Journal of Coordination Chemistry

ISSN: 0095-8972 (Print) 1029-0389 (Online) Journal homepage: http://www.tandfonline.com/loi/gcoo20

Interaction of Ru^{III}(EDTA) with cellular thiols and O_2: biological implications thereof

Debabrata Chatterjee, Namita Jaiswal, Jagadeesh C. Bose K, Ram Balak Mahato & Diptendu Bhattacharyya

To cite this article: Debabrata Chatterjee, Namita Jaiswal, Jagadeesh C. Bose K, Ram Balak Mahato & Diptendu Bhattacharyya (2015) Interaction of Ru^{III}(EDTA) with cellular thiols and O_2: biological implications thereof, Journal of Coordination Chemistry, 68:17-18, 3229-3235, DOI: 10.1080/00958972.2015.1056172

To link to this article: http://dx.doi.org/10.1080/00958972.2015.1056172

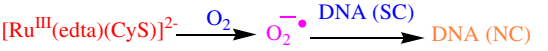
Journal of Coordination Chemistry, 2015
Vol. 68, Nos. 17–18, 3229–3235, http://dx.doi.org/10.1080/00958972.2015.1056172

Interaction of Ru^{III}(EDTA) with cellular thiols and O_2: biological implications thereof

DEBABRATA CHATTERJEE*†, NAMITA JAISWAL†, JAGADEESH C. BOSE K‡, RAM BALAK MAHATO‡ and DIPTENDU BHATTACHARYYA§

†Chemistry & Biomimetics Group, CSIR-Central Mechanical Engineering Research Institute, Durgapur, India
‡Department of Biotechnology, National Institute of Technology, Durgapur, India
§Department of Chemistry, CSIR-Indian Institute of Chemical Biology, Kolkata, India

(*Received 8 February 2015; accepted 2 May 2015*)



Reaction of $[Ru^{III}(EDTA)(CyS)]^{2-}$ ($edta^{4-}$ = ethylenediaminetetraacetate; CySH = cysteine) with molecular oxygen (O_2) has been studied as a function of pH (4.0–8.0) and cysteine concentration (0.2–2.0 mM) at room temperature (25 °C). Biological activities of the [Ru(EDTA)]/CySH/O_2 system pertaining to cleavage of supercoiled plasmid DNA to its nicked open circular form has been explored in this work. Results are discussed in regard to the reaction of the ruthenium(III)-complex with molecular oxygen) and a working mechanism is proposed for the biological activities of the ruthenium(III)-complex in the presence of O_2.

Keywords: Ru(III)-EDTA complex; Cysteine; Redox reaction; DNA cleavage

1. Introduction

The potential of $[Ru^{III}(EDTA)]$ complexes toward biological applications had been reported [1]. $EDTA^{4-}$ is like many metalloenzymes in its donor character, utilizing carboxylate or amine donors of amino acids to bind to the metal. The reasons that $[Ru^{III}(EDTA)]$ complexes should be of interest in potential biological applications are because of the number of stable and accessible oxidation states they possess, their rapid rate of ligand exchange, and their ability to bind to certain biological molecules. Furthermore, $[Ru^{III}(EDTA)]$ complexes exhibit catalytic properties, in homogeneous conditions in the presence of oxygen donors that mimic the biological enzymatic oxidation [1]. The reactions of Ru^{III}(EDTA) complexes with biologically important molecules such as DNA constituents, amino acids, thiols, NO, and H_2O_2 are referred herein [2–8]. Studying the reaction of $[Ru^{III}(EDTA)]$ complexes with cysteine (CySH), a cellular thiol has been our enduring interest. Although cysteine is the least utilized amino acid (protein-bound, peptide-bound, and free) in biology [9, 10], it is one of the most conserved amino acids in proteins fulfilling very important

