Electronic Supplementary Material (ESI) for New Journal of Chemistry.

Supplementary Information for:

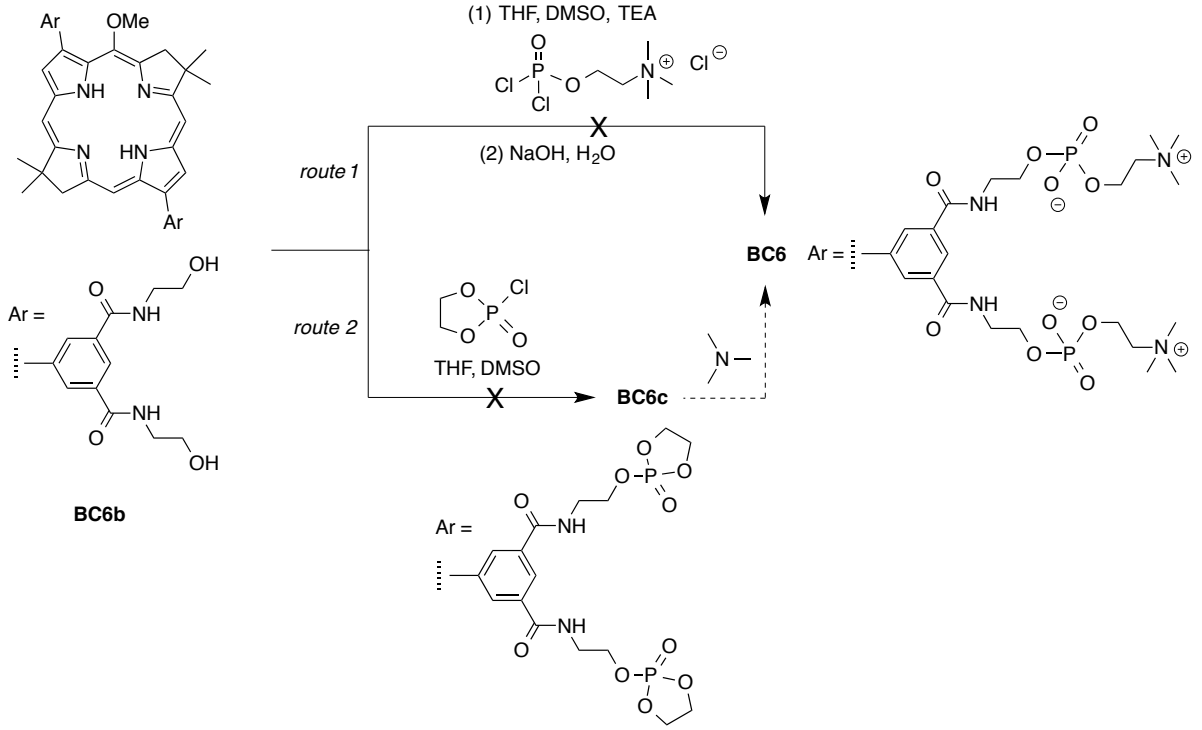
Synthetic Bacteriochlorins Bearing Polar Motifs (Carboxylate, Phosphonate, Ammonium and a Short PEG). Water-solubilization, Bioconjugation, and Photophysical Properties

Jianbing Jiang, Eunkyung Yang, Kanumuri Ramesh Reddy, Dariusz M. Niedzwiedzki, Christine Kirmaier, David F. Bocian, Dewey Holten and Jonathan S. Lindsey

Table of Contents

1. Attempted synthesis of a phosphatidylcholine bacteriochlorin.

Attempts to incorporate the 1,3,2-dioxaphospholane 2-oxide unit onto the tetrahydroxybacteriochlorin **BC6b** failed to give the desired compound **BC6c** in clean fashion (Scheme S1). While peaks corresponding to the mono-, di-, tri-phosphorylated and desired products were observed upon MALDI-MS analysis (Figure S1), the reaction could not be driven to completion, despite use of large quantities (up to 40 equiv) of the 2-chloro-1,3,2-dioxaphospholane 2-oxide reagent at 40 °C. Attempted analysis by TLC failed to separate the desired product from the incompletely derivatized intermediates.



Scheme S1. Attempted synthesis of bacteriochlorin-phosphatidylcholine **BC6**.

