Monatshefte für Chemie 135, 1023–1031 (2004)
DOI 10.1007/s00706-004-0178-6

Monatshefte für Chemie
Chemical Monthly
Printed in Austria

DNA Hydrolysis by Homo- and Heteronuclear Cu(II)–Ni(II) Complexes of Two Diester-type Ligands

Nagihan Saglam[1], **Ahmet Colak**[1], **Kerim Serbest**[2], **Serdar Karaböcek**[1], and **Saadettin Güner**[1,*]

[1] Department of Chemistry, Karadeniz Technical University, TR-61080 Trabzon, Turkey
[2] Department of Chemistry, Karadeniz Technical University, Rize Faculty of Arts and Sciences, TR-53100 Rize, Turkey

Received November 11, 2003; accepted (revised) February 10, 2004
Published online May 21, 2004

Summary. Nucleolytic activities of novel mononuclear Cu(II), homo- and heterodinuclear Cu(II)–Ni(II) complexes with two diester-type ligands were investigated on pCYTEXP by neutral agarose gel electrophoresis. The analyses of the cleavage products obtained electrophoretically indicate that the examined complexes induce very similar conformational changes on supercoiled *DNA* by converting supercoiled form to nicked form. At concentrations greater than 100 μM, the complexes possessed effective nucleolytic activities for 10 min of incubation time. However, their nucleolytic activities did not increase significantly with longer periods of incubation. The *pH*-nucleolytic activity profiles of the complexes differed significantly. Metal complex induced *DNA* cleavage was also tested for inhibition by various radical scavengers. It could be proposed from the data that diffusible intermediate oxidants are not involved in these reactions or they are not necessary for *DNA* cleavage since none of anti-oxidants inhibited *DNA* cleaving activities of the complexes.

Keywords. *DNA* cleavage; Chemical nuclease; Nucleolytic activity; Copper; Nickel.

Introduction

Hydrolytic or oxidative cleavage of nucleic acids are of increasing importance in biological processes and pharmacology. The non-degradative and site specific scission of nucleic acids has offered many applications for the investigation of *DNA* and *RNA* structure, the design of new chemotherapeutic agents, and the manipulation of genes [1]. Conventional enzymatic nucleases such as restriction endonucleases and topoisomerases have been used widely in molecular biology applications [2]. In recent years, the interest in modeling and synthesis of chemical nucleases or nuclease mimics, and the use of these

* Corresponding author. E-mail: sguner@ktu.edu.tr

agents in pharmacology and biotechnology have been increased [3, 4]. Chemical nucleases have been reported to possess some advantages over conventional enzymatic nucleases in that they have smaller size and thus can reach more sterically hindered regions of a macromolecule. In addition, these compounds can be manipulated and derivatized to obtain effective chemical nucleases for specific purposes such as catalysis or chemotherapy. These abilities and advantages made chemical nucleases valuable tools for several biological processes [3, 5, 6].

Nucleolytic activities of several transition metal complexes have been extensively studied [7–9]. These redox active compounds cleave the phosphodiester backbone of *DNA* molecules under physiological *pH* and temperature [9, 10]. Among these compounds, copper(II) complexes have a wide range of biological activity and some of these complexes have been known to be antitumour, antiviral, and anti-inflammatory agents. In addition, since copper(II) complexes especially with *Schiff* base ligands are models of physical and chemical behaviour of biological copper systems, considerable attention has been focused on these compounds [11]. The copper(II) complex of 1,10-phenathroline was the first synthetic transition metal complex effectively exhibiting nucleolytic activity [7]. Several other compounds such as bleomycine- [12], pyrrole- [13, 14], thioether- [15, 16], oxime- [17], peptide- [18], and imidazole-type [19] ligands which were complexed with copper ions also exhibited *DNA*-cleaving activities.

