ChemComm

Two-Photon, Red Light Uncaging of Alkyl Radicals from Organorhodium(III) Phthalocyanine Complexes

Journal:	*ChemComm*
Manuscript ID	CC-COM-07-2022-003672.R1
Article Type:	Communication

COMMUNICATION

Two-Photon, Red Light Uncaging of Alkyl Radicals from Organorhodium(III) Phthalocyanine Complexes

Received 00th January 20xx,
Accepted 00th January 20xx

DOI: 10.1039/x0xx00000x

Kei Murata*, Yuki Saibe, Mayu Uchida, Mizuki Aono, Ryuji Misawa, Yoshiho Ikeuchi and Kazuyuki Ishii*

A stepwise two-photon, red light excitation of organorhodium(III) phthalocyanine complexes was found to induce the activation of the axial metal–carbon bond to generate alkyl radicals/aldehydes. The cooperative action of the photouncaging reaction and the photochemical generation of reactive oxygen species were indicated to induce the cell deaths.

Photodynamic therapy (PDT) has attracted much attention as a minimally invasive cancer therapy based on tumor-selective cell deaths.[1] In this method, the therapeutic effect is achieved via the photochemical formation of reactive oxygen species (ROS) such as singlet oxygen by a photosensitizer accumulated in tumor cells. On the basis of this principle, various types of photosensitizers have been developed to improve the applicability of PDT.[2] However, in conventional PDT, only ROS are available as active species, and the treatment under hypoxic condition still remains a challenge. To address these issues, the use of a photouncaging system has emerged as a promising alternative,[3] in which the tumor-selective release of functional, long-lived active agents is expected to enhance the therapeutic effect of the treatment. Alkyl radicals are plausible candidates as active agents in such systems because they are readily oxidized and transformed to terminal aldehydes, which act as a bioactive group in various functional molecules.[4] In addition, alkyl radicals participate in various free-radical reactions with biological tissues, inducing cell deaths under both normoxic and hypoxic conditions.[5] With regard to the photochemical generation of alkyl radicals, organocobalt(III) and organorhodium(III) porphyrin derivatives are known to release alkyl radicals by homolytic cleavage of an axial metal–carbon bond via single-photon excitation.[3d,6] However, these reactions are usually conducted under visible-light (≤560 nm) irradiation, and the introduction of an antenna chromophore is required to utilize low-energy photons with high penetration ability into biological tissues, such as red light (≥650 nm). Moreover, since these molecules can be easily activated by natural light, careful treatment under dark condition is required. Therefore, the development of a stable reaction platform that can be activated by artificial light is highly desirable for the construction of easy-to-handle uncaging systems.[7]

Herein, we describe a new platform based on organorhodium(III) phthalocyanine (Pc) complexes for the photochemical generation of an alkyl radical/aldehyde via a stepwise two-photon excitation under red light irradiation (Fig. 1). These complexes were stable during synthetic, purification, and measurement processes but could be activated by a nanosecond pulsed laser. As Pc ligands absorb red light owing to its large π-conjugated system, they have been often used in PDT as second-generation photosensitizers.[8] However, to the best of our knowledge, organometallic Pc complexes have never been applied in a phototherapeutic system. Our study provides a simple, easy-to-handle approach for uncaging an alkyl radical as an active agent in biological environments.

Methylrhodium(III) (**1**) and butylrhodium(III) (**2**) tetra-*tert*-butyl Pcs were synthesized and characterized by various spectroscopic methods. During the synthesis and purification processes, these complexes were sufficiently stable and tolerated ambient light and room temperature conditions. The electronic absorption, emission and magnetic circular dichroism (MCD) spectra of complex **1** are shown in Fig. 2. A sharp, intense Q-band originated from the π–π* transition of the Pc ring was observed at 651 nm. The extinction coefficient (ε) of the Q-band reaches a value of 2×10^5 M^{-1} cm^{-1}, indicating that complex **1** absorbs red light efficiently, as typically

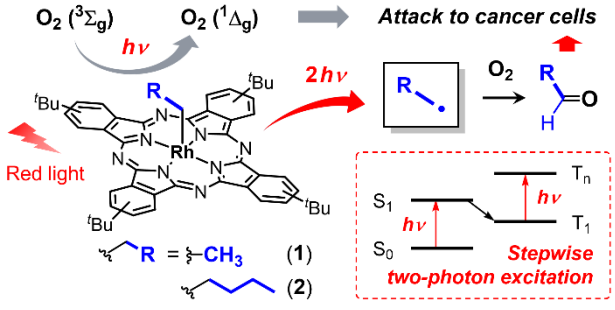


Fig. 1 Schematic representation of the uncaging system used in this study and molecular structures of complexes **1** and **2**.

