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Characterization of the Structural and Electronic Properties of Spin-Coupled Dinuclear Copper(II) Centers by Proton NMR Spectroscopy

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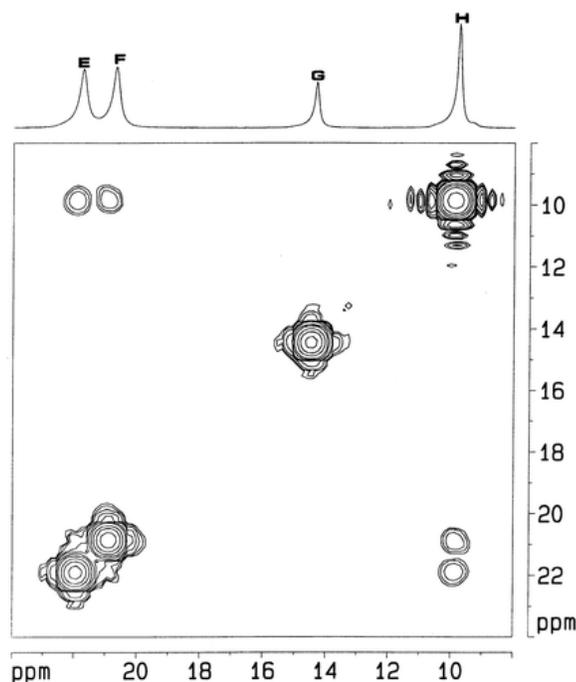
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SUBJECTS:

Crystallography, X-rays, Pyridines, Ions,

Abstract



The ^1H NMR spectra of a series of well-characterized μ -phenoxo and μ -alkoxo spin-coupled dicopper(II) complexes have been investigated. The complexes studied were $[\text{Cu}_2(\text{BPMP})(\text{OH})]^{2+}$ (**1**) (BPMP = 2,6-bis[[bis(2-pyridylmethyl)amino]methyl]-4-methylphenol), $[\text{Cu}_2(\text{CH}_3\text{HXTA})(\text{OH})]^{2-}$ (**2**) (CH_3HXTA = N,N' -(2-hydroxy-5-methyl-1,3-xylylene)bis(N -carboxymethylglycine)), $[\text{Cu}_2(m\text{-XYL})(\text{OH})]^{2+}$ (**3**) ($m\text{-XYL}$ = 2,6-bis[[bis(2-pyridylethyl)amino]methyl]phenol), and $[\text{Cu}_2(\text{TBHP})(\text{OAc})]^{2+}$ (**4**) (TBHP = N,N,N',N' -tetrakis[(2-benzimidazolyl)methyl]-2-hydroxy-1,3-diaminopropane). The magnetic interactions of these complexes range from strongly antiferromagnetically to weakly ferromagnetically coupled. Both one- and two-dimensional (COSY) ^1H NMR methods were used to facilitate the assignment of the hyperfine shifted ^1H NMR signals of each complex. COSY experiments provide clear cross signals for resonances <200 Hz wide. These data have facilitated the assignment of the hyperfine shifted ^1H NMR signals and have verified that the solid state structures exist in solution for each system studied. Assuming a paramagnetic dipolar relaxation mechanism, the crystallographically determined Cu–H distance (r) is proportional to $T_1^{1/6}$. All calculated Cu–H distances for **1–4** are within *ca.* 20% of the Cu–H distances derived from X-ray crystallography. These data indicate that a paramagnetic dipolar relaxation mechanism is the dominant proton relaxation pathway for spin-coupled dicopper(II) centers. Our results indicate that ^1H NMR spectroscopy is an excellent tool with which to probe the solution structures of spin-coupled dicopper(II) centers in model complexes as well as biological systems.

Synopsis

One- and two-dimensional ^1H NMR spectra of both antiferromagnetically and ferromagnetically coupled μ -phenoxo and μ -alkoxo dicopper(II) complexes have been recorded. Two-dimensional COSY experiments provide clear cross signals for resonances <200 Hz wide and establish spin–spin connectivities. These data have facilitated the assignment of hyperfine shifted ^1H NMR signals in dicopper(II) complexes and have verified that the solid state structures exist in solution for each system studied. In addition, relaxation data coupled with X-ray crystallographic results indicate that a paramagnetic dipolar relaxation mechanism is the dominant proton relaxation pathway.

Introduction

Proton NMR spectroscopy has been widely used to study the structural and electronic properties of spin-coupled diiron, iron–sulfur cluster, and heterobimetallic systems such as those prepared from superoxide dismutase.^{1–5} ^1H NMR spectroscopy is an extremely sensitive tool for the study of these systems since only protons proximate to a paramagnetic center are affected, resulting in a fingerprint of the active site. In addition, a wealth of magnetic information on spin-coupled systems (*e.g.*, 4Fe–4S clusters) has been obtained by measuring the temperature dependence of the isotropically shifted signals.^{3,5} These studies allow local spin magnetization to be characterized without interference from the bulk susceptibility. Despite these successes, ^1H NMR spectroscopy has been largely overlooked as a probe of the electronic properties of other spin-coupled transition metal ions (*e.g.*, copper) in biological systems. The characterization of the structure and function of dicopper(II) centers in biological systems is a problem of outstanding importance since these enzymes play many varied roles in nature including dioxygen activation and reversible dioxygen binding.^{6–10} Therefore, a thorough understanding of the ^1H NMR spectra of simple biomimetic complexes must be developed before ^1H NMR spectroscopy can be established as a probe of the structural and magnetic properties of spin-coupled dinuclear Cu(II) centers in biological systems.

The synthesis and characterization of dinuclear Cu(II) complexes has received a great deal of attention because of the role of dicopper centers in biological processes.^{6,10–12} As a result, these biomimetic dicopper(II) complexes have been extensively studied using several physical techniques such as electronic absorption spectroscopy, EPR, X-ray crystallography, and magnetic susceptibility.¹⁰ ^1H NMR spectroscopy has only recently emerged as a window into the structural and magnetic properties of dinuclear Cu(II) complexes. It has been proposed that relatively sharp hyperfine shifted ^1H NMR signals are observed due to the proximity of the ground and first excited states, which are separated by the magnitude of the exchange constant, $-2J$.^{13,14} ^1H NMR spectra have been reported for antiferromagnetically and ferromagnetically coupled systems and both provide relatively sharp, hyperfine shifted ^1H NMR signals.^{13,15–23} However, the majority of the studies reported to date have relied on laborious synthetic methods such as substitution or deuteration to assign only a portion of the observed isotropically shifted signals. Therefore, ^1H NMR spectroscopy has been of little utility as a structural or magnetic probe of dicopper(II) centers.