In the present paper, we report the potentials for nucleolytic activity of mononuclear copper(II), homodinuclear copper(II) and heterodinuclear copper(II)-nickel(II) complexes with two ester-type ligands, 1,9-dioxa-3,6-dithiacyclotridecane-10,12-dione (**1**) and 1,4-dioxa-7,10-dithiacyclododecane-2,3-dione (**2**).

Results and Discussion

It was previously observed in our laboratories that several copper complexes prepared from oxime-, tetramin-, tetrathioether-, and *Schiff* base-type ligands had potential superoxide dismutase- or nuclease-mimicking activities [15, 17, 20–22]. These observations have prompted us to synthesize various homo- or heteronuclear copper complexes with novel ligands having the requirements for such enzyme mimicking activities.

The ligands **1** and **2**, and their Cu(II)/Ni(II) complexes were prepared and characterized as reported previously [23]. *DNA* cleavage activities of Cu(II) and Ni(II) complexes with ligands **1** and **2** were electrophoretically investigated with the relaxation of supercoiled plasmid (form I) *DNA* to nicked (form II) and to linear (form III) *DNA* and/or smaller fragments. Reactions were carried out by using supercoiled form of pCYTEXP (5 kb). Of the synthesized 10 complexes with either **1** or **2**, only three complexes (mononuclear Cu(II) complex (**3**) with **1**, homodinuclear Cu(II) complex (**4**) with **2** and heterodinuclear Cu(II)–Ni(II) complex (**5**) with **1**) showed effective *DNA*-cleaving activity. Control experiments carried out with supercoiled DNA in the absence of the complexes showed no background cleavage, and perchlorates of copper(II) or nickel(II) and ligands at concentrations where complexes showed cleavage of *DNA* were ineffective.

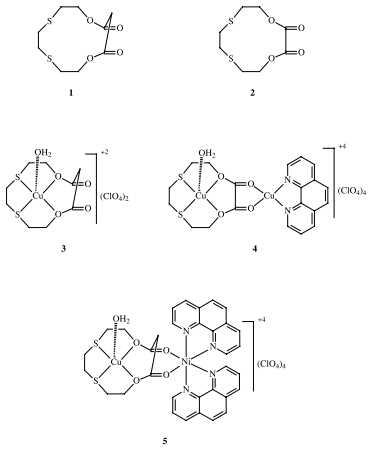

Fig. 1. Proposed structures for the mononuclear copper (**3**), dinuclear copper (**4**), and dinuclear copper/nickel (**5**) complexes of the oxime ligands (**1** or **2**)

Concentration dependency of nucleolytic efficiencies of the complexes were examined at their concentrations between 0.1–1000 μM for 10 and 60 min of reaction period using 52 $\mu g/cm^3$ *DNA* in phosphate buffer, *pH* 7.0 (Fig. 2). 100 μM **3**, 100 μM **4**, or 500 μM **5** induce single strand cleavage of pCYTEXP and convert the circular supercoiled *DNA* (form I) to nicked *DNA* (form II) within the 10 min incubation period (Fig. 2A, 2B, and 2C). At 500 μM **3**, a small fragment of form II is converted to form III. 1000 μM **3** and 500 and 1000 μM **4** completely degraded *DNA* into smaller fragments. **3** had no such degredation activity up to 1000 μM concentration within short period of time.

