DOI: 10.1002/chem.201101123

Red-Emitting Dyes with Photophysical and Photochemical Properties Controlled by pH

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Abstract: New unsymmetrical zinc azaphthalocyanines, bearing one substituted aniline as a peripheral substituent, were prepared by using a statistical condensation approach. Both fluorescence and singlet oxygen quantum yields were extremely low in DMF ($\Phi_F < 0.01$, $\Phi_\Delta < 0.02$, respectively), but increased after the addition of sulfuric acid, reaching values comparable to controls without aniline substituents ($\Phi_F = 0.22$–0.29, $\Phi_\Delta = 0.40$–0.59, respectively). This behavior was attributed to the deactivation of excited states by intramolecular charge transfer from a donor site (aniline), which was blocked after protonation in acidic media. In the protonated form, all of the compounds efficiently emitted light with λ_{em} in the region of 662–675 nm. The investigated compounds were anchored to dioleoylphosphatidylcholine (DOPC) unilamellar vesicles and showed response to buffer pH. They were highly fluorescent at low pH values and almost nonfluorescent in neutral solutions. The pK_a values were determined in DOPC vesicles and ranged between 2.2 and 4.2.

Keywords: dyes/pigments · fluorescence · liposomes · phthalocyanines · sensors · singlet oxygen

Introduction

Changes in photophysical and photochemical parameters of different dyes depending on the surrounding environment has been widely utilized in last few decades for the development of various sensors or imaging agents.[1,2] In particular, reversibly switching between ON and OFF states based on pH changes has recently gained attention in the visualization or more selective treatment of tumors using photodynamic therapy (PDT).[3–6] Generally, two mechanisms leading to the ON–OFF switching were recently used for the design of new pH dependent probes. The first one employs static quenching (OFF) between two molecules of a chromophore[5] or between the chromophore and a quencher.[4] The subsequent changes in the pH lead to an increase in the distance between these dyes and the loss of quenching (ON state). Another approach is based on photoinduced electron transfer (PET) from amines to aromatic system of the chromophore that quenches the excited states.[3,6,7] The wavelength of excitation and emission of the dyes employed in these applications are optimally over 640 nm, because of deeper penetration of the light through tissues and the elimination of possible autofluorescence of the endogenous chromophores, for example hemoglobin.[1]

Phthalocyanines (Pc) and their aza analogues, azaphthalocyanines (AzaPc), are characterized by strong absorptions, which are usually over 650 nm, good chemical stability, low photobleaching, and sufficient quantum yields of fluorescence (Φ_F) and singlet oxygen (Φ_Δ).[8,9] Singlet oxygen produced after excitation of a photosensitizer is essential for phototoxic effects in PDT.[10] For these reasons, Pc and AzaPc represent ideal chromophore systems for the above-mentioned applications. Despite this, the first and only Pc with pH controlled photochemical and photophysical properties was developed only recently.[6,11] A likely explanation for a lack of extensive studies in this area is that Pc macrocycles behave mostly as electron donors and not as acceptors.[12] The switching between ON and OFF states in sensors is usually based on electron transfer from a donor (usually amine) to an acceptor (a macrocycle dye), as in the case of the silicon Pc substituted axially with aliphatic polyamine chains.[6,11] Recently, we independently showed that intramolecular charge transfer (ICT) from peripherally substituted aromatic amines in AzaPc strongly influences Φ_F and Φ_Δ values.[13,14] The ICT occurs when the donor amines are conjugated with AzaPc macrocycle. The ICT in AzaPc was also shown to be easily controlled by environmental properties,[13,14] similarly as was observed for PET in porphyrinoid systems.[15,16]

In the presented work, several new AzaPc compounds which emit in the red portion of the UV/Vis spectrum were

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Supporting information for this article is available on the WWW under http://dx.doi.org/10.1002/chem.201101123.

prepared, and their fluorescence and singlet oxygen production with regards to pH is described. The aim of this work was to develop compounds that extend the group of Pc and AzaPc dyes to also be sensitive to lower pH values, which may not be always achievable with strategies using more basic aliphatic amines.[17] The sensitivity to lower pH that occurs in lysosomes was recently successfully applied in the imaging of viable cancer cells in vivo with BODIPY (BODIPY = borondipyrromethene) antibody conjugates with pK_a values in the 4.4–5.8 range.[3]

Results and Discussion

Synthesis: The design of the molecules was based on the following rational considerations. The photophysical and photochemical properties of AzaPc or Pc may be controlled by PET or ICT as shown previously.[6,14] According to our experience, ICT, in which the donor is directly conjugated with macrocyclic system, is more efficient than PET, in which the electron must "jump" through space. In particular, the distance plays a crucial role in the PET quenching of the excited states.[18] The high efficiency of ICT is supported by the fact that only one donor center in ICT is enough to efficiently switch off the excited states.[14] Since the probes are intended to be used at lower pH values, the substituted aniline moiety with generally lower and substituent tunable pK_a values seems to be well suited to carry the donor. Besides the pH sensitive moiety, bulky substituents should be introduced to the periphery of the macrocycle to prevent a self-aggregation that is a well-known phenomenon of planar aromatic systems and decreases the photophysical and photochemical properties without any control. *tert*-Butylsulfanyl substituents have been shown to efficiently inhibit the aggregation both in organic solvents and liposomal membranes.[19,20] Zinc(II) as the central cation is known to increase triplet state lifetimes of Pc and AzaPc and gives the macrocycle reasonable fluorescence and singlet oxygen quantum yields.[8] All of the above-mentioned conditions were combined into a model compound depicted in Figure 1 in which substituents R^1 and R^2 are intended to modulate the basicity of the donor. The substituent R^3 is present only for synthetic reasons and does not play an important role in the mechanism of pH sensitivity.

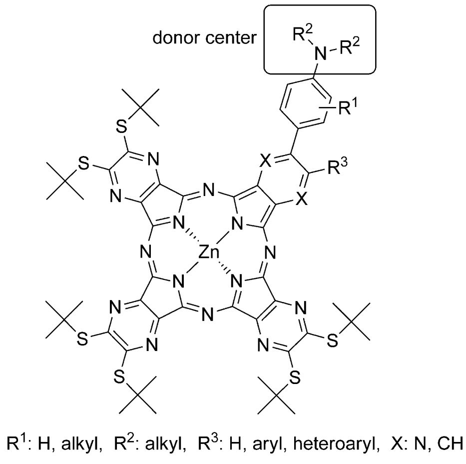
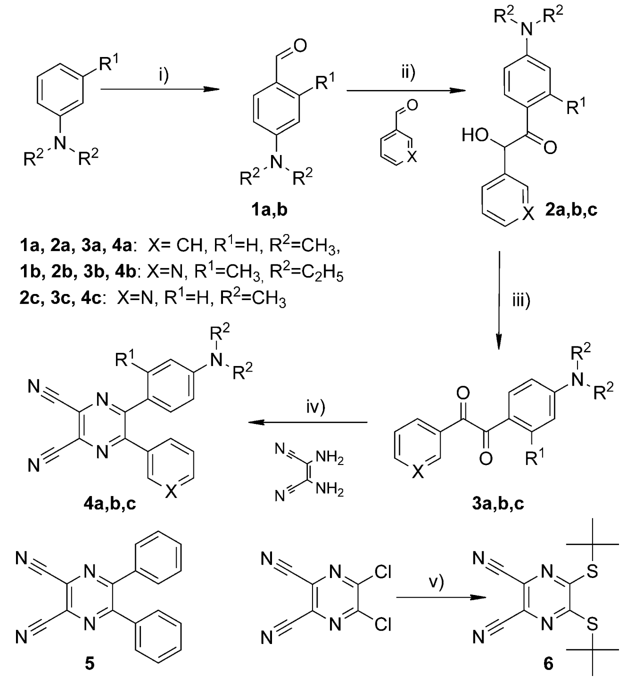


Figure 1. Models of pH sensitive compounds investigated in this work.

