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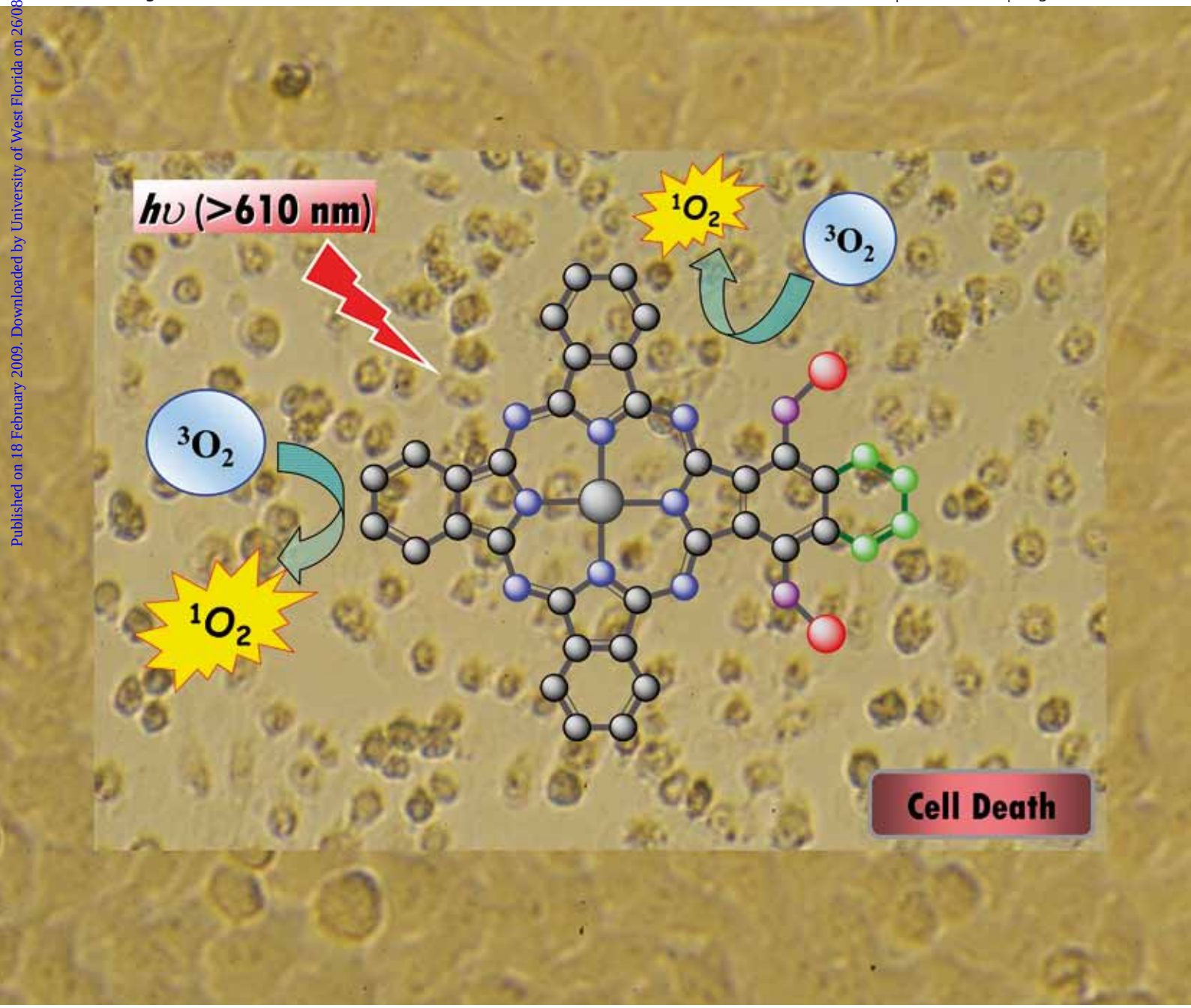

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HOT ARTICLE
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Infrared spectra and density functional theory calculations of coinage metal disulfide molecules and complexes

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Synthesis and *in vitro* photodynamic activities of di-α-substituted zinc(II) phthalocyanine derivatives†

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A new series of di-α-substituted zinc(II) phthalocyanine derivatives have been prepared by mixed cyclisation of the corresponding 1,4-disubstituted phthalonitriles or naphthalonitriles with an excess of unsubstituted phthalonitrile in the presence of $Zn(OAc)_2 \cdot 2H_2O$ and 1,8-diazabicyclo[5.4.0]undec-7-ene. Having a large hydrophobic macrocyclic core substituted with two hydrophilic triethylene glycol chains or glycerol moieties, these compounds are amphiphilic in nature. They are highly soluble and remain non-aggregated in DMF as shown by the intense and sharp Q-band absorption. Compared with the unsubstituted zinc(II) phthalocyanine, these di-α-substituted analogues exhibit a red-shifted Q band (at 689–701 nm), a relatively weaker fluorescence emission, and a higher efficiency to generate singlet oxygen. Upon illumination, these compounds are highly cytotoxic towards HT29 human colorectal carcinoma and HepG2 human hepatocarcinoma cells with IC_{50} values as low as 0.06 μM. The high photocytotoxicity of these compounds can be attributed to their high cellular uptake and low aggregation tendency in the biological media, leading to a high efficiency to generate reactive oxygen species inside the cells.

Introduction

Photodynamic therapy (PDT) has emerged as a non-invasive modality for the treatment of malignant tumours and wet age-related macular degeneration.[1] It involves the combination of visible light and a photosensitiser. Both components are harmless by themselves, but in combination with oxygen, they result in the generation of reactive oxygen species (ROS) leading to oxidative cell damage. Fig. 1 depicts the basic mechanism of action of PDT. For cancer treatment, PDT has several potential advantages including its non-invasive nature, tolerance of repeated doses, and high specificity that can be achieved through precise application of the light.[1b] Porphyrin derivatives are traditional photosensitisers. These compounds, however, generally induce sustained skin photosensitivity, show weak absorptions in the body's therapeutic window (650–800 nm), and require long drug-to-light intervals (48–96 h), making them far from ideal for the use in PDT.[2] Therefore, there is a great impetus for the development of non-porphyrin photosensitisers, which have stronger absorptions in the red visible region and improved photophysical and biological properties.[3] Phthalocyanines, generally having these desirable characteristics, are promising second-generation photosensitisers, which have received considerable attention over the last decade.[4] We have been interested in the use of these functional dyes in this avenue, focusing on their structure-property-activity relationships.[5] Various chemical modifications have been performed on the macrocyclic core with a view to enhancing the therapeutic outcome. In this report, we describe a novel series of di-α-substituted zinc(II) phthalocyanine derivatives. The two substituents adding to the α positions of the macrocycles not only shift their Q-band absorption further to the red (*ca.* 700 nm), allowing a deeper light penetration into tissues, but also reduce their aggregation tendency, thereby promoting the generation of singlet oxygen. To our knowledge, this class of disubstituted phthalocyanines remains rare.[5b,6] We report herein the synthesis, spectroscopic and photophysical characteristics, as well as the *in vitro* photodynamic activities of these compounds.

