ChemComm



COMMUNICATION

View Article Online



Cite this: *Chem. Commun.,* 2020, **56**. 2320

Received 6th October 2019, Accepted 18th December 2019

DOI: 10.1039/c9cc07845b

rsc.li/chemcomm

Biotinylated photoactive Pt(IV) anticancer complexes†

Huayun Shi, (1) Cinzia Imberti, (1) Huaiyi Huang, (1) Ian Hands-Portman (1) and Peter J. Sadler (1) *

Novel biotinylated diazido-Pt(|v|) complexes exhibit high visible light photocytotoxicity while being stable in the dark. Photocytotoxicity and cellular accumulation of all-trans-[Pt(py)₂(N_3)₂(biotin)(OH)] (2a) were enhanced significantly when bound to avidin; irradiation induced dramatic cellular morphological changes in human ovarian cancer cells treated with 2a.

Tumour-targeting drugs can improve selective drug delivery to cancer cells and reduce undesirable side effects. 1-4 Biotin (vitamin H or B7) plays an important role as cellular growth promoter and co-enzyme for carboxylase enzymes.⁵ Exogenous biotin is taken up via a high-affinity biotin transporter and a sodium-dependent multivitamin transporter (SMVT).^{6,7} Notably, cancer cells overexpress SMVT, thus making biotin a tumour-targeting vector.8-10 Biotin possesses a high affinity specific for tetrameric egg white avidin through noncovalent bonding with $K_{\rm d}$ values of 10^{-13} to 10^{-15} M.¹¹ The avidin–biotin complex induces a relatively low immune response in vivo and has been widely used in cancer-targeted therapy. 5,12-15 Avidin displays strong adherence to cells in vitro, and can be administered as a nano-carrier for delivery of biotinylated drugs. 16 Biotinylation of drugs is therefore a feasible strategy to improve their cancer-cell accumulation and selectivity, 17,18 and has also been applied in the design of photoactive drugs. 19-22

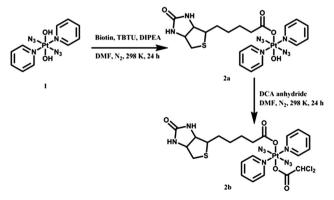
Photoactive Pt agents provide spatial control of cytotoxicity with novel mechanisms of action and lack of cross-resistance with cisplatin. 23,24 Diazido Pt(IV) complexes are potent photoactive prodrugs with high dark stability due to their kinetic inertness, $^{25-27}$ and photocytotoxicity exerted by the combined effects of Pt(II) products, azidyl radicals and reactive oxygen species (ROS) formed on photoactivation. $^{28-30}$ *Trans,trans,trans*[Pt(N₃)₂(OH)₂(py)₂] (1) kills cancer cells upon irradiation with

 $\label{lem:power_loss} \begin{tabular}{ll} Department of Chemistry, University of Warwick, Coventry, CV4 7AL, UK. \\ E-mail: p.j.sadler@warwick.ac.uk \end{tabular}$

high photocytotoxicity (dark *versus* light) indices. ²⁷ Axial OH ligands enhance aqueous solubility of Pt(IV) complexes and stabilise the Pt^{IV} oxidation state. ³¹ Derivatisation of the releasable axial ligand can improve the pharmacological properties without altering the mechanism of action. ^{32–34}

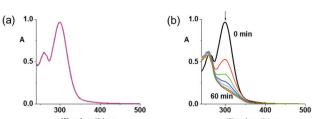
Herein, we have prepared two novel biotinylated diazido Pt(rv) complexes $trans, trans, trans-[Pt(py)_2(N_3)_2(biotin)(OH)]$ (2a) and $trans, trans-[Pt(py)_2(N_3)_2(biotin)(DCA)]$ (2b, Scheme 1). The pyruvate dehydrogenase kinase (PDK) inhibitor dichloroacetate (DCA) can be delivered to cancer cells effectively as an axial ligand in Pt(rv) prodrugs. $^{35-37}$ Dark stability, photodecomposition, photoreactions with 5'-GMP and DNA, and interaction with avidin were investigated for 2a and 2b, and their photocytotoxicity and cellular accumulation compared to those of avidin-complex adducts. Cellular morphological changes upon treatment of cancer cells with 2a were examined by confocal microscopy.

