TETRAPYRROLIC GLYCOSYLATED MACROCYCLES FOR AN APPLICATION IN PDT

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ABSTRACT

The synthesis and characterization of amphiphilic glycoconjugated porphyrins, benzochlorin, and azaporphyrins were reported. Among these molecules, several were found to be efficient photosensitizers in an in vitro assay using the human tumoral cell line HT29. Moreover, glycosylated benzochlorin and azaporphyrins, whose absorption bands in the red region of the visible spectrum are substantially increased as compared to porphyrins, display a good photocytotoxicity on tumor cells after irradiation with wavelength above 590 nm.

1 INTRODUCTION

Although the photodynamic properties of hematoporphyrin derivative (HpD) were first described by Lipson and Baldes in 1960, its utilization for the photodynamic therapy (PDT) of human cancers was first mentioned by Dougherty in 1978. In spite of considerable efforts devoted to the development of PDT for the treatment of human malignancies, this procedure remains largely underestimated by physicians, mainly because HpD and its active fraction, Photofrin®, are complex mixtures which are retained for a long time in normal tissues, inducing long-lasting light hypersensitivity.

The search for new, well-characterized photosensitizers has become a major goal for the scientific community engaged in this field and several research groups have focused their attention on the synthesis of new tetrapyrrolic macrocycles with improved distribution kinetics and biological activities. Actually, the design of new photosensitizers having well-defined structure with great selectivity for tumor cells, fast elimination from healthy tissues, and strong light absorption in the red region of the visible spectrum is an important challenge for chemists. Thus, synthesis of many tetrapyrrolic compounds such as purpurins, chlorins, phthalocyanins, and benzochlorins has developed.

For the last few years, we were engaged in the preparation of neutral glycoconjugated porphyrins derived from the 5,10,15,20-tetraarylporphyrins in which mono- or disaccharides were linked directly at the phenyl groups. The study of this series of neutral water-soluble glycosylated porphyrins as photosensitizing dyes allowed us to define the effect of structural and chemical modifications and of the balance between hydrophilic glycosyl groups and hydrophobic substituents on their photocytotoxic properties. The resulting structure–activity relationships suggest that both planar structure and amphiphilic character are essential factors for photodynamic activity on human tumoral KB cell line in vitro as exemplified by the high photosensitizing properties of tris(5-glucosylphenyl) phenyl porphyrin [TPP(GluOH)₃] and the relative inefficiency of tetrakis(5-glucosylphenyl) porphyrin [TPP(GluOH)₄].

In this paper, we report the synthesis of meso tetraakis and tris-glycoconjugated phenylporphyrins 1–5 (Figure 1) bearing monosaccharides linked, via an alkoxy spacer, to meso-phenyl groups directly from meso-(p-hydroxyphenyl) porphyrins. These molecules were designed to study the influence of the alkoxy spacer on the in vitro photocytotoxic properties.

During the course of this work, P. Krausz and collaborators described the synthesis of 13 new meso-mono or tetrakis-glycosylated phenylporphyrins where the carbohydrate moieties were also linked to the phenyl group by an alkoxy spacer; however, these molecules were obtained either by direct condensation of glycosides on hydroxyalkoxarylporphyrin or by condensation of the corresponding glycosylated benzaldehydes with pyrrole or meso-(p-tolyl)dipyrromethane.
We also described glycoconjugated benzochlorin 6, azaporphyrins 7–9, and the corresponding porphyrins 10–12 (Figure 1) to study the influence of the absorption intensity in the red region of the visible spectrum on the photodynamic properties. In vitro photocytotoxic properties were evaluated on the human colon adenocarcinoma cell line HT29. Several new molecules were found equally or more efficient than Photofrin® in this experimental model.

2 RESULTS AND DISCUSSION

2.1 SYNTHESIS

The synthesis of glycosylated meso-tetraarylporphyrins usually required the condensation of pyrrole and glycosylated benzaldehyde under Lindsey’s conditions. However, many trials have been performed in an attempt to link directly a glycoside to porphyrin. Thus condensation of bromoalkanes on meso-(hydroxyphenyl)porphyrins under Little’s conditions gives alkoxy derivatives in very good yields. Using the same synthetic method, condensation of 1-bromoalkoxy-per-acetylglycosides I–IV on meso-tetrakis-(p-hydroxyphenyl)porphyrin V or on meso-tris-(p-hydroxyphenyl)phenylporphyrin VI following by transesterification gave meso-(alkoxy-glycoside phenyl)porphyrins 1–5 in 65%–70% yields (Scheme 1). Meso-tris-(p-hydroxyphenyl)phenylporphyrin VI was obtained by condensation of pyrrole (4 equiv.), benzaldehyde (1 equiv.), and para-methoxybenzaldehyde (3 equiv.) under Lindsey’s conditions following by demethylation with BBr3 in dry methylene chloride. Preparation of 1-bromoalkoxy-per-acetylglycosides were performed by condensation of per-acetylated sugars on bromo alcohol using the boron-etherate method in dry methylene chloride.

Synthesis of glycosylated benzochlorins derived from 5-meso-aryl octaethylporphyrin was performed from nickel (II) porphyrin by electrophilic substitution with 3-(dimethylamino)acrolein under Vilsmeier’s conditions, which led to the two isomeric nickel (II) complexes 14a and 14b (total yield 85%, ratio 14a/14b, 85.5/14.5) in which the 2-formylvinyl group is linked either at the adjacent meso-carbon (C10) in 14a or at the opposite (C15) to the meso-aryl position in 14b. Treatment of porphyrin 14a, by trifluoroacetic acid under argon atmosphere at room temperature, afforded nickel (II) benzochlorin in

Fig. 1 Structures of glycoconjugated tetraaryl macrocycles.
58% yield. HPLC analysis and $^1$H nuclear magnetic resonance (NMR) studies showed the presence of a single compound corresponding exclusively to one of the two possible nickel monoarylbenzochlorins. Dealkylation by boron tribromide in dry methylene chloride afforded nickel complex. Demetallation of 16 in concentrated sulfuric acid gave the metal-free benzochlorin which was glycosylated, in dimethylformamide in the presence of potassium carbonate, by 1-bromoethoxy-per-acetylmaltose to benzochlorin. Transesterification of 18 afforded the maltose deacetylated glycoconjugated derivative in quantitative yield.

Azaporphyrin 7 bearing two sugar moieties directly linked to $\beta$-pyrrolic positions was prepared from glycosylated pyrrole 21, obtained by the Barton and Zard’s method from 1,2,3,4-di-O-isopropyliden-5-formyl-$\alpha$-D-galactopyranose 19 via the nitro derivative in 75% yield. Condensation of its benzyl ester 22 with dimethoxymethane in the presence of potassium carbonate, by 1-bromoethoxy-per-acetylmaltose to benzochlorin 18. Transesterification of 18 afforded the maltose deacetylated glycoconjugated derivative in quantitative yield.

Glycoconjugated azaporphyrins 8–9, bearing two glucose or maltose moieties via an aryl spacer, were prepared from 3-(3′-nitro-4′-hydroxyphenyl)-4-methyl-2-ethoxycarbonyl pyrrole 30 obtained by the method of Barton et al. 19 from 3-nitro-4-hydroxybenzaldehyde in 73% yield. Condensation of this pyrrole on dimethoxymethane led quantitatively to dipyrromethane 31. This last compound was hydrolyzed by NaOH/MeOH, almost quantitatively, to dicarboxylic dipyrromethane 32. Coupling 32 with 2-formyl-3,4-diethyl pyrrole afforded 2, 8-di-(1′, 2′, 3′, 4′-di-O-isopropylidene-$\alpha$-D-galactosyl)-3, 7-dimethyl-12, 13, 17, 18-tetraethylbiladien ac-c hydrobromide 27. Cyclization of 27 in methanol, in presence of K$_3$FeCN$_6$ and ammonium hydroxide, followed by treatment with a mixture of trifluoroacetic acid, water (9:1, v/v) led to the expected azaporphyrin 7. Condensation of the glycosylated dicarboxylic dipyrromethane 24 with dialdehyde dipyrromethane 25 in presence of catalytic amount of para-toluenesulfonic acid gave, after deprotection of sugars by a mixture of trifluoroacetic acid water (9:1, v/v), the glycosylated porphyrin 10 (Scheme 3).

Scheme 1 Synthesis of compounds 1–5.

Reagents: (i) 3-(dimethylamino)acrolein/POCl₃, (ii) CF₃CO₂H/Ar, (iii) BBr₃/dry CH₂Cl₂, (iv) H₂SO₄, (v) Bromoethyl peracetylmaltose and K₂CO₃ in DMF/60°C, (vi) MeONa/MeOH.

Conditions used for the synthesis of 28. Glycosylation with α-1-bromo-per-acetyl-sugars in a mixture of acetonitrile and triethanol amine (TEA)²² and deprotection by Zemplen’s method afforded azaporphyrins 8 and 9. The corresponding porphyrins 11–12 were obtained from biladien 34 and formaldehyde in methanol/HBr followed by glycosylation with α-1-bromo-per-acetyl-glucose or maltose in a mixture of acetonitrile and TEA then deprotected by Zemplen’s method (Scheme 4).

2.2 SPECTROSCOPIC PROPERTIES

Absorption properties of compounds 1–12 and 17, in different solvents according to their solubility, are shown in Table 1. The electronic spectra of all meso substituted porphyrins 1–5 are very similar to those of known free base meso-5,10,15,20-tetrakisphenylporphyrins with a Soret band around 420 nm and four less intense Q bands near 520, 550, 590 and 650 nm. The UV-visible spectra of porphyrins bearing substituents on β-pyrrolic positions, 10–12, are of the “etio” type, characterized by a
Scheme 4 Synthesis of $\beta$-glycoconjugated diphenyl azaporphyrins 8 and 9 and porphyrins 11 and 12.

