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FULL PAPER

Synthesis and J-Dimer Formation of Tetrapyrazinoporphyrazines with Different Functional Groups for Potential Biomolecular Probe Applications

Jiri Demuth, Miroslav Miletin, Matej Machan, Michal Kantor, Petr Zimcik* and Veronika Novakova*

Abstract: Despite the fact that tetrapyrazinoporphyrazines (TPyzPzs) are generally presented as universal dark quenchers for oligonucleotide probes, the availability of TPyzPzs bearing different functional groups suitable for attachment to 3´, and 5´ ends or intrastrand positions remains rather limited. Therefore, a synthetic route to hexa(bis(2-methoxyethyl)amino) or hexa(diethylamino) TPyzPzs functionalized by an azide, hydroxy, or carboxy group or their combinations was developed. Studies of self-assembly into J-dimers in nonpolar solvents and their stability upon titration with pyridine (association constants, K_P values, ranging 0.32-12.7×10^2 M^{-1}) revealed that smaller peripheral substituents and functionalization of TPyzPzs improves the stability of J-dimers. Φ_Δ and Φ_F were low for the monomers (Φ_F < 0.0001, Φ_Δ < 0.006, DMF) due to quenching by intramolecular charge transfer; however, they increased in nonpolar solvents and after self-assembly into J-dimer (up to Φ_F = 0.027, Φ_Δ = 0.28).

Introduction

The binding of synthetic molecules to various biomolecules and their subsequent use in different fields, such as biochemistry, molecular biology, medicinal chemistry, and environmental analysis, represent a current trend in basic research and in practice.[1, 2] Detailed understanding of the structure of DNA and the elucidation of its functions have led to the development of many helpful biochemical assays. Among them, quantitative real-time PCR employing oligodeoxynucleotide (ODN) probes has become a powerful tool in molecular diagnostics.[3] Such ODN probes typically consist of a fluorophore and a quencher separated by a sequence of bases complementary to a target section of DNA.[4, 5]

In the past decade, dialkylamine substituted tetrapyrazinoporphyrazines (TPyzPzs) have been developed into efficient dark quenchers. Thanks to their panchromatic absorption, they are universal for many types of fluorophores. Generally, the quencher in the probe may be attached to the ODN at the 3´or 5´end; its incorporation into the middle of the ODN has also been reported.[6, 7] Amidation, click or phosphoramidite chemistry belong among the typical reaction used for this purpose. Furthermore, binding of various modifiers to quenchers was found to increase the melting temperature of duplexes of ODN probes with target strand that subsequently, improved the signal-to-noise ratio during application. Different methods of attachment of quencher to ODNs (and the potential binding of modifiers directly to the quencher) require different functional groups on the quencher. The range of different functional groups in TPyzPzs quenchers, however, remains rather limited.

This project aims to describe the synthesis of a series of TPyzPzs bearing various functional groups suitable for different binding modes of the quencher to the ODN, namely, to the 3´ or 5´ends or an intrastrand position. Furthermore, the role of peripheral dialkylamine groups on TPyzPzs as a function of their bulkiness and physicochemical properties (*i.e.*, solubility) is investigated. Emphasis is also placed on the formation of J-dimers of TPyzPzs because the self-assembly of ODN-TPyzPz conjugates into J-dimers has been recently found to positively affect the application of these probes in quantitative PCR or it may lead to the development of biomolecular logic gates.[8]

Results and Discussion

Design of target derivatives

TPyzPzs suitable for use as quenchers of the fluorescence of ODN probes must meet several key requirements. First, peripheral substituents must be attached to the macrocycle through the nitrogens to enable intramolecular charge transfer (ICT) as a quenching principle.[9] Diethylamino and bis(2-methoxyethyl)amino substituents, which differ in hydrophilicity and in bulkiness, were included to allow the effects of these parameters on the aggregation and solubility of target derivatives to be studied. The presence of only one or two functional groups for definable binding to the ODN probe is essential. As a result, target TPyzPzs (**1**-**8**, Figure 1) are of low-symmetrical character with only one quarter decorated with functional groups. Regarding the functional groups, carboxy group, azide moiety and hydroxy group were chosen for this series based on the typical procedures for binding molecules to ODN probes.[2, 5, 9] For example, azide moiety in TPyzPzs **3** and **7** may be used to attach the quencher to a solid phase (CPG) which may be followed by ODN synthesis resulting in an ODN probe with a

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quencher at its 3´end. The azide-alkyne cycloaddition (*i.e.*, click chemistry) may be used for binding a modifier; such modified quencher may be then attached to 5´ end of ODN using phosphoramidite chemistry. Click chemistry may be employed also for binding the quencher into intrastrand position when commercially available deoxythymidine modified with dibenzoazacyclooctyne (DBCOdT) is incorporated into ODN sequence. In addition, 5´ end click binding of quencher may be employed when ODN synthesis is terminated by the DBCOdT. Intrastrand binding of the quencher may be achieved also by phosphoramidite chemistry with TPyzPz **8** bearing "ZEN motive", that may substitute one base in the ODN chain as a non-nucleosidic building block and may even increase the stability and T_m of the nucleic acid duplexes.[6, 10] Finally, amidation of ODN probes modified by a commercially available aminolinkers with quenchers bearing carboxy groups may result into probes with quenchers at both 3´ and 5´end as well as inside of the ODN sequence. An illustrative scheme of possible binding of involved TPyzPzs to different parts of a ODN probe is shown in Figure 1b.

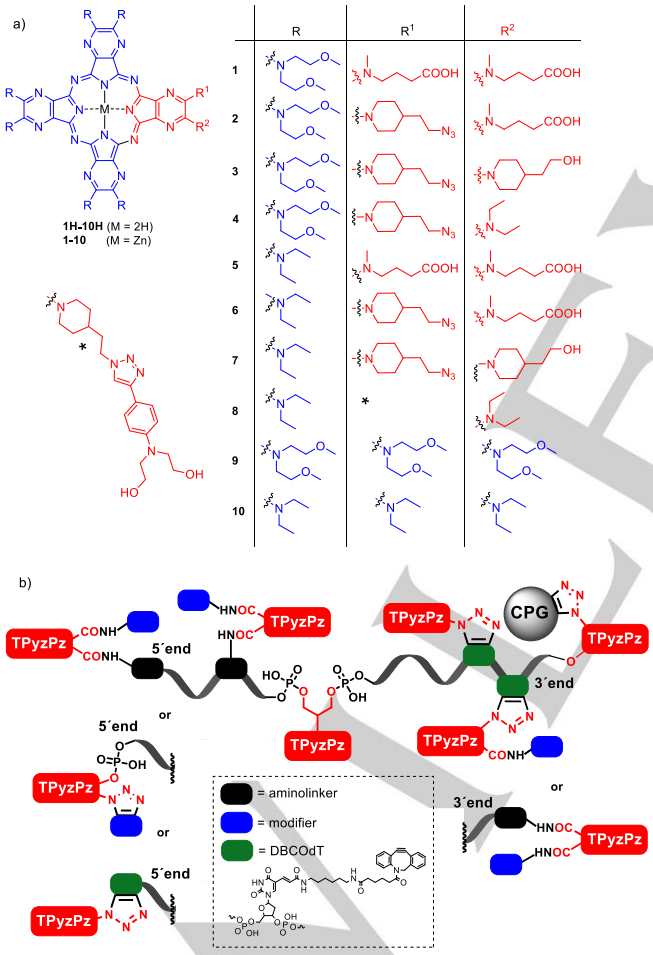

Figure 1. Structure of target TPyzPzs involved in the study (a) and illustrative scheme showing the possible binding of target quenchers to an ODN probe (for detail description see the text) (b)

TPyzPzs may be metal-free (M = 2H, see Figure 1) or may coordinate different central atoms, of which zinc (II) was chosen to preclude unpredictable chelation of cations during ODN synthesis of the corresponding metal-free forms that has been reported in the literature.[11, 12] Two symmetrical TPyzPzs **9** and **10** were added to this series as model unfunctionalized compounds.

Synthesis of precursors and target TPyzPzs

Although several synthetic approaches for accessing low-symmetry TPyzPzs have been published,[13] the statistical condensation of two different precursors, *i.e.,* 5,6-disubstituted pyrazine-2,3-dicarbonitriles, remains the most commonly used method. Suitable precursors were prepared from 5,6-dichloropyrazine-2,3-dicarbonitrile (**11**) by nucleophilic substitution using the appropriate nucleophiles (Scheme 1). Symmetrical precursors **23** and **24** were prepared in high yields in one step by heating **11** with excess of the appropriate amines. Similar reaction conditions with excess of *N*-methyl-4-aminobutanoic acid (**29**) did not, however, provide symmetrical precursor **12** in a reasonable yield as many side products were generated. After optimization, compound **12** was prepared in 24 % yield with triethylamine as the base by stirring at room temperature, although this reaction temperature necessitated a long reaction time. The synthesis of unsymmetrical precursors **14**, **17**, **18**, and **22** required a multistep procedure involving nucleophilic substitution followed usually by modification of the terminal functional groups. Generally, chlorines in strongly electron-deficient **11** readily react with (di)alkylamines.[13, 14] To efficiently achieve only monosubstitution by one dialkylamine on pyrazine-2,3-dicarbonitrile **11** (synthesis of **13**, **15** and **19**), equimolar amounts were necessary during the first nucleophilic substitution and sometimes even cooling of the reaction mixture was required. Because installation of the first alkylamine disfavors subsequent nucleophilic substitution with another dialkylamine, stirring at room temperature (synthesis of **16**) or even heating (synthesis of **14**) is typically employed for the second substitution. In the case of precursor **14**, azide nucleophile **28** with azide was employed. However, due to the necessity of a multistep preparation of **28** and the low yield of the final deprotection step (see Scheme 2 and the text below), a different approach was employed in the case of precursors **17** and **22**. Namely, 2-(piperidine-4-yl)ethan-1-ol was used as the nucleophile, and this was followed by the conversion of the OH to the corresponding methyl sulfonate by treatment with MsCl in the presence of triethylamine as a nonnucleophilic base. Subsequent conversion to the azide *via* treatment with NaN_3 led to desired **17** and **22** in high yields (over 80 %). Finally, a copper(I)-catalyzed azide-alkyne Huisgen cycloaddition between azide on compound **17** and the terminal alkyne on **27** provided precursor **18** in a high yield (84 %). Intermediates **28**-**30** have been prepared based on procedures described previously in literature[15-17] (Scheme 2). For the synthesis of **28**, the published procedure[15] was slightly modified, and unlike the referenced publication, final product **28** was characterized. When employing the conditions from the literature[17] for the synthesis of **30**, we were not able to achieve the published yields (*i.e.*, 71 %, 91 % and 90 % for steps vi), vii)

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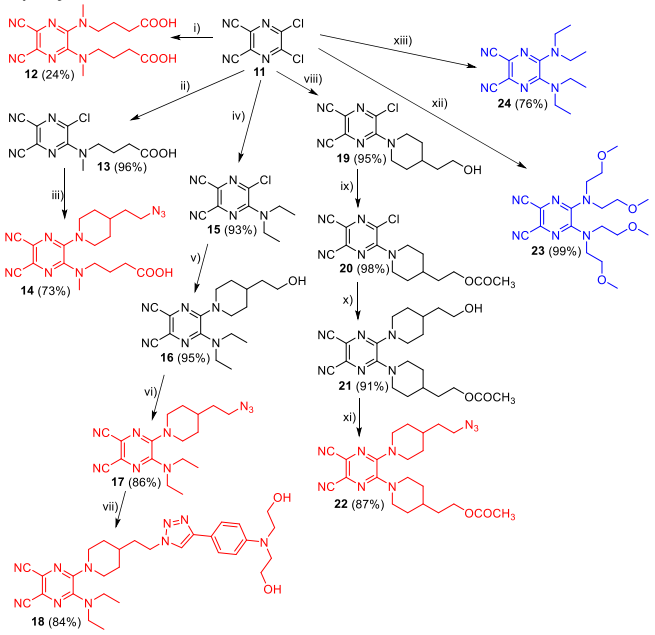
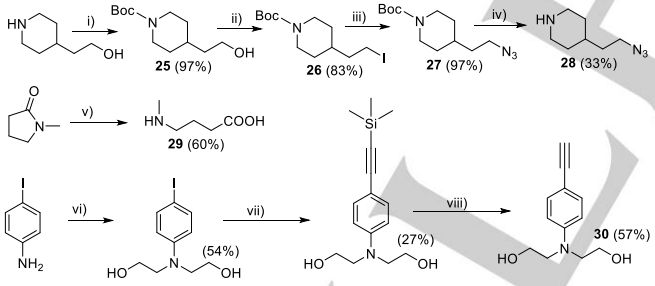
and viii), respectively). Notably, final **30** had to be stored in freezer, in the dark under an inert atmosphere because it decomposed rapidly.