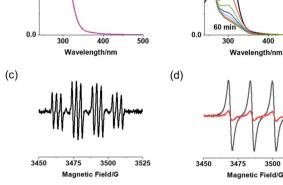
The synthetic routes for photoactive Pt(IV) complexes 2a and 2b are summarised in Scheme 1. Mono-substituted 2a was obtained by combining 1 with biotin using TBTU/DIPEA as coupling agents, while the bi-substituted 2b was synthesised by stirring 2a with DCA anhydride. Both complexes gave satisfactory elemental analyses and were also characterised by ESI-HRMS, NMR and UV-vis spectroscopy, and for purity by HPLC (Fig. S1, ESI†).



Scheme 1 Synthetic routes for biotinylated complexes 2a and 2b.

 $[\]dagger$ Electronic supplementary information (ESI) available: NMR spectra, photo-decomposition, UV-vis titration, and experimental details. See DOI: 10.1039/c9cc07845b





Communication

Fig. 1 (a) UV-vis spectral changes for 2a (50 μ M) in RPMI-1640 in the dark for 2 h, and (b) in H₂O upon 1 h irradiation with blue light (420 nm); (c) EPR spectra for 2a (2.5 mM) with DMPO in H₂O after irradiation (463 nm), and (d) with TEMP in CH₃CN in the dark (–) and after irradiation (463 nm, –).

3525

3500

The ¹H and ¹³C NMR data are consistent with the proposed structures of the complexes. The Pt-coordinated pyridine has characteristic ¹H NMR doublets with ¹⁹⁵Pt satellites at *ca.* 8.8 ppm, and the triplets at ca. 8. 3 and 7.9 ppm, with ¹³C NMR resonances at ca. 150, 143 and 127 ppm (Fig. S2-S5, ESI†). Two typical singlets at 6.38 and 6.35 ppm assignable to NHC(O)NH of biotin were detected for both complexes. The electronic absorption spectra of 2a and 2b in aqueous solution were similar, although 2b exhibited a slight red shift of the LMCT ($N_3 \rightarrow Pt$) transition and stronger absorption at wavelengths <270 nm due to the presence of the DCA ligand.

Complexes 2a and 2b exhibited excellent dark stability in RPMI-1640/DMSO (95%/5%, v/v) monitored by UV-vis spectroscopy (Fig. 1a and Fig. S6a, ESI†). When irradiated with blue light (420 nm), the LMCT bands of both complexes at ca. 300 nm decreased in intensity, indicating release of the azide ligands (Fig. 1b and Fig. S6b, ESI†).27 Notably, 2b exhibited a larger decrease at the absorption maximum, probably due to release of the DCA ligand. Released azidyl radicals and singlet oxygen were trapped by DMPO and TEMP, respectively, for 2a upon irradiation (463 nm; Fig. 1c and d).

The photoreactions of 2a/2b with 5'-GMP were analysed by LC-MS. Pt(IV) complexes (30 μM) were incubated with 5'-GMP (2 mol equiv.) at 310 K in the dark for 1 h, then irradiated by blue light (420 nm) for 1 h (Fig. S7, ESI†). For both complexes, $[Pt^{II}(CH_3CN)(py)_2(GMP-H)]^+$ (G1, 756.48 m/z) was detected as the major Pt-GMP product, together with two minor adducts $[Pt^{II}(HCOO)(py)_2(GMP)]^+(G2, 762.18 \, m/z)$ and $[Pt^{II}(N_3)(py)_2(GMP)]^+$ (G3, 758.23 m/z). These results were similar to those obtained for 1, suggesting a negligible effect of axial substituents on the photoreaction with guanine.38

