Tyrosinase Models. Synthesis, Structure, Catechol Oxidase Activity, and Phenol Monooxygenase Activity of a Dinuclear Copper Complex Derived from a Triamino Pentabenzimidazole Ligand

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The dicopper(II) complex with the ligand *N*,*N*,*N*′,*N*′,*N*″-pentakis[(1-methyl-2-benzimidazolyl)methyl]dipropylenetriamine (LB5) has been synthesized and structurally characterized. The small size and the quality of the single crystal required that data be collected using synchrotron radiation at 276 K. $[Cu_2(LB5)(H_2O)_2][ClO_4]_4$: platelet shaped, $P\bar{1}$, $a = 11.028$ Å, $b = 17.915$ Å, $c = 20.745$ Å, $\alpha = 107.44°$, $\beta = 101.56°$, $\gamma = 104.89°$, $V = 3603.7$ Å^3, $Z = 2$; number of unique data, $I \geq 2\sigma(I) = 3447$; number of refined parameters = 428; $R = 0.12$. The ligand binds the two coppers nonsymmetrically; Cu1 is coordinated through five N donors and Cu2 through the remaining three N donors, while two water molecules complete the coordination sphere. Cu1 has distorted TBP geometry, while Cu2 has distorted SP geometry. Voltammetric experiments show quasireversible reductions at the two copper centers, with redox potential higher for the CuN_3 center (0.40 V) and lower for the CuN_5 center (0.17 V). The complex binds azide in the terminal mode at the CuN_3 center with affinity lower than that exhibited by related dinuclear polyaminobenzimidazole complexes where this ligand is bound in the bridging mode. The catechol oxidase activity of $[Cu_2(LB5)]^{4+}$ has been examined in comparison with that exhibited by $[Cu_2(L\text{-}55)]^{4+}$ (L-55 = α,α′-bis{bis[(1-methyl-2-benzimidazolyl)methyl]amino}-*m*-xylene) and $[Cu_2(L\text{-}66)]^{4+}$ (L-66 = α,α′-bis{bis[2-(1-methyl-2-benzimidazolyl)ethyl]amino}-*m*-xylene) by studying the catalytic oxidation of 3,5-di-*tert*-butylcatechol in methanol/aqueous buffer pH 5.1. Kinetic experiments show that $[Cu_2(L\text{-}55)]^{4+}$ is the most efficient catalyst (rate constant 140 M^{-1} s^{-1}), followed by $[Cu_2(LB5)]^{4+}$ (60 M^{-1} s^{-1}), in this oxidation, while $[Cu_2(L\text{-}66)]^{4+}$ undergoes an extremely fast stoichiometric phase followed by a slow and substrate-concentration-independent catalytic phase. The catalytic activity of $[Cu_2(L\text{-}66)]^{4+}$, however, is strongly promoted by hydrogen peroxide, because this oxidant allows a fast reoxidation of the dicopper(I) complex during turnover. The activity of $[Cu_2(LB5)]^{4+}$ is also promoted by hydrogen peroxide, while that of $[Cu_2(L\text{-}55)]^{4+}$ is little affected. The phenol monooxygenase activity of $[Cu_2(LB5)]^{2+}$ has been compared with that of $[Cu_2(L\text{-}55)]^{2+}$ and $[Cu_2(L\text{-}66)]^{2+}$ by studying the ortho hydroxylation of methyl 4-hydroxybenzoate to give methyl 3,4-dihydroxybenzoate. The LB5 complex is much more selective than the other complexes since its reaction produces only catechol, while the main product obtained with the other complexes is an addition product containing a phenol residue condensed at ring position 2 of the catechol.

Introduction

A large number of dinuclear copper complexes have been reported recently as model compounds for the active sites of type 3 copper proteins like hemocyanin and tyrosinase.[1,2] In general, complexes derived from dinucleating ligands providing symmetrically arranged, three-coordinate copper centers with nitrogen donors have been used, because this arrangement simulates the coordination environment present in hemocyanin[3]

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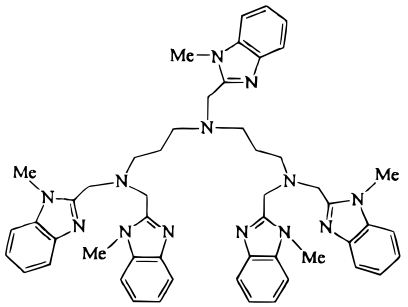
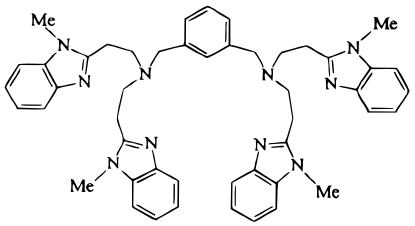
and tyrosinase,[4] although in the latter case the different accessibility and reactivity of the two copper centers may even reflect coordination asymmetry.[5] For this reason some of the biomimetic models reported recently use nonsymmetric dinucleating ligands to produce nonsymmetric dicopper complexes.[2de,6] We have recently developed a series of dinuclear copper complexes derived from *m*-xylyl tetrabenzimidazole ligands which act as functional models for tyrosinase.[7] In fact, their reduced forms are able to mediate the regiospecific ortho hydroxylation of exogenous phenols to catechols in the presence of dioxygen. In order to broaden the scope of our approach we are exploring the potentialities of other biomimetic systems where some structural variation is introduced in the ligand. In the present paper we report the biomimetic chemistry of a new polyaminobenzimidazole ligand, LB5 (Chart 1). We describe its synthesis, protonation equilibria, and copper(II) complexation properties and the preparation, X-ray structural characterization, electrochemistry, and reactivity of the dinuclear complex $[Cu^{II}_2(LB5)(H_2O)_2]^{4+}$. In particular, we report here comparative studies on the catechol oxidase activity and phenol hydroxylase activity between the present dicopper−LB5 system and the dicopper−L-55 and −L-66 systems reported previously.[7]

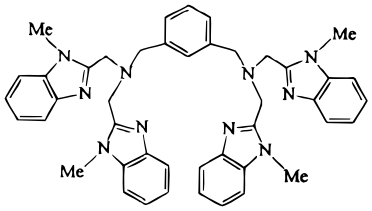
Chart 1



LB5



L-66



L-55

Experimental Section

General Procedures. All reagents and solvents were of commercially available reagent quality unless otherwise stated. Acetonitrile (spectral grade) was distilled from potassium permanganate and dry potassium carbonate; then, it was stored over calcium hydride and distilled prior to use under an inert atmosphere. Dioxygen was dried over a column of 3-Å molecular sieves. Lactoperoxidase was obtained from Sigma as a freeze-dried powder (RZ = 0.81). The ligands L-55 and L-66, as well as their copper complexes, methyl 4-hydroxybenzoate, and methyl 3,4-dihydroxybenzoate were prepared as described previously.[7d] 2-(Chloromethyl)-1-methylbenzimidazole was prepared following a known procedure.[7a] Preparation and handling of air-sensitive or moisture-sensitive materials were carried out under argon with standard Schlenk techniques.

Elemental analyses were from the microanalytical laboratory of the Chemistry Department in Milano. NMR spectra were recorded on a Bruker AC-200 spectrometer operating at 200 MHz. EPR spectra were measured in frozen solutions using a Varian E-109 spectrometer operating at X-band frequencies. UV−visible spectra were measured on a HP 8452A diode array spectrophotometer. Infrared spectra were recorded on a Jasco FT-IR 5000 instrument. Analyses of the organic mixtures resulting from the copper-mediated oxygenation of methyl 4-hydroxybenzoate were performed with a Perkin-Elmer 1020 LC PLUS HPLC, equipped with a LC 235C diode array detector, using a Merck LiChrosorb RP-18 column. The detector was set at 260 nm, and the eluent was a 60:40 (v/v) mixture of methanol containing 1% acetic acid and water. Using 4-hydroxybenzoic acid as standard for quantitative analysis, with a flow rate of 0.75 mL/min, the retention times were as follows: 4-hydroxybenzoic acid, 6 min; methyl 3,4-dihydroxybenzoate, 9 min; methyl 4-hydroxybenzoate, 13 min.

CAUTION! *While none of the perchlorate complexes reported in this paper have proven to be heat- or shock-sensitive, only small*

(1) (a) *Bioinorganic Chemistry of Copper*; Karlin, K. D., Tyeklàr, Z., Eds.; Chapman and Hall: New York, 1993. (b) Kitajima, N.; Moro-oka, Y. *Chem. Rev.* **1994**, *94*, 737. (c) Karlin, K. D.; Fox, S.; Nanthakumar, A.; Murthy, N. N.; Wei, N.; Obias, H. V.; Martens, C. F. *Pure Appl. Chem.* **1995**, *67*, 289. (d) Karlin K, D.; Tyeklàr, Z. In *Advances in Inorganic Biochemistry*; Eichhorn, G. L., Marzilli, L. G., Eds.; Prentice Hall: Englewood Cliffs, NJ, 1994; Vol. 9, p 123.

