Dinickel complexes of disubstituted benzoate polydentate ligands: mimics for the active site of urease†

Way-Zen Lee,*[a] Huan-Sheng Tseng,[a] Meng-Yu Ku[a] and Ting-Shen Kuo[b]

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Two dinickel mimics, $[LNi_2(DMF)_4](ClO_4)_3$ (1) and $[L'Ni_2(CH_3CN)_4](ClO_4)_3$ (3), for the active site of urease supported by a disubstituted benzoate polydentate ligand were synthesized and fully characterized, subsequently addition of urea afforded two urea adducts, $[LNi_2(urea)_4](ClO_4)_3$ (4) and $[L'Ni_2(urea)_4](ClO_4)_3$ (5).

Urease, a historic landmark in enzymology, was the first enzyme to be crystallized.[1] Its rapid catalysis for the hydrolysis of urea to ammonia and carbon dioxide plays an essential role in agriculture and human health.[2,3] The active site of the enzyme (Fig. 1), determined by X-ray crystallography,[4] contains two nickel ions bridged by a carbamylated lysine and a hydroxide ion. Each nickel coordinates two histidine residues and a water molecule. The coordination sphere of one nickel center, Ni(2), is completed by an additional terminally bound aspartate resulting in a pseudo-octahedral ligand environment; whereas another nickel ion, Ni(1), having a vacant site possesses a distorted square pyramidal geometry. It has been confirmed that carbon dioxide is required for nickel binding to apo-urease.[5] The active site structure reveals that a lysine residue reacts with carbon dioxide and converts to a carbamate which captures the nickel ions into the active site. Many model complexes have been synthesized using phthalazine-, phenolate-, or alkyloxide-based polydentate ligands.[6–8] However, to our knoweldge, the synthetic mimics supported by a benzoate-based ligand, which can mimic the carbamate in the active site of urease, have not been reported in the literature. Herein we wish to demonstrate the first example of dinickel mimics supported by a disubstituted benzoate polydentate ligand.

Recently, we have reported a dinickel complex supported by the tripodal ligand bis(1-methylbenzimidazolyl-2-methyl)amine.[9] Urea is found not to bind to the nickel centers of the complex due to the coordinate saturation of the dinickel complex by three acetates. In order to generate vacant sites on dinickel mimics which also have a similar coordination environment to the active site of urease, 2,6-bis[di(pyridinyl-2-methyl)amino-methyl]benzoic acid (H**L**) and 2,6-bis[di(1-methylbenzimidazolyl-2-methyl)aminomethyl]benzoic acid (H**L**′), where a benzoate group is fused with two tripodal ligands, were synthesized and

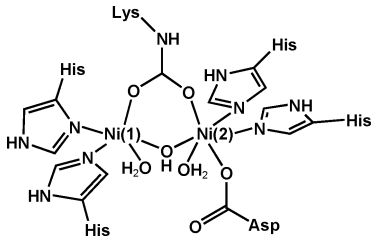
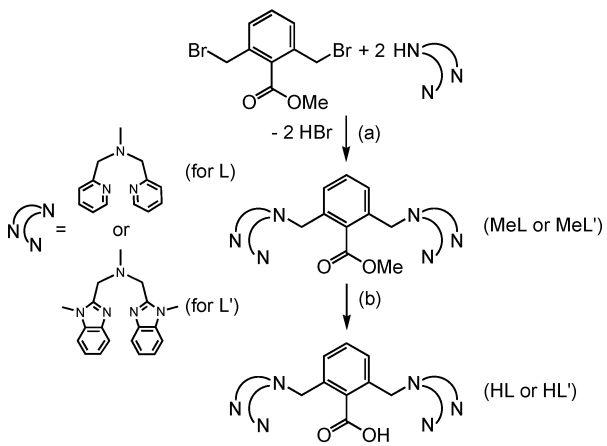


Fig. 1 Schematic representation of the active site of urease.

characterized by ^1H and ^{13}C NMR spectroscopy and elemental analysis (see ESI†). The synthesis of the benzoate-based ligands is outlined in Scheme 1. Methyl 2,6-bis(bromomethyl)benzoate was prepared according to a literature method.[10] Reflux of methyl 2,6-bis(bromomethyl)benzoate and two equivalents of dipicolyl-amine in the presence of triethylamine in THF for 3 d afforded methyl 2,6-bis[di(pyridinyl-2-methyl)aminomethyl]benzoate (Me**L**). H**L** was then obtained from the hydrolysis of Me**L** by refluxing with potassium hydroxide in ethanol. The overall yield of the two steps for preparing H**L** is about 84%. The 1-methylbenzimidazolyl derivative, H**L**′, was prepared using a similar procedure with a yield of 88%.