The *DNA* cleaving activity of **3**, **4**, and **5** for 60 min incubation period was also investigated for complex concentration ranging 0.1–1000 μM in phosphate buffer, pH 7.0 (Fig. 3). At 100 μM concentration of either **3** (Fig. 3A) or **4** (Fig. 3B), supercoiled *DNA* is converted to nicked *DNA* form. At concentrations of **3** and **4** greater than 100 μM, both complexes completely degraded *DNA* forms. However, **5** with greater concentrations produced only form II and the *DNA* was not degraded completely (Fig. 3C). At lower concentrations (up to 100 μM), none of the complexes showed effective *DNA* nicking activity. No linear *DNA* except in the presence of 100 μM **3** was formed within 10 min or 60 min incubation periods at 0.1–1000 μM complex. These results indicate that scission activity of the complexes is both concentration- and time-dependent (Table 1). It is clear from these electrophoretic data that the complexes are not able to carry out double strand *DNA* scission on nearby sites and therefore supercoiled *DNA* is converted to nicked DNA at lower complex concentrations, and finally to small *DNA* fragments at higher concentrations. The complexes may possibly induce conformational changes on plasmid *DNA* by making single strand nicking, therefore converting supercoiled form to nicked form. There is a significant increase to the conversion of supercoiled *DNA* to nicked DNA especially by **3** and **4** when the reaction period is increased from 10 to 60 min (Table 1). These data indicate the production of intermediates responsible for the cleavage of *DNA* upon increasing the reaction period. Moreover, formation of linear form was not observed (Figs. 2 and 3).

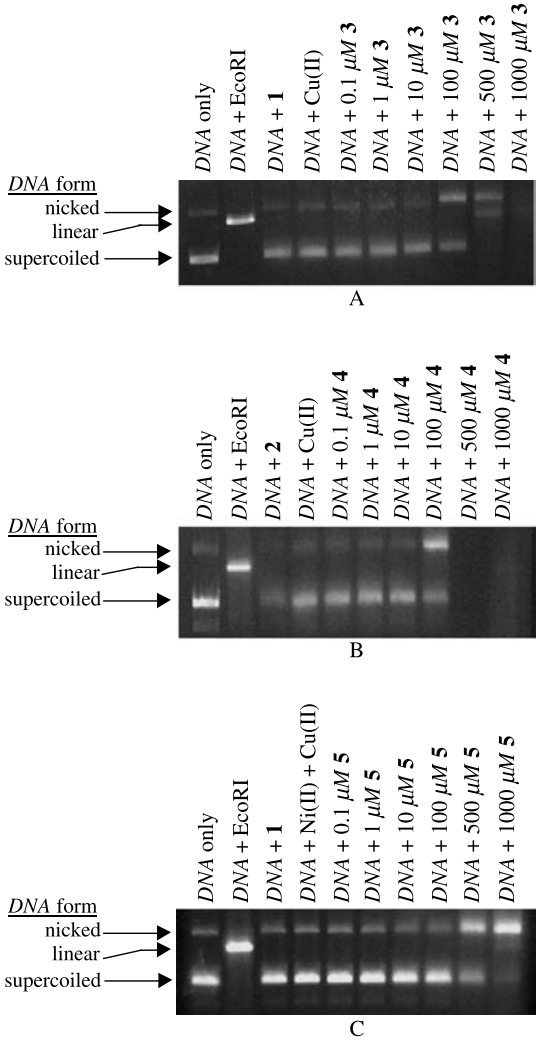

Fig. 2. *DNA* cleavage as a function of increasing concentrations of complexes **3** (Fig. 2A), **4** (Fig. 2B), and **5** (Fig. 2C) for 10 min of reaction period as described under Experimental Section

pH dependencies of the interaction of the complexes with plasmid *DNA* were performed in appropriate buffers for 10 min of reaction period. *pH* was varied over a *pH* range of 6.0–7.5 in 50 m*M* phosphate buffer and *pH* 8.0–10.0 in 20 m*M* trisacetate buffer. The effect of complexes on supercoiled form of *DNA* between *pH* 6.0–10.0 was observed electrophoretically by monitoring the *DNA* forms to be produced and the data were analyzed by plotting *pH* changes versus percentage of *DNA* form II present per lane (Fig. 4). All complexes had different *DNA* scission activity at different *pH* values. **3** was nucleolytically active especially at either acidic or basic *pH* values. In contrast, **4** had nucleolytic activity at a wide range of *pH* values. **5** behaved differently from those of **3** and **4**. The nucleolytic activity of **5** was extremely low at *pH* 7.0–8.0 and values greater than *pH* 9.0.

