High-yielding syntheses of hydrophilic conjugatable chlorins and bacteriochlorins†

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Next-generation photodynamic therapy agents based upon the conjugation of multiple photosensitizers to a targeting backbone will allow for more efficacious light-based therapies. To this end, we have developed glucose-modified chlorins and bacteriochlorins featuring a reactive carboxylic acid linker for conjugation to targeting moieties. The photosensitizers were synthesized in relatively high yields from *meso*-tetra(*p*-aminophenyl)porphyrin, and resulted in neutral, hydrophilic chromophores with superb absorption profiles in the far-red and near-infrared portions of the electromagnetic spectrum. In addition, conjugation of these photosensitizers to a model nanoscaffold (crosslinked dextran-coated nanoparticles) demonstrated that the inclusion of hydrophilic sugar moieties increased the number of dyes that can be loaded while maintaining suspension stability. The described compounds are expected to be particularly useful in the synthesis of a number of targeted nanotherapeutic systems.

Introduction

Far-red and near infrared absorbing photosensitizers (PS) have been extensively investigated for use in photodynamic therapy. These second-generation PS are substantially improved *versus* their first-generation analogues, including the clinically utilized Photofrin®.[1,2] First-generation agents generally suffer from short wavelengths of absorption and prolonged photosensitivity when administered *in vivo*. Second-generation PS, based upon modified tetrapyrrolic macrocycles, possess significantly stronger absorption profiles at longer wavelengths, thus increasing their efficiency. Second-generation PS include porphyrinoids such as chlorins, bacteriochlorins, benzoporphyrin derivatives, and (na)phthalocyanines.[2,3]

While more efficient PS will allow for increased generation of cytotoxic singlet oxygen, the ability to localize these molecules to sites of interest is also necessary.[3] Such third-generation PS have been targeted by a variety of means, including antibodies,[4–7] peptides,[8] and nanoparticles.[9–14] One of the advantages of these targeted systems is that they can allow for the delivery of multiple PS per molecule of agent. Aside from delivery schemes where the PS is encapsulated within a matrix, such as a silica or polymeric nanoparticle, there are a number of requirements for the synthesis of targeted-PS, including (1) availability of a conjugatable handle, (2) hydrophilicity under physiological conditions, (3) retained singlet oxygen generation in the conjugated state and (4) excellent biocompatibility (*i.e.* absence of dark toxicity). From a chemistry perspective, the second requirement is particularly important for the development of high-payload agents in order to prevent aggregation which has deleterious effects on singlet oxygen quantum yields.

Non-polar porphyrin chromophores have been rendered more hydrophilic by attaching polyethylene glycol,[15,16] cationic substituents,[8,17–19] and anionic substituents, such as sulfonates.[17] One class of substituents that has been highly studied is carbohydrates. Glycosylated porphyrins are water soluble, yet maintain a neutral charge.[20] Sugar modified porphyrins can also be recognized by cell surface carbohydrate receptors expressed in malignant cancers, and thus enable a degree of targeting. Despite previous reports, there continues to be a need for simple, conjugatable glucose modified PS.

Herein, we detail the high yielding syntheses of hydrophilic, conjugatable, glucose modified *meso*-tetraphenylporphyrin (TPP) derivatives, including the corresponding chlorin and bacteriochlorin analogues, from *meso*-tetra(*p*-aminophenyl)porphyrin. Furthermore, we illustrate the increased loading potential of the hydrophilic PS on a representative dextran-coated nanoparticle scaffold *versus* the corresponding hydrophobic derivative.

Results and discussion

In order to synthesize glucose modified porphyrin derivatives, it is important to start with high yielding materials. To this end, we chose *meso*-tetra(*p*-aminophenyl)porphyrin **1**, which is readily obtained *via* the condensation reaction of nitrobenzaldehyde and pyrrole, followed by $SnCl_2 \cdot 2H_2O$-mediated reduction of the nitro groups to the corresponding amines, as previously reported.[21] Concomitantly, glucuronic acid was acetylated to protect the secondary hydroxyl groups, and then reacted with oxalyl chloride to give the corresponding acid chloride.[22,23]

The sugar modified porphyrin was synthesized using a one pot, two step methodology at millimole scale (Scheme 1). To porphyrin **1** in tetrahydrofuran at 0 °C was added triethylamine and 1.1 equivalents of methyl glutaryl chloride, in order to introduce a

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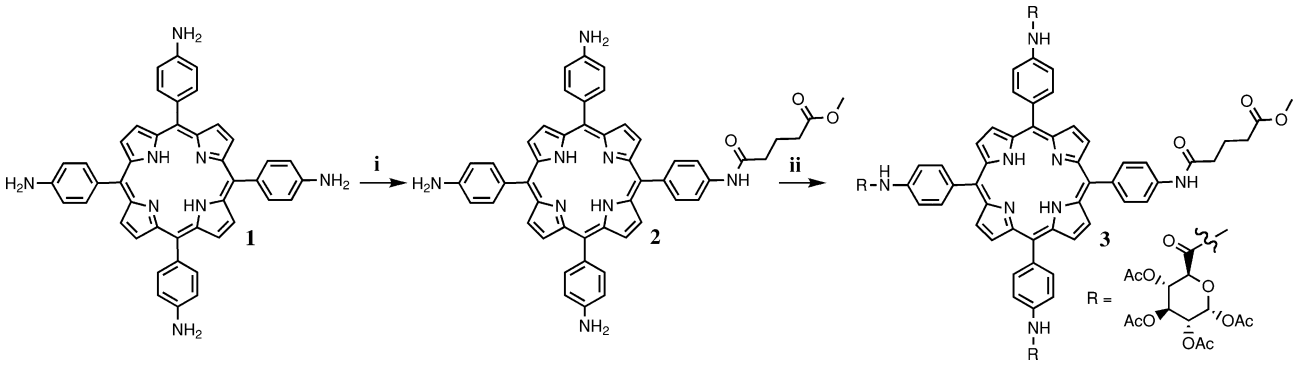