(2) See, for instance: (a) Martens, C. F.; Klein Gebbink, R. J. M.; Feiters, M. C.; Nolte, R. J. M. *J. Am. Chem. Soc.* **1994**, *116*, 5667. (b) Zeng, W. F.; Cheng, C. P.; Wang, S. M.; Cheng, M.-C.; Lee, G.-H.; Wang, Y. *Inorg. Chem.* **1995**, *34*, 728. (c) Müller, E.; Bernardinelli, G.; Reedijk, J. *Inorg. Chem.* **1995**, *34*, 5979. (d) Satcher, J. H.; Droege, M. W.; Weakley, T. J. R.; Taylor, R. T. *Inorg. Chem.* **1995**, *34*, 3317. (e) Lubben, M.; Hage, R.; Meetsma, A.; Byma, K.; Feringa, B. L. *Inorg. Chem.* **1995**, *34*, 2217. (f) Lee, D. H.; Wei, N.; Murthy, N. N.; Tyeklàr, Z.; Karlin, K. D.; Kaderli, S.; Jung, B.; Zuberbühler, A. D. *J. Am. Chem. Soc.* **1995**, *117*, 12498. (g) Zippel, F.; Ahlers, F.; Werner, R.; Haase, W.; Nolting, H.-F.; Krebs, B. *Inorg. Chem.* **1996**, *35*, 3409. (h) Brudenell, S. J.; Spiccia, L.; Tiekink, E. R. T. *Inorg. Chem.* **1996**, *35*, 1974. (i) Koolhaas, G. J. A. A.; Driessen, W. L.; van der Plas, J. L.; de Graaf, R. A. G.; Gatteschi, D.; Kooijman, H.; Spek, A. L. *Inorg. Chem.* **1996**, *35*, 1509. (j) Zeng, W. F.; Cheng, C. P.; Wang, S. M.; Lee, G.-H. *Inorg. Chem.* **1996**, *35*, 2259. (k) Jung, B.; Karlin, K. D.; Zuberbühler, A. D. *J. Am. Chem. Soc.* **1996**, *118*, 3763. (l) Ghosh, D.; Lal, T. K.; Ghosh, S.; Mukherjee, R. *J. Chem. Soc., Chem. Commun.* **1996**, 13. (m) Lee, D. H.; Murthy, N. N.; Karlin, K. D. *Inorg. Chem.* **1996**, *35*, 804. (n) Kamaras, P.; Cajulis, M. C.; Rapta, M.; Brewer, G. A.; Jameson, G. B. *J. Am. Chem. Soc.* **1994**, *116*, 10334.

(3) (a) Magnus, K. A.; Ton-That, H.; Carpenter, J. E. *Chem. Rev.* **1994**, *94*, 727. (b) Magnus, K. A.; Hazes, B. Ton-That, H.; Bonaventura, C.; Bonaventura, J.; Hol, W. G. J. *Proteins: Struct. Funct. Genet.* **1994**, *19*, 302.

(4) (a) Robb, D. A. In *Copper Proteins and Copper Enzymes*; Lontie, R., Ed.; CRC Press: Boca Raton, FL, 1984; Vol. 2, p 207. (b) Lerch, K. *Methods Enzymol.* **1987**, *142*, 165. (c) Sánchez-Ferrer, A.; Rodriguez-Lopez, J. N.; Garcia-Cánovas, F.; Garcia-Carmona, F. *Biochim. Biophys. Acta* **1995**, *1247*, 1.

(5) Yong, G.; Leone, C.; Strothkamp, K. G. *Biochemistry* **1990**, *29*, 9684.

(6) (a) Nasir, M. S.; Karlin, K. D.; McGowty, D.; Zubieta, J. *J. Am. Chem. Soc.* **1991**, *113*, 698. (b) Mahroof-Tahir, M.; Murthy, N. N.; Karlin, K. D.; Blackburn, N. J.; Shaikh, S. N.; Zubieta, J. *Inorg. Chem.* **1992**, *31*, 3001. (c) Adams, H.; Candeland, G.; Crane, J. D.; Fenton, D. E.; Smith, A. J. *J. Chem. Soc., Chem. Commun.* **1990**, 93. (d) Crane, J. D.; Fenton, D. E.; Latour, J. M.; Smith, A. J. *J. Chem. Soc., Dalton Trans.* **1991**, 2979.

(7) (a) Casella, L.; Gullotti, M.; Radaelli, R.; Di Gennaro, P. *J. Chem. Soc., Chem. Commun.* **1991**, 1611. (b) Casella, L.; Carugo, O.; Gullotti, M.; Garofani, S.; Zanello, P. *Inorg. Chem.* **1993**, *32*, 2056. (c) Casella, L. Gullotti, M. In ref. 1a, p 292. (d) Casella, L.; Monzani, E.; Gullotti, M.; Cavagnino, D.; Cerina G.; Santagostini, L.; Ugo, R. *Inorg. Chem.* **1996**, *35*, 7516.

amounts of materials should be prepared and handled. Care is recommended.[8]

***N,N,N′,N′,N″*-Pentakis[(1-methyl-2-benzimidazolyl)methyl]dipropylenetriamine (LB5).** A mixture of dipropylenetriamine (0.26 mL, 1.85 mmol), 2-(chloromethyl)-1-methyl benzimidazole (2.08 g, 11.53 mmol), anhydrous sodium carbonate (1.6 g), and dry acetonitrile (100 mL) was refluxed with stirring in the dark for 48 h. After cooling to room temperature, the precipitate was filtered off and washed several times with small amounts of acetonitrile. The solid was then poured into water (20 mL) and stirred in order to dissolve the inorganic salts. The undissolved white solid was collected by filtration and dried under vacuum (yield 70%). Anal. Calcd for $C_{51}H_{57}N_{13}\cdot 1.5H_2O$: C, 69.67; H, 6.88; N, 20.72. Found: C, 69.25; H, 6.73; N, 20.80. Mp: 215 °C dec; the mp did not change by further crystallization of the compound. IR (Nujol mull), cm^{-1}: 1615, 1513, 1402, 1331, 1288, 1258, 1240, 1209, 1177, 1150, 1141, 1121, 1101, 1052, 988, 769, 751, 736, 666. ^1H NMR ($CDCl_3$): δ 1.47 (m, 4H, CCH_2C), 2.25 (t, 4H, CH_2N), 3.36 (s, 3H, CH_3N), 3.39 (s, 12H, CH_3N), 3.62 (s, 2H, NCH_2-benzimidazole), 3.83 (s, 8H, NCH_2-benzimidazole), 7.0−7.4 (m, 15H, benzimidazolyl-H), 7.6−7.7 (m, 5H, benzimidazolyl-H).

$[Cu_2(LB5)(H_2O)_4][ClO_4]_4$. The ligand LB5 (0.31 g, 0.35 mmol) was dissolved in methanol (∼15 mL) with stirring. Then, $Cu(ClO_4)_2.6H_2O$ (8 mmol) dissolved in water (2 mL) was added. A green-blue precipitate immediately formed. The mixture was concentrated to about half-volume under vacuum, and the product was collected by filtration, washed with small amounts of water and diethyl ether, and dried (yield > 90%). Anal. Calcd for $C_{51}H_{57}N_{13}Cu_2Cl_4O_{16}\cdot 4H_2O$: C, 42.27; H, 4.52; N, 12.57. Found: C, 41.89; H, 4.36; N, 12.61. IR (Nujol mull), cm^{-1}: 3450, 1618, 1504, 1328, 1296, 1262, 1250, 1095, 1011, 975, 940, 932, 917, 843, 747, 723, 625. UV−vis [MeCN; λ_{max}, nm (ϵ, M^{-1} cm^{-1})]: 248 (43 000), 274 (44 000), 280 (40 500), 320 sh (3700), 670 (300), 795 (320).

$[Cu_2(LB5)(N_3)][ClO_4]_3.2H_2O$. This adduct was obtained by adding a solution of sodium azide (0.04 mmol) in a few milliliters of methanol to $[Cu_2(LB5)(H_2O)_4][ClO_4]_4$ (0.04 mmol) dissolved in a 2.5:1.0 (v/v) mixture of methanol/acetonitrile (30 mL) with stirring. The solution turned immediately to dark green. It was concentrated to a small volume, and diethyl ether was added to form a precipitate. This was filtered off, washed with small amounts of water, and dried under vacuum (yield ∼ 75%). Anal. Calcd for $C_{51}H_{57}N_{16}Cu_2Cl_3O_{12}\cdot 2H_2O$: C, 45.19; H, 4.54; N, 16.53. Found: C, 45.29; H, 4.38; N, 16.31. IR (Nujol mull), cm^{-1}: 3500, 2077, 1615, 1501, 1344, 1324, 1294, 1248, 1096, 1010, 982, 939, 932, 913, 774, 741, 723, 666. UV−vis [MeCN; λ_{max}, nm (ϵ, M^{-1} cm^{-1})]: 274 (44 500), 280 (41 000), 388 (1850), 680 sh (330), 780 (350).

Potentiometric Determinations. Potentiometric determinations of LB5 in the absence and in the presence of copper(II) ions were performed with apparatus and methods described elsewhere[9] in 50 mL of an 80:20 (v/v) dioxane/water mixture made 0.1 M in ionic strength with $NaClO_4$ at 298 K. The electrodes were dipped for $^1/_2$ h in the above solvent mixture before the system was standardized, by a procedure described elsewhere.[9] Nernst's equation was strictly obeyed in the range of $-Log[H^+]$ of the experiment to be performed. The HYPERQUAD[10] program was used to process the data to calculate both the protonation and stability constants.

