Published in final edited form as:

Bioconj Chem. 2018 February 21; 29(2): 306–315. doi:10.1021/acs.bioconjchem.7b00636.

Carbon-1 versus Carbon-3 Linkage of D-Galactose to Porphyrins: Synthesis, Uptake, and Photodynamic Efficiency

Patrícia M. R. Pereira^{#†‡§¶}, Waqar Rizvi^{#§⊥}, N. V. S. Dinesh K Bhupathiraju[§], Naxhije Berisha[§], Rosa Fernandes^{†,‡}, João P.C. Tome^{†,⊖}, and Charles Michael Drain^{*,§}

[†]QOPNA, Department of Chemistry, University of Aveiro, 3810-193 Aveiro, Portugal

[‡]Institute for Biomedical Imaging and Life Sciences (IBILI), Faculty of Medicine, University of Coimbra, 3000-548 Coimbra, Portugal

[¶]Centre for Neuroscience and Cell Biology, Institute for Biomedical Imaging and Life Sciences (CNC.IBILI), Research Consortium, University of Coimbra, 3004-504 Coimbra, Portugal

[§]Department of Chemistry and Biochemistry, Hunter College of the City University of New York, New York, New York 10065, United States

[⊥]Ph.D. Program in Chemistry, The Graduate Center of the City University of New York, New York, New York 10016, United States

[⊖]CQE, Departamento de Engenharia Química, Instituto Superior Tecnico, Universidade de Lisboa, 1049-001 Lisboa, Portugal

[#] These authors contributed equally to this work.

Abstract

The use of glycosylated compounds is actively pursued as a therapeutic strategy for cancer due to the overexpression of various types of sugar receptors and transporters on most cancer cells. Conjugation of saccharides to photosensitizers such as porphyrins provides a promising strategy to improve the selectivity and cell uptake of the photosensitizers, enhancing the overall photosensitizing efficacy. Most porphyrin–carbohydrate conjugates are linked via the carbon-1 position of the carbohydrate because this is the most synthetically accessible approach. Previous studies suggest that carbon-1 galactose derivatives show diminished binding since the hydroxyl group in the carbon-1 position of the sugar is a hydrogen bond acceptor in the galectin-1 sugar binding site. We therefore synthesized two isomeric porphyrin–galactose conjugates using click chemistry: one linked via the carbon-1 of the galactose and one linked via carbon-3. Free base and zinc analogs of both conjugates were synthesized. We assessed the uptake and photodynamic therapeutic (PDT) activity of the two conjugates in both monolayer and spheroidal cell cultures of four different cell lines. For both the monolayer and spheroid models, we observe that the uptake

*Corresponding Author cdrain@hunter.cuny.edu.

[¶]Present Address P.M.R.P.: Department of Radiology, Memorial Sloan Kettering, New York, NY 10065, USA.

Notes

The authors declare no competing financial interest.

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: [10.1021/acs.bioconjchem.7b00636](https://doi.org/10.1021/acs.bioconjchem.7b00636). NMR spectra, UV–visible absorbance spectra, fluorescence emission spectra, mass spectra, DLS, uptake, and dark toxicity (PDF)

of both conjugates is proportional to the protein levels of galectin-1 and the uptake is suppressed after preincubation with an excess of thiogalactose, as measured by fluorescence spectroscopy. Compared to that of the carbon-1 conjugate, the uptake of the carbon-3 conjugate was greater in cell lines containing high expression levels of galectin-1. After photodynamic activation, MTT and lactate dehydrogenase assays demonstrated that the conjugates induce phototoxicity in both monolayers and spheroids of cancer cells.

Graphical Abstract

INTRODUCTION

Galectins are animal lectins with high affinity for β -galactose-containing oligosaccharides.¹ Galectin-1, a member of this family, has a key role in different events associated with cancer biology including tumor transformation. Galectin-1 has been exploited as a target for anticancer drugs, since its expression is higher in tumors compared to normal tissues.²

Drugs and other molecules can be taken up by cells via active or passive transport mechanisms or combination of both.³ Passive mechanisms involve simple diffusion of the molecule through the cell membrane and depend on the molecular hydrophobicity,⁴ for example, the octanol/water partition coefficient, while active uptake requires the target molecules to be recognized by specific receptors and other intermolecular interactions. Porphyrinoids and other photosensitizers (PS) can be targeted toward cell receptors by appending appropriate targeting moieties. For example, chromophores appended with carbohydrates are directed to specific cell surface carbohydrate receptors or carbohydrate-binding proteins overexpressed in cancer cells. After light activation, glyco-PS can sensitize the formation of reactive oxygen species and induce cytotoxicity.^{5–16} Simple click-type reactions were reported to append polyglycerol dendrimers, glucose, poly(ethylene glycol)s (PEGs), polyamines, and polysaccharides to porphyrinoids.^{5,17–20} Since most glycosylated porphyrinoids have *O*-linkages where hydrolysis diminishes *in vivo* effectiveness, nonhydrolyzable linkages can be more effective.⁵

PS such as porphyrins are commonly used in photodynamic therapy (PDT).^{6–9,11,16,21} PDT is a rapidly growing modality to treat age-related macular degeneration, cancer, and various skin disorders using visible light.^{4,21,22} Previously, our group appended PEGs, polyamines, lysines, alkanes, fluorinated alkanes, sugars, and nucleotides with *S*-, *O*-, and *N*-linkages to

porphyrins.^{5,23} The PDT activity of many of these derivatives was studied with a variety of cell lines, and the glycosylated compounds affect both necrosis and apoptosis depending on PS concentration and light flux.²³ We reported the synthesis of nonhydrolyzable tetrathioglucose and tetrathiogalactose porphyrins²¹ connected through carbon-1 of the sugar (Figure 1). The glucose derivative proved to be selective toward cancer cells overexpressing glucose transporters and an effective PDT agent *in vitro* using cell lines such as MDA-MB-231 human breast cancer, whereas the galactose derivative was much less effective.²¹ In addition to upregulation of glucose transporters expression, such as GLUT1, many cancer cells exhibit overexpression of galectin-1. Galactose derivatives linked at the carbon-1 position bind to galectins 200-fold less than corresponding carbon-3 derivatives.²⁴

Galactosylated photosensitizers have demonstrated an excellent potential in PDT due to their ability to target galactose-binding proteins such as galectin-1.²⁵ Photosensitizers linked to galactose via N-, S-, C-, and O-bonds at carbon-1 (anomeric) or at carbon-6 of the sugar to the porphyrinoids are reported.⁶ To improve affinity for galactose-binding proteins, galactosylated dendritic motifs appended to porphyrinoids were developed to enable multivalent interactions.^{26,27} Monovalent galactose derivatives attached at carbon-3 yielded compounds with greater affinity to galectin-1 than the corresponding carbon-1 derivatives.²⁴ Therefore, we hypothesized that attachment of a porphyrin through the carbon-3 of galactose will result in improved uptake to cancer cells overexpressing galectin-1 protein.²⁸ This report is the first direct comparison of the uptake and PDT efficiency of carbon-1 versus carbon-3 linked porphyrin–galactose conjugates.