Fischer and Fridrich have shown that introduction of one nitrogen atom at a meso position of tetrapyrrolic macrocycles (monoazaporphyrins) increases absorbance in the red region (610–615 nm). Actually, azaporphyrins 8 and 9 have molecular absorption coefficient $\epsilon$ between 23 and 24 L mmol$^{-1}$ cm$^{-1}$. In contrast, for compound 7, intensity of the band at 607 nm is decreased to $\epsilon = 7.3$ L mmol$^{-1}$ cm$^{-1}$ (Figure 3).

2.3 $^1$H NMR CHARACTERIZATION

$^1$H NMR spectroscopy (200 MHz) was used for the characterization of protected and unprotected com-
Assignment of the resonances to individual protons are based on integration and selective homonuclear decoupling experiments. The general aspect of the spectra of glycoconjugated porphyrins 1–5 derived from meso-5,10,15,20-tetrakisphenylporphyrin is similar to that of the para-glycoconjugated porphyrins previously studied.8 These spectra show six groups of resonance. The NMR spectral properties of these molecules are governed by symmetry characteristic. Because of the $D_{2h}$ symmetry of meso-tetraarylporphyrins, the resonance of pyrrolic protons appears as single peaks at 8.85 ppm in CDCl$_3$ and near 9.1 ppm in DMF $d_7$ solution. The aromatic protons appear between 8.2 and 7.2 ppm, “ose” protons of protected and unprotected glycosylated compounds between 5 and 3 ppm, acetyl protons as singlets around 2 ppm and pyrrolic NH at −2.7 ppm. Protons of spacer appear at 4.4 (triplet) and 4.15 (triplet) ppm for −OCH$_2$CH$_2$O− and at 4.4, 4.1 (triplet) and 2.3 ppm (multiplet) for −OCH$_2$CH$_2$CH$_2$O− parts. Furthermore, the resonance of the anomeric proton of glycosyl groups in all protected and unprotected glycosylated porphyrins appears as well-defined doublet near 4.9 ppm.

### Table 1
Electronic spectra of glycoconjugated porphyrins, benzochlorin, and monoazaporphyrins, solvent: (a) pyr, (b) pyr/MeOH 1/24, (c) MeOH, (d) THF, (e) CH$_2$Cl$_2$.

<table>
<thead>
<tr>
<th>Compound</th>
<th>Soret band $\lambda$ (nm) $\epsilon$ (L mmol$^{-1}$ cm$^{-1}$)</th>
<th>Visible bands $\lambda$ (nm) $\epsilon$ (L mmol$^{-1}$ cm$^{-1}$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1a</td>
<td>425 (337)</td>
<td>520.5(15.8), 558(13), 596.5(7.7), 653(8.2)</td>
</tr>
<tr>
<td>2b</td>
<td>418.5 (435)</td>
<td>516.5(18), 553.5(14), 593(8.3), 650(8.6)</td>
</tr>
<tr>
<td>3b</td>
<td>417.5 (385)</td>
<td>515(17), 552(12.4), 591(8.4), 648(8)</td>
</tr>
<tr>
<td>4b</td>
<td>417 (363)</td>
<td>515.5(15.2), 551(10.6), 591(6.3)</td>
</tr>
<tr>
<td>5b</td>
<td>417.5 (361)</td>
<td>515.5(14.5), 551.5(9.9), 591.5(6), 648(5.5)</td>
</tr>
<tr>
<td>6c</td>
<td>415 (69)</td>
<td>546 (shoulder), 582(10.2), 618(11.3), 672(24.3)</td>
</tr>
<tr>
<td>7d</td>
<td>379 (41)</td>
<td>500(2.9), 534(8.2), 557(3.3), 607(7.3)</td>
</tr>
<tr>
<td>8d</td>
<td>381 (119)</td>
<td>507(8.1), 537(24.9), 563(8.9), 614(24.7)</td>
</tr>
<tr>
<td>9d</td>
<td>382 (118)</td>
<td>504(8.5), 538(25.2), 563(9.1), 614(24.7)</td>
</tr>
<tr>
<td>10e</td>
<td>397 (105)</td>
<td>498(6.5), 532(5.1), 566(4.3), 618(1.6)</td>
</tr>
<tr>
<td>11d</td>
<td>407 (160)</td>
<td>504(13.4), 539(10.3), 572(6.7), 627(4.1)</td>
</tr>
<tr>
<td>12d</td>
<td>408 (162)</td>
<td>504(13.6), 540(11.5), 573(7), 627(3.8)</td>
</tr>
<tr>
<td>17e</td>
<td>418 (95)</td>
<td>548.5(7.4), 581.5(9.6), 618(11), 673(26.1)</td>
</tr>
</tbody>
</table>

**Fig. 2** Electronic spectra of oxyhemoglobin in water (A), derivative 5 (B), and maltosylbenzochlorin (C) in methanol.

**Fig. 3** Electronic spectra of protected porphyrin 11 OAc (A), protected azaporphyrin 80 Ac (B) in a THF solution.
with \( J = 7.5 \text{ Hz} \) (1,3) and \( J = 1.5 \text{ Hz} \) (2, 4, and 5). These coupling constants are indicative of a pure configuration for the anomeric carbon: \( \beta \) for glucosylated compounds 1 and 3, and \( \alpha \) for mannose derivatives 2, 4, and 5.

1D and homonuclear 2D \(^1\text{H} \) NMR studies confirm the structure of benzochlorins 6 and 17. NOESY cross-correlation peaks were seen between ethyl groups carried by carbon 7 and the two \textit{ortho} protons of the \textit{meso}-phenyl group (Figure 4). Moreover \(^1\text{H} \) NMR 2D spectra of benzochlorin 6 showed NOE interactions between the ten protons (1.96 ppm CH\(_2\)-ethyl and −0.02 ppm CH\(_3\)) of the C\(_7\) ethyls and H\(_8\) and H\(_{10}\), \textit{ortho} protons of the \textit{meso}-phenyl group (7.79 ppm). Such a behavior corresponds to a cyclization of the vinylformyl group on the C\(_8\) atom with ethyl migration from C\(_8\) to C\(_7\) atom. The resonance of the C\(_1\) proton of the maltosyl group which appears as a doublet (\( J = 7.5 \text{ Hz} \)) indicates a pure \( \beta \)-configuration of the anomeric carbon of maltose.

The \(^1\text{H} \) NMR spectra of porphyrins 10–12 and azaporphyrins 7–9 show five resonance groups: aromatic protons between 7 and 9 ppm; from 4 to 0 ppm, \( \beta \)-pyrrolic alkyl substituents; acetyl protecting groups at 2.1 ppm and isopropylidene moieties from 1.8 to 1.2 ppm; between 10 and 11.7 ppm, \textit{meso} protons, and pyrrolic NH from −3 to −3.7 ppm for porphyrins 10–12 and from −1.8 to −2.9 ppm for azaporphyrins 7–9. The resonance of the anomeric proton of glycosyl groups in compounds 7–12 appears as a well-defined doublet with \( J = 3 \text{ Hz} \) for 7 and 10 (\( \alpha \) anomeric configuration) and \( J = 8 \text{ Hz} \) for 8, 9, 11, and 12, respectively (\( \beta \) anomeric configuration).

2.4 PARTITION PROPERTIES

Amphiphilic property is a characteristic of dyes which may be decisive for photosensitizing activity since this parameter may influence their ability to cross cell membrane as well as their localization within the cell. The partition between 2-octanol and PBS buffer at pH 7.4, determined by equilibrating equal parts of PBS and 2-octanol at 20 °C allows us to define the partition coefficient (PC) which is dependent on the amphiphily of the molecule. Optical density (OD) was measured between 400 and 450 nm and PC was calculated as the ratio of OD(2-octanol)/OD(PBS). Except for compounds 1 and 2, the repartition of hydrophilic and lipophilic substituents around the macrocycles confers a variable amphiphilic character to the molecules, confirmed by the values of partition coefficient; however, no direct correlation was found with the \textit{in vitro} photocytotoxicity (Table 2).

2.5 \textit{IN VITRO} PHOTOCYTOTOXICITY

All these compounds were evaluated \textit{in vitro} on the human colonic adenocarcinoma cell line HT29. None of them were found cytotoxic in absence of light at the tested concentrations (up to 10 \( \mu \text{g/mL} \)). Photo-activation was performed using a home made “light box” giving a fluence of 3.8 mW/cm\(^2\) on the whole visible spectrum. Irradiation with red light was carried out using the same device fitted with an orange filter (0% \( T \) at 520 nm and 80% \( T \) at 590 nm and above) leading to a fluence of 2 mW/cm\(^2\). As previously observed with glycophenyl porphyrins,\(^9\) tetrakis derivatives 1 and 2 were found inefficient as photosensitizers while trisubstituted compounds 3–5, which are amphiphilic molecules, display good photocytotoxic properties equal to or better than \textit{Photofrin}³⁰ in this experimental model. The best compound of these series was the trismannosyloxypropylphenyl derivative 5 which displayed an activity equal to that of TPP(\textit{GluOH})\(_3\) (Figure 5).
As shown in Figure 6, azaporphyrins were also found good in vitro sensitizers. In spite of a relatively high partition coefficient (log PC = 1.48), which is indicative of its low water solubility, compound 7, which has two adjacent glucose residues on β-pyrrolic positions, gives a regular dose response curve from 90% survival at 0.1 mg/mL to 13% at 10 mg/mL for a light dose of 2.3 J/cm². Regarding the nitrophenyl glycoconjugated molecules, activities are also relevant: however, the glucosylated dye 11 is more efficient than the maltosyl derivative 12 as well as than Photofrin® (respectively 63%, 100%, and 85% survival at 0.5 μg/mL for a light dose of 0.6 J/cm²). The corresponding azaporphyrins 8 and 9 are fairly less active with around 40% survival at 10 μg/mL and 0.6 J/cm².

