DALTON
FULL PAPER

New H-bond accepting tris(pyrazolyl)borates: stabilization of metal aquo species as models for the vicinal oxygen chelate enzyme superfamily

Brian S. Hammes, Mary W. Carrano and Carl J. Carrano *

Department of Chemistry and Biochemistry, Southwest Texas State University, San Marcos, TX 78666 USA. E-mail: cc05@swt.edu

Received 30th November 2000, Accepted 6th February 2001
First published as an Advance Article on the web 10th April 2001

The hydrogen-bond accepting ligand K[$Tp^{CO_2Et,Me}$] was prepared and its complexes with divalent Ni, Co, Mn and Cu reveal a stabilization of penta- or hexa-coordinate metal aquo complexes of the general form [$Tp^{CO_2Et,Me}M(H_2O)_x$] ($x = 2$ or 3) which are unprecedented for the alkyl substituted Tp analogs. These complexes make good structural models for many of the enzymes of the vicinal oxygen chelate superfamily.

Introduction

The resting state of the catalytically active metal center in many metalloenzymes contains one or more water molecules in addition to the ligands supplied by the protein backbone. These ubiquitous metal bound water molecules generally provide one of two major functions: (1) a ligand that is labile, and thus easily replaceable for inner sphere coordination at the metal by incoming substrates; (2) as a nucleophile in the actual catalytic reaction. The number of metalloenzymes containing such sites are extremely numerous and include enzymes involved in DNA (phosphodiester), amide, or ester hydrolysis, oxygen activation, isomerizations *etc.* This diversity is well illustrated by the so called vicinal oxygen chelate (VOC) superfamily.[1] The enzymes of this family use a variety of different divalent metal ions in catalysis (Co, Ni, Zn, Mn and Fe) but from an inorganic perspective, the common thread in this structurally related group is that they all provide a metal coordination environment with two or three "open" (*i.e.* water containing) coordination sites, which promote direct electrophilic participation by the metal ion in catalysis. While mechanisms of action for many of these enzymes are readily proposed, little hard data concerning the various steps centered at the metal ion are available.

One of the well established methods to study the reactivity inherent in a particular structure and/or ligand environment of complex molecules such as metalloenzymes is the synthesis, structural characterization, and reactivity studies of small synthetic analogs to these proteins *i.e.* the so called "model compound" approach. This approach has been highly successful in generating spectroscopic, structural and reactivity models for many metalloproteins.[2,3] At the heart of the model compound approach is the design and synthesis of creative ligands to mimic metal ion binding sites. Good models for enzymes of the VOC superfamily could be based on a facially coordinating tripodal chelate which leaves two or three sites open at the metal center where water could be bound. However such water molecules would normally be extremely labile, making isolation and characterization of such species problematic. Nature herself often stabilizes such reactive or labile structures through various secondary interactions, the most important of which is hydrogen bonding. While the use of sterically restricted ligands to control the size and shape of metal binding cavities is well established, it has proven more difficult, despite its importance, to incorporate hydrogen bonding into synthetic small molecule binding site analogs.[4,5] Thus despite the importance of metal aquo complexes, as structural and/or reactivity models for metalloenzymes, surprisingly few well characterized systems of the kinetically labile divalent first row transition metals containing multiple adjacent water molecules are known.

Based on the enormous success of the tris(pyrazolyl)borate, Tp^R, platform[6,7] as a ligand for metalloenzyme modeling studies, we describe here a first generation of a simple H-bond accepting analog which simultaneously provides both steric bulk and hydrogen bonding groups which can be directed towards the center of a metal binding cavity. The presence of strong intramolecular H-bonding between the ligand and water molecules does indeed lead to the stabilization of discrete, well characterized, divalent metal aquo complexes which are both, unprecedented in Tp chemistry, and good structural models for many of the enzymes of the VOC superfamily.

Results and discussion

Based on ease of synthesis and the desire to stabilize the H-bond donating ligand, water, we chose ester substituted tris(pyrazolyl)borates as our target. Thus the carboxyethyl substituted, three-fold symmetric, $Tp^{CO_2Et,Me}$ ligand **1**, has been prepared from the corresponding pyrazole and potassium borohydride in one step using standard melt conditions.[6] The ligand has been completely characterized by standard methodology and its structure verified in the metal complexes reported below.