DNA melting experiments monitored by UV-vis were carried out in phosphate buffer (1 mM, pH = 7.9) to investigate DNA binding with a drug/base pair ratio of 0.2 (Table S1, ESI†). Similar to parent complex 1, 2a and 2b interact with ct-DNA

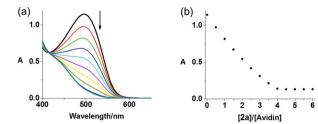


Fig. 2 (a) UV-vis spectral changes for avidin-HABA in PBS solution upon addition of complex 2a in DMSO, (b) absorbance change at 496 nm in HABA displacement titrations of biotinylated complexes 2a

only weakly ($\Delta T_{\rm m} < 2$ K) in the dark, while upon irradiation with blue light (420 nm) an apparent interaction was observed $(\Delta T_{\rm m} \ ca. \ 5 \ K)$. The increase in the DNA melting temperature suggests that 2a and 2b might form inter-strand cross-links after irradiation, similar to 1.

The binding affinity of avidin towards 2a and 2b in PBS was assessed by a displacement titration using HABA (2-(4-hydroxyphenylazo)benzoic acid) and monitored by UV-vis spectroscopy (Fig. 2). The avidin-HABA adduct displays an absorption band at ca. 496 nm, which decreases when HABA is replaced by biotin.³⁹ On mixing, a gradual decrease of the absorbance at 496 nm was observed upon addition of either complex to a solution containing HABA (160 μM) and avidin (8 μM), indicating their stronger binding to avidin compared with HABA (dissociation constant $K_d = 10^{-6} \text{ M}$).³⁹ A sharp end point and same absorbance changes ($\Delta A = 1.01$) with biotin were observed for 2a and 2b, indicating their similar affinity towards avidin as unmodified biotin. These results suggested the possibility of using avidin as a nanocarrier for delivery of biotinylated complexes to cancer cells.

The IC₅₀ values of 2a and 2b in human A2780 ovarian, A549 lung and PC3 prostate cancer cell lines were determined by the sulforhodamine B (SRB) colorimetric assay (Table 1), using parent complex 1 and CDDP (cisplatin) as references. Both complexes were relatively non-toxic towards all cancer cells and healthy MRC-5 lung fibroblasts in the dark with IC50 values > 50/100 μ M, but exhibited promising photocytotoxicity with high photocytotoxicity indices (PI). In all the cancer cell lines studied, di-substituted 2b (1.3-5.9 µM) is at least twice as phototoxic as the mono-substituted 2a (11.7-21.1 μM), and $5\times$ more toxic than 1 (7.1-55.6 μ M). Under the same conditions, cisplatin exhibited very low cytotoxicity in all cell lines $(IC_{50} > 100 \mu M)$ due to the short incubation time (2 h).

In A2780 ovarian cancer cells, 2a (IC₅₀ = 11.7 μ M) was slightly less active than 1 (IC₅₀ = 7.1 μ M), while 2b was more potent (IC₅₀ = 1.3 μ M), potentially attributable to the synergistic anticancer activity of the released DCA ligand. In order to investigate the effect of avidin on activity, 2a was mixed with avidin (2a: avidin = 4:1) prior to addition to A2780 cells following the same protocol used for 2a alone. Notably, the avidin-2a complex exhibited good dark stability, being potently photocytotoxic in A2780 cells with an IC₅₀ value of 4.4 μ M, 2.7 \times lower than that of 2a, and $1.6 \times$ more toxic than 1. In contrast, the photocytotoxicity of 2b was not enhanced in the presence of avidin.