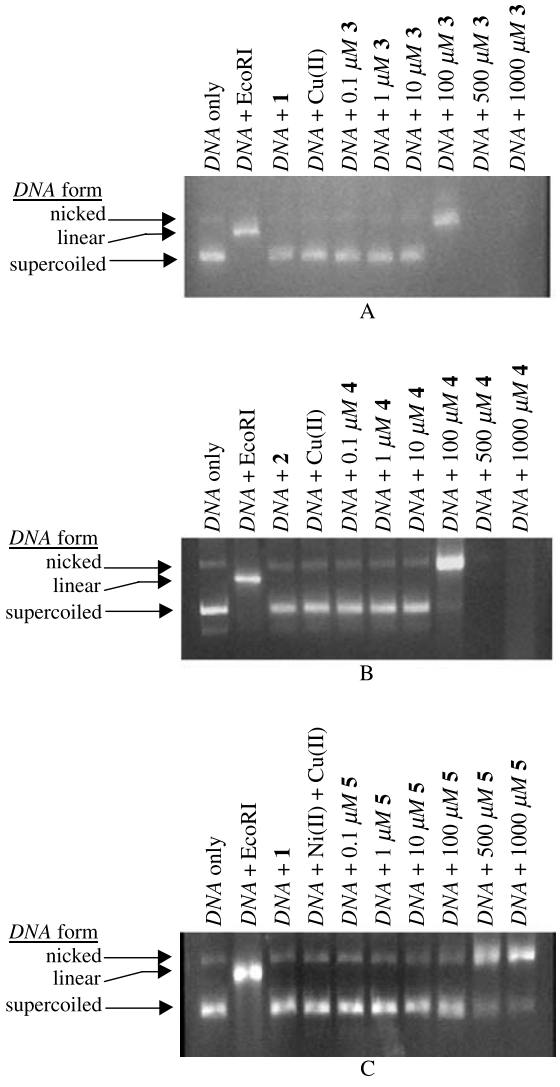

Fig. 3. *DNA* cleavage as a function of increasing concentrations of complexes **3** (Fig. 3A), **4** (Fig. 3B), and **5** (Fig. 3C) for 60 min of reaction period as described under Experimental Section

Table 1. Time-dependencies of *DNA* cleavage by 100 μM **3**, 100 μM **4**, and 500 μM **5** for 10 or 60 min reaction period; the amount of form II produced in the presence of the complexes was determined by BioDoc Analyze System from the data in Figs. 2 and 3

Complex	The percentage of form II/lane	
	10 min	60 min
3	60	100
4	65	95
5	60	75

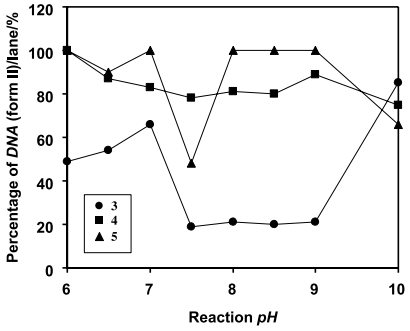

Fig. 4. A plot of the reaction *pH* versus the percentage of form II *DNA*; form III *DNA* was not formed; the amount of form II produced in the presence of the oxime complexes **3**, **4**, and **5** was determined by BioDoc Analyze System

Metal complex induced *DNA* cleavage was also tested for inhibition by various radical scavengers as superoxide dismutase, sodium azide, thiourea, and potassium iodide. Sodium azide is a singlet oxygen scavenger, superoxide dismutase (*SOD*) removes superoxide anion, and potassium iodide and thiourea eliminate hydroxyl radicals. It is clear from the data (Fig. 5) that none of the radical scavengers inhibited the metal complex-induced *DNA* cleavage since similar *DNA* cleavage profiles were seen with the complexes or with the complexes and radical scavengers. The data observed when the complexes and these antioxidants are combined indicates generation of either the same activated intermediates or different intermediates possessing similar or greater *DNA* cleaving specificities. These results also suggest that diffusible intermediate oxidants are not involved in these reactions or not necessary for *DNA* cleavage [24–27].

