Journal of Carbohydrate Chemistry, 25:345–360, 2006

ISSN: 0732-8303 print 1532-2327 online
DOI: 10.1080/07328300600770527

Synthesis of New Glucosylated Porphyrins Bearing an α-D-Linkage

Vincent Sol, Alexandre Charmot, and Pierre Krausz

Laboratoire de Chimie des Substances Naturelles, Université de Limoges-Faculté des Sciences et Techniques, Limoges Cedex, France

Stéphane Trombotto

Laboratoire d'Ingénierie des Matériaux Polymères, CNRS UMR 5627, Université Claude Bernard Lyon 1, ISTIL, Villeurbanne Cedex, France

Yves Queneau

Laboratoire de Chimie Organique, INSA Lyon, (UMR 5181 CNRS Université Lyon 1-INSA Méthodologies de Synthèse et Molécules Bioactives), Villeurbanne Cedex

This paper presents the synthesis of two new glucosyl tritolylporphyrins in which the carbohydrate moiety is connected through a carboxymethyl glycosidic α-D-linkage. These compounds have been obtained by reaction between porphyrins bearing an amino function with a lactone prepared from the available disaccharide isomaltulose. The photocytotoxicity of these compounds against K562 human chronic myelogenous leukemia cells has been evaluated in comparison to Photofrin II.

Keywords Porphyrins, α-D-glucose, Photodynamic therapy, Isomaltulose

INTRODUCTION

The chemistry of porphyrins and chlorins bearing glycosylated groups has made rapid progress during the last few years owing to the development of new photosensitizers designed for cancer photochemotherapy.[1–11] Indeed, sugar moieties not only modulate the amphiphilicity of the photosensitizers, but also may be involved in specific membrane interactions.[12–14] Moreover,

Received February 12, 2005; accepted May 4, 2006.
Address correspondence to Vincent Sol, Laboratoire de Chimie des Substances Naturelles, Université de Limoges-Faculté des Sciences et Techniques, 123 Avenue Albert Thomas, 87060, Limoges Cedex, France. E-mail: vincent.sol@unilim.fr

the presence of carbohydrate moieties on porphyrins is known to increase their plasmatic life time[15] and to allow cancer cell surface targeting through specific binding to membrane receptors.[16] Use of synthetic carbohydrates as carriers in directed drug delivery may be an interesting approach in cancer cell targeting. In the present work, which is part of an ongoing research program on glycosylated porphyrins, we report the synthesis of two mono-α-D-glucosyl porphyrins (**7a**,**b**). The synthesis (Sch. 1) involves 3,4,6-tri-*O*-acetyl carboxymethyl-α-D-glucopyranoside-2-*O*-lactone **5** as the carbohydrate moiety supplier. This lactone is obtained from the available disaccharide isomaltulose [6-*O*-(α-D-glucopyranosyl)-β-D-fructofuranose] in a straightforward oxidation-acetylation sequence. It was shown to act as an efficient glucoside provider in the synthesis of various derivatives such as surfactants and polymerisable derivatives, as well as new pseudo-glycopeptides.[17,18]

RESULTS AND DISCUSSION

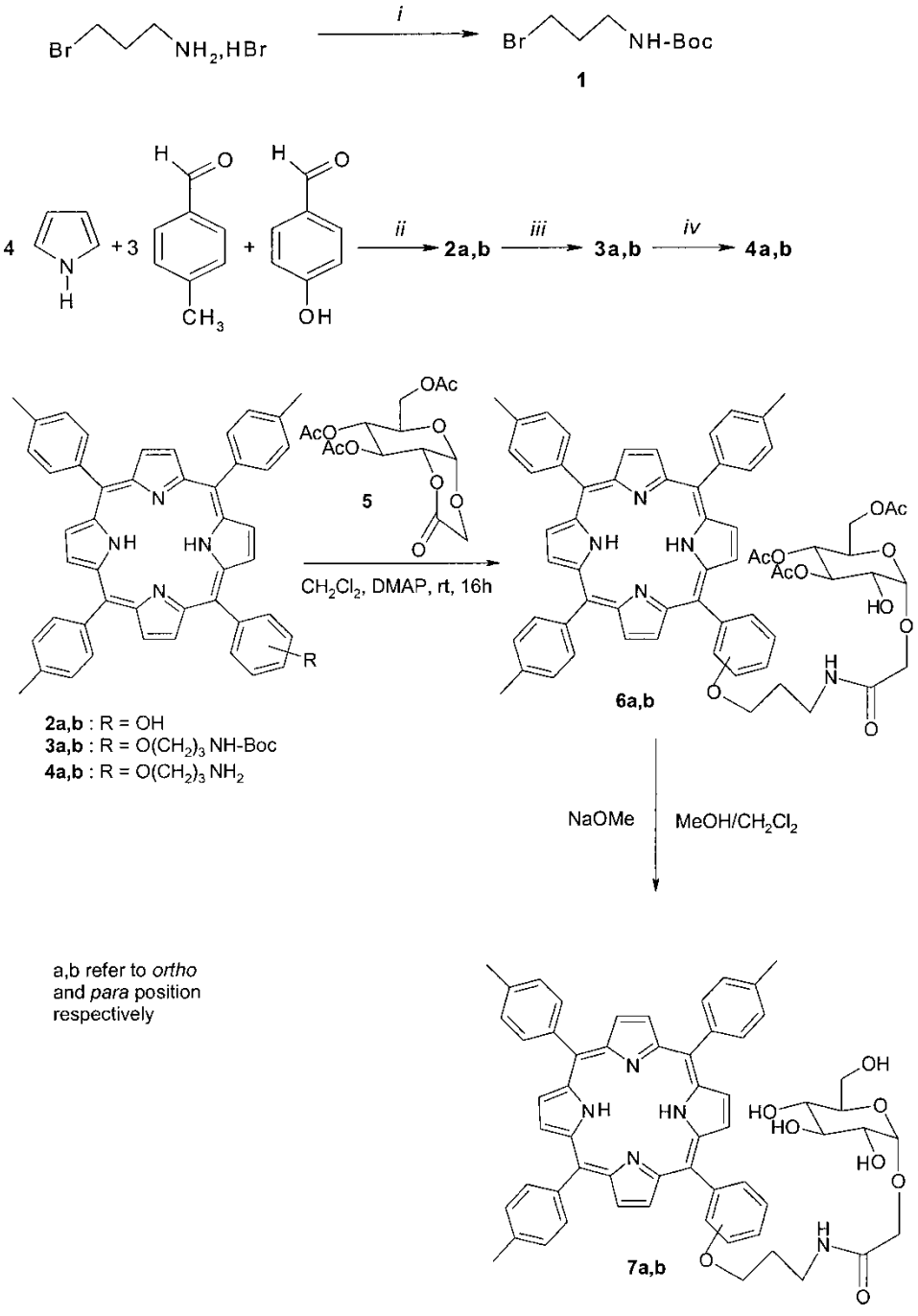
Monohydroxyphenyltritolylporphyrins **2a,b** were prepared according to the Little method[19] by condensation of pyrrole (4 equiv.) with *para*tolualdehyde (3 equiv.) and *ortho*-or *para*hydroxybenzaldehyde (1 equiv.) in propionic acid under reflux. Only *o*- and *p*-monohydroxyphenyltritolylporphyrins **2a,b** were isolated after purification, in 5% to 6% yield, respectively. The amine-Boc protected spacer arm **1** then reacted, in five fold excess, with monohydroxytritolylporphyrin **2a,b** to yield **3a,b** (95%). Acidic removal of the Boc groups gave compound **4a,b** respectively (85%).