Institute of Industrial Science, The University of Tokyo, 4-6-1 Komaba, Meguro-ku, Tokyo, 153-8505, Japan. E-mail: murata-k@iis.u-tokyo.ac.jp, k-ishii@iis.u-tokyo.ac.jp
Electronic Supplementary Information (ESI) available. See DOI: 10.1039/x0xx00000x

observed in Pc analogues.[9] In the MCD spectrum, a dispersion-type Faraday *A* term is observed in the Q-band region, indicating the degeneracy of the lowest excited singlet (S_1) state, which possesses D_{4h} symmetry.[10] Moreover, complex **1** exhibited a relatively sharp phosphorescence band at 962 nm. Time-resolved emission spectroscopy demonstrated that the excited lifetime was 3.6 μs (Fig. 2 inset). As can be extracted from Fig. S1, the photophysical properties of complex **2** were analogous to those of complex **1**.

The photoreactivities of complexes **1** and **2** were investigated in organic solvents under O_2 atmosphere by means of ^{1}H NMR spectroscopy. When a C_6D_6 solution of complex **1** was irradiated by a continuous wave (CW) of red light ($\lambda_{irr.}$ > 640 nm, metal halide lamp), no reaction occurred. Meanwhile, when the solution was subjected to irradiation with a nanosecond pulsed laser of red light ($\lambda_{irr.}$ = 650 nm) for 3 h, complex **1** fully converted, and formaldehyde was generated as a major photoproduct (≥34% yield)[‡] together with methanol as a byproduct (14% yield). This reaction also proceeded under air to furnish the same products, albeit with slightly lower yields (Table S1). These results indicated that the axial rhodium–carbon bond was cleaved upon photoexcitation, uncaging a methyl radical whose subsequent reaction with O_2 produced its oxidized products (Scheme 1a). The release of the methyl radical was confirmed by conducting the light irradiation reaction in the presence of an excess amount of 2,2,6,6-tetramethylpiperidine1-oxyl (TEMPO) under N_2 atmosphere, which produced the methyl radical adduct of TEMPO in addition to ethane as a homocoupling product. Similarly, complex **2** was stable under CW red light irradiation, whereas it reacted upon irradiation with a nanosecond pulsed laser of red light under O_2 atmosphere for 3 h to furnish butyraldehyde and 1-butanol in 50% and 13% yields, respectively (Scheme 1b). 1-Butene was also obtained (≥21% yield), indicating that the *β*-hydride elimination competes with the oxidation when the axial ligand possesses a hydrogen atom at the *β*-position to the metal centre. The photochemical behaviors of complexes **1** and **2** demonstrated that organorhodium Pc complexes bearing a simple alkyl ligand could be activated for the uncaging of an alkyl radical when excited by a nanosecond pulsed laser of red light.

According to the reaction mechanism previously proposed for organometallic porphyrin complexes,[6d-f] the photochemical aldehyde formation proceeded via the homolytic cleavage of a

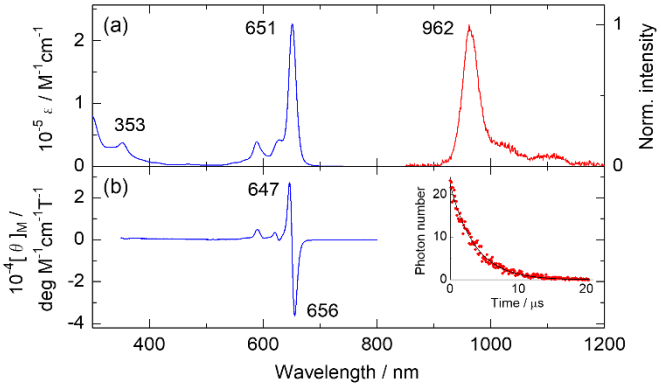
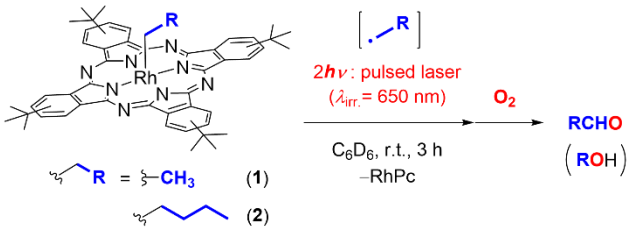