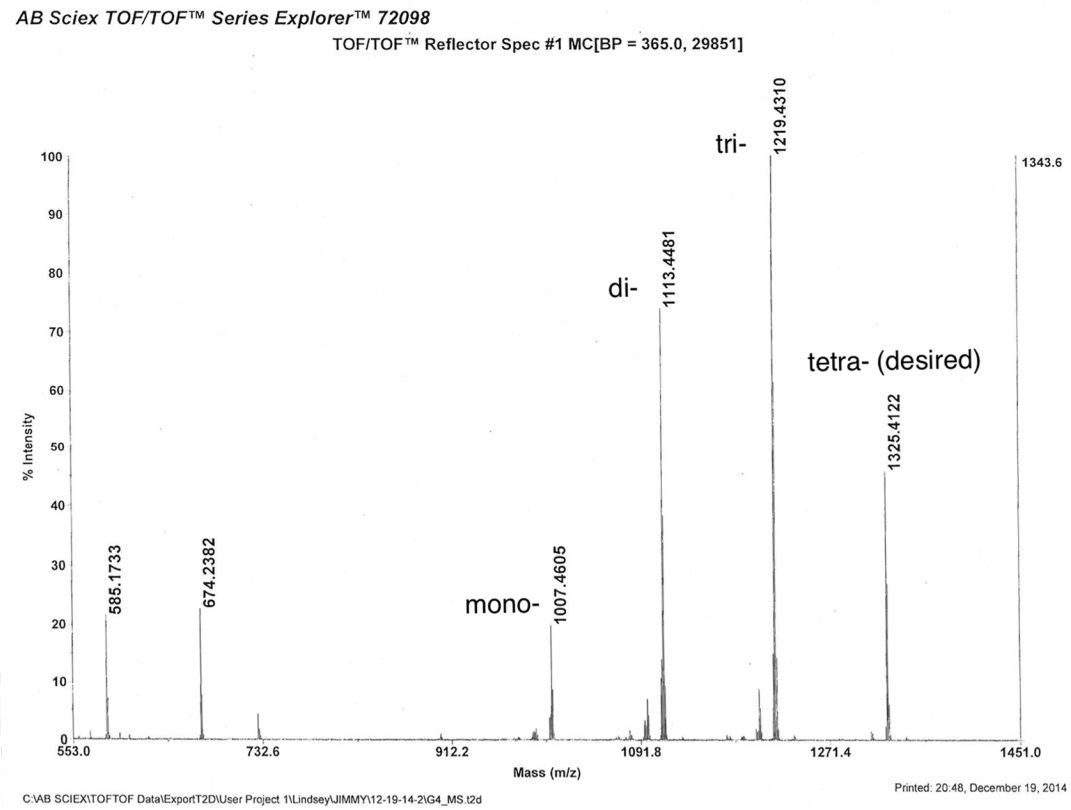


Figure S1. MALDI-MS spectrum in the attempted synthesis of bacteriochlorin **BC6c**. The spectrum was recorded after 2 h of reaction of the bacteriochlorin (10 mM in THF) with 8 equiv of 2-chloro-1,3,2-dioxaphospholane 2-oxide and 8 equiv of triethylamine. The mono-, di-, and tri- labels refer to bacteriochlorin **BC6b** bearing 1–3 dioxaphospholane 2-oxide units.

Alternatively, reaction of bacteriochlorin **BC6b** with phosphorylcholine dichloride followed by hydrolysis but the bacteriochlorin could not be purified to homogeneity (Scheme S1). The crude reaction mixture was dialyzed (regenerated cellulose membrane, dialysis cassette, 2 K molecular weight cutoff) overnight in water. However, the resulting product contained substantial amounts of unidentified impurities, as determined by ^{1}H NMR spectroscopy. The target bacteriochlorin **BC6** was not detected by MALDI-MS or ESI-MS analysis. Also, the absorption spectra of the crude product show a broader absorption band than its counterparts bearing different water-solubilizing groups (Figure S2). The fwhm value of the crude **BC6** is 28 or 38 nm in DMF or PB, respectively, to be compared with the PEGylated bacteriochlorin **BC5** with fwhm value or 22 or 23 nm in DMF or water, respectively. On the basis of these results, we ceased pursuit of the target bacteriochlorin **BC6**.

