ChemPubSoc Europe

DOI: 10.1002/chem.201502533

CHEMISTRY A European Journal Full Paper

Fluorescence |*Hot Paper*|

Phenol-Substituted Tetrapyrazinoporphyrazines: pH-Dependent Fluorescence in Basic Media

Veronika Novakova,*[a] Miroslava Lásková,[b] Hana Vavřičková,[b] and Petr Zimcik*[b]

Abstract: Tetrapyrazinoporphyrazines (TPyzPzs) bearing one, two, four or eight 3,5-di(*tert*-butyl)-4-hydroxyphenol moieties were synthesized as zinc(II) complexes and metal-free derivatives. The deprotonation of the phenol using tetrabutylammonium hydroxide induced the formation of a strong donor for intramolecular charge transfer that switched OFF the red fluorescence ($\lambda_F \sim 660$ nm) of the parent zinc TPyzPzs. The changes were fully reversible for TPyzPzs with one to four phenolic moieties, and an irreversible modification was observed for TPyzPzs substituted with eight phenols. The sensors were anchored to lipophilic particles in water, and a pK_a approximately 12.5–12.7 was determined for the phenolic hydroxyl based on fluorescence changes in different buffers. In addition, a novel concept for fluorescence OFF-ON-OFF switching in metal-free TPyzPzs bearing phenolic moieties upon addition of specific amounts of base was demonstrated.

Introduction

Tetrapyrazinoporphyrazines (TPyzPzs) are the most widely investigated group of phthalocyanine (Pc) aza-analogues. In particular, TPyzPzs have received increasing attention in recent years due to their promising fluorescent,[1] optical limiting,[2] catalytic[3] and liquid crystalline properties[4] as well as their ability to form nanoporous materials.[5] In addition, TPyzPzs are able to efficiently produce singlet oxygen upon irradiation.[6] Therefore, TPyzPzs have been investigated as photosensitizers in photodynamic therapy (PDT) with good efficiency, which has been confirmed in vitro on cells.[7] Aza-substitution in benzene rings of Pcs imparts TPyzPz with significant electron deficient properties,[8] which results in a macrocycle core that is a good electron acceptor that can undergo intramolecular charge transfer (ICT) or photoinduced electron transfer (PET) after combination with a suitable donor moiety.[9] Both processes can be utilized in sensing because they are competitive relaxation pathways to fluorescence and can be easily switched ON and OFF upon binding of an analyte.[1b,10]

Recently, a series of red fluorescent TPyzPz sensors for cations or pH detection have been reported.[1b,10,11] These sensors contained various amines that serve as donors in ICT, and the fluorescence was switched ON upon binding of a cation or by protonation in acidic pH. Similarly, amines have also been utilized as donors in Pcs for the development of pH sensitive photosensitizers in PDT.[12] The use of phenol or phenolate in TPyzPzs or Pcs as the donor that can switch between ON and OFF states in more basic pH or react in the presence of basic cations has received limited attention[13] even though this type of donor is often used in other types of fluorescent molecules.[14] In particular, the 2,6-di(*tert*-butyl)phenol moiety has been primarily studied as a substituent on a series of porphyrins for colorimetric detection.[15] A study on the use of the phenol/phenolate couple for fluorescence sensing in TPyzPzs that possess significant electron-deficient character, photostability and strong fluorescence in the red portion of the emission spectra[1a] is lacking.

The aim of this study was to investigate the possibility of using a phenolate anion to act as a donor for ICT in TPyzPzs and switch OFF their fluorescence. Zinc (**1Zn–4Zn**) and metal-free (**1H–4H**) TPyzPzs (Figure 1) bearing one, two, four or eight phenol moieties were synthesized and investigated to elucidate the influence of the phenol/phenolate couple on the resulting photophysics with the potential for switching between ON (phenol) and OFF (phenolate) states. To reduce or eliminate undesirable aggregation of planar TPyzPz molecules, the compounds were designed to contain bulky substituents in both the nondonor (*tert*-butylsulfanyls) and donor moieties (2,6-di(*tert*-butyl)phenol). Aggregation nonspecifically decreases fluorescence and may lead to a misleading interpretation of photophysical data. The data were compared with negative controls **5H**, **5Zn** and **6Zn** (Figure 1), which have a similar structure but lack a phenol group.

[a] *Dr. V. Novakova*
Department of Biophysics and Physical Chemistry
Faculty of Pharmacy in Hradec Kralove, Charles University in Prague
Heyrovskeho 1203, Hradec Kralove, 50005 (Czech Republic)
E-mail: veronika.novakova@faf.cuni.cz

[b] *M. Lásková, H. Vavřičková, Dr. P. Zimcik*
Department of Pharmaceutical Chemistry and Drug Control
Faculty of Pharmacy in Hradec Kralove, Charles University in Prague
Heyrovskeho 1203, Hradec Kralove, 50005 (Czech Republic)
E-mail: petr.zimcik@faf.cuni.cz

Supporting information for this article is available on the WWW under http://dx.doi.org/10.1002/chem.201502533.

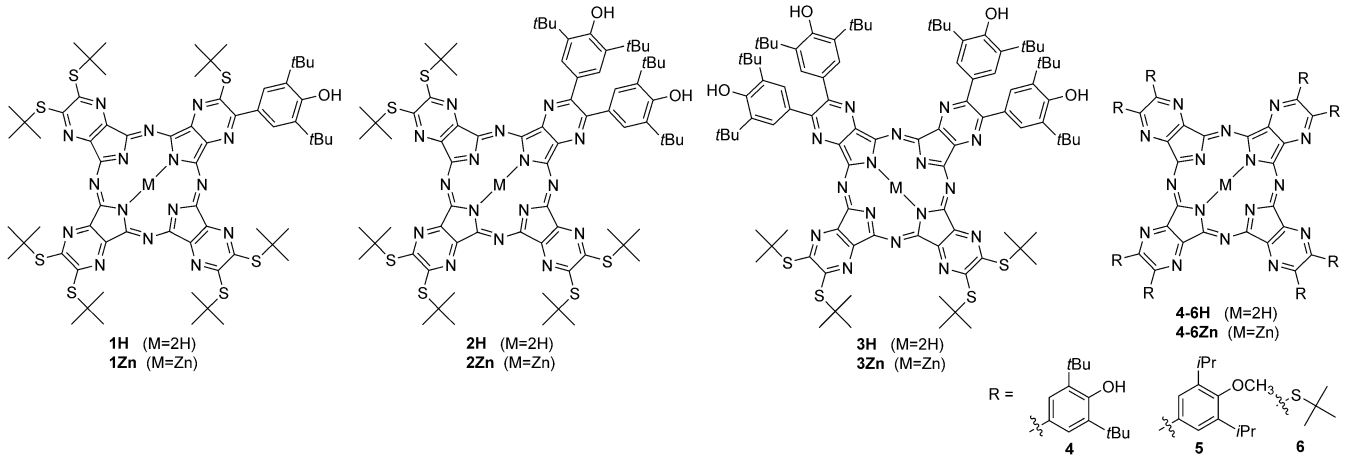


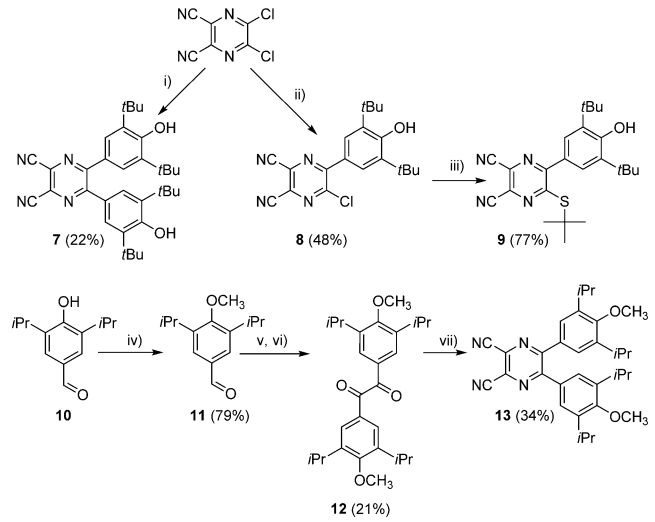
Figure 1. Structures of studied TPyzPzs.

Results and Discussion

Synthesis

The synthesis of TPyzPzs is typically performed using cyclotetramerization of precursors—suitably substituted pyrazine-2,3-dicarbonitriles. The synthesis of precursors containing phenol moieties started from commercially available 5,6-dichloropyrazine-2,3-dicarbonitrile. This compound was reported[13a] to undergo nucleophilic substitution with 2,6-di(*tert*-butyl)phenol to **7** and **8**. In our case, a slightly modified procedure was used with 22 and 48% yields, respectively (Scheme 1).

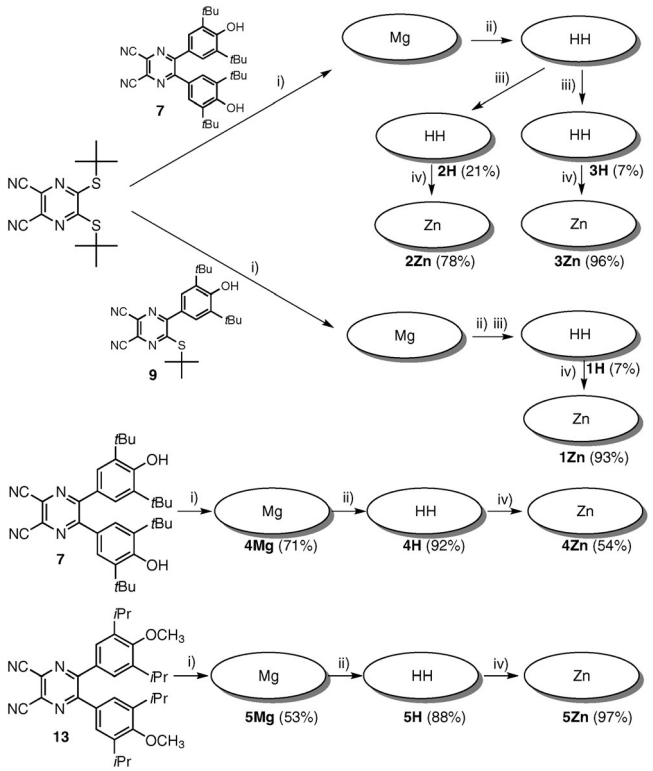
Interestingly, 2,6-di(*tert*-butyl)phenol acts as a C-nucleophile in this reaction. The O-nucleophilic center is sterically hindered by bulky *tert*-butyl groups, and no phenoxy-substituted pyrazine was detected. A similar C-nucleophile behavior for this phenol was reported during the synthesis of monosubstituted phthalonitriles.[16] The second electron-deficient center in monosubstituted **8** was further modified by nucleophilic substitution using bulky *tert*-butyl thiolate to afford precursor **9** in 77% yield. A different approach using condensation of a suitably substituted diketone with diaminomaleonitrile was applied to synthesize pyrazine **13**. First, 2,6-diisopropylphenol was converted via a Duff reaction with methenamine to **10** (76% yield), which was alkylated by dimethylsulfate to **11** (79% yield). Subsequent benzoin condensation of aldehyde **11** in presence of the 5-(2-hydroxyethyl)-3,4-dimethylthiazolium iodide catalyst yielded the corresponding acyloin. However, this acyloin was unstable and gradually oxidized in solution after the condensation and on silica during purification. Therefore, this acyloin was directly oxidized to diketone **12** without isolation, and the product was obtained in 21% yield from two steps. Condensation of **12** with diaminomaleonitrile in acetic acid afforded the required pyrazine precursor **13** in 34% yield.