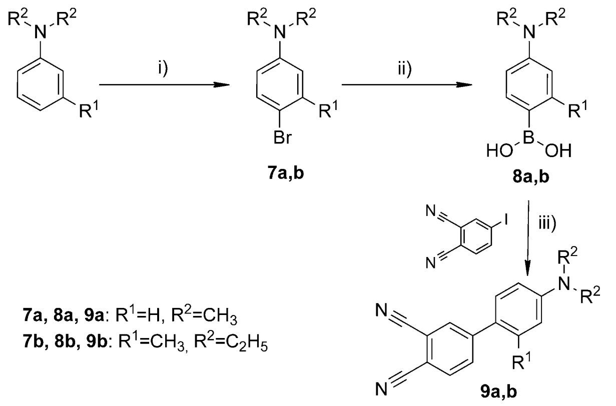
The general methods leading to Pc and AzaPc core formations employed cyclotetramerization of the corresponding precursors, substituted phthalonitriles and pyrazine-2,3-dicarbonitriles, respectively.[21] Because the compounds involved in this study combined both Pc and AzaPc units, different synthetic routes had to be considered in the syntheses of the precursors. The 5,6-disubstituted pyrazine-2,3-dicarbonitriles can be prepared either from 5,6-dichloropyrazine-2,3-dicarbonitrile by nucleophilic substitution[22] or by condensation of the appropriate diketone with diaminomaleonitrile (DAMN).[23] The former procedure is suitable for the alkylheteroatom-substituted pyrazines and was used for synthesis of **6** (Scheme 1). The latter method was applied for the pyrazines with the substituents connected through C−C bonds (**4a–c** and **5**) following the synthesis of the appropriate diketones **3a–c** (Scheme 1). Thus, Vilsmeier–Haack formylation was used for the synthesis of the aldehyde **1b**,[24] which was then treated with nicotinaldehyde under benzoin condensation conditions to form unsymmetrical acyloin **2b** in a moderate yield of 20% (Scheme 1). Similarly, commercially available **1a** gave acyloins **2a** and **2c** after condensation with benzaldehyde or nicotinaldehyde, respectively (Scheme 1).[13] Oxidation of the acyloins with copper(II) gave diketones **3a–c** that reacted with DAMN to produce the desired pyrazine-2,3-dicarbonitriles **4a–c**. Similarly, the



Scheme 1. Reaction conditions: i) $POCl_3$, DMF, RT; ii) KCN, EtOH/water, reflux; iii) $Cu(CH_3COO)_2$, NH_4NO_3, acetic acid, reflux; iv) acetic acid, reflux; and v) NaOH/water, THF, $(CH_3)_3CSH$, RT.

condensation of benzil with DAMN gave compound **5** (Scheme 1).[25]

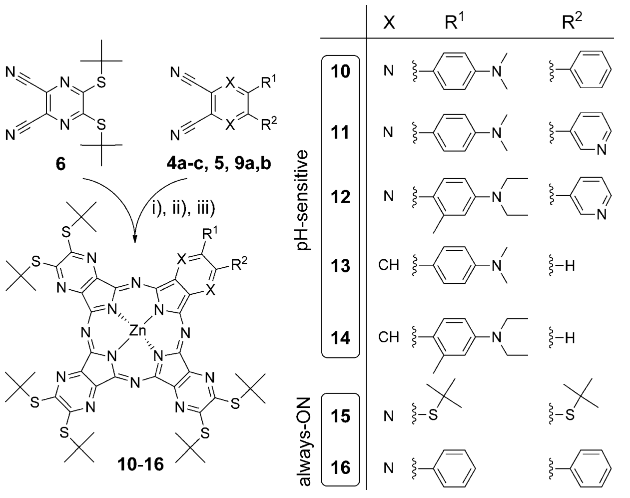
The electronic effects of benzene are dissimilar to a pyrazine ring and that is why different procedures had to be applied for the synthesis of 4-substituted phthalonitriles **9a** and **9b** (Scheme 2). Thus, bromination of *N,N*-diethyl-3-methylaniline with *N*-bromosuccinimide (NBS) gave the compound **7b** in excellent yield of 93% (Scheme 2). Compounds **7a** (available commercially) and **7b** were subsequently converted to phenylboronic acids **8a** and **8b**, which underwent Suzuki coupling with 4-iodophthalonitrile to give the desired **9a** and **9b** in good yields of 78 and 56%, respectively (Scheme 2). Originally, we intended to include also 4′-(diethylamino)-3′-methylbiphenyl-3,4-dicarbonitrile (see Supporting Information) into the study. However, all of the tested routes to 4-halogeno-*N,N*-diethyl-2-methylaniline, which is a key precursor of the substituted phenylboronic acid, failed. These routes are widely discussed in the Supporting Information and involved the bromination and iodination of *N,N*-diethyl-2-methylaniline with various agents or several alkylation attempts of 4-bromo-2-methylaniline.



Scheme 2. Reaction conditions: i) NBS, DMF, RT; ii) triisopropylborate, THF, BuLi/hexane, −78°C; and iii) K_2CO_3, $[Pd(PPh_3)_4]$, THF/water, reflux.

The above precursors were used for the synthesis of AzaPc compounds **10**–**16** (Scheme 3). In the case of the unsymmetrical Pc and AzaPc derivatives, two different units (A and B) are involved in the cyclization. Their random combination during the reaction gives a statistical mixture consisting of AAAA, ABBB, ABAB, AABB, AAAB, and BBBB congeners. The desired congener was then separated by chromatographic methods. Although some selective methods have been published,[26] statistical condensation still remains a useful and reliable tool for preparation of the ABBB congener.[27]

Thus, the statistical condensation of the precursors **4a**–**c**, **5**, and **9a**–**b** (A) with **6** (B, Scheme 3) in a 1:3 molar ratio in butanol with magnesium(II) butoxide gave a statistical mixture of the magnesium(II) complexes. Subsequent removal of the central magnesium(II) by *p*-toluenesulfonic acid afforded metal-free dyes that were separated over silica, and the ABBB congeners were isolated. The isolation of the desired congener was performed in the metal-free form, because of strong tailing and overlapping of the fractions of the metal complexes (both magnesium(II) and zinc(II)). The last step involved chelation of zinc(II) into the center of the complex by heating the metal-free derivative in DMF with anhydrous zinc acetate. The overall yields of the statistical condensation, isolation and metal exchange in AzaPc were in a range of 7–14%. The symmetrical compound **15** was prepared by simple cyclotetramerization of **6** as previously described.[20] Compounds **15** and **16** were used as “always-ON” controls. Since they did not contain a donor center for ICT, they were expected not to respond to the pH changes.



Scheme 3. Reaction conditions: i) Mg, butanol, reflux; ii) *p*-toluenesulfonic acid, THF, RT; and iii) $Zn(CH_3COO)_2$, DMF, reflux.

Photophysical and photochemical characterization: All of the prepared AzaPc compounds showed absorption spectra typical for Pc and AzaPc compounds with a high-energy B band in region of 377–390 nm and a low-energy Q-band in the region of 655–670 nm with two vibration bands in region of 600–640 nm (Table 1, Figure 2). The absorption spectra indicated the presence of only the monomeric form; no aggregation was detected during the whole study in coordinating solvents (THF, DMF). Despite the unsymmetrical composition of the macrocycle, the Q-bands were not split (Figure 2). This is due to similar electronic effects of the alkylsulfanyl and aryl substituents in the compounds **10**–**12** and **16**. Only a broadening of the Q-band was observed in the cases of **13** and **14** with one phthalonitrile moiety in the macrocycle. In these cases, the unsymmetrical composition of the macrocyclic core (three pyrazines and one benzene ring) may influence the shape of the Q-band, but the positions of the Q_x and Q_y bands were apparently close to each other, this leading to the broadening and not to splitting (Figure 2). Conjugation of the lone-pair of the donor center with a macrocyclic system was indicated by very small red-shift of λ_{max} of pH sensitive compounds (e.g., **10**–**12**) when compared with compounds without the donor (**15**, **16**). This

Table 1. Photophysical and photochemical data of compounds **10**–**16** in DMF and DMF with sulfuric acid (SA).

	Absorption λ_{max} (DMF/DMF+SA)	Emission λ_{max}	Φ_F (DMF)	Φ_F (DMF+SA)[a]	Φ_F (toluene)	Factor of increase (Φ_F)[b]	Φ_Δ (DMF)[c]	Φ_Δ (DMF+SA)[a,c]	Factor of increase (Φ_Δ)[b]	pK_a[d]
10	660/656	663	0.0036	0.29	0.0032	81	0.023	0.42	18	2.6
11	660/658	666	0.0061	0.29	0.0020	48	0.014	0.40	29	2.7
12	658/658	666	0.0050	0.26	0.010	52	0.018	0.59	33	3.4
13	671/664	675	0.0050	0.22	0.11	44	0.005	0.55	110	2.2
14	669/664	675	0.010	0.25	0.15	25	0.015	0.58	39	4.2
15	654/654	662	0.36	0.34	–	0.94	0.60	0.56	0.94	–
16	655/655	662	0.30	0.31	–	1.03	0.60	0.55	0.92	–

[a] DMF with 5% (v/v) sulfuric acid. [b] Calculated as follows: (value in DMF+SA)/(value in DMF). [c] ZnPc as reference in DMF ($\Phi_\Delta = 0.56$) or **15** as reference in DMF+SA ($\Phi_\Delta = 0.56$). [d] Determined in DOPC liposomes.

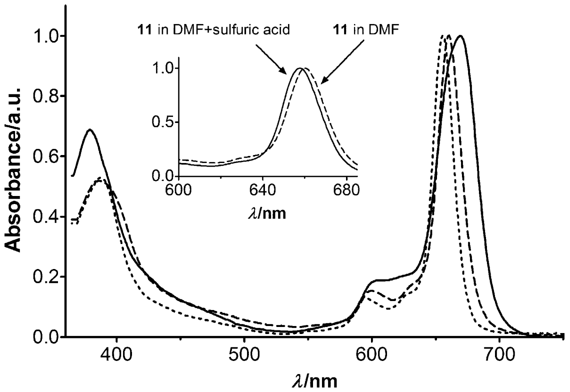


Figure 2. Absorption spectra of **11** (-----), **14** (——) and **16** (•••••) in DMF. Spectra were normalized to the same absorption in the Q-band. Inset: Normalized absorption spectra of **11** in DMF (-----) and DMF with 5% sulfuric acid (——).

