Modeling the Active Site in Hemocyanin: Synthesis and Reactivity of Binuclear Copper Complexes

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Abstract

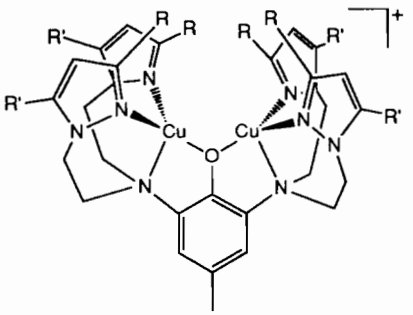
Binucleating ligands that provide three nitrogen donors, and in some cases a phenolate group, to each copper ion have been synthesized. Introduction of substituents to provide steric shielding of the presumed dioxygen-binding site in a previously-described complex prevents reaction of these new species with dioxygen. That these changes are not a direct consequence of steric effects is demonstrated by the synthesis of a Cu(II) derivative having a bridging acetate group. Thus, seemingly simple structural modifications of model systems for the hemocyanin active site may cause substantial changes in reactivity.

Introduction

The synthesis of stable copper(I) complexes ligated by heterocyclic nitrogen donors has led to the generation [1] and isolation [2] of binuclear dioxygen adducts that mimic the oxygenated form of hemocyanin to a certain degree [3]. The rarity of copper–dioxygen complexes can be attributed to the side reaction that results in the four-electron reduction of dioxygen to water, probably by the interaction of four Cu(I) ions with the dioxygen molecule.

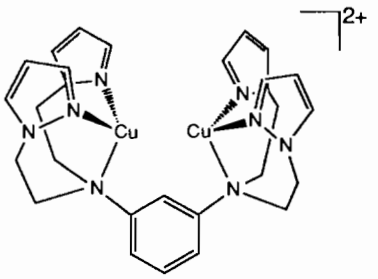
Several years ago, we reported the preparation of a binuclear complex $Cu_2(bpeac)^+$ (**1**) which positions two copper ions at an optimal distance to promote the binding of oxygen in a μ-1,2 manner while preventing the formation of a μ-1,1 bridge [4]. The spectroscopic properties of the azido-bridged copper(II) derivative [5] closely mimic those for the azide derivative of the oxidized form of hemocyanin; and the copper–copper separation of 3.77 Å is comparable to the value of 3.66 Å calculated from EXAFS data of metazidohemocyanin [6]. However, copper(I) complex **1** does not reversibly bind oxygen [4]. Instead, manometric measurements indicate a stoichiometry of two molecules of complex to one molecule of dioxygen; and the clean isosbestic behavior observed in the electronic spectra during the oxygenation reaction suggests a rapid bimolecular decomposition of a copper peroxo species. The stability of the peroxide in oxyhemocyanin may result, in part, from site isolation of the bound dioxygen group by the protein milieu [7].

We reasoned that a sterically hindered analog of the ligand Hbpeac might prevent the bimolecular reduction of oxygen, increasing the stability of the peroxo species; and on the basis of that hypothesis, we undertook the synthesis of binucleating ligands related to Hbpeac [8]. We report here the synthesis of several copper(I) complexes (**2** and **3**) and their reactions with dioxygen. The failure to generate stable dioxygen adducts in these systems illustrates



1 R = R' = H

2a R = R' = CH_3
2b R = phenyl, R' = H
2c R = *t*-butyl, R' = H



3

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that the stability of the complex is undoubtedly related to many factors, not all addressed by these simple models.

Experimental

All reagents and solvents were purchased from commercial sources and used as received unless otherwise noted. The following solvents were distilled and stored under nitrogen: tetrahydrofuran (from sodium benzophenone ketyl), methanol (from $Mg(OMe)_2$), and acetonitrile (from CaH_2). Manipulations involving copper(I) complexes were performed in a Vacuum Atmosphere glovebox, operating with less than 1 ppm of O_2 and H_2O. Melting points were obtained with use of a Fisher-Johns apparatus and are uncorrected. Flash chromatography was performed using the procedure of Still *et al.* [9]. Thin layer chromatography was performed using Analtech precoated (0.25 mm) silica gel plates. Microanalyses were determined by Desert Analytics, Tucson, AZ.

^1H and ^{13}C NMR spectra were recorded on an IBM AC 200 instrument at 200.132 MHz or on a Bruker WM 250 instrument at 250.13 MHz. Chemical shifts are reported in parts per million (ppm) relative to an internal standard of tetramethylsilane. Infrared spectra were recorded on a Beckman IR 4250 spectrophotometer and peaks are reported in cm^{-1}.

