

Synthesis and Characterization of Copper(II) Complexes of Cysteinyl-dopa and Benzothiazine Model Ligands Related to Pheomelanin

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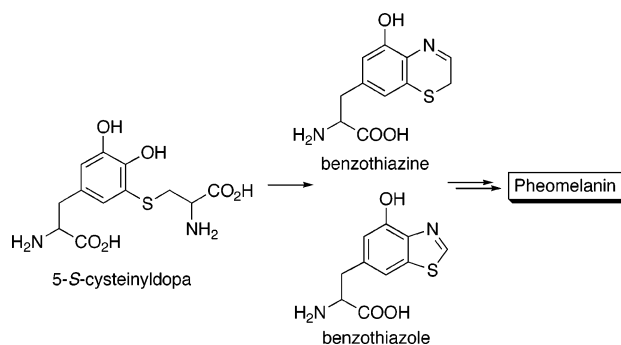
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A new cysteinyl-dopa model ligand **Cydo** {3-[(2-aminoethyl)sulfanyl]-4,6-di-*tert*-butylbenzene-1,2-diol} was prepared and its reactivity with Cu^{II} explored. Under anaerobic conditions, tetranuclear [Cu₄(Cydo)₄] is isolated, but in the presence of O₂, a benzothiazine intermediate accumulates that is trapped as the Cu^{II} complex [Cu(zine)₂]. Under slightly different reaction conditions, the benzothiazine further oxidizes to benzothiazole (**zole**). All three compounds were characterized by X-ray crystallography, and the reactions were monitored spectrophotometrically.

Melanins are ubiquitous biological pigments found, for example, in skin, hair, and bird feathers.¹ The brown-black eumelanins are polymers composed primarily of 5,6-dihydroxyindoles, whereas the reddish pheomelanins are composed of benzothiazine and benzothiazole units that derive from cysteinyl-dopa (Scheme 1).² A third class of melanin includes neuromelanin, a pigment that contains features of both eumelanin and pheomelanin³ and that coats the neurons of the substantia nigra that are susceptible to cell death in Parkinson's disease.^{4,5}

The varied and sometimes controversial biological roles of melanins include UV photoprotection, camouflage, radical scavenging, and metal ion sequestration.⁶ Melanins accumulate relatively high concentrations of metal ions in vivo and have an even higher chelating capacity in vitro.⁷ Metals attenuate the redox behavior of melanins, perhaps affecting whether they act as pro- or antioxidants,^{8,9} and may modulate

Scheme 1



the biosynthesis of the pigments. For example, Fe^{III}, Cu^{II}, and Zn^{II} differentially affect the rates and product distribution of cysteinyl-dopa oxidation reactions,^{10–13} suggesting that metal ions may be regulatory factors that contribute to the variety of natural pheomelanins and mixed melanins.¹¹

Potential metal-binding ligands found in melanins include the carboxylic acid, catechol, and quinone functionalities of the dihydroxyindoles as well as the benzothiazine and benzothiazole units that contain N/O bidentate chelating motifs similar to that of the well-known chelator 8-hydroxyquinoline. Most attention has been paid to the catechol-type ligands found in eumelanin, whereas little is known about metal complexes of the pheomelanin units, even though red-hair melanosomes accumulate 4 times more Fe^{III} than black-hair melanosomes, with similar amounts of Cu^{II} and Zn^{II}.¹⁴

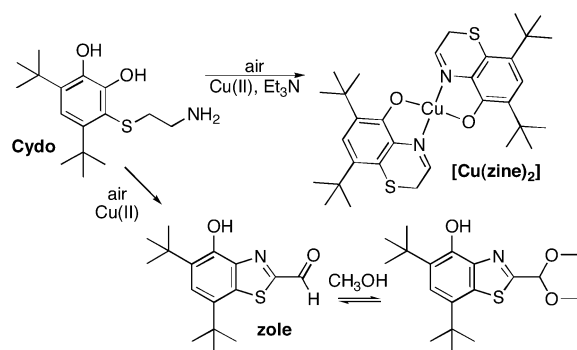
We are interested in studying the structure and reactivity of transition-metal ions in coordination environments such as those provided by eumelanin, pheomelanin, and neu-

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Scheme 2



romelanin.¹⁵ Here, we present the reactivity and coordination complexes of a 5-cysteinyldopa model ligand **Cydo** with Cu^{II} (Scheme 2).