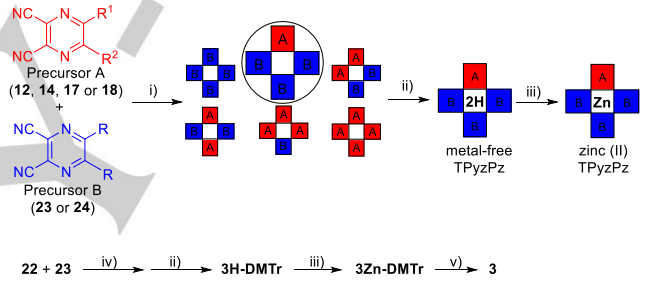


Scheme 1. Synthesis of precursors. i) cpd. **29**, TEA, NMP, rt, 48 h; ii) cpd. **29**, THF, rt, 3 days; iii) cpd. **28**, THF, reflux, 24 h; iv) *N,N*-diethylamine, THF, -12 °C, 20 min; v) 2-(piperidine-4-yl)ethan-1-ol, THF, rt, 90 min; vi) 1. MsCl, TEA, anh. DCM, rt, 1h; 2. NaN_3, anh. DMF, 60 °C, 90 min; vii) cpd. **30**, CuI, DIPEA, anh. DMF, argon, reflux, 15 h; viii) 2-(piperidine-4-yl)ethan-1-ol, THF, 0 °C; ix) AcCl, AcONa, THF, rt; x) 2-(piperidine-4-yl)ethan-1-ol, THF, rt; xi) 1. DCM, DIPEA, MsCl, rt; 2. DMF, NaN_3, 100 °C; xii) bis(2-methoxy)ethylamine, THF, reflux, 5 h; xiii) *N,N*-diethylamine, THF, reflux, 6 h.



Scheme 2. Synthesis of compounds **25**-**30**. i) $(Boc)_2O$, DCM, rt, 4 h; ii) Ph_3P, I_2, pyridine, toluene, 80 °C, 3 h; iii) NaN_3, DMSO, rt, 60 h; iv) TFA, DCM, rt, 3 h; v) $Ba(OH)_2$, water, reflux, 4 h; vi) 2-chloroethanol, K_2CO_3, Ar, 55 °C, 10 h; vii) (trimethylsilyl)acetylene, $PdCl_2(PPh_3)_2$, CuI, TEA, dioxane, argon, 50-55 °C, 14 h; viii) K_2CO_3, MeOH, rt, 1 h.

The statistical condensation of a precursor A (**12**, **14**, **17**, **18** or **22**) and a precursor B (**23** or **24**) under Linstead conditions with lithium butoxide as the reaction initiator (Scheme 3) provided mixtures of six metal-free congeners (AAAA, ABBB, ABAB, etc.) from which the desired ABBB congener was separated by chromatographic methods. Advantageously, the hydroxy group of precursor **22** was quantitatively deprotected by attack of excess of alkoxide during cyclotetramerization and provided a free OH group in final TPyzPzs **3** and **7**.[18] The different lipophilicities of precursors A and B led to significant differences in the retention factors of particular congeners, thus enabling facile separation of the desired ABBB congener (see photo of a typical TLC plate with a mixture of congeners after statistical condensation, Fig. S1). However, the combination of precursors **22** and **23** gave rise to a mixture of congeners with almost identical retention factors in all tested mobile phases. Therefore, the mixture of metal-free congeners was in this case treated with 4,4′-dimethoxytrityl chloride to directly protect the hydrophilic OH groups, and the desired ABBB species (**3H-DMTr**) was isolated as the fifth spot on TLC. Deprotection of **3-DMTr** to afford target TPyzPz **3** was achieved after coordination of zinc (II) to the center of **3H-DMTr**, which was performed by treatment of the metal-free derivative with zinc(II) acetate in pyridine, similar to all other target zinc(II) TPyzPzs. Symmetrical TPyzPzs **9** and **10** were synthesized under Linstead conditions similar to those described for the low-symmetry TPyzPzs above starting from precursors **23** or **24**, respectively.



Scheme 3. Synthesis of target TPyzPzs. i) Li, BuOH, reflux, 30 min, 7-20 %; ii) chromatographic separation; iii) $Zn(OAc)_2$, pyridine, reflux, 3 h, 50-88 %; iv) 1. Li, BuOH, reflux, 30 min; 2. DMTrCl, DMAP, anh. pyridine, rt. 48h, 8 %; v) trichloroacetic acid, DCM, rt. 2 h, 88 %.

Solubility of target TPyzPzs

Good solubility of TPyzPz in organic solvents, and specifically polar organic solvents, is essential for efficient binding of the quencher to a polar ODN strand or for the attachment of TPyzPz to a solid phase. In any case, the reaction runs in a heterogeneous system (the ODN to be modified is typically attached to a solid phase[8, 12, 18, 19]), where a high concentration of the reactants increases the chance of successful labeling. Therefore, we used symmetrical TPyzPzs bearing either diethylamino (**10**, **10H**) or more polar bis(2-methoxyethyl)amino groups (**9**, **9H**) as model compounds and compared their solubilities in a series of organic solvents of different polarities (Fig. 2). Bis(2-methoxyethyl)amino substitution increased the solubility of the compound in all the studied solvents due to its polar and bulky character. The effect was more pronounced in hydrophilic solvents such as MeOH, DMF and DMSO, in which the solubility was 100-1000 times higher than the solubility of derivatives with diethylamino groups.

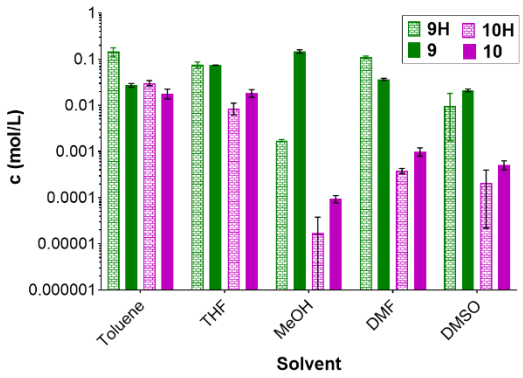


Figure 2. Comparison of solubility of model TPyzPzs **9H**, **9**, **10H** and **10** in organic solvents of different polarity.

J-Dimer formation

As strong absorption at suitable wavelengths is crucial for the efficient quenching of the emission of a fluorophore, and no intrinsic emission of the quencher is desirable, the spectral and photophysical properties of the prepared TPyzPzs were investigated (Fig. S2, S3 and Table 1). In coordinating solvents, all the prepared derivatives showed characteristic low-energy Q-bands at 660 nm and high-energy B-bands at 380 nm with extinction coefficients exceeding 150 000 L mol^{-1} cm^{-1} in the Q-band (an example shown in Fig. 3, red line). In a noncoordinating solvents (*e.g.* toluene), the spectra showed broad features of the Q-band with a new, bathochromically shifted band (an example shown in Fig. 3, blue line). As recently reported,[8] this is a typical spectral feature indicating the controlled self-assembly of TPyzPzs into J-dimers. In contrast to the most common H-dimers formed in TPyzPzs and phthalocyanines only through π-π stacking between planar molecules, J-dimers are formed by coordination of the peripheral amino substituent to the central cation, which may be further supported by π-π stacking between macrocycles. Despite the lower number of literature reports on J-dimers of phthalocyanines and their analogues in comparison to H-dimers, J-dimers have recently been shown to have important implications for ODN probes, in energy transfer systems and in material chemistry.[8, 23]

The stabilities of the J-dimers of TPyzPzs **1**-**10** were studied by titration of their toluene solutions with pyridine, as it is a Zn-coordinating solvent. As a result, the dissociation of the J-dimers into monomers occurred, which was easily monitored by the gradual changes in the absorption spectra, namely, the decrease in the J-dimer band (at 706 nm) with a concomitant increase in the band belonging to monomer (660 nm) (Fig. 3a). The observed isosbestic points provided clear evidence of the transition of one form (J-dimer) into another (monomer). The calculated K_P values for compounds bearing bulky bis(methoxyethyl)amino substituents ranged between 2.1-7.9 × 10^2 M^{-1} (see Table 1), indicating that the stabilities of the J-dimers decreased in the following order **1**~**3**~**2**>**4**. The presence of functional groups evidently increases the stability of J-dimers because symmetrical control compound **9** possessed a significantly higher K_P value (12.7 × 10^2 M^{-1}). If less sterically demanding *N,N*-diethylamino groups were employed instead of bis(2-methoxyethyl)amino substituents, the stability of the J-dimers was improved, which was evident from the K_P values of **6**-**8**, which ranged from 0.32-0.60 × 10^2 M^{-1}, *i.e.*, approximately an order of magnitude lower. Similar to the above-mentioned series, symmetrical control compound **10** showed higher K_P value (1.9×10^2 M^{-1}) indicating weaker binding of the symmetrical molecules in J-dimers. The apparent influence of the bulkiness of the peripheral substituents as well as the suggested notable role of the peripheral functional groups in the J-dimer assembly will be studied in a follow-up project.

Photophysics

Most zinc TPyzPzs are able to emit fluorescence and/or produce singlet oxygen, which is used, *e.g.*, in photodynamic therapy[24] and fluorescence sensing[25]. The introduction of a (di)alkylamine moiety on the periphery leads, however, to efficient quenching of excited states due to ultrafast intramolecular charge transfer (ICT) between the peripheral amine (*i.e.*, donor for the ICT) and the macrocyclic core (*i.e.*, acceptor for the ICT).[26] The attachment of four or more donors for ICT typically results in molecules with extremely low quantum yields of fluorescence (Φ_F) and singlet oxygen production (Φ_Δ), regardless of the surrounding environment. Such compounds are even able to quench the fluorescence of other compounds, thus serving as dark quenchers.[9] However, self-assembly into J-dimers may affect ICT and consequently also influence the photophysical properties of TPyzPzs.[27] Therefore, the effects of J-dimer formation of target TPyzPzs **1**-**10** were evaluated.