3-Phenylpyrazole (4)

A slurry of sodium hydride (2.7 g, 0.11 mol) and anhydrous ether (250 ml) was mechanically stirred under nitrogen. A mixture of acetophenone (13.5 g, 0.11 mol) and ethyl formate (12.0 ml, 0.15 mol) was added dropwise. The reaction mixture was heated to reflux for 4.5 h and vigorously stirred overnight at room temperature. The precipitate was collected, washed with anhydrous ether, and dissolved in water (100 ml). Hydrazine hydrate (12 ml) was added, and the mixture was cooled in an ice bath. The resultant mixture was acidified to pH 3 by the dropwise addition of concentrated sulfuric acid. The reaction was neutralized with 20% NaOH and extracted with ether (3 × 50 ml). The extracts were dried ($MgSO_4$) and evaporated to dryness. The residue was recrystallized from benzene to give white prisms (7.2 g, 44%); melting point (m.p.) 77–78 °C (lit. [10] 77–78 °C). ^1H NMR ($CDCl_3$): δ 6.60 (d, J = 2 Hz, 1 H, pyrazolyl C–H), 7.23–7.38 (m, 3 H, Ar–H), 7.58 (d, J = 2 Hz, 1 H, pyrazolyl C–H), 7.74–7.78 (m, 2 H, Ar–H), 11.0 (br s, 1 H, N–H). ^{13}C NMR ($CDCl_3$): δ 102.59(d), 125.90(d), 127.97(d), 128.74(d), 132.13(s), 134.40(s), 150.20(s).

3-t-Butylpyrazole (5) [10]

Under a dinitrogen atmosphere, a mixture of 37.5 g (0.375 mol) of pinacolone and 31.5 g (0.438 mol) of ethyl formate was added to a slurry of 19.5 g (0.381 mol) of sodium methoxide in 750 ml of anhydrous ether. The reaction mixture was heated under reflux for 6 h, then extracted with three 100-ml portions of water. To this solution was added 29.3 g (0.375 mol) of 64% hydrazine hydrate in water. The solution was slowly acidified with concentrated sulfuric acid. The yellow oil which first separated dissolved as the solution became acidic. The pH of the solution was readjusted to neutral, and the product extracted with three 100-ml portions of ether. The combined extracts were dried over $MgSO_4$, filtered, and concentrated to yield 20.5 g (44%) of a colorless oil. ^1H NMR ($CDCl_3$): δ 1.35 (s, 9 H, t-butyl), 6.1 (d, 1 H, pyrazolyl C–H), 7.5 (d, 1 H, pyrazoyl C–H).

3,5-Diacetamido-4-methoxytoluene (7)

Compound **6** [5] (41.0 g, 0.11 mol) was dissolved in a solution of potassium hydroxide (13.0 g) in methanol (500 ml). Methyl iodide (30 ml, 0.48 mol) was added in one portion and the flask was sealed with a septum. The mixture was stirred at room temperature for 3 days and evaporated to dryness. The residue was dissolved in 10% sodium hydroxide (100 ml) and extracted with methylene chloride (5 × 200 ml). The combined extracts were dried ($MgSO_4$) and evaporated under reduced pressure. The crude product was recrystallized from 95% ethanol to yield 26.7 g (64%) of white needles, m.p. 204–206 °C. ^1H NMR ($CDCl_3$); δ 2.2 (s, 6 H, $CH_3C{=}O$), 2.3 (s, 3 H, ArMe), 3.7 (s, 3 H, –OMe), 7.6 (s, 2 H, Ar–H), 7.8 (s, 2 H, NHC=O).

3,5-Diamino-4-methoxytoluene (8)

A mixture of 7 (17.6 g, 0.75 mol), potassium hydroxide (150 g) and methanol (500 ml) was allowed to reflux for 12 h under nitrogen. The solvent was evaporated. The residue was dissolved in water (200 ml) and extracted with methylene chloride (5 × 200 ml). The combined extracts were dried ($MgSO_4$) and concentrated to afford 4.8 g (90%) of a pale yellow oil which was used without further purification. ^1H NMR ($CDCl_3$): δ 2.1 (s, 3 H, Ar–Me), 3.7 (s, 3 H, –OMe), 3.8 (s, 4 H, ArNH), 5.9 (s, 2 H, Ar–H).

3,5-Di[bis-(2-hydroxyethyl)amino]-4-methoxytoluene (9)

3,5-Diamino-4-methoxytoluene (10.6 g, 0.070 mol) was dissolved in 1:1 methanol–water (80 ml) and cooled with an ice bath. Concentrated hydrochloric acid (2 ml) and ethylene oxide (35 ml) were added, and the flask was sealed with a septum. The reaction mixture was allowed to warm slowly to room temperature. After 3 days, the solvent was evaporated to dryness. The residue was dissolved in water, neutralized with 10% sodium hydroxide,

saturated with sodium chloride, and extracted with methylene chloride (5 X 200 ml). The extracts were dried ($MgSO_4$) and evaporated to a viscous, colorless oil (21.5 g, 94%). ^1H NMR ($CDCl_3$): δ 2.2 (s, 3 H, Ar–Me), 3.2 (t, J = 5.1 Hz, 8 H, $ArNCH_2$), 3.5 (br s, 4 H, –OH), 3.6 (t, J = 5.1 Hz, 8 H, $–OCH_2$), 3.8 (s, 3 H, –OMe), 6.7 (s, 2 H, Ar–H). ^{13}C NMR ($CDCl_3$): δ 21.15, 56.03, 59.90, 60.01, 119.52, 134.00, 143.74, 147.00.

