Glycosylated zinc(II) phthalocyanines as efficient photosensitisers for photodynamic therapy. Synthesis, photophysical properties and *in vitro* photodynamic activity†

Chi-Fung Choi,[a] Jian-Dong Huang,[a,b] Pui-Chi Lo,[a] Wing-Ping Fong[c] and Dennis K. P. Ng*[a]

Received 8th February 2008, Accepted 17th March 2008
First published as an Advance Article on the web 18th April 2008
DOI: 10.1039/b802212g

Treatment of 3- or 4-nitrophthalonitrile with 1,2:5,6-di-*O*-isopropylidene-α-D-glucofuranose or 1,2:3,4-di-*O*-isopropylidene-α-D-galactopyranose in the presence of K_2CO_3 gave the corresponding glycosubstituted phthalonitriles. These precursors underwent self-cyclisation, or mixed-cyclisation with the unsubstituted phthalonitrile, to afford the tetra- or mono-glycosylated zinc(II) phthalocyanines, respectively. As shown by absorption spectroscopy, these compounds were not significantly aggregated in organic solvents, giving a weak to moderate fluorescence emission. Upon irradiation these compounds could sensitise the formation of singlet oxygen in DMF, with quantum yields in the range of 0.40–0.66. The *in vitro* photodynamic activities of these compounds against HepG2 human hepatocarcinoma and HT29 human colon adenocarcinoma cells were also studied. The mono-glycosylated phthalocyanines exhibited significantly higher photocytotoxicity compared with the tetra-α-glycosylated analogues, having IC_{50} values down to 0.9 μM. The tetra-β-glycosylated counterparts were essentially inactive. The lower photocytotoxicities of the tetra-glycosylated phthalocyanines are in line with their lower cellular uptake and/or higher aggregation tendency as reflected by weaker intracellular fluorescence, and lower efficiency at generating intracellular reactive oxygen species. For the mono-glycosylated phthalocyanines, the higher uptake can be attributed to their hydrophilic saccharide units, which increase the amphiphilicity of the macrocycles.

Introduction

Phthalocyanines represent an important class of functional dyes.[1] In addition to their use as advanced materials in various fields, these macrocyclic compounds have also found application in medicine. Owing to their strong and long-wavelength absorptions, high efficiency at generating reactive oxygen species (ROS), and ease of chemical modification, phthalocyanines have emerged as a promising class of second-generation photosensitisers for photodynamic therapy (PDT).[2] Over the last decade, a substantial number of phthalocyanine-based photosensitisers have been prepared and evaluated for their photodynamic activity, with the focus on silicon, zinc and aluminium analogues as a result of their desirable photophysical properties. To date, several phthalocyanine systems such as the silicon(IV) phthalocyanine Pc4 and a liposomal preparation of zinc(II) phthalocyanine have been in clinical trials. Photosense®, which is a mixture of sulfonated aluminium(III) phthalocyanines, is clinically used in Russia for the treatment of a range of cancers.[2c]

One of the major challenges in PDT is to develop selective photosensitisers which can preferentially accumulate in malignant tissues relative to normal tissues. Although a level of selectivity can be achieved by the confined illumination of the target area, the use of selective photosensitisers, which can greatly reduce the side effects and enhance the therapeutic outcomes, is still important. Various approaches have been explored to enhance the photosensitiser concentrations in target tissues, and include encapsulation in colloidal carriers such as liposomes and polymeric micelles,[3] and conjugation to tumour-specific carrier molecules such as antibodies, synthetic peptides, epidermal growth factor and adenoviruses, *etc*.[4] For the latter approach, only a limited target specificity has been achieved so far.

In addition to these biomolecules, carbohydrates are also promising candidates for bioconjugation to photosensitisers to achieve targeted PDT. It has long been known that cancer cells have increased levels of glucose uptake and glycolysis in order to provide sufficient metabolic energy to sustain their proliferation.[5] The transport of glucose across the cell membrane is mediated by glucose transporter proteins, which are over-expressed in a variety of human carcinomas.[6] By taking advantage of this, glycoconjugation of various photosensitisers such as porphyrins,[7] chlorins,[8] pyropheophorbides[9] and hypocrellins[10] has been carried out with a view to enhancing their cellular uptake and eventually the PDT efficacy. In a number of cases, particularly for the unsymmetrically conjugated analogues, promising results have been observed.[7d,8a,c,9]

In contrast to carbohydrate–porphyrin conjugates, glycoconjugated phthalocyanines are extremely rare despite their great

[a]*Department of Chemistry and Centre of Novel Functional Molecules, The Chinese University of Hong Kong, Shatin, N.T., Hong Kong, China. E-mail: dkpn@cuhk.edu.hk; Fax: +852 2603 5057; Tel: +852 2609 6375*
[b]*College of Chemistry and Chemical Engineering, Fuzhou University, Fuzhou 350002, China*
[c]*Department of Biochemistry and Centre of Novel Functional Molecules, The Chinese University of Hong Kong, Shatin, N.T., Hong Kong, China*
† Electronic supplementary information (ESI) available: ^{1}H–^{1}H COSY and HMQC with BIRD spectra of **18** and UV–vis spectra of **6**, **8** and **13**. See DOI: 10.1039/b802212g

potential in PDT.[11] We have recently prepared a novel series of silicon(IV) phthalocyanines with isopropylidene-protected glucose and galactose moieties as the axial substituents.[12] These compounds exhibit a high cellular uptake and are highly potent toward several cancer cell lines. The IC_{50} values, defined as the dye concentration required to kill 50% of the cells, are as low as 6 nM. Herein we describe an extension of this work, focusing on zinc(II) analogues, which are generally more stable than their silicon(IV) counterparts. Although the synthesis and characterisation of several glycosylated zinc(II) phthalocyanines have been reported,[11] their photobiological properties remain unexplored. In this paper, we report the synthesis, spectroscopic characterisation and photophysical properties of a new series of mono- and tetra-glycosylated phthalocyanines. The *in vitro* photodynamic activities of this class of photosensitisers are also reported for the first time.

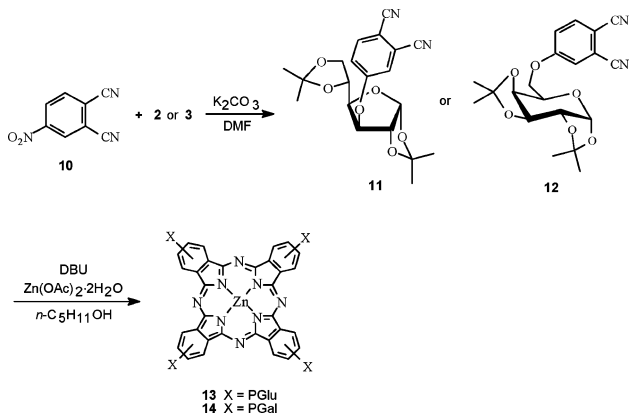
Results and discussion

Synthesis and characterisation

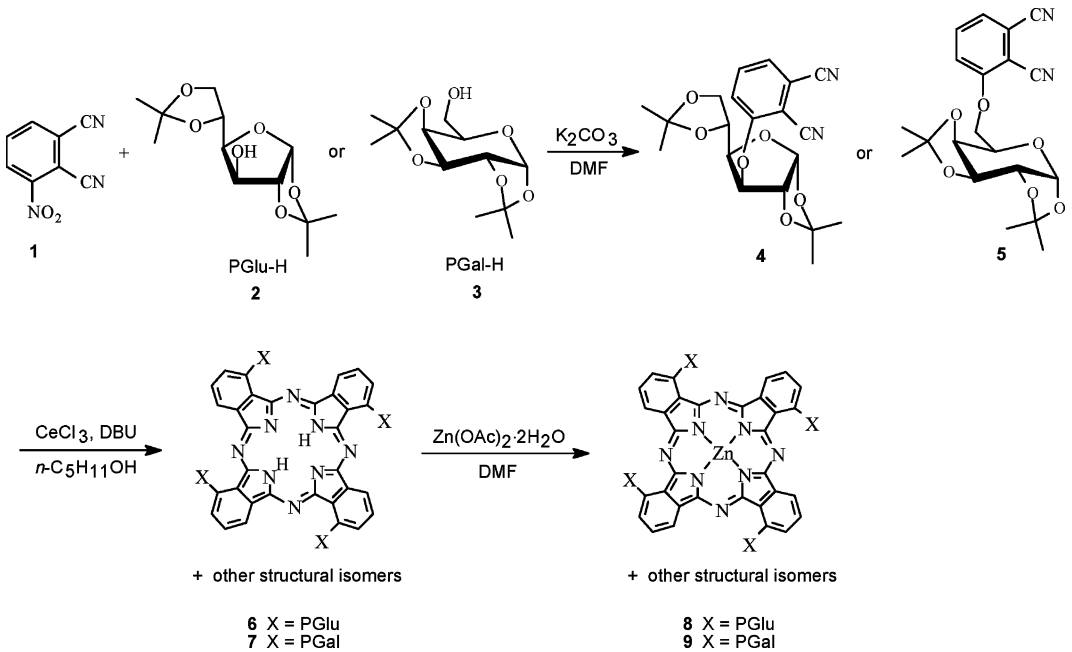
Scheme 1 shows the synthetic route to tetra-α-glycosylated phthalocyanines **6–9**. Treatment of 3-nitrophthalonitrile (**1**) with the isopropylidene-protected glucofuranose **2** or galactopyranose **3** in the presence of K_2CO_3 in DMF led to nucleophilic aromatic substitution, giving the corresponding glycosylated phthalonitrile **4** or **5**. These compounds then underwent a cerium-promoted cyclisation reaction[13] to afford the metal-free phthalocyanines $H_2Pc(\alpha\text{-PGlu})_4$ (**6**) and $H_2Pc(\alpha\text{-PGal})_4$ (**7**) in moderate yields (47% and 58%, respectively). By using the phthalonitrile with a bulky 2,4-dimethyl-3-pentoxy group at the α-position, we have found that the 1,8,15,22- (or C_4) isomer is predominantly formed, which can be isolated by column chromatography followed by recrystallisation.[13,14] However, the tetra-α-glycosylated phthalocyanines **6** and **7** exhibited excellent solubility in most organic solvents. It was difficult to find an appropriate solvent system to isolate the C_4 isomers by recrystallisation. Hence, compounds **6** and **7** were prepared as mixtures of structural isomers.

