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Effective Monofunctional Azaphthalocyanine Photosensitizers for Photodynamic Therapy

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In this work we present a rational design of the active part of third generation photosensitizers for photodynamic therapy based on phthalocyanine and an azaphthalocyanine core. The preferred zinc complexes of the AAAB type that contain bulky *tert*-butylsulfanyl substituents (A) and one carboxy group (B) have been synthesized by statistical condensation and fully characterized. The tetramerization was performed using magnesium(II) butoxide followed by demetalation and insertion of Zn^{II}. Compound **1** synthesized from 4,5-bis(*tert*-butylsulfanyl)phthalonitrile (A) and 2,3-dicyanoquinoxaline-6-carboxylic acid (B) exerted very promising photophysical properties (Q-band absorption at 726 nm, $\varepsilon = 140000\,M^{-1}\,cm^{-1}$), which allowed strong absorption of light at long wavelengths where the penetration of the light through human tissues is deeper. The very high singlet oxygen quantum yield of **1** ($\Phi_\Delta = 0.80$) assures efficient photosensitization. As a result of bulky peripheral substituents, compound **1** shows good solubility in organic solvents with a low degree of aggregation, which makes it potentially viable for non-complicated modification. One carboxy group in the final structure of **1** allows simple binding to possible carriers. This compound is suitable for binding to targeting moieties to form the highly active part of a third-generation photosensitizer.

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Introduction

Photodynamic therapy (PDT) is a well established method for cancer treatment that combines high efficiency with minimal side effects.[1–3] It is based on the absorption of light by a photosensitizer (PS) and transfer of the energy to surrounding molecules, mainly oxygen. Cytotoxic species produced after illumination, the most important being singlet oxygen ($^1O_2$), cause damage to surrounding subcellular compartments, which leads to cell death.[4]

The PSs now used can be divided into two or three generations. The first involves porfimers, which are a complex mixture of several oligomers and absorb at low wavelengths ($\lambda_{max} \sim 630$ nm), where the light penetration through the tissues is not so deep. Moreover, the porfimer absorption at this wavelength is only weak ($\varepsilon \sim 2000\,M^{-1}\,cm^{-1}$). The second generation compounds are defined chemical individuals with absorption at longer wavelengths (close to 700 nm), most of them being from the chlorin family. Although their spectroscopic properties seem to be much better, the targeting of these compounds or their pharmacokinetic properties are often far from optimal. One of the approaches to solve this problem is conjugation of the efficient PS to biomolecules or other carriers that influence the above-mentioned characteristics.[5] Such compounds are sometimes called third generation PSs.

Phthalocyanines (Pcs) are well known organic dyes that have attracted the interest of scientists in several fields.[6,7] PDT applications using Pc derivatives have been developed widely in Russia and a mixture of sulfonated aluminium Pcs (Photosens) have been in clinical practice since 2001.[8] Our research has been focussed on the properties of aza-analogues of Pcs – azaphthalocyanines (AzaPcs) – which appear to be slightly more soluble than the parent Pc. Recently, we have prepared compounds with one carboxy group suitable to bind a targeting moiety,[9] but their properties, in particular their singlet oxygen quantum yield and Q-band absorption maximum ($\Phi_\Delta = 0.36$, $\lambda_{max} = 654$ nm), were not as good as desired. To improve these deficiencies, in this work we present the rational design, synthesis, and properties of improved PSs that can be considered as very promising active parts of third generation PSs.

Results and Discussion

Design of the Molecule

We have designed simple AzaPc molecules that bear one functional group suitable for future derivatization with predicted high Φ_Δ values, strong absorption at longer wavelengths, low degree of aggregation in solution, and good solubility in organic solvents. We have based our design on the following formerly uncovered structure–activity relationships:

(1) Both Pcs and AzaPcs, especially if unsubstituted, suffer from relatively low solubility and high aggregation caused by π–π interactions of the planar macrocyclic molecules. The aggregation of PSs is undesirable because the absorbed energy is dissipated by internal conversion to the ground

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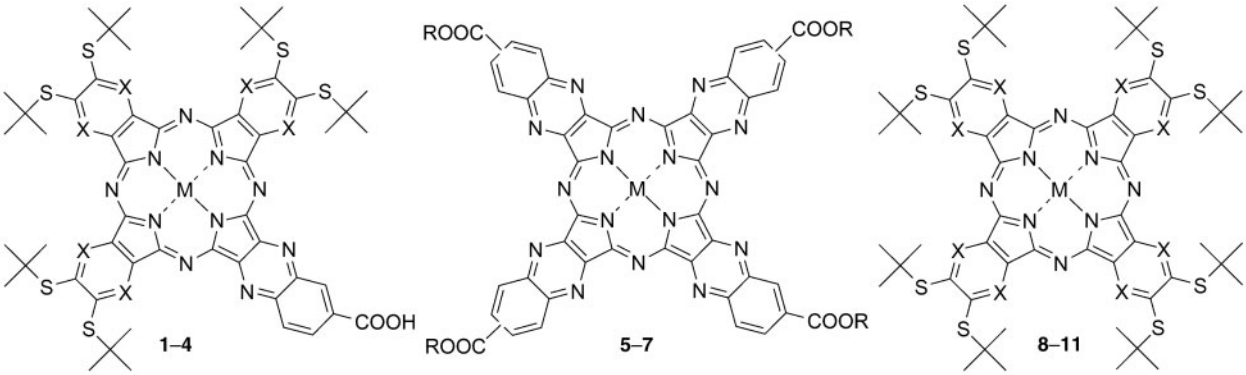


	M	X
1	Zn	CH
2	Zn	N
3	Mg	CH
4	Mg	N

	M	R
5	Mg	H
6	Mg	*n*-butyl
7	Zn	*n*-butyl

	M	X
8	Mg	CH
9	Mg	N
10	Zn	CH
11	Zn	N

Fig. 1. Structures of compounds involved in the study.

state rather than through triplet formation with subsequent singlet oxygen production. The best strategy as to how to decrease the tendency to aggregation is the introduction of peripheral substituents, either long alkyl chains or branched bulky substituents, which prevent the stacking of planar molecules.[10–12] In our recent study on the liposomal model of a biological membrane[13] we found bulky *tert*-butyl groups were preferable for our intended compounds as opposed to long alkyl chains.

(2) Formerly, we have investigated the relationships between the efficiency of singlet oxygen production and the heteroatom that connects the peripheral chain and macrocycle.[14] Nitrogen was found to be quite unsuitable as it inhibits singlet oxygen formation, most likely as a result of intramolecular photoinduced electron transfer. Comparing oxygen and sulfur, the latter one shows a better singlet oxygen production, whereas the former one induces stronger fluorescence. This behaviour may be concerned with the well known heavy atom effect.[15] Similarly, singlet oxygen production increases with the molecular weight of the isosteric heteroatom in the series of xanthenes, thioxanthenes, and selenoxanthenes.[16–18]

(3) The central metal is also significant. The effect of zinc(II) on the longer triplet-state lifetimes and triplet states quantum yields is well known.[19] That is why it is the metal of choice.

