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SYNTHETIC MODELS FOR BINUCLEAR COPPER PROTEINS

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1. INTRODUCTION

The field of bioinorganic chemistry is still a rapidly growing area for research, which, despite its title, encompasses many disciplines of chemistry and biology; synthetic organic chemists have an opportunity to make substantial contributions to the field.

The purpose of this review is to summarize work of the past decade aimed at elucidating the active site structure and reactivity of the binuclear copper proteins, hemocyanin and tyrosinase. A recent article has reviewed the inorganic chemistry of copper proteins and dioxygen binding by copper complexes,[1] so the emphasis of this review will be on the aspects of synthetic organic chemistry that have been utilized in those studies.

Before addressing the subject of copper proteins and their associated models, it seems worthwhile to summarize the strategies involved in using synthetic compounds to mimic biological molecules.

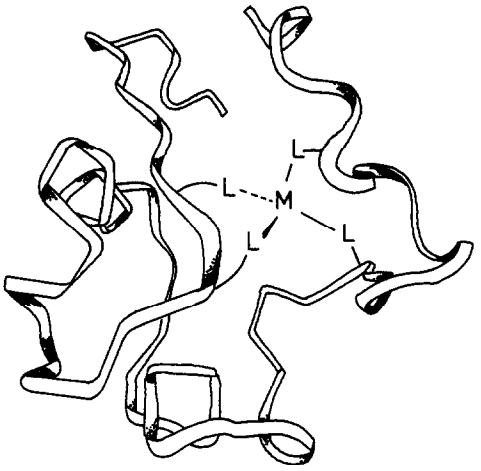
There are many facets of bioinorganic chemistry, and several of these are summarized in Table I. Certainly, the most work in this field has concerned the isolation and characterization of metalloproteins (Table I.1). As illustrated in Figure 1, metalloproteins can be considered, in the simplest way, as large coordination complexes. Thus, characterization of metalloproteins by spectroscopic or physico-chemical methods is little different from the analogous studies carried out by inorganic chemists on low-MW species, with the following exception: X-ray crystallography of metalloproteins will never provide the precision usually associated with, and expected for, small molecule structures. This limitation of studying the biological system itself has led naturally to studying synthetic *analogs* or *models* for metalloproteins (Table I.2). As noted by Reed,[2] the term "synthetic analog" is used for those complexes which provide a very close structural facsimile of the metal coordination in a metalloprotein with regard to ligand type, geometry, and physical properties while a "synthetic model" may mimic certain aspects only.

Table I. Aspects of Bioinorganic Chemistry

1. Metalloproteins as coordination complexes
   a) spectroscopic and physical properties
   b) catalysis
   c) electron transfer mechanisms
2. Synthetic models for metalloproteins[a]
   a) define intrinsic properties of the active site
   b) probe environmental effects
   c) prepare low-MW catalysts
3. Interaction of metal-ion complexes with biomolecules
   a) non-covalent interactions[b]
   b) covalent attachment of metal complexes[c]
4. Mutagenesis of metalloproteins[d]
   a) probe environmental effects
   b) generate unnatural coordination environments
   c) define ranges for natural function
   d) change function

[a]reference 2 [b]reference 3 [c]reference 4 [d]references 7 and 8

Figure 1. Schematic representation of a metalloprotein. L symbolizes the amino acid side-chain donors; no specific geometry or coordination number is intended.



The synthetic analog approach, reviewed several years ago by Ibers and Holm,[3] is based on the premise that the chemistry of the metal-binding site is dependent, for the most part, on the immediate coordination environment of the metal ion. For most metalloproteins, the immediate coordination environment consists of donors from the side-chains of amino acids, summarized in Table II. Sometimes, a prosthetic group (e.g., a porphyrin ring) completes the coordination sphere of the metal ion.

There are two principal strategies used for preparing biomimics: self-assembly and total synthesis. In the self-assembly approach, the metal ions and ligand-donor mimics are mixed together, and the complex is isolated. Compounds made by this method can be used to assess whether the properties of a protein active site are *intrinsic* to the metal ion environment, since the ligands impose no constraints on the coordination geometry. The other strategy employs the technique of total synthesis to prepare an organic molecule (ligand) capable of binding the metal ion in a prescribed way. Subsequent addition of the metal ion gives the desired complex. The advantage of the latter approach is that steric, hydrophobic, or hydrophilic constraints can be incorporated into the complex; conceptually, the polypeptide loops and linkers illustrated in Figure 1 have been replaced by simpler organic fragments, holding the donors in the desired manner.

The synthesis of biomimics normally follows a procedure like that outlined in Table III.[6] After something is known about the active-site structure of the metalloprotein itself, including at least minimal spectroscopic characterization, there must be a design stage in which one considers what ligating groups to use and what, if any, geometric constraints need to be incorporated in the chelator. One then carries out the synthesis, isolation, and characterization of the compound(s), and compares the properties of the analog with those for

Table II. Common amino acids with the potential for ligating metal ions in metalloproteins.

Coordinating atom	*Amino acid*	*Side chain, R* $[RCH(NH_3^+)CO_2^-]$
O	Serine (Ser)*	$-CH_2OH$
	Threonine (Thr)*	$-CHOH$ $\quad\quad\vert$ $\quad\quad CH_3$
	Tyrosine (Tyr)*	$-CH_2-C_6H_4-OH$
	Aspartic acid (Asp)*	$-CH_2COOH$
	Glutamic acid (Glu)*	$-CH_2CH_2COOH$
N	Lysine (Lys)	$-(CH_2)_4-NH_2$
	Arginine (Arg)	$-(CH_2)_3-NHC(=NH)-NH_2$
	Tryptophan (Trp)*	$-CH_2-C_8H_6N$ (indole)
	Histidine (His)	$-CH_2-C_3H_3N_2$ (imidazole)
O, N	Asparagine (Asn)	$-CH_2-C(=O)-NH_2$
	Glutamine (Glu)	$-CH_2CH_2-C(=O)-NH_2$
S	Cysteine (Cys)*	$-CH_2SH$
	Methionine (Met)	$-CH_2CH_2SCH_3$

*will most likely coordinate to a metal ion in its deprotonated form

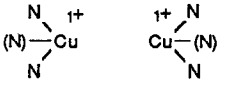
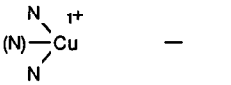
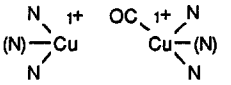
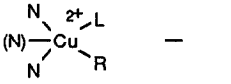
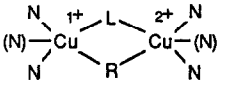
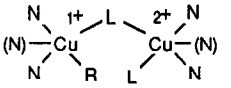
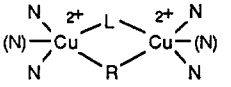
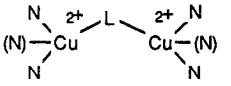
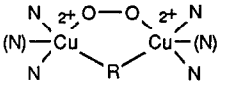
Table III. A Strategy for Modeling the Active Site in Functional Metalloenzymes

1. Isolation and purification of the metalloenzyme
2. Measurement of the detailed physical properties and preliminary characterization of components at the active site
3. Design of ligands
4. Synthesis and characterization of model compounds
5. Comparison of the physical properties of model compounds with those of the purified metalloprotein
6. Structural analysis of model compounds
7. Investigation of the chemical reactivity of the models

the protein. If there are few similarities, then one may conclude that the model is poor and return to the design stage to refine it. If the mimic is a good one based on the observed spectroscopic or physical properties, then it is important that its crystal structure be determined. Precise structural data along with the spectroscopic information may be useful for elucidating structures of less well characterized proteins. With a good structural analog, one can begin to probe the reaction chemistry. For example, if the goal were to mimic a metalloprotein that reversibly binds O_2, then a compound that proves to be a good structural model might also bind dioxygen, by analogy to the protein. If the reactivity profile of the complex does not match that of the protein, then one would return to the design stage to incorporate other structural elements into the molecule which could influence its reactions, without altering its spectroscopic properties.

The strategy in Table III has been rarely realized for several reasons. The first is, not surprisingly, that a protein is not as simple as the schematic of Figure 1 depicts. Environmental effects of the polypeptide may be quite complicated and far-reaching; and it may not be apparent, for example, that an amino acid residue 10 Å from the active site makes an important contribution to the reactivity. The second reason is that it may be nearly impossible to change the structure of a synthetic compound by small increments. For instance, one might want to increase a metal-ligand distance by only 0.1 Å to study its effect on the absorption spectrum. How can one design and control such a small structural change while keeping the rest of the molecule fixed? A third problem is that even when a synthetic compound *could* be made to mimic a protein, it may be more tedious to make and perhaps even more complicated than the protein itself. As mentioned above, one principal advantage of studying low-MW mimics is that precise structural information can be obtained by X-ray crystallography. A model that is too complex or will not crystallize is of little advantage in that regard.

Figure 2. Derivatives of hemocyanin. N = histidine; (N) = histidine, H_2O, or vacant; L = exogenous anion; R = endogenous ligand

Derivative	*Proposed Substructure*	*Abbreviation*
deoxy		deoxyHc
half-apo		half-apoHc
carbonyl		HcCO
met-apo		met-apoHc(L)
half-met-1		half-metHc(L)
half-met-2		half-metHc$(L)_2$
met (epr non-detectable)		metHc(L)
dimer (epr detectable)		dimerHc(L)
oxy		oxyHc

The problem of making "analogs" that are as complex as the protein itself presents an intriguing idea, outlined in section 4 of Table 1. Instead of building a model with small organic molecules, one may be able to modify a protein in specific ways using techniques of molecular-biology and/or chemical synthesis. In this way one could either generate a number of closely related species or prepare "protein analogs" of other metalloproteins (i.e., change the structure or function completely). Although that topic is beyond the scope of this review, such strategies are being explored and have begun to produce results.[7,8]

2. BINUCLEAR COPPER PROTEINS

2.1. Hemocyanin

While heme proteins perform the function of molecular oxygen transport in vertebrates, the binuclear copper protein hemocyanin fills this role in many species of invertebrates belonging to the phyla of Mollusca and Arthropoda.[9]

The hemocyanins are highly aggregated molecules that bind dioxygen cooperatively, and they contain one active site per subunit of molecular weight approximately 50,000 (molluscan) to 75,000 (arthropodan). The total molecular weight for the functional protein can be over a million daltons.

Undoubtedly because of the large size of these molecules, attempts to elucidate the active site structure of the hemocyanins have been generally unproductive, until the last decade. Table IV outlines an historical summary of the work that has led to the present understanding of the active site structure. This historical perspective is important for seeing how the design of synthetic biomimics has progressed concomitantly with new data about the protein structure.

Characterization of the hemocyanin active site was accelerated during the late 1970's by the discovery that a variety of derivatives of the protein could be generated (Figure 2).[23-30] These were extensively studied by several instrumental methods, leading to the proposal of a "spectroscopically-effective" active-site structure (Figure 3). Although a crystal structure of

Figure 3. Spectroscopically effective active site structure for oxyhemocyanin (from reference 28).

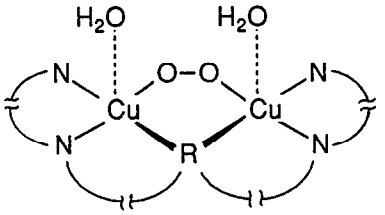


Table IV. Studies Related to the Elucidation of the Active-site Structure of Hemocyanin

Date	*Result or Proposal*	*Reference*
1928	stoichiometry of O_2 binding (1:2 O_2:Cu)	10
1934	stoichiometry of CO binding (1:2 CO:Cu)	11
1955	proposal of -SH ligation from cysteine	12
1958	rebuttal of proposed -SH ligation; evidence for imidazole ligands from histidine	13
1970	measurement of CO stretching frequency	14
1972	formation of a half-met derivative by reaction with NO	15
1973	magnetic susceptibility: oxyHc is diamagnetic	16
1974	proposal of Cu(II)-peroxide unit	17
	pH titrations show 2 histidine residues per copper ion	18
	resonance Raman spectroscopy used to demonstrate that O_2 is bound as peroxide	19,20
1975	preparation of metHc(azide)	21
1977	proposal for thioether ligation and phenolate bridging based on absorption spectra of synthetic complexes	22
1978	preparation and full spectroscopic characterization of half-met, met-apo, and met derivative	23-30
1979	transition dipole vector coupling model of oxyHc: dioxygen is bound as a cis-μ-1,2-peroxo moiety "spectroscopically-effective active site structure"	31
1980	EXAFS spectra: 3-coordinate Cu ions in deoxyHc; confirmation of histidine ligation; Cu-Cu distance of oxyHc = 3.67 Å	32
	resonance Raman spectra confirm histidine ligation	33
	luminescence of HcCO	34-37
1981	EXAFS spectra: 2-coordinate Cu ions in deoxyHc; Cu-Cu distance of oxyHc = 3.55 Å	38
1984	X-ray structure of deoxyHc; 3-coordinate Cu ions ligated only by histidine; no phenolate bridge	39-41
	pH titration study shows endogenous bridge with $pK_a > 7$	42
	EXAFS spectra of metHc(azide); Cu-Cu distance = 3.66 Å	43
1986	spectroscopic analysis of metHc(zaide): μ-1,3-azide	44
1988	luminescence of deoxyHc	45

the deoxy form of the protein is now available (Figure 4),[39-41] many of the early model studies relied on Solomon's proposed model, for which several features are noteworthy:

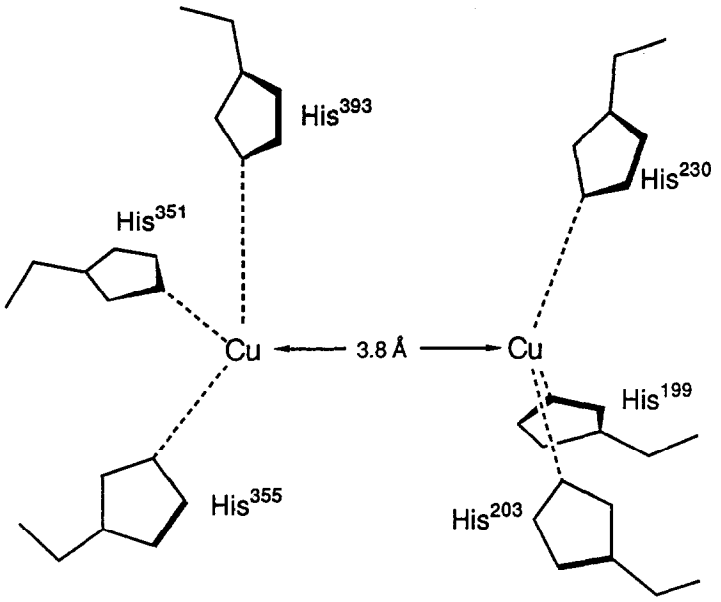
1) the copper ions are coordinated to two or three histidyl-imidazole ligands;

2) the copper ions in the oxy form are in the oxidized (+2) state, bonded to dioxygen in the peroxide oxidation state, and their geometries are tetragonal;

3) the peroxide ion bridges the copper ions in a cis-μ-1,2 fashion and gives rise to intense ligand-to-metal charge transfer (LMCT) bands at 345 nm (ε=20,000 M^{-1} cm^{-1}) and 570 nm (ε=1000 M^{-1} cm^{-1}), and a strong circular dichroism (CD) feature at 485 nm;

4) the copper ions are bridged by an endogenous ligand that results in a diamagnetic, hence strongly antiferromagnetically coupled, binuclear unit.

Figure 4. Schematic representation of the active site of deoxyHc according to X-ray crystallography (from reference 40).



Details about the coordination of the copper ions in the deoxy form and the distance between the copper ions were not available until about 1980 with the use of EXAFS spectroscopy.[32,38] Thus, it is not surprising to find that mononuclear complexes were utilized for the early modeling work. Mononuclear complexes are still useful as models for probing some of the specific properties of the active site (*vide infra*), as well as for modeling the half-apo and half-met forms of the protein (Figure 2).

2.2. Tyrosinase

Closely related to hemocyanin in structure is tyrosinase,[47] which functions primarily as a monooxygenase rather than as an oxygen-carrier. It catalyzes the formation of melanin pigments by performing the hydroxylation of monophenols (creolase activity: Figure 5) and the oxidation of *o*-diphenols to *o*-quinones (catecholase activity: Figure 6). For each scheme, note the involvement of the species labeled "OXY", which is apparently identical in form to oxyhemocyanin (Figure 3). Differences between hemocyanin and tyrosinase are most obvious in terms of binding substrate molecules, relating to their different biological functions.

Many of the derivatives of hemocyanin shown in Figure 2 have also been generated

using tyrosinase. The spectroscopic properties of those are very similar to the properties of the Hc analogs, with a notable exception. The half-apo and half-met derivatives of tyrosinase cannot be made because it is not possible to remove only one copper ion. However, using site-directed mutagenesis, a histidine residue that has been proposed as a copper ligand can be replaced by asparagine. The resulting *inactive* protein binds only a single copper ion, providing an entry into mono-metal derivatives of tyrosinase (cf. Table I.4).[48]

The most important work with tyrosinase has been that aimed at elucidating its mechanism of action by examining the interactions of the enzyme with substrate analogs and inhibitors.[47] Second in importance has been the study of sequence homology among the different species. The principal conclusion is that one of the copper sites (Cu_B) has been highly conserved throughout evolution, whereas the structure of the Cu_A site is quite variable.[49] In fact, for some species, there are only two histidine residues that one can reasonably propose as ligands for Cu_A. For modeling studies, this result suggests that unsymmetrical, binuclear complexes are desirable synthetic targets, an area that is largely unexplored at present.

Figure 5. Mechanistic scheme for the creolase activity of tyrosinase (adapted from reference 46). The oxidation state of copper is +2 except for the form labeled "deoxy" in which it is +1.

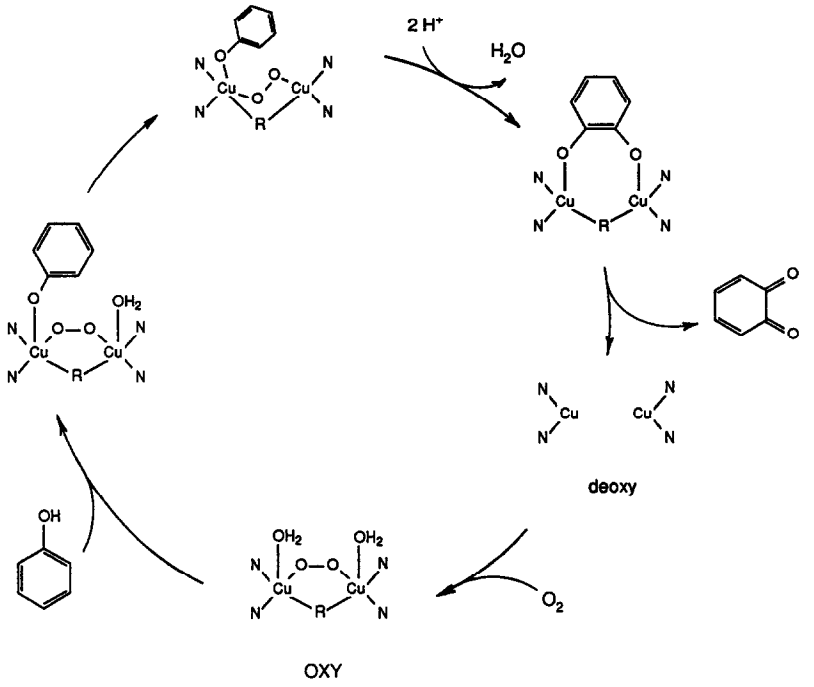
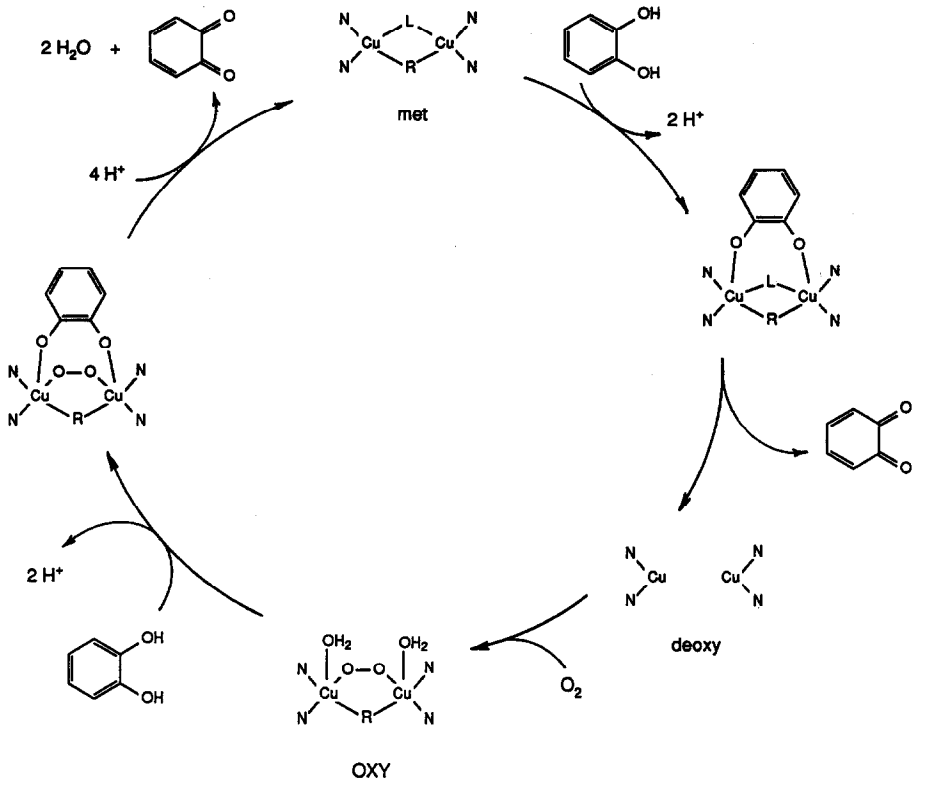


Figure 6. Mechanistic scheme for the catecholase activity of tyrosinase (adapted from reference 46). The oxidation state of copper is +2 except for the form labeled "deoxy" in which it is +1.



2.3. General considerations for modeling copper proteins

The complexes that have been prepared highlight some of the questions that workers in this area have obviously been attempting to answer with the use of models, and this review has been arranged accordingly:

1) What is the structure of deoxyHc, and how can we explain that it binds only one molecule of CO per binuclear copper unit?

