Chemical Synthesis and Medicinal Applications of Glycoporphyrins

Claire Moylan¹, Eoin M. Scanlan², Mathias O. Senge¹,³*

¹ School of Chemistry, SFI Tetrapyrrole Laboratory, Trinity Biomedical Sciences Institute, 152-160 Pearse Street, Trinity College Dublin, The University of Dublin, Dublin 2, Ireland; ² School of Chemistry, Trinity Biomedical Sciences Institute, 152-160 Pearse Street, Trinity College Dublin, The University of Dublin, Dublin 2, Ireland; ³ Medicinal Chemistry, Institute of Molecular Medicine, Trinity Centre for Health Sciences, Trinity College Dublin, St James’s Hospital, Dublin 8, Ireland.

Abstract: This review presents an in-depth overview of the modification of porphyrins with bioconjugates and their applications in medicine today. Porphyrin bioconjugates ranging from nucleotides to steroids are under active scrutiny. However, carbohydrates have been at the forefront of such research in recent years and offer significant potential. This is attributed to their own selectivity to lectins on the surface of cancer cells and their influence on the amphiphilicity of the porphyrin macrocycle. These characteristics and the tendency of porphyrin photosensitizers to accumulate in tumor tissues make glycoporphyrins promising candidates for use as photosensitizers. Thus, a detailed overview of the synthesis and biological evaluation of glycoporphyrins is given with a particular focus on their applications in photodynamic therapy and their future prospects as drug candidates have been reported.

Keywords: Carbohydrates, Glycoporphyrins, Porphyrins, Photodynamic Therapy, Sugars and Tetrapyrroles.

1. INTRODUCTION

1.1 Porphyrins and PDT

Porphyrins are ubiquitous in nature and are an essential class of pigments in biological systems. They are found in cytochromes, peroxidases and catalases and carry out a variety of functions in respiration, electron transfer, catalysis and photosynthesis [¹]. Porphyrins are energy excitable chromophores and have many functions in biochemistry and industrial applications [²]. They have a wide range of applications in modeling energy transfer, in supramolecular chemistry and in solar cells [³], but specifically in biological system they are involved in oxygen transport, photosynthesis, and catalysis. In clinical terms they are involved in genetic disorders such as porphyrias, play a role in signaling and other areas of photomedicine [⁴,⁵].

Due to their biological relevance and involvement in many clinical problems much research has focused on the development of synthetic porphyrin derivatives for medical research. Medical applications of porphyrin derivatives have focused on both photodynamic detection (PDD) and photodynamic therapy (PDT). Photodynamic detection is a diagnostic technique where a specific light activating molecule, i.e. a photosensitizer (PS) agent is administered to the targeted cells. These cells are then exposed to short wavelength light which produces emission of light at a lower energy wavelength. This light is detected and analyzed spectroscopically [⁶]. PDD refers to the diagnosis, while PDT refers to the actual therapy of malignant and nonmalignant diseases [⁷,⁸].

PDT is a non-invasive cancer therapy which can allow for more selectivity in destroying tumor cells compared to conventional methods with significantly less side effects. It involves the administration of a PS agent either intravenously or topologically to the body which can accumulate in cancer tissue largely due to the enhanced permeability and retention (EPR) effect [⁹-¹¹]. These PS agents can be activated with specific wavelengths to produce reactive oxygen species (ROS), often singlet oxygen, which in turn induces irreversible cancer tissue damage. Accumulation of PS agents in normal tissue and the inability of light to penetrate deeply into the skin are just two of the drawbacks which highlight the need for continuous research in this field [⁷,¹²,¹³].

PDT has shown significant advances in recent years, from the synthesis of first generation PSs, e.g., the haematoporphyrin derivative Photofrin® to second generation systems, such as Temoporfin® [¹⁴]. Syntheses of new derivatives focusing on eradicating the problems observed in the first generation derivatives have been investigated extensively in recent times [¹⁵]. Efforts to improve the selectivity of PS agents have led to the incorporation of targeting agents including bioconjugates which have selectivity for receptors over-expressed on tumor tissue, thus, resulting in the study of third generation PSs. Commercially available PS formulations such as Photofrin® and Foscan® exhibit passive selectivity for malignant tissue modulated by increased metabolic activity of cancer cells.

*To whom correspondence should be addressed Fax: +353-1-896-8536; E-mail: sengem@tcd.ie.
Therapeutically this lack of selectivity implicates the dosage of PS administered to the patient so as to obtain significant therapeutic effect. The pharmacokinetics and biodistribution of PS must be tackled to overcome these undesirable characteristics with the goal to provide beneficial palliative treatment.

1.2 Carbohydrates

Oligosaccharides play an important role in many biological functions such as cell adhesion, metastasis, signaling, communication, and as growth signals[16,17]. The interactions between the receptors and the substrates are very specific, indicating their potential as targets for drug delivery. Complex carbohydrates mediate a wide variety of biological effects ranging from basic energy requirements to cell recognition, cell-cell communication, fertilization, hormone activity, viral and bacterial infections and tumor cell metastasis[18]. Thus, glycochemistry involves a cornucopia of hormone activity, viral and bacterial infections and tumor cell recognition, cell-cell communication, fertilization, delivery. Complex carbohydrates mediate a wide variety of specific, indicating their potential as targets for drug interactions between the receptors and the substrates are very specific, indicating their potential as targets for drug delivery. Complex carbohydrates mediate a wide variety of biological effects ranging from basic energy requirements to cell recognition, cell-cell communication, fertilization, hormone activity, viral and bacterial infections and tumor cell metastasis[18]. Thus, glycochemistry involves a cornucopia of

Most carbohydrates have a corresponding receptor on the cell surface, i.e. a binding protein receptor called a lectin. Lectins are non-enzymatic proteins which carry out cellular functions such as trafficking, adhesion and signaling through deciphering the “glycocode”[17,20]. Many changes in cell surface lectins are brought about by oncogenic transformation which provides impetus for future research in cancer detection and treatment. For example, galectin and glucose transporters[21] are found to be over-expressed on tumor cells[22-24]. As a result, noteworthy research has been carried out on galectin-3 as its expression has been biologically evaluated and used to identify the surface binding receptor targets. The interaction between carbohydrates and lectins over-expressed on the surface of cancer cells can be taken advantage of in drug development. For the last couple of decades, carbohydrate modified porphyrins have been explored for use as PDT agents. Glycoporphyrins were envisaged to tackle the obstacles of first and second generation PS agents. Even with the many advances in basic porphyrin functionalization, dedicated reactions for bioconjugates with carbohydrate units are only slowly emerging[29]. This is despite the excellent water solubility of glycoporphyrins, a significant improvement over established PSs.

Various methods of linking carbohydrates to porphyrin scaffolds, for example by direct glycosylation to the porphyrin[30], amide[31], or triazole linkages[32] are available. The number and regiochemical arrangement of the sugar units gives scope for fine-tuning the hydrophobicity and hydrophilicity. The major problems with these methods are the low yielding synthesis with condensation methods and the use of very strong Lewis acids, bases and coupling reagents which means that the carbohydrate has to be suitably protected. This is evident in the numerous glycoporphyrins substituted with the simple monosaccharide D-glucose. When introducing more expensive and synthetically relevant carbohydrates, it is preferably done in the later steps ensuring stability of the glycosidic linkage on large oligosaccharides. For example, our groups have published work using the CuAAC reaction under microwave and significantly less harsh conditions which are high yielding for the synthesis of homo- and heteroatom substituted tetraphenylporphyrins[32]. This allowed the synthesis of an extensive glycoporphyrin library which has been biologically evaluated and used to identify the intracellular localization of glycoporphyrins depending on the carbohydrate unit present[33]. For example, these preliminary results in biological testing showed that triglycosyl-substituted porphyrins lack the ability to be taken up by esophageal cancer cells possibly due to their high water solubility and reinforced that amphiphilicity of such systems is key to their ability to cross the lipid membrane[34-42].
1.3 Porphyrin Bioconjugates

The significant medical potential of porphyrins has raised interest in related bioconjugates. It is hoped to increase targeting to tumor tissue, enhance the solubility of the porphyrin scaffold and to lower the photosensitivity experienced by patients with current clinically approved PSs. In a conceptual sense the general structure of porphyrin-bioconjugates consists of the subunits porphyrin-linker-bioconjugate group. The linker between the porphyrin and the bioconjugate can be introduced into either the meso or β positions by condensation methods or through substitution of pre-synthesized porphyrin scaffolds (Figure 1). It can range from polyethylene glycol (PEG) chains, amide bonds, triazole rings, ether linkages, etc., all discussed below.

![Figure 1. Illustration of porphyrin-bioconjugate systems.](image)

As an established area on its own, there are many examples of such bioconjugates now available [43]. These include porphyrins with steroids, lipids, peptides, carbohydrates, lectins and nucleotides. Especially for porphyrins with signaling peptides a significant body of information is now available [44-48]. Porphyrins appended with amino acids have been prepared to model haemoproteins [49,50] to investigate ligand binding and oxygenative catalysis and energy transfer in photosynthesis [51]. Likewise, the difference in the expression of aminopeptidases in tumor vasculature versus normal vasculature has been identified in some organs [52]. Berger et al. synthesized amino acid ALA (δ-aminolevulinic acid) derivatives which have the potential to act as substrates for aminopeptidase expressed in lung tumor and endothelial cell lines, thus, increasing the accumulation of ALA in malignant tissue and potentially Pp IX (Protoporphyrin IX) [53].

Porphyrin-antibody conjugates have also been studied in depth [54] as well as their non-covalent interactions [55]. Levy and coworkers in the 1980’s reported the first conjugation of an antibody to a porphyrin macrocycle. The antibody-haematoporphyrin conjugates showed significant in vitro destruction of cells [56]. Lipid-porphyrins have also been synthesized, initially for an investigation of their electron transfer properties [57,58]. Current interest in the synthesis of lipid porphyrins aims at the construction of self-organized porphyrin assemblies for modeling electron transfer processes and oxygen transport [59-62]. The inclusion of porphyrins into monolayer, bilayer and micellar lipid aggregates forms supramolecular structures with unique physical-chemical properties [63,64] as do nanoformulations [65]. Also, phospholipid vesicles containing porphyrins have been prepared as model systems for haemoproteins for investigating O2 transport systems [66] and electron transfer reactions [67]. Such vesicles have been observed under electron microscopes to be 100-150 nm in diameter [68].

Initial work on porphyrin conjugates focused on steroids [69]. For example, the first investigation was aimed at studying the effect of steroid hormones such as estrone and progesterone for targeting an excess of receptors in breast cancer cells [70]. A typical example is the synthesis of an estrone derivative 2 covalently linked through an alkenyl unit via a Heck type reaction with a meso-iododiphenylporphyrin 1 (Scheme 1) [71]. In contrast to these areas, the chemistry of the related carbohydrate conjugates is only slowly emerging in recent years.

![Scheme 1. Synthesis of an estrone-porphyrin conjugate.](image)

Very few examples of naturally occurring glycoporphyrins have been described. One example is a chlorophyll c2-monogalactosyldiacylglyceride ester 3, which was extracted from *Emiliania huxleyi* and identified using mass spectrometry [72]. Similar compounds have also been identified in *Chrysochroulina polyepis* [73,74]. The best known example is perhaps tolyporphin A 4, one of a series of bacteriochlorin pigments [75,76]. It is the first glycoporphyrin isolated in nature from blue-green algae and was the target of a total synthesis (vide supra) [77]. Another such example includes the fluorescent glycosylated chlorophyll linear tetrpyrrole catabolite 5 which accumulates in senescent, yellow banana leaves. The structure was elucidated using
NMR and mass spectrometry and proved to comprise a 6-α-galactopyranosyl-(1→6)-β-galactopyranosyl-(1→1)-glyceryl unit [78]. The physiological function of these fluorescent chlorophyll catabolites is unknown, however, they are a source of blue luminescence in senescent banana leaves.

Porphyrin cofactors exhibit a structural partnership with proteins which provide vital roles in biochemistry. Note, that porphyrins are bound and transported in the serum by carbohydrate proteins and hemopoxin [79]. This is important for the tissue distribution and uptake of potential PS and has implications for the anti-angiogenic properties of PSs [80]. Porphyrins such as the heme cofactor of the glycoprotein hemoglobin are essential for gaseous transport [81], while the glycoprotein transcobalamin provides facile transport of cofactor vitamin B₁₂ (cobalamin or hydroxocobalamin) into the body [82]. The detailed interactions of glycoproteins and porphyrin PSs are beyond the scope of this review, however, several documents focusing on this area have been published [83-85]. One such example are the direct inhibition of ATPase activity of P-glycoprotein by porphyrin PSs [83] and the interaction of sulfonated porphyrins with HIV glycoproteins gp120 causing photodamage and inhibition of antibody binding [84]. One highlight of this research has focused recently on the fructation of hemoglobin (i.e. protein glycation with the monosaccharide fructose) which promotes the formation of endogenous reactive oxygen species resulting in the degradation of the heme macrocycle. Such research can provide insight into the effect of the accumulation of heme degradation products for hyperglycemia in diabetics [86].

Similar to interactions with glycoproteins, porphyrins have been shown to exhibit other physiological interactions with, for example, lectins, i.e., specific carbohydrate recognition proteins. Several examples exist of metalloporphyrins binding to plant lectins where the association constant (Kₐ) and stoichiometry of binding have been determined [87-89]. In most cases the addition of the binding sugar appears to have no effect on the binding of the porphyrin, i.e., their binding sites are different. The studies into their non-covalent interactions lead to the investigation of porphyrin-lectin conjugates as drug delivery systems. Lectins are not just found on the surface of cells but are easily taken up by cells as well. While tumors over-express lectins, solid tumors have also shown an abundance of O-glycosyl moieties such as T or Tn antigens [90,91]. Very few examples of lectins covalently bound to porphyrins exist. One such compound is 5-(4-(5-carboxy-1-butoxy)phenyl)-10,15,20-tris(4-N-methyl)-pyridiniumyl)-porphyrin covalently coupled with the plant lectin Morniga G, a galactose specific binding lectin which exhibited an increased photocytotoxicity (at 10 nM concentration) compared to the free porphyrin in vitro through an O-glycan dependent process using Tn-positive Jurkat leukemia cells as well as targeting glycan alterations on tumor cells [92,93].
1.4 Porphyrin Carbohydrate Interactions

Although the focus of the present work is on covalently linked porphyrin-sugar systems, the possibility for non-covalently bound systems exists [94,95]. For example, boronic acid porphyrins have been used for sugar sensing [96-105], discrimination of sugars [106,107], and have been used to control sugar-DNA interactions [108]. Boronic acid porphyrins have also been employed in glycosylated steroid sensing [109] and the detection of carbohydrates on the surface of cells using a photochemical approach [110]. Other studies included selective saccharide recognition using naphthyl substituted porphyrins [111,112], quinoline substituted porphyrins [113], steroid derivatives [114-117], selective maltotetraose binding by meso-meso linked bisporphyrins [118,119], and a Ce(IV) double-decker system for saccharide binding [119,120]. Dimeric porphyrins have been exploited for their hydrogen bonding interactions with oligosaccharides for sensory applications [121]. Non-covalent host-guest interactions were also observed between cyclic porphyrin-cryptand conjugates sandwiched together and saccharides, i.e., the guest molecule which occupied the cavity between the assemblies in water [122]. Studies have also been carried out using aspartate ureaporphyrins [123] and urea functionalized porphyrins with amino acid residues [124] as artificial carbohydrate receptors. Urea appended porphyrins incorporated into molecular imprinted polymers displayed differentiation properties between closely related carbohydrates [125].

TPP substituted with boronic acid groups, NH₄⁺ and SO₄⁻ groups in solution and immobilized on cellulose fibers were used to identify and quantify rhamnose and other sugars which were known to be contained in the exosporium of bacterial endospores of some Clostridium and Bacillus species [126]. Picket fence type αααα-TPP’s containing urea, carbamate, or amide groups provided carbohydrate recognition through hydrogen-bonding and CH-π interactions [127,128]. Several studies have been carried out using bile acid conjugate porphyrins for carbohydrate recognition [129-131], one particular example exhibits the potential of such derivatives for direct targeting of bile acid porphyrins to sugar moieties which are expressed on malignant tumors. Both in vivo and in vitro studies displayed significant potential for PDT [129]. Likewise, positively charged amphiphilic cyclodextrins were investigated as supramolecular aggregates for the delivery of PSs to bacterial cells for antimicrobial PDT. Here the focus was on targeting methicillin-resistant Staphylococcus aureus (MRSA) and Escherichia coli Gram-positive and Gram-negative bacteria [132]. Supramolecular control of π-stacked aqueous phthalocyanines via host guest interactions with per-O-methylated α-cyclodextrin were also achieved and observed by enhanced fluorescence [133]. Another example of non-covalently bound systems includes the use of chitosan-based nanoparticles as PS delivery vehicles which proved successful in sustained release of the PS in vitro and in vivo [134].

2 SYNTHESIS OF GLYCOPORPHYRINS

2.1 Porphyrin Chemistry

Porphyrins are a large group of organic compounds containing four pyrrole rings connected to form a highly conjugated, aromatic ring system. The 4N-core is able to complex various metal ions with cobalt, nickel, magnesium, iron and zinc complexes found in nature. They have absorption maxima in the UV-visible spectrum at ~ 400 nm (Soret band) and several smaller absorption peaks from 500-800 nm (Q bands). The optical, chemical and photophysical properties can be varied significantly through substitution and/or macrocycle manipulation [135].

Since the landmark synthesis of protoporphyrin IX by Fischer significant advances have been made in their synthesis. For aldehyde-pyrrole condensation reactions, this ranges from the early Rothemund condensation [136] to the now standard Lindsey condensation [137]. Partial and total syntheses were facilitated first by the MacDonald condensation, a [2+2] condensation utilizing dipyrromethenes, dipyrroyl ketones and dipyrromethanes [138]. Condensation methods are continuously improving and almost any desired meso or β-substituted porphyrin can be prepared today [139-142]. In parallel the functionalization of porphyrins through functional group interconversions and direct substitution reactions has progressed dramatically in the past decades [140,143]. This is mainly a result of the use of organolithium [144,145] and transition metal catalyzed reactions which now allows the preparation of unsymmetrical ABCD-type meso substituted porphyrins and arrays [139,147,148].

Reports on the synthesis of glycoporphyrins mimic this progress. Thus, initial studies on rather simple glycoporphyrins have now progressed to more complex systems. Still, advances in porphyrin chemistry must be accompanied by progress in carbohydrate chemistry and the necessary linker chemistry.

Strategically glycoporphyrins may be synthesized via the following general methods:

- Condensation reactions using pyrrole and a protected carbohydrate aldehyde.
- Total synthesis with a protected carbohydrate unit linked to a β-pyrrole position.
- Attachment of carbohydrate units to porphyrin linker groups.

With regard to the porphyrin macrocycle the linker unit can be attached to either the meso or β positions or, in principle, through the metal center via axial ligation. Additionally, the number and regiochemical arrangement of the sugar units gives scope in fine tuning the hydrophobicity and hydrophilicity of these molecules [149,150].
2.2 Total Synthesis of Tolyporphin

Tolyporphin A (4) is a bacteriochlorin, which is not involved in photosynthesis. It was isolated from the blue-green microalga *Tolypothrix nodosa* by Moore *et al.* and was the first natural glycoporphyrin to be identified and fully characterized by $^1$H NMR, $^{13}$C NMR and extensively studied by 2D NMR [75,77]. In addition, the isolation and structural identification of ten other tolyporphins B-K has been reported [75,76]. Compound 4 was found to be a multidrug resistant (MDR) reversing agent. For example, it increased the cytotoxicity of adriamycin or vinblastine, an intercalating chemotherapy drug, in vinblastine-resistant human ovarian adenocarcinoma and breast cell lines with doses as low as 1 μg.mL$^{-1}$ [77,151].

Next to heme and chlorophyll a, tolyporphin A presents one of the few natural porphyrins whose constitution was confirmed by total synthesis. Tolyporphins are comprised of an unsymmetrical hydroproporphyrin macrocycle (dioxobacteriochlorin), β-linked to C-glycosides at C-2 and C-12, which have hydroxyl or acetyl groups. A retrosynthetic analysis of the macrocyclic system 6 established the construction of tolyporphin via four monocyclic precursors (Scheme 2) where the 8 and 9 precursors were identical. The feasibility of this approach was demonstrated by Kishi's group through a total synthesis [152,153]. Compound 21 was synthesized via octahydroporphyrin 19, conversion to the bacteriochlorin 20.

Scheme 3. Synthesis of the tolyporphin chromophore I. Conditions: i) NIS (1 equiv.), DBU (2 equiv.), CH$_3$CN, 3 h, r.t.; ii) (EtO)$_3$P, xylenes (1:4), 23 h, 125 °C; iii) t-BuOK/t-BuOH, 3 h, 85 °C; iv) Lawesson's reagent, toluene, 3 h, 80 °C; v) NIS (1 equiv.), DBU (4 equiv.), CH$_3$CN, 3 h, r.t.; vi) CDCl$_3$, NaHCO$_3$, PPh$_3$, CH$_3$CN, 4 h, r.t.; vii) KCN, MeOH, 10 min, r.t.; (vii(h)) t-BuOK/t-BuOH, 15 min., 80 °C; (ix) Zn(ClO$_4$)$_2$, MeOH, r.t.; (x) Et$_3$OBF$_4$, CH$_2$Cl$_2$, i-Pr$_2$NEt, 20 h, r.t.; (xi) a: TFA, anisole, 1 h, r.t.; b: MeOH, 10 min; c: t-BuOK/t-BuOH, 15 min, d: 20 % HCl, 1 min, r.t.; (xii) CrO$_3$·dimethyl pyrazole, CH$_2$Cl$_2$, r.t.
by a double retroaldol/autoxidation reaction followed by site-specific oxidation. Thus, with several modifications, the Eschenmoser sulfide contraction/iminoster cyclization method was incorporated in the assembly of the four monocyclic pyrrole precursors (11, 12, 14, 15) to yield 19. Establishing protecting groups X at C-7 and C-13 allowed for double elimination yielding the tetrahydroporphyrin and upon oxidation gave the chromophore 21 (Scheme 3) [159].

Two years later Kishi et al. synthesized (+)-tolyporphin-O,O-diacetate derivatives using a similar methodology as reported above, however, replacing precursors 11, 15 with 7, 9 [159]. However, the structure of the final product did not match the comparative NMR of the natural (+)-tolyporphin A ultimately leading to a revised structure of the original natural product. Using extensive NOE experiments the true structure of the synthetic tolyporphin O,O-diacetate derivative was confirmed as 6 which is different from the natural tolyporphin derivative. Re-examination of the NMR data obtained for the natural (+)-tolyporphin A O,O-diacetate showed the NOE experiments to be identical. The only difference between the two derivatives was at the quaternary centers C-2 and C-12 resulting in four possible structures, depending on the orientation of the methyl and glycosyl units. Based on spectroscopic data and analysis of the various early syntheses 4 was proposed as the structure of natural (+)-tolyporphin A and this was finally confirmed by total synthesis [158,159]. These landmark syntheses are now classic examples of porphyrin total synthesis. However, in practical terms such approaches are not suitable for synthetic applications. In recent years, the total synthesis of structural models via [3+1] condensation reactions was attempted with only a tricyclic fragment as a precursor for tolyporphin [158].

2.3 Synthesis via Condensation Reactions

The synthesis of porphyrins via pyrrole condensation reactions is the most widely used method reported in the literature. Condensation reactions, notably using Lindsey conditions [137] offer a simple entry into porphyrin systems and have been widely used, especially for meso substituted systems. This requires a combination of pyrrole (delivering the β-pyrrole units) and aldehyde (delivering the meso carbon unit) under acid-catalyzed conditions and obviously is a convenient method for the preparation of Α₂-type porphyrins with the same residue in all meso positions. For glycoporphyrins the utility of this method is limited to acid-stable derivatives.

One of the oldest examples of glycoporphyrins synthesis involved the preparation of a β-glycopyrropyphyrin. Based on his interest in etio-type porphyrin systems, Ono et al. prepared a Knorr-type pyrrole using a Barton-Zard reaction (22→23) to yield the protected 3-glycopyrrole 23 and related systems in 50-70 % yield (Scheme 4) [159]. Reduction to the pyrrole carbinal and followed by acid-catalyzed tetramerization then gave the etioporphyrin 24 in 18 % yield. Due to the steric hindrance imparted on the system through the sugar units no scrambling was observed in this case. Porphyrin 24 could easily be deprotected to the respective water-soluble derivative. Using a singlet oxygen trapping reaction the photosensitizing activity of the deprotected system was shown to be similar to Rose Bengal and haematoporphyrin, indicating its potential as a PS.

![Scheme 4](https://example.com/scheme4.png)

Scheme 4. Synthesis of β-substituted systems. Conditions: i) CNCH₂CO₂Et, DBU, THF, r.t., 8 h; ii) LiAIH₄, 0 °C→r.t., 2 h; iii) pTsOH, r.t., 10 h; iv) p-chloranil, r.t., 3 h.

2.3.1 Α₂-type Porphyrins

Due to the ease of synthesis most of the early reports focused on the synthesis of meso-aryl substituted glycoporphyrins. We have attempted to compile most of these syntheses into Table 1 (see Figure 2 for carbohydrate abbreviations used). For example, in one of the earlier studies condensation of tetraacetyl-β-β-glucose-2-benzaldehyde with pyrrole gave tetrasubstituted glycoporphyrin 25 in 42 % yield. The compound was obtained as three atropisomers (αββα, ααββ and αααβ) in a ratio of 1:2:4 all of which were converted to the water soluble glycoporphyrins 26. The substitution of the glycosyl group on the ortho position of the meso phenyl was...
investigated for its steric hindrance, solubility in water, and atropisomerism. Later studies on the use of these compounds for the asymmetric oxidation of alkenes showed significant potential for the \( \alpha \alpha \alpha \alpha \) atropisomer [160] which could be prepared via atropisomerization [161]. The same methods are suitable for preparation of both glucosyl (26) and maltosyl (28) substituted TPPs. The substitution of the glycosyl group on the \( \alpha \alpha \alpha \alpha \) position of the \( \alpha \alpha \alpha \alpha \) phenyl caused steric hindrance to metallation with zinc [160].

In extension of this work Maillard et al. synthesized a range of tetraglycosylated TPP’s containing glucopyranosyl, galactopyranosyl, maltosyl, and glucosaminosyl residues O-linked at the \( \alpha \alpha \alpha \alpha \) position under Lindsey condensation conditions (29-36) [162]. Similar compounds were prepared to determine the effect the glycosyl unit had on the singlet oxygen production for possible application in asymmetric photooxygenation. Compounds 30 and 38 were synthesized via trifluoride etherate catalyzed condensation of acetylated carbohydrate-substituted benzaldehydes and pyrrole with subsequent deprotection of the acetyl groups [163]. Their efficiency of singlet oxygen production was measured monitoring the oxidation of TEMP (2,2,6,6-tetramethyl-4-piperidone) to TEMPO using ESR spectroscopy and compared to the non-glycosylated derivative 37. Higher reaction rates were observed for the sugar derivatives in the order 37 < 38 < 30. Both the glucosyl and celllobiosyl porphyrins exhibited longer triplet lifetimes compared to 37. Preliminary photooxygenation experiments with the ene reaction of ethyl trans-2-methyl-2-butenolate with 38 embedded in polystyrene matrices were promising, but only low ee values were observed.

Related compounds [164] prepared via condensation methods include a series of 5,10,15,20-tetrakis[3- or 4-(\( \beta \)-\( \beta \)-glycopyranosyloxy)phenyl]porphyrins (30, 32, 39-44) and 45-52 [165]. The latter were prepared by condensation of pyrrole with \( p \)- or \( m \)-peracetlyl-glycosyl benzaldehydes via modification of Lindsey’s method where Zn(CH\(_3\)CO\(_2\))\(_2\)•2H\(_2\)O was used as a templating agent affording the acetylated derivatives in 53-93 % yield, followed by demetallation to the free bases in 36-86 % yield. Both the metallated and demetallated derivatives were deprotected. The hydrophobicity parameters (Log P) of the 16 acetylated derivatives of these systems were determined via reverse phase HPLC and ranged from 4.8 to 7.8 [166].

Simple variation of the aldehyde in porphyrin condensation reactions allows the preparation of porphyrins with more than four carbohydrate moieties. For example, Mikata et al. used a 3,5-dii-O-glycopyranosylbenzaldehyde to generate the porphyrin 53 with eight sugar units after deprotection [167].

The first example of directly \( \alpha \alpha \alpha \alpha \) linked glycosyl porphyrins were synthesized by Momontea et al. via Lindsey condensation methods in 1992 [168]. The four protected sugar substituents were directly linked to the \( \alpha \alpha \alpha \alpha \) positions via condensation with pyrrole and their pre-formed aldehyde analogues. Porphyrins (54-56) bearing xylofuranose, glucosfuranose and galactopyranose were isolated in 4 % yield compared to the porphyrins (57, 58) bearing \( O \)-linked glucopyranose and galactopyranose, which were not stable.

Subsequently, these studies were extended by Cornia et al. to the synthesis of \( \alpha \alpha \alpha \alpha \)-tetraglycosylated porphyrins with varying degree of lipophilicity and hydrophilicity. Using a slightly modified Lindsey procedure, the acid catalyzed condensation of pyrrole with sugar alddehydes was achieved at high dilution using BF\(_3\)•OEt\(_2\), affording an easy route to \( \alpha \alpha \alpha \alpha \)-glycosylated pyrrole atroposomers. Pyrrole condensed with enantiopure 2,3,5-\( \alpha \)-isopropylidene-\( \alpha \)-glyceraldehyde yielded two major atropisomers, \( \alpha \alpha \beta \beta \) 59 and \( \alpha \alpha \beta \beta \) 60 in 3 % and 4 % yield, respectively. This reaction was applied to different carbohydrates, e.g., 1,2-\( \alpha \)-isopropylidene-3-\( \alpha \)-methyl-\( \alpha \)-xylo-pentodialdofuranose yielding 61 as a single \( \alpha \beta \alpha \beta \) isomer in 6 % and both enantiomers of 2,3,4,5-di-\( \alpha \)-isopropylidenearabinose produced three major porphyrin compounds as the same \( \alpha \beta \alpha \beta \)-isomer. These were the expected fully protected porphyrin 62 in 2 % yield, accompanied by monodeprotected porphyrin in 1 % and the bisdeprotected porphyrin in 4 % yield. The other isomers and derivatives (63-65) were synthesized in similar yields. All derivatives were fully deprotected yielding water soluble compounds for possible use in chiral recognition and asymmetric catalysis in aqueous medium [169].

Several other examples were reported. These include the benzyl protected derivatives 66 and 67 [170] and condensation products of commercially available di-aldose and pyrrole [171]. Metallation and deprotection of the glycosyl units provided compounds 68 and 69 in yields ranging from 63-90 %. The latter compounds were used for DNA photocleavage studies with the Pd(II) complex 69 exhibiting the best activity [171]. More details on other examples of \( \alpha \alpha \alpha \alpha \)-type glycoporphyrins (70-108) will be given below and are listed in Table 1 [172-183].

Pyrrole condensation reaction may also be employed for the preparation of dodecasubstituted porphyrins. Note, these highly substituted porphyrins are nonplanar and thus have bathochromically shifted absorption bands and altered excited state properties [135]. For example, the tetrabenzoporphyrin 113 bearing glucosyl moieties on the \( \alpha \alpha \alpha \alpha \) positions was synthesized from tetrahydroisoindole and glucosylaldehyde in 36 % yield [184]. Followed by zinc metal complexation (62 %) and oxidative aromatization with DDQ to afford 112, this could be demetallated and deacetylated to 113 (Scheme 5). In addition, 5,10,15,20-tetraphenylecarboxytetrabenzo[porphyrin 109b] was glycosylated via addition of 2,20-(ethylenedioxy)-bis-ethylamine as a linker which was substituted with triacetyl lactone and subsequently deprotected affording 111. All compounds display a high degree of photostability at light of fluence rate 2.5 mW/cm\(^2\) and absence of photobleaching after 1 h. The glycobenzoporphyrins 111 and 113 were found to be as efficient as HP in producing \( 1O_2 \) [184].
Figure 2.
Table 1. Symmetric A₄-type (5,10,15,20-tetrasubstituted) glycoporphyrins and reference compounds.

<table>
<thead>
<tr>
<th>Entry #</th>
<th>Metal</th>
<th>Comments</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>2H</td>
<td>Condensation of pyrrole and 2-(AcOGlcO)-C₆H₄CHO, CH₂Cl₂, BF₃•OEt₃, then DDQ; Obtained as αβαβ, αβαβ, ααβα isomers in 2, 7, and 17 %, respectively.</td>
<td>[160,255]</td>
</tr>
<tr>
<td>2</td>
<td>2H</td>
<td>From 25 NaOMe/MeOH, Obtained as αβαβ, αβαβ, ααβα isomers in 90, 80 and 100 %, respectively.</td>
<td>[160]</td>
</tr>
<tr>
<td>3</td>
<td>2H</td>
<td>Condensation of pyrrole and 2-(AcOMalO)-C₆H₄CHO, CH₂Cl₂, BF₃•OEt₃, then of p-chloranil; 10 %.</td>
<td>[160]</td>
</tr>
<tr>
<td>4</td>
<td>2H</td>
<td>From 27 NaOMe/MeOH; 90 %.</td>
<td>[160]</td>
</tr>
<tr>
<td>5</td>
<td>2H</td>
<td>Condensation of pyrrole and 4-(AcOGlcO)-C₆H₄CHO, CH₂Cl₂, BF₃•OEt₃, then p-chloranil; best: 28 %.</td>
<td>[162,167,225, 207]</td>
</tr>
<tr>
<td>6</td>
<td>2H</td>
<td>From 29 NaOMe/MeOH or condensation and in situ cleavage; 64-88 %.</td>
<td>[162,160, 163,165, 342]</td>
</tr>
<tr>
<td>7</td>
<td>2H</td>
<td>Condensation of pyrrole and 4-(AcOGalO)-C₆H₄CHO, CH₂Cl₂, BF₃•OEt₃, then p-chloranil; 20 %.</td>
<td>[162,167]</td>
</tr>
<tr>
<td>8</td>
<td>2H</td>
<td>From 31 NaOMe/MeOH; 88 %.</td>
<td>[162,165,167]</td>
</tr>
<tr>
<td>9</td>
<td>2H</td>
<td>Condensation of pyrrole and 4-(AcOMalO)-C₆H₄CHO, CH₂Cl₂, BF₃•OEt₃, then addition of p-chloranil; 10 %.</td>
<td>[162]</td>
</tr>
<tr>
<td>10</td>
<td>2H</td>
<td>From 33 NaOMe/MeOH; 96 %.</td>
<td>[162]</td>
</tr>
<tr>
<td>11</td>
<td>2H</td>
<td>Condensation of pyrrole and 4-(AcGlcNaeO)-C₆H₄CHO, CH₂Cl₂, BF₃•OEt₃, then p-chloranil; 15 %.</td>
<td>[162]</td>
</tr>
<tr>
<td>12</td>
<td>2H</td>
<td>From 35 NaOMe/MeOH; 97 %.</td>
<td>[162,167]</td>
</tr>
<tr>
<td>13</td>
<td>2H</td>
<td>From 37 NaOMe/MeOH; 97 %.</td>
<td>[162]</td>
</tr>
<tr>
<td>14</td>
<td>2H</td>
<td>Condensation of pyrrole and 4-(AcOcelO)-C₆H₄CHO, CH₂Cl₂, BF₃•OEt₃, then p-chloranil, then NaOMe/MeOH.</td>
<td>[163]</td>
</tr>
<tr>
<td>15</td>
<td>2H</td>
<td>Condensation of pyrrole and 3-(AcOGlcO)-C₆H₄CHO, CHCl₃, BF₃•OEt₃, Zn(OAc)₂, then p-chloranil and 4M HCl; 68 %. NaOMe/MeOH → 39; 96 %.</td>
<td>[163,342]</td>
</tr>
<tr>
<td>16</td>
<td>2H</td>
<td>Like entry 15 with 3-(AcGlcNaeO)-C₆H₄CHO; 57 %. NaOMe/MeOH → 40; 76 %.</td>
<td>[165]</td>
</tr>
<tr>
<td>17</td>
<td>2H</td>
<td>Like entry 15 with 3-(AcOxylo)-C₆H₄CHO; 86 %. NaOMe/MeOH → 41; 91 %.</td>
<td>[165]</td>
</tr>
<tr>
<td>18</td>
<td>2H</td>
<td>Like entry 15 with 4-(AcOxylo)-C₆H₄CHO; 64 %. NaOMe/MeOH → 42; 94 %.</td>
<td>[163,342,160]</td>
</tr>
<tr>
<td>19</td>
<td>2H</td>
<td>Like entry 15 with 3-(AcOaraO)-C₆H₄CHO; 37 %. NaOMe/MeOH → 43; 98 %.</td>
<td>[165]</td>
</tr>
<tr>
<td>20</td>
<td>2H</td>
<td>Like entry 15 with 4-(AcOaraO)-C₆H₄CHO; 63 %. NaOMe/MeOH → 44; 96 %.</td>
<td>[165]</td>
</tr>
<tr>
<td>21</td>
<td>2H</td>
<td>Condensation of pyrrole and 4-(AcOGlcO)-C₆H₄CHO, CHCl₃, BF₃•OEt₃, Zn(OAc)₂, then p-chloranil; 94 %. NaOMe/MeOH → 45; 93 %.</td>
<td>[163]</td>
</tr>
<tr>
<td>22</td>
<td>2H</td>
<td>Like entry 21 with 3-(AcOgalaC)-C₆H₄CHO; 93 %. NaOMe/MeOH → 46; 89 %.</td>
<td>[165]</td>
</tr>
<tr>
<td>23</td>
<td>2H</td>
<td>Like entry 21 with 3-(AcOGalaO)-C₆H₄CHO; 53 %. NaOMe/MeOH → 47; 90 %.</td>
<td>[165]</td>
</tr>
<tr>
<td>24</td>
<td>2H</td>
<td>Like entry 21 with 3-(AcOGalaO)-C₆H₄CHO; 72 %. NaOMe/MeOH → 48; 90 %.</td>
<td>[165]</td>
</tr>
<tr>
<td>25</td>
<td>2H</td>
<td>Like entry 21 with 3-(AcOGalaO)-C₆H₄CHO; 84 %. NaOMe/MeOH → 49; 98 %.</td>
<td>[165]</td>
</tr>
<tr>
<td>26</td>
<td>2H</td>
<td>Like entry 21 with 3-(AcOGalaO)-C₆H₄CHO; 84 %. NaOMe/MeOH → 50; 92 %.</td>
<td>[165]</td>
</tr>
<tr>
<td>27</td>
<td>2H</td>
<td>Like entry 21 with 4-(AcOGalaO)-C₆H₄CHO; 63 %. NaOMe/MeOH → 51; 98 %.</td>
<td>[165]</td>
</tr>
<tr>
<td>28</td>
<td>2H</td>
<td>Like entry 21 with 3-(AcOCelO)-C₆H₄CHO; 60 %. NaOMe/MeOH → 52; 96 %.</td>
<td>[165]</td>
</tr>
<tr>
<td>29</td>
<td>2H</td>
<td>Modified Lindsey condensation with 3,5-(di-AcOGlcO)-C₆H₄CHO in 32 %; deprotection with NaOMe/MeOH → 53; 100 %.</td>
<td>[167]</td>
</tr>
<tr>
<td>30</td>
<td>2H</td>
<td>Condensation of pyrrole and (Bn-isop-Xyl-Fur)-CHO, CH₂Cl₂, BF₃•OEt₃, then p-chloranil, 4 % yield.</td>
<td>[168]</td>
</tr>
<tr>
<td>31</td>
<td>2H</td>
<td>Like entry 30 with (Bn-isop-Glc-Fur)-CHO; 4 %.</td>
<td>[168]</td>
</tr>
<tr>
<td>32</td>
<td>2H</td>
<td>Like entry 30 with (GlcO)-C₆H₄CHO; 16 %.</td>
<td>[168]</td>
</tr>
<tr>
<td>33</td>
<td>2H</td>
<td>Unstable, not characterized.</td>
<td>[168]</td>
</tr>
<tr>
<td>34</td>
<td>2H</td>
<td>Unstable, not characterized.</td>
<td>[168]</td>
</tr>
<tr>
<td>35</td>
<td>2H</td>
<td>Pyrrole, 2,3-O-isopropylidene-o-glyceraldehyde, BF₃•OEt₃, CH₂Cl₂, then DDQ; 3 % αβαβ.</td>
<td>[169]</td>
</tr>
<tr>
<td>36</td>
<td>2H</td>
<td>Like entry 35; 4 %, αβαβ.</td>
<td>[169]</td>
</tr>
<tr>
<td>37</td>
<td>2H</td>
<td>Like entry 35 with (MeO-isop-Xyl-Fur)-CHO; 6 %, αβαβ.</td>
<td>[169]</td>
</tr>
<tr>
<td>38</td>
<td>2H</td>
<td>Like entry 35 with 2,3,4,5-di-O-isopropylidene-o-arabinose-tetritol-1-yl, 2 %, αβαβ.</td>
<td>[169]</td>
</tr>
</tbody>
</table>

XXX-XXX/14 $58.00 + 0.00 © 2014 Bentham Science Publishers
From 64: 50 % aq. TFA, CH₂Cl₂, r.t., sonication, 2 h; 90 %, αβαβ.