Scheme 1. Synthesis of precursors for cyclotetramerization. i) 2,6-Di(*tert*-butyl)phenol (3 equiv), K_2CO_3, anhydr. MeCN, Ar, reflux, 24 h; ii) 2,6-di(*tert*-butyl)phenol (1.1 equiv), K_2CO_3, anhydr. MeCN, Ar, RT, 2.5 h; iii) 2-methylpropane-2-thiol, pyridine, reflux, 1 h; iv) dimethylsulfate, THF, K_2CO_3, reflux, 21 h; v) 5-(2-hydroxyethyl)-3,4-dimethylthiazolium iodide, DBU, anhydr. EtOH, reflux, 3 h; vi) NH_4NO_3, $Cu(CH_3COO)_2$, AcOH, reflux, 4 h; vii) diaminomaleonitrile, AcOH, reflux, 3 h.

The synthesis of symmetrical complexes **4H**, **4Zn**, **5H**, and **5Zn** was performed via magnesium butoxide induced cyclotetramerization of the corresponding precursors **7** or **13** (Scheme 2). The reaction primarily led to magnesium complexes **4Mg** and **5Mg**, which were subsequently demetalated by *p*-toluenesulfonic acid (TsOH) to metal-free **4H** and **5H**. The metal-free derivatives were converted to zinc complexes **4Zn** and **5Zn** with $Zn(CH_3COO)_2$ in pyridine. Both the demetalation reaction and complexation with the zinc cation were nearly quantitative for both derivatives with the exception of the synthesis of **4Zn**, where only 54% yield was obtained due to losses on the silica column during purification. The synthesis of compound **6Zn** has been previously reported.[17]

Unsymmetrical TPyzPzs were obtained by mixed cyclotetramerization (statistical condensation) of precursors **7** or **9** with 5,6-bis(*tert*-butylsulfanyl)pyrazine-2,3-dicarbonitrile induced by magnesium butoxide (Scheme 2). This approach led to a mixture of six different congeners (i.e., AAAA, AAAB, ABAB, AABB, ABBB and BBBB) that were chromatographically separated. However, the mixture of magnesium complexes was insepara-



Scheme 2. Synthesis of TPyzPzs. i) Mg, BuOH, reflux; ii) TsOH, THF, RT; iii) separation of congeners; iv) $Zn(CH_3COO)_2$, pyridine, reflux.

ble due to the strongly overlapping and tailing spots of the congeners during TLC. The conversion to metal-free derivatives by treating the mixture with TsOH enabled separation of the individual congeners. In the metal-free form, nicely separated round spots appeared during TLC, and **1H**–**3H** were successfully isolated. TPyzPzs **2H** and **3H** were isolated from one reaction, and the other possible congeners in this reaction appeared as only minor products based on the weak spots that were visible by TLC. The unsymmetrical metal-free TPyzPzs were subsequently complexed nearly quantitatively by zinc cations using $Zn(CH_3COO)_2$ in pyridine. It is important to note that the A_2B_2 congener can potentially exist as two positional isomers [i.e., adjacent (drawn on Figure 1, i.e., AABB type) and opposite (i.e., ABAB type)] that are typically extremely difficult to separate. The NMR spectroscopy data for **3Zn** (Figure S1 in the Supporting Information) confirmed that its structure can be attributed primarily to the adjacent isomer based on the presence of two equal sets of signals corresponding to all of the protons. Only one signal for each of the protons would be expected for the opposite isomer type, which appears to be present as a very minor component.

Absorption spectra

The absorption spectra for all of the studied compounds were typical for TPyzPzs. These spectra were characterized by two important bands including a high energy B-band (~375 nm) and a low energy Q-band (~650 nm; Figure 2).

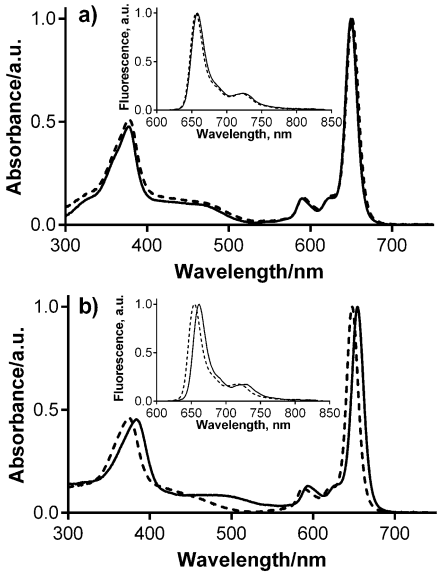


Figure 2. Normalized absorption and fluorescence emission (inset) spectra of studied TPyzPz in THF. a) **1Zn** (full line), **6Zn** (dashed line). b) **4Zn** (full line), **5Zn** (dashed line). Emission spectra were corrected for detector response.

A single Q-band (ε typically over 300000 $M^{-1}cm^{-1}$) was observed for the zinc complexes, and due to lowered symmetry, splitting was observed for the metal-free derivatives (see also Figure S2 in the Supporting Information). All of the spectra in THF as well as in other studied solvents (i.e., toluene, dioxane, MeCN, DMF, DMSO, DMAC) exhibited the typical shape of monomeric species and the presence of aggregates was not detected at the studied concentrations (up to 5 μM). The effect of the substituted phenyl rings and *tert*-butylsulfanyl substituents on the position of the Q-band was essentially the same (Table 1). The only difference was a slight red shift of a few nanometers as the number of phenols in the molecule increased (Figure S3 in the Supporting Information), which may be due to contributions from the hydroxyl lone pairs. Therefore, the phenolic OH groups are conjugated to the TPyzPz macrocycle despite the slight rotation of the phenyl rings from the pyrazine plane, as shown in the recently reported X-ray crystal structure of **7**.[13a] The electronic coupling of the phenolic hydroxyl with the TPyzPz core was also confirmed by the significant increase in the n–π transition at approximately 500 nm in the absorption spectrum of **4Zn** compared to that of **5Zn** (Figure 2b). The coupling is an essential requirement for efficient ICT because the donor and acceptor must be in conjugation.[9a]

Photophysical characterization

The fluorescence emission spectra (example for THF: Figure 2, insets), which were obtained for all of the studied solvents, mirrored the absorption spectra with only small Stokes shift. The excitation spectra overlapped the absorption spectra, which further confirmed the presence of only monomers and excluded the influence of aggregation on the photophysical data.

Table 1. Absorption and photophysical data of studied TPyzPz in THF.[a]

Compound	λ_{max} [nm]	λ_F [nm]	Φ_F[b]	Φ_Δ[b]	$\Phi_F+\Phi_\Delta$
1H	672, 642	676	0.036[c]	0.056	0.092
2H	673, 643	678	0.020[c]	0.028	0.048
3H	674, 647	677	0.006[c]	0.012	0.018
4H	675, 650	663	0.002	0.005	0.007
5H	668, 639	673	0.25	0.10	0.35
1Zn	651	658	0.28	0.58	0.86
2Zn	651	658	0.26	0.60	0.86
3Zn	653	659	0.24	0.52	0.76
4Zn	654	661	0.25	0.44	0.71
5Zn	648	654	0.32	0.56	0.88
6Zn	650	656	0.30	0.55	0.85

[a] Absorption maximum at the Q-band (λ_{max}), fluorescence emission maximum (λ_F), fluorescence quantum yield (Φ_F), singlet oxygen quantum yield (Φ_Δ). [b] With unsubstituted ZnPc in THF as reference ($\Phi_F=0.32$, $\Phi_\Delta=0.53$). [c] $\Phi_{F(1H)}$ in MeCN = 0.005, $\Phi_{F(2H)}$ in MeCN = 0.002, $\Phi_{F(3H)}$ in MeCN = 0.002

The photophysical data were primarily collected for all of the compounds to characterize the properties of the phenol form (i.e., the ON state in the potential sensor). Both the fluorescence (Φ_F) and singlet oxygen quantum yields (Φ_Δ) in THF were determined by comparative methods using unsubstituted ZnPc as a reference. The data are summarized in Table 1. The fluorescence and singlet oxygen production represent two major deactivation pathways for the excited states of TPyzPzs. The sum of these quantum yields ($\Phi_F+\Phi_\Delta$) in TPyzPzs reaches typical values close to 1 when no other relaxation pathways are available.[9a] The $\Phi_F+\Phi_\Delta$ sum in THF for the zinc complexes without any possible donors (**5Zn**, **6Zn**) and those with just one (**1Zn**) and two (**2Zn**) phenolic groups were comparable (i.e., typically more than 0.85). However, a decrease in the $\Phi_F+\Phi_\Delta$ sum of more than 10% was observed for **3Zn** and **4Zn** bearing four and eight phenolic groups, respectively. The $\Phi_F+\Phi_\Delta$ sum for the metal-free derivatives in this study was unusually low (Table 1). This observation, which is not yet well explained, is typical for various metal-free TPyzPz irrespective of the presence or absence of donors for ICT/PET on the periphery of the macrocycle[1a] and is apparently not connected with the ICT from peripheral substituents. Despite the low values in the metal-free derivatives, the same trend with a decrease in the $\Phi_F+\Phi_\Delta$ sum as the number of phenolic groups increased was observed (Table 1).

