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Thiomethyl Substituted Dicopper Complexes: Attempts to Reproduce the Asymmetry of the Active Site from Type 3 Copper Enzymes

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Abstract. Two new dinucleating phenol-based ligands (*m*-HL_{SMe} and *p*-HL_{SMe}) bearing pyridine-containing pendant arms with a SMe group on one pyridine (*meta* or *para* position relative to the pyridine nitrogen atom) have been synthesized. After coordination by two copper(II) ions, the corresponding μ-phenoxido, μ-hydroxido dicopper(II) complexes were isolated and characterized by UV/Vis, EPR spectroscopy, single-crystal X-ray analysis (for the complex with the SMe substituent at the *meta* position) and electrochemistry. The presented compounds mimic the active site of type 3 copper enzymes and in particular the distinct environments of the copper ions.Both complexes are active as catalysts for the oxidation of 3,5-di-*tert*-butylcatechol to the respective quinone. The catalytic properties of the complexes depend on substrate binding, as reflected by the K_M values determined for the complexes in presence of 3,5-dtbc and are not correlated directly with the redox properties of the dicopper center.

Introduction

Catechol oxidases (COs)[1] belong to the family of type 3 copper enzymes, which also includes tyrosinases (Tys).[2]A vast body of literature concerning plant and fungal enzymes use the designation of polyphenol oxidases (PPOs) without distinguishing between Ty and CO. Both catalyze the oxidation of *o*-diphenols to *o*-quinones in the presence of dioxygen, while tyrosinases can also convert monophenols to *o*-diphenols. These enzymes are characterized by a dinuclear copper active site in a native *met* Cu^{II}–Cu^{II} form that is EPR-silent due to a strong antiferromagnetic coupling between the bridged copper(II) ions. The crystal structure of catechol oxidase from sweet potatoes (*Ipomoea batatas,* ibCO) has been reported in different states: the oxidized Cu^{II}–Cu^{II} *met* form, the reduced *deoxy* Cu^{I}–Cu^{I} form, and the species with bound phenylthiourea (ptu) inhibitor.[3] In the *met* state, both metal binding sites involve three histidine ligands, and the two cupric ions, which are likely bridged by a hydroxide ion, are 2.87 Å apart in a trigonal pyramidal arrangement (Figure 1A). In the reduced *deoxy* state, the copper atoms are not bridged ($Cu^{I}\cdots Cu^{I}$ distance = 4.4 Å) and a water molecule is coordinated to Cu_A. The coordination sphere of Cu_A is distorted trigonal pyramidal, whereas the coordination sphere of Cu_B, which is bonded to three histidine ligands, can be described as square planar with a vacant coordination site.

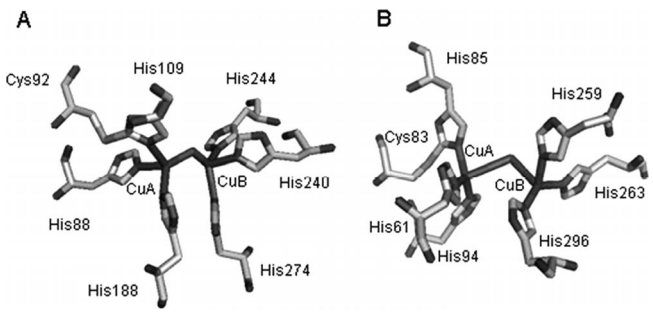

Figure 1. The active site of (A) *Ipomoea batatas* catechol oxidase (*met* form - PDB code: 1BT3)[3] and (B) *Agaricus bisporus* mushroom tyrosinase (reduced form - PDB code: 2y9w).[4]

An interesting feature of the dinuclear metal center is a covalent thioether bond between a carbon atom of His109, which is a ligand on Cu_A, and the sulfur atom of Cys92 (Figure 1A). The recent X-ray structure of reduced *Vitis vinifera* PPO[5] reveals that this covalent bond puts additional structural restraints on the ligand sphere of the Cu_A center. A similar type of bond was reported some time ago in the sequence homology of *Neurospora crassa* tyrosinase[6] and more recently in the solved three-dimensional structure of mushroom *Agaricus bisporus* tyrosinase (reduced form, Figure 1B).[4] The absence of such a Cys-His bridge in bacterial and human tyrosi-

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nase indicates that thioether modification does not play a direct role in the functional activity of the enzymes and only imposes structural constraints (i.e., ensuring trigonal pyramidal geometry) around the Cu_A copper center. This thioether bond may also prevent both the displacement of the His bound to the Cys and bidentate binding of the substrate to a single Cu^{II} ion. This may, in turn, optimize the redox potential of the metal for catechol substrate oxidation and allow rapid electron transfer during redox processes. Despite clarification of the active-site structures of different forms of CO and Ty by X-ray crystallography, the role of this thioether bond at the binuclear copper center remains unclear.[7]

