Published as:
Daly, R.; Vaz, G.; Davies, A. M.; Senge, M. O.; Scanlan, E. M. (2012): Synthesis and Biological Evaluation of a Library of Glycoporphyrin Compounds.
Chemistry – A European Journal **18**, 14671–14679.

CHEMISTRY
A EUROPEAN JOURNAL

FULL PAPER

DOI: 10.1002/chem.200((will be filled in by the editorial staff))

Synthesis and Biological Evaluation of a Library of Glycoporphyrin Compounds

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Abstract: A library of glycosylated porphyrins (Glycoporphyrins) was prepared and evaluated for their PDT activity against oesophageal squamous cell carcinoma cell line OE21 *in vitro*. Synthetic methodology was developed to allow incorporation of biologically active carbohydrates including the histo blood group antigen trisaccharide, *Lewis x* onto the porphyrin backbone. The effect of the carbohydrate group and substitution pattern on the PDT activity, cell uptake and subcellular localisation of the glycoporphyrin compounds is reported.

Keywords: Carbohydrates • Porphyrinoids • Photodynamic therapy • Cell Study • Microwave synthesis

Introduction

Carbohydrates are important biomolecules that play a fundamental role in many biological processes including fertilization, neuronal development, cell-cell communication, hormone activities and inflammatory responses.[1] Synthetic oligosaccharides and glycoconjugates offer fascinating applications for medicinal chemistry as anti-adhesion agents, drug delivery systems, imaging agents and vaccines.[2] Their potent biological activity is in part due to their ability to bind specific host lectins and to modulate host immune response.[3] Altered glycosylation is a universal feature of cancer cells and certain glycan structures are well known biomarkers for tumour progression.[4] As well as displaying altered glycosylation patterns, the surface of cancer cells are known to over express certain carbohydrate binding lectins.[5] Specific carbohydrate-lectin interactions allow for the direct targeting of glycosylated therapeutics specifically to cancer cells. The ability to target therapeutics to a specific cell type or organ is of significant clinical importance, in particular for cancer treatments such as Photo Dynamic Therapy (PDT),[6] where current clinically approved drugs such as Foscan® and Photofrin® are nonspecific and can result in destruction of healthy tissue.

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As part of our ongoing research program into the development of novel glycotherapeutics we are interested in the synthesis of tetrapyrrolic macrocycle cores displaying biologically active synthetic carbohydrates.[7] These compounds have an obvious application in cancer therapies such as photodynamic therapy (PDT) and due to their exquisite photophysical properties they may also function as useful tools for glycobiology. The synthesis of glycosylated tetrapyrroles has been pursued vigorously by a number of research groups over the past decade[8] and there is now a significant body of evidence in the literature supporting the hypothesis that glycosylation of tetrapyrrole core structures such as porphyrins,[9] phthalocyanines[10] and chlorins[11] can offer an improvement in the cell uptake and photocytotoxicity of these compounds over the nonfunctionalised systems. Variation of the carbohydrate moiety has been demonstrated to have a significant impact the biological behaviour of these compounds, both in terms of cell uptake[9-12] and subcellular localisation.[9b,12a,13] The observation that variation in biological behaviour can be observed between two different monosaccharide units, varying only in the orientation of a single hydroxyl group, clearly demonstrates that this is not simply a lipophylic effect resulting from the introduction of a hydrophilic carbohydrate group, but is the result of a biological distinction between two carbohydrate groups.[14] Recent lectin binding studies have demonstrated that these compounds can indeed interact with lectins and form strong binding interactions. [15]
The interaction of these compounds with cells is complex and despite several intensive studies, the exact mode of endocytosis remains unclear.[9-16] Both the tetrapyrrole and the carbohydrate units appear to play a role in endocytosis and several different uptake pathways have been reported to be involved.[9a, 10a, 14b, 17] Different types of cancer cells have been shown to display different behaviour towards glycosylated photosensitisers. [10, 11, 12, 16]
While there has been a significant synthetic focus on varying the tetrapyrrolyic core structure to improve the photophysics and consequently the photocytotoxicity of these compounds,[9-11] there has not been such an intense focus on variation of the carbohydrate moiety. Dendrimeric clusters have been employed to enhance lectin binding[15] but synthetic oligosaccharide conjugates have not yet been studied. Variation in the substitution pattern, the number of carbohydrate groups, the nature and length of the linker and the