*3,5-Di[bis(2-chloroethyl)amino]-4-methoxytoluene (**10**)*

A solution of tetraol **9** (4.9 g, 15 mmol) in methylene chloride (20 ml) was added dropwise to thionyl chloride (32.0 g, 269 mmol) in methylene chloride (20 ml). After stirring at room temperature for 9.5 h, the reaction mixture was poured into ice and neutralized with 10% sodium hydroxide. The layers were separated and the aqueous layer was extracted with methylene chloride (2 X 100 ml). The combined organic extracts were dried (Na_2SO_4). The solvent was evaporated to an oil under reduced pressure while maintaining the bath temperature below 50 °C. The crude product was dissolved in warm methanol (20 ml) and slowly cooled to give yellow needles (2.2 g, 36%), m.p. 77–79 °C. This compound was stored in a desiccator at –20 °C. ^1H NMR ($CDCl_3$): δ 2.2 (s, 3 H, Ar–Me), 3.6 (s, 16 H, NCH_2CH_2Cl), 3.7 (s, 3 H, –OMe), 6.5 (s, 2 H, Ar–H).

*3,5-Di{bis-[2-(3,5-dimethyl-1-pyrazolyl)ethyl]-amino}-4-methoxytoluene (**11a**)*

A solution of 3,5-dimethylpyrazole (2.0 g, 20 mmol) in DMF (10 ml) was added to sodium hydride (1.0 g, 42 mmol) in DMF (10 ml) under nitrogen. The slurry was stirred at room temperature for 1 h. Tetrachloride **10** (2.0 g, 4.9 mmol) in DMF (10 ml) was added and the reaction mixture was heated to 70 °C. After 46 h, water (10 ml) was carefully added to quench the reaction. The solution was evaporated to dryness, treated with 10% sodium hydroxide (10 ml) and extracted with benzene (3 X 250 ml). The extracts were dried (Na_2SO_4) and evaporated to an oil. The product was isolated by flash chromatography with ethyl acetate–methanol (9:1), R_f = 0.32. ^1H NMR ($CDCl_3$): δ 2.20 (s, 12 H, Pz–Me), 2.32 (s, 12 H, Pz–Me), 3.25 (s, 3 H, Ar–Me), 3.42 (s, 3 H, –OMe), 3.53 (t, J = 6.3 Hz, 8 H, $ArNCH_2$), 4.00 (t, J = 6.3 Hz, 8 H, $PzCH_2$), 5.72 (s, 4 H, Pz–H), 6.40 (s, 2 H, Ar–H).

*2,6-Di{bis-[2-(3,5-dimethyl-1-pyrazolyl)ethyl]-amino}-p-cresol (**12a**)*

The methyl protected ligand **11a** (1.87 g, 3 mmol) and sodium methyl mercaptide (1.0 g, 14 mmol) in DMF (400 ml) were allowed to reflux under nitrogen. After 5 h, the reaction mixture was evaporated to dryness. The residue was dissolved in water (50 ml), neutralized with 5% hydrochloric acid and extracted with benzene (3 X 150 ml). The extracts were dried (Na_2SO_4) and evaporated to an oil which was purified by flash chromatography with 95:5 ethyl acetate–methanol to yield 1.76 g (96%), R_f = 0.29. ^1H NMR ($CDCl_3$): δ 1.78 (s, 12 H, Pz–Me), 1.88 (s, 12 H, Pz–Me), 1.95 (s, 3 H, Ar–Me), 3.10 (t, J = 6.5 Hz, 8 H, $ArNCH_2$), 3.65 (t, J = 6.5 Hz, 8 H, $PzCH_2$), 5.48 (s, 4 H, Pz–H), 6.05 (s, 1 H, Ar–OH), 7.1 (s, 2 H, Ar–H).

*3,5-Di{bis-[2-(3-phenyl-1-pyrazolyl)ethyl]amino}-4-benzyloxytoluene (**11b**)*

3,5-Di[bis-(2-hydroxyethyl)amino]-4-benzyloxytoluene [5] (1.59 g, 3.9 mmol) was added to thionyl chloride (4.5 g, 38 mmol) in methylene chloride (50 ml). The reaction mixture was stirred at room temperature for 20 h and was evaporated under a stream of nitrogen. After all of the excess thionyl chloride had been removed, the crude chloride was dissolved in DMF (20 ml) and added to a solution of 16 mmol of sodium 3-phenylpyrazolate [prepared from 2.3 g of 3-phenylpyrazole and excess NaH (1.0 g) in DMF (20 ml)]. After stirring for 3 days at room temperature the reaction was quenched with water (20 ml) and evaporated to dryness. The residue was treated with 10% sodium hydroxide and extracted with benzene (5 X 100 ml). The extracts were dried ($MgSO_4$) and evaporated to a pale oil. The product, 0.85 g (24%), was isolated by flash chromatography using 3:1 hexane–ethyl acetate as the eluent (R_f = 0.15). ^1H NMR ($CDCl_3$): δ 2.16 (s, 3 H, Ar–Me), 3.67 (t, J = 5.9 Hz, 8 H, $ArNCH_2$), 4.12 (t, J = 5.9 Hz, 8 H, $PzCH_2$), 4.36 (s, 2 H, $PhCH_2O$), 6.35 (d, J = 1.9 Hz, 4 H, pyrazolyl C–H), 6.38 (s, 2 H, Ar–H), 7.08 (d, J = 1.9 Hz, 4 H, pyrazolyl C–H), 7.35 (t, J = 7.2 Hz, 8 H, Ar–H), 7.25 (t, J = 7.2 Hz, 4 H, Ar–H), 7.73 (d, J = 7.2 Hz, 8 H, Ar–H).