Scheme 1 Two-step, one-pot synthesis of the sugar modified porphyrin, **3**. (i) Methyl glutaryl chloride, triethylamine, THF, 0 °C; (ii) 1,2,3,4-tetra-*O*-acetyl-β-D-glucopyranuronoyl chloride, triethylamine.

single protected carboxylic acid functionality. After 2 h, additional triethylamine was added with subsequent addition of the protected β-D-glucopyranuronoyl chloride, which was allowed to react for another hour. This second addition of base is required due to the innate acidity of the acid chlorides. In the absence of excess base, the porphyrin becomes protonated and precipitates from solution, thereby decreasing the yield of the desired product. The glucose-derivatized porphyrin **3** was purified by flash chromatography using a gradient from 2% to 4% methanol in methylene chloride. Importantly, all fractions not containing the product can be recovered and hydrolyzed back to the starting porphyrin **1** (see ESI† for hydrolysis conditions), so this reaction methodology is essentially lossless.

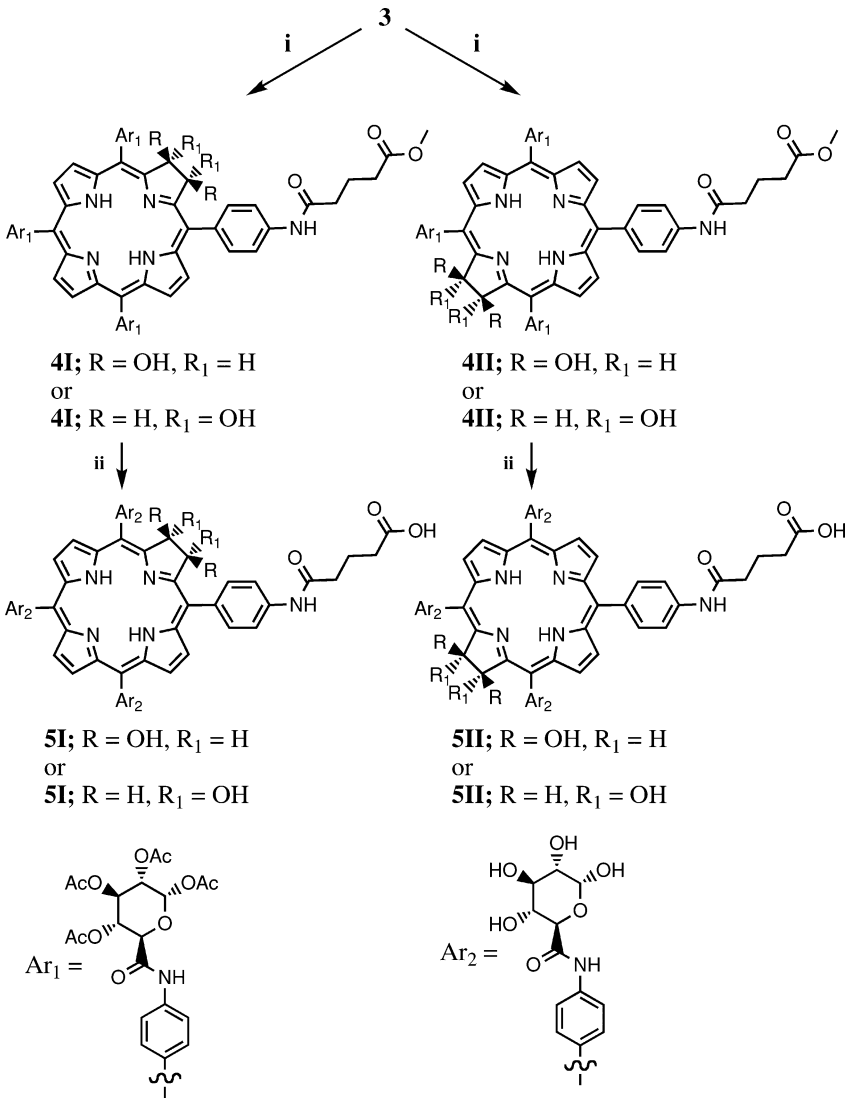
The identity of porphyrin **3** was confirmed by NMR spectroscopy and high-resolution electrospray mass spectrometry (HRMS). The ^{1}H NMR illustrated the inclusion of the three sugar moieties and the methyl glutarate, with signals equivalent to 45 protons between 2.0 and 2.3 ppm corresponding to the sugar acetyl groups (36 H), and the methoxy (3 H) and methylene (6 H) protons of the methyl glutarate (see ESI† for spectra). In addition, the protons of the hexose ring were observed with the expected couplings between 4.3 and 6.0 ppm. The expected peaks were also observed in the ^{13}C NMR, including signals corresponding to the four amide and one ester carbonyl groups (164–175 ppm).

