HHS Public Access

Author manuscript

Eur J Med Chem Rep. Author manuscript; available in PMC 2022 December 22.

Published in final edited form as:

Eur J Med Chem Rep. 2022 August ; 5: . doi:10.1016/j.ejmcr.2022.100047.

Impact of mono- and di-β-galactose moieties in *in vitro* / *in vivo* anticancer efficacy of pyropheophorbide-carbohydrate conjugates by photodynamic therapy

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Abstract

To investigate the impact of mono- and di-β-galactose moieties in tumor uptake and photodynamic therapy (PDT) efficacy, HPPH [3-(1′-hexyloxy)ethyl-3-devinylpyropheophorobide-a], the meso pyropheophorbide-a [3-ethyl-3-devinyl-pyropheophorbide-a], and the corresponding 20-benzoic acid analogs were used as starting materials. Reaction of the intermediates containing one or two carboxylic acid functionalities with 1-aminogalactose afforded the desired 17^2- or 20(4′)- mono- and 17^2, 20(4′)-di galactose conjugated photosensitizers (PSs) with and without a carboxylic acid group. The overall lipophilicity caused by the presence of galactose in combination with either an ethyl or (1′-hexyloxy)ethyl side chain at position-3 of the macrocycle made a significant difference in *in vitro* uptake by tumor cells and photoreaction upon light exposure. Interestingly, among the PSs investigated, compared to HPPH **1** the carbohydrate conjugates **2** and **11** in which β-galactose moieties are conjugated at positions 17^2 and 20(4′) of meso-pyro pheophorbide-a showed similar *in vitro* efficacy in FaDu cell lines, but in SCID mice bearing FaDu tumors (head & neck) Ps **11** gave significantly improved long-term tumor cure.

Keywords

Photodynamic therapy; Photosensitizer; Cancer imaging

[1]Summer Research Program.

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Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ejmcr.2022.100047.

1. Introduction

Since the approval of Photofrin for the treatment of cancer by photodynamic therapy (PDT), a wide variety of porphyrins and non-porphyrin-based photosensitizers have been reported [1–4]. Efforts have also been made to investigate the tumor targeting ability of photosensitizers by conjugating certain small molecules and peptides to target receptors known for their over-expression in tumors [5,6]. Among these targeting moieties, carbohydrates-especially β-galactose have gained attention [7–11]. The other strategy, which has also made a significant impact in developing improved agent(s) for PDT is the structure activity relationship (SAR) approach, and some of the agents, e. g. HPPH (Photochlor) [12], Foscan [13], Tookad [14], Talaporphin [15], Photobac [16], benzoporphyrin derivative (BPD) [17] and (PC4, a Phthalocyanine) [18] are at various stages of human clinical trials for the use in oncologic and non-oncological applications. Another approach, which has been equally useful in selecting effective agents before evaluating them for *in vivo* efficacy is to define the cell-specificity of PSs in various tumor cell types, especially PS uptake and retention [19].

Most of the porphyrin-based compounds currently being investigated for the use in PDT show poor water solubility. The common substituents that have been introduced in tetrapyrrolic systems (porphyrins, phthalocyanines or expanded porphyrins) are amine, carboxylic acid, sulfonic acid and ethylene glycol side chains, which do not show much impact in water solubility [20,21]. However, some of the glycosylated porphyrin systems may improve the hydrophilic nature by attaching the sugar moiety at the peripheral positions of the macrocycle. In general, (Glycan-protein) lectin-type receptors are highly expressed in some type of malignant cells, which specifically interact with carbohydrate-conjugated tetrapyrroles [22]. Among certain proteins the galectins are highly conserved carbohydrate recognition domain and exhibit high affinity for β–galactoside [23]. Galectins are known to involve in the modulation of cell adhesion [24], cell growth [25], immune response [26] and angiogenesis [27] and changes in their expression play a critical role in tumor progression.

In our attempt to enhance the efficacy and selectivity of HPPH [3-(1′-hexyloxy)ethyl-3-devinylpyropheophorbide-a], currently under human clinical trials[1,28,29], was conjugated with a series of mono- di- and tetrasaccharides, with the goal to target carbohydrate-binding proteins, especially galectin-1 and galectin-3. Among the compounds investigated HPPH-Gal in which a β-galactose moiety was conjugated with HPPH at position 17^2 showed the best efficacy [30]. The increased activity (*both in vitro and in vivo*) could not be confirmed on the basis of its binding to cellular galectins. However, the overall lipophilicity and the lack of transport by the ABCG2 transporter may contribute to improved efficacy [31].

In a parallel study, to determine the effect of substituents at various peripheral positions of the HPPH moiety, the 20-phenyl substituted analogs were of particular interest due to their higher tumor-cell selectivity. The meso-phenyl substituted analog provides a unique opportunity not only to introduce the targeting moieties at two different positions (17 and 20) of the molecule, but also to investigate the impact of overall charge in tumor cell-uptake and retention. Therefore, in present study, a series of HPPH-based PSs containing mono- and di-galactose moiety with and without the presence of hexyl ether side chain at position

3(1′) of the macrocycle (**4–6** & **9–11**) were synthesized. The *in vitro* PDT efficacy of these compounds were compared with known HPPH analogs **1–3** & **7,8** [30] with and without the hexyl ether side chain [31].

2. Results and discussion

2.1. Chemistry

The photosensitizers investigated herein (Fig. 1) were prepared either from HPPH **1**, methyl pyropheophorbide-a **12** or methyl meso pyropheophorbide-a **13** (Scheme 1) obtained from chlorophyll-a by following the reported methods [32].

2.1.1. Synthesis of 20-phenyl-β-galactose conjugated pyropheopheophorbides—

For the synthesis of the title compounds, **12** and **13** were separately reacted with pyridinium tribromide [33], the 20-bromo derivatives **14** and **15** were then converted into 20-phenyl-*p*-*tert*-butoxy analogs **16** and **17** by reacting with 4-(*tert*-butoxycarbonyl) phenylboronic acid pinacol ester and tetrakis(triphenylphosphine)palladium as catalyst [33]. Further treatment of these intermediates with trifluoroacetic acid (TFA) at room temperature gave the desired carboxylic acid chlorins **18** and **19**. These chlorins were reacted with 1-β-aminogalactose tetraacetate, and the galactose protected compounds **20** and **21** were isolated in 57% and 47% yield, which on reacting with aqueous lithium hydroxide at room temperature gave PSs **5** (61% yield) and **10** (52% yield) respectively. (Scheme 1).

2.1.2. Synthesis of 20-phenyl-p-carboxylic acid 17^2-β-galactose conjugated meso pyropheophorbides—

Compound **17** containing a methyl ester functionality was hydrolyzed to the corresponding carboxylic acid **22** under mild basic conditions in quantitative yield, on reacting with aqueous lithium hydroxide at room temperature. Compound **22** was then reacted with 1- β–aminogalactose tetraacetate and the carbohydrate conjugate **23** thus obtained in 43% yield on treating with TFA at room temperature afforded 20-phenyl-p-carboxylic acid analog **24** in quantitative yield. In the last step of synthesis, reaction of **24** with sodium methoxide gave the desired 20-phenyl-β-galactose conjugate **9** in 43% yield (Scheme 2).

2.1.3. Synthesis of 17^2- and 20-phenyl-β-galactose conjugates meso pyropheophorbides—

20-Phenyl-*p*-carboxylic acid meso pyropheophorbide-a methyl ester **19** was first converted into corresponding 17, 20-dicarboxylic acid **25** in quantitative yield on mild base hydrolysis. The dicarboxylic acid derivative was then reacted with 1-β–aminogalactose tetraacetate to provide 17^2, 20-disubstituted acetylated galactose derivate **26**. Finally, after deprotection of galactose, moieties with sodium methoxide, the desired di-β–galactose meso pyropheophorbide **11** was isolated in 46% yield (Scheme 3).

2.1.4. Synthesis 17^2- and 20-phenyl-β-galactose conjugated 3-(1′-hexyloxy) ethyl-3-devinylpyropheo- phorbide-a—

For the preparation of di-β-galactose HPPH analog, a similar approach as discussed for compound **11** was followed. In brief, 20-bromo HPPH **27** prepared by following our own methodology [25,26] was first converted

into 17–β-amino-galactose tetraacetate **28** in 69% yield. It was then reacted with 4-(*tert*-butoxycarbonylphenyl)boronic acid pinacol ester in the presence of palladium catalyst, and the intermediate **29** was obtained in 59% yield. The *tert*-butyl ester functionality was removed under acidic condition and the corresponding carboxylic acid analog **30** was obtained in quantitative yield. It was again reacted with 1- β-amino-galactose tetraacetate to produce compound **31**, which, as well as compound **30**, on deprotection under basic conditions gave the desired bis- and mono-galactose HPPH analogs **6** and **4** respectively, each in 50% yield (Scheme 4). All the intermediates and final products were characterized by NMR, UV-vis and mass spectrometry analysis, and the purity of the galactose derivatives was also confirmed by HPLC analyses.

2.1.5. HPLC analysis of carbohydrate conjugates—HPLC chromatograms for compounds **4**, **5**, **6**, **9**, **10** and **11** were obtained using a Waters Delta 600 System consisting of: a 600 Controller, 600 Fluid Handling Unit and a 996 Photodiode Array Detector. A Waters Symmetry C18 column (4.6 × 150 mm, 5 μm particle size) was used to investigate the purity of the compounds before biological evaluation. An isocratic mobile phase consisting of 0.5% (v/v) acetic acid in methanol, at a flow rate of 1.0 ml/min was used. Absorption range was selected between 350 and 800 nm; data were processed at 362 nm. The purity ascertained by HPLC, and retention times of the conjugates are depicted in Fig. 2 and Table 1 respectively.

The ^{1}H and ^{13}C NMR spectra (1D and 2D) are consistent with the proposed structures. However, the ^{13}C spectrum of **4** exhibits some unusual features that are worth noting. Each of the carbons of **4** is expected to generate up to two signals in the 1D ^{13}C spectrum, because **4** exists as two diastereomers due to the chiral center at position 3^{1}. Nearly all carbons of **4** were observed as either one or two peaks, exactly as expected. However, four carbons exhibited unexpected, additional peaks. Each of these four carbons produced either three or four roughly equally spaced peaks. These appear at: 198.63, 198.61, 198.59, and 198.57 ppm for 13^{1}-C (i.e., the keto C=O); 149.22, 149.18, and 149.15 ppm for 14-C; 131.33, 131.30, 131.27, and 131.24 ppm for 13-C; and 107.29, 107.27, 107.24, and 107.21 ppm for 15-C. HMBC data firmly assign three of these four carbons as indicated above. The assignment of the cluster of peaks at ~131.3 ppm is less certain, but HMBC data indicate that the 13-position carbon is a likely source of these signals.

All of these four carbons are members of the photosensitizer E-ring. The only E-ring carbon that failed to generate observable signals that exceeded the expected number of peaks is 13^{2}-C, which could not be observed at all in the 1D ^{13}C spectrum due to severe overlap with the solvent (CD_3OD) resonance. This carbon may, in fact, generate additional (unexpected) peaks. If it does, however, these would simply not be observable in the 1D carbon spectrum because of the solvent interference. The HSQC spectrum shows a clear cross peak at the proton and carbon chemical shifts expected for the 13^{2}methylene group, but HSQC is not able to resolve the individual carbon resonances that compose this cross peak, preventing a peak count for this carbon. The location of these four carbons suggests that some kind of heterogeneity associated with the E-ring is responsible for producing the “extra” peaks. Interestingly, no extra peaks were observed in the ^{13}C spectra of any of the other compounds

examined here. Even protected conjugates **29** and **30**, which are pre-cursors of **4**, fail to exhibit the additional peaks despite the fact that each of these is structurally similar to **4**.

These facts lead us to the following hypothesis. Hydrogen bonding – from a hydroxyl group of the unprotected galactose to the E-ring's keto oxygen – may be responsible for the presence of the unexpected carbon resonances observed in **4**. The additional carbon peaks could arise due to the presence of two states of each diastereomer: hydrogen bound, and non-hydrogen bound. This would account for our observation of up to four peaks for each of the affected carbons. The lack of extra peaks in **29** and **30** is consistent with this hypothesis because the acetyl-protected galactose moieties in these two precursors lack the hydroxyl groups required for hydrogen bonding to the keto oxygen. The localized nature of this effect (E-ring only) is also consistent with the hydrogen bonding scenario because the influence on carbon chemical shifts is likely to be greatest near the site of the hydrogen bond.

Although plausible, the hydrogen bond explanation fails to account for some of our observations. No other mono- or di-galactose-containing conjugate examined here exhibits the same effect in its E-ring carbons. One might expect **6** and **9** to behave like **4** because each also has an unprotected galactose at position-17, and (relative to **4**) each has a similar general structure. Conjugate **6** differs from **4** only in its additional galactose linked through position-20. Conjugate **9** differs from **4** in its lack of the O-hexyl group at position-3 [1]. To be consistent with our hydrogen bonding explanation, we must assume that the O-hexyl group and second galactose moiety somehow prevent hydrogen bond formation, which would account for the behavior of **6** and **9**. However, we recognize that the unusual behavior of the E-ring carbons of conjugate **4** may be due to some other source. For example, it might be due to two different ring conformations, or two different stacking geometries. Each of these could manifest itself in two distinct structural states (per diastereomer) resulting in two distinct magnetic environments (per diastereomer) in the vicinity of the E-ring. This could account for the number of carbon peaks observed, up to four per E-ring carbon. Proof of any of these proposed explanations would obviously require further studies.

2.2. Biological studies

2.2.1. In vitro PDT efficacy—The PDT efficacy of the galactose conjugated compounds was investigated in FaDu, and Colon 26 tumor cell lines known for overexpression of galectin-3. A standard MTT assay [34] was performed to determine the IC_{50} value and percentage cell viability of each compound at variable concentrations after exposing to 1 J/cm^2 of light at 665 nm. Briefly, cells were seeded into a 96 well plate at 5000 cells per well and allowed to adhere. Photosensitizer was added to the media at two-fold dilutions with a top concentration of 1600 nM and incubated with cells for 24 h. After incubation, the cells were washed with media exposed to appropriate wavelength of light (1 J/cm^2). At 48 h after PDT, the MTT assay was performed, and cell viabilities was determined. The IC_{50} values of all the compounds are listed in Table 2.

The PDT efficacy of galactose analogs varied depending on tumor cell type, but compound **2** in which the galactose moiety conjugated at position 17[2] of HPPH and the di-galactose analog of mesopyropheophorbide-a **11** showed significant efficacy (percentage cell survival)

in both cell types. The efficacy of PS **11** was reduced on replacing the ethyl group at position 3 with 3 (1′-hexyloxy)ethyl substituent. The *in vitro* efficacy (percentage cell survival) results of PS **2–6** and **9–11** in both FaDu and Colon26 cell lines are shown in Fig. 3.

2.2.2. A difference in mitochondrial and lysosomal localization of HPPH and the corresponding carbohydrate analogs—The importance of subcellular localization of a photosensitizer and its impact on the destruction of tumor cells by reactive oxygen species (mainly singlet oxygen) produced after exposing the tumors/cells with an appropriate wavelength of light has been a subject of discussion from a long time [35,36]. However, it is now well accepted that most of the porphyrin-based mainly localize in mitochondria and lysosomes [37]. Depending on the type of PS, both diffusion and endocytosis play an important role in cell-uptake. It is now also well accepted that apoptosis [38] and necrosis [39] are the two main mechanisms of cell death. The *in vivo* studies have suggested that vascular shutdown play crucial role in reducing the survivability of tumor cells as it starves them to nutrients and oxygen [40]. However, direct cell cytotoxicity has not been ruled [41] out in less extent, and the overall mechanism of cell death by PDT is complex. In any event PSs localize either in mitochondria or lysosomes (or both) are reported to be effective, and the tumor specificity seems to depend on over all lipophilicity, charge and the nature of substituents present at peripheral position of the PDT agent.

In our present study, we compared the subcellular localization characteristics of HPPH **1**, with its mono- and di-galactose conjugates **2** and **11** respectively. Cells were plated in 6 well plate and allowed to adhere before 1 μM of PS **1** was added. After 24 h, Lysotracker Green DND-26 and Mitotracker Red CmxRos were added to enable fluorescent labeling of the lysosome or mitochondria, respectively. The images were analyzed using Amnis IDEAS v6.2 where the bright detail similarity score was generated by comparing co-localization between the photosensitizer and the organelle Results depicted in Fig. 4 indicate that PS **2** (non-galactose analog) shows the preference to mitochondria over the lysosome, whereas the mono- and di-galactose analogs show preference to lysosome with almost similar localization pattern.

2.2.3. Comparative tumor uptake and PDT efficacy PSs 1, 2 and 11—We have previously shown that conjugation of β-galactose at position 17^2- HPPH enhances PDT efficacy of mice bearing BALB/c tumors. In present study, the tumor-uptake and PDT efficacy of HPPH **1** was compared with the corresponding mono- and di-b-galactose conjugates **2** and **11** (at a dose of 0.47 mmol/kg) in SCID mice bearing FaDu tumors. and imaged at various time points using IVIS Spectrum. The fluorescence intensity of tumor, liver and skin was measured at variable time points and the results are shown in Fig. 5. Interestingly, both β-galactose conjugated PSs in SCID mice bearing FaDu tumors showed similar pattern of uptake in tumor, liver and skin from 1 to 8 h post-injection. However, compared to **2**, the uptake of PS **11** in tumor was slightly higher at 8 h. A significant clearance from tumor, liver and skin at 24 h post-injection was observed by both the PSs. In contrast to carbohydrate analogs, the uptake and biodistribution characteristics of HPPH was significantly different. For example, the tumor-uptake increased with time, and maximum uptake was observed at 24 h post injection, with a significant clearance from both liver and

skin. The high uptake of mono and di-galactose analogs in liver could be due to hepatic uptake and metabolism of galactose moiety (see Fig. 6).

2.2.4. Comparative in vivo PDT efficacy of HPPH (1) with mono- and di-galactose conjugates (2 and 11)—The SCID mice bearing FaDu (Head & Neck tumors) were selected to study the antitumor activity of PSs **1, 2** and **11**. When the tumors were of treatment size (4–5 mm in diameter), the mice were injected with PS formulated in 1% Tween 80/5% dextrose solution at a dose of 0.47 μmol/kg. The tumors were exposed to light (665 nm, 135 J/cm^2, 75 mW/cm^2) at 24 h post-injection for PS **1** and 8 h post-injection for the galactose analogs **2** and **11** (due to their optimal tumor uptake at those time points), and tumor growth was measured daily. The PS **2** and **11** injected intravenously in SCID mice bearing FaDu tumors gave complete response (CR) 50% (3/6 mice were tumor free on day 60) and 77.5% (7/9 mice were tumor free on day 60) respectively on day 60, whereas HPPH, at similar drug and light dose was 60% effective (3/5 mice were tumor free on day 60). The statistical evaluation of the survival curves was analyzed by Mantel-Cox test.