*2,6-Di{bis-[2-(3-phenyl-1-pyrazolyl)ethyl]amino}-p-cresol (**12b**)*

Compound **11b** (0.700 g, 0.7 mmol) was allowed to reflux in 20% aqueous hydrobromic acid (35 ml) for 19 h. The reaction mixture was neutralized with 10 M sodium hydroxide and extracted with methylene chloride (5 X 50 ml). The organic extracts were dried (Na_2SO_4) and concentrated. The crude product was purified by flash chromatography using 55:45 hexanes–ethyl acetate as the eluent to afford 0.240 g (38%) of product (R_f = 0.34). ^1H NMR ($CDCl_3$): δ 2.17 (s, 3 H, Ar–Me), 3.43 (t, J = 5.8 Hz, 8 H, $ArNCH_2$), 4.05 (t, J = 5.8 Hz, 8 H, $PzCH_2$), 6.34 (d, J = 1.4 Hz, 4 H, pyrazolyl C–H), 6.61 (s, 2 H, Ar–H), 7.16 (d, J = 1.4 Hz, 4 H, pyrazolyl C–H), 7.26 (t, J = 7.0 Hz, 4 H, Ar–H), 7.36 (t, J = 7.0 Hz, 8 H, Ar–H), 7.76 (d, J = 7.0 Hz, 8 H, Ar–H), 8.12 (s, 1 H, Ar–OH).

3,5-Di{bis-[2-(3-t-butyl-1-pyrazolyl)ethyl]amino}-4-benzyloxytoluene (11c)

The same procedure as outlined for the preparation of compound **11b** was used for 0.9 g (2.23 mmol) of 3,5-di[bis-(2-hydroxyethyl)amino]-4-benzyloxytoluene [5] and 1.1 g (8.9 mmol) of 3-t-butylpyrazole. The product (0.49 g, 27%) was isolated by flash chromatography using 2:1 ethyl acetate–hexanes (R_f = 0.33). ^{1}H NMR ($CDCl_3$): δ 1.28 (s, 36 H, t-butyl), 2.30 (s, 3 H, Ar–CH_3), 3.54 (t, 8 H, ArNCH_2), 4.02 (t, 8 H, PzCH_2), 4.38 (s, 2 H, Ar–CH_2–Ph), 5.99 (d, 4 H, pyrazolyl-H), 6.38 (s, 2 H, Ar–H), 7.0 (d, 4 H, pyrazolyl-H), 7.18–7.40 (m, 5 H, phenyl).

3,5-Di{bis-[2-(3-t-butyl-1-pyrazolyl)ethyl]amino}-p-cresol (12c)

Compound **11c** (0.49 g, 0.6 mmol) was deprotected using the same procedure used to prepare **12b**. Flash chromatography with 3:2 ethyl acetate–hexanes afforded 0.25 g (56%) of product. ^{1}H NMR ($CDCl_3$): δ 1.29 (s, 36 H, t-butyl), 2.21 (s, 3 H, Ar–CH_3), 3.33 (t, 8 H, ArNCH_2), 3.98 (t, 8 H, PzCH_2), 6.0 (s, 2 H, pyrazolyl-H), 6.58 (s, 2 H, Ar–H), 7.07 (s, 4 H, pyrazolyl C–H).

1,3-Di[bis-(2-hydroxyethyl)amino]benzene (13)

m-Phenylenediamine (3.4 g, 0.031 mol) was dissolved in 1:1 methanol–water (100 ml) and cooled in an ice bath. Concentrated hydrochloric acid (1 ml) and ethylene oxide (10 ml) were added, and the flask was sealed with a septum. The reaction was stirred at room temperature for 3–4 days until only one spot was observed by thin layer chromatography (95:5 ethyl acetate–methanol R_f = 0.28). After evaporation of the methanol at reduced pressure, the reaction mixture was neutralized with aqueous sodium bicarbonate and continuously extracted with ethyl acetate for 24 h. The extracts were dried (Na_2SO_4) and evaporated to a pale, viscous oil (8.8 g, 99%). ^{1}H NMR ($CDCl_3$): δ 3.49 (t, J = 5.6 Hz, 8 H, ArNCH_2), 3.71 (t, J = 5.6 Hz, 8 H, –CH_2OH), 4.38 (br s, 4 H, –OH), 6.06 (d, J = 7.6 Hz, 2 H, Ar–H), 6.08 (s, 1 H, Ar–H), 6.9 (t, 1 H, 7.6 Hz, 1 H, Ar–H); ^{13}C NMR 55.31, 60.51, 97.43, 101.91, 130.19, 149.93.

