Azaphthalocyanines Containing Pyrazine Rings with Focus on the Alkylheteroatom, Aryl and Heteroaryl Substitution and Properties Important in Photodynamic Therapy

Petr Zimcik,[@] Veronika Novakova, Miroslav Miletin, and Kamil Kopecky

Department of Pharmaceutical Chemistry and Drug Control, Faculty of Pharmacy in Hradec Kralove, Charles University in Prague, Heyrovskeho 1203, Hradec Kralove, 50005, Czech Republic
[@] Corresponding author E-mail: petr.zimcik@faf.cuni.cz

In this work, we summarize the recent knowledge about the syntheses, UV-vis absorption properties, aggregation, protonation and deprotonation of macrocycle and singlet oxygen and fluorescence quantum yields in the group of azaphthalocyanines bearing pyrazine rings in their conjugated system. Precursors (pyrazine-2,3-dicarbonitriles) for tetramerization are usually built up by condensation from aliphatic starting materials thus enabling peripheral substitutions sometimes not available for phthalocyanines (Pc). Conditions of tetramerizations are discussed in the article too. Although tetrapyrazinoporphyrazines (TPyzPA) suffer from the hypsochromic shift comparing to corresponding Pc, several types of peripheral substituents can induce important bathochromic shift and eliminate this disadvantage. TPyzPA macrocycle tends to higher aggregation comparing to Pc molecules and a lot of aggregation inhibiting substituents have been developed. Protonation of the TPyzPA macrocycle in acidic media starts on azomethine nitrogens and at higher concentrations of acid also pyrazine nitrogens accept the proton. The central hydrogens in metal-free compounds are acidic (N-acids) and the macrocycle can be deprotonated in basic solutions. Singlet oxygen production as well as fluorescence emission are strongly dependent on peripheral substituents, namely on the heteroatoms connecting the core with the substituents.

Introduction

Phthalocyanines (Pc) are well-known organic dyes which have attracted steady attention of researchers for a number of years.[1,2] Azaphthalocyanines (AzaPc) are aza-analogues of Pc with some carbon atoms in macrocyclic core replaced by nitrogens. According to number and position of the nitrogens, the macrocycles of tetrapyridino porphyrazine, tetrapyridazinoporphyrazine, tetrapyrazino-porphyrazine (TPyzPA) *etc.* type may originate. Our research work has been focused on compounds containing pyrazine rings in their structure (Figure 1) and aimed mainly on properties important for photodynamic therapy (PDT). Insertion of four benzene rings to TPyzPA macrocycle results in tetra-2,3-quinoxalinoporphyrazines (2,3-TQP) or their isomers tetra-6,7-quinoxalino-porphyrazines (6,7-TQP) according to position of the added benzene rings, either to the periphery of TPyzPA

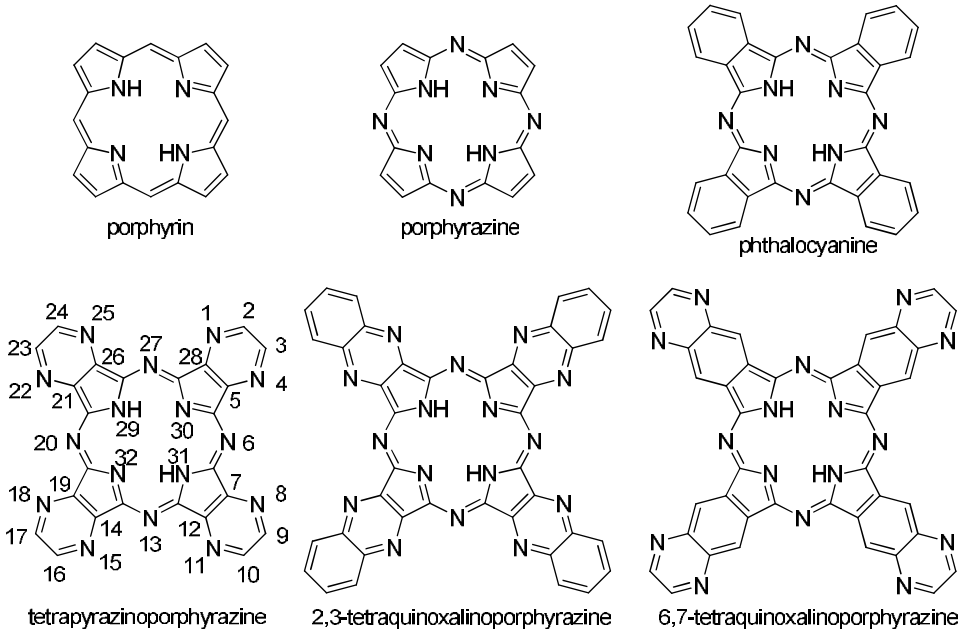


Figure 1. Structures of basic macrocycles based on porphyrin or porphyrazine core.

or between porphyrazine macrocycle and pyrazine rings. The TQPs may be also considered as aza-analogues of naphthalocyanines.

