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Cationic Axial Ligands on Sulfur Substituted Silicon(IV) Phthalocyanines: Improved Hydrophilicity and Exceptionally Red-shifted Absorption into the NIR Region

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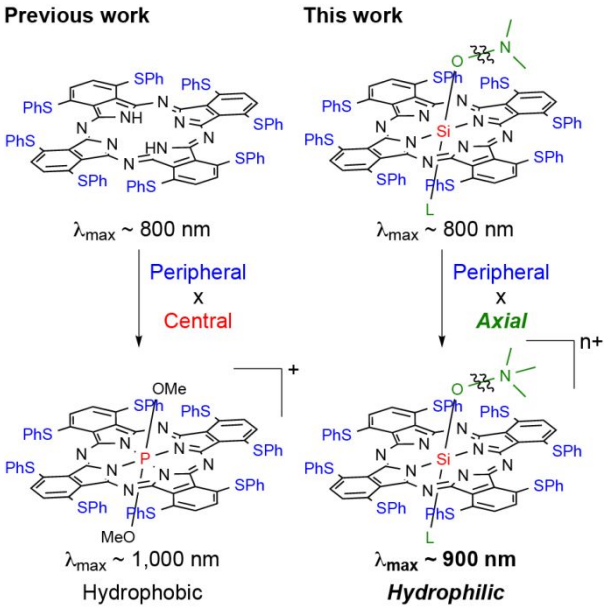
Herein, we report the exceptionally red-shifted absorption of sulfur-substituted silicon(IV) phthalocyanines upon introduction of cationic axial ligands. The Q band was red-shifted to approximately 900 nm with improved hydrophilicity by the combination of peripheral sulfur substituents and axial ammonium ligands. One such phthalocyanine exhibited remarkable photocytotoxicity upon irradiation with NIR light (~ 810 nm) in live cells.

Near-infrared (NIR) light (specifically 700–1000 nm) displays high penetration in human tissue (the so-called "therapeutic windows"). This characteristic is accompanied by relatively low toxicity and high selectivity for biological applications. Therefore, organic dyes and pigments that absorb in this spectral region are promising candidates for photodynamic therapy (PDT) photosensitizers.[1] Such photosensitizers generate singlet oxygen ($O_2(^1\Delta_g)$) under irradiation with light, which ultimately leads to cell death. Silicon(IV) phthalocyanines (SiPcs) represent one of the most promising classes of materials for this purpose.[2] Pcs exhibit an intense absorption band (the so-called "Q-band") in the far-red to NIR (650–700 nm) region, which can be fine-tuned via appropriate peripheral modifications.[3] SiPcs bear two axial ligands, whose modifications are well established.[4] For the design and development of novel PDT photosensitizers, multiple functional features, such as intense light absorption, the ability to generate high levels of singlet oxygen, hydrophilicity, and recognition of therapeutic sites, are simultaneously required in one single molecule. SiPcs are thus an appropriate "platform" for this purpose. However, most reported SiPcs absorb light < 700 nm, for which the penetration in human tissue is low. Although NIR dyes, such as cyanine derivatives[5] and modified porphyrinoids,[6] are good candidates to overcome this problem, complex modifications are usually required, and these usually proceed via multi-step syntheses and thus afford low yields.



Scheme 1 General design of hydrophilic phthalocyanines absorbing in the NIR range.

Recently, we have developed phosphorus(V) Pcs (PPcs), which are able to absorb in the NIR (> 1000 nm) region.[7] This exceptional red-shift is induced by a synergistic effect of peripheral electron-rich sulfur atoms and the central electron-deficient phosphorus(V) ion (Scheme 1). However, the prepared PPcs were relatively hydrophobic, which limited their biological applications.[8] In this communication, we describe how cationic axial ligands for SiPcs can simultaneously improve the hydrophilicity of the resulting compounds and red-shift the absorption band (Q-band) in synergy with the aforementioned

