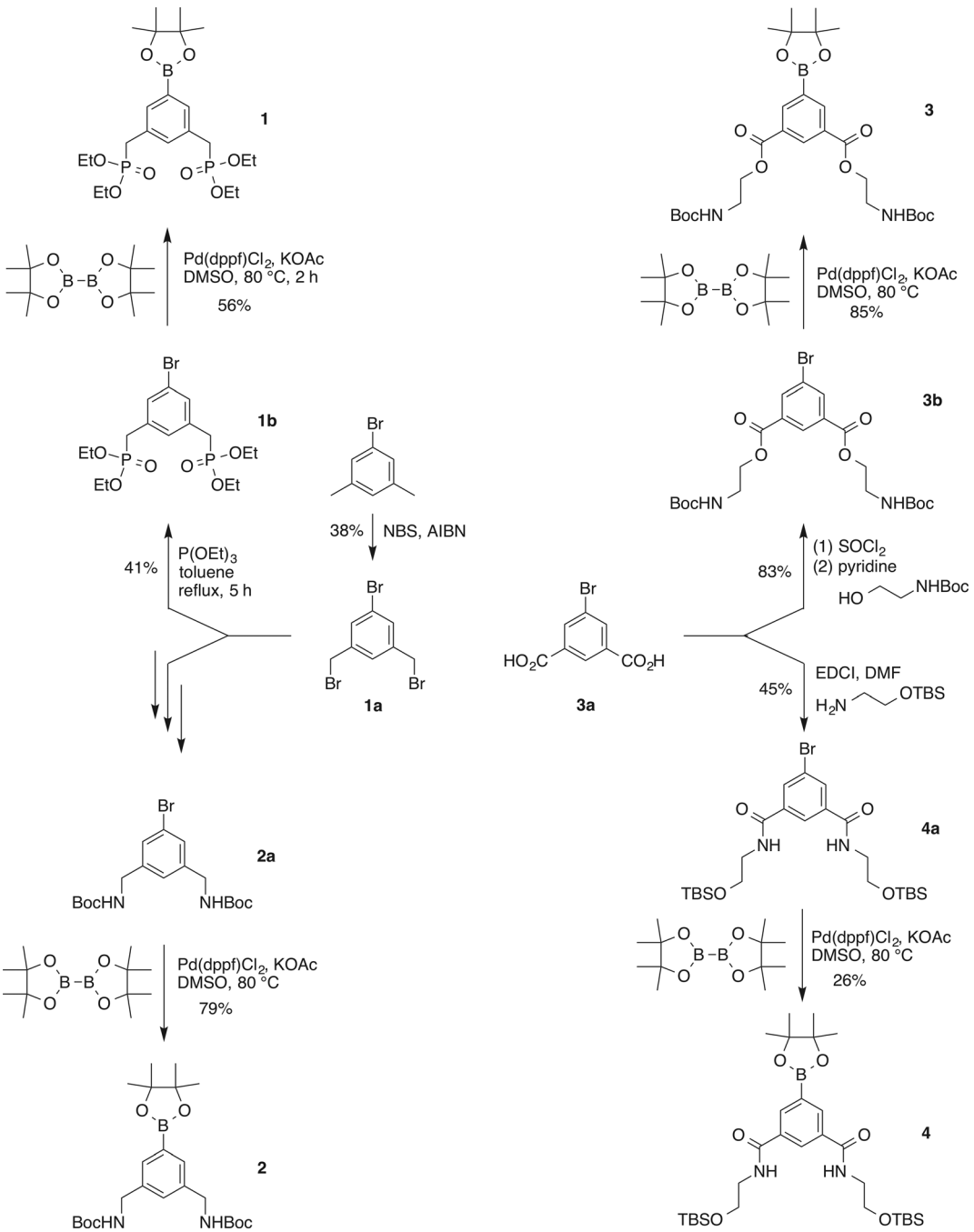
Herein, the synthesis of bacteriochlorins **BC1** and **BC3**–**BC5** are reported; the synthesis of **BC2** was described earlier.[42] The five bacteriochlorins are employed in a comprehensive comparison regarding synthetic amenability, photophysical properties [absorption/fluorescence bandwidths, fluorescence yield (Φ_f), singlet excited-state lifetime (τ_S)], stability and ease of derivatization. The derivatization process of interest entails selective bromination at the 15-position (enabled by the distal 5-methoxy group)[43] followed by Suzuki coupling to install a bioconjugatable tether.

The synthetic strategy, evaluation methods, and results obtained should be applicable across the tetrapyrrole family of macrocycles.

Results

I. Synthesis

A. Suzuki coupling partners. The synthesis of all the bacteriochlorins entails Suzuki coupling of the dibromobacteriochlorin[44] **BC-Br**[3,13] with an aryl boronate ester. Four Suzuki coupling partners were synthesized as is shown in Scheme 1. 1-Bromo-3,5-dimethylbenzene was dibrominated with *N*-bromosuccinimide (NBS) in the presence of azobis(isobutyronitrile) (AIBN) to give the known 1-bromo-3,5-bis(bromomethyl)benzene (**1a**),[45] which was prepared here at 14-fold larger scale, isolated without chromatography, and fully characterized. Treatment of **1a** with triethyl phosphite in toluene afforded the corresponding phosphonate **1b** in 41% yield. Pd-mediated coupling[46] of **1b** with bis(pinacolato)diboron gave diethyl phosphonate **1** in 56% yield. Pd-mediated coupling of the known *tert*-butoxycarbonyl-protected



Scheme 1 Synthesis of Suzuki coupling partners.

3,5-bis(aminomethyl)bromobenzene **2a** (derived from **1a**)[47] with bis(pinacolato)diboron gave the Boc-protected Suzuki coupling partner **2** in 79% yield. The Suzuki coupling reactions were carried out with the palladium dichloride reagent containing a 1,1′-bis(diphenylphosphino)ferrocene (dppf) ligand in the presence of dimethylsulfoxide (DMSO).

Treatment of commercially available 5-bromoisophthalic acid (**3a**) with thionyl chloride afforded 5-bromoisophthaloyl dichloride, which was directly treated with *N*-(*tert*-butoxycarbonyl)-ethanolamine in pyridine at 0 °C to give the Boc-protected 3,5-diester-5-bromobenzene **3b** in 83% yield for two steps. Pd-mediated coupling of **3b** with bis(pinacolato)diboron gave Suzuki coupling partner **3** in 85% yield.

5-Bromoisophthalic acid (**3a**) and 2-(*tert*-butyldimethylsiloxy)ethylamine[48] were combined in the presence of 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide methiodide (EDCI) to afford the amide product **4a** in 45% yield. Pd-mediated coupling of **4a** with bis(pinacolato)diboron gave Suzuki coupling partner **4** in 26% yield. This relative low coupling yield is presumably due to removal of the *tert*-butyldimethylsilyl (TBS) group under basic conditions at high temperature (80 °C).

B. Hydrophilic bacteriochlorins. The synthesis of the set of hydrophilic bacteriochlorins relied on the following strategy: (1) introduction of the protected polar motif by Suzuki coupling reaction of **BC-Br**[3,13] with the above-mentioned coupling partners **1–4**; and (2) removal of the protecting group to give the hydrophilic bacteriochlorins directly (for **BC1** and **BC2**), or followed by quaternization (for **BC3** and **BC4**) or PEGylation (for **BC5**).

Suzuki coupling[49] of **BC-Br**[3,13] with **1** gave the protected phosphono bacteriochlorin **BC1a** in 81% yield. The ethyl protecting groups were removed by treatment with 80 equiv. of bromotrimethylsilane (TMSBr)[18,39] in $CHCl_3$ followed by hydrolysis in methanol and water (Scheme 2). In this manner, **BC1** was obtained from the respective protected counterparts in 90% yield. The chemoselectivity and essentially quantitative nature of the protecting group cleavage reactions enabled the resulting bacteriochlorin **BC1** to be characterized and used directly without purification.

Suzuki reaction with coupling partner **2** gave diarylbacteriochlorin **BC3a**, which upon exposure to trifluoroacetic acid (TFA) in CH_2Cl_2 gave bacteriochlorin **BC3b** in 78% yield (Scheme 2). The tetraaminobacteriochlorin provided a common precursor to both **BC3** and **BC5**. Quaternization with iodomethane in the presence of tributylamine (Bu_3N) gave ammoniobacteriochlorin **BC3** in 81% yield. Bu_3N serves to (i) neutralize the protonated tetraamine derived from TFA, and (ii) sponge up the alkylation byproduct HI.[50] Bu_3N was chosen *versus* triethylamine because the protonated form of the former is more easily removed upon washing with tetrahydrofuran (THF). Bacteriochlorin **BC3b** also was subjected to PEGylation to give bacteriochlorin **BC5** (to be reported elsewhere). Suzuki reaction with **3** gave the 3,13-bis-(aryl-3,5-diester)bacteriochlorin **BC4a** in 79% yield. Following the same approach as with **BC3a**, deprotection of **BC4a** and subsequent quaternization afforded **BC4** in 86% and 75% yield for the two sequential steps.

We also attempted to prepare a bacteriochlorin that bears phosphatidylcholine substituents as shown in Scheme 2. Suzuki coupling reaction of **BC-Br**[3,13] with compound **4** afforded the TBS-protected tetrahydroxy-bacteriochlorin (**BC6a**) in 81% yield. Treatment of **BC6a** with tetrabutylammonium fluoride (TBAF) unveiled all four hydroxy groups (**BC6b**) but attempts to incorporate the 1,3,2-dioxaphospholane 2-oxide unit gave incomplete reaction on the path to the desired compound **BC6** (see ESI,† Fig. S1). Alternative reaction with phosphorylcholine dichloride[51] did not lead to the target bacteriochlorin **BC6** (see ESI†).

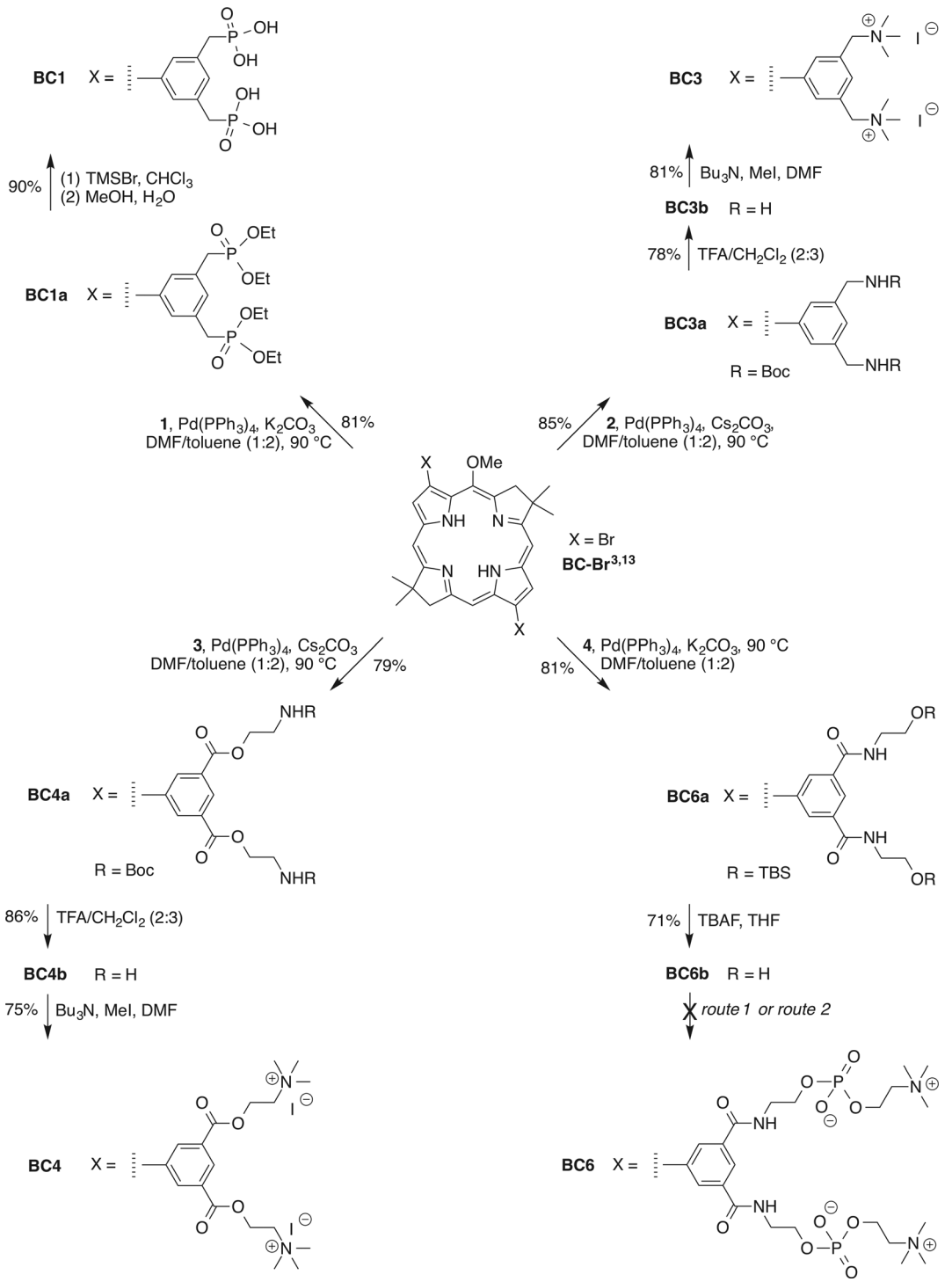
C. Characterization. The bacteriochlorins typically were characterized by absorption and fluorescence spectroscopy, ^{1}H NMR spectroscopy, ^{13}C NMR spectroscopy (where quantity and solubility allowed), ^{31}P NMR spectroscopy (where applicable), matrix-assisted laser desorption ionization mass spectrometry (MALDI-MS), and electrospray ionization mass spectrometry (ESI-MS). Exceptions include **BC1**, for which MALDI-MS spectra could not be obtained, but each gave a clean ^{1}H NMR spectrum and characteristic bacteriochlorin absorption and emission spectra. The observed charge state for the various target bacteriochlorins upon ESI-MS analysis ranged from 1–4 (see Table S1 in ESI†).

