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De Novo Generation of Singlet Oxygen and Ammine Ligands by Photoactivation of a Platinum Anticancer Complex**

Yao Zhao, Nicola J. Farrer, Huilin Li, Jennifer S. Butler, Ruth J. McQuitty, Abraha Habtemariam, Fuyi Wang,* and Peter J. Sadler*

The potential for spacial selectivity, as offered by photoactivation, together with novel excited-state chemistry and accompanying mechanisms of action make exploration of photoactivated metal chemotherapeutic complexes attractive for cancer therapy.^[1] We have been studying Pt^{IV}-diazidodihydroxido anticancer complexes, [Pt(N₃)₂(OH)₂(Am₁)(Am₂)] (Am₁/Am₂ = am(m)ines),^[2] which, in the absence of light, exhibit minimal cytotoxicity towards cancer cells and do not react with glutathione (GSH), 5'-guanosine monophosphate (5'-GMP), or DNA in either cell-free media or aqueous solutions. By contrast, upon irradiation with UVA or visible (blue/green) light, these complexes display potent cytotoxicity towards a range of cancer cell lines. The reported photodecomposition products include azide anions (N₃⁻), azidyl radicals (N₃[•]), nitrogen gas (N₂), and oxygen gas (O₂).^[3] A particularly potent photocytotoxic anticancer complex is *trans,trans,trans*-[Pt(N₃)₂(OH)₂(MA)(Py)] (**1**, MA = methylamine, Py = pyridine).^[4] Herein we report the unprecedented oxidation of 5'-GMP by **1** upon irradiation with UVA and the identification of some unexpected reaction pathways involving singlet oxygen (¹O₂) and nitrene (Pt-N) intermediates. The source of the ¹O₂ was also investigated.

The photoreaction of **1** with 5'-GMP upon irradiation with blue light with a wavelength of 450 nm for 1 h gave (*SP*-4-2)-

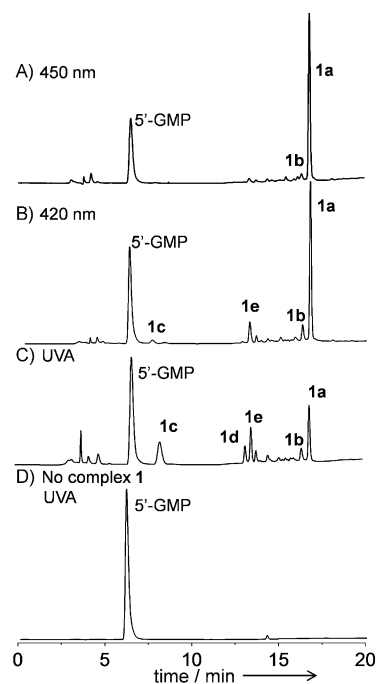


Figure 1. Chromatograms for the photoreactions of **1** (0.67 mM) with 5'-GMP (1.0 mM) in aqueous solution upon irradiation with A) 450 nm light, 50 mWcm⁻², 60 min; B) 420 nm, 4.3 mWcm⁻², 30 min; C) UVA (365 nm), 3.5 mWcm⁻², 15 min; D) 5'-GMP (1.0 mM) only, UVA, 15 min.

[Pt(N₃)(MA)(Py)(5'-GMP)]⁺ (**1a**⁺) as the major product and *trans*-[Pt(MA)(Py)(5'-GMP)₂]²⁺ (**1b**²⁺) as the minor product (Figure 1 A). A similar result has been reported for **1** under slightly different conditions, and for related compounds.^[2a,4,5] However, when the reaction mixture was irradiated at 420 nm for 30 min, two new photoproducts, **1c** and **1e**, were observed by HPLC (Figure 1 B). When the sample was irradiated with UVA (365 nm) for 15 min, one more species, **1d**, was found (Figure 1 C). The isotopic distributions observed in the ESI-MS analysis revealed that the singly charged cations of **1c** (*m/z*=718.1), **1d** (*m/z*=684.1), and **1e** (*m/z*=700.1) all contain a Pt atom. A control experiment with 5'-GMP in the absence of **1** and UVA irradiation for 15 min showed no reaction (Figure 1 D), thus suggesting that 5'-GMP is stable under these conditions. The photoreaction of **1** and 5'-GMP under an atmosphere of argon gave similar results, thereby excluding the possibility that dissolved oxygen is the oxidant. Hence, **1c**, **1d**, and **1e** are all produced by the photoreaction of **1**.

