Supplementary Information for:

Hydrophilic tetracarboxy bacteriochlorins for photonics applications

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1. X-ray crystal structure of pyrrole 8

Data Collection and Processing. For the sample **8** (NCSU number rds002), all X-ray measurements were made on a Bruker-Nonius X8 Kappa APEX II CCD system equipped with a graphite monochromator. The frames were integrated with the Bruker SAINT software package using a SAINT algorithm. The integration of the data using a triclinic unit cell yielded a total of 9158 reflections to a maximum θ angle of 26.37° (0.80 Å resolution), of which 2021 were independent (average redundancy 4.531, completeness = 98.2%, R_{int} = 1.82%, R_{sig} = 1.50%) and 1968 (97.38%) were greater than $2\sigma(F^2)$. The final cell constants of <u>a</u> = 7.3702(2) Å, <u>b</u> = 7.6424(3) Å, <u>c</u> = 9.3429(3) Å, α = 95.852(2)°, β = 105.3520(10)°, γ = 93.7530(10)°, volume = 502.48(3) Å³, are based upon the refinement of the XYZ-centroids of reflections above 20 $\sigma(I)$.

Structure Solution and Refinement. The structure was solved and refined using the Bruker SHELXTL Software Package, using the space group P -1, with Z = 2 for the formula unit, $C_8H_9BrN_2O_4$. The final anisotropic full-matrix least-squares refinement on F² with 137 variables converged at R1 = 2.15%, for the observed data and wR2 = 6.10% for all data. The goodness-of-fit was 1.077. The largest peak in the final difference electron density synthesis was 0.908 e⁻/Å³ and the largest hole was -0.291 e⁻/Å³ with an RMS deviation of 0.076 e⁻/Å³. On the basis of the final model, the calculated density was 1.831 g/cm³ and F(000), 276 e⁻. The ORTEP drawing is provided in Fig. S1.

Fig. S1 ORTEP drawing of 3bromo-4-(methoxycarbonyl)-2-(2-nitroethyl)pyrrole (8). Ellipsoids are at the 50% probability level and hydrogen atoms were drawn with arbitrary radii for clarity.



2. X-ray crystal structure of pyrrole 13

Data Collection and Processing. For the sample **13** (NCSU number rds060), all X-ray measurements were made on a Bruker-Nonius X8 Kappa APEX II CCD system equipped with a graphite monochromator. The frames were integrated with the Bruker SAINT software package using a SAINT algorithm. The integration of the data using an orthorhombic unit cell yielded a total of 43538 reflections to a maximum θ angle of 33.29° (0.65 Å resolution), of which 5415 were independent (average redundancy 8.040, completeness = 99.5%, R_{int} = 2.70%, R_{sig} = 2.46%) and 5009 (92.50%) were greater than $2\sigma(F^2)$. The final cell constants of <u>a</u> = 9.5461(4) Å, <u>b</u> = 9.6709(4) Å, <u>c</u> = 15.4442(7) Å, volume = 1425.80(11) Å³, are based upon the refinement of the XYZ-centroids of 9874 reflections above 20 $\sigma(I)$ with 4.969° < 2 θ < 66.43°.

Structure Solution and Refinement. The structure was solved and refined using the Bruker SHELXTL Software Package, using the space group $P2_12_12_1$, with Z = 4 for the formula unit, $C_{13}H_{13}BrN_2O_4S$. The final anisotropic full-matrix least-squares refinement on F² with 192 variables converged at R1 = 1.92%, for the observed data and wR2 = 4.91% for all data. The goodness-of-fit was 1.057. The largest peak in the final difference electron density synthesis was 0.390 e⁻/Å³ and the largest hole was -0.505 e⁻/Å³ with an RMS deviation of 0.065 e⁻/Å³. On the basis of the final model, the calculated density was 1.739 g/cm³ and F(000), 752 e⁻. The ORTEP drawing is provided in Fig. S2.

