Supplementary Information for:

Near-Infrared Tunable Bacteriochlorins Equipped for Bioorthogonal Labeling

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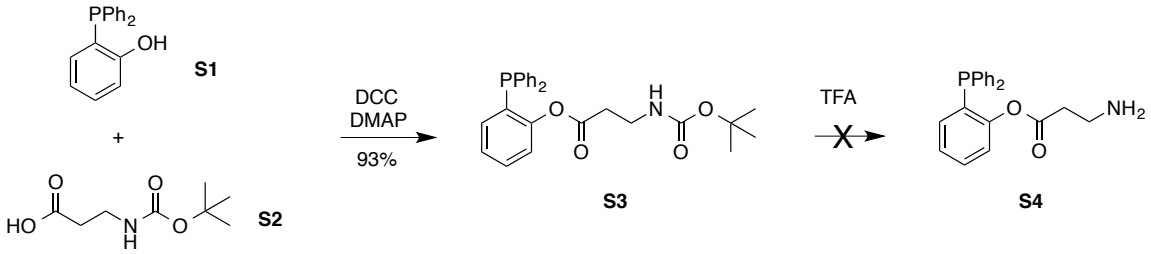
1. Synthesis of a phosphorane intermediate.

An aminoalkyl unit containing a phosphine moiety (**S4**) was designed for incorporation into a carboxy-bacteriochlorin for use in Staudinger ligation (Scheme S1). Treatment of (2-hydroxyphenyl)diphenylphosphine (**S1**) with Boc-protected β-alanine (**S2**) in the presence of DCC and DMAP afforded compound **S3** in 93% yield. However, cleavage of the Boc protecting group of **S3** failed to give **S4**, perhaps due to oxidation of phosphine or the ligation between the phosphine and nitrogen atoms. We turned to use of the borane-blocked phosphine (**4**, Scheme 5) as described in the body of the paper.



Scheme S1. Synthesis of a phosphorane intermediate.

2-Diphenylphosphinophenyl 3-(*tert*-butoxycarbonylamino)propanoate (S3). A mixture of (2-hydroxyphenyl)diphenylphosphine (**S1**, 100 mg, 359 μmol), 3-[(*tert*-butoxycarbonyl)amino]propanoic acid (**S2**, 81.6 mg, 431 μmol) and DMAP (4.40 mg, 36.0 μmol) in CH_2Cl_2 (1.80 mL) was treated with DCC in CH_2Cl_2 (1.80 mL). The mixture was stirred at room temperature for 1.5 h. The mixture was filtered to remove insoluble material. The filtrate was concentrated and chromatographed (silica, CH_2Cl_2) to yield a viscous colorless liquid (150 mg, 93%): ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.44 (s, 9H), 2.48 (t, J = 5.7 Hz, 2H), 3.27–3.32 (m, 2H), 5.06 (br, 1H), 6.82–6.87 (m, 1H), 7.13–7.18 (m, 2H), 7.28–7.41 (m, 11H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 28.6, 34.9, 36.1, 79.8, 122.7, 126.6, 128.8, 128.9, 129.4, 130.2, 134.0, 134.3, 135.4, 135.5, 156.0, 170.7; ^{31}P NMR (121 MHz, $CDCl_3$) –16.0; ESI-MS obsd 450.1834, calcd 450.1756 [$(M + H)^+$, M = $C_{26}H_{28}NO_4P$].

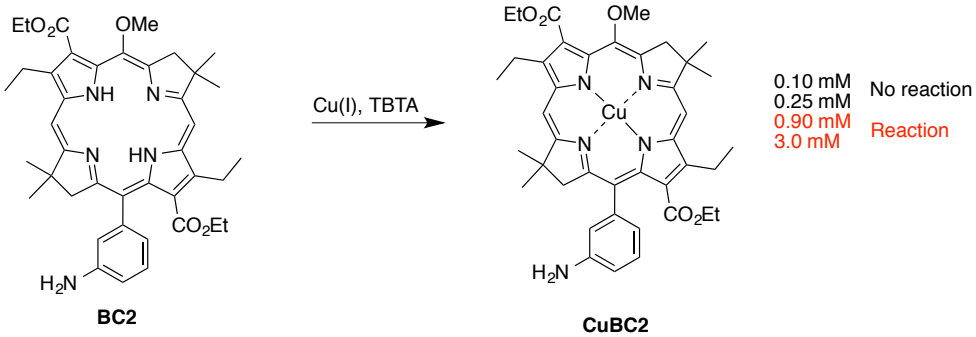
2. Test reactions for copper insertion.