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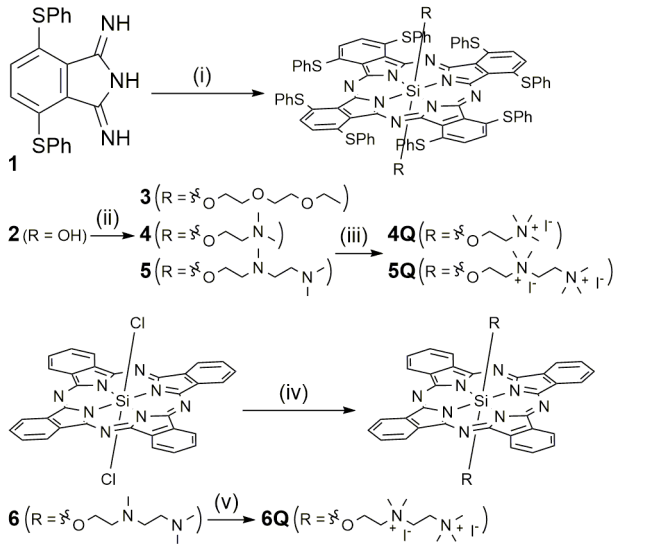
peripheral sulfur-substitution effects. If two functions are imparted by one fragment of the SiPc, i.e., the axial ligands, the remaining fragment, i.e., the phthalocyanine, can be employed to further improve the PDT efficacy. The effect of axial ligands on the Q-band position is usually small,[2d] while the use of electron-deficient cationic axial ligands can be expected to generate an electron-deficient silicon center whose absorption should be further red-shifted as a central phosphorus of PPcs. Introducing axial substituents into Pcs may thus be considered as a more general strategy to tailor the absorption properties of Pcs in the NIR region.



Scheme 2 Synthesis of silicon(IV) phthalocyanines (SiPcs). *Reagents and conditions*: (i) $SiCl_4$, quinoline, 180 °C, 24 h, 84%; (ii) diethylene glycol monoethyl ether (for **3**), 2-dimethylaminoethanol (for **4**), 2-[2-(dimethylamino)ethylamino]ethanol (for **5**), toluene, reflux, 12 h, 74% (for **3**), 91% (for **4**), 76% (for **5**); (iii) iodomethane (excess), CH_2Cl_2, rt, 12 h (for **4Q**), 4 d (for **5Q**), 87% (for **4Q**), 40% (for **5Q**); (iv) 2-[2-(dimethylamino)ethylamino]ethanol, pyridine, toluene, reflux, 36 h, 81%; (v) iodomethane (excess), DMF, rt, 5 h, 93%.

The synthetic route to SiPcs with and without peripheral sulfur-based substituents is shown in Scheme 2. Hydroxy-substituted **2** was obtained by a template method from 3,6-disubstituted pyrroline-diimine derivative **1**. Glycol- (**3**) and aminoalkyl-based (**4** and **5**) ligands were introduced by dehydrative condensation between **2** and the corresponding alcohols. Cationic ligands (**4Q** and **5Q**) were introduced by *N*-methylation of **4** and **5**. All SiPcs were fully characterized by ^1H and ^{29}Si NMR spectroscopy and HR-MALDI-FT-ICR mass spectrometry. Single crystals for an X-ray diffraction analysis were obtained by diffusion of methanol into a chloroform solution of **4**. Unfortunately, the axial ligands were replaced by methanol during the recrystallization (Fig. S1). The Pc macrocycle adopts a slightly waved structure,[9] similar to the previously reported solid-state structures of SiPcs.[2e] The hydrophilicity of the SiPcs was markedly improved after the introduction of hydrophilic axial glycol or ammonium moieties. For example, SiPcs bearing neutral aminoalkyl ligands could not be dissolved in hydrophilic media (DMSO/PBS buffer = 1:1, v/v), but their solubility was markedly improved after *N*-methylation of the axial ligands (Fig. S2).