In general, perchlorate salts of metals possess extremely high ionic characters due to the resonance stabilization of perchlorate anion. One of the reasons for the preparation and study of perchlorate salts for *DNA* hydrolysis is that the cationic part of complexes **3**, **4**, and **5** stays free in aqueous medium. In this case, the interaction efficiency of these cations with *DNA* is greatly enhanced. Therefore, the interaction with *DNA* might involve ionic species rather than oxygen radicals as observed by insensitivity of various radical scavengers to nucleolytic activity of complexes. This is well consistent with previous information of hydrolytic attack of the cationic metal complex on the phosphodiester backbone of *DNA* [24, 26]. Copper(II) and/or nickel(II) centers as strong Lewis acids in such complexes facilitate deprotonation of the coordinated water molecule and produce a hydroxide nucleophile to form the active catalyst [1, 26]. One of the possible active species is the hydroxo form $[Cu(L)OH]^+$ of the complexes formed when a proton is removed from the metal-coordinated water molecule in all three complexes.

The present study has established nucleolytic activities of novel mononuclear Cu(II), homo- and heterodinuclear Cu(II)–Ni(II) complexes with two diester-type ligands. The *DNA* cleavage activities of the examined complexes are concentration-, *pH*-, and time-dependent. The complexes possessed effective nucleolytic

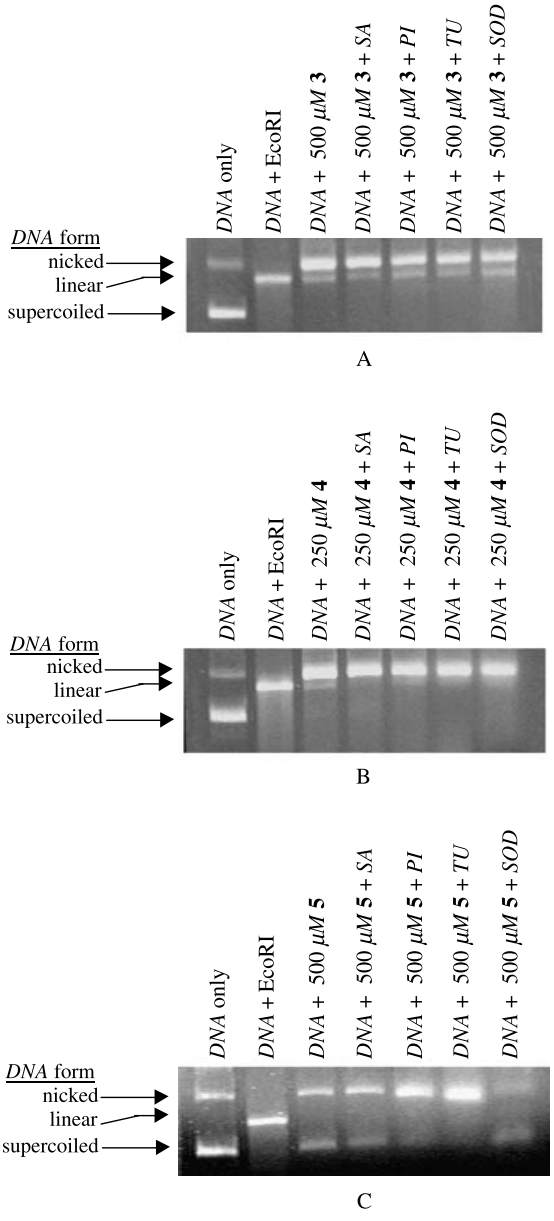

Fig. 5. Effect of various radical scavengers on *DNA* cleavage induced by the oxime complexes **3** (Fig. 5A), **4** (Fig. 5B), and **5** (Fig. 5C) with 52 $\mu g/cm^3$ *DNA* in phosphate buffer, *pH* 7.0; the final concentrations of all the scavengers, sodium azide (*SA*), potassium iodide (*PI*), and thiourea (*TU*), were 50 m*M* except that superoxide dismutase (*SOD*) was 0.1 mg/cm^3

activities for shorter periods of reaction times which indicate the generation of some activated intermediates. It could be proposed from metal complex induced *DNA* cleavage data obtained in the presence of radical scavengers that diffusible intermediate oxidants are not involved in these reactions or not necessary for *DNA* cleavage.