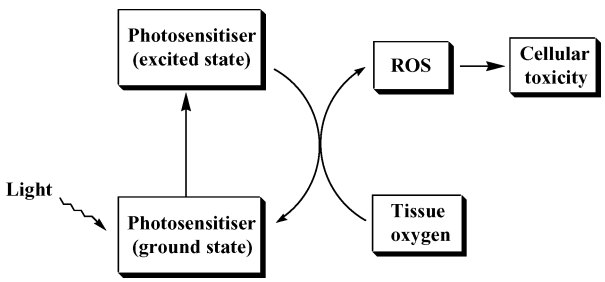

Fig. 1 Basic mechanism of the action of PDT.

Results and discussion

Synthesis and characterisation

Scheme 1 shows the synthetic route used to prepare the 1,4-di-α-substituted phthalocyanines **6a–6c**. Firstly, the alcohols **1a–1c** were converted to the corresponding tosylates **2a–2c**,

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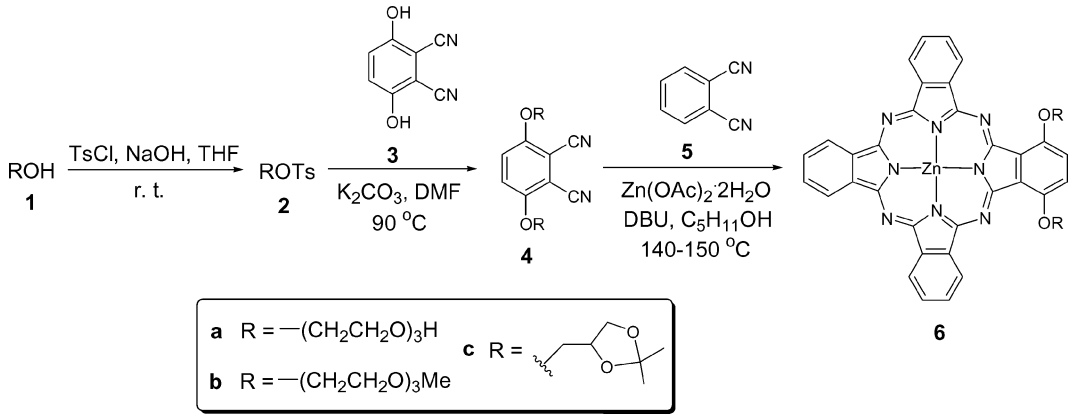
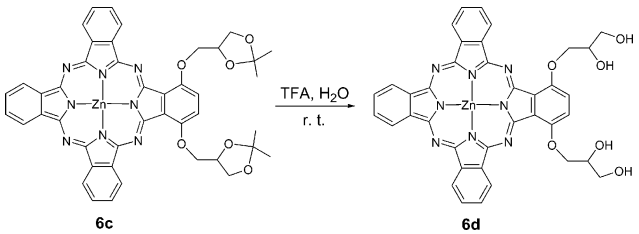
† Electronic supplementary information (ESI) available: ^{1}H NMR spectrum of **9a**, UV-vis spectra of **6a** and **9a** in the RPMI medium, fluorescence images of HT29 after incubation with **6a–6d** and **9a–9b**, and ^{1}H and $^{13}C\{^1H\}$ NMR spectra of all the new compounds. See DOI: 10.1039/b817940a



Scheme 1 Synthesis of 1,4-disubstituted zinc(II) phthalocyanines **6a–6c**.

which then underwent nucleophilic substitution with 2,3-dicyanohydroquinone (**3**) to give the disubstituted phthalonitriles **4a–4c**. Mixed cyclisation of these compounds with an excess of unsubstituted phthalonitrile (**5**) (9 equiv.) in the presence of $Zn(OAc)_2 \cdot 2H_2O$ and 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU) in 1-pentanol afforded the corresponding "3 + 1" cyclised products **6a–6c** as a blue solid. These oxygen-rich substituents were used to enhance the solubility and reduce the aggregation of these macrocycles, facilitating their separation from the other cyclised side products, particularly the unsubstituted zinc(II) phthalocyanine (ZnPc), which was found to be the major product. Hence, these 1,4-di-α-substituted phthalocyanines **6a–6c** could be isolated readily by silica gel column chromatography followed by size exclusion chromatography in 12–18%. Polyethylene glycols are also known to be excellent pharmaceutical vehicles, which can prolong circulating half-life, minimise non-specific uptake, and enable specific tumour-targeting through the enhanced permeability and retention (EPR) effect.[7] In addition, these hydrophilic moieties adding to the hydrophobic core will make the molecules amphiphilic in character, which is a desirable characteristic for efficient photosensitisers.[8]

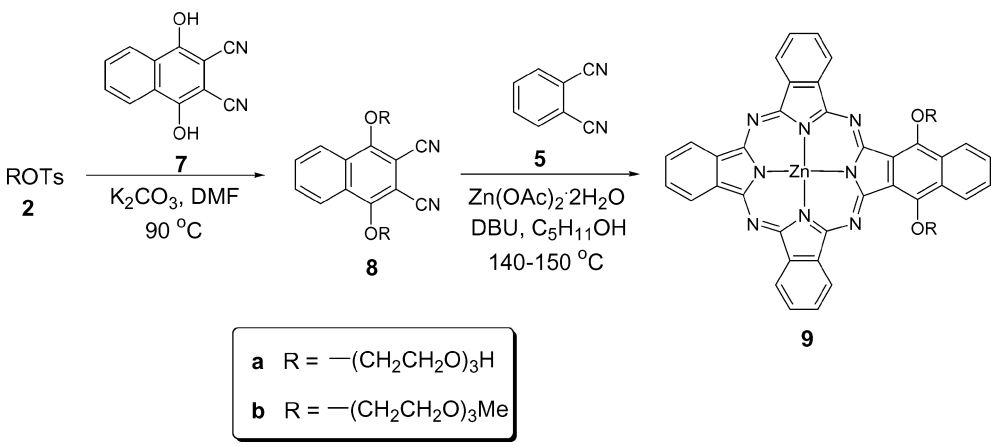
The two isopropylidene groups of **6c** could be removed readily upon treatment with a mixture of trifluoroacetic acid (TFA) and H_2O (9 : 1 v/v) giving the tetrahydroxy phthalocyanine **6d** in 85% yield (Scheme 2). This compound has a very high polarity and could not be purified by column chromatography. It was simply isolated by precipitation. The sample was found to be essentially pure.



Scheme 2 Synthesis of tetrahydroxy zinc(II) phthalocyanine **6d**.