Fig. 2 (a) Electronic absorption and phosphorescence spectra and (b) MCD spectrum of complex **1** (toluene with 1% pyridine, r.t.). Inset: phosphorescence decay and curve fitting (τ = 3.6 ± 0.1 μs).



Scheme 1 Photouncaging reactions of complexes **1** and **2**.

rhodium–carbon bond, insertion of molecular oxygen, and oxygen–oxygen bond cleavage followed by abstraction of a hydrogen atom (Fig. S2). In general, the initial homolytic cleavage of the metal–carbon bond is induced by the removal of an electron from the bonding σ(M–C) orbital or the injection of an electron to the antibonding σ*(M–C) orbital upon photoexcitation. In the present system, the nanosecond pulsed laser light was required to activate the axial rhodium–carbon bond in the Pc ring. To clarify the mechanism for the activation with the pulsed laser excitation, the photophysical process of complex **1** was investigated during excitation with the pulsed laser. The conversion rate of complex **1** was nearly proportional to the square of the laser power in a range of 0.4–1.0 mJ pulse^{-1} (Fig. S3), indicating that the reaction involved a two-photon excitation process. Since the photon density of the nanosecond pulsed laser is about 10^{25} photons cm^{-2} s^{-1}, a stepwise two-photon excitation process could occur under our experimental conditions.[11] In addition, a μs-order lifetime was observed for the lowest excited triplet (T_1) state in the time-resolved phosphorescence measurement, whereas a much shorter lifetime was indicated for the S_1 state of a related porphyrinic compound owing to the heavy atom effect of the rhodium ion.[12] Therefore, the second excitation could occur at the T_1 state during exposure to the nanosecond pulsed laser light. These factors suggest that the initial $S_0 \rightarrow S_1$ excitation generated the T_1 state via the fast intersystem crossing, and the second excitation at the T_1 state produced the higher-lying excited triplet states (T_n; n > 1) corresponding to the reactive state for the activation of the rhodium–carbon bond.

To analyze the stepwise two-photon excitation process of the rhodium Pc complexes, a theoretical study was conducted by DFT/TD-DFT calculations using a model of complex **1** bearing hydrogen atoms instead of the *tert*-butyl groups on the Pc ring (**1'**; Fig. S10, Table S2). First, the initial excitation process was analyzed based on the optimized structure of the ground (S_0) state. In this geometry, the S_1 state mainly comprised the HOMO (a_{1u}(Pc)) to LUMO (e_{gy}(Pc)) / LUMO+1 (e_{gx}(Pc)) transitions, that is, the π–π* transitions of the Pc ring. The σ*(Rh–C) orbital mainly contributed to LUMO+2, to which the electronic transition occurred in the S_5 state. The calculated excitation energy of the $S_0 \rightarrow S_5$ transition (2.79 eV) was much higher than that of $S_0 \rightarrow S_1$ (2.07 eV), indicating that a single-photon excitation with red light cannot induce the activation of the rhodium–carbon bond. Meanwhile, the T_1 state (1.30 eV) was predominantly composed of the HOMO to LUMO / LUMO+1 transitions corresponding to the π–π* transitions of the Pc ring. Second, the subsequent excitation process was analyzed based on the optimized structure of the T_1 state considering the structural change after the intersystem crossing. The σ*(Rh–C) orbital contributed mainly to LUMO+1 in this geometry (Fig. 3). The electronic transition from SOMO (e_{gx}(Pc)) to LUMO+1 was found in

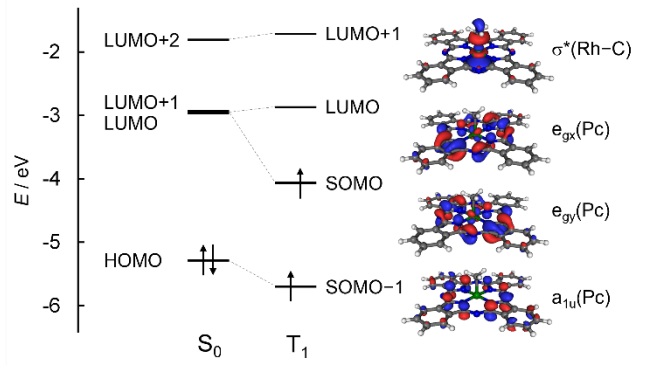


Fig. 3 Electron configurations of complex **1**′ in the S_0 and T_1 optimized geometries and selected molecular orbitals in the T_1 state. DFT calculation: B3LYP/Def2-TZVPP level with PCM (benzene).