41 65 (BnGal)-CH₂  

2H

Pyrrole, (BnGal)-CH₂-CHO, BF₃·OEt₂, CH₂Cl₂, then DDQ, 18 %. [170]

42 66 (BnGluc)-CH₂  

2H

Like entry 42 with (BnGluc)-CHO; 36 %. [170]

43 67 (MeO-Xyl)-Fur  

2H

From 61: 75 % aq. TFA, CH₂Cl₂, sonication, 3 h; 90 %. [171]

44 68 (MeO-Xyl)-Fur  

M(II)

From 61: M(OAc), CH₂OH/CH₃Cl, (1:1, v/v), sonication, then 75 % aq. TFA, CH₂Cl₂, r.t., 3 h; 63-90 %; M = Pd(II), Cu(II), Ni(II), Zn(II).

45 69 (MeO-Xyl)-Fur  

2H

From 61: H₂, Pd 10 % on C, EtOAc–MeOH, r.t., 16 h; 98 %. [172]

46 70 4-(BnGluc)-C₆H₄  

2H

Pyrrole and 4-(BnGluc)-CH₂-CHO, BF₃·OEt₂, NaCl, r.t., 5 h, then DDQ, r.t., 30 min; 53 %. [172]

47 71 4-(Glc)-C₆H₄  

2H

From 61: H₂, Pd 10 % on C, EtOAc–MeOH, r.t.; 70 %. [172]

48 72 4-(Glc)-C₆H₄  

Cu(II)

From 61: H₂, Pd 10 % on C, EtOAc–MeOH, r.t.; 40 %. [172]

49 73 4-Pyridyl  

2H

Pyrrole, pentfluorobenzaldehyde, CH₂Cl₂, BF₃·OEt₂, 4 h then p-chloranil, reflux, 12 h. Zn(OAc)₂, MeOH, CH₂Cl₂, reflux, 10 h; 77 %. [177]

50 85 C₆F₅  

Pd(II)

From 83: 2,3,4,6-Tetra-O-acetyl-1-thio-β-β-glucopyranosyranoside, diethylamine, DMF, r.t., 24 h; 74 %. [178,177,206,296]

51 86 C₆F₅  

Pd(II)

From 83: 2,3,4,6-Tetra-O-acetyl-1-thio-β-β-glucopyranosyranoside, diethylamine, DMF, r.t., 24 h; 74 %. [178,177,206,296]

52 87 4-(AcOGlSc)-C₆F₅  

2H

From 83: 2,3,4,6-Tetra-O-acetyl-1-thio-β-β-glucopyranosyranoside, diethylamine, DMF, r.t., 24 h; 74 %. [178,177,206,296]

53 88 4-(AcOGlSc)-C₆F₅  

Zn(II)

From 84: Like entry 63; 74 %. [177]

54 89 4-(AcOGlSc)-C₆F₅  

Pd(II)

From 85: Like entry 63; 83 %. [177]

55 90 4-(AcOGlSc)-C₆F₅  

Pd(II)

From 86: Like entry 63; 85 %. [177]

56 91 4-(GlSc)-C₆F₅  

2H

From 87: NaOMe, MeOH, CH₂Cl₂, 43-50 °C, 1 h; 69 %. [178,177,206,296]

57 92 4-(GlSc)-C₆F₅  

Zn(II)

From 88: NaOMe, MeOH, CH₂Cl₂, 43-50 °C, 1 h; 81 %. [177]

58 93 4-(GlSc)-C₆F₅  

Pd(II)

From 89: Like entry 69; 68 %. [177]

59 94 4-(GlSc)-C₆F₅  

Pd(II)

From 90: Like entry 69; 70 %. [177]

60 95 4-(GlSc)-C₆F₅  

2H

From 91: NaOMe, MeOH, CH₂Cl₂, 43-50 °C, 1 h; 80 %. [178,247]

61 96 4-(AcOGal)-C₆F₅  

Zn(II)

Pyrrole, pentfluorobenzaldehyde, CH₂Cl₂, BF₃·OEt₂, 4 h then p-chloranil, reflux, 12 h. Zn(OAc)₂, MeOH, CH₂Cl₂, reflux, 10 h; 77 %. [177]

62 85 C₆F₅  

Pd(II)

From 83: 2,3,4,6-Tetra-O-acetyl-1-thio-β-β-glucopyranosyranoside, diethylamine, DMF, r.t., 24 h; 74 %. [178,177,206,296]

63 86 C₆F₅  

Pd(II)

From 83: 2,3,4,6-Tetra-O-acetyl-1-thio-β-β-glucopyranosyranoside, diethylamine, DMF, r.t., 24 h; 74 %. [178,177,206,296]

64 87 4-(AcOGlSc)-C₆F₅  

2H

From 83: 2,3,4,6-Tetra-O-acetyl-1-thio-β-β-glucopyranosyranoside, diethylamine, DMF, r.t., 24 h; 74 %. [178,177,206,296]

65 88 4-(AcOGlSc)-C₆F₅  

Zn(II)

From 84: Like entry 63; 74 %. [177]

66 89 4-(AcOGlSc)-C₆F₅  

Pd(II)

From 85: Like entry 63; 83 %. [177]

67 90 4-(AcOGlSc)-C₆F₅  

Pd(II)

From 86: Like entry 63; 85 %. [177]

68 91 4-(GlSc)-C₆F₅  

2H

From 87: NaOMe, MeOH, CH₂Cl₂, 43-50 °C, 1 h; 69 %. [178,177,206,296]

69 92 4-(GlSc)-C₆F₅  

Zn(II)

From 88: NaOMe, MeOH, CH₂Cl₂, 43-50 °C, 1 h; 81 %. [177]

70 93 4-(GlSc)-C₆F₅  

Pd(II)

From 89: Like entry 69; 68 %. [177]

71 94 4-(GlSc)-C₆F₅  

Pd(II)

From 90: Like entry 69; 70 %. [177]

72 95 4-(GlSc)-C₆F₅  

2H

From 91: NaOMe, MeOH, CH₂Cl₂, 43-50 °C, 1 h; 80 %. [178,247]

73 96 4-(AcOGal)-C₆F₅  

Zn(II)

Pyrrole, pentfluorobenzaldehyde, CH₂Cl₂, BF₃·OEt₂, 4 h then p-chloranil, reflux, 12 h. Zn(OAc)₂, MeOH, CH₂Cl₂, reflux, 10 h; 77 %. [177]
2.3.2 Unsymmetrically Substituted Glycoporphyrins

Mixed condensations have been widely used for the construction of unsymmetrical glycoporphyrins of the A3B-, A2B2-, A2BC and ABCD-type (Figure 3) [160,185]. Most of the relevant compounds are listed in Table 2. Condensations with two or more different aldehydes with pyrrole can yield a variety of porphyrins including all the A3B-, A2B2-, A2BC and ABCD-types. Selectivity for a particular array can be achieved using specific equivalents of aldehyde versus pyrrole. Reactions using Lindsey’s [2+2] and [3+1] methods can afford large scale syntheses of all types of substituted porphyrins [137,139,186].

A classic study in this context is Maillard’s work from 1995 [162]. After the synthesis of the symmetric compounds 29-36 the importance of the amphiphilicity of such structures was becoming apparent and their synthetic studies then focused on mixed meso (glycosylated aryl) aryl porphyrins and mixed meso (glycosylated aryl) alky porphyrins bearing the same glycosyl moieties as the tetrasubstituted TPP’s studied earlier (114-119). Optimized condensation conditions provided primarily the di- and triglycosylated derivatives and minimized the tetrasubstituted compounds.

The behavior of such tetra-, tri- and diglycosylated meso-substituted porphyrins 114-119 and 30 were examined in aqueous media by absorbance and fluorescence spectroscopy [162,187]. All dimerized in aqueous solutions with the dimers stability dependent upon the substitution pattern. The diglycosylated derivative 115 was the most stable dimer with an offset stacked geometry similar to that reported for the dimer of an aminoglucosamide protoporphyrin in solution [31,187]. The fluorescence of all dimers was quenched, except for the 5,10-diglycosylated derivative 115. A linear dependence was determined between the number of the aryl groups of the glucosylated porphyrins and the singlet oxygen formation, with yields of singlet oxygen in the range 0.54-0.81.

A similar study by Gaud et al. [183] provided 13 meso glycosylporphyrins using mixed pyrrole condensation reactions and/or direct glycosylations of α- and ρ-hydroxyalkoxyarylporphyrins. The series included AB3- (120-127), A3B- (128), 5,10-A2B2- (129), 5,15-A2B2- (130), and A4-type (107,108) porphyrins with A being either glucose, ribose, maltose or lactose residues. In all cases a β-configuration was found after basic deprotection. Due to the use of mixed condensation reactions for the underlying porphyrin framework yields were limited. Nevertheless, this presented a significant body of work and gave the first possibility for QSAR studies investigating either the influence of different sugar units and the regiochemistry and number thereof. Glycoporphyrins derived from simple mixed condensation can also serve as precursors for more complex

![Scheme 5. Synthesis of glycoconjugated tetrabenzoporphyrins.](image-url)
Figure 3. Synthesis of unsymmetric ABCD-type glycoporphyrins via mixed condensation reactions.

target systems. For example, following on from their work on glycosylated haematoporphyrins \[188\], \[see below\], Krausz et al. synthesized a range of amphiphilic cationic glycoporphyrins of the \(A_3B\)-type \[189\]. Here, the amphiphilicity was introduced via varying lipophilic \(N\)-substituted entities including methyl, isopropyl, \(n\)-octyl groups. First, 5-(2-tetraacetyl-\(\beta\)-D-glucopyranosylphenyl)-10,15,20-tris(4-pyridyl)porphyrin 131 was synthesized via condensation of glycosyl benzaldehyde, 4-pyridinecarboxaldehyde and pyrrole in 7 % yield. Next, compound 131 was alkylated at the pyridyl-\(N\) using large excesses of methyl, isopropyl and \(n\)-octyl iodides affording 132-135 as the triiodides in 85, 90 and 75 % yield, respectively. Finally, all derivatives were deprotected providing 135-138.

This work was later extended to the synthesis of a whole range of unsymmetrical free base cationic \(O\)-linked glycoporphyrins based on 5,10,15-tris(4-alkyl...
Table 2. Unsymmetric ABCD-type (5-A,10-B,15-C,20-D-substituted) glycoporphyrins and reference compounds.

<table>
<thead>
<tr>
<th>Entry</th>
<th>A substituent</th>
<th>B substituent</th>
<th>C substituent</th>
<th>D substituent</th>
<th>Metal</th>
<th>Comments</th>
</tr>
</thead>
</table>
| 1     | 4-(GlcO)-C₆H₄- | 4-(GlcO)-C₆H₄- | 4-(GlcO)-C₆H₄- | Phenyl        | 2H    | a) Pyrrole, 4-(AcOGlC)=C₆H₄-CHO, benzaldehyde, CHCl₃, BF₃•OEt₂, r.t., 24 h, then p-chloranil, 1 h, reflux; 10 %; b) NaOMe, MeOH, 1 h, r.t.; 83 %.
| 2     | 4-(GlcO)-C₆H₄- | 4-(GlcO)-C₆H₄- | n-Butyl       | n-Butyl       | 2H    | Like entry 1 with n-pentanal. a) 3 %; b) 82 %.
| 3     | 4-(GlcO)-C₆H₄- | 4-(GlcO)-C₆H₄- | 4-(GlcO)-C₆H₄- | C₆F₅-        | 2H    | Like entry 1 with C₆F₅-CHO. a) 3 %; b) 87 %.
| 4     | 4-(GlcO)-C₆H₄- | 4-(GlcO)-C₆H₄- | n-Butyl       | n-Butyl       | 2H    | Like entry 1 with n-pentanal. a) 1 %; b) 78 %.
| 5     | 4-(GlcO)-C₆H₄- | 4-(GlcO)-C₆H₄- | n-Butyl       | n-Butyl       | 2H    | Like entry 1 with n-pentanal. a) 2 %; b) 82 %.
| 6     | 4-(GlcO)-C₆H₄- | 4-(GlcO)-C₆H₄- | n-Butyl       | n-Butyl       | 2H    | Like entry 1 with n-pentanal. a) 2 %. b) NaOMe, MeOH, CH₂Cl₂, 1 h; 80 %.
| 7     | 2-[GlcO-(CH₂)₃-O]-C₆H₄- | 4-Me-C₆H₄- | 4-Me-C₆H₄- | 2H | Like entry 1 with 5-[2-(3-Hydroxypropoxyphenyl)-10,15,20-tritolyporphyrin, 1,2,3,4,6-penta-O-acetyl-β-α-gluconopyranosyl, SnCl₃, CH₂Cl₂, 18 h, 35 %; b) NaOMe, MeOH, CH₂Cl₂, 1 h; 80 %.
| 8     | 4-(GlcO)-(CH₂)₃-O)-C₆H₄- | 4-Me-C₆H₄- | 4-Me-C₆H₄- | 2H    | Like entry 7 with 5-[4-(3-hydroxypropoxyphenyl)-10,15,20-tritolyporphyrin. a) 2 h; 60 %; b) 80 %.
| 9     | 2-[RbOH-(CH₂)₃-O]-C₆H₄- | 4-Me-C₆H₄- | 4-Me-C₆H₄- | 2H    | Like entry 7 with O-acetyl-2,3,5-tribenzoate-β-α-ribofurano. a) 16 h; 60 %; b) 85 %.
| 10    | 4-[RbOH-(CH₂)₃-O]-C₆H₄- | 4-Me-C₆H₄- | 4-Me-C₆H₄- | 2H    | Like entry 9 with 5-[4-(3-hydroxypropoxyphenyl)-10,15,20-tritolyporphyrin. a) 2 h; 75 %; b) 85 %.
| 11    | 2-[MaltO-(CH₂)₃-O]-C₆H₄- | 4-Me-C₆H₄- | 4-Me-C₆H₄- | 2H    | Like entry 7 with 2,3,6,2,3,4,5,6-hepta-O-acetyl-β-α-maltopyranosyl. a) 24 h; 12 %; b) 85 %.
| 12    | 4-[MaltO-(CH₂)₃-O]-C₆H₄- | 4-Me-C₆H₄- | 4-Me-C₆H₄- | 2H    | Like entry 8 with 2,3,6,2,3,4,5,6-hepta-O-acetyl-β-α-maltopyranosyl. a) 24 h; 6 %; b) 90 %.
| 13    | 2-[LacO-(CH₂)₃-O]-C₆H₄- | 4-Me-C₆H₄- | 4-Me-C₆H₄- | 2H    | Like entry 7 with 2,3,6,2,3,4,5,6-hepta-O-acetyl-β-α-lactopyranosyl. a) 24 h; 21 %; b) 85 %.
| 14    | 4-[LacO-(CH₂)₃-O]-C₆H₄- | 4-Me-C₆H₄- | 4-Me-C₆H₄- | 2H    | Like entry 8 with 2,3,6,2,3,4,5,6-hepta-O-acetyl-β-α-lactopyranosyl. a) 6 h; 42 %; b) 85 %.
| 15    | 2-[GlcO-(CH₂)₃-O]-C₆H₄- | 4-(GlcO)-(CH₂)₃-O)-C₆H₄- | 4-(GlcO)-(CH₂)₃-O)-C₆H₄- | 2H | a) 2 × Pyrrole, 3 × 4-(AcOGLc)=C₆H₄-CHO, 1 × 4-Me-C₆H₄-CHO, EtCOOH/AcO, 3 %; b) NaOMe, MeOH, CH₂Cl₂, 1 h; 80 %.
| 16    | 2-[GlcO-(CH₂)₃-O]-C₆H₄- | 4-(GlcO)-(CH₂)₃-O)-C₆H₄- | 4-(GlcO)-(CH₂)₃-O)-C₆H₄- | 2H | Like entry 7 with 5-[2-(3-Hydroxypropoxyphenyl)-10,15,20-tritolyporphyrin, 1,2,3,4,6-penta-O-acetyl-β-α-gluconopyranosyl, SnCl₃, CH₂Cl₂, 18 h, 35 %; b) NaOMe, MeOH, CH₂Cl₂, 1 h; 80 %.
| 17    | 4-(GlcO)-(CH₂)₃-O)-C₆H₄- | 4-Me-C₆H₄- | 4-Me-C₆H₄- | 2H    | Like entry 6; 2 % mixture of 5,10 and 5,15 regioisomers b) NaOMe, MeOH, CH₂Cl₂, 1 h; 75 %.
| 18    | 2-[AcOGLc)=C₆H₄- | 4-Pyrindyl | 4-Pyrindyl | 2H    | Pyrrole, 4-pyridinecarboxaldehyde, 2-(AcOGLc)=C₆H₄-CHO, EtCOOH/AcO, reflux; 7 %.
| 19    | 2-[AcOGLc)=C₆H₄- | 4-N-Me-4-pyrindyl | N-Me-4-pyrindyl | 2H | From 131: Mel, DMF, Δ; 85 %.
| 20    | 2-[AcOGLc)=C₆H₄- | 4-N-Pr-4-pyrindyl | N-Pr-4-pyrindyl | 2H | From 131: Isopropyl iodide, DMF, Δ; 90 %.
| 21    | 2-[AcOGLc)=C₆H₄- | 4-N-Octyl-4-pyrindyl | N-Octyl-4-pyrindyl | 2H | From 131: α-octyl iodide, DMF, Δ; 75 %.
| 22    | 2-[AcOGLc)=C₆H₄- | 4-N-Me-4-pyrindyl | N-Me-4-pyrindyl | 2H | From 132: EtN-MeOH-H₂O (10/10/1, v/v/v), 0 °C.
| 23    | 2-[AcOGLc)=C₆H₄- | 4-N-Pr-4-pyrindyl | N-Pr-4-pyrindyl | 2H | From 133: Like entry 22.
| 24    | 2-[GlO)=C₆H₄- | 4-N-Octyl-4-pyrindyl | N-Octyl-4-pyrindyl | 2H | From 134: Like entry 22.
| 25    | 2-[GlO)=C₆H₄- | 4-Pyrindyl | 4-Pyrindyl | 2H | From 135: Like entry 22.
| 26    | 4-[GlO)=C₆H₄- | 4-Pyrindyl | 4-Pyrindyl | 2H | From 136: Like entry 22.
| 27    | 4-[GlO)=C₆H₄- | 4-Pyrindyl | 4-Pyrindyl | 2H | From 137: Like entry 22.
| 28    | 4-[GlO)=C₆H₄- | 4-Pyrindyl | 4-Pyrindyl | 2H | From 138: Like entry 22.
| 29    | 4-[GlO)=C₆H₄- | 4-Pyrindyl | 4-Pyrindyl | 2H | From 139: Like entry 22.
| 30    | 4-[GlO)=C₆H₄- | 4-Pyrindyl | 4-Pyrindyl | 2H | From 139: Like entry 22.
| 31    | 4-[GlO)=C₆H₄- | 4-Pyrindyl | 4-Pyrindyl | 2H | From 139: Like entry 22.
| Entry | Compound 1 | Compound 2 | Compound 3 | Yield (%)
|-------|------------|------------|------------|---------
| 32    | 145 4-(GlcO)-C₆H₄-N\(^{\text{N}}\)-Pr-4-pyridyl | 4-(GlcO)-C₆H₄-N\(^{\text{N}}\)-Octyl-4-pyridyl | 4-(GlcO)-C₆H₄-N\(^{\text{N}}\)-Octyl-4-pyridyl | 91 |
| 33    | 146 4-(GlcO)-C₆H₄-N\(^{\text{N}}\)-Octyl-4-pyridyl | 4-(GlcO)-C₆H₄-N\(^{\text{N}}\)-Octyl-4-pyridyl | 4-(GlcO)-C₆H₄-N\(^{\text{N}}\)-Octyl-4-pyridyl | 90 |
| 34    | 147 2-(AcOMalO)-C₆H₄-4-Pyridyl | 4-Pyridyl | 4-Pyridyl | 7 |
| 35    | 148 4-(AcOMalO)-C₆H₄-4-Pyridyl | 4-Pyridyl | 4-Pyridyl | 7 |
| 36    | 149 2-(MalO)-C₆H₄-4-Pyridyl | 4-Pyridyl | 4-Pyridyl | 92 |
| 37    | 150 4-(MalO)-C₆H₄-4-Pyridyl | 4-Pyridyl | 4-Pyridyl | 92 |
| 38    | 151 2-(AcOLacO)-C₆H₄-4-Pyridyl | 4-Pyridyl | 4-Pyridyl | 6 |
| 39    | 152 4-(AcOLacO)-C₆H₄-4-Pyridyl | 4-Pyridyl | 4-Pyridyl | 7 |
| 40    | 153 2-(LacO)-C₆H₄-4-Pyridyl | 4-Pyridyl | 4-Pyridyl | 92 |
| 41    | 154 4-(LacO)-C₆H₄-4-Pyridyl | 4-Pyridyl | 4-Pyridyl | 92 |
| 42    | 155 2-(AcOMalO)-C₆H₄-4-Pyridyl | N-Me-4-pyridyl | N-Me-4-pyridyl | 93 |
| 43    | 156 4-(AcOMalO)-C₆H₄-4-Pyridyl | N-Me-4-pyridyl | N-Me-4-pyridyl | 93 |
| 44    | 157 2-(MalO)-C₆H₄-4-Pyridyl | N-Me-4-pyridyl | N-Me-4-pyridyl | 94 |
| 45    | 158 4-(MalO)-C₆H₄-4-Pyridyl | N-Me-4-pyridyl | N-Me-4-pyridyl | 94 |
| 46    | 159 2-(AcOLacO)-C₆H₄-4-Pyridyl | N-Me-4-pyridyl | N-Me-4-pyridyl | 94 |
| 47    | 160 4-(AcOLacO)-C₆H₄-4-Pyridyl | N-Me-4-pyridyl | N-Me-4-pyridyl | 94 |
| 48    | 161 2-(LacO)-C₆H₄-4-Pyridyl | N-Me-4-pyridyl | N-Me-4-pyridyl | 94 |
| 49    | 162 4-(LacO)-C₆H₄-4-Pyridyl | N-Me-4-pyridyl | N-Me-4-pyridyl | 94 |
| 50    | 163 4-(AcOGlO)-C₆H₄-4-Pyridyl | 4-(AcOGlO)-C₆H₄-4-Pyridyl | 4-(AcOGlO)-C₆H₄-2HO-C₆H₄-2H | 92 |
| 51    | 164 4-(AcOGlO)-C₆H₄-4-Pyridyl | 4-(AcOGlO)-C₆H₄-4-Pyridyl | 4-(AcOGlO)-C₆H₄-4-HO-C₆H₄-2H | 92 |
| 52    | 165 4-(AcOGlO)-C₆H₄-4-Pyridyl | 4-(AcOGlO)-C₆H₄-4-Pyridyl | 2-(diCH₂O)-O-C₆H₄-2H | 80-85 |
| 53    | 166 4-(AcOGlO)-C₆H₄-4-Pyridyl | 4-(AcOGlO)-C₆H₄-4-Pyridyl | 4-(diCH₂O)-O-C₆H₄-2H | 80-85 |
| 54    | 167 4-(AcOGlO)-C₆H₄-4-Pyridyl | 4-(AcOGlO)-C₆H₄-4-Pyridyl | 4-(AcOGlO)-C₆H₄-4-Pyridyl | 92 |
| 55    | 168 4-(GlcO)-C₆H₄-4-Pyridyl | 4-(GlcO)-C₆H₄-4-Pyridyl | 4-(GlcO)-C₆H₄-4-Pyridyl | 92 |
| 56    | 169 4-(AcOLacO)-C₆H₄-4-Pyridyl | H | 4-(AcOLacO)-C₆H₄-4-Pyridyl | 92 |
| 57    | 170 4-MeO-C₆H₄-4-Pyridyl | H | 4-MeO-C₆H₄-4-Pyridyl | 92 |
| 58    | 171 4-(AcOLacO)-C₆H₄-4-Pyridyl | H | 4-(AcOLacO)-C₆H₄-4-Pyridyl | 78 |
| 59    | 172 4-MeO-C₆H₄-4-Pyridyl | H | 4-MeO-C₆H₄-4-Pyridyl | 78 |
| 60    | 173 4-(LacO)-C₆H₄-4-Pyridyl | H | 4-(LacO)-C₆H₄-4-Pyridyl | 69 |
| 61    | 174 1,2,4-Tris-2-benzyl-2,2,6,7-tetramethoxybiphenyl-1-yl | 4-F-C₆H₄-2H | 1,2,4-tris-2-benzyl-2,2,6,7-tetramethoxybiphenyl-1-yl | 80 |
| 62    | 175 (α-BnOGal)-CH₂-C₂F₇ | C₂F₇ | (α-BnOGal)-CH₂-C₂F₇ | 96 |
| 63    | 176 (α-BnOGlcC)-C₂F₇ | C₂F₇ | (α-BnOGlcC)-C₂F₇ | 96 |
| 64    | 177 (α-Gal)-CH₂-C₂F₇ | C₂F₇ | (α-Gal)-CH₂-C₂F₇ | 96 |
65 178 4-(β-BnOGlcC)-C6H4-Phenyl Pheny1 Phenyl Phenyl 2H 4 × Pyrrole + 3 benzaldehyde + 1.4-(β-BnOGlcC)-C6H4-CHO, BF3·OEt2, NaCl, r.t., 5 h, then DDQ, r.t., 30 min.; 15 %.

From 178: H2, Pd 10 % on carbon, EtOAc–MeOH, r.t., 16 h; 98 %.

[172]

66 179 4-(β-GlcC)-C6H4-4-Me-C6H4-4-Me-C6H4-2H 4 × Pyrrole + 3 benzaldehyde + 1.4-(β-BnOGlcC)-C6H4-CHO, BF3·OEt2, NaCl, r.t., 5 h, then DDQ, r.t., 30 min.; 15 %.

From 178: MeLi, Gal-isop-H, THF, 8 h, 80–90 °C; 38 %.

[197]

67 180 4-(β-GlcC)-C6H4-4-Me-C6H4-4-Me-C6H4-2H 4 × Pyrrole + 3 benzaldehyde + 1.4-(β-BnOGlcC)-C6H4-CHO, BF3·OEt2, NaCl, r.t., 5 h, then DDQ, r.t., 30 min.; 15 %.

From 180: MeLi, Gal-isop-H, THF, 5 h, 80–90 °C; 38 %.

From 181: MeLi, Gal-isop-H, THF, 2.5 h, 80–90 °C; 50 %.

[197]

68 181 4-(β-GlcC)-C6H4-4-Me-C6H4-4-Me-C6H4-2H 4 × Pyrrole + 3 benzaldehyde + 1.4-(β-BnOGlcC)-C6H4-CHO, BF3·OEt2, NaCl, r.t., 5 h, then DDQ, r.t., 30 min.; 15 %.

From 181: MeLi, Gal-isop-H, THF, 2.5 h, 80–90 °C; 50 %.

[197]

69 182 4-(β-GlcC)-C6H4-4-Me-C6H4-4-Me-C6H4-2H 4 × Pyrrole + 3 benzaldehyde + 1.4-(β-BnOGlcC)-C6H4-CHO, BF3·OEt2, NaCl, r.t., 5 h, then DDQ, r.t., 30 min.; 15 %.

From 182: Like entry 73; 28 %.

[197]

70 183 4-(β-GlcC)-C6H4-4-Me-C6H4-4-Me-C6H4-2H 4 × Pyrrole + 3 benzaldehyde + 1.4-(β-BnOGlcC)-C6H4-CHO, BF3·OEt2, NaCl, r.t., 5 h, then DDQ, r.t., 30 min.; 15 %.

From 183: Like entry 73, 5 h; 7 %.

[197]

71 184 4-(β-GlcC)-C6H4-4-Me-C6H4-4-Me-C6H4-2H 4 × Pyrrole + 3 benzaldehyde + 1.4-(β-BnOGlcC)-C6H4-CHO, BF3·OEt2, NaCl, r.t., 5 h, then DDQ, r.t., 30 min.; 15 %.

From 184: Like entry 73, 4.5 h, 80–90 °C; 5 %.

[197]

72 185 4-(β-GlcC)-C6H4-4-Me-C6H4-4-Me-C6H4-2H 4 × Pyrrole + 3 benzaldehyde + 1.4-(β-BnOGlcC)-C6H4-CHO, BF3·OEt2, NaCl, r.t., 5 h, then DDQ, r.t., 30 min.; 15 %.

From 185: Like entry 73, 5 h; 7 %.

[197]

73 186 4-(β-GlcC)-C6H4-4-Me-C6H4-4-Me-C6H4-2H 4 × Pyrrole + 3 benzaldehyde + 1.4-(β-BnOGlcC)-C6H4-CHO, BF3·OEt2, NaCl, r.t., 5 h, then DDQ, r.t., 30 min.; 15 %.

From 186: Like entry 73, 4.5 h, 80–90 °C; 5 %.

[197]
214 4-HO-C6H4- 4-Me-C6H4- 4-Me-C6H4- 4-Me-C6H4- 2H From 101
215 2-(OC3H6-NH-Boc)-C6H4- 4-Me-C6H4- 4-Me-C6H4- 4-Me-C6H4- 2H From 102
216 4-(OC3H6-NH-Boc)-C6H4- 4-Me-C6H4- 4-Me-C6H4- 4-Me-C6H4- 2H From 103
217 2-(OC3H6-NH3)-C6H4- 4-Me-C6H4- 4-Me-C6H4- 4-Me-C6H4- 2H From 104
218 4-(OC3H6-NH)-C6H4- 4-Me-C6H4- 4-Me-C6H4- 4-Me-C6H4- 2H From 105
219 2-OglC(OAc-2'-OH)-C6H4- 4-Me-C6H4- 4-Me-C6H4- 4-Me-C6H4- 2H From 106
220 4-(OglC(OAc-2'-OH)-C6H4- 4-Me-C6H4- 4-Me-C6H4- 4-Me-C6H4- 2H From 107
221 2-OglC(OAc-2'-OH)-OglC(OH)-2'(OH)-C6H4- 4-Me-C6H4- 4-Me-C6H4- 4-Me-C6H4- 2H From 108
222 4-(OglC(OAc-2'-OH)-OglC(OH)-2'(OH)-C6H4- 4-Me-C6H4- 4-Me-C6H4- 4-Me-C6H4- 2H From 109
223 2-(Br(CH3)-O)-C6H4- 4-Me-C6H4- 4-Me-C6H4- 4-Me-C6H4- 2H From 110
224 4-Bt(CH3)-O)-C6H4- 4-Me-C6H4- 4-Me-C6H4- 4-Me-C6H4- 2H From 111
225 2-[AcOGalS-(CH2)3-O]-C6H4- 4-Me-C6H4- 4-Me-C6H4- 4-Me-C6H4- 2H From 112
226 4-[AcOGalS-(CH2)3-O]-C6H4- 4-Me-C6H4- 4-Me-C6H4- 4-Me-C6H4- 2H From 113
227 2-[AcOGalS-(CH2)3-O]-C6H4- 4-Me-C6H4- 4-Me-C6H4- 4-Me-C6H4- 2H From 114
228 4-[AcOGlcS-(CH2)3-O]-C6H4- 4-Me-C6H4- 4-Me-C6H4- 4-Me-C6H4- 2H From 115
229 2-[AcOGlcS-(CH2)3-O]-C6H4- 4-Me-C6H4- 4-Me-C6H4- 4-Me-C6H4- 2H From 116
230 4-[AcOManS-(CH2)3-O]-C6H4- 4-Me-C6H4- 4-Me-C6H4- 4-Me-C6H4- 2H From 117
231 4-[AcOManS-(CH2)3-O]-C6H4- 4-Me-C6H4- 4-Me-C6H4- 4-Me-C6H4- 2H From 118
232 2-[GalS-(CH2)3-O]-C6H4- 4-Me-C6H4- 4-Me-C6H4- 4-Me-C6H4- 2H From 119
233 2-[GalS-(CH2)3-O]-C6H4- 4-Me-C6H4- 4-Me-C6H4- 4-Me-C6H4- 2H From 120
234 2-[GlcS-(CH2)3-O]-C6H4- 4-Me-C6H4- 4-Me-C6H4- 4-Me-C6H4- 2H From 121
235 2-[GlcS-(CH2)3-O]-C6H4- 4-Me-C6H4- 4-Me-C6H4- 4-Me-C6H4- 2H From 122
236 2-[ManS-(CH2)3-O]-C6H4- 4-Me-C6H4- 4-Me-C6H4- 4-Me-C6H4- 2H From 123
237 2-[AcOGlc(S)-Cf2]-Cf2- 2-[AcOGlc(S)-Cf2]-Cf2- 2-[AcOGlc(S)-Cf2]-Cf2- 2H From 124
238 4-(GlcS)-Cf2- 4-(GlcS)-Cf2- 4-(GlcS)-Cf2- 2H Like entry 125; 19 %. [200]
239 4-(AcOGlcS)-Cf2- 4-(AcOGlcS)-Cf2- 4-(AcOGlcS)-Cf2- 2H Like entry 126; 19 %. [200]
240 4-(GlcS)-Cf2- 4-(GlcS)-Cf2- 4-(GlcS)-Cf2- 2H Like entry 127; 19 %. [200]
241 4-(AcOGlcS)-Cf2- 4-(AcOGlcS)-Cf2- 4-(AcOGlcS)-Cf2- 2H Like entry 128; 19 %. [200]
242 4-(GlcS)-Cf2- 4-(GlcS)-Cf2- 4-(GlcS)-Cf2- 2H Like entry 129; 19 %. [200]
243 4-(AcOGlcS)-Cf2- 4-(AcOGlcS)-Cf2- 4-(AcOGlcS)-Cf2- 2H Like entry 130; 19 %. [200]
244 4-(GlcS)-Cf2- 4-(GlcS)-Cf2- 4-(GlcS)-Cf2- 2H Like entry 131; 19 %. [200]
245 3,5-(di-GalCONH-O)-C6H4- Phenyl Phenyl Phenyl Phenyl 2H From 132
246 3,5-(di-GlCCONH-O)-C6H4- Phenyl Phenyl Phenyl Phenyl 2H From 133
247 4-(AcOGlcNH-CO)-C6H4- Phenyl Phenyl Phenyl Phenyl 2H From 134
248 4-(AcOGlcNH-CO)-C6H4- Phenyl Phenyl Phenyl Phenyl 2H From 135
249 4-(BnOGlc-6-NH)-Cf2- 4-(BnOGlc-6-NH)-Cf2- 4-(BnOGlc-6-NH)-Cf2- 2H Like entry 136; 14 %. [200]
<table>
<thead>
<tr>
<th>Entry</th>
<th>Formula</th>
<th>Product</th>
<th>Note</th>
</tr>
</thead>
<tbody>
<tr>
<td>138</td>
<td>C6F4-</td>
<td>C6F4-</td>
<td>2H</td>
</tr>
<tr>
<td>139</td>
<td>4-(BnOGlc-6-NH)-C6F4-</td>
<td>C6F4-</td>
<td>Like entry 136; 7 %</td>
</tr>
<tr>
<td>140</td>
<td>4-(BnOGlc-6-NH)-C6F4-</td>
<td>C6F4-</td>
<td>Like entry 136; 8 %</td>
</tr>
<tr>
<td>141</td>
<td>4-(BnOGlc-6-NH)-C6F4-</td>
<td>C6F4-</td>
<td>Zn(II) Zn(II)TPPF2s, methyl 6-amino-2,3,4-tri-O-benzyl-6-deoxy-a-L-glucopyranoside, 1,2,4-trichlorobenzene, reflux, 5 h; 43 %</td>
</tr>
<tr>
<td>142</td>
<td>4-(BnOGlc-6-NH)-C6F4-</td>
<td>C6F4-</td>
<td>Like entry 140; 5 %</td>
</tr>
<tr>
<td>143</td>
<td>4-(BnOGlc-6-NH)-C6F4-</td>
<td>C6F4-</td>
<td>Like entry 140; 1 %</td>
</tr>
<tr>
<td>144</td>
<td>4-(N-(GalO)-4'-(OCH2)-1',2',3'-(triazol-1'-yl)-C6H4)-C6H4</td>
<td>4-HO-C6H4</td>
<td>i) Propargyl bromide, K₂CO₃, 60 °C, 24 h; ii) Zn(OAc)₂, CH₃OH,CHCl₃, reflux 1 h; 28 %; iii) Gal-N₃, CuSO₄•5H₂O, sodium ascorbate, t-BuOH/H₂O (1:1,v/v), 70 °C, 24 h; 92 %</td>
</tr>
<tr>
<td>145</td>
<td>4-(N-(LacO)-4'-(OCH2)-1',2',3'-(triazol-1'-yl)-C6H4)-C6H4</td>
<td>4-HO-C6H4</td>
<td>Similar to entry 144; 88 %</td>
</tr>
<tr>
<td>146</td>
<td>4-(4'-(AcOGlcO-CH2)-1'N,1',2',3'-triazol-1'-yl)-C6H4- C6H5- C6H5- C6H5- Zn(II)</td>
<td>5-(4-Azido-phenyl),10,15,20-triphenylporphyrin, CuCl, (AcOGlcO)₂CNHCOCH₂NHCO</td>
<td>50 %</td>
</tr>
<tr>
<td>147</td>
<td>4-(4'-(ManO-CH2)-1'N,1',2',3'-triazol-1'-yl)-C6H4- C6H5- C6H5- C6H5- Zn(II)</td>
<td>Similar to entry 146 with (ManO)-CH₂CCH; 61 %</td>
<td></td>
</tr>
<tr>
<td>148</td>
<td>4-(4'-(AcOGlcO-CH2)-1'N,1',2',3'-triazol-1'-yl)-C6H4- C6H5- C6H5- C6H5- Zn(II)</td>
<td>5,10,15-Tris(4-azidophenyl)-20-phenylporphyrin, CuCl, (AcOGlcO)₂CNHCOCH₂NHCO</td>
<td>91 %</td>
</tr>
<tr>
<td>149</td>
<td>4-(4'-(AcOGlcO-CH2)-1'N,1',2',3'-triazol-1'-yl)-C6H4- C6H5- C6H5- C6H5- Zn(II)</td>
<td>5,10,15,20-Tetrakis(4-azidophenyl)porphyrin, CuCl, (AcOGlcO)₂CNHCOCH₂NHCO</td>
<td>50 %</td>
</tr>
<tr>
<td>150</td>
<td>4-(4'-(AcOGlcO-CH2)-1'N,1',2',3'-triazol-1'-yl)-C6H4- C6H5- C6H5- C6H5- Zn(II)</td>
<td>From 266: NaOMe, MeOH, THF; quantitative</td>
<td></td>
</tr>
<tr>
<td>151</td>
<td>4-(4'-(AcOGlcO-CH2)-1'N,1',2',3'-triazol-1'-yl)-C6H4- C6H5- C6H5- C6H5- Zn(II)</td>
<td>From 267 like entry 155</td>
<td></td>
</tr>
<tr>
<td>152</td>
<td>4-(4'-(AcOGlcO-CH2)-1'N,1',2',3'-triazol-1'-yl)-C6H4- C6H5- C6H5- C6H5- Zn(II)</td>
<td>From 270: NaOMe, MeOH, CH₂Cl₂; 89 %</td>
<td></td>
</tr>
<tr>
<td>153</td>
<td>4-(4'-(AcOGlcO-CH2)-1'N,1',2',3'-triazol-1'-yl)-C6H4- C6H5- C6H5- C6H5- Zn(II)</td>
<td>From 282: Methylglutaryl chloride, triethylamine, THF, 0 °C, 2 h</td>
<td></td>
</tr>
</tbody>
</table>
160 273 4-[CH$_2$OOC(CH$_2$)$_3$CONH]-C$_6$H$_4$- 4-[AcOGlc-6'-CO- (NH)]-C$_6$H$_4$- 4-[AcOGlc-6'-CO- (NH)]-C$_6$H$_4$- 4-[AcOGlc-6'-CO- (NH)]-C$_6$H$_4$- 2H From 272: 1,2,3,4-Tetra-acetyl-β-D-glucopyranosyl chloride, triethylamine, 1 h; 33 %. [206]