We attached galactose units through carbon-3 or carbon-1 to a Zn(II) porphyrin by click chemistry to yield (C1-Gal)₄-ZnPor and (C3-Gal)₄-ZnPor, respectively. Removing the metal ion yields the (C3-Gal)₄-Por and (C1-Gal)₄-Por derivatives (Scheme 1). Porphyrins conjugated with galactose through the carbon-3 of the sugar have not been previously explored in PDT. The main focus of the present research was to study the photophysical properties, *in vitro* cell uptake, and photo-dynamic efficiency of the carbon-1 versus the carbon-3 conjugates. *In vitro* cell studies were performed using HCT-116 colon cancer cells, MCF-7 breast cancer cells, UM-UC-3 bladder cancer cells, and HeLa cervical cancer cells, which exhibit different levels of the galectin-1 protein (HeLa > UM-UC-3 > HCT-116 > MCF-7 cells). In addition to the monolayer cancer cell culture studies, we performed studies using 0.05 mm³ three-dimensional spheroids because they more closely mimic the tumor microenvironment and diffusion processes that take place in solid tumors; this 3D-spheroid culture model enables more accurate predictions of *in vivo* of photosensitizer efficacy.^{29–31} Recently, we have observed that monolayer cultures and tumor spheroids differ in glucose metabolism, endogenous reactive oxygen species (ROS), and galectin-1 and GLUT1 protein levels.²⁸ Here, we report that even though the cell uptake for carbon-3 conjugates is significantly greater than the carbon-1 derivatives and proportional to the protein levels of galectin-1, the PDT activities are about the same.

RESULTS AND DISCUSSION

Synthesis and Characterization of the Conjugates.

The synthesis and characterization of porphyrin 1 is typical for tetraaryl porphyrins and proceeds in a ca. 33% overall yield. The zinc complex was made to avoid copper metalation of the macrocycle during the next step wherein copper salts were used for the click reaction, because copper(II) porphyrins are difficult to demetalate and have complex photophysics.³² Appending the commercially available or synthesized azido sugars to compound 1 were carried out using standard click chemistry conditions in 85–90% yields of compounds 2 and 5 without transmetalation of the macrocycle. Deprotection by removal of the acetate groups on the sugars was efficiently accomplished with sodium methoxide in methanol. Herein, a large excess of the base was avoided to prevent hydrolysis of the sugars, and Sephadex columns were used for purification. The zinc was removed using 1:1 methanol/TFA in about 6 h without hydrolysis of the sugars.

The ¹H NMR characterization of porphyrins 2 and 3 showed characteristic peaks for β pyrrolic porphyrin at ~8.5 ppm, 2,6- and 3,5-phenyl protons at ~8.1 and 7.2 ppm, galactopyranose peaks around 4.0–5.6 ppm, and the acetate peaks were found between 1.8 and 2.2 ppm. Due to the anomeric galactopyrano-side (1,2,4,6-tetra-*O*-acetyl-3-azido-3-deoxy-*D*-galactopyranose), porphyrin 5 has a distinct multiplicity compared to porphyrin 2. ¹³C NMR confirmed the formation of the products where the carbonyl carbon peaks of acetates were found around 170 ppm, and porphyrin aromatic peaks were found between 110 and 158 ppm. HRMS is consistent with the reported structures.

After deprotection of the sugar hydroxyl groups on porphyrins 2 and 5, no peaks between 1.8 and 2.2 ppm in the ¹H NMR and no carbonyl peaks in the ¹³C NMR were observed, indicating that the acetate groups were effectively removed. The free base porphyrins are characterized by the UV–visible spectra, the observation of the pyrrole N–H at –2.7 ppm, and HRMS.

Photophysics.

The photophysical properties of chromophores such as porphyrins with polar substituents depend on the solvent. The amphipathic nature of sugar substituted porphyrin allows it to partition into lipophilic, hydrophilic, or amphipathic cellular structures.⁵ Therefore, we have studied the photophysical properties in DMSO, ethanol, and DPBS (pH = 7.4). The electronic spectra of the free base and metalated derivatives are significantly different. Zn analogues show red-shifted Soret bands and the typical two Q-bands between the 500–700 nm, while free base analogues show the expected four Q bands. The absorbance and fluorescence emission spectra for the four compounds are correspondingly different and have a strong solvent dependence (Table 1).

Compared to the fluorescence in DMSO, the fluorescence emission of both the free bases and the zinc complexes are ca. 80–90% quenched in PBS. There are negligible Stokes shifts for all compounds in DMSO and ethanol solvents, indicating minimal specific solvent–solute interactions with the macro-cycle effecting vibrational processes. Excitation spectra

of all compounds indicate the presence of only one species (data not shown). The 0.05 and 0.06 fluorescence quantum yields for (C1-Gal)₄-Por (4) and (C3-Gal)₄-Por (7) are similar to other free base glycosylated porphyrins and almost twice those of the zinc complexes.^{5,33} Because of the orthogonal phenyl groups, the 1,2,3-triazole and hydrophilic sugar molecules have minimal effect on photophysical properties, but their presence modulates the amphipathicity to promote solvent dependence. The split Soret bands in the UV–visible spectra in PBS at ca. 0.1 μ M of all four conjugates (Supporting Information) indicate some degree of aggregation even at this low concentration. Thus, some of the quenching observed in PBS is due to the internal shading and the branching deactivation of aggregates.

All deprotected compounds show normal UV–visible spectra in DMSO as expected for typical solvated porphyrins (Supporting Information). In ethanol, the peaks are somewhat broadened indicating some level of aggregation. In PBS buffer, (C1-Gal)₄-ZnPor (3), (C1-Gal)₄-Por (4), and (C3-Gal)₄-Por (7) are broadened and have shoulders to the blue and red of the main absorption in the UV–visible spectra indicating both J and H aggregates. The exception is (C3-Gal)₄-ZnPor (6), for which the electronic spectra indicate only J aggregates in PBS. To investigate the aggregation, DLS studies were done in PBS buffer and ethanol. All compounds in PBS buffer yield 16–30 nm diameter aggregates. Larger 44–80 nm aggregates are observed in ethanol (Supporting Information). Aggregates less than ca. 50 nm are known to be taken up by cancer cells.⁶

Upon irradiation with 550 ± 10 nm light, DMF solutions of galactose-conjugated porphyrins catalyze the formation of singlet oxygen (¹O₂) as indicated by the ¹O₂ scavenger 1,3-diphenylisobenzofuran (DPBF) to a similar extent as the reference tetraphenylporphyrin (TPP) (Supporting Information).³⁴ Consistent with similar porphyrinic compounds in this solvent the Zn(II) complexes (C1-Gal)₄-ZnPor and (C3-Gal)₄-ZnPor have about the same ¹O₂ yields as the free base compounds (C1-Gal)₄-Por and (C3-Gal)₄-Por measured by this method even though the heavy atom effect increases intersystem crossing to the triplet state.³⁵ Importantly, there is no significant difference between the C1 and the C3 isomers. Though there are different values in the literature, both TPP and ZnTPP have ¹O₂ quantum yield (ϕ) of ca. 0.52 ± 0.12 in DMF.^{34–36} Thus, both the free base and the Zn(II) C1 and C3 porphyrins have about the same ϕ as the TPP reference in DMF.

Uptake/Binding.

It is generally accepted that there is a correlation between the expression level of receptors on the cell membrane and the uptake and binding of drugs appended to the ligands for the receptor. However, nonspecific partition into the membrane is also possible with hydrophobic or amphipathic molecules. Targeted porphyrin conjugates increase the local concentration of the dye around cancer cells, and when the binding constant is not large, there is an equilibrium between the free and bound conjugate. For dyes weakly bound to receptors, such as the compounds reported herein, the high local concentration of unbound dye around the cells increases passive diffusion, so uptake also depends on the hydrophobicity of the molecules.³⁷

Octanol–PBS Partition Coefficient Values.

Octanol–PBS partition coefficient values were determined for (C1-Gal)₄-ZnPor, (C1-Gal)₄-Por, (C3-Gal)₄-ZnPor, and (C3-Gal)₄-Por using the standard shake flask method.⁵ Data show that the values of log *P* are somewhat greater for (C3-Gal)₄-Por compared to (C1-Gal)₄-Por (Table 2), indicating that (C1-Gal)₄-Por has somewhat less water solubility compared with the corresponding (C3-Gal)₄-Por. As observed for many other tetraarylporphyrins, the partition coefficients were greater for the free base porphyrins compared with Zn-porphyrin indicating that the free bases are ca. 33% more soluble in PBS.⁴ These differences in PS intrinsic lipophilicity can result in different subcellular localization and mechanisms of cell death after photoactivation.

