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Synthesis and photophysical properties of thioglycosylated-chlorins, isobacteriochlorins and bacteriochlorins for bioimaging and diagnostics

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Abstract

The facile synthesis and photophysical properties of three non-hydrolysable thioglycosylated porphyrinoids are reported. Starting from meso perfluorophenylporphyrin, the non-hydrolysable thioglycosylated porphyrin (PGlc₄), chlorin (CGlc₄), isobacteriochlorin (IGlc₄), and bacteriochlorin (BGlc₄) can be made in 2–3 steps. The ability to append a wide range of targeting agents onto the perfluorophenyl moieties, the chemical stability, and the ability to fine-tune the photophysical properties of the chromophores make this a suitable platform for development of biochemical tags, diagnostics, or as photodynamic therapeutic agents. Compared to the porphyrin in phosphate buffered saline, CGlc₄ has a markedly greater absorbance of red light near 650 nm and a 6-fold increase in fluorescence quantum yield; whereas IGlc₄ has broad Q bands and a 12-fold increase in fluorescence quantum yield. BGlc₄ has a similar fluorescence quantum yield to PGlc₄, (<10%) but the lowest energy absorption/emission peaks of BGlc₄ are considerably red shifted to near 730 nm with a nearly 50-fold greater absorbance, which may allow this conjugate to be an effective PDT agent. The uptake of CGlc₄, IGlc₄, and BGlc₄ derivatives into cells such as human breast cancer cells MDA-MB-231 and K:MoLv NIH 3T3 mouse fibroblast cells can be observed at nM concentrations. Photobleaching under these conditions is minimal.

Introduction

Chlorins are porphyrinoids with one pyrrole double bond missing. Isobacteriochlorins and bacteriochlorins are porphyrinoids with two pyrrole double bonds missing on adjacent or opposite pyrroles, respectively. Oxidative or reductive transformations of porphyrins can yield these chromophores. Porphyrins, chlorins, bacteriochlorins, and isobacteriochlorins each have unique photophysical properties that are exploited by nature and can be used for

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Supporting Information Available: Additional spectroscopic data including ¹H NMR, ¹³C NMR, ¹⁹F NMR, and HRMS of all the protected and deprotected glycosylated porphyrinoids, UV-visible and fluorescence spectra in three different solvents: PBS, ethanol, and ethyl acetate, diastereomeric structures of CF₂₀ and IF₂₀, CD spectra of CF₂₀, IF₂₀, and BF₂₀ in chloroform, CD spectra of CGlc₄, IGlc₄, and BGlc₄ in methanol, Cell uptake fluorescence images using MDA-MB-231 breast cancer cell line, singlet oxygen quantum yield data, and photobleaching experiment of IGlc₄ in sunlight. Spectroscopic characterization of the compounds, and the substitution of a diol chlorine outlined. This information is available free of charge via the Internet at <http://pubs.acs.org/>.

diverse applications (1,2). Compared to the parent tetraarylporphyrin, the intensity of the lowest energy UV-visible absorption band near 650 nm can increase by ~20 fold for chlorins, and by ~5 fold for the isobacteriochlorins. For bacteriochlorins the absorption band near 650 nm is similar to porphyrins, but an additional high intensity absorption band appears near 730 nm. The excited state lifetimes, singlet and triplet quantum yields and distortion upon metal ion binding are significantly different for these three types of macrocycles (3,4).

For diagnostic and photodynamic therapeutic (PDT) applications, the enhanced intensity of the low energy absorption bands allows more efficient use of wavelengths of light that penetrate further into tissues (5–8). An example of a chlorin PDT photosensitizer is meso-tetrakis(3'-hydroxyphenyl)chlorin (m-THPC) and its derivatives (9). Other water soluble chlorins are reported (10–12). For therapeutic uses of PDT agents, high triplet quantum yields are desirable to photosensitize the formation of singlet oxygen and other reactive oxygen species, which then cause damage to diverse cellular components. For diagnostics the dyes appended with appropriate targeting motifs need to have greater fluorescence quantum yields for use as fluorescent tags or trackers (13). Fluorescent dyes with targeting motifs can be used in fluorescence guided surgery (14).

Porphyrins, chlorins, isobacteriochlorins, bacteriochlorins, and phthalocyanines with 1–8 appended sugar moieties can have cytotoxic activity because the sugars can direct the chromophore to some cancer cells (15–27). However, most reported compounds have O-glyco linkages where hydrolysis may diminish in vivo effectiveness compared to corresponding non-hydrolysable derivatives. The number, type, and position of the sugar moieties have long been known to effect cell uptake and photochemical properties (16,21,28). The synthesis of a polyglycerol dendrimer with a porphyrin core is reported (29), click reactions have been used to glycosylate chlorin e_6 (30), and polysaccharides have been appended to porphyrins (31).

Previously, we reported the synthesis of non-hydrolysable thioglycosylated tetraarylporphyrins and showed that the tetraglucose derivative (PGlc₄, Scheme 1 **1b**) is a selective and effective PDT agent in vitro using several cell lines, such as MDA-MB-231 human breast cancer cells, whereas the galactose derivative is much less effective (32). Also the glycosylated compounds were taken up by transformed mouse 3Y1^{vSrc} cells but not taken up by normal mouse fibroblast cells (32,33). We first reported the synthesis and ground state electronic properties of a glycosylated chlorin formed using a dipolar addition to TPPF₂₀ to append a fused N-methyl-pyrrolidine moiety and the same chemistry to append the sugars (Scheme 2) (34).

One advantage of using the tetra-perfluorophenylporphyrin (TPPF₂₀) is that it can serve as a core platform for a host of materials (35,36) and biochemical applications because the para fluoro group can be routinely substituted with a variety of nucleophiles to form bioconjugates, biocompatible compounds, and combinatorial libraries (33,37–41). For example, we have appended ethyleneglycols, polyamines, lysines, alkanes, fluorinated alkanes, sugars and nucleotides using both primary amines and thiols. Subsequently, glycols with an oxygen linkage were also reported (42). The PDT activity of many of these derivatives has been studied with a variety of cell lines, and the glycosylated compounds effect apoptosis in several cases (22,42–44). Also, the 16 fluoro groups make this compound amenable for in vivo ¹⁹F NMR studies of localization (45).