161 274 2-[AcOGlcO]-C$_6$H$_4$- 4-NO$_2$-C$_6$H$_4$- 4-NO$_2$-C$_6$H$_4$- 4-NO$_2$-C$_6$H$_4$- 2H From 274: H$_2$, 10 % Pd-C, THF, r.t., 5 h; 60 %. [207]

162 275 4-[AcOGlcO]-C$_6$H$_4$- 4-NO$_2$-C$_6$H$_4$- 4-NO$_2$-C$_6$H$_4$- 4-NO$_2$-C$_6$H$_4$- 2H From 275: Like entry 167; 56 %. [207]

163 276 4-[AcOGlcO]-C$_6$H$_4$- 4-NO$_2$-C$_6$H$_4$- 4-NO$_2$-C$_6$H$_4$- 4-NO$_2$-C$_6$H$_4$- 2H From 276: Like entry 167, 62 %. [207]

164 277 4-[AcOGlcO]-C$_6$H$_4$- 4-[AcOGlcO]-C$_6$H$_4$- 4-[AcOGlcO]-C$_6$H$_4$- 4-[AcOGlcO]-C$_6$H$_4$- 2H From 277: Like entry 167, 64 %. [207]

165 278 4-[AcOGlcO]-C$_6$H$_4$- 4-[AcOGlcO]-C$_6$H$_4$- 4-[AcOGlcO]-C$_6$H$_4$- 4-[AcOGlcO]-C$_6$H$_4$- 2H From 278: Like entry 167, 76 %. [207]

166 279 4-[AcOGlcO]-C$_6$H$_4$- 4-[AcOGlcO]-C$_6$H$_4$- 4-[AcOGlcO]-C$_6$H$_4$- 4-[AcOGlcO]-C$_6$H$_4$- 2H From 279: Like entry 167, 60 %. [207]

167 280 4-[AcOGlcO]-C$_6$H$_4$- 4-[AcOGlcO]-C$_6$H$_4$- 4-[AcOGlcO]-C$_6$H$_4$- 4-[AcOGlcO]-C$_6$H$_4$- 2H From 280: Like entry 167, 54 %. [207]

168 281 4-[AcOGlcO]-C$_6$H$_4$- 4-[AcOGlcO]-C$_6$H$_4$- 4-[AcOGlcO]-C$_6$H$_4$- 4-[AcOGlcO]-C$_6$H$_4$- 2H From 281: CH$_2$Cl$_2$, DCC, Fmoc-iso-ala, 15 h; 89 %. [207]

169 282 4-[AcOGlcO]-C$_6$H$_4$- 4-[AcOGlcO]-C$_6$H$_4$- 4-[AcOGlcO]-C$_6$H$_4$- 4-[AcOGlcO]-C$_6$H$_4$- 2H From 282: Like entry 174; 88 %. [207]

170 283 4-[AcOGlcO]-C$_6$H$_4$- 4-[AcOGlcO]-C$_6$H$_4$- 4-[AcOGlcO]-C$_6$H$_4$- 4-[AcOGlcO]-C$_6$H$_4$- 2H From 283: Like entry 174, 85 %. [207]

171 284 4-[AcOGlcO]-C$_6$H$_4$- 4-[AcOGlcO]-C$_6$H$_4$- 4-[AcOGlcO]-C$_6$H$_4$- 4-[AcOGlcO]-C$_6$H$_4$- 2H From 284: Like entry 174, 90 %. [207]

172 285 4-[AcOGlcO]-C$_6$H$_4$- 4-[AcOGlcO]-C$_6$H$_4$- 4-[AcOGlcO]-C$_6$H$_4$- 4-[AcOGlcO]-C$_6$H$_4$- 2H From 285: Like entry 174, 85 %. [207]

173 286 4-[AcOGlcO]-C$_6$H$_4$- 4-[AcOGlcO]-C$_6$H$_4$- 4-[AcOGlcO]-C$_6$H$_4$- 4-[AcOGlcO]-C$_6$H$_4$- 2H From 286: Morpholine, then NaOMe/MeOH/CH$_2$Cl$_2$; 71 %. [207]

174 287 4-[AcOGlcO]-C$_6$H$_4$- 4-[AcOGlcO]-C$_6$H$_4$- 4-[AcOGlcO]-C$_6$H$_4$- 4-[AcOGlcO]-C$_6$H$_4$- 2H From 287: Like entry 179; 71 %. [207]

175 288 4-[AcOGlcO]-C$_6$H$_4$- 4-[AcOGlcO]-C$_6$H$_4$- 4-[AcOGlcO]-C$_6$H$_4$- 4-[AcOGlcO]-C$_6$H$_4$- 2H From 288: Like entry 179, 75 %. [207]

176 289 4-[AcOGlcO]-C$_6$H$_4$- 4-[AcOGlcO]-C$_6$H$_4$- 4-[AcOGlcO]-C$_6$H$_4$- 4-[AcOGlcO]-C$_6$H$_4$- 2H From 289: Like entry 179, 67 %. [207]

177 290 4-[AcOGlcO]-C$_6$H$_4$- 4-[AcOGlcO]-C$_6$H$_4$- 4-[AcOGlcO]-C$_6$H$_4$- 4-[AcOGlcO]-C$_6$H$_4$- 2H From 290: Like entry 179, 64 %. [207]

178 291 4-[AcOGlcO]-C$_6$H$_4$- 4-[AcOGlcO]-C$_6$H$_4$- 4-[AcOGlcO]-C$_6$H$_4$- 4-[AcOGlcO]-C$_6$H$_4$- 2H From 291: Like entry 179, 71 %. [207]

179 292 2-[GlcO]-C$_6$H$_4$- 4-[(NH$_2$)(CH$_3$)CHCON H]-C$_6$H$_4$- 4-[(NH$_2$)(CH$_3$)CHCON H]-C$_6$H$_4$- 4-[(NH$_2$)(CH$_3$)CHCON H]-C$_6$H$_4$- 2H From 292: Like entry 179, 71 %. [207]

180 293 4-[GlcO]-C$_6$H$_4$- 4-[GlcO]-C$_6$H$_4$- 4-[GlcO]-C$_6$H$_4$- 4-[GlcO]-C$_6$H$_4$- 2H From 293: Like entry 179, 75 %. [207]

181 294 4-[GlcO]-C$_6$H$_4$- 4-[GlcO]-C$_6$H$_4$- 4-[GlcO]-C$_6$H$_4$- 4-[GlcO]-C$_6$H$_4$- 2H From 294: Like entry 179, 67 %. [207]

182 295 4-[GlcO]-C$_6$H$_4$- 4-[GlcO]-C$_6$H$_4$- 4-[GlcO]-C$_6$H$_4$- 4-[GlcO]-C$_6$H$_4$- 2H From 295: Like entry 179, 64 %. [207]

183 296 4-[GlcO]-C$_6$H$_4$- 4-[GlcO]-C$_6$H$_4$- 4-[GlcO]-C$_6$H$_4$- 4-[GlcO]-C$_6$H$_4$- 2H From 296: Like entry 179, 57 %. [207]
BF₃•OEt₂, 18 h, then p-chloranil, 1 h; 15 %. [207]

Like entry 187 with 4-(NO₂)-C₆H₄-CHO; 15 %. [207]

From 300: SnCl₂/HCl reflux, 1 h, 15 %. [207]

From 301: Like entry 189, 90 %. [207]

From 302: DCC, (AcOGl)Ser-Fmoc, 15 h, r.t.; 60 %. [207]

BF₃•OEt₂, 18 h, then p-chloranil, 1 h; 15 %. [207]

Like entry 187 with 4-(NO₂)-C₆H₄-CHO; 15 %. [207]

From 300: SnCl₂/HCl reflux, 1 h, 15 %. [207]

From 301: Like entry 189, 90 %. [207]

From 302: DCC, (AcOGl)Ser-Fmoc, 15 h, r.t.; 60 %. [207]

BF₃•OEt₂, 18 h, then p-chloranil, 1 h; 15 %. [207]

Like entry 187 with 4-(NO₂)-C₆H₄-CHO; 15 %. [207]

From 300: SnCl₂/HCl reflux, 1 h, 15 %. [207]

From 301: Like entry 189, 90 %. [207]

From 302: DCC, (AcOGl)Ser-Fmoc, 15 h, r.t.; 60 %. [207]
<table>
<thead>
<tr>
<th>Entry</th>
<th>Reaction</th>
<th>Product</th>
<th>Yield</th>
</tr>
</thead>
<tbody>
<tr>
<td>211</td>
<td>4-(ManO-CH2)-1'-N',1'-2',3'-triazol-1'-yl]-C6H4-CH2)-1'N',1'-2',3'-triazol-1'-yl]-C6H4-CH2)-1'N',1'-2',3'-triazol-1'-yl]-C6H4-CH2)</td>
<td>4-(ManO-CH2)-1'N',1'-2',3'-triazol-1'-yl]-C6H4-CH2)-1'N',1'-2',3'-triazol-1'-yl]-C6H4-CH2)</td>
<td>CaH4-</td>
</tr>
<tr>
<td>212</td>
<td>4-[LacNacO-CH2]-1'-N',1'-2',3'-triazol-1'-yl]-C6H4-</td>
<td>4-[LacNacO-CH2]-1'-N',1'-2',3'-triazol-1'-yl]-C6H4-</td>
<td>CaH4-</td>
</tr>
<tr>
<td>213</td>
<td>4-[GlcoO-CH2]-1'-N',1'-2',3'-triazol-1'-yl]-C6H4-</td>
<td>4-[GlcoO-CH2]-1'-N',1'-2',3'-triazol-1'-yl]-C6H4-</td>
<td>CaH4-</td>
</tr>
<tr>
<td>214</td>
<td>4-[ManO-CH2]-1'-N',1'-2',3'-triazol-1'-yl]-C6H4-</td>
<td>4-[ManO-CH2]-1'-N',1'-2',3'-triazol-1'-yl]-C6H4-</td>
<td>CaH4-</td>
</tr>
<tr>
<td>215</td>
<td>4-[ManO-CH2]-1'-N',1'-2',3'-triazol-1'-yl]-C6H4-</td>
<td>4-[ManO-CH2]-1'-N',1'-2',3'-triazol-1'-yl]-C6H4-</td>
<td>CaH4-</td>
</tr>
<tr>
<td>216</td>
<td>4-[ManO-CH2]-1'-N',1'-2',3'-triazol-1'-yl]-C6H4-</td>
<td>4-[ManO-CH2]-1'-N',1'-2',3'-triazol-1'-yl]-C6H4-</td>
<td>CaH4-</td>
</tr>
<tr>
<td>217</td>
<td>4-[ManO-CH2]-1'-N',1'-2',3'-triazol-1'-yl]-C6H4-</td>
<td>4-[ManO-CH2]-1'-N',1'-2',3'-triazol-1'-yl]-C6H4-</td>
<td>CaH4-</td>
</tr>
<tr>
<td>218</td>
<td>4-[ManO-CH2]-1'-N',1'-2',3'-triazol-1'-yl]-C6H4-</td>
<td>4-[ManO-CH2]-1'-N',1'-2',3'-triazol-1'-yl]-C6H4-</td>
<td>CaH4-</td>
</tr>
<tr>
<td>219</td>
<td>4-[GlcoO-CH2]-</td>
<td>4-[GlcoO-CH2]-</td>
<td>4-[GlcoO-CH2]-</td>
</tr>
<tr>
<td>220</td>
<td>4-[AcOGlc-(NHCSNH)-C6H4-</td>
<td>4-[AcOGlc-(NHCSNH)-C6H4-</td>
<td>4-[AcOGlc-(NHCSNH)-C6H4-</td>
</tr>
<tr>
<td>221</td>
<td>4-[AcOGlc-(NHCSO)-C6H4-</td>
<td>4-[AcOGlc-(NHCSO)-C6H4-</td>
<td>4-[AcOGlc-(NHCSO)-C6H4-</td>
</tr>
<tr>
<td>222</td>
<td>4-[AcOGlc-(NHCOO)-C6H4-</td>
<td>4-[AcOGlc-(NHCOO)-C6H4-</td>
<td>4-[AcOGlc-(NHCOO)-C6H4-</td>
</tr>
<tr>
<td>223</td>
<td>3-(GlcO)-C6H4-</td>
<td>3-(GlcO)-C6H4-</td>
<td>3-(GlcO)-C6H4-</td>
</tr>
<tr>
<td>224</td>
<td>3-(GlcO)-C6H4-</td>
<td>3-(GlcO)-C6H4-</td>
<td>3-(GlcO)-C6H4-</td>
</tr>
<tr>
<td>225</td>
<td>3-(GlcO)-C6H4-</td>
<td>3-(GlcO)-C6H4-</td>
<td>3-(GlcO)-C6H4-</td>
</tr>
<tr>
<td>226</td>
<td>3-(GlcO)-C6H4-</td>
<td>3-(GlcO)-C6H4-</td>
<td>3-(GlcO)-C6H4-</td>
</tr>
<tr>
<td>227</td>
<td>4-(AcOGlcO)-C6H4-</td>
<td>4-(AcOGlcO)-C6H4-</td>
<td>4-(AcOGlcO)-C6H4-</td>
</tr>
<tr>
<td>228</td>
<td>4-(AcOGlcO)-C6H4-</td>
<td>4-(AcOGlcO)-C6H4-</td>
<td>4-(AcOGlcO)-C6H4-</td>
</tr>
</tbody>
</table>

**Note:** For entries 211-219, the yields are given for the microwave reaction at 110 °C, 20 min. For entries 220-228, the yields are given for the reaction with p-chloranil at reflux. Additional reagents and conditions are noted for each entry.
Note: all are Beta isomers (apart from mannose which is alpha) unless stated as alpha (or beta for mannose) or a mixture of the two.

pyridiniumyl) porphyrin [190]. The respective A,B neutral precursor porphyrins with one glycoside residue (glucoside, maltoside and lactoside) (131, 139, 147, 148, 151, 152) were synthesized via the Little method [191] by condensation of pyrrole, 4-pyridinecarboxaldehyde, and ortho- or para-peracetylated glycosylbenzaldehyde derivatives in 6-7 % yield. Again, addition of methyl, isopropyl, n-octyl groups provided the cationic glycosylated N-alkylpyridinium porphyrins 132-134, 141-143, 151-155. Deacetylation then gave 135-138, 140, 149, 150, 153, 154, and 144-146, 155, 156 in quantitative yield.

Similar strategies were also employed to construct dimeric systems. Here, condensation reactions with a mixture of 4-(2',3',4',6'-teta-O-acetyl-β-D-glucopyranosyl)- benzaldehyde, ortho- or para-hydroxybenzaldehyde and pyrrole via Lindsey conditions, followed by oxidation with p-chloranil, afforded the neutral tri-O-glycosyl porphyrins 163 and 164 in 5-13 % yield [192]. Alternatively, use of the Little method gave the same porphyrins in yields of 5-7 %.

The neutral porphyrin dimers 364o,p were prepared via two step ether linkage with good yields, followed by quantitative deprotection to provide 365o,p. Related cationic dimers were prepared via Fleischer's method [193] in which the porphyrins were linked by a 1,3-dipyrindiniumyl propane linkage. Here, the Little method was adapted to yield 167 in 7 %. The 1,3-dipyrindiniumylpropane-linked bisporphyrin 366 was synthesized in one step with 1,3-diiodopropane in 14 % yield. Subsequently the sugar units were deprotected quantitatively yielding 367. Compounds 368o,p and 369 differed by the o,p ortho- or para-substitution and exhibited significant fluorescence quenching in aqueous solutions due to aggregation [194].

The same building blocks could also be used for the synthesis of mixed glycosylated neutral and cationic porphyrin dimers prepared with a flexible hydrocarbon chain linked at the meso position [194]. Within the series the two hydrophilic compounds 365o,p differing by the ortho or para-substitution and an amphiphilic derivative 368 were synthesized. The second part of the series consisted of the synthesis of two O-glycosyl cationic dimers where the linkage at the pyridine nitrogen afforded its mono-cationic nature. Again, the monomer structures were synthesized by Lindsey’s method via condensation of pyrrole, 4-pyridine carboxaldehyde and 4-(2',3',4',6'-teta-O-acetyl-β-D-glucopyranosyl) benzaldehyde with propionic acid. The previously synthesized monomers were also incorporated into 369 and 370 which allowed for covalent attachment on one side via eth ether linkage and alkylation of the
Formula 2.
pyridine nitrogen on the other side. Deacetylation afforded the mono-cationic derivatives 369 and 370.

Mixed condensations may also be used as an entry for subsequent solid phase syntheses. For example, Krausz et al. carried out the solid phase synthesis of two mono-RGD-triglucosylporphyrins where RGD is a tripeptide sequence of arginine-glycine-aspartate \[^{[195]}\]. Using Little’s method the protected derivatives of glycoporphyrins 168 were synthesized through condensation of pyrrole, glucosylaldehyde and either para-hydroxybenzaldehyde or salicylaldehyde in propionic acid in 6 and 4 %, while the use of Lindsey’s method doubled the yields. Conversion to the carboxylic acid provided the porphyrin scaffold for amide coupling to a solid phase Wang resin functionalized with a RGD residue. The porphyrin derivatives 168b,p were obtained after detachment from the Wang resin and subsequent deprotection.

An example for the preparation of 5,15-disubstituted porphyrins involved the condensation under Lindsey conditions of tetraacetyl-\(\beta\)-\(D\)-lactosyl-benzaldehyde with dipyrromethane to afford 5,15-bis(\(p\)-per-acetyl-\(\beta\)-lactosylphenyl)porphyrin 169 after oxidation with \(p\)-chloranil \[^{[29]}\]. The metallated porphyrin 171 was obtained after treatment with FeCl\(_2\) and followed by deacetylation yielded 173. The compound was tested in a range of solvents and was found to be mono-dispersed in aqueous DMF but formed aggregates in aqueous NMP (\(N\)-methylpyrrolidone). It showed an unusual colorimetric response with the addition of calcium ions in tris-HCl buffer (50 mM, pH 9.6, 50 v/v% NMP) changing the color from orange to pink over a 10 h equilibration period. The same response was not observed for any other ion including potassium, sodium or magnesium. The calcium ion response was dependent on the pH and the co-solvent used. A comparison with chloro(2,3,7,8,12,13,17,18-octaethylporphyrinato)iron(III) and the non-conjugated derivative 172, both of which displayed no response, confirmed the carbohydrate units being responsible for the response \[^{[29]}\].

Naturally, mixed condensations may also be employed for the synthesis of unsymmetrical \(C\)-glycosylated porphyrins. Often, this involves initial multi-step syntheses of appropriate dipyrromethanes. A typical example is the preparation of \(\alpha,\alpha^\prime\)-dipyrrylalditols (371) via condensation of pyrrole with glyceraldehyde, arabinofuranose or glucopyranose using a mixture of EtMgBr and TiCl\(_4\). This yields synthetically useful precursors for the synthesis of glycoporphyrins, incorporating carbon-linked sugar moieties (Scheme 6). Condensation of dipyrryl derivative 371b with \(p\)-fluorobenzaldehyde was achieved in 20 % yield with a single isomer identified to yield the corresponding \(A_2B_2\) porphyrin 174 \[^{[196]}\].

Other dipyrrylglycosides (e.g., 372 and 373 were easily prepared by reaction of pyrrole with aldoses in 30-40 % yield \[^{[216]}\]. Standard [2+2] condensation with aryl aldehydes then yielded the respective \(meso\)-glycoconjugated porphyrins in 6-16 % yield. Note, that the presence of branched \(meso\)
alkyl residues from the sugar units results in conformationally constrained systems [217]. Here, a dynamic saddle conformation was proposed to exist in solution [216]. These studies were later extended to the related A₄-type systems (namely the four atropisomers of 5,10,15,20-tetrakis-(1,2-O-isopropylidene-1,2-dihydroxyethyl)-porphyrins) which were investigated in detail with regard to their atropisomerism [218]. Selective deprotection pathways were found for the acetonide precursor systems which allowed conversion of the lipophilic protected systems into amphiphilic, partially deprotected and water soluble, fully deprotected systems. A unique method for the synthesis of C-dipyrrolyglycosides incorporates the use of dithioacetal glycosyl derivatives by reaction with pyrrole to afford mono- and disaccharidic C-1-deoxyalditol moieties [219]. The glycosyl starting materials D-galactose, D-mannose, D-glucose, D-lactose and agarose underwent a mercaptolysis-acetylation to form the peracetylated diethyl dithioacetals. The carbonyl of these residues were deprotected and subsequently reacted via pyrrole condensation to afford 375-379 in an overall yield of 22-49 % [219].

In extension of the A₄ C-glycosyl porphyrins 66 and 67 diglycosylated porphyrins were synthesized to investigate their ability to form chiral supramolecular structures in different aqueous-organic solvent mixtures [170]. The 5,15-substitution pattern was achieved using the [2+2] MacDonald type procedure where the dipyrryl derivatives were prepared by condensing the respective aldehydes with pyrrole affording 373 and 374 in 49 and 84 % yield, respectively. Condensation with pentafluorobenzaldehyde gave 175 in 22 %. The same reaction was carried out for the glucose derivative 176. An alternative method used a condensation of pentafluoroethyl-dipyrromethane with the sugar aldehydes which afforded 175 in a higher yield. Debenzylation was achieved by hydrogenation over Pd/C giving the hydroxylated derivative 177 in 63 % yield. Note, these structures form chiral super-structures due to self-aggregation in aqueous-organic solvent mixtures with the morphology of the aggregates depending on the porphyrin structure and the bulk conditions of aggregation [170].

Another example of C-glycosylated tetraarylporphyrins was reported by Drain and coworkers [172]. Their synthesis of the glycosyl benzaldehyde required several steps to convert the initial S-glycosyl linkage to the more stable C-linkage. α,α'-Dibromo-p-xylene was reacted with 2,3,4,6-tetra-O-acetyl-1-thio-β-D-glucopyranoside providing the thioglucoside 380 which was converted to the silyl derivative 381 and the protecting groups were changed from the acetyl to benzyl groups for subsequent steps. Next, the sulfone of compound 383 was synthesized and used in a Ramberg-Bäcklund synthesis of the exo-glucal 384 (two isomers with a Z/E ratio of 8:2). Subsequent hydrogenation afforded the β-C-glycoside 385 followed by cleavage of the silyl protecting group and Swern oxidation to the required aldehyde (Scheme 7). The aldehyde 386 was then used to synthesize 70 and 178 in 53 and 15 % yields, respectively, using Lindsey conditions. Hydrogenolysis of the benzyl protecting groups provided 71 and 179 in quantitative yields [172].

Scheme 7. Synthesis of mono- and tetra-C-glycosyl substituted TPP. i) NaH, THF, r.t., 2 h, 90 %; ii) TBDMSOH, Ag(OTf), 2,6-di-tert-butylpyridine, CH₂Cl₂, r.t., 3 h, 60 %; iii) MeONa, MeOH, r.t., 3 h; iv) NaH, BnBr, Bu₄NI, THF–DMF, r.t., 8 h, 80 % for 2 steps; v) MMPP, THF–EtOH–H₂O, 60 ºC, 2 h, 87 %; vi) CBr₂F₂, KOH 25 % on alumina, CH₂Cl₂–t-BuOH, 0 ºC to r.t., 3 h, 88 %; vii) H₂, Pd 5 % on alumina, EtOAc, r.t., 12 h, 95 %; viii) Bu₄NF, THF, r.t., 2 h, 98 %; ix) oxalyl chloride, DMSO, CH₂Cl₂, r.t., then Et₃N, -78 ºC to r.t., 1.5 h, 85 %.
Condensation reactions can also be employed to construct geometrically more complex structures. For example Krausz and coworkers synthesized glycosyl strapped porphyrins to determine the effect of strapping on its efficacy as a PS (Scheme 8) [220]. The synthesis of the two glycosyl strapped porphyrin 389 (5,15, 'trans') and 391 (5,10, 'cis') used standard Lindsey conditions with meso-(p-tolyl)dipyrromethane (2 equiv.) (388) and glycosyl bisaldehyde 6,6'-di-O-(3-formyl)benzoyl,2,3,4,2',3',4'-hexa-O-trimethylsilyl-α,α-trehalose (1 equiv.) (387) to yield 389 and 391 which were subsequently deprotected giving 390 and 392 in 8 % and 5 % yield, respectively.

2.4 Synthesis via Functionalization Reactions

Due to the low stability of carbohydrates under many of the conditions used for porphyrin synthesis the preparation of more complex systems is better achieved by first constructing the porphyrin macrocycle and then appending the desired sugar units. The most basic approach is the use of porphyrins with simple functional groups such as –OH, -NH₂, -COOH and standard coupling methods [221]. Functionalization of porphyrins by functional group interconversion and direct substitution of the porphyrin scaffold have facilitated the assembly of several glycoporphyrins via different ligation strategies including, glycosylation, olefin metathesis, amide coupling, organometallic coupling and are summarized in Figure 4. All of which afford O-, N-, S- C-linkage of the glycoconjugate for A3B-, A2B2-, A2BC and ABCD-type porphyrins including β-substituted and dimeric porphyrins, too.

2.4.1 β-Substituted Porphyrins

2.4.1.1 Glycosidation Reactions

Diglucoside 396 and digalactoside 397 derivatives of isohematoporphyrin were the first water soluble glycoporphyrins to be synthesized as efficient PSs (Scheme 9) [222]. Both 394 and 395 were synthesized via diastereoselective O-glycosylations using a solid surface active silver catalyst and acetyl groups for neighboring group participation yielding the β-diglycosides in 51 % and 85 % yields, respectively. The glycosylations with α-D-acetobromoglucose or α-D-acetobromogalactose were carried out at room temperature over 1-7 days followed by deprotection of the acetyl groups yielding the stable bis(D-glucosyl)isohematoporphyrin 396 and the bis(D-galactosyl)isohematoporphyrin 397. A similar approach to amphiphilic porphyrins was taken with hematoporphyrin dimethyl ester. It underwent glycosylation with 2,3,4,6-tetra-O-acetyl-α-D-glucopyranosyl bromide in the presence of mercuric salts (HgBr₂ and Hg(CN)₂) for 6 h to yield the mono-glycosylated porphyrin in 30 % when using two equivalents of the glucosyl residue while using 4 equivalents

Scheme 8. Synthesis of glycosyl-strapped porphyrins. i) BF₃•OEt₂, p-chloranil, dichloromethane; ii) TBAF.
Figure 4. Synthesis of glycoporphyrins via porphyrin FGI.
of the sugar unit afforded the diglycosylated derivative in 52 % yield. Deprotection of the acetyl groups using either KCN or NaOMe could be achieved in 70-80 % yields [188].

\[ \text{Scheme 9. Synthesis of bis(\text{o-glucosyl})isohematoporphyrin and bis(\text{o-galactosyl})isohematoporphyrin. i) \alpha-\text{AcOGlcOAc or } \alpha-\text{AcOGalOAc, Ag}_2\text{CO}_3, \text{Na}_2\text{SO}_4, \text{CH}_2\text{Cl}_2, 20 ^\circ\text{C, 1-7 days; ii) KOH, MeOH, 100 ^\circ\text{C, 24 h.}} ]

Naturally, the functional group used for coupling may also be positioned on a \textit{meso} substituent. Hombrecher reported the synthesis of several galatopyranosyl substituted porphyrins using different procedures, the first of which was the treatment of porphyrinic carboxylic esters (180-184) with 1,2,3,4-di-\text{O}-isopropylidene-\text{\textalpha}-\text{D}-galactopyranose. The reaction proceeded in moderate yields of 28-50 % for porphyrins 185-187. Compounds 188 and 189, however, were produced in 7 % yield due to increased formation of the carboxylic acid [197]. Alternative routes were explored using condensation reactions which produced compound 189 in a yield of 20 % by using the 4-[\text{di-\text O}-isopropylidene-\text{\textalpha}-\text{D}-galactopyranosyl]benzaldehyde. They also used Lindsey’s procedure, treating the aldehyde in a mixed condensation with \text{p}-tolyl aldehyde and pyrrole which proved unsuccessful in increasing the yields [197]. Hombrecher later extended his studies with the porphyrin framework shown for 402. After preparation of the hydroxmethylphenylporphyrin 399 this was reacted with a variety of glycosyl imidates 398 to yield the nickel(II) porphyrins 400. These were then deprotected and demetallated to the free bases 401 (Scheme 10). Notably, this was one of the earlier papers that attached disaccharides to a porphyrin core [223]. In aqueous solution these systems formed very stable aggregates. However, the porphyrins could be incorporated easily as monomers into membrane model systems (liposomes).

\[ \text{Scheme 10. Synthesis of porphyrins with a carbohydrate unit appended to the } \text{\textit{meso} position. } \text{Conditions: i) } \text{CH}_2\text{Cl}_2, \text{ZnCl}_2, 22-33 \%; \text{ii) NaOMe, MeOH, 5 min.; iii) TFA, 1,3-propanediol, 61-99 \%; } R^1 = \text{OH, HOAc; } R^2 = H, \text{OH, NHAc, OAc; } R^3 = H, \text{OH, OAc; } R^4 = H, \text{OAc, \text{\textalpha}(1,4)Glc(OSOAc), } \beta(1,4)\text{Glc(OSOAc), } \text{\textalpha}(1,4)\text{Glc, } \beta(1,4)\text{Gal, } \beta(1,4)\text{Glc.} \]
2.4.1.2 Organometallic Coupling Reactions

Organometallic couplings can also be used to introduce bioconjugate groups in the β-position. One example comprises the synthesis of β-substituted glycoporphyrins using second generation Grubbs catalyst, vinylporphyrins, and allylic acetonide carbohydrate units \[224\]. The Zn(II) complexes of 2-vinyl-5,10,15,20-tetraphenylporphyrin 403a and protoporphyrin-IX dimethyl ester (403b) were reacted with allylic acetonides of the D-ribose (404c), D-galactose (404d), D-glucose (404e), and two isomeric derivatives of D-fructose (404f,g) using 25 mol% of Grubbs catalyst. The yields for 405a-(c-g) were quantitative, however the yields for 405b-(c-g) varied from 74-93 % with no dimerization of either starting substrate observed. The reaction displayed a significantly high E-stereoselectivity due to steric hindrance provided by the large macrocycle (Scheme 11).

Another example is the one pot synthesis of a water soluble (octa-β-lactosylated porphyrinato)copper(II) 72 via copper-catalyzed Husigen click chemistry using an octaalkyne porphyrin and β-lactosyl azide \[173\]. Its spatial structure and lectin affinity were determined. It was observed that the eight β-lactoside-appendages formed a thick hydrophilic shell which shielded the hydrophobic surface of porphyrin-core forming the most stable spherical conformation. These (porphyrinato)copper(II) derivatives have poor singlet oxygen yields and are not suitable for PDT \[173\].

2.4.2 meso-Substituted Porphyrins

The most significant body of work is available for derivatives of 5,10,15,20-tetrasubstituted porphyrins. The ease of their synthesis makes them suitable precursors for glycosylation and functionalization reactions.

2.4.2.1 Glycosidation Reactions

O-linked systems: A typical example is the analysis of two series of glycosylated porphyrins which were synthesized and studied using solid secondary ion mass spectroscopy (SIMS) \[189,225,226\]. The first series of monosubstituted tristolyl glycoporphyrins were prepared starting from the mono-hydroxyphenyl-tolyl porphyrins obtained with propionic acid via the Little method followed by substitutions with 3-bromo-propan-1-ol and glycosylation with four protected mono- and disaccharides affording \[408-411\] in 20-70 % yield (Scheme 12) \[226\]. The second series, unsymmetrical pyridyl mono-glycosylated porphyrins \[131, 139, 147, 148, 151, 152\] substituted with protected mono- and disaccharides, were synthesized according to the Adler-Long method via glycosylated benzaldehydes in ~ 6 % yield \[189,190\]. For a comparative study the symmetric tetraglucosyl derivative 29 was also synthesized \[225\]. SIMS was evaluated as a good study for the rapid screening of compounds during synthesis. It avoids problems displayed by FABMS (fast atom bombardment mass spectroscopy) which can result in contamination of the ion source and chemical reactions occurring in a matrix. Positive and negative spectra were obtained of all glycoporphyrins. The simple spectra identified the molecular weight and showed the groups appended at the meso positions unlike DCI-CID (desorption chemical ionization – collision-induced dissociation) which does not yield mass spectra of higher mass compounds \[225\].

Similarly, four series of meso-tetraaryl-glycoporphyrins were synthesized with protected and de-protected hydroxyl groups to evaluate their photo-inactivation of HSV-1 and HSV-2 herpes simplex virus \[30\]. Three different classes of porphyrins \[83, 190, 197\] were prepared by Rothemund or cross-Rothemund reactions and porphyrin 190 was reacted with hexafluorobenzene affording 198 in addition to the series. The first two series of glycoporphyrins were synthesized via the coupling of tetraphenylporphyrins with a hydroxyl group \[190\] or a carboxylic group \[197\] with 1-bromo-2,3,4,6-tetra-O-acetyl-α-D-glucopyranose and 6-iodo-1,2,3,4-di-O-isopropylidene-α-D-galactopyranose, respectively affording 191 and 193 in 66 % and 50 % yields. The other two series were synthesized via substitution of one
evaluated their antiviral activity [229]. The porphyrin starting glycosylated neutral and cationic tripyridylporphyrins and derivatives 199 for detailed electrospray tandem mass spectrometry [227, 228].

cells. These compounds also proved to be suitable materials (HSV-1 and HSV-2) [30] Tomé which displayed good inactivation of herpes simplex virus previous work on the synthesis of neutral glycoporphyrins unsymmetrical cationic glycoporphyrins. In an extension of converted to the methyl galactopyranosides CH2Cl2.

Similar concepts can also be employed to prepare para fluorine atom on 83 or on a meso-tetraphenylporphyrin containing a pentafluorobenzene spacer (198) with 1,2,3,4-di-O-isopropylidene-α-D-galactopyranosyl containing 201 and 199. All series were deprotected yielding the amphiphilic derivatives 192, 194, 200 and 202. Compound 194 was converted to the methyl galactopyranosides 195 and 196 in 43 % and 40 % yields, respectively [30]. All porphyrin derivatives were tested against HSV-1 and HSV-2 in Vero cells. These compounds also proved to be suitable materials for detailed electrospray tandem mass spectrometry [227,228].

Similar concepts can also be employed to prepare unsymmetrical cationic glycoporphyrins. In an extension of previous work on the synthesis of neutral glycoporphyrins which displayed good inactivation of herpes simplex virus (HSV-1 and HSV-2) [30] Tomé et al. synthesized glycosylated neutral and cationic tripyridylporphyrins and evaluated their antiviral activity [229]. The porphyrin starting material 412 was synthesized via crossed Rothemund reaction which underwent a one-pot conversion to the activated ester 413 by reaction with thionyl chloride and addition of N-hydroxysuccinimide. Coupling of the activated ester afforded the glycoporphyrin 414 which was subsequently deprotected to give 415. Treatment with methyl iodide afforded the tris(N-methylpyridinium)porphyrins 416a-c (Scheme 13). All compounds are good singlet oxygen generators, however, the cationic porphyrin derivatives are slightly better than the neutral ones.

Related studies targeted cationic galactosyl porphyrins and their inactivation potential of Micrococcus sp. [Gram (+)] and Pseudomonas sp. [Gram (−)] which were resistant to UV inactivation [230]. Glycoporphyrins 416b,c [229] and the non-glycoconjugate 73 [175] served as controls. The latter compound was used as a precursor for the synthesis of the mono-charged derivatives 209 and 210 and tetra-charged porphyrin derivatives (74, 211, 212). Compound 209 was synthesized via N-alkylation of 73 with 6-iodo-1,2,3,4-di-O-isopropylidene-α-D-galactopyranosyl which was subsequently deprotected to afford the α/β mixture of galactoporphyrin 210 in 90 % yield. To form the cationic derivatives 74, 211 and 212 methylation of the pyridyl groups of 73 and 209 and 210 was carried out [230]. All of the galactoporphyrin derivatives proved to be more efficient at singlet oxygen production compared to 73. The tri-cationic derivatives 416b,c displayed higher production than the tetra-cationic ones. The unprotected PSs 416c and 212 have a higher efficiency to generate singlet oxygen compared to the protected derivatives 416b and 211 [230].

Other linker units may be employed as well. Thus, a library of diethylene glycol linked glycoporphyrins TPP (204-206) and their S-analouges (207, 208) were synthesized and their PDT efficacy tested against retinoblastoma cells [198]. All O-glycosylated diethylene glycol porphyrin and the reference compound (203) were synthesized from 5,10,15-tris(p-phenoxy)-20-phenylporphyrin and reacted with the bromo-diethylene glycol substituted glycosides followed by deacetylation or with 2-(2-chloroethoxy)ethanol for 203. The thiosugar derivatives 207 and 208 were obtained by reacting 5,10,15-tris(p-phenoxy)-20-phenylporphyrin with 1-bromo-2-(2-bromo-ethoxy)-ethane which was subsequently condensed with 1-thio-tetraacetate-β-D-galactose and 1-thio-tetraacetate-α-D-mannose, followed by deacetylation.

Diethylene glycol linkers also found use in Maillard’s synthesis of a range of glycosylated zinc dimer/trimer derivatives (418-421) for application in 2-photon absorption PDT [231]. All derivatives were substituted with three α-mannose units on each porphyrin in the hope of targeting tumor cells which contained over-expressed lectin-type membrane receptors. The four oligomers (418-421) were all synthesized from a single parent monomer 417 with varying π-conjugation linkers between the porphyrin moieties. The parent monomer 417 was pre-formed in five steps and the trimethylsilyl group was deprotected for oligomer synthesis. The butadiyne core dimer 418 was synthesized via Glaser-
Scheme 13. Synthesis of glycosyl tris(N-methylpyridinium)porphyrins. i) SOCl₂, pyridine, 50 °C, 30 min., then N-hydroxysuccinimide, 50 °C, 3 h; ii) 1,2:3,4-di-O-isopropylidene-α-D-galactopyranose, NaH, toluene, r.t., 90 min.; iii) TFA, H₂O, r.t., 30 min.; iv) CH₃I, DMF, 40 °C, 3 h.
Hay oxidative coupling in 72 % yield. The dimers 419, 420 and trimer 421 were prepared via Heck cross-coupling reactions with the corresponding halogenated derivatives in 31 %, 42 % and 80 % yields, respectively. Compounds 418 and 421 were deprotected using Zemplén conditions which proved impossible to characterize via NMR and photophysical data due to solubility problems.

Next, the photophysical properties of glycoporphyrins (418, 419, 421) were compared to data of 1PA (one-photon absorption) and 2PA (two-photon absorption) non-glycosylated derivatives (422) [231-233]. The 1PA absorption studies of 418 and 419 are very similar to that of 422 and 423, however, all the transitions of the former were slightly blue-shifted compared to the latter pair. This is indicative of a weaker conjugation along the π-system of the glycosylated derivatives. Compound 431 also displayed similar results to dimer 419 [232]. The carbohydrate moieties caused a twisted conformation of the dimers which subsequently decreased the conjugation and the 2PA cross-section, but this interpretation needs further proof. Looking at the 2PA properties of the trimer 421 it exhibits two fluorescence emission bands which show different behavior upon 2PA excitation, i.e., they have two different 2PA excitation pathways. The 2PA cross sections of 418 and 421 are above the average cross section of typical 2PA PSs [232].

Subsequent extension of this work by Maillard focused on

Formula 4.
Scheme 14. Synthesis of glycoconjugated porphyrin dimers. i) bromo-carbohydrate derivatives, CsCO₃, DMF, r.t., 24 h; ii) NBS, CHCl₃, pyridine, 15 min., 0 °C; iii) Zn(OAc)₂, MeOH, CHCl₃, reflux, 1 h; iv) TMSA, Cul, Pd(PPh₃)₄Cl₂, THF, NEt₃, -180 °C→r.t., 12 h; v) TBAF 1 M, Cul, Pd(PPh₃)₄Cl₂, CH₂Cl₂, THF, NEt₃, r.t., 18 h; vi) NaOMe 0.1 M, MeOH, THF, r.t., 1 h.
derivatives with carbohydrate variation and with different polypeethylene glycol linkers, which is illustrated in Scheme 14 [234]. The one-photon PDT efficiency was examined and compared to the free base monomer 206 which is used for *in vivo* PDT treatment [234-236]. All glycoporphyrins were synthesized from 424 which underwent a Williamson reaction with various bromo-polypeethylene glycol protected glycosyl units affording 425a-d in yields ranging from 54-78 %. Mono-bromination was carried out on the trisubstituted macrocycles in 83-97 % yields followed by metallation with zinc acetate in quantitative yield affording 427a-d. Sonogashira cross coupling reactions were carried out to provide 428a-d in 49-78 %. The trimethylsilyl group was subsequently removed and self-dimerization was carried out using a palladium catalyst yielding 429a-d in 38-69 %. Zénplen deprotection was performed to provide the final dimers 430a-d.