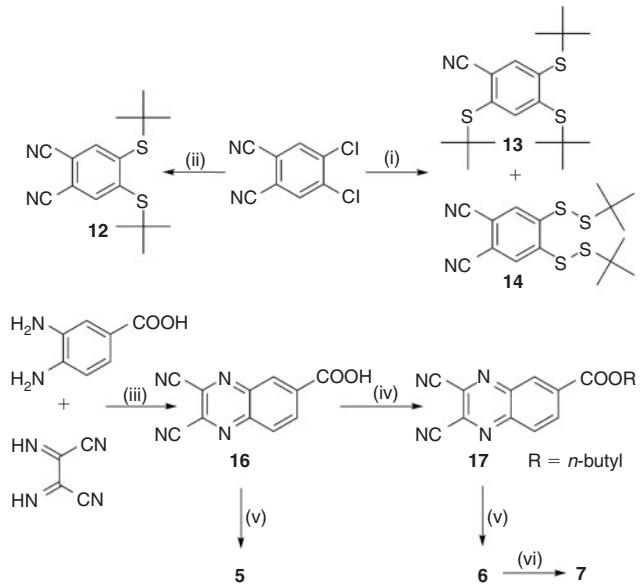
(4) Unsubstituted ZnAzaPc absorbs only at 636 nm. The presence of sulfur as the connecting heteroatom (in addition to a positive effect on Φ_Δ) shifts the Q-band maximum bathochromically and hyperchromically (656 nm, ε close to 300000 M^{-1} cm^{-1}). This is another good reason for choosing the alkylsulfanyl derivatives because both alkyl (634 nm) and alkoxy (624 nm) substitutions do not induce a red-shift.[20] However, the most intensive red-shift is caused by linear annulation of benzene rings onto the periphery of the macrocycle.[21–23] Also, because the presence of nitrogens in the basic macrocycle of AzaPc causes an undesirable blue-shift in comparison to Pc,[10] the presence of some Pc subunits in the structure will have a positive effect on the Q-band position.

(5) The carboxy group is perhaps the most suitable derivatizable substituent that can be used for further modifications. It allows binding of molecules through, e.g., an amide bond, whose chemistry is well investigated and a lot of efficient coupling activators are known from peptide chemistry.[24] The presence of only one carboxy group in the structure is desired as it limits the possibility of polymerization during connecting the PS to a biomolecule. It also gives a possibility of binding of suitable spacers, e.g., for very efficient 'click' chemistry.[25]

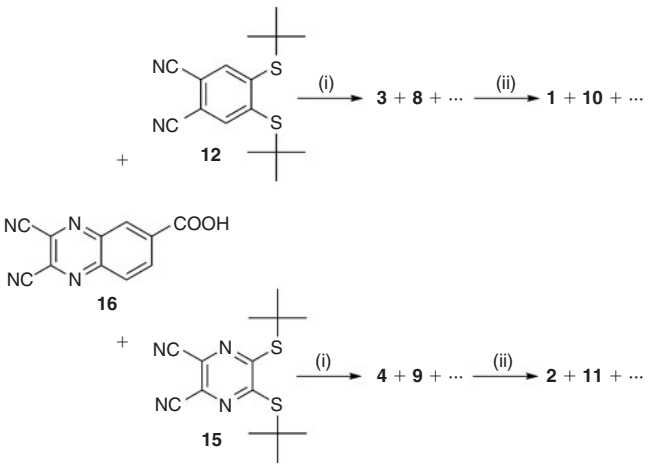
Combining all the above-mentioned facts we decided to prepare AzaPcs **1** (preferred) and **2** (Fig. 1) that meet the outlined requirements. Other compounds **3**–**11** were also investigated in this work for comparative purposes.

Synthesis of the Precursors

The synthesis of Pc and AzaPc molecules is usually performed from the starting aromatic *ortho*-dicarbonitriles. The phthalonitrile derivative **12** has been prepared by us but only in 25% yield.[10] The method described by Choi et al. states 93% yield for a similar *n*-butyl derivative.[26] Surprisingly, by applying their procedure to our substituents we did not receive the desired compound **12**. Instead, only low amounts of two derivatives, which were later characterized as **13** and **14**, appeared in the reaction mixture (Scheme 1). Attempts to tetramerize the dicarbonitrile **14** to Pc failed. After several attempts, our originally published procedure[10] for the synthesis of **12** appeared to be the most suitable. We were able to increase the yield up to 65% by careful treatment and quantitative extraction of the crude reaction mixture. Similarly, we increased the yield of 5,6-bis(*tert*-butylsulfanyl)pyrazine-2,3-dicarbonitrile (**15**) up to 93% (previous work 74%[10]). Synthesis of quinoxaline derivative **16** (Scheme 1) was published over 30 years ago by Rothkopf et al.[27] The described simple crystallization of the crude product from ethanol did not afford the pure compound in our case. Consequently we purified the product using column chromatography on silica with a yield of only 38% (Lit. 65%). Esterification of **16** to **17** through acylchloride was achieved in 89%. Surprisingly, no bands that corresponded to CN vibrations



Scheme 1. Reaction conditions: (i) NaH, Cu_2O, DMF, *t*BuSH, 170°C, 45 min, (ii) NaH, Cu_2O, DMF, *t*BuSH, 90°C, 30 min, (iii) TFA, room temperature, overnight, (iv) $SOCl_2$, THF, toluene, 100°C, 3 h, toluene, pyridine, butanol, 120°C, 3 h, (v) Mg, butanol, I_2, reflux, 24 h, (vi) *p*TSA, room temperature, 90 min, $Zn(CH_3COO)_2$, pyridine, 2 h, reflux.



Scheme 2. Reaction conditions: (i) Mg, butanol, I_2, reflux, overnight, (ii) *p*TSA, room temperature, 90 min, $Zn(CH_3COO)_2$, pyridine, 2 h, reflux.

were observed in the IR spectrum of compounds **16** and **17**, although other data confirmed the right structure and two ^{13}C NMR signals at 114 ppm were attributed to CN carbons.

Synthesis of the Dyes

Although selective methods were developed,[28] in our opinion, the statistical condensation is still the most convenient approach to give reasonable yields of AAAB type unsymmetrical Pc and AzaPc derivatives, which are the aim of this work.

Heating of the precursors **12** and **15**–**17** in *N*,*N*-dimethylformamide (DMF), *N*,*N*-dimethylaminoethanol (DMAE), or quinoline with the zinc(II) acetate template did not afford satisfactory results. The magnesium(II) butoxide method gave good yields for tetramerization so was, therefore, chosen instead.[10] Thus compound **16** reacted with **12** or **15** (Scheme 2) to produce

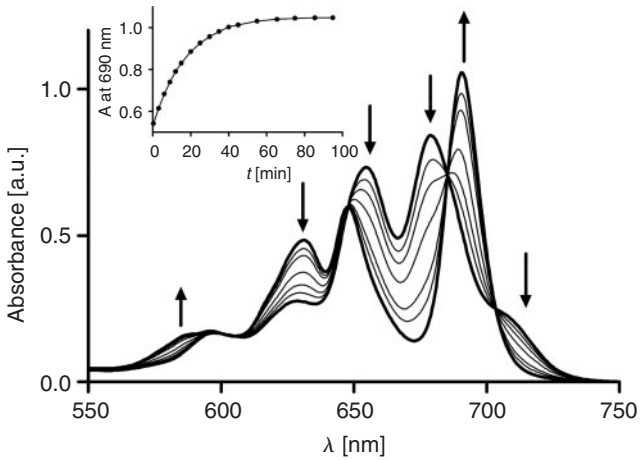


Fig. 2. UV-Vis spectroscopic changes of a THF solution of **4** during demetalation by *p*TSA. Time from 0 to 90 min. Inset: progress of the reaction monitored at 690 nm.

a statistical mixture of six possible dyes in both cases. The mixtures were purified to obtain magnesium(II) complexes **3** and **8** or **4** and **9**.