Given the diversity of the targeting moieties on the core platform, it is desirable to be able to tune the photophysical properties of the chromophore for these assorted applications, and an obvious means to accomplish this is to adjust the number of double bonds in the macrocycle

as is done in nature (46). There are many strategies to make porphyrins lacking one or two double bonds using oxidative and reductive strategies (5,47). The formation of the perfluorophenylchlorin (CF₂₀), perfluorophenylisobacteriochlorin (IF₂₀), and perfluorophenylbacteriochlorin (BF₂₀) by 1,3-dipolar additions were reported by the Cavaleiro group (48–52). The advantage of this route is that it does not use toxic reagents and the products are reasonably stable chemically and to photobleaching. Vicente and coworkers exploited the thio coupling chemistry on CF₂₀ to append carboranyl groups (53) and Hirohara has appended sugars to free base and Pt derivatives (54,55) for potential therapeutic applications. We now report the synthesis, photophysical properties, and relative cell uptake of the glycosylated chlorin **2b** (CGlc₄), isobacteriochlorin **3b** (IGlc₄) and bacteriochlorin **4b** (BGlc₄) analogues (Scheme 2).

Experimental Procedure

Materials and methods

General—¹H and ¹³C NMR spectra were recorded in a Bruker Avance 500 MHz spectrometer and the ¹⁹F spectra in a JEOL 400 MHz spectrometer. Electrospray ionization mass spectrometric analyses were performed at the CUNY Mass Spectrometry Facility at Hunter College using an Agilent Technologies HP-1100 LC/MSD instrument. The Electrospray ionization was run in methanol, with 0.1% formic acid. UV-visible spectra were recorded on a Varian Bio3 spectrophotometer. Steady-state fluorescence (emission) spectra were measured with a Fluorolog τ3, Jobin-SPEX Instruments S.A., Inc. All reagents were obtained from commercial sources and used without further purification. TPPF₂₀ porphyrin was obtained from Frontier Scientific. Dulbecco's Modified Eagle Medium (DMEM), trypsin-EDTA and antimycotic for cell culture were obtained from GibcoBRL. Hanks' balanced salt solution was obtained from Cellgro (Mediatech). Bovine calf serum was obtained from HyClone. Phosphate buffered saline (PBS) was purchased from Invitrogen. Flash column chromatography was performed using silica gel-60, and the analytical TLC was carried out on precoated sheets with silica gel (0.2 mm thick) both from Sorbent Technologies. The octanol/water partition coefficients were determined by saturating 1:1 (v:v) mixtures of the solvents with the porphyrinoid, waiting 8–10 h, and measuring the Soret and first Q band intensities. This data was confirmed by saturating each solvent separately before mixing equal volumes.

Synthesis

Improved synthesis PGlcAc₄ (**1a**)

To a solution of TPPF₂₀, **1** (50 mg, 51 μmol) and 2,3,4,6-tetra-*O*-acetyl-glucosylthioacetate (90 mg, 233 μmol, 4.6 equiv) in DMF (5 mL) diethyl amine (1 mL) was added. The reaction mixture was stirred at room temperature for 1.5 h. Then the product was precipitated with methanol/water and the solid filtered through a short column of Celite and washed with water. The crude mixture was recovered in dichloromethane and purified by flash chromatography (silica gel) using a mixture of ethyl acetate/hexanes (3:1) as eluent. The glycoporphyrin **1a** (110 mg, 92%) was obtained after crystallization in dichloromethane/hexane, as a red powder.

Synthesis of Porphyrin PGlc₄, (**1b**)

Porphyrin **1a** (50 mg, 21 μmol) was dissolved in methanol/dichloromethane (3:1, 8 mL) and treated with sodium methoxide (0.5 M solution in methanol, 1 mL). The reaction mixture was stirred at room temperature for 1.5 h and then neutralized by an aqueous citric acid solution. The mixture was filtered through a Waters Sep-Pak C₁₈ 35cc reverse phase prep column and washed with water. The deprotected glycoporphyrin **1b** was then purified on

flash chromatography (silica gel) eluted using a mixture of ethyl acetate/methanol (3:1) and crystallized in methanol/CH₂Cl₂ (33.5 mg, 96%). Mp > 250 °C. ¹⁹F NMR (DMSO-d₆): δ -133.82 to -132.57 (m, 8F, Ar-*m*-F), -139.75 to -139.50 (m, 8F, Ar-*o*-F). ¹H NMR (DMSO-d₆): δ 9.33 (bs, 8H, pyrrolic β-H), 5.78 (bs, 4H, Glc-H), 5.10–5.29 (m, 13H, Glc-H), 4.72 (bs, 4H, Glc-H), 3.82–3.84 (m, 5H, Glc-H), 3.52–3.55 (m, 6H, Glc-H), 3.18–3.23 (m, 12H, Glc-H), -3.17 (s, 2H, NH).

Synthesis of protected thioglycosylated chlorin, CGlcAc₄ (2a)

Chlorin **2** was prepared as previously reported method (48,49). To a solution of chlorin **2** (25 mg, 24 μmol) and 2,3,4,6-tetra-*O*-acetyl-glucosylthioacetate (42 mg, 109 μmol, 4.5 equiv) in DMF (2.5 mL) diethyl amine (0.5 mL) was added. The reaction mixture was stirred at room temperature for 1h. Then the reaction mixture was precipitated with methanol/water and the solid was filtered through a short column of Celite and washed with water. The crude mixture was recovered in CH₂Cl₂ and purified by flash chromatography (silica gel) using a mixture of ethyl acetate/hexanes (3:2) as eluent. Chlorin **2a** (48 mg, 82%) was obtained after crystallization in CH₂Cl₂/hexanes, as a green powder. Because the *N*-methylpyrrolidine points to one face of the chlorin and the stereo centers on the sugars, **2a** is made as a mixture of diastereomers at the β pyrrole positions bearing the *N*-methyl pyrrolidine moieties. These are seen in some of the resonances in the ¹H NMR and especially the complexity of the phenyl region of the ¹³C NMR, which show the coupling to ¹⁹F and the diastereomers. The peaks in the ¹⁹F NMR spectrum are somewhat broader than the achiral starting material. Mp > 250 °C. ¹⁹F NMR (DMSO-d₆): δ -130.09 to -129.81, -130.56 to -130.25 (2m, 4F, Ar-*m*-F), -131.69 to -131.54 (m, 4F, Ar-*m*-F), -137.10 to -136.78 (m, 2F, Ar-*o*-F), -138.98 to -138.82 (m, 6F, Ar-*o*-F). ¹H NMR (CDCl₃): δ 8.82–8.84, 8.55–8.57, 8.48–8.50 (3m, 6H, pyrrolic β-H), 5.11–5.40 (3m, 18H, 2H β-H [C (sp³)] and 16H, Glc-H), 4.26–4.35 (m, 8H, Glc-H), 3.88–3.91 (m, 4H, Glc-H), 2.58 and 3.16 (2t, 4H, pyrrolidine-H), 2.22 (s, 3H, NCH₃), 2.07–2.10 (m, 48H, acetyl-H), -1.74 (s, 2H, NH). ¹³C NMR (CDCl₃): δ 20.6 and 20.7 (CH₃CO₂), 41.2 (NCH₃), 53.1 and 53.2 (C2 and C3), 61.7 and 61.9 (Glc), 63.0 (CH₂), 68.0, 68.1, 70.7, 73.9, 84.5 and 84.6 (Glc), 97.5, 106.7, 106.8, 111.6, 111.8, 122.1, 122.4, 124.0, 124.3, 128.2, 128.3, 132.5, 134.9, 135.0, 140.0, 141.1, 144–149 (C₆F₄), 152.3, 168.6, 168.8, 169.4 (CH₃CO₂), 170.2 and 170.7 (CH₃CO₂). HRMS calcd for C₁₀₃H₉₅F₁₆N₅O₃₆S₄ (M+2H)⁺ 2409.4384, found 2409.4318.