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Because one aim of this work was to improve photosensitization to red light, which is the only one able to enter deeply in living tissues, we compared the activity ofaza derivatives and of benzochlorins following whole spectrum irradiation (2.3 J/cm², fluence 3.8 mW/cm²), or red light irradiation (λ > 590 nm, 2.5 J/cm², fluence 2 mW/cm²). Data are given in Figure 7 with those obtained under the same conditions with Photofrin® and TPP(GluOH)₃ used as standards. As expected, because of the relatively low absorption in the red, Photofrin® is less active with light above 590 nm [Figure 7(A)]. For TPP(GluOH)₃, this difference appears only at low dose (0.5 μg/mL) since this compound displays outstanding activity [Figure 7(B)]. Activity of azaporphyrins 7–9 did not show significant variation because of the strong increase of absorbance in the red region [Figures 7(E)–7(G)]. Benzochlorin 17 did not elicit any photosensitizing property, probably because of its very high hydrophobicity [Figure 7(C)], while its maltosyl derivative 6, although poorly hydrophilic (log PC > 3), exhibits significant photodynamic activity above 5 μg/mL with light above 590 nm [Figure 7(D)].

### Table 2  
Log (PC) and survival fraction of HT29 tumor cells after irradiation. HT29 cells were grown in DMEM supplemented with 10% FCS. Surviving fraction was estimated using the MTT assay.

<table>
<thead>
<tr>
<th>Compound</th>
<th>Log (P.C.)</th>
<th>Surviving Fraction, after irradiation (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>TPP(GluOH)₄</td>
<td>0.3</td>
<td>70</td>
</tr>
<tr>
<td>1</td>
<td>0,032</td>
<td>100</td>
</tr>
<tr>
<td>2</td>
<td>0.18</td>
<td>70</td>
</tr>
<tr>
<td>3</td>
<td>1.4</td>
<td>62</td>
</tr>
<tr>
<td>TPP(GluOH)₃</td>
<td>1.8</td>
<td>13</td>
</tr>
<tr>
<td>4</td>
<td>0.78</td>
<td>10</td>
</tr>
<tr>
<td>5</td>
<td>1.6</td>
<td>14</td>
</tr>
<tr>
<td>benzochlorin 17</td>
<td>&gt;3</td>
<td>100, (85°)</td>
</tr>
<tr>
<td>6</td>
<td>&gt;3</td>
<td>75, (55°)</td>
</tr>
<tr>
<td>7</td>
<td>1.48</td>
<td>70, (59°)</td>
</tr>
<tr>
<td>8</td>
<td>1</td>
<td>47, (40°)</td>
</tr>
<tr>
<td>9</td>
<td>0.43</td>
<td>47, (73°)</td>
</tr>
<tr>
<td>10</td>
<td>1.48</td>
<td>70</td>
</tr>
<tr>
<td>11</td>
<td>0.65</td>
<td>30</td>
</tr>
<tr>
<td>12</td>
<td>1</td>
<td>90</td>
</tr>
<tr>
<td>Photofrin²</td>
<td>...</td>
<td>45</td>
</tr>
</tbody>
</table>

a Dose 1 μg/mL.  
b Dose 5 μg/mL.  
c Whole spectrum irradiation, total light dose 2.3 J/cm², fluence 3.8 mW/cm².  
d Red light irradiation (λ > 590 nm): orange filter 520 nm 0% T, 590 nm 80% T, light dose 2.5 J/cm², fluence 2 mW/cm².

As shown in Figure 6, azaporphyrins were also found good in vitro sensitizers. In spite of a relatively high partition coefficient (log PC = 1.48), which is indicative of its low water solubility, compound 7, which has two adjacent glucose residues on β-pyrrolic positions, gives a regular dose response curve from 90% survival at 0.1 μg/mL to 13% at 10 μg/mL for a light dose of 2.3 J/cm². Regarding the nitrophenyl glycoconjugated molecules, activities are also relevant: however, the glucosylated dye 11 is more efficient than the maltosyl derivative 12 as well as than Photofrin® (respectively 63%, 100%, and 85% survival at 0.1 μg/mL for a light dose of 0.6 J/cm²). The corresponding azaporphyrins 8 and 9 are fairly less active with around 40% survival at 10 μg/mL and 0.6 J/cm². Because one aim of this work was to improve photosensitization to red light, which is the only one able to enter deeply in living tissues, we compared the activity of aza derivatives and of benzochlorins following whole spectrum irradiation (2.3 J/cm², fluence 3.8 mW/cm²), or red light irradiation (λ > 590 nm, 2.5 J/cm², fluence 2 mW/cm²). Data are given in Figure 7 with those obtained under the same conditions with Photofrin® and TPP(GluOH)₃ used as standards. As expected, because of the relatively low absorption in the red, Photofrin® is less active with light above 590 nm [Figure 7(A)]. For TPP(GluOH)₃, this difference appears only at low dose (0.5 μg/mL) since this compound displays outstanding activity [Figure 7(B)]. Activity of azaporphyrins 7–9 did not show significant variation because of the strong increase of absorbance in the red region [Figures 7(E)–7(G)]. Benzochlorin 17 did not elicit any photosensitizing property, probably because of its very high hydrophobicity [Figure 7(C)], while its maltosyl derivative 6, although poorly hydrophilic (log PC > 3), exhibits significant photodynamic activity above 5 μg/mL with light above 590 nm [Figure 7(D)].

### Fig. 5  
In vitro response of HT29 cells to glycoconjugated alkoxy TPP 1–5 following 24 h incubations and irradiation with white light (0.6 J/cm²).

### Fig. 6  
In vitro response of HT29 cells to azaporphyrins 7, 8, and 9, and porphyrins 11 and 12 following 24 h incubations and irradiation with white light (0.6 J/cm²). Photofrin® data are given for comparison.
2.6 CONCLUSION

Various new glycoconjugated tetrapyrrolic macrocycles have been described and characterized. Preliminary in vitro biological data confirm previous observations suggesting the requirement of amphiphilic for efficient photodynamic activity. Glycoconjugation is obviously a good mean to introduce such a balance between hydrophilicity and hydrophobicity: however, the nature of the sugar residues seems to take a significant part in the photosensitizing properties and remains to be elucidated. This should be undertaken in the light of the

Fig. 7 Survival of HT29 cells treated with various compounds (24 h incubations) without irradiation (filled) or after whole spectrum (dotted, 2.3 J/cm², 3.8 mW/cm²) or red light irradiation (shaded, 2.5 J/cm², 2 mW/cm²). Panel A: photofrin, B: TPP(GluOH)₃, C: benzochlorin 17, (D): maltosyl benzochlorin 6, E: di-β-glucosyl porphyrin 10, F: di-β-glucosyl phenyl porphyrin 8, G: di-β-maltosyl phenyl porphyrin 9.
knowledge of the various lectines occurring at the surface of the cell membrane and of their implication in glycoconjugated dyes internalization. As expected, increase of light absorption above 590 nm may improve the photosensitizing properties; this is particularly true in the benzochlorin series for which the maltosyl derivative displays, for a constant energy exposition, a higher activity when irradiated with red light than with all the visible spectrum. This last compound is however too poorly water soluble to be considered as a good candidate for PDT; further synthesis of di or tri glycoconjugated analogs should be considered to reach highly efficient molecules.

3 EXPERIMENTAL SECTION

3.1 GENERAL

All chemicals used were of reagent grade and were purchased from Aldrich or Fluka. Merck silica gel 60 (0.040–0.060 mm) was used for column chromatography. Macherey–Nagel precoated plates (SIL G-200, 2 mm) were used for preparative thin layer chromatography. Elemental analysis were carried out by the “Service Central de Microanalyse du CNRS.” 1H NMR spectra were obtained in the indicated deuterated solvents with Brucker AM-200 and AM-400 instruments. Acidic impurities of chloroform-d3 were removed with anhydrous K2CO3. Chemical shift values were given in ppm relative to TMS. Coupling constants were given in Hz. Optical spectra were recorded using a Varian DMS 200 spectrometer.

Isomeric ratios were determined by HPLC analysis which was performed with a Gilson apparatus with a dynamic mixer module Gilson 811, a monometric module Gilson 802, a pump Gilson 303 and a holochrom module Gilson (detection at 420 nm). Column:Hibar Lichrosorb SI 60, 7 mm Merck, mobile phase, gradient heptane/methylene chloride (1.5 mL/min, start at 80% heptane, then 50% at 15 min and 80% at 49 min).

3.2 GENERAL PROCEDURE FOR THE PREPARATION OF BROMO ALKOXY PERACETYLATED-D-GLYCOSIDES

To a cooled solution of per acetylated glycoside (25 mmol) and 2-bromo ethanol or 3-bromo propanol (30 mmol) in dry methylene chloride (50 mL) was added, drop by drop (15 min), boron trifluoride etherate complex (15.4 mL, 125 mmol). The solution was stirred 1 h at 0 °C then at room temperature overnight. The crude solution was poured into ice water. The aqueous solution was extracted with methylene chloride. The organic phase was washed with water, diluted sodium hydrogen carbonate, water, dried over sodium sulfate, filtered and concentrated. The yellow syrup was chromatographed on silica gel column eluted by a mixture of methylene chloride/ethanol (10:1, v/v). The first fraction was title compound.

Compounds I–IV were synthesized by this method:

2-bromoethoxy 2, 3, 4, 6-tetra-O-acetyl-β-D-glucose I. This compound crystallized in white needles from ethyl acetate/isooctane, yield 40%.

Anal. Calcd for C16H23O10Br: C 42.21; H, 5.09; Br, 17.55. Found: C, 42.52; H, 5.07; Br, 16.39. m.p.
116 °C. 1H RMN (CDCl3), δ (ppm): 5.06 (2H, H “ose”), 5.01 (3H, H C2 “ose”), 4.55 (2H, H C1 “ose,” J = 7.8 Hz), 4.15 (3H, H “ose” and CH2α), 3.70, 3.80 (2H, H C6 “ose”), 3.43 (2H, CH2β), 2.06 (3H, AcO), 2.04 (3H, AcO), 1.99 (3H, AcO), 1.98 (3H, AcO).