2) What is the nature of the endogenous protein bridge (phenoxide, alkoxide, hydroxide, nothing)?

3) What is the nature and geometry of the Cu_2O_2 unit (does the cis-μ-1,2 peroxo unit account for the spectroscopic properties of oxyHc?)

4) What factors account for the reversibility of dioxygen binding and for the stability of dioxygen adducts?

5) How does tyrosinase function to activate the dioxygen molecule?

Based on Figure 3, modeling the active site of hemocyanin requires the synthesis of binucleating ligands with 4-6 nitrogen-containing donors.[50] Because of the substitution lability of copper ions in both biologically-relevant oxidation states (+1 and +2), chelating ligands are necessary to enforce, insofar as possible, the desired coordination environments of the metal ions. In addition, previous modeling studies of hemoglobin[6] point to the use of ligands that can impose various steric and/or environmental influences to the proximity of the metal ion binding site. Therefore, it is not surprising that organic synthesis has played a vital role in the design of hemocyanin analogs.

However, in designing ligands for modeling copper proteins, it is important that the issue of rigidity and/or steric effects not be overemphasized. Copper(I) ions prefer low coordination numbers (2, 3, and 4) and assume linear, trigonal, and tetrahedral geometries, respectively, in the absence of other influences. On the other hand, the copper(II) ion is usually four-, five-, or six-coordinate and will be square planar, square pyramidal (tetragonal), trigonal bipyramidal, or octahedral (the latter geometry is distorted because of Jahn-Teller effects). Thus, the ligands utilized must be flexible enough to accommodate different geometries, especially if there is a valence change, as is the case for dioxygen binding.

The nitrogen-atom donors needed for binding the copper ions have been provided by several different strategies, summarized in Figure 7.

Certainly the simplest types of ligands from a synthetic standpoint are those having only amino groups, for which there are literally dozens of preparative routes available. Unfortunately, amino groups are poor mimics of imidazole ligation found in proteins because they are highly basic and non-aromatic, features that might affect the spectroscopic properties of the resulting copper complexes.

Imines (Schiff bases) are easy to prepare from amines and carbonyl compounds. The sp^2 nature of the imine nitrogen atom makes this group electronically more similar to imidazole than an amine.

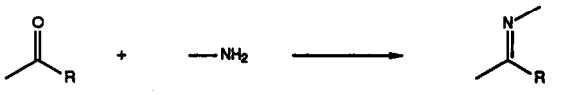
Nitrogen-containing heterocycles have also been used extensively in ligands. Pyridine is incorporated into chelates using either vinyl pyridine[51] or 2-chloromethylpyridine, precursors which unfortunately limit possibilities for derivatizing the heterocycle. The basicity of pyridine is similar to that of the imidazole group of histidine (Table V), and models having pyridine have been very successful in mimicking the reactivity of the copper proteins.

The imidazole ring itself, which should be the best mimic of histidine, has been incorporated most often by a Schiff-base condensation using histidine or histamine. The nucleophilic addition of 2-lithio-1-alkylimidazole to carbonyl compounds is also widely used.[52] The latter reaction is limited by the strongly basic conditions, but derivatives like (bisimidazolyl)nitromethane provide another entry into ligands with the imidazole group.[53]

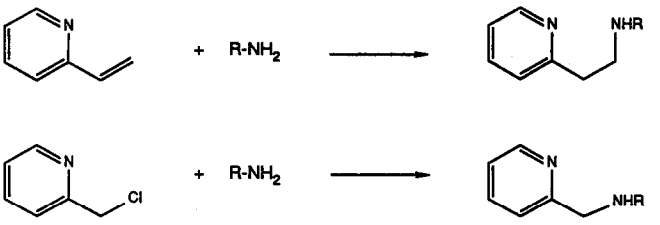
Imidazole derivatives like benzimidazole can also be utilized. They are easy to synthesize and derivatize,[54] are more stable than simple imidazoles, and have increased steric bulk which might be useful for controlling the metal ion stereochemistry. A drawback to using benzimidazole is that the aromatic ring fused to the imidazole nucleus can affect the spectroscopic properties of the resulting metal complex and can also mask the UV region, a point that is important for studies of copper(I) complexes.

Figure 7. Mimics of histidyl-imidazole ligation used in chelating ligands.

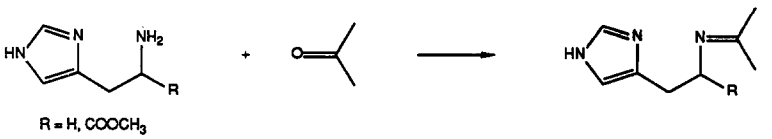
Imines

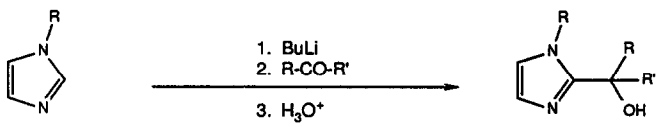


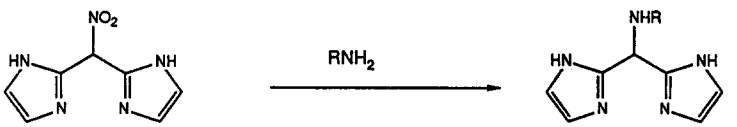
Pyridines



Imidazoles







Benzimidazoles

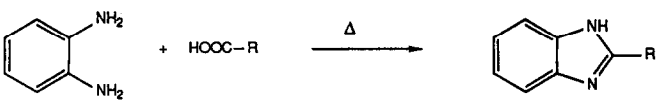
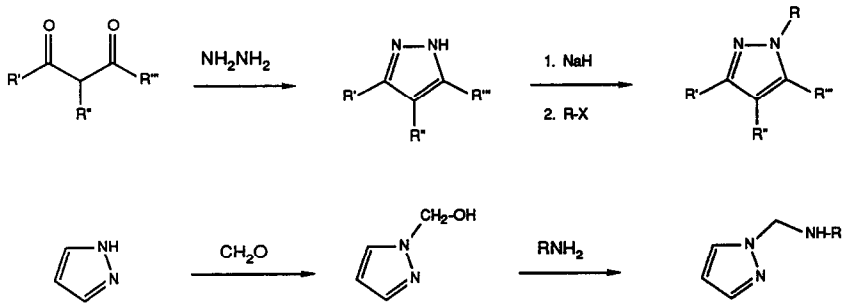


Figure 7. (continued)

Pyrazoles



Finally, pyrazole has been used for several ligands. Its size (5-membered ring), ease of preparation (from 1,3-diketones), and simple incorporation into chelating ligands (via an S_N2 reaction) are positive features.[55,56] The principal, and unfortunately major, drawback is that its basicity is very different from that of imidazole. Thus, the reactivity of copper complexes made with pyrazole-containing ligands has been found to be quite different from the reactivity observed for the proteins being modeled.

Table V. Basicity of nitrogen-donors used in chelating ligands

Donor	pK_b
alkylamine	3.0
arylamine	9.4
benzimidazole	8.5
histidine	8.0
imidazole	7.0
pyrazole	11.5
pyridine	8.7

reference: *CRC Handbook of Chemistry and Physics, 62nd Ed.*, Robert C. Weast, Ed, CRC Press, Inc., Boca Raton, 1981, pp D139-141.

3. MODELS FOR THE REDUCED FORMS OF HEMOCYANIN

Few attempts have been made to synthesize copper(I) complexes that reproduce the specific properties of deoxyHc and its carbonyl derivative, other than dioxygen binding (Section 5). Certainly the reason for this is the lack of spectroscopic handles for the colorless, diamagnetic (d^{10} electron configuration) copper(I) ion. At present, the goal of modeling deoxyHc may seem pointless because the crystal structure has given us a fairly accurate picture of the active site.[41] This was not the case several years ago, however, so the goal of preparing structural models was of value. Furthermore, the protein structure still provides no explanation for the properties of the carbonyl derivative.

There are three characteristics of the reduced forms of hemocyanin (*and* tyrosinase) that provide an entry into assessing the suitability of a model:

1) DeoxyHc reacts with carbon monoxide in the ratio of 2:1 Cu:CO, the same as that with dioxygen.[11] From infrared measurements,[14] it is clear that, unlike O_2, CO does not bridge between the copper ions.

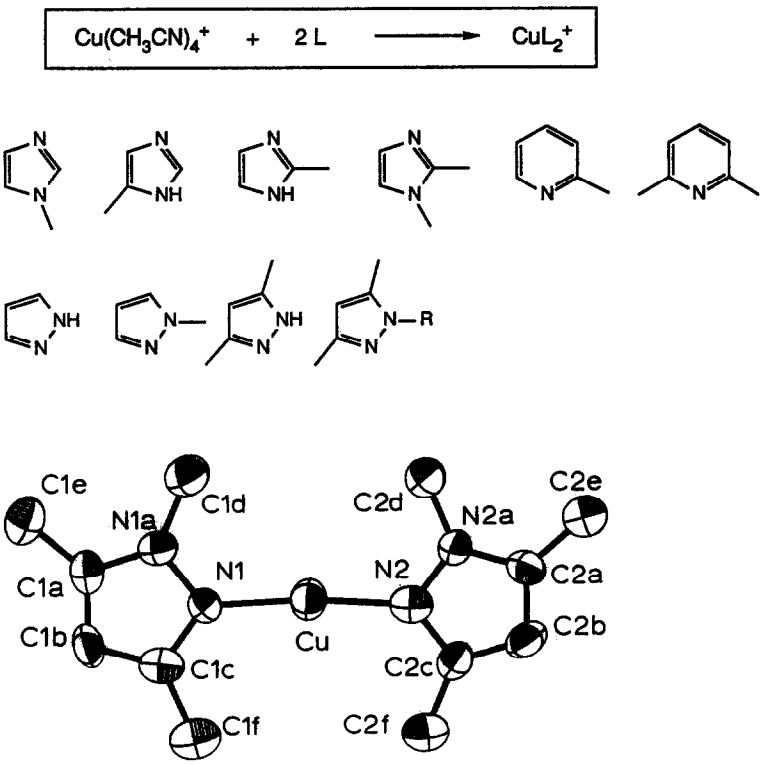
2) Binding of carbon monoxide results in a slight increase in absorption at approximately 300 nm;[57] and excitation with light in the region of 300 nm gives rise to an intense luminescence at about 550 nm.[34-37]

3) DeoxyHc itself luminesces (600-650 nm) when irradiated at approximately 300 nm.[45] This property was actually discovered *after* studying a model complex (*vide infra*).

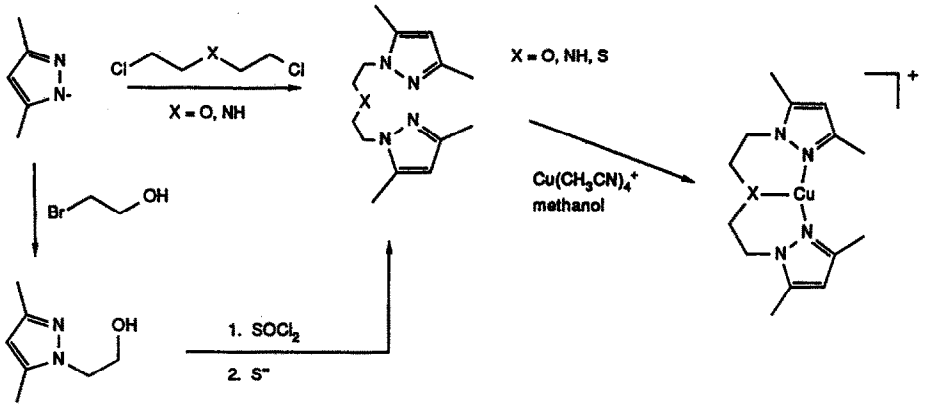
We began our work in this area with the study of two-coordinate copper(I) complexes[58] because of the results of an EXAFS study,[38] and the notion that such 14e$^-$ species might be highly reactive. We were able to prepare several complexes (Figure 8) without resorting to the synthesis of a chelating ligand, and we were surprised to find that bis(imidazole)copper(I) complexes are inert toward reaction with carbon monoxide. The low reactivity is presumably a result of the stable linear arrangement of ligands and very short Cu-N bonds (1.87 Å). The result prompted us to propose an explanation for the observed stoichiometry of CO binding by the protein, namely that one of the copper ions is essentially two-coordinate, hence unreactive toward CO, while the other copper ion is three-coordinate, and readily binds carbon monoxide. The argument was strengthened by the synthesis of a series of three-coordinate copper(I) complexes (Scheme 1) and the demonstration that they react readily with CO.[59] Additional work by Thompson[59a] and Floriani[59b] support these results. In view of the structural variability of the binding site for Cu_A (*vide supra*)[49], the likelihood is high that one of the copper ions is two-coordinate in hemocyanin or tyrosinase from certain species.

To probe the properties of tris(imidazole)copper(I) complexes required the synthesis of a tridentate chelate. Breslow[52] has reported the synthesis of a number of such ligands; and we have adapted those routes for the synthesis of timm (**1**), shown in Scheme 2.[60] Ligand **1** reacts with $Cu(CH_3CN)_4^+$ to form a dimeric product $[Cu(timm)]_2^{2+}$ (**2**), demonstrating that even when one designs a ligand to coordinate in a certain fashion, one isolates the most thermodynamically favored (least soluble) product, whether that fits the design or not. This is a good example of the substitution lability of Cu(I), demonstrated by the facile equilibrium between dimer and monomer in solution.

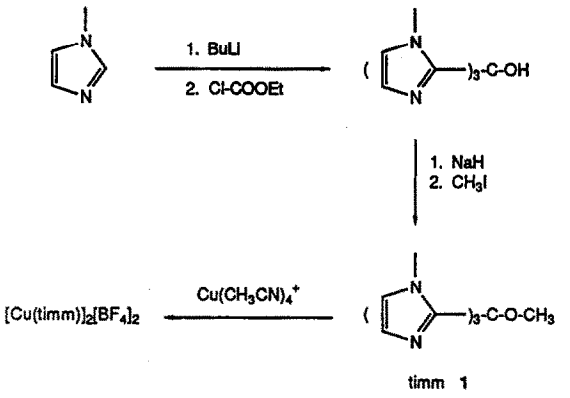
Figure 8. Two-coordinate Cu(I) complexes prepared as synthetic models for deoxyHc and the crystal structure of bis(trimethylpyrazole)copper(I) tetrafluoroborate. Cu-N distances: Cu-N1, 1.873 Å; Cu-N2, 1.879 Å (from reference 58).

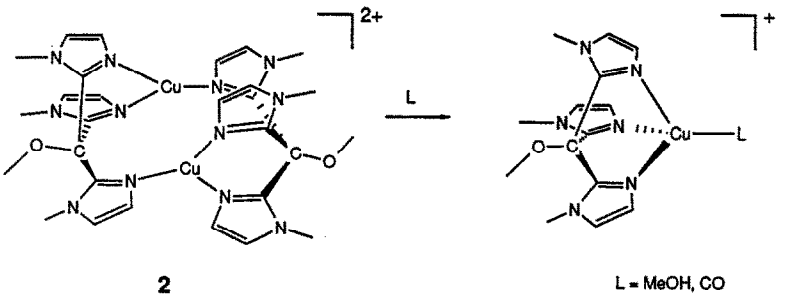


Complex **2** is important as a model for deoxyHc for two reasons: first, it readily forms a carbonyl derivative which has the same luminescence properties as HcCO. The emission results from a ^{3}d-π^* MLCT state as shown by measurements of the lifetimes of the excited state. Because the energies of the ligand orbitals are important, it is necessary to examine models having imidazole ligands. Second, in apparent contradiction of earlier studies with the protein,[34,36] the synthetic complex luminesces even in the *absence* of CO, which led us to reexamine the proteins themselves, and to find that certain species of deoxyHc and deoxyTyr have a low intensity luminescence at low energy.[45] Thus, model studies can sometimes reveal properties of the protein that may have been overlooked. Along the same line, a comprehensive study of two-, three-, and four-coordinate Cu(I) pyrazole and imidazole complexes revealed that such complexes have intense metal-to-ligand charge transfer bands in the UV region.[61] Although that region is usually obscured in proteins by absorptions from the aromatic amino acids, such bands undoubtedly exist in the biological systems as well, even if they have never been observed.



Scheme 1





Scheme 2

Because the active site of deoxyHc and deoxyTyr is fairly well-defined, we have been able to prepare models for the two structural possibilities that may exist for each copper(I) ion. The carbon monoxide reactivity and physico-chemical properties of two- and three-coordinate copper-imidazole complexes provide data consistent with the observed properties of the proteins, including the crystallographic results. Note, however, that the complexes discussed above are not synthetic *analogs*: as yet, there is no binuclear copper(I) complex that binds only a single molecule of CO and has the appropriate luminescense properties.

4. THE NATURE OF THE ENDOGENOUS BRIDGE

The study of the reactions of copper(I) complexes with dioxygen is central to modeling, or at least assessing the accuracy of analogs of, the active site of hemocyanin (Section 5). However, at least half of the papers in the field have dealt with *structural* analogs for hemocyanin. In particular, many studies have focused on the magnetic properties of copper(II) dimers that may be related structurally to the binuclear copper proteins. One impetus for such work has been to elucidate the nature, or even presence, of the "endogenous" bridging ligand at the active site (Figure 2).

The evidence for an endogenous bridge is based on the observation that oxyHc and metHc(azide) are diamagnetic at room temperature.[16,28-30] Based on the known magnetic properties of dimeric copper(II) complexes, it seemed unlikely that a single bridging ligand (i.e., O_2^{2-} in oxyHc) could mediate the strong antiferromagnetic coupling necessary to render the active site diamagnetic. Interconversions between epr active and inactive forms of oxidized hemocyanin (met and dimer, Figure 2) by simple protonation also provide evidence for a bridge that is endogenous to the protein.

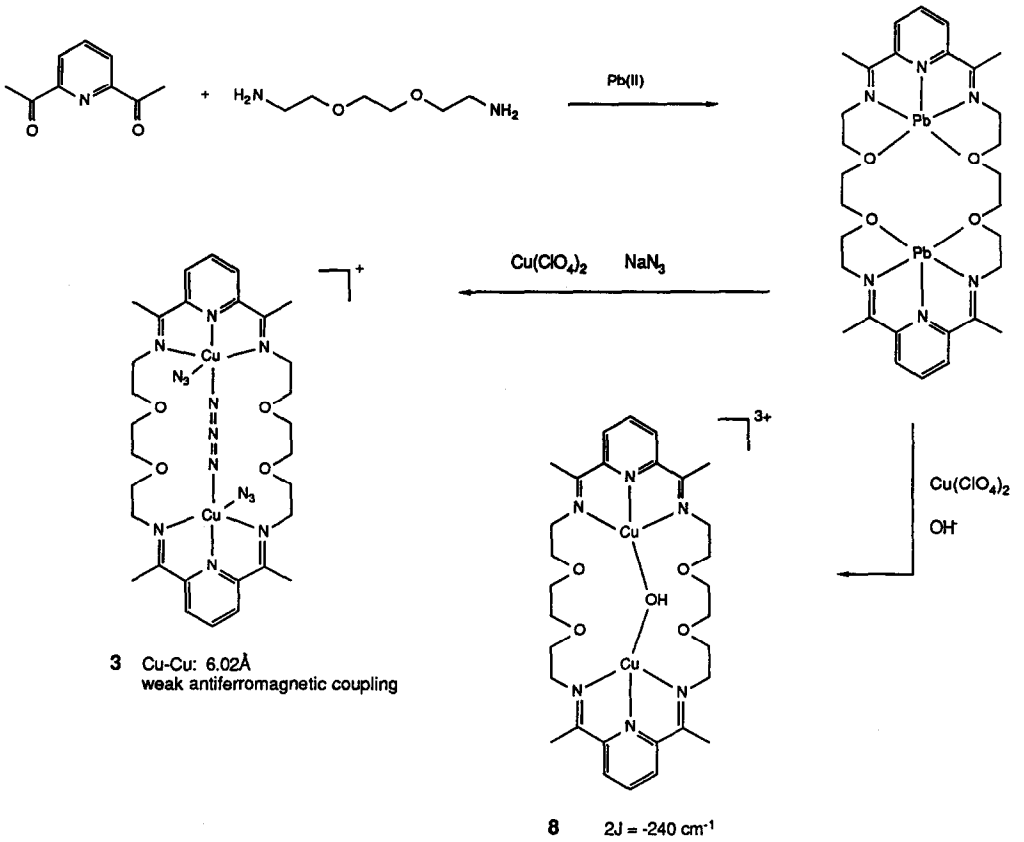
There are a number of ligands that are candidates for the endogenous bridge, so several types of complexes have been prepared to elucidate its identity. The X-ray crystal structure of deoxyHc[41] has subsequently ruled out the possibility of phenolate (from tyrosine) or alkoxide (from serine or threonine) ligation; however, literally hundreds of μ-phenolato complexes were prepared prior to 1985 because that group was consistently mentioned as a likely candidate.[22,42] On the other hand, proponents of the idea that no endogenous bridge exists point to the characterization of some dioxygen adducts (Section 5) that are diamagnetic at temperatures as high as -40° suggesting, perhaps, that the single bridge in oxyHc or in metHc(azide) (O_2^{2-} or N_3^-) may be sufficient to promote the strong antiferromagnetic coupling observed for the protein.

For the purposes of this section, the different types of complexes are discussed according to the nature of the endogenous bridging group. Not all the complexes bear a strong structural similarity to the hemocyanin active site, and those that are substantially different have been excluded. As much as possible, the discussion is aimed at synthetic azide complexes which might be suitable mimics of metHc(azide) since these can be easily characterized by UV-vis and IR spectroscopy as well as by magnetic susceptibility measurements. For the latter, 2J is used to indicate the singlet (no unpaired electrons)--triplet (two unpaired electrons) energy separation. A negative value indicates that the singlet state is lower in energy, so the magnetic interaction is antiferromagnetic.

4.1. No endogenous bridge

Most of the complexes in this category are based on macrocyclic ligands, many of which are synthesized by high-dilution or template methods.

