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Thioglycosylated cationic porphyrins—convenient synthesis and photodynamic activity in vitro

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Abstract—A convenient and flexible synthesis of *meso*-tetraaryl porphyrins substituted with three thioglycosyl units, and also bearing one pyridyl substituent is reported. Quaternisation of the pyridine nitrogen with alkyl iodides gives access to a range of water soluble glycosyl cationic porphyrins. Screening for photodynamic activity against human colorectal adenocarcinoma cells (HT-29) indicates that all the glycosyl cationic porphyrins made in this way are active photosensitisers, but direct comparison with a cationic porphyrin bearing no sugar residues indicates an important role for these groups in reducing generalised 'dark' toxicity.

Photodynamic therapy (PDT) is a treatment for the eradication of unwanted tissue that utilises a combination of photosensitiser, visible or near infrared light and oxygen to induce necrosis and/or apoptosis in the target tissue.[1–3] The vast majority of photodynamic sensitisers are porphyrin-based compounds, the macrocyclic core providing the required photoactivity, while peripheral substituents are used to control biodistribution and pharmacokinetics.[4] It is desirable, for ease of systemic administration that the porphyrin has sufficient water solubility, and it has also been shown that those bearing one positive charge tend to localise around the mitochondria of the cell.[5] Photodynamic damage to the mitochondria has been implicated in rapid induction of apoptosis, and this subcellular site is therefore a major target for PDT. Cationic water-soluble porphyrins, such as the commercially available 5,10,15,20-tetrakis(*N*-methyl-4-pyridyl)porphine and 5,10,15,20-tetrakis[4-(trimethylammonio)phenyl]porphine are powerful photosensitisers; however they have also demonstrated significant toxicity in the absence of light, often referred to as 'dark' toxicity. Recently, interest has been shown in porphyrins bearing both sugar residues and positively charged groups as PDT sensitisers, this combination of functionalities allows water solubility, but also greater membrane permeability, compared with porphyrins solubilised solely by multiple charged groups.[6,7] Previously, glycosyl cationic porphyrins have been synthesised by condensing mixtures of sugar substituted benzaldehydes with 4-pyridinecarboxaldehyde and pyrrole in propionic acid at reflux, followed by chromatographic purification of the required porphyrins from the resulting statistical mixture.[6] Subsequent quaternisation of the purified products gave the required compounds. We now wish to report a synthetic method, which allows introduction of the sugar residues after porphyrin formation, and under exceptionally mild conditions. We have used this method to generate a small series of 5-(*N*-alkyl-4-pyridyl)-10,15,20-tris(4-thioglycosyl-2,3,5,6-tetrafluorophenyl)porphines and explore structure–photodynamic activity relationships for this new class of PDT agent against human colorectal adenocarcinoma cells (HT-29).

In order to allow attachment of the glycosyl residues to the preformed porphyrin we chose to exploit a reaction previously developed in our laboratory for substituting the *para*-fluorine from *meso*-pentafluorophenyl porphyrins using sulfur nucleophiles;[8,9] application of this methodology for attachment of sugars to porphyrins has also been reported.[10] The synthetic route thus required the synthesis of the key intermediate 5-(4-pyridyl)-10,15,20-tris(pentafluorophenyl)porphyrin **1**. Using Adler–Longo conditions it was found that **1** could easily be synthesised on a gram scale from 4-pyridinecarboxaldehyde, pentafluorobenzaldehyde and pyrrole. Stirring **1** with 2,3,4,6-tetra-*O*-acetyl-β-D-thioglucopyranose in DMF at room temperature for 16 h followed by

