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Synthesis and Biological Evaluation of Thioglycosylated Porphyrins for an Application in Photodynamic Therapy

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Abstract—The aim of this work is the synthesis of a new family of glycosylated porphyrins in which the sugar moieties are linked to the tetrapyrrole ring by a thioglycosidic bond. Two series have been designed. The first one corresponds to *meso*-aryl porphyrin derivatives. The second one has been obtained from protoporphyrin IX derivatization. Aryl-porphyrins were prepared from tristolyl *o*- and *p*-hydroxyporphyrins followed by bromoallylation and thioglycosylation with *per*acetylated S-glucose, mannose and galactose and deprotection. The other series has been synthesized from protoporphyrin IX dimethylester with a regioselective glycosylation of terminal alkenyl carbon. The UV-visible, NMR and MALDI mass spectra are presented. Photocytotoxicities of the synthesized compounds against K562 chronic leukaemia cell line has been evaluated.

Introduction

Photodynamic therapy (PDT) is a type of cancer therapy in which a photosensitizer localizes selectively in tumor tissues and elicites a photodynamic effect upon action of penetrating light. Tumor necrosis is initiated presumably through formation of singlet oxygen. Derived from hemoglobin, Photofrin® (Pf) is currently the most widely used photosensitizer in PDT. It has received approval for clinical use in a few countries (Canada, Japan, USA) for the treatment of several cancers. However, this product is a complex mixture containing various monomeric porphyrins along with dimers and higher oligomers responsible for tumor localization and photosensitization. Thus, little is known about its mode of action. In addition, Pf has demonstrated important drawbacks. Because of its low selectivity it enters almost every tissue and its slow clearance leads to a long lasting photosensitivity. As a result, new photosensitizers having well-defined structure and exhibiting both higher selectivity for neoplasic cells as well as fast elimination from healthy tissues and strong light absorption in the red region of the visible spectrum are desired.

Although the exact mechanism of sensitizer uptake by tumor cells is still unknown, there is evidence that, owing to their hydrophobic or amphiphilic character, injected porphyrins are carried by plasma lipoproteins and more precisely by low-density lipoproteins (LDL). LDL easily enter tumor cells through receptor-mediated endocytosis since cancer cells contain high levels of LDL receptors.[1,2] Furthermore, a specific affinity of several carbohydrates for cancer cells has been reported.[3] Therefore, many amphiphilic porphyrins linked to sugar moieties have been synthesized and notably porphyrins bearing one or two carbohydrate substituents gave promising results.[4] Finally, such conjugates increase the water solubility of the parent porphyrin which contribute to their elimination from the organism after treatment.

As part of our research program on the synthesis of such compounds,[5] we became interested in the preparation and biological evaluation of thioglycosylated porphyrins obtained from synthetic or natural precursors. In comparison with *O*-glycosylated porphyrins, [6] *S*-glycosyl bonds should resist endogenous hydrolysis catalysed by glycosidases.[7] In the present paper, we report the synthesis of two series

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of neutral thioglycosylated porphyrins, the first one derived from *meso*-monohydroxyphenylporphyrin (**9a,b**–**11a,b**), the second from Protoporphyrin IX (**18**–**23**) and an evaluation of their photocytotoxic activity compared to Pf.

Results and Discussion

Synthesis

The *meso*-monothioglycosylated porphyrins (Fig. 1) were prepared according to the general route shown in

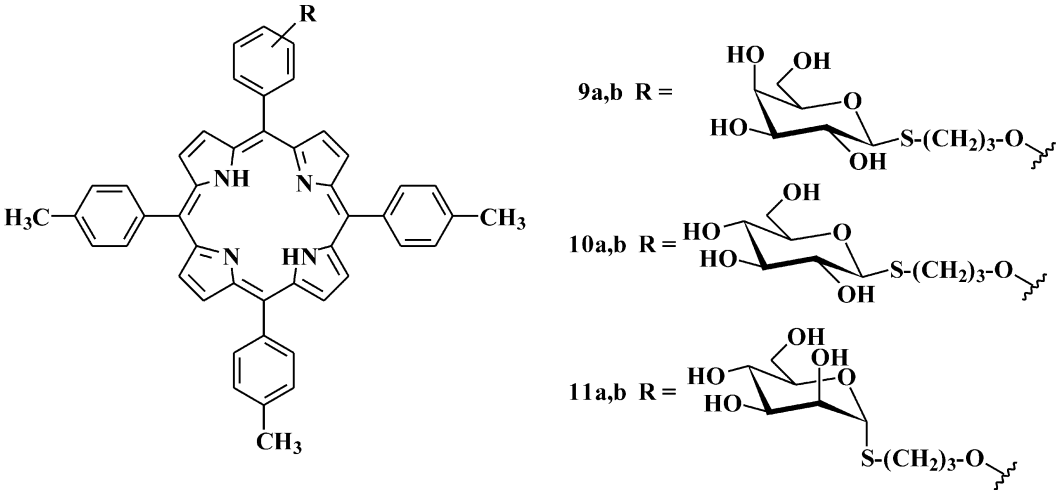
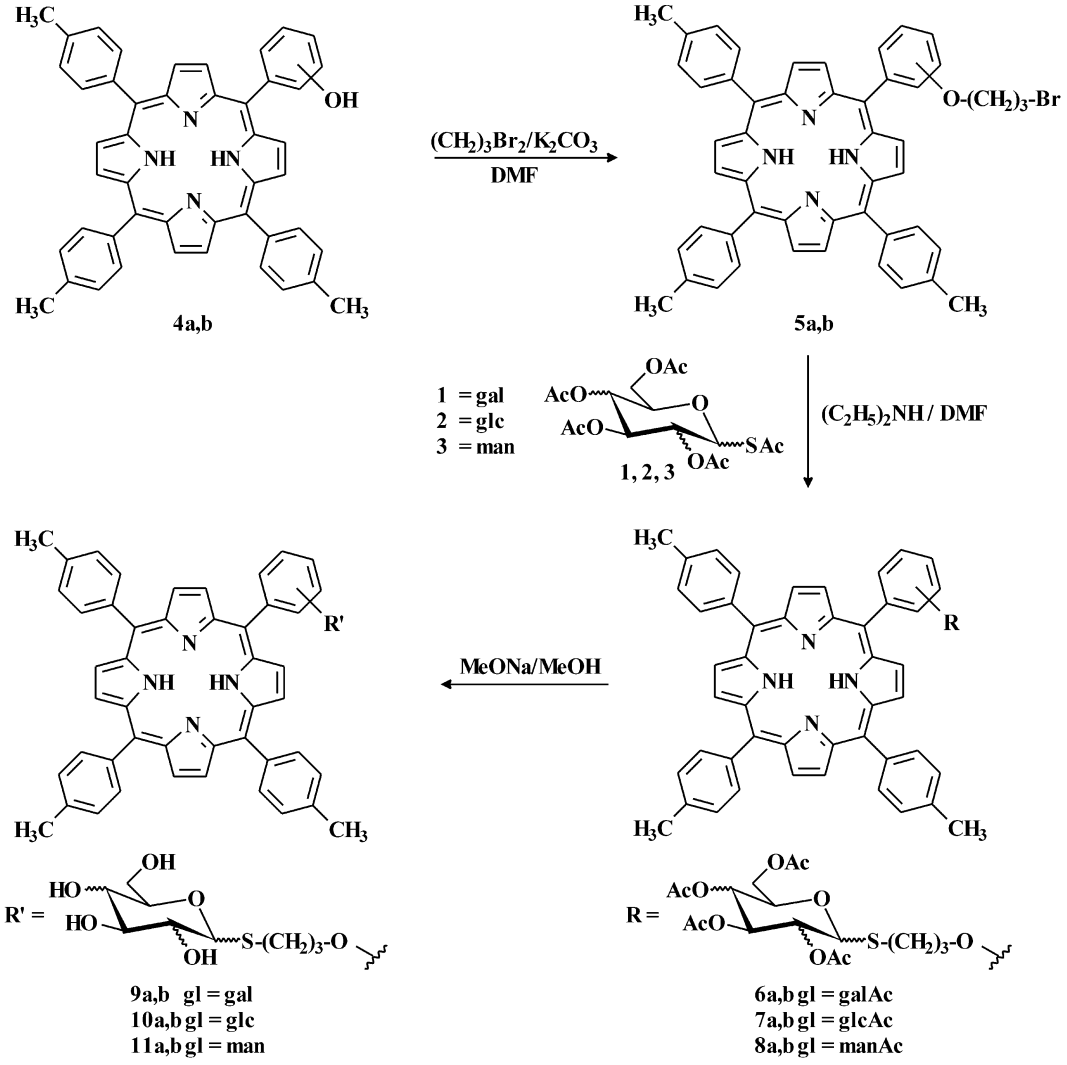


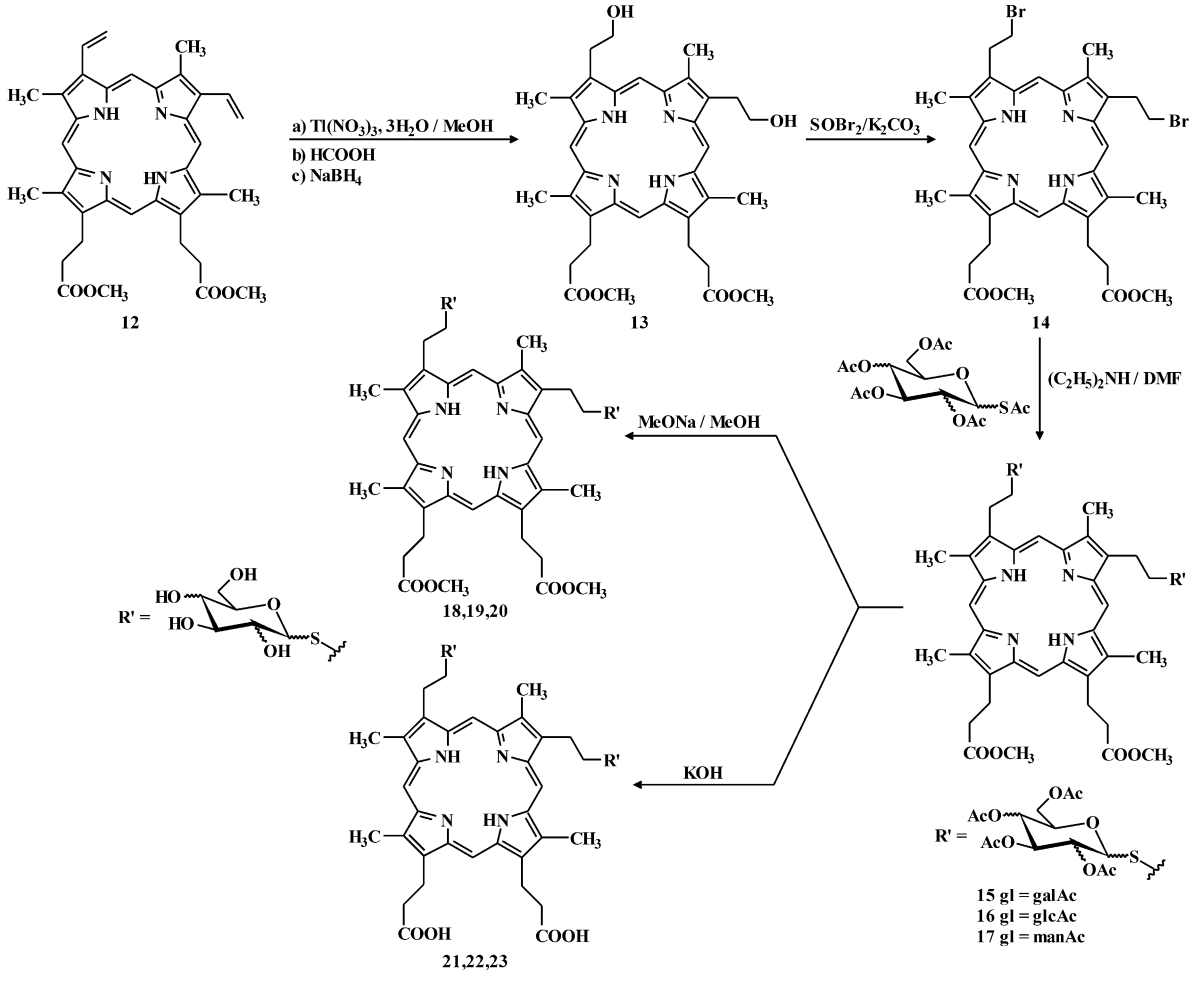
Figure 1. Structures of thioglycosylated *meso*porphyrins. a and b refer to *ortho* and *para* positions, respectively.



Scheme 1.