This synthesis can also be accomplished stepwise, although the separation of the methyl glutaryl porphyrin **2** is rather difficult by flash chromatography, largely due to smearing caused by the presence of the amines which undergo protonation–deprotonation due to the acidity of the silica gel. Additionally, alternate protected acid moieties can be introduced, such as methyl 4-chloro-4-oxobutyrate, methyl adipoyl chloride, or glutaric acid monoethyl ester chloride. The use of succinic or glutaric anhydride could also be envisioned, but products bearing non-protected acids complicate the subsequent reactions due to the formation of side products, making the purifications difficult.

Conversion of the porphyrin **3** to the corresponding chlorins and bacteriochlorins was effected by the osmium tetroxide-mediated dihydroxylation (Schemes 2 and 3, respectively). Reaction of **3** with 2 equivalents of OsO_4 resulted in the formation of four osmate ester isomers; two sets of regioisomers (adjacent to and opposite to the methyl glucaryl group), each of which consists of two stereoisomers. At this stage, the chlorins were separated by column chromatography into the respective regioisomers, as the stereoisomers are of identical polarity, and are thus inseparable by this method. The regioisomers were then treated with hydrogen sulfide to yield the vic-dihydroxychlorins **4I** and **4II**, which were further purified by column chromatography. As with the porphyrin, the presence of glucose and methyl glutaryl groups was observed by ^{1}H and ^{13}C NMR spectroscopy. In addition, signals corresponding to the hydroxyl protons (3.45 ppm) and 2,3-β hydrogens (6.26 ppm) were also observed. Hydrolysis of the sugar acetyl ester and glutaryl methyl ester of **4I** and **4II** with 0.3 M LiOH yields the conjugatable, hydrophilic chlorins **5I** and **5II**. These products were readily purified by HPLC and characterized by NMR and HRMS. Since it has proven difficult to fully resolve the identity of the isomers, they have been labeled with Roman numerals based upon their polarity, with the least polar labeled I and the most polar labeled II.

The 2,3,12,13-bis-(vic-dihydroxy)bacteriochlorins, **6**, were synthesized as described above for the chlorins, except for the addition of a large excess of OsO_4 due to the need to include two osmate esters per porphyrin. Reaction of **3** with 6 equivalents of OsO_4 resulted in the formation of four isomers (Scheme 3) which were separable by column chromatography into two components. Each purified mixture is expected to be comprised of two isomers, likely the co-facial or anti-facial isomers, respectively. The purified products were then treated with hydrogen sulfide to yield the bis-(vic-dihydroxy)bacteriochlorins **6I** and **6II**, which were further purified by column chromatography. The NMR spectra of the isomeric mixtures were highly similar. In fact, the similarity also extended to the spectra of **4I** and **4II**, since the chlorins and bacteriochlorins bear the same symmetry point group and aminophenyl modifications. The main differences were seen in the aromatic region, where the bacteriochlorins possess 2 fewer protons, and in the aliphatic region, where the additional β-protons and hydroxyl protons are present. Each of the acetyl protected bacteriochlorins was further treated with a 0.3 M LiOH solution in order to remove the acetyl protecting groups and at the same time to cleave the ester group in the side chain, as described above for the chlorins. The crude deprotected bacteriochlorins were purified by C-18 reverse phase chromatography to afford sugar-modified bacteriochlorins **7I** and **7II**.

When the UV-vis absorption spectra of the starting porphyrins were examined (Fig. 1), a large increase in the extinction coefficient (10-fold) and the hypsochromic shift (10 nm) of the Soret band were observed upon conjugation of the methyl



Scheme 2 Synthesis of vic-dihydroxychlorins **5I** and **5II**. (i) 1. OsO_4, pyridine; 2. H_2S; (ii) 0.3 M LiOH, 2 h.

glutaryl chloride and the protected sugar moieties to *meso*-tetra(*p*-aminophenyl)porphyrin **1**. Interestingly, the extinction coefficient of **1** is also significantly lower than that of TPP, illustrating the effect the presence of amine moieties has on the photophysics of the chromophore, although they do not directly partake in its conjugation. Amide bond formation, on the other hand, seems to negate the presence of the amines and enhances its absorption profile. Conversion of porphyrin **3** to chlorins **4I** and **4II** results in the expected broadening of the Soret band, and an increase in the extinction coefficient of the farthest red side band (~3-fold *versus* the porphyrin). Deprotection of **4I** and **4II** unfortunately yields chlorins with decreased extinction coefficients in DMF, likely due to differences in solubility and solvation. Conversion of the porphyrin to bacteriochlorins **6I** and **6II** results in significantly altered absorption spectra, as demonstrated previously.[24] The main advantage of bacteriochlorins is that the longest wavelength absorption is shifted to 713 nm, 65 nm further red than the respective chlorins, with comparable extinction coefficients. At longer wavelengths, light is able to penetrate deeper into tissues, maximizing the therapeutic potential of light activated therapies.[25]