Coupling of lactone **5** and the primary amine of porphyrin **4a,b** gave **6a,b** (95%). Finally, conventional transesterification of the acetyl groups gave compounds **7a,b** in 95% yield.

UV-visible absorption spectra of these compounds (**7a,b**) in $CHCl_3$/MeOH (8/2) showed the expected bands: the Soret band around 420 nm and the four less intense visible Q bands between 520 and 650 nm; all the compounds displayed the *etio* spectra ($\varepsilon_{IV} > \varepsilon_{III} > {}_{II} > \varepsilon_I$) characteristic of $\ll$ free-base $\gg$ porphyrins. In THF/H_2O (8/2), the molar absorption coefficients of the two derivatives decrease as shown in Table 1 and the Soret band are broadening and splitting. These results are due to the combination of cofacial and edge-to-edge interaction of self-assembled aggregates.[2,7,20]

All fluorescence spectra of porphyrins **7a,b** in THF were charaterized by two emission bands in the 550 to 750-nm region. The fluorescence emission wavelengths in H_2O/THF (8/2) were identical to those obtained in THF, but the emission was strongly quenched. This decay of fluorescence can be explained by the formation of aggregates[21] and supports the results observed in the UV-visible spectra.

Mass spectrometry analysis of all glucosylated porphyrins was performed using the MALDI (matrix-assisted laser desorption ionization) technique.



Scheme 1: (*i*) DIBOC (2 eq), NEt_3 (1.1 eq), CH_2Cl_2, rt, 1 h 30 min; (*ii*) EtCOOH, 1 h, reflux; (*iii*) $Br(CH_2)_3NH$-Boc (5 eq.) **2**, K_2CO_3 (20 eq.), DMF, 18 h,rt; (*iv*) CH_2Cl_2/TFA (80/20, v/v), 3 h, rt.

Table 1: UV-visible spectra of porphyrin derivatives **7a,b** in various solvents.

		Soret 1		Soret 2		IV		III		II		I	
Derivatives	**Solvents**	λ (nm)	ε (M^{-1} cm^{-1})	λ (nm)	ε (M^{-1} cm^{-1})	λ (nm)	ε (M^{-1} cm^{-1})	λ (nm)	ε (M^{-1} cm^{-1})	λ (nm)	ε (M^{-1} cm^{-1})	λ (nm)	ε (M^{-1} cm^{-1})
7a	$CHCl_3$/MeOH (8/2)	418	361.6×10^3			516	13.4×10^3	552	7.8×10^3	590	4.3×10^3	648	3.9×10^3
	H_2O/THF (2/8)	418	126.9×10^3	442	167.9×10^3	519	5.6×10^3	551	4.3×10^3	592	3.4×10^3	648	2.8×10^3
7b	$CHCl_3$/MeOH (8/2)	418	322.4×10^3			516	12.2×10^3	552	7.1×10^3	592	4.0×10^3	648	3.1×10^3
	H_2O/THF (2/8)	418	110.6×10^3	449	114.6×10^3	524	2.7×10^3	555	2.0×10^3	596	1.5×10^3	653	1.3×10^3

Positive ion mass spectra exhibited a base peak corresponding to the intact porphyrin, and no fragment ions were detected. The isotopic analysis indicated the presence of a protonated species $(M + H)^+$ with a minor contribution of the radical cation $M^{+\cdot}$, allowing the determination of the molecular mass with an accuracy close to 0.001%.[7]

^{1}H NMR spectra of these compounds showed the expected signals. More precisely, analysis of the sugar moieties showed the expected H1′-H2′ coupling interaction corresponding to the awaited α anomer, with $J_{1'\text{-}2'} = 3.5$ Hz and $J_{1'\text{-}2'} = 3.8$ Hz for **6a** and **6b**, respectively. For the *para* compounds **2b**, **3b**, **4b**, **6b,** and **7b**, the resonances of the β-pyrrolic protons appear as a single peak between 8.80 and 8.85 ppm. For *ortho* compounds **6a** and **7a**, all the sugar protons experience a pronounced shielding from −0.43 ppm (H-6′$_a$) to −3.02 ppm (H-2'). These nuclei are obviously located inside the shielding cone of the porphyrin macrocycle. Moreover, the β-pyrrole proton signals of **6a** appear as four doublets ($J = 4.8$ Hz) between 8.92 and 8.77 ppm, and one singlet at 8.90 ppm. According to the literature,[7,10] these phenomena, that is, a shielding of *ortho* substituents and a splitting of β-pyrrolic protons signals, reflect a reduced symmetry induced by the orientation of the substituent that in turn leads to an unsymmetrical folded structure because of steric hindrance.

In Vitro Photocytotoxicity

Experiments were conducted with K562 chronic leukemia cell line. Figure 1 displays dead cell counts and the subsequent changes following a further 24 h incubation in the dark. The results obtained with these synthetic porphyrins have been compared with those observed with Photofrin. These data show that *ortho* porphyrin **7a** is much more active than *para* porphyrin **7b**, although less active than Photofrin at the same ponderal concentration. In addition, a further 24-h incubation does not result in a significant increase in dead cell percentage, contrary to Photofrin; porphyrin **7a** then probably induces early necrotic death and the cellular damages do not lead to secondary necrosis that could be attributed to the induction of apoptosis. It should be noted that these results are reminiscent of those previously observed with *ortho* and *para* β-glycosyl porphyrins.[10] Within the latter series, photoactivity displayed by the *ortho* compounds was always significantly stronger than their *para* counterparts. However, the structural basis of this biological discrepancy is presently unknown.