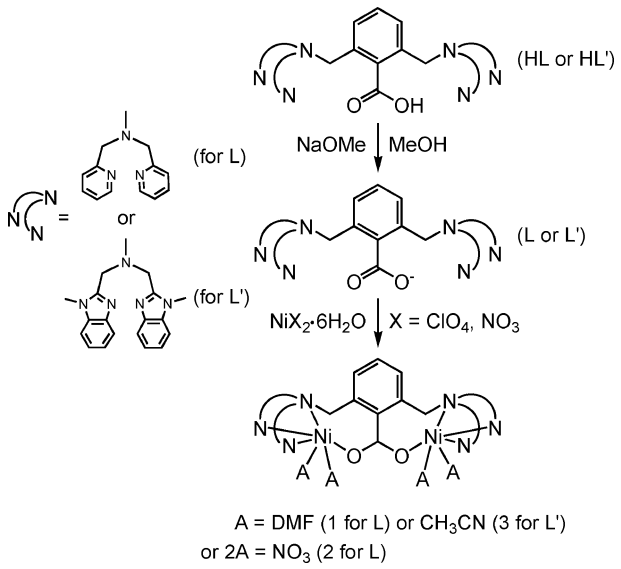
(a) NEt_3, THF, reflux 3 days for HL; Na_2CO_3, DMF, 80 °C, 3 days for HL'.
(b) KOH, EtOH or DMF, reflux 36 hrs.

Scheme 1

H**L** was deprotonated by sodium methoxide in methanol to form a pale-yellow solution, subsequently transferred to an acetonitrile solution of $Ni(ClO_4)_2 \cdot 6H_2O$ to form a light blue solution of $[LNi_2(DMF)_4](ClO_4)_3$ (**1**, Scheme 2). After purification, complex

[a]*Department of Chemistry, National Taiwan Normal University, 88 Sec. 4 Ting-Chow Rd., Taipei, 11677, Taiwan. E-mail: wzlee@ntnu.edu.tw; Fax: +886 2 29324249; Tel: +886 2 29318166*
[b]*Instrumentation Center, Department of Chemistry, National Taiwan Normal University, 88 Sec. 4 Ting-Chow Rd., Taipei, 11677, Taiwan*

† Electronic supplementary information (ESI) available: Experimental details, UV-vis spectra of all complexes, and ^1H NMR spectra of complexes **1**, **3**, **4**, and **5**. CCDC reference numbers 667937–667941. For ESI and crystallographic data in CIF or other electronic format see DOI: 10.1039/b803094b



