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PREPARATION OF AN N_3S TETRADENTATE LIGAND AND VARIOUS RELATED LIGANDS AND THEIR REACTION WITH CUPRIC SALTS--TOWARD A MODEL FOR THE BLUE COPPER PROTEINS

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ABSTRACT

PREPARATION OF AN N_3S TETRADENTATE LIGAND AND VARIOUS RELATED LIGANDS AND THEIR REACTION WITH CUPRIC SALTS--TOWARD A MODEL FOR THE BLUE COPPER PROTEINS

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Methods for preparing a model for the blue copper proteins have been investigated. These have included the synthesis of tetradentate ligands containing the following donor sets: N_2(imidazole), N(amine), S(aromatic thioether); N_2(imidazole), N(amine), S(alipathic thioether); N_2(imidazole), N(amine), S(aromatic thiol); and N(imidazole), N(amine), S(aliphatic thiol), S(alphatic thioether). The synthesis of these ligands is reported, in addition to the results of the reactions of these ligands with various cupric salts. It has not been possible to duplicate the unusual properties of the blue copper proteins with these ligands, partially because of retention of a solvent molecule as a fifth ligand. Plans for overcoming this difficulty are discussed.

For Kitty, who helped.

ACKNOWLEDGMENTS

I would like to extend my thanks to Bruce Averill for his help and guidance in this project, and for sharing his understanding of its difficult aspects. I would also like to thank Dr. Achyuta Chaudhuri for his companionship while this project was underway, and for the teaching he always gave. Also, I am especially grateful for my parents' and my family's support during this time.

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DCCI -- Dicyclohexylcarbodimide

DMF -- N,N-dimethylformamide

EPR -- Electron paramagnetic resonance

HMPA -- Hexamethylphosphorotriamide

NHBT -- N-hydroxybenzotriazole

THF -- Tetrahydrofuran

INTRODUCTION

One of the most important aspects of biological chemistry is the branch that deals with the mechanisms living systems have devised to contain the potentially destructive forces generated by the processes of energy utilization, and to channel these forces into useful and constructive pathways.

These mechanisms are often the result of the activity of compounds with unusual, even unique, properties. Of these compounds, the copper-containing proteins are among the most interesting. This thesis describes an attempt to model some aspects of the copper proteins. This was done by the synthesis and characterization of low molecular weight cupric complexes which contained some of the structural features of one of the types of copper found in proteins, the so-called "blue," or Type 1 copper, and comparison of the properties of the complexes with those of the proteins.

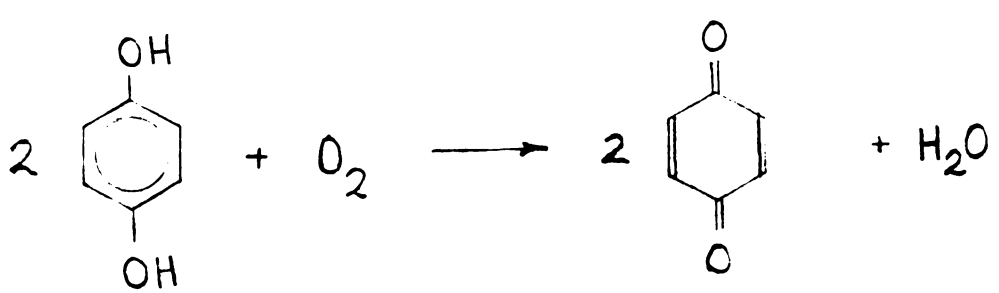
The fact that copper is an essential component of certain enzymes was first recognized in the 1930s by Kubowitz[1] and by Keilin and Mann,[2,3]. These enzymes had earlier been identified as involved in the catalytic

oxidation of phenols by Bertrand,[4] and had been given the general term "oxidases."

The copper-containing enzymes now appear to have four major metabolic roles:

1. Oxidation-reduction catalysts
2. Oxygen transport
3. Superoxide dismutases
4. High protential electron transfer

1. Oxidation-reduction catalysts--Among the enzymes serving as oxidation-reduction catalysts are one of the "oxidases" discovered by Bertrand, tyrosinase, which is found in the fungus Neurospora crassa,[5] and the laccases from the fungus Polyphorus versicolor and the laquer tree Rhus verncifera, which catalyze the oxidation of p-diphenols[6,7].



2. Oxygen transport--Copper proteins are also active in oxygen transport. Hemocyanin, found in arthropods, serves a function analogous to that of hemoglobin in mammals.[8]

3. Superoxide dismutases--Superoxide dismutases, found in a wide variety of organisms, are responsible for removal of toxic superoxide anion, which is known to be generated by physiological processes.[9] These enzymes contain copper and zinc in a 1:1 ratio. Superoxide dismutases which contain manganese and iron are also known.

4. High potential electron transfer--Azurin, from bacteria of the genus Pseudomonas,[10] stellacyanin, from Rhus vernicifera,[11] and plastocyanin, found in the chloroplast of green algae,[12] spinach,[13] French bean,[14] and parsley,[15] are examples of the fourth type of copper protein. Plastocyanin is responsible for election transfer from photosystem I to photosystem II in green plants. The other members of this group may function as high potential electron transfer agents. These proteins, as well as some of the others mentioned, are distinguished by an intense blue color.

The copper in the copper containing oxidases and supposed election transfer enzymes appears in three quite distinct types. The first of these is "blue" copper, generally referred to as Type 1 copper. This is the copper that gives the electron transfer proteins and some of the oxidases their blue color, a color about 100 times as intense as that found in almost all other known copper (II) complexes.

The Type 1 copper exists as the cupric ion in the resting enzyme. Its properties are the most striking of the three types. First, there is the intensely dark color. This results from an absorption at about 600 nm having an extinction coefficient of 3,000 - 5,000 $M^{-1}cm^{-1}$ per copper. In addition, Type 1 copper has an EPR spectrum which shows an unusually small hyperfine coupling constant $A_{||}$, and a reduction potential of +180 to +770 mV, values higher than the +158 mV potential of the aqueous Cu^{++}/Cu^{+} couple.[16]

The EPR and optical properties of Type 2 copper are more reminiscent of the properties of small copper complexes. Type 2 is sometimes called "colorless" copper, although it is colorless only in comparison with Type 1. Some of the oxidases, such as laccase, contain both Type 1 and Type 2 copper, while others, such as tyrosinase and galactose oxidase, contain only Type 2. Some investigators claim to see significant differences in the chemistry of the two instances of Type 2 copper, and subdivide the classification accordingly.

The oxidases containing both Type 1 and Type 2 also contain the third type of copper, Type 3, as do the oxygen transport proteins. This third type is essential for oxygen reduction. It acts as a 2 electron donor, absorbs at 330nm, and is EPR silent. This type apparently

consists of either two antiferromagnetically coupled Cu^{2+} ions or a $2Cu^{1+}$-RSSR center.

Some of the copper containing proteins, their functions, and their specific copper content, are listed in Table 1.

Because of its unusual properties, the Type 1, or "blue" copper center has attracted interest. Studies have generally advanced along three fronts: investigations of the ligand environment of the ion, analysis of the optical properties, and EPR.

In the first area, Williams, writing in 1973, ascribed the intense blue color to a metal to thiolate ligand charge transfer.[17] Solomon and Gray, in 1975, also predicted the presence of a thiolate ligand, based on X-ray photoelectron spectroscopy,[18] and this conclusion has been supported by chemical evidence in the case of azurin[19] and plastocyanin.[20] König and Brown have demonstrated that the cupric ion does not have a coordination site open to water.[21]

Additionally, Vänngard,in an electron nuclear double resonance (ENDOR) experiment, found at least one nitrogen coordinated to copper in stellacyanin,[22] and Mims and Peisach conducted a pulsed EPR study suggesting that the nitrogen is from an imidazole.[23] Nuclear magnetic resonance studies by Hill on azurin,[24] and Krogman on

TABLE 1.--Copper Proteins

		Copper Content			
		Total	Type 1	Type 2	Type 3
1.	*Polyporus* laccase	4	1	1	2
	Rhus laccase	4	1	1	2
	Ascorbate oxidase	8	2	2	4
	Galactose oxidase	1	0	1	0
	Dopamine-β-hydroxylase	4-7	0	100%	0
2.	Hemocyanin	2	0	0	2
3.	Superoxide dismutase	2+2 Zn	--	--	--
4.	Stellacyanin	1	1	0	0
	Azurin	1	1	0	0
	Plastocyanin	2	2	0	0

Note: The table lists some representative copper enzymes from the four functional groups, along with their total copper content, and their content of each type of copper. (1) Oxidation-reduction catalysts; (2) Oxygen transport enzymes, (3) Superoxide dismutase, and (4) High potential electron transfer enzymes.

plastocyanin,[25] indicate the coordination of two imidazole nitrogen atoms. Shapiro, using resonance Raman spectroscopy, predicted a trigonal bipyramidal structure around copper with sulfur and two nitrogens in the equatorial plane and nitrogen or oxygen in weak axial positions,[26] but Siiman, also using resonance Raman data, predicted a distorted tetrahedral geometry, and amide nitrogen coordination.[27]

The optical studies of Type 1 copper have primarily been directed toward elucidiating the geometry of the copper environment. Williams, for example, concluded in 1963 that because of its intensity, the absorption band at around 600 nm is due to metal to ligand charge transfer, and that this is most likely in a geometry lacking a center of inversion.[28] Blumberg, in 1966, formulated an optical spectra similar to that of Type 1 copper from calculations based on a distorted square planar geometry.[29] In 1968 Brill and Bryce, beginning with the idea that the cupric ion lies in a low-symmetry environment, assigned all the observable optical bands solely to d-d transitions resulting from a D_2 symmetry and 3d, 4s, and 4p hybridization.[30] They then found it possible, by assigning parameters to the resulting wavefunctions, to develop a theoretical model whose transitions correspond to those actually observed in the optical spectra of the

proteins. This model places the cupric ion in a D_2 environment that is distorted about 10° from square planer.

More recently, the same approach has been followed to a more definitive conclusion for stellacyanin, plastocyanin, and azurin. This study, by Solomon and Gray, used CD spectra, which revealed absorption bands not apparent in simple optical spectra.[31] As a result, it was possible to identify charge-transfer and d-d transitions and develop a structure whose predicted absorption bands are consistent with those observed in the proteins. Like the one proposed by Brill and Bryce, this structure has D_2 symmetry, but it is more nearly tetrahedral, with the ligands lying about 30° out of plane. The corresponding angle for a tetrahedral structure is 35.25°. Finally, with cobalt (II) derivatives of stellacyanin,[32a] and of azurin and plastocyanin,[32b] Gray found an optical spectra characteristic of tetrahedral cobalt(II).

Lastly, studies done on various small cupric complexes have demonstrated that EPR values, especially the value of $A_{||}$, are sensitive to ligand geometry.[33,34] In tetrahedral complexes $A_{||}$ values are decidedly lower than those of corresponding square planar complexes, but not as low as those of Type 1 copper.

From these studies a consensus emerged regarding the structure of Type 1 copper: it was seen as occupying

a site having a distorted tetrahedral geometry, ligated to two imidazole nitrogen atoms, a cysteine thiol, and possibly an amide nitrogen. This structure has recently been confirmed in its essential details by crystallographic structure analysis of azurin[35a] and plastocyanin,[35b] with one notable difference being found in the fact that the fourth ligand is a methionine thioether instead of an amide nitrogen. The properties of Type 1 copper are seen as resulting from the following causes: (1) the intense color is ascribed to a thiolate ligand to metal charge transfer, (2) the EPR parameters result from a combination of near-tetrahedral geometry and delocalization of spin density onto the thiolate, and (3) the increased reduction potential is a result of two factors which stabilize copper(I) relating to copper(II)--the presence of the thiol, with its tendency to reduce copper(II), and again, the near tetrahedral structure. This last effect is due to the fact that while copper(II), a d^9 system, is subject to Jahn-Teller distortions and thus is more stable as a square planar or D_2 structure, copper(I) is stable in a tetrahedral environment[36] (Figure 1).