We were extremely gratified to find that simple cationic, metal aquo-complexes of the general type [$Tp^{CO_2Et,Me}M(H_2O)_x$]$^+$ (M = Ni(II) **2**, Co(II) **3**, Mn(II) **4** or Cu(II) **5**) where $x = 3$ for octahedral favoring Ni(II), Co(II) or Mn(II) and $x = 2$ for square pyramidal Cu(II), were in fact formed when first row transition metal salts of poorly coordinating counter ions were reacted with the ligand in MeOH or THF. The perchlorate salts of complexes **2**–**5** have all been crystallographically characterized and the water molecules in these complexes are strongly hydrogen bonded to the ester carbonyl moieties, although the exact H-bonding pattern is different for each. The structure of the representative octahedral Ni(II) complex **2** is shown in Fig. 1 where all the water hydrogens were found on the difference map. Important bond lengths and angles in the coordination sphere of the Ni are shown in the figure caption. One hydrogen of each water molecule is hydrogen bonded to an ester carbonyl oxygen while the other is hydrogen bonded to an oxygen of the perchlorate group (not shown). The resulting rather tight ion pair accounts for the high solubility of this complex in polar

 DOI: 10.1039/b009621k

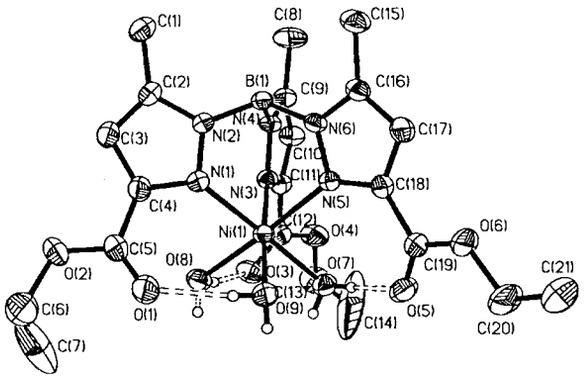

Fig. 1 Thermal ellipsoid diagram of the cationic portion of **2**, $[(Tp^{CO_2Et,Me})Ni(H_2O)_3]^+$. The ellipsoids are drawn at the 30% probability level and hydrogens, except those involved in H-bonding are removed for clarity. Selected bond distances (Å) and angles (°): Ni(1)–N(1) 2.083(4), Ni(1)–N(3) 2.085(4), Ni(1)–N(5) 2.111(4), Ni(1)–O(7) 2.082(4), Ni(1)–O(8) 2.091(4), Ni(1)–O(9) 2.064(4); N(1)–Ni(1)–N(3) 90.4(2), N(1)–Ni(1)–N(5) 88.0(2), N(1)–Ni(1)–O(7) 177.8(2), N(1)–Ni(1)–O(8) 88.4(2), N(1)–Ni(1)–O(9) 92.2(2), N(3)–Ni(1)–N(5) 87.3(2), N(3)–Ni(1)–O(7) 89.0(2), N(3)–Ni(1)–O(8) 91.3(2), N(3)–Ni(1)–O(9) 176.9(2), N(5)–Ni(1)–O(7) 93.6(2), N(5)–Ni(1)–O(8) 176.5(2), N(5)–Ni(1)–O(8) 91.0(2), O(7)–Ni(1)–O(8) 89.6(2), O(7)–Ni(1)–O(9) 88.5(2), O(8)–Ni(1)–O(9) 90.6(2).