Synthesis of thioglycosylated chlorin, CGlc₄ (2b)

This details what we previously reported (34), and later modified by Hirohara et al (54). Glycochlorin **2a** (30 mg, 12.4 μmol) was dissolved in methanol/CH₂Cl₂ (3:1, 4 mL) and treated with sodium methoxide (0.5 M solution in methanol, 1 mL). The reaction mixture was stirred at room temperature for 1 h and then neutralized by an aqueous citric acid solution. The mixture is filtered through Waters Sep-Pak C₁₈ 35cc reverse phase prep column and washed with water. The deprotected glycochlorin **2b** was then eluted with methanol and purified by flash chromatography (silica gel) using a mixture of ethyl acetate/methanol (3:2) as eluent. Chlorin **2b** (20 mg, 93%) was obtained after crystallization in methanol/CH₂Cl₂, as a green powder. **2b** is also a mixture of diastereomers. These are not well resolved in the ¹H NMR but are observed especially the complexity of the phenyl region of the ¹³C NMR which show the coupling to ¹⁹F and the diastereomers. Mp > 250 °C. ¹⁹F NMR (DMSO-d₆): δ -132.67 to -132.28 (m, 4F, Ar-*m*-F), -133.79 to -133.64 (m, 4F, Ar-*m*-F), -137.90 to -137.53 (m, 2F, Ar-*o*-F), -139.93 to -139.82 (m, 6F, Ar-*o*-F). ¹H NMR (DMSO-d₆): δ 9.14, 8.81 and 8.74 (3bs, 6H, pyrrolic β-H), 5.02–5.10, 5.28 and 5.75 (1m and 2bs, 18H, 2H β-H [C (sp³)] and 16H, Glc-H), 4.55–4.68 (m, 4H, Glc-H), 4.10 (m, 2H, Glc-H), 3.73–3.78, 3.51, 3.18 and 3.04 (1m and 3bs, 26H, 4H pyrrolidine-H and 22H Glc-H), 2.05 (s, 3H, NCH₃), -1.97 (s, 2H, NH). ¹³C NMR (DMSO-d₆): δ 41.0 (NCH₃), 49.1 and 53.1 (C2 and C3), 61.7, 61.71 and 61.9 (Glc), 62.9 (CH₂), 70.57, 70.60, 70.68, 70.78,

70.8, 75.1, 75.2, 78.51, 78.54, 78.6, 79.6, 82.2, 82.3, 84.9, 85.0 and 85.2 (Glc), 97.8, 106.8, 113.8, 113.9, 114.1, 114.2, 119.5, 119.6, 119.8, 120.0, 120.2, 126.6, 129.8, 133.5, 135.1, 140.3, 143–149 (C₆F₄), 152.4, 170.2. HRMS calcd. for C₇₁H₆₂F₁₆N₅O₂₀S₄ (M+H)⁺ 1736.2615, found 1736.2603.

Synthesis of isobacteriochlorin (3) and bacteriochlorin (4)

This compound is made using modifications of the previously reported procedure (49) and scaled up. A toluene (20 mL) solution of TPPF₂₀ (100 mg, 0.1 mmol), *N*-methylglycine (30 mg, 1 mmol) and paraformaldehyde (20 mg, 0.22 mmol) was heated at reflux for ca. 12 h under a nitrogen atmosphere. Further portions of *N*-methylglycine (30 mg) and paraformaldehyde (20 mg) were again added and the resulting mixture was refluxed for a total of 24 h (2 × 12 h). The compounds were separated by flash chromatography (silica gel) using hexane/CH₂Cl₂ (50:50 v/v) to elute the negligible amount of unreacted porphyrin **1**, followed by CH₂Cl₂ to get the chlorin **2** (45 mg, 42%) and then CH₂Cl₂/acetone (95:5) to get the isobacteriochlorin **3** (40 mg, 34%). However, we found that a small amount (ca. 10–15%) of the bacteriochlorin consistently elutes with the isobacteriochlorin under these conditions. Thus, compounds **2**, **3** and **4** were separated using a petroleum ether/ethylacetate gradient on a flash silica gel column. From 100 mg TPPF₂₀ (102.6 μmol) is obtained 70 mg of the chlorin compound **2** (67.89 μmol, 66 % yield), 15 mg of the anti isobacteriochlorin compound **3** (13.77 μmol, 13 % yield), and 5 mg of both the syn and anti bacteriochlorin compound **4** (4.6 μmol, 5 % yield). The diastereomeric anti form of compound **3** is preferentially formed, conversely the syn form of compound **4** is the major product (3:2 syn:anti), and the NMR, HRMS, and UV-visible spectra for **3** and **4** were consistent with previous the report (49).