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Table 1: Positive ions for complexes **1a**, **1c**, **1d**, and **1e** observed by HRMS and the corresponding assignments.

Found m/z	710.1205	718.1337	684.1228	700.1235
Proposed chemical structure				
Theoretical m/z	710.1164	718.1314	684.1260	700.1209
Error [ppm]	5.8	3.2	4.7	3.7

High-resolution MS of **1a**, **1c**, **1d**, and **1e**, as well as their tandem MS (CID), were performed to examine further the structure of each species (see Figures S1 and S2 in the Supporting Information). They are all assigned as Pt^{II} complexes, and their structures are listed in Table 1, while fragment ions are listed in Tables S1–S4 in the Supporting Information, together with their assignments.

Curiously, species **1c**, **1d**, and **1e** all have an NH₃ ligand, which was not present in the reactants. A reasonable source of the NH₃ is the {Pt-N₃} fragment, which can lose N₂ upon irradiation with light to form a {Pt-N} nitrene intermediate.^[6] This postulation was verified by using **1***, azide *trans,trans-trans*-[Pt(N₃^{*})₂(OH)₂(MA)(Py)], where N₃^{*} = [¹⁵N=¹⁴N=¹⁴N], and hence each bound N atom from the azide is now 50% ¹⁵N. The photoreaction of **1*** with 5'-GMP, carried out under identical conditions, gave an identical chromatogram as that shown in Figure 1C. The ESI-MS spectra for **1a***, **1c***, **1d***, and **1e***, which have the same retention times as **1a**, **1c**, **1d**, and **1e**, are shown in Figure S5 in the Supporting Information. The molecular weight of compound **1a*** was 1 Da larger than **1a**, thus suggesting that it has an intact N₃⁻ ligand. The isotope distributions of **1c***, **1d***, and **1e*** indicate that they are all 50%/50% mixtures of [M] and [M + 1], so they are all considered as {Pt-NH₃^{*}} fragments derived from {Pt-N₃^{*}} (NH₃^{*} = 50% ¹⁵NH₃/50% ¹⁴NH₃). This result suggests that N₂ gas is released directly from {Pt-N₃}, thereby generating a {Pt-N} intermediate.

The release of N₂ on photolysis of **1** was verified by ¹⁴N NMR spectroscopy (see Figure S6 in the Supporting Information); signals for free azide (N₃⁻) were also detected (see the Supporting Information for details). N₂ may be released directly from {Pt-N₃} and also may be formed from the recombination of the azidyl radicals (N₃[•]) generated in this photoreaction.^[7] The release of N₃[•] was confirmed by EPR spectroscopy by using 5,5-dimethylpyrroline-*N*-oxide (DMPO) as the spin trap. Signals for the DMPO-¹⁴N₃ spin adduct were detected (see Figure S7 in the Supporting Information), but hydroxyl radicals OH[•] were not trapped. It is evident that 5'-GMP is not oxidized by N₃[•]; as its presence did not affect the trapping of N₃[•] radicals (see the Supporting Information for details).

Species **1e** contains an 8-hydroxyguanine (8-OH-G) fragment, equivalent to 8-oxoguanine (8-oxo-G), which is one of the most common products of DNA oxidation.^[8] The

possibility that the oxidation of guanine by photoactivated **1** involved singlet oxygen or nitrene intermediates was investigated. Product **1c** has a similar structure as **1e**, but the 8-OH-G is replaced by RedSp (*N*-formylamidoiminohydantoin),^[8a] hydrolyzed 8-OH-G (shown in Scheme S1 in the Supporting Information).

