ChemComm
Chemical Communications

Accepted Manuscript

This article can be cited before page numbers have been issued, to do this please use: S. Dutta, J. Rühle, M. Schikora, N. Deussner-Helfmann, M. Heilemann, T. S. Zatsepin, P. Duchstein, D. Zahn, G. Knör and A. Mokhir, *Chem. Commun.*, 2020, DOI: 10.1039/D0CC03086D.

This is an Accepted Manuscript, which has been through the Royal Society of Chemistry peer review process and has been accepted for publication.

Accepted Manuscripts are published online shortly after acceptance, before technical editing, formatting and proof reading. Using this free service, authors can make their results available to the community, in citable form, before we publish the edited article. We will replace this Accepted Manuscript with the edited and formatted Advance Article as soon as it is available.

You can find more information about Accepted Manuscripts in the Information for Authors.

Please note that technical editing may introduce minor changes to the text and/or graphics, which may alter content. The journal's standard Terms & Conditions and the Ethical guidelines still apply. In no event shall the Royal Society of Chemistry be held responsible for any errors or omissions in this Accepted Manuscript or any consequences arising from the use of any information it contains.

View Article Online
DOI: 10.1039/D0CC03086D

COMMUNICATION

Red light-triggered photoreduction on a nucleic acid template

Subrata Dutta,[a] Jennifer Rühler,[a] Margot Schikora,[a†] Nina Deussner-Helfmann,[b] Mike Heilemann,[b] Timofei Zatsepin,[c,d] Patrick Duchstein,[e] Dirk Zahn,[e] Günther Knör[f] and Andriy Mokhir*[a]

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

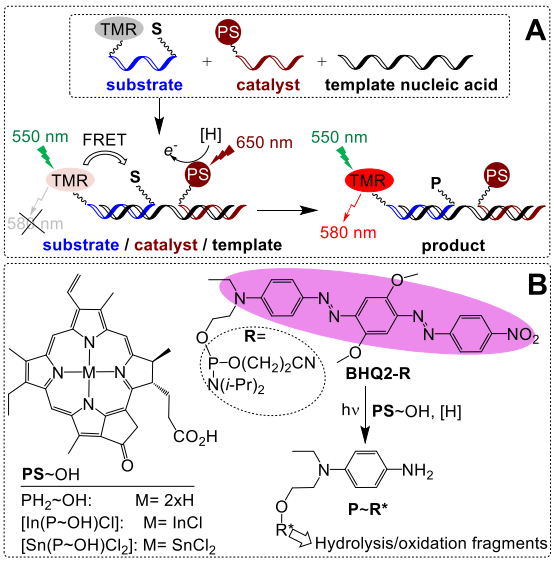
DOI: 10.1039/x0xx00000x

Conjugate Sn(IV)(pyropheophorbide)dichloride~(peptide nucleic acid) catalyzes reduction of azobenzene derivatives in the presence of complementary nucleic acid (NA) upon irridiation with red light (660 nm). This is the first red light-induced NA-templated photoreduction. It is highly sensitive to single mismatches in the NA-template and can detect down to 5 nM NAs.

Templated reactions are applied for detection of NAs in various bioanalytical assays.[1] Photochemical reactions of this type have an advantage of the possibility of temporal and spatial control.[2,3] Numerous UV-light driven reactions are known.[1-3] However, their usefulness is limited by the toxicity of UV-light to cells.[4-6] This issue stimulated the research towards development of systems sensitive to visible light. Important known examples include (a) photoreduction of organic azides and N-alkylpyridinium salts mediated by a [Ru(bpy)$_2$phen] in the presence of reducing agents triggered by 455 nm-light,[7-11] (b) Cy3-sensitized oxidation of radical species derived from dyes that is triggered by 532 nm-light,[12,13] and (c) oxidation of organic substrates mediated by singlet oxygen ($^1O_2$).[14-17] The latter reaction is photosensitizer (PS) dependent. For example, we have demonstrated that In(pyropheophorbide-a)chloride ([In(P~OH)Cl], Scheme 1) is an efficient catalyst, whose activity can be triggered by ~650 nm light. The $^1O_2$-mediated chemistry has an intrinsic drawback of generating a toxic mediator $^1O_2$. Due to its rather long lifetime of 3-4 μs in aqueous solution,[18,19] $^1O_2$ can migrate ≥100 nm thereby damaging biomolecules. A solution of this problem could be in using a mediator with a shorter lifetime, e.g. a photochemically generated electron. Templated reactions mediated by electron transfer have been developed by Winssinger group.[7-11] These are catalyzed exclusively by a [Ru(bpy)$_2$phen], which is excited by 455 nm light. The latter trigger is still toxic to cells,[4-6] that can limit applications of these reactions.