ChemComm

Table 1	IC_{50}	values	and	photocytot	toxicity	indices	(PI)	for	2a	and	2b
obtained	after	1 h inci	ubati	on, 1 h irrac	liation ((465 nm)	anc	124	h re	ecov	ery,
in comparison with the parent complex 1 and CDDP (cisplatin) ³⁴											

		Cell line/IC $_{50}$ (μM) a			
Comp.		A2780	A549	PC3	MRC5
2a	Dark Irrad PI	>100 11.7 ± 0.3 >8.5	>100 13.3 ± 0.7 >7.5	>100 21.1 ± 0.4 >4.7	>100
2a -avi (4:1)	Dark Irrad PI	>100 4.4 ± 0.3 >22.7		·	
2b	Dark Irrad PI	>50 1.3 ± 0.2 >38.5	>100 5.9 ± 0.6 >16.9	>100 3.0 ± 0.1 >33.3	>100
2b -avi (4:1)	Dark Irrad PI	> 50 > 50 2.45 ± 0.01 > 20.4	7 10.3	2 00.0	
1	Dark Irrad PI	> 100 7.1 \pm 0.4 > 14.1	>100 51.9 ± 2.5 >1.9	>100 55.6 ± 0.9 >1.8	>100
CDDP	Dark Irrad	>100 >100 >100	>100 >100	>100 >100 >100	>100

^a Data are from three independent experiments.

Since biotin is expected to exhibit a preference towards cancer cells due to overexpressed receptors, 5 ICP-MS was used to quantify and compare the cellular uptake of biotinylated complexes 2a and 2b as well as the unsubstituted 1. A2780 ovarian cancer cells were treated with 10 µM prodrugs in the dark for 1 h, then the Pt content of cells was analysed by ICP-MS (Table 2). The mono-substituted 2a exhibited lower Pt accumulation (0.4 ng per 10⁶ cells) than 1 (0.64 ng per 10⁶ cells) after 1 h incubation, while accumulation of di-substituted **2b** (21.1 ng per 10⁶ cells) was 33× higher than that of 1 and 53× higher than 2a. These results showed that conjugation with biotin alone did not increase the accumulation of diazido Pt(IV) complexes in A2780 cells, while the substitution of the second axial ligand with DCA resulted in significant increase in Pt cellular accumulation, perhaps due to higher lipophilicity of 2b, consistent with its longer HPLC retention time (12.3 min for 2a, 19.7 min for 2b, Fig. S1, ESI†). Notably, the amount of Pt taken up by A2780 cells incubated with 1, 2a and 2b was inversely proportional to their IC50 values. These results suggested that cellular accumulation of Pt(IV) prodrugs plays an important role in their antiproliferative activity. Since the avidin-2a complex exhibited improved photocytotoxicity, the effect of avidin on the uptake of 2a was also investigated.

Table 2 Cellular Pt accumulation in A2780 ovarian cancer cells after exposure to complexes 2a, 2b and 1, and the effect of avidin (10 μM Pt complex. 1 h incubation in the dark)

Pt accumulation (ng per 10 ⁶ cells) ^a						
Complex		Complex with av	idin			
2a	0.40 ± 0.15*	2a-avi (4:1)	4.1 ± 0.7*			
2b	$21.1 \pm 2.0***$	2 b -avi (4:1)	$20.0 \pm 1.5***$			
1	$0.64 \pm 0.07***$	1 -avi (4:1)	$0.56 \pm 0.09**$			

^a All experiments were conducted in triplicate. Comparison with untreated controls was performed by a two-tail t-test with unequal variances. *p < 0.05, **p < 0.01, ***p < 0.005.

The accumulation of Pt after exposure of A2780 cells to the mixture of 10 μ M 2a and avidin (4:1) was 10× higher than 2a alone, which correlates with the higher photocytotoxicity of 2a-avidin complex. However, Pt accumulation for 1 and 2b was not affected by avidin, since 1 does not contain biotin and 2b is mainly taken up by passive diffusion due to its high lipophilicity.