II. Incorporation of a bioconjugatable tether

A. 15-Bromination study. A potential application of hydrophilic bacteriochlorins entails the introduction of a conjugatable tether for attachment to proteins or surfaces. One strategy in this regard relies on bromination at the 15-position of the macrocycle,[43] which is known to occur with a high degree of regioselectivity for many but not all 5-methoxybacteriochlorins. The known examples of 15-bromination of 5-methoxybacteriochlorins bearing diverse substituents at the β-positions (2, 12, 3, 13 positions) have been summarized.[42] Here, bromination was carried out for a set of 3,13-diarylbacteriochlorins to gauge their suitability for subsequent installation of a bioconjugatable tether. The results are shown in Table 1.

The parent 5-methoxybacteriochlorin bearing phenyl groups at the 3,13-positions (**BC-Ph**[3,13]) smoothly gave the 15-bromobacteriochlorin as did **BC2a**, both of which have been examined previously.[42] **BC1a** under similar or somewhat more forcing bromination conditions (50 °C, 12 h) afforded only a trace of the desired product **BC1a-Br** (detected by MALDI-MS), whereas the majority was unreacted material. **BC3a** exhibited better activity and gave an almost 1 : 1 mixture of brominated product **BC3a-Br** and starting material, as measured by MALDI-MS assuming equal ionization efficiencies of these two species; however, the reaction mixture could not be separated. Both **BC4a** and **BC6a** showed similar activities to that of **BC2a**, and the desired 15-bromobacteriochlorins **BC4a-Br** and **BC6a-Br** were isolated in good yields.

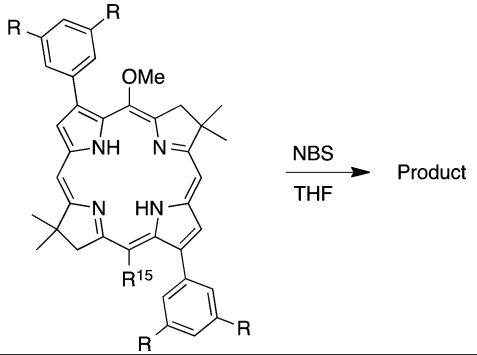
B. Bioconjugatable bacteriochlorins. Bacteriochlorins **BC3** and **BC4** were selected for further elaboration (*via* **BC3a-Br** and **BC4a-Br**) to the bioconjugatable bacteriochlorins **BC7** and **BC8**, respectively. Each target bacteriochlorin bears an NHS ester as the bioconjugatable tether with potential application for amino-protein labeling. The syntheses are displayed in Scheme 3.

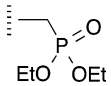
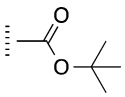
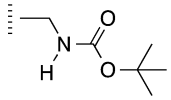
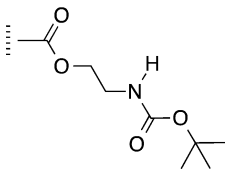
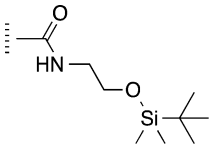


Scheme 2 Diversification of dibromobacteriochlorin **BC-Br**[3,13].

The mixture obtained upon bromination of bacteriochlorin **BC3a** with NBS (estimated 1 : 1 ratio of **BC3a-Br** and **BC3a**, see Table 1) could not be separated by column chromatography and was used directly in the Suzuki coupling reaction. Thus, reaction with known partner **5**[52] gave the corresponding 3,13-diaryl-15-(carboxyalkylaryl)bacteriochlorin (**BC7a**) in 31% yield for two steps. The four Boc and one *tert*-butyl groups were removed upon treatment with 40% TFA in CH_2Cl_2 to yield **BC7b** in 99% yield. The quaternization of bacteriochlorin **BC7b** with excess methyl iodide in *N*,*N*-dimethylformamide (DMF) containing tributylamine[50] at room temperature for 24 h afforded the ammoniobacteriochlorin **BC7c** in 73% yield. The cationic bacteriochlorin was precipitated by addition of diethyl ether, following by washing the crude precipitate with THF and

Table 1 Bromination of 3,13-diarylbacteriochlorins[a]



Compound	R (R^{15} = H)	Product (R^{15} = Br)	Yield (%)
BC-Ph^{3,13}		**BC-Ph^{3,13}-Br**	54[b]
BC1a		**BC1a-Br**	Trace
BC2a		**BC2a-Br**	70[b]
BC3a		**BC3a-Br**	50[c]
BC4a		**BC4a-Br**	76
BC6a		**BC6a-Br**	70

[a] Reactions were done at 0.5 mM in THF at room temperature with 1–1.1 equiv. of NBS for 15–60 min. [b] Ref. 42. [c] Not isolated. Estimated to consist of **BC3a** and **BC3a-Br** in 1 : 1 ratio by MALDI-MS assuming equal desorption and ionization efficiencies.

diethyl ether. Coupling of the carboxylic acid of **BC7c** and *N*-hydroxysuccinimide (HOSu) in the presence of *N*,*N*′-dicyclohexylcarbodiimide (DCC) and 4-(dimethylamino)pyridine (DMAP) afforded **BC7** in 80% yield (~90% purity). The same strategy (Suzuki coupling reaction at the 15-position, deprotection to remove the Boc group, introduction of the ammonium group, and NHS ester formation) was applied to **BC4a-Br** to afford the final target bacteriochlorin NHS ester **BC8**.

Given the excellent results observed with the ammonium-substituted bacteriochlorins, comparable if not better results were anticipated with the phosphatidylcholine-substituted bacteriochlorin. Hence, in parallel with the studies of **BC6a** → **BC6b** → **BC6**, bacteriochlorin **BC6a-Br** also was coupled with Suzuki partner **5** to give the product **BC9a** (39% yield; see ESI,† Scheme S2); however, the (surprising) difficulty in fully installing the phosphatidylcholine units (to form **BC6**) led this promising route to be discontinued.

III. Photophysical properties

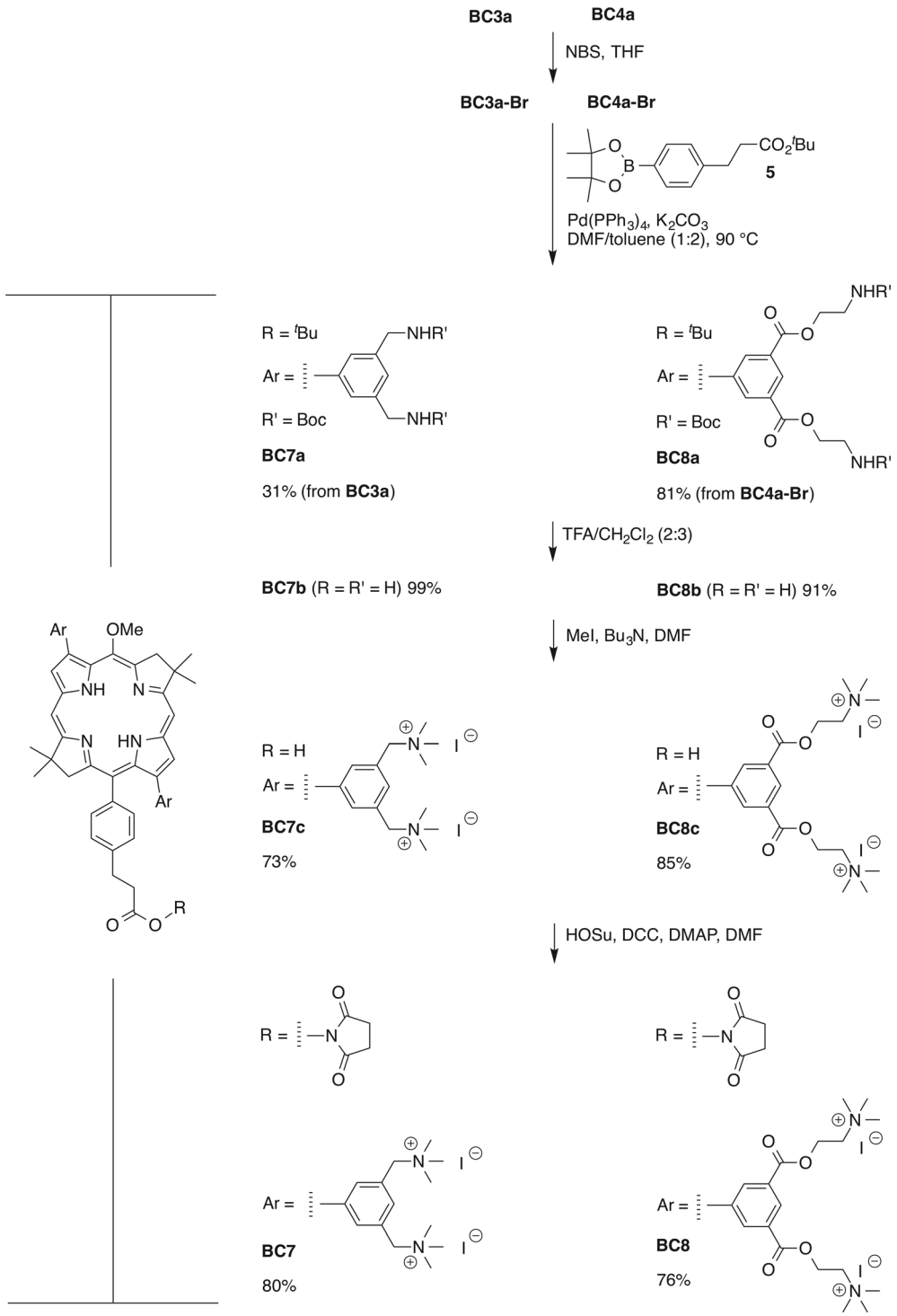
A. Spectral characteristics. Absorption and fluorescence emission spectra were collected for each bacteriochlorin in DMF, standard aqueous phosphate buffered saline (PBS; pH 7.4), and 0.5 M aqueous potassium phosphate buffer (PB; pH 7.0). Such studies typically utilized bacteriochlorin concentrations of ~1 μM (*e.g.*, $A = 0.12$ with $\varepsilon \sim 120\,000\ M^{-1}\ cm^{-1}$ for the Q_y band in a 1 cm path); studies over a range of concentrations are described below. All the bacteriochlorins exhibit similar absorption spectra in these media. The spectra show characteristic bacteriochlorin features[53] including a strong Soret feature (with partially resolved B_y and B_x components) in the near-ultraviolet, a modest Q_x band in the green-yellow region, and intense Q_y band in the NIR region. Representative spectra for **BC3** in PBS and DMF are shown in Fig. 1. Data are not given for **BC1** in DMF due to minimal solubility in that solvent. Spectral data for the bacteriochlorins in DMF and PBS are summarized in Table 2 and in all media in Table S2 (ESI†). As noted in the ESI† (Fig. S3), samples of the bacteriochlorins in PBS were allowed to equilibrate after preparation prior to quantitative fluorescence studies.

For each bacteriochlorin the Q_x band is at the same position to within 1 nm in DMF, PB, and PBS, and the Q_y band to within 2 nm; the Q_y full-width-at-half-maximum (fwhm) is the same to within ~15%. In general, the Q_y absorption band of the bacteriochlorins in the organic and aqueous media is quite sharp and in the range 22–26 nm. One exception is **BC4**, for which the Q_y fwhm increases from a typical 23 nm in DMF to 30–40 nm in aqueous media.

The Q_y fluorescence band of each bacteriochlorin is also quite sharp and is typically in the range 23–26 nm (including **BC4**). The fluorescence maximum is shifted from the Q_y absorption maximum by 6–8 nm (Table 2), corresponding to a very small Stokes shift (Fig. 1). A weak fluorescence vibronic satellite, the $Q_y(0,1)$ feature, appears as a shoulder ~55 nm (~800 cm^{-1}) to lower energy than the $Q_y(0,0)$ fluorescence maximum.

The effect of a bioconjugatable tether on the spectral properties of the bacteriochlorins was examined for two representative cases. **BC7c** (the immediate precursor to bioconjugatable, hydrophilic bacteriochlorin **BC7**, which adds the tether to **BC3**) has Q_y absorption and fluorescence band positions (and fwhm) of 725 nm (25 nm) and 732 nm (27 nm) in DMF, and 729 nm (27 nm) and 735 nm (27 nm) in PBS. **BC8c** (the immediate precursor to bioconjugatable, hydrophilic bacteriochlorin **BC8**, which adds the tether to **BC4**) has Q_y absorption and fluorescence band positions (and fwhm) of 730 nm (25 nm) and 736 nm (27 nm) in DMF, and 727 nm (37 nm) and 736 nm (27 nm) in PBS. Comparison with the properties of **BC3** and **BC4** in Table 2 indicates that the tether at the 15-position affects the spectral positions by ≤6 nm and the bandwidths by <10%.

B. Singlet excited-state lifetime and decay-pathway yields. The lowest singlet excited state (S_1,Q_y) decays by $S_1 \rightarrow S_0$ fluorescence, $S_1 \rightarrow T_1$ intersystem crossing and $S_1 \rightarrow S_0$ internal conversion with yields Φ_f, Φ_{isc} and Φ_{ic} and rate constants k_f,



Scheme 3 Synthesis of hydrophilic bacteriochlorins bearing NHS esters.

k_{isc} and k_{ic}. All of these quantities were determined for the new bacteriochlorins in DMF (except **BC1**) and PBS (Table 2).

The hydrophilic bacteriochlorins and the corresponding hydrophobic precursors have similar Φ_f values in DMF, indicating that the introduction of the polar motifs to bacteriochlorins does not impart a significant adverse effect on the excited-state properties (Table 2). The Φ_f of bacteriochlorins **BC2**, **BC3**, and **BC5** are reduced by 20–50% in PBS relative to that in DMF (the same disparity is observed for **BC1a**; **BC1** does not dissolve in DMF hence the comparison could not be made). The Φ_f values for three representative cases (PBS *vs.* DMF) are **BC2** (0.14 *vs.* 0.20), **BC3** (0.12 *vs.* 0.19) and **BC5** (0.16 *vs.* 0.20). A similar diminution was observed with hydrophilic chlorins[39] and bacteriochlorins[42] in aqueous solutions. A greater decrease in Φ_f is found for **BC4** (0.04 *vs.* 0.18).