3. Conclusions

In this study, a series of pyropheophorbides containing β-galactose moiety at position-17 [2] and/or position-20(4′) with variable lipophilicity by altering the substituents at position-3 were synthesized. The compounds were characterized by NMR (^{1}H & ^{13}C), and the purity was ascertained by HPLC. These compounds were insoluble in water and formulated in 1% Tween 80/5% dextrose (D5W) solution [27] The *in vitro* PDT efficacy of the conjugates was determined in two tumor cell lines: FaDu (head & neck) and Colon16. In both cell lines, PS **2** in which galactose moiety was conjugated at position-17 [2] of HPPH and compound **11** containing β-galactose moieties at positions 17^2 and 20(4′) of meso pyropheophorbide-a showed similar efficacy than the non-galactose analog **1** (HPPH). The PS **1** (HPPH) and the corresponding carbohydrate analogs **2** and **11** showed significant tumor uptake in mice bearing FaDu tumors. However, as expected compared to PS **1**, the PSs **2** and **11** showed higher uptake in liver, which could be due to higher hepatic uptake/metabolism of β-galactose. Among the compounds tested for *in vivo* efficacy, the di- β-galactose conjugate **11** gave the best long-term tumor cure in SCID mice bearing FaDu tumors (7/9 mice = 77.7% tumor free on day 30). The higher efficacy of PS **11** over **2** could be due to (a) the difference in biodistribution of the PS across the tumor, (b) the consequences of PDT (immunological impact) after light treatment, and (c) the role of ABCG 2 pump in efflux of the PS (with and without the galactose moiety. These studies using a larger group of mice are currently underway, and hope will help to address these questions. Compared to PS **1** (HPPH), the carbohydrate analogs **4,5,6,** and **10** were significantly less effective than the non-carbohydrate analog **1** *in vitro*, and were not evaluated for *in vivo* efficacy.

3.1. Experimental section

3.1.1. Chemistry—Chemicals used for the synthesis were purchased from Aldrich and Synthose and were reagent grade unless otherwise specified. All reactions were carried out in heat gun-dried glassware under an atmosphere of argon and magnetic stirring. Thin layer chromatography (TLC) was performed on pre-coated silica gel sheets (layer thickness 0.2

mm). Column chromatography was performed using silica gel 60 (70–230 mesh) purchased from Merk. Preparative TLC was also used for the purification of compounds (Analtec, 20 × 20 cm, silica gel GF, 1000 μm). Purity of the compounds was ascertained by TLC, NMR and HPLC. All compounds including the intermediates were >95% pure. UV-visible spectra were recorded on an UV-visible spectrophotometer using DCM and MeOH as solvents. Mass spectrometry analyses were performed at the Mass Spectrometry Facility, University of Buffalo, NY.

NMR data were acquired at 28 °C on a Bruker Avance III HD NMR spectrometer equipped with a 9.4 T narrow-bore magnet, a 5-mm BBO Z-gradient probe, and Topspin 3.2 software.

Frequencies for ^{1}H and ^{13}C observations were 400 MHz and 100 MHz, respectively. Chemical shifts were calibrated to the residual solvent resonance and are reported relative to tetramethylsilane (TMS) at 0.00 ppm. ^{1}H multiplicities are reported as follows: s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet, br = broad.

3.1.2. General procedure for the synthesis of compounds 16, 17, and 29 (Suzuki coupling)—To the solution of starting material (1 eq.) and K_3PO_4 (6 eq.) in 15 ml of dry THF, 4-(*tert*-butoxycarbonyl)phenylboronic acid pinacol ester (12 eq.) and Pd $(PPh_3)_4$ (0.3 eq.) were added. After refluxing overnight under Ar atmosphere, the reaction mixture was cooled to room temperature and filtered through celite to remove excess salts. Solvent was removed under reduced pressure, residue was redissolved in DCM (50 ml), washed with sat. $NaHCO_3/H_2O$/Brine (50 ml x 1 each) and dried over Na_2SO_4. After filtration, solvent was evaporated and resulting crude product was purified by silica column chromatography, using Ethyl Acetate: Hexane (1:4) as eluent for compounds **16** and **17**. Compound **29** was purified by three separate runs of preparative TLC using silica plates and the following eluents: (1) 4% MeOH in DCM, (2) 3% MeOH in DCM, and (3) 3.5% MeOH in DCM.

3.1.3. General procedure for deprotection of tert-butyl esters for synthesis of compounds 18, 19, 24, and 30—Starting material (15–25 mg) was stirred in 3 ml of 70% solution of trifluoroacetic acid in DCM for 25 min under Ar atmosphere at room temperature. The reaction mixture was then diluted with 20 ml of DCM, washed with H_2O (4 × 20 ml, until wash water pH > 6.5), dried over Na_2SO_4, and filtered. After removing the solvent, clean product was obtained in quantitative yield.

3.1.4. General procedure for the synthesis of the compounds 20, 21, 26, and 31—Starting material (1 eq.), 1-β-amino-galactose tetraacetate (2.5 eq.) and benzotriazol-1-yloxytris(dimethylamino) phosphonium hexafluorophosphate (2.5 eq.) were dissolved in dry DMF (7 ml) under Ar atmosphere. To this solution, triethylamine (2.5 eq.) was added, and the reaction mixture was stirred overnight (for compounds **26** and **31** for 32 h) at room temperature. After this, 20 ml of DCM was added to the reaction mixture, and it was washed with H_2O (3 × 20 ml) and dried over Na_2SO_4. After filtration, the solvent was removed under reduced pressure. The resulting residue was purified by three separate runs of preparative TLC using silica plates and the following eluents: (1) 4% MeOH in DCM, and (2 & 3) 3% MeOH in DCM.

3.1.5. General procedure for the synthesis of the compounds 5, 10, 22, and 25—Starting material (1 eq.) was dissolved in 10–20 ml of a degassed (3 times, vacuum – Ar) mixture of MeOH and THF (1:1). LiOH monohydrate (15–25 eq.) was dissolved in 5–10 ml of H_2O and degassed (3 times, vacuum – Ar). Then, the solution of LiOH was added to the solution of starting material and the reaction mixture was degassed (5 times, vacuum – Ar) and stirred overnight at room temperature. The reaction mixture was then neutralized with a cold (0 °C) 5% aqueous solution of CH_3COOH. The product was extracted from the reaction mixture with DCM (50 ml). DCM solution was washed with H_2O (5 × 50 ml). The organic layer was then dried over Na_2SO_4, filtered, and solvent was removed under reduced pressure. Yield was quantitative for compounds **22** and **25**. Compounds **5** and **10**, however, required purification by silica column chromatography, using gradient 30–90% MeOH in DCM as eluent.

3.1.6. General procedure for the synthesis of the compounds 23 and 28—Starting material (1 eq.), 1-β-amino-galactose tetraacetate (2 eq.), 1-Ethyl-3-(3-dimethylaminopropyl)carbodiimide (2 eq.), and 4-Dimethylaminopyridine (2.2 eq.) were dissolved in dry DCM (15 ml) and stirred overnight under Ar atmosphere. After this, the reaction mixture was diluted with DCM (15 ml) and washed with H_2O (3 × 30 ml). Then, the organic layer was dried over Na_2SO_4, filtered, and the solvent was evaporated under reduced pressure. The product was purified by three separate runs of preparative TLC using silica plates and the following eluents: (1) 3% MeOH in DCM, (2) 4% MeOH in DCM, and (3) 3% MeOH in DCM.

3.1.7. General procedure for the deacetylation of galactose moieties for obtaining compounds 4, 6, 9, and 11—Starting material (1 eq.) was dissolved in a mixture of dry DCM (10 ml) and MeOH (1.0 ml). Sodium methoxide in MeOH (0.5 M, 250 μL) was added to this solution, and the reaction mixture was stirred for 25 min under Ar atmosphere at room temperature. It was then neutralized with a solution of 5% acetic acid in DCM and diluted with DCM to 50 ml total volume. The reaction mixture was then washed with H_2O (3 × 50 ml), dried over Na_2SO_4, filtered, and solvents were evaporated under vacuum. The crude product was purified by silica column chromatography, using gradient 35–95% MeOH in DCM as eluent.

Compound 4.: 14 mg of starting photosensitizer **30** were used for reaction by following the method described above, and the title compound was obtained in 50% yield (6 mg). UV-Vis λ_{max} (MeOH): 671 (rel. intensity 0.149), 615 (0.026), 548 (0.044), 513 (0.037), 412 (0.319); ^{1}H NMR (400 MHz, CD_3OD, δ ppm): 10.07/10.03 (1H, s, PS 5-H), 9.15/9.01 (1H, s, 10-H), 8.41 (1H, dd, J = 7.8, 1.7 Hz, 20^{3a}-H), 8.25 (1H, dd, J = 7.8, 1.7 Hz, 20^{3b}-H), 8.18 (1H, dd, J = 7.8, 1.7 Hz, 20^{2a}-H), 7.66/7.65 (1H, dd, J = 7.8, 1.7 Hz, 20^{2b}-H), 5.85/5.84 (1H, q, J = 6.8 Hz, 3^1-H), 5.12 (1H, d, J = 20.1 Hz, 13^2-C*H*H), 5.11/5.08 (1H, d, J = 20.1 Hz, 13^2-CH*H*), ~4.81 (1H, d, J ~8 Hz, Gal 1-H), 4.34/4.33 (1H, q, J = 7.2 Hz, 18-H), 4.10 (1H, m, 17-H), 3.834/3.832 (1H, dd, J = 1.1, ~3 Hz, Gal 4-H), ~3.72/3.59 (1H, m, −OC*H*H$(CH_2)_4CH_3$), ~3.56–3.69 (2H, m, Gal 6-CH_2), ~3.65/3.38 (1H, m, −OCH*H*$(CH_2)_4CH_3$), ~3.57 (1H, m, Gal 5-H), ~3.48/3.40 (2H, m, 8-CH_2CH_3), ~3.45 (1H, m, Gal 3-H), ~3.43 (1H, m, Gal 2-H), ~3.32/3.25 (3H, s, 12-CH_3), 3.19/3.16

(3H, s, 7-CH_3), ~2.48 (1H, m, 17-CH_2CHH−), 2.40/2.39 (3H, s, 2-CH_3), 2.33 (1H, m, 17-$CHHCH_2$−), 2.17/1.99 (3H, d, J = 6.7 Hz, 3^1-CH_3), ~2.01 (1H, m, 17-$CHHCH_2$−), ~1.96 (1H, m, 17-CH_2CHH−), ~1.72/1.63 (1H, m, −$OCH_2CHH(CH_2)_3CH_3$), ~1.72/1.53 (1H, m, −$OCH_2CHH(CH_2)_3CH_3$), 1.58/1.53 (3H, t, J = 7.6 Hz, 8-CH_2CH_3), ~1.42/1.34 (1H, m, −$O(CH_2)_2CHH(CH_2)_2CH_3$), ~1.42/1.23 (1H, m, −$O(CH_2)_2CHH(CH_2)_2CH_3$), ~1.17/1.06 (2H, m, −$O(CH_2)_4CH_2CH_3$), ~1.17/1.03 (2H, m, −$O(CH_2)_3CH_2CH_2CH_3$), 1.08/1.05 (3H, d, J = 7.1 Hz, 18-CH_3), 0.69/0.56 (3H, distorted t, J ~ 7 Hz, −$O(CH_2)_5CH_3$; ^{13}C NMR (100 MHz, CD_3OD, δ ppm): 198.63/198.61/198.59/198.57#, 176.4/176.3, 175.1, 174.1, 162.2/162.1, 155.44/155.36, 152.9/152.7, 149.22/149.18/149.15#, 146.0/145.9, 144.11/144.09, 143.0/142.7, 140.8/140.7, 139.6/139.5, 139.23/139.21, 137.7/137.6, 135.23/135.14, 135.21/134.77, 134.90/134.66, 133.0/132.9, 131.33/131.30/131.27/131.24#, 130.5, 129.9, 129.3/129.2, 113.5, 107.29/107.27/107.24/107.21#, 104.4/104.3, 99.8/99.7, 81.6/81.5, 78.2, 75.8/75.7, 74.2/74.1, 71.2, 70.6/70.3, 70.4, 62.5, 53.2, 49.6*, ~48.9*, 33.7/33.6, 32.8/32.6, 31.3/31.2, 31.12/31.05, 27.2/27.1, 25.3/24.9, 23.6/23.5, 21.4, 20.0/19.9, 17.65/17.61, 14.2/14.1, 14.2/14.0, 11.61/11.56, 11.31/11.28. # See Results & Discussion. * From HSQC. HRMS (ESI) calculated for $C_{52}H_{64}N_5O_{10}$[MH^+] 918.4653, found 918.47026. HPLC retention time: 4.15 min (see Supporting Information, Fig. S43, purity >96.0%).

Compound 5.: 16 mg of starting photosensitizer **20** were used for reaction by following the method described above, and the title compound was obtained in 61% yield (8 mg). UV-Vis λ_{max} (MeOH): 670 (rel. intensity 0.252), 613 (0.045), 549 (0.076), 515 (0.049), 413 (0.541); ^{1}H NMR (400 MHz, CD_3OD, δ ppm): 10.13/10.08 (1H, s, PS 5-H), 9.03 (1H, br s, 10-H), 8.38 (1H, dd, J = 7.9, 1.9 Hz, 20^{3a}-H), 8.19 (2H, m, 20^{2a}-H & 20^{3b}-H), 7.74 (1H, m, 20^{2b}-H), 5.85/5.84 (1H, q, J = 6.7 Hz, 3^1-H), 5.280/5.277 (1H, d, J = 9.1 Hz, Gal 1-H), 4.95 (1H, d, J = 20.2 Hz, 13^2-CHH), ~4.80* (1H, m, 13^2-CHH), 4.11/4.09 (1H, q, J = 7.2 Hz, 18-H), 4.01 (1H, d, J = 3.2 Hz, Gal 4-H), 3.92 (1H, t, J = 9.3 Hz, Gal 2-H), ~3.77–3.88 (2H, m, Gal 6-CH_2), ~3.87 (1H, m, 17-H), ~3.79 (1H, m, Gal 5-H), ~3.61–3.74/3.59,3.43 (2H, m, −$OCH_2(CH_2)_4CH_3$), ~3.69 (1H, m, Gal 3-H), ~3.46 (2H, m, 8-CH_2CH_3), 3.23/3.21 (3H, s, 7-CH_3), ~3.18/3.16 (3H, br s, 12-CH_3), 2.39/2.38 (3H, s, 2-CH_3), ~2.26 (1H, m, 17-CH_2CHH−), 2.14/2.02 (3H, d, J = 6.7 Hz, 3^1-CH_3), ~1.84 (2H, m, 17-$CHHCHH$−), 1.64–1.78/1.49–1.70 (2H, m, −$OCH_2CH_2(CH_2)_3CH_3$), 1.56 (3H, t, J = 7.6 Hz, 8-CH_2CH_3), ~1.47 (1H, m, 17-$CHHCH_2$−), ~1.30–1.48/1.20–1.40 (2H, m, −$O(CH_2)_2CH_2(CH_2)_2CH_3$), ~1.16/1.04 (4H, m, −$O(CH_2)_3(CH_2)_2CH_3$), 0.89 (3H, m, 18-$CH_3$), 0.68/0.58 (3H, distorted t, J ~ 7 Hz, −$O(CH_2)_5CH_3$); ^{13}C NMR (100 MHz, CD_3OD, δ ppm): 198.3/198.2, 177.8, 173.22/173.20, 170.8, 162.7/162.2, 155.27/155.26, 152.9, 149.0, 146.18/146.02, 146.01, 143.2/142.9, 140.4, 139.6, 137.7, 135.8, 135.2, 134.7, 134.1/134.0, 133.7, 131.3, 129.6, 128.8, 128.47/128.45, 112.53/112.50, 107.1, 104.5, 100.1, 82.5, 78.6, 75.9, 74.3/74.1, 71.3, 70.7, 70.6/70.4, 62.7, 53.38/53.36, 49.4*, 49.1*, 32.8, 32.7, 31.3/31.2, 30.8, 27.2/27.1, 25.2/24.9, 23.6/23.5, 21.3, 19.98/19.96, 17.7, 14.5/14.1, 14.23/14.19, 11.49/11.48, 11.34/11.31. * From HSQC. HRMS (ESI) calculated for $C_{52}H_{64}N_5O_{10}$ [MH^+] 918.4653, found 918.4704. HPLC retention time: 4.69 min (see Supporting Information, Fig. S43, purity >97.3%).