To further extend the Q-band absorption to the red, we also prepared the benzo-fused analogues **9a** and **9b** according to Scheme 3. The tosylates **2a** and **2b** were treated with 1,4-dihydroxy-2,3-naphthalonitrile (**7**) and K_2CO_3 to give the disubstituted products **8a** and **8b**, respectively. Similarly, mixed cyclisation of these naphthalonitriles with **5** (9 equiv.) in the presence of $Zn(OAc)_2 \cdot 2H_2O$ and DBU led to the formation of **9a** and **9b** as a green solid in 13–15% yield.

All of the zinc(II) phthalocyanines **6a–6d** and **9a–9b** are soluble in common organic solvents and possess a high general stability. They were fully characterised with various spectroscopic methods and elemental analysis. The NMR spectra were recorded in



Scheme 3 Synthesis of benzo-fused zinc(II) phthalocyanines **9a** and **9b**.

$CDCl_3$ with a trace amount of pyridine-d_5, which can reduce the aggregation of these compounds. The only exception is the tetrahydroxy analogue **6d**, of which the spectra were recorded in DMSO-d_6. Fig. 2 shows the ^{1}H-^{1}H COSY spectrum of **6d** to illustrate the general spectral features of these compounds. The signals for the six phthalocyanine α ring protons overlap as a multiplet at δ 9.32–9.41. For compounds **6a** and **6c**, a multiplet and a doublet (in 2 : 1 integration ratio) were observed instead for these protons. The phthalocyanine β ring protons of **6d** resonate as a multiplet (at δ 8.19–8.26, 6 H) and a singlet (at δ 7.77, 2 H). The doublet at δ 5.56 and triplet at δ 4.96 can easily be assigned to the secondary and primary hydroxy groups, respectively. The other signals can also be unambiguously assigned as shown in the figure with the aid of the connectivity revealed by the COSY experiment.

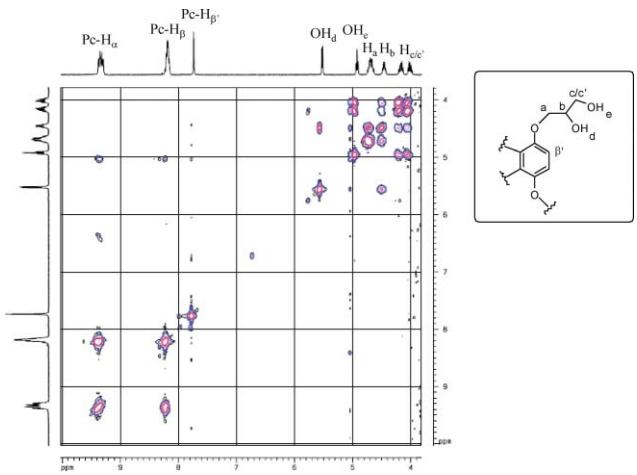


Fig. 2 ^{1}H-^{1}H COSY spectrum of **6d** in DMSO-d_6.

For **9a** and **9b**, the signals for the naphthyl α and β ring protons were also seen adjacent to the respective phthalocyanine ring protons' signals. In the spectrum of **9b** (ESI, Fig. S1†), four well-resolved doublets of doublets (at δ 9.41, 9.37, 9.29 and 9.08) were observed for the four different sets of α ring protons. The remaining four different sets of β ring protons resonated as two overlapping doublets of doublets (at δ 8.09–8.15) and another two doublets of doublets (at δ 8.04 and 7.91). Six well-separated virtual triplets or multiplets (in the region δ 3.6–5.7) and a singlet (at δ 3.41) were also seen for the six sets of methylene protons and the methyl groups, respectively, of the chains.

The ^{13}C{^{1}H} NMR spectra of these phthalocyanines showed clearly the expected number of signals for the substituents, but for the phthalocyanine ring, some of the signals were overlapped. All of these compounds were further characterised with ESI mass spectrometry. The isotopic distribution as well as the exact mass for the $[M + H]^+$ or $[M + Na]^+$ species were in good agreement with the calculated ones.

Electronic absorption and photophysical properties

The electronic absorption spectra of phthalocyanines **6a–6d** in DMF are very similar and are typical for non-aggregated phthalocyanines. It is likely that DMF, being a coordinating solvent, binds axially to these zinc(II) phthalocyanines, reducing their aggregation tendency. Taking the spectrum of **6b** (Fig. 3) as an example, it shows a broad Soret band peaking at 342 nm, an intense and sharp Q band at 689 nm, together with a vibronic band at 621 nm. The Q band strictly follows the Lambert Beer's law suggesting that aggregation of this compound is not significant. Compared with ZnPc, the mono-α-alkoxy analogues have a red-shifted Q band (from 670 to 672–679 nm).[5d,9] The di-α-substitution in **6a–6d** further shifts the Q band to the red (689–692 nm), but the positions are still not as far as those of the tetra- (696 nm) and octa-α-butoxy analogues (758 nm).[10] It has been shown that attachment of an electron-releasing alkoxy group at the α position destabilises the HOMO level more than the LUMO level.[10] As a result, the HOMO–LUMO gap is reduced upon α-substitution, which can explain the red shift in this series of compounds. Introduction of an additional fused benzene ring in **9a–9b** extends the π-conjugated system, leading to a further red-shift of the Q band to 701 nm. These data are summarised in Table 1.

Table 1 Electronic absorption and fluorescence data for **6a–6d** and **9a–9b** in DMF

Compound	λ_{max}/nm (log ε)	λ_{em}/nm[a]	Φ_F[b]
6a	341 (4.71), 621 (4.51), 689 (5.27)	698	0.17
6b	342 (4.69), 621 (4.47), 689 (5.24)	698	0.19
6c	341 (4.78), 621 (4.58), 689 (5.34)	697	0.19
6d	344 (4.77), 623 (4.57), 692 (5.32)	699	0.18
9a	343 (4.76), 632 (4.59), 701 (5.24)	720	0.12
9b	343 (4.78), 632 (4.60), 701 (5.25)	720	0.14

[a] Excited at 621 (for **6a–6d**) or 632 nm (for **9a–9b**). [b] Using ZnPc in DMF as the reference ($\Phi_F = 0.28$).

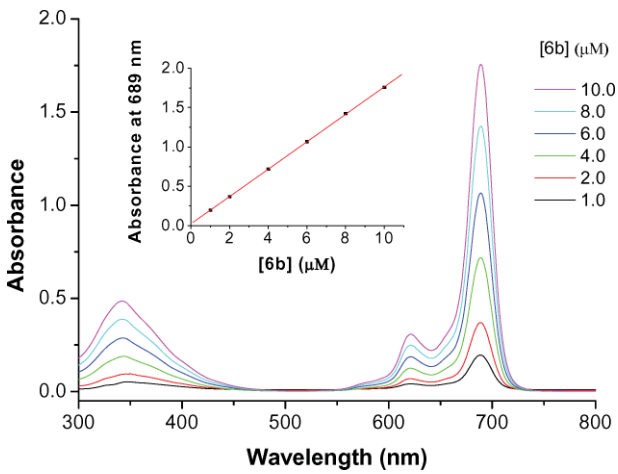


Fig. 3 Electronic absorption spectra of **6b** at various concentrations in DMF. The inset plots the Q-band absorbance *versus* the concentration of **6b**.