During the photoreaction of **1** and 5'-GMP with irradiation at 450 nm the yellow color of the solution became darker, and gas bubbles formed (see Figure S8 in the Supporting Information). Similar results were obtained when the reaction was triggered with UVA. The evolution of the gases O₂ and N₂ was verified by GC-MS performed in ¹⁸O-labeled water under an argon atmosphere, with the aim also of examining the source of the O atoms in the generated O₂. After the photolysis of **1**, the gas phase was analyzed by GC-MS (see Figure S9 and the Supporting Information for details), and ¹⁶O₂ ($m/z = 32$) and N₂ ($m/z = 28$) were both found. No ¹⁸O-substituted O₂ was detected. This result verified the release of N₂ and O₂ and, moreover, provided evidence that the two oxygen atoms in the generated O₂ are both from **1** rather than from the solvent.

The nature of the released oxygen was investigated using a fluorescence probe for singlet oxygen: SOSG. SOSG is a highly selective sensor for ¹O₂ without any appreciable response to hydroxyl radicals or superoxide.^[9] In the absence of ¹O₂, SOSG exhibits low fluorescence, but in the presence of ¹O₂, strong green fluorescence can be observed with $\lambda_{\text{ex}} = 504$ nm and $\lambda_{\text{em}} = 525$ nm. Solutions containing **1** and SOSG were stable in the dark or even upon irradiation at $\lambda \geq 504$ nm. However, when exposed to weak irradiation at 365 nm (21 $\mu\text{W cm}^{-2}$), the intensity of the fluorescence at 525 nm increased rapidly, thus indicating that ¹O₂ was generated (Figure 2). The dose-dependent efficiency of generating ¹O₂ upon irradiation was higher the shorter the wavelength (UVA > 420 nm > 450 nm, see Figure S10 in the Supporting Information). Control experiments carried out in the dark or in the absence of **1** showed no change in the fluorescence intensity (see Figure S10 in the Supporting Information). A sample saturated with argon was irradiated at 365 nm and gave stronger fluorescence (Figure 2). This result revealed that the ¹O₂ was not generated from the dissolved O₂ through energy transfer from a photosensitizer. N₂ had a similar effect as argon. Neither argon nor N₂ itself could trigger the fluorescence of SOSG. The release of singlet

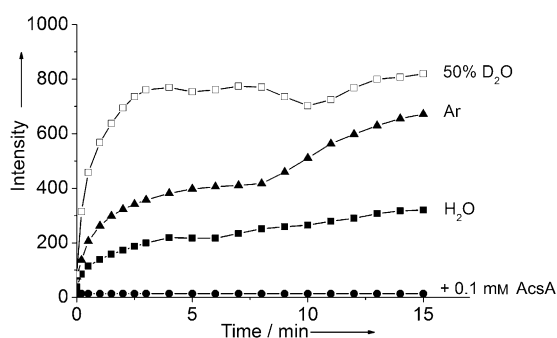


Figure 2. Time-dependent fluorescence ($\lambda_{\text{ex}}/\lambda_{\text{em}} = 504/525$ nm) from **1** ($50 \mu\text{M}$) and SOSG ($1 \mu\text{M}$) in H_2O (3% MeOH) upon weak irradiation at 365 nm ($21 \mu\text{Wcm}^{-2}$) (293 K). ■: no additive; □: 50% D_2O ; ▲: saturated with argon; ●: 0.1 mM L-ascorbic acid (AscA). All the data points were the average of 2–4 independent experiments.

oxygen from a Pt^{IV} -diazidodihydroxido complex upon irradiation with light in the absence of any exogenous source of oxygen gas appears to be unprecedented.

The lifetime of $^1\text{O}_2$ in D_2O is known to be much longer than that in H_2O .^[10] Indeed, the fluorescence intensity arising from the reaction of **1** with SOSG in 50% D_2O with $\lambda_{\text{irr}} = 365$ nm was three- to fourfold higher than that of the reaction carried out in H_2O alone (Figure 2). The reaction was repeated in the presence of $100 \mu\text{M}$ L-ascorbic acid (AscA) as a $^1\text{O}_2$ scavenger. Complex **1** does not react with AscA in the absence of light, even though AscA is a strong reductant. However, upon irradiation at 365 nm, the fluorescence was totally quenched (Figure 2). These results again confirmed the generation of $^1\text{O}_2$.