2-bromoethoxy 2, 3, 4, 6-tetra-O-acetyl-α-D-mannose II. This compound crystallized in white needles from ethyl acetate/isooctane, yield 63%.

Anal. Calcd for C16H23O10Br: C, 45.23; H, 5.29; Br, 21.80, amorphous. 1H RMN (CDCl3), δ (ppm): 5.31 (s, 1H, H C2 “ose”), 5.23 (t, 2H, CH2α), 4.80 (d, 1H, H C1 “ose,” J = 1.1 Hz), 4.28 (d, 1H, “ose”), 4.22 (d, 1H, “ose”), 4.13 (m, 2H, “ose”), 3.88 (m, 2H, H C6 “ose”), 3.50 (m, 2H, CH2β), 2.13 (3H, AcO), 2.08 (3H, AcO), 1.97 (3H, AcO).

3-bromopropoxy 2, 3, 4, 6-tetra-O-acetyl-α-D-mannose III. Yield 77%. Anal. Calcd for C16H23O10Br: 0.5 BrCH2CH2CH2OH: C, 41.24; H, 5.33; Br, 22.25. Found: C, 40.85; H, 5.27; Br, 21.80, amorphous. 1H RMN (CDCl3), δ (ppm): 5.37 (s, 1H, H C2 “ose”), 5.25 (t, 2H, CH2α), 4.85 (d, 1H, H C1 “ose,” J = 1.4 Hz), 4.30 (d, 1H, “ose”), 4.24 (d, 1H, “ose”), 4.11 (m, 2H, “ose”), 3.92 (m, 2H, H C6 “ose”), 3.50 (m, 4H, CH2β, and CH2α), 2.15 (3H, AcO), 2.10 (3H, AcO), 2.04 (3H, AcO), 1.99 (3H, AcO).

2-bromoethoxy 2, 3, 6-2’, 3’, 4’, 6’-hepta-O-acetyl-β-D-maltose IV. Yield 70%. Anal. Calcd for C25H46O18Br: C, 45.23; H, 5.29; Br 10.75. Found: C, 45.53; H, 5.32; Br, 10.10. m.p. 74 °C, pasty. 1H RMN (CDCl3), δ (ppm): 5.38 (d, 1H, H C1, “ose,” J = 3.8 Hz), 5.25 (2H, CH2α, J = 10 Hz), 5.02 (t, 1H, “ose,” J = 9.8 Hz), 4.48 (m, 2H, “ose”), 4.56 (d, 1H, H C1 “ose,” J = 7.9 Hz), 4.46 (dd, 1H, “ose,” J = 2.5 and 12 Hz), 4.27–3.70 (8H, M, “ose”), 3.42 (2H, CH2β, J = 5 Hz), 2.19 (3H, AcO), 2.11 (3H, AcO), 2.07 (3H, AcO), 2.04 (3H, AcO), 2.01 (3H, AcO), 1.99 (3H, AcO), 1.97 (3H, AcO).

5, 10, 15-tri(4-methoxyphenyl)-20-phenyl porphyrin (methyl ether of VI). A solution of pyrrole (15.66 mL, 226 mmol), benzaldehyde (6 g, 56.6 mmol), and 4-methoxybenzaldehyde (23 g, 169 mmol) in propionic acid (250 mL) were refluxed during 30 min. The crude solution was concentrated under vacuum. The black crystals were purified by silica gel chromatography eluting with a mixture of methylene chloride/heptane (3/1, v/v).
The first red band corresponded to 5,10,15,20-tetrakis(4-methoxyphenyl)porphyrin (methyl ether of V), (2.6 g, yield 6.3%), the second one was title compound and the other red bands corresponded to meso tetraphenyl porphyrin and its mono and dimethoxy analogs (3.3 g). The trimethoxy compound was crystallized from a mixture of methylene chloride/methanol (2 g, yield 4.8%).

Anal. Calcld. for C₄₄H₃₀N₄O₃·H₂O: C, 77.63; H, 4.74; N, 8.23. Found: C, 77.32; H, 4.78; N, 7.88. ¹H RMN (MeOD), δ (ppm): 8.82 (s broad, 6H, pyrrole), 8.73 (s broad, 2H, pyrrole), 8.09 (m, 2H, phenyl), 7.93 (d, 6H, phenyl, J = 8 Hz), 7.73 (m, 3H, phenyl), 7.14 (d, 6H, phenyl, J = 8 Hz). UV-visible spectrum in CH₂Cl₂: λₘₐₓ nm (ε, L mmol⁻¹ cm⁻¹): 420.5 (522.8), 517 (21.4), 592.5 (8.4), 649 (9).

3.3 GENERAL PROCEDURE FOR THE PREPARATION OF PER-O-ACETYLATED GLYCOSYLATED PORPHYRINS

meso-5, 10, 15, 20-tetakis(4-hydroxyphenyl) porphyrin V or meso-5,10,15,20-tetakis(4-hydroxyphenyl)-20-phenylporphyrin VI (1 g, 1.412 mmol) in dry methyl acetate was slowly added to the porphyrin solution. This solution was heated at 60 °C and vigorously stirred during three days. The crude solution was concentrated under vacuum. The residue was dissolved in methylene chloride, washed with water. The organic phase was dried over sodium sulfate, filtered and the solvent was evaporated. The crude product was purified by silica gel chromatography eluting with a mixture of methylene chloride/acetic acid (10:1, v/v) and crystallized from a mixture of methylene chloride/heptane or methylene chloride/methanol.

The following porphyrins were synthesized by this method:

meso-5,10,15,20-tetakis[4-(2-ethoxy-2',3',4',6'-tetr-O-acetyl-β-D-glucosyloxy)phenyl]porphyrin, 1-OAc. Crystallized from a mixture of methylene chloride/heptane, yield 62%. Anal. Calcld. for C₁₀₈H₁₁₈N₄O₃₂·2H₂O: C, 58.57; H, 5.55; N, 2.48. ¹H RMN (CDCl₃), δ (ppm): 8.86 (s, 8H, pyrrole), 8.13 (d, 8H, ortho-phenyl, J = 8 Hz), 7.28 (d, 8H, meta-phenyl, J = 8 Hz), 5.46 (m, 4H, H C₃ ‘‘ose’’), 5.39 (m, 4H, H C₄ ‘‘ose’’), 5.30 (m, 4H, H C₅ ‘‘ose’’), 5.08 (d, 4H, H C₁ ‘‘ose’’), J = 1 Hz), 4.45 (m, 8H, CH₂CD₃), 4.28 (m, 8H, H C₆ ‘‘ose’’), 4.10 (t, 8H, CH₂CD₃), 2.21 (s, 12H, AcO), 2.16 (s, 12H, AcO), 2.05 (s, 12H, AcO), 2.02 (s, 12H, AcO), 2.02 (s, 12H, AcO), −2.78 (s, 2H, NH). UV-visible spectrum in CH₂Cl₂: λₘₐₓ nm (ε, L mmol⁻¹ cm⁻¹): 422.5 (433), 519 (16.5), 556 (12), 594 (9), 650 (7.2).

meso-5,10,15,20-tetakis[4-(2-ethoxy-2',3',4',6'-tetr-O-acetyl-β-D-mannosyloxy)phenyl]porphyrin, 2-OAc. Crystallized from a mixture of methylene chloride/methanol, yield 61%. Anal. Calcld. for C₁₀₈H₁₁₈N₄O₃₄·2H₂O: C, 58.61; H, 5.60; N, 2.53. Found: C, 58.57; H, 5.55; N, 2.48. ¹H RMN (CDCl₃), δ (ppm): 8.86 (s, 8H, pyrrole), 8.13 (d, 8H, ortho-phenyl, J = 8 Hz), 7.28 (d, 8H, meta-phenyl, J = 8 Hz), 5.46 (m, 4H, H C₃ ‘‘ose’’), 5.39 (m, 4H, H C₄ ‘‘ose’’), 5.30 (m, 4H, H C₅ ‘‘ose’’), 5.08 (d, 4H, H C₁ ‘‘ose’’), J = 1 Hz), 4.45 (m, 8H, CH₂CD₃), 4.28 (m, 8H, H C₆ ‘‘ose’’), 4.10 (t, 8H, CH₂CD₃), 2.21 (s, 12H, AcO), 2.16 (s, 12H, AcO), 2.05 (s, 12H, AcO), 2.02 (s, 12H, AcO), 2.02 (s, 12H, AcO), −2.78 (s, 2H, NH). UV-visible spectrum in CH₂Cl₂: λₘₐₓ nm (ε, L mmol⁻¹ cm⁻¹): 422.5 (506.5), 490 (6), 519 (19.2), 556 (12.9), 593 (7.1), 650 (7.2).

