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Microwave-mediated 'click-chemistry' synthesis of glycoporphyrin derivatives and in vitro photocytotoxicity for application in photodynamic therapy

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ABSTRACT

In this paper, we report the synthesis of a series of porphyrins, designed as photodynamic therapy (PDT) agents, substituted by three glycosyl units linked by a triazole group to chromophore in the aim to target tumor cells overexpressing lectin-type membrane receptors, by 'click-chemistry' under microwave heating.

1. Introduction

Photodynamic therapy (PDT) is a non-invasive treatment based on the combined action of three components, a photosensitizer, light, and oxygen. The photosensitizer upon absorption of specific light reacts with surrounding molecular oxygen to produce lethal reactive oxygen species as singlet oxygen. Currently, this therapy is recommended in the treatment of dermatological or ophthalmic diseases, and oncology.[1] However, this method has some limitations among which only cancers, which are affected by light (skin, airway, prostate, bladder or intestine cancers) can be treated. Unfortunately, the low selectivity of approved photosensitizers as Photofrin® and Foscan® for tumor tissues generates a skin photosensitivity and causes damages to normal tissue irradiated during PDT.[2] Thus an effective targeting inducing efficient penetration of photosensitizers inside tumor cells is a crucial parameter of their effectiveness. Diffusion or surface proteins may mediate penetration mechanism of drug in tumor cells. Active targetings toward over-expressed membrane receptors in tumor tissue have been intensively studied in recent years.[3] For a PDT use, various targeting vectors[4] have been combined with tetrapyrrolic macrocycles as serum proteins (albumin, lipoproteins LDL, transferrin), steroids, toxins, carbohydrates, polyamines, antibodies, folic acid, peptides, epithelial growth factor (EGF), and functionalized nanoparticles. The addition of sugar moieties on photosensitizers can increase their selectivity toward tumor cells, which over-express specific membrane lectins and facilitate penetration.[5] In last 10 years we have shown that an amphiphilic structure of the glycoconjugated photosensitizers in peculiar for porphyrins and chlorins bearing three monosaccharides, induced the best photocytotoxicity in vitro.[6] In addition, the metabolic stability of the glycosyl part of glycoconjugated photosensitizers plays an important role. Indeed, the degradation and elimination of photosensitizers should not be immediate in order to get a good response to an efficient photochemical treatment.[7] It appears in the literature that the triazole nucleus is relatively stable with respect to metabolic degradation while not posing any particular problem of toxicity.[8] In the aim to decrease a possible degradation of glycoconjugated photosensitizers, we designed new glycoconjugated porphyrin derivatives having a triazole core linking the chromophore and the sugar. These compounds were obtained by microwave-assisted 'click-chemistry'.[9] This one has been previously used to functionalization of tetrapyrrolic macrocycles.[10,14] Compared with literature data, in

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this work, we varied several parameters, such as the nature of the sugar, the length of the spacer arm and the position and orientation of triazole core (Fig. 1).

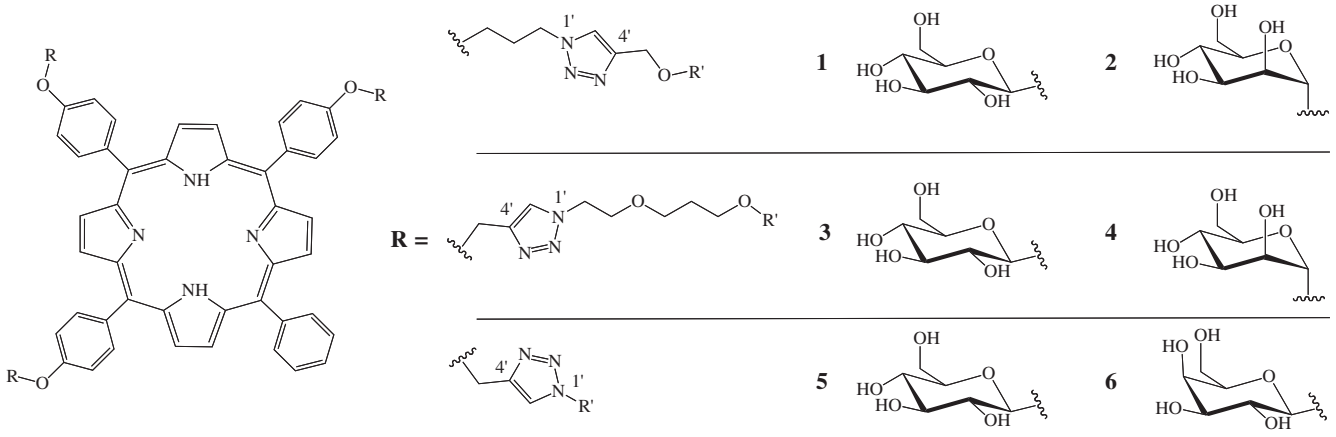


Fig. 1. Structure of glycoconjugated photosensitizers obtained by click-chemistry.

2. Results and discussion

2.1. Chemistry

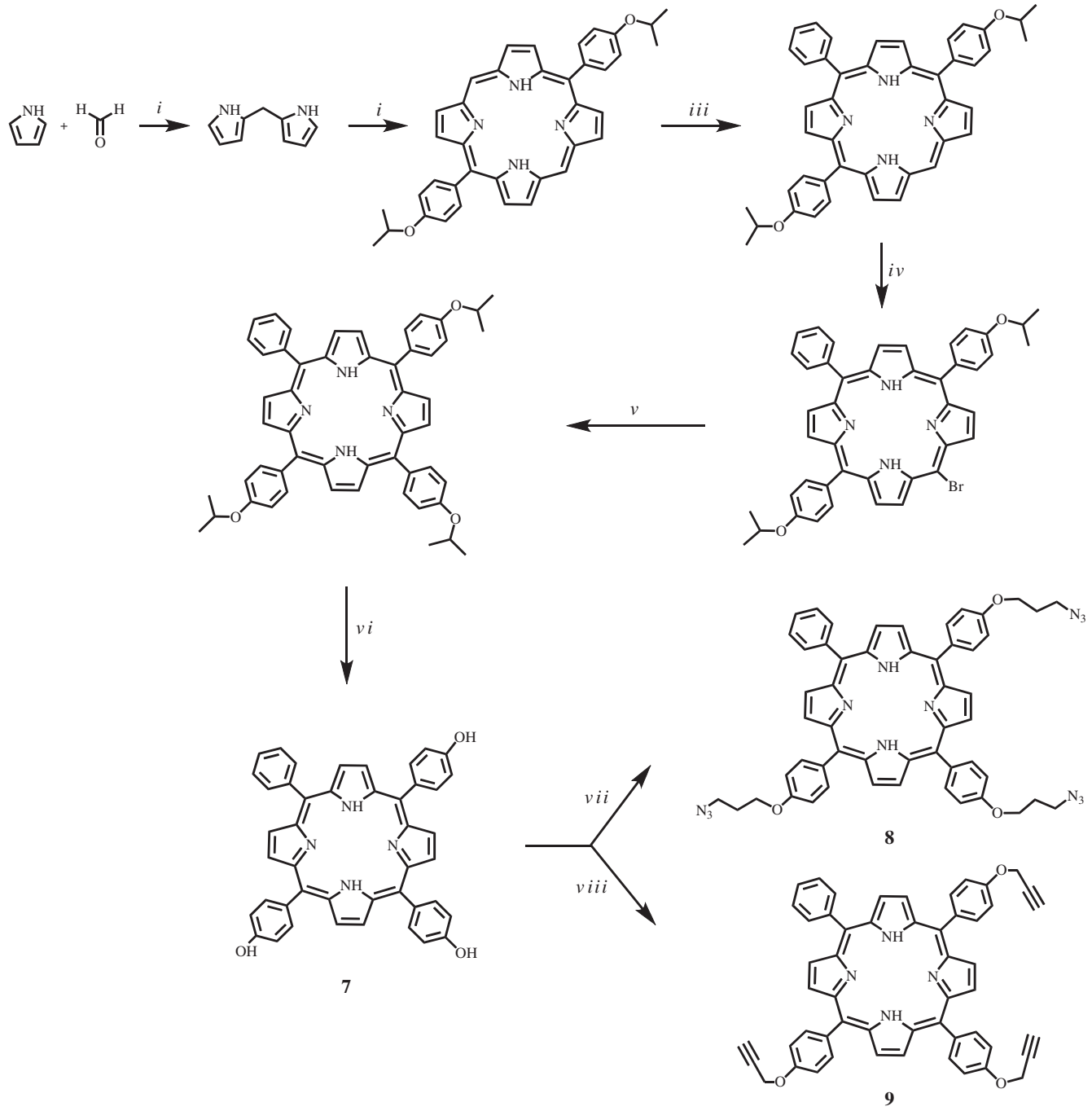
Porphyrin **7** is the starting material for the synthesis of all glycoconjugated derivatives **1**–**6** (Scheme 1). This compound was obtained by modified Senge's method[11] as described in a previous paper.[12] Two types of sugar–porphyrin conjugates can be obtained depending the presence of azido or propargyl functions on macrocyclic precursors **8** and **9**. Porphyrin **7** was condensed with 1,3-dibromopropane by Williamson's protocol and then the terminal bromines were substituted by azido groups to obtain **8**. Tetrapyrrolic macrocycle **9** was obtained from **7** and propargyl bromide in the presence of potassium carbonate in dry dimethylformamide using the same method. The presence of these two different substitutions on porphyrins **8** and **9** allows to prepare compounds **Zn-10**–**Zn-15** and thus to study the influence of triazole nucleus orientation between the macrocycle and the sugar moieties on photobiological properties (Scheme 2).