Synthesis of protected thioglycosylated isobacteriochlorin, IGlcAc₄ (3a)

To a solution of isobacteriochlorin **3** (25 mg, 23 μmol) and 2,3,4,6-tetra-*O*-acetylglucosylthioacetate (40 mg, 103 μmol, 4.5 equiv) in DMF (2.5 mL), diethyl amine (0.5 mL) was added. The reaction mixture was stirred at room temperature for 4 h. The reaction mixture was precipitated with methanol/H₂O and the solid filtered through a short column of Celite and washed with water. The crude mixture was recovered in CH₂Cl₂ and purified by flash chromatography (silica gel) using a mixture of ethyl acetate/hexanes (3:1) as eluent. Isobacteriochlorin **3a** (49 mg, 86%) was obtained after crystallization in CH₂Cl₂/hexanes, as a pink powder. Because the *N*-methylpyrrolidines points to opposite faces of the isobacteriochlorin and of the stereo centers on the sugars, **3a** is made as a mixture of two sets of diastereomers on the four β pyrrole positions bearing the *N*-methyl pyrrolidine moieties. These are seen in some of the resonances in the ¹H NMR and especially the complexity of the phenyl region of the ¹³C NMR, which shows the coupling to ¹⁹F and the multiplicity due to the adjacent pair of diastereomers. Mp > 250 °C. ¹⁹F NMR (DMSO-*d*₆): δ –128.46 to –128.35, –128.81 to –128.70 (2m, 2F, Ar-*m*-F), –130.11 to –129.88, –130.45 to –130.35, –130.91 to –130.81 and –131.62 to –131.41 (4m, 6F, Ar-*m*-F), –136.79 to –136.64, –137.35 to –137.17, –139.42 to –139.23 and –140.04 to –139.94 (4m, 8F, Ar-*o*-F). ¹H NMR (CDCl₃): δ 7.64, 7.63, 7.22, 7.17 (4d, *j* = 5 Hz, 4H, pyrrolic β-H), 5.03–5.32 (m, 16H, Glc-H), 4.45–4.50 and 4.16–4.25 (2m, 14H, 4H β-H [C (sp³)], 2H NH and 8H Glc-H), 3.83(d, *j* = 5 Hz, 4H, Glc-H), 2.70 and 2.88 (2t, *j* = 8.2 Hz, 4H, pyrrolidine-H), 2.06–2.31 (m, 58H, 4H pyrrolidine-H, 6H NCH₃, 48H, acetyl-H). ¹³C NMR (CDCl₃): δ 20.6 (CH₃CO₂), 41.0 (NCH₃), 47.8 and 51.7 (C2, C3, C7 and C8), 61.4, 61.8, 62.5 (CH₂ and Glc), 68.0, 70.2, 70.5, 73.81, 73.83, 76.3, 84.5 and 84.6 (Glc), 92.2, 98.2, 111.0, 111.1, 111.3, 113.6, 121.4, 121.5, 121.6, 128.5, 128.6, 144.4–148.7 (C₆F₄), 169.37, 169.38, 169.41, 170.16 and 170.62 (CH₃CO₂). HRMS calcd. for C₁₀₆H₁₀₀F₁₆N₆O₃₆S₄ (M)⁺⁺ 2464.4806, found 2464.4700.

Synthesis of thioglycosylated isobacteriochlorin, IGlc₄ (3b)

Isobacteriochlorin **3a** (30 mg, 12.1 μ mol) was dissolved in methanol/CH₂Cl₂ (3:1, 4 mL) and treated with sodium methoxide (0.5 M solution in methanol, 1 mL). The reaction mixture was stirred at room temperature for 1 h and then neutralized by an aqueous citric acid solution. The mixture was filtered through Waters Sep-Pak C₁₈ 35cc reverse phase prep column and washed with water. The deprotected chlorin **3b** was eluted with methanol and purified by flash chromatography (silica gel) using a mixture of ethyl acetate/methanol (3:2) as eluent. Isobacteriochlorin **3b** (19 mg, 88%) was obtained after crystallization in methanol/CH₂Cl₂, as a pink powder. **3b** is likewise a mixture of two sets of diastereomers. These are not resolved in the ¹H NMR, but are observed especially in the complexity of the phenyl region of the ¹³C NMR which shows the coupling to ¹⁹F and the multiplicity due to the adjacent pair of diastereomers. Mp > 250 °C. ¹⁹F NMR (DMSO-d₆): δ -131.31 to -131.01, -132.98 to -132.25 and -133.68 to -133.43 (3m, 8F, Ar-*m*-F), -138.2 to -137.59, -140.13 to -140.04, -140.37 to -140.31 and -140.89 to -140.79 (4m, 8F, Ar-*o*-F). ¹H NMR (DMSO-d₆): δ 7.78 and 7.30 (2s, 4H, pyrrolic β -H), 5.67 (m, 4H, Glc-H), 5.21–5.24, 5.02–5.06 and 4.91–4.95 (3m, 12H, Glc-H), 4.40–4.44 and 4.05–4.13 (2m, 12H, 4H β -H [C (sp³)], 2H NH, 8H Glc-H), 3.16–3.26 (m, 20H), Glc-H), 2.70, 2.57, 2.19 and 2.18 (4bs, 8H, pyrrolidine-H), 2.01 (s, 6H, NCH₃). ¹³C NMR (DMSO-d₆/methanol-d₄): δ 40.6 (NCH₃), 47.7, 49.06 (C2, C3, C7 and C8), 61.5, 61.6, 61.7, 62.5, 70.48, 70.52, 75.0, 75.1, 78.44, 78.46, 78.48, 84.8, 85.0 (CH₂ and Glc), 92.2, 97.8 113.2, 113.3, 113.36, 113.38, 113.61, 119.0, 119.2, 146.0–148.6 (C₆F₄). HRMS calcd. for C₇₄H₆₉F₁₆N₆O₂₀S₄ (M+H)⁺ 1793.3194, found 1793.3197.

Synthesis of protected thioglycosylated bacteriochlorin, BGlcAc₄ (4a)