Due to their interesting physical, photophysical and chemical properties, TPyzPAs were investigated in several applications. They show red fluorescence and can be used as red- emitters.[3,4] Non-linear optical behavior has been observed for TPyzPA in several cases.[5-8] The electron deficient properties of TPyzPA macrocycle have been studied using electrochemical measurements.[8-10] The chelated central atom (*e.g.* Co, Fe) may take part in oxidation of organic substrates[11-13] or other catalysis.[14] Herein, we would like to summarize the results reached in the field of TPyzPA and related compounds up to now in relation to their application in PDT. This review will concern mostly with syntheses, UV-vis absorption, aggregation, protonization and fluorescence and singlet oxygen quantum yields of these interesting organic compounds.

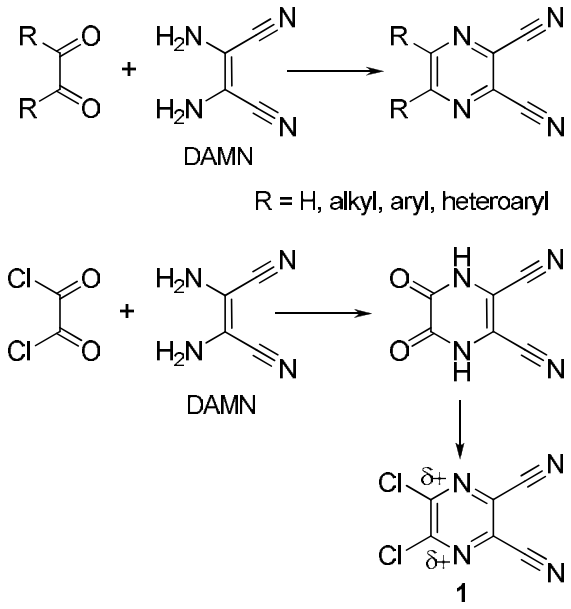
Synthesis

Syntheses of Precursors

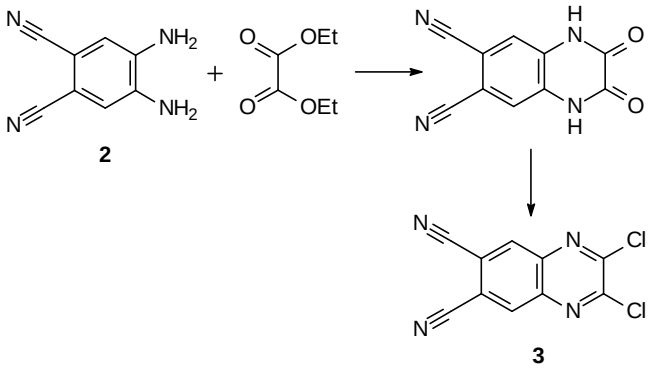
Due to similar structure with Pc, syntheses of TPyzPA proceed in the similar way. TPyzPA are prepared mostly by cyclization reactions of substituted precursors pyrazine-2,3-dicarbonitriles. Contrary to Pc, these precursors are built up *de novo* from aliphatic starting materials – diaminomaleonitrile (DAMN) and vicinal diketons. The substituents which are to be placed on the periphery of final TPyzPA macrocycle are substituents of the diketon (Scheme 1).[15-18] The condensation of DAMN with appropriate diketon is facilitated in acidic medium (acetic acid or catalytic amount of HCl). If the peripheral chain is intended to be bound through heteroatom, the 5,6-dichloropyrazine-2,3-dicarbonitrile (**1**) should be synthesized as intermediate in two steps (Scheme 1).[19,20] Carbons in pyrazine ring next to chlorine atoms in **1** are electrondeficient and are readily displaced by various nucleophiles such as amines,[21-23] alkoxides[24,25] and thiolates.[26,27] In some cases, especially for amines, it is possible to substitute **1** in two steps with two different substituents[23,28] to obtain an unsymmetrically substituted precursor.

Pyrazine-2,3-dicarbonitriles are not the only precursors used for cyclization reaction. Other functional derivatives of pyrazine-2,3-dicarboxylic acids such as amides or free acids undergo tetramerization but this procedure requires a donor of ammonia (urea or an ammonium salt) and purity of product is often lower than in the case of dicarbonitriles.[29] Less reactive pyrazine-2,3-dicabonitriles can be also converted to aza-analogues of isoindoline-diimines which are then more reactive.[21]

Substituted quinoxaline-2,3-dicarbonitriles, precursors for synthesis of 2,3-TQPs, are built up either from DAMN and *o*-quinones with weak acid catalysis[30] or by condensation of diiminosuccinonitrile (DISN) with substituted aromatic *o*-diamines under strongly acidic conditions of trifluoroacetic acid.[31,32] Isomeric 6,7-TQPs were developed only recently mainly due to complicated availability of suitable precursor 4,5-diaminophthalonitrile (**2**) (Scheme 2) obtainable in low yields.[33,34] However, recently improved methods allowed synthesis of this precursor in reasonable yields.[35,36] Similarly to DAMN, **2** may be condensed with suitable vicinal diketons[37,38] or *o*-quinones[33] to yield derivatives of quinoxaline-6,7-dicarbonitriles. For synthesis of heteroatom substituted 6,7-TQPs, we have developed a simple synthetic route to 2,3-dichloroquinoxaline-6,7-dicarbonitrile (**3**) which undergoes similar nucleophilic substitutions as its pyrazine homologue **1**.[39]



Scheme 1. Synthesis of pyrazine-2,3-dicarbonitrile.