protecting group pattern of the carbohydrates have all been reported to have an effect on the biological activity of these compounds.[9-12, 18] but the carbohydrates investigated to date remain largely limited to those that are commercially available. These include simple monosaccharides such as glucose, mannose and galactose and the disaccharide lactose which have been studied in depth. The introduction of more complex synthetic oligosaccharides to the tetrapyrrolic core offers the possibility of targeting very specific carbohydrate-lectin interactions that have not yet previously been investigated. For example, the *selectins* are a group of important cell adhesion glycoproteins that mediate leukocyte trafficking. Each selectin has affinity for the ligand NeuAcα2-3Galβ1-4(Fucα1-3)GlcNAcβ1-R, also known as the sialyl Lewis^x antigen or SLe^x. In addition, P- and L-selectin bind to some forms of the oligosaccharide heparin/heparin sulfate. [19] It has been reported that P-selectins are overexpressed on the surface of pancreatic cancer cells.[20] The galactose binding lectin Gal-9 has been reported to be overexpressed on breast cancer cells[21] and certain melanomas.[22] These lectins bind to defined complex oligosaccharides and therefore offer potential new targets for glycosylated PDT reagents. There is also a significant opportunity to target bacterial lectins involved in the early stages of infection[23] and to apply PDT to bacteria and other carbohydrate binding pathogens. There can be little doubt that the most effective PDT reagents will possess significant synthetic complexity in terms of both the tetrapyrrole core and the carbohydrate unit.

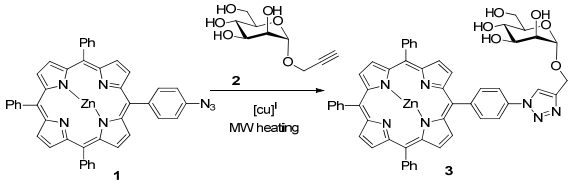
In our current study we present the synthesis of a library of glycosylated porphyrins (Glycoporphyrins) displaying monosaccharides and for the first time synthetic disaccharides and a synthetic trisaccharide. Both homogeneous and bis-modified systems are reported. The activity of these compounds against the human oesophageal squamous cell carcinoma cell line OE21 is also reported.

Results and Discussion

A number of methodologies for the functionalisation of porphyrins with carbohydrates have been investigated[8] including the use of Sonogashira[24] and olefin metathesis cross coupling. [25] The Cu(I)-catalysed 1,3-dipolar 'click' reaction has previously been applied to the functionalisation of porphyrins, but to date there are few literature examples describing a 'click' reaction between a carbohydrate and a porphyrin. Recently, Vicente and co-workers have employed a 'click' methodology for the preparation of glycoporphyrins displaying either one or four galactose or lactose moieties.[26] Hasegawa and co-workers have applied 'click' chemistry to the preparation of octa-glycosylated porphyrins.[27] In our previous synthetic studies of glycosylated porphyrins we reported how the Cu(I)-catalysed 1,3-dipolar 'click' reaction could be employed as a robust methodology to access highly defined, multifunctionalised glycoporphyrin compounds.[7a] The methodology was optimised for fully protected monosaccharides displaying a propargyl group at the anomeric position. A single example of conjugation using a fully deprotected mannosyl monosaccharide was also reported, albeit in lower yields.

Synthetic studies:

Herein we report the synthesis of a library of fully deprotected glycoporphyrin compounds displaying a wide range of carbohydrate groups. The number and relative orientation of the carbohydrates was varied and a bis-modified glycoporphyrin displaying two different types of carbohydrate is reported. A significant highlight of this study was the incorporation of a synthetic trisaccharide (Lewis x) onto a glycoporphyrin structure. We reasoned that the most efficient route to a synthetic library of glycoporphyrins was to carry out the ligation reaction between the azidoporphyrin and the fully deprotected propargylic carbohydrate at the final step. This strategy is favourable in that the final synthetic step is a high yielding ligation reaction. This approach is advantageous when ligating more complex, synthetically challenging carbohydrates. With this in mind we set about optimising the reaction conditions for the copper catalysed 'click' reaction between a fully deprotected propargyl mannose and an azido porphyrin.



Scheme 1. Reaction between azidoporphyrin **1** and propargyl mannose **2** to furnish the desired glycoporphyrin.

The ligation conditions previously developed for the protected carbohydrates allowed for efficient conjugation reactions to proceed in yields of >90%, however, it was determined that these yields diminished rapidly when applied to fully deprotected systems (Entry 1). Optimisation studies were carried out in order to improve the efficiency of the conjugation reaction between azidoporphyrins and the fully deprotected carbohydrates. The results of these optimisation studies using mannose as a model system are presented in Table 1.