Our laboratory recently communicated that two-dimensional (COSY) ^1H NMR techniques can be performed on dicopper(II) complexes facilitating the assignment of observed hyperfine shifted ^1H NMR resonances.²⁰ We report here one- and two-dimensional ^1H NMR studies on a series of magnetically diverse μ -phenoxo and μ -alkoxo dicopper(II) complexes (Figure 1). These complexes, $[\text{Cu}_2(\text{BPMP})(\text{OH})]^{2+}$ (**1**), $[\text{Cu}_2(\text{CH}_3\text{HXTA})(\text{OH})]^{2+}$ (**2**), $[\text{Cu}_2(m\text{-XYL})(\text{OH})]^{2+}$ (**3**), and $[\text{Cu}_2(\text{TBHP})(\text{OAc})]^{2+}$ (**4**), have been thoroughly characterized by X-ray crystallography as well as several spectroscopic methods.^{20,21,24–28} The spin-coupling interactions for these complexes, determined from SQUID susceptibility measurements, range from strongly antiferromagnetically to weakly ferromagnetically coupled. Clear COSY cross signals are observed between hyperfine shifted ^1H NMR resonances for both types of spin-coupled complexes. These data have facilitated the assignment of the hyperfine shifted ^1H NMR signals and have verified that the solid state structures exist in solution for each system studied. Combining signal assignment with X-ray crystallographic results and T_1 information has allowed us to address the mechanism of relaxation for spin-coupled dicopper(II) centers. Our data indicate that ^1H NMR spectroscopy is an excellent tool with which to probe the structural and magnetic properties of dicopper(II) centers in both model complexes and biological systems.

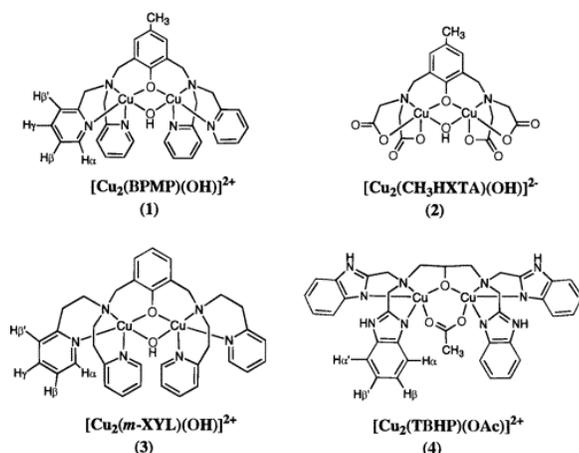


Figure 1 Schematic representations of $[\text{Cu}_2(\text{BPMP})(\text{OH})]^{2+}$ (**1**), $[\text{Cu}_2(\text{CH}_3\text{HXTA})(\text{OH})]^{2-}$ (**2**), $[\text{Cu}_2(m\text{-XYL})(\text{OH})]^{2+}$ (**3**), and $[\text{Cu}_2(\text{TBHP})(\text{OAc})]^{2+}$ (**4**).

Experimental Methods

Synthetic Methods.

All chemicals were purchased commercially and used as received unless otherwise stated. The dinucleating ligand 2,6-bis[[bis(2-pyridylmethyl)amino]methyl]-4-methylphenol (BPMP) was prepared according to the method of Suzuki *et al.*^{29,30} with revisions as reported by Borovik *et al.*³¹ N,N,N',N' -tetrakis[(2-benzimidazolyl)methyl]-2-hydroxy-1,3-diaminopropane (TBHP) was obtained by condensing 1,2-diaminobenzene with 1,3-diamino-2-hydroxypropane- N,N,N',N' -tetraacetic acid according to the method of McKee *et al.*²⁵ with minor revisions. N,N' -(2-Hydroxy-5-methyl-1,3-xylylene)bis(N -carboxymethylglycine) (CH_3HXTA) was synthesized from *p*-cresol, iminodiacetic acid, and formaldehyde according to the method of Schwarzenbach *et al.*³² with minor revisions as reported by Murch *et al.*³³

$[\text{Cu}_2(\text{BPMP})(\text{OH})](\text{ClO}_4)_2$ (**1**) was prepared by the method of Berands *et al.*³⁴ To a stirred methanol solution (20 mL) containing (0.64 g; 1.2 mmol) HBPMP, 1.2 g (2.4 mmol) of $\text{Cu}(\text{ClO}_4)_2 \cdot 6\text{H}_2\text{O}$ dissolved in 2 mL of methanol was added. Triethylamine (1 equiv) was added to the resulting brown solution, upon which the solution turned a deep green. After filtration, **1** was recrystallized by vapor diffusion with diethyl ether. The analytical purity of **1** was checked by elemental analysis (Atlantic Microlab, Inc.). Calculated for $[\text{Cu}_2(\text{BPMP})(\text{OH})](\text{ClO}_4)_2$ (**1**) ($\text{C}_{33}\text{H}_{34}\text{N}_6\text{O}_{10}\text{Cl}_2\text{Cu}_2$): C, 45.42; H, 3.93; N, 9.63. Found: C, 45.52; H, 3.98; N, 9.57. $\text{Na}_2[\text{Cu}_2(\text{CH}_3\text{HXTA})(\text{OH})]$ (**2**) was synthesized as previously described, and $[\text{Cu}_2(m\text{-XYL})(\text{OH})](\text{PF}_6)_2$ (**3**) was provided as a kind gift by Dr. Kenneth Karlin.

$[\text{Cu}_2(\text{TBHP})(\text{OAc})](\text{ClO}_4)_2$ (**4**) was prepared by the method of McKee *et al.*²⁵ with minor revisions as reported by Satcher and Balch.²² The electronic absorption spectrum of **4** in acetonitrile solution was identical to that reported by McKee *et al.*²⁵ The analytical purity of **4** was determined by elemental analysis (Atlantic Microlab, Inc.). Calculated for $[\text{Cu}_2(\text{TBHP})(\text{OAc})](\text{ClO}_4)_2$ ($\text{C}_{36}\text{H}_{36}\text{N}_{10}\text{O}_{11}\text{Cl}_2\text{Cu}_2$): C, 44.64; H, 3.65; N, 14.08. Found: C, 44.37; H, 3.78; N, 13.94. The benzoate derivative of **4** $[\text{Cu}_2(\text{TBHP})(\text{OBz})](\text{ClO}_4)_2$ (**5**) was synthesized identically to that of **4**. The electronic absorption spectrum of **5** in acetonitrile solution shows absorbance maxima at 795 and 1045 nm. The analytical purity of **5** was determined by elemental analysis (Atlantic Microlab, Inc.). Calculated for $[\text{Cu}_2(\text{TBHP})(\text{OBz})](\text{ClO}_4)_2$ ($\text{C}_{41}\text{H}_{38}\text{N}_{10}\text{O}_{11}\text{Cl}_2\text{Cu}_2$): C, 47.13; H, 3.67; N, 13.40. Found: C, 46.56; H, 3.79; N, 13.21.