A2780 ovarian cancer cells were treated with 2a (1 or 2× photo IC₅₀ concentration) in the presence and absence of light and live-imaged using confocal microscopy and flow cytometry to investigate changes in cell morphology (Fig. 3 and Fig. S8, S10, ESI†). The cell permeant dye SYTO™ 17 was used to stain the nuclei. Without irradiation, 2a exhibited low cytotoxicity $(IC_{50} > 100 \mu M)$ and, accordingly, no changes in cellular morphology were observed when cells were treated with 2a for 2 h in the dark. In these conditions, the cells appeared healthy with welldefined plasma membranes and intact nuclei. In contrast, A2780 cells treated with 2a exhibited dramatic morphological changes after 1 h irradiation with blue light (465 nm). The cells rounded up and the nuclei were fragmented into pieces. Damaged membranes and copious cell debris were observed when cells were treated with 2a at $2 \times IC_{50}$ concentration (Fig. 3 and Fig. S10, ESI†). DNA is usually regarded as the major target of platinum anticancer drugs, so the ability of 2a to fragment cell nuclei exclusively upon irradiation, indicated its potential as a photoactive prodrug with a novel mechanism of action. Complex 2a at higher concentration induced more morphological changes upon irradiation, while irradiation alone did not result in significant effects on cell morphology (Fig. S9 and S10, ESI†).

Cellular ROS generation for 2a, 2b and 1 was monitored in A549 lung cancer cells by DCFH-DA, which exhibits switch-on fluorescence in the presence of ROS. Cells treated with complexes (10 µM) in the dark showed no apparent change, but 2b induced an increased DCF fluorescence upon irradiation, indicating its ability to generate ROS (Table S2, ESI†).

In summary, two novel biotinylated Pt(IV) complexes trans, $trans, trans-[Pt(py)_2(N_3)_2(biotin)(OH)]$ (2a) and trans, trans, trans-[Pt(py)₂(N₃)₂(biotin)(DCA)] (2b) have been synthesised and characterised. They exhibited good dark stability and promising

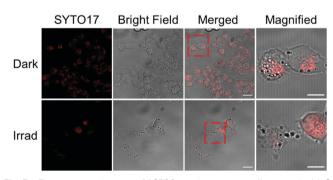


Fig. 3 Fluorescence images of A2780 ovarian cancer cells treated with 2a (2 \times photo IC $_{50}$ concentration) in the dark and after irradiation with blue light (λ = 465 nm). Cells were stained by SYTOTM 17 (2.5 μ M, $\lambda_{ex}/\lambda_{em}$ = 633/638-759 nm) for 30 min. Scale bar = 20 μ m (10 μ m in magnification). Fragmented nuclei and damaged membranes of A2780 cells treated with 2a were observed upon irradiation, while in the dark, cells were intact and well-defined.

Communication ChemComm

photoactivity, releasing azidyl radicals and generating singlet oxygen. Photoreactions with 5'-GMP resulted in the formation of [Pt^{II}(CH₃CN)(py)₂(GMP-H)]⁺ as a major product. An increase in ct-DNA melting temperature suggested formation of inter-strand crosslinks. Promising photocytotoxicity of 2a and 2b towards human A2780 ovarian, A549 lung and PC3 prostate cancer cells was observed upon irradiation with low-dose blue light (465 nm, 4.8 mW cm⁻², 1 h), with high photocytotoxicity indices. The di-substituted 2b exhibited much higher photocytotoxicity and cellular Pt accumulation, compared with mono-substituted 2a and unsubstituted 1. The strong interaction of 2a and 2b with avidin was confirmed by HABA displacement from avidin-HABA. When A2780 ovarian cancer cells were incubated with 1:4 avidin: 2a, both photocytotoxicity and cellular accumulation increased significantly. Dramatic morphological changes in A2780 cells were observed after irradiation with 2a, consistent with its promising photocytotoxicity.

