

# Molecular Heme–Cyanide–Copper Bridged Assemblies: Linkage Isomerism, Trends in $\nu_{\text{CN}}$ Values, and Relation to the Heme- $a_3$ /Cu<sub>B</sub> Site in Cyanide-Inhibited Heme–Copper Oxidases

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Bovine heart cytochrome *c* oxidase and related heme copper oxidases are inhibited by cyanide, which binds at the binuclear heme- $a_3$ /Cu<sub>B</sub> site where dioxygen is reduced to water. To determine the mode of cyanide binding, heme-based binuclear complexes containing iron–cyanide–copper bridges in different oxidation states have been prepared by the reaction of [(py)(OEP)Fe(CN)] with Cu(II,I) precursors and structurally characterized by X-ray methods. Structures of two precursor complexes and two binuclear Cu<sup>I</sup>–CN–Cu<sup>I</sup> species are reported. The assembly [(py)(OEP)Fe–CN–Cu(Npy<sub>3</sub>)]<sup>2+</sup> has a nearly linear Fe<sup>III</sup>–CN–Cu<sup>II</sup> bridge containing low-spin Fe(III). The assemblies [(OEP)Fe–NC–Cu(MeNpy<sub>2</sub>)]<sup>+</sup> and [(OEP–CH<sub>2</sub>CN)Fe–NC–Cu(Npy<sub>3</sub>)]<sup>+</sup> exhibit the high-spin bridges Fe<sup>III</sup>–NC–Cu<sup>I</sup> and Fe<sup>II</sup>–NC–Cu<sup>I</sup>, respectively. These are the first title bridges in these oxidation states. Bridge atom sequences are obtained from structural refinements of both linkage isomers; those for the reduced bridges are consistent with the soft-acid nature of Cu(I). Cyanide stretching frequencies respond to metal oxidation state and bridge geometry and, using data for solution and solid states, fall into the following ranges: Fe<sup>III</sup>–CN–Cu<sup>II</sup>, 2120–2184 cm<sup>-1</sup> (11 examples); Fe<sup>III</sup>–NC–Cu<sup>I</sup>, 2072–2100 cm<sup>-1</sup> (2 examples); Fe<sup>II</sup>–NC–Cu<sup>I</sup>, 2099–2107 cm<sup>-1</sup> (1 example). These data are compared with  $\nu_{\text{CN}}$  values for the enzymes in different oxidation states. A nonlinear Fe<sup>III</sup>–CN–Cu<sup>II</sup> bridge (Cu–N–C = 150–160°) is consistent with the 2146–2152 cm<sup>-1</sup> range found for the fully oxidized enzymes. Bands that can be assigned with some certainty as Fe–CN vibrations in partially and fully reduced enzymes do not appear to correspond to Fe<sup>III</sup>–NC–Cu<sup>I</sup> and Fe<sup>II</sup>–NC–Cu<sup>I</sup> bridges but rather to Fe<sup>II</sup>–CN modes. The current work complements and extends our previous investigation (Scott and Holm, *J. Am. Chem. Soc.* **1994**, *116*, 11357) of linear and nonlinear Fe<sup>III</sup>–CN–Cu<sup>II</sup> bridges and is part of an investigation directed at providing a molecular basis of cyanide toxicity. (MeNpy<sub>2</sub> = bis(2-(2-pyridylethyl))-methylamine; Npy<sub>3</sub> = tris(2-pyridylmethyl)amine; OEP = octaethylporphyrinate(2-), OEP–CH<sub>2</sub>CN = *N*-(cyanomethyl)octaethylporphyrinate(1-).)

## Introduction

Heme–copper terminal oxidases catalyze the reduction of dioxygen to water in a process whose free energy is used to translocate protons across cell membranes, generating proton and electric field gradients that are used in ATP synthesis.<sup>1</sup> Protein crystallography<sup>2,3</sup> has confirmed the existence of a binuclear heme- $a_3$ /Cu<sub>B</sub> site, at which substrate reduction occurs. In the oxidized forms of the enzymes from *Paracoccus denitrificans* and bovine heart, the site consists of a high-spin Fe(III) atom with an axial N•His ligand distal to a Cu(II) atom which is implicated in Cu(N•His)<sub>3</sub> coordination. The Fe•••Cu separations are 5.2<sup>2</sup> and 4.5 Å,<sup>3</sup> in the bacterial and bovine enzymes, respectively. However, the bridging ligand or ligands, whose presence is strongly inferred from magnetic and spectroscopic properties,<sup>1</sup> have not yet been identified. In an attempt

to clarify the expected properties of the putative Fe<sup>III</sup>–X–Cu<sup>II</sup> bridges in the oxidases, we have prepared a set of oxidized binuclear heme–copper complexes with X = O<sup>2-</sup>, OH<sup>-</sup>, and RCO<sub>2</sub><sup>-4-7</sup> and demonstrated antiferromagnetic coupling mediated by these bridges.<sup>8</sup> Exogenous small ions and molecules such as cyanide, isonitriles, carbon monoxide, nitric oxide, and azide have been used as spectroscopic probes of the binuclear site. Among these species, cyanide binds to the oxidized and reduced enzymes and is an irreversible inhibitor of enzyme action,<sup>1,9</sup> accounting for its toxicity.<sup>10</sup> Cyanide ligation at the

- (1) (a) Malmström, B. G. *Chem. Rev.* **1990**, *90*, 1247. (b) Babcock, G. T.; Wikström, M. *Nature* **1992**, *356*, 301. (c) García-Horsman, J. A.; Barquera, B.; Rumley, J.; Ma, J.; Gennis, R. B. *J. Bacteriol.* **1994**, *176*, 5587. (d) Ferguson-Miller, S.; Babcock, G. T. *Chem. Rev.* **1996**, *96*, 2889.
- (2) (a) Iwata, S.; Ostermeier, C.; Ludwig, B.; Michel, H. *Nature* **1995**, *376*, 660. (b) Ostermeier, C.; Harrenga, A.; Ermiler, U.; Michel, H. *Proc. Natl. Acad. Sci. U.S.A.* **1997**, *90*, 1247.
- (3) (a) Tsukihara, T.; Aoyama, H.; Yamashita, E.; Tomizaki, T.; Yamaguchi, H.; Shinzawa-Ito, K.; Nakashima, R.; Yaono, R.; Yoshikawa, S. *Science* **1995**, *269*, 1069. (b) Tsukihara, T.; Aoyama, H.; Yamashita, E.; Tomizaki, T.; Yamaguchi, H.; Shinzawa-Ito, K.; Nakashima, R.; Yaono, R.; Yoshikawa, S. *Science* **1996**, *272*, 1136.

- (4) Lee, S. C.; Holm, R. H. *J. Am. Chem. Soc.* **1993**, *115*, 5833, 11789.
- (5) Scott, M. J.; Zhang, H. H.; Lee, S. C.; Hedman, B.; Hodgson, K. O.; Holm, R. H. *J. Am. Chem. Soc.* **1995**, *117*, 568.
- (6) Scott, M. J.; Goddard, C. A.; Holm, R. H. *Inorg. Chem.* **1996**, *35*, 2558.
- (7) Oxo- and hydroxo-bridged systems have also been prepared by Karlin and co-workers: Fox, S.; Nanthakumar, A.; Wikström, M.; Karlin, K. D.; Blackburn, N. J. *J. Am. Chem. Soc.* **1996**, *118*, 24 and references therein.
- (8) Kauffmann, K. E.; Goddard, C. A.; Zang, Y.; Holm, R. H.; Münck, E. *Inorg. Chem.* **1997**, *36*, 985.
- (9) (a) Baker, G. M.; Noguchi, M.; Palmer, G. *J. Biol. Chem.* **1987**, *267*, 595. (b) Schoonover, J. R.; Palmer, G. *Biochemistry* **1991**, *30*, 7541. (c) Day, E. P.; Peterson, J.; Sendova, M. S.; Schoonover, J.; Palmer, L. *Biochemistry* **1993**, *32*, 7855. (d) Lodder, A. L.; Van Gelder, B. F. *Biochim. Biophys. Acta* **1994**, *1186*, 67. (e) Wilson, M. T.; Antonio, G.; Malatesta, F.; Sarti, P.; Brunori, M. *J. Biol. Chem.* **1994**, *269*, 24114. (f) Fabian, M.; Palmer, G. *Biochemistry* **1995**, *34*, 1534. (g) Panda, M.; Robinson, N. C. *Biochemistry* **1995**, *34*, 10009.
- (10) Lablanca, D. A. *J. Chem. Educ.* **1979**, *56*, 788.

binuclear site blocks dioxygen binding and thus the reaction  $O_2 + 4H^+ + 4e^- \rightarrow 2H_2O$ , the final step in aerobic metabolism.

The mode of cyanide binding to the binuclear sites of heme–copper oxidases is unresolved, a matter raised recently by Palmer.<sup>11</sup> Proposals have included terminal binding to either metal, bridging to both metals, and binding at the heme group with the nitrogen end of the ligand subject to hydrogen-bonding interactions. That cyanide invades the binuclear site in the oxidized form is based, among other evidence, on conversion of heme *a*<sub>3</sub> from a five-coordinate high-spin configuration to a low-spin state, strongly implying six-coordinate Fe(III), and strong modification of magnetic coupling between heme *a*<sub>3</sub> and Cu<sub>B</sub>.<sup>9c</sup> Structural propositions have been based largely on the position and intensity of cyanide stretching frequencies ( $\nu_{CN}$ ) of bovine heart cytochrome *c* oxidase.<sup>12–17</sup> Similar data have been reported for several bacterial ubiquinol oxidases.<sup>18–20</sup>

Toward the end of ultimately providing a molecular basis for cyanide toxicity by defining the structures of cyanide-bound binuclear enzyme sites, we have prepared and structurally characterized a set of heme–copper complexes containing the bridge [Fe<sup>III</sup>–CN–Cu<sup>II</sup>].<sup>21,22</sup> These complexes have afforded a necessary database of bridge structures and values of  $\nu_{CN}$  as dependent on the metric parameters of the bridges. Bridge Cu–N bond lengths and Cu–N–C angles vary within the set, allowing a correlation between these parameters and  $\nu_{CN}$  and leading to the conclusion that a (nonlinear) bridge structure in the enzymes is possible.<sup>22</sup> A vibrational analysis of a species with a nearly linear bridge strengthens the bridge structure proposal and further suggests that the binuclear site is sufficiently flexible structurally to accommodate other exogenous ligands.<sup>23</sup> In addition to the fully oxidized Fe<sup>III</sup>/Cu<sup>II</sup> form, the binuclear site can be adjusted to the half-reduced and fully reduced states Fe<sup>III</sup>/Cu<sup>I</sup> and Fe<sup>II</sup>/Cu<sup>I</sup>, respectively, with which cyanide also reacts. There being no structural or vibrational information on heme-based cyanide-bridged molecules with these lower oxidation states, we have synthesized several examples of such bridged assemblies and determined their structures for the purpose of defining the nature of their bridges. Whereas the fully oxidized complexes possess the foregoing bridge atom sequence,<sup>21,22</sup> the possibility of linkage isomerism, viz., Fe–CN–Cu vs Fe–NC–Cu, appears in the reduced forms and in any enzyme bridge in these oxidation states. The results of our expanded investigation of cyanide-bridged heme–copper assemblies in three different oxidation states are reported herein.