*Corresponding author. Email: deb_cmeri@yahoo.com

functions in cells. Oxidation of cysteine is of significance with regard to the regulation of many protein functions. While a new paradigm has emerged, in which oxidative stress and cysteine modifications appear to be prominent features of many acute and chronic diseases, as well as the normal aging process, recent upsurge in research interest toward elucidation of the role of cysteine oxidation in an array of physiological processes is evident in the literature [11]. It had been reported that $[Ru^{III}(EDTA)(H_2O)]^-$ reacts with cysteine to form the red $[Ru^{III}(EDTA)(CyS)]^{2-}$ complex [3], and inhibits cysteine protease [3] and protein tyrosine phosphatase activity [12] in a mechanism that involves the binding of the cysteine residue of the catalytic domain of the enzymes. Our recent studies revealed that $[Ru(EDTA)(H_2O)]^-$ can catalyze the oxidation of cysteine, which occurs by direct attack of H_2O_2 on the S of coordinated cysteine in $[Ru^{III}(EDTA)(CyS)]^{2-}$ [6]. Very recently, we have shown that $[Ru^{III}(EDTA)]$ could mediate O-atom transfer from nitrite (NO_2^-) to cysteine (CySH) leading to formation of $[Ru^{III}(EDTA)(NO)]$ and cysteine sulfenic acid (CySOH) [7]. At lower pH (4.5) coordinated cysteine in $[Ru^{III}(EDTA)(CyS)]^{2-}$ undergoes S-nitrosylation in the presence of nitrite [13].

In this work we have studied the reaction of $[Ru^{III}(EDTA)(CyS)]^{2-}$ and molecular oxygen, and explored the DNA cleavage activity of the $[Ru^{III}(EDTA)]$/cysteine/O_2 system at physiological pH. We report herein the results of spectral and kinetic studies of the reaction of $[Ru^{III}(EDTA)(CyS)]^{2-}$ with molecular oxygen O_2 along with the results of the biological investigations.

2. Experimental

2.1. *Materials*

$K[Ru^{III}(HEDTA)Cl]\cdot 2H_2O$ was synthesized according to the published procedure [14] and characterized (see Supplementary information for micro-analysis and spectral data). All other chemicals used were of A.R grade. Doubly distilled H_2O was used throughout the experiments.

2.2. *Instrumentation*

A Perkin–Elmer 240C elemental analyzer was used to obtain microanalytical (C, H, N) data. UV–vis spectra were recorded on a Varian Model Cary 100 spectrophotometer. IR spectra were recorded (using KBr pellets) on a Perkin–Elmer Model Lambda 783. The ESI MS studies were performed (in CSIR-IICB, Kolkata) on a Q-TOF micromass (waters), capable of a resolution of 5000 FWHM. Detection was done in positive ion mode and the capillary voltage was 3.0 kV. The flow rate was 10–15 μL min^{-1}. The drying gas (N_2) to aid solvent removal was kept at 125 °C. The source temperature was 80 °C. Reaction of $[Ru^{III}(EDTA)(CyS)]^{2-}$ with O_2 was performed with atmospheric oxygen at normal temperature and pressure.

2.3. *DNA cleavage studies*

Cleavage of supercoiled plasmid cDNA3 was carried out at pH 7.2 (1 mM phosphate buffer). The buffer, $[Ru(EDTA)(H_2O)]^-$, CySH, and sufficient water were premixed in an

Eppendrof vial, DNA was added subsequently to the reaction mixture, and the reaction was allowed to proceed for 30 min at 37 °C before loading onto agarose gel. The conversion of supercoiled (Form I) into open circular (Form II) was followed by agarose gel electrophoresis of plasmid DNA performed at 50 V using 1% slab gels containing 0.5 μg mL^{-1} ethidium bromide. DNA was visualized by photographing the fluorescence of intercalated ethidium bromide under UV illumination.

3. Results and discussion

The "$HEDTA^{3-}$" ligand in K[Ru(HEDTA)Cl] is pentadentate, confirmed by crystallographic evidence [15]. $K[Ru^{III}(HEDTA)Cl)]$ rapidly hydrolyzes to the corresponding aqua complex $[Ru^{III}(HEDTA)(H_2O)]$ (**1**) when dissolved in water [16, 17]. The sixth coordination site of the $Ru^{III}(EDTA)$ complex is occupied either by a water molecule at low pH or by a hydroxide at high pH.