We acknowledge financial support from the EPSRC (EP/G006792, EP/F034210/1 to PJS), University of Warwick (Chancellor's International PhD Scholarship for HS), Royal Society (grant no. NF160307, Newton International Fellowship for HH), and Wellcome Trust (grant no. 209173/Z/17/Z, Si Henry Wellcome Fellowship for CI).

Conflicts of interest

There are no conflicts to declare.

Notes and references

- 1 D. L. Ma, D. S. H. Chan and C. H. Leung, Chem. Soc. Rev., 2013, 42, 2130-2141.
- 2 L. Cai, Z. Gu, J. Zhong, D. Wen, G. Chen, L. He, J. Wu and Z. Gu, Drug Discovery Today, 2018, 23, 1126-1138.
- 3 H. Ijaz, J. Qureshi, U. R. Tulain, F. Iqbal, Z. Danish, A. Fayyaz and A. Sethi, Bioinspired, Biomimetic Nanobiomater., 2018, 7, 109-121.
- 4 E. R. Ruskowitz and C. A. DeForest, Nat. Rev. Mater., 2018, 17087.
- 5 W. X. Ren, J. Han, S. Uhm, Y. J. Jang, C. Kang, J. H. Kim and J. S. Kim, Chem. Commun., 2015, 51, 10403-10418.
- 6 J. Zempleni and D. M. Mock, Am. J. Physiol., 1998, 275, C382-C388. 7 K. Balamurugan, A. Ortiz and H. M. Said, Am. J. Physiol., 2003, 285,
- 8 P. D. Prasad, H. Wang, W. Huang, Y. J. Fei, F. H. Leibach,
- L. D. Devoe and V. Ganapathy, Arch. Biochem. Biophys., 1999, 366, 95 - 106
- 9 S. Luo, V. S. Kansara, X. Zhu, D. Pal and A. K. Mitra, Mol. Pharming, 2006, 3, 329-339.
- 10 H. Wang, W. Huang, W. Fei, H. Xia, T. L. Y. Feng, F. H. Leibac, L. D. Devoe, V. Ganapathy and P. D. Prasad, J. Biol. Chem., 1999, 274, 14875-14883.
- 11 N. M. Green, Adv. Protein Chem., 1975, 29, 85-133.