The smaller $\Phi_F+\Phi_\Delta$ sum for TPyzPzs bearing more phenol groups may indicate that phenol also acts as a weak donor for ICT in these molecules. Indeed, phenols have been reported to serve as donors for ICT or PET processes in various fluorophores.[18] However, in the design of fluorescence sensors, the phenol form in the phenol/phenolate pair is expected to be the fluorescent one (i.e., in ON state). From this point of view, any excessive quenching of fluorescence by a phenolic hydroxyl donor would reduce the usability of this pair in sensing applications, especially in more polar solvents as both PET and ICT are more efficient in polar solvents.[19] Therefore, the influence of the phenolic hydroxyl on the photophysical properties in the ON state was investigated by determining the Φ_F values for selected compounds in solvents with different polarities (Table 2). The Φ_F values for TPyzPzs without any phenol groups (**5Zn**, **6Zn**) were independent of the solvent polarity. The Φ_F values for **1Zn** and **4Zn** were fully comparable to those of the controls (**5Zn**, **6Zn**) in nonpolar solvents (dioxane, toluene) where the ICT is not the preferred process. Because the thermodynamic feasibility of ICT increases in polar solvents, the quenching of excited states becomes more effective. This behavior was observed as a drop in the fluorescence quantum yields of **1Zn** and **4Zn** in solvents with a higher relative permittivity (ε_r) with much steeper decrease for the latter (compare, e.g., data in DMF, DMAC or DMSO). The experiments supported the hypothesis that the phenolic hydroxyl is really a donor for ICT in TPyzPzs but its effect is very weak and may only have a significant effect on the deactivation process in combination of a large number of phenolic groups (e.g., **4Zn**) and polar solvents. For comparison, the ICT in structurally similar TPyzPzs with only one diethylamino substituent as a donor is way much stronger (e.g., $\Phi_{F(THF)}=0.013$, $\Phi_{F(MeCN)}=0.0013$)[9b] than the effect of eight phenols in **4Zn**. Therefore, the phenol form was not excluded from use in the fluorescent form in the phenol/phenolate pair.

Table 2. Fluorescence quantum yields (Φ_F) and fluorescence emission maxima (λ_F) of studied TPyzPz in solvents of different polarity.[a,b,c]

Solvent	ε_r	**1Zn**	**4Zn**	**5Zn**	**6Zn**
dioxane	2.2	0.25 (659)	0.26 (661)	0.27 (654)	0.27 (658)
toluene	2.4	0.25 (662)	–[d]	–[d]	0.27 (660)
THF	7.4	0.28 (658)	0.25 (661)	0.32 (654)	0.30 (656)
DMF	36.7	0.16 (664)	0.017 (668)	0.25 (663)	0.23 (662)
MeCN	37.5	0.22 (661)	0.15 (665)	0.24 (661)	0.23 (659)
DMAC	37.8	0.14 (664)	0.013 (668)	0.26 (663)	0.26 (661)
DMSO	48.9	0.04 (666)	0.002 (670)	0.30 (664)	0.26 (664)

[a] Expressed as Φ_F (λ_F [nm]). [b] Relative permittivity of the solvent (ε_r), *N*,*N*-dimethylacetamide (DMAC), acetonitrile (MeCN). [c] With unsubstituted ZnPc in THF as reference ($\Phi_F=0.32$). [d] Insoluble in toluene.

ON-OFF switching of fluorescence

The conversion of phenol to phenolate by deprotonation in basic media was expected to induce a strong donor center in the molecule, which should be able to efficiently quench the excited states. Therefore, this effect may be used for switching OFF the fluorescence of TPyzPzs in basic media. This premise was tested using titration experiments with tetrabutylammonium hydroxide (TBAH) in acetonitrile (MeCN). Stepwise addition of TBAH (up to 500 μM) into a solution containing **1Zn**–**3Zn** (1 μM) induced small hypso- and hypochromic shifts of the absorption Q-band (Figure 3a, and Figures S5 and S6 in the Supporting Information). Simultaneously, the fluorescence intensity decreased with no changes in the shape of the emission spectra (Figure 3a, inset). The progress of the changes in the absorption and emission spectra corresponded to each other and stopped when **1Zn**–**3Zn** were fully deprotonated on the phenolic hydroxyls (Figure 3c, and Figures S5 and S6). Excess TBAH did not lead to any further changes (Figure 3b). Acidifi-

cation of the solution at the end of the titration by addition of excess acetic acid resulted in the full recovery of both the absorption spectra and fluorescence intensity (Figures S4–S6 in the Supporting Information).

Similarly, the titrations of **4Zn** containing eight donor centers induced a decrease in the fluorescence intensity. However, the absorption spectra exhibited a different behavior than **1Zn**–**3Zn**. The hypso- and hypochromic shift of the Q-band continued with increasing amounts of TBAH even when the fluorescence reached a minimum. Ultimately, a completely different absorption spectrum was obtained at large excesses of TBAH (Figure 3d and e). Notably, acidification of the solution at the end of the titration of **4Zn** with acetic acid did not restore the Φ_F value (Table 3), and the absorption spectrum was less intense in the Q-band and blue shifted by 7 nm (Figure 3e and Figure S4 in the Supporting Information). This result suggests an irreversible change in the structure of **4Zn**. Any attempt to characterize the product mixture (e.g., by mass spectrometry) failed. However, a reasonable explanation was presented by Clarkson et al., who observed similar broad and flat absorption spectra after addition of TBAH and a hypsochromic shift after subsequent acidification for metal-free Pcs substituted with four 2,6-di(*tert*-butyl)phenol moieties.[16a] The 2,6-di(*tert*-butyl)phenol moieties may undergo oxidation to quinone and/or other well-known transformations including the formation of endoperoxides and/or aromatic ring contraction.[20] Alternatively, the oxidation of the 2,6-di(*tert*-butyl)phenol moiety to *ortho*-quinone may also be involved, as recently suggested by a study on porphyrins *meso*-substituted with 2,6-di(*tert*-butyl)phenols.[21]

The changes in the absorption spectra or Φ_F values of controls **5Zn** and **6Zn** after addition of TBAH were negligible (Figure 3c and f). A very small red shift (~3 nm) of the Q-band and a slight decrease in the Φ_F values were observed after the first addition of TBAH, and then, the spectral shape and fluorescence intensity remained constant as the amount of TBAH increased. Both the Q-band and Φ_F returned to their original shape and values after acidification. These small changes may be explained in terms of the complexation of the hydroxyl anion to the central zinc cation after the first addition of TBAH and its removal after acidification. This behavior also most likely occurs for **1Zn**–**4Zn** but the effect on the properties is small and obscured by the changes induced by the formation of the phenolate ion (Figure 4).

Compounds **1Zn**–**3Zn** bear suitable structural features for anchoring in the lipophilic particles in water with the hydrophobic moiety (TPyzPz with *t*BuS substituents) buried in the hydrophobic core and with the more hydrophilic phenol oriented out of the particle. This arrangement also allows for the assessment of the sensing potential in water-based medium and determination of the pK_a. Compound **4Zn** was not included in these experiments due to the irreversible changes observed after addition of base (see above). Therefore, **1Zn**–**3Zn** and control **6Zn** were integrated into particles in a microemulsion prepared from medium-chain triglycerides (MCT) stabilized with the Cremophor EL emulsifier, and their responses to different pH values of the buffer were evaluated. It is important to note that the TPyzPzs **1Zn**, **2Zn** and **6Zn** were incorporated into particles in a monomeric form based on the shape of their absorption spectra (Figure S7 in the Supporting

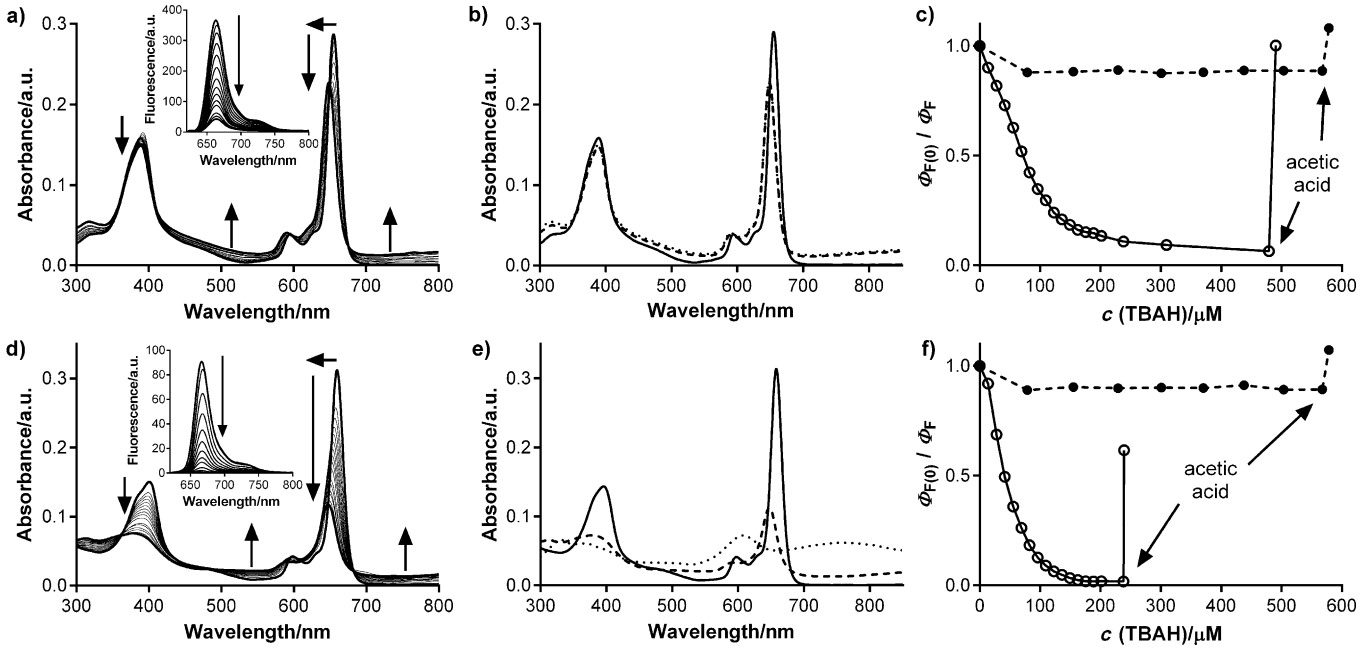


Figure 3. Changes in the absorption spectra of: a) **1Zn**, and b) **4Zn** in MeCN ($c = 1$ μM) after addition of TBAH (up to 240 μM). Insets a, d): corresponding changes in the fluorescence emission spectra ($\lambda_{exc} = 603$ and 597 nm for **1Zn** and **4Zn**, respectively). Spectra of: b) **1Zn**, and e) **4Zn** in MeCN ($c = 1$ μM) before (full line) and after addition of TBAH ($c = 240$ μM, complete deprotonation; dashed line) and after addition of a large excess of TBAH (dotted). All of the absorption spectra were corrected for dilution. c, f) Dependence of $\Phi_{F(0)}/\Phi_F$ on the concentration of TBAH in the solution for **1Zn** (c, open symbols), **4Zn** (f, open symbols), **5Zn** (f, full symbols) and **6Zn** (c, full symbols). The addition of excess acetic acid that neutralized TBAH is indicated by the arrows (c, f).

