Highly photocytotoxic 1,4-dipegylated zinc(II) phthalocyanines. Effects of the chain length on the *in vitro* photodynamic activities†

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A new series of 1,4-dipegylated zinc(II) phthalocyanines have been synthesised and spectroscopically characterised. The derivatives ZnPc[O(CH_2CH_2O)$_n$Me]$_2$ (n = 2, 4, *ca.* 12) have been prepared by mixed cyclisation of the corresponding disubstituted phthalonitriles with an excess of unsubstituted phthalonitrile in the presence of Zn(OAc)$_2$·2H$_2$O and 1,8-diazabicyclo[5.4.0]undec-7-ene. The other two analogues ZnPc[O(CH_2CH_2O)$_n$Me]$_2$ (n = 6, 8) have been prepared by chain elongation reaction of ZnPc[O(CH_2CH_2O)$_4$H]$_2$. These macrocycles are highly soluble and remain non-aggregated in DMF as shown by the sharp Q-band absorption. Compared with the unsubstituted zinc(II) phthalocyanine, these di-α-substituted analogues have a red-shifted Q band (at 689 vs. 670 nm) and exhibit a relatively weaker fluorescence emission and a higher efficiency at generating singlet oxygen. Upon illumination, these compounds are highly cytotoxic toward HT29 human colorectal carcinoma and HepG2 human hepatocarcinoma cells. The compounds with medium-length substituents are particularly potent, having IC_{50} values as low as 0.02 μM. The high photodynamic activity of these compounds can be attributed to their high cellular uptake and low aggregation tendency in the biological media, which promote the generation of reactive oxygen species inside the cells. The effects of the chain length on the aggregation behaviour, photophysical properties, cellular uptake and *in vitro* photodynamic activities of this series of compounds have also been examined.

Introduction

Phthalocyanines are macrocyclic compounds containing four *N*-fused isoindole units. The highly conjugated π systems absorb strongly in the red visible region. This property, together with the extraordinary stability of these compounds, renders them able to be used as classical blue pigments.[1] Recently, the applications of these compounds have been greatly extended, ranging from materials science, catalysis, nanotechnology to medicine,[2] mainly as a result of their versatile characteristics. Through rational modification, the intrinsic properties as well as the molecular stacking of the macrocycles can be adjusted. This facilitates the optimisation of their performance as advanced functional materials.[3]

In biomedical applications, phthalocyanines are promising second-generation photosensitisers for photodynamic therapy (PDT) as a result of their strong absorption of tissue-penetrating red light and high efficiency at generating singlet oxygen.[4] Hydrophilic moieties can also be incorporated readily into the macrocycles making the molecules amphiphilic in nature, which is also a desirable characteristic for efficient photosensitisers.[5] Classical phthalocyanine-based photosensitisers include liposomal zinc(II) phthalocyanine, sulfonated zinc(II) and aluminum(III) phthalocyanines and the silicon(IV) phthalocyanine Pc4 developed by Kenney and co-workers. Over the last few years, we[6] and others[7] have greatly extended this series of compounds. A substantial number of phthalocyanine derivatives have been prepared and evaluated for their photodynamic activities, focusing on the structure-property-activity relationships. As a continuing effort in this endeavour, we report herein a new series of zinc(II) phthalocyanines having two diethylene glycol to polyethylene glycol chains at the 1,4-di-α-positions, including their synthesis, spectroscopic and photophysical properties, as well as their *in vitro* photodynamic activities. The effects of the chain length on these properties have also been examined.

Results and discussion

Molecular design and synthesis

Although a vast number of phthalocyanines have been reported, to our knowledge, these 1,4-disubstituted analogues remain relatively rare.[6b,8] Compared with the unsubstituted zinc(II) phthalocyanine (ZnPc), they exhibit a red-shifted Q band (at *ca.* 690 vs. 670 nm for ZnPc), which allows deeper light penetration into tissues.[9] The two substituents added near the macrocyclic core can also effectively enhance the solubility and reduce the aggregation tendency of the macrocycles. This strategy is different from those reported in the literature such as the introduction of bulky substituents at the axial or peripheral positions,[10] and the use of perfluorinated substituents.[11] All of these characteristics are beneficial for PDT

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† Electronic supplementary information (ESI) available: Electronic absorption spectra of **5a** and **5f** in thin films; comparison of the rates of decay of DPBF in DMF using phthalocyanines **5a–5c**, **5e–5f** and ZnPc as the photosensitisers; electronic absorption spectra of **5a–5c** and **5e–5f** in the RPMI medium; ^{1}H and ^{13}C{^{1}H} NMR spectra of all the new compounds. See DOI: 10.1039/b814627f