Viewed simply as a chemical entity, this arrangement must be seen as a rather unlikely one, for two reasons: the instability inherent in the tetrahedral environment, and the instability arising from the presence

Figure 1.--Figure 1 illustrates how the Jahn-Teller effect operates in d^9 complexes such as that of the cupric ion. (a) In an octahedral field the degenerate set of five d orbitals in a free ion is split into an e_g set of higher energy and a t_{2g} set of lower energy. However, the total energy of the five orbitals remains the same. If one axis of the octrahedron is elongated, the symmetry is lowered to tetragonal, and further splitting of the orbitals occurs. Again, the total energy of the five orbitals is the same, but since the orbitals of a d^9 system are not fully occupied, a net reduction of energy takes place. For this reason an octahedral cupric complex is unstable relative to a tetragonal complex. (b) The same effect destabilizes a tetrahedral complex relative to a flattened tetrahedral or square planar complex.

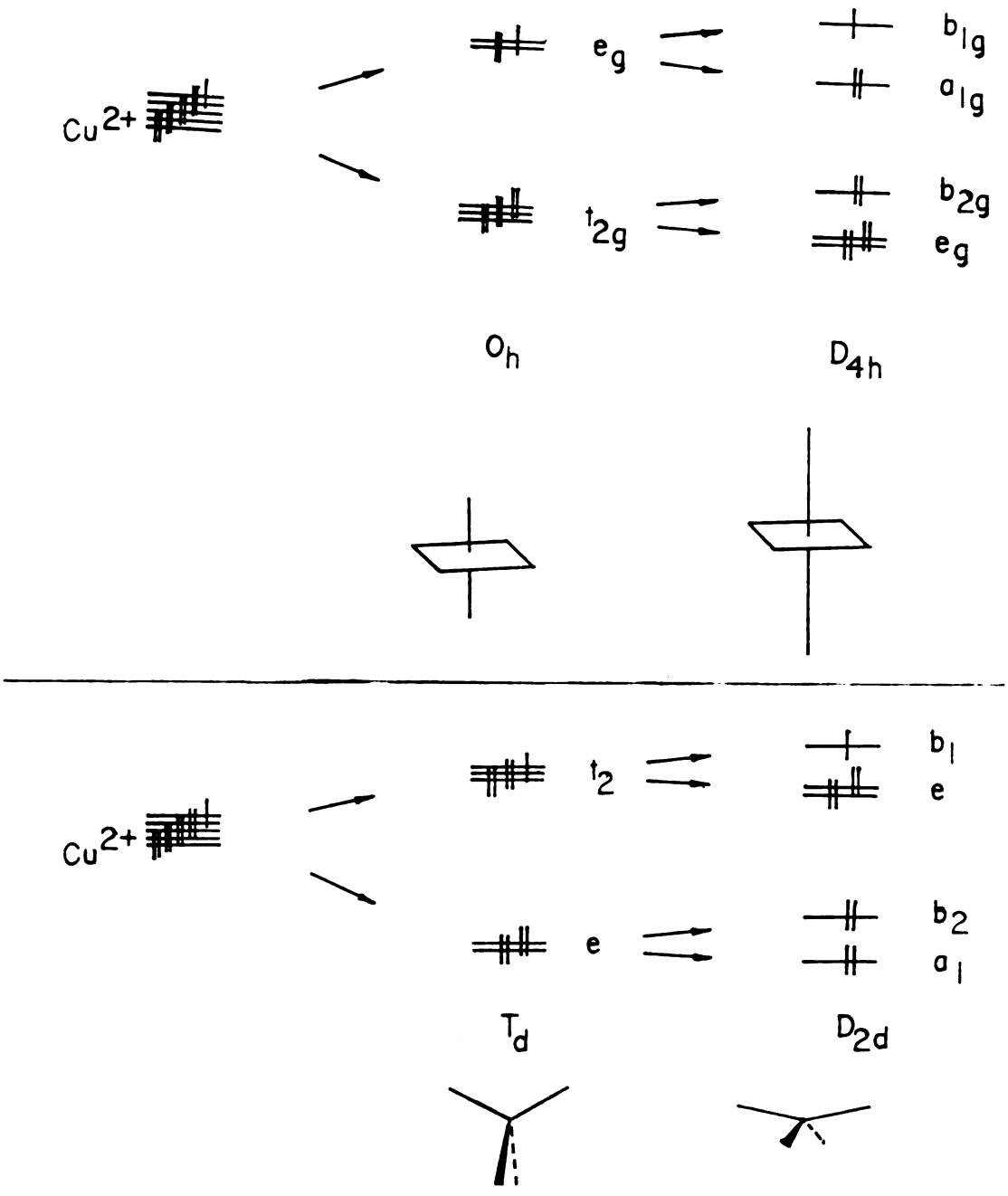


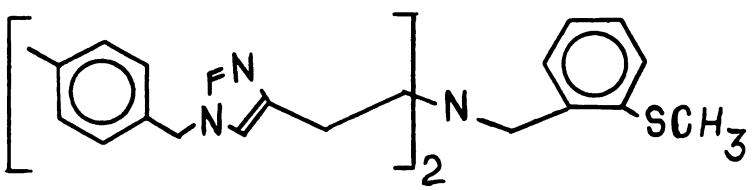
Figure 1

of the thiol and its tendency to cause reduction. Yet the Type 1 copper has somehow been stabilized. The mechanism by which this is accomplished is itself potentially of interest. Additionally, the use of this structure implies that it offers some unique properties which are biologically valuable and which cannot be obtained through another, simpler route. Consequently, the copper proteins offer an opportunity to study the relationship between the structure and the properties of an unusual transition metal complex, and also an opportunity to study how these properties may become part of a useful biological mechanism.

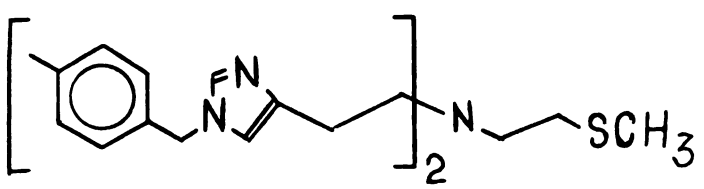
This paper describes the synthesis of four ligands which are suitable for the preparation of low molecular cupric complexes in which, as a first condition, the cupric ion is forced to accept a nearly tetrahedral geometry, and which also contain some combination of the donor atoms found in the proteins. Three such ligands (L-1, L-2, and L-3) have been prepared, and their synthesis is described in this report. The synthesis of a fourth ligand (L-4) has been completed except for the final stages (see Figures 2 and 3). A fifth ligand (L-5) has been described previously.[37]

In addition, this paper described the reaction of these ligands with various cupric salts, and compares the properties of the reaction products with those of the blue copper proteins.

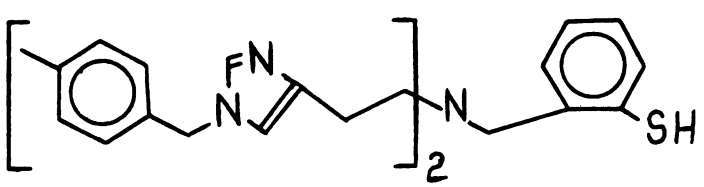
Figure 2.--The four ligands whose snythesis is reported here. All are tertiary amines which contain donor atoms in their side chains. L-1 and L-2 both contain two imidzole nitrogen donors and a thioether donor. L-3 contains two imidazole nitrogens and a thiol. L-4 contains an imidazole nitrogen, a thioether, and a thiol, and differs from the ligand set of the proteins only in that an amine nitrogen replaces one of the imidazoles present in the protein. In each of these, the amine nitrogen is expected to act as a donor atom.



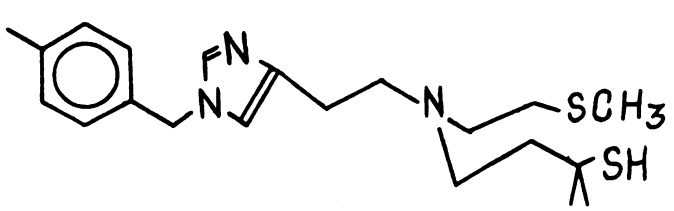
L-1



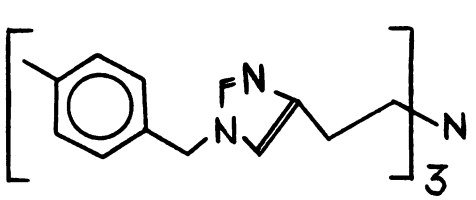
L-2



L-3



L-4



L-5

Figure 2

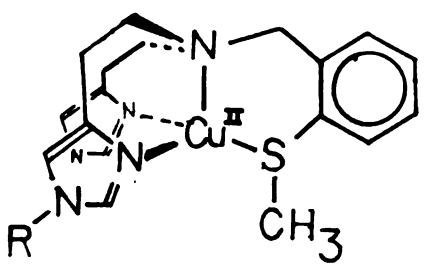


Figure 3

EXPERIMENTAL

Methods and Materials

All solvents were distilled prior to use, except for water and methylene chloride used in extractions, recystallization and chromatography solvents, and ammonia, which was dried by addition of an alkali metal. Tetrahydrofuran was distilled from sodium benzophenone ketyl. Acetonitrile was distilled from CaH_2 and P_2O_5, and hexamethylphosphortrimide and methylene chloride were subjected to simple distillation. All starting materials were reagent grade. Liquid reagents were distilled, and both liquids and solids were examined by NMR prior to use.

All coupling reactions involving DCCI and all reductions involving diborane were conducted under anerobic, anhydrous conditions, using distilled and dried solvents. All reactions of ligands with copper salts were conducted in an inert argon atmosphere, except for the formation of C-1, which was done under nitrogen. Melting points are corrected.

Spectrophotometric analyses were done on a varian T-60 NMR Spectrophotometer, a Perkin-Elmer 457 IR Grating Spectrophotomer, a Varian Cary 219 Spectrophotometer, and a Varian E-4 EPR Spectrometer. Elemental analyses were performed by Galbraith Laboratories, Knoxville, Tennessee.

II 2-(4-(1-para-methylbenzyl) imidazolyl)ethylamine monohydrochloride

To 25.0g. (0.137 mol.) of histamine dihydrochloride (Aldrich reagent) dissolved in about 1 l. of ammonia at -78° was added 10.4g. (0.411 mol.) of sodium, so that an intense blue color persisted. The solution was allowed to reflux for two hours, with additional small pieces of sodium being added as necessary to maintain the blue color. Then 25.0 ml. of para-methylbenzyl chloride (0.187 mol., sp. gr. = 1.05) was added dropwise. The blue yielded to a brilliant rose color, which faded to colorless with continuing addition of reageant. The solution was allowed to evaporate overnight. The resulting product was taken up in water, acidified with 6N HCl, filtered, and extracted with CH_2Cl_2. The pH was taken to 12 by addition of solid Na_2CO_3, and the solution was extracted three times with CH_2Cl_2. The combined CH_2Cl_2 fractions were dried with $MgSO_4$ and evaporated, producing an oil, which was purified as the monohydrochloride by crystallization from methanol/toluene. Yield: 13%. NMR (D_2O/t-BuOH): δ2.27(s,3H), 2.90(t,2H,J=6Hz), 2.97(t,2H,J=6Hz), 4.97(s,2H), 6.83(s,1H), 7.00(s,4H), 7.53(s,1H). Elemental analysis: calculated for $C_{13}H_{18}N_3Cl$: C 62.02, H 7.22, N 16.88, Cl 14.08%; found: C 61.43, H 7.22, N 16.37, Cl 14.08%. m.p.: 164-166°.