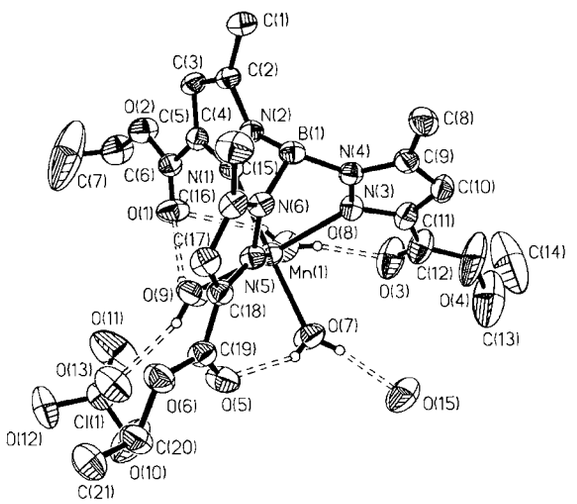

Fig. 2 Thermal ellipsoid diagram of $[(Tp^{CO_2Et,Me})Mn(H_2O)_3]ClO_4 \cdot H_2O$ **4**. The ellipsoids are drawn at the 30% probability level and hydrogens, except those involved in H-bonding are removed for clarity. Selected bond distances (Å) and angles (°): Mn(1)–N(1), 2.254(4), Mn(1)–N(3) 2.273(4), Mn(1)–N(5) 2.307(4), Mn(1)–O(8) 2.165(4), Mn(1)–O(9) 2.151(4), Mn(1)–O(7) 2.156(4); N(1)–Mn(1)–N(3) 83.6(1), N(1)–Mn(1)–N(5) 83.36(12), N(1)–Mn(1)–O(8) 93.5(2), N(1)–Mn(1)–O(9) 92.8(2), N(3)–Mn(1)–N(5) 85.90(13), N(3)–Mn(1)–O(8) 92.3(2), N(3)–Mn(1)–O(9) 175.5(2), N(5)–Mn(1)–O(8) 176.6(2), N(5)–Mn(1)–O(9) 91.0(2), O(8)–Mn(1)–O(9) 90.5(2), O(9)–Mn(1)–O(7) 89.6(2), O(7)–Mn(1)–O(8) 93.2(2).

organic solvents and its low solubility in water. The average $O_{carbonyl}$–O_{water} distance of 2.69 Å and the average $O_{carbonyl}$–H_{water}–O_{water} angle of 165.4° are commensurate with strong hydrogen bonding between the water ligands and the Tp ester carbonyls. The fact that all of the ester carbonyls are pointed into the metal binding cavity gives the tripodal ligand in this complex a rather open or "splayed out" conformation as compared to that seen in structures lacking the H-bonding interaction where the carbonyls are all pointed out. Despite this, no evidence for the bis-ligand, "sandwich" complex is observed. The perchlorate salt of the Co complex **3** is isomorphous and isostructural with the Ni analog.

Mn(II) also forms an octahedral complex with three facially coordinated water molecules (Fig. 2). Again all the relevant hydrogens were located on the difference map and these were refined independently but constrained to have similar bond

