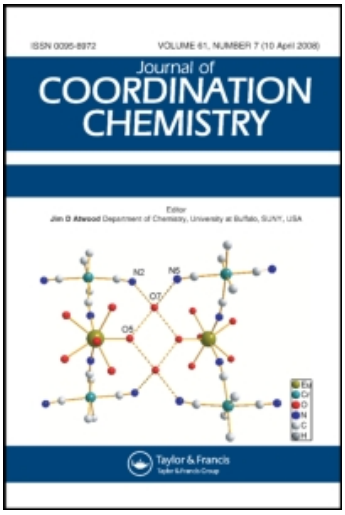

Journal of Coordination Chemistry
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713455674

Reactivity of polyaminocarboxylatoruthenium(III) complexes with serine and their protease inhibition

Debabrata Chatterjee[a]; Ayon Sengupta[a]; Anannya Mitra[a]; Susan Basak[a]; Reema Bhattacharya[b]; Debasish Bhattacharyya[b]
[a] Chemistry Group, Central Mechanical Engineering Research Institute, Durgapur - 713209, India [b] Indian Institute of Chemical Biology, Jadavpur, Kolkata - 700032, India

To cite this Article Chatterjee, Debabrata , Sengupta, Ayon , Mitra, Anannya , Basak, Susan , Bhattacharya, Reema and Bhattacharyya, Debasish(2005) 'Reactivity of polyaminocarboxylatoruthenium(III) complexes with serine and their protease inhibition', Journal of Coordination Chemistry, 58: 18, 1703 — 1711
To link to this Article: DOI: 10.1080/00958970500247487
URL: http://dx.doi.org/10.1080/00958970500247487

Journal of Coordination Chemistry
Vol. 58, No. 18, December 2005, 1703–1711

Taylor & Francis
Taylor & Francis Group

Reactivity of polyaminocarboxylatoruthenium(III) complexes with serine and their protease inhibition

DEBABRATA CHATTERJEE*†, AYON SENGUPTA†, ANANNYA MITRA†, SUSAN BASAK†, REEMA BHATTACHARYA‡ and DEBASISH BHATTACHARYYA‡

†Chemistry Group, Central Mechanical Engineering Research Institute, Durgapur – 713209, India
‡Indian Institute of Chemical Biology, Jadavpur, Kolkata – 700032, India

(*Received in final form 29 March 2005*)

Reaction of $[Ru(edta)(H_2O)]^-$ ($edta^{4-}$ = ethylenediaminetetraacetate), $[Ru(pdta)(H_2O)]^-$ ($pdta^{4-}$ = propylenediaminetetraacetate) and $[Ru(hedtra)(H_2O)]$ ($hedtra^{3-}$ = *N*-hydroxyethylethylenediaminetriacetate) with *S*-serine (Ser) was studied spectrophotometrically and kinetically. Serine protease inhibition studies were performed with the three complexes using the serine protease enzymes chymotrypsin and subtilisin with azoalbumin as substrate. Results are discussed in terms of the reactivity of the Ru-pac (pac = polyaminopolycarboxylates) complexes with serine. The order of protease inhibition efficacy of the Ru-pac complexes is $[Ru(pdta)(H_2O)]^- > [Ru(edta)(H_2O)]^- \gg [Ru(hedtra)(H_2O)]$, in good agreement with the observed reactivity of Ru-pac complexes with serine.

Keywords: Ruthenium-pac complexes; Chymotrypsin; Subtilisin; Serine protease inhibitors

1. Introduction

The serine proteases comprise a major family of proteolytic enzymes, which play key roles in blood coagulation cascade, complement activation, bacterial pathogenesis and fibrinolysis [1]. Serine proteases are implicated in a number of diseases [2] and inhibition of these enzymes is an intense field of study. Recently, the role of serine proteases in breast cancer [3,4], hepatitis C [5] and inflammation and vascular immune phenomena [6] were reported. Enzyme inhibitors in therapeutic use consist mainly of organic compounds. Most of these interact with their targets through the weak hydrogen bonding or van der Waals contacts. Thus the drug molecule resides at the target for a short time, allowing non-specific interactions with non-target molecules that result in undesirable side effects. However, the coordination ability