In order to obtain glycoconjugated porphyrins **1**–**6**, general conditions and methodology of copper-catalyzed azide–alkyne cycloadditions (click reaction[13]) were used and optimized depending of the nature of substituents on the initial porphyrin core. The Cu-catalyzed Huisgen 1,3-dipolar cycloaddition of terminal alkynes and azides is now the most widely used click reaction. As described in literature,[14] it is necessary to protect the porphyrins **8** and **9** by zinc(II) complexation before performing cycloaddition reaction in the presence of copper salts. Porphyrins **8** and **9** were quantitatively converted to metalated porphyrins **Zn-8** and **Zn-9** using zinc acetate in solution of a mixture of chloroform/methanol at reflux. Zinc(II) cation in porphyrin **8**–**9** is sufficiently stable under click reaction conditions to avoid replacement by a copper(II) ion.

Two systems of copper catalyst were used to realize this reaction under microwave activation. Coupling porphyrin **Zn-8** with propargyl glycosides[15] was realized in presence of CuCl in toluene. Mixtures were incubated in a microwave oven in sealed cap reactors. After purification, glycoporphyrins **Zn-10** and **Zn-11** were isolated with relatively good yield (65 and 68%, respectively). This same catalyst system was used to synthesize porphyrin derivatives **Zn-12**–**15** from **Zn-9** and azidoglycosides[16] but very low yields (<10%) were obtained for every derivative. Instead, $CuSO_4$/sodium L-ascorbate catalyst system was used to condensed propargyl porphyrin derivative **Zn-9** with azidoglycosides. Products were placed in THF/*t*-BuOH/water and the mixture reacted under microwaves in sealed cap reactors. The presence or not of spacer arm on glycosides was used to show the significance of steric disturbance of the macrocycle during the reaction. Porphyrin derivatives **Zn-12**–**15** were obtained with variable yields (45–80%) without any significant effect of steric hindrance due to the spacer arms. Zinc removing from glycoporphyrins **Zn-10**–**15** was quantitatively performed by acidic treatment with trifluoroacetic acid. To obtain final compounds, acetyl protecting groups on sugar moieties are fully removed by Zemplén's transesterification.[17] After neutralization by ion-exchange resin, neutral glycoconjugated porphyrins **1**–**6** were obtained quantitatively without further purification (Scheme 2). All synthesized protected and unprotected glycoporphyrins were characterized by ^1H and ^{13}C NMR analysis (300 MHz and 75.3 MHz, respectively) in $CDCl_3$ or pyridine d_5. The detailed resonance assignments are based on integration and 2D homonuclear COSY experiments. The characteristic signal of the proton on triazole ring appears as a singlet between 7 and 8.5 ppm depending on the nature of spacer arm and sugar. The resonance of anomeric proton of compounds **1**, **3**, **5**, **6**, **10-Zn**, **12-Zn**, **14-Zn**, and **15-Zn** appears as a well-defined doublet between 6.46 and 4.57 ppm (J=7.6–9.0 Hz, characteristic of β anomeric configuration) for β-glucosylated and β-galactosylated compounds and as a narrow doublet (J<2 Hz, α anomeric configuration) for compounds **2**, **4**, **11-Zn**, and **13-Zn** for the α-mannosylated derivatives. UV–vis spectra of all porphyrins are characteristic of *meso*-substituted porphyrin macrocycles and show an intense Soret band near 420 nm and two less intense visible bands for metalated porphyrins or four less intense visible bands (Q bands) for free base ones. Mass spectrometry was performed using the MALDI-TOF technique. All final compound spectra show isotopic profiles indicating the presence of charged species $[M+H]^+$ and cation species $[M+Na]^+$ and $[M+K]^+$. In some cases, in peculiar for protected compounds **Zn-10**–**Zn-15**, we note the loss of metal ion due to ionization conditions.

2.2. Partition coefficient determination

In medicinal chemistry, amphiphilicity is often well correlated with the bioactivity of drugs. Amphiphilicity is evaluated by the logarithm of a partition coefficient, (log *P*), which reflects the partitioning equilibrium of a molecule between a non polar and a polar phase, such as the 2-octanol/water system. Log *P* was estimated according to the literature procedure.[18] Porphyrins were diluted in DMF. DMF solution was added to a mixture of 2-octanol/PBS (pH=7.4) in a reactor. The two phases were decanted, separated, and diluted in MeOH. Log *P* was obtained as log ($A_{2\text{-octanol}}/A_{PBS}$),

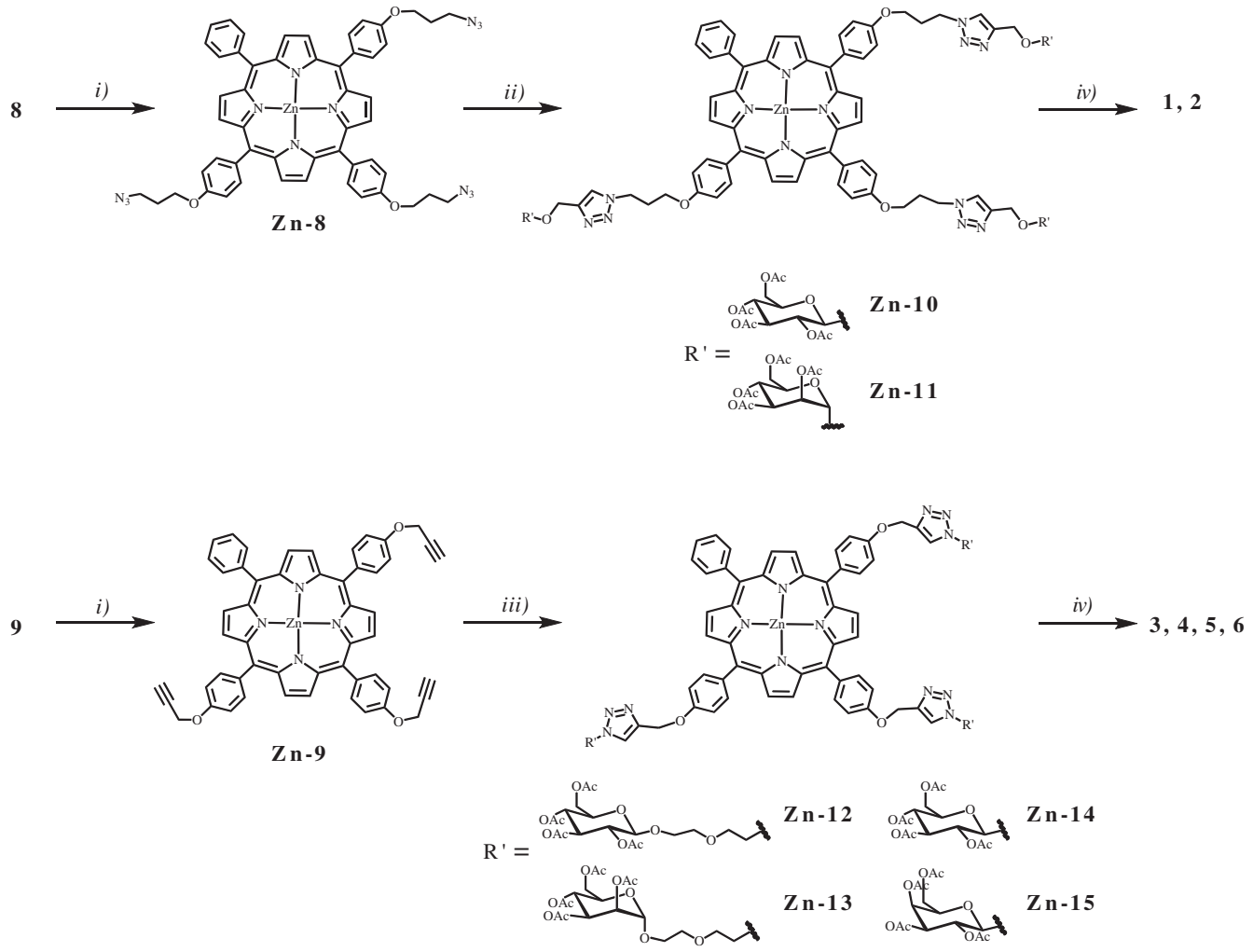


Scheme 1. (i) (a) $InCl_3$, 55 °C, 2 h 30 min, (b) NaOH, rt, 1 h, 96%; (ii) (a) TFA, dry CH_2Cl_2, rt, 18 h, (b) DDQ, rt, 1 h, 54%; (iii) PhLi, dry THF, rt, 2 h 30 min, 78%; (iv) NBS, $CHCl_3$/pyridine, 0 °C, 15 min, quantitative yield; (v) Phenyl boronic acid, K_2CO_3, $Pd(PPh_3)_4$, dioxane/H_2O, 100 °C, 2 h 30 min, 86%; (vi) BBr_3, CH_2Cl_2, rt, 16 h, quantitative yield; (vii) 1,3-dibromopropane, K_2CO_3, dry DMF, rt, 60 h, 77%, then NaN_3, dry DMF, rt, 16 h, 59%; (viii) propargyl bromide, K_2CO_3, dry DMF, rt, 18 h, 98%.

absorbance (A) in 2-octanol and PBS, respectively, measured at 417 nm. Results (Table 1) show that compounds **1**–**6** possess amphiphilic characters ($-0.1 > \log P > 0.4$) and thus can be used in a biological protocol applied to PDT.