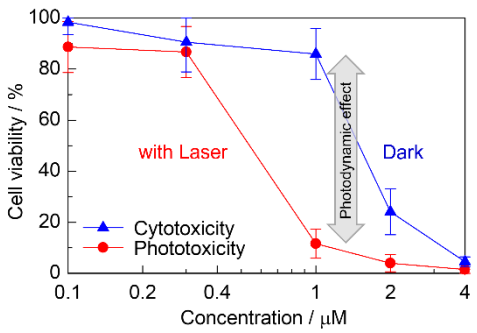


Fig. 4 Cytotoxicity and phototoxicity response of complex **1** for HeLa cells at a concentration of 0.1–4 μM.

the T_5 state, and the calculated excitation energy of the $T_1 \rightarrow T_5$ transition (1.48 eV) was lower than that of red light. These theoretical results demonstrated that a stepwise two-photon excitation with a pulsed laser of red light opens the access to the higher-lying excited triplet state that activates the rhodium–carbon bond, which is not possible for the single-photon excitation with CW red light. The corresponding theoretical study on a model of complex **2** (**2'**) demonstrated that the excited-state properties were similar in the presence of an axial butyl ligand instead of a methyl ligand (Fig. S11, Table S3), which suggests the generality of this approach for organorhodium Pc complexes possessing a simple alkyl ligand.

To investigate the feasibility of the photouncaging reaction in a biological environment, small unilamellar liposomes of **1** were prepared by treating a lipid bilayer membrane of 1,2-dipalmitoyl-sn-glycero-3-phosphocholine (DPPC) according to previously reported methods,[13] since complex **1** was insoluble in aqueous media. In the electronic absorption spectrum of liposomal **1**, the Q-band was observed at 653 nm (Fig. S4), confirming the encapsulation of complex **1** by DPPC. A slight broadening of the absorption band suggested the aggregation of complex **1** in the composite. An aqueous solution of liposomal **1** was then subjected to irradiation with a nanosecond pulsed laser of red light ($\lambda_{irr.}$ = 653 nm) under air atmosphere. To detect the formation of formaldehyde in the aqueous solution, *O*-(2,3,4,5,6-pentafluorobenzyl)hydroxylamine hydrochloride (PFBHA) was used as a trapping agent. After irradiation for 1 h, a formaldehyde–PFBHA adduct was obtained in 40% yield, which demonstrated that the activation of the rhodium–carbon bond in complex **1** also occurred in a biomimetic environment.

Finally, the photodynamic effects of complex **1** toward HeLa cells were examined. To perform cytotoxicity and phototoxicity tests, monolayer cultures of HeLa cells (ATCC CCL-2) were treated with a phosphate-buffered saline solution of liposomal **1** and then incubated. After medium exchange, complex **1**-uptaken HeLa cells were placed in the dark or irradiated with a nanosecond pulsed laser of red light ($\lambda_{irr.}$ = 653 nm) for 30 min. The cells were then subjected to formazan colour reaction to evaluate the cell viability. Fig. 4 shows the cytotoxicity and phototoxicity as a function of the concentration of complex **1** (0.1–5 μM). No significant cell viability loss was observed upon treatment of the cell cultures with complex **1** at a concentration of ≤1 μM in the absence of light. In contrast, the pulsed laser irradiation resulted in a high phototoxicity; complex **1** caused >90% loss of HeLa cell viability at concentrations of ≥1 μM. These results demonstrated that complex **1** showed photodynamic effect on HeLa cells at concentrations around 1 μM (Fig. S5).

