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Synthesis of glycoporphyrin derivatives and their antiviral activity against herpes simplex virus types 1 and 2

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Abstract—Studies on the synthesis, structural elucidation, and antiviral evaluation of several carbohydrate-substituted *meso*-tetraarylporphyrins against herpes simplex virus type 1 (HSV-1) and type 2 (HSV-2) are described. The potential of those photosensitizers, and of their precursors, on the photoinactivation of HSV-1 and HSV-2 was examined in Vero cells. Their virucidal and viral replication effects were assessed under white light, at their maximum noncytotoxic concentrations. The highest inhibitory effects on viral replication, for both viruses, were obtained with the glycoporphyrins where the sugar moiety bears unprotected hydroxyl groups. Strong inhibition of virus yield was observed even at concentrations much lower than their maximum noncytotoxic concentrations. These compounds can be postulated to be useful as potential drugs for the treatment of herpes simplex viruses infections.
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1. Introduction

Herpesviruses are responsible for a wide range of human diseases, of special concern in immunocompromised individuals, namely AIDS, hematological malignancies, after organ bone marrow transplantation, fulminant hepatitis, and infections in transplant recipients.[1–4] They have also been implicated in the development of several malignancies of epithelial or lymphatic origin.[5]

The transmission of pathogenic viruses by blood transfusion is still a subject of concern, since absolute safety has not yet been achieved. Sterilization of blood and its components seems to be the best way to obtain safe products. However, the sterilization of cellular blood components presents a unique challenge, since cell structure and function are easily disrupted.[6] The photochemical sterilization of such products using effective photosensitizers might be a solution to this problem.[7]

The fact that few antiviral drugs of proven effectiveness exist has prompted the search for new drugs and methodologies to inactivate viruses. Photodynamic therapy (PDT) can become a promising method. It is being used in the treatment of cancer, psoriasis, age-related macular degeneration, and also in the inactivation of microorganisms and viruses.[8–14] This type of therapy requires a combination of a photosensitizer, oxygen, and light. The mechanism by which the cell destruction occurs is thought to involve mainly the disruption of the cellular, mitochondrial, or nuclear membranes by cytotoxic agents such as singlet oxygen.[15] The main advantages associated with the use of PDT over conventional chemotherapy are: (a) the action of the pharmacophore can be 'turned on or off', since no reaction occurs in the absence of light; (b) the duration time of the action can be controlled, and (c) both the photosensitizer and light can be selectively directed to the locations to be treated.[15]

Amongst the various types of photosensitizers used in PDT, the porphyrins are the most extensively studied and are the ones approved so far for clinical use.[9,16–19] Several groups are involved in structural modification of porphyrins in order to generate compounds with the photophysical and hydrophobic/hydrophilic properties required for an ideal photosensitizer. Porphyrins with carbohydrate moieties have been described as efficient photosensitizers to be used in PDT.[18,20–28] That

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is mainly due to the specific affinity of several carbohydrates for cancer cells.[29,30] Following our interest on the development of porphyrins with potential use in medicine,[31–33] we have set up a work program on the synthesis of carbohydrate-substituted porphyrins and the study of their efficiency for the photo-inactivation of HSV-1 and HSV-2. Our interest in finding new drugs to inactivate herpes simplex virus is related to the fact that some viral strains are very often resistant to drugs commonly employed in the treatment of herpetic infections.[34–37] Acyclovir[38] and foscarnet[36] are often employed to treat infections such as herpetic encephalitis. However, these antiviral agents are not able to inhibit resistant viruses and can lead to hemotoxicity.[38]

In this article, we describe the synthesis of four series of porphyrins containing carbohydrate moieties with protected and unprotected hydroxyl groups. The antiviral activity of these compounds, and of the corresponding porphyrin precursors, was examined against HSV-1 and HSV-2 in different situations of cell culture infection. At the same time, the influence of the amphiphilic character of the porphyrins on the inhibitory capacity was evaluated. We have found that under the weak white light exposure of the laminar flow cabinet (3 mW/cm^{2}during 15 min), some of the synthesized glycoporphyrins show similar efficiency to the currently used drugs acyclovir and foscarnet.

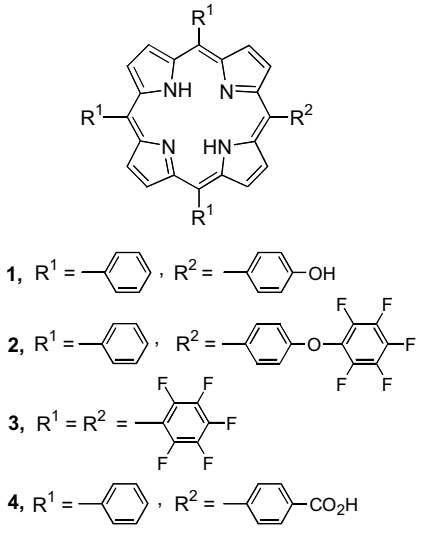
2. Results and discussion

2.1. Chemistry

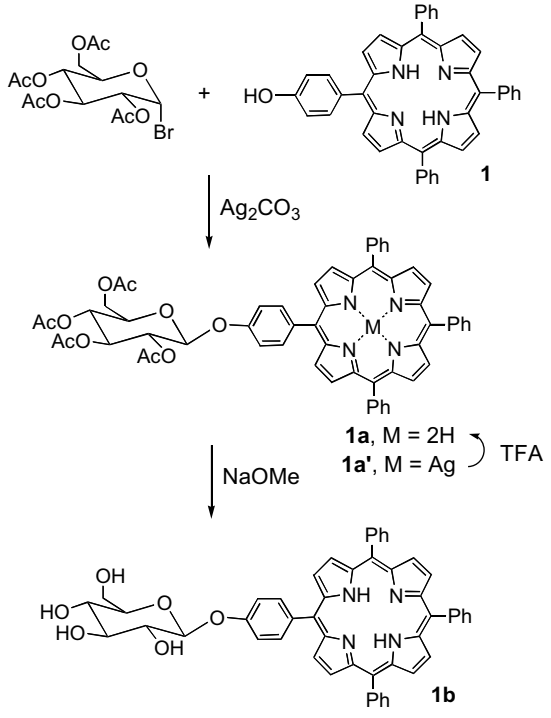
Porphyrins **1**, **3**, and **4** (Scheme 1), key compounds in the synthesis of the glycoporphyrins, were obtained from Rothemund and crossed-Rothemund reactions using the appropriate benzaldehydes and pyrrole in refluxing acetic acid and nitrobenzene.[39] Porphyrin **2** was obtained in 85% yield by coupling porphyrin **1** with hexafluorobenzene, in DMF, in the presence of sodium hydride.

The monosaccharide derivatives selected for this work were 1-bromo-2,3,4,6-tetra-*O*-acetyl-α-D-glucopyranose and 1,2:3,4-di-*O*-isopropylidene-α-D-galactopyranose. These are commercially available starting materials, with protecting groups which can be easily and selectively removed in the presence of other functional groups, leading to the desired glycoporphyrins with the sugar moiety bearing free hydroxyl groups. The 1,2:3,4-di-*O*-isopropylidene-α-D-galactopyranose was also used to prepare 6-iodo-1,2:3,4-di-*O*-isopropylidene-α-D-galactopyranose,[40,41] a useful compound for the synthesis of other glycoporphyrins. Two series of porphyrins were obtained by coupling these sugars with *meso*-tetraphenylporphyrins containing an hydroxyl group (**1**) or a carboxylic group (**4**); the other two series were obtained by direct substitution of a fluorine atom on *meso*-tetrakis(pentafluorophenyl)porphyrin (**3**) or on a *meso*-tetraphenylporphyrin containing a pentafluorobenzene spacer (**2**).

Glycoporphyrin **1a** was obtained from the reaction of porphyrin **1** with 1-bromo-2,3,4,6-tetra-*O*-acetyl-α-D-glucopyranose (Scheme 2). The reaction was carried out at room temperature in the presence of freshly prepared silver carbonate. After the work up, the reaction mixture was separated by column chromatography affording a mixture of the desired porphyrin **1a** with the corresponding silver complex **1a′** (Scheme 2). Treatment of this mixture with trifluoroacetic acid (TFA) gave, after purification, pure porphyrin **1a** in 66% yield. The carbohydrate protection groups were removed by



Scheme 1. Key compounds for the synthesis of the glycoporphyrins.