Scheme 2

1 was recrystallized by slow diffusion of diethyl ether into a DMF solution of the product. Nice needle-like crystals were obtained in a yield of 83%. The molecular structure of complex **1**, determined by X-ray analysis,‡ reveals a dinickel complex, in which two nickel ions are bridged by the benzoate group of **L**. The C–C bond between the phenyl and the carboxylate group is twisted with a torsion angle of 63°. Each nickel ion is facially coordinated by three nitrogen donors, *i.e.* one amine and two pyridines, from a side arm of **L**, and the coordination sphere of each nickel center is completed by two DMF molecules (Fig. 2a). Due to the short length of the side arms and the torsion of the benzoate group in **L**, the two nickel ions and the bridging benzoate form a W-shaped dinuclear core, a rare arrangement for a bridging carboxylate, with a Ni···Ni separation of 6.0 Å, longer than that of urease (3.5 Å). When **L** was treated with an alternate nickel source, $Ni(NO_3)_2 \cdot 6H_2O$, in acetonitrile, a light blue complex of $[LNi_2(NO_3)_2](NO_3)$ (**2**) was produced. The X-ray structure of **2** (Fig. 2b) displays a dinickel complex similar to complex **1**. Interestingly, the NO_3^- anions coordinate to the nickel centers occupying two coordination sites. Moreover, both nickels are coordinated by the N_3 donor side arm of **L** but in different conformations, one in a meridional and the other in a facial fashion. The coordination of NO_3^- implies that the dinickel moiety of $[LNi_2]^{3+}$ is very electron deficient. The average bond length of $Ni–O_{DMF}$ in **1** is 0.070 Å shorter than those of $Ni–O_{NO_3^-}$ in **2**, and the average Ni–N distance in **1** is 0.033 Å longer than that in **2**. The difference between Ni–N and Ni–O bond lengths in complexes **1** and **2** illustrates that the coordination of the DMF molecules to the nickel centers is stronger than that of the NO_3^- anions. The 1-methyl-benzimidazolyl derivative of **1**, $[L'Ni_2(CH_3CN)_4](ClO_4)_3$ (**3**), was also prepared in the same manner. The molecular structure of complex **3** is similar to that of **1** except for the bound solvent molecules of acetonitrile (Fig. 2c).

The electronic absorption spectra of **1**, **2**, and **3** displayed similar absorption bands consisting of three weak d–d transitions in the visible region (see ESI† Fig. S1). The coordination environment of complexes **1** and **3** is related to the active site of urease. The polydentate ligands, **L** and **L′**, provide coordinating groups

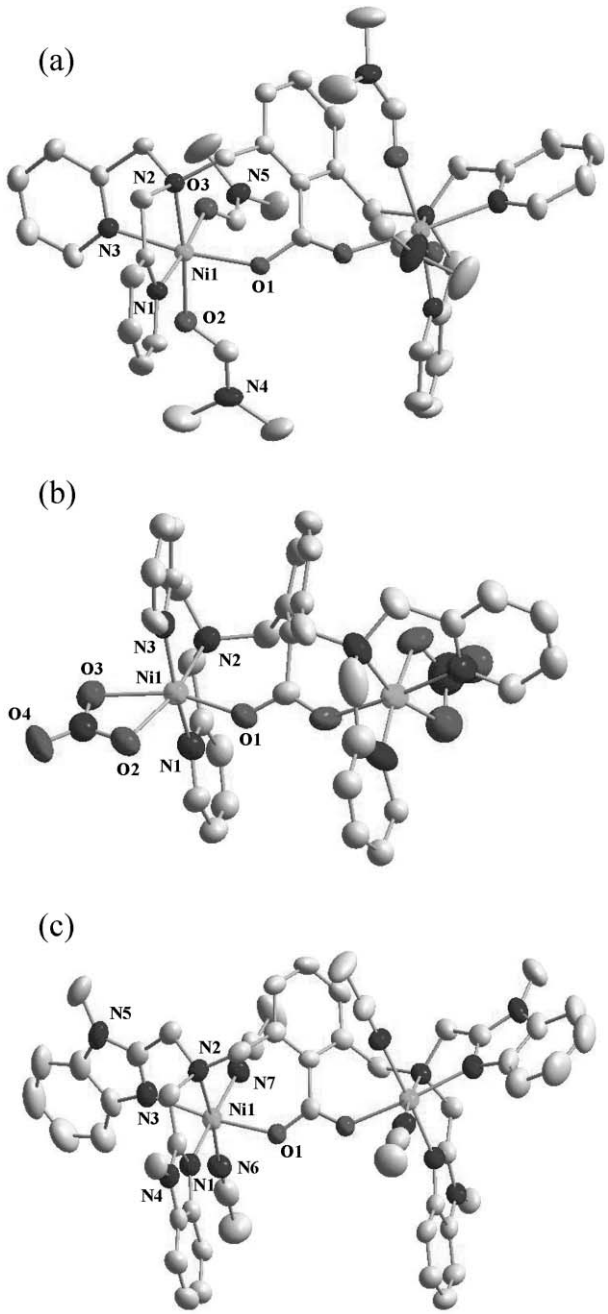


Fig. 2 Thermal ellipsoid representation of (a) $[LNi_2(DMF)_4](ClO_4)_3$ (**1**), (b) $[LNi_2(NO_3)_2](NO_3)$ (**2**), and (c) $[L'Ni_2(CH_3CN)_4](ClO_4)_3$ (**3**) at 50% probability level. Hydrogen atoms, solvent molecules, and the counter anions of **1**, **2**, and **3** are omitted for clarity.