Well-suited biomimetic models can be used to elucidate the likely behavior of the two copper atoms in distinct environments and the involvement of the Cys-His thioether bond in the catalytic activity of the enzyme. A large number of dinuclear model complexes of CO and Ty are described in literature,[8] but few complexes that mimic the Cys-His thioether bond have been reported.[9] The rational design of a dinucleating ligand that induces controlled synthesis of the targeted unsymmetrical complexes is a prerequisite for such models.[10] Dinucleating phenol-based ligands have previously been successfully applied as structural and functional models of the CO active site;[8a,11] the introduction of a thioether group to one arm of this series of ligands (Scheme 1) may provide new model complexes. Such unsymmetrical ligands have contributed heavily to improved understanding of the spectroscopic properties and reactivity of corresponding homo- or heterodinuclear complexes with respect to their symmetrical analogs.[12] In this paper, we describe the synthesis and catalytic activities of two new models with unsymmetrical dinuclear copper chelating ligands (Scheme 1). The asymmetry arises from the presence of a thiomethyl substituent introduced at the *para* or *meta* position relative to the pyridine nitrogen atom on one arm of the ligand. In this way, the SMe group can influence the electron density on the nitrogen atom coordinated to the metal ion via the electron donating (*p*-SMe) or electron withdrawing (*m*-SMe) effect.

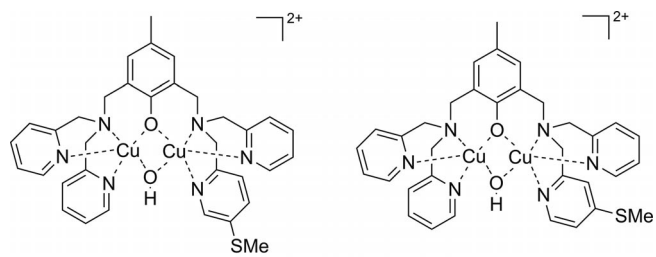
Results and Discussion

Synthesis

The new unsymmetrical ligands, *m*-HL_{SMe} and *p*-HL_{SMe}, were synthesized from intermediate **1** (Scheme 1) according a known procedure.[13] They possess a thiomethyl group in the *meta* or *para* position, respectively with respect to the pyridine nitrogen atom. The general synthetic pathways for *m*-HL_{SMe} and *p*-HL_{SMe} are depicted in Scheme 1. Details of the syntheses of the *m*-HL_{SMe} and *p*-HL_{SMe} ligands and secondary amines **2** and **3** are given in the Experimental Section and Supporting Information, respectively.

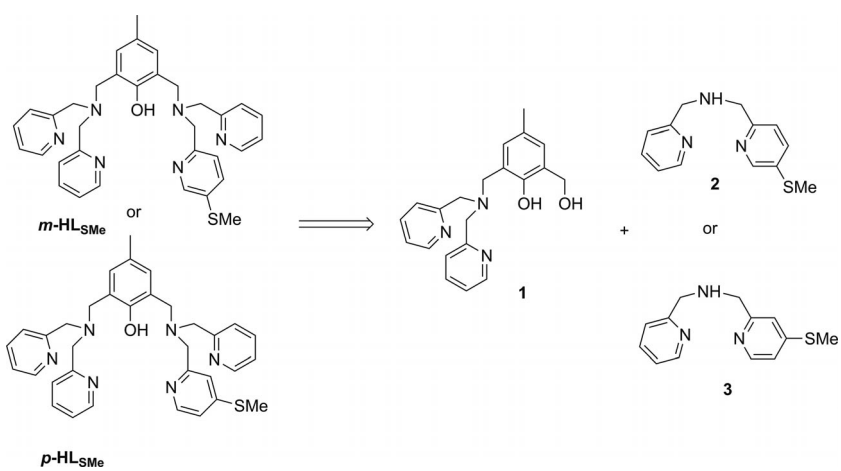
Synthesis and Characterization of the Dinuclear Copper(II) Complexes

The synthesis of the doubly bridged dinuclear copper(II) complex $[Cu_2(L)(\mu\text{-}OH)](ClO_4)_2$ from the symmetric HL ligand was described previously by us.[14] A similar procedure was applied using *m*-HL_{SMe} and *p*-HL_{SMe} (Scheme 2). Two μ-phenoxido, μ-hydroxido dicopper(II) complexes were prepared via the addition of two equivalents of $Cu(ClO_4)_2 \cdot 6H_2O$ to a solution of *m*-HL_{SMe} or *p*-HL_{SMe} in acetonitrile containing three equivalents of triethylamine. The complexes (Scheme 2) were isolated in the solid state as green powders. Crystals of



$[Cu_2(m\text{-LSMe})(\mu\text{-OH})]^{2+}$ $[Cu_2(p\text{-LSMe})(\mu\text{-OH})]^{2+}$

Scheme 2. Dicopper(II) complexes included in this work.



Scheme 1. Dinucleating ligands included in this work and the synthetic strategy.

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$[Cu_2(m\text{-}L_{SMe})(\mu\text{-}OH)](ClO_4)_2$ suitable for X-ray single-crystal analysis were obtained by slow diffusion of tetrahydrofuran into an acetonitrile solution.