Table 3. Fluorescence quantum yields (Φ_F) in MeCN and pK_a values in microemulsions of studied TPyzPz.[a,b]

	$\Phi_{F(phenol)}$	$\Phi_{F(phenolate)}$[c]	$\Phi_{F(AcOH)}$[d]	Decrease[e]	pK_a[f]
1Zn	0.22	0.015	0.24	15	12.5
2Zn	0.18	0.03	0.22	6	12.6
3Zn	0.17	0.0030	0.20	57	12.7
4Zn	0.15	0.0026	0.09	58	–
5Zn	0.24	0.22	0.25	1.1	–
6Zn	0.23	0.20	0.25	1.2	–

[a] Tetrabutylammonium hydroxide (TBAH), acetic acid (AcOH). [b] [TPyzPz] = 1 μM. [c] Fully deprotonated form ([TBAH] = 240 μM). [d] After acidification ([AcOH] ~ 120 mM) of the solution of fully deprotonated form. [e] Calculated as follows: Φ_F(phenol)/Φ_F(phenolate). [f] Determined in microemulsion made from MCT/Cremophor EL in water.

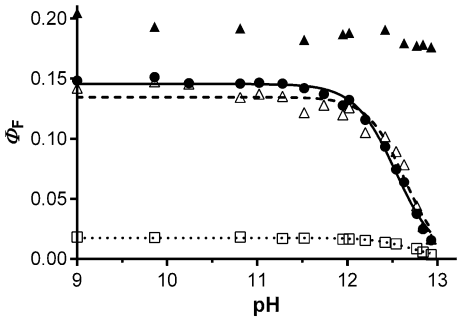


Figure 4. Dependence of the Φ_F value on the pH of the buffer solution for **1Zn** (dots, full line), **2Zn** (open triangles, dashed line), **3Zn** (open squares, dotted line) and **6Zn** (full triangles) incorporated in the microemulsion. Symbols represent experimental data, and the lines are the best fit.

Information) and the perfect match with the corresponding excitation spectra. Compound **3Zn** slightly aggregated (Figure S7c). A change in the buffer pH from 9 to 13 induced similar changes in the absorption spectra of **1Zn**–**3Zn** as seen in MeCN upon titration with TBAH. The fluorescence signal decreased at a higher pH due to the deprotonation of the phenolic OH (Figure 4). No changes were detected for control **6Zn**, which did not contain a phenol, over the studied pH range. Φ_F of **1Zn** and **2Zn** in the microemulsion in the ON state (below pH 11) reached values of 0.15 and 0.14 (comparable to $\Phi_F = 0.19$ of **6Zn** in a microemulsion), respectively, and these values decreased by more than 1 order of magnitude at pH 13. However, the initial Φ_F value for **3Zn** was only 0.02, which was most likely due to the aggregation of this TPyzPz in the microemulsion. The pK_a values, which were determined from the dependence of Φ_F on the pH of the buffer, were similar for all of the sensor compounds (**1Zn**–**3Zn**) and reached values of approximately 12.5–12.7 (Table 3). Interestingly, the pK_a value corresponded well to the calculated value for 2,6-di(*tert*-butyl)phenol (pK_a = 12.16, calculated using ACD/Labs Software V11.02).

OFF-ON-OFF switching of fluorescence

The low fluorescence of metal-free TPyzPz and the results from several experiments where the fluorescence increased after deprotonation of the central NH groups led us to evaluate the novel concept of sensing based on OFF-ON-OFF switching.

The concept was tested on **1H** in MeCN (1 μM). After the addition of a small amount of TBAH, the absorption spectra changed from a split Q-band to a single unsplit one (Figure S8 in the Supporting Information). This change is indicative of deprotonation of both central NH groups.[22] The deprotonation was accompanied by a 2.5-times increase in the fluorescence (Figure 5). The [**1H**]/[TBAH] ratio was 2 for the break point of the change in the absorption and fluorescence spectra (Figure 5, inset), and this result further supports the simultaneous deprotonation of both central NH groups, which is typical of TPyzPzs and structurally similar Pcs.[22b,23] Further addition of a small amount of TBAH did not change the spectra or the fluorescent properties. However, addition of larger amounts (over 10 μM) induced spectral changes similar to those observed during titration of **1Zn** upon deprotonation of phenol with TBAH (Figure S9 in the Supporting Information). The phenol deprotonation in **1H** was also accompanied by a decrease in its fluorescence (Figure 5). Similar changes were also observed for **2H** and **3H** (Figures S10 and S11 in the Supporting Information). This result confirmed that the switching between OFF-ON-OFF states was driven by the addition of specific amounts of base. Metal-free TPyzPzs are in the OFF state in their neutral form, switched ON after addition of a small amount of base (TBAH) due to deprotonation of the central NH groups and switched OFF after addition of a larger amount of base, which deprotonates the phenolic group (Figure 5).

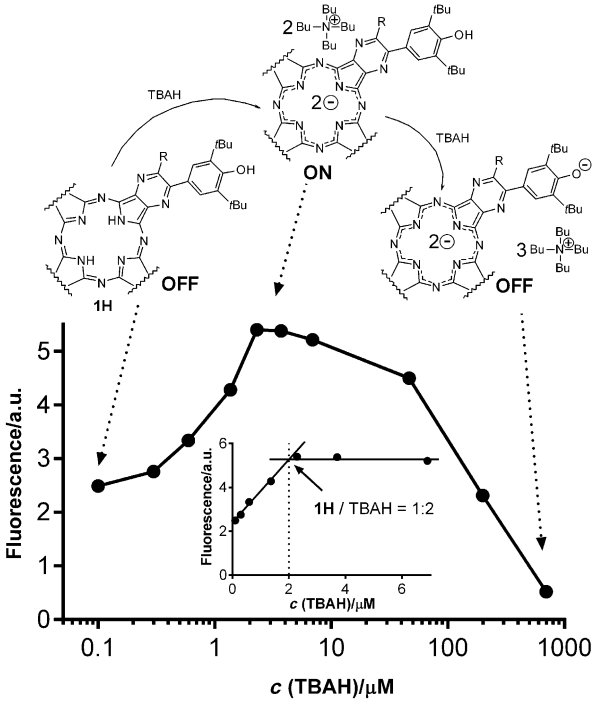


Figure 5. Concept of OFF-ON-OFF switching. Changes in the fluorescence of **1H** in MeCN upon addition of TBAH; c(**1H**) = 1 μM, λ_{exc} = 600 nm, λ_{em} = 669 nm. Inset: enlarged part of the graph at lower TBAH concentrations.

Conclusion

In conclusion, new metal-free and zinc complexes of TPyzPzs bearing phenol(s) as peripheral substituents were synthesized. The deprotonation of phenol to phenolate by TBAH induced a strong donor center for intramolecular charge transfer in the molecule, leading to a significant decrease in the Φ_F values of the zinc complexes. The changes induced by addition of base led to irreversible modification of **4Zn** bearing eight phenolic moieties. However, fully reversible switching was observed for **1Zn**–**3Zn** bearing up to four phenols. **1Zn**–**3Zn** were also successfully anchored into the lipophilic core of a MCT microemulsion, their fluorescence responded well to changes in the buffer pH, and the pK_a was determined to be 12.6. This study confirmed that TPyzPzs bearing phenol moieties may be suitable sensors for basic media and should stimulate future research in this area (e.g., manipulation of the acidic properties of the donor by suitable substitution patterns). The advantage of the TPyzPzs for use in sensing applications is their strong absorption ($\lambda_{max} > 650$ nm, $\varepsilon > 300\,000\ M^{-1}cm^{-1}$) and emission in the red portion of the spectrum ($\lambda_{em} > 655$ nm). Living tissues are more permeable at these wavelengths, and the light is less scattered. In addition, autofluorescence of endogenous chromophores is reduced. A novel fluorescence off-on-off switch using metal-free TPyzPzs bearing phenolic moieties based on the addition of specific amounts of base was demonstrated for the first time.

Experimental Section

General

All organic solvents used for synthesis were of analytical grade. Anhydrous butanol for cyclotetramerization was freshly distilled from magnesium. All chemicals for synthesis were purchased from certified suppliers (Sigma–Aldrich, TCI Europe, Acros, Merck) and used as received. 5,6-Dichlorpyrazine-2,3-dicarbonitrile was obtained from TCI Europe, unsubstituted zinc phthalocyanine was obtained from Sigma–Aldrich. TLC was performed on Merck aluminum sheets coated 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 Engineeering, Southend-on-Sea, Essex, Great Britain). The infrared spectra were obtained on a Nicolet 6700 spectrometer in ATR mode. ^{1}H and ^{13}C NMR spectra were recorded on a Varian Mercury Vx BB 300 NMR spectrometer or VNMR S500 NMR spectrometer. Chemical shifts are given relative to $Si(CH_3)_4$ and were locked to the signal of the solvent. The UV/Vis spectra were recorded using a Shimadzu UV-2600 spectrophotometer. The steady-state fluorescence spectra were measured using an AMINCO-Bowman Series 2 luminescence spectrometer. The MALDI-TOF mass spectra were recorded in positive reflectron mode on a 4800 MALDI TOF/TOF mass spectrometer (AB Sciex, Framingham, MA, USA) in *trans*-2- [3-(4-*tert*-butylphenyl) -2-methyl-2-propenylidene] -malononitrile as a matrix. The instrument was calibrated externally with a five-point calibration using a peptide calibration Mix1 kit (LaserBio Laboratories, Sophia-Antipolis, France). High resolution mass spectra (HR MS) were measured using a UHPLC system Acquity UPLC I-class (Waters, Millford, USA) coupled to a high resolution mass spectrometer Synapt G2Si (Waters, Manchester, UK) based on Q-TOF. The chromatography for this HR MS measurement was performed using an Acquity UPLC BEH300 C4 (2.1×50 mm, 1.7 μm) column with isocratic elution consisting of MeCN and 10 mM ammonium formate buffer at a pH of 3 (90:10) and a flow rate of 0.4 mL min^{-1}. Electrospray ionization was operated in positive mode. The ESI spectra were recorded in the range 200–2000 *m/z* using glu-fibrinopeptide B as a lock mass reference and sodium iodide for calibration. Slightly modified procedures to syntheses of compounds that have already been published (**7**, **8**, **10**, **11**) are mentioned in the Supporting Information. Synthesis of compound **6Zn** was performed according to literature and is not reported here.[17]