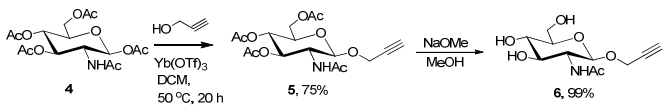
Table 1. Optimisation table for Cu(I) catalysed 'click' reaction.

Entry	Catalyst eq	Temp (^{o}C)[b]	Solvent	Yield (%)[e]
1	CuCl (0.3)	140	$PhCH_3$:MeOH[c]	5
2	$Cu(MeCN)_4PF_5$ (0.50)	140	$PhCH_3$:MeOH[c]	60
3	$Cu(MeCN)_4PF_5$ (0.10)	120	$PhCH_3$:MeOH[c]	87
4	$Cu(MeCN)_4PF_5$ (0.10)[a]	100	$PhCH_3$:MeOH[c]	61
5	$Cu(MeCN)_4PF_5$ (0.15)[a]	110	$PhCH_3$:MeOH[c]	93
6	$Cu(MeCN)_4PF_5$ (0.15)[a]	110	$PhCH_3$:MeOH[c]	93
7	$Cu(MeCN)_4PF_5$ (0.15)	110	$PhCH_3$:MeOH[c]	90
8	$Cu(MeCN)_4PF_5$ (0.15)	115	$PhCH_3$:MeOH[c]	91
9	$Cu(MeCN)_4PF_5$ (0.10)	110	$PhCH_3$:MeOH[c]	91
10	$Cu(MeCN)_4PF_5$ (0.10)	110	$PhCH_3$:MeOH[d]	90

[a] THPTA ligand included [b] Temperatures were obtained under microwave heating for 20 min [c] Solvent ratio (3:1) [d] Solvent ratio (3:5) [e] Isolated yield.

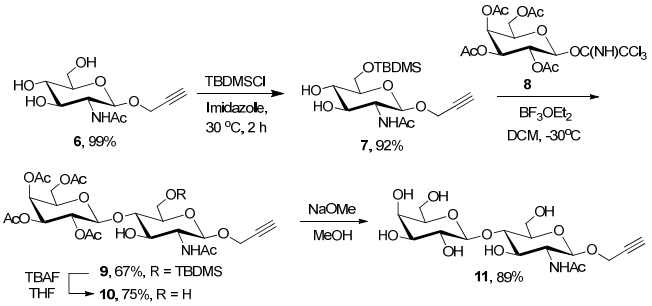
Our previous microwave 'click' conditions required toluene/H_2O as a solvent system. The water solubility of deprotected sugars requires a monophasic system for porphyrins and carbohydrates to react efficiently. Thus, the solvent system was changed to toluene/MeOH a homogeneous system that would solubilise both carbohydrate and porphyrin. CuCl, a copper catalyst we previously found to be the most efficient provided the required glycoporphyrin in a low yield under these conditions. $Cu(MeCN)_4PF_5$ which is soluble in polar organic solvents was investigated as a source of Cu(I). The required glycoporphyrin was isolated, however, we found that at temperatures above 115 ^{o}C, Cu(II) insertion into the tetrapyrrole ring became a competing reaction, with up to 30 % isolated product (Entry 2). A ligand, THPTA, was added as a Cu(I) stabilising ligand to avoid formation of this side product.[28] Whilst this ligand was initially successful in improving yields, further experimentation

proved that it was not required below 120 °C. We also investigated the tolerance of our system to solvent polarity, with a view towards the synthesis of highly polar glycoporphyrins. As can be seen in Entry 10, increased ratios of MeOH are well tolerated. Finally, we were able to reduce the catalyst loading to a more efficient 10 mol%. These improved conditions allow lower equivalents of carbohydrate, lower temperatures and reduced catalyst loadings to be employed with both deprotected and protected carbohydrates (see supporting info). The reaction conditions were found to be extremely efficient and yields of up to 93% were obtained. The optimised copper catalysed 'click' reaction conditions provided a general synthetic strategy for ligation of the deprotected carbohydrate units with azidoporphyrins. In order to fully exploit this methodology and to access a library of glycoporphyrins for biological screening a number of biologically important carbohydrate systems suitable for 'click' reactions were prepared. Propargyl glucose and propargyl mannose were prepared according to literature procedures[7a] and the desired stereochemistry at the anomeric position was readily achieved using neighbouring group participation. The synthesis of the N-Ac glucosamine containing derivatives was more challenging. The use of lewis acids such $SnCl_4$ and BF_3OEt_2 resulted in diminished yields and the formation of significant quantities of byproducts, however, employing ytterbium triflate[29] as a lewis acid furnished the desired product in good yield (75%). Zemplen deprotection furnished the fully deprotected product **6** in quantitative yield. Scheme 2