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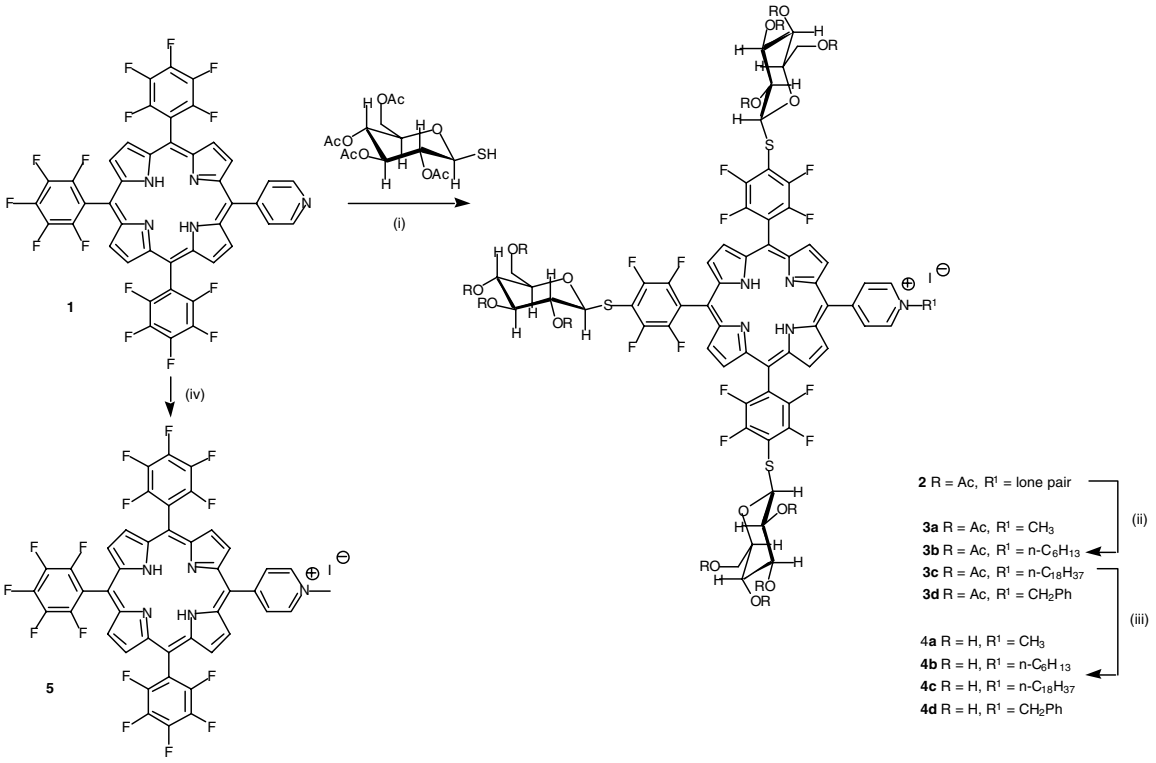

Scheme 1. Reagents and conditions: (i) DMF, rt, 16 h; (ii) R^1I, DMF, rt, 16 h; (iii) NaOMe, MeOH, rt, 1 h; (iv) MeI, rt, 16 h.

chromatographic purification resulted in 5-(4-pyridyl)-10,15,20-tris[4-(2′,3′,4′,6′-tetra-*O*-acetyl-β-D-glucopyranosylthio)-2,3,5,6-tetrafluorophenyl)]porphine **2** in 72% yield (Scheme 1). Treatment of **2** with a small range of alkyl iodides gave the cationic 5-(*N*-alkyl-4-pyridyl) analogues of **2**, as their iodide salts, bearing methyl **3a**, *n*-hexyl **3b**, *n*-octadecyl **3c** and benzyl **3d** groups on the pyridine nitrogen in yields ranging from 39% to 71% (Scheme 1). Finally, the sugar residues were deprotected by stirring with sodium methoxide in methanol at room temperature for 1 h, to give 5-(*N*-alkyl-4-pyridyl)-10,15,20-tris[4-(β-D-glucopyranosylthio)-2,3,5,6-tetrafluorophenyl)]porphines **4a**–**d** (Scheme 1). All compounds were shown to be single compounds by TLC and were characterised by ^{1}H NMR, MALDI-MS and UV–vis spectroscopy.

Compounds **4a**–**d** were then investigated for their ability to photodynamically inactivate human colorectal adenocarcinoma cells. Briefly, this consisted of incubating HT-29 cells with **4a**–**d** for 1 h followed by washing to remove photosensitiser, which had failed to associate with the cells. Cells were then plated in 96 well plates in quadruplicate and irradiated with cooled filtered red light (630 nm; 3.6 J cm^{-2}). After irradiation, 5 μL FBS was added to each well and the cells were incubated overnight. Finally, cell survival was determined at 24 h post-irradiation by MTT assay.[11]

Comparison of concentrations required to kill 90% of cells (LD_{90}; SD < 0.05) for the four glycosyl cationic porphyrins indicated that all compounds were active to this level in the micromolar range with **4c** the most active ($LD_{90} = 25$ μM) and **4d** the least active ($LD_{90} = 50$ μM), **4a** and **4b** exhibited intermediate activity of 35 and 45 μM, respectively. All compounds showed negligible toxicity to cells in the absence of light at the highest concentration used (50 μM).

In order to investigate the effect of the cationic porphyrin in the absence of sugar residues 5-(*N*-methyl-4-pyridyl)-tris(pentafluorophenyl)porphine iodide salt **5** was synthesised and its photodynamic potential was also determined. Interestingly, **5** was more active than any of the glycosyl cationic porphyrins with an LD_{90} value of 5 μM, however in this case significant 'dark' toxicity was encountered, with 80% of cells killed in the absence of light at 50 μM.

In conclusion, we have demonstrated a flexible, and mild, synthetic method for accessing fully water-soluble porphyrins bearing three glycosyl units and one positive charge, which are potent photodynamic sensitisers in vitro. The presence of the glycosyl substituents play an important role in moderation of the 'dark toxicity' associated with many cationic PDT agents.

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