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Bacteriochlorin Dyads as Solvent Polarity Dependent Near-Infrared Fluorophores and Reactive Oxygen Species Photosensitizers

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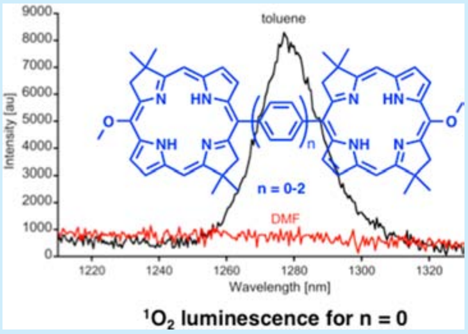
S *Supporting Information*

ABSTRACT: Symmetrical, near-infrared absorbing bacteriochlorin dyads exhibit gradual reduction of their fluorescence (intensity and lifetime) and reactive oxygen species photosensitization efficiency (ROS) with increasing solvent dielectric constant ε. For the directly linked dyad, significant reduction is observed even in solvents of moderate ε, while for the dyad containing a 1,4-phenylene linker, reduction is more parallel to an increase in solvent ε. Bacteriochlorin dyads are promising candidates for development of environmentally responsive fluorophores and ROS sensitizers.



Photonic agents with photochemical properties responsive to the local microenvironment are of great interest since they can function, for example, as fluorescent probes that monitor a variety of biological and biochemical processes[1] or photosensitizers of reactive oxygen species (ROS) selectively activated by a specific microenvironment.[2−4] Dielectric properties expressed by the dielectric constant ε vary significantly between intracellular organelles[5,6] and macrobiomolecules.[6−8] Recent findings also suggest that the local polarity (which is a function of ε) in the mitochondria of cancerous cells is lower compared to that of healthy ones.[9] Therefore, the local ε can be a potential target for activation of imaging or therapeutic agents. Although there is a plethora of solvatochromic fluorophores,[1,9,10] only a few of them have been utilized for determination of intracellular ε,[5,8] and there are only a handful of ROS photosensitizers which respond in predictable manner to the local dielectric properties.[2−4] However, the majority of ε-responsive fluorophores and photosensitizers absorb and emit at $\lambda < 600$ nm, while those with excitation/emission at $\lambda > 650$ nm, suitable for deep-tissue applications, are less common.[4,10] Moreover, such ε-responsive agents reported so far can usually perform either fluorescence or ROS sensitization functions separately. Bacteriochlorins, strongly near-infrared (near-IR) absorbing tetrapyrrolic macrocycles, exhibit relatively high both fluorescence quantum yield (Φ_f) and quantum efficiency of ROS photosensitization (Φ_{ROS}, ROS include singlet oxygen $^1O_2$, and superoxide radical $O_2^{\bullet-}$).[11,12] The relatively high Φ_{ROS} for bacteriochlorins originates from the high yield of intersystem crossing (Φ_{ISC}), leading to the long-lived triplet state capable of transferring either energy or electron to oxygen to produce $^1O_2$ or $O_2^{\bullet-}$, respectively.[12] Thus, bacteriochlorins are utilized as near-IR fluorophores, ROS photosensitizers for photodynamic therapy, and both simultaneously.[12,13] Recently, we found that strongly conjugated bacteriochlorin dyads exhibit a significant reduction in both Φ_f and τ_f in solvents of high ε due to the greatly enhanced internal conversion.[14,15] We reasoned that similar dependence should be true for Φ_{ROS}, thus allowing bacteriochlorin dyads to function as ε-dependent ROS photosensitizers. However, strongly conjugated bacteriochlorin dyads exhibit a low Φ_{ISC} (i.e., 0.09−0.39)[15] and, consequently, low Φ_{ROS} even in nonpolar solvents (see the Supporting Information for an example). Therefore, here we describe a new series of weakly conjugated bacteriochlorin arrays where bacteriochlorin subunits are connected either directly (**BC1**) or through 1,4-phenylene (**BC2**) or 4,4′-biphenylene (**BC3**) linkers. Our hypothesis is that such constructs should exhibit a relatively high Φ_f and Φ_{ROS} in solvents of low ε, and both of them will be reduced with increasing solvent polarity. As benchmarks, we include bacteriochlorin monomer **BC4** and directly linked chlorin dyad **C1**, analogous to **BC1**, to determine to what extent observed properties are specific to bacteriochlorin dyads (Figure 1).