Subsequent metallation of these compounds with $Zn(OAc)_2\cdot 2H_2O$ in DMF gave the corresponding zinc(II) complexes **8** and **9** in good yields (75% and 79%, respectively). It was found that, upon treatment with $Zn(OAc)_2\cdot 2H_2O$ and DBU, phthalonitriles **4** and **5** could be directly converted to zinc phthalocyanines **8** and **9** respectively, but this one-step procedure seemed to produce more side products, which slightly hindered the purification process.

By contrast, this one-step cyclisation method could be readily employed to prepare the tetra-β-substituted analogues $ZnPc(\beta\text{-PGlu})_4$ (**13**) and $ZnPc(\beta\text{-PGal})_4$ (**14**). As shown in Scheme 2, treatment of 4-nitrophthalonitrile (**10**) with protected monosaccharide **2** or **3** gives the substituted product **11** or **12**, which undergoes a DBU-promoted self-cyclisation reaction using $Zn(OAc)_2\cdot 2H_2O$ as the template to give phthalocyanine **13** or **14**. These compounds, again as mixtures of structural isomers, could be readily purified by



Scheme 2

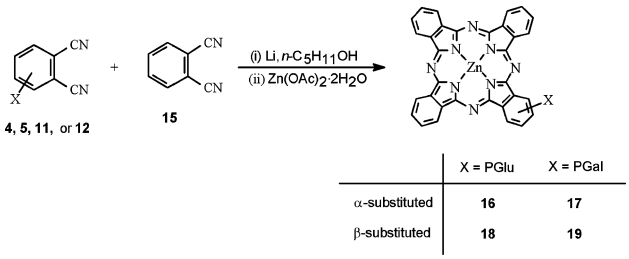


Scheme 1

This journal is © The Royal Society of Chemistry 2008

silica-gel column chromatography. The glycosylated phthalonitrile **11** and phthalocyanine **13** have been described previously, but only the UV–vis data have been given.[11a]

To enhance the amphiphilicity of the molecules, which is generally believed to be an advantageous characteristic in photosensitisers,[15] we also prepared the mono-glycosylated analogues **16–19**. These compounds were prepared (in 25–43% yields) by mixed cyclisation of the glycosylated phthalonitrile **4**, **5**, **11** or **12** with an excess of the unsubstituted phthalonitrile **15** (9 equiv.) using lithium as the template, followed by metallation with $Zn(OAc)_2 \cdot 2H_2O$ (Scheme 3). These A_3B-type cyclised products could be separated from the other side products by silica-gel column chromatography followed by size exclusion chromatography.



Scheme 3

Attempts were made to remove the isopropylidene protecting groups of the zinc(II) phthalocyanines ZnPc(α-PGlu)$_4$ (**8**) and ZnPc(α-PGlu) (**16**) by treatment with trifluoroacetic acid and water (v/v 9 : 1). Difficulties were encountered in the purification of the products either by chromatography or recrystallisation. The ^{1}H NMR signals of the crude products in DMSO-d$_6$ were significantly broadened and were difficult to assign. It is likely that the deprotected sugar moieties promote the aggregation of the macrocycles through hydrogen bonding formation. This possible phenomenon, together with the anomerisation of the sugar moieties, complicates the spectra, hindering the characterisation of these compounds. Due to these difficulties, deprotection of these glycosylated phthalocyanines was not pursued further.

All the new compounds were unambiguously characterised by various spectroscopic methods. For the tetra-β-glycosylated phthalocyanines **13** and **14**, although they exist as a mixture of structural isomers, the ^{1}H NMR spectra recorded in $CDCl_3$ in the presence of a trace amount of pyridine-d$_5$ are simpler than expected. Three well-separated multiplets in the region δ 7.7–9.5 were observed for the three sets of phthalocyanine ring protons. The sugar protons resonated as six (for **13** at δ 4.2–6.2) or three (for **14** at δ 4.4–5.8) multiplets, while the isopropylidene methyl groups resonated as several singlets at δ 1.3–1.9. The assignment could be readily made with the aid of ^{1}H–^{1}H COSY spectra. Fig. 1 shows the spectrum of **13** for illustration.

The NMR spectra for the mono-glycosylated phthalocyanines **16–19** were also informative. Taking the ^{1}H NMR spectrum of **18** as an example, it showed a multiplet at δ 9.06–9.30 (7 H) and a broad signal at δ 8.85 (1 H) for the eight phthalocyanine α ring protons. The seven β ring protons resonated as a multiplet at δ 7.93–8.11 (6 H) and a doublet at δ 7.67 (1 H). The signals for the protected glucose appeared as three doublets (for H1, H2 and H3 of the glucofuranose ring), one doublet of doublets (for H4), two multiplets (for H5 and H6) and four singlets (for the

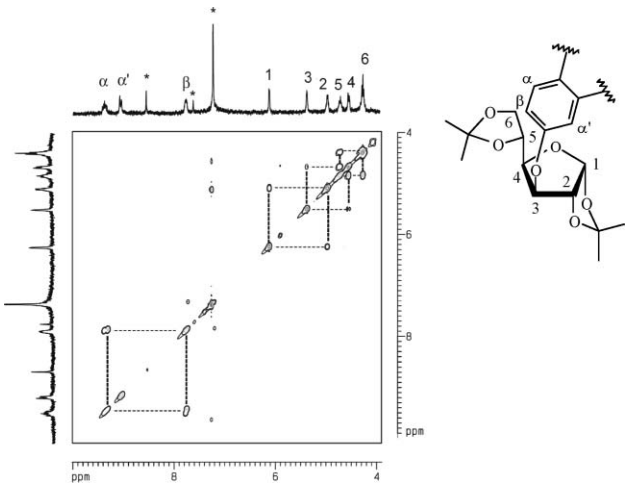


Fig. 1 ^{1}H–^{1}H COSY spectrum of ZnPc(β-PGlu)$_4$ (**13**) in $CDCl_3$ with a trace amount of pyridine-d$_5$ (*ca.* 1% v/v); * indicates signals for residual solvents.

four methyl groups). The assignment was supported by its ^{1}H–^{1}H COSY spectrum (Fig. S1†). Due to the low symmetry of these compounds, the ^{13}C{^{1}H} NMR signals for the phthalocyanine ring were split, but extensively overlapped. Nevertheless, some of the phthalocyanine α- and β-ring carbon signals, as well as those for the sugar ring could be identified using HMQC with BIRD spectra [see the spectrum for **18** (Fig. S2†) given as an example].

The ESI mass spectra of all the phthalocyanines showed the protonated molecular ion [(M + H)$^+$] signal as the base peak, of which the isotopic distribution was in good agreement with the simulated pattern. The identity of these species was also confirmed by accurate mass measurements.