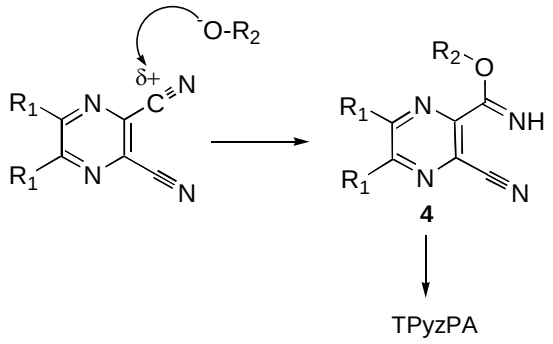
Scheme 2. Synthesis of 2,3-dichloroquinoxaline-6,7-dicarbonitrile.

Syntheses of Macrocycles

After appropriate precursor is synthesized, it undergoes cyclization reactions. The conditions of this reaction depend on the intended peripheral substitution of the macrocycle as well as on the metal to be inserted. Generally, electron-withdrawing substituents increase the reactivity of carbonitrile groups of precursor[24,40] and the tetramerization to metal-free TPyzPA may proceed even by simple heating of the precursor in high boiling solvent, *e.g.* quinoline.[4] On the other hand, electron-donating alkylamino groups fairly lowers the reactivity and long reaction times[24] or more reactive intermediates have to be used.[21]

An excellent review of the synthetic methods has been performed by Stuzhin and Ercolani in The Porphyrin Handbook.[41] Therefore, in this part we would like to summarize only the general methods pointing out to recent efforts in the synthesis of TPyzPA.

Metal free TPyzPA can be easily prepared by heating of pyrazine-2,3-dicarbonitriles with the aliphatic alkoxides (attempts to use phenoxides failed[4]) of low molecular weight (up to octanol). These alkoxides are generated either by strong base 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU)[9,17,38,42-44] or by alkali metals (lithium[22-24,30,39] or sodium[31,45]) and alkaline earth metals (magnesium [16,18,19,22,46,47]). Both groups of metals above mentioned are coordinated only weakly and can be easily removed with the use of weak acids (*e.g.* acetic acid for lithium) or stronger acids (*e.g. p*-toluenesulphonic acid for magnesium). The reaction mechanism of the cyclization is initialized by attack of the electrondeficient carbonitrile carbon by alkoxide anion and the first intermediate– alkylcarboximidate (**4**) – has been isolated (Scheme 3).[25]



Scheme 3. Mechanism of alkylcarboximidate formation.

A stability of peripheral chains bound through heteroatom may constitute a certain problem during cyclization reaction. While alkylamino derivatives are stable, alkylsulfanyl[19,24] and alkoxy[24,25] substituents may be replaced by nucleophilic alkoxide used as initiator of the tetramerization. Alkylsulfanyl derivatives are stable in magnesium but not in lithium alkoxide, alkoxy derivatives were transetherified also in less reactive magnesium alkoxide. The use of bulky substituents (2,6-di-*iso*-propyl-phenoxy- or 2,6-diphenyl-phenoxy) hindering the reactive centre did not improve the stability.[4]

The centre of TPyzPA is able to coordinate almost any metal. The simplest method to prepare metal dyes is to insert metal to metal-free TPyzPA macrocycle. This reaction is usually performed by heating metal-free dye with suitable metal salt in high boiling solvent.[22,47] However, the most common method for syntheses of metal TPyzPA is heating of appropriate pyrazine-2,3-dicarbonitrile in high boiling solvent such as trichlorobenzene,[31] dichlorobenzene,[43] dimethylformamide,[48] dimethylaminoethanol,[38] pyridine,[49] or quinoline[30,50] with template of metal salts (anhydrous chlorides or acetates) or metals alone[51] sometimes with the addition of urea[16,30,52] or catalytic amount of ammonium molybdate.[15,31,43] Interesting and efficient tetramerization reagent, $Zn(quinoline)_2Cl_2$ complex, has been developed by Mørkved *et al.*[53]

Bauer *et al*. brought quite new synthetic approach for substituted TPyzPA.[54] They built the TPyzPA macrocycle by condensation of benzil with octaaminoporphyrazine prepared previously by simple opening of selenodiazole rings in tetrakis(selenodiazole)porphyrazine.