Physical Methods.

Electronic absorption spectra were recorded on a Shimadzu UV-3101PC spectrophotometer. Elemental analyses were performed by Atlantic Microlabs, Inc. (Norcross, GA). All ^1H NMR spectra were recorded on a Bruker ARX-

400 spectrometer at 400.13 MHz. All one-dimensional spectra were recorded with a spectral width between 125 and 83 kHz with a repetition rate of 3 s⁻¹. Chemical shifts (in ppm) are reported with respect to the residual solvent signals at 1.92 ppm for CH₃CN and 4.7 for H₂O. Spectra were routinely Fourier transformed with an exponential weighting function and a 30 Hz line broadening. Longitudinal relaxation times (*T*₁) were measured by the use of an inversion–recovery pulse sequence (180°–τ–90°). Plots of ln(*I*₀–*I*_τ) vs τ for each signal provided a straight line over all τ values investigated. Magnitude ¹H COSY spectra were obtained at 400.13 MHz on **1**, **3**, **4**, and **5** in CD₃CN solutions. These spectra were obtained at various temperatures and acquisition times depending on the signal widths. These spectra were typically obtained with 512 or 1024 blocks of 1024 or 2048 complex points, respectively. An unshifted sine-bell-squared weighting function was applied prior to Fourier transformation followed by baseline correction in both dimensions and symmetrization.

Results and Discussion

A fundamental and, as yet, largely unexplored issue is the determination of the structural and magnetic properties of dinuclear Cu(II) centers in essentially arbitrary environments using ¹H NMR spectroscopy. Both antiferromagnetically and ferromagnetically coupled dicopper(II) systems have been shown to provide relatively sharp, hyperfine shifted ¹H NMR signals.^{13,15-23} We have synthesized a series of well-characterized, magnetically diverse, μ-phenoxo and μ-alkoxo dicopper(II) complexes with either ferromagnetic or antiferromagnetic properties (Figure 1). Having this series has enabled us to explore the effects of magnetic coupling on their ¹H NMR spectral properties as well as the use of two-dimensional (COSY) ¹H NMR techniques for signal assignment. In addition, the determination of *T*₁ values for each observed ¹H NMR resonance has allowed us to address the mechanism of relaxation in both ferromagnetically and antiferromagnetically coupled dicopper(II) centers.

Antiferromagnetically Coupled Systems.

[Cu₂(BPMP)(OH)]²⁺ (**1**) is an antiferromagnetically coupled, crystallographically characterized (μ-phenoxo)(μ-hydroxo)dicopper(II) complex with a –2*J* value of 187 cm⁻¹ (Figure 1).³⁵ The magnetic properties of **1** compare well with several related (μ-phenoxo)(μ-hydroxo)dicopper(II) complexes, all of which exhibit moderate to strong antiferromagnetic coupling between the Cu(II) centers with –2*J* values greater than 100 cm⁻¹.^{27,36-38} The Evans susceptibility method^{39,40} provides a room temperature magnetic moment (μ_{eff}/Cu) of 1.27 BM which gives the number of unpaired electrons (*n*/Cu) as 0.62. These data indicate that the Cu(II) ions in **1** are moderately antiferromagnetically coupled in acetonitrile solution. Complex **1** exhibits several sharp, isotropically shifted ¹H NMR signals in acetonitrile solution at 25 °C in the 90 to –50 ppm chemical shift range.²⁰ Upon increasing the temperature, all of the isotropically shifted signals sharpen and shift toward the diamagnetic region. A new broad signal at ~130 ppm appears as the temperature is increased to 60 °C (Figure 2A, Table 1).

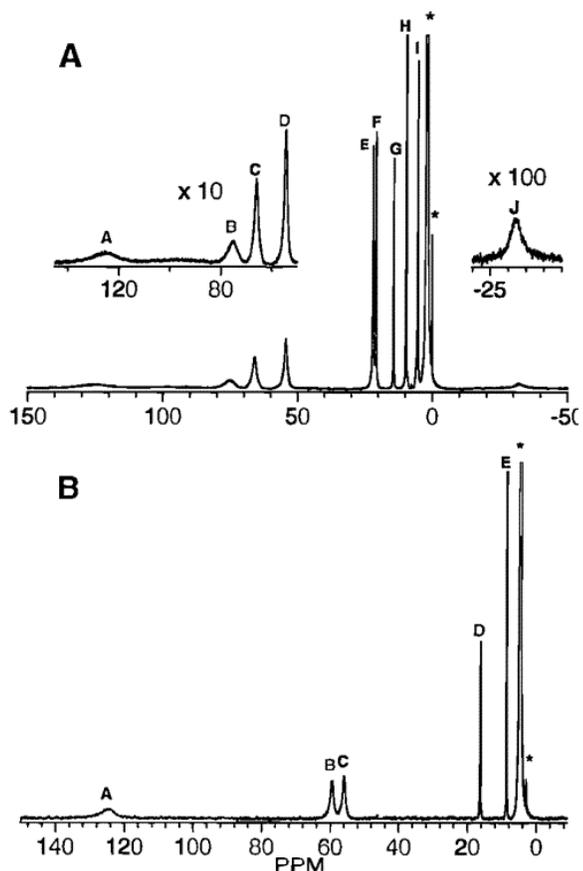


Figure 2 ^1H NMR spectra: (A) spectrum of **1** in CD_3CN at 60°C and (B) spectrum of **2** in D_3O at 90°C . Spectra were referenced to the residual protic solvent signals (*) at 1.92 and 4.7 ppm for acetonitrile and water, respectively.