Voltammetric Measurements. Cyclic voltammograms were obtained by an AMEL model 473 multipolarograph equipped with a three-electrode system, a 3-mm gold electrode as the working electrode, a platinum wire as the auxiliary electrode, and a Ag/Ag^+ [11] electrode as the reference electrode. Complex solutions (0.5 mM) were prepared by dissolving the ligand (L) and copper(II) perchlorate in Cu:L ratios of 1:1 and 2:1 in dry acetonitrile, using 100 mM tetraethylammonium perchlorate (TEAP) as the supporting electrolyte. Cyclic voltammograms at 25 ± 0.1 °C were recorded in the region from +1 to −1 V versus Ag/Ag^+, the sweep rate being varied from 10 to 200 mV s^{-1}, in order to gain information on the reversibility and the number of electrons of the electron-transfer process. The electrode processes are diffusion-controlled, as was ascertained from the variation of the current peak as a function of temperature, which gave temperature coefficients lower than 2%.

Single-Crystal X-ray Structure Determination of $[Cu_2(LB5)(H_2O)_2][ClO_4]_4$. The crystals of the dicopper(II) complex of LB5 used in X-ray analysis were grown by slow evaporation of a methanol solution of the complex. In spite of many attempts, we were not able to obtain single crystals of a size suitable for collecting data using conventional X-ray sources. Furthermore, the crystals lose crystallinity under long exposure to the X-ray beam, even when sealed in a glass capillary tube. These problems were overcome by collecting data using synchrotron radiation at the ELETTRA X-ray diffraction beam-line. The rotation method (60 frames of 3°) was used. A platelet-shaped light-blue crystal of dimensions 0.1 × 0.1 × 0.02 mm was sealed in a thin glass capillary tube, and data were collected at 276 K with a wavelength of 0.80 Å and a Mar Research imaging plate (18 cm) at a distance of 70 cm. The exposure time for each frame was 60 s. A total of 6929 reflections were collected, 3447 of which, having $I > 2\sigma(I)$, were used in the subsequent calculations. A lowering of the reflection intensity with increasing θ Bragg angle was apparent. Unit cell parameters are $a = 11.028$, $b = 17.9l5$, $c = 20.745$ Å, $\alpha = 107.44°$, $\beta = 101.56°$, $\gamma = 104.89°$ for the $P\bar{1}$ space group, $V = 3603.7$ $Å^3$, $Z = 2$, $M_w = 1574$, $D_{calc} = 1.45$ $g\cdot cm^{-3}$. Data were reduced by the program DENZO.[12] The structure was solved by conventional Patterson and Fourier methods and refined by the least-squares method.[13] The final refinement of 428 parameters, treating anisotropically only the Cu and Cl species and including the H atom contribution, held constant, gave an R index of 0.12 ($R_w = 0.16$). Complete tables of atomic parameters and bond lengths and angles are included as Supporting Information.

Ligand-Binding Studies. Spectrophotometric titrations of the binding of azide to $[Cu_2(LB5)]^{4+}$ were performed by adding small amounts of a concentrated solution of the ligand in methanol to a solution of the complex in 8:1 (v/v) methanol/acetonitrile. The data were analyzed as described previously.[7b,14] Similar titration experiments were carried out using methanolic sodium hydroxide solutions.

Kinetics of 3,5-Di-*tert*-Butylcatechol Oxidation. The kinetic experiments were followed spectroscopically using a magnetically stirred, thermostated, 1-cm-path-length cell and a HP8452 A diode array spectrophotometer. The temperature during the measurement was kept constant at 20 ± 0.1 °C. The solvent employed for the kinetic reactions was a 30:1 (v/v) mixture of methanol/aqueous phosphate buffer (50 mM) pH 5.1, saturated with atmospheric oxygen. The concentration of the copper complexes $[Cu^{II}_2(LB5)]^{4+}$, $[Cu^{II}_2(L\text{-}55)]^{4+}$, $[Cu^{II}_2(L\text{-}66)]^{4+}$, and $[Cu^{II}(ClO_4)_2]$ during the catalytic measurements was kept at 1.3×10^{-5} M while that of the substrate was varied from 0.07 to 15 mM. The formation of quinone was followed by observing the increase of the characteristic quinone absorption band around 400 nm. In order to decrease the effect of noise in the absorbance readings during the kinetic determinations, it was found convenient to monitor the difference between the absorbance at 396 nm and that at a wavelength where the absorbance remains negligible during the assay (700 nm). For the dicopper(II) complexes of LB5, L-66, and L-55, the reaction of which follows a biphasic behavior, the reaction rate was obtained from the slope in both parts of the trace at 396 nm. The conversion of the reaction rate units from ΔA/s to M/s was done using $\epsilon = 1500$ M^{-1} cm^{-1} for DTBQ in methanol. In a separate set of experiments, the

(8) (a) Wolsey, W. C. *J. Chem. Educ.* **1973**, *50*, A335. (b) Raymond, K. N. *Chem Eng. News* **1983**, *61* (Dec 5), 4. (c) *Chem. Eng. News* **1963**, *41* (July 8), 47.
(9) Fabbrizzi, L.; Kaden, T.; Perotti, A.; Seghi, B.; Siegfried, L. *Inorg. Chem.* **1986**, *25*, 807.
(10) Sabatini, A.; Vacca, A.; Gans, P. *Coord. Chem. Rev.* **1992**, *120*, 389.
(11) Sawyer, D. T.; Roberts, J. L. *Experimental Electrochemistry for Chemists*; Wiley: New York, 1974; p. 54.
(12) Otwinowski, B. *Oscillation Data Reduction Program*. In *Proceedings of the CCCP4 Study Weekend: Data Collection and Processing*, 29−30 January 1993; compiled by Sawer, L., Isaacs, N., Bailey, S.; SERC Daresbury Lab.: Daresbury, England; pp 56−62.
(13) Frenz, B. A. *An Interactive Structure Solution Procedure*; Enraf-Nonius: Delft, The Netherlands, 1990.
(14) Casella, L.; Gullotti, M.; Pallanza G.; Buga, M. *Inorg. Chem.* **1991**, *30*, 221.

kinetic determinations were performed using the methanol/buffer solution saturated with 1 atm of oxygen.

Detection of Hydrogen Peroxide in the Catalytic Reactions. To detect the formation of hydrogen peroxide during the catalytic reaction, the development of the characteristic I_3^- band ($\lambda_{max} = 353$ nm; $\epsilon = 26\,000$ M^{-1} cm^{-1}), upon reaction with I^-, was monitored. The oxidation reaction of DTBC in the presence of the different catalysts was carried out as in the kinetic experiments. When the absorbance at 396 nm, due to the formation of DTBQ, reached a value of 0.1−0.4 AU (using a path length of 1 cm), an equal volume of water was added. The quinone formed was extracted three times using dichloromethane (removal of the quinone is necessary since it can oxidize iodide). The water layer was acidified with H_2SO_4 to pH 2, and one-third volume of a solution of KI (50 g/L) in water and 100 nM lactoperoxidase (to accelerate the oxidation of iodide by hydrogen peroxide)[15] were added. In the presence of hydrogen peroxide, a band due to the formation of I_3^- develops. Since atmospheric oxygen can oxidize I^-, blank experiments (without catalyst or without catechol) were also performed. For the catalytic reactions performed in the presence of $[Cu_2(L\text{-}55)]^{4+}$, $[Cu_2(L\text{-}66)]^{4+}$, or $[Cu_2(LB5)]^{4+}$, no appreciable hydrogen peroxide accumulation was found. Instead, when the reaction was performed in the presence of copper(II) salt, the assay revealed a concentration of hydrogen peroxide corresponding to above 80% of the quinone formed in the catalytic reaction.

Effect of Hydrogen Peroxide on the Kinetics of DTBC Oxidation. The effect of H_2O_2 on the catalytic oxidation of DTBC was studied through observation of how the reaction rates, under the conditions used for the kinetic measurements, were altered by the presence of hydrogen peroxide in the reaction mixture. The concentration of DTBC, unless otherwise stated, was kept at 5 mM, while that of hydrogen peroxide was varied from 0 to 50 mM. The formation of DTBQ versus time always followed a biphasic behavior. For the dicopper(II) complexes of LB5 and L-55 the reaction rates were obtained for both of the phases, while for $[Cu_2(L\text{-}66)]^{4+}$ only the rate in the second (slow) phase could be obtained.

Study of the Catalase Activity of the Complexes. To observe whether the dicopper(II) complexes of LB5, L-66, and L-55 exhibited catalase activity, a 0.1 M solution of hydrogen peroxide in methanol was divided into four parts. One part was used as a blank, while the others were incubated for 2 h each in the presence of a 15 μM concentration of one of the dicopper(II) complexes. At the end of the incubation time, 5 μL of the incubated solutions were added to 2 mL of a water solution acidified to pH 2 with H_2SO_4 and containing 5 g/L of KI and 100 nM lactoperoxidase. After a 15-min incubation time, the spectra of the solutions were recorded using a 1-mm-path-length cuvette. The intensity of the I_3^- band developed at 353 nm was the same as that of the blank for all three complexes, indicating negligible catalase activity.