*Corresponding author. Email: dchat57@hotmail.com

Journal of Coordination Chemistry
ISSN 0095-8972 print: ISSN 1029-0389 online © 2005 Taylor & Francis
DOI: 10.1080/00958970500247487

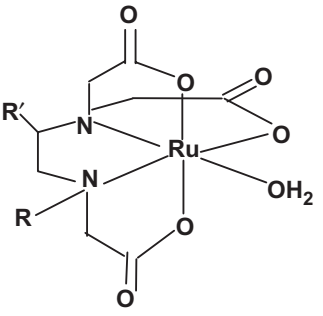

Figure 1. Pictorial representation of complexes **1**–**3**. $R' = H$, $R = -COO^-$, pac = $edta^{4-}$ (ethylenediaminetetraacetate); $R' = CH_3$, $R = -COO^-$, pac = $pdta^{4-}$ (propylenediaminetetraacetate); $R' = H$, $R = -CH_2CH_2OH$, pac = $hedtra^{3-}$ (*N*-hydroxyethylethylenediaminetriacetate).

of metals towards active site residues (to block substrate interaction) or residues outside the active-site (to affect structural integrity) holds the attractive promise of forming stronger covalent and ionic bonds.

Use of coordination complexes of cobalt [7–10], boron [11, 12] and zinc [13–15] are known, but no report on the use of ruthenium complexes in serine proteases inhibition is available in the literature [16]. The import of ruthenium-based drugs has been well established in recent review articles [17, 18]. In this regard the potential of Ru-pac complexes (pac = polyaminopolycarboxylates) as metallopharmaceuticals has been documented. The polyaminopolycarboxylate ligand is somewhat comparable in its donor character to many metallo-enzymes, which make use of carboxylate and amine donors from amino acids to bind to a metal centre. Pac ligands form very stable 1 : 1 metal complexes with ruthenium. It was shown earlier [19–21] and later confirmed by crystallographic studies [22–26] that pac ligands function as pentadentates, as represented in figure 1 in Ru-pac complexes. The sixth coordination site is occupied by a water molecule at low pH or by a hydroxide ion at high pH. Thus, the authors have been engaged in exploring the potential of Ru-pac complexes in biological processes [27, 28] and, very recently, discovered the cysteine protease inhibition activity of the Ru-edta complex ($edta^{4-}$ = ethylenediaminetetraacetate) [28]. The present work stems from an interest to examine the ability of other Ru-pac complexes to inhibit serine protease. The following have been chosen, namely ethylenediaminetetraacetate ($edta^{4-}$), propylenediaminetetraacetate ($pdta^{4-}$) and *N*-hydroxyethylenediaminetriacetate ($hedtra^{3-}$) for the preparation of ruthenium(III) complexes. All the three form pentacoordinate complexes of ruthenium(III). Here the reactivity of $[Ru^{III}(pac)(H_2O)]$ complexes with serine, together with results of protease inhibition studies using two serine protease enzymes, chymotrypsin and subtilisin are reported.

2. Experimental

$K_2[RuCl_5(OH_2)]$ was prepared by a reported method [29]. All other chemicals used were of AR grade and multi-distilled H_2O was used for preparing all solutions.

2.1. *K[Ru(Hedta)(Cl)] (1)*

K[Ru(Hedta)Cl] was prepared by modifying a published procedure [30]. To a hot solution (10 cm^3) of $K_2[RuCl_5H_2O]$ (1 mmol) in $HClO_4$ (1 mM), Na_2H_2edta (1 mmol) dissolved in 10 cm^3 of $HClO_4$ (1 mM) was slowly added with continuous stirring. The reaction mixture was refluxed for 2 h and the volume of the resulting pale yellow solution reduced to 5 cm^3. Addition of cold ethanol precipitated the pale-yellow product, which was filtered off, washed several times with water/acetone (1 : 9) to remove excess chloride, and dried under vacuum. Anal. Calcd for $C_{10}H_{14}ClKN_2O_9Ru$ (%): C, 24.0; H, 3.42; N, 5.59. Found: C, 23.9; H, 3.38; N, 5.61. IR, ν/cm^{-1}: 1720 (COOH), 1650 (COO^-). UV-vis: λ_{max}/nm ($\varepsilon_{max}/M^{-1}\,cm^{-1}$): 283 ($2800 \pm 50$), 350s ($680 \pm 10$).