Considering that both methyl radical/formaldehyde and singlet oxygen could contribute to the phototoxicity of complex **1**,[§] the mechanism of cell death was investigated. The cellular uptake of complex **1** was determined to be about 5.0×10^{-17} mol cell^{-1} after treating the cells with 1 μM solution of the complex.[14] According to the reported value of the minimal effective concentration of formaldehyde, the cellular uptake of complex **1** was sufficiently high to kill the cells by the action of formaldehyde.[15] In the case of complex **1**, the apoptotic cell ratio in the dead cells was about 18% when the cells were treated with 1 μM solution followed by irradiation with the pulsed laser of red light (Table S2). To further clarify the phototoxic effect of complex **1**, the photodynamic effect of zinc(II) Pc, which also possesses hydrophobic *tert*-butyl groups and can generate singlet oxygen (Φ_Δ = 0.54)[16] but not an alkyl radical/aldehyde under light irradiation, was investigated. The apoptotic cell ratio was about 7% under similar conditions, indicating that ROS mainly induced necrotic cell death under the present conditions.[13c,17] Thus, the higher apoptotic cell ratio observed for complex **1** compared with zinc(II) Pc indicated that methyl radical/formaldehyde could contribute to the apoptotic cell death. This is consistent with previous reports demonstrating that both methyl radical and formaldehyde can work as an apoptosis inducer.[18] These results indicate that the photochemical formation of alkyl radical/aldehyde from organorhodium Pc also occurs in cancer cells.

In conclusion, we developed a photouncaging system to release an alkyl radical from organorhodium(III) Pc complexes induced by irradiation with a nanosecond pulsed laser of red light. The experimental and theoretical results demonstrated that the axial rhodium–carbon bond was activated by a stepwise two-photon excitation process, whereas it remained stable upon single-photon excitation with CW red light. A phototoxicity experiment revealed that the methylrhodium(III) Pc complex exhibited photodynamic effect toward HeLa cells.

The combination of the organorhodium(III) centre and the Pc ring provides a new platform for the photouncaging of an alkyl radical under artificial pulsed laser of red light.[§§] The complex was stable under ambient light and underwent activation upon irradiation with a nanosecond pulsed laser, which is easy-to-handle compared with a femtosecond pulsed laser. This principle could be applied to the

photochemical generation of a wide variety of alkyl radicals/aldehydes, realizing the site-selective release of various bioactive molecules. Furthermore, our system could induce effective cell death based on the cooperative action of the photouncaging of an alkyl radical and the photogeneration of ROS. Since the alkyl radical formation is not affected by the oxygen tension, this system could be used to kill hypoxic cells in tumor tissues where conventional PDT fails. On the basis of these advantages, our strategy could open up a new avenue for cancer phototherapy.

K. I. acknowledges support from JSPS KAKENHI Grant Number JP17H06375. K. M. acknowledges supports from JST, ACT-X Grant Number JPMJAX191H and JSPS KAKENHI Grant Number JP19K15582. The computation was performed using Research Center for Computational Science, Okazaki, Japan (Project: 21-IMS-C083).

Conflicts of interest

There are no conflicts to declare.

Notes and references

‡ For gaseous products, their yields were calculated based on those dissolved in the solution, which were detectable by ^{1}H NMR spectroscopy.

§ The singlet oxygen quantum yield (Φ_Δ) of complex **1** was determined to be 0.43 using DPBF method (Fig. S6, DPBF = 1,3-diphenylisobenzofuran).

§§ The introduction of the rhodium(III) centre enabled to stabilize the organometallic Pc complex. Although we synthesized methyl tetra-*tert*-butyl cobalt(III) Pc, the photouncaging reaction was not successful due to the instability of the complex.

1 (a) *Photodynamic Tumor Therapy: 2nd and 3rd Generation Photosensitizers*, ed. J. G. Moser, Harwood Academic Publishers, Amsterdam, 1998; (b) *Photodynamic Therapy*, ed. T. Patrice, The Royal Society of Chemistry, London, 2004.