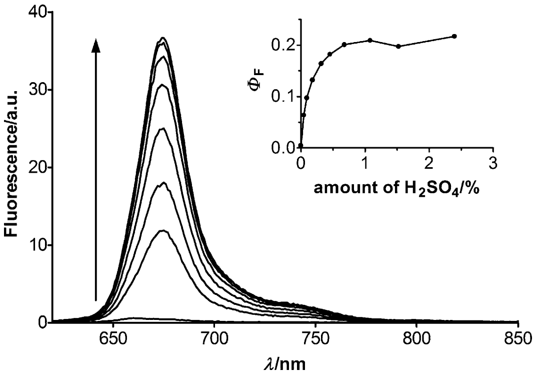


Figure 3. Fluorescence emission spectra of **13** in DMF with the addition of sulfuric acid up to 1% ($\lambda_{exc} = 603$ nm). This represents typical behavior of compounds **10**–**14**. Inset: Dependence of Φ_F value of **13** in DMF on sulfuric acid added.

redshift was partially removed by protonation of the amino moiety after the addition of sulfuric acid (5%) into the DMF solution (Table 1, Figure 2 inset).

In noncoordinating solvents, the peripheral amino substituents or pyridyl rings may be susceptible to coordination with the central zinc(II) to form supramolecular assemblies. The spectra in toluene (Supporting Information, Figure S3) showed that this happened only when the whole macrocycle was composed of pyrazine rings (compounds **10**, **11**, and **12**). The absorption spectra were typical for the formation of slipped J-dimers recently described.[14,28] In the case of compound **12**, the spectra indicated an equilibrium between the monomer and J-dimer, most likely due to steric hindrance caused by methyl in the *ortho*-position on the peripheral benzene ring. However, J-dimers were not detected in the spectra of **13** and **14**.

After excitation, the investigated compounds **10**–**16** in DMF emitted fluorescence with emission maxima in region of 663–675 nm, although the signal was very weak for compounds **10**–**14**. The fluorescence emission spectra were typical for Pc, AzaPc, and similar compounds (Figure 3).[29] Fluorescence quantum yields (Φ_F) of **10**–**14** in DMF (unsubstituted zinc(II) phthalocyanine (ZnPc) as reference, $\Phi_{F(chloronaphthalene)} = 0.30$[30]) were extremely low owing to ultrafast ICT that efficiently quenched the excited states (Table 1). As mentioned above, the compounds were in the monomeric form and, therefore, aggregation cannot account for the low quantum yields. Stepwise addition of sulfuric acid into solutions of **10**–**14** in DMF led to rapid increase of the Φ_F values as a consequence of inhibition of the ICT by protonation of the peripheral amino groups (Figure 3, Figure 4). A shift in the emission maxima or changes in the

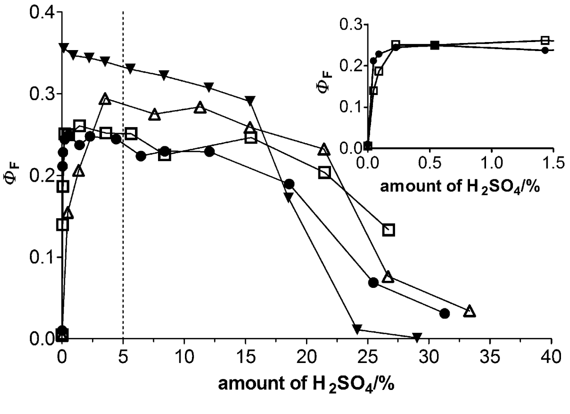


Figure 4. Dependence of Φ_F values of **10** (△), **12** (□), **14** (●), and **15** (▼) in DMF on sulfuric acid added. Inset: enlarged part of the graph for compounds **12** (□) and **14** (●).

shapes of the emission spectra were not observed during the titrations (Figure 3). All of the compounds reached a plateau for the Φ_F values, apparently after full protonation of the aniline moiety when a donor center is no longer available for the ICT. It is worth noting that Φ_F increased by almost two orders of magnitude and reached the values comparable with the always ON controls (see also Table 1). Compounds **15** and **16** in DMF showed the high Φ_F values typical for ZnAzaPc. Very slow decreases in Φ_F, which became steep after approximately 15% of the sulfuric acid was added to the solution, were observed upon acidification. This behavior may be attributed to the protonation of azomethine nitrogen atoms as deduced from changes in the absorption spectra (see Supporting Information, Figure S2). Protonation of these sites in the Pc or AzaPc macrocycles has been widely discussed in previous works and was shown to significantly decrease the fluorescence and singlet oxygen quantum yields.[13,31] Similar to **15** and **16**, protonation of the azomethine nitrogen atoms was also detected for **10**–**14**, but at higher sulfuric acid concentrations (generally above 20%, Figure 4). This does not represent an obstacle in the development of pH sensitive dyes because the basicity of the azomethine nitrogen atoms is low, and these nitrogen atoms were protonated only in strongly acidic media.

In toluene, a significant increase of Φ_F, one order of magnitude, was observed for compounds **13** and **14** (Table 1) due to decreased feasibility of ICT in the less polar solvent, which is consistent with the findings published in the literature.[14,15] A significant influence of the decreased polarity was not observed in compounds **10** and **11** that formed J-dimers in this noncoordinating solvent. Compound **12** showed only a moderate increase of Φ_F due to equilibrium between the monomer and J-dimer. The results indicated that formation of J-dimers also deactivated the excited states.

Singlet oxygen quantum yields were determined in DMF using decomposition of a specific singlet oxygen scavenger, diphenylisobenzofuran (DPBF), with ZnPc as a reference ($\Phi_{\Delta(\mathrm{DMF})} = 0.56$[32]). Similar to what was observed for fluorescence in DMF, the Φ_Δ of compounds **10**–**14** reached very low values and the controls (**15** and **16**) showed values typical for ZnAzaPc.[33,34] After acidification (5% sulfuric acid in solution), the Φ_Δ values of all of the compounds were comparable due to the inhibition of the ICT in the pH sensitive derivatives (Table 1). Singlet oxygen production for the pH sensitive derivatives increased several times (by a factor of 18–110) upon changing the acidity of the solutions. The concentration of the sulfuric acid was chosen on the basis of the fluorescence experiments, in which 5% sulfuric acid in DMF was shown to be enough to restore the fluorescence quantum yields of all of the compounds (**10**–**14**) to normal, and, at the same time, the changes could not be attributed to significant protonation of azomethine nitrogen atoms (Figure 4; Supporting Information, Figure S1). Despite this, determination of the Φ_Δ values in the acidified solution was complicated by the stronger basicities of azomethine nitrogen atoms of the reference ZnPc, which was extensively protonated at this concentration of sulfuric acid (Supporting Information, Figure S1). For this reason, compound **15** was used as the reference in DMF with 5% sulfuric acid. The $\Phi_\Delta = 0.56$ was calculated on the basis of "factor of increase" from fluorescence measurements (Table 1).

In order to mimic the pH sensitivity in a biologically relevant environment, the investigated compounds were anchored to dioleoylphosphatidylcholine (DOPC) large unilamellar vesicles (liposomes). Liposomes, particularly unilamellar, are considered to be simple models of biological membranes.[35] That is why, DOPC large unilamellar vesicles were prepared by the extrusion method, and compounds **10**–**16** were incorporated into a lipidic bilayer.[19] The dependence of the fluorescence signal on the pH was measured in buffer solutions with pH values ranging from 2.6 to 7.4. It must be noted that the absorption spectra of AzaPc in liposomes were of monomeric character, and J-dimers contributions were not observed (Supporting Information, Figure S4). Thus, the changes in fluorescence intensity can be attributed solely to ICT and the block thereof after protonation. As anticipated, the pH sensitive compounds became highly fluorescent at low pH levels and almost nonfluorescent at higher pH levels. A quite steady fluorescence signal which was not dependent on pH was detected for the always ON controls (Figure 5). The factor of increase for AzaPc (**10**–**14**) was generally over 20, but reached 70 for compound **14**. This increase suggests a good signal-to-noise ratio between the ON and OFF states. The changes in fluorescence intensities allowed the determination of the pK_a of the donor center by applying the Henderson–Hasselbalch equation. The pK_a values (Table 1) ranged between 2.2 and 4.2 and corresponded well with electron donating effects of alkyl substituents on the aniline moiety. Compounds **12** and **14**, containing the 4-(diethylamino)-2-methylphenyl substituent, belonged among the most basic in the study.

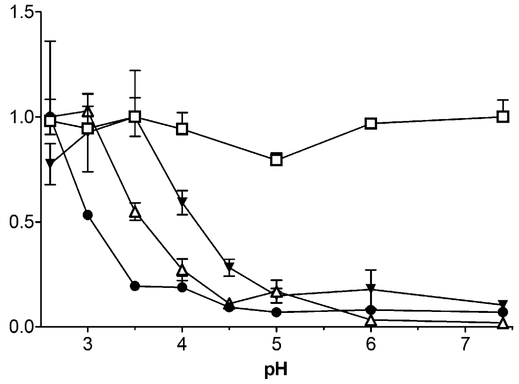


Figure 5. Changes in the fluorescence intensity of compounds **12** (△), **13** (●), **14** (▼), and **16** (□) in DOPC liposomes dependent on the pH of the buffer. F is the fluorescence intensity at the actual pH, F_{max} is the maximum value from all pH values for the respective compound ($\lambda_{exc} = 382$ nm). The mean of three measurements is plotted, and error bars represent the standard deviation.