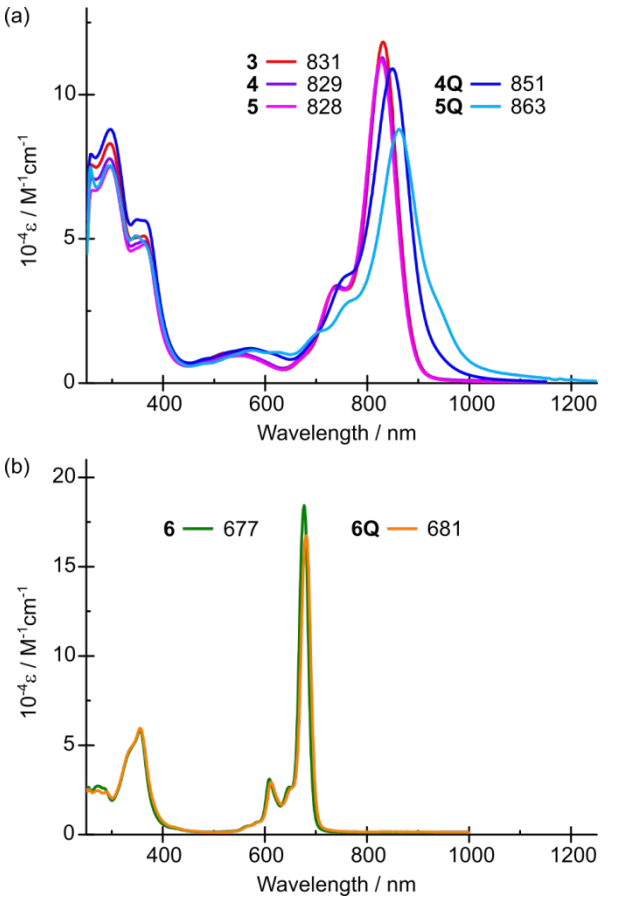


Fig. 1 (a) UV-vis-NIR absorption spectra of **3** (red), **4** (purple), **4Q** (blue), **5** (pink), and **5Q** (light blue) in DMSO. (b) UV-vis-NIR absorption spectra of **6** (green) and **6Q** (orange) in DMSO.

The absorption spectra of the prepared SiPcs in DMSO are shown in Fig. 1. As discussed in a previous paper, electron-donating sulfur substituents at the α-positions of the Pc platform shift the Q-band bathochromically due to destabilization of the HOMO level.[7] Hence, the Q-bands of **3–5** (831–828 nm) appear in the NIR region (> 800 nm). The presence of neutral axial ligands does not affect the position of the Q-band. Interestingly, a further red-shift was observed after *N*-methylation of the axial ligands in **4** to give **4Q** (22 nm, 310 cm^{-1}) and in **5** to give **5Q** (35 nm, 490 cm^{-1}), while the Q-band position of **6** (677 nm) and **6Q** (681 nm) remained almost constant (4 nm, 90 cm^{-1}) as reported previously.[2e] The *N*-methylation-induced shift was found to depend not only on the peripheral substituents, but also on the number of cations, i.e., the Q-band of tetracation **5Q** (863 nm) appears at longer wavelengths than that of dication **4Q** (851 nm). An unusual red shift induced by the presence of cationic axial ligands was also observed in other solvents. Although the peak position after *N*-methylation depends on the solvent, a significant shift was observed in all solvents that dissolve both neutral and cationic SiPcs (Fig. S3). The absorption spectra are typical of non-aggregated Pcs, which strictly follow the Beer-Lambert law (Fig. S4).

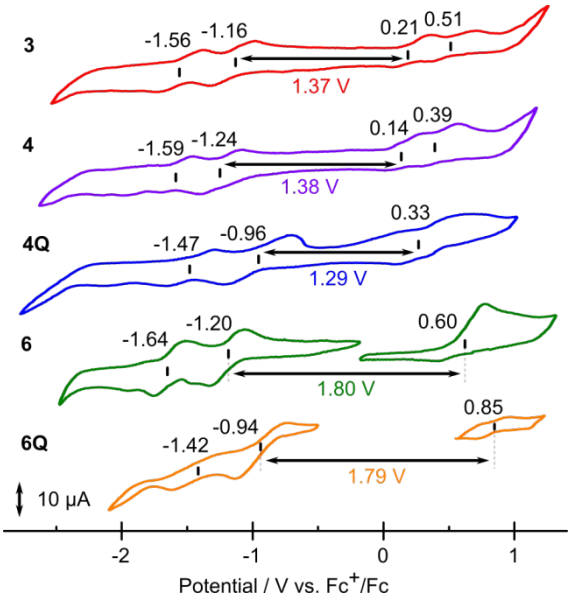


Fig. 2 Cyclic voltammograms of **3** (red), **4** (purple), **4Q** (blue), **6** (green), and **6Q** (orange) recorded from 1.0 mM solutions of the analytes in $[^nBu_4N]ClO_4$/*o*-DCB. Ferrocene was used as the internal standard and the Fc/Fc^+ couple was set to 0 V.