- (11) Palmer, G. J. *Bioenerg. Biomembr.* **1993**, *25*, 145.  
 (12) Yoshikawa, S.; Caughey, W. S. *J. Biol. Chem.* **1990**, *265*, 7945.  
 (13) Caughey, W. S.; Dong, A.; Sampath, S.; Yoshikawa, S.; Zhao, X.-J. *J. Bioenerg. Biomembr.* **1993**, *25*, 81.  
 (14) Tsubaki, M.; Yoshikawa, S. *Biochemistry* **1993**, *32*, 164.  
 (15) Li, W.; Palmer, G. *Biochemistry* **1993**, *32*, 1833.  
 (16) Yoshikawa, S.; Mochizuki, M.; Zhao, X.-J.; Caughey, W. S. *J. Biol. Chem.* **1995**, *270*, 4270.  
 (17) Hirota, S.; Ogura, T.; Shinzawa-Itoh, K.; Yoshikawa, S.; Kitagawa, T. *J. Phys. Chem.* **1996**, *100*, 15274.  
 (18) Tsubaki, M.; Mogi, T.; Anraku, Y.; Hori, H. *Biochemistry* **1993**, *32*, 6065.  
 (19) Tsubaki, M.; Mogi, T.; Hori, H.; Sato-Watanabe, M.; Anraku, Y. *J. Biol. Chem.* **1996**, *271*, 4017.  
 (20) Tsubaki, M.; Matsushita, K.; Adachi, O.; Hirota, S.; Kitagawa, T.; Hori, H. *Biochemistry* **1997**, *36*, 13034.  
 (21) (a) Lee, S. C.; Scott, M. J.; Kauffmann, K.; Münck, E.; Holm, R. H. *J. Am. Chem. Soc.* **1994**, *116*, 401. (b) Scott, M. J.; Lee, S. C.; Holm, R. H. *Inorg. Chem.* **1994**, *33*, 4651.  
 (22) Scott, M. J.; Holm, R. H. *J. Am. Chem. Soc.* **1994**, *116*, 11357.  
 (23) Gardner, M. T.; Deinum, G.; Kim, Y.; Babcock, G. T.; Scott, M. J.; Holm, R. H. *Inorg. Chem.* **1996**, *35*, 6878.

### Chart 1. Designation of Complexes

[Cu(Npy <sub>3</sub> )(MeCN)] <sup>2+</sup>	1 <sup>7</sup>
[Cu(Npy <sub>3</sub> )(MeCN)] <sup>+</sup>	2 <sup>25</sup>
[Cu(Npy <sub>3</sub> )(CN)] <sup>+</sup>	3
[Cu(MeNpy <sub>2</sub> )(OCIO <sub>3</sub> )]	4
[Cu <sub>2</sub> (MeNpy <sub>2</sub> ) <sub>2</sub> (CN)] <sup>+</sup>	5
[Cu <sub>2</sub> (Npy <sub>3</sub> ) <sub>2</sub> (CN)] <sup>+</sup>	6
[Fe(OEP)(py)(CN)]	7 <sup>21</sup>
[(py)(OEP)Fe–CN–Cu(Npy <sub>3</sub> )] <sup>2+</sup>	8
[(py)(OEP)Fe–CN–Cu(Me <sub>6</sub> tren)] <sup>2+</sup>	9 <sup>21</sup>
[(OEP)Fe–NC–Cu(MeNpy <sub>2</sub> )] <sup>+</sup>	10
[(OEP)Fe–NC–Cu(Me <sub>5</sub> dien)] <sup>+</sup>	11 <sup>22</sup>
[(OEP–CH <sub>2</sub> CN)Fe–NC–Cu(Npy <sub>3</sub> )] <sup>+</sup>	12

### Experimental Section<sup>24</sup>

**Designation of Complexes.** Complexes 1–12 are of principal interest in this work and are numerically designated in Chart 1. Of these, 1, 2, 7, 9, and 11 have been previously prepared.

**Preparation of Compounds.** Except for those of Cu(II) complexes, all preparations were carried out under a pure dinitrogen atmosphere using Schlenk or standard glovebox techniques. Solvents were dried and degassed by standard methods.

**Caution!** Although we have experienced no difficulty with the compounds described here, perchlorate salts are potentially explosive and should be handled accordingly.

**(a) Copper Complexes. [Cu(Npy<sub>3</sub>)(CN)](ClO<sub>4</sub>) ([3](ClO<sub>4</sub>)).** A solution of 27 mg (0.41 mmol) of KCN in 15 mL of methanol was added to a solution of 0.25 g (0.40 mmol) of [Cu(Npy<sub>3</sub>)(MeCN)](ClO<sub>4</sub>)<sub>2</sub><sup>7</sup> in 5 mL of methanol. The reaction mixture was stirred overnight and filtered, and the filtrate was reduced to dryness in vacuo. The solid residue was dissolved in 5 mL of acetonitrile, and the solution was filtered. Ether (20 mL) was slowly added to the filtrate, and the mixture was allowed to stand for 6 h. A blue crystalline solid was collected by filtration and washed with ether to afford 86 mg (45%) of product. IR (Nujol mull):  $\nu_{CN}$  2143 cm<sup>-1</sup>. Anal. Calcd for C<sub>19</sub>H<sub>18</sub>ClCuN<sub>5</sub>O<sub>4</sub>: C, 47.67; H, 3.78; N, 14.61. Found: C, 47.48; H, 3.78; N, 14.53.

**[Cu<sub>2</sub>(Npy<sub>3</sub>)<sub>2</sub>(CN)](ClO<sub>4</sub>) ([6](ClO<sub>4</sub>)).** A solution of 0.14 g (0.98 mmol) of (Et<sub>4</sub>N)(CN) in 5 mL of acetonitrile was added to a solution of 0.50 g (1.0 mmol) of [Cu(Npy<sub>3</sub>)(MeCN)](ClO<sub>4</sub>)<sub>2</sub><sup>25</sup> in 5 mL of acetonitrile. The mixture was stirred for 10 min, resulting in a white suspension. The solvent was removed in vacuo, and the solid residue was partially dissolved in a minimal volume of THF. The solution was filtered, and the filtrate was reduced to dryness in vacuo to give a yellow solid. This material was dissolved in 5 mL of acetonitrile, and 20 mL of ether was added to the solution. After several days, the product was obtained as 0.17 g (20%) of a yellow solid. FAB-MS: *m/z* 732 (M<sup>+</sup>), 379 (M<sup>+</sup> – Npy<sub>3</sub>), 353 (M<sup>+</sup> – Npy<sub>3</sub> – CN). IR (Nujol):  $\nu_{CN}$  2107 cm<sup>-1</sup>. The THF-insoluble solid is formulated as the polymer [Cu(Npy<sub>3</sub>)(CN)]<sub>x</sub>. FAB/MS: *m/z* 379 (M<sup>+</sup>), 353 (M<sup>+</sup> – CN). The polymer was also prepared by the reaction of CuCN with Npy<sub>3</sub> in THF in 80% yield.

**[Cu<sub>2</sub>(MeNpy<sub>2</sub>)<sub>2</sub>(CN)](ClO<sub>4</sub>) ([5](ClO<sub>4</sub>)).** [Cu(MeNpy<sub>2</sub>)(OCIO<sub>3</sub>)] was prepared in a procedure analogous to that for [Cu(MeNpy<sub>2</sub>)](PF<sub>6</sub>)<sub>2</sub><sup>26</sup> using MeNpy<sub>2</sub><sup>26</sup> and [Cu(MeCN)<sub>4</sub>](ClO<sub>4</sub>)<sub>2</sub>.<sup>27</sup> The compound was

- (24) Abbreviations: hs, high-spin; ls, low-spin; Me<sub>5</sub>dien, 1,1,4,7,7-pentamethyldiethylenetriamine; 1-MeIm, 1-methylimidazole, MeNpy<sub>2</sub>, bis(2-(2-pyridylethyl)methylamine); Me<sub>6</sub>tren, tris(2-(dimethylamino)ethyl)amine; Npy<sub>3</sub>, tris(2-pyridylmethyl)amine; OEP, octaethylporphyrinate(2-); OEP–CH<sub>2</sub>CN, *N*-(cyanomethyl)octaethylporphyrinate(1-); TPP, 5,10,15,20-tetraphenylporphyrinate(2-); TTP, 5,10,15,20-tetra-*p*-tolylporphyrinate(2-). Note that the ligand Npy<sub>3</sub> (so named here for comparison with MeNpy<sub>2</sub>) has been previously designated as TMPA<sup>7</sup> and originally as TPA (Anderegg, G.; Wenk, F. *Helv. Chim. Acta* **1967**, *50*, 2330).  
 (25) Tyeklár, Z.; Jacobson, R. R.; Wei, N.; Murthy, N. N.; Zubieta, J.; Karlin, K. D. *J. Am. Chem. Soc.* **1993**, *115*, 2677.  
 (26) Sanyal, I.; Mahroof-Tahir, M.; Nasir, M. S.; Ghosh, P.; Cohen, B. I.; Gultneh, Y.; Cruse, R. W.; Farooq, A.; Karlin, K. D.; Liu, S.; Zubieta, J. *Inorg. Chem.* **1992**, *31*, 4322.  
 (27) Hemmerich, P.; Sigwart, C. *Experientia* **1963**, *19*, 488.

**Table 1.** Crystallographic Data<sup>a</sup> for the Copper Complexes

	[1](ClO <sub>4</sub> ) <sub>2</sub> ·MeCN	[2](ClO <sub>4</sub> )	[5](ClO <sub>4</sub> )	[6](ClO <sub>4</sub> )
formula	C <sub>22</sub> H <sub>24</sub> Cl <sub>2</sub> CuN <sub>6</sub> O <sub>8</sub>	C <sub>20</sub> H <sub>21</sub> ClCuN <sub>5</sub> O <sub>4</sub>	C <sub>31</sub> H <sub>38</sub> ClCu <sub>2</sub> N <sub>7</sub> O <sub>4</sub>	C <sub>37</sub> H <sub>36</sub> ClCu <sub>2</sub> N <sub>9</sub> O <sub>4</sub>
fw	634.92	494.42	735.24	833.30
cryst syst	monoclinic	orthorhombic	monoclinic	monoclinic
space group	<i>P2<sub>1</sub>/c</i>	<i>Fdd2</i>	<i>P2<sub>1</sub>/n</i>	<i>C<sub>2</sub>/c</i>
Z	4	16	4	4
a, Å	18.832(4)	26.209(5)	11.9295(4)	14.723(3)
b, Å	10.778(2)	26.379(5)	19.2561(3)	11.548(2)
c, Å	14.566(3)	12.550(2)	14.5407(3)	22.256(5)
β, deg	110.18(3)		96.60(3)	97.62(2)
V, Å <sup>3</sup>	2775(1)	8677(3)	3318(1)	3750(1)
T, K	223	223	223	298
d <sub>calc</sub> , g/cm <sup>3</sup>	1.520	1.514	1.433	1.476
μ, mm <sup>-1</sup>	1.04	1.17	1.41	1.26
R (wR <sub>2</sub> ), <sup>b</sup> %	3.69 (10.18)	5.14 (6.18)	5.73 (11.92)	5.37 (12.92)

<sup>a</sup> Obtained with graphite-monochromatized Mo Kα radiation (λ = 0.710 73 Å). <sup>b</sup> R = Σ||F<sub>o</sub> - |F<sub>c</sub>||/Σ|F<sub>o</sub>|; wR<sub>2</sub> = {Σ[w(F<sub>o</sub><sup>2</sup> - F<sub>c</sub><sup>2</sup>)]/Σ[w(F<sub>o</sub><sup>2</sup>)]}<sup>1/2</sup>.