Despite extremely low fluorescence emission of **1**-**10**, the emission maxima of the J-dimers in toluene were detected and found significantly red-shifted up to 760 nm with Stokes shifts over 50 nm, which are atypically large for phthalocyanines. Upon monomerization (either by addition of 0.1 M pyridine or in DMF), spectra typical for monomeric species of phthalocyanines and related compounds were observed (Fig. 4). The shapes of the excitation fluorescence spectra of both the J-dimers (in toluene) and monomers (in toluene with 0.1 M pyridine or in DMF) resembled those of the corresponding absorption spectra confirming the origin of the emission.

ICT, as a quenching process, is highly efficient in polar solvents, while its efficacy decreases in nonpolar solvents (e.g., toluene). Therefore, the values of Φ_F and Φ_Δ were determined in several solvents to study the properties of the dimer (toluene) and monomer (either in polar solvent, *i.e.*, in DMF, or in a nonpolar solvent, *i.e.*, toluene with 0.1 M pyridine). As expected, both quantum yields were close to zero for the monomeric species in DMF (Φ_F < 0.0001, Φ_Δ < 0.006, see Table 1). However, the values for the monomer increased by approximately an order of magnitude in the nonpolar solvent (*i.e.*, toluene with 0.1 M pyridine), which may be simply explained by a decrease of thermodynamic feasibility of ICT in the nonpolar medium.[28] The most pronounced hindering of the ICT was achieved spatially after assembly into J-dimers, which was evident by the relatively high Φ_F and Φ_Δ values in toluene (Φ_F = 0.012-0.027; Φ_Δ = 0.029-0.28).

Table 1. Photophysical properties of studied TPyzPzs in different solvents[a].

Cpd.				Φ_Δ (tol)	Φ_F (tol)			Φ_Δ (tol+pyr)	Φ_F (tol+pyr)		Φ_Δ (DMF)	Φ_F (DMF)
	Toluene (J-dimers)					Toluene + 0.1 M pyridine (monomers)				DMF (monomers)		
	K_P (M^{-1})	λ, nm	λ_F, nm	Φ_Δ	Φ_F	λ, nm	λ_F, nm	Φ_Δ	Φ_F	λ, nm	Φ_Δ	Φ_F
1	2.1×10^2	709	766	0.029	0.0012	662	671	0.023	0.00031	660	0.0076	0.00003
2	3.1×10^2	708	765	0.063	0.0027	662	670	0.026	0.00040	660	0.0078	0.00004
3	2.2×10^2	711	763	0.12	0.0075	660	669	0.028	0.0011	659	0.0078	0.00005
4	7.9×10^2	708	762	0.13	0.0071	661	669	0.030	0.0011	660	0.0076	0.00005
5	0.55×10^2	700	758	0.055	0.0015	660	669	0.025	0.0010	659	0.0058	0.00006
6	0.32×10^2	704	755	0.12	0.0086	660	668	0.047	0.0014	659	0.0055	0.00008
7	0.48×10^2	705	752	0.24	0.022	659	667	0.056	0.0032	658	0.0051	0.00010
8	0.60×10^2	701	752	0.22	0.020	660	668	0.049	0.0033	660	0.0057	0.000004
9[8]	12.7×10^2	706	768	0.090	0.00035	661	665	0.029	0.0009	661	< 0.005	< 0.0001
10[8]	1.9×10^2	699	750	0.28	0.027	659	665	0.056	0.00043	661	< 0.005	< 0.0001

[a] absorption maximum at Q-band (λ), equilibrium constant involving monomerization and association with pyridine (KP), quantum yield of singlet oxygen production (Φ_Δ), fluorescence quantum yield (Φ_F). Literature values used for reference compound, unsubstituted ZnPc (Φ_F = 0.32 (THF)[20], Φ_Δ = 0.56 (DMF)[21], 0.58 (toluene)[22].

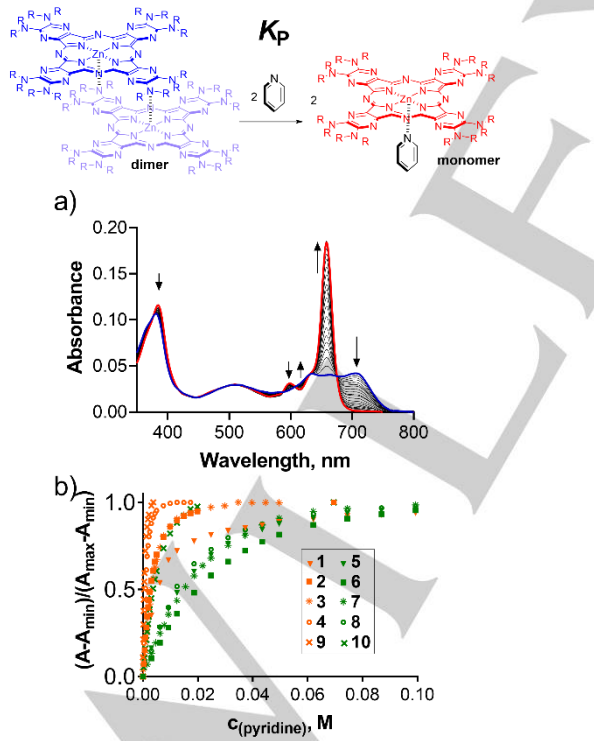


Figure 3: Study of J-dimer stability: a) absorption spectrum of TPyzPz **7** in toluene (1 μM, blue spectrum, dimer) and changes upon addition of pyridine. Red spectrum corresponds to monomer, blue spectrum to J-dimer; b) Comparison of monomerization profiles of target TPyzPzs **1**-**10** (analyzed at absorption maximum of a monomer).

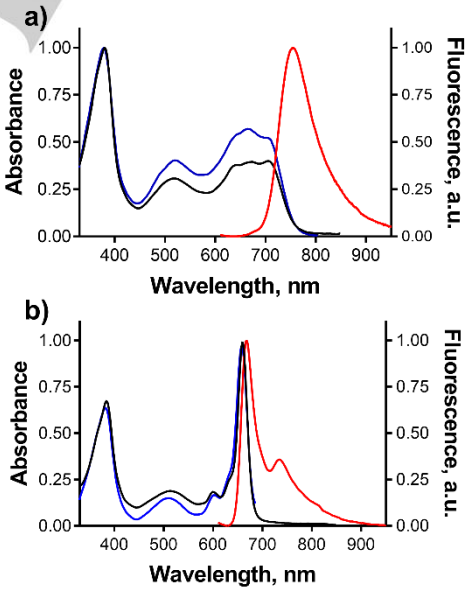


Figure 4: Normalized absorption (black), emission (red) and excitation (blue) spectra of TPyzPz **6** in toluene (dimer, graph a) and in toluene with 0.1M pyridine (monomer, graph b).

Notably, the low quantum yields determined for the monomers are highly desirable when using the TPyzPzs as dark quenchers in ODN probes because intrinsic fluorescence could interfere with the fluorescence of the fluorophore, and singlet oxygen could destroy the ODN probe and DNA template in course of the rt-PCR

experiment. In the case of dimers, the observed fluorescence and singlet oxygen production in organic solvents does not limit the application of these quenchers because the final ODN probes work in water. Water, as an extremely polar solvent, improves ICT, which results in efficient quenching of excited states and suppresses fluorescence and singlet oxygen production.

Conclusions

A series of low-symmetry dialkylamino TPyzPzs possessing different types of functional groups were synthesized. The panchromatic absorption of all the prepared TPyzPzs covering 300-700 nm with Φ_F and Φ_Δ values lower than 0.001 is a key prerequisite for use as universal dark quenchers in ODN probes. Azide, hydroxy, carboxy groups or their combinations were used as the target functional groups because these groups are typically employed in the binding of quenchers to ODN either to the 3´ end, the 5´end or the intrastrand position. Now, synthetic strategies for accessing suitably functionalized TPyzPzs are available, and the newly developed building blocks may facilitate the future synthesis of target TPyzPzs. Considering the other three quarters of the TPyzPz macrocycle, bis(2-methoxyethyl)amino substitution was found to be advantageous with respect to providing high solubility in all tested organic solvents tried, especially in polar solvents, which are typically used in ODN synthesis. On the other hand, this bulkier substituent disfavor self-assembly into *J*-dimers. The determined K_P values showed that the stability of the J-dimers increases substantially with sterically less-demanding substituents, as well as when going from symmetrical to unsymmetrical derivatives. ICT, the quenching process of the excited states of TPyzPzs, was sterically hindered in J-dimers, which resulted in the restoration of the fluorescence emission and production of singlet oxygen with quantum yields up to $\Phi_F = 0.027$ and $\Phi_\Delta = 0.28$. Because J-dimer formation has previously been shown to influence the quenching of fluorescence in ODN probes[8], studies on this extraordinarily interesting topic are of great interest, and the results of this project will be helpful for follow-up studies on this topic.

Experimental Section

General

All organic solvents were of analytical grade. Anhydrous butanol used for the cyclotetramerization was freshly distilled from magnesium prior to use. All other chemicals for the syntheses were purchased from certified suppliers (i.e., Sigma-Aldrich, TCI Europe, Acros, and Merck) and used as received. TLC was performed on Merck aluminum sheets coated with silica gel 60 F254. Merck Kieselgel 60 (0.040−0.063 mm) was used for column chromatography. The infrared spectra were measured using a Nicolet 6700 spectrometer in the ATR mode. The ^{1}H and ^{13}C NMR spectra were recorded on a Varian VNMR S500 NMR spectrometer. The chemical shifts are reported as δ values in ppm and are indirectly referenced to $Si(CH_3)_4$ via the signal from the solvent. J values are given in Hz. Elemental analysis was carried out using a Vario Micro Cube Elemental Analyzer (Elementar Analysensysteme GmbH, Hanau, Germany). The UV−Vis spectra were recorded using a Shimadzu UV-2600 spectrophotometer.. HRMS spectra were measured at UHPLC system Acquity UPLC I-class (Waters, Millford, USA) coupled to high resolution mass spectrometer (HRMS) Synapt G2Si (Waters, Manchester, UK) based on Q-TOF were used for HRMS spectra measurement. Chromatography was carried out using Acquity UPLC Protein BEH C4 (2.1 x 50mm, 1.7 µm, 300 Å) column using gradient elution with ACN and 0.1% formic acid at flow-rate 0.4 ml/min. Electrospray ionization was operated in positive ion mode. The ESI spectra were recorded in the range 50 - 5000 m/z using leucine-enkefaline as a lock mass reference and sodium iodide for external calibration or in the range 50 - 1200 m/z using leucine-enkefaline as a lock mass reference and sodium formate for external calibration. Following compounds were prepared according to published procedures: TPyzPzs **7H**,[8] **7**,[8] **9H**,[8] **9**,[8] **10H**,[29] **10**,[29] 4-((3-chloro-5,6-dicyanopyrazin-2-yl)(methyl)amino)butanoic acid (**13**)[12], 5-chloro-6-(diethylamino)pyrazine-2,3-dicarbonitrile (**15**)[30], 5-chloro-6-(4-(2-hydroxyethyl)piperidin-1-yl)pyrazine-2,3-dicarbonitrile (**19**)[18], 2-(1-(3-chloro-5,6-dicyanopyrazin-2-yl)piperidin-4-yl)ethyl acetate (**20**)[18], 2-(1-(5,6-dicyano-3-(4-(2-hydroxyethyl)piperidin-1-yl)pyrazin-2-yl)piperidin-4-yl)ethyl acetate (**21**)[18], 2-(1-(3-(4-(2-azidoethyl)piperidin-1-yl)-5,6-dicyanopyrazin-2-yl)piperidin-4-yl)ethyl acetate (**22**)[18], 5,6-bis(bis(2-methoxyethyl)amino)pyrazine-2,3-dicarbonitrile **(23)[8],** 5,6-bis(diethylamino)pyrazine-2,3-dicarbonitrile (**24**)[31], 4-(methylamino)butanoic acid (**29**)[16], *N*,*N*-di(2-hydroxyethyl)-4-ethynylaniline (**30**)[17].