- 12 H. P. Lesch, M. U. Kaikkonen, J. T. Pikkarainen and S. Ylä-Herttuala, Expert Opin. Drug Delivery, 2010, 7, 551-564.
- 13 X. Zeng, Y. Sun, X. Zhang and R. Zhuo, Org. Biomol. Chem., 2009, 7, 4201-4210.
- 14 A. Jain and K. Cheng, J. Controlled Release, 2017, 245, 27-40.
- 15 S. C. Park, Y. M. Kim, N. H. Kim, E. J. Kim, Y. H. Park, J. R. Lee and M. K. Jang, Macromol. Res., 2017, 25, 882-889.
- 16 L. Chen, B. Schechter, R. Arnon and M. Wilchek, Drug Dev. Res., 2000, 50, 258-271.
- 17 N. Muhammad, N. Sadia, C. Zhu, C. Luo, Z. Guo and X. Wang, Chem. Commun., 2017, 53, 9971-9974.
- 18 S. Maiti, N. Park, J. H. Han, H. M. Jeon, J. H. Lee, S. Bhuniya, C. Kang and J. S. Kim, J. Am. Chem. Soc., 2013, 135, 4567-4572.
- 19 K. Li, L. Qiu, Q. Liu., G. Lv, X. Zhao, S. Wang and J. Lin, J. Photochem. Photobiol., B, 2017, 174, 243-250.
- 20 B. Siewert, M. Langerman, A. Pannwitz and S. Bonnet, Eur. J. Inorg. Chem., 2018, 4117-4124.
- 21 J. Li, L. Zeng, K. Xiong, T. W. Rees, C. Jin, W. Wu, Y. Chen, L. Jia and H. Chao, Chem. Commun., 2019, 55, 10972-10975.
- 22 B. Ghazal, E. N. Kaya, A. Husain, A. Ganesan, M. Durmuş, S. Makhseed and J. Porphyr, Phthalocyanines, 2019, 23, 46-55.
- 23 S. Bonnet, Dalton Trans., 2018, 47, 10330-10343.
- 24 A. Bjelosevic, B. J. Pages, L. K. Spare, K. M. Deo, D. L. Ang and J. R. Aldrich-Wright, Curr. Med. Chem., 2018, 25, 478-492.
- 25 P. Müller, B. Schröder, J. A. Parkinson, N. A. Kratochwil, R. A. Coxall, A. Parkin, S. Parsons and P. J. Sadler, Angew. Chem., Int. Ed., 2003, 42, 335-339.
- 26 F. S. Mackay, J. A. Woods, P. Heringová, J. Kašpárková, A. M. Pizarro, S. A. Moggach, S. Parsons, V. Brabec and P. J. Sadler, Proc. Natl. Acad. Sci. U. S. A., 2007, 104, 20743-20748.
- 27 N. J. Farrer, J. A. Woods, L. Salassa, Y. Zhao, K. S. Robinson, G. Clarkson, F. S. Mackay and P. J. Sadler, Angew. Chem., Int. Ed., 2010, 49, 8905-8908.
- 28 Y. Zhao, N. J. Farrer, H. Li, J. S. Butler, R. J. McQuitty, A. Habtemariam, F. Wang and P. J. Sadler, Angew. Chem., Int. Ed., 2013, 52, 13633-13637.
- 29 J. S. Butler, J. A. Woods, N. J. Farrer, M. E. Newton and P. J. Sadler, J. Am. Chem. Soc., 2012, 134, 16508-16511.
- 30 J. Pracharova, L. Zerzankova, J. Stepankova, O. Novakova, N. J. Farrer, P. J. Sadler, V. Brabec and J. Kasparkova, Chem. Res. Toxicol., 2012, 25, 1099-1111.
- 31 M. D. Hall and T. W. Hambley, Coord. Chem. Rev., 2002, 232, 49-67.
- 32 A. Gandioso, E. Shaili, A. Massaguer, G. Artigas, A. González-Cantó, J. A. Woods, P. J. Sadler and V. Marchán, Chem. Commun., 2015, 51, 9169-9172.
- 33 E. Shaili, M. Fernández-Giménez, S. Rodríguez-Astor, A. Gandioso, L. Sandín, C. García-Vélez, A. Massaguer, G. J. Clarkson, J. A. Woods, P. J. Sadler and V. Marchán, Chem. - Eur. J., 2015, 21, 18474-18486.
- 34 H. Shi, Q. Wang, V. Venkatesh, G. Feng, L. S. Young, I. Romero-Canelón, M. Zeng and P. J. Sadler, Dalton Trans., 2019, 48, 8560-8564.
- 35 E. Wexselblatt, E. Yavin and D. Gibson, Angew. Chem., Int. Ed., 2013, **52**, 6059-6062.
- 36 S. Dhara and S. J. Lippard, Proc. Natl. Acad. Sci. U. S. A., 2009, 106, 22199-22204.
- 37 E. Petruzzella, J. P. Braude, J. R. Aldrich-Wright, V. Gandin and D. Gibson, Angew. Chem., Int. Ed., 2017, 56, 11539-11544.
- 38 R. R. Vernooij, T. Joshi, M. D. Horbury, B. Graham, E. I. Izgorodina, V. G. Stavros, P. J. Sadler, L. Spiccia and B. R. Wood, Chem. - Eur. J., 2018, 24, 5790-5803.
- 39 N. M. Green, Biochem. J., 1965, 94, 23c-24c.