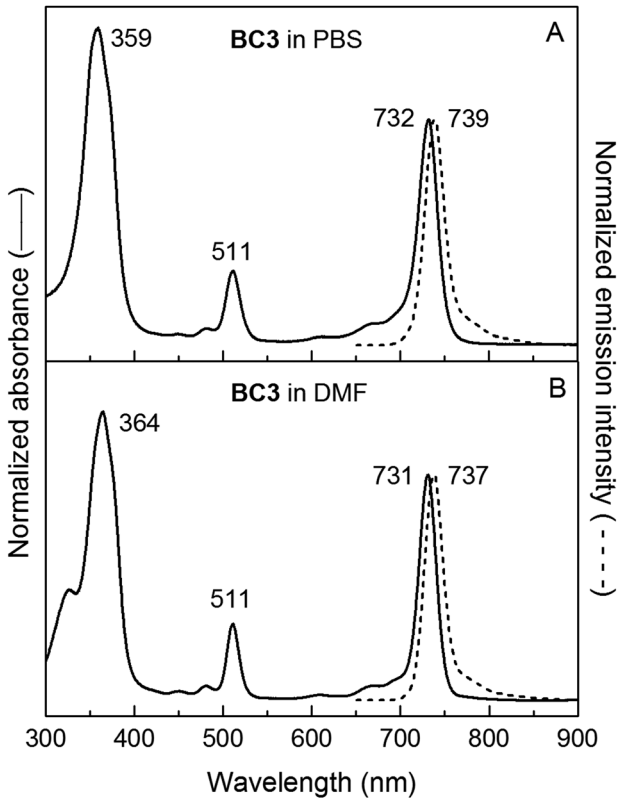


Fig. 1 Normalized absorption spectra (solid) and emission spectra (dashed) of **BC3** in PBS (A) or DMF (B) at room temperature.

The singlet lifetime τ_S of **BC1–BC5** in DMF is in the range 1.9–3.2 ns. These values are generally similar to those for a number of hydrophobic free base bacteriochlorins that have Q_y maximum in the same wavelength range (720–740 nm).[54] The τ_S value for each bacteriochlorin is reduced in PBS *versus* DMF in parallel with the Φ_f values. The τ_S values for three representative cases (PBS *vs.* DMF) are **BC2** (2.1 *vs.* 3.1 ns), **BC3** (2.2 *vs.* 3.1 ns) and **BC5** (2.7 *vs.* 3.2 ns). The τ_S for **BC4** is reduced even further (1.0 *vs.* 2.9 ns).

To understand the origin of the reduced Φ_f and τ_S values, the yield of $S_1 \rightarrow T_1$ intersystem crossing (Φ_{isc}), commonly called the triplet yield, was measured using ultrafast transient absorption spectroscopy. Representative data for **BC5** and **BC4** in DMF and PBS are shown in Fig. 2. The Φ_{isc} is slightly reduced for the bacteriochlorins in aqueous solution *versus* DMF (Table 2). The Φ_{isc} values for (PBS *vs.* DMF) are as follows for **BC2** (0.43 *vs.* 0.44), **BC3** (0.32 *vs.* 0.36) and **BC5** (0.40 *vs.* 0.42). A larger reduction in Φ_{isc} is seen for **BC4** (0.22 *vs.* 0.39). The yield of $S_1 \rightarrow S_0$ internal conversion (Φ_{ic}) is obtained by difference: $\Phi_{ic} = 1 - \Phi_f - \Phi_{isc}$. The Φ_{ic} values (PBS *vs.* DMF) are as follows for **BC2** (0.43 *vs.* 0.36), **BC3** (0.56 *vs.* 0.35) and **BC5** (0.44 *vs.* 0.38). There is also substantial increase in Φ_{ic} for **BC4** (0.74 *vs.* 0.53).

C. Singlet excited-state decay-pathway rate constants. The singlet excited-state lifetime (τ_S) and decay yields (Φ_f, Φ_{isc}, Φ_{ic}) afford the corresponding rate constants (k_f, k_{isc}, k_{ic}) *via* the formula $k_x = \Phi_x/\tau_S$, where x = f, ic, isc. The latter values are listed in the last three columns of Table 2 as the time constants (k^{-1}, in nanoseconds). The main conclusions drawn from the values for the bacteriochlorins in PBS *versus* DMF are as follows:

(1) The k_f values do not change appreciably. The rate constant for $S_1 \rightarrow S_0$ spontaneous emission is related to the rate constant for $S_0 \rightarrow S_1$ stimulated absorption *via* the Einstein coefficients.[55] As noted above (Fig. 1), each bacteriochlorin has a similar absorption spectrum with generally similar ratios of absorption bands in PBS and DMF, implying similar oscillator strength of the Q_y ($S_0 \rightarrow S_1$) absorption. As such one would expect little change in k_f for a bacteriochlorin in the two media, as is observed.

(2) The k_{isc} values do not change appreciably for a given bacteriochlorin in PBS *versus* DMF. This result is consistent with the expectation that the media employed should not result in an appreciable increase in spin–orbit coupling (*e.g.*, due to an external heavy-atom effect). **BC4** shows evidence for enhanced (PBS *vs.* DMF) intermolecular interactions that could affect k_{isc} (and k_{ic}). In addition to bacteriochlorin–bacteriochlorin interactions, hydrogen bonding between water and the ester-keto moieties of the polar groups of **BC4** could alter k_{isc}. However, a decrease rather than an increase in the rate constant would be expected if electron-withdrawing effects were the principal contributor. The result warrants further examination.

Table 2 Absorption and fluorescence properties of bacteriochlorins[a]

Cmpd	Solvent	λ_{abs} (nm)	fwhm abs (nm)	λ_{em} (nm)	fwhm em (nm)	Φ_f	Φ_{isc}	Φ_{ic}	τ_S (ns)	$(k_f)^{-1}$ (ns)	$(k_{isc})^{-1}$ (ns)	$(k_{ic})^{-1}$ (ns)
BC1a	DMF	729	21	736	23	0.20						
BC1	PBS	729	26	736	30	0.10	0.37	0.53	1.9	19	5	4
BC2[b]	DMF	731	24	738	25	0.18						
BC2	DMF	728	22	735	24	0.20	0.44	0.36	3.1	15	7	9
BC2	PBS	729	26	736	26	0.14	0.43	0.43	2.1	15	5	5
BC3a	DMF	729	21	734	23	0.20						
BC3	DMF	731	23	737	25	0.19	0.46	0.35	3.1	16	7	9
BC3	PBS	732	26	739	26	0.12	0.32	0.56	2.2	18	7	4
BC4a	DMF	730	23	738	23	0.19						
BC4	DMF	731	23	739	25	0.18	0.29	0.53	2.9	16	10	6
BC4	PBS	731	40	739	26	0.040	0.22	0.74	1.0	24	4	1
BC5	DMF	729	22	735	24	0.20	0.42	0.38	3.2	16	8	9
BC5	PBS	727	22	733	23	0.16	0.40	0.44	2.7	17	7	6

[a] All data were acquired at room temperature. The typical errors (percent of value) of the photophysical properties are as follows: τ_S (±7%), Φ_f (±5%), Φ_{isc} (±15%), Φ_{ic} (±20%), k_f (±10%), k_{isc} (±20%), k_{ic} (±25%). The error bars for τ_S, Φ_f, and Φ_{isc} were determined from select repeat measurements over a broad range of values and those for the Φ_{ic}, k_f, k_{isc} and k_{ic} were obtained from propagation of errors. [b] Data from ref. 42.

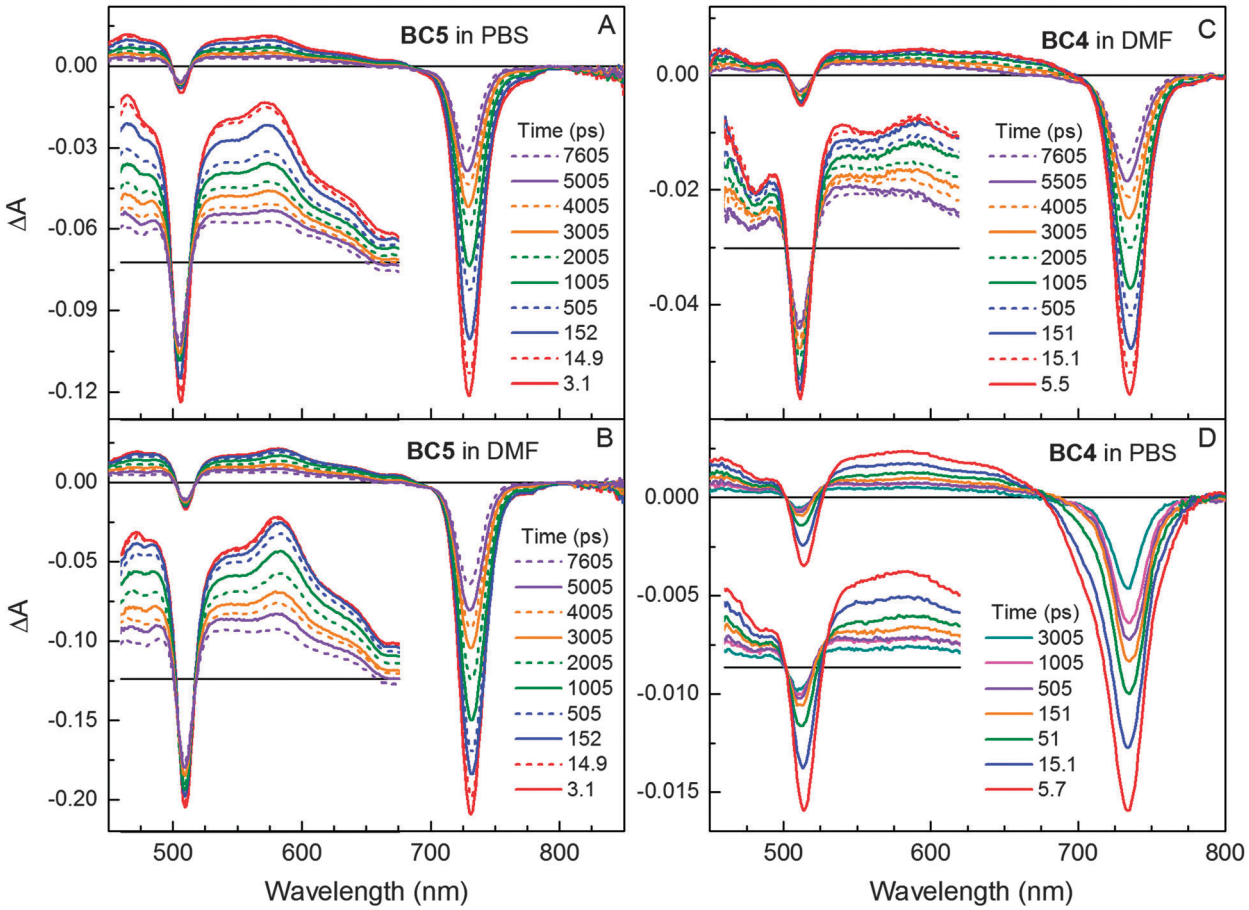


Fig. 2 Representative time-resolved absorption data for **BC5** (A, B) and **BC4** (C, D) obtained using 0.5 μJ, 100 fs excitation flashes at 730 nm.

(3) The k_{ic} values are greater [the $(k_{ic})^{-1}$ smaller] by two-fold on average for the bacteriochlorins in PBS *versus* DMF. An increase in k_{ic} would result from inter-bacteriochlorin interactions in the aqueous medium (in the ground or excited states), although such interactions are exhibited mainly by **BC4**. Although a bathochromic shift in the Q_y band would increase k_{ic},[56,57] **BC1–BC5** have similar Q_y positions and thus S_1 energies. Regardless, the combined results suggest that enhanced $S_1 \rightarrow S_0$ internal conversion primarily underlies the modest decrease in Φ_f and τ_S for the bacteriochlorins in aqueous media *versus* organic solvents. Further studies are required to elucidate the mechanism(s) at play.

D. Effect of concentration on spectral properties. All spectral measurements performed above were carried out using the typical low (micromolar) concentrations of bacteriochlorins required for such studies. Absorption *versus* concentration studies also were conducted to assess the aqueous solution properties of the bacteriochlorins over a greatly expanded concentration range of 1000-fold (~200–600 μM to ~0.2–0.6 μM). This type of study has been explained in detail,[42] and the same approach was adopted herein. The experimental approach entails reciprocal variation of concentration and cuvette pathlength (0.1–10 cm), as outlined in Fig. 3 (panel A). PBS was used for **BC1–BC4**, and neat water was used for **BC5**. The initial dissolution was facilitated by use of 5% DMSO. The DMSO co-solvent was not needed in all cases, but was included in each sample for consistency.

The spectra for each bacteriochlorin are shown in Fig. 3 (panels B–F). While **BC2** and **BC4** displayed obvious band broadening indicative of some degree of aggregation, **BC1**, **BC3** and **BC5** exhibited almost unchanged spectroscopic properties regardless of the 1000-fold change of concentration. At all concentrations for all five bacteriochlorins, the samples remained clear upon visual inspection indicating the absence of precipitates.