1,3-Di[bis-(2-methanansulfonatoethyl)amino]benzene (14)

Tetraol **13** (6.0 g, 21 mmol) was dissolved in triethylamine (200 ml) and methylene chloride (50 ml). The solution was stirred under nitrogen and cooled to –30 °C. Methanesulfonyl chloride (8.5 ml) in methylene chloride (20 ml) was added dropwise over 40 m. The reaction mixture was allowed to warm to 0 °C over a period of 0.5 h and was poured into a mixture of saturated aqueous sodium bicarbonate and ice. The layers were separated rapidly, and the organic phase was dried (Na_2SO_4). Evaporation under reduced pressure at room temperature afforded a pale oil (11.7 g, 93%), R_f = 0.67 (ethyl acetate). This compound was used immediately without purification. ^{1}H NMR ($CDCl_3$): δ 3.0 (s, 12 H, –OMs), 3.68 (t, 8 H, ArNCH_2), 4.40 (t, 8 H, CH_2O–), 6.15 (s, 1 H, Ar–H), 6.17 (d, 2 H, Ar–H), 7.08 (t, 1 H, Ar–H).

1,3-Di{bis-[2-(1-pyrazolyl)ethyl]amino}benzene (15)

Pyrazole (3.55 g, 52 mmol) in DMF (50 ml) was added to a cooled slurry of sodium hydride (1.4 g, 12.5 mmol) in DMF (25 ml) under nitrogen. When the gas evolution was complete, tetramesylate **14** (7.5 g, 12.0 mmol) in DMF (100 ml) was added dropwise over 1 h with cooling. The reaction was stirred at room temperature for 1 day, quenched with water (10 ml) and evaporated to dryness. The residue was triturated with absolute ethanol and filtered. After evaporation of the solvent, the crude product was purified by flash chromatography with 5:95 methanol–ethyl acetate (R_f = 0.34) to afford 4.2 g (66%) of a pale oil. ^{1}H NMR ($CDCl_3$): δ 3.58 (t, J = 6.3 Hz, 8 H, ArNCH_2), 4.14 (t, J = 6.3 Hz, 8 H, PzCH_2), 6.08 (s, 1 H, Ar–H), 6.16 (d, J = 8.1 Hz, 2 H, Ar–H), 6.20 (t, J = 2.0 Hz, 4 H, pyrazolyl C–H), 7.11 (t, J = 8.1 Hz, 1 H, Ar–H), 7.25 (d, J = 2.0 Hz, 4 H, pyrazolyl C5–H), 7.53 (d, 1.0 Hz, 4 H, pyrazolyl C3–H). ^{13}C NMR ($CDCl_3$): 49.23, 51.60, 96.54, 101.85, 105.22, 129.96, 130.35, 139.58, 147.82.

{2,6-Di[bis-2-(3,5-dimethyl-1-pyrazolyl)ethyl]-amino-p-cresolato}dicopper(I) Tetrafluoroborate (2a)

Ligand **12a** (780 mg, 1.2 mmol) was dissolved in methanol (3 ml) and treated with a solution of potassium methoxide (1.2 mmol) in methanol (5 ml) under an inert atmosphere. Tetrakis(acetonitrile)-copper(I) tetrafluoroborate (782 mg, 2.4 mmol) in methanol (15 ml) was added and the resultant mixture was filtered. The filtrate was evaporated to dryness under reduced pressure, dissolved in a minimum amount of methanol and filtered. Orange crystals were obtained by slow diffusion of THF into the methanol solution. ^{1}H NMR (DMSO-d_6): δ 2.06 (s, 12 H, Pz–Me), 2.12 (s, 15 H, Pz–Me, Ar–Me), 3.05 (m, 4 H), 3.40 (m, 4 H), 3.62 (m, 4 H), 3.96 (m, 4 H), 5.88 (s, 4 H, pyrazolyl C–H), 6.83 (s, 2 H Ar–H). *Anal.* Calc. for $C_{35}H_{49}N_{10}OBF_4$: C, 50.11; H, 5.89; N, 16.70. Found: C, 48.58; H, 5.68; N, 16.77%

{2,6-Di{bis-[2-(3-phenyl-1-pyrazolyl)ethyl]amino}-p-cresolato}dicopper(I) Tetrafluoroborate (2b)