Synthesis and characterization

Synthesis of 2-[3,5-di(*tert*-butyl)-4-hydroxyphenyl]-3,9,10,16,17,23,24-heptakis(*tert*-butylsulfanyl)-1,4,8,11,15,18,22,25-(octaaza)phthalocyanine (1H): Magnesium turnings (680 mg, 28 mmol) were refluxed with a small crystal of iodine in anhydr. butanol (65 mL) for 3 h. After completion of the magnesium butoxide formation, compound **9** (423 mg, 1 mmol) and 5,6-bis(*tert*-butylsulfanyl)pyrazine-2,3-dicarbonitrile[24] (918 mg, 3 mmol) were added, and the refluxing was continued for an additional 19 h. After cooling, the entire reaction was poured into a mixture (300 mL) consisting of MeOH/water/acetic acid (10:10:1, v/v) and stirred at RT for 30 min. The precipitate was collected and washed thoroughly with water and air-dried. Then, the mixture of magnesium congeners was demetalated. The entire product mixture was dissolved in $CHCl_3$ (50 mL), and TsOH monohydrate (1.9 g, 10 mmol) dissolved in THF (50 mL) was added. The mixture was stirred at RT for 4 h. The solvent was evaporated, and the residue was washed with water. The crude product was purified by column chromatography on silica with toluene/$CHCl_3$ (3:1) as the eluent, and the second intense green fraction was collected. The purified product was washed gently with MeOH to yield a dark green solid. Yield 90 mg (7%). ^{1}H NMR (300 MHz, $CDCl_3$, 25 °C, TMS): $\delta = 8.38$ (s, 2H; ArH), 5.66 (s, 1H; OH), 2.14 (s, 27H, $SCCH_3$), 2.11 (s, 18H, $SCCH_3$), 2.10 (s, 18H, $SCCH_3$), 1.68 (s, 18H, $ArCCH_3$) and −1.73 ppm (s, 2H, NH); ^{13}C NMR (75 MHz, $CDCl_3$, 25 °C, TMS): $\delta = 160.67$, 160.31, 159.81, 159.16, 159.10, 156.03, 155.19, 135.54, 128.79, 127.78, 124.44, 123.96, 51.85, 51.75, 51.71, 51.64, 51.61, 50.85, 34.82, 30.79, 30.67, 30.57, 30.52, 30.18, 29.69 ppm (some aromatic carbon signals were not detected); IR (ATR): $\tilde{\nu} = 3630$ (OH), 3302 (centr NH), 2957, 2901, 1522, 1452, 1362, 1313, 1279, 1232, 1141, 1081, 1011, 971 cm^{-1}; UV/Vis (THF, 1 μM): λ_{max} (ε) = 673 (135 100), 642 (110 000), 621 (36 700), 590 (26 100), 480 (49 400), 367 nm (103 500 mol^{-1} cm^{-1} L); MS (MALDI): *m/z*: 1342.4 $[M]^+$, HR MS (ESI): *m/z* 1343.5281 $[M+H]^+$, calcd for $C_{66}H_{87}N_{16}OS_7$: 1343.5288.

Synthesis of 2-[3,5-di(*tert*-butyl)-4-hydroxyphenyl]-3,9,10,16,17,23,24-heptakis(*tert*-butylsulfanyl)-1,4,8,11,15,18,22,25-(octaaza)phthalocyaninato zinc(II) (1Zn): Metal-free **1H** (35 mg, 26 μmol) was dissolved in pyridine (2 mL) and anhydr. $Zn(CH_3COO)_2$ (48 mg, 0.26 mmol) was added. The mixture was refluxed for 1 h. The solvent was evaporated, and the residue was washed with water. The crude product was purified by column chromatography on silica with toluene/$CHCl_3$ 1:1 as the eluent. The purified product was washed gently with MeOH to afford dark green solid. Yield 34 mg (93%). ^{1}H NMR (300 MHz, $CDCl_3$/[D_5]pyridine, 25 °C, TMS): $\delta = 8.53$ (s, 2H; ArH), 7.04 (s, 1H; OH), 2.27 (s, 27H, $SCCH_3$), 2.25 (s, 18H, $SCCH_3$), 2.23 (s, 9H, $SCCH_3$), 2.21 (s, 9H, $SCCH_3$), 1.79 ppm (s, 18H, $ArCCH_3$); ^{13}C NMR (75 MHz, $CDCl_3$/[D_5]pyridine, 25 °C, TMS): $\delta = 159.84$, 159.02, 158.55, 158.51, 158.29, 156.50, 154.79, 151.97, 151.64, 151.54, 151.50, 151.43, 151.11, 146.85, 145.19, 144.85, 144.68, 144.59, 144.57, 144.55,

137.00, 129.29, 127.78, 51.52, 51.49, 51.46, 51.41, 50.76, 35.21, 31.07, 30.96, 30.88, 30.83, 30.78, 29.88 ppm (some aromatic carbon signals were not detected); IR (ATR): $\tilde{\nu}=2968$, 2915, 1518, 1477, 1454, 1391, 1362, 1321, 1252, 1146, 976 cm^{-1}; UV/Vis (THF, 1 μM): λ_{max} (ε) = 651 (290900), 591 (39100), 378 nm (148900 $mol^{-1}cm^{-1}L$); MS (MALDI): *m/z*: 1404.3 $[M]^+$. HR MS (ESI): *m/z* 1405.4415 $[M+H]^+$, calcd for $C_{66}H_{85}N_{16}OS_7Zn$: 1405.4423.

Synthesis of 2,3-bis[3,5-di(*tert*-butyl)-4-hydroxyphenyl]-9,10,16,17,23,24-hexakis(*tert*-butylsulfanyl)-1,4,8,11,15,18,22,25-(octaaza)phthalocyanine (2H): Magnesium turnings (803 mg, 33 mmol) were refluxed with a small crystal of iodine in anhydr. butanol (65 mL) for 3 h. After completion of the magnesium butoxide formation, compound **7** (638 mg, 1.2 mmol) and 5,6-bis(*tert*-butylsulfanyl)pyrazine-2,3-dicarbonitrile (1.09 g, 3.6 mmol) were added, and the refluxing was continued for an additional 6 h. After cooling, the entire reaction was poured into a mixture (300 mL) consisting of MeOH/water/acetic acid (10:10:1, v/v) and stirred at RT for 30 min. The precipitate was collected and washed thoroughly with water and air-dried. Then, the mixture of magnesium congeners was demetalated. The entire product mixture was dissolved in THF (100 mL), and TsOH monohydrate (2.3 g, 12 mmol) dissolved in THF (50 mL) was added. The mixture was stirred at RT for 4 h. The solvent was evaporated, and the residue was washed with water. The crude product was purified by gradient column chromatography on silica with toluene/$CHCl_3$ (3:1) up to 1:1 as the eluent, and the second intense green fraction ($R_f=0.16$ in toluene/$CHCl_3$ (3:1); $R_f=0.27$ in toluene/$CHCl_3$ 1:1) was collected (**2H**). The third most intense green fraction ($R_f=0.05$ in toluene/$CHCl_3$ 3:1; $R_f=0.12$ in toluene/$CHCl_3$ 1:1) was also collected (**3H**). Both fractions were repurified by repeated column chromatography using toluene/$CHCl_3$ (3:1) as the eluent. After evaporation of the pure fractions, the obtained dark green solids were washed gently with a small amount of hexane. Yield of **2H**: 340 mg (21%). ^{1}H NMR (500 MHz, $CDCl_3$/[D_5]pyridine, 25 °C, TMS): $\delta=8.29$ (s, 4H; ArH), 6.86 (s, 2H; OH), 2.29 (s, 18H, $SCCH_3$), 2.25 (s, 18H, $SCCH_3$), 2.23 (s, 18H, $SCCH_3$), 1.70 (s, 36H, $ArCCH_3$), −1.80 ppm (s, 2H, NH); ^{13}C NMR (125 MHz, $CDCl_3$/[D_5]pyridine, 25 °C, TMS): $\delta=160.66$, 160.33, 159.13, 156.05, 155.70, 137.42, 131.35, 128.17, 51.88, 51.85, 51.63, 35.00, 30.98, 30.80, 30.72 ppm (some aromatic carbon signals were not detected); IR (ATR): $\tilde{\nu}=3630$ (OH), 3295 (centr NH), 2959, 2919, 1518, 1444, 1362, 1317, 1233, 1142, 1081, 1015, 972 cm^{-1}; UV/Vis (THF, 1 μM): λ_{max} (ε) = 674 (153600), 643 (121800), 619 (43300), 590 (30400), 481 (53700), 370 nm (110200 $mol^{-1}cm^{-1}L$); MS (MALDI): *m/z*: 1458.5 $[M]^+$. HR MS (ESI): *m/z* 1459.6466 $[M+H]^+$, calcd for $C_{76}H_{99}N_{16}O_2S_6$: 1459.6456.

Synthesis of 2,3-bis[3,5-di(*tert*-butyl)-4-hydroxyphenyl]-9,10,16,17,23,24-hexakis(*tert*-butylsulfanyl)-1,4,8,11,15,18,22,25-(octaaza)phthalocyaninato zinc (2Zn): The same method used for the synthesis of **1Zn** was applied but starting from **2H** (280 mg, 0.19 mmol) and anhydr. $Zn(CH_3COO)_2$ (246 mg, 1.34 mmol). Mobile phase: $CHCl_3$. Yield 229 mg (78%). ^{1}H NMR (300 MHz, $CDCl_3$/[D_5]pyridine, 25 °C, TMS): $\delta=8.22$ (s, 4H; ArH), 6.87 (s, 2H; OH), 2.263 (s, 18H, $SCCH_3$), 2.256 (s, 18H, $SCCH_3$), 2.247 (s, 18H, $SCCH_3$), 1.63 ppm (s, 36H, $ArCCH_3$); ^{13}C NMR (75 MHz, $CDCl_3$/[D_5]pyridine, 25 °C, TMS): $\delta=158.93$, 158.53, 158.31, 155.58, 155.30, 151.58, 151.53, 151.44, 151.38, 147.35, 144.83, 144.66, 144.55, 137.43, 131.28, 127.93, 123.84, 51.49, 51.43, 51.40, 34.91, 31.07, 30.85, 30.83, 30.63 ppm; IR (ATR): $\tilde{\nu}=3632$ (OH), 2960, 2919, 1519, 1445, 1392, 1362, 1320, 1250, 1141, 1106, 976 cm^{-1}; UV/Vis (THF, 1 μM): λ_{max} (ε) = 651 (308900), 591 (40400), 380 (148500 $mol^{-1}cm^{-1}L$); MS (MALDI): *m/z*: 1520.4 $[M]^+$. HR MS (ESI): *m/z* 1521.5571 $[M+H]^+$, calcd for $C_{76}H_{97}N_{16}O_2S_6Zn$: 1521.5591.