Investigations of the influence of peripheral substituents on TPyzPA properties or fine tuning of desired properties require often synthesis of unsymmetrical derivatives. Although many selective synthetic approaches have been developed for preparation of desired unsymmetrical Pc,[55] the statistical condensation is the most often used method, especially when all congeners (AAAA, AAAB, ABAB, AABB, ABBB and BBBB) are of investigation interest. The statistical condensation, however, requires chromatographic separation of arising mixture of at least six different macrocycles. Due to well-known aggregation phenomenon (see below), the precursors must be designed to eliminate more or less this inconvenient feature.[23,27,38-40,48]

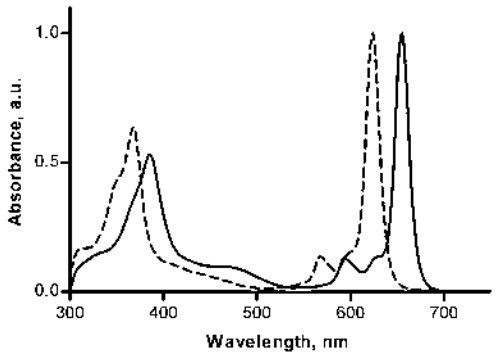


Figure 2. Normalized UV-vis absorption spectra of compounds **12** (dashed) and **13** (full) in pyridine.

UV-vis Absorption

Absorption spectrum seems to be the most important characteristic of Pc and related compounds. Since the penetration of light through human tissues increases at longer wavelengths, the absorption spectrum determines the therapeutic depth of the treatment in PDT.

TPyzPA possesses significant *Q*-band in the far-red end of the visible spectrum near 660 nm with quite high extinction coefficients (usually over 200 000 M^{-1} cm^{-1}) and *B*-band, called Soret band, about 370 nm in blue area of visible spectrum (Figure 2). Both of these bands are attributed to allowed π-π^* transition. For alkylheteroatom substituted TPyzPA another band (or sometimes only a shoulder) arises in the 420 – 520 nm region (Figure 2). Presence of this new band is ascribed to *n*-π^* transition of the lone pair electron in the non-bonding orbital of the heteroatom.[40] This band is more significant by metal free dyes what induces the hypothesis that lone pairs of central nitrogens N^{30} and N^{32} may contribute to the intensity of this band too.[56]

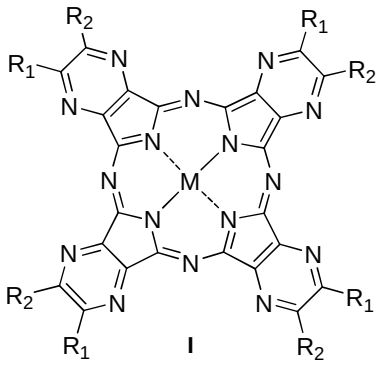
Isosteric substitution of CH groups in Pc macrocycle for nitrogens causes significant hypsochromic shift of the *Q*-band position in the range 40-60 nm.[26] Apart from the solvatochromic effect that causes the red shift of Pc and similar macrocycles in dependence on increasing refractive index of the solvent[57] and can be easily characterized by

"polarization red shift", the *Q*-band position may be influenced by several structural factors which are discussed in the following paragraphs (see also Tables 1 and 2).

Central atom may influence the *Q*-band position. For unsubstituted TPyzPA or those substituted with the same peripheral pattern the order $ZrCl_2$ > V=O > $SiCl_2$ ~ $HfCl_2$ ~ Cu > Zn ~ Mg > Co of red shift has been observed.[24,50,58] The difference between the marginal metals (*e.g.* V=O and Co) may reach almost 30 nm.

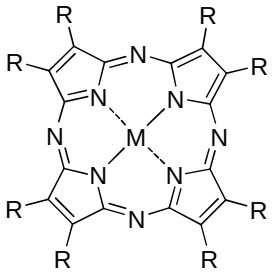
The *Q*-band position depends on atom(s) by which peripheral chain is attached to TPyzPA's core (Table 1). Aliphatic carbon as the first atom of the chain almost does not contribute to red shift and TPyzPA modified with such substitution absorbs at the same wavelength as unsubstituted TPyzPA[58] (compare **5** and **6** or **7**, **8** and **9**). Heteroatoms influence the *Q*-band only when directly attached to the macrocycle since only then they can contribute to conjugation with their free electron pairs. Oxygen causes blue shift of the *Q*-band comparing to unsubstituted or carbon substituted TPyzPAs (compare **8** or **9** with **10** or **11**). On the other hand, both sulfur (**13**) and nitrogen (**14**) as connecting atoms shift the absorption spectrum bathochromically (compare with **12**, see also Figure 2). Carbonyl group of *e.g.* ester (**15**) may participate in conjugated system and shifts the spectrum significantly to higher wavelengths.

We have also shown that the aromatic peripheral substituents may be partially conjugated with the aromatic π-system of TPyzPA even if they are partially rotated out of the macrocycle plane. Thus six-membered substituents modified TPyzPA (phenyl **16** and pyridin-2-yl **17**) absorb at 30 nm longer wavelengths and five-membered modified ones (furan-2-yl **18** and thiophen-2-yl **19**) at 50 nm longer



I

(for compounds **5-23** and **45-49**)



II

(for compounds **24-28**)

Figure 3. Basic macrocyclic structures **I** and **II**.