In order to gain insight into the origin of this exceptional red-shift, cyclic voltammograms of the SiPcs were recorded in *o*-dichlorobenzene (*o*-DCB) (Fig. 2). Neutral **3** and **4** as well as dication **4Q** exhibit one and two oxidation/reduction processes, respectively. The first redox potentials of **3** and **4** are comparable, confirming that the effect of neutral axial ligands on the energy level of the frontier orbitals is small. On the other hand, the E_{1ox}–E_{1red} value decreased from **4** (1.38 V) to **4Q** (1.29 V), as anticipated based on the bathochromically shifted absorption upon *N*-methylation of the ligand. In the case of **4Q**, both redox potentials were shifted anodically. The first reduction potential of **4Q** appears at –0.96 V, which corresponds to an anodic shift of 0.28 V upon *N*-methylation, while the first oxidation potential appears at 0.33 V, with an anodic shift of 0.19 V. The redox data indicate that although both the HOMO and LUMO are stabilized upon *N*-methylation, the stabilization of the LUMO is greater than that of the HOMO. Anodic shifts of the redox potentials were also observed for peripherally unsubstituted **6** and **6Q**. However, the extent of the anodic shift is similar for the first oxidation (0.25 V) and reduction (0.26 V) potential, respectively, as expected from the absorption spectra. Therefore, the marked anodic shift of the reduction potential upon *N*-methylation depends on the peripheral substituents.

For a better understanding of the effects of peripheral and axial substitution, molecular orbital (MO) calculations were performed on these SiPcs. Since the effect of sulfur atoms is much higher than that of substituent groups on sulfur atoms, the close Q band positions have been observed between $(alkylthio)_8Pc$[10] and $(arylthio)_8Pc$.[7] Hence model structures **4'** and **4Q'**, where the phenyl groups on the sulfur atoms had been replaced by methyl groups, were used, as phenyl groups barely affect the absorption spectra. For peripherally unsubstituted SiPcs, dication **7Q** and the corresponding neutral species **7** were used as model structures in order to reduce the calculation time. The partial MO energy diagrams of the model structures and

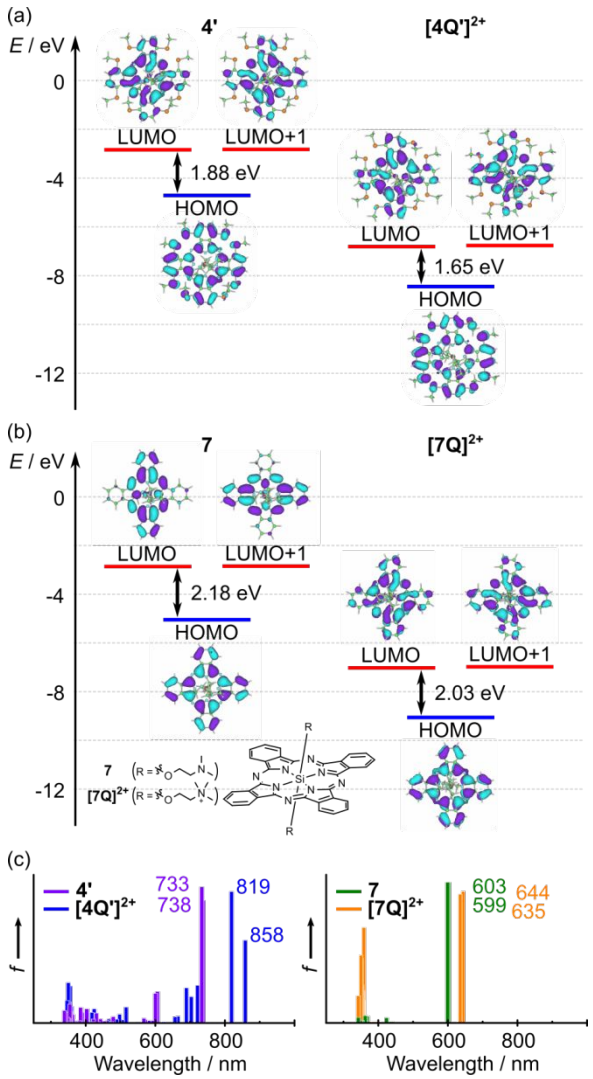


Fig. 3 Partial molecular energy diagram and orbitals of (a) peripherally substituted $(MeS)_8SiPcs$ (**4'** and **[4Q']$^{2+}$**) and (b) peripherally unsubstituted SiPcs (**7** and **[7Q]$^{2+}$**), as well as (c) their calculated absorption spectra. Calculations were carried out at the B3LYP/6-31G* level of theory (for details, see the ESI).