In this paper we describe the first red light-triggered NA-templated photoreduction (Scheme 1A). We selected [In(P~OH)Cl] and [Sn(P~OH)Cl$_2$] (Scheme 1B) as possible photocatalysts based on the following considerations. Pyropheophorbide a (PH$_2$~OH) is an accessible chlorin derived from chlorophyll. It has a strong absorbance band in the spectral region between 600 and 700 nm. Chlorophyll metabolites are known to catalyse photoreduction of organic compounds, e.g. ubiquinone in blood plasma.[20] Moreover, chlorophylls linked to a CO_2-reducing complex catalyse photoreduction of CO_2.[21] PH$_2$~OH as well as its known Mg(II) and Zn(II) complexes are photounstable, whereas its Pd(II) and Pt(II) complexes

[a] Department of Chemistry and Pharmacy, Organic Chemistry II, Friedrich-Alexander-University of Erlangen-Nürnberg (FAU), 91058 Erlangen, Germany.
[b] Institute of Physical and Theoretical Chemistry, Johann Wolfgang Goethe-University, 60438 Frankfurt, Germany.
[c] Skolkovo Institute of Science and Technology, Moscow, 126046, Russia.
[d] Chemistry Department, M.V.Lomonosov Moscow State University, Leninskie gory, 1-3,Moscow 119992, Russia.
[e] Computer-Chemistry-Center, Department of Chemistry and Pharmacy, Friedrich-Alexander University Erlangen-Nürnberg (FAU), 91052 Erlangen, Germany
[f] Johannes Kepler University Linz, Institute of Inorganic Chemistry, Austria.
Electronic Supplementary Information (ESI) available: [details of any supplementary information available should be included here]. See DOI: 10.1039/x0xx00000x



Scheme 1. **A**: A nucleic acid-templated photoreduction reaction triggered by red light; Substrate: an oligodeoxyribonucleotide (ODN) conjugate carrying 3'-TMR (N,N,N',N'-tetramethylrhodamine) and 5'-S, where S is an azobenzene dye (e.g. S1 and S2 in Scheme 2); Catalyst: conjugate of ODN or peptide nucleic acid (PNA) with a photosensitizer PS (its structure is shown in inset B). **B**: Reduction of azobenzene derivative BHQ2~R catalyzed by a photosensitizer PS~OH upon irradiation with red light (hν) with formation of a colourless product P-R*; [H]: bulk reducing agent (sodium ascorbate).

are prone to aggregation and their synthesis is low yielding.[23-25] In contrast, both In(III) and Sn(IV) complexes of PH_2~OH are substantially more photostable and can be obtained in relatively high yield. [In(P~OH)Cl] has been previously studied in the group of Mokhir as a photosensitizer for $^1O_2$ generation.[23-29] Furthermore, Knör and co-workers have demonstrated that Sn(chlorin)X_2 (X= OH, Cl) generated *in situ* from the corresponding Sn-porphyrins can act as light-harvesting sensitizers by recycling rhodium hydride $[Cp^*Rh(bpy)H]^+$.[22]

We prepared [In(P~OH)Cl] as previously described.[23] The protocol for synthesis of [Sn(P~OH)Cl_2] is provided in the supporting information (*SI*). Analogous Sn complexes with chlorine e6[30] and related ligands[31] were known.