Two sets of studies were carried out concerning copper insertion in bacteriochlorins. The first set employed copper(II) without a nitrogenous ligand and is described in the main body of the paper. The relevant spectra are displayed below. The reactions were carried out in DMSO/H_2O (3:1) with copper(II) acetate. The absorption spectra provide a quantitative measure of metalation, where the diagnostic feature is the bathochromic shift and broadening of the Q_y band. The absorption spectra for **BC1** show only a hint of metalation after 21 h for the more dilute reaction (0.2 mM) as shown in Figure S1-A, whereas no change was noted at all in the reaction at 5 mM (Figure S1-B). The corresponding MALDI-MS data are consistent with the absorption spectra (Figures S2-A,B). Note that the peak intensity in the MALDI-MS data cannot be directly compared in the absence of knowledge concerning ionization efficiencies in the mass spectrometry experiment.

The reaction of **BC2** show extensive metalation after 4 h for the more dilute reaction (0.2 mM) as shown in Figure S1-C, as well as for the reaction at 5 mM (Figure S1-D). The corresponding MALDI-MS data are consistent with the absorption spectra (Figures S2-C,D).

While the overall results are quite clear – that **BC1** is relatively slow to metalate whereas **BC2** is relatively fast to metalate for a given set of conditions – comparisons at different concentrations may need to take into consideration the possibility of intermolecular aggregation of the bacteriochlorins in the reaction medium. Aggregation at higher concentration could in fact lead to a less effective reaction. In each of the reactions examined in this second set of studies (for **BC1** and **BC2**), the reaction medium appeared somewhat cloudy upon addition of the copper solution [in DMSO/H_2O (1:1)] to the bacteriochlorin solution (in DMSO). Control experiments showed that addition of copper(II) acetate alone to the reaction medium [DMSO/H_2O (3:1)] afforded a heterogeneous mixture. Whether other species also are insoluble remains uncertain. The minimum conclusion is that the studies reported herein on copper insertion were carried out under heterogeneous conditions.

The second set concerned the use of Cu(I) in the presence of a nitrogenous ligand and is described as follows. The test metalation of bacteriochlorin **BC2** (precursor of **BC8**) with Cu(I) (5.0 equiv relative to bacteriochlorin) was conducted at different bacteriochlorin concentrations (0.10 mM, 0.25 mM, 0.90 mM and 3.0 mM) as shown in Scheme S2. When the reaction concentration was above 0.9 mM, copper metalation occurred, which necessitates the protection of **BC8** from copper metalation. Zinc metalation was done to protect **BC8** from copper insertion (**ZnBC8**).



Scheme S2. Model study for copper metalation.