2 (a) M. Mitsunaga, M. Ogawa, N. Kosaka, L. T. Rosenblum, P. L. Choyke and H. Kobayashi, *Nat. Med.*, 2011, **17**, 1685; (b) W. Piao, K. Hanaoka, T. Fujisawa, S. Takeuchi, T. Komatsu, T. Ueno, T. Terai, T. Tahara, T. Nagano and Y. Urano, *J. Am. Chem. Soc.*, 2017, **139**, 13713; (c) T. Hirayama, A. Mukaimine, K. Nishigaki, H. Tsuboi, S. Hirosawa, K. Okuda, M. Ebihara and H. Nagasawa, *Dalton Trans.*, 2017, **46**, 15991; (d) X. Zhao, J. Liu, J. Fan, H. Chao and X. Peng, *Chem. Soc. Rev.*, 2021, **50**, 4185; (e) G.-X. Xu, E. C.-L. Mak and K. K.-W. Lo, *Inorg. Chem. Front.*, 2021, **8**, 4553.

3 (a) N. Umeda, H. Takahashi, M. Kamiya, T. Ueno, T. Komatsu, T. Terai, K. Hanaoka, T. Nagano and Y. Urano, *ACS Chem. Biol.*, 2014, **9**, 2242; (b) A. P. Gorka, R. R. Nani, J. Zhu, S. Mackem and M. J. Schnermann, *J. Am. Chem. Soc.*, 2014, **136**, 14153; (c) T. Šolomek, J. Wirz and P. Klán, *Acc. Chem. Res.*, 2015, **48**, 3064; (d) T. A. Shell and D. S. Lawrence, *Acc. Chem. Res.*, 2015, **48**, 2866; (e) P. P. Goswami, A. Syed, C. L. Beck, T. R. Albright, K. M. Mahoney, R. Unash, E. A. Smith and A. H. Winter, *J. Am. Chem. Soc.*, 2015, **137**, 3783; (f) E. D. Anderson, A. P. Gorka and M. J. Schnermann, *Nat. Commun.*, 2016, **7**, 1.

4 (a) K. M. Schelkle, C. Schmid, K. Yserentant, M. Bender, I. Wacker, M. Petzoldt, M. Hamburger, D.-P. Herten, R. Wombacher, R. R. Schröder and U. H. F. Bunz, *Angew. Chem. Int. Ed.*, 2017, **56**, 4724; (b) G. Quash, A. M. Roch, J. Chantepie, Y. Michal, G. Fournet and C. Dumontet, *Biochem. J.*, 1995, **305**, 1017; (c) X. He, C. Wu, Y. Cui, H. Zhu, Z. Gao, B. Li, J. Hua and B. Zhao, *Oncotarget*, 2017, **8**, 100128.

5 (a) Z. Chen, Y. Saito, Y. Yoshida and E. Niki, *Biosci. Biotechnol. Biochem.*, 2008, **72**, 1491; (b) S. Shen, C. Zhu, D. Huo, M. Yang, J. Xue and Y. Xia, *Angew. Chem. Int. Ed.*, 2017, **56**, 8801; (c) R. Xia, X. Zheng, X. Hu, S. Liu and Z. Xie, *ACS Appl. Mater. Interfaces*, 2019, **11**, 5782; (d) X. Liu, Y. Yang, M. Ling, R. Sun, M. Zhu, J. Chen, M. Yu, Z. Peng, Z. Yu and X. Liu, *Adv. Funct. Mater.* 2021, 2101709.

6 (a) D. Dolphin, A. W. Johnson and R. Rodrigo, *Ann. N. Y. Acad. Sci.*, 1964, **112**, 590; (b) D. Dolphin, A. W. Johnson and R. Rodrigo, *J. Chem. Soc.*, 1964, 3186; (c) M. Hoshino, K. Yasufuku, H. Seki and H. Yamazaki, *J. Phys. Chem.*, 1985, **89**, 3080; (d) M. J. Kendrick, W. Al-Akhdar, *Inorg. Chem.*, 1987, **26**, 3971; (e) T. Hayashi, K. Okazaki, N. Urakawa, H. Shimakoshi, J. L. Sessler, E. Vogel and Y. Hisaeda, *Organometallics*, 2001, **20**, 3074; (f) K. Imabeppu, H. Kuwano, E. Yutani, H. Kitagishi and K. Kano, *Eur. J. Inorg. Chem.*, 2016, 1784.