**Table 2.** Crystallographic Data<sup>a</sup> for the Iron–Copper Cyanide-Bridged Assemblies

	[8](ClO <sub>4</sub> ) <sub>2</sub> ·2MeCN·Et <sub>2</sub> O	[10](ClO <sub>4</sub> )·2MeCN	[12](ClO <sub>4</sub> )·MeCN	[12](ClO <sub>4</sub> )·EtCN
formula	C <sub>68</sub> H <sub>83</sub> Cl <sub>2</sub> CuFeN <sub>12</sub> O <sub>9</sub>	C <sub>56</sub> H <sub>69</sub> ClCuFeN <sub>10</sub> O <sub>4</sub>	C <sub>59</sub> H <sub>67</sub> ClCuFeN <sub>11</sub> O <sub>4</sub>	C <sub>60</sub> H <sub>69</sub> ClCuFeN <sub>11</sub> O <sub>4</sub>
fw	1402.79	1101.08	1149.10	1163.13
cryst syst	orthorhombic	monoclinic	monoclinic	monoclinic
space group	<i>Pca2<sub>1</sub></i>	<i>P2<sub>1</sub>/n</i>	<i>P2<sub>1</sub>/c</i>	<i>P2<sub>1</sub>/c</i>
Z	4	4	4	4
a, Å	35.469(2)	18.8322(3)	12.079(9)	12.1215(2)
b, Å	12.9779(8)	15.1367(1)	33.79(2)	33.9151(4)
c, Å	15.6382(9)	19.9600(2)	15.04(1)	15.0970(2)
β, deg		103.473(1)	110.35(3)	110.395(1)
V, Å <sup>3</sup>	7198.4(7)	5533.2(1)	5754(7)	5817.3(1)
T, K	213	213	213	213
d <sub>calc</sub> , g/cm <sup>3</sup>	1.294	1.322	1.327	1.329
μ, mm <sup>-1</sup>	0.63	0.75	0.72	0.72
R (wR <sub>2</sub> ), <sup>b</sup> %	6.81 (12.75)	6.45 (17.72)	7.79 (13.37)	5.21 (12.14)

<sup>a</sup> Obtained with graphite-monochromatized Mo Kα radiation (λ = 0.710 73 Å). <sup>b</sup> R = Σ||F<sub>o</sub> - |F<sub>c</sub>||/Σ|F<sub>o</sub>|; wR<sub>2</sub> = {Σ[w(F<sub>o</sub><sup>2</sup> - F<sub>c</sub><sup>2</sup>)]/Σ[w(F<sub>o</sub><sup>2</sup>)]}<sup>1/2</sup>.

obtained in 50% yield as a pale yellow solid. A solution of 0.10 g (0.25 mmol) of [Cu(MeNpy<sub>2</sub>)(OCIO<sub>3</sub>)] in 25 mL of acetonitrile was treated with 16 mg (0.25 mmol) of KCN. The reaction mixture was stirred overnight and filtered. The collected solid was dissolved in 20 mL of dichloromethane, and the solution was filtered. Ether (50 mL) was added to the filtrate, resulting in the separation of the product as 0.11 g (60%) of a yellow microcrystalline solid. IR (Nujol): ν<sub>CN</sub> 2103 cm<sup>-1</sup>. Anal. Calcd for C<sub>31</sub>H<sub>38</sub>ClCu<sub>2</sub>N<sub>7</sub>O<sub>4</sub>: C, 50.64; H, 5.21; N, 13.34. Found: C, 50.53; H, 5.18; N, 13.29. The compound has also been identified by an X-ray structure determination.

**(b) Iron–Copper Bridged Assemblies.** [(py)(OEP)Fe–CN–Cu(Npy<sub>3</sub>)](ClO<sub>4</sub>)<sub>2</sub>·0.5Et<sub>2</sub>O ([8](ClO<sub>4</sub>)<sub>2</sub>·0.5Et<sub>2</sub>O). A solution of 16 mg (27 μmol) of [Cu(Npy<sub>3</sub>)(MeCN)](ClO<sub>4</sub>)<sub>2</sub><sup>7</sup> in 0.5 mL of acetonitrile was added slowly to a solution of 19 mg (28 μmol) of [Fe(OEP)(CN)(py)]<sup>21</sup> in 0.5 mL of acetonitrile. The dark red solution was stirred for 20 min and filtered. Several volume equivalents of ether were introduced into the filtrate by vapor diffusion. The product was collected by filtration, washed with ether, and obtained as 25 mg (74%) of red-violet crystals. Absorption spectrum (dichloromethane): λ<sub>max</sub> (ε<sub>M</sub>) 397 (86 000), 521 (8400), 560 (5400), 623 (1700), 832 (770) nm. IR (Nujol): ν<sub>CN</sub> 2175 cm<sup>-1</sup>. <sup>1</sup>H NMR (Me<sub>2</sub>CO), δ: 10.10, 10.21 (CH<sub>2</sub>); 3.31 (CH), 1.24 (CH<sub>3</sub>) (OEP). The product analyzed satisfactorily as an ether hemisolvate. Anal. Calcd for C<sub>62</sub>H<sub>72</sub>Cl<sub>2</sub>CuFeN<sub>10</sub>O<sub>8.5</sub>: C, 58.01; H, 5.65; Cu, 4.95; Fe, 4.35; N, 10.91. Found: C, 57.72; H, 5.48; Cu, 5.03; Fe, 4.41; N, 10.29. This compound has also been identified by an X-ray structure determination. The <sup>13</sup>CN-labeled compound was prepared in the same way using [Fe(OEP)(<sup>13</sup>CN)(py)]. ν<sub>CN</sub> (Nujol): 2135 cm<sup>-1</sup>.

[(OEP)Fe–NC–Cu(MeNpy<sub>2</sub>)](ClO<sub>4</sub>)<sub>2</sub>·2MeCN ([10](ClO<sub>4</sub>)<sub>2</sub>·2MeCN). A solution of 8.4 mg (21 μmol) of [Cu(MeNpy<sub>2</sub>)(OCIO<sub>3</sub>)] in 0.5 mL of acetonitrile was added to a solution of 13 mg (19 μmol) of [Fe(OEP)(CN)(py)] in 0.5 mL of acetonitrile. The reaction mixture was stirred for 2 h and filtered. Several volume equivalents were introduced

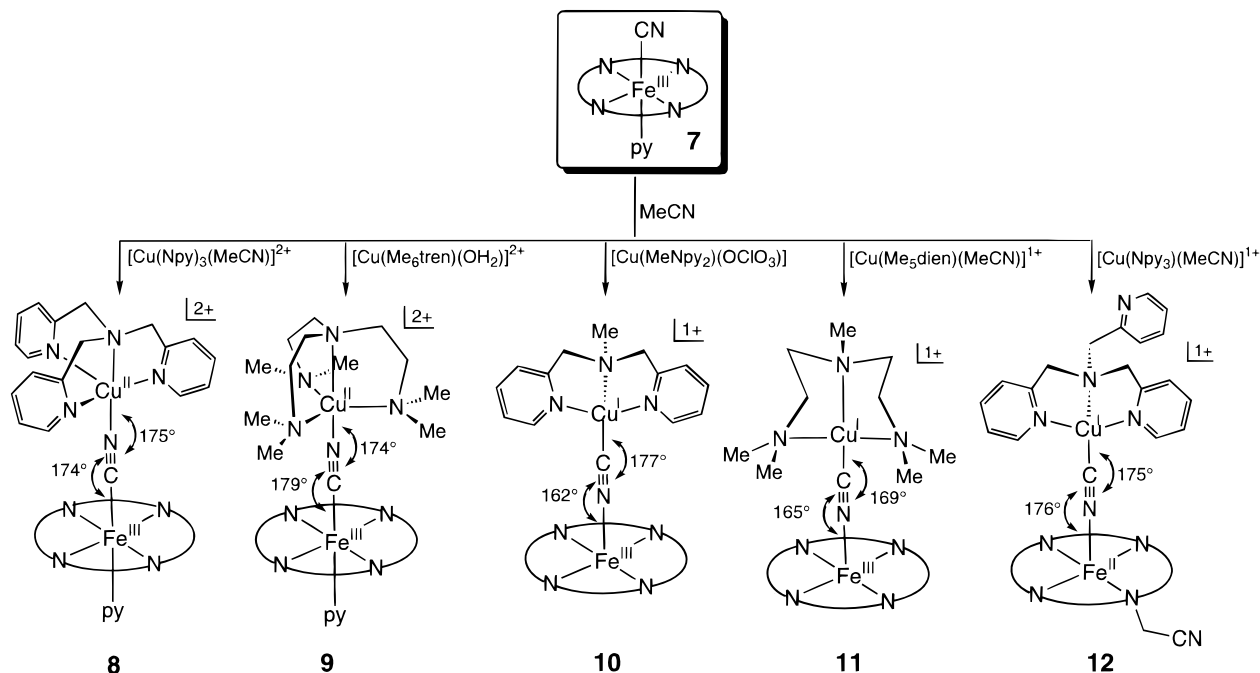
into the filtrate by vapor diffusion of pentane/ether (1:1 v/v). The product was collected by filtration, washed with ether, and obtained as 12 mg (57%) of black crystals. Absorption spectrum (dichloromethane): λ<sub>max</sub> (ε<sub>M</sub>) 379 (110 000), 503 (9900), 639 (3600), 830 (1700). IR (Nujol): ν<sub>CN</sub> 2072 cm<sup>-1</sup>. <sup>1</sup>H NMR (Me<sub>2</sub>CO), δ: 54.2, 44.8 (CH<sub>2</sub>); 7.80 (CH<sub>3</sub>) (OEP). Anal. Calcd for C<sub>56</sub>H<sub>69</sub>ClCuFeN<sub>10</sub>O<sub>4</sub>: C, 61.09; H, 6.32; Cu, 5.77; Fe, 5.07; N, 12.72. Found: C, 61.19; H, 6.26; Cu, 5.83; Fe, 4.94; N, 12.63.

[(OEP–CH<sub>2</sub>CN)Fe–NC–Cu(Npy<sub>3</sub>)](ClO<sub>4</sub>)·MeCN ([12](ClO<sub>4</sub>)·MeCN). A solution of 20 mg (39 μmol) of [Cu(Npy<sub>3</sub>)(MeCN)](ClO<sub>4</sub>)<sup>25</sup> in 0.5 mL of acetonitrile was slowly added to a solution of 26 mg (38 μmol) of [Fe(OEP)(CN)(py)] in 0.5 mL of dichloromethane. The dark brown solution was stirred for 10 min and filtered. Several volume equivalents were introduced into the filtrate by vapor diffusion of pentane/ether (1:1 v/v). The product was collected by filtration, washed with ether, and obtained as 12 mg (28%) of black needlelike crystals. IR (Nujol mull): ν<sub>CN</sub> 2099 cm<sup>-1</sup>. <sup>1</sup>H NMR (Me<sub>2</sub>CO), δ: 28.66, 25.03, 21.68, 20.60 (CH<sub>2</sub>); 0.88, -0.36 (CH<sub>3</sub>) (OEP–CH<sub>2</sub>CN). The bulk sample contained an ca. 20 mol % impurity of [Fe(OEP)Cl]; all efforts to remove this material failed. For this reason, the preparation was not analyzed. This compound has also been identified by an X-ray structure determination.