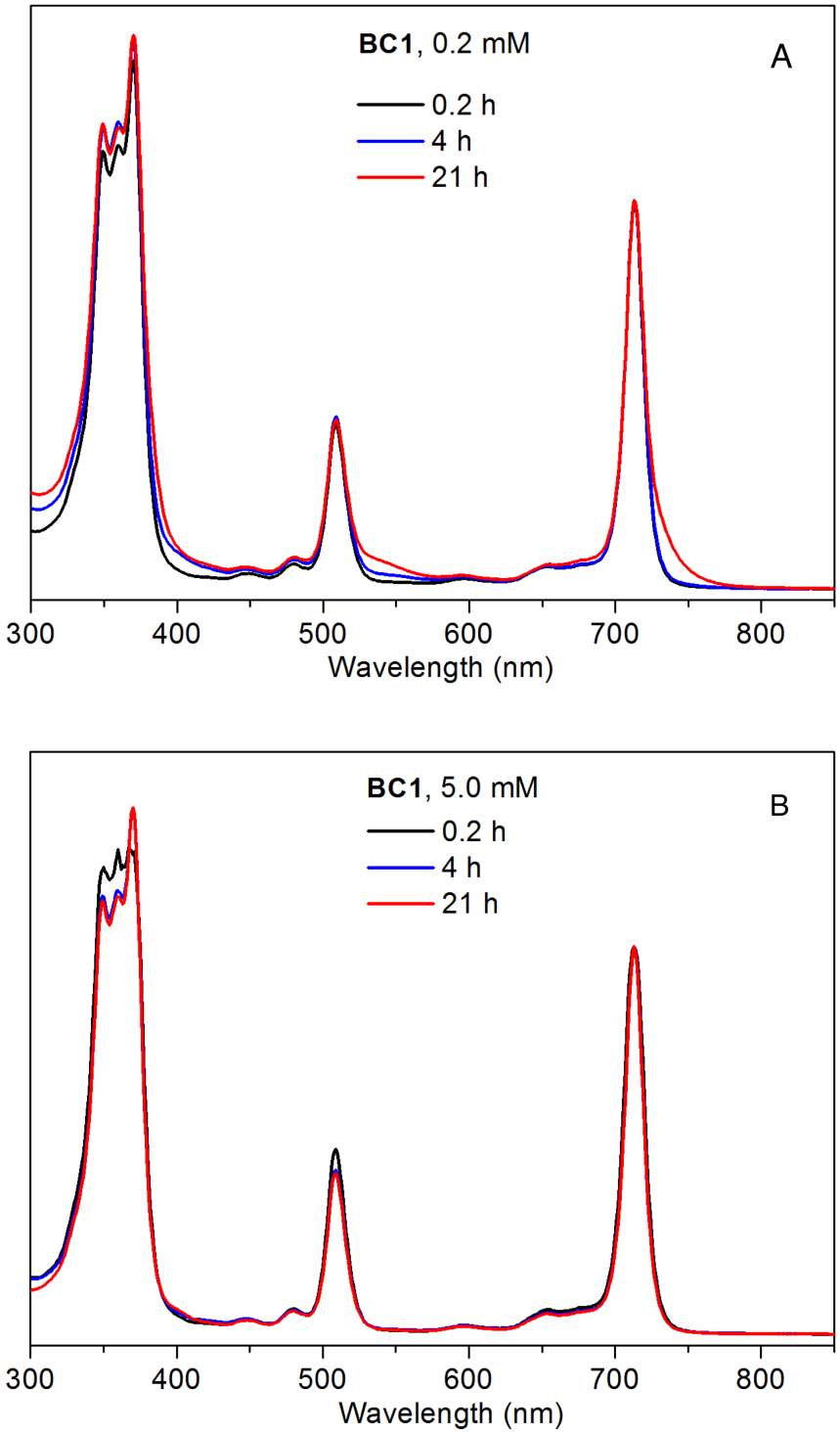


Figure S1 (A, B). Absorption spectra of bacteriochlorins **BC1** and **BC2** for the copper metalation study reported in the body of the paper. All spectra were recorded in DMSO at room temperature. Spectra in panel A and B normalized at the bacteriochlorin Q_y band. Absorption spectra in panels C and D are displayed at arbitrary intensities.