In order to evaluate the influence of glucosyl units on cell viability, we irradiated K562 cells in the presence of nonglucosylated compound **3a**; 120 min of irradiation was followed by only 15% cell death. This result clearly shows that introduction of a sugar unit as peripherical substituent improves the photoactivity of porphyrin derivatives. Moreover, by comparison with porphyrin

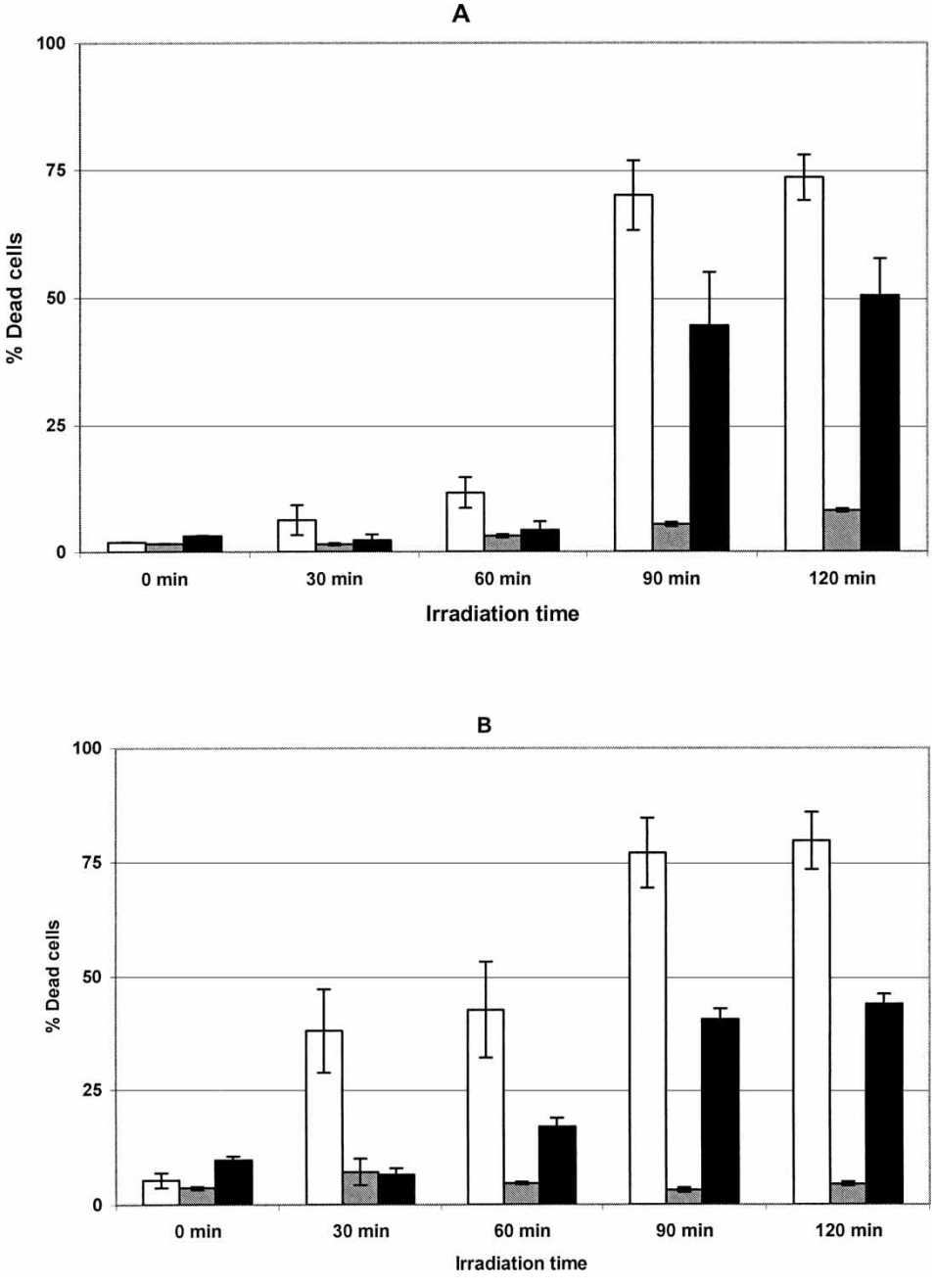


Figure 1: Phototoxicity of α-D-glucosyl porphyrins **7a** and **7b**. The figure shows the percentage of dead cells (PI stained) vs. irradiation time; void bars: effect of Photofrin, hatched bars, porphyrin **7b**; solid bars, porphyrin **7a**; **A**: Dead cell count just after irradiation; **B**: Dead cell count after a further 24 h incubation in the dark. Error bars are based upon standard deviations.

conjugate of comparable structure, previously synthetized in our laboratory,[10] compound **7a** shows similar in vitro photocytotoxicity. The exact role of the sugar residue in the photosensitizing properties remains to be elucidated. Further comparisons with similar prophyrins bearing carbohydrate moieties having either α- or β-anomeric carboxymethyl linkages and based on various mono-or oligosaccharides will be performed.

EXPERIMENTAL

General

All solvents and reagents were purchased from Aldrich, Prolabo, or Acros. Pyrrole was distilled over CaH_2 under reduced pressure immediately before use. Dimethylformamide was distilled over CaH_2 under reduced pressure and stored under Argon. Methylene chloride (CH_2Cl_2) was distilled over P_2O_5 and then CaH_2. Analytical thin-layer chromatography (TLC) was performed on silica gel Merck $60F_{254}$. Column chromatography was carried out with silica gel (60 ACC; 15–40 μm, Merck). ^{1}H NMR spectroscopy was performed with a Brucker DPX-400 spectrometer. Chemical shifts are reported as δ ppm, downfield from internal TMS, and are listed according to the standard numbering of meso-arylporphyrins. UV-visible spectra were recorded on a Perkin Elmer Lambda 25 double-beam spectrophotometer using 10- or 50-mm quartz cells. Infrared spectra were recorded on a Perkin Elmer spectrum 1000 with KBr pellets. MALDI-TOF mass spectra were recorded with a Voyager Elite (Framingham, MA, USA) time-of-flight mass spectrometer equipped with a 337-nm nitrogen laser (VSL 337ND). It was operated in the reflectron delayed extraction mode at an acceleration voltage of 20 kV. Internal standards (peptides) were used to calibrate the mass scale with the two-point calibration Software version 3.07.1 from PerSeptive Biosystems. One microliter of an aceton solution of matrix (α-cyano-4-hydroxycinnamic acid) and compounds at concentrations of 0.1 M and 0.01 mM, respectively, was deposited onto the stainless steel sample slide and dried in air.

N-Tert-butoxycarbonyl-3-bromopropylamine (1)

3-Bromopropylamine hydrobromide (1 g, 4.57 mmol, 1 equiv.) was dissolved in 8 mL of distilled CH_2Cl_2. Triethylamine (0.7 mL, 5.03 mmol, 1.1 equiv.) and diterbutyl dicarbonate (2.1 g, 9.57 mmol, 2 equiv.) were added and the reaction was carried out under an argon atmosphere and stirring during 1 h 30 min at rt. After evaporation under reduced pressure, residue was redissolved in CH_2Cl_2 (20 mL) and washed with water (3 × 20 mL). The organic layer was separated and dried over anhydrous magnesium sulfate, filtered,

concentrated, and subjected to flash chromatography (CH_2Cl_2/EtOH, 100:0 to 50:50) to give compound **1**, which was recrystallized in petroleum ether as white crystals (910.2 mg, 84%). mp: 35–37°C. R_f 0.48 ($CHCl_3$/EtOH 98/2). IR (KBr), ν (cm^{-1}): 3370 (NH); 3000 (CH); 1686 (C=O). ^{1}H NMR (400.13 MHz, $CDCl_3$), δ 1.44 (s, 9H, CH_3); 2.05 (q, 2H, $J = 6.5$ Hz, Br–CH_2–C$\underline{H}_2$–CH_2–NHCO); 3.27 (bt, 2H, $J = 6.5$ Hz, Br–CH_2–CH_2–C$\underline{H}_2$–NHCO); 3.44 (t, 2H, $J = 6.5$ Hz, Br–C$\underline{H}_2$–CH_2–CH_2–NHCO); 4.64 (bs, 1H, NH).