Synthesis of **BC1** starts from Miyaura borylation of known 15-bromobacteriochlorin **BC5**,[16] which provides a boronic ester **BC6** in excellent yield (92%) (Scheme 1). Subsequent Suzuki coupling of **BC6** with **BC5** provides **BC1** in 62% yield.[17] Suzuki reaction of **BC5** with 1,4-bis(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)benzene or 4,4′-bis(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)-1,1′-biphenyl provides **BC2**

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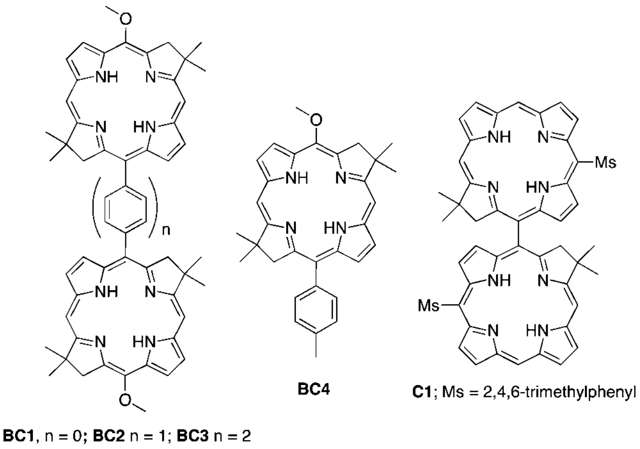
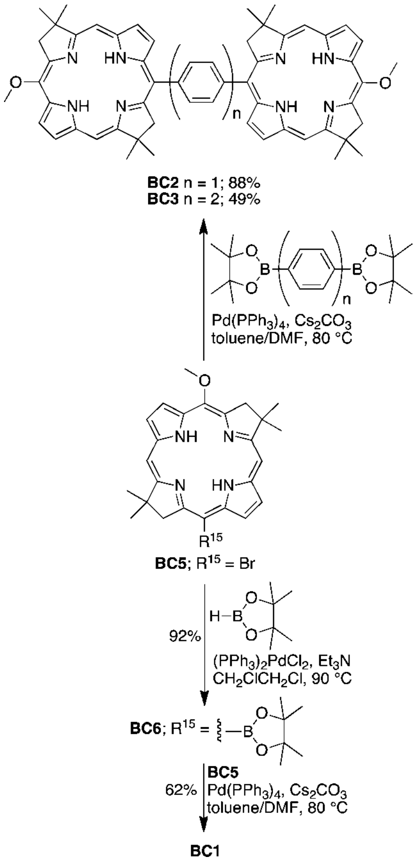


Figure 1. Structures of compounds **BC1–4** and **C1**.

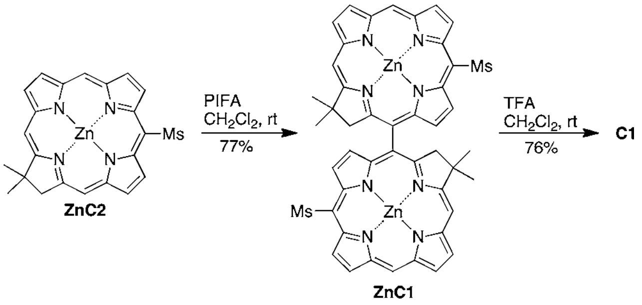
Scheme 1. Synthesis of Dyads BC1–3



(88% yield) or **BC3** (49% yield), respectively. Monomer **BC4** was synthesized in a similar Suzuki reaction (Scheme S1). Chlorin dyad **C1** was prepared following a published procedure[18] via PIFA-mediated oxidative coupling of monomer **ZnC2** and subsequent demetalation of resulted complex **ZnC1** (Scheme 2). All new compounds show ^{1}H, ^{13}C NMR, and MS data consistent with expected structures. Note that both **BC1** and **C1** are axially chiral, and accordingly, their ^{1}H NMR spectra show distinctive resonances of diastereotopic protons.