IV Cyanomethylimidazole

To 200g. (0.953 mol.) of L-histidine monohydrochloride (Sigma reagent) slurried in about 50 ml. of water was added dropwise 2310 ml. of NaOCl (5.25% water solution, sp. gr. = 1.05, 1.96 mol.) while the temperature was maintained at 0-5°. The pH was taken to 8 by addition of solid Na_2CO_3, and the solution was evaporated and vacuum dried. The residue was crushed to <#8 mesh and extracted five times with refluxing ethyl acetate. The combined ethyl acetate fractions were evaporated, giving an amber solid, which was recrystallized from ethyl acetate. Yield: 55%. NMR (D_2O/t-BuOH): δ3.60 (s,2H), 6.80(s,1H), 7.40(s,1H). m.p.: 133-136°d (literature:[38] 138°.)

V 4-imidazolylacetic acid

To 50g. (0.468 mol.) of IV was added 300 ml. of 2N LiOH, and the resulting solution was refluxed for 90 min. The pH was then adjusted to 5.5 by addition of 6N HCl. The solution was chilled to 0°, causing precipitation of fine white crystals, which were recrystallized from water solution by addition of acetone. Yield: 65%. NMR (D_2O/t-BuOH): δ3.57(s,2H), 7.06(s,1H), 8.33(s,1H). m.p.: 218-220° (literature:[38] 221-223°).

VI 4-(1-para-methylbenzyl)imidazolylacetic acid

To 25.0g. (0.197 mol.) of V dissolved in about 1 l. of ammonia at -78° was added 2.1g. (0.394 mol.) of lithium, so that an intense blue color persisted. The solution was allowed to reflux for four hours, with additional small pieces of lithium being added as necesary to maintain the blue color. Then 29.0 ml. of para-methylbenzyl chloride (0.217 mol.) was added dropwise. As before, the blue yielded to a brilliant rose color, which faded to colorless with continuing addition of reageant. The solution was allowed to evaporate overnight. The resulting product was taken up in about 200 ml. of water. The pH was taken to 5.5 by addition of 6N HCl. The solution was filtered, extracted with CH_2Cl_2, and evaporated to a white solid containing a mixture of product, starting material, and inorganic salts. A pure sample was crystallized from water by addition of acetone. NMR(D_2O)/t-BuOH): δ2.33(s,3H), 3.83 (d,2H,J=1 Hz), 5.32(s,2H), 7.30(m,4H), 7.35(d,1H, J=1.5Hz), 8.72(d,1H,J=1.7Hz). IR (nujol mull): ν3.20, 1680 cm^{-1}. m.p.: 158-162°.

VII Methyl(4-(1-para-methylbenzyl) imidazolyl) acetate

To the crude, vacuum dried VI from the previous step, in 500 ml. of methanol, was added dropwise 21.8 ml.

of $SOCl_2$ (0.300 mol., sp. gr. = 1.65). The resulting solution was refluxed for 90 min., and evaporated. The residue was dissolved in water and the pH was taken to 12 by addition of solid Na_2CO_3. Extraction with ethyl acetate and evaporation of the ethyl acetate solution gave a brown oil. NMR demonstrated that this was VII, free of the corresponding methyl ester of V. The oil was vacuum dried, weighted carefully, and exactly an equivalent amount of tetraethylammonium hydroxide (titrated aqueous solution) was added to its solution in about 200 ml. of methanol. The solution was refluxed for 90 min., and evaporated. Water was added, and exactly an equivalent amount of HCl (titrated aqueous solution) was added. The resulting solution was evaporated, vacuum dried to a brown gum, and weighed. NMR revealed the presence of a mixture of VI and Et_4NCl. Yield: 90% from V.

IX S-methyl-ortho-mercaptobenzoic acid

To 10.0g. (64.8 mmol.) of o-mercaptobenzoic-acid (Eastman reagent) dissolved in 20 ml. of water at pH = 12 and 40 ml. of DMF was added 4.4 ml. (71 mmol., sp. gr. = 2.28) of CH_3I. The solution was stirred for 1 hour, then evaporated and vacuum dried 6 hours. The resulting brown solid was dissolved in water and the solution was acidified. The solid that formed was recovered by filtration and recrystallized from ethanol in

quantitative yield. NMR ($CDCl_3$/TMS): δ2.40(s,3H), 7.20(m,4H), 8.00(dd,1H,J=7,2Hz). IR (nujol mull): ν2870s, 1690, 1380, 1310, 1285, 1270, 1250, 865, 730 cm^{-1}. m.p.: 167-169°.

X N-[2-(4-(1-para-methylbenzyl)imidazolyl)ethyl)-(4-(1-para-methylbenzyl)imidazolyl)acetamide

To 5.00g. (19.9 mmol.) of II dissolved in 40 ml. of HMPA, under nitrogen, was added 2.76 ml. of triethylamine (19.9 mmol./sp. gr. = 0.73) an equivalent weight of VI and Et_4NCl in 10 ml. of HMPA, and 2.95 g (21.9 mmol.) of NHBT in 10 ml. HMPA. The resulting solution was chilled to 5° and stirred 15 min. At the end of this time, 4.51g. (21.9 mmol.) of DCCI in 10 ml. HMPA was added. The solution was stirred overnight. The HMPA was removed by vacuum distillation at 80°, and 50 ml. of a 3:1 water: acetic acid solution was added. The mixture was stirred until all solids were either dissolved or in suspension, filtered, and extracted with CH_2Cl_2. The pH was taken to 12 by addition of solid Na_2CO_3, and the solution was extracted three times with CH_2Cl_2. The combined CH_2Cl_2 fractions were dried with $MgSO_4$ and evaporated, and the resulting oil was crystallized from CH_2Cl_2/hexanes. Yield: 85%. NMR ($CDCl_3$/TMS): δ2.33(s,6H), 2.77(t,2H), J=6Hz), 3.50(m,2H), 3.47(s,2H), 5.00(s,2H), 5.03(s,2H), 6.64(s,1H), 6.73(s,1H), 7.13(m,8H), 7.37(d,2H,J=1.2Hz). IR (nujol mull): ν3250, 3130, 1655, 1545, 1350, 1295,

1230, 1155, 985, 980, 800, 760, 750, 660 cm^{-1}. m.p.: 132-134°.

XI Bis[2-(4-(1-para-methylbenzyl)imidazolyl) ethyl]amine hydrochloride

To 5.0g (11.7 mmol.) of X in 19 ml. of THF, under nitrogen, was added 110 ml. (1M in THF, 0.110 mol.) of BH_3. The solution was refluxed for 90 min., followed by careful addition of 50 ml. of 6N HCl. Reflux, with evaporation of the THF, was continued for an additional 90 min. The solution was cooled to 15° and extracted with CH_2Cl_2. The pH was taken to 12 by addition of solid Na_2CO_3, and the solution was extracted three times with CH_2Cl_2. The combined CH_2Cl_2 fractions were dried with $MgSO_4$ and evaporated, producing an oil, which was purified as the monohydrochloride by crystallization from methanol/ toluene. Yield: 90%. NMR (as free base, $CDCl_3$/TMS): δ2.30(s,3H), 3.17(m,4H,J=4Hz), 5.17(s,2H), 7.17(s,5H, 8.33 (d,1H,J=1Hz). Elemental analysis (as free base): calculated for $C_{26}H_{31}N_5$: C 75.54, H 7.50, N 16.95%; found: C 75.93, H 7.59, N 16.53%. m.p.: 182-183°.

XII N,N-Bis[2-(4-(1-para-methylbenzyl) Imidazolyl)ethyl]-(S-methyl)ortho-mercapto)benzamide

To 1.3g. (3.1 mmol.) of XI, as the free base, in 10 ml. of THF, under nitrogen, was added 0.52g. (3.1 mmol.) of IX, and 0.46g. (3.4 mmol.) of NHBT, each dissolved in

5 ml. of THF. The solution was stirred for 15 min. at 5°, 0.70g. (3.4 mmol.) of DCCI in 5 ml. THF was added, and the solution was stirred overnight at room temperature. A solution of 5 ml. of glacial acetic acid in 20 ml. of water was then added, the solution was filtered, and the THF was evaporated. The solution was extracted repeatedly with CH_2Cl_2, until foaming no longer occurred. The combined CH_2Cl_2 fractions were evaporated and the resulting brown oil was taken up in 20 ml. of water. The pH was taken to 12 by addition of solid Na_2CO_3. The solution was extracted 3 times with CH_2Cl_2. The combined CH_2Cl_2 fractions were dried with $MgSO_4$ and evaporated. The product was purified by elution with 1% CH_3OH in CH_2Cl_2 from an alumina column. The product was slightly air sensitive. Yield: 80%. NMR ($CDCl_3$/TMS): δ1.97(s,3H), 2.03(s,6H), 2.47 (t,4H,J=6Hz), 3.30(m,4H), 4.57(d,4H,J-3Hz), 6130(s,2H), 6.67(s,8H), 6.90(m,4H), 6.97 (s,2H). IR (neat): ν3380, 2300, 1570, 1450, 1375, 1300, 1145, 1050, 1040, 855, 835, 735, 720cm^{-1}. TLC: 1 spot, R_f = 0.14-0.35 (1% CH_3OH in CH_2Cl_2/alumina).

XIII (L-1) Bis[2-(4-(1-para-methylbenzyl) imidazolyl)ethyl]-(S-methyl-ortho-mercapto-benzyl)amine

To 1.1g. (2.2 mmol.) of XII in 5 ml. of THF, under nitrogen, was added 25 ml. (1M in THF, 25 mmol.) of BH_3. The solution was refluxed for 90 min., and 30 ml. of 6M HCl

was added slowly. The solution was reluxed an additional 90 min., with evaporation of the THF. The pH was taken to 12 with addition of solid Na_2CO_3, and the solution was extracted three times with CH_2Cl_2. The combined CH_2Cl_2 fractions were dried with $MgSO_4$ and evaporated to a pale brown oil, which was purified by elution with 1% CH_3OH in CH_2Cl_2 from an alumina column. The product was slightly air sensitive. Yield: 90%. NMR ($CDCl_3$/TMS): δ2.23 (s,6H), 2.30(s,3H), 2.77(s,8H), 3.67(s,2H), 4.77(s,4H), 6.43(s,2H), 6.83(m,12H), 7.77(s,2H). Elemental analysis: Calculated for $C_{34}H_{39}N_5S$: C 74.26, H 7.16, N 12.74, S 5.83%; found: C 72.01, H 6.53, N 12.40, S 5.70%. TLC: 1 spot, R_f = 0.30-0.60 (1% CH_3OH in CH_2Cl_2/alumina).

XV S-Methyl Mercaptoacetic Acid

To 9.2g. (0.10 mol.) of XIV dissolved in about 20 ml. of water was added 8.0g. (0.20 mol.) of NaOH in water solution and about 50 ml. of DMF, along with 7.5 ml. (0.12 mol., sp. gr. = 2.28) of methyl iodide. The mixture was stirred 1 hour, evaporated, and vacuum dried for 6 hours. Water was then added, the solution was made acidic by addition of HCl, and extracted five times with CH_2Cl_2. The combined CH_2Cl_2 fractions were dried with $MgSO_4$ and evaporated and the resulting yellow oil was vacuum distilled to give a colorless oil (b.p.: 55° at 0.05 mm Hg). Yield: 70%. NMR ($CDCl_3$/TMS): δ2.27(s,3H), 3.27(s,2H), 11.33(s,1H). IR (neat): ν3000, 1700, 1425, 1290, 1130 cm^{-1}.