Fig. S2 ORTEP drawing of 3-bromo-2-(2-nitroethyl)-*N*-tosylpyrrole (13). Ellipsoids are at the 50% probability level and hydrogen atoms were drawn with arbitrary radii for clarity.



3. Synthesis and experimental procedures

The Suzuki coupling reaction of **BC-9** with **4** afforded mono-coupled or di-coupled product, depending on the equivalents of **4** and Pd catalyst (Fig. S3). Reaction with 3 equivalents of **4** and 0.3 equivalents of Pd catalyst (relative to **BC-9**) afforded mono-coupled product **mono/pro-BC-6** in 25% yield with 33% starting material recovered. Further coupling of the latter gave the di-coupled product **pro-BC-6** in 56% yield. This is the first time to observe the selectivity for Suzuki coupling reaction of **BC-9**. Increasing the amount of **4** to 6 equivalents and Pd catalyst to 0.6 equivalents under the same coupling conditions afforded the di-coupled product in 62% yield.



Fig. S3 Synthesis of a 3,13-diarylbacteriochlorin.

3-Bromo-13-[3,5-bis(tert-butoxycarbonyl)phenyl]-5-methoxy-8,8,18,18-

tetramethylbacteriochlorin (mono/pro-BC-6). A mixture of **BC-9** (15 mg, 26.9 µmol), **4** (32.6 mg, 80.6 µmol), Pd(PPh₃)₄ (9.3 mg, 8.1 µmol), K₂CO₃ (44.6 mg, 322.4 µmol), and DMF/toluene [2.7 mL, (1:2), degassed by bubbling with argon] in a Schlenk flask was deaerated by three freeze-pump-thaw cycles with argon. The reaction mixture was stirred at 90 °C for 18 h. The reaction mixture was cooled to room temperature and concentrated to dryness. The residue was diluted with CH₂Cl₂ and washed with saturated aqueous NaHCO₃. The organic layer was separated, dried (Na₂SO₄) and concentrated. Column chromatography [silica, CH₂Cl₂/hexanes (7:3)] first provided unreacted **BC-9** (5.0 mg) followed by the title compound as a greenish solid (5.0 mg, 25%): ¹H NMR (400 MHz) δ –1.81 (brs, 1H), –1.53 (brs, 1H), 1.70 (s, 18H), 1.93 (s, 6H), 1.97 (s, 6H), 4.35 (br, 5H), 4.43 (s, 2H), 8.51(s, 1H), 8.63 (s, 1H), 8.67–8.70 (m, 2H), 8.80–8.83 (m, 2H), 8.91–8.93 (m, 2H); MALDI-MS obsd 756.2; ESI-MS obsd 754.2725, calcd 754.2730 (C₄₁H₄₇BrN₄O₅); λ_{abs} (CH₂Cl₂) 361, 508, 727 nm.

3,13-Bis[3,5-bis(tert-butoxycarbonyl)phenyl]-5-methoxy-8,8,18,18-

tetramethylbacteriochlorin (pro-BC-6). A mixture of mono/pro-BC-6 (4.5 mg, 5.9 μ mol), 4 (12.0 mg, 29.7 μ mol), Pd(PPh₃)₄ (2.7 mg, 2.3 μ mol), K₂CO₃ (10 mg, 72.4 μ mol) and DMF/toluene [0.8 mL, (1:2), degassed by bubbling with argon] in a Schlenk flask was deaerated by three freeze-pump-thaw cycles with argon. The reaction mixture was stirred at 90 °C for 18 h. The reaction mixture was cooled to room temperature and concentrated to dryness. The

residue was diluted with CH_2Cl_2 and washed with saturated aqueous NaHCO₃. The organic layer was separated, dried (Na₂SO₄) and concentrated. Column chromatography [silica, CH_2Cl_2 /hexanes (3:1)] provided the title compound as a greenish solid (3.2 mg, 56%). The characterization data were essentially identical to those obtained upon the dicoupling reaction to prepare **pro-BC-6**.