The **Cydo** ligand is readily obtained in good yield by reacting an excess of 2-aminoethanethiol·HCl with 3,5-di-*tert*-butyl-*o*-benzoquinone at room temperature.¹⁶ The crude product can be used without further purification. Isolated as the HCl salt, solid **Cydo** is stable in air, slowly decomposes in an aerated solution, but more rapidly oxidizes in a basic solution¹⁷ to give a complex mixture in which **zole** and **zine** can be identified by mass spectrometry.

The 1:1 anaerobic reaction of **Cydo** and CuSO₄·5H₂O in the presence of Et₃N results in the formation of tetranuclear **[Cu₄Cydo₄]**, the structure of which is shown in Figure 1a with selected bond distances and angles given in Table 1.¹⁸

[Cu₄Cydo₄] is the first structurally characterized example of an aminothioether-appended catechol bound to a metal. Each of the four distorted trigonal-bipyramidal Cu centers is connected by bridging catechol O atoms (O1) to form a saddle-shaped eight-membered ring. This core is very similar to a known tetranuclear copper(II) catecholate pyridine cluster.¹⁹ In addition to two bridging catechol (O1) atoms from adjacent **Cydo** ligands, each Cu center is also coordinated by a terminal catechol (O2), a pendant amine (N1), and a thioether (S1) in the apical position with a Cu1–S1 distance of 2.613(1) Å.

The C–O bond lengths of 1.336(5) and 1.361(4) Å match other catecholate complexes and confirm the oxidation state as copper(II) catecholate as opposed to copper(I) semi-

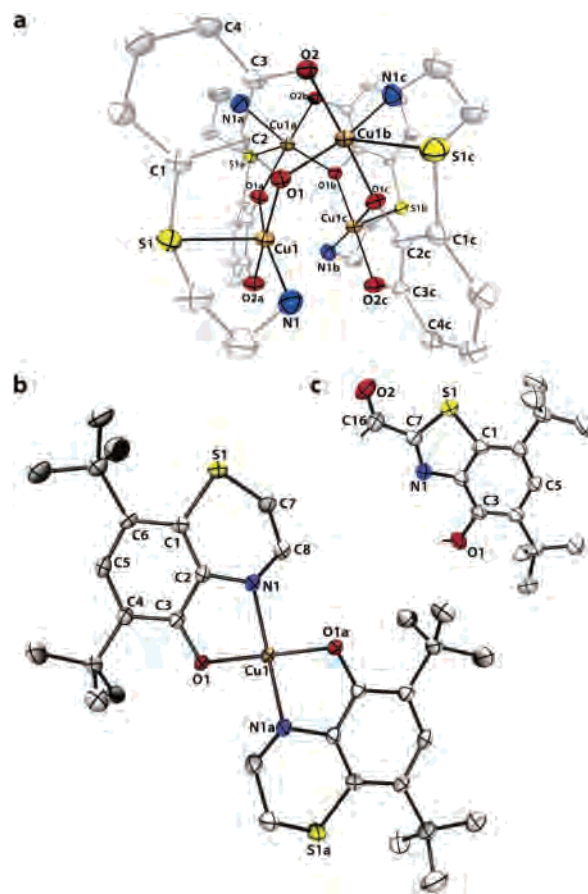


Figure 1. X-ray structures of (a) **[Cu₄Cydo₄]** (*tert*-butyl groups were removed for clarity), (b) **[Cu(zine)₂]**, and (c) **zole**, shown as ORTEP diagrams with 50% thermal ellipsoids. The only H atoms shown are those of the alcohol and aldehyde on **zole** (c).

Table 1. Selected Bond Lengths (Å) and Angles (deg) for **[Cu₄Cydo₄]** and **[Cu(zine)₂]**

[Cu₄Cydo₄]			
Cu1–S1	2.613(1)	O1–Cu1–O1a	92.8(1)
Cu1–O1	1.959(3)	O1a–Cu1–O2a	85.3(1)
Cu1–O1a	1.951(3)	O2a–Cu1–N1	89.3(1)
Cu1–O2a	1.891(3)	N1–Cu1–O1	92.2(1)
Cu1–N1	2.019(3)	O1–Cu1–O2a	177.5(1)
N1–C8	1.461(6)	N1–Cu1–O1a	165.6(1)
O1–C2	1.361(4)	S1–Cu1–O1	79.2(1)
O2–C3	1.336(5)	S1–Cu1–O1a	108.1(1)
C1–C2	1.405(5)	S1–Cu1–N1	86.2(1)
C3–C4	1.397(5)	S1–Cu1–O2a	102.9(1)
[Cu(zine)₂]			
Cu1–O1	1.894(1)	O1–Cu1–N1	84.21(7)
Cu1–N1	1.964(2)	O1–Cu1–N1a	95.79(7)
O1–C3	1.320(2)	O1–Cu1–O1a	180.0
N1–C2	1.415(3)	N1–Cu1–N1a	180.0
N1–C8	1.281(3)		

quinone.²⁰ Furthermore, the C–C bonds adjacent to the C–O groups [$d_{C1-C2} = 1.405(5)$ Å; $d_{C3-C4} = 1.397(5)$ Å] are shorter than the 1.44-Å value of coordinated semiquinones. The Cu^{II}–O bond distances are typical for Cu^{II} and are shorter for the terminal O [1.891(3) Å] compared to the bridging O [1.959(3) Å], as expected.¹⁹

