REVIEW

J. Chem. Research (S), 2002, 95–98
J. Chem. Research (M), 2002, 0301–0315

Copper complexes with N-donor ligands as models of the active centres of nitrite reductase and related enzymes

Raymond L. Richards[a]* and Marcus C. Durrant[b]

[a]*School of Chemistry, Physics and Environmental Science, University of Sussex, Brighton, East Sussex BN1 9QJ, UK*
[b]*Computational Biology Unit, John Innes Centre, Norwich Research Park, Colney Lane, Norwich NR4 7UH, UK*

The chemistry of copper complexes that are designed to model aspects of the structure and function of the copper-containing enzyme nitrite reductase (CuNiR) is reviewed.

Keywords: copper, nitrite reductase, pyridyl ligands

1. Introduction

Denitrification, the enzymatic reduction of nitrate (NO_3^-) and nitrite (NO_2^-) to gaseous NO, N_2O and/or N_2, is part of the nitrogen cycle which is essential to life. Studies of denitrification have gained impetus in recent years because of environmental concerns over the widespread agricultural use of potentially polluting nitrate and nitrite, and the damage to the atmosphere from NO and N_2O that can be produced by their degradation.[1]

Denitrification is mediated by a number of metalloenzymes; in particular we concentrate in this article on the reduction of nitrite, which is catalysed by two distinct types of nitrite reductase enzyme. One type involves a multi-haeme system and the second, upon which we shall focus, is a multicopper enzyme (CuNiR).[2]

X-ray studies of CuNiR from a number of sources have shown that the enzyme is a homotrimer in which each monomer contains a so-called Type 1 copper, with a $(His)_2(Cys)(Met)$ ligand set, linked by sequential protein residues (CysHys) to a Type 2 copper, bound at the interface between subunits of the protein by a facial array of three histidine residues. The two copper centres are around 12.6 Å apart. A fourth ligand, water or hydroxide, completes the pseudo-tetrahedral coordination of the Type 2 copper which is situated in a hydrophobic pocket, some 12 Å from the protein surface (Fig. 1).[3–6]

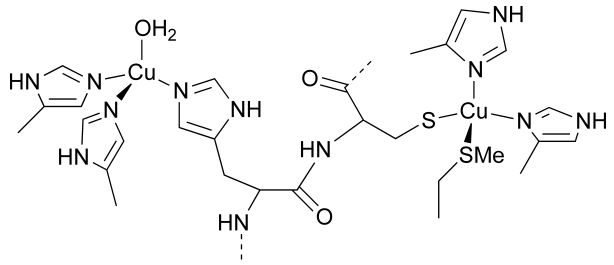
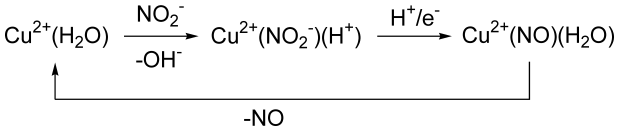


Fig. 1

A combination of X-ray crystallographic and spectroscopic studies has established that the Type 2 copper is the site of binding of NO_2^- and reduction.[3–6] Uptake of nitrite at the Type 2 copper appears to involve displacement of water (hydroxide).[2,6] The overall reduction process to give NO is outlined in Scheme 1. The detailed mechanism is suggested to proceed by attack of protons, mediated by amino-acid residues, on Cu-bound NO_2^-, with concomitant electron transfer from the Type 1 copper, *via* the protein, during the reduction process.[2,6,7] The precise detail within the steps shown in this scheme remains to be established. A second process catalysed by CuNiR is the formation of N_2O, which might occur by attack of NO or NO_2^- on the nitrosyl intermediate.[8]

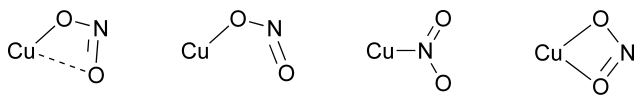


Scheme 1

It has been the aim of studies using copper complexes to model the structure and function of the CuNiR reaction centre and thereby not only to help establish the detailed mechanism of reduction of nitrite, but also to derive simple, robust and effective catalytic methods to remove nitrite pollutant from water supplies.