2.3. Cellular phototoxicity

Cytotoxicity and phototoxicity of photosensitizers in HT29 (colorectal adenocarcinoma cell line) and Y79 (human retinoblastoma cell line) were determined by cell survival fraction measurements using the 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyl tetrazolium bromide (MTT) assay[19] (Table 2). Toxicity in darkness was found to be negligible in all cases, with a survival fraction close to 100%. It is now established that some tumor cells over-express lectin-type membrane receptors, specific for certain carbohydrates and then facilitate penetration of glycophotosensitizers.[5] It is known, in particular, that colorectal adenocarcinoma cell line (HT29) over-express membrane lectins specific for β-glucose and retinoblastoma cells (Y79) over-express membrane lectins specific for α-mannose and β-galactose. The first point revealed by this study is that, whatever the nature of carbohydrates, all compounds **1**–**6** exhibit a photocytotoxicity in Y79 superior to HT29 one. But the relationship between structure and activity is very difficult to establish in this study.



Scheme 2. (i) ZnOAc, MeOH, reflux, 1 h, quantitative yield; (ii) CuCl, propargyl glycosyl derivatives, toluene, microwave irradiation (100 W, 140 °C, 20 min), 65%; (iii) $CuSO_4$, sodium L-ascorbate, glycosyl derivatives, THF/*t*-BuOH/H_2O, microwave irradiation (80 W, 85 °C, 3 min); (iv) TFA (demetalation) and then MeONa/MeOH, CH_2Cl_2, 1 h, quantitative yield.

Table 1
Log *P* of photosensitizers at 20 °C±1°

Compound	**1**	**2**	**3**	**4**	**5**	**6**
log *P* (±0.3)	−0.1	−0.1	0.4	0.4	0.1	0.0

Table 2
Phototoxicity of photosensitizers with red light illumination (2 J/cm^2, $\lambda \geq 590$ nm), incubation 24 h

Compound	Phototoxicity IC_{50} (μM)	
	HT29	Y79
1	>15	15
2	>15	1.2
3	nd	2
4	11	0.4
5	>15	0.5
6	>15	1.5

The nature of the glycosyl units (β-glucosyl, β-galactosyl, and α-mannosyl) does not influence the phototoxicity. Porphyrin **4** is an analogous molecule of the previously tested glycoporphyrin **A** (Fig. 2) that shows a very interesting in vitro IC_{50} (0.43 and 0.35 μm) on HT 29 and Y79 cell lines.[5] Although compound **4** exhibits an interesting photodynamic activity it is lower than this one observed with the molecule **A** in peculiar for HT29 cell line.

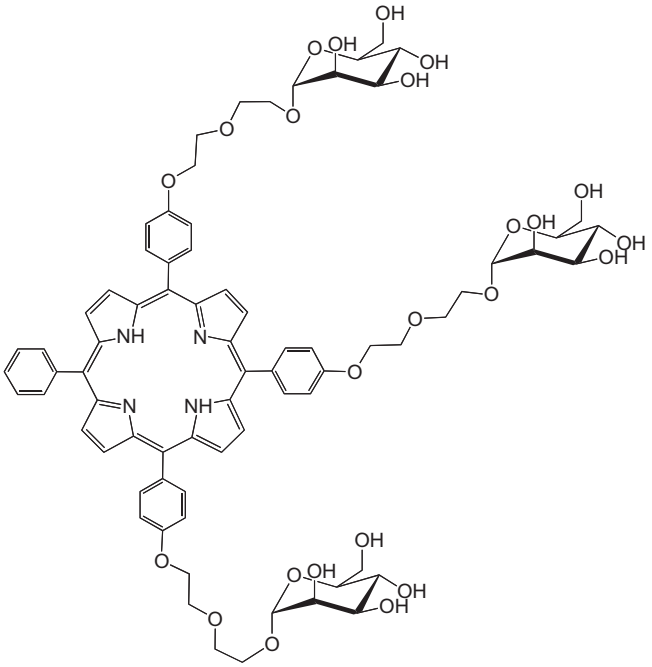


Fig. 2. Structure of porphyrin **A**.

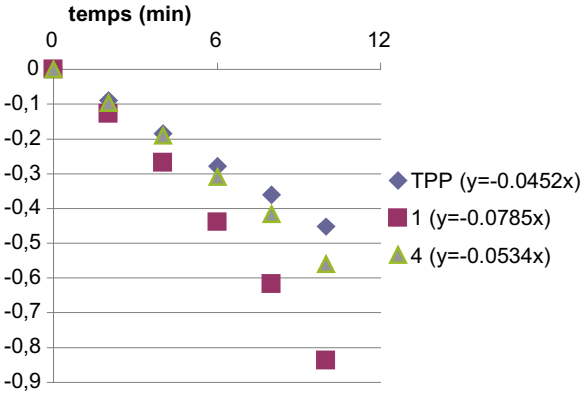


Fig. 3. $^1O_2$ production of glycoporphyrins **1**, **4**, and TPP as reference.

Glycoporphyrins **1**–**3**, **5**, and **6** exhibit little or no activity compared to compounds **4** and **A**.[5] These results indicate that triazole core decreases in vitro phototoxicity independently of its position in spacer arm or its orientation (porphyrin→1′,4′-triazole or →4′,1′-triazole, see Fig. 1).

2.4. Singlet oxygen production

A high singlet oxygen yield production is one of the conditions of efficient photodynamic activity. To verify that the photosensitizers produce effectively singlet oxygen, we estimated this production only for two compounds, the most phototoxic and the least phototoxic. The production of $^1O_2$ by glycoporphyrins **1** and **4** was determinated from evolution of UV–vis absorption of 1,3-diphenylisobenzofuran (DPBF) solution at 411 nm in DMF by a modified method described by Carloni et al.[20] and Gorman et al.,[21] and compared to results obtained with a known $^1O_2$ producer (TPP).[22] An air-saturated solution of DPBF and porphyrins **1** and **4** was irradiated with white light and monitored by the variation of intensity of the absorption band of DPBF over time. UV–vis measurements were done for the two porphyrins **1** and **4** to estimate a percentage of $^1O_2$ production compare with that of TPP[22] (Fig. 3). It results that products **1** and **4** present a good singlet oxygen production and appear as potential candidates for application in PDT.

3. Conclusions

In summary in this paper we have described the synthesis of a series of glycoporphyrin conjugates possessing a triazole core between chromophore and glycosyl moieties. To this goal, click-chemistry cycloaddition was used and we showed that good yields can be obtained by microwave activation. Spectroscopic characteristics were determined and good singlet oxygen production was demonstrated for some of the final products, confirming the potential use of these glycoporphyrins for PDT. Phototoxicity was determined on two cell lines and some compounds exhibit a good activity in peculiar against Y79 cell line (compounds **4** and **5**). Although triazole core could play a stabilizing role in metabolic degradation, photocytotoxicity of the best compounds is decreased compared to previously published molecule.[5] Metabolic stability of these new glycoconjugated photosensitizers is currently under investigation in our laboratory.

4. Experimental section

4.1. General information

All solvents (reagent grade and dry grade) were used without purification. Column chromatography was performed with the indicated solvents using E. Merck silica gel 60 (particle size 0.035–0.070 mm). Macherey–Nagel precoated plates (SIL G-200, 2 mm) were used for preparative thin-layer chromatography. Yields refer to chromatographically and spectroscopically pure compounds. ^1H and ^{13}C NMR spectra were recorded on a Bruker AC-300 spectrometer at room temperature using an internal deuterium lock. Chemical shift values are given in parts per million relative to tetramethyl silane (TMS). Acidic impurities in $CDCl_3$ were removed by treatment with anhydrous K_2CO_3. Quantitative UV–vis spectra were recorded with a UVIKON xm SECOMAM spectrometer (molar extinction coefficient values are given in L mmol^{-1} cm^{-1}). The MALDI-TOF mass spectra were performed with MALDI-TOF Voyager Spec equipped with a N_2 Laser emitting at 337 nm: Matrix: 2,5-Dihydroxybenzoic acid (2,5-DHB) in THF (20 mg/mL), and porphyrins in THF. CEM Discover microwave apparatus was used for 'click-chemistry' synthesis in sealed cap reactors.