Table 1. ^1H NMR Data for $[\text{Cu}_2(\text{CH}_3\text{HXTA})(\text{OH})]^{2-}$ in D_2O Solution at $90\text{ }^\circ\text{C}$ and $[\text{Cu}_2(\text{BPMP})(\text{OH})]^{2+}$ in CD_3CN Solution at 40 and $60\text{ }^\circ\text{C}$

	$[\text{Cu}_2(\text{CH}_3\text{HXTA})(\text{OH})]^{2-}$					$[\text{Cu}_2(\text{BPMP})(\text{OH})]^{2+}$				
	assignment	chemical shift ^a	relative area	line width ^b (Hz)	T_1 (ms)	assignment	chemical shift ^a	relative area	line width ^b (Hz)	T_1 (ms)
A	Ph- CH_2 -N	130	~4	~4500	1	Ph- CH_2 -N	130	~4	~4000	5
B	N- CH_2 - COO^-	61	~4	~1500	1	Py α -H	77	~4	1500	3
C	N- CH_2 - COO^-	59	~4	~1400	1	N- CH_2 -Py	68	4	900	4
D	Ph β -H	16.4	2	130	25	N- CH_2 -Py	56	4	475	2
E	Ph- CH_3	8.8	3	70	7	Py β' -H	22.4	4	77	24
F						Py β -H	21.3	4	66	24
G						Ph β -H	14.5	2	40	22
H						Py γ -H	9.8	4	32	56
I						Ph- CH_3	5.5	3	23	103
J						μ -OH	-32	~1	850	2

^a All shifts are in ppm relative to the residual solvent signals at 4.7 and 1.92 ppm for H_2O and CH_3CN , respectively. ^b The line widths are full width at half-maximum.

Several of the isotropically shifted ^1H NMR signals observed for **1** can be initially assigned by inspection of their peak areas. Signals E (22.4 ppm), F (21.3 ppm), G (14.5 ppm), H (9.8 ppm), and I (5.5 ppm) integrate to 4:4:2:4:3 protons, respectively (Table 1). These data, taken together with the crystallographic results, suggest that signals E, F, and H arise from pyridyl protons while signals G and I are due to the *m*-phenol and the *p*-methylphenol protons, respectively. Definitive assignment of each of these signals was made by two-dimensional ^1H NMR techniques. A magnitude COSY spectrum of **1** was recorded at 25 °C and clearly shows cross signals between resonances E and H and also between resonances F and H (Figure 3). These signals can be unequivocally assigned to the pyridine β -H (E or F), β' -H (E or F), and γ -H (H) protons, respectively.

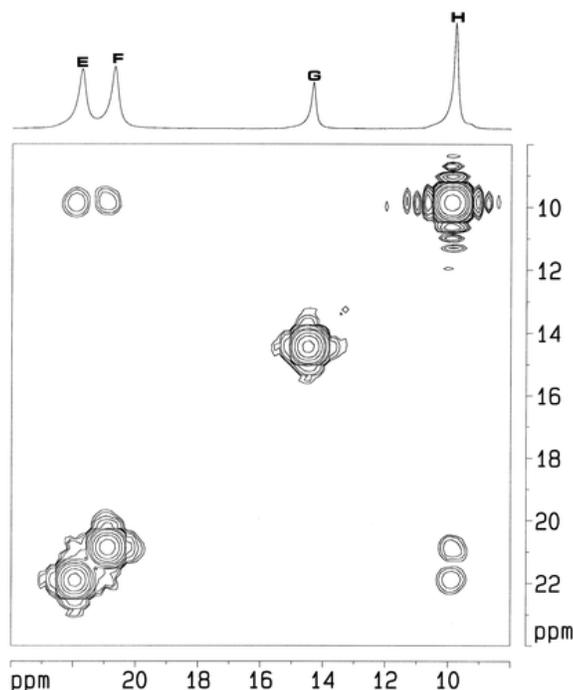


Figure 3 Magnitude ^1H COSY spectrum of **1** obtained at 400.13 MHz (Bruker ARX-400) at 60 °C in CD_3CN solution. The spectrum was referenced to the residual protic solvent signal at 1.92 ppm. This spectrum was obtained with an acquisition time of 6 ms and 128 data points in the F1 dimension and 256 data points in the F2 dimension. An unshifted sine-bell-squared weighting function and zero-filling to 512 data points were applied prior to Fourier transformation in both dimensions followed by baseline correction in both dimensions and symmetrization.

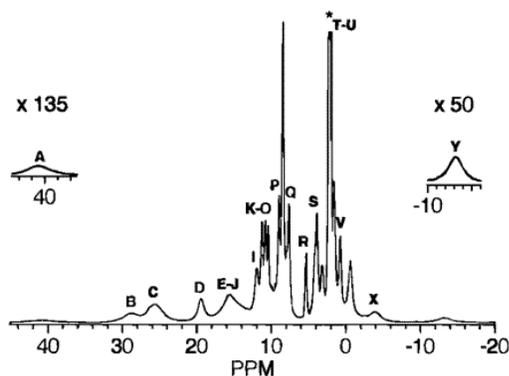
Assignment of the remaining signals of **1** comes from T_1 values and comparison of the spectrum of **1** with that of a related complex $[\text{Cu}_2(\text{CH}_3\text{XTA})(\text{OH})]^{2-}$ (**2**) where the methylpyridyl ligands have been replaced by acetate moieties (Figure 1).²¹ The ^1H NMR spectrum of **2** shows five isotropically shifted signals at 60 °C in D_2O solution and pH 10 (Figure 2B). The assignment of each of the observed hyperfine shifted signals for **2** has been previously reported (Table 1).²¹ Comparison of the chemical shift, T_1 values, and relative integrations of signals B (61 ppm) and C (59 ppm) of complex **2** (which were assigned to the diastereotopic methylene- CH_2 acetate protons) with signals C (68 ppm) and D (56 ppm) of **1** are consistent with the assignment of these signals to the diastereotopic methylene- CH_2 protons of the pyridylmethyl moiety. Comparison of signal A (130 ppm) of **2** with signal A (130 ppm) of **1** is consistent with this signal resulting from the methylene- CH_2 protons of the phenol-methylamine linkage.

Signals B (77 ppm) and J (−32 ppm) are the only remaining unassigned signals in the ^1H NMR spectrum of **1**. The only protons in **1** not assigned are the pyridine α -H protons and the μ -hydroxo OH proton. Signal J can be assigned to the μ -hydroxo OH proton since the addition of a small amount of D_2O causes this signal to disappear.^{19,20} The corresponding ^2H NMR experiment reveals a resonance at the appropriate chemical shift,

thus allowing unambiguous assignment of J as the μ -hydroxo OH proton. Moreover, the T_1 value is ~ 1 ms, which is consistent with the short crystallographically determined Cu–H distance (2.54 Å). From X-ray diffraction studies on **1**, the Cu d_{z^2} orbital, which contains the unpaired electron, is directed along the Cu–O μ -hydroxo bond.²⁰ Therefore, a spin polarization mechanism would cause the μ -hydroxo proton to be shielded and thus shifted upfield, consistent with its assignment to signal J. Signal B can be assigned to the pyridine α -H protons by default, and this assignment is consistent with T_1 values, chemical shift, and relative integrations.