The fluorescence emission spectra of these compounds were also recorded in DMF. Upon excitation at 621 nm, compounds **6a–6d** showed a fluorescence emission at 697–699 nm with a quantum yield (Φ_F) of 0.17–0.19, which is substantially lower than that of ZnPc ($\Phi_F = 0.28$). For the benzo-fused analogues **9a–9b**, upon excitation at 632 nm, their fluorescence emission was red-shifted to 720 nm with an even lower Φ_F value (0.12–0.14) (Table 1). This is in accord with the general observation that the lower the energy of the Q band, the smaller the Φ_F value.[10] It has been suggested

that the excited state becomes unstable as the HOMO–LUMO gap decreases, probably due to the ease of electron transfer.

The efficiency of these compounds in generating singlet oxygen, as reflected by the rate of decay of the singlet oxygen quencher 1,3-diphenylisobenzofuran (DPBF), was also compared. As shown in Fig. 4, all of these phthalocyanines can induce the photo-bleaching of DPBF and the efficiency follows the order **9a–9b** > **6a–6d** > ZnPc. This suggests that as the HOMO–LUMO gap becomes smaller, the singlet excited state has a higher tendency to undergo intersystem crossing to generate singlet oxygen. This can also explain the opposite trend observed for the Φ_F values.

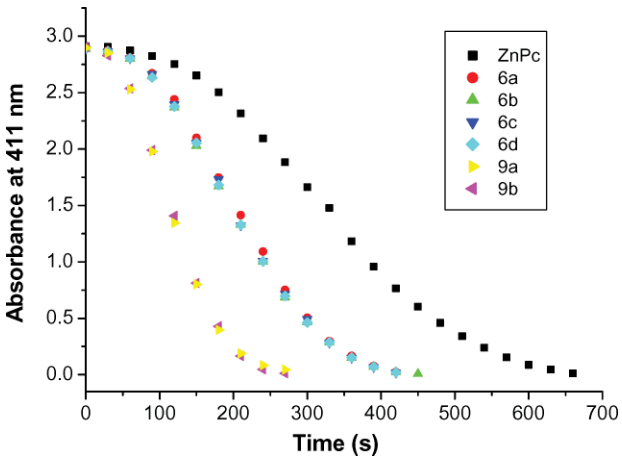


Fig. 4 Comparison of the rates of decay of DPBF in DMF (initial concentration = 1.85×10^{-4} M), as monitored spectroscopically at 411 nm, using phthalocyanines **6a–6d** and **9a–9b** as the photosensitisers and ZnPc as the reference.

In vitro photodynamic activities

The *in vitro* photodynamic activities of compounds **6a–6d** and **9a–9b** in Cremophor EL emulsions were investigated against two different cell lines, namely HT29 human colorectal carcinoma and HepG2 human hepatocarcinoma cells. Fig. 5 shows the dose-dependent survival curves for **6a** and **9a** towards HT29 given for exemplification. It can be seen that both compounds are essentially non-cytotoxic in the absence of light (up to 4 and 8 μM, respectively). However, they become cytotoxic upon illumination with red light (λ > 610 nm). Compound **6a** is significantly more potent with an IC_{50} value of 0.41 μM (*vs.* 2.93 μM for **9a**). Only *ca.* 1 μM of dye is required to essentially kill all the cells (*vs.* > 8 μM for **9a**). The photocytotoxicity of **6b–6d** is comparable with that of **6a**, which is significantly higher than that of **9a–9b**. The corresponding IC_{50} values are compiled in Table 2. Among these compounds, **6b** exhibits the highest photocytotoxicity with IC_{50} values down to 0.06 μM. This compound is among the most potent zinc(II) phthalocyanines prepared in our laboratory so far.[5b,5d,9,11] Its *in vitro* photocytotoxicity almost reaches the level attained by some of the very potent silicon(IV) analogues.[5a,5e,12] As shown by the IC_{50} values for **6c** and **6d** (Table 2), removing the isopropylidene protecting groups of **6c** does not exert a significant effect on the photocytotoxicity.

It is worth noting that although the benzo-fused phthalocyanines **9a–9b** exhibit a higher efficiency in generating singlet oxygen

Table 2 IC_{50} values of **6a–6d** and **9a–9b** against HT29 and HepG2 cells

	IC_{50}/μM	
Compound	For HT29	For HepG2
6a	0.41	0.16
6b	0.13	0.06
6c	0.40	0.40
6d	0.38	0.17
9a	2.93	2.76
9b	5.18	5.84

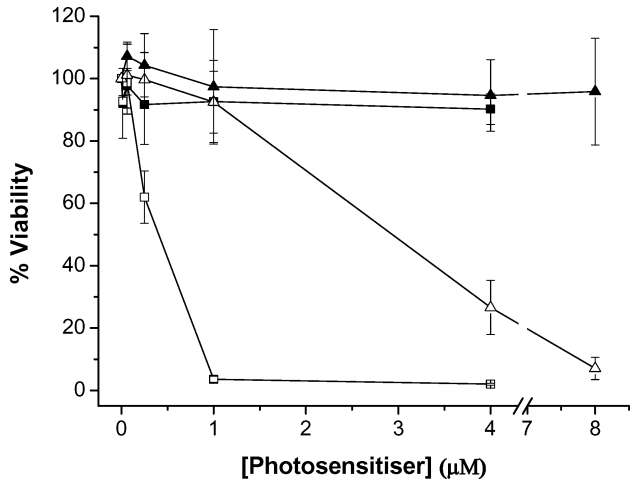


Fig. 5 Effects of **6a** (squares) and **9a** (triangles) on HT29 in the absence (closed symbols) and presence (open symbols) of light (λ > 610 nm, 40 mW cm^{-2}, 48 J cm^{-2}). Data are expressed as mean values ± S.E.M. of three independent experiments, each performed in quadruplicate.

in DMF, their photocytotoxicity is significantly lower than that of **6a–6d**. To account for these results, the absorption spectra of these compounds in the culture media were examined. Fig. 6 shows the spectra of **6a** and **9a** in the DMEM medium (used for HT29), which are typical for these two groups of compounds. It can be seen that the Q band for **6a** remains sharp and intense, while that for **9a** is significantly weaker and broadened. Similar results were observed in the RPMI medium (used for HepG2) (ESI, Fig. S2†).