The absorption spectra of **BC1–4** (Figure 2 and Table 1) contain features typical for synthetic bacteriochlorins reported previously,[11] i.e., strong Q_y-type bands in the near-IR spectral window (712–738 nm), Q_x-type bands in the visible window (500–510), and B-type bands in the UV range (365–370 nm). Note that the Q_y band for **BC1** is split with the second maximum at 706 nm. Emission spectra in toluene consist of narrow 0–0 bands with a small (<7 nm) Stokes' shift versus the Q_y-type band. The maxima of both Q_y-type absorption and emission for dyads are shifted bathochromically, compared to corresponding bands in monomer **BC4**; the most pronounced shift is observed for **BC1** (365 and 436 cm^{-1} for absorption and emission, respectively) and is much lower for **BC2** (98 and 116 cm^{-1}) and **BC3** (39 cm^{-1} for both absorption and emission). The positions of absorption and emission maxima vary only slightly in solvents of different polarities (Table S1). The linker-dependent, bathochromic shifts of both the Q_y absorption and emission bands are indicative of increasing electronic communications between bacteriochlorin subunits in dyads with decreasing distance between them.

Scheme 2. Synthesis of Dyad C1



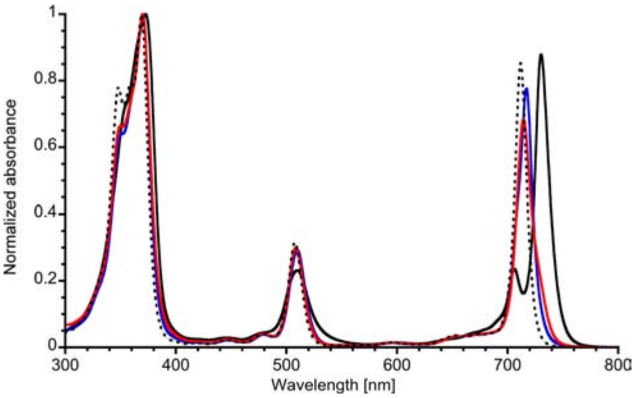


Figure 2. Absorption spectra of **BC1–4** in toluene: **BC1** (black, solid), **BC2** (blue), **BC3** (red), and **BC4** (black, dotted). All spectra are normalized at the *B*-band maxima.

Fluorescence properties of **BC1–4** and **C1** (Table 1, Figures S1–S3) were determined in an array of aprotic and protic solvents of broad ε range: toluene ($\varepsilon = 2.38$), THF ($\varepsilon = 7.58$), dichloromethane (CH_2Cl_2, $\varepsilon = 8.93$), PhCN ($\varepsilon = 26.0$), MeOH ($\varepsilon = 32.7$), and DMF ($\varepsilon = 36.7$). In toluene, **BC1** shows markedly higher Φ_f (0.28) than **BC4** (0.22), whereas both **BC2** and **BC3** show Φ_f in toluene comparable with that observed for **BC4**. For **BC1**, both Φ_f and τ_f diminish when solvent ε increases, resulting in a significant reduction of fluorescence in solvents of moderate ε (THF, CH_2Cl_2, PhCN) and almost no fluorescence in MeOH and DMF. A similar trend was observed for **BC2**; however, the reduction of both Φ_f and τ_f is less dramatic and more parallel with an increase in ε of the solvents. The τ_f in **BC2** decreases nearly linearly when ε of the solvent increases (Figure S5), which suggests that this dyad can be useful as a fluorescence lifetime probe for determination of