To a solution of bacteriochlorin **4** (7 mg, 6.4 μ mol) and 2,3,4,6-tetra-*O*-acetylglucosylthioacetate (12 mg, 29 μ mol, 4.5 equiv) in DMF (0.5 mL) diethyl amine (0.1 mL) was added. The reaction mixture was stirred at room temperature for 4h. Then the reaction mixture was precipitated with methanol/H₂O and the solid was filtered through a short column of Celite and washed with water. The crude mixture was recovered in CH₂Cl₂ and purified by flash chromatography (silica gel) using a mixture of ethyl acetate/hexanes (3:1) as eluent. Bacteriochlorin **4a** (13 mg, 86%) was obtained after crystallization in CH₂Cl₂/hexanes, as a green powder. Because of the stereo centers on the sugars, **4a** is made as a mixture of two sets of diastereomers. Mp > 250 °C. ¹⁹F NMR (CDCl₃): δ -129.05 to -128.15 (4m, 8F, Ar-*m*-F), -132.80 to -132.40 (m, 4F, Ar-*o*-F), -134.95 to -134.45 (m, 4F, Ar-*o*-F). ¹H NMR (CDCl₃): δ 8.30 (m, 4H, pyrrolic β -H), 5.12–5.23 (m, 20H, 16H Glc-H, 4H, β -H [C (sp³)], 4.15–4.30 (m, 8H Glc-H), 3.88 (m, 4H, Glc-H), 3.05–3.10 (m, 4H, pyrrolidine-H), 2.45–2.46 (m, 4H pyrrolidine-H), 2.06–2.31 (m, 54H, 6H NCH₃, 48H, 48H, acetyl-H), -1.75 (s, 2H, NH). ¹³C NMR (CDCl₃): δ 20.6 (CH₃CO₂), 41.2 (NCH₃), 52.4 and 52.5 (C2, C3, C7 and C8), 61.7, 61.8 (CH₂ and Glc), 68.0, 70.6, 73.88, 76.70, 77.0, 77.3, 84.6 and 84.7 (Glc), 92.2, 99.7, 107.7, 111.4, 111.5, 115.5, 115.6, 122.6, 122.7, 136.0, 136.1, 144.4–148.7 (C₆F₄), 164.0, 169.38, 169.40, 170.19 and 170.64 (CH₃CO₂). HRMS calcd. for C₁₀₆H₁₀₀F₁₆N₆O₃₆S₄ (M)⁺⁺ 2464.4806, found 2464.4813.

Synthesis of thioglycosylated bacteriochlorin, BGlc₄ (4b)

Bacteriochlorin **4a** (10 mg, 4.0 μ mol) was dissolved in methanol/CH₂Cl₂ (3:1, 1.5 mL) and treated with sodium methoxide (0.5 M solution in methanol, 0.2 mL). The reaction mixture was stirred at room temperature for 1 h and then neutralized by an aqueous citric acid solution. The mixture is filtered through Waters Sep-Pak C₁₈ 35cc reverse phase prep column and washed with water. Though **4** has a plane of symmetry, because of the chiral centers on the sugars on **4a** and **4b**, these are prepared as a set of two diastereomers. The deprotected bacteriochlorin **4b** was eluted with methanol and purified by flash chromatography (silica gel) using a mixture of ethyl acetate/methanol (3:2) as eluent.

Bacteriochlorin **4b** (6.5 mg, 88%) was obtained after crystallization in methanol/CH₂Cl₂, as a green powder. Mp > 250 °C. ¹⁹F NMR (MeOD): δ −134.0 to −132.5 (4m, 8F, Ar-*m*-F), −137.2 to −136.0 (m, 4F, Ar-*o*-F), −140.0 to −139.0 (m, 4F, Ar-*o*-F). ¹H NMR (MeOD): δ 8.42 (bs, 4H, pyrrolic β-H), 5.30–5.45 (m, 4H, Glc-H), 5.05–5.14 (m, 4H, β-H [C (sp³)]), 3.73–3.74 and 3.63–3.65 (2m, 8H, Glc-H), 3.49–3.54 and 3.41–3.43 (2m, 16H, 8H and 8H Glc-H), 3.15–3.18 (m, 4H, pyrrolidine-H), 2.79–2.82 (m, 4H, pyrrolidine-H), 2.28 (s, 6H, NCH₃). ¹³C NMR (MeOD): δ 39.8 (NCH₃), 51.8, 51.9 (C2, C3, C7 and C8), 61.6, 70.5, 74.57, 74.59, 78.3, 81.36, 81.37, 84.9, 85.12 (CH₂ and Glc), 99.91, 99.94 100.0, 103.9, 113.32, 113.34, 113.45, 113.50, 120.86, 121.0, 136.2, 136.3, 136.4, 146.0–148.6 (C₆F₄). HRMS calcd. for C₇₄H₆₉F₁₆N₆O₂₀S₄ (M+H)⁺ 1793.3194, found 1793.3179.

UV-visible, fluorescence spectroscopy and quantum yield calculations

UV-visible and fluorescence measurements were performed on dilute solutions, typically ~2 μM, of compounds in ethanol, phosphate buffered saline (PBS) and ethyl acetate. The UV-visible spectra were obtained from 330 nm to 800 nm using 1 cm quartz cuvettes. For steady state fluorescence spectroscopy, samples were excited at 509 nm for ethyl acetate, 512 nm for PBS and ethanol where absorbencies ≤ 0.1. For emission spectra both the excitation and detection monochromators had a band pass of 1 nm. The corrected emission (for instrument response) and absorption spectra were used to calculate the quantum yield. Fluorescence quantum yields were determined for chlorin, isobacteriochlorin, and bacteriochlorin solutions relative to TPP in toluene, which has a fluorescence quantum yield of 0.11 (57,58). The quantum yields were measured indirectly using TPP, thus these values may have some systematic error. All experiments were carried out on the same day, using identical concentrations to minimize any experimental errors. These three glycosylated conjugates are quite stable towards photobleaching. When ethanolic solution of IGlc₄ is exposed to sunlight with a power of ca 40–80 W/m² for ca. 2 hours we found only 10.5% of the compound decomposed (See Supporting Information).

Quantum yield of singlet oxygen production (Φ_Δ)

Φ_Δ were determined on a relative basis by using *meso*-Tetrakis(4-sulfonatophenyl)porphine dihydrochloride (TSPP) as a reference sensitizer (Φ_{Δ,TSPP} = 0.7 in methanol) (56). A time-resolved Nd:YAG laser (Polaris II, Electro Scientific Industries, Inc.) equipped with low temperature cooled Ge detector (Applied Detector Corporation) was employed as an excitation source at 532 nm. All of the experiments were carried out in deuterium methanol-d₁. The absorbances from samples and TSPP at 532 nm were controlled between 0.1–0.5. ¹O₂ luminescence was monitored as a function of sensitizer absorbance. Slopes were analyzed from a plot of ¹O₂ intensity via absorbance. Φ_Δ can be calculated according to following equation.

$$\Phi_{\Delta \text{ sample}} / \Phi_{\Delta \text{ reference}} = \text{slope}_{\text{sample}} / \text{slope}_{\text{reference}}$$