meso-5, 10, 15, 20-tetakis[4-(2-ethoxy-2',3',4',6'-tetr-O-acetyl-α-D-mannosyloxy)phenyl]porphyrin, 3-OAc. Crystallized from a mixture of methylene chloride/heptane, yield 66%. Anal. Calcld. for C₁₀₈H₁₁₈N₄O₃₄·2H₂O: C, 58.61; H, 5.60; N, 2.53. Found: C, 58.57; H, 5.55; N, 2.48. ¹H RMN (CDCl₃), δ (ppm): 8.86 (s, 8H, pyrrole), 8.13 (d, 8H, ortho-phenyl, J = 8 Hz), 7.28 (d, 8H, meta-phenyl, J = 8 Hz), 5.46 (m, 4H, H C₃ ‘‘ose’’), 5.39 (m, 4H, H C₄ ‘‘ose’’), 5.30 (m, 4H, H C₅ ‘‘ose’’), 5.08 (d, 4H, H C₁ ‘‘ose’’), J = 1 Hz), 4.45 (m, 8H, CH₂CD₃), 4.28 (m, 8H, H C₆ ‘‘ose’’), 4.10 (t, 8H, CH₂CD₃), 2.21 (s, 12H, AcO), 2.16 (s, 12H, AcO), 2.05 (s, 12H, AcO), 2.02 (s, 12H, AcO), 2.02 (s, 12H, AcO), −2.78 (s, 2H, NH). UV-visible spectrum in CH₂Cl₂: λₘₐₓ nm (ε, L mmol⁻¹ cm⁻¹): 422.5 (506.5), 490 (6), 519 (19.2), 556 (12.9), 593 (7.1), 650 (7.2).
Found: C, 59.65; H, 5.52; N, 2.84. 1H RMN (CDCl₃, δ (ppm)): 8.86 (s, 4H, pyrrole), 8.86 (d, 2H, pyrrole), 8.81 (d, 2H, pyrrole), 8.20 (dd, 2H, ortho-phenyl, J = 7.5 Hz), 8.12 (d, 6H, ortho-phenyl, J = 7.8 Hz), 7.75 (m, 3H, phenyl), 7.8 (dd, 6H, meta-phenyl, J = 8 Hz), 5.49 (dd, 3H, H C₅ “ose”), 5.38 (t, 3H, H C₄ “ose”, J = 10.3 Hz), 5.08 (d, 3H, H C₁ “ose”, J = 1.4 Hz), 4.44 (m, 6H, CH₂₃), 4.49 (dd, 3H, H C₆ “ose”), 4.22 (dd, 3H, H C₆ “ose”), 4.28 (m, 3H, H C₆ “ose”), 4.09 (m, 6H, CH₂₃), 2.20 (s, 9H, AcO), 2.15 (s, 9H, AcO), 2.05 (s, 9H, AcO), 2.01 (s, 9H, AcO), −2.78 (s, 2H, NH). UV-visible spectrum in CH₂Cl₂: λ_max, nm (ε, L mmol⁻¹ cm⁻¹): 421.5 (450), 489 (5.4), 518 (17.6), 555 (10.9), 592.5 (6.5), 649 (6.1).

**meso - 5, 10, 15 - tri[4 - (3-propoxy-2', 3', 4', 6'-tetra-O-acetyl-α-D-mannosylphenyl)-20-phenyl porphyrin, 5-OAc.** Crystallized from a mixture of methylene chloride/heptane, yield 43%. Anal. Calcld for C₅₉H₁₀₂N₄O₃₅: H₂O: C, 61.82; H, 5.68; N, 3.04. Found: C, 61.71; H, 6.01; N, 2.55. 1H RMN (CDCl₃, δ (ppm)): 8.86 (s, 6H, pyrrole), 8.81 (d, 2H, pyrrole), 8.18 (m, 2H, ortho-phenyl, J = 8.4 Hz), 8.12 (d, 6H, ortho-phenyl, J = 8.6 Hz), 7.76 (m, 3H, phenyl), 7.28 (d, 6H, meta-phenyl), 5.36 (m, 10H, “ose”), 4.96 (d, 3H, H C₅ “ose”), 4.33 (m, 10H, “ose” and CH₂₃), 4.10 (m, 10 H, “ose”), 3.84 (m, 6H, CH₂₃), 2.30 (t, 6H, CH₂₃), 2.18 (s, 9H, AcO), 2.16 (s, 9H, AcO), 2.15 (s, 9H, AcO), −2.77 (s, 2H, NH). UV-visible spectrum in CH₂Cl₂: λ_max, nm (ε, L mmol⁻¹ cm⁻¹): 421 (450), 518 (18.1), 554.5 (12.2), 594 (7.1), 650 (7.3).

**3.4 GENERAL PROCEDURE FOR THE PREPARATION OF GLYCOCONEJUGATED PORPHYRINS**

Sodium methanolate in dry methanol (100 μL, 0.1 N) was added to a solution of protected glycosylated porphyrin (2×10⁻⁵ mol) in dry methanol (10 mL). The solution was stirred at room temperature for 60 min. Amberlite MB3 (200 mg) was added to the solution which was stirred 15 min then filtered. The resin was washed with methanol. The solution was concentrated under vacuum. The crude product was crystallized from MeOH, 1,2-dichloroethane and used without purification.

The following glyconjugated porphyrins were synthesized by this method:

**meso - 5, 10, 15, 20 - tetrakis[4 - (2 - ethoxy - β-D-glucosyl)phenyl]porphyrin, 1.** Yield 100%. Anal. Calcld for C₆₈H₇₀N₄O₂₅, 10H₂O: C, 55.88; H, 6.35; N, 3.83. Found: C, 55.22; H, 5.30; N, 3.85. 1H RMN (DMF-d₇, δ (ppm)): 8.98 (s, 4H, pyrrole), 8.98 (d, 2H, pyrrole, J = 4.4 Hz), 8.90 (d, 2H, pyrrole, J = 4.4 Hz), 8.31 (dd, 2H, ortho-phenyl, J = 8 Hz), 8.21 (d, 6H, ortho-phenyl, J = 8 Hz), 7.89 (dd, 3H, phenyl, J = 8 Hz), 7.46 (d, 6H, meta-phenyl, J = 8 Hz), 5.04 (s broad, 3H, OH C₅ “ose”), 5.29 (s broad, 6H, OH C₅ and OH C₆ “ose”), 5.43 (d, 3H, H C₅ “ose”), 4.76 (s broad, 3H, OH C₆ “ose”), 4.53 (d, 3H, H C₄ “ose”), 4.53 (m, 6H, CH₂₃), 4.41 and 4.12 (q, 6H, CH₂₃), 3.93 and 3.72 (m, 6H, H C₆ “ose”), 3.46 (m, 6H, H C₅, and H C₄ “ose”), 3.37 (m, 3H, H C₅ “ose”), 3.30 (t, 3H, H C₃ “ose”), −2.69 (s, 2H, NH). UV-visible spectrum in pyridine/MeOH (1/24, v/v): λ_max, nm (ε, L mmol⁻¹ cm⁻¹): 417.5 (384.9), 515 (17), 552 (12.4), 591 (8.4), 648 (8).

**meso - 5, 10, 15 - tri[4 - (2 - ethoxy - α-D-mannosyl)phenyl]20-phenylporphyrin, 4.** Yield 85%. Anal. Calcld for C₆₉H₇₁N₄O₂₅, 10H₂O: C, 55.88; H, 6.35; N, 3.83. Found: C, 55.22; H, 5.30; N, 3.85. 1H RMN (DMF-d₇, δ (ppm)): 8.98 (s, 4H, pyrrole), 8.98 (d, 2H, pyrrole, J = 4.4 Hz), 8.90 (d, 2H, pyrrole, J = 4.4 Hz), 8.31 (dd, 2H, ortho-phenyl, J = 8 Hz), 8.21 (d, 6H, ortho-phenyl, J = 8 Hz), 7.89 (dd, 3H, phenyl, J = 8 Hz), 7.46 (d, 6H, meta-phenyl, J = 8 Hz), 5.04 (s broad, 3H, OH C₅ “ose”), 4.99 (d, 3H, H C₅ “ose”), 4.95 (s broad, 3H, OH C₅ “ose”), 4.64 (broad, 6H, OH “ose”), 4.54 (m, 6H, CH₂₃), 4.23 and 4.03 (m, 6H, CH₂₃), 3.93 (m, 3H, H C₅ “ose”), 3.91 (m, 3H, H C₅ “ose”), 3.80 (m, 3H, H C₅ “ose”), 3.76–3.73 (m, 9H, H C₃ and H C₆ “ose”), −2.71 (s, 2H, NH). UV-visible spectrum in pyridine/MeOH (1/24, v/v): λ_max, nm (ε, L mmol⁻¹ cm⁻¹): 417.5 (384.9), 515 (17), 552 (12.4), 591 (8.4), 648 (6.2).

**meso - 5, 10, 15 - tri[4 - (3-propoxy-α-D-mannosyl)phenyl]20-phenylporphyrin, 5.** Yield 100%. Anal. Calcld for C₇₁H₇₈N₄O₂₆, 5H₂O: C, 60.33; H, 6.28; N, 3.96. Found: C, 60.38; H, 6.52; N, 3.81. 1H RMN (DMF-d₇, δ (ppm)): 8.99 (s, 4H, pyrrole), 8.98 (d, 2H,
pyrrole, $J = 4.4 \text{ Hz}$), 8.91 ($d$, 2H, pyrrole, $J = 4.4 \text{ Hz}$), 8.32 ($dd$, 2H, ortho-phenyl, $J = 8$ and 3 Hz), 8.22 ($d$, 6H, ortho-phenyl, $J = 8.3 \text{ Hz}$), 7.89 ($dd$, 3H, phenyl, $J = 8$ and 3 Hz), 7.47 ($d$, 6H, meta-phenyl, $J = 8.3 \text{ Hz}$), 4.99 ($s$ broad, 3H, OH C$_5$ “ose”), 4.89 ($d$, 3H, H C$_1$ “ose”), 4.87 ($s$ broad, 3H, OH C$_3$ “ose”), 4.75 ($s$ broad, 3H, OH C$_4$ “ose”), 4.55 (broad, 3H, OH “ose”), 4.45 ($t$, 6H, CH$_2$, $J = 6 \text{ Hz}$), 4.09 and 3.77 ($m$, 6H, CH$_2$), 3.89 ($m$, 6H, H C$_2$ and H C$_3$ “ose”), 3.71 ($m$, 3H, H C$_4$ “ose”), 3.72 ($m$, 9H, H C$_5$ and H C$_6$ “ose”), 2.27 ($m$, 6H, CH$_2$), $-2.69$ ($s$, 2H, NH).