Scheme 1. *Ortho* and *para*-hydroxyphenyl precursors **4a** and **4b** were obtained by condensation of pyrrole (4 equiv), *ortho* or *para*-hydroxybenzaldehyde (1 equiv) and *para*-tolualdehyde (3 equiv) under Little's conditions.[8] The yields of chromatographically pure porphyrins were about 3 and 6% for the *ortho* and *para* derivatives, respectively. They were converted into **5a** and **5b** derivatives in dry DMF by action of a large excess of 1, 3-dibromopropane in presence of potassium carbonate.[9] In both cases, after purification, we observed on TLC the presence of two products that were the expected compound and a small proportion of its zinc complex. This was confirmed by UV–visible, NMR and Mass spectroscopy. So treatment of crude **5a** and **5b** with HCl resulted in demetalation with global yields of 91 and 84%, respectively. 2,3,4,6-tetra-*O*-acetyl-1*S*-acetyl-1-thio-β-D-galactopyranose, -β-D-glucopyranose and -α-D-mannopyranose were synthesized as already described in the literature[10] by reaction of thioacetic acid with the corresponding 1,2-*trans* per-*O*-acetylated β-D-galacto-, β-D-gluco- and α-D-mannopyranose. The reaction was carried out in dry dichloromethane at 50 °C using zirconium chloride as a Lewis acid. Preparation of thioglycosylated porphyrins **6a,b**–**8a,b** was performed by condensation of these 1-thioacetylated sugars with monobromotritolylporphyrins **5a** and **5b** in dry DMF.[11] The reaction products were purified by silica gel chromatography using dichloromethane/ethanol as eluent (yields 66–84%). The acetal groups were removed by treatment with sodium methanolate in methanol[12] (yield 96%).

Preparation of the second series of thioglycosylated porphyrins (shown in Scheme 2), was achieved using Protoporphyrin IX as the starting material. Protoporphyrin IX dimethylester **12** was synthesized by esterification with 5% H_2SO_4/methanol for 18 h at −10 °C in the dark[13] (yield 95%). Direct conversion[14] of **12** to isohematoporphyrin **13** without isolation of aldehydic intermediate was realized by modifying the procedures of Snow[15] and Kenner.[16] Treatment of **12** with thallium (III) nitrate in methanol gave the bis-(2, 2-dimethoxyethyl) deuteroporphyrin which was reacted with 88% formic acid. The resulting residue was washed with water and reacted with sodium borohydride in $MeOH/CH_2Cl_2$. This procedure avoids ester cleavage and the need of reesterification. Bromation of **13** was carried out at room temperature by reaction of isohematoporphyrin with thionyl bromide in presence of potassium carbonate for 5 h.[14] Compound **14** was obtained after purification on TLC and demetalation with HCl in 75% yield.



Scheme 2.

The three different thioglycosides already presented **1**, **2** and **3** (4 equiv) were added to **14** in DMF according to Bennett's method.[11] The obtained carbohydrate-disubstituted porphyrins **15**–**17** were purified by chromatography on silica gel (yields 64–93%). Conversion to the water-soluble, free diglycosides **21**–**23** (Fig. 2) was accomplished by alkaline saponification. Reaction was complete after refluxing in dichloromethane/methanol: 5:5 with KOH for 4 h (yield 94%). To selectively remove the acetal groups, we realized the treatment with sodium methanolate in methanol (yield 92%) (Scheme 2).

Mass characterization

Mass spectrometry of the various derivatives was carried out using the MALDI-TOFMS (matrix-assisted laser desorption ionization-time of flight mass spectrometry) technique.[17] Positive ion mass spectra exhibited a base peak corresponding to the intact porphyrin and no fragment ion was detected. The analysis of the isotopic cluster showed the presence of the protonated species $(M+H)^+$ with a minor contribution of the radical cation $M^{+\cdot}$ allowing the determination of the molecular mass with an accuracy around 0.001% (Fig. 3).

NMR characterization

^1H and ^{13}C NMR spectroscopies (recorded at 400 MHz) were used for the characterization of protected compounds **6a,b**–**8a,b** and **15**–**17** in $CDCl_3$. Assignments of the resonances to individual protons were based on 2-D homonuclear COSY and HMQC experiments.

Spectra of *meso*-thioglycosylated porphyrins **6a,b**–**8a,b** derived from *meso*-5, 10, 15, 20-tetrakisphenylporphyrin show six groups of resonance. For the first one, contrary to the spectra of mono *para O*-glycosylated analogues[4] the resonance of pyrrolic protons of **6b**, **7b** and **8b** appears as a single peak near 8.8 ppm (Fig. 4).

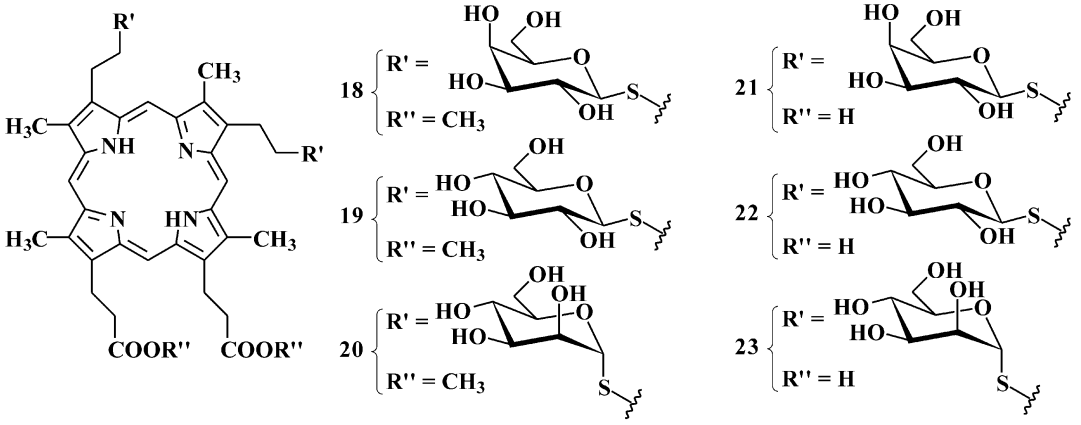


Figure 2. Structures of thioglycosylated porphyrins **18**–**23**.

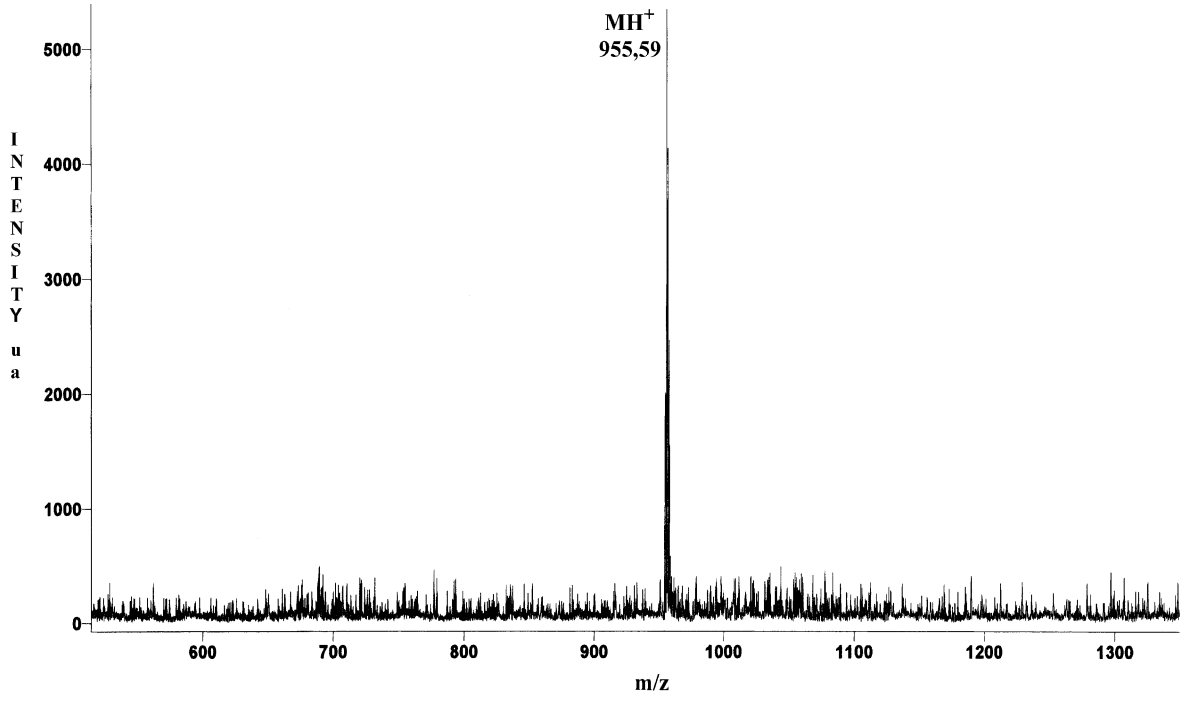


Figure 3. MALDI spectra of compound **22**.

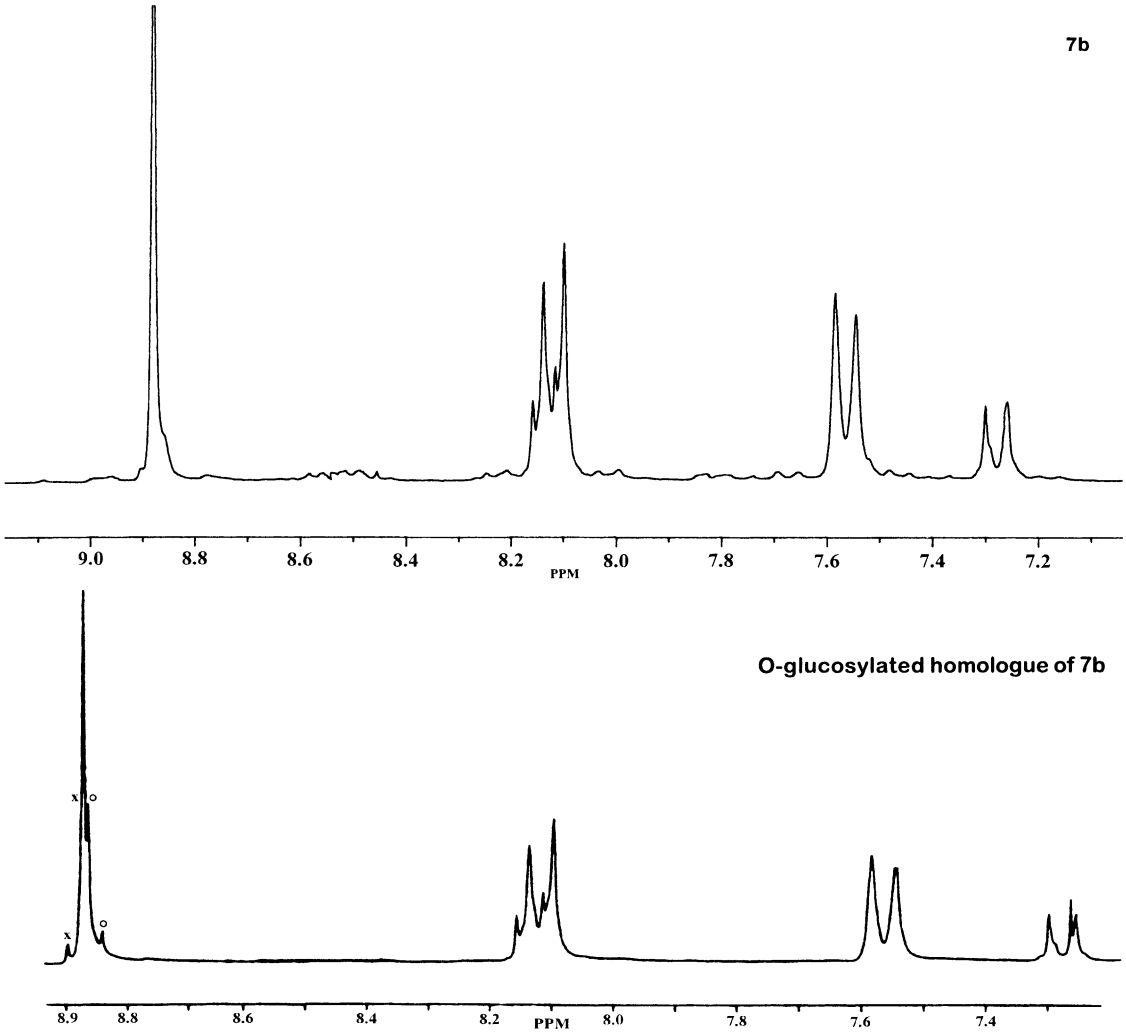


Figure 4. ^{1}H NMR spectra in $CDCl_3$ of compound **7b** and its *O*-glucosylated homologue.