mimicking the bound amino acid residues around the active site of urease, and the benzoate models the carbamylated lysine. The nickel centers in complexes **1** and **3** all have a N_3O donor set, and each nickel is coordinated with two labile solvent molecules, suggesting the coordination of urea to the nickel centers of the dinickel mimics. As excess urea was added to the acetonitrile solution of **1** and **3**, two urea adducts, $[LNi_2(urea)_4](ClO_4)_3$ (**4**) and $[L'Ni_2(urea)_4](ClO_4)_3$ (**5**) were obtained. The X-ray structures of **4** and **5** (Fig. 3a and 3b) reveal that urea molecules are coordinated to the nickel centers of the complexes through the oxygen atom of

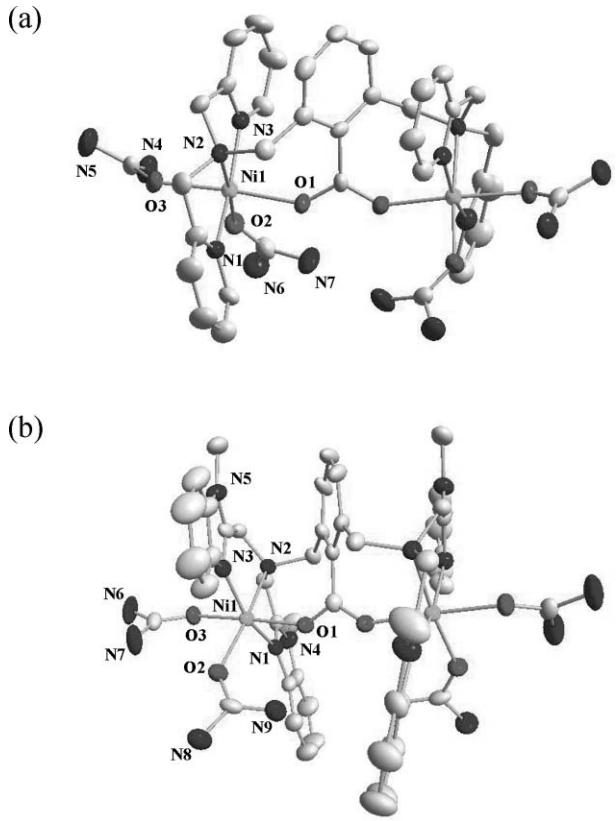


Fig. 3 Thermal ellipsoid representation of (a) $[LNi_2(urea)_4](ClO_4)_3$ (**4**) and (b) $[L'Ni_2(urea)_4](ClO_4)_3$ (**5**) at 50% probability level. Hydrogen atoms, solvent molecules, and the counter anion of **4** and **5** are omitted for clarity.

the carbonyl group with a Ni–O bond length of 2.084 and 2.092 Å, respectively. The solid FTIR spectra (KBr pellets) of **4** and **5** also exhibit a shift in the carbonyl stretching frequency of urea from 1690 to 1664 and 1668 cm^{-1}, respectively. We have also taken the ^1H NMR spectra of complexes **1**, **3**, **4**, and **5** (see ESI† Fig. S2), which exhibited relatively sharp resonances for high spin six-coordinated dinickel(II) species.[11] From a comparison of the spectra for **4** and **5** to those for **1** and **3**, pronounced signals for the bound urea molecules were seen in the spectra of **4** and **5** at 5.65 (CD_3OD) and 5.25 ppm (CD_3CN), respectively, downfield shifted from free urea at 4.81 ppm (CD_3CN). In addition, the ESI-MS spectra of **4** and **5** indicate that the urea molecules remain coordinated to the nickel centers of the complexes in the solution. (ESI-MS (*m*/*z*, amu): 260 for $[LNi_2(urea)_2]^{3+}$, 240 for $[LNi_2(urea)]^{3+}$, and 311 for $[L'Ni_2(urea)_2]^{3+}$.)