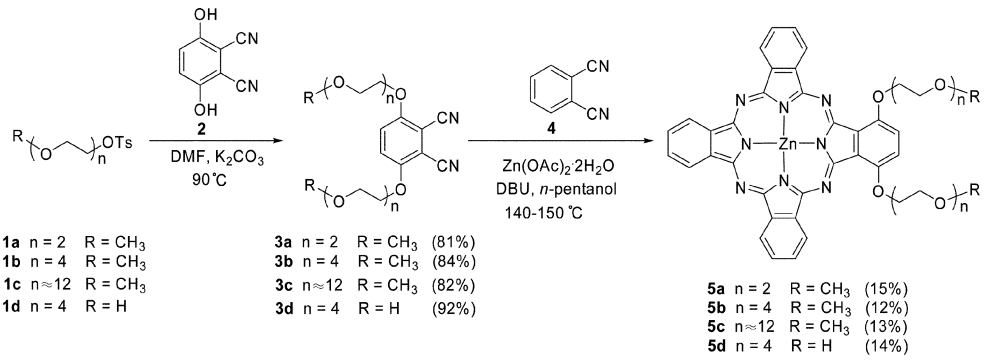
application. Zinc(II) ion was selected as the metal centre because of the general robustness and the desirable photophysical properties of these metallo-phthalocyanines.[12] Polyethylene glycols are also excellent pharmaceutical carriers which can prolong the drugs' circulating half-life, minimise their non-specific uptake and enable specific tumour-targeting through the enhanced permeability and retention (EPR) effect.[13] Addition of these hydrophilic chains to the hydrophobic macrocyclic core also imparts a high amphiphilicity to the photosensitisers. Hence, we believed that these specially designed molecules, which fulfil most of the criteria for superior photosensitisers,[14] would behave ideally. Substituents having different numbers of oxyethylene units were introduced with a view to fine-tuning the photodynamic activities. It is worth noting that although various pegylated photosensitisers have been reported,[15] the effects of the chain length on their photodynamic activities remain little studied.

Scheme 1 shows the synthetic pathway used to prepare phthalocyanines $ZnPc[O(CH_2CH_2O)_nMe]_2$ [n = 2 (**5a**), 4 (**5b**), *ca.* 12 (**5c**)], which contain two diethylene glycol to polyethylene glycol methyl ether chains at the 1,4-positions. The synthesis first involves the nucleophilic substitution reaction of tosylates **1a–1c** with 2,3-dicyanohydroquinone (**2**) to afford the disubstituted phthalonitriles **3a–3c**. The tosylate **1c** was prepared from the commercially available polyethylene glycol methyl ether having an average molecular weight of 550. These phthalonitriles then underwent mixed cyclisation with an excess of unsubstituted phthalonitrile (**4**) (9 equiv.) in the presence of $Zn(OAc)_2{\cdot}2H_2O$ and 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU) in *n*-pentanol to give the respective phthalocyanines **5a–5c** in 12–15% yield. Although these reactions also afforded other cyclised products, particularly ZnPc, these disubstituted analogues were readily isolated by column chromatography followed by size exclusion chromatography as a result of their high solubility and low aggregation tendency in common organic solvents.

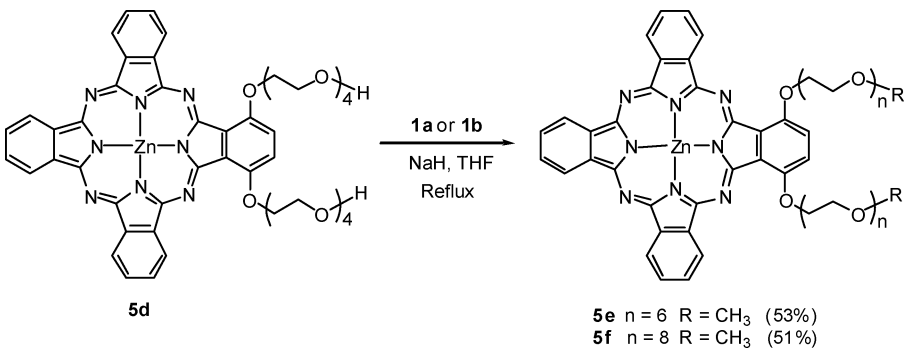
This synthetic route can also be applied to prepare the non-methyl protected analogue **5d** (Scheme 1). Starting from the tetraethylene glycol monotosylate (**1d**) and following this reaction sequence, the dihydroxy phthalocyanine $ZnPc[O(CH_2CH_2O)_4H]_2$ (**5d**) was obtained with comparable yield. The hydroxy groups could tolerate the conditions for these substitution and cyclisation reactions.

This compound was found to be an excellent precursor for other 1,4-disubstituted analogues through transformations of the terminal hydroxy groups. Thus treatment of **5d** with tosylate **1a** or **1b** in the presence of NaH led to chain elongation giving $ZnPc[O(CH_2CH_2O)_nMe]_2$ [n = 6 (**5e**), 8 (**5f**)] in moderate yield (Scheme 2). With these two procedures, a series of 1,4-disubstituted zinc(II) phthalocyanines having two diethylene glycol to polyethylene glycol methyl ether chains were prepared.

All of the new compounds were fully characterised with various spectroscopic methods. The ^1H NMR spectra of phthalocyanines **5a–5f** were recorded in $CDCl_3$ with a trace amount of pyridine-d_5 added to reduce the aggregation of these compounds. Generally, the spectra showed one to two multiplet(s) at the most downfield position (at *ca.* δ 9.4) for the phthalocyanine α ring protons, and one multiplet (at *ca.* δ 8.2, 6 H) and a singlet (at *ca.* δ 7.6, 2 H) for the phthalocyanine β ring protons. For the chains' methylene protons near the phthalocyanine ring, the signals, mostly in a triplet form, were shifted downfield significantly (up to *ca.* δ 5.0) by the ring current. As expected, the length of the chains



Scheme 1



Scheme 2

did not exert a significant influence on the positions of these signals.

The FAB or ESI mass spectra of all of these compounds showed the molecular ion signals. Accurate mass measurements were also performed to confirm the identity of these compounds. In the ESI mass spectrum of **5c**, two major envelopes for the protonated $[M + H]^+$ and sodiated $[M + Na]^+$ molecular ions were observed. For both of them, the clusters are separated by 44 mass units corresponding to the molecular mass of the repeating unit of polyethylene glycol.