XVI N,N-di[2-(4-(1-para-methylbenzyl) imidazolyl)ethyl]-(S-methyl)mercapto-acetamide

To 9.45g. (1.1 mmol.) of XI, as the free base, under nitrogen, in 5 ml. of THF, was added 0.11g. (1.1 mmol.) of XV and 9.15g. (1.2 mmol) of NHBT, each in 5 ml. of THF. The solution was stirred for 15 min. at 5°, 0.22g. (1.2 mmol.) of DCCI was added, and the solution was stirred overnight at room temperature. A solution of 5 ml. of glacial acetic acid in 20 ml. of water was added, the THF was evaporated, and the solution was filtered. The solution was then extracted repeatedly with CH_2Cl_2, until foaming no longer occurred. The combined CH_2Cl_2 fractions were evaporated and 20 ml. of water was added to the resulting brown oil. The pH was taken to 12 by addition of solid Na_2CO_3, and the solution was extracted three times with CH_2Cl_2. The combined CH_2Cl_2 fractions were dried with $MgSO_4$ and evaporated to a light brown oil which required no purification. Yield: 55%. NMR ($CDCl_3$/TMS): δ1.97(s,3H), 2.20(s,6H), 2.67(m,4H,J=4Hz), 2.93(s,2H), 3.37(m,4H,J=6Hz), 4.80(s,4H), 6.43(d,2H,J=3Hz), 6.87(s,8H), 7.10(s,2H). TLC: 1 spot, R_f = 0.23-0.30 (2% CH_3OH in CH_2/alumina).

XVII (L-2) Bis[2-(4-(1-para-methylbenzyl) imidazolyl)ethyl]-(S-methyl-2-mercapto-ethyl)amine

To 0.30g. (0.60 mmol.) of XVI in 5 ml. THF, under nitrogen was added 6.0 ml. (1 M in THF, 6.0 mmol.) of BH_3. THF, 6.0 mmol.) of BH_3. The solution was refluxed for 90 min., and 30 ml. of 6M HCl was added slowly. The solution was refluxed an additional 90 min., with evaporation of the THF. The pH was taken to 12 by addition of solid Na_2CO_3, and the solution was extracted three times with CH_2Cl_2. The combined CH_2Cl_2 fractions were dried with $MgSO_4$ and evaporated to a pale brown oil, which was purified by elution with 1% CH_3OH in CH_2Cl_2 from an alumina column. The product was slightly air sensitive. Yield: 90%. NMR ($CDCl_3$/TMS): δ1.97(s,3H), 2.23(s,6H), 2.67(m,12H), 4.80 (s,4H), 6.47(s,2H), 6.90(s,8H), 7.10(s,2H). IR (neat: ν3340, 2900, 2180, 1490, 1430, 1350, 1300, 1220, 1145, 1105, 1025, 1105, 965, 800, 735, 610 cm^{-1}. TLC: 1 Spot, R_f = 0.30-0.67 (1% CH_3OH in CH_2Cl_2).

XVIII Dithiodi-(ortho-benzoic acid)

To 10.0g (64.8 mmol) of VIII (Eastman reagent) in 200 ml. of ethanol, was added 5.19g. (0.129 mol.) of NaOH in 10 ml. of water. A 4% solution of I_2 in ethanol was added slowly until a brown color persisted in the solution. The solvent was evaporated, and the resulting residue was dissolved in water. On acidification with HCl a pale brown

solid appeared, which was collected by filtration. Quantitative yield. NMR(d_6-DMSO): δ9.3-8.0(m). IR(nujol mull): ν2890, 1680, 1590, 1415, 1310, 1290, 1270, 1260, 1150, 1030, 735, 690, 650 cm^{-1}. m.p.: 293-295° (302-305° Sadtler #10396).

XIX Dithiodi-ortho-[N-N-di(2-(4-para-methylbenzyl)imidazolyl)ethyl) benzamide

To 0.44g. (106 mmol.) of XI, as the free base, under nitrogen, in 10 ml. THF was added 0.16g. (0.53 mmol.) of XVIII and 9.14g. (1.06 mmol.) of NHBT, each in 5 ml. of THF. The solution was stirred 15 min. at 5°, and 0.22g. (1.06 mmol.) of DCCI in 5 ml. of THF was added. The solution was warmed to room temperature and stirred overnight. A solution of 5 ml. of glacial acetic acid in 20 ml. of water was added, the THF was evaporated, and the solution was filtered. The solution was extracted four times with CH_2Cl_2 and the combined CH_2Cl_2 fractions were evaporated. To the resulting oil was added 20 ml. of water, and the pH was taken to 12 by addition of solid Na_2CO_3. This solution was extracted three times with CH_2Cl_2, the combined CH_2Cl_2 fractions were dried with $MgSO_4$ and evaporated, giving a pale brown oil, which was purified by elution with 1% CH_3OH in CH_2Cl_2 from an alumina column. Yield: 77%. NMR ($CDCl_3$/THF): δ2.30(s,6H), 3.10(m,8H), 4.87(s,4H), 6.20 (m,4H), 6.67(s,2H), 7.00(s,8H), 7.37(s,2H). IR (neat):

ν2920, 2200, 1620, 1425, 1350, 1290, 1230, 1150, 1130, 1020, 970, 905, 810, 735, 615 cm^{-1}. TLC: 1 spot, R_f = 0.0-0.18 (1% CH_3OH in CH_2Cl_2/alumina).

XX (L-3) Sodium [bis(2-(4-1-para-methyl-benzyl)imidazolyl)ethyl)-ortho-mercapto benzyl)amine]

To 0.45g. (0.41 mmol.) of XIX, under nitrogen, in 5 ml. of THF was added 4 ml. (1M in THF, 4 mmol.) of BH_3. The solution was refluxed for 90 min., and 30 ml of 6M HCl was added slowly. The solution was refluxed an additional 90 min., with evaporation of the THF. The pH was taken to 12 by addition of solid Na_2CO_3, and the solution was extracted three times with CH_2Cl_2. The combined CH_2Cl_2 fractions were dried with $MgSO_4$ and evaporated to a colorless oil, a sample of which was found to decolorize an iodine solution in ethanol. The compound was not characterized. Instead, it was immediately used for reaction with a copper salt.

XXII 3-mercapto-3-methylbutyric acid

A solution of 5.8g. (58 mmol.) of XXXI (Pfaltz and Bauer reagent) in 30 ml. of conc. H_2SO_4 was stirred for 30 min. H_2S was then bubbled through the solution for 10 min., and 200 ml. of water was added very slowly (over about 3 hours). The solution was then stirred overnight. The pH was taken to 3 by addition of solid Na_2CO_3, and the

solution was filtered and evaporated. The solid white residue was extracted with CH_2Cl_2, which was evaporated to a pale yellow oil. Yield: 25%. NMR ($CDCl_3$/TMS): δ2.27 (s,3H), 3.27(s,2H), 11.33(s,2H). IR (neat): ν3000, 1700, 1425, 1290, 1130cm^{-1}.

XXIII 3,3'-Dithiodi(3-methylbutyric acid)

To 2.0g. (15 mmol.) of XXXII in 10 ml. of water was added 1.98g. (8.5% pure, 30 mmol.) of KOH in 10 ml. of water. The solution was titrated with a standard I_2 solution until a brown color persisted. The solution was evaporated to remove the excess I_2, and 20 ml. of water was added to the yellow solid that resulted. The pH was found to be neutral. This solution was filtered, and evaporated to give a solid which contained a known weight of the potassium salt of the product. The yield was presumed to quantitative (see preparation of XVIII). NMR (D_2O/t-BuOH): δ1.57 (s,3H), 2.60(s,1H). IR (nujol mull): ν3000, 1715, 1390, 1320, 1210, 1155, 895 cm^{-1}.

XXIV N-[2-(4-(1-para-methylbenzyl)imidazolyl) ethyl]-(S-methyl)mercapto-acetamide

To 1.82g. (7.99 mmol.) of II, as the free base, under nitrogen, was added 0.85g. (7.99 mmol.) of XV, and 1.35g. (19 mmol.) of NHBT each in 5 ml. of THF. The solution was stirred for 15 min. at 5°, 2.06g. (19 mmol.) of

DCCI in 5 ml. THF was added, and the solution was stirred overnight at room temperature. A solution of 1.5 ml. of glacial acetic acid in 40 ml. of water was then added, the THF was evaporated, and the solution was filtered. The solution was then extracted repeatedly with CH_2Cl_2, until foaming no longer occurred. The combined CH_2Cl_2 fractions were evaporated and 20 ml. of water was added to the resulting brown oil. The pH was taken to 12 by addition of solid $NaCO_3$, and the solution was extracted three times with CH_2Cl_2. The combined CH_2Cl_2 fractions were evaporated to a nearly colorless oil which on recrystallization from CH_2Cl_2/hexanes gave an oil. After several hours of stirring, the oil gave white needle-like cyrstals. Yield: 75%. NMR ($CDCl_3$/TMS): δ1.97(s,3H), 2.30(s,3H), 2.63(q,2H,J=6Hz), 3.07(s,2H), 3.53(q,2H,J=6Hz) 4.93(s,2H), 6.57(s,1H), 6.97 (s,4H), 7.30(s,1H). IR (neat): ν3230, 3060, 2950, 1690, 1570, 1490, 1310, 1220, 1190, 970, 810, 740 cm^{-1}. m.p.: 88-89°.

XXV [2-(4-(1-para-methylbenzyl)imidazolyl)ethyl]-(S-methyl-2-mercaptoethyl)amine

To 1.3g. (4.3 mmol.) of XXIV in 5 ml. THF, under nitrogen, was added 5 ml. (1M in THF, 5 mmol.) of BH_3. The solution was refluxed for 3 hr., and 30 ml. of 6M HCl was added slowly. The solution was refluxed an additional 90 min., with evaporation of the THF. The pH was taken to 12

by addition of solid $NaCO_3$, and the solution was extracted three times with CH_2Cl_2. The combined CH_2Cl_2 fractions were dried with $MgSO_4$ and evaporated to a pale brown oil. Purification appears possible through chromatography on alumina. In some instances no purification is required. Yield: 90%. NMR: ($CDCl_3$/TMS): δ2.00(s,3H), 2.17(s,3H), 2.70(m,8H), 4.90(s,2H), 6.53(s,1H), 6.97(s,4H), 7.27 (s,1H).

XXVI 3,3'-Dithiodi [[N-(2-(4-(1-para-methylbenzyl)imidazolyl)ethyl)-N-(2-(S-methyl) mercaptoethyl)]-3-methylbutyramide

To 0.18g. (0.62 mmol.) of XXV as the monohydrochloride (weight as free base) and 0.082g. (0.31 mmol.) of XXIII as the potasium salt in 20 ml. of THF, under nitrogen, was added 0.09g. (0.66 mmol.) of NHBT in 5 ml. of THF. The solution was stirred for 45 min. at 5°, 0.15g. (0.73 mmol.) of DCCI in 5 ml. of THF was added, and the solution was stirred overnight at room temperature. A solution of 5 ml. of glacial acetic acid in 20 ml. of water was added, the THF was evaporated, and the solution was filtered. The pH was taken to 12 by addition of solid Na_2CO_3, and the solution was extracted three times with CH_2Cl_2. The combined CH_2Cl_2 fractions were dried with $MgSO_4$ and evaporated to a colorless oil. Yield: 90%. NMR ($CDCl_3$/TMS): δ1.23(d,6H,J=3Hz), 2.10(d,3H,J=2Hz),

2.37(s,5H), 2.67 (q,4H,J=6Hz), 3.50(q,4H,J=6Hz), 4.93 (s,2H), 6.57(s,1H), 7.00(s,4H), 7.33(s,1H).