Electronic absorption and photophysical properties

The electronic absorption and basic photophysical data of these glycosylated phthalocyanines are summarised in Table 1. The absorption and fluorescence spectra of the tetra-substituted phthalocyanines were recorded in chloroform, but for the mono-substituted analogues, due to their limited solubility in neat chloroform, the spectra were taken in THF. The UV–vis spectra of these compounds were typical for phthalocyanines with a low aggregation tendency, showing one (for zinc(II) analogues) or two (for metal-free analogues) intense Q band(s) at the red visible region. The Q bands for the α-substituted zinc(II) phthalocyanines were significantly red-shifted by 18–20 nm (for tetra-substituted analogues) or 5–6 nm (for mono-substituted analogues) compared with those for the β-substituted counterparts [see the spectra of **6**, **8** and **13** (Fig. S3†)]. α-Glycosylation also led to a weakening of the fluorescence emission. For the tetra-substituted series (**8**, **9**, **13** and **14**), the change from β to α substitution decreased the fluorescence quantum yield by about four-fold (Table 1). These results are in accord with the observations and theoretical calculations reported previously for a series of metal-free and zinc(II) phthalocyanines.[16] The effects of the sugar moieties are insignificant both on the absorption and fluorescence emission properties.

The singlet oxygen quantum yields (Φ_Δ) of these glycosylated phthalocyanines were also determined in DMF using 1,3-diphenylisobenzofuran (DPBF) as the scavenger. The

Table 1 Electronic absorption and photophysical data for the glycosylated phthalocyanines[a]

Compound	λ_{max}/nm (log ε)	λ_{em}/nm[b]	Φ_f[c]	Φ_Δ[d]
$H_2Pc(\alpha\text{-PGlu})_4$ (**6**)	315 (4.68), 353 (4.65), 626 (4.43), 660 (4.59), 690 (5.06), 724 (5.11)	724	0.03	0.12
$H_2Pc(\alpha\text{-PGal})_4$ (**7**)	315 (4.68), 352 (4.59), 626 (4.42), 659 (4.56), 691 (5.05), 724 (5.10)	726	0.05	0.14
$ZnPc(\alpha\text{-PGlu})_4$ (**8**)	319 (4.57), 350 (4.56), 629 (4.49), 669 (4.41), 698 (5.20)	705	0.07	0.66
$ZnPc(\alpha\text{-PGal})_4$ (**9**)	319 (4.56), 350 (4.58), 629 (4.54), 667 (4.44), 699 (5.31)	706	0.06	0.52
$ZnPc(\beta\text{-PGlu})_4$ (**13**)	350 (4.82), 612 (4.38), 648 (4.31), 680 (5.09)	687	0.23	0.41
$ZnPc(\beta\text{-PGal})_4$ (**14**)	351 (4.89), 613 (4.48), 647 (4.43), 679 (5.15)	689	0.24	0.40
ZnPc(α-PGlu) (**16**)	343 (4.88), 607 (4.65), 645 (4.62), 673 (5.40)	679	0.20	0.47
ZnPc(α-PGal) (**17**)	341 (4.76), 608 (4.53), 645 (4.49), 672 (5.28)	678	0.21	0.44
ZnPc(β-PGlu) (**18**)	344 (4.85), 603 (4.59), 640 (4.53), 667 (5.38)	673	0.26	0.47
ZnPc(β-PGal) (**19**)	344 (4.92), 603 (4.65), 639 (4.59), 667 (5.42)	673	0.26	0.45

[a] Recorded in chloroform (for **6–9** and **13–14**) or THF (for **16–19**) unless otherwise stated. [b] Excited at 610 nm (for **13–14** and **16–19**) or 625 nm (for **6–9**). [c] Φ_f: fluorescence quantum yield relative to unsubstituted zinc(II) phthalocyanine (ZnPc) ($\Phi_f = 0.30$ in 1-chloronaphthlene). [d] Φ_Δ: singlet oxygen quantum yield measured in DMF relative to ZnPc ($\Phi_\Delta = 0.56$).

concentration of the quencher was monitored spectroscopically at 411 nm along with time, from which the values of Φ_Δ could be determined by the method described previously.[17] These data are also compiled in Table 1. It can be seen that the tetra-α-substituted zinc(II) phthalocyanines **8** and **9** give the highest Φ_Δ values (0.66 and 0.52, respectively), while the metal-free phthalocyanines **6** and **7** are the least efficient at the generation of singlet oxygen ($\Phi_\Delta =$ 0.12 and 0.14, respectively). This is in line with the higher triplet quantum yields generally observed for zinc(II) phthalocyanines compared with the metal-free analogues.[18]

In vitro photodynamic activities

The photodynamic activities of all the glycoconjugated zinc(II) phthalocyanines in Cremophor EL emulsions were investigated using two different cell lines, namely HT29 human colon adenocarcinoma and HepG2 human hepatocarcinoma cells. In the absence of light, all these compounds were essentially non-toxic to the cells. Upon illumination, these compounds exhibited different degrees of photocytotoxicity. While the tetra-β-glycosylated phthalocyanines **13** and **14** remained non-cytotoxic up to 8 μM, the other phthalocyanines, particularly the mono-substituted analogues **16–19**, were photodynamically active. Fig. 2 shows the dose response curves for the galactosylated phthalocyanines $ZnPc(\alpha\text{-PGal})_4$ (**9**), $ZnPc(\beta\text{-PGal})_4$ (**14**), ZnPc(α-PGal) (**17**) and ZnPc(β-PGal) (**19**) against HT29. A similar trend was observed for the glucosylated series and for the HepG2 cells. The IC_{50} values of the tetra-α- (**8** and **9**) and mono-substituted (**16–19**) photosensitisers against the two cell lines are compiled in Table 2. It can be seen that the mono-glycosylated analogues generally exhibit higher photocytotoxicity, with IC_{50} values down to 0.9 μM. Apparently, the photocytotoxicity of these compounds is higher than that of the tetrasulfonated zinc(II) phthalocyanine and comparable if not better than that of the tetrasulfonated aluminium(III) phthalocyanine and Photosense®, which are all common phthalocyanine-based photosensitisers.[19] Nevertheless, compared with the silicon(IV) analogues prepared by us previously, whose IC_{50} values could be as low as 6 nM,[12] these zinc(II) glycosylated phthalocyanines are substantially less photocytotoxic, probably due to the higher intrinsic stacking tendency of these non-axially substituted macrocycles. As shown in Table 2, the sugar moieties do not have a significant effect on the photodynamic activities of this series of compounds.

Table 2 IC_{50} values of the glycosylated zinc(II) phthalocyanines **8**, **9** and **16–19** against HepG2 and HT29 cells[a]

Compound	For HepG2/μM	For HT29/μM
$ZnPc(\alpha\text{-PGlu})_4$ (**8**)	4.7	4.0
$ZnPc(\alpha\text{-PGal})_4$ (**9**)	5.1	3.5
ZnPc(α-PGlu) (**16**)	1.1	1.0
ZnPc(α-PGal) (**17**)	1.7	1.7
ZnPc(β-PGlu) (**18**)	1.8	2.0
ZnPc(β-PGal) (**19**)	1.5	0.9

[a] Defined as the dye concentration required to kill 50% of the cells.

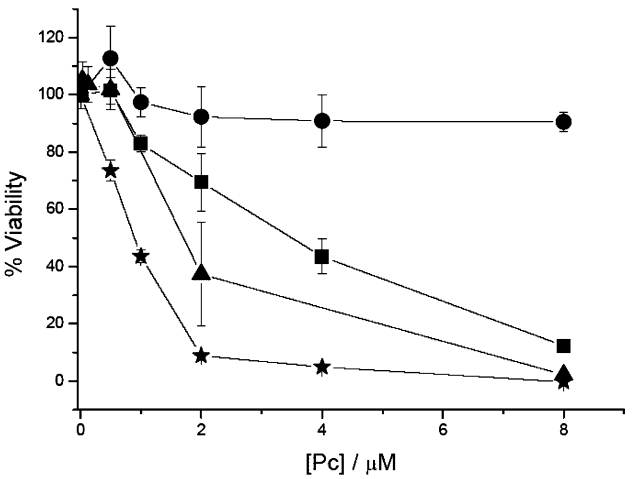


Fig. 2 Effects of $ZnPc(\alpha\text{-PGal})_4$ (**9**) (■), $ZnPc(\beta\text{-PGal})_4$ (**14**) (●), ZnPc(α-PGal) (**17**) (▲) and ZnPc(β-PGal) (**19**) (★) on HT29 cells in the presence of light (λ >610 nm, 40 mW cm^{-2}, 48 J cm^{-2}). Data are expressed as mean values ± S. E. M. of three independent experiments, each performed in quadruplicate.