Scheme 2.

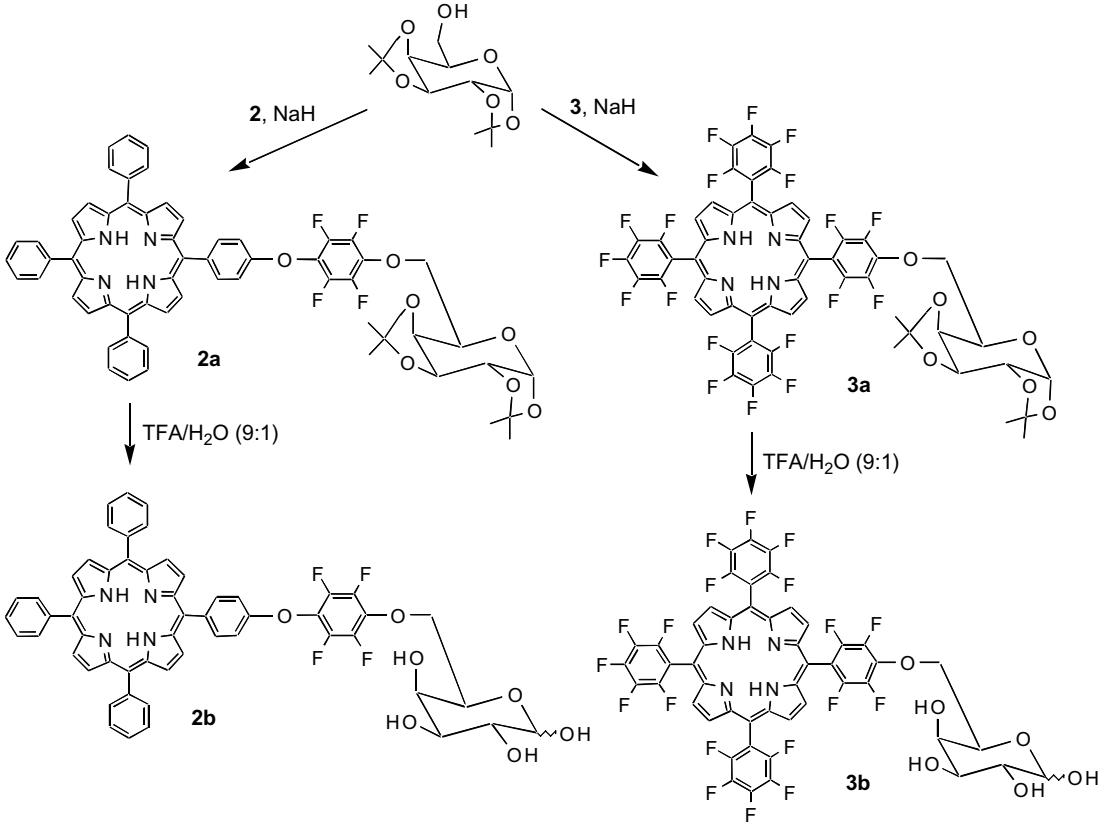
treating **1a** with sodium methoxide in dry methanol; the glycoporphyrin **1b** was obtained in 80% yield.

Glycoporphyrins **2a** and **3a** were obtained, respectively, from the reaction of porphyrins **2** and **3** with 1,2:3,4-di-*O*-isopropylidene-α-D-galactopyranose (Scheme 3). Both compounds were formed by nucleophilic substitution of a fluorine atom by the alkoxide generated from the reaction of the carbohydrate with sodium hydride. The fluorobenzene bridge was introduced in order to evaluate any effect on the separation of the carbohydrate unit from the porphyrin macrocycle.

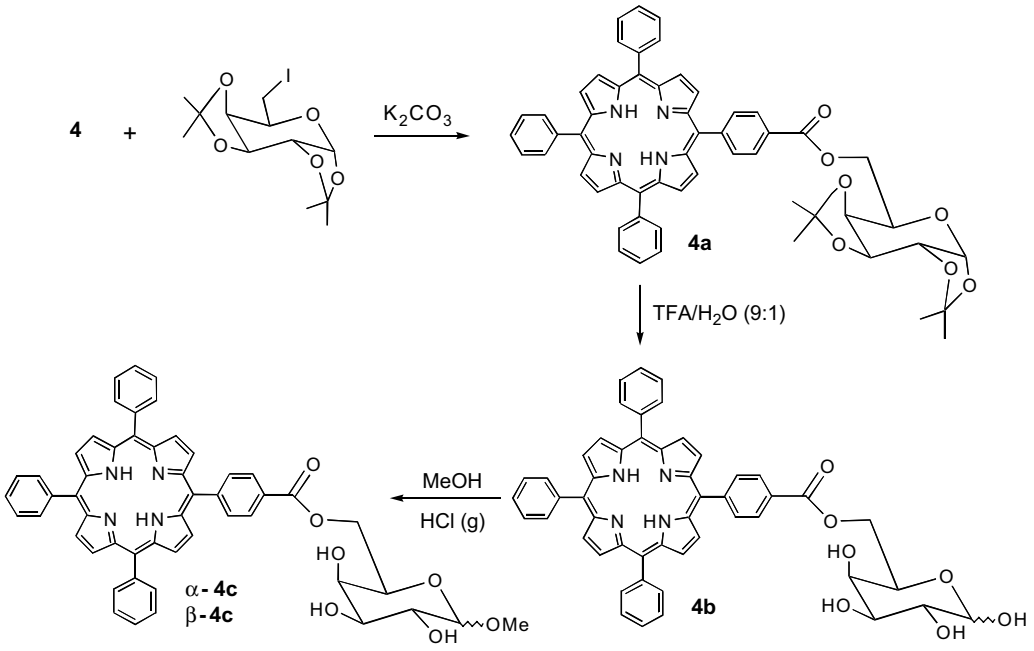
Attempts to couple 1,2:3,4-di-*O*-isopropylidene-α-D-galactopyranose with porphyrin **4**, in the presence of *N*,*N*-dicyclohexylcarbodiimide (DCC) and 4-pyrrolopyridine, did not lead to the expected compound **4a** but to the corresponding *N*-acylurea derivative.[42] However, we were able to obtain **4a** in 50% yield from the reaction of **4** with 6-iodo-1,2:3,4-di-*O*-isopropylidene-α-D-galactopyranose in the presence of potassium carbonate (Scheme 4). The removal of the carbohydrate protection groups in compounds **2a**, **3a**, and **4a** to the corresponding porphyrins **2b**, **3b**, and **4b** was performed with aqueous TFA at room temperature.

The methyl galactopyranosides α-**4c** and β-**4c** were prepared by bubbling gaseous hydrogen chloride through a solution of **4b** in dry methanol and chloroform at room temperature; purification by preparative TLC afforded the α and β anomers in 43% and 40% yields, respectively.

The structures of all porphyrins were confirmed by NMR, UV–vis, mass spectrometry, and HRMS or elemental analysis. The ^{1}H NMR spectra of porphyrins **2a**, **3a**, and **4a** show two distinct regions: the signals at lower field (between 7 and 9 ppm) are due to the resonances of the protons of the porphyrinic moiety and the ones at higher field (between 1 and 6 ppm) are due to the resonances of the protons of the carbohydrate unit. Each spectrum shows four singlets, between 1.3 and 1.6 ppm, due to the isopropylidene protons; the resonances of the other protons of the carbohydrate unit appear between 4 and 6 ppm. The resonances of the anomeric protons of the three compounds appear as doublets at 5.54, 5.70, and 5.66 ppm, respectively. For each one of these derivatives, the multiplet centered at ca. 8.9 ppm was assigned to the β-pyrrolic protons; the resonances of the protons of the nonsubstituted phenyl groups appear at ca. 8.2 ppm (*ortho* protons) and at 7.7 ppm (*meta* and *para* protons). The resonances of the protons of the *para*-substituted phenyl groups appear as two doublets at 7.35 and 8.16 ppm (for **2a**) and at 8.30 and 8.45 ppm (for **4a**). The ^{19}F NMR spectrum of **2a** confirmed the substitution of the *para*-fluorine atom by the sugar unit. The resonances of the *ortho*- and *meta*-fluorine atoms appear as two doublets (J = 22.6 Hz) at −151.91 and −152.40 ppm, respectively. The ^{1}H NMR spectra of **2b**, **3b**, and **4b** confirmed



Scheme 3.