Crystal Structure Description

The solid-state structure of $[Cu_2(m\text{-}L_{SMe})(\mu\text{-}OH)](ClO_4)_2$ contains one associated THF solvent molecule. An ORTEP view of dicationic complex is shown in Figure 2 for the majority form (see the Experimental Section for details on the crystal structure parameters). Selected bond lengths and angles are given in Table S1. The crystal structure reveals that the two copper atoms are doubly bridged by phenoxido and hydroxido groups. Cu1 and Cu2 are pentacoordinated by the tertiary amine, two pyridine nitrogen atoms, and two bridging oxygen atoms. Similar to the coordination geometry of each copper center in $[Cu_2(L)(\mu\text{-}OH)](ClO_4)_2$,[14] the geometries of the copper ions are distorted trigonal bipyramidal (with σ values[15] of 0.85 and 0.69 for Cu1 and Cu2, respectively).

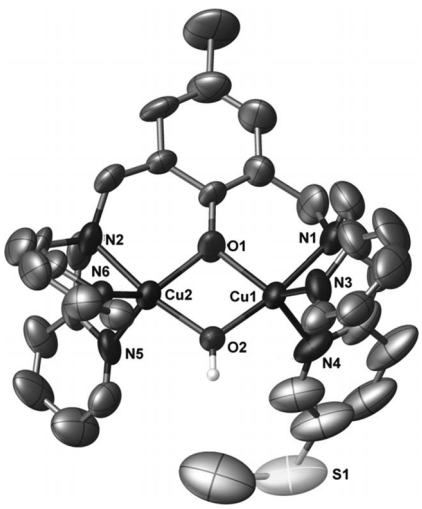

Figure 2. Plot of dication $[Cu_2(m\text{-}L_{SMe})(\mu\text{-}OH)]^{2+}$ in its majority form (see Exp. Sect.). Hydrogen atoms (except for the hydroxido bridge) and ClO_4^- counteranions were omitted for clarity. Ellipsoids are drawn at the 30 % probability level.

In this structure, the Cu1–O1–Cu2 bond angle is 100.95°. The Cu–O distances in the Cu_2O_2 unit are in the range of 1.884–1.938 Å and are slightly more asymmetric than the Cu–O distances in $[Cu_2(L)(\mu\text{-}OH)](ClO_4)_2$ (1.928–1.961 Å). The four Cu1, Cu2, O1, and O2 atoms are in the same plane. The Cu1–O1–Cu2, Cu1–O2–Cu2, O1–Cu1–O2, and O1–Cu2–O2 angles are 100.99, 104.23, 77.49, and 76.99°, respectively, leading to a Cu1···Cu2 distance of 2.9755(8) Å, which is similar to the intermetallic distance (2.966 Å) in $[Cu_2(L)(\mu\text{-}OH)](ClO_4)_2$.[14]

Characterization in Solution

UV/Vis spectra of $[Cu_2(m\text{-}L_{SMe})(\mu\text{-}OH)](ClO_4)_2$ and $[Cu_2(p\text{-}L_{SMe})(\mu\text{-}OH)](ClO_4)_2$ recorded in acetonitrile at 298 K show two transitions in the regions of 440–460 and 785–820 nm (Table 1). The first transition corresponds to charge transfer from the phenoxido and hydroxido bridges to the copper(II) ions, whereas the second one is assigned to the d–d transition of the Cu^{II} ions.[16] These transitions are slightly affected by the position of the electron-donating SMe substituent (i.e., *para* or *meta*) with respect to the corresponding pyridine nitrogen atom.

As previously observed with $[Cu_2(L)(\mu\text{-}OH)](ClO_4)_2$,[14] the two μ-phenoxido, μ-hydroxido complexes are EPR silent in frozen solutions, which indicates a strong antiferromagnetic coupling ($S = 0$) ground spin state between the two copper(II) atoms. This observation is consistent with a doubly bridged structure and the short copper–copper distance evidenced in the solid state for $[Cu_2(L)(\mu\text{-}OH)](ClO_4)_2$[14] and $[Cu_2(m\text{-}L_{SMe})(\mu\text{-}OH)](ClO_4)_2$.

The electrochemical behavior of the series of complexes was investigated by cyclic voltammetry (CV) in acetonitrile solution, with tetra-*n*-butylammonium perchlorate (TBAP) as supporting electrolyte (0.1 M). The potentials were referenced to a Ag/10 mM $AgNO_3$ + CH_3CN + 0.1 M TBAP reference electrode (regular ferrocene/ferrocenium redox couple = +0.089 V under the experimental conditions). The CV curves of both complexes are very similar and close to the one published for the $[Cu_2(L)(\mu\text{-}OH)](ClO_4)_2$,[14] suggesting that the addition of a SMe group on one pyridine of the ligand in a *m*- or *p*-position does not affect the electrochemical behavior of the corresponding di-nuclear complex. The CV curves are characterized by three electrochemical signals (Table 1, Figure 3). In the negative region of the potential, the first wave at –0.95V and –0.98 V for the *p*- and *m*-substituted complexes, respectively, is reversible and corresponds to a one-electron exchange leading to a mixed valence Cu^{II}–Cu^{I} species. This signal is followed by a second one which is irreversible at the time scale of the CV and leading to the Cu^{I}–Cu^{I} complex at E_{pc} = –1.26 V

Table 1. UV/Vis spectroscopic data, electrochemistry, catalytic behavior of complexes $[Cu_2(L)(\mu\text{-}OH)](ClO_4)_2$; $[Cu_2(m\text{-}L_{SMe})(\mu\text{-}OH)](ClO_4)_2$ and $[Cu_2(p\text{-}L_{SMe})(\mu\text{-}OH)](ClO_4)_2$.