It is noteworthy that the conformation of each nickel center in **4** and **5** changes to a meridional fashion from a facial arrangement in **1** and **3**. The rearrangement of the N_3 side arm coordination is due to the hydrogen bond interactions between two urea molecules and the bridging benzoate oxygen coordinated on the same nickel ion. The hydrogen bond interactions cause the two urea oxygens and the bridging benzoate oxygen to lie on a meridional plane (Fig. 4). The hydrogen bond interactions were proposed to be a significant factor in the catalytic cycle of urease.[12] Such hydrogen bond interactions have also been seen in other enzymes such as phosphotriesterase (PTE).[13]

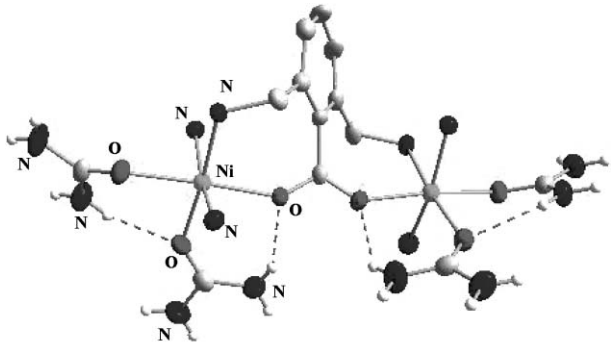


Fig. 4 Thermal ellipsoid representation of hydrogen bond inter-actions (dashed lines) between the bound urea molecules and the bridging benzoate group in **4** and **5**.

In summary, we have successfully prepared the first example of dinickel mimics, in which two nickel ions are bridged by a disubstituted benzoate polydentate ligand, for the active site of urease. Coordination of urea to the dinickel mimics was characterized by FTIR, ^1H NMR, and ESI-MS spectroscopies, and X-ray crystallography. Two isolated urea adducts, complexes **4** and **5**, represent the initial intermediate in the urease catalytic cycle. Both ^1H NMR and ESI-MS spectroscopies illustrate that the bound urea molecules in **4** and **5** do not dissociate from the nickel centers of the complexes in solution. The hydrolysis of urea by our dinickel mimics is currently undergoing further investigation.