Ligand **12b** (240 mg, 0.3 mmol) was dissolved in methanol (5 ml) and treated with potassium methoxide (0.3 mmol) in methanol (1.2 ml) in an inert atmosphere box. A solution of tetrakis(acetonitrile)-

copper(I) tetrafluoroborate (190 mg, 0.6 mmol) in methanol (10 ml) was added. After 10 min, a tan precipitate was collected, dissolved in a minimum amount of acetonitrile and filtered. The complex crystallized by slow diffusion of THF into the acetonitrile solution. ^{1}H NMR (DMSO-d_6): δ 2.17 (s, 3 H, $ArCH_3$), 3.10–3.20 (m, 4 H, $PzCH_2$), 3.40–3.60 (m, 4 H, $PzCH_2$), 3.91–4.08 (m, 4 H, $ArNCH_2$), 4.16–4.28 (m, 4 H, $ArNCH_2$), 6.58 (t, J = 7.6 Hz, 8 H, Ar–H), 6.76 (d, J = 2.4 Hz, 4 H, pyrazolyl C–H), 6.89 (t, J = 7.6 Hz, 4 H, Ar–H), 7.00 (s, 2 H, Ar–H), 7.76 (d, J = 2.4 Hz, 4 H, pyrazolyl C–H), 8.04 (d, J = 7.6 Hz, 8 H, Ar–H). *Anal.* Calc. for $C_{41}H_{49}N_{10}OCu_2BF_4$: C, 59.35; H, 4.80; N, 13.58. Found: C, 59.05; H, 4.47; N, 13.74%.

{2,6-Di{bis-[2-(3-t-butyl-1-pyrazolyl)ethyl]amino}-p-cresolato}dicopper(I) Tetrafluoroborate (2c)

A solution of 0.126 (0.17 mmol) of ligand **12c** was treated with an equivalent of KH dissolved in methanol under dinitrogen. To this solution was added 0.11 g (0.34 mmol) of $Cu(CH_3CN)_4BF_4$. After stirring for several minutes, the solution was evaporated to dryness, then crystallized from methanol–THF. ^{1}H NMR ($CDCl_3$): δ 1.24 (s, 36 H, t-butyl), 2.20 (s, 3H, $ArCH_3$), 3.16 (m, 4 H, CH_2), 3.36 (m, 4 H, CH_2), 4.06 (m, 4 H, CH_2), 4.21 (m, 4 H, CH_2), 6.19 (d, 4 H, pyrazolyl C–H), 6.84 (s, 2 H, Ar–H), 7.32 (d, 4 H, pyrazolyl C–H). *Anal.* Calc. for $C_{43}H_{65}BCu_2F_4N_{10}O$: C, 54.25; H, 6.88; N, 14.71. Found: C, 54.58; H, 7.09; N, 14.08%.

μ-Acetato{2,6-di{bis-[2-(3,5-dimethyl-1-pyrazolyl)-ethyl]amino}-p-cresolato}dicopper(II) Bis(perchlorate) Monohydrate (16)

A solution of 203 mg (0.33 mmol) of ligand **12a** in methanol (5 ml) was treated with $Cu(ClO_4)_2 \cdot 6H_2O$ (230 mg, 0.62 mmol). The resulting purple–brown solution was treated with sodium acetate (50 mg, 0.83 mmol). Isopropanol (50 ml) was added to the olive green solution. After stirring at room temperature overnight, the crude product was collected by filtration and dissolved in a minimum amount of acetone. From the filtered solution, green prisms were obtained by allowing THF to slowly diffuse into the acetone solution. *Anal.* Calc. for $C_{37}H_{54}N_{10}O_{12}Cu_2Cl_2$: C, 43.19; H, 5.10; N, 13.36; Cl, 6.89. Found: C, 43.20; H, 5.24; N, 12.42; Cl, 7.21%.

{1,3-Di{bis-[2-(1-pyrazolyl)ethyl]amino}benzene}-dicopper(I) Bis(tetrafluoroborate) (3)

Ligand **15** (808 mg, 1.67 mmol) in methanol (150 ml) was added to a solution of $Cu(CH_3CN)_4BF_4$ (1.05 g, 3.3 mmol) in methanol (100 ml) in an inert atmosphere box. The solvent was evaporated to dryness at reduced pressure, and the residue was recrystallized from methanol to yield 720 mg (55%) of colorless crystals. ^{1}H NMR (DMSO-d_6): δ 3.56 (m, 8 H, $ArNCH_2$), 4.38 (m, 8 H, $PzCH_2$), 5.51 (s, 1 H, Ar–H), 5.87 (d, J = 8.5 Hz, 2 H, Ar–H), 6.41 (t, J = 2 H, 4 H, pyrazolyl C–H), 6.60 (t, J = 8.5 Hz, 1 H, Ar–H), 7.64 (d, J = 2 Hz, 4 H, pyrazolyl C–H), 7.92 (d, J = 2 Hz, 4 H, pyrazolyl C–H). *Anal.* Calc. for $C_{26}H_{32}N_{10}Cu_2B_2F_8$: C, 39.77; H, 4.11; N, 17.84. Found: C, 40.23; H, 4.35; N, 17.71%.

μ-Pyrazolato-{1,3-di{bis-[2-(1-pyrazolyl)ethyl]-amino}benzene}dicopper(II) Tris(tretrafluoroborate) Hemi(tetrahydrofuranate) (17)

Ligand **15** (528 mg, 1.1 mmol) in methanol (3 ml) and pyrazole (62 mg, 1.0 mmol) were added to a slurry of tetrakis(acetonitrile)copper(II) bis(tetrafluoroborate) [11] (873 mg, 2.2 mmol) in methanol (5 ml) in an inert atmosphere box. The mixture was immediately treated with potassium methoxide (1.0 mmol) in methanol (2 ml), and the green solution was filtered. Slow diffusion of THF into the methanol solution gave turquoise crystals. *Anal.* Calc. for $C_{31}H_{39}N_{12}O_{1/2}Cu_2B_3F_{12}$: C, 38.19; H, 4.03; N, 17.24. Found: C, 37.91; H, 4.13; N, 17.14%.