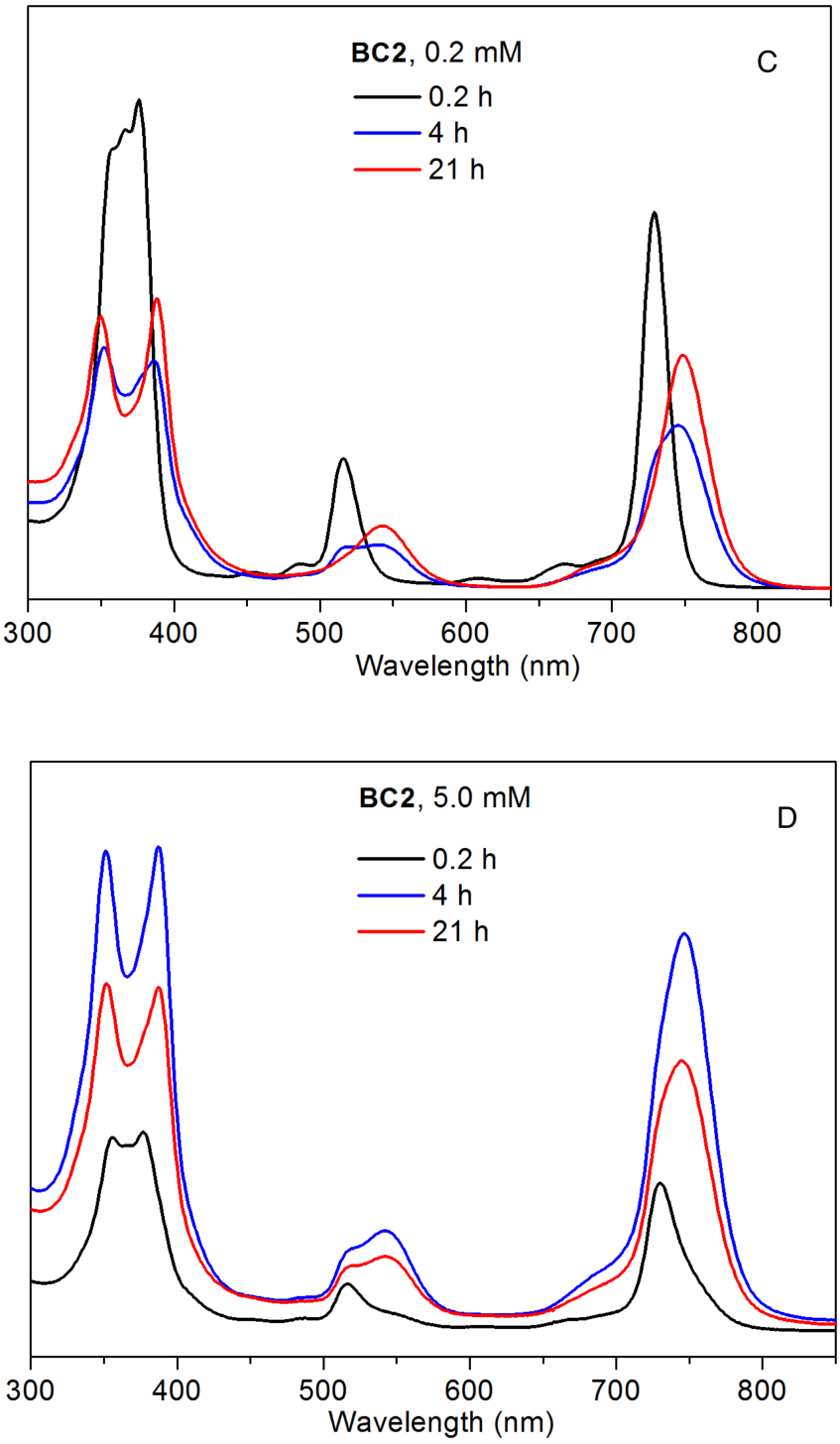


Figure S1 (C, D). Absorption spectra of bacteriochlorins **BC1** and **BC2** for the copper metalation study reported in the body of the paper. All spectra were recorded in DMSO at room temperature. Spectra in panel A and B normalized at the bacteriochlorin Q_y band. Absorption spectra in panels C and D are displayed at arbitrary intensities.