Scheme 4.

the deprotection of the carbohydrate moieties with the disappearance of the four singlets due to the isopropylidene protons. The spectra show several multiplets between 3 and 6 ppm due to the resonances of the protons of the sugar units. As expected, there are no significant differences between the lower field regions of the ^{1}H NMR spectra of porphyrins **2b**, **3b**, and **4b** and that of the corresponding precursors. Each ^{1}H NMR spectrum of porphyrins α-**4c** and β-**4c** shows a singlet due to the methoxy group at 3.47 and 3.59 ppm, respectively. The resonance of the anomeric proton appears as a singlet at 5.02 ppm for the α anomer and as a doublet at 4.94 ppm (J = 4.8 Hz) for the β anomer.

The UV–vis spectra of the glycoporphyrin derivatives show, as expected, spectroscopic features analogous to those of the starting porphyrins. The linking of carbohydrate units to the *meso*-aryl groups of the porphyrins does not change the macrocycle conjugation in order to affect the absorption properties of the new derivatives.

3. Biological evaluation

3.1. Results and discussion

All the described porphyrin derivatives, at their maximum noncytotoxic studied concentration (MNCSC), were tested against HSV-1 and HSV-2 in Vero cells. Studies were carried out at room temperature under the white light of a laminar flow cabinet type B, corresponding to a fluence rate of 3 mW/cm^2, for 15 min. Under these conditions, the intensity of the Soret band of each porphyrin did not show any decrease, indicating that the compounds are photostable under such conditions. To evaluate the cytotoxic effect of the porphyrin derivatives, monolayers of Vero cells were cultured for 72 h in the presence of different concentrations of the drugs, and then the cell viability was determined. These studies indicated that the cytotoxic effects of the porphyrin derivatives, as well as acyclovir and foscarnet (antiviral controls), are concentration dependent. The values of the MNCSC for each compound are listed in Table 1.

The antiviral activity of the porphyrin derivatives was determined by the observed virucidal effect inhibition and the decrease on the virus yield (Table 1). Porphyrins with the sugar moiety bearing unprotected hydroxyl

Table 1. Effect of the synthesized compounds on HSV-1 and HSV-2 infectivity

Compound	MNCSC[a] (μg/mL)	Virucidal effect inhibition (% control)[b]		Replication cycle inhibition (% control)[b]	
	Vero cells	HSV-1	HSV-2	HSV-1	HSV-2
1	30	0	0	100	40
1a	30	0	0	40	20
1b	15	ND	ND	100	100
2	50	ND	ND	42	33
2a	50	ND	ND	50	14
2b	50	57	25	80	86
3	40	ND	ND	30	50
3a	50	ND	ND	60	80
3b	15	60	50	90	99
4	5	85	60	30	25
4a	50	30	50	35	50
4b	10	70	82	100	100
α-**4c**	50	15	26	100	97
β-**4c**	25	20	31	99	98
Acyclovir	100	45	0	99	99
Foscarnet	1000	20	0	99	99

ND—not done.

[a] Maximum noncytotoxic studied concentration.

[b] Each value represents the average of three independent experiments.

groups (**1b**, **2b**, **3b**, and **4b**) and the methyl galactopyranosides α-**4c** and β-**4c** showed virus yield inhibitions comparable to the ones obtained with the control compounds (Fig. 1a). The virus yield inhibition, quite similar for both viruses, must be specific of the viral cycle, because no significant virucidal effect was observed with the same porphyrins (Fig. 1b).

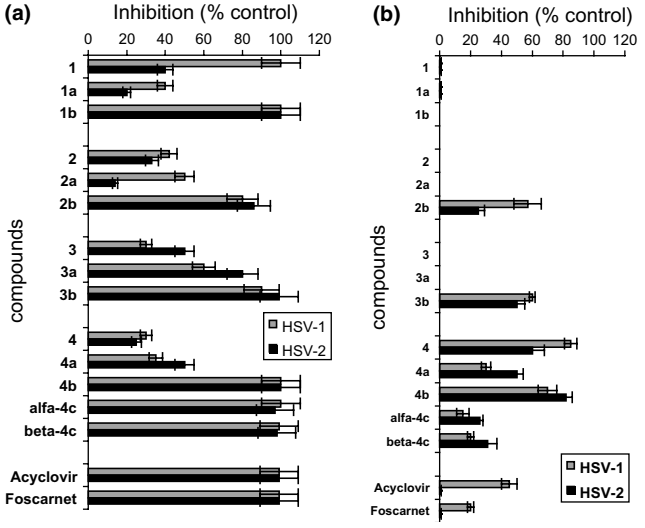


Figure 1. (a) Viral replication effect and (b) virucidal effect of the HSV-1 and HSV-2 incubated with the compounds at their MNCSC, and exposed for 15 min to 3 mW/cm^2 of white light. The inhibition values (in percentage) were calculated in relation to the control. Data are reported as the means of three independent assays, each run in duplicate. Error bars represent standard deviations.

From such results, it can be stated that the glycoporphyrins **1b**, **2b**, **3b**, **4b**, α-**4c**, and β-**4c** are more effective than their precursors in the inactivation of both viruses. This structure–activity relationship indicates that the sugar moiety plays an important role in the interaction of the photosensitizer with the biological target.

The antiviral activity of the most severe inhibitors was also determined at concentrations lower than the MNCSC. The results show that all these compounds continue to be highly effective against both viruses even at concentrations much lower than the MNCSC (Fig. 2). Porphyrins **1b**, **3b**, and **4b** show antiviral effects higher than 40% even at 0.01 μg/mL.

Methyl glycosides α-**4c** and β-**4c** show similar inhibitions as porphyrin **4b** (Fig. 1). These results indicate that it is not required to have a reducing carbohydrate moiety for a good antiviral activity. This is also confirmed with the results from porphyrin **1b**.

Adsorption and post-adsorption assays for HSV-1 were carried out with compound **1b** at concentrations of 15 and 3 μg/mL. Despite no inhibition being observed in the adsorption assay using compound **1b** at 15 μg/mL, a total inhibition (100%) in the post-adsorption test was reached even when a lower concentration (3 μg/mL) of the drug was used. These results are consistent with the hypothesis that the viral inhibition occurs in a late step of the viral cycle.

When the effect on the viral replication was studied with **1b**, at the MNCSC, under careful exclusion of light, the virus titration showed a much lower inhibition (40%) than the one obtained under the currently used conditions, that is, with exposure to the light of the laminar