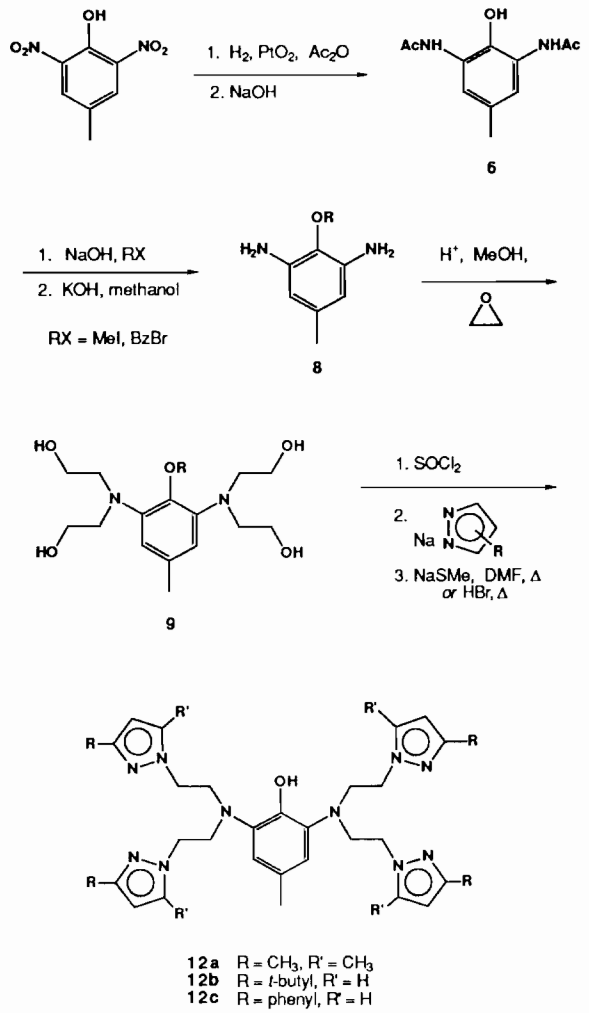
Low Temperature Absorption Measurements

Absorption spectra were recorded on a Hewlett-Packard 8540A or 8541A diode array spectrometer fitted with an Oxford Instruments DN 1704 variable temperature Dewar. Samples were prepared in a 1 cm quartz cell and sealed under nitrogen with a septum. Oxygen was purified by passage through a column of $CaSO_4$ and added to the atmosphere above the sample by slow passage through a syringe needle.

Results and Discussion

The synthetic sequences used for the preparation of Hbpeac derivatives are shown in Schemes 1 and 2. The first type differed by the use of pyrazole derivatives in the sequence published previously [5]. We found that the use of a methyl protecting group for intermediates 7–**11** substantially increased the yield and stability of the intermediates in the synthesis, although the *O*-benzyl intermediate was also used. The tetraol was converted to tetrachloride **10** which is relatively unstable but can be isolated in moderate yield. Nucleophilic displacement with substituted pyrazolate anions gave the protected ligands. Deprotection of the phenol oxygen atom was readily accomplished with sodium methylmercaptide in refluxing DMF.

There are two possible products formed in the alkylation of a pyrazolate anion because reaction can occur at either N_1 or N_2. This synthetic route to Hbpeac derivatives is thereby limited to pyrazoles that are either symmetrical or in which the steric and electronic factors prevent alkylation of the nitrogen

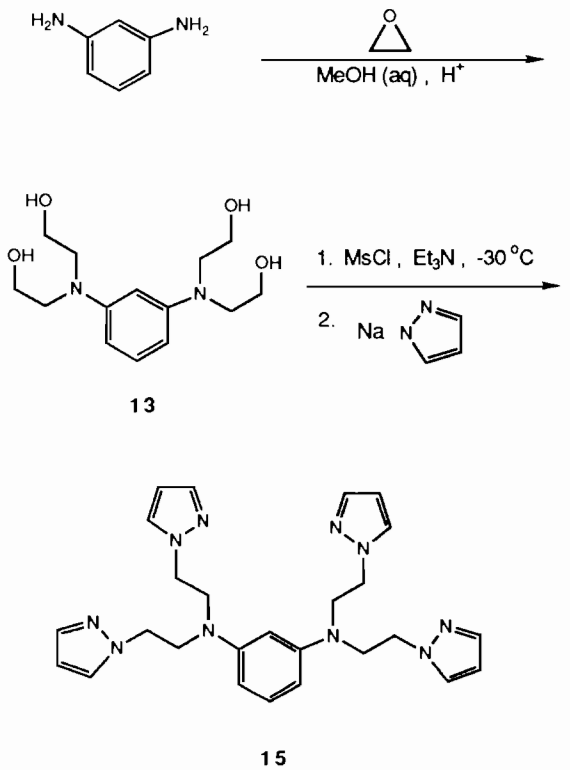


Scheme 1.