To account for the different photodynamic activities of these compounds, their aggregation behaviour in the culture media was examined by absorption and fluorescence spectroscopic methods. Fig. 3a shows the UV–vis spectra of the galactosylated phthalocyanines **9**, **14**, **17** and **19** in the DMEM medium (for HT29 cells). It can be seen that the Q bands of **9**, **17** and **19** remain sharp and intense, indicating that these compounds are not significantly aggregated in the medium. By contrast, for the tetra-β-substituted analogue $ZnPc(\beta\text{-PGal})_4$ (**14**), the Q band is

 This journal is © The Royal Society of Chemistry 2008

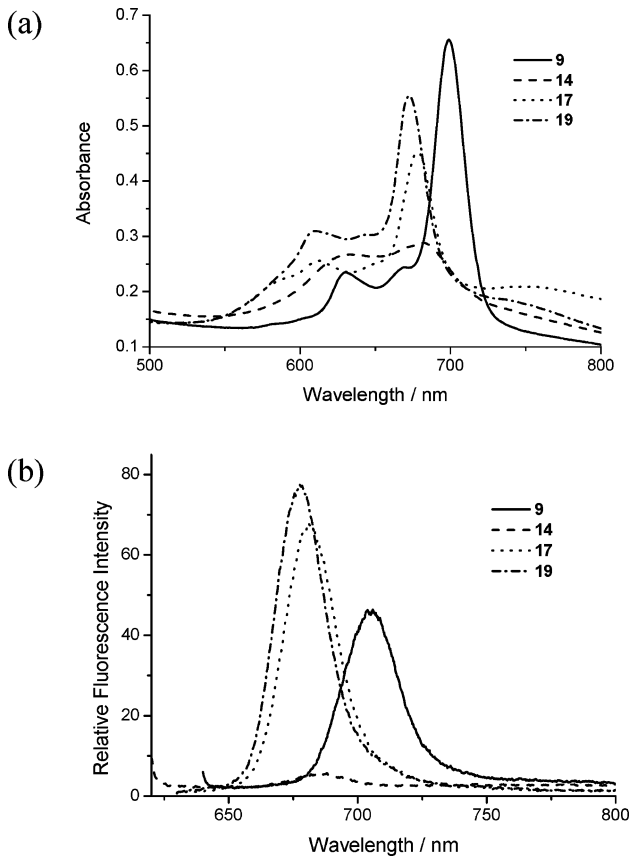


Fig. 3 (a) UV–vis and (b) fluorescence spectra of ZnPc(α-PGal)$_4$ (**9**), ZnPc(β-PGal)$_4$ (**14**), ZnPc(α-PGal) (**17**) and ZnPc(β-PGal) (**19**), formulated with Cremophor EL, in the DMEM medium. The concentrations of the phthalocyanines were fixed at 4 μM.

much weaker and significantly broadened. This indicates that this compound is highly aggregated in the medium, which can explain that even though it has a reasonably high singlet oxygen quantum yield in DMF (Table 1), it shows no photocytotoxicity. The higher aggregation tendency of **14** compared with the other three compounds was in accord with its much weaker fluorescence in the culture medium (Fig. 3b). Similar results were obtained for the glucosylated counterparts and in the RPMI medium (for HepG2 cells).

In addition to the cell viability studies, we also employed fluorescence microscopy to investigate the uptake of the galactosylated phthalocyanines **9**, **14**, **17** and **19** by HT29 cells. After incubation with these compounds (formulated with Cremophor EL) for 2 h, and upon excitation at 630 nm, fluorescence images of the HT29 cells were captured. It was found that for the tetra-β-substituted analogue ZnPc(β-PGal)$_4$ (**14**), no fluorescence was observed. This indicated that the cellular uptake is negligible and/or the compound is highly aggregated within the cells, both of which disfavour photodynamic action. Hence, this compound is not photocytotoxic. By contrast, the mono-substituted analogues **17** and **19** gave strong intracellular fluorescence throughout the cytoplasm. The intensity was stronger than that caused by the tetra-α-substituted analogue ZnPc(α-PGal)$_4$ (**9**) as shown in Fig. 4. These results suggested that the mono-substituted analogues **17** and **19** have a better cellular uptake, probably due to their amphiphilic character,[15] and lower aggregation tendency within

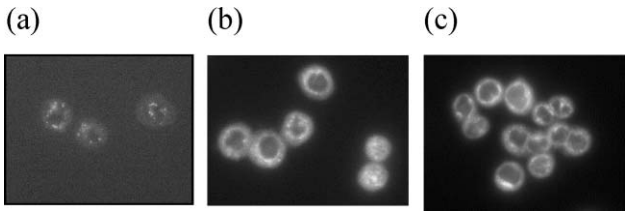


Fig. 4 Fluorescence microscopic images of HT29 cells after being incubated with (a) ZnPc(α-PGal)$_4$ (**9**), (b) ZnPc(α-PGal) (**17**) and (c) ZnPc(β-PGal) (**19**) (all at 8 μM) for 2 h.

the cells. The results can account for the trend in photocytotoxicity: **17** ≈ **19** > **9** > **14**. A higher potency has also been observed for unsymmetrical tetrapyrrolic photosensitisers having three saccharide units compared with the symmetrical analogues with four saccharide substituents.[7d,8a]

The intracellular production of ROS by these compounds in HT29 cells was also studied using 2′,7′-dichlorodihydrofluorescein diacetate (DCFDA) as the quencher. As shown in Fig. 5, all the compounds cannot generate ROS in the absence of light. Upon illumination, the efficiency at generating ROS follows the order: **19** > **17** > **9** > **14**, which is in accord with the trend in photocytotoxicity.

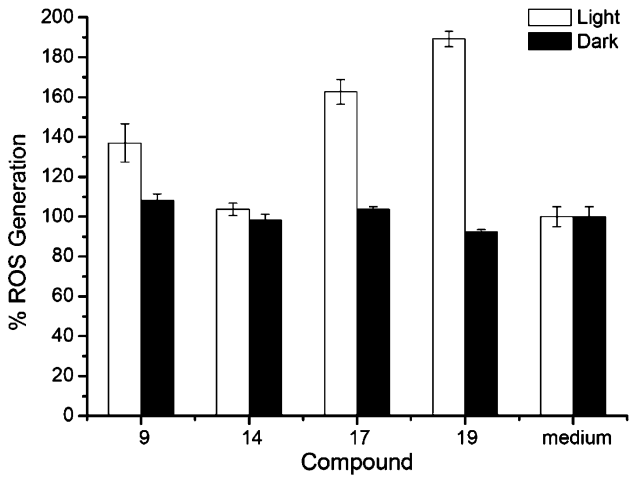


Fig. 5 ROS production induced by ZnPc(α-PGal)$_4$ (**9**), ZnPc(β-PGal)$_4$ (**14**), ZnPc(α-PGal) (**17**) and ZnPc(β-PGal) (**19**) in HT29 cells (all at 8 μM). Each data point represents the mean value ± S. E. M. of three independent experiments, each performed in quadruplicate.

Conclusions

In summary, we have prepared and characterised a new series of glycosylated zinc(II) phthalocyanines. Compared with the tetra-substituted analogues, the mono-glycosylated phthalocyanines **16–19** exhibit a higher cellular uptake, lower aggregation tendency and higher efficiency at generating intracellular ROS, leading to higher *in vitro* photocytotoxicity. Their IC_{50} values against HepG2 and HT29 cells are as low as 0.9 μM. For the tetra-substituted series, the position of the substituents (α or β) also exerts a great effect on their photodynamic activity. Although the tetra-β-glycosylated phthalocyanines **13** and **14** are efficient singlet oxygen

generators in DMF, their high aggregation tendency in the culture media means they have no photodynamic activity.

Experimental

Experimental details regarding the purification of solvents, instrumentation and *in vitro* studies are described elsewhere.[12b] Singlet oxygen quantum yields (Φ_Δ) were measured in DMF by the method of chemical quenching of DPBF using ZnPc as a reference ($\Phi_{\Delta(\mathrm{ref})} = 0.56$).[20] A mixture of DPBF (0.24 mM, 1 mL) and the photosensitiser (the absorbance at the excitation position was adjusted to *ca.* 0.1, 1 mL) in DMF was illuminated with red light coming from a 200 W halogen lamp after passing through a water tank for cooling and a colour glass filter (Newport, cut-on at 610 nm). The decay of the DPBF, as shown by the decrease in absorbance at 411 nm, was monitored along with time. The singlet oxygen quantum yield of the photosensitiser was estimated using the relationship: $\Phi_{\Delta(\mathrm{sample})} \approx \Phi_{\Delta(\mathrm{ref})}(W_{\mathrm{sample}}/W_{\mathrm{ref}})$, where W represents the photobleaching rate of DPBF, taken as the slope of the central linear region of the decay curve.[17]

General procedure for the preparation of glycosylated phthalonitriles 4, 5, 11 and 12

A mixture of 3- or 4-nitrophthalonitrile (1 equiv.), protected monosaccharide **2** or **3** (1.5 equiv.) and K_2CO_3 (3 equiv.) in DMF was stirred at 60 °C for 2 days (for **2**) or room temperature for 4 days (for **3**). The volatiles were evaporated *in vacuo*, then the residue was dissolved in chloroform (250 mL). The solution was washed with water (125 mL × 2), then dried over anhydrous $MgSO_4$. After evaporation under reduced pressure, the residue was purified by silica-gel chromatography using ethyl acetate–*n*-hexane (v/v 1 : 2) as the eluent. The crude product was further purified by recrystallisation from chloroform layered with *n*-hexane.