$[\text{Cu}_2(m\text{-XYL})(\text{OH})]^{2+}$ (**3**) contains two strongly coupled Cu(II) ions with a $-2J$ value, determined from SQUID susceptibility measurements, of 600 cm^{-1} (Figure 1).^{26,27} From the Evans susceptibility method, the room temperature magnetic moment ($\mu_{\text{eff}}/\text{Cu}$) of **3** in acetonitrile solution is 0.67 BM, which gives the number of unpaired electrons (n/Cu) as 0.33. These data confirm that the Cu(II) ions in **3** are strongly coupled in acetonitrile solution. Interestingly, the ^1H NMR spectrum of **3** at 25 °C shows 20 isotropically shifted signals in the 50 to -20 ppm chemical shift range (Figure 4A). As the temperature is decreased, all of the observed signals sharpen and shift toward the diamagnetic region following non-Curie law behavior. The temperature dependence of **3** is exactly opposite to that observed for **1** and **2**. The non-Curie law behavior of strongly coupled dicopper(II) systems has previously been shown to be a function of the magnitude of $-2J$.³⁵

A



B

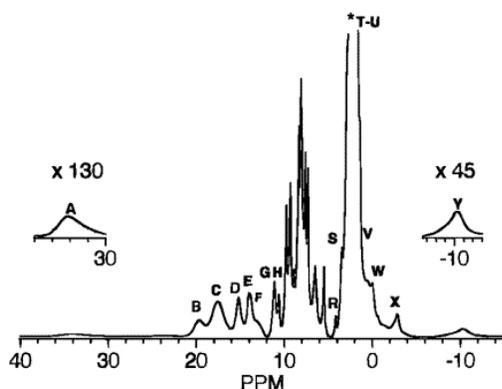


Figure 4 ^1H NMR spectra: (A) spectrum of **3** in CD_3CN at 25 °C and (B) spectrum of **3** in CD_3CN at -20 °C. Spectra were referenced to the residual protic solvent signals (*) at 1.92 ppm for acetonitrile. The * notes a small impurity as well as the position of the transmitter. Additional peak labels for the 10–0 ppm region appear in Figure 5.

At -20 °C, 25 sharp hyperfine shifted ^1H NMR resonances are observed (Figure 4B). The large number of sharp hyperfine shifted ^1H NMR resonances observed for **3** suggest asymmetry in the solution structure of **3**. Inspection of the X-ray crystal structure of **3** indicates two distinct sets of pyridine rings.²⁶ Two of the pyridine rings occupy axial positions while the remaining two are in equatorial positions of the square pyramidal Cu(II)

centers. This difference in environments is reflected in the ^1H NMR spectrum in that 40 protons exist in **3** and 25 resonances are observed. If the pyridyl arms were in identical environments in acetonitrile solution, a maximum of 13 resonances would be expected. Tentative assignment of several of the observed hyperfine shifted resonances can be made from inspection of their peak areas and T_1 values (Table 2); however, definitive assignment of several observed hyperfine shifted resonances can be obtained from a magnitude COSY spectrum.

Table 2. ^1H NMR Data for $[\text{Cu}_2(m\text{-XYL})(\text{OH})]^{2+}$ in CD_3CN Solution at $-20\text{ }^\circ\text{C}$

	assignment	chemical shift ^a	relative area	line width ^b (Hz)	T_1 (ms)
A		34	1	1200	1
B		20	1	650	1
C		17.8	4	600	1
D		15.6	2	260	1
E		14.3	2	180	14
F		13	1	500	1
G		11.6	2	170	4
H		11.1	2	60	39
I	Py $\beta\text{-H}$	10.3	2	50	50
J		9.9		100	22
K	Py $\beta'\text{-H}$	9.8	2	70	48
L	Py $\gamma\text{-H}$	8.9	2	40	110
M	Py $\gamma\text{-H}$	8.6		40	83
N	Py $\beta\text{-H}$	8.5		50	47
O	Ph $\beta\text{-H}$	8.1	2	35	59
P	Py $\beta'\text{-H}$	7.8	2	30	66
Q	Ph $\gamma\text{-H}$	6.0	1	30	95
R		4.0	2	140	5
S		3.4	4	120	8
T		2.0	2	30	43
U		1.8	2	50	6
V		1.0	1	350	40
W		0.5	1	230	4
X		-2.3	1	160	5
Y	$\mu\text{-OH}$	-10	~ 1	530	1

^a All shifts are in ppm relative to the residual solvent signal at 1.92 ppm for CH_3CN . ^b The line widths are full width at half-maximum.

Clear COSY cross signals are observed between signals L (8.9 ppm) and K (9.8 ppm), and between signals L and I (10.3 ppm) (Figure 5). In addition, clear COSY cross signals are observed between signals M (8.6 ppm) and P (7.8 ppm), and between signals M and N (8.5 ppm). The only sets of three protons in **3** that are spin-coupled are those of the pyridine rings. Therefore, signals L and M are assigned to the $\gamma\text{-H}$ protons of inequivalent pyridine rings. Resonances K and I are the corresponding $\beta\text{-H}$ protons of the pyridine rings containing proton L. Likewise, N and P are the $\beta\text{-H}$ protons of the pyridine rings containing proton M. Clear COSY cross signals are also observed between signals O (8.1) and Q (6.0 ppm) (Figure 5). Signals O and Q can be assigned to the *m*-phenol and *p*-phenol protons, respectively (Table 2). This assignment is also consistent with the integrations and T_1 values of these two resonances. Signal O integrates to two protons while Q integrates to one proton. Moreover, signal Q has a T_1 value of 95 ms compared to 59 ms for O, consistent with Q being the *p*-phenol proton which is farther from the paramagnetic dicopper(II) center.