*4.1.1. 5,10,15-Tris(4-hydroxyphenyl)-20-phenylporphyrin (**7**).* This compound was synthesized according to the literature as described in a previous paper.[12]

*4.1.2. 5,10,15-Tris[4-(3-azidopropanoxy)phenyl]-20-phenylporphyrin (**8**).* To a solution of 5,10,15-tris[4-(3-bromopropanoxy)phenyl]-20-phenylporphyrin[5] (40 mg, 39 μmol, 1 equiv) in anhydrous DMF (4 mL), NaN_3 (76 mg, 117 μmol, 30 equiv) was added and the mixture was stirred overnight at round temperature. The solution was concentrated under vacuum, and then CH_2Cl_2 was added to the crude. Organic layer obtained was washed with water (3×), dried over $MgSO_4$, filtered, and concentrated under reduced pressure. The crude product was purified by thin-layer chromatography (CH_2Cl_2/cyclohexane, 8/2, v/v, twice elutions) to give the expected product **8** (21 mg, 59%) as a purple-red powder. ^1H NMR (300 MHz, $CDCl_3$): δ 8.86 (br s, 8H, H pyrr.), 8.21 (br d, 2H, J=5.6 Hz, H *o*-phenyl), 8.12 (d, 6H, J=8.3 Hz, H *o*-phenoxy), 7.75 (br d, 3H, J=6.4 Hz, H *m*-phenyl and *p*-phenyl), 7.27 (d, 6H, J=8.4 Hz, H *m*-phenoxy), 4.35 (t, 6H, J=5.8 Hz, OCH_2), 3.70 (t, 6H, J=6.6 Hz, CH_2N_3), 2.25 (m, 5H, CH_2), −2.76 (s, 2H, NH). ^{13}C NMR (75.3 MHz, $CDCl_3$): δ 158.5 (C4 phenoxy), 142.2 (C1 phenyl), 135.6 (C phenoxy), 134.6 (C phenyl), 127.7 (C pyrr.), 126.7 (C pyrr.), 119.9 (C phenyl), 112.7 (C phenoxy), 64.7 (OCH_2), 48.4 (CH_2N_3), 29.0 (CH_2). UV–vis spectrum in CH_2Cl_2: λ_{max} nm (ε, L mmol^{-1} cm^{-1}) 420 (474.5), 518 (17.9), 554 (11.4), 592 (6.2), 649 (6.2). Anal. Calcd for ($C_{53}H_{45}N_{13}O_3$) $5H_2O$: C, 63.52; H, 5.53; N, 18.17. Found: C, 63.31; H, 5.94; N, 11.97.

*4.1.3. 5,10,15-Tris[4-(3-propynyloxy)phenyl]-20-phenylporphyrin (**9**).* Porphyrin **7** (150 mg, 226 μmol, 1 equiv) was dissolved in anhydrous DMF (30 mL) under argon, then K_2CO_3 (1g, 724 μmol, 32 equiv) and propargyl bromide (900 μL, 837 μmol, 37 equiv) were added. The mixture was stirred overnight at room temperature. The mixture was concentrated under vacuum and then CH_2Cl_2 was added to the crude. Organic layer obtained was washed with water (3×), dried over $MgSO_4$, filtered, and concentrated under reduced pressure, giving a solid residue that was purified by flash chromatography over silica gel (CH_2Cl_2) to give 173 mg (98%) of **9** as a purple-red powder. ^1H NMR (300 MHz, $CDCl_3$): δ 8.87 (br s, 8H, H pyrr.), 8.21 (br d, 2H, J=5.7 Hz, H *o*-phenyl), 8.14 (d, 6H, J=8.4 Hz, H *o*-phenoxy), 7.75 (d, 3H, J=6.7 Hz, H *m*-phenyl and *p*-phenyl), 7.36 (d, 6H, J=8.4 Hz, H *m*-phenoxy), 4.98 (d, 6H, J=2.1 Hz, OCH_2), 2.70 (br t, 3H, H propargyl), −2.77 (s, 2H, NH). ^{13}C NMR (75.3 MHz, $CDCl_3$): δ 157.4 (C4 phenoxy), 142.2 (C1 phenyl), 135.5 (C phenoxy), 134.6 (C phenyl), 127.7 (C pyrr.), 126.7 (C pyrr.), 119.7 (C phenyl), 113.1 (C phenoxy), 78.7 (C), 75.9 (CH), 56.1 (CH_2). UV–vis spectrum in CH_2Cl_2: λ_{max} nm (ε, L mmol^{-1} cm^{-1}) 420 (402.7), 517 (15.8), 553 (9.2), 592 (5.1), 648

(4.8). Anal. Calcd for ($C_{53}H_{36}N_4O_3$) $4H_2O$: C, 74.98; H, 5.22; N, 6.60. Found: C, 74.39; H, 4.98; N, 6.06.

4.2. General procedure followed for metalation of porphyrins 8 and 9

To a well-stirred solution of porphyrin (223 μmol, 1 equiv) in $CHCl_3$ (50 mL) was added zinc acetate (1.11 mmol, 5 equiv) in solution of methanol (25 mL) and the mixture was refluxed for 1 h. CH_2Cl_2 was added to the crude. The organic layer was washed with water (3×), dried over $MgSO_4$, filtered, and concentrated under reduced pressure, giving **Zn-8** and **Zn-9** as a purple powder in quantitative yield. **Zn-8** and **Zn-9** were used without further purification. **Zn-8**: UV–vis spectrum in CH_2Cl_2: λ_{max} nm (normalized absorbance) 423 (1), 553 (0.07), 590 (0.05). **Zn-9**: UV–vis spectrum in CH_2Cl_2: λ_{max} nm (normalized absorbance) 422 (1), 551 (0.09), 594 (0.02).

*4.2.1. 5,10,15-Tris{4-(2′,3′,4′,6′-tetra-O-acetyl-β-D-glucopyranosyloxymethylenetriazolyl)-[N-(4″-propyloxy-phenyl)]}-20-phenylporphyrin zinc complex (**Zn-10**).* In a reactor were introduced solutions of **Zn-8** (15 μmol, 15 mg, 1 equiv) and 2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyloxypropynyl (90 μmol, 35 mg, 7 equiv) in toluene (1 mL) and copper(I) chloride (4.5 μmol, 0.45 mg, 0.3 equiv). The mixture was heated by microwave irradiation (100 W, 140 °C, 20 min) then allowed to cool to room temperature. The mixture was concentrated under vacuum and then purified by flash chromatography over silica gel (CH_2Cl_2/MeOH, 99/1, v/v) to give 21 mg (65%) of **Zn-10** as a purple powder. ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.93 (s, 8H, H pyrr.), 8.26 (br s, 2H, *o*-phenyl), 8.12 (d, 6H, *J*=7.7 Hz, *o*-phenoxy), 7.77 (br s, 3H, *m*-phenyl and *p*-phenyl), 7.16 (d, 6H, *J*=7.7 Hz, *m*-phenoxy), 7.07 (s, 3H, H triazole), 5.14 (t, 3H, *J*=9.4 Hz, H3 sugar), 5.01 (t, 3H, *J*=9.5 Hz, H4 sugar), 4.84 (br t, 3H, H2 sugar), 4.30–3.50 (m, 6H, CH_2–O–sugar), 4.26 (br s, 6H, CH_2), 4.23 (d, *J*=8.5 Hz, H1 sugar), 4.20 (br dd, 3H, *J*=3.2 and 11.0 Hz, H6′ sugar), 4.00 (br d, 3H, *J*=12.0 Hz, H6 sugar), 3.81 (br s, 6H, CH_2), 3.54 (m, 3H, H5 sugar), 2.24 (br s, 6H, CH_2), 2.02 (s, 9H, acetyl), 2.00 (s, 9H, acetyl), 1.97 (s, 9H, acetyl), 1.92 (s, 9H, acetyl). ^{13}C NMR (75.3 MHz, $CDCl_3$): δ 170.6 (C=O acetyl), 170.2 (C=O acetyl), 169.4 (C=O acetyl), 169.3 (C=O acetyl), 158.0 (C4 phenoxy), 135.6 (C1 phenyl), 134.7 (C phenoxy), 131.7 (C pyrr.), 126.5 (C phenyl), 122.5 (C triazole), 112.4 (C phenoxy), 100.1 (C1 sugar), 72.6 (C3 sugar), 71.8 (C5 sugar), 71.1 (C2 sugar), 68.1 (C4 sugar), 63.8 (C6 sugar), 62.2 (C–O–sugar), 61.6 (CH_2), 46.8 (CH_2), 29.4 (CH_2), 20.7 (C acetyl), 20.6 (C acetyl), 20.6 (C acetyl). UV–vis spectrum in CH_2Cl_2: λ_{max} nm (ε, L mmol^{-1} cm^{-1}) 423 (471.1), 551 (19.7), 594 (8.2). MALDI-TOF MS calcd for $C_{104}H_{109}N_{13}O_{33}Zn$, 2132.66 (*m*/*z* 100%), found 2071.70 $[M+H-Zn]^+$. Anal. Calcd for ($C_{104}H_{109}N_{13}O_{33}Zn$): C, 58.52; H, 5.29; N, 8.20. Found: C; 59.08; H; 5.29; N, 8.20.