Anhydrous Copper (II) Salts

To 6.1g. (50 mmol.) of $CuCO_3$ (Baker and Adamson reagent) in a plastic flask was added 15 ml. of 65% HPF_6, using a plastic tube. The resulting dark blue solution was evaporated to near dryness, and 125 ml. of acetonitrile was added. The solution was chilled to 5° and filtered. In a dry box, about 5g. of P_2O_5 was added. The solution was stirred and again filtered, and the process was repeated until the added P_2O_5 remained in a fine granular state. The solvent was then evaporated under vacuum until crystals appeared, and the crystals were recovered by anerobic filtration and stored in a dry box. Yield: 10%. IR revealed the presence of PF_6^- [39], CH_3CN, and the absence of water. The formula was taken to be $Cu(II)(CH_3CN)_4\ (PF_6)_2$ by analogy with the known formulas of the BF_4^- and ClO_4^- salts[40]. IR: (Nujol mull): ν2320, 1145, 1040, 860-800, 560 cm^{-1}.

Hydrated cupric BF_4^- and ClO_4^- salts are commercially available. (Alfa reagent). These were dehydrated in a manner similar to that used for the PF_6^- salt.

C-1. Reaction of Cu(II)$(BF_4)_2$ with XIII (L-1)

To a solution of 0.321g. (0.583 mmol.) of L-1 in 10 ml. of methanol under nitrogen was added 0.16g. (0.64 mmol.) of $Cu(NO_3)_2 \cdot 3H_2O$ in 5 ml. of water, which caused the solution to turn dark. To this was added 0.42g. (3.5 mmol.) of $NaBF_4$ in 5 ml. of water. The methanol was removed by slow evaporation, causing the precipitation of dark blue-green crystals. These were dissolved in CH_2Cl_2, which caused a further color change from blue-green to blue. The volume was reduced to 5-7 ml. and crystallization was induced by addition of 1-2 drops of ether and chilling to -20°. Optical specta revealed peaks at (ε = 87 $m^{-1}cm^{-1}$) and 800nm (ε = 40 m^{-1} sec^{-1}). EPR ($CHCl_3$, liquid N_2): $A_{||}$ = 1.68 x $10^{-2}cm^{-1}$, $q_{\perp}$ = 2.05, $q_{||}$ = 2.22. Elemental analysis: calculated for $C_{34}H_{59}N_6SCuBF_4O_3$: C 53.57, H 5.17, N 11.03, S 4.20, Cu 8.33, B 1.42, F 9.97, O 6.30%; found: C 51.15, H 4.99, N 9.93, S 3.68, Cu 8.26, F 9.37%.

C-2. Reaction of Cu(II$(CH_3CN)_4(BF_4)_2$ with XIII (L-1)

To 0.056g. (0.10 mmol.) of XII in 2 ml. of CH_3CN, under nitrogen, was added 0.050g. (0.10 mmol.) of Cu(II) $(BF_4)_2(CH_3CN)_4$. The color changed from pale blue to green. The solvent was evaporated and the residue was vacuum

dried 12 hr. and taken up in 4 ml. CH_2Cl_2. Attempts at crystallization proved unsuccessful. Optical spectra revealed peaks at 625nm (ε = 67 $m^{-1}cm^{-1}$) and 440 (ε = 300 - 350 $m^{-1}cm^{-1}$). EPR (CH_2Cl_2, liquid N_2): $A_{||}$ = 1.64 x 10^{-2} cm^{-1}, $g_{||}$ = 2.06, $q_{||}$ = 2.23.

C-3. Reaction of Cu(II)$(CH_3CN_4)(ClO_4)_2$ with XIII (L-1)

To 0.080g. (0.14 mmol.) of XIII in 5 ml. of CH_3CN under nitrogen was added 0.062g. (0.14 mmol.) of $Cu(ClO_4)_2(CH_3CN)_4$ in 5 ml. of CH_3CN. The solution immediately turned dark green. The solvent was evaporated and the green residue was vacuum dried 12 hrs. The residue was then dissolved in 4 ml. CH_2Cl_2 but all attempts to induce crystallization failed.

C-4. Reaction of Cu(II)$(CH_3CN)_4(ClO_4)_2$ with XIII (L-1)

To 0.080g. (0.14 mmol.) of XIII in 5 ml. of CH_3CN, under nitrogen, was added 0.075g. (0.14 mmol.) of Cu $(PF_6)_2(CH_3CN)_4$ in 5 ml. of CH_3CN. The solution immediately turned dark green. The solvent was evaporated and the green residue was vacuum dried 12 hrs. The residue was then dissolved in 30 ml. CH_2Cl_2, and on cooling to -20° the solution yielded about 100 mg. of green crystals, which were collected by filtration under nitrogen.

C-5. Reaction of $Cu(II)(CH_3CN)_4(PF_6)_2$ with XVII (L-2)

To 0.050g. (0.10 mmol.) of XVII in 2 ml. CH_3CN under nitrogen, was added 0.053g. (0.10 mmol.) of $Cu(II)(PF_6)_2(CH_3CN)_4$. The solution immediately turned dark green. The solvent was evaporated and the green residue was vacuum dried 12 hrs. The residue was then dissolved in 10 ml. of CH_2Cl_2, but all attempts to induce crystallization failed. EPR (CH_2Cl_2, liquid nitrogen 1.36 mM): $A_{||} = 2.70 \times 10^{-2} cm^{-1}$, $g_{||} = 2.05$, $g_{\perp} = 2.01$ (only 6% of the expected EPR intensity, in comparison with a standard $CuSO_4$ solution, was found).

C-6. Reaction of $Cu(II)(CH_3CN)_4(PF_6)_2$ with XX (L-3)

To 0.33g. (0.58 mmol.) of XX in 5 ml. of CH_3CN, under nitrogen, was added 0.30g. (0.58 mmol.) of $Cu(II)(PF_6)_2(CH_3CN)_4$ in 5 ml. of CH_3CN. The solution immediately turned dark green. The solvent was evaporated and the green residue was vacuum dried 12 hrs. Over a period of about 2 weeks in an inert atmosphere, the green color gradually faded to a whitish-gray.

RESULTS

Organic Synthesis

Synthesis of an N_3S Aromatic Thioether Ligand (L-1)

Preparation of this ligand centered about a coupling reaction that fuses a carboxylic acid and an amine into an amide, followed by a reduction. In this manner it was possible to progress from a primary to a secondary to a tertiary amine in a stepwise fashion.

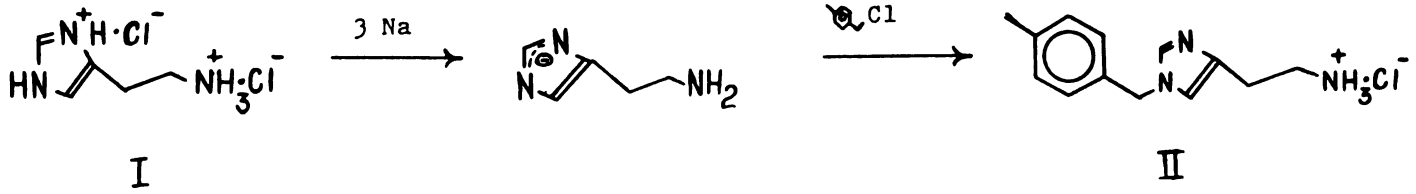
The synthesis began with histamine, I, a primary amine containing an imidazole. Two of the nitrogen atoms in this compound will ultimately become donor atoms, the amino nitrogen and the π-imidazole nitrogen.

The first step involves placement of an appropriate blocking group on the τ-imidazole nitrogen (N-3) to prevent its interaction with copper. This was done by deprotonation of histamine dihydrochloride with three equivalents of sodium in liquid ammonia followed by addition of p-methylbenzyl chloride, as shown in Scheme 1. It was expected that the benzyl chloride would add to the τ-nitrogen rather than the π-nitrogen of the imidazole anion for steric reasons. In fact, a significant amount of π-benzylated product did appear, but purification of

the τ-isomer was easily accomplished by recrystallization of the hydrochloride salt (Scheme 1). The fact that the correct isomer was actually obtained was established by NMR, which showed a cross-ring coupling constant of 1.2 Hz consistent with τ-benzylation.[41]

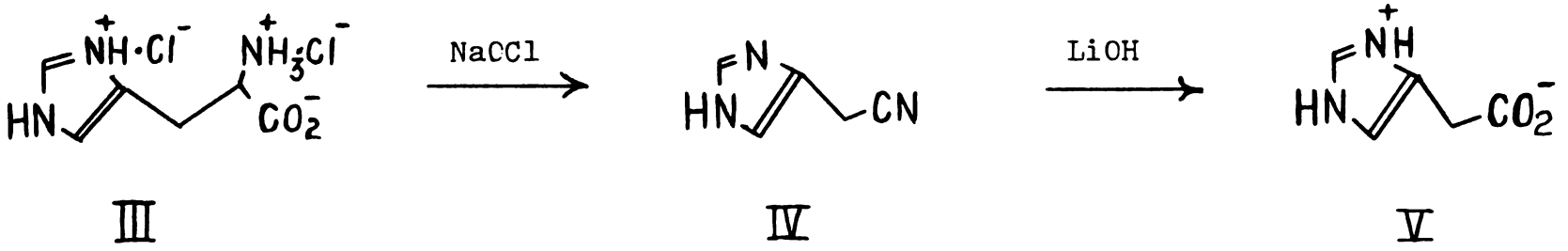
With histidine, III, as a starting material, the corresponding carboxylic acid was produced. This was done by treatment of histidine with hypochlorite, generating cyanomethylmidazole, IV, which on basic hydrolysis produced imidazoleacetic acid, V.[39] A repetition of the reduction in liquid ammonia, using lithium because of the limited solubility of the sodium salt, required about four hours during which lithium was added periodically to maintain the blue color. The length of time required was apparently due to the slow rate of deprotonation of the imidazoleacetic acid by alkali metals. This was followed by addition of para-methylbenzyl chloride which gave VI, the τ-nitrogen benzylated compound, exclusively. VI was converted to its methyl ester for purposes of separation from unreacted V, and then converted back to the acid form with tetraethylammonium hydroxide. This series of reactions is outlined in Scheme 2.

Finally, the third component of the ligand was formed by the methylation of ortho-mercaptobenzoic acid with methyl iodide, Scheme 3.

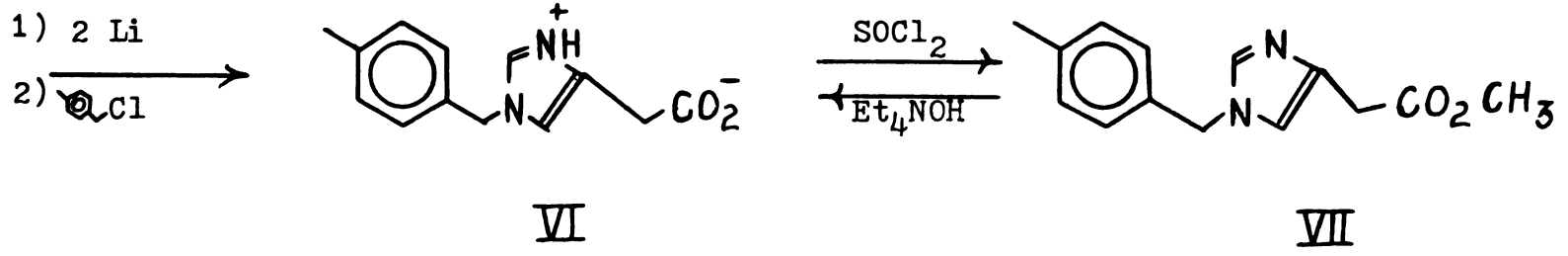


I II

Figure 4. Scheme 1

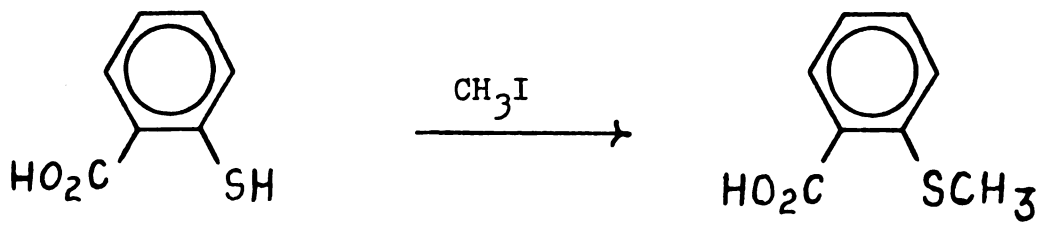


III IV V



VI VII

Figure 5. Scheme 2



VIII IX

Figure 6. Scheme 3

Following these initial steps, the synthesis proceeded along the squence outlined in Scheme 4. First, II and VI were coupled using dicyclohexylcarbodiimide, DCCI, and excess N-hydroxybenzotriazole, NHBT, which is known to catalyze peptide bond formation, and also, although it is not an issue here, to reduce concurrent racemization.[42] The resulting amide X was reduced to the secondary amine XI with diborane.[43] The coupling and reduction process was then repeated with the thioether carboxylic acid IX, giving the secondary amide XII and the tertiary amine XIII.