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Table 1. Photophysical Properties of BC1−4 and C1 in Solvents of Different Dielectric Constants[a]

solvent	Φ_f	τ_f (ns)	ϕ_{ROS}[b]
BC1: λ_{Qy} = 706, 731 nm, λ_{em} = 738 nm (in toluene)			
toluene	0.28	3.6	1.00
THF	0.20	3.1	0.87
CH_2Cl_2	0.037	0.48	0.097
PhCN	0.031	<0.4[c]	nd[d]
MeOH[e]	0.007[f]	<0.4[c]	nd[d]
DMF	0.003[f]	<0.4[c]	0.008
BC2: λ_{Qy} = 717 nm, λ_{em} = 721 nm (in toluene)			
toluene	0.22	4.1	1.00
THF	0.20	4.2	1.08
CH_2Cl_2	0.17	3.6	0.94
PhCN	0.11	2.4	nd[d]
MeOH[e]	0.069	2.3	nd[d]
DMF	0.066	1.6	0.39
BC3: λ_{Qy} = 714 nm, λ_{em} = 717 nm (in toluene)			
toluene	0.22	4.1	1.00
THF	0.19	4.1	1.06
CH_2Cl_2	0.18	3.8	0.91
PhCN	0.18	3.9	nd[d]
MeOH[e]	0.15	3.7	nd[d]
DMF	0.16	3.7	0.81
BC4: λ_{Qy} = 712 nm, λ_{em} = 715 nm (in toluene)			
toluene	0.22	4.4	
THF	0.19	4.5	
CH_2Cl_2	0.19	4.2	
PhCN	0.22	4.6	
MeOH[e]	0.15	4.2	
DMF	0.20	4.6	
C1: λ_{Qy} = 650 nm, λ_{em} = 654 nm (in toluene)			
toluene	0.31	5.9	nd[d]
DMF	0.29	6.0	nd[d]

[a] All data were determined in air-equilibrated solvents. For Φ_f and τ_f determination samples were excited at the maxima of their Q_x band and 375 nm, respectively. Fluorescence quantum yields were determined with respect to *meso*-tetraphenylporphyrin (TPP, Φ_f = 0.070 in nondegassed toluene).[11] The estimated error in Φ_f, τ_f, and ϕ_{ROS} determination is ±10%. [b] For details of ϕ_{ROS} determination, see the SI. [c] The τ_f is too short to be accurately determined by our experimental setup. [d] Not determined. [e] 5% of THF (v/v) was used as a cosolvent. [f] The weak emission signal at 736 nm overlaps with the second weak emission peak at 717 nm of unknown origin; therefore, the Φ_f is approximate.

local dielectric constants. **BC3** shows marked reduction of Φ_f in DMF only. Both **BC4** and **C1** show virtually no dependence of both Φ_f and Φ_f on solvents ε.

These results indicate that for bacteriochlorin dyads a new and efficient process for deactivation of the excited state, which competes with fluorescence, becomes accessible in polar solvents. The efficiency of this process clearly depends on ε of the solvent. Moreover, the efficiency of this new process appears to depend on the electronic conjugation between macrocycles in dyads, as the most extensive reduction of fluorescence in given solvent is observed for **BC1** (where electronic communication between macrocycles is presumed to be the strongest). Importantly, this process is specific only for bacteriochlorin dyads since fluorescence properties for the monomer **BC4** and for the chlorin dyad **C1** are nearly solvent-independent. To the best of our knowledge, analogous porphyrin dyads show only a slight dependence of their photophysical properties on solvent ε.[19]