UV-visible spectra of PH_2~OH, [In(P~OH)Cl] and [Sn(P~OH)Cl_2] dissolved in CH_3CN are shown in Figure S17 (left plot, *SI*). They all feature a strong absorbance band at ~655 nm and, therefore, were expected to be well responsive to red light. We found that the fluorescence from the complexes was weaker than that of the ligand under similar conditions (Figure S17, right plot). This indicates the higher triplet (^{3}PS*) quantum yield in [In(P~OH)Cl] and [Sn(P~OH)Cl_2]. UV-visible spectra of the complexes are practically not changed when CH_3CN solvent is replaced with aqueous buffered at pH 7 solution (Figure S18). However, the dependences of intensities of absorbance bands at 415 and 655 nm from the concentration of the complexes PS~OH deviate significantly from Beer-Lambert's law at [PS~OH]> 2.5 μM. These data indicate aggregation of these coordination compounds. Therefore, when possible, all experiments described in this paper were conducted at [PS~OH]< 2.5 μM.

Photoreductive properties of PS~OH's rely on the formation of their long-lived triplet state (^{3}PS*) upon the excitation with red light (Figure S19). In the absence of bulk reducing agents the ^{3}PS* is converted back to the ground state mainly by the energy transfer to $^3O_2$ leading to formation of $^1O_2$. Thus, the population of ^{3}PS* can be estimated by quantification of $^1O_2$. We observed that both [Sn(P~OH)Cl_2] and [In(P~OH)Cl] are efficient catalysts for $^1O_2$ generation (Figure S19) indicating that their triplet states are populated upon their excitation with red light.

Next, we investigated photocatalytic properties of [Sn(P~OH)Cl_2] in aqueous buffered solutions containing sodium ascorbate (10 mM) as a bulk reducing agent. We selected azo dye BHQ2-R (Scheme 1B) as an organic substrate. In control experiments we confirmed that in aqueous solutions BHQ2-R (10 μM) is photostable when irradiated with red light for 30 min (Figure S20). [Sn(P~OH)Cl_2] is partially (~25 %) bleached under these conditions (Figure S21). A mixture of BHQ2-R (10 μM) and [Sn(P~OH)Cl_2] (2 μM) was found to be stable for at least 30 min when kept in the dark. However, when the latter mixture was irradiated with red light, BHQ2-R was fully decomposed in 15 min (plot "BHQ2-R + [Sn(P~OH)Cl_2] / +hν", Figure S20). The complete conversion of BHQ2-R under these conditions indicates that [Sn(P~OH)Cl_2] can reach at least 5 catalytic turnovers. We found that another azo dye BHQ1-R is also photoreduced as efficiently as its analogue BHQ2-R (Figure S22). Unexpectedly, [In(P~OH)Cl] is not a catalyst of BHQ1-R and BHQ2-R photoreduction.

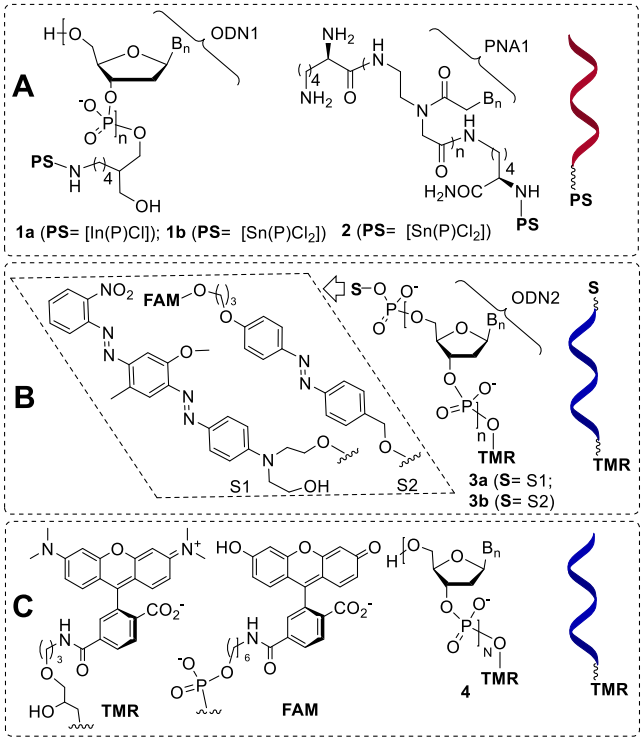
According to the study of PS~OH/BHQ1 interactions by molecular dynamics simulations (Figures S23, S24), [In(P~OH)Cl] can form a non-covalent complex with the substrate due to hydrophobic segregation and π-π-stacking interactions. However, the aromatic systems of the catalyst and the substrate are laterally shifted leading to less efficient overlap (Figure S23, left). In contrast, in the modelled [Sn(P~OH)Cl_2]/BHQ-1 complex both aromatic systems stack over one another that should enable the efficient photoinduced electron transfer from the excited state of [Sn(P~OH)Cl_2] to the substrate (Figure S23, right). Furthermore, we observed that ascorbate inhibits [Sn(P~OH)Cl_2] much more efficiently than [In(P~OH)Cl] in the reaction of $^1O_2$ formation (Figure S25). Correspondingly, photoreduction pathway in the former case is more preferable than $^1O_2$ generation. A possible reason for that is stronger binding of ascorbate to the more electron deficient Sn(IV) centre.