Phenol Hydroxylation. The procedure followed basically that reported for the studies employing the dicopper(I) complexes of L-55 and L-66.[7d] The dicopper(I) complex of LB5 was prepared in situ by mixing and heating under an inert atmosphere the ligand (0.05 mmol) and $[Cu(MeCN)_4][ClO_4]$ (0.1 mmol) in dry acetonitrile (30 mL). After cooling, a solution of tetra-*n*-butylammonium 4-carbomethoxyphenolate, or methyl 4-hydroxybenzoate, (0.05 mmol) in dry, degassed acetonitrile (~3 mL) was added via gas-tight syringe. The solution of the ligand, in the presence of the copper(I) complex, became yellow. Then, the solution was exposed to dioxygen (1 atm) at room temperature, with stirring. Samples (1 mL) for HPLC analysis were withdrawn at various times. Each sample was evaporated to dryness and decomposed with 2 M aqueous H_2SO_4 (4 mL). The phenolic derivatives were extracted with chloroform (3 × 5 mL). The combined organic extracts were washed with water saturated with Na_2SO_4 (2 mL) and rotary-evaporated to dryness. The residue was then dissolved in a few milliliters of methanol, a known amount of 4-hydroxybenzoic acid as internal standard was added, and the solution was analyzed by HPLC. The actual recovery of organic material was never above about 80% of the amount of phenol initially reacted. The figures reported as yields are normalized to 100% recovery.

(15) Jenzer, H.; Jones, W.; Kohler, H. *J. Biol. Chem.* **1986**, *261*, 15550.

Results and Discussion

Ligand Synthesis and Binding Properties. The synthetic route to LB5 is very simple, since it is based on the condensation reaction between dipropylenetriamine and 2-(chloromethyl)-1-methylbenzimidazole in the presence of base. The original scope of producing a ligand with eight nitrogen donors stemmed from the attempt to obtain trinuclear copper(II) complexes which could act as synthetic models for the as yet incompletely understood type 2−type 3 trinuclear cluster present in the blue copper oxidases,[16] where a total of eight histidine imidazole groups provide the protein ligands for the metal centers. It soon became clear that, as described below, only two copper(II) ions are tightly bound to LB5 and occupy all the available ligand donor atoms. In spite of its symmetric structure, LB5 binds the two metal ions asymmetrically, providing an example of ligand coordination asymmetry.[2d] It has been noticed that this type of coordination asymmetry may be important in determining the peculiar reactivity of a number of dinuclear metalloproteins.[2d]

Protonation and Stability Constants. In the range of $-\text{Log}[H^+]$ explored (from 3 to 9), seven protonation steps have been detected for LB5, with the cumulative Log β of formation constants of 9.33 (0.01), 12.01 (0.01), 17.10 (0.01), 21.25 (0.02), 24.65 (0.5), 27.55 (0.5), and 30.03 (0.5), respectively (the eighth protonation step should have a pK less than 2). The ligand easily incorporates two copper(II) ions in a stepwise process, and with different formation constants, according to the equilibria

$$Cu^{2+} + L \rightleftharpoons [CuL]^{2+} \qquad \text{Log } \beta_{110} = 9.85$$

$$2Cu^{2+} + L \rightleftharpoons [Cu_2L]^{4+} \qquad \text{Log } \beta_{120} = 17.40$$

$$(Cu^{2+} + [CuL]^{2+} \rightleftharpoons [Cu_2L]^{4+} \qquad \text{Log } K = 7.55)$$

showing, as it has been demonstrated by the crystal structure in the solid state (see below), that the two copper ions have different coordination environments. Successively, an equilibrium corresponding to the deprotonation of a coordinated water molecule,

$$[Cu_2L(H_2O)]^{4+} \rightleftharpoons [Cu_2L(OH)]^{3+} + H^+$$

occurs with a pK of about 4. On the basis of the crystal structure, it is likely that this equilibrium involves the copper(II) center coordinated to three nitrogen donors of LB5 and two water molecules. The rather low pK value, similar to that reported for the formation of a bridging dicopper(II)−hydroxide complex,[17] can be tentatively attributed to the high positive charge of the complex and the low basicity of the benzimidazole ligands involved in the coordination of this CuN_3 unit.

X-ray Structure of $[Cu_2(LB5)(H_2O)_2][ClO_4]_4$. The crystal of the complex of formula $[Cu_2(LB5)(H_2O)_2][ClO_4]_4 \cdot 7.6H_2O \cdot 0.8CH_3OH$ is built up by $[Cu_2(LB5)(H_2O)_2]^{4+}$ cations, perchlorate anions, crystallization water, and CH_3OH molecules. A side view of the cation, together with the numbering scheme for Cu and the coordinated donors, is given in Figure 1. LB5 acts as an octadentate dinucleating ligand, coordinating Cul through five N donors and Cu2 through the remaining three N donors. Two water molecules complete the Cu2 coordination

(16) (a) Solomon, E. I.; Baldwin, M. J.; Lowery, M. D. *Chem Rev.* **1992**, *92*, 521. (b) Messerschmidt, A. *Adv. Inorg. Chem.* **1993**, *40*, 121.
(17) Menif, F.; Reibenspies, J.; Martell, A. E. *Inorg. Chem.* **1991**, *30*, 3446.

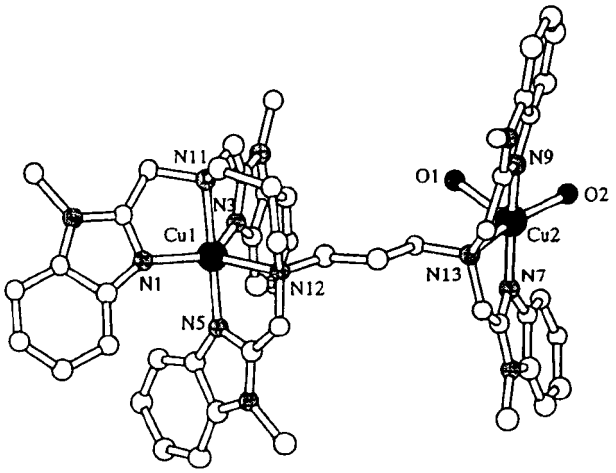


Figure 1. Side view of the cation $[Cu_2(LB5)]^{4+}$ showing the coordination geometry about the two copper ions. The numbering scheme for copper and coordinated donors is also shown.

sphere. Thus, both copper ions are five-coordinated. The approach of Mutterties and Guggenberger,[18] which uses the shape-determining dihedral angle e_3, is the best way to decide the actual geometry of the five-coordination sphere about the metal center. The angle e_3 has a value of 53.1° for the ideal trigonal bipyramid (TBP) and 0.0° for the square pyramid (SP). For Cu1 this value is 44°, showing that Cul has a distorted TBP geometry with two benzimidazole residues (Nl, N3) and the tertiary amine N12 donor in the equatorial positions, whereas a third benzimidazole residue (N5) and the tertiary amine N11 donor occupy the axial positions (Figure 1). Equatorial bond angles deviate slightly from the expected 120°, the largest deviation being 13°, whereas the N5−Cu−N11 is 177.6(5)°. The sum of the equatorial bond angles at Cul is 358.5°, showing that the metal ion is essentially in the mean plane of the equatorial donors. For the same kind of donor, the axial distances are significantly shorter than the equatorial ones. In fact, the Cul−N5 axial distance of l.95(1) Å is shorter than the equatorial Cul−N1 and Cu1−N3 distances, which are 2.03(1) and 2.05(1) Å, respectively. Analogously, the axial Cul−N11 and the equatorial Cul−N12 distances are 2.09(2) and 2.20(1) Å, respectively. The e_3 value of 3° indicates that the coordination geometry about Cu2 is distorted SP, where two benzimidazole residues, the amine N13 donor and the O2 water molecule, occupy the basal positions, whereas the O1 water molecule occupies the apical position (Figure 1). The basal donors are coplanar within ±0.03 Å, and the Cu2 displacement out of their mean plane toward O1 is 0.245(1) Å. The apical Cu2−Ol distance of 2.27(1) Å is significantly longer than the basal Cu2−O2 one of 2.05(2) Å. The Cu2−N(benzimidazole) distances are 1.93(1) and 1.95(1) Å, close to the corresponding axial distance Cul−N5, whereas the Cu2−N13 is 2.13(1) Å. The propylene spacer between N12 and N13 atoms has a nearly zigzag conformation leading the two copper ions 8.019(3) Å away.

Most of the dinucleating ligands for copper(II) reported in the literature have a spacer, either possessing or not possessing a further coordinating group, and separating two structurally identical arms, so that the two resulting copper(II) centers have the same set of donors.[1,6b,19] The coordination geometry is very often distorted SP, and only rarely is a distorted TBP geometry observed.[20] However, recently, dicopper complexes of *N,N,N′,N′*-tetrakis[(2-benzimidazolyl)methyl]-2-hydroxy-1,3-diaminopropane, containing either chloride or thiocyanate counterions, have been reported, where the hydroxy group does not lose the proton.[2j] In these complexes, the hydroxy group acts as either a terminal or a bridging ligand, depending upon the nature of the accompanying anion(s). In some of the dinuclear complexes in this series, significant differences between the coordination geometries about the two Cu centers are found. Recently, only a few examples of nonsymmetric dinucleating ligands have been reported, which provide different sets of N donors and, hence, coordination asymmetry in the dinuclear model (see, e.g., refs 2d,e). However, in all of the above dinuclear complexes, both copper(II) ions need exogenous ligands for completing their coordination sphere. In this respect, the structure of the $[Cu_2(LB5)(H_2O)_2]^{4+}$ cation is the first example where one metal ion completes its coordination only with five endogenous donors.