General synthesis of metal-free TPyzPzs: Precursors A bearing functional groups (*i.e.*, **12**, **14**, **17**, **18**, **22**) and a precursor B (*i.e.*, **23** or **24**) were dissolved in anhydrous butanol in ratio 1:3 (A:B), heated to reflux and 28 equivalents of metal lithium was added. Reaction mixture was refluxed for 30 minutes. Butanol was evaporated under reduce pressure and a solid residue was dissolved in a mixture of dichloromethane/~5% HCl 1:1 (pH of aqueous phase should be bellow 5.0). Aqueous phase was washed three times with dichloromethane. Organic layer was collected and evaporated under reduce pressure with addition of toluene to remove traces of water. Desired congener of a metal-free TPyzPz was isolated and purified by column chromatography on silica (mobile phases are mentioned at each compound below. Finally, product was dissolved in minimal amount of dichloromethane (0.5 mL – 1mL), dropped into hexane (100 mL) and let precipitate in a freezer for 24 hours. Solid crystals were collected and dried under vacuum.

9,10,16,17,23,24-hexakis[bis(2-methoxyethyl)amino]-2,3-bis(3-carboxypropyl-methylamino)-1,4,8,11,15,18,22,25-octaazaphthalocyanine (1H): Prepared according to general procedure described above using precursors **12** (400 mg, 1.11 mmol), **23** (1307 mg, 3.33 mmol), lithium (233 mg, 28.57 mmol), eluent: dichloromethane/MeOH/acetic acid 50:3:2 (R_f=0.24). Yield: purple solid (200 mg, 12 %). ^{1}H NMR (500 MHz, Pyridine-d_5, 278 K): δ(ppm, *J*(Hz)): 4.41 (t, *J* = 5.4 Hz, 24H), 4.08 (t, *J* = 7.2 Hz, 4H), 3.77 – 3.71 (m, 24H), 3.37 (s, 6H), 3.22 (s, 36H), 2.65 (d, *J* = 6.6 Hz, 4H), 2.30 – 2.24 (m, 4H), -1.52 (s, 2H); ^{13}C NMR (126 MHz, Pyridine-d_5, 278 K): δ(ppm, *J*(Hz)): 152.7, 152.2, 142.6, 142.4, 72.4, 59.8, 51.8, 50.4, 38.7, 24.8, 2.71. HR MS (ESI): (*m/z*): 770.4179 $[M+2H]^{2+}$ (teor.: 770.4182). IR (ATR): $\bar{\nu}$= 3305 (central H), 2888, 2826, 1469, 1351, 1194, 1111 cm^{-1}. HR MS (ESI): (*m/z*): 1539.8289 $[M+H]^+$ (teor.: 1539.8291). UV/Vis (DMF): λ_{max} (ε)= 684 (64 900), 657 (59 500), 534 (64 100), 371 nm (98 200 $mol^{-1}dm^3cm^{-1}$).

9,10,16,17,23,24-hexakis[bis(2-methoxyethyl)amino]-3-[4-(2-azidoethyl)piperidin-1-yl]-2-(3-carboxypropyl-methylamino)-1,4,8,11,15,18,22,25-octaazaphthalocyane (2H): Prepared according to general procedure described above using precursors **14** (340 mg, 0.86 mmol), **23** (1007 mg, 2.57 mmol), lithium (177 mg, 25.29 mmol), eluent dichloromethane/MeOH 12:1 (R_f=0.41). Yield: purple solid (101 mg, 7 %); ^{1}H NMR (500 MHz, Pyridine-d_5, 278 K): δ(ppm, *J*(Hz)): 4.51 (d, *J* =

12.5 Hz, 2H), 4.46 – 4.36 (m, 24H), 4.24 (t, *J* = 7.2 Hz, 2H), 3.77-3.72 (m, 19H), 3.70 (t, *J* = 5.3 Hz, 5H), 3.51 (s, 3H), 3.30 (t, *J* = 7.1 Hz, 2H), 3.23 (d, *J* = 1.2 Hz, 24H), 3.22 (s, 6H), 3.22 (s, 6H), 3.03 (t, *J* = 11.8 Hz, 2H), 2.69 (t, *J* = 7.1 Hz, 2H), 2.31 (p, *J* = 7.1 Hz, 2H), 1.81 (d, *J* = 10.6 Hz, 2H), 1.58 – 1.46 (m, 5H), -1.49 (s, 2H); ^{13}C NMR (126 MHz, Pyridine-d_5, 278 K): δ(ppm, *J*(Hz)): 175.5, 151.5, 151.1, 151.04, 150.98, 150.96, 150.94, 150.8, 149.7, 141.6, 140.9, 135.5, 135.3, 123.5, 123.3, 71.2, 71.1, 71.11, 71.08, 58.5, 50.3, 49.2, 49.1, 49.0, 48.1, 37.7, 35.4, 33.8, 32.5, 32.1, 23.4; IR (ATR): $\tilde{\nu}$= 3305 (central H), 2929, 2096 (N_3), 1470, 1421, 1113 cm$^{-1;}$ HR MS (ESI): (*m/z*): 1576.8663 [M+H]$^+$ (teor.: 1576.8720). UV/Vis (DMF): λ_{max} (*ε*)= 685 (66 100), 656 (60 300), 534 (63 800), 370 nm (99 800 mol^{-1}dm^{3}cm^{-1}).

2-[4-(2-azidoethyl)piperidin-1-yl]-3-[4-(2-dimethoxytrityloxyethyl)-piperidin-1-yl]-9,10,16,17,23,24-hexakis[bis(2-methoxyethyl)amino]-1,4,8,11,15,18,22,25-octaazaphthalocyanine (3H-DMTr): Prepared according to general procedure described above using precursors **22** (1.0 g, 2.22 mmol), **23** (2.61 g, 6.66 mmol), lithium (460 mg, 66.6 mmol). However, metal-free congeners have very similar retention on silica. Therefore, mixture of congeners with R_f around 0.47 (dichloromethane/MeOH 12:1) were separated by column chromatography on silica. This mixture of congeners (1.67 g, around 1 mmol) and 4,4′-dimethoxytrityl chloride (4.31 g, 12.72 mmol) were dissolved in anhydrous pyridine and few crystals of DMAP were added. Reaction mixture was stirred for 48 hours at rt. After this modification, desired congener was isolated by column chromatography on silica with dichloromethane/MeOH 15:1 as a mobile phase (R_f=0.38). Yield: purple solid (320 mg, 8 %).^{1}H NMR (500 MHz, Pyridine-d_5, 278 K): δ(ppm, *J*(Hz)): 7.84 (d, 2H), 7.73 – 7.66 (m, 4H), 7.51 (t, *J* = 7.7 Hz, 2H), 7.37 (t, *J* = 7.3 Hz, 1H), 7.15 – 7.08 (m, 4H), 4.76 (dd, *J* = 22.7, 12.2 Hz, 4H), 4.42 (t, *J* = 5.5 Hz, 16H), 4.37 (d, *J* = 4.9 Hz, 8H), 3.77 (s, 6H), 3.75 (t, *J* = 5.3 Hz, 16H), 3.69 (q, *J* = 4.9 Hz, 8H), 3.47 – 3.41 (m, 2H), 3.35 (t, *J* = 7.1 Hz, 2H), 3.23 (d, *J* = 2.6 Hz, 24H),3.22 (s, 6H), 3.21 (s, 6H), 3.07 (t, *J* = 12.3 Hz, 4H), 1.98 – 1.78 (m, 8H), 1.59 – 1.43 (m, 6H), -1.49 (s, 2H); ^{13}C NMR (126 MHz, Pyridine-d_5, 278 K): δ(ppm, *J*(Hz)): 158.9, 151.2, 151.1, 150.9, 150.8, 150.6, 146.2, 137.1, 130.5, 128.6, 128.1, 127.0, 113.5, 86.3, 70.97, 70.94, 70.85, 70.56, 70.47, 61.3, 58.3, 58.3, 58.2, 55.1, 49.01, 48.89, 48.83, 48.79, 48.40, 48.38, 47.8, 47.5, 36.9, 35.2, 33.7, 33.5, 32.6, 32.1, 31.5; IR (ATR): $\tilde{\nu}$= 3307 (central H), 2922, 2096 (N_3), 1438, 1113 cm$^{-1;}$ HR MS (ESI): (*m/z*): 1891.0325 [M+H]$^+$ (teor.: 1891.0391). UV/Vis (DMF): λ_{max} (*ε*)= 684 (73 400), 655 (68 000), 532 (68 700), 372 nm (109 100 mol^{-1}dm^{3}cm^{-1}).

9,10,16,17,23,24-hexakis[bis(2-methoxyethyl)amino]-3-diethylamino-2-[4-(2-azidoethyl)piperidin-1-yl]-1,4,8,11,15,18,22,25-octaazaphthalocyanine (4H): Prepared according to general procedure described above using precursors **17** (500 mg, 1.41 mmol), **23** (1.67 g, 4.25 mmol), lithium (277 mg, 39.57 mmol),eluent: dichloromethane/MeOH 18:1 (R_f=0.30). Yield: purple solid (330 mg, 15 %). ^{1}H NMR (500 MHz, Pyridine-d_5, 278 K): δ(ppm, *J*(Hz)): 4.60 (d, *J* = 12.4 Hz, 2H), 4.45 – 4.33 (m, 24H), 4.06 (q, *J* = 7.2 Hz, 4H), 3.77 – 3.66 (m, 24H), 3.33 (td, *J* = 7.2, 2.4 Hz, 2H), 3.25 – 3.19 (m, 36H), 3.10 – 3.01 (m, 2H), 1.84 (d, *J* = 12.2 Hz, 2H), 1.65 – 1.56 (m, 1H), 1.56 – 1.44 (m, 4H), 1.29 (d, *J* = 7.2 Hz, 6H), -1.47 (s, 2H); ^{13}C NMR (126 MHz, Pyridine-d_5, 278 K): δ(ppm, *J*(Hz)): 151.5, 151.03, 150.97, 150.9, 150.8, 150.5, 140.8, 71.2, 71.13, 71.09, 71.05, 58.5, 49.21, 49.15, 49.08, 48.99, 48.0, 42.6, 35.4, 33.9, 32.2, 13.2; HR MS (ESI): (*m/z*): 1532.8800 [M+H]$^+$ (teor.: 1532.8822). UV/Vis (DMF): λ_{max} (*ε*)= 684 (74 300), 657 (67 500), 537 (71 000), 370 nm (108 000 mol^{-1}dm^{3}cm^{-1}).