3-(1,2:5,6-Di-*O*-isopropylidene-α-D-glucofuranosyl)phthalonitrile (4)

According to the general procedure, 3-nitrophthalonitrile (**1**) (1.08 g, 6.2 mmol) was treated with protected glucose **2** (2.42 g, 9.3 mmol) in DMF (10 mL) to give **4** as a white crystalline solid (1.57 g, 65%). ^{1}H NMR: δ 7.67–7.72 (m, 1 H, ArH), 7.42–7.46 (m, 2 H, ArH), 6.01 (d, J = 3.9 Hz, 1 H, H1), 4.82 (d, J = 3.0 Hz, 1 H, H3), 4.59 (d, J = 3.9 Hz, 1 H, H2), 4.49 (ddd, J = 4.8, 6.0, 8.7 Hz, 1 H, H5), 4.26 (dd, J = 3.0, 8.7 Hz, 1 H, H4), 4.19 (dd, J = 6.0, 8.7 Hz, 1 H, H6), 4.07 (dd, J = 4.8, 8.7 Hz, 1 H, H6), 1.55 (s, 3 H, Me), 1.41 (s, 3 H, Me), 1.32 (s, 3 H, Me), 1.30 (s, 3 H, Me). ^{13}C{^{1}H} NMR: δ 159.7, 134.6, 126.2, 118.1, 117.2, 115.0, 112.6 (two overlapping signals), 109.5, 106.1, 105.3, 82.4, 82.2, 80.4, 71.7, 67.5, 26.9, 26.6, 26.2, 25.0. MS (FAB): m/z 387 [20%, (M + H)$^+$]. HRMS (FAB): m/z calc. for $C_{20}H_{23}N_2O_6$ (M + H)$^+$, 387.1551; found, 387.1551. Anal. calcd for $C_{20}H_{22}N_2O_6$: C 62.17, H 5.74, N 7.25; found: C 62.13, H 5.63, N 7.09.

3-(1,2:3,4-Di-*O*-isopropylidene-α-D-galactopyranosyl)phthalonitrile (5)

According to the general procedure, 3-nitrophthalonitrile (**1**) (2.30 g, 13.3 mmol) was treated with protected galactose **3** (5.10 g, 19.6 mmol) in DMF (20 mL) to give **5** as a white crystalline solid (3.39 g, 66%). ^{1}H NMR: δ 7.61–7.66 (m, 1 H, ArH), 7.30–7.37 (m, 2 H, ArH), 5.53 (d, J = 4.8 Hz, 1 H, H1), 4.68 (dd, J = 2.4, 7.8 Hz, 1 H, H3), 4.43 (dd, J = 1.2, 7.8 Hz, 1 H, H4), 4.35 (dd, J = 2.4, 4.8 Hz, 1 H, H2), 4.23–4.32 (m, 3 H, H5 and H6), 1.55 (s, 3 H, Me), 1.44 (s, 3 H, Me), 1.35 (s, 3 H, Me), 1.34 (s, 3 H, Me). ^{13}C{^{1}H} NMR: δ 161.1, 134.5, 125.4, 117.3, 116.9, 115.3, 112.8, 109.5, 109.1, 105.3, 96.2, 70.6 (3 overlapping signals), 68.3, 66.1, 26.1, 25.9, 24.9, 24.3. MS (ESI): m/z 409 [100%, (M + Na)$^+$]. HRMS (ESI): m/z calc. for $C_{20}H_{22}N_2NaO_6$ (M + Na)$^+$, 409.1370; found, 409.1374. Anal. calcd for $C_{20}H_{22}N_2O_6$: C 62.17, H 5.74, N 7.25; found: C 62.10, H 5.73, N 6.93.

4-(1,2:5,6-Di-*O*-isopropylidene-α-D-glucofuranosyl)phthalonitrile (11)

According to the general procedure, 4-nitrophthalonitrile (**10**) (3.14 g, 18.1 mmol) was treated with protected glucose **2** (6.39 g, 24.5 mmol) in DMF (20 mL) to give **11** as a white crystalline solid (5.14 g, 73%). ^{1}H NMR: δ 7.75 (d, J = 8.7 Hz, 1 H, ArH), 7.41 (d, J = 2.7 Hz, 1 H, ArH), 7.31 (dd, J = 2.7, 8.7 Hz, ArH), 5.95 (d, J = 3.9 Hz, 1 H, H1), 4.79 (d, J = 3.0 Hz, 1 H, H3), 4.54 (d, J = 3.9 Hz, 1 H, H2), 4.28–4.31 (m, 1 H, H5), 4.24 (dd, J = 3.0, 8.7 Hz, H4), 4.15 (dd, J = 5.7, 8.7 Hz, 1 H, H6), 4.06 (dd, J = 4.5, 8.7 Hz, 1 H, H6), 1.56 (s, 3 H, Me), 1.42 (s, 3 H, Me), 1.33 (s, 3 H, Me), 1.28 (s, 3 H, Me). ^{13}C{^{1}H} NMR: δ 159.8, 134.8, 126.2, 118.1, 117.2, 115.0, 112.6, 105.9, 105.2, 82.7, 82.1, 79.0, 68.2, 64.0, 26.6, 26.2 (some of the signals are overlapped). MS (FAB): m/z 387 [15%, (M + H)$^+$]. HRMS (FAB): m/z calc. for $C_{20}H_{23}N_2O_6$ (M + H)$^+$, 387.1551; found, 387.1562.

4-(1,2:3,4-Di-*O*-isopropylidene-α-D-galactopyranosyl)phthalonitrile (12)

According to the general procedure, 4-nitrophthalonitrile (**10**) (2.56 g, 14.8 mmol) was treated with protected galactose **3** (5.39 g, 20.7 mmol) in DMF (20 mL) to give **12** as a white crystalline solid (3.32 g, 58%). ^{1}H NMR: δ 7.70 (d, J = 8.7 Hz, 1 H, ArH), 7.33 (d, J = 2.4 Hz, 1 H, ArH), 7.25 (dd, J = 2.4, 8.7 Hz, 1 H, ArH), 5.56 (d, J = 5.1 Hz, 1 H, H1), 4.67 (dd, J = 2.4, 7.8 Hz, 1 H, H3), 4.37 (dd, J = 2.4, 5.1 Hz, 1 H, H2), 4.32 (dd, J = 1.2, 7.8 Hz, 1 H, H4), 4.18–4.25 (m, 3 H, H5 and H6), 1.54 (s, 3 H, Me), 1.47 (s, 3 H, Me), 1.35 (s, 6 H, Me). ^{13}C{^{1}H} NMR: δ 161.8, 135.1, 119.9, 119.5, 117.4, 115.6, 115.2, 109.8, 108.9, 107.5, 96.3, 70.8, 70.6, 70.3, 68.1, 66.2, 26.0, 25.9, 24.8, 24.4. MS (ESI): m/z 409 [100%, (M + Na)$^+$]. HRMS (ESI): m/z calc. for $C_{20}H_{22}N_2NaO_6$ (M + Na)$^+$, 409.1370; found, 409.1374. Anal. calcd for $C_{20}H_{22}N_2O_6$: C 62.17, H 5.74, N 7.25; found: C 62.13, H 5.68, N 7.42.

1(4),8(11),15(18),22(25)-Tetrakis(1,2:5,6-di-*O*-isopropylidene-α-D-glucofuranosyl)phthalocyanine (6)

A mixture of phthalonitrile **4** (1.33 g, 3.4 mmol) and $CeCl_3$ (0.2 equiv.) in *n*-pentanol (6 mL) was heated to 100 °C, then 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU) (0.8 mL) was added. The mixture was heated further, to 150 °C, and kept at this temperature overnight with stirring. The volatiles were evaporated *in vacuo*, then the residue was purified by silica-gel chromatography using ethyl acetate–*n*-hexane (changing gradually from v/v 1 : 2 to 1 : 1) as the eluent to give **6** as a green solid (0.62 g, 47%). ^{1}H NMR: δ 9.09–9.27 (m, 4 H, Pc-H$_\alpha$), 8.14–8.22 (m, 4 H, Pc-H$_\beta$), 7.81–7.94

This journal is © The Royal Society of Chemistry 2008

(m, 4 H, Pc-H_β), 6.50–6.64 (m, 4 H, H1), 4.22–5.88 (m, 24 H, CH), 1.39–1.72 (m, 48 H, Me). MS (ESI): an isotopic cluster peaking at *m/z* 1547 [100%, $(M + H)^+$]. HRMS (ESI): *m/z* calc. for $C_{80}H_{91}N_8O_{24}$ $(M + H)^+$, 1547.6141; found, 1547.6151. Anal. calcd for $C_{80}H_{90}N_8O_{24}$: C 62.09, H 5.86, N 7.24; found: C 62.36, H 6.00, N 6.84.