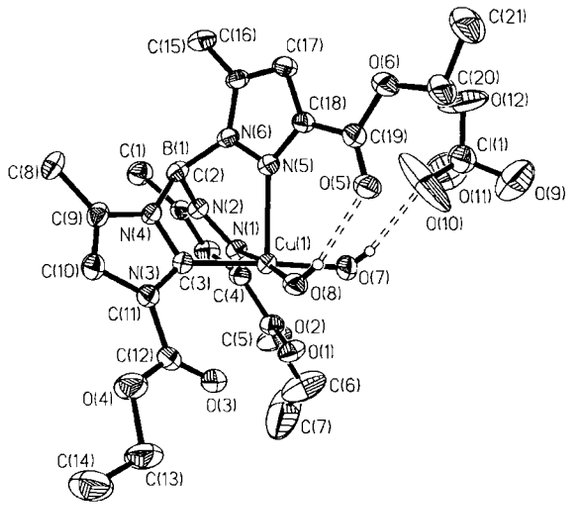

Fig. 3 Thermal ellipsoid diagram of $[(Tp^{CO_2Et,Me})Cu(H_2O)_2]ClO_4$ **5**. The ellipsoids are drawn at the 30% probability level and hydrogens, except those involved in H-bonding are removed for clarity. Selected bond distances (Å) and angles (°): Cu(1)–N(1) 1.981(5), Cu(1)–N(3) 1.991(4), Cu(1)–N(5) 2.322(4), Cu(1)–O(7) 1.986(5), Cu(1)–O(8) 1.981(5); N(1)–Cu(1)–N(3) 85.1(2); N(1)–Cu(1)–N(5) 94.7(2), N(1)–Cu(1)–O(7) 93.0(2), N(3)–Cu(1)–N(5) 86.1(2), O(8)–Cu(1)–N(1) 175.2(2), O(8)–Cu(1)–N(5) 88.7(2), O(7)–Cu(1)–N(5) 96.8(2), O(8)–Cu(1)–O(7) 89.9(2), O(8)–Cu(1)–N(3) 91.7(2), O(7)–Cu(1)–N(3) 176.7(2).

lengths. In **4** one water molecule (O8) is H-bonded to two ester carbonyl oxygens (O3 and O1), another water (O7) is H-bonded to ester carbonyl O5 and a solvent water (O15) while the final water (O9) interacts with ester carbonyl O1 and perchlorate oxygen O13. The average $O_{carbonyl}$–O_{water} distance is 2.71 Å and $O_{carbonyl}$–H–O_{water} angle is 153.1°. The structure of the square pyramidal Cu complex **5** is shown in Fig. 3. In this case only one of the water molecules (O8) is H-bonded to an ester carbonyl (O5) with an $O_{carbonyl}$–O_{water} distance of 2.69 Å and a $O_{carbonyl}$–H_{water}–O_{water} angle of 162.4°. The other water is H-bonded only to the perchlorate.

These complexes are good structural models for some of the members of the VOC enzyme family. The Ni complex is a model for *Escherichia coli* glyoxalase I, the Mn complex a model for the fosfomycin resistance protein and a Mn extradiol dioxygenase and the Co complex for methylmalonyl-CoA epimerase.[1] In these enzymes the metal is typically bound to two histidine nitrogens, one or two aspartate carboxyl oxygens and two or three mutually *cis* waters in an overall octahedral geometry. The Cu complex is an interesting structural model for the binding site in copper amine oxidases, whose square pyramidal Cu(II) ion is surrounded by three histidine nitrogens and two water molecules.[8]

To be other than structural models for enzymes it is important that the water molecules be easily displaced by "substrate" ligands. In solution there is evidence that the water molecules on these metal complexes are in fact labile. Thus in pure frozen CH_2Cl_2 the Mn complex **4** is not very soluble but shows a six line pattern indicative of a single Mn environment. Addition of acetonitrile to increase the solubility gives the frozen solution EPR spectrum shown in Fig. 4 where both a major and a minor species can be observed. Other preliminary results indicate that the waters are easily displaced by bidentate ligands such as catechol, amino acids *etc.* to give stable "enzyme-substrate" analogs as well. Details of these solution based studies will be reported in full later.

In conclusion, a series of divalent metal aquo complexes have been prepared that are stabilized by internal H-bonding with a tris(pyrazolyl)borate based tripodal ligand. These complexes are unprecedented for simple Tp ligands which almost invariably yield tetrahedral species, TpMX, if the ligand is highly sterically

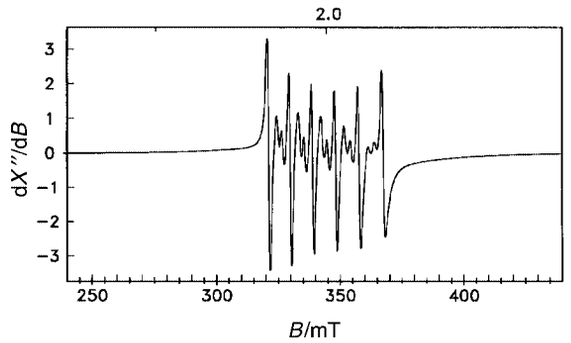

Fig. 4 Frozen solution X-band (9.6435 GHz) EPR spectrum of **4** in mixed CH_2Cl_2–CH_3CN solvent, 10 K, 20 μW/40 dB, 2 G mod. amp.

encumbered or simple $(Tp)_2M$ "sandwich" complexes if not.[6] They represent good structural models for enzymes of the VOC superfamily.