It had been reported earlier that at pH 5.1 (acetate buffer) $[Ru^{III}(EDTA)(H_2O)]^-$ rapidly reacts ($k = 170\ M^{-1}\ s^{-1}$ at 25 °C) with cysteine (CySH) to form the red $[Ru^{III}(EDTA)(CyS)]^{2-}$ complex characterized by a band at 510 nm, $\varepsilon_{max} = 3330\ M^{-1}\ cm^{-1}$, with a complex-formation constant value of $3 \times 10^5\ M^{-1}$ at 25 °C and pH 5.0 [3]. The red $[Ru^{III}(EDTA)(CyS)]^{2-}$ complex was unstable when exposed to oxygen. Addition of oxygen saturated solution in phosphate buffer (1 mM) to the red solution of $[Ru^{III}(EDTA)(CyS)]^{2-}$ (prepared by mixing the solutions of $[Ru^{III}(EDTA)(H_2O)]^-$ and cysteine in an equimolar ratio at pH 7.1) caused gradual collapse of the spectral features (as shown typically in figure 1(a) characteristic of $[Ru^{III}(EDTA)(CyS)]^{2-}$, and ultimately resulted in a colorless solution. The effect of the cysteine concentration on the kinetic profile (time *versus* absorbance traces recorded at 510 nm) is displayed in figure 1(b).

ESI-MS studies of the resultant colorless solution so obtained from the reaction of $[Ru^{III}(EDTA)(CyS)]^{2-}$ (performed by reacting 0.2 mM $[Ru^{III}(EDTA)(H_2O)]^-$ with 2.0 mM cysteine) with O_2 revealed that the signal at $m/z = 510.43$ characteristic of the red $[Ru^{III}(EDTA)(CyS)]^{2-}$ complex completely disappeared (see figure S1 [see online supplemental material at http://dx.doi.org/10.1080/00958972.2015.1056172]). It is noteworthy here that the recorded spectrum for the resultant solution did not exhibit the characteristic signal for $[Ru^{III}(edta)]$ (at $m/z = 389.91$) also. Absence of $[Ru^{III}(EDTA)(H_2O)]^-$ in the resultant colorless solution was further evident as the addition of fresh solution of cysteine to the colorless solution did not regenerate the red $[Ru^{III}(EDTA)(CyS)]^{2-}$ complex as evident by spectral measurements. Above results signify decomposition of $[Ru^{III}(EDTA)]$ under specified conditions. However, the presence of free cysteine was clearly evidenced by reappearance of $[Ru^{III}(EDTA)(CyS)]^{2-}$ when a freshly prepared solution of $[Ru^{III}(EDTA)]$ was added to the colorless reaction mixture obtained at the end of the reaction of $[Ru^{III}(EDTA)(CyS)]^{2-}$ and O_2 under specified conditions. Based on the above findings and considering the earlier reports on the transition metal complexes catalyzed oxidation of cysteine by molecular oxygen [18–20], the following working mechanism is proposed (scheme 1) for the overall oxidation reaction.

In the proposed mechanism (scheme 1), $[Ru^{III}(EDTA)(CyS)]^{2-}$ rapidly formed in the reaction of $[Ru^{III}(EDTA)(H_2O)]^-$ with cysteine [equation (1)] is vulnerable ($E_{1/2}$ corresponding to $Ru^{III/II}$ couple is −0.26 V *versus* SCE [3]) to oxidation by O_2 [equation (2)]