The pyrrolic proton resonances of the compounds with *ortho* substitution are more complex and depend on the nature of the linked sugar. The aromatic protons appear between 8.2 and 7.2 ppm, 'ose' protons between 5 and 1 ppm, acetyl protons as singlets around 2 ppm and pyrrolic NH at −2.7 ppm. Protons of spacer arms appear between 4 and 1 ppm. Furthermore, the resonance of the anomeric proton of glycosyl groups appears as a well-defined doublet near 4.5 ppm for *para* substitution and between 2.9 and 1.3 ppm for *ortho* substitution. Coupling constants (compounds **6a,b**; **7a,b** $J = 10.0$ Hz and compound **8a,b** $J = 1.0$ Hz) are marks of a pure configuration of the anomeric carbon: β for thiogalactose and thioglucose derivatives **6a,b** and **7a,b** and α for thiomannose derivatives **8a,b**. Therefore, for porphyrins which possess a glycosyl substituent in *ortho* position (**6a**, **7a** and **8a**), we observe an obvious change in the chemical shifts of most of the nuclei. For example, all the sugar and spacer arm protons experience an unusually pronounced shielding from −3.6 ppm (H1′ thiomannosyle) to −0.3 ppm (Hα thioglucosyle or thiomannosyle). The same is true for acetyl groups. The shielding effect on these protons results from ring current located within the macrocycle.

The ^{1}H spectra of dithioglycosylated porphyrins **15**–**17** show six resonance groups: *meso* protons near 10 ppm; protons of glycosyl groups between 6 and 3 ppm; ethyl chain protons between 4.5 and 3 ppm; β-pyrrolic methyl and methyl ester protons near 3.5 ppm; acetyl protons near 2 ppm and pyrrolic NH at −3.8 ppm. The resonances of the anomeric protons of thioglycosyl groups display the same coupling constants as for the *meso* porphyrins. Apart from the porphyrin plane, Protoporphyrin IX dimethyl ester and its derivatives have no symmetry element. So, precise assignment within certain groups of substituents (β-pyrrole, methyl groups) is a difficult task. ^{13}C spectroscopy and HMQC has allowed us to assign alkyl side chain protons for compounds **15**–**17** and their precursors.

UV–visible characterization

Absorption properties of compounds **6a,b**–**11a,b** and **15**–**23** in different solvents according to their solubility are shown in Table 1. The electronic spectra of all *meso*-substituted porphyrins **6a,b**–**11a,b** are similar to those of known free base *meso*-5, 10, 15, 20-tetrakisphenyl-

porphyrins with a Soret band around 420 nm and four less intense Q bands near 520, 550, 590 and 650 nm. However, the relative intensities of the Q (I) and Q (II) bands of the *ortho* derivatives **6a**, **7a**, **8a**, **9a**, **10a**, **11a** show a small difference ($\varepsilon_{II}/\varepsilon_{I} < 1$). The UV–visible spectra of porphyrins bearing substituents on β-pyrrolic positions **15**–**23** are also of the 'etio' type characterized by a Soret band near 400 nm and four weak Q bands near 500, 530, 570 and 620 nm. In aqueous solutions, the *meso*-substituted porphyrins **9**–**11** exhibit a significantly broadened and less intense Soret band. The Soret band of β-substituted porphyrins **18**–**23** is blue-shifted and also less intense. Both results indicate some degree of porphyrin aggregation in this solvent (Fig. 5).[18]

Resistance of β-thioglucoside to β-glucosidase

Thioglycosylated porphyrins **10a** and **10b** were tested for enzymatic hydrolysis of β-glucosidic bond by β-glucosidase as described in the Experimental. No cleavage of these compounds has been observed, contrary to the *O*-glycosylated counterparts, namely 5-[2-[3-(β-D-glucosyloxy)propyloxy]phenyl]-10,15,20-tritolylporphyrin and 5-[4-[3-(β-D-glucosyloxy)propyloxy]phenyl]-10,15,20-tritolylporphyrin, where we observed formation of glucose.

In vitro photocytotoxicity

Figure 6 displays, in function of irradiation time, dead cell counts and the subsequent increase following a

Table 1. UV–vis spectra of porphyrin derivatives in various solvents

Compd	λ_{nm} ($\varepsilon\times10^{-3}$, cm^{-1}, mol^{-1}, L)					
Solvent:[a]	Soret	IV	III	II	I	Ratio $\varepsilon_{II}/\varepsilon_{I}$
6a (a)	420 (430.9)	516 (15.7)	550 (8.5)	592 (4.9)	648 (6.0)	0.8
6b (a)	420 (471.0)	516 (14.3)	552 (9.4)	592 (4.7)	648 (4.2)	1.1
7a (a)	420 (413.0)	516 (13.9)	550 (7.1)	592 (4.0)	648 (5.3)	0.8
7b (a)	420 (433.0)	516 (13.7)	552 (8.7)	592 (4.4)	648 (4.6)	1
8a (a)	420 (308.3)	516 (12.6)	550 (6.4)	592 (3.7)	648 (4.8)	0.8
8b (a)	420 (325.0)	516 (11.9)	552 (7.5)	592 (3.8)	648 (3.5)	1.1
9a (b)	418 (208.4)	514 (08.5)	548 (4.3)	592 (2.5)	650 (3.2)	0.8
9b (b)	418 (319.4)	514 (13.2)	550 (7.9)	592 (3.8)	650 (3.8)	1
10a (b)	418 (370.8)	514 (13.5)	548 (7.1)	592 (4.1)	650 (5.2)	0.8
10b (b)	418 (294.6)	514 (11.2)	550 (6.7)	592 (3.3)	650 (3.2)	1
11a (b)	418 (425.0)	514 (17.7)	548 (9.1)	592 (5.2)	650 (6.8)	0.8
11b (b)	418 (453.2)	514 (17.0)	550 (10.4)	592 (5.1)	650 (4.8)	1.1
15 (a)	400 (208.5)	498 (15.8)	532 (11.3)	568 (8.1)	622 (5.0)	1.6
16 (a)	400 (255.8)	498 (18.6)	532 (14.4)	568 (10.9)	622 (6.0)	1.8
17 (a)	400 (246.5)	498 (18.8)	532 (14.0)	568 (10.1)	622 (6.2)	1.6
18 (b)	400 (128.4)	498 (10.4)	530 (8.2)	568 (6.0)	622 (3.9)	1.5
18 (c)	372 (73.0)	512 (8.4)	550 (6.9)	580 (6.5)	636 (3.4)	1.9
19 (b)	400 (198.8)	498 (16.9)	530 (12.3)	568 (8.4)	622 (6.3)	1.3
19 (c)	372 (72.6)	512 (6.9	550 (5.8)	574 (5.0)	628 (2.1)	2.3
20 (b)	400 (195.9)	498 (11.3)	530 (8.4)	568 (5.7)	622 (4.8)	1.2
20 (c)	372 (75.4)	516 (8.9)	550 (8.8)	574 (8.5)	628 (3.9)	2.2
21 (d)	396 (131.0)	498 (15.9)	530 (11.7)	566 (8.1)	620 (5.3)	1.5
21 (c)	372 (118.1)	506 (6.8)	540 (6.9)	566 (6.5)	618 (3.0)	2.2
22 (d)	396 (176.2)	498 (12.4)	530 (11.2)	566 (8.2)	620 (4.0)	2
22 (c)	372 (109.1)	506 (5.5)	540 (4.8)	566 (4.3)	618 (2.0)	2.1
23 (d)	396 (99.3)	498 (8.3)	530 (6.3)	566 (4.6)	620 (3.1)	1.5
23 (c)	372 (79.8)	506 (5.2)	540 (4.9)	566 (4.6)	618 (2.6)	1.8

[a]The solvents used are as follows: (a) CH_2Cl_2, (b) THF/H_2O 8:2, (c) aqueous solution, (d) acetone/H_2O 6:4.

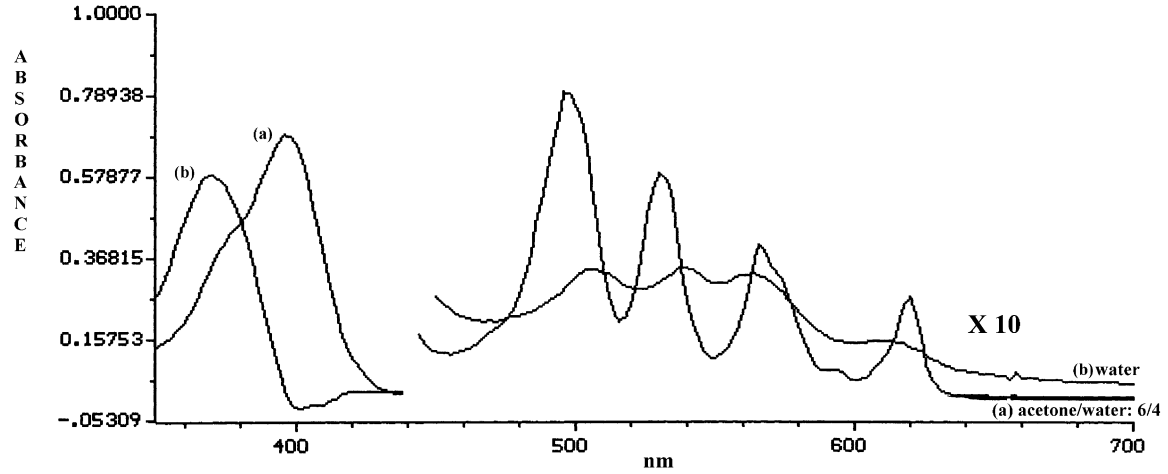


Figure 5. Electronic spectra of compound **21** in acetone/water: 6:4 (a) and in water (b).

further 24 h incubation in the dark. The results obtained with these synthetic porphyrins have been compared with those observed with Pf. In every cases, the subsequent 24 h incubation in the dark results in an increase in dead cell count. The immediate effect (J0) accounts for early necrotic death; the delayed post-irradiation death is likely the result of secondary necrosis following irradiation-induced apoptosis.

Among the thioglycosylated *meso*-arylporphyrins, all the *ortho* isomers **9a**, **10a** and **11a** are photoactive. Yet only one *para* isomer (**10b**) is found as being photocytotoxic (Fig. 7A). The immediate dead cell counts (J0) are lower than those observed with Pf. However, the delayed effects, observed after a 24 h incubation in the dark at 37 °C (J1), are in every case comparable to those observed with Pf provided that the irradiation time is longer than 60 min. This remark holds especially for porphyrins **9a**, **10a** and **10b**.

Among the derivatives of isohematoporphyrin, only compounds **18**, **19** and **20** have been found photoactive (Fig. 7B). Moreover, they were found to be more efficient than Pf. Hydrolysis of methyl ester functions

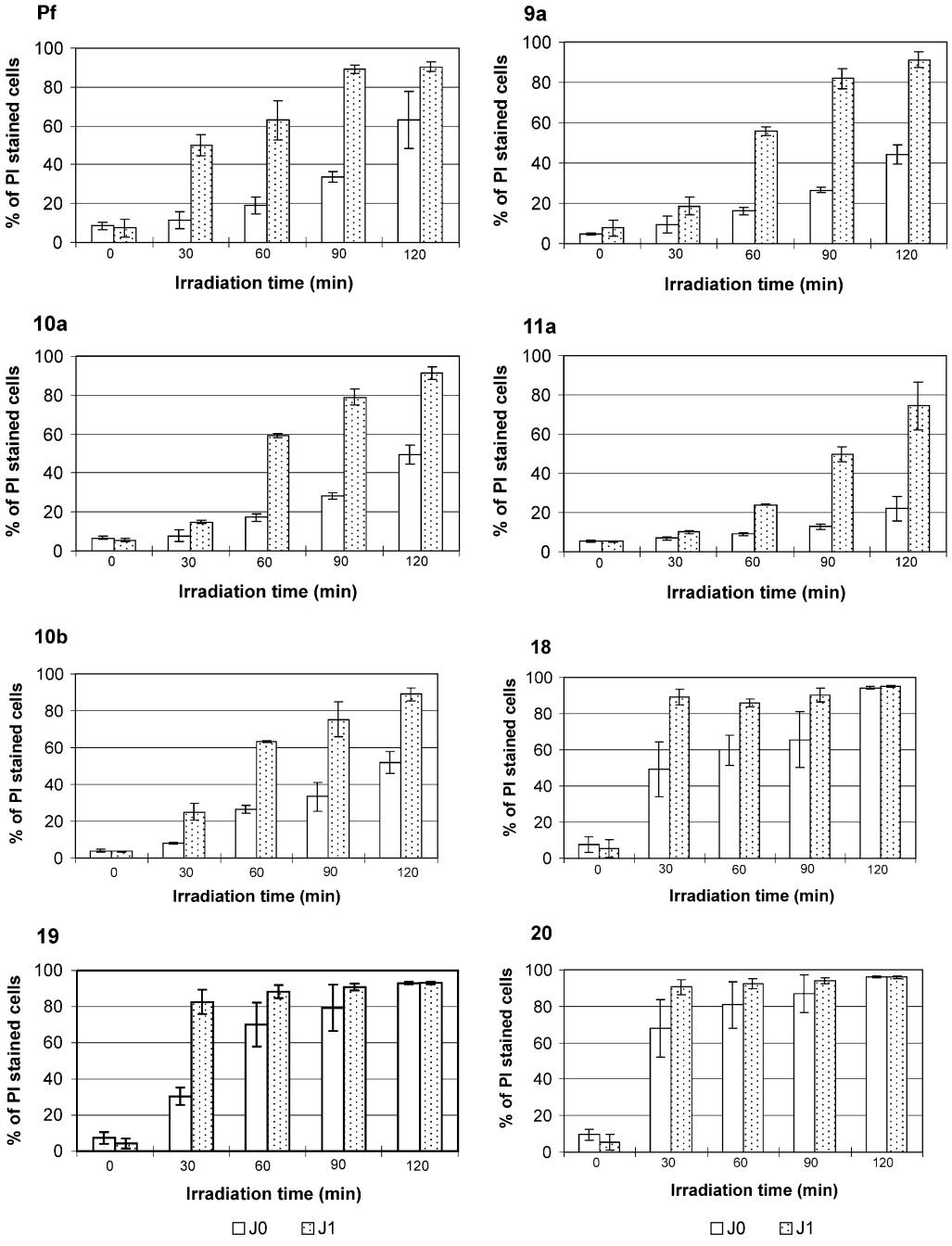


Figure 6. Percentage of PI stained K 562 cells treated with various compounds versus time. Void bars: dead cell count after indicated irradiation time (J0). Solid bars: dead cell count after a further 24 h incubation in the dark (J1). Each bar represents the mean of 3–5 independent experiments. **Pf**: photofilm, **9a**: *meso-ortho*galactosyl porphyrin, **10a**: *meso-ortho*glucosyl porphyrin, **11a**: *meso-ortho*mannosyl porphyrin, **10b**: *meso-para*glucosyl porphyrin, **18** di-β-galactosyl porphyrin diester, **19** di-β-glucosyl porphyrin diester, **20** di-α-mannosyl porphyrin diester.