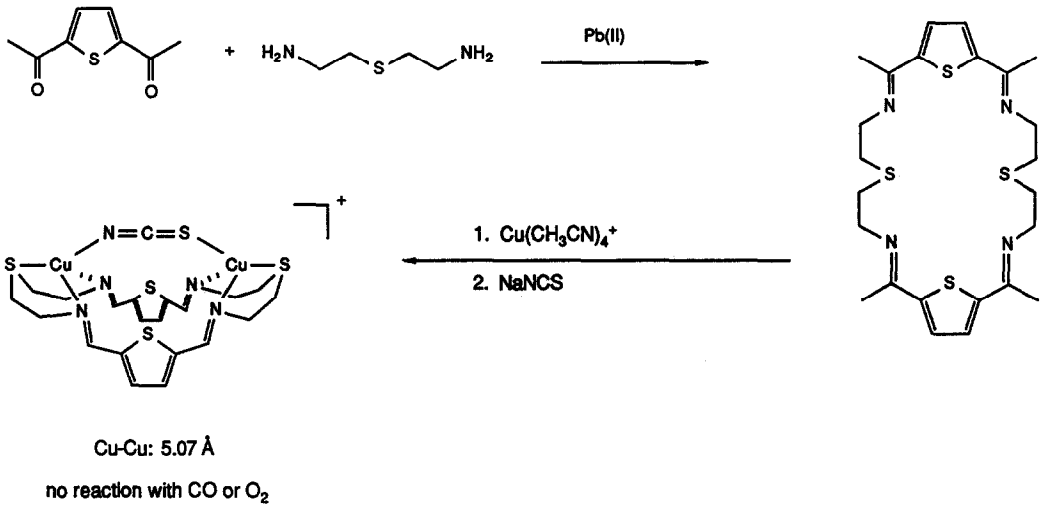
The late Martin Nelson and co-workers have prepared several large-ring macrocycles, usually by template-induced condensation reactions, in an effort to synthesize binuclear Cu(II) complexes. The mono-μ-azido complex **3** was prepared by the route shown in Scheme 3.[62] The copper ions, which are separated by 6.02 Å, are only weakly magnetically coupled. A related complex, prepared as shown in Scheme 4, is a copper(I) dimer; unfortunately, the copper(II) derivative was not reported.[63] An additional example of a mono-μ-azido species is presented in Scheme 10, below.



3 Cu-Cu: 6.02Å
weak antiferromagnetic coupling

8 $2J = -240\ cm^{-1}$

Scheme 3



Scheme 4

Agnus and Weiss, using methodology devised for the synthesis of crown-ethers, have isolated a bis(μ-azido)copper(II) dimer that is diamagnetic, like metHc(azide) (Scheme 5).[64,65] However, the Cu•••Cu distance in **4** is 5.145 Å, much longer than the 3.66 Å observed for metHc(azide) by EXAFS spectroscopy.[43] Furthermore, the complex contains sulfur donors, not present in Hc.

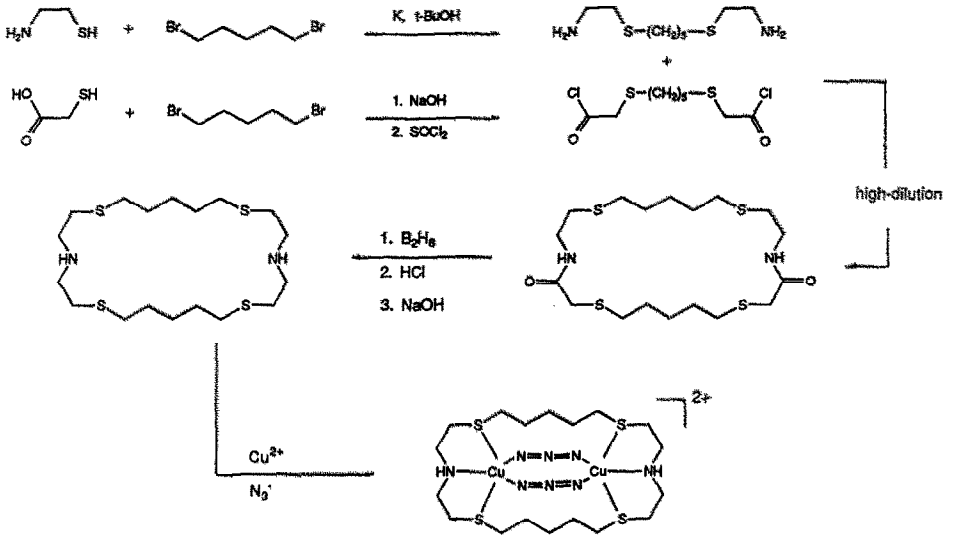
The synthetic complexes without an endogenous bridge which are probably most closely related to Hc are the μ-azido species prepared from dimerization of a monomeric copper(II) precursor (Scheme 6).[66] The copper ions are bound by three nitrogen donors (besides azide), have the required tetragonal geometry, and are very strongly antiferromagnetically coupled. However, the Cu•••Cu separations are much larger than that in metHc(azide),[43] and each copper atom binds two N_3^- anions.

4.2. Hydroxide-bridged complexes

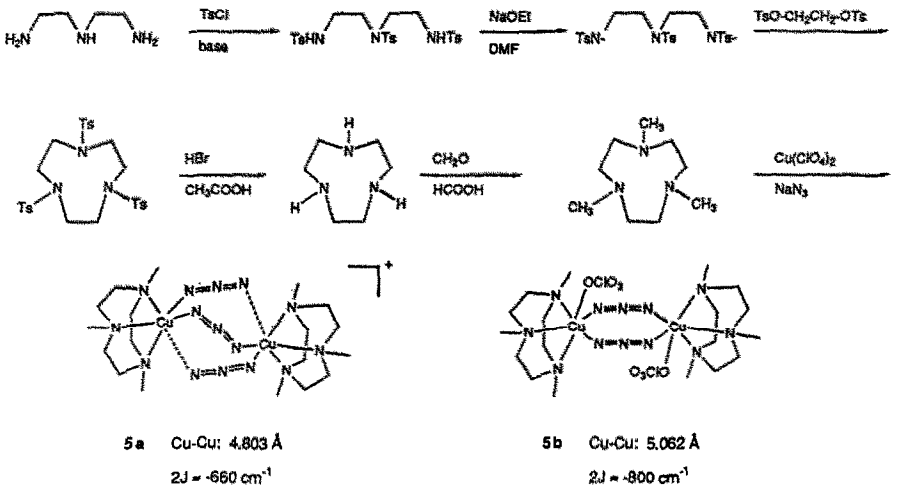
Bis(μ-hydroxy)copper(II) dimers represent one of the classic types of compounds studied for correlation of magnetic and structural properties.[67] Models for hemocyanin must have only a single hydroxy bridge because the other bridging position would be occupied by peroxide (in oxyHc) or another anion (in metHc).

Lippard was the first to formally propose that the endogenous bridge in hemocyanin might be the hydroxide ion.[68] The crystal structure of deoxyHc[41] points to the apparent correctness of this proposal, assuming that an endogenous bridge exists.

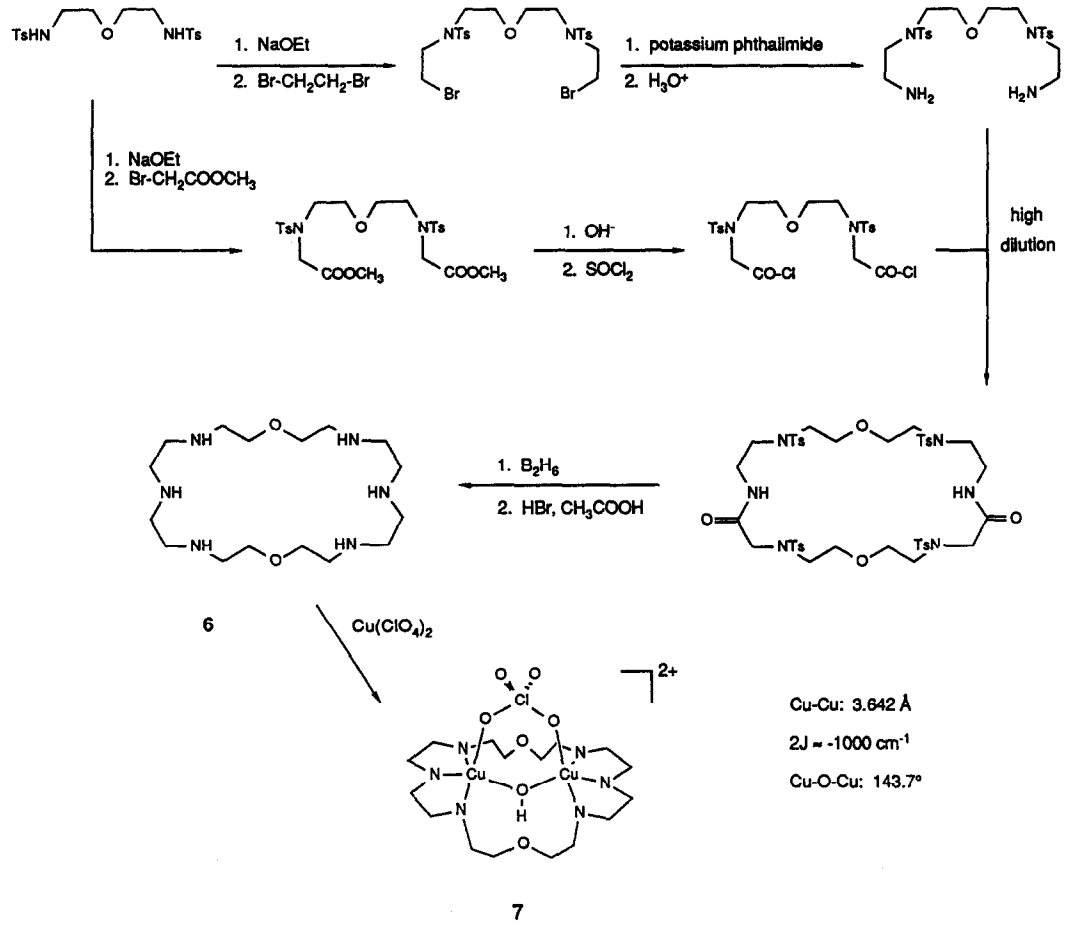
The ligand employed by Lippard is synthesized by the high-dilution method shown in Scheme 7.[69,70] The resulting μ-OH complex, **7**, in which the copper ions are also bridged by a perchlorate group through the axial positions, is presumably very similar in structure to that of



Scheme 5



Scheme 6

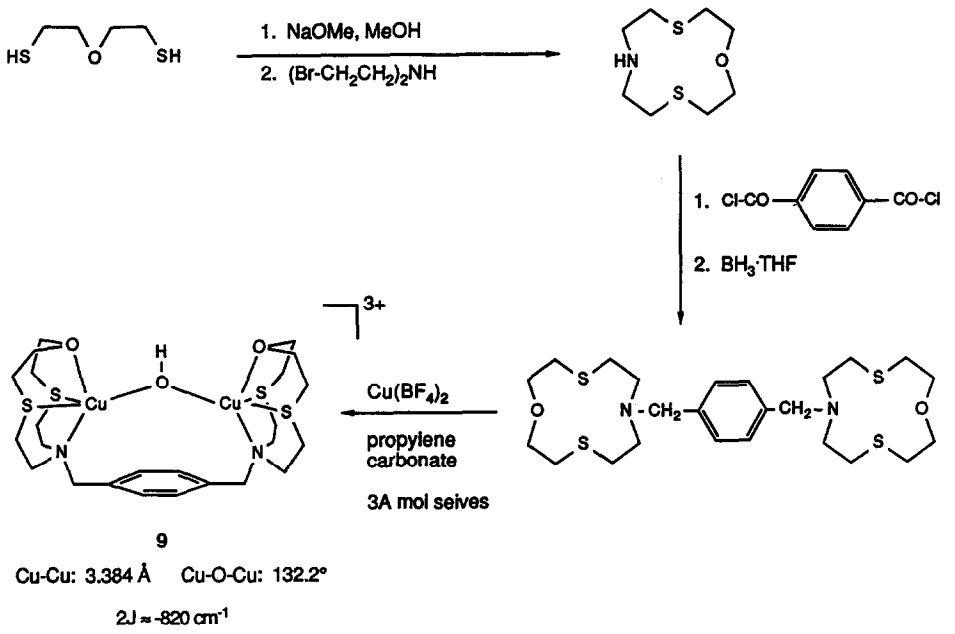


Scheme 7

metHc: the copper ions are coordinated to three nitrogen donors and the OH bridge, the Cu•••Cu separation is 3.64 Å, and 2J is -1000 cm^{-1}.

Structurally related to **7** is complex **8**, prepared by Nelson, *et al.*[71] The ligand synthesis has been shown already in Scheme 3. Treatment of the complex with hydroxide instead of azide ion gives the μ-OH complex **8** (Scheme 3) (Cu•••Cu = 3.57 Å), which, unlike Lippard's compound, shows only moderate coupling (2J = -240 cm^{-1}).

Osborn, *et al.*, using the "earmuff complex" presented in Scheme 8, was actually the first to prepare a structurally-characterized mono-μ-hydroxy complex.[72] The hydroxy group binds in an equatorial position of the tetragonal copper(II) ions which leads to a value of 2J = -820 cm^{-1}. The Cu•••Cu separation in **9** is 3.384 Å.



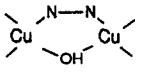
Scheme 8

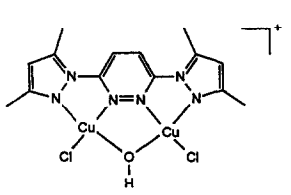
Finally, L. Thompson and co-workers have prepared a large number of μ-hydroxo copper(II) dimers that are also bridged by a nitrogen heterocycle, functioning as a 1,2-bridging unit, analogous to peroxide.[73-81] Several of these complexes are shown in Figure 9 along with the associated 2J values. The magnetic couplings vary considerably and have been recently correlated with structure.[81] In several cases, extremely strong antiferromagnetic coupling is observed. The utility of this class of ligands may be to stabilize unusual binuclear geometries for copper(I) complexes that may subsequently form stable dioxygen adducts, although this use has not yet been reported. The syntheses of two representative ligands are shown in Scheme 9.

4.3. Alkoxide-bridged complexes

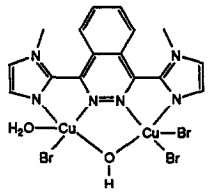
Two types of μ-alkoxy complexes can be imagined, and both have been studied: those in which the alcoholate ion is part of the ligand backbone, and those in which it is not. In general, ligands having an endogenous RO^- group have proven more useful because they can be systematically varied more easily. In fact, one reason that μ-alkoxy- and μ-phenoxy complexes have been studied so much with regard to magnetic-structural correlations is because the bridge is incorporated into the chelating structure, and unexpected structural anomalies can be minimized.

Figure 9. Representative hydroxy-bridged complexes of the form:

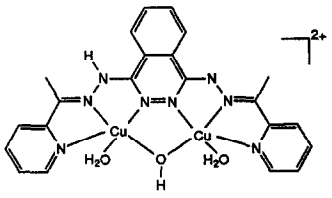




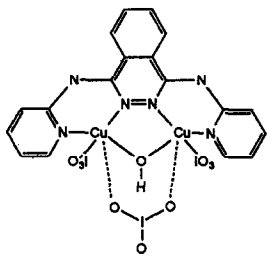
Cu-Cu: 3.384 Å diamagnetic



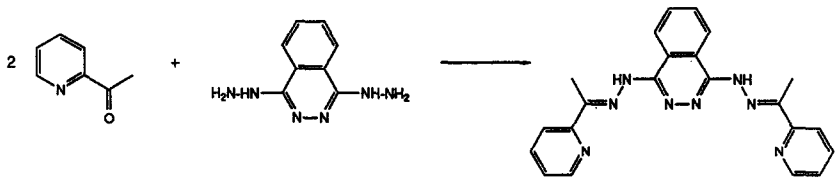
Cu-Cu: 3.420 Å $2J = -992\ cm^{-1}$

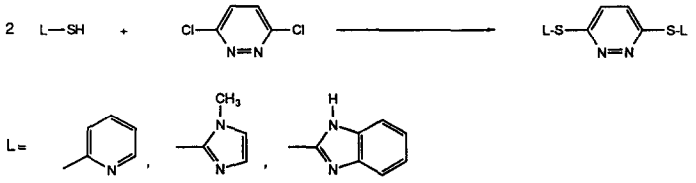


Cu-Cu: 3.295 Å $2J = -990\ cm^{-1}$



Cu-Cu: 3.165 Å $2J = -283\ cm^{-1}$





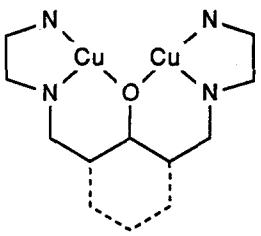
Scheme 9

In designing ligands of this type, one must consider the size of the chelate rings within the ligand framework. Some of the symmetrical sets are shown schematically in Figure 10 and apply to both μ-alkoxy and μ-phenoxy copper dimers. Synthetic sequences that can be varied to produce different chelation modes are particularly useful for systematic variation of the structure.

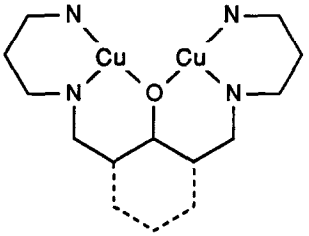
There are two relevant complexes in which the alkoxy group is *not* part of the ligand. Nelson reported a copper(II) dimer, presumably containing a single methoxy bridge (Scheme 10).[82] The magnetic coupling is only moderate and may reflect a distortion of the site that prevents maximal orbital overlap. The ring size seems small for the binding of two copper ions, but a crystal structure of a μ-OH-μ-H_2O derivative confirms its binuclearity. Complex **10** reacts with azide ion to give what is presumed to be a μ-1,1-azido complex, **11**, which is weakly ferromagnetic.

The other complex of this type was reported by Lippard. It is a μ-methoxy-μ-acetato copper dimer which is weakly coupled ($2J = -70$ cm^{-1}).[83] The synthesis utilized an unusual ligand called a "tropocoronand" (Scheme 11). Binucleating ligands of this type[84,85] might be considered for the synthesis of dioxygen complexes, but this use has not yet been reported.

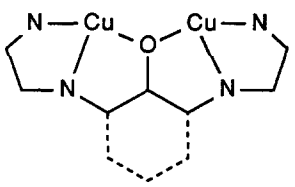
Figure 10. Representative chelate ring sizes for ligands providing an alkoxide or phenoxide bridge.



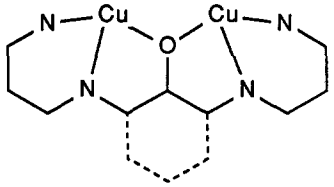
5,6,6,5



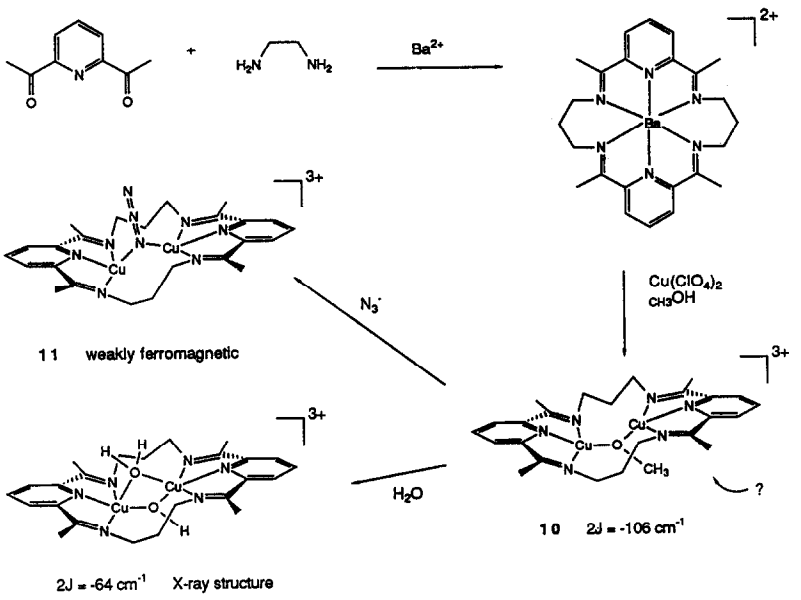
6,6,6,6



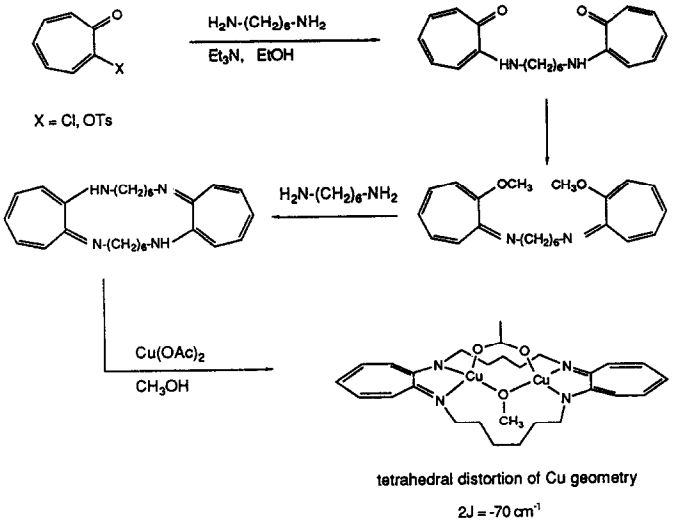
5,5,5,5



6,5,5,6



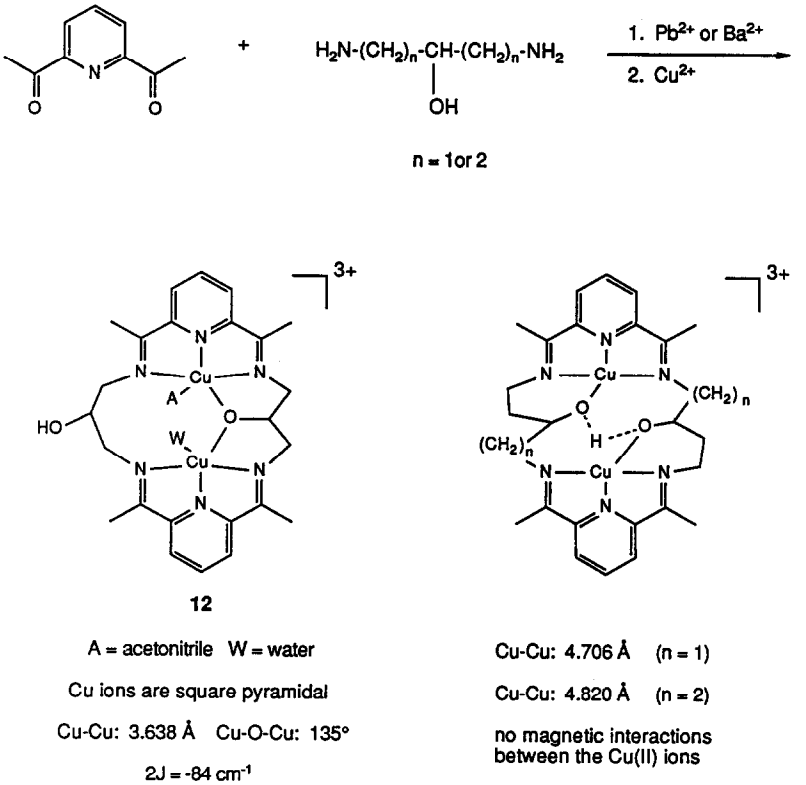
Scheme 10



Scheme 11

Ligands which incorporate the alkoxy bridge in the ligand framework have usually been "open" systems rather than macrocyclic. However, there is one macrocycle of interest, shown in Scheme 12.[86] The synthesis makes use of a template effect, followed by transmetallation to generate the copper complex. Complex **12** is the first example of a structurally-characterized copper dimer with a single RO^- bridge. The magnetic coupling between the copper ions is surprisingly weak given their tetragonal geometries and the presumably favorable orbital overlap through the alkoxy group.