Discussion

Tetrapyrrole macrocycles are inherently hydrophobic, yet many applications require their incorporation in aqueous solutions. Molecular design strategies that equip tetrapyrroles for solubilization in water remain under active development.[1–5] The conceptual underpinning of the design explored herein is to shield the intrinsically hydrophobic faces of the tetrapyrrole π system from aqueous solution, by dint of steric bulk and/or electrostatic repulsion of appended polar motifs, and thereby suppress aggregation. The resulting architecture contains a

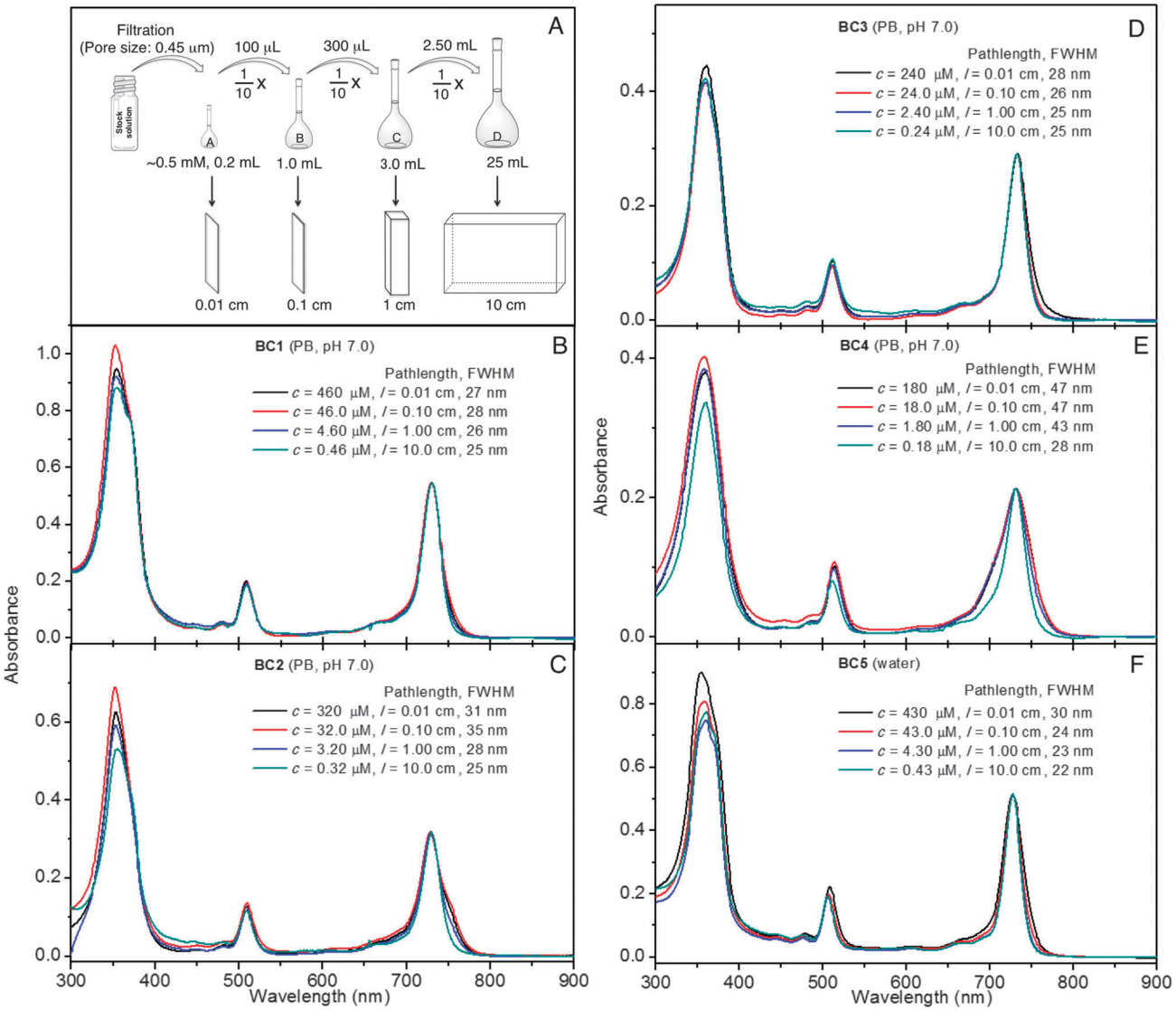


Fig. 3 Flowchart for absorption *versus* concentration study (panel A). Absorption *versus* concentration of **BC1–BC5** each over a range of 1000-fold (panels B–F). All spectra were normalized at the Q_y band. The concentration was calculated based on absorption in the 1 cm cuvette, assuming $\varepsilon(Q_y) = 120\,000\ M^{-1}\ cm^{-1}$.[58]

hydrophilic exterior and a hydrophobic core, and as such, exhibits a polarity profile somewhat akin to that of a micelle. Of equal importance are synthesis capabilities that together with molecular design enable the desired solubility and photophysical features to be attained. In the following section, we evaluate the five solubilization motifs employed with **BC1–BC5** with respect to these attributes.

The suitability of the solubilization motifs incorporated in **BC1–BC5** for bioconjugation studies depends on a host of factors including synthetic accessibility and photophysical parameters. Five features were considered in this regard. The features include (1) synthesis yield from the common precursor **BC-Br**[3,13] and ease of purification, (2) ease of 15-bromination, (3) water solubility, (4) fwhm of the long-wavelength (Q_y) absorption and fluorescence bands, τ_s and Φ_f, and (5) stability upon standing in the dark or in the light.

Synthetic amenability is measured here by the total yield beginning with the Suzuki coupling reaction of the 3,13-dibromobacteriochlorin (**BC-Br**[3,13]) and all subsequent steps. Methods for purification of compounds **BC1–BC5** vary with the nature of the polar motifs. The rationale for emphasis on the 15-bromination is because low yields in this step present a difficult separation problem; hence, 15-bromination can be a key bottleneck in preparation of bioconjugatable analogues.[42] Bacteriochlorin stability is measured by the extent of bacteriochlorin (%) remaining after standing in PBS for 48 h at 4 °C in the dark, determined by the Q_y absorption intensity. Bacteriochlorin stability in the light is assessed similarly in PBS during several hours of steady-state and time-resolved absorption and fluorescence studies. The results for the five bacteriochlorins assessed against these criteria are listed in Table 3.

Little distinction was observed for synthesis yields, with the range of 51–73% likely subject to improvement for any of the

Table 3 Properties of bacteriochlorins **BC1–BC5**

Test	Description	BC1	BC2	BC3	BC4	BC5
1a	Synthesis yield[a]	73%	55%	54%	51%	53%
1b	Purification methods[b]	C18 chromatography	Ppt, washing with hexanes/methanol (49 : 1)	Ppt, washing with Et_2O/THF (1 : 1)	Same as **BC3**	Ppt, washing with hexanes/CH_2Cl_2 (19 : 1)
2	Derivatization[c]	No	Yes	Yes, mixture	Yes	Yes, mixture
3	Water-solubility[d]	E	G	E	F	E
4a	fwhm (abs, flu)[e] (nm)	26, 30	26, 26	26,26	40, 26	22, 23
4b	Φ_f[e]	0.10	0.14	0.12	0.04	0.16
4c	τ_S[e] (ns)	2.4	2.3	2.6	0.9	3.2
5a	Stability in dark[e] (%)	85	96	82	96	95
5b	Stability in light[e]	>95	>95	>95	>95	>95

[a] Product of yields from all steps beginning with **BC-Br**[3,13] until the end of the synthesis. [b] For the final step of the synthesis. [c] Assessed by 15-bromination of the relevant precursors (**BC1a**, **BC2a**, **BC3a**, **BC4a**). [d] As measured in aqueous media by the absorption upon reciprocal change in concentration and pathlength (E, G, F, P = excellent, good, fair, poor). [e] For the bacteriochlorin at ~1 μM in PBS.

bacteriochlorins *via* further optimization. The purification procedures for the final compounds vary considerably, and while such consideration might seem unimportant, dyes with charged groups can present significant difficulties with regards to purification. The final step in the synthesis of **BC1** entailed TMSBr-mediated cleavage of the ethyl phosphonate protecting groups. **BC1** was purified by deprotonation of the phosphonic acid with sodium hydroxide solution (so as to make the compound more polar) followed by reversed phase column chromatography (C18, 5 cm length and 1 cm diameter) using H_2O/MeOH (from 99 : 1 to 99 : 5) as the co-eluent. The final step in the synthesis of **BC2** entailed TFA-mediated cleavage of the *tert*-butyl protecting groups of the carboxylate units. **BC2** was purified by partitioning into the organic phase (from aqueous acid) followed by washing the solid with hexanes/methanol (49 : 1).[42] The final step in the syntheses of ammonium bacteriochlorins **BC3** and **BC4** does not entail deprotection but rather quaternization of the free amines with methyl iodide. **BC3** and **BC4** were purified by addition of diethyl ether/THF (1 : 1) followed by sonication, centrifugation and decanting of the supernatant to remove excessive methyl iodide and protonated tributylamine. The final step in the synthesis of **BC5** also does not entail deprotection, but rather amidation of the free amines with the PEG–NHS ester.

Chromatography is often regarded as the least attractive method of purification (with regards to expense and lack of scalability) but is widely employed in basic research. Chromatography was employed in the final step for **BC1** but not for **BC2–BC5**. Consideration of the intrinsic polarity of the compounds suggests chromatography could have been done if needed for **BC2** and **BC5**, but would be difficult for **BC3** and **BC4** given the presence of the permanently charged ammonium groups.

Considering all of the factors, **BC1** shows a relatively broad Q_y band, a lower Φ_f value and stability than others, a modest τ_S, and most importantly, the precursor of **BC1** is the only bacteriochlorin examined herein that could not be brominated. Until methods of 15-bromination or alternative strategies for incorporation of a bioconjugatable tether are identified, this latter result at present disqualified the phosphonate motif for a variety of applications. **BC2** has high stability, one of the largest Φ_f values and a modest τ_S, and its precursor (an aryldiester) affords facile 15-bromination. **BC4** has similarly facile 15-bromination and stability, the broadest Q_y absorption yet normal emission width, and the lowest Φ_f and τ_S values. These characteristics do not bode well for use of **BC4** for quantitative photochemical studies. **BC3** and **BC5** gave the narrowest absorption and fluorescence band shapes and greatest Φ_f and τ_S values. **BC3** ranks slightly below **BC5** in composite characteristics. **BC3** and **BC5** share a common precursor (**BC3a**), which upon 15-bromination yields a mixture of starting material and the desired product. **BC3** has spectral bandwidths similar to **BC1** and **BC2**, an intermediate Φ_f and slightly larger τ_S, and slightly lower stability. **BC5** has the narrowest absorption and emission widths, the greatest Φ_f and τ_S, and high stability. In general, the properties of **BC5** in PBS are the closest to those found in DMF.

Conclusions

The ammonium-, carboxylate-, and PEG-substituted bacteriochlorins all appear suitable for use with bioconjugatable tethers. The particular choice depends, of course, on application. For applications where fluorescence (Φ_f, sharpness of emission) is an important parameter, the nonionic yet polar PEG–bacteriochlorin appears superior, with the incomplete 15-bromination as the only drawback if this route is employed to install a bioconjugatable tether.

Much remains to be done in the area of molecular design and synthetic methodology for rational and streamlined access to water-soluble tetrapyrrole macrocycles. Ultimately one goal is not only to solubilize bacteriochlorin monomers but to be able to create multipigment architectures containing bacteriochlorins that are water-soluble for use in diverse scientific applications.[25,26,49,59–63] The design, synthesis, and physicochemical characterization of the substituted bacteriochlorins described herein provide a small step toward this goal. Yet, the 3,5-disubstituted aryl groups employed herein constitute a compromise between design objectives and synthetic capabilities. One potential drawback is the low torsional barrier for rotation about the aryl-bacteriochlorin C–C single bond, which enables motions of the polar groups toward conformations more coplanar with the bacteriochlorin π framework, thereby opening the bacteriochlorin faces to

interact with each other, or if present, with surfaces or hydrophobic patches on proteins. More attractive designs – beyond present synthesis capabilities – would suppress or avoid such torsional motions and thereby provide more extensive facial encumbrance.

Experimental section

A. Materials and methods

^{1}H NMR and ^{13}C NMR spectroscopies were performed at room temperature. ^{31}P NMR (162 MHz) chemical shifts are reported for the sample in $CDCl_3$ *versus* the resonance of phosphoric acid (H_3PO_4) as an external reference (insert tube). In ^{13}C NMR spectroscopy of selected compounds, not all quaternary carbons were observed. MALDI-MS was performed with the matrix 1,4-bis(5-phenyl-2-oxaxol-2-yl)benzene (POPOP) for bacteriochlorins,[64] except that α-cyano-4-hydroxycinnamic acid (CHCA) was used for **BC5**. Silica gel (40 μm average particle size) was used for column chromatography. All solvents were reagent grade and were used as received unless noted otherwise. THF was freshly distilled from sodium/benzophenone ketyl. $CHCl_3$ was stabilized with amylenes (≤1%). Sonication was carried out in a benchtop sonication bath. Known compounds **1a**,[45] **2a**,[47] **5**,[52] **BC-Br**[3,13],[44] **BC2a**,[42] and **BC2**[42] were prepared following literature procedures. All other compounds were used as received from commercial sources. For ammoniobacteriochlorins **BC3** and **BC4**, yield calculations assumed the presence of the tetraiodide salt.

B. Syntheses leading to bacteriochlorins BC1–BC5, BC6

1-Bromo-3,5-bis(bromomethyl)benzene (1a). Following a general procedure,[45] a mixture of 1-bromo-3,5-dimethylbenzene (14.8 g, 80.0 mmol), NBS (28.5 g, 160 mmol), and AIBN (0.657 g, 4.00 mmol, 5 mol%) in acetonitrile (400 mL) was refluxed under argon for 5 h. The mixture was concentrated. CCl_4 (75 mL) was added with heating to dissolve the crude product. The reaction mixture was allowed to cool and then filtered to remove any undissolved succinimide. The filtrate was concentrated to afford the crude product, which upon crystallization from ethanol afforded white crystals (10.5 g, 38%): m.p. 91–93 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 4.40 (s, 4H), 7.32–7.35 (m, 1H), 7.47 (d, J = 1.8 Hz, 2H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 31.7, 122.9, 128.5, 132.2, 140.5; anal. calcd C, 28.03; H, 2.06. Found C, 28.03; H, 1.98.

1-Bromo-3,5-bis(diethylphosphonomethyl)benzene (1b). Following a general procedure,[45] a solution of **1a** (19.0 g, 55.0 mmol) and triethyl phosphite (18.6 g, 100 mmol) in toluene (30.0 mL) was refluxed under argon for 5 h. The reaction mixture was allowed to cool to room temperature whereupon toluene was removed by rotary evaporation. The resulting oily liquid was dissolved in $CHCl_3$, washed with water, dried (Na_2SO_4), concentrated and purified by chromatography on a short column [silica, hexanes/ethyl acetate (1 : 1)] to obtain a colorless liquid (10.5 g, 41%): ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.27 (t, J = 7.2 Hz, 12H), 3.09 (t, J = 22.2 Hz, 4H), 3.94–4.10 (m, 8H), 7.14–7.18 (m, 1H), 7.33–7.37 (m, 2H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 16.5–16.6 (m with peaks at 16.51, 16.53, 16.56), 33.4 (d, J = 138 Hz), 62.4–62.5 (m with peaks at 62.42, 62.46, 62.49), 122.5, 130.0–130.3 (m with peaks at 130.04, 130.10, 130.17), 131.2–131.5 (m with peaks at 131.38, 131.44, 131.48), 134.0–134.4 (m); ^{31}P NMR δ 25.86; ESI-MS obsd 457.0540, calcd 457.0539 [$(M + H)^+$, M = $C_{16}H_{27}BrO_6P_2$].