Conclusion

Several new unsymmetrical ZnAzaPc compounds with absorption and emission maxima in the red portion of the UV/Vis spectrum were synthesized from the corresponding precursors. Substituted anilino moieties in AzaPc served as the donor for ICT, a process that efficiently quenched the excited states in DMF, leading to very low Φ_Δ and Φ_F values. Protonation of the donor center by the addition of sulfuric acid led to strong increases in both parameters and to values that were comparable with always ON controls. All of the compounds were incorporated into DOPC liposomes, and AzaPc (**10**–**14**) showed fluorescence signal responses relative to the pH of the buffer solution with pK_a values in the range 2.2–4.2. All of the above-mentioned measurements unequivocally demonstrated that photophysical and photochemical properties of the concerned compounds are easily modified by acidification and that they may serve as sensors or pH controlled photosensitizers in areas for which low pH is appreciated and light activation at higher wavelengths desirable.

Experimental Section

General: All of the organic solvents used were of analytical grade. Anhydrous butanol was stored over magnesium and distilled prior to use. All of the chemicals for synthesis were obtained from established suppliers (Aldrich, Acros, Merck) and used as received. Sulfuric acid used for titrations was obtained from Penta (Czech Republic) and contained 96% sulfuric acid and 4% water. Zinc phthalocyanine (ZnPc) was purchased from Aldrich. TLC was performed on Merck aluminum sheets with silica gel 60 F254. Merck Kieselgel 60 (0.040–0.063 mm) was used for column chromatography. Melting points were measured on an Electrothermal IA9200 Series Digital Melting Point apparatus (Electrothermal Engineering Ltd., Southend-on-Sea, Essex, Great Britain). Infrared spectra were measured on a Nicolet Impact 400 IR spectrometer (KBr) or Nicolet 6700 (ATR mode). ^{1}H and ^{13}C NMR spectra were recorded on a Varian Mercury Vx BB 300 or VNMR S500 NMR spectrometer. The reported chemical shifts are given relative to $Si(CH_3)_4$ and were locked to the signal of the solvent. The elemental analysis was carried out on Automatic Microanalyser EA1110CE (Fisons Instruments S.p.A., Milano, Italy). The UV/Vis spectra were recorded using a Shimadzu UV-2401PC spectrophotometer. The fluorescence spectra were obtained by an AMINCO-Bowman Series 2 luminescence spectrometer. Matrix assisted laser desorption ionization - time of flight (MALDI-TOF) mass spectra were recorded in positive reflectron mode on a Voyager-DE STR mass spectrometer (Applied Biosystems, Framingham, MA, USA) in *trans*-2-[3-(4-*tert*-butylphenyl)-2-methyl-2-propenylidene]-malononitrile as the matrix. The instrument was calibrated externally with a five-point calibration using Peptide Calibration Mix1 (LaserBio Labs, Sophia-Antipolis, France). Electrospray ionization mass spectroscopic (ESI MS) experiments were performed using LCQ Advantage Max (Thermo Finnigan, San Jose, USA) equipped with an ESI source. The data were analyzed by Thermo Finnigan Xcalibur software. Mass spectra of the samples were obtained by direct infusion of each standard in a methanol (MeOH) and water solution into the detector. The compounds **1b**,[24] **4a**, **4c** (and their intermediates **2a**,**c** and **3a**,**c**),[13] **5**,[25,33] **6**,[14,20] and **15**[20] were prepared according to the literature. Compounds **1a** and **7a** are available commercially.

1-[4-(Diethylamino)-2-methylphenyl]-2-hydroxy-2-(pyridin-3-yl)ethanone (2b): This compound was prepared as published before for similar compounds[13,36] by the condensation of pyridine-3-carboxaldehyde (1.89 g, 0.01 mmol) and **1b** (2.6 g, 0.025 mmol) with 10% aqueous potassium cyanide solution (1 mL) in ethanol (5 mL). The solution was heated at reflux for 48 h. Ethanol was evaporated, and the mixture was extracted with ethylacetate/water. The organic layer was dried over anhydrous Na_2SO_4 and evaporated. The crude product was purified over silica with ethylacetate as an eluent to yield a yellow oil (580 mg, 20%, **2b** and 180 mg, 6%, **3b**). ^{1}H NMR (300 MHz, [D$_6$]acetone): δ=1.13 (t, J=7 Hz, 6H; CH_2CH_3), 2.47 (s, 3H; CH_3), 3.42 (q, J=7 Hz, 4H; CH_2CH_3), 6.07 (s, 1H; CHOH), 6.48–6.54 (m, 2H; ArH(aniline)), 7.26 (dd, J_1=8 Hz, J_2=5 Hz, 1H; ArH(aniline)), 7.68 (dt, J_1=8 Hz, J_2=2 Hz, 1H; ArH-(pyridyl)), 7.85 (d, J=9 Hz, 1H; ArH(pyridyl)), 8.41 (dd, J_1=5 Hz, J_2=2 Hz, 1H; ArH(pyridyl)), 8.64 ppm (d, J=2 Hz, 1H, ArH(pyridyl)); ^{13}C NMR (75 MHz, [D$_6$]acetone): δ=12.73, 23.30, 44.77, 73.64, 108.47, 114.75, 120.18, 124.32, 134.21, 135.14, 138.05, 144.32, 149.66, 149.93, 151.66, 197.56 ppm; IR (ATR): $\tilde{\nu}$=2973, 2929, 1706, 1650, 1596, 1540, 1470, 1454, 1426, 1396, 1378, 1356, 1328, 1289, 1258, 1195, 1135, 1073, 1026, 1001, 965, 940, 843, 790, 753 cm^{-1}.

1-[4-(Diethylamino)-2-methylphenyl]-2-(pyridin-3-yl)ethane-1,2-dione (3b): Compound **2b** (580 mg, 1.94 mmol), ammonium nitrate (195 mg, 2.43 mmol) and copper(II) acetate (3.5 mg, 0.019 mmol) were dissolved in 80% (v/v) acetic acid (20 mL) and then heated to reflux for 2 h. The mixture was concentrated and extracted three times with ethylacetate/water. The organic layer was dried over anhydrous Na_2SO_4, and the solvent was evaporated. The crude product was purified by column chromatography over silica with ethylacetate as an eluent to yield a yellow oil (507 mg, 88%). ^{1}H NMR (500 MHz, [D$_6$]DMSO): δ=1.10 (t, J=7 Hz, 6H; CH_2CH_3), 2.61 (s, 3H; CH_3), 3.42 (q, J=7 Hz, 4H; CH_2CH_3), 6.53 (dd, J_1=9 Hz, J_2=3 Hz, 1H; ArH(aniline)), 6.64 (d, J=2 Hz, 1H; ArH-(aniline)), 7.36 (d, J=9 Hz, 1H; ArH(aniline)), 7.62 (ddd, J_1=1 Hz, J_2=5 Hz, J_3=8 Hz, 1H; ArH(pyridyl)), 8.23 (dt, J_1=2 Hz, J_2=8 Hz, 1H; ArH(pyridyl)), 8.86 (dd, J_1=5 Hz, J_2=2 Hz, 1H; ArH(pyridyl)), 8.99 ppm (dd, J_1=1 Hz, J_2=2 Hz, 1H; ArH(pyridyl)); ^{13}C NMR (75 MHz, [D$_6$]DMSO): δ=12.56, 22.87, 44.10, 108.42, 114.21, 117.19, 124.64, 129.00, 136.60, 137.04, 144.19, 150.46, 151.74, 154.84, 192.23, 194.86 ppm; IR (ATR): $\tilde{\nu}$=2974, 1680, 1639, 1587, 1537, 1460, 1405, 1378, 1354, 1309, 1269, 1224, 1205, 1169, 1138, 1078, 1049, 1027, 1014, 878, 843, 789, 766, 748 cm^{-1}.