If the 1:1 reaction of **Cydo** with CuSO₄·5H₂O and Et₃N is carried out in air, the mononuclear copper complex **[Cu-**

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(18) X-ray data collection was performed on a Bruker Kappa ApexII CCD diffractometer with Mo K α radiation (0.710 73 Å) at 174 K. **[Cu₄Cydo₄]**: C₆₄H₁₀₀Cu₄N₄O₈S₄, fw = 1435.88, tetragonal, space group I4₁/a, $a = b = 13.2334(2)$ Å, $c = 38.5792(9)$ Å, $V = 6756.1(2)$ Å³, $Z = 8$, $\mu = 1.420$ mm⁻¹, $\rho_{\text{calcd}} = 1.412$ Mg/m³, $R_1 = 0.0460$, $wR_2 = 0.1149$, GOF = 1.015. **[Cu(zine)₂]**: C₃₂H₄₄CuN₂O₂S₂, fw = 616.35, monoclinic, space group P2₁/c, $a = 12.6592(7)$ Å, $b = 8.9229(4)$ Å, $c = 14.7668(8)$ Å, $\beta = 109.399(4)^\circ$, $V = 1573.3(1)$ Å³, $Z = 2$, $\mu = 0.857$ mm⁻¹, $\rho_{\text{calcd}} = 1.301$ Mg/m³, $R_1 = 0.0359$, $wR_2 = 0.0862$, GOF = 1.004. **zole**: C₁₆H₂₁NO₂S, fw = 291.40, triclinic, space group P1, $a = 9.9417(5)$ Å, $b = 11.8736(6)$ Å, $c = 13.7756(7)$ Å, $\alpha = 89.784(1)^\circ$, $\beta = 82.815(1)^\circ$, $\gamma = 86.064(1)^\circ$, $V = 1609.5(1)$ Å³, $Z = 4$, $\mu = 0.202$ mm⁻¹, $\rho_{\text{calcd}} = 1.203$ Mg/m³, $R_1 = 0.0472$, $wR_2 = 0.1184$, GOF = 1.114.

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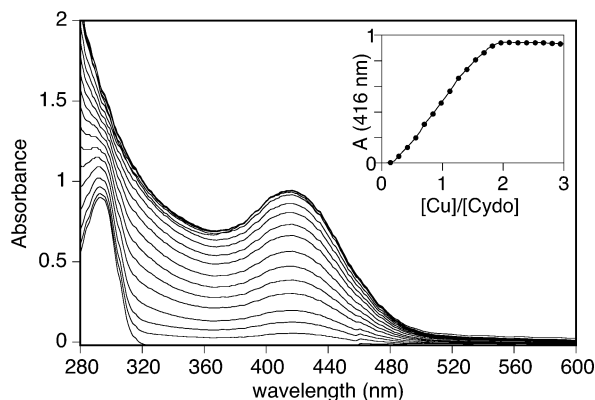


Figure 2. UV-vis spectra of a CH₃OH solution of **Cydo** upon incremental addition of up to 3 equiv of Cu^{II} in air. The inset shows an increase of the quinone band at 416-nm plateaus after the addition of 2 equiv of Cu^{II}.

(**zine**)₂] is isolated in good yield. As shown by the structure in Figure 1b, the reaction results in intramolecular condensation of **Cydo** to benzothiazine (**zine**).¹⁸ Similar transformations of 5-*S*-cysteinyl-dopa to 1,4-benzothiazines upon oxidation by K₃Fe(CN)₆ or peroxidase/H₂O₂ and during oxidation in the presence of transition metals have been reported, but no metal complexes were characterized in those studies.^{11,12,21}

The asymmetric unit of the [Cu(**zine**)₂] structure consists of a benzothiazine coordinated via the phenolate O and imine N to Cu^{II}, which sits on a crystallographic inversion center that generates the square-planar N₂O₂ coordination environment. The Cu–N and Cu–O bond lengths of 1.964(2) and 1.894(1) Å, respectively, are consistent with those found in Cu^{II} complexes of 8-hydroxyquinoline derivatives.²²

