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Heteroatom-substituted tetra(3,4-pyrido)porphyrazines: A stride toward near-infrared-absorbing macrocycles

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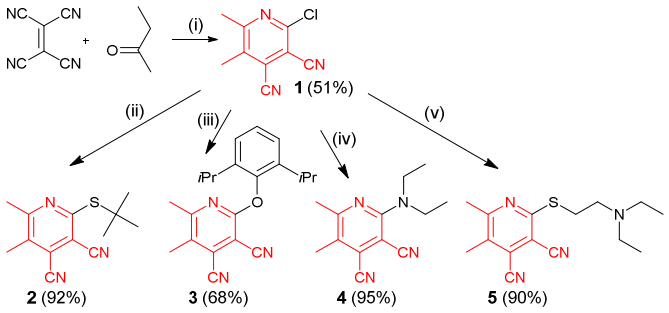
A synthesis procedure for heteroatom-substituted tetra(3,4-pyrido)porphyrazines that absorb light near 800 nm was developed. Based on the observed relationships between the structure and photophysical parameters, a novel highly photodynamically active (IC_{50} = 0.26 μM) compound was synthesized and biologically characterized.

Tetrapyridoporphyrazines (TPyPzs) are a class of phthalocyanine (Pc) aza analogs in which all four benzene rings are replaced by pyridines. In general, the two types of TPyPz derivatives, namely tetrapyrido[3,4-*b*:3',4'-*g*:3'',4''-*l*:3''',4'''-*q*]porphyrazines (tetra(3,4-pyrido)porphyrazines) and tetrapyrido[2,3-*b*:2',3'-*g*:2'',3''-*l*:2''',3'''-*q*]porphyrazines (tetra(2,3-pyrido)porphyrazines), are distinguished by the nitrogen position in the pyridine ring. The main Q-band absorption peak of the 2,3-isomer undergoes an undesirable blue shift relative to that of the parent Pc.[1, 2] On the other hand, the absorption properties of the former one remain unchanged after isosteric replacement of CH for nitrogen in Pc benzene ring.[1, 2] Recently, 3,4-isomers have received much attention for use in many applications because of their intriguing electrocatalytical,[1, 3] photosensitizing,[4] and surface-binding properties.[5] Surprisingly, although TPyPzs were among the first Pc analogs described by Linstead in 1937,[6] their structural modifications have been limited to central cation exchange and quaternization of the pyridine nitrogen till now.[7] Very few reports on alkyl-substituted derivatives have appeared in the literature.[8]

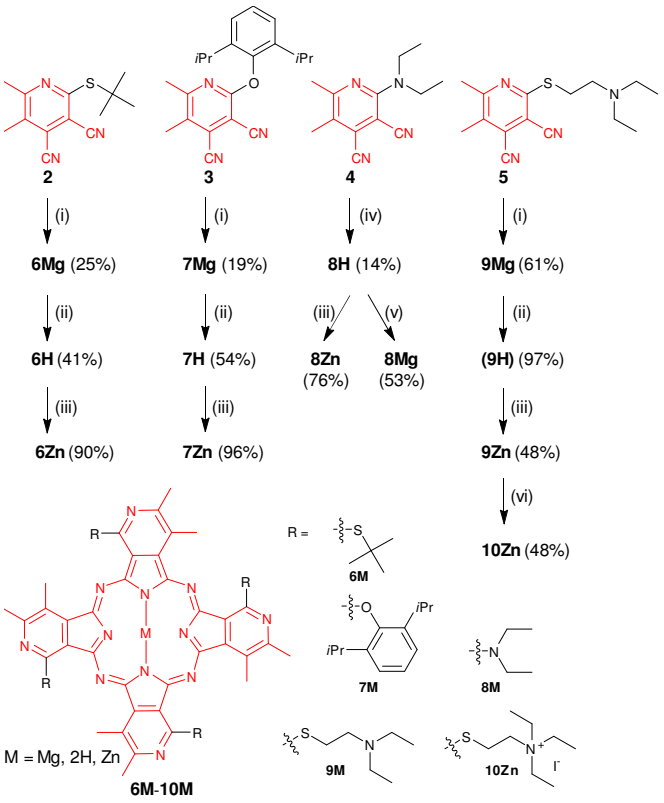
It is well known that heteroatoms, such as O, S and N, substantially influence both the spectral and photophysical properties of Pc-type macrocycles when they are attached directly to the core,[9] particularly in non-peripheral (α) positions.[10, 11] This work therefore focuses on developing the first heteroatom-substituted TPyPzs and investigating their properties to determine their potential for use in photodynamic therapy (PDT). The substituents in this study were chosen not only to study the effects of different connecting heteroatoms (O, S, N) but also to ensure that their bulkiness prevented aggregation of the TPyPzs in solution. Thus, precursors **2**, **3** and **4** (Scheme 1) were selected for this study. Zinc (II) and Mg (II) were used as the central cations in the target TPyPzs due to their suitable photophysical properties.