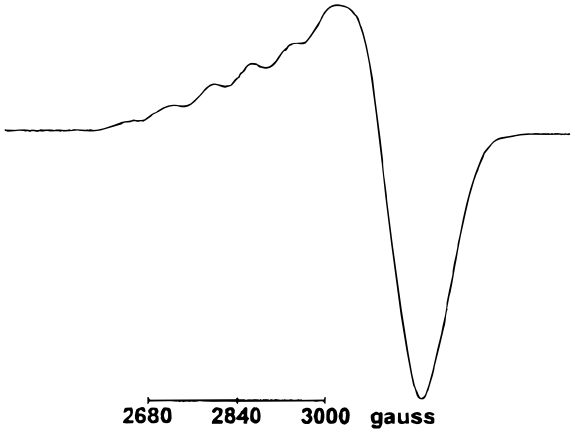


Figure 2. ESR spectrum of $[Cu_2(LB5)]^{4+}$ recorded in frozen acetonitrile solution at −150 °C.

Spectroscopic and Ligand-Binding Properties of $[Cu_2(LB5)(H_2O)_2]^{4+}$. The electronic spectrum of the $[Cu_2(LB5)]^{4+}$ complex recorded in CH_3CN solution exhibits, besides the intense UV absorptions below 300 nm, due to benzimidazole $\pi \rightarrow \pi^*$ transitions, a poorly defined absorption of moderate intensity between 300 and 400 nm, which can be attributed to σ(amino) → Cu(II)[21] and π(benzimidazole) → Cu(II)[22] LMCT transitions, and two weaker and partially resolved bands in the visible region, near 670 and 800 nm. The presence of two d−d bands in this range agrees[23] with coordination geometries close to trigonal bipyramidal for the Cu(II) center bound to five LB5 nitrogen donors and to square pyramidal for the Cu(II) center bound to three LB5 donors, with acetonitrile replacing the water molecules bound in the solid-state structure. The EPR spectrum of the complex in frozen CH_3CN solution is interesting, since it exhibits a resolved hyperfine structure in the $g_{||}$ region, with six discernible lines with an average splitting of 76 G (Figure 2). This feature may arise from the overlap of two sets of peaks, corresponding to two different, but similar, Cu(II) centers in tetragonal environments, with the following simulated EPR parameters: (a) $g_{||} = 2.232(5)$, $|A_{||}| = 137(5) \times 10^{-4}$ cm^{-1}; (b)

(18) Mutterties, E. L.; Guggenberger, L. J. *J. Am. Chem. Soc.* **1974**, *96*, 1748.
(19) See, for instance: Paul, P. P.; Tyeklàr, Z.; Farooq, A.; Karlin, K. D.; Liu, S.; Zubieta, J. *J. Am. Chem. Soc.* **1990**, *112*, 2430 and references therein.
(20) Oberhausen, K. J.; Richardson, J. F.; Buchanan, R. M.; McCusker, J. M.; Hendrickson, D. N.; Latour, J. *Inorg. Chem.* **1991**, *30*, 1357.
(21) Lever, A. B. P. In *Inorganic Electronic Spectroscopy*, 2nd ed.; Elsevier: Amsterdam, 1984; p 355.
(22) Dagdigian, J. V.; McKee, V.; Reed, C. A. *Inorg. Chem.* **1982**, *21*, 1332.
(23) Hathaway, B. J. *Struct. Bonding* **1984**, *57*, 55. Yokoi, H. *Chem. Lett.* **1973**, 1023.

$g_{\|} = 2.276(5)$, $|A_{\|}| = 140(5) \times 10^{-4}$ cm^{-1}. However, this would imply a change in stereochemistry for the CuN_5 center, from TBP to SP, and therefore it is to be considered that, alternatively, the EPR signal may originate from a weak exchange interaction between the Cu(II) centers.[24,25] The hyperfine pattern would result from coupling with two Cu nuclei, with the seventh line overlapped with the broad $g_{\|}$ envelope near 3100 G. EPR spectra similar to that of the $[Cu_2(LB5)]^{4+}$ cation have been observed for other dinuclear Cu(II) complexes with Cu−Cu separations of 7.3−8.0 Å (in the solid state);[26] this seems to support the dimer interpretation of the signal. Even though we did not observe the forbidden $\Delta m = 2$ transition at half-field, this transition may be difficult to detect when the coupling is weak.

The addition of azide to an 8:1 methanol/acetonitrile solution of $[Cu_2(LB5)(H_2O)_2][ClO_4]_4$ (the complex is poorly soluble in pure methanol) produces the characteristic appearance of LMCT bands in the near-UV spectral region. At low $[N_3^-]$:$[Cu_2]$ ratios, up to about 1:1, the λ_{max} of the LMCT band is located at 396 nm ($\Delta\epsilon \sim 2200$ M^{-1} cm^{-1}). An adduct with this stoichiometry has been isolated also in the solid state. At higher azide concentrations the λ_{max} of the LMCT band progressively shifts to 392 nm, with a parallel increase of intensity. An isosbestic point at about 336 nm is maintained in the binding of the first, high-affinity azide molecule. The binding constant $K_1 = 3 \times 10^4$ M^{-1} is lower than those found for the dinuclear complexes derived from L-55 and L-66,[7b] likely because, as shown by the symmetric shape of the LMCT band, azide behaves as a terminal ligand to a single copper(II) center of $[Cu_2(LB5)]^{4+}$, while it binds as a bridging ligand to the dicopper(II) complexes of L-55 and L-66. There is little doubt that azide is bound to the copper(II) center carrying only three nitrogen donors of LB5; both the Lewis acidity and the accessibility of this center are much higher than those of the other copper(II) ion. The LF spectrum of the azide adduct confirms that no significant structural change has occurred with respect to the precursor complex.

The different behavior toward azide between $[Cu_2(LB5)]^{4+}$ and $[Cu_2(L\text{-}55)]^{4+}$ or $[Cu_2(L\text{-}66)]^{4+}$ is reproduced in titrations with OH^- to yield dicopper(II)−hydroxo species. In the present case, spectrophotometric titration of the complex with methanolic sodium hydroxide gives rise to the appearance of a single hydroxide-to-Cu(II) LMCT band at 356 nm, while two LMCT bands characterized the bis(μ-hydroxo) arrangement attained by the adduct formed by $[Cu_2(L\text{-}55)]^{4+}$ and $[Cu_2(L\text{-}66)]^{4+}$.[7b]

Voltammetric Data. The reduction processes of the LB5 complexes, studied in 1:1 and 2:1 Cu(II):ligand ratios, were electrochemically quasireversible, the anodic peak being far away from the theoretical value of 60 mV for a one-electron transfer and the cathodic and anodic potentials varying with the sweep rates. Additional complications arise for the appearance of absorption peaks on the gold electrode surface. The reduction processes involve the transfer of one electron for each step, as indicated by the variation of the peak current as a function of the square root of the potential sweep rate. Anodic peaks with comparable currents were detected at more positive potentials, which are not the potential of free copper(II), thus avoiding the complications due to dismutation reactions of the reduced copper(I) complexes. In addition, successive scans did not reveal a different behavior so that these peaks refer to the reduction of copper(II) and to the oxidation of copper(I) species. As shown by the data in Table 1, where the redox potentials toward NHE are reported, E°_{Ag} (acetonitrile) vs NHE (water) being 0.637,[27] and from Figure 3, the $[Cu(LB5)]^{2+}$ complex gives a single reduction peak at relatively low potential, while the $[Cu_2(LB5)]^{4+}$ complex gives an additional peak at more positive potential. It is important to note that the potentials pertaining to the second peak of the $[Cu_2(LB5)]^{4+}$ voltammogram are very similar to those of the single peak present in the voltammogram of $[Cu(LB5)]^{2+}$, which means that the copper(II) ion involved in the five-nitrogen arrangement is always reduced at the same potentials and is only slightly influenced by the second copper(II) ion in the three-nitrogen environment. In Table 1, the calculated $E^\circ(Cu^{2+}/Cu^+)$ values for the mono- and dinuclear LB5 complexes are also reported, assuming their redox transfers as reversible.[28]

Table 1. Voltammetric Data at 25 °C for 0.5 mM Copper(II) Complexes with LB5, in Copper(II):Ligand Ratios of 1:1 and 2:1, in Acetonitrile Solution, with 100 mM TEAP as Supporting Electrolyte, and at 50 mV s^{-1}

	$[Cu(LB5)]^{2+}$	$[Cu_2(LB5)]^{4+}$	
Ep_c, V vs NHE[a]	+0.103	+0.313	+0.113
Ip_c, μA	2.7	2.6	2.2
Ep_a, V vs NHE[a]	+0.242	+0.483	+0.230
Ip_a, μA	2.3	0.8	2.0
$E^\circ = (Ep_c + Ep_a)/2$[b]	0.173	0.398	0.172

[a] The maximum error is ±0.007 V on peak potentials and ±10% on peak currents. [b] Considering the redox electronic transfers as they were reversible.