[(OEP–CH<sub>2</sub>CN)Fe–NC–Cu(Npy<sub>3</sub>)](ClO<sub>4</sub>)·EtCN ([12](ClO<sub>4</sub>)·EtCN). This compound was prepared by an analogous procedure but with propionitrile instead of acetonitrile. It was obtained in a yield comparable to that of the preceding compound and also contained an impurity of [Fe(OEP)Cl]. This compound has also been identified by an X-ray structure determination.

**X-ray Structure Determinations.** Structures were determined for the eight compounds in Tables 1 and 2, which contain cell parameters and refinement results. Crystals of [1](ClO<sub>4</sub>)<sub>2</sub>·MeCN, [2](ClO<sub>4</sub>), [6](ClO<sub>4</sub>), [8](ClO<sub>4</sub>)<sub>2</sub>·2MeCN·Et<sub>2</sub>O, and [10](ClO<sub>4</sub>)<sub>2</sub>·2MeCN were grown by vapor diffusion of ether into saturated acetonitrile solutions. Crystals





**Figure 1.** Synthesis of heme–copper bridged assemblies utilizing heme complex **7** and the indicated Cu(II,I) precursors: Fe<sup>III</sup>/Cu<sup>II</sup>, **8**, **9**; Fe<sup>III</sup>/Cu<sup>I</sup>, **10**, **11**; Fe<sup>II</sup>/Cu<sup>I</sup>, **12**. The crystal structures of all assemblies have been determined in this and previous work;<sup>21–23</sup> probable bridge atom sequences for **10–12** are indicated.

of [5](ClO<sub>4</sub>) were obtained by vapor diffusion of ether into a saturated dichloromethane solution. Crystals of [12](ClO<sub>4</sub>)·RCN (R = Me, Et) were grown by vapor diffusion of ether/pentane (1:1 v/v) into a saturated solution in acetonitrile/dichloromethane (1:1 v/v) or propionitrile/dichloromethane (1:1 v/v). Crystals were mounted in Paratone-N oil on the ends of glass capillaries and frozen under a dinitrogen cold stream. Data for [6](ClO<sub>4</sub>) were obtained at ambient temperature because of the cracking of crystals at low temperature. Data were collected on a Nicolet P3F for copper complexes and on a Siemens SMART CCD Area Detector System for iron–copper complexes. Cell parameters were determined from a least-squares analysis of more than 30 reflections with 12° ≤ 2θ ≤ 24° for the copper complexes. None of the compounds showed significant decay over the course of data collection. Peak profile fitting was performed by the program XDISK, and absorption corrections were applied using ψ-scan techniques for [1](ClO<sub>4</sub>)<sub>2</sub>·MeCN, [2](ClO<sub>4</sub>), [5](ClO<sub>4</sub>), and [6](ClO<sub>4</sub>). Raw intensity data for [8](ClO<sub>4</sub>)<sub>2</sub>·2MeCN·Et<sub>2</sub>O, [10](ClO<sub>4</sub>)·2MeCN, and [12](ClO<sub>4</sub>)·RCN were integrated to structure factor amplitudes and their esd's by the program SAINT, and absorption corrections were applied by SADABS. Space groups were confirmed by systematic absences and by successful structural refinements. Structures were solved by direct methods and refined on F<sup>2</sup> using the programs SHELXTL 5.03 or SHELXTL 97. All non-hydrogen atoms except the disordered carbon and nitrogen bridge atoms in [5](ClO<sub>4</sub>) were described anisotropically; hydrogen atoms were placed in calculated positions. One methyl group of a porphyrin ethyl substituent in [10](ClO<sub>4</sub>)·2MeCN is disordered over two positions and was refined with occupancy factors of 0.7 and 0.3. The bridge atoms in [6](ClO<sub>4</sub>) are disordered across the inversion center and were refined with the site occupancies 0.5C + 0.5N. In [5](ClO<sub>4</sub>), the Cu(1)–N–C–Cu(2) and disordered Cu(1)–N/C–C/N–Cu(2) bridge atom sequences refined to the same agreement factors; the bridge is concluded to be disordered. Both isomeric bridge atom sequences were examined in the final refinement of the Fe–Cu compounds. The following R factors were obtained for Fe–CN–Cu/Fe–NC–Cu refinements. [8](ClO<sub>4</sub>)<sub>2</sub>·2MeCN·Et<sub>2</sub>O: 6.81/6.83. [10](ClO<sub>4</sub>)·MeCN: 6.62, 6.44. [12](ClO<sub>4</sub>)·EtCN: 5.44, 5.21. The Fe–CN–Cu sequence is preferred for **8** and the Fe–NC–Cu sequence for **10** and **11** on the basis of R factors and reasonable values of thermal parameters (C, N); these preferences were supported by the Hamilton test<sup>28</sup> at the 1% significance level. In the final cycles of refinement of all structures, all parameters shifted by <1% of their esd's and the

final difference Fourier maps showed no significant electron density. Final agreement factors are contained in Tables 1 and 2.<sup>29</sup>

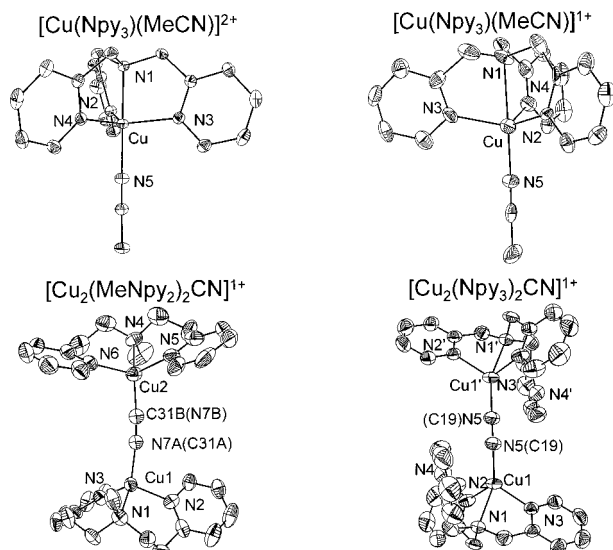
**Other Physical Measurements.** <sup>1</sup>H NMR spectra were obtained with a Bruker AM-500 spectrometer. Infrared spectra were recorded on a Nicolet IR/42 FT-IR spectrometer. Absorption spectra were recorded on a Perkin-Elmer Lambda 4C spectrophotometer.

## Results and Discussion

**Preparation of Compounds.** In our earlier investigations of cyanide-bridged binuclear heme–copper complexes, emphasis was placed on the fully oxidized Fe<sup>III</sup>–CN–Cu<sup>II</sup> bridge. Some 10 species containing this bridge were prepared.<sup>21,22</sup> These were sought primarily to understand the effect of variant bridge Cu–N distances and Cu–N–C angles on the vibrational properties of the bridge, especially the cyanide stretching frequencies.<sup>22,23</sup> In this work, we have prepared one additional example of this bridge and have sought reduced forms of the bridge that might correspond to other states of heme–copper oxidases reported to bind cyanide. Methods of synthesis of the cyanide-bridged iron–copper assemblies **8**, **10**, and **12** are outlined in Figure 1. The preparations of **9**<sup>21</sup> and **11**<sup>22</sup> have been described previously. All are derived from heme complex **7** by reaction with the appropriate Cu(II,I) precursor in acetonitrile. The latter contain labile ligands that are displaced by the cyanide group of **7**. The synthesis of **8**, obtained in 74% yield from **7** and **1**, closely parallels that of **9** and other Fe<sup>III</sup>–CN–Cu<sup>II</sup> assemblies.<sup>21,22</sup> The Fe<sup>III</sup>–NC–Cu<sup>I</sup> complex **10** was prepared by reaction of **7** with the preformed Cu(I) complex **4** and isolated in 57% yield. It is preceded only by **11**, having a different copper ligand and, despite multiple attempts, obtained only in very small yields. The Fe<sup>II</sup>–NC–Cu<sup>I</sup> complex **12** is formed by the reaction of **7** and Cu(I) complex **2** in acetonitrile/dichloromethane. While the bridge oxidation level is that desired, the N-cyanomethylation of the OEP ligand was

(28) W. C. Hamilton, W. C. *Acta Crystallogr.* **1965**, *18*, 502.

(29) See paragraph at the end of this article concerning Supporting Information available.



**Figure 2.** Structures of Cu(II) (**1**) and Cu(I) (**2**) precursors to bridged assemblies (upper) and two binuclear cyanide-bridged Cu(I) complexes (**5**, **6**) (lower), showing atom-labeling schemes and 50% probability ellipsoids.

unexpected. When the reaction was conducted in propionitrile/dichloromethane, the same complex was obtained, showing that the source of the cyanomethyl group is coordinated acetonitrile. *N*-Alkylporphyrins<sup>30</sup> and their metal complexes, including those of Fe(II,III),<sup>30–33</sup> are well-known, but we are unaware of any prior alkylation reaction of a metalloporphyrin by coordinated or free acetonitrile. Two solvates, [**12**](ClO<sub>4</sub>)·RCN (R = Me, Et), were obtained after recrystallization. While neither compound could be obtained in bulk form completely free of [Fe(OEP)Cl], a persistent contaminant that we were unable to separate, single crystals of both solvates suitable for X-ray analysis were obtained.

**Structures of Cu(II,I) Complexes.** We note briefly the structures of four complexes, two of which are synthetic precursors (**1**, **2**) and two of which are bridged species (**5**, **6**) useful in comparison of  $\nu_{\text{CN}}$  values with those of other complexes. Structures are depicted in Figure 2, and metric data, some of them given as ranges of values, are collected in Table 3. Complexes **1** and **2** provide a structural comparison of different oxidation states with the same ligand set. Copper(II) complex **1** has a somewhat distorted trigonal bipyramidal shape and closely resembles [Cu(Npy<sub>3</sub>)Cl]<sup>+</sup>.<sup>34</sup> The Cu(II) atom is displaced by 0.28 Å from the equatorial plane N(2–4) toward the axial acetonitrile ligand, accounting for the angles N(1)–Cu–N(2–4) being less than 90°. The principal elements of distortion from idealized C<sub>3v</sub> symmetry are the unequal Cu–N(2–4) bond lengths, which vary over 0.041 Å, and the N(2)–Cu–N(3) angle, which is 7.0° smaller than the other two equatorial bond angles. Copper(I) complex **2** also approaches trigonal symmetry and is nearly identical to a [Cu(Npy<sub>3</sub>)–

(MeCN)]<sup>+</sup> derivative in which one pyridyl group carries a –CO<sub>2</sub>Me substituent.<sup>25</sup> In **2**, however, the Cu(I) atom lies 0.55 Å below the equatorial plane, placing the N(1)–Cu–N(2–4) angles at ca. 75°. The displacement is in the direction of the axial acetonitrile ligand whose Cu–N(5) bond length is 1.99–(1) Å. This results in an axial Cu–N(1) bond length of 2.43–(1) Å, as compared with equatorial values in the range 2.07(1)–2.12(1) Å. The pattern of Cu–N distances is an obvious manifestation of the preference of Cu(I) for tetrahedral rather than five-coordinate stereochemistry, even with the semirigid trigonal ligand Npy<sub>3</sub> which can present four donor sites within normal bonding distances.