Experimental

Materials

Circularly closed superhelical plasmid (pCYTEXP) was a gift from Dr. *J.E.G. McCarthy* (Biomolecular Sciences, UMIST, UK). *E. coli* JM101 cells harbouring the plasmid were grown in LB media for overnight [28] and purified by the Promega Corporation Wizard Plus SV Minipreps DNA Purification Systems (Madison, USA). All commercial reagents were of reagent quality.

Preparation of the Ligands and their Metal Complexes

1,9-Dioxa-3,6-dithiacyclotridecane-10,12-dione (**1**) and 1,4-dioxa-7,10-dithiacyclododecane-2,3-dione (**2**) ligands and their mononuclear copper(II) (**3**), homodinuclear copper(II) (**4**), and heterodinuclear copper(II)–nickel(II) (**5**) complexes (Fig. 1) were prepared as reported [23].

DNA Cleavage Studies

Stock solutions of the complexes were prepared dissolving the complexes in 100 mm^3 of dimethylsulfoxide and dilution to 1 cm^3 with Milli-Q water. *DNA* cleaving activities of all complexes were assayed with supercoiled plasmid pCYTEXP in potassium phosphate or trisacetate buffer at 37°C. Reaction mixtures contained individual metal complexes, 50 m*M* potassium phosphate or 20 m*M* trisacetate buffer, pCYTEXP (52 $\mu g/cm^3$), and pure water at various conditions. In experiments to determine the effect of concentrations of the complexes, *pH* of medium and incubation time on *DNA* cleavage, supercoiled *DNA* was treated with 0.1–1000 μM solutions of the complexes in a *pH* range of 6.0–7.5 in 50 m*M* potassium phosphate buffer or *pH* 8.0–10.0 in 20 m*M* trisacetate buffer. Reaction mixtures were incubated for 5, 10, 20, 30, 40, 50, and 60 min at 37°C. Reactions were terminated by the addition of 5 mm^3 of the terminating agent containing 10 m*M* β-mercaptoethanol, 20% glycerol, 25 m*M EDTA*, and 0.05% bromophenol blue:xylene cyanol (1:1) after an appropriate incubation period as described previously [15]. *DNA* strand cleavage was investigated on 0.7% neutral agarose gels including 0.5 mg/cm^3 ethidium bromide by conversion of the supercoiled plasmid (form I) *DNA* initially to the nicked (form II) and finally to linear (form III) plasmids. The gels were viewed on a transilluminator, and photographed by using a BioDoc Analyze System. Band intensities on agarose gels were determined by using the same system.

Effect of Radical Scavengers on DNA Breakage

The nucleolytic properties of **3**, **4**, and **5** on pCYTEXP in the presence of various radical scavengers were assayed electrophoretically. pCYTEXP (52 $\mu g/cm^3$) was incubated in a reaction mixture (10 mm^3) containing effective concentrations of individual metal complexes and various radical scavengers as sodium azide, potassium iodide, thiourea, and superoxide dismutase (*SOD*) in 50 m*M* potassium phosphate buffer, *pH* 7.5 at 37°C. Reactions were terminated by the addition of 5 mm^3 of a terminating agent containing 20% glycerol, 25 m*M EDTA*, 10 m*M* β-mercaptoethanol, and 0.05% bromophenol blue:xylene cyanol (1:1) after 1 h of incubation. *DNA* strand cleavage was estimated on 0.7% neutral agarose gel including 0.5 mg/cm^3 ethidium bromide. Quantitative analysis of cleavage products was performed by BioDoc Analyze System.