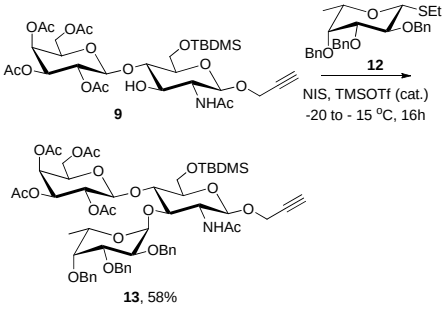
Scheme 2. Synthesis of *O*-propargyl glucosamine **6**.

The *N*-Acetylated glucosamine compound **6** was further modified in order to convert it into acetylated lactosamine **11**, an amido derivative of lactose that appears in many biologically important glycoconjugates. A regioselective glycosylation reaction between a galactose trichloroacetimidate donor **8** and the glucosamine acceptor **7** was employed to give the desired disaccharide. The trichloroacetimidate (TCA) donor **8** was prepared from the corresponding hemiacetal using an excess of trichloroacetonitrile in the presence of catalytic DBU. The acceptor was prepared in a single step from propargyl glucosamine **6**. Selective protection of the primary hydroxyl group was achieved through slow addition of TBDMS-Cl in excess imidazole. Regioselective glycosylation of the OH-4 over OH-3 in TBDMS protected GlcNAc has previously been reported in the literature.[30] A modification of the reported literature procudeure furnished the desired disaccharide product **9** in an improved yield of 67 %. The beta glycoside linkage was confirmed by detailed NMR studies. Subsequent deprotection of the disaccharide over two steps led to formation of compounds **10** and **11**. The overall synthesis is outlined in Scheme 3.



Scheme 3. Synthesis of *O*-propargyl lactosamine **11**.

In order to fully investigate the synthetic scope of the carbohydrate-porphyrin ligation methodology and to demonstrate that it could be applied to a synthetic oligosaccharide, we further modified disaccharide **9** to the histo blood group antigen trisaccharide, Lewis x.[31] The disaccharide was glycosylated with a benzyl protected fucosyl thioglycoside donor **12** and the Lewis X trisaccharide was isolated in a yield of 58%. Scheme 4. 2 equivalents of donor **12** were required in order to ensure complete consumption of the unreactive acceptor **9**. In an attempt to lower the amount of donor required, a highly reactive and unstable fucosyl TCA donor was employed. This however did not improve the yields (see supporting info)



Scheme 4. Synthesis of Lewis x trisaccharide **13**.

With the propargyl sugars in hand, synthetic efforts focused on the synthesis of a glycoporphyrin library to include mono-, di- and trisubstituted systems. A major goal of this research was to investigate how variation in the nature and distribution of carbohydrates on the porphyrin surface would affect their biological properties. Azidoporphyrins were prepared according to previously described literature procedures.[7a] Figure 1 shows the monosubstituted glycoporphyrin compounds that were prepared. Yields correspond to the isolated yield of the copper catalysed 'click' reaction between the carbohydrate and the porphyrin.

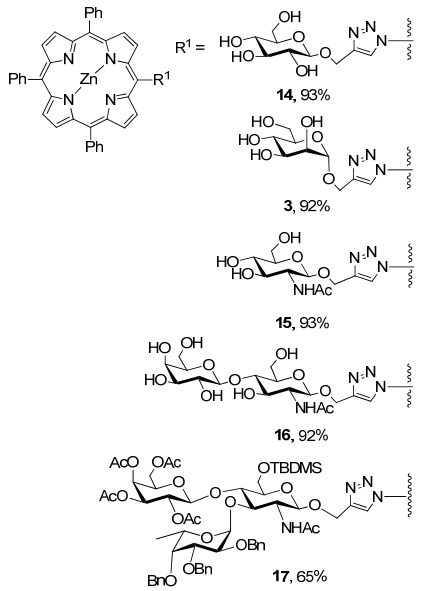


Figure 1. Monofunctionalised glycoporphyrin compounds.