2. Mononuclear models of the Type 2 copper site

A number of questions have been posed as a result of the above biochemical studies. The first concerns the mode of binding of nitrite to the Type 2 copper. Structural studies have shown that nitrite can have a variety of coordination modes to a single copper(II) centre, as shown in Scheme 2. X-ray structural studies of nitrite-soaked, oxidised CuNiR have shown that nitrite binds to the Type 2 copper via its oxygens in an asymmetric, pseudo-chelated mode, with one oxygen of the nitrite close to the copper (at about 2.1 Å) and the other more distant (at about 2.4Å) (**1a** in Scheme 2).[2–7] Given that the catalytic cycle most likely involves reduction of the initial Cu^{II} nitrite adduct to a transient Cu^{I} nitrite, it was an early aim of chemists to synthesise nitrite complexes of both Cu oxidation



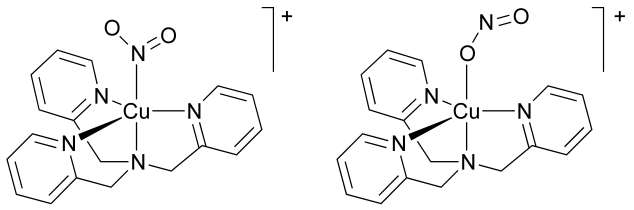
1a **1b** **1c** **1d**

Scheme 2

* To receive any correspondence.

states, to explore the type of binding that nitrite could show at such a centre, and to examine the reactivity of bound nitrite.

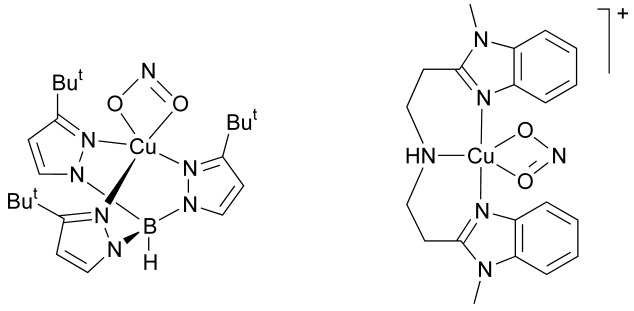
Such studies have generally involved nitrogen-donor, chelating ligands which model the histidine environment of the Type 2 copper. A typical study, illustrated by Figure 2, used the tris-(2-pyridylmethyl)amine (tpa) ligand.[9] This type of ligand has had extensive use in the synthesis of copper complexes which model other copper enzyme centres, particularly in O_2-activating enzymes such as haemocyanin and peptylglycine monooxygenase.[10] In the work outlined in Fig. 2, it was shown that nitrite could bind to copper(II) either through essentially one oxygen or through the nitrogen; there appeared to be little energy difference between the two forms, and there is an observable equilibrium between them in solution.[9] An analogue of this complex having ethylene rather than methylene groups linking the amino-nitrogen to the pyridyls also shows **1a**-type binding.[11] Further evidence that the O- and N-bound nitrite linkage isomers are generally close in energy is provided by the complex $[Cu(NO_2)_2(tpzm)]$ [tpzm = tris-(pyrazol-1-yl)methane], whose X-ray crystal structure reveals one nitrite ligand of each type coordinated to the Cu^{II} centre.[12]