Guanine and the other nucleobases may be oxidatively damaged by reactive oxygen species (ROS), radicals, and ionizing/UVA radiation.^[11] The oxidation of guanine usually leads to DNA damage so as to cause lethality, aging, and mutagenicity.^[8] Although it has been reported that several

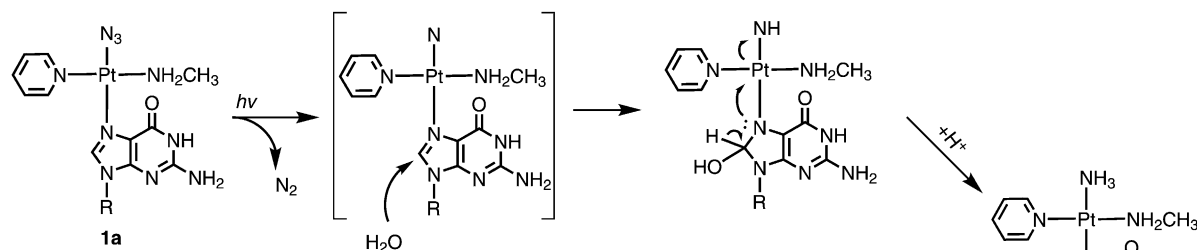
Pt^{IV} -tetrachlorido complexes can directly oxidize guanine,^[12] we report here for the first time that the photodecomposition of a Pt^{IV} -diazidodihydroxido complex can oxidize guanine. Complexes containing Pt^{II} and oxidized guanine as 8-OH-G and RedSp were detected (Table 1).

Two pathways can be proposed for the oxidation of guanine. The first involves a nitrene intermediate (Scheme 1, Mechanism 1). This can arise from loss of N_2 from the $\{\text{Pt}-\text{N}_3\}$ fragment upon irradiation with UVA.^[6] Two electrons are transferred from guanine to the nitrene, and the guanine itself is oxidized to give 8-OH-G on addition of H_2O . The nitrene is reduced and finally forms the ammine adduct $\{\text{Pt}-\text{NH}_3\}$. Another possible oxidant is singlet oxygen. The $^1\text{O}_2$ generated by photolysis of **1** can oxidize guanine to 8-OH-G by a direct [4+2] cycloaddition (Scheme 1, Mechanism 2).^[8a]

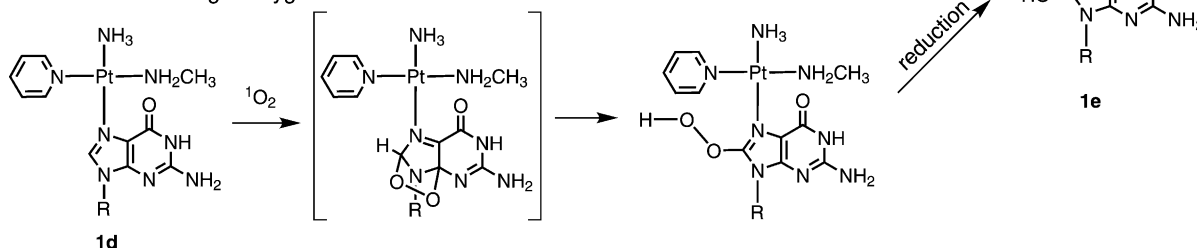
Singlet oxygen ($^1\text{O}_2$) is a highly reactive and toxic species in biological systems. It is considered the principal antiproliferative species in photodynamic therapy (PDT), in which it reacts with many biomolecules, including nucleic acids, proteins, and lipids, thereby causing cancer cell death.^[8,13] A common method to generate $^1\text{O}_2$ is photosensitization, which produces $^1\text{O}_2$ by energy transfer from a photoexcited sensitizer to ground-state triplet oxygen ($^3\text{O}_2$). This strategy is the basis of current PDT, but requires the presence of oxygen at the target site. However, tumor cells are often hypoxic. In contrast, the generation of $^1\text{O}_2$ from *trans,trans,trans*- $[\text{Pt}(\text{N}_3)_2(\text{OH})_2(\text{MA})(\text{Py})]$ upon irradiation with UVA/blue light does not require any exogenous source of oxygen gas (Figure 2). This feature may be beneficial for the potential clinical application of **1** and killing of hypoxic cancer cells. Moreover, the wavelength can be tuned to control its photocytotoxicity, as shorter wavelengths generate more $^1\text{O}_2$.