Several non-macrocyclic μ-alkoxy complexes have been characterized, and it is with such species that the magnitude of coupling approaches that observed for hemocyanin. Murray and co-workers have reported a series of complexes in which the chelate ring sizes (cf. Figure 10) are varied.[87,88] The ligands are prepared by a Schiff-base condensation of an aldehyde with a diaminoalcohol (Scheme 13) which, upon addition of a copper(II) salt, gives the desired complexes (Figure 11). The magnetic coupling in one case is very strong and shows that an alkoxide bridge is able to produce diamagnetic copper(II) dimers.



12

A = acetonitrile W = water

Cu ions are square pyramidal

Cu-Cu: 3.638 Å Cu-O-Cu: 135°

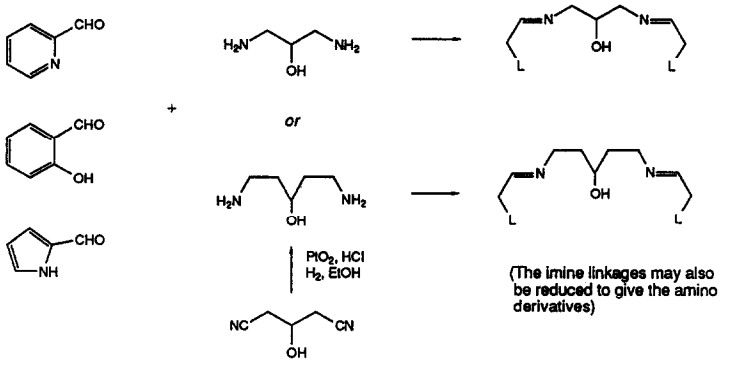
$2J = -84\ cm^{-1}$

Cu-Cu: 4.706 Å (n = 1)

Cu-Cu: 4.820 Å (n = 2)

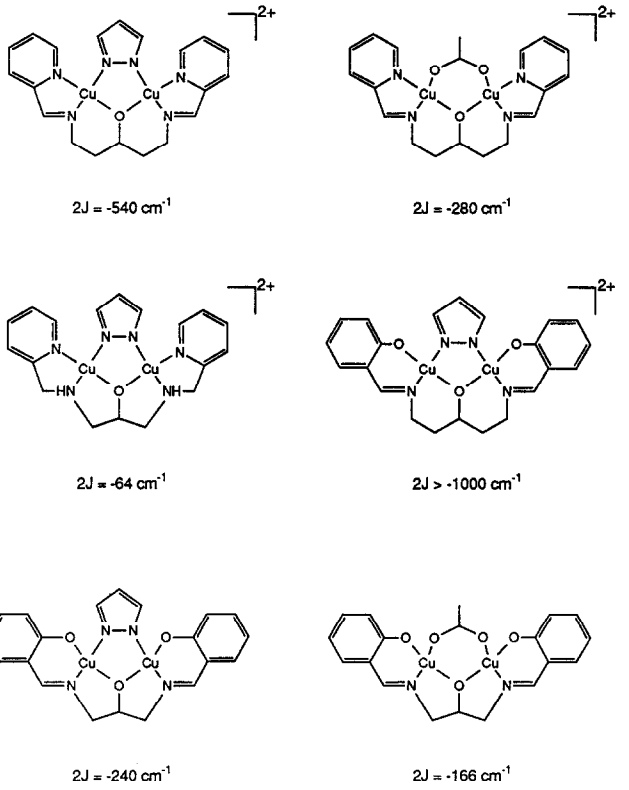
no magnetic interactions between the Cu(II) ions

Scheme 12

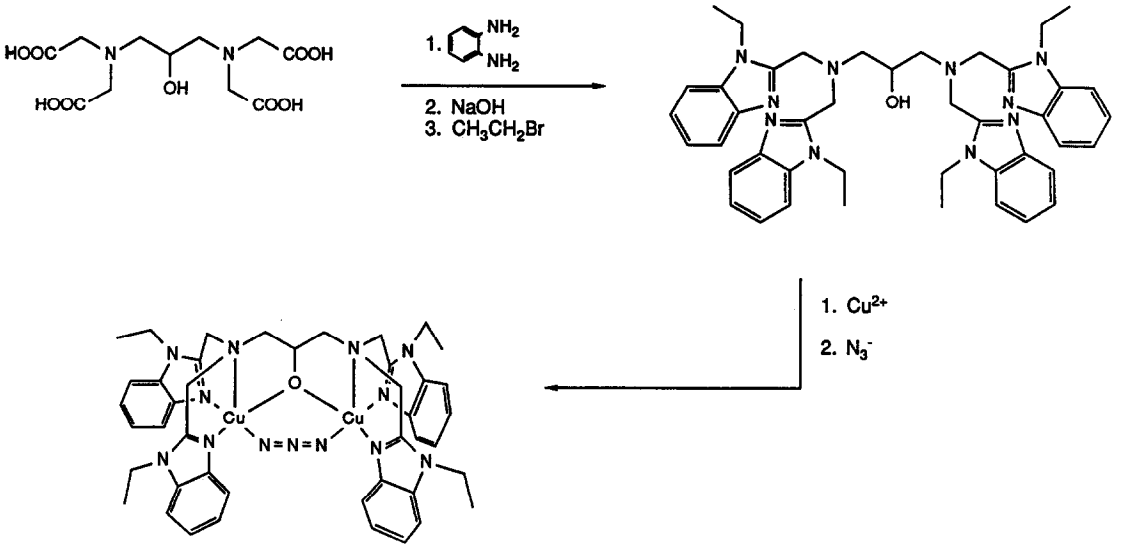


Scheme 13

Figure 11. Examples of μ-alkoxy copper(II) dimers



The best work in this area, related to preparing a synthetic analog of metHc, is that reported by Reed and co-workers.[89-91] Assuming the proposed site model for metHc(azide) to comprise two tetragonal Cu(II) ions bound to three N-donors, a bridging alkoxide group, and a bridging azide ion, Reed was able to synthesize complex **13** by the route shown in Scheme 14. Two other complexes were isolated and structurally characterized. Those contained either a μ-acetato or a μ-nitrito group. The azido complex mimics several properties of metHc quite well (*vide infra*) and is therefore an excellent structural analog. The other two complexes are interesting from the standpoint of their magnetic behavior, which is used to define the concept of "orbital complimentarity", describing how two copper ions may interact magnetically. The same concept has been discussed by Nishida and Kida as a result of their studies of μ-alkoxy copper(II) dimers.[92]



13

Cu-Cu: 3.615 Å

2J > -1100 cm^{-1}

Scheme 14

4.4. *Phenoxide-bridged complexes*

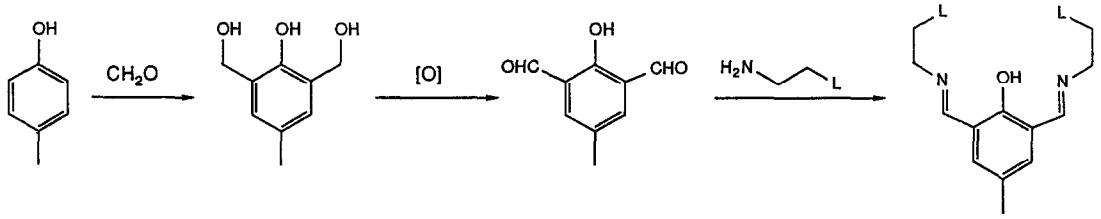
Of all types of copper(II) dimers having an oxygen-atom bridge, the phenolate complexes are the most common and probably best studied. One reason for this is that phenolate was considered for many years as the most likely candidate for the endogenous bridging ligand in hemocyanin.[22] The apparent pK_a of the protein bridging ligand, determined by Solomon several years after the initial proposal,[42] is also consistent with that hypothesis. Another reason for the plethora of μ-phenolate complexes is their ready synthesis and subsequent utility for the study of magnetic interactions between two Cu(II) ions.

Robson is generally credited with the first syntheses of the classic Schiff-base ligands used to prepare binuclear μ-phenolate complexes (Scheme 15).[93-99] Subsequently, a large variety of complexes having different donors and geometries have been prepared and characterized,[100-113] and representative complexes are shown in Figure 12 along with their associated magnetic properties. Note that the chelate ring sizes of (4,6,6,4), (5,6,6,5), and (6,6,6,6) are all represented, although a direct correlation of structure and magnetic coupling is not readily made because the actual geometry of the copper atoms varies, as does the orbital overlap between the phenolate and copper ions.

The use of these complexes as hemocyanin analogs is limited by the fact that the ligands provide only two nitrogen donors to each copper atom. Many of the complexes of this type are not structurally characterized, so magneto-structural correlations have not always been made. Moreover, few azido derivatives have been prepared.

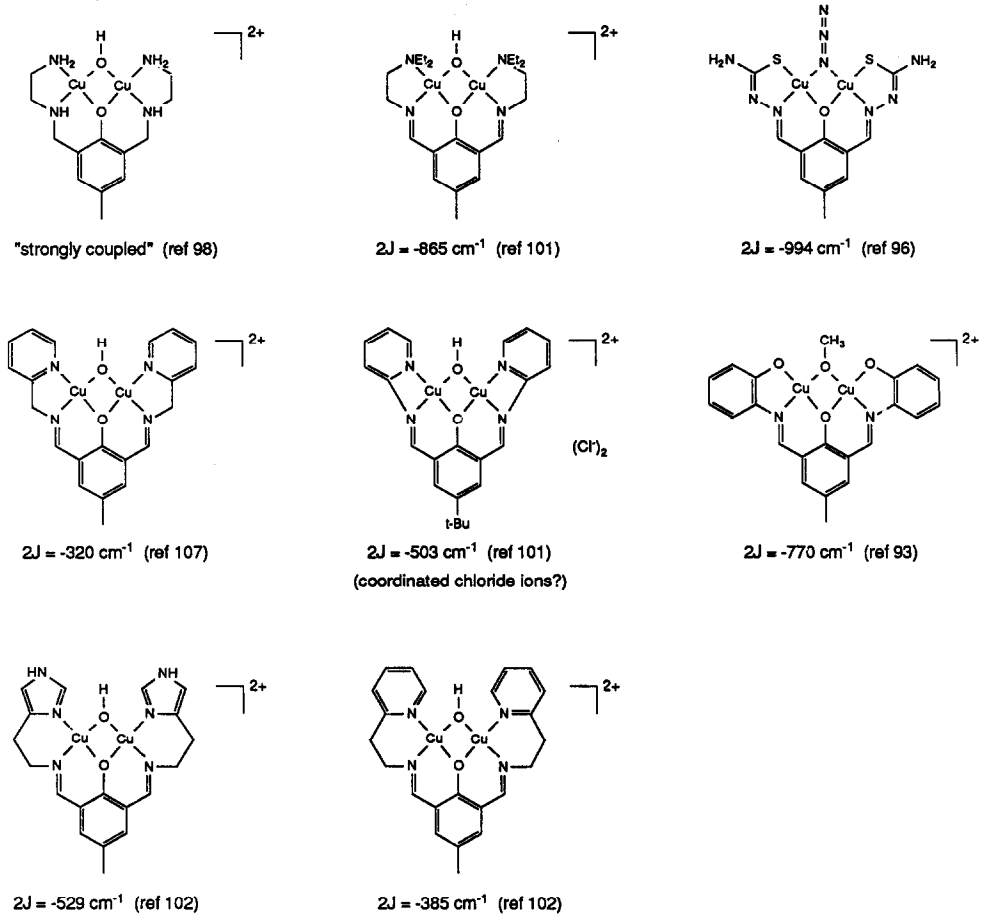
As an aside, both Gagne[104] and Urbach[103] utilized the Robson-type ligands in attempts to prepare copper(I) complexes. However, they are not accessible because of disproportionation of Cu(I) to Cu(II) and copper metal, or loss of a metal ion to give a mononuclear complex. The disproportionation problem could be circumvented by the addition of a bridging ligand like pyrazole, but those complexes are irreversibly oxidized by dioxygen.[104]

Beginning in the early 1980's, several groups began to prepare ligands that could provide three nitrogen donors to each metal ion. Those ligands are not accessible by the Schiff-base route, and involve the coupling of two $(N)_3$ units to an appropriate aryl precursor.



Scheme 15

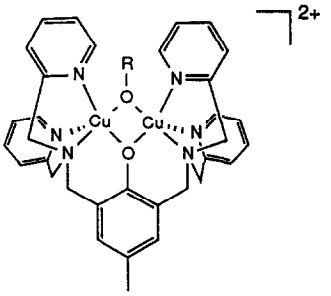
Figure 12. Representative phenolate-bridged copper dimers prepared via imine condensation of 2,6-diformylphenols



A number of workers have treated bis(chloromethyl)-*p*-cresol) with bis(pyridylmethyl)amine or bis(benzimidazolylmethyl)amine to give ligands in which the N-N-N chelates are 5-membered rings (Figure 13).[114-120]

The Cu(II) complexes prepared using these ligands show only modest magnetic coupling in most cases, and the copper ion geometry is sometimes trigonal bipyramidal. With complexes of these ligands having two, single-atom bridges (**14**), there is apparently some associated strain which can be relieved in solution, generating a complex having a single

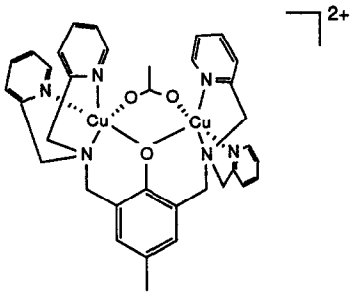
Figure 13. Phenolate-bridged copper(II) dimers having five-membered N⌒N⌒N chelates



14 Cu-Cu: 2.989 Å

Cu ion geometry - trigonal bipyramidal
phenolate equatorial to each Cu ion

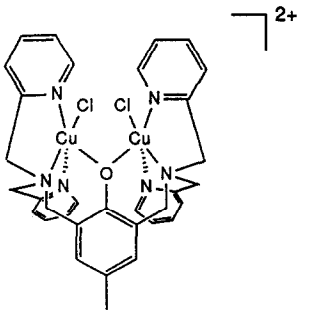
no magnetic data



Cu-Cu: 3.549 Å

Cu ion geometry - square pyramidal
phenolate - equatorial to Cu1
axial to Cu2

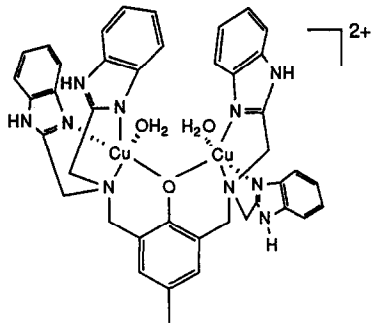
$2J = -80\ cm^{-1}$



Cu-Cu: 4.128 Å

Cu ion geometry - square pyramidal
phenolate axial to each Cu ion

$2J = 0\ cm^{-1}$



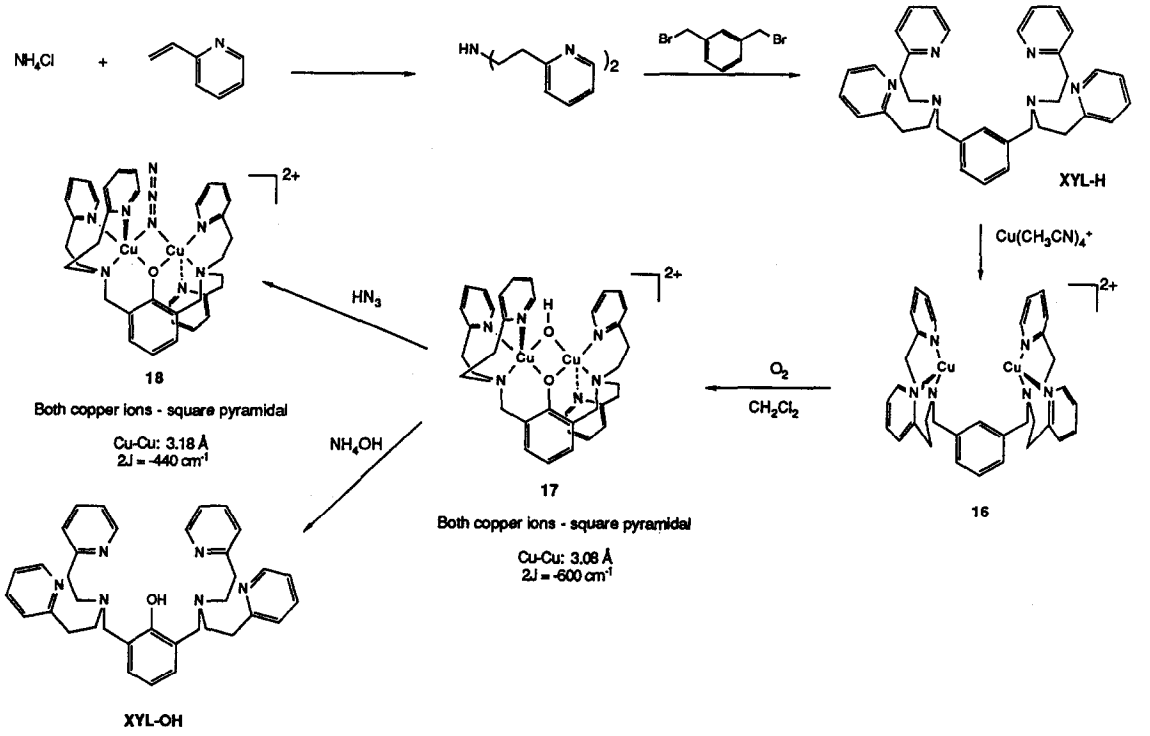
15 Cu-Cu: 3.785 Å

Cu ion geometry - square pyramidal
phenolate - equatorial to Cu1
axial to Cu2

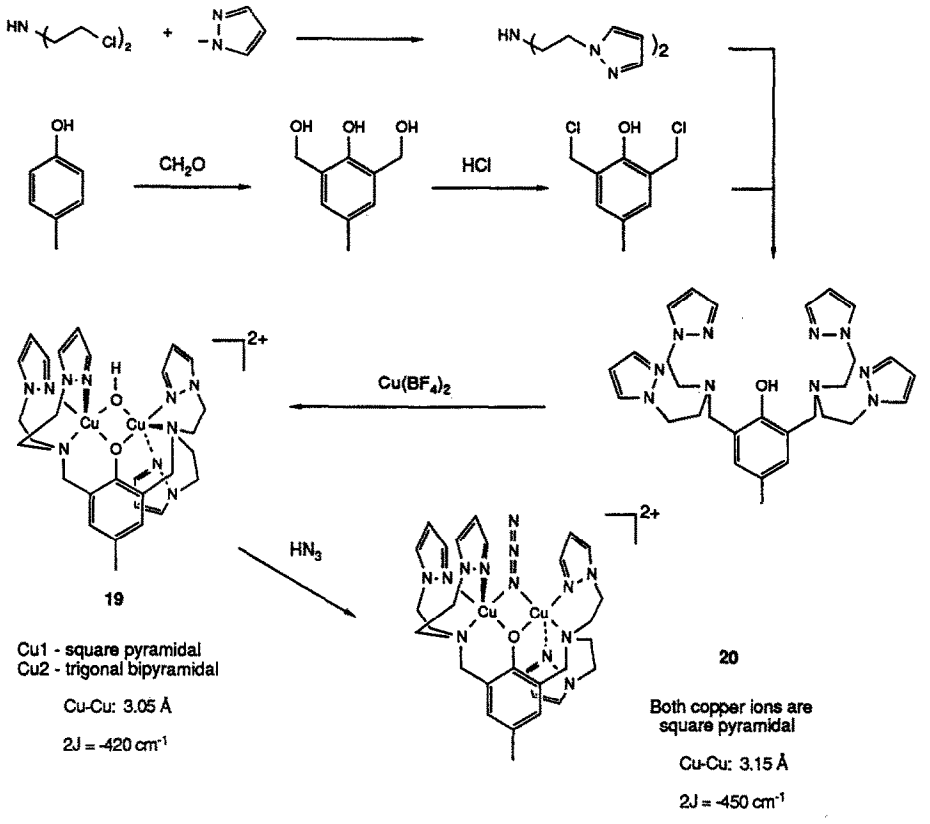
$2J = +4.2\ cm^{-1}$

phenolate bridge.[118] The bis(aquo) complex **15** prepared by Stephan is the only structurally-characterized copper(II) dimer having a phenolate bridge without another bridging group (or halide ions). Having the bound water molecules makes this complex potentially a good model for metHc(aquo), except that the phenolate ion is equatorially bound to one copper ion and axially coordinated to the other. As a result, the magnetic interaction is actually slightly ferromagnetic rather than antiferromagnetic. The azido derivative of **15** was also prepared but apparently has two different types of bound azide so was not characterized magnetically.