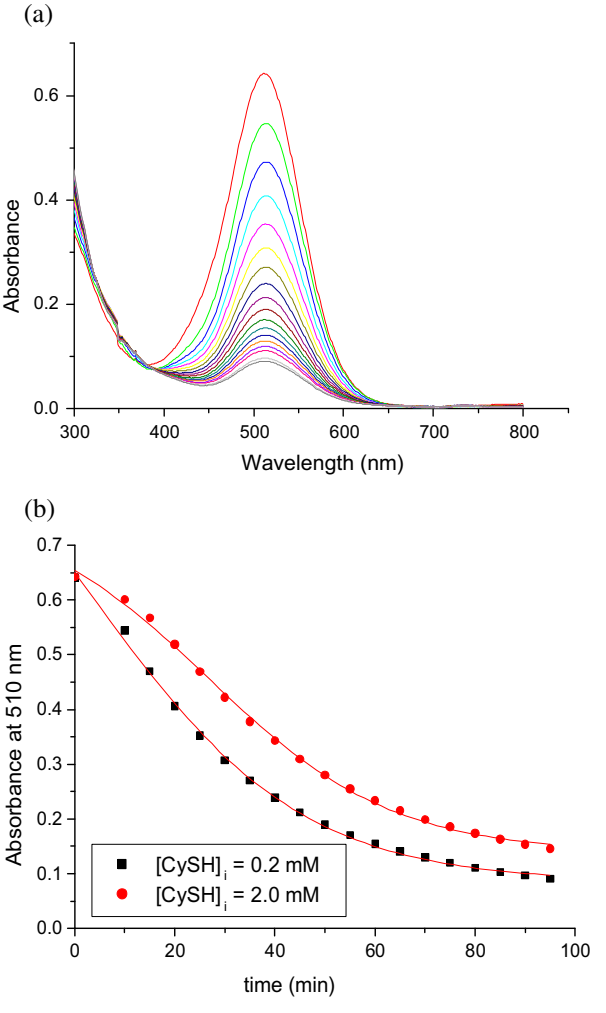


Figure 1. (a) Spectral changes for the reaction of $[Ru^{III}(EDTA)(CyS)]^{2-}$ (performed by reacting 0.2 mM $[Ru^{III}(EDTA)(H_2O)]^{-}$ with 2.0 mM cysteine) with O_2 at 25 °C and pH 7.1 (1 mM phosphate buffer); (b) effect of cysteine concentration on the time *vs* absorbance trace at 510 nm.

leading to formation of $[Ru^{IV}(EDTA)(CyS)]^{-}$ and superoxide radical ion ($O_2^{\cdot-}$). The $[Ru^{IV}(EDTA)(CyS)]^{-}$ could undergo intramolecular electron transfer to produce an intermediate $[Ru^{III}(EDTA)(CyS^{\cdot})]^{-}$ species. The cysteinyl radicals ($CyS^{\cdot}$) formed either by hydrolysis of $[Ru^{III}(EDTA)(CyS^{\cdot})]^{-}$ [equation (3)] or via oxidation of unreacted cysteine by the superoxide radical ion [equation (4)] react together [equation (5)] to produce cystine (CySSCy) as evidenced by ESI-MS studies (see figure S1 in Supplementary information). However, at high pH superoxide radical ion ($O_2^{\cdot-}$) could react with hydroxide ion (OH^{-}) to produce highly oxidizing $OH^{\cdot}$ radical species [equation (6)], which decomposes the red $[Ru^{III}(EDTA)(CyS)]^{2-}$ complex [equation (7)]. Probably this could be the reason for observing higher rate of decay of 510 band at higher pH (figure 2).

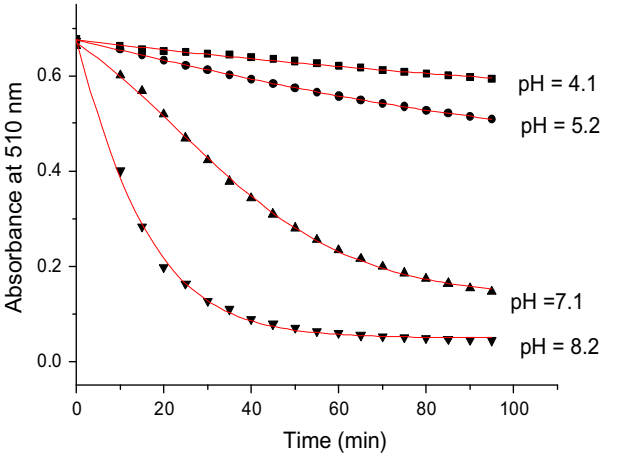


Figure 2. Effect of pH on the time *vs* absorbance traces (at 510 nm) for the reaction of $[Ru^{III}(EDTA)(CyS)]^{2-}$ (performed by reacting 0.2 mM $[Ru^{III}(EDTA)(H_2O)]^{-}$ with 2.0 mM cysteine) with O_2 at 25 °C.

The potential of $[Ru^{III}(EDTA)(SCy)]^{2-}$ toward DNA cleavage was examined by gel electrophoresis using supercoiled plasmid cDNA3 at pH 7.2 (1.0 mM phosphate buffer). DNA cleavage efficiency of $[Ru^{III}(EDTA)(SCy)]^{2-}$ was monitored by observing the conversion of supercoiled (form I) plasmid DNA to the nicked open circular form (form II).

Controlled experiments revealed the inability of cysteine or $[Ru^{III}(EDTA)]$ alone to effect the cleavage of supercoiled plasmid cDNA3 under employed conditions. However, $[Ru^{III}(EDTA)]$/cysteine/O_2 together could cleave the plasmid DNA as shown in figure 3. It is suggested that the superoxide radical [equation (4) in scheme 1] and hydroxide radical [equation (6) in scheme 1] produced in the reaction of $[Ru^{III}(EDTA)(CyS)]^{2-}$ with O_2 (scheme 1) through their oxidative attack (though indiscriminate) presumably causes single-strand scission by attacking the deoxyribose unit leading to sugar fragmentation, followed by base release and then DNA cleavage [21, 22].