2-[3,5-Bis(diethylphosphonomethyl)phenyl]-3,3,4,4-tetramethyl-1,3,2-dioxaborolane (1). Following a general procedure,[46] a mixture of Pd(dppf)Cl_2 (0.132 g, 0.180 mmol, 3 mol%), KOAc (1.76 g, 18.0 mmol) and bis(pinacolato)diboron (1.67 g, 6.60 mmol) in a Schlenk flask was deareated under high vacuum for 20 min. Then, a solution of DMSO (18 mL) containing **1b** (2.74 g, 6.00 mmol) (degassed for 10 min) was added under argon, and the reaction mixture was degassed by three freeze–pump–thaw cycles. The mixture was stirred at 80 °C for 2 h. The starting material and product co-chromatographed upon TLC analysis [silica, ethyl acetate/hexanes (1 : 1)]. Hence, the reaction progress was monitored by ^{1}H NMR spectroscopy (the aromatic protons of the product are deshielded compared with those of the starting material) whereupon a new peak was observed at δ 1.33 ppm. The reaction mixture was allowed to cool to room temperature. The mixture was diluted with ethyl acetate, washed with water, dried (Na_2SO_4), and concentrated. The resulting residue was purified by column chromatography [silica, hexanes/ethyl acetate (1 : 1)] to obtain a light brown liquid (1.7 g, 56%): ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.22–1.29 (m, 12H), 1.33 (s, 12H), 3.14 (d, J = 21.6 Hz, 4H) 3.95–4.10 (m, 8H), 7.34–7.38 (m, 1H), 7.58–7.64 (m, 2H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 16.5 (d, J = 6.8 Hz), 25.0, 33.6 (d, J = 137.2 Hz), 62.3 (d, J = 6.8 Hz), 84.0, 131.5, 133.8–134.2 (m with peaks at 133.97, 134.03, 134.09), 134.8–135.1 (m with peaks at 134.92, 134.97, 135.02); ^{31}P NMR, δ 26.91; ESI-MS obsd 505.2288, calcd 505.2286 [$(M + H)^+$, M = $C_{22}H_{39}BO_8P_2$].

2-[3,5-Bis(*tert*-butoxycarbonylaminomethyl)phenyl]-3,3,4,4-tetramethyl-1,3,2-dioxaborolane (2). Following a general procedure,[46] samples of **2a** (1.25 g, 3.00 mmol), bis(pinacolato)diboron (761 mg, 3.00 mmol), Pd(dppf)Cl_2 (65.9 mg, 90.0 μmol), KOAc (883 mg, 9.00 mmol), and DMSO (20.0 mL, deaerated by bubbling with argon for 30 min) were added to a Schlenk flask. The reaction mixture was deaerated by three freeze–pump–thaw cycles. The reaction mixture was stirred at 80 °C under argon for 16 h. The reaction mixture was cooled to room temperature, diluted with ethyl acetate and washed with brine. The organic layer was separated, dried (Na_2SO_4) and concentrated. Column chromatography [silica, hexanes/CH_2Cl_2 (3 : 2)] provided a viscous liquid (1.10 g, 79%): ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.34 (s, 12H), 1.46 (s, 18H), 4.32 (d, J = 5.2 Hz, 4H), 4.82 (br, 2H), 7.31 (s, 1H), 7.61 (s, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 25.1, 28.6, 44.8, 84.2, 129.8, 133.0, 138.8, 156.1; ESI-MS obsd 485.2792, calcd 485.2793 [$(M + Na)^+$, M = $C_{24}H_{39}BN_2O_6$].

Bis[2-(*tert*-butoxycarbonylamino)ethyl] 5-bromoisophthalate (3b). A sample of 5-bromoisophthalic acid (**3a**, 490 mg, 2.00 mmol) in $SOCl_2$ (5.00 mL) was stirred under argon at 65 °C for 16 h. The solvent was removed under vacuum. The residue was slowly treated with a solution of *N*-(*tert*-butoxycarbonyl)ethanolamine (800 mg, 5.00 mmol) in pyridine (4.50 mL) in an ice bath. The reaction mixture was stirred under argon at room temperature for 16 h. The reaction mixture was diluted with ethyl acetate,

and then washed with 1 N HCl solution and brine. The aqueous solution was extracted three times with ethyl acetate. The combined organic extract was dried (Na_2SO_4), concentrated and chromatographed [silica, CH_2Cl_2/ethyl acetate (9 : 1)] to afford a white sticky solid (882 mg, 83%): ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.44 (s, 18H), 3.53–3.59 (m, 4H), 4.42 (t, J = 5.2 Hz, 4H), 5.20 (br, 2H), 8.32 (d, J = 1.6 Hz, 2H), 8.58 (s, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 28.6, 39.8, 65.4, 79.8, 122.8, 129.6, 132.3, 136.9, 156.1, 164.6; ESI-MS obsd 553.1148, calcd 553.1156 [(M + Na)$^+$, M = $C_{22}H_{31}O_8N_2Br$].

Bis[2-(*tert*-butoxycarbonylamino)ethyl] 5-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)isophthalate (3). Following a general procedure,[46] samples of **3b** (0.593 g, 1.12 mmol), bis(pinacolato)-diboron (283 mg, 1.12 mmol), Pd(pddf)Cl_2 (40.8 mg, 55.8 μmol), KOAc (329 mg, 3.35 mmol), and DMSO (5.6 mL, deaerated by bubbling with argon for 30 min) were added to a Schlenk flask. The reaction mixture was deaerated by three freeze–pump–thaw cycles. The reaction mixture was stirred at 80 °C for 4 h. The reaction mixture was cooled to room temperature, diluted with ethyl acetate and washed with brine. The organic layer was separated, dried (Na_2SO_4) and concentrated. Column chromatography [silica, CH_2Cl_2/ethyl acetate (17 : 3)] provided a white sticky solid (548 mg, 85%): ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.34 (s, 12H), 1.43 (s, 18H), 3.58 (m, 4H), 4.45 (t, J = 5.2 Hz, 4H), 5.42 (s, 2H), 8.61 (d, J = 2.0 Hz, 2H), 8.74 (t, J = 2.0 Hz, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 25.0, 28.5, 39.8, 64.9, 79.5, 84.6, 130.0, 133.6, 140.2, 156.1, 165.9; ESI-MS obsd 601.2908, calcd 601.2903 [(M + Na)$^+$, M = $C_{28}H_{43}BN_2O_{10}$].

Bis[2-(*tert*-butyldimethylsiloxy)ethyl] 5-bromoisophthalate (4a). A sample of 5-bromoisophthalic acid (**3a**, 1.96 g, 8.00 mmol), 2-(*tert*-butyldimethylsiloxy)ethylamine (4.21 g, 24.0 mmol) and EDCI (4.60 g, 24.0 mmol) in a mixed solvent of DMF and CH_2Cl_2 (10 mL, 1 : 1) was stirred at room temperature for 16 h. The reaction mixture was diluted with CH_2Cl_2, and then washed with brine for 6 times. The organic solution was dried (Na_2SO_4), concentrated and chromatographed [silica, CH_2Cl_2/ethyl acetate (7 : 3)] to afford a white sticky solid (2.00 g, 45%): ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.05 (s, 12H), 0.88 (s, 18H), 3.51–3.56 (m, 4H), 3.76 (t, J = 5.7 Hz, 4H), 6.80 (t, J = 4.8 Hz, 2H), 7.98 (d, J = 1.8 Hz, 2H), 8.06 (t, J = 1.8 Hz, 1H); ^{13}C NMR (75 MHz, $CDCl_3$) δ −5.1, 18.5, 26.1, 42.6, 61.8, 123.1, 124.2, 133.1, 137.0, 165.5; ESI-MS obsd 559.2016, calcd 559.2018 [(M + H)$^+$, M = $C_{24}H_{43}BrN_2O_4Si_2$].

Bis[2-(*tert*-butyldimethylsiloxy)ethyl] 5-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)isophthalate (4). Following a general procedure,[46] samples of **4a** (2.00 g, 3.57 mmol), bis(pinacolato)-diboron (907 mg, 3.57 mmol), Pd(pddf)Cl_2 (131 mg, 179 μmol), KOAc (1.05 g, 10.7 mmol), and DMSO (17.8 mL, deaerated by bubbling with argon for 30 min) were added to a Schlenk flask. The reaction mixture was deaerated by three freeze–pump–thaw cycles. The reaction mixture was stirred at 80 °C for 16 h. The reaction mixture was cooled to room temperature, diluted with ethyl acetate and washed with brine. The organic layer was separated, dried (Na_2SO_4) and concentrated. Column chromatography [silica, CH_2Cl_2/ethyl acetate (7 : 3)] provided a white sticky solid (569 mg, 26%): ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.08 (s, 12H), 0.91 (s, 18H), 1.34 (s, 12H), 3.55–3.61 (m, 4H), 3.79 (t, J = 5.7 Hz, 4H), 6.65 (t, J = 4.8 Hz, 2H), 8.26 (d, J = 1.8 Hz, 2H), 8.37 (t, J = 1.8 Hz, 1H); ^{13}C NMR (75 MHz, $CDCl_3$) δ −5.1, 18.5, 25.1, 26.2, 42.4, 62.0, 84.6, 129.0, 134.7, 135.6, 166.7; ESI-MS obsd 607.3762, calcd 607.3765 [(M + H)$^+$, M = $C_{30}H_{55}BN_2O_6Si_2$].

3,13-Bis[3,5-bis(diethylphosphonomethyl)phenyl]-5-methoxy-8,8,18,18-tetramethylbacteriochlorin (BC1a). Following a general procedure,[49] samples of **BC-Br**[3,13] (45 mg, 80 μmol), Pd(PPh_3)$_4$ (28 mg, 24 μmol) and anhydrous K_2CO_3 (0.13 g, 0.96 mmol) were placed in a Schlenk flask and dried under high vacuum for 1 h. Toluene/DMF [8.0 mL (2 : 1), degassed by bubbling with argon for 10 min] was added along with **1** (0.12 g, 0.24 mmol), and the resulting reaction mixture was degassed by three freeze–pump–thaw cycles. The reaction mixture was heated at 90 °C for 18 h. After allowing to cool to room temperature, the toluene was removed by rotary evaporation. The resulting residue was diluted with CH_2Cl_2, and the resulting solution was washed with aqueous $NaHCO_3$ solution. The organic layer was separated, dried (Na_2SO_4) and concentrated. The residue was purified by column chromatography [silica, CH_2Cl_2/MeOH (24 : 1)] to afford a green solid (75 mg, 81%): ^{1}H NMR (400 MHz, $CDCl_3$) δ −1.93 (brs, 1H), −1.67 (brs, 1H), 1.30–1.40 (m, 24H), 1.95 (s, 6H), 1.97 (s, 6H), 3.37 (d, J = 15.2 Hz, 4H), 3.42 (d, J = 15.6 Hz, 4H), 3.66 (s, 3H), 4.04–4.24 (m, 16H), 4.35 (s, 2H), 4.39 (s, 2H), 7.43 (s, 1H), 7.48 (s, 1H), 7.94 (d, J = 1.6 Hz, 2H), 8.02 (s, 2H), 8.58 (d, J = 2.0 Hz, 1H), 8.61 (s, 1H), 8.64 (s, 1H), 8.76 (d, J = 2.0 Hz, 1H), 8.80 (s, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 16.7 (d, J = 3.0 Hz), 31.3, 31.4, 34.0 (dd, J = 138.2 Hz & 14.5 Hz), 45.7, 45.8, 47.8, 52.1, 62.4 (dd, J = 16.0 Hz & 6.8 Hz), 63.3, 96.9, 122.3, 122.6, 127.4, 129.9, 130.5, 131.2, 131.5, 132.9 (d, J = 11. 3 Hz), 133.3, 134.0, 135.2, 135.5, 135.8, 136.3, 137.0, 138.8, 154.0, 160.6, 169.1, 169.72; ^{31}P NMR δ 26.9, 27.1; MALDI-MS obsd 1153.3; ESI-MS obsd 577.2407, calcd 577.2409 [(M + 2H)$^{2+}$, M = $C_{57}H_{80}N_4O_{13}P_4$], λ_{abs} (CH_2Cl_2) 363, 510, 730 nm.

2,12-Bis[3,5-bis(phosphonomethyl)phenyl]-5-methoxy-8,8,18,18-tetramethylbacteriochlorin (BC1). Following a reported procedure,[39] a solution of **BC1a** (3.1 mg, 2.7 μmol) in anhydrous $CHCl_3$ (0.14 mL) was treated with TMSBr (29 μL, 0.22 mmol, 80 equiv.) under argon, and the reaction mixture was stirred in the dark for 16 h. Methanol (0.30 mL) was then added, and the mixture was stirred for 3 h. The solvent was removed by rotary evaporation, and the residue was dried under high vacuum for 1 h. A solution of 0.25 M NaOH (1.0 mL) was added to the dried reaction mixture to give a green solution. The solution was concentrated to dryness. The residue was dissolved in a minimum amount of water and chromatographed [C18 silica, H_2O/MeOH (from 99 : 1 to 99 : 5)] to afford a dark green solid (2.5 mg, 90%): ^{1}H NMR [300 MHz, $CDCl_3$/CD_3OD (1 : 1); two pyrrolic-NH and eight phosphonic acid protons were not observed] δ 1.81 (s, 6H), 1.87 (s, 6H), 2.96–3.06 (m, 8H), 3.63 (s, 3H), 4.32 (s, 2H), 4.51 (s, 2H), 7.41 (s, 1H), 7.47 (s, 1H), 7.73 (s, 2H), 7.93 (s, 2H), 8.69 (s, 1H), 8.73 (s, 1H), 8.75 (s, 1H), 8.85 (s, 1H), 8.95 (s, 1H); λ_{abs} (PB) 353, 508, 730 nm.