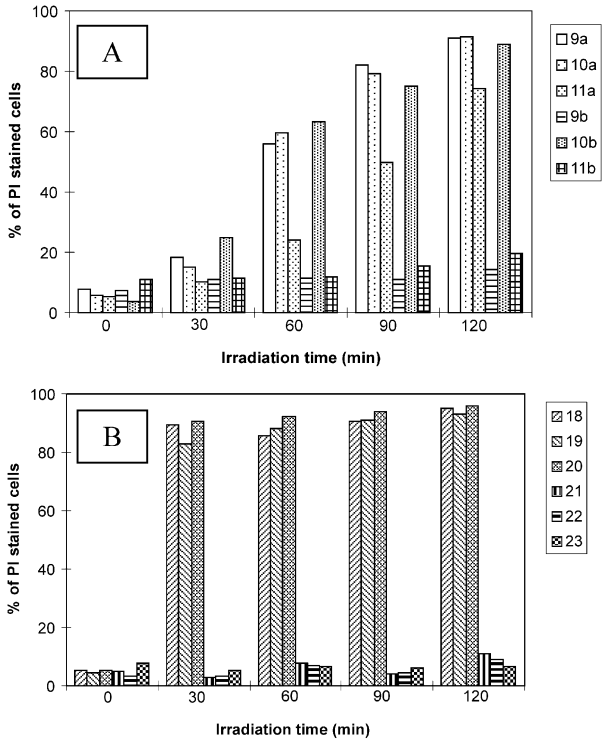


Figure 7. Summary of phototreatment efficacy of synthetic porphyrins. K562 cells were incubated for the indicated times in presence of 2×10^{-6} M synthetic porphyrins and incubated for a further 24 h as described in Experimental. Dead cells were then detected and counted by flow cytometry. (A) treatment with *meso*-*S*-glycosylporphyrins; (B) treatment with glycosylisohematoporphyrins. Numbers refer to porphyrins described in the text.

leading to compounds **21**, **22** and **23** results in their inactivation (Fig. 7B). One can assume that photoactivity of the esterified compounds is correlated to their lack of ionized groups, thus facilitating their diffusion into cells. Photocytotoxic activity of these compounds is thus more strongly related to their hydrophobic/hydrophylic character than to the nature of the glucidic moiety.

Experimental

General

Para-hydroxybenzaldehyde, *para*-tolualdehyde and pyrrole were purchased from Aldrich and distilled prior to use. All other reagents were purchased from Aldrich or Sigma and used without further purification. Solvents were dried when necessary using standard techniques. Removal of solvents was performed under reduced pressure. Organic phases were dried over $MgSO_4$. Preparative thin-layer chromatography (TLC) was performed on Merck silica gel 60 F_{254}, 2 mm thickness. Column chromatography was carried out with silica gel (60 ACC, 15–40 μm, Merck) or with Sephadex LH20 (Pharmacia). MALDI mass spectra were obtained on a mass spectrometer using α-cyano-4-hydroxycinnamic acid (Sigma, France) as matrix and UV–vis spectra on a spectrophotometer using 0.1 or 1 cm quartz cells. ^{1}H and ^{13}C NMR spectra were recorded using a Bruker DPX-400 spectrometer for solution in deuterochloroform with tetramethylsilane as internal standard and assignments were confirmed by double irradiation. The chemical shifts are given in ppm (s, singlet; d, doublet; dd, doublet of doublets; ddd, doublet of doublet of doublets; t, triplet; dt, doublet of triplets; q, quadruplet, m, multiplet) and coupling constants are in Hertz. MALDI mass spectra were obtained on a Voyager Elite (Framingham, MA, USA) time-of-flight mass spectrometer. Elemental analyses were carried by the Service Régional de Microanalyse de l'Université Pierre et Marie Curie, Paris.

Synthesis

O-Acetylated-1-*S*-acetyl-1-thioglycoses **1**, **2** and **3** were synthesized according to the literature.[10] 5-(2-Hydroxyphenyl)-10,15,20-tritolylporphyrin **4a** and 5-(4-hydroxyphenyl)-10,15,20-tritolyl-porphyrin **4b** were synthesized according to the literature.[6,8]

5-(2-(3-Bromo-1-propanoxy)phenyl)-10,15,20-tritolylporphyrin (5a). Compound **5a** was synthesized according to the literature[9] The crude product was purified on TLC (CH_2Cl_2/petroleum ether, 5:5) to give, after demetalation with 10% concentrated hydrochloric acid in chloroform, 108 mg (91%) of pure **5a**. R_f 0.57 (CH_2Cl_2/petroleum ether, 5:5). UV–vis (CH_2Cl_2) λ ($\varepsilon\times10^{-3}$) 420 (310.3) 516 (12.6) 552 (6.5) 592 (4.0) 646 (4.9). ^{1}H NMR ($CDCl_3$, 200 MHz) δ −2.70 (2H, br s, NH); 0.89 (2H, m, H-β); 2.26 (2H, t, $J=6.2$ Hz, H-γ); 2.70 (9H, s, CH_3 tolyl); 4.03 (2H, t, $J=5.5$ Hz, H-α); 7.32 (1H, br d, $J=8.2$ Hz, H-3 aryl); 7.37 (1H, t, $J=7.5$ Hz, H-5 aryl) 7.55 (6H, d, $J=7.6$ Hz, H-3, 5 tolyl); 7.75 (1H, dt, $J=7.9$-1.5 Hz, H-4 aryl); 8.06 (6H, m, H-2, 6 tolyl); 8.10 (1H, dd, $J=7.5$–1.5 Hz, H-6 aryl); 8.80 (2H, d, $J=4.8$ Hz, H β pyrrole); 8.86 (2H, d, $J=4.8$ Hz, H β pyrrole); 8.87 (4H, s, H-7, 8, 12, 13 β pyrrole). MS (MALDI) m/z 795 ($[M+H]^+$ monoisotopic).

5-(4-(3-Bromo-1-propanoxy)phenyl)-10,15,20-tritolylporphyrin (5b). The porphyrin **5b** was prepared by a procedure analogous to that given for **5a**. The yield was 84%. R_f 0.42 (CH_2Cl_2/petroleum ether, 5:5). UV–visible (CH_2Cl_2) λ ($\varepsilon\times10^{-3}$) 420 (363.1) 516 (12.9) 552 (7.2) 592 (3.7) 646 (3.7). ^{1}H NMR ($CDCl_3$, 200 MHz) δ −2.70 (2H, br s, NH); 2.51 (2H, br quint, $J=6.1$ Hz, H-β); 2.72 (9H, s, CH_3 tolyl); 3.77 (2H, t, $J=6.2$ Hz, H-γ); 4.35 (2H, t, $J=5.7$ Hz, H-α); 7.26 (2H, d, $J=8.5$ Hz, H-3, 5 aryl); 7.56 (6H, d, $J=7.8$ Hz, H-3, 5 tolyl); 8.12 (2H, d, $J=8.5$ Hz H-2, 6 aryl); 8.12 (6H, d, $J=7.8$ Hz, H-2, 6 tolyl); 8.89 (8H, s, H β pyrrole). MS (MALDI) m/z 795 ($[M+H]^+$ monoisotopic).

General procedure for thioglycosylation of monobromopropanoxyphenyltritolyl-porphyrins

To a solution of porphyrin **5a** or **5b** (40 mg, 0.050 mmol) and acetylated 1-thiosugar (31 mg, 0.076 mmol, 1.5 equiv), in dry DMF (6 mL) at room temperature under a nitrogen atmosphere, was added diethylamine (0.10 mL, 1.01 mmol, 20 equiv). After stirring for several hours, diethylamine and dimethylformamide were

removed in vacuo. Purification by TLC on silica gel (CH_2Cl_2/EtOH) gave the desired compound.

5-[2-(1-*S*-(2,3,4,6-tetra-*O*-acetyl-1-thio-β-D-galactopyranosyl)-3-thio-propanoxy)-phenyl]-10,15,20-tritolylporphyrin (6a). Compound **5a** and *O*-acetylated-1-*S*-acetyl-1-thio-β-D-galacto-pyranose (**1**) gave, after 15 h of reaction, 36 mg of **6a** (66%). R_f 0.40 (CH_2Cl_2/EtOH, 99:1). UV–vis (CH_2Cl_2) λ (ε×10^{-3}) 420 (430.9) 516 (15.7) 550 (8.5) 592 (4.9) 646 (6.0). ^{1}H NMR ($CDCl_3$, 400 MHz) δ −2.70 (2H, br s, NH); 1.23 (2H, m, H-β); 1.36 (2H, m, H-γ); 1.57 (3H, s, OAc); 1.68 (1H, m, H-5′ ose); 1.77, 1.86, 1.87 (9H, s, OAc); 2.71 (9H, s, CH_3 tolyl); 2.81 (1H, dd, J = 11.1–6.6 Hz, H-6_a′ ose); 2.90 (1H, d, J = 10.2 Hz, H-1′ ose); 2.93 (1H, dd, J = 11.1–7.3 Hz, H-6_b′ ose); 3.85 (1H, dd, J = 10.2-3.3 Hz, H-3′ ose); 3.97 (2H, m, H-α); 4.37 (1H, d, J = 3.3 Hz, H-4′ ose); 4.59 (1H, t, J = 10.2 Hz, H-2′ ose); 7.30 (1H, br d, J = 8.2 Hz, H-3 aryl); 7.38 (1H, ddd, J = 8.2–7.4–0.5 Hz, H-5 aryl); 7.57 (4H, d, J = 7.8 Hz, H-3, 5 tolyl); 7.59 (2H, d, J = 7.8 Hz, H-3, 5 tolyl); 7.76 (1H, br td, J = 8.0–1.7 Hz, H-4 aryl); 8.08 (1H, dd, J = 7.4–1.7 Hz, H-6 aryl); 8.16 (6H, m, H-2, 6 tolyl); 8.80, 8.83, 8.90 (4H, s, H-2, 3, 7, 8 β pyrrole); 8.88, 8.92 (4H, s, H-12, 13, 17, 18 β pyrrole). ^{13}C NMR ($CDCl_3$, 100 MHz) δ 20.1, 20.3, 20.4, 20.5 (4C, *C*H_3CO); 21.4 (3C, CH_3 tolyl); 27.7 (1C, C-γ); 29.7 (1C, C-β); 60.4 (1C, C-6′ ose); 66.3 (1C, C-α); 66.4 (1C, C-4′ ose); 67.2 (1C, C-2′ ose); 71.0 (1C, C-3′ ose); 72.7 (1C, C-5′ ose); 84.4 (1C, C-1′ ose); 111.9 (1C, C-3 aryl); 115.9 (1C, C *meso*); 119.8 (1C, C-5 aryl); 120.0, 120.1, 120.5 (3C, C *meso*); 127.5, 127.6 (6C, C-3, 5 tolyl); 130.0 (1C, C-4 aryl); 131.1 (8C, C β pyrrole); 131.4 (1C, C-1 aryl); 134.7, 134.8 (6C, C-2, 6 tolyl); 135.6 (1C, C-6 aryl); 137.5 (3C, C-4 tolyl); 139.3, 139.4 (3C, C-1 tolyl); 145.0–150.0 (8C, C α pyrrole); 158.7 (1C, C-2 aryl); 169.4, 169.5, 170.0, 170.1 (4C, CH_3*C*O). MS (MALDI) m/z 1077 ($[M+H]^+$ monoisotopic). Anal. calcd for $C_{64}H_{60}N_4O_{10}S$: C, 71.36; H, 5.61; N, 5.20. Found: C, 71.22; H, 5.72; N, 5.17.