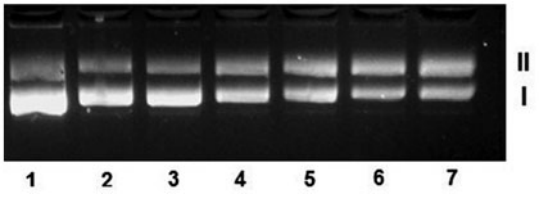


Figure 3. $[Ru^{III}(EDTA)(SCy)]^{2-}$ mediated cleavage of supercoiled plasmid cDNA3 in 1-mM phosphate buffer (pH 7.2) at 37 °C. Lane 1: plasmid DNA only, forms I and II are present, maximum is form I; Lane 2: Ru(EDTA) (250 μM) + CySH (0.2 mM) causes DNA nicking as more form II present; Lane 3: Ru(EDTA) (250 μM) + CySH (0.3 mM) causes increased DNA nicking as more form II present; Lane 4: Ru(EDTA) (250 μM) + CySH (0.4 mM) causes further increase in DNA nicking as more form II present; Lane 5: Ru(EDTA) (250 μM) + CySH (0.5 mM) causes further increase in DNA nicking as more form II present; Lane 6: Ru(EDTA) (250 μM) + CySH (0.6 mM) causes further increase in DNA nicking as more form II present; Lane 7: Ru(EDTA) (250 μM) + CySH (1.0 mM) causes further increase in DNA nicking as more form II present.

$$[Ru^{III}(edta)(H_2O)]^- + CySH \longrightarrow [Ru^{III}(edta)(CyS)]^{2-} + H_2O \quad (1)$$

$$[Ru^{III}(edta)(CyS)]^{2-} + O_2 \longrightarrow [Ru^{IV}(edta)(CyS)]^- / [Ru^{III}(edta)(C\dot{y}S)]^- + \bar{O}_2^{\bullet} \quad (2)$$

$$[Ru^{III}(edta)(C\dot{y}S)]^- \xrightarrow{H_2O} [Ru^{III}(edta)(H_2O)]^- + C\dot{y}S \quad (3)$$

$$\bar{O}_2^{\bullet} + CyS^- \longrightarrow O_2^{2-} + C\dot{y}S \quad (4)$$

$$C\dot{y}S + C\dot{y}S \longrightarrow CySSCy \quad (5)$$

$$\bar{O}_2^{\bullet} + OH^- \longrightarrow O_2^{2-} + OH^{\bullet} \quad (6)$$

$$[Ru^{III}(edta)(H_2O)]^- + OH^{\bullet} \longrightarrow \longrightarrow \text{Decomposition of Ru(III) complex} \quad (7)$$

Scheme 1. Mechanism for $[Ru^{III}(EDTA)]$ mediated oxidation of cysteine by O_2.

4. Conclusion

The results of present studies clearly demonstrate that $[Ru^{III}(EDTA)]$ complex in presence of cysteine and molecular oxygen cleave supercoiled plasmid DNA to nicked circular form. Although bio-activity of ruthenium(II) complexes, especially with regard to DNA cleavage are well documented in the literature [23–28], our results on the reaction of $[Ru^{III}(EDTA)(CyS)]^{2-}$ with O_2 may shed light on a mechanistic understanding of many important biological redox processes of cellular thiols such as cysteine and glutathione. Although reports on the interaction of ruthenium(II) complexes containing polypyridyl [23, 24], ethylenediamine [25], dmp/dip [26] with DNA is well documented in the literature, examples of ruthenium(III)-complexes [27, 28] showing biological activity augment the results of our present study.

Acknowledgement

This article is a small tribute to Rudi's immense contributions to the mechanistic chemistry. I would like to express my sincere gratitude for the warm response and valued guidance I received during the last one and half decade. I really hope that Rudi will enjoy reading this article which is closely related to his area of research. Let me wish him good health and much success in his further scientific pursuits.

Disclosure statement

No potential conflict of interest was reported by the authors.