in the 2-position. Alkylation of the sterically-hindered 3-phenyl- and 3-t-butylpyrazolate ions resulted only in generation of the N_1-substituted isomer. 3,5-Dimethylpyrazole is symmetrical and therefore can give only a single product. Several other pyrazoles with polar amide groups were prepared by standard methods [12] from aminopyrazoles or 3,5-pyrazoledicarboxylic acid, but the nucleophilic displacement with those derivatives failed. This may be a result of charge delocalization, decreasing the reactivity of the anion, or to a chelate effect. In any case, the desired (amidopyrazole bpeac ligands could not be obtained.

The three pyrazoles which did react gave ligands that we could use to probe the effect on dioxygen reactivity by varying the steric bulk at the reactive face of the resulting copper(I) complex. The copper(I) complexes of ligands **12** were prepared by treating their potassium salts with tetrakis(acetonitrile)-copper(I) tetrafluoroborate and recrystallization from appropriate solvents. Surprisingly, the copper(I) complexes (**2**) are inert to dioxygen in a variety of aprotic solvents, even at room temperature.

To determine whether the lack of reactivity toward O_2 is a result of steric or electronic effects, we prepared the Cu(II) derivative $Cu_2(Me_8bpeac)(OAc)^{2+}$ (**16**), the least sterically-hindered of the complexes. Just as we found previously for $Cu_2(bpeac)(OAc)^{2+}$, the complex is not magnetically coupled (C. J. O'Connor, unpublished results). The acetate ion bridges between the copper(II) ions, as judged by infrared spectroscopy. Treatment of the acetate complex with a ten-fold excess of azide ion gave the corresponding azido complex characterized by appearance of azide-to-copper charge transfer bands in the visible region of the spectrum [λ_{max} = 297 nm (ϵ = 5500 M^{-1} cm^{-1}), 415 (4100), and 720 (300)]. Attempts to isolate this product as a solid were unsuccessful. Since both acetate and azide ions are apparently able to bind to the copper centers, we think that steric effects cannot account for the lack of reactivity of the copper(I) complexes (**2**) toward dioxygen. Instead, we believe that introduction of substituents on the pyrazole rings raises the oxidation potential, stabilizing the reduced valence. We demonstrated several years ago that such a phenomenon is observed for mononuclear copper(I) complexes [13]; and the present results are consistent with that observation.



Scheme 2.

A second modification of the parent Hbpeac ligand that we made was the removal of the phenolic oxygen group at position 1 of the benzene ring. The rationale for this comes from a consideration of results of the crystal structure of deoxyhemocyanin which shows no tyrosine residue within 17 Å of the active site [14]. Ligand **15** was prepared from *o*-phenylenediamine according to Scheme 2. The diamine was treated with ethylene oxide in acidic methanol to give the tetraol **14** which can be converted to the tetramesylate at −30 °C with methanesulfonyl chloride and triethylamine. Subsequent nucleophilic displacement with sodium pyrazolate gave the desired ligand. The copper(I) complex was again isolated as a white crystalline solid by treatment of the ligand with $Cu(CH_3CN)_4BF_4$.

The copper(I) complex (**3**) reacts rapidly at room temperature with oxygen in methanol to form an intensely violet-colored species with λ_{max} = 390 nm (ϵ = 9000 M^{-1} cm^{-1}) and 550 nm (2000). Measurement of oxygen uptake by manometric methods was impossible because of the low solubility of the complex. The intact ligand could not be reisolated after the reaction with oxygen. Instead, a violet-colored organic compound was isolated, after removal of the copper ions by extraction with ammonium hydroxide. This compound has not been thoroughly characterized; however, the intense color and changes in the aromatic region of the ^1H and ^{13}C NMR spectra suggest that the phenyl ring of the ligand has undergone some type of oxidation.

We were also unable to isolate the copper(II) complex of ligand **15** by the treatment of the ligand with $Cu(BF_4)_2 \cdot 6H_2O$. However, the copper(II)–pyrazolate complex (in which the pyrazolate may be bridging the copper ions, by analogy with known complexes [15–17]) was prepared by treatment of the ligand with tetrakis(acetonitrile)copper(II) bis(tetrafluoroborate) [11] and potassium pyrazolate. This complex was stable in the solid state; however, when redissolved in methanol, the color gradually changed from green to violet with an electronic spectrum nearly identical to that observed for the reaction of **3** with dioxygen. Thus, it appears that the copper(I) complex (**3**) reacts with O_2 to form a copper(II) complex which in turn oxidizes the ligand.

Conclusions

Binuclear copper complexes having different steric and/or electronic constraints built into the ligand are either inert to dioxygen or react by simple oxidation of the metal centers. Thus, modification of ligands on the heterocyclic ring to introduce steric hindrance is likely to influence the electronic properties of the metal ions as well, changing the overall reactivity of the complex. Alternate strategies may be necessary to prepare copper complexes able to bind dioxygen at ambient temperature.

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