General Procedure for the Synthesis of Monohydroxyphenylporphyrins

These compounds were synthesized according to the Little method.[19] 2- or 4-hydroxybenzaldehyde (1 equiv.) and 4-tolualdehyde (3 equiv.) for porphyrins **2a,b** were dissolved in propionic acid. The mixture was stirred under reflux for 30 min. Pyrrole (4 equiv.) was then added dropwise over the course of 5 min and the mixture was stirred under reflux for a further 1 h 30 min. After cooling, a mixture of *meso* phenylporphyrins crystallized. This mixture was filtered and then the crude compounds **2a,b** were purified by silica gel column chromatography.

5-(2-Hydroxyphenyl)-10,15,20-Tris(4-Methylphenyl) Porphyrin (2a)

Reaction of 2-hydroxybenzaldehyde (3 mL, 28.0 mmol, 1 eq.), 4-tolualdehyde (10.1 mL, 85.0 mmol, 3 equiv.), and pyrrole (8.0 mL, 114.0 mmol, 4 equiv.) led to pure product **2a** (940 mg, 5%). R_f 0.70 (CH_2Cl_2); UV-visible spectrum in CH_2Cl_2: λ_{max}, (ε,10^{-3} L mol^{-1} cm^{-1}): 418 (339.2), 516 (14.4), 552 (6.7), 592 (4.5), 648 (4.1); ^{1}H NMR (400 MHz, $CDCl_3$, 25°C): $\delta = -2.7$ (s, 2H, NH), 2.7 (s, 9H, CH_3), 7.2 (m, 2H, H-3,5 Ar), 7.5 (d, 6H, $J_{H,H} = 7.2$ Hz, H-3,5 tolyl), 7.7 (m, 1H, H-4 Ar), 7.9 (m, 1H, H-6 Ar), 8.1 (d, 6H, $J_{H,H} = 7.2$ Hz, H-2,6 Tolyl), 8.8 (s, 8H, H_β pyr). MS (MALDI) m/z = 673.6 ($[M + H]^+$ monoisotopic calc 672.3).

5-(4-Hydroxyphenyl)-10,15,20-Tris(4-Methylphenyl) Porphyrin (2b)

Reaction of 4-hydroxybenzaldehyde (3.4 g, 28.0 mmol, 1 equiv.), 4-tolualdehyde (10.1 mL, 85.0 mmol, 3 equiv.), and pyrrole (8.0 mL, 114.0 mmol, 4 equiv.) gave, after purification by column chromatography ($CHCl_3$/petroleum ether 8/2 to 10/0), pure product **2b** (1.12 g, 6%). R_f 0.33 (CH_2Cl_2); UV-visible spectrum in CH_2Cl_2: λ_{max}, (ε,10^{-3} L mol^{-1} cm^{-1}): 418 (363.0), 516 (13.5), 552 (7.4), 592 (4.0), 648 (4.3); ^{1}H NMR (400 MHz, $CDCl_3$, 25°C): $\delta = -2.76$ (bs, 2H, NH), 2.69 (s, 9H, CH_3), 7.15 (d, 2H, $J = 8.2$ Hz, H-3,5 Aryl), 7.53 (d, 6H, $J = 7.7$ Hz, H-3,5 tolyl), 8.03 (d, 2H, $J = 8.2$ Hz, H-2,6 Aryl), 8.08 (d, 6H, $J = 7.7$ Hz, H-2,6 tolyl), 8.84 (bs, 6H, H_β pyr), 8.93 (d, 2H, $J = 1.2$ Hz, H_β pyr); MS (MALDI) m/z = 673.6 ($[M + H]^+$ monoisotopic calc 672.3).

General Procedure for the Synthesis of Monoaminopropyloxyphenylporphyrins 4a,b

Porphyrins **2a** and **2b** (1 equiv.) were dissolved in dry DMF (10 mL) with a large excess of K_2CO_3 (20 equiv.). The mixtures were stirred for 15 min at rt. Compound **1** (5 equiv.) was added and the solutions were stirred at rt overnight in the dark. After reaction, DMF was evaporated under vacuum and the crude product was dissolved in CH_2Cl_2. The organic layer was washed several times with water, dried ($MgSO_4$), and then evaporated to give, after purification by preparative TLC, the pure porphyrins **3a,b**.

Tritolylporphyrin derivatives **3a** or **3b** were dissolved in CH_2Cl_2 (8 mL) and trifluoroacetic acid (2 mL) was added. The mixture was stirred at rt for 2 h. Following completion of the reaction, CH_2Cl_2 (30 mL) was added, followed by $NaHCO_3$-saturated aqueous solution until pH = 7. The organic layer was washed with water and dried over magnesium sulfate. Porphyrins **4a,b** were obtained pure as seen by TLC ($CHCl_3$/EtOH/NH_3, 80/20/1).

5-(2-[N-Terbutoxycarbonyl-3-Aminopropyloxy]Phenyl)-10,15,20-Tris[4-Methylphenyl] Porphyrin (3a)

Reaction of porphyrin **2a** (113 mg, 0.17 mmol, 1 equiv.), compound **1** (200 mg, 0.84 mmol, 5 equiv.), and K_2CO_3 (475 mg, 3.44 mmol, 20 equiv.) led to pure product **3a** (132.3 mg, 95%). R_f 0.39 (CH_2Cl_2); UV-visible spectrum in $CHCl_3$: λ_{max}, (ε,10^{-3} L mol^{-1} cm^{-1}): 419 (531.8), 516 (18.8), 552 (9.1), 592 (5.8), 647 (4.3). ^{1}H NMR (400.13 MHz, $CDCl_3$, 25°C): $\delta = -2.73$ (s, 2H, NH), 1.06 (s, 9H, CH_3 tertiobutyl), 1.16 (q, 2H, $J = 5.7$ Hz, O–C$\underline{H}_2$–CH_2–CH_2NHCO), 1.91 (q, 2H, $J = 5.7$ Hz, –C$\underline{H}_2$–NHCO), 2.69 (s, 9H, CH_3), 3.56 (t, 1H, $J = 4.5$ Hz, –N$\underline{H}$–CO), 3.92 (t, 2H, $J = 5.8$ Hz, –O–C$\underline{H}_2$–CH_2–), 7.32 (d, 1H, $J = 8.3$ Hz, H-3 aryl), 7.35 (td, 1H, $J = 1.6$-8.3 Hz, H-5 aryl), 7.54 (bd, 6H, $J = 7.8$ Hz, H-3,5 tolyl), 7.70 (td, 1H, $J = 1.6$-7.9 Hz, H-4 aryl), 8.04 (dd, 1H, $J = 1.5$-7.3 Hz, H-6 aryl), 8.12 (bd, 6H, $J = 7.6$ Hz, H-2,6 tolyl), 8.79 (d, 2H, $J = 4.4$ Hz, H_β pyr), 8.83 (d, 2H, $J = 4.8$ Hz, H_β pyr), 8.84 (s, 4H, H_β pyr). MS (MALDI) m/z = 831.31 ($[M + H]^+$ monoisotopic calc: 830.1).