5-[4-(Diethylamino)-2-methylphenyl]-6-(pyridin-3-yl)pyrazine-2,3-dicarbonitrile (4b): Diaminomaleonitrile (237 mg, 2.2 mmol) was added to a solution of **3b** (500 mg, 1.7 mmol) in glacial acetic acid (15 mL). The mixture was sonicated for 15 min and then heated to reflux for 48 h. The solvent was evaporated. The crude product was purified over silica with ethylacetate/chloroform 2:1 as an eluent and recrystallized in EtOH/H_2O to yield an orange-purple solid (370 mg, 60%). M.p. 174.4–175.6°C; ^{1}H NMR (300 MHz, [D$_6$]acetone): δ=1.14 (t, J=7 Hz, 6H; CH_3), 2.84 (s, 3H; CH_3), 3.42 (q, J=7 Hz, 4H; CH_2CH_3), 6.52–6.61 (m, 2H; ArH-(aniline)), 7.07 (d, J=9 Hz, 1H; ArH(aniline)), 7.40 (ddd, J_1=1 Hz, J_2=6 Hz, J_3=8 Hz, 1H; ArH(pyridyl)), 7.96 (ddd, J_1=2 Hz, J_2=2 Hz, J_3=8 Hz, 1H; ArH(pyridyl)), 8.60 (dd, J_1=2 Hz, J_2=5 Hz, 1H; ArH-(pyridyl)), 8.70 ppm (dd, J_1=1 Hz, J_2=2 Hz, 1H; ArH(pyridyl)); ^{13}C NMR (75 MHz, [D$_6$]acetone): δ=12.74, 20.60, 44.73, 110.11, 114.15, 114.81, 114.92, 122.36, 124.12, 129.97, 131.34, 133.40, 133.73, 137.29, 138.90, 150.24, 150.70, 151.69, 154.16, 158.28 ppm. IR (ATR): $\tilde{\nu}$=2974, 2932, 2232, 1603, 1552, 1524, 1498, 1469, 1404, 1372, 1361, 1286, 1265, 1242, 1198, 1145, 1123, 1092, 1079, 1023, 1000, 949, 851, 829, 814, 787 cm^{-1}; MS-ESI: m/z (%): 369 (100) $[M+H]^+$; MS/MS (49 eV): m/z (%): 341 (100) $[M-C_2H_4]^+$, 369 (4.5) $[M+H]^+$; elemental analysis calcd (%) for $C_{22}H_{20}N_6$: C 71.72, H 5.47, N 22.81; found: C 71.79, H 5.72, N 23.29.

4-Bromo-*N*,*N*-diethyl-3-methylaniline (7b): NBS (2.2 g, 12 mmol) in DMF (10 mL) was added dropwise to a solution of *N*,*N*-diethyl-3-methylaniline (2 g, 12 mmol) in DMF (4 mL). After 5 min of stirring, water was added (50 mL), and the mixture was extracted three times with ethylacetate. The organic layer was dried over anhydrous Na_2SO_4, and the solvent was evaporated. The crude product was purified over silica with hexane/ethyl acetate 100:3 as an eluent to yield a yellow oil (2.76 g, 93%); ^{1}H NMR (500 MHz, $CDCl_3$): δ=1.16 (t, J=7 Hz, 6H; CH_3CH_2N), 2.36 (s, 3H; CH_3), 3.33 (q, J=7 Hz, 4H; CH_3CH_2N), 6.40 (dd, J_1=9 Hz, J_2=3 Hz, 1H; ArH), 6.56 (d, J=3 Hz, 1H; ArH) and 7.30 ppm (d, J=9 Hz, 1H; ArH); ^{13}C NMR (75 MHz, CDCl$_3$): $\delta = 12.43$, 23.40, 44.38, 109.82, 111.16, 114.08, 132.53, 137.98 and 147.03 ppm; IR (ATR): $\tilde{\nu} = 2970$, 2929, 2871, 1597, 1562, 1489, 1467, 1449, 1420, 1390, 1375, 1355, 1290, 1262, 1196, 1150, 1094, 1077, 1032, 1017, 921, 834 and 791 cm^{-1}; MS-ESI: *m/z* (%): 242 (91) $[M+H]^+$, 244 (100) $[M+2+H]^+$.

4-(Dimethylamino)phenylboronic acid (8a): 4-Bromo-*N*,*N*-dimethylaniline **7a** (1 g, 5 mmol) was dissolved in anhydrous THF (15 mL) under an argon atmosphere and cooled to −78 °C. After cooling, a solution of butyl lithium (BuLi, 3.44 mL, 5.5 mmol) in hexane (1.6 M) was added dropwise, and the solution was stirred at −78 °C for 60 min. Triisopropyl borate (1.4 g, 7.5 mmol) was slowly added, and the solution turned yellow. After stirring at −78 °C for 60 min, the solution was allowed to slowly warm to RT. Water (15 mL) was added to the solution, and the white precipitate that formed immediately after addition disappeared after a few minutes. The pH was adjusted to 6 by using 18 % (v/v) HCl, and the solution was stirred for 18 h at RT. The organic layer was extracted three times with 4 % NaOH (10 mL). The water extracts were collected, and the pH was adjusted to 7 using concentrated HCl. The precipitate that formed was collected and washed with water to obtain a pink solid (275 mg, 33 %). ^{1}H NMR (300 MHz, [D$_6$]DMSO): $\delta = 2.89$ (s, 6H; CH$_3$), 6.58–6.86 (m, 2H; ArH), 7.52–7.76 ppm (m, 2H, ArH); ^{13}C NMR (75 MHz, [D$_6$]DMSO): $\delta = 39.98$ (NCH$_3$, overlapped by solvent residual peak), 111.24, 116.10, 129.08, 135.64 ppm. The NMR spectroscopic data were essentially the same as those given elsewhere.[37]

4-(Diethylamino)-2-methylphenylboronic acid (8b): The same procedure described for compound **8a** was used but with **7b** (3 g, 12 mmol), butyl lithium (8.5 mL, 13.6 mmol) in hexane (1.6 M), and triisopropyl borate (3.5 g, 18 mmol) to a give brown solid (686 mg, 27 %). M.p. >400 °C′; ^{1}H NMR (300 MHz, [D$_4$]methanol): $\delta = 1.06$ (t, $J = 7$ Hz, 6H; CH$_2$C*H*$_3$), 2.20 (s, 3H; CH$_3$), 3.27 (q, $J = 7$ Hz, 4H; C*H*$_2$CH$_3$), 6.39 (d, $J = 7$ Hz, 1H; ArH), 6.43–6.50 (m, 1H; ArH), 6.93–7.01 (m, 1H; ArH); ^{13}C NMR (75 MHz, [D$_4$]methanol): $\delta = 12.81$, 22.02, 45.65, 111.36, 114.69, 129.99, 134.13, 139.66, 149.15 ppm. IR (ATR): $\tilde{\nu} = 3332$, 2972, 1600, 1580, 15447, 1498, 1391, 1374, 1355, 1295, 1274, 1199, 1176, 1146, 1093, 1076, 1030, 917, 822, 779 cm^{-1}; elemental analysis calcd (%) for C$_{11}$H$_{18}$BNO$_2$: C 63.80, H 8.76, N 6.76; found C 64.20, H 8.54, N 6.77.

4′-(Dimethylamino)biphenyl-3,4-dicarbonitrile (9a): Compound **8a** (205 mg, 1.24 mmol) and 4-iodophthalonitrile (210 mg, 0.83 mmol) were dissolved in THF (10 mL) together with a solution of K$_2$CO$_3$ (2 M) in water (1.49 mL, 2.98 mmol). The mixture was heated to 40 °C and tetrakis(triphenylphosphine)palladium(0) (96 mg, 0.083 mmol) was added. The solution was heated at reflux for 3 h. After cooling, water (100 mL) was add to the solution, and the mixture was extracted three times with ethylacetate. The organic layer was dried over anhydrous Na$_2$SO$_4$, and the solvent was evaporated. The crude product was purified over silica with toluene/triethylamine 25:1 as an eluent and recrystallized from ethanol (EtOH) to obtain an orange solid (158 mg, 78 %). M.p. 193.5–195.8 °C; ^{1}H NMR (300 MHz, CDCl$_3$): $\delta = 3.06$ (s, 6H; CH$_3$), 6.82 (d, $J = 9$ Hz, 2H, ArH(aniline)), 7.49–7.54 (m, 2H; ArH(aniline)), 7.76 (d, $J = 8$ Hz, 1H; ArH), 7.85 (dd, $J_1 = 8$ Hz, $J_2 = 2$ Hz, 1H; ArH), 7.94 ppm (d, $J = 2$ Hz, 1H; ArH); ^{13}C NMR (75 MHz, CDCl$_3$): $\delta = 40.52$, 111.67, 112.97, 115.78, 115.93, 116.24, 128.00, 129.60, 130.43, 133.80, 146.13, 150.94 ppm; IR (ATR): $\tilde{\nu} = 2914$, 2822, 2224, 1612, 1582, 1561, 1534, 1492, 1444, 1399, 1339, 1310, 1283, 1267, 1230, 1201, 1178, 1113, 1071, 999, 949, 881, 888, 838, 809, 755 cm^{-1}; elemental analysis calcd (%) for C$_{16}$H$_{13}$N$_3$: C 77.71, H 5.30, N 16.99; found: C 76.41, H 5.46, N 16.61.