Compound 6.: 13 mg of starting photosensitizer **31** were used for reaction by following the method described above, and the title compound was obtained in 50% yield (5 mg). UV-Vis λ_{max} (MeOH): 671 (rel. intensity 0.107), 616 (0.020), 547 (0.034), 515 (0.024), 412 (0.237); ^{1}H NMR (400 MHz, 95:5 $CD_3OD/CDCl_3$, δ ppm): 10.11/10.07 (1H, s, PS 5-H), 9.49/9.48 (1H, s, 10-H), 8.38 (1H, d, J = 7.9 Hz, 20^{3a}-H), 8.34/8.33 (1H, dd, J = 7.9, 1.5 Hz, 20^{2a}-H), 8.204/8.198 (1H, dd, J = 7.9, ~1.6 Hz, 20^{3b}-H), 7.78/7.76 (1H, dd, J = 8.0, 1.6 Hz, 20^{2b}-H), 5.84/5.83 (1H, q, J = 6.8 Hz, 3^1-H), 5.27 (1H, d, J = 9.1 Hz, 20-Gal 1-H), 5.18 (2H, m, 13^2-CH_2), ~4.81* (1H, d, J ~ 9 Hz, 17-Gal 1-H), 4.304/4.300 (1H, q, J = 7.1 Hz, 18-H), 4.13 (1H, m, 17-H), 4.01 (1H, d, J ~ 3.4 Hz, 20-Gal 4-H), 3.89 (1H, t, J = 9.2 Hz, 20-Gal 2-H), ~3.84 (1H, m, 17-Gal 4-H), 3.76–3.86 (2H, m, 20-Gal 6-CH_2), ~3.78 (1H, m, 20-Gal 5-H), ~3.62–3.72/3.57,3.38 (2H, m, −OC$H_2$$(CH_2)_4CH_3$), ~3.69 (1H, dd, J = 9.4, 3.4 Hz, 20-Gal 3-H), ~3.67 (2H, m, 8-C$H_2$$CH_3$), 3.56–3.69 (2H, m, 17-Gal 6-CH_2), 3.564/3.560 (3H, s, 12-CH_3), ~3.56 (1H, m, 17-Gal 5-H), ~3.48 (1H, m, 17-Gal 3-H), ~3.41 (1H, m, 17-Gal 2-H), 3.244/3.242 (3H, s, 7-CH_3), ~2.49 (1H, m, 17-CH_2CHH−), ~2.38 (1H, m, 17-CHHCH_2−), 2.38/2.37 (3H, s, 2-CH_3), ~2.20 (1H, m, 17-CH$H$$CH_2$−), 2.14/1.98 (3H, d, J = 6.7 Hz, 3^1-CH_3), ~2.05 (1H, m, 17-CH_2CHH−), 1.68 (3H, t, J = 7.6 Hz, 8-CH_2CH_3), ~1.71/1.60 (2H, m, −OCH_2C$H_2$$(CH_2)_3CH_3$), ~1.42/1.33 (2H, m, −O$(CH_2)_2CH_2$$(CH_2)_2CH_3$), ~1.18/1.06 (4H, m, −O$(CH_2)_3$(C$H_2$$)_2CH_3$), 1.03/1.02 (3H, d, J = 7.0 Hz, 18-CH_3), 0.71/0.59 (3H, distorted t, J ~ 7 Hz, −O$(CH_2)_5$CH_3); ^{13}C NMR (100 MHz, 95:5 $CD_3OD/CDCl_3$, δ ppm): 198.6, 176.2, 173.3*, 170.6, 162.0*, 155.4, 153.0, 149.4*, 146.1, 146.0, 143.0/142.7*, 140.5*, 139.8, 137.7*, 135.7*, 134.9*, 134.6*, 134.0*, 133.7*, 131.4*, 129.77/129.74, 128.9, 128.3, 112.4, 107.33/107.32, 104.81/104.78, 100.0, 82.3, 81.4, 78.4, 78.0, 75.8, 75.6, 74.0, 71.2, 71.1, 70.6/70.3, 70.5, 70.2, 62.6, 62.4, 53.3, 49.4*, 49.3*, 33.9, 32.7/32.6, 31.2/31.1, 30.9*, 27.1/27.0, 25.2/24.9, 23.5/23.4, 21.3, 20.1, 17.7, ~14.2, 14.2/14.1, 11.9, 11.4. * From HSQC or HMBC. HRMS (ESI) calculated for $C_{58}H_{75}N_6O_{14}$ [MH^+] 1079.5341, found 1079.5372. HPLC retention time: 3.03 min (see Supporting Information, Fig. S43, purity >96.0%).

Compound 9.: 14 mg of starting photosensitizer **24** were used for reaction by following the method described above, and the title compound was obtained in 43% yield (5 mg). UV-Vis λ_{max} (MeOH): 665 (rel. intensity 0.105), 608 (0.020), 546 (0.032), 512 (0.026), 412 (0.245); ^{1}H NMR (400 MHz, CD_3OD, δ ppm): 9.43 (1H, s, 10-H), 9.30 (1H, s, 5-H), 8.41 (1H, dd, J = 7.9, 1.7 Hz, 20^{3a}-H), 8.25 (1H, dd, J = 7.8, 1.7 Hz, 20^{3b}-H), 8.20 (1H, dd, J = 7.9, 1.7 Hz, 20^{2a}-H), 7.67 (1H, dd, J = 7.8, 1.7 Hz, 20^{2b}-H), 5.20, 5.17 (2H, ABq, J_{AB} = 20.3 Hz, 13^2-CH_2), 4.81* (1H, Gal 1-H), 4.33 (1H, q, J = 7.1 Hz, 18-H), 4.15 (1H, dd, J ~ 8.8, 2.9 Hz, 17-H), 3.83 (1H, d, J ~ 2.7 Hz, Gal 4-H), 3.79 (2H, q, J = 7.7 Hz, −C$H_2$$CH_3$), 3.59–3.69 (4H, m, Gal 6-CH_2 & −C$H_2$$CH_3$), 3.56 (3H, s, 12-$CH_3$), ~3.55 (1H, m, Gal 5-H), 3.39–3.47 (2H, m, Gal 2-H & Gal 3-H), 3.20 (3H, s, 7-CH_3), ~2.50, ~2.43, ~2.14, ~2.03 (each 1H, 4 × m, 17-CH_2CH_2−), 2.32 (3H, s, 2-CH_3), 1.66 (3H, t, J = 7.6 Hz, −CH_2CH_3), 1.65 (3H, t, J = 7.6 Hz, −CH_2CH_3), 1.09 (3H, d, J = 7.0 Hz, 18-CH_3); ^{13}C {^{1}H} NMR (100 MHz, CD_3OD, δ ppm): 198.7, 176.4, 174.8, 174.5, 161.8, 155.7, 152.5, 149.4, 146.1, 145.3, 144.3, 141.8, 139.4, 137.3, 136.6, 135.1, 133.5, 133.0, 131.2, 130.9, 130.5, 129.8, 129.0, 113.3, 107.3, 104.6, 97.2, 81.5, 78.2, 75.8, 71.2, 70.4, 62.5, 53.1, 49.6*, 49.2*, 36.5, 33.8, 21.4, 20.1, 20.0, 17.7, 17.1, 14.2, 11.7, 11.0. * From HSQC. MS (ESI)

calculated for $C_{46}H_{52}N_5O_9[MH^+]$ 818.3765, found 818.3756. HPLC retention time: 6.17 min (see Supporting Information, Fig. S44, purity >97.1%).

Compound 10.: 14 mg of starting photosensitizer **21** were used for reaction by following the method described above, and the title compound was obtained in 52% yield (6 mg). UV-Vis λ_{max} (MeOH): 667 (rel. intensity 0.101), 612 (0.019), 546 (0.029), 511 (0.020), 412 (0.230); ^{1}H NMR (400 MHz, CD_3OD, δ ppm): 9.22 (1H, s, PS 5-H), 9.13 (1H, s, 10-H), 8.37 (1H, dd, J = 7.9, 1.9 Hz, 20^{3a}-H), 8.23 (1H, dd, J = 7.9, 1.6 Hz, 20^{2a}-H), 8.17 (1H, dd, J = 7.9, 1.9 Hz, 20^{3b}-H), 7.69 (1H, dd, J = 7.9, 1.6 Hz, 20^{2b}-H), 5.27 (1H, d, J = 9.1 Hz, Gal 1-H), 5.04 (2H, m, 13^2-CH_2), 4.19 (1H, q, J = 7.1 Hz, 18-H), 4.01 (1H, d, J = 3.2 Hz, Gal 4-H), 3.97 (1H, dd, J ~ 9.4, 3.2 Hz, 17-H), 3.93 (1H, t, J = 9.3 Hz, Gal 2-H), 3.76–3.88 (2H, m, Gal 6-CH_2), 3.78 (1H, m, Gal 5-H), 3.74 (2H, q, J = 7.7 Hz, 3-CH_2CH$_3$), 3.69 (1H, dd, J = 9.6, 3.4 Hz, Gal 3-H), 3.44 (2H, q, J = 7.7 Hz, 8-CH_2CH$_3$), 3.37 (3H, s, 12-CH_3), 3.12 (3H, s, 7-CH_3), ~2.34 (1H, m, 17-CH_2C*H*H−), 2.29 (3H, s, 2-CH_3), ~2.11 (1H, m, 17-C*H*HCH_2−), ~1.96 (1H, m, 17- CH_2CH*H*−), ~1.79 (1H, m, 17-CH*H*CH_2−), 1.64 (3H, t, J = 7.6 Hz, 3-CH_2CH_3), 1.57 (3H, t, J = 7.6 Hz, 8-CH_2CH_3), 0.94 (3H, d, J = 7.1 Hz, 18-CH_3); ^{13}C {^{1}H} NMR (100 MHz, CD_3OD, δ ppm): 198.6, 179.8, 173.9, 170.8, 162.4, 155.6, 152.5, 149.4, 146.2, 146.1, 145.4, 141.5, 139.4, 137.3, 136.4, 135.8, 135.1, 133.8, 132.9, 131.2, 129.2, 128.7, 128.4, 112.3, 107.2, 104.7, 97.5, 82.5, 78.6, 75.9, 71.3, 70.7, 62.7, 53.7, 49.6*, 49.2*, 34.8, 32.0, 21.3, 20.1, 20.0, 17.7, 17.2, 14.4, 11.7, 11.0. * From HSQC. HRMS (ESI) calculated for $C_{46}H_{52}N_5O_9$ $[MH^+]$ 818.3765, found 818.3753. HPLC retention time: 4.18 min (see Supporting Information, Fig. S44, purity >95.4%).

Compound 11.: 12 mg of starting photosensitizer **26** were used for reaction by following the method described above, and the title compound was obtained in 46% yield (5 mg). UV-Vis λ_{max} (MeOH): 667 (rel. intensity 0.097), 610 (0.012), 545 (0.024), 514 (0.014), 412 (0.224); ^{1}H NMR (400 MHz, 99:1 $CD_3OD/CDCl_3$, δ ppm): 9.27 (1H, s, 10-H), 9.20 (1H, s, PS 5-H), 8.36 (1H, dd, J = 7.9, 1.9 Hz, 20^{3a}-H), 8.25 (1H, dd, J = 7.9, 1.6 Hz, 20^{2a}-H), 8.17 (1H, dd, J = 7.9, 1.9 Hz, 20^{3b}-H), 7.69 (1H, dd, J = 7.9, 1.6 Hz, 20^{2b}-H), 5.28 (1H, d, J = 9.1 Hz, 20-Gal 1-H), ~5.1 (2H, m, 13^2-CH_2), ~4.82 (1H, d, J ~ 9 Hz, 17-Gal 1-H), 4.20 (1H, q, J = 7.0 Hz, 18-H), 4.04 (1H, dd, J ~ 8.9, 3.7 Hz, 17-H), 4.01 (1H, d, J = 3.3 Hz, 20-Gal 4-H), 3.90 (1H, t, J = 9.3 Hz, 20-Gal 2-H), ~3.85 (1H, m, 17-Gal 4-H), ~3.76–3.87 (2H, m, 20-Gal 6-CH_2), ~3.78 (1H, m, 20-Gal 5-H), ~3.71 (2H, m, 3-CH_2CH$_3$), ~3.69 (1H, m, 20-Gal 3-H), ~3.58–3.70 (2H, m, 17-Gal 6-CH_2), ~3.57 (1H, m, 17-Gal 5-H), ~3.54 (2H, m, 8-CH_2CH$_3$), 3.49 (1H, dd, J = 9.5, 3.2 Hz, 17-Gal 3-H), 3.47 (3H, s, 12-CH_3), 3.43 (1H, dd, J ~ 9.4, 8.9 Hz, 17-Gal 2-H), 3.13 (3H, s, 7-CH_3), ~2.44 (1H, m, 17-CH_2C*H*H−), ~2.31 (1H, m, 17-C*H*HCH_2−), 2.27 (3H, s, 2-CH_3), ~2.07 (1H, m, 17-CH*H*CH_2−), ~2.03 (1H, m, 17-CH_2CH*H*), 1.61 (6H, t, J = 7.5 Hz, 3-CH_2CH_3& 8-CH_2CH_3), 0.97 (3H, d, J = 7.0 Hz, 18-CH_3); ^{13}C {^{1}H} NMR (100 MHz, 99:1 $CD_3OD/CDCl_3$, δ ppm): 198.6, 176.2, 173.7, 170.6, 161.6, 155.6, 152.4, 149.4, 146.1, 146.0, 145.3, 141.5, 139.4, 137.2, 136.3, 135.6, 134.9, 133.7, 132.8, 131.2, 129.2, 128.8, 128.2, 112.1, 107.0, 104.7, 97.4, 82.3, 81.4, 78.4, 77.9, 75.8, 75.6, 71.2, 71.0, 70.5, 70.3, 62.6, 62.4, 53.2, ~49.4*, ~48.8*, 33.9, 30.9, 21.3, 20.0, 19.9, 17.7, 17.1, 14.3, 11.8, 11.1. * From HSQC. HRMS (ESI) calculated for $C_{52}H_{63}N_6O_{13}[MH^+]$ 979.4453, found 979.4444. HPLC retention time: 2.29 min (see Supporting Information, Fig. S44, purity >96.9%)

Compound 16.: 40 mg of starting photosensitizer **14** were used for reaction by following the method described above, and the title compound was obtained in 69% yield (31 mg). UV-Vis λ_{max} (CH_2Cl_2): 670 (rel. intensity 0.303), 615 (0.050), 550 (0.099), 514 (0.059), 417 (0.728); ^{1}H NMR (400 MHz, $CDCl_3$, δ ppm): 10.17/10.12 (1H, s, 5-H), 9.554/9.547 (1H, s, 10-H), 8.41 (1H, dd, J = 7.9, 1.8 Hz, 20^{3a}-H), 8.26/8.25 (1H, dd, J = 7.9, 1.8 Hz, 20^{3b}-H), 8.21/8.20 (1H, dd, J = 7.9, ~1.8 Hz, 20^{2a}-H), 7.73/7.71 (1H, dd, J = 7.9, 1.7 Hz, 20^{2b} -H), 5.830/5.825 (1H, q, J = 6.7 Hz, 3^1-H), 5.22 (2H, m, 13^2-CH_2), 4.28/4.26 (1H, q, J = 7.1 Hz, 18-H), 4.11 (1H, dd, J = 8.4, 3.3 Hz, 17-H), 3.739/3.736 (2H, q, J = 7.7 Hz, 8-CH_2CH$_3$), 3.703/3.698 (3H, s, 12-CH_3), 3.67[@] (1H, t, J = 6.7 Hz, −OCH_2(CH$_2$)$_4$CH$_3$), 3.591/3.587 (3H, s, −COOCH$_3$), 3.55[@] (0.5H, dt, J = 9.0, 6.5 Hz, −OCHH(CH$_2$)$_4$CH$_3$), 3.45[@] (0.5H, dt, J = 9.0, 6.9 Hz, −OCHH(CH$_2$)$_4$CH$_3$), 3.310/3.306 (3H, s, 7-CH_3), ~2.56 (1H, m, 17-CH$_2$CHH−), ~2.47 (1H, m, 17-CHHCH$_2$−), 2.37/2.36 (3H, s, 2-CH_3), ~2.24 (1H, m, 17-CHHCH$_2$−), ~2.22 (1H, m, 17-CH$_2$CHH−), 2.14/2.00 (3H, d, J = 6.7 Hz, 3^1-CH_3), 1.761 (9H, s, −COOC(CH_3)$_3$), ~1.76/~1.65 (2H, m, −OCH$_2$CH_2(CH$_2$)$_3$CH$_3$), 1.740/1.738 (3H, t, J = 7.7 Hz, 8-CH$_2$CH_3), ~1.22–1.48 (2H, m, −O(CH$_2$)$_2$CH_2(CH$_2$)$_2$CH$_3$), ~1.24/~1.16 (4H, m, −O(CH$_2$)$_3$(CH_2)$_2$CH$_3$), 1.05/1.04 (3H, d, J = 7.0 Hz, 18-CH_3), 0.80/0.73 (3H, distorted t, J ~ 7 Hz, −O(CH$_2$)$_5$CH_3), −1.51/−1.57 (1H, br s, core NH); ^{13}C {^{1}H} NMR (100 MHz, $CDCl_3$, δ ppm): 196.2, 173.4, 171.12/171.09, 165.7, 160.2/160.1, 153.7, 151.6, 148.4, 145.6, 144.6/144.5, 141.8/141.4, 139.25/139.14, 139.14/139.11, 136.83/136.82, 134.5/134.3, 133.9/133.6, 132.7/132.6, 132.2/132.0, 131.7, 131.28/131.26, 129.4, 129.0, 128.9/128.8, 111.0, 106.3, 103.75/103.70, 99.25/99.17, 81.6, 72.9/72.8, 69.7/69.5, 52.24/52.21, 51.6, 48.6, 48.30/48.26, 31.75/31.65, 31.23/31.21, 30.3/30.1, 29.8, 28.3 (3C), 26.1/26.0, 25.0/24.6, 22.6/22.5, 21.0, 19.5, 17.4, 14.1/13.8, 14.0/13.9, 12.1, 11.3. Note: [@] See spectrum & notes in Supporting Information. HRMS (ESI) calculated for $C_{51}H_{63}N_4O_6$ [MH^+] 827.4748, found 827.4793.

Compound 17.: 40 mg of starting photosensitizer **15** were used for reaction by following the method described above, and the title compound was obtained in 71% yield (33 mg). UV-Vis λ_{max} (CH_2Cl_2): 667 (rel. intensity 0.306), 612 (0.053), 547 (0.082), 516 (0.055), 416 (0.736); ^{1}H NMR (400 MHz, $CDCl_3$, δ ppm): 9.50 (1H, s, 10-H), 9.37 (1H, s, 5-H), 8.39 (1H, dd, J = 7.9, 1.8 Hz, phenyl H), 8.25 (1H, dd, J = 7.8, 1.8 Hz, phenyl H), 8.17 (1H, dd, J = 7.9, 1.7 Hz, phenyl H), 7.71 (1H, dd, J = 7.9, 1.7 Hz, phenyl H), 5.20, 5.18 (2H, ABq, J_{AB} = 19.9 Hz, 13^2-CH_2), 4.22 (1H, q, J = 7.1 Hz, 18-H), 4.09 (1H, dd, J ~ 8.3, 3.5 Hz, 17-H), 3.79 (2H, q, J = 7.6 Hz, 3-CH_2CH$_3$), 3.71 (2H, q, J = 7.7 Hz, 8-CH_2CH$_3$), 3.68 (3H, s, 12-CH_3), 3.57 (3H, s, −COOCH$_3$), 3.29 (3H, s, 7-CH_3), ~2.54 (1H, m, 17-CH$_2$CHH−), ~2.45 (1H, m, 17-CHHCH$_2$−), 2.31 (3H, s, 2-CH_3), ~2.14–2.27 (2H, m, 17-CHHCHH−), 1.76 (9H, s, −COOC(CH_3)$_3$), 1.72 (3H, t, J = 7.7 Hz, 8-CH$_2$CH_3), 1.65 (3H, t, J = 7.6 Hz, 3-CH$_2$CH_3), 1.05 (3H, d, J = 7.1 Hz, 18-CH_3), −1.45 (1H, s, core NH); ^{13}C {^{1}H} NMR (100 MHz, $CDCl_3$, δ ppm): 196.2, 173.4, 171.4, 165.8, 159.8, 153.8, 151.1, 148.5, 145.6, 144.7, 144.0, 140.2, 138.8, 136.1, 135.0, 134.3, 132.1, 131.7, 131.6, 131.0, 129.3, 128.8, 128.6, 110.8, 106.2, 103.9, 96.8, 81.5, 52.1, 51.6, 48.6, 48.3, 31.2, 29.9, 28.3 (3C), 21.1, 19.50, 19.46, 17.4, 16.8, 14.1, 12.1, 11.3. HRMS (ESI) calculated for $C_{45}H_{51}N_4O_5$ [MH^+] 727.3859, found 727.3869.