Experimental

All syntheses were carried out in air and the reagents and solvents purchased from commercial sources and used as received unless otherwise noted. Methanol was distilled under argon over CaH_2. Ethyl 2,4-diketopentanoate was prepared using previously reported procedures. **CAUTION!** The perchlorate salts used in this study are potentially explosive and should be handled with due caution.

3-Methyl-5-carboxyethylpyrazole

Ethyl 2,4-diketopentanoate (85.5 g, 0.54 mol) was dissolved in 400 mL of MeOH and treated with an excess (48 g, 0.70 mol) of aqueous hydrazine monohydrochloride. The reaction mixture was stirred at room temperature for 15 min and extracted with CH_2Cl_2, dried over $MgSO_4$ and volatiles removed under reduced pressure. The crude product was recrystallized from boiling hexane to yield 45 g, 54% of 3-methyl-5-carboxyethylpyrazole. ^{1}H NMR ($CDCl_3$) δ 6.60 (s, 1 H, PzH), 4.35 (q, 2 H, $J = 7$ Hz, –OCH_2–), 2.40 (s, 3 H, Pz-CH_3), 1.34 (t, 3 H, $J = 7$ Hz, –CH_3). ^{13}C NMR ($CDCl_3$) δ 161.16, 143.19, 141.04, 107.41, 61.10, 14.13, 11.32.

Potassium tris(3-methyl-5-carboxyethyl)pyrazolylborate (1)

A total of 8 g (0.052 mol) of 3-methyl-5-carboxyethylpyrazole was mixed with 0.93 g (0.0173 mol) of potassium borohydride and heated slowly to 150 °C until hydrogen generation ceased. The melt temperature was then raised to 170 °C and the temperature maintained until hydrogen evolution again ceased and the initial fluid reaction mixture had solidified. The mixture was cooled briefly and quenched with toluene. Filtration of the white solid, brief washing with toluene, followed by hexane, and drying gave 6.2 g (70%) of the desired product. ^{1}H NMR ($CDCl_3$) δ 6.30 (s, 1 H, PzH), 4.26 (q, 2 H, $J = 7$ Hz, –OCH_2–), 2.42 (s, 3 H, Pz-CH_3), 1.32 (t, 3 H, $J = 7$ Hz, –CH_3). ^{13}C NMR ($CDCl_3$) δ 164.78, 144.22, 142.66, 106.13, 60.18, 14.36, 12.66. FTIR (KBr, cm^{-1}): $\nu = 2523$ (B–H), 1715 (C=O). MS (ESI, methanol) m/z 471 (M^-).

$[(Tp^{CO_2Et,Me})Ni(H_2O)_3]ClO_4$ (2)

A slurry of potassium tris(3-methyl-5-carboxyethyl)pyrazolylborate **1** (0.32 g, 0.63 mmol) in 20 mL of CH_3OH was treated dropwise with a solution of $Ni(ClO_4)_2 \cdot 6H_2O$ (0.23 g, 0.63 mmol) in CH_3OH. The resulting solution was stirred for 6 h and dried under reduced pressure. The light blue solid was taken up into CH_3CN and filtered to remove a small amount of white solid. Crystals for an X-ray diffraction study were grown by the slow diffusion of diethyl ether into a CH_3CN solution. FTIR (KBr, cm^{-1}): $\nu = 2559$ (B–H), 1708 (C=O). λ_{max}/nm (ε/M^{-1} cm^{-1}) (CH_3CN): 386 (10), 612 (10). Magnetism (solid state, room temperature): $\mu_{eff} = 2.9\ \mu_B$.