9,10,16,17,23,24-hexakis(diethylamino)-2,3-bis(3-carboxypropyl-methylamino)-1,4,8,11,15,18,22,25-octaazaphthalocyane (5H): Prepared according to general procedure described above using precursors **12** (360 mg, 1.00 mmol), **24** (816 mg, 3.00 mmol), lithium (210 mg, 30.00mmol), eluent: first – dichloromethane/MeOH 30:1, second - ethyl acetate/acetic acid 15:1 (R_f= 0.33). Yield: purple solid (167 mg, 14 %). ^{1}H NMR (500 MHz, Pyridine-d_5, 278 K): δ(ppm, *J*(Hz)): 4.12 (s, 4H), 4.07 – 3.92 (m, 24H), 3.42 (s, 6H), 2.68 (t, *J* = 8.9 Hz, 4H), 2.39 – 2.22 (m, 4H), 1.35 – 1.23 (m, 36H), -1.38 (s, 2H); ^{13}C NMR (126 MHz, Pyridine-d_5, 278 K): δ(ppm, *J*(Hz)): 151.6, 150.9, 141.1, 140.3, 50.8, 43.1, 37.4, 33.9, 23.7, 13.0; IR (ATR): $\tilde{\nu}$= 3305 (central H), 2969, 2932, 2872, 1423, 1286, 1159 cm^{-1}; HR MS (ESI): (*m/z*): 1179.7026 [M+H]$^+$ (teor.: 1179.7024). UV/Vis (DMF): λ_{max} (*ε*)= 684 (69 100), 658 (61 900), 531 (64 200), 369 nm (101 800 mol^{-1}dm^{3}cm^{-1}).

9,10,16,17,23,24-hexakis(diethylamino)-3-[4-(2-azidoethyl)piperidin-1-yl]-2-(3-carboxypropyl-methylamino)-1,4,8,11,15,18,22,25-octaazaphthalocyane (6H): Prepared according to general procedure described above using precursors **14** (700 mg, 1.76 mmol), **24** (1434 mg, 5.27 mmol), lithium (255 mg, 36.43 mmol), eluent: dichloromethane/MeOH/acetone 30:1:1 (R_f=0.39). Yield: 225 mg (12 %) of purple solid. ^{1}H NMR (500 MHz, Pyridine-d_5, 278 K): δ(ppm, *J*(Hz)): 4.46 (d, *J* = 12.5 Hz, 2H), 4.21 (t, *J* = 7.0 Hz, 2H), 3.96 – 3.85 (m, 24H), 3.46 (s, 3H), 3.28 (t, *J* = 7.1 Hz, 2H), 2.98 (t, *J* = 11.7 Hz, 2H), 2.69 (t, *J* = 7.3 Hz, 2H), 2.30 (p, *J* = 7.2 Hz, 2H), 1.78 (d, *J* = 11.2 Hz, 2H), 1.55 – 1.45 (m, 5H), 1.25 – 1.15 (m, 36H), -1.24 (s, 2H); ^{13}C NMR (126 MHz, Pyridine-d_5, 278 K): δ(ppm, *J*(Hz)): 175.5, 151.5, 151.1, 150.8, 150.7, 141.4, 141.2, 50.4, 49.2, 48.1, 43.1, 43.0, 42.9, 37.6, 35.4, 33.8, 32.6, 32.1, 23.4, 13.2, 13.14, 13.11.; HR MS (ESI): (*m/z*): 1216.7462 [M+H]$^+$ (teor.: 1216.7452). UV/Vis (DMF): λ_{max} (*ε*)= 684 (66 700), 658 (60 300), 531 (59 700), 371 nm (99 100 mol^{-1}dm^{3}cm^{-1}).

3,9,10,16,17,23,24-heptakis(diethylamino)-2-(4-(2-(4-(4-(bis(2-hydroxyethyl)amino)phenyl)-1H-1,2,3-triazol-1-yl)ethyl)piperidin-1-yl)-1,4,8,11,15,18,22,25-octaazaphthalocyane (8H): Prepared according to general procedure described above using precursors **18** (99 mg, 0.18 mmol), **24** (149 mg, 0.55 mmol), lithium (37 mg, 5.3 mmol), eluent: dichloromethane/MeOH/acetone 15:1:1 (R_f=0.40). Yield: 36 mg (20 %) of purple solid. ^{1}H NMR (500 MHz, Pyridine-d_5, 278 K): δ(ppm, *J*(Hz)): 8.39 (s, 1H), 8.14 (d, *J* = 8.4 Hz, 2H), 7.03 (d, *J* = 9.3 Hz, 2H), 4.57 – 4.46 (m, 4H), 4.07 (t, *J* = 5.8 Hz, 4H), 4.01 (q, *J* = 7.1 Hz, 4H), 3.90 (p, *J* = 6.6 Hz, 24H), 3.81 (t, *J* = 5.9 Hz, 4H), 2.94 (t, *J* = 11.8 Hz, 2H), 1.87 (d, *J* = 12.9 Hz, 4H), 1.61 – 1.41 (m, 4H), 1.27 (t, *J* = 7.0 Hz, 9H), 1.24 – 1.18 (m, 28H), 1.18 – 1.14 (m, 6H), -1.22 (s, 2H); 13C NMR (126 MHz, Pyridine-d_5, 278 K): δ(ppm, *J*(Hz)): 151.46, 150.82, 150.80, 150.73, 150.71, 150.46, 149.68, 148.76, 148.71, 135.5, 135.3, 127.3, 123.8, 123.5, 123.3, 119.5, 119.2, 112.6, 59.8, 55.0, 48.0, 47.9, 43.0, 42.9, 42.7, 37.1, 33.6, 32.0, 13.2, 13.1; IR (ATR): $\tilde{\nu}$= 3305 (central H), 2969, 2931, 2872, 1423, 1285 cm^{-1}; HR MS (ESI): (*m/z*): 1377.8655 [M+H]$^+$ (teor.: 1377.8657). UV/Vis (DMF): λ_{max} (*ε*)= 684 (66 900), 658 (60 700), 536 (59 900), 372 nm (100 400 mol^{-1}dm^{3}cm^{-1}).

2,3,9,10,16,17,23,24-octakis[bis(2-methoxyethyl)amino]-1,4,8,11,15,18,22,25-octaazaphthalocyanine (9H): Synthesis and characterization has been published before.[8] UV/Vis (DMF): λ_{max} (*ε*)= 686 (74 400), 659 (66 300), 535 (72 300), 372 nm (107 700 mol^{-1}dm^{3}cm^{-1}).

General synthesis of zinc(II) TPyzPzs: Metal-free TPyzPz was dissolved in pyridine in a round-bottomed flask and 6 equivalents of zinc (II) acetate was added. Reaction was refluxed for 3 hours. Then, pyridine was evaporated and zinc(II) TPyzPz was purified by column chromatography on silica (mobile phases ae mentioned at each compound below).

9,10,16,17,23,24-hexakis[bis(2-methoxyethyl)amino]-2,3-bis(3-carboxypropyl-methylamino)-1,4,8,11,15,18,22,25-octaazaphthalocyaninato zinc(II) (1): Prepared according to general method described above using TPyzPz **1H** (190 mg, 0.12 mmol), zinc (II) acetate (135 mg, 0.74 mmol); eluent: dichloromethane/MeOH/acetic acid 13:1:1 (R_f=0.27). Yield: dark blue crystals (105 mg, 53 %). ^{1}H NMR (500

MHz, Pyridine-d_5, 278 K): δ(ppm, *J*(Hz)): 4.35 (s, 28H), 3.69 (s, 24H), 3.18 (s, 42H), 2.66 (br s, 4H), 2.11 (br s, 4H). ^{13}C NMR (126 MHz, Pyridine-d_5, 278 K): δ(ppm, *J*(Hz)): 151.7, 142.7, 71.2, 71.0, 58.4, 51.3, 49.1, 37.5, 36.0, 24.3; IR (ATR): $\tilde{\nu}$= 2979, 2933, 1560, 1425, 1113 cm^{-1}; HR MS (ESI): (*m/z*): 1601.7394 $[M+H]^+$ (teor.: 1601.7426). Q39-1Zn UV/Vis (DMF): λ_{max} (*ε*)= 659 (171030), 600 (33870), 523 (35110), 383 nm (121350 mol^{-1}dm^{3}cm^{-1}).

9,10,16,17,23,24-hexakis[bis(2-methoxyethyl)amino]-3-[4-(2-azidoethyl)piperidin-1-yl]-2-(3-carboxypropyl-methylamino)-1,4,8,11,15,18,22,25-octaazaphthalocyaninato zinc(II) (2): Prepared according to general method described above using TPyzPz **2H** (90 mg, 0.06 mmol), zinc (II) acetate (63 mg, 0.34 mmol); eluent: dichloromethane/MeOH 14:1 (R_f=0.45). Yield: dark blue solid (63 mg, 67 %). ^{1}H NMR (500 MHz, Pyridine-d_5, 278 K): δ(ppm, *J*(Hz)): 4.49 (d, *J* = 12.3 Hz, 2H), 4.45 – 4.33 (m, 24H), 4.22 (t, *J* = 7.2 Hz, 2H), 3.77 – 3.70 (m, 20H), 3.69 (t, *J* = 5.4 Hz, 4H), 3.48 (s, 3H), 3.29 (t, *J* = 7.0 Hz, 2H), 3.22 (s, 24H), 3.21 (s, 6H), 3.20 (s, 6H), 3.01 (t, *J* = 11.5 Hz, 2H), 2.68 (t, *J* = 7.1 Hz, 2H), 2.29 (p, *J* = 7.1 Hz, 2H), 1.79 (d, *J* = 10.1 Hz, 2H), 1.57 – 1.45 (m, 5H); ^{13}C NMR (126 MHz, Pyridine-d_5, 278 K): δ(ppm, *J*(Hz)): 175.5, 151.5, 151.4, 151.3, 151.22, 151.16, 151.02, 150.93, 143.8, 142.89, 142.86, 142.8, 142.3, 71.22, 71.20, 71.18, 71.14, 58.5, 58.4, 50.3, 49.2, 49.1, 49.0, 48.1, 37.7, 35.4, 33.8, 32.6, 32.1, 23.4.; IR (ATR): $\tilde{\nu}$= 2927, 2096 (N_3), 1463, 1418, 1112 cm^{-1}; HR MS (ESI): (*m/z*): 1638.7856$[M+H]^+$ (teor.: 1638.7855). UV/Vis (DMF): λ_{max} (*ε*)= 660 (182780), 601 (36320), 522 (34720), 383 nm (125810 mol^{-1}dm^{3}cm^{-1}).