Next, we confirmed that the activity of [Sn(P~OH)Cl_2] is not limited to photoreduction of azo dyes. This catalyst can induce conversion of fluorescein to its leuco form and transform aromatic azides to amines (Figure S26). However, one has to apply a substantial excess of the catalyst (10 eq) to enable these reactions, whereas the reduction of BHQ2-R and BHQ1-R occurs even in the presence of only 0.2 eq catalyst.

To evaluate whether the photoreduction of azo dyes could be conducted in the nucleic acid-templated fashion, we prepared conjugates of the catalysts and the substrates with either oligodeoxyribonucleotides (ODNs) or their analogues peptide nucleic acids (PNAs). Both ODNs and PNAs are able to bind to target nucleic acids in a sequence specific manner thereby supporting the templated reactions (Scheme 1A). First, we synthesized conjugate **1a** containing [In(P)Cl] attached to the 3'-terminus of ODN1 (Schemes 1, 2) as described elsewhere.[23-25]

View Article Online DOI: 10.1039/D0CC05086D

This compound was used as a negative control. Attempts to obtain analogous conjugate containing [Sn(P)Cl$_2$] (**1b**) were not successful due to oxidative decomposition of [Sn(P)Cl$_2$] during the synthesis. We solved this problem by replacing ODN1 for peptide nucleic acid PNA1. Resulting conjugate **2** was successfully obtained by solid phase synthesis as described in the *SI*. Next, we prepared two substrates **3a** and **3b**, where azobenzene derivatives S1 and S2 were attached to the 5'-terminus of ODN2 (Schemes 2, 3)



Scheme 2. Structures of catalysts (inset **A**), substrates (inset **B**) as well as controls (inset C) used in this study. Structures of dyes TMR and FAM are shown in inset C. Sequences of ODN1 and ODN2 as well as PNA1 are given in Scheme 3.

	ODN2:	3'- TGTTACTTCT
	PNA1:	C-teminus- AGTTCT
Templates	DNA2:	5'- ACAATGAAGATCAAGA
	DNA2-T:	5'- ACAATGAAGA *T* TCAAGA
	DNA2-TT:	5'- ACAATGAAGA *TT* TCAAGA
	DNA2-TTT:	5'- ACAATGAAGA *TTT* TCAAGA
	DNA2-mm1:	5'- ACAAT *C* AAGATCAAGA
	DNA2-mm2:	5'- ACAATGAA *C* ATCAAGA
	DNA1:	5'- ACAATGAAGATCAAGATCAAGATCATTGCT
	ODN1:	3'- AGTTCTAGTAACGA

Scheme 3. Sequences of ODN1 and ODN2 as well as PNA1 and DNA templates aligned to indicate sequence regions, which complementary to each other.

and N,N,N',N'-tetramethylrhodamine (TMR) – to its 3'-terminus. Substrates S1 and S2 are both azobenzene derivatives, one of which is more electron deficient (S1) than another (S2). TMR was used as a fluorescent probe. S1 is a strong quencher, whose visible light absorbance band overlaps with the emission of TMR. Therefore, we expected that the TMR dye in intact **3a** will be quenched, but recover its fluorescence upon photoreduction of S1. The azobenzene fragment S2 is not a quencher of TMR. Therefore, we added a fluorescein (FAM) residue to S2 to enable the efficient quenching of TMR in intact **3b**. As a control we used conjugate **4** lacking any 5'-modification (Scheme 2, 3). Synthetic details of conjugate preparation are provided in the *SI*. >90 % Purity of all conjugates was confirmed by HPLC and their identity - by MALDI-TOF mass spectrometry (*SI*).