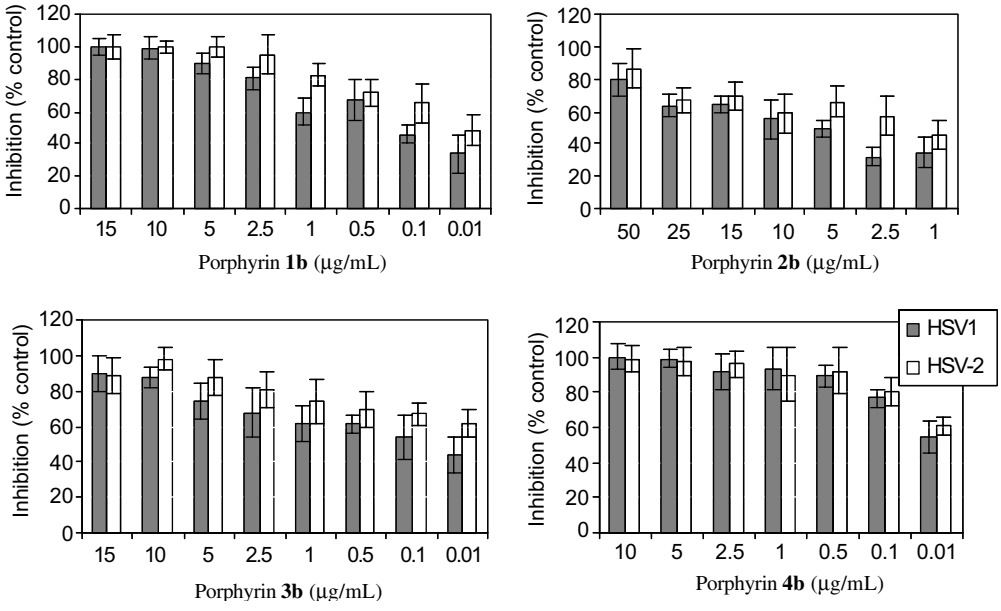


Figure 2. Viral replication effect of the HSV-1 and HSV-2 incubated with the compounds at concentrations lower than MNCSC and exposed for 15 min to 3 mW/cm^2 of white light. The inhibition values (in percentage) were calculated in relation to the control. Data are reported as the means of three independent assays, each run in duplicate. Error bars represent standard deviations.

flow cabinet. Although several reports show that certain porphyrins under dark conditions can have cytotoxic effects in cancer cells[43] and on HIV-1,[44] our results suggest that the antiviral activity of our compounds is significantly enhanced by light.

4. Conclusion

We have demonstrated that glycoporphyrins can significantly inhibit the infection of Vero cells by HSV-1 and HSV-2. Our results show that glycoporphyrins **1b** and **4b** (with a sugar moiety bearing unprotected hydroxyl groups) have a similar inhibitory capacity in the formation of infectious virions as acyclovir or foscarnet. The glycoporphyrins are active against both viruses; only marginal differences were observed, in all cases, for the inhibition of HSV-1 and HSV-2. Post-adsorption studies with glycoporphyrin **1b** showed a reduction on the replication cycle by 6 log in relation with the control without the drug (data not shown). This might mean that a specific inhibitory action occurs during the formation of new viral particles. Some of the tested compounds showed strong virus yield inhibition, even at concentrations much lower than the MNCSC, and so they may be useful for the treatment of herpesvirus infections.

5. Experimental

5.1. General

^{1}H, ^{13}C, and ^{19}F solution NMR spectra were recorded on a Bruker AMX 300 spectrometer at 300.13, 75.47, and 282.38 MHz, respectively. Tetramethylsilane was used as internal reference. Mass spectra and HRMS were recorded on VG AutoSpec Q and M mass spectrometers using chloroform as solvent and NBA as matrix. Elemental analyses were performed with a Leco 932 CHNS analyzer. The UV–vis spectra were recorded on an Uvikon spectrophotometer using dichloromethane or chloroform as solvent. Melting points were measured on a Reichert Thermovar apparatus fitted with a microscope and are uncorrected. Column chromatography was carried out in silica gel (Merck, 35–70 mesh). Preparative thin-layer chromatography was carried out on 20×20 cm glass plates coated with silica gel (1 mm thick, Merck). Analytical TLC was carried out on precoated sheets with silica gel (0.2 mm thick, Merck).

5.2. Synthesis

5.2.1. 5-(4-Hydroxyphenyl)-10,15,20-triphenylporphyrin (1). 4-Hydroxybenzaldehyde (1.07 g, 8.8 mmol, 1.2 equiv) and benzaldehyde (2.20 mL, 21.7 mmol, 3 equiv) were added to a refluxing mixture of glacial acetic acid (200 mL) and nitrobenzene (150 mL). Pyrrole (2.00 mL, 28.9 mmol, 4 equiv) was then added dropwise over 15 min and the mixture was refluxed for a further 1 h. After cooling to room temperature, the acetic acid, and nitrobenzene were distilled under reduced pressure. The crude material was taken into chloroform and submitted to column chromatography (silica gel) using a mixture of chloroform–petroleum ether (1:1) as eluent. The first fraction was identified by TLC as 5,10,15,20-tetraphenylporphyrin (TPP). The desired porphyrin **1** was then eluted with chloroform. Evaporation of the solvent and recrystallization from chloroform/petroleum ether gave purple crystals (300 mg, 7% yield). Mp >300 °C. ^{1}H NMR ($CDCl_3$): δ −2.78 (s, 2H, NH), 7.15 (d, $J = 8.5$ Hz, 2H, 5-Ar-*m*-H), 7.72–7.78 (m, 9H, 10,15,20-Ar-*m*- and *p*-H), 8.06 (d, $J = 8.5$ Hz, 2H, 5-Ar-*o*-H), 8.20–8.23 (m, 6H, 10,15,20-Ar-*o*-H), 8.84–8.88 (m, 8H, β-H). ^{13}C NMR ($CDCl_3$): δ 113.6, 119.8, 120.1, 126.7, 127.7, 131.0, 131.1, 134.5, 134.7, 135.7, 142.2, 155.4. Anal. Calcd for $C_{44}H_{30}N_4O \cdot 5/2H_2O$: C, 78.20; H, 5.22; N, 8.29. Found: C, 78.41; H, 4.97; N, 8.41. UV–vis ($CHCl_3$): λ_{max} (log ε): 419 (5.65), 515 (4.24), 551 (3.92), 591 (3.73), 647 (3.67) nm. MS (FAB^+) *m*/*z*: 631 $(M+H)^+$.

5.2.2. 5-[4-(Pentafluorophenyloxy)phenyl]-10,15,20-triphenylporphyrin (2). To a suspension of porphyrin **1** (50.0 mg, 80.9 μmol) in dimethylformamide (2 mL) was added an excess of sodium hydride (≈25 mg) and the mixture was heated at 70 °C during approximately 10 min. Hexafluorobenzene (1 mL, 8.7 mmol, 107 equiv) was then added and the mixture was maintained at that temperature for 4 h in the dark and under nitrogen. After cooling to room temperature, the reaction mixture was neutralized with a saturated aqueous citric acid solution, and the resulting porphyrin was extracted with chloroform. The organic layer was washed with water (2×100 mL), dried (Na_2SO_4), and evaporated to dryness. The residue was purified by column chromatography on silica using chloroform–petroleum ether (70:30) as eluent. The major fraction afforded product **2** (55 mg, 85% yield) after recrystallization from chloroform/methanol. Mp 295–296 °C. ^{1}H NMR ($CDCl_3$): δ −2.80 (s, 2H, NH), 7.33 (d, $J = 8.6$ Hz, 2H, 5-Ar-*m*-H), 7.73–7.78 (m, 9H, 10,15,20-Ar-*m*- and *p*-H), 8.17 (d, $J = 8.6$ Hz, 2H, 5-Ar-*o*-H), 8.20–8.23 (m, 6H, 10,15,20-Ar-*o*-H), 8.82–8.87 (m, 8H, β-H). ^{13}C NMR ($CDCl_3$): δ 113.7, 118.6, 120.25, 120.3, 126.7, 127.7, 131.2, 134.6, 135.7, 138.0, 142.1, 157.0. ^{19}F NMR ($CDCl_3$): δ −158.10 (t, $J = 21.2$ Hz, 2F, Ar-*m*-F), −155.88 (t, $J = 21.2$ Hz, 1F, Ar-*p*-F), −149.91 (d, $J = 16.9$ Hz, 2F, Ar-*o*-F). Anal. Calcd for $C_{50}H_{29}F_5N_4O \cdot 3H_2O$: C, 70.58; H, 4.15; N, 6.58. Found: C, 70.36; H, 3.91; N, 6.24. UV–vis ($CHCl_3$) λ_{max} (log ε): 420 (5.71), 516 (4.30), 551 (3.92), 591 (3.76), 646 (3.63) nm. MS (FAB^+) *m*/*z*: 797 $(M+H)^+$.