The alkaline earth metals are not complexed strongly in the centre of the Pc or AzaPc macrocycle and they can be easily removed using stronger acids (e.g., *p*-toluenesulfonic acid, *p*TSA). The removal of the central magnesium(II) is usually accompanied by a change of absorption spectrum. Observing the changes in the spectrum we studied the kinetics of the demetalation of isolated magnesium(II) complexes (example for **4** in Fig. 2). From the data obtained it was obvious that 90 min treatment with *p*TSA was enough to remove all magnesium(II) from the centre of AzaPc **4**. Similar reaction times were obtained also for other magnesium(II) complexes in the study. The transformation to metal-free derivatives was quantitative.

Zinc(II) was introduced into the centre of the macrocycles in pyridine (Scheme 2). Similarly to demetalation, the kinetics of the zinc(II) insertions were also studied and 2 h was found to be sufficient to complete the insertion. The crude mixtures of zinc(II) complexes were chromatographically separated to obtain desired compounds **1** and **2** along with symmetrical products **10** and **11**, which were eluted from the column as the first fractions. Chromatographic isolation of the desired AzaPcs **1** and **2** (as well as their magnesium(II) analogues **3** and **4**) was slightly problematic because they showed very strong silica-binding properties (for more see Experimental section).

Single tetramerization of **16** in magnesium(II) butoxide (Scheme 1) gave compound **5** which was, however, absolutely insoluble in organic solvents (including pyridine, DMF, and dimethyl sulfoxide (DMSO)). The only way to dissolve this compound was by the use of aqueous NaOH, but the character of the spectrum indicated strong aggregation (see Accessory Publication). Reprecipitation by HCl gave again the insoluble free acid of uncertain composition (Mg^{II} or metal-free). Because of this insolubility, the compound could not be used for subsequent investigations and was of low research interest. In order to also study the behaviour and properties of the tetra[2,3]quinoxalinoporphyrazine macrocycle we prepared *n*-butylesters **6** and **7**. Butanol was chosen as the alcoholic part of the ester because it has been shown that it is sufficiently long to allow good solubility.[29] Another reason for this substitution is the transesterification that usually appears during

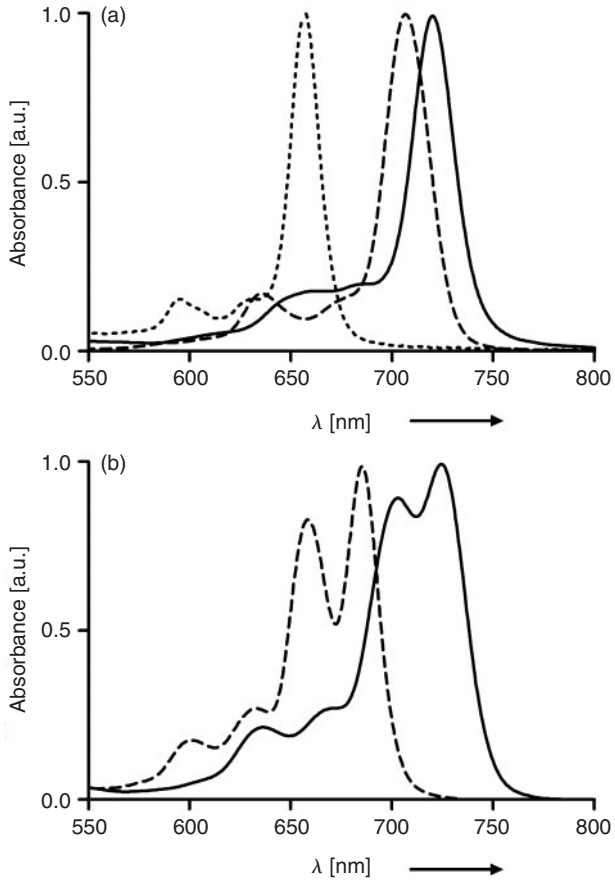


Fig. 3. Normalized UV-vis spectra of (a) **11** (dotted line), **10** (dashed line), and **7** (full line), and (b) **2** (dashed line) and **1** (full line).

tetramerization in alkoxides. In this case, the transesterification can be omitted due to accordance of alkoxide with the ester.

Characterization of the Dyes

All spectroscopic data confirmed the structure and purity of the samples. An interesting feature appeared in the ^{1}H NMR spectra of the dyes **1**–**4**. Comparing to dinitrile **16**, the aromatic signals of the quinoxaline ring are shifted to lower fields (see Accessory Publication). Similar observations of the deshielding effect of the macrocycle were described for peripheral pyridyl substituents by Donzello et al.[30] As a result of the enlarged planar system and not ideal aggregation-inhibiting substituents, compounds **6** and **7** exerted a much higher tendency towards aggregation. That is why their aromatic signals in both the ^{1}H and ^{13}C NMR spectra were not detected. As **6** and **7** are prepared from an unsymmetrical precursor, the final product most likely contains four positional isomers. However, due to the aforementioned aggregation phenomenon, they were not detected by TLC or in the NMR spectrum.

Compounds **1**–**4** showed very good solubility in a wide range of common organic solvents (acetone, chloroform, pyridine, toluene, DMF, and tetrahydrofuran (THF)). As anticipated, the pyrazine analogues **2** and **4** were slightly more soluble. This is a good prerequisite for further modifications, which usually occur in concentrated solutions.

UV-Vis absorption spectra of the symmetrical compounds showed a non-split Q-band (Fig. 3a). The position is dependent on the macrocyclic system (see also Table 1). While tetrapyrazinoporphyrazine (TPyzPA) **11** absorbs at 656 nm, its

Table 1. Photophysical data of synthesized compounds in pyridine

Sample	Metal	Q-band λ_{max} [nm]	Emission λ_{max} [nm]	Φ_{Δ}[B]	Φ_{F}[B]
1	Zn^{II}	726	734	0.80	0.06
2	Zn^{II}	685	691	0.70	0.14
3	Mg^{II}	724	735	0.20	0.06
4	Mg^{II}	686	693	0.33	0.25
6	Mg^{II}	722	731	0.28	0.09
7	Zn^{II}	720	727	0.75	0.05
10	Zn^{II}	706	717	0.72	0.10
11	Zn^{II}	656	663	0.66	0.22
ZnPc[A]	Zn^{II}	674	680	0.61	0.20

[A] Used as ref. [43].
[B] Mean of three independent measurements, estimated error ± 10%.