UV-visible spectrum in THF/H$_2$O (4/1, v/v): $\lambda_{max}$, nm ($\epsilon$, L mmol$^{-1}$ cm$^{-1}$): 420 (298.7), 516.5 (12.1), 553 (8.5), 594 (4.6), 651 (4.8).

meso-5-(2'-formylvinyl)-10-(4-methoxyphenyl)-2, 3, 7, 8, 12, 13, 17, 18-octaethylporphyrin nickel, 14a and meso-5-(2'-formylvinyl)-15-(4-methoxyphenyl)-2, 3, 7, 8, 12, 13, 17, 18-octaethylporphyrin nickel, 14b. To a stirred suspension of 3-dimethylamino acrolein (2.10 mL) in dry dichloromethane (77.1 mL) under an argon atmosphere was added trifluoroacetic acid (6 mL) in dry dichloromethane (77.1 mL). The mixture was stirred for 1 h, the color of solution changed from green/orange. Dichloromethane was added after evaporation of acid, and the mixture was washed with water and neutralized with saturated sodium bicarbonate solution. The organic layer was washed with water and dried over sodium sulfate. The residue obtained after filtration and evaporation was chromatographed on silica gel eluted with CH$_2$Cl$_2$/heptane (5/1). The first green fraction was collected and evaporated to give a green powder (57 mg, yield 58%).

Anal. Calcd for C$_{46}$H$_{52}$N$_4$O$_2$Ni: C, 75.11; H, 7.12; N, 6.90. $^1$H NMR (CDCl$_3$), $\delta$ (ppm): 8.75 ($m$, 2H, H meso, and H benzo), 8.40 ($s$, 1H, H meso), 7.62 ($d$, 2H, ortho-phenyl, $J = 8 \text{ Hz}$), 7.64 ($m$, 2H, H$_{meso}$ and H$_{benzo}$), 6.96 ($d$, 2H, meta-phenyl, $J = 8 \text{ Hz}$), 3.96 ($s$, 3H, OCH$_3$), 3.40 ($m$, 8H, CH$_2$), 2.11 ($g$, 2H, CH$_3$), 1.88 ($g$, 2H, CH$_2$), 1.55 ($m$, 12H, CH$_3$), 0.89 ($t$, 3H, CH$_3$), 0.63 ($t$, 3H, CH$_3$), 0.06 ($t$, 6H, CH$_3$).

UV-visible spectrum in CH$_2$Cl$_2$: $\lambda_{max}$, nm ($\epsilon$, L mmol$^{-1}$ cm$^{-1}$): 429.5 (70), 525.5 (shoulder), 642 (shoulder), 693.5 (31.1).

meso-5-(4-hydroxyphenyl)-2, 3, 7, 8, 12, 13, 17, 18-octaethyl-8-10-benzochlorin nickel, 16. A solution of meso-5-(para-methoxyphenyl)-2, 3, 7, 8, 12, 13, 17-octaethyl-8-10-benzochlorin Nickel 15 (171 mg, 2.31$x10^{-4}$ mol) in dry methylene chloride (30 mL) was cooled to $-20 \text{ °C}$ under argon. Bore tribromide (1.665 mL, 15 equiv.) was slowly added to the solution which was stirred at $-20 \text{ °C}$ for 30 min then heated to room temperature overnight. The green crude solution was diluted in ice and neutralized by a sodium bicarbonate solution then extracted by methylene chloride. The organic phase was washed with water (2x), dried over sodium sulfate, filtered and concentrated under vacuum. The benzochlorin was chromatographed on silica gel eluted with CH$_2$Cl$_2$/heptane (5/1) then crystallized from methylene chloride/methanol (141 mg, 84%).

Anal. Calcd for C$_{48}$H$_{52}$N$_4$ONi, 2H$_2$O: C, 71.34; H, 7.18; N, 7.40. Found: C, 71.34; H, 6.79; N, 7.21. UV-visible spectrum in CH$_2$Cl$_2$: $\lambda_{max}$, nm ($\epsilon$, L mmol$^{-1}$ cm$^{-1}$): 429.5 (92.7), 523.5 (shoulder), 647 (shoulder), 693 (43.2).

meso-5-(4-hydroxyphenyl)-2, 3, 7, 8, 12, 13, 17, 18-octaethyl-8-10-benzochlorin nickel, 17. Solid nickel benzochlorin 16 (110 mg, 1.5$x10^{-4}$ mol) was dissolved in concentrated sulfuric acid (8 mL) and stirred at room temperature for 1/2 hour. Dichloromethane...
and water was slowly added and the solution was neutralized with saturated hydrogen carbonate solution. The organic layer was dried over sodium sulfate. The residue obtained after filtration and evaporation was chromatographed on silica gel with CH₂Cl₂/acetonate (100/1, v/v). The title compound as green powder (71 mg) was collected (yield: 73%).

Anal. Calcld. for C₇₃H₉₀N₄O₁₉·7H₂O: C, 60.16; H, 7.98; N, 8.05. ¹H NMR (CDCl₃, δ (ppm): 9.50 (d, 1H, H benzono), 9.26 (s, 1H, H meso), 8.68 (s, 1H, H meso), 8.12 (t, 1H, H benzono), 7.96 (d, 2H, ortho-phenyl), 7.89 (d, 1H, H benzono, J = 8.3 Hz), 7.81 (d, 1H, H benzono, J = 8.3 Hz), 6.93 (d, 2H, meta-phenyl, J = 8 Hz), 4.07 (d, 2H, NH), 3.68 (m, 4H, CH₂), 3.46 (t, 6H, CH₃J, J = 7.6 Hz), 2.70 (broad, H, OH), 2.21 (m, 3H, CH₂), 1.96 (m, 4H, CH₂), 1.73 (t, 3H, CH₃J, J = 7.4 Hz), 1.59 (m, 2H, CH₂), 0.92 (t, 3H, CH₃J, J = 7.2 Hz), −0.02 (t, 6H, CH₃J, J = 7.2 Hz).

UV-visible spectrum in CH₂Cl₂: λ max, nm (ε, L mmol⁻¹ cm⁻¹): 418 (74.5), 548.5 (shoulder), 581.5 (7.9), 618.5 (9), 673 (22.7).

meso-5-[4-(2-ethoxy-β-D-maltosyl)phenyl]-2, 3, 7, 8, 12, 13, 17,17-octaethyl-8,10-benzochlorin, 6. Sodium methanolate in dry methanol (100 µL, 0.1 N) was added to a solution of protected glycosylated benzochlorin 18 (10 mg, 0.7×10⁻⁵ mol) in dry methanol (10 mL). The solution was stirred at room temperature for 60 min. Amberlite MB3 (100 mg) was added to the solution which was stirred 15 min then filtered. The resin was washed with methanol. The solution was concentrated under vacuum. The crude product was crystallized in a mixture MeOH/1-2 dichloroethane/heptane and used without purification (8 mg, yield 100%).

Anal. Calcld. for C₇₃H₉₀N₄O₁₉·7H₂O: C, 68.58; H, 7.41; N, 5.42. Found: C, 68.27; H, 7.12; N, 5.15. ¹H NMR (pyridine d₅), δ (ppm): 9.50 (d, 1H, H benzono), 9.26 (s, 1H, H meso), 8.68 (s, 1H, H meso), 8.12 (t, 1H, H benzono), 7.96 (d, 2H, ortho-phenyl), 7.89 (d, 1H, H benzono), 7.49 (m, 2H, CH₂ and C₃), 7.47 (t, 1H, OH C₇), 7.28 (d, 2H, meta-phenyl), 7.09 (m, 2H, OH C₃, and C₄), 6.38 (t, 2H, OH C₆), 6.32 (t, 2H, OH C₇), 5.95 (d, 1H, H C₅, “ose”, J = 4 Hz), 4.61 (dd, 1H, H C₇, “ose”), 4.95 (d, 1H, H C₇, “ose”, J = 8 Hz), 4.51 (dd, 2H, H C₅, “ose”), 4.54 (t, 1H, H C₃ “ose”), 4.47 (t, 2H, CH₂), 4.20 (t, 1H, H C₄ “ose”), 4.40 (m, 2H, H C₃ and H C₄ “ose”), 4.39 (t, 2H, CH₂), 4.09 (dd, 1H, H C₂ “ose”), 4.17 (dd, 1H, H C₇, “ose”), 0.05 (dd, 1H, H C₆ “ose”), 0.03 (dd, 1H, H C₆ “ose”), 3.87 (m, 1H, H C₅, “ose”), 3.67 (q, 2H, CH₂C₇), 3.63 (q, 2H, CH₂), 3.49 (q, 4H, CH₂), 3.42 (q, 2H, CH₂), 2.38 (q, 4H, CH₂C₂ and C₇), 2.13 (q, 2H, CH₂C₂), 1.72 (t, 3H, CH₃C₁₂), 1.66 (t, 3H, CH₃), 1.65 (t, 3H, CH₃C₁₂), 1.58 (t, 6H, CH₃), 1.57 (t, 3H, CH₃C₄), 0.15 (t, 6H, CH₃C₇). UV-visible spectrum in MeOH: λ max, nm (ε, L mmol⁻¹ cm⁻¹): 415 (68.9), 546 (shoulder), 582 (10.2), 618 (11.3), 672 (24.3).

1, 2, 3, 4-di-O-isopropylidene-6-(1-acetoxymethyl-2-nitro-2-nitro-α-D-galactose, 20. A solution of formyl protected galactose 19 (1.98 g, 7.68 mmol) and dimethylamino pridine (31 mg, 0.26 mmol) in nitromethane (1.1 mL) was stirred under reflux and argon for 48 h. 1.5 mL of acetic anhydride (15 mmol) in 5.5 mL of methylene chloride was added. The solution was stirred during 24 h. The crude mixture was quenched by a solution of aqueous sodium hydrogen carbonate (3 g in 15 mL) and then the aqueous phase was extracted by methylene chloride. The organic layers were dried over sodium sulfate, filtered and evaporated. The title product was purified by silica gel chromatography eluted with methylene chloride (2.61 g, yield 91%) and used without other characterization.

2-ethoxy carbonyl-3- (1', 2', 3', 4' - di-O-isopropylidene-α-D-galactosyl)-4-methyl-pyrrole, 21. Compound 20 (2.24 g, 5.96 mmol) was dissolved in isopropanol THF (1/1, v/v, 4.4 mL). This solution...
was added to 1,8-diazabicyclo(5.4.0)undec-7-en (DBU) (1.95 g), ethyl isocyanate (5.2 mL, 0.65 mmol) in isopropanol THF (12.5 mL, 1/1, v/v) at 0 °C. The solution was stirred 48 h at room temperature then was concentrated under vacuum. The crude product was purified by silica gel chromatography eluted by methylene chloride. The title product (1.89 g) was obtained in 83% yield.