Electronic absorption and photophysical properties

The electronic absorption spectra of $ZnPc[O(CH_2CH_2O)_nMe]_2$ (n = 2, 4, 6, 8, *ca.* 12) **5a–5c** and **5e–5f** were recorded in DMF. The spectra showed typical features of non-aggregated phthalocyanines. They displayed a Soret band at 340 nm, an intense and sharp Q band at 689 nm, together with a vibronic band at 621–622 nm. For all of these compounds, the Q band strictly followed the Lambert-Beer law (up to 5 μM), indicating that these compounds are essentially free from aggregation in DMF. The spectrum of **5a** is given as an example (Fig. 1). As shown in Table 1, the data are virtually the same for this series of compounds showing that the length of the chains does not affect the π macrocyclic system.

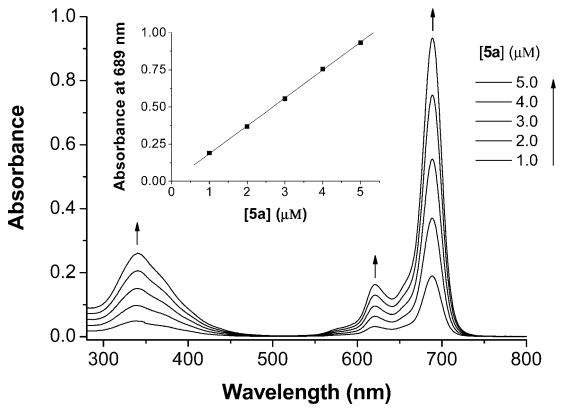


Fig. 1 Electronic absorption spectra of **5a** at different concentrations in DMF. The insert plots the Q-band absorbance at 689 nm *versus* the concentration of **5a**.

For comparison, the absorption spectra of **5a** and **5f** in thin solid films were also recorded (Fig. S1†). The Q bands were significantly broadened with a shoulder at *ca.* 650 nm. However, the absorption maxima (at *ca.* 700 nm) were not significantly shifted compared with those in DMF (689 nm). It suggested that the two α-substituents can somewhat reduce the stacking tendency of these compounds even in the solid state.

Compared with ZnPc, these di-α-substituted analogues exhibit a red-shifted Q band (at 689 vs. 670 nm for ZnPc). It has been found that attachment of electron-releasing alkoxy groups at the α positions of phthalocyanines destabilises their HOMO level more than the LUMO level.[16] As a result, the HOMO-LUMO gap is reduced upon α-substitution, which can explain the red shift observed for this series of compounds.

Upon excitation at the vibronic band, these compounds showed a fluorescence emission at 698 nm with a quantum yield (Φ_F) of 0.18–0.19 in DMF (Table 1) relative to ZnPc ($\Phi_F = 0.28$).[17] The weaker fluorescence of these compounds is in accord with the general observation that the lower the energy of the Q band, the smaller the Φ_F value.[16] It is likely that as the HOMO-LUMO gap decreases, electron transfer may take place more readily which lowers the stability of the excited state.

The singlet oxygen generation efficiency of these di-α-substituted phthalocyanines was also examined in DMF by a steady-state method using 1,3-diphenylisobenzofuran (DPBF) as the scavenger. The concentration of the quencher was monitored spectroscopically at 411 nm over time, from which the singlet oxygen quantum yields (Φ_Δ) were determined by the previously described method.[18] Fig. S2† compares the rates of decay of DPBF using **5a–5c**, **5e–5f** and ZnPc as the photosensitisers. The values of Φ_Δ are also compiled in Table 1. It can be seen that all of these di-α-substituted phthalocyanines are highly efficient singlet oxygen generators having comparable quantum yields ($\Phi_\Delta = 0.81$–0.83). Their efficiency is significantly higher than that of ZnPc, which was used as the reference.

In vitro photodynamic activities

The *in vitro* photodynamic activities of these phthalocyanines in Cremophor EL emulsions were investigated against two different cell lines, namely HT29 human colorectal carcinoma and HepG2 human hepatocarcinoma cells. Fig. 2 compares the effects of these compounds on the two cell lines both in the absence and presence of light. It can be seen that all of these compounds are essentially non-cytotoxic in the dark, but upon illumination, they exhibit substantial cytotoxicity. The corresponding IC_{50} values, defined as the dye concentration required to kill 50% of the cells, are summarised in Table 2. For both of the cell lines, the photocytotoxicity of these compounds depends on the length of the substituents. Fig. 3 depicts the variation of the IC_{50} values with the number of oxyethylene unit in the chains. For HepG2, the dyes with shorter chains (n = 2, 4, 6) are generally more potent than the analogues with longer substituents (n = 8, *ca.* 12). For HT29,

Table 1 Electronic absorption and photophysical data for phthalocyanines **5a–5c** and **5e–5f** in DMF

Compound	λ_{max} (nm) (log ε)	λ_{em} (nm)[a]	Φ_F[b]	Φ_Δ[c]
$ZnPc[O(CH_2CH_2O)_2Me]_2$ (**5a**)	340 (4.73), 621 (4.52), 689 (5.27)	698	0.18	0.83
$ZnPc[O(CH_2CH_2O)_4Me]_2$ (**5b**)	340 (4.77), 621 (4.58), 689 (5.33)	698	0.18	0.83
$ZnPc[O(CH_2CH_2O)_6Me]_2$ (**5e**)	340 (4.77), 622 (4.57), 689 (5.31)	698	0.18	0.83
$ZnPc[O(CH_2CH_2O)_8Me]_2$ (**5f**)	340 (4.73), 621 (4.53), 689 (5.28)	698	0.18	0.82
$ZnPc[O(CH_2CH_2O)_nMe]_2$ (n = *ca.* 12) (**5c**)	340 (4.71), 622 (4.48), 689 (5.23)	698	0.19	0.81

[a] Excited at 621 nm. [b] Relative to ZnPc ($\Phi_F = 0.28$ in DMF). [c] Relative to ZnPc ($\Phi_\Delta = 0.56$ in DMF).