Synthesis of an N_3S Aliphatic Thioether Ligand (L-2)

This synthesis proceeded in an analogous manner, differing only in the fact that an aliphatic thioether, rather than an aromatic thioether was the final product. To this end, XV was prepared from mercaptoacetic acid and methyl iodide, and this compound was coupled to the secondary amine XI, giving XVI. XVI was then reduced, giving XVII. This series of reactions is shown in Scheme 5.

Synthesis of an N_3S Aromatic Thiol Ligand (L-3)

In an effort to prepare a complex containing one of the most essential features of the blue copper center, a ligand containing a thiol group was prepared. An

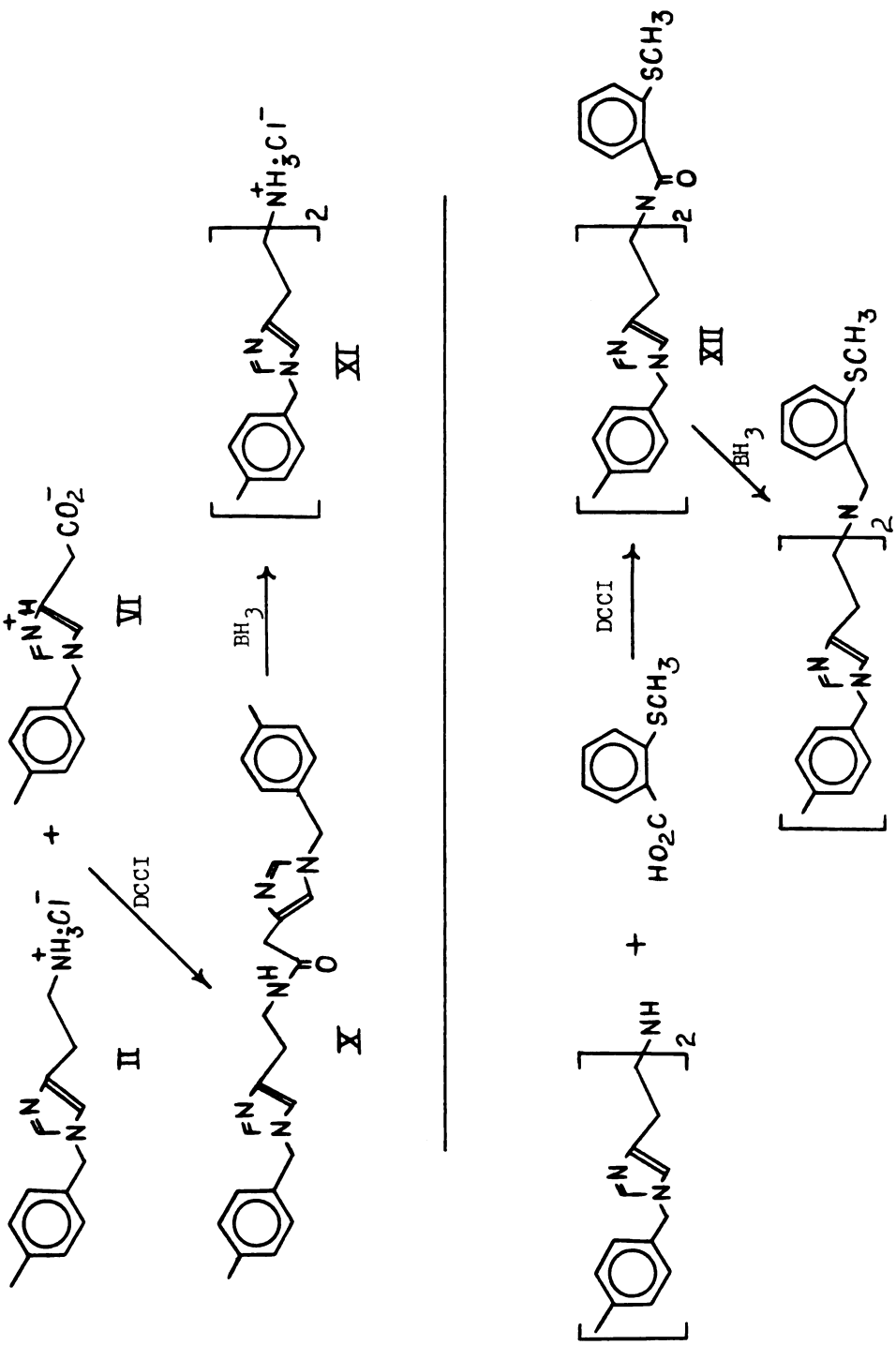


Figure 7. Scheme 4.

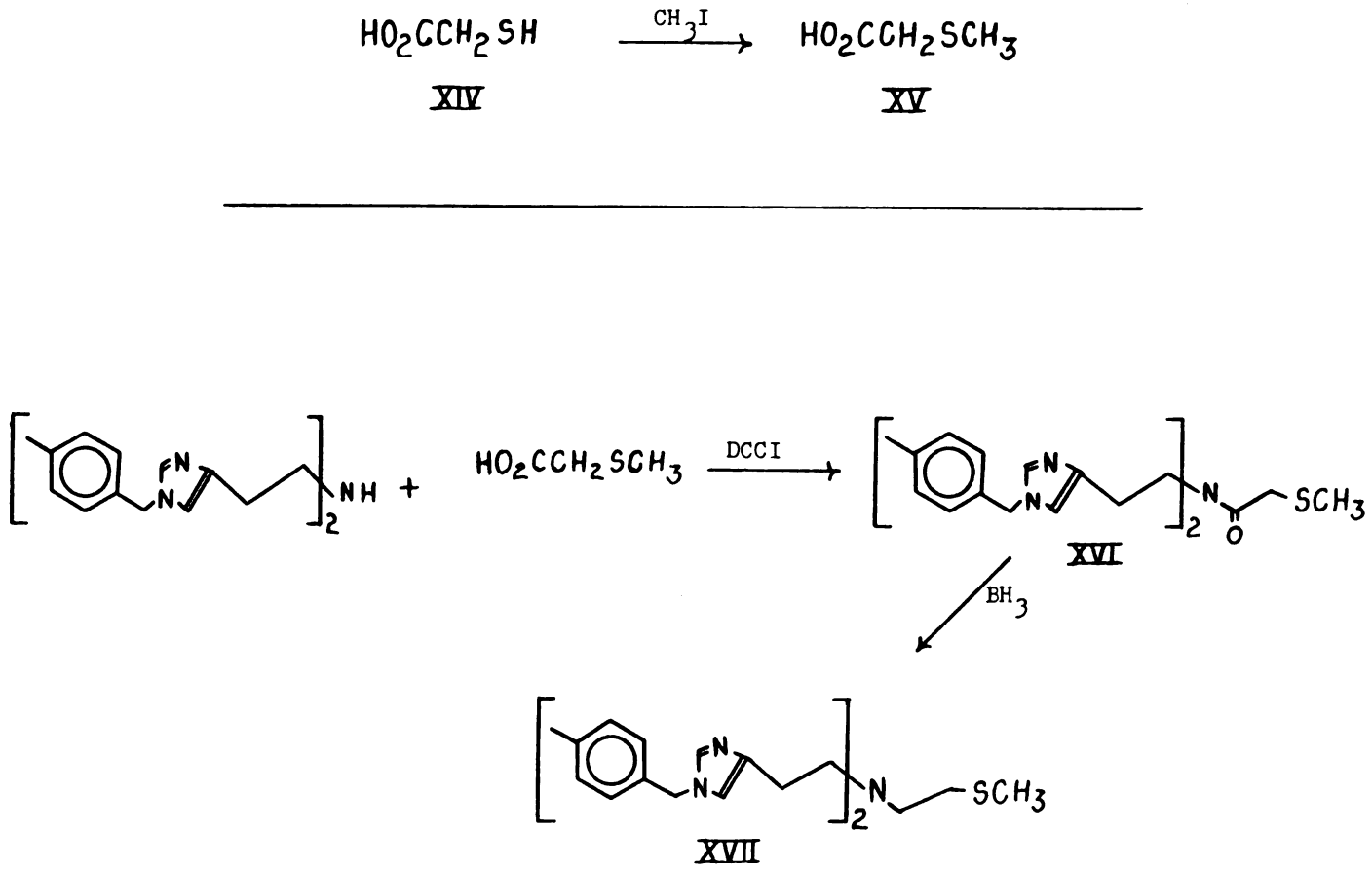


Figure 8. Scheme 5.

aromatic thiol was selected for this, as it was thought that aromatic delocalization of the thiolate electron might diminish the tendency for the complex to undergo oxidation by copper with intermolecular disulfide formation.

For this compound, the disulfide of o-mercaptobenzoic acid was prepared from the thiol using iodine and this was coupled to the secondary amine XI as in the previous procedure, giving XIX. Disulfide formation was necessary because thiols must be protected from DCCI, with which they react. Disulfides in essence allow the thiol to protect itself, and they are easily cleaved later on. Reduction of XIX with borane reduced the amide to an amine, and also cleaved the disulfide bond as evidenced by the ability of the product to decolorize iodine. In fact, a large number of closely related reducing agents are known to break disulfide bonds.[44] The compound was extracted from a water solution at high pH as the thiolate salt. This series of reactions is outlined in Scheme 6.