Compound 18.: 25 mg of starting photosensitizer **16** were used for reaction by following the method described above, and the title compound was obtained in quantitative yield (23 mg). UV-Vis λ_{max} (CH_2Cl_2): 669 (rel. intensity 0.276), 613 (0.039), 548 (0.074), 515 (0.042), 416 (0.687); ^{1}H NMR (400 MHz, $CDCl_3$, δ ppm): 10.18/10.14 (1H, s, 5-H), 9.57/9.56 (1H, s, 10-H), 8.59 (1H, dd, J = 7.9, 1.8 Hz, phenyl H), 8.43/8.42 (1H, dd, J = 7.8, 1.9 Hz, phenyl H), 8.33/8.32 (1H, dd, J = 7.8, ~2.0 Hz, phenyl H), 7.83/7.80 (1H, dd, J = 7.9, 1.7 Hz, phenyl H), 5.849/5.846 (1H, q, J = 6.8 Hz, 3^1-H), 5.24 (2H, m, 13^2-CH_2), 4.31/4.29 (1H, q, J = 7.1 Hz, 18-H), 4.15 (1H, dd, J ~ 8.2, 3.1 Hz, 17-H), 3.74 (2H, q, J = 7.6 Hz, 8-CH_2CH$_3$), 3.714/3.710 (3H, s, 12-CH_3), 3.69[@] (1H, t, J = 6.7 Hz, −OCH_2(CH$_2$)$_4$CH$_3$), 3.600/3.597 (3H, s, −COOCH$_3$), 3.57[@] (0.5H, dt, J = 9.1, 6.4 Hz, −OCHH(CH$_2$)$_4$CH$_3$), 3.47[@] (0.5H, dt, J = 9.1, 6.8 Hz, −OCHH(CH$_2$)$_4$CH$_3$), 3.313/3.312 (3H, s, 7-CH_3), ~2.57 (1H, m, 17-CH$_2$CHH−), ~2.49 (1H, m, 17-CHHCH$_2$−), 2.40/2.39 (3H, s, 2-CH_3), ~2.18–2.34 (2H, m, 17-CHHCHH−), 2.15/2.02 (3H, d, J = 6.7 Hz, 3^1-CH_3), ~1.76/1.66 (2H, m, −OCH$_2$CH_2(CH$_2$)$_3$CH$_3$), 1.744/1.742 (3H, t, J = 7.6 Hz, 8-CH$_2$CH_3), ~1.23–1.49 (2H, m, −O(CH$_2$)$_2$CH_2(CH$_2$)$_2$CH$_3$), ~1.24/~1.17 (4H, m, −O(CH$_2$)$_3$(CH_2)$_2$CH$_3$), 1.08/1.07 (3H, d, J = 7.1 Hz, 18-CH_3), 0.80/0.74 (3H, distorted t, J ~ 7 Hz, −O(CH$_2$)$_5$CH_3), −1.49/−1.55 (1H, br s, core NH); ^{13}C {^{1}H} NMR (100 MHz, $CDCl_3$, δ ppm): 196.2, 173.4, 170.9, 170.8/170.7, 160.2/160.1, 153.8, 151.7, 148.5, 147.2, 144.64/144.63, 142.0/141.6, 139.24/139.22, 139.16/139.04, 136.94/136.92, 134.9/134.7, 133.8/133.5, 132.6/132.5, 132.4/132.3, 131.33/131.32, 130.2, 129.63/129.61, 129.2, 129.0, 110.6, 106.3, 103.91/103.87, 99.5/99.4, 73.0/72.9, 69.8/69.6, 52.31/52.28, 51.7, 48.7, 48.35/48.32, 31.8/31.7, 31.29/31.27, 30.3/30.1, 29.9, 26.1/26.0, 25.0/24.6, 22.6/22.5, 21.02/21.01, 19.5, 17.4, 14.1/13.8, 14.0/13.9, 12.2, 11.4. Note: [@] See spectrum & notes in Supporting Information. HRMS (ESI) calculated for $C_{47}H_{55}N_4O_6$ [MH^+] 771.4122, found 771.4109.

Compound 19.: 25 mg of starting photosensitizer **17** were used for reaction by following the method described above, and the title compound was obtained in quantitative yield (23 mg). UV-Vis λ_{max} (CH_2Cl_2): 668 (rel. intensity 0.346), 614 (0.065), 549 (0.093), 514 (0.062), 417 (0.751); ^{1}H NMR (400 MHz, $CDCl_3$, δ ppm): 9.52 (1H, s, meso H), 9.40 (1H, s, meso H), 8.58 (1H, dd, J = 7.9, 1.7 Hz, phenyl H), 8.41 (1H, dd, J = 7.8, 1.7 Hz, phenyl H), 8.28 (1H, dd, J = 7.9, 1.5 Hz, phenyl H), 7.80 (1H, dd, J = 7.9, 1.5 Hz, phenyl H), 5.23, 5.21 (2H, ABq, J_{AB} = 20.0 Hz, 13^2-CH_2), 4.26 (1H, q, J = 7.1 Hz, 18-H), 4.12 (1H, dd, J ~ 8.4, 3.5 Hz, 17-H), 3.82 (2H, q, J = 7.6 Hz, 3-CH_2CH$_3$), 3.72 (2H, q, J = 7.6 Hz, 8-CH_2CH$_3$), 3.69 (3H, s, 12-CH_3), 3.59 (3H, s, −COOCH$_3$), 3.30 (3H, s, 7-CH_3), ~2.55 (1H, m, 17-CH$_2$CHH−), ~2.48 (1H, m, 17-CHHCH$_2$−), 2.33 (3H, s, 2-CH_3), ~2.16–2.32 (2H, m, 17-CHHCHH−), 1.72 (3H, t, J = 7.6 Hz, 8-CH$_2$CH_3), 1.67 (3H, t, J = 7.6 Hz, 3-CH$_2$CH_3), 1.08 (3H, d, J = 7.1 Hz, 18-CH_3), −1.41 (1H, br s, core NH); ^{13}C {^{1}H} NMR (100 MHz, $CDCl_3$, δ ppm): 196.3, 173.4, 171.1, 170.9, 159.8, 153.8, 151.2, 148.5, 147.2, 144.8, 144.2, 140.2, 138.9, 136.2, 135.0, 134.8, 132.5, 131.3, 131.1, 130.1, 129.6, 128.9, 128.8, 110.5, 106.3, 104.0, 97.1, 52.2, 51.7, 48.6, 48.4, 31.3, 29.9, 21.0, 19.5 (2C), 17.4, 16.9, 14.1, 12.1, 11.3. HRMS (ESI) calculated for $C_{41}H_{43}N_4O_5$ [MH^+] 671.3233, found 671.3210.

Compound 20.: 20 mg of starting photosensitizer **18** were used for reaction by following the method described above, and the title compound was obtained in 57% yield (16 mg).

UV-Vis λ_{max} (CH_2Cl_2): 668 (rel. intensity 0.906), 612 (0.143), 545 (0.269), 513 (0.177), 416 (2.182); ^{1}H NMR (400 MHz, $CDCl_3$, δ ppm): 10.17/10.13 (1H, s, PS 5-H), 9.562/9.555 (1H, s, 10-H), 8.253/8.249 (1H, dd, J = 7.9, ~1.6 Hz, 20^{2a}-H), 8.18/8.17 (1H, dd, J = 7.9, 2.1 Hz, 20^{3a}-H), 8.04/8.03 (1H, dd, J = 7.9, 2.0 Hz, 20^{3b}-H), 7.76/7.73 (1H, dd, J = 7.9, 1.7 Hz, 20^{2b}-H), 7.34 (1H, d, J = 9.1 Hz, amide NH), 5.81 (1H, q, J = 6.7 Hz, 3^1-H), 5.58 (1H, dd, J = 9.2, 8.7 Hz, Gal 1-H), 5.57 (1H, d, J = 3.1 Hz, Gal 4-H), 5.37 (1H, dd, J = 10.4, 8.7 Hz, Gal 2-H), 5.32 (1H, dd, J = 10.3, 3.1 Hz, Gal 3-H), ~5.21 (2H, m, 13^2-CH_2), ~4.18–4.32 (4H, m, 18-H, Gal 5-H & Gal 6-CH_2), 4.12 (1H, dd, J = 8.1, 3.3 Hz, 17-H), 3.74 (2H, q, J = 7.6 Hz, 8-C*H*$_2$CH$_3$), 3.703/3.699 (3H, s, 12-CH3), 3.66[@] (~1H, t, J = 6.8 Hz, −OC*H*$_2$(CH$_2$)$_4$CH$_3$), 3.570/3.566 (3H, s, −COOCH$_3$), 3.54[@] (~0.5H, dt, J = 9.0, 6.4 Hz, −OC*H*H(CH$_2$)$_4$CH$_3$), 3.44[@] (~0.5H, dt, J = 9.1, 6.8 Hz, −OCH*H*(CH$_2$)$_4$CH$_3$), 3.299/3.297 (3H, s, 7-CH$_3$), ~2.50 (2H, m, 17-C*H*HC*H*H−), 2.32/2.31 (3H, s, 2-CH$_3$), ~2.25 (1H, m, 17-CH*H*CH$_2$−), 2.220/2.218, 2.20, 2.11, 2.07 (each 3H, 4 × s, 4 × CH$_3$C(=O)O−), ~2.19 (1H, m, 17-CH$_2$CH*H*−), 2.13/1.99 (3H, d, J = 6.7 Hz, 3^1-CH$_3$), ~1.74/1.64 (2H, m, −OCH$_2$C*H*$_2$(CH$_2$)$_3$CH$_3$), 1.734/1.731 (3H, t, J = 7.6 Hz, 8-CH$_2$C*H*$_3$), ~1.21–1.47 (2H, m, −O(CH$_2$)$_2$C*H*$_2$(CH$_2$)$_2$CH$_3$), ~1.24 (1H, br s, core NH), ~1.23/1.15 (4H, m, −O(CH$_2$)$_3$(C*H*$_2$)$_2$CH$_3$), 1.03/1.02 (3H, d, J = 7.0 Hz, 18-CH$_3$), 0.79/0.72 (3H, distorted t, J ~ 7 Hz, −O(CH$_2$)$_5$C*H*$_3$), −1.53/−1.59 (1H, br s, core NH); ^{13}C {^{1}H} NMR (100 MHz, $CDCl_3$, δ ppm): 196.1, 173.4, 172.17/172.16, 170.8, 170.4, 170.0, 169.8, 167.02/167.00, 160.2/160.0, 153.7, 151.7, 148.4, 145.7, 144.6, 142.0/141.6, 139.21/139.07, 139.19, 136.9, 135.0/134.8, 133.8/133.4, 132.7/132.5, 132.61/132.59, 132.4/132.2, 131.4/131.3, 129.2, 127.18/127.15, 126.7, 110.5, 106.3, 103.9/103.8, 99.43/99.40, 79.5, 72.91/72.86, 72.5, 70.8, 69.8/69.6, 68.8, 67.3, 61.2, 52.3/52.2, 51.6, 48.7, 48.32/48.28, 31.7/31.6, 31.20/31.18, 30.3/30.1, 29.8, 26.1/26.0, 25.0/24.6, 22.6/22.5, 21.05/21.04, 20.98, 20.8, 20.65, 20.62, 19.5, 17.4, 14.03/ 13.74, 13.98/13.90, 12.2, 11.3. [@] See spectrum & notes in Supporting Information. HRMS (ESI) calculated for $C_{61}H_{74}N_5O_{14}$ [MH^+] 1100.5232, found 1100.5211.

Compound 21.: 20 mg of starting photosensitizer **19** were used for reaction by following the method described above, and the title compound was obtained in 47% yield (14 mg). UV-Vis λ_{max} (CH_2Cl_2): 669 (rel. intensity 0.643), 614 (0.102), 543 (0.192), 515 (0.126), 415 (1.558); ^{1}H NMR (400 MHz, $CDCl_3$, δ ppm): 9.51 (1H, s, 10-H), 9.38 (1H, s, PS 5-H), 8.23 (1H, dd, J = 7.9, 1.6 Hz, 20^{2a}-H), 8.17 (1H, dd, J = 7.9, 1.8 Hz, 20^{3a}-H), 8.04 (1H, dd, J = 7.9, 1.8 Hz, 20^{3b}-H), 7.75 (1H, dd, J = 7.9, 1.6 Hz, 20^{2b}-H), 7.36 (1H, d, J = 9.1 Hz, amide NH), 5.55–5.62 (2H, m, Gal 1-H & Gal 4-H), 5.38 (1H, dd, J = 10.3, 9.0 Hz, Gal 2-H), 5.33 (1H, dd, J = 10.3, 3.2 Hz, Gal 3-H), 5.20, 5.18 (2H, ABq, J_{AB} = 20.0 Hz, 13^2-CH_2), 4.21–4.27 (3H, m, Gal 5-H & Gal 6-CH_2), 4.19 (1H, q, J = 7.1 Hz, 18-H), 4.10 (1H, dd, J = 8.4, 3.5 Hz, 17-H), 3.79 (2H, q, J = 7.7 Hz, −C*H*$_2$CH$_3$), 3.71 (2H, q, J = 7.7 Hz, −C*H*$_2$CH$_3$), 3.68 (3H, s, 12-CH$_3$), 3.56 (3H, s, −COOCH$_3$), 3.29 (3H, s, 7-CH$_3$), ~2.51 (1H, m, 17-CH$_2$C*H*H−), ~2.46 (1H, m, 17-C*H*HCH$_2$−), 2.26 (3H, s, 2-CH$_3$), ~2.23 (1H, m, 17-CH*H*CH$_2$−), 2.22, 2.21, 2.12, 2.08 (each 3H, 4 × s, 4 × CH$_3$C(=O)O−), ~2.18 (1H, m, 17-CH$_2$CH*H*−), 1.72 (3H, t, J = 7.6 Hz, −CH$_2$C*H*$_3$), 1.65 (3H, t, J = 7.6 Hz, −CH$_2$C*H*$_3$), 1.32 (1H, br s, core NH), 1.04 (3H, d, J = 7.0 Hz, 18-CH$_3$), −1.45 (1H, s, core NH); ^{13}C {^{1}H} NMR (100 MHz, $CDCl_3$, δ ppm): 196.1, 173.4, 172.1, 171.1, 170.4, 170.0, 169.8, 167.1, 159.7, 153.8, 151.2, 148.5, 145.8, 144.8, 144.1, 140.2, 138.8, 136.2, 134.94, 134.88, 132.61, 132.56, 131.2, 131.1, 128.8, 127.1, 126.7, 110.3, 106.3, 104.0, 97.0, 79.5, 72.5,

70.8, 68.8, 67.3, 61.2, 52.1, 51.6, 48.6, 48.4, 31.2, 29.9, 21.03, 21.01, 20.75, 20.64, 20.61, 19.49, 19.45, 17.4, 16.9, 14.0, 12.1, 11.3. HRMS (ESI) calculated for $C_{55}H_{60}N_5O_{13}$ [MH^+] 1000.4344, found 1000.4349.

Compound 22.: 25 mg of starting photosensitizer **17** were used for reaction by following the method described above, and the title compound was obtained in quantitative yield (24 mg). UV-Vis λ_{max} (CH_2Cl_2): 669 (rel. intensity 0.313), 615 (0.047), 546 (0.096), 514 (0.059), 417 (0.724); ^{1}H NMR (400 MHz, $CDCl_3$, δ ppm): 9.47 (1H, s, meso H), 9.34 (1H, s, meso H), 8.38 (1H, dd, *J* = 7.9, 1.7 Hz, phenyl H), 8.23 (1H, dd, *J* = 7.9, 1.8 Hz, phenyl H), 8.15 (1H, dd, *J* = 7.9, 1.6 Hz, phenyl H), 7.70 (1H, dd, *J* = 7.9, 1.6 Hz, phenyl H), 5.19, 5.16 (2H, AB_q, J_{AB} = 19.9 Hz, 13^2-CH_2), 4.22 (1H, q, *J* = 7.1 Hz, 18-H), 4.09 (1H, m, 17-H), 3.77 (2H, q, *J* = 7.6 Hz, 3-C*H*$_2$CH$_3$), 3.69 (2H, q, *J* = 7.7 Hz, 8-C*H*$_2$CH$_3$), 3.64 (3H, s, 12-CH_3), 3.27 (3H, s, 7-CH_3), ~2.56 (1H, m, 17-CH_2C*H*H−), ~2.44 (1H, m, 17-CHHCH_2−), ~2.12–2.32 (2H, m, 17-CH*H*CH*H*−), 2.29 (3H, s, 2-CH_3), 1.73 (9H, s, −COOC(C*H*$_3$)$_3$), 1.70 (3H, t, *J* = 7.7 Hz, 8-CH_2C*H*$_3$), 1.64 (3H, t, *J* = 7.6 Hz, 3-CH_2C*H*$_3$), 1.04 (3H, d, *J* = 7.1 Hz, 18-CH_3), −1.44 (1H, s, core NH); ^{13}C {^{1}H} NMR (100 MHz, $CDCl_3$, δ ppm): 196.4, 176.9, 171.4, 165.8, 159.6, 153.9, 151.2, 148.5, 145.6, 144.8, 144.1, 140.3, 138.8, 136.1, 135.1, 134.3, 132.1, 131.7, 131.6, 131.0, 129.4, 128.8, 128.6, 110.8, 106.3, 103.9, 96.8, 81.5, 52.0, 48.5, 48.3, 30.8, 29.5, 28.3 (3C), 21.1, 19.5, 19.4, 17.4, 16.8, 14.1, 12.1, 11.3. HRMS (ESI) calculated for $C_{44}H_{49}N_4O_5$ [MH^+] 713.3703, found 713.3689.