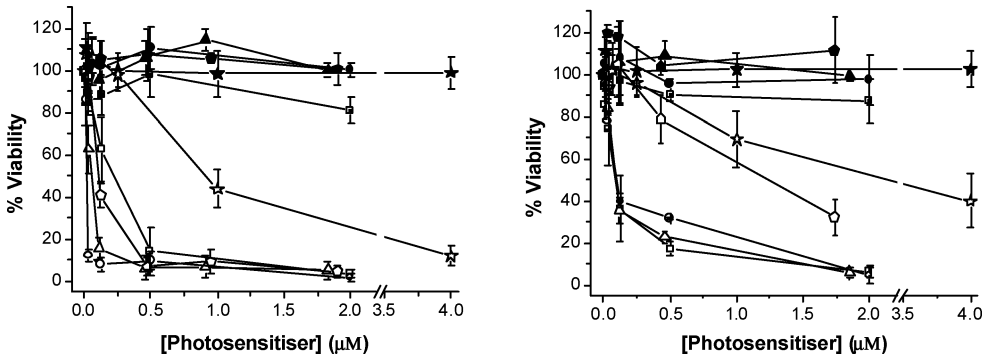


Fig. 2 Effects of **5a** (squares), **5b** (triangles), **5c** (stars), **5e** (circles) and **5f** (pentagons) on HT29 (left) and HepG2 (right) in the absence (closed symbols) and presence (open symbols) of light (λ >610 nm, 40 mW cm^{-2}, 48 J cm^{-2}). Data are expressed as mean values ± standard error of the mean of three independent experiments, each performed in quadruplicate.

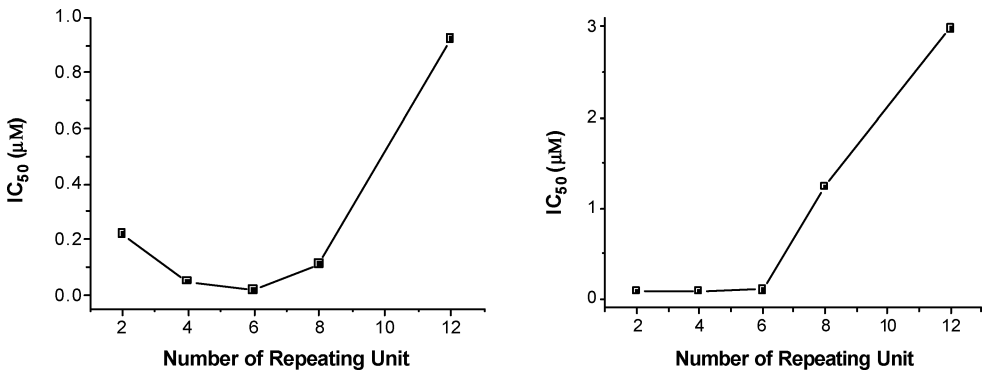


Fig. 3 The effects of chain length on the photocytotoxicity of phthalocyanines **5a–5c** and **5e–5f** for HT29 (left) and HepG2 (right) cells.

Table 2 Comparison of the IC_{50} values of phthalocyanines **5a–5c** and **5e–5f** against HT29 and HepG2

Photosensitiser	For HT29 (μM)	For HepG2 (μM)
$ZnPc[O(CH_2CH_2O)_2Me]_2$ (**5a**)	0.22	0.09
$ZnPc[O(CH_2CH_2O)_4Me]_2$ (**5b**)	0.05	0.09
$ZnPc[O(CH_2CH_2O)_6Me]_2$ (**5e**)	0.02	0.10
$ZnPc[O(CH_2CH_2O)_8Me]_2$ (**5f**)	0.11	1.24
$ZnPc[O(CH_2CH_2O)_nMe]_2$ (n = *ca.* 12) (**5c**)	0.92	2.98

compound **5e** (with n = 6) shows the highest photocytotoxicity. A further increase in the chain length results in a substantial increase in the IC_{50} value. The *in vitro* photocytotoxicity attained by **5e** (IC_{50} = 0.02 μM for HT29) is in fact very high compared with that of the classical photosensitiser porfimer sodium (IC_{50} = 7.5 μg mL^{-1} vs. 23.3 ng mL^{-1} for **5e**),[19] pheophorbide *a* (IC_{50} = 0.5 μM),[19] and other mono- and tetrasubstituted zinc(II) phthalocyanines prepared by us recently.[6b,d,20] It is believed that the high potency of **5e** is also related to its unique 1,4-di-α-substitution pattern.

To account for the different photodynamic activities of these compounds, their aggregation behaviour in the culture media was examined by absorption spectroscopy. Fig. 4 shows the electronic absorption spectra of these compounds in the Dulbecco's modified Eagle's medium (DMEM) used for HT29 cells. It can be seen that the Q bands of $ZnPc[O(CH_2CH_2O)_4Me]_2$ (**5b**) and $ZnPc[O(CH_2CH_2O)_6Me]_2$ (**5e**) remain sharp and intense, indicating that these compounds are not significantly aggregated in the medium. The spectrum of $ZnPc[O(CH_2CH_2O)_2Me]_2$ (**5a**)