Scheme 1. Reaction conditions: i) HCl, dioxane, 65°C. ii) *t*BuSH, aq. NaOH, THF, rt. iii) 2,6-Di(isopropyl)phenol, CsF, anhydr. DMF, Ar, rt. iv) Diethylamine, THF, reflux. v) Diethylaminoethanethiol, K_2CO_3, DMSO, rt.

TPyPzs are typically synthesized by cyclotetramerization of pyridine-3,4-dicarbonitrile. The synthesis of the precursors **2**-**4** was achieved by high-yielding nucleophilic substitutions with thiolate, phenolate or amine in **1** (**1** was synthesized according to literature method[12]) due to the strongly electron-deficient character of its $C_{(2)}$ (Scheme 1). The precursor **1** was smoothly converted to *tert*-butylsulfanyl derivative **2** by reaction with 2-methylpropane-2-thiol in THF in a presence of aqueous NaOH in yield of 92% (other bases can be used as well, e.g. K_2CO_3). The aryloxy derivative **3** can be synthesized using only CsF in anhydrous DMF. Attempts to apply other bases (NaOH, triethylamine, pyridine, K_2CO_3) did not lead to **3** at all. Compound **4** was received almost quantitatively by reaction of **1** with excess of diethylamine.†

None of the tested template methods for the cyclotetramerization of precursors to yield TPyPz (with zinc acetate in DMF, pyridine, or quinoline or with $Zn(quinoline)_2Cl_2$ in a melt) gave the desired product. The TPyPz ring was only formed when alkoxides were used as initiators



Scheme 2. i) Mg, anhydr. BuOH, reflux. ii) TsOH, $CHCl_3$ or THF/MeOH, rt. iii) $Zn(CH_3COO)_2$, pyridine, reflux. iv) Li, anhydr. BuOH, reflux. v) $Mg(CH_3COO)_2$, pyridine, reflux. vi) CH_3CH_2I, NMP, rt.

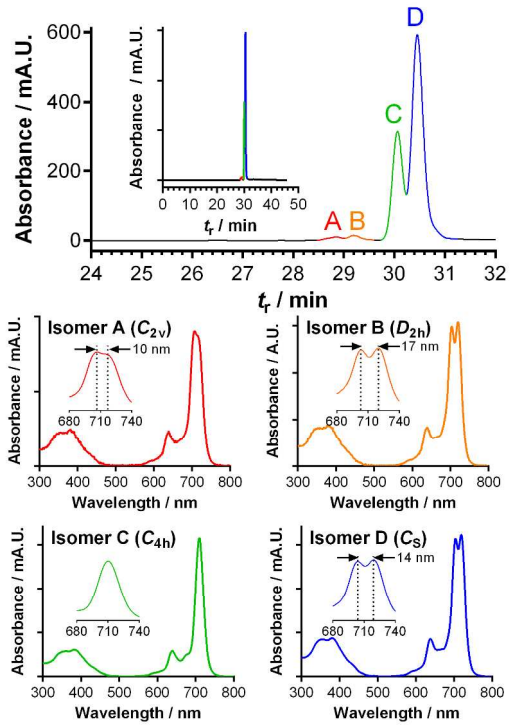


Figure 1. HPLC chromatogram (λ = 710 nm) of **7Mg** regioisomers (inset: full chromatogram) with the absorption spectra collected at the HPLC peak maxima of isomers A-D. The detailed Q-bands are presented in the insets.