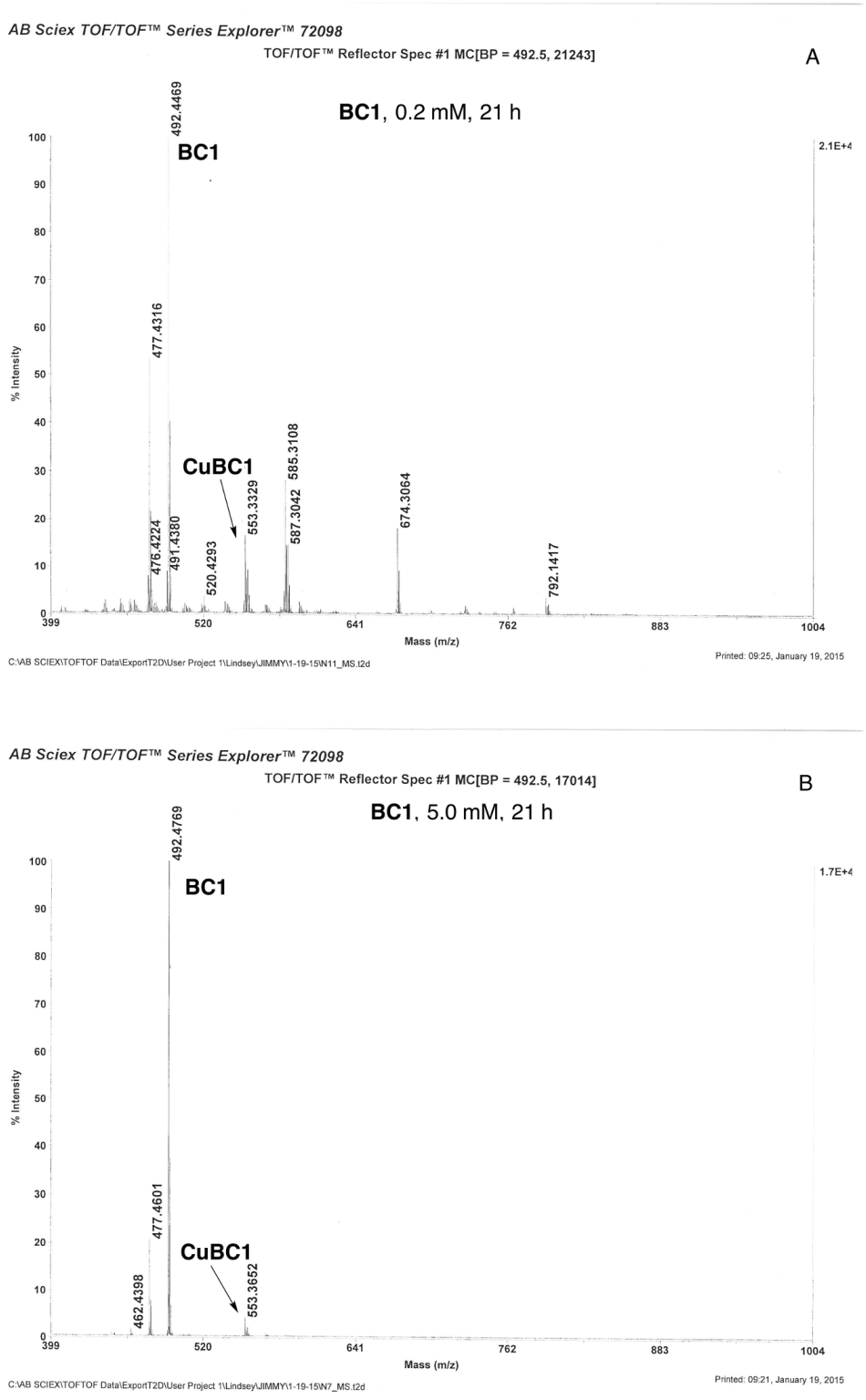
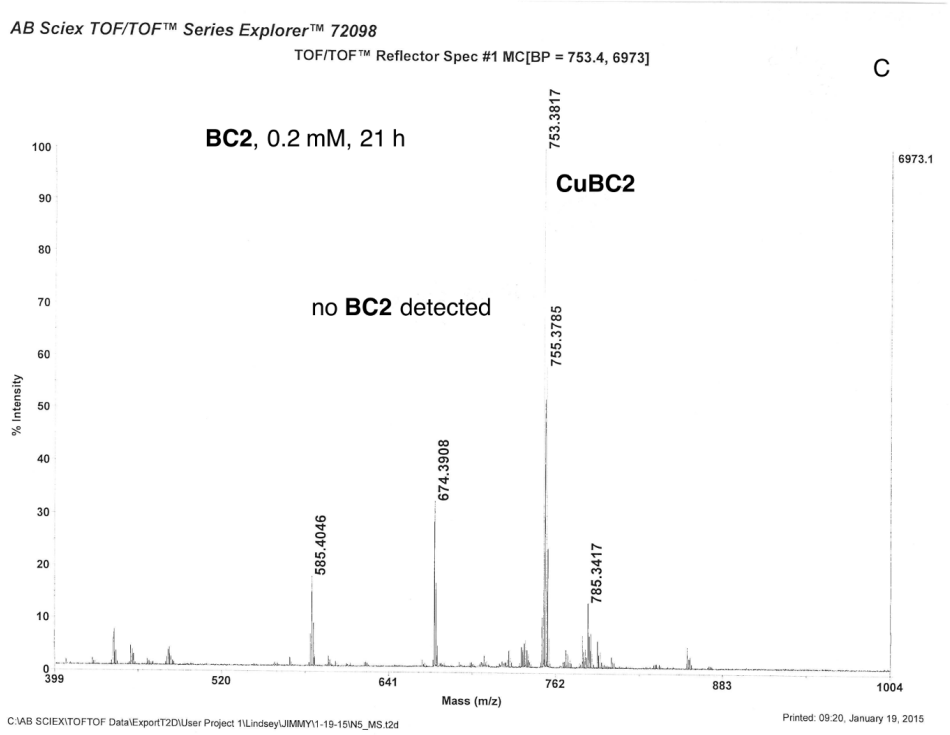


Figure S2 (A, B). MALDI-MS data of bacteriochlorins **BC1** and **BC2** for the copper metalation study reported in the body of the paper.