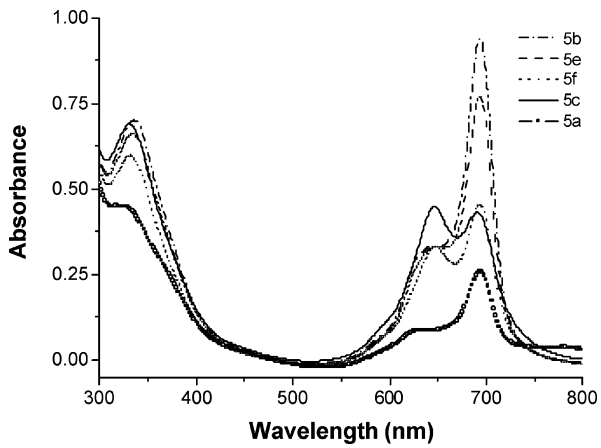


Fig. 4 Electronic absorption spectra of **5a–5c** and **5e–5f**, formulated with Cremophor EL, in DMEM (all at 8 μM).

also has similar spectral features, but the intensity of the Q band is substantially weaker due to its lower solubility in this medium. In fact, precipitation occurred after leaving the solution for *ca.* 2 h. For $ZnPc[O(CH_2CH_2O)_8Me]_2$ (**5f**) and $ZnPc[O(CH_2CH_2O)_nMe]_2$ (n = *ca.* 12) (**5c**), in addition to the Q band at *ca.* 700 nm, the blue-shifted Q band at *ca.* 650 nm attributable to the H-aggregates[21] become more prominent. Hence, for this series of compounds, when the number of oxyethylene unit is larger than 6, the compounds tend to aggregate in the culture medium, probably due to the stronger dipole-dipole interactions among the side

chains. Aggregation provides an efficient non-radiative relaxation pathway thereby reducing the population of the triplet state and the singlet oxygen generation efficiency.[22] The different solubility and aggregation tendencies of this series of compounds in DMEM can well explain the observed trend of photocytotoxicity (Table 2 and Fig. 3a), despite the fact that these compounds have virtually the same singlet oxygen quantum yield in DMF (Table 1). Similar results were observed in the RPMI medium 1640 used for HepG2 (Fig. S3†).

In addition to the cell viability studies, we also employed confocal microscopy to investigate the uptake of these photosensitisers by HT29 cells. After incubation with these compounds (formulated with Cremophor EL) for 2 h and upon excitation at 630 nm, the HT29 cells showed intracellular fluorescence throughout the cytoplasm as shown in Fig. 5, indicating that there was a substantial uptake of the dyes. The intensity generally decreases as the length of the substituent increases, *i.e.* **5a** > **5b** > **5e** > **5f** > **5c**. The results suggest that as the length of the substituent increases, the compounds show a lower cellular uptake and/or higher aggregation tendency, both of which are undesirable for photosensitisation. This can also explain the trend of photocytotoxicity observed for this series of compounds (Table 2).

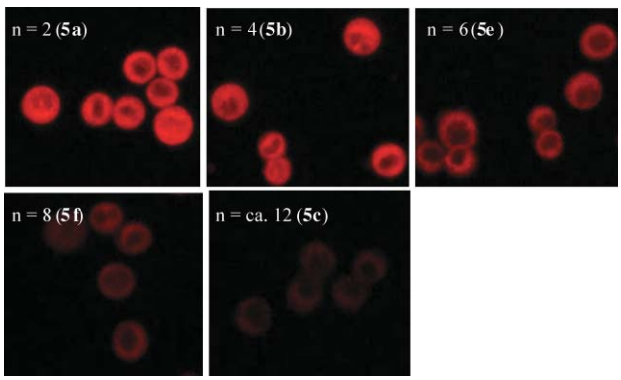


Fig. 5 Fluorescence microscopic images of HT29 after incubation with $ZnPc[O(CH_2CH_2O)_nMe]_2$ (**5a–5c** and **5e–5f**) at a concentration of 8 μM for 2 h.

Conclusions

In summary, we have prepared and characterised a new series of zinc(II) phthalocyanines with two diethylene glycol to polyethylene glycol methyl ether chains at the 1,4-di-α-positions. This unique substitution pattern shifts the Q-band absorption to the red and reduces the aggregation of the macrocycles. All of these compounds are photocytotoxic against HT29 and HepG2 cells. The solubility and aggregation behaviour in the culture media, cellular uptake and eventually the potency of these compounds greatly depend on the chain length. Optimal results can be achieved for compounds **5b** and **5e**, which respectively have two tetra- or hexaethylene glycol methyl ether chains.

Experimental

Experimental details regarding the purification of solvents, instrumentation and *in vitro* studies are described elsewhere.[6a] Tosylates **1a**,[23] **1b**,[23] **1c**[24] and **1d**[25] were prepared as described.

General procedure for the preparation of phthalonitriles 3a–3d

A mixture of 2,3-dicyanohydroquinone (**2**) (1 equiv.), tosylates **1a–1d** (2 equiv.) and K_2CO_3 (2 equiv.) in DMF (20–30 mL) was stirred at 90 °C under an atmosphere of nitrogen for 24 h. The volatiles were removed *in vacuo*, then the residue was mixed with water (100 mL) and extracted with CH_2Cl_2 (80 mL × 3). The combined organic extracts were dried over anhydrous $MgSO_4$. After evaporation under reduced pressure, the residue was purified by silica gel column chromatography using $CHCl_3/CH_3OH$ (100:1 v/v) as the eluent.