(*i.e.*, original Linstead conditions, Scheme 2). A milder initiator, magnesium butoxide, was employed for the cyclotetramerizations of **2** and **3** to avoid possible exchange reactions with the peripheral substituents in the aryloxy and alkylsulfanyl derivatives, which was reported for some Pc aza-analogs synthesized using a lithium alkoxide initiator.[13] In contrast, a lithium alkoxide initiator was necessary for the cyclotetramerization of **4** because the strongly electron-donating diethylamino substituent hindered the reaction and no product was detected with magnesium butoxide. The reaction with lithium butoxide gave the metal-free derivative **8H**. The magnesium complexes **6Mg** and **7Mg** (obtained from reaction with magnesium butoxide) were subsequently demetalated with a strong organic acid, and the metal-free derivatives **6H**-**8H** were converted into zinc or magnesium complexes using the corresponding acetates in pyridine to afford **5Zn**-**8Zn** and **8Mg**.

Each TPyPz **6**-**8** could potentially exist as four positional isomers (Figure S34) due to the unsymmetrical structures of the starting dicarbonitriles. The isomer distribution is theoretically predicted to be 12.5% of the D_{2h} and C_{4h} isomers, 25% of the C_{2v} isomer, and 50% of the C_s isomer.[14] Several isomers were detected using HPLC (Table S1), but only the **7Mg** isomers could be identified based on their absorption spectra. Four peaks denoted A (C_{2v}), B (D_{2h}), C (C_{4h}) and D (C_s) with the distribution 1%, 1.5%, 30.5% and 67%, respectively, were detected in the **7Mg** HPLC chromatogram (Figure 1). The peaks were assigned to the different isomers based on the Q-band splitting, which varies substantially for isomers with different symmetries as recently predicted by TD-DFT theoretical calculations.[15] The obvious deviations from the theoretical isomer distribution can be explained by the significant steric hindrance of the bulky 2,6-diisopropylphenoxy substituents in **7Mg**. Thus, the percent of the least sterically hindered C_{4h} isomer increased by almost 2.5 times, while only 1.5% of the most sterically hindered D_{2h} isomer was detected. In addition to the steric effects, electronic effects might play a role in the observed isomer distribution because the steric hindrance does not explain the marked difference in the amounts of the similarly sterically hindered C_s and C_{2v} isomers, similarly as reported.[16] The isomers could not be isolated due to strongly overlapping TLC spots. Thus, all the samples were isomer mixtures, and the NMR spectra were very complex (Figures S11-S19). Despite this complexity, the **7Mg** ^{1}H NMR results (Figure S14) indicated that the C_s isomer was the predominant species, which is consistent with the HPLC data.

The absorption spectra shapes for the TPyPzs **6**-**8** (regioisomer mixtures) in THF (Figures 2 and S35) were similar to those for the Pcs and their aza analogs, and a high-energy B-band and low-energy Q-band were observed in the ranges of 315–390 nm and 710–806 nm, respectively (Table 1). As expected, the positions of the Q-band maxima were affected considerably by the nature of the heteroatom. Unsubstituted zinc tetra(3,4-pyrido)porphyrazine in pyridine was reported to absorb at 676 nm.[17] The presence of an aryloxy substituent induced a significant red shift of 36 nm in the Q-band position (λ_{max} (**7Zn**) = 712 nm). Larger bathochromic shifts were observed for the alkylsulfanyl-substituted (λ_{max} (**6Zn**) = 730 nm) and dialkylamino-substituted (λ_{max} (**8Zn**) = 772 nm) complexes.

Importantly, the absorption band of the metal-free dialkylamino-substituted TPyPz was observed at an even longer wavelength (λ_{max} (**8H**) = 806 nm) and is comparable to those of recently synthesized near-infrared-absorbing metal-free Pcs that have non-peripheral chalcogen substituents.[11] However, the ability of an amine to induce a red shift is apparently stronger than that of a chalcogen as demonstrated by the fact that the four amino substituents in **8H** induced the same red shift in the Q-band as eight chalcogen substituents. This result indicated that amino substituents in non-peripheral positions on the macrocycle are highly promising for the development of near-infrared-absorbing Pcs and their analogs. However, the synthesis of Pcs with amino substituents in non-peripheral positions has been challenging due to the limited number of synthesis procedures for suitably substituted phthalonitriles; very few reports can be found in the literature.[8, 18] Amino-substituted TPyPzs are therefore a suitable alternative because their synthesis procedures are simple and their precursors are easily prepared in high yields.†