5-[4-(1-*S*-(2,3,4,6-tetra-*O*-acetyl-1-thio-β-D-galactopyranosyl)-3-thio-propanoxy)-phenyl]-10,15,20-tritolylporphyrin (6b). Compound **5b** and *O*-acetylated-1-*S*-acetyl-1-thio-β-D-galacto-pyranose (**1**) gave, after 6 h of reaction, 37 mg of **6b** (67%). R_f 0.68 (CH_2Cl_2/EtOH, 98:2). UV–vis (CH_2Cl_2) λ (ε×10^{-3}) 420 (471.0) 516 (14.3) 552 (9.4) 592 (4.7) 650 (4.2). ^{1}H NMR ($CDCl_3$, 400 MHz) δ −2.77 (2H, br s, NH); 2.00, 2.05, 2.11, 2.19 (12H, s, OAc); 2.28 (2H, m, H-β); 2.72 (9H, s, CH_3 tolyl); 3.01 (1H, dt, J = 13.1–6.2 Hz, H-γ); 3.11 (1H, dt, J = 13.1–5.9 Hz, H-γ); 3.99 (1H, td, J = 6.7–1.0 Hz, H-5′ ose); 4.16 (1H, dd, J = 11.3–6.5 Hz, H-6_a′ ose); 4.33 (1H, dd, J = 11.3–6.8 Hz, H-6_b′ ose); 4.34 (2H, t, J = 6.0 Hz, H-α); 4.60 (1H, d, J = 10.0 Hz, H-1′ ose); 5.10 (1H, dd, J = 10.0–3.4 Hz, H-3′ ose); 5.33 (1H, t, J = 10.0 Hz, H-2′ ose); 5.47 (1H, dd, J = 3.4–1.0 Hz, H-4′ ose); 7.25 (1H, d, J = 8.7 Hz, H-3 aryl); 7.28 (1H, d, J = 8.6 Hz, H-5 aryl); 7.53 (6H, d, J = 7.8 Hz, H-3, 5 tolyl); 8.08 (6H, d, J = 7.8 Hz, H-2, 6 tolyl); 8.10 (2H, d, J = 8.6 Hz H-2, 6 aryl); 8.84 (8H, s, H β pyrrole). ^{13}C NMR ($CDCl_3$, 100 MHz) δ 20.6, 20.7 (4C, *C*H_3CO); 21.5 (3C, CH_3 tolyl); 27.3 (1C, C-γ); 29.5 (1C, C-β); 61.5 (1C, C-6′ ose); 66.4 (1C, C-α); 66.7 (1C, C-2′ or 4′ ose); 67.3 (1C, C-2′ or 4′ ose); 71.9 (1C, C-3′ ose); 74.6 (1C, C-5′ ose); 84.5 (1C, C-1′ ose); 112.7 (2C, C-3, 5 aryl); 119.6 (1C, C *meso*); 120.1 (3C, C *meso*); 127.3 (6C, C-3, 5 tolyl); 131.0 (8C, C β pyrrole); 134.5 (6C, C-2, 6 tolyl); 134.6 (1C, C-1 aryl); 135.6 (2C, C-2, 6 aryl); 137.3 (3C, C-4 tolyl); 139.3 (3C, C-1 tolyl); 145.0–150.0 (8C, C α pyrrole); 158.6 (1C, C-4 aryl); 169.8, 170.0, 170.2, 170.4 (4C, CH_3*C*O). MS (MALDI) m/z 1078 ($[M+H]^+$ monoisotopic). Anal. calcd for $C_{64}H_{60}N_4O_{10}S$: C, 71.36; H, 5.61; N, 5.20. Found: C, 71.25; H, 5.72; N, 5.08.

5-[2-(1-*S*-(2,3,4,6-Tetra-*O*-acetyl-1-thio-β-D-glucopyranosyl)-3-thio-propanoxy)-phenyl]-10,15,20-tritolylporphyrin (7a). Compound **5a** and *O*-acetylated-1-*S*-acetyl-1-thio-β-D-gluco-pyranose (**2**) gave, after 15 h of reaction, 46 mg of **7a** (84%). R_f 0.54 (CH_2Cl_2/EtOH, 96:4). UV–vis (CH_2Cl_2) λ (ε×10^{-3}) 420 (413.0) 516 (13.9) 550 (7.1) 590 (4.0) 648 (5.3). ^{1}H NMR ($CDCl_3$, 400 MHz) δ −2.74 (2H, br s, NH); 0.87 (1H, m, H-γ); 0.98 (2H, m, H-β); 1.17 (1H, ddd, J = 9.2–4.8–2.5 Hz, H-5′ ose); 1.32 (1H, dd, J = 12.5–4.8 Hz, H-6_a′ ose); 1.47, 1.51 (6H, s, OAc); 1.66 (1H, d, J = 9.7 Hz, H-1′ ose); 1.72, 1.77 (6H, s, OAc); 1.78 (1H, m, H-γ); 1.81 (1H, m, H-6_b′ ose); 2.69 (9H, s, CH_3 tolyl); 2.87 (1H, m, H-3′ ose); 3.84 (1H, m, H-α); 3.82 (1H, t, J = 9.3 Hz, H-4′ ose); 4.01 (1H, m, H-2′ ose); 4.01 (1H, m, H-α); 7.24 (1H, dd, J = 7.6–0.5 Hz, H-3 aryl); 7.45 (1H, ddd, J = 8.1–7.4–0.5 Hz, H-5 aryl); 7.57 (4H, d, J = 7.8 Hz, H-3, 5 tolyl); 7.60 (2H, d, J = 7.8 Hz, H-3, 5 tolyl); 7.72 (1H, ddd, J = 8.2–7.6–1.7 Hz, H-4 aryl); 8.06 (1H, dd, J = 7.4–1.7 Hz H-6 aryl); 8.20 (6H, m, H-2, 6 tolyl); 8.73 (1H, d, J = 4.8 Hz, H β pyrrole);8.82 (2H, d, J = 4.8 Hz, H β pyrrole);8.85, 8.87, 8.88, 8.92, 8.96 (5H, d, J = 4.8 Hz, H β pyrrole). ^{13}C NMR ($CDCl_3$, 100 MHz) δ 20.3, 20.4, 20.6, 20.7 (4C, *C*H_3CO); 21.7 (3C, CH_3 tolyl); 27.7 (1C, C-γ); 29.9 (1C, C-β); 59.0 (1C, C-6′ ose); 66.1 (1C, C-4′ ose); 66.7 (1C, C-α); 70.0 (1C, C-2′ ose); 73.0 (1C, C-3′ ose); 77.0 (1C, C-5′ ose); 83.3 (1C, C-1′ ose); 112.0 (1C, C-3 aryl); 116.1 (1C, C *meso*); 119.9 (1C, C-5 aryl); 120.2, 120.3, 120.8 (3C, C *meso*); 127.5, 127.6, 127.8 (6C, C-3, 5 tolyl); 130.1 (1C, C-4 aryl); 131.1 (8C, C β pyrrole); 131.5 (1C, C-1 aryl); 134.7, 134.9, 135.0 (6C, C-2, 6 tolyl); 135.3 (1C, C-6 aryl); 137.5, 137.6 (3C, C-4 tolyl); 139.3, 139.4, 139.5 (3C, C-1 tolyl); 145.0–150.0 (8C, C α pyrrole); 159.0 (1C, C-2 aryl); 168.9, 169.2, 169.4, 170.0 (4C, CH_3*C*O). MS (MALDI) m/z 1078.8 ($[M+H]^+$ monoisotopic). Anal. calcd for $C_{64}H_{60}N_4O_{10}S$: C, 71.36; H, 5.61; N, 5.20. Found: C, 71.27; H, 5.65; N, 5.15.

5-[4-(1-*S*-(2,3,4,6-tetra-*O*-acetyl-1-thio-β-D-glucopyranosyl)-3-thio-propanoxy)-phenyl]-10,15,20-tritolylporphyrin (7b). Compound **5b** and *O*-acetylated-1-*S*-acetyl-1-thio-β-D-gluco-pyranose (**2**) gave, after 6 h of reaction, 43 mg of **7b** (79%). R_f 0.68 (CH_2Cl_2/EtOH, 97:3). UV–vis (CH_2Cl_2) λ (ε×10^{-3}) 420 (433.0) 516 (13.7) 552 (8.7) 592 (4.4) 648 (4.6). ^{1}H NMR ($CDCl_3$, 400 MHz) δ −2.71 (2H, br s, NH); 2.06, 2.14, 2.15 (12H, s, OAc); 2.30 (2H, m, H-β); 2.72 (9H, s, CH_3 tolyl); 3.05 (2H, m, H-γ); 3.77 (1H, ddd, J = 9.2–4.6–2.4 Hz, H-5′ ose); 4.16 (1H, dd, J = 12.5–2.5 Hz, H-6_a′ ose); 4.32 (1H, dd, J = 12.5–4.8 Hz, H-6_b′ ose); 4.35 (2H, br t, J = 6.9 Hz, H-α); 4.62 (1H, d, J = 9.7 Hz, H-1′ ose); 5.15 (1H, dd, J = 9.7–8.7 Hz, H-2′ ose); 5.21 (1H, t, J = 9.1 Hz, H-4′ ose); 5.30

(1H, t, $J=9.1$ Hz, H-3′ ose); 7.28 (2H, d, $J=8.5$ Hz, H-3, 5 aryl); 7.56 (6H, d, $J=7.8$ Hz, H-3, 5 tolyl); 8.12 (6H, d, $J=7.8$ Hz, H-2, 6 tolyl); 8.14 (2H, d, $J=8.6$ Hz H-2, 6 aryl); 8.88 (8H, s, H β pyrrole). ^{13}C NMR ($CDCl_3$, 100 MHz) δ 20.7, 20.8 (4C, *C*H_3CO); 21.5 (3C, CH_3 tolyl); 27.1 (1C, C-γ); 29.5 (1C, C-β); 62.2 (1C, C-6′ ose); 66.3 (1C, C-α); 68.4 (1C, C-2′ or 4′ ose); 70.0 (1C, C-2′ or 4′ ose); 73.9 (1C, C-3′ ose); 76.0 (1C, C-5′ ose); 84.0 (1C, C-1′ ose); 112.7 (2C, C-3, 5 aryl); 119.7 (1C, C *meso*); 120.1 (3C, C *meso*); 127.4 (6C, C-3, 5 tolyl); 131.1 (8C, C β pyrrole); 134.5 (6C, C-2, 6 tolyl); 134.6 (1C, C-1 aryl); 135.6 (2C, C-2, 6 aryl); 137.3 (3C, C-4 tolyl); 139.3 (3C, C-1 tolyl); 145.0–150.0 (8C, C α pyrrole); 158.6 (1C, C-4 aryl); 169.4, 170.2, 170.6 (4C, CH_3*C*O). MS (MALDI) *m*/*z* 1078 ($[M+H]^+$ monoisotopic). Anal. calcd for $C_{64}H_{60}N_4O_{10}S$: C, 71.36; H, 5.61; N, 5.20. Found: C, 71.42; H, 5.59; N, 5.18.