Anal. Calcd for C_{10}H_{22}O_{2}: C, 59.83; H, 7.14. Found: C, 59.93; H, 7.07. 1H NMR (CDCl3, δ (ppm): 8.74 (s, 1H, NH), 6.64 (d, 1H, H-C5), 5.75 (d, 1H, H-C5, “ose”), 5.67 (d, 1H, H-C1, “ose”), J = 4 Hz), 4.69 (dd, 1H, H-C4, “ose”), 4.45 (dd, 1H, H-C5, “ose”), 4.30 (q, 2H, OCH2CH3), 4.25 (d, 1H, H-C3, “ose”), 2.24 (s, 3H, 4-CH3), 1.57–1.37–1.28 (s, 12H, isopropylidene), 1.30 (t, 3H, OCH2CH3).

2- benzoxycarbonyl-3′-(1′, 2′, 3′, 4′-di-O-isopropylidene-α-D-galactosyl)-4-methyl-pyrrole, 22. To a solution of sodium (30 mg, 1.29 mmol) dissolved in anhydrous methanol (6 mL) pyrrole 21 (1.9 g, 49.8 mmol) was added and the mixture was warmed to 100 °C during 4 h under a pressure of 10 mm Hg. After cooling, the solvent was evaporated and the residue was dissolved in toluene, washed with acidic water (pH = 5) with neutral water, then dried on sodium sulfate, filtered and evaporated. The crude product was purified by silica gel chromatography (methylene chloride/ether: 2/1, v/v). Title compound was obtained as yellow crystals (2.15 g, yield 87%).

Anal. Calcd for C_{25}H_{20}NO_{2}: C, 64.98; H, 6.62; N, 3.25. Found: C, 64.98; H, 6.59; N, 3.16. 1H NMR (CDCl3, δ (ppm): 8.87 (s, 1H, NH), 7.36 (m, 5H, CO2CH2Ph), 6.60 (d, 1H, H-C5), 5.76 (d, 1H, H-C5, “ose”), 5.66 (d, 1H, H-C1, “ose”), J = 5 Hz), 5.39–5.33 (d, 2H, CO2CH2Ph), 4.61 (dd, 1H, H-C4, “ose”), 4.30 (d, 1H, H-C4, “ose”), 3.24 (s, 3H, 4-CH3), 1.52–1.50–1.33–1.25 (s, 12H, isopropylidene).

3,3′-dimethyl-4,4′-di(1′, 2′, 3′, 4′-di-O-isopropylidene-α-D-galactosyl)-5′,5′-dibenzocarbonyl dipyrromethane, 23. Pyrrole 22 (500 mg, 1.13 mmol) and methylale (250 μL) were stirred in dichloromethane (6 mL) at room temperature during four days under argon. Every morning and evening, methylale (250 μL) was added. The reaction was controlled by thin layer chromatographic analysis until vanishing of pyrrole. The solution was washed by water than a saturated solution of sodium hydrogenocarbonate. The pure title compound was obtained as yellow crystals by silica gel chromatography with a mixture of methylene chloride/ether (10/1, v/v) (275 mg, yield 54%).

Anal. Calcd for C_{49}H_{58}N_{4}O_{10}: C, 68.85; H, 7.33; N, 5.18. Found: C, 68.28; H, 7.19; N, 5.98. 1H NMR (CDCl3, δ (ppm): 10.31 (s, 2H, H-C5,15 meso), 10.18–10.04 (s, 1H, H-C10 meso), 6.80 (s, 2H, H-C5, “ose”), J = 1.5 Hz), 6.21 (dd, 2H, H-C5, “ose”), J = 5 Hz), 5.05 (dd, 4H, H-C5, “ose” and C4, “ose”), 4.75 (dd, 2H, H-C5, “ose”), 4.09 (q, 2H, OCH2CH3), 3.80 (s, 6H, CH3 pyr), 1.96–1.90–1.86–1.25 (s, 24H, isopropylidene), 1.73–1.57 (t, 12H, CH2CH3), –3.67 (s, 2H, NH). UV-visible spectrum in CH2Cl2: λ_{max} nm (ε, L mmol⁻¹ cm⁻¹): 402 (168.5), 500 (13.7), 534 (8.6), 569 (6.3), 622 (3.6).

2, 8-di(α-D-galactosyl)-3, 7-dimethyl-12, 13, 17, 18-tetraethylporphyrin, 26. A solution of dipyrromethane 23 (250 mg, 0.28 mmol) and palladium 10% on activated carbon (33 mg) in tetrahydrofuran (4 mL) was stirred under hydrogen. The end of reaction was controlled by thin layer chromatographic analysis. The crude solution was filtered on celite, evaporated, and used quickly without purification (200 mg, yield 100%). 1H NMR(CDCl3, δ (ppm): 11.10 (s, 2H, NH), 6.89 (s, 2H, H-pyr), 5.60 (d, 2H, H-C1, “ose”), J = 5 Hz), 5.38 (d, broad, 2H, H-C5, “ose”), J = 5 Hz), 4.62 (dd, 2H, H-C3, “ose”), 4.35 (dd, 2H, H-C1, “ose”), 4.33 (dd, 1H, H-C5, “ose”), 3.67 (dd, 2H, CH2 pyr), 2.18 (s, 6H, CH3 pyrrole), 1.49–1.42–1.34–1.27–1.21–1.17 (s, 24H, isopropylidene).

2, 8-di(1′, 2′, 3′, 4′-di-O-isopropylidene-α-D-galactosyl)-3, 7-dimethyl-12, 13, 17, 18-tetraethylporphyrin, 26. A solution of dipyrromethane 23 (164 mg, 0.223 mmol) and 3,3′, 4, 4′-tetraethyl-5, 5′-diformyl-dipyrromethane18 25 (84 mg, 0.267 mmol) in methylene chloride/methanol (8 mL) was diluted in a mixture of para-toluene sulfonic acid (2.4 mg) in methylene chloride (20 mL) and methanol (1 mL) and was stirred at room temperature for 48 h. The mixture was evaporated, dissolved in methylene chloride and washed threefold with water. The organic phase was dried over sodium sulfate, filtered, and concentrated to dryness. The crude product was purified by silica gel chromatography eluted by methylene chloride/ether (100/5, v/v), then by gel filtration on LH20 eluted with methanol. The title compound was obtained in 14% yield (28 mg).

Anal. Calcd for C_{52}H_{66}N_{4}O_{10}: C, 68.85; H, 7.33; N, 5.18. Found: C, 68.28; H, 7.19; N, 5.98. 1H NMR (CDCl3, δ (ppm): 10.31 (s, 2H, H-C5,15 meso), 10.18–10.04 (s, 1H, H-C10 meso), 6.80 (s, 2H, H-C5, “ose”), J = 1.5 Hz), 6.21 (dd, 2H, H-C5, “ose”), J = 5 Hz), 5.05 (dd, 4H, H-C5, “ose” and C4, “ose”), 4.75 (dd, 2H, H-C5, “ose”), 4.09 (q, 2H, OCH2CH3), 3.80 (s, 6H, CH3 pyr), 1.96–1.90–1.86–1.25 (s, 24H, isopropylidene), 1.73–1.57 (t, 12H, CH2CH3), –3.67 (s, 2H, NH). UV-visible spectrum in CH2Cl2: λ_{max} nm (ε, L mmol⁻¹ cm⁻¹): 402 (168.5), 500 (13.7), 534 (8.6), 569 (6.3), 622 (3.6).
Anal. Calcd for C_{40}H_{50}N_{4}O_{10}: C, 64.30; H, 6.75; N, 7.7. 1H NMR (pyridine d_{5}), δ (ppm): 11.7–11.6 (s, 2H, C_{15–15} meso), 10.28 (m, 2H, H C_{10–20} meso), 6.88–6.21–5.05–4.75 (m, 10H, H “ose”), 4.05 (q, 8H, CH_{2}CH_{3}), 3.79 (s, 6H, CH_{3} pyr), 1.84 (t, 12H, CH_{2}CH_{3}), −3.67 (s, 2H, NH). UV-visible spectrum in CHCl_{3}: λ_{max}, nm (ε, L mmol⁻¹ cm⁻¹): 397 (104.8), 498 (6.5), 532 (5.1), 566 (4.3), 618 (1.6).

2. 8-di(1’, 2’, 3’, 4’-di-O-isopropylidene-α-D- galactosyl)-3, 7-dimethyl-12, 13, 18-tetraethylbiladien bromide, 27. A mixture of dipyrromethane 24 (0.4 g, 0.577 mmol) and 2-formyl-3,4-diethylylpyrroloid 168 mg, 1.11 mmol) was warmed to 100 °C during 15 min under argon. After cooling 66% bromohydrin acid aqueous solution (0.577 mL) was added and the solution was stirred for 5 min. The crude mixture was used immediately without further purification.

2. 8-di(1’, 2’, 3’, 4’-di-O-isopropylidene-α-D-galactosyl)-3, 7-dimethyl-12, 13, 18-tetraethyl-15-azaporphyrin, 28. To the crude solution of previous biladien bromide 27, in methanol (320 mL), potassium ferricyanide (250 mg, 0.76 mmol) were dissolved in tetrahydrofurane/dimethoxymethane (2.3 mL, 30 mmol) were dissolved in methylene chloride, washed with water evaporation to dryness the crude product was dissolved in methylene chloride, washed with water, dried over sodium sulfate, filtered, and concentrated under vacuum. The pure azaporphyrin was obtained by a column silica gel chromatography eluted with methylene chloride/ether (100/5, v/v). The crude mixture was used immediately without further purification.