	UV/Vis, λ_{max}/nm (ε/M^{-1}·cm^{-1})[a]		Electrochemistry[b] Peaks potential/V	Catalytic activity			
	LMCT	d-d	$E_{pc}/E_{1/2}/E_{pa}$	V_{max}[c] 10^{-6} M^{-1}·s^{-1}	K_M[c] mM	k_{cat}[c] 10^{-3} s^{-1}	TON[d] (10 min)
$[Cu_2(L)(\mu\text{-}OH)](ClO_4)_2$[14]	445(480)	785(280)	–1.24/–0.96/+0.82	1.92	2.46	7.68	2.01
$[Cu_2(p\text{-}L_{SMe})(\mu\text{-}OH)](ClO_4)_2$	454(342)	819(208)	–1.26/–0.95/+0.79	1.94	2.38	7.76	2.20
$[Cu_2(m\text{-}L_{SMe})(\mu\text{-}OH)](ClO_4)_2$	459(490)	816(260)	–1.29/–0.98/+0.76	1.70	4.24	6.80	1.34

a) Recorded at 25 °C in CH_3CN; b) 1 mM, in CH_3CN + 0.1 M TBAP, WE: glassy carbon (ϕ = 5 mm), v = 0.1 V·s^{-1}, E vs. $Ag/AgNO_3$; c) Determined at 25 °C in CH_3CN from Lineweaver–Burk plot, [3,5-dtbc] = 2.5–60 mM, [complex] = 0.25 mM; d) [3,5-dtbc] = 25 mM, [complex] = 0.25 mM.

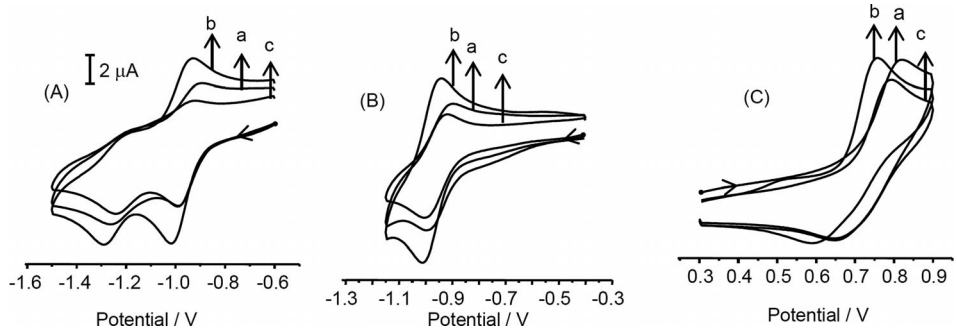

Figure 3. Voltammetric curves of $[Cu_2(L)(\mu\text{-}OH)](ClO_4)_2$ (a); $[Cu_2(m\text{-}L_{SMe})(\mu\text{-}OH)](ClO_4)_2$ (b) and $[Cu_2(p\text{-}L_{SMe})(\mu\text{-}OH)](ClO_4)_2$ (c) (1 mM) in CH_3CN + TBAP 0.1 M solution recorded at a stationary vitreous carbon working electrode (Ø = 5 mm); ν = 0.1 V·s^{-1}; E vs. Ag/Ag$^+$ 10^{-2} M; (A) scan between –0.6 V and –1.5 V; (B) scan between –0.4 V and –1.15 V; (C) scan between +0.3 V and 0.9 V.

and –1.29 V for the *p*- and *m*-substituted complex, respectively. As previously described[17] OH^- bridges have a poor ability to bind Cu^I atoms and tend to dissociate upon reduction, causing the irreversibility of the electrochemical system. The anodic part of the CV curves (Figure 3) displays one fully irreversible electrochemical signal at around +0.8 V, which likely results from oxidation centered on the phenol ligand.