2a **2b**

Fig. 2

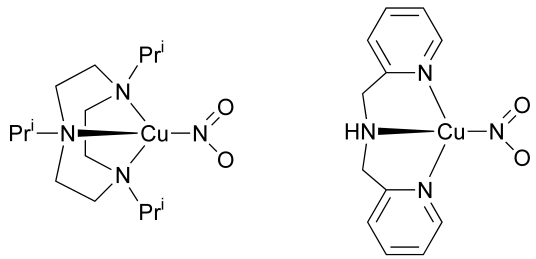
Nitrite binding as in Scheme 2, **1d** is shown in Fig. 3. These two examples also show two other common types of ligand used to mimic histidine binding, the pyrazolyl borates[13] [tris(3-R,5-R′-pyrazolyl)hydroborate] (*e.g.* **3a**, R = Bu^t, R′ = H) and benzimidazole-type ligands (bim) (see **3b**).[14] Here, in each case, the copper is bound by only three nitrogen donors so that chelation of nitrite gives a favoured 5-coordinate structure.



3a **3b**

Fig. 3

Some of the questions raised by the biochemical mechanism of nitrite reduction shown in Scheme 1 have been addressed at least partially by these model studies. The first concerns the catalytically productive mode of binding of nitrite at copper. The binding of nitrite to the oxidised enzyme appears to be of the **1a** type and this also has been established in Cu^{II} model complexes. Nevertheless, the difference in energy between the various modes of nitrite binding in Scheme 2 is small. The catalytically productive mode of binding would appear to be attained at the Cu^{I} stage; here relatively few nitrite complexes have been obtained, and only mode **1c** has been structurally established so far. Two such Cu^{I} complexes are shown in Figure 4; **4a** contains a triazacyclononane ligand, 1,4,7-triisopropyl-1,4,7-triazacyclononane,[15] whilst **4b** contains bis-(6-methyl-2-pyridylmethyl)amine.[16] The Cu^{II} analogue of **4b** has an octahedral geometry with O-coordinated nitrite.[16] Although NO can be produced from complexes **4a** and **4b**, at present the mode of binding of nitrite immediately prior to its conversion to NO remains the subject of debate.[17]



4a **4b**

Fig. 4

The binding and reduction of nitrite at copper complexes $[CuL]^{2+}$, where L is a range of tridentate N-donor ligands as shown in Fig. 5, has been studied.[18,19]

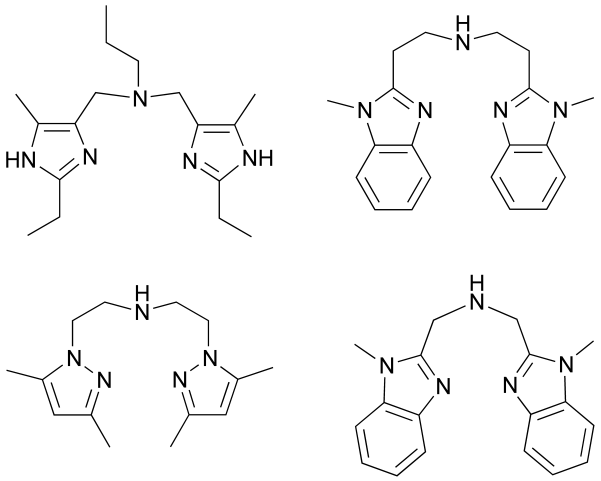


Fig. 5

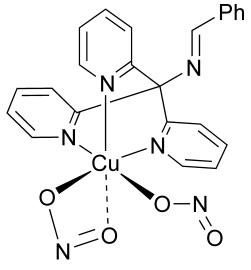
The equilibrium constants for binding of NO_2^- are in the range of $7.5 \times 10^4 - 2.6 \times 10^6$ M^{-1} for the Cu^{II} complexes (in methanol), compared to $30 - 210$ M^{-1} for the Cu^{I} complexes (in 1:1 acetonitrile:methanol), and the Cu^{II}/Cu^{I} couple is shifted to lower potentials on binding nitrite. It was suggested that although Cu-coordinated nitrite is a very poor base, electron transfer between Cu^{I} and coordinated nitrite may be facilitated by protonation of the substrate.[19] This would be consistent with X-ray crystallographic studies on mutant forms of the enzyme, which implicated His and Asp amino acid sidechains in controlling the protonation state of bound nitrite.[6]