In Vitro Studies with Monolayer and Spheroids.

Despite the fact that the uptake of carbon-3 conjugates is greater relative to the carbon-1 conjugates in the four cell lines, compounds 6 and 7 are not significantly better PDT agents in both monolayer and spheroid cell cultures. Since the free base (C1-Gal)₄-Por and (C3-Gal)₄-Por and the zinc complexes (C1-Gal)₄-ZnPor and (C3-Gal)₄-ZnPor have similar ¹O₂ quantum yields (vide supra),^{38,39} these observations suggest that there are important differences in uptake and intracellular transport between the carbon-1 and carbon-3 conjugates.

Previous studies reported this lack of association between uptake and phototoxicity of galactose-conjugated PS.²⁶ Factors such as different amounts of intracellular ROS or induction of a protective cellular mechanism after PDT can explain the reasons by which PSs with different uptake exhibit similar phototoxicity after PDT.²⁶ Additionally, differences in the intracellular localization of photosensitizer galactose-conjugated sugars can result in different cell death processes.⁴⁰

Carbon-1 and Carbon-3 Galactose–Porphyrins Accumulate in Cancer Cells Growing as Monolayer and Spheroid Cultures and Are Nontoxic in the Dark.

To evaluate the photodynamic potential of the newly synthesized carbon-1 and carbon-3 galactose-porphyrins, we first evaluated their accumulation in monolayer and spheroid cultures by fluorescence spectroscopy and microscopy (Figures S32–S36) and dark cytotoxicity (Figures S37–S44). Monolayer cultures of cancer cells were incubated with increasing concentrations (2.25, 4.5, and 9 μM) of (C1-Gal)₄-ZnPor, (C1-Gal)₄-Por, (C3-Gal)₄-ZnPor, and (C3-Gal)₄-Por in PBS containing a maximum of 0.5% v/v dimethyl sulfoxide, DMSO, for up to 4 h. Cancer cell-derived spheroids were incubated with the highest concentration (9 μM) of each photosensitizer.

No toxicity was observed in untreated cells (up to 4 h) in the presence of 0.5% (v/v) DMSO in the incubation medium²⁸. Moreover, the galactose-porphyrins showed no significant dark cytotoxicity in monolayer and spheroid cultures at the 9 μM maximum concentration tested (Figures S37–S44).

The uptake by HCT-116, UM-UC-3, and HeLa cancer cells growing as monolayers or spheroids was greater for carbon-3 porphyrins than for carbon-1 porphyrins (Figures 2 and

3). The uptake is proportional to the expression of the receptors, and competition experiments demonstrated that a preincubation with thiogalactose decreased the uptake of both the carbon-1 and carbon-3 galactose-porphyrins in UM-UC-3 cell cultures (Supporting Information).⁴¹ Data showed that the reduction in uptake was higher for carbon-3 when compared with carbon-1 galactose-porphyrins. The uptake of galactose-porphyrins was greater in HCT-116, HeLa, or UM-UC-3 cancer cells growing as monolayers than with monolayers of MCF-7 breast cancer cells. MCF-7 breast cancer cells have lower levels of galectin-1 protein compared to HCT-116, UMUC-3, and HeLa cancer cells.²⁸ When spheroids were used as cell culture model, a decrease in the uptake of galactose-porphyrins was observed in HeLa cancer cells.

Galactose-Porphyrins Induce Cytotoxicity in Mono-layer and Spheroid Cultures after Photodynamic Activation.

After performing uptake assays, we investigated the effect of galactose-porphyrin mediated PDT efficacy in monolayer and spheroid cultures. The cells were treated 48 h after plating with 9 μ M galactosylated porphyrins for 4 h and then exposed to light (420–700 nm) delivered at 0.44 mW/cm² during 30 min (0.792 J/cm²). The 48 h time point following cell seeding was chosen based on the time needed for spheroids of the different cell lines to form and achieve reproducible and similar compactness (based on spheroid volume and shape) in each well.²⁸ In all monolayer cultures, the light activation of galactose-porphyrins induced reduction of cell viability.

As shown in Figure 4, the phototoxicity of carbon-3 porphyrins in cancer cells growing as monolayers was similar to that of carbon-1 porphyrins. As expected, the phototoxicity was greater in monolayer cultures of HCT-116, UM-UC-3, and HeLa compared to MCF-7 cells because of the reduced galectin-1 expression level in the latter.²⁸ When PDT assays used spheroid cultures, the release of LDH into the culture medium was increased compared with control cells (Figure 5). After PDT, LDH released by HCT-166, UM-UC-3, and HeLa spheroids was greater than that by MCF-7 spheroid cultures. However, the amount of LDH leakage into the extracellular medium was lower for all cancer spheroid cultures when compared to monolayer counter cultures. In spheroid cultures of HCT-166, UM-UC-3, and HeLa, the PDT efficacy is slightly greater for the carbon-3 derivative than for the carbon-1 derivatives, and again there is little effect on spheroids of MCF-7.

CONCLUSION

Carbon-1 and carbon-3 galactose-porphyrins demonstrate high accumulation and phototoxicity in cell lines (monolayers or spheroids) containing high expression of galectin-1 protein. The octanol/PBS partition coefficients indicate that the carbon-1 compounds are somewhat more lipophilic than the carbon-3 derivatives and indicate facilitated diffusion as a mode of uptake. Carbon-1 and carbon-3 galactose-porphyrins demonstrated low uptake in MCF-7 cancer cells, which contain low levels of galectin-1 protein. In cell lines containing high levels of galectin-1 (HCT-116, HeLa, and UM-UC-3), carbon-3 compounds demonstrated greater uptake compared with carbon-1 galactose-porphyrins. The greater uptake of carbon-3 compared with carbon-1 galactose-porphyrins

can be explained by its higher water solubility, as well as the availability of the hydroxyl group at carbon-1 to bind the carbohydrate-recognition domain of galectin-1 protein.²⁴ The similar photodynamic efficiency of carbon-1 and carbon-3 galactose-porphyrins in cell lines containing different levels of galectin-1 protein can be a result of differences in intracellular localization, as well as differences in cellular oxygenation, cell death mechanism, and cellular antioxidant responses upon light activation.

EXPERIMENTAL PROCEDURES

Commercially available reagents such as trifluoroacetic acid (TFA), sodium methoxide, and solvents such as methanol, DMSO, and CH₂Cl₂, were purchased from Sigma-Aldrich, Fisher Scientific, or Acros Organics and used without further purification. 1,2,4,6-Tetra-*O*-acetyl-3-azido-3-deoxy- β -D-galactopyranose was prepared using the published procedure.⁴² 5,10,15,20-Tetrakis(4-propargyloxyphenyl)porphyrin was synthesized using a known procedure.⁴³ Analytical thin-layer chromatography (TLC) was performed on polyester-backed TLC plates 254 (precoated, 200 μ m, Sorbent Technologies), and silica gel 60 (70–230 mesh, Merck) was used for column chromatography. ¹H and ¹³C NMR spectra were obtained using a Bruker Avance III 400 MHz and Bruker Avance DRX 500 MHz; chemical shifts are expressed in ppm relative to CDCl₃ (7.26 ppm, ¹H; 77.0 ppm, ¹³C), (CD₃)₂CO (2.05 ppm, ¹H; 29.84 and 206.26 ppm, ¹³C), or CD₃OD (3.31 and 4.78 ppm, ¹H; 49.2 ppm, ¹³C). Mass analyses were conducted at the CUNY Mass Spectrometry Facility at Hunter College on an Agilent iFunnel 6550 Q-ToF LC/MS System (for HRMS-ESI). The electrospray ionization was run in methanol, with 0.1% (v/v) formic acid. UV–visible spectra were recorded on a Lambda 35 PerkinElmer UV/vis spectrophotometer. Steady-state fluorescence (emission) spectra were measured with a Fluorolog τ -3, Jobin-SPEX Instruments S.A., Inc.