Since neither of the oxygen atoms in the released $^1\text{O}_2$ originate from water, the most plausible source is the OH groups of **1**. Furthermore, solvent substitution at Pt^{IV} is not likely to occur before its reduction to Pt^{II} . A possible photolysis mechanism is given in Scheme 2. Upon irradiation

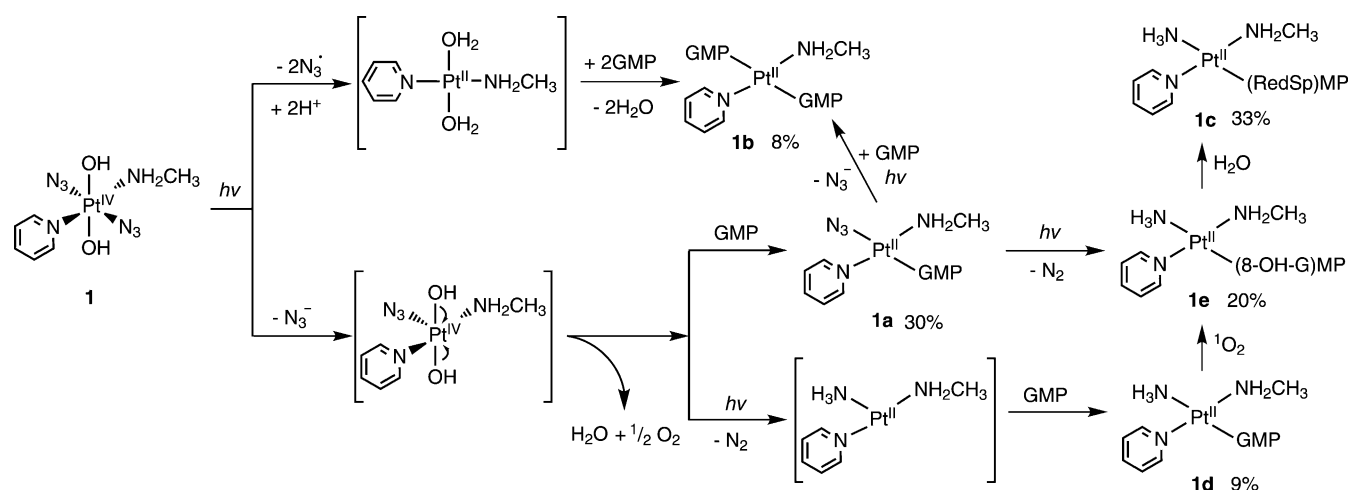
Mechanism 1 – Nitrene



Mechanism 2 – Singlet oxygen



Scheme 1. Two possible mechanisms for the oxidation of 5'-GMP. Charges are omitted for clarity.



Scheme 2. Possible mechanisms for the photoreaction of **1** with 5'-GMP upon irradiation with UVA. Species in square brackets are unstable intermediates. Charges are omitted for clarity. Species percentages are average HPLC integrations for four experiments with UVA irradiation (Figure 1 C).

with short-wavelength light, such as UVA, photodecomposition of **1** in the presence of 5'-GMP may occur through two pathways. In the first, two azidyl radicals (N₃[•]) are lost and Pt^{IV} is reduced to Pt^{II}. The OH groups are protonated and thus are substituted by 5'-GMP to give product **1b**. The second pathway is more complicated, but perhaps more likely. The integration of the HPLC peaks in Figure 1 C suggested that about 92% of the Pt species were produced through this pathway. Complex **1** loses one azide ligand (N₃⁻) and the OH ligands donate one electron each to reduce the Pt^{IV} to Pt^{II}; they then rapidly recombine to generate oxygen gas, as singlet oxygen, through H₂O₂ formation.^[14] Then the binding of 5'-GMP to Pt produces **1a**, which can be further transformed to **1e** by loss of N₂ gas from the N₃ ligand, as in Scheme 1. Alternately, if N₂ is expelled from the N₃ ligand before 5'-GMP binds, **1d** is produced, which can also be transformed to **1e** by reacting with ¹O₂ (Scheme 1).