7 J. V. Garcia, F. Zhang and P. C. Ford, *Phil. Trans. R. Soc. A*, 2012, **371**, 20120129.

8 (a) T. T. Nyokong, *Pure Appl. Chem.*, 2011, **83**, 1763; (b) J. T. Ferreira, J. Pina, C. A. F. Ribeiro, R. Fernandes, J. P. C. Tomé, M. S. Rodríguez-Morgade and T. Torres, *Chem. Eur. J.*, 2020, **26**, 1789; (c) P.-C. Lo, M. S. Rodriguez-Morgade, R. K. Pandey, D. K. P. Ng, T. Torres and F. Dumoulin, *Chem. Soc. Rev.*, 2020, **49**, 1041; (d) A. Galstyan, *Chem. Eur. J.*, 2021, **27**, 1903.

9 Y. Kitagawa and K. Ishii, *Handbook of Porphyrin Science*, *Vol.* 32, eds. K. M. Kadish, K. M. Smith and R. Guilard, World Scientific Pub Co Inc, Singapore, 2014, pp. 173–270.

10 (a) R. Gale, A. J. McCaffery and M. D. Rowe, *J. Chem. Soc. Dalton Trans.*, 1972, 596; (b) J. Mack and M. J. Stillman, *Coord. Chem. Rev.*, 2001, **219**–**221**, 993; (c) J. Mack, M. J. Stillman and N. Kobayashi, *Coord. Chem. Rev.*, 2007, **251**, 429.

11 R. M. Wilson and K. A. Schnapp, *Chem. Rev.*, 1993, **93**, 223.

12 τ (S_1) of chloro rhodium(III) tetraphenylporphyrin (ClRhTPP) was reported to be < 1 ns. K. Kalyanasundaram, *Chem. Phys. Lett.*, 1983, **104**, 357.

13 (a) V. Cuomo, G. Jori, B. Rihter, M. E. Kenney and M. A. J. Rodgers, *Br. J. Cancer*, 1990, **62**, 966; (b) D. Wörle, M. Shopova, S. Mqller, A. D. Milev, V. N. Mantareva and K. K. Krastev, *J. Photochem. Photobiol. B. Biol.*, 1993, **21**, 155; (c) K. Ishii, A. Takayanagi, S. Shimizu, H. Abe, K. Sogawa and N. Kobayashi, *Free Radic. Biol. Med.*, 2005, **38**, 920; (d) K. Ishii, M. Shiine, Y. Shimizu, S. Hoshino, H. Abe, K. Sogawa and N. Kobayashi, *J. Phys. Chem. B*, 2008, **112**, 3138.

14 The hydrophobic Pc photosensitizers have been shown to localize in membranous organelles such as mitochondria and Golgi apparatus. (a) G. H. Rodal, S. K. Rodal, J. Moan and K. Berg, *J. Photochem. Photobiol. B*, 1998, **45**, 150; (b) N. S. Trivedi, H. -W. Wang, A. -L. Nieminen, N. L. Oleinick and J. A. Izatt, *Photochem. Photobiol.*, 2000, **71**, 634.

15 The concentration of **1** in HeLa cells after cultivation was calculated to be about 19 μM when the cell volume is 2.6×10^3 μm^3; L. Zhao, C. D. Kroenke, J. Song, D. Piwnica-Worms, J. J. H. Ackerman and J. J. Neil, *NMR Biomed.*, 2008, **21**, 159. Formaldehyde is reported to express cytotoxicity when the intracellular concentration is about 1 μM; Y. J. Ke, X. D. Qin, Y. C. Zhang, H. Li, R. Li, J. L. Yuan, X. Yang and S. M. Ding, *Hum. Exp. Toxicol.*, 2014, **33**, 822.

16 S. M. Bishop, A. Beeby, A. W. Parker, M. S. C. Foley and D. Phillips, *J. Photochem. Photobiol. A Chem.*, 1995, **90**, 39.

17 P. Mroz, A. Yaroslavsky, G. B. Kharkwal and M. R. Hamblin, Cancers (Basel), 2011, **3**, 2516.

18 Alkyl radical is known to induce apoptosis by causing oxidative damage to lipids and proteins even in a hypoxic conditions; *see* ref. 5. Formaldehyde is also known to induce apoptosis by nucleophilic alkylation and crosslinking of biological tissues; (a) B. Szende and E. Tyihák, *Cell Biol. Int.*, 2010, **34**, 1273; (b) G. L. Finch and L. A. Burns-Naas, Cancer Chemotherapeutic Agents in *Encyclopaedias of Toxicology 3rd edition*, ed. P. Wexler, Academic Press, Cambridge, 2014, pp. 630–641.