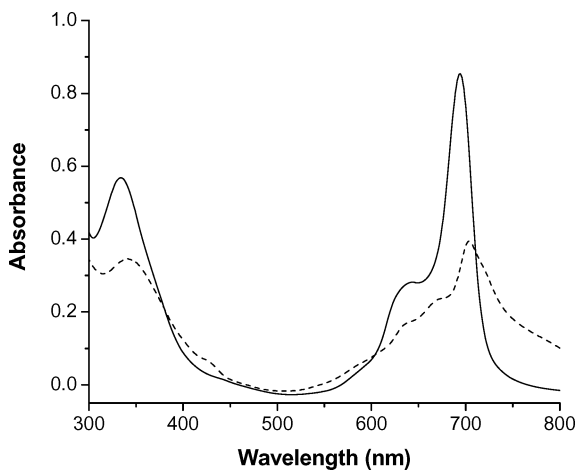


Fig. 6 Electronic absorption spectra of **6a** (—) and **9a** (---), formulated with Cremophor EL, in the DMEM culture medium (both at 8 μM).

The results indicate that compound **9a** (as well as **9b**), having a larger π-conjugated system, is more aggregated than **6a** (as well as **6b–6d**) in the culture media, thereby reducing its photosensitising efficiency.

The cellular uptake of these phthalocyanines was also investigated by fluorescence microscopy. After incubation with **6a–6d** for 2 h, the HT29 cells showed a strong intracellular fluorescence image throughout the cytoplasm with similar brightness, indicating that there was substantial uptake of these dyes. However, the intensity was much weaker when the dyes **9a–9b** were used (ESI, Fig. S3†). Hence, the lower photocytotoxicity of **9a–9b** may be attributed to their higher aggregation tendency in the biological media and/or lower cellular uptake.

Conclusions

In summary, we have prepared and characterised a new series of di-α-substituted zinc(II) phthalocyanine derivatives. These rare disubstituted phthalocyanines possess a high solubility, low aggregation tendency, and high efficiency in generating singlet oxygen, rendering them to be useful as photosensitisers. The two substituents adding at the α positions also shift the Q-band absorption to the red, which is desirable for PDT application. Addition of a fused benzene ring, however, promotes aggregation of the macrocycles in biological media, resulting in a lower photocytotoxicity.

Experimental

The experimental details regarding the purification of solvents, instrumentation, photophysical measurements, and *in vitro* studies were described previously.[5a] The tosylates **2a**,[13] **2b**[14] and **2c**,[15] and 1,4-dihydroxy-2,3-naphthalonitrile (**7**)[16] were prepared as described.

3,6-Bis(8-hydroxy-3,6-dioxaoctoxy)phthalonitrile (4a)

A mixture of 2,3-dicyanohydroquinone (**3**) (2.40 g, 15.0 mmol), tosylate **2a** (9.12 g, 30.0 mmol) and K_2CO_3 (4.14 g, 30.0 mmol) in DMF (20 mL) was stirred at 90 °C under an atmosphere of nitrogen for 24 h. The volatiles were removed *in vacuo*. The residue was mixed with water (100 mL), then extracted with CH_2Cl_2 (3 × 80 mL). The combined organic extracts was dried over anhydrous $MgSO_4$ and then evaporated to dryness under reduced pressure. The residue was chromatographed on a silica gel column using $CHCl_3$–CH_3OH (100 : 1 v/v) as the eluent. The product was obtained as a white solid (5.02 g, 79%). M.p. 78.4–79.0 °C. ^{1}H NMR (300 MHz, DMSO-d_6): δ 7.64 (s, 2 H, ArH), 4.58 (t, J = 5.4 Hz, 2 H, OH), 4.30 (vt, J = 4.5 Hz, 4 H, CH_2), 3.77 (vt, J = 4.5 Hz, 4 H, CH_2), 3.59–3.62 (m, 4 H, CH_2), 3.51–3.54 (m, 4 H, CH_2), 3.46 (t, J = 4.8 Hz, 4 H, CH_2), 3.39–3.42 (m, 4 H, CH_2). ^{13}C{^{1}H} NMR (75.4 MHz, $CDCl_3$): δ 155.4, 119.4, 113.0, 105.4, 72.4, 71.1, 70.3, 70.1, 69.4, 61.6. MS (ESI): m/z 447 (100%, $[M + Na]^+$). HRMS (ESI) calcd for $C_{20}H_{28}N_2NaO_8$ $[M + Na]^+$ 447.1738, found: 447.1745. Anal. calcd for $C_{21}H_{32}N_2O_9$ (**4a**·CH_3OH): C 55.25, H 7.07, N 6.14. Found: C 55.33, H 6.73, N 6.30.

3,6-Bis(3,6,9-trioxadecoxy)phthalonitrile (4b)

According to the above procedure, 2,3-dicyanohydroquinone (**3**) (1.60 g, 10.0 mmol) was treated with tosylate **2b** (6.36 g, 20.0 mmol) and K_2CO_3 (2.76 g, 20.0 mmol) to give phthalonitrile **4b** as a white solid (3.81 g, 84%). M.p. 49.6–50.2 °C. ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.25 (s, 2 H, ArH), 4.24 (t, J = 4.8 Hz, 4 H, CH_2), 3.90 (t, J = 4.8 Hz, 4 H, CH_2), 3.74–3.77 (m, 4 H, CH_2), 3.64–3.69 (m, 8 H, CH_2), 3.54–3.57 (m, 4 H, CH_2), 3.38 (s, 6 H, CH_3). ^{13}C{^{1}H} NMR (75.4 MHz, $CDCl_3$): δ 155.3, 119.2, 112.9, 105.5, 71.9, 71.0, 70.6, 70.5, 70.1, 69.4, 59.0. MS (ESI): m/z 475 (100%, $[M + Na]^+$). HRMS (ESI) calcd for $C_{22}H_{32}N_2NaO_8$ $[M + Na]^+$ 475.2051, found: 475.2046. Anal. calcd for $C_{22}H_{32}N_2O_8$: C 58.40, H 7.13, N 6.19. Found: C 58.60, H 7.06, N 6.15.

3,6-Bis(2,2-dimethyl-1,3-dioxol-4-ylmethoxy)phthalonitrile (4c)

According to the above procedure, 2,3-dicyanohydroquinone (**3**) (4.27 g, 26.7 mmol) was treated with tosylate **2c** (15.28 g, 53.4 mmol) and K_2CO_3 (7.45 g, 53.9 mmol) to give **4c** as a white solid (8.97 g, 86%). M.p. 174.5–175.4 °C. ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.24 (s, 2 H, ArH), 4.44–4.53 (m, 2 H, CH), 4.13–4.22 (m, 4 H, CH), 4.05–4.10 (m, 2 H, CH), 3.97–4.02 (m, 2 H, CH), 1.44 (s, 6 H, CH_3), 1.39 (s, 6 H, CH_3). ^{13}C{^{1}H} NMR (75.4 MHz, $CDCl_3$): δ 155.0, 118.9, 112.6, 110.0, 105.8, 73.5, 70.2, 66.4, 26.7, 25.2. MS (ESI): m/z 411 (100%, $[M + Na]^+$). HRMS (ESI) calcd for $C_{20}H_{24}N_2NaO_6$ $[M + Na]^+$ 411.1527, found: 411.1532. Anal. calcd for $C_{20}H_{24}N_2O_6$: C 61.85, H 6.23, N 7.21. Found: C 61.80, H 6.33, N 6.96.