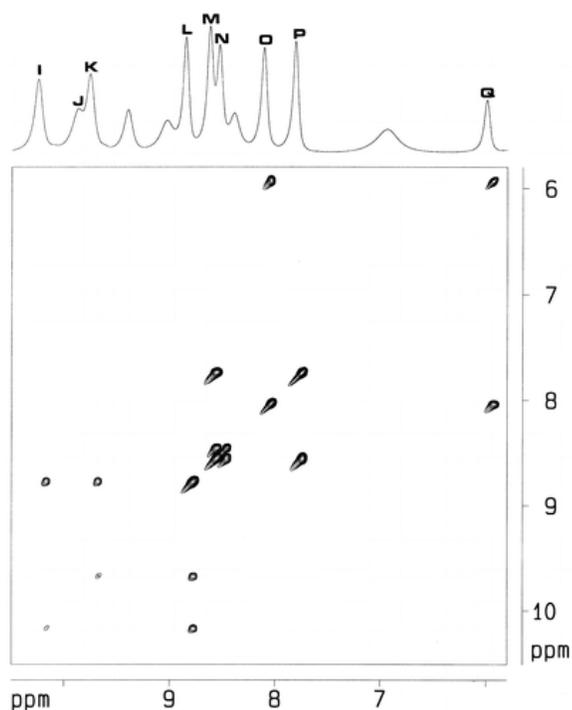


Figure 5 Magnitude ^1H COSY spectrum of **3** obtained at 400.13 MHz (Bruker ARX-400) at $-20\text{ }^\circ\text{C}$ in CD_3CN solution. The spectrum was referenced to the residual protic solvent signal at 1.92 ppm. This spectrum was obtained with an acquisition time of 150 ms and 1536 data points in the F1 dimension and 3072 data points in the F2 dimension. An unshifted-sine-bell squared weighting function and zero-filling to 4096 data points were applied prior to Fourier transformation in both dimensions followed by baseline correction in both dimensions and symmetrization.

The ^1H NMR spectrum of **3** at $-20\text{ }^\circ\text{C}$ reveals an upfield shifted signal at -10 ppm (Y). In **1**, a similar resonance was assigned to the μ -hydroxo OH proton by substitution with deuterium. Upon the addition of a drop of D_2O to an acetonitrile solution of **3**, signal Y disappears. ^2H NMR experiments show the corresponding resonance at the appropriate chemical shift value, thus substantiating the assignment of Y to the μ -hydroxo OH proton. In addition, the T_1 value for Y is ~ 1 ms, consistent with the crystallographically determined short Cu–H distance of 2.56 \AA . The only remaining protons in **3** that are unassigned reside on the ethylene and methylene linkages as well as the α -pyridine protons. These protons are expected to be closer to the Cu(II) ions ($< 3.5\text{ \AA}$) causing severe line broadening of the observed ^1H NMR resonances. Of the remaining unassigned signals, nearly all have line widths of 150 Hz or greater. Since the observation of COSY cross signals is a function of T_2 , it is not surprising that no additional cross signals are observed.

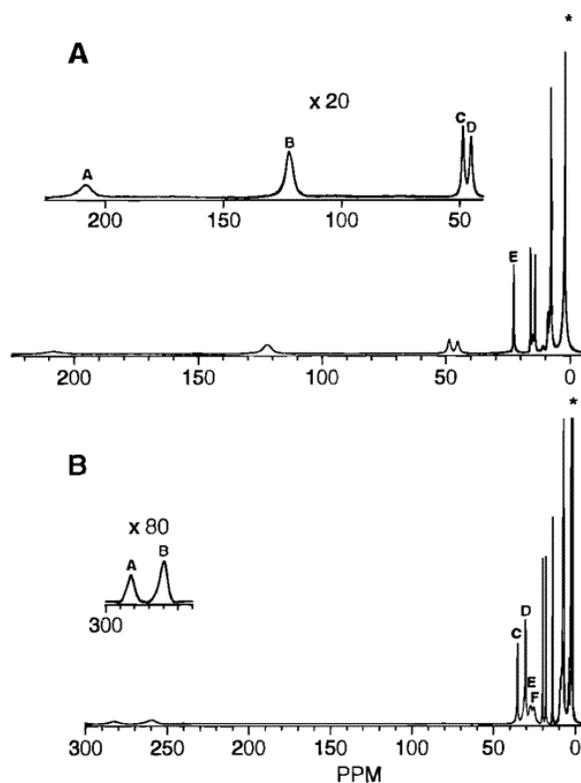


Figure 6 ^1H NMR spectra: (A) spectrum of **4** in CD_3CN at $55\text{ }^\circ\text{C}$ and (B) spectrum of **5** in CD_3CN at $-35\text{ }^\circ\text{C}$. Spectra were referenced to the residual protic solvent signals (*) at 1.92 ppm for acetonitrile. The * notes a small impurity as well as the position of the transmitter. Additional peak labels for the 20–0 ppm region appear in Figure 7 or Figure 1S (see supporting information).

Table 3. ^1H NMR Data for $[\text{Cu}_2(\text{TBHP})(\text{OAc})]^{2+}$ in CD_3CN Solution at $60\text{ }^\circ\text{C}$

	assignment	chemical shift ^a	relative area	line width ^b (Hz)	T_1 (ms)
A	N- CH_2 -CH	210	~2	2700	~1
B	N- CH_2 -Bz	120	4	1500	1
C	N- CH_2 -Bz	48	2	540	2
D	N- CH_2 -Bz	45	2	520	2
E	OOC- CH_3	22.4	3	110	3
F	Bz β - or α' -H	15.7	2	65	45
G	N-H	15	2	415	2
H	Bz β' -H	13.8	2	80	29
I	N- CH_2 -CH	11	~2	480	2
J	Bz α -H	8.7	2	215	3
K	Bz α -H	8.1	2	245	4
L	Bz β - and α' -H	7.5	4	90	44
M	Bz β - or α' -H	7.3	2	80	42
N	Bz β' -H	7.2	2	100	51

^a All shifts are in ppm relative to the residual solvent signals at 1.92 ppm for CH_3CN . ^b The line widths are full width at half-maximum.

Ferromagnetically Coupled Systems.