Cell Culture

3T3 K:MOLV NIH cells maintained in DMEM, 10% BCS, 1% antimycotic at 37 °C and in 5% CO₂ atmosphere were plated onto coverslips in cell culture dishes. PGlc₄, CGlc₄ and BGlc₄ dissolved in methanol were added to the cell cultures to a final concentration of 1 or 2.5 μM such that there was never more than 0.5% methanol in the solution. After 20 h incubation, cells were washed with PBS 3 – 5 times and fixed in 4% paraformaldehyde solution for 10 minutes at room temperature. The cells were then washed with PBS 3 times. The cells were visualized using a Nikon Optiphot 2 fluorescence microscope. Images were

captured as JPEG files at 10x magnification, with a 505 – 565 nm excitation band pass filter and a 565 – 685 nm emission band pass filter. For each set of experiments, cells were cultured and the fluorescence images were taken under identical culture and microscopic conditions. For quantitative studies, the image intensities of the cells in the fluorescence micrographs were calculated by FLim program available at: <http://nathan.instras.com/projects/FLim/index.html>

Confocal microscopy

Cells were plated onto cover slips in cell culture dishes. BGlc₄ dissolved in DMSO was added to the cultures to a final concentration of 10 μ M (DMSO concentrations were < 0.2%). After incubation for 24 hours the cells were rinsed three times with PBS and incubated with a 4% paraformaldehyde solution for 15 minutes at 37 °C under cell growth conditions. Cells were then washed three times with PBS, mounted in Dako fluorescence mounting medium, and visualized using a Zeiss LSM510 laser scanning confocal microscope where images were captured with excitation at 514 nm, emission used a 710–750 nm band pass filter.

Results and Discussion

The reduction of TPPF₂₀ is carried out by modifications of a reported 1,3-dipolar cycloaddition reaction of an azomethine ylide with TPPF₂₀, **1** to produce chlorin **2**, isobacteriochlorin **3**, and bacteriochlorin **4** (Scheme 2) (48,49,51). The reaction is scaled up by 5-fold, and the chlorin, bacteriochlorin and isobacteriochlorin products were separated by column chromatography using a petroleum ether/ethylacetate gradient as eluent. When compound **3** is the target, the products are formed in yields of ca. 66% for **2**, 13% yield of the anti isomer of **3**, and 5% yields for the syn and anti isomers of the bacteriochlorin **4**. These yields are somewhat different from those reported, and reflect the yield of the isobacteriochlorin to the bacteriochlorin is ca. 5:1 rather than a few % of the latter.

The mechanism of these dipolar additions has been discussed (51). Interestingly, chiral HPLC assays and CD spectra indicate that a trace quantity of what is likely the product of a non-concerted addition reaction in the crude reaction mixture in the synthesis of the chlorin. This may arise because of the stability of an intermediate conferred by the macrocycle (59,60), but we did not pursue this. Statistically, the second dipolar addition reaction should prefer the adjacent pyrrole 2:1 over the opposite pyrrole, but IF₂₀ is formed 3:1. As with previous reports, for IF₂₀ the anti structure (Scheme 2) forms predominantly by ca. 7-fold (48,49), and our preliminary dynamics calculations indicate steric crowding between the N-methylpyrrolidine methylenes and adjacent fluorophenyls results in distortion of the macrocycle and somewhat blocking the syn face. In the case of the BF₂₀ the syn isomer is the major product. The dipolar addition mechanism in terms of MO calculations was discussed (51).

The principal goal was to obtain the four compounds in one-step and in sufficient quantity for the next reactions. NMR and mass spectrometry are consistent with the previous report and attest to the purity of these porphyrinoid-F₂₀ intermediates. The nucleophilic substitution of the thioglucose at the para position of the perfluorophenyl substituents of these porphyrinoids is a modification of that previously reported (33). Specifically, 4.5 equivalents of 1-thioacetate-2,3,4,6-tetra-O-acetyl- β -D-glucopyranose, a greater quantity of diethylamine (DEA), in 1 h reactions yield PGlcAc₄ **1a**. Thus, **2a** (CGlcAc₄), **3a** (IGlcAc₄) and **4a** (BGlcAc₄), are obtained from **2**, **3**, and **4** respectively (Scheme 2). More than four thiosugars can be added with greater equivalents and longer reaction times. Flash chromatography yields the protected glycosylated derivatives as the mixture of diastereomers in ca. 85% yields. The carbohydrate protection groups were removed by

treating **1a**, **2a**, **3a**, and **4a** with sodium methoxide in dry methanol to obtain the glycoporphyrinoids **1b**, **2b**, **3b**, and **4b** in ca. 95–98% yield (Scheme 2) (see Supporting Information).

Since the N-methylpyrrolidine points toward one face of the macrocycle, addition of moieties with chiral centers such as the sugars to chlorin **2** results in diastereomers at the N-methylpyrrolidine β -carbons for **2a**, **2b**, even though **2** has a plane of symmetry. Thus **2a** and **2b** are 2R,3S diastereomers, which was not previously recognized. As pointed out in the synthesis (51), there is a set of enantiomers at the β -carbons for the anti conformer of **3** thus the addition of the sugars further differentiates these in **3a** and **3b** into diastereomers 2R,3S, 7S,8R and 2S,3R,7R,8S. Both the syn and anti forms of bacteriochlorin **4** have a plane of symmetry, but addition of the sugars results in a set of diastereomers, 2R,3S,12S,13R and 2S,3R,12R,13S.

The structures of all porphyrinoids were confirmed by NMR, UV–visible, mass spectrometry and HRMS analysis. The ^1H NMR spectra of **2a**, **3a** and **4a** show the porphyrinoid core pyrrole β protons near 8.9 ppm and the pyrrole NH protons near -2 ppm. The ^1H NMR spectrum of all three compounds show resonance at 2.1 and 2.3 ppm, due to the acetyl protons, and the resonances of the other protons of the carbohydrate unit appear between 4 and 6 ppm. The resonances of the anomeric protons appear as doublets at 5.03 ppm to 5.40 ppm. The N-methyl groups are observed in the 2.22 ppm for **2a** and are obscured by the acetyl groups in **3a** and **4a**. While the diastereomers are observed for some resonances for the protected derivatives, they are not well resolved for **2b**, **3b**, and **4b**.

The ^{19}F NMR spectra confirm the substitution of the para-fluorine atom by the sugar unit; showing the disappearance of the resonances due to the para-fluorine atoms in **2**, **3** and **4** at -150 ppm. An important diagnostic is the signal due to the meta-fluorine atoms shifts from -160 ppm in **2**, **3**, and **4** to -130 ppm for **2a**, **3a** and **4a**. The ortho-fluorine atoms resonances remain near -140 ppm. The ^{19}F NMR spectra do not exhibit marked differences between **2a** and **2b**, **3a** and **3b**, or **4a** and **4b**, though these peaks are broadened due to the diastereomeric centers. The ^{13}C NMR clearly indicates the multiple resonances arising from the diastereomers, especially for the phenyl carbons, which remain coupled to the F-atoms, and are closest to the chiral centers of **2a**, **2b**, **3a**, **3b**, **4a**, and **4b**.