*4.2.2. 5,10,15-Tris{4-(2′,3′,4′,5′-tetra-O-acetyl-α-D-mannopyranosyloxymethylenetriazolyl)-[N-(4″-propyloxyphenyl)]}-20-phenylporphyrin zinc complex (**Zn-11**).* Prepared as compound **Zn-10**. In presence of 2,3,4,6-tetra-*O*-acetyl-α-D-mannopyranosyloxypropynyl (7 equiv). Porphyrin **Zn-11** (22 mg (68%)) was obtained as a purple powder. ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.94 (s, 4H, H pyrr.), 8.92 (s, 4H, H pyrr.), 8.26 (br s, 2H, *o*-phenyl), 8.13 (d, 6H, *J*=7.1 Hz, *o*-phenoxy), 7.76 (br s, 3H, *m*-phenyl and *p*-phenyl), 7.35 (m, 3H, H triazole), 7.20 (d, 6H, *J*=7.1 Hz, *m*-phenoxy), 5.14 (br s, 3H, H3 sugar), 5.01 (br s, 3H, H4 sugar), 4.84 (br s, 3H, H2 sugar), 4.23 (s, 3H, H1 sugar), 4.73 (br s, 6H, CH_2–O–sugar), 4.21–4.26 (dd, 3H, *J*=4.3 and 12.1 Hz, H6′ sugar), 4.15–3.90 (m, 15H, CH_2, and H6 sugar), 3.85 (m, 3H, H5 sugar), 2.37 (br s, 6H, CH_2), 2.12 (s, 9H, acetyl), 2.08 (s, 9H, acetyl), 1.98 (s, 9H, acetyl), 1.92 (s, 9H, acetyl). ^{13}C NMR (75.3 MHz, $CDCl_3$): δ 170.7 (C=O acetyl), 170.0 (C=O acetyl), 169.9 (C=O acetyl), 169.7 (C=O acetyl), 158.0 (C4 phenoxy), 150.3 (C1 phenyl), 150.3 (C phenoxy), 136.2 (C phenyl), 131.7 (C pyrr.), 122.9 (C triazole), 112.4 (C phenoxy), 96.9 (C1 sugar), 69.3 (C2 sugar), 68.9 (C3 sugar), 68.6 (C5 sugar), 65.9 (C4 sugar), 64.0 (C–O–sugar), 62.3 (C6 sugar), 60.4 (CH_2), 47.1 (CH_2), 29.9 (CH_2), 29.6 (C acetyl), 20.9 (C acetyl), 20.8 (C acetyl), 20.6 (C acetyl), 20.6 (C acetyl). UV–vis spectrum in CH_2Cl_2: λ_{max} nm (ε, L mmol^{-1} cm^{-1}) 423 (439.0), 551 (17.4), 594 (6.9). MALDI-TOF MS calcd for $C_{104}H_{109}N_{13}O_{33}Zn$, 2132.66 (*m*/*z* 100%), found 2133.61 $[M+H]^+$ and 2156.59 $[M+Na]^+$. Anal. Calcd for ($C_{104}H_{109}N_{13}O_{33}Zn$) $1H_2O$: C, 58.03; H, 5.20; N, 8.46. Found: C, 58.02; H, 5.26; N, 8.02.

*4.2.3. 5,10,15-Tris{4-[N-(2′,3′,4′,6′-tetra-O-acetyl-β-D-glucopyranosyloxyethoxy]-(4″-methylenoxytriazole)-phenyl}-20-phenylporphyrin zinc complex (**Zn-12**).* In a reactor were introduced solutions of **Zn-9** (24 μmol, 20 mg, 1 equiv) and [(2-azido-ethoxy) ethoxy]-2′,3′,4′,6′-tetra-*O*-acetyl-β-D-glucose (140 μmol, 65 mg, 7 equiv) in THF (1 mL), copper(II) sulfate (12 μmol, 1.9 mg, 0.5 equiv), and sodium L-ascorbate in *t*-BuOH (0.5 mL) and water (0.25 mL). The mixture was heated by microwave irradiation (80 W, 85 °C, 3 min) then allowed to cool to room temperature. The mixture was concentrated under vacuum and then purified by thin-layer chromatography (CH_2Cl_2/EtOH, 96/4, v/v) to give the product **Zn-12** (30 mg, 57%) as a purple powder. ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.91 (s, 4H, H pyrr.), 8.88 (s, 4H, H pyrr.), 8.20 (br s, 2H, *o*-phenyl), 8.07 (br d, 6H, *o*-phenoxy), 7.80 (s, 3H, H triazole), 7.72 (br s, 3H, *m*-phenyl and *p*-phenyl), 7.16 (br d, 6H, *m*-phenoxy), 5.05–4.92 (m, 6H, H2 sugar, H3 sugar and H4 sugar), 4.57 (d, 3H, *J*=7.6 Hz, H1 sugar,), 4.35 (m, 3H, H6 sugar), 4.11–4.00 (m, 15H, CH_2, H6′ sugar and CH_2–Otriazole) 3.59–3.29 (m, 24H, CH_2), 3.42 (m, 3H, H5 sugar), 2.06 (s, 9H, acetyl), 2.03 (s, 9H, acetyl), 1.99 (s, 9H, acetyl), 1.94 (s, 9H, acetyl). ^{13}C NMR (75.3 MHz, $CDCl_3$): δ 169.9 (C=O acetyl), 169.5 (C=O acetyl), 169.1 (C=O acetyl), 168.3 (C=O acetyl), 161.4 (C4 phenoxy), 149.3(C1 phenyl), 134.7 (C phenoxy), 133.6 (C phenyl), 130.6 (C pyrr.), 125.3 (C triazole), 119.2 (C phenyl), 111.7 (C phenoxy), 99.7 (C1 sugar), 71.6 (C2 sugar or C3 sugar), 70.7 (C2 sugar or C3 sugar), 70.2 (C5 sugar), 69.1 (O–*C*–triazole), 67.8 (C6 sugar), 67.2 (C4 sugar), 60.7 (CH_2), 49.5 (CH_2), 19.6 (C acetyl), 19.5 (C acetyl). UV–vis spectrum in CH_2Cl_2: λ_{max} nm (ε, L mmol^{-1} cm^{-1}) 423 (453.1), 553 (18.0), 596 (7.5). MALDI-TOF MS calcd for $C_{107}H_{115}N_{13}O_{36}Zn$, 2222.69 (*m*/*z* 100%), found 2161.71 $[M+H-Zn]^+$. Anal. Calcd for ($C_{107}H_{115}N_{13}O_{36}Zn$) $1H_2O$: C, 57.31; H, 5.26; N, 8.12. Found: C, 57.31; H, 5.16; N, 8.78.

*4.2.4. 5,10,15-Tris[4-(N-(2′,3′,4′,6′-tetra-O-acetyl-α-D-mannopyranosyloxyethoxy)ethoxy)-(4″-methylenoxytriazole)-phenyl]-20-phenylporphyrin zinc complex (**Zn-13**).* Prepared as compound **Zn-12**. In presence of [(2-azido-ethoxy)ethoxy]-2′,3′,4′,6′-tetra-*O*-acetyl-α-D-mannose. After purification by thin-layer chromatography (CH_2Cl_2/EtOH, 96/4, v/v), 24 mg (45%) of porphyrin **Zn-13** was obtained as a purple powder. ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.95 (s, 4H, H pyrr.), 8.91 (s, 4H, H pyrr.), 8.25 (br s, 2H, *o*-phenyl), 8.09 (d, 6H, *J*=8.0 Hz, *o*-phenoxy), 7.76 (br s, 3H, *m*-phenyl and *p*-phenyl), 7.13 (d, 6H, *J*=8.0 Hz, *m*-phenoxy), 7.39 (s, 3H, H triazole), 5.28 (m, 3H, H2 sugar or H3 sugar or H4 sugar), 5.27 (m, 3H, H2 sugar or H3 sugar or H4 sugar), 5.21 (m, 3H, H2 sugar or H3 sugar or H4 sugar), 4.82 (s, 3H, H1 sugar), 4.25–4.05 (m, 18H, CH_2), 4.17–4.00 (m, 6H, CH_2–O–triazole), 3.99 (m, 3H, H6′ sugar), 3.68 (m, 3H, H6 sugar), 3.68–3.54 (m, 18H, CH_2), 3.42 (m, 3H, H5 sugar), 2.13 (s, 9H, acetyl), 2.05 (s, 9H, acetyl), 2.03 (s, 9H, acetyl), 1.98 (s, 9H, acetyl). ^{13}C NMR (75.3 MHz, $CDCl_3$): δ 170.6 (C=O acetyl), 170.1 (C=O acetyl), 169.9 (C=O acetyl), 169.7 (C=O acetyl), 157.6 (C4 phenoxy), 143.1 (C1 phenyl), 134.7 (C phenoxy), 131.6 (C pyrr.), 126.3 (C phenyl), 123.2 (C triazole), 112.7 (C phenoxy), 100.1 (C1 sugar), 70.0 (CH_2), 69.5 (C5 sugar), 69.2 (C6 sugar), 68.9 (C4 sugar), 68.5 (C3 sugar), 67.1 (C2 sugar), 66.1 (O–*C*–triazole), 62.4 (CH_2), 50.1 (CH_2), 20.9 (C acetyl), 20.1 (C acetyl). UV–vis spectrum in CH_2Cl_2:

λ_{max} nm (ε, L mmol^{-1} cm^{-1}) 423 (494.1), 552 (20.3), 595 (9.5). MALDI-TOF MS calcd for $C_{107}H_{115}N_{13}O_{36}Zn$, 2222.69 (*m*/*z* 100%), found 2161.82 $[M+H-Zn]^+$. Anal. Calcd for ($C_{107}H_{115}N_{13}O_{36}Zn$): C, 57.77; H, 5.21; N, 8.19. Found: C, 57.57; H, 5.21; N, 8.19.

*4.2.5. 5,10,15-Tris[4-N-(2′,3′,4′,6′-tetra-O-acetyl-β-D-glucopyranosyl)-(4″-methylenoxytriazole)-phenyl]-20-phenylporphyrin zinc complex (**Zn-14**).* Prepared as compound **Zn-12**. In presence of 1-azido-(2′,3′,4′,6′-tetra-*O*-acetyl)-β-D-glucose. After purification by thin-layer chromatography (CH_2Cl_2/EtOH, 96/4, v/v), 37 mg (80%) of porphyrin **Zn-14** was obtained as a purple powder. ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.97 (s, 4H, H pyrr.), 8.93 (s, 4H, H pyrr.), 8.24 (br s, 2H, *o*-phenyl), 8.11 (br d, 6H, *J*=6.4 Hz, *o*-phenoxy), 7.75 (s, 3H, *m*-phenyl and *p*-phenyl), 7.49 (s, 3H, H triazole), 7.17 (d, 6H, *J*=7.4 Hz, *m*-phenoxy), 5.32 (m, 3H, H1 sugar), 5.23 (m, 6H, H2 sugar and H3 sugar), 5.13 (m, 3H, H4 sugar), 4.45–4.12 (m, 9H, CH_2–O–triazole and H6 sugar), 3.92 (br d, 3H, *J*=11.9 Hz, H6′ sugar), 3.68 (br s, 3H, H5 sugar), 2.03 (s, 18H, acetyl), 1.99 (s, 9H, acetyl), 1.73 (s, 9H, acetyl). ^{13}C NMR (75.3 MHz, $CDCl_3$): δ 170.4 (C=O acetyl), 169.9 (C=O acetyl), 169.3 (C=O acetyl), 168.7 (C=O acetyl), 157.5 (C4 phenoxy), 144.2 (C1 phenyl), 136.3 (C phenyl), 135.7 (C phenoxy), 131.7 (C pyrr.), 120.4 (C triazole), 112.8 (C phenoxy), 85.4 (C1 sugar), 74.8 (C5 sugar), 72.4 (C2 sugar or C3 sugar), 70.0 (C2 sugar or C3 sugar), 67.4 (C4 sugar), 61.2 (C6 sugar), 61.1 (O–*C*–triazole), 20.6 (C acetyl), 20.4 (C acetyl), 20.0 (C acetyl). UV–vis spectrum in CH_2Cl_2: λ_{max} nm (ε, L mmol^{-1} cm^{-1}) 421 (574.9), 549 (23.4), 589 (7.8). MALDI-TOF MS calcd for $C_{95}H_{91}N_{13}O_{30}Zn$ (MH^+), 1958.53 (*m*/*z* 100%), found 1897.62 $[M+H-Zn]^+$. Anal. Calcd for ($C_{95}H_{91}N_{13}O_{30}Zn$) $9H_2O$: C, 53.76; H, 5.18; N, 8.58. Found: C, 53.89; H, 4.77; N, 7.69.

*4.2.6. 5,10,15-Tris[4-N-(2′,3′,4′,6′-tetra-O-acetyl-β-D-galactopyranosyl)-(4″-methylenoxytriazole)-phenyl]-20-phenylporphyrin zinc complex (**Zn-15**).* Prepared as compound **Zn-12**. In presence of 1-azido-(2′,3′,4′,6′-tetra-*O*-acetyl)-β-D-galactose. After purification by thin-layer chromatography (CH_2Cl_2/EtOH, 96/4, v/v), 31 mg (67%) of porphyrin **Zn-15** was obtained as a purple powder. ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.98 (s, 4H, H pyrr.), 8.95 (d, 2H, *J*=4.7 Hz, H pyrr.), 8.93 (d, 2H, *J*=4.7 Hz, H pyrr.), 8.25 (br s, 2H, *o*-phenyl), 8.13 (d, 6H, *J*=7.9 Hz, *o*-phenoxy), 7.77 (br s, 3H, *m*-phenyl and *p*-phenyl), 7.68 (br s, 3H, H triazole), 7.20 (d, 6H, *J*=7.9 Hz, *m*-phenoxy), 5.49–5.15 (m, 12H, H1 sugar, H2 sugar, H3 sugar, and H4 sugar), 4.46 (br d, 3H, H6 sugar), 4.14–4.08 (m, 12H, CH_2–O–triazole, H6′ sugar and H5 sugar), 2.19 (s, 9H, acetyl), 2.04 (s, 9H, acetyl), 2.00 (s, 9H, acetyl), 1.75 (s, 9H, acetyl). ^{13}C NMR (75.3 MHz, $CDCl_3$): δ 170.3 (C=O acetyl), 169.9 (C=O acetyl), 169.8 (C=O acetyl), 168.9 (C=O acetyl), 157.5 (C4 phenoxy), 144.2 (C1 phenyl), 135.7 (C phenoxy), 131.7 (C pyrr.), 126.4 (C phenyl), 120.4 (C triazole), 112.8 (C phenoxy), 86.1 (C1 sugar), 73.9 (C5 sugar), 70.6 (C2 sugar or C3 sugar or C4 sugar), 67.5 (C2 sugar or C3 sugar or C4 sugar), 66.7 (C2 sugar or C3 sugar or C4 sugar), 61.3 (C6 sugar), 61.1 (O–*C*–triazole), 20.6 (C acetyl), 20.4 (C acetyl), 20.1 (C acetyl). UV–vis spectrum in CH_2Cl_2: λ_{max} nm (ε, L mmol^{-1} cm^{-1}) 421 (515.3), 549 (20.9), 589 (6.9). MALDI-TOF MS calcd for $C_{95}H_{91}N_{13}O_{30}Zn$ (MH^+), 1958.53 (*m*/*z* 100%), found 1897.65 $[M+H-Zn]^+$. Anal. Calcd for ($C_{95}H_{91}N_{13}O_{30}Zn$) $10H_2O$: C, 53.31; H, 5.23; N, 7.85. Found: C, 53.06; H, 4.58; N, 7.75.

4.3. General procedure followed for syntheses of porphyrins 1–6 (demetalation and deacetylation of porphyrins Zn-10–Zn-15)

To a solution of metaled porphyrin (50 mg) in CH_2Cl_2 (100 mL), trifluoroacetic acid (4 mL) was added. After 5 min at room temperature with vigorous stirring, the organic layer was washed with saturated aqueous $NaHCO_3$, water (3×), dried over $MgSO_4$, and filtered. After evaporation to dryness under high vacuum, porphyrin was obtained with quantitative yield as a red-purple powder and used without further purification. To a solution of demetalled porphyrin (50 mg) in dry MeOH (10 mL) and dry CH_2Cl_2 (10 mL) was added a solution of MeONa in MeOH (100 μL, 1 M) and the mixture was stirred for 1 h 30 min at room temperature. MB3 ion-exchange resin (1 g) was then added and gentle stirring was continued for 30 min. The reaction mixture was filtered and the resin was washed with MeOH and pyridine. The combined filtrates and washings were then evaporated to dryness. Porphyrins **1**–**6** were obtained with quantitative yields as a red-purple powder.