Phthalonitrile 3a

According to the general procedure, 2,3-dicyanohydroquinone (**2**) (2.40 g, 15.0 mmol) was treated with tosylate **1a** (8.22 g, 30.0 mmol) and K_2CO_3 (4.14 g, 30.0 mmol) to give **3a** as a pale white solid (4.42 g, 81%). ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.23 (s, 2 H, ArH), 4.25 (t, J = 4.8 Hz, 4 H, CH_2), 3.90 (t, J = 4.8 Hz, 4 H, CH_2), 3.73–3.76 (m, 4 H, CH_2), 3.55–3.58 (m, 4 H, CH_2), 3.39 (s, 6 H, CH_3). ^{13}C{^{1}H} NMR (75.4 MHz, $CDCl_3$): δ 155.4, 119.1, 112.9, 105.6, 71.9, 71.1, 70.1, 69.4, 59.0. MS (ESI): m/z 387 {100%, $[M + Na]^+$}. HRMS (ESI) calcd for $C_{18}H_{24}N_2NaO_6$ $[M + Na]^+$ 387.1527, found: 387.1535. Anal. calcd for $C_{18}H_{24}N_2O_6$: C, 59.33; H, 6.64; N, 7.69. Found: C, 59.50; H, 6.82; N, 7.55.

Phthalonitrile 3b

According to the general procedure, 2,3-dicyanohydroquinone (**2**) (0.48 g, 3.0 mmol) was treated with tosylate **1b** (2.17 g, 6.0 mmol) and K_2CO_3 (0.83 g, 6.0 mmol) to give **3b** as a pale white solid (1.36 g, 84%). ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.27 (s, 2 H, ArH), 4.25 (t, J = 4.2 Hz, 4 H, CH_2), 3.90 (t, J = 4.2 Hz, 4 H, CH_2), 3.71–3.76 (m, 4 H, CH_2), 3.63–3.68 (m, 16 H, CH_2), 3.53–3.58 (m, 4 H, CH_2), 3.37 (s, 6 H, CH_3). ^{13}C{^{1}H} NMR (75.4 MHz, $CDCl_3$): δ 155.3, 119.2, 112.9, 105.3, 71.8, 71.0, 70.5 (three overlapping signals), 70.4, 70.0, 69.3, 58.9. MS (FAB): m/z 541 {100%, $[M + H]^+$}. HRMS (FAB) calcd for $C_{26}H_{41}N_2O_{10}$ $[M + H]^+$ 541.2756, found: 541.2742.

Phthalonitrile 3c

According to the general procedure, 2,3-dicyanohydroquinone (**2**) (2.00 g, 12.5 mmol) was treated with tosylate **1c** (18.51 g, 26.3 mmol) and K_2CO_3 (3.63 g, 26.3 mmol) to give **3c** as a pale yellow oil (12.57 g, 82%). ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.26 (s, 2 H, ArH), 4.24 (t, J = 4.5 Hz, 4 H, CH_2), 3.90 (t, J = 4.5 Hz, 4 H, CH_2), 3.61–3.76 (m, *ca.* 84 H, CH_2), 3.53–3.56 (m, 4 H, CH_2), 3.38 (s, 6 H, CH_3). MS (ESI): m/z 1267 {52%, $[M + Na]^+$ for n = 12}.

Phthalonitrile 3d

According to the general procedure, 2,3-dicyanohydroquinone (**2**) (1.91 g, 11.9 mmol) was treated with tosylate **1d** (8.28 g, 23.8 mmol) and K_2CO_3 (3.28 g, 23.7 mmol) to afford **3d** as a pale white solid (5.62 g, 92%). ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.29 (s, 2 H, ArH), 4.26 (t, J = 4.5 Hz, 4 H, CH_2), 3.89 (t, J = 4.5 Hz, 4 H, CH_2), 3.73–3.77 (m, 4 H, CH_2), 3.64–3.71 (m, 16 H, CH_2), 3.59–3.62 (m, 4 H, CH_2), 2.86 (br s, 2 H, OH). ^{13}C{^{1}H} NMR (75.4 MHz, $CDCl_3$): δ 155.3, 119.3, 113.0, 105.2, 72.4, 70.9, 70.5, 70.4, 70.1, 70.0, 69.3,

61.5. MS (ESI): *m*/z 535 {100%, $[M + Na]^+$}. HRMS (ESI) calcd for $C_{24}H_{36}N_2NaO_{10}$ $[M + Na]^+$ 535.2262, found: 535.2255.

General procedure for the preparation of phthalocyanines 5a–5d

A mixture of phthalonitriles **3a–3d** (1 equiv.), unsubstituted phthalonitrile (**4**) (9 equiv.) and $Zn(OAc)_2{\cdot}2H_2O$ (2.5 equiv.) in *n*-pentanol (25 mL) was heated to 100 °C, then a small amount of DBU (1 mL) was added. The mixture was stirred at 140–150 °C for 24 h. After a brief cooling, the volatiles were removed under reduced pressure. The residue was dissolved in $CHCl_3$ (200 mL), then the solution was filtered to remove the ZnPc formed. The filtrate was collected and evaporated to dryness *in vacuo.* The residue was purified by silica gel column chromatography using $CHCl_3/CH_3OH$ (30:1 v/v) as the eluent, followed by size exclusion chromatography using THF as the eluent. The crude product was further purified by recrystallisation from a mixture of THF and hexane to give the product as a blue solid or oil.