5,10,15,20-Tetrakis(4-propargyloxyphenyl) porphyrin was synthesized from commercially available 4-propargyloxy benzaldehyde and pyrrole.⁴³ Zn complexation was carried out using Zn(OAc)₂ in CH₂Cl₂ at room temperature overnight resulting in 98% overall yield of the metalloporphyrin 1. 1,2,4,6-Tetra-*O*-acetyl-3-azido-3-deoxy- β -D-galactopyranose was prepared using the published procedure,⁴² and characterization was in agreement with the published spectra. The 1-azido-1-deoxy- β -D-galactopyranoside tetraacetate is commercially available. Either 1,2,4,6-tetra-*O*-acetyl-3-azido-3-deoxy- β -D-galactopyranose or 2,3,4,6-tetra-*O*-acetyl-1-azido-1-deoxy- β -D-galactopyranose (2.6 equiv) was then refluxed overnight with 1 equiv of porphyrin 1 using 1 equiv Cu(0), 0.2 equiv of CuSO₄·5H₂O, and 0.5 equiv of Na-ascorbate dissolved in a mixture of THF/ water resulting in porphyrins 2 and 5 in good yields after silica column purification using CH₂Cl₂/EtOAc (80:20) as eluent.

5,10,15,20 - Tetrakis(4-propargyloxyphenyl)-porphyrinato Zn(II), Compound 1.

To 100 mg (0.12 mmol) of 5,10,15,20-tetrakis(4-propargyloxyphenyl)porphyrin in 20 mL of CH₂Cl₂ was added 300 mg of Zn(OAc)₂. The reaction mixture was stirred at room temperature overnight. The obtained mixture was evaporated using rotary evaporator under reduced pressure followed by a silica chromatography with CH₂Cl₂/EtOAc (95:5) as eluent resulting in compound 1 in 98% yield (105 mg, 0.117 mmol). ¹H NMR (500 MHz, CDCl₃)

δ 8.97 (s, 8H), 8.13 (d, $J = 8.35$ Hz, 8H), 8.35 (d, $J_1 = 8.35$ Hz, 8H), 4.98 (s, 8H), 2.69 (s, 4H); ^{13}C NMR (125 MHz, CDCl_3) δ 156.4, 134.5, 134.4, 118.5, 112.0, 77.6, 74.8, 55.1.

5,10,15,20-Tetrakis[*N*-(2',3',4',6'-*O*-acetyl-1'- β -D-gal-actopyranosyl)-(4''-methylenoxytriazole)-phenyl]-porphyrinato Zn(II), Compound 2.

Compound 1 (52.5 mg, 0.058 mmol), 1-azido-1-deoxy- β -D-galactopyranoside tetraacetate (56.24 mg, 0.15 mmol), and Cu(0) (3.6 mg, 0.058 mmol) were dissolved in a mixture of THF/water (6 mL, 3:1), under inert gas atmosphere. To the above mixture, a solution of $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$ (2.9 mg, 0.0116 mmol) and Na-ascorbate (5.75 mg, 0.029 mmol) in THF/water (6 mL, 3:1) was added, and the reaction mixture was refluxed overnight. Then the reaction mixture was cooled to room temperature and extracted using ethyl acetate and brine. The organic layer was washed twice with brine, the combined organic layers were dried over Na_2SO_4 , and the solvent was evaporated under reduced pressure. The crude product was then purified by silica chromatography. First using CH_2Cl_2 as eluent to remove any unreacted starting materials then followed by 80:20 (CH_2Cl_2 /EtOAc) to obtain the desired product in 90% yield (125 mg, 0.052 mmol). ^1H NMR (500 MHz, CDCl_3) δ 8.95 (s, 8H), 8.13 (d, $J = 7.8$ Hz, 8H), 7.77 (s, 4H), 7.24 (d, $J = 7.8$ Hz, 8H), 5.59 (t, $J = 9.2$ Hz, 4H), 5.53 (t, $J = 2.9$ Hz, 4H), 5.42–5.45 (m, 4H), 5.21 (dd, $J_1 = 2.95$, $J_2 = 2.95$ Hz, 4H), 4.67 (s, 8H), 4.16–4.20 (m, 8H), 4.09–4.13 (m, 4H), 2.20 (s, 12H), 2.05 (s, 12H), 2.00 (s, 12H), 1.80 (s, 12H). ^{13}C NMR (125 MHz, CDCl_3) δ 170.3, 170.1, 169.9, 169.3, 157.7, 150.4, 144.5, 136.4, 135.7, 131.7, 120.9, 120.3, 112.9, 88.2, 86.1, 72.8, 70.7, 68.1, 66.9, 61.2, 61.2, 20.6, 20.6, 20.6, 20.9. HRMS (ESI) m/z calcd for $\text{C}_{112}\text{H}_{112}\text{N}_{16}\text{O}_{40}\text{Zn}$ ($[\text{M} + 2\text{H}]^{2+}$) 1194.3352, found 1194.3325.

5,10,15,20-Tetrakis[*N*-(1'- β -D-galactosyl)-(4''-methylenoxytriazole)-phenyl]porphyrinato Zn(II), Compound 3.

Compound 2 (62.5 mg, 0.026 mmol) was dissolved in methanol/ CH_2Cl_2 (3:1, 10 mL). To this mixture was added sodium methoxide (0.5 M solution in methanol, 3 mL). The reaction mixture was stirred at room temperature for 4 h, and then the solvent was evaporated using rotary evaporator. The resulting crude product was passed through Sephadex LH-20 using MeOH as eluent to yield (C1-Gal) $_4$ -ZnPor (3) in 84% yield (37.39 mg, 0.021 mmol). ^1H NMR (400 MHz, CD_3OD) δ 8.85 (s, 8H), 8.54 (s, 4H), 8.13 (d, $J = 7.92$ Hz, 8H), 7.51 (d, $J = 8$ Hz, 8H), 5.62 (d, $J = 9.04$ Hz, 4H), 5.51 (s, 8H), 4.20 (t, $J = 9.12$ Hz, 4H), 3.81 (bs, 4H), 3.81–3.82 (m, 4H), 3.63–3.68 (m, 12H). ^{13}C NMR (100 MHz, CD_3OD) δ 158.3, 150.11, 143.5, 136.0, 135.7, 131.9, 124.1, 120.3, 113.4, 88.8, 79.0, 74.4, 70.0, 69.1, 62.1, 61.1. HRMS (ESI) m/z calcd for $\text{C}_{80}\text{H}_{80}\text{N}_{16}\text{O}_{24}\text{Zn}$ ($[\text{M} + \text{H}]^+$) 1715.4905, found 1715.4893.

5,10,15,20-Tetrakis[*N*-(1'- β -D-galactosyl)-(4''-methylenoxytriazole)-phenyl]porphyrin, Compound 4.

(C1-Gal) $_4$ -ZnPor (3) (37.39 mg, 0.021 mmol) was dissolved in methanol/TFA (1:1, 10 mL). The reaction mixture was stirred at room temperature overnight, and then the solvent was evaporated using rotary evaporator. The resulting crude product was washed with CH_2Cl_2 and acetone several times to yield (C1-Gal) $_4$ -Por (4) in 90% yield (31.18 mg, 0.018 mmol) as green solid. ^1H NMR (500 MHz, CDCl_3) δ 8.87 (bs, 8H), 8.60 (s, 4H), 8.18 (bs, 8H), 7.53 (bs, 8H), 5.72–5.76 (m, 8H), 5.49–5.62 (m, 8H), 4.47–4.59 (m, 4 H), 4.13–4.28 (m,

8H), 3.80–3.87 (m, 8H). ^{13}C NMR (125 MHz, CDCl_3) δ 158.1, 156.5, 156.2, 142.7, 124.0, 123.9, 113.1, 88.2, 87.6, 78.5, 74.4, 73.7, 73.0, 69.3, 68.9, 68.6, 68.5, 68.0, 61.3, 60.5, 54.9. HRMS (ESI) m/z calcd for $\text{C}_{80}\text{H}_{82}\text{N}_{16}\text{O}_{24}$ ($[\text{M} + \text{H}]^+$) 1652.5791, found 1652.5771.