Catalytic Activities

The catalytic activity of $[Cu_2(m\text{-}L_{SMe})(\mu\text{-}OH)](ClO_4)_2$ and $[Cu_2(p\text{-}L_{SMe})(\mu\text{-}OH)](ClO_4)_2$ for the oxidation of 3,5-di-*tert*-butylcatechol (3,5-dtbc) was studied and compared to that of $[Cu_2(L)(\mu\text{-}OH)](ClO_4)_2$. The kinetic studies were performed in acetonitrile solution saturated with dioxygen; the initial rate method was used and involved monitoring the development of the UV/Vis band at 400 nm, which corresponds to the absorption of the quinone (ε = 1640 M^{-1}· cm^{-1}) produced over time. Upon treating a 0.25 mM solution of the complexes with 100 equivalents of 3,5-dtbc, $[Cu_2(m\text{-}L_{SMe})(\mu\text{-}OH)](ClO_4)_2$ and $[Cu_2(p\text{-}L_{SMe})(\mu\text{-}OH)](ClO_4)_2$ exhibited a catechol oxidase catalytic activity in the same range as that observed with $[Cu_2(L)(\mu\text{-}OH)](ClO_4)_2$ (Table 1).The initial rate of oxidation was also measured as a function of the substrate concentration (2.5–60 mM) and shown that the reaction follows Michaelis–Menten behavior. Lineweaver–Burk treatment of the data gave the V_{max}, K_M and k_{cat}values presented in Table 1. The catecholase activity of the μ-hydroxido complexes is slightly modulated by the introduction of the thiomethyl group on one pyridine arm. In the *para* position of the pyridine coordinated to the copper ion, the SMe group moderately increases the activity, while substitution in the *meta* position decreases the activity. These results provide evidence that the catalytic properties of the complexes depend on substrate binding, which is reflected by the K_M value of the μ-OH dicopper(II) complexes (Table 1) in the presence of 3,5-dtbc.

Conclusions

Two new phenol-based ligands, *m*-HL_{SMe} and *p*-HL_{SMe}, bearing pyridine-containing pendant arms with one SMe group were synthesized. The corresponding doubly bridged dinuclear copper(II) complexes were isolated and characterized by UV/Vis and EPR spectroscopy, single-crystal X-ray analysis (of the complex with the SMe substituent at the *meta* position), and electrochemistry. The presented compounds mimic the active site of type 3 copper enzymes and, in particular, the distinct environments of the metal ions in relation with a thiomethyl group on the pyridine arm. To date, the experimental results from models are not sufficiently clear to enable definitive and general conclusions. Nevertheless, some features can be identified: both complexes are active catalysts for the oxidation of 3,5-di-*tert*-butylcatechol to the corresponding quinone, and the catalytic properties of the complexes depend on substrate binding, as reflected by the K_M values determined for the complexes in the presence of 3,5-dtbc. The K_M values are associated with the ability of the complex to form an adduct with the substrate and do not directly correlate with the redox properties of the dicopper center. As this type of sulfur ligation in copper proteins is unusual, a sound understanding via biomimetic models remains an open research area.

Experimental Section

General: All reagents were purchased from commercial sources and used as received. Solvents were purified by standard methods before use.

Caution: Although no problems were encountered, suitable care and precautions should be taken when handling the perchlorate salts.

Elemental analyses were performed by the CNRS Microanalysis Laboratory of Lyon, France. ESI mass spectra were recorded with an Esquire 300 plus Bruker Daltonics with nanospray inlet. EPR spectra were recorded at 100 K with a Bruker ESP 300 spectrometer operating at 9.4 GHz (X-band) in H_2O/DMSO (1/1 v/v, 1 mM).

UV/Vis spectra were obtained using a Perkin–Elmer Lambda 2 spectrophotometer operating in the 200–1100 nm range with quartz cells. Temperature was maintained at 298 K with a temperature control unit. ^{1}H NMR spectra were recorded with a Bruker Advance 300 spectrometer at 298 K with the deuterated solvent as lock.

The syntheses of the secondary amines **2** and **3** are described in the Supporting Information.