5.2.3. *meso*-Tetrakis(pentafluorophenyl)porphyrin (3). Pentafluorobenzaldehyde (4 mL, 32 mmol) was added to a refluxing mixture of glacial acetic acid (200 mL) and nitrobenzene (150 mL). Pyrrole (2.5 mL, 36 mmol) was then added dropwise over 15 min and the mixture was refluxed for a further 1 h. After cooling to room temperature, the acetic acid and nitrobenzene were distilled under reduced pressure to dryness. The crude material was taken into chloroform and submitted to column chromatography (silica gel) using a mixture of chloroform–petroleum ether (1:1) as eluent. After evaporation of the solvent, the desired porphyrin **3** was recrystallized from dichloromethane/petroleum ether

(15% yield). ^{1}H NMR ($CDCl_3$): δ −2.93 (s, 2H, NH), 8.92 (s, 8H, β-H). Anal. Calcd for $C_{44}H_{10}F_{20}N_4 \cdot 3/2H_2O$: C, 52.76; H, 1.31; N, 5.59. Found: C, 52.74; H, 0.90; N, 5.98. UV–vis ($CHCl_3$) λ_{max} (log ε): 411 (5.36), 504 (4.28), 579 (3.83), 632 (2.96) nm. MS (FAB^+) *m/z*: 976 $(M+2H)^+$.

5.2.4. 5-(4-Carboxyphenyl)-10,15,20-triphenylporphyrin (4). Methyl 4-formylbenzoate (1.44 g, 8.77 mmol, 1.2 equiv) and benzaldehyde (2.20 mL, 21.7 mmol, 3 equiv) were added to a refluxing mixture of glacial acetic acid (200 mL) and nitrobenzene (150 mL). After complete dissolution of methyl 4-formylbenzoate, pyrrole (2.00 mL, 28.9 mmol, 4 equiv) was then added dropwise and the mixture was refluxed for 1 h. The solvents were then distilled under reduced pressure and the crude material was taken into chloroform and submitted to column chromatography (silica gel) using a mixture of chloroform–petroleum ether (1:1) as eluent. The first fraction was identified as 5,10,15,20-tetraphenylporphyrin (TPP) and the second one as the methyl ester of porphyrin **4** (evaporation of the solvent and recrystallization from chloroform/methanol gave the pure compound in 13% yield). Mp 268–270 °C. ^{1}H NMR ($CDCl_3$): δ −2.79 (s, 2H, NH), 4.11 (s, 3H, CO_2CH_3), 7.72–7.81 (m, 9H, 10,15,20-Ar-*m*- and *p*-H), 8.20–8.22 (m, 6H, 10,15,20-Ar-*o*-H), 8.31 (d, J = 8.9 Hz, 2H, 5-Ar-*o*-H), 8.44 (d, J = 8.9 Hz, 2H, 5-Ar-*m*-H), 8.78–8.87 (m, 8H, β-H). HRMS (FAB^+): *m/z* calcd for $C_{46}H_{32}N_4O_2$ $(M+H)^+$: 673.2604. Found: 673.2594.

The previous porphyrin (162 mg, 0.241 mmol) was then dissolved in tetrahydrofuran (4 mL) and pyridine (0.8 mL). KOH (4.00 g, 71.3 mmol) dissolved in methanol (40 mL) was added to this solution and the mixture was refluxed for 38 h. After cooling to room temperature, the mixture was neutralized with a saturated aqueous citric acid solution and the resulting suspension extracted with chloroform–methanol (85:15). The organic layer was washed with water (2 × 100 mL), dried (Na_2SO_4), and evaporated to dryness. Porphyrin **4** (151 mg, 95% yield) was obtained after recrystallization in chloroform–methanol (85:15)/petroleum ether. Mp > 300 °C. ^{1}H NMR ($CDCl_3/CD_3OD$): δ 7.73–7.80 (m, 9H, 10,15,20-Ar-*m*- and *p*-H), 8.21–8.24 (m, 6H, 10,15,20-Ar-*o*-H), 8.32 (d, J = 8.2 Hz, 2H, 5-Ar-*o*-H), 8.46 (d, J = 8.2 Hz, 2H, 5-Ar-*m*-H), 8.87 (br s, 8H, β-H). UV–vis ($CHCl_3$): λ_{max} (log ε): 418 (5.78), 513 (4.40), 549 (4.07), 588 (3.94), 649 (3.81) nm. Anal. Calcd for $C_{45}H_{30}N_4O_2$: C, 82.05; H, 4.59; N, 8.51. Found: C, 82.22; H, 4.77; N, 8.25.

5.2.5. 5-[4-(2,3,4,6-Tetra-*O*-acetyl-β-D-glucopyranosyloxy)-phenyl]-10,15,20-triphenylporphyrin (1a).[45] To a solution of porphyrin **1** (101 mg, 160 μmol) in dry dichloromethane (150 mL), sodium sulfate (1.84 g, 13.0 mmol, 81.2 equiv), freshly prepared silver carbonate (0.95 g, 3.4 mmol, 21.2 equiv), and 1-bromo-2,3,4,6-tetra-*O*-acetyl-α-D-glucopyranose (1.00 g, 2.43 mmol, 15 equiv) were added. The reaction mixture was stirred for 24 h at room temperature, in the dark and under nitrogen. The reaction mixture was then filtered and the organic phase was washed with water (2 × 50 mL). The organic extracts were dried (Na_2SO_4) and evaporated under reduced pressure. The resulting residue was taken into chloroform and purified by column chromatography (silica gel) using the same solvent as eluent. The first fraction was identified as the silver complex of the starting porphyrin **1** and the second one as a mixture of porphyrin **1a** and its silver complex **1a′**. After evaporation of the eluent, the second fraction was stirred with TFA (5 mL) for 10 min at room temperature. Chloroform (5 mL) was then added and after 5 min the solution was neutralized with saturated aqueous sodium carbonate. The organic phase was dried (Na_2SO_4) and the solvent removed under vacuum. The residue was purified by column chromatography (silica gel) using chloroform as eluent. The desired porphyrin was then crystallized from chloroform/petroleum ether (101 mg, 66% yield). Mp >300 °C. ^{1}H NMR ($CDCl_3$): δ −2.79 (s, 2H, NH), 2.10, 2.11, 2.12, 2.22 (4s, 12H, 4 × acetyl), 4.06 (ddd, J = 9.6, 5.4, 2.4 Hz, 1H, Glc-H5), 4.30 (dd, J = 12.3, 2.4 Hz, 1H, Glc-H6), 4.42 (dd, J = 12.3, 5.4 Hz, 1H, Glc-H6), 5.30 (t, J = 9.6 Hz, 1H, Glc-H4), 5.42–5.50 (m, 3H, Glc-H1, H2, H3), 7.38 (d, J = 8.7 Hz, 2H, 5-Ar-*m*-H), 7.73–7.79 (m, 9H, 10,15,20-Ar-*m*- and *p*-H), 8.14 (d, J = 8.7 Hz, 2H, 5-Ar-*o*-H), 8.20–8.23 (m, 6H, 10,15,20-Ar-*o*-H), 8.85 (s, 8H, β-H). ^{13}C NMR: δ 20.6 (CH_3CO_2), 20.7 (CH_3CO_2), 20.8 (2 × CH_3CO_2), 62.1 (Glc-C6), 68.4, 71.4, 72.3, 72.9 (Glc-C2, 3, 4, 5), 99.2 (Glc-C1), 115.0, 119.2, 120.2, 126.7, 127.7, 131.2, 134.5, 135.6, 137.3, 142.1, 156.6, 169.5 (2 × CH_3CO_2), 170.3 (CH_3CO_2), 170.6 (CH_3CO_2). Anal. Calcd for $C_{58}H_{48}N_4O_{10} \cdot 1/2H_2O$: C, 71.81; H, 5.09; N, 5.78. Found: C, 71.74; H, 5.21; N, 5.73. UV–vis ($CHCl_3$) λ_{max} (log ε): 418 (5.68), 515 (4.28), 550 (3.93), 590 (3.75), 645 (3.62) nm. MS (FAB^+) *m/z*: 961 $(M+H)^+$.