During this same period, our group and Karlin's group independently synthesized ligands providing three nitrogen donors to each copper ion. For these ligands, all of the chelate rings are 6-membered, which allows the copper(II) ions to attain a tetragonal geometry without strain. The synthesis of the pyridine-containing ligand is shown in Scheme 16 (to be discussed later),[121,122] and the synthesis of the pyrazole analog is presented in Scheme 17.[123,124]



Scheme 16

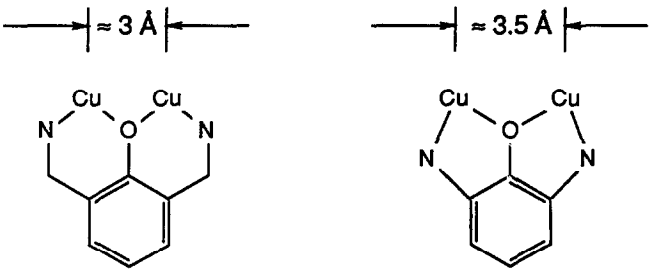


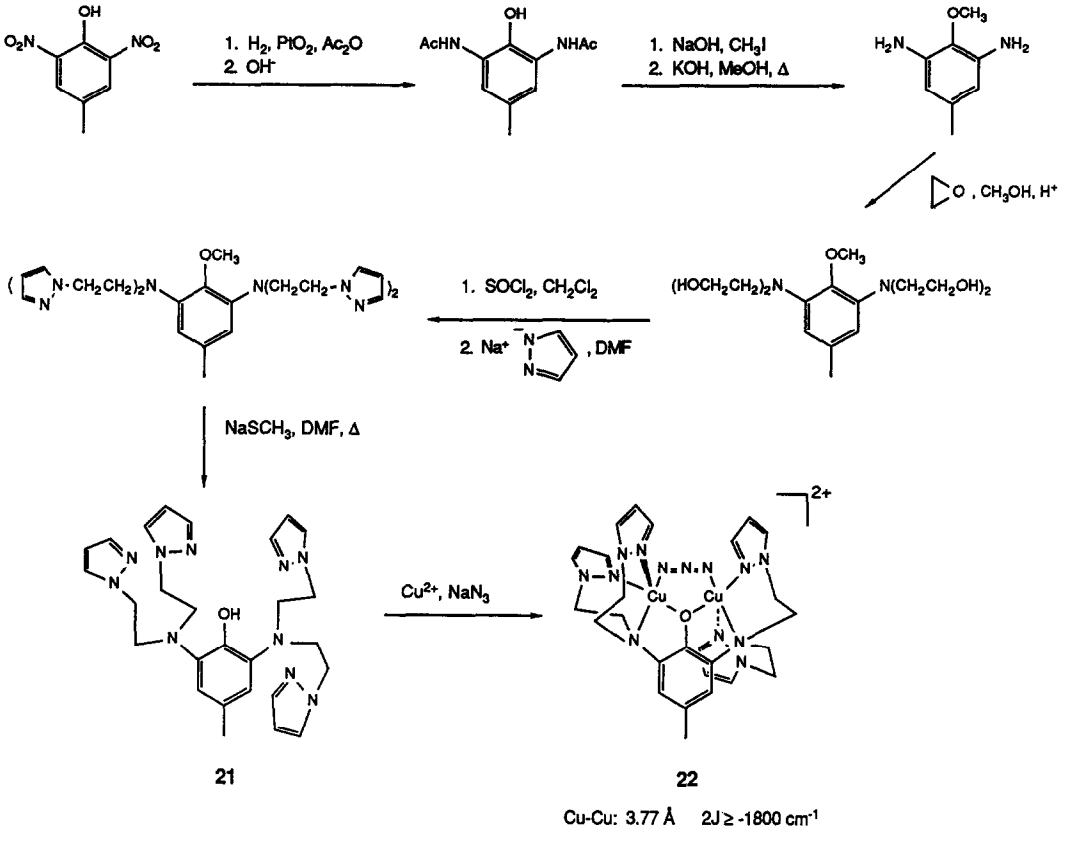
Scheme 17

The azido derivatives **18** and **20** are structurally and magnetically similar, in contrast to the situation observed for the μ-OH derivatives, **17** and **19**. For complex **19**, the copper ions actually have different geometries, which probably accounts for its weaker coupling compared to **17**, in which each copper(II) ion is tetragonal. Since the μ-1,1 bridging mode for azide leads to a ferromagnetic interaction,[125] the coupling in the azide complex should be less than for the hydroxy species. These results illustrate the importance of having a series of complexes that retain specific geometrical features when comparing a given physical or spectroscopic property.

Because the azido complexes are not diamagnetic, in contrast both to metHc(azide) and Reed's μ-alkoxy complex **13**, we considered how we could change the bridging mode of the azide. By incorporating 5-membered chelates into the ligand backbone, we reasoned that the copper-copper separation would be increased and the azide would be forced to bridge in a 1,3-fashion (Figure 14). The synthesis of the desired ligand **21** was accomplished by the route shown in Scheme 18,[126] and the resulting azido complex **22** was subsequently generated by the addition of Cu(II) and azide ions. A crystal structure showed that the

Figure 14. Relation of the Cu-Cu distance to the mode of chelation in phenolate-bridged dimers.





21

22

Cu-Cu: 3.77 Å $2J \geq -1800\ cm^{-1}$

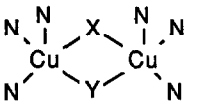
Scheme 18

Cu•••Cu separation had increased, relative to that in **20**, to 3.77 Å. Magnetic susceptibility measurements showed that the complex was diamagnetic at room temperature ($2J > -1800$ cm^{-1}), thus demonstrating that phenolate was a reasonable candidate for the role of the endogenous bridging ligand. That the fifth (axial) ligand has little effect on the physical properties of the binuclear copper unit was demonstrated by synthesizing the ligand **23** and complex **24** shown in Scheme 19.[127]

4.5. Biological relevance

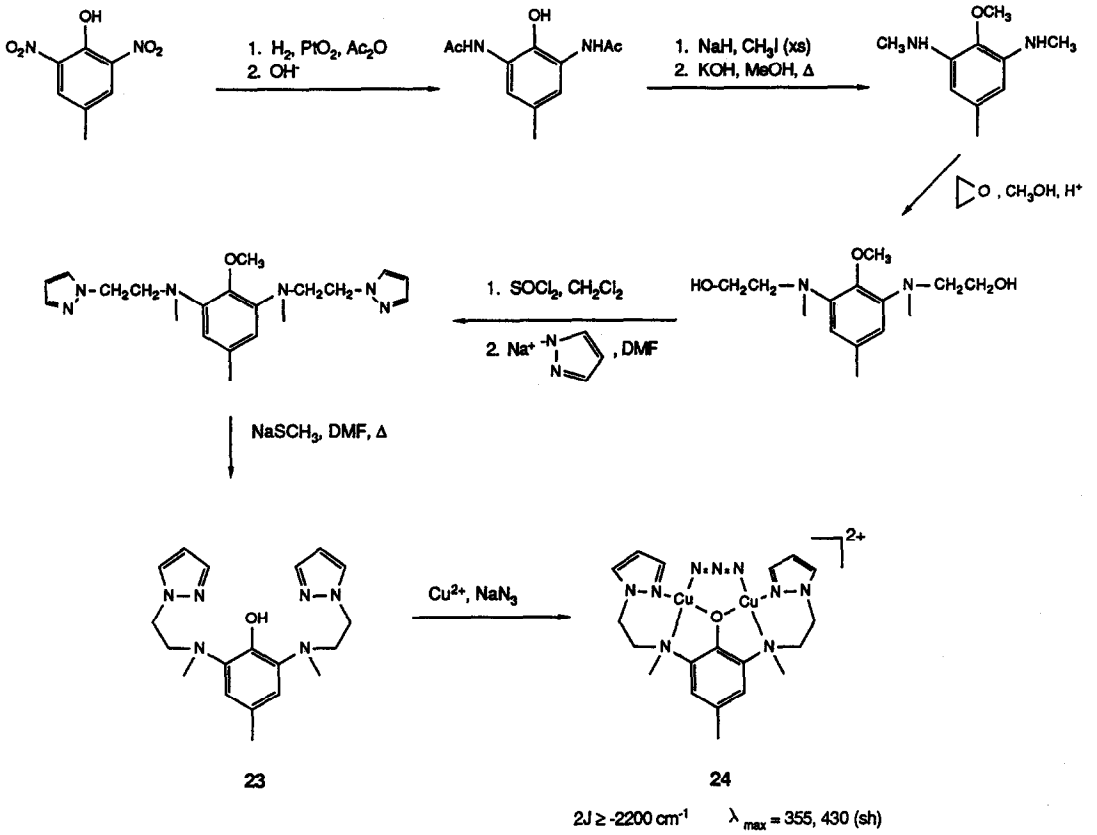
Table VI summarizes the properties for several of the model complexes discussed above, showing how they compare to those of the protein. It is clear that by assessing only the magnetic data, any oxygen-containing ligand might function as the proposed endogenous bridge in hemocyanin. Using other criteria, complexes with alkoxide and phenoxide bridges appear to be superior analogs; however, the X-ray structure rules out the possibility of either an alkoxide or phenoxide endogenous bridge.[41] An example of a μ-OH-μ-1,3-N_3 copper dimer having heterocyclic ligands would be a welcome addition to this table. Such a complex presents a challenging synthetic target because of the difficulty associated with binding two different exogenous anions. The synthesis of unsymmetrically-bridged complexes of that type is not without precedent, however, and Kahn has studied a μ-OH-μ-1,1-N_3 copper dimer.[125]

Table VI. Properties of synthetic analogs for metHc of the form:



Complex	X	Y	λ_{max}, nm (ϵ, $M^{-1}cm^{-1}$)[a]	N_3, cm^{-1}	-2J, cm^{-1}	Cu-Cu, Å
5b	1,3-N_3^-	1,3-N_3^-	-	2030, 2000	800	5.06
7	OH^-	-[b]	-	-	1000	3.64
12	OR^-	-	-	-	84	3.64
13	OR^-	1,3-N_3^-	365 (2380) 415 (sh)	2020	>1100	3.62
18	OAr^-	1,1-N_3^-	370 (2600)	2068	440	3.18
20	OAr^-	1,1-N_3^-	364 (2500)	2065	450	3.15
22	OAr^-	1,3-N_3^-	376 (4600) 444 (sh)	2032	>1800	3.77
metHc(azide) Busycon	?	1,3-N_3^-	380 (1200) 450 (sh)	2040	>1000	3.66

[a]azide-to-copper charge transfer bands; [b]no other bridging group in magnetically interacting orbitals



Scheme 19

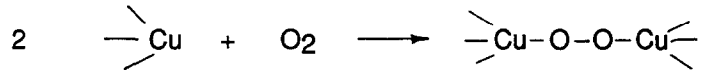
5. DIOXYGEN COMPLEXES

The interaction of copper(I) ions and dioxygen has been reviewed recently,[1] and interested readers are encouraged to consult that reference with regard to the redox chemistry of dioxygen and its interaction with metal ions. Only those systems that are of interest in the context of hemocyanin will be mentioned here. In that regard, there are two reasons for preparing dioxygen complexes of copper: 1) to study the possible geometries and spectroscopic and physico-chemical properties associated with the Cu_XO_2 substructure; and 2) to probe what features promote *reversible* binding of molecular oxygen to copper.

5.1. Mononuclear precursors

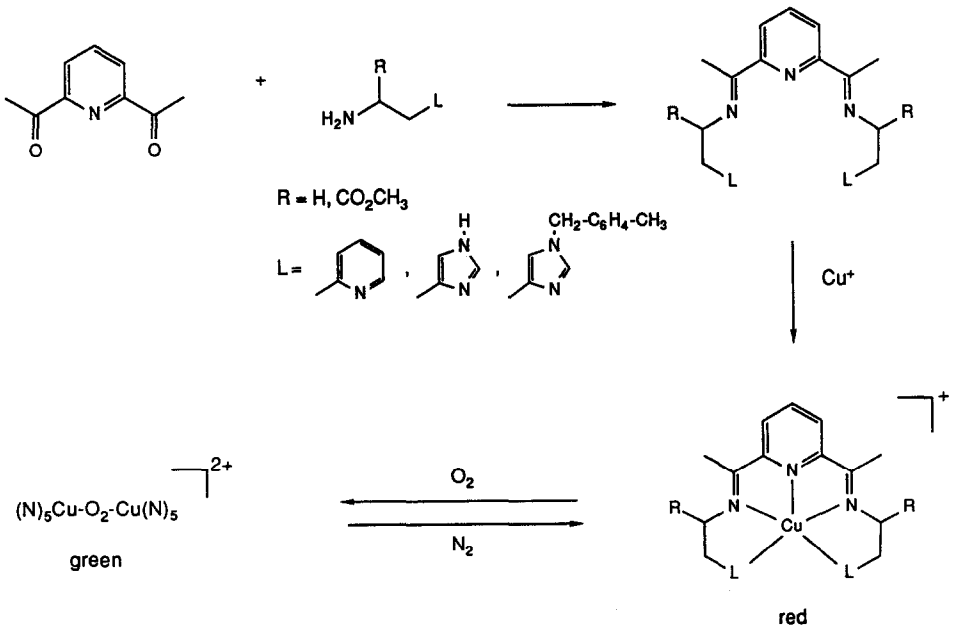
Although it is now certain that the active site in hemocyanin is binuclear, one might assume that there is no direct interaction between the copper ions in the absence of O_2;

therefore, bringing together two mononuclear complexes with an oxygen molecule might give an accurate structural model for oxyHc:

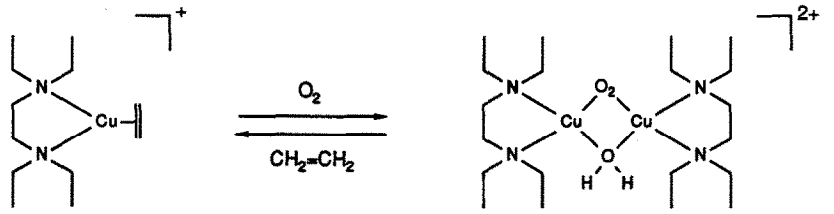


One of the earliest reported systems of this kind was prepared by Wilson and co-workers[128-131] and subsequently by Casella[132] (Scheme 20). The precursor is a five-coordinate copper(I) complex having only nitrogen donors, and two of the ligating groups can be easily changed to examine the effects of donor basicity. In those ligands having appended imidazole groups, the initially red copper(I) complex reacts with O_2 in a stoichiometry of 2:1 $Cu:O_2$, even at room temperature, to form a green product formulated as a binuclear Cu(II)-peroxide species. The reaction is partially reversed by purging the solution with nitrogen, but it has not been possible to confirm dioxygen coordination by spectroscopic methods. In particular, resonance Raman spectra, which probably provides the most direct indication of a Cu_2O_2 unit, showed no enhancement of a band assignable as an O-O stretch.

Thompson has utilized a very simple mononuclear complex to generate a μ-peroxo-Cu(II) complex.[133] N,N,N',N'-tetraethylethylenediamine and ethylene form a stable copper(I) adduct that reacts with dioxygen at low temperature in dry methanol containing a slight excess of water to generate the complex formulated as **25** (Scheme 21). The reaction can be



Scheme 20



25

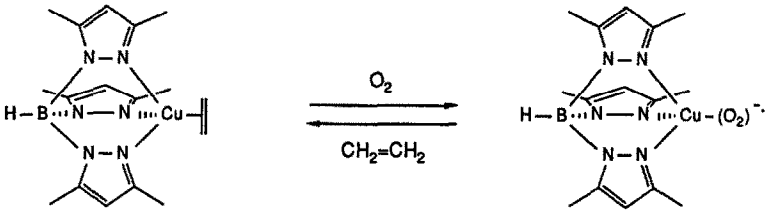
Scheme 21

reversed by displacement of the oxygen with ethylene. The IR spectrum shows that the O_2 group is coordinated as peroxide; and apparently, there is a bridging water molecule completing each copper coordination sphere. While the proposed structure of this complex is clearly similar to that proposed for oxyHc (Figure 3), unfortunately, there are no structural data available for **25**.

Using the same strategy of displacing ethylene from a copper(I) precursor, Thompson has also been able to prepare a monomeric Cu(II)-O_2^- (superoxo) complex, **26** (Scheme 22).[134] That result bears little on the structure of oxyHc, except that Kitajima *et al.* have recently reported the generation of a binuclear peroxy complex **27** by a different route using the same system (Scheme 23).[135] The resonance Raman spectrum of the complex shows a peroxo O-O stretch at 725 cm^{-1}; but the absorption spectrum, touted to be like that for oxyHc, is apparently identical to that of Thompson's superoxo complex **25**.[136] Because there are no structural data available, we must await further characterization of the putative peroxo species before it can be evaluated as a model for oxyHc.

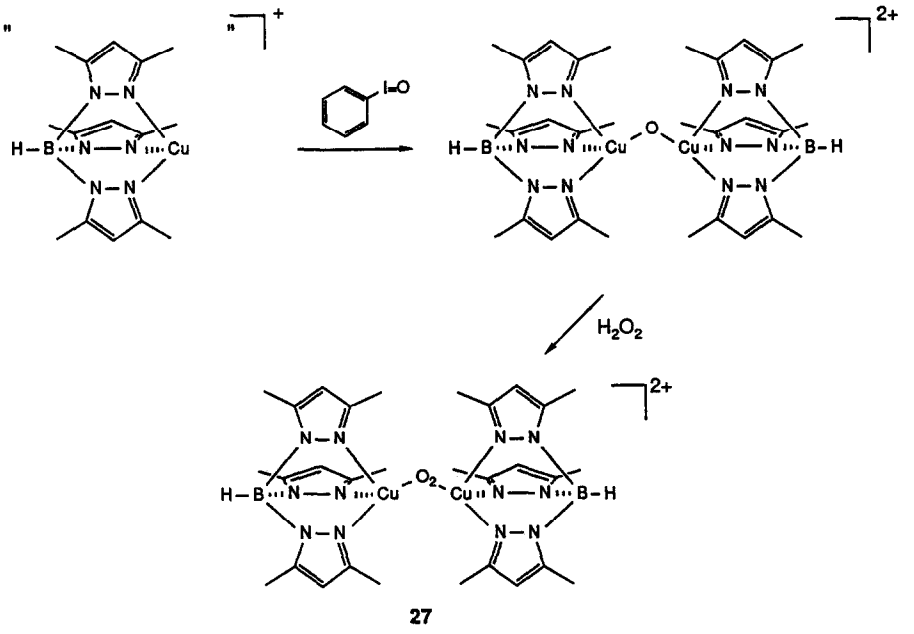
Finally, Karlin, *et al.* have prepared and structurally characterized a binuclear copper(II)-peroxo complex (**28**) starting from a mononuclear precursor.[137] This complex is the first crystallographically-characterized copper-dioxygen adduct. The ligand is made by a straightforward procedure (Scheme 24) and has been known for many years.[138]

Although **28** is important as the first structurally-characterized copper dioxygen complex, its absorption spectrum is very different from that for oxyHc, and it is therefore not a structural analog. There are at least two important differences: 1) the copper ion geometry is trigonal

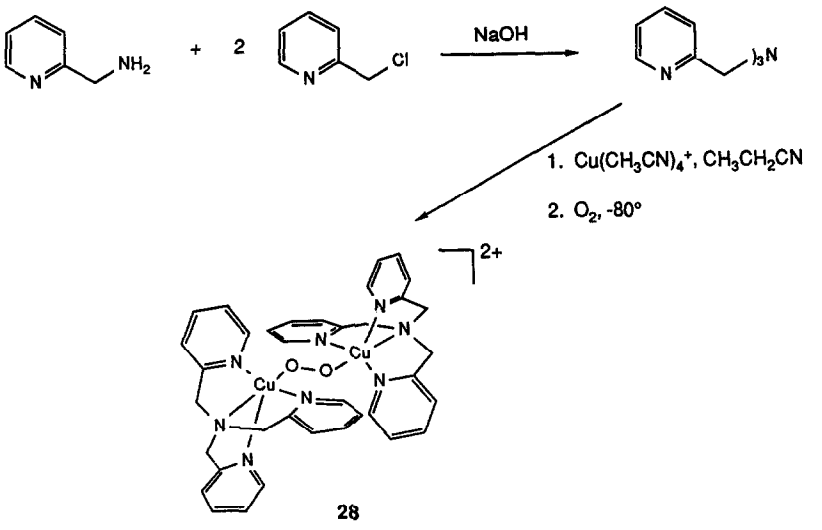


26

Scheme 22



Scheme 23



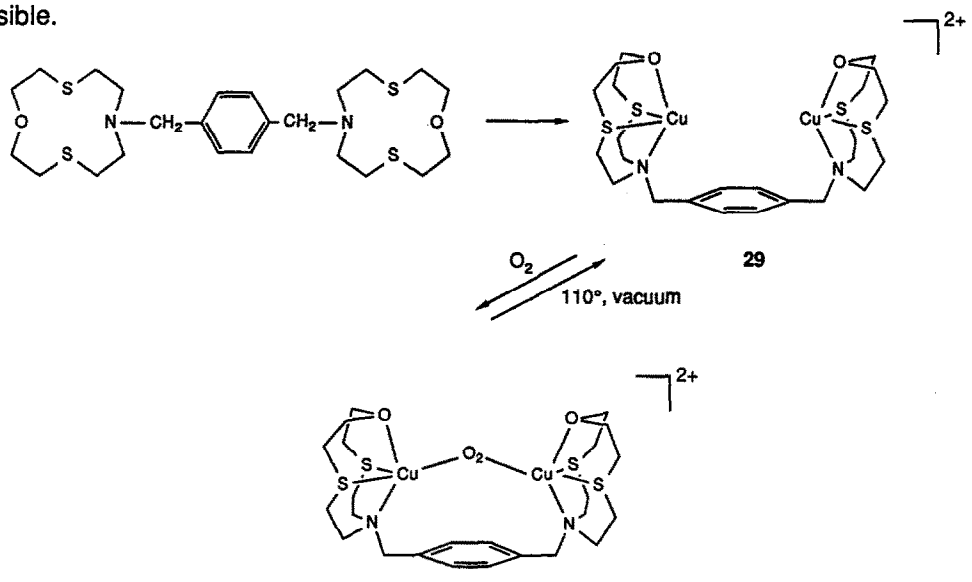
Scheme 24

bipyramidal, whereas it is thought to be tetragonal in the protein; and 2) the geometry of the peroxo bridge is μ-1,2-trans instead of the proposed μ-1,2-cis for oxyHc. The latter situation will undoubtedly obtain for dioxygen adducts prepared from mononuclear precursors since there is no steric constraint preventing the preferred anti-conformation. The case is analogous to that observed in 1,2-diphenylethane, for example, in which the anti-conformation predominates in order to minimize the steric repulsion of the bulky substituents. It is interesting that **28** is moderately magnetically-coupled, since this supports the contention that a single bridging ligand may be sufficient to account for the observed diamagnetism of oxyHc (Section 4).