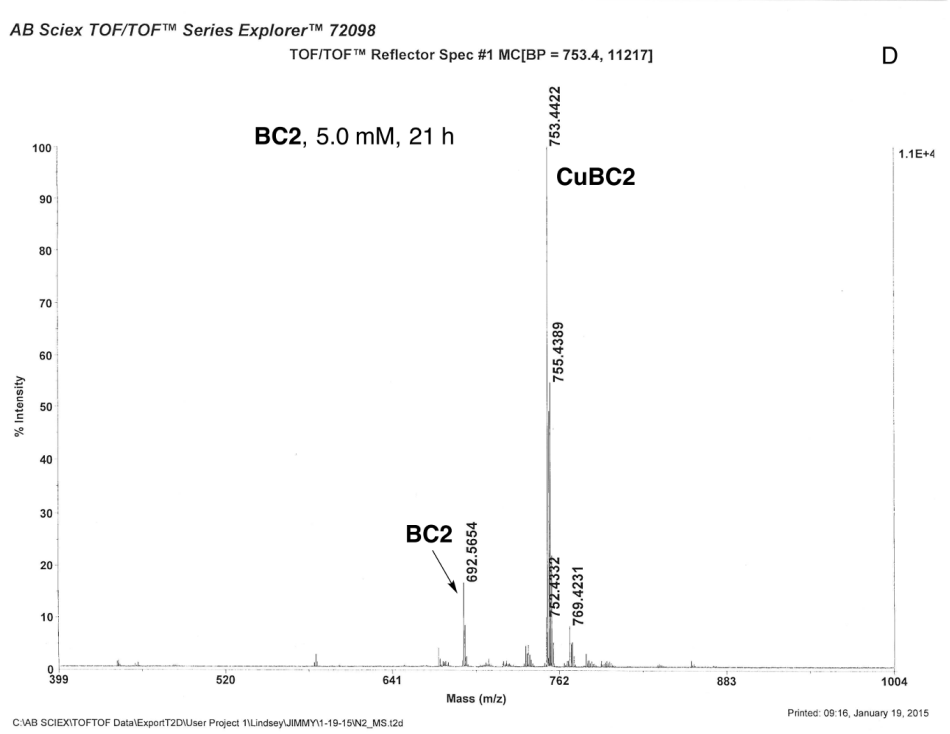
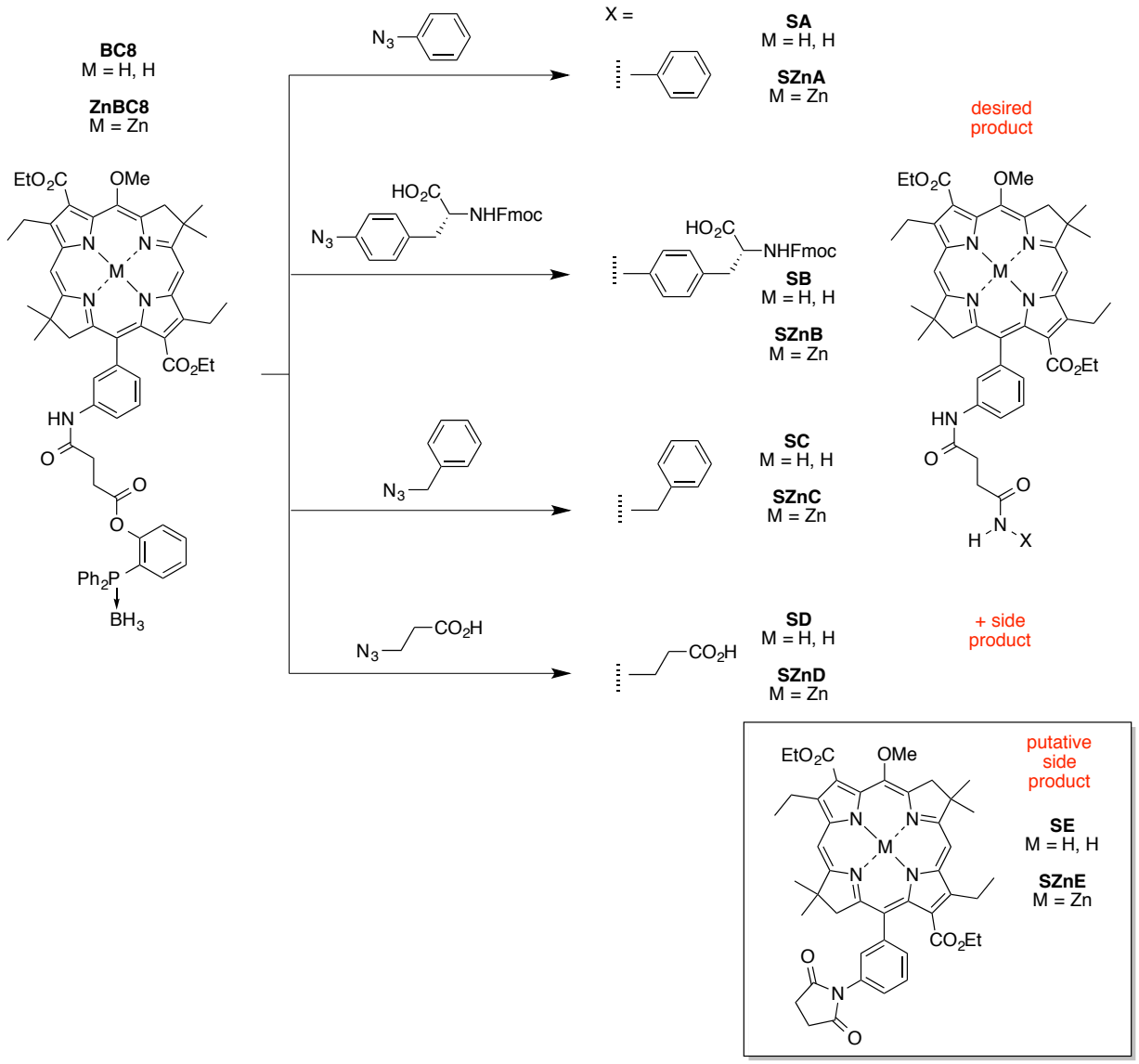