2-[4-(2-azidoethyl)piperidin-1-yl]-3-[4-(2-dimethoxytrityloxyethyl)-piperidin-1-yl]-9,10,16,17,23,24-hexakis[bis(2-methoxyethyl)amino]-1,4,8,11,15,18,22,25-octaazaphthalocyaninato zinc(II) (3-DMTr): Prepared according to general method described above using TPyzPz **3H-DMTr** (290 mg, 0.15 mmol), zinc (II) acetate (169 mg, 0.92 mmol), eluent: dichloromethane/MeOH 22:1 (R_f=0.37). Yield: dark blue crystals (150 mg, 50 %). ^{1}H NMR (500 MHz, Pyridine-d_5, 278 K): δ(ppm, *J*(Hz)): 7.86 – 7.81 (m, 2H), 7.71 – 7.67 (m, 4H), 7.52 (t, *J* = 7.8 Hz, 2H), 7.40 – 7.35 (m, 1H), 7.14 – 7.09 (m, 4H), 4.75 (dd, *J* = 23.6, 12.3 Hz, 4H), 4.42 (t, *J* = 5.4 Hz, 16H), 4.38 (t, *J* = 5.6 Hz, 8H), 3.78 (s, 6H), 3.77 – 3.72 (m, 16H), 3.70 (t, *J* = 5.2, 3.5 Hz, 8H), 3.44 (t, *J* = 6.4 Hz, 2H), 3.37 – 3.32 (m, 2H), 3.23 (s, 24H), 3.21 (s, 6H), 3.20 (s, 6H), 3.14 – 3.03 (m, 4H), 1.97 – 1.78 (m, 8H), 1.58 – 1.42 (m, 6H); ^{13}C NMR (126 MHz, Pyridine-d_5, 278 K): δ(ppm, *J*(Hz)): 159.12, 151.52, 151.43, 151.29, 151.27, 151.02, 150.88, 150.85, 146.4, 143.12, 143.09, 142.9, 142.83, 142.79, 137.3, 130.7, 128.8, 128.3, 127.2, 113.7, 86.4, 71.22, 71.19, 71.1, 61.5, 58.5, 58.4, 55.3, 49.21, 49.16, 49.1, 49.0, 48.0, 47.7, 37.1, 35.4, 33.9, 33.7, 32.8, 32.3; IR (ATR): $\tilde{\nu}$= 2924, 2096 (N_3), 1509, 1418, 1112 cm^{-1}; HR MS (ESI): (*m/z*): 1952.9515 $[M+H]^+$ (teor.: 1952.9526). UV/Vis (DMF): λ_{max} (*ε*)= 659 (199740), 602 (38700), 522 (36520), 382 nm (137670 mol^{-1}dm^{3}cm^{-1}).

2-[4-(2-azidoethyl)piperidin-1-yl]-3-[4-(2-hydroxyethyl)-piperidin-1-yl]-9,10,16,17,23,24-hexakis[bis(2-methoxyethyl)amino]-1,4,8,11,15,18,22,25-octaazaphthalocyaninato zinc(II) (3): TPyzPz **3-DMTr** (50 mg, 0.03 mmol) was dissolved in dichloromethane and trichloroacetic acid (8 mg, 0.05 mmol) was added. Reaction mixture was stirred for 2 hours at rt. Then, it was diluted with dichloromethane and washed by 5% $NaHCO_3$ (3×20 mL). Organic phase was dried with anhydrous Na_2SO_4, filtered and evaporated to dryness. Final compound was purified by column chromatography on silica with dichloromethane/MeOH 15:1 as a mobile phase (R_f=0.31). Yield: dark blue crystals (37 mg, 88 %); ^{1}H NMR (500 MHz, Pyridine-d_5, 278 K): δ(ppm, *J*(Hz)): 4.78 (br s, 4H), 4.41 (s, 16H), 4.36 (t, *J = 5.1 Hz*, 8H), 4.04 (t, *J = 6.6 Hz*, 2H), 3.74 (q, *J = 5.1 Hz*, 16H), 3.70 – 3.66 (m, 8H), 3.34 (p, *J = 7.1 Hz*, 2H), 3.23 (s, 24H), 3.21 (s, 6H), 3.20 (s, 6H), 3.15-3.03 (m, 4H), 2.07 (d, *J* = 11.4 Hz, 2H), 2.06-1.79 (m, 6H), 1.67-1.44 (m, 6H). ^{13}C NMR (126 MHz, Pyridine-d_5, 278 K): δ(ppm, *J*(Hz)): 151.5, 151.4, 151.3, 150.9, 149.7, 143.2, 143.1, 142.9, 142.8, 135.5, 135.3, 123.5, 123.3, 71.2, 71.1, 59.7, 58.5, 49.2, 49.1, 49.0, 48.1, 47.8, 40.5, 35.4, 33.9, 33.5, 33.0, 32.3; IR (ATR): $\tilde{\nu}$= 2926, 2095 (N_3), 1420, 1112 cm^{-1}; HR MS (ESI): (*m/z*): 1650.8181 $[M+H]^+$ (teor.: 1650.8219), 826.4205 $[M+2H]^{2+}$ (teor.: 826.4183); UV/Vis (DMF): λ_{max} (*ε*)= 659 (180160), 601 (35060), 514 (33380), 382 nm (123140 mol^{-1}dm^{3}cm^{-1}).

9,10,16,17,23,24-hexakis[bis(2-methoxyethyl)amino]-3-diethylamino-2-[4-(2-azidoethyl)piperidin-1-yl]-1,4,8,11,15,18,22,25-octaazaphthalocyaninato zinc(II) (4): Prepared according to general method described above using TPyzPz **4H** (285 mg, 0.19 mmol), zinc (II) acetate (205 mg, 1.12 mmol); eluent: dichloromethane/MeOH 20:1 (R_f=0.42). Yield: dark blue solid (213 mg, 72 %). ^{1}H NMR (500 MHz, Pyridine-d_5, 278 K): δ(ppm, *J*(Hz)): 4.58 (d, *J* = 12.4 Hz, 2H), 4.43 – 4.32 (m, 24H), 4.04 (q, *J* = 7.1 Hz, 4H), 3.76 – 3.70 (m, 20H), 3.67 (t, *J* = 5.4 Hz, 4H), 3.33 (t, *J* = 7.0 Hz, 2H), 3.22 (s, 30H), 3.20 (s, 6H), 3.03 (t, *J* = 11.9 Hz, 2H), 1.82 (d, *J* = 12.1 Hz, 2H), 1.63 – 1.56 (m, 1H), 1.56 – 1.45 (m, 4H), 1.27 (t, *J* = 7.0 Hz, 6H); ^{13}C NMR (126 MHz, Pyridine-d_5, 278 K): δ(ppm, *J*(Hz)): 151.47, 151.44, 151.40, 151.32, 151.27, 151.14, 151.05, 150.95, 143.60, 142.90, 142.87, 142.85, 142.82, 142.56, 71.23, 71.20, 71.17, 71.13, 58.47, 58.44, 49.21, 49.16, 49.11, 48.99, 48.04, 42.55, 35.40, 33.89, 32.19, 13.17; IR (ATR): $\tilde{\nu}$= 2927, 2096 (N_3), 1460, 1420, 1112 cm^{-1}; HR MS (ESI): (*m/z*): 1594.7903 $[M+H]^+$ (teor.: 1594.7957). Q41-1Zn UV/Vis (DMF): λ_{max} (ε)= 659 (199740), 602 (38700), 522 (36520), 382 nm (137670 mol^{-1}dm^{3}cm^{-1}).

9,10,16,17,23,24-hexakis(diethylamino)-2,3-bis(3-carboxypropyl-methylamino)-1,4,8,11,15,18,22,25-octaazaphthalocyaninato zinc(II) (5): Prepared according to general method described above using TPyzPz **5H** (170 mg, 0.14 mmol), zinc (II) acetate (154 mg, 0.84 mmol); eluent: dichloromethane/MeOH/acetic acid 100:5:2 (R_f=0.39). Yield: 135 mg (75 %) of dark blue solid. ^{1}H NMR (500 MHz, Pyridine-d_5, 278 K): δ(ppm, *J*(Hz)): 4.03 (t, *J* = 7.2 Hz, 4H), 3.94 – 3.84 (m, 24H), 3.31 (s, 6H), 2.66 (t, *J* = 7.3 Hz, 4H), 2.25 (q, *J* = 7.4 Hz, 4H), 1.23 – 1.14 (m, 36H); ^{13}C NMR (126 MHz, Pyridine-d_5, 278 K): δ(ppm, *J*(Hz)): 175.7, 151.5, 151.4, 150.8, 143.0, 142.9, 50.7, 43.0, 37.4, 32.7, 23.4, 13.2, 13.1; IR (ATR): $\tilde{\nu}$= 2967, 1423, 1254, 1160 cm^{-1}; HR MS (ESI): (*m/z*): 1241.6151 $[M+H]^+$ (teor.: 1241.6159). UV/Vis (DMF): λ_{max} (*ε*)= 659 (191640), 599 (38940), 514 (37490), 382 nm (137970 mol^{-1}dm^{3}cm^{-1}).

9,10,16,17,23,24-hexakis(diethylamino)-3-[4-(2-azidoethyl)piperidin-1-yl]-2-(3-carboxypropyl-methylamino)-1,4,8,11,15,18,22,25-octaazaphthalocyaninato zinc(II) (6): Prepared according to general method described above using TPyzPz **6H** (160 mg, 0.13 mol), zinc (II) acetate (145 mg, 0.79 mmol); eluent: dichloromethane/MeOH 18:1 (R_f=0.46). Yield: dark blue solid (126 mg, 75 %). ^{1}H NMR (500 MHz, Pyridine-d_5, 278 K): δ(ppm, *J*(Hz)): 4.45 (d, *J* = 12.4 Hz, 2H), 4.19 (t, *J* = 7.3 Hz, 2H), 3.97 – 3.84 (m, 24H), 3.45 (s, 3H), 3.29 (t, *J* = 7.0 Hz, 2H), 2.99 (t, *J* = 12.0 Hz, 2H), 2.70 (t, *J* = 7.1 Hz, 2H), 2.30 (p, *J* = 7.3 Hz, 2H), 1.77 (d, *J* = 10.7 Hz, 2H), 1.54 – 1.46 (m, 5H), 1.25 – 1.12 (m, 36H); ^{13}C NMR (126 MHz, Pyridine-d_5, 278 K): δ(ppm, *J*(Hz)): 175.6, 151.61, 151.56, 151.52, 151.49, 151.41, 151.37, 151.16, 150.99, 143.8, 143.1, 143.00, 142.96, 142.4, 50.5, 49.2, 48.1, 42.99, 42.97, 42.77, 37.6, 35.4, 33.8, 32.7, 32.1, 23.4, 13.2, 13.1, 13.1; IR (ATR): $\tilde{\nu}$= 2968, 2931, 2872, 2095 (N_3), 1423, 1255, 1160 cm^{-1}; HR MS (ESI): (*m/z*): 1278.6575 $[M+H]^+$ (teor.: 1278.6587). UV/Vis (DMF): λ_{max} (*ε*)= 659 (171030), 600 (33870), 523 (35110), 383 nm (121350 mol^{-1}dm^{3}cm^{-1}).