phthalocyanine analogue **10** is red-shifted to 706 nm. Linear annulation of a benzene ring to each pyrazine in TPyzPA caused a red shift up to 720 nm as observed for compound **7**. These results are expected and have been discussed briefly in the paragraph concerning the design of the molecules. The absorption in the Q-band area usually contains two weak well-resolved vibrational bands in addition to the main band. These were observed for all dyes tested. In the spectrum of **6** and **7** (see Fig. 3a for **7**), these vibrational bands had a somewhat broader character, which may indicate a possible presence of aggregates. However, perfect accordance of the fluorescence excitation spectrum and the absorption spectrum of **6** and **7** (see Accessory Publication) revealed that this atypical character is attributed purely to monomeric species and may be concerned with the presence of positional isomers. The Q-band for dyes **1** and **2** (and also for their magnesium(II) analogues) is split because of the unsymmetrical composition of the whole macrocycle (Fig. 3b). The position of the λ_{max} of **2** is red-shifted in comparison to **11** because of annulation of one benzene ring to the TPyzPA macrocycle. Compound **1** is composed of moieties found in the symmetrical **7** and **10**, so its λ_{max} position is expected to lie somewhere between these two derivatives. However, splitting of the Q-band caused another red-shift for **1** and its λ_{max} appeared at 726 nm, which is even more than for compound **7**. This is a good result and the spectroscopic properties of **1** are very promising for application in PDT. Splitting of the Q-band also has an undesirable effect in decreasing the strength of absorption. The ε values of **1** and **2** are approximately half ($\varepsilon \sim 135000\ M^{-1}\ cm^{-1}$) of those obtained for symmetrical derivatives **10** and **11** ($\varepsilon \sim 280000\ M^{-1}\ cm^{-1}$). However, the absorption is still much higher than for compounds of the chlorin family recently widely investigated as PS in PDT ($\varepsilon \sim 40000\ M^{-1}\ cm^{-1}$). It means that more photons (energy) will be absorbed per one molecule.

We noticed another small band (more aptly described as a shoulder) at higher wavelengths in the absorption spectrum of the magnesium(II) complexes during demetalation by *p*TSA in THF (710 nm for **4** on Fig. 2). This band is not observed when the magnesium(II) complexes are dissolved in THF alone. The band disappears along with demetalation and is not observed in the arising metal-free derivatives. According to our previous observations and literature sources, this band is caused by protonation of one azomethine nitrogen in some of the dye molecules present in solution.[31,32] The basicity of the azomethine nitrogens is higher for metal complexes, especially for the magnesium(II) ones.[33,34] Metal-free derivatives are not sufficiently basic to be protonated under the same conditions.

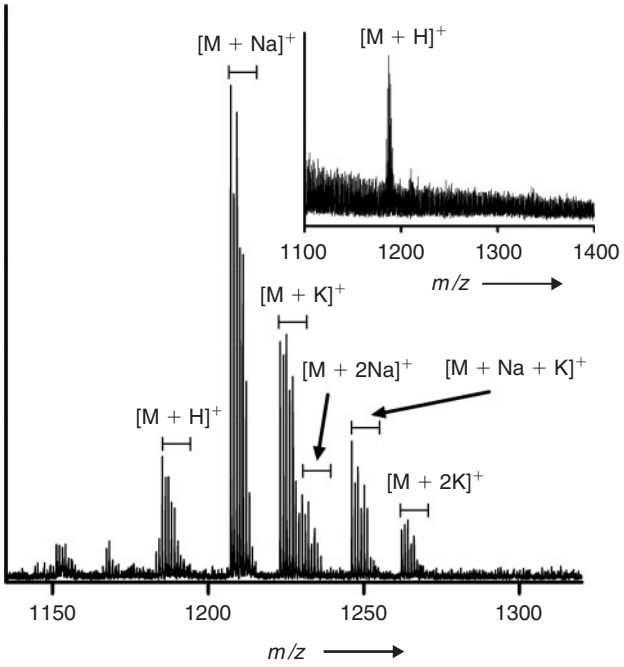


Fig. 4. Part of the MALDI-TOF spectra of **7** taken with TFA (inset) or without TFA added to the matrix.

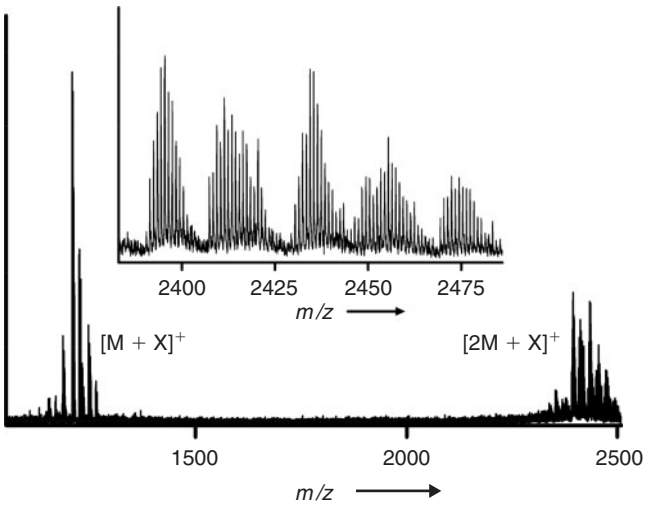


Fig. 5. MALDI-TOF spectrum of **7** taken without TFA added to the matrix with signals that correspond to monomer and dimer. X represents Na or K. Inset: enlarged area of dimer.

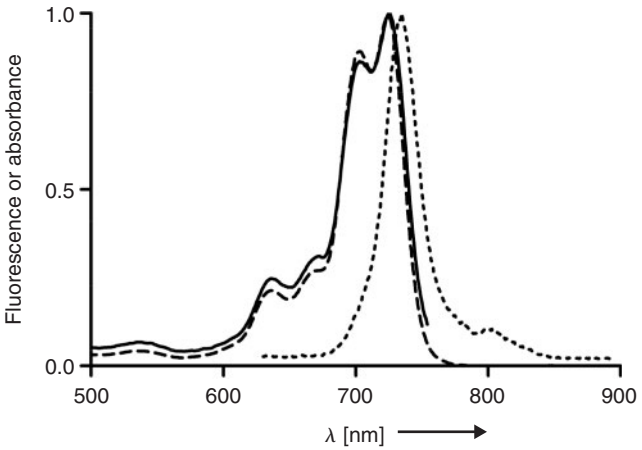


Fig. 6. Normalized UV-vis (dashed line), fluorescence excitation (full line), and fluorescence emission (dotted line) spectra of **1** in pyridine.