2.2. *K[Ru(Hpdta)Cl] (2)*

K[Ru(Hpdta)Cl] complex was prepared by reacting $K_2[RuCl_5H_2O]$ with H_2Na_2pdta according to the procedure described for **1**. Anal. Calcd for $C_{11}H_{16}ClKN_2O_9Ru$ (%): C, 24.8; H, 3.94; N, 5.26. Found: C, 24.5; H, 3.98; N, 5.19. IR, ν/cm^{-1}: 1730 (COOH), 1652 (COO^-). UV-vis: λ_{max}/nm ($\varepsilon_{max}/M^{-1}\,cm^{-1}$): 285 ($2650 \pm 60$), 366s ($630 \pm 15$).

2.3. *K[Ru(hedtra)Cl] (3)*

K[Ru(hedtra)Cl] was isolated by reacting $K_2[RuCl_5H_2O]$ with H_3hedtra by the procedure described for **1**. Anal. Calcd for $C_{10}H_{15}ClKN_2O_7Ru$ (%): C, 26.6; H, 3.34; N, 6.2. Found: C, 26.3; H, 3.53; N, 6.1. IR, ν/cm^{-1}: 1635 (COO^-). UV-vis: λ_{max}/nm ($\varepsilon_{max}/M^{-1}\,cm^{-1}$): 285 ($2280 \pm 20$), 360s ($780 \pm 10$).

2.4. *Instrumentation*

Electronic spectra were recorded using a GBC Cintra 10 spectrophotometer. IR spectra were recorded on a Perkin–Elmer 783 spectrophotometer using KBr pellets. A Perkin–Elmer 240C elemental analyser was used to collect microanalytical data. Kinetic measurements were carried out with a SF-61SX2 (HI-TECH) stopped-flow spectrophotometer coupled with an on-line data analyser (KinetAsyst3). Solution temperature was maintained to within $\pm 0.1^\circ C$ using a circulating water bath. Rate constant data were measured under pseudo-first-order conditions with excess (10–50 fold) serine at pH 4.5 (acetate buffer). The pH measurements were carried out with a Elico L1 127 pH meter. Rate constant data (k_{obs}) are an average of several kinetic runs and reproducible within $\pm 4\%$.

2.5. *Protease inhibition studies*

Chymotrypsin and subtilisin were used. A mixture of buffer [sodium phosphate buffer (0.2 M) containing 0.001 M ethylenediaminetetra-acetic acid (EDTA) and 0.05 M β-mercaptol], substrate (1% azoalbumin) and enzyme (with or without inhibitor) was incubated at 37°C for 1 h. The pH of the reaction mixture was 7.5. The reaction was ended by adding 0.5 cm^3 of 5% trichloroacetic acid, the mixture kept at room temperature for 30 min and then centrifuged (4000 rpm) for 15 min. The supernatant (0.5 cm^3) was added to 0.5 cm^3 of 0.5 M NaOH and absorbance recorded at 440 nm.

3. Results and discussion

As confirmed by earlier studies, all three pac ligands form very stable pentacoordinate complexes with Ru(III) with the sixth coordination site occupied by a water molecule at low pH or by a hydroxide ion at high pH. Chloroaminopolycarboxylatoruthenium(III) complexes, $[Ru(pac)Cl]^-$, rapidly convert to $[Ru(pac)(H_2O)]^{-/0}$ when dissolved in water [19–21]. The feature that dominates the chemistry of such complexes is the lability of the water molecule towards substitution, which affords the advantage of facile and straightforward binding of incoming ligand [26]. The substitution follows the interchange associative (I_a) pathway. The lability of $[Ru(pac)(H_2O)]^-$ decreases in the order $[Ru(pdta)(H_2O)]^-$ (**2**) $\geq$ $[Ru(edta)(H_2O)]^-$ (**1**) $\gg$ $[Ru(hedtra)(H_2O)]$ (**3**) [26]. The high reactivity of $[Ru(edta)(H_2O)]^-$ and $[Ru(pdta)(H_2O)]^-$ toward substitution reaction is induced by an internal I_a pathway via a pendant carboxyl group [26]. The slightly higher reactivity observed for $[Ru(pdta)(H_2O)]^-$ as compared to $[Ru(edta)(H_2O)]^-$ could be due to the presence of an electron donating methyl group in the ethylene backbone of the pdta ligand [24].