Next, we determined the influence of solvent ε on ROS photosensitization for **BC1−4** using 2,5-diphenylisobenzofuran (DPBF). DPBF reacts with $^1O_2$ and $O_2^{\bullet-}$, causing a decay of DPBF absorbance at 414 nm.[20,21] The comparison of Φ_{ROS} for a given photosensitizer in different solvents is more complex than in the case of Φ_f discussed above. Φ_{ROS} depends on both the properties of the photosensitizer excited states (i.e., Φ_{ISC} and triplet state lifetime) as well as solvent properties such as oxygen solubility, rate of oxygen diffusion, rate of bimolecular quenching of triplet state by oxygen, etc.[22] Thus, influence of a solvent on Φ_{ROS} is observed, to some extent, for many photosensitizers.[23] Moreover, rate of DPBF degradation depends not only on the amount of singlet oxygen generated by the photosensitizer but also on the lifetime of singlet oxygen in given solvents and bimolecular rate constants for reaction of singlet oxygen with DPBF, both of which vary in different solvents.[24] Hence, to evaluate the influence of solvent ε on ROS photosensitization of dyads, we determined how different the influence of solvent ε on ROS photosensitization is for a given dyad compared to that of the benchmark monomer **BC4**. Quantitatively, this influence was expressed through the relative quantum yield of ROS photosensitization ϕ_{ROS} (see the SI for the exact definition and measurement methodology). The ϕ_{ROS} were determined for dyads **BC1−3** in four solvents: toluene (as a reference solvent), THF, CH_2Cl_2, and DMF. The plots of rate of DPBF absorbance decays for **BC1−4** are shown in Figure S8, and the resulting ϕ_{ROS} are given in Table 1. For **BC1**, we observed a dramatic reduction of ϕ_{ROS} in both CH_2Cl_2 (10-fold) and DMF (125-fold) compared to that in toluene. For **BC2** and **BC3**, ϕ_{ROS} exhibit a lesser reduction than **BC1**, with the exception of DMF, for which ϕ_{ROS} is significantly (2.5-fold) lower for **BC2** but only slightly (1.2-fold) lower for **BC3**, compared to toluene. The influence of solvent ε on ϕ_{ROS} reduction was further confirmed by determining the rate of DPBF decay in the presence of **BC1** in a series of toluene/DMF mixtures with increasing DMF concentration. The ϕ_{ROS} gradually decreases with increasing ratio of DMF in the mixture (Figure S9).

The quenching of $^1O_2$ photosensitization of **BC1** in DMF was further confirmed by monitoring $^1O_2$ luminescence at 1270 nm, which in toluene is observed for both **BC1** and **BC4**, but in DMF it is observed only for **BC4** and is completely absent for **BC1** (Figure S7). The quantum yields of $^1O_2$ photosensitization (Φ_Δ) in air-equilibrated toluene (determined by comparing the intensity of $^1O_2$ luminescence generated by bacteriochlorins, to those generated by TPP) are 65% for both **BC1** and **BC4**. Φ_Δ corresponds well with Φ_{ISC} determined previously for various *meso*-substituted bacteriochlorins, (i.e., Φ_{ISC} = 0.54−0.71).[11]

In summary, fluorescence and ROS photosensitization ability in bacteriochlorin dyads are reduced when the solvent ε increases. The degree of quenching of photochemical activity in these dyads appears to be dictated by the strength of electronic interaction between bacteriochlorin subunits. This indicates that the degree of the response of photochemical properties to the solvent ε in dyads can be adjusted for desired applications and polarity ranges by proper molecular design (i.e., selecting the linker between bacteriochlorin subunits). These properties, along with high Φ_f and relatively long τ_f of near-IR emission, as well as high Φ_Δ in nonpolar environment, allow bacteriochlorin dyads to be promising platforms for development of smart

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fluorophores and ROS photosensitizers activated in an environment of low ε and fluorescence lifetime probes for determination of local dielectric properties.

ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.orglett.6b02237.

> Additional absorption and emission spectra, plots of DPBF decay in various solvents, experimental details, and NMR spectra for new compounds (PDF)

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Notes

The authors declare no competing financial interest.

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(20) Bacteriochlorins can photosensitize both $^1O_2$ and $O_2^{\bullet-}$, depending on their exact structures.[12] We confirmed that **BC1−4** photosensitize $^1O_2$ (by determining luminescence at 1270 nm); however, we did not make any efforts to determine whether or not any of the bacteriochlorins examined here photosensitizes $O_2^{\bullet-}$.

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