5.2. Binuclear precursors

Because the hemocyanin active site contains two copper ions, stable O_2 adducts might be readily obtained using binuclear copper complexes. For the design of such systems, the principal criterion is the copper-copper distance which must be small enough to allow the dioxygen moiety to coordinate to each metal ion. Different research groups have employed various ligands in attempting to hold two copper ions within the desired 3-4 Å separation.

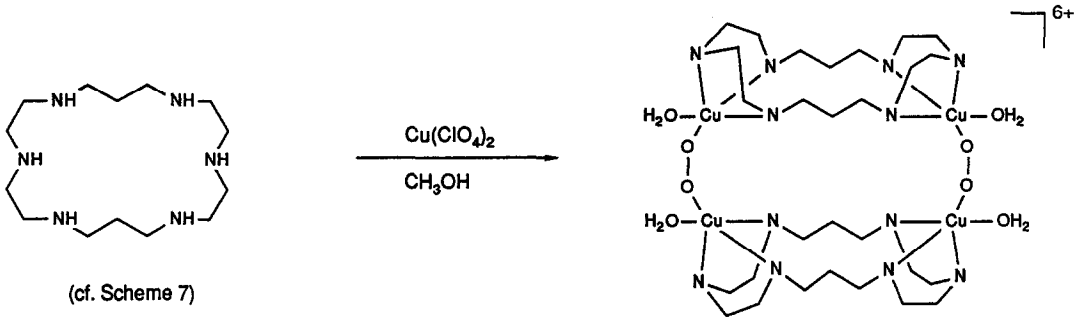
Some of the early approaches employed macrocyclic ligands, and the most successful of those was first reported in 1977.[139] The macrocyclic ligand is of the crown-ether type[140] and contains sulfur donors (Scheme 25), chosen as a model of the possible copper environment in hemocyanin. Recall that as late as 1977, there was some thought that sulfur ligation of the copper ions was likely.[22] In the solid state, complex **29** reacts with dioxygen to form a pale green species that is epr active, hence paramagnetic. The color change can be reversed by heating the product at 110° under vacuum. In solution, the reaction with O_2 is irreversible.



Scheme 25

The ligand for macrocyclic complex **30** (Scheme 26)[141] is prepared by reactions presented earlier (cf. Scheme 7), and a dioxygen adduct was isolated and structurally characterized. However, the structure is only marginally well-defined, and the charge balance requires that the O_2 units are actually bound as superoxide rather than peroxide; therefore, the relationship of **30** to oxyHc is questionable.

Beginning in the early 1980's, much of the work aimed at modeling oxyHc focused on the use of "open" (non-macrocyclic) ligands. By that time, further characterization of hemocyanin had revealed that there were two or three nitrogen ligands, and probably an oxygen-containing bridging group, coordinated to each copper ion (cf. Table IV).



(cf. Scheme 7)

30

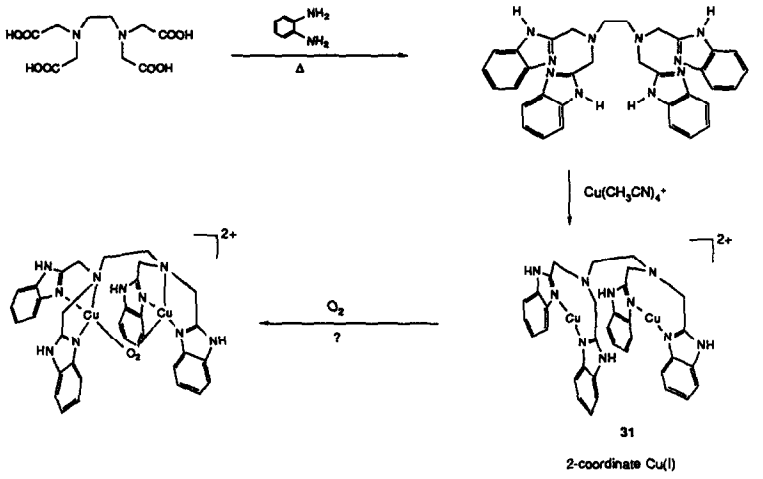
Scheme 26

Reedijk and co-workers reported the synthesis of a novel binuclear, two-coordinate Cu(I) dimer, **31**, and its reaction with dioxygen (Scheme 27).[142,143] An intriguing aspect of this complex is that while the copper ions are only two-coordinate in the reduced state, there is another potential donor for coordination in the oxidized form. The reaction of **31** with O_2 proceeds with a stoichiometry of $Cu:O_2 = 2:1$. However, no O-O stretch could be observed, and the reaction was apparently irreversible.

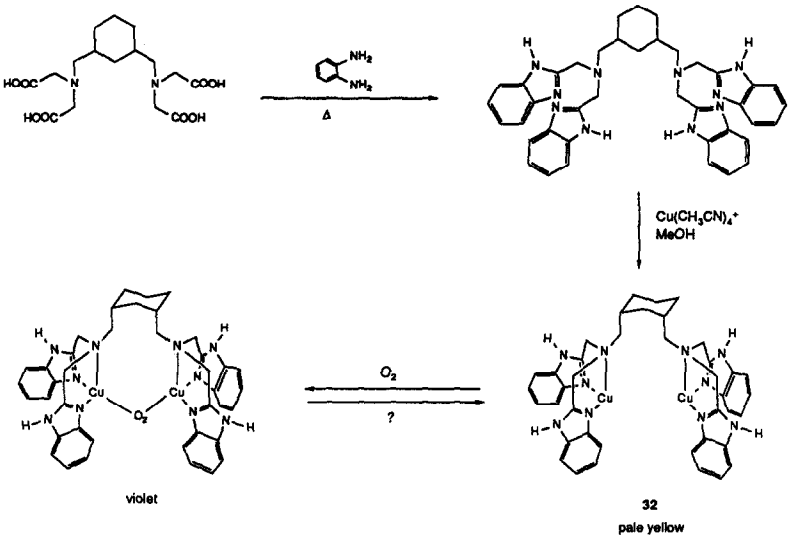
About the same time, Nishida and co-workers prepared a binuclear copper(I) complex which had three nitrogen donors bound to each metal ion (Scheme 28).[144] Complex **32** reacts semireversibly with dioxygen in methanol-acetonitrile at 5°, changing in color from pale yellow to deep violet. Unfortunately, no additional data about the nature of the O_2 adduct are given.

Closely related in structure to complex **32** are the binuclear complexes prepared by Karlin and co-workers. These species are the best reactivity models for hemocyanin studied so far. The systems have two, three-coordinate copper(I) ions ligated by nitrogen donors; and one type of complex also contains a bridging phenolate group.

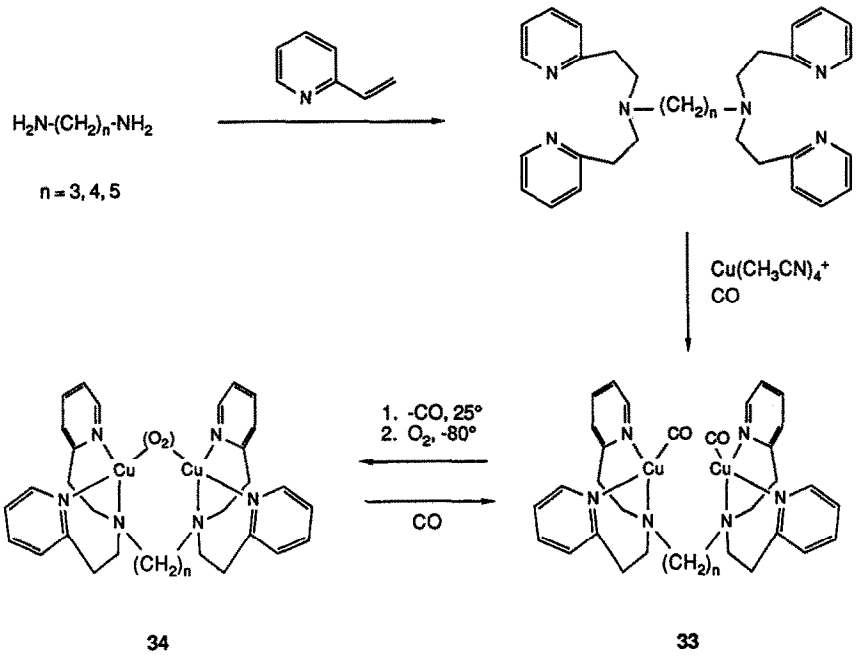
All of the ligands prepared by Karlin's group are based on the reaction of amines with vinylpyridine according to Figure 7.[51] The simplest systems have two $Cu(N)_3$ units linked by a number of methylene groups; and with these, Karlin has been able to generate dioxygen adducts that mimic the spectroscopic features of oxyHc to a certain degree.[145] The synthesis of the ligands follows that summarized in Scheme 29 using $H_2N\text{-}(CH_2)_n\text{-}NH_2$ ($n = 3,4,5$). The



Scheme 27



Scheme 28

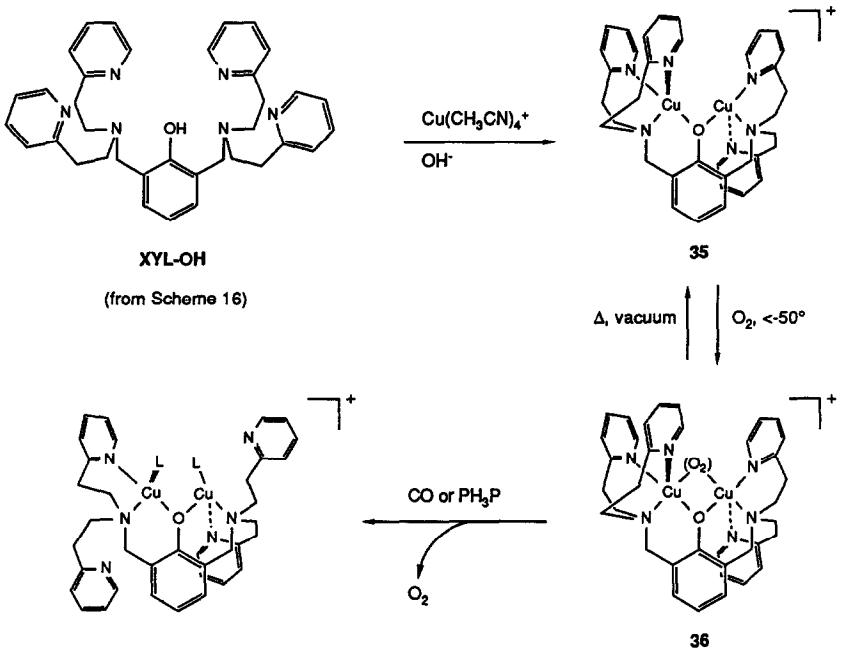


Scheme 29

resulting binuclear copper(I) complexes (**33**) react reversibly with O_2 at -80° in CH_2Cl_2 to give products characterized by manometric uptake and UV/vis spectra as copper(II)-peroxide species, **34**. The spectral similarity to oxyHc is quite good; the only shortcoming is that no crystallographic data are available yet. However, the stability of the adducts at low temperatures should permit the eventual isolation and structural characterization of the complexes.

More thoroughly characterized is the dioxygen complex prepared from a μ-phenolato copper dimer (Scheme 30).[146] The ligand in this case is prepared by a copper-mediated hydroxylation of an aromatic ring according to Scheme 16.[147]

Complex **35** reacts with dioxygen below -50° in dichloromethane to give an intensely-colored violet solution. Resonance Raman spectroscopy demonstrates that the complex **36** is certainly a $[Cu(II)]_2$-$O_2^=$ adduct, and the binding of dioxygen is reversible if the solution is warmed under vacuum. Carbon monoxide and triphenylphosphine displace coordinated O_2, even at low temperatures, forming four-coordinate Cu(I) derivatives. Because the UV/vis spectrum of **36** is so different from that of oxyHc, the structure is also probably quite different. Analysis of the electronic, vibrational, resonance Raman[148] and EXAFS[149] spectra is consistent with the notion that the peroxo ligand is essentially coordinated in a terminal fashion to one copper ion. An asymmetric μ-1,2 bridge cannot be ruled out, especially if the peroxo group is coordinated in an equatorial position of one copper ion and in an axial position of the other. Again, the lack of a crystal structure has frustrated a detailed comparison with the proposed structure of oxyHc.



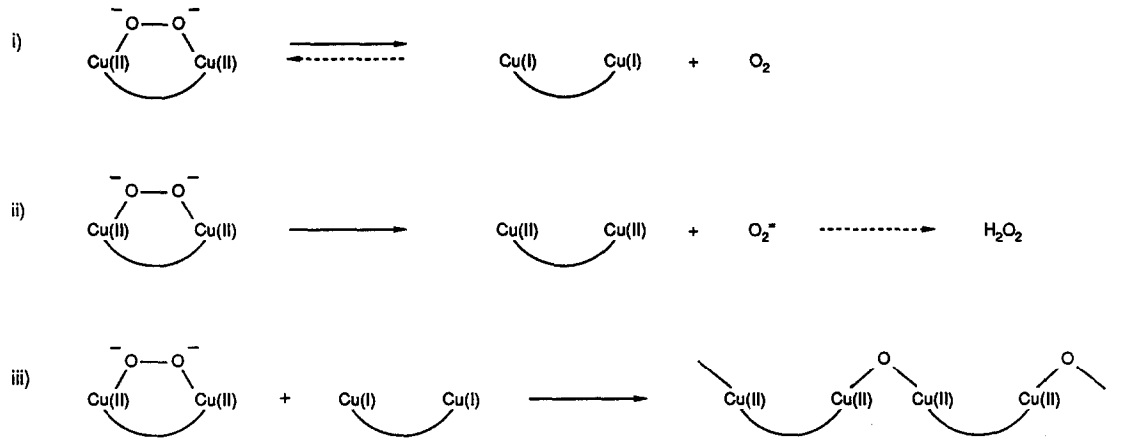
Scheme 30

5.3. *Future prospects*

Although there is now at least one structurally characterized copper-dioxygen adduct,[137] much work still needs to be done in order to model oxyHc. Based on the results summarized above, we can certainly rule out the possibility that the copper ions in oxyHc are trigonal-bipyramidal with the peroxo group coordinated through the axial positions.[137] We can also disregard the notion that the peroxy ligand is bound in a terminal fashion to only one copper ion.[148] However, we still need to generate structurally characterized complexes having tetragonal copper(II) ions with the peroxo unit in the cis-μ-1,2 geometry. It is fairly certain that this will require binucleating ligands because of the probability that mononuclear precursors will lead to the formation of the sterically less-hindered trans-μ-1,2-peroxo conformation.

Even when we understand how the dioxygen is bound and can correlate the spectroscopic properties of synthetic complexes with those for oxyHc, we still will not have begun to understand how the protein is able to facilitate *reversible* binding of O_2. So far, low temperatures have been used to prevent the side reactions which are summarized in Scheme 31; however, other ways need to be developed if a functional hemocyanin analog is to be made.

The simplest process to be prevented is the loss of dioxygen, that is, the reverse of O_2-binding. Low temperatures apparently favor the formation of the Cu(II)-peroxo species, since

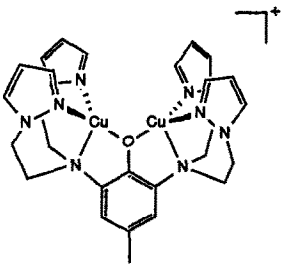


Scheme 31

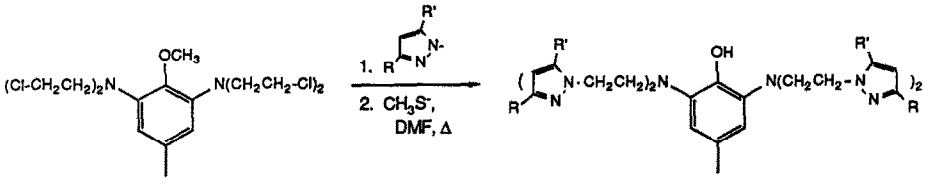
it is necessary to warm the O_2 complexes to above ambient temperatures to form the deoxy species in those systems reported by Karlin.[145,146] Another problem is the dissociation of the peroxide ion and its subsequent decomposition, perhaps via protonation to form hydrogen peroxide. Finally, the bimolecular redox reaction that results in the 4e$^-$ reduction of dioxygen must be stopped. If the complexes (e.g. **33**) that bind O_2 at low temperature are exposed to dioxygen at room temperature, then a stoichiometry of 4:1 Cu:O_2 is observed. Presumably, steric bulk must be included within the ligand to prevent the bimolecular reaction, analogous to the situation discovered in porphyrin chemistry a decade ago.[150]

Unfortunately, the solution to this last problem is not as straightforward as simply including sterically-demanding substituents in the ligand, and one example from our own work illustrates this. The binuclear complex **37** (Scheme 32) was synthesized using the ligand prepared by the route shown in Scheme 18. Like complex **35** prepared by Karlin, the ligand used to prepare **37** has two segments providing three nitrogen donors to each metal ion and a potential bridging phenolate group. Complex **37** does not form a dioxygen adduct, even at low temperature, because of the 4e$^-$ reduction of O_2.[151]

However, we planned the ligand synthesis so that structural modifications could be easily performed. The synthesis of derivatives proved to be straightforward, and the unsymmetrical sterically-hindered pyrazoles gave only the single products shown (Scheme 32).[152] Unexpectedly, the copper(I) adducts (**38**) of the resulting ligands were inert to dioxygen, even at room temperature. Thus, incorporation of larger substituents on the pyrazole groups also affected the electronic features of the ligand.



37

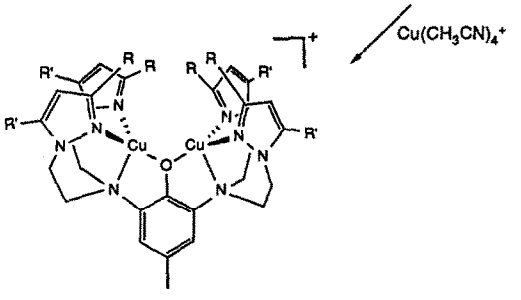


(Scheme 18)

R = t-Bu, R' = H

R = Ph, R' = H

R = CH_3, R' = CH_3



38

Scheme 32

We showed several years ago that hydrophobic effects could influence the redox potentials of copper complexes significantly.[153] Since the binding of dioxygen by copper(I) is a redox process, it is possible that the Cu(I)/Cu(II) potentials have been shifted enough to prevent the reaction of complexes **38** with O_2. Syntheses of new types of ligands are probably necessary to solve the problems associated with preparing binuclear copper complexes capable of reversibly binding dioxygen at room temperature.

6. MONOOXYGENASE MODELS

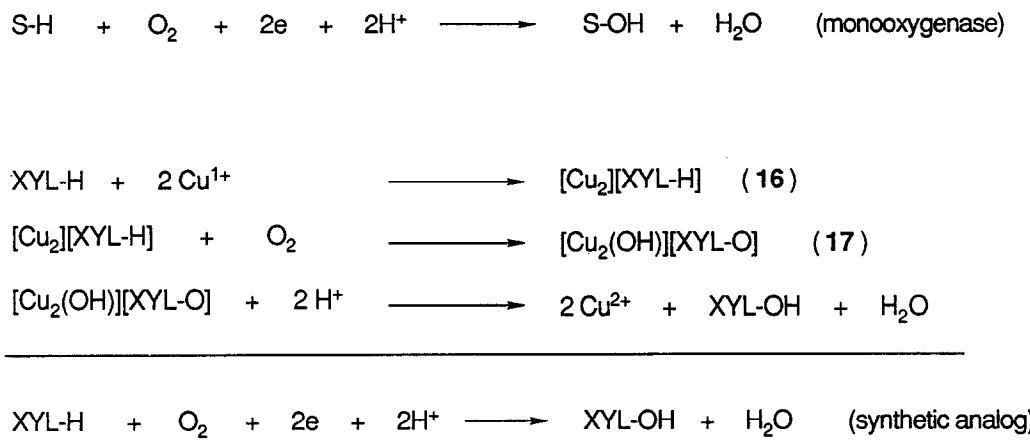
6.1 Synthetic complexes

As discussed in the introduction, tyrosinase is a monooxygenase having a binuclear copper-containing active site that is very similar to that in hemocyanin. Spectroscopic characterization of the oxy form of the enzyme indicates that dioxygen binds in the same μ-1,2-peroxo configuration. Apparently, the active site of oxyTyr is more accessible than that in oxyHc, and this fact accounts for its function as an oxygenase.

To date, the best model system for tyrosinase is that prepared by Karlin.[147] As was the case for modeling hemocyanin, the strategy for preparing a tyrosinase mimic involves the synthesis of a binucleating ligand that can position two copper ions within *ca.* 3.5 Å of each other while providing two or three nitrogen donors to each metal ion.

The system investigated by Karlin is based on the *m*-xylyl derivative shown in Scheme 16. The dicopper(I) complex **16** reacts with dioxygen in DMF or CH_2Cl_2 to give the μ-phenoxo-μ-hydroxo complex **17**. The use of $^{18}O_2$ in the reaction shows that both the phenolate and the hydroxide oxygen atoms come from dioxygen. The stoichiometry is identical to that of monooxygenase-catalyzed reactions (Scheme 33), in which one atom of the oxygen molecule is incorporated into substrate, and the other is converted to water. In this case, the "water molecule" binds to the copper ions as hydroxide ion; but upon demetallation of the ligand, the hydroxide group is liberated as water.