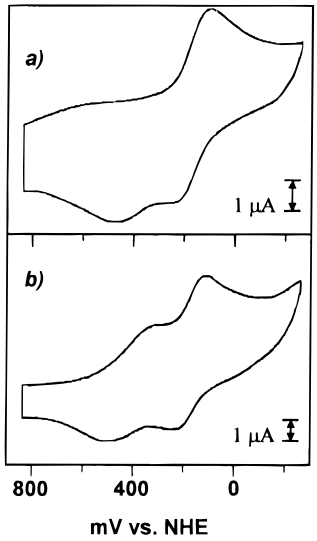


Figure 3. Cyclic voltammograms of 0.5 mM copper(II) complexes with LB5 in copper(II):ligand ratios of (a) 1:1 and (b) 2:1 in acetonitrile solution, with 100 mM TEAP as supporting electrolyte, at 50 mM s^{-1}, and recorded at 25 °C.

Catechol Oxidase Activity. The catalytic oxidation of catechol and, in particular, 3,5-di-*tert*-butylcatechol (DTBC) has been widely studied as a model reaction for the catecholase activity of tyrosinase.[2g,29,30] When the reactions were performed using methanol saturated with atmospheric oxygen as solvent, at 20 °C, the dicopper(II) complexes of LB5, L-55, and L-66

(24) Yokoi, H. *Chem. Lett.* **1973**, 1023.
(25) (a) Felthouse, T. R.; Laskowski, E. J.; Hendrickson, D. N. *Inorg. Chem.* **1977**, *16*, 1077. (b) Felthouse, T. R.; Hendrickson, D. N. *Inorg. Chem.* **1978**, *17*, 444. (c) Haddad, M. S.; Hendrickson, D. N. *Inorg. Chem.* **1978**, *17*, 2622.
(26) (a) Mandal, S. K.; Thompson, L. K.; Newlands, M. J.; Gabe, E. J.; Lee, F. L. *Inorg. Chem.* **1990**, *29*, 3556. (b) Tandon, S. S.; Thompson, L. K.; Bridson, J. N.; Dewan, J. C. *Inorg. Chem.* **1994**, *33*, 54.
(27) Kratochvil, B.; Lorah, E.; Garber, C. *Anal. Chem.* **1969**, *41*, 1793.
(28) Bond, A. M. *Modern Polarographic Methods in Analytical Chemistry*; Marcel Dekker: New York and Basel, 1980; p 182.

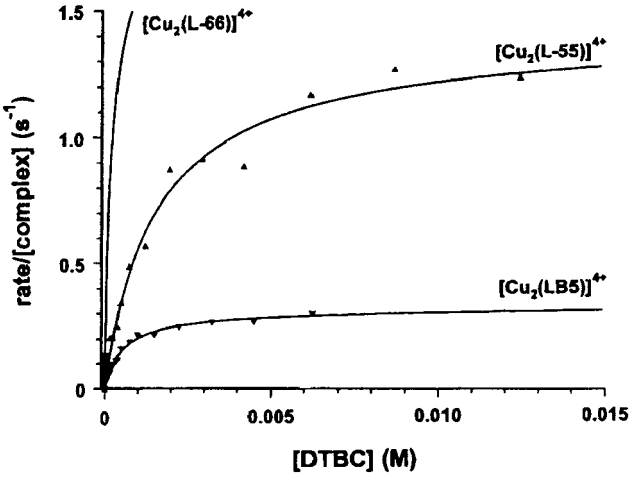


Figure 4. Rates in the first phase of DTBQ production at different DTBC concentrations. The reactions were performed in a 30:1 (v/v) mixture of methanol/aqueous phosphate buffer (50 mM) pH 5.1 at 20 ± 0.1 °C. The concentration of the copper(II) complexes was 13 μM.

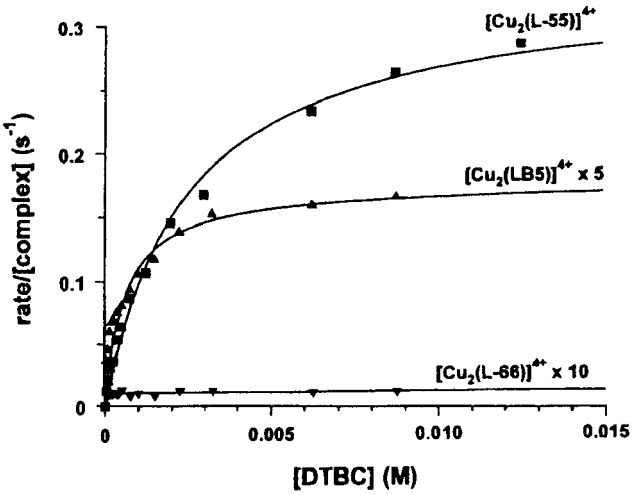


Figure 5. Rates in the second phase of DTBQ production at different DTBC concentrations. The reactions were performed in a 30:1 (v/v) mixture of methanol/aqueous phosphate buffer (50 mM) pH 5.1 at 20 ± 0.1 °C. The concentration of the copper(II) complexes was 13 μM.

reacted with DTBC stoichiometrically, or with extremely low catalytic efficiency after the stoichiometric reaction. In order to observe catalytic rather than stoichiometric reactions, a small amount of an aqueous buffer had to be added to the methanol solution. An aqueous buffer at pH 5.1 was used, since at pH near neutrality we found that the contribution of the noncatalytic oxidation of DTBC became nonnegligible. For all three dicopper(II) complexes the quinone produced versus time curve follows a biphasic behavior. The reactions occur with a first stoichiometric fast phase followed by a slower reaction. For both phases, it is possible to obtain the reaction rates and observe the rate dependence on the concentration of DTBC, as shown in Figures 4 and 5, respectively. Applying the Michaelis−Menten approach it is possible to characterize the kinetic behavior in most cases using the parameters k_{cat}, K_M, and k_{cat}/K_M, the values of which are reported in Table 2. The dicopper(II) complex of L-66 differs significantly from the other catalysts since its reaction rate in the first step is extremely fast and can be evaluated only at low DTBC concentration (Figure 4). This prevents a good estimation of K_M and k_{cat}, so that only the ratio k_{cat}/K_M is reported in Table 2. Moreover, with $[Cu_2(L\text{-}66)]^{4+}$, the rate in the second phase is low and independent of substrate concentration in the [DTBC] range 0.1−15 mM (Figure 5). For $[Cu_2(LB5)]^{4+}$ and $[Cu_2(L\text{-}55)]^{4+}$, a clean substrate saturation behavior could be observed in both phases. These complexes are much more efficient catalysts for catechol oxidation than simple copper(II) perchlorate salt (Table 2). However, the reaction of $[Cu_2(LB5)]^{4+}$ showed an indication of substrate inhibition at very high DTBC concentration.

For the three dinuclear complexes the DTBC oxidation rate was found to depend linearly on the catalyst concentration, while for simple mononuclear complexes the rate depends on the square of the copper(II) concentration.[29f,31] This shows that the reaction needs the cooperation of two close copper centers, quite likely for the need to bind the catechol substrate in a bridging mode. Such adducts have been isolated previously for the L-55 and L-66 complexes,[7d] but we have been unable to obtain them in sufficiently pure form for the LB5 complex so far. In the copper-catalyzed oxidation of catechols, dioxygen is often reduced to hydrogen peroxide.[30a,31] The following mechanisms were proposed by Chyn and Urbach to explain their experimental data.[30a]

mechanism I

$$Cu^{II}\cdots Cu^{II} + DTBC \rightarrow Cu^{I}\cdots Cu^{I} + DTBQ + 2H^{+} \quad (1)$$

$$Cu^{I}\cdots Cu^{I} + O_2 \rightarrow Cu^{II}(O_2)^{2-}Cu^{II} \quad (2)$$

$$Cu^{II}(O_2)^{2-}Cu^{II} + 2\,H^{+} \rightarrow Cu^{II}\cdots Cu^{II} + H_2O_2 \quad (3)$$

mechanism II

$$\text{initial step: } Cu^{II}\cdots Cu^{II} + DTBC \rightarrow Cu^{I}\cdots Cu^{I} + DTBQ + 2H^{+} \quad (1)$$

$$\text{redox cycle: } Cu^{I}\cdots Cu^{I} + O_2 \rightarrow Cu^{II}(O_2)^{2-}Cu^{II} \quad (2)$$

$$Cu^{II}(O_2)^{2-}Cu^{II} + DTBC \rightarrow Cu^{I}\cdots Cu^{I} + DTBQ + H_2O_2 \quad (4)$$

Formation of hydrogen peroxide during the catalytic turnover can provide a further route to the catalytic reaction since it can compete with dioxygen in the reoxidation of the dicopper(I) complex. The different mechanisms can be grouped together, as shown in Scheme 1. This scheme also reports a different cycle (path c), which resembles that of the enzyme tyrosinase, in which the oxidation of two molecules of DTBC by dioxygen occurs without release of hydrogen peroxide. What discriminates between pathways a and b is the reactivity of the $Cu^{II}(O_2)^{2-}Cu^{II}$ intermediate, since it can react with protons, according to pathway a (first mechanism), or with the catechol, according to pathway b (second mechanism).