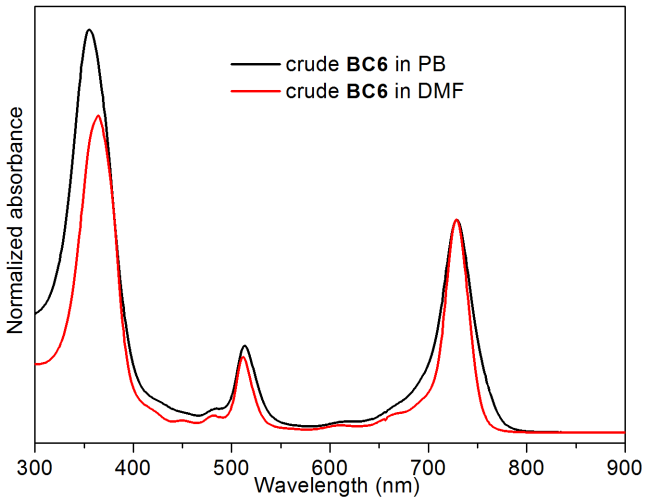
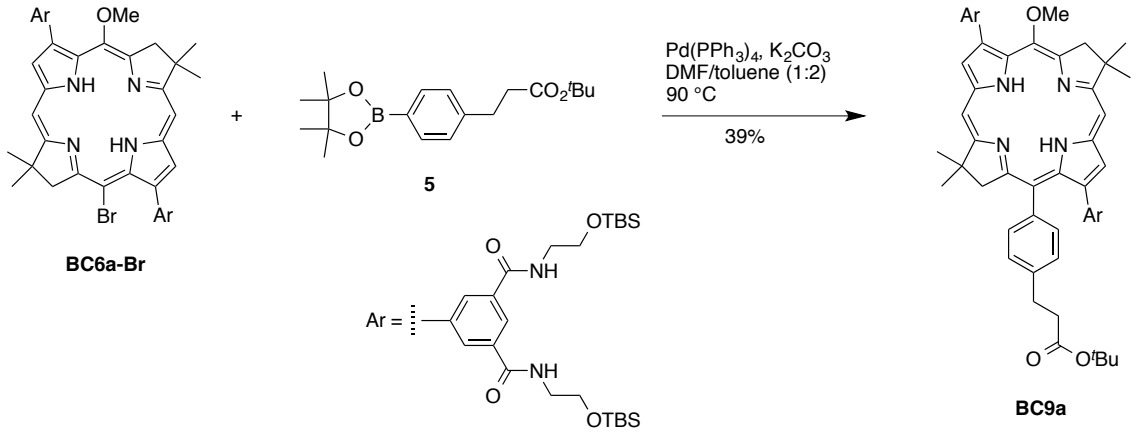


Figure S2. Absorption spectra of crude **BC6** in DMF and in PB. The spectra were normalized at the Q_y band. The concentrations are ~4 μM, calculated based on absorption in the 1-cm cuvette, assuming $\varepsilon(Q_y) = 120{,}000\ M^{-1}cm^{-1}$.[184]

The 15-bromo derivative of bacteriochlorin **BC6a** (**BC6a-Br**) was coupled with **5** to give the corresponding bacteriochlorin **BC9a** as shown in Scheme S2. The incomplete incorporation of the phosphatidylcholine moiety to prepare **BC6** (Scheme S1) prompted the parallel work on bioconjugation to be discontinued.



Scheme S2. Attachment of a bioconjugatable tether to bacteriochlorin **BC6a-Br**.

2. Mass spectrometry analysis for bacteriochlorins BC1–BC5.

The five water-solubilizing motifs exhibit potentially distinct behavior upon mass spectrometric analysis given the presence or absence of charged moieties. Thus, the tetraphosphonic acid **BC1** can in principle exhibit 0–8 negative charges, the tetracarboxylic acid **BC2** can in principle exhibit 0–4 negative charges, the tetraammonium **BC3** and **BC4** each bear four cationic charges, and the PEGylated **BC5** is neutral. Hence, it is of interest to comment on the observed charged state upon mass spectrometry analysis. Tetrapyrroles typically afford much higher quality mass spectra upon positive-ion rather than negative-ion analysis. The results upon positive-ion mass spectrometry are summarized in Table S1.