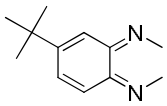
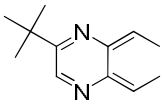
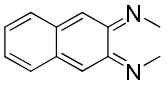
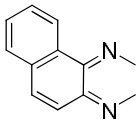
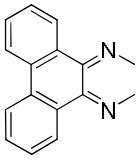
Table 1. *Q*-band positions of TPyzPAs in dependence on peripheral substitution and central metal. Structures are based on macrocycle **I** (Figure 3).

Compound	R_1	R_2	Metal	Solvent	Polarization red shift λ (nm)[a]	*Q*-band, λ (nm)	Ref.
5	H	H	Co	DMSO	12	615	[58]
6	*t*-Bu	H	Co	DMSO	12	616	[58]
7	*t*-Bu	H	Cu	$CHCl_3$	13	631	[58]
8	*n*-$C_{12}H_{25}$	*n*-$C_{12}H_{25}$	Cu	$CHCl_3$	13	634	[42]
9	*n*-C_4H_9	*n*-C_4H_9	Mg	CH_2Cl_2	11	634	[16]
10	O-*n*-C_3H_7	O-*n*-C_3H_7	Cu	pyridine	14	626	[25]
11	O-*n*-C_4H_9	O-*n*-C_4H_9	Mg	pyridine	14	626	[24]
12	O-*n*-C_4H_9	O-*n*-C_4H_9	Zn	pyridine	14	624	[24]
13	S-*n*-C_4H_9	S-*n*-C_4H_9	Zn	pyridine	14	655	[24]
14	NH-*n*-C_4H_9	NH-*n*-C_4H_9	Zn	pyridine	14	651	[24]
15	COOEt	COOEt	Zn	$CHCl_3$	13	658	[50]
16	phenyl	phenyl	Zn	pyridine	14	657	[59]
17	pyridin-2-yl	pyridin-2-yl	Zn	pyridine	14	657	[59]
18	furan-2-yl	furan-2-yl	Zn	pyridine	14	676	[59]
19	thiophen-2-yl	thiophen-2-yl	Zn	pyridine	14	673	[59]
20	thiophen-3-yl	thiophen-3-yl	Zn	pyridine	14	661	[60]
21	benzo[*b*]thiophen-3-yl	benzo[*b*]thiophen-3-yl	Zn	pyridine	14	664	[60]
22	-C≡C-Si(*i*-Pr)$_3$	-C≡C-Si(*i*-Pr)$_3$	Zn	THF	8	676	[16]
23	4-methylstyryl	4-methylstyryl	Zn	pyridine	14	687	[45]

[a] Mentioned for possible corrections of solvatochromic shift. Data taken from ref. [57]

than unsubstituted dyes. The red shift of five-membered against six-membered rings was explained by less sterical strain between two *ortho*-substituted aromatic rings and thus better conjugation of substituents with macrocycle system.[59] Interestingly, also position of the heteroatom in the aromatic peripheral ring plays a role. TPyzPA with thiophen-3-yl substituents (**20**) is 12 nm blue shifted comparing to its 2-yl isomer **19**. Further addition of benzene rings to peripheral aromatic substituents (**21**) does not influence the *Q*-band position significantly.

Table 2. *Q*-band positions of TPyzPAs in dependence on enlargement of the core with benzene rings. Structures are based on macrocycle **II** (Figure 3).

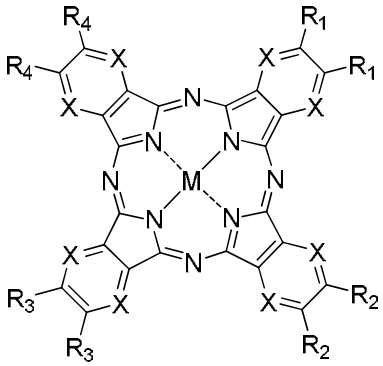
Compound	R	Metal	*Q*-band, λ (nm)[a]	Ref.
24		Cu	712	[58]
25		Cu	755	[58]
26		Zn	806	[31]
27		Cu	689	[30]
28		Cu	684	[30]

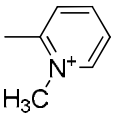
[a] Solvent quinoline

An interesting approach of increasing absorption wavelength was introduced by Faust *et al.*[16] Conjugation of acetylenic units enlarges the chromophore (**22**) and induces important bathochromic shift for almost 40 nm comparing to carbon substituted TPyzPAs **9**. Similar strategy has been used recently by Russian authors. The styryl peripheral substituents (**23**) expanded the π-conjugated system and induced the strongest bathochromic shift from all used peripheral substituents till this time.[45]

The second and more pronounced possibility how to shift absorbance towards the higher wavelengths is the enlargement of basic TPyzPA core by insertion of another benzene ring producing the TQPs. The four benzene rings condensed onto periphery (2,3-TQPs) cause bathochromic shift of approximately 80 nm (compare **7** and **24**), but insertion of the benzene rings between the pyrazine and porphyrazine rings (6,7-TQPs, **25**) leads to more than 120 nm red shift. We have investigated the influence of stepwise addition of benzene rings between pyrazine and porphyrazine rings leading to series of pyrazinoquinoxalinoporphyrazines with different number of pyrazine and 6,7-quinoxaline subunits.[39] The position of *Q*-band centre was increasing linearly with 22 nm bathochromic shift for each inserted benzene ring. An approach combining both insertion of benzene rings (6,7-TQP) and conjugation of peripheral acetylenic substituents have been shown to efficiently increase the *Q*-band position up to 774 nm.[37] Further linear condensation of benzene rings to 2,3-TQP's produces tetrabenzo[*g*]quinoxaline-2,3-porphyrazines (**26**) absorbing over 800 nm.[31] On the other hand, condensation of next benzene rings to TQPs angularly causes hypsochromic shift (compounds **27** and **28**).[30,52]