Partial Synthesis of an N_2S_2 Aliphatic Thiol-Thioether Ligand (L-4)

In an effort to prepare a ligand having the closest possible match to the ligand environment of the blue copper, the synthesis of a ligand having an imidazole, a thiol, and a thioether was undertaken. The first steps of the synthesis were devoted to preparing a suitable

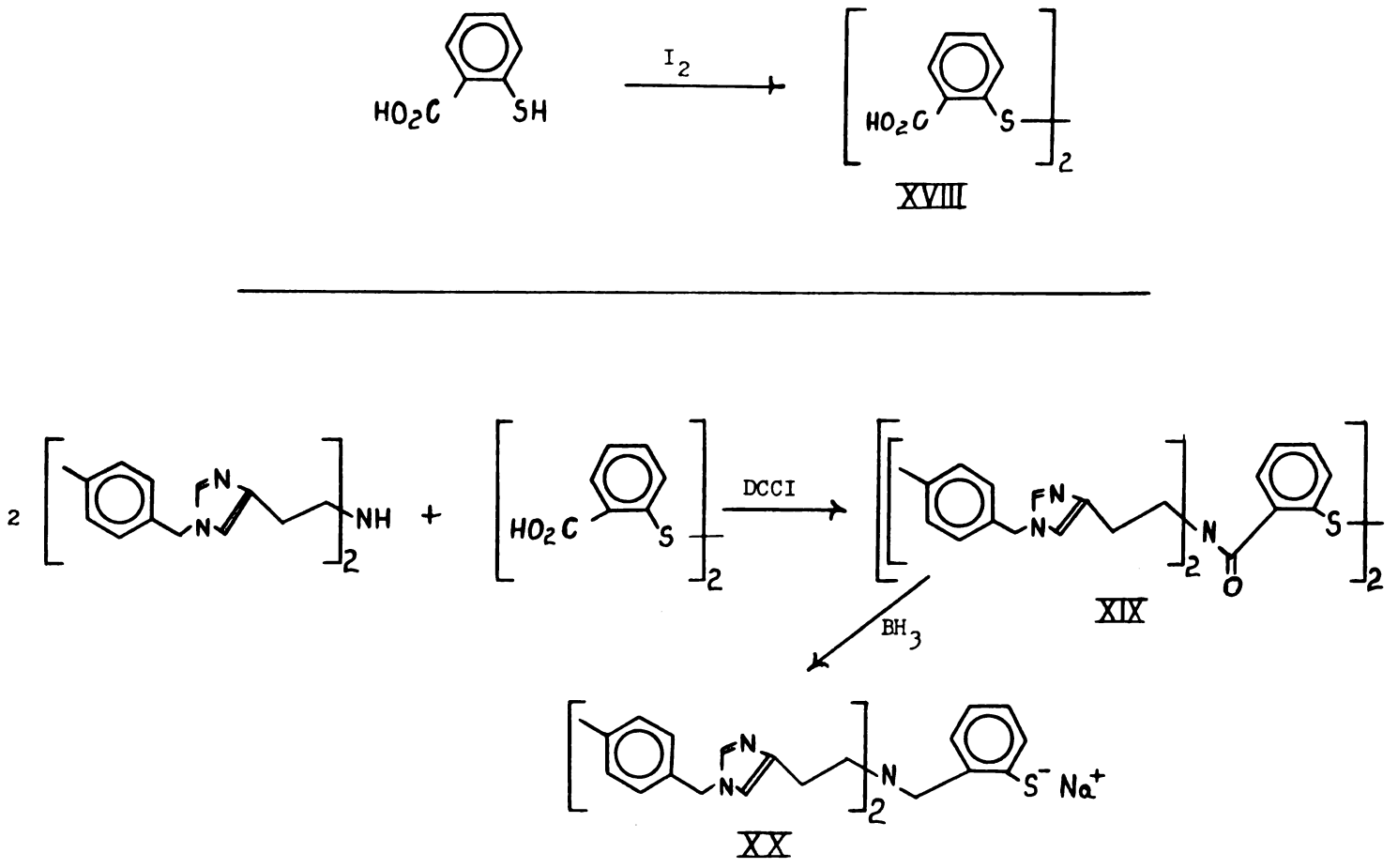


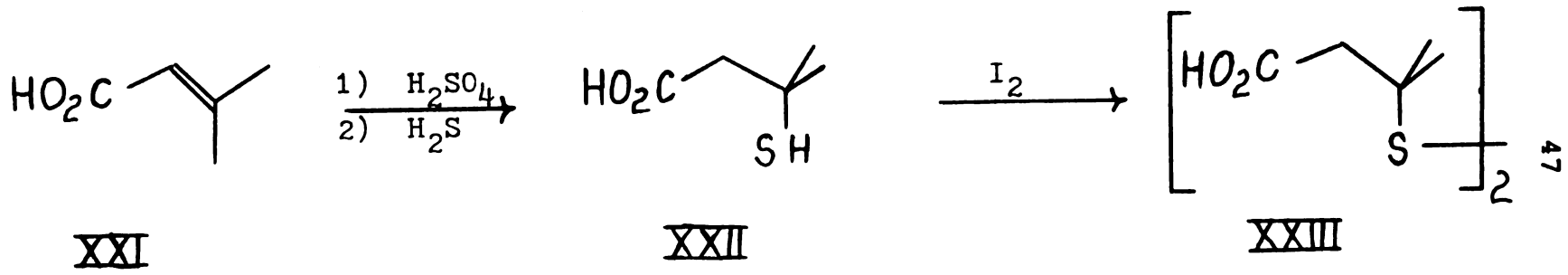
Figure 9. Scheme 6.

thiol, XXII, in which the thiol group was attached to a carbon atom bearing two methyl groups. This was done in the hope that disulfide formation, and consequent copper reduction, would be blocked by unfavorable steric interactions.

For this preparation, β-methylcrotonic acid, XXI, was dissolved in concentrated sulfuric acid and H_2S was bubbled through the solution. Water was then added slowly and the desired acid, XXII, was obtained (Scheme 7). This reaction evidently proceeds through an intermediate in which H_2SO_4 has added across the double bond, followed by nucleophilic displacement when the addition of water liberates H_2S from its salt with sulfuric acid.[45] Elimination, to give a starting material, is a competing reaction. Ironically, an alternative method, in which H_2S is dissolved in a strong base, successful for the closely related trans-crotonic acid, fails completely for this compound.

Addition of iodine to a fully deprotonated aqueous solution of XXII produces the disulfide XXIII. As the disulfide bond in this compound proved to be sensitive to acid cleavage, the compound was kept as the potassium salt.

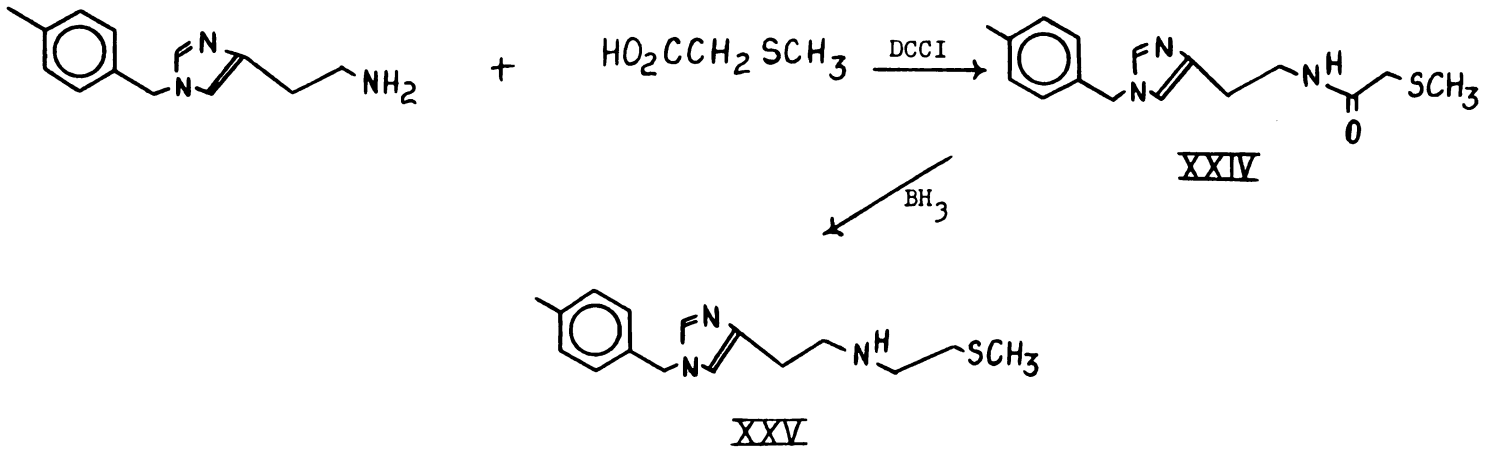
The second aspect of this synthesis involved the preparation of a suitable secondary amine, that is, one



XXI XXII XXIII

47

Figure 10. Scheme 7



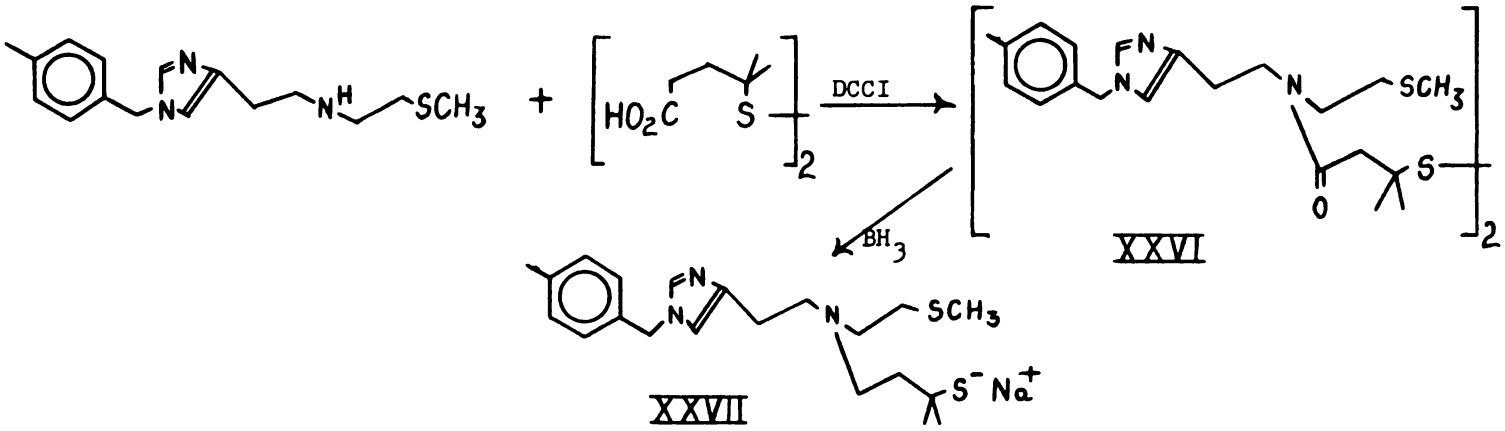


Figure 11. Scheme 8.

containing an imidazole and a thioether. This was done by coupling an amine, II, with a thioether-containing acid, XV, to give an amide, XXIV, and then reducing the amide to an amine, XXV, in a series of reactions similar to those followed earlier (Scheme 8). The hydrochloride salt of XXV was then coupled with the potassium salt of XXIII to give the amide XXVI. Subsequent reduction to the amine XXVII (L-4) and formation of the cupic complex remain to be done.

Preparation and Characterization of Cupric Complexes

The final step in the preparation of a model for the blue copper center is the formation of the cupric complex. In principle, this is done simply by addition of a stoichiometric amount of an anhydrous copper salt to the ligand, using a suitable solvent. In practice, the formation of a complex having the desired properties has been far more difficult, apparently because of the elusive nature of the tetrahedral structure.

This has led, in at least some cases, to the formation of a five-coordinate complex, where the fifth donor atom has been supplied in some fashion or other by the anion of the curpic salt used. For example, reaction of the tris(imidazole)amine, L-5, with $CuSO_4 \cdot 5H_2O$ and an excess of $NaBF_4$ gave a crystalline fluoride-bridged dimer,

$[Cu(II)_2(L\text{-}5)_2F](BF_4)_3$.[46] The source of the fluoride apparently was the $NaBF_4$, which was found to contain fluoride ion as a 2% impurity. The same result followed from an attempt to prepare the Co(II) complex of L-5. A complex of L-5 and anhydrous $Cu(ClO_4)_2$ gave EPR and UV spectra indicative of a 5-coordinate complex, apparently as a result of a copper-perchlorate interaction.

Complexes of the N_3S Aromatic Thioether (L-1)

In an initial attempt to prepare a tetrahedral cupric complex with L-1, an aqueous solution of $Cu(NO_3)_2$ was added to a methanol solution of L-1. An excess of $NaBF_4$ in water was added and the methanol was removed by evaporation. This caused the precipitation of dark blue crystals, C-1, which proved to be a mixed nitrate/tetrafluoroborate salt with EPR parameters characteristic of those of a 5-coordinate species (see Table 2). A second attempt, using anhydrous $Cu(BF_4)_2(CH_3CN)_4$, gave a green amorphous solid on evaporation of solvent (C-2). Again, the EPR was not indicative of a tetrahedral complex. Evidence of a copper-sulfur interaction was seen in the optical spectra with a shoulder located at about 800nm ($\varepsilon \approx 350\ M^{-1}cm^{-1}$). This compares with a band occurring in the blue copper proteins at about 550nm, which has been attributed to thioether to copper charge transfer.[31] This shoulder disappeared on addition of water.