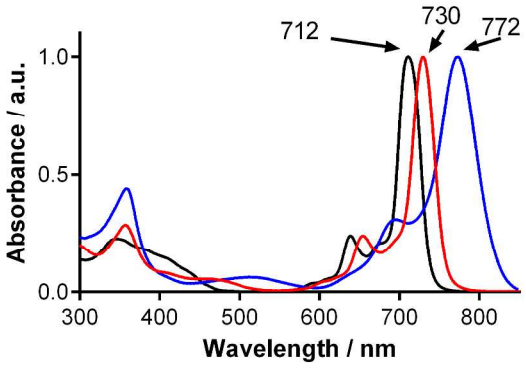


Figure 2. Normalized absorption spectra of **6Zn** (red), **7Zn** (black) and **8Zn** (blue) in THF.

Table 1 Photophysical data of the studied TPyPzs in THF.[a]

Cpd.	X	λ_{max} [nm] (ε [dm^3·mol^{-1}·cm^{-1}])	λ_F [nm]	$\Delta\lambda$ [cm^{-1}]	Φ_Δ[b]	Φ_F[b]
6Mg	S	728 (225 000)	739	204	0.36	0.38
6H[c]	S	743	771	489	0.10	0.11
6Zn	S	730 (154 000)	742	222	0.69	0.14
7Mg	O	712 (193 000)	721	175	0.29	0.46
7H	O	741 (89 000)	748	126	0.14	0.26
7Zn	O	712 (126 000)	724	233	0.58	0.23
8Mg	N	771 (95 000)	802	501	0.18	0.10[d]
8H	N	806 (86 000)	825	286	0.01	0.02[d]
8Zn	N	772 (94 000)	802	485	0.41	0.06[d]
10Zn[e]	S	722 (207 500)	735	245	0.61	0.10

[a] Connecting heteroatom (X), Q-band absorption maximum (λ_{max}), extinction coefficient (ε), emission maximum (λ_F), Stokes shift ($\Delta\lambda$), singlet oxygen quantum yield (Φ_Δ), fluorescence quantum yield (Φ_F). [b] Determined using ZnPc as a reference ($\Phi_{F(THF)}$ = 0.32, $\Phi_{\Delta(THF)}$ = 0.53, $\Phi_{\Delta(DMF)}$ = 0.56), three independent experiments, estimated error ±15%. [c] Poor solubility, partially aggregated. [d] Estimated error ±50%. [e] In DMF.

The shapes of the fluorescence emission spectra and the small Stokes shifts observed in this study were typical of those of Pc and its analogs (Figure S35, Table 1). Excitation spectra were collected and they corresponded well to the absorption spectra. It indicated that the compounds did not aggregate during the measurements (only **6H** formed aggregates) (Figure S35). The photophysical data (Φ_Δ and Φ_F, Table 1) can be interpreted in several ways. The sum of Φ_Δ and Φ_F, the two most important relaxation pathways for the excited state, was low for all the metal-free TPyPzs regardless of the type of substituent. These results are consistent with those for metal-free Pcs and tetrapyrazinoporphyrazines, which were attributed to other relaxation pathways that have not yet been completely elucidated.[9] For the dialkylamino derivatives (**8**), another excited state deactivation process, namely intramolecular charge transfer (ICT), must also be considered due to presence of donor amino substituents. The Φ_Δ+Φ_F values for the amino TPyPz metal complexes (**8Zn**, **8Mg**) were lower than those for the corresponding alkylsulfanyl (**6Mg, 6Zn**) and aryloxy (**7Zn, 7Mg**) TPyPzs, indicating that ICT influenced the photophysical properties of **8**. However, its effect was rather limited compared to that observed for tetrapyrazinoporphyrazines, for which ICT is a major relaxation pathway.[19] The aryloxy and alkylsulfanyl TPyPz metal complexes had Φ_Δ+Φ_F values of up to 0.80, suggesting that other relaxation pathways were not significant. The compounds differed only in the ratio of the fluorescence to singlet oxygen production. Strong fluorescence was observed for the aryloxy derivatives and magnesium complexes, while the singlet oxygen production of the alkylsulfanyl derivatives and zinc complexes was high. This can be interpreted to result from a two-dimensional heavy atom effect due to the central metal (first dimension) and the substituents (second dimension), which is consistent with a recently published study.[20]