ZnPc[O(CH_2CH_2O)$_2$Me]$_2$ (5a)

According to the general procedure, phthalonitrile **3a** (0.73 g, 2.0 mmol) was treated with unsubstituted phthalonitrile (**4**) (2.31 g, 18.0 mmol) and $Zn(OAc)_2{\cdot}2H_2O$ (1.10 g, 5.0 mmol) to give **5a** as a blue solid (0.24 g, 15%). ^{1}H NMR (300 MHz, $CDCl_3$ with a trace amount of pyridine-d_5): δ 9.41–9.49 (m, 6 H, Pc–H_α), 8.14–8.19 (m, 6 H, Pc–H_β), 7.57 (s, 2 H, Pc–H_β), 4.99 (t, J = 5.1 Hz, 4 H, CH_2), 4.57 (t, J = 5.1 Hz, 4 H, CH_2), 4.15 (t, J = 4.8 Hz, 4 H, CH_2), 3.76 (t, J = 4.8 Hz, 4 H, CH_2), 3.44 (s, 6 H, CH_3). ^{13}C{^{1}H} NMR (75.4 MHz, DMSO-d_6): δ 152.6, 152.5, 152.4, 151.9, 149.8, 138.3, 138.0, 137.9, 129.3, 129.2, 126.2, 122.5, 122.3, 115.4, 71.8, 70.4, 70.3, 69.1, 58.4 (some of the Pc signals are overlapped). MS (ESI): an isotopic cluster peaking at *m*/z 813 {100%, $[M + H]^+$}. HRMS (ESI) calcd for $C_{42}H_{37}N_8O_6Zn$ $[M + H]^+$ 813.2122, found: 813.2121. Anal. calcd for $C_{42}H_{36}N_8O_6Zn$: C, 61.96; H, 4.46; N, 13.76. Found: C, 62.45; H, 4.83; N, 13.47.

ZnPc[O(CH_2CH_2O)$_4$Me]$_2$ (5b)

According to the general procedure, phthalonitrile **3b** (0.54 g, 1.0 mmol) was treated with unsubstituted phthalonitrile (**4**) (1.15 g, 9.0 mmol) and $Zn(OAc)_2{\cdot}2H_2O$ (0.55 g, 2.5 mmol) to give **5b** as a blue solid (0.12 g, 12%). ^{1}H NMR (300 MHz, $CDCl_3$ with a trace amount of pyridine-d_5): δ 9.34–9.40 (m, 4 H, Pc-H_α), 9.25–9.27 (m, 2 H, Pc–H_α), 8.07–8.15 (m, 6 H, Pc-H_β), 7.42 (s, 2 H, Pc-H_β), 4.92 (t, J = 5.1 Hz, 4 H, CH_2), 4.52 (t, J = 5.1 Hz, 4 H, CH_2), 4.15 (t, J = 4.8 Hz, 4 H, CH_2), 3.87 (t, J = 4.8 Hz, 4 H, CH_2), 3.72–3.75 (m, 4 H, CH_2), 3.60–3.67 (m, 8 H, CH_2), 3.49–3.52 (m, 4 H, CH_2), 3.33 (s, 6 H, CH_3). ^{13}C{^{1}H} NMR (75.4 MHz, $CDCl_3$ with a trace amount of pyridine-d_5): δ 153.9, 153.8, 153.6, 152.6, 150.4, 138.9, 138.5, 129.0, 127.4, 122.7, 122.5, 115.0, 71.8, 71.1, 70.8, 70.6, 70.5 (two overlapping signals), 70.4, 69.2, 58.9 (some of the Pc signals are overlapped). MS (FAB): an isotopic cluster peaking at *m*/z 989 {100%, $[M + H]^+$}. HRMS (FAB) calcd for $C_{50}H_{53}N_8O_{10}Zn$ $[M + H]^+$ 989.3171, found: 989.3149.

ZnPc[O(CH_2CH_2O)$_n$Me]$_2$ (n = *ca.* 12) (5c)

According to the general procedure, phthalonitrile **3c** (0.57 g, 0.47 mmol) was treated with unsubstituted phthalonitrile (**4**) (0.53 g, 4.14 mmol) and $Zn(OAc)_2{\cdot}2H_2O$ (0.25 g, 1.14 mmol) to give **5c** as a blue oil (0.10 g, 13%). ^{1}H NMR (300 MHz, $CDCl_3$ with a trace amount of pyridine-d_5): δ 9.41–9.49 (m, 6 H, Pc-H_α), 8.15–8.18 (m, 6 H, Pc–H_β), 7.58 (s, 2 H, Pc–H_β), 4.97 (t, J = 5.1 Hz, 4 H, CH_2), 4.56 (t, J = 5.1 Hz, 4 H, CH_2), 4.16 (t, J = 4.8 Hz, 4 H, CH_2), 3.87 (t, J = 4.8 Hz, 4 H, CH_2), 3.69–3.72 (m, 4 H, CH_2), 3.57–3.68 (m, *ca.* 72 H, CH_2), 3.51–3.56 (m, 4 H, CH_2), 3.35–3.38 (m, 6 H, CH_3). MS (ESI): an isotopic cluster peaking at *m*/z 1715 {13%, $[M + Na]^+$ for n = 12}.

ZnPc[O(CH_2CH_2O)$_4$H]$_2$ (5d)