The second question which models can be used to address is whether nitrite degrades to give a copper-bound NO ligand immediately prior to NO release, and if so, how is the NO bound? The conversion of nitrite to NO has been achieved in a number of systems, but intermediate NO complexes have not been characterised. Thus the complex $[Cu(tpa)(H_2O)]ClO_4$

catalyses the electrochemical reduction of nitrite to NO (minor product) and N_2O (major product) presumably via complex **2a** or **2b**.[9] Copper(I) complexes related to **3b**, $[Cu(bim)]^+$, react slowly with $NaNO_2$ in MeOH as proton source, to produce NO and $[Cu(bim)]^{2+}$ species; protonation and dehydration of nitrite was deduced to be faster than its subsequent reduction. Again no intermediate complexes were characterised, but presumably the first step would be the production of a Cu^I analogue of **3b**.[14] Complexes **4a** and **4b** react with protic acids to give NO, but again no NO-bound intermediate has been reported to date. Although transient Cu–ON species have been proposed for the biochemical mechanism,[2,6] it is most likely that should a copper nitrosyl species of any stability be formed, the NO would be N-bound, since this is the normal mode of binding of this ligand at mononuclear sites, as seen for example in the analogue of **3a** in which NO replaces NO_2^-.[20] Reaction of NO with Cu^I complexes of other substituted tris(pyrazolyl)hydroborate ligands also gave N-bound NO complexes, which were fully characterised.[21] The NO ligand in these complexes is relatively weakly bound, and could be displaced by application of vacuum or the addition of MeCN or CO.

An additional open question is the mechanism of formation of N_2O. A number of nitrito-complexes of copper can be used to generate N_2O. Thus complex **2a** (or **2b**) catalyses[9] the electrochemical reduction of nitrite to N_2O as the major product, as well as NO. An analogue of **3a**, [Cu{tris(3-Ph,5-Ph-pyrazolyl)hydroborate}(MeCN)], under an excess of NO, initially forms NO adducts that then cleanly convert to Cu^{II} nitrite complexes and one equivalent of N_2O,[20] and NO is reduced to N_2O by alcoholic solutions of $[Cu(Pr^i_3 9[ane]N_3)(MeCN)][PF_6]$.[15] Mechanisms for these reactions involving intermediates having bis(nitrosyl) ligation or N_2O_2 groups have been proposed,[20] but these have not been isolated and detailed mechanistic study is required to establish mechanisms.

A further class of tripodal ligand based on tris(2-pyridyl)methylamine (tpm) has been developed with the aim of providing both a Type 2 environment for copper and a potential means of linking to a second, preferably Type 1, copper.[22] The Cu^{II} complexes of tpm and its derivatives react with nitrite to give a range of complexes, such as the Schiff base complex **6a** in Fig. 6. The use of derivatives of tpm to give dinuclear complexes is discussed below.



6a

Fig. 6

3. Dinuclear models

Here the aim of model studies has been to prepare complexes containing two copper atoms, separated by an organic linking group at a distance close to 12.6 Å, with the copper atoms having environments which mimic those found in nitrite reductase (Fig. 1). In one such systematic study,[23] identical Cu^{II} atoms carrying terminal tridentate ligands such as diethylenetriamine (dien) have been linked using bridging bidentate ligands such as 3,6-bis(imidazolyl)pyridazoline (bimpydz), conferring Type 2 ligation on the coppers. Treatment of the complex $[\{Cu(dien)\}_2(\mu\text{-bimpydz})][BF_4]_4$ with $NaNO_2$ then gave a polymeric complex, $[\{Cu(dien)\}_2(\mu\text{-bimpydz})][NO_2][BF_4]_3$. The crystal structure of this complex, outlined in Fig. 7, is notable for two features. The first is that in the $\{Cu_2(\mu\text{-bimpydz})\}$ unit two Type 2 coppers are held apart at 12.88 Å, and the second is the binding of the NO_2 group, which bridges two of the dinuclear copper cations by mixed N- and O-ligation (μ-NO_2-κ-O:κ-N) to give a one-dimensional chain. The binding of NO_2 to copper is somewhat unusual in that it occupies an essentially axial, rather than the more usual equatorial coordination site.[23] The same bridging linkage is found in the complex array $[\{Cu(bdmppy)(NO_2)\}_2(\mu\text{-}NO_2\text{-}\kappa\text{-}:\kappa\text{-}N)]_2$- $[Cu(bdmppy)(NO_2)_2]_2[Cu(NO_2)_4]$ (bdmppy = 2,6-bis[(3,5-dimethyl)pyrazol-1-yl]pyridine.[24]