1(4),8(11),15(18),22(25)-Tetrakis(1,2:3,4-di-*O*-isopropylidene-α-D-galactopyranosyl)phthalocyanine (7)

According to the above procedure, cyclisation of phthalonitrile **5** (1.28 g, 3.3 mmol) gave **7** as a green solid (0.74 g, 58%). ^{1}H NMR: δ 8.95–9.18 (m, 4 H, Pc-H_α), 8.06–8.16 (m, 4 H, Pc-H_β), 7.62–7.94 (m, 4 H, Pc-H_β), 4.30–5.85 (m, 28 H, CH), 1.21–1.59 (m, 48 H, Me). MS (ESI): an isotopic cluster peaking at *m/z* 1547 [100%, $(M + H)^+$]. HRMS (ESI): *m/z* calc. for $C_{80}H_{91}N_8O_{24}$ $(M + H)^+$, 1547.6141; found, 1547.6132. Anal. calcd for $C_{80}H_{90}N_8O_{24}$: C 62.09, H 5.86, N 7.24; found: C 62.46, H 5.98, N 6.93.

[1(4),8(11),15(18),22(25)-Tetrakis(1,2:5,6-di-*O*-isopropylidene-α-D-glucofuranosyl)phthalocyaninato]zinc(II) (8)

A solution of the metal-free phthalocyanine **6** (50 mg, 32 μmol) and $Zn(OAc)_2{\cdot}2H_2O$ (2 equiv.) in DMF (5 mL) was stirred at 150 °C overnight. The solvent was evaporated *in vacuo*, then the residue was purified by silica-gel chromatography using ethyl acetate–*n*-hexane (changing gradually from v/v 1 : 2 to 2 : 1) as the eluent to give **8** as a green solid (39 mg, 75%). ^{1}H NMR: δ 9.16–9.25 (m, 4 H, Pc-H_α), 8.06–8.14 (m, 4 H, Pc-H_β), 7.76–7.87 (m, 4 H, Pc-H_β), 6.48–6.62 (m, 4 H, H1), 4.09–6.01 (m, 24 H, CH), 1.24–1.68 (m, 48 H, Me). MS (ESI): an isotopic cluster peaking at *m/z* 1609 [100%, $(M + H)^+$]. HRMS (ESI): *m/z* calc. for $C_{80}H_{89}N_8O_{24}Zn$ $(M + H)^+$, 1609.5276; found, 1609.5271.

[1(4),8(11),15(18),22(25)-Tetrakis(1,2:3,4-di-*O*-isopropylidene-α-D-galactopyranosyl)phthalocyaninato]zinc(II) (9)

According to the above procedure, metallation of the metal-free phthalocyanine **7** (50 mg, 32 μmol) with $Zn(OAc)_2{\cdot}2H_2O$ (2 equiv.) gave **9** as a green solid (41 mg, 79%). ^{1}H NMR: δ 9.13–9.21 (m, 4 H, Pc-H_α), 8.05–8.12 (m, 4 H, Pc-H_β), 7.65–7.88 (m, 4 H, Pc-H_β), 4.24–5.83 (m, 28 H, CH), 1.21–1.53 (m, 48 H, Me). MS (ESI): an isotopic cluster peaking at *m/z* 1609 [100%, $(M + H)^+$]. HRMS (ESI): *m/z* calc. for $C_{80}H_{89}N_8O_{24}Zn$ $(M + H)^+$, 1609.5276; found, 1609.5264. Anal. calcd for $C_{80}H_{88}N_8O_{24}Zn$: C 59.65, H 5.51, N 6.96; found: C 59.87, H 5.73, N 6.52.

[2(3),9(10),16(17),23(24)-Tetrakis(1,2:5,6-di-*O*-isopropylidene-α-D-glucofuranosyl)phthalocyaninato]zinc(II) (13)[11a]

A mixture of phthalonitrile **11** (0.61 g, 1.6 mmol) and $Zn(OAc)_2{\cdot}2H_2O$ (2 equiv.) in *n*-pentanol (5 mL) was heated to 100 °C, then DBU (0.8 mL) was added. The mixture was heated further, to 150 °C, and kept stirring at this temperature overnight. The volatiles were then evaporated *in vacuo* and the residue was purified by silica-gel chromatography using ethyl acetate–*n*-hexane (changing gradually from v/v 1 : 3 to 1 : 1) as the eluent. The product **13** was isolated as a blue solid (0.37 g, 58%). ^{1}H NMR: δ 9.35–9.45 (m, 4 H, Pc-H_α), 9.06–9.10 (m, 4 H, Pc-H_α), 7.76–7.83 (m, 4 H, Pc-H_β), 6.13–6.16 (m, 4 H, H1), 5.39–5.42 (m, 4 H, H3), 4.97–5.03 (m, 4 H, H2), 4.72–4.78 (m, 4 H, H5), 4.56–4.60 (m, 4 H, H4), 4.27–4.35 (m, 8 H, H6), 1.35–1.70 (m, 48 H, Me). MS (ESI): an isotopic cluster peaking at *m/z* 1609 [100%, $(M + H)^+$]. HRMS (ESI): *m/z* calc. for $C_{80}H_{89}N_8O_{24}Zn$ $(M + H)^+$, 1609.5276; found, 1609.5273.

[2(3),9(10),16(17),23(24)-Tetrakis(1,2:3,4-di-*O*-isopropylidene-α-D-galactopyranosyl)phthalocyaninato]zinc(II) (14)

According to the above procedure, cyclisation of phthalonitrile **12** (1.34 g, 3.5 mmol) gave **14** as a blue solid (1.08 g, 77%). ^{1}H NMR: δ 9.30–9.39 (m, 4 H, Pc-H_α), 8.98–9.02 (m, 4 H, Pc-H_α), 7.77–7.80 (m, 4 H, Pc-H_β), 5.72–5.74 (m, 4 H, H1), 4.62–4.81 (m, 16 H, CH), 4.43–4.54 (m, 8 H, CH), 1.41–1.83 (m, 48 H, Me). MS (ESI): an isotopic cluster peaking at *m/z* 1609 [100%, $(M + H)^+$]. HRMS (ESI): *m/z* calc. for $C_{80}H_{89}N_8O_{24}Zn$ $(M + H)^+$, 1609.5276; found, 1609.5272. Anal. calcd for $C_{80}H_{88}N_8O_{24}Zn$: C 59.65, H 5.51, N 6.96; found: C 59.62, H 5.48, N 7.05.

General procedure for the preparation of mono-glycosylated zinc(II) phthalocyanines 16–19

Lithium (0.2 g, 29 mmol) was first dissolved in *n*-pentanol (20 mL) at 100 °C, then a solution of glycosylated phthalonitrile **4**, **5**, **11**, or **12** (1 equiv.) and unsubstituted phthalonitrile (**15**) (9 equiv.) in a minimum amount of tetrahydrofuran (THF) (*ca.* 10 mL) was added. The resulting mixture was stirred at 120 °C for 5 h, then $Zn(OAc)_2{\cdot}2H_2O$ (3 equiv.) was added. The resulting mixture was stirred at this temperature overnight, then the volatiles were removed under reduced pressure. The residue was redissolved in THF and the suspension was filtered to remove some of the unsubstituted ZnPc formed. The filtrate was adsorbed onto silica gel which was then loaded onto a silica-gel column and eluted with THF–*n*-hexane (changing gradually from v/v 1 : 2 to 1 : 1). The crude product obtained was further purified by size exclusion chromatography using THF as the eluent. A green band was first developed, which was followed by a blue band containing the desired A_3B-type product. The product was further purified by recrystallisation from THF layered with *n*-hexane.

[1-(1,2:5,6-Di-*O*-isopropylidene-α-D-glucofuranosyl)phthalocyaninato]zinc(II) (16)

According to the general procedure, phthalonitrile **4** (0.16 g, 0.4 mmol) reacted with **15** to give **16** as a blue solid (0.09 g, 26%). ^{1}H NMR: δ 9.39–9.49 (m, 6 H, Pc-H_α), 9.20 (d, $J = 7.5$ Hz, 1 H, Pc-H_α), 8.11–8.20 (m, 7 H, Pc-H_β), 7.79 (d, $J = 8.1$ Hz, 1 H, Pc-H_β), 6.52 (d, $J = 3.6$ Hz, 1 H, H1), 5.91–5.99 (m, 1 H, H5), 5.57 (d, $J = 2.4$ Hz, 1 H, H3), 5.19 (d, $J = 3.6$ Hz, 1 H, H2), 4.69–4.73 (m, 1 H, H4), 4.46–4.58 (m, 2 H, H6), 1.69 (s, 3 H, Me), 1.41 (s, 3 H, Me), 1.35 (s, 3 H, Me), 0.90 (s, 3 H, Me). $^{13}C\{^1H\}$ NMR: δ 154.2, 153.5, 153.4, 153.2, 152.8, 141.1, 139.0, 138.4, 138.1, 130.2 (Pc-C_β), 129.2 (Pc-C_β), 128.8 (Pc-C_β), 122.4 (Pc-C_α), 122.3 (Pc-C_α), 116.5 (Pc-C_α), 113.7 (Pc-C_β), 112.3, 109.2, 106.0 (C1), 82.9 (C2), 81.6 (C3 or C4), 81.4 (C3 or C4), 72.5 (C5), 67.7 (C6), 26.9 (two overlapping signals, Me), 26.3 (Me), 25.0 (Me) (some of the aromatic signals are overlapped). MS (ESI): an isotopic cluster peaking at *m/z* 835 [100%, $(M + H)^+$]. HRMS (ESI): *m/z* calc. for $C_{44}H_{35}N_8O_6Zn$ $(M + H)^+$, 835.1966; found, 835.1960. Anal. calcd

for $C_{44}H_{34}N_8O_6Zn$: C 63.20, H 4.10, N 13.40; found: C 62.69, H 4.72, N 13.48.