5.2.6. 5-(4-β-D-Glucopyranosyloxyphenyl)-10,15,20-triphenylporphyrin (1b).[45] To porphyrin **1a** (24.9 mg, 25.9 μmol) in dichloromethane (6 mL) and methanol (6 mL) was added a catalytic amount of sodium methoxide in methanol (0.1 mol dm^{-3}) (57.6 μL, 5.76 μmol, 0.22 equiv). The reaction mixture was stirred in the dark at room temperature under nitrogen for 6 h. Water was then added and the mixture was extracted with dichloromethane. The organic extracts were washed again with water and dried over Na_2SO_4. The solvent was evaporated under reduced pressure to dryness and the product was crystallized from chloroform/petroleum ether (16.4 mg, 80% yield). Mp >300 °C. ^{1}H NMR (DMSO-d_6): δ −2.92 (s, 2H, NH), 3.52–3.57 (m, 4H, Glc-OH), 3.59–3.61 (m, 2H, Glc-H), 4.74 (br s, 1H, Glc-H), 5.14 (d, J = 6.0 Hz, 1H, Glc-H), 5.23 (br s, 2H, Glc-H), 5.55 (s, 1H, Glc-H), 7.48 (d, J = 8.3 Hz, 2H, 5-Ar-*m*-H), 7.84 (m, 9H, 10,15,20-Ar-*m*- and *p*-H), 8.14 (d, J = 8.3 Hz, 2H, 5-Ar-*o*-H), 8.22 (m, 6H, 10,15,20-Ar-*o*-H), 8.83–8.88 (m, 8H, β-H). ^{13}C NMR (DMSO-d_6): δ 60.8 (Glc-C6), 69.8, 73.5, 76.7, 77.2 (Glc-C2, 3, 4, 5), 100.5 (Glc-C1), 114.6, 119.9, 120.0, 127.0, 128.1, 131.4, 134.2, 134.6, 135.3, 141.2, 157.5. Anal. Calcd for $C_{50}H_{40}N_4O_6 \cdot 4H_2O$: C, 69.43; H, 5.59; N, 6.48. Found: C, 69.41; H, 5.42; N, 6.46. UV–vis ($CHCl_3$) λ_{max} (log ε): 418 (5.64), 515 (4.23), 550 (3.88), 590 (3.70), 645 (3.57) nm. MS (FAB^+) *m/z*: 793 $(M+H)^+$.

5.2.7. 5-{4-[4-(1,2:3,4-Di-*O*-isopropylidene-α-D-galactopyranosyl-6-oxy)tetrafluorophenyloxy]phenyl}-10,15,20-triphenylporphyrin (2a). A mixture of 1,2:3,4-di-*O*-isopropylidene-α-D-galactopyranose (4.5 mg, 17.3 μmol) and porphyrin **2** (20.0 mg, 25.1 μmol, 1.45 equiv) in DMF (2.5 mL) was heated at 70 °C until total dissolution of reagents (approximately 10 min). An excess of sodium hydride (≈25 mg) was then added and the resulting mixture was maintained at that temperature for 1.5 h in the dark and under nitrogen. After cooling to room temperature, the mixture was neutralized with a saturated aqueous citric acid solution, and extracted with chloroform. The organic layer was washed with water (2 × 100 mL), dried (Na_2SO_4), and the solvent removed. The residue was purified by column chromatography (silica gel) using chloroform–petroleum ether (1:1) as eluent. The major fraction afforded, after recrystallization from chloroform/petroleum, the desired product (12.5 mg, 70% yield). Mp 131–133 °C. ^{1}H NMR ($CDCl_3$): δ −2.80 (s, 2H, NH), 1.33, 1.35, 1.47, 1.55 [4s, 12H, $C(CH_3)_2$], 4.20 (dt, *J* = 5.7, 2.0 Hz, 1H, Gal-H5), 4.34 (dd, *J* = 5.2, 2.6 Hz, 1H, Gal-H2), 4.37 (dd, *J* = 8.0, 2.0 Hz, 1H, Gal-H4), 4.47 (br d, *J* = 5.7 Hz, 2H, Gal-H6), 4.66 (dd, *J* = 8.0, 2.6 Hz, 1H, Gal-H3), 5.54 (d, *J* = 5.2 Hz, 1H, Gal-H1), 7.35 (d, *J* = 8.6 Hz, 2H, 5-Ar-*m*-H), 7.73–7.80 (m, 9H, 10,15,20-Ar-*m*- and *p*-H), 8.16 (d, *J* = 8.6 Hz, 2H, 5-Ar-*o*-H), 8.20–8.23 (m, 6H, 10,15,20-Ar-*o*-H), 8.84–8.86 (m, 8H, β-H). ^{13}C NMR ($CDCl_3$): δ 24.4, 24.9, 25.9, 25.9 [4 × C(*C*H_3)$_2$], 67.0 (Gal-C6), 70.4, 70.7, 71.0 (Gal-C2, 3, 4, 5), 96.2 (Gal-C1), 108.8, 109.6 [2 × *C*$(CH_3)_2$], 113.6, 118.9, 120.2, 126.7, 127.7, 134.6, 135.6, 137.5, 142.1, 157.3. ^{19}F NMR ($CDCl_3$): δ −152.40 (d, *J* = 22.6 Hz, 2F, Ar-*m*-F), −151.91 (d, *J* = 22.6 Hz, 2F, Ar-*o*-F). Anal. Calcd for $C_{62}H_{48}F_4N_4O_7$: C, 71.81; H, 4.67; N, 5.40. Found: C, 71.82; H, 5.01; N, 5.07; UV–vis ($CHCl_3$): λ_{max} (log ε): 420 (5.67), 516 (4.25), 551 (3.89), 590 (3.73), 646 (3.56) nm. MS (FAB^+) *m*/*z*: 1037 $(M+H)^+$.

5.2.8. 5-{4-[4-(α/β-D-Galactopyranosyl-6-oxy)tetrafluorophenyloxy]phenyl}-10,15,20-triphenylporphyrin (2b). Porphyrin **2a** (15.0 mg, 14.5 μmol) was dissolved in TFA–water (9:1) (3 mL); the mixture was stirred in the dark at room temperature for 30 min. Then, chloroform (10 mL) and water (20 mL) were added and the mixture was neutralized with aqueous sodium carbonate. The organic layer was separated, washed with water (100 mL), and dried over Na_2SO_4. The solvent was evaporated under reduced pressure to dryness and the residue was crystallized from chloroform/petroleum ether (12.9 mg, 93% yield). Mp 215–216 °C. ^{1}H NMR ($CDCl_3$): δ −2.94 (br s, 2H, NH), 3.63–5.42 (m, 11H, Gal-H and OH), 6.90–8.03 (m, 19H, 5,10,15,20-Ar-H), 8.52–8.66 (m, 8H, β-H). ^{19}F NMR ($CDCl_3$): δ −153.38 (br s, 2F, Ar-*m*-F), −151.26 (br s, 2F, Ar-*o*-F). UV–vis ($CHCl_3$) λ_{max} (log ε): 420 (5.66), 518 (4.24), 551 (3.98), 591 (3.76), 646 (3.55) nm. HRMS (FAB^+) *m*/*z*: Calcd for $C_{56}H_{41}F_4N_4O_7$ $(M+H)^+$: 957.2911. Found: 957.2915.