3,9,10,16,17,23,24-heptakis(diethylamino)-2-(4-(2-(4-(4-(bis(2-hydroxyethyl)amino)phenyl)-1H-1,2,3-triazol-1-yl)ethyl)piperidin-1-yl)-1,4,8,11,15,18,22,25-octaazaphthalocyaninato zinc (II) (8): Prepared according to general method described above using TPyzPz **8H** (25 mg, 0.02 mmol), zinc (II) acetate (20 mg, 0.11 mmol); eluent dichloromethane/MeOH 13:1 (R_f=0.30). Yield: 18 mg (70%) of dark blue

solid. ^{1}H NMR (500 MHz, Pyridine-d_5, 278 K): δ(ppm, *J*(Hz)): 8.40 (s, 1H), 8.16 (d, *J* = 9.2 Hz, 2H), 7.04 (d, *J* = 8.9 Hz, 2H), 4.56 – 4.47 (m, 4H), 4.12 – 4.06 (m, 4H), 4.01 (q, *J* = 7.0 Hz, 4H), 3.90 (p, *J* = 6.9 Hz, 24H), 3.82 (t, *J* = 5.9 Hz, 4H), 2.95 (d, *J* = 11.3 Hz, 2H), 1.91 – 1.82 (m, 4H), 1.53 – 1.43 (m, 4H), 1.25 (t, *J* = 7.1 Hz, 9H), 1.23 – 1.17 (m, 28H), 1.15 (t, *J* = 7.1 Hz, 6H); ^{13}C NMR (126 MHz, pyridine) δ 150.9, 142.3, 126.6, 118.5, 111.9, 59.1, 54.3, 47.3, 47.2, 42.3, 42.1, 41.9, 36.4, 31.3, 12.5, 12.4; IR (ATR): $\bar{\nu}$= 2968, 2931, 2871, 1423, 1254, 1160 cm^{-1}; HR MS (ESI): (*m/z*): 1439.7747 [M+H]$^+$ (teor.: 1439.7792). UV/Vis (DMF): λ_{max} (*ε*)= 660 (169490), 601 (32770), 511 (31440), 382 nm (119280 mol^{-1}dm^{3}cm^{-1}).

2,3,9,10,16,17,23,24-octakis[bis(2-methoxyethyl)amino]-1,4,8,11,15,18,22,25-octaazaphthalocyaninato zinc (II) (9): Synthesis and characterization has been published before.[8] UV/Vis (DMF): λ_{max} (*ε*)= 660 (200550), 600 (38150), 516 (40490), 382 nm (132230 mol^{-1}dm^{3}cm^{-1}).

4,4'-((5,6-dicyanopyrazine-2,3-diyl)bis(methylazanediyl))dibutyric acid (12): 5,6-dichloropyrazine-2,3-dicarbonitrile (2.63 g, 13.22 mmol) and compound **29** (4.62 g, 39.49 mmol) were dissolved in *N*-methl-2-pyrrolidone (30 mL) and TEA (11 mL, 78.86 mmol) was added. Reaction mixture was stirred at rt for 48 hours. Solvent was evaporated, dry residue was dissolved in ethyl acetate and washed by water (3×80 mL). Organic layer was dried by anhydrous Na_2SO_4, filtered, and evaporated to dryness. Product was purified by column chromatography on silica with hexane/ethyl acetate/acetic acid 5:5:1 as an eluent (R_f=0.29). Yield: yellow oil (1.14 g, 24 %). ^{1}H NMR (500 MHz, DMSO-d_6, 278 K): δ(ppm, *J*(Hz)): 3.45 (t, *J* = 7.1 Hz, 4H), 2.94 (s, 6H), 2.08 (t, *J* = 7.1 Hz, 4H), 1.70 (p, *J* = 7.3 Hz, 4H); ^{13}C NMR (126 MHz, DMSO-d_6, 278 K): δ(ppm, *J*(Hz)): 174.2, 146.9, 119.1, 115.7, 49.5, 36.6, 31.4, 22.1; HR MS (ESI): (*m/z*): 361.1627 [M+H]$^+$ (teor.: 361.1619).

4-((3-(4-(2-azidoethyl)piperidin-1-yl)-5,6-dicyanopyrazin-2-yl)(methyl)amino)butanoic acid (**14**): Compounds **13** (828 mg, 2.9 mmol) and **29** (1596 mg, 10.36 mmol) were dissolved in THF (25 mL) and refluxed for 24 hours. The reaction was checked by TLC with hexane/ethyl acetate/acidic acid 8:3:1 as an eluent. R_f of product (0.43) is very close to R_f of **13**, but color under UV 366 nm is completely different (orange-yellow and bright yellow for **13** and **14**, respectively). Solvent was evaporated, dry residue was dissolved in ethyl acetate and washed with 1% solution of hydrochloric acid (3×50 mL). Organic phase was collected and dried by anhydrous $NaSO_4$. Final product was purified by column chromatography on silica with hexane/ethyl acetate/acidic acid 8:3:1 as an eluent. Yield: yellow oil (840 mg, 73 %). ^{1}H NMR (500 MHz, DMSO-d_6, 278 K): δ(ppm, *J*(Hz)): 3.90 (d, *J* = 13.6 Hz, 2H), 3.55 (s, 2H), 3.38 (t, *J* = 7.0 Hz, 2H), 3.01 (s, 3H), 2.78 (t, *J* = 12.4 Hz, 2H), 2.07 (t, *J* = 7.2 Hz, 2H), 1.75 (dd, *J* = 13.7, 3.4 Hz, 2H), 1.69 (p, *J* = 7.1 Hz, 2H), 1.64 – 1.53 (m, 1H), 1.49 (q, *J* = 7.0 Hz, 2H), 1.23 (qd, *J* = 12.5, 3.9 Hz, 2H); ^{13}C NMR (126 MHz, DMSO-d_6, 278 K): δ(ppm, *J*(Hz)): 175.1, 149.8, 146.4, 121.6, 118.5, 115.5, 51.1, 48.3, 46.3, 36.6, 34.9, 32.8, 31.8, 30.9, 21.8; IR (ATR): $\bar{\nu}$= 2930, 2226 (CN), 2096 (N_3), 1706, 1519, 1492, 1447, 1408, 1373, 1258 cm^{-1}; HR MS (ESI): (*m/z*): 398.2049 [M+H]$^+$ (teor.: 398.2047).

5-(diethylamino)-6-(4-(2-hydroxyethyl)piperidin-1-yl)pyrazine-2,3-dicarbonitrile (16): Compound **15** (2.78 g, 10.82 mmol) and 2-(piperidin-4-yl)ethan-1-ol (4.00 g, 30.96 mmol) were dissolved in THF (50 mL) in a round-bottomed flask. Reaction mixture was stirred at rt for 90 minutes. It was followed by evaporation of solvent. Dry residue was dissolved in ethyl acetate and washed by 1% solution of hydrochloric acid (3×50 mL). Organic layer was dried by anhydrous Na_2SO_4, filtered, and evaporated to dryness. Product was purified by column chromatography on silica with hexane/ethyl acetate – 1:2 as an eluent (R_f = 0.23). Yield: yellow oil (3.57 g, 95 %). ^{1}H NMR (500 MHz, $CDCl_3$, 278 K): δ(ppm, *J*(Hz)): 4.04 (d, *J* = 13.3 Hz, 2H), 3.73 (d, *J* = 6.1 Hz, 2H), 3.56 (q, *J* = 7.0 Hz, 4H), 2.76 (dt, *J* = 12.9, 2.5 Hz, 2H), 1.84 (d, *J* = 12.3 Hz, 2H), 1.78 – 1.68 (m, 1H), 1.55 (q, *J* = 6.6 Hz, 2H), 1.33 – 1.20 (m, 3H), 1.11 (t, *J* = 7.1 Hz, 6H); ^{13}C NMR (126 MHz, $CDCl_3$, 278 K): δ(ppm, *J*(Hz)): 147.6, 146.0, 122.1, 119.8, 114.9, 115.0, 58.7, 48.8, 43.8, 39.5, 33.4, 31.8, 30.9; IR (ATR): $\bar{\nu}$= 2931, 2227 (CN), 1712, 1518, 1487, 1435, 1373, 1270 cm^{-1}; HR MS (ESI): (*m/z*): 329.2091 [M+H]$^+$ (teor.: 329.2084).

5-(4-(2-azidoethyl)piperidin-1-yl)-6-(diethylamino)pyrazine-2,3-dicarbonitrile (17): Compound **16** (3.5 g, 10.87 mmol) was dissolved in anhydrous dichloromethane (80 mL) in a round-bottomed flask. Then, triethylamine (3.18 mL, 22.80 mmol) and methanesulfonyl chloride (1.01 mL, 13.04 mmol) were added. The reaction mixture was stirred at rt for 1 h. After that, reaction was washed three times with brine (3×60mL). The organic phase was dried by anhydrous Na_2SO_4, filtered and evaporated to dryness. The dry residue was dissolved in anhydrous DMF (25 mL), then sodium azide (3.53 g, 54.30 mmol) was added and the reaction mixture was stirred for 90 min at 60°C. The solvent was evaporated under reduced pressure and the product was dissolved in chloroform and wash with water (3×50mL). The organic layer was dried with Na_2SO_4, filtered and evaporated under reduce pressure. Crude product was purified by column chromatography on silica with hexane/ethyl acetate 5:2 as an eluent (R_f=0.41). Yield: viscous yellow oil (3.3 g, 86 %). ^{1}H NMR (300 MHz, $CDCl_3$, 278 K): δ(ppm, *J*(Hz)): 4.05 (d, *J* = 13.3,Hz, 2H), 3.56 (q, *J* = 7.1 Hz, 4H), 3.35 (t, *J* = 6.8 Hz, 2H), 2.75(dt, 2H), 1.90 – 1.75 (m, 2H), 1.73-1.62 (m, 1H), 1.57 (q, *J* = 6.5 Hz, 2H), 1.26 (qd, *J* = 12.8, 3.9 Hz, 2H), 1.10 (t, *J* = 7.1 Hz, 6H); ^{13}C NMR (75 MHz, $CDCl_3$, 278 K): δ(ppm, *J*(Hz)): 146.7, 146.0, 121.3, 119.7, 114.9, 114.8, 48.7, 46.9, 35.0, 33.2, 31.4, 12.8; IR (ATR): $\bar{\nu}$= 3350, 2933, 2881, 2225 (CN), 1617, 1508, 1455, 1434, 1265 cm^{-1}; HR MS (ESI): (*m/z*): 354.2147 [M+H]$^+$ (teor.: 354.2149).