Conjugation to nanoparticles

In order to demonstrate the advantage of the sugar-modified chlorins in the conjugation to targeting and delivery platforms, we modified crosslinked dextran-coated nanoparticles with chlorin **5I**. The conjugation efficiency of our chlorin was compared to that of chlorin e_6, which is commercially available and has been commonly used in a number of photodynamic therapy applications.[26–28] Twenty four to 138 PS were appended to the particle surface *via* 1-ethyl-3-[3-dimethylaminopropyl]carbodiimide (EDC)-mediated amide bond formation. At all PS loadings, the particles modified with **5I** remained in suspension, with almost quantitative recovery of the products. In contrast, at higher loadings of chlorin e_6 (101 and 87 PS), 50% of the particles flocculated and settled out of suspension within 16 h. At 48 h after purification, all chlorin e_6-modified particles demonstrated some degree of precipitation, with the highest loadings completely absent from suspension. In contrast, all nanoagents synthesized with the sugar-modified chlorins were stable for more than one month, with no observed flocculation.

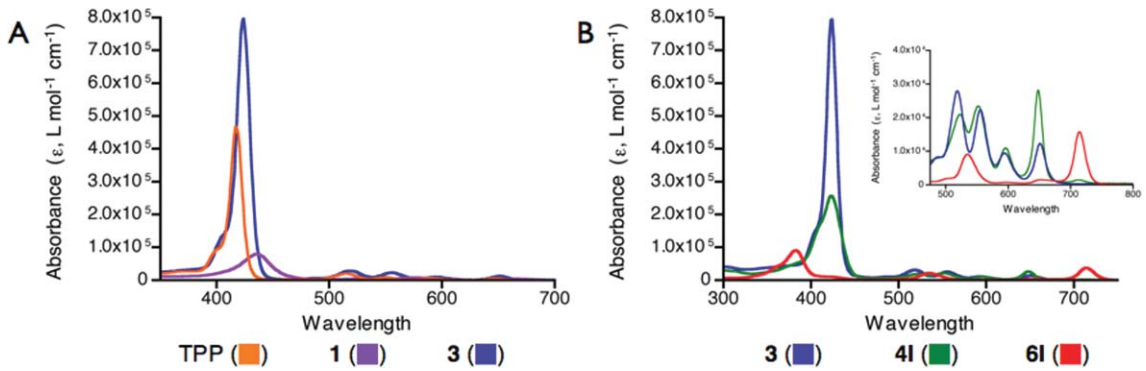
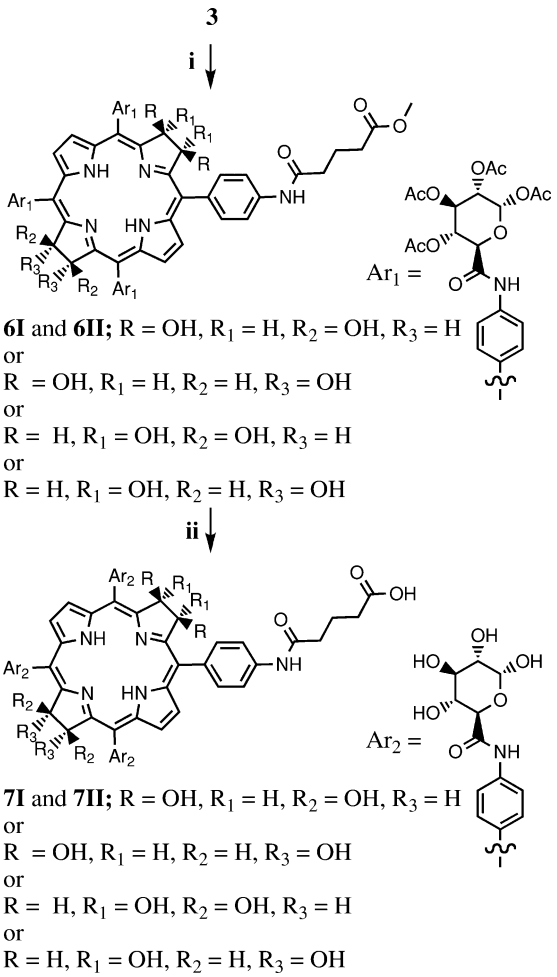


Fig. 1 UV-vis absorption spectra graphed *versus* the extinction coefficient of the chromophores. A. *meso*-Tetraphenylporphyrin (TPP), *meso*-tetra(*p*-aminophenyl)porphyrin **1**, and sugar-modified porphyrin **3**. B. Sugar-modified porphyrin **3**, sugar-modified chlorin **4I**, and sugar-modified bacteriochlorin **6I**.



Scheme 3 Synthesis of bis-(vic-dihydroxy)bacteriochlorins **7**. (i) 1. OsO_4, pyridine; 2. H_2S; (ii) 0.3 M LiOH, 2 h.