The click reactions for the monosubstituted porphyrins proceeded smoothly and in high yield. The fact that the glycoporphyrins are easily visible on the chromatography column facilitated the rapid and simple purification of the glycoconjugates displaying fully deprotected sugar units. Gratifyingly, both the synthetic disaccharide **11** and synthetic trisaccharide **13** were both tolerant of the MW heating conditions. The trisaccharide was ligated to the azidoporphyrin in its protected form. Studies are currently ongoing to introduce fully deprotected oligosaccharides onto the porphyrin core structure. The 'click' reaction proceeded smoothly to furnish the glycoporphyrin **17** in good yield (65%) thereby demonstrating the broad scope of this method for glycoporphyrin synthesis. The incorporation of a biologically relevant trisaccharide onto a glycoporphyrin represents the most complex example of a synthetic glycoporphyrin reported to date.

For the disustituted azidoporphyrin compounds both the 5,10- and 5,15-disubstituted systems were prepared.[32] Both substitution patterns were prepared in order to investigate if varying the substitution pattern of the carbohydrate group would affect the biological properties of the glycoporphyrin. The disubstituted compounds are illustrated in Figure 2.

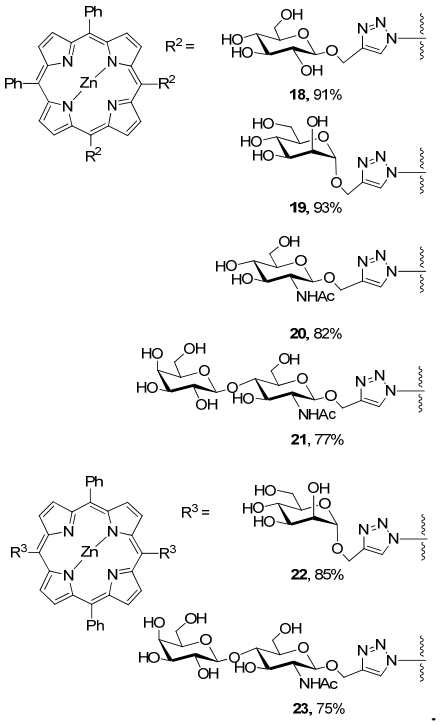


Figure 2. Disubstituted glycoporphyrin compounds.

A number of trisubstituted examples were also prepared. It has previously been demonstrated that trisubstituted glycoporphyrins are efficiently uptaken by cancer cells[12a] and we were interested in investigating how variation in the carbohydrate group would modulate cellular uptake on the OE21 cell line. The ligation reactions proceeded in good yield and demonstrated that the click reaction could be used to readily access highly substituted glycoporphyrins. The slightly lower yield observed for the trilactosamine derivative **27** was due to difficulty in purification of this highly polar molecule. These compounds are illustrated in Figure 3

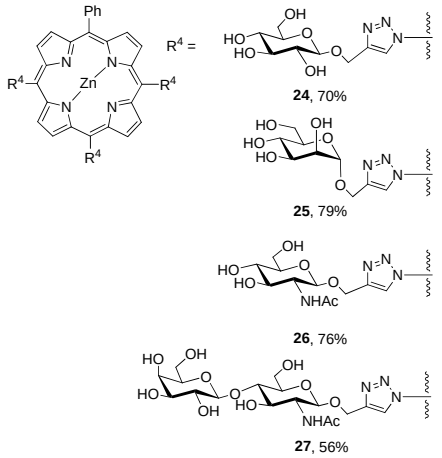
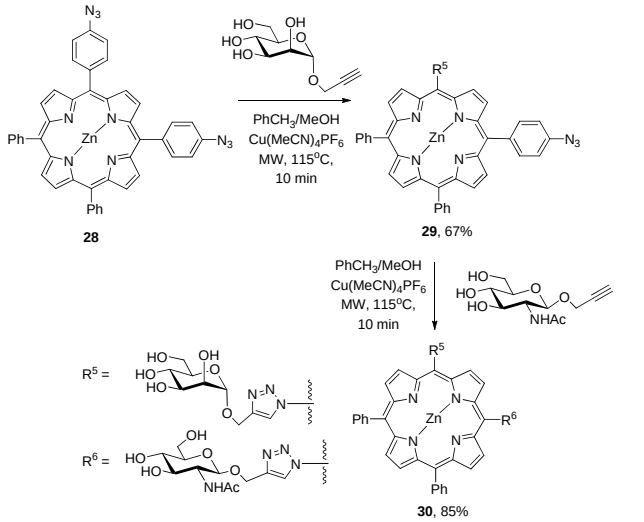