Complexes **5** and **6** evidence distorted tetrahedral Cu(I) coordination and nearly linear four-atom bridges (bridge angles 174 and 178°). In **5**, with two chemically equivalent but crystallographically inequivalent Cu(MeNpy<sub>2</sub>) portions, refinement indicates a disordered bridge. Because of the centrosymmetry imposed upon **6**, its bridge is necessarily disordered. Note that, in **6**, the potentially tetradentate Npy<sub>3</sub> ligand functions in a tridentate mode with one pyridyl group uncoordinated, another indication of the four-coordinate preference of Cu(I). Tricoordinate ligands are useful for the preparation of the precursors [Cu(Me<sub>5</sub>dien)(MeCN)]<sup>+</sup><sup>22</sup> and **4** (Figure 1) and for the stabilization of Cu(I) in bridged assemblies.

**Structures of Cyanide-Bridged Heme–Copper Assemblies.** Three new complexes of this type—**8**, **10**, and **12**—have been prepared. Their X-ray structures have been determined and are presented in Figures 3–5. Selected interatomic distances and angles are summarized in Table 4. The structure of heme precursor complex **7** is described elsewhere.<sup>35</sup>

(a) **Fe<sup>III</sup>–CN–Cu<sup>II</sup> Bridged Assembly.** Complex **8** is our latest addition to this set of species and is best compared with **9**<sup>21</sup> (Figure 1). It features a low-spin six-coordinate ferriheme group, a nearly linear Fe–C–N–Cu bridge (Fe–C–N 173.7–(7)°, Cu–N–C 174.5(8)°), and a distorted trigonal pyramidal Cu<sup>II</sup>(Npy<sub>3</sub>) fragment (Figure 3). While there are some small differences between this fragment and initial complex **1**, most notably a decrease in the axial Cu–N distance from 2.104(2) to 2.007(7) Å upon cyanide binding, dimensions are very similar. The comparison extends to a 0.26 Å displacement of Cu(II) toward cyanide with the same pattern of axial–equatorial bond angles found in **1**. As in **9** and related complexes, there is no large structural change in the Cu(II) fragment upon formation of the cyanide bridge. All previous heme Fe<sup>III</sup>–CN structures, of which **7** is but one example,<sup>35</sup> have been finally refined with the indicated atom sequence. We note that linear and nonlinear four-atom bridges can be distinguished by EXAFS owing to the enhancement of multiple-scattering intensity when the bridge approaches linearity.<sup>36</sup>

(b) **Fe<sup>III</sup>–NC–Cu<sup>I</sup> Bridged Assembly.** Bridged assembly **10** is composed of a five-coordinate ferriheme group, a nonlinear bridge, and an irregular Cu<sup>I</sup>(MeNpy<sub>2</sub>) fragment (Figure 4). The mean Fe–N bond length (2.051(2) Å) and displacement of the iron atom from the porphyrin mean plane (0.38 Å) are consistent with high-spin Fe(III)<sup>37</sup> (vide infra). The Fe–N–C–Cu bridge atom sequence is preferable from the X-ray refinement. The bridge angle Fe–N–C = 162.1(3)° is comparable with the value of 165.3(4)° in **11**;<sup>22</sup> however, the bridge departs from linearity to a smaller extent in **10** (176.7(3)°) than in **11** (168.9(4)°) on the basis of the indicated Cu–C–N angles.

(30) Lavalley, D. K. *The Chemistry and Biochemistry of N-substituted Porphyrins*; VCH Publishers: New York, 1987.

(31) Anderson, O. P.; Kopelove, A. B.; Lavalley, D. K. *Inorg. Chem.* **1980**, *19*, 2101.

(32) (a) Balch, A. L.; La Mar, G. N.; Latos-Grażyński, L.; Renner, M. W. *Inorg. Chem.* **1985**, *24*, 2432. (b) Balch, A. L.; Cornman, C. R.; Latos-Grażyński, L.; Olmstead, M. M. *J. Am. Chem. Soc.* **1990**, *112*, 7552.

(33) (a) Wyslouch, A.; Latos-Grażyński, L.; Grzeszczak, M.; Drabent, K.; Bartczak, T. *J. Chem. Soc., Chem. Commun.* **1988**, 1377. (b) Bartczak, T. J.; Latos-Grażyński, L.; Wyslouch, A. *Inorg. Chim. Acta* **1990**, *171*, 205.

(34) Karlin, K. D.; Hayes, J. C.; Juen, S.; Hutchinson, J. P.; Zubieta, J. *Inorg. Chem.* **1982**, *21*, 4108.

(35) Scheidt, W. R.; Hatano, K. *Acta Crystallogr.* **1991**, *C47*, 2201.

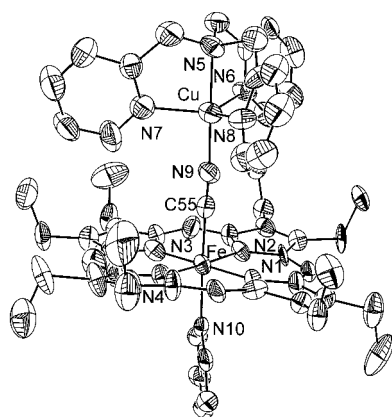
(36) Zhang, H. H.; Filippini, A.; Di Cicco, A.; Scott, M. J.; Holm, R. H.; Hedman, B.; Hodgson, K. O. *J. Am. Chem. Soc.* **1997**, *119*, 2470.

(37) Scheidt, W. R.; Reed, C. A. *Chem. Rev.* **1981**, *81*, 543.

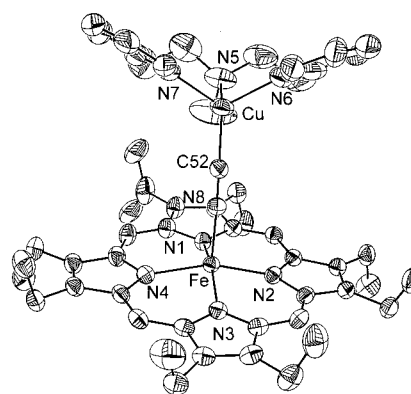
**Table 3.** Selected Interatomic Distances (Å) and Angles (deg) of Cu(II,I) Complexes **1**, **2**, **5**, and **6**

	[Cu(Npy <sub>3</sub> )(MeCN)] <sup>2+</sup> ( <b>1</b> )		[Cu(Npy <sub>3</sub> )(MeCN)] <sup>+</sup> ( <b>2</b> )	
Cu–N(1)	2.104(2)		2.43(1)	
Cu–N(2)	2.074(2)		2.08(1)	
Cu–N(3)	2.057(2)		2.12(1)	
Cu–N(4)	2.033(2)		2.07(1)	
Cu–N(5)	1.967(2)		1.99(1)	
N(1)–Cu–N(2–4)	81.58(9)–82.46(9)		74.4(5)–75.1(5)	
N(1)–Cu–N(5)	178.3(1)		179.5(5)	
N(1)–Cu–N(2)	81.58(9)		75.1(4)	
N(1)–Cu–N(3)	82.39(9)		75.1(5)	
N(1)–Cu–N(4)	82.46(9)		74.4(5)	
N(5)–Cu–N(2–4)	96.9(1)–98.9(1)		104.5(5)–106.0(6)	
N(2)–Cu–N(3)	113.5(1)		117.6(4)	
N(2)–Cu–N(4)	120.5(1)		107.9(4)	
N(3)–Cu–N(4)	120.4(1)		114.8(4)	
	[Cu <sub>2</sub> (MeNpy <sub>2</sub> ) <sub>2</sub> CN] <sup>+</sup> ( <b>5</b> ) <sup>a</sup>			
Cu(1)–N(1)	2.162(7)	Cu(2)–N(4)	2.145(8)	
Cu(1)–N(2)	2.055(8)	Cu(2)–N(5)	2.059(7)	
Cu(1)–N(3)	2.006(7)	Cu(2)–N(6)	2.070(8)	
Cu(1)–N/C	1.93(1)	Cu(1)–N/C–C/N	174.2(9)	
C/N–N/C	1.15(1)	Cu(2)–C/N–N/C	174.0(9)	
Cu(2)–C/N	1.87(1)			
	[Cu <sub>2</sub> (Npy <sub>3</sub> ) <sub>2</sub> CN] <sup>+</sup> ( <b>6</b> ) <sup>b</sup>			
Cu(1)–N(1)	2.314(5)	Cu(1)–N/C	1.865(4)	
Cu(1)–N(2)	2.054(5)	Cu(1)–N/C–C/N	178.4(7)	
Cu(1)–N(3)	2.064(5)	C–N/N–C	1.180(8)	

<sup>a</sup> C = C(31), N = N(7). <sup>b</sup> C = C(19), N = N(5).

[(py)(OEP)Fe–CN–Cu(Npy<sub>3</sub>)]<sup>2+</sup>**Figure 3.** Structure of Fe<sup>III</sup>/Cu<sup>II</sup> bridged assembly **8**, showing the atom-labeling scheme and 50% probability ellipsoids.

(c) **Fe<sup>II</sup>–NC–Cu<sup>I</sup> Bridged Assembly.** Two crystal structures were solved containing bridged assembly **12** (Table 1), which were essentially identical. Results for the propionitrile adduct are reported.<sup>29</sup> The complex contains a five-coordinate N-alkylated iron(II) porphyrin, a nonlinear bridge, and an irregular three-coordinate Cu<sup>I</sup>(Npy<sub>3</sub>) fragment with one unbound pyridyl group (Figure 5).<sup>38</sup> Here also, the X-ray refinement favors the Fe–N–C–Cu bridge. The porphyrin is configured such that the cyanomethyl group is distal to the bridging cyanide ligand. Atom N(1), to which the methyl group is bound, is displaced 0.086 Å below the porphyrinic N(2–4) plane, i.e., away from the axial ligand. Analogous stereochemistry is found with [Fe<sup>II</sup>(N-MeTPP)Cl],<sup>31</sup> [Fe<sup>III</sup>(N-MeTTP)Cl]<sup>+</sup>,<sup>32b</sup> and related five-coordinate complexes.<sup>30</sup> Other structural features of **12** closely follow those of square pyramidal [Fe<sup>II</sup>(N-MeTPP)Cl], which has been shown to contain high-spin Fe(II).<sup>31</sup> The Fe–NMe bond length of 2.360(3) Å and the mean Fe–N(2–4) bond

[(OEP)Fe–NC–Cu(MeNpy<sub>2</sub>)]<sup>1+</sup>**Figure 4.** Structure of Fe<sup>III</sup>/Cu<sup>I</sup> bridged assembly **10**, showing the atom-labeling scheme and 50% probability ellipsoids.