While efficient PS conjugation is important, so are the excited state processes of the conjugated chlorins, including singlet oxygen generation and fluorescence emission. In order to determine this efficiency, the relative fluorescence quantum yield of the chromophore at each nanoparticle loading was determined. As compared to the free glucose-modified chlorin **5I**, the comparative fluorescence quantum yield of the conjugates was approximately 25%, regardless of the number conjugated. Alternately, the chlorin e_6 conjugates displayed only 5% of the fluorescence quantum yield of the free PS, also regardless of the number of chlorins per particle. This decrease in fluorescence may be attributed to a number of factors, including the aforementioned dye–dye interactions, as well as a difference in the local environment between the free dye and the conjugate. An additional confounding factor is the light scattering that may be introduced by the nanoparticles, further decreasing the observed quantum yields.

Conclusions

We have demonstrated the syntheses of hydrophilic, conjugatable chlorins and bacteriochlorins based upon the modification of *meso*-tetra(*p*-aminophenyl)porphyrin. These PS bear glucose moieties in order to increase the polarity of the relatively hydrophobic porphyrin, while also maintaining a neutral overall charge. In addition, a carboxylic acid functionalized linker is also appended to the macrocycle in order to allow for facile conjugation to biomolecules and nanoagents. Conjugation of the chlorins to a model nanoscaffold illustrated that the PS do not exhibit appreciable differences in excited state quenching at any loading while simultaneously demonstrating excellent suspension stability, as compared to chlorin e_6. Thus, we believe that these PS are expected to be useful in the synthesis of the next generation of targeted nanoagents for the therapy and treatment of a number of diseases, including cancer and atherosclerosis.

Experimental section

General

All chemicals and solvents were purchased from Fisher or Sigma Aldrich and used as received without further purification. Silica gel (Sorbent Technologies, 60 Å, 40–63 μm, 230 × 400 mesh) was used for column chromatography. UV-vis spectra were recorded on a Varian Cary 50 UV-vis spectrophotometer. Fluorescence data were collected from a Varian Cary Eclipse fluorescence spectrophotometer. Absorption and fluorescence spectra were collected in DMF at room temperature unless noted otherwise. LCMS data were collected from a Water 2695 HPLC equipped with a 2996 diode array detector, a Micromass ZQ4000 ESI-MS

module, and a Grace-Vydac RPC18 column (model 218TP5210) at a flow rate of 0.3 mL min^{-1}. Gradients were run with buffer A (H_2O–0.1% trifluoroacetic acid (TFA)) and buffer B (90% acetonitrile–10% H_2O–0.1% TFA). For analytical HPLC a C-18 reverse phase column (Varian) was used with dimensions of 250 mm × 4.6 mm. For semi-preparative HPLC a C-18 reverse phase column (Varian) was used with dimensions of 250 mm × 21.2 mm. High-resolution eletrospray ionization (ESI) mass spectra were obtained from a Bruker Daltonics APEXIV 4.7 T Fourier transform ion cyclotron resonance spectrometer (FT-ICR-MS) in the Department of Chemistry Instrumentation Facility (DCIF) at the Massachusetts Institute of Technology. All ^{1}H NMR spectra (500 MHz) and ^{13}C NMR spectra (125 MHz) were collected in the solvents noted. Porphyrin **1**[21] and β-D-glucopyranuronoyl chloride[22,23] were synthesized as described previously. Iron oxide nanoparticles were obtained from the chemistry core at the Center for Molecular Imaging Research.

5,10,15-Tris(4-1′,2′,3′,4′-*O*-acetyl-glucopyranuron-*N*-phenylamide)-20-[4-(5′-methoxy-1′,5′-dioxopentyl)aminophenyl]porphyrin, 3

To 1.04 g of porphyrin **1** (1.54×10^{-3} mol) in tetrahydrofuran (300 mL) at 0 °C was added triethylamine (Et_3N, 5 equiv, 1.08 mL), and methyl glutaryl chloride (1.1 equiv, 233 μL). The reaction was allowed to proceed at 0 °C for 2 h, at which time an additional 5 equiv of Et_3N were added (1.08 mL). To this solution was then added β-D-glucopyranuronoyl chloride (4 equiv, 2.34 g), and the reaction mixture was allowed to warm to room temperature over the course of 1 h. The reaction mixture was then concentrated to approximately 50 mL and then diluted with CH_2Cl_2 (100 mL). The solution was washed once with water, once with saturated aq $NaHCO_3$, and once with water again. The organic layer was subsequently dried over anhydrous $MgSO_4$ and evaporated to dryness. The product was purified by flash chromatography (silica gel, CH_2Cl_2–MeOH, gradient from 98 : 2 to 96 : 4). All fractions containing the product were combined and evaporated to dryness, redissolved in CH_2Cl_2, and precipitated with hexanes to give the product in 33% recovered yield (0.92 g). UV-vis (DMF) λ_{max} (log ε): 424 (5.90), 519 (4.44), 556 (4.36), 595 (3.99), 648 (4.09) nm; ^{1}H NMR (500 MHz, $CDCl_3$, δ) −2.84 (s, 2H), 2.08–2.28 (m, 41H), 2.57 (m, 4H), 4.38 (d, 3H, J = 9.5), 5.26 (t, 3H, J = 8.0), 5.44 (t, 3H, J = 9.0), 5.50 (t, 3H, J = 9.5), 5.94 (d, 2H, J = 8.0), 7.87 (br m, 8H), 8.14 (br m, 9H), 8.31 (s, 3H), 8.82 (br m, 8H) ppm; ^{13}C NMR (125 MHz, $CDCl_3$, δ) 20.7, 20.7, 20.8, 21.0, 21.0, 33.0, 33.1, 36.8, 51.9, 69.1, 70.6, 72.1, 73.6, 91.8, 118.0, 118.8, 135.2, 136.4, 139.0, 164.4, 169.0, 169.1, 169.4, 169.9, 170.0, 174.1 ppm; +ESI-MS (30 V, CH_3CN–0.1% TFA) m/z = 1837.2 (MH^+), 919.0 (MH^{2+}); HR-MS (ESI of MH+, CH_3CN): m/z calc'd for $C_{92}H_{91}N_8O_{33}$: 1836.5761, found: 1836.5729; HPLC t_R = 11.40 min (using a gradient of 60% to 0% of buffer A over 25 min).