Compound 23.: 24 mg of starting photosensitizer **22** were used for reaction by following the method described above, and the title compound was obtained in 43% yield (15 mg). UV-Vis λ_{max} (CH_2Cl_2): 670 (rel. intensity 0.389), 615 (0.058), 543 (0.119), 514 (0.073), 416 (0.897); ^{1}H NMR (400 MHz, $CDCl_3$, δ ppm): 9.51 (1H, s, 10-H), 9.39 (1H, s, PS 5-H), 8.42 (1H, dd, *J* = 7.9, 1.8 Hz, phenyl H), 8.24 (1H, dd, *J* = 7.9, 1.8 Hz, phenyl H), 8.19 (1H, dd, *J* = 7.9, 1.6 Hz, phenyl-H), 7.70 (1H, dd, *J* = 7.9, 1.6 Hz, 20^{2b}-H), 6.03 (1H, d, *J* = 9.3 Hz, amide NH), 5.38 (1H, d, *J* = 3.3 Hz, Gal 4-H), 5.22, 5.19 (2H, ABq, J_{AB}= 19.8 Hz, 13^2-CH_2), 5.16 (1H, t, *J* = 9.3 Hz, Gal 1-H), 5.07 (1H, dd, *J* = 10.3, 3.4 Hz, Gal 3-H), 4.94 (1H, dd, *J* = 10.3, 9.3 Hz, Gal 2-H), 4.22 (1H, q, *J* = 7.0 Hz, 18-H), 4.13 (1H, dd, *J* = 8.0, 3.6 Hz, 17-H), 3.94–4.07 (3H, m, Gal 5-H & Gal 6-CH_2), 3.80 (2H, q, *J* = 7.6 Hz, −C*H*$_2$CH$_3$), 3.72 (2H, q, *J* = 7.7 Hz, −C*H*$_2$CH$_3$), 3.67 (3H, s, 12-CH_3), 3.29 (3H, s, 7-CH_3), ~2.47, ~2.38, ~2.20, ~1.90 (each 1H, 4 × m, 17-CH_2CH_2−), 2.31 (3H, s, 2-CH_3), 2.05, 1.97, 1.95, 1.91 (each 3H, 4 × s, 4 × CH_3C(=O)O−), 1.75 (9H, s, −C(=O)OC(C*H*$_3$)$_3$), 1.72 (3H, t, *J* = 7.6 Hz, −CH_2C*H*$_3$), 1.65 (3H, t, *J* = 7.6 Hz, −CH_2C*H*$_3$), 1.32 (1H, br s, core NH), 1.05 (3H, d, *J* = 7.1 Hz, 18-CH_3), −1.46 (1H, s, core NH); ^{13}C {^{1}H} NMR (100 MHz, $CDCl_3$, δ ppm): 196.1, 172.3, 171.4, 171.3, 170.3, 170.0, 169.7, 165.8, 159.5, 153.8, 151.1, 148.5, 145.6, 144.8, 144.1, 140.3, 138.8, 136.1, 135.1, 134.4, 132.1, 131.7, 131.6, 131.1, 129.4, 128.8, 128.7, 110.8, 106.3, 103.9, 96.9, 81.5, 78.5, 72.2, 70.8, 68.4, 67.1, 61.0, 52.0, 48.6, 48.3, 33.2, 30.0, 28.4 (3C), 21.1, 20.7, 20.6, 20.52, 20.49, 19.51, 19.47, 17.4, 16.8, 14.1, 12.1, 11.3. HRMS (ESI) calculated for $C_{58}H_{68}N_5O_{13}$ [MH^+] 1042.4814, found 1042.4578.

Compound 24.: 15 mg of starting photosensitizer **23** were used for reaction by following the method described above, and the title compound was obtained in quantitative yield

(14 mg). UV-Vis λ_{max} (CH_2Cl_2): 669 (rel. intensity 0.413), 613 (0.077), 547 (0.111), 514 (0.081), 416 (0.902); ^{1}H NMR (400 MHz, $CDCl_3$, δ ppm): 9.56 (1H, s, 10-H), 9.43 (1H, s, PS 5-H), 8.60 (1H, dd, J = 7.9, 1.7 Hz, 20^{3a}-H), 8.38 (1H, dd, J = 7.8, 1.7 Hz, 20^{3b}-H), 8.33 (1H, dd, J = 7.9, ~1.5 Hz, 20^{2a}-H), 7.80 (1H, dd, J = 7.8, ~1.5 Hz, 20^{2b}-H), 6.14 (1H, d, J = 9.2 Hz, amide NH), 5.39 (1H, d, J = 3.3 Hz, Gal 4-H), 5.24, 5.206 (2H, ABq, J_{AB} = 19.8 Hz, 13^2-CH_2), 5.210 (1H, t, J = 9.4 Hz, Gal 1-H), 5.14 (1H, dd, J = 10.3, 3.4 Hz, Gal 3-H), 4.96 (1H, dd, J = 10.2, 9.3 Hz, Gal 2-H), 4.24 (1H, q, J ~ 7.1 Hz, 18-H), 4.14 (1H, dd, J ~ 8.3, 3.1 Hz, 17-H), 3.98–4.09 (3H, m, Gal 5-H & Gal 6-CH_2), 3.82 (2H, q, J = 7.5 Hz, 3-CH_2CH$_3$), 3.74 (2H, q, J = 7.7 Hz, 8-CH_2CH$_3$), 3.70 (3H, s, 12-CH_3), 3.31 (3H, s, 7-CH_3), ~2.50, (1H, m, 17-CHHCH$_2$−), ~2.47 (1H, m, 17-CH$_2$CHH−), 2.33 (3H, s, 2-CH_3), ~2.28 (1H, m, 17-CHHCH$_2$−), 2.07, 1.99, 1.97, 1.92 (each 3H, 4 × s, 4 × CH_3C(=O)O−), ~1.94 (1H, m, 17-CH$_2$CHH), 1.73 (3H, t, J = 7.6 Hz, 8-CH$_2$CH_3), 1.67 (3H, t, J = 7.5 Hz, 3-CH$_2$CH_3), ~1.3 (1H, br s, core NH), 1.06 (3H, d, J = 6.9 Hz, 18-CH_3), −1.43 (1H, br s, core NH); ^{13}C {^{1}H} NMR (100 MHz, $CDCl_3$, δ ppm): 196.0, 172.5, 171.3, 171.2, 170.3*, 170.0, 169.9, 169.3, 159.8*, 148.7*, 146.9, 144.7, 144.3, 140.3*, 138.9, 136.1, 135.2, 134.7, 132.8, 131.5, 131.2, 130.3, 129.4, 129.0, ~110.5*, 106.5*, 104.0, 97.1, 78.5, 72.2, 70.8, 68.4, 67.1, 61.0, 52.5, 48.6, 48.1, 33.5, 29.7, 21.1, 20.7, 20.6, 20.5 (2C), 19.5 (2C), 17.4, 16.9, 14.1, 12.2, 11.3. See spectrum & notes in Supporting Information regarding three missing ^{13}C peaks. * From HMBC. MS (ESI) calculated for $C_{54}H_{60}N_5O_{13}$[MH^+] 986.4, found 986.4; HRMS (ESI) calculated for $C_{54}H_{59}N_5O_{13}Na$[MNa^+] 1008.4007, found 1008.4020.

Compound 25.: 20 mg of starting photosensitizer **19** were used for reaction by following the method described above, and the title compound was obtained in quantitative yield (19 mg). UV-Vis λ_{max} (CH_2Cl_2): 668 (rel. intensity 0.375), 615 (0.056), 547 (0.115), 514 (0.071), 417 (0.869); ^{1}H NMR (400 MHz, CD_3OD, δ ppm): 9.21 (1H, s, 5-H), 9.10 (1H, s, 10-H), 8.41 (1H, dd, J = 7.9, 1.8 Hz, phenyl H), 8.22 (1H, dd, J = 7.9, 1.8 Hz, phenyl H), 8.09 (1H, dd, J = 7.9, 1.7 Hz, phenyl H), 7.59 (1H, dd, J = 7.9, 1.7 Hz, phenyl H), 5.00, 4.88 (2H, ABq, J_{AB}= 20.0 Hz, 13^2-CH_2), 4.08 (1H, q, J = 7.2 Hz, 18-H), 3.93 (1H, dd, J = 9.7, 2.9 Hz, 17-H), 3.72 (2H, q, J = 7.6 Hz, −CH_2CH$_3$), ~3.42 (2H, m, −CH_2CH$_3$), 3.33 (3H, s, ring CH_3), 3.10 (3H, s, ring CH_3), ~2.35, ~2.01, ~1.99, ~1.65 (each 1H, 4 × m, 17-CH_2CH_2−), 2.24 (3H, s, 2-CH_3), 1.63 (3H, t, J = 7.6 Hz, −CH$_2$CH_3), 1.55 (3H, t, J = 7.6 Hz, −CH$_2$CH_3), 0.91 (3H, d, J = 7.1 Hz, 18-CH_3); ^{13}C {^{1}H} NMR (100 MHz, CD3OD, δ ppm): 198.4, 176.8, 173.5, 169.9, 161.7, 155.6, 152.5, 149.2, 147.0, 146.1, 145.5, 141.4, 139.4, 137.3, 136.3, 135.7, 133.7, 132.9, 132.0, 131.1, 130.7, 130.1, 129.3, 112.3, 107.1, 104.7, 97.5, 53.2, 49.6*, 49.1*, 32.1, 30.6, 21.2, 20.1, 19.9, 17.6, 17.1, 14.2, 11.6, 11.0. * From HSQC. HRMS (ESI) calculated for $C_{40}H_{41}N_4O_5$ [MH^+] 657.3077, found 657.3070.

Compound 26.: 20 mg of starting photosensitizer **25** were used for reaction by following the method described above, and the title compound was obtained in 30% yield (12 mg). UV-Vis λ_{max} (CH_2Cl_2): 670 (rel. intensity 0.374), 615 (0.059), 545 (0.111), 514 (0.073), 417 (0.901); ^{1}H NMR (400 MHz, $CDCl_3$, δ ppm): 9.52 (1H, s, 10-H), 9.40 (1H, s, PS 5-H), 8.24 (1H, dd, J = 7.8, 1.6 Hz, 20^{2a}-H), 8.19 (1H, dd, J = 7.9, 1.8 Hz, 20^{3a}-H), 8.03 (1H, dd, J = 7.9, 1.8 Hz, 20^{3b}-H), 7.73 (1H, dd, J = 7.9, 1.6 Hz, 20^{2b}-H), 7.43 (1H, d, J = 9.0 Hz, 20-amide NH), 5.95 (1H, d, J = 9.2 Hz, 17-amide NH), 5.59 (1H, t, J = 9.0 Hz, 20-Gal 1-H), 5.57 (1H, d, J ~ 3.2 Hz, 20-Gal 4-H), 5.38 (1H, dd, J = 10.3, 8.8 Hz, 20-Gal 2-H),

5.37 (1H, d, J = 3.4 Hz, 17-Gal 4-H), 5.32 (1H, dd, J = 10.3, 3.2 Hz, 20-Gal 3-H), 5.21, 5.19 (2H, ABq, J_{AB} = 20.0 Hz, 13^2-CH_2), 5.17 (1H, t, J = 9.3 Hz, 17-Gal 1-H), 5.07 (1H, dd, J = 10.3, 3.4 Hz, 17-Gal 3-H), 4.90 (1H, dd, J = 10.3, 9.3 Hz, 17-Gal 2-H), 4.18–4.27 (3H, m, 20-Gal 5-H & 20-Gal 6-CH_2), 4.21 (1H, q, J ~ 7.1 Hz, 18-H), 4.15 (1H, dd, J = 7.3, 3.9 Hz, 17-H), 3.96–4.04 (3H, m, 17-Gal 5-H & 17-Gal 6-CH_2), 3.80 (2H, q, J = 7.6 Hz, 3-CH_2CH$_3$), 3.72 (2H, q, J = 7.6 Hz, 8-CH_2CH$_3$), 3.68 (3H, s, 12-CH_3), 3.30 (3H, s, 7-CH_3), 2.50 (1H, m, 17-CHHCH$_2$−), ~2.31 (1H, m, 17-CH$_2$CHH−), 2.27 (3H, s, 2-CH_3), 2.23, 2.20, 2.11, 2.07, 2.02, 1.98, 1.94, 1.91 (each 3H, 8 × s, 8 × CH_3C(=O)O−), ~2.21 (1H, m, 17-CHHCH$_2$−), 1.84 (1H, m, 17-CH$_2$CHH), 1.72 (3H, t, J = 7.6 Hz, 8-CH$_2$CH_3), 1.65 (3H, t, J = 7.6 Hz, 3-CH$_2$CH_3), ~1.3 (1H, br s, core NH), 1.03 (3H, d, J = 7.0 Hz, 18-CH_3), −1.47 (1H, s, core NH); ^{13}C {^{1}H} NMR (100 MHz, $CDCl_3$, δ ppm): 196.1, 172.3, 172.1, 171.19, 171.16, 170.4, 170.3, 170.0, 169.9, 169.8, 169.7, 167.2, 159.4, 153.8, 151.2, 148.4, 145.6, 144.8, 144.2, 140.2, 138.9, 136.2, 135.0, 134.9, 132.7, 132.6, 131.3, 131.1, 128.8, 127.3, 126.7, 110.3, 106.3, 104.0, 97.1, 79.5, 78.4, 72.5, 72.1, 70.8, 70.7, 68.7, 68.4, 67.3, 67.0, 61.2, 60.9, 52.1, 48.6, 48.2, 33.1, 29.9, 21.1 (2C), 20.8, 20.67, 20.66, 20.64, 20.62, 20.50, 20.49, 19.51, 19.47, 17.4, 16.9, 14.1, 12.1, 11.3. HRMS (ESI) calculated for $C_{68}H_{78}N_6O_{21}Na$ [MNa^+] 1337.5118, found 1337.5147.

Compound 28.: 40 mg of starting photosensitizer **27** were used for reaction by following the method described above, and the title compound was obtained in 69% yield (26 mg). UV-Vis λ_{max} (CH_2Cl_2): 673 (rel. intensity 0.498), 615 (0.063), 551 (0.144), 518 (0.084), 415 (1.048); ^{1}H NMR (400 MHz, $CDCl_3$, δ ppm): 10.233/10.225 (1H, s, 5-H), 9.579 (1H, s, 10-H), 6.00 (1H, q, J = 6.6 Hz, 3^1-H), ~5.98 (1H, m, amide NH), 5.38 (1H, d, J = 3.3 Hz, Gal 4-H), ~5.26 (2H, m, 13^2-CH_2), 5.18/5.17 (1H, t, J = 9.3 Hz, Gal 1-H), 5.060/5.058 (1H, dd, J = 10.3, 3.3 Hz, Gal 3-H), 4.930/4.925 (1H, dd, J = 10.2, 9.4 Hz, Gal 2-H), 4.88/4.87 (1H, q, J = 7.0 Hz, 18-H), 4.31 (1H, m, 17-H), ~4.04 (2H, m, Gal 6-CH_2), 3.98 (1H, m, Gal 5-H), 3.73 (2H, q, J = 7.6 Hz, 8-CH_2CH$_3$), 3.52–3.72 (2H, m, −OCH_2(CH$_2$)$_4$CH$_3$), 3.68 (3H, s, ring CH_3), 3.62 (3H, s, ring CH_3), 3.31 (3H, s, 7-CH_3), 2.55 (1H, m, 17-CHHCH$_2$−), 2.41 (1H, m, 17-CH$_2$CHH−), 2.23 (1H, m, 17-CHHCH$_2$−), 2.16/2.11 (3H, d, J = 6.7 Hz, 3^1-CH_3), 2.052/2.049, 1.99, 1.94, 1.90 (each 3H, 4 × s, 4 × CH_3C(=O)O−), ~1.82 (1H, m, 17-CH$_2$CHH−), ~1.74 (2H, m, −OCH$_2$CH_2(CH$_2$)$_3$CH$_3$), 1.72 (3H, t, J = 7.6 Hz, 8-CH$_2$CH_3), 1.59/1.58 (3H, d, J = 6.9 Hz, 18-CH_3), ~1.28–1.46 (2H, m, −O(CH$_2$)$_2$CH_2(CH$_2$)$_2$CH$_3$), 1.20 (4H, m, −O(CH$_2$)$_3$(CH_2)$_2$CH$_3$), 0.77/0.75 (3H, distorted t, J ~ 7 Hz, −O(CH$_2$)$_5$CH_3), −1.81/−1.85 (1H, s, core NH); ^{13}C {^{1}H} NMR (100 MHz, $CDCl_3$, δ ppm): 195.9, 172.3, 171.6/171.5, 171.2, 170.3, 169.9, 169.7, 160.6/160.5, 153.5, 152.2, 148.0, 144.6, 142.2/142.0, 139.74/139.72, 138.2/138.1, 137.3, 133.3/133.2, 132.9/132.8, 131.7, 129.7, 106.7, 103.83/103.80, 99.67/99.65, 94.5/94.4, 78.4, 73.2/73.1, 72.2, 70.8, 69.8/69.7, 68.3, 67.1, 61.1, 51.8, 51.6, 48.6, 32.7, 31.73/31.70, 30.24/30.19, 29.9, 26.1/26.0, 25.0/24.9, 22.6/22.5, 20.8, 20.7, 20.6, 20.51, 20.48, 19.5, 17.4, 17.04/16.97, 13.95/13.93, 12.2, 11.3. HRMS (ESI) calculated for $C_{53}H_{67}N_5O_{12}Br$ [MH^+] 1044.3970, found 1044.3998.