[1,4-Bis(8-hydroxy-3,6-dioxaoctoxy)phthalocyaninato]zinc(II) (6a)

A mixture of phthalonitrile **4a** (0.50 g, 1.2 mmol), unsubstituted phthalonitrile (**5**) (1.36 g, 10.6 mmol), and $Zn(OAc)_2{\cdot}2H_2O$ (0.65 g, 3.0 mmol) in 1-pentanol (15 mL) was heated to 100 °C, then 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 $CHCl_3$ (150 mL), then filtered to remove part of 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 [Bio-Rad Bio-Beads S-X1 beads (200–400 mesh)] using THF as the eluent. The crude product was further purified by recrystallisation from a mixture of THF and hexane to give phthalocyanine **6a** as a blue solid (0.19 g, 18%). ^{1}H NMR (300 MHz, $CDCl_3$ with a trace amount of pyridine-d_5): δ 9.37–9.41 (m, 4 H, Pc–H_α), 9.25 (d, J = 6.0 Hz, 2 H, Pc–H_α), 8.06–8.15 (m, 6 H, Pc–H_β), 7.39 (s, 2 H, Pc–H_β), 4.91 (t, J = 4.8 Hz, 4 H, CH_2), 4.49 (t, J = 4.8 Hz, 4 H, CH_2), 4.11–4.15 (m, 4 H, CH_2), 3.85–3.88 (m, 4 H, CH_2), 3.68–3.71 (m, 4 H, CH_2), 3.63–3.66 (m, 4 H, CH_2). ^{13}C{^{1}H} NMR (75.4 MHz, $CDCl_3$ with a trace amount of pyridine-d_5): δ 153.8, 153.7, 153.5, 152.4, 150.2, 138.7, 138.4, 128.9, 128.8, 127.2, 122.6, 122.4, 114.9, 72.7, 71.0, 70.4, 69.1, 61.3 (some of the Pc and chain signals are overlapped). MS (ESI): an isotopic cluster peaking at m/z 873 (100%, $[M + H]^+$). HRMS (ESI) calcd for $C_{44}H_{41}N_8O_8Zn$ $[M + H]^+$ 873.2333, found: 873.2342. Anal. calcd for $C_{44}H_{40}N_8O_8Zn$: C 60.45, H 4.61, N 12.82. Found: C 60.49, H 4.24, N 12.83.

[1,4-Bis(3,6,9-trioxadecoxy)phthalocyaninato]zinc(II) (6b)

According to the above procedure, phthalonitrile **4b** (0.50 g, 1.1 mmol) was treated with unsubstituted phthalonitrile (**5**) (1.28 g, 10.0 mmol) and $Zn(OAc)_2{\cdot}2H_2O$ (0.61 g, 2.8 mmol) to give **6b** as a blue solid (0.12 g, 12%). ^{1}H NMR (300 MHz, $CDCl_3$ with a trace amount of pyridine-d_5): δ 9.41–9.49 (m, 6 H, Pc–H_α), 8.15–8.18 (m, 6 H, Pc–H_β), 7.58 (s, 2 H, Pc–H_β), 4.98 (t, J = 5.1 Hz, 4 H, CH_2), 4.56 (t, J = 5.1 Hz, 4 H, CH_2), 4.15–4.18 (m, 4 H, CH_2), 3.86–3.89 (m, 4 H, CH_2), 3.69–3.72 (m, 4 H, CH_2), 3.52–3.55 (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, 153.4, 153.3, 152.2, 150.0, 138.7, 138.3, 138.2, 128.7, 127.0, 122.5, 122.3, 114.6, 71.8, 71.0, 70.7, 70.5 70.4, 68.9, 58.9 (some of the Pc signals are overlapped). MS (ESI): an isotopic cluster peaking at m/z 901 (100%, $[M + H]^+$). HRMS (ESI) calcd for $C_{46}H_{45}N_8O_8Zn$ $[M + H]^+$ 901.2646, found: 901.2645. Anal. calcd for $C_{46}H_{44}N_8O_8Zn$: C 61.23, H 4.92, N 12.42. Found: C 61.21, H 5.02, N 12.04.

[1,4-Bis(2,2-dimethyl-1,3-dioxol-4-ylmethoxy)phthalocyaninato]zinc(II) (6c)

According to the above procedure, phthalonitrile **4c** (0.50 g, 1.3 mmol) was treated with unsubstituted phthalonitrile (**5**) (1.49 g, 11.6 mmol) and $Zn(OAc)_2{\cdot}2H_2O$ (0.71 g, 3.2 mmol) to give phthalocyanine **6c** as a blue solid (0.17 g, 16%). ^{1}H NMR (300 MHz, $CDCl_3$ with a trace amount of pyridine-d_5): δ 9.32–9.37 (m, 4 H, Pc–H_α), 9.13 (d, J = 7.2 Hz, 2 H, Pc–H_α), 8.00–8.13 (m, 6 H, Pc–H_β), 7.22 (d, J = 8.1 Hz, 2 H, Pc–H_β), 5.14–5.24 (m, 2 H, CH), 4.73–4.85 (m, 2 H, CH), 4.57–4.72 (m, 4 H, CH), 4.45–4.51 (m, 2 H, CH), 1.72 (s, 6 H, CH_3), 1.66 (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, 153.4, 153.0, 151.7, 149.7, 138.7, 138.3, 128.7, 126.8, 122.4, 122.3, 122.2, 114.0, 113.9, 109.8, 74.8, 70.3, 67.3, 27.0, 25.6 (some of the Pc signals are overlapped). MS (ESI): an isotopic cluster peaking at m/z 837 (100%, $[M + H]^+$). HRMS (ESI) calcd for $C_{44}H_{37}N_8O_6Zn$ $[M + H]^+$ 837.2122, found: 837.2133. Anal. calcd for $C_{44}H_{36}N_8O_6Zn$: C 63.05, H 4.33, N 13.37. Found: C 63.32, H 4.49, N 13.05.