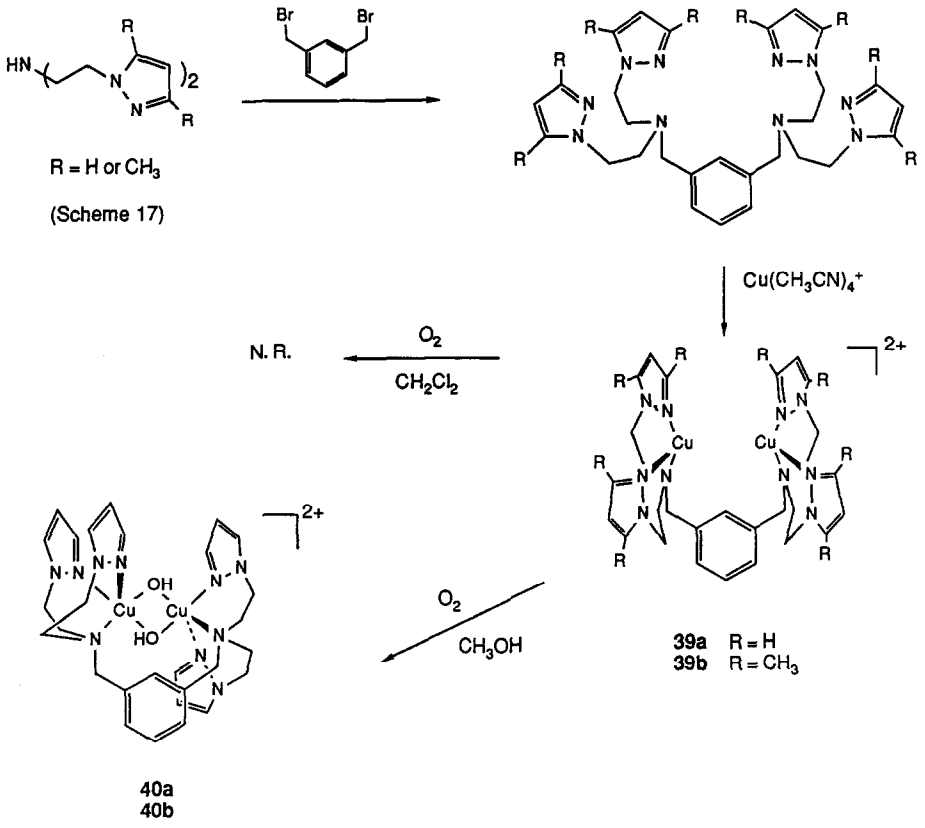
To understand how either tyrosinase or complex **16** is able to activate dioxygen requires knowing about the nature of the active intermediate. The synthetic system lends itself to systematic modification and so has proven a fertile area for work aimed at probing the mechanism.



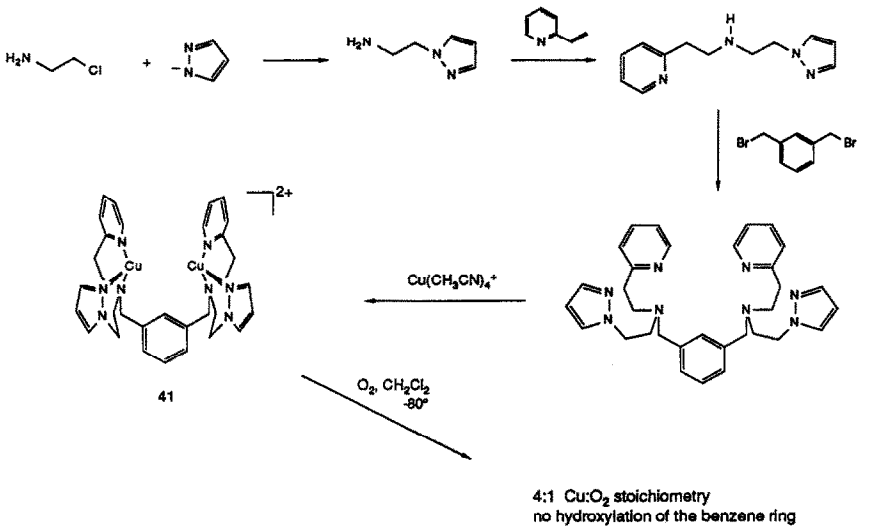
Scheme 33

One obvious modification that can be imagined is the substitution of pyridine by another heterocycle. Our own group prepared the pyrazole and dimethylpyrazole analogs (**39**) according to Scheme 34.[154] Not only do the Cu(I) complexes not catalyze the hydroxylation of the aromatic ring, they do not even react with dioxygen in CH_2Cl_2. In methanol, the products of **39** and O_2 are the bis(μ-hydroxo) species **40**. Probing further, we prepared the mixed pyridine-pyrazole complex **41** (Scheme 35).[155] In this case, the reaction with dioxygen proceeded smoothly (in a 4 Cu: 1 O_2 stoichiometry) but again, no hydroxylation of the aromatic ring occurs. The results suggest that electronic factors are very important; in particular, the much lower basicity of pyrazole relative to pyridine (Table V) must have a substantial influence on the reactivity.

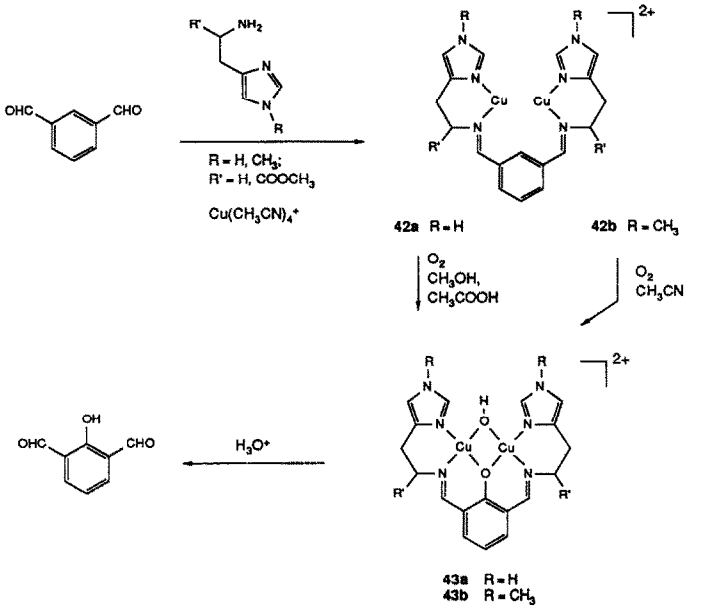
Interestingly, the substitution of imidazole for pyridine, although with a ligand that provides only one heterocyclic donor to each copper ion, also led to arene hydroxylation. Casella and co-workers prepared the Schiff-base ligand shown in Scheme 36.[156] Complex



Scheme 34



Scheme 35



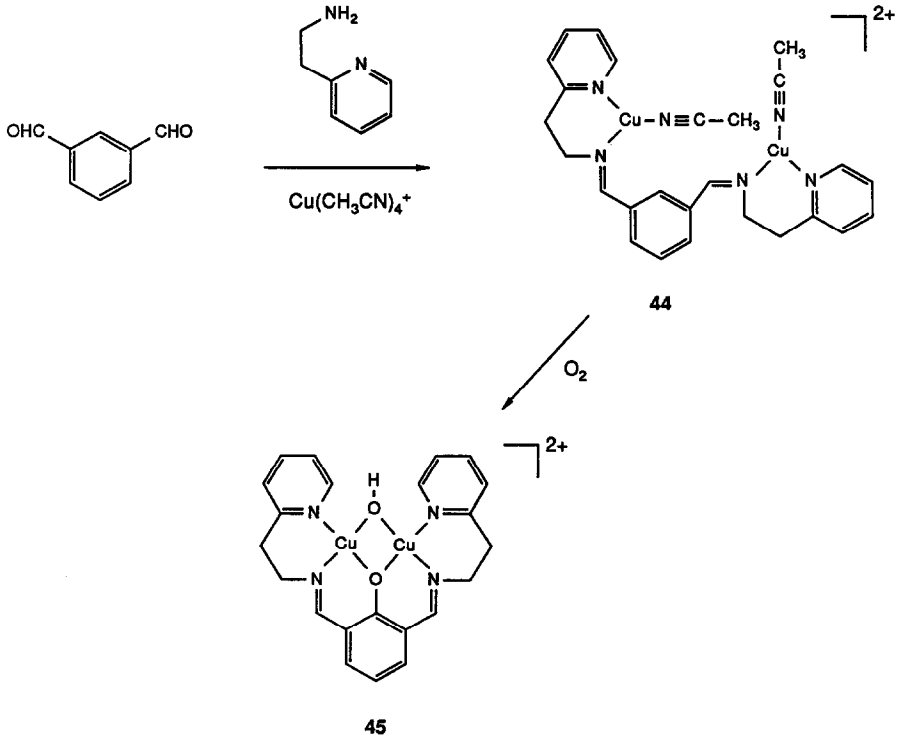
Scheme 36

42b (R = CH_3) which is reported to be two-coordinate, reacts with dioxygen in dry DMF or CH_3CN in the ratio 2:1 Cu:O_2 to give the hydroxylated complex **43**, whereas **42a** gives the hydroxylated product in good yield only in a protic medium. In aprotic solvents, **42a** reacts with dioxygen in a 4:1 Cu:O_2 ratio, resulting only in the $4e^-$ reduction of the oxygen molecule. Hydrolysis and demetallation of **43** produces the hydroxylated dialdehyde.

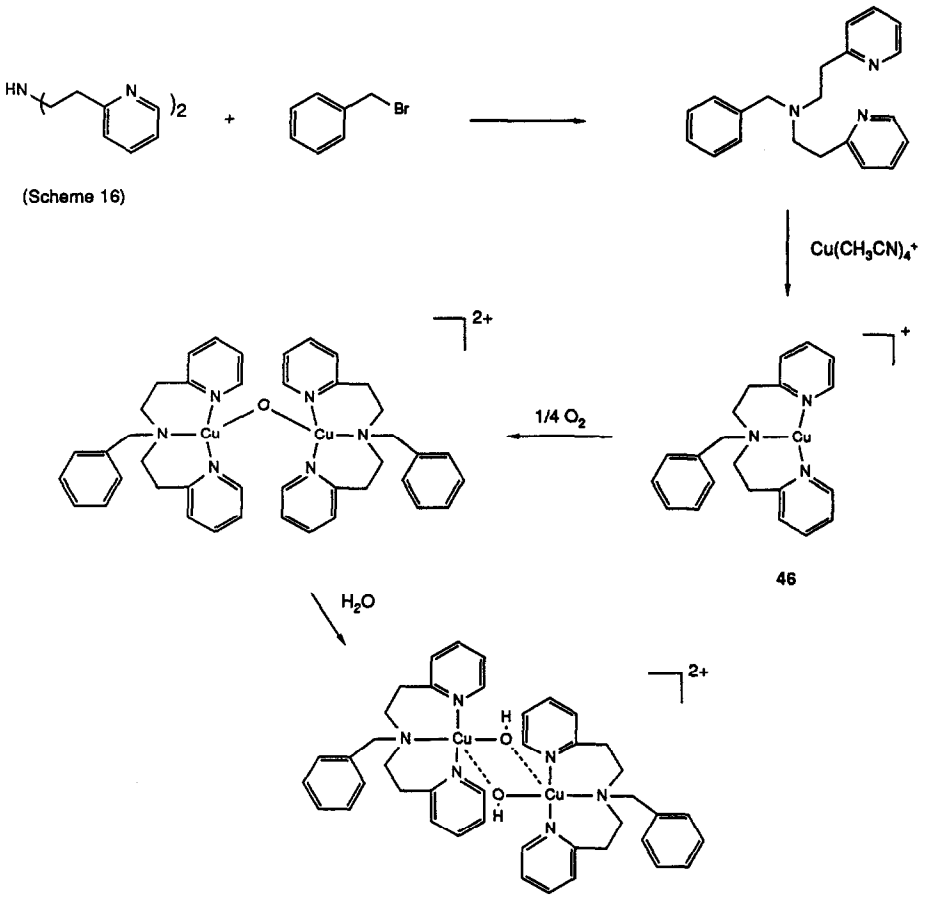
Related to **42** is the complex **44** which has pyridine instead of imidazole (Scheme 37).[157] In an analogous way, reaction of three-coordinate **44** with dioxygen gives the hydroxylated arene complex **45**.

While the study of ligating groups other than pyridine (in complexes **38**, **41**, and **42**) has given us little information about the nature of the active oxidant, modifications of the aryl portion of complex **16**, keeping the bis(pyridylethylamine) groups constant, have been revealing.

The simplest modification that Karlin performed was the removal of one of the copper-ion units (Scheme 38).[158,159] The mononuclear complex **46** reacts with dioxygen in a 4:1 Cu:O_2 ratio (although at low temperature), and no hydroxylation of the aromatic ring occurs. This experiment suggests that two things must happen in the hydroxylation reaction: 1) a peroxo intermediate must be formed; and 2) the arene and O_2 units must be in the proper orientation.



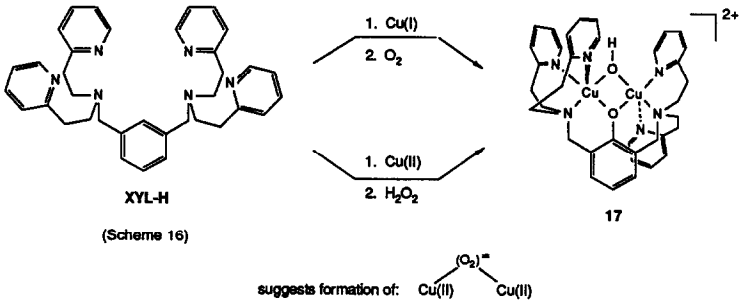
Scheme 37



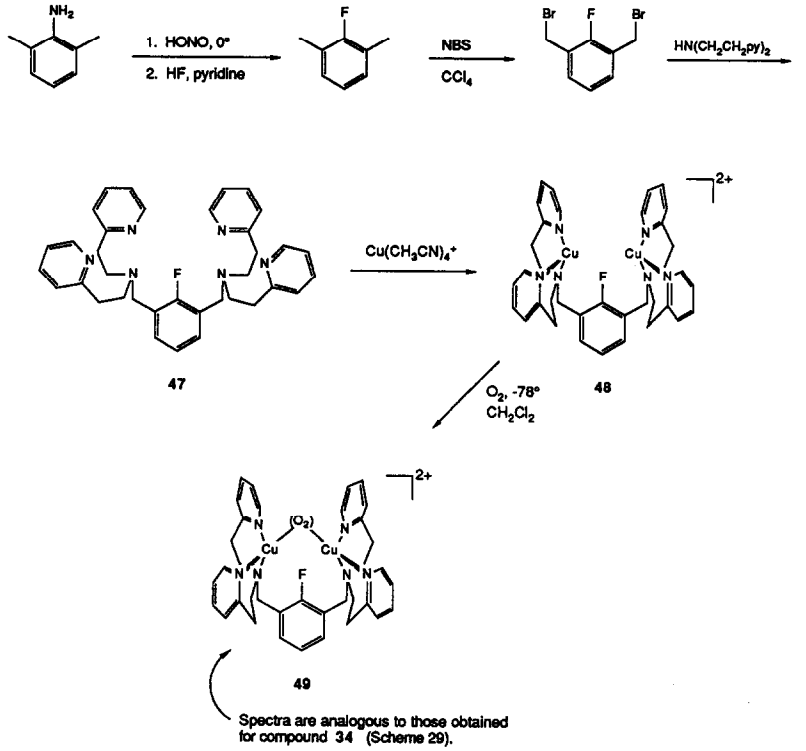
Scheme 38

The first criterion is more easily probed. When the Cu(II) derivative of the binucleating ligand XYL-H (Scheme 16) is treated with H_2O_2, the same reaction occurs as that when the Cu(I) species **16** is treated with O_2 (Scheme 39). However, recent kinetic studies reveal that the mechanism must be different because 4 copper ions and hydroperoxide are involved when starting with the copper(II) dimer, whereas hydroxylation utilizing the copper(I) precursor involves only an intramolecularly-bound oxygen moiety.[159b]

More direct evidence for a peroxo intermediate in the hydroxylation using molecular oxygen comes from the following experiments. Using the fluoro-substituted binucleating ligand **47**,[160] prepared by the route shown in Scheme 40,[161] Karlin was able to prepare the corresponding Cu(I) derivative **48**. Complex **48** reacts with O_2 at -80° in CH_2Cl_2 to give a species formulated as the peroxy adduct **49** based on the reaction stoichiometry (2:1 Cu:O_2) and the similarity of the UV-vis spectrum to that observed for the binuclear Cu(II)-peroxo

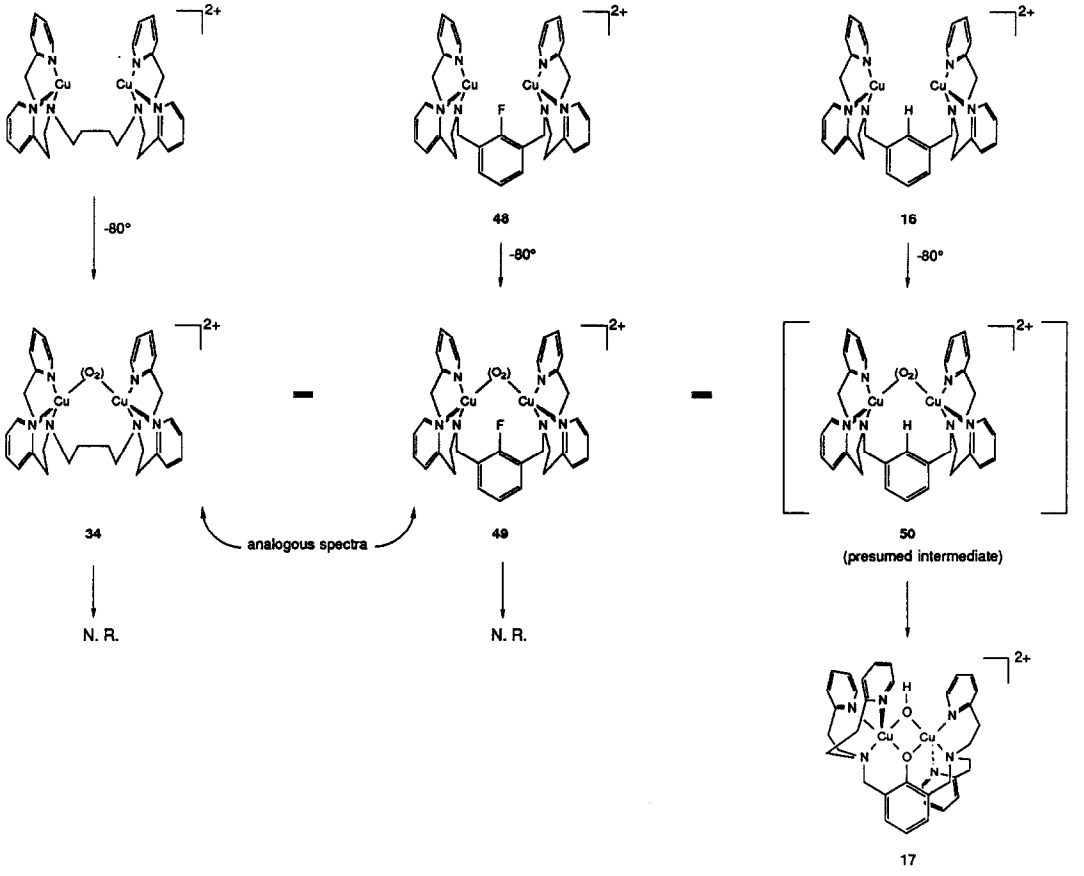


Scheme 39



Scheme 40

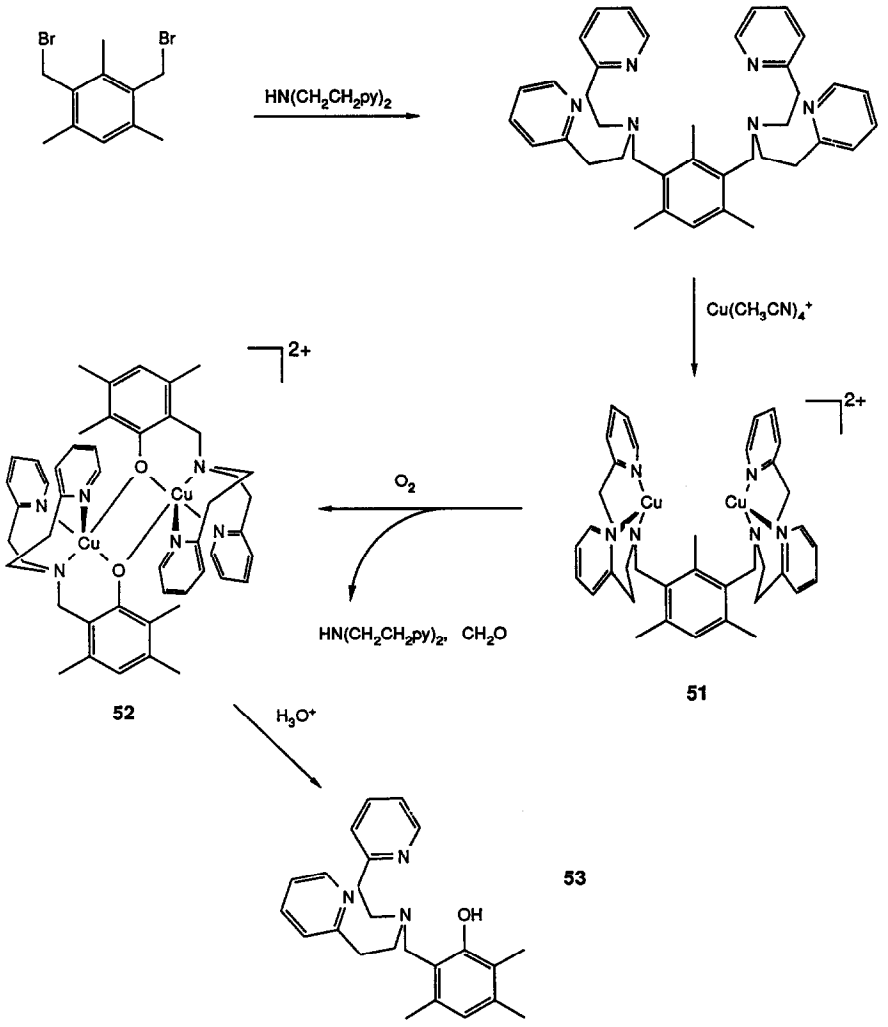
species **34** prepared as oxyHc mimics (see Scheme 29). The assumption is made that complex **16** and **48** should form identical products with dioxygen (**50** and **49**, respectively; Scheme 41). However, only **16** is able to undergo subsequent hydroxylation. Since the m-XYL-F complex apparently forms a peroxo adduct, by analogy to the characterized aliphatic species **34**, then so must the XYL-H complex. It is peroxo intermediate **50** then that is responsible for the observed hydroxylation.