Based on the photophysical data, the most promising compounds for PDT are the zinc complexes of alkylsulfanyl TPyPzs. They had the highest production of singlet oxygen, the most important cytotoxic species in PDT, and exhibit an important red shift in the Q-band maximum to λ_{max} ~ 730 nm. Thus, water-soluble compound **10Zn** was designed, synthesized (Scheme 2) and photophysically characterized (Table 1). Both the fluorescence and singlet oxygen quantum yields were similar to those of the model compound **6Zn**, confirming the observed relationships between the structure and photophysical properties. The photodynamic activity of compound **10Zn** was subsequently evaluated using the HeLa human cervix carcinoma cell line to demonstrate the potential of TPyPzs in PDT. The dark toxicity (without light activation) was low (TC_{50} = 105±9.5 μM). Activating the compound with light (λ > 570 nm, 12.4 mW·cm^{-2}, 11.2 J·cm^{-2}) resulted in a lethal photodynamic effect on the HeLa cells with IC_{50} = 0.26±0.089 μM (Figure S37), which is comparable to the IC_{50} value obtained when HeLa cells were treated with octacationic Pcs under the same conditions.[21] The high ratio of the toxic concentration to the active concentration (TC_{50}/IC_{50} ~400) suggested that this compound is a promising photosensitizer. To obtain deeper insight into its mechanism of action, the subcellular localization of **10Zn** was studied by fluorescence microscopy (Figure 3). The punctate red fluorescence of TPyPz **10Zn** in the HeLa cells colocalized exclusively with lysosomes, indicating that they are the primary singlet oxygen targets. The morphological changes observed in different organelles after irradiation (lysosome rupture, TPyPz diffusion into the cytosol followed by destruction of the mitochondria and, finally, interactions between the cationic TPyPz and nuclear DNA) were consistent with the behavior induced by octacationic Pcs,[21] suggesting a similar sequence of events leading primarily to necrotic cell death.

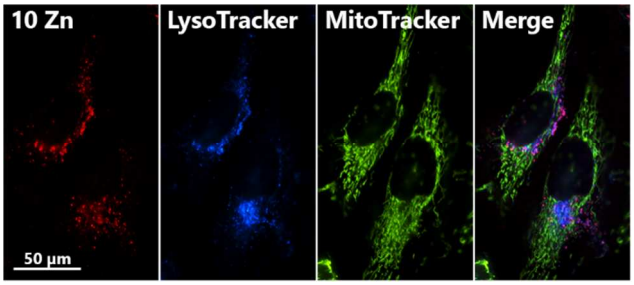


Figure 3. Subcellular localization of **10Zn** (red) in HeLa cells visualized by fluorescence microscopy after co-incubation with the organelle-specific fluorescent probes MitoTracker (green) and LysoTracker (blue).

In summary, a versatile procedure for synthesizing heteroatom-substituted TPyPzs was developed. This paved the interesting way for further functionalization of TPyPz macrocycles, the modifications of which were strongly limited until now. The basic photophysical relationships of the series were determined and can be used to rationally design TPyPzs with specific photophysical properties. The dialkylamino and alkylsulfanyl derivatives seem to be particularly promising for further development. The dialkylamino derivatives were characterized by an unusually large red shift (almost 100 nm) in the Q-band maximum, which was induced by just four substituents. The relatively straightforward synthesis make them promising candidates in the development of near-infrared-absorbing macrocycles. The alkylsulfanyl derivatives were characterized by high singlet oxygen production, making them suitable for use as photosensitizers for PDT. The potential of alkylsulfanyl TPyPzs in this application was demonstrated by the high photodynamic activity of cationic, water-soluble **10Zn**.

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Notes and references

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† The reaction of **1** with other amines also gave high yields that were typically greater than 70% (not shown), and **1** reacted also readily with residual traces of *N,N*-dimethylamine in DMF.

Electronic Supplementary Information (ESI) available: experimental procedures and NMR, MS, absorption and fluorescence spectra. See DOI: 10.1039/c000000x/

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