Anal. Calcd for C_{29}H_{28}N_{4}O_{10}: C, 58.78; H, 4.53; N, 9.68. Found: C, 58.21; H, 5.06; N, 9.37. 1H NMR (CDCl_{3}), δ (ppm): 10.60 (s, 1H, OH), 9.09 (s, 1H, NH), 8.10 (d, 1H, phenyl), 7.60 (dd, 1H, phenyl), 7.17 (d, 1H, phenyl), 6.79 (s, 1H, H_{5}), 4.18 (q, 2H, CO_{2}CH_{2}CH_{3}), 2.00 (s, 3H, CH_{3}).

3.3’-dimethyl-4,4’-di(3’-nitro-4’-hydroxyphenyl)-5’,5’-diethoxycarbonyl-dipyrromethane, 31. The previous nitroalkene 29 (1.2 g, 5.6 mmol) and ethyl isocyanate (1.15 mL, 10.5 mmol) were dissolved in tetrahydrofurane/isopropanol (28 mL, 1/1, v/v). DBU (1.5 mL, 10 mmol) was added slowly and the solution was stirred for a day at room temperature. The solvent was evaporated and the crude residue was purified by silica gel chromatography eluted with methylene chloride. The pure product was obtained as yellow crystals (1.44 g, yield 70%).

Anal. Calcd for C_{40}H_{52}N_{4}O_{10}: C, 58.78; H, 4.76; N, 9.45. Found: C, 58.42; H, 5.15; N, 8.83. 1H NMR (CDCl_{3}), δ (ppm): 10.60 (s, 2H, NH), 9.08 (s, 2H, NH), 8.08 (d, 2H, phenyl), 7.60 (dd, 2H, phenyl), 7.17 (d, 1H, phenyl), 6.79 (s, 1H, H_{5}), 4.18 (q, 2H, CO_{2}CH_{2}CH_{3}), 1.96 (s, 3H, CH_{3}), 1.14 (t, 3H, CO_{2}CH_{2}CH_{3}).
3, 3'-dimethyl-4-4'-di(3'-nitro-4'-hydroxyphenyl)-5, 5'-dicarboxy dipyrromethane. Previous dipyrromethane 30 (0.2 g, 0.338 mmol) was dissolved in methanol (3 mL) containing sodium hydroxide (52 mg in 650 μL of water). The solution was kept under reflux for 3 h. Then, the cold solution was concentrated under vacuum. The residue was dissolved in water, acidified by acetic acid until pH = 4. The precipitate was filtered, then dried (181 mg, yield 100%).

1H NMR (CDCl₃), δ (ppm): 10.60-10.55 (s, 2H, OH), 9.00 (s, 2H, NH), 8.08 (d, 2H, phenyl), 7.60 (dd, 2H, phenyl), 7.13 (d, 2H, phenyl), 6.8 (2H, H_pyr), 3.99 (s, 2H, pyr-CH₂-pyr), 2.21 (s, 6H, CH₃).

2, 3, 17, 18-tetraethyl-7, 13-di(3'-nitro-4'-hydroxyphenyl)-8, 12-dimethyl-biladien dibromide. A solution of 2-formyl-3,4-diethyl pyrrole 33 (113 mg, 1.3 mmol) and ammoniac (11 mL) were added. The solution was kept under reflux for 3 h. Then the cold solution was concentrated under vacuum. The residue was dissolved in methanol (10 mL) previously degassed with argon, aqueous solution of 66% bromhydric acid (0.75 mL) was added. The mixture was stirred overnight. The brown precipitate was filtered (100 mg, yield 34%) and used immediately without purification.

1H NMR (CDCl₃), δ (ppm): 13.70 (s, 2H, NH), 13.43 (s, 2H, NH), 11.23 (s, 1H, OH), 10.59 (s, 1H, OH), 8.06 (d, 2H, phenyl), 7.80 (s, 2H, C=CH_pyr), 7.54 (d, 2H, phenyl), 7.22 (dd, 2H, phenyl), 7.00 (s, 2H, C=CH), 4.48 (s, 2H, pyr-CH₂-pyr), 2.50 (q, 8H, CH₂CH₃), 2.13 (s, 3H, CH₃), 1.21-1.12 (t, 12H, CH₂CH₃).

2, 3, 17, 18-tetraethyl-7, 13-di(3'-nitro-4'-hydroxyphenyl)-8, 12-dimethyl-20-azaporphyrin. Biladien 34 (0.1 g, 0.118 mmol) was dissolved in methanol (190 mL). Potassium ferricyanide (52 mg in 650 μL of water) and ammoniac (11 mL) were added. The solution was kept under reflux for 100 °C for 10 min and stirred at room temperature for 1 day. The solvent was removed and the crude product was dissolved in methylene chloride then filtered. The crystals were washed with methylene chloride until colorless solvent. The organic solution was concentrated under vacuum. The title compound was obtained by silica gel chromatography eluted with methylene chloride/methanol to give purple crystals (34 mg, 10%).

Anal. Calcd for C₈₃H�ประทับใจ₃N₇O₆: C, 80.54; H, 7.78; N, 6.95. 1H RMN (CDCl₃), δ (ppm): 10.15 (s, 1H, H_meso), 9.61 (s, 2H, H_meso), 8.53 (s, 2H, phenyl), 8.21 (dd, 2H, phenyl), 7.79 (d, 2H, phenyl), 5.47 (m, 7H, H ‘ose”), 4.37 (s, 4H, H ‘ose”), 3.59 (8H, CH₂CH₃), 2.2-2.15-2.11 (s, 24H, CH₂CH₄), 1.86 (t, 12H, CH₂CH₄), −2.20 (s, 2H, NH). UV-visible spectrum in CH₂Cl₂: λ_max, nm (ε, L mmol⁻¹ cm⁻¹): 383 (101.7), 508 (7.3), 539 (20.3), 561 (8.7), 612 (18.3).
2, 3, 17, 18-tetraethyl-7, 13-di[3-nitro-4’-(2’, 3’, 4’, 6’ - tetra-O-acetyl - β-D - glucosyl]-phenoxy]-8, 12-dimethyl-porphyrin, 11. 30 mg, yield 83%. Anal. Calc for C_{70}H_{76}N_{14}O_{22} C: 56.81; H: 5.79; N: 7.13. Found: C: 56.84; H: 5.78; N: 7.12. 1H NMR (CDCl3), δ (ppm): 10.70 (s, 1H, H meso), 10.40 (s, 1H, H meso), 8.90 (d, 2H, phenyl), 8.35 (d, 4H, phenyl), 4.77-4.4 (m, 14H, H “ose”), 3.95 (q, 8H, CH$_2$CH$_3$), 3.72 (s, 6H, CH$_3$), 1.87 (t, 12H, CH$_2$CH$_3$), −2.93 (s, 2H, NH). UV-visible spectrum in THF: $\lambda_{max}$ nm (ε, L mmol$^{-1}$ cm$^{-1}$): 407 (160), 504 (13.4), 539 (10.3), 572 (6.7), 627 (4.1).

2, 3, 17, 18-tetraethyl-7, 13-di[3 ‘-nitro-4’- β-D-maltosyl-phenoxy]-8, 12-dimethyl-porphyrin, 12. 14 mg, yield 71%. Anal. Calc for C_{76}H_{80}N_{26}O_{26} C: 57.72; H: 5.87; N: 6.12. Found: C: 53.73; H: 5.56; N: 5.89. 1H NMR (Pyridine d$_5$), δ (ppm): 10.71 (s, 1H, H meso), 10.55 (s, 1H, H meso), 10.36 (s, 2H, H meso), 8.90 (s, 2H, phenyl), 8.41 (d, 4H, phenyl), 6.02-3.73 (m, 8H, CH$_2$CH$_3$+H “ose”), 3.43 (s, 6H, CH$_3$), 1.85 (t, 12H, CH$_2$CH$_3$), −2.92 (s, 2H, NH). UV-visible spectrum in THF: $\lambda_{max}$ nm (ε, L mmol$^{-1}$ cm$^{-1}$): 408 (161.8), 504 (13.6), 540 (11.5), 573 (7), 627 (3.8).

3.6 PARTITION MEASUREMENTS

The partition coefficient of the compounds between 2-octanol and buffer at pH 7.4 was determined by equilibrating equal parts of PBS and 2-octanol at 20 °C. Optical density (OD) of each phase was measured between 400 and 450 nm and the log(partition coefficient) [log (PC)] was calculated as the log(OD(2-octanol)/OD(PBS)).

3.7 IN VITRO PHOTOCYTOTOXICITY TESTS

Photodynamic activity of the glycoconjugated tetrapyrryl macrocycles have been estimated using the viability of a human colic adenocarcinoma cell line HT29 (ATCC, HTB 38) after 24 h incubation with the tested compounds followed by visible light irradiation.

HT29 cells were cultivated in Dulbecco’s MEM supplemented with 10% fetal calf serum (FCS). Cells from log-phase culture were seeded in 24-microwell plates (1 mL−5×10$^4$ cells/well) and kept at 37 °C in a water-jacketed incubator for 2 days under an air/CO$_2$ atmosphere (5% CO$_2$). Tested compounds, in DMSO solution, were added under the minimum volume (5 μL) to reach a concentration ranging from 0.1 to 10 μg/mL. Controls cells received 5 μL of DMSO free of dye. Plates were incubated 24 h, then medium was removed and the cells were washed twice with phosphate buffered saline (PBS) before addition of fresh medium free of.
drug and irradiation with visible light using a home made “light box” giving a fluence of 3.8 mW/cm² on the whole visible spectrum. Irradiation with red light was carried out using the same device fitted with an orange filter (0% T at 520 nm and 80% T at 590 nm) leading to a fluence of 2 mW/cm².

Plates were reincubated for 3 days before evaluation of the cell survival using the MTT assay using 30 min incubation with 100 μg well of 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT, Sigma). After removal of the medium, formazan crystals were taken up with 100 μL of DMSO and absorbance at 540 nm was measured with a Bio-Rad microplate reader (model 450); survival was expressed as % of untreated controls.

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REFERENCES