5-(4-[N-Terbutoxycarbonyl-3-Aminopropyloxy]Phenyl)-10,15,20-Tris[4-Methylphenyl] Porphyrin (3b)

Reaction of porphyrin **2b** (100 mg, 0.15 mmol, 1 equiv.), compound **1** (178 mg, 0.74 mmol, 5 equiv.), and K_2CO_3 (410 mg, 2.97 mmol, 20 equiv.) led to pure product **3b** (88 mg, 82%). R_f 0.52 (CH_2Cl_2); UV-visible spectrum in $CHCl_3$: λ_{max}, (ε,10^{-3} L mol^{-1} cm^{-1}): 421 (259.3), 518 (7.9), 554 (4.7), 592 (2.5), 648 (2.3); ^{1}H NMR (400.13 MHz, $CDCl_3$, 25°C): $\delta = -2.76$ (s, 2H, NH), 1.49 (s, 9H, CH_3 tertiobutyl), 2.03 (q, 2H, $J = 6.3$ Hz, OCH_2–C$\underline{H}_2$–CH_2CO–), 2.69 (s, 9H, CH_3), 3.42 (t, 2H, $J = 6.5$ Hz, –C$\underline{H}_2$–NHCO), 4.29 (t, 2H, $J = 5.5$ Hz, –O–C$\underline{H}_2$–), 7.24 (d, 2H, $J = 8.4$ Hz, H-3,5 Aryl), 7.54 (d, 6H,

$J = 7.7$ Hz, H-3,5 tolyl), 8.08 (d, 6H, $J = 7.7$ Hz, H-2,6 tolyl), 8.10 (d, 2H, $J = 8.3$ Hz, H-2,6 Aryl), 8.84 (s, 8H, H_β pyr). SM (MALDI) m/z = 831.22 ($[M + H]^+$ monoisotopic calc: 830.1).

5-(2-[3-Aminopropoxy]Phenyl)-10,15,20-Tris[4-Methylphenyl] Porphyrin (4a)

Reaction of porphyrin **3a** (132 mg, 0.16 mmol) led to pure product **4a** (100 mg, 86%). $R_f = 0.85$ ($CHCl_3$/EtOH/NH_3, 80/20/1); UV-visible spectrum in $CHCl_3$: λ_{max}, (ε,10^{-3} L mol^{-1} cm^{-1}): 419 (219.0), 516 (20.0), 552 (10.1), 590 (7.0), 645 (5.0). ^{1}H NMR (400.13 MHz, $CDCl_3$, 25°C): $\delta = -2.74$ (s, 2H, NH), 1.09, (quint, 2H, $J = 6.2$ Hz, O–H_2C–C$\underline{H}_2$–CH_2–NH_2); 1.68 (t, 2H, $J = 6.5$ Hz, O–H_2C–CH_2–C$\underline{H}_2$–NH_2); 2.69 (s, 9H, CH_3 tolyl), 3.93 (t, 2H, $J = 5.8$ Hz, O–$\underline{H}_2$C–CH_2–CH_2–NH_2), 7.31 (dd, 1H, $J = 8.4$-0.8 Hz, H-3 aryl), 7.34 (td, 1H, $J = 7.3$ Hz, H-5 aryl), 7.53 (bd, 6H, $J = 7.5$ Hz, $H_{3,5}$ tolyl), 7.70 (td, 1H, $J = 7.6$-1.6 Hz, H-4 aryl), 8.01 (dd, 1H, $J = 7.4$-1.6 Hz, H-6 aryl), 8.07 (d, 2H, $J = 6.9$ Hz, H-2,6 tolyl), 8.09 (d, 2H, $J = 7.0$ Hz, H-2,6 tolyl), 8.11 (d, 2H, $J = 7.1$ Hz, H-2,6 tolyl), 8.77 (d, 2H, $J = 4.8$ Hz, Hβ pyrrole); 8.82 (d, 2H, $J = 4.9$ Hz, Hβ pyrrole); 8.84 (s, 4H, Hβ pyrrole). MS (MALDI) m/z = 730.95 ($[M + H]^+$ monoisotopic calc: 729.8).

5-(4-[3-Aminopropoxy]phenyl)-10,15,20-Tris[4-Methylphenyl] Porphyrin (4b)

Reaction of porphyrin **3b** (132 mg, 0.16 mmol) led to pure product **4b** (98 mg, 85%). $R_f = 0.55$ ($CHCl_3$/EtOH/NH_3, 80/20/1); UV-visible spectrum in $CHCl_3$: λ_{max}, (ε, 10^{-3} L mol^{-1} cm^{-1}): 420 (467.6), 517 (13.6), 554 (7.6), 592 (4.2), 648 (3.8). ^{1}H NMR (400.13 MHz, $CDCl_3$, 25°C): $\delta = -2.77$ (s, 2H, NH), 2.02 (m, 2H, O–H_2C–CH_2–C$\underline{H}_2$–NH_2); 2,62 (m, 2H, O–H_2C–C$\underline{H}_2$–CH_2–NH_2); 2.67 (s, 9H, CH_3 tolyl), 2.99 (bs, 2H, NH_2), 7.25 (d, 2H, $J = 8.4$ Hz, H-3,5 aryl), 7.53 (d, 6H, $J = 7.7$ Hz, H-3,5 tolyl), 8.08 (d, 6H, $J = 7.8$ Hz, H-2,6 tolyl), 8.10 (d, 2H, $J = 8.4$ Hz, H-2,6 aryl), 8.85 (s, 8H, Hβ pyrrole). MS (MALDI) m/z = 730.85 ($[M + H]^+$ monoisotopic calc: 729.8).

Triacetyl Lactone (5)

Lactone (**5**) was prepared in two steps[17,18] by treating isomaltulose with excess aqueous hydrogen peroxide under acidic catalysis to yield carboxymethyl glucoside, followed by acetylation (and lactone formation). This last step could be either performed by treatment with acetic anhydride in pyridine or in anhydrous DMF or by acetyl chloride in a dichloromethane-pyridine mixture.

General Procedure for Coupling of Triacetyl Lactone 5

Crude porphyrins **4a,b** (1 equiv.) were dissolved in CH_2Cl_2 and lactone **5** (3 equiv.) and DMAP (2.5 equiv.) were added. The mixture was stirred 16 h in the dark, at rt. The reaction was monitored by TLC ($CHCl_3$/EtOH, 95/5).

The solution was concentrated *in vacuo* and purification of the residue by preparative TLC gave pure products **6a,b**.