Analogously to unconjugated substrates azobenzene derivatives in both **3a** and **3b** are susceptible to the photoreduction in the presence of [Sn(P)Cl$_2$], but not [In(P)Cl] (Figure 2A). In solution substrate **3a** (100 nM) exhibits low emission intensity of 4 $\pm$ 2 a.u. (λ_{ex}= 550 nm, λ_{em}= 580 nm) indicating strong quenching of the TMR dye due to its interaction with substrate S1 (Figure 1B, trace 1). Addition of the catalyst **2** (1 eq, trace 2) to **3a** practically does not affect the emission. In contrast, addition of complementary DNA2 (1 eq) to solution of **3a** or to the equimolar mixture of **3a** and **2** leads to the 7.7-fold fluorescence increase in both cases indicating formation of duplexes. Irradiation of the equimolar mixture **3a**/**2**/DNA2 with red light induces the reduction of substrate S1 in **3a** that is reflected in the fluorescence increase (trace 4) that is 12-fold faster than in the absence of the template (trace 3). The fluorescence of control mixture **4**/**2**/DNA2 remains stable upon its irradiation with red light (trace 5). To evaluate whether the distance between the substrate and the catalyst in the templated reaction affects its rate, we introduced to DNA2 gaps of 1, 2 and 3 "T"-residues (Figure 1C, Scheme 3). We observed the clear drop in the reaction rate with increasing the distance between the reactants that is an expected outcome for the templated reaction mediated by photoinduced electron transfer.[32] Furthermore, we confirmed that the reaction is highly sensitive to even single mismatches in the template (Figure 1D).

In contrast to **3a**, **3b** was found to be inactive in the templated reaction despite the fact that S1 and S2 exhibit comparable reactivity in the presence of [Sn(P~OH)Cl$_2$] (Figure 1A). This might be an indication that the mutual orientation of the catalyst and the substrate on the template required for the electron transfer cannot be achieved for S2.

Finally, we tested whether the photoreduction occurs at lower concentrations of the reagents (Figure S36, Table S1, *SI*). We observed that at 20 nM of **2** and **3a** the reaction is still accelerated by 3.6 fold in the presence of 1 eq DNA2. The decreasing of the template-induced acceleration can be explained by limited DNA binding affinity of catalyst **2**, which contains only a 6-mer PNA sequence. This is confirmed by the fact that the templated reaction at 5 nM of the reagents is not significantly faster than the non-templated reaction. However, at the conditions forcing the binding of catalyst **2** to DNA2 (2-fold excess of **2**) the templated reaction is 5.2-fold faster than the non-templated one (Table S2). These data indicate that further improvement of the sensitivity of the newly developed templated photoreduction can be achieved by increasing affinity of catalyst **2** to DNA templates. That would be possible e.g. by using longer PNA sequences.

In summary, we developed the first red light dependent nucleic acid templated photoreduction, which is highly sequence specific, provides for ~12-fold template-induced rate acceleration and allows detecting down to 20 nM nucleic acids. Best known photochemical templated reactions triggered by

visible light are similarly sequence specific, can reach over 100-fold template-induced rate acceleration and are able to detect down to 0.1 pM nucleic acids.[33,34] We envision that after improving sensitivity limit (e.g. by using catalysts with longer PNA sequences) and rate acceleration (e.g. by optimization of reducible substrates and substrate/catalyst orientation on the template), our reaction can become a highly useful addition to the currently available repertoire of photochemical nucleic acid-templated reactions. It will offer a critical advantage over the known systems of using mild and, therefore, non-toxic trigger: red light.