5.2.9. 5-[4-(1,2:3,4-Di-*O*-isopropylidene-α-D-galactopyranosyl-6-oxy)tetrafluorophenyl]-10,15,20-tris(pentafluorophenyl)porphyrin (3a). To a mixture of 1,2:3,4-di-*O*-isopropylidene-α-D-galactopyranose (5.5 mg, 21.1 μmol) and *meso*-tetrakis(pentafluorophenyl)porphyrin (30.0 mg, 30.8 μmol, 1.46 equiv), in dry toluene (2.5 mL), was added an excess of sodium hydride (≈25 mg). The reaction mixture was refluxed for 24 h in the dark and under nitrogen. After cooling at room temperature, the solution was neutralized with saturated aqueous citric acid and the resulting porphyrin was extracted with chloroform. The organic layer was washed with water (2 × 100 mL), dried (Na_2SO_4), and the solvent removed. The residue was purified by column chromatography (silica gel) using chloroform–petroleum ether (1:1) as eluent. The major fraction afforded, after crystallization from chloroform/petroleum ether, porphyrin **3a** (10.1 mg, 40% yield). Mp 171–174 °C. ^{1}H NMR ($CDCl_3$): δ −2.92 (s, 2H, NH), 1.44, 1.45, 1.56, 1.67 [4s, 12H, $C(CH_3)_2$], 4.40–4.53 (m, 3H, Gal-H), 4.75–4.78 (m, 3H, Gal-H), 5.70 (d, *J* = 3.0 Hz, 1H, Gal-H1), 8.89–9.02 (m, 8H, β-H). ^{19}F NMR ($CDCl_3$): δ −157.95 to −157.75 (m, 6F, 10,15,20-Ar-*m*-F), −152.62 (dd, *J* = 22.6, 8.5 Hz, 2F, 5-Ar-*m*-F), −147.90 to −147.73 (m, 3F, 10,15,20-Ar-*p*-F), −135.80 to −135.68 (m, 2F, 5-Ar-*o*-F), −133.01 to −132.90 (m, 6F, 10,15,20-Ar-*o*-F). UV–vis ($CHCl_3$) λ_{max} (log ε): 414 (5.54), 507 (4.39), 585 (3.89) nm. HRMS (FAB^+) *m*/*z*: Calcd for $C_{56}H_{30}F_{19}N_4O_6$ $(M+H)^+$: 1215.1862. Found: 1215.1866.

5.2.10. 5-[4-(α/β-D-Galactopyranosyl-6-oxy)tetrafluorophenyl]-10,15,20-tris(pentafluorophenyl)porphyrin (3b). A mixture of TFA–water (9:1, 4 mL) was added to porphyrin **3a** (20.0 mg, 16.5 μmol). The resulting mixture was stirred in the dark at room temperature for 30 min. Chloroform (20 mL) and water (20 mL) were then added and the mixture was neutralized with aqueous sodium carbonate. The organic layer was separated, washed with water (100 mL) and dried over Na_2SO_4. The solvent was evaporated under reduced pressure to dryness and the residue was crystallized from chloroform/petroleum ether (16.8 mg, 90% yield). Mp >300 °C. ^{1}H NMR ($CDCl_3$): δ −3.04, −3.01 (2s, 2H, NH), 3.05–3.55, 4.23–4.53, 4.79–5.10 (3m, 10H, Gal-H and OH), 5.70 (s, 1H, Gal-H1), 8.73–8.89 (m, 8H, β-H). ^{19}F NMR ($CDCl_3$): δ −158.41 to −157.82 (m, 6F, 10,15,20-Ar-*m*-F), −153.58 to −153.21 (m, 2F, 5-Ar-*m*-F), −148.37 to −147.78 (m, 3F, 10,15,20-Ar-*p*-F), −135.00 to −134.91 (m, 2F, 5-Ar-*o*-F), −133.44 to −133.00 (m, 6F, 10,15,20-Ar-*o*-F). Anal. Calcd for $C_{50}H_{21}F_{19}N_4O_6$: C, 52.92; H, 1.87; N, 4.94. Found: C, 52.91; H, 2.40; N, 4.57. UV–vis ($CHCl_3$) λ_{max} (log ε): 414 (5.56), 507 (4.40), 585 (3.89) nm. MS (FAB^+) *m*/*z*: 1135 $(M+H)^+$.

5.2.11. 5-[4-(1,2:3,4-Di-*O*-isopropylidene-α-D-galactopyranosyl-6-oxycarbonyl)phenyl]-10,15,20-triphenylporphyrin (4a). To a solution of porphyrin **4** (50 mg, 76 μmol) and 6-iodo-1,2:3,4-di-*O*-isopropylidene-α-D-galactopyranose (176 mg, 475 μmol, 6.2 equiv) in dry dimethylformamide (10 mL) was added potassium carbonate (125 mg, 0.9 mmol, 11.9 equiv). The reaction mixture was stirred for 30 h at 100 °C in the dark and under nitrogen. After cooling to room temperature, the solution was neutralized with aqueous saturated citric acid, and the resulting porphyrin extracted with chloroform. The organic phase was washed with water

(2 × 100 mL), dried (Na_2SO_4), and the solvent removed. The residue was purified by column chromatography (silica gel) using chloroform as eluent. The major fraction afforded, after crystallization from chloroform/methanol, porphyrin **4a** (34 mg, 50% yield). Mp >300 °C. ^{1}H NMR ($CDCl_3$): δ −2.79 (s, 2H, NH), 1.38, 1.42, 1.55, 1.63 [4s, 12H, C(CH_3)$_2$], 4.35–4.49, 4.61–4.75 (2m, 6H, Gal-H2, H3, H4, H5, H6), 5.66 (d, J = 5.1 Hz, 1H, Gal-H1), 7.73–7.79 (m, 9H, 10,15,20-Ar-*m*- and *p*-H), 8.20–8.23 (m, 6H, 10,15,20-Ar-*o*-H), 8.30 (d, J = 8.4 Hz, 2H, 5-Ar-*o*-H), 8.45 (d, J = 8.4 Hz, 2H, 5-Ar-*m*-H), 8.78–8.88 (m, 8H, β-H). ^{13}C NMR ($CDCl_3$): δ 24.6, 25.0, 26.0, 26.1 [4 × C(CH_3)$_2$], 64.2 (Gal-C6), 66.2 (Gal-C5), 70.6, 70.8 (Gal-C3, 4), 71.2 (Gal-C2), 96.4 (Gal-C1), 108.9, 109.8 [2 × *C*(CH_3)$_2$], 118.5, 120.4, 120.5, 126.7, 127.8, 128.0, 129.4, 134.5, 142.0, 147.1, 166.7 (CO). UV–vis ($CHCl_3$) λ_{max} (log ε): 420 (5.73), 516 (4.30), 551 (3.92), 591 (3.74), 646 (3.60) nm. HRMS (FAB$^+$) *m*/*z*: Calcd for $C_{57}H_{49}N_4O_7$ (M+H)$^+$: 901.3601. Found: 901.3590.

5.2.12. 5-[4-(α/β-D-Galactopyranosyl-6-oxycarbonyl)phenyl]-10,15,20-triphenylporphyrin (4b). Porphyrin **4a** (15.0 mg, 16.5 μmol) in TFA–water (9:1) (3 mL) was stirred in the dark at room temperature for 30 min. Chloroform (20 mL) and water (20 mL) were then added and the mixture was neutralized with aqueous sodium carbonate. The mixture was extracted with chloroform–methanol (85:15), and then the organic phase was washed with water (100 mL) and dried over Na_2SO_4. The solvent was evaporated under reduced pressure to dryness and the residue was crystallized from chloroform/methanol (12.9 mg, 94% yield). Mp >300 °C. ^{1}H NMR ($CDCl_3$/CD_3OD): δ 3.52–3.70, 3.90–4.15, 4.50–4.68, 4.68–4.75, and 5.32 (4m and 1d, J = 3.2 Hz, 11H, Gal-H and OH), 7.74–7.81 (m, 9H, 10,15,20-Ar-*m*-H and *p*-H), 8.21–8.23 (m, 6H, 10,15,20-Ar-*o*-H), 8.33 (d, J = 8.1 Hz, 2H, 5-Ar-*o*-H), 8.47 (d, J = 8.1 Hz, 2H, 5-Ar-*m*-H), 8.88 (br s, 8H, β-H). UV–vis [$CHCl_3$–MeOH (85:15)] λ_{max} (log ε): 420 (5.52), 518 (4.12), 551 (3.75), 590 (3.60), 645 (3.41) nm. HRMS (FAB$^+$) *m*/*z*: Calcd for $C_{51}H_{41}N_4O_7$ (M+H)$^+$: 821.2975. Found: 821.2988.