Several other influences may contribute to red shift of the *Q*-band position. It includes protonization of azomethine nitrogens[56] (see also below), unsymmetrical composition of the macrocycle observed by metal-free TPyzPA[22,24] or unsymmetrical structure of the whole macrocycle.[39]



29 M=Zn, X=N, R= S-*t*Bu
30 M=Zn, X=CH, R= S-*t*Bu
31 M=2H, X=N, R= S-*t*Bu
32 M=Zn, X=N, R= $N(CH_2CH_3)_2$
33 M=2H, X=N, R= $N(CH_2CH_3)_2$
34 M=Mg, X=N, R= S-*t*Bu
35 M=Mg, X=N, R= S-*n*-C_8H_{17}
36 M=Mg, X=CH, R=S-*t*Bu
37 M=Mg, X=CH, R= S-*n*-C_8H_{17}
38 M=2H, X=N, R= 

$R=R_1=R_2=R_3=R_4$

39 M=Zn, X=N, $R_1=R_2=R_3$= S-*t*Bu, R_4= $SCH_2CH_2N(CH_2CH_3)_2$.HCl
40 M=Zn, X=N, $R_1=R_2$= S-*t*Bu, $R_3=R_4$= $SCH_2CH_2N(CH_2CH_3)_2$.HCl
41 M=Zn, X=N, $R_1=R_3$= S-*t*Bu, $R_2=R_4$= $SCH_2CH_2N(CH_2CH_3)_2$.HCl
42 M=Zn, X=N, $R_1=R_2=R_3=R_4$= $SCH_2CH_2N(CH_2CH_3)_2$.HCl
43 M=Zn or Cu, X=N, $R_1=R_2=R_3=R_4$= COO^-Na^+
44 M=Fe or Co or Cu or Ni, X=N, $R_1=R_2=R_3=R_4$= 4-sulfophenyl

Figure 4. Structure of compounds **29**-**44**.

Acid-base Properties

TPyzPA macrocycle is basic due to presence of azomethine and pyrazine nitrogens. Metal-free TPyzPA possesses also acid central hydrogens and may behave as *N*-acids. We have studied the amphoteric character of compounds **29**-**33** (Figure 4) by means of UV-vis spectroscopy.[56] Protonation of the TPyzPA macrocycle **29**, achieved by addition of trifluoroacetic acid (TFA) or sulphuric acid, starts on one azomethine nitrogen and similarly to Pc[61] it causes splitting of the *Q*-band and its bathochromic shift (Figure 5). The second azomethine nitrogen and one pyrazine nitrogen are protonated in the second step. Data were supported by quantitative titration data (Figure 6). Comparing ZnTPyzPA **29** and ZnPc **30**, the TPyzPA porphyrazine ring is less basic most likely due to electron-withdrawing effect of the condensed pyrazine rings. Metal-free TPyzPA **31** is even less basic and the protonation of the first azomethine nitrogen starts at higher concentration of acids simultaneously with protonation of pyrazine nitrogen which may be the primary target of protons. Similar observations were made by other group for metal free octaphenylsubstituted TPyzPA and its Lu^{III} complex. They showed that acid-base interactions of metal-free compound involved primarily nitrogen atoms in pyrazine fragment while its Lu^{III} complex underwent protonation at the *meso*-nitrogen atoms.[62] In the case, when another basic nitrogens are introduced to the macrocycle periphery (**32**, **33**) they become the primary targets of protons using even week acids (acetic acid).

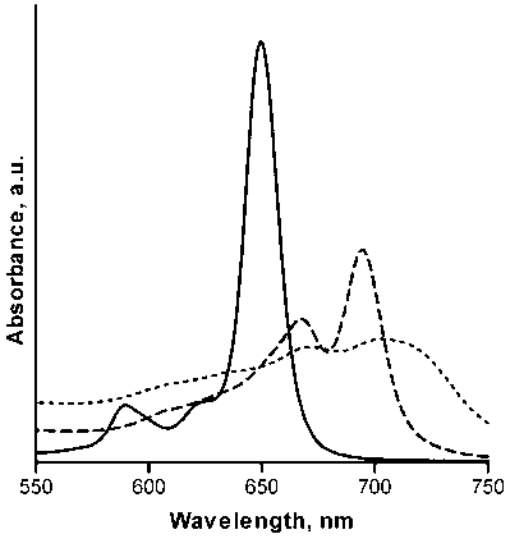


Figure 5. Spectra of neutral (full line) and protonated forms (one protonated azomethine nitrogen – dashed line, two protonated azomethine nitrogens – dotted line) of compound **29**.