3,13-Bis[3,5-bis(*tert*-butoxycarbonylaminomethyl)phenyl]-5-methoxy-8,8,18,18-tetramethylbacteriochlorin (BC3a). Following a general procedure,[49] samples of **BC-Br**[3,13] (44.6 mg, 80.0 μmol), **2** (81.4 mg, 176 μmol), Pd(PPh_3)$_4$ (55.6 mg, 48.1 μmol), Cs_2CO_3

(156 mg, 480 μmol) and toluene/DMF [4.00 mL (2 : 1), deaerated by bubbling with argon for 45 min] were added to a Schlenk flask. The mixture was deaerated by three freeze–pump–thaw cycles. The reaction mixture was stirred at 90 °C for 18 h. The reaction mixture was cooled to room temperature, concentrated to dryness, diluted with CH_2Cl_2 and washed with saturated aqueous $NaHCO_3$. The organic layer was separated, dried (Na_2SO_4), concentrated and chromatographed [silica, CH_2Cl_2/ethyl acetate (4 : 1)]. The resulting solid was treated with hexanes/ethyl acetate (4 : 1), sonicated, and centrifuged. The supernatant was discarded to afford a green solid (72.7 mg, 85%): ^{1}H NMR (300 MHz, $CDCl_3$) δ −1.92 (s, 1H), −1.66 (s, 1H), 1.50 (s, 36H), 1.95 (s, 6H), 1.97 (s, 6H), 3.62 (s, 3H), 4.37 (s, 2H), 4.39 (s, 2H), 4.54–4.61 (m, 8H), 5.05 (br, 4H), 7.38 (s, 1H), 7.44 (s, 1H), 7.94 (s, 2H), 7.98 (s, 2H), 8.59–8.64 (m, 3H), 8.73 (d, J = 5.7 Hz, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 28.70, 28.76, 31.33, 31.41, 44.67, 44.79, 45.00, 45.09, 45.76, 45.90, 47.9, 52.2, 63.4, 79.75, 79.82, 79.95, 84.2, 97.0, 113.6, 117.7, 122.45, 122.53, 125.2, 125.5, 125.7, 127.5, 128.4, 129.2, 129.5, 130.2, 130.8, 133.6, 134.1, 134.86, 134.92, 134.98, 135.26, 135.32, 135.39, 135.6, 136.2, 136.4, 137.3, 138.8, 139.1, 140.2, 140.4, 140.9, 150.0, 141.7, 154.2, 156.30, 156.40, 156.47, 157.6, 160.8, 169.2, 169.9; MALDI-MS obsd 1070.7; ESI-MS obsd 1068.6043, calcd 1068.6043 (M^+, M = $C_{61}H_{80}N_8O_9$); λ_{abs} (CH_2Cl_2) 363, 510, 729 nm.

3,13-Bis[3,5-bis(aminomethyl)phenyl]-5-methoxy-8,8,18,18-tetramethylbacteriochlorin (BC3b). A solution of **BC3a** (20.4 mg, 19.1 μmol) in CH_2Cl_2 (1.15 mL) was stirred under argon for 2 min, followed by addition of TFA (768 μL). After 20 min, the reaction mixture was diluted with $CHCl_3$ and dried under an argon flow. Tributylamine (77.8 mg, 0.420 mmol) was added to the solid residue, and the mixture was sonicated for 3 min. A mixture of THF and hexanes (1 : 1) was then added. The resulting suspension was sonicated for 5 min followed by centrifugation. The supernatant was discarded to afford a green solid (9.9 mg, 78%): ^{1}H NMR (300 MHz, CD_3OD, the four primary amine protons and two pyrrolic-NH protons were not observed) δ 1.99 (s, 6H), 2.01 (s, 6H), 3.70 (s, 3H), 4.40 (s, 2H), 4.41 (s, 4H), 4.45 (s, 2H), 4.47 (s, 4H), 7.72 (s, 1H), 7.80 (s, 1H), 8.28 (d, J = 1.8 Hz, 2H), 8.34 (d, J = 1.8 Hz, 2H), 8.74 (s, 1H), 8.77 (s, 1H), 8.83 (s, 1H), 8.87 (s, 1H), 8.99 (s, 1H); MALDI-MS obsd 668.7; ESI-MS obsd 669.4026, calcd 669.4024 [$(M + H)^+$, M = $C_{41}H_{48}N_8O$]; λ_{abs} (methanol) 360, 507, 726 nm.

3,13-Bis[3,5-bis(*N*,*N*,*N*-trimethylammoniomethyl)phenyl]-5-methoxy-8,8,18,18-tetramethylbacteriochlorin diiodide (BC3). A mixture of **BC3b** (4.0 mg, 6.0 μmol) and tributylamine (44 mg, 0.24 mmol) in DMF (0.20 mL) was stirred under argon for 2 min, followed by addition of iodomethane (45 μL, 0.72 mmol). After 16 h, diethyl ether/THF (1 : 1) was added to the reaction mixture to precipitate the crude product. The suspension was sonicated for 3 min followed by centrifugation. The supernatant was discarded to afford a green solid. This procedure (diethyl ether and THF addition/sonication/centrifugation) was carried out three additional times to afford a green solid (6.5 mg, 81%): ^{1}H NMR (300 MHz, DMSO-d_6, the ammoniomethyl peaks were overlapped with that from the moisture in the solvent) δ −1.93 (s, 1H), −1.69 (s, 1H), 1.91 (s, 6H), 1.93 (s, 6H), 3.25 (s, 36H), 3.58 (s, 3H), 4.30 (s, 2H), 4.37 (s, 2H), 4.81 (s, 4H), 4.87 (s, 4H), 7.84 (s, 1H), 7.90 (s, 1H), 8.40 (s, 2H), 8.56 (s, 2H), 8.80 (s, 1H), 8.87–8.90 (m, 2H), 8.95 (s, 1H), 9.16 (s, 1H); obsd 210.1528, calcd 210.1530 [$(M - 4I)^{4+}$, M = $C_{53}H_{76}I_4N_8O$]; λ_{abs} (PB) 363, 519, 731 nm.

3,13-Bis[3,5-bis(2-(*tert*-butoxycarbonylamino)ethoxycarbonyl)-phenyl]-5-methoxy-8,8,18,18-tetramethylbacteriochlorin (BC4a). Following a general procedure,[49] samples of **BC-Br**[3,13] (44.6 mg, 80.0 μmol), **3** (44.5 mg, 134 μmol), $Pd(PPh_3)_4$ (12.4 mg, 10.7 μmol) and Cs_2CO_3 (34.9 mg, 107 μmol) were placed in a Schlenk flask and dried under high vacuum for 30 min. Toluene/DMF [2.70 mL (2 : 1), deaerated by bubbling with argon for 45 min] was added to the Schlenk flask under argon and deaerated by three freeze–pump–thaw cycles. The reaction mixture was stirred at 90 °C for 13 h. The reaction mixture was allowed to cool to room temperature, then concentrated to dryness, diluted with CH_2Cl_2 and washed with saturated aqueous $NaHCO_3$. The organic layer was separated, dried (Na_2SO_4), concentrated and chromatographed [silica, CH_2Cl_2/ethyl acetate (4 : 1)]. Treatment of the resulting solid with hexanes/CH_2Cl_2 (4 : 1) afforded a suspension, which was sonicated followed by centrifugation. The supernatant was discarded to afford a green solid (82.3 mg, 79%): ^{1}H NMR (300 MHz, $CDCl_3$) δ −1.80 (s, 1H), −1.56 (s, 1H), 1.42 (s, 36H), 1.96 (s, 6H), 1.98 (s, 6H), 3.62–3.66 (m, 11H), 4.38 (s, 2H), 4.40 (s, 2H), 4.50–4.60 (m, 8H), 5.02–5.07 (m, 4H), 8.66 (s, 1H), 8.69 (s, 3H), 8.87 (s, 2H), 8.92 (s, 1H), 9.02–9.04 (m, 4H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 28.6, 31.30, 31.38, 40.0, 45.8, 46.0, 47.8, 52.2, 63.4, 65.21, 65.32, 79.9, 96.6, 97.41, 97.49, 122.6, 123.1, 127.3, 128.3, 129.5, 129.9, 130.1, 131.44, 131.46, 134.18, 134.23, 135.1, 135.6, 136.32, 136.39, 136.8, 137.6, 139.3, 154.6, 156.1, 161.3, 166.1, 166.4, 169.8, 170.5; MALDI-MS obsd 1302.7; ESI-MS obsd 1301.6335, calcd 1301.6340 [$(M + H)^+$, M = $C_{69}H_{88}N_8O_{17}$]; λ_{abs} (CH_2Cl_2) 364, 511, 732 nm.

3,13-Bis[3,5-bis(2-aminoethoxycarbonyl)phenyl]-5-methoxy-8,8,18,18-tetramethylbacteriochlorin (BC4b). A solution of **BC4a** (42.0 mg, 32.3 μmol) in CH_2Cl_2 (1.94 mL) was stirred under argon for 2 min, followed by addition of TFA (1.29 mL). After 1 h, the reaction mixture was diluted with $CHCl_3$ and dried under an argon flow. Tributylamine (300 μL) was added to the solid residue, and the resulting suspension was sonicated for 3 min. A mixture of THF and diethyl ether (1 : 1) was then added, and the resulting suspension was sonicated for 5 min followed by centrifugation. The supernatant was discarded to afford a green solid (25 mg, 86%): ^{1}H NMR (300 MHz, CD_3OD/$CDCl_3$; the eight primary amine protons and two pyrrolic-NH protons were not observed) δ 1.98 (s, 6H), 2.01 (s, 6H), 3.44–4.51 (m, 8H), 3.64 (s, 3H), 4.40 (s, 2H), 4.42 (s, 2H), 4.70–4.77 (m, 8H), 8.74 (s, 1H), 8.80 (d, J = 2.7 Hz, 2H), 8.87 (s, 1H), 8.99 (t, J = 1.5 Hz, 1H), 9.03 (t, J = 1.5 Hz, 1H), 9.06 (s, 1H), 9.10 (d, J = 1.5 Hz, 2H), 9.15 (d, J = 1.5 Hz, 2H); MALDI-MS obsd 900.4; obsd 301.1462, calcd 301.1463 [$(M + 3H)^{3+}$, M = $C_{49}H_{56}N_8O_9$]; λ_{abs} (methanol) 361, 508, 727 nm.

3,13-Bis[3,5-bis(2-(*N*,*N*,*N*-trimethylammonio)ethoxycarbonyl)-phenyl]-5-methoxy-8,8,18,18-tetramethylbacteriochlorin diiodide (BC4). A mixture of **BC4b** (6.2 mg, 6.9 μmol) and tributylamine (51 mg, 0.28 mmol) in DMF (0.20 mL) was stirred under argon

for 2 min, followed by addition of iodomethane (51 μL, 0.83 mmol). After 16 h, diethyl ether/THF (1 : 1) was added to the reaction mixture to precipitate the crude product. The suspension was sonicated for 3 min followed by centrifugation. The supernatant was discarded to afford a green solid. This procedure (diethyl ether and THF addition/sonication/centrifugation) was carried out three more times to afford a green solid (8.2 mg, 75%): ^{1}H NMR (300 MHz, DMSO-d_6) δ −1.87 (s, 1H), −1.62 (s, 1H), 1.91 (s, 6H), 1.95 (s, 6H), 3.25 (s, 36H), 3.54 (s, 3H), 3.91 (br, 8H), 4.29 (s, 2H), 4.34 (s, 2H), 4.86 (br, 8H), 8.72–8.82 (m, 3H), 8.90 (s, 1H), 8.94–9.04 (m, 6H), 9.20 (s, 1H); obsd 268.1584, calcd 268.1585 [(M − 4I)$^{4+}$, M = $C_{61}H_{84}I_4N_8O_9$]; λ_{abs} (PB) 363, 519, 731 nm.

3,13-Bis[3,5-bis(2-(*tert*-butyldimethylsiloxy)ethylaminocarbonyl)-phenyl]-5-methoxy-8,8,18,18-tetramethylbacteriochlorin (BC6a). Following a general procedure,[49] samples of **BC-Br^{3,13}** (168 mg, 300 μmol), **4** (401 mg, 660 μmol), Pd(PPh$_3$)$_4$ (104 mg, 90.0 μmol) and K_2CO_3 (249 mg, 1.80 mmol) were placed in a Schlenk flask and dried under high vacuum for 30 min. Toluene/DMF [15.0 mL (2 : 1), deaerated by bubbling with argon for 45 min] was added to the Schlenk flask under argon and deaerated by three freeze–pump–thaw cycles. The reaction mixture was stirred at 90 °C for 16 h. The reaction mixture was cooled to room temperature, concentrated to dryness, diluted with CH_2Cl_2 and washed with saturated aqueous $NaHCO_3$. The organic layer was separated, dried (Na_2SO_4), concentrated and chromatographed [silica, CH_2Cl_2/ethyl acetate (9 : 1 to 13 : 7)]. Treatment of the resulting solid with hexanes/CH_2Cl_2 (9 : 1) afforded a suspension, which was sonicated followed by centrifugation. The supernatant was discarded to afford a green solid (330 mg, 81%): ^{1}H NMR (300 MHz, $CDCl_3$) δ −1.85 (s, 1H), −1.60 (s, 1H), 0.09 (s, 12H), 0.10 (s, 12H), 0.88 (s, 18H), 0.90 (s, 18H), 1.96 (s, 6H), 1.99 (s, 6H), 3.59 (s, 3H), 3.67–3.74 (m, 8H), 3.86–3.91 (m, 8H), 4.37 (s, 2H), 4.39 (s, 2H), 6.82 (t, J = 4.8 Hz, 4H), 8.40 (t, J = 1.5 Hz, 1H), 8.43 (t, J = 1.5 Hz, 1H), 8.65 (s, 1H), 8.67 (s, 1H), 8.69–8.72 (m, 6H), 8.82 (d, J = 2.4 Hz, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ −5.02, −5.01, 18.55, 18.57, 26.16, 26.18, 31.37, 42.68, 42.71, 45.8, 45.9, 47.9, 52.2, 62.05, 62.12, 63.3, 96.7, 97.3, 122.84, 122.88, 124.3, 124.4, 127.3, 132.0, 132.3, 132.6, 134.3, 134.6, 134.7, 135.1, 135.6, 136.1, 136.3, 137.8, 139.3, 154.5, 161.2, 166.9, 167.2, 169.7, 170.2; MALDI-MS obsd 1357.2; ESI-MS obsd 1357.7680, calcd 1357.7702 [(M + H)$^+$, M = $C_{73}H_{112}N_8O_9Si_4$]; λ_{abs} (CH_2Cl_2) 364, 511, 732 nm.

3,13-Bis[3,5-bis(2-hydroxyethoxycarbonyl)phenyl]-5-methoxy-8,8,18,18-tetramethylbacteriochlorin (BC6b). A solution of **BC6a** (120 mg, 88.0 μmol) in THF (88.0 mL) was stirred under argon for 2 min, followed by addition of 1 M TBAF solution in THF (528 μL, 528 μmol). After 40 min, the solvent was removed under vacuum, and the reaction residue was chromatographed [silica, CH_2Cl_2/methanol (9 : 1 to 7 : 3)] to afford a green solid (56.0 mg, 71%): ^{1}H NMR (300 MHz, CD_3OD/$CDCl_3$; the four OH, four amido NH, and two pyrrolic NH protons were not observed) δ 1.97 (s, 6H), 2.00 (s, 6H), 3.64 (s, 3H), 3.65–3.71 (m, 8H) 3.83–3.88 (m, 8H), 4.37 (s, 2H), 4.43 (s, 2H), 8.49 (s, 1H), 8.52 (s, 1H), 8.68 (s, 1H), 8.72 (s, 2H), 8.75 (s, 2H), 8.79 (s, 1H), 8.84 (s, 2H), 8.93 (s, 1H); MALDI-MS obsd 900.3; ESI-MS obsd 451.2159, calcd 451.2158 [(M + 2H)$^{2+}$, M = $C_{49}H_{56}N_8O_9$]; λ_{abs} (methanol) 361, 508, 727 nm.