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Synthesis

***m*-HL$_{SMe}$:** To a solution of **1** (0.35 g, 1 mmol) in dry dichloromethane (10 mL) at 0 °C $SOCl_2$ (221 μL, 3 mmol) in dry dichloromethane (2 mL) was added dropwise under nitrogen. The resulting suspension was stirred for 3 h at 0 °C and the solvents evaporated to dryness under reduced pressure. The resulting residue was washed with dry pentane leading to a solid residue. At 0 °C under nitrogen, NaH (0.1 g, 2.5 mmol, 60 % dispersion in mineral oil) washed with dry pentane and amine **2** (0.247 g, 1.0 mmol) dissolved in dry CH_2Cl_2 (5 mL) were slowly mixed and the solution was stirred for 2 h at 0 °C. The mixture was then added dropwise to a solution of the former residue 2-(*N*,*N*-bis(2-methylpyridyl)aminomethyl)-6-(chloromethyl)-4-methylphenol in dry dichloromethane (8 mL) and triethylamine (0.43 mL, 3.0 mmol). The resulting solution was stirred at room temperature for 2 days and afterwards cooled in 0 °C and methanol (25 mL) was added. The solution was evaporated to dryness under reduced pressure, and the residue was redissolved in dichloromethane. The pH of the solution was adjusted to about 8–9 by addition of $NaHCO_3$. The slightly basic solution was extracted four times with dichloromethane. The extracts were combined, washed with brine and dried with anhydrous Na_2SO_4. The solvent was removed in vacuo. The residue was purified by column chromatography on silica gel (acetone) to give the ligand ***m*-HL$_{SMe}$** (0.355 g, 61 %) as a yellow oil. **^{1}H NMR** (300 MHz, $CDCl_3$, Me_4Si): δ = 10.80 (s, 1 H, O*H*), 8.68 (s, *J* = 1.9 Hz, 1 H, Py(SMe)-*oH*), 8.60 (d, *J* = 4.2 Hz, 3 H, Py-*oH*), 7.55 (d, *J* = 8.2 Hz 1 H, Py(SMe)-*mH*), 7.51 (td, *J* = 7.5 Hz, *J* = 7.4 Hz, 3 H, Py-*mH*), 7.36–7.48 (m, 4 H, Py(SMe)-*pH* and Py-*pH*), 7.11 (t, *J* = 6.2 Hz, 3 H, Py-*mH*), 6.98 (2, 2 H, Ph-*H*), 3.87(s, 6 H, N-CH_2-Py), 3.82 (s, 2 H, N-CH_2-Py(SMe), 3.78 (s, 4 H, Ph-CH_2-N), 2.44 (s, 3 H, S-CH_3) and 2.23 (s, 3 H, CH_3). **^{13}C NMR** (75.5 MHz, $CDCl_3$, Me_4Si): δ = 159.5, 156.5, 153.8, 149.1, 147.4, 136.7, 135.5, 133.4, 130.0, 127.5, 124.0, 123.9, 123.1, 122.1, 60.0, 59.5, 55.1, 55.0, 20.8 and 16.3. **MS** (DCI): *m*/*z* (%) = 577(100 %) (M^+).

***p*-HL$_{Sme}$:** This ligand was prepared following a similar procedure as described for *p*-HL$_{Sme}$; however, the amine **3** was used instead of amine **2**. Yield (50 %) **^{1}H NMR** (300 MHz, $CDCl_3$, Me_4Si): δ = 10.80 (s, 1 H, O*H*), 8.64 (d, *J* = 4.2 Hz, 3 H, Py-*oH*), 8.54 (d, *J* = 1.9 Hz, 1 H, Py(SMe)-*oH*), 7.82 (td, *J* = 7.5 Hz, *J* = 7.3 Hz, 3 H, Py-*pH*), 7.58 (d, *J* = 5.2 Hz, 3 H, Py-*mH*), 7.50 (d, *J* = 8.2 Hz, 1 H, Py(SMe)-*mH*), 7.29 (td, 3 H, *J* = 6.2 Hz, *J* = 7.5 Hz, Py-*mH*), 6.50 (s, 2 H, Ph-*H*), 3.95 (s, 6 H, N-CH_2-Py), 3.91 (s, 2 H, N-CH_2-Py(SMe), 3.88 (s, 4 H, Ph-CH_2-N), 2.47 (s, 3 H, S-CH_3) and 2.35 (s, 3 H, CH_3). **MS** (DCI): *m*/*z* (%) = 577(100 %) (M^+).

[Cu$_2$(*m*-L$_{SMe}$)(μ-OH)](ClO$_4$)$_2$: To *m*-HL$_{SMe}$, (288 mg, 0.5 mmol) dissolved in CH_3CN (15 mL), a solution of $Cu(ClO_4)_2 \cdot 6H_2O$ (378 mg, 1 mmol) in CH_3CN (5 mL) and Et_3N (210 μL, 1.12 mmol) were added dropwise. The solution turned green and was stirred for 1 h at room temperature. The solvent was partially removed under reduced pressure and the resulting solution (3 mL) after addition of THF was allowed to stand at –20 °C for four days. A green powder (321 mg) was collected by filtration (70 %). Crystals of X-ray quality were obtained by vapor diffusion of THF in a CH_3CN solution. **ESI-MS:** *m*/*z*: *z* = 1, 819 = [M – ClO_4^-]; *z* = 2, 360 = [M – 2ClO_4^-]. **UV/Vis** (CH_3CN): λ (ε, $M^{-1} \cdot cm^{-1}$) = 459(490) and 816(260) nm. $C_{34}H_{36}N_6O_{10}SCu_2Cl_2 \cdot C_4H_8O$; C 45.55 (calc 46.06); H 4.48 (4.47); N 8.48 (8.51) %.

[Cu$_2$(*p*-L$_{SMe}$)(μ-OH)](ClO$_4$)$_2$: This complex was prepared by a similar procedure as described for [Cu$_2$(*m*-L$_{SMe}$)(μ-OH)](ClO$_4$)$_2$; however, the ligand *p*-HL$_{Sme}$ was used instead of *m*-HL$_{SMe}$. An oily compound was obtained. After dissolution in a small amount of CH_3CN and dropwise addition to a large amount of ethyl ether, the green precipitate [Cu$_2$(*p*-L$_{SMe}$)(μ-OH)](ClO$_4$)$_2$ was obtained (yield = 48 %). For analytic purposes the crude product was purified on a Sephadex LH-20 column in dichloromethane. **ESI-MS:** *m*/*z*: *z* = 1, 819 = [M – ClO_4^-]; *z* = 2, 360 = [M – 2ClO_4^-]. **UV/Vis** (CH_3CN): λ (ε, $M^{-1} \cdot cm^{-1}$) = 454(342) and 819(208) nm. $C_{34}H_{36}N_6O_{10}SCu_2Cl_2 \cdot 5H_2O$; C 40.11 (calcd. 40.48); H 3.56 (4.60); N 8.25 (8.33) %.