5,10,15,20-Tetrakis[*N*-(1',2',4',6'-*O*-acetyl-3'- β -D-gal-actopyranosyl)-(4"-methylenoxytriazole)-phenyl]-porphyrinato Zn(II), Compound 5.

Compound 1 (52.5 mg, 0.058 mmol), 1,2,4,6-tetra-*O*-acetyl-3-azido-3-deoxy-D-galactopyranose (56.24 mg, 0.15 mmol), and Cu(0) (3.6 mg, 0.058 mmol) were dissolved in a mixture of THF/water (6 mL, 3:1), under inert gas atmosphere. To the above mixture, a solution of $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$ (2.9 mg, 0.0116 mmol) and Na-ascorbate (5.75 mg, 0.029 mmol) in THF/water (6 mL, 3:1) was added, and the reaction mixture was refluxed overnight. Then the reaction mixture was cooled to room temperature and extracted using ethyl acetate and brine. The organic layer was washed twice with brine, the combined organic layers were dried over anhydrous Na_2SO_4 , and the solvent was evaporated under reduced pressure. The crude product was then purified by silica chromatography, first using CH_2Cl_2 as eluent to remove any unreacted starting materials then followed by $\text{CH}_2\text{Cl}_2/\text{EtOAc}$ (80:20) to obtain the desired product in 84% yield (116 mg, 0.048 mmol). ^1H NMR (500 MHz, CDCl_3) δ 8.93 (bs, 8H), 8.11 (bs, 8H), 7.42–7.49 (m, 4H), 7.12–7.27 (m, 8H), 5.09–5.53 (m, 12H), 4.65–4.72 (m, 8H), 3.86–4.34 (m, 12H), 1.67–2.14 (m, 48H). ^{13}C NMR (100 MHz, CDCl_3) δ 170.6, 169.5, 169.4, 168.6, 162.8, 143.7, 142.8, 132.0, 132.0, 130.5, 126.0, 123.3, 115.1, 115.0, 90.1, 72.0, 71.5, 68.6, 66.0, 63.0, 62.0, 61.9, 60.4, 57.9, 20.98, 20.8, 20.4, 20.2, 20.2. HRMS (ESI) m/z calcd for $\text{C}_{112}\text{H}_{112}\text{N}_{16}\text{O}_{40}\text{Zn}$ ($[\text{M} + 2\text{H}]^{+2}$) 1216.3161, found 1216.4143.

5,10,15,20-Tetrakis[*N*-(3'- β -D-galactosyl)-(4"-methyle-noxytriazole)-phenyl]porphyrinato Zn(II), Compound 6.

Compound 5 (62.5 mg, 0.026 mmol) was dissolved in methanol/ CH_2Cl_2 (3:1, 10 mL). To this mixture was added sodium methoxide (0.5 M solution in methanol, 3 mL). The reaction mixture was stirred at room temperature for 4 h, and then the solvent was evaporated using rotary evaporator. The resulting crude product was passed through Sephadex LH-20 using MeOH as eluant to give (C3-Gal) $_4$ -ZnPor (6) in 79% yield (35.16 mg, 0.020 mmol). ^1H NMR (500 MHz, CD_3OD) δ 8.81 (bs, 8H), 8.02 (bs, 8H), 7.65 (bs, 4H), 7.28 (bs, 8H), 5.45–5.47 (m, 4H), 5.31–5.33 (m, 8H), 4.90–5.12 (m, 24H). ^{13}C NMR (125 MHz, CD_3OD) δ 159.8, 151.7, 141.6, 137.3, 136.6, 132.5, 131.3, 121.5, 113.8, 63.4. HRMS (ESI) m/z calcd for $\text{C}_{80}\text{H}_{80}\text{N}_{16}\text{O}_{24}\text{Zn}$ ($[\text{M} + \text{H}]^+$) 1715.4905, found 1715.4864; calcd for $\text{C}_{80}\text{H}_{80}\text{N}_{16}\text{O}_{24}\text{Zn}$ ($[\text{M} + \text{Na}]^+$) 1736.4745, found 1736.4662.

5,10,15,20-Tetrakis[*N*-(3'- β -D-galactosyl)-(4"-methyle-noxytriazole)-phenyl]porphyrin, Compound 7.

(C3-Gal) $_4$ -ZnPor (6) (35.16 mg, 0.020 mmol) was dissolved in methanol/TFA (1:1, 10 mL). The reaction mixture was stirred at room temperature overnight, and then the solvent was evaporated using rotary evaporator. The resulting crude product was washed with CH_2Cl_2 and acetone several times resulting in (C3-Gal) $_4$ -Por (7) in 87% yield (28.71 mg, 0.017 mmol) as a green solid. ^1H NMR (500 MHz, $\text{DMSO}-d_6$) δ 8.86 (s, 8H), 8.14 (s, 12H), 7.47 (bs, 8H), 5.54 (s, 8H), 3.76–4.79 (m, 28H), –2.82 (bs, 2H). ^{13}C NMR (500 MHz, $\text{DMSO}-d_6$) δ 158.5, 158.2, 158.1, 158.0, 157.7, 135.4, 133.8, 120.3, 119.6, 117.9, 115.6, 113.2, 76.5,

72.9. HRMS (ESI) m/z calcd for $C_{80}H_{82}N_{16}O_{24}$ ($[M + H]^+$) 1652.5791, found 1652.5735; calcd for $C_{80}H_{82}N_{16}O_{24}$ ($[M + Na]^+$) 1674.5610, found 1674.5545.

Photophysics.

UV–visible and fluorescence measurements were performed on diluted solutions, typically about 0.1 μ M, of compounds in ethanol, dimethyl sulfoxide (DMSO), and Dulbecco's phosphate buffered saline (DPBS without $CaCl_2$ and $MgCl_2$, pH = 7.4, with 2% v/v DMSO). The UV–visible spectra were obtained from 350 to 750 nm using 1 cm quartz cuvettes. For steady-state fluorescence spectroscopy, samples were excited at 425 nm for all solvents at concentrations where absorbancies are less than 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 (C3-Gal)₄-Por (7), (C3-Gal)₄-ZnPor (6), (C1-Gal)₄-Por (4), and (C1-Gal)₄-ZnPor (3) solutions relative to a tetraphenylporphyrin standard in toluene, which has a fluorescence quantum yield of 0.11 in this and the other solvents.^{5,44} All experiments were carried out on the same day, using identical concentrations to minimize any experimental errors (Supporting Information).

Dynamic light scattering (DLS) was used to determine aggregate size in the different solvents using a Malvern nano Zetasizer, modeled as a sphere and reported as the diameter. Samples were sonicated for about a minute and filtered with hydrophilic 200 nm filters before measurements.

The partition coefficient (P) values were measured in an n-octanol/PBS (1:1, v/v) mixture at a concentration of 10 μ M. After shaking the solution at room temperature, the two different phases were obtained by centrifugation, and the concentration of the PS in each phase was determined by UV–vis using the molar absorptivity values.

For the singlet oxygen experiments, DMF solutions containing 50 μ M DPBF and 5 μ M porphyrins were irradiated at room temperature and under gentle magnetic stirring. DPBF depletion was monitored by measuring the decrease in absorbance (415 nm) at 2, 4, 6, 8, and 10 min after light irradiation. Irradiation with 0.49 mW/cm² light (measured at the front of the cuvette) was from a xenon arc lamp in Horiba SPEX fluorometer with the monochromator set at 550 nm, and the band-pass was set at 10 nm to minimize absorption by DPBF.