5-[2-(1-*S*-(2,3,4,6-tetra-*O*-acetyl-1-thio-α-D-mannopyranosyl)-3-thio-propanoxy)-phenyl]-10,15,20-tritolylporphyrin (8a). Compound **5a** and *O*-acetylated-1-*S*-acetyl-1-thio-β-D-manno-pyranose (**3**) gave, after 15 h of reaction, 41 mg of **8a** (75%). R_f 0.55 (CH_2Cl_2/EtOH, 97:3). UV–vis (CH_2Cl_2) λ (ε×10^{-3}) 420 (308.3) 516 (12.6) 550 (6.4) 592 (3.7) 650 (4.8). ^{1}H NMR ($CDCl_3$, 400 MHz) δ −2.70 (2H, br s, NH); 0.80 (1H, m, H-5′ ose); 1.30 (2H, m, H-β); 1.30 (2H, m, H-γ); 1.34 (1H, d, $J=1.0$ Hz, H-1′ ose); 1.66, 1.69, 1.83, 1.94 (12H, s, OAc); 2.64 (2H, m, H-6′ ose); 2.72 (9H, s, CH_3 tolyl); 3.38 (1H, dd, $J=10.2$–3.4 Hz, H-3′ ose); 4.00 (2H, m, H-α); 4.44 (1H, t, $J=10.2$ Hz, H-4′ ose); 4.79 (1H, dd, $J=3.2$–1.0 Hz, H-2′ ose); 7.31 (1H, br d, $J=8.2$ Hz, H-3 aryl); 7.40 (1H, br td, $J=7.8$–0.5 Hz, H-5 aryl); 7.57 (4H, d, $J=7.8$ Hz, H-3, 5 tolyl); 7.60 (2H, d, $J=7.8$ Hz, H-3, 5 tolyl); 7.77 (1H, br td, $J=8.0$–1.7 Hz, H-4 aryl); 8.07 (1H, dd, $J=7.4$–1.7 Hz H-6 aryl); 8.10 (4H, d, $J=7.8$ Hz, H-2, 6 tolyl); 8.19 (2H, d, $J=7.8$ Hz, H-2, 6 tolyl); 8.80 (1H, d, $J=4.8$ Hz, H-2, 3, 7 or 8 β pyrrole); 8.86 (1H, d, $J=4.8$ Hz, H-2, 3, 7 or 8 β pyrrole); 8.88 (4H, s, H-12, 13, 17, 18 β pyrrole); 8.92 (1H, d, $J=4.8$ Hz, H-2, 3, 7 or 8 β pyrrole); 8.94 (1H, d, $J=4.8$ Hz, H-2, 3, 7 or 8 β pyrrole). ^{13}C NMR ($CDCl_3$, 100 MHz) δ 20.3, 20.4 (4C, *C*H_3CO); 21.5 (3C, CH_3 tolyl); 28.0 (1C, C-γ); 29.8 (1C, C-β); 60.5 (1C, C-6′ ose); 64.3 (1C, C-4′ ose); 66.1 (1C, C-α); 69.6 (1C, C-2′ ose); 71.0 (1C, C-3′ ose); 74.6 (1C, C-5′ ose); 82.7 (1C, C-1′ ose); 111.9 (1C, C-3 aryl); 116.0 (1C, C *meso*); 119.7 (1C, C-5 aryl); 120.0, 120.1, 120.3 (3C, C *meso*); 127.4 (6C, C-3, 5 tolyl); 130.0 (1C, C-4 aryl); 131.1 (8C, C β pyrrole); 131.4 (1C, C-1 aryl); 134.6 (6C, C-2, 6 tolyl); 135.4 (1C, C-6 aryl); 137.3 (3C, C-4 tolyl); 139.1, 139.2, 139.3 (3C, C-1 tolyl); 145.0–150.0 (8C, C α pyrrole); 158.6 (1C, C-2 aryl); 169.0, 169.1, 169.9, 170.0 (4C, CH_3*C*O). MS (MALDI) *m*/*z* 1078 ($[M+H]^+$ monoisotopic). Anal. calcd for $C_{64}H_{60}N_4O_{10}S$: C, 71.36; H, 5.61; N, 5.20. Found: C, 71.40; H, 5.63; N, 5.21.

5-[4-(1-*S*-(2,3,4,6-tetra-*O*-acetyl-1-thio- α-D-mannopyranosyl)-3-thio-propanoxy)-phenyl]-10,15,20-tritolylporphyrin (8b). Compound **5b** and *O*-acetylated-1-*S*-acetyl-1-thio-β-D-manno-pyranose (**3**) gave, after 6 h of reaction, 42 mg of **8b** (76%). R_f 0.60 (CH_2Cl_2/EtOH, 98:2). UV–vis (CH_2Cl_2) λ (ε×10^{-3}) 420 (325.0) 516 (11.9) 552 (7.5) 592 (3.8) 648 (3.5). ^{1}H NMR ($CDCl_3$, 400 MHz) δ −2.70 (2H, br s, NH); 2.03, 2.07, 2.15, 2.27 (12H, s, OAc); 2.33 (2H, m, H-β); 2.72 (9H, s, CH_3 tolyl); 3.08 (2H, br t, $J=7.0$ Hz, H-γ); 3.78 (1H, ddd, $J=10.0$–5.9–2.6 Hz, H-5′ ose); 4.16 (1H, dd, $J=12.2$–6.0 Hz, H-6$_a$′ ose); 4.23 (1H, dd, $J=12.2$–2.6 Hz, H-6$_b$′ ose); 4.30 (2H, m, H-α); 4.91 (1H, d, $J=1.0$ Hz, H-1′ ose); 5.14 (1H, dd, $J=10.1$–3.7 Hz, H-3′ ose); 5.34 (1H, t, $J=10.1$ Hz, H-4′ ose); 5.64 (1H, dd, $J=3.7$–1.0 Hz, H-2′ ose); 7.26 (2H, d, $J=8.6$ Hz, H-3, 5 aryl); 7.57 (6H, d, $J=7.8$ Hz, H-3, 5 tolyl); 8.12 (6H, d, $J=7.8$ Hz, H-2, 6 tolyl); 8.14 (2H, d, $J=8.6$ Hz H-2, 6 aryl); 8.89 (8H, s, H β pyrrole). ^{13}C NMR ($CDCl_3$, 100 MHz) δ 20.5, 20.6, 20.8 (4C, *C*H_3CO); 21.5 (3C, CH_3 tolyl); 28.7 (1C, C-γ); 29.8 (1C, C-β); 62.9 (1C, C-6′ ose); 66.1 (1C, C-α); 65.9 (1C, C-4′ ose); 70.6 (1C, C-2′ ose); 71.9 (1C, C-3′ ose); 76.8 (1C, C-5′ ose); 83.4 (1C, C-1′ ose); 112.7 (2C, C-3, 5 aryl); 119.6 (1C, C *meso*); 120.0 (3C, C *meso*); 127.4 (6C, C-3, 5 tolyl); 131.1 (8C, C β pyrrole); 134.5 (6C, C-2, 6 tolyl); 134.7 (1C, C-1 aryl); 135.6 (2C, C-2, 6 aryl); 137.3 (3C, C-4 tolyl); 139.3 (3C, C-1 tolyl); 145.0–150.0 (8C, C α pyrrole); 158.5 (1C, C-4 aryl); 169.6, 170.0, 170.2, 170.6 (4C, CH_3*C*O). MS (MALDI) *m*/*z* 1078.3 ($[M+H]^+$ monoisotopic). Anal. calcd for $C_{64}H_{60}N_4O_{10}S$: C, 71.36; H, 5.61; N, 5.20. Found: C, 71.42; H, 5.49; N, 5.11.

Protoporphyrin IX was converted to its dimethyl ester (**12**) by the method of Smith.[19]

2, 4-Bis (2-hydroxyethyl)-6,7-bis[2-(methoxycarbonyl)-ethyl]-1,3,5,8-tetramethylporphyrin (13) was synthesized according to the literature.[13] R_f 0.47 (CH_2Cl_2/MeOH, 9:1). UV–vis (CH_2Cl_2) λ (ε×10^{-3}) 400 (159.6) 498 (9.2) 532 (7.9) 568 (6.3) 620 (2.7). ^{1}H NMR ($CDCl_3$, 400 MHz) δ −4.23 (2H, br s, NH); 3.54, 3.56, 3.57, 3.58 (18H, s, COOCH_3 and CH_3 β pyrrole); 3.25, 3.27 (4H, t, $J=7.8$ Hz, CH_2–COOCH$_3$); 4.16, 4.20 (8H, m, CH_2–CH_2–OH); 4.28, 4.31 (4H, t, $J=7.8$ Hz, CH_2–CH_2–COOCH$_3$); 10.02, 10.07, 10.09 (4H, s, H *meso*). ^{13}C NMR ($CDCl_3$, 100 MHz) δ 11.2, 11.3, 11.4, 11.5 (4C, CH_3 β pyrrole); 21.2 (2C, *C*H_2–CH_2–COOCH$_3$); 30.1 (2C, *C*H_2–CH_2–OH); 36.4 (2C, CH_2–*C*H_2–COOCH$_3$); 51.4 (2C, COO*C*H$_3$); 63.0 (2C, CH_2–*C*H_2–OH); 96.4, 96.6, 96.8, 96.9 (4C, C *meso*); 136–148 (16C, C α and β pyrrole); 173.0 (2C, *C*OOCH$_3$). MS (MALDI) *m*/*z* 627.49 ($[M+H]^+$ monoisotopic).

2,4-Bis (2-bromoethyl)-6,7-bis[2-(methoxycarbonyl)-ethyl]-1,3,5,8-tetramethylporphyrin (14) was synthesized according to the literature.[14] The porphyrin was purified by preparative TLC on silica gel with 3% EtOH/CH_2Cl_2 and demetalated with 10% concentrated HCl in chloroform. The solution was washed with saturated sodium hydrogencarbonate and the solvent removed. 90 mg of **14** were obtained (75%). R_f 0.42 (CH_2Cl_2/EtOH, 97:3). UV–vis (CH_2Cl_2) λ (ε×10^{-3}) 402 (331.3) 498 (24.7) 532 (18.5) 568 (13.7) 622 (8.0). ^{1}H NMR ($CDCl_3$, 400 MHz) δ −4.25 (2H, br s, NH); 3.20, 3.22 (4H, t, $J=7.8$ Hz, CH_2–COOCH$_3$); 3.34, 3.40, 3.47, 3.51 (12H, s, CH_3 β pyrrole); 3.64, 3.66 (6H, s, COOCH_3); 3.95, 4.02 (4H, t, $J=7.9$ Hz, CH_2–CH_2–Br); 4.24, 4.26 (4H, t, $J=7.8$ Hz, CH_2–CH_2–COOCH$_3$); 4.30, 4.35 (4H, t, $J=7.9$ Hz, CH_2–CH_2–Br); 9.58, 9.70, 9.75, 9.85 (4H, s, H *meso*).

^{13}C NMR ($CDCl_3$, 100 MHz) δ 11.6, 11.7, 11.8 (4C, CH_3 β pyrrole); 21.7 (2C, CH_2–CH_2–$COOCH_3$); 30.1, 30.2 (2C, CH_2–CH_2–Br); 32.9 (2C, CH_2–CH_2–Br); 36.4 (2C, CH_2–CH_2–$COOCH_3$); 51.4 (2C, $COOCH_3$); 96.4, 96.6, 96.8, 96.9 (4C, C *meso*); 136–148 (16C, C α and β pyrrole); 173.0 (2C, $COOCH_3$). MS (MALDI) *m/z* 627.49 ($[M+H]^+$ monoisotopic). Anal. calcd for $C_{36}H_{40}N_4O_4Br_2$: C, 57.46; H, 5.36; N, 7.43. Found: C, 57.41; H, 5.41; N, 7.37.

General procedure for thioglycosylation of 2,4-bis (2-bromoethyl)deuteroporphyrin dimethyl ester

2,4-Bis (2-bromoethyl)deuteroporphyrin dimethyl ester **14** (70 mg, 0.093 mmol) was dissolved in dry DMF (10 mL). Then acetylated 1-thiosugar (152 mg, 0.372 mmol, 4 equiv) and diethylamine (0.385 mL, 3.72 mmol, 40 equiv) were added at room temperature under a nitrogen atmosphere. The mixing was stirred for several hours. Diethylamine and dimethylformamide were removed in vacuo. Purification by TLC on silica gel (CH_2Cl_2/EtOH or toluene/acetone) gave the desired compound.

2,4-Bis[2-(1-*S*-(2,3,4,6-tetra-*O*-acetyl-1-thio-β-D-galacto-pyranosyl))ethyl]-6,7-bis-[2-(methoxycarbonyl)-ethyl]-1,3,5,8-tetramethylporphyrin (15). After 4 h of reaction, 99 mg of **15** are obtained (80%). R_f 0.60 (CH_2Cl_2/EtOH, 96:4). UV–vis (CH_2Cl_2) λ ($\varepsilon\times10^{-3}$) 400 (208.5) 498 (15.8) 532 (11.3) 568 (8.1) 622 (5.0). ^{1}H NMR ($CDCl_3$, 400 MHz) δ −3.81 (2H, br s, NH); 1.82, 1.83, 1.98, 1.99, 2.00, 2.01, 2.02 (24H, s, OAc); 3.28, 3.29 (4H, t, $J=7.5$ Hz, CH_2–$COOCH_3$); 3.58 (4H, m, CH_2–CH_2–S); 3.63, 3.66, 3.67, 3.68 (18H, s, CH_3 β pyrrole and $COOCH_3$); 3.91 (1H, td, $J=6.6-<1.0$ Hz, H-5′ ose); 3.95 (1H, td, $J=6.6-<1.0$ Hz, H-5′ ose); 4.10 (1H, dd, $J=11.4$–6.5 Hz, H-6_a′ ose); 4.11 (1H, dd, $J=11.4$–6.5 Hz, H-6_a′ ose); 4.14 (1H, dd, $J=11.5$–6.8 Hz, H-6_b′ ose); 4.15 (1H, dd, $J=11.5$–6.8 Hz, H-6_b′ ose); 4.33 (4H, m, CH_2–CH_2–S); 4.41 (4H, m, CH_2–CH_2–$COOCH_3$); 4.67 (1H, d, $J=10.1$ Hz, H-1′ ose); 4.70 (1H, d, $J=10.1$ Hz, H-1′ ose); 5.05 (1H, dd, $J=10.1$–3.3 Hz, H-3′ ose); 5.08 (1H, dd, $J=10.1$–3.3 Hz, H-3′ ose); 5.43 (2H, m, H-4′ ose); 5.43 (1H, t, $J=10.1$ Hz, H-2′ ose); 5.47 (1H, t, $J=10.1$ Hz, H-2′ ose); 10.04, 10.06, 10.07, 10.09 (4H, s, H *meso*). ^{13}C NMR ($CDCl_3$, 100 MHz) δ 11.7, 11.8, 11.9, 12.0 (4C, CH_3 β pyrrole); 20.5, 20.6, 20.8 (8C, CH_3CO); 21.9 (2C, CH_2–CH_2–$COOCH_3$); 27.4 (2C, CH_2–CH_2–S); 32.1 (2C, CH_2–CH_2–S); 36.9, 37.1 (2C, CH_2–CH_2–$COOCH_3$); 51.7 (2C, $COOCH_3$); 61.5 (2C, C-6′ ose); 67.2 (2C, C-2′ or 4′ ose); 67.3 (2C, C-2′ or 4′ ose); 71.9 (2C, C-3′ ose); 74.7 (2C, C-5′ ose); 84.3 (2C, C-1′ ose); 96.5, 96.6, 96.7, 97.0 (4C, C *meso*); 136–148 (16C, C α and β pyrrole); 169.6, 170.0, 170.2, 170.3 (8C, CH_3CO); 173.6 (2C, $COOCH_3$). MS (MALDI) *m/z* 1320.08 ($[M+H]^+$ monoisotopic). Anal. calcd for $C_{64}H_{78}N_4O_{22}S_2$: C, 58.26; H, 5.96; N, 4.24. Found: C, 58.20; H, 5.93; N, 4.15.