Figure S2 (C, D). MALDI-MS data of bacteriochlorins **BC1** and **BC2** for the copper metalation study reported in the body of the paper.

3. Staudinger ligations.

To test the Staudinger reaction of **BC8** and **ZnBC8**, a series of test reactions was done using different azido compounds (Scheme S3). The expected product is shown (**SA**, **SB**, **SZnB**, **SC**, **SZnC**, **SD, SZnD**), each of which differs only in the nature of the free base or zinc bacteriochlorin and the amide substituent. In each case, a common side product was observed. A proposed structure for the common side product, **SE** or **SZnE**, is also shown in Scheme S3.



Scheme S3. Model studies of Staudinger ligation.

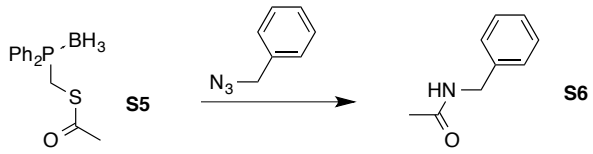
The particular conditions and the results of the reactions shown in Scheme S3 and displayed in Table S1. Little of the expected product was observed for **BC8** (entries 1–5) and for **ZnBC8** (entry 6). The formation of the side product may stem from the aromatic amide hydrogen attacking the iminophosphorane intermediate.

Table S1. Test reactions for Staudinger ligation.