calculated absorption spectra are shown in Fig. 3, while the results of time-dependent density functional theory (TD-DFT) calculations are summarized in Table S2. The HOMO–LUMO energy gap (ΔHL) decreases from neutral **4'** (1.88 eV) to dication **4Q'** (1.65 eV), while smaller ΔHL values were obtained for **7** (2.18 eV) and **7Q** (2.03 eV). When cationic axial ligands are introduced in sulfur-substituted Pcs, the LUMO stabilization is higher (ΔLUMO: –3.83 eV) than that of the HOMO (ΔHOMO: –3.62 eV), which is consistent with the experimental results of the optical and electrochemical measurements. A small MO contribution at the central silicon is observed for all the frontier orbitals of all the calculated SiPcs. The calculated charges (Table S3) and the experimental ^{29}Si NMR spectra (Fig. S5) also support the hypothesis that the electron density at the central silicon does not change upon *N*-methylation. The destabilization of

Pc's LMOs may enhance the electronic interaction with cationic axial ligands, leading the further exceptional red-shift as PPcs.[7]

Finally, we investigated the potential of the present compounds to serve as photocytotoxic agents under NIR light irradiation. First, the photosensitizing efficiency of SiPcs in DMSO was determined by a steady-state method using red light (680 nm) and methylene blue as the standard. The singlet oxygen quantum yields of the cationic compounds (**4Q**:Φ_Δ = 0.09; **5Q**: 0.02; in DMSO) were smaller than those of the neutral compounds (**4**: Φ_Δ = 0.32; **5**: 0.14), as previously reported SiPcs.[11] Although the yields are lower than that of indocyanine green (Φ_Δ = 0.120), which shows high PDT efficiency upon irradiation of light at ~ 800 nm,[12] the generation of singlet oxygen under NIR irradiation was confirmed (Fig. S6). Subsequently, we evaluated the photocytotoxity of the hydrophilic SiPcs. Unfortunately, dication **4Q** could not be dissolved under the conditions required for an evaluation of the photocytotoxicity, and so glycol-derived **3** and ammonium-derived **5Q** were used. Initially, we evaluated the photocytotoxicity of **3** and **5Q** toward HEK293T cells using the MTT assay (WST-8). NIR light (810 nm) was chosen as the light source as both SiPcs absorb strongly in this region. The cytotoxicity of **3** and **5Q** was low at a concentration of 10 μM (cell viabilities were over 90% after two days of incubation), while **5Q** exhibited high photocytotoxicity after 15 min of irradiation at 100 mW cm^{-2} (Fig. S7). Under these conditions, only a few cells survived after two days of incubation. Next, we evaluated the dependence of the **5Q** photocytotoxicity on the light intensity (Fig. 4). A clear correlation was observed between the light intensity (5–20 mW cm^{-2}) and the cell viability. The low cytotoxicity of tetracation **5Q** was confirmed upon incubation in the absence of light irradiation, suggesting promising potential of this compound for practical applications in PDT using NIR light.

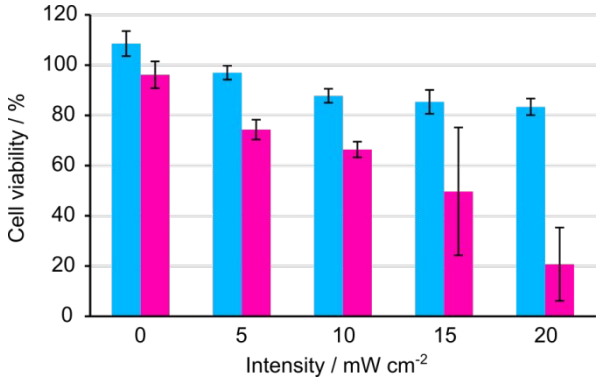


Fig. 4 Photocytotoxicity of **5Q** (10 μM, 0.1% DMSO) on HEK293T cells after 15 min of irradiation (λ_{ex} = 810 nm). The MTT assay was performed soon after irradiation (blue) and after 2 days of incubation (red). Data are presented as mean values ± SD (n = 3).

In summary, an exceptional red-shift of the absorption of sulfur-substituted SiPcs was achieved upon *N*-methylation of aminoalkyl ligands in axial positions. The introduction of cationic axial ligands not only enhanced the hydrophilicity, but also induced a shift of the Q-band toward the NIR region. Cyclic voltammetry data and theoretical calculations clarified the effect of the peripheral and axial substituents. Hydrophilic SiPcs were applied in photocytotoxicity assays, and efficient PDT activity against HEK293T cells was observed under irradiation with NIR light (810 nm). The combination of peripheral sulfur and axial cationic ligands, obtained by simple synthetic procedures, is crucial to achieve these unique NIR properties. The present strategy will be applied to fine-tune the optical properties in the NIR range (800–1000 nm) and further modify the peripheral substituents to improve the practical PDT efficiency.