References

[1] D. Chatterjee, A. Mitra, G.S. De. *Platinum Met. Rev.*, **50**, 2 (2006).
[2] D. Chatterjee, A. Mitra, M.S.A. Hamza, R. van Eldik. *J. Chem. Soc., Dalton Trans.*, 962 (2002).
[3] D. Chatterjee, M.S.A. Hamza, A. Mitra, M.M. Shoukry, S. Deshmukh, R. van Eldik. *J. Chem. Soc., Dalton Trans.*, 203 (2003).
[4] D. Chatterjee, A. Mitra, R. van Eldik. *Dalton Trans.*, 943, (2007).
[5] D. Chatterjee, K.A. Nayak, E. Ember, R. van Eldik. *Dalton Trans.*, **39**, 1695 (2010).
[6] D. Chatterjee, E. Ember, U. Pal, S. Ghosh, R. van Eldik. *Dalton Trans.*, **40**, 10997 (2011).
[7] D. Chatterjee, A. Franke, M. Oszajca, R. van Eldik. *Dalton Trans.*, **43**, 3087 (2014).
[8] D. Chatterjee, N. Jaiswal, A. Franke, R. van Eldik. *Chem. Commun.*, **50**, 14562 (2014).
[9] A. Martínez-Ruiz, S. Lamas. *Cardiovasc. Res.*, **75**, 220 (2007).

[10] N. Gould, P.T. Doulias, M. Tenopoulou, K. Raju, H. Ischiropoulo. *J. Biol. Chem.*, **288**, 2647 (2013).
[11] B. Lima, M.T. Forrester, D.T. Hess, J.S. Stamler. *Circ. Res.*, **106**, 633 (2010).
[12] D. Chatterjee, A. Mitra, A. Levina, P.A. Lay. *Chem. Commun.*, 2864 (2008).
[13] D. Chatterjee, N. Jaiswal, M. Schmeisser, R. van Eldik. *Dalton Trans.*, **43**, 18042 (2014).
[14] A.A. Diamantis, J.V. Dubrwaski. *Inorg. Chem.*, **20**, 1142 (1981).
[15] M.M. Taqui Khan, D. Chatterjee, R.R. Merchant, P. Paul, S.H.R. Abidi, D. Srinivas, M.R.H. Siddiqui, M.A. Moiz, M.M. Bhadbhade, K. Venkatasubramanian. *Inorg. Chem.*, **31**, 2711 (1992).
[16] T. Matsubara, C. Creutz. *Inorg. Chem.*, **18**, 1956 (1979).
[17] H.C. Bajaj, R. van Eldik. *Inorg. Chem.*, **27**, 4052 (1988).
[18] E.T. Adman. *Adv. Protein Chem.*, **42**, 145 (1991).
[19] H. Beinert, M.C. Kennedy, C.D. Stout. *Chem. Rev.*, **96**, 2335 (1996).
[20] G.A. Bagiyan, I.K. Koroleva, N.V. Soroka, A.V. Ufimtsev. *Russ. Chem. Bull. Int. Ed.*, **52**, 1075 (2003).
[21] R.P. Hertzberg, P.B. Dervan. *J. Am. Chem. Soc.*, **104**, 313 (1982).
[22] R.P. Hertzberg, P.B. Dervan. *Biochemistry*, **23**, 3934 (1984).
[23] J. Sun, W.-X. Chen, X.-D. Song, X.-H. Zhao, A.-Q. Ma, J.-X. Chen. *J. Coord. Chem.*, **68**, 308 (2015).
[24] H. Paul, T. Mukherjee, M. Mukherjee, T.K. Mondal, A. Moirangthem, A. Basu, E. Zangrando, P. Chattopadhyay. *J. Coord. Chem.*, **66**, 2747 (2013).
[25] M. Shilpa, C. Shobha Devi, P. Nagababu, J.N.L. Latha, R. Pallela, V.R. Janapala, K. Aravind, S. Satyanarayana. *J. Coord. Chem.*, **66**, 1661 (2013).
[26] Y.-Y. Xie, G.-B. Jiang, J.-H. Yao, G.-J. Lin, H.-L. Huang, X.-Z. Wang, Y.-J. Liu. *J. Coord. Chem.*, **66**, 2423 (2013).
[27] J. Wang, X. Zhao, F. Yan, L. Wei, Q. Pan, F. Zhang, P. Yang. *J. Coord. Chem.*, **66**, 3848 (2013).
[28] S. Sathiyaraj, R.J. Butcher, C. Jayabalakrishnan. *J. Coord. Chem.*, **66**, 580 (2013).