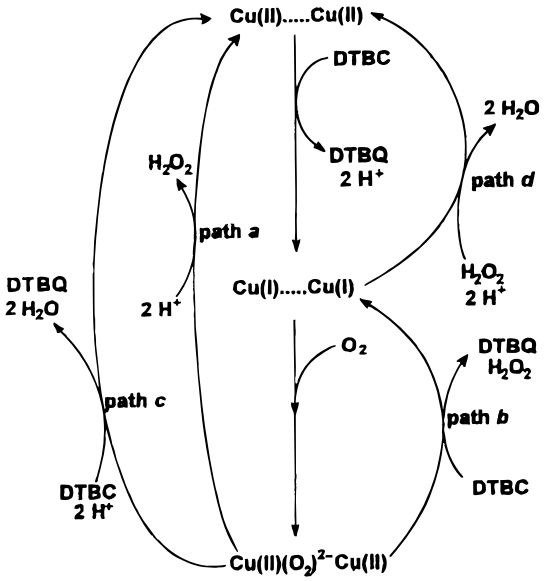
The presence of a fast initial phase agrees with both mechanisms, since it is due to the high reactivity of the copper(II) complex toward DTBC. The kinetic parameters for this phase (Table 2) show that the order of reactivity of the

(29) (a) Oishi, N.; Nishida, Y.; Ida, K.; Kida, S. *Bull. Chem. Soc. Jpn* **1980**, *53*, 2847. (b) Demmin, T. R.; Swerdloff, M. D.; Rogič, M. M. *J. Am. Chem. Soc.* **1981**, *103*, 5795. (c) Casellato, U.; Tamburini, S.; Vigato, P. A.; De Stefani, A.; Vidali, M.; Fenton, D. E. *Inorg. Chim. Acta* **1983**, *69*, 45. (d) Casella, L.; Gullotti, M.; Pessina, C.; Pintar, A.; *Gazz. Chim. Ital.* **1986**, *116*, 41. (e) Malachowski, M. R.; Davidson, M. G. *Inorg. Chim. Acta* **1989**, *163*, 199. (f) Speier, G. *J. Mol. Catal.* **1986**, *37*, 259.
(30) (a) Chyn, J. P.; Urbach, F. L. *Inorg. Chim. Acta* **1991**, *189*, 157. (b) Rockcliffe, D. A.; Martell, A. E. *Inorg. Chem.* **1993**, *32*, 3143.
(31) Balla, J.; Kiss, T.; Jameson, R. F. *Inorg. Chem.* **1992**, *31*, 58

Table 2. Kinetic Parameters for the Catalytic Oxidation of DTBC in Methanol/Aqueous Buffer pH 5.1 at 20 °C

	first step			second step		
complex	k_{cat} (s^{-1})	k_{cat}/K_M (M^{-1} s^{-1})	K_M (mM)	k_{cat} (s^{-1})	k_{cat}/K_M (M^{-1} s^{-1})	K_M (mM)
$[Cu_2(LB5)]^{4+}$	0.31 ± 0.01	590 ± 30	0.52 ± 0.04	0.035 ± 0.001	60 ± 5	0.59 ± 0.05
$[Cu_2(L\text{-}55)]^{4+}$	1.40 ± 0.06	900 ± 90	1.5 ± 0.2	0.33 ± 0.01	140 ± 10	2.4 ± 0.2
$[Cu_2(L\text{-}66)]^{4+}$		9300 ± 800				
$[Cu]^{2+}$					2	

Scheme 1



complexes is $[Cu_2(L\text{-}66)]^{4+} \gg [Cu_2(L\text{-}55)]^{4+} > [Cu_2(LB5)]^{4+}$. The very high reactivity of the L-66 complex is due to its high redox potential. In fact, the Cu(II)/Cu(I) redox potentials in acetonitrile solution are 0.64 V for L-66,[7b] 0.39 V for L-55[7b], and 0.40 and 0.17 V for LB5. The lower reactivity of $[Cu_2(LB5)]^{4+}$ with respect to $[Cu_2(L\text{-}55)]^{4+}$ is probably due also to the necessity to release one of the coordinated benzimidazole ligands at the CuN_5 center in the binding of catechol.

The reactivities in the second phase are lower. For $[Cu_2(L\text{-}55)]^{4+}$ and $[Cu_2(LB5)]^{4+}$, the reaction rates depend on the catechol concentration, indicating that the substrate reacts in the rate-determining step (rds) of the reaction. This excludes path a, where the steps after the fast reduction of the dicopper(II) species are independent of DTBC. Considering that pathway b is valid, the rds is step 4. For $[Cu_2(L\text{-}66)]^{4+}$, the rate independence of substrate concentration indicates that the rds is the formation of the dioxygen adduct (reaction 2). This is confirmed by the strong dependence of the reaction rate of this complex on the oxygen concentration. Using a methanol/buffer solution saturated with 1 atm of oxygen, instead of air oxygen, the reaction rate was found to increase 3-fold, while for $[Cu_2(L\text{-}55)]^{4+}$ and $[Cu_2(LB5)]^{4+}$ the rate increase was less than 10%. The low rate of formation of the dioxygen adduct of the L-66 complex is probably due to its high redox potential, which indicates that the Cu(I) state is more stable.

In order to study the effect of hydrogen peroxide in the catechol oxidation reaction, the kinetics were followed under conditions close to DTBC saturation in the presence of variable amounts of H_2O_2. The rates for the second phase of the reaction as a function of hydrogen peroxide concentration are reported in Figure 6. For $[Cu_2(L\text{-}66)]^{4+}$ the rate is strongly affected by H_2O_2, and a linear dependence between the rate and H_2O_2 concentration was found. The estimated k_{app} for this process is 50 ± 1 M^{-1} s^{-1}. This behavior can be associated with the slow reoxidation reaction of the dicopper(I) intermediate, which

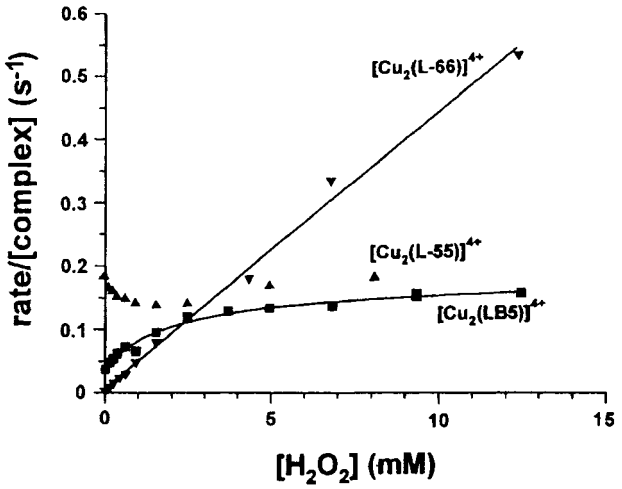


Figure 6. Dependence of the reaction rates in the second phase of DTBQ formation on the concentration of hydrogen peroxide. The reactions were performed in a 30:1 (v/v) mixture of methanol/aqueous phosphate buffer (50 mM) pH 5.1 at 20 ± 0.1 °C. The concentration of the copper(II) complexes was 13 μM while that of DTBC was 5 mM.

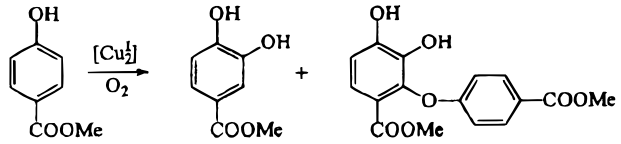
instead of reacting with dioxygen prefers to react with hydrogen peroxide, following path d. For $[Cu_2(L\text{-}55)]^{4+}$, the reaction rate is slightly reduced in the presence of hydrogen peroxide, at least at relatively low oxidant concentration. Probably the oxygenation of the dicopper(I) complex is very fast and, therefore, the intermediate prefers to react with O_2 (path b) rather than with H_2O_2 (path d). The decrease in the observed rate could be due to the partial transformation of the dicopper(II) complex into the less reactive peroxide adduct, according to the reaction (reverse of path a)

$$Cu^{II}\cdots Cu^{II} + H_2O_2 \rightarrow Cu^{II}(O_2)^{2-}Cu^{II} + 2H^+ \qquad (5)$$

The rate of DTBC oxidation in the presence of $[Cu_2(LB5)]^{4+}$ as catalyst shows H_2O_2 saturation behavior (Figure 6). This is likely due to a change in the rds of the reaction. At low H_2O_2 concentration, the reaction follows the normal cycle, since the contribution of the route provided by this oxidant (path d) is low. As the hydrogen peroxide concentration increases, the importance of this path and, hence, the rate progressively increase. When the hydrogen peroxide concentration is higher than 5 mM, reoxidation of the dicopper(I) complex by H_2O_2 is faster than catechol oxidation by the dicopper(II) complex. Thus, the latter process becomes rate-limiting and the rate becomes independent of H_2O_2 concentration. The observation that at high H_2O_2 concentration the rate depends on the catechol concentration also in the range above 5 mM where, without H_2O_2, DTBC is under saturation conditions (Figure 5) agrees with the hypothesis of a change in the rds of the reaction. The estimate of k_{app} for the catalytic oxidation of DTBC by H_2O_2 is possible at low oxidant concentration and gives the value 73 ± 8 M^{-1} s^{-1}.