[1,4-Bis(2,3-dihydroxypropoxy)phthalocyaninato]zinc(II) (6d)

A solution of phthalocyanine **6c** (60 mg, 0.07 mmol) in TFA–H_2O (9 : 1 v/v) (4 mL) was stirred at room temperature for 30 min. The volatiles were then removed under reduced pressure. The residue was dissolved in a mixture of THF (2 mL) and CH_3OH (4 mL), then hexane (8 mL) was added to induce precipitation. The mixture was filtered to give a blue solid, which was dried *in vacuo* (46 mg, 85%). ^{1}H NMR (300 MHz, DMSO-d_6): δ 9.32–9.41 (m, 6 H, Pc–H_α), 8.19–8.26 (m, 6 H, Pc–H_β), 7.77 (s, 2 H, Pc–H_β), 5.56 (d, J = 4.5 Hz, 2 H, OH), 4.96 (t, J = 5.7 Hz, 2 H, OH), 4.66–4.77 (m, 4 H, CH), 4.46–4.51 (m, 2 H, CH), 4.16–4.24 (m, 2 H, CH), 4.00–4.08 (m, 2 H, CH). ^{13}C{^{1}H} NMR (75.4 MHz, DMSO-d_6): δ 153.1, 152.9, 152.7, 150.6, 138.4, 138.1, 138.0, 129.5, 129.6, 129.7, 126.7, 123.1, 122.6, 116.5, 71.7, 70.9, 63.4 (some of the Pc signals are overlapped). MS (ESI): an isotopic cluster peaking at m/z 757 (100%, $[M + H]^+$). HRMS (ESI) calcd for $C_{38}H_{29}N_8O_6Zn$ $[M + H]^+$ 757.1496, found: 757.1480.

1,4-Bis(8-hydroxy-3,6-dioxaoctoxy)-2,3-naphthalonitrile (8a)

A mixture of 1,4-dihydroxy-2,3-naphthalonitrile (**7**) (1.26 g, 6.0 mmol), tosylate **2a** (3.65 g, 12.0 mmol), and anhydrous K_2CO_3 (1.66 g, 12.0 mmol) in DMF (12 mL) was stirred vigorously at 90 °C for 24 h. The volatiles were then removed under reduced pressure. The residue was mixed with water (60 mL) and the mixture was extracted with chloroform (3 × 60 mL). The combined organic extracts was dried over anhydrous $MgSO_4$ and evaporated to dryness under reduced pressure. The residue was subject to silica gel column chromatography using $CHCl_3$–CH_3OH (60 : 1 v/v) as the eluent. Compound **8a** was obtained as a brown oil (2.53 g, 89%). ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.38 (dd, J = 3.3, 6.3 Hz, 2 H, ArH), 7.79 (dd, J = 3.3, 6.3 Hz, 2 H, ArH), 4.57–4.60 (m, 4 H, CH_2), 3.96–3.99 (m, 4 H, CH_2), 3.68–3.77 (m, 12 H, CH_2), 3.58–3.61 (m, 4 H, CH_2), 2.49 (br s, 2 H, OH). ^{13}C{^{1}H} NMR (75.4 MHz, $CDCl_3$): δ 157.5, 130.7, 130.3, 123.9, 114.2, 99.6, 75.1, 72.4, 70.7, 70.2, 70.1, 61.6. MS (ESI): m/z 497 (100%, $[M + Na]^+$). HRMS (ESI) calcd for $C_{24}H_{30}N_2NaO_8$ $[M + Na]^+$ 497.1894, found: 497.1893.

1,4-Bis(3,6,9-trioxadecoxy)-2,3-naphthalonitrile (8b)

According to the above procedure, 1,4-dihydroxy-2,3-naphthalonitrile (**7**) (0.63 g, 3.0 mmol) was treated with tosylate **2b** (1.91 g, 6.0 mmol) and K_2CO_3 (0.83 g, 6.0 mmol) to give **8b** as a brown oil (1.31 g, 87%). ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.41 (dd, J = 3.3, 6.3 Hz, 2 H, ArH), 7.78 (dd, J = 3.3, 6.3 Hz, 2 H, ArH), 4.58 (vt, J = 4.5 Hz, 4 H, CH_2), 3.96 (vt, J = 4.5 Hz, 4 H, CH_2), 3.74–3.77 (m, 4 H, CH_2), 3.64–3.70 (m, 8 H, CH_2), 3.53–3.57 (m, 4 H, CH_2), 3.38 (s, 6 H, CH_3). ^{13}C{^{1}H} NMR (75.4 MHz, $CDCl_3$): δ 157.6, 130.6, 130.4, 124.1, 114.3, 99.6, 75.2, 71.9, 70.8, 70.6, 70.5, 70.1, 59.0. MS (ESI): m/z 525 (100%, $[M + Na]^+$). HRMS (ESI) calcd for $C_{26}H_{34}N_2NaO_8$ $[M + Na]^+$ 525.2207, found: 525.2210.

Benzo-fused phthalocyanine 9a

According to the procedure described for **6a**, naphthalonitrile **8a** (0.60 g, 1.3 mmol) was treated with unsubstituted phthalonitrile (**5**) (1.46 g, 11.4 mmol) and $Zn(OAc)_2{\cdot}2H_2O$ (0.70 g, 3.2 mmol) to give **9a** as a green solid (0.17 g, 15%). ^{1}H NMR (300 MHz, $CDCl_3$ with a trace amount of pyridine-d_5): δ 9.20–9.35 (m, 6 H, Pc–H_α), 9.02–9.06 (m, 2 H, Np–H_α), 7.98–8.09 (m, 6 H, Pc–H_β), 7.87–7.90 (m, 2 H, Np–H_β), 5.63 (vt, J = 4.5 Hz, 4 H, CH_2), 4.45 (vt, J = 4.5 Hz, 4 H, CH_2), 4.02–4.05 (m, 4 H, CH_2), 3.90–3.94 (m, 4 H, CH_2), 3.81–3.85 (m, 4 H, CH_2), 3.74–3.77 (m, 4 H, CH_2). ^{13}C{^{1}H} NMR (75.4 MHz, $CDCl_3$ with a trace amount of pyridine-d_5): δ 154.1, 154.0, 152.6, 152.3, 148.5, 138.3, 138.2, 137.9, 130.8, 129.0, 128.8, 128.5, 127.3, 125.6, 124.3, 122.5, 122.4, 122.2, 74.2, 72.8, 71.4, 71.0, 70.6, 61.6. MS (ESI): an isotopic cluster peaking at m/z 945 (100%, $[M + Na]^+$). HRMS (ESI) calcd for $C_{48}H_{42}N_8NaO_8Zn$ $[M + Na]^+$ 945.2309, found: 945.2297. Anal. calcd for $C_{49}H_{46}N_8O_9Zn$ (**9a**·CH_3OH): C 61.54, H 4.85, N 11.72. Found: C 61.66, H 4.80, N 11.67.