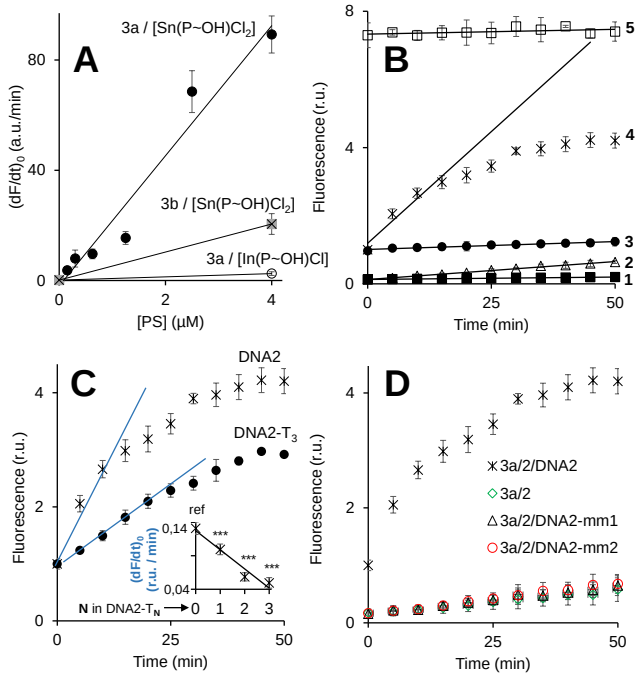


Figure 1. **A**: Dependence of initial rate of photoreduction of **3a** and **3b** upon irradiation with red light ($(dF/dt)_{t=0}$, where F is emission intensity, λ_{ex}= 550 nm, λ_{em}= 580 nm) from [PS~OH]. Buffer: phosphate, 10 mM, pH 7, NaCl, 150 mM, sodium ascorbate, 10 mM, 1% DMSO (v/v). **B**: Dependence of fluorescence (F/F_0, where F_0 is the initial fluorescence of equimolar mixture of **3a**, **2** and DNA2, each 100 nM; F/F_0 is expressed in relative units r.u.) from time of irradiation with red light of either solutions of **3a** (100 nM) (trace 1) containing **2** (100 nM) (trace 2); DNA2 (100 nM) (trace 3); **2** (100 nM), DNA2 (100 nM) (trace 4) or control solution containing **4** (100 nM), **2** (100 nM), DNA2 (100 nM) (trace 5). **C**: Dependence of photoreduction rate of **3a** (100 nM) in the presence of **2** (100 nM) and different DNA templates DNA2-T_N (100 nM), where N=0, 1, 2, 3; Student's t test, ***: p< 0.0001; **D**: Effects of mismatches in the DNA template on the photoreduction rate of **3a** (100 nM) in the presence of **2** (100 nM). Buffer conditions in **B**, **C** and **D** are as in inset **A**.

Conflicts of interest

There are no conflicts to declare.

Notes and references

‡ This project is funded by German Research Council (DFG, MO1418/8-1). Support of G.K. by the Austrian Science Fund (FWF project W-1250 DK9: "Photochemical Control of Cellular Processes") is also gratefully acknowledged. The partial support was provided by DFG/RSF (DFG MO 1418/11-1, RSF 19-44-04111(DNA template azo dye reduction)) and Emerging Field Initiative of Friedrich-Alexander-University of Erlangen-Nürnberg, project "Chemistry in live cells".