The mass spectra were obtained using the matrix-assisted laser desorption–ionization time-of-flight (MALDI-TOF) technique. They confirmed the right mass, the peaks of which appeared as a typical cluster that corresponded well to the calculated isotope distribution (see Accessory Publication). The presence of *tert*-butylsulfanyl substituents can be detected in the mass spectrum as a loss of isobutenes (*m/z* 56) from the macrocycle periphery.[35] This behaviour was observed for the prepared compounds that contained these substituents and it confirmed further the structure. Interesting and unusual observations were made for compounds **6** and **7**. Their mass spectra were also determined without the addition of trifluoroacetic acid (TFA), originally due to possible cleavage of the ester bond. Surprising adducts with sodium or potassium were observed in the case when TFA was not added to the matrix (see Fig. 4 and Accessory Publication). The adducts with these ions are not unusual but their formation with two monovalent ions per one molecule with a *m/z* signal that corresponds to only one positive charge is uncommon. Thus strong signals that correspond to *m/z* $[M+H]^+$, $[M+Na]^+$ (strongest), $[M+K]^+$, but also $[M+2Na]^+$, $[M+Na+K]^+$, and $[M+2K]^+$ were detected in the mass spectrum. A maximum of two ions were observed to join one molecule of the macrocycle. Another interesting feature was that dimers of **6** and **7** were also detected in the mass spectrum, with the same ions, again with a maximum two ions per one molecule of macrocycle and with only one positive charge. It means that signals corresponding to *m/z* $[2M+Na]^+$, $[2M+K]^+$, but also, e.g., $[2M+2Na+K]^+$, $[2M+4Na]^+$ etc., were observed (Fig. 5). No such adducts were detected when the mass spectrum was obtained with TFA added to support ionization (Fig. 4, inset). Instead, the only signal that appeared was attributed to *m/z* $[M+H]^+$. No such adducts were detected for the other derivatives studied. At this moment, we do not have a sufficient explanation for this behaviour but this problem is worthy of further investigation. One of the explanations may be concerned with the remarkable electron-deficient properties of AzaPc macrocycles.[30,36] The electrochemistry of Pc and AzaPc is well investigated.[6] It has been shown by several examples that the macrocyclic ligand can be reduced in several steps to yield anions.[37,38] In the case when the central metal is redox inactive (Zn^{II}, Mg^{II}) the reduction is only ligand-centred. Such a reduction of the macrocycle may also occur during the MALDI-TOF measurements and the negative charge on the ligand may compensate one of the positive charges of the cation adducts. However, we are still far away from the final explanation and this behaviour should be investigated more closely.

Fluorescence and Singlet Oxygen

Fluorescence spectra of the prepared compounds in pyridine were of a typical shape for Pc or AzaPc derivatives (Fig. 6). They showed only a small Stokes shift that did not exceed 10 nm (Table 1). The excitation spectra were always taken and compared with the absorption spectra and perfect accordance has been found in every case (Fig. 6, for more spectra see Accessory Publication). This confirms the purity and exclusively monomeric character of the dissolved dyes. The dimers or higher aggregates that can be formed in solution would alter significantly

Table 2. Photophysical data of 1 and 2 compared with photosensitizers in clinical practice

Compound	Q-band λ_{max} [nm]	ε [M^{-1} cm^{-1}]	Φ_Δ	Solvent	Ref.
1	726	139000	0.80	Pyridine	
2	685	130000	0.70	Pyridine	
Verteporfin	686	34000	0.76	MeOH	[44]
Temoporfin	650	30000	0.43	MeOH	[45]
Talaporfin	654	40000	0.77	PBS	[46]
Photosens	675	200000	0.42	PBS	[8]

the absorption spectrum. The fluorescence excitation spectrum is not influenced because the aggregates usually do not fluoresce besides a few exceptions of J-dimers.[39,40] The presence of only monomers is also an important prerequisite for proper measurement of the quantum yields, which could be influenced by possible aggregate formation.

The singlet oxygen quantum yields (Φ_Δ) were determined by a comparative method using decomposition of a chemical trap – 1,3-diphenylisobenzofuran (DPBF). Results are summarized in Table 1. Zinc(II) complexes always showed much higher Φ_Δ values than the corresponding magnesium(II) complexes. However, the magnesium(II) derivatives are stronger fluorophores. Similarly to previous observations,[41] magnesium(II) complexes, therefore, release more energy through fluorescence emission than the zinc(II) derivatives. Interestingly, in the case of compounds that bear pyrazine rings in the basic macrocycle, the singlet oxygen quantum yields were lower than those composed mostly or exclusively of benzene rings (compare **1** with **2**, or **10** with **11**). The pyrazine analogues, however, showed stronger fluorescence. It seems, therefore, that the presence of pyrazine in the basic macrocycle leads to a slightly stronger release of energy through fluorescence emission on the account of singlet oxygen production. Both observations may be used in the future design of photosensitizers (preferred Zn^{II} complexes of Pc) or efficient fluorophores (preferred Mg^{II} complexes of AzaPc).

The larger systems have a usually higher probability of dissipating the energy from the excited singlet state through collisions with surrounding molecules and subsequently the singlet oxygen quantum yields are decreased. Contrary to the enlarged π-system of compound **7**, its quantum yield is not decreased and its $\Phi_\Delta = 0.75$ is comparable with other zinc(II) derivatives in the study. This also means that the quinoxaline moiety shall not influence negatively the quantum yields of the composed dyes (e.g., **1** or **2**). The highest $\Phi_\Delta = 0.80$ in the present work was measured for the preferred compound **1**, which was designed to be the optimal active part of a third generation PS. Its photophysical properties, therefore, fulfilled the expectations more than sufficiently. Its aza-analogue **2** also exerted strong singlet oxygen production ($\Phi_\Delta = 0.70$) satisfactory for a good PS, but it suffered from a hypsochromic shift of the Q-band of 40 nm in comparison to **1**. However, the magnesium(II) complex of the same ligand (**4**) possesses strong fluorescence and it may find a place in superficial photodetection where this wavelength is optimal. Its emission is still in an area that is visible for human eyes. The emission at longer wavelengths, however strong, will suffer from low resolution by human sight.

For comparison, the photophysical data of **1** and **2** together with those of PSs in clinical practice are shown in Table 2. It is clearly demonstrated that compound **1** has the strongest singlet oxygen production, it absorbs at a much longer wavelength than the other PSs, and has three to four times stronger Q-band absorption than the chlorin derivatives. Compound **1** appears to be a very efficient PS more suitable for future binding to biomolecules than any of the approved PSs.

Conclusions

Based on previously uncovered relationships we have designed and synthesized a new promising active part of a third generation PS. Compound **1** is a very efficient singlet oxygen producer ($\Phi_\Delta = 0.80$) with strong absorption ($\varepsilon = 139000\ M^{-1}\ cm^{-1}$) at long wavelengths ($\lambda_{max} = 726$ nm), which thus exceeds the photophysical properties of successful PSs established in clinical practice. Because of the presence of aggregation-inhibiting substituents, **1** is well soluble in a wide range of organic solvents, which makes it potentially useful for non-complicated treatment by further chemical modification. Although the photophysical and photochemical properties of this PS are very promising, this is only one part of efficient PDT treatment. The pharmacokinetics of the PSs also play a very important role. As a consequence, compound **1** alone need not be an efficient PS for clinical use. However, its potential lies in its modifiable carboxy group. This PS can, therefore, be connected to a wide range of active-targeting biomolecules, such as steroidal hormones, cholesterol, monoclonal antibodies, nucleic acids, polypeptides, and sugars, or to biodegradable polymers that utilize passive-targeting principles. The targeting moiety will assure much better localization in target tissues whereas the active part (compound **1**) will subsequently guarantee very efficient photosensitization. Anchored to different surfaces it can also be used in other applications that are connected with singlet oxygen production, e.g., decomposition of waste water.