Benzo-fused phthalocyanine 9b

According to the procedure described for **6a**, naphthalonitrile **8b** (0.44 g, 0.9 mmol) was treated with unsubstituted phthalonitrile

(**5**) (1.01 g, 7.9 mmol) and $Zn(OAc)_2 \cdot 2H_2O$ (0.48 g, 2.2 mmol) to give **9b** as a green solid (0.11 g, 13%). ^{1}H NMR (300 MHz, $CDCl_3$ with a trace amount of pyridine-d_5): δ 9.41 (dd, J = 2.7, 5.7 Hz, 2 H, Pc–H_α), 9.37 (dd, J = 2.7, 5.7 Hz, 2 H, Pc–H_α), 9.29 (dd, J = 3.0, 5.4 Hz, 2 H, Pc–H_α), 9.08 (dd, J = 3.3, 6.3 Hz, 2 H, Np–H_α), 8.09–8.15 (m, 4 H, Pc–H_β), 8.04 (dd, J = 2.7, 5.7 Hz, 2 H, Pc–H_β), 7.91 (dd, J = 3.3, 6.3 Hz, 2 H, Np–H_β), 5.64 (vt, J = 4.5 Hz, 4 H, CH_2), 4.46 (vt, J = 4.5 Hz, 4 H, CH_2), 4.04–4.07 (m, 4 H, CH_2), 3.91–3.94 (m, 4 H, CH_2), 3.79–3.82 (m, 4 H, CH_2), 3.61–3.65 (m, 4 H, CH_2), 3.41 (s, 6 H, CH_3). $^{13}C\{^1H\}$ NMR (75.4 MHz, $CDCl_3$ with a trace amount of pyridine-d_5): δ 154.3, 154.2, 152.8, 152.4, 148.7, 138.4, 138.3, 137.9, 130.9, 129.0, 128.9, 128.6, 127.3, 125.7, 124.4, 122.6, 122.5, 122.2, 74.3, 71.9, 71.3, 71.0, 70.8, 70.6, 59.0. MS (ESI): an isotopic cluster peaking at m/z 951 (100%, $[M + H]^+$). HRMS (ESI) calcd for $C_{50}H_{47}N_8O_8Zn$ $[M + H]^+$ 951.2803, found: 951.2805. Anal. calcd for $C_{51}H_{50}N_8O_9Zn$ (**9b**·CH_3OH): C 62.23, H 5.12, N 11.38. Found: C 61.69, H 4.90, N 11.44.

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References

1 (*a*) D. E. J. G. J. Dolmans, D. Fukumura and R. K. Jain, *Nat. Rev. Cancer*, 2003, **3**, 380; (*b*) S. B. Brown, E. A. Brown and I. Walker, *Lancet Oncol.*, 2004, **5**, 497; (*c*) A. P. Castano, P. Mroz and M. R. Hamblin, *Nat. Rev. Cancer*, 2006, **6**, 535; (*d*) B. C. Wilson and M. S. Patterson, *Phys. Med. Biol.*, 2008, **53**, R61.
2 G. Jori, *J. Photochem. Photobiol. A: Chem.*, 1992, **62**, 371.
3 (*a*) M. R. Detty, S. L. Gibson and S. J. Wagner, *J. Med. Chem.*, 2004, **47**, 3897; (*b*) E. S. Nyman and P. H. Hynninen, *J. Photochem. Photobiol. B: Biol.*, 2004, **73**, 1.
4 (*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.
5 See for example: (*a*) P.-C. Lo, C. M. H. Chan, J.-Y. Liu, W.-P. Fong and D. K. P. Ng, *J. Med. Chem.*, 2007, **50**, 2100 and references cited therein; (*b*) J.-Y. Liu, X.-J. Jiang, W.-P. Fong and D. K. P. Ng, *Metal-Based Drugs*, 2008, Article ID 284691; (*c*) S. C. H. Leung, P.-C. Lo, D. K. P. Ng, W.-K. Liu, K.-P. Fung and W.-P. Fong, *Br. J. Pharm.*, 2008, **154**, 4; (*d*) C.-F. Choi, J.-D. Huang, P.-C. Lo, W.-P. Fong and D. K. P. Ng, *Org. Biomol. Chem.*, 2008, **6**, 2173; (*e*) P.-C. Lo, W.-P. Fong and D. K. P. Ng, *ChemMedChem*, 2008, **3**, 1110.
6 (*a*) T. Fukuda, S. Homma and N. Kobayashi, *Chem.–Eur. J.*, 2005, **11**, 5205; (*b*) W. Maqanda, T. Nyokong and M. D. Maree, *J. Porphyrins Phthalocyanines*, 2005, **9**, 343; (*c*) H. Li, F. R. Fronczek and M. G. H. Vicente, *Tetrahedron Lett.*, 2008, **49**, 4828; (*d*) J.-Y. Liu, X.-J. Jiang, W.-P. Fong and D. K. P. Ng, *Org. Biomol. Chem.*, 2008, **6**, 4560.
7 (*a*) S. Stolnik, L. Illum and S. S. Davis, *Adv. Drug Deliv. Rev.*, 1995, **16**, 195; (*b*) V. P. Torchilin, *Pharm. Res.*, 2007, **24**, 1; (*c*) L. E. van Vlerken, T. K. Vyas and M. M. Amiji, *Pharm. Res.*, 2007, **24**, 1405.
8 I. J. MacDonald and T. J. Dougherty, *J. Porphyrins Phthalocyanines*, 2001, **5**, 105.
9 P.-C. Lo, B. Zhao, W. Duan, W.-P. Fong, W.-H. Ko and D. K. P. Ng, *Bioorg. Med. Chem. Lett.*, 2007, **17**, 1073.
10 (*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.
11 C.-F. Choi, P.-T. Tsang, J.-D. Huang, E. Y. M. Chan, W.-H. Ko, W.-P. Fong and D. K. P. Ng, *Chem. Commun.*, 2004, 2236.
12 (*a*) P.-C. Lo, J.-D. Huang, D. Y. Y. Cheng, E. Y. M. Chan, W.-P. Fong, W.-H. Ko and D. K. P. Ng, *Chem.–Eur. J.*, 2004, **10**, 4831; (*b*) P.-C. Lo, S. C. H. Leung, E. Y. M. Chan, W.-P. Fong, W.-H. Ko and D. K. P. Ng, *Photodiag. Photodyn. Ther.*, 2007, **4**, 117.
13 X.-Z. Zhu and C.-F. Chen, *J. Am. Chem. Soc.*, 2005, **127**, 13158.
14 A. W. Snow and E. E. Foos, *Synthesis*, 2003, 509.
15 J. S. Bradshaw, P. Huszthy, C. W. McDaniel, C.-Y. Zhu, N. K. Dalley and R. M. Izatt, *J. Org. Chem.*, 1990, **55**, 3129.
16 G. A. Reynolds and J. A. VanAllan, *J. Org. Chem.*, 1964, **29**, 3591.