*4.3.1. 5,10,15-Tris{(β-D-glucopyranosyloxymethylenetriazolyl)-[N-(4-propyloxy-phenyl)]}-20-phenyl-porphyrin (**1**).* ^{1}H NMR (300 MHz, pyridine-d_5): δ 9.19 (4H, H pyrr.), 9.16 (d, 2H, *J*=4.8 Hz, H pyrr.), 9.09 (d, 2H, *J*=4.6 Hz, H pyrr.), 8.40 (m, 2H, *o*-phenyl), 8.31 (br s, 6H, *o*-phenoxy), 7.83 (m, 3H, *m*-phenyl and *p*-phenyl), 7.43 (d, 6H, *J*=8.3 Hz, *m*-phenoxy), 7.29 (s, 3H, H triazole), 5.47 (d, 3H, *J*=12.2 Hz, O–CH_2–triazole), 5.25 (d, 3H, *J*=12.2 Hz, O–CH_2–triazole), 5.16 (d, 3H, *J*=7.6 Hz, H1 sugar), 4.77 (t, 6H, *J*=6.4 Hz, CH_2), 4.62 (m, 3H, H6 sugar), 4.44 (m, 3H, H6′ sugar), 4.32–4.27 (m, 12H, CH_2, H3 sugar and H4 sugar), 4.16 (m, 3H, H2 sugar), 4.05 (m, 3H, H5 sugar), 2.53 (m, 6H, CH_2), −2.25 (2H, NH). ^{13}C NMR (75.3 MHz, pyridine-d_5): δ 160.7 (C4 phenoxy), 147.1 (C1 phenyl), 137.8 (C phenoxy), 137.6 (C phenyl), 136.6 (C phenyl), 126.1 (C triazole), 115.1 (C phenoxy), 105.9 (C1 sugar), 80.3 (C5 sugar), 80.2 (C4 sugar), 76.8 (C2 sugar), 73.3 (C3 sugar), 66.8 (CH_2), 64.8 (C–O–sugar), 64.4 (C6 sugar), 49.0 (CH_2), 32.1 (CH_2). UV–vis spectrum in MeOH: λ_{max} nm (ε, L mmol^{-1} cm^{-1}) 417 (300.1), 516 (11.7), 552 (10.5), 598 (6.7), 647 (5.3). MALDI-TOF MS calcd for $C_{80}H_{87}N_{13}O_{21}$ 1565.61 (*m*/*z* 100%), found 1566.64 $[M+H]^+$, 1588.62 $[M+Na]^+$, and 1604.59 $[M+K]^+$. Anal. Calcd for ($C_{80}H_{87}N_{13}O_{21}$) not obtained.

*4.3.2. 5,10,15-Tris{(α-D-mannopyranosyloxymethylenetriazolyl)-[N-(4-propyloxy-phenyl)]}-20-phenyl-porphyrin (**2**).* ^{1}H NMR (300 MHz, pyridine-d_5): δ 9.17 (4H, H pyrr.), 9.14 (m, 2H, *J*=4.7 Hz, H pyrr.), 9.07 (d, 2H, *J*=4.7 Hz, H pyrr.), 8.38 (m, 2H, *o*-phenyl), 8.29 (d, 6H, *J*=7.6Hz, *o*-phenoxy), 8.21 (s, 3H, H triazole), 7.81 (m, 3H, *m*-phenyl and *p*-phenyl), 7.40 (d, 6H, *J*=7.6Hz, *m*-phenoxy), 5.66 (s, 3H, H1 sugar), 5.30 (d, 3H, *J*=12.1 Hz, O–CH_2–triazole), 5.07 (d, 3H, *J*=12.1 Hz, O–CH_2–triazole), 4.77 (t, 6H, *J*=6.7 Hz, CH_2), 4.71–4.60 (m, 12H, H2 sugar, H3 sugar, H4 sugar, and H6 sugar), 4.52–4.41 (m, 6H, H5 sugar and H6′ sugar), 4.25 (m, 6H, CH_2), 2.51 (m, 6H, CH_2), −2.28 (2H, NH). ^{13}C NMR (75.3 MHz, pyridine-d_5): δ 161.1 (C4 phenoxy), 138.2 (C1 phenyl), 137.0 (C phenyl), 129.3 (C phenyl), 126.3 (C triazole), 126.0 (C phenoxy) 115.5 (C phenoxy), 103.2 (C1 sugar), 77.8 (C5 sugar), 80.2 (C2 sugar or C3 sugar or C4 sugar), 76.8 (C2 sugar or C3 sugar or C4 sugar), 73.3 (C2 sugar or C3 sugar or C4 sugar), 67.2 (CH_2), 65.1 (C6 sugar), 62.7 (C–O–sugar), 49.4 (CH_2), 32.5 (CH_2). UV–vis spectrum in MeOH: λ_{max} nm (ε, L mmol^{-1} cm^{-1}) 417 (343.3), 515 (13.2), 551 (8.7), 592 (4.9), 648 (4.7). MALDI-TOF MS calcd for $C_{80}H_{87}N_{13}O_{21}$ 1565.61 (*m*/*z* 100%), found 1566.65 $[M+H]^+$, 1588.62 $[M+Na]^+$, and 1604.60 $[M+K]^+$. Anal. Calcd for ($C_{80}H_{87}N_{13}O_{21}$) $4H_2O$: C, 58.64; H, 5.84; N, 11.11. Found: C, 58.61; H, 6.11; N, 9.87.

*4.3.3. 5,10,15-Tris{[N-(β-D-glucopyranosyloxyethoxy)ethoxy]-(4-methyleneoxytriazole)phenyl}-20-phenylporphyrin (**3**).* ^{1}H NMR (300 MHz, pyridine-d_5): δ 9.17 (6H, H pyrr.) 9.07 (d, 2H, *J*=4.2 Hz, H pyrr.), 8.65 (s, 3H, H triazole), 8.32 (m, 2H, *o*-phenyl), 8.30 (d, 6H, *J*=7.7Hz, *o*-phenoxy), 7.83 (m, 3H, *m*-phenyl and *p*-phenyl), 7.65 (d, 6H, *J*=8.3 Hz, *m*-phenoxy), 5.70 (s, 6H, O–CH_2–triazole), 4.94 (d, 3H, *J*=7.7 Hz, H1 sugar), 4.68 (m, 6H, *J*=4.7 Hz, CH_2), 4.70–4.30 (m, 12H, H2 sugar, H3 sugar, H4 sugar, and H5 sugar), 4.26 (m, 6H, CH_2), 4.20–3.94 (m, 12H, H6 sugar and CH_2) 3.74 (m, 6H, CH_2), −2.30 (2H, NH). ^{13}C NMR (75.3 MHz, pyridine-d_5): δ 160.7 (C4

phenoxy), 145.7 (C1 phenyl), 137.6 (C phenoxy), 137.5 (C phenoxy) 136.9 (C phenyl), 133.4 (C pyrr.), 128.7 (C phenyl), 127.3 (C triazole), 115.2 (C phenoxy), 106.4 (C1 sugar), 80.1 (C2 sugar or C3 sugar or C4 sugar or C5 sugar), 79.4 (C2 sugar or C3 sugar or C4 sugar or C5 sugar), 76.8 (C2 sugar or C3 sugar or C4 sugar or C5 sugar), 72.3 (CH_2), 71.2 (CH_2), 70.8 (C2 sugar or C3 sugar or C4 sugar or C5 sugar), 64.4 (C6), 64.1 (O–*C*–triazole), 63.5 (CH_2), 52.2 (CH_2). UV–vis spectrum in MeOH: λ_{max} nm (ε, L mmol^{-1} cm^{-1}) 417 (318.3), 515 (12.8), 552 (8.7), 592 (5.1), 648 (4.7). MALDI-TOF MS calcd for $C_{83}H_{93}N_{13}O_{24}$ 1655.65 (*m*/*z* 100%), found 1656.62 $[M+H]^+$. Anal. Calcd for ($C_{83}H_{93}N_{13}O_{24}$) not obtained.