3.1. *Reaction of Ru-pac complexes with serine*

All three complexes (**1**–**3**) have been characterized by analysis and spectroscopic studies. Electronic and IR data (see Experimental; only major bands are given) are comparable to those reported earlier [19–26]. Typical spectroscopic changes associated with the addition of serine to an aqueous solution of Ru-pac are shown in figure 2. Preliminary kinetic experiments revealed that added buffer components and medium

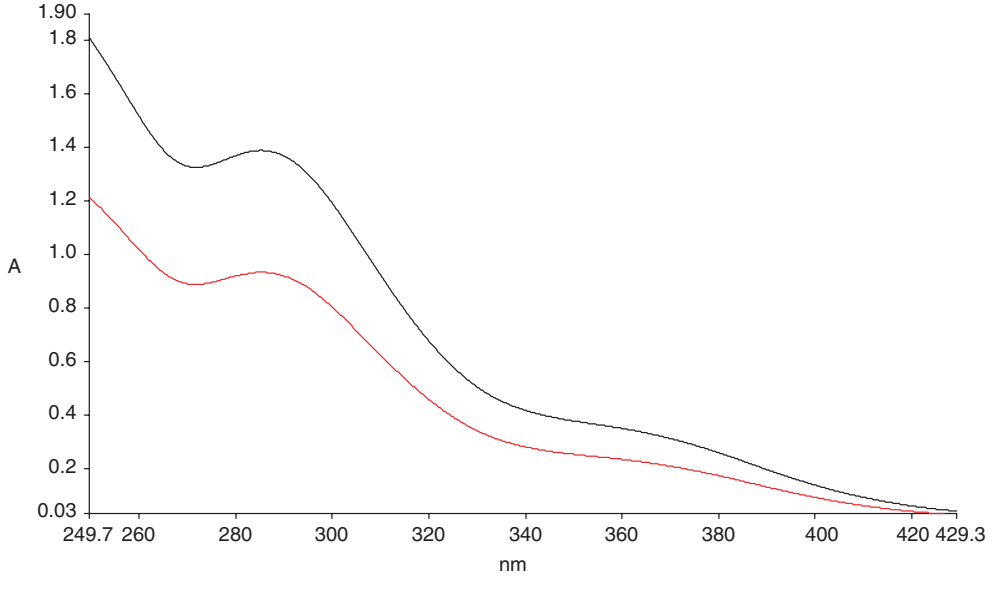

Figure 2. Spectra of (a) **2** and (b) **2** + serine in water at 25°C; $[\mathbf{2}] = 5 \times 10^{-4}$ M, [serine] $= 5 \times 10^{-3}$ M, pH = 4.5, $I = 0.1$ M.

selected to adjust ionic strength of the solution did not interfere with the substitution. The kinetic trace obtained by mixing complex **1** with serine under specified conditions is shown in figure 3. The observed rate constant (k_{obs}) increased linearly with increasing concentration of serine as shown in figure 4. The noticeable intercept suggests that the reverse aquation reaction adds significantly to the observed kinetics.