Notes and references

‡ Crystal data for **1**·DMF·MeOH: $Ni_2C_{49}H_{70}N_{11}Cl_3O_{20}$, $M = 1356.93$, $T = 200$ K, monoclinic, $P2_1/n$, $a = 13.4365(3)$, $b = 34.4541(8)$, $c = 14.4644(4)$ Å, $\beta = 97.1670(10)°$, $V = 6643.9(3)$ Å^3, $Z = 4$, $D_c = 1.357$ Mg m^{-3}, $\mu = 0.761$ mm^{-1}, 0.45 × 0.35 × 0.20 mm, GoF = 1.056, $R_1 = 0.0615$, $wR_2 = 0.1724$ ($I > 2\sigma(I)$). Crystal data for **2**·CH_3CN·H_2O: $Ni_2C_{35}H_{36}N_{10}O_{12}$, $M = 906.16$, $T = 200$ K, triclinic, $P\bar{1}$, $a = 8.6704(4)$, $b = 14.8713(6)$, $c = 15.0500(8)$ Å, $\alpha = 81.222(2)$, $\beta = 82.826(2)$, $\gamma = 89.510(2)°$, $V = 1902.70(15)$ Å^3, $Z = 2$, $D_c = 1.582$ Mg m^{-3}, $\mu = 1.067$ mm^{-1}, 0.11 × 0.10 × 0.04 mm, GoF = 1.003, $R_1 = 0.0693$, $wR_2 = 0.1682$ ($I > 2\sigma(I)$). Crystal data for **3**·2CH_3CN·0.5H_2O: $Ni_2C_{57}H_{62}N_{16}Cl_3O_{14.5}$, $M = 1427.00$, $T = 200$ K, monoclinic, $C2/c$, $a = 21.9140(7)$, $b = 26.5340(11)$, $c = 13.9360(5)$ Å, $\beta = 119.740(2)°$, $V = 7036.0(4)$ Å^3, $Z = 4$, $D_c = 1.347$ Mg m^{-3}, $\mu = 0.719$ mm^{-1}, 0.22 × 0.12 × 0.11 mm, GoF = 1.050, $R_1 = 0.0760$, $wR_2 = 0.1999$ ($I > 2\sigma(I)$), with crystallographically imposed twofold symmetry. Crystal data for **4**·2MeOH·urea: $Ni_2C_{40}H_{59}N_{16}Cl_3O_{21}$, $M = 1323.80$, $T = 200$ K, monoclinic, Cc, $a = 13.6282(4)$, $b = 18.4465(5)$, $c = 22.9362(8)$ Å, $\beta = 101.0440(10)°$, $V = 5659.2(3)$ Å^3, $Z = 4$, $D_c = 1.554$ Mg m^{-3}, $\mu = 0.895$ mm^{-1}, 0.16 × 0.14 × 0.04 mm, $R_1 = 0.0598$, $wR_2 = 0.1547$ ($I > 2\sigma(I)$). Crystal data for **5**: $Ni_2C_{49}H_{59}N_{18}Cl_3O_{18}$, $M = 1411.91$, $T = 200$ K, monoclinic, $C2/c$, $a = 18.4897(4)$, $b = 15.2943(3)$, $c = 24.4927(6)$ Å, $\beta = 110.4140(10)°$, $V = 6491.2(2)$ Å^3, $Z = 4$, $D_c = 1.445$ Mg m^{-3}, $\mu = 0.783$ mm^{-1}, 0.50 × 0.28 × 0.12 mm, $R_1 = 0.0778$, $R_w = 0.2235$ ($I > 2\sigma(I)$), with crystallographically imposed twofold symmetry.

1 J. B. Sumner, *J. Biol. Chem.*, 1926, **69**, 435–441.
2 R. L. Mulvaney and J. M. Bremner, in *Soil Biochemistry*, ed. E. A. Paul and J. N. Ladd, Marcel Dekker, Inc., New York, 1981, pp. 153–196.
3 H. L. T. Mobley, M. D. Island and R. P. Hausinger, *Microbiol. Rev.*, 1995, **59**, 451–480.
4 (*a*) E. Jabri, M. B. Carr, R. P. Hausinger and P. A. Karplus, *Science*, 1995, **268**, 998–1004; (*b*) I.-S. Park, L. O. Michel, M. A. Pearson, E. Jabri, P. A. Karplus, S. Wang, J. Dong, R. A. Scott and B. P. Koehler, *J. Biol. Chem.*, 1996, **271**, 18632–18637; (*c*) M. A. Pearson, L. O. Michel, R. P. Hausinger and P. A. Karplus, *Biochemistry*, 1997, **36**, 8164–8172; (*d*) S. Benini, W. R. Rypniewski, K. S. Wilson, S. Ciurli and S. Mangani, *JBIC, J. Biol. Inorg. Chem.*, 1998, **3**, 268–273; (*e*) S. Benini, W. R. Rypniewski, K. S. Wilson, S. Miletti, S. Ciurli and S. Mangani, *Structure*

(London), 1999, **7**, 205–216; (*f*) N. C. Ha, S. T. Oh, J. Y. Sung, K. A. Cha, M. H. Lee and B. H. Oh, *Nat. Struct. Biol.*, 2001, **8**, 505–509; (*g*) S. Benini, W. R. Rypniewski, K. S. Wilson, S. Ciurli and S. Mangani, *JBIC, J. Biol. Inorg. Chem.*, 2001, **6**, 778–790.