5.2.13. 5-[4-(α-Methyl-D-galactopyranosyl-6-oxycarbonyl)-phenyl]-10,15,20-triphenylporphyrin (α-4c) and 5-[4-(β-methyl-D-galactopyranosyl-6-oxycarbonyl)phenyl]-10,15,20-triphenylporphyrin (β-4c). Porphyrin **4b** (22 mg, 26.8 μmol) in anhydrous methanol (10 mL) and chloroform (4 mL) was saturated with gaseous hydrogen chloride. The mixture was stirred for 4 h in the dark and at room temperature. It was then neutralized with aqueous sodium carbonate. The organic layer was separated, washed with water (100 mL), and dried over Na_2SO_4. The two anomers α-**4c** and β-**4c** were separated by preparative TLC using $CHCl_3$–MeOH (95:5) as eluent; the one with higher R_f (0.39) was identified by NMR as the α-anomer. The two methyl glycosides were crystallized from chloroform/petroleum ether.

Anomer α-4c: 9.5 mg (43% yield), mp 241–243 °C. ^{1}H NMR ($CDCl_3$): δ −2.79 (s, 2H, NH), 2.93 (d, J = 11.3 Hz, 1H, Gal-OH), 3.47 (s, 3H, Gal-OMe), 3.56 (d, J = 3.7 Hz, 1H, Gal-OH), 3.98 (d, J = 10.2 Hz, 1H, Gal-OH), 4.07, 4.17, and 4.32 (2d and 1s, J = 9.9, 11.2 Hz, 3H, Gal-H2,3,4), 4.39–4.41 (m, 1H, Gal-H5), 4.46 (dd, J = 11.7, 3.7 Hz, 1H, Gal-H6), 4.78 (dd, J = 11.7, 7.9 Hz, 1H, Gal-H6), 5.02 (s, 1H, Gal-H1), 7.73–7.79 (m, 9H, 10,15,20-Ar-*m*-H and *p*-H), 8.20–8.23 (m, 6H, 10,15,20-Ar-*o*-H), 8.33 (d, J = 8.1 Hz, 2H, 5-Ar-*o*-H), 8.46 (d, J = 8.1 Hz, 2H, 5-Ar-*m*-H), 8.77–8.88 (m, 8H, β-H). HRMS (FAB$^+$) *m*/*z*: Calcd for $C_{52}H_{43}N_4O_7$ (M+H)$^+$: 835.3132. Found: 835.3099. UV–vis ($CHCl_3$) λ_{max} (log ε): 418 (5.68), 514 (4.28), 550 (3.92), 589 (3.76), 645 (3.60) nm.

Anomer β-4c: 9 mg (40% yield), mp 210–212 °C. ^{1}H NMR ($CDCl_3$): δ −2.79 (s, 2H, NH), 2.48 (d, J = 9.9 Hz, 1H, Gal-OH), 2.61 (s, 1H, Gal-OH), 2.77 (d, J = 6.8 Hz, 1H, Gal-OH), 3.59 (s, 3H, Gal-OMe), 4.15–4.21 (m, 3H, Gal-H2, H3, H4), 4.35–4.38 (m, 1H, Gal-H5), 4.59 (dd, J = 11.5, 4.8 Hz, 1H, Gal-H6), 4.67 (dd, J = 11.5, 6.1 Hz, 1H, Gal-H6), 4.94 (d, J = 4.8 Hz, 1H, Gal-H1), 7.73–7.79 (m, 9H, 10,15,20-Ar-*m*-H and *p*-H), 8.20–8.23 (m, 6H, 10,15,20-Ar-*o*-H), 8.31 (d, J = 8.2 Hz, 2H, 5-Ar-*o*-H), 8.46 (d, J = 8.2 Hz, 2H, 5-Ar-*m*-H), 8.77–8.87 (m, 8H, β-H). HRMS (FAB$^+$) *m*/*z*: Calcd for $C_{52}H_{43}N_4O_7$ (M+H)$^+$: 835.3132. Found: 835.3152. UV–vis ($CHCl_3$) λ_{max} (log ε): 418 (5.62), 515 (4.22), 550 (3.89), 589 (3.71), 645 (3.53) nm.

5.2.14. Photosensitizers. Each compound stock solution was prepared at a concentration of 8 mg/mL in dimethyl sulfoxide (DMSO) and diluted to the final concentration in Dulbecco's modified Eagle's medium (DMEM) or in phosphate buffer solution (PBS).

5.2.15. Photostability of the compounds. The photostability of the photosensitizers was determined by keeping 1 μM solutions of the various porphyrin samples under the white light of the laminar flow cabinet (3 mW/cm^2) at room temperature, with magnetic stirring, for 30 min. At fixed intervals of time, the intensity of the Soret band of each porphyrin was monitored by visible absorption spectrophotometry.

5.2.16. Cells and virus. African green monkey kidney (Vero) cells were grown as monolayers in DMEM supplemented with 10% fetal calf serum (FCS) and gentamycin (50 μg/mL), both purchased from Gibco, Scotland, and incubated at 37 °C in a humidified atmosphere containing 5% CO_2. HSV-1 strain SC16 and HSV-2 strain HD from ATCC (USA), adapted to grow in Vero cells, were produced in the same conditions of cells.[46]

5.2.17. Photocytotoxicity. Confluent monolayer Vero cells in 96-well plates with 100 μL of solutions with decreasing concentrations of the compounds (prepared in DMEM from the stock solutions) were kept under the white light of the laminar flow cabinet for 15 min at room temperature, and then incubated for 72 h at 37 °C in a humidified atmosphere containing 5% CO_2. The dye uptake assay[47,48] was then performed to determine the percentage of living cells related to the control (cells incubated without drug and kept under the same conditions). The maximum noncytotoxic studied

concentration (MNCSC) is the highest concentration of the drug, which gives identical results as the control after 24, 48, and 72 h.

5.2.18. Virucidal effect. HSV-1 and HSV-2 (10^7 PFU/mL) were incubated with the compounds, at their MNCSC in PBS, for 1 h at 37 °C and then kept for 15 min under the laminar flow cabinet light. The drugs were then removed by ultracentrifugation (44,000*g*). Controls were similarly processed. Virucidal effect was determined by plaque titration: confluent Vero cells in a 24-well plate were infected with 100 μL of serial dilutions of the viruses in PBS. After 2 h of adsorption at 37 °C the inoculum was removed, the cells were washed and covered with 2% of Sephadex in DMEM with 2% FCS. On the fourth day post-infection, the cells were fixed with 10% formaldehyde in ethanol and stained with a solution of 0.1% crystal violet. Viral plaques were counted under the microscope. The inhibitory activity of each compound was calculated in relation to the control.

5.2.19. Viral replication effect. To evaluate the effect of compounds on the viral replication cycle, Vero cell monolayers were incubated for 30 min at 37 °C with the compounds at their MNCSC and, when specified, at lower concentrations. Then, the compounds were removed and the cells were washed with PBS or DMEM with 2% FCS at the same temperature. Treated and untreated (control) cell monolayers were then infected with HSV-1 or HSV-2, at a multiplicity of infection (MOI) of approximately 1 PFU per cell. After adsorption of the viruses for 2 h at 37 °C, the unbound viruses were removed by washing the cells with PBS. DMEM 2% with or without drug was then added and the plates were kept for 15 min under the laminar flow cabinet light. Such plates were incubated at 37 °C until an extensive cytopathic effect was observed in the control infected cells, normally 24 h post-infection. The cells were collected and the extracellular infectious virions were evaluated by standard plaque assay on Vero cells.

5.2.20. Effect of glycoporphyrin 1b on viral adsorption and post-adsorption. Confluent Vero cells in 35 mm diameter dishes were cooled to 4 °C for 15 min and then infected with an ice-cold suspension of HSV-1 or HSV-2 (10^7 PFU/mL), at a MOI of approximately 2 PFU per cell, with 15 μg/mL of compound **1b**. The dishes were then gently shaken for 2 h at 4 °C. The free viruses were then removed and the cell monolayers were washed twice with cold PBS. New medium without drug was added. The virus yield was determined 24 h post-infection, when extensive cytopathic effect was observed in the control cells. The viral post-adsorption assay was carried out in the same way, but during the virus adsorption the drug was not present.

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