Complexes were also formed using $Cu(ClO_4)_2(CH_3CN)_4$ (C-3), and $Cu(PF_6)_2(CH_3CN)_4$ (C-4). Both were dark green. The first complex, C-3, could not be crystallized but the second, C-4, gave crystals from a methylene chloride solution at -20°. EPR of C-4 again indicated that a tetrahedral geometry had not been obtained.

Complexes of the N_3S Aliphatic Thioether (L-2) and the N_3S Aromatic Thiol (L-3)

The cupric complexes of these ligands have been dealt with only briefly. For L-2, a green cupric complex was formed using the PF_6^- salt (C-5). EPR indicated that not only were the properties of a tetrahedral complex absent, but that the signal had only 6% of the expected intensity. Evidently the remainder of the signal was lost through intermolecular coupling, perhaps as a result of dimer formation, but this phenomenon has not yet been explored. It was not possible to crystallize this substance.

A cupric complex of L-3 was also prepared, again using the PF_6^- salt (C-6). The expected dark color failed to develop. Instead, the green color of the complex appeared very similar to the colors of the thioether complexes. This compound was unstable, with the green color fading to a whitish-gray after about 2 weeks in

an argon atmosphere at room temperature. If this color change is due to cupric reduction and disulfide formation, then for a compound containing a cupric-thiol coordinate bond, this substance is actually relatively stable. For example, a similar complex, using a hydrotris(pyrazole)borate ligand plus para-nitrothiophenol, was found to decompose above -30°.[47] However, it has not yet been possible to characterize this complex more fully.

The EPR and optical properties of these complexes are tabulated in Table 2.

DISCUSSION

The primary method of characterization of any potential model for the blue copper center is EPR spectroscopy. This is so because the EPR values of the blue copper proteins, especially the unusually low values of the hyperfine coupling constant, $A_{||}$, place these proteins in what is essentially a unique class of cupric complexes. So far, it has not been possible to duplicate the values for $A_{||}$ found in the proteins in any model compound.

It can be demonstrated theoretically that the value of $A_{||}$ will be lower in a distorted tetrahedral complex than in a corresponding square planar complex,[48,49] and this effect has been shown to occur in a pseudo-tetrahedral $Cu(II)S_4$ complex[34] and in a tetrahedral $Cu(II)N_2O_2$.[33] Empirical studies have found that a positive charge on a $Cu(II)X_4$ center reduces $A_{||}$ and increases $g_{||}$, and that $A_{||}$ and $g_{||}$ decrease along the donor series $N_4 > N_2O_2 > O_4 > N_2S_2 > S_4$.[50] Again, it has not been possible to account for the observed values of $A_{||}$ through theoretical or empirical studies. However, it does appear that a reduced value for $A_{||}$ is indicative of a tetrahedral or near tetrahedral geometry. By this standard, the

complexes formed in this study are not tetrahedral (see Table 2).

For at least some of the reaction products this seems to be a result of the fact that a solvent molecule, acetonitrile, is retained as an additional ligand, or as a ligand that displaces one of the donor atoms, probably sulfur, of the principal ligand, or both. This is revealed in the IR spectra of the various reaction products. The IR of the reaction product of $Cu(CH_3CN)_4)(PF_6)_2$ and L-1 (C-4) shows absorption at 2270 and 2298 cm^{-1}, both in a region characteristic of the C ≡ N stretching frequency. The acetonitrile in this material remains in spite of the fact that C-4 was crystallized from methanol.

Similarly, the product of the reaction of $Cu(CH_3CN)_4(BF_4)_2$ and L-1 (C-2) shows an absorption shoulder in the region of 2400-2250 cm^{-1} that might be attributed to acetonitile, and C-5, the reaction product of $Cu(CH_3CN)_4$ $(PF_6)_2$ and L-2, shows clear absorptions at 2300 and 2280 cm^{-1}. In each case the substance in question was recovered from a solution of acetonitrile by evaporation and vacuum drying for 12 hours, and then by evaporation and vacuum drying after attempts to crystallize them from methylene chloride failed.

The final substance resulting from the reaction of $Cu(PF_6)_2(CH_3CN)_4$ and the only thiol ligand, L-3 (C-6) has

TABLE 2.--EPR and optical properties of some cupric complexes and some blue copper proteins

	EPR			Optical	
	$A_{\|\|}$ $\times 10^{-2}$ cm^{-1}	$g_{\|\|}$	$g_{\perp}$	λ (nm)	ε ($M^{-1}cm^{-1}$)
$Cu(II)_2(L\text{-}5)_2(BF_4)_3F$					
$Cu(II)(1\text{-}5)(ClO_4)_c$					
$Cu(II)(L\text{-}1)(BF_4)(NO_3)$	1.68	2.22	2.05	600	87
C-1				800	40
$Cu(II)(L\text{-}1)(BF_4)_2$	1.64	2.23	2.06	625	67
C-2				440	300-350
$Cu(II)(L\text{-}1)(ClO_4)_2$					
C-3					
$Cu(II)(L\text{-}1)(PF_6)_2$					
C-4					
$Cu(II)(L\text{-}2)(PF_6)_2$	2.70*	2.05*	2.01*		
C-5					
$Cu(II)(L\text{-}3)(PF_6)_2$					
C-6					
Plastocyanin	0.63	2.33	2.05	460	1180
Spinach[a]				597	9800
				770	3300
Stellacyanin	0.31	2.29	2.08	448	554
Rhus vernifica				604	3820
				845	700
Azurin	0.58	2.26	2.05	459	285
Pseudomonas				625	3500
fluorescens				781	320
Bis(N-i-propyl-2-methyl-salicylaliminato)(Cu(II)[b]	1.20	2.27	2.03		
$Cu(S{=}C(CH_3)NH_2)_4^{2+}$[c]	0.87	2.15			

[a] R. Malkin and B. G. Malmstron, Adv. Enzymol. 33 177 (1970).

[b] V. Sakaguchi and A. W. Addison, J. Amer. Chem. Soc. 99 5189 (1977).

[c] I. Bertini, G. Canti, R. Grassi, A. Scozzafara, Inorganic Chemistry 19 2198 (1980).

*Only 6% of expected EPR activity was found for C-5.

an IR spectrum which shows no acetonitrile, while the first product, the one characterized as a mixed nitrate-tetrafluoroborate salt, shows IR absorptions at 1445, 1285, and 1010 cm^{-1}, close those assigned to unidentate nitrate complexes. No evidence of the presence of water can be seen in the IR. The IR for the reaction product of $Cu(ClO_4)_2(CH_3CN)_4$ and L-1 (C-4) was not done. (See Appendix A for IR spectra.)

It seems clear, then, that the nature of the solvent itself is a serious impediment to attaining a tetrahedral copper complex, and that a solvent with a non-complexing nature must be selected. The obvious choice is methylene chloride, but the problem then becomes one of getting the hydrated copper salts to dissolve. It may be possible to add the hydrated salts to a methylene chloride solution of ligand and then, if the copper can be induced to dissolve by forming a hydrated complex, attempt to dehydrate the complex by additions of a drying agent such as P_2O_5. Failing this, it may be possible to prepare soluble copper salts with a method similar to that described in the experimental section but using a solvent that is a weaker ligand, such as ether or tetrahydrofuran.

If this can be done, then, with the synthesis of the ligands essentially complete, it should be possible to model the blue copper center, and also, by using ligands

that contain only one or two of the features found in blue copper, to find the exact source of the unusual properties it displays.

Final Remarks

There has been considerable speculation in the literature as to the exact purpose of this near-tetrahedral structure found in the blue copper proteins. The question is of special interest in light of the known instability of the tetrahedral structure--a fact confirmed by every attempt, including this one, to duplicate its properties--and also in light of the biological principle that, as a whole, evolution is efficient, and does not produce or maintain functions it does not need.

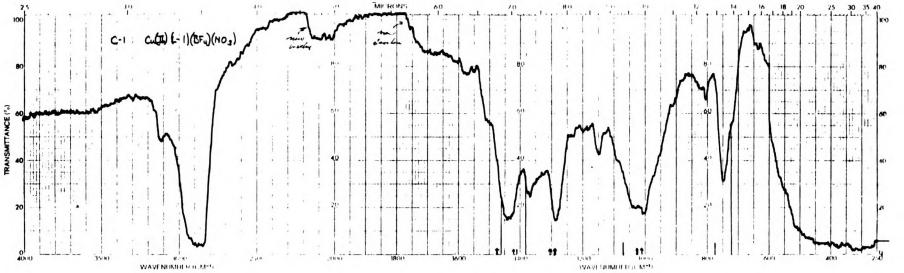
Some speculations into the reason the near-tetrahedral structure exists are possible. One suggestion is that the blue copper proteins meet a physiological need for an electron transfer enzyme that is a relatively strong oxidant, and this need is met by a tetrahedral structure, which destabilizes the oxidized state of the enzyme. It is also possible that the structure confers another advantageous prosperity to the enzyme. Freeman has suggested that by having a tetrahedral structure built-in, the need for conformational changes from tetrahedral to square-planar structure is eliminated, and as a result, the kinetics is governed by the rate of

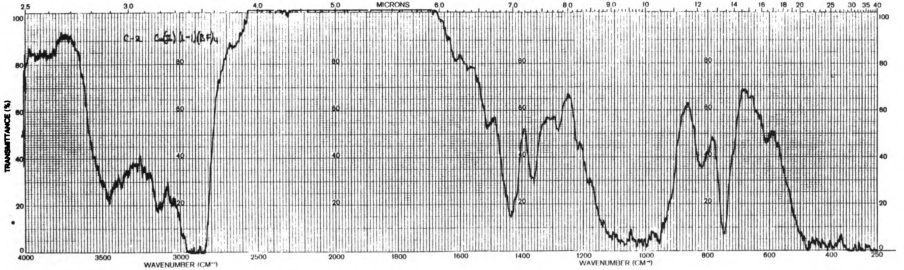
electron transfer, which is orders of magnitude faster.[51] It may be that two effects, one thermodynamic, the other kinetic, are provided simultaneously by the tetrahedral structure, and that is the reason it exists in the blue copper proteins.

APPENDIX

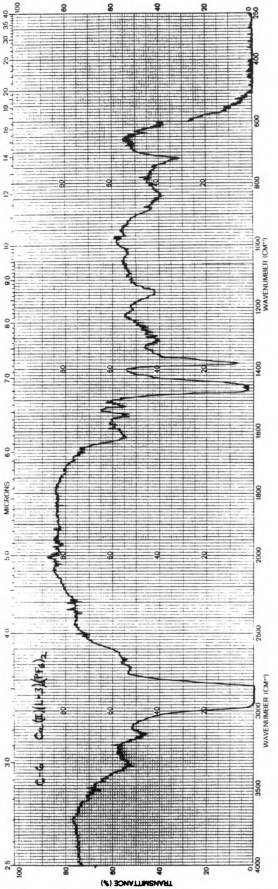
APPENDIX A

IR SPECTRA OF CUPRIC COMPLEXES


C-1 Cu(II)(L-1)(BF4)(NO3)
TRANSMITTANCE (%)
WAVENUMBER (CM-1)
MICRONS


C-2 Cu(II)(L-1)(BF4)2
TRANSMITTANCE (%)
WAVENUMBER (CM-1)
MICRONS

C-4 Co(II)(L-1)(Fe)4
TRANSMITTANCE (%)
MICRONS
WAVENUMBER (CM-1)
C-5 Co(II)(L-2)(Fe)4
TRANSMITTANCE (%)
MICRONS
WAVENUMBER (CM-1)


TRANSMITTANCE (%)
WAVENUMBER (CM⁻¹)
MICRONS
WAVENUMBER (CM⁻¹)

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