Figure 3. Trifunctionalised glycoporphyrin compounds

In the preliminary communication we described a novel, sequential 'double click' strategy for preparing mixed glycoporphyrin systems. A single example of a bis-modified glycoporphyrin displaying a protected glucose and a protected mannose group was reported. The methodology required a significant excess of porphyrin for the first step and the yield of the second step was relatively low. In our current study we reinvestigated this reaction using our modified

procedure to allow incorporation of two different deprotected sugar units. Glycoconjugates in nature are often heterogeneous and contain different types of carbohydrates so we were interested in preparing heterogeneous glycoporphyrins to see how this would modulate behaviour in a biological system. It may also be possible to target different cellular uptake mechanisms with the same molecule. The synthesis of the bis-modified glycoporphyrin is outlined in Scheme 4.



Scheme 4. Synthesis of heterogeneous difunctionalised glycoporphyrin using two step , sequential 'double click' reaction.

Three equivalents of 5,10 diazido porphyrin were used to yield the monofunctionalised intermediate **29**. 1.7 equivalents of starting material porphyrin and 25 % of difunctionalised material were also recovered. The intermediate can be isolated, purified and further reacted to give the bis-modified compound **30**, in 85 % yield. Thus we completed the synthesis of the glycoporphyrin library containing in all 17 new glycoporphyrins display a range of biologically relevant carbohydrates. These compounds were then tested for their biological uptake in cell studies. Results of this study are reported in the following section.

Biological Studies: In order to assess the potential of the synthetic glycoporphyrins for medical applications initial *in vitro* studies were performed. Based on our current interest in PDT for oesophageal cancer we choose the human oesophageal squamous cell carcinoma cell line OE21 for *in vitro* studies.[33] As a PDT reference compound the well established second generation photosensitiser Foscan® (Temoporfin) [5,10,15,20-tetrakis(3-hydroxyphenyl)chlorin] was used.[34] As a representative sample of the present library of compounds the glycoporphyrins **18**-**27**,**30** were used for standard MTT and HCA analysis. The monoglycoylated porphyrins were found to be too insoluble for *in vitro* study.

The MTT assay showed a decrease in cell viability upon plate illumination with increasing Foscan® concentrations (Figure S1) indicating the validity of the high content assay (HCA) system for PDT. However, no such significant decrease was obtained for cells treated with any of the glycoporphyrins tested at any of the concentrations tested. Results obtained from a two way ANOVA statistical analysis confirmed that there is no difference between illuminated and dark cultures when treated with any of the eleven glycoporphyrins tested (see Table S1). As expected there was a significant difference in toxicity in cells treated with Foscan, where proliferation significantly decreased when the cultures were illuminated (Figure S1). Thus, these glycoporphyrins do not show significant toxicity in the chosen cell line under the experimental conditions used. The photolysis time of 2 mins was chosen as a clinically relevant timeframe. Based on the photophysical differences we expected less PDT activity for the glycoporphyrins compared to Foscan, however, the absence of any activity is surprising. Note, that other sugar porphyrin bioconjugates have shown PDT activity in other cell lines albeit with longer photolysis times, up to 120 minutes in some cases.[9-17]

This raised the question whether the lack of toxicity was due to the fact that the compounds were being taken up by the cells and then rapidly expelled or if the dyes are not taken up at all. Thus, uptake studies were performed. Of the compounds studied Foscan and six glycoporphyrins **18**, **19**, **20**, **21**, **22** and **30** were taken up by OE21 cells. Staining results for these compounds are shown in Figure 4. It is clear from the images that these glycoporphyrin occupy a finite space within the cytoplasm of the cell. Furthermore, there is evidence that the intracellular fluorescence pattern of these compounds (Figure 4) is very similar to that exhibited by fluorescence probes targeted to the golgi apparatus.[35] From this, it is possible to infer that the glycoporphyrins accumulate, most likely, in this organelle. This will be investigated further in ongoing studies.