5 I.-S. Park and R. P. Hausinger, *Science*, 1995, **267**, 1156–1158.

6 (*a*) A. M. Barrios and S. J. Lippard, *J. Am. Chem. Soc.*, 1999, **121**, 11751–11757; (*b*) A. M. Barrios and S. J. Lippard, *J. Am. Chem. Soc.*, 2000, **122**, 9172–9177; (*c*) A. M. Barrios and S. J. Lippard, *Inorg. Chem.*, 2001, **40**, 1250–1255; (*d*) S. V. Kryatov, E. V. Rybak-Akimova, F. Meyer and H. Pritzkow, *Eur. J. Inorg. Chem.*, 2003, 1581–1590; (*e*) S. Buchler, F. Meyer, E. Kaifer and H. Pritzkow, *Inorg. Chim. Acta*, 2002, **337**, 371–386; (*f*) F. Meyer, E. Kaifer, P. Kircher, K. Heinze and H. Pritzkow, *Chem.–Eur. J.*, 1999, **5**, 1617–1630; (*g*) M. Konrad, F. Meyer, A. Jacobi, P. Kircher, P. Rutsch and L. Zsolnai, *Inorg. Chem.*, 1999, **38**, 4559–4566; (*h*) F. Meyer and H. Pritzkow, *Chem. Commun.*, 1998, 1555–1556.

7 (*a*) R. M. Buchanan, M. S. Mashuta, K. J. Oberhausen, J. F. Richardson, Q. Li and D. N. Hendrickson, *J. Am. Chem. Soc.*, 1989, **111**, 4497–4498; (*b*) T. Koga, H. Furutachi, T. Nakamura, N. Fukita, M. Ohba, K. Takahashi and H. Okawa, *Inorg. Chem.*, 1998, **37**, 989–996; (*c*) H. Carlsson, M. Haukka and E. Nordlander, *Inorg. Chem.*, 2002, **41**, 4981–4983; (*d*) H. Carlsson, M. Haukka, A. Bousseksou, J. Latour and E. Nordlander, *Inorg. Chem.*, 2004, **43**, 8252–8262; (*e*) H. Adams, S. Clunas, D. E. Fenton and S. E. Spey, *Dalton Trans.*, 2003, 625–630; (*f*) S. Uozumi, H. Furutachi, M. Ohba, H. Okawa, D. E. Fenton, K. Shindo, S. Murata and D. J. Kitko, *Inorg. Chem.*, 1998, **37**, 6281–6287.

8 (*a*) K. Yamaguchi, S. Koshino, F. Akagi, M. Suzuki, A. Uehara and S. Suzuki, *J. Am. Chem. Soc.*, 1997, **119**, 5752–5753; (*b*) D. Volkmer, A. Hoerstmann, K. Griesar, W. Haase and B. Krebs, *Inorg. Chem.*, 1996, **35**, 1132–1135; (*c*) D. Volkmer, B. Hommerich, K. Griesar, W. Haase and B. Krebs, *Inorg. Chem.*, 1996, **35**, 3792–3803.

9 W.-Z. Lee, H.-S. Tseng and T.-S. Kuo, *Dalton Trans.*, 2007, 2563–2570.

10 N. Martin, S. M. Stephen and J. C. Donald, *J. Am. Chem. Soc.*, 1977, **99**, 6405–6410.

11 C. Belle, C. Bougault, M.-T. Averbuch, A. Durif, J.-L. Pierre, J.-M. Latour and L. Le Pape, *J. Am. Chem. Soc.*, 2001, **123**, 8053–8066.

12 (*a*) P. A. Karplus, M. A. Pearson and R. P. Hausinger, *Acc. Chem. Res.*, 1997, **30**, 330–337; (*b*) M. A. Pearson, I.-S. Park, R. A. Schaller, L. O. Michel, P. A. Karplus and R. P. Hausinger, *Biochemistry*, 2000, **39**, 8575–8584; (*c*) A. Warshel, M. Strajbl, J. Villa and J. Florian, *Biochemistry*, 2000, **39**, 14728–14738; (*d*) G. Estiu and K. M. Merz Jr., *J. Am. Chem. Soc.*, 2004, **126**, 11832–11842.

13 (*a*) S. D. Aubert, Y. Li and F. M. Raushel, *Biochemistry*, 2004, **43**, 5707–5715; (*b*) J. B. Thoden, R. Marti-Arbona, F. M. Raushel and H. M. Holden, *Biochemistry*, 2003, **42**, 4874–4882.