Compound 29.: 30 mg of starting photosensitizer **28** were used for reaction by following the method described above, and the title compound was obtained in 59% yield (19 mg). UV-Vis λ_{max} (CH_2Cl_2): 670 (rel. intensity 0.181), 615 (0.027), 547 (0.054), 513 (0.033),

415 (0.427); ^{1}H NMR (400 MHz, $CDCl_3$, δ ppm): 10.17/10.12 (1H, s, PS 5-H), 9.55 (1H, s, 10-H), 8.43 (1H, dd, J = 7.9, 1.8 Hz, 20^{3a}-H), 8.25/8.24 (1H, dd, J = 7.8, 1.8 Hz, 20^{3b}-H), 8.22/8.21 (1H, dd, J = 8.0, 1.6 Hz, 20^{2a}-H), 7.71/7.68 (1H, dd, J = 7.9, 1.7 Hz, 20^{2b}-H), 6.03/6.01 (1H, d, J = 9.2 Hz, amide NH), 5.82/5.81 (1H, q, J = 6.7 Hz, 3^{1}-H), 5.38 (1H, d, J = 3.3 Hz, Gal 4-H), ~5.22 (2H, m, 13^{2}-CH_2), 5.17/5.16 (1H, t, J = 9.3 Hz, Gal 1-H), 5.072/5.070 (1H, dd, J = 10.3, 3.3 Hz, Gal 3-H), 4.942/4.935 (1H, dd, J = 10.3, 9.3 Hz, Gal 2-H), 4.26/4.24 (1H, q, J = 7.2 Hz, 18-H), 4.15 (1H, m, 17-H), 3.94–4.07 (3H, m, Gal 5-H & Gal 6-CH_2), 3.74 (2H, q, J = 7.6 Hz, 8-C*H*$_2$CH$_3$), 3.69 (3H, s, 12-CH_3), 3.67[@] (~1H, t, J = 6.6 Hz, −OC*H*$_2$(CH$_2$)$_4$CH$_3$), 3.54[@] (~0.5H, dt, J = 9.1, 6.4 Hz, −OC*H*H(CH$_2$)$_4$CH$_3$), 3.44[@] (~0.5H, dt, J = 9.0, 6.8 Hz, −OC*H*H(CH$_2$)$_4$CH$_3$), 3.30 (3H, s, 7-CH_3), ~2.49 (1H, m, 17-C*H*HCH$_2$−), ~2.39 (1H, m, 17-CH$_2$C*H*H−), 2.36/2.35 (3H, s, 2-CH_3), 2.21 (1H, m, 17-CH*H*CH$_2$−), 2.13/1.99 (3H, d, J = 6.6 Hz, 3^{1}-CH_3), 2.053/2.049, 1.97, 1.95, 1.93 (each 3H, 4 × s, 4 × CH_3C(=O)O−), ~1.91 (1H, m, 17-CH$_2$CH*H*−), ~1.76/1.64 (2H, m, −OCH$_2$C*H*$_2$(CH$_2$)$_3$CH$_3$), 1.75 (9H, s, −C(=O)OC(C*H*$_3$)$_3$), 1.73 (3H, t, J = 7.6 Hz, 8-CH$_2$C*H*$_3$), ~1.2–1.5 (2H, m, −O(CH$_2$)$_2$C*H*$_2$(CH$_2$)$_2$CH$_3$), ~1.23/1.17 (2H, m, −O(CH$_2$)$_4$C*H*$_2$CH$_3$), ~1.23/1.16 (2H, m, −O(CH$_2$)$_3$C*H*$_2$CH$_2$CH$_3$), ~1.22 (1H, br s, core NH), 1.031/1.025 (3H, d, J = 7.0 Hz, 18-CH_3), 0.79/0.73 (3H, distorted t, J ~ 7 Hz, −O(CH$_2$)$_5$C*H*$_3$), −1.54/−1.60 (1H, s, core NH); ^{13}C NMR (100 MHz, $CDCl_3$, δ ppm): 196.1, 172.3, 171.29/171.28, 171.0, 170.3, 169.9, 169.7, 165.7, 159.9/159.7, 153.8, 151.6, 148.4, 145.6, 144.60/144.58, 141.9/141.5, 139.3, 139.2/139.1, 136.88/136.86, 134.5/134.3, 133.8/133.5, 132.7/132.6, 132.2/132.0, 131.7, 131.31/131.30, 129.5, 129.0, 128.9/128.8, 110.9, 106.3, 103.8/103.7, 99.3/99.2, 81.6, 78.5, 72.9/72.8, 72.2, 70.7, 69.8/69.5, 68.4, 67.1, 61.0, 52.2/52.1, 48.6, 48.21/48.17, 33.27/33.25, 31.8/31.7, 30.3/30.1, 30.0, 28.3 (3C), 26.1/26.0, 25.0/24.6, 22.6/22.5, 21.1/21.0, 20.7, 20.6, 20.51, 20.49, 19.5, 17.4, 14.1/13.8, 14.0/13.9, 12.1, 11.3. [@] See spectrum & notes in Supporting Information. HRMS (ESI) calculated for $C_{64}H_{80}N_5O_{14}$ [MH^+] 1142.5702, found 1142.5747.

Compound 30.: 15 mg of starting photosensitizer **29** were used for reaction by following the method described above, and the title compound was obtained in quantitative yield (14 mg). UV-Vis λ_{max} (CH_2Cl_2): 671 (rel. intensity 0.577), 615 (0.085), 547 (0.170), 514 (0.108), 415 (1.355); ^{1}H NMR (400 MHz, $CDCl_3$, δ ppm): 10.19/10.15 (1H, s, PS 5-H), 9.56 (1H, s, 10-H), 8.64 (1H, dd, J = 7.9, 1.7 Hz, 20^{3a}-H), 8.41/8.40 (1H, dd, J = 7.8, 1.8 Hz, 20^{3b}-H), 8.373/8.368 (1H, dd, J = 7.9, 1.8 Hz, 20^{2a}-H), 7.81/7.78 (1H, dd, J = 7.9, 1.7 Hz, 20^{2b}-H), 6.16/6.14 (1H, d, J = 9.2 Hz, amide NH), 5.85/5.84 (1H, q, J = 6.7 Hz, 3^{1}-H), 5.40 (1H, d, J = 3.2 Hz, Gal 4-H), ~5.24 (2H, m, 13^{2}-CH_2), 5.224/5.218 (1H, t, J = 9.3 Hz, Gal 1-H), 5.140/5.136 (1H, dd, J = 10.3, 3.3 Hz, Gal 3-H), 4.97/4.96 (1H, dd, J = 10.3, 9.3 Hz, Gal 2-H), 4.29/4.27 (1H, q, J = 7.1 Hz, 18-H), 4.17 (1H, m, 17-H), ~3.97–4.09 (3H, m, Gal 5-H & Gal 6-CH_2), 3.74 (2H, q, J = 7.6 Hz, 8-C*H*$_2$CH$_3$), 3.693 (3H, s, 12-CH_3), 3.688[@] (~1H, t, J = 6.8 Hz, −OC*H*$_2$(CH$_2$)$_4$CH$_3$), 3.57[@] (~0.5H, dt, J = 9.1, 6.4 Hz, −OC*H*H(CH$_2$)$_4$CH$_3$), 3.47[@] (~0.5H, dt, J = 9.0, 6.8 Hz, −OCH*H*(CH$_2$)$_4$CH$_3$), 3.31 (3H, s, 7-CH_3), ~2.50 (2H, m, 17-C*H*HCHH−), 2.40/2.39 (3H, s, 2-CH_3), ~2.29 (1H, m, 17-CH*H*CH$_2$−), 2.15/2.02 (3H, d, J = 6.7 Hz, 3^{1}-CH_3), 2.061/2.057, 1.99, 1.974/1.973, 1.952/1.950 (each 3H, 4 × s, 4 × CH_3C(=O)O−), ~1.93 (1H, m, 17-CH$_2$CH*H*), ~1.77/1.66 (2H, m, −OCH$_2$C*H*$_2$(CH$_2$)$_3$CH$_3$), 1.74 (3H, t, J = 7.6 Hz, 8-CH$_2$C*H*$_3$), ~1.22–1.49 (2H, m, −O(CH$_2$)$_2$C*H*$_2$(CH$_2$)$_2$CH$_3$), ~1.25/1.17 (4H, m, −O(CH$_2$)$_3$(C*H*$_2$)$_2$CH$_3$), 1.059/1.055

(3H, d, J = 7.1 Hz, 18-CH_3), 0.80/0.74 (3H, distorted t, J ~ 7 Hz, −O$(CH_2)_5$CH_3), −1.51/−1.57 (1H, br s, core NH); ^{13}C {^{1}H} NMR (100 MHz, $CDCl_3$, δ ppm): 196.1, 172.6, 171.37/171.36, 170.8, 170.3, 169.99, 169.96, 169.8, 159.9/159.8, 153.7, 151.6, 148.5, 146.9, 144.7/144.6, 142.0/141.6, 139.22/139.20, 139.19/139.06, 136.94/136.92, 134.8/134.6, 133.8/133.5, 132.8/132.6, 132.5/132.3, 131.3, 130.3, 129.51/129.49, 129.2, 129.1, 110.6, 106.4, 103.91/103.87, 99.5/99.4, 78.5, 72.95/72.88, 72.3, 70.8, 69.8/69.6, 68.4, 67.1, 61.0, 52.51/52.49, 48.6, 48.1, 33.5, 31.8/31.7, 30.3/30.1, 29.8, 26.1/26.0, 25.0/24.6, 22.6/22.5, 21.08/21.07, 20.7, 20.6, 20.5 (2C), 19.5, 17.4, 14.1/13.8, 14.0/13.9, 12.2, 11.4. @ See spectrum & notes in Supporting Information. HRMS (ESI) calculated for $C_{60}H_{72}N_5O_{14}$[MH^+] 1086.5076, found 1086.5063;

Compound 31.: 20 mg of starting photosensitizer **30** were used for reaction by following the method described above, and the title compound was obtained in 50% yield (13 mg). UV-Vis λ_{max} (CH_2Cl_2): 671 (rel. intensity 0.241), 614 (0.036), 546 (0.074), 513 (0.046), 415 (0.557); ^{1}H NMR (400 MHz, $CDCl_3$, δ ppm): 10.18/10.13 (1H, s, PS 5-H), 9.561/9.559 (1H, s, 10-H), 8.27/8.26 (1H, dd, J = 7.9, ~1.6 Hz, 20^{2a}-H), 8.21/8.20 (1H, dd, J = 7.9, ~2.0 Hz, 20^{3a}-H), 8.03/8.02 (1H, dd, J = 7.9, 2.0 Hz, 20^{3b}-H), 7.73/7.70 (1H, dd, J = 7.9, 1.7 Hz, 20^{2b}-H), 7.43/7.42 (1H, d, J = 9.1 Hz, 20-amide NH), 5.97/5.96 (1H, d, J = 9.3 Hz, 17-amide NH), 5.813/5.807 (1H, q, J = 6.7 Hz, 3^1-H), 5.58 (1H, dd, J = 9.2, 8.7 Hz, 20-Gal 1-H), 5.57 (1H, d, J ~ 3.2 Hz, 20-Gal 4-H), 5.373 (1H, dd, J = 10.3, 8.7 Hz, 20-Gal 2-H), 5.366 (1H, d, J ~ 3.3 Hz, 17-Gal 4-H), 5.32 (1H, dd, J = 10.3, 3.2 Hz, 20-Gal 3-H), ~5.22 (2H, m, 13^2-CH_2), 5.17/5.16 (1H, t, J = 9.3 Hz, 17-Gal 1-H), 5.070/5.066 (1H, dd, J = 10.4, 3.4 Hz, 17-Gal 3-H), 4.90/4.89 (1H, dd, J = 10.4, 9.3 Hz, 17-Gal 2-H), ~4.18–4.28 (4H, m, 18-H, 20-Gal 5-H & 20-Gal 6-CH_2), 4.16 (1H, m, 17-H), ~3.94–4.04 (3H, m, 17-Gal 5-H & 17-Gal 6-CH_2), 3.74 (2H, q, J = 7.6 Hz, 8-C$H_2$$CH_3$), 3.70 (3H, s, 12-$CH_3$), 3.67@ (~1H, t, J = 6.7 Hz, −OC$H_2$$(CH_2)_4CH_3$), 3.55@ (~0.5H, dt, J = 9.0, 6.4 Hz, −OCHH$(CH_2)_4CH_3$), 3.44@ (~0.5H, dt, J = 9.0, 6.8 Hz, −OCH$H$$(CH_2)_4CH_3$), 3.30 (3H, s, 7-$CH_3$), 2.51 (1H, m, 17-C$HHCH_2$−), ~2.32 (1H, m, 17-$CH_2CH$H−), 2.32/2.31 (3H, s, 2-CH_3), 2.230/2.227, 2.20, 2.11, 2.07, 2.023/2.020, 1.976/1.975, 1.939, 1.924/1.922 (each 3H, 8 × s, 8 × CH_3C(=O)O−), ~2.22 (1H, m, 17-CH$H$$CH_2$−), 2.13/1.99 (3H, d, J = 6.7 Hz, 3^1-CH_3), 1.85 (1H, m, 17-CH_2CHH−), ~1.75/1.64 (2H, m, −OCH_2C$H_2$$(CH_2)_3CH_3$), 1.73 (3H, t, J = 7.6 Hz, 8-CH_2CH_3), ~1.21–1.46 (2H, m, −O$(CH_2)_2$C$H_2$$(CH_2)_2CH_3$), ~1.26 (1H, br s, core NH), ~1.24/1.16 (4H, m, −O$(CH_2)_3$(C$H_2$$)_2CH_3$), 1.02/1.01 (3H, d, J = 7.0 Hz, 18-CH_3), 0.79/0.72 (3H, distorted t, J ~ 7 Hz, −O$(CH_2)_5$CH_3), −1.55/−1.61 (1H, br s, core NH); ^{13}C {^{1}H} NMR (100 MHz, $CDCl_3$, δ ppm): 196.1, 172.3, 172.1, 171.2, 170.81/170.79, 170.4, 170.34/170.33, 170.0, 169.9, 169.8, 169.7, 167.1, 159.7, 153.8, 151.7, 148.4, 145.6, 144.7/144.6, 142.0/141.6, 139.23/139.07, 139.20, 137.0/136.9, 135.0/134.8, 133.7/133.4, 132.72/132.71, 132.67/132.54, 132.4/132.3, 131.4, 129.17/129.16, 127.4/127.3, 126.7, 110.4, 106.3, 103.91/103.86, 99.5/99.4, 79.5, 78.4, 72.93/72.86, 72.5, 72.1, 70.8, 70.7, 69.8/69.6, 68.8, 68.4, 67.3, 67.0, 61.2, 60.9, 52.2, 48.7, 48.2, 33.1, 31.8/31.7, 30.3/30.1, 29.9, 26.1/26.0, 25.0/24.7, 22.6/22.5, 21.1 (2C), 20.75, 20.69, 20.66, 20.64, 20.62, 20.5 (2C), 19.5, 17.4, 14.04/13.74, 13.98/13.91, 12.1, 11.3. Note: @ See spectrum & notes in Supporting Information. HRMS (ESI) calculated for $C_{74}H_{91}N_6O_{22}$ [MH^+] 1415.6186, found 1415.6445.

3.1.8. Method to determine in vitro PDT efficacy (MTT assay)—Cells were plated in 96 well plate between 5×10^4 and 10×10^4 cells per well. The cells were allowed to adhere to the plates then photosensitizer was added. The maximum dose was 1.6 μM and the minimum was 6.25 nM. The cells incubated with the photosensitizer for 24 h then exposed to light at the appropriate wavelength. After 48 h, the cell viability was read using an MTT assay and the results were analyzed and plotted using Graphpad Prism software.

3.1.9. Method of tumor-implantation—Colon 26 and FaDu tumors were transplanted in 6–8-week-old Female Balb/c (NCI Balb/cAnNCr) and SCID mice (Strain C.B Igh-1b Icr Tac Prkdc Scid) respectively and were maintained in the animal facility. Mice bearing an established tumor (~ 7 days after implantation) were treated with the PDT. Tumor size was measured on two axes (in millimeters; L, longest axis, and W, shortest axis) with the aid of Vernier calipers. Tumor size (mm^3) was calculated by using the formula: tumor weight = 1/2 ($L \times W^2$). All studies were performed in accordance with protocols approved by the institutional animal care and use committee at Roswell Park Cancer Institute.

3.1.10. Determination of in vivo tumor uptake and PDT efficacy—SCID mice with FaDu tumors and Colon 26 tumors of 200–250 mm^3 were injected intravenously (i.v) with photosensitizer PS 2 and PS 11 at dose of 0.47 μmol/kg in Tween formulations. The PS uptake in FaDu tumors was determined by fluorescence imaging using a PerkinElmer IVIS Spectrum at variable timepoints, and the maximum uptake was observed post-injection. At this timepoint the tumors were irradiated with light (fluence:135 J/cm^2; fluence rate: 75 mW/cm^2) for 30 min at 665 nm using a Lightwave™ laser diode. Mice were restrained without anesthesia in plexiglass holders designed to expose only the tumor and a 2–4 mm annular margin of skin to light. The tumor assessment and measurements were taken daily, then three times a week for 4 weeks, and twice a week thereafter for a total of 30 days post treatment. Tumor volume (mm^2) was estimated using a formula: tumor volume = ½ ($L \times W^2$). Two axes (mm) of tumor (L, longest axis; W, shortest axis) were measured with the aid of a Vernier caliper. The complete tumor regression (CR) was defined as the inability to detect tumor by palpation at the initial site of tumor appearance for more than two-month post-therapy. The Partial tumor regression (PR) was defined as ≥ 50% reduction in initial tumor size. The edema, erythema, and scar formation in the treatment field was observed and recorded. Tumor response for each treatment was evaluated fot the tumor response.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

The authors are highly thankful to NIH for the financial support (partial) through a program project grant (PO1 CA55791), Photolitec, LLC, Buffalo and the shared resources of the Roswell Park Comprehensive Center, Buffalo, NY, United States Support Grant (P30 CA016056). The authors are also thankful to Mr. Eric Jensen and Dr. Valerie A. Frerichs, Mass spectrometry Facility, SUNY, Buffalo.

ABBREVIATIONS

PDT	photodynamic therapy

PS	photosensitize
HSQC	heteronuclear single quantum coherence
ROS	Reactive oxygen species