[1-(1,2:3,4-Di-*O*-isopropylidene-α-D-galactopyranosyl)phthalocyaninato]zinc(II) (17)

According to the general procedure, phthalonitrile **5** (0.35 g, 0.9 mmol) was treated with **15** to give **17** as a blue solid (0.19 g, 25%). ^{1}H NMR: δ 9.42–9.50 (m, 1 H, Pc-H$_\alpha$), 9.25–9.34 (m, 5 H, Pc-H$_\alpha$), 9.09 (d, J = 7.5 Hz, 1 H, Pc-H$_\alpha$), 8.02–8.18 (m, 7 H, Pc-H$_\beta$), 7.66 (d, J = 7.8 Hz, 1 H, Pc-H$_\beta$), 5.86 (d, J = 4.8 Hz, 1 H, H1), 5.40 (dd, J = 1.2, 7.8 Hz, 1 H, H4), 4.90–5.11 (m, 4 H, H3, H5 and H6), 4.61 (dd, J = 2.4, 4.8 Hz, 1 H, H2), 1.57 (s, 3 H, Me), 1.55 (s, 3 H, Me), 1.41 (s, 3 H, Me), 1.28 (s, 3 H, Me). ^{13}C{^{1}H} NMR: δ 155.5, 152.9, 152.8, 140.7, 138.9, 138.3, 138.0, 137.8, 130.2 (Pc-C$_\beta$), 128.9 (Pc-C$_\beta$), 128.7 (Pc-C$_\beta$), 128.6 (Pc-C$_\beta$), 122.7 (Pc-C$_\alpha$), 122.5 (Pc-C$_\alpha$), 122.1 (Pc-C$_\alpha$), 122.0 (Pc-C$_\alpha$), 121.9 (Pc-C$_\alpha$), 115.8 (Pc-C$_\alpha$), 112.9 (Pc-C$_\beta$), 109.4, 109.0, 96.8 (C1), 71.4 (C2, C3 or C4), 71.0 (C2, C3 or C4), 70.9 (C2, C3 or C4), 67.3 (C5 or C6), 66.8 (C5 or C6), 26.4 (Me), 26.1 (Me), 25.1 (Me), 24.6 (Me) (some of the aromatic signals are overlapped). MS (ESI): an isotopic cluster peaking at m/z 835 [100%, (M + H)$^+$]. HRMS (ESI): m/z calc. for $C_{44}H_{35}N_8O_6Zn$ (M + H)$^+$, 835.1966; found, 835.1968. Anal. calcd for $C_{44}H_{34}N_8O_6Zn$: C 63.20, H 4.10, N 13.40; found: C 63.23, H 4.92, N 13.93.

[2-(1,2:5,6-Di-*O*-isopropylidene-α-D-glucofuranosyl)phthalocyaninato]zinc(II) (18)

According to the general procedure, phthalonitrile **11** (0.15 g, 0.4 mmol) was treated with **15** to give **18** as a blue solid (0.14 g, 43%). ^{1}H NMR: δ 9.06–9.30 (m, 7 H, Pc-H$_\alpha$), 8.85 (br s, 1 H, Pc-H$_\alpha$), 7.93–8.11 (m, 6 H, Pc-H$_\beta$), 7.67 (d, J = 7.5 Hz, 1 H, Pc-H$_\beta$), 6.17 (d, J = 3.6 Hz, 1 H, H1), 5.42 (d, J = 2.4 Hz, 1 H, H3), 5.04 (d, J = 3.6 Hz, 1 H, H2), 4.80–4.86 (m, 1 H, H5), 4.63 (dd, J = 2.4, 7.5 Hz, 1 H, H4), 4.29–4.39 (m, 2 H, H6), 1.72 (s, 3 H, Me), 1.58 (s, 3 H, Me), 1.42 (s, 3 H, Me), 1.41 (s, 3 H, Me). ^{13}C{^{1}H} NMR: δ 158.3, 152.8 (br s), 140.0, 137.9, 132.3, 128.6 (Pc-C$_\beta$), 123.6 (Pc-C$_\alpha$), 122.2 (Pc-C$_\alpha$), 118.1 (Pc-C$_\beta$), 112.4, 109.3, 107.3 (Pc-C$_\alpha$), 105.5 (C1), 82.6 (C2), 80.9 (C4), 80.5 (C3), 72.5 (C5), 67.3 (C6), 27.0 (two overlapping signals, Me), 26.4 (Me), 25.4 (Me) (some of the aromatic signals are overlapped). MS (ESI): an isotopic cluster peaking at m/z 835 [100%, (M + H)$^+$]. HRMS (ESI): m/z calc. for $C_{44}H_{35}N_8O_6Zn$ (M + H)$^+$, 835.1966; found, 835.1973. Anal. calcd for $C_{44}H_{34}N_8O_6Zn$: C 63.20, H 4.10, N 13.40; found: C 63.05, H 4.45, N 14.26.

[2-(1,2:3,4-Di-*O*-isopropylidene-α-D-galactopyranosyl)phthalocyaninato]zinc(II) (19)

According to the general procedure, phthalonitrile **12** (1.06 g, 2.7 mmol) was treated with **15** to give **19** as a blue solid (0.91 g, 40%). ^{1}H NMR: δ 8.95–9.10 (m, 6 H, Pc-H$_\alpha$), 8.79 (d, J = 8.1 Hz, 1 H, Pc-H$_\alpha$), 8.43 (d, J = 2.1 Hz, 1 H, Pc-H$_\alpha$), 7.93–8.00 (m, 6 H, Pc-H$_\beta$), 7.49 (dd, J = 2.1, 8.1 Hz, 1 H, Pc-H$_\beta$), 5.80 (d, J = 5.1 Hz, H1), 4.86 (dd, J = 2.1, 7.8 Hz, 1 H, H3), 4.66–4.73 (m, 3 H, H4 and H6), 4.57–4.63 (m, 1 H, H5), 4.51 (dd, J = 2.1, 5.1 Hz, 1 H, H2), 1.76 (s, 3 H, Me), 1.67 (s, 3 H, Me), 1.53 (s, 3 H, Me), 1.46 (s, 3 H, Me). ^{13}C{^{1}H} NMR: δ 160.1, 153.1, 152.9, 152.4, 140.1, 138.1, 131.5, 128.6 (Pc-C$_\beta$), 123.2 (Pc-C$_\alpha$), 122.2 (Pc-C$_\alpha$), 118.1 (Pc-C$_\beta$), 109.6, 108.9, 105.6 (Pc-C$_\alpha$), 96.6 (C1), 71.2 (C4 or C6), 70.8 (two overlapping signals, C2 and C3), 67.2 (C4 or C6), 66.6 (C5), 26.3 (Me), 26.2 (Me), 25.0 (Me), 24.6 (Me) (some of the aromatic signals are overlapped). MS (ESI): an isotopic cluster peaking at m/z 835 [100%, (M + H)$^+$]. HRMS (ESI): m/z calc. for $C_{44}H_{35}N_8O_6Zn$ (M + H)$^+$, 835.1966; found, 835.1959. Anal. calcd for $C_{44}H_{34}N_8O_6Zn$: C 63.20, H 4.10, N 13.40; found: C 63.45, H 4.85, N 12.66.

Acknowledgements

We thank Prof. Wing-Hung Ko for technical support in the fluorescence imaging study. This work was supported by a grant from the Research Grants Council of the Hong Kong Special Administrative Region, China (Project No. 402505) and a strategic investments scheme administered by The Chinese University of Hong Kong.