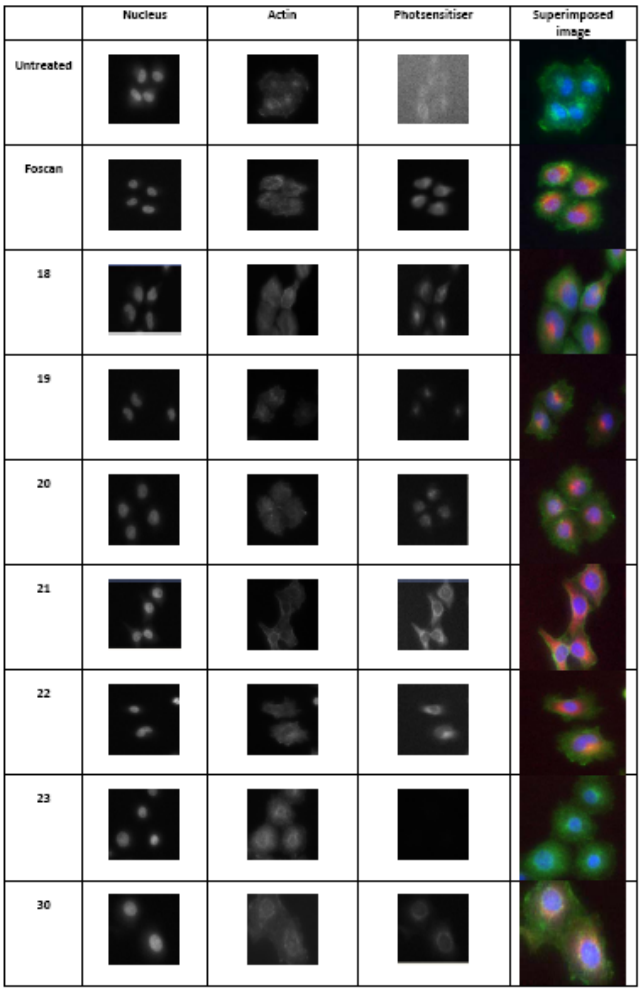


Figure 4. Examples of images collected with the InCell imaging system for the uptake of glycoporphyrins in OE21 cells after 24 h. The images represents the Hoechst stained nucleus, phalloidin stained F-actin and photosensitisers.

Uptake of porphyrin dyes in cells can easily be established through their inherent fluorescence. Only six out of the eleven porphyrins evaluated showed any intracellular fluorescence and this

was confirmed by the images collected with a InCell 1000 system (see right panel in Figure 4). Foscan showed the strongest intracellular fluorescence intensity after 4 hours of incubation and the intensity increased exponentially up to 24 hours. The glycoporphyrins exhibited a lower intracellular intensity at all time points (see supplementary material). Clearly, the uptake of these compounds by the cells is much slower. Nevertheless, a structure-uptake comparison of the compounds tested reveals some interesting differences. Three different types of glycoporphyrins were investigated: 5,10-disubstituted, 5,15-disubstituted, and 5,10,15-trisubstituted. Of these a larger number of the 5,10-disubstituted showed uptake. The results suggest a relationship between structure of the glycoporphyrins and cellular uptake, an important structural design criterion for the development of new photosensitisers. More extensive biological tests with these and related compounds and other cell lines must now be performed and we will report on these in due course. For the OE21 cells the absence of phototoxic effects indicates the potential of the 5,10-disubstituted glycoporphyrins as possible imaging dyes that could be used under normal day light conditions without adverse effects.

Conclusion

We have investigated the Cu(I)-catalysed 1,3-dipolar 'click' methodology as an efficient method for the synthesis of a library of glycosylated porphyrin compounds. Conditions were optimised to allow efficient ligation of both fully deprotected and protected carbohydrates with the porphyrin. For the first time, synthetic carbohydrates including N-Ac lactosamine and the histo blood group antigen trisaccharide, Lewis x, were coupled to a porphyrin. The methodology allowed for the synthesis of a range of compounds of varying synthetic complexity displaying both variation in the carbohydrate unit and in the presentation pattern. The compounds were screened for their PDT activity against human oesophageal cancer cell lines. Variation in the carbohydrate group appeared to modulate the biological behaviour of the glycoporphyrins. Further studies will target different malignant cell lines and changing the tetrapyrrole core to a chlorin to potentially improve these compound's photosensitising properties.[36]

Experimental Section

Syntheis: Full experimental details and characterisation data for all reported compounds can be found in the Supplementary information.

Cell cultures: The human oesophageal squamous cell carcinoma cell line OE21 was purchased from the Health Protection Agency (HPA), these cells were routinely cultured according to suppliers instructions. For photodynamic treatment and uptake studies 3×10^3 cells per well were plated in 96-well plates (Nunc, Denmark) and treated with either 3 μM, 2 μM, 1 μM, or 0.5 μM of the glycoporphyrin solutions or Foscan® for toxicity studies. For uptake studies the cells were incubated with 5 μM of the compounds. Plates were then incubated in the dark for up to 24 hours at 37 ºC and 5% CO_2. Control cells were treated with vehicle alone and Foscan® was used as positive control. The dye solutions were prepared by dissolving in ethanol:propylene glycol = 3:2 (v/v).