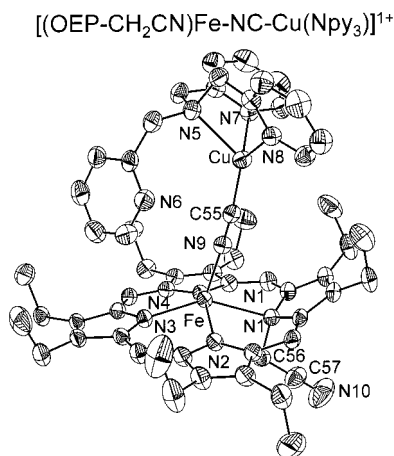
length of 2.09(3) Å are in good agreement with the corresponding values (2.329(2), 2.11(2) Å) for the latter complex. Both [Fe<sup>II</sup>(N-MeTPP)Cl] and **12** have identical displacements of 0.62 Å from the porphyrinic N<sub>3</sub> plane. Given the well-known effects of metal spin state and radius on heme stereochemistry,<sup>37</sup> it is clear that **12** contains high-spin Fe(II).

**Linkage Isomerism.** Historically, cyano (M–CN) vs isocyano (M–NC) bonding has been differentiated by neutron<sup>39</sup> and (in favorable cases) X-ray<sup>40</sup> diffraction results usually

- (39) (a) Curry, N. A.; Runciman, W. A. *Acta Crystallogr.* **1958**, *12*, 674. (b) Sequeira, A.; Chidambaram, R. *Acta Crystallogr.* **1966**, *20*, 910. (c) Seccombe, R. C.; Kennard, C. H. *J. Organomet. Chem.* **1969**, *18*, 243.
- (40) (a) Ozbirn, W.; Jacobson, R. A. *Inorg. Chim. Acta* **1970**, *4*, 377. In this study of [Co(NH<sub>3</sub>)<sub>5</sub>(CN)](ClO<sub>4</sub>)<sub>2</sub>·0.5H<sub>2</sub>O, conventional and weighted *R*-factors for the Co–C–N (*R* = 0.082, *R<sub>w</sub>* = 0.119) and Co–N–C (*R* = 0.085, *R<sub>w</sub>* = 0.123) models were considered decisive for the cyano isomer. For more recent examples (but without the results of alternative refinements), cf.: (b) Figgis, B. N.; Reynolds, P. A. *J. Chem. Soc., Dalton Trans.* **1987**, 1747. (c) Griffith, W. P.; Mockford, M. J.; Skapski, A. C. *Inorg. Chim. Acta* **1990**, *175*, 161.

(38) An uncoordinated pyridyl group has been observed in [Cu(Npy<sub>3</sub>)(PPh<sub>3</sub>)]<sup>+</sup>.<sup>25</sup>





**Figure 5.** Structure of Fe<sup>II</sup>/Cu<sup>I</sup> bridged assembly **12**, showing the atom-labeling scheme and 50% probability ellipsoids.

refined in alternative bonding models. The classic work of Jones,<sup>41</sup> involving full vibrational analyses of isotopomers of [M(CN)<sub>2</sub>]<sup>-</sup> (M = Ag, Au) and Hg(CN)<sub>2</sub> and a judgment of the reasonableness of certain force constants derived therefrom, led to the conclusion that these were cyano species. Our assignments of bridge atom sequences in cyanide-bridged assemblies **8–12** rests mainly on the results of crystal structure refinements.

Prior to consideration of the relation of bridge structure and values of  $\nu_{\text{CN}}$ , we note certain other matters bearing on linkage isomerism. The Fe<sup>III</sup>-CN group in all hemes<sup>21,35,42</sup> and in **8**, **9**, and seven additional bridged assemblies<sup>21,22</sup> closely approaches or achieves linearity, the range of angles being 175–180°. If in the generalized bridge Fe<sup>III</sup>-X-Y-M, the angle involving the iron atom is very close to or within this range and the angle involving atom M is not, then the bridge is most likely Fe-C-N-M. If the converse is true, the bridge is probably Fe-N-C-M, a situation that for M = Cu(I) is reinforced by the usual close approach to or achievement of linearity by the Cu<sup>I</sup>-CN unit. Application of this test to **10** and **11** affords the crystallographically preferred structures, although **11** presents a close call (Figure 1). Here, the Fe-N-C-Cu arrangement would rest more heavily on the Fe-N-C angle (165.4(3)°<sup>22</sup>). In addition to structural features, there are prior reactivity observations that suggest a reasonable probability for the preferred Fe<sup>III</sup>-N-C-Cu<sup>I</sup> atom sequence in **10** and **11**. In the aquation reactions of [Cr(OH<sub>2</sub>)<sub>5</sub>(CN)]<sup>2+</sup> and [Cr(OH<sub>2</sub>)<sub>5</sub>(NC)]<sup>2+</sup> in the presence of Hg<sup>2+</sup>, the rate of reaction of the isocyano isomer is much faster because the formation of the [Cr-CN-Hg]<sup>4+</sup> intermediate and its linkage isomerization to [Cr-NC-Hg]<sup>4+</sup> are obviated.<sup>43</sup> Reaction of the cyano complex [Cr(en)<sub>2</sub>(CN)<sub>2</sub>]<sup>+</sup> with 1 equiv of Ag<sup>+</sup> induces linkage isomerization and the formation of the Cr-N-C-Ag bridge, as inferred from band shifts in absorption spectra.<sup>44</sup> These results are demonstrations of the affinity of soft acids for the carbon end of cyanide in systems where that affinity is expressed by linkage isomerization. Copper(I), also being a soft acid, has a similar affinity, sufficiently pronounced so as to induce isomerization of cyanide bound to Fe(III) in **7** in the

course of forming **10** and **11**. While a similar event presumably occurs in the preparation of **12**, the reaction sequence is complicated by N-cyanoalkylation and reduction of Fe(III). The reductant has not been identified.

**Solution Properties.** The absorption spectra of heme complex **7**, low-spin bridged assemblies **8** and **9**, and high-spin assemblies **10** and **11**, all in dichloromethane, are presented in Figures 6 and 7. Soret maxima are summarized in Table 5. The Soret bands of **8** (397 nm) and **9** (404 nm) are blue-shifted relative to that of **7** (407 nm), while those of **10** (379 nm) and **11** (380 nm) occur at shorter wavelengths. It is immediately apparent that the two types of assemblies can be distinguished by their Soret features and also by bands at lower energies (500–700 nm). Within each type, the different copper fragments have little effect on the electronic features of the heme centers. When examined by <sup>1</sup>H NMR, the OEP methylene protons of **8** (10.10, 10.21 ppm) and **9** (8.10, 11.66 ppm) and of **10** (44.8, 54.2 ppm) and **11** (45.3, 57.0 ppm) show the expected diastereotopic splittings with chemical shifts consistent with low-spin and high-spin heme configurations, respectively.<sup>45</sup> The spin state of **10** has been further confirmed by EPR; in dichloromethane at 4.2 K, this complex affords a nearly axial, typical high-spin spectrum with  $g_{\perp} = 5.81$  and  $g_{\parallel} = 2.00$  (not shown). By these techniques, we have found no evidence for dissociation of bridged assemblies in acetone or dichloromethane solutions.

**Cyanide Stretching Frequencies.** Values of  $\nu_{\text{CN}}$  relative to the free cyanide ion are set by three main factors: (i) electron donation from the filled 5 $\sigma^*$  MO of cyanide to the metal, strengthening the C-N bond and raising  $\nu_{\text{CN}}$ , effects that increase with increasing metal oxidation state; (ii) electron donation from the metal to the vacant 2 $\pi^*$  MO's of cyanide, with consequences opposite to those in (i); (iii) kinematic coupling, which results in constraints on the motion of cyanide and is expected to increase  $\nu_{\text{CN}}$ .<sup>46</sup> When cyanide is bridged, the additional metal should enhance both (i) and (ii) because of increased charge removal; (iii) applies only to bridged species. Further consideration of these factors and relevant examples are available.<sup>47,48</sup> Summarized in Table 5 are  $\nu_{\text{CN}}$  data for free cyanide, copper-cyanide complexes, bridged assemblies, and oxidases in different oxidation states. Values for uncoordinated cyanide vary with the compound and the medium; 2080 cm<sup>-1</sup> is a reasonable comparative value. For bridged complexes **5–12**, the small differences between solid and solution values indicate that the bridged structures are maintained in solution.

(a) **Cu<sup>II</sup>-I-CN and Cu<sup>I</sup>-I-CN-Cu<sup>I</sup>.** In mononuclear Cu(II) complex **3**, the frequency is increased over that of free cyanide, as expected. The only structurally characterized molecular Cu<sup>I</sup>-CN species is [Cu(HB(Me<sub>2</sub>pz)<sub>3</sub>(CN))]<sup>-</sup>,<sup>51</sup> in which the metal has trigonally distorted tetrahedral coordination. The frequency shift

- (41) Jones, L. H. *J. Chem. Phys.* **1957**, *26*, 1578; **1957**, *27*, 468, 665.  
 (42) Additional examples: (a) [Fe(TPP)(CN)(py)]<sup>-</sup>-Scheidt, W. R.; Lee, Y. J.; Luangdilok, W.; Haller, K. J.; Anzai, K.; Hatano, K. *Inorg. Chem.* **1983**, *22*, 1516. (b) [Fe(TPP)(CN)<sub>2</sub>]<sup>-</sup>-Scheidt, W. R.; Haller, K. J.; Hatano, K. *J. Am. Chem. Soc.* **1980**, *102*, 3017.  
 (43) (a) Birk, J. P.; Espenson, J. H. *Inorg. Chem.* **1968**, *7*, 991. (b) Espenson, J. H.; Bushey, W. R. *Inorg. Chem.* **1971**, *10*, 2457.  
 (44) Heatherington, A.; Oon, S. M.; Vargas, R.; Kane-Maguire, N. A. P. *Inorg. Chim. Acta* **1980**, *44*, L279.

- (45) Comparative methylene chemical shifts in acetone (298 K): **7** (low-spin), 5.77, 6.46 ppm; [Fe(OEP)Cl] (high-spin), 39.5, 41.8 ppm. The spectra of **8–10** were recorded in acetone-*d*<sub>6</sub>, and the spectrum of **11** was recorded in dichloromethane-*d*<sub>2</sub>.  
 (46) Dows, D. A.; Haim, A.; Wilmarth, W. K. *J. Inorg. Nucl. Chem.* **1961**, *21*, 33.  
 (47) (a) Bignozzi, C. A.; Argazzi, R.; Schoonover, J. R.; Gordon, K. C.; Dyer, R. B.; Scandola, F. *Inorg. Chem.* **1992**, *31*, 5260. (b) Dunbar, K. R.; Heintz, R. A. *Prog. Inorg. Chem.* **1997**, *45*, 283. (c) Laidlaw, W. M.; Denning, R. G. *J. Chem. Soc., Dalton Trans.* **1994**, 1987.  
 (48) Nakamoto, K. *Infrared and Raman Spectra of Inorganic and Coordination Compounds*, 4th ed.; Wiley-Interscience: New York, 1986; pp. 272–280.  
 (49) Penneman, R. A.; Jones, L. H. *J. Chem. Phys.* **1956**, *24*, 293.  
 (50) (a) Lipschutz, B. H.; James, B. *J. Org. Chem.* **1994**, *59*, 7585. (b) Lipschutz, B. H.; Sharma, S.; Ellsworth, E. L. *J. Am. Chem. Soc.* **1990**, *112*, 4032.