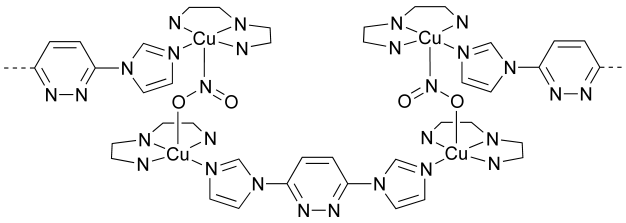


Fig. 7

A related complex in which two Cu^I centres are linked by a nitrite bridge, namely $[\{Cu(Pr^i_3 9[ane]N_3)\}_2(\mu\text{-}NO_2\text{-}\kappa\text{-}O_2:\kappa\text{-}N)][PF_6]$, was obtained by treatment of the mononuclear complex $[Cu(Pr^i_3 9[ane]N_3)(MeCN)][PF_6]$ with $NaNO_2$. Upon one-electron oxidation it gave the compound, $[\{Cu(Pr^i_3 9[ane]N_3)\}_2(\mu\text{-}NO_2\text{-}\kappa\text{-}O_2:\kappa\text{-}N)][PF_6]_2$, in which the two coppers are bridged by a nitrite which binds one copper through its N-atom and chelates the second through its two oxygens.[15] Although species having nitrite bridging between two coppers can be excluded from the enzyme mechanism, these bridging structures could perhaps relate to the hydrogen bonding of copper-bound nitrite.

A second type of systematic approach to the synthesis of binuclear complexes uses derivatisation of the NH_2 group of the tpm ligand. Thus, for example, conversion of this NH_2 group to $NHCO(CH_2)_2CO_2H$ affords a ligand which can bind copper by the pyridyl nitrogens, leaving the carboxylate group in the resulting complex to be used to bind further copper. A tricopper complex unit, in which two such copper units bind a

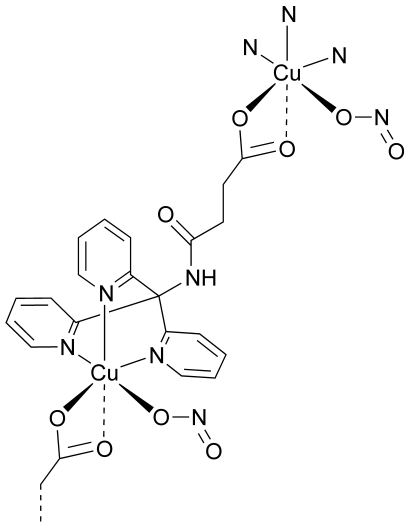


Fig. 8

central copper by their carboxylate groups, has been obtained.[22] An example of this type of complex in which nitrite also binds copper is shown in Fig. 8; the X-ray crystal structure of this complex shows it to have a chain polymeric structure.[25]

Although the coppers in these dinuclear and polynuclear complexes are still far from an accurate mimic of nitrite reductase, routes have been established for synthesis of such complexes, which in principle could be adapted to give models for the enzyme site in which model Type 1 and Type 2 copper centres are linked. Another approach to the preparation of linked Type 1/Type 2 analogues is suggested by the ligand-peptide conjugate shown in Fig. 9.[26] We await a full report on this work with interest.