The metal-free TPyzPA macrocycles (*e.g.* **31** and **33**) may lose their central hydrogens in basic media. Losing the hydrogen they acquire symmetric structure (D_{4h}) and the UV-vis spectrum corresponds to metal TPyzPA. Both central hydrogens are removed at once producing directly dianion without detectable amount of monoanionic form.[56,63] Metal-free TPyzPA may also produce so called "proton-transfer complex"[23,56] with weak bases (pyridine, DMF, amines) as suggested also for metal-free Pc.[64-66] In this case, the central hydrogen is not completely removed. Electron-donating substituents (*e.g.* diethylamino, **33**) decrease the acidity of the central hydrogens and thus **31** is stronger acid than **33** which is reflected also in the speed of the proton-transfer complex formation (formation is faster for **31**).

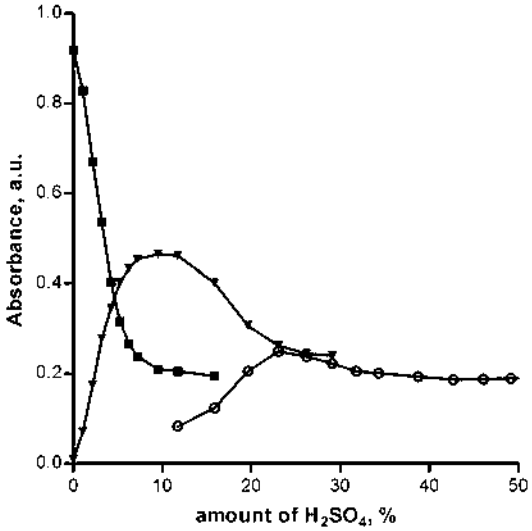


Figure 6. Changes in absorption of TPyzPA **29** after addition of H_2SO_4 into its THF solution. (■) Corresponds to nonprotonated form (650 nm). (▼) Corresponds to monoprotonated form (695 nm). (○) Corresponds to diprotonated form (719 nm).

Aggregation

Due to planar structure of basic macrocycle, TPyzPA as well as other AzaPc and Pc strongly tend to aggregate. It is one of the most undesirable properties which decreases solubility, causes problems during purification and shortens triplet state lifetimes which are crucial for the effect of TPyzPA in PDT. After excitation, aggregates lose their energy mostly through the heat being photodynamically inactive. This problem is usually solved by introduction of optimal substituents onto periphery of a TPyzPA core which suppress the aggregation. We compared the suitability of long (**35**, **37**) and bulky (**34**, **36**) alkyl chains to induce monomerization as well as susceptibility of TPyzPA (**34**, **35**) and Pc (**36**, **37**) core to aggregation.[26] From dimerization constants (K_d) measured in toluene it is clearly obvious that bulky substituents are more suitable to induce monomerization and that TPyzPA macrocycle aggregates more than Pc core. The propensity to aggregation decreased in order **35** > **37** > **34** > **36**. The differences between the K_d values in toluene were in several orders. The biological consequence of such finding has been revealed recently.[67] Incorporation of compound **35** into liposomal bilayer, a model of biological membrane, was not achieved contrary to **34** and similar compound with bulky substituents. Compound **35**, with the highest lipophilicity in that series which predestinates it to be well soluble in lipid bilayer, stayed only aggregated in water medium and did not enter the lipids contrary to **34** or more hydrophilic TPyzPA with bulky substituents. For PDT purposes, compounds like **35** seem to be useless since they

will not enter the cell membrane at all and they cannot exhibit their activity. It shows that the inhibition of aggregation must be really efficient to allow lipophilic compounds to serve as photosensitizers in PDT in biological medium.

tert-Butyl substituents[27,30,43,47] are not the only one which can serve as efficient inhibitors of aggregation. Phenoxy groups disubstituted in *ortho* positions to ether linkage to macrocycle were used as substituents preventing association even in solid films.[4] Also substitution of the central silicon by two alkylsiloxy substituents brings very good inhibition of aggregation.[49,52]

The bulkiness of the peripheral substituents plays an important role only in organic solutions. The aggregation in water medium must be inhibited using other approaches, mainly by introduction of cationic or anionic ionized peripheral substituents. The TPyzPA with eight quarternized pyridyl substituents on periphery **38**[63] and its metalated derivatives (Mg, Co, Cu and Zn)[10] have shown very good solubility in water, but they were strongly aggregated. The disaggregation appeared only after addition of concentrated HCl. We have prepared and studied compounds bearing different number of aliphatic tertiary amino groups (**39**, **40**, **41** and **42**) which were quarternized by transformation to their hydrochlorides.[48] It was demonstrated that two charges in molecule (**39**) were not enough for water-solubility, while four charges (both isomers **40** and **41**) have already led to good solubility but did not hinder the aggregation. Simple transition from dimer to monomer for these two compounds was accomplished after DMF addition into water solution. The compound with eight charged amino groups (**42**) stayed monomeric in water solution. It is therefore evident that eight charges in molecule are necessary for complete monomerization of such compounds. Eight negative charges were also used successfully to inhibit aggregation in water. Compounds with both carboxy **43**[50] and sulfo **44**[68] groups with different central metals showed excellent solubility in water and UV-vis spectra typical for presence of only monomers.