Scheme 41

Although a peroxy intermediate is implicated as the active species in the experiments summarized above, those results are inconsistent with the observed chemistry, which seemingly requires the presence of electrophilic oxygen. Another modification of the parent ligand shows that the copper-peroxo species formed in these XYL systems is electrophilic in nature.

The reaction of 2,4-bis(chloromethyl)mesitylene with bis(pyridylethyl)amine (Scheme 42) followed by treatment with $Cu(CH_3CN)_4^+$ gives the binuclear copper(I) complex **51**, in which the hydroxylation site in the parent compound has been blocked with a methyl group.[162] For **51**, reaction with dioxygen gives a mixture, from which complex **52** is isolated in >68% yield (formaldehyde is detected in 81% yield). Alternatively, the product may be demetallated to give compound **53**. The products obtained arise from migration of the methyl group, from the position that is hydroxylated, to the *ortho*-position, with concomitant loss of one side-arm. The latter hydrolyzes to CH_2O and bis(pyridylethyl)amine under the conditions of the reaction.

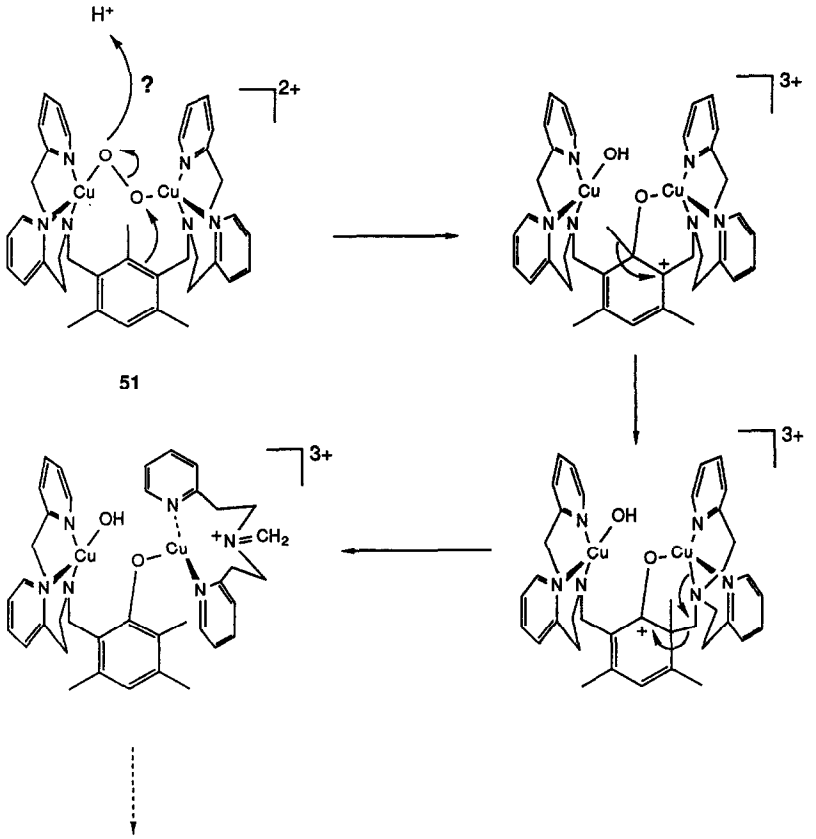


Scheme 42

The reaction that occurs between **51** and O_2 is reminiscent of the NIH shift,[163] which suggests the presence of an oxo-intermediate with some electrophilic character. Perhaps the reaction proceeds by a process like that shown in Scheme 43.

The studies reported by Karlin raise an important question: how can a peroxo intermediate, which is presumably nucleophilic, act as an electrophilic oxidant? The answer to this question is relevant to the action of tyrosinase as well.

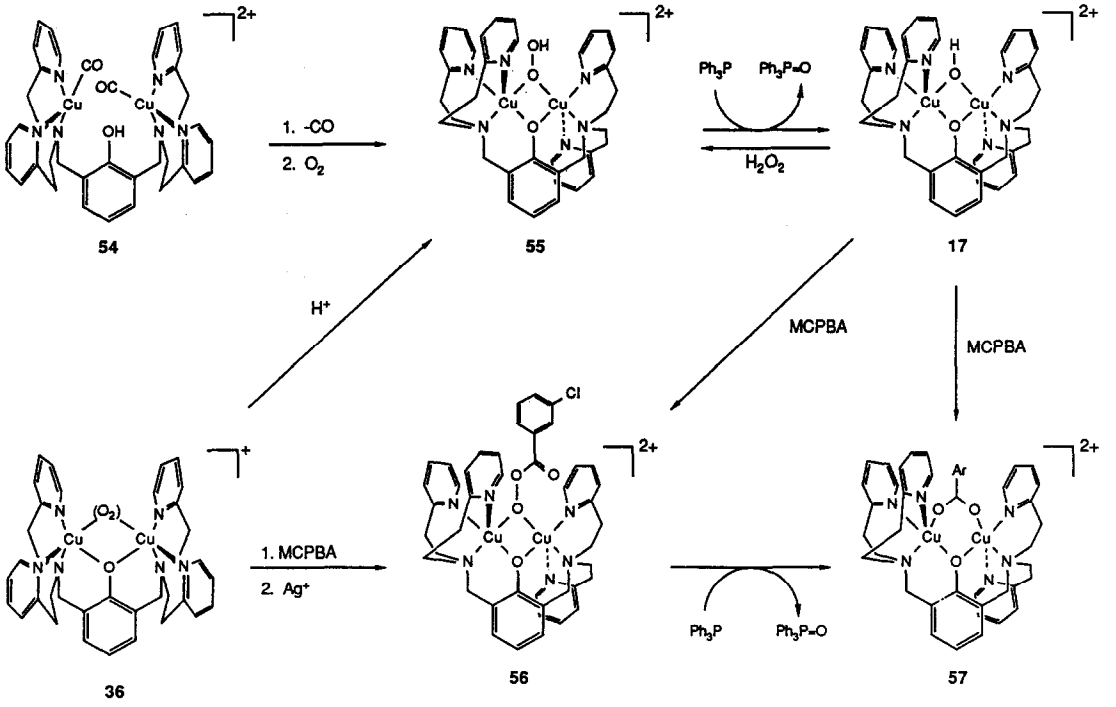
One possibility is that the reactions are assisted by protons (see the "?" in Scheme 43). Certainly in the system studied by Casella,[156] a proton source is necessary in certain cases for complete hydroxylation of the aromatic ring. Too, the reaction of [Cu(II)]2[XYL-H] with H_2O_2 apparently occurs *via* a hydroperoxide complex.[159b] However, at least two of Karlin's systems require no apparent external proton source, so perhaps there are two different mechanisms that can lead to hydroxylation.



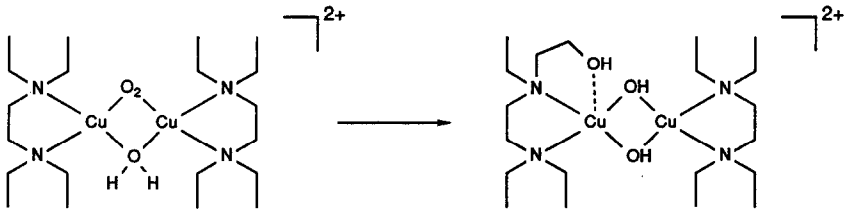
Scheme 43

Karlin has begun to probe the point of hydroperoxide intermediates with the synthesis of hydroperoxo-(**55**)[164] and acylperoxo-(**56**) [165] copper dimers (Scheme 44). Reaction of the hydroperoxo- or acylperoxo-complex with Ph_3P gives $Ph_3P{=}O$, showing that the protonated (or acylated) oxo-species is clearly electrophilic. This result may be used in the future to address the issue of substrate-oxidant orientation that was mentioned earlier.

In connection with both issues of proton assistance and orientation effects, one other complex deserves comment. The ligand of dioxygen adduct **25** prepared by Thompson (Scheme 21) undergoes oxidation upon standing at room temperature (Scheme 45),[133] and the product has been characterized by X-ray crystallography. The reaction is carried out in a protic solvent (methanol containing water slightly in excess of complex), so proton assistance may play a role in the mechanism. Analogs of complex **25** could prove useful for probing the effects of orientation of the C-H bond and the peroxo group.



Scheme 44



25 58

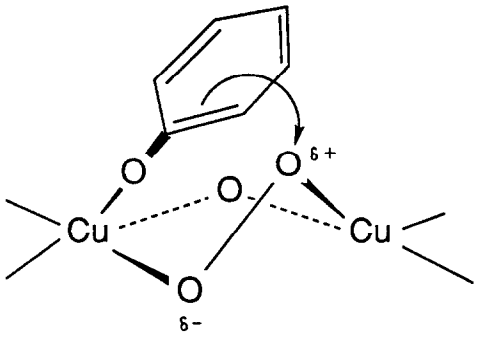
Scheme 45

6.2. A proposed mechanism for the hydroxylation of arenes by binuclear copper systems

As noted above, the function of tyrosinase, as well as the model systems, presents a striking dichotomy: how can a peroxo intermediate, which is presumably nucleophilic, act as an electrophilic oxidant? The possibility that protons assist the reaction has been mentioned in connection with Schemes 36, 39, and 43 and in reference 46. However, that explanation may not suffice in every case: for example, it appears that the hydroxylation of the aromatic ring in Karlin's model complex **16** proceeds in dichloromethane *without* proton assistance.

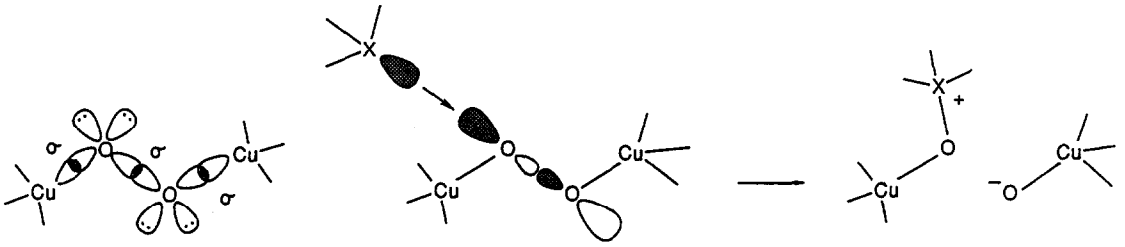
An alternative has been suggested by Solomon, *et al*,[46] and embraces the notion that one oxygen atom of the peroxide unit acquires partial electrophilic character which is situated for attack by the substrate (Figure 15). This postulate is based on the observation that the coordination geometry of one of the copper(II) ions in tyrosinase changes from tetragonal toward trigonal bipyramidal, with concomitant labilization of the peroxide moiety. Calculations on iron porphyrin dioxygen complexes are used by analogy to suggest that the oxygen atom bound to the nonlabilized copper ion will have a partial positive charge.[46]

Figure 15. Structural mechanism for the hydroxylation of a phenolic substrate by oxytyrosinase (from reference 46).



A simpler interpretation, which circumvents the disquieting thought that a peroxide ion might acquire even a *partial* positive charge, is provided by the illustration in Scheme 46. In this representation, the electron pair of the substrate molecule approaches along the axis of the O-O bond, colinear with the outer lobe of the σ*-orbital of the peroxide ion. Donation of two electrons into that orbital populates the antibonding energy level, causing rupture of the O-O bond and formation of the X-O bond.

In the model systems described by Karlin, triphenylphosphine is rarely a good substrate, a surprising result when one considers that an aromatic ring (in **16**) is readily oxidized, even at -80°. However, triarylphosphines are fairly bulky nucleophiles, and the peroxy-copper dimers are hindered, especially if they have the proposed[145] "butterfly" structure shown in Figure 16a. Even if those compounds are μ-1,2-peroxy species, the reaction coordinate must be along a path that is probably blocked by the ligand (Figure 16b). Consistent with this notion, the polymethylene-bridged complexes (e.g., **34**, Scheme 29), which are the least-hindered of the copper peroxo adducts, are the most reactive toward triphenylphosphine.

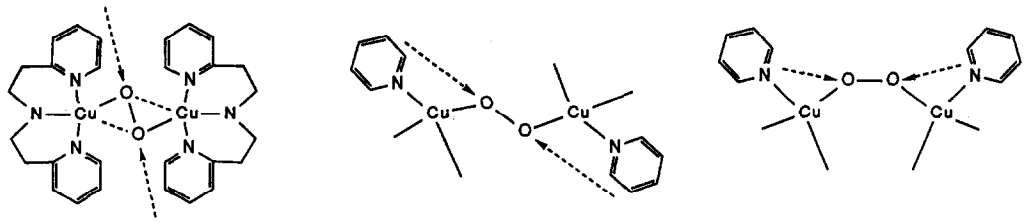


bonding and non-bonding orbitals of the peroxide group in Cu_2O_2

antibonding orbital of the peroxide group in Cu_2O_2

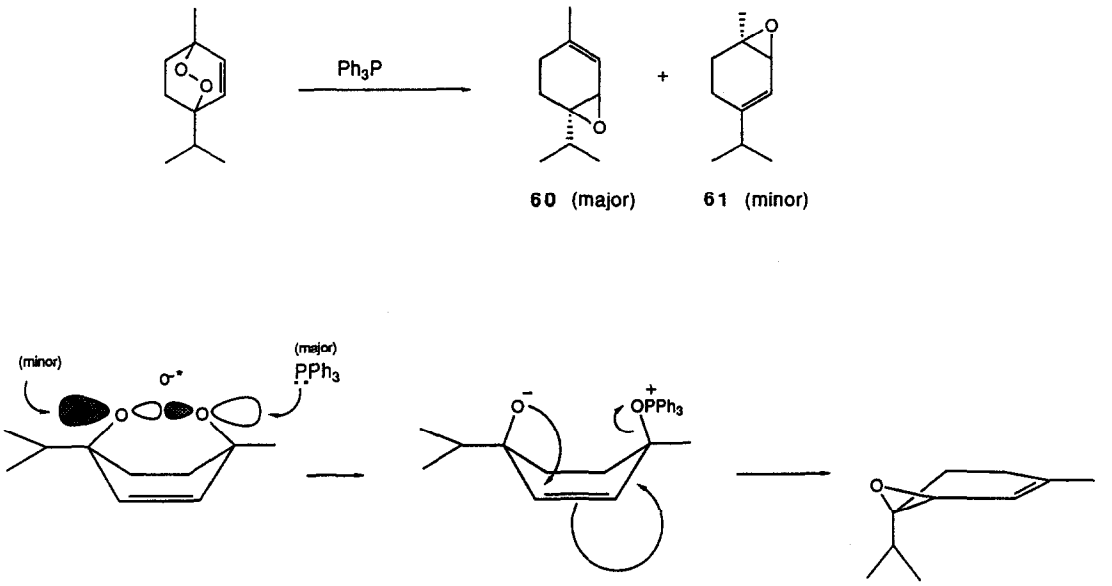
Scheme 46

Figure 16. Approach vectors for a nucleophile reacting with binuclear copper-peroxide complexes at the O-O σ* orbital.



(a) (b)

Steric effects are observed even for relatively simple organic peroxides, as illustrated in Scheme 47.[166] In the case of ascaridole, **59**, the major product of the reaction, **60**, results by attack from the side of the less-hindered, methyl-substituted carbon atom. Thus, it is not surprising that the copper-peroxide adducts are not attacked by triphenylphosphine.

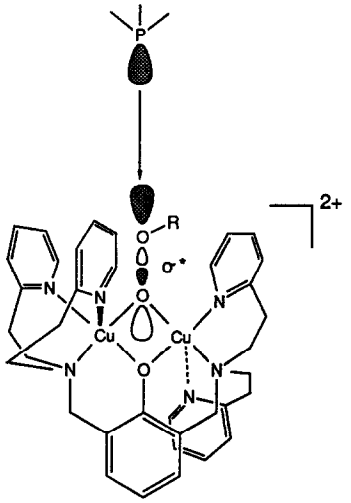


Scheme 47

However, the hydroperoxide (**55**) or acylperoxide (**56**) species, both of which *do* oxidize triphenylphosphine, have a μ-1,1 geometry as illustrated in Figure 17a. Now the attack vector lies normal to the Cu-Cu bond and should not be affected by the ligand bulk. The only apparent enigma is the peroxy complex **36**, which is proposed to have a terminal, or asymmetrically-bound, peroxide ligand (Figure 17b).[148] While the peroxide group may be accessible, it will carry a full negative charge on the terminal atom, which could shield the σ*-orbital from attack by repulsion of the nucleophile lone-pair.

Phosphines and sulfides are actually not very good substrates to probe the reactivity of the peroxy-copper complexes because decomposition products resulting from protonation of the peroxide ligand can give hydrogen peroxide which itself is able to oxidize these substrates in solution. Thus, the structurally-characterized complex **28** (Scheme 24), which is inert to triphenylphosphine, does oxidize Ph_3P in solution upon addition of acid, probably by forming H_2O_2.[137]

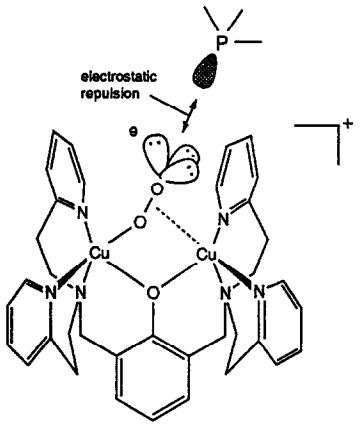
Figure 17. Oxidation of triphenylphosphine by $Cu_2(OOR)$ complexes



55 R=H

56 R = CO-C_6H_4-Cl

(a)



36

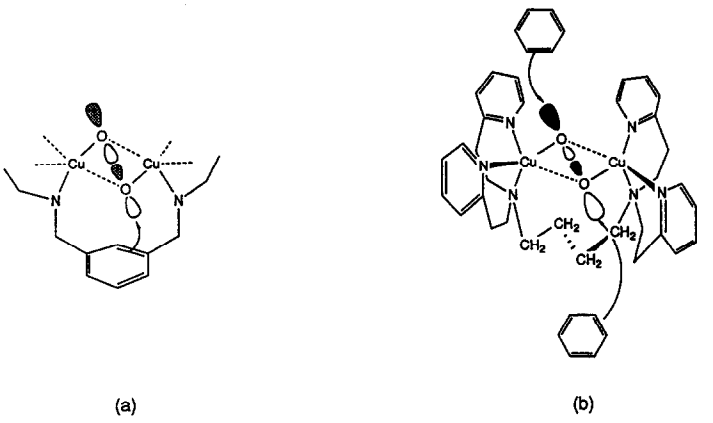
(b)

On the other hand, arenes are ideal substrates to probe the oxidation process because they are generally not attacked by hydrogen peroxide in solution, therefore their oxidation must result from interaction with the copper-peroxide unit. In that regard then, the observed hydroxylation of complexes **16** (Scheme 16), **42b** (Scheme 36), and **44** (Scheme 37) most likely results from attack of the arene π-electrons on the σ*-orbital of the coordinated peroxide as shown in Figure 18a. The fact that the peroxide adducts **34** (Scheme 29) and **49** (Scheme 40) do not oxidize arenes via an *intermolecular process in solution* supports the notion that the peroxide group is inaccessible because of the steric hindrance imposed by the ligand (Figure 18b).

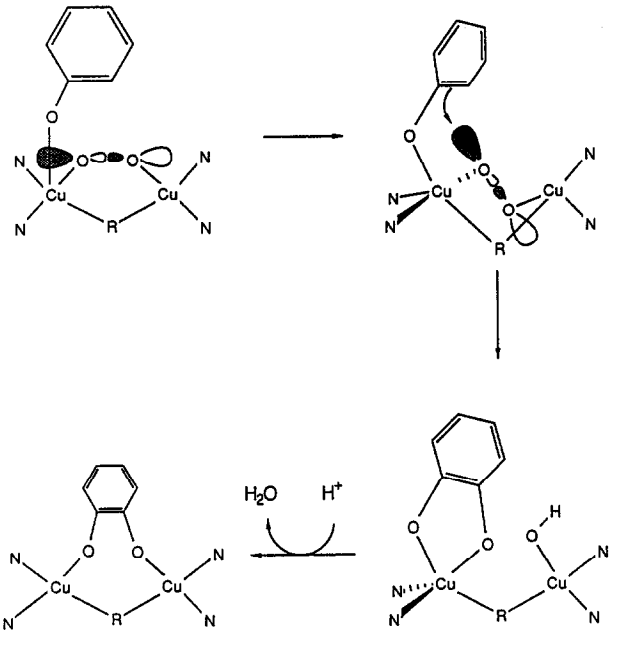
The action of tyrosinase can also be explained by the concept of arene attack on the peroxide σ*-orbital. In this case, the phenolate coordinates in the axial position of the tetragonal copper(II) ion, as illustrated in Scheme 48. The coordination environment then undergoes a distortion to the trigonal bipyramidal geometry, as described previously by Solomon,[46] aligning the phenol π-system with the O-O bond axis. The remaining steps occur as shown in Figure 5.

To investigate this proposal further will require the synthesis of structurally characterized peroxy-copper complexes with aromatic residues fixed in different positions with respect to the O-O bond axis. Organic synthesis of the requisite ligands will certainly play a significant role in such studies.

Figure 18. Hydroxylation of arene-containing ligands by copper-peroxide adducts



(a) (b)



Scheme 48

7. CONCLUSIONS

Research aimed at modeling the active site in the binuclear copper proteins hemocyanin and tryosinase will continue. There have been many exciting results that indicate that it is possible to prepare synthetic analogs that bind and activate dioxygen as well as mimic the spectroscopic and physico-chemical properties of various derivatives of these proteins. The range of binucleating ligands summarized in this review is broad; but certainly, other metal-ion geometries and orientations, as well as donor groups can be explored. Ligands that incorporate imidazole residues are especially desirable and still largely unexplored, and might lead to the synthesis of better biomimics.

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