**Table 4.** Selected Interatomic Distances (Å) and Angles (deg) of Bridged Assemblies: Fe<sup>III</sup>–CN–Cu<sup>II</sup> (**8**), Fe<sup>III</sup>–NC–Cu<sup>I</sup> (**10**), Fe<sup>II</sup>–CN–Cu<sup>I</sup> (**12**)

		[(py)(OEP)Fe–CN–Cu(Npy <sub>3</sub> )] <sup>2+</sup> ( <b>8</b> )		
bridge	Fe–C(55)	1.91(1)	Fe–C(55)–N(9)	173.7(7)
	C(55)–N(9)	1.157(9)	Cu–N(9)–C(55)	174.5(8)
heme	Cu–N(9)	1.906(8)	Fe···Cu	4.96
	Fe–N(1)	1.979(7)	Fe–N(10)	2.043(6)
	Fe–N(2)	2.014(8)	Fe···Cr <sup>d</sup>	0.017
	Fe–N(3)	1.979(9)	C(55)–Fe–N(1–4)	85.3(5)–92.3(3)
	Fe–N(4)	1.992(8)		
Cu fragment	mean of 4	1.99(2)		
	Cu–N(5)	2.007(7)	N(5)–Cu–N(6–8)	81.1(3)–84.7(5)
	Cu–N(6)	2.01(1)	N(9)–Cu–N(6–8)	95.0(5)–101.1(3)
	Cu–N(7)	2.06(1)	N(5)–Cu–N(9)	177.1(4)
	Cu–N(8)	2.084(8)	N(6)–Cu–N(7)	128.0(4)
	Cu–N(9)	1.906(8)	N(6)–Cu–N(8)	119.5(4)
		N(7)–Cu–N(8)	107.6(4)	
		[(OEP)Fe–NC–Cu(MeNpy <sub>2</sub> )] <sup>+</sup> ( <b>10</b> )		
bridge	Fe–N(8)	1.997(3)	Fe–N(8)–C(52)	162.1(3)
	C(52)–N(8)	1.151(5)	Cu–C(52)–N(8)	176.7(3)
heme	Cu–C(52)	1.870(4)	Fe···Cu	4.95
	Fe–N(1)	2.053(3)	Fe···Cr <sup>d</sup>	0.38
	Fe–N(2)	2.048(3)	N(8)–Fe–N(1–4)	97.0(1)–103.9(1)
	Fe–N(3)	2.050(3)		
	Fe–N(4)	2.053(3)		
Cu fragment	mean of 4	2.051(2)		
	Cu–N(5)	2.163(5)	N(5)–Cu–N(6)	95.4(2)
	Cu–N(6)	2.048(4)	N(5)–Cu–N(7)	97.5(2)
	Cu–N(7)	2.052(4)	N(6)–Cu–N(7)	108.3(2)
		C(52)–Cu–N(5–7)	114.7(2)–118.6(2)	
		[(OEP–CH <sub>2</sub> CN)Fe–NC–Cu(Npy <sub>3</sub> )] <sup>+</sup> ( <b>12</b> ) <sup>b</sup>		
bridge	Fe–N(9)	2.033(3)	Fe–N(9)–C(55)	175.6(2)
	C(55)–N(9)	1.156(4)	Cu–C(55)–N(9)	174.7(3)
heme	Cu–C(55)	1.869(3)	Fe···Cu	5.05
	Fe–N(1)	2.360(3)	Fe···Cr <sup>c</sup>	0.62
	Fe–N(2)	2.101(2)	N(9)–Fe–N(1)	92.4(1)
	Fe–N(3)	2.062(2)	N(9)–Fe–N(2)	101.6(1)
	Fe–N(4)	2.116(2)	N(9)–Fe–N(3)	122.6(1)
	N(1)–Fe–N(2)	82.16(9)	N(9)–Fe–N(4)	109.9(1)
	N(2)–Fe–N(3)	88.02(9)		
	N(3)–Fe–N(4)	87.41(9)		
Cu fragment	N(4)–Fe–N(1)	81.99(9)		
	Cu–N(5)	2.273(3)	N(5)–Cu–N(7)	76.4(1)
	Cu–N(7)	2.116(3)	N(5)–Cu–N(8)	80.4(1)
	Cu–N(8)	2.051(3)	N(7)–Cu–N(8)	105.2(1)
			C(55)–Cu–N(5)	126.8(1)
		C(55)–Cu–N(7)	117.2(1)	
		C(55)–Cu–N(8)	133.1(1)	

<sup>a</sup> Displacement (Å) from porphyrin mean plane toward py (**8**), NC (**10**). <sup>b</sup> Data refer to [**12**](ClO<sub>4</sub>)·EtCN. <sup>c</sup> Displacement (Å) from plane N(2,3,4); N(1) is displaced –0.086 Å from this plane.

is essentially zero because the  $\pi$ -bonding effect (ii) compensates for the  $\sigma$ -bonding effect (i). In the bridged linear Cu(I) complexes **5** and **6** (Figure 2), the effect of two metals raises  $\nu_{\text{CN}}$  by 30–40 cm<sup>-1</sup>, presumably because of the combined dominance of factors (i) and (iii). These results are consistent with those for other bridged Cu(I) species,<sup>52</sup> with the value for solid CuCN ( $\nu_{\text{CN}}$  2172 cm<sup>-1</sup><sup>49</sup>) being an apparent upper limit.

(b) **Fe<sup>III</sup>–CN–Cu<sup>II</sup>**. Data are now available for some 11 linear and nonlinear bridged assemblies containing this unit. Attachment of Cu(II) to the nitrogen end of cyanide in heme

precursor **7** to afford the linear assemblies **8** and **9**<sup>21</sup> raises  $\nu_{\text{CN}}$  by ca. 50–60 cm<sup>-1</sup> through the operation of factors (i) and (iii). From a normal-coordinate analysis of [(1-Melm)(OEP)Fe–CN–Cu(Me<sub>6</sub>tren)]<sup>2+</sup>, these vibrational modes consist entirely of the C–N stretching coordinate.<sup>23</sup> When the Cu–N–C angle is decreased to near 150°, the Cu–N bond length increases and  $\nu_{\text{CN}}$  decreases. At the small-angle limit, stretching frequencies closely approach the 2146–2152 cm<sup>-1</sup> values found for the fully oxidized enzymes (Table 5).

(c) **Fe<sup>III</sup>–NC–Cu<sup>I</sup>**. Two examples of this type of assembly, **10** and **11**,<sup>22</sup> both with bridges that depart from strict linearity, are available. In the solid state,  $\nu_{\text{CN}}$  values differ by 28 cm<sup>-1</sup>, but by only 2 cm<sup>-1</sup> in solution. Perhaps **11** relaxes to a bridge geometry closer to that of **10** when placed in solution. Values of  $\nu_{\text{CN}}$  in solution are used for comparison. The bridge has isomerized upon coordination of Cu(I), whose  $\pi$ -bonding to cyanide via the carbon end results in frequencies that are ca. 40 cm<sup>-1</sup> lower than that of **7** and ca. 90 cm<sup>-1</sup> below those of the linear or slightly bent Fe<sup>III</sup>–CN–Cu<sup>II</sup> assemblies. The

(51) The compound (Et<sub>4</sub>N)[Cu(HB(Me<sub>2</sub>p<sub>z</sub>)<sub>3</sub>)(CN)] was prepared by the reaction of CuCN and Na(HB(Me<sub>2</sub>p<sub>z</sub>)<sub>3</sub>) in THF followed by addition of (Et<sub>4</sub>N)(BF<sub>4</sub>). It crystallizes in trigonal space group *P1* with *a* = 9.961(2) Å, *b* = 10.845(2) Å, *c* = 14.585(3) Å,  $\alpha$  = 86.55(3)°,  $\beta$  = 83.62(3)°,  $\gamma$  = 63.04(3)°, *Z* = 2, and *V* = 1395.6(5) Å<sup>3</sup>. With use of 4921 unique data, the structure was refined to *R*<sub>1</sub>/*wR*<sub>2</sub> = 4.67/12.13%. Selected parameters: Cu–C, 1.872(3) Å; C–N, 1.143(4) Å; mean Cu–N, 2.10(4) Å, N–Cu–C, 119.9(1)–135.5(1)°. (HB(Me<sub>2</sub>p<sub>z</sub>)<sub>3</sub>) = hydrotris(3,5-dimethylpyrazolyl)borate (1–).

(52) Huang, H.; Alvarez, K.; Liu, Q.; Barnhart, T. M.; Snyder, J. P.; Penner-Hahn, J. E. *J. Am. Chem. Soc.* **1996**, *118*, 8808.





**Table 5.** Cyanide Stretching Frequencies and Soret Band Maxima

group/bridge	compd	$\nu_{\text{CN}}, \text{cm}^{-1}$		$\lambda_{\text{max}}, ^b \text{nm}$	ref
		solid <sup>a</sup>	soln <sup>b</sup>		
CN <sup>-</sup>	NaCN	2080	2080 <sup>d</sup>		48,49
	(Et <sub>4</sub> N)CN	2085			<i>e</i>
	(Bu <sub>4</sub> N)CN	2070	2080 <sup>f</sup>		50a
Cu <sup>II</sup> –CN	[ <b>3</b> ](ClO <sub>4</sub> )	2143	2142 <sup>c</sup>		<i>e</i>
Cu <sup>I</sup> –CN	<i>g</i>	2083	2077		<i>e</i>
Cu <sup>I</sup> –CN–Cu <sup>I</sup>	[ <b>5</b> ](ClO <sub>4</sub> )	2103	2103		<i>e</i>
	[ <b>6</b> ](ClO <sub>4</sub> )	2107	2109		<i>e</i>
Fe <sup>III</sup> –CN	[ <b>7</b> ]	2129	2121	407	21, <i>e</i>
Fe <sup>III</sup> –CN–Cu <sup>II</sup>	[ <b>8</b> ](ClO <sub>4</sub> ) <sub>2</sub>	2175	2172	397	<i>e</i>
	[ <b>9</b> ](ClO <sub>4</sub> ) <sub>2</sub>	2177	2179 <sup>h</sup>	404	21
Fe <sup>III</sup> –NC–Cu <sup>I</sup>	[ <b>10</b> ](ClO <sub>4</sub> ) <sub>2</sub>	2072	2080	379	<i>e</i>
	[ <b>11</b> ](ClO <sub>4</sub> ) <sub>2</sub>	2100	2082	380	<i>e</i>
Fe <sup>II</sup> –NC–Cu <sup>I</sup>	[ <b>12</b> ](ClO <sub>4</sub> ) <sub>2</sub>	2099	2107	<i>i</i>	<i>e</i>
	ox. CcO (bovine heart) <sup>j</sup>		2150–2152		12, 14, 16, 17
	ox. cytochrome <i>bo</i> ( <i>E. coli</i> )		2146		18
	ox. cytochrome <i>a</i> <sub>1</sub> , <i>o</i> ( <i>A. acetii</i> )		2147, 2146		20
	partially red. CcO (bovine heart)		2132, 2093		12, 14, 16
	fully red. CcO (bovine heart)		2058, 2045, 2037		14, 16
	fully red. cytochrome <i>bo</i> ( <i>E. coli</i> )		2035		19
	fully red. cytochrome <i>a</i> <sub>1</sub> ( <i>A. acetii</i> )		2051		20