General synthesis of 2,3-vic-dihydroxy-5,10,15-tris(4-1′,2′,3′,4′-*O*-acetyl-glucopyranuron-*N*-phenylamide)-20-[4-(5′-methoxy-1′,5′-dioxopentyl)aminophenyl]chlorins, 4I and 4II

To a stirring solution of **3** (~0.8 g) in $CHCl_3$–pyridine (4 : 1, 150 mL) was added OsO_4 (2.0 equiv.). The reaction was allowed to proceed for 48 hours, at which time it was evaporated to dryness. The two isomers of the osmate ester were separated by flash chromatography (silica gel, CH_2Cl_2–MeOH, 96 : 4), with fractions containing the respective isomers combined and evaporated to dryness. Each isomer was then dissolved in CH_2Cl_2 (20 mL) and H_2S was bubbled through the solution for 5 min. The reaction mixtures were stoppered and reacted for 45 min, at which time they were blown dry with a stream of nitrogen. The crude products were then dissolved in CH_2Cl_2 and passed through celite to remove any residual solids, and were again evaporated to dryness. The final products were obtained by flash chromatography using the solvent systems detailed below. Alternatively, the reaction can be monitored by UV-vis spectroscopy in order to determine the extent of reaction, allowing for the adjustment of reaction time.

4I. Flash chromatography (silica gel, CH_2Cl_2–MeOH, gradient from 97.5 : 2.5 to 95 : 5). Fractions containing the product were combined and evaporated to dryness, dissolved in a minimal volume of CH_2Cl_2 and precipitated with petroleum ether to give chlorin **4I** in 8% yield (0.07 g). UV-vis (DMF) λ_{max} (log ε): 424 (5.52), 523 (4.36), 552 (4.42), 597 (4.08), 648 (4.51) nm; ^{1}H NMR (500 MHz, $CDCl_3$, δ) −1.87 (s, 2H), 2.12–2.24 (br m, 41H), 2.57 (br, 4H), 3.4 (br, 2H), 4.42 (br m, 3H), 5.23 (br m, 3H), 5.49 (br m, 6H), 5.96 (br m, 3H), 6.25 (br 2H), 7.90 (br m, 17 H), 8.39 (br m, 11 H) ppm; ^{13}C NMR (125 MHz, $CDCl_3$, δ) 20.5, 20.6, 20.7, 20.8, 20.8, 33.0, 51.7, 68.8, 70.3, 71.9, 73.5, 91.6, 112.4, 117.9, 119.3, 119.8, 124.1, 127.9, 132.5, 134.4, 136.1, 140.6, 164.2, 164.3, 169.0, 169.3, 169.6, 169.7, 169.9, 170.0, 173.9 ppm; +ESI-MS (30 V, CH_3CN–0.1% TFA) m/z = 1871.2 (MH^+); HR-MS (ESI of MH+, CH_3CN): m/z calc'd for $C_{92}H_{93}N_8O_{35}$: 1870.5816, found: 1870.5810; HPLC t_R = 12.52 min (using a gradient of 60% to 0% of buffer A over 25 min).

4II. Flash chromatography (silica gel, CH_2Cl_2–MeOH, 96 : 4). Fractions containing the product were combined and evaporated to dryness, dissolved in a minimal volume of CH_2Cl_2 and precipitated with petroleum ether to give chlorin **4II** in 12% yield (0.10 g). UV-vis (DMF) λ_{max} (log ε): 424 (5.41), 523 (4.28), 552 (4.34), 597 (4.02), 648 (4.45) nm; ^{1}H NMR (500 MHz, $CDCl_3$, δ) −1.90 (s, 2H), 2.15 (m, 41H), 2.54 (s, 4H), 2.96 (br s, 2H), 4.42 (m, 3H), 4.59 (m, 6H), 5.30 (m, 3H), 5.96 (m, 3H), 6.30 (br s, 2H), 7.87 (br, 17H), 8.45 (m, 11H) ppm; ^{13}C NMR (125 MHz, $CDCl_3$, δ) 20.6, 20.8, 33.0, 36.5, 51.7, 68.9, 70.4, 71.9, 73.6, 91.7, 118.8, 122.2, 124.3, 128.0, 132.6, 134.4, 135.5, 136.2, 137.8, 140.7, 152.9, 161.5, 164.4, 169.0, 169.3, 169.7, 169.9, 170.8, 173.9 ppm; +ESI-MS (30 V, CH_3CN–0.1% TFA) m/z = 1871.3 (MH^+); HR-MS (ESI of MH+, CH3CN): m/z calc'd for $C_{92}H_{93}N_8O_{35}$: 1870.5816 found: 1870.5889; HPLC t_R = 12.61 min (using a gradient of 60% to 0% of buffer A over 25 min).