Experimental

All organic solvents used for the synthesis were of analytical grade. Anhydrous DMF was purchased from Acros, and 1,3-diphenylisobenzofuran (DPBF) was from Aldrich. Zinc(II) phthalocyanine (ZnPc) was obtained from Eastman Organic Chemicals (New York, USA). All chemicals were used as received except for zinc(II) acetate dihydrate (Lachema, Czech Republic), which was dried at 78°C under reduced pressure (13 mbar) for 5 h. TLC was performed on Merck aluminium sheets with silica gel 60 F_{254}. Merck Kieselgel 60 (0.040–0.063 mm) was used for column chromatography. Melting points were measured on Electrothermal IA9200 Series Digital Melting point Apparatus (Electrothermal Engineering Ltd, Southend-on-Sea, Essex, UK) and are uncorrected. Infrared spectra were measured in KBr pellets on a IR-Spectrometer Nicolet Impact 400. ^1H and ^{13}C NMR spectra were recorded on Varian Mercury Vx BB 300 (299.95 MHz for ^1H and 75.43 MHz for ^{13}C). The chemical shifts reported are given relative to internal $Si(CH_3)_4$. UV-Vis spectra were recorded on a UV-2401PC spectrophotometer from Shimadzu Europa GmbH (Duisburg, Germany). MALDI-TOF mass spectra were recorded in positive reflectron mode on a Voyager-DE STR mass spectrometer (Applied Biosystems, Framingham, MA, USA). For each sample, 0.5 μL of the mixture was spotted onto the target plate, air-dried, and covered with 0.5 μL of a matrix solution that consisted of 10 mg of α-cyano-4-hydroxycinnamic acid in 100 μL of 50% ACN in 0.1% trifluoroacetic acid. The instrument was calibrated externally with a five-point calibration using Peptide Calibration Mix1 (LaserBio Laboratories, Sophia-Antipolis, France). Diiminosuccinonitrile (DISN) was prepared according

to published procedures from DAMN.[42] Compounds **12** and **15** were prepared before in our laboratory.[10] For improved procedures see the Accessory Publication.

Synthesis of the Precursors

*2,4,5-Tris(*tert-*butylsulfanyl)benzonitrile (**13**) and 4,5-bis(*tert-*butyldisulfanyl)phthalonitrile (**14**)*

NaH (60% mineral oil dispersion, 2.46 g, 61 mmol) was washed from the mineral oil with dry hexane and dried using an argon stream. Anhydrous DMF (100 mL) was added and the dispersion was cooled to 0°C with water/ice. 2-Methylpropan-2-thiol (54 mmol, 6.1 mL) was added dropwise under an argon atmosphere. Upon the cessation of gas evolution, Cu_2O (52 mmol, 7.38 g) and 4,5-dichlorophthalonitrile (25 mmol, 4.84 g) were added and the mixture was heated for 45 min at 170°C. After cooling, the mixture was poured into ice-cold water (500 mL). The suspension was extracted by diethylether (6 × 150 mL) and the combined organic fractions were washed by concentrated ammonia (25%, 3 × 300 mL) and water (2 × 300 mL). The organic layer was dried (Na_2SO_4) and purified by column chromatography on silica (toluene). Two important fractions that corresponded to **13** (yellow solid, 200 mg, 2.2%) and **14** (off-white solid, 190 mg, 2.1%) were obtained. Compound **13**: $R_f = 0.48$ (toluene). mp 77.5–79.0°C. (Found: C 61.85, H 7.81, N 4.19. $C_{19}H_{29}NS_3$ requires C 62.07, H 7.95, N 3.81%.) ν_{max} (KBr)/cm^{-1} 2961, 2939, 2920, 2896, 2872, 2221s (C≡N), 1567, 1471, 1458, 1444, 1397, 1368, 1253, 1164, 1099, 915 cm^{-1}. δ_H ($CDCl_3$) 1.33 (s, 9H, CH_3), 1.41 (s, 9H, CH_3), 1.52 (s, 9H, CH_3), 7.70 (s, 1H, arH), 7.85 (s, 1H, arH). δ_C ($CDCl_3$) 30.06, 31.08, 31.15, 35.57, 48.03, 48.57, 108.36, 119.69, 131.55, 134.63, 143.82, 148.54, 152.55. Compound **14**: $R_f = 0.26$ (toluene). mp 136°C. (Found: C 51.88, H 5.47, N 7.52. $C_{16}H_{20}N_2S_4$ requires C 52.14, H 5.47, N 7.60%.) ν_{max} (KBr)/cm^{-1} 3064, 2965, 2926, 2898, 2763, 2229s (C≡N), 1568, 1446, 1364, 1347, 1214, 1163, 907. δ_H ($CDCl_3$) 1.35 (s, 18H, CH_3), 8.16 (s, 2H, arH). δ_C ($CDCl_3$) 29.7, 51.0, 112.5, 115.3, 130.4, 144.4.

*2,3-Dicyanoquinoxaline-6-carboxylic Acid (**16**)*

Diiminosuccinonitrile (50.4 mmol, 5.35 g) was dissolved in trifluoroacetic acid (80 mL) under argon atmosphere and 3,4-diamninobenzoic acid (50.4 mmol, 7.67 g) was added in several small portions. The reaction was stirred at room temperature overnight. The solvent was evaporated and the solid residue was dissolved in a small amount of hot ethanol and precipitated with water. The solid was collected, washed with water, dried, and purified by column chromatography on silica (CH_2Cl_2/ethyl acetate/acetic acid, 25/10/1) to receive an off-white solid (4.3 g, 38%). A sample for analysis was recrystallized from ethanol to give white crystals. Mp 218°C (lit.[27] 220°C). (Found: C 58.56, H 1.91, N 24.62. $C_{11}H_4N_4O_2$ requires C 58.94, H 1.80, N 24.99%.) ν_{max} (KBr)/cm^{-1} 3432, 3098, 3068, 2936, 1697s (C=O), 1623, 1556, 1439, 1352, 1312, 1271, 1182, 1107, 914, 861, 761. δ_H ([D$_6$]-acetone) 8.43 (dd, 1H, J_1 0.5, J_2 9, H8), 8.68 (dd, 1H, J_1 2, J_2 9, H7), 8.85 ppm (dd, J_1 0.5, J_2 2, H5). δ_C ([D$_6$]-acetone) 114.67, 114.70, 131.0, 132.2, 133.0, 134.8, 136.7, 141.6, 143.6, 165.4.

*Butyl 2,3-Dicyanoquinoxaline-6-carboxylate (**17**)*

Compound **16** (1 mmol, 224 mg) was dissolved in anhydrous THF (5 mL) and anhydrous toluene (25 mL) and $SOCl_2$ (0.75 mL, 10 mmol) were added after full dissolution. The mixture was heated at 100°C for 3 h and then evaporated. Anhydrous toluene (20 mL), anhydrous pyridine (1.25 mmol, 0.1 mL), and anhydrous butanol (10 mmol, 0.92 mL) were added and the mixture was heated at 120°C for 3 h. The reaction mixture was then washed three times with water and once with brine, dried over Na_2SO_4, and evaporated. The crude product was purified by column chromatography on silica (toluene/DCM, 2/3) to give an off-white solid (250 mg, 89%). Mp 104.6–105.8°C. (Found: C 63.97, H 4.35, N 20.08. $C_{15}H_{12}N_4O_2$ requires C 64.28, H 4.32, N 19.99%.) ν_{max} (KBr)/cm^{-1} 3085, 3063, 2980, 2963, 2868, 1718s (C=O), 1621, 1557, 1462, 1438, 1350, 1229, 1259, 1187, 1141, 1096, 999, 967, 943, 913, 857, 764. δ_H ($CDCl_3$) 1.16 (t, 3H, J 7, CH_3), 1.53 (sex, 2H, J 7, CH_2), 1.84 (p, 2H, J 7, CH_2), 4.47 (t, 2H, J 7, OCH_2), 8.33 (dd, 1H, J_1 0.5, J_2 9, H8), 8.66 (dd, 1H, J_1 2, J_2 9, H7), 8.91 (dd, J_1 0.5, J_2 2, H5). δ_C ($CDCl_3$) 13.7, 19.2, 30.6, 113.03, 113.06, 130.3, 131., 131.9, 134.5, 136.4, 141.2, 143.1, 164.1.