C. Bacteriochlorin bromination and bioconjugation

Attempted synthesis of 15-bromo-3,13-bis[3,5-bis(diethylphosphonomethyl)phenyl]-5-methoxy-8,8,18,18-tetramethylbacteriochlorin (BC1a-Br). Following a general procedure,[49] a solution of **BC1a** (10.0 mg, 8.70 μmol) in THF (4.35 mL) was treated with NBS (1.54 mg, 8.70 μmol) in THF (2.17 μL) at room temperature for 1.5 h. MALDI-MS and absorption spectroscopy showed no detectable product. The reaction was then heated to 50 °C for 12 h, whereupon a trace amount of product was detected by MALDI-MS, but could not be isolated from the reaction mixture.

15-Bromo-3,13-bis[3,5-bis(2-(*tert*-butoxycarbonylamino)ethoxycarbonyl)phenyl]-5-methoxy-8,8,18,18-tetramethylbacteriochlorin (BC4a-Br). Following a general procedure,[49] a solution of **BC4a** (42.6 mg, 32.7 μmol) in THF (65.4 mL) was treated with NBS (5.80 mg, 32.7 μmol) in THF (327 μL) at room temperature for 15 min. The reaction mixture was diluted with CH_2Cl_2 and washed with saturated aqueous $NaHCO_3$. The organic layer was dried (Na_2SO_4), concentrated and chromatographed [silica, CH_2Cl_2/ethyl acetate (7 : 3)] to afford a red solid (34.3 mg, 76%): ^{1}H NMR (300 MHz, $CDCl_3$) δ −1.63 (s, 1H), −1.33 (s, 1H), 1.40 (s, 18H), 1.42 (s, 18H), 1.96 (s, 6H), 1.97 (s, 6H), 3.57–3.64 (m, 11H), 4.37 (s, 2H), 4.41 (s, 2H), 4.48–4.54 (m, 8H), 4.98 (br, 2H), 5.07 (br, 2H), 8.63 (s, 1H), 8.67 (s, 1H), 8.69 (d, J = 2.4 Hz, 1H), 8.74–8.76 (m, 3H), 8.89 (t, J = 1.5 Hz, 2H), 8.89 (d, J = 1.5 Hz, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 28.63, 28.65, 31.5, 31.7, 40.1, 45.6, 45.9, 48.2, 54.8, 63.7, 65.3, 79.9, 97.2, 97.5, 99.0, 125.1, 126.2, 129.4, 129.87, 129.97, 130.12, 130.3, 130.9, 132.6, 133.6, 133.8, 135.4, 136.2, 136.7, 137.0, 138.6, 140.4, 156.2, 157.8, 160.3, 166.3, 168.7, 172.0; ESI-MS obsd 1379.5458, calcd 1379.5445 [(M + H)$^+$, M = $C_{69}H_{87}BrN_8O_{17}$]; MALDI-MS obsd 1382.6; λ_{abs} (CH_2Cl_2) 368, 523, 729 nm.

15-Bromo-3,13-bis[3,5-bis(2-(*tert*-butyldimethylsiloxy)ethylaminocarbonyl)phenyl]-5-methoxy-8,8,18,18-tetramethylbacteriochlorin (BC6a-Br). Following a general procedure,[49] a solution of **BC6a** (47.5 mg, 35.0 μmol) in THF (70.0 mL) was treated with NBS (6.85 mg, 38.5 μmol) in THF (38.5 μL) at room temperature for 15 min. The reaction mixture was diluted with CH_2Cl_2 and washed with saturated aqueous $NaHCO_3$. The organic layer was dried (Na_2SO_4), concentrated and chromatographed [silica, CH_2Cl_2/ethyl acetate (7 : 3)] to afford a red solid (35.0 mg, 70%): ^{1}H NMR (400 MHz, $CDCl_3$) δ −1.68 (s, 1H), −1.37 (s, 1H), 0.07 (s, 12H), 0.09 (s, 12H), 0.86 (s, 18H), 0.87 (s, 18), 1.96 (s, 6H), 1.97 (s, 6H), 3.61 (s, 3H), 3.64–3.72 (m, 8H), 3.83–3.89 (m, 8H), 4.36 (s, 2H), 4.40 (s, 2H), 6.79 (t, J = 5.6 Hz, 2H), 6.86 (t, J = 5.6 Hz, 2H), 8.41 (s, 2H), 8.44 (s, 1H), 8.61 (s, 1H), 8.66 (s, 1H), 8.67 (d, J = 2.4 Hz, 1H), 8.69 (s, 2H), 8.73 (d, J = 2.4 Hz, 2H); MALDI-MS obsd 1439.8; ESI-MS obsd 1435.6798, calcd 1435.6807 [(M + H)$^+$, M = $C_{73}H_{111}BrN_8O_9Si_4$]; λ_{abs} (CH_2Cl_2) 368, 523, 729 nm.

3,13-Bis[3,5-bis(*tert*-butoxycarbonylaminomethyl)phenyl]-15-[4-(2-(*tert*-butoxycarbonyl)ethyl)phenyl]-5-methoxy-8,8,18,18-tetramethylbacteriochlorin (BC7a). Following a general procedure,[49] a solution of **BC3a** (88.0 mg, 82.3 μmol) in THF (165 mL) was treated with NBS (17.6 mg, 98.8 μmol) in THF (988 μL) at room temperature for 1.5 h. The reaction mixture was diluted with

CH_2Cl_2 and washed with saturated aqueous $NaHCO_3$. The organic layer was dried (Na_2SO_4), concentrated and chromatographed [silica, hexanes/CH_2Cl_2 (1 : 4)] to afford a red solid. TLC analysis was uninformative concerning the progress of the reaction, as no new species appeared distinct from that of the starting material; however, MALDI-MS analysis showed the presence of the starting material and the brominated product thereof (**BC3a-Br**): MALDI-MS obsd 1147.1, calcd 1146.5 ($C_{61}H_{79}BrN_8O_9$); obsd 1069.2, calcd 1068.6 ($C_{61}H_{80}N_8O_9$). Given the inability to purify the brominated bacteriochlorin, the entire crude product was transferred to a Schlenk flask. Samples of **5** (67.8 mg, 0.204 mmol), $Pd(PPh_3)_4$ (19.2 mg, 16.6 μmol), and Cs_2CO_3 (82.2 mg, 0.252 mmol) were placed in the Schlenk flask, and dried under high vacuum for 30 min. Toluene/DMF [4.2 mL (2 : 1), deaerated by bubbling with argon for 30 min] was added to the Schlenk flask under argon, and the mixture was deaerated by three freeze–pump–thaw cycles. The reaction mixture was stirred at 90 °C for 19 h. The reaction mixture was cooled to room temperature, concentrated to dryness, diluted with ethyl acetate and washed with saturated aqueous $NaHCO_3$. The organic layer was separated, dried (Na_2SO_4), concentrated and chromatographed [silica, hexanes/ethyl acetate (8 : 2 to 7 : 3)] to provide a green solid (33.0 mg, 31%): ^{1}H NMR (400 MHz, $CDCl_3$) δ −1.61 (s, 1H), −1.24 (s, 1H), 1.48 (s, 18H), 1.50 (s, 18H), 1.52 (s, 9H), 1.83 (s, 6H), 1.97 (s, 6H), 2.59 (t, J = 7.6 Hz, 2H), 2.93 (t, J = 7.6 Hz, 2H), 3.66 (s, 3H), 3.89 (s, 2H), 4.27 (d, J = 5.6 Hz, 4H), 4.37 (s, 2H), 4.56 (d, J = 5.6 Hz, 4H), 4.90 (br, 2H), 5.07 (br, 2H), 6.98 (s, 1H), 7.04 (d, J = 7.6 Hz, 2H), 7.11 (s, 2H), 7.39–7.42 (m, 3H), 7.97 (s, 2H), 8.57 (d, J = 2.4 Hz, 1H), 8.62–8.64 (m, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 28.4, 28.7, 30.0, 30.9, 31.3, 36.9, 44.7, 45.1, 45.2, 45.9, 47.8, 52.3, 63.5, 79.7, 80.8, 97.0, 97.5, 113.8, 123.1, 124.0, 125.2, 126.5, 127.2, 128.2, 129.2, 129.5, 133.6, 133.8, 134.0, 134.2, 136.2, 136.9, 137.7, 138.8, 138.9, 139.2, 139.3, 154.9, 156.1, 156.2, 160.9, 168.9, 172.7; MALDI-MS obsd 1274.9; ESI-MS obsd 648.3581, calcd 648.3582 [$(M + H + Na)^{2+}$, M = $C_{74}H_{96}N_8O_{11}$]; λ_{abs} (CH_2Cl_2) 367, 517, 729 nm.

15-[4-(2-Carboxyethyl)phenyl]-3,13-bis[3,5-bis(aminomethyl)-phenyl]-5-methoxy-8,8,18,18-tetramethylbacteriochlorin (BC7b). A solution of **BC7a** (15.5 mg, 12.2 μmol) in CH_2Cl_2 (730 μL) was stirred under argon for 2 min, followed by addition of TFA (470 μL). After 1 h, the reaction mixture was diluted with $CHCl_3$ and dried under an argon flow. Tributylamine (200 μL, 840 μmol) was added to the solid residue and sonicated for 3 min. A mixture of THF and hexanes (1 : 1) was then added, and the resulting suspension was sonicated for 5 min followed by centrifugation. The supernatant was discarded to afford a green solid (9.90 mg, 99%): ^{1}H NMR (300 MHz, CD_3OD, the four amine protons, two pyrrolic-NH protons and one carboxylic acid proton were not observed) δ 1.82 (s, 6H), 1.98 (s, 6H), 2.57 (br, 2H), 2.89 (br, 2H), 3.69 (s, 3H), 3.88 (s, 2H), 4.06 (s, 4H), 4.28 (s, 4H), 4.37 (s, 2H), 7.12 (d, J = 8.1 Hz, 2H), 7.28 (s, 1H), 7.37 (s, 2H), 7.44 (d, J = 8.1 Hz, 2H), 7.64 (s, 1H), 8.20 (s, 2H), 8.67 (s, 1H), 8.74 (s, 2H), 8.77 (s, 1H); MALDI-MS obsd 816.0; ESI-MS obsd 409.2308, calcd 409.2311 [$(M + 2H)^{2+}$, M = $C_{50}H_{56}N_8O_3$]; λ_{abs} (methanol) 363, 515, 726 nm.

15-[4-(2-Carboxyethyl)phenyl]-3,13-bis[3,5-bis(*N*,*N*,*N*-trimethylammoniomethyl)phenyl]-5-methoxy-8,8,18,18-tetramethylbacteriochlorin tetraiodide (BC7c). A mixture of **BC7b** (12.3 mg, 15.0 μmol) and tributylamine (112 mg, 602 μmol) in DMF (300 μL) was stirred under argon for 2 min, followed by addition of iodomethane (256 μL, 1.81 mmol). After 24 h, diethyl ether and THF (1 : 1) was added to the reaction mixture to precipitate the crude product. The suspension was sonicated for 3 min followed by centrifugation. The supernatant was discarded to afford a green solid. This procedure (diethyl ether and THF addition/sonication/centrifugation) was carried out three more times to afford a green solid (16.4 mg, 73%): ^{1}H NMR (300 MHz, CD_3OD, the carboxylic acid proton and two pyrrolic-NH protons were not observed, and resonances from the four ethylene protons were overlapped with the solvent peak) δ 1.84 (s, 6H), 2.01 (s, 6H), 3.21 (s, 18H), 3.41 (s, 18H), 3.72 (s, 3H), 3.88 (s, 2H), 4.37 (s, 2H), 4.93 (s, 8H), 7.18 (d, J = 6.0 Hz, 2H), 7.50 (d, J = 6.0 Hz, 2H), 7.60 (s, 1H), 7.75 (d, J = 6.0 Hz, 2H), 8.12 (s, 1H), 8.55 (s, 2H), 8.78–8.91 (m, 4H); ESI-MS obsd 247.1662, calcd 247.1661 [$(M - 4I)^{4+}$, M = $C_{62}H_{84}I_4N_8O_3$]; λ_{abs} (0.5 M phosphate buffer, pH 7.0) 362, 519, 731 nm.

3,13-Bis[3,5-bis(*N*,*N*,*N*-trimethylammoniomethyl)phenyl]-5-methoxy-15-[4-(2-(*N*-succinimidooxycarbonyl)ethyl)phenyl]-8,8,18,18-tetramethylbacteriochlorin tetraiodide (BC7). A mixture of **BC7c** (9.8 mg, 6.5 μmol), DCC (6.7 mg, 33 μmol), and DMAP (0.20 mg, 1.64 μmol) in DMF (654 μL) was treated with HOSu (3.8 mg, 33 μmol) and stirred under argon at room temperature. After 12 h, diethyl ether was added to the reaction mixture to precipitate the crude product. The suspension was sonicated for 3 min followed by centrifugation. The supernatant was discarded to afford a green solid. This procedure (diethyl ether addition/sonication/centrifugation) was carried out three more times to afford a green solid (8.3 mg, ∼90% purity, 80% yield): ^{1}H NMR (300 MHz, CD_3OD, the two pyrrolic-NH protons were not observed, and the four ethylene protons were overlapped with the solvent peak) δ 1.86 (s, 6H), 2.01 (s, 6H), 3.16 (s, 4H), 3.21 (s, 18H), 3.41 (s, 18H), 3.72 (s, 3H), 3.89 (s, 2H), 4.38 (s, 2H), 4.98 (s, 8H), 7.20 (d, J = 6.0 Hz, 2H), 7.50 (d, J = 6.0 Hz, 2H), 7.60 (s, 1H), 7.75 (d, J = 6.0 Hz, 2H), 8.12 (s, 1H), 8.55 (s, 2H), 8.78–8.91 (m, 4H); λ_{abs} (PB) 363, 519, 731 nm.