Cell Lines and Culture.

UM-UC-3 bladder cancer cell line, HeLa cervical cancer cell line, HCT-116 colon cancer cell line, and MCF-7 breast cancer cell line were obtained from the American Type Culture Collection (ATCC, Manassas, VA, USA). All batches of culture media were supplemented with 10% (v/v) of fetal bovine serum (Life Technologies, Carlsbad, CA, USA), 100 U/mL penicillin, 100 μ g/mL streptomycin, and 1.25 μ g/mL amphotericin B (Sigma). HCT-116 colon cancer cells, MCF-7, and MDA-MB-231 breast cancer cells were cultured in Dulbecco's Modified Eagle's Medium (DMEM, Sigma). UM-UC-3 bladder cancer cells were cultured in Eagle's Minimum Essential Medium (EMEM; Corning, NY, USA) with 1.5 g/L sodium bicarbonate, nonessential amino acids, L-glutamine, and sodium pyruvate. HeLa

cervical cancer cells were cultured in DMEM (Corning) with 4.5 g/L glucose, and L-glutamine without sodium pyruvate. All cells were maintained at 37 °C in a 5% CO₂ humidified atmosphere. Agarose-coated 96-well plates were used for spheroid cultures as previously reported in the literature.²⁸ Briefly, 5000 HCT-116 cells per well, 15000 MCF-7 cells per well, 20000 UM-UC-3 cells per well, and 20000 HeLa cells per well were plated on agarose-coated microplates and left for 48 h undisturbed to obtain viable spheroids with volume ≈ 0.05 mm.³

Cellular Uptake.

After incubation of monolayers or spheroids of cancer cells with the photosensitizer in the dark, the cells were washed with PBS buffer (pH 7.60, 10 mM NaH₂PO₄, 70 mM Na₂HPO₄, and 145 mM NaCl) and scrapped in 1% (m/v) sodium dodecyl sulfate (SDS; Sigma) in PBS buffer at pH 7.0. The concentration of the porphyrins was determined by spectrofluorimetry using a microplate reader (Gemini EM Microplate Spectrofluorometer) with the excitation and emission filters set at 410 and 702 nm, respectively. The results were normalized for protein concentration using the bicinchoninic acid reagent (Pierce, Rockford, IL, USA). For the competition experiments, monolayers of UM-UC-3 cells were preincubated with the thiogalactose sugar (100 μ M or 1.00 mM) for 1 h. Next, those cells were incubated for 3 h with Pors at a concentration of 9 μ M.

For evaluation of cellular uptake by fluorescence microscopy, cells were grown on coated glass coverslips with poly(L-lysine) (Sigma). The cells were incubated with 9 μ M porphyrins for 4 h, at 37 °C. After incubation, cells were washed twice with PBS and fixed with 4% paraformaldehyde (PFA; Merck, Darmstadt, Germany) for 10 min at room temperature. The samples were then rinsed in PBS and mounted in Vecta SHIELD mounting medium containing 4',6-diamidino-2-phenylindole (DAPI; Vector Laboratories, CA, Burlingame) for visualization under a confocal microscope (Nikon Eclipse Ti With Ultra High Speed Wavelength Source, Molecular Devices) equipped with Andor iXon EMCCD camera. For DAPI detection, the specimen was excited at 405 nm and light emitted was collected with a 460/50 nm band-pass filter. Porphyrin fluorescence was obtained using 640 nm excitation and a 685/40 nm emission band-pass filter.

PDT Assays.

After monolayers or spheroid cultures were incubated with 9 μ M of porphyrins for 4 h, the cultures were gently rinsed with PBS, and then PDT assays were performed. The cells were exposed to light (420–700 nm) emitted by OLED Lumiblade Brite FL300-wm Level 4 (OLED Works, Rochester, NY, USA) delivered at 0.44 mW/cm² during 30 min (0.792 J/cm²). The controls were performed by keeping monolayers or spheroids in the dark for the same durations and under the same conditions as the irradiated cells. As a control, all cell lines were incubated under identical light conditions without the PS and cells were similarly viable as those incubated in the dark. Triplicate wells were established under each experimental condition, and each experiment was repeated at least three times. Cytotoxicity was determined in monolayer or spheroid cultures 24 h after treatment using the 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide (MTT, Sigma) and lactate dehydrogenase (LDH) assays, respectively.²⁸

MTT and Lactate Dehydrogenase Assays.

The MTT assay was used to determine metabolic activity of cells growing in monolayers by measuring using a microplate reader (PowerWave HT Microplate Spectrophotometer) their ability to reduce the yellow-colored MTT to a colored formazan. The data were expressed in percentage of control (i.e., optical density of formazan from control cells). The CytoTox 96 Non-Radioactive Cytotoxicity Assay (Promega, Madison, WI, USA) was used to determine cytotoxicity in spheroid cultures. The LDH activity was normalized to protein concentration, and the data were normalized to the maximal LDH release.

Statistical Analysis.

Statistical analysis was performed using GraphPad Prism. Student's *t*-test was used to compare the treatment effects with that of control. *P*-value was considered at the 5% level of significance of the data. All graphs and statistics were prepared using the GraphPad Prism 5.0 software.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

ACKNOWLEDGMENTS

We thank Professor Eileen Jaffer, Department of Molecular Therapeutics at Fox Chase Cancer Center of Temple University Health Systems, for reading the manuscript and insightful comments. This work was supported by the National Science Foundation CHE-1610755, Hunter College science infrastructure is supported by the NSF, the National Institute on Minority Health and Health Disparities 8G12 MD007599, and the City University of New York. The authors also acknowledge Faculdade de Ciencias e Tecnologia, Universidade Nova de Lisboa, for the doctoral research SFRH/BD/85941/ 2012 (to P.M.R.P.). Thanks are due to FCT/MEC for the financial support to QOPNA (FCT UID/QUI/00062/2013), IBILI (FCT UID/NEU/04539/2013), and CQE (FCT UID/QUI/0100/2013) research units, through national funds and where applicable cofinanced by the FEDER, within the PT2020 Partnership Agreement.

ABBREVIATIONS

GLUT1	glucose transporter 1
PDT	photodynamic therapy
PS	photosensitizer
ROS	reactive oxygen species
DMS	Dimethyl sulfoxide

REFERENCES

- (1). Liu F-T, and Rabinovich GA (2005) Galectins as modulators of tumour progression. *Nat. Rev. Cancer* , 29–41.
- (2). Rabinovich GA (2005) Galectin-1 as a potential cancer target. *Br. J. Cancer* 92, 1188–1192. [PubMed: 15785741]
- (3). Kell DB, and Oliver SG (2014) How drugs get into cells: tested and testable predictions to help discriminate between transporter-mediated uptake and lipoidal bilayer diffusion. *Front. Pharmacol* 5, 31. [PubMed: 24624087]