Deprotection of chlorins 4I and 4II. A sample of **4I** or **4II** (~20 mg) in THF–MeOH (1 : 1, 3 mL) was cooled to 0 °C. A cold solution of LiOH in water (1.5 mL, 0.8 M) was then slowly added. The reaction mixture was stirred at 0 °C. The reaction was monitored by HPLC. Upon complete hydrolysis of the protecting groups (~2 h) the reaction mixture was poured into 30 mL cold water. The pH of the resulting solution was raised to 4 by slow addition of cold 0.1 M HCl. The resulting mixture was purified by reverse phase column chromatography (C-18 cartridge, eluted with a gradient of 100 to 0 of buffer A) followed by preparative HPLC

(using a gradient of 100 to 0 of buffer A, flow rate = 21 mL min^{-1}). The fractions containing the desired product were combined and evaporated to dryness affording a green solid:

5I. Yield 10 mg, 73%; UV-vis (DMF) λ_{max} (log ε): 424 (5.18), 522 (3.94), 552 (3.99), 595 (3.68), 647 (4.04) nm; UV-vis (PBS) λ_{max} (log ε): 406 (4.65), 526 (3.49), 556 (3.54), 597 (3.33), 649 (3.59) nm; +ESI-MS (30 V, CH_3CN–0.1% TFA) $m/z = 1351$ (MH^+); HR-MS (ESI of MH+, CH_3CN): m/z calc'd for $C_{67}H_{66}N_8O_{23}$: 1351.4314, found: 1351.4371; HPLC $t_R = 9.61$ min (using a gradient of 100% to 0% of buffer A over 20 min).

5II. Yield 11 mg, 80%; UV-vis (DMF) λ_{max} (log ε): 424 (5.07), 522 (3.86), 552 (3.92), 595 (3.64), 647 (4.00) nm; +ESI-MS (30 V, CH_3CN–0.1% TFA) $m/z = 1351$ (MH^+); HR-MS (ESI of MH+, CH_3CN): m/z calc'd for $C_{67}H_{66}N_8O_{23}$: 1351.4314, found: 1351.4328; HPLC $t_R = 9.57$ min (using a gradient of 100% to 0% of buffer A over 20 min).

General synthesis of 2,3,12,13-bis-(vic-dihydroxy)-5,10,15-tris(4-1′,2′,3′,4′-*O*-acetyl-glucopyranuron-*N*-phenylamide)-20-[4-(5′-methoxy-1′,5′-dioxopentyl)aminophenyl]bacteriochlorins, 6I and 6II

To a stirring solution of porphyrin **3** (0.30 g, 0.16 mmol) in anhydrous $CHCl_3$–pyridine (4 : 1, 50 mL) was added a solution of OsO_4 (0.25 g, 6.0 equiv., in 2.5 mL $CHCl_3$–pyridine). The reaction was allowed to proceed for 4 days, at which time it was evaporated to dryness. The bacteriochlorin osmate ester was purified by flash chromatography (silica gel, CH_2Cl_2–MeOH, 96 : 4 to 95 : 5), with fractions containing each of the isomers combined and evaporated to dryness. Each isomer was then dissolved in CH_2Cl_2 (5 mL) and H_2S was bubbled through the solution for 5 min. The reaction mixture was stoppered and reacted for 45 min, at which time it was blown dry with a stream of nitrogen. The crude product was then dissolved in CH_2Cl_2–MeOH (50 : 1 to 25 : 1) and passed through celite to remove any residual solids, and was again evaporated to dryness. The final products were obtained by flash chromatography using the solvent systems detailed below. Alternatively, the reaction can be monitored by UV-vis spectroscopy in order to determine the extent of reaction, allowing for the adjustment of reaction time.

6I. Flash chromatography (silica gel, CH_2Cl_2–MeOH, gradient from 97.5 : 2.5 to 92 : 8). Fractions containing the product were combined and evaporated to dryness affording a pink solid in 10% yield (30 mg). UV-vis (DMF) λ_{max} (log ε): 382 (4.93), 533 (4.30), 713 (4.52) nm; ^{1}H NMR (500 MHz, CD_2Cl_2–CD_3OD 9 : 1, δ) 2.18 (m, 42H), 2.50 (m, 4H), 3.60 (br, 2H), 3.61 (s, 2H), 4.37 (d, $J = 9.0$, 3H), 5.24 (t, $J = 8.0$, 3H), 5.43 (m, 6H), 5.91 (m, 3H), 6.00 (m, 3H), 7.70 (br, 12H), 7.94 (br, 4H), 8.14 (m, 4H) ppm; ^{13}C NMR (125 MHz, CD_2Cl_2, δ) 20.9, 20.9, 21.0, 21.1, 21.4, 33.6, 36.7, 51.4, 69.5, 70.6, 72.4, 73.5, 74.2, 92.1, 115.9, 123.7, 132.7, 136.4, 137.6, 138.8, 159.4, 165.2, 169.8, 169.9, 170.3, 170.5, 174.5 ppm; +ESI-MS (30 V, CH_3CN–0.1% TFA) $m/z = 1903$ (MH^+); HR-MS (ESI of MH^++Na, CH_3CN): m/z calc'd for $C_{92}H_{95}N_8O_{37}Na$: 1926.5696, found: 1926.5702; HPLC $t_R = 14.12$ min (using a gradient of 60% to 0% of buffer A over 25 min).