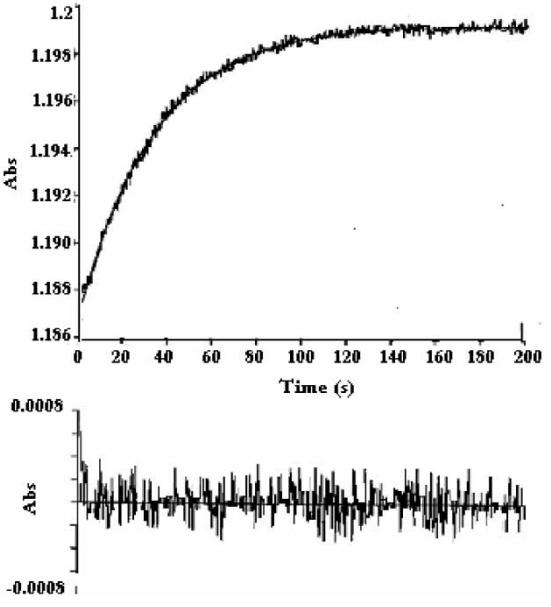

Figure 3. Typical kinetic trace for the reaction between **1** (5×10^{-4} M) and serine (0.025 M) at pH 4.5 and 25°C. The trace was fitted to a single exponential by following the growth in absorbance at 305 nm. The lower trace represents the difference between the experimental and calculated curves.

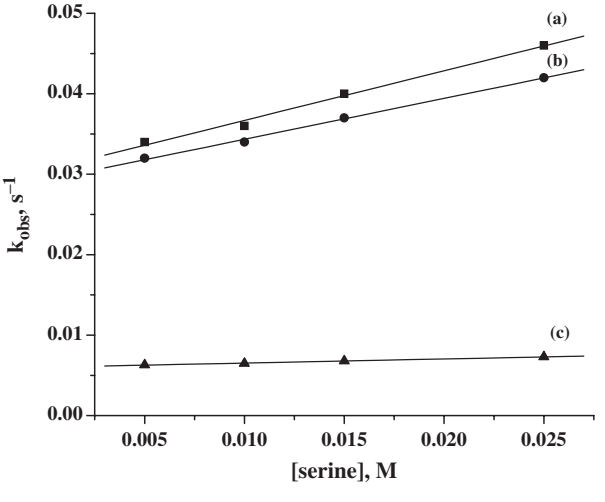

Figure 4. Plot of k_{obs} versus [serine] for the reaction of (a) $[Ru(pdta)H_2O]^-$ with serine, (b) $[Ru(edta)H_2O]^-$ with serine and (c) $[Ru(hedtra)H_2O]$ with serine at 25°C. Experimental conditions: $[Ru^{III}] = 5 \times 10^{-4}$ M, pH 4.5, $I = 0.1$ M ($NaClO_4$).

Table 1. Protocols for serine protease inhibition studies.

					Absorption at 420 nm							
					Chymotrypsin				Subtilisin			
						With inhibitor				With inhibitor		
No.	Buffer added (μL)	Substrate added (μL)	Enzyme added (μL)	Conc. of enzyme (ppm)	Without inhibitor	**1**	**2**	**3**	Without inhibitor	**1**	**2**	**3**
1	500	500	0	0	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
2	490	500	10	5.0	0.447	0.105	0.098	0.445	0.275	0.002	0.001	0.215
3	485	500	15	7.5	0.675	0.235	0.149	0.665	0.434	0.005	0.0016	0.412
4	480	500	20	10.0	0.951	0.291	0.207	0.895	0.566	0.009	0.0027	0.556
5	475	500	25	12.5	1.167	0.358	0.257	1.162	0.716	0.010	0.0035	0.708
6	470	500	30	15.0	1.401	0.405	0.309	1.360	0.908	0.019	0.0038	0.897
7	460	500	40	20.0	1.825	0.585	0.402	1.791	1.172	0.027	0.0040	1.124