Synthesis of 2,3,9,10-tetrakis[3,5-di(*tert*-butyl)-4-hydroxyphenyl]-16,17,23,24-tetrakis (*tert*-butylsulfanyl)-1,4,8,11,15,18,22,25-(octaaza)phthalocyanine (3H): The compound was isolated as the third most intense green fraction from the column chromatography in the synthesis of **2H**. Yield (142 mg, 7%). ^{1}H NMR (500 MHz, $CDCl_3$/[D_5]pyridine, 25 °C, TMS): $\delta=8.26$ (s, 4H; ArH), 8.14 (s, 4H; ArH), 6.81 (s, 2H; OH), 6.75 (s, 4H; OH), 2.30 (s, 18H, $SCCH_3$), 2.29 (s, 18H, $SCCH_3$), 1.71 (s, 36H, $ArCCH_3$), 1.67 (s, 36H, $ArCCH_3$), −1.02 ppm (s, 2H, NH); ^{13}C NMR (125 MHz, $CDCl_3$/[D_5]pyridine, 25 °C, TMS): $\delta=157.11$, 155.56, 141.61, 137.42, 137.07, 131.36, 129.20, 128.48, 128.42, 128.21, 126.70, 125.50, 51.71, 51.57, 35.02, 35.00, 31.06, 30.90, 30.77, 30.72, 30.69 ppm (some aromatic carbon signals were not detected); IR (ATR): $\tilde{\nu}=3630$ (OH), 3301 (centr NH), 2959, 2926, 2873, 1522, 1443, 1393, 1361, 1319, 1282, 1235, 1148, 1113, 1081, 1023, 986, 970 cm^{-1}; UV/Vis (THF, 1 μM): λ_{max} (ε) = 675 (134000), 646 (102400), 620 (37900), 593 (27500), 483 (41600), 374 nm (93300 $mol^{-1}cm^{-1}L$); MS (MALDI): *m/z*: 1690.7 $[M]^+$. HR MS (ESI): *m/z* 1691.8831 $[M+H]^+$, calcd for $C_{96}H_{123}N_{16}O_4S_4$: 1691.8791.

Synthesis of 2,3,9,10-tetrakis[3,5-di(*tert*-butyl)-4-hydroxyphenyl]-16,17,23,24-tetrakis(*tert*-butylsulfanyl)-1,4,8,11,15,18,22,25-(octaaza)phthalocyaninato zinc (3Zn): The same method used for the synthesis of **1Zn** was applied but starting from **3H** (100 mg, 5.9 μmol) and anhydr. $Zn(CH_3COO)_2$ (76 mg, 41 μmol). Mobile phase: toluene/$CHCl_3$/THF 20:10:1. Yield 100 mg (96%). ^{1}H NMR (300 MHz, $CDCl_3$/[D_5]pyridine, 25 °C, TMS): $\delta=8.21$ (s, 4H; ArH), 8.05 (s, 4H; ArH), 6.85 (s, 2H; OH), 6.75 (s, 2H; OH), 2.27 (s, 18H, $SCCH_3$), 2.25 (s, 18H, $SCCH_3$), 1.63 (s, 36H, $ArCCH_3$), 1.61 ppm (s, 36H, $ArCCH_3$); ^{13}C NMR (75 MHz, $CDCl_3$, 25 °C, TMS): $\delta=159.06$, 158.34, 156.12, 155.55, 155.36, 151.70, 151.59, 151.35, 147.83, 144.82, 144.65, 137.53, 137.22, 131.64, 130.97, 128.17, 127.86, 51.44, 51.40, 34.91, 34.89, 31.07, 30.83, 30.66, 30.63 ppm (some aromatic carbon signals were not detected); IR (ATR): $\tilde{\nu}=3632$ (OH), 2958, 2927, 2871, 1599, 1535, 1443, 1391, 1360, 1320, 1254, 1240, 1200, 1145, 1105, 1026, 1042, 980 cm^{-1}; UV/Vis (THF, 1 μM): λ_{max} (ε) = 653 (280 300), 593 (37 400), 380 (133900 $mol^{-1}cm^{-1}L$); MS (MALDI): *m/z*: 1752.6 $[M]^+$. HR MS (ESI): *m/z* 1753.7892 $[M+H]^+$, calcd for $C_{96}H_{121}N_{16}O_4S_4Zn$: 1753.7926.

Synthesis of 2,3,9,10,16,17,23,24-octakis[3,5-di(*tert*-butyl)-4-hydroxyphenyl]-1,4,8,11,15,18,22,25-(octaaza)phthalocyaninato magnesium(II) (4Mg): Magnesium turnings (316 mg, 13 mmol) were refluxed with a small crystal of iodine in anhydr. butanol (65 mL) for 3 h. After completion of the magnesium butoxide formation, compound **7** (1 g, 1.86 mmol) was added, and the refluxing was continued for an additional 19 h. After cooling, the entire reaction was poured into a mixture (300 mL) consisting of MeOH/water/acetic acid (10:10:1, v/v) and stirred at RT for 30 min. The precipitate was collected and washed thoroughly with water and MeOH. The crude product was purified by column chromatography on silica with $CHCl_3$/THF (20:1) as the eluent. The purified product was dissolved in a minimal amount of $CHCl_3$ and dropped into hexane. A fine precipitate was collected and dried to yield a dark green solid. Yield 714 mg (71%). ^{1}H NMR (300 MHz, $CDCl_3$/[D_5]pyridine, 25 °C, TMS): $\delta=8.03$ (s, 16H; ArH), 1.61 ppm (s, 144H, CH_3); ^{13}C NMR (75 MHz, $CDCl_3$/[D_5]pyridine, 25 °C, TMS): no signals were detected. IR (ATR): $\tilde{\nu}=3630$ (OH), 2956, 1636, 1437, 1393, 1355, 1319, 1239, 1198, 1159, 11010, 1004 cm^{-1}; UV/Vis (THF, 1 μM): λ_{max} (ε) = 657 (364900), 596 (45600), 465 (27000), 391 nm (171000 $mol^{-1}cm^{-1}L$); MS (MALDI): *m/z*: 2177.1 $[M]^+$. HR MS (ESI): *m/z* 2178.3208 $[M+H]^+$, calcd for $C_{136}H_{169}MgN_{16}O_8$: 2178.3154.

Synthesis of 2,3,9,10,16,17,23,24-octakis[3,5-di (*tert*-butyl)-4-hydroxyphenyl]-1,4,8,11,15,18,22,25-(octaaza)phthalocyanine (4H): Magnesium complex **4Mg** (175 mg, 80 μmol) was dissolved in THF

(10 mL), and TsOH monohydrate (153 mg, 0.8 mmol) dissolved in THF (10 mL) was added. The mixture was stirred at RT for 4 h. The solvent was evaporated, and the residue was washed with water. The crude product was purified by column chromatography on silica with $CHCl_3$/THF (50:1) as the eluent. The purified product was washed thoroughly with hexane. A fine precipitate was collected and dried to afford a black solid. Yield 159 mg (92%). ^{1}H NMR (300 MHz, $CDCl_3$/[D_5]pyridine, 25 °C, TMS): $\delta = 8.07$ (s, 16H; ArH), 6.78 (bs, 8H, OH), 1.64 (s, 144H, CH_3), −0.69 ppm (bs, 2H, NH); ^{13}C NMR (75 MHz, $CDCl_3$/[D_5]pyridine, 25 °C, TMS): $\delta = 158.0$, 155.7, 146.2, 137.5, 131.0, 128.2, 34.9, 30.7 ppm (one aromatic carbon was not detected); IR (ATR): $\tilde{\nu} = 3633$ (OH), 3301 (NH), 2955, 2871, 1635, 1598, 1541, 1434, 1400, 1353, 1319, 1255, 1238, 1223, 1150, 1110, 1015 cm^{-1}; UV/Vis (THF, 1 μM): λ_{max} (ε) = 676 (174500), 650 (128300), 621 (47200), 599 (38400), 519 (45600), 384 nm (118400 mol^{-1} cm^{-1} L); MS (MALDI): *m/z*: 2155.2 [*M*]$^+$; HR MS (ESI): *m/z* 2156.3438 [*M*+H]$^+$, calcd for $C_{136}H_{171}N_{16}O_8$: 2156.3460. The data corresponded well with those published by Hill et al.[13a]

Synthesis of 2,3,9,10,16,17,23,24-octakis[3,5-di(*tert*-butyl)-4-hydroxyphenyl]-1,4,8,11,15,18,22,25-(octaaza)phthalocyaninato zinc(II) (4Zn): Metal-free **4H** (78 mg, 36 μmol) was dissolved in pyridine (5 mL) and anhydr. $Zn(CH_3COO)_2$ (66 mg, 0.36 mmol) was added. The mixture was refluxed for 1 h. The solvent was evaporated, and the residue was washed with water. The crude product was purified by column chromatography on silica with $CHCl_3$/THF (25:1) as the eluent. The purified product was washed thoroughly with hexane. A fine precipitate was collected and dried to afford a black solid. Yield 43 mg (54%). ^{1}H NMR (300 MHz, $CDCl_3$/[D_5]pyridine, 25 °C, TMS): $\delta = 8.06$ (s, 16H; ArH), 7.49 (s, 8H, OH), 1.63 ppm (s, 144H, CH_3); ^{13}C NMR (75 MHz, $CDCl_3$/[D_5]pyridine, 25 °C, TMS): $\delta = 34.4$, 30.1 ppm (aromatic carbon signals were not detected). IR (ATR): $\tilde{\nu} = 3639$ (OH), 2956, 2871, 1635, 1601, 1540, 1440, 1356, 1318, 1238, 1159, 1103, 1005 cm^{-1}; UV/Vis (THF, 1 μM): λ_{max} (ε) = 654 (325500), 595 (42300), 460 (28300), 384 nm (147800 mol^{-1} cm^{-1} L); MS (MALDI): *m/z*: 2217.1 [*M*]$^+$; HR MS (ESI): *m/z* 2218.2534 [*M*+H]$^+$, calcd for $C_{136}H_{169}N_{16}O_8Zn$: 2218.2595.