Acknowledgements

This work was financially supported by The Research Fund of Karadeniz Technical University (P.Nr. 20.111.002.3 to *S. G.*). The authors thank Drs. *S. Canakci* and *A.O. Beldüz* (Karadeniz Technical

University, Department of Biology) for their kind support and Dr. *I. Degirmencioglu* for critical reading of the manuscript.

References

[1] Cowan JA (2001) Curr Op Chem Biol **5**: 634
[2] Branum ME, Que L (1999) J Biol Inorg Chem **4**: 593
[3] Papavassiliou AG (1995) Biochem J **305**: 345
[4] Wiseman H, Halliwell B (1996) Biochem J **313**: 17
[5] Blackburn GM, Gait MJ (eds) (1990) Reversible Interactions of Nucleic Acids with Small Molecules. In: Nucleic Acids in Chemistry and Biology, Oxford University Press, Oxford, p 295
[6] Kaim W, Schwederski B (eds) (1994) Chemotherapy with Compounds of Some Nonessential Elements. In: Bioinorganic Chemistry: Inorganic Elements in The Chemistry of Life, John Wiley and Sons, Chichester, p 363
[7] Pope LE, Sigman DS (1984) Proc Natl Acad Sci USA **81**: 3
[8] Thomas AM, Nethaji M, Mahadevan S, Chakravarty AR (2003) J Inorg Biochem **94**: 171
[9] McLachlan GA, Muller JG, Rokita SE, Burrows C (1996) J Inorg Chim Acta **251**: 193
[10] Sigman DS, Graham DR, D'Aurura V, Stern AM (1979) J Biol Chem **254**: 12269
[11] Reddy KH, Reddy PS (2000) Transition Met Chem **25**: 505
[12] Kane SA, Hecht MS (1994) Prog Nucleic Acid Res Mol Biol **49**: 313
[13] Borah S, Melvin MS, Lindquist N, Manderville RA (1998) J Am Chem Soc **120**: 4557
[14] Asad SF, Singh S, Ahmad A, Hadi SM (1999) Biochim Biophys Acta **1428**: 201
[15] Dülger S, Saglam N, Beldüz AO, Güner S, Karaböcek S (2000) Biometals **13**: 261
[16] Athar F, Arjmand F, Tabassum S (2001) Transition Met Chem **26**: 426
[17] Saglam N, Colak A, Serbest K, Dülger S, Güner S, Karaböcek S, Beldüz AO (2002) Biometals **15**: 357
[18] Garcia-Raso A, Fiol JJ, Adrover B, Moreno V, Mata I, Espinosa E, Molins E (2003) J Inorg Biochem **95**: 77
[19] Gonzalez-Alvarez M, Alzuet G, Borras J, Macias B, del Olmo M, Liu-Gonzalez M, Sanz F (2002) J Inorg Biochem **89**: 29
[20] Güner S, Karaböcek S (1998) J Biochem Mol Toxicol **12**: 53
[21] Güner S, Karaböcek S, Kaklikkaya I (1999) Bioorg Med Chem **7**: 329
[22] Serbest K, Colak A, Güner S, Karaböcek S (2001) Trans Metal Chem **26**: 625
[23] Karaböcek S, Değirmencioglu I, Karaböcek N, Serbest K (2003) Transition Met Chem **28**: 529
[24] Gravert DJ, Griffin JH (1993) J Org Chem **58**: 820
[25] Santra BK, Reddy PAN, Neelakanta G, Mahadevan S, Nethaji M, Chakravarty AR (2002) J Inorg Biochem **89**: 191
[26] Rossi LM, Neves A, Hörner R, Terenzi H, Szpoganicz B, Sugai J (2002) Inorg Chim Acta **337**: 366
[27] Sugden KD (1999) J Inorg Biochem **77**: 177
[28] Belev TN, Singh M, McCarthy JEG (1991) Plasmid **26**: 147