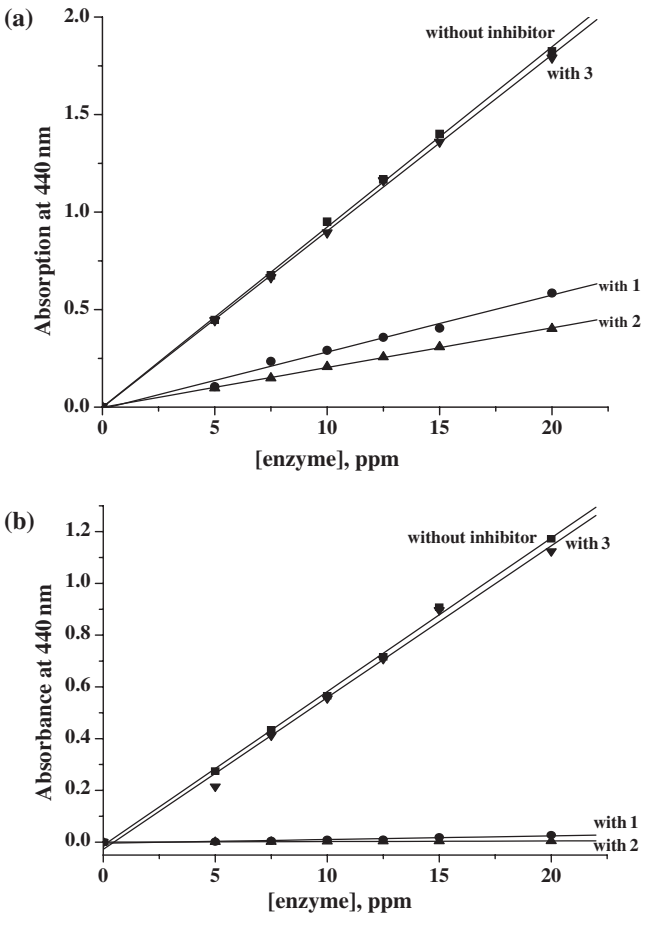

Figure 5. Inhibition of serine protease activity of (a) chymotrypsin and (b) subtilisin by Ru^{III}-pac complexes.

The kinetic behaviour can be rationalized in terms of reaction (1), for which (2) is derived (Ser = serine).

$$[Ru(pac)(H_2O)] + Ser \underset{k_r}{\overset{k_f}{\rightleftharpoons}} [Ru^{III}(pac)(Ser)] + H_2O \quad (1)$$

$$k_{obs} = k_f[Ser] + k_r. \quad (2)$$

Values of k_f and k_r estimated from the plots of k_{obs} *versus* [serine] (figure 4) are $0.50\,M^{-1}\,s^{-1}$ and $0.023\,s^{-1}$ for **1**, $0.62\,M^{-1}\,s^{-1}$ and $0.03\,s^{-1}$ for **2** and $0.05\,M^{-1}\,s^{-1}$ and $0.006\,s^{-1}$ for **3** at 25°C. The reactivity order is $[Ru(pdta)(H_2O)]^- > [Ru(edta)(H_2O)]^- \gg [Ru(hedtra)(H_2O)]$.

3.2. *Serine protease inhibition*

Serine protease inhibition studies using all complexes **1**–**3** as inhibitors were carried out with chymotrypsin and subtilisin with azoalbumin as substrate. Enzyme inhibition

was followed as per the protocol described in table 1. The results (figure 5) clearly demonstrate that complexes **1** and **2** effectively inhibit protease activity of both enzymes under investigation, whereas **3** failed to do so under the specified conditions. Protease inhibition of Ru-pac complexes is believed to occur by ruthenium(III) coordination to the active site of the enzyme. The order of protease inhibition efficacy of Ru-pac complexes, $\mathbf{2} > \mathbf{1} \gg \mathbf{3}$, is in good agreement with the observed reactivity of Ru-pac complexes with serine. The pendant carboxylate arm of **1** and **2** could play a key in the enhanced inhibition activity of **1** and **2**. The inability of **3** to inhibit chymotrypsin and subtilisin is suggested to be due to its slower rate of reaction with serine.

3.3. *Conclusions*

The results clearly demonstrate the ability of **1** and **2** to effectively inhibit the serine protease activity of chymotrypsin and subtilisin. Ru-pac complexes could be valuable additions to the current repertoire of serine protease inhibitors. Peptide conjugates of the complexes would be of interest in studying selectivity of such systems. Work pertinent to this issue is in progress.

Acknowledgements

We thank Dr G.P. Sinha, Director of this Institute, for his encouragement. DC gratefully acknowledges financial support from the DST, India (Grant SP/S1/F35/99). AM thanks the CSIR for RA.