Structure Determination and Refinement: A single crystal of the complex [Cu$_2$(*m*-L$_{SMe}$)(μ-OH)](ClO$_4$)$_2$·THF was mounted on a Kappa CCD Nonius diffractometer equipped with graphite-monochromated Mo-K_α radiation (λ = 0.71073 Å) at 200 K.

$C_{34}H_{36}N_6O_{10}SCu_2Cl_2 \cdot C_4H_8O$: *M* = 990.83 g·mol^{-1}, emerald green needle (0.36 × 0.16 × 0.12 mm), orthorhombic, space group *Pna*2$_1$, *a* = 19.098(4) Å, *b* = 17.566(4) Å, *c* = 12.358(3) Å, $\alpha = \beta = \gamma = 90.00°$, *V* = 4145.7(14) Å^3, D_c = 1.587 g·cm^{-3}, *Z* = 4, μ(Mo-K_α) = 1.272 mm^{-1}, 40615 reflections measured [R_{int} = 0.0363], 10105 unique (Friedel's included), 6648 with $F > 2\sigma$ and final *R* values R_1 = 0.0711 [$F > 2\sigma$]; wR_2 = 0.1636 (all data).The goodness of fit on F^2 was 1.071.

The structure was solved by direct methods implemented by SIR-92.[18] Refinement was performed using SHELXL[19] run under OLEX2.[20] C, N, O, S, Cl, and Cu atoms were refined anisotropically by the full-matrix least-squares method. Hydrogen atoms were geometrically placed and constrained to ride on their bearing atoms.

The space group determination led to the non centrosymmetric *Pna*2$_1$ group. The obtained model displays one formula unit within the asymmetric cell. The structure refinement was not satisfying due to a racemic twinning with two domains in a 1:1 ratio. The model was then refined according the twin law but despite a strong improvement, the result was still not sufficient. Three different kinds of disorder had to be treated to reach the final model. The first was for the two different positions for the thiomethyl group, which were observed on the sides of either Cu1 or Cu2 with an approximate 3:1 ratio, respectively. The second disorder was trickier, it could be best described as follows: half of the ligand surrounding Cu2 could be positioned on two different positions affected from a rotation of 60° around the Cu2–N2 axis. These two positions were roughly in a 2:1 ratio. The third disorder came from the THF solvent molecule which appeared in two different positions in a 1:1 ratio. Crystallographic data (excluding structure factors) for the structure reported in this paper have been deposited with the Cambridge Crystallographic Data Centre as supplementary publication no. CCDC-906835. Copies of the data can be obtained free of charge on application to CCDC, 12 Union Road, Cambridge CB2 1EZ, UK [Fax: + 44 1223/336-033; E-mail: deposit@ccdc.cam.ac.uk].

Electrochemistry: Electrochemical experiments were carried out using a PAR model 273 potentiostat equipped with a Kipp-Zonen x-y recorder. All experiments were run at room temperature under argon. A standard three-electrode cell was used. 0.1 M tetra-*n*-butylammonium perchlorate (TBAP) in CH_3CN was used as supporting electrolyte. All potentials are referred to an Ag/10 mM $AgNO_3$ + CH_3CN + 0.1 M TBAP reference electrode. The redox potential of the regular ferrocene/ferrocenium redox couple used as an internal reference was +0.089 V under experimental conditions. The working electrode was a vitreous carbon disc electrode (5 mm diameter) polished with 1 μm diamond paste prior to each record.

Catecholase Activity: The catecholase activity of complexes was evaluated by reaction with 3,5-di-*tert*-butylcatechol (3,5dtbc) at 25 °C.

The absorption at 400 nm, characteristic of the formed quinone (3,5-di-*tert*-butyl-*o*-benzoquinone) was measured as a function of time. The experiments were run in acetonitrile saturated with dioxygen. The kinetic parameters were determined for 2.5×10^{-4} M solutions of complexes and 2.5 mM–60 mM solutions of the substrate.

Supporting Information (see footnote on the first page of this article): Description of the syntheses of amines **2** and **3**, bond lengths and angles for $[Cu_2(m\text{-}L_{SMe})(\mu\text{-}OH)](ClO_4)_2 \cdot THF$.