5-(4-(2-(4-(4-(bis(2-hydroxyethyl)amino)phenyl)-1H-1,2,3-triazol-1-yl)ethyl)piperidin-1-yl)-6-(diethylamino)pyrazine-2,3-dicarbonitrile (18): Compounds **17** (174 mg, 0.49 mmol), **30** (250 mg, 1.22 mmol) and copper (I) iodide (8.5 mg, 0.04 mmol) were put into a round-bottomed flask with condenser and septum. Reaction system was evacuated and filled with argon. Then, anhydrous THF (5 mL) was added, reaction was heated to refluxed and DIPEA (2.3 ml, 13.15 mmol) was added by syringe. Reaction was refluxed for 15 hours. After that, it was filtrated, filtrate was evaporated to dryness and purified by column chromatography on silica with ethyl acetate/MeOH 19:1 as an eluent. Yield: yellow crystals (225 mg, 84%). Melting point 180.0-181.9 °C. ^{1}H NMR (500 MHz, DMSO-d_6, 278 K): δ(ppm, *J*(Hz)): 8.32 (s, 1H), 7.58 (d, *J* = 8.9 Hz, 2H), 6.73 (d, *J* = 9.1 Hz, 2H), 4.75 (t, *J* = 4.9 Hz, 2H), 4.40 (t, *J* = 7.2 Hz, 2H), 3.94 (d, *J* = 13.0 Hz, 2H), 3.59 – 3.48 (m, 8H), 3.43 (t, *J* = 6.4 Hz, 4H), 2.78 (t, *J* = 12.5 Hz, 2H), 1.86 – 1.77 (m, 4H), 1.54 – 1.43 (m, 1H), 1.25 (dq, *J* = 12.6, 3.8 Hz, 2H), 1.05 (t, *J* = 7.0 Hz, 6H); ^{13}C NMR (126 MHz, DMSO-d_6, 278 K): δ(ppm, *J*(Hz)): 147.8, 147.1, 146.6, 145.8, 126.4, 120.0, 119.3, 118.7, 118.0, 115.6, 115.5, 111.5, 58.3, 53.4, 47.1, 46.6, 42.2, 36.0, 32.6, 30.8, 12.7.

***t*-butyl 4-(2-hydroxyethyl)piperidine-1-carboxylate (25):** In round-bottomed flask, 2-(piperidin-4-yl)ethan-1-ol (6.0 g; 46.0 mmol) was dissolved in dichloromethane and reaction mixture was cooled to 0 °C. Then, di-*tert*-butyl dicarbonate (10.14 g; 46.0 mmol) was dissolved in dichloromethane and added dropwise to reaction mixture. When addition of di-tert-butyl dicarbonate was finished, reaction mixture was warm to rt and stirred for 4 hours. Reaction mixture was transferred into separation funnel, washed by 10% solution of $KHSO_4$ (3×50 ml), dried with anhydrous Na_2SO_4 and evaporated. Yield: 10.33 g (97%) as colorless oil. TLC Hex:EAC 6:4; ^{1}H NMR (500 MHz, $CDCl_3$, 278 K): δ(ppm, J(Hz)): 4.07 (br s, 2H,), 3.70 (t, *J* = 6.6 Hz, 2H), 2.69 (t, *J* = 12.4 Hz, 2H), 1.67 (d, *J* = 12.8 Hz, 2H), 1.56-1.47 (m, 3H), 1.44 s, 9H), 1.12 (qd, *J* = 12.5, 4.4 Hz, 2H); ^{13}C NMR (126 MHz, $CDCl_3$, 278 K): δ(ppm, J(Hz)): 154.9, 79.2, 60.2, 43.9, 39.2, 32.5, 32.1, 28.4.

***t*-butyl 4-(2-iodoethyl)piperidine-1-carboxylate (26):** In round-bottomed flask, iodine (13.72 g; 54.06 mmol) and triphenylphosphine (14.18 g; 54.06 mmol) were dissolved in toluene, stirred for 5 minutes at 80 °C, pyridine (8.7 ml; 108.1 mmol) was added and stirring was continues for next 5 minutes. Followed by addition of compound **25** (10.33 g; 45.05 mmol) in toluene (30 ml). Reaction mixture was stirred at 80 °C for 3 hours and then other 16 hours at rt. Reaction mixture was filtrated, precipitate was washed by toluene and filtrate was concentrated. Iodine derivate was purified by column chromatography (hexane: ethyl acetate – 9:1). Yield: 12.7 g (83%) as yellow oil. ^{1}H NMR (500 MHz, $CDCl_3$, 278 K): δ(ppm, *J*(Hz)): 4.09 (br s, 2H), 3.22 (t, *J* = 7.2 Hz, 2H), 2.70 (t, *J* = 12.0 Hz. 2H), 1.78 (q, *J* = 7.1 Hz, 2H), 1.66 (d, *J* = 12.6 Hz, 2H), 1.62-1.54 (m, 1H), 1.45 s, 9H, CCH_3), 1.11 (qd, J = 12.4, 4.4 Hz, 2H); ^{13}C NMR (126 MHz, $CDCl_3$, 278 K): δ(ppm, J(Hz)): 154.8, 79.3, 43.8, 39.9, 36.6, 31.2, 28.4, 3.9.

***t*-butyl 4-(2-azidoethyl)piperidine-1-carboxylate (27):** Compound **26** (12.7 g; 37.4 mmol) was dissolved in DMSO and sodium azide (4.87 g; 74.8 mmol) was added. Reaction mixture was stirred for 60 hours at rt. Reaction mixture was poured into ethyl acetate (200 ml), washed with brine (3×100 ml), dried with Na_2SO_4 and evaporated. Yield: 9.2 g (97%) as colorless oil. ^{1}H NMR (500 MHz, $CDCl_3$, 278 K): δ(ppm, J(Hz)): 4.09 (br s, 2H,), 3.33 (t, *J* = 6.6 Hz, 2H), 2.69 (t, *J* = 13.0 Hz. 2H), 1.66 (d, *J* = 13.0, 2H), 1.57-1.52 (m, 3H), 1.46 (s, 9H), 1.12 (qd, J = 12.5, 4.5 Hz, 2H); ^{13}C NMR (126 MHz, $CDCl_3$, 278 K): δ(ppm, J(Hz)): 154.8, 79.3, 48.8, 43.8, 35.2, 33.3, 31.8, 28.4.

4-(2-azidoethyl)piperidine (28): Compound **27** (6.0 g; 23.6 mmol) was dissolved in dichloromethane (40 ml) and cooled to 0 °C. TFA (20 ml) was added dropwise into reaction over 30 min. The reaction mixture was let to warm at rt and stirred for 2.5 hours. Volatile components were evaporated from reaction. Oily residue was dissolved in ethyl acetate (100 ml) and wash with 1M solution of NaOH until aqueous phase had pH around 9. Organic phase was dried by anhydrous Na_2SO_4 and evaporated. Final compound was purified by column chromatography on neutral Al_2O_3. Yield: 1.2 g (33%) as very bright yellow oil. ^{1}H NMR (500 MHz, $CDCl_3$, 278 K): δ(ppm, J(Hz)): 3.45 (s, 1H,), 3.30 (t, *J* = 6.7 Hz, 2H), 3.11 (d, *J* = 12.5 Hz, 2H), 2.61 (td, *J* = 12.3, 2.6 Hz. 2H), 1.68 (d, *J* = 12.9 Hz, 2H), 1.56-1.48 (m, 3H), 1.45-1.25 (m, 2H); ^{13}C NMR (126 MHz, $CDCl_3$, 278 K): δ(ppm, J(Hz)): 48.3, 43.9, 34.5, 31.3, 28.3.

Solubility of symmetrical TPyzPzs: The studied TPyzPz (50-100 mg) was placed in a 1.5 mL vial, and a particular solvent (500 µL) (methanol, dimethyl sulfoxide, THF, dimethylformamide or toluene) was added. The vial was closed with a stopper, sealed with parafilm and subjected to ultrasonication for 10 minutes at rt. After that, the vial was shaken for 5 hours at rt, then subjected again to ultrasonication for 5 minutes at rt and shaken for another 12 hours at rt. Then, the sample was left to stand for 15 minutes, the supernatant (300 µL) was collected and centrifuged for 15 minutes (10 000 rpm, rt). 200 µL of supernatant was collected and centrifuged for another 20 minutes (10 000 rpm, rt). Then, 10 µL of the supernatant was diluted to 5 mL in THF (in the case of metal-free TPyzPz) or pyridine (for zinc(II) TPyzPz) to give a stock solution. Finally, the absorption spectrum of the stock solution was measured. If needed, the stock solution was further diluted in THF (metal-free TPyzPz) or pyridine (zinc(II)TPyzPz), and the absorption spectrum was corrected for dilution. The concentration of the saturated solution was calculated from the extinction coefficient of particular TPyzPz. All measurements were performed in triplicate, and the data in Fig. 2 represent the mean of these measurements.

Study of the stability of J-dimers: Zinc(II) TPyzPz in toluene (1 µM) was transferred to a cuvette, and its absorbance was measured. After that, pyridine was stepwise added up to a total concentration of 2.5×10^{-1} M of pyridine, and the absorption spectrum was taken after each addition. The apparent association constant (K_P) was calculated by non-linear regression using GraphPad Prism 8 software from a plot of dependence of amount of monomer (expressed as $(A-A_{min})/(A_{max}-A_{min})$) on concentration of pyridine.

Fluorescence quantum yields (Φ_F) were determined on a FLS 1000 spectrofluorometer (Edinburg Instruments) by the comparative method[27] using unsubstituted zinc(II) phthalocyanine as a reference compound (Φ_F = 0.32 (THF)[20]. Excitation wavelength 600 nm (for TPyzPzs **1**,**2**, **4**-**8**) or 607 nm (for TPyzPz **3**). The determination of Φ_F values was performed in triplicate, and the data represent the mean of these measurements. The estimated experimental error was ±10%. Absorption of the samples at the excitation wavelength was kept below 0.05 and at a Q band maximum below 0.1 to avoid the inner-filter effect. The results of Φ_F were corrected for the refractive indices of the solvents. In the case of the toluene/pyridine mixture, 1% pyridine solution in toluene was used, which corresponds to 0.124 M pyridine in toluene.

Quantum yields of singlet oxygen production (Φ_Δ) were determined by the comparative method based on the decomposition of a chemical trap for singlet oxygen (1,3-diphenylisobenzofuran) and using unsubstituted zinc(II) phthalocyanine as a reference compound (Φ_Δ = 0.56 (DMF)[21], 0.58 (toluene)[22]). Details of the method are described elsewhere.[32] All the determinations were performed in triplicate, and the data represent the mean of the measurements. The estimated experimental error was ±10%. In the case of the toluene/pyridine mixture, 1% pyridine solution in toluene was used, which corresponds to 0.124 M pyridine in toluene (unsubstituted zinc(II) phthalocyanine in toluene was used as a reference).

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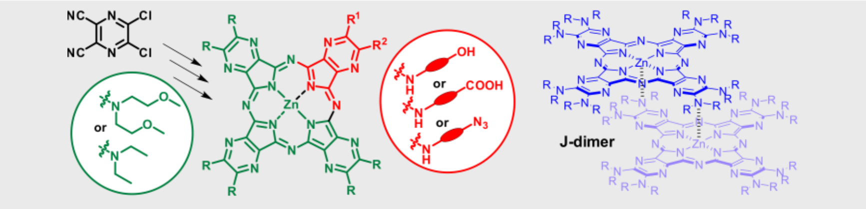

Dark quencher for 3´, 5´or intrastrand position of oligonucleotide probe available: Synthetic strategy for accessing suitably functionalized azaphthalocyanines is described and their self-assembly into J-dimers compared. Stability of J-dimers improved with less bulky substituents and low-symmetrical character of molecule. Quantum yields of fluorescence lower than 0.006 are optimal for target applications.

Jiri Demuth, Miroslav Miletin, Matej Machan, Michal Kantor, Petr Zimcik and Veronika Novakova**

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Synthesis and J-Dimer Formation of Tetrapyrazinoporphyrazines with Different Functional Groups Suitable for Biomolecular Probe Applications