Since hydrogen peroxide is not decomposed by the $[Cu_2(L\text{-}55)]^{4+}$, $[Cu_2(L\text{-}66)]^{4+}$, and $[Cu_2(LB5)]^{4+}$ complexes, its presence

Scheme 2



in the catalytic cycle when dioxygen is used as the oxidant should be detectable if it accumulates. As shown in Scheme 1, hydrogen peroxide is formed when DTBC oxidation occurs according to pathway a or b, but it can be consumed in the reoxidation of the dicopper(I) complex (path d). In order for hydrogen peroxide to be detected, path d must be slower than either path a or b. The I_3^- assays performed on samples taken from the catalytic reactions of $[Cu_2(L\text{-}55)]^{4+}$, $[Cu_2(L\text{-}66)]^{4+}$, and $[Cu_2(LB5)]^{4+}$ were never significantly different from blank experiments, indicating that hydrogen peroxide does not accumulate. Thus, either the catalytic reactions occur through the tyrosinase cycle (pathway c) or the H_2O_2 produced is consumed in a fast reoxidation of the dicopper(I) complex (path d). By contrast, as for the oxidation of catechol in water,[31] production of hydrogen peroxide by the I_3^- assay was confirmed by us in the oxidation of DTBC by copper(II) salt in the methanol/buffer pH 5.1 medium.

Phenol Monooxygenase Activity. The most important biomimetic reaction characterizing tyrosinase models is the hydroxylation reaction of exogenous phenols. Although a few copper systems capable of performing this transformation have been reported,[32] the only dinuclear copper complexes with relevance to tyrosinase for which the reaction has been characterized in some detail are those derived from L-55 and L-66.[7d] The model reaction studied was the oxygenation of methyl 4-hydroxybenzoate (**P**) to methyl 3,4-dihydroxybenzoate (**C**), because the presence of an electron-withdrawing substituent on the phenol nucleus stabilizes the dicopper(II)−catecholate product of the reaction to internal electron transfer, to yield dicopper(I) and quinone, thereby preventing further redox and condensation reactivity. The only drawback of the reaction mediated by the dicopper(I)−L-55 and dicopper(I)−L-66 complexes was the finding that the bound catechol intermediate can undergo a formal Michael addition process with unreacted phenol, to give methyl 2-[4-(carbomethoxy)phenoxy]-3,4-dihydroxybenzoate (**M**) (Scheme 2),[32c] unless the reaction is carried out at low temperature.[7d] Since the present LB5 system exhibits catechol oxidase activity, it was of interest to investigate its monooxygenase activity in the same reaction mediated by the parent L-55 and L-66 systems.

The results of experiments carried out under the same conditions as those reported for the L-55 and L-66 systems[7d] are striking. The oxygenation of the tetra-*n*-butylammonium salt of methyl 4-hydroxybenzoate mediated by the dicopper(I) complex of LB5 and dioxygen proceeds rapidly and selectively to give only catechol, with no trace of the Michael adduct, even when the reaction is performed at room temperature (Table 3). Formation of the latter adduct becomes apparent only after reaction times of the order of 20 h and occurs with depletion of catechol, which remains the major product. The dicopper(I)−LB5 system, therefore, appears to be much more selective than the parent L-55 and L-66 systems in the monooxygenase reaction. Also interesting is the observation that some monooxygenase activity is observed when simply the phenol, instead of its tetra-*n*-butylammonium salt, is used (Table 3). This reaction does not occur with $[Cu_2(L\text{-}55)]^{2+}$ or $[Cu_2(L\text{-}66)]^{2+}$ and can be accounted for by considering that the ligand LB5 has nitrogen donors that are not involved in the complexation to the copper(I) centers and can be used as basic groups to deprotonate the phenol.

Table 3. Oxygenation of Methyl 4-Hydroxybenzoate Mediated by Dicopper(I) Complexes

complex	temp (°C)	time (h)	**P** (%)	**C** (%)	**M** (%)
		Using Phenolate			
$[Cu_2(LB5)]^{2+}$	20	0.2	56	44	
	20	3	51	49	
	20	20	50	38	12
$[Cu_2(L\text{-}66)]^{2+}$ [a]	23	2	1	4	95
	−40	26	70	30	trace
$[Cu_2(L\text{-}55)]^{2+}$ [a]	23	2	40	2	58
		Using Phenol			
$[Cu_2(LB5)]^{2+}$					
(1:1)[b]	20	1	83	17	
	20	20	82	12	6
(2:1)[b]	20	1	80[c]	20	
	20	14	73[c]	13	14

[a] Reference 7d. [b] Molar ratio of [phenol]:[Cu_2] complex. [c] Value neglecting excess phenol.

The limitations to the reactivity of $[Cu_2(LB5)]^{2+}$ seem to be connected with a distribution of the copper(I) centers among several donor environments. Assuming two- or three-coordinated copper(I) centers, as is usually the case with ligands similar to the coordinating arms present in LB5,[33] there are several possible isomeric forms of $[Cu_2(LB5)]^{2+}$. The presence of these isomers is indicated, for instance, by the ^{1}H NMR spectrum of the complex (in CD_3CN) showing a downfield shift (0.2−0.5 ppm) for all the CH_2 and NCH_3 groups of the ligand. In the ^{1}H NMR spectrum of the adduct between the dicopper(I) complex of LB5 and methyl 4-hydroxybenzoate, three methyl signals of comparable intensity for the phenolate ion can be observed. This indicates that two or three types of phenolate adducts are present in solution, not all of them being suitable to undergo the ortho hydroxylation by reaction with molecular oxygen.

Conclusion

In the present work we have reported a new dinuclear complex characterized by nonsymmetric binding of the ligand to the two copper(II) centers and expanded upon the biomimetic chemistry of a related series of dinuclear complexes with polyaminobenzimidazole donor sets in the context of the activity of tyrosinase. These systems represent suitable models of the enzyme for their capacity to perform regiospecific ortho hydroxylation of exogenous phenols and to carry out complete reduction of dioxygen to water in the catalytic oxidation of catechols. Also the latter feature is a characteristic of tyrosinase catalysis,[34] while with many synthetic copper catalysts dioxygen

(32) (a) Capdevielle, P.; Maumy, M.; *Tetrahedron Lett.* **1982**, *23*, 1573, 1577. (b) Chioccara, F.; Di Gennaro, P.; La Monica, G.; Sebastiano, R.; Rindone, B. *Tetrahedron* **1991**, *47*, 4429. (c) Sayre, L. M.; Nadkarni, D. V. *J. Am. Chem. Soc.* **1994**, *116*, 3157. (d) Spodine, E.; Manzur, J. *Coord. Chem. Rev.* **1991**, *119*, 171. (e) Simándi, L. I. *Catalytic Activation of Dioxygen by Metal Complexes*; Kluwer: Dordrecht, 1992; Chapter 5.

(33) (a) Hendriks, H. M. J.; Birker, P. J. M. W. L.; van Rijn, J.; Verschoor, G. C.; Reedijk, J. *J. Am. Chem. Soc.* **1982**, *104*, 3607. (b) Patch, M. G.; Choi, H.; Chapman, D. R.; Bau, R.; McKee, V.; Reed, C. A. *Inorg. Chem.* **1990**, *29*, 110.

(34) (a) Solomon, E. I.; Lowery, M. D. *Science* **1993**, *259*, 1575. (b) Sanchez-Ferrer, A.; Rodriguez-Lopez, J. G.; Garcia-Canovas, F.; Garcia-Carmona, F. *Biochim. Biophys. Acta* **1995**, *1247*, 1.

is reduced to hydrogen peroxide. The details of the biomimetic oxidations mediated by LB5, L-55, and L-66 complexes show several differences in the behavior of the complexes, depending upon various factors, which include the redox potential of the Cu(II)/Cu(I) couples, the binding affinity, and possibly the binding mode of the two substrates of the reactions, the phenolic compound and dioxygen, to the dicopper units. The various complexes bear advantages and disadvantages in individual aspects of their tyrosinase-like activities, and the relative importance of the factors affecting these activities begins to emerge from the behavior of the complexes in the catechol oxidation reaction. Thus, the complex $[Cu_2(L\text{-}55)]^{4+}$ is most efficient in the catalytic oxidation of DTBC, followed by $[Cu_2(LB5)]^{4+}$, while $[Cu_2(L\text{-}66)]^{4+}$ exhibits very low activity. This low activity is due to the high redox potential of the complex, since with a stronger oxidant than dioxygen, that allows fast reoxidation of copper(I) during turnover, also $[Cu_2(L\text{-}66)]^{4+}$ becomes an efficient catalyst of DTBC oxidation. The difference between $[Cu_2(L\text{-}55)]^{4+}$ and $[Cu_2(LB5)]^{4+}$ is ascribable to more subtle factors. In fact, the high denticity of the LB5 ligand moiety at one of the copper(II) centers does not seem to prevent binding of the catechol, or other ligands, at this center, and the redox potential should even favor $[Cu_2(LB5)]^{4+}$ over $[Cu_2(L\text{-}55)]^{4+}$. Thus, the different efficiency of the two systems depends on the mode of binding of dioxygen to the reduced species and the reactivity of the resulting adducts toward the catechol. On the other hand, $[Cu_2(L\text{-}66)]^{2+}$ is more effective than $[Cu_2(L\text{-}55)]^{2+}$ in mediating the phenol ortho hydroxylation by dioxygen, while $[Cu_2(LB5)]^{2+}$ is much more selective than both the other complexes; but the phenol hydroxylation has been studied only stoichiometrically so far. More detailed mechanistic studies on this reaction, which are currently in progress in our laboratory, will improve our understanding of the structure−function relationships ruling tyrosinase-catalyzed reactions.

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Supporting Information Available: Atomic coordinates, thermal parameters, and bond distances and angles (5 pages). Ordering information is given on any current masthead page.

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