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References

[1] C. Gerdemann, C. Eicken, B. Krebs, *Acc. Chem. Res.* **2002**, *35*, 7019.
[2] E. I. Solomon, U. M. Sudaram, T. E. Mackonkin, *Chem. Rev.* **1996**, *96*, 2563.
[3] T. Klabunde, C. Eicken, J. C. Sacchettini, B. Krebs, *Nat. Struct. Biol.* **1998**, *5*, 1084.
[4] W. T. Ismaya, H. J. Rozeboom, A. Weijn, J. J. Mes, F. Fusetti, H. J. Wichers, B. W. Dijkstra, *Biochemistry* **2011**, *50*, 5477.
[5] V. M. Virador, J. P. R. Grajeda, A. Blanco-Labra, E. Mendiola-Olaya, G. M. Smith, A. Moreno, J. R. Whitaker, *J. Agric. Food Chem.* **2010**, *58*, 1189.
[6] K. Lerch, *J. Biol. Chem.* **1982**, *257*, 6414.
[7] A. W. J. W. Tepper, E. Lonardi, L. Bubacco, G. W. Canters, in *Handbook of metalloproteins* (Ed.: A. Messerschmidt), John Wiley, Chicherster, **2010**, p. DOI: 10.1002/0470028637.met265.
[8] a) I. A. Koval, P. Gamez, C. Belle, K. Selmeczi, J. Reedijk, *Chem. Soc. Rev.* **2006**, *35*, 814; b) M. Rolff, J. Schottenheim, H. Decker, F. Tuczek, *Chem. Soc. Rev.* **2011**, *40*, 4077.
[9] a) I. A. Koval, M. Huisman, A. F. Stassen, P. Gamez, O. Roubeau, C. Belle, J. L. Pierre, E. Saint Aman, M. Lüken, B. Krebs, M. Lutz, A. L. Spek, J. Reedijk, *Eur. J. Inorg. Chem.* **2004**, 4036; b) M. Merkel, N. Möller, M. Piacenza, S. Grimme, A. Rompel, B. Krebs, *Chem. Eur. J.* **2005**, *11*, 1201.
[10] a) D. E. Fenton, *Chem. Soc. Rev.* **1999**, *28*, 159; b) C. Belle, J. L. Pierre, *Eur. J. Inorg. Chem.* **2003**, 4137.
[11] C. Belle, K. Selmeczi, S. Torelli, J. L. Pierre, *C. R. Chim.* **2007**, *10*, 271.
[12] Recent representative examples: a) C. Bochot, E. Favre, C. Dubois, B. Baptiste, L. Bubacco, P.-A. Carrupt, G. Gellon, R. Hardré, D. Luneau, Y. Moreau, A. Nurisso, M. Réglier, G. Serratrice, C. Belle, H. Jamet, *Chem. Eur. J.* **2013**, *19*, 3655; b) S. J. Smith, R. A. Peralta, R. Jovito, A. Horn, A. J. Bortoluzzi, C. J. Noble, G. R. Hanson, R. Stranger, V. Jayaratne, G. Cavigliasso, L. R. Gahan, G. Schenk, O. R. Nascimento, A. Cavalett, T. Bortolotto, G. Razzera, H. Terenzi, A. Neves, M. J. Riley, *Inorg. Chem.* **2012**, *51*, 2065; c) F. Heims, V. Mereacre, A. Ciancetta, S. Mebs, A. K. Powell, C. Greco, K. Ray, *Eur. J. Inorg. Chem.* **2012**, 4565; d) M. Carboni, M. Clémancey, F. Molton, J. Pécaut, C. Lebrun, L. Dubois, G. Blondin, J. M. Latour, *Inorg. Chem.* **2012**, *51*, 10447; e) F. R. Xavier, A. J. Bortoluzzi, A. Neves, *Chem. Biodiversity* **2012**, *9*, 1794; f) M. Jarenmark, M. Haukka, S. Demeshko, F. Tuczek, L. Zuppiroli, F. Meyer, E. Nordlander, *Inorg. Chem.* **2011**, *50*, 3866.
[13] C. Belle, G. Gellon, C. Scheer, J. L. Pierre, *Tetrahedron Lett.* **1994**, *35*, 7019.
[14] S. Torelli, C. Belle, I. Gautier Luneau, J. L. Pierre, E. Saint Aman, J. M. Latour, L. Le Pape, D. Luneau, *Inorg. Chem.* **2000**, *39*, 3526.
[15] A. W. Addison, T. N. Rao, J. Reedijk, J. van Rijn, G. Verschoor, *J. Chem. Soc., Dalton Trans.* **1984**, 1349.
[16] B. J. Hathaway, *Struct. Bonding (Berlin)* **1984**, *57*, 55.
[17] J. Reim, B. Krebs, *J. Chem. Soc., Dalton Trans.* **1997**, 3793.
[18] A. Altomare, G. Cascarano, C. Giacovazzo, A. Guagliardi, *J. Appl. Crystallogr.* **1993**, *26*, 343.
[19] G. M. Sheldrick, *Acta Crystallogr., Sect. A* **2008**, *64*, 112.
[20] O. V. Dolomanov, L. J. Bourhis, R. J. Gildea, J. A. K. Howard, H. Puschmann, *J. Appl. Crystallogr.* **2009**, *42*, 339.

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