- (4). Drain CM, and Mauzerall DC (1992) Photogating of ionic currents across lipid bilayers. Hydrophobic ion conductance by an ion chain mechanism. *Biophys. J* 63, 1556–1563. [PubMed: 1489913]
- (5). Singh S, Aggarwal A, Thompson S, Tomé JPC, Zhu X, Samaroo D, Vinodu M, Gao R, and Drain CM (2010) Synthesis and photophysical properties of thioglycosylated-chlorins, isobacteriochlorins and bacteriochlorins for bioimaging and diagnostics. *Bioconjugate Chem* 21, 2136–2146.
- (6). Singh S, Aggarwal A, Bhupathiraju NVSDK, Arianna G, Tiwari K, and Drain CM (2015) Glycosylated Porphyrins, Phthalocyanines, and Other Porphyrinoids for Diagnostics and Therapeutics. *Chem. Rev* 115, 10261–10306. [PubMed: 26317756]
- (7). Thompson S, Chen X, Hui L, Toschi A, Foster DA, and Drain CM (2008) Low concentrations of a non-hydrolysable tetra-S-glycosylated porphyrin and low light induces apoptosis in human breast cancer cells via stress of the endoplasmic reticulum. *Photochem. Photobiol. Sci* 7, 1415–1421.
- (8). Csík G, Balog E, Voszka I, Tölgyesi F, Oulmi D, Maillard P, and Mometeau M (1998) Glycosylated derivatives of tetraphenyl porphyrin: photophysical characterization, self-aggregation and mem-brane binding. *J. Photochem. Photobiol., B* 44, 216–224.
- (9). Obata M, Hirohara S, Sharyo K, Alitomo H, Kajiwarra K, Ogata S, Tanihara M, Ohtsuki C, and Yano S (2007) Sugar-dependent photodynamic effect of glycoconjugated porphyrins: a study on photocytotoxicity, photophysical properties and binding behavior to bovine serum albumin (BSA). *Biochim. Biophys. Acta, Gen. Subj* 1770, 1204–1211.
- (10). Hirohara S, Obata M, Ogata S, Ohtsuki C, Higashida S, Ogura S, Okura I, Takenaka M, Ono H, Sugai Y, Mikata Y, Tanihara M, and Yano S (2005) Cellular uptake and photo-cytotoxicity of glycoconjugated chlorins in HeLa cells. *J. Photochem. Photobiol., B* 78, 7–15. [PubMed: 15629244]
- (11). Maillard P, Loock B, Grierson DS, Laville I, Blais J, Doz F, Desjardins L, Carrez D, Guerin-Kern JL, and Croisy A (2007) In vitro phototoxicity of glycoconjugated porphyrins and chlorins in colorectal adenocarcinoma (HT29) and retinoblastoma (Y79) cell lines. *Photodiagn. Photodyn. Ther* 4, 261–268.
- (12). Zorlu Y, Ermeýdan MA, Dumoulin F, Ahsen V, Savoie H, and Boyle RW (2009) Glycerol and galactose substituted zinc phthalocyanines. Synthesis and photodynamic activity. *Photochem. Photobiol. Sci* 8, 312–318. [PubMed: 19255671]
- (13). Okada M, Kishibe Y, Ide K, Takahashi T, and Hasegawa T (2009) Convenient Approach to Access Octa-Glycosylated Porphyrins via “Click Chemistry. *Int. J. Carbohydr. Chem* 2009, 305276.
- (14). Iqbal Z, Hanack M, and Ziegler T (2009) Synthesis of an octasubstituted galactose zinc(II) phthalocyanine. *Tetrahedron Lett* 50, 873–875.
- (15). Soares ARM, Tome JPC, Neves MGPM, Tome AC, Cavaleiro JAS, and Torres T (2009) Synthesis of water-soluble phthalocyanines bearing four or eight d-galactose units. *Carbohydr. Res* 344, 507–510. [PubMed: 19114279]
- (16). Hirohara S, Nishida M, Sharyo K, Obata M, Ando T, and Tanihara M (2010) Synthesis, photophysical properties and photo-cytotoxicity of mono-, di-, tri- and tetra-glucosylated fluorophenylporphyrins. *Bioorg. Med. Chem* 18, 1526–1535. [PubMed: 20097078]
- (17). Pasetto P, Chen X, Drain CM, and Franck RW (2001) Synthesis of hydrolytically stable porphyrin C- and S-glycoconjugates in high yields. *Chem. Commun*, 81–82.
- (18). Samaroo D, Soll CE, Todaro LJ, and Drain CM (2006) Efficient microwave-assisted synthesis of amine-substituted tetrakis-(pentafluorophenyl)porphyrin. *Org. Lett* 8, 4985–4988. [PubMed: 17048824]
- (19). Samaroo D, Vinodu M, Chen X, and Drain CM (2007) meso-Tetra(pentafluorophenyl)porphyrin as an Efficient Platform for Combinatorial Synthesis and the Selection of New Photodynamic Therapeutics using a Cancer Cell Line. *J. Comb. Chem* 9, 998–1011. [PubMed: 17877415]
- (20). Bhupathiraju NVSDK, Rizvi W, Batteas JD, and Drain CM (2016) Fluorinated porphyrinoids as efficient platforms for new photonic materials, sensors, and therapeutics. *Org. Biomol. Chem* 14, 389–408. [PubMed: 26514229]

- (21). Chen X, Hui L, Foster DA, and Drain CM (2004) Efficient synthesis and photodynamic activity of porphyrin-saccharide conjugates: targeting and incapacitating cancer cells. *Biochemistry* 43, 10918–10929. [PubMed: 15323552]
- (22). Josefsen LB, and Boyle RW (2008) Photodynamic therapy: novel third-generation photosensitizers one step closer? *Br. J. Pharmacol* 154, 1–3. [PubMed: 18362894]
- (23.) Kralova J, Briza T, Moserova I, Dolensky B, Vasek P, Pouckova P, Kejik Z, Kaplanek R, Martasek P, Dvorak M, and Kral V (2008) Glycol porphyrin derivatives as potent photodynamic inducers of apoptosis in tumor cells. *J. Med. Chem* 51, 5964–5973. [PubMed: 18788727]
- (24). van Hattum H, Branderhorst HM, Moret EE, Nilsson UJ, Leffler H, and Pieters RJ (2013) Tuning the Preference of Thiodigalactoside- and Lactosamine-Based Ligands to Galectin-3 over Galectin-1. *J. Med. Chem* 56, 1350–1354. [PubMed: 23281927]
- (25). Pereira PMR, Silva S, Ramalho JS, Gomes CM, Girao H, Cavaleiro JAS, Ribeiro CAF, Tome JPC, and Fernandes R (2016) The role of galectin-1 in in vitro and in vivo photodynamic therapy with a galactodendritic porphyrin. *Eur. J. Cancer* 68, 60–69. [PubMed: 27718388]
- (26). Pereira PMR, Silva S, Cavaleiro JAS, Ribeiro CAF, Tome JPC, and Fernandes R (2014) Galactodendritic Phthalocyanine Targets Carbohydrate-Binding Proteins Enhancing Photodynamic Therapy. *PLoS One* 9, No. e95529.
- (27). Silva S, Pereira PMR, Silva P, Almeida Paz FA, Faustino MAF, Cavaleiro JAS, and Tome JPC (2012) Porphyrin and phthalocyanine glycodendritic conjugates: synthesis, photophysical and photochemical properties. *Chem. Commun* 48, 3608–3610.
- (28). Pereira PMR, Berisha N, Bhupathiraju NVSDK, Fernandes R, Tome JPC, and Drain CM (2017) Cancer cell spheroids are a better screen for the photodynamic efficiency of glycosylated photosensitizers. *PLoS One* 12, No. e0177737.
- (29). Breslin S, and O'Driscoll L (2013) Three-dimensional cell culture: the missing link in drug discovery. *Drug Discovery Today* 18, 240–249. [PubMed: 23073387]
- (30). Perche F, and Torchilin VP (2012) Cancer cell spheroids as a model to evaluate chemotherapy protocols. *Cancer Biol. Ther* 13, 1205–1213. [PubMed: 22892843]
- (31). Zaroni M, Piccinini F, Arienti C, Zamagni A, Santi S, Polico R, Bevilacqua A, and Tesei A (2016) 3D tumor spheroid models for in vitro therapeutic screening: a systematic approach to enhance the biological relevance of data obtained. *Sci. Rep* 6, 19103. [PubMed: 26752500]
- (32). Hao E, Jensen TJ, and Vicente MGH (2009) Synthesis of porphyrin-carbohydrate conjugates using "click" chemistry and their preliminary evaluation in human HEp2 cells. *J. Porphyrins Phthalocyanines* 13, 51–59.
- (33). Boisbrun M, Vanderesse R, Engrand P, Olie A, Hupont S, Regnouf-de-Vains J-B, and Frochot C (2008) Design and photophysical properties of new RGD targeted tetraphenylchlorins and porphyrins. *Tetrahedron* 64, 3494–3504.
- (34). Venkatesan R, Periasamy N, and Srivastava TS (1992) Singlet molecular oxygen quantum yield measurements of some porphyrins and metalloporphyrins. *Proc. Indian Acad. Sci. - Chem. Sci* 104, 713–722.
- (35). Wilkinson F, Helman WP, and Ross AB (1995) Rate Constants for the Decay and Reactions of the Lowest Electronically Excited Singlet State of Molecular Oxygen in Solution. An Expanded and Revised Compilation. *J. Phys. Chem. Ref. Data* 24, 663–677.
- (36). Spiller W, Kliesch H, Wöhrle D, Hackbarth S, Röder B, and Schnurpfeil G (1998) Singlet oxygen quantum yields of different photosensitizers in polar solvents and micellar solutions. *J. Porphyrins Phthalocyanines* 2, 145–158.
- (37). Mishra PP, Patel S, and Datta A (2006) Effect of Increased Hydrophobicity on the Binding of Two Model Amphiphilic Chlorin Drugs for Photodynamic Therapy with Blood Plasma and Its Components. *J. Phys. Chem. B* 110, 21238–21244. [PubMed: 17048951]
- (38). Garcia G, Naud-Martin D, Carrez D, Croisy A, and Maillard P (2011) Microwave-mediated 'click-chemistry' synthesis of glycoporphyrin derivatives and in vitro photocytotoxicity for application in photodynamic therapy. *Tetrahedron* 67, 4924–4932.
- (39). Lafont D, Zorlu Y, Savoie H, Albrieux F, Ahsen V, Boyle RW, and Dumoulin F (2013) Monoglycoconjugated phthalocyanines: Effect of sugar and linkage on photodynamic activity. *Photodiagn. Photodyn. Ther* 10, 252–259.