4. Absorption versus concentration study

Concentration (µM)	Solution	Pathlength (mm)	Calcd Abs ^a
500	A	0.1	0.5
50	В	1	0.5
5	С	10	0.5
0.5	D	100	0.5

^{*a*}Assuming a molar absorption coefficient of $10^5 \text{ M}^{-1} \text{ cm}^{-1}$.



[@]Stock solution contains DMSO (5%, v/v) [#]Buffer: Potassium phosphate (0.5 M, pH = 7.0)

Fig. S4 Flowchart for absorption versus concentration study. The concentration of DMSO in each solution is as follows: 5% (A), 0.5% (B), 0.05% (C), and 0.005% (D).



Fig. S5 Concentration study with normalized spectra for BC-7 and BC-8.





Fig. S6 Concentration study with raw spectra (not normalized) for BC-6, BC-7, and BC-8.

5. HPLC method for purification of β(-14Cys)BC-16

Analytical HPLC was performed on a Hewlett-Packard 1100 series instrument using a C4 column (Vydac, 10 μ m, 300 Å, 150 mm X 4.6 mm) in series with a guard column. The HPLC solvent system consisted of eluant A (0.1% TFA in water) as the aqueous solvent and eluant B (0.1 % TFA in 1:2 acetonitrile/2-propanol) as the organic solvent. The initial composition of A/B was 50/50 and the final composition of A/B at 48 min was 30/70. In other words, the HPLC solvent was initially H₂O (50%), CH₃CN (16.7%), isopropanol (33.3%), and TFA (0.1%), which has pH 2.01. The use of a gradient afforded a final composition of H₂O (30%), CH₃CN (23.3%), isopropanol (46.6%), and TFA (0.1%) at 48 min. The flow rate was 0.8 mL/min.

The HPLC traces of the initial peptide β (-14Cys), crude conjugate, and purified conjugate β (-14Cys)BC-16 are displayed in Fig. S7.

The absorption spectra of the peptide β (-14Cys), maleimido-bacteriochlorin BC-16, and conjugate β (-14Cys)BC-16 in the HPLC solvent mixture are displayed in Fig. S8.



Fig. S7 HPLC traces of β (-14Cys), crude mixture and purified β (-14Cys)BC-16 conjugate, detected at 220 nm (A) and 729 nm (B). The free bacteriochlorin elutes near the solvent front.



Fig. S8 Absorption spectra of peptide $\beta(-14Cys)$ (red), maleimido-bacteriochlorin BC-16 (black), and conjugate $\beta(-14Cys)BC-16$ (blue) in the HPLC solvent mixture (isopropanol, 38.6%; H₂O, 42%; acetonitrile, 19.3%; and TFA 0.1%). The spectra of BC-16 (black) and the conjugate $\beta(-14Cys)BC-16$ (blue) are normalized at the Q_y band. The spectrum of the peptide $\beta(-14Cys)$ (red) is shown at arbitrary scale to correspond to the corresponding peak in the spectrum of the conjugate $\beta(-14Cys)BC-16$.

6. Spectral data



S9





S11







Ψ

CO₂Me















































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1		731.0	6.3346E-2
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S38



Spectrum Report

S39



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S59





S61



















Date 8/13/2013 Time 10:13:07 Page 1 of 1 Spectrum/Peak Report Method file : <untitled> Default Method Information : C:\Chem32\1\DATA\JIMMY\pro-C-NH2.SD Created : 4/11/13 19:47:27 Data File : Overlaid Spectra: ő 0.4 0.3 Absorbance (AU) 0.2 0.1 0 700 Wavelength (nm) 400 500 600 Abs(AU) # Name Peaks(nm) _____ _____ ___ 0.44637 365.0 1 728.0 0.35965 1 517.0 8.4251E-2 1 Signature: Report generated by : Lindsey Lab _____ *** End Spectrum/Peak Report *** _____ _____












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