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Notes and references

1 T. J. Dougherty, C. J. Gomer, B. W. Henderson, G. Jori, D. Kessel, M. Korbelik, J. Moan and Q. Peng, *J. Natl. Cancer Inst.*, 1998, **90**, 889-905.

2 (*a*) K. Sato, K. Ando, S. Okuyama, S. Moriguchi, T. Ogura, S. Totoki, H. Hanaoka, T. Nagaya, R. Kokawa, H. Takakura, M. Nishimura, Y. Hasegawa, P. L. Choyke, M. Ogawa and H. Kobayashi, *ACS Cent. Sci.*, 2018, **4**, 1559-1569; (*b*) M. Mitsunaga, M. Ogawa, N. Kosaka, L. T. Rosenblum, P. L. Choyke and H. Kobayashi, *Nat. Med. (N. Y., NY, U. S.)*, 2011, **17**, 1685-1691; (*c*) N. Ahmad, D. K. Feyes, R. Agarwal and H. Mukhtar, *Proc. Natl. Acad. Sci. USA*, 1998, **95**, 6977-6982; (*d*) X.-J. Jiang, P.-C. Lo, S.-L. Yeung, W.-P. Fong and D. K. P. Ng, *Chem. Commun.*, 2010, **46**, 3188-3190; (*e*) P.-C. Lo, J.-D. Huang, D. Y. Y. Cheng, E. Y. M. Chan, W.-P. Fong, W.-H. Ko and D. K. P. Ng, *Chem.-Eur. J.*, 2004, **10**, 4831-4838.

3 (*a*) T. Fukuda and N. Kobayashi, in *Handbook of Porphyrin Science*, ed. K. M. Kadish, K. M. Smith and R. Guilard, World Scientific, Singapore, 2010, vol. 9, pp. 1-650; (*b*) Y. Rio, M. S. Rodríguez-Morgade and T. Torres, *Org. Biomol. Chem.*, 2008, **6**, 1877-1894.

4 (*a*) E. van de Winckel, R. J. Schneider, A. de la Escosura and T. Torres, *Chem.-Eur. J.*, 2015, **21**, 18551-18556; (*b*) J. T. F. Lau, X.-J. Jiang, D. K. P. Ng and P.-C. Lo, *Chem. Commun.*, 2013, **49**, 4274-4276.

5 J. T. Alander, I. Kaartinen, A. Laakso, T. Patila, T. Spillmann, V. V. Tuchin, M. Venermo and P. Välisuo, *Int. J. Biomed. Imaging*, 2012, 940585.

6 P. Pushpanandan, Y. K. Maurya, T. Omagari, R. Hirosawa, M. Ishida, S. Mori, Y. Yasutake, S. Fukatsu, J. Mack, T. Nyokong and H. Furuta, *Inorg. Chem.* 2017, **56**, 12572-12580.

7 T. Furuyama, K. Satoh, T. Kushiya and N. Kobayashi, *J. Am. Chem. Soc.*, 2014, **136**, 765-776.

8 Y. Zhou, D. Wang, Y. Zhang, U. Chitgupi, J. Geng, Y. Wang, Y. Zhang, T. R. Cook, J. Xia and J. F. Lovell, *Theranostics*, 2016, **6**, 688-697.

9 R. Harada, Y. Matsuda, H. Okawa, R. Miyamoto, S. Yamauchi and T. Kojima, *Inorg. Chim. Acta.*, 2005, **358**, 2489-2500.

10 N. Kobayashi, H. Ogata, N. Nonaka and E. A. Luk'yanets, *Chem.-Eur. J.*, 2003, **9**, 5123-5134.
11 İ. Ömeroğlu, E. N. Kaya, M. Göksel, V. Kussovski, V. Mantareva and M. Durmuş, *Bioorg. Med. Chem.* 2017, **25**, 5415-5422.
12 (*a*) J. A. Cardillo, R. Jorge, R. A. Costa, S. M. T. Nunes, D. Lavinsky, B. D. Kuppermann, A. C. Tedesco and M. E. Farah, *Br. J. Ophthalmol.*, 2008, **92**, 276-280; (*b*) L. Jiao, F. Song, J. Cui and X. Peng, *Chem. Commun.*, 2018, **54**, 9198-9201.