4′-(Diethylamino)-2′-methylbiphenyl-3,4-dicarbonitrile (9b): Essentially the same procedure as described for compound **9a** was used but with **8b** (100 mg, 0.48 mmol), 4-iodophthalonitrile (81.3 mg, 0.32 mmol), a 2 M solution of K$_2$CO$_3$ in water (0.58 mL, 1.2 mmol), tetrakis(triphenylphosphine)palladium(0) (37 mg, 0.032 mmol). The reaction was heated at reflux for 8 h. Water (100 mL) was added, and the mixture extracted three times with ethylacetate. The organic layer was dried over anhydrous Na$_2$SO$_4$, and the solvent was evaporated. The crude product was purified over silica with toluene/chloroform 1:1 as an eluent and recrystallized from EtOH/H$_2$O to obtain an orange solid (78 mg, 56 %). M.p. 99.5–100.3 °C; ^{1}H NMR (500 MHz, CDCl$_3$): $\delta = 1.20$ (t, $J = 7$ Hz, 6H; C*H*$_3$CH$_2$N), 2.29 (s, 3H; CH$_3$), 3.40 (q, $J = 7$ Hz, 4H; CH$_3$C*H*$_2$N), 6.55–6.62 (m, 2H; ArH), 7.06 (d, $J = 9$ Hz, 1H; ArH), 7.67 (dd, $J_1 = 8$ Hz, $J_2 = 2$ Hz, 1H; ArH), 7.75 (d, $J = 2$ Hz, 1H; ArH), 7.78 ppm (d, $J = 8$ Hz, 1H; ArH); ^{13}C NMR (75 MHz, CDCl$_3$): $\delta = 12.54$, 21.12, 44.28, 109.60, 112.07, 113.31, 115.47, 115.75, 115.86, 124.35, 130.91, 133.09, 133.69, 134.10, 136.08, 148.00, 148.21 ppm; IR (ATR): $\tilde{\nu} = 2967$, 2928, 2871, 2231, 1609, 1591, 1558, 1542, 1518, 1486, 1450, 1427, 1399, 1374, 1353, 1307, 1295, 1283, 1259, 1199, 1154, 1128, 1097, 1078, 1038, 917, 898, 852, 804, 784 cm^{-1}; elemental analysis calcd (%) for C$_{19}$H$_{19}$N$_3$: C 78.86, H 6.62, N 14.52; found: C 78.30, H 6.88, N 14.53.

General procedure for the synthesis of AzaPc: Magnesium (28 equiv) and a small crystal of iodine were heated to reflux for 3 h in anhydrous butanol. Precursor A (1 equiv) and precursor B (3 equiv) were added all at once or precursor B was divided into four parts and added subsequently within 2 h (mentioned for each compound below). The reflux continued for the next 6 h. The mixture was left to cool and was poured into water/methanol/acetic acid 5:5:1 and stirred for 45 min at RT. The dark solid was collected and washed thoroughly with water and slightly with methanol. Part of the symmetrical magnesium(II) congener BBBB was in some cases partially separated (and discarded) by column chromatography over silica (mentioned for each compound below). Subsequently, the mixture of AzaPc congeners (1 equiv) was dissolved in THF and stirred at RT for 45 min with *p*-toluenesulfonic acid (20 equiv). The mixture was concentrated, and water was added. The precipitate was collected and washed with 1 % NaHCO$_3$ and then with water. The product was dried thoroughly. The desired congener (ABBB) was isolated by column chromatography over silica using the eluents mentioned below. As the final step, the solution of the metal-free ABBB congener (1 equiv) was heated with anhydrous zinc(II) acetate (7 equiv) in DMF at 160 °C for 2 h. Water was added, the precipitate was collected and washed thoroughly with water and slightly with methanol. The crude product was purified by column chromatography over silica. The pure compounds were washed with methanol (**10**, **11**) or were dissolved in a small amount of pyridine and added dropwise to methanol (**12**, **13**, and **16**). The precipitate was collected and put into a drying pistol to remove any residual solvent. Compound **14** dissolves in methanol; therefore, the final purification step was omitted.

2-[4-(Dimethylamino)phenyl]-3-(phenyl)-9,10,16,17,23,24-hexakis(*tert*-butylsulfanyl)-1,4,8,11,15,18,22,25-(octaaza)phthalocyaninezinc(II) (10): Precursor A (**4a**, 90 mg, 0.28 mmol), precursor B (**6**, 254 mg, 0.83 mmol) were added all at once. The other materials used were: Mg (188 mg, 7.74 mmol); the mobile phase for metal-free dye: toluene/chloroform/pyridine 15:10:1 ($R_f = 0.24$) and then toluene/chloroform/ethyl acetate 14:6:1 ($R_f = 0.33$); mobile phase for zinc(II) complex: chloroform/acetone/pyridine 20:3:1 ($R_f = 0.40$). Green solid (28 mg, 8 %); ^{1}H NMR (300 MHz, [D$_5$]pyridine): $\delta = 2.02$ (s, 9H; SC(CH$_3$)$_3$), 2.09 (s, 9H; SC(CH$_3$)$_3$), 2.27 (s, 18H; SC(CH$_3$)$_3$), 2.28 (s, 18H; SC(CH$_3$)$_3$), 2.83 (s, 6H; NCH$_3$), 6.88–6.93 (m, 2H; ArH), 7.52–7.65 (m, 3H; ArH, overlapped by solvent residual peak), 8.18–8.25 ppm (m, 4H; ArH); ^{13}C NMR (75 MHz, [D$_5$]pyridine): $\delta = 29.98$, 30.74, 30.79, 39.83, 51.19, 51.23, 51.29, 51.30, 51.45, 112.15, 124.03, 127.05, 128.92, 129.12, 130.88, 132.52, 135.99, 141.64, 144.98, 145.00, 145.18, 145.41, 145.47, 147.60, 149.28, 151.38, 151.53, 151.68, 151.71, 151.74, 151.85, 151.87, 152.06, 153.95, 154.85, 158.13, 158.16, 158.75, 158.76, 159.63, 159.72 ppm (some of the signals fused together); IR (KBr): $\tilde{\nu} = 3442$, 2960, 2919, 2862, 1608, 1541, 1534, 1499, 1477, 1458, 1400, 1363, 1349, 1251, 1195, 1143, 1110, 975, 947, 847, 783, 754, 704, 693 cm^{-1}; UV/Vis (THF): λ_{max} (ε) = 654 (273 600), 628 sh, 595 (44 500), 377 nm (154 100 mol^{-1} m^3 cm^{-1}); MS (MALDI-TOF): *m/z*: 1308 $[M+H]^+$, 1330 $[M+Na]^+$, 1346 $[M+K]^+$, 1252 $[M+H-C_4H_8]^+$, 1196 $[M+H-2\,C_4H_8]^+$, 1140 $[M+H-3\,C_4H_8]^+$; elemental analysis calcd (%) for C$_{62}$H$_{69}$N$_{17}$S$_6$Zn$\cdot$2H$_2$O: C 55.32, H 5.47, N 17.69; found C 55.34, H 5.38, N 17.56.

2-[4-(Dimethylamino)phenyl]-3-(pyridin-3-yl)-9,10,16,17,23,24-hexakis-(*tert*-butylsulfanyl)-1,4,8,11,15,18,22,25-(octaaza)phthalocyaninezinc(II) (11): Precursor A (**4c**, 110 mg, 0.34 mmol) and precursor B (**6**, 315 mg, 1.03 mmol) were added all at once. The other materials used were: Mg (230 mg, 9.47 mmol); mobile phase for magnesium(II) complex: toluene/chlororform/pyridine 20:10:1 ($R_f = 0.08$) and after symmetrical congener BBBB was eluted mobile phase was changed to chloroform/pyridine 20:1

(R_f=0.52); mobile phase for metal-free dye: chloroform/ethyl acetate 5:1 (R_f=0.35); mobile phase for zinc(II) complex: chloroform/THF 7:1. (R_f=0.31). Green solid (33 mg, 7%); ^{1}H NMR (300 MHz, [D$_5$]pyridine): δ=2.02 (s, 9H; SC(CH$_3$)$_3$), 2.07 (s, 9H; SC(CH$_3$)$_3$), 2.26 (s, 18H; SC(CH$_3$)$_3$), 2.28 (s, 18H; SC(CH$_3$)$_3$), 2.84 (s, 6H; NCH$_3$), 6.89–6.99 (m, 2H; ArH), (ddd, J_1=8 Hz, J_2=5 Hz, J_3=1 Hz, 1H; ArH) and 8.10–8.16 (m, 2H; ArH), 8.42 (dt, J_1=8 Hz, J_2=2 Hz, 1H; ArH), 8.91 (dd, J_1=5 Hz, J_2=2 Hz, 1H, ArH), 9.63 ppm (dd, J_1=2 Hz, J_2=1 Hz, 1H, ArH); ^{13}C NMR (75 MHz, [D$_5$]pyridine): δ=14.28, 22.94, 29.52, 29.61, 29.91, 29.98, 30.72, 30.77, 32.11, 39.80, 51.19, 51.22, 51.31, 51.46, 112.26, 126.37, 132.56, 137.70, 144.67, 145.03, 145.16, 145.44, 147.68, 151.44, 151.65, 151.89, 152.07, 158.28, 158.32, 158.80, 159.66 ppm (some of the signals fused together); IR (KBr): $\tilde{\nu}$=3441, 2923, 2853, 1737, 1608, 1544, 1529, 1460, 1362, 1255, 1142, 974, 783, 693 cm^{-1}; UV/Vis (THF): λ_{max} (ε)=655 (248000), 627 sh, 596 (38400), 480 sh, 377 nm (136700 mol^{-1}m^{3}cm^{-1}); MS (MALDI-TOF): *m/z*: 1309 [*M*+H]$^+$, 1331 [*M*+Na]$^+$, 1347 [*M*+K]$^+$; elemental analysis calcd (%) for $C_{61}H_{68}N_{18}S_6Zn\cdot 2H_2O$: C 54.39, H 5.39, N 18.72; found C 54.33, H 5.54, N 17.53.