5-[2-(3,4,6-Tri-O-Acetyl-α-D-Glucopyranosyloxymethylcarbonyl-3-aminopropoxy)Phenyl] -10,15,20-Tritolylporphyrine (6a)

Porphyrin **4a** (99 mg, 0.14 mmol, 3 equiv.) reacted with lactone **5** (15.7 mg, 0.04 mmol, 1 equiv.) and DMAP (13.8 mg, 0.11 mmol, 2.5 equiv.) in CH_2Cl_2 (13 mL) to afford pure product **6a** (58.2 mg, 99%). R_f 0.54 ($CHCl_3$/EtOH, 95/5); UV-visible spectrum in$CHCl_3$: λ_{max}, (ε,10^{-3} L mol^{-1} cm^{-1}): 420 (335.4), 516 (12.7), 552 (8.4), 590 (5.0), 648 (5.8). ^{1}H NMR ($CDCl_3$, 400.13 MHz): $\delta = -2.87$ (s, 2H, NH-pyrrole), 0.76 (dd, 1H, J = 3,1-9,9Hz, H-2'), 0.89 (m, 2H, $OH_2C-C\underline{H}_2-CH_2NHCO$), 1.23 (s, 3H, acetyl), 1.46 (m, 2H, $O-C\underline{H}_2-CH_2-C\underline{H}_2NHCO$), 1.79 (s, 3H, acetyl), 1.99 (s, 3H, acetyl), 2.56 (d, 1H, J = 3.5 Hz, H-1'), 2.46 (bd, 2H, J = 15.8 Hz, OC-$C\underline{H}_2-O$), 2.69 (s, 3H, CH_3 tolyl), 2.70 (s, 3H, CH_3 tolyl), 2.71 (s, 3H, CH_3 tolyl), 3.16 (ddd, 1H, J = 9.8-4.0-1.8 Hz, H-5'), 3.61 (dd, 1H, J = 1.8-12.2 Hz, H-6b'), 3.81 (m, 1H, $OC\underline{H}_2-CH_2-CH_2NH$), 3,86 (dd, 1H, J = 4.4-12.4 Hz, H-6a'), 3.93 (m, 1H, $OC\underline{H}_2-CH_2-CH_2NH$), 4.00 (t, 1H, J = 9.7 Hz, H-3'), 4.12 (t, 1H, J = 9.8 Hz, H-4'), 7,29 (d, 1H, J = 8.2 Hz, H-3 aryl), 7,41 (t, 1H, J = 7.4 Hz, H-5 aryl), 7.56 (bs, 4H, H-3,5 tolyl), 7.63 (d, 2H, J = 7.5 Hz, H-3,5 tolyl); 7.76 (td, 1H, J = 7.9-1.4 Hz, H-4 aryl), 8.01 (d, 2H, J = 7.4 Hz, H-2,6 tolyl), 8.05 (d, 2H, J = 8.4 Hz, H-2,6 tolyl), 8.13 (dd, 1H, J = 7.3-1.5 Hz, H-6 aryl), 8.10 (d, 2H, J = 7.9 Hz, H-2,6 tolyl), 8.77 (d, 1H, J = 4.8 Hz, Hβ pyrrole), 8.87 (d, 1H, J = 4.8 Hz, Hβ pyrrole), 8.89 (d, 1H, J = 4.8 Hz, Hβ pyrrole), 8.90 (s, 4H, Hβ pyrrole), 8.92 (d, 1H, J =4.8 Hz, Hβ pyrrole). MS (MALDI) m/z = 1076.4 ($[M + H]^+$ monoisotopic calc: 1075.4).

5-[4-(3,4,6-Tri-O-Acetyl-α-D-Glucopyranosyloxymethylcarbonyl-3-aminopropoxy)Phenyl] -10,15,20-Tritolylporphyrine (6b)

Porphyrin **4b** (67.1 mg, 0.10 mmol, 3 equiv.) reacted with lactone **5** (11.2 mg, 0.03 mmol, 1 equiv.) and DMAP (9.9 mg, 0.08 mmol, 2.5 equiv.) in CH_2Cl_2 (9 mL) to afford pure product **6b** (33 mg, 95%). R_f 0.47 ($CHCl_3$/EtOH, 95/5); UV-visible spectrum in $CHCl_3$: λ_{max}, (ε,10^{-3} L mol^{-1} cm^{-1}): 420 (321.3), 516 (13.1), 552 (6.5), 592 (4.9), 648 (4.1). ^{1}H NMR ($CDCl_3$, 400.13 MHz): $\delta = -2.77$ (s, 2H, NH-pyrrole) 1.99 (s, 3H, acetyl), 2.00 (s, 3H, acetyl), 2.09 (s, 3H, acetyl), 2.20 (quint, 2H, J = 6.3 Hz, $OH_2C-C\underline{H}_2-CH_2N$), 2.69 (s, 9H, CH_3 tolyl), 3.65 (m, 2H, CH_2N), 3.78 (dd, 1H, J =3.7-9.9 Hz, H-2′), 4.07 (ddd, 1H, J = 9.8-1.8-4.6 Hz, H-5′), 4.09 (d, 1H, J = 12.5 Hz, CH_2), 4.13 (d, 1H, J = 14.5 Hz, CH_2), 4.27 (dd, 1H, J = 2.4-9.6 Hz, H-6′b), 4.28 (t, 2H, J = 6.3 Hz, CH_2O), 4.29 (dd, 1H, J = 3.3-9.6 Hz, H-6'a), 4.91 (d, 1H, J = 3.8 Hz, H-1′), 5.02 (t, 1H, J = 9.8 Hz, H-4′), 5.30 (t, 1H, J = 9.6 Hz, H-3′), 7.25 (d, 2H, J = 8.4 Hz, H-3,5 aryl), 7.39 (t, 1H, J = 5.6 Hz, NH), 7.53 (d, 6H, J = 7.7 Hz, H-3,5 tolyl), 8.08

(d, 6H, $J = 7.8$ Hz, H-2,6 tolyl), 8.10 (d, 2H, $J = 8.4$ Hz, H-2,6 aryl), 8.85 (s, 8H, Hβ pyrrole). MS (MALDI) m/z = 1076.5 ($[M + H]^+$ monoisotopic calc: 1075.4).

General Procedure for Removal of Acetate Protective Groups

Crude porphyrins **6a** and **6b** were dissolved in CH_2Cl_2/MeOH (80/20, v/v) (5 mL) and then sodium methoxide (0.5M in MeOH, 3 equiv. per acetate groups) was added. The mixture was stirred for 1 h in the dark. After reaction, resulting porphyrins **7a** and **7b** were quantitatively precipitated by addition of petroleum ether (30 mL) and then filtered on sintered glass disk (porosity 4) and washed with MeOH and CH_2Cl_2.