Table S1. Detected charge states of hydrophilic bacteriochlorins upon MALDI-MS and ESI-MS analysis.

Compound	MALDI-MS charge state	ESI-MS charge state
BC1	N.O.[a]	N.O.
BC2	+1	+1
BC3	N.O.	+4
BC4	N.O.	+4
BC5	+1	+1
BC7	N.O.	N.O.
BC8	N.O.	+4

[a]Not observed.

BC1 did not give a detectable signal for either MALDI-MS or ESI-MS analysis. **BC2** and **BC5** each displayed a monocationic species upon both MALDI-MS and ESI-MS analysis. **BC3** and **BC4** gave no signal was obtained upon MALDI-MS analysis using various matrices (POPOP, 4-hydroxy-α-cyanocinnamic acid or sinapic acid). On the other hand, a quadruply charged species was observed upon ESI-MS analysis (obsd *m/z* = 210.1528 for **BC3**, obsd *m/z* = 268.1584 for **BC4**, respectively, where z = 4).

3. Photophysical properties of bacteriochlorins.

Spectral data for the bacteriochlorins in DMF, PBS and PB are summarized in Table S2.

Table S2. Absorption and fluorescence properties of bacteriochlorins.[a]

cmpd	solvent	λ_{abs} (nm)	fwhm abs (nm)	λ_{em} (nm)	fwhm em (nm)
BC1a	DMF	729	21	736	23
BC1	PBS	729	26	736	30
BC1	PB	730	29	735	28
BC2a[b]	DMF	731	24	738	25
BC2	DMF	728	22	735	24
BC2	PBS	729	26	736	26
BC2[b]	PB	730	26	736	26
BC3a	DMF	729	21	734	23
BC3	DMF	731	23	737	25
BC3	PBS	732	26	739	26
BC3	PB	732	25	739	26
BC4a	DMF	730	23	738	23
BC4	DMF	731	23	739	25
BC4	PBS	731	40	739	26

BC4	PB	731	31	738	24
BC5	DMF	729	22	735	24
BC5	PBS	727	22	733	23
BC5	Water	728	23	733	23
BC7c	DMF	725	25	732	27
BC7c	PBS	729	25	735	27
BC8c	DMF	730	25	736	27
BC8c	PBS	727	37	736	27

[a]All data were acquired at room temperature. [b]Data from ref. 312.

Figure S3A shows the absorption spectrum of **BC5** in PBS taken at various times during the course of routine sample preparation and analysis using static and time-resolved fluorescence measurements. Panels B-F of the figure focuses on the Q_y region for bacteriochlorins **BC1–BC5**. The line/color coding for all panels is that shown at the top right of panel A. In each case the sample was prepared by adding solid bacteriochlorin to PBS with no pre-solubilization with DMF.

The data show that some "equilibration" is typically required for the bacteriochlorins after sample preparation before making quantitative spectroscopic measurements. The effect does not reflect chemical or photo-induced instability but rather an aspect of this class of compounds. The data in Figure S3 show an average 15% diminution in the intensity of the overall spectrum between initial sample preparation (black), sitting in the dark undisturbed for 30 min (red), and then again after purging with argon (which produces large hydrophobic bubbles) for 30 min (blue). The insets for the Soret, Q_x and Q_y bands show that no subsequent change within experimental error is observed after the sample sits for another 30 min prior to making fluorescence measurements (dashed magenta) or several hours later after static and time-resolved fluorescence measurements are complete (dotted green). Such spectral changes were not observed for **BC2 – BC4** in DMF (**BC1** is not soluble in DMF) or for hundreds of such prior studies for hydrophobic bacteriochlorins in organic solvents.

Bacteriochlorin **BC8c** behaves similarly to its analog **BC4** (which lacks the 15-bioconjugatable linker). Bacteriochlorin **BC7c** showed a diminution of absorption intensity of ~50% during sample preparation and handling, over twice that for its analog **BC3**. [In this case, the solid was pre-solubilized with DMF (1% of final volume) or else the compound was insoluble in PBS.] Thus, the addition of the benzoic-acid-terminated precursor of the bioconjugatable tether of **BC7** appears to render this construct less soluble in the aqueous medium.