2-[4-(Diethylamino)-2-methylphenyl]-3-(phenyl)-9,10,16,17,23,24-hexakis(*tert*-butylsulfanyl)-1,4,8,11,15,18,22,25-(octaaza)phthalocyaninezinc(II) (12): Precursor A (**4b**, 250 mg, 0.68 mmol) and precursor B (**6**, 625 mg, 2.0 mmol) were added all at once. The other materials used were: Mg (450 mg, 18.8 mmol); mobile phase for metal-free dye: chloroform/ethylacetate 10:1 (R_f=0.45); mobile phase for zinc(II) complex: toluene/THF/pyridine 30:4:1 (R_f=0.29). Yield green solid (126 mg, 14%); ^{1}H NMR (300 MHz, CDCl$_3$/[D$_5$]pyridine 3:1): δ=0.88 (t, J=7 Hz, 6H; NCH$_2$C*H*$_3$), 1.74 (s, 9H; SC(CH$_3$)$_3$), 1.81 (s, 9H; SC(CH$_3$)$_3$), 1.85 (s, 36H; SC(CH$_3$)$_3$), 2.15 (s, 3H; ArCH$_3$), 3.07 (q, J=7 Hz, 4H; NC*H*$_2$CH$_3$), 6.30–6.36 (m, 2H; ArH), 7.02 (ddd, J_1=8 Hz, J_2=5 Hz, J_3=1 Hz, 1H; ArH), 7.20–7.25 (m, 1H; ArH), 7.97 (dt, J_1=8 Hz, J_2=2 Hz, 1H; ArH), 8.34 (dd, J_1=5 Hz, J_2=2 Hz, 1H; ArH), 9.16 ppm (dd, J_1=2 Hz, J_2=1 Hz, 1H; ArH); ^{13}C NMR (75 MHz, CDCl$_3$/[D$_5$]pyridine 3:1): δ=11.89, 20.32, 29.90, 29.99, 30.05, 43.47, 50.48, 50.51, 50.60, 108.90, 112.83, 124.72, 132.40, 135.31, 136.38, 137.19, 143.78, 143.88, 143.92, 146.61, 147.57, 147.78, 149.77, 149.83, 150.40, 150.69, 150.85, 150.94, 150.97, 151.09, 151.15, 155.53, 157.51, 157.57, 157.84, 158.38, 158.45 ppm (some of the signals overlapped); IR (ATR): $\tilde{\nu}$=2963, 2919, 1606, 1519, 1479, 1453, 1398, 1362, 1349, 1248, 1144, 1109, 1092, 1059, 1024, 975, 953, 848, 782, 746 cm^{-1}; UV/Vis (THF): λ_{max} (ε)=653 (252100), 626 sh, 594 (39900), 376 nm (147800 mol^{-1}m^{3}cm^{-1}); MS (MALDI-TOF): *m/z*: 1350 [*M*]$^+$, 1373 [*M*+Na]$^+$, 1322 [*M*−C$_2$H$_4$]$^+$, 1294 [*M*−2C$_2$H$_4$]$^+$, 2701 [2*M*]$^+$; elemental analysis calcd (%) for $C_{64}H_{74}N_{18}S_6Zn\cdot 2H_2O$: C 55.33, H 5.66, N 18.15; found C 55.26, H 5.60, N 18.05.

23-[4-(Dimethylamino)phenyl]-2,3,9,10,16,17-hexakis(*tert*-butylsulfanyl)-1,4,8,11,15,18-(hexaaza)phthalocyaninezinc(II) (13): Precursor A (**9a**, 200 mg, 0.81 mmol) and precursor B (**6**, 745 mg, 2.43 mmol) were added in four parts over 2 h. The other materials used were: Mg (538 mg, 22.1 mmol); mobile phase for metal-free dye: toluene/chloroform/THF 10:3:0.3 (R_f=0.34); mobile phase for zinc(II) complex: toluene/THF 100:4 (R_f=0.40). Green solid (136 mg, 14%); ^{1}H NMR (300 MHz, [D$_5$]pyridine): δ=2.15 (s, 9H; SC(CH$_3$)$_3$), 2.20 (s, 9H; SC(CH$_3$)$_3$), 2.25 (s, 18H; SC(CH$_3$)$_3$), 2.27 (s, 18H; SC(CH$_3$)$_3$), 2.90 (s, 6H; NCH$_3$), 7.06 (d, J=9 Hz, 2H; ArH), 8.18 (d, J=9 Hz, 2H; ArH), 8.55 (dd, J_1=8 Hz, J_2=1 Hz, 1H; ArH), 9.79 (d, J=8 Hz, 1H; ArH), 10.04 ppm (s, 1H; ArH); ^{13}C NMR (75 MHz, [D$_5$]pyridine): δ=30.75, 30.80, 40.15, 51.10, 51.31, 51.34, 113.53, 128.80, 128.84, 137.25, 140.94, 144.34, 144.38, 144.46, 144.52, 145.36, 151.05, 152.21, 152.36, 157.68, 157.87, 157.89, 158.20, 158.21, 159.17, 159.30 ppm (some of the signals fused together); IR (ATR): $\tilde{\nu}$=2960, 2918, 1602, 1523, 1479, 1446, 1390, 1362, 1333, 1249, 1192, 1144, 1111, 1092, 1057, 975, 915, 846, 811, 782, 774, 748 cm^{-1}; UV/Vis (THF): λ_{max} (ε)=668 (189400), 612 (37000), 471 sh, 426 sh, 373 nm (137800 mol^{-1}m^{3}cm^{-1}); MS (MALDI-TOF): *m/z*: 1229 [*M*]$^+$, 1252 [*M*+Na]$^+$, 1268 [*M*+K]$^+$, 2458 [2*M*]$^+$; elemental analysis calcd (%) for $C_{58}H_{67}N_{15}S_6Zn$: C 56.54, H 5.48, N 17.05; found: C 57.06, H 5.56; N 16.74.

23-[4-(Diethylamino)-2-methylphenyl]-2,3,9,10,16,17-hexakis(*tert*-butylsulfanyl)-1,4,8,11,15,18-(hexaaaza)phthalocyaninezinc(II) (14): Precursor A (**9b**, 200 mg, 0.69 mmol) and precursor B (**6**, 477 mg, 1.56 mmol) were added all at once. The other materials used were: Mg (468 mg, 19.26 mmol); water/methanol/acetic acid 5:5:1 was not added to the mixture of magnesium complexes, but the butanol was evaporated and mixture was extracted with chloroform. After that the procedure continued according to general procedure; mobile phase for metal-free dye: toluene/chloroform/pyridine 15:5:0.3 (R_f=0.34); mobile phase for zinc(II) complex: toluene/THF 100:5 (R_f=0.45). Green solid (95 mg, 13%); ^{1}H NMR (300 MHz, [D$_5$]pyridine): δ=1.15 (t, J=7 Hz, 6H; NCH$_2$C*H*$_3$), 2.06 (s, 9H; SC(CH$_3$)$_3$), 2.18 (s, 9H; SC(CH$_3$)$_3$), 1.08–1.14 (m, 36H; SC(CH$_3$)$_3$), 2.68 (s, 3H; CH$_3$), 3.34 (q, J=7 Hz, 4H; NC*H*$_2$CH$_3$), 6.83–6.91 (m, 2H; ArH), 7.69 (d, J=9 Hz, 2H; ArH), 8.31 (d, J=8 Hz, 1H; ArH), 9.78 (d, J=7 Hz, 1H; ArH), 9.85 ppm (s, 1H; ArH); ^{13}C NMR (75 MHz, [D$_5$]pyridine): δ=12.89, 21.71, 30.61, 30.75, 30.80, 44.80, 51.05, 51.10, 51.31, 51.35, 51.44, 110.59, 114.29, 124.03, 129.30, 131.98, 132.43, 135.99, 136.70, 137.43, 140.19, 144.36, 144.38, 144.40, 144.51, 144.93, 145.25, 145.32, 145.78, 148.11, 151.67, 152.24, 152.39, 157.82, 157.86, 157.87, 157.90, 158.20, 158.22, 158.62, 159.10 ppm; IR (ATR): $\tilde{\nu}$=2962, 2920, 1602, 1519, 1455, 1391, 1362, 1335, 1251, 1196, 1146, 1093, 1055, 1028, 976, 915, 846, 803, 781 cm^{-1}; UV/Vis (THF): λ_{max} (ε)=665 (185400), 601 (37200), 471 sh, 418 (38000) and 373 nm (142900 mol^{-1}m^{3}cm^{-1}); MS (MALDI-TOF): *m/z*: 1271 [*M*]$^+$, 1294 [*M*+Na]$^+$, 1310 [*M*+K]$^+$, 1243 [*M*−C$_2$H$_4$]$^+$, 2542 [2M]$^+$, 2565 [2*M*+Na]$^+$, 2581 [2*M*+K]$^+$; elemental analysis calcd (%) for $C_{61}H_{73}N_{15}S_6Zn$: C 57.50, H 5.77, N 16.49; found: C 57.38, H 6.04, N 16.45.