$[(Tp^{CO_2Et,Me})Co(H_2O)_3]NO_3$ (3)

A slurry of potassium tris(3-methyl-5-carboxyethyl)pyrazolylborate **1** (1 g, 2.1 mmol) and $Co(NO_3)_2 \cdot 6H_2O$ (0.6 g, 2.1 mmol) in 100 mL of THF was stirred for 6 h and dried under reduced pressure. The purple-pink solid was taken up into CH_2Cl_2 and extracted with water. The organic layer was dried and the solvent removed by rotary evaporation. Crystals for an X-ray diffraction study were grown by the slow evaporation of an acetone solution (pink) containing the Co(II) complex. Anal. Calc. (found) for $[(Tp^{CO_2Et,Me})Co(H_2O)_3]NO_3$, $C_{21}H_{34}N_7O_{12}BCo$: C, 39.02 (39.30); H, 5.31 (5.21); N, 15.17 (15.23%). FTIR (KBr, cm^{-1}): $\nu = 2563$ (B–H), 1708 (C=O). λ_{max}/nm (ε/M^{-1} cm^{-1}): 504 (20). Magnetism (solid state, room temperature): $\mu_{eff} = 4.8\ \mu_B$. The perchlorate salt can be prepared using cobalt(II) perchlorate as the metal source.

$[(Tp^{CO_2Et,Me})Mn(H_2O)_3]ClO_4$ (4)

A slurry of potassium tris(3-methyl-5-carboxyethyl)pyrazolylborate **1** (0.31 g, 0.61 mmol) in 20 mL of CH_3OH was treated dropwise with a solution of $Mn(ClO_4)_2 \cdot 6H_2O$ (0.22 g, 0.61 mmol) in CH_3OH. The resulting solution was stirred for 6 h and dried under reduced pressure. The off-white solid was taken up into CH_3CN and filtered to remove a small amount of white solid. Crystals for an X-ray diffraction study were grown by the slow diffusion of diethyl ether into a CH_3CN solution. FTIR (KBr, cm^{-1}): $\nu = 2575$ (B–H), 1715 (C=O). Magnetism (solid state, room temperature): $\mu_{eff} = 6.27\ \mu_B$.

$[(Tp^{CO_2Et,Me})Cu(H_2O)_2]ClO_4$ (5)

A slurry of potassium tris(3-methyl-5-carboxyethyl)pyrazolylborate **1** (0.31 g, 0.61 mmol) in 20 mL of CH_3OH was treated dropwise with a solution of $Cu(ClO_4)_2 \cdot 6H_2O$ (0.23 g, 0.61 mmol) in CH_3OH. The resulting solution was stirred for 6 h and dried under reduced pressure. The blue-green solid was taken up into CH_3CN and filtered to remove a small amount of white solid. Crystals for an X-ray diffraction study were grown by the slow diffusion of diethyl ether into a CH_3CN solution of $[(Tp^{CO_2Et,Me})Cu(H_2O)_2]ClO_4$. Anal. Calc. (found) for $[(Tp^{CO_2Et,Me})Cu(H_2O)_2]ClO_4 \cdot 0.25CH_3CN$, $C_{21.5}H_{35.5}N_{6.25}O_{12}BClCu$: C, 37.94 (38.12); H, 4.86 (4.81); N, 12.86 (12.65%). FTIR (KBr, cm^{-1}): $\nu = 2531$ (B–H), 1735 (C=O), 1701 (C=O). λ_{max}/nm (ε/M^{-1} cm^{-1}) (CH_3CN): 736 (34). Magnetism (solid state, room temperature): $\mu_{eff} = 1.54\ \mu_B$.

Physical methods

Elemental analyses were performed on all compounds by Quantitative Technologies, Inc., Whitehouse, NJ. All samples were dried *in vacuo* prior to analysis. The presence of solvates was corroborated by FTIR, ^{1}H NMR or X-ray crystallography. ^{1}H and ^{13}C NMR spectra were collected on a Varian UNITY INOVA 400 MHz NMR spectrometer. Chemical shifts are reported in ppm relative to an internal standard of TMS. The ^{13}C quaternary carbon peaks that are not observed are a result of either poor solubility and/or overlapping signals. IR spectra were recorded from KBr disks on a Perkin-Elmer 1600 Series FTIR spectrometer and are reported in wavenumbers. Electronic spectra were recorded using a Hewlett-Packard 8452A diode array spectrophotometer. Room-temperature magnetic susceptibility measurements of the metal complexes were determined using a magnetic susceptibility balance MSB-1 manufactured by Johnson Matthey and calibrated with mercury(II) tetrathiocyanatocobaltate(II) ($\chi_g = 16.44(8) \times 10^{-6}$ cm^3 g^{-1}).[3] Diamagnetic corrections were taken from those reported by O'Connor.[9] Low temperature EPR spectra were obtained on a Bruker X-band spectrometer with Oxford helium cryostat

in the Department of Biophysics at the Medical University of Lübeck, Germany. We thank Dr Volker Schünemann for assistance in acquiring these spectra.