Considering the large dependence of the photophysical properties of polar chromophores on the solvent matrix and on other experimental parameters, it is important to develop a self-consistent set of data for accurate comparison of these properties and assessment of potential applications. Since the dye can partition into lipophilic, hydrophilic or amphipathic cellular structures, we have assayed the photophysical properties in three different solvents. Thus, PBS at pH = 7.4, ethanol, and ethyl acetate solvents were used to probe the photophysical properties (the partition coefficients and photophysical properties are summarized in Tables 1 and 2). While the initial association with the cell is mediated by glucose receptors and the four glycosyl groups on the chromophore, some non-specific partition into the membrane is also indicated. Because of the size of the molecules, uptake is unlikely to proceed by active or passive transport, but the high local concentration around the cell increases diffusion. Thus uptake is also dependent on the relative hydrophobicity of the compounds (61). The octanol/water partition coefficient and R_f for the glycosylated compounds are listed in Table 1.

The electronic spectra of **2**, **3**, and **4** and the glycosylated derivatives are significantly different from the TPPF₂₀. The lowest energy Q band of chlorin, CGlc₄ in PBS buffer at 649 nm has about 25-fold greater intensity than corresponding Q band of porphyrin PGlc₄ at 646 nm. For isobacteriochlorin, IGlc₄ in the same buffer, the lowest energy Q band at 645 nm is

5-fold more intense than the porphyrin at the same wavelength. For BGlc₄ the strong lowest energy peak is located at 730 nm improving the absorption in the red region of the spectrum. The fluorescence emission spectra for the four glycosylated porphyrinoids are correspondingly different and have a strong solvent dependence (Table 2).

There are negligible Stokes shifts for PGlc₄, CGlc₄ and BGlc₄ in the three solvents, indicating minimal specific solvent-solute interactions with the macrocycle effecting vibrational processes (62). Notably, for the IGlc₄ in PBS, the strongest fluorescence band λ_{max} is at 606 nm with a weaker emission centered at 650 nm while for BGlc₄ the peak is presented at 725 nm. Excitation spectra all indicate the presence of only the given compounds. The 0.17 and 0.36 fluorescence quantum yields for CGlc₄ and for IGlc₄ in PBS are 6 times and 12 times respectively that of the porphyrin analogue PGlc₄ while for BGlc₄ is 0.03, similar to the PGlc₄. There is a small decrease in fluorescence quantum yield upon replacement of the para F atom by the thiocarbohydrate group due to both electronic and heavy atom effects. The greater fluorescence quantum yield of **2b** than m-THPC (13) or a water soluble di-meso substituted chlorin (10) likely arises from the presence of the 16 F groups and relative energies of the molecular orbitals, and is consistent with the quantum yield of singlet oxygen generation by **2** (63). However, tetraarylporphyrins with a tripeptide (64) and other meso aryl chlorins (65) have similar photophysical properties to what we find. The sugars have little direct effect on the photophysics but modulate the amphipathicity and enable the observed solvent dependence.

The quantum yields of singlet oxygen formation for three of the compounds in methanol d₁ are listed in Table 2. Since the porphyrin ring is the reactive site for ¹O₂ production, the frequency of collisions that result in the formation of ¹O₂ is related to the probability that an ³O₂ molecule makes physical contact with the chromophore in the excited, triplet state. The additional steric effect of the pyrrolidine-fused rings on CGlc₄ and IGlc₄ could result in reduced collision frequencies and a lower Φ_{Δ} compared to PGlc₄. It is reasonable to expect that internal conversion is somewhat consistent between the compounds, so the increased fluorescence quantum yield means a decrease in the amount of triplet formed. The Φ_{Δ} data dovetails with the observed fluorescence yields, but the Φ_{Δ} for IGlc₄ in methanol is more consistent with the PBS buffer data.

Because our previous studies with thioglycosylated porphyrin PGlc₄ indicated good uptake and photodynamic effects on several cancer cell lines, K:Molv NIH 3T3 mouse fibroblasts (Figure 2) and MDA-MB-231 breast cancer cell line were used to evaluate the cellular uptake of the glycosylated derivatives. Our initial hypothesis was that the N-methylpyrrolidine moieties used to make and stabilize the chlorin, bacteriochlorin, and isobacteriochlorin (as well as the stereo centers at the β positions), would have minimal effect on cell uptake, because these are sandwiched between the glycosylated tetrafluorophenyl groups. K:Molv NIH 3T3 cells were incubated identically with 2.5 μ M concentrations of PGlc₄, CGlc₄ and IGlc₄, rinsed with buffer, and the relative uptake was quantified by comparison of images taken by fluorescence microscopy under identical settings (38). Confocal microscopy using 10 μ M BGlc₄ was used because the red absorption and emission are outside the range of our fluorescence microscope (Figure 3). The electronic spectra, fluorescence, and fluorescence microscopy studies all show that these compounds are robust to photobleaching. The remaining 16 F groups impart oxidative stability and further enhance the photonic properties.

At 2.5 μ M, the relative integrated intensities for PGlc₄, CGlc₄ and IGlc₄ in the fluorescence micrographs of the K:Molv NIH 3T3 are 1:2:7, respectively (Figure 2). Taking into consideration both the partition coefficients and the fluorescence quantum yields, one would expect that the relative uptake of these compounds into the cells measured by fluorescence

to be ca. 1:4:2 for the PGlc₄:CGlc₄:IGlc₄, respectively. The disparity may arise from differences in cell specificity or the degree of aggregation of the compounds in the PBS buffer and/or aggregation as the local concentration of the dyes around the cell increases (17). The propensity of aggregation inside the cell may also vary. Also, some diastereomers may be taken up preferentially over others. The mechanism of uptake and photodynamic effects in this and other cell lines will be reported elsewhere.

Though there may be differences in uptake between these compounds, we have shown that the PGlc₄ compound localizes in the endoplasmic reticulum because of the metabolic needs of this organelle (43). The significantly greater fluorescence quantum yield of the IGlc₄ system means that ca. 25 nM concentrations can be used to follow receptors and/or glycolysis processes in the cell (Figure 4). Thus, IGlc₄ is better suited for tagging and sensor applications. For PDT applications, sensitizers with strong red absorptions are generally considered better because these wavelengths penetrate deeper into tissue (6,7). The optical cross section of CGlc₄ in the red region is significantly larger than the porphyrin or isobacteriochlorin analogues. Thus, if red light is used to activate the second generation dye CGlc₄, the increased light absorptivity more than compensates the reduced triplet quantum yield. Given the very strong 730 nm absorption, the low fluorescence quantum yield, and greater triplet quantum yield of BGlc₄, this may be the best photosensitizer for PDT of the compounds described herein. However, a more efficient synthesis and purification of the isomers and diastereomers needs to be developed for the bacteriochlorin compounds to be used. The detailed photophysics of these compounds, including that in biological systems will be the subject of future studies.