References

[1] E. Tsilikounas, C. Kettner, W. Bachovchin. *Biochem.*, **31**, 12839 (1992).
[2] R.E. Babine, S.L.Bender. *Chem. Rev.*, **97**, 1359 (1997).
[3] D. Kidd, Y. Liu, B.F. Cravatt. *Biochemistry*, **40**, 4005 (2001).
[4] N. Jessani, Y. Liu, M. Humphrey, B.F. Cravatt. *Proc. Nat. Acad. Sci. USA*, **99**, 10335 (2002).
[5] N. Kakiuchi, K. Fukuda, F. Nishikawa, S. Nishikawa, K. Shimotohno. *Combinatorial Chemistry High Throughput Screening*, **6**, 155 (2003).
[6] T.V. Bilfinger, G.B. Stefano. *Current Pharmacruticals Design*, **8**, 505 (2002).
[7] A. Bottcher, T. Takeuchi, K. Hardcastle, T.J. Meade, H.B. Gray, D. Cwikel, M. Kapon, Z. Dori. *Inorg. Chem.*, **36**, 2498 (1997).
[8] S. Bagger, K.Wagner. *J. Peptide. Res.*, **52**, 273 (1998).
[9] T. Takeuchi, A. Bottcher, C.M. Quezada, T.J. Meade, H.B. Gray. *J. Am. Chem. Soc.*, **120**, 8555 (1998).
[10] T. Takeuchi, A. Bottcher, C.M. Quezada, T.J. Meade, H.B. Gray. *Bioorg. Med. Chem.*, **6**, 1 (1999).
[11] E. Tsilikounas, C. Kettner, W. Bachovchin. *Biochem.*, **32**, 12651 (1993).
[12] B. Katz, J. Finer-Moore, R. Mortezael, R. Stroud. *Biochem.*, **34**, 8204 (1995).
[13] J. Geratz, R. Tidwell, J. Schwab, S. Anderie, K. Pryzwansky. *Am. J. Pathol.*, **136**, 909 (1990).
[14] B. Katz, J. Clark, J. Finer-Moore, T. Jenkins, C. Johnson, M. Ross, C. Loung, W. Moore, R. Stroud. *Nature*, **391**, 608 (1998).
[15] H.H. Thorpe. *Chem. Biol.*, **5R**, 125 (1998).
[16] A.Y. Louie, T.J. Mead. *Chem. Rev.*, **99**, 2711 (1999).
[17] M.J. Clarke, F. Zhu, D.R. Fraska. *Chem. Rev.*, **99**, 2511 (1999).
[18] M.J. Clarke. *Coord. Chem. Rev.*, **232**, 69 (2002).
[19] T. Matsubara, C. Creutz. *Inorg. Chem.*, **18**, 1956 (1979).
[20] H.C. Bajaj, R. van Eldik. *Inorg. Chem.*, **28**, 1980 (1989).
[21] H.C. Bajaj, R. van Eldik. *Inorg. Chem.*, **29**, 2855 (1990).
[22] 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).
[23] M.M. Taqui Khan, H.C. Bajaj, Z. Shirin, K. Venkatasubramanian. *Ind. J. Chem. (A)*, **31**, 303 (1992).

[24] M.M. Taqui Khan, H.C. Bajaj, Z. Shirin, K. Venkatasubramanian. *Polyhedron*, **11**, 1059 (1992).
[25] M.M. Taqui Khan, D. Chatterjee, S. Sanal Kumar, Z. Shirin, M.A. Moiz, S.D. Bhatt. *Proc. Ind. Acad. Sci. (Chem. Sci.)*, **106**, 717 (1994).
[26] D. Chatterjee, *Coord. Chem. Rev.*, **16**, 8273 (1998) and references therein.
[27] D. Chatterjee, A. Mitra, M.S.A. Hamza, R. van Eldik. *J. Chem. Soc., Dalton Trans.*, 962 (2002).
[28] D. Chatterjee, M.S.A. Hamza, M.M. Shoukry, A. Mitra, S. Deshmukh, R. van Eldik. *J. Chem. Soc., Dalton Trans.*, 203 (2003).
[29] E.E. Mercer, R.R. Buckley. *Inorg. Chem.*, **41**, 692 (1965).
[30] A.A. Diamantis, J.V. Dubrwaski. *Inorg. Chem.*, **20**, 1142 (1981).