There are a number of reports of the chemical reduction of Pt^{IV} to Pt^{II}, and it is widely accepted that a concerted two-electron transfer from, for example, ascorbate, GSH, or guanine, to Pt^{IV} is involved.^[12,15] However, the photoreduction of **1** may not follow the above pathway. Pt^{IV} is more likely to gain one electron from each of the two N₃ or two OH ligands and give rise to N₃[•] or OH[•] radicals, respectively. We were not able to trap OH[•] radicals, perhaps because their lifetime is too short.

The in situ formation of an NH₃ ligand can give rise to potential hydrogen-bonding interactions with DNA. DNA adducts of the type **1d**, for example, may strongly inhibit RNA polymerase II and nucleotide excision repair.^[16] Nitrenes are highly reactive intermediates and are reported to be responsible for a wide range of DNA lesions.^[17] N₃[•] is a relatively mild and selective oxidant that can oxidize amino acids such as tryptophan.^[3b] N₃⁻ is a mitochondrial inhibitor, and a myeloperoxidase and catalase inhibitor. These species, together with ¹O₂, could all contribute to the potent photoantiproliferative effect of **1** on cancer cells.

In summary, we observed the unexpected oxidation of guanine during the photoreaction of complex **1** (*trans,trans*-[Pt(N₃)₂(OH)₂(MA)(Py)]) with 5'-GMP. The photodecomposition of **1** involves Pt-nitrene intermediates and formation of singlet oxygen, free azide, azidyl radicals, and nitrogen gas. The oxidation of guanine is likely to arise from reactions of singlet oxygen and nitrene intermediates. The generation of singlet oxygen in the absence of oxygen gas and the oxidative damage to guanine may contribute to the potent photocytotoxic effects of this complex.

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- [1] a) A. Kastl, S. Dieckmann, K. Wähler, T. Völker, L. Kastl, A. L. Merkel, A. Vultur, B. Shannan, K. Harms, M. Ocker, W. J. Parak, M. Herlyn, E. Meggers, *ChemMedChem* **2013**, *8*, 924–927; b) Z. Li, S. J. Burya, C. Turro, K. R. Dunbar, *Philos. Trans. R. Soc. London Ser. A* **2013**, *371*, 20120128; c) S. Banerjee, P. Prasad, A. Hussain, I. Khan, P. Kondaiah, A. R. Chakravarty, *Chem. Commun.* **2012**, *48*, 7702–7704; d) W. Vanderlinden, M. Blunt, C. C. David, C. Moucheron, A. Kirsch-De Mesmaeker, S. De Feyter, *J. Am. Chem. Soc.* **2012**, *134*, 10214–10221; e) F. Schmitt, P. Govindaswamy, G. Süß-Fink, W. H. Ang, P. J. Dyson, L. Juillerat-Jeanneret, B. Therrien, *J. Med. Chem.* **2008**, *51*, 1811–1816; f) D. Crespy, K. Landfester, U. S. Schubert, A. Schiller, *Chem. Commun.* **2010**, *46*, 6651–6662; g) B. S. Howerston, D. K. Heidary, E. C. Glazer, *J. Am. Chem. Soc.* **2012**, *134*, 8324–8327; h) P.-K. Lee, W. H.-T. Law, H.-W. Liu, K. K.-W. Lo, *Inorg. Chem.* **2011**, *50*, 8570–8579; i) S. L. H. Higgins, A. J. Tucker, B. S. J. Winkel, K. J. Brewer, *Chem. Commun.* **2012**, *48*, 67–69; j) N. A. Smith, P. J. Sadler, *Philos. Trans. R. Soc. London Ser. A* **2013**, *371*, 20120519.
- [2] a) F. S. Mackay, J. A. Woods, P. Heringova, J. Kasparkova, A. M. Pizarro, S. A. Moggach, S. Parsons, V. Brabec, P. J. Sadler, *Proc. Natl. Acad. Sci. USA* **2007**, *104*, 20743–20748; b) N. J. Farrer,