Though the fluorescence of CGlc₄ and IGlc₄ is greater than the parent porphyrin, there is still sufficient triplet formation to effect cell death via necrosis and/or apoptosis. This indicates that these compounds can be used as dual function agents that both locate and treat cancerous cells. To demonstrate PDT activity, CGlc₄ and IGlc₄ were tested under the same conditions as PGlc₄, (10 μ M concentration and 10 minutes irradiation with white light from a 13W fluorescent bulb). CGlc₄ and IGlc₄ induce cell death only after twice the light power is used (data not shown).

We have also made the chlorin via a gemdiol on one pyrrole unit of TPPF₂₀ and the corresponding tetraglycosylated derivative (47,66), but the yield and stability are not as good as the compounds described herein (see Supporting Information). Similar to the parent TPPF₂₀, preliminary studies indicate the same high yield substitution chemistry can yield an array of conjugates on these core platforms to target various tissues (2,34,38), cell types, and cellular structures (67).

In conclusion, these core platforms will enable rapid development of new multifunctional imaging, sensing, and therapeutic agents (68) for specific targets, and the ability to assess the effectiveness appended motifs in targeting. These platforms will also facilitate the development of effective probes for fundamental biochemical/biophysical studies (44,69). Both CGlc₄ and IGlc₄ have significantly enhanced fluorescence quantum yields compared to the PGlc₄, while the BGlc₄ derivative has a fluorescence quantum yield that is similar to the parent porphyrin. Thus IGlc₄ has greatest potential use as fluorescent tags, diagnostics, or imaging agents. The intermediate fluorescence of the CGlc₄ system may well serve as a dual purpose agent for targeting, detecting, and treating diseased tissues. The BGlc₄ system has near optimal properties as the PDT agent, but new synthetic strategies are needed to make these compounds.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Figure 1.

Top: UV-Visible spectra of the compounds, 2 μ M in ethanol. Bottom: emission spectra of the compounds in ethanol; excitation at 512 nm where the absorbance is ca. 0.05 for each compound.

Figure 2. Fluorescence microscopy of K:MOLV NIH 3T3 cells treated with 2.5 μ M **PGlc₄** (**1b**), **CGlc₄** (**2b**), and **IGlc₄** (**3b**). K:Molv NIH 3T3 cells were incubated for 20 hrs with porphyrinoids, followed by removal of unbound dye from the cell culture by repeated rinsing with PBS, and the cells were imaged under identical microscope settings and not enhanced; magnification 10X.

Figure 3.

K:Molv NIH 3T3 cells were incubated with 10 μ M BGlc₄ for 24 hrs., rinsed three times with PBS buffer, and fixed with 4% paraformaldehyde solution. Confocal microscope excitation at 514 nm, emission monitored with a 710–750 band pass filter. No images are observed using a 610–650 nm emission band pass filter, so fluorescence does not arise from the one of the other dye systems. The image is not enhanced, magnification is 60x.

Figure 4. Fluorescence image of a MDA-MB-231 breast cancer cell treated with 25 nM IGlc₄, and rinsed three times with BPS buffer to remove unbound dye (see Supporting Information for comparisons to the other porphyrinoids).

Scheme 1.

Synthesis of PGlc₄ from tetrakis(pentafluorophenyl)porphyrin (TPPF₂₀)

Scheme 2.
Synthesis of CGlc₄, IGlc₄ and BGlc₄

1 (2*R*,3*S*) - meso when R is achiral

2 (2*R*,3*S*) - one optically active diastereomer when R^{*} is chiral

Scheme 3.
CGlc₄ diastereomers (see Supporting Information).

Table 1

Partition Coefficients

Cpd.	octanol/water	R _f ^a
PGlc ₄	43.9 (4.8)	0.6 (5.5)
CGlc ₄	28.5 (3.13)	0.37 (3.4)
IGlc ₄	9.1 (1)	0.11 (1)
BGlc ₄	12.7 (1.4)	0.13 (1.2)

^a normalized values in parentheses, see experimental section

Table 2

Photophysical Properties of Glycosylated Porphyrinoid Derivatives.

Cpd.	Solvent	UV-visible ^a	Emission λ_{max}	Φ_F^b	$^1O_2 \Phi_A$
TPPF₂₀	DMSO	412, 504, 538, 580, 632	637, 702	--	
CF₂₀	DMSO ^c	408, 504, 536, 598, 652	656	--	
m-THPC	methanol ^d		653, 720	0.09	0.43
m-THPBC	methanol ^d		612, 653, 746	0.11	0.43
PGlc₄	ethanol	412, 505, 535, 586, 653	653, 702	0.05	0.85 ^f
	PBS buffer	410, 510, ---, 576, 646(1) ^a	646, 692	0.03	
	ethylacetate	412, 507, 450, 583, 655	649, 701	0.05	
	DMSO ^c	415, 507, 539, 581, 636	640, 705	0.06	
CGlc₄	ethanol	408, 507, 534, 599, 653	653, 715	0.43	0.32 ^f
	PBS buffer	409, 506, 533, 597, 649(25) ^a	649, 707	0.17	
	ethylacetate	406, 504, 531, 597, 651	653, 712	0.39	
	DMSO ^e	412, 506, 537, 598, 652	652	--	0.28
BGlc₄	ethanol	353, 374, 505, 732	729	0.047	--
	PBS buffer	357, 508, 730	725	0.03	
	ethylacetate	352, 379, 508, 730	730	0.055	
IGlc₄	ethanol	385, 513, 548, 588, 643	596, 646, 705	0.70	0.59 ^f
	PBS buffer	385, 513, 548, 593, 645(5) ^a	606, 650, 711	0.36	
	ethylacetate	384, 510, 548, 589, 653	596, 646, 700	0.60	

^a relative intensity of lowest energy Q bands,

^b fluorescence experiments done in air,

^c taken from reference (55),

^d m-THPC and the bacteriochlorin m-THPBC taken from reference (13),

^e taken from reference (54),

^f excited at 532 nm in methanol d1