An example for the use of amide linker units was reported in 2006. Here two new mono-glucosyl tritolylporphyrins (221,222) were synthesized with the glycosyl moiety linked via a carboxymethyl glycosidic α-D-linkage. The monohydroxyphenyltritolylporphyrins 213/214 were prepared by Little’s method [191] through condensation of pyrrole with *p*-tolualdehyde and the *ortho* or *para*-hydroxybenzaldehyde in propionic acid. Incorporation of a terminal amine (217/218) allowed for coupling with 3,4,6-tri-*O*-acetyl-carboxymethyl-α-D-glucopyranoside-2-*O*-lactone and transesterification afforded 221,222 [237].

In addition to the increase in water solubility glycoporphyrins also offer an entry into amphiphilic systems. A typical example is a study on amphiphilic porphyrins with three maltolhexaose units and a single alkyl chain (ethyl, *n*-butyl, *n*-hexyl, *n*-decyl and hexadecyl) [231a]. Here 3-iodopropyl nonadeca-*O*-acetyl-β-D-maltohexaoside was reacted with 5,10,15,20-tetrakis(4-hydroxyphenyl)porphyrin (*m*THPP) to yield trisugar substituted porphyrins followed by alkylation of the remaining free HO-group. Alternatively, monoalkylation of one OH group followed by introduction of three carbohydrates was possible as well. Deprotection then provided a library of amphiphilic porphyrins (431). The derivatives exhibited high water solubility, however, the Soret band broadening was dependent on the concentration in solution compared to solutions of DMSO which exhibited sharp Soret bands and inactive CD. The aggregation of the derivatives was examined by absorbance and circular dichroism (CD) spectra. Trismaltohexaoseylated porphyrins with long alkyl chains formed stable aggregates in water and did not exhibit concentration dependency. In aqueous media, the intensity of the CD cotton effect signals decreased with increasing alkyl chain length. A chiral face to face aggregation was observed for short alkyl chain derivatives while an edge to edge aggregation was observed in the long alkyl chain derivatives. These observations show the potential to create photofunctional nanomaterials [231a].

Similar strategies were used for the derivatization of *m*THPP. *m*THPP itself was identified by Bonnet *et al.* to be a promising PS. A significant screen was carried out showing *m*THPP to be 25-30 times better than HpD in tumor models as a PS [238,239]. Several *in vitro* and *in vivo* studies have been carried out displaying the advantages of *m*THPP [239-242]. The derivatization of *m*THPP with 3-iodopropyl/peracetylmaltohexaoside yielded a series of mono-, di-, tri- and tetra-maltohexaose substituted *m*THPP (432-436) as highly water soluble PSs for PDT applications [202a]. All derivatives were deprotected affording 437-441 either in form of the free base of the zinc(II) complexes (Scheme 15). The water solubility of which rose with the increasing number of sugar moieties while the zinc derivatives had higher water solubility compared to the corresponding free base derivatives.

A robust methodology for direct glycosylation of a range of different mono-hydroxyl porphyrins with trichloroacetimidate glycosyl donors afforded high yields and faster reaction times [214].
achieved via direct glycosylation in the presence of BF\textsubscript{3}\cdot\text{OEt}\textsubscript{2} as the Lewis acid producing the alkyl and aryl derivatives in high yields 84-92 % followed by subsequent deprotection in 94-97 % yield. This procedure was also extended to tri-hydroxyl porphyrins and gave the glycosyl derivative 340 in 90 % yield \cite{214}.

S-linked systems: In comparison to the \(\text{O}\)-linked glycoporphyrins \(\text{S}\)-glycosyl bonds might be more resistant towards endogenous hydrolysis by glycosidases. A typical synthesis is outlined in Scheme 16. It involved the addition of a 1,3-dibromopropane linker unit to monohydroxytolylporphyrins (442) in 90 % and 85 % yields for 443, respectively \cite{243}. Glycosylation using a method from Bennett et al. \cite{244} gave 444-446 in 60-80 % yields. Quantitative deacetylation gave 447-449. Similarly, a range of \textit{meso}-aryl porphyrins and protoporphyrin IX derivatives appended with deprotected \(\text{S}\)-glucose, mannose and galactose residues were synthesized \cite{199}. Series one was synthesized from preformed \textit{ortho}- and \textit{para}-hydroxyphenyl precursors (213, 214) using Little conditions, to yield 223 and 224 in 91 % and 84 % yields when reacted with 1,3, dibromopropane and subsequently underwent glycosylation with 2,3,4,6-tetra-\textit{O}-acetyl-1-\textit{S}-acetyl-1-thio-\(\beta\)-\(\text{D}\)-galactopyranose, \(\beta\)-\(\text{D}\)-glucopyranose and \(\alpha\)-\(\text{D}\)-mannopyranose in 64-84 %, followed by deacetylation in quantitative yields producing 230-236. The second series of \(\text{thio}\)-glycosylated porphyrins was synthesized from protoporphyrin IX dimethyl ester 450 which was converted to the isohematoporphyrin 451 via modifications using methods from Smith’s group \cite{245,246} (Scheme 17). The latter was brominated to form 452 and was subsequently glycosylated yielding 453-455 in 64-93 % yield. This was followed by full deprotection (456a-458a) and partial deprotection (456b-458b) in quantitative yields. The relative stability of \(\beta\)-\text{thioglycosides} versus \(\beta\)-\text{glucosides} towards hydrolysis was tested by \(\beta\)-glycosidases finding \(\beta\)-\text{thioglucoside} to be a more stable residue \cite{199}.

5,10,15,20-Tetra(pentafluorophenyl)porphyrin (TPPF\textsubscript{20}) is a useful porphyrin precursor as the \(\text{p}\)-position of the pentafluorophenyl residue is susceptible to nucleophilic substitution. Thus, it can be used directly in glycosylation reactions without prior activation. For example, Drain et al. synthesized nonhydrolyzable tetrasubstituted glycoporphyrins \(\text{e.g.}, 87, 95\) in high yields from TPPF\textsubscript{20} in a nucleophilic substitution reaction with \(\text{thio}\)-glucose and \(\text{thio}\)-galactose derivatives to decrease the acid hydrolysis of the glycosyl bond using \(\text{C}\)- or \(\text{S}\)-glycoside linkages to reduce drug dosage requirements \cite{172,247}. \(\text{S}\)- or \(\text{C}\)-glycoside type \text{PSs} were previously synthesized in relatively low yields \cite{171,199,218,243}. However, significantly higher yields > 85 % were observed with both the acetyl protected or the free hydroxyl sugar present with such an approach \cite{172,247}.

These strategies can also be employed for cationic water soluble systems. Here, an example is Boyle et al.’s synthesis of a series of 5-\((\text{N-alkyl-4-pyridyl})-\text{-10,15,20-tris(4-thioglycosyl-2,3,5,6-tetrafluorophenyl)porphyrins (Scheme 18)} \))\cite{246}. The synthesis of the key intermediate, 5-(4-pyridyl)-
Scheme 16. Synthesis of thioglycosyl tolylporphyrins. (i) Br-(CH)\(_3\)Br, DMF, r.t., 20 h. ii) 2,3,4,6 tetra-O-acetyl-1S-acetyl-thio-β-D-galactopyranose, β-D-glucopyranose, or α-D-mannopyranose, (C\(_2\)H\(_5\))\(_2\)NH, DMF, r.t., 6 h, 60-80%; iii) MeONa/MeOH, r.t., 1 h.
Scheme 17. Synthesis of thioglycosylated isoheptaporphyrin derivatives. i) a) Ti(NO$_3$)$_3$, 3H$_2$O, MeOH, b) HCOOH c) NaBH$_4$; ii) SOBr$_2$/K$_2$CO$_3$; iii) 2,3,4,6-tetra-O-acetyl-1-S-acetyl-1-thio-β-D-galactopyranose, β-D-glucopyranose and α-D-mannopyranose (C$_2$H$_5$)NH, DMF; iv) NaOMe, MeOH or v) KOH.

synthesized via combinatorial synthesis and identified by HPLC and mass spectrometry. From the series conjugates with the most promising PDT efficacy were identified by \textit{in vitro} analysis. The viable derivatives (341-363) were subject to direct synthesis and further testing \cite{215}.

\textbf{N-linked systems:} A range of N-linked glycoporphyrins have been prepared as well. For example, amide-linked di- and octagalactosyl and glucosyl tetraphenylporphyrins (78, 79, 245, 246) were synthesized for a comparative study of their uptake in hepatocytes which have receptors (asialoglycoproteins) for terminal galactose residues. The highly water-soluble amide-linked octagalactose and octaglucose derivatives of TPP were synthesized by reacting lactonolactone or maltonolactone with octaamine TPP \text{75}, which was formed from the octa-ol TPP \text{77} \textit{via} the octanitrile TPP \text{76} \cite{175}.

Aminoglycosamide TPP and chlorin were studied for the effect symmetric or asymmetric glycoconjugation had on the photophysical properties, photosensitivity and cellular internalization compared to the parent porphyrin TPP in HT29 human adenocarcinoma cells \cite{201}. Synthesis of \text{247} was carried out by coupling O-acetylated glucosamine with 5-(4-carboxyphenyl)-10,15,20-triphenyl-porphyrin which was previously prepared via the Little condensation conditions in 7 % yield \cite{191}. The corresponding monocarboxyphenyl chlorin was prepared by the diimide reduction of 5-(4-carboxyphenyl)-10,15,20-triphenylporphyrin in 70 % yield which was subsequently coupled with \textit{O}-acetylated glucosamine affording the inseparable isomeric 2,3- and 7,8- monoglucosylated chlorins. The \textit{trans}-biglucoconjugated porphyrin \text{248} was synthesized from glucosylated dipyrromethane and benzaldehyde. The number of glycosylated groups altered...
Scheme 18. Synthesis of a series of 5-(N-alkyl-4-pyridyl)-10,15,20-tris(4-thioglycosyl 2,3,5,6-tetrafluorophenyl)porphyrins. i) DMF, r.t., 16 h; ii) R', DMF, r.t., 16 h; iii) NaOMe, MeOH, r.t., 1 h.

the singlet oxygen quantum yield showing the effect of structural changes on the triplet quantum yield.

In a more exhaustive study a range of glucosylated meso-tetraarylporphyrins were synthesized from meso-aminophenyl-substituted, β-amino-substituted and pentafluorophenyl-substituted porphyrin derivatives. Amide coupling of 5-(4-aminophenyl)-10,15,20-triphenylporphyrin with glucuronic acid afforded the N-linked glycoconjugate 462 after deprotection. Due to difficulty in hydrogenation of the benzyl protecting groups small amounts of monobenzylated derivatives were still present. A similar coupling was carried out via β-amino substituted porphyrins using nickel complexes which provided easier deprotection of the benzyl groups affording 463. Another approach for the coupling of glycosyl units was achieved via nucleophilic aromatic substituted with meso-pentafluorophenyl-substituted porphyrin selectively at the para position affording a mixture of products 249-252 in 40%, 7%, 14% and 8%, respectively. In addition, zinc derivatives 253-259 provided similar results.

Thiourea, thiocarbamate and carbamate linkages have been investigated for their chemical stability and PDT efficiency in the form of glycoconjugates 333-335. They were prepared from the amino or hydroxyl substituted tetraaryl porphyrins and per-O-acetyl-β-D-glucopyranosyl isothiocyanate under reflux or ultrasonication in good yields 72%, 23% and 49%, respectively. Examining the photophysical properties displayed the best singlet oxygen production in the range 335 > Photofrin™ > 333 > 334 while the photostability increased in the same order. In conclusion, the carbamate derivative 335 has the most promising potential.

2.4.2.2 Organometallic Coupling Reactions

The use of “click chemistry” as a mode of chemoselective linkage of azido appended polysaccharides and alkyne terminated porphyrins was successfully carried out and has now turned into a mainstay of carbohydrate porphyrin
chemistry. The native linear (1→3)-β-D-glucan (curdlan, 464) was converted to the 6-bromo-6-deoxycurdlan 465 with subsequent azidation afforded 6-azido-6-deoxycurdlan 466. Coupling of 466 with the alkyne porphyrin was achieved using CuBr₂ and ascorbic acid (Scheme 19) [250]. By now, this method has been extended to generate a wide variety of glycoporphyrins. These include systems with either one or four galactose or lactose units (257, 258) from Vicente's group [203]. Note, the click reaction can also be used to prepare suitable aldehydes for condensation reactions as exemplified in the synthesis of 472 (Scheme 20) [203].

A collaboration between our groups focused on the development of robust methodology for the synthesis of mono-, di-, tri- and tetra-modified glycoporphyrins (259-268) [32]. TPP was functionalized with an azido moiety and subsequently reacted with commercially available β-propargyl glucose and α-propargyl mannose either with acetylated or free hydroxyl groups under conventional and microwave-mediated heating conditions. The microwave conditions reduced reaction times from 3 days to 20 min. and was optimized to allow functionalization with both protected and deprotected carbohydrates in high yields. A sequential “double-click” process was used to obtain a new class of bis-modified 5,10-diglycoporphyrins (263) with heterogeneous carbohydrates. Reacting a propargyl glucoside with three equivalents of 5,10-di(4-azido-phenyl)-15,20-diphenylporphyrin provided 262 as the only product in 87 % yield and was subsequently reacted with propargyl mannanside via a second ‘click’ reaction affording 263 in 53 % yield.

In continuation of this initial work, a significantly large library of mono-, di- and trideprotected glycoporphyrins (316-331,261) were synthesized with monosaccharides and for the first time, synthetic disaccharides and trisaccharide [33]. These studies also provide the first examples of bis-modified glycosylated porphyrins and a significant highlight was the ligation of the synthetic trisaccharide LewisX, a histo-blood group antigen. Optimization studies were conducted to achieve a high yield ligation with the unprotected saccharide units all synthesized with a propargyl unit at the anomeric position. Again TPP was functionalized with an azido moiety and subsequently reacted with synthetic mono-, di- and tri-saccharide via optimized Huisgen ‘click’ cycloaddition reaction in very high yields. With a sequential “double-click” process used again to obtain bis-modified 5,10-diglycoporphyrins (331). This study was the first example of synthetic carbohydrates including N-Ac-lactosamine and LewisX conjugated to the porphyrin scaffold [33].

Similar work by Maillard prepared a series of porphyrins with three glycosyl units linked via triazole groups using microwave heating conditions [251]. Several parameters were examined including the sugar type, the length of the spacer, and the position of the triazole ring for changes in photobiological properties. All derivatives were synthesized from the precursor 473 which was synthesized previously by

![Scheme 19. Synthesis of (1→3)-β-D-glucan conjugated methoxycarbonylphenyl-porphyrin. i) Triphenylphosphine, DMF, LiCl, r.t., 3 h, and then, carbon tetrabromide, 60 °C, 24 h; ii) sodium azide, Me₂SO, 80 °C, 36 h; iii) alkyneterminated functional modules, CuBr₂, ascorbic acid, propylamine, r.t., 12 h, Me₂SO or NMP.](image-url)
Scheme 20. Synthesis of tetrasubstituted glycoconjugated porphyrins. i) Pyrrole, BF$_3$•OEt$_2$, DDQ; ii) 0.5 M CH$_3$ONa/MeOH; iii) Zn(OAc)$_2$; iv) tetrahydroisoindole, BF$_3$•OEt$_2$; v) DDQ; vi) Pd(OAc)$_2$; DDQ; vii) 0.5 M CH$_3$ONa/MeOH.

a modified Senge procedure \cite{144,252}. It was substituted with 1,3-dibromo propane which was converted to the azido groups or propargyl bromide to provide two different scaffolds 474, 475 for glycosyl conjugations. The compounds were protected by zinc(II) complexation before carrying out the Cu-catalyzed Huisgen 1,3-dipolar cycloaddition with copper salts affording 476, 477 in quantitative yields. Likewise, 478, 479, 482-484 were obtained via copper-catalyzed azide-alkyne cycloadditions with optimized conditions for the different substituents on the porphyrins 476, 477. Coupling of 476 with propargyl glycosides was carried out using CuCl affording 478, 479 in 65 % and 68 % yield while coupling of 477 with azido glycosides required a CuSO$_4$/sodium L-ascorbate catalyst system producing 482-485 in 40-80 %. Zinc was removed from all glycoporphyrins quantitatively and followed by deacetylation to afford 480, 481, 486-489 (Scheme 21).

A series of dendritic glycoporphyrins (491a-494a) and 491b-494b) were obtained from a multi-step procedure via coupling of the glycodendritic precursors and the alkyn-functionalized porphyrin core by a convergent methodology using click chemistry (Scheme 22). The porphyrin core was obtained via Lindsey-type condensation and subsequent functionalization with propargyl group and metallation with zinc. This porphyrin core was coupled through Cu(I)-catalyzed click reactions with the azidic glycodendritic
Scheme 21. Synthesis of tri-substituted glycoporphyrins via Huisgen 'click' chemistry. i) 1,3-Dibromopropane, K₂CO₃, dry DMF, r.t., 60 h, 77 %, then NaN₃, dry DMF, r.t., 16 h, 59 %; ii) propargyl bromide, K₂CO₃, dry DMF, r.t., 18 h, 98 %; iii) ZnOAc, MeOH, reflux, 1 h; iv) CuCl, propargyl glycosyl derivatives, toluene, microwave irradiation (100 W, 140 °C, 20 min.); v) TFA (demetalation) and then MeONa/MeOH, CH₂Cl₂, 1 h; (vi) CuSO₄, sodium L-ascorbate, glycosyl derivatives, THF/t-BuOH/H₂O, microwave irradiation (80 W, 85 °C, 3 min.).
Scheme 22. Synthesis of tetra- and octa-substituted glycoporphyrins via Huisgen ‘click’ chemistry. i) CuSO₄, sodium L-ascorbate, glycosyl dendron, THF/H₂O, 18 h r.t.; ii) MeONa/MeOH, CH₂Cl₂, overnight.
precursors using CuSO₄ and sodium L-ascorbate to form the dendritic glycoporphyrins (491a-494a) in 40-60 % yield followed by deprotection to afford the water soluble derivatives 491b-494b [253].

2.4.2.3 Dendritic Systems

The first glycoporphyrin with branched carbohydrate units was reported by Stoddart and coworkers using amide bond forming reactions [254]. Highly water soluble, tetrasubstituted porphyrins with peracetylated and perbenzylated β-D-glucopyranosyl modalities were synthesized in 16 % and 39 % yield and then deprotected under Zemplén conditions to 495 and 496.

In order to gain an entry into amphiphilic dendritic porphyrins Maillard prepared two amphiphilic glycodendrimeric TPPs bearing only one glycodendrimer moiety with variable linkers [204]. The glycodendrimeric derivatives 266 and 267 were synthesized via amide coupling of preformed glycodendrimers with 5-(p-benzoic acid)-10,15,20-triphenylporphyrin in good yields of 86 % and 57 %, respectively. De-acetylation afforded amphiphilic glycodendrimeric porphyrins 268 and 269 [204].

Maillard’s group also synthesized two families of glycoporphyrins, the first with sterically hindered sugar moieties with reduced mobility (271) and the second (499b-511b) with flexible linkers reducing constraints observed in 271 [205]. Compound 271 with reduced mobility was synthesized by coupling 5-(4-benzoic acid)-10,15,20-triphenylporphyrin with the aminoglycodendrimer using EDC-HOBt in 49 % yield. Compound 270 subsequently underwent deacetylation in quantitative yield. The second family (499b-511b, Scheme 23) were prepared by condensing porphyrin 497 with several aminoglycodendrimers using EDC-HOBt as a coupling agent in ~ 40 % yield followed by quantitative deacetylation. The non-glycosylated dendron was also coupled with 497 affording 512a,b for comparative biological studies. Taking into account their previous work on glycoporphyrins [160,162,198,204,205] they concluded that the best compounds appeared to have three α-glycosyl groups on the para-position of the meso-phenyl ring via a flexible linker. However, the presence of three sugars via a glycodendrimer decreased the phototoxicity [256].

2.4.3 Chlorins

Chlorins and bacteriochlorins having strong absorption in the red region of the visible spectrum are ideal candidates for deep tissue penetration with light (Figure 5) [257].

In order to gain an entry into amphiphilic dendritic porphyrins Maillard prepared two amphiphilic glycodendrimeric TPPs bearing only one glycodendrimer moiety with variable linkers [204]. The glycodendrimeric derivatives 266 and 267 were synthesized via amide coupling of preformed glycodendrimers with 5-(p-benzoic acid)-10,15,20-triphenylporphyrin in good yields of 86 % and 57 %, respectively. De-acetylation afforded amphiphilic glycodendrimeric porphyrins 268 and 269 [204].

Maillard’s group also synthesized two families of glycoporphyrins, the first with sterically hindered sugar moieties with reduced mobility (271) and the second (499b-511b) with flexible linkers reducing constraints observed in 271 [205]. Compound 271 with reduced mobility was synthesized by coupling 5-(4-benzoic acid)-10,15,20-triphenylporphyrin with the aminoglycodendrimer using EDC-HOBt in 49 % yield. Compound 270 subsequently underwent deacetylation in quantitative yield. The second family (499b-511b, Scheme 23) were prepared by condensing porphyrin 497 with several aminoglycodendrimers using EDC-HOBt as a coupling agent in ~ 40 % yield followed by quantitative deacetylation. The non-glycosylated dendron was also coupled with 497 affording 512a,b for comparative biological studies. Taking into account their previous work on glycoporphyrins [160,162,198,204,205] they concluded that the best compounds appeared to have three α-glycosyl groups on the para-position of the meso-phenyl ring via a flexible linker. However, the presence of three sugars via a glycodendrimer decreased the phototoxicity [256].

2.4.3 Chlorins

Chlorins and bacteriochlorins having strong absorption in the red region of the visible spectrum are ideal candidates for deep tissue penetration with light (Figure 5) [257].

2.4.3.1 Reduction

The simplest method for the preparation of chlorins is reduction of a parent porphyrin. However, yields can be low
and for unsymmetrical porphyrins different regioisomers will be formed. For example, Mikata et al. synthesized chlorin derivatives via diimide reduction from their previously synthesized acetylated-glycosyl porphyrins 29-32 and 53 \([167,258]\). The tetra- and octaglycosyl-tetraphenylchlorins 513a-c were obtained in 57-73 % yield and deprotected to the water soluble derivatives 514a-c. A similar synthesis of the N-acetylglucosamine derivative \([258,259]\) could not be isolated due to solubility problems with the product. Similar mTHPC glycoconjugates were prepared as illustrated in Scheme 24. However, reduction of the trisubstituted porphyrin yielded two inseparable isomers 2,3- and 7,8-meta-triglycosylated chlorins (516, 517) while the meta-substituted tetraglycosylated chlorin 514f was formed as a mixture of four atropisomers at r.t. \([260]\). By now, many other glychlorins have been prepared using the Whitlock method, including a series of 5,10,15,20-tetrakis[3- or 4-(β-D-glycopyranosyloxy)phenyl]chlorins with glucopyranosyl, galactopyranosyl, xylopyranosyl and arabinopyranosyl groups (514a,b,d-i) \([261,262]\). Note, that reduction can afford a mixture of chlorins and bacteriochlorins which was the case in this study. Purification of the glychlorins required reverse phase-TLC to give the glychlorin derivatives in 14-49 % yield. UV-vis spectroscopy of the crude mixture showed for the meta-substituted glycoporphyrins the yield of the bacteriochlorins to be considerably higher than the chlorins unlike the para-substituted derivatives \([261]\).
Scheme 24. Synthesis of carbohydrate derivatives of mTHPC. i) (a) toluene-4 sulfonohydrazide, anhydrous K$_2$CO$_3$, dry pyridine, 100 °C – 110 °C, (b) o-chloranil, ethyl acetate or benzene, 20 °C; ii) CHCl$_3$/MeONa/MeOH, 20 °C.

2.4.3.2 Dihydroxylation

More synthetically useful is the 2,3-dihydroxylation of porphyrins and follow-up chemistry [263-265]. Some of the earlier examples of hydroxychlorins derived from octaethylporphyrin were substituted with glycerol, D-glucose and D-mannitol via ether linkages as outlined in Scheme 25 [39,266]. The 2,3-dihydroxychlorin 520 undergoes a pinacol
rearrangement yielding the oxochlorin 521 in 75 % yield. This is an advantageous reaction as the geminal dimethyl unit stabilizes the system and prevents reoxidation to the porphyrin. Reduction in an excess of sodium borohydride in ethanol yielded the hydroxochlorin 522 in 87 % yield with conversion to the bromide derivative which was reacted immediately with the desired alcohol. Glycerol 524 and D-mannitol 526 derivatives yielded in both cases two diastereoisomers while the D-glucose derivative 525 yielded four diastereoisomers due to the C-2 on the chlorin and the anomeric carbon of the sugar [99].

The method can also be applied to meso-substituted porphyrins as was exemplified by functionalization of 5,10,15,20-tetra(p-aminophenyl)porphyrin 82 [206]. The glucosyl TPP derivative 273 was synthesized from the monosubstituted protected carboxylic acid functionality (272) and subsequent addition of the β-D-glucopyranuronoyl chloride and additional base afforded the desired compound in 33 % yield. Conversion to the corresponding chlorins and bacteriochlorins was determined by the equivalents of osmium tetroxide-mediated dihydroxylation. Reacting porphyrin 273 with two equivalents of OsO₄ afforded four osmate ester isomers, two sets of regiosomers each of which consisted of two stereoisomers. The regiosomers were separated and subsequently treated with hydrogen sulfide affording vic-dihydroxychlorins 527a/a’ and 527b/b’ (Scheme 26). Following with hydrolysis of the sugar acetyl groups and glutaryl methyl ester of 527a/a’ and 527b/b’ afforded the hydrophilic chlorins 528a/a’ and 528b/b’. The related 2,3,12,13-(vic-dihydroxy)bacteriochlorins were synthesized with a large excess of OsO₄ from porphyrin 273 affording four isomers which were separated into two components. The two fractions consisted of two isomers which were treated with hydrogen sulphide providing bis(vic-dihydroxy)bacteriochlorins 529 which were subsequently hydrolyzed to the deprotected bacteriochlorins 530. The carboxylic acid functionality allows for conjugation to biomolecules and nanoscaffolds which was displayed by the conjugation of the chlorins to a dextran-coated nanoscaffold. It displayed good suspension stability and did not show differences in excited state quenching at any loading compared to chlorin e₅ [206].

2.4.3.3 1,3-Dipolar Cycloadditions

More significant structural variations to the porphyrin core can be introduced with 1,3-dipolar cycloaddition reactions at the β-pyrrole positions [267,268].

In this context a typical example are pyrroldinoporphyrin glycoconjugates with N- or C-linked galactose derivatives prepared using the 1,3-dipolar cycloaddition strategy with sugar azomethine ylides by Cavaleiro and coworkers [269]. A typical example is the synthesis of glycosylated isoxazolidine-fused chlorins and bacteriochlorins via 1,3-dipolar cycloaddition reactions of TPPF₂₀ and glycosyl nitrones [270]. A mixture of porphyrin TPPF₂₀ and an excess of the galactosyl nitrone were reacted at 60 °C in a concentrated solution in toluene for 5 days and afforded a mixture of three compounds. The major product, chlorin 531a in 66 %, displayed endo addition. Two other products were the result of bis addition to the porphyrin affording the bacteriochlorins 532a/b or 533a/b in 21 % and 9 % yields, respectively. The relative yield was dependent on the the reaction time [270]. The cycloaddition of other sugar

![Scheme 25](image_url)

**Scheme 25.** Synthesis of hydroxochlorins derived from 2,3,7,8,12,13,17,18-octaethylporphyrin substituted with glycerol, D-glucose and D-mannitol via ether linkages. i) Fuming H₂SO₄; ii) sodium borohydride, 1 h, EtOH/H₂O; iii) 50 % HBr-HOAc for 1 h at 20 °C; iv) glycerol; v) D-glucose; vi) D-mannitol.
Scheme 26. Synthesis of vic-dihydroxychlorins and -bacteriochlorins. i) excess OsO₄; ii) 0.3 M LiOH, 2 h; iii) (a) OsO₄, pyridine; b) H₂S; iv) 0.3 M LiOH, 2 h.

residues yielded the chlorin 531 and in some cases the bacteriochlorins. Reacting ribosyl nitroxy at 60 °C for 5 days provided only the chlorin derivative 531b in 30 % yield. The xylose derivative reacting at 100 °C for 10 h yielded the chlorin 531c in 37 % and only traces of the bacteriochlorins.

The lyxosyl nitroxy gave a single bacteriochlorin (532) in 61 % yield and the chlorin 531d in 29 %. All glycosylated chlorins and bacteriochlorins isopropylidene acetals could be deprotected in quantitative yields.
Subsequently, this work was extended to azomethine ylides and diazoacetates \[271\]. A new method for the synthesis of the glyco-conjugates \((536-539)\) was developed using \(\text{CuCl}\) to catalyze the cyclopropanation reactions of \(\{5,10,15,20\text{-tetra(pentafluorophenyl)porphyrinato}\text{zinc(II)}\) \((534)\) with \(\alpha\)-diazoacetates \((535a-d)\) of glucofuranose, fructopyranose, galactopyranose and xylofuranose (Scheme 27). It was found that the distribution of products \((536-539)\) was dependent upon the carbohydrate moiety with the trans-chlorin \(536\) being the major product in all cases. The highest selectivity was obtained with \(535a\). The new chlorins \(536a-d\) and the isobacteriochlorin \(538b\) were characterized while the other structures present in trace amounts were only identified by UV-vis spectra. Removal of the protecting groups and metal complexes of \(536a-d\) was carried out in aq. TFA affording \(540a-d\). Singlet oxygen production studies showed the chlorins \(540a-d\) to be better than the well-known singlet oxygen producer methylene blue with compound \(540b\) providing the best production. No significant difference in Log P was observed, but chlorins \(540a\) and \(540c\) were only slightly more hydrophilic than \(540b, d\) \[271\].

Similar methods were used by Hirohara et al. to study a range of \(O\)-glycosylated tetraarylporphyrins and -chlorins, which showed several advantages including reduced dark cytotoxicity, improved water-solubility, better cellular uptake, and sugar-dependent photocytotoxicity \[165,261,262,272,273\]. The \(O\)-glycosylated chlorins had promising photocytotoxicity in HeLa cells but were difficult to purify. In expansion of these results, research focused on the photochemical evaluation of \(S\)-glycosylated chlorins. Eight

XXX-XXX/14 $58.00+0.00 © 2014 Bentham Science Publishers
Scheme 27. Synthesis of deprotected glyco-chlorins and bacteriochlorins. i) CuCl (2 mol %), CH₂Cl₂, 40 °C; ii) TFA/H₂O (9:1), r.t., 30 min.

S-glycosylated TPPF₂₀’s (87, 91, 95, 96) and their 1,3-dipolar cycloaducts, i.e. chlorins 542a’, 542b’, 542a and 542b were synthesized by nucleophilic substitution of the pentafluorophenyl groups (83) with S-glycosides. S-glycosylated porphyrins 87 and 96 and chlorins 542a’ and 542b’ were isolated in excellent yields of 74 %, 86 %, 94 % and 82 %, respectively. Subsequent removal of the acetyl groups via alkaline hydrolysis afforded the free-hydroxyl derivatives (91, 95, 542a and 542b). The fused chlorins were found to be significantly more stable than those prepared via diimide reduction. The photophysical properties showed the S-glycosylated chlorins to have molar extinction coefficients of the Q band twice that of the corresponding unconjugated derivative. These compounds were also investigated for their in vitro photocytotoxicity in HeLa cells [178].

In a similar way, Drain and coworkers extended from previous work on the synthesis of thioglycosylated porphyrins (95 and 91) [247] to form their 1,3-dipolar cycloaducts, i.e. chlorins (542a,a’), isobacteriochlorins (545a,a’) and bacteriochlorins (546a,a’) via the same procedure [274]. The photophysical properties showed that the lowest energy Q band of 542a is 25 times greater than that of the corresponding porphyrin 91 while that of the isobacteriochlorin 545a is only 5 times greater than 91. All these compounds were evaluated against MDA-MB-231 and K: Molv NIB 3T3 mouse fibroblast cells [274].
2.4.3.4 Benzochlorins

A more 'classic' approach is the preparation of benzochlorins [275,276]. Maillard et al. regioselectively synthesized an amphiphilic glycoconjugated meso-monoaryl-benzochlorin from the meso-monoaryl-nickel(II) porphyrin [277]. This involved a somewhat regioselective Vilsmeier reaction of $547$ to provide two isomeric nickel(II) porphyrins $548$ and $549$ (85:14) in 85 % yield. The former was subjected to inert acid conditions and underwent cyclization yielding the nickel(II) benzochlorin $550$ in 58 %. Followed by dealkylation of the methoxy group, demetallation and subsequent glycosylation of the free hydroxyl gave the glycosylated benzochlorin $552a$. The amphiphilic benzochlorin $552b$ was obtained after quantitative deacetylation under Zemplén conditions (Scheme 28).

Pandey et al. expanding on their previous work [278] synthesized an extended library of $569a,b$ and $570$ incorporating compounds which are known for their photoactivity. A benzochlorin derivative of octaethylporphyrin (OEP) condensed with a glucosamine, galactosamine, and lactosamine were investigated for their galectin-binding ability [279]. The carboxylic acid functionality on the fused exocyclic benzene ring of the benzochlorin $553a$ was substituted with acetylated galactosamine, glucosamine, and lactosamine affording the glycoconjugate derivatives $554a$, $555a$ and $556a$ in 40 %, 97 % and 43 % yields, respectively. All of which were deprotected in quantitative yields and the free base derivatives were converted into the Zn(II) analogues ($554b$, $555b$, $556b$) as they had been previously shown to have better PDT efficacy [280]. Studies have shown that the C-glycosyl derivatives are more stable than the O- or N-glycosyl compounds [281]. To determine the difference in PDT efficacy a benzochlorin-C-galactose conjugate was prepared as well ($557$) [279].
Scheme 28. Synthesis of an amphiphilic glycosylated benzochlorin. i) 3-(Dimethylamino)acrolein/POCl₃; ii) CF₃CO₂H/Ar; iii) BBr₃/dry CH₂Cl₂; iv) H₂SO₄; v) K₂CO₃ in DMF/60 °C; vi) MeONa/MeOH.
2.4.3.5 Chlorophyll, Phytochlorin, and Rhodochlorin Derivatives

Chlorophyll derivatives offer another intriguing avenue for new PS \[150,282,283\]. Their reduced character makes them promising candidates for PDT, although this is somewhat counteracted by their low stability. Mironov used his expertise in chlorophyll chemistry to prepare pheophorbide a 559 derivatives, where the sugar moiety was linked via the 3-vinyl group \[284\]. Synthetically this required transformation of the vinyl group in 559 into the 2-bromoethyl derivatives with HBr, which were reacted in situ with 1,2,3,4-di-O-isopropylidenegalactose to yield, for example, the glycolpheophorbide a 560. These compounds were insoluble in nonpolar solvents, displayed good solubility in alcohol and limited solubility in water \[284\].

Naturally, the C17 side chain can be used for conjugation as well. Pyropheophorbide 2-deoxyglucosamide 563 was synthesized for applications in fluorescence imaging and for PDT. It was obtained through pyropheophorbide succinimidyl ester 562 by reacting the parent pyropheophorbide 561 with N-hydroxysuccinimide which subsequently underwent glycosylation with D-glucosamine affording the bioconjugate in an overall yield of 50 % (Scheme 29). Synthesized as a glucose transporter targeting PS it can take advantage of the intracellular trapping mechanism displayed by some tumor cells for 2-deoxyglucosamide \[285\].

The carboxylic acid function also offers a convenient handle for the improvement of "established" PSs. In the phytochlorin series one such compound is HPPH (Photochlor) which displays strong absorptions at 408 nm and 665 nm, the latter of which is important for its PDT efficacy \[286\]. HPPH is under investigation in several clinical trials for esophageal cancer, non-small cell lung cancer, basal cell skin cancer, head and neck cancer. HPPH, like Photofrin\[\text{®}\], is lipophilic with slow clearance from the body \[286\]. 3-Devinyl-3-(1'-hexyloxyethyl) pyropheophorbide-a (HPPH) was conjugated with mono-, di-, or tetra-saccharides to determine the effect the carbohydrate unit had on the PDT efficacy of the basic macrocycle \[287\]. To determine the effect β-galactose carbohydrates have on tumor-selectivity and its dependence on galectin 3 binding both galactose conjugates (galactose and lactose) and non-galactose conjugates (glucose and cellobiose) of HPPH were synthesized. The first series of HPPH derivatives were synthesized by coupling HPPH with 1-aminotetra-acetogalactose, 1-aminotetra-acetoglucose, 1-aminohexa-acetolactose and 1-aminohexapacetocellobiose and deacetylated affording 558a-d. The second series synthesized incorporated mono- and multivalent rigidified lactose units which were coupled to HPPH with an extended amine linker (558e) followed by

Formula 11.
deacetylation afforded 558-h. All were tested for their affinity for galectins overexpressed on cancer cells [287].

Similar systems were also employed in a study on the effect of the carbohydrate unit on PET imaging and PDT efficacy. A range of glycosylated phytochlorin derivatives were synthesized. Galactose conjugated HPPH had previously shown higher photosensitizing efficacy than HPPH in mice bearing RIF tumors [285,287]. Following this 3-(1′-m-iodobenzyloxyethyl)pyropheophorbide-a methyl ester (564), 3-(1′-m-iodobenzyloxyethyl)-17′-{(2-deoxy)glucose}pyropheophorbide-a (565), and 3-(1′-m-iodobenzyloxyethyl)-17′-{(1-deoxy)-galactose} pyropheophorbide-a (566) were synthesized and converted into the corresponding 124I-labeled analogues via reacting trimethyltin analogues with Na124I. All pyropheophorbide-a analogues 564-566 were examined in vivo for PET imaging, biodistribution and therapeutic potential in a comparative study in BALB/c mice bearing Colon-26 tumors [288].
Pandey's group also spent considerable efforts on the preparation of purpurin derivatives. Purpurin-18 is a derivative of chlorophyll which can be readily isolated from spinach [289]. It has three distinct features: a vinyl group, a fused anhydride ring and a propanoic acid side chain. Thus, upon modification it can provide a series of PSs including purpurinimides [35]. Pandey's group first synthesized β-galactose conjugated purpurinimides (569a,b, 570) via enyne metathesis for more specific targeting to the Gal-1 (galectin-1) receptor. Molecular modeling studies indicated that once the PS was connected via the anomeric position it had no effect on the galectin-carbohydrate recognition. The quantitative structure–activity relationship evaluation of the galactosylated derivatives identified that the presence and positioning of the sugar moiety was important for photobiological activity [278].

Subsequently, purpurinimides were substituted at varying positions with different linkers and glycosyl units to determine their effect on the PDT efficacy *in vitro* and *in vivo* [290]. The lactose unit was linked with a diene (569b), an amide (574), an ethylene (575) or was regioselectively introduced at positions 3, 8 and 12 forming the positional isomers (571b, 572, 573). Compounds 571c and 571d were prepared for comparison. A facile methodology allowed for the synthesis of both N-alkyl/aryl analogues of monosaccharide-purpurinimides with varying lipophilicity.

Other chlorophyll derivatives potentially suitable for PDT are the rhodochlorsins, notably chlorin e6. For example, Mironov and coworkers used 1,3-dipolar cycloaddition Huisgen click chemistry for the synthesis of a glycoconjugate of chlorin e6 providing an amphiphilic PS for application in PDT. The synthesis of 577a was achieved by conversion of methyl pheophorbide a (576) to the chlorin e6 derivative *via* ring-opening of the pentanone exocycle using propargylamine forming the terminal triple bond (Scheme 30) [291]. The ethynyl derivative 577a underwent a
cycloaddition reaction with 1-O-(2-azidoethyl)-β-D-lactose peracetate using copper(I) iodide as a catalyst affording the glycochlorin 578a in 82% yield. The removal of the copper from the macrocycle caused destruction of the glycochlorin. Therefore, the Zn(II) complex 577b was prepared to protect the macrocycle and was easily removed following the cycloaddition reaction. Compound 578e was subsequently deacetylated to give the water soluble glycochlorin 578d. The photophysical properties of 578c were examined and the quantum yields of fluorescence (Φ ~ 0.25) and singlet oxygen (Φ'O2 ~ 0.75) were not changed by addition of the sugar residue compared to 577a. This work was later extended to prepare a series of derivatives with glucose, galactose and lactose residues (579, 578b, d) as well as the chlorine p6 glycoconjugate 581. In addition glycoconjugates of a bacteriochlorin cyclic imide were prepared from bacteriopurpurin yielding the glycosylated bacteriochlorophyll a (580a, 582) derivatives. Quantum yields of fluorescence and singlet oxygen of all three 579a,c and 578b derivatives were Φ ~ 0.25 and Φ'O2 ~ 0.75.