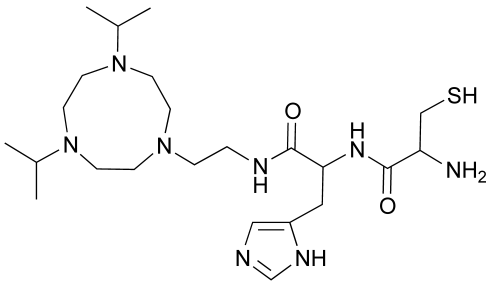


Fig. 9

With regard to models for the Type 1 copper centre,[27] it is not the purpose of this brief article to review such centres (which are found in a variety of copper metalloproteins other than nitrite reductase, such as blue copper oxidases,[28] and plastocyanins[29]), other than to point out that many model studies of copper complexes with N_2S_2 ligation have been carried out.[30,31] They have highlighted the remarkably flexible nature of the copper with regard to its coordination geometry and the difficulty of synthesis of copper(II) thiolate complexes, owing to the tendency of the copper to be reduced to Cu^I with concomitant oxidation of thiolate to disulfide. Nevertheless the versatility of ligand systems such as **6a**, which can be derivatised to give thiolate/thioether substituents,[22] and the preparation of potentially binucleating ligands as shown in Fig. 9,[26] holds promise for the synthesis of asymmetric binuclear complexes containing Type 2 and Type 1 coppers.

4. Conclusions

The foregoing discussion has shown that much remains to be done before the detailed mechanism of the conversion of NO_2^- to NO and N_2O at a Type 2 copper site is fully understood, and before a close model for the active centre of nitrite reductase is synthesised. Nevertheless, such conversions of nitrite have been achieved in model complexes and synthetic routes to interesting and relevant binuclear complexes are emerging.

It was mentioned at the beginning of this article that an aim of chemical studies was to find a simple catalytic means of removing nitrite pollutant from water supplies. Recent research has highlighted the potential of electrochemical methods using films of elemental copper to detect and discriminate between nitrate and nitrite.[32] An alternative approach is the preparation of discrete copper complexes, anchored to an electrode surface so that nitrite from aqueous solution could bind the copper and then be reductively cleaved using electrons supplied from the electrode and protons from solvent. Complexes of the **6a** type might prove amenable to such anchoring after appropriate derivatisation of the tpm ligand.