- J. A. Woods, V. P. Munk, F. S. Mackay, P. J. Sadler, *Chem. Res. Toxicol.* **2010**, *23*, 413–421; c) A. F. Westendorf, J. A. Woods, K. Korpis, N. J. Farrer, L. Salassa, K. Robinson, V. Appleyard, K. Murray, R. Grünert, A. M. Thompson, P. J. Sadler, P. J. Bednarski, *Mol. Cancer Ther.* **2012**, *11*, 1894–1904.
- [3] a) L. Ronconi, P. J. Sadler, *Dalton Trans.* **2011**, *40*, 262–268; b) J. S. Butler, J. A. Woods, N. J. Farrer, M. E. Newton, P. J. Sadler, *J. Am. Chem. Soc.* **2012**, *134*, 16508–16511.
- [4] Y. Zhao, J. A. Woods, N. J. Farrer, K. S. Robinson, J. Pracharova, J. Kasparkova, O. Novakova, H. Li, L. Salassa, A. M. Pizarro, G. J. Clarkson, L. Song, V. Brabec, P. J. Sadler, *Chem. Eur. J.* **2013**, *19*, 9578–9591.
- [5] N. J. Farrer, J. A. Woods, L. Salassa, Y. Zhao, K. S. Robinson, G. Clarkson, F. S. Mackay, P. J. Sadler, *Angew. Chem.* **2010**, *122*, 9089–9092; *Angew. Chem. Int. Ed.* **2010**, *49*, 8905–8908.
- [6] J. Šima, *Coord. Chem. Rev.* **2006**, *250*, 2325–2334.
- [7] S. J. David, R. D. Coombe, *J. Phys. Chem.* **1986**, *90*, 3260–3263.
- [8] a) G. Pratiel, B. Meunier, *Chem. Eur. J.* **2006**, *12*, 6018–6030; b) S. Kanvah, J. Joseph, G. B. Schuster, R. N. Barnett, C. L. Cleveland, U. Landman, *Acc. Chem. Res.* **2010**, *43*, 280–287.
- [9] A. Gollmer, J. Arnbjerg, F. H. Blaikie, B. W. Pedersen, T. Breitenbach, K. Daasbjerg, M. Glasius, P. R. Ogilby, *Photochem. Photobiol.* **2011**, *87*, 671–679.
- [10] J. R. Hurst, J. D. McDonald, G. B. Schuster, *J. Am. Chem. Soc.* **1982**, *104*, 2065–2067.
- [11] J. Cadet, T. Douki, J. L. Ravanat, *Acc. Chem. Res.* **2008**, *41*, 1075–1083.
- [12] S. Choi, R. B. Cooley, A. S. Hakemian, Y. C. Larrabee, R. C. Bunt, S. D. Maupas, J. G. Muller, C. J. Burrows, *J. Am. Chem. Soc.* **2004**, *126*, 591–598.
- [13] P. R. Ogilby, *Chem. Soc. Rev.* **2010**, *39*, 3181–3209.
- [14] J. Wahlen, S. De Hertogh, D. E. De Vos, V. Nardello, S. Bogaert, J.-M. Aubry, P. L. Alsters, P. A. Jacobs, *J. Catal.* **2005**, *233*, 422–433.
- [15] J. Z. Zhang, E. Wexselblatt, T. W. Hambley, D. Gibson, *Chem. Commun.* **2012**, *48*, 847–849.
- [16] a) G. Zhu, M. Myint, W. H. Ang, L. Song, S. J. Lippard, *Cancer Res.* **2012**, *72*, 790–800; b) J. Pracharova, L. Zerzankova, J. Stepankova, O. Novakova, N. J. Farrer, P. J. Sadler, V. Brabec, J. Kasparkova, *Chem. Res. Toxicol.* **2012**, *25*, 1099–1111.
- [17] Z. Guo, J. Xue, Z. Ke, D. L. Phillips, C. Zhao, *J. Phys. Chem. B* **2009**, *113*, 6528–6532.