Entry	Cmpd[b]	Reagent	Solvent	Temp (°C)	Time (h)	Expected product	Results
1	**BC8**	Phenyl azide, 10 eq	CH_3CN	rt	2.0	**SA**	No reaction detected by TLC analysis
2	**BC8**	Phenyl azide, 10 eq DABCO, 1 eq	CH_3CN	rt	16	**SA**	Major product **SE** detected by TLC analysis
3	**BC8**	Fmoc-4-azido-L-phenylalanine, 1 eq DABCO, 1 eq	CH_3CN/H_2O (4:1)	rt	5.0	**SB**	Major product **SE** detected by MALDI- and ESI-MS
4	**BC8**	Benzyl azide, 3 eq DABCO, 1 eq	CH_3CN/H_2O (4:1)	rt	4.0	**SC**	Major product **SE** detected by ESI-MS
5	**BC8**	3-Azidopropionic acid, 3 eq	DMSO	60	7.0	**SD**	Major product **SE** detected by TLC analysis
6	**ZnBC8**	Benzyl azide, 5 eq DABCO, 1 eq	$DMSO/H_2O$ (4:1)	rt	4.5	**SZnC**	Major product **SZnE** detected by TLC analysis
7[a]	**S5**	DABCO, 3 eq	$CDCl_3$	60	3.0	**S5 – BH_3**	BH_3 removed as detected by ^{1}H NMR spectroscopy
8[a]	**S5**	Benzyl azide, 1 eq DABCO, 3 eq	$CDCl_3$	60	3.0	**S6**	BH_3 removed and product peak **S6** detected by ^{1}H NMR spectroscopy

[a]Reactions were conducted in an NMR tube. [b]Concentrations are 2–3 mM.

A model study of thioester **S5** with phenyl azide was examined and found to proceed well to give the desired product **S6** (Scheme S4, Table S1 entry 8). This observation indicated the preparation of thioester-substituted bacteriochlorins (**BC9** and **BC10**) were likely viable for traceless Staudinger ligation.

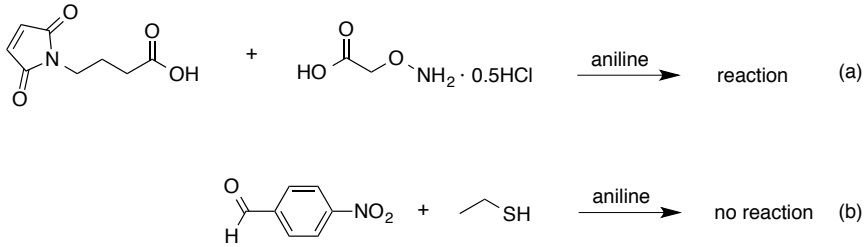


Scheme S4. Model studies of Staudinger ligation.

4. Study on bioorthogonality.

Reactions were conducted to test the cross-reactivity of the maleimido and formyl groups (Scheme S5a). Compounds 3-carboxypropylmaleimide and *p*-nitrobenzaldehyde were used for these model studies. Samples of aminooxy-containing *o*-(carboxymethyl)hydroxylamine hemihydrochloride (5.4 mg, 50 μmol) and *N*-(3-carboxypropyl)maleimide (9.1 mg, 50 μmol) were dissolved in a mixed solvent of aqueous potassium phosphate (0.3 M, pH 7.0, 7.2 mL) and acetonitrile (7.2 mL), followed by addition of aniline (4.6 mL, 50 mmol, 1000 equiv). After 4 h, the reaction mixture was washed with 1N HCl solution, and extracted with ethyl acetate. The organic extract was dried over $MgSO_4$, and the solvent was removed under vacuum. The product was used for further analysis without purification (no amount was recorded). ^{1}H NMR analysis showed the disappearance of the maleimido proton signals.

In a separate experiment (Scheme S5b), samples of ethanethiol (3.1 mg, 50 μmol) and *p*-nitrobenzaldehyde (7.5 mg, 50 μmol) were dissolved in a mixed solvent of aqueous potassium phosphate (0.3 M, pH 7.0, 7.2 mL) and acetonitrile (7.2 mL), followed by addition of aniline (4.6 mL, 50 mmol). The workup procedure was the same as that in Scheme S5a. The product was used for further analysis without purification (no amount was recorded). ^{1}H NMR analysis showed no detectable product.



Scheme S5. Study on bioorthogonality.

The results show that the maleimide group reacts with the aminooxy group in the presence of excess aniline at a dilute concentration (2.5 mM for maleimide reagent). In comparison, the aldehyde is not susceptible to thiol groups, though an equilibrium of hemithioacetal or thioacetal formation might be present in this test reaction. The findings necessitate a prior aldehyde-aminooxy (oxime formation) reaction to consume the aminooxy group, which could interfere with the maleimide-thiol reaction.