References

1 (*a*) N. B. McKeown, *Phthalocyanine Materials: Synthesis, Structure and Function*, Cambridge University Press, Cambridge, UK, 1998; (*b*) *The Porphyrin Handbook*, ed. K. M. Kadish, K. M. Smith, and R. Guilard, Academic Press, San Diego, 2003, vol. 19; (*c*) Y. Chen, M. Hanack, Y. Araki and O. Ito, *Chem. Soc. Rev.*, 2005, **34**, 517; (*d*) G. de la Torre, C. G. Claessens and T. Torres, *Chem. Commun.*, 2007, 2000; (*e*) Y. Chen, X. Zhuang, W. Zhang, Y. Liu, Y. Lin, A. Yan, Y. Araki and O. Ito, *Chem. Mater.*, 2007, **19**, 5256; (*f*) Y. Chen, Y. Lin, M. E. El-Khouly, X. Zhuang, Y. Araki, O. Ito and W. Zhang, *J. Phys. Chem. C*, 2007, **111**, 16096.

2 (*a*) H. Ali and J. E. van Lier, *Chem. Rev.*, 1999, **99**, 2379; (*b*) E. A. Lukyanets, *J. Porphyrins Phthalocyanines*, 1999, **3**, 424; (*c*) C. M. Allen, W. M. Sharman and J. E. van Lier, *J. Porphyrins Phthalocyanines*, 2001, **5**, 161; (*d*) A. C. Tedesco, J. C. G. Rotta and C. N. Lunardi, *Curr. Org. Chem.*, 2003, **7**, 187.

3 N. Nishiyama, W.-D. Jang and K. Kataoka, *New J. Chem.*, 2007, **31**, 1074.

4 (*a*) W. M. Sharman, J. E. van Lier and C. M. Allen, *Adv. Drug Deliv. Rev.*, 2004, **56**, 53; (*b*) N. Solban, I. Rizvi and T. Hasan, *Lasers Surg. Med.*, 2006, **38**, 522; (*c*) S. Verma, G. M. Watt, Z. Mai and T. Hasan, *Photochem. Photobiol. Sci.*, 2007, **83**, 996; (*d*) J.-P. Taquet, C. Frochot, V. Manneville and M. Barberi-Heyob, *Curr. Med. Chem.*, 2007, **14**, 1673.

5 O. Warburg, *Science*, 1956, **123**, 309.

6 R. E. Airley and A. Mobasheri, *Chemotherapy (Basel, Switz.)*, 2007, **53**, 233.

7 (*a*) A. A. Aksenova, Yu. L. Sebyakin and A. F. Mironov, *Russ. J. Bioorg. Chem.*, 2003, **29**, 210; (*b*) X. Chen and C. M. Drain, *Drug Design Rev.–Online*, 2004, **1**, 215; (*c*) X. Chen, L. Hui, D. A. Foster and C. M. Drain, *Biochemistry*, 2004, **43**, 10918; (*d*) I. Laville, S. Pigaglio, J.-C. Blais, F. Doz, B. Loock, P. Maillard, D. S. Grierson and J. Blais, *J. Med. Chem.*, 2006, **49**, 2558; (*e*) M. Obata, S. Hirohara, K. Sharyo, H. Alitomo, K. Kajiwara, S.-i. Ogata, M. Tanihara, C. Ohtsuki and S. Yano, *Biochim. Biophys. Acta*, 2007, **1770**, 1204.

8 (*a*) I. Laville, T. Figueiredo, B. Loock, S. Pigaglio, Ph. Maillard, D. S. Grierson, D. Carrez, A. Croisy and J. Blais, *Bioorg. Med. Chem.*, 2003, **11**, 1643; (*b*) S. Hirohara, M. Obata, S.-i. Ogata, K. Kajiwara, C. Ohtsuki, M. Tanihara and S. Yano, *J. Photochem. Photobiol., B*, 2006, **84**, 56; (*c*) S. K. Pandey, X. Zheng, J. Morgan, J. R. Missert, T.-H. Liu, M. Shibata, D. A. Bellnier, A. R. Oseroff, B. W. Henderson, T. J. Dougherty and R. K. Pandey, *Mol. Pharm.*, 2007, **4**, 448.

9 M. Zhang, Z. Zhang, D. Blessington, H. Li, T. M. Busch, V. Madrak, J. Miles, B. Chance, J. D. Glickson and G. Zheng, *Bioconjugate Chem.*, 2003, **14**, 709.

10 (*a*) Y.-Y. He, J.-Y. An and L.-J. Jiang, *Biochim. Biophys. Acta*, 1999, **1472**, 232; (*b*) L.-J. Jiang and Y.-Y. He, *Chin. Sci. Bull.*, 2001, **46**, 6.

11 (*a*) P. Maillard, J.-L. Guerquin-Kern, M. Momenteau and S. Gaspard, *J. Am. Chem. Soc.*, 1989, **111**, 9125; (*b*) X. Alvarez-Mico, M. J. F. Calvete, M. Hanack and T. Ziegler, *Tetrahedron Lett.*, 2006, **47**, 3283; (*c*) A. O. Ribeiro, J. P. C. Tomé, M. G. P. M. S. Neves, A. C. Tomé,

 This journal is © The Royal Society of Chemistry 2008

J. A. S. Cavaleiro, Y. Iamamoto and T. Torres, *Tetrahedron Lett.*, 2006, **47**, 9177; (*d*) X. Álvarez-Micó, M. J. F. Calvete, M. Hanack and T. Ziegler, *Carbohydr. Res.*, 2007, **342**, 440; (*e*) M. R. Reddy, N. Shibata, H. Yoshiyama, S. Nakamura and T. Toru, *Synlett*, 2007, 628; (*f*) X. Álvarez-Micó, M. J. F. Calvete, M. Hanack and T. Ziegler, *Synthesis*, 2007, 2186.

12 (*a*) P. P. S. Lee, P.-C. Lo, E. Y. M. Chan, W.-P. Fong, W.-H. Ko and D. K. P. Ng, *Tetrahedron Lett.*, 2005, **46**, 1551; (*b*) P.-C. Lo, C. M. H. Chan, J.-Y. Liu, W.-P. Fong and D. K. P. Ng, *J. Med. Chem.*, 2007, **50**, 2100; (*c*) P.-C. Lo, S. C. H. Leung, E. Y. M. Chan, W.-P. Fong, W.-H. Ko and D. K. P. Ng, *Photodiagn. Photodyn. Ther.*, 2007, **4**, 117; (*d*) P.-C. Lo, W.-P. Fong and D. K. P. Ng, *ChemMedChem*, 2008, DOI: 10.1002/cmdc.200800042.

13 C.-H. Lee and D. K. P. Ng, *Tetrahedron Lett.*, 2002, **43**, 4211.

14 W. Liu, C.-H. Lee, H.-S. Chan, T. C. W. Mak and D. K. P. Ng, *Eur. J. Inorg. Chem.*, 2004, 286.

15 I. J. MacDonald and T. J. Dougherty, *J. Porphyrins Phthalocyanines*, 2001, **5**, 105.

16 (*a*) N. Kobayashi, N. Sasaki, Y. Higashi and T. Osa, *Inorg. Chem.*, 1995, **34**, 1636; (*b*) N. Kobayashi, H. Ogata, N. Nonaka and E. A. Luk'yanets, *Chem.–Eur. J.*, 2003, **9**, 5123.

17 M. D. Maree, N. Kuznetsova and T. Nyokong, *J. Photochem. Photobiol., A*, 2001, **140**, 117.

18 K. Ishii, and N. Kobayahsi, in *The Porphyrin Handbook*, ed. K. M. Kadish, K. M. Smith, and R. Guilard, Academic Press, San Diego, 2003, vol. 16, pp. 1–42.

19 For a rough comparison, the IC_{50} value of tetrasulfonated zinc(II) phthalocyanine against SiHa human cervical carcinoma cells is 0.2 mM using a 665 nm diode laser with a light dose of 3 J cm^{-2} (S. L. Haywood-Small, D. I. Vernon, J. Griffiths, J. Schofield and S. B. Brown, *Biochem. Biophys. Res. Commun.*, 2006, **339**, 569). The values for tetrasulfonated aluminium(III) phthalocyanine against several squamous cell carcinoma range from 0.7 μM to >1 μM using a 675 nm dye laser with a light dose of 25 J cm^{-2} (M. B. Vrouenraets, G. W. M. Visser, M. Stigter, H. Oppelaar, G. B. Snow and G. A. M. S. van Dongen, *Int. J. Cancer*, 2002, **98**, 793). For Photosense® towards OAT-75 human small cell lung carcinoma, at a dye concentration of 3.75 μM, the light dose required to kill 50% of the cells is 93 J cm^{-2} (I. G. Meerovich, V. V. Jerdeva, V. M. Derkacheva, G. A. Meerovich, E. A. Lukyanets, E. A. Kogan and A. P. Savitsky, *J. Photochem. Photobiol., B*, 2005, **80**, 57).

20 W. Spiller, H. Kliesch, D. Wöhrle, S. Hackbarth, B. Röder and G. Schnurpfeil, *J. Porphyrins Phthalocyanines*, 1998, **2**, 145.