3,13-Bis[3,5-bis(2-(*tert*-butoxycarbonylamino)ethoxycarbonyl)-phenyl]-15-[4-(2-(*tert*-butoxycarbonyl)ethyl)phenyl]-5-methoxy-8,8,18,18-tetramethylbacteriochlorin (BC8a). Following a general procedure,[49] samples of **BC4a-Br** (37.0 mg, 26.8 μmol), **5** (56.3 mg, 0.257 mmol), $Pd(PPh_3)_4$ (23.7 mg, 20.6 μmol), and Cs_2CO_3 (101 mg, 0.308 mmol) were placed in a Schlenk flask, and dried under high vacuum for 30 min. Toluene/DMF [5.1 mL (2 : 1), deaerated by bubbling with argon for 30 min] was added to the Schlenk flask under argon, and the mixture was deaerated by three freeze–pump–thaw cycles. The reaction mixture was stirred at 90 °C for 18 h. The reaction mixture was cooled to room temperature, concentrated to dryness, diluted with ethyl acetate and washed with saturated aqueous $NaHCO_3$. The organic layer was separated, dried (Na_2SO_4), concentrated and chromatographed [silica, CH_2Cl_2/ethyl acetate (24 : 1)] to provide a red solid (32.7 mg, 81%): ^{1}H NMR (300 MHz, $CDCl_3$) δ −1.51 (s, 1H), −1.15 (s, 1H), 1.41 (s, 18H), 1.43 (s, 18H), 1.52 (s, 9H), 1.85 (s, 6H),

1.99 (s, 6H), 2.53 (t, J = 8.7 Hz, 2H), 2.84 (t, J = 8.7 Hz, 2H), 3.59–3.66 (m, 11H), 3.92 (s, 2H), 4.40 (s, 2H), 4.70 (t, J = 8.4 Hz, 4H), 4.53 (t, J = 8.4 Hz, 4H), 5.10–5.13 (m, 4H), 6.95 (d, J = 7.8 Hz, 2H), 7.43 (d, J = 7.8 Hz, 2H), 8.15 (s, 2H), 8.51 (s, 1H), 8.68 (s, 3H), 8.73 (d, J = 2.4 Hz, 1H), 8.88 (s, 1H), 8.89 (d, J = 1.5 Hz, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 28.4, 28.6, 30.9, 31.32, 31.37, 36.9, 40.0, 45.2, 46.1, 47.7, 52.3, 65.0, 65.2, 79.8, 80.9, 97.5, 97.9, 113.7, 123.2, 126.9, 127.5, 128.0, 128.3, 129.3, 129.5, 130.2, 131.4, 131.6, 133.5, 134.0, 134.2, 135.0, 136.02, 136.13, 136.4, 136.8, 138.8, 139.0, 139.4, 139.6, 155.2, 156.1, 161.3, 165.9, 166.3, 169.46, 169.51, 172.7; MALDI-MS obsd 1507.3; ESI-MS obsd 1505.7499, calcd 1505.7491 $[(M + H)^+$, M = $C_{82}H_{104}N_8O_{19}]$; λ_{abs} (CH_2Cl_2) 368, 518, 730 nm.

3,13-Bis[3,5-bis(2-aminoethoxycarbonyl)phenyl]-15-[4-(2-carboxyethyl)phenyl]-5-methoxy-8,8,18,18-tetramethylbacteriochlorin (BC8b). A solution of **BC8a** (23.7 mg, 15.7 μmol) in CH_2Cl_2 (315 μL) was stirred under argon for 2 min, followed by addition of TFA (210 μL). After 1.5 h, the reaction mixture was diluted with $CHCl_3$ and dried under an argon flow. Tributylamine (400 μL) was added to the solid residue. The resulting suspension was sonicated for 3 min. A mixture of THF and diethyl ether (1 : 1) was then added. The resulting suspension was sonicated for 5 min followed by centrifugation. The supernatant was discarded to afford a green solid (15.0 mg, 91%): ^{1}H NMR (300 MHz, $CD_3OD/CDCl_3$, the CO_2H, NH_2 and pyrrolic-NH protons were not observed) δ 1.86 (s, 6H), 2.02 (s, 6H), 2.61 (t, J = 7.5 Hz, 2H), 2.87 (t, J = 7.5 Hz, 2H), 3.46–3.50 (m, 8H), 3.67 (s, 3H), 3.94 (s, 2H), 4.40 (s, 2H), 4.68–4.76 (m, 8H), 7.04 (d, J = 7.8 Hz, 2H), 7.45 (d, J = 7.8 Hz, 2H), 8.30 (d, J = 1.5 Hz, 2H), 8.64 (t, J = 1.2 Hz, 1H), 8.79–8.83 (m, 4H), 9.00 (d, J = 1.2 Hz, 1H), 9.12 (d, J = 1.5 Hz, 2H); MALDI-MS obsd 1050.2; ESI-MS obsd 1049.4776, calcd 1049.4767 $[(M + H)^+$, M = $C_{58}H_{64}N_8O_{11}]$; λ_{abs} (methanol) 364, 515, 726 nm.

3,13-Bis[3,5-bis(2-(*N*,*N*,*N*-trimethylammonio)ethoxycarbonyl)-phenyl]-15-[4-(2-carboxyethyl)phenyl]-5-methoxy-8,8,18,18-tetramethylbacteriochlorin tetraiodide (BC8c). A mixture of **BC8b** (19.0 mg, 18.1 μmol) and tributylamine (33.6 mg, 181 μmol) in DMF (1.81 μL) was stirred under argon for 2 min, followed by addition of iodomethane (257 μL, 1.81 mmol). After 44 h, a solution of diethyl ether and THF (1 : 1) was added to the reaction mixture to precipitate the crude product. The suspension was sonicated for 3 min followed by centrifugation. The supernatant was discarded to afford a green solid. This procedure (diethyl ether and THF addition/sonication/centrifugation) was carried out three more times to afford a green solid (27.0 mg, 85%): ^{1}H NMR (300 MHz, DMSO-d_6; the CO_2H was not observed, and two ethylene protons were overlapped with the solvent peak) δ −1.59 (s, 1H), −1.20 (s, 1H), 1.82 (s, 6H), 1.99 (s, 6H), 2.74 (t, J = 7.5 Hz, 2H), 3.18 (s, 18H), 3.28 (s, 18H), 3.61 (s, 3H), 3.89–3.96 (m, 10H), 4.30 (s, 2H), 4.82–4.89 (m, 8H), 7.01 (d, J = 7.8 Hz, 2H), 7.43 (d, J = 7.8 Hz, 2H), 8.11–8.19 (m, 2H), 8.27 (s, 1H), 8.76 (s, 1H), 8.94–9.03 (m, 6H); ESI-MS obsd 305.1711, calcd 305.1716 $[(M - 4I)^{4+}$, M = $C_{70}H_{92}N_8O_{11}I_4]$; λ_{abs} (PB) 363, 519, 731 nm.

3,13-Bis[3,5-bis(2-(*N*,*N*,*N*-trimethylammonio)ethoxycarbonyl)-phenyl]-5-methoxy-15-[4-(2-(*N*-succinimidooxycarbonyl)ethyl)-phenyl]-8,8,18,18-tetramethylbacteriochlorin tetraiodide (BC8). A mixture of **BC8c** (8.0 mg, 4.6 μmol), DCC (6.7 mg, 33 μmol), and DMAP (0.20 mg, 1.6 μmol) in DMF (654 μL) was treated with HOSu (3.8 mg, 33 μmol) and stirred under argon at room temperature. After 8 h, diethyl ether was added to the reaction mixture to precipitate the crude product. The suspension was sonicated for 3 min followed by centrifugation. The supernatant was discarded to afford a green solid. This procedure (diethyl ether addition/sonication/centrifugation) was carried out three more times to afford a green solid (6.4 mg, 76%): ^{1}H NMR (300 MHz, DMSO-d_6, the CO_2H was not observed, two ethylene protons were overlapped with the solvent peak) δ −1.60 (s, 1H), −1.24 (s, 1H), 1.80 (s, 6H), 1.96 (s, 6H), 2.54–2.60 (m, 6H), 2.71 (t, J = 7.5 Hz, 2H), 3.16 (s, 18H), 3.27 (s, 18H), 3.57 (s, 3H), 3.88–3.91 (m, 10H), 4.30 (s, 2H), 4.81–4.84 (m, 8H), 6.97 (d, J = 7.5 Hz, 2H), 7.40 (d, J = 7.5 Hz, 2H), 8.09 (s, 2H), 8.35 (s, 1H), 8.73 (s, 1H), 8.94–9.02 (m, 6H); ESI-MS obsd 329.4254, calcd 329.4257 $[(M - 4I)^{4+}$, M = $C_{74}H_{95}I_4O_{13}N_9]$; λ_{abs} (PB) 362, 522, 729 nm.

15-[4-(2-(*tert*-Butoxycarbonyl)ethyl)phenyl]-3,13-bis[3,5-bis(2-(*tert*-butyldimethylsiloxy)ethylaminocarbonyl)phenyl]-5-methoxy-8,8,18,18-tetramethylbacteriochlorin (BC9a). Following a general procedure,[49] samples of **BC6a-Br** (35.0 mg, 24.4 μmol), **5** (40.5 mg, 121 μmol), $Pd(PPh_3)_4$ (11.3 mg, 9.70 μmol), K_2CO_3 (20.2 mg, 146 μmol) and toluene/DMF [2.40 mL (2 : 1), deaerated by bubbling with argon for 45 min] were added to a Schlenk flask and deaerated by three freeze–pump–thaw cycles. The reaction mixture was stirred at 90 °C for 16 h. The reaction mixture was allowed to cool to room temperature, then concentrated to dryness, diluted with CH_2Cl_2 and washed with saturated aqueous $NaHCO_3$. The organic layer was separated, dried (Na_2SO_4) and concentrated. Column chromatography [twice, silica, CH_2Cl_2/ethyl acetate (7 : 3 to 11 : 9)] afforded a green solid (15.0 mg, 39%): ^{1}H NMR ($CDCl_3$, 400 MHz) δ −1.55 (br, 1H), −1.19 (br, 1H), 0.086 (s, 12H), 0.093 (s, 12H), 0.871 (s, 18H), 0.879 (s, 18H), 1.50 (s, 9H), 1.85 (s, 6H), 1.99 (s, 6H), 2.55 (t, J = 5.4 Hz, 2H), 2.86 (t, J = 5.4 Hz, 2H), 3.63 (s, 3H), 3.69–3.73 (m, 8H), 3.85–3.91 (m, 11H), 4.37 (s, 2H), 6.68 (t, J = 3.6 Hz, 2H), 6.88 (t, J = 3.6 Hz, 2H), 7.01 (d, J = 5.7 Hz, 2H), 7.43 (d, J = 5.7 Hz, 2H), 7.82 (s, 2H), 8.05 (s, 1H), 8.44 (s, 1H), 8.65–8.66 (m, 3H), 8.72 (s, 3H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ −5.030, −5.015, 18.54, 18.56, 26.2, 28.4, 30.9, 31.32, 31.36, 36.8, 42.61, 42.68, 45.2, 46.0, 47.8, 52.3, 62.1, 62.2, 63.4, 80.7, 97.4, 97.8, 113.7, 123.2, 123.3, 124.4, 127.1, 127.6, 128.0, 131.9, 132.6, 133.4, 133.8, 134.0, 134.1, 134.2, 134.8, 135.5, 136.1, 138.96, 139.00, 139.4, 139.8, 155.1, 161.2, 166.6, 167.1, 169.3, 169.4, 172.6; MALDI-MS obsd 1560.0945; ESI-MS obsd 803.4280, calcd 803.4282 $[(M + 2Na)^{2+}$, M = $C_{86}H_{128}N_8O_{11}Si_4]$; λ_{abs} (CH_2Cl_2) 367, 518, 731 nm.

D. Spectroscopy

Solvents. The solvent systems employed herein are as follows: DMF of ≥99.8% purity; PBS (phosphate buffered saline) is aqueous 137 mM NaCl, 2.7 mM KCl, 10 mM Na_2HPO_4 and 1.8 mM KH_2PO_4 at pH 7.4; PB is aqueous potassium phosphate buffer (0.5 M, pH 7.0); and deionized water.

Absorption *versus* concentration study. For each bacteriochlorin, four different solutions (**A–D**) in PB were prepared. The concentration of solution **A** (~500 μM) afforded absorbance (A) ~ 0.5 for the Q_y transition measured with a 0.01 cm path-length cuvette. Successive serial dilution (10 times each) with PB

gave bacteriochlorin concentrations as follows: [solution **A**] = 10 × [solution **B**] = 100 × [solution **C**] = 1000 × [solution **D**].

A typical experiment proceeded as follows:[42] a stock solution was prepared by adding a small amount of DMSO (12.5–37.5 μL, to facilitate dissolution) to a bacteriochlorin (~0.1–0.3 mg; ~0.1–0.3 μM) in a small vial followed by addition of PB (237.5–712.5 μL). The resulting sample was sonicated for one minute and then filtered (poly-vinylidene difluoride high-volume low pressure filter, pore size 0.45 μm) to obtain solution **A** (~500 μM). The absorbance of solution **A** was measured in a 0.01 cm pathlength cuvette. Solution **B** was obtained by mixing solution **A** (100–200 μL) with PB (900–1800 μL) in a small vial. The absorbance of solution **B** was measured in a 0.1 cm pathlength cuvette. For further dilution, solution **B** (300 μL) was mixed with PB (2.70 mL) in a 1 cm pathlength cuvette to obtain solution **C** and the absorbance was measured. Solution **C** (2.50 mL) was transferred into a 25 mL volumetric flask and made up to the mark with PB. The absorbance of the resulting solution **D** was measured in a 10 cm pathlength cuvette. The procedure was followed for each bacteriochlorin **BC1–BC5**. Note that the range of volumes employed depends on the initial quantity of bacteriochlorin, which was added to the initial vial without weighing.

Photophysical studies. Photophysical measurements were performed as described previously[54,65] and employed dilute (μM) Ar-purged toluene solutions at room temperature. Samples for Φ_f measurements had an absorbance <0.12 at λ_{exc} and in the Q_y band. The Φ_f values were measured with respect to one or two standards including free base tetraphenylporphyrin (Φ_f = 0.070 in nondegassed toluene). The τ_S values were the average of results determined using (1) a time-resolved fluorescence stroboscope with a Gaussian instrument response function with ~1 ns width and (2) transient absorption studies with 0.5 μJ, ~100 fs excitation pulses. The latter apparatus was used to determine Φ_{isc} values *via* the extent of ground-state bleaching due to lowest singlet excited state (at early times) compared with that due to the lowest triplet excited state (at the asymptote of the singlet decay).

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