- (40). Pereira PMR, Silva S, Bispo M, Zuzarte M, Gomes C, Girao H, Cavaleiro JAS, Ribeiro CAF, Tome JPC, and Fernandes R (2016) Mitochondria-Targeted Photodynamic Therapy with a Galactodendritic Chlorin to Enhance Cell Death in Resistant Bladder Cancer Cells. *Bioconjugate Chem* 27, 2762–2769.
- (41). Adlercreutz D, Yoshimura Y, Mannerstedt K, Wakarchuk WW, Bennett EP, Dovichi NJ, Hindsgaul O, and Palcic MM (2012) Thiogalactopyranosides are Resistant to Hydrolysis by α -Galactosidases. *ChemBioChem* 13, 1673–1679. [PubMed: 22740420]
- (42). Lowary TL, and Hindsgaul O (1994) Recognition of synthetic O-methyl, epimeric, and amino analogues of the acceptor α -L-Fuc p-(1 $\rightarrow$ 2)- β -D-Gal p-OR by the blood-group A and B gene-specified glycosyltransferases. *Carbohydr. Res* 251, 33–67. [PubMed: 8149379]
- (43). Lindsey JS, Schreiman IC, Hsu HC, Kearney PC, and Marguerettaz AM (1987) Rothmund and Adler-Longo reactions revisited: synthesis of tetraphenylporphyrins under equilibrium conditions. *J. Org. Chem* 52, 827–836.
- (44). Seybold PG, and Gouterman M (1969) Porphyrins. *J. Mol. Spectrosc* 31, 1–13.

Figure 1.
Tetrathioglycosylated porphyrins.

Figure 2.

Intracellular uptake of galactose-porphyrins by monolayer cultures of HCT-116, MCF-7, UM-UC-3, or HeLa cancer cells. The concentration of porphyrins was determined by fluorescence spectroscopy after incubation of cancer cells with 9 μ M of galactose-porphyrins for 4 h, and the results were normalized to protein quantity. Data are the means $\pm$ SD of at least three independent experiments performed in triplicate. ** $p < 0.001$, significantly different from the corresponding C3-Gal₄-Por.

Figure 3.

Intracellular uptake of galactose-porphyrins by spheroid cultures of HCT-116, MCF-7, UM-UC-3, or HeLa cancer cells. The concentration of porphyrins was determined by fluorescence spectroscopy after incubation of cancer cells with 9 μ M of galactose-porphyrins for 4 h, and the results were normalized to protein quantity. Data are the means $\pm$ SD of at least three independent experiments performed in triplicate. * $p < 0.05$, ** $p < 0.001$, significantly different from the corresponding C3-Gal₄-Por.

Figure 4.

Cytotoxicity at 24 h after PDT with galactose-porphyrins in cancer cells growing in monolayers, determined using the MTT assay. Cells were incubated with 9 μM PS for 4 h, and PDT was performed during 30 min at 0.44 mW/cm^2 . Data are means $\pm$ SD of at least three independent experiments performed in triplicate. *** $p < 0.0001$, significantly different from control cells.

Figure 5.

Cytotoxicity at 24 h after PDT with galactose–porphyrins in cancer cells growing in spheroids, determined using the LDH assay. Spheroids were incubated with 9 μ M PS for 4 h, and PDT was performed during 30 min at 0.44 mW/cm². Data are means $\pm$ SD of at least three independent experiments performed in triplicate. * p < 0.05, *** p < 0.0001, significantly different from control cells.

Scheme 1.
Synthesis of Porphyrin–Galactose Conjugates

Table 1.

Photophysical Properties of C-1 and C-3 Porphyrin Derivatives

compound	solvent	UV-visible ^a λ_{max} (nm)	emission ^a λ_{max} (nm) 424 nm excitation	Φ_F ^b	DLS (PDI) mean diameter and polydispersity index ^c (nm)
(C1-Gal) ₄ -ZnPor (3)	DMSO	431, 568, 608	609, 656	0.035	
	ethanol	425, 564, 604	604, 655	0.03	74 (0.39)
	PBS buffer	410, 425, 446, 571, 613	655, 718	0.002	16, 38 (0.39)
(C3-Gal) ₄ -ZnPor (6)	DMSO	430, 568, 608	608, 660	0.035	
	ethanol	425, 564, 604	602, 651	0.034	79, 164 (0.23)
	PBS buffer	424, 440, 453, 454, 573, 617	655, 723	0.003	24 (0.33)
(C1-Gal) ₄ -Por (4)	DMSO	424, 523, 559, 588, 614	610, 654, 715	0.05	
	ethanol	419, 523, 562, 599, 651	604, 653, 715	0.06	80 (0.30)
	PBS buffer	402, 429, 530, 556, 603, 655	658, 734	0.01	24 (0.34)
(C3-Gal) ₄ -Por (7)	DMSO	424, 524, 560, 588, 613	608, 654, 714	0.06	
	ethanol	419, 522, 563, 602, 657	603, 653, 714	0.05	67 (0.23)
	PBS buffer	410, 427, 530, 567, 602, 659	657, 718	0.008	28 (0.33)

^aSpectra in Supporting Information^b Φ_F are $\pm 10\%$.^cDLS histograms in Supporting Information.

Table 2.Partition Coefficient (log *P* *n*-octanol/PBS) of the Galactose Conjugates

compound	log <i>P</i> ± SD
(C1Gal) ₄ -Por (4)	-1.36 ± 0.05
(C3Gal) ₄ -Por (7)	-1.50 ± 0.12
(C1Gal) ₄ -ZnPor (3)	-0.99 ± 0.01
(C3Gal) ₄ -ZnPor (6)	-1.10 ± 0.10