References

1 C.C. Delwiche, Ed., *Denitrification, nitrification and atmospheric nitrous oxide*, Wiley, New York, 1981.
2 S. Suzuki, K. Kataoka and K. Yamaguchi, *Acc. Chem. Res.*, 2000, **33**, 728 and references therein.
3 E.T. Adman, J.W. Godden and S. Turley, *J. Biol. Chem.*, 1995, **270**, 27458.
4 F.E. Dodd, S.S. Hasnain, Z.H. Abraham, R.R. Eady and B.E. Smith, *Acta. Crystallogr.*, 1997, **D53**, 406.
5 T. Inoue, M. Gotowda, Deligeer, M. Katoaka, K. Yamaguchi, S. Suzuki, H. Watanabe, M. Gohow and Y. Kai, *J. Biochem.*, 1998, **124**, 876.
6 M.J. Boulanger, M. Kukimoto, N. Nishiyama, S. Horinouchi and M.E.P. Murphy, *J. Biol. Chem.*, 2000, **275**, 23957.
7 R.W. Strange, L.M. Murphy, F.E. Dodd, Z.H. Abraham, R.R. Eady, B.E. Smith and S.S. Hasnain, *J. Mol. Biol.*, 1999, **287**, 1001.
8 E. Weeg-Aerssens, J. M. Tiedje and B. A. Averill, *J. Am. Chem. Soc.*, 1987, **109**, 7214.
9 N. Komeda, H. Nago, Y. Kushi, G. Adachi, M. Suzuki, A. Uehara and K. Tanaka, *Bull. Chem. Soc.* Japan, 1995, **68**, 581.
10 C. X. Zhang, H.-C. Liang, K. J. Humphreys and K. D. Karlin, in L. Simandi, Ed., *Copper-dioxygen Complexes and their Roles in Biomimetic Oxidation Reactions*, Dordrecht, The Netherlands, 2001.
11 F. Jiang, *J. Am. Chem. Soc.*, 1993, **115**, 2093.
12 K.-Y. Wong and K.-W. Yeung, *J. Inorg. Biochem.*, 2001, **86**, 481.
13 W.B. Tolman, *Inorg. Chem.*, 1991, **30**, 4877.
14 L. Casella, O. Carugo, M. Gullotti, S. Doldi and M. Frassoni, *Inorg. Chem.*, 1996, **35**, 1101.
15 J.A. Halfen, S. Mahapatra, E.C. Wilkinson, A. J. Gegenbach, V.G. Young, L. Que and W.B. Tolman, *J. Amer. Chem. Soc.*, 1996, **118**, 763.
16 K. Yamaguchi, H. Yokoyama and S. Suzuki, *J. Inorg. Biochem.*, 2001, **86**, 485.
17 M. E. Murphy, S. Turley and E. T. Adman, *J. Biol. Chem.*, 1997, **272**, 28455.
18 M. Beretta, E. Bouwman, L. Casella, B. Douziech, W.L. Driessen, L. Gutierrez-Soto, E. Monzani and J. Reedijk, *Inorg. Chim. Acta*, 2000, **310**, 41.
19 E. Monzani, G.J.A.A. Koolhaas, A. Spandre, E. Leggieri, L. Casella, M. Gullotti, G. Nardin, L. Randaccio, M. Fontani, P. Zanello and J. Reedijk, *J. Biol. Inorg. Chem.*, 2000, **5**, 251.
20 C.E. Ruggiero, S.M. Carrier and W.B. Tolman, *Angew. Chem. Int. Ed. Engl.*, 1994, 33, 895.
21 C.E. Ruggiero, S.M. Carrier, W.E. Antholine, J.W. Whittaker, C.J. Cramer and W.B. Tolman, *J. Am. Chem. Soc.*, 1993, **115**, 11285.
22 P.J. Arnold, S.C. Davies, J.R. Dilworth, M.C. Durrant, D.V. Griffiths, D.L. Hughes, R.L. Richards and P.C. Sharpe, *J. Chem. Soc.*, Dalton Trans., 2001, 736.
23 M.J. Begley, P. Hubberstey and J. Stroud, *J. Chem. Soc., Dalton Trans.*, 1996, 4295.
24 A J. Blake, S.J. Hill and P. Hubberstey, *Chem. Commun.*, 1998, 1587.
25 P.J. Arnold, S.C. Davies, M.C. Durrant, D.V. Griffiths, D.L. Hughes, R.L. Richards and P.C. Sharpe, unpublished results.
26 R.P. Houser, E.L. Klein and A. Mondal, *J. Inorg. Biochem.*, 2001, **86**, 270.
27 W. Kaim and J. Rall, *Angew. Chem., Int. Ed. Engl.*, 1996, **35**, 43.
28 A. Messerschmidt, *Adv. Inorg. Chem.*, 1993, **40**, 121.
29 A.G. Sykes, *Struct. Bonding (Berlin)*, 1991, **75**, 175.
30 J. Gazo, I. B. Bersuker, J. Garaj, M. Kabesova, J. Kohout, H. Langfelderova, M. Melnik, M. Serator and F. Valach, *Coord, Chem. Rev.*, 1976, **19**, 253.
31 E. Bouwman, W. L. Driessen and J. Reedijk, *Coord. Chem. Rev.*, 1990, **104**, 143.
32 J. Davis, M. J. Moorcroft, S. J. Wilkins, R. G. Compton and M. F. Cardosi, *Analyst*, 2000, **125**, 737.