5-[2-(α-D-Glucopyranosyloxymethylcarbonyl-3-Aminopropoxy)Phenyl]-10,15,20-Tritolyl Porphyrine (7a)

37.6 mg (95%), R_f 0.68 ($CHCl_3$/MeOH, 7/3); UV-visible spectrum in $CHCl_3$/MeOH (8/2,v/v): λ_{max}, (ε,10^{-3} L mol^{-1} cm^{-1}): 418 (361.6), 516 (13.4), 552 (7.8), 590 (4.3), 648 (3.9).^{1}H NMR (DMSO d_6, 400.13 MHz)): $\delta = -2.87$ (s, 2H, NH-pyrrole) 2.65 (s, 9H, CH_3 tolyl), 2.95 (bt, 1H, $J = 9.3$ Hz, H-3′), 3.00 (dd, 1H, $J = 3.6$-9.6 Hz, H-2′), 3.20 (bt, 1H, $J = 9.7$ Hz, H-5′), 3.35 (dd, 1H, $J = 5.7$–11.8 Hz, H-6′$_a$), 3.52 (bt, 1H, $J = 2.0$-12.1, H-6′$_b$), 4.42 (d, 1H, $J = 3.6$ Hz, H-1′), 7.38 (t, 1H, $J = 7.4$ Hz, H-5 aryl), 7.52 (d, 1H, $J = 7.5$ Hz, H-3 aryl), 7.60 (d, 6H, $J = 7.6$ Hz, H-3,5 tolyl), 7.82 (t, 1H, $J = 7.6$ Hz, H-4 aryl), 7.96 (d, 1H, $J = 7.3$ Hz, H-6 aryl), 8.06 (bd, 6H, $J = 7.5$ Hz, H-2,6 tolyl), 8.78(d, 2H, $J = 4.6$ Hz, H-β pyrrole), 8.79 (d, 2H, $J = 4.7$ Hz, Hβ pyrrole), 8.81 (s, 4H, Hβ pyrrole). MS (MALDI) m/z = 950.3 ($[M + H]^+$ monoisotopic calc: 949.3).

5-[4-(α-D-Glucopyranosyloxymethylcarbonyl-3-Aminopropoxy)Phenyl]-10,15,20-Tritolyl Porphyrine (7b)

16.4 mg (95%); R_f 0.52 ($CHCl_3$/MeOH, 7/3); UV-visible in $CHCl_3$/MeOH (8/2,v/v): $\lambda_{max,}$(ε,10^{-3} L mol^{-1} cm^{-1}): 418 (322.4), 516 (12.2), 552 (7.1), 592 (4.0), 648 (3.1). ^{1}H NMR (DMSO d_6, 400.13 MHz): $\delta = -2.88$ (s, 2H, NH), 2.11 (bt, 2H, $J = 5.4$ Hz, OH_2C—$C\underline{H_2}$—CH_2N), 2.65 (s, 9H, CH_3 tolyl.), 3.13 (m, 1H, H-6′b), 3.25 (bs, 1H, H-6′a), 3.26 (bd, 1H, $J = 7.0$ Hz, H-2′), 3.46 (t, 1H, $J = 9.1$ Hz, H-5′), 3.48 (m, 2H, —CH_2N), 3.56 (bt, 1H, $J = 9.3$ Hz, H-3'), 3.66 (bd, 1H, $J = 10.2$ Hz, H-4′), 3.94 (d, 1H, $J = 16.2$ Hz, CH_2), 4.30 (bs, 2H, –CH_2O), 4.12 (d, 1H, $J = 14.8$ Hz, CH_2), 4.77 (bs, 1H, H-1′), 7.35 (d, 2H, $J = 7.0$ Hz, H-3,5 aryl), 7.59 (d, 6H, $J = 5.0$ Hz, H-3,5 tolyl), 8.06 (d, 6H, $J = 6.3$ Hz, H-2,6 tolyl), 8.09 (d, 2H, $J = 7.0$ Hz, H-2,6 aryl), 8.81 (bs, 8H, Hβ pyrrole);. MS (MALDI), m/z 950.4 ($[M + H]^+$ monoisotopic calc: 949.3).

Cell Culture

Phototoxicities of the synthesized compounds against K562 chronic leukemia cell line have been evaluated. K562 cells were suspended in HEPES-buffered RPMI 1640 medium (Sigma, R4130) pH = 7.0 $\pm$0.3 containing 2 mM *L*-glutamine supplemented with 2 g L^{-1} $NaHCO_3$, 50 U mL^{-1} penicillin, 50 mg mL^{-1} streptomycin (Sigma, P0906), and 10% (v/v) fetal bovine serum (Biochrom KG, Polylabo 60810). Cultures were incubated at 37°C in a fully humidified atmosphere containing 5% CO_2 in air. Cells were picked during exponential growth and washed and diluted to 10^6 cells mL^{-1} with fresh RPMI medium. This suspension was then distributed in 24 well plates (2 mL/well). Porphyrins (final concentration 2.10^{-6} M) or Photofrin (final concentration 1.25 μg mL^{-1}) were added to the wells and the plates were then incubated 18 h in the dark before illumination; without this preincubation these synthetic porphyrins did not display any detectable photocytotoxicity. Moreover, none of the compounds studied exhibited cytotoxicity in the dark.

Flow Cytometry

Dead cells were identified as propidium iodide (PI) (Sigma) permeable ones and the dead cell counts were measured by flow cytometry. Samples were analyzed with a Coulter Epics XL System II, immediately after each illumination time (J0); then cell suspensions were incubated in the dark at 37°C for 24 h and the dead cell counts were estimated thereafter (J1). A stock solution of PI was prepared in distilled water, filtered, sterilized, and used at 10^{-4} M.

Partition Coefficient Measurements

Lipophylicity, often well correlated with the bioactivity of drugs, can be indicated, for example, by the logarithm of a partition coefficient, log *P*, that reflects the equilibrium partitioning of a molecule between a nonpolar and a polar phase, such as an *n*-octanol/water system. For tritolylporphyrin **7a,b,** calculated log *P*—as $\log([\text{Porphyrin}]_{n\text{-octanol}}/[\text{Porphyrin}]_{\text{water}})$—is >3, which indicates that these compounds are very nearly insoluble in water. Determinations were repeated three times.

Singlet Oxygen Production

In order to determine the photosensitizing properties of porphyrins **7a** and **7b,** trapping reactions of $^1O_2$ with ergosterol acetate were carried out.[22–24] Reference experiments with eosin, rose bengal, or hematoporphyrin (HP), known singlet oxygen producers, gave ergosterol acetate epidioxide with nearly quantitative yields. In the same experimental conditions, our porphyrins had the same efficiency for $^1O_2$ production than HP.

CONCLUSION

We have demonstrated a simple synthetic method for accessing porphyrins bearing a glucosyl unit, by the way of an α-D-carboxymethyl linkage. The carbohydrate moiety is connected by reaction with a bicyclic lactone prepared from the available disaccharide isomaltulose. Preliminary biological results have showed that the *ortho* compound was more active than the *para* porphyrin, and significantly more active than the nonglycosylated analog. However, activity was shown to be lower than that of Photofrin, suggesting that only one sugar unit in **7a** is not enough to increase significantly the amphiphilic character for efficient photodynamic activity.[2,7] Similar porphyrins incorporating α- or β-carboxymethyl mono-or oligosaccharides will be studied. Such variations will allow to discriminate between specific interactions with the carbohydrate parts of the molecules, the issue of the stability of the glycosidic linkage allowing longer lifetime of the compounds, or physicochemical considerations such as variations in their polarity. This might notably be undertaken in light of the knowledge of the various lectins at the surface of cell membranes and their implication in glycoconjugated dyes internalization.