2,3-Bis(phenyl)-9,10,16,17,23,24-hexakis(*tert*-butylsulfanyl)-1,4,8,11,-15,18,22,25-(octaaza)phthalocyaninezinc(II) (16): Precursor A (**5**, 50 mg, 0.18 mmol) and precursor B (**6**, 163 mg, 0.53 mmol) were added all at once. The other materials used were: Mg (121 mg, 5.0 mmol); mobile phase for metal free dye: toluene/chloroform/pyridine 50:25:1 (R_f=0.18); mobile phase for zinc(II) complex: chloroform/toluene/pyridine 50:25:1 (R_f=0.39). Green solid (24 mg, 11%); ^{1}H NMR (300 MHz, [D$_5$]pyridine): δ=2.01 (s, 18H; SC(CH$_3$)$_3$), 2.26 (s, 18H; SC(CH$_3$)$_3$), 2.28 (s, 18H; SC(CH$_3$)$_4$), 7.49–7.55 (m, 6H; ArH; signal is overlapped by solvent residual peak), 8.08–8.13 ppm (m, 4H; ArH); ^{13}C NMR (75 MHz, [D$_5$]pyridine): δ=30.71, 30.76, 51.20, 51.30, 51.47, 128.79, 131.18, 140.50, 145.09, 145.18, 151.30, 154.46, 158.26, 159.78 ppm; IR (ATR): $\tilde{\nu}$=2959, 2922, 2855, 1518, 1454, 1401, 1362, 1350, 1253, 1234, 1140, 1114, 1068, 1036, 974, 949, 848, 783 cm^{-1}; UV/Vis (THF): λ_{max} (ε)=650 (227200), 623 sh, 589 (29800), 478 sh, 377 nm (115900 mol^{-1}m^{3}cm^{-1}); MS (MALDI-TOF): *m/z*: 1264 [*M*]$^+$, 1287 [*M*+Na]$^+$, 1303 [*M*+K]$^+$, 2529 [2*M*]$^+$.

Singlet oxygen measurements: All of the samples for both singlet oxygen and fluorescence quantum yield determinations were further purified by TLC to ensure absolute purity of the samples. The samples were eluted over silica TLC plates (Silufol Kavalier Czechoslovakia, without fluorescence indicator) with eluents corresponding to the eluents used for the purification of zinc(II) complexes of studied dyes. Corresponding parts of the TLC were scrapped and AzaPc compounds were extracted from silica using THF. Quantum yields of singlet oxygen (Φ_Δ) were determined according to a previously published procedure[38] using the decomposition of chemical trap 1,3-diphenylisobenzofuran (DPBF). Two solvents were considered: DMF and DMF with 5% H$_2$SO$_4$ (v/v). Zinc phthalocyanine (ZnPc) was used as a reference for DMF measurement (Φ_Δ=0.56 in DMF[32,39]) and compound **15** for DMF with 5% H$_2$SO$_4$ because of the strong protonation of ZnPc under such conditions (Supporting Information, Figure S1). In detail, the procedure was as follows. A stock solution of DPBF in DMF (2.5 mL, 5×10^{-5} M) was transferred into a 10×10 mm quartz optical cell and bubbled with oxygen for 1 min. Sulfuric acid (132 μL) was added (or not in the case of DMF measurements), and the solution was mixed thoroughly and left to cool to RT (10 min). A defined amount of concentrated stock solution of the tested compound (**10**–**16**) in DMF (usually 30 μL) was then added. Absorbance of the final solution in the Q band maximum was always about 0.1. The solution was stirred and irradiated for defined times using a halogen lamp (Tip, 300 W). Incident light was filtered through a water filter (6 cm) and an orange HOYA G filter to remove heat and light under 506 nm, respectively. The decrease of DPBF in solution over the time of irradiation was monitored at 415 nm (Supporting Information, Figure S5). All of the experiments were performed three times, and the data presented in the paper represent a mean of these three experiments. The estimated error is ±10%. All of the calculations were performed according to a previously published procedure.[38]

Fluorescence measurements: Fluorescence quantum yields (Φ_F) were determined by the comparative method[40] using ZnPc as a reference ($\Phi_F = 0.30$ in chloronaphthalene[30]). In the case of titrations with sulfuric acid, a stock solution of the studied compound (**10–16**) in DMF (2.2 mL, 2.9 μm) was transferred to a quartz optical cell (10×10 mm), and a definite amount of concentrated sulfuric acid was added. The absorption and emission spectra were collected. Both the reference and the sample were excited at 603 nm (**12–15**) or at 596 nm (**10**, **11** and **16**) for measurements in DMF. The excitation wavelength in toluene was 605 nm for all of the compounds. The Φ_F was calculated using Equation (1) in which F is the integrated area under the emission spectrum, A is absorbance at the excitation wavelength, and n is the refractive index of the solvent. Superscripts R and S correspond to the reference and sample, respectively.

$$\Phi_F^S = \Phi_F^R \left(\frac{F^S}{F^R}\right) \left(\frac{1-10^{-A^R}}{1-10^{-A^S}}\right) \left(\frac{n^S}{n^R}\right)^2 \qquad (1)$$

ZnPc as a reference was measured only in DMF without the addition of sulfuric acid. The refractive index for solutions of the samples in DMF with increasing amounts of sulfuric acid was determined for a series of concentrations [from 0% to 35% (v/v)] and calculated from a nonlinear regression of experimentally measured dependence between refractive index and the amount of sulfuric acid in DMF.[13] All of the experiments presented in Table 1 were performed three times and the data represent a mean of these three experiments. The estimated error is ±15%.

Liposome preparation and incorporation of the dyes: Large unilamellar vesicles formed by the extrusion technique (LUVETs) were prepared from a suspension of multilamellar vesicles (MLVs) using a procedure similar to one previously published.[19] The lipids (1,2-dioleoylphosphatidylcholine, DOPC, from Lipoid GmbH, 25.0 mg, 31.8 mmol) were dissolved in chloroform (10 mL) and evaporated in a 100 mL round-bottom flask in water bath with the temperature set to 37°C. The thin lipid film was further left on the water bath for 30 min at a pressure of 5 mbar to remove all traces of organic solvents. A citrate-phosphate buffer (2.5 mL) of the appropriate pH (2.6, 3.0, 3.5, 4.0, 4.5, 5.0, 6.0, or 7.4) was added, and the lipids were removed from the flask walls by gentle shaking. The suspension was then vortexed for 3 min to form MLVs and left to stand for 3 h at room temperature to allow complete swelling. LUVETs were prepared from this MLV suspension using a hand extruder LiposoFast Basic (Avestin, Canada).[41] The suspension was passed back and forth twenty-one times through two stacked polycarbonate filters (pore diameter 100 nm) at RT and subsequently diluted to the final concentration using the appropriate buffer. The vesicles (at buffer pH 3, 5, and 7) were characterized using a photon correlation spectroscope (Zetasizer Nano-ZS, Malvern, UK) at a LUVETs concentration of 150 μm. The average size was determined to be 154.7 nm (polydispersity index 0.227), 132.2 nm (polydispersity index 0.106) and 131.5 nm (polydispersity index 0.074) for pH 3, 5, and 7, respectively. Dye incorporation was performed with following procedure. Generally, 3 μL of the THF stock solution (200 μm) of compounds **10–16** were added to 3 mL of LUVETs (lipid concentration 0.5 m). The mixture was stirred for 15 min, intensively vortexed for 1 min, and then shaken on orbital shaker for 12 h, again vortexed for 1 min, and shaken for 20 h. The final dye concentration was 0.2 μm; the lipid-to-dye ratio was 2500. The final amount of THF [0.1% (v/v)] is believed to not disturb the phospholipid bilayer to a significant extent.[42] Fluorescence emission spectra were collected after excitation at 382 nm and monitored at the emission maximum of each compound. All of the experiments were performed in triplicate, and the data presented in the paper (Figure 5) represent a mean of these three experiments.

The absorption spectra of compounds **10–13** in liposomes were obtained at higher concentrations, and this is why the dye had to be incorporated into LUVETs in a different manner. The method was similar to that described above, but a proper amount of the stock solution of the dyes in THF was added to a chloroform solution of the lipids before evaporation to a thin film. All of the subsequent treatments, including swelling and extrusion, were performed with the dye already incorporated into the LUVETs ($c_{dye} = 25$ μm). The final lipid-to-dye ratio was 1000. The absorption spectra were measured after the appropriate dilution of the suspension of liposomes by a buffer of pH 7.4.

Acknowledgements

The work was supported by Czech Science Foundation, grant P207/11/1200. The authors would like to thank Jiří Kuneš for the NMR measurements, Vojtěch Tambor, Petra Kovaříková, and Ivana Pasáková for the MS measurements, and Pavel Berka for determination of the LUVETs properties. A kind donation of the phospholipids by Lipoid GmbH is gratefully acknowledged.

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Received: April 12, 2011