$[\text{Cu}_2(\text{TBHP-Et})(\text{OAc})]^{2+}$ is a ferromagnetically coupled, crystallographically characterized (μ -alkoxo)(μ -acetato)dicopper(II) complex with a $-2J$ value of -24 cm^{-1} .²⁵ We have synthesized the N-protonated derivative

$[\text{Cu}_2(\text{TBHP})(\text{OAc})]^{2+}$ (**4**) and characterized it by elemental analysis and electronic absorption spectroscopy (Figure 1).²⁵ The Evans susceptibility method was used to determine the room temperature magnetic moment of **4** ($\mu_{\text{eff}}/\text{Cu}$) which is 1.79 BM, giving the number of unpaired electrons (n/Cu) as 1.05.^{39,40} These data are consistent with the fact that the Cu(II) ions in **4** are weakly ferromagnetically coupled in acetonitrile solution. Complex **4** exhibits 14 relatively sharp, isotropically shifted ^1H NMR resonances in acetonitrile solution at 25 °C in the 300–0 ppm chemical shift range. Upon increasing the temperature, nearly all of the isotropically shifted signals sharpen and shift toward the diamagnetic region following Curie law behavior. The signals between 7 and 9 ppm are clearly resolved at 60 °C (Figure 6A); thus, all of our NMR studies were performed at this temperature (Table 3). The ^1H NMR spectrum of the N-methylated dicopper(II) complex $[\text{Cu}_2(\text{TBHP-Me})(\text{OAc})]^{2+}$ in acetonitrile solution at 23 °C was recently reported by Satcher and Balch.²² The ^1H NMR spectrum of **4** is qualitatively similar to that reported with the exception of the N-methylated methyl protons that resonated at ~5 and ~3 ppm.

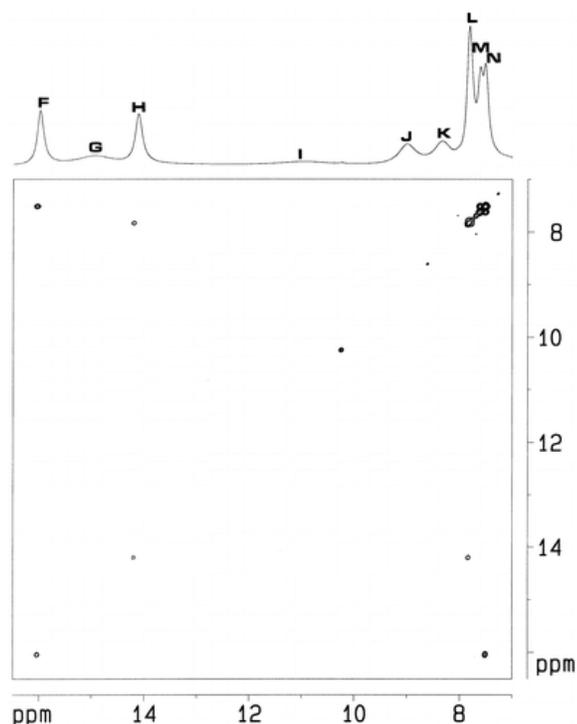


Figure 7 Magnitude ^1H COSY spectrum of **4** obtained at 400.13 MHz (Bruker ARX-400) at 55 °C in CD_3CN solution. The spectrum was referenced to the residual protic solvent signal at 1.92 ppm. This spectrum was obtained with an acquisition time of 125 ms and 1024 data points in the F1 dimension and 2048 data points in the F2 dimension. An unshifted sine-bell-squared weighting function and zero-filling to 3072 data points were applied prior to Fourier transformation in both dimensions followed by baseline correction in both dimensions and symmetrization.

Table 4. Average Cu–H Distances Determined from X-ray Crystal Structures of 1–4 and the Calculated Cu–H Distances Determined from T_1 Data for Assigned ^1H NMR Resonances

	$[\text{Cu}_2(\text{CH}_3\text{HXTA})(\text{OH})]^{2-}$		$[\text{Cu}_2(\text{BPMP})(\text{OH})]^{2+}$		$[\text{Cu}_2(m\text{-XYL})(\text{OH})]^{2+}$		$[\text{Cu}_2(\text{TBHP})(\text{OAc})]^{2+}$	
	average ^a Cu–H (Å)	calcd ^b Cu–H (Å)	average ^a Cu–H (Å)	calcd ^b Cu–H (Å)	average ^a Cu–H (Å)	calcd ^b Cu–H (Å)	average ^a Cu–H (Å)	calcd ^b Cu–H (Å)
A	3.46	4.2	3.42	4.4			3.3	3.7
B	3.73	4.2	3.16	4.1			3.6	3.7
C	2.98	4.2	3.68	4.3			3.8	4.1
D	5.07	5.7	3.17	3.8			4.1	4.1
E	7.14	–	4.89	5.7			4.7	4.4
F			5.11	5.7			5.9	6.9
G			5.13	5.7			5.0	4.1
H			5.75	6.6			6.0	6.4
I			7.31	–	5.07	5.6	3.9	4.1
J			2.54	3.8			3.6	4.4
K					4.99	4.9	3.5	4.6
L					5.77	6.4	6.5	6.9
M					5.77	5.8	6.5	6.8
N					5.07	5.9	7.0	–
O					5.19	6.0		
P					4.99	3.6		
Q					6.47	–		
Y					2.56	4.5		

^a Crystallographically determined average distances. All Cu–H average distances are taken as the arithmetic average of equivalent protons to each Cu(II) ion. ^b Distances calculated from $r_i = r_{\text{ref}}(T_{1i}/T_{1\text{ref}})^{1/6}$ assuming that r_{ref} is purely dipolar in nature.

Satcher and Balch assigned only three of the approximately 15 observed resonances of $[\text{Cu}_2(\text{TBHP-Me})(\text{OAc})]^{2+}$ by deuteration of the TBHP ligand.²² Their results indicate that signals A (210 ppm) and I (11 ppm) are due to the diastereotopic methylene protons of the isopropyl alcohol linkage. The signal observed at 26.5 ppm (22.5 ppm for **4**; E) was assigned to the acetate protons which was confirmed by deuteration experiments. Specific assignments of the remaining signals were not reported. We confirmed the assignment of signal E as the acetate protons by substitution of the acetate bridge with benzoate $[\text{Cu}_2(\text{TBHP})(\text{OBz})](\text{ClO}_4)_2$ (**5**). The signal at 22.5 ppm disappears and is replaced by three new resonances at 27.5, 14.0 and 7.2 ppm (Figure 6B). The remainder of the ^1H NMR spectrum of **5** is qualitatively similar to that of **4**. A magnitude COSY spectrum of **5** reveals a clear cross signal between the two resonances at 14.0 and 7.2 ppm (see supporting information). These signals are assigned to the *m*-H and *p*-H protons of the benzoate ring moiety, respectively. This assignment is also consistent with the integrations and T_1 values determined for these resonances (see supporting information).