2,4-Bis[2-(1-*S*-(2,3,4,6-tetra-*O*-acetyl-1-thio-β-D-gluco-pyranosyl))ethyl]-6,7-bis-[2-(methoxycarbonyl)-ethyl]-1,3,5,8-tetramethylporphyrin (16). After 4 h of reaction, 78 mg of **16** are obtained (64%). R_f 0.48 (CH_2Cl_2/EtOH, 96:4). UV–vis (CH_2Cl_2) λ ($\varepsilon\times10^{-3}$) 402 (255.8) 498 (18.6) 532 (14.4) 568 (10.9) 622 (6.0). ^{1}H NMR ($CDCl_3$, 400 MHz) δ −3.90 (2H, br s, NH); 1.80, 1.92, 1.93, 1.98, 2.00, 2, .01 (24H, s, OAc); 3.27, 3.28 (4H, t, $J=7.5$ Hz, CH_2–$COOCH_3$); 3.52 (4H, m, CH_2–CH_2–S); 3.61, 3.65, 3.67 (18H, s, CH_3 β pyrrole and $COOCH_3$); 3.63 (1H, m, H-5′ ose); 3.71 (1H, ddd, $J=9.8$–4.8–2.3 Hz, H-5′ ose); 4.09 (1H, dd, $J=12.5$–2.2 Hz, H-6_a′ ose); 4.12 (1H, dd, $J=12.5$–2.2 Hz, H-6_a′ ose); 4.17 (1H, dd, $J=12.5$–4.9 Hz, H-6_b′ ose); 4.22 (1H, dd, $J=12.5$–4.9 Hz, H-6_b′ ose); 4.30 (4H, m, CH_2–CH_2–S); 4.38 (2H, t, $J=7.5$ Hz, CH_2–CH_2–$COOCH_3$); 4.40 (2H, t, $J=7.5$ Hz, CH_2–CH_2–$COOCH_3$); 4.63 (1H, d, $J=9.6$ Hz, H-1′ ose); 4.69 (1H, d, $J=9.6$ Hz, H-1′ ose); 5.10 (2H, H-4′ ose); 5.23 (4H, m, H-2′ and 3′ ose); 10.00, 10.01, 10.02, 10.05 (4H, s, H *meso*). ^{13}C NMR ($CDCl_3$, 100 MHz) δ 11.6, 11.7, 11.8, 11.9 (4C, CH_3 β pyrrole); 20.5, 20.6 (8C, CH_3CO); 21.8 (2C, CH_2–CH_2–$COOCH_3$); 27.2, 27.3 (2C, CH_2–CH_2–S); 32.1, 32.3 (2C, CH_2–CH_2–S); 36.9 (2C, CH_2–CH_2–$COOCH_3$); 51.7 (2C, $COOCH_3$); 62.0, 62.1 (2C, C-6′ ose); 68.3, 68.4 (2C, C-4′ ose); 69.7, 69.8 (2C, C-2′ ose); 73.8, 73.9 (2C, C-3′ ose); 76.0, 76.1 (2C, C-5′ ose); 83.8, 84.0 (2C, C-1′ ose); 96.5, 96.6, 97.0 (4C, C *meso*); 136–145 (16C, C α and β pyrrole); 169.4, 170.1, 170.6 (8C, CH_3CO); 173.6 (2C, $COOCH_3$). MS (MALDI) *m/z* 1320.04 ($[M+H]^+$ monoisotopic). Anal. calcd for $C_{64}H_{78}N_4O_{22}S_2$: C, 58.26; H, 5.96; N, 4.24. Found: C, 58.18; H, 5.95; N, 4.12.

2,4-Bis[2-(1-*S*-(2,3,4,6-tetra-*O*-acetyl-1-thio-α-D-manno-pyranosyl))ethyl]-6,7-bis-[2-(methoxycarbonyl)-ethyl]-1,3,5,8-tetramethylporphyrin (17). After 2 h of reaction, 113 mg of **17** are obtained (92%). R_f 0.60 (CH_2Cl_2/EtOH, 96:4). UV–vis (CH_2Cl_2) λ ($\varepsilon\times10^{-3}$) 400 (246.5) 498 (18.8) 532 (14.0) 568 (10.1) 622 (6.2). ^{1}H NMR ($CDCl_3$, 400 MHz) δ −3.79 (2H, br s, NH); 2.00, 2, .01, 2.06, 2.09 (24H, s, OAc); 3.27, 3.29 (4H, t, $J=7.5$ Hz, CH_2–$COOCH_3$); 3.43 (2H, m, CH_2–CH_2–S); 3.52 (2H, m, CH_2–CH_2–S); 3.61, 3.63, 3.65, 3.67 (18H, s, CH_3 β pyrrole and $COOCH_3$); 4.09 (1H, dd, $J=12.1$–2.1 Hz, H-6_a′ ose); 4.11 (1H, dd, $J=12.1$–2.1 Hz, H-6_a′ ose); 4.26 (1H, dd, $J=12.1$–5.9 Hz, H-6_b′ ose); 4.27 (1H, dd, $J=12.1$–5.9 Hz, H-6_b′ ose); 4.38 (4H, m, CH_2–CH_2–S); 4.39 (2H, t, $J=7.5$ Hz, CH_2–CH_2–$COOCH_3$); 4.40 (2H, t, $J=7.5$ Hz, CH_2–CH_2–$COOCH_3$); 4.52 (2H, ddd, $J=9.9$–6.0–2.1, H-5′ ose); 5.30 (1H, t, $J=9.9$ Hz, H-4′ ose); 5.31 (1H, t, $J=9.9$ Hz, H-4′ ose); 5.35 (1H, dd, $J=9.9$–3.2 Hz, H-3′ ose); 5.36 (1H, dd, $J=9.9$–3.2 Hz, H-3′ ose); 5.46 (1H, dd, $J=3.2$–1.0 Hz, H-2′ ose); 5.47 (1H, dd, $J=3.2$–1.0 Hz, H-2′ ose); 5.57 (1H, d, $J=1.0$ Hz, H-1′ ose); 5.61 (1H, d, $J=1.0$ Hz, H-1′ ose); 10.01, 10.03, 10.04, 10.07 (4H, s, H *meso*). ^{13}C NMR ($CDCl_3$, 100 MHz) δ 11.6, 11.7, 11.8, 11.9 (4C, CH_3 β pyrrole); 20.2, 20.3, 20.6, 20.7, 20.8, 20.9 (8C, CH_3CO); 21.8 (2C, CH_2–CH_2–$COOCH_3$); 27.2, 27.3 (2C, CH_2–CH_2–S); 33.6 (2C, CH_2–CH_2-S); 36.8, 36.9 (2C, CH_2–CH_2–$COOCH_3$); 51.7 (2C, $COOCH_3$); 62.6 (2C, C-6′ ose); 66.5 (2C, C-4′ ose); 69.2 (2C, C-5′ ose); 69.5 (2C, C-3′ ose); 71.1 (2C, C-2′ ose); 83.0, 83.1 (2C, C-1′ ose); 96.4, 96.5, 96.6, 97.1 (4C, C *meso*); 136–145 (16C, C α and β pyrrole); 169.7, 169.8, 169.9, 170.0, 170.4, 170.5 (8C, CH_3CO); 173.5, 173.6 (2C, $COOCH_3$). MS (MALDI) *m/z* 1320.25 ($[M+H]^+$ monoisotopic). Anal. calcd for $C_{64}H_{78}N_4O_{22}S_2$: C, 58.26; H, 5.96; N, 4.24. Found: C, 58.23; H, 5.92; N, 4.27.

General procedure for deacetylation of mono and diglycosylated porphyrins 6–8 and 15–17

Porphyrins **6a**, **b**, **7a**, **b**, **8a**, **b**, **15**, **16** and **17** (30 mg) were dissolved into CH_2Cl_2/MeOH, 7:3 and a solution of sodium methoxide in methanol was added. After 1 h at room temperature, the desired porphyrin was precipitated by addition of petroleum ether and filtered.

5-[2-(1-*S*-1-Thio-β-D-galactopyranosyl-3-thio-propanoxy)-phenyl]-10,15,20-tritolyl-porphyrin (9a). 24 mg obtained (96%). R_f 0.53 (CH_2Cl_2/MeOH, 9:1). UV–vis (THF/H_2O, 8:2) λ (ε×10^{-3}) 418 (208.4) 514 (8.5) 548 (4.3) 594 (2.5) 650 (3.2). MS (MALDI) *m*/*z* 911.0 ($[M+H]^+$ monoisotopic). Anal. calcd for $C_{56}H_{52}N_4O_6S$: C, 73.99; H, 5.76; N, 6.16. Found: C, 74.02; H, 5.70; N, 6.10.

5-[4-(1-*S*-1-Thio-β-D-galactopyranosyl-3-thio-propanoxy)-phenyl]-10,15,20-tritolyl-porphyrin (9b). 24 mg obtained (96%). R_f 0.48 (CH_2Cl_2/MeOH, 9:1). UV–vis (THF/H_2O, 8:2) λ (ε×10^{-3}) 418 (319.4) 516 (13.2) 550 (7.9) 592 (3.8) 650 (3.8). MS (MALDI) *m*/*z* 910.4 ($[M+H]^+$ monoisotopic). Anal. calcd for $C_{56}H_{52}N_4O_6S$: C, 73.99; H, 5.76; N, 6.16. Found: C, 74.14; H, 5.69; N, 6.07.

5-[2-(1-*S*-1-Thio-β-D-glucopyranosyl-3-thio-propanoxy)-phenyl]-10,15,20-tritolyl-porphyrin (10a). 24 mg obtained (96%). R_f 0.36 (CH_2Cl_2/MeOH, 9:1). UV–vis (THF/H_2O, 8:2) λ (ε×10^{-3}) 418 (370.8) 514 (13.5) 548 (7.1) 592 (4.1) 650 (5.2). MS (MALDI) *m*/*z* 911.0 ($[M+H]^+$ monoisotopic). Anal. calcd for $C_{56}H_{52}N_4O_6S$: C, 73.99; H, 5.76; N, 6.16. Found: C, 73.87; H, 5.70; N, 6.12.

5-[4-(1-*S*-1-Thio-β-D-glucopyranosyl-3-thio-propanoxy)-phenyl]-10,15,20-tritolyl-porphyrin (10b). 24 mg obtained (96%). R_f 0.51 (CH_2Cl_2/MeOH, 9:1). UV–vis (THF/H_2O, 8:2) λ (ε×10^{-3}) 418 (294.6) 514 (11.2) 550 (6.7) 592 (3.3) 650 (3.2). MS (MALDI) *m*/*z* 910.9 ($[M+H]^+$ monoisotopic). Anal. calcd for $C_{56}H_{52}N_4O_6S$: C, 73.99; H, 5.76; N, 6.16. Found: C, 73.94; H, 5.72; N, 6.04.

5-[2-(1-*S*-1-Thio-α-D-mannopyranosyl-3-thio-propanoxy)-phenyl]-10,15,20-tritolyl-porphyrin (11a). 24 mg obtained (96%). R_f 0.51 (CH_2Cl_2/MeOH, 9:1). UV–vis (THF/H_2O, 8:2) λ (ε×10^{-3}) 418 (425.0) 514 (17.7) 548 (9.1) 592 (5.2) 650 (6.8). MS (MALDI) *m*/*z* 910.3 ($[M+H]^+$ monoisotopic). Anal. calcd for $C_{56}H_{52}N_4O_6S$: C, 73.99; H, 5.76; N, 6.16. Found: C, 74.05; H, 5.66; N, 6.05.