ACKNOWLEDGEMENTS

Part of this work was achieved in the former CNRS Beghin Say research facility in Villeurbanne. Financial support from these two institutions and ANRT for a grant to ST as well as from the "Conseil Régional du Limousin" are gratefully acknowledged. Dr Jean Claude Blais is also warmly acknowledged for routine MS MALDI analysis.

REFERENCES

[1] Cornia, M.; Casiraghi, G.; Binacchi, S.; Zanardi, F.; Rassu, G. Facile entry to 5,10,15,20-tetra-C-glycosylporphyrins. J. Org. Chem. **1994**, *59*, 1226–1230.

[2] Momenteau, M.; Oulmi, D.; Maillard, P.; Croisy, A. In vitro photobiological activity of a new series of photosensitizers: the glycoconjugated porphyrins. SPIE, Photodynamic Therapy Cancer II. **1994**, *2325*, 13–23.

[3] Hombrecher, H.K.; Ohm, S.; Koll, D. Synthesis of galactopyranosyl substituted porphyrins. Tetrahedron **1996**, *52*, 5441–5448.

[4] Sol, V.; Blais, J.C.; Bolbach, G.; Carré, V.; Granet, R.; Guilloton, M.; Spiro, M.; Krausz, P. Toward glycosylated peptidic porphyrins: a new strategy for PDT? Tetrahedron Lett. **1997**, *38*, 6391–6394.

[5] Mikata, Y.; Onchi, Y.; Tabata, K.; Ogura, S.; Okura, I.; Ono, H.; Yano, S. Sugar-dependent photocytotoxic property of tetra- and octa-glycoconjugated tetraphenylporphyrins. Tetrahedron Lett. **1998**, *39*, 4505–4508.

[6] Schell, C.; Hombrecher, H.K. Synthesis, self-assembly properties and incorporation of carbohydrate-substituted porphyrins into cell membrane models. Chem. Eur. J. **1999**, *5*, 587–598.

[7] Sol, V.; Blais, J.C.; Carré, V.; Granet, R.; Guilloton, M.; Spiro, M.; Krausz, P. Synthesis, spectroscopy and photocytotoxicity of glycosylated amino acid porphyrins derivatives as promising molecules for cancer phototherapy. J. Org. Chem. **1999**, *64*, 4431–4444.

[8] Zheng, G.; Graham, A.; Shibata, M.; Missert, J.R.; Oseroff, A.R.; Dougherty, T.J.; Pandey, R.K. Synthesis of β-galactose-conjugated chlorins derivated by enyme metathesis as galectin-specific photosensitizers for photodynamic therapy. J. Org. Chem. **2001**, *66*, 8709–8716.

[9] Laville, I.; Figueiredo, T.; Loock, B.; Pigaglio, S.; Maillard, P.; Grierson, D.S.; Sarrez, C.D.; Croisy, A.; Blais, J. Synthesis, cellular internalization and photodynamic activity of glucoconjugated derivated of tri and tetra(meta-hydroxyphenyl)-chlorins. Bioorg. Med. Chem. **2003**, *11*, 1643–1652.

[10] Sylvain, I.; Benhaddou, R.; Carré, V.; Cottaz, S.; Driguez, H.; Granet, R.; Guilloton, M.; Krausz, P. Synthesis and biological evaluation of thioglycosylated meso-arylporphyrins. J. Porphyrins Phthalocyanines **1999**, *3*, 1–4.

[11] Chaleix, V.; Sol, V.; Huang, Y-M.; Guilloton, M.; Granet, R.; Blais, J.C.; Krausz, P. RGD-porphyrin conjugates: synthesis and potential application in photodynamic therapy. Eur. J. Org. Chem. **2003**, 1486–1493.

[12] Kieda, C.; Monsigny, M. Involvement of membrane sugar receptors and membrane glycoconjugates in the adhesion of 3LL cell subpopulations to cultured pulmonary cells. Invasion Metastasis **1986**, *6*, 347–366.

[13] Monsigny, M.; Roche, A.C.; Midoux, P.; Kieda, C.; Mayer, R. *Lectins and Glycoconjugates in Oncolog: Structure, Function, Clinical Application*. Gabius, H.J. and Nagel, G.A. Ed: Spinger-Verlag, Heidelberg., 1988, 1.

[14] Fujimoto, K.; Miyata, T.; Aoyama, Y. Saccharide-directed cell recognition and molecular delivery using macrocyclic saccharide clusters: masking of hydrophobicity to enhance the saccharide specificity. J. Am. Chem. Soc. **2000**, *122*, 3358–3359.

[15] Ono, N.; Bougauchi, M.; Maruyama, K. Water-soluble porphyrins with four sugar molecules. Tetrahedron. Lett. **1992**, *33*, 1629–1632.

[16] Lee, Y.C.; Lee, R.T. Affinity enhancement by multivalent lectin-carbohydrate interaction Glycoconjug. J. **2000**, *17*, 543–551.

[17] Trombotto, S.; Bouchu, A.; Descotes, G.; Queneau, Y. Hydrogen peroxide oxidation of palatinose and trehalulose: direct preparation of carboxymethyl α-D-glucopyranoside. Tetrahedron Lett. **2000**, *41*, 8273–8277.

[18] Trombotto, S.; Danel, M.; Fitremann, J.; Bouchu, A.; Queneau, Y. Straightforward route for anchoring a glucosyl moiety onto nucleophilic species: reaction of amines and alcohols with carboxymethyl 3,4,6 tri-*O*-acetyl-α-D-glucopyranoside 2-*O*-lactone. J. Org. Chem, **2003**, *68*, 6672–6678.

[19] Little, R.G.; Anton, J.A.; Loach, P.A.; Ibers, J-A. The synthesis of some substituted tetraalkylporphyrins. J. Heterocycl. Chem. **1975**, *12*, 345–349.

[20] Furhop, J.H.; Demoulin, C.; Boettcher, C.; Koning, J.; Siggel, U. Chiral micellar porphyrin fibers with 2-aminoglycosamide head groups. J. Am. Chem. Soc. **1992**, *114*, 4159–4165.

[21] Barber, D.C.; Freitag-Beeston, R.A.; Whitten, D.G. Atropisomer-specific formation of premicellar porphyrin J-aggregates in aqueous surfactant solutions. J. Phys. Chem. **1991**, *95*, 4074–4086.

[22] Ohloff, G. Singlet oxygen: a reagent in organic synthesis Pure. Appl. Chem. **1975**, *43*, 481–502.

[23] Albro, P.W.; Corbett, J.T.; Shroeder, J.L. Doubly allylic hydroperoxide formed in the reaction between sterol 5,7 dienes and singlet oxygen. Photochem. Photobiol. **1994**, *60*, 310–315.

[24] Böcking, T.; Barrow, K.D.; Netting, A.G.; Chilcott, T.C.; Coster, H.G.; Höfer, M. Effect of singlet oxygen on membrane sterols in the yeast *saccharomyces cerevisiae*. Eur. J. Biochem. **2000**, *267*, 1607–1618.