Syntheses of Magnesium(II) Complexes

*Compound **3***

Anhydrous butanol (20 mL) was refluxed with magnesium (28 mmol, 672 mg) and a small crystal of iodine for 3 h. Compounds **12** (3 mmol, 906 mg) and **16** (1 mmol, 224 mg) were added and the mixture was refluxed overnight. After cooling, the mixture was diluted with chloroform and filtered. The undissolved solid on the filter was extracted several times with chloroform also using sonication. Two-thirds of the chloroform solution were put aside and used later for transformation to zinc(II) complexes. The rest was evaporated and purified by column chromatography on silica using a step gradient. The first eluent was chloroform/pyridine, 8/1. After the green fraction of symmetrical compound **8** (110 mg, 28%) was eluted, the eluent was changed to chloroform/pyridine (2/1) to obtain a small amount of **3**. Most of the desired product, however, stayed on the column. The column was emptied, and the part that contained the product was carefully separated and the product was washed from silica using pyridine/methanol, 2/1. The crude product **3** was purified once more using column chromatography on silica (chloroform/pyridine, 2/1), again followed by isolation of the rest of the product from silica using the above-mentioned procedure. After evaporation, the product was washed with hexane to give a green-blue solid (51 mg, 14%). (Found: C 59.11, H 5.87, N 11.35. $C_{59}H_{64}MgN_{10}O_2S_6{\cdot}2H_2O$ requires C 59.16, H 5.72, N 11.69%.) ν_{max} (KBr)/cm^{-1} 2961, 1896, 2861, 1718w (C=O), 1596, 1483, 1472, 1390, 1365, 1240, 1163, 1099, 1067, 942, 785, 752, 697. λ_{max} (pyridine)/nm (ε/M^{-1} cm^{-1}) 724 (116100), 705 (115100), 669 (34000), 549sh, 637 (27700), 374 (81600). δ_H ([D$_5$]-pyridine) 1.54 (s, 9H, CH_3), 1.56 (s, 9H, CH_3), 1.66 (s, 18H, CH_3), 1.67 (s, 18H, CH_3), 8.96 (d, 1H, J 9, arH), 9.02 (d, 1H, J 9, arH), 9.97 (s, 1H, arH), 10.12–10.25 ppm (m, 6H, arH). δ_C ([D$_5$]-pyridine) 31.4, 48.7, 48.8, 131.08, 131.15, 131.22, 131.44, 132.70, 133.76, 134.68, 138.81, 138.88, 138.97, 141.53, 141.67, 142.33, 142.38, 142.89, 142.95, 142.98, 145.18, 151.07, 151.34, 154.31, 154.48, 155.29, 155.42, 155.64, 155.70, 168.55 (some signals are overlapped by signals of the solvent or fused together). *m/z* (MALDI-TOF) 1161 $[M+H]^+$, 1105 $[M-C_4H_8+H]^+$, 1049 $[M-2C_4H_8+H]^+$, 993 $[M-3C_4H_8+H]^+$, 937 $[M-4C_4H_8+H]^+$.

*Compound **4***

This compound was prepared and purified following the above-mentioned procedure for compound **3** but starting from

compound **15** (3 mmol, 918 mg) instead of **12**. The procedure gave compound **9** (127 mg, 33%) and the desired product **4** as a green solid (37 mg, 10%). (Found: C 52.31, H 5.20, N 18.35. $C_{53}H_{58}MgN_{16}O_2S_6 \cdot 3H_2O$ requires C 52.10, H 5.28, N 18.34%.) ν_{max} (KBr)/cm^{-1} 2961, 2920, 2861, 1636, 1527, 1457, 1363, 1259, 1234, 1143, 1094, 974, 787, 751, 711, 693. λ_{max} (pyridine)/nm (ε/M^{-1} cm^{-1}) 686 (104900), 660 (94500), 632 (29700), 602 (20900), 383 (97000). δ_H ([D_5]-pyridine) 2.07 (s, 18H, CH_3), 2.25 (s, 18H, CH_3), 2.27 (s, 18H, CH_3), 8.86–8.96 (m, 2H, arH), 9.88 (s, 1H, arH). δ_C ([D_5]-pyridine) 30.77, 30.80, 30.91, 51.02, 51.06, 51.27, 51.35, 128.27, 131.28, 133.74, 142.88, 145.04, 145.56, 145.62, 145.64, 146.02, 151.45, 151.58, 151.88, 152.21, 157.76, 157.82, 158.31 (some signals are most likely overlapped by signals of the solvent or fused together). *m/z* (MALDI-TOF) 1167 $[M+H]^+$, 1111 $[M-C_4H_8+H]^+$.

*[2,11(12),20(21),29(30)-Tetrakis(butoxycarbonyl) tetra[2,3]quinoxalinoporphyrazinato] Magnesium(II) (**6**)*

Anhydrous butanol (10 mL) was refluxed with magnesium (6 mmol, 146 mg) and a small crystal of iodine for 3 h. Compound **17** (0.89 mmol, 249 mg) was added and the reflux continued for the next 24 h. Solvent was evaporated and the green solid was stirred with 50% acetic acid (50 mL) for 30 min. The crude product was filtered off and washed thoroughly and sonicated with 50% acetic acid, water, acetone, and hexane. The final product appeared as a blue solid (197 mg, 80%). (Found: C 60.68, H 4.87, N 18.47. $C_{60}H_{48}MgN_{16}O_8 \cdot 2H_2O$ requires C 61.00, H 4.44, N 18.97%.) ν_{max} (KBr)/cm^{-1} 2959, 2872, 1719s, 1636, 1558, 1458, 1309, 1255, 1225, 1173, 1088, 966, 849, 754, 710. λ_{max} (pyridine)/nm (ε/M^{-1} cm^{-1}) 727 (133000), 660sh, 494 (20000), 390 (78800), 360 (82300). δ_H ([D_5]-pyridine) 0.74–1.19 (m, 12H, CH_3), 1.30–1.60 (m, 8H, CH_2), 1.63–2.03 (m, 8H, CH_2), 4.20–4.62 (m, 8H, O–CH_2) (aromatic signals not detected). δ_C ([D_5]-pyridine) 14.0, 19.6, 30.9, 65.8 (aromatic signals not detected). *m/z* (MALDI-TOF with TFA) 1145 $[M+H]^+$. *m/z* (MALDI-TOF without TFA) 1111 $[M-C_4H_9+Na]^+$, 1145 $[M+H]^+$, 1167 $[M+Na]^+$, 1183 $[M+K]^+$, 1190 $[M+2Na]^+$, 1206 $[M+Na+K]^+$, 1222 $[M+2K]^+$, 2311 $[2M+Na]^+$, 2327 $[2M+K]^+$, 2334 $[2M+2Na]^+$, 2350 $[2M+Na+K]^+$, 2366 $[2M+2K]^+$, 2389 $[2M+2K+Na]^+$.