1 A. P. Silverman, E. T. Kool, *Chem. Rev.*, 2006, **106**, 3775.
2 K. Gorska, N. Winssinger, *Angew. Chem. Int. Ed.*, 2013, **57**, 6820.
3 A. Shibata, H. Abe, Y Ito, *Molecules*, 2012**,** **17**, 2446.
4 J.-R. Meunier, A. Sarasin, L. Marrot, *Photochem. Photobiol.*, 2007, **75**, 437.
5 A. Khodjakov, C. L. Rieder, *Methods*, 2006, **38**, 2.
6 E. M. M. Manders, H. Kimura, P. R. Cook, *J. Cell Biol.*, 1999, **144**, 813.
7 L. Holtzer, I. Oleinich, M. Anzola, E. Lindberg, K. K. Sadhu, M. Gonzalez-Gaitan, N. Winssinger, *ACS Cent. Sci.*, 2016, **2**, 394.
8 K. K. Sadhu, N. Winssinger, *Chem. Eur. J.,* 2013, **19**, 8182.
9 M. Rothlingshofer, K. Gorska, N. Winssinger, *Org. Lett.*, 2012, **14**, 482.
10 D. Chang, K. T. Kim, E. Lindberg, N. Winssinger, *Bioconj. Chem.*, 2018, **29**, 158.
11 D. Chang, E. Lindberg, N. Winssinger, *J. Am. Chem. Soc.*, 2017, **139**, 1444.
12 M. Bates, T. R. Blosser, X. Zhuang, *Phys. Rev. Lett.*, 2005, **94**, 108101.
13 M. Bates, B. Huang, G. T. Dempsey, X. Zhuang, *Science*, 2007, **317**, 1749.
14 A. Fülöp, X. Peng, M. M. Greenberg, A. Mokhir, *Chem. Comm.*, 2010, **46**, 5659.
15 S. Dutta, B. Flottmann, M. Heilemann, A. Mokhir, *Chem. Comm.*, 2012, **48**, 9664.
16 S. Dutta, A. Fülöp, A. Mokhir, *Bioconj. Chem.*, 2013, **24**, 1533.
17 M. Schikora, S. Dutta, A. Mokhir, *Histochem. Cell Biol.*, 2014, **142**, 103.
18 E. F. da Silva, B. W. Pedersen, T. Breitenbach, R. Toftegaard, M. K. Kuimova, L. G. Arnaut, P. R. Ogilby, *J. Phys. Chem. B*, 2012, **116**, 445.
19 C. Schweitzer, R. Schmidt, *Chem. Rev.*, 2003, **103**, 1685.
20 J. Qu, L. Ma, J. Zhang, S. Jockusch, I. Washington, *Photochem. Photobiol.*, 2013, **89**, 310.
21 Y. Kitigawa, S. Ogasawara, D. Kosumi, H. Hashimoto, H. Tamiaki, *J. Photochem. Photobiol. A: Chemistry*, 2015, **311**, 104.
22 K. T. Oppelt, E. Wöß, M. Stiftinger, W. Schöfberger, W. Buchberger, G. Knör, *Inorg. Chem.*, 2013, **52**, 11910.
23 D. Arian, E. Clo, K. V. Gothelf, A. Mokhir, *Chem. Eur. J.*, 2010, **16**, 288.
24 D. Arian, L. Kovbasyuk, A. Mokhir, *J. Am. Chem. Soc.*, 2011, **133**, 3972.
25 D. Arian, L. Kovbasyuk, A. Mokhir, *Inorg. Chem.*, 2011, **50**, 12010.
26 A. Meyer, A. Mokhir, *Angew. Chem. Int. Ed.*, 2014, **53**, 12840.
27 A. Meyer, M. Schikora, V. Starkuviene, A. Mokhir, *Photochem. Photobiol. Sci.*, 2016, **15**, 1120.
28 S. G. Konig, A. Mokhir, *Bioorg. Med. Chem. Lett.*, 2013, **23**, 6544.
29 A. Meyer, M. Schikora, A. Mokhir, *Chem. Comm.*, 2015, **51**, 13324.
30 V. A. Ol'shevskaya, A. N. Savchenko, A. V. Zaitsev, E. G. Kononova, P. V. Petrovskii, A. A. Ramonova, V. V. Tatarskii, O. V. Uvarov, M. M. Moisenovich, V. N. Kalinin, A. A. Shtil, *J. Organomet. Chem.*, 2009, **694**, 1632.
31 B. C. Robinson, B. A. Garcia, *U.S. Patent* US 20020137924 A1 20020926, 2002.
32 F. D. Lewis, T. Wu, Y. Zhang, R. L. Letsinger, S. R. Greenfield, M. R. Wasielewski, *Science*, 1997, **277**, 673.
33 M. Anzola, N. Winssinger, *Chem. Eur. J*, 2019, **25**, 334.
34 S. Angerani, N. Winssinger, *Chem. Eur. J.*, 2019, **25**, 6661.

View Article Online
DOI: 10.1039/D0CC03086D

Graphical Abstract