Synthesis of 2,3,9,10,16,17,23,24-octakis(3,5-diisopropyl-4-methoxyphenyl)-1,4,8,11,15,18,22,25-(octaaza)phthalocyaninato magnesium(II) (5Mg): Magnesium complex **5Mg** was prepared in the same way as **4Mg** from magnesium (86 mg, 3.5 mmol) and precursor 13 (257 mg, 0.5 mmol). $CHCl_3$/THF (50:1) was used as the eluent for column chromatography, and the final product was only washed with hexane without dissolving in $CHCl_3$. Yield 137 mg (53%). ^{1}H NMR (300 MHz, $CDCl_3$/[D_5]pyridine, 25 °C, TMS): $\delta = 7.83$ (s, 16H; ArH), 3.89 (s, 24H, OCH_3), 3.50 (hept, $J = 6.8$ Hz, 16H, CH), 1.31 ppm (d, $J = 6.8$ Hz, 96H, CH_3); ^{13}C NMR (75 MHz, $CDCl_3$/[D_5]pyridine, 25 °C, TMS): $\delta = 156.0$, 155.3, 151.2, 148.9, 142.1, 136.1, 127.3, 62.4, 26.7, 24.3 ppm. IR (ATR): $\tilde{\nu} = 2962$, 2871, 2828, 1632, 1536, 1462, 1366, 1311, 1286, 1254, 1236, 1197, 1168, 1099, 1061, 1013 cm^{-1}; UV/Vis (THF, 1 μM): λ_{max} (ε) = 651 (385400), 624 (43600), 591 (46400), 383 nm (176100 mol^{-1} cm^{-1} L); MS (MALDI): *m/z*: 2065.0 [*M*]$^+$; HR MS (ESI): *m/z* 2066.1924 [*M*+H]$^+$, calcd for $C_{128}H_{153}MgN_{16}O_8$: 2066.1902.

Synthesis of 2,3,9,10,16,17,23,24-octakis(3,5-diisopropyl-4-methoxyphenyl)-1,4,8,11,15,18,22,25-(octaaza)phthalocyanine (5H): Metal-free complex **5H** was prepared in the same way as **4H** from magnesium complex **5Mg** (88 mg, 43 μmol) and TsOH monohydrate (82 mg, 0.43 mmol). Toluene/$CHCl_3$/THF (25:25:1) was used as the eluent for column chromatography, and the final product was only washed with MeOH. Yield 77 mg (88%). ^{1}H NMR (300 MHz, $CDCl_3$/[D_5]pyridine, 25 °C, TMS): $\delta = 7.84$ (s, 16H; ArH), 3.88 (s, 24H, OCH_3), 3.49 (hept, $J = 6.9$ Hz, 16H, CH), 1.30 (d, $J = 6.9$ Hz, 96H, CH_3), −0.77 ppm (bs, 2H, NH); ^{13}C NMR (75 MHz, $CDCl_3$/[D_5]pyridine, 25 °C, TMS): $\delta = 157.8$, 155.7, 146.7, 142.3, 135.6, 127.3, 62.4, 26.8, 24.2 ppm (one aromatic carbon was not detected). IR (ATR): $\tilde{\nu} = 3305$ (NH), 2964, 2870, 2829, 1633, 1543, 1460, 1364, 1311, 1286, 1225, 1200, 1169, 1011 cm^{-1}; UV/Vis (THF, 1 μM): λ_{max} (ε) = 668 (146400), 639 (121700), 612 (41200), 588 (31800), 470 (42400), 369 nm (113700 mol^{-1} cm^{-1} L); MS (MALDI): *m/z*: 2043.1 [*M*]$^+$; HR MS (ESI): *m/z* 2044.2190 [*M*+H]$^+$, calcd for $C_{128}H_{155}N_{16}O_8$: 2044.2208.

Synthesis of 2,3,9,10,16,17,23,24-octakis(3,5-diisopropyl-4-methoxyphenyl)-1,4,8,11,15,18,22,25-(octaaza)phthalocyaninato zinc(II) (5Zn): Zinc complex **5Zn** was prepared in the same way as **4Zn** from metal-free **5H** (35 mg, 17 μmol) and anhydr. $Zn(CH_3COO)_2$ (32 mg, 0.17 mmol). Toluene/$CHCl_3$/THF (25:25:1) was used as the eluent for column chromatography, and the final product was only washed with MeOH. Yield 32 mg (97%). ^{1}H NMR (300 MHz, $CDCl_3$/[D_5]pyridine, 25 °C, TMS): $\delta = 7.79$ (s, 16H; ArH), 3.84 (s, 24H, OCH_3), 3.53–3.36 (m, 16H, CH), 1.25 ppm (d, $J = 6.8$ Hz, 96H, CH_3); ^{13}C NMR (75 MHz, $CDCl_3$/[D_5]pyridine, 25 °C, TMS): 156.4, 155.3, 151.7, 148.3, 142.0, 135.9, 127.2, 62.3, 26.6, 24.1 ppm; IR (ATR): $\tilde{\nu} = 3962$, 2870, 2831, 1633, 1537, 1461, 1369, 1310, 1286, 1236, 1197, 1168, 1099, 1061, 1012 cm^{-1}; UV/Vis (THF, 1 μM): λ_{max} (ε) = 648 (330600), 588 (42000), 375 nm (153200 mol^{-1} cm^{-1} L); MS (MALDI): *m/z*: 2105.0 [*M*]$^+$; HR MS (ESI): the compound did not ionize sufficiently in ESI.

Synthesis of 5-*tert*-butylsulfanyl-6-[3,5-di(*tert*-butyl)-4-hydroxyphenyl]pyrazine-2,3-dicarbonitrile (9): Compound **8** (1 g, 2.7 mmol) was dissolved in pyridine (30 mL) to yield a red solution. 2-Methylpropan-2-thiol (292 mg, 365 μL, 3.2 mmol) was added, and the mixture was refluxed for 1 h. The solvent was removed under reduced pressure, and the solid residue was washed with water and air-dried. The crude product was purified by column chromatography on silica with hexane/ethylacetate (15:1) as the eluent. The purified product was crystallized from chloroform/hexane to yield yellow crystals. Yield 0.88 g (77%); m.p. 143.7–144.6 °C; ^{1}H NMR (300 MHz, $CDCl_3$, 25 °C, TMS): $\delta = 7.67$ (s, 2H; ArH), 5.69 (s, 1H; OH), 1.62 (s, 9H, $SCCH_3$), 1.48 ppm (s, 18H, CH_3); ^{13}C NMR (75 MHz, $CDCl_3$, 25 °C, TMS): $\delta = 162.5$, 157.1, 156.2, 136.1, 127.8, 126.8, 126.1, 125.1, 113.7, 113.6, 51.3, 34.6, 30.1, 29.5 ppm; IR (ATR): $\tilde{\nu} = 2963$, 2874, 2225 (CN), 1489, 1379, 1363, 1222, 1139, 999 cm^{-1}.

Synthesis of 1,2-bis(3,5-diisopropyl-4-methoxyphenyl)ethane-1,2-dione (12): Benzaldehyde **11** (6.6 g, 30 mmol) was dissolved in absolute EtOH (75 mL), and 5-(2-hydroxyethyl)-3,4-dimethylthiazolium iodide (427 mg, 1.5 mmol) and 1,8-diazabicyclo[5.4.0]undec-7-ene (227 mg, 227 μL, 1.5 mmol) were added. The reaction was heated under reflux for 3 h, and the color changed from red to yellow-orange. The solvent was removed under reduced pressure, and the residue was suspended in water. The suspension was extracted three times with ethyl acetate, and the organic layers were dried over anhydr. Na_2SO_4 and evaporated to dryness. The acyloin was directly oxidized to a diketone after dissolution in acetic acid (50 mL) using $Cu(CH_3COO)_2$ (30 mg, 0.16 mmol) and NH_4NO_3 (1.4 g, 17.5 mmol). The mixture was refluxed for 4 h, and the solvent was evaporated under reduced pressure. The residue was suspended in water and extracted three times with ethyl acetate. The organic layers were dried over anhydr. Na_2SO_4 and evaporated to dryness. The crude product was purified by column chromatography on silica with toluene/$CHCl_3$ (4:1) as the eluent. The purified product was crystallized from $CHCl_3$/hexane (the crystals appeared after couple of days in the freezer) to yield colorless crystals. Yield 1.4 g (21%); m.p. 162.6–163.9 °C; ^{1}H NMR (300 MHz, $CDCl_3$, 25 °C, TMS): $\delta = 7.75$ (s, 4H, ArH), 3.80 (s, 6H, OCH_3), 3.34 (hept, $J = 6.9$ Hz, 4H, CH), 1.24 ppm (d, $J = 6.9$ Hz, 24H, CH_3); ^{13}C NMR (75 MHz, $CDCl_3$, 25 °C, TMS): $\delta = 194.1$, 160.5, 142.9, 129.8, 126.7, 62.3, 26.7,

23.8 ppm; IR (ATR): $\tilde{\nu}$ = 2966, 2931, 2871, 1663 (CO), 1593, 1461, 1418, 1364, 1305, 1163, 1112, 1005 cm^{-1}.

Synthesis of 5,6-bis (3,5-diisopropyl-4-methoxyphenyl)pyrazine-2,3-dicarbonitrile (13): Dione **12** (1.4 g, 2.7 mmol) was dissolved in acetic acid (25 mL), and diaminomaleonitrile (1.4 g, 13 mmol) was added. The mixture was refluxed for 3 h, and then, the solvent was evaporated under reduced pressure. The residue was extracted with acetone and filtered, and the filtrate was evaporated. The crude product was purified by column chromatography on silica with hexane/ethyl acetate (9:1) as the eluent. The purified product was crystallized from $CHCl_3$/hexane to afford fine yellow needles. Yield 555 mg (34%); m.p. 241.0–242.3 °C; ^{1}H NMR (500 MHz, [D_6]acetone, 25 °C, TMS): δ = 7.27 (s, 4H, ArH), 3.72 (s, 6H, OCH_3), 3.28 (hept, J = 6.9 Hz, 4H, CH), 1.07 ppm (d, J = 6.9 Hz, 24H, CH_3); ^{13}C NMR (125 MHz, [D_6]acetone, 25 °C, TMS): δ = 157.38, 157.36, 143.04, 133.21, 130.54, 127.05, 114.88, 62.54, 27.14, 24.06 ppm; IR (ATR): $\tilde{\nu}$ = 2964, 2873, 2240 (CN), 1603, 1516, 1474, 1382, 1327, 1301, 1279, 1250, 1194, 1167, 1102, 1003 cm^{-1}.

Singlet oxygen and fluorescence quantum yields

For singlet oxygen and fluorescence determination as well as the titration experiments, the dye samples were purified by preparative TLC on Merck aluminum sheets coated with silica gel 60 F254 to ensure high purity. Both the Φ_Δ and Φ_F values were determined by comparative methods[25] using unsubstituted zinc phthalocyanine (ZnPc) as a reference ($\Phi_{\Delta(THF)}$ = 0.53,[26] $\Phi_{F(THF)}$ = 0.32[1a]). All of the experiments were performed in triplicate, and the data represent the means of the measurements (estimated error ±10%). For Φ_F determination, the sample and reference were excited at 599 nm, and the absorbance at the Q-band maximum was maintained at less than 0.05 to avoid the inner filter effect. The emission spectra were corrected for the instrument response. The Φ_F values were corrected for the refractive indices of the appropriate solvents.