6II. Flash chromatography (silica gel, CH_2Cl_2–MeOH, gradient from 95 : 5 to 90 :10). Fractions containing the product were combined and evaporated to dryness affording a pink solid in 18% yield (55 mg). UV-vis (DMF) λ_{max} (log ε): 381 (4.96), 535 (4.31), 714 (4.55) nm; ^{1}H NMR (500 MHz, CD_2Cl_2–CD_3OD 9:1, δ) 2.10 (m, 50H), 2.51 (m, 5H), 3.53 (m, 2H), 3.70 (s, 3H), 4.37 (d, $J = 9.0$ Hz, 3H), 5.23 (m, 3H), 5.43 (m, 6H), 5.90 (m, 3H). 6.11 (br, 4H), 7.78 (br m, 14H), 8.10 (br, 6H) ppm; ^{13}C NMR (125 MHz, CD_2Cl_2, δ) 17.7, 20.9, 26.4, 31.4, 33.6, 36.6, 52.1, 92.2, 115.9, 120.2, 123.8, 132.7, 135.1, 136.5, 137.5, 138.9, 159.7, 165.3, 170.0, 170.4, 170.6, 174.7 ppm; +ESI-MS (30 V, CH_3CN–0.1% TFA) $m/z = 1903$ (MH^+); HR-MS (ESI of MH^++Na, CH_3CN): m/z calc'd for $C_{92}H_{95}N_8O_{37}Na$: 1926.5696, found: 1926.5696; HPLC $t_R = 15.52$ min (using a gradient of 100% to 0% of buffer A over 20 min).

Deprotection of bacteriochlorins 6I and 6II. A sample of **6** (~10 mg) in THF–MeOH (1 : 1, 2 mL) was cooled to 0 °C. A cold solution of LiOH in water (1 mL, 0.6 M) was then slowly added. The reaction mixture was stirred at 0 °C. The reaction was monitored by HPLC analysis. Upon complete hydrolysis of all the protecting groups (~2 h) the organic solvents (THF and MeOH) were removed. The resulting solution was diluted with water and poured into a reverse-phase column (C-18 cartridge). The solid residue was washed with plenty of water to remove all inorganic salts. The residue was then eluted with MeOH affording the deprotected bacteriochlorin as a pink solid.

7I. Yield 9 mg, 93%; UV-vis (DMF) λ_{max} (log ε): 384 (4.3), 540 (3.8), 715 (4.0) nm; +ESI-MS (30 V, CH_3CN–0.1% TFA) $m/z = 1385.5$ (MH^+); HR-MS (ESI of $[M-H]^-$, CH_3CN): m/z calc'd for $C_{67}H_{67}N_8O_{25}$: 1383.4217, found: 1383.4209; HPLC $t_R = 9.97$ min (using a gradient of 100% to 0% of buffer A over 20 min).

7II. Yield 6 mg, 87%; UV-vis (DMF) λ_{max} (log ε): 383 (4.5), 537 (3.8), 714 (4.1) nm; +ESI-MS (30 V, CH_3CN–0.1% TFA) $m/z = 1385.3$ (MH^+); HR-MS (ESI of $[M-H]^-$, CH_3CN): m/z calc'd for $C_{67}H_{67}N_8O_{25}$: 1383.4217, found: 1383.4219; HPLC $t_R = 10.08$ min (using a gradient of 100% to 0% of buffer A over 20 min).

Conjugation to crosslinked dextran-coated iron oxide nanoparticles

Stock solutions of chlorin **5I** (2.6 mg, 52 μL) and chlorin e_6 (3.5 mg, 70 μL) were made up in DMSO. To 1 mg crosslinked dextran-coated iron oxide nanoparticles in phosphate buffered saline (12.12 mg Fe mL^{-1}) was added a varying amount of chlorin (1 mg to 0.05 mg) and 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide (EDC) (1 mg). The solutions were allowed to react for 16 h, at which time they were purified by filtration through Sephadex G-25. The volumes of the resulting solutions were measured. The concentration of the particles in suspension was calculated from the optical density (OD) of the iron at 300 nm, as compared to the starting suspension. The amount of chlorin conjugated was calculated using the extinction coefficient and the OD of the farthest red side band. Assuming 8000 Fe per particle, the molecular weight of the iron in each particle is 446 400 amu. The number of chlorins per particle is thus calculated from this. The extinction coefficient of chlorin e_6 in water is 25 000 L mol^{-1} cm^{-1} at 660 nm.

Relative fluorescence quantum yields

The determination of relative fluorescence quantum yields was performed as described previously.[29]

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