Crystallography

Crystal data for 2. $C_{21}H_{34}BClN_6O_{13}Ni$, $M = 683.51$, $a = 9.419(2)$, $b = 18.220(2)$, $c = 18.166(2)$ Å, $\beta = 93.378(12)°$, $V = 3112.1(7)$ Å^3, monoclinic, space group $P2_1/c$, $Z = 4$, $T = 293(2)$ K, final $R1 = 0.0525$, $wR2 = 0.1391$, GOF (on F^2) = 0.976.

Crystal data for 4·CH_2Cl_2·H_2O. $C_{22}H_{34}BCl_3N_6O_{14}Mn$, $M = 778.65$, $a = 11.8559(12)$, $b = 12.9935(13)$, $c = 12.9957(13)$ Å, $\alpha = 72.828(9)$, $\beta = 67.473(10)$, $\gamma = 187.706(9)°$, $V = 1760.7(3)$ Å^3, triclinic, space group $P\bar{1}$, $Z = 2$, $T = 293(2)$ K, final $R1 = 0.0579$, $wR2 = 0.1656$, GOF (on F^2) = 1.043.

Crystal data for 5·$2H_2O$. $C_{21}H_{30}BClN_6O_{15}Cu$, $M = 716.31$, $a = 12.106(3)$, $b = 12.6519(11)$, $c = 13.012(2)$ Å, $\alpha = 106.982(11)$, $\beta = 108.75(3)$, $\gamma = 103.97(2)°$, $V = 1676.7(5)$ Å^3, triclinic, space group $P\bar{1}$, $Z = 2$, $T = 293(2)$ K, final $R1 = 0.0602$, $wR2 = 0.1766$, GOF (on F^2) = 1.131.

CCDC reference numbers 159090, 161749 and 161750.

See http://www.rsc.org/suppdata/dt/b0/b009621k/ for crystallographic data in CIF or other electronic format.

Acknowledgements

This work was supported in part by Grants AI-1157 from the Robert A. Welch Foundation and CHE-9726488 from the NSF. The NSF-ILI program grant USE-9151286 is acknowledged for partial support of the X-ray diffraction facilities at Southwest Texas State University.

References

1 N. Armstrong, *Biochemistry*, 2000, **39**, 13625.
2 N. Kitajima, K. Fujisawa, C. Fujimoto, Y. Moro-oka, S. Hashimoto, T. Kitagawa, K. K. Toriumi, M. Tatsumi and A. Nakamura, *J. Am. Chem. Soc.*, 1992, **114**, 1277.
3 N. Kitajima, K. Fujisawa, M. Tanaka and Y. Moro-oka, *J. Am. Chem. Soc.*, 1992, **114**, 9232.
4 B. S. Hammes, V. G. Young and A. S. Borovik, *Angew. Chem., Int. Ed.*, 1999, **38**, 666.
5 L. M. Berreau, R. A. Allred, M. M. Makowska-Grzyska and A. M. Arif, *Chem. Commun.*, 2000, 1423.
6 S. Trofimenko, *Scorpionates: The Coordination Chemistry of Polypyrazolylborate Ligands*, Imperial College Press, London, UK, 1999.
7 G. Parkin, *Chem. Commun.*, 2000, 1971.
8 M. C. Wilce, D. M. Dooley, H. C. Freeman, J. M. Guss, H. Matsunami, W. S. McIntire, C. E. Ruggiero, K. Tanizawa and H. Yamaguchi, *Biochemistry*, 1997, **36**, 16116.
9 C. J. O'Connor, *Prog. Inorg. Chem.*, 1982, **29**, 203.