Illumination protocol: Following the removal of medium with compound and addition of fresh, pre-heated, supplemented RPMI supplemented with foetal bovine serum (Hyclone, USA), 1 % penicillin/streptomycin (Hyclone, USA) solution, the incubated plates were separated into two groups. Group 1 was illuminated for two minutes using an illumination box, consisting of Luxeon High Power LEDs (LXHL-BW03) as light sources, emitting white light with a total fluence rate of 1.7 $mW.cm^{-2}$.[37] Group 2 was kept in the dark under the same conditions as group 1. The cells were allowed to recover for four hours in the dark at 37 °C and 5% CO_2 in a humidified atmosphere.

Cell proliferation assay: Two plates (one illuminated and one non-illuminated) were used for a 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay (Promega, USA), according to manufacturer's instructions.[38] Immediately following illumination 15 μL of MTT dye was added to each well and kept for four hours at 37 °C and 5% CO_2 in a humidified atmosphere in the dark. The formatazan crystals were allowed to dissolve overnight at room temperature and absorbance was measured at 540 nm with a Wallac Victor2 plate reader (Perkin Elmer, Singapore).

High content imaging and analysis for uptake studies: Following 0, 2, 4, 8, 12 and 24 hours incubation plates were fixed with 4 % paraformaldehyde solution and further stained with FITC-labeled phalloidin (Dyomics, Germany), an F-actin stain, and Hoechst (Invitrogen, USA), a nuclear stain. Images were collected using the InCell 1000 high content system (GE Healthcare, USA). A total of ten fields per well were imaged under 10× magnification using three separate filters to capture the nucleus (blue, excitation 345 nm, emission 435 nm), F-actin (green, excitation 475 nm, emission, 535 nm) and photosensitiser (red, excitation 535 nm, emission 620 nm). Image analysis was performed using the InCell 1000 image analyser using a dual area object analysis algorithm for the determination of intracellular photosensitiser intensity (average fluorescence intensity of pixels within the cytoplasmic region of the red PS). The image analysis software detects cells nuclear area/cell number by nuclear dye uptake with quantifications of cell morphologies and PS parameters determined by F-actin stain and Foscan®. Stain parameters such as morphology and intensity of fluorescence were logged numerically for individual cells in every field as well as averages for each field and well.[39] Each experiment was repeated a minimum of three times and results were normalised to untreated controls. Averages and standard error of the mean (SEM) of each well were plotted using Graphpad Prism version 5.0 (GraphPad Software, USA). Differences in the cell proliferation data were evaluated by 2-way ANOVA with Bonferroni comparison using Graphpad Prism version 5.0.

Acknowledgements ((optional))

This work was supported by grants from the School of Chemistry (TCD), the Health Research Board (translational research award 2007 TRA/2007/11), Science Foundation Ireland (SFI P.I. 09/IN.1/B2650) Fundação da Ciência e Tecnologia (SFRH / BD / 62364 / 2009) and IRCSET.

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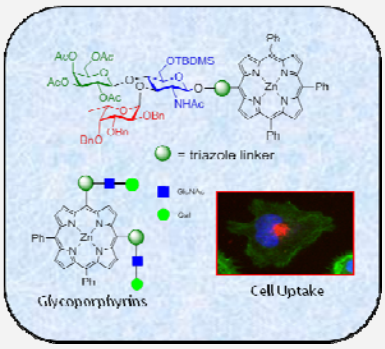
Received: ((will be filled in by the editorial staff))
Revised: ((will be filled in by the editorial staff))
Published online: ((will be filled in by the editorial staff))

All Sweetness and Light

Robin Daly, Gisela Vaz, Anthony M. Davies, Mathias O. Senge and Eoin M. Scanlan*

Synthesis and Biological Evaluation of a Library of Glycoporphyrin Compounds



A library of mono-, di- and trisubstituted glycoconjugated porphyrin compounds was prepared. A number of biologically active synthetic carbohydrates were conjugated to the porphyrin core. Cellular uptake, photoactivity and subcellular localisation have been examined in human oesophageal cell carcinoma cell line OE21. The nature of the carbohydrate group and the substitution pattern was found to modulate biological behaviour *in vitro*.