Fluorescence and Singlet Oxygen Production

As mentioned at the beginning, Pc and related compounds have attracted a great attention in PDT. For example, sulfonated aluminium phthalocyanine has been approved in Russia since 2001 for PDT treatment of several cancers under the name Photosens. The PDT principle is based on the combination of photosensitizer, light and oxygen. Singlet oxygen is the most important cytotoxic agent involved in PDT. The effectiveness of photosensitizer is therefore characterized by singlet oxygen quantum yield (Φ_Δ), the amount of singlet oxygen molecules ($^1O_2$) formed per absorbed energy quantum. Emission of the photon from singlet excited state of photosensitizer, known as fluorescence, may help in treatment too, since it allows detection of tumor or quantification of the amount of the dye absorbed by target cells.

Singlet oxygen and fluorescence studies of the TPyzPA are quite rare and to the best of our knowledge only one group except us has performed some studies with this kind of organic dyes.[16,37,47] The quantum yields are influenced mainly by the central metal and peripheral substitution. Coordination of zinc leads to the high Φ_Δ and lower fluorescence quantum yields (Φ_F). On the other hand, TPyzPA with magnesium as the central metal are strongly fluorescent while being only moderate producers of singlet oxygen (Table 3). Metal-free compounds release the absorbed energy through competitive processes (*e.g* collisions) and both quantum yields are lowered. Concerning the peripheral substitution, the heteroatoms play an important role. As it has been shown on the series of compounds with different connecting heteroatom,[24] alkylsulfanyl substituents (**13**) are the most suitable for PDT due to the highest singlet oxygen production followed by alkoxy derivatives (**12**). Alkalymino derivatives (**14**) are characterized by very low quantum yields which are most likely caused by intramolecular photoinduced electron transfer from donor nitrogen atoms to conjugated system (Zimcik *et. al*, unpublished results). Similar conclusions as in the case of aliphatic substitution can be deduced also for heteroaromatic derivatives (Table 3, compare compounds **16**-**19**).[59] The best producer is again compound containing sulfur in the structure (**19**). The furan-2-yl derivative (**18**) showed instability after irradiation which may partially explain its low quantum yields. These results were further supported by measurements of unsymmetrically substituted TPyzPA (different R_1 and R_2, compounds **47**-**49**).[60] As anticipated, combination of thiophen-2-yl with alkylsulfanylsubstituents (**47**, **48**) with positive effect on singlet oxygen production resulted in high Φ_Δ with values over 0.60.

Table 3. Quantum yields of some TPyzPAs. Structures are based on macrocycle **I** (Figure 3).

Compound	R_1	R_2	Metal	Solvent	Φ_Δ	Φ_F	Ref.
16	phenyl	phenyl	Zn	pyridine	0.49	0.24	[59]
17	pyridin-2-yl	pyridin-2-yl	Zn	pyridine	0.53	0.26	[59]
18	furan-2-yl	furan-2-yl	Zn	pyridine	0.38[a]	0.14[a]	[59]
19	thiophen-2-yl	thiophen-2-yl	Zn	pyridine	0.64	0.18	[59]
29	S-*t*-Bu	S-*t*-Bu	Zn	DMF	0.66	0.22	[48]
32	$N(CH_2CH_3)_2$	$N(CH_2CH_3)_2$	Zn	pyridine	0.02	n.d.[b]	[69]
45	thiophen-2-yl	thiophen-2-yl	Mg	pyridine	0.31	0.32	[59]
46	pyridin-2-yl	pyridin-2-yl	Mg	pyridine	0.25	0.50	[59]
47	thiophen-2-yl	S-*t*-Bu	Zn	pyridine	0.67	0.16	[60]
48	thiophen-2-yl	S-(4-chlorobenzyl)	Zn	pyridine	0.70	0.18	[60]
49	thiophen-2-yl	thiomorpholine	Zn	pyridine	0.11	0.02	[60]

[a] decomposition, [b] n.d.=not detected

On the other hand, when nitrogen was introduced as the connecting heteroatom of the half of the substituents (thiomorpholine, **49**), both quantum yields decreased rapidly. In the case when only nitrogen is used as the connecting heteroatom (**32**), the Φ_Δ is close to zero and fluorescence has not been detected at all.

Conclusions

In comparison to Pc, the main advantages of TPyzPA's are in relatively simple preparation of precursors, wide range of available peripheral substitutions which are not sometimes available for Pc rings and better solubility. Unfortunately, increased density of electrons on pyrazine nitrogens causes stronger π-π interactions between two molecules of the dye. As a consequence, TPyzPA macrocycle tend to stronger aggregation. Furthermore, the absorption maximum of TPyzPA is shifted hypso-chromically for almost 50 nm comparing to corresponding Pc. However, both of these undesirable properties can be easily overwhelmed using suitable substituents and TPyzPA could find their use in many areas (including PDT) either similar to Pc or a new ones.

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