<sup>a</sup> Nujol mull or KBr. <sup>b</sup> Dichloromethane. <sup>c</sup> Acetonitrile. <sup>d</sup> Aqueous. <sup>e</sup> This work. <sup>f</sup> THF; also reported at 2057 cm<sup>-1</sup>.<sup>50b</sup> <sup>g</sup> (Et<sub>4</sub>N)[Cu(HB(Me<sub>2</sub>p<sub>z</sub>)<sub>3</sub>CN)].<sup>51</sup> <sup>h</sup> Nitromethane. <sup>i</sup> Not isolated in pure form. <sup>j</sup> All enzymes in aqueous buffer.

resonance Raman, and Mössbauer spectroscopies.<sup>54,55</sup> All enzymes show a sharp, intense band in the narrow interval 2146–2152 cm<sup>-1</sup>. For bovine heart CcO, it has been established that one cyanide binds per binuclear site.<sup>16</sup> Structural interpretations have varied from terminal Fe<sup>III</sup>–CN binding with a possible hydrogen-bonding interaction at the nitrogen end<sup>16</sup> to the bridged structure Fe<sup>III</sup>–CN–Cu<sup>II</sup>, but without specification of bridge geometry. The possible validity of a bridge structure was reinforced by the MCD determination of an *S* = 1 ground state for bovine heart CcO,<sup>56</sup> a result obtained well before IR data were available. Presumably, a bridging ligand propagates the ferromagnetic coupling required to effect this state. Our earlier work showed that the enzyme  $\nu_{\text{CN}}$  values can be achieved with a nonlinear Fe<sup>III</sup>–CN–Cu<sup>II</sup> bridge having a linear Fe–C–N portion with normal bond distances, a Cu–N bond distance of 2.1–2.2 Å, and a Cu–N–C bond angle of 150–160°. Note that a nearly linear structure such as those of **8** and **9** generates  $\nu_{\text{CN}}$  values that are about 25–30 cm<sup>-1</sup> too high (Table 5). The bridge structure based on IR results now enjoys a level of acceptance.<sup>14,17,19</sup> Finally, **9** has an integer spin ground state (*S* ≥ 1)<sup>57</sup> as do the oxidases,<sup>55,56</sup> a seemingly fortuitous finding if a bridge structure does not obtain in the enzymes.

**(b) Partially Reduced.** Addition of less than 4 equiv of electrons to the fully oxidized form of bovine heart CcO affords the partially reduced form, in which electrons are equilibrated over the four redox centers. In the binuclear site, the oxidation

state is ls-Fe<sup>III</sup>/Cu<sup>I</sup> with perhaps a minor amount of ls-Fe<sup>II</sup>/Cu<sup>I</sup>.<sup>56a,c,58</sup> With no cyanide present, the iron centers are high-spin.<sup>54–56</sup> Two bands appear at 2132 and 2093 cm<sup>-1</sup>, and that near 2150 cm<sup>-1</sup> is abolished. The 2132 cm<sup>-1</sup> feature has been assigned to Fe<sup>III</sup>–CN, unbridged to the copper site.<sup>12,14,16</sup> This frequency is close to that of **7** and protein-bound oxidized heme cyanides.<sup>59</sup> The 2093 cm<sup>-1</sup> band has been associated with terminal Cu<sup>I</sup>–CN coordination.<sup>14,16</sup> This value is near, but higher than,  $\nu_{\text{CN}}$  for our Cu<sup>I</sup>–CN reference complex. It is also close to the range for the hs-Fe<sup>III</sup>–NC–Cu<sup>I</sup> bridged assemblies **10** and **11** and the hs-Fe<sup>II</sup>–NC–Cu<sup>I</sup> assembly **12**. Intervention of these bridge types would require an enzyme EPR spectrum similar to those of **10** and **11** for the former and evidence for high-spin Fe(II) for the latter. We are unaware of spectral features possibly corresponding to these bridges in partially reduced enzymes treated with cyanide.

**(c) Fully Reduced.** This state is reached by a four-electron reduction of bovine heart CcO and has also been produced for two ubiquinol oxidases. The binuclear site is ls-Fe<sup>II</sup>/Cu<sup>I</sup>. Three cyanide stretching frequencies have been detected for CcO in addition to a residual 2093 cm<sup>-1</sup> band. The 2058 and 2045 cm<sup>-1</sup> bands are suppressed by CO and thus assigned to heme-bound ligands. Heterogeneity at the binuclear site has been proposed, such that the 2058 cm<sup>-1</sup> absorption arises from the weak interaction Fe<sup>II</sup>–CN···Cu<sup>I</sup> and the 2045 cm<sup>-1</sup> feature from a weaker interaction, or no interaction, with Cu(I).<sup>14</sup> Perturbation of  $\nu_{\text{CN}}$  by hydrogen bonding has been discounted owing to the absence of an appreciable isotope effect. Assignment of the 2037 cm<sup>-1</sup> band is less certain and may arise from the Cu<sup>I</sup>–(CN)<sub>2</sub> group of doubly substituted Cu<sub>B</sub>.<sup>14</sup> Bands for the two bacterial oxidases have been assigned to the Fe<sup>II</sup>–CN group.<sup>19,20</sup>

**Summary.** The following are the principal results and conclusions of this investigation.

(1) Heme-based molecular assemblies containing the bridges ls-Fe<sup>III</sup>–CN–Cu<sup>II</sup> (**8**, **9**), hs-Fe<sup>III</sup>–NC–Cu<sup>I</sup> (**10**, **11**), and hs-Fe<sup>II</sup>–NC–Cu<sup>I</sup> (**12**) have been prepared by the reaction of [Fe(OEP)(py)(CN)] with appropriate Cu(II,I) precursors in this and

- (54) (a) Babcock, G. T.; Vickery, L. E.; Palmer, G. *J. Biol. Chem.* **1976**, *251*, 7907. (b) Babcock, G. T.; Callahan, P. M.; Ondrias, M. R.; Salmeen, I. *Biochemistry* **1981**, *20*, 959. (c) Sherman, D.; Kotake, S.; Ishibe, N.; Copeland, R. A. *Proc. Natl. Acad. Sci. U.S.A.* **1991**, *88*, 4265.
- (55) (a) Kent, T. A.; Münck, E.; Dunham, W. R.; Filter, W. F.; Findling, K. L.; Yoshida, T.; Fee, J. A. *J. Biol. Chem.* **1982**, *257*, 12849. (b) Kent, T. A.; Young, L. J.; Palmer, G.; Fee, J. A.; Münck, E. *J. Biol. Chem.* **1983**, *258*, 8543.
- (56) (a) Thomson, A. J.; Johnson, M. K.; Greenwood, C.; Gooding, P. E. *Biochem. J.* **1981**, *193*, 687. (b) Thomson, A. J.; Eglinton, D. G.; Hill, B. C.; Greenwood, C. *Biochem. J.* **1982**, *207*, 167. (c) Hill, B. C.; Brittain, T.; Eglinton, D. G.; Gadsby, P. M. A.; Greenwood, C.; Nicholls, P.; Peterson, J.; Thomson, A. J.; Woon, T. C. *Biochem. J.* **1983**, *215*, 57.
- (57) Kauffmann, K. E.; Day, E. P.; Münck, E.; Lee, S. C.; Holm, R. H. Unpublished results.

(58) Nicholls, P.; Chanady, G. A. *Biochem. J.* **1982**, *203*, 541.

(59) Yoshikawa, S.; O'Keefe, D. H.; Caughey, W. S. *J. Biol. Chem.* **1985**, *260*, 3518.

prior work.<sup>21,22</sup> The last bridge type occurs in a species in which one porphyrin nitrogen atom is cyanomethylated by coordinated acetonitrile in the precursor Cu(I) complex.

(2) In the complexes of (1), bridges are nearly linear (**8**, **9**, **12**) or somewhat bent (**10**, **11**) but with no bridge angle  $< 162^\circ$ . Structures of the Fe(III,II) sites are typical of high- or low-spin states. The indicated bridge atom sequences are preferred from X-ray structure refinements; the Cu<sup>I</sup>–CN linkage is consistent with the soft acid character of Cu(I). From this and earlier studies,<sup>21,22</sup> there are nine structurally established heme-based assemblies with ls-Fe<sup>III</sup>–CN–Cu<sup>II</sup>, two with hs-Fe<sup>III</sup>–NC–Cu<sup>I</sup>, and one with hs-Fe<sup>II</sup>–NC–Cu<sup>I</sup>. These constitute a (partial) basis set for the investigation of cyanide stretching frequencies as dependent on bridge structure.

(3) From the basis set of bridged assemblies, ranges of  $\nu_{\text{CN}}$  values are defined from solid and solution IR data. Values for ls-Fe<sup>III</sup>–CN–Cu<sup>II</sup> are well above that for uncoordinated cyanide because of  $\sigma$ -bonding and kinematic effects. Those for hs-Fe<sup>III</sup>–NC–Cu<sup>I</sup> and hs-Fe<sup>II</sup>–NC–Cu<sup>I</sup> are below that of free cyanide, primarily because of Cu<sup>I</sup>–CN  $\pi$ -bonding. These  $\nu_{\text{CN}}$  ranges may not, of course, encompass all subsequent examples of heme-based assemblies with the same bridge types.

(4) Values of  $\nu_{\text{CN}}$  in fully oxidized cyanide-inhibited heme–copper oxidases fall into a narrow ( $6 \text{ cm}^{-1}$ ) range and are entirely consistent with ls-Fe<sup>III</sup>–CN–Cu<sup>II</sup> having a linear Fe–C–N and a nonlinear (ca.  $160^\circ$ ) Cu–N–C fragment at the heme- $a_3$ /Cu<sub>B</sub> site. Data for cyanide-inhibited partially and fully reduced enzymes fall below the ranges for hs-Fe<sup>III</sup>–NC–Cu<sup>I</sup>,

and hs-Fe<sup>II</sup>–NC–Cu<sup>I</sup> and have been assigned by others to Fe–CN vibrations. There is no clear evidence that these bridges exist in the reduced enzymes where heme  $a_3$  appears to be low-spin in the presence of cyanide. However,  $\nu_{\text{CN}}$  data are lacking for the bridges ls-Fe<sup>III,II</sup>–CN/NC–Cu<sup>I</sup>, which thus far we have been unable to prepare.

To define the structural nature of the binuclear site in cyanide-inhibited enzymes, a desirable next step is a correlated vibrational and X-ray absorption study of an enzyme lacking Cu<sub>A</sub>. From examination of structurally defined bridged assemblies, iron and copper EXAFS criteria have been developed for linear and nonlinear bridges.<sup>36</sup> However, EXAFS cannot distinguish bridge linkage isomers, the possible existence of which has gone largely unnoticed in analyses of enzyme vibrational spectral results.

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**Supporting Information Available:** Tables of crystal and intensity collection data, positional and thermal parameters, and interatomic distances and angles for the compounds in Tables 1 and 2 (86 pages). Ordering information is given on any current masthead page.

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