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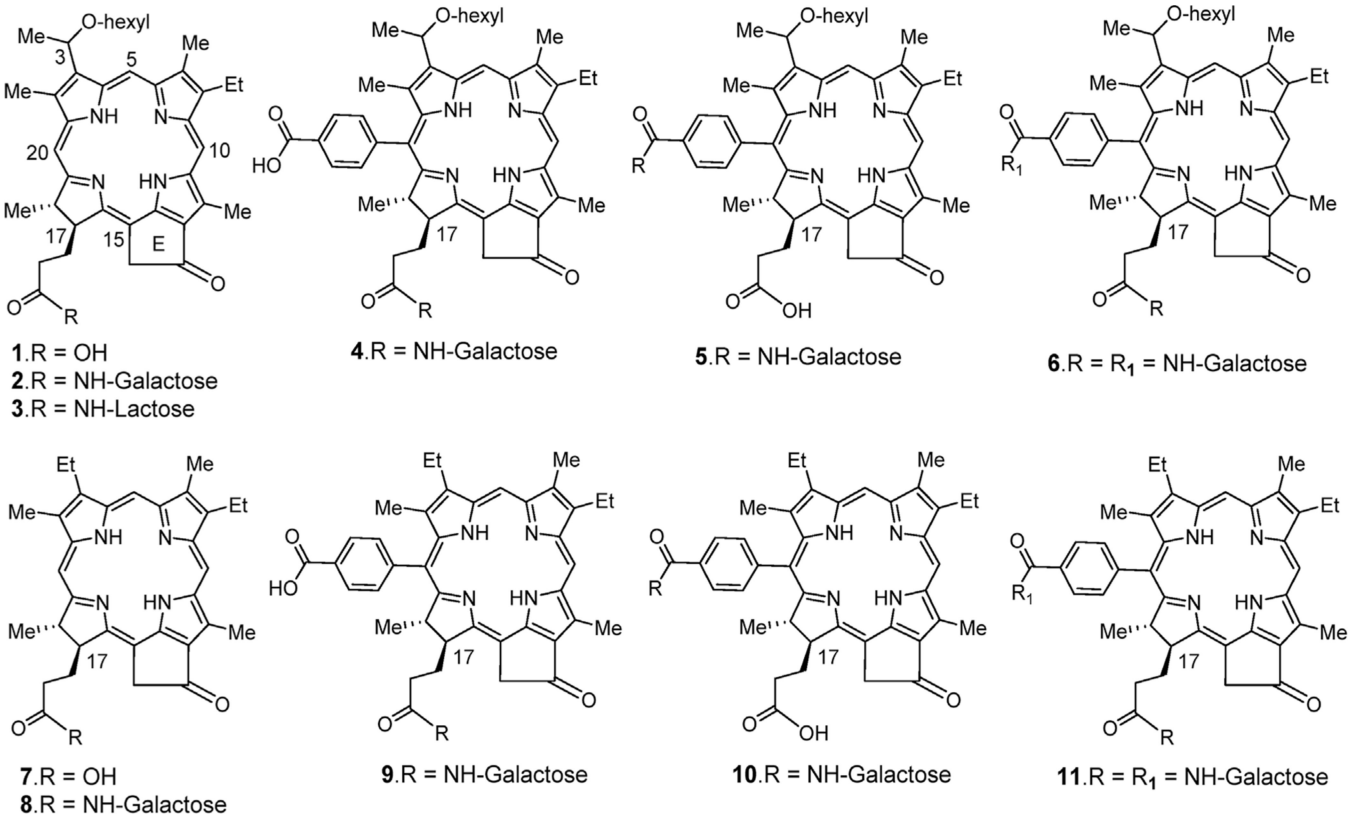
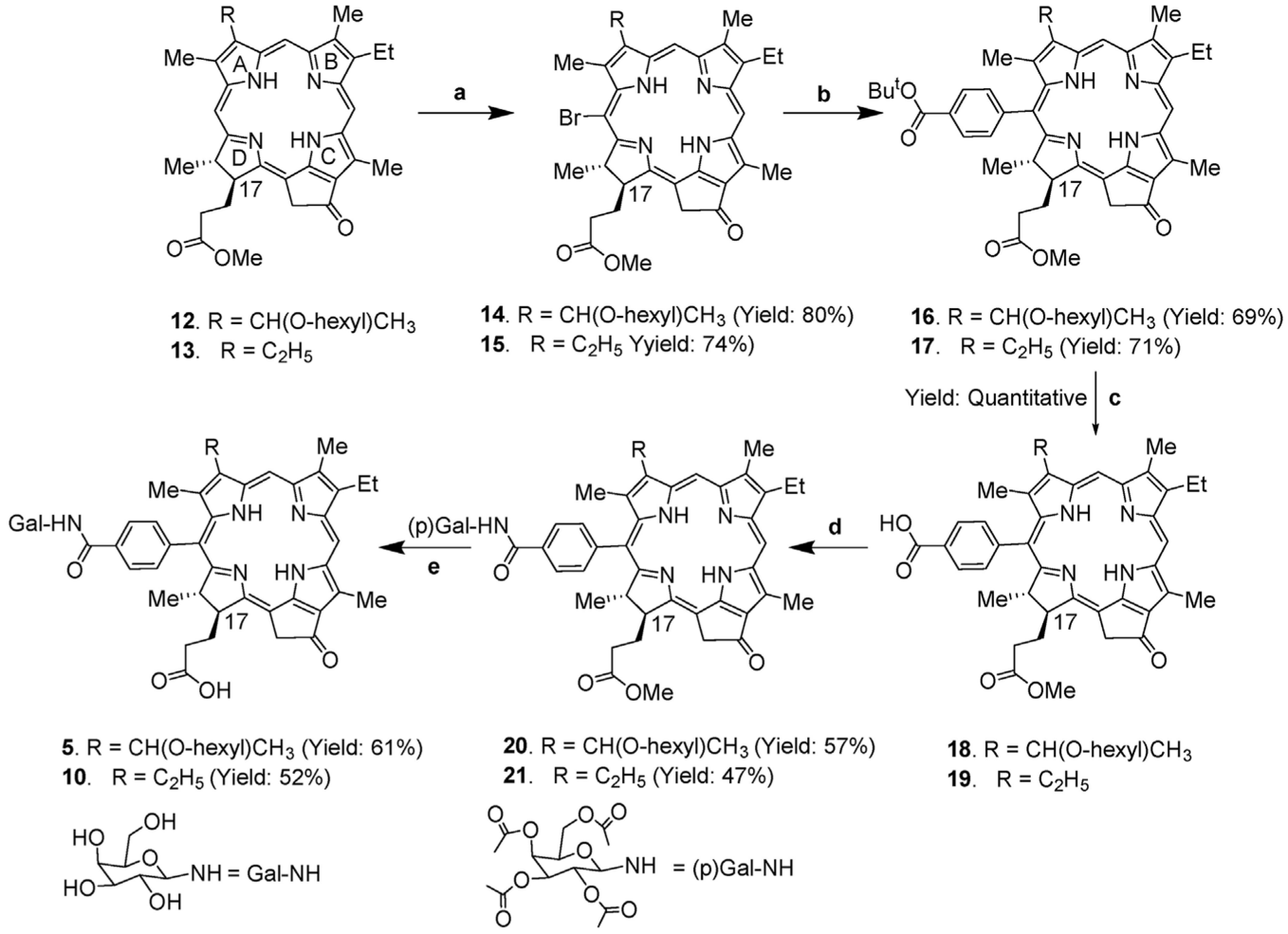


Fig. 1.

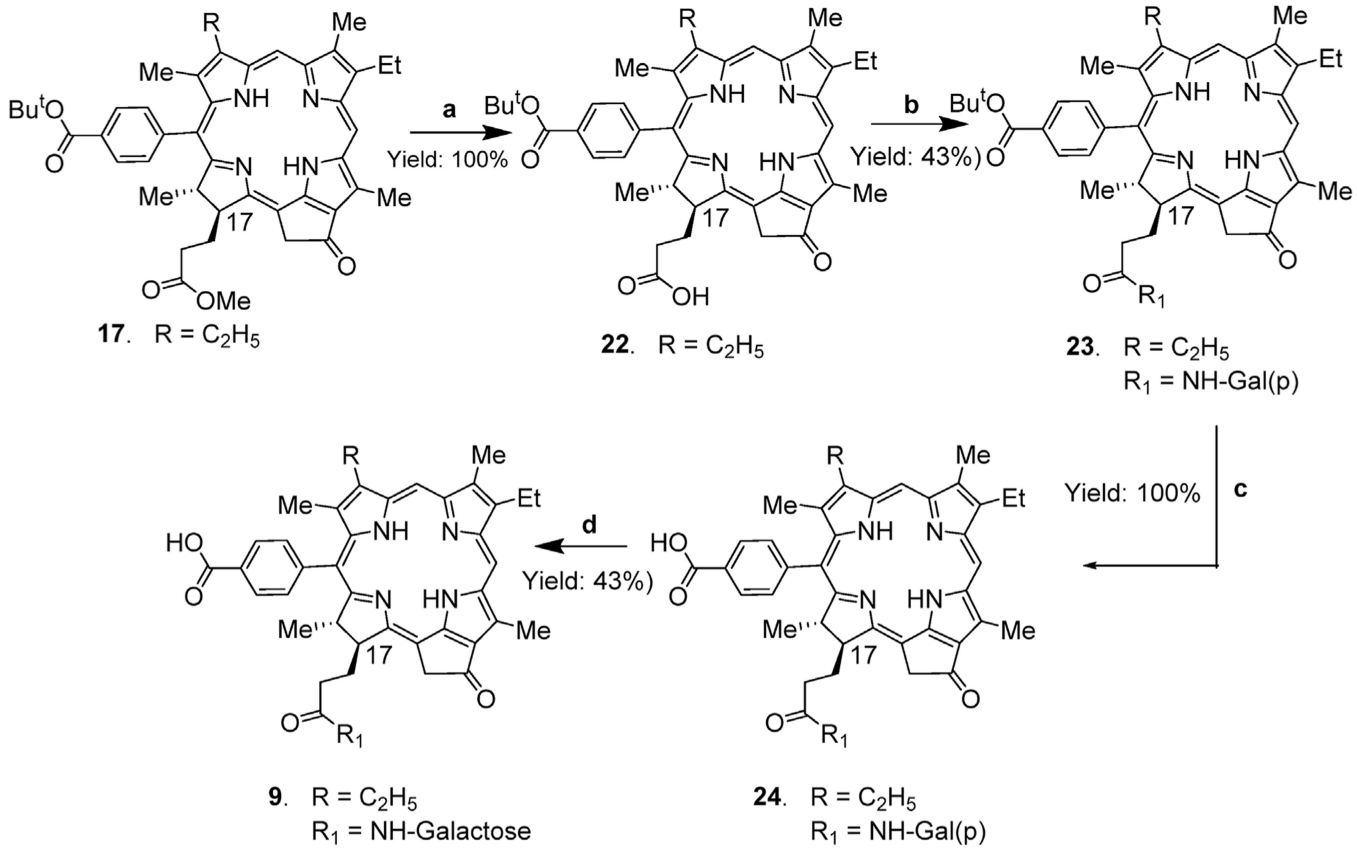
Structures of photosensitizer-carbohydrate conjugates investigated in current study.



a) Pyridinium tribromide, CH_2Cl_2, **b)** 3-(*tert*-Butoxycarbonyl)phenylboronic acid pinacol ester, $Pd(PPh_3)_4$, K_3PO_4, THF, reflux; **c)** 70% TFA/CH_2Cl_2; **d)** 1-β-aminogalactose tetraacetate, PyBOP, Et_3N, DMF; **e)** LiOH in H_2O, MeOH, THF

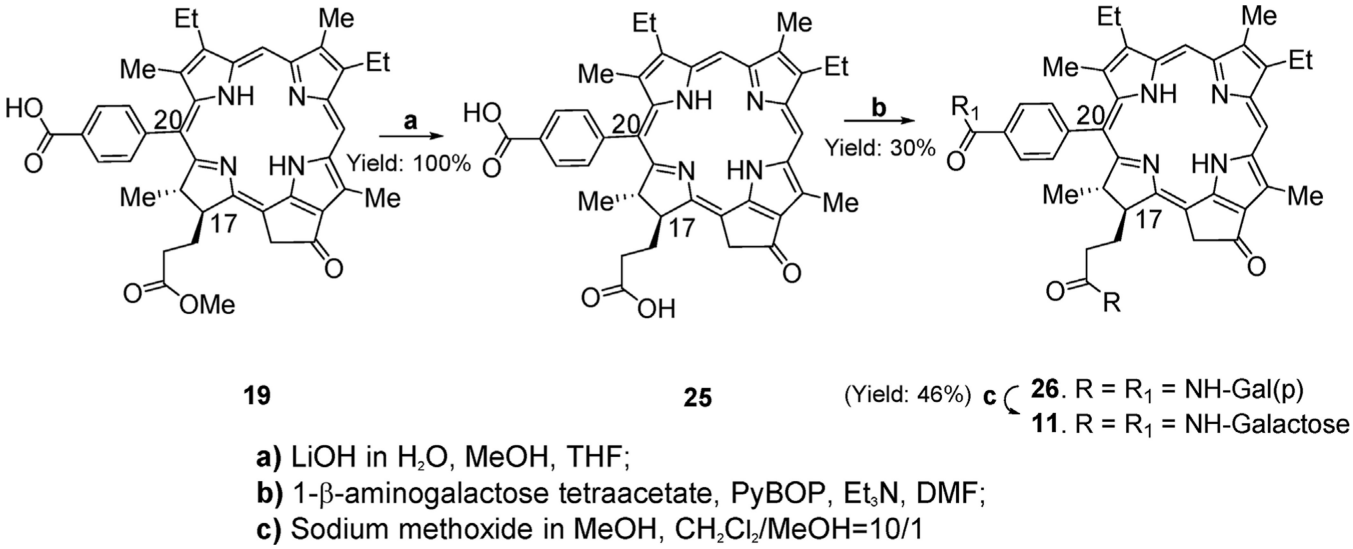
Scheme 1.

Synthesis of 20-substituted galactose conjugates of pyropheophorbide-a analogs.

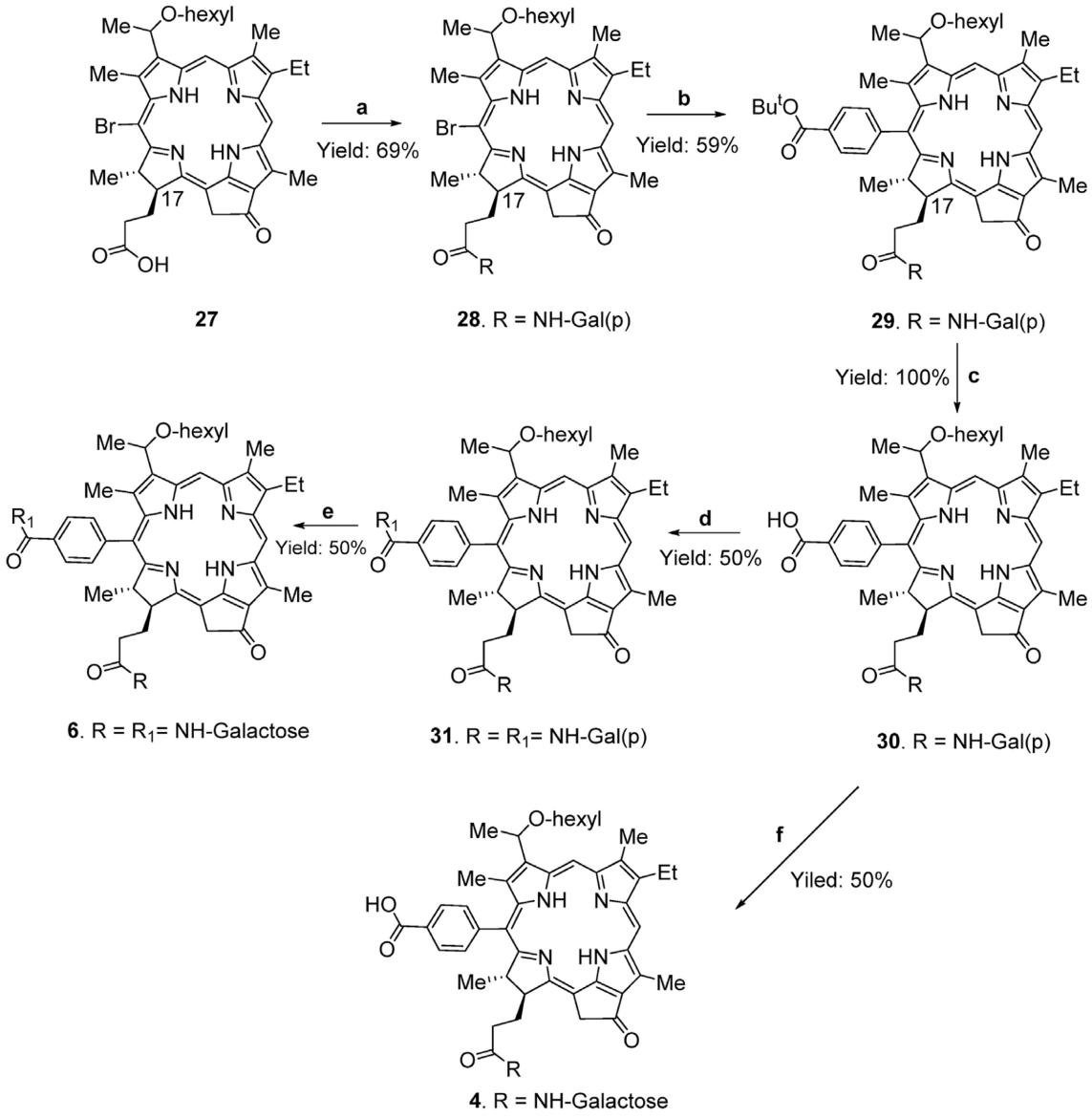


a) LiOH in H_2O, MeOH, THF; **b)** 1-β-aminogalactose tetraacetate, EDC, DMAP, CH_2Cl_2
c) 70% TFA/CH_2Cl_2 **d)** Sodium methoxide in MeOH, CH_2Cl_2/MeOH=10/1

Scheme 2.
Synthesis of 17-substituted galactose conjugate of 20-benzoic acid substituted meso pyropheophorbide-a.



Scheme 3.
Synthesis of 17[2], 20-disubstituted galactose conjugate of meso pyropheophorbide-a.



a) 1-β-aminogalactose tetraacetate, EDC, DMAP, CH_2Cl_2
b) 3-(tert-Butoxycarbonyl)phenylboronic acid pinacol ester, $Pd(PPh_3)_4$, K_3PO_4, THF, reflux
c) 70% TFA/CH_2Cl_2
d) 1-β-aminogalactose tetraacetate, PyBOP, Et_3N, DMF
e), **f)** Sodium methoxide in MeOH, CH_2Cl_2/MeOH=10/1

Scheme 4.

Synthesis of 17-substituted and 17, 20-disubstituted galactose conjugates of HPPH (Photochlor).

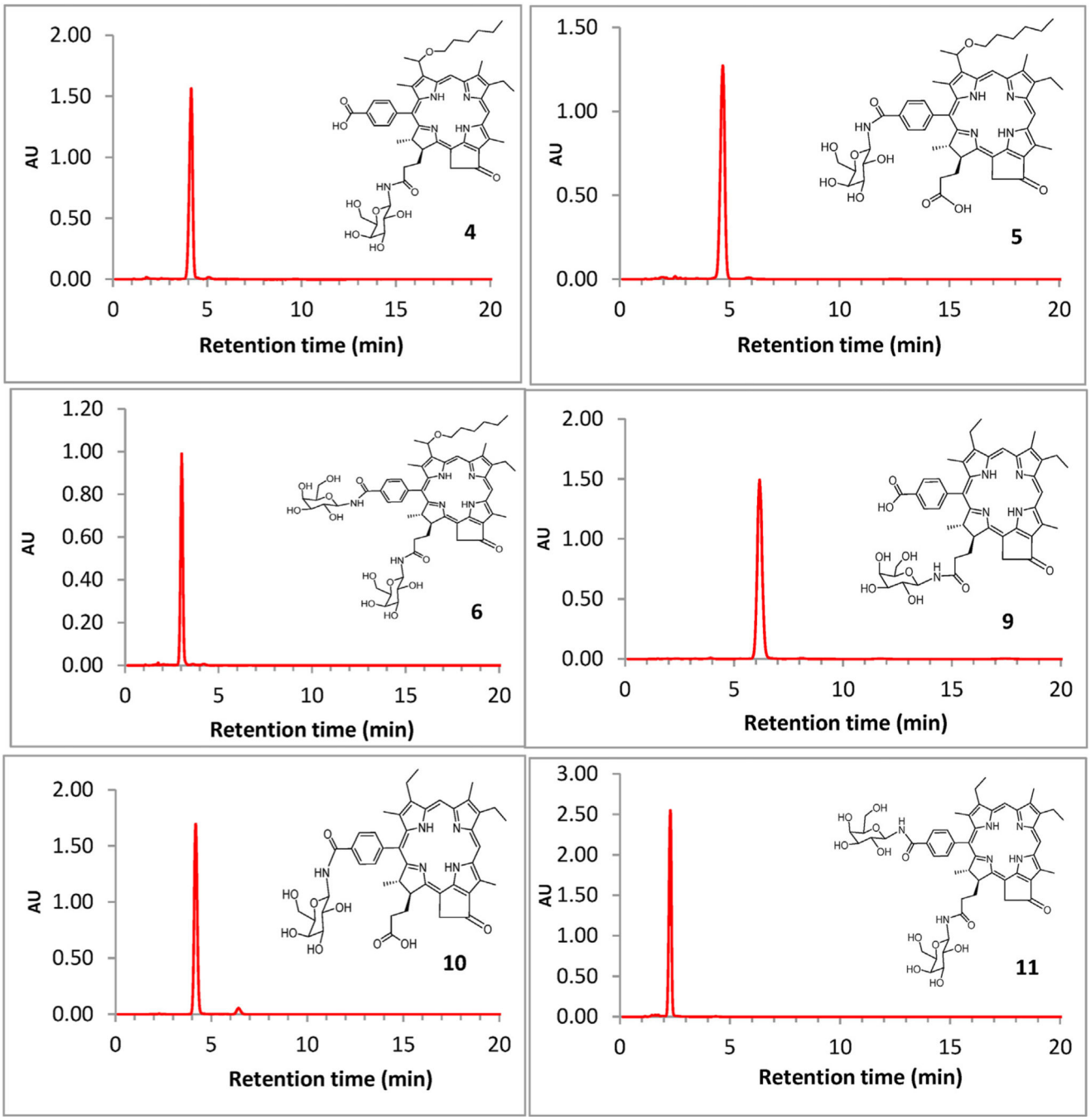


Fig. 2.