A magnitude COSY spectrum of **4**, obtained at 60 °C in acetonitrile solution, shows clear cross signals between several resonances (Figure 7). Clear COSY cross signals are observed between signals N (7.2 ppm) and M (7.3 ppm), and signals N and F (15.7 ppm) (Figure 7). These signals can be unequivocally assigned to a set of benzimidazole ring α' -H (F or M), β -H (F or M), and β' -H (N) protons, respectively (Figure 1). A second clear COSY cross signal is observed between signals L (7.5 ppm) and H (13.8 ppm). Inspection of the integrations for these two resonances indicates that L has twice the area of H. These data suggest that H is a benzimidazole β' -H proton while L is the benzimidazole α' - and β -H protons. Similar to **3**, **4** has benzimidazole rings in different environments in acetonitrile solution. Examination of the X-ray crystallographic data for the N-ethylated derivative of **4** reveals that both Cu(II) ions reside in trigonal bipyramidal environments with inequivalent benzimidazole groups.²⁵ This observation is consistent with the ^1H spectrum of **4** and verifies the solid state structure is maintained in solution.

The remaining observed ^1H NMR resonances for **4** that are unassigned are signals B (120 ppm), C (48 ppm) D (45 ppm), G (15 ppm), J (8.7), and K (8.1) (Table 3). The observation of the methine proton is unlikely due to its close proximity ($< 2.5 \text{ \AA}$) to the paramagnetic Cu(II) ions. Therefore, only the N-H and α -H benzimidazole protons and the methylene-CH₂ protons of the benzimidazole methyl groups remain unaccounted for. Signal G is assigned to the benzimidazole N-H protons since the addition of a drop of D₂O causes it to disappear in 24 h. Similarly, the broad signal observed at 35 ppm for **5** can be exchanged for deuterium upon the addition of D₂O. ^2H NMR experiments confirm these assignments. Since many dinuclear enzyme active sites contain histidine residues, the chemical shift of the N-H exchangeable protons in **4** and **5** provide essential chemical shift information for these types of protons. On the basis of a series of model complexes, Maekawa *et al.*¹⁷ and, more recently, Satcher and Balch²² have correlated isotropic shift with the spin-coupling constant $-2J$. Both of these studies showed that the larger the isotropic shift the larger $\mu_{\text{eff}}/\text{Cu}$ and, consequently, smaller $-2J$ values. Since **4** and **5** are both ferromagnetically coupled, the chemical shifts of the N-H protons may represent the upper limit for these types of protons.

Assignment of the remaining five signals is difficult due to their large line widths and identical integrations of two protons each; however, by default the only unassigned protons in **4** are the α -H benzimidazole protons and the methylene-CH₂ protons of the benzimidazole methyl moieties (Table 3). It seems likely that the farthest downfield shifted signals B, C, and D are due to the diastereotopic methylene-CH₂ protons of the benzimidazole methyl groups similar to signals A and I, previously assigned to the diastereotopic methylene protons of the isopropyl alcohol linkage. Therefore, signals J and K can be tentatively assigned to the α -H benzimidazole protons. Assuming a predominant contact shift mechanism, the chemical shift of the benzimidazole α -H protons would be expected to be less than those of the methylene-CH₂ protons of the benzimidazole methyl moieties. This is also consistent with our assignments.

Relaxation Properties.

The assignment of the ^1H NMR spectra of these complexes combined with X-ray crystallographic results and T_1 values allows the dominant proton relaxation pathway to be determined. Assuming a paramagnetic dipolar relaxation mechanism for coupled dinuclear Cu(II) complexes, the Cu–H distance (r) should be proportional to $T_1^{1/6}$ (Table 4).⁴ Using the equation $r_i = r_{\text{ref}}(T_{1i}/T_{1\text{ref}})^{1/6}$ where r_i is the Cu–H_{*i*} distance, r_{ref} is the Cu–H_{ref} distance, T_{1i} is the relaxation time of proton *i*, and $T_{1\text{ref}}$ is the relaxation time of a reference proton, distances of each proton from the Cu(II) center can be estimated. If r_{ref} is taken as the arithmetic average of equivalent protons to each Cu(II) ion for the proton with the greatest distance from the copper ions, the remaining distances of all of the protons in the complexes can be calculated (Table 4). All of the calculated average Cu–H distances from definitively assigned protons of **1–4** are within *ca.* 20% of the average Cu–H distances derived from X-ray crystallographic data (Table 4). These data indicate, contrary to the suggestion by Satcher and Balch,²² that a paramagnetic dipolar relaxation mechanism is the dominant relaxation pathway in spin-coupled dicopper(II) complexes.

Conclusions

Isotropically shifted ^1H NMR signals can be easily observed for both antiferromagnetically and ferromagnetically coupled dicopper(II) complexes, and both one- and two-dimensional ^1H NMR techniques can be performed. The exchangeable N–H and μ -hydroxo O–H protons of **1**, **3**, **4**, and **5** provide important chemical shift and T_1 information for similar protons residing in metalloprotein active sites. Two-dimensional COSY experiments provide clear cross signals for resonances <200 Hz wide and establish spin–spin connectivities. Our studies show that COSY data facilitate the assignment of hyperfine shifted ^1H NMR signals in dicopper(II) complexes and that these studies verify that the solid state structure exists in solution for each system studied. Furthermore, the relaxation process for dicopper(II) systems is primarily regulated by a dipolar mechanism. This allows T_1 values to be used as a measure of the distance any given proton resides from a dicopper(II) center. These data, taken collectively, suggest that ^1H NMR spectroscopy is an excellent structural and magnetic probe of dicopper(II) complexes in solution. The application of both one- and two-dimensional ^1H NMR methods to dicopper(II) metalloprotein active sites is currently under investigation in our laboratory.

Supporting Information Available

A table of ^1H NMR data for $[\text{Cu}_2(\text{TBHP})(\text{OBz})]^{2+}$ (**5**) and a magnitude COSY spectrum of **5** in acetonitrile solution at $-35\text{ }^\circ\text{C}$ (3 pages). Ordering information is given on any current masthead page.

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- 24 Abbreviations: $\text{CH}_3\text{HXTA} = N,N'$ -(2-hydroxy-5-methyl-1,3-xylylene)bis(*N*-carboxymethylglycine); BPMP = 2,6-bis[[bis(2-pyridylmethyl)amino]methyl]-4-methylphenol; *m*-XYL = 2,6-bis[[bis(2-pyridylethyl)amino]methyl]phenol; TBHP = *N,N,N',N'*-tetrakis[(2-benzimidazolyl)methyl]-2-hydroxy-1,3-diaminopropane.
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