To gain more insight into the **Cydo** to **zine** transformation, we monitored the reaction spectrophotometrically. Figure 2 shows the changes in the UV-vis spectra of a CH₃OH solution of **Cydo**, which has a single absorption maximum at 293 nm, upon titration with CuSO₄ in air. A new absorption band at 416 nm increases in intensity until 2 equiv of Cu^{II} have been added; no changes are apparent with the addition of more Cu^{II} during the ~30-min time frame of the experiment (Figure 2, inset). The 416-nm band is attributed to the two-electron-oxidized *o*-quinone version of **Cydo**. A similar experiment performed under N₂ does not reveal a band at 416 nm, indicating that the Cu-mediated oxidation of **Cydo** requires O₂. An O₂ dependence on Cu-catalyzed oxidations of catechols and hydroquinones has been observed previously.^{23,24}

The 416-nm band is not stable indefinitely, as shown by the time-dependent spectra in Figure 3. The first spectrum recorded (thick red line) is that of a 1:1 solution of **Cydo** and Cu^{II}. The quinone signal at 416 nm initially increases in intensity before decreasing and shifting to 410 nm over 4 h. During this time, a new band at 318 nm replaces the

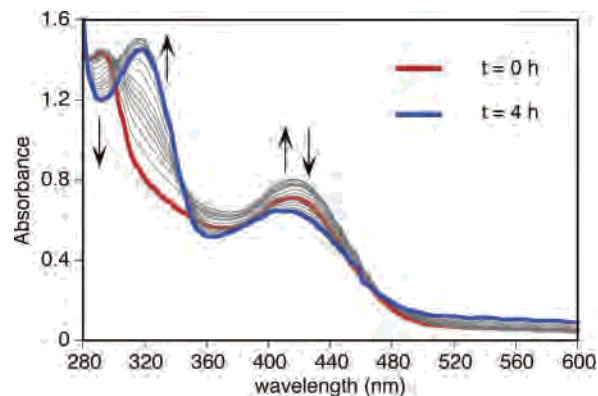


Figure 3. UV-vis spectra of **Cydo** with 1 equiv of Cu^{II} in CH₃OH in air measured over 4 h. The red line corresponds to the first time point. The decay of the 293-nm band signifies the disappearance of **Cydo**, while a new band appears at 318 nm. The band at 416 nm increases before losing intensity and shifting to 410 nm, indicating the formation of the dimethoxy derivative of **zole** (blue line).

original **Cydo** feature at 293 nm. Analysis by electrospray mass spectrometry of the final product, represented by the thick blue line with $\lambda_{\text{max}} = 318$ and 410 nm, indicates 5,7-di-*tert*-butyl-2-dimethoxybenzothiazol-4-ol ($m/z = 338$), which forms in situ from CH₃OH and the **zole** aldehyde (Scheme 2). Evaporation of CH₃OH and recrystallization from CH₃CN provide bright-yellow crystals of **zole**; the structure is shown in Figure 1c.¹⁸

If Et₃N is added to the reaction after the 416-nm quinone peak forms but before the 320-nm **zole** peak appears, [Cu(**zine**)₂] precipitates as the major product. The Et₃N facilitates metal complexation by deprotonating the phenolic O atom of benzothiazine. Once formed, [Cu(**zine**)₂] is stable in air, as tetrahydrofuran solutions show no signs of decomposition when monitored spectrophotometrically for 48 h. Benzothiazines are unstable, and prior studies relied on reduction to more stable dihydrobenzothiazines in order to confirm their presence after cysteinyl-dopa oxidation.¹⁰ The current work shows that coordination to a metal center stabilizes the benzothiazine intermediate from further oxidation and ring contraction to benzothiazole.

Of the many mechanisms that have been proposed for Cu-catalyzed oxidations of catechols and hydroquinones,^{23,24} two that are consistent with the observations in this study include the formation of a [Cu^IO₂]⁺ species as the active catalyst,²³ or alternatively, direct reaction of O₂ with a Cu-bound semiquinone intermediate.²⁴ Both mechanisms rely on the ability of the supporting ligand environment to modulate the Cu^{II}/Cu^I redox potential to support Cu^I. The pendant S and N ligands on **Cydo** could provide the softer environment favored by Cu^I. Further studies to probe these mechanistic hypotheses are underway.

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Supporting Information Available: X-ray structural information for [Cu₄Cydo₄], [Cu(**zine**)₂], and **zole** (CIF) and full experimental and synthetic details. This material is available free of charge via the Internet at <http://pubs.acs.org>.

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