*4.3.4. 5,10,15-Tris{[N-(α-D-mannopyranosyloxyethoxy)ethoxy]-(4-methyleneoxytriazole)phenyl}-20-phenylporphyrin (**4**).* ^{1}H NMR (300 MHz, pyridine-d_5): δ 9.14 (s, 6H, pyrr.), 9.06 (d, 2H, *J*=4.7 Hz, pyrr.), 8.55 (s, 3H, H triazole), 8.38 (m, 2H, *o*-phenyl), 8.29 (d, 6H, *J*=6.6 Hz, *o*-phenoxy), 7.83 (m, 3H, *m*-phenyl and *p*-phenyl), 7.43 (d, 6H, *J*=8.2 Hz, *m*-phenoxy), 5.74 (s, 6H, O–CH_2–triazole), 5.41 (s, 3H, H1 sugar), 4.68 (t, 6H, *J*=4.7 Hz, CH_2), 4.70–4.35 (m, 12H, H2 sugar, H3 sugar, H4 sugar, and H5 sugar), 4.68 (m, 6H, CH_2), 4.11 (m, 3H, H6′ sugar), 3,87 (t, 6H, *J*=4.1 Hz, CH_2), 3.73 (m, 3H, H6 sugar), 3.66 (m, 6H, CH_2), −2.30 (2H, NH). ^{13}C NMR (75.3 MHz, pyridine-d_5): δ 160.7 (C4 phenoxy), 145.7 (C1 phenyl), 137.8 (C phenoxy), 137.6 (C phenoxy), 136.6 (C phenyl), 136.1 (C pyrr.), 129.0 (C phenyl), 127.1 (C triazole), 115.5 (C phenoxy), 103.3 (C1 sugar), 77.1 (C2 sugar or C3 sugar or C4 sugar or C5 sugar), 74.7 (C2 sugar or C3 sugar or C4 sugar or C5 sugar), 72.1 (C2 sugar or C3 sugar or C4 sugar or C5 sugar), 72.8 (CH_2), 71.2 (CH_2), 70.8 (C2 sugar or C3 sugar or C4 sugar or C5 sugar), 68.2 (C6), 64.9 (CH_2), 64.1 (O–*C*–triazole), 52.1 (CH_2). UV–vis spectrum in MeOH: λ_{max} nm (ε, L mmol^{-1} cm^{-1}) 417 (310.1), 515 (11.8), 552 (8.6), 594 (4.8), 648 (4.1). MALDI-TOF MS calcd for $C_{83}H_{93}N_{13}O_{24}$ 1655.65 (*m*/*z* 100%), found 1656.67 $[M+H]^+$. Anal. Calcd for ($C_{83}H_{93}N_{13}O_{24}$) $3H_2O$: C, 58.27; H, 5.83; N, 10.64. Found: C, 58.56; H, 5.73; N, 10.08.

*4.3.5. 5,10,15-Tris{[N-(β-D-glucosyl)]-(4-methyleneoxytriazole)phenyl}-20-phenylporphyrin (**5**).* ^{1}H NMR (300 MHz, pyridine-d_5): δ 9.17 (s, 4H, H pyrr.), 9.14 (d, 2H, *J*=4.8 Hz, H pyrr.), 9.08 (d, 2H, *J*=4.5 Hz, H pyrr.), 8.93 (s, 3H, H triazole), 8.39 (br s, 2H, *o*-phenyl), 8.32 (br d, 6H, *o*-phenoxy), 7.82 (s, 3H, *m*-phenyl and *p*-phenyl), 7.63 (d, 6H, *J*=8.5 Hz, *m*-phenoxy), 6.51 (d, 3H, *J*=9.2 Hz, H1 sugar), 5.68 (m, 6H, O–CH_2–triazole), 4.91 (t, 3H, *J*=8.8 Hz, H2 sugar), 4.61 (d, 3H, *J*=11.6 Hz, H6′ sugar), 4.52 (d, 3H, *J*=8.6 Hz, H6 sugar), 4.50–4.41 (m, 6H, H3 sugar and H4 sugar), 4.32 (m, 3H and H5 sugar), −2.28 (s, 2H, NH). ^{13}C NMR (75.3 MHz, pyridine-d_5): δ 161.1 (C4 phenoxy), 146.22 (C1 phenyl), 138.2 (C phenoxy), 137.0 (C phenyl), 130.3 (C pyrr.), 126.1 (C triazole), 115.7 (C phenoxy), 91.8 (C1 sugar), 84.0 (C5 sugar), 81.0 (C3 sugar or C4 sugar), 76.0 (C2 sugar), 73.0 (C3 sugar or C4 sugar), 64.6 (C6 sugar), 64.3 (O–*C*–triazole). UV–vis spectrum in MeOH: λ_{max} nm (ε, L mmol^{-1} cm^{-1}) 417 (377.1), 515 (15.2), 551 (10.2) 592 (6.2), 647 (5.9). MALDI-TOF MS calcd for $C_{71}H_{69}N_{13}O_{18}$ 1391.49 (*m*/*z* 100%), found 1392.54 $[M+H]^+$. Anal. Calcd for ($C_{71}H_{69}N_{13}O_{18}$) $6H_2O$: C, 56.76; H, 5.57; N, 12.12. Found: C, 56.88; H, 5.66; N, 10.07.

*4.3.6. 5,10,15-Tris{[N-(β-D-galactosyl)]-(4-methyleneoxytriazole)phenyl}-20-phenylporphyrin (**6**).* ^{1}H NMR (300 MHz, pyridine-d_5): δ 9.17 (s, 4H, H pyrr.), 9.14 (br d, 2H, H pyrr.), 9.08 (d, 2H, *J*=4.2 Hz, H pyrr.), 8.83 (s, 3H, H triazole), 8.39 (br s, 2H, *o*-phenyl), 8.32 (d, 6H, *J*=7.5 Hz, *o*-phenoxy), 7.82 (s, 3H, *m*-phenyl and *p*-phenyl), 7.60 (d, 6H, *J*=8.1 Hz, *m*-phenoxy), 6.46 (d, 3H, *J*=9.0 Hz, H1 sugar), 5.63 (s, 6H, O–CH_2–triazole), 4.91 (br t, 3H, H2 sugar), 4.76 (s, 3H, H3 sugar or H4 sugar), 4.56 (br d, 3H, H6′ sugar), 4.48 (m, 12H, H6 sugar, H3 sugar or H4 sugar, and H5 sugar), −2.28 (s, 2H, NH). ^{13}C NMR (75.3 MHz, pyridine-d_5): δ 160.7 (C4 phenoxy), 145.6 (C phenyl), 144.2 (C1 phenyl), 137.8 (C phenoxy), 129.0 (C phenyl), 125.7 (C pyrr.), 125.4 (C triazole), 115.3 (C phenoxy), 92.1 (C1 sugar), 82.2 (C5 sugar), 77.4 (C3 sugar or C4 sugar), 73.1 (C2 sugar), 72.0 (C3 sugar or C4 sugar), 64.2 (C6 sugar), 64.0 (O–*C*–triazole). UV–vis spectrum in MeOH: λ_{max} nm (ε, L mmol^{-1} cm^{-1}) 417 (392.3), 515 (15.6), 551 (10.26) 591 (5.9), 647 (5.6). MALDI-TOF MS calcd for $C_{71}H_{69}N_{13}O_{18}$ 1391.49 (*m*/*z* 100%), found 1392.49 $[M+H]^+$. Anal. Calcd for ($C_{71}H_{69}N_{13}O_{18}$) not obtained.

4.4. Partition coefficient determination

Partition coefficients were determined using Chemspeed ASW 2000 system. Porphyrins are diluted in DMF (100 μL, 5–6 μM). DMF solution (10 μL) was added to a mixture of 2-octanol/PBS (pH=7,4) (1 mL/1 mL) in a reactor (13 mL). The mixture was stirred at 20 °C±1 °C during 30 min then the two phases were decanted, separated, and diluted in MeOH (1/1, v/v). Log *P* was obtained as log [absorbance in 2-octanol/absorbance in PBS ($A_{2\text{-octanol}}/A_{PBS}$)] measured at 417 nm.

4.5. In vitro phototoxicity

Photodynamic activity of the tetrapyrrolic macrocycles was estimated after 24 h incubation with the tested compounds followed by visible red illumination ($\lambda \geq 590$ nm) for 15 min (2 J/cm^2). Suspension of Y79 cells from log-phase culture was placed into 24-microwell plates (5×10^5 cells/well) and kept at 37 °C in Dulbecco's medium in a water-jacketed incubator for 2 days under an air/CO_2 atmosphere (5% CO_2). Cell culture conditions of adherent human colorectal adenocarcinoma cells (HT29) have been previously described.[23] Tested compounds (5 μL), in DMSO solution, were added at a final concentration ranging from 0.1 to 15 μM. Control cells received 5 μL of DMSO free of dye. After incubation and before irradiation with light, Y79 cells were centrifuged, washed twice with phosphate buffered saline (PBS), and resuspended in fresh medium free of drug. After medium was removed and before irradiation with light, adherent HT29 cells were washed twice with PBS before addition of fresh medium free of drug. After irradiation with light, plates were left to incubate in the dark for 3 days before evaluation of the cell viability using the MTT assay, i.e., 30 min incubation with 100 μg of 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyl tetrazolium bromide per well (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 percent of untreated controls, IC_{50} values corresponding to the concentration of drug leading to 50% survival were interpolated from the dose response curves and are expressed in μM. Experiments have been triplicated and the experimental error was within 10%.

4.6. Comparative singlet oxygen production

An aerated solution of 1,3-diphenylisobenzofuran (DPBF) (5×10^{-5} M) and photosensitizer (10^{-6} M) in dimethylformamide (50 mL) was irradiated with white light (home made source) at 25 °C for 10 min. Aliquots (3 mL) were removed from the solution at 2 min intervals and UV–vis spectrum was recorded. Reaction of DPBF with $^1O_2$ was monitored by the reduction of intensity of the absorption band at 411 nm over time. Irradiation of aerated DPBF solution without photosensitizer gave no reduction in intensity of the 411 nm absorption band.

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Supplementary data

Characterization data including ^{1}H, ^{13}C NMR, and MALDI-TOF spectra of compounds **1**–**6** are available. Supplementary data associated with this article can be found in online version at doi:10.1016/j.tet.2011.04.080.

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