According to the general procedure, phthalonitrile **3d** (0.50 g, 0.98 mmol) was treated with unsubstituted phthalonitrile (**4**) (1.13 g, 8.82 mmol) and $Zn(OAc)_2{\cdot}2H_2O$ (0.54 g, 2.46 mmol) to give **5d** as a blue solid (0.13 g, 14%). ^{1}H NMR (300 MHz, $CDCl_3$ with a trace amount of pyridine-d_5): δ 9.43–9.49 (m, 4 H, Pc-H_α), 9.39–9.41 (m, 2 H, Pc–H_α), 8.12–8.18 (m, 6 H, Pc–H_β), 7.58 (s, 2 H, Pc–H_β), 4.97 (t, J = 4.8 Hz, 4 H, CH_2), 4.54 (t, J = 4.8 Hz, 4 H, CH_2), 4.14 (t, J = 4.5 Hz, 4 H, CH_2), 3.84 (t, J = 4.5 Hz, 4 H, CH_2), 3.58–3.68 (m, 12 H, CH_2), 3.51 (t, J = 4.5 Hz, 4 H, CH_2). ^{13}C{^{1}H} NMR (75.4 MHz, $CDCl_3$): δ 153.0, 152.8, 152.7, 150.9, 148.4, 138.4, 138.3, 138.2, 128.5, 128.3, 128.2, 125.1, 123.0, 122.3, 121.9, 112.3, 70.8, 70.6, 70.2 (two overlapping signals), 70.0, 69.1, 67.9, 59.4. UV-Vis (DMF) [λ_{max} (nm), (log ε)]: 341 (4.72), 621 (4.53), 689 (5.28). MS (ESI): an isotopic cluster peaking at *m*/z 961 {100%, $[M + H]^+$}. HRMS (ESI) calcd for $C_{48}H_{49}N_8O_{10}Zn$ $[M + H]^+$ 961.2858, found: 961.2848. Anal. calcd for $C_{48}H_{48}N_8O_{10}Zn$: C, 59.91; H, 5.03; N, 11.64. Found: C, 59.49; H, 5.17; N, 11.16.

ZnPc[O(CH_2CH_2O)$_6$Me]$_2$ (5e)

Phthalocyanine **5d** (96 mg, 0.10 mmol) was added to a suspension of NaH (60% in mineral oil, 40 mg, 1.00 mmol) in THF (8 mL). After the evolution of gas bubbles had ceased, a solution of the monotosylate **1a** (110 mg, 0.40 mmol) in THF (2 mL) was added slowly. The resulting mixture was refluxed overnight. A few drops of water were then added to quench the reaction. The volatiles were removed under reduced pressure. The residue was mixed with water (20 mL) and the mixture was extracted with $CHCl_3$ (20 mL x 3). The combined organic extracts were dried over anhydrous $MgSO_4$ and evaporated under reduced pressure. The residue was chromatographed on a silica gel column using $CHCl_3/CH_3OH$ (20:1 v/v) as the eluent. The product was obtained as a blue solid (62 mg, 53%). ^{1}H NMR (300 MHz, $CDCl_3$ with a trace amount of pyridine-d_5): δ 9.40–9.51 (m, 6 H, Pc–H_α), 8.12–8.20 (m, 6 H, Pc–H_β), 7.58 (s, 2 H, Pc–H_β), 4.97 (t, J = 4.8 Hz, 4 H, CH_2), 4.56 (t, J = 4.8 Hz, 4 H, CH_2), 4.15 (t, J = 4.8 Hz, 4 H, CH_2), 3.87 (t, J = 4.8 Hz, 4 H, CH_2), 3.69–3.73 (m, 4 H, CH_2), 3.57–3.65 (m, 24 H, CH_2), 3.47–3.52 (m, 4 H, CH_2), 3.32 (s, 6 H, CH_3). ^{13}C{^{1}H} NMR (75.4 MHz, $CDCl_3$ with a trace amount of pyridine-d_5): δ 154.0, 153.8, 153.7, 152.7, 150.4, 138.9, 138.5, 129.0, 127.4, 122.7, 122.5, 115.0, 71.8, 71.1, 70.7, 70.6, 70.5, 69.2, 58.9 (some of the signals are overlapped). MS (ESI): an isotopic cluster peaking at *m*/z 1165 {98%, $[M + H]^+$}. HRMS (ESI) calcd for $C_{58}H_{69}N_8O_{14}Zn$ $[M + H]^+$ 1165.4219, found: 1165.4209.

ZnPc[O(CH$_2$CH$_2$O)$_8$Me]$_2$ (5f)

According to the above procedure, treatment of phthalocyanine **5d** (96 mg, 0.10 mmol) with tosylate **1b** (145 mg, 0.40 mmol) and NaH (60% in mineral oil, 40 mg, 1.00 mmol) afforded **5f** as a blue solid (68 mg, 51%). ^{1}H NMR (300 MHz, $CDCl_3$ with a trace amount of pyridine-d_5): δ 9.43–9.47 (m, 4 H, Pc–H$_\alpha$), 9.37–9.40 (m, 2 H, Pc–H$_\alpha$), 8.11–8.16 (m, 6 H, Pc–H$_\beta$), 7.53 (s, 2 H, Pc–H$_\beta$), 4.96 (t, J = 5.1 Hz, 4 H, CH_2), 4.55 (t, J = 5.1 Hz, 4 H, CH_2), 4.16 (t, J = 4.8 Hz, 4 H, CH_2), 3.87 (t, J = 4.8 Hz, 4 H, CH_2), 3.70–3.73 (m, 4 H, CH_2), 3.59–3.66 (m, 40 H, CH_2), 3.50–3.53 (m, 4 H, CH_2), 3.35 (s, 6 H, CH_3). ^{13}C{^{1}H} NMR (75.4 MHz, $CDCl_3$ with a trace amount of pyridine-d_5): δ 153.5, 153.3, 153.2, 152.2, 150.0, 138.7, 138.3, 138.2, 128.7, 126.9, 122.5, 122.3, 114.5, 71.8, 71.0, 70.7, 70.5, 70.4, 68.9, 58.9 (some of the signals are overlapped). MS (FAB): an isotopic cluster peaking at m/z 1342 {100%, [M + H]$^+$}. HRMS (FAB) calcd for $C_{66}H_{85}N_8O_{18}Zn$ [M + H]$^+$ 1341.5268, found: 1341.5282.

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