Syntheses of Zinc(II) Complexes

*Compound **1***

A chloroform solution of magnesium(II) complexes (approx. 0.66 mmol of macrocycles, see preparation of **3**) was evaporated and dissolved in chloroform/THF (1/1, v/v, 80 mL), and *p*-toluenesulfonic acid (6.6 mmol, 1.25 g) in THF (20 mL) was added. The reaction was stirred at room temperature for 90 min and then the solvents were evaporated. The resulting dark solid was washed with water and methanol and air-dried. The crude mixture of metal-free complexes was then dissolved in pyridine (80 mL) and anhydrous zinc(II) acetate (6.6 mmol, 1.2 g) was added. The solution was refluxed for 2 h, evaporated, and the residue dissolved in chloroform. The chloroform solution was washed three times with 1% HCl, three times with water, and once with brine. The organic layer was dried (Na_2SO_4), evaporated, and purified by column chromatography on silica using the same conditions as in the case of **3**. The procedure gave compound **10** (200 mg, 25%) and the desired product **1** as a green-blue solid (85 mg, 11%). (Found: C 57.17, H 5.49, N 11.18. $C_{59}H_{64}N_{10}O_2S_6Zn \cdot 2H_2O$ requires C 57.20, H 5.53, N 11.30%.) ν_{max} (KBr)/cm^{-1} 2960, 2895, 2861, 1708w (C=O), 1595, 1487, 1390, 1373, 1239, 1163, 1102, 1065, 942, 784, 746, 694. λ_{max} (pyridine)/nm (ε/M^{-1} cm^{-1}) 726 (139400), 704 (128100), 669 (39000), 636 (31200), 540sh, 377 (86200). δ_H ([D_5]-pyridine) 1.53 (s, 9H, CH_3), 1.56 (s, 9H, CH_3), 1.69 (s, 18H, CH_3), 1.70 (s, 18H, CH_3), 8.97 (s, 2H, arH), 9.92 (s, 1H, arH), 10.05–10.24 (m, 6H, arH). δ_C ([D_5]-pyridine) 31.43, 48.70, 48.73, 48.90, 130.52, 130.66, 131.13, 132.41, 133.79, 137.99, 138.11, 138.27, 141.54, 141.75, 142.57, 142.65, 143.08, 143.34, 143.46, 145.26, 150.86, 154.45, 154.62, 155.31, 155.43, 155.64, 155.70, 168.51 (some signals are most likely overlapped by signals of the solvent or fused together). *m/z* (MALDI-TOF) 1201 $[M+H]^+$.

*Compound **2***

This compound was prepared similarly to the above-mentioned procedure for compound **1**. It gave compound **11** (228 mg, 28%) and the desired compound **2** as a green solid (64 mg, 8%). (Found: C 50.51, H 5.21, N 18.01. $C_{53}H_{58}N_{16}O_2S_6Zn \cdot 3H_2O$ requires C 50.40, H 5.11, N 17.74%.) ν_{max} (KBr)/cm^{-1} 3441, 2960, 2924, 1719, 1628, 1518, 1457, 1384, 1363, 1258, 1234, 1146, 1093, 973, 787, 709. λ_{max} (pyridine)/nm (ε/M^{-1} cm^{-1}) 685 (130800), 659 (112500), 633 (37900), 600 (25600), 385 (123900). δ_H ([D_5]-pyridine) 2.09 (s, 18H, CH_3), 2.28 (s, 36H, CH_3), 8.97 (s, 2H, arH), 9.95 (s, 1H, arH). δ_C ([D_5]-pyridine) 29.98, 30.76, 30.84, 51.17, 51.35, 51.42 (aromatic signals not detected). *m/z* (MALDI-TOF) 1207 $[M+H]^+$, 1151 $[M-C_4H_8+H]^+$.

*[2,11(12),20(21),29(30)-Tetrakis(butoxycarbonyl) tetra[2,3]quinoxalinoporphyrazinato] Zinc(II) (**7**)*

Compound **6** (0.035 mmol, 41 mg) was dissolved in chloroform (20 mL) and THF (20 mL) was added. *p*-Toluenesulfonic acid (0.70 mmol, 133 mg) in THF (10 mL) was added and the reaction was stirred at room temperature for 90 min. The solvent was evaporated and the solid residue was washed with water and dried. Metal-free macrocycle was then refluxed in pyridine (10 mL) with anhydrous zinc(II) acetate (0.35 mmol, 64 mg) for 2 h. Pyridine was evaporated and the residue was washed thoroughly with water, acetone, and diethylether (also using sonication). Compound **7** was obtained as a blue solid (30 mg, 73%). (Found: 58.07, H 4.79, N 18.30. $C_{60}H_{48}N_{16}O_8Zn \cdot 3H_2O$ requires C 58.09, H 4.39, N 18.06%.) ν_{max} (KBr)/cm^{-1} 2962, 2945, 2872, 1720s (C=O), 1627, 1608, 1501, 1465, 1401, 1382, 1310, 1253, 1226, 1174, 1089, 772, 754, 709. λ_{max} (pyridine)/nm (ε/M^{-1} cm^{-1}) 720 (140000), 682sh, 655sh, 485 (11000), 369 (83600). δ_H ([D_5]-pyridine) 0.68–1.11 (m, 12H, CH_3), 1.14–1.51 (m, 8H, CH_2), 1.59–1.95 (m, 8H, CH_2), 4.04–4.59 (m, 8H, O-CH_2) (aromatic signals not detected). δ_C ([D_5]-pyridine) 13.9, 19.5, 30.8, 65.7 (aromatic signals not detected). *m/z* (MALDI-TOF with TFA) 1185 $[M+H]^+$. *m/z* (MALDI-TOF without TFA) 1151 $[M-C_4H_9+Na]^+$, 1167 $[M-C_4H_9+K]^+$, 1185 $[M+H]^+$, 1207 $[M+Na]^+$, 1223 $[M+K]^+$, 1230 $[M+2Na]^+$, 1246 $[M+Na+K]^+$, 1262 $[M+2K]^+$, 2385 $[2M+H]^+$, 2391 $[2M+Na]^+$, 2407 $[2M+K]^+$, 2414 $[2M+2Na]^+$, 2430 $[2M+Na+K]^+$, 2437 $[2M+3Na]^+$, 2446 $[2M+2K]^+$, 2453 $[2M+2Na+K]^+$, 2460 $[2M+4Na]^+$, 2469 $[2M+2K+Na]^+$.

Singlet Oxygen and Fluorescence

Singlet oxygen quantum yields were determined according to a previously published procedure using decomposition of a chemical trap of singlet oxygen 1,3-diphenylisobenzofuran

(DPBF).[35] Absorption of the dyes in the Q-band area during measurements was approx. 0.1. ZnPc was used as the reference ($\Phi_\Delta = 0.61$ in pyridine[43]). Fluorescence quantum yields were also determined by a comparative method using ZnPc as reference ($\Phi_F = 0.20$ in pyridine[43]). Absorption of the dyes in the Q-band area was approx. 0.05, with an excitation wavelength of 375 nm.

Accessory Publication

Synthesis details, NMR spectra, UV-Vis absorption, fluorescence emission and fluorescence excitation spectra, MS (MALDI-TOF) spectra are available from the Journal's website.

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