HPLC chromatograms of photosensitizer-galactose conjugates **4, 5, 6,9,10** and **11.** For details (HPLC system, column, eluting solvents, absorption wavelength) see the text.

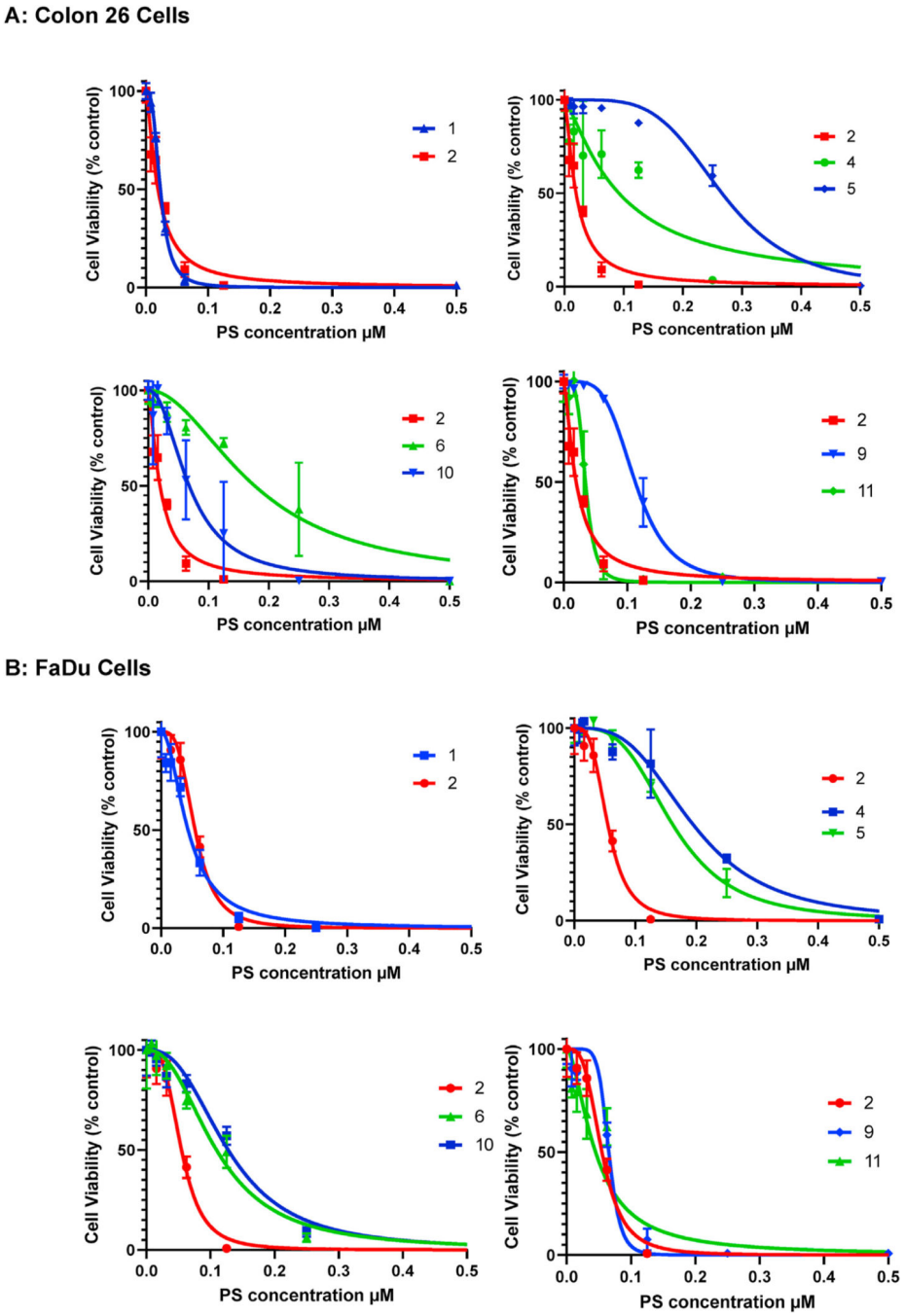


Fig. 3.

The *in vitro* photosensitizing efficacy (MTT assay) of PS-carbohydrate conjugates in FaDu and Colon 26 tumor cells under similar treatment parameters. The PDT efficacy pf PSs was compared at variable concentrations and single light dose: 1 J/cm^2 at 665 +/5 nm. Among the compounds evaluated, PSs **2** and **11** were almost equally effective in both cell line.

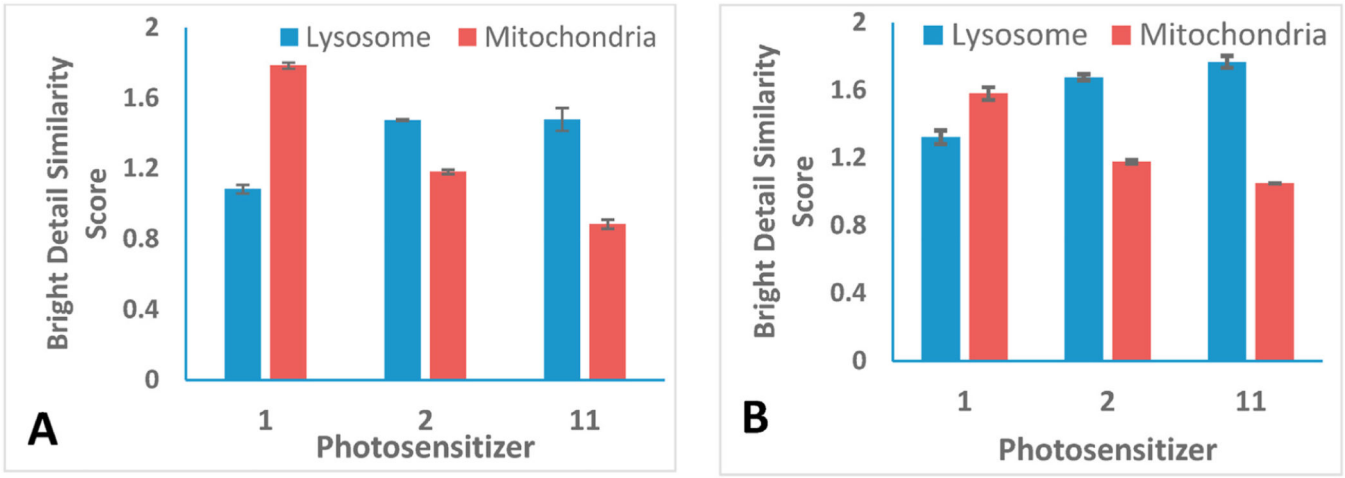
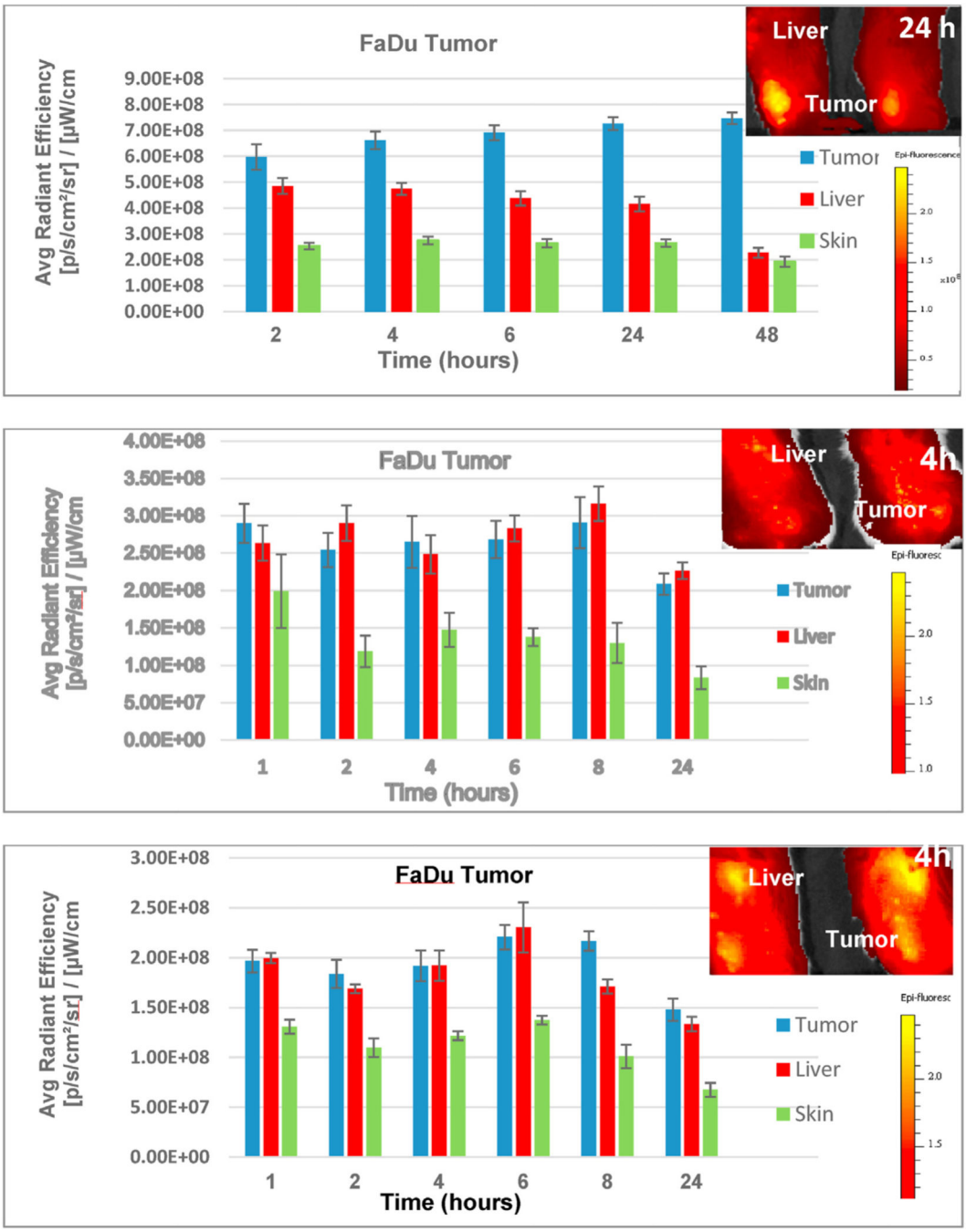
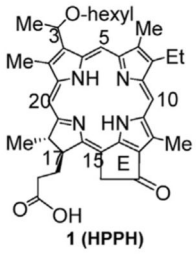


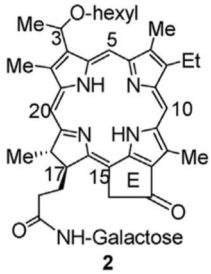
Fig. 4.

Localization of Photosensitizer **1, 2** and **11** in FaDu (**A**) and Colon 26 cells (**B**). Photosensitizer (I μM) was added to cells with mitotracker red or lysotracker Green then imaged using Imagestream MkII. In both cell lines, the addition of a galactose moiety induces enhanced uptake in subcellular localization from mitochondria to lysosome. For images of cells labeled with Lysotracker GreenDND-26, Mitotracker Red CmxRos and the PSs (**HPPH, 2 &11**), see Figs. S43–S45 (Supplementary Material Information).

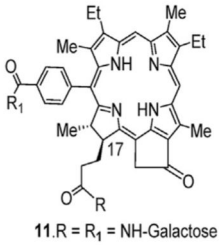




1 (HPPH)



2



11.R = R_1 = NH-Galactose

Fig. 5.

PS uptake: SCID mice bearing FaDu tumors (3 mice/group) were injected with PS **1** (HPPH), **2** and **11** at a dose of 0.47 μmol/kg. The tumor vs liver and skin (adjacent to tumor) uptake were measured by fluorescence at variable timepoints using IVIS spectrum (excitation wavelength: 665 nm, emission: > 720 nm). Tumor *vs* liver and skin uptake was determined based on the fluorescence intensity measured by IVIS Spectrum, and was plotted against time. Tumor Images with PS **1, 2 & 3** (2/3 mice/group) are shown at the time of their optimal uptake in tumors.

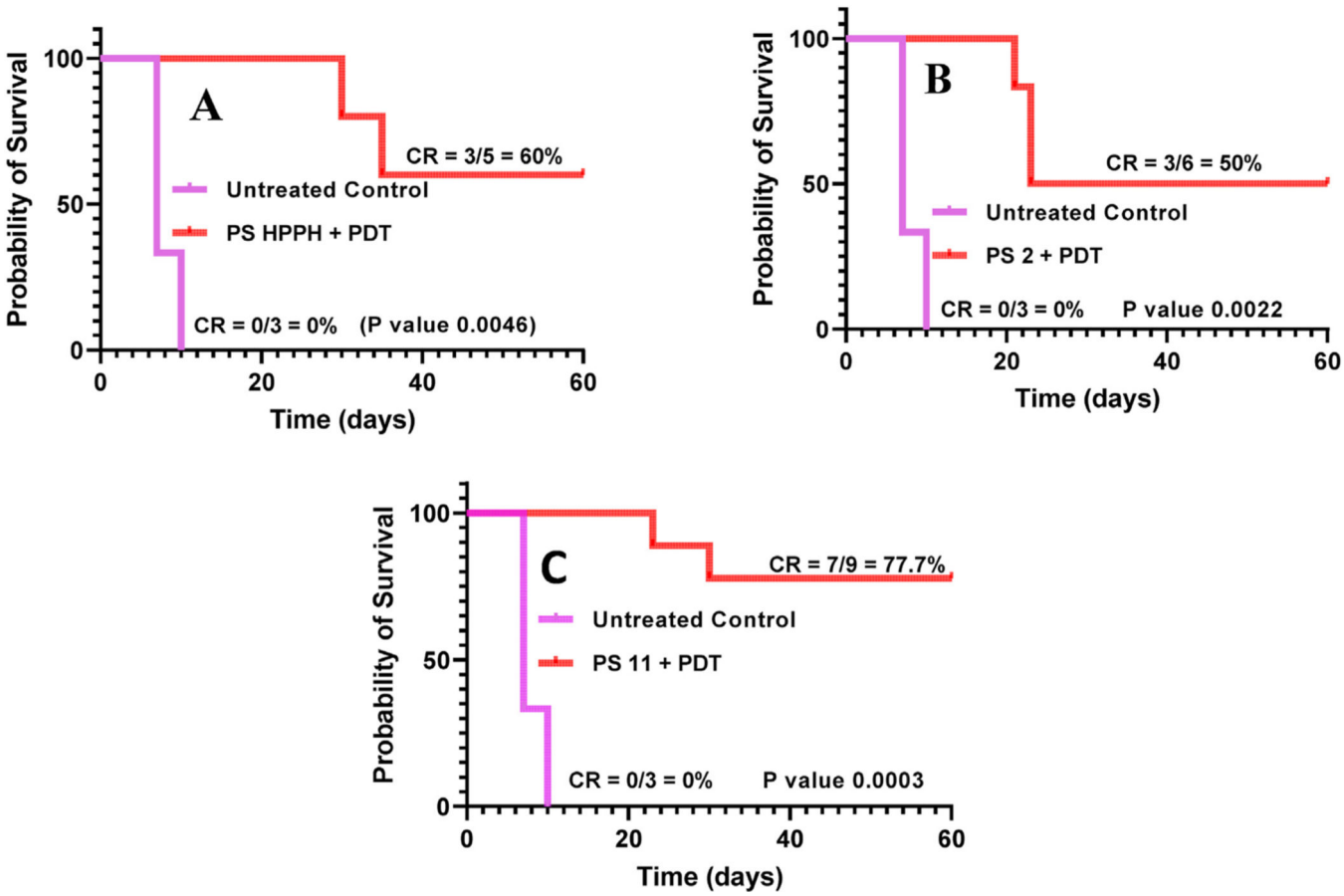


Fig. 6.

Comparative *In vivo* anti-cancer activity of (**A**): HPPH **1**, (**B**) PS 2 and (**C**): **PS11** in SCID mice bearing FaDu tumors (head & neck) under similar PS dose: 0.47 μmol/kg, and light dose (665 nm, 135 J/cm^2, 75 mW/cm^2). In case of PS1 injected mice, the tumors were exposed to light at 24 h and for PSs **2** and **11** at 8 h post-injection of the PS (the timepoint of maximal PS uptake). Tumor growth was measured. Among the compounds, PS **11** (di-*β*-galactose analog) showed the best anticancer activity. Note: None of the mice after the PDT treatment. The mice with tumor regrowth (>400 mm^3) were euthanized following the IACUC approved animal protocol.

Table 1

Retention time and the purity of carbohydrate conjugates determined by HPLC analysis.

Compound	**4**	**5**	**6**	**9**	**10**	**11**
Retention time (min)	4.15	4.69	3.03	6.17	4.18	2.29
Purity, %	96.07	97.32	96.06	97.11	95.45	96.97

Table 2

Comparative *in vitro* photosensitizing efficacy (IC_{50} values) of photo sesitizers.

Photosensitizer	IC50 Values FaDu cells	IC50 Values Colon 26 cells
1	0.044 ± 0.005 μM	0.023 ± 0.001 μM
2	0.055 ± 0.006 μM	0.019 ± 0.003 μM
4	0.195 ± 0.020 μM	0.266 ± 0.012 μM
5	0.161 ± 0.009 μM	0.092 ± 0.037 μM
6	0.109 ± 0.017 μM	0.177 ± 0.029 μM
9	0.066 ± 0.010 μM	0.113 ± 0.005 μM
10	0.128 ± 0.013 μM	0.068 ± 0.010 μM
11	0.039 ± 0.006 μM	0.034 ± 0.002 μM