5-[4-(1-*S*-1-Tthio-α-D-mannopyranosyl-3-thio-propanoxy)-phenyl]-10,15,20-tritolyl-porphyrin (11b). 24 mg obtained (96%). R_f 0.56 (CH_2Cl_2/MeOH, 9:1). UV–vis (THF/H_2O, 8:2) λ (ε×10^{-3}) 418 (453.2) 516 (17.0) 550 (10.4) 594 (5.1) 650 (4.8). MS (MALDI) *m*/*z* 910.4 ($[M+H]^+$ monoisotopic). Anal. calcd for $C_{56}H_{52}N_4O_6S$: C, 73.99; H, 5.76; N, 6.16. Found: C, 73.98; H, 5.72; N, 6.13.

2,4-Bis[2-(1-*S*-1-Thio-β-D-galactopyranosyl)ethyl]-6,7-bis-[2-(methoxycarbonyl)-ethyl]-1,3,5,8-tetramethylporphyrin (18). 21 mg obtained (92%). R_f 0.72 (THF/MeOH/H_2O, 8:1:1). UV–vis (THF/H_2O, 8:2) λ (ε×10^{-3}) 400 (128.4) 498 (10.4) 530 (8.2) 568 (6.0) 622 (3.9). UV–vis (H_2O) λ (ε×10^{-3}) 374 (73.0) 512 (8.4) 550 (6.9) 580 (6.5) 636 (3.4). MS (MALDI) *m*/*z* 983.46 ($[M+H]^+$ monoisotopic). Anal. calcd for $C_{48}H_{62}N_4O_{14}S_2$: C, 58.64; H, 6.36; N, 5.70. Found: C, 58.69; H, 6.30; N, 5.71.

2,4-Bis[2-(1-*S*-1-thio-β-D-glucopyranosyl)ethyl]-6,7-bis-[2-(methoxycarbonyl)-ethyl]-1,3,5,8-tetramethylporphyrin (19). 21 mg obtained (92%). R_f 0.68 ($CHCl_3$/MeOH, 7:3). UV–vis (THF/H_2O, 8:2) λ (ε×10^{-3}) 400 (198.8) 498 (16.9) 530 (12.3) 568 (8.4) 622 (6.3). UV–vis (H_2O) λ (ε×10^{-3}) 372 (72.6) 512 (6.9) 550 (5.8) 574 (5.0) 626 (2.1). MS (MALDI) *m*/*z* 983.26 ($[M+H]^+$ monoisotopic). Anal. calcd for $C_{48}H_{62}N_4O_{14}S_2$: C, 58.64; H, 6.36; N, 5.70. Found: C, 58.67; H, 6.28; N, 5.65.

2,4-Bis[2-(1-*S*-1-thio-α-D-mannopyranosyl)ethyl]-6,7-bis-[2-(methoxycarbonyl)-ethyl]-1,3,5,8-tetramethylporphyrin (20). 21 mg obtained (92%). R_f 0.70 ($CHCl_3$/MeOH, 7:3). UV–vis (THF/H_2O, 8:2) λ (ε×10^{-3}) 400 (195.9) 498 (11.3) 530 (8.4) 568 (5.7) 622 (4.8). UV–vis (H_2O) λ (ε×10^{-3}) 374 (74.0) 516 (8.9) 550 (8.8) 574 (8.5) 628 (3.9). MS (MALDI) *m*/*z* 984.26 ($[M+H]^+$ monoisotopic). Anal. calcd for $C_{48}H_{62}N_4O_{14}S_2$: C, 58.64; H, 6.36; N, 5.70. Found: C, 58.58; H, 6.35; N, 5.68.

General procedure for total deprotection of diglycosylated porphyrins 15–17

Porphyrins **15**, **16** and **17** (60 mg) were dissolved in CH_2Cl_2/MeOH, 5:5 and a solution of potassium hydroxide in MeOH/H_2O, 6:4 was added. The mixture was refluxed 4 h then neutralized with glacial acetic acid. The desired porphyrin was filtered and purified by gel filtration on a Sephadex LH20 column eluted with acetone/MeOH/H_2O, 3:1.5:5.5.

2,4-Bis[2-(1-*S*-1-thio-β-D-galactopyranosyl)ethyl]-6,7-bis-[2-(carboxyethyl]-1,3,5,-8-tetramethylporphyrin (21). 41 mg of **21** were obtained (94%). R_f 0.61 (acetone/MeOH/H_2O, 6:3:1). UV–vis (acetone/H_2O, 6:4) λ (ε×10^{-3}) 396 (131.0) 496 (15.9) 530 (11.7) 566 (8.1) 620 (5.3). UV–vis (H_2O) λ (ε×10^{-3}) 372 (118.1) 506 (6.8) 540 (6.9) 564 (6.5) 616 (3.0). MS (MALDI) *m*/*z* 955.65 ($[M+H]^+$ monoisotopic). Anal. calcd for $C_{46}H_{58}N_4O_{14}S_2$: C, 57.85; H, 6.12; N, 5.86. Found: C, 57.77; H, 6.27; N, 5.83.

2,4-Bis[2-(1-*S*-1-thio-β-D-glucopyranosyl)ethyl]-6,7-bis-[2-(carboxyethyl]-1,3,5,8-tetramethylporphyrin (22). 41 mg of **22** were obtained (94%). R_f 0.59 (acetone/MeOH/H_2O, 6:3/1). UV–vis (acetone/H_2O, 6:4) λ (ε×10^{-3}) 398 (176.2) 498 (12.4) 534 (11.2) 566 (8.2) 620 (4.0). UV–vis (H_2O) λ (ε×10^{-3}) 372 (109.1) 506 (5.5) 540 (4.8) 566 (4.3) 618 (2.0). MS (MALDI) *m*/*z* 983.26 ($[M+H]^+$ monoisotopic). Anal. calcd for $C_{46}H_{58}N_4O_{14}S_2$: C, 57.85; H, 6.12; N, 5.86. Found: C, 57.91; H, 6.10; N, 5.88.

2,4-Bis[2-(1-*S*-1-thio-α-D-mannopyranosyl)ethyl]-6,7-bis-[2-(carboxyethyl]-1,3,5,-8-tetramethylporphyrin (23). 39 mg of **23** were obtained (90%). R_f 0.56 (THF/MeOH/

H_2O, 8:1:1). UV–vis (acetone/H_2O, 6:4) λ (ε×10^{-3}) 396 (99.3) 498 (8.3) 530 (6.3) 566 (4.6) 620 (3.1). UV–vis (H_2O) λ (ε×10^{-3}) 372 (79.8) 506 (5.2) 540 (4.9) 566 (4.6) 616 (2.6). MS (MALDI) *m/z* 984.26 ($[M+H]^+$ monoisotopic). Anal. calcd for $C_{46}H_{58}N_4O_{14}S_2$: C, 57.85; H, 6.12; N, 5.86. Found: C, 57.80; H, 6.23; N, 5.90.

Biochemicals

A stock solution of propidium iodide (PI) (Sigma) was prepared in distilled water, filter sterilized and used at 1×10^{-4}M. Almond beta-glucosidase (2 units/mg) was purchased from Sigma.

In vitro photocytotoxicity

Phototoxicity of synthetic porphyrins was tested against K562 human chronic myelogenous leukemia cell line. Porphyrin concentration of 2×10^{-6} M was chosen because it did not affect cellular growth for 3 days in the dark. Photofrin® was tested at 1.2 μg/ml; this ponderal concentration corresponds to ca. 2.10^{-6} M of hematoporphyrin.

Cell culture

K562 cells were suspended in HEPES-buffered RPMI 1640 medium (Sigma, R4130) pH = 7.0 ± 0.3 containing 2 mM L-glutamine supplemented with 2 g/L $NaHCO_3$, 50 U/mL penicillin, 50 mg/mL streptomycin (Sigma, P0906), and 10% (v/v) fetal bovine serum (Biochrom KG, Polylabo 60810). Cells were picked during exponential growth, washed and diluted to 10^6 cells/mL with fresh RPMI medium. This dilution was then distributed in 24-well plates (2 mL/well). Porphyrins (final concentration 2×10^{-6} M) and Photofrin® (final concentration 1.2 μg/mL) were added to wells before illumination. The cultures were incubated at 37 °C in a humidified atmosphere containing 5% CO_2 in air.

Cell illumination

Cells were irradiated with white bulbs (fluence rate: 50 W/m^2) for 0 to 120 min.

Flow cytometry

Dead cells were identified as propidium iodide (PI) permeable ones and the counts were measured by flow cytometry. Samples were analyzed in a Coulter Epics XL System II™, immediately after each illumination time (J0). Cell suspensions were then incubated in the dark at 37 °C for 24h and the dead cell counts were estimated thereafter (J1).

Glucosidase assay

Almond glucosidase from Sigma (3 units) was added to 2×10^{-6} M glucosyl- and thioglucosyl-porphyrins dissolved in 0.067 M *o*-phtalate buffer pH 5.0 at 37 °C. Incubation was conducted during 15 min and reactions products were characterized by TLC.

References and Notes

1. Kessel, D. *Cancer Lett*. **1986**, *33*, 183.
2. Shulock, J. R.; Wade, M. H.; Lin, C.-W. *Photochem. Photobiol*. **1990**, *51*, 451.
3. (a) Kieda, C.; Monsigny, M. *Invasion Metastasis* **1986**, *6*, 347. (b) Monsigny, M.; Roche, A.C.; Midoux, P.; Kieda, C.; Mayer, R. In *Lectins and Glycoconjugates in Oncology: Structure, Function, Clinical Application*; Gabius, H.J.; Nagel, G.A., Eds; Springer: Heidelberg, 1988, 1. (c) Sharon, N.; Lis, H. *Science* **1989**, *246*, 227.
4. Schell, C.; Hombrecher, H. K. *Chem. Eur. J*. **1999**, *5*, 587.
5. (a) Sol, V.; Blais, J. C.; Carré, V.; Granet, R.; Guilloton, M.; Spiro, M.; Krausz, P. *J. Org. Chem*. **1999**, *64*, 4431. (b) Davoust, E.; Granet, R.; Carré, C.; Guilloton, M.; Krausz, P. *Tetrahedron Lett*. **1999**, *40*, 2513. (c) Kaldapa, C.; Blais, J.-C.; Carré, C.; Granet, R.; Sol, V.; Guilloton, M.; Spiro, M.; Krausz, P. *Tetrahedron Lett*. **2000**, *41*, 331.
6. Gaud, O.; Granet, R.; Kaouadji, M.; Krausz, P.; Blais, J. C.; Bolbach, G. *Can. J. Chem*. **1996**, *74*, 481.
7. Defaye, J.; Gelas, J. In *Studies in Natural Products Chemistry*; Atta-ur-Rhaman, Ed.; Elsevier: Amsterdam 1991; Vol. 8, p 315.
8. Little, R. G.; Anton, J. A.; Loach, P. A.; Ibers, J. A. *J. Heterocycl. Chem*. **1975**, *12*, 343.
9. Little, R. G. *J. Heterocycl. Chem*. **1978**, *15*, 203.
10. Defaye, J.; Driguez, H.; Ohleyer, E.; Orgeret, C.; Viet, C. *Carbohydrate Research* **1984**, *130*, 317.
11. Bennett, S.; Von Itzstein, M.; Kiefel, M. J. *Carbohydrate Research* **1994**, *259*, 293.
12. Wolfrom, M. L.; Thompson, M. In *Methods in Carbohydrate Chemistry*; Whistler, R. L., Wolfrom, M. L., BeMiller, J. N., Shafizadeh, F. Eds. Academic: New York 1962; Vol. 1, p 334.
13. Smith, K.M. In *Porphyrins and Metalloporphyrins*; Smith, K.M. Ed.; Elsevier: New York, 1975, p 835.
14. Kalh, S. B.; Schaeck, J. J.; Koo, M. S. *J. Org. Chem*. **1997**, *62*, 1875.
15. Snow, K. M.; Smith, K. M. *J. Org. Chem*. **1989**, *54*, 3270.
16. Kenner, G. W.; McCombie, S. W.; Smith, K. M. *Liebigs Ann. Chem*. **1973**, 1329.
17. Karas, M.; Hillenkamp, F. *Anal. Chem*. **1988**, *60*, 2299.
18. (a) Furhop, J. H.; Demoulin, C.; Boettcher, C.; Koning, J.; Siggel, U. *J. Am. Chem. Soc*. **1992**, *114*, 4159. (b) Nagata, T.; Osuka, A.; Maruyama, K. *J. Am. Chem. Soc*. **1990**, *112*, 3054.
19. Smith, K.M. In *Porphyrins and Metalloporphyrins*; Smith, K.M., Ed.; Elsevier: New York, 1975; p 835.