Titrations

A stock solution of **1Zn** (100 μM) was prepared in MeCN, and stock solutions of **2Zn**–**6Zn** were prepared in THF (100 μM). The stock solutions were diluted with MeCN to prepare 1 μM solutions. An aliquot (2.5 mL) of the 1 μM solution was transferred into a quartz cuvette and titrated with small additions of TBAH (5 mM stock solution in MeCN, typical additions 7–40 μL), and the absorption and fluorescence spectra were collected after each addition (λ_{ex} = 603 nm for **1Zn** or 597 nm for **4Zn**–**6Zn** and 595 nm for **2Zn** and **3Zn**). All of the titrations were ended by acidification of the solution by addition of excess acetic acid (7 μL, final concentration ~120 mM). Based on the shape of the absorption spectra, the acidification did not lead to protonization of azomethine nitrogen atoms, which is the most basic center in TPyzPz. A diluted TBAH solution (50 μM) was also used for titrations of **1H**–**3H** (λ_{ex} = 600 nm) to achieve lower TBAH concentrations.

For measurements in an aqueous medium, the microemulsion stock solutions of **1Zn**–**3Zn** and **6Zn** (50 μM) were prepared (see below) and diluted (at least 100 times) in a buffer with the corresponding pH. The Britton–Robinson buffer (0.04 M H_3PO_4, 0.04 M CH_3COOH, 0.04 M H_3BO_3 adjusted to selected pH by 0.2 M NaOH) was used for the pH range of 7.0–11.7, and the Bates and Bower buffer (0.2 M KCl/0.2 M NaOH) was used for the pH range of 11.8–13.0. The pH was measured using a Hanna pH209 pH meter calibrated with a two-point calibration (pH 7.00 and pH 4.00) prior to use.

Preparations of the microemulsions

Compounds **1Zn**–**3Zn** and **6Zn** (0.25 nmol), Cremophor EL (BASF, 72 mg) and medium-chain triglycerides (MCT, Ecogreen oleochemicals, meeting specifications of Ph.Eur.) (29 mg) were dissolved in THF. The solvent was evaporated under reduced pressure, and the flask was maintained under deep vacuum (5 mbar) for 30 min at 40 °C to remove all traces of solvent. Then, water was added to a total volume of 5.0 mL, and the mixture was thoroughly shaken on a vortex and orbital shaker. The particles were characterized using a photon correlation spectroscope Zetasizer Nano-ZS (Malvern, UK). Their size was determined to be approximately 300 nm. The microemulsion was stable for at least two weeks without any significant change in the particle size.

Acknowledgements

The work was financially supported by the Czech Science Foundation (14-02165P). The authors wish to thank Jiří Kuneš for NMR spectroscopy measurements, Juraj Lenčo for MALDI-TOF measurements and Lucie Nováková for HR MS data.

Keywords: azaphthalocyanines · fluorescence · intramolecular charge transfer · pH sensors · phthalocyanines

[1] a) P. Zimcik, V. Novakova, K. Kopecky, M. Miletin, R. Z. Uslu Kobak, E. Svandrlikova, L. Váchová, K. Lang, *Inorg. Chem.* **2012**, *51*, 4215–4223; b) V. Novakova, L. Lochman, I. Zajícová, K. Kopecky, M. Miletin, K. Lang, K. Kirakci, P. Zimcik, *Chem. Eur. J.* **2013**, *19*, 5025–5028.

[2] a) D. Dini, M. Hanack, M. Meneghetti, *J. Phys. Chem. B* **2005**, *109*, 12691–12696; b) M. Villano, V. Amendola, G. Sandona, M. P. Donzello, C. Ercolani, M. Meneghetti, *J. Phys. Chem. B* **2006**, *110*, 24354–24360.

[3] a) E. A. Vlasova, N. Hessenauer-Ilicheva, D. S. Salnikov, E. V. Kudrik, S. V. Makarov, R. van Eldik, *Dalton Trans.* **2009**, 10541–10549; b) V. N. Shishkin, E. V. Kudrik, S. V. Makarov, G. P. Shaposhnikov, *Kinet. Catal.* **2007**, *48*, 660–663.

[4] a) K. Ohta, S. Azumane, W. Kawahara, N. Kobayashi, I. Yamamoto, *J. Mater. Chem.* **1999**, *9*, 2313–2320; b) S. V. Efimova, O. I. Koifman, V. V. Bykova, I. Y. Lukyanov, V. V. Sotsky, N. V. Usol'tseva, *Mol. Cryst. Liq. Cryst.* **2012**, *553*, 66–71.

[5] S. Makhseed, F. Ibrahim, J. Samuel, M. Helliwell, J. E. Warren, C. G. Bezzu, N. B. McKeown, *Chem. Eur. J.* **2008**, *14*, 4810–4815.

[6] a) F. Mitzel, S. FitzGerald, A. Beeby, R. Faust, *Eur. J. Org. Chem.* **2004**, 1136–1142; b) G. De Mori, Z. Fu, E. Viola, X. H. Cai, C. Ercolani, M. P. Donzello, K. M. Kadish, *Inorg. Chem.* **2011**, *50*, 8225–8237.

[7] P. Zimcik, M. Miletin, H. Radilova, V. Novakova, K. Kopecky, J. Svec, E. Rudolf, *Photochem. Photobiol.* **2010**, *86*, 168–175.

[8] C. Bergami, M. P. Donzello, C. Ercolani, F. Monacelli, K. M. Kadish, C. Rizzoli, *Inorg. Chem.* **2005**, *44*, 9852–9861.

[9] a) V. Novakova, P. Hladik, T. Filandrova, I. Zajicova, V. Krepsova, M. Miletin, J. Lenco, P. Zimcik, *Phys. Chem. Chem. Phys.* **2014**, *16*, 5440–5446; b) V. Novakova, P. Zimcik, M. Miletin, L. Vachova, K. Kopecky, K. Lang, P. Chábera, T. Polívka, *Phys. Chem. Chem. Phys.* **2010**, *12*, 2555–2563.

[10] V. Novakova, M. Miletin, K. Kopecky, P. Zimcik, *Chem. Eur. J.* **2011**, *17*, 14273–14282.

[11] L. Lochman, J. Svec, J. Roh, V. Novakova, *Dyes Pigm.* **2015**, *121*, 178–187.

[12] a) X. J. Jiang, P. C. Lo, Y. M. Tsang, S. L. Yeung, W. P. Fong, D. K. P. Ng, *Chem. Eur. J.* **2010**, *16*, 4777–4783; b) X. J. Jiang, S. L. Yeung, P. C. Lo, W. P. Fong, D. K. P. Ng, *J. Med. Chem.* **2011**, *54*, 320–330.

[13] a) J. P. Hill, N. K. Subbaiyan, F. D'Souza, Y. S. Xie, S. Sahu, N. M. Sanchez-Ballester, G. J. Richards, T. Mori, K. Ariga, *Chem. Commun.* **2012**, *48*, 3951–3953; b) X. F. Zhang, *J. Fluoresc.* **2011**, *21*, 1559–1564.

[14] a) T. Jokic, S. M. Borisov, R. Saf, D. A. Nielsen, M. Kuhl, I. Klimant, *Anal. Chem.* **2012**, *84*, 6723–6730; b) K. Waich, T. Mayr, I. Klimant, *Talanta* **2008**, *77*, 66–72.

[15] a) S. Ishihara, J. P. Hill, A. Shundo, G. J. Richards, J. Labuta, K. Ohkubo, S. Fukuzumi, A. Sato, M. R. J. Elsegood, S. J. Teat, K. Ariga, *J. Am. Chem. Soc.* **2011**, *133*, 16119 – 16126; b) A. Shundo, S. Ishihara, J. Labuta, Y. Onuma, H. Sakai, M. Abe, K. Ariga, J. P. Hill, *Chem. Commun.* **2013**, *49*, 6870 – 6872.

[16] a) G. J. Clarkson, P. Humberstone, N. B. McKeown, *Chem. Commun.* **1997**, 1979 – 1980; b) M. Brewis, G. J. Clarkson, P. Humberstone, S. Makhseed, N. B. McKeown, *Chem. Eur. J.* **1998**, *4*, 1633 – 1640.

[17] M. Kostka, P. Zimcik, M. Miletin, P. Klemera, K. Kopecky, Z. Musil, *J. Photochem. Photobiol. A* **2006**, *178*, 16 – 25.

[18] a) N. Barman, K. Sahu, *RSC Adv.* **2014**, *4*, 58299 – 58306; b) N. Barman, D. Singha, K. Sahu, *J. Phys. Chem. A* **2013**, *117*, 3945 – 3953.

[19] a) L. Fajari, P. Fors, K. Lang, S. Nonell, F. R. Trull, *J. Photochem. Photobiol. A* **1996**, *93*, 119 – 128; b) M. Quintiliani, A. Kahnt, T. Wolfle, W. Hieringer, P. Vázquez, A. Gorling, D. M. Guldi, T. Torres, *Chem. Eur. J.* **2008**, *14*, 3765 – 3775.

[20] a) A. Nishinaga, T. Itahara, T. Matsuura, A. Rieker, D. Koch, K. Albert, P. B. Hitchcock, *J. Am. Chem. Soc.* **1978**, *100*, 1826 – 1834; b) A. Nishinaga, T. Shimizu, T. Matsuura, *J. Org. Chem.* **1979**, *44*, 2983 – 2988.

[21] J. P. Hill, W. Van Rossom, S. Ishihara, N. Subbaiyan, F. D'Souza, Y. Xie, N. M. Sanchez-Ballester, K. Ariga, *J. Porphyrins Phthalocyanines* **2014**, *18*, 173 – 181.

[22] a) P. Petrik, P. Zimcik, K. Kopecky, Z. Musil, M. Miletin, V. Loukotova, *J. Porphyrins Phthalocyanines* **2007**, *11*, 487 – 495; b) P. A. Stuzhin, *J. Porphyrins Phthalocyanines* **1999**, *3*, 500 – 513.

[23] J.-B. Liu, Y. Zhao, F.-Q. Zhao, F.-S. Zhang, Y.-W. Tang, X.-q. Song, F.-T. Zhou, *Acta Phys. Chim. Sin.* **1996**, *12*, 202 – 207.

[24] P. Zimcik, M. Miletin, V. Novakova, K. Kopecky, M. Nejedla, V. Stara, K. Sedlackova, *Aust. J. Chem.* **2009**, *62*, 425 – 433.

[25] V. Novakova, M. Miletin, T. Filandrová, J. Lenčo, A. Růžička, P. Zimcik, *J. Org. Chem.* **2014**, *79*, 2082 – 2093.

[26] L. Kaestner, M. Cesson, K. Kassab, T. Christensen, P. D. Edminson, M. J. Cook, I. Chambrier, G. Jori, *Photochem. Photobiol. Sci.* **2003**, *2*, 660 – 667.

Received: June 29, 2015
Published online on August 26, 2015