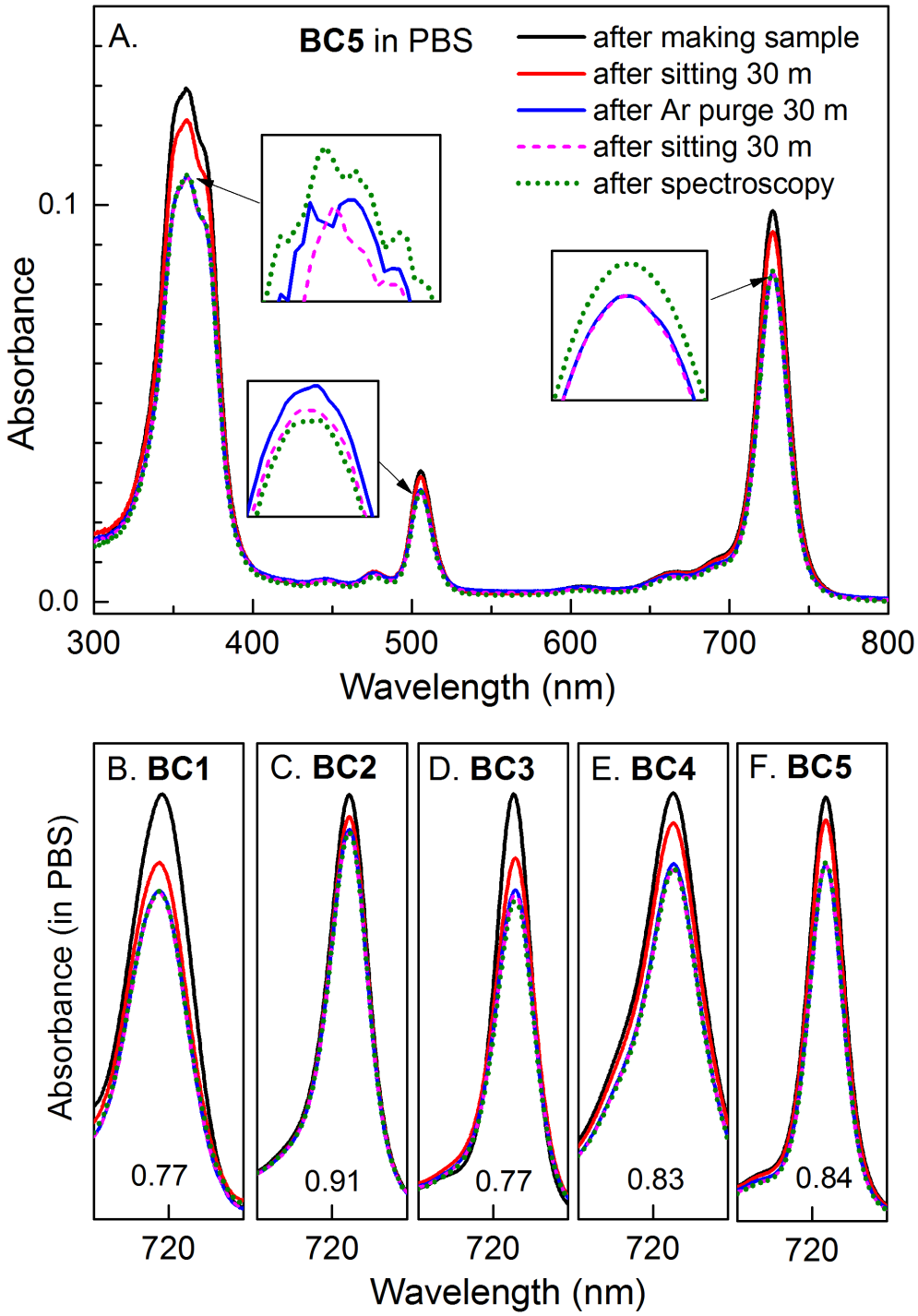


Figure S3. (A) Absorption spectrum of **BC5** in PBS taken at various points during the course of sample preparation and routine static and time-resolved fluorescence measurements. The insets show that the spectrum remains unchanged for the last three scans, namely after final sample preparation and over the course of the spectroscopic studies. Panels B-F show a focused view of the Q_y region for **BC1** - **BC5** in PBS for the same five absorption scans. The value at the bottom is the ratio of the peak absorption prior to (and after) spectral studies (e.g., blue trace) to that obtained immediately when sample was first prepared (black trace).