Scheme 30. Synthesis of chlorin e6 glycoconjugates and metal complexes. i) Propargylamine, CH2Cl2; ii) Zn(OAc)2, CHCl3–MeOH; iii) CuI, MeCN, Et3N; iv) HCl; v) MeONa, MeOH.
compared to meso-tetakis(4-sulfophenyl)porphyrin \(^{292,293}\).

More complex carbohydrates have also been appended to chlorophyll derivatives. For example, a significantly advanced glycosyl unit in the form of hyaluronic acid, consisting of repeating disaccharide units of N-acetyl-D-glucosamine and D-glucuronic acid was adhered to several Pheophorbide a PSs. The synthesis involved acetylation of hyaluronic acid with acetic anhydride, followed by conjugation with pheophorbide a via carbodiimide coupling reaction to yield 583 \(^{294}\). In addition a similar polysaccharide nanogel was synthesized containing pullulan/folate conjugated to Pheophorbide a PSs \(^{295}\). Pullulan is a maltotriose isolated from the fungus Aureobasidium pullulans and can be used as a drug carrier and, combined via DCC or DMAP-mediated ester formation with folate which can target overexpressed receptors on the surface of several human cancer cells, provides a self-organized nanogel. When coupled with pheophorbide a again with a carbodiimide reaction they afford nanostructures ~170 nm in size with three derivatives isolated with varying degrees of PS attached to the sugar chain \(^{295}\).
2.5 Related Dyes

2.5.1 Pyrrole Derivatives

Many other dye systems have been investigated for PDT. With the exception of systems such as hypericin, most of these are chemically related to porphyrins. Consequently some examples of expanded, contracted and isomeric glycoporphyrins have been investigated as PS. However, synthetic examples of carbohydrate conjugates for these systems are limited.

One such example is 585a, a sapphyrin derivative, which exhibited a singlet oxygen quantum yield of 0.18 and showed phototoxicity against *Staphylococcus aureus* similar to that of HpD. Although initially promising, interest in PDT use of sapphryins has waned in recent years [296]. Král et al. also synthesized similar expanded dyes (587 and 586b) via preformed sapphryins functionalized with glycosyl residues by amine coupling using DMAP as well as the disubstituted glyco-protoporphyrin 587 which were tested in vivo [297,298].

Two water soluble glucosamine sapphyrin derivatives were synthesized via EDC-mediated coupling of sapphyrin diacid 586a and either D-glucosamine (586d, 13 %) or N-methyl-D-glucosamine (586c, 10 %) to investigate their anion binding properties [299]. The compounds formed H-aggregates in neutral aqueous solutions and the extent of aggregation was anion dependent with the prototated sapphyrin acting as an anion binding agent. In particular, addition of phosphate anions at pH 7 afforded considerable formation of monomeric species. The concentration dependent behavior of this effect indicates the possible use of glycosylated sapphryins as fluorescent phosphate anion sensors. Several examples of mono-, di- and triglycosylated fluorophenyl corroles were synthesized via combinatorial approaches with a total of 24 products [215].

An example of a deprotected mono-glycosylated calix[4]pyrrole derivative was synthesized by Drašer et al. via C-glycosyl linkage to the meso-positions of oligopyrrole. The synthesis of the protected mono- and diglycosylated calix[4]pyrrole derivatives was achieved in a two-step procedure (Scheme 31). First, acid catalyzed condensation of pyrrole and the carbohydrate ketone derivative 588a gave the dipyrryl derivative 589a and secondly, acid-catalyzed MacDonald reaction with acetone provided a mixture of diastereoisomers of 5,15-syn and 5,15-anti calix[4]pyrrol-10,20-diyl-bis-D-glycero-D-gulo-heptitols (591) and monosubstituted calix[4]pyrrole (590a) in 16 % yield. In a similar procedure, the deprotected carbohydrate ketone 588b was used with methane sulfonic acid as a catalyst affording the mono-substituted 590b in 11 % with no disubstituted product found after purification. Ketone 588b and 5,5-dimethylpyrromethane gave 590b in 10 % yield. Usually dipyrrylmethane derivatives provide the symmetric...
disubstituted oligopyroles in 2+2 additions; however, a 3+1 addition forming the mono-substituted calix[4]pyrrole is usually only achieved by mixed condensations.

2.5.2 Phthalocyanines

Phthalocyanines (5,10,15,20-tetraazabenzoporphyrins) have also found wide interest as potential PSs. They are normally more photostable than porphyrins but the synthesis of unsymmetrically substituted phthalocyanines lags behind that of the porphyrins. The synthesis of their glycoconjugates follows similar strategies to those employed for porphyrins. However, as the meso position is now a nitrogen atom the carbohydrate unit is solely introduced at the β-pyrrole positions and often there is ambiguity as to the exact regiochemistry. For condensation reactions this requires the use of dedicated precursors. For example, the phthalocyanine derivative was synthesized in 61% yield from 592a and zinc acetate and was subsequently deprotected to afford 593. A good example of the synthesis of glycoconjugates follows similar strategies to those employed for porphyrins.

With the choice of the right precursors unsymmetrically glycosylated phthalocyanines are accessible through condensation reactions as well. Such an amphiphilic glycoporphyrine was synthesized via the cross-condensation of tetrakis(1,2,3,4-di-O-isopropylidene-α-D-galactopyranos-6-yl)phthalonitrile with phthalocyanine in 30% though cross-condensation with zinc chloride. Deprotection yielded the amphiphilic phthalocyanine. Amphiphilic derivatives
can be prepared as well, for example by cross-condensation of 1,2,5,6-di-O-isopropylidene-\(\alpha\)-\(D\)-glucofuranosyl phthalonitrile and octadecyloxyl phthalonitrile in the presence of zinc chloride with subsequent deprotection in 25% yield to afford an amphiphilic derivative with three sugar units and a C18 side chain \[305\].

Unprotected phthalonitriles can be employed in such condensation reactions as well. In a novel glycosylation reaction nucleophilic aromatic substitution was used to synthesize the first glycopthalonitrilite linked via the anomeric position of the sugar unit in high yields \[306\].

Starting from 4-nitrophthalonitrile, which underwent glycosylation with 2,3,4,6-tetra-\(O\)-benzyl-, benzoyl- and acetyl-protected glucopyranoses using NaH for \[599a\] and K\(_2\)CO\(_3\) for \[599b\,c\], afforded preferentially the \(\alpha\) anomers in \[600a\] (\(\alpha/\beta = 9/1\)), \[600b\] (\(\alpha/\beta = 10/1\)) and \[600c\] (\(\alpha/\beta = 10/1\)) \(\text{(Scheme 34)}\). Use of classical template synthesis with these protected glycopthalonitriles \(600a\,c\), followed by deprotection, gave poor conversion. However, preliminary deprotection of the tetraacetylated derivative \[600c\] under Zemplén conditions to \[601a\] and template synthesis with zinc acetate gave \[602a\] in 51% \[307\]. A similar method of direct
anomeric linkage was applied by Boyle et al. to synthesize a
tetragalactose-substituted Zn(II) phthalocyanine (602b) \[308\].
Similarly, water soluble glycopthalocyanines with four or
eight unprotected D-galactose moieties were synthesized for
application in PDT \[309\]. The glycopthalonitrile 603 was
tetramerized in the presence of ZnCl₂, affording
phthalocyanine 604 in 43 % yield. Compound 604 had been
previously synthesized via DBU-promoted self-cyclisation in
the presence of zinc acetate \[310\]. The glycopthalocyanine
with eight D-galactose units 608 was synthesized using a
similar route with glycopthalonitrile 606, whereby
phthalocyanine 607 was obtained in 62 % yield. Treatment
of compound 604 and 607 with aqueous TFA gave the
unprotected compounds 605 and 608 (Scheme 35).
Phthalocyanine 607 was later also synthesized through
cyclotetramerization of the isoindoline derivative of 4,5-
bis(1,2,3,4-di-O-isopropylidene-α-D-galactopyranos-6-
yl)phthalonitrile in presence of zinc acetate and DMAE in 29
% yield and subsequent deprotection \[311\].
In addition to the octa substituted glycopthalocyanine
600c, a highly glycosylated dendritic glycopthalocyanine
601a containing 16 glycosyl units was synthesized by
diglycosylation of 2,4,6-trichloro-1,3,5-triazine followed by
substitution with 1,3-dimercaptopropane and subsequent
nucleophilic substitution with (hexadecafluorophthalocyanato)zinc(II) in 81 % yield. The
porphyrin derivatives (610a) was synthesized in a similar
way using TPPF₂₉. Hydrolysis of both produced an α/β
mixture of the unprotected glycodendrimer conjugates 610b
or 611b (Scheme 36) \[312\].

\[Scheme 33. Synthesis of tetrasubstituted galactosyl phthalocyanine. i) (Gal-isop)-OH, NaH, toluene, N₂, 70 °C; ii) ZnCl₂, DMAE, N₂, reflux; iii) TFA/H₂O (9:1), r.t.\]

\[Scheme 34. Synthesis of tetra-substituted glucosylated phthalocyanine. i) For 599a: NaH (1 equiv.), dry DMF, r.t., 1 h; for 599bc: K₂CO₃ (6 equiv.), DMF, r.t.,
12 h; for 599d: K₃CO₃, DMF, r.t.; ii) for 600a and 600d: cat. NaOMe, dry MeOH, r.t., 1 h; iii) DMAE/n-butanol (2:1), dry zinc acetate (Zn(OAc)₂), 100 °C, 24 h.\]
Scheme 35. Synthesis of glycoconjugated phthalocyanines. i) ZnCl₂, DMAE, N₂, 140 °C; ii) TFA/H₂O (9:1), r.t.

Scheme 36. Synthesis of glycoconjugated phthalocyanines. i) TPPF₂₀, N,N'-diisopropylethylamine, DMF, 50 °C, N₂, 24 h, 78 %; ii) (hexadecafluorophthalocyaninato)zinc(II), N,N'-diisopropylethylamine, DMF, 50 °C, N₂, 24 h, 81 %; iii) TFA/H₂O (9:1), 78-80 %.
Organometallic couplings can also be employed for these tetrapyroles. For example, the synthesis of the deoxyribose–phthalocyanine conjugate 612, where the sugar moiety is connected via a C-C bond to the phthalocyanine, was achieved via Sonogashira coupling of (tetraiodophthalocyaninato)zinc(II) with {2-deoxy-3,5-bis[O-(p-toluoyl)]-α,β-D-ribofuranosyl}ethyne which was subsequently p-toluoyl deprotected affording the free hydroxyl derivative. The sugar unit improved water solubility without decreasing the photophysical properties.[313] Other organometallic couplings were used in the synthesis of tetruglucosyl zinc phthalocyanine (613) via click chemistry.[314] Likewise, axial coordination can be used for binding of the carbohydrate units. This is illustrated in a series of galactose conjugated silicon(IV) phthalocyanines which were synthesized via substitution of dichloro(phthalocyaninato)silicon(IV) 616 with 1,2,3,4-di-O-isopropylidene-α-D-galactopyranose using NaH in toluene for the disubstitution product (617, 38 %) or a mixed substitution of 1,2,3,4-di-O-isopropylidene-α-D-galactopyranose and several alcohols for the unsymmetric derivatives (618, 11-18 %, Scheme 37).

![Chemical structures](image-url)
In a similar manner, the glucosylated silicon(IV) phthalocyanines derivatives 614a,b were synthesized via two axial 1,2:5,6-di-O-isopropylidene-α-D-glucofuranose linked by a tetraethylene glycol chain to increase water solubility. The dichloro derivative 614b was synthesized by a 3+1 mixed cyclization of 1,3-diminoisoindoline and dichloro-1,3-diminoisoindoline in the presence of SiCl₄, subsequently was treated directly with 1,2:5,6-di-O-isopropylidene-α-D-glucofuranose with a tetraethylene glycol chain [315]. In addition, the galactose derivatives 615b,c were prepared with di- and octachloro substitution via mixed- and self-cyclization with dichloro 1,3-diminoisoindoline and 1,3-diminoisoindoline in the presence of SiCl₄ and subsequent additions of the protected galactose with NaH in toluene at 120°C for two days. Increasing the temperature to 170°C for four days the mono-substituted derivative 615a could be formed in 4 % yield [316]. All derivatives displayed significant PDT activity against HepG2 human hepatocarcinoma [315,317,318]. Larger constructs are also accessible, for example in the form of cyclodextrin-phthalocyanine dyads. This involved the synthesis of 4-(β-cyclodextrin) phthalonitrile by coupling β-cyclodextrin with 4-nitrophthalonitrile, followed by cross-condensation of 619 with excess of phthalonitrile or 4,5-dibutoxyphthalonitrile affording 620a and 620b (Scheme 38) [319].

**Scheme 37.** Synthesis of amphiphilic silicon(IV) glycosylated phthalocyanines. i) (Gal-isop)-OH, NaH, toluene, reflux, 24 h; ii) (a) (Gal-isop)-OH, NaH, toluene, reflux, 24 h (b) NaOC₂H₅ or NaOC₅H₁₁ or NaOC₈H₁₇, toluene.

**Scheme 38.** Synthesis of the first cyclodextrin phthalocyanine dyad. i) DMF, K₂CO₃, r.t.; ii) DMAE, ZnCl₂, reflux.
With the success of the Si-phthalocyanine derivatives focus turned to the synthesis of the more stable tetra- and monoglycosylated zinc(II) phthalocyanines (Scheme 39). Both galactosyl (also reported by Soares et al. [309], 604) and glucosyl (622) tetra-substituted derivatives were synthesized via DBU-promoted self-cyclisation in the presence of zinc acetate. In addition selectivity for the 1,8,15,22 isomer was observed when bulky groups were placed in the α position affording 625a and 625b from cerium promoted cyclisation reactions followed by metallation by zinc. Mixed condensations with the glycosubstituted phthalonitriles and phthalonitrile afforded AB3 type substituted derivatives 627a-d deprotection of the isopropylidene protecting groups proved problematic and was not carried out [310].

Scheme 39. Synthesis of glycoconjugated phthalocyanines. i) Zn(OAc)2, DBU, n-C5H11OH; ii) CeCl3, DBU, n-C5H11OH; iii) Zn(OAc)2, DMF; iv) Li, n-C5H11OH; v) Zn(OAc)2.
In addition, mono-β, di-α-, di-β- and tetra-substituted glycosylated zinc(II) phthalonitriles (627-630) were synthesized with tetraethylene-glycol-linked saccharide units via self- and mixed cyclisation of glycosyl phthalonitrile and phthalonitrile in the presence of zinc acetate and DBU. Both the di- and tetra- derivatives were converted to the water-soluble conjugates 628b-630b and investigated to determine the effect of the number and position of glycosyl units on PDT activity.

Organometallic couplings and glycosylations were also used for the synthesis of two series of amphiphilic nickel(II) phthalocyanines (631, 632) displaying an AB3 substitution pattern. A common hydroxylated phthalocyanine–nickel(II) was substituted with an ethyleneglycol spacer (633), a tetraethyleneglycol spacer (631) or an azidotetraethyleneglycol spacer (632). The both former underwent direct glycosylation in 70-90 % yield to afford glucose, galactose, mannose and lactose glycoconjugates.
which were subsequently deprotected. With the benzoylated imidates providing better yields then the corresponding acetylated derivatives. The latter azido functionality underwent a click cycloaddition reaction with propargyl sugar units with subsequent deprotection providing 632a-d \[321,322\]. In expansion of this work, a series of water soluble asymmetric AB₃ type glycothiacyanines were synthesized with a single glycosyl unit and three glycerol moieties using the same click cycloaddition reaction. Glycoconjugates 633a-d were prepared in ~95% yield and subsequently deprotected \[323\].

In extension of such work, Dumoulin \textit{et al.} also synthesized two sets of zinc metallated glycosylated phthalocyanines to determine the difference in PDT efficacy of the glycosyl linked sugar unit versus the triazole bridge (Scheme 40 and 41) \[324\]. The compounds were synthesized from mixed condensation of solketal and tetraethyleneglycol substituted phthalonitriles in the presence of \(\text{Zn(OAc)}_2\) affording 634 which could be converted to the alkyne derivative 636 for the triazole linkage. Glycosylation of 634 with 2,3,4,6-tetra-O-benzoyl-\(\alpha\)-D-galactopyranosyl trichloroacetimidate, 2,3,4,6-tetra-O-benzoyl-\(\alpha\)-D-mannopyranosyl trichloroacetimidate and 4-O-(2,3,4,6-tetra-O-benzoyl-\(\beta\)-D-galactopyranosyl)-2,3,6-tri-O-benzoyl-\(\alpha\)-D-glucopyranosyl trichloroacetimidate was carried out in the presence of trimethylsilyl trifluoromethanesulfonate and subsequent deprotection affording 635a-c. Compounds 637a-c were synthesized via similar procedure by Lafont \textit{et al.} \[321,324\]. Another example of post-cyclization functionalization includes the synthesis of octasubstituted carbamoyl glucosyl phthalocyanines and 1,4-substituted triazole glucosyl and galactosyl phthalocyanines all of which retained their \(\beta\)-configuration at the anomeric centre of the sugar units \[325\].

![Scheme 40. Synthesis of glycoconjugated phthalocyanines.](image)

![Scheme 41. Synthesis of glycosylated phthalocyanines via triazole linkage.](image)
2.6 Dual Modality Systems

Increasingly attention is focused on dual modality systems where two or more different bioconjugate groups of different properties are present which have intrigued a number of researchers, including ourselves (Figure 6) [326]. As mentioned, some of the earlier studies on bioconjugates aimed to target porphyrins to steroid receptors. A classic example is Hombrecher's work to increase the degree of amphiphilicity in the target systems through preparation of a porphyrin with both a galactopyranosyl and a cholesterin unit. The porphyrin 402 was prepared in 5% yield using a mixed 2+2 condensation of a sugar- and steroid dipyrromethane [68]. The porphyrin was easily incorporated into phosphatidyl ethanolamine vesicles and formed vesicles or aggregates in water [69].

The first ever porphyrin-carbohydrate bioconjugate synthesized for a specific medicinal application remains to date one of the most complex and elaborate systems. Rakestraw et al. covalently linked a Sn(IV) chlorin e6 derivative to monoclonal antibody using a dextran linker group [327]. They first prepared a dextran carrier system through synthesis of a dextran carbazate with a terminal hydrazide which was functionalized by reaction with a hydroxybenzotriazole active ester of (chlorinato e6)tin(IV). The tetrapyrrole unit was trace-labelled with 113Sn to facilitate quantitation and the chlorin-dextran carrier system with a terminal hydrazide group was then linked to anti-melanoma and anti-lymphoma monoclonal antibodies. The system showed excellent dose-dependent phototoxicity against SKMEL-2 human melanoma cells an effect only observed upon antibody binding to the cells. Note, this immunoconjugate system is also an efficient PS delivery system as about 10-15 tetrapyrrole units were bound per conjugate.

Clearly the use of targeting peptides in conjunction with carbohydrate units presents an intriguing possibility for the construction of new drugs. The first comprehensive study of this approach was provided by Sol et al. [207]. They prepared a series of porphyrins with meso glycosyl-aryl and alanylphenyl residues (292-297) and a number of compounds where the sugar unit was separated from the aryl residue by a serine unit (298, 299). In the first approach, the nitrophenylporphyrin derivatives (274-279) were synthesized via Lindsey condensation, followed by oxidation with p-chloranil. Use of p-nitrobenzaldehyde and 2- or 4- (2',3',4',6'-tetra-O-acetyl-β-D-glucopyranosyloxy)benzaldehyde gave 274 and 275 in 14% and 16% yield, respectively. Different ratios of reagents, pyrrole, nitrobenzaldehyde and 4-(2',3',4',6'-tetra-O-acetyl-β-D-glucopyranosyloxy)benzaldehyde afforded maximum yields of compounds 275 (16%), 276 (14%), 277 (10%) and 278 (16%). Compound 29 was isolated as a side product in a yield of 11%. The nitro functionalities were reduced using H2/Pd-C yielding glycosylated aminoporphyrins 280-285 in 56-76% yields followed by coupling with N-Fmoc-L-alanine provided 286-291. Deprotection of Fmoc and acetate groups yielded the respective mono-, di- and triamino acid glycoporphyrins 292-297 in 75-64% yield. A second series of glycosylated amino acid porphyrins was synthesized where two tristolylporphyrins were substituted with glycosylated serine [259]. The synthesis of the mono-nitrophenyltristolylporphyrins (300, 301) was carried out via condensation of pyrrole and p-tolylaldehyde with 2- or 4-nitrobenzaldehyde in a ratio of 4/3/1 to yield the mononitro derivatives in 14 and 15% yield. The nitro moieties were reduced (302, 303) and coupled with an O-glycosylated Ser-Fmoc derivative, followed by deprotection of Fmoc and acetate groups providing monoglycosylamino acid porphyrins (298, 299) [207,259].

![Figure 6. Dual modality systems.](image-url)
3 MEDICINAL APPLICATIONS

3.1 Photodynamic Cancer Therapy (PDT)

3.1.1 Basic Principle of PDT

Photodynamic therapy is used for the selective treatment of tumor cells. Intravenous or topological administration of a tumor-specific PS drug, e.g., porphyrin derivatives, causes moderate retention of the drug in tumor tissue. The uptake of such PSs into neoplastic tissue is often greater than that of non-neoplastic tissue due to the EPR effect, previously discussed. Intracellular localization of PSs can be determined by fluorescence microscopy and some have been identified to localize in the mitochondria, endoplasmic reticulum, lysosomes, nuclei, plasma membranes and tumor vasculature. Several documented cases suggest destruction of tumor vascular systems is of vital importance to the efficacy of PDT. Most PDT PSs tend not to localize in the nuclei, reducing the risk of DNA damage and future carcinomas. The localization of each individual PS is different. THPC has been identified to localize in the nuclear envelope while δ-aminolevulinic acid (ALA) localizes in the mitochondria and methylene blue in lysosomes.

The mechanism of uptake of PSs has not been clarified. It has been reported that the excess of low density lipoproteins (LDL) receptors on cancer cells, suggest LDL easily enter tumor cells. Due to the amphiphilic character of porphyrins it is known that they are carried by plasma lipoproteins. After localization the PS can be activated with visible or near IR light. De-excitation can then occur either via relaxation to the ground state (fluorescence, which may be used for diagnostic purposes) or via a photosensitization reaction with oxygen. The latter yields singlet oxygen (and other reactive oxygen species) which can damage cells within about 0.03 nm radius and has a lifetime of ~ 3 μs. Singlet oxygen is a highly reactive species which causes irreversible photodamage to proteins, lipids, and nucleic acids in the affected cells, and plays a major role in vascular breakdown and immune response. Porphyrins derivatives are ideal PSs as they can produce singlet oxygen quite efficiently and absorb in the red region of the electromagnetic spectrum.

The method is non-invasive, mostly non-scarring, can be applied in an outpatient setting, and may be used in conjunction with other treatments such as surgery, radio- or chemotherapy. Side effects are often pain and prolonged photosensitivity. Its historical development is well documented and the potential of this approach is excellent.

At present PDT is an approved modality for the treatment of non small cell lung, cervical, bladder, head and neck, gastric and esophageal cancers. It also has applications in the treatment of acne, skin cancer, cosmetic skin improvements, wrinkles and psoriasis. Some examples of approved drugs for PDT include intravenous drugs Photofrin® (porfirmer sodium), Foscan® (mTHPC) and topically drugs Leuvan (ALA), Metvix (methyl aminolevulinate). In addition, PDT applications are currently under investigation in clinical trials for cancers of the prostate gland, cervix, head and neck, bladder, larynx, abdominal cavity, basal cell carcinoma, and brain with a range of second generation PSs.

Porphyrin based PSs traditionally dominated the field of PDT. Yet, the first generation PSs have many disadvantages including poor light absorption, long retention times, low tumor-tissue ratios, low water solubility and cause prolonged photosensitization of patients. As a result an expansion of the field to second and third generation PSs has ensued to tackle the efficacy of PDT.

With regards to the development of new PSs specific characteristics apply to an ‘ideal’ PS which must be considered. The chemical purity and stability of a PS must be determined, i.e., the exact composition of the substance must be known, unlike first generation Photofrin® which consists of mixtures of monomers, dimers and oligomers, where only some of the components are biologically effective. Knowledge of the optimum absorption, distribution, metabolism and excretion (ADME) properties are desirable. Minimal dark toxicity should be observed and the PS should only be toxic when activated by light of specific wavelengths. The PS should not produce toxic metabolites in vivo which itself could cause mutagenicity or carcinomas. Fast clearance from cells and the body is optimum to reduce severe photosensitization side effects that can be quite debilitating to patients.

The diffusion of singlet oxygen is predicted as 2-4 × 10^-6 cm² s⁻¹, therefore, tissue penetration of the light is extremely important for effective PDT treatment. As the absorption of light by tissue increases with wavelength decrease, longer excitation wavelengths are essential. However, wavelengths beyond 800 nm are not desirable as the formation of singlet oxygen is difficult. 700-800 nm is the optimum range of wavelengths to increase light penetration, reduce light scattering and provide sufficient energy for singlet oxygen formation. Porphyrins have 22 π-electrons in an aromatic macrocycle which exhibit π-π* transition around 400 nm (Soret bands) and Q bands in the visible region. Two of the double bonds are not involved in the aromatic system and can therefore be reduced providing chlorins and bacteriochlorins. This change in symmetry causes a bathochromic shift of the Q bands which exhibit higher extinction coefficients. Such shifts can be seen in the tolorphrin spectrum explained earlier. Bacteriochlorins such as tolporphyrin have long wavelengths of 700-800 nm but they have the disadvantage
that they are quite susceptible to oxidation making them the least stable derivative. In vivo, exposure to light can cause oxidation of these compounds making the chlorin derivative and changing the absorption of the chromophore leading to reduced efficiency of PDT.

Also, the PS must produce high singlet oxygen quantum yields for production of singlet oxygen and other ROS but undergo minimal photobleaching. Changes of the absorption of the molecules can be easily modified, however, the bioavailability and pharmacokinetics of such molecules is much harder to control [15]. For the development of a drug it is necessary to identify a target which will improve the therapeutic action of that drug. New PSs are required which exhibit significant selectivity for neoplastic tissue in high concentration leading to the development of third generation PSs i.e. target specific PSs. In the synthesis of such derivatives care must be taken not to lose the pharmacological properties of both moieties.

The solubility of PSs in aqueous media is essential for administration, however, the importance of amphiphilicity of such derivatives is related to their photodynamic activity. It has been proven by several groups that amphiphilic molecules versus hydrophilic and hydrophobic molecules show a greater degree of activity and can localize at the interface of hydrophobic-hydrophilic membranes [306,323]. Amphiphilicity can also affect the aggregation of PSs and in turn affect the photophysical properties of a PS [12].

Subcellular localization within organelles like the mitochondria is beneficial to obtain apoptotic instead of necrotic cell death which is favored.

The search for a novel PS that meets all the above chemical, physical and biological criteria is still ongoing. Also keeping in mind its production should be inexpensive and starting materials commercially available so as to sustain widespread production.

### 3.1.2 Glycoporphyrins in PDT

At a first glance one might assume that peripheral binding of sugar units to a porphyrin should not drastically alter its photophysical properties while gaining the benefit of increase water solubility and/or amphiphilicity. As always the reality is more complex. For example one of the earlier studies with a series of glucose or galactose (2- or 4-O) appended porphyrins of the A3B- or A4-type showed that the singlet-state properties are affected by the configuration of the molecules. Likewise aggregation properties and hydrophobicity are influenced significantly. Even more importantly for drug delivery with liposomes an amphiphilic character was found to be beneficial. Thus, the A3B systems are clearly superior for incorporation into liposomes [341].

Clearly, both photophysics, uptake and photobiological studies must go hand in hand.

Some insight into the PDT utility of new compounds can also be gained from membrane incorporation experiments. Thus, during the initial phase of investigations on glycoporphyrins several studies investigated their incorporation into model membranes, notably into liposomes. For example, a comparative study by Voszka et al. utilized a series of glyco-tetraphenylporphyrin derivatives with glycosyl (114, 116 and 30) or xylosyl (42) residues [342]. The compounds differed in their degree of symmetry and hydrophobic character. Utilizing either neutral or negatively charged liposomes they found a strong effect of the liposomal surface charge and the negative surface charges of DMPC/DMPG liposomes helped with the association of the symmetrically substituted hydrophilic porphyrins to the liposomes. As expected, the association constants of the various compounds with liposomes followed their lipophilicity.

Likewise, tetra- and tri-meta-substituted TPP (39, 306) were investigated for their interaction with cell membranes compared to the non-glycoconjugate parent derivative 37 [208]. They were tested with a range of phospholipids spread at the air-water interface or grafted onto an RP-LC column. In the absence of phospholipids all porphyrin derivatives were able to form monolayers at the air-water surface interface. Although 39 and 306 have completely different chemical structures both lowered the surface tension to the same extent. However, 306 displayed reduced stability over time with an increase in surface tension observed after 10 h as a result of rearrangement of its asymmetric structure. For 39 the decrease in surface tension was very low compared to the other derivatives but appeared more stable over time compared to 306. The interfacial behavior of the three porphyrin derivatives were very different in their interactions in mixtures with diestearylphosphatidylcholine (DSPC) and diarachidoylphosphatidylcholine (DAPC) phospholipids and the phospholipid monolayer model is a good initial method to determine cellular uptake of such glycoporphyrins [208].

Many of the more basic PDT-related studies have been described above. However, as a natural compound tolyporphin A deserves a special mention in investigations of glycoporphyrins as PSs. It was identified to be potent against EMT-6 tumor cell lines, where it localizes in the endoplasmic reticulum (ER), and also in vivo in tumors implanted on the backs of immunodeficient mice [343]. Its long wavelength absorption at 675 nm and its relatively good water solubility made it an excellent candidate for PDT. It showed exceptional activity and produced more effective photosensitization compared to Photofrin® both in vitro and in vivo. The higher photocytotoxicity was not attributed to a higher quantum yield of singlet oxygen or absorbance, as they are similar, if not the same as Photofrin®, therefore, other factors played a role i.e. the localization of the compound within the cell [344]. Irradiation for 1 h at 0.1-0.5 J/cm² at drug dose concentrations of 0.02-0.1 μM showed Tolyporphin to be ~ 5000 times more effective in PDT then Photofrin®. Photofrin® localizes in the plasma membrane, lysosomes and mitochondria compared to tolyporphin which is exclusively in the ER and nuclear membrane [341].

As well as its exceptional activity in vivo with regard to PDT, also highlighted was the unusual biodistribution of tolyporphin.
compared to Photofrin® or other second generation PS. It displayed higher delivery to tumor tissue compared to significantly less extraction by the liver where the opposite applies to Photofrin®. Its amphiphilicity is also essential for significantly less extraction by the liver where the opposite displayed higher delivery to tumor tissue compared to Photofrin® or other second generation PS. It further investigations into the use of tolyporphin derivatives for PDT are limited due to the tedious preparation via total synthesis.

3.1.3 In Vitro Studies

A significant number of in vitro studies have been reported for glycoporphyrins and related bioconjugates. The main results of the individual studies are compiled in Table 3 and, for ease of access, have been sorted by cell line. For the subsequent discussion an ordering by basic porphyrin type have been choosen.

### Table 3. In vitro PDT studies with glycoporphyrin conjugates.

<table>
<thead>
<tr>
<th>Cell line</th>
<th>Compounds</th>
<th>Comments</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td>HeLa</td>
<td>Aβ-glycoporphyrins: 29, 32, 35, 36 and 53</td>
<td>PDT: 8 min. irradiation, λ &gt; 500 nm, 65 mW/cm², 2 h incubation and 24 h post-treatment. 15 % cell survival at 0.14 x 10⁻⁴ M.</td>
<td>[167]</td>
</tr>
<tr>
<td></td>
<td>53</td>
<td>Inactive.</td>
<td></td>
</tr>
<tr>
<td>HeLa</td>
<td>Aβ-glycohlorins: 513a-c and 514a-c</td>
<td>All similar O₂ production. Higher activity for 514ab free OH derivatives unlike 53 and 29. 514c exhibited poor uptake and phototoxicity. Compound 30 displayed highest activity in the series.</td>
<td>[258]</td>
</tr>
<tr>
<td>HeLa</td>
<td>44, 30 and 5,10,15,20-tetrakis(4-sulfonatophenyl)porphyrin</td>
<td>PDT: at 5 x 10⁻⁶ M, 16 J/cm², λ &gt; 500 nm, 24 h incubation post-treatment. % cell survival: 44 (27.6 %), 30 (35.6 %) and 5,10,15,20-tetrakis(4-sulfonatophenyl)porphyrin (69.9 %). 44 &gt; 30 &gt; 5,10,15,20-tetrakis(4-sulfonatophenyl)porphyrin.</td>
<td>[165]</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Cellular uptake study: 44 &gt; 30 &gt; 5 &gt; 31 &gt; 42 &gt; 52 &gt; 50 &gt; 62 &gt; 49 &gt; 46 &gt; 47 &gt; 45 &gt; 48 &gt; 40 &gt; 5,10,15,20-tetrakis(4-sulfonatophenyl)porphyrin.</td>
<td></td>
</tr>
<tr>
<td>HeLa</td>
<td>44, 30, 32, 42</td>
<td>PDT: at 1-7 µ M, 16 J/cm², λ &gt; 500 nm, 24 h incubation post-treatment. IC₉₀ = 44 (&gt; 4 µM) &lt; 30 (4 µM) &lt; 32 (2.5 µM) &lt; 42 (1 µM).</td>
<td>[272]</td>
</tr>
<tr>
<td>HeLa</td>
<td>87-94</td>
<td>PDT: @ 0.09-0.5 µM, 24 h incubation, 16 J/cm². 91-93 with EC₉₀ (90% effective concentration) = 0.2 µM and 87-90 and 94 inactive. Compared to the O-glycosylated 44, 30, 32, 42 with EC₉₀ &gt; 5 µM. ROS inhibitors PDT: @ 0.1 µM (91-93) and 0.2 µM (94), with NaN₃ (91-94 significantly decreased PDT effect) or D-mannitol (small increase in cell survival). O₂ dominant ROS.</td>
<td>[177]</td>
</tr>
<tr>
<td>HeLa</td>
<td>Aβ-glycochlorins and Aβ-glycoporphyrins: 91, 93, 542a, 543a</td>
<td>Cellular uptake: @ 0.1 and 0.5 µ M, 24 h incubation, 91, 93, 542a, 543a have 4 times greater uptake then 543. No preference for the chromophore: PDT: @ 0.05-0.5 µ M, 16 J/cm², 100 W lamp, λ &gt; 500 nm, 24 h incubation post treatment, no dark toxicity, EC₉₀ (median effective concentration) = 0.09 (542a) ≈ 0.1 (543a) &lt; 0.15 (91, 93) &lt; 5 (543) µ M. chlorins (542a, 543a) 1.5 fold greater than porphyrins (91, 93) due to higher f₅₀/O₂ value. ROS: Both type I and type II photoreactions occur, Φ₅₀ values: 0.17 (542a) &gt; 0.25 (91), 0.28 (543a), 0.52 (93), 0.89 (543) and Φ₅₀ value: 0.92 (542a), 1.2 (91), 6.3 (543a), 14.6 (93).</td>
<td>[177]</td>
</tr>
<tr>
<td>HeLa</td>
<td>238, 240, 242, 244, 91</td>
<td>No dark toxicity, 238, 240, 242, 244, 91 significantly higher than 5,10,15,20-tetrakis(4-sulfonatophenyl)porphyrin with EC₉₀ 242 ( &lt; 5 nM), 244 (80 nM), 87, 238, 240, 90 (nM). 242 – 2 fold greater efficacy.</td>
<td>[200]</td>
</tr>
<tr>
<td>HeLa</td>
<td>437-441 and Zn(II)437-441</td>
<td>PDT: @ 0-25 µ M, 2 h incubation, 8 min. irradiation with 500 W halogen lamp. 437 and Zn-437 had much greater PDT effect compared to 438-441 and Zn-438-441 and 30 and 53. 438 &gt; Zn-439 opposite to that of 241. Cellular uptake study: 437 accumulates in the</td>
<td>[202a]</td>
</tr>
<tr>
<td></td>
<td>Compared to 53 and 30</td>
<td></td>
<td>[167]</td>
</tr>
</tbody>
</table>
HeLa Cyclodextrinporphyrins: 638d and 638a

cytoplasm. 441 displayed no uptake. Compounds 438 showed considerable uptake.

HeLa Aγ-glycochlorins: 514d-g

OΔ2-Dp: 1.3 to 0.6.

PDT: @ 0.2 μM, 16 J/cm², 100 W lamp, λ > 500 nm, 24 h incubation, no dark toxicity. 514f > 514d > 514e > 514g, 5,10,15,20-tetakis(4-sulfonatophenyl)porphyrin 514a

HeLa Aγ-glycochlorins: 514a,b, 514d-i

Cellular uptake studies: @ 5 μM, 24 h incubation, 514d > 514i > 514e > 514a > 514b > 514h > 514f > 514g, 5,10,15,20-tetakis(4-sulfonatophenyl)porphyrin. 514d 50 times greater than 5,10,15,20-tetakis(4-sulfonatophenyl)porphyrin.

PDT: 514d,f Aγ-glycoporphyrin

30 = 514f

PDT: 30, 32, 39-44, (514a,b, 514d-i), 45-52 and 5,10,15,20-tetakis(4-sulfonatophenyl)porphyrin

HeLa Aγ-glycochlorins and glycoporphyrins: 542a, 542b, 542a', 542b' and 87, 91, 95, 96

Glycopeconjugates > 541 > 84

87, 96 and 542a' 542b' – inactive

514d-g proved potent @ 5 μM.

EC50 proves potent (30 @ 0.5 μM) < para-position (30 @ 4 μM).

514a molar extinction co-efficient 3.7 fold greater than 30.

O2 dominant ROS.

514a – 3 derivatives of varying content of Porphophoride a

Autoquenching in aqueous medium: - decrease with increased pheophorbide a content.

ΦΔ = 0.5 similar for all in DMF, 0 in aqueous medium.

Cellular uptake: 6 h incubation: very rapid uptake, forming perinuclear spots in cytoplasm; possible internalization via endocytosis.

Addition of a hyaluronic acid polymer reduced 583 uptake; hyaluronic acid receptor mediated uptake. Fluorescence increases over time; 583 disintegrated by enzymes and release of Porphophoride a.

PDT: @ 0.0025- 2.5 μg/mL, 1 mW/cm², 10 min.
HeLa cells – STxB receptor (Gb3) 114, 332-STxB and STxB-Cys cell line

Human breast adenocarcinoma Tetrabenzoporphyrins: 111 and 113

Human breast adenocarcinoma MCF-7 – drug sensitive

Human breast adenocarcinoma 4 MCF-7/ADR – drug resistant

Human breast cancer cell lines 91 and 95 MDA-MB-231

irradiation, slight dark toxicity, $583 \approx$ Pheophorbide a.

Autoquenching in aqueous medium:

In organic solvent: - fluorescence similar to Pheophorbide a

Aqueous medium: Autoquenching

Addition of esterase to aqueous medium: 8.5 U/mL, surfactant (0.1 % Tween 80); increase fluorescence.

Cellular uptake: 30 min. - 24 h: increase fluorescence over time; enzymatic cleavage.

Localization: @ 1.5 µg/mL of Pheophorbide a content, 1, 6, 12 h, 37 °C; $584 \approx$ endosomes to lysosomes

PDT: @ 0.125 - 1.25 µg/mL of Pheophorbide a content, 12 h incubation, 1.2 J/cm², 670 nm; $584 \approx$ Pheophorbide a, IC$_{50} = ~ 0.2$ µg/mL. Apoptotic cell death.

|$\Phi\Delta = 0.13 (332$-STxB), 0.4 (114); $332$-STxB 4 times lower.

PDT: @ 1,6 µM, $\lambda > 540$ nm, 15 min. irradiation, 1.8 J/cm², 2 mW/cm², 24 h incubation, no dark toxicity; LD$_{50} = 0.6 (332$-STxB), 3 (114) µM. $332$-STxB > 114: Golgi apparatus – higher PDT.

Presence of Gb3 inhibitor (1-phenyl-2-hexadecanoyl-amino-3-morphorpholin-1-propanol (PPMP)): 114 – no change $332$-STxB – inactive. Presence of brefeldin A (induces the accumulation of STxB in early endosomes): reduced PDT of $332$-STxB - early endosomes

|$111 <$ Photofrin; $113$ ineffective.

Chemosensitization by 4 and verapamil:

Presence of anticancer drugs (daunomycin, taxol, actinomycin D, colchicine, cisplatin, melphalan): 0, 1, 2 µM of 4 compared to 10 µM of verapamil – no significant effects on the IC$_{50}$ values.

|$[3H]$-vinblastine transport:

Human breast adenocarcinoma MCF-7/ADR – drug resistant

Chemosensitization by 4 and verapamil:

Presence of anticancer drugs (daunomycin, taxol, actinomycin D, colchicine, cisplatin, melphalan): 0, 1, 2 µM of 4 compared to 10 µM of verapamil – significantly reduced the resistance for daunomycin, taxol, actinomycin D and colchicine. No increased toxicity for cisplatin and melphalan.

|$[3H]$-vinblastine transport:

Human breast cancer cell lines 91 and 95 MDA-MB-231

Cellular uptake study: @ 10 µM, 24 h incubation, 91 > 95, 2.3 fold greater.

|$91$ PDT: @ 10 or 20 µM, ~ 11.3 kJ/m² (20 min. at 0.94 mW/cm²): 20 % and > 60 % of the cells, respectively, were necrotic immediately and 100 % at 24 h via apoptosis.

|$95$ PDT: @ 20 µM, ~ 1.6 kJ/m² (10 min. at 0.27 mW/cm²) cell death in part by apoptosis.

Cell migration study: @ 10 µM, 0.75 kJ/m² (5 min. at 0.25 mW/cm²) – migration was reduced. @ 1 µM –
| Human breast cancer cell lines | Aγ-glycochlorins: 542a.a'  
| MDA-MB-231  
| Aγ-glycosobacteriochlorins: 545a.a'  
| Aγ-glycobacteriochlorins: 546a.a | no necrosis or apoptosis however, significantly less aggressive in cell migration. Fluorescence imaging: @ 25 nM of 546a, high fluorescence quantum yield. φf = 0.28 (542a, DMSO) < 0.32 (542a, ethanol) < 0.59 (545a) < 0.85 (91). |
| Human chronic leukemia K562  
| Human keratinocyte cell line HaCaT Tetrabenzoporphyrins: | Cellular uptake: @ 1-5 µM each porphyrin, 100 µM total porphyrin content, 24 h incubation, up-taken porphyrins were isolated. Fluorescence imaging of 91, 341, 342, 350, 351: @ 2.5 or 10 µM, 24 h incubation, @ 24 h and 1 week imaging @ 10 µM 1 day and 1 week 91 > 350 > 341. Poor uptake of 342, 351: @ 2.5 µM, 24 h imaging: all weak fluorescence @ 2.5 µM, 1 week imaging: 91 > 350 > 341. Conjugates display initial uptake by aggregation. PDT of 91, 350, 341: @ 2.5 or 10 µM, 20 h incubation, 20 min. irradiation, 138 fluorescent bulb, 0.9 mW/cm², 10.8 J/cm², @ 10 µM: 91, 350 25 % immediate necrotic cell death, after 24 h: 91, 350 > 80 % apoptotic cell death and 341 < 60 %. @ 2.5 µM, 91, 350 40 % necrotic, > 60 % apoptotic. Cellular uptake of 352-363, 91: @ 5 µM, 20 h incubation, 361, 91 – best uptake: cationic species better binders. |
| Human chronic leukemia K562  
| Normal 3Y1 rat fibroblasts, partially transformed 3Y1 overexpressing c-Src (3Y1-vSrc) and fully transformed 3Y1 cells (3Y1-vSrc) | Cellular uptake study 91: no uptake in 3Y1, increase in the order 3Y1 < 3Y1-vSrc < 3Y1-vSrc with affinity 1:2:3:3:2. 91 PDT: 7 min., λ > 500 nm, 0.84 mW/cm² (3.5 J/cm²), 24 h incubation with 91, both apoptosis and necrosis in the order 3Y1-vSrc > 3Y1-vSrc > 3Y1, PDT: 2 µM, 50 W/m², 24 h incubation - no effect due to poor cell permeability. PDT: 2 µM, 30, 60, 90 and 120 min., 10 mW/cm², 24 h incubation, Photofrin® = 368 > 370. 365o,p and 369 – low activity. Similar O₂ generation as HP. |
| Human chronic leukemia K562  
| Human chronic leukemia K562 O-Glucosyl porphyrin dimers: 365o,p and 367  
| Human chronic leukemia K562 O-Glucosyl porphyrin dimers: 365o,p, 368-370 | PDT activity: @ 2 µM (221, 222) or 1.25 µg/mL (Photofrin II®), 18 h incubation, white light, 0-120 minutes. Photofrin® > 221 > 222. No dark toxicity and early necrosis observed but absence of late necrosis/apoptosis |
| Human chronic leukemia K562 A,B,A,B; with amino acids: 292-299 | PDT: @ 2×10⁻⁶ M, 50 W/m², 24 h incubation, irradiation time 0-100 min., less effective than Photofrin. O₂ production similar to HP. Primarily, apoptotic cell death. |
| Human chronic leukemia K562 A,B with tripeptide: 168o,p | PDT: @ 2 µM, 10 mW/cm², 30, 60, 90, 120 min., 0 h and 24 h incubation, at 120 min. proved less effective than Photofrin; induced apoptosis. |
| Human chronic leukemia K562 meso-Strapped glycoporphyrins: 390 and 392 | PDT: @ 2×10⁻⁶ M, 50 W/m², 0-100 min., 24 h incubation, HP > 390 > 392 |
| Human chronic leukemia K562 231-236 and 456b-458b | PDT: @ 2×10⁻⁶ M (231-236 and 456b-458b) and 1.2 µg/mL (Photofrin®), white bulb, 60 W/m², 0-120 min., 24 h incubation, 231, 233, 235 all comparable to Photofrin® when irradiation time > 60 min. 456b-458b > Photofrin®. |
| Human keratinocyte cell line HaCaT  
| Tetrabenzoporphyrins: 111 and 113 | Early necrosis and induced apoptosis. |
| Human keratinocyte cell line HaCaT  
| Glycophthalocyanines: 598 | PDT: @ 5 (598), 5-30 min, λ = 636 nm, 5.58 J/cm², no dark toxicity. |
| Human keratinocyte cell line HaCaT  
| Purpurin imides: 569a/b, 570 | Low activity with 598, Activity of 569a/b, 570 could be suppressed by pre-incubation with lactose. |
| Human leukemia T-cells Mol4 |  |
|  |  |

**Title of the Article**

Journal Name, 2014, Vol. 0, No. 0 73
| Type of Study | Cell Line | Glycoporphyrins | PDT: λ > 610 nm, 40 mW/cm², 48 J/cm² | IC₅₀ = 0.1 (617, 618a) 0.28 (618b), 0.79 (618c) μM. | FD₃O₂ = 0.79 – 0.94. | Human esophageal squamous-cell carcinoma cell line OE21 320-331, 261 | Human ovarian carcinoma SKOV3 4 - drug sensitive | Chemosensitization by 4. Absence of anticancer drugs: IC₅₀ = 0.8 μM | Presence of anticancer drugs (daunomycin, taxol, cisplatin): 0, 0.1, 0.25, 0.5 μM of 4 - only slight effects on the IC₅₀ values. | [1H]-vinblastine transport: 60 min. incubation - control. 60 min. incubation, 2 or 5 μM of 4 or 10 μM of verapamil. – no increase. | Human retinoblastoma cells Y79 203-208, 315 | Cellular uptake study: @ 4 μM, 4 h incubation. Intracellular concentration ranging from 0.78 ± 0.05 × 10⁻⁶ to 0.26 ± 0.03 × 10⁻⁶ nmol/cell for 206 (highest) to 203 (lowest) and 315 poor internalization. Absence of glucose inhibited uptake by 40-45 % for 204 and 206 – uptake via active mechanism. | Human ovarian carcinoma SKVLB1 4 - drug resistant | Chemosensitization by 4. Absence of anticancer drugs: IC₅₀ = 4 μM; only ~ 5 fold less sensitive to resistance cell lines. Presence of anticancer drugs (daunomycin, taxol, cisplatin): 0, 0.1, 0.25, 0.5 μM of 4 - significantly reduced the resistance for daunomycin and taxol. No increase sensitivity was observed for Cisplatin, i.e. non P-glycoprotein transport drug. | [1H]-vinblastine transport: 60 min. incubation – significantly lower concentration of [1H]-vinblastine. 60 min. incubation, 2 or 5 μM of 4 or 10 μM of verapamil. 4 > verapamil (10 fold) @ 2 μM (4) and 10 μM (verapamil) – total inhibition of efflux. | Human retinoblastoma cells Y79 499b-511b, 271 | PDT: @ λ > 540 nm, 2 J/cm², 24 h incubation, no dark toxicity, LD₃₀ = 0.43 (206), 2.5-2.7 (499b, 501b, 503b), 3.4-5 (500b, 506b,508b, 511b), 4-9.6 (502b, 504b, 505b, 509b, 512b), >10 (271) μM. | Human retinoblastoma cells Y79 480, 481, 486-489 | -0.1 > Log P > 0.4 | Human retinoblastoma cells Y79 A₄, AB₄-glycoporphyrins - 30, 39, 35, 114, 203-206, 306-308 | PDT: @ 0.1-15 μM, 24 h incubation, 15 min. @ 1.8 J/cm², λ > 540, 3 day incubation post treatment. | Human retinoblastoma cells Y79 A₄, AB₄-glycoclorins - 516 517,516c,d, 514f, 518/519 | Cytotoxicity: IC₅₀ = 3-6 (516, 517, 517, 306, 39, 35, 203, 514f, mTHPC), > 10 (204-206,308), > 15 (mTHPP, 307, 114, 518/519) μM. Phototoxicity: IC₅₀ = 0.05 (205), 0.35 (206), 0.5-0.7 (mTHPP, 308, 204, mTHPC, 518/519), 0.8-1.9 (203, 114, 307, 39, 306), 2-3.8 (35, 516, 517, 30, 514f, 516c/d) μM. | Human retinoblastoma cells Y79 A₄-glycoporphyrins dimers - 430-d deacetylated version of 418 | PDT: 0.15 - 7.5 μM, λ > 540 nm, 11 min., 2 J/cm², 3 μM/cell, 3 day incubation, all displaced dark toxicity IC₅₀ = 0.5 (430a), 4 (430d), 7.5 (430a-h, deacetylated-418) - all inactive PDT. Cellular uptake of 430d: 4 μM, 24 h incubation, poor uptake of 430d due to solubility. | Human colorectal adenocarcinoma cells HT 29 A₄-glycoporphyrins dimers - 430-d deacetylated version of 418 | PDT: 0.15 - 7.5 μM, λ > 540 nm, 11 min., 2 J/cm², 3 μM/cell, 3 day incubation, all displaced dark toxicity IC₅₀ = 0.5 (430a), 4 (430d), 7.5 (430a-h, deacetylated-418).
Title of the Article

Journal Name, 2014, Vol. 0, No. 0 75

Human colorectal adenocarcinoma cells HT-29

Benzochlorins: 552b

Human colorectal adenocarcinoma cells HT-29

203-208, 315

Human colorectal adenocarcinoma cells HT-29

Glycophthalocyanines

A4 AB 3-glycochlorins -

and

516c/d

and

203

517

315

m

m

THPP (16 ± 2 %), 514f (22 ± 4 %), photoactivity of 514f is poor.

PDT of metabolic 307: 0.5 -2 mM, 3 h incubation, λ = 514 nm, 5 J/cm², LD₅₀ = 0.7 μM of both 307 and 516, 517 but cellular drug conc. 307 (5.9()0.8) × 10⁻⁶ μmol/μg) > 516, 517 (2.5()0.8) × 10⁻⁶ μmol/μg).

λ > 540 nm, 24 h incubation post treatment, cytotoxicity: IC₅₀ = 6.8 (non-glycoconjugates: 35, mTHPP, 307, 203, 518-519), > 10 (308, 203-206), > 15 (mTHPP, 30, 39, 514f, 306, 114, 516, 517, 516/d) μM.

Phototoxicity: IC₅₀ = 0.1 (205), 0.4 (206), 0.5 (203), 0.7 (204), 0.8 (mTHPP), 1.2-2.2 (mTHPP, 308, 114, 518-519, 35), 3.5-4.8 (514f, 516, 517, 306-307), 7.8 (516/c/d), > 10 (30, 39) μM.

Human colorectal adenocarcinoma cells HT-29

A₅, AB₂-glycoporphyrins - 30, 39, 35, 114, 203-206, 306-308

A₅, AB₂-glycoporphyrins - 516, 517, 516/d, 514f, 518/519

deacetylated-418 and 430b – slightly PDT active but dark toxicity – unviable.

PDT: IC₅₀ = 8 μM (white light); IC₅₀ = 5.7 μM (λ > 590 nm). 552b - active

Cellular uptake study: @ 4 μM, 4 h incubation. Highest uptake by 206 compared to 208 and 203. The low cellular uptake of 205 (log P = 0.5) and 208 (log P = 0.46) is clearly related to their higher lipophilicity compared to those with high amphiphility, e.g., 206 (log P = 0.02).

PDT: @ 2 μM, λ = 514 nm, 5 J/cm², 4 h incubation, no dark toxicity, LD₅₀ = 0.1 (205), 0.43 (206), 0.68 (204), 0.85 (315), 2 (207, 208) μM. All similar O₂ quantum yields.

PDT: @ λ > 540 nm, 2 J/cm², 24 h incubation, no dark toxicity, LD₅₀ = 0.35 (206), 3(503b), 3.7 (499b, 501b), 5-6 (500b, 504b-508b, 511b, 512b) > 10 (502b, 509b, 510b, 271) μM.

Human colorectal adenocarcinoma cells HT-29

271 and 499b-512b

Human colorectal adenocarcinoma cells HT-29

461a-d

Human colorectal adenocarcinoma cells HT-29

480, 481, 486-489

Human colorectal adenocarcinoma cells HT-29

A₅, AB₂-glycoporphyrins - 30, 39, 35, 114, 203-206, 306-308

A₅, AB₂-glycoporphyrins - 516, 517, 516/d, 514f, 518/519

Metabolite: 307

Cellular uptake: 2 h incubation saturation after @ 6 μM, 3 h incubation, drug concentrations 3.57 × 10⁻⁶ (mTHPC), 2.4 × 10⁻⁶ (516, 517) and 1.03 × 10⁻⁵ (514f) μmol/μg. mTHPC > 516, 517 (30 % less) > 514f (80 % less). 516, 517 temperature depend, active process. 516, 517 uptake more energy consuming then mTHPC tested via inhibition of mitochondrial respiratory chain via NaN₃.

516, 517 and 514f localization in mitochondria.

PDT: @ 514 nm, 5, 10, 25 J/cm², no dark toxicity for 516, 517 however, 514f, exhibited dark toxicity dependent on FCS concentration (@ 4 μM, 3 h incubation, 2 % FCS in the dark ~ 51 % cell survival, compared to 100 % @ 10 % FCS).

@ 2 μM, 5 J/cm², cell survival fraction for 516, 517 (2 ± 0.6 %), mTHPC (16 ± 2 %), 514f (22 ± 4 %), photoactivity of 514f is poor.


Human colorectal adenocarcinoma cells HT-29

AB₁, AB₂-glycoporphyrins - 247, 248 and 514a,b

Cellular uptake study: @ 10 μM, 24 h incubation. 247 > 514a,b > 248 > TPP. 247 11.5 fold greater than TPP.

ΨO₂ = 0.73 (514a,b), 0.55 (247, TPP), 0.33 (248).

Cytotoxicity: @ 0.5 to 30 μM, 24 h incubation, 247 and 514a,b no dark toxicity ≤ 10, 248 slight cytotoxicity.

Phototoxicity: @ 1 μM, 24 h incubation, λ = 650 nm, 4.54 mW/cm², LD₅₀ = 4.4 (247), 5.2 (514a,b), 11.1 (248), 30.2 (TPP) μJ/cm².

247: Localization in the ER

PDT: λ > 610 nm, 40 mJ/cm², 20 min., 48 J/cm², no dark toxicity, IC₅₀ = 28 mJ (617) > 7.5 μg/mL (Photofrin®)

Localization: granular spots through-out the cytoplasm.

Human colorectal adenocarcinoma Glyco-Si-phthalocyanines

PDT: λ > 610 nm, 40 mJ/cm², 48 J/cm², no dark toxicity IC₁₀ = 28 mJ (617) > 7.5 μg/mL (Photofrin®)
cells HT-29

Glyco-Si-phthalocyanines: 615a-c, 617

Toxicity, IC50 = 6 nM (614a), 17 nM (614b), 7.5 µg/mL (Photofrin®).

614a = 614b > Photofrin®

@ 8 J/cm²; IC50 = 7 nM (614a).
≥ 2 J/cm²; IC50 = 46 nM (614a).
ΦΔ = 0.32 (614a) - 0.41 (614b)

Presence of α-glucose did not impede uptake – not transported via glucose uptake.

Localization: lysosomes (614a)

615a-c inactive

Φ5 = 0.08 (615c), 0.35 (615f), 0.37 (615a), 0.41 (615b).

Localization: 617 in lysosomes.

Human colorectal adenocarcinoma cells HT-29

Glycophthalocyanines: 624a-b, 625a-b, 604, 622, 627a-d

628-630b

PDO: 0.8 µM, λ > 610 nm, 40 mW/cm², 48 J/cm², no dark toxicity, IC50 = 0.9 (627c), 1 (627b), 1.7 (627a), 2 (627d), 3.5 (625a), 4 (625b).

604, 622 inactive. PDO efficacy > Photosense®

Cellular uptake: 604, 622 inactive.

627a > 627c > 627a > 625a.

ROS generation: 627c > 627a > 625a > 604.

PDO ≤ 100 µM, 3.6 J/cm², 24 h incubation,

604b inactive and no cellular uptake.

Human colorectal adenocarcinoma cells HT-29

Glycophthalocyanines: 627-630, 628-630b

Cellular uptake: 8 µM, 2 h, 629a-b inactive.

630a > 629a > 630b.

PDO: 0.8 µM, λ > 610 nm, 40 mW/cm², 48 J/cm², no dark toxicity, IC50 = 0.03 (630a), 0.26 (629a), 1.38 (627), 2.97 (630b).

629ab inactive.

Φ5 = 0.43 (627) - 0.86 (630a)

Cellular uptake: 635b < 635a ≈ 637a < 637b < 635ab-

c < 635c ≈ 637c.

PDO: 1-500 µM, µM, λ > 600 nm, 3.6 J/cm², 4.6 mW/cm², 24 h incubation post treatment, LD50 = 110 (635b), 219 (635a), 300 (637a), 386 (637b), 428 (637c) > 500 (635c).

635 - inactive @ 500 µM.

Human colorectal adenocarcinoma cells HT-29

Glycophthalocyanines: 635a-c and 637a-c

PDO: λ > 610 nm, 40 mW/cm², 20 min., 48 J/cm², IC50 = 68 nM (617) > 7.5 µg/mL (Photofrin®).

472-5-fold higher accumulation compared to

257 ≥ 258 = 470.

PDO: 257, 258, 470 – all inactive @ 100 µM.

257, 258 - localization in ER and endosomes.

Human colorectal adenocarcinoma cells HT-29

Glycophthalocyanines: 637a

PDO: λ > 610 nm, 40 mW/cm², 20 min., 48 J/cm², IC50 = 68 nM (617) > 7.5 µg/mL (Photofrin®).

472-472 - localization in lysosomes

257, 258, 470 and 472

PDO: λ > 610 nm, 40 mW/cm², 20 min., 48 J/cm², IC50 = 6 nM (614a), 21 nM (614b), 7.5 µg/mL (Photofrin®).

Human hepatocarcinoma cells HepG2

Glyco-Si-phthalocyanines: 614a-b

PDO: λ > 610 nm, 40 mW/cm², 20 min., 48 J/cm², no dark toxicity, IC50 = 6 nM (614a), 21 nM (614b), 7.5 µg/mL (Photofrin®).

Human hepatocarcinoma cells HepG2

Glyco-Si-phthalocyanines: 615a-c, 617

PDO: λ > 610 nm, 40 mW/cm², 20 min., 48 J/cm², no dark toxicity, IC50 = 0.1 (617), 0.4 (615a), 1.0 (615b) µM.

615c inactive

Φ5 = 0.08 (615c), 0.35 (617), 0.37 (615a), 0.41 (615b).

Human hepatocarcinoma cells HepG2

Glycophthalocyanines: 624a-b, 625a-b, 604, 622 and 627a-d

PDO: 0.8 µM, λ > 610 nm, 40 mW/cm², 48 J/cm², no dark toxicity, IC50 = 1.1 (627b), 1.5-1.8 (627a-c,d), 4.7 (625b), 5.1 (625a)

604, 622 inactive. PDO efficacy > Photosense®

Human hepatocarcinoma cells HepG2

Glycophthalocyanines: 627-630, 628-630b

PDO: 0.8 µM, λ > 610 nm, 40 mW/cm², 48 J/cm², no dark toxicity, IC50 = 0.04 (630a), 0.28 (629a), 1.52 (627), 2.48 (630b). 628a-b inactive. PDO efficacy: 630a (50 ng/mL) > Photofrin® (4.5 µg/mL).

Human chronic myelogenous leukaemia K562

Cyclodextrinophorphyins: 638d and 638a

PDO @ 5 and 10 µM, 0-4.2 J/cm², 24 h incubation, no dark toxicity. EC50 = 5 µM @ 2.5 J/cm² (638d), 10 µM @ 3.4 J/cm² (638a) and 5 µg/mL @ 3.4 J/cm² (Photofrin®). Via apoptotic cell death.

Murine melanocyte cells B16

203-208, 315

Cellular uptake study: @ 4 µM, 4 h incubation. Highest uptake by 206 compared to 208 and 203. The low cellular uptake of 205 (log P = 0.5) and 208 (log
Melanoma cells B16- Highly metastatic - B16-BL6 Weakly metastatic - B16-F1 Radiation-induced fibrosarcoma (RIF) tumor cells

OEP-benzochlorins: 553-556b, 557

Galectin binding studies: ELISA showed 553-556b, 557 bind 141-387 times greater than lactose (IC₅₀ = 1200 μM). IC₅₀ = 3.1 (557), 4.5 (556b), 5.1 (555b), 6.2 (553b), 8.5 (554b), 1200 (lactose) μM. 

Radiation-induced fibrosarcoma (RIF) tumor cells

Aβ-glycochlorins 542a, 543a

PDT: @ 0.05 - 0.1 μM, IC₅₀ = 0.5 μM in B16-BL6 542a > 543a [354]

Radiation-induced fibrosarcoma (RIF) tumor cells

Purpurin imides: 570, 569c,571a-d, 572-575

PDT: @ 1 J/cm², 572 – dark toxicity > 0.5 μM.

Murine radiation-induced fibrosarcoma (RIF) tumor cells

HPPH-glyco-conjugates: 558a-h

558f > 558g, h.

558a > HPPH – due to HPPH efflux ABCG2 pump. 

PDT: @ 0-1 μM, 24 incubation λ = 665 nm, 48 h incubation post treatment, no dark toxicity, 558a,b > 558c,d > HPPH.

558c > 558f > HPPH.

558g and 558h – inactive

No improvement of efficacy due to galectin binding. 

Localization: HPPH – mitochondria, 558a,c,d,f and lysosomes, 558c – Golgi apparatus and lysosomes

558g,h – mitochondria, Golgi apparatus and lysosomes

Mouse mammary carcinoma 4T1

Cyclodextrinporphyrins: 638d and 638a

PDT: @ 5 and 10 μM, 0.4-2 J/cm², 24 h incubation, no dark toxicity. EC₅₀ = 5 μM @ 2.5 J/cm² (638d), 10 μM @ 3.4 J/cm² (638a) and 5 μg/mL @ 3.4 J/cm² (Photofrin®). Via both apoptosis and necrosis. 

Mouse mammary carcinoma 4T1

Aβ-glycochlorins 542a, 543a

PDT: @ 0.05 - 0.1 μM, 542a > 543a. [354]

Cyclodextrinporphyrins: 638d and 638a

PDT: @ 5 and 10 μM, 0.4-2 J/cm², 24 h incubation, no dark toxicity. 638d > 638a. [351]

Cyclodextrinporphyrins: 638a-d

PDT: 638a-c complexation with particular drugs, 1 h, r.t., 4 J/cm², 24 h incubation, dark toxicity due to conjugated drug. 638a,b conjugates with taxanes gave increase cell mortality 5-8 % compared to β-cyclodextrin/drug. 

Combined therapy observed in most except kinase activation inhibitors (sunitinib, sunitimbi) and approved advantageous.

small molecule delivery; @ pH 7 – strong binding of the drug complex versus pH 5 – significantly diminished binding.

638a and 638c >> 638b > 638d.
Mouse colon adenocarcinoma
Colon26

78a-h

Cellular uptake: @ 3 h or 24 h 558a = HPPH.
PDT: @ 0-1 μM, 24 h incubation, λ = 665 nm, 48 h incubation post treatment, no dark toxicity, @ 24 h incubation: 558a = 558b = HPPH @ 4 h incubation: 558a > 558b > HPPH.

Mouse fibroblasts K.Molv NIH 3T3

Aγ-glycobacteriochlorins: 542a, 542a’
Aγ-glycosobacteriochlorins: 545a, a’
Aγ-glycobacteriochlorins: 546a.

M. Saccharomyces cerevisiae FL100

Rat hepatoma liver cells RLC-16
ABCG2 expressing

245, 246
Pyrophereophorbides, HPPH and purpurinimides: 558a-d, 558f-h, 559, 570, 569c, 571a-d, 572-575, 565-569,

Murine mammary adenocarcinoma
4T1 containing a subset of ABCG2 expressing side population (SP) of cells versus no expression.

HPPH and 558a

SP analysis: cells treated with imatinib mesylate and fumitremorgin found 4-15 % of side population present.
PDT for 4T1: @ 800 nM, 1 J/cm², 3.2 nW/cm², presence or absence of 10 μM imatinib mesylate (ABCG2 inhibitor), 48 h incubation, for HPPH and 558a.
Absence of inhibitor: HPPH displayed an increase of 10-105 nM, 7.5 min., 16 h incubation, for HPPH and 558a.
No photobleaching.

Rat hepatoma liver cells RLC-16
Transformed human embryonic kidney cells HEK-293 482R –
ABCG2 expressing

245, 246
Pyrophereophorbides, HPPH and purpurinimides: 558a-d, 558f-h, 559, 570, 569c, 571a-d, 572-575, 565-569,

Transformed human embryonic kidney cells HEK-293 PcDNA –
ABCG2 non-expressing.

Saccharomyces cerevisiae, strain FL100
Thio-glycosylated tolylporphyrins 247-249

Jurkat T leukemia Tn-positive cells

TrMPyP-MorG

TrMPyP

MorG

PDT: 1 × 10^-5 M, 150 W/cm²; delayed effect compared to Photofrin. β-derivatives more effective than p.

Cellular uptake: 30 min., 4 °C, very high uptake,
1 μM Bovine submaxillary mucin (glycoprotein containing high-density O-linked cryptic polyvalent T /Tn glycotopes) – complete inhibition.
PDT: 10^-10 nM, 7.5 min., 16 h incubation, No dark toxicity ≤ 10^-5 nM, TrMPyP @ 10^-10 nM – active
3.1.3.1 meso-Substituted Porphyrins

Due to their easier synthesis the first PDT studies on glycoporphyrins used symmetrically meso substituted systems, e.g., A$_2$-type porphyrins. However, it became apparent quickly that the nature, number and linkage position of the glycosyl unit, i.e., their amphiphilic character is of critical importance for their PDT efficacy \[242\]. In preliminary investigation of their PDT efficacy, the amphiphilic molecules 114-116 were found to be better than Hpd, i.e. those with strong amphiphilic character \[162,345\]. Still, symmetric systems should not be discounted nor should the protected "precursor" compounds. The latter became clear in an early study from 1998 where the meso substituted porphyrins 29-32, 35-36 and 53 were studied in HeLa cells \[162,167,345\]. Interestingly, in all cases the acetylated, i.e. protected derivatives, showed significantly higher photocytotoxicity than the deprotected species. The octaglycosylated derivative 53 gave no improvement; rather its effect was weaker. In general more globular water soluble systems appear to be disfavored for uptake by the cells. "Flatter", more hydrophobic derivatives such as 29 appear to be superior. The singlet oxygen quantum yield was comparable in all compounds and thus, the PDT effect is mainly the result of different uptake \[167\].

The first comprehensive study of 30, 32, 39-52 in HeLa cells showed that all 16 glycoporphyrins displayed better cellular uptake compared to 5,10,15,20-tetrakis(4-sulfonatophenyl)porphyrin except 41 and 32 which exhibited precipitation. The para-substituted porphyrins showed much higher uptake compared to the meta-substituted derivatives. Improved uptake was shown for the β-D-xylopyranosyl and β-D-arabinopyranosyl groups compared to β-D-glucopyranosyl and β-D-galactopyranosyl groups \[165\]. The order of photocytotoxicity was 44 > 30 > 5,10,15,20-
tetrakis(4-sulfonatophenyl)porphyrin in agreement with the order of cellular uptake.

Subsequently, these derivatives were further investigated to determine the role of the sugar moiety and their photocytotoxicity was found to follow the order 44 < 30 < 32 < 42 where the IC$_{50}$ of 44 was 5 times that of 42 \[272\]. The photophysical properties of the glycoconjugated porphyrins are also dependent on the sugar moieties in physiological media such as phosphate buffered saline (PBS) containing 10 wt.% bovine serum albumin (BSA). The oscillator strength of the PS alone: (tetrakis(4-sulfonatophenyl)porphyrin-MorG, 1/1), 25 (tetrakis(4-sulfonatophenyl)porphyrin-MorG, 3/1), 12 (tetrakis(4-sulfonatophenyl)porphyrin-MorG, 1/1), 7 (chlorin e$_6$-MorG, 1/1), 5 (AlPcS$_4$-MorG, 10/1), 3 (5,10,15,20-tetrakis(4-sulfonatophenyl)porphyrin-MorG, 1/1), 2 (chlorin e$_6$-MorG, 9/1), 1 (AlPcS$_4$-MorG, 9/1). Ratio of PS/MorG: white light: LD$_{50}$ = 2 (5,10,15,20-tetrakis(4-sulfonatophenyl)porphyrin-MorG, 3/1), 4 (tetrakis(4-sulfonatophenyl)porphyrin-MorG, 1/1), 7 (chlorin e$_6$-MorG, 9/1), < 10 (AlPcS$_4$-MorG, 1/1), 16 (chlorin e$_6$-MorG, 1/1), 35 (AlPcS$_4$-MorG, 10/1), 4 (AlPcS$_4$-MorG, 1/1). Photosensitizers and the photochemical properties and phototoxicity but clearly the photophysical properties of the glycoporphyrins are strongly dependent on the physiological media resulting in the sugar-dependent photocytotoxicity \[272\].

It is worth noting however, carbohydrate units do not necessarily always improve on PDT activity. For example, a study of thioglycosylated tolylporphyrins (247-249) against yeast Saccharomyces cerevisiae, showed that the photocytotoxicity was un-related to the specific glycosyl unit \[243\].

Rather detailed investigations focused on the S-glycosylated A$_2$-type porphyrins 85 and 87. In comparison to...
5,10,15,20-tetakis(4-methoxyphenyl)porphyrin they exhibited enhanced cellular binding in human breast cancer cell lines (MDA-MB-231). The glucose derivative showed higher uptake and enhanced PDT activity and induced both apoptosis and necrosis \cite{247}. The PDT activity was dependent upon the drug dosage, length of irradiation and the degree of uptake with the glucose derivative 85 displaying the best activity. Characteristically malignant cells have increased uptake of glucose for metabolic activity and human breast cancer cells have a higher expression of glucose transporters then normal cells which could be attributed to the higher uptake of 85. The migration of cancerous cells was significantly reduced and thus reducing the aggressiveness of the cancer when exposed to low levels of PDT. It was also determined that a normal rat fibroblast cell line (3Y1) exhibited less uptake of the conjugates than those overexpressing tyrosine kinase transformed version of the cell line. Showing a $\geq 3$-fold affinity for cancer cells over non-cancerous ones reinforcing the results obtained in the MDA MB-231 cell line \cite{207,236}. Subsequent localization studies showed that $\approx 90\%$ of the PS accumulated in the ER and caused ER-stress inducing apoptosis upon irradiation. This caused the release of free calcium, followed by release of cytochrome c from the mitochondria, caspase 3 activation, PARP cleavage and chromation condensation; all of which are indicative of the apoptosis cascade process \cite{346}. A similar PARP cleavage and chromation condensation; all of which were destroyed. Glycoconjugate PSs characteristically undergo apoptotic cell death as was reiterated here \cite{200}

A series maltohexaose substituted nTHPP derivatives (437-441 and Zn(II)437) proved interesting with regard to the number of sugar units required for PDT effect. Investigations, again in HeLa cells, revealed that porphyrins containing a single maltohexaose (free base and Zn(II)) had significantly higher phototoxicity compared to all other maltohexaose derivatives and 30 and 53 \cite{167,202a}. The 5,10-disubstituted free base derivative 438 was more active than the 5,15-derivative 439. On the other hand, the singlet oxygen production of the free bases and zinc(II) complexes were comparable. Thus, the better PDT efficiency of 437 is the result of higher cellular uptake and accumulation in the cytoplasm.

For amide linked AB$_3$-type systems (221, 222) the ortho-substituted derivative 221 displayed much higher activity compared to the para derivative 222 but was still less active then Photofrin$^\text{\textregistered}$ against K562 human chronic myelogenous leukemia cells. Here, the glucosyl unit was essential for the PDT efficacy as the non-glycosyl derivative 215 displayed significantly less activity \cite{237}. Also, compounds 221 and 222 showed similar singlet oxygen production to HP.

The in vitro behavior of N-linked glycoporphyrins 78, 79 in rat hepatoma (liver cancer) cells (RLC-16) was determined in comparison to non-glycosyl water soluble porphyrin meso-tetakis(4-sulfopheny1)porphyrin and 74 and the slightly water soluble digalactose and diglucose derivatives 245 and 246. Octaglucosyl TPP lacked cellular affinity while the galactose derivative displayed significant affinity which was related to saccharide specificity for hepatocytes of the galactose residue \cite{175}.

A number of A$_3$B-type systems with diethyleneglycol (203, 204-208) and monoethylene glycol (315) linkages were investigated for their cellular uptake, localization and photoactivity in Y79, HT-29 and B16 cell lines. The extent of cellular uptake was dependent on the sugar residue (206 > 204, 205, 207), its anomic configuration (204/207 > 205) and the linker (206 > 315) with the unconjugated derivative 203 exhibiting poor internalization in comparison \cite{198}. These cell lines over-express lectins for specific glycosides but the uptake of the glycoporphyrins was independent of such receptors. Initial work carried out on the pre-incubation of the cells in glycosylated albumin showed a 40-45% inhibition of uptake for 204 and 206 indicating a cell-sugar-receptor saturation. With regard to phototoxicity of the PSS
(203-208, 315), the most significant result was the high uptake of 206 and its good photoactivity (LD$_{50}$ = 0.35 µM) compared to the parent compound 203. Interestingly, 205 which only exhibited twice the internalization as 203 showed a significantly high phototoxicity with LD$_{50}$ = 0.05 µM in the Y79 cell line [198] due to the variation in localization within a cellular structure playing a major role in photodynamic efficiency. Cellular metabolic degradation of all compounds was minimal however, possible in vivo degradation can not be excluded.

It might appear logical to extend the carbohydrate units for improved biological effects. Here, a study of glycodendrimeric A$_3$B porphyrins (499-512b and 271) used HT 29 and Y79 cell lines with > 540 nm light with a fluence of 2 J/cm$^2$ and 24 h incubation of the PS [205]. The glycodendritic porphyrins 499b, 501b and 502b had the highest LD$_{50}$ (2.5 to 2.7 µM) in HT29 cells and 503b had an LD$_{50}$ = 3 µM, in Y79 cells. However, in comparison to the previously synthesized glycoporphyrin 206 the compounds were six times less phototoxic in HT29 cells and 10 times less phototoxic in Y79 cell lines; clearly more is not always better [201].

Cationic porphyrins of the A$_3$B-type (461a-d) showed good PDT activity against HT-29 cells with LD$_{50}$ ranging from 25 (461c) to 50 µM (461d). Here the carbohydrate units proved to be important for lowering the dark toxicity, as comparable systems without the sugar unit had much higher LD$_{50}$ (5 µM) indicated the importance of the sugar unit in reducing dark toxicity [246].

Another series of A$_3$B porphyrins (480, 481, 486-489) substituted with three glycosyl units was based on ‘click-chemistry’ and was used to investigate the effect of the sugar, the length of the spacer arm and the position and orientation of the triazole core on photoactivity [271]. Cytotoxicity and phototoxicity were examined in HT29 and Y79 cells. All compounds exhibited better phototoxicity in Y79 cells than HT29 and SAR were difficult to establish. The nature of the glycosyl units appeared to have no effect on the phototoxicity. Porphyrin 489 (IC$_{50}$ = 11 and 0.4 µM on HT 29 and Y79 cell lines) is an analogy of a compound previously synthesized 206 which showed good PDT efficacy in vitro (IC$_{50}$ = 0.43 and 0.35 µM on HT29 and Y79 cell lines, respectively) [198]. Although 489 exhibited good photodynamic activity it was still lower than 206 in HT29 cell lines. The other glycoporphyrins exhibited little or no activity compared to the reference compounds. In summary, the triazole core decreased in vitro phototoxicity independently of the spacer or its orientation (1',4'-triazole versus 4',1'-triazole).

Attempts to utilize both carbohydrate and amino acid residues in bioconjugates failed. For example, studies on porphyrins (292-299) bearing both carbohydrate and amino acid residues in K562 leukemia cells gave discouraging results. All compounds showed lower phototoxicity than hematoporphyrin. Only at very long illumination times and with a significant delay (24 h) did the cell killing count reach that of Hp [207,259]. A similar study employed A$_3$B-type glycoporphyrins carrying the RGD tripeptide as unit b (168o,p). The compounds exhibited a similar singlet oxygen production to that of hematoporphyrin. However, their phototoxic activity reached that of Photofrin$^\circledR$ only at very long irradiation times of 2 h. Unlike Photofrin$^\circledR$, they did not induce immediate cell death but delayed apoptotic cell death [195].

A large library of A$_3$B, A$_3$B$_2$ and A$_3$B$_3$ deprotected glycoporphyrins (316-331, 261) was synthesized to investigate the nature and distribution of carbohydrates on the periphery of the porphyrin and how this affected their biological properties on esophageal squamous-cell carcinoma cell line OE21 [33]. The different carbohydrate units controlled the biological behavior. All glycoconjugates were tested for their PDT efficacy except for the mono-substituted derivatives (316-319, 261) which proved too insoluble for the study. None of the glycoporphyrins showed PDT efficacy under these experimental conditions. Similar results for triazole linked glycoporphyrins have been reported [251,324], while no biological data are available for related cationic bioconjugates [347]. However, the D$_2$-symmetric 5α,10β,15α,20β-tetrakis(1,2,3,4-di-O-isopropyldene-a-D-galactopyranos-6-y)porphyrin localized in lysosomes and caused cell death by apoptosis via caspase-dependent apoptotic pathways [348].

Cyclodextrin-porphyrin systems have elicited significant interest as drug carriers and for binding studies. For example, β-cyclodextrin sandwiched porphyrins were synthesized to mimic holohemoproteins [349]. This involved the synthesis of atropoisomers of 5,10,15,20-tetrakis(o-thioacetoxyphenyl)porphyrins and linkage with A,D-diiodo-β-cyclodextrin (10-15 % yield) [349,350]. A first detailed PDT study involved mono- (638d) and bis-cyclodextrin-appended (638a) tetrakis(pentafluorophenyl)porphyrins derivatives, which were tested on several tumor cell lines. Both PSs localized in lysosomes and caused delayed cell death by apoptosis in HL-60 cells compared to 4T1 cell lines which were subject to both apoptotic and necrotic cell death. The mono-cyclodextrin porphyrin derivative 638d showed higher in vitro phototoxicity than the bis-cyclodextrin derivative 638a [351].

Further studies using the β-cyclodextrin conjugates 638a,b,d focused on paclitaxel delivery while the γ-cyclodextrin conjugate 638c was used for doxorubicin transport. Thus, these systems were designed to act as dual-modality systems with the cyclodextrin functioning as a drug delivery unit while the porphyrin fragment acted as a PDT agent. It was anticipated that the pH-dependent cyclodextrin binding could be used to release the small molecule drugs in the slightly acidic cancer cells. The monosubstituted derivative 638d exhibited low binding affinity and was not studied further. The tetrasubstituted compound 638b was found to be more effective in vitro when tested against 4T1 and K562 cells compared to the same animal model as used.
before. Best results were obtained with the disubstituted derivatives carrying two β- (638a) or γ-cyclodextrins (638c) which gave very good in vitro and in vivo PDT activity combined with drug complexation at physiological pH conditions and release in cancer cells [351,352].

An alternative method to present carbohydrate units is through strapping of two meso residues. A comparison of 5,10- and 5,15-strapped compounds (390 and 392) showed that 390 is significantly more active than 392 when tested on K562 human chronic myelogenous leukemia cells. The 5,15-strapped porphyrin 390 showed significant delayed activity compared to Hp [220].

3.1.3.2 Hydroporphyrins

Many studies focusing on chlorins utilized the basic framework of mTHPC, i.e., a m-tetra-"O"-phenylporphyrin core [153]. For example, the glycosylated tetraphenylchlorins 513a-c and 514a-c were tested for their PDT ability against HeLa cells [258]. They were more effective in generating singlet oxygen compared to 639a while within the series no marked difference in singlet oxygen production was observed. However, clear differences in cellular uptake were found. The highest activity was observed for the deprotected chlorins 514a,b in marked contrast to the respective parent porphyrin derivatives [167,258]. Interestingly, the octaglycosylated derivative 514c exhibited poor uptake and minimal PDT activity due to its high hydrophilicity.

Both tri- and tetra-glucosyl mTHPC conjugates (514f, 516, 517) were investigated for their photoactivity and internalization compared to mTHPC in HT29 human colorectal adenocarcinoma cells. The glycosyl substitution modified the photoactivity of mTHPC. Typically the symmetrical tetra-glycosylated chlorin 514f displayed poor internalization and photoactivity compared to the asymmetric, more amphiphilic derivatives (516, 517) which showed better photoactivity compared to mTHPC with light doses of 5-25 J/cm². Maximum phototoxicity in HT29 cells was observed with concentrations as low as 2 μM of 516, 517 at light doses of 5-25 J/cm² compared to m-THPC required four times the concentration to achieve the same toxicity. Surprisingly, the cellular uptake of 516, 517 was 30 % lower than mTHPC and exhibited saturation. This increase in toxicity was due to the higher accumulation in mitochondria, approximately a 2-fold increase in mitochondrial affinity for the glycoconjugated derivative 516, 517 [260].

Next to the basic PDT activity the metabolism of the PS must be considered as well. Depending on the glycosyl unit these may be cleaved in vivo resulting in changes in the biodistribution, amphiphilicity and drug-cell interaction and possibly their PDT activity. Thus, the possible enzymatic metabolism of TPC(m-O-Glc)₃ (516, 517) was investigated in vitro in HT29 cells using MALDI-TOF mass spectrometry and HPLC [209]. This chlorin is four times more photoactive
in vitro than Foscarné itself and thus of potential interest in the PDT treatment of tumors [260]. The three sugar units of 516, 517 were shown to undergo sequential hydrolysis with subsequent oxidation of the chlorin to the corresponding porphyrins. A cellular extract treated with 516 and 517 showed deglycosylation indicating that the cellular metabolism was independent of the substitution pattern. The cellular metabolism clearly effects the PDT activity of 516, 517 with the final metabolite 307 requiring 2-3 times the concentration to provide the same PDT effect [209].

A comprehensive study expanding on work on 516, 517 [260] and 206 [198] studied a range of related hydroxyphenylporphyrins and -chlorins and their glycoconjugated counterpart in HT29 and human retinoblastoma Y79 cell lines. The glycoconjugated derivatives had lower PDT activity compared to mTHPC as well as their respective parent compounds, displaying IC50 = 2.4-0.05 μM against Y79 cells. Among all the derivatives studied the tris-p-O-β-glucose porphyrin derivative 114 was found to have the best photoxicity versus cytotoxicity activity compared to the parent porphyrin system with an IC50 = 0.9 μM [210]. However, this compound is less PDT active compared to the diethyleneglycol porphyrin conjugates described before (204-206) [188] including the glucose derivative 308. The extension of the linker between the glycoconjugate and the chromophore causes a significant increase in the PDT activity. Also highlighted was the difference in the anomic configuration the p-O-Deg-O-α-Gal compound (205, IC50 = 0.05 μM) [198] which shows a 10-fold greater photoxicity than the β-glucose conjugate (308, IC50 = 0.6 μM) in Y79 cells as well as mTHPC IC50 = 0.6 μM. Here, porphyrins 205 and 114 were identified as good PDT PS [210].

Similarly, the photochemical properties and the in vitro photocytotoxicity in HeLa cells of the four chlorin derivatives 514d-g were investigated [262]. Fluorescence intensities in phosphate-buffered saline of 514f and 514g (hexose groups) were ~ 2-times greater than 514d and 514e (pentose groups) proving a sugar-dependent difference of aggregation behavior. All the glycochlorins except 514g were spread throughout the cytoplasm. Compound 514f displayed the highest photocytotoxicity in HeLa cells while no phototoxicity was observed for 514g.

Uptake studies in HeLa cells had shown that the chlorins 514a,b,d-i exhibited higher uptake then 639a and either equal or higher uptake then the corresponding porphyrin derivatives [261]. E.g., the 5,10,15,20-tetrakis[3-[β-D-xlylopyranosyloxy]phenyl]chlorin 514d showed 50 times higher uptake than 639a and the overall order of uptake was 514d > 514i > 514e > 514a > 514b > 514h > 514f > 514g > 639a. The photocytotoxicity was unrelated to the cellular uptake as evidenced by the fact that the photocytotoxicity of the glycoconjugated chlorin 514f (LD50 ~ 2×10–7 M) is almost 25 times that of the porphyrin 30 (LD50 = 5×10–6 M) [165] even though the cellular uptake of the two compounds are the same and there is no difference in their Φ1O2.

Other studies looked at the impact of physiological properties (i.e. cellular uptake and ROS) and structural diversity of the sugar unit [273]. The photocytotoxicity profiles poorly related to the amount of cellular uptake of the PSs. The cytotoxicity of 24 glycoconjugated PSs (30, 32, 39-52, 524a,b,d,i and 639a) was evaluated in HeLa cells at a drug dose of 5 μM, irradiation with 16 J/cm² followed by 24 h incubation post treatment. All derivatives displayed no dark cytotoxicity and better photocytotoxicity than 639a. For 15 of the PSs (39-43, 46, 49, 50, 52, 514a,b, 514d-g) the EC50 was less than 5 μM. At 0.5 μM, the meta-substituted chlorins 514d-g still showed potent photocytotoxicity. EC50 values varied from about 0.5 μM for 51 and 30 to less than 0.2 μM for 514f.

Examining the structural units the sugar moieties, the light-absorbing moiety and the substitution position of the sugar moiety in detail. The PSs bearing D-xylose residues usually showed higher photocytotoxicity than the other PSs, while those with D-arabinose units usually showed lower photocytotoxicity. Looking at the light-absorbing moiety the photocytotoxicity increased in the order of zinc porphyrin < porphyrin < chlorin. For example, taking EC50 values 514a (0.7 μM) < 30 (4.0 μM) < 45 (5.0 μM) this showed the chlorin to be almost 6 times more phototoxic than the porphyrins [273]. On the other hand, no difference in the quantum yields of the porphyrin and chlorin derivatives were identified. However the molar extinction coefficient of 514a (ε = 2.4×104 M–1.cm–1) at 652 nm which is 3.7 times that of 30 (ε = 0.67×10–1 M–1.cm–1 at 647 nm). The meta-substitution appears to increase photocytotoxicity for almost all PSs. Regarding the EC50 values for 30 at 4 μM and 39 at 0.5 μM, switching the substitution position from para to meta changes the photocytotoxicity ~ 8-fold.

Significant results can also be obtained with one carbohydrate unit in chlorins. For example, a study with 247, 248 and 514a,b in HT29 cells clearly indicated that unsymmetric and less hydrophobic glucosylated PSs are superior to TPP [201]. The chlorin 514a,b gave the best PDT activity, probably due to its good singlet oxygen quantum yield (Φ1O2 = 0.73 compared to 0.55 in 247, 0.33 in 248 and 0.55 for TPP). However, porphyrin 247 displayed 11.5 fold higher cellular uptake compared to TPP while the uptake of the chlorin was only five times better. Compound 247 localized in the ER. In general, the compounds with asymmetric substitution of saccharide units are more phototoxic than symmetrical compounds [201].

An example of a different class of meso substituted chlorins are compounds 541a’, 542b’, 542a and 542b which were tested for their photocytotoxicity in HeLa cells [178]. No dark toxicity was found at 0.5 μM. Only the deprotected PSs 91, 95, 542a and 542b were photocytotoxic. The S-glucosylated PSs 91 and 542a showed higher photoactivity than the S-galactosylated compounds 95 and 542b. The cellular uptake of the former increased up to 24 h, while that of 95 and 542b was saturated by 12 h. The photocytotoxicity of 542a was higher than that of 542b even at the same level
of cellular uptake; clearly the glucose unit plays an important role in PDT effect.

Wavelengths > 500 nm are usually employed for PDT treatment, therefore the \(\delta\)-glycosylated chlorins have better photophysical properties for PDT compared to the \(\O\)-glycosylated TPCs. The difference in photoactivity is not attributed to the photophysics as all the compounds have similar properties therefore is due to biological effect. Overall, the EC\(_{50}\) values of the chlorins 542a,b were half or one-fifth of the porphyrins 91 and 95. Also, the \(\beta\)-\(d\)-glucosyl-thio derivatives (91) showed a better photodynamic effect compared to \(\beta\)-\(d\)-galactosyl-thio systems in MDA-MB-231 human breast cancer and 3Y1 rat fibroblasts observed by Drain et al. \[247\].

In addition \(\alpha\) substituted chlorins (542a,a'), isobacteriochlorins \(545a,a'\) and bacteriochlorin \(546a,a'\) were evaluated against human breast cancer cells MDA-MB-231 and K-Molv NIB 3T3 mouse fibroblast cell lines \[274\]. Compound 542a showed a significant red shift compared to 91 however, the bacteriochlorins 546a displayed the greatest red shift near 730 nm and 50 times greater absorbance optimum for PDT.

Following from this, a comprehensive cellular uptake study of compounds 543, 543a,a', 542a, 93 and 91 was carried out showing that the glyco-conjugates had four times the uptake of 543 and with no difference in cellular uptake between the porphyrin and the chlorin macrocycles in HeLa cells \[177,354\]. The photocytotoxicity of the glycoconjugated PSs were 40 times greater than 543 where the EC\(_{50}\) values decreased in the order of 543 (5.0 \(\mu\)M) > 93 and 91 (0.15 \(\mu\)M) > 543a (0.1 \(\mu\)M) \(\approx\) 542a (0.09 \(\mu\)M). The photocytotoxicity of the chlorin derivatives 543 and 542a was \~1.5 times greater than the corresponding porphyrin derivatives 93 and 91 \[354\]. Tests of 542a and 543a were also performed with B16-BL6 melanoma cells, weakly metastatic B16F1 cells and metastatic 4T1 breast cancer cells. The free base chlorins gave the best results in all cell lines. Thus, any heavy atom effect on the photocytotoxicity was negligible. While often heavy atom effects are noted in solution of \(\alpha\) porphyrins 189 \[307\]. It is more efficient to attempt to improve the PS efficacy through the sugar unit \[177,178,354\].

Benzochlorins have been of interest in PDT for a significant time \[355\]. The amphiphilic benzochlorin 552b displayed good photocytotoxicity in \(\alpha\) vitro against HT29 tumor cells when irradiated either with white light and a better one with red light irradiation to be more efficient. In comparison the parent, un conjugated derivative 551b exhibited no photocytotoxicity \[277\]. HT29 is also susceptible to a range of other amphiphilic porphyrins, benzochlorins and azaporphyrins; again glycosylated benzochlorin and azaporphyrins displayed the best photocytotoxicity when irradiated at 590 nm \[556\].

In another study on OEP-type benzochlorins, all PSs \(553-556b, 557\) were found to be active in \(\alpha\) vitro on radiation-induced fibrosarcoma (RIF) tumor cells. These cells are known for their galectin-3 (Gal-3) expression. In control experiments the tumor cells were pre-incubated with lactose to block the \(\beta\)-galactoside binding sites before treatment with the PS. Both the galactose- and lactose-benzochlorin conjugates \(555b, 556b, 557\) exhibited 100 % loss of their PDT efficacy in the presence of free lactose, whereas 553b retained its activity. This indicates a possible \(\beta\)-galactoside-recognized protein specificity for 555b, 556b and 557 \[279\].

Due to their excellent uptake and photophysical properties phyto- and rhodochlorin derivatives have remained at the forefront of efforts to develop better PS \[283\]. Thus they present ideal candidates to investigate the influence of sugar units. For example, initial studies on purpurin imide conjugates compared the free purpurin imide to those conjugated with galactose (569a, 570) and lactose (Gal(\(\beta\)-1-4)-Glc) \(569b\) which showed a significant increase in photosensitizing efficacy in \(\alpha\) vitro under similar light and drug doses in Molt-4 cells with the conjugate derivatives. The lactose derivative gave an increase in PDT efficacy compared to the galactose one \[278\]. Pre-incubation of the cell line with lactose showed a significant decrease in photoactivity, highlighting target-specificity in relation to the carbohydrate derivatives.

Expanding from these previous studies \[278\], the effect of glycosyl regiochemistry, linker and glycosyl unit in \(\alpha\) hexyl mesopurpurimide glycoconjugates \(569b,c, 571-575\) was tested in radiation-induced fibrosarcoma (RIF) tumor cell lines \[290\]. A comparison of the three isomers 571b, 572, 573 indicated that the 3-lactose purpurin-18-\(\alpha\)-hexylamide \(571b\) provided the best efficacy; it was \~4 times more potent than 572 and 573. The effect of different linker units was examined with the lactose derivatives bound by an amide bond at 17^{2} \(574\), an \(\alpha\)-benzyl group via an alkyne at 13^{2} \(575\), 569b and the non-galactose purpurimide \(569c\). These showed poor \(\alpha\) vitro photoactivity compared to 571b. Changing the sugar unit at position 3 showed the glucose conjugate 571c to be better than 571b and 571d. Overall the conjugate with lactose at position 3 (571b) proved the most effective \[290\]. Molecular modeling studies showed favorable binding affinity interactions of 3- and 12-lactose-purpurimide analogues with both galectin-1 and galectin-3. An ELISA study of the lactose conjugates compared to free lactose showed binding of all carbohydrate purpurimides to both gal-1 and gal-3 galectins however there was a significant variation between the batches of galectins therefore requiring additional investigations.

One of the currently most promising candidates under trials is 2-(1'-hexyloxyethyl) pyropheophorbide a (HPPH), an effective PSs itself in phase I/II clinical trials \[286\]. Here, the dependence on galectins-3 binding was evaluated on both galactose conjugates \(558a, 558c\) and nongalactose conjugates \(558b, 558d\) of HPPH \[287\]. All \(\alpha\) vitro tests were carried out in murine radiation-induced fibrosarcoma (RIF) and colon carcinoma (Colon26) cell lines; both express similar levels of the \(\beta\)-galactose-recognizing galectin-1 and
gallactin-3. They differ, however, in the levels of the ATP-dependent transporter ABCG2 which is responsible for the removal of HPPH in the cells. RIF cells express high levels of the protein, while Colon26 cells have no ABCG2 expression. All conjugates gave higher PDT activity than HPPH in RIF cells and 558a,b were twice as effective as 558c,d.

Notably, the introduction of a rigid linker in 558f proved to be several times less effective than amide bound lactose analogue 558c but similar to that of non glycosylated HPPH. Increasing the saccharide moiety (558g,h) still proved ineffective. No difference was observed between the glucose (558a) and galactose (558b) derivatives therefore ruling out any contribution to uptake with the presence of galectins-3. Lower activity for HPPH was observed in RIF cells which was attributed to the action of the ABCG2 pump for which HPPH is a substrate. Both 558c,d and 558f,h all displayed lower activity. This was attributed to the larger carbohydrate units and the rigidity of the latter structures affecting the mode of cellular uptake and intracellular distribution. While 558c localized in the Golgi apparatus and lysosomes, 558g,h was also present in mitochondria as well. Several of the carbohydrate conjugates exhibited superior PDT activity which may be determined by subcellular localization and the signaling mechanisms initiated by PDT [357].

The role of the ATP-dependent transporter ABCG2 was investigated in some detail. It is a multidrug resistant pump expressed at variable levels in cancerous cells and can cause efflux of a range of PS, thus lowering intracellular accumulation of the PS in the tumor tissue. Pandey’s group investigated structural factors that affect the affinity of a PS for ABCG2 in an ABCG2-expressing cell line (HEK 293 482R) and a non-expressing cell line (HEK-293 PcDNA) with the goal of improving accumulation and photocytotoxicity in ABCG2 expressing cells [337]. A series of conjugated PS with different groups attached at different positions of the macrocycle were examined, to test whether a change in affinity for the pump occurred. PSs without substitutions including phorphobrides (559) HPPH and purpurinimides (569c, 571a) were good substrates for ABCG2 efflux. However, carbohydrate groups conjugated at positions 8 (572), 12 (573), 13 (569b, 575), and 17 (565, 566, 558a-d, 558f-h, 574) but not at position 3 repelled ABCG2 interactions independent of the structure/ type of the attached sugar unit [278,287,288,290,357]. At position 3, affinity for ABCG2 was maintained with the addition of iodoobenzene (569), alkyl chains (567, 568) and monosaccharides (571c,d), but not with disaccharides (571b) [278,290,357]. These results highlighted the possible importance of characteristics of position 3 influencing binding to ABCG2 [357].

Self organized nanogels of 583 ~ 125 nm in size were evaluated for the auto-quenching properties and in vitro PDT efficacy against HeLa cells [294]. The auto-quenching of these materials decreased with increase Porphorbide a content. Internalization of 583 was mediated via hyaluronic acid receptors with subsequent cleavage by intracellular enzymes releasing the free Pheophorbide a. Compound 583 exhibited similar PDT efficacy as the lone Pheophorbide a [294]. Similar results were obtained for compound 584, the fluorescence and singlet oxygen production were measured in DMF and aqueous medium where self-quenching was evident only in the latter. 584 was internalized via folate receptors and the PS cleaved by intracellular enzymes or extracellular matrix producing an active PS. Fluorescence was detected 30 minutes after injection and increased over 12 h period thus exhibiting similar phototoxic properties as the free phophorbide a [293].

3.1.3.3 Related Systems

Similar to that observed with the efflux of PSs by the ABCG2 expressing cell lines [357], P-glycoproteins also act as energy dependent drug efflux pumps causing multi-drug resistance. β-glycosylated Tolyporphin (S) was investigated for its reversal of multidrug resistance of daunomycin, taxol and cisplatin in human ovarian (SKOV3 and SKVLB1) and breast cancer (MCF-7 and MCF-7/ADR) cell lines, the latter two of which over-express P-Glycoprotein [151]. Tolyporphin chemosensitizes SKVLB1 and MCF-7/ADR cells to drugs effluxed by P-glycoprotein but not those nontransported drugs. Similar to other reversing agents including verapamil, Tolyporphin acts as an antagonist binding to P-glycoprotein and inhibiting the removal of cytotoxic drugs. In comparison to verapamil for example Tolyporphin is significantly more potent at lower concentrations and the efficacy of both Tolyporphin and verapamil vary depending on the drug tested. Tolyporphin is particularly promising as a potential MDR agent [151].

Although Hp and similar systems have often been used for comparison in the studies outlined here only one paper appears to have directly compared meso and β-substituted glycoporphyrins. Sylvain et al. determined the photocytotoxicities of two compound series (231-236 and 456b-458b) against K562 chronic leukaemia cells with Photofrin® as a reference [199]. The compounds required 24 h post-treatment incubation in the dark at 37 °C and only with irradiation times of > 60 minutes were they comparable to Photofrin®. They initially displayed necrosis but time delayed apoptosis gave further cell death over the post treatment incubation period. All the thioglycosylated mesoaryl porphyrins ortho isomers (231, 233, 235) and only the glucose para isomer 234 were found to be photocytotoxic. The partially deprotected isohematoporphyrin derivatives (456b-458b) were also found to be photocytotoxic and were more efficient then Photofrin® indicating that their PDT effect is dependent upon their amphiphilicity rather than the glycosyl moiety [199].

One of the first few examples of sugar-containing phthalocyanines tested for their photocactivity were 617 and 618a-e which were studied on HepG2 human hepatocarcinoma cells [317]. They displayed very good singlet oxygen quantum yields ranging from 0.79 to 0.94. The axially bisglycosylated compound 617 showed high
photocytotoxicity providing IC$_{50}$ value of 0.10 µM. The unsymmetric analogues (618) gave similar results and showed a slight decrease in PDT activity with the alkyloxy chain length providing IC$_{50}$ values ranging from 0.10-0.79 µM. Si(IV)Pc(O(C$_2$H$_5$)$_2$) was tested for its PDT activity for comparison and showed no cytotoxicity. Thus, the PDT activity of 618 is primarily dependent upon the galactose residue and higher cellular uptake. Compound 617 was also examined in HT29 and T84 human colon adenocarcinoma cells with the IC$_{50}$ showing it to more effective in the HT29 cells and significantly better than Photofrin® [318].

Two glucosylated silicon(IV) phthalocyanines derivatives (614a,b) were examined against two different cell lines HT29 human colorectal carcinoma and HepG2 human hepatocarcinoma cells for their PDT efficacy [315]. Due to the heavy atom effect the dichloro glucosylated silicon(IV) phthalocyanine derivative (614b) displayed greater singlet oxygen generation but lower fluorescence emission compared to that of the non-chlorinated derivative (614a). Compound 614a showed higher photocytotoxicity with IC$_{50}$ values of 6 nM compared to 17-21 nM for 614b with the effects greater in HT29 cells compared to HepG2 [315]. The photocytotoxicity of 614a,b were significantly higher compared to the PSs Photofrin® and photophorbide a [358] with 614a highlighted as an excellent potential PS [315]. A comparative study of the galactose derivatives 617 and 615 against HT29 human colon adenocarcinoma and HepG2 human hepatocarcinoma cells was also carried out. It was observed that the octa-substituted derivative 615c displayed no photocytotoxicity while the IC$_{50}$ values ranged from 0.1-1.05 µM (HepG2) and 0.03-0.87 µM (HT29) for the other analogues in the series 617 < 615a < 615b. Concluding the chloro-derivatives are less potent than 617 [316].

Glycophthalocyanines 605, 608 and 598 [304,309] were investigated for their PDT effect and influence on cell death of HeLa and HaCaT cells [335]. The symmetric glycoporphyrin 598 showed selective targeting with significant higher photocytotoxicity in the cancer cell line compared to non-malignant HaCaT cells. Compounds 605 and 608, even at higher concentrations, displayed very low PDT efficacy. The extent of cell damage was dependent upon both concentration and light dosage. The difference in PDT efficacy was presumed to be due to the difference in the behavior i.e., aggregation of the glycoporphyrinlines 605 and 608 in solution [359]. The tetra galactose-substituted Zn(II) phthalocyanine 602b was also tested against HT-29 human adenocarcinoma cells but provided no LD$_{50}$ values at all concentrations as high as 100 µM [108].

The o-substituted zinc(II) phthalocyanines (625a,b) gave higher singlet oxygen quantum yields ranging from 0.40-0.66 compared to the metal free derivatives (624a,b, $\Phi_\Sigma = 0.12-0.14$) [310]. The glycoconjugated zinc(II) phthalocyanines 625a/b, 604, 622 and 627a-d were investigated for their PDT efficacy against HT29 human colon adenocarcinoma and HepG2 human hepatocarcinoma cells. The tetra-β-substituted glycosylated phthalocyanines (604,622) were not cytotoxic up to 8 µM while 625a/b and in particularly the mono-substituted analogues (627a-d) proved good PSs with IC$_{50}$ values ranging from 2.0-0.9 µM [110]. Unfortunately, 625a/b and 627a-d were less effective compared to the silicon(IV) analogues prepared previously [315,317,318].

Zinc (II) glucosyl phthalonitrides (627-630) were studied for their PDT activity against HT29 human colon adenocarcinoma and HepG2 human hepatocarcinoma cells [120]. The mono- and di-glycosyl derivatives 627, 629a/b and 630a/b exhibited significant phototoxicity with the order of 630a > 629a > 627 > 630b compared to Photofrin®. The isopropyl derivative 630a proved significantly potent displaying IC$_{50}$ values of 0.03 and 0.04 against HT29 human colon adenocarcinoma and HepG2 human hepatocarcinoma cells, respectively.

Glycosylated phthalocyanines were investigated for their PDT efficacy against human colon adenocarcinoma cells (HT-29) [224]. The most potent derivative was the mannosne conjugate 635b with an LD$_{50}$ value of 110 µM compared to 635c which failed to give an LD$_{50}$ value even at concentrations as high as 500 µM. In comparing the linkage of glycosyl substitution versus triazole linkage, it was observed that a significant decrease in PDT activity was displayed by the 637 derivatives especially for the mannosne conjugate 637b with a greater than three fold decrease in activity despite having similar cellular uptake [224]. This is in accordance with previous work carried out on triazole linkages for PDT by Maillard et al. [221] and Daly et al. [13].

The highly glycosylated phthalocyanine (611b) and its porphyrin derivative 610b were evaluated for their photostability, singlet oxygen generation and their interaction with human serum albumin (HSA) [312]. Both displayed significant photostability over irradiation period of 30 minutes and singlet oxygen production similar to TPP. Fluorescence quenching of the intrinsic tryptophan fluorescence of HSA on addition of the two compounds was indicative of PS binding sites. In DMSO, 611b forms unusual needle like structures and under the electron microscopy, they self-assemble in irregular fashion forming sponge like material [312].

Glucosyl-tetraaryl tetrasporbenzoporphyrin conjugates (111 and 113) were investigated for their photocytotoxicity against the human cell lines HaCaT and MCF-7. Compound 113 was less active than Photosofrin® and 111 proved to be an inefficient PS against both cell lines [184]. Tetrabenzenporphyrins also formed part of a study on triazole-linked glycoconjugates. The cellular uptake kinetics of 257, 258, 470 and 472 were evaluated in HEp2 cells. The tetrabenzenophosphyrin conjugate 472 displayed a ~ 5-fold higher accumulation compared to the TPP conjugates at all time points. However, no conjugates exhibited toxicity up to 100 µM concentrations [203].

The O-glucosyl porphyrin dimers 3650,p and 367 were tested for their photocytotoxicity against the promyelocytary cell line K562 and compared to haematoporphyrin.
However, no significant cell death was observed with all symmetrical glycosylated neutral and cationic porphyrin dimers possibly due to poor cellular permeability [192]. Subsequent studies on 365o.p, 368-370 confirmed this. This series of porphyrins showed the same efficiency for \( ^1O_2 \) production as haematoporphyrin. The more hydrophilic dimers 365o.p, 369 exhibited very low photocytotoxic activity. The more amphiphilic compounds 368 and 370 were active against K562 cells. The former was the most active but still only equivalent to Photofrin® [194].

Another study found that all oligomeric derivatives (418-421) displayed exceptionally high two-photon absorption at wavelengths suitable for biological tissues as well as significantly high singlet oxygen quantum yields (\( \Phi_1 \) from 0.43 up to 0.75) which make them promising PSs for two-photon excited PDT to treat tumor cells with overexpressing lectin-type receptors [231]. The rate of \(^1O_2 \) generation in a quenching of DPBF in DMF. Apart from the deacetylated version of 418 all dimers produced \(^1O_2 \) more or less twice compared to 206 [234]. Thus, conjugation had no effect on the \(^1O_2 \) generation as it was dependent on the number of macrocycles in the species. PDT studies with HT29 and Y79 cells revealed that the deacetylated version of 418 and 430b were only slightly phototoxic at high concentrations toward the Y79 cell line but also exhibited dark toxicity. The HT29 cell line overexpresses lectins for \( \beta \)-glucose residues and Y79 overexpresses lectins for \( \alpha \)-mannose residues. Here, no correlation was observed between the molecular structure and the lectins overexpressed. Additionally, these compounds have low solubility and are difficult to handle [234].

The most complex carbohydrate unit being linked to a porphyrin involved the plant lectin Morniga G (MorG). This is a galactose specific binding lectin which was covalently bound to 5-(4-(5-carboxy-1-butoxy)-phenyl)-10,15,20-tris(4-N-methyl)-pyridiniumyl)-porphyrin (TrMPyP) [92]. This system was used to determine its tumor-associated Tn antigen specificity in Tn-positive (Jurkat lymphoid leukemia) and CTC-PD efficacy. Several tumors show over-expression of O-glycans such as T (CD176) (Galβ1-3GalNAcα1-O-Ser/Thr) or Tn (CD175) (GalNAcα1-O-Ser/Thr) antigens.

A similar study focused on the PS 332-STxB for its receptor binding and PDT efficacy in STxB receptor cell line HeLa cells. It was found to have significantly higher PDT efficacy than 114 in such cell lines [212]. Concluding STxB conjugation offers an interesting tool for delivery of PSs.

### 3.1.4 In Vivo Studies

Similar to other porphyrin classes in vivo PDT results are more limited. Often different animal models and/or illumination protocols are used and thus a comprehensive QSAR analysis of in vivo data from different research groups is not possible.

For meso substituted glycoporphyrins initial in vivo studies on pharmacokinetics and biodistribution were performed in healthy rats with the triglucoc conjugated porphyrin 306 (0.25-1 mg/kg). mTHPC (0.3 mg/kg) was used as a control [200,360]. The glycoporphyrin was cleared three times faster from the animals than mTHPC with mean residence times of 5 and 20 h, respectively. In contrast to in vitro studies [200] no deglycosylated or oxidized metabolites were found. In vivo, compound 306 was primarily concentrated in lung, liver and spleen, i.e. reticuloendothelial cells. The maximum PS concentration was observed at 14 h post-injection and elimination from the organs was complete within 48 h. As glycosylation per se should not affect the photophysical properties this study underlines one of the more fundamental properties of glycoporphyrins in PDT, namely their rapid elimination. In a clinical setting this would shorten the post-treatment time of photosensitivity.

A similar study focused on the PS 206 which had shown affinity for human retinoblastoma cells and is specifically activated by green light at 514 nm [198]. The flexible linkers increased the photodynamic activity in both HT29 and Y79 cell lines exhibiting a particularly high phototoxicity in human retinoblastoma cells Y79 and very low doses of the drug are required for a sufficient photobiological effect [198]. The compound was investigated in a mouse model of subcutaneously implanted retinoblastomas and \(^1\)H and \(^23\)Na magnetic resonance imaging (MRI) were used to monitor...
Comprehensive \textit{in vivo} studies were also reported for glycosylated pyropheophorbides \cite{564-566}. Again, using C3H mice bearing RIF tumors it was observed that in fact the non-carbohydrate analogue 654 was more effective \textit{in vivo}, completely contrasting the \textit{in vitro} findings which showed galactose and glucose derivatives to have enhanced cell killing compared to the parent compound. At the lowest drug dose (0.50 \text{mM/kg}), 565 showed one out of five mice to be tumor-free compared to the non-conjugate 564 and galactose conjugate 566 which gave no tumor response. Increasing the dose to 1.5 \text{mM/kg}, 564 showed an increase in photosensitizing activity where four out of five mice were tumor-free at day 60. At 1 \text{mM/kg} 566 was too toxic, killing the mice after 24 h post-treatment and at 1.5 \text{mM/kg} 565 showed some toxicity with two mice dying within 24 h after PDT and two out of the remaining three mice being tumor free by day 60. Clearly, 564 has better PDT activity than the glycosylated derivatives \cite{558f}. The \textit{in vivo} biodistribution of the three compounds were measured at 24, 48, 72, 96 h post-injection. Compound 564 gave the best uptake into the tumor tissue at 24 h and 48 h with 4.1 \% IDg (percent injected dose per unit weight) at 24 h post-injection. However, 566 showed higher uptake at 72 h and 96 h but exhibited high liver and spleen uptake at these times, too. Lastly, 565 provided the worst uptake at all times. The poor accumulation of the glycosylated derivatives makes it unlikely that these PS will find application in PDT. Compound 564 was also tested in BALB/c mice bearing Colon-26 tumors. It showed higher tumor accumulation in Colon-26 tumors than the RIF tumors at every time point. The clearance rate was higher in RIF tumors and the PS showed slightly higher blood uptake in BALB/c than C3H mice \cite{558f}.

Pandey et al. synthesized purpurinimides substituted at varying positions with different linkers and glycosyl units to determine their effect on the PDT efficacy. \textit{In vivo} testing in C3H mice transplanted with RIF tumors showed the non-conjugated derivative to be ineffective at drug dosage of 5 \text{ mm/kg} and light dosage of 130 J/cm² treated 24 h post-injection and under similar conditions after 30 days the carbohydrate derivatives 569b and 570 provided 50 \% of mice free of tumors \cite{558f}.

The hydroxylated chlorin derivatives 524-526 were subject to an \textit{in vivo} bioassay for tumors photonecrosis in PC6 myeloma. The most hydroxylated compounds, the tetrahydroxybacteriochlorin and the mannityl chlorin ether were the most effective in promoting tumors photonecrosis compared to Photofrin. The chlorin-glucose derivative is an effective PS but showed no selectivity for the neoplastic tissue \textit{in vivo}. \cite{558f}

A detailed \textit{in vivo} analysis of the mono-cyclodextrin porphyrin derivative 638d showed higher \textit{in vitro} phototoxicity than the bis-cyclodextrin derivative 638a. The opposite result was obtained \textit{in vivo} in BALB/c mice bearing subcutaneously transplanted syngeneic mouse mammary carcinoma 4T1. Compound 638a exhibited faster and more
selective tumor uptake and caused tumor eradication [331]. Clearly, even within a set study the in vivo activity of PS cannot simply be predicted from in vitro data. Another study of β- and γ-cyclodextrins were investigated for their drug carrying ability with In vivo studies of 638 complexed with specific drugs carried out on BALB/c mice bearing subcutaneously growing mammary carcinoma, 4T1, and the nude mouse model with human amelanotic melanoma C32 at 100 J/cm² after 6 h incubation. The most efficient PDT combined therapy was observed in mice treated with 638a-paclitaxel or 638c-doxorubicin however, tumor relapse occurred after 10 days. Repeating the therapy after 1 week had a higher therapeutic effect [352].

In vivo study was carried out to determine if the ester cleavage of 583 to produce the free PS Pheophorbide a observed in vitro occurred in non-tumor-bearing Balb/C-nude mice [295]. It was demonstrated that the initial release of Pheophorbide a was slow but over time increased with the maximum achieved after 12 h incubation in agreement with the in vitro study. The retention of 583 was greater than for Pheophorbide a alone its presence was still observed 30 days after injection. The quantum yield of singlet oxygen was maintained at 0.43 for 30 days with irradiation at 1, 7 and 30 days carried out [295].

A glucosylated zinc phthalocyanine 613 was investigated for its potential as a near-infrared imaging agent with liver tumor-bearing athymic nude mice. The compound displayed a relatively high photostability. Significant luminescence was observed 12 h post injection with 2 x 10⁻⁴ M of 613 in the lung, kidney and liver i.e., the main metabolic organs. Tumor fluorescence was substantial and no toxicity was observed confirming the dye as a potential near infrared fluorescent probe [314].

Král et al. found that sapphyrins bearing neutral water solubilizing groups were the most selective for pancreatic carcinoma tissue in a xenographic murine model and showed the best tumor to liver ratio in vivo including the glyco-conjugate 586b when compared against a disubstituted glyco-protoporphyrin 587 [297]. The high tumor to liver ratio of 585a and 586b could be due to the faster metabolism of the sugar unit causing faster clearance. In further studies, 585a was found to display the highest uptake in intact mitochondria in vitro compared to a series of six expanded porphyrins (metalloxaphyrins and free-base sapphyrins) and 5,10,15,20-tetrakis(4-sulfonatophenyl)porphyrin [298].

The photochemical properties of 586 were investigated in aqueous media determining their self-aggregation and also interactions with target receptors. It displayed absorbance in the far visible region (> 600 nm) which allowed for deeper interactions with target receptors. It displayed absorbance in aqueous media determining their self-aggregation and also fluorescence in aqueous media at concentrations ranging from 0.15 nmol/L to 2.5 μmol/L showed a significant change in the Soret bands, indicative of dissociation from self-aggregates in dilute solutions [361]. Addition of a large excess of BSA caused monomerization of the aggregated species while at low concentrations highly aggregated dimeric and monomer BSA bound derivatives were evident. Its photochemical activity was low; on irradiation it was unable to damage BSA, poly(Trp) and GMP in aqueous media due to self-aggregation. Only in the presence of a large excess of BSA and calf thymus DNA did compound 586b result in effective photodamage.

The glycosapphrin 586c [299] was investigated for its localization in normal and tumor human pancreatic adenocarcinoma-bearing mice using resonance raman (RR) and UV-Visible spectroscopy [362]. These methods offer a means of identifying localization without tissue destruction and were used to identify the presence and to estimate the relative content of sapphrin in various tissues at various times post-injection. A maximum preferential localization of sapphrin in tumor tissue was observed at 6 days post-infection and sapphrin metabolites were identified in the liver and kidneys.

3.2 Other Photomedical Uses

3.2.1 Antiviral and Antifungal PDT

Several of the compounds described so far have been tested for their antiviral or antifungal PDT activity [363,364]. These include sapphyrins [296] and various porphyrin derivatives. An example for the latter was a study by Carré et al. who investigated the antifungal properties of various meso aryl glycoporphyrins the synthesis of which was described previously [183,365]. Similar to the results found in PDT studies the amphiphilic derivatives (129, 121) showed much better uptake by and phototoxicity for Saccharomyces cerevisia.

Another study investigated the antiviral activity of 191, 193, 194, 199, 200, 201, 202 and 195, 196 against herpes simplex virus type 1 (HSV-1) and type 2 (HSV-2) in Vero cells at different stages of cell culture infection in comparison to acyclovir and foscarnet (antiviral controls) [30]. The antiviral activity of the porphyrin derivatives was determined by the virucidal effect inhibition and the decrease of the virus yield. The highest inhibitory effect on viral yield for both viruses was displayed by the deprotected porphyrin derivatives 192, 194, 200, 202 and the methyl galactoporphyrin 195 and 196 which were comparable to those of the controls. The strong inhibition of viral yields was observed at concentrations lower than their maximum non-cytotoxic concentrations. Compounds 192 and 194 in particularly have very similar inhibitory effects as acyclovir or foscarnet. These compounds display significant inhibitory effects and offer potential for the treatment of herpes virus infections [30].

Another broad study compared the antiviral effect of neutral porphyrins (412, 414, 415) and their cationic analogues (416a-c) against HSV-1 [220]. With the exception of 416a all compounds gave IC₅₀ > 0.5 μM where the neutral
porphyrin displayed higher toxicity with IC₅₀ values just below 1.0 μM possibly due to different interactions with the viral envelope. The neutral compounds (412, 414, 415) were more effective than the cationic ones, with 90% viral inactivation at irradiation for 15 minutes and 50 mW cm⁻². Earlier it had been shown that the fully deprotected derivative have the best activity [30]. Here, both 416c and 415 significantly reduced the HSV-1 yield in vero cells; additionally the cationic derivative exhibited dark toxicity at all times of the viral replication cycle [229].

Another approach used supramolecular aggregates of positively charged amphiphilic cyclodextrins with 5-[4-(1-dodecanoylpyridinium)]-10,15,20-triphenylporphyrin (TDPyP). The cyclodextrin complexes displayed a very high singlet oxygen quantum yield (Φₛ = 0.90) and gave good inactivation of the Gram-positive methicillin-resistant bacterium Staphylococcus aureus and Gram-negative bacterial pathogens, such as Escherichia coli. The monomeric porphyrin-cyclodextrin complex showed excellent stability with no release of the porphyrin species from the host-guest system. The anti-bacterial PDT results indicate the utility of positively charged carrier systems for PSs, resulting in faster diffusion to intracellular compartments. Photokilling of antibiotic-resistant pathogens could be achieved at concentrations of 5 μM upon irradiation compared to the uncomplexed PS which proved ineffective [132].

Cationic porphyrins 416b and 416c, 209-212, 74 were also examined for their potential photodynamic inactivation of Micrococcus sp. and all were found to cause inactivation at drug dosage of 0.2 μmol/L¹ and inactivation values ranging between 4.0 and 8.0 log after 15 minutes of irradiation. PSSs 74 and 211 showed the lowest inactivation values compared to 212, 416b and 416c which were significantly more effective. Compound 416b gave the best results as it was able to inactivate Micrococcus sp. at 10 minutes. Inactivation of Pseudomonas sp. was also achieved by all the PSSs however, the rate of inactivation was considerably lower after 15 minutes irradiation with the inactivation values ranging between 3.4-6.0 log. The porphyrins 212 and 416b were the best against Pseudomonas sp. causing similar inactivation after 15 minutes with inactivation values of 5.3 and 6.0 log [230]. The best inactivation was displayed by 416b in both bacteria strains exhibiting the higher ¹O₂ production and cellular binding. In conclusion the PSSs can be used against multi-drug resistant and UV-B resistant bacteria.

### 3.2.2 DNA Cleavage

DNA cleavage studies have been reported with some compounds which were prepared to simultaneously behave as nucleic acid binders and destructive entities. The deprotected sugar units in close proximity to the porphyrin scaffold provides non covalent binding to the nucleic acids and the porphyrin scaffold upon irradiation causes selective double stranded DNA cleavage to form II nicked, circular DNA [171]. Notably, the Pd(II) complex (69, M = Pd(II)) showed high activity in this regard.

DNA photosensitization by pyropheophorbide derivatives has not been extensively studied. One example, the watersoluble cationic pyropheophorbide 642, was investigated as a photonuclease on both single and double stranded oligodeoxynucleotides (ODN), it was confirmed that singlet oxygen caused damage to sites in the DNA mainly guanine residues. Also observed were the Frank scissions of the ODN from a radical process through hydrogen extraction and sugar oxidation initiated by the photodegradation of the pyropheophorbide derivative. The photosensitization of ODN causes two lesions, base oxidation and sugar oxidation [166].

![Formula 19](image)

The study of porphyrins DNA binding capacity is extensive [167-169], especially in the area of cationic porphyrins since the discovery of their ability to form several different complexes with DNA [170]. In recent years this research has expanded to incorporate bioactive functionalities for targeting. One such example was the introduction of amino acids/peptide conjugates to either increase cellular uptake of the porphyrin derivative or delivery of the peptidic units to nucleic acids [24,371-374]. One such recent example includes cationic porphyrins with Mn complexation which have been covalently linked with peptide cell signaling sequences. This conjugated derivative displaced significantly enhanced cellular uptake and DNA cleavage compared to the non-conjugated derivative [169].

Some studies have also reported on the synthesis of water soluble porphyrinyl nucleosides. For example, tri-, di- and mono-p-phenylene-5′-O-uridine substituted meso-(4-pyridyl) porphyrins were synthesized through conversion of the meso-(4-pyridyl)₄(N-p-methoxyphenyl)₁-porphyrin to the p-hydroxyl derivatives. Next coupling with the uridine nucleoside gave the meso-(4-pyridyl)₄(N-(5′-O-p-phenylene-2′-3′-O-isopropylidene-uridine)₁-porphyrin which underwent N-methylation to afford the desired products 643-645 [175]. The first examples of per(trifluoroethoxy) phthalocyanine–deoxyribonucleoside conjugates 646a, 67a were synthesized and evaluated as PDT agents. The twelve trifluoroethoxy substitutions prevent aggregation of the
phthalocyanine and it was hoped that the nucleoside unit provides a key role for binding at the DNA recognition sites and induces radical selective cleavage of the DNA strand in the tumor cells under irradiation. The deoxyribonucleoside unit was connected to the phthalocyanine ring via an ethynyl linker as ethynyl-purine and pyrimidine nucleosides have significant potential due to their biological activities. The synthesis of 646 and 647 was achieved using Sonogashira cross-coupling of iodides and terminal alkynes in good yields. Additionally, the fluorinated conjugates can be advantageous in vivo studies using $^{19}$F NMR techniques. Pasetto et al. investigated a different approach to DNA photocleavage using C- and S- linked glycosylated tetraarylporphyrins. DNA photocleavage assays were used to evaluate the photoactivity of several porphyrins and 95 all of which exhibited poor activity compared to 5,10-bis(4-methylpyridinium)-15-(4-methylphenyl)-20-(4-hydroxyphenyl)porphyrin (DiMePy'MeOHP).
4. Other Applications of Glycoporphyrins

4.1 Membrane and Protein Interactions

PSs have been shown to bind to several plasma proteins depending on their amphiphilicity \[377\]. The more hydrophobic PSs bind to lipoproteins \[378\] while hydrophilic PSs bind to albumin \[379\]. Albumin is the most important drug carrier protein. Preliminary observations from cellular uptake and phototoxicity tests indicate that 516, 517 showed more intermolecular association with albumin compared to mTHPC which is an important parameter to consider for in vivo testing \[260\].

Membrane interaction studies are also important, as they can serve as models for more complex biological systems. For example, \(269\) and newly synthesized \(649\) prepared in the same manner were compared against the non-glycosylated dendrimeric porphyrin \(648\) for their interactions with biomimetic models of retina cancerous cell membranes which were absent of any lectin receptors on the surface and had increasing cholesterol content. It was observed that increasing the cholesterol content had no impact on the penetration of the PS into the lipid layers which was studied by surface pressure measurements and fluorescence spectroscopy \[380\]. The non-glycosylated derivative penetrated to a greater extent into the membranes at high lipid/porphyrin ratios only. The presence of the sugar moiety played an important role as the glycosylated derivatives could penetrate into the membranes even at low lipid/porphyrin ratios. Interactions of the sugar moiety with the polar phospholipid head groups limit their penetration depth. This work highlighted that glycoporphyrins can penetrate by passive diffusion independent of specific lectin interactions.

Another example is the use of glycosylated protoporphyrin IX derivatives as micellar fibers \[31\]. The amphiphilic glycosylated porphyrins \(652-655\) were synthesized from the porphyrin-formic acid mixed anhydride \((651a)\) and the acetyl-2-deoxy-2-aminopyranosides which on subsequent deprotection afforded the desired amphiphilic compounds. All four compounds, as well as the racemate \(653a\), \(654a\) showed a broad and unsymmetric Soret band as a clear indication of some porphyrin aggregation, i.e. colloidal formation. These colloidal aqueous solutions showed significant stability over several months. Circular dichroism spectra revealed the more stable aggregate \(652a\) to have greater rotational strength than the less stable aggregates of \(653a\) and \(654a\) thus providing strong evidence of only one dominant type of porphyrin aggregate. Electron micrography identified ribbons for \(652a\) and \(653a\), \(654a\) with approximate widths of 4 nm and lengths of 20 nm and 5 µm, respectively. On mixing \(652b\) with photoactive dimeric porphyrins the aggregates do not quench their fluorescence which makes them candidates for electron acceptor or donor systems. The type and stability of molecular aggregates of glycoporphyrins strongly depends on the type and number of sugar units \[381\].

Amphiphilic dendritic porphyrins \(268\) and \(269\) were tested for their interaction with phospholipids at the air-water interface and liposome bilayers were studied. Both compounds were incorporated in liposome membranes when...
Compound 269 mixed to a better extent than 268 and led to larger vesicles than DMPC alone with higher stability over time. Porphyrin 269 and DMPC mixed liposomes aggregated upon addition of α-mannose specific concanavalin A suggesting that the macrocycle was embedded into the phospholipid bilayer while the sugar unit extended out into the aqueous phase [204].

A range of hydroxylated (\textit{m}THPP, \textit{p}THPP, 473, 512b) and glycosylated (30, 39, 206, 504b) derivatives of TPP were also examined for the effect their different structural characteristics had on their binding affinity to DMPC liposomes and HSA [382]. The glycosyl derivatives displayed greater solubility in aqueous medium but such conjugation decreased the affinity for the phospholipid bilayer of DMPC in all cases but increased binding to HSA with 39 displaying the best results. The hydroxylated derivatives proved to have the best affinity for DMPC liposomes with \textit{m}THPP and \textit{p}THPP affording the highest affinity. The dendrimeric structures 512b and 504b displayed significant self-aggregation and low affinity to both DMPC and HSA. Those conjugates with intermediate lipophilicity had greater affinity for DMPC and those of higher polarity displayed larger binding to HSA. The extent of penetration into the phospholipid bilayer of DMPC was dependent on the number of phenyl groups present and the distance of the glycosyl unit from the macrocycle. The dendrimeric structures showed the deepest penetration while the presence of diethylene glycol spacer increased the insertion of macrocycle compared to those without the linker [382].

The incorporation of glycosyl units into a hemoprotein is quiet rare. One such example is the substitution of β-N-galactosyl dendritic units on the propionates of a ferric heme macrocycle (652b) with the ultimate goal of insertion into
sperm whale apomyoglobin [383]. Porphyrin 652b was synthesized via an alaining type linkor attached to the propionates of Pp IX which was coupled with O-acetylated l-amino-D-galactose by BOP in 42 % yield. Subsequent insertion of iron and deprotection of the acetyl groups afforded the highly water soluble 652b. The glycosylated ferric PpIX was inserted into sperm whale apomyoglobin with a yield of 40 % and was purified by Sephadex G-25 gel filtration. The incorporation of 652b in the apomyoglobin was monitored by the UV-visible spectroscopy where the band shift at 396 nm to 408 nm is indicative of such events and ES-MS spectrum which identified 19,065 ± 1 as the reconstituted myoglobin containing 652b [383]. The immunoprecipitation of 652b was tested in comparison to the wild-type myoglobin with biotin-labelled peanut lectin specific for β-galactose on streptavidin-modified sepharose. A decrease in heme content was observed for lectin combined with 652b whereas not significant change was displayed for the wild-type myoglobin [390].

One paper investigated the interaction of glycophorphyrins with toxins. Porphyrin 332 was covalently linked to the nontoxic homopentamer STxB subunit B of the bacterial Shiga toxins and verotoxins to take advantage of its retrograde delivery route. Shiga toxins and verotoxins comprise of an enzymatic component i.e. subunit A which inhibits protein biosynthesis and nontoxic homopentamer STxB i.e. Subunit B which promotes plasma membrane binding and internalization of subunit A [212]. STxB delivers holotoxin from the plasma membrane to the endoplasmic reticulum by endosomes and Golgi apparatus escaping degradation i.e. the retrograde route. Porphyrin 332 was synthesized via condensation of pyrrole with 4-O-(2',3',4',6'-tetraacetyl-l-β-D-glucopyranosylxy)benzaldehyde and 4-bromomethylbenzaldehyde using BF3·OEt2 in 31 % yield and subsequent deprotection. It was then coupled with STxB-Cys via a cysteine residue [212]. The conjugate 332-STxB was evaluated for its receptor binding and PDT efficacy in STxB receptor cell line HeLa cells [212].

4.2 Lectin Binding

The interaction of porphyrin carbohydrate bioconjugates with lectins presents one of the more fundamental aspects of clinically relevant research in this area. Thus, many related studies have been reported. A classic topic is the use of glycosylated porphyrins (587, 101) and sapphyrins (585b, 586b) for their non-covalent reversible binding to concanavalin A as a monitor of saccharide-lectin interactions. The addition of a natural substrate (e.g., D-mannose), which has a higher affinity for Con A compared to the non-covalent complex of lectin-glycoconjugate causes the release of the weakly bound glycoporphyrin. This could be monitored by fluorescence spectroscopy due to quenching of fluorescence upon aggregation of the macrocycles. Here, compounds 586b, 587 and 101 showed good results for noncovalent fluorescent labeling of proteins [381]. In addition, the lectin-affinity of 73 was determined and exhibited strong specific binding with suppression of fluorescent intensity of fluorescein isothiocyanate (FITC)-labeled lectins, FITC-RCA120 (Recticus comunis agglutinin, Lac-specific) while no change was observed with the α-mannose and α-glucose specific lectin FITC-ConA (Concanavalin A) [173].

In a more recent study glycodendrimeric porphyrins (269, 649) were examined for their ability to be recognized by Con A. Their interactions were monitored by surface pressure measurements, fluorescence spectroscopy, dynamic light scattering, and QCM-D [384,385]. Binding capacity to Con A was dependent on the chemical structure with porphyrins 649 and 269 showing the highest binding constants. Immobilization of Con A as a monolayer covering a QCM-D gold sensor with addition of porphyrin-bearing liposomes determined the specific interaction via the sugar moiety [384,385].

Note, that previously glycodendrimeric porphyrins had given poor results in PDT as a result of their tendency to aggregate and to interact with blood proteins [108,204,205,235,236,380,384]. Such glycodendrimers have been also tested by dynamic light scattering and QCM-D for their affinity for a synthetic biomimetic lipid bilayer membrane of similar composition to retinoblastoma cell membranes containing Con A. The interactions proved promising affording specificity for mannosylated porphyrins with the spacer length between a porphyrin core and the mannose moiety playing an important role in their interaction [384,386]. Such models afforded better mimics of in vivo conditions and insight into the mechanisms of interaction between the glycoconjugates and retinoblastoma cells.

Expanding on the grafting of Con A bilayer systems [386] a detailed study was carried out to evaluate the interaction of porphyrins with classical Y79 cell lines and the adhesion of porphyrin-bearing liposomes to Y79 cells immobilized onto the sensor of a quartz crystal microbalance (QCM-D) in the absence of fetal calf serum [387]. QCM-D allowed for analysis of the mechanism of interaction of the glycodendrimeric porphyrins with the lectins which the classical cell cultures were not. The mannosylated porphyrins were able to interact specially with the mannose receptors similar to the results with Con A grafted onto artificial lipid bilayers [386,387].

Noncovalent porphyrin protein systems have also been suggested as carrier vehicles. Thus, legume lectins, which show preferentially binding to tumor cells were found to bind simple porphyrins such as 5,10,15,20-tetrakis(4-sulfonatophenyl)porphyrin with a binding constant of about 1.2–6.3×10⁴ M⁻¹ [388]. Binding occurred in a binding site distant from the carbohydrate binding region. Studies with the galactose-specific lectin from snake gourd revealed two porphyrin binding sites with association constants of 1.7×10⁴ - 6.2×10⁴ M⁻¹ with higher affinities for metalloporphyrins compared to free base systems [389]. The binding appears to be predominantly through hydrophobic interactions as both cationic and anionic porphyrins were bound. Similar results were found for Jacalin, which recognizes the tumor
associated T-antigenic structure Galβ1→3GalNAc and could bind one porphyrin\textsuperscript{[89]}.

Another example is the interaction of water soluble free-base and metalloporphyrins (639a/b, 640a/b, 641a and 74) with Momordica charantia (bitter gourd) lectin (MCL), a tetrameric galactose-specific glycoprotein. Here, significant changes in the Soret band region of the electronic absorption spectra of the porphyrins were observed upon binding to MCL\textsuperscript{[390]}. The association constants for the interaction of different porphyrins with MCL at 25 °C varied between $5 \times 10^3$ M\textsuperscript{-1} and $10^5$ M\textsuperscript{-1}. Porphyrin binding affinity to MCL was not affected by addition of galactose, indicating that porphyrin and carbohydrate ligands bind at different sites. Likewise porphyrin binding did not significantly change the secondary or tertiary structures. 640b, 74 and 641a had significant affinity for MCL which identified MCL as a potential carrier for targeting these porphyrins to tumor tissues\textsuperscript{[390]}.

The 639a-peanut lectin (PNA) interaction was analyzed crystallographically and provided considerable information on the porphyrin protein interaction\textsuperscript{[301]}. The PNA-porphyrin-lactose ternary complex and the PNA-porphyrin binary complex were determined at 3.10 Å and 3.12 Å resolutions, respectively. PNA is a tetrameric lectin, however, the four subunits are not related by 4-fold symmetry but do have similar tertiary structures and have equivalent carbohydrate binding sites which are specific for galactopyranose units at the monosaccharide level. The porphyrin binding partially overlaps with the carbohydrate binding site of two subunits while the two other subunits remain free for carbohydrate binding. Eleven molecules of 639a bind to the tetramer of PNA in five different stacking arrangements in both the PNA-639a binary complex and PNA-639a-lactose ternary complex. A comparison of the two structures revealed conformational changes in the presence and absence of lactose in the carbohydrate-binding site of PNA. Further details are given in a comprehensive review of carbohydrate-lectin non-covalent interactions (Figure 7)\textsuperscript{[392]}.

Thermodynamic studies were carried out on the interaction of water soluble free base and metalloporphyrins with pea (Pisum sativum) lectin (PSL). Here, cationic and anionic porphyrins displayed similar affinity while 639b showed the strongest affinity for PSL compared to other porphyrin derivatives, possibly due to axial interactions of

---

**Figure 7.** Crystal structures of (a) 11 H\textsubscript{2}TPPS (black) molecules interacting with tetramer PNA (in grey) and (b) the H\textsubscript{2}TPPS–PNA–lactose ternary complex. Lactose (black) occupies the carbohydrate-binding site in subunits C and D of the PNA ternary complex but not in subunits A and B. Reproduced from\textsuperscript{[397]}.

(Copyright, 2005, American Chemical Society).
the Zn(II) metal ion with the protein. Introduction of the methyl-a-D-mannopyranoside, a specific substrate for PSL, showed no effect on the porphyrin binding determining the presence of different binding sites on the lectin. The temperature dependence of the $K_a$ values were used to evaluate the thermodynamic forces that influence the interaction. It was concluded that the porphyrin binding to PSL was driven mainly by enthalpic forces with the entropic contribution being negative. Enthalpy-entropy compensation was observed in some interaction and highlighted the role of water structures in the overall binding process. Circular dichroism and differential scanning calorimetric studies showed porphyrin binding caused no change in the lectin structure or thermal stability however, carbohydrate binding has a stabilizing effect on the protein structure.

Several investigations addressed the interaction of glycoporphyrins with galectins. For example using the enzyme-linked immunosorbent assay it was shown that compounds 553-556b, 557 bind to Galectin with an enhanced affinity 141-387 times that of lactose 50 % inhibition concentration (IC$_{50}$) = 1.2 mM which is the standard $\beta$-galactoside used as a reference. The benzochlorin galactose conjugates (555b, IC$_{50}$ = 5.1 $\mu$M and 557, IC$_{50}$ = 3.1 $\mu$M) and the benzochlorin lactose conjugate (556b, IC$_{50}$ = 4.3 $\mu$M) had a two-fold advantage over the benzochlorin glucose conjugate 554b and the non-conjugated derivative 553b. This suggests a specificity of galactose and lactose conjugates for proteins that are known for their $\beta$-galactose recognition and overexpression on tumor cells.

In another study the binding activity of the tumor specific lectin-recombinant human galectin-1 (hGal-1) to three simple porphyrin compounds (639b, 641b,c) was reported. High binding affinities were observed with dissociation constants of 0.6-1.5 nM, similar to that for Con A and porphyrin binding. This reaffirms previous crystallographic studies which indicated that galectin and legume lectins such as Con A have similar topologies even though they have different sequences. Experiments with human galectin-3 (hGal-3) gave similar results with 639b (KD = 0.18-0.2 $\mu$M, both with and without lactose present). Thus, addition of lactose showed no inhibitory effect on binding showing the carbohydrate binding domain to be separate from non-carbohydrate binding sites. Circular dichroism spectra exhibited that binding of such hydrophobic compounds changed the hGal-3 secondary structure. As hGal-3 binds to important cancer carbohydrate antigens, it has the potential to operate as a target delivery molecule for anti-cancer therapy.

Contemporary studies focus on related but more complex systems. For example, a series of tetravalent lactosylated glycoconjugates were synthesized via Cu(I)-catalyzed azide–alkyne cycloaddition in high yields. The glycoconjugates consist of a glycoporphyrin (657a) and several calix[4]arenes which were prepared from propargyl mTHPP and three topological isomers of tetra-O-propargylated calix[4]arenes, respectively via microwave mediated azide–alkyne cycloaddition with an acetylated azido-lactose derivative which was subsequently deprotected to afforded the free hydroxyl conjugates. They were evaluated for their binding to two lectins, ECA a lactose/fucosylactose specific lectin from the legume plant Erythrina cristagalli and recombinant human galectin-1(gal-1). The inhibitory properties of these multivalent glycoconjugates were compared to the monovalent species (658) to determine selectivity of the glycoconjugates between the two lectins based on their topology. The glycoconjugates displayed high affinity for ECA and Galectin-1 with the porphyrin derivative 657a providing the best results for both in hemagglutination assays (HIA), only for ECA in enzyme-linked lectin assays experiments. A high selectivity was observed for the porphyrin derivative under HIA analysis but the same was not observed with surface plasmon resonance experiments. In conclusion this approach was not fully appropriate for multivalent ligands.

An intriguing but complex approach promotes the use of phycocyanin in PDT. Phycocyanin (Pc) is a non-toxic, non-carcinogenic, water soluble phycobiliprotein present in the microalgae, Spirulina platensis. It is a pigment protein with a similar structure to bilirubin and is involved in light harvesting in photosynthesis of cyanobacteria, red alga and cryptomonads with potential in PDT. It was shown that the tumor-specific lectin jacalin, binds to Pc at a site independent of the carbohydrate active site and with affinities greater than that observed for porphyrins. Thus, the lectin has the potential to be a carrier for Pc for direct targeting of tumor tissue.

Another approach focused on the use of carbon nanotubes (CNTs) for the electronic detection of carbohydrate-protein interactions. It is based on the effect that a single walled carbon nanotube field-effect transistor (NTFET) device can electronically transduce interactions between proteins and other biomolecules for example carbohydrates. The NTFET were functionalized non-covalently with glycoporphyrins which were obtained using “click” azide-alkyne chemistry. The specific binding of two bacterial lectins PA-IL, PA-IIIL from Pseudomonas aeruginosa and a plant lectin Con A to three glycoporphyrins substituted with
β-D-galactose (657b), α-L-fucose (657d) and α-D-mannose (657b), respectively, were examined by changes in electrical conductance where the SWNT networks acted as conducting channels which transduced the binding between glycoporphyrins and lectins into electrical signals. Non-covalent functionalization of the devices with the glycoporphyrins prevented non-specific binding of the proteins with significant selective lectin carbohydrate binding. For example, the binding of PA-IL to galactosylated NTFETs was very sensitive (2 nM) with a measured dissociation constant \( K_d = 6.8 \mu M \) \[400\]. Further investigations utilized both SWNTs and chemically converted graphene (CCG) as nano based biosensors \[401\].

4.3 Catalysis

Metalloglycoporphyrins have been investigated in some detail as potential catalysts. While catalytic applications have no direct relevance for biomedical uses such studies do indicate the degree to which porphyrin-conjugates can be fine-tuned and modified for a specific purpose, such as substrate or receptor binding. The catalytic ability of metalloporphyrins is well known \[402-404\] and use of glycoporphyrins might result in either water soluble catalysis and/or enhance catalyst selectivity. Such studies can also be of relevance for understanding natural porphyrin based oxidations.

A typical example is the use of chloro[tetra(o-2,3,4,6-tetraacetyl-β-D-glucopyranosyl-1-O-phenyl)porphyrinato]iron(III) \[405\]. The acetylated sugar derivatives exhibited improved turn-over-numbers and better oxidation stability in the cyclohexane oxidation with PhIO compared to the nonglycosylated ones. Likewise, related chloro-manganese(III) derivatives were investigated for the catalytic asymmetric epoxidation of p-chlorostyrene \[406\]. The steric hindrance induced by the protected sugar units prohibited a close contact between two porphyrins and allowed for stereoselective oxidation of the alkene. While all compounds induced asymmetric epoxidation it was lower
than with reference compounds and the conversion to the epoxide decreased as the catalytic environment became asymmetric.

Detailed studies were performed with manganese and iron complexes of \( \text{O}-\)acetylated sugar TPP derivatives which provided stereoselective epoxidation of 4-chlorostyrene [160,406]. Initial catalysts were susceptible to \( \text{H}_2\text{O}_2 \) and thus (chloro)(5,10,15,20-tetrakis[2-chloro-6-(2,3,4,6-tetraacetyl-\( \text{O}\)-\( \beta\)-glucosyl)-phenyl]porphyrinato)manganese derivatives (661a-c) were synthesized (Scheme 42) [408]. Use of Lindsey conditions gave \( \text{660a} \) (\( \alpha\beta\alpha\beta \)), \( \text{660b} \) (\( \alpha\alpha\beta\beta \)) and \( \text{660c} \) (\( \alpha\alpha\alpha\beta \)) in 1, 3 and 5 % yields, respectively. Attempts at thermal atropisomerisation to form \( \alpha\alpha\alpha\beta \) atropisomer were unsuccessful in contrast to the previously synthesized \( \text{ortho} \) glycosylated compounds without the chloro group [161].

Conversion to the manganese complexes 661a-c was achieved via treatment with \( \text{MnCl}_2 \) and 4-nitrophenol. The glycosyl group at the \( \text{ortho} \) position provides the chiral environment while the strong electron withdrawing groups provide stability to the catalyst. Using PhIO in the reactions provided 4-chlorostyrene epoxide in yields similar to the ones with nonchlorinated glycoporphyrins tested previously. Catalytic oxidation of 4-chlorostyrene was examined by two methods using either 4-\( \text{tert} \)-butylpyridine as axial ligand and benzoic acid as cocatalyst or using 2-methylimidazole as an axial ligand. Both required addition of a base to enhance the activity [408,409]. The epoxidation of 4-chlorostyrene by \( \text{H}_2\text{O}_2 \) with 4-\( \text{tert} \)-butylpyridine gave the epoxide in 25-30 % yield, similar to the unchlorinated glycoporphyrins [182]. In contrast, epoxidation with \( \text{H}_2\text{O}_2 \) with 2-methylimidazole afforded very low yields 4-5 %. The presence of the chloro groups did however, reduce the rapid autooxidation observed by the unchlorinated glycoporphyrins [160,408]. Generally speaking, the glycosyl groups of such derivatives provide a chiral environment and steric hindrance on one or both faces of the porphyrin which influenced the complexes catalytic properties. The enantioselectivity of the epoxidation was moderate and was dependent on the position of the chiral sugar unit. The \( m \)-glycosylated derivatives (104-106) are further from the porphyrin metal center and do not produce significant ee’s. As the \( \text{ortho} \) substituted derivatives (102, 103) are closer to the active center of the porphyrin they afford higher enantioselectivity. The best result was obtained using \( \text{H}_2\text{O}_2 \) at low temperatures achieving enantiomeric excess of 20 % [182].

A significant body of work on catalysis used cyclodextrin derivatives. One of the first examples of regioselective and stereoselective oxidation of linoleic acid via cyclodextrin sandwiched thioporphyrin mimicked lipoxygenase activity provided the stepping stone for the following work [410,411]. As well as initial work on selective electron transfer between cyclodextrin sandwiched thioporphyrin and quinone [412].

Tetra- and dicycldextrin substituted Mn(III) porphyrins were synthesized to investigate their ability to bind substrates for selective catalytic oxidation [179]. The starting material 97 was synthesized from \( p \)-methylthiobenzaldehyde by oxidation to the sulfoxide and followed by condensation with pyrrole. Compound 97 underwent Pummerer rearrangement and decacylation to yield the tetrathiol which was coupled with 6-iodo-6-deoxycycloheptamylene to afford the tetra-cyclodextrin derivative 98. Compound 309 was synthesized from dipyrrylmethane and condensed with \( p \)-methylthiobenzaldehyde which was subjected to the same coupling conditions as 97. Scrambling of the reaction of 309 afforded some of the isomer 310. All of the free base porphyrins were subjected to binding studies with a variety of stilbene substrates and depending on the substrates a 2/1 or 1/1 ratio with 98 and a 1/1 ratio with 309 was found. Conversion to their Mn(III) complexes allowed for selective oxidation of the substrates in good conversions, especially for 99 and 311 [179].

Studies with these and related catalysts (e.g., 662-665, 100) or P450 mimics [413] focused on several different substrates. For example, catalyst 662 was used for studies on steroid hydroxylation [414] and comparative reactions with
stilbenes \[^{179,415}\]. Pioneering studies by Breslow indicated that catalyst \(662\) provided the best results for hydroxylation at C-9 of steroids with 90 turnovers \[^{416}\]. Later on, an improved catalyst (\(663\)) was synthesized which incorporated the pyridine ring within its structure and was found to provide a turnover of 2000. The precursor \(664\) performed the 6\(\alpha\)-hydroxylation of a steroid ligand with even better turnovers of 3000 \[^{417}\]. In the natural P-450 enzymes the iron porphyrin is axially coordinated by a cysteine thiolate sulfur and the oxygen adds to the sixth coordination position. Initially, imidazole had been incorporated as the fifth ligand in such P450 mimics \[^{180,414,415,418}\]. Inclusion of thiolates in the P450 mimic either covalently attached (see \(665\)) or via hydrogen bonding to the catalyst allowed the use of H\(_2\)O\(_2\) as oxidant \[^{419}\]. However, the turnovers were modest (15 with NaOCl, 10 with hydrogen peroxide), which may be due to the incorporation of only two perfluorinated phenyl rings. Also the thiol group itself is an oxidizable point in the catalyst, too.

Intriguingly porphyrin cycloextrin conjugates (\(313,314\)) were also used as synthetic receptors for \(\beta,\beta\)-carotene and for the regioselective catalytic cleavage of the C15-C15\(^{\prime}\) bond to afford retinol (vitamin A). The ruthenium derivative \(314\) was chosen to provide better cleavage capacity compared to zinc(II). The first step in the cleavage of the double bond is epoxide formation followed by tert-butyl hydroperoxide / ruthenium porphyrin mediated fragmentation. The ruthenium catalyst afforded cleavage at C15-C15\(^{\prime}\), C12\(^{-}\)-C11\(^{\prime}\), and at C10\(^{-}\)-C9\(^{\prime}\) in an overall yield of 30 \%. However, the selective cleavage of C15-C15\(^{\prime}\) was observed when one of the cyclohexene end groups of \(\beta,\beta\)-carotene was replaced by an ortho-dimethylphenyl group \[^{211}\].
Other catalytic investigations targeted the electrochemical oxidation of sugars for applications as fuel in fuel cells. This has now expanded into the use of Rh porphyrins on carbon black as a catalyst to promote such oxidation [420]. Certain types of Rh porphyrins were found to be good catalysts for oxidizing aldose in low-potential regions and exhibited higher activity for aldose than ketose derivatives. Also investigated for such application were cobalt phthalocyanines [420-424].

4.4 Other uses of glycoporphyrins

Carbohydrate appended porphyrins have also been used to construct biomimetic electron transfer (ET) systems. Examples are porphyrin-saccharide-anthraquinone systems, where a change in the sugar unit was found to affect the ET properties [425]. The porphyrin cyclodextrin conjugate 638d was used for photophysical and ET studies [426]. Resonance light scatter experiments in aqueous media showed that exciton coupling occurred between adjacent porphyrin units due to the formation of chiral assemblies. The cyclodextrins are of crucial importance for the overall structure and properties. For example, addition of cyclodextrin inclusion compounds (e.g., 1,4-benzoquinone) resulted in a quenching of fluorescence. This could be partially reversed through addition of 1-adamantancarboxylic acid, which is known to have a high affinity for the cyclodextrin cavity but has no significant effect on the fluorescence. Fluorescence monitoring of the displacement of quenchers allowed a determination of the oxidative and reductive electron transfer potential of benzoquinone and others [426].

Also reported was the immobilization of 638d on a solid surface of 3-aminopropylated silica particles and its interactions with several achiral aromatic compounds investigated. The influence of the achiral porphyrin spacer on the chiral recognition of binaphthyl derivatives by the β-cyclodextrin was investigated [427]. Other examples of applications include use as potential antioxidants [176] molecular receptors [428] or as imaging [429] and gelator materials [430,431].

Superoxide dismutase mimic

Mn-porphyrin bioconjugates were synthesized as antioxidants for selective targeting of ROS overproducing sites. They had been previously identified to mimic superoxide dismutase (SOD) but they lack the selectivity to target cells that overproduce ROS [432]. The maltose derivative 80 was synthesized via connecting a maltose derivative to the Mn-porphyrin via the Maillard reaction with little information on the selectivity at the anomic position of maltose in the paper. Compound 80 showed effective SOD activity due to the cationic pyridyl group and carbohydrate recognition with Con A, a lectin specific for α-D-glucosyl or α-D-mannosyl residues [176]. Likewise, a Mn porphyrin lactose conjugate was synthesized which could also facilitated carbohydrate receptor binding. For example, a triantennary carbohydrate which has three terminal β-galactose residues showed significant affinity for a hepatic lectin asialoglycoprotein receptor (ASGP-R) [433]. Introducing several β-galactose residues around the periphery of the SOD mimic could allow for recognition of the ASGP-R expressed on hepatocytes. Hence the Mn porphyrin lactose conjugate 81 was synthesized via the initial introduction of cationic pyridyl groups connected to primary amino groups which subsequently underwent reductive amination between the amino group of the porphyrin scaffold and the reducing end of the lactose residue followed by Mn insertion [434]. Compound 81 showed significant SOD activity despite the addition of the targeting ligands. It displayed no cytotoxicity at 100 μM concentration and showed cell recognition on a human hepatoma cell line (Hep G2) which expresses ASGP-R on the cell surface. In the presence of free lactose residues the recognition of the lactose Mn porphyrin derivative was decreased showing inhibition of the derivatives binding when in competition with lactose molecules [414].

5. Outlook

Porphyrin bioconjugates specifically carbohydrate conjugates have remained at the forefront of third generation PS research. It has been established that porphyrins and carbohydrates play a key role in nature. Both of which can serve several medicinal applications. Carbohydrate biological research still lags behind that of porphyrins in understanding their complex biological function. Only with advances in the knowledge of both the chemical and biological properties of such building blocks, a possible key glycoporphyrin derivative will emerge.

This review offers an extremely detailed look at the synthesis as well as the biological evaluation of such derivatives. The range of synthetic conjugations of these molecules follows that of the evolution of porphyrin and carbohydrate chemistry concluding that the post-functionalization of the carbohydrate unit on the porphyrin scaffold is the most advantageous method developed in recent times. Offering high yields with the stability of sensitive carbohydrate units remaining intact. The main synthetic focus has been that of mono- to tetrasubstitution at the meso positions with few examples of β-substitutions explored on the porphyrin periphery. One of the significant highlights of carbohydrate-lectin interaction is that of the cluster effect, while interesting comparisons of the effect the distance of meso di-substituted derivatives versus those glycodendrimer derivatives have on the binding potential to lectins is yet to be fully understood.

The emphasis of this review is that of PDT efficacy of glycoporphyrins, however for such conjugates to be efficient PDT agents their targeting ability and amphiphilic characteristics also needs to be examined in detail alongside any PDT applications. In summary, glycoporphyrins offer a range of tetrapyrrolic systems with quite varying degrees of
aqueous solubility. In general, those derivatives with the greatest water solubility can afford poor PDT activity while those with amphiphilic character offer more scope as PDT agents. For systemic PDT a water soluble formulation is the prime desirable development as well as improved selectivity. Glycoporphyrins have the potential for such criteria. Not only has the carbohydrate residue the potential for improved amphiphilic character of the desired porphyrin scaffold, the unit itself can pose as a potential targeting agent. At a cellular level specific transporters and proteins can be targeted. Little understanding of lectin carbohydrate interactions exist and more precisely those interactions of glycosyl units with protein receptors over-expressed on malignant tissue. Specifically such third generation PSs have excellent potential in the PDT field however, only with significant advances in the understanding of the oncogenic transformations of surface lectins and glycobiology can such a field advance to surpass those of the current PDT standards while eradicating the problems observed in 1st and 2nd generations. With PSs displaying ideal photophysical characteristics including high singlet oxygen production, absorption in the near infra-red and requirements for a good delivery agent are all essential.

No reported glycoporphyrin has excelled nor shown true potential for medicinal trials or such advances, however, these derivatives have an excellent potential in the field with some fine-tuning of biological procedures. As seen from the analysis of the biological literature in Table 3 on in vitro testing, no standard protocols have been outlined. It is difficult to draw comparisons from one glycoporphyrin to another due to variance in light influence, irradiation time and incubation. Derivatives prove considerably difficult to evaluate as some contradictions apply to several of the same glycoporphyrin conjugates due to diverse variance in glycoporphyrin biological protocol. Most are reported in comparison to their non-conjugated parent analogues which has very little stance in PDT efficacy even if they display higher PDT activity. A detailed protocol is essential for such a field with direct comparison to approved PDT agents similar to procedures carried out in our group. Each PDT agent in clinical use has their own protocols to afford the best results for said agent. It is noteworthy that very few examples have been examined with endogenous biomolecules e.g., albumin. Such interactions have a clear impact on the photophysical properties in physiological media for such glycoporphyrins and approved PSs and are an essential investigation.

One of the main highlights includes tolyporphin which is the gold standard for this field. Localization in the ER and nuclear membrane it affords excellent targeting and cell death 5000 times greater than Temoporfin through apoptotic cell death characteristic of most glycoporphyrins. Again this is an excellent example of the inconsistency of biological testing with no direct comparison to clinical conditions investigated as an excess of 1 h irradiation was required to achieve these results. A standard procedure similar to that outlined here for many chemical syntheses could afford a fitting protocol for such biological studies. There is significant scope in this area for research with the development of the correct protocols affording direct comparisons. Amphiphilic structures similar to that of tolyporphin could provide interesting results but with preparations less tedious as that of the total synthesis of such a complex molecule. It can be seen through the synthetic pathway that the nature, number and linkage positions of the glycosyl unit is extremely important affording amphiphilic character. Those issues highlighted if addressed by future research have the potential to afford an excellent third generation PS.

LIST OF ABBREVIATIONS
ALA - δ-aminolevulinic acid; BOP – Benzotriazol-1-yl-oxy)tris(dimethylamino)phosphoniumhexafluorophosphate; DMPC – dimyristoylphosphatidylcholine; DPBF – 1,3-diphenyldisbenzofuran; DCC – N,N'-dicyclohexylcarbodiimide; DCI-CID - desorption chemical ionization – collision-induced dissociation; DMAE – dimethylaminoethanol; EC50 - median effective concentration; EC90 - 90% effective concentration; EDC/HOBt - 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide/ Hydroxybenzotriazole; ELISA – enzyme linked immunosorbent assay; FCS – fetal calf serum; Fmoc – Fluorenylmethyloxycarbonyl; Glc – glucosyl; Glc – glucosyl; Hp – haematoporphyrin; HpD, haematoporphyrin derivative; HPPH – 3-Devinyl-3-(1'-galactosyl; Glc – glucosyl; Hp – haematoporphyrin; HpD, haematoporphyrin derivative; HPPH – 3-Devinyl-3-(1'-hexyloxyethyl) pyropheophorbide-a; HSA- human serum albumin; HSV - herpes simplex virus; MDR – multidrug resistant; NAS- nucleophilic aromatic substitution; PA - photon absorption; PC – Phycocyanin, PS – photosensitizer; PDT – Photodynamic therapy; PDD – Photodynamic detection; Pp IX – Protoporphyrin IX; PTT – 5,10,15,20-tetrakis(4-hydroxyphenyl)porphyrin; PPR – enhanced permeability and retention; ROS – reactive oxygen species; ER – endoplasmic reticulum; TPP – 5,10,15,20-tetraphenylporphyrin; TPPF20 – 5,10,15,20-tetra(2-naphthalenyl)porphyrin; DMF – N,N'-dimethylformamide; NMP – N-methylpyrrolidone; QCM-D – quartz crystal microbalance with dissipation; SAR – structure-activity relationship.

ACKNOWLEDGEMENTS
Our work was supported by grants from Science Foundation Ireland (SFI P.I. 09/IN.1/B2650, 12/TIDA/B2381, and IvP 13/1A/1894) and the School of Chemistry (TCD).

REFERENCES


Ferro, S.; Jori, G.; Sorinto, S.; Stancanelli, R.; Nikolov, P.; Tognon, G.; Ricchelli, F.; Mazzaglia, A. Inclusion of 5-[4-(1-


Sugasaki, A.; Ikeda, M.; Takeuchi, M.; Kouro, M.; Shinkai, S. The first example of positive allosterism in an aqueous saccharide-binding system designed on a Ce(IV) bis(bisporphyrin) double decker scaffold. Tetrahedron, 2000, 56, 4717-4723.


Casiraghi, G.; Cornia, M.; Rassu, G.; Del Sante, C.; Spanu, P. 1493.

51-59.


galactopyranos-6-yl)-phthalocyaninato]zinc(II): a water-soluble α

Ribeiro, A.O.; Tomé, J.P.C.; Neves, M.G.P.M.S.; Tomé, A.C.; Cavaleiro, J.A.S.; Serra, O.A.; Torres, T. First phthalocyanine-β-


Plant and human lectins.


Title